ChemPubSoc Europe

DOI: 10.1002/cmdc.201300053

An Efficient Bioorthogonal Strategy Using CuAAC Click Chemistry for Radiofluorinations of SNEW Peptides and the Role of Copper Depletion**

Marc Pretze,[a, b] Manuela Kuchar,[a, b] Ralf Bergmann,[a] Jörg Steinbach,[a, b] Jens Pietzsch,[a, b] and Constantin Mamat*[a, b]

The EphB2 receptor is known to be overexpressed in various types of cancer and is therefore a promising target for tumor cell imaging by positron emission tomography (PET). In this regard, imaging could facilitate the early detection of EphB2-overexpressing tumors, monitoring responses to therapy directed toward EphB2, and thus improvement in patient outcomes. We report the synthesis and evaluation of several fluorine-18-labeled peptides containing the SNEW amino acid motif, with high affinity for the EphB2 receptor, for their potential as radiotracers in the non-invasive imaging of cancer using PET. For the purposes of radiofluorination, EphB2-antagonistic SNEW peptides were varied at the C terminus by the introduction of L-cysteine, and further by alkyne- or azide-modified amino acids. In addition, two novel bifunctional and bioorthogonal labeling building blocks [^{18}F]AFP and [^{18}F]BFP were applied, and their capacity to introduce fluorine-18 was compared with that of the established building block [^{18}F]FBAM. Copper-assisted Huisgen 1,3-dipolar cycloaddition, which belongs to the set of bioorthogonal click chemistry reactions, was used to introduce both novel building blocks into azide- or alkyne-modified SNEW peptides under mild conditions. Finally, the depletion of copper immediately after radiolabeling is a highly important step of this novel methodology.

Introduction

Eph receptors have recently gained much attention in cancer research owing to their dysregulation in various tumor entities.[1–3] Representing the largest family of receptor tyrosine kinases (RTK), Eph receptors play important roles in carcinogenesis, tumor angiogenesis, metastasis, and apoptosis.[4,5] In particular, the EphB2 receptor is overexpressed in various solid tumor entities including gastric,[6] colorectal,[7] ovarian,[8] breast,[9] prostate,[10] and brain.[11] EphB2 receptor expression levels differ depending on the tumor stage. Early-stage tumors show a substantially higher expression of EphB2;[12] in contrast, the expression of EphB2 is down-regulated in later stages. Imaging of the EphB2 receptor is therefore considered to be an important tool in the diagnosis of early-stage tumor entities including metastasis as well as for monitoring anticancer therapy.

There are two general approaches for targeting the EphB2 receptor. One involves an intracellular tyrosine kinase domain, on which the receptor can be inhibited, particularly through the use of small organic kinase inhibitors.[13–16] The other involves an extracellular domain, which is available to bind ephrin ligands (B1, B2, and A5 in the case of EphB2). In almost the same manner, small organic molecules[17,18] as well as peptides[19] (e.g., with the key amino acid sequence SNEW) have been identified as high-affinity antagonist ligands.[20,21]

In principle, the radiolabeling of high-molecular-weight compounds such as peptides, proteins, or antibodies with fluorine-18 poses a considerable challenge. A radiolabeling strategy involving ^{18}F-labeled prosthetic groups, also known as ^{18}F-labeling building blocks, is necessary owing to the harsh reaction conditions required for the direct introduction of [^{18}F]fluoride into these biomacromolecules at high specific activity levels. Because of this and the high number of functional groups present in the peptides, a labeling strategy for nearly every peptide must be developed. For this purpose, the conventional building block *N*-(6-(4-[^{18}F]fluorobenzylidene)aminooxyhexyl)-maleimide ([^{18}F]FBAM) was initially chosen.[22–24] [^{18}F]FBAM is available in high radiochemical yield using a remotely controlled synthesis module. Based on radiolabeling results with [^{18}F]FBAM, the search for viable alternatives based on bioorthogonal reactions such as Staudinger ligation,[25,26] tetrazine click,[27] or copper-mediated Huisgen click reaction[28–31] was the main subject of the research effort reported herein.

Two novel and versatile labeling building blocks, 1-(but-3-ynyl)-4-(3-[^{18}F]fluoropropyl)piperazine ([^{18}F]BFP) and 1-(3-azidopropyl)-4-(3-fluoropropyl)piperazine ([^{18}F]AFP),[32,33] were introduced for our purposes, and subsequent radiofluorinations were carried out with the 1,3-dipolar Huisgen cycloaddition

[a] M. Pretze, M. Kuchar, Dr. R. Bergmann, Prof. Dr. J. Steinbach, Prof. Dr. J. Pietzsch, Dr. C. Mamat
Institut für Radiopharmazeutische Krebsforschung
Helmholtz-Zentrum Dresden-Rossendorf
Bautzner Landstraße 400, 01328 Dresden (Germany)
E-mail: c.mamat@hzdr.de

[b] M. Pretze, M. Kuchar, Prof. Dr. J. Steinbach, Prof. Dr. J. Pietzsch, Dr. C. Mamat
Fachrichtung Chemie und Lebensmittelchemie
Technische Universität Dresden, 01062 Dresden (Germany)

[**] Parts of this work were presented as a Highlight Presentation under the topic "Radiochemie" at the 51st annual congress of the DGN 2013.

(click approach).[34] It is mandatory for all subsequent labeling methods that the peptide SNEWILPRLPQH be exclusively altered at the C terminus to maintain high affinity for the EphB2 receptor, as the ligand–receptor interaction is associated with the key amino acid sequence SNEW, located at the N terminus of the peptide.[21]

The work reported herein comprises the preparation and modification of new SNEW peptides as well as their radiolabeling with fluorine-18 using two different labeling strategies, including the application of novel fluorine-18-containing building blocks. Thus, the variation of peptides was achieved either by introduction of L-cysteine or by a connection of amino acids containing an alkyne or an azide residue. Further, the broad adaptability of [^{18}F]BFP and [^{18}F]AFP as labeling building blocks for radiofluorinations was analyzed. Importantly, a strategy for the thorough removal of copper species is essential for adjacent biological investigations. Finally, the feasibility of these fluorine-18-labeled SNEW peptides as potent and versatile imaging agents was evaluated by using small-animal PET in a biological pilot study.

Results and Discussion

Radiolabeling of cysteine-containing SNEW peptide 2 with [^{18}F]FBAM

In our first approach, the maleimide-based building block [^{18}F]FBAM was applied. [^{18}F]FBAM ([^{18}F]**1**) is well established, readily accessible, and in current use for the regioselective radiofluorination of cysteine-containing peptides. Cysteine-containing SNEW peptide **2** was prepared, and it reacted readily with non-radioactive FBAM (**1**) to yield reference peptide **3** in high yield (72%, $t_R = 11.1$ min).

For radiofluorinations, [^{18}F]FBAM was prepared by automated module synthesis.[35] Next, a solution of **2** in Sørensen phosphate buffer was added to the [^{18}F]FBAM-containing solution, and the mixture was incubated at 60 °C. After 60 min, a new peak at $t_R = 11.2$ min was observed in analytical radio-HPLC, indicating the formation of [^{18}F]**3** (Figure 1). The preparation of

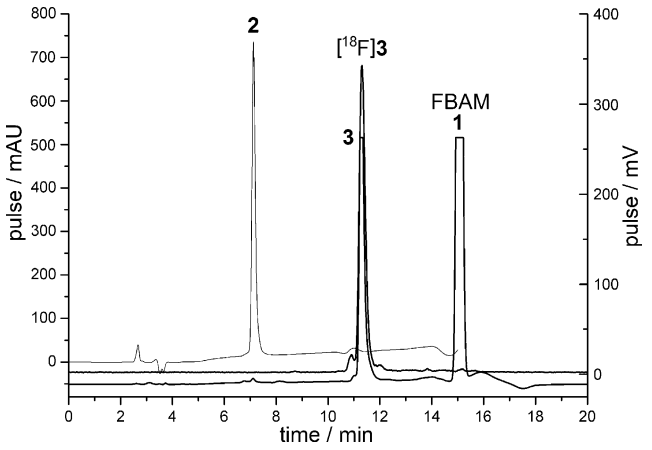
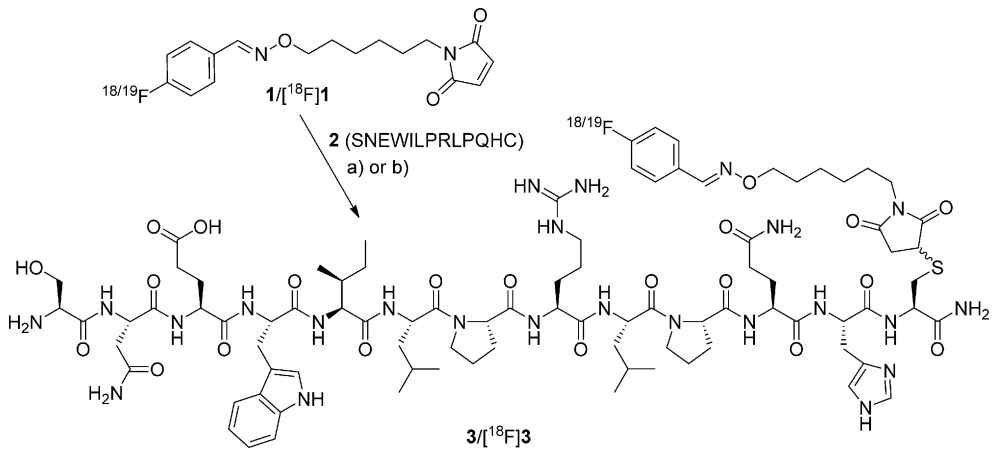


Figure 1. HPLC traces of starting peptide **2**, FBAM-labeled peptide **3**, FBAM **1**, and radiolabeled peptide [^{18}F]**3**.

reference **3** and tracer [^{18}F]**3** is outlined in Scheme 1. Optimizations were achieved, with the result that ~500 μg peptide **2** are required for an almost complete conversion of [^{18}F]FBAM after 60 min.

The stability of radiolabeled peptide [^{18}F]**3** was determined by treatment of [^{18}F]**3** with rat plasma, trypsin, and glycine·HCl buffer (pH 2.0), respectively, at 37 °C for 1 h. The metabolites of peptides are usually more hydrophilic than the parent peptide; however, it was found that most of the activity (>85%) had converted into a more lipophilic byproduct at $t_R = 14.3$ min relative to [^{18}F]**3** ($t_R = 11.2$ min) and [^{18}F]FBAM ($t_R = 14.7$ min), as determined by radio-HPLC analyses. Moreover, the same conversion was observed in tests of the non-radioactive peptide **3**. A signal at $m/z = 638$, which indicated twice the mass of FBAM **1**, was observed in ESI-MS analyses. Moreover, the Michael addition has been reported to be a reversible process.[36] Owing to the reversibility and instability of [^{18}F]**3**, it was necessary to evaluate an alternative, convenient and non-reversible strategy for the radiofluorination of SNEW peptides.

In addition, cysteine-containing starting peptide **2** was stored at −65 °C over a period of three months. Afterward,

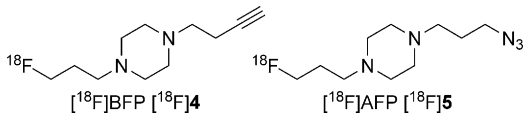


Scheme 1. Synthetic pathway to $^{18/19}$F-labeled SNEW peptide **3**/[^{18}F]**3** radiolabeled with [$^{18/19}$F]FBAM **1**/[^{18}F]**1**. *Reagents and conditions*: a) Sørensen phosphate buffer, CH_3CN, 60 °C, 2 h; *radiolabeling*: b) Sørensen phosphate buffer, CH_3CN, 60 °C, 1 h.

a solution of this stored peptide was prepared and analyzed by HPLC. Interestingly, one UV peak with a retention time 2 min later than the original peptide **2** was observed under the same HPLC conditions. Furthermore, reaction of [^{18}F]FBAM with the stored precursor **2** showed no formation of [^{18}F]**3**. Peptide **2** appeared to dimerize due to oxidization of the cysteine residues. To circumvent this, the solution was treated with tris(2-carboxyethyl)phosphine (TCEP) at ambient temperature for 1 h. After this procedure, the desired SNEW peptide **2** was observed by HPLC and was successfully applied as precursor.

Evaluation of an effective click radiolabeling strategy

The Cu^{I}-mediated azide–alkyne cycloaddition (CuAAC) reaction belongs to the set of bioorthogonal labeling reactions. Recently, the radiolabeling of human serum albumin as well as functionalized phosphopeptides and oligonucleotides was described.[28,30] To further improve this approach, [^{18}F]BFP ([^{18}F]**4**) and [^{18}F]AFP ([^{18}F]**5**)[33] were prepared as novel fluorine-18-containing building blocks with favorable properties, based on the piperazine skeleton. As an advantage, spiro-salts were used as precursors,[37] facilitating separation from the desired radiolabeled building block through the application of cartridges. Moreover, the labeling process can be performed in water or



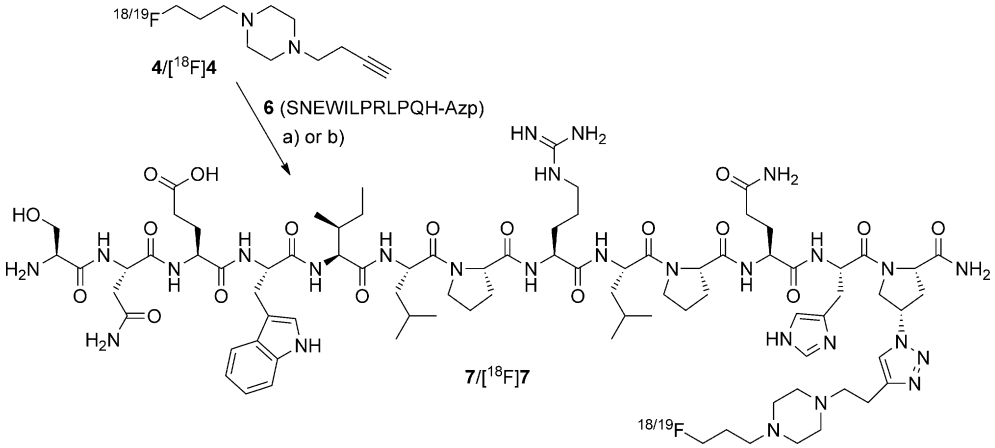
[^{18}F]BFP [^{18}F]**4** [^{18}F]AFP [^{18}F]**5**

buffer owing to the high hydrophilicity (log $D = 0.31$ for [^{18}F]**4**, 0.70 for [^{18}F]**5**).[33] Separation of the radiolabeled product from the starting peptide by size-exclusion chromatography (SEC) was made amenable due to the medium molar mass of these building blocks. An automated procedure was recently evaluated by using a remotely controlled synthesis module (TRACERlab Fx$_{FN}$) for the synthesis of [^{18}F]BFP and [^{18}F]AFP.[32,33] This allows radiofluorinations of the chosen modified SNEW peptides with high specific activities.

The following click labeling strategy was first evaluated with the alkyne-containing building block [$^{18/19}$F]BFP **4**/[^{18}F]**4**. A SNEW peptide was functionalized with *N*-Fmoc-(*S*)-4-azido-L-proline at the C terminus to obtain **6**. Afterward, the non-radioactive reference peptide **7** was prepared in 98% yield by the click reaction of BFP **4** with SNEW peptide **6** in Sørensen phosphate buffer and ethanol for 30 min at ambient temperature using sodium ascorbate and $CuSO_4$. Purification was carried out by preparative HPLC. For radiofluorination purposes, tris(benzyltriazolylmethyl)amine (TBTA), $CuSO_4$, and sodium ascorbate (6:1:10 equiv) were added to a mixture of SNEW peptide **6** (1 equiv) in Sørensen phosphate buffer at pH 7.2 and [^{18}F]BFP. Optimized conditions were found to be 0.3–0.5 mg mL^{-1} peptide at 60 °C for 60 min. More insoluble residues were found in the reaction mixture at higher peptide concentrations, which are thought to be an unknown triazole–copper species.[38] The purification of [^{18}F]**7** from starting peptide **6** was accomplished by semi-preparative HPLC followed by SEC to separate [^{18}F]**4** from [^{18}F]**7** due to the similar t_R values. Finally, [^{18}F]**7** was obtained in 62% RCY with >95% RCP ($n = 3$).

Metabolite analyses in rats indicated that most of the activity of [^{18}F]**7** resided in blood and furthermore substantially accumulated in liver and in lungs (PET data not shown). Moreover, the biodistribution showed that [^{18}F]**7** does not behave like a "normal" peptide, e.g., by showing a more prominent renal elimination. It was assumed that [^{18}F]**7**, as well as reference **7**, form aggregates with the copper species used for the click reaction. In this regard, subsequent MS analyses (Figure 2) of the respective reference peptide **7** gave evidence that the copper species used for the click reaction was partly bound by the peptide ($m/z = 943$ $[M + Cu]^{2+}$). Consequently, [^{18}F]**7** was considered inappropriate for further biological investigations.

This could also explain the low retention time of **7**/[^{18}F]**7**. Unfavorable strong copper complexes were formed with the SNEW peptide due to such residues as serine, histidine, and arginine.[39] This implies that the copper was not removed by



Scheme 2. Synthetic pathway to $^{18/19}$F-containing SNEW peptides **7**/[^{18}F]**7** radiolabeled with [$^{18/19}$F]BFP **4**/[^{18}F]**4**. *Reagents and conditions*: a) DMF, sodium ascorbate, $CuSO_4 \cdot 5H_2O$, 40 °C, 16 h; *radiolabeling*: b) DMF/Sørensen phosphate buffer, TBTA, sodium ascorbate, $CuSO_4 \cdot 5H_2O$, 50 W, 1 h.

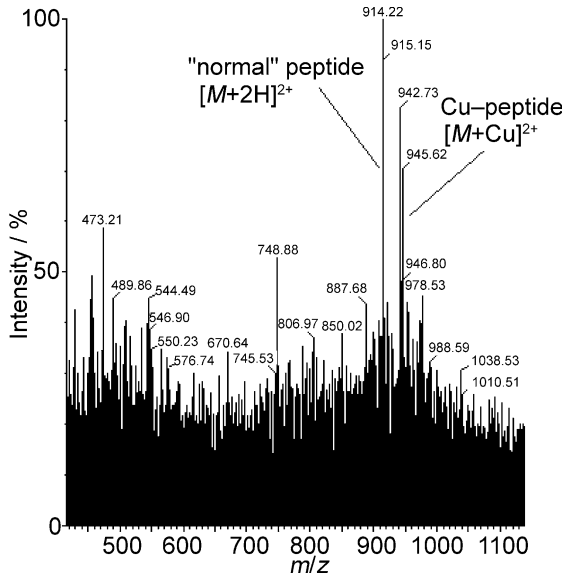


Figure 2. Part of the mass spectrum of **7** prepared in solution, indicating the formation of the Cu–peptide species.

HPLC or SEC purifications. Therefore, it was important to develop a new strategy that includes separation of the copper species from the desired peptide. On that account, peptide **7** was directly synthesized on solid support by incubating resin-bound **6** with BFP **4** under standard conditions, followed by cleavage from the resin at the end of the procedure. Unfortunately, subsequent HPLC analysis showed no differences in the retention time, still indicating the presence of copper species. However, the lyophilized product was colorless, and no copper impurities were found by ESI-MS analyses. Based on these findings, resin-bound peptide **7** was treated and incubated with strong chelating agents such as EDTA and bispidine (BIS),[40] respectively, after the click reaction with BFP **4**. Fortunately, peptide **7** showed a higher retention time ($t_R = 14.1$ min) after incubation with BIS and subsequent cleavage from resin; EDTA had no effect. Synthesis of reference peptide **7** on solid support showed high-yielding conversion from precursor peptides **6** by using BFP/azide/sodium ascorbate/$CuSO_4$ (1.5:1:55:36 *v/v*) in DMF after stirring at 40 °C for 16 h (98% yield). Moreover, the absence of copper was proven by ICP-MS analyses, determining the copper concentration in all reference peptide preparations; the results are summarized in Scheme 2 and Table 1.

Based on these results, an adequate radiofluorination strategy was developed, including the click labeling of resin-bound peptide **6** with [^{18}F]BFP, followed by a washing step with DMF and H_2O, incubation with BIS (2 equiv relative to $CuSO_4$) in methanol and DMF, a second washing step with DMF and H_2O, and final cleavage from the solid support with TFA/TIS (9:1 *v/v*). Nevertheless, radiosyntheses with [^{18}F]BFP showed very low yield for [^{18}F]**7** (0.4%, d.c.) in contrast to the non-radioactive reference **4** (98% yield). A higher reaction temperature (90 °C), microwave conditions (50, 90 W) for an electrostatic fixed peptide backbone,[41] longer reaction times (60, 120 min), and higher starting activities (3–6 GBq [^{18}F]BFP) gave no significant changes in the RCY.

Table 1. Purification, HLPC, ESI-MS and ICP-MS analyses of synthesized peptides **7**, **9**, **11**, and **14**.[a]

Peptide	Purification	t_R [min]	ESI-MS [*m/z*]	[Cu] [μg g^{-1}]	$n_{Cu}/n_{pept.}$
7 (soln)	HPLC	4.1	943 $[M+Cu]^{2+}$	ND[b]	ND
7 (resin)	HPLC	4.1	1825 $[M+H]^+$	ND[c]	ND
7 (resin)	HPLC + BIS	15.0	1825 $[M+H]^+$	611	1:57
9 (resin)	HPLC + BIS	15.2	1841 $[M+H]^+$	692[d]	1:50
11 (resin)	HPLC + BIS	15.4	1813 $[M+H]^+$	501	1:70
14 (resin)	HPLC + BIS	13.9	1927 $[M+H]^+$	426	1:77

[a] ND: not determined. [b] Pale green. [c] Colorless. [d] Dissolved in H_2O $c = 1$ g mL^{-1}.

This prompted us to reconsider the position of the azide moiety of the starting peptide. The azide residue of peptide precursor **6** is very close to the Rink amide resin. Thus, the sterically hindered 4-azidoproline moiety of **6** could be the reason for the low yield of [^{18}F]**7**. Therefore, two modified SNEW peptide precursors **8** and **10** were prepared (Scheme 3). Peptide **8** contains an azidonorleucine moiety at the C terminus, whereas peptide **10** has an azidopentanamido moiety at the N terminus for an enhanced distance between the azide group and the Rink amide resin, but certainly a decrease in affinity for the EphB2 receptor. Unfortunately, both radiolabeled peptides [^{18}F]**9** and [^{18}F]**11** showed no significantly higher decay-corrected radiochemical yields ([^{18}F]**9**: 0.5–1.4%, [^{18}F]**11**: 1.8%). Results of the radiolabeling are summarized in Scheme 3 and Figure 3.

Further investigations revealed an unknown byproduct in all labeling reactions with [^{18}F]BFP ($R_f = 0.58$ in methanol) in addition to the desired peptides. In this case we found that Glaser

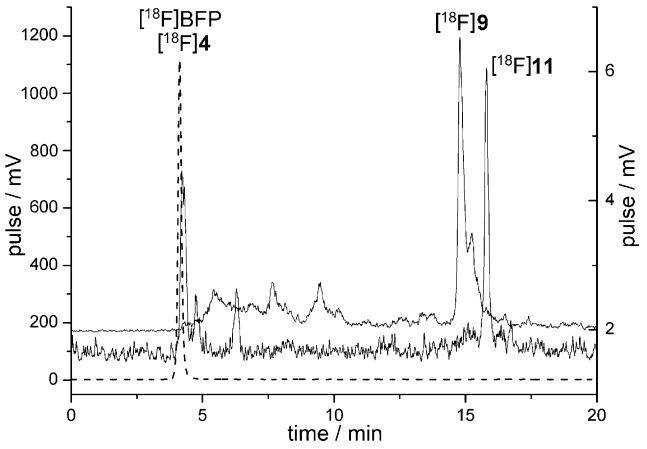
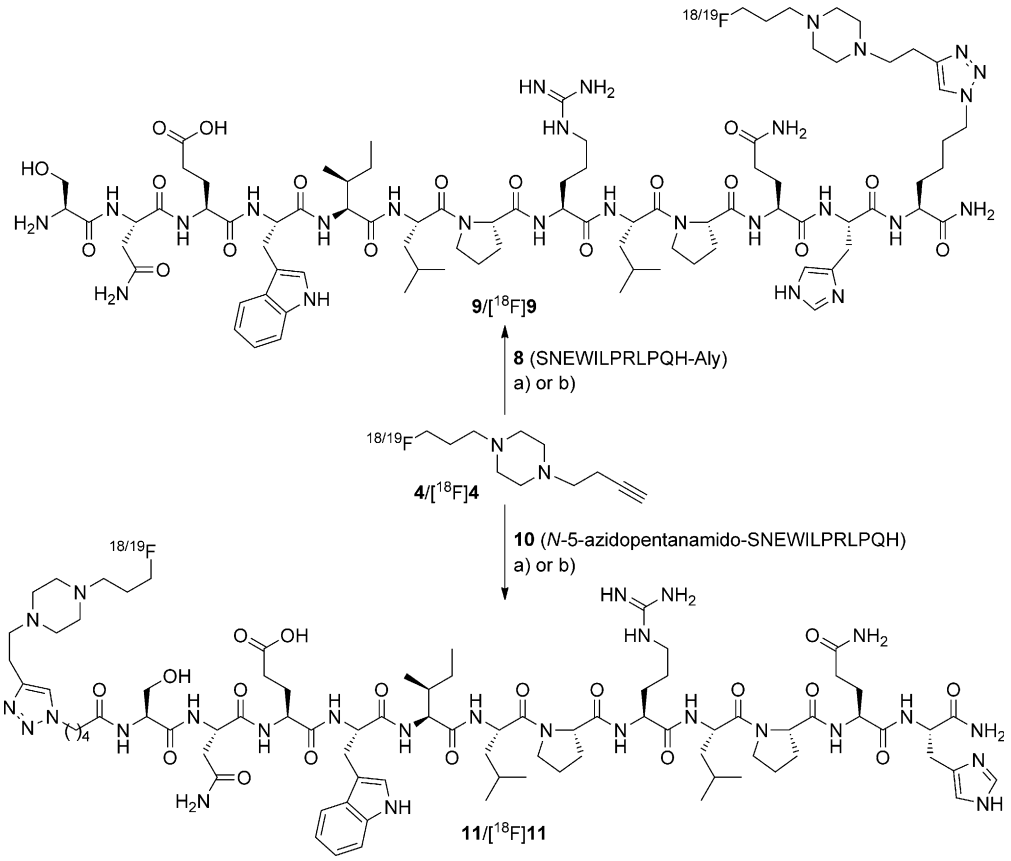
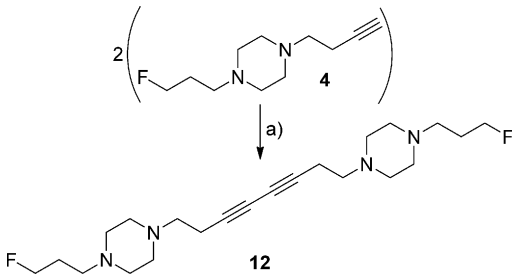


Figure 3. HPLC traces of [^{18}F]**4**, crude [^{18}F]**9**, and crude [^{18}F]**11**.

coupling[42] became the main reaction pathway instead of the Huisgen click reaction at very low concentrations of the alkyne [^{18}F]BFP, which generally occur in these radiosyntheses. To prove this assumption, non-radioactive BFP **4** was reacted in the absence of azides and yielded diyne **12** (Scheme 4). Subsequently, compound **12** ($R_f = 0.27$ in methanol) was compared with the unknown byproduct from all labeling reactions by means of (radio-)TLC to identify this byproduct as [^{18}F]**12**. In contrast, [^{18}F]BFP has an R_f value of 0.58 in methanol. HPLC



Scheme 3. Synthetic pathway to the ^{18}F-labeled SNEW peptides [^{18}F]**9** and [^{18}F]**11** as well as references **9** and **11** using [$^{18/19}$F]BFP **4**/[^{18}F]**4**. *Reagents and conditions*: a) DMF/H_2O, TBTA, sodium ascorbate, $CuSO_4$·5 H_2O, 50 °C, 3 days; *radiolabeling*: b) DMF/Sørensen phosphate buffer, TBTA, sodium ascorbate, $CuSO_4$·5 H_2O, 90 °C, 1 h or 50 W, 1.5 h.



Scheme 4. Glaser coupling of BFP **4** for the identification of byproduct **12**. *Reagents and conditions*: a) $CuSO_4$·5 H_2O, DMF, 40 °C, 3 days.

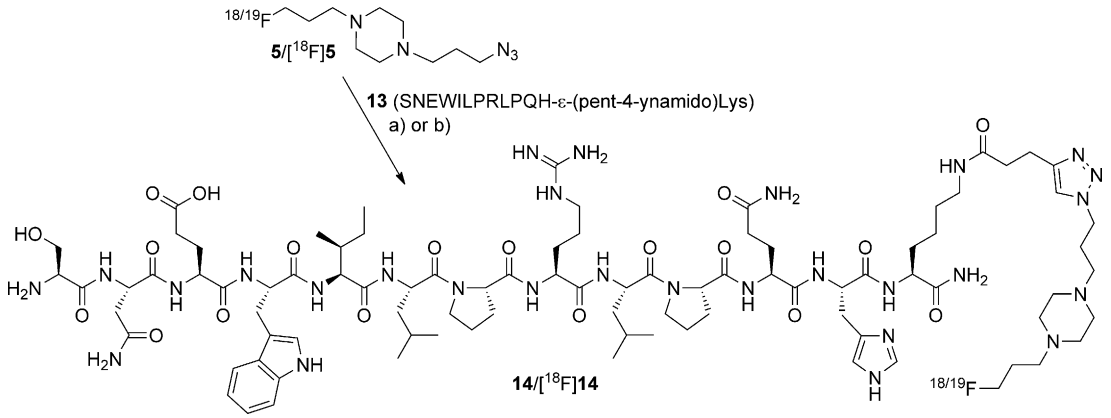
analyses of both compounds revealed t_R values of 3.3 min for the dimer **12** and 4.9 min for compound **4**.[43]

All these results prompted us to switch the functionalities of the peptide precursor and of the radiolabeling building block. Therefore, [^{18}F]AFP, similar to [^{18}F]BFP, was applied, and peptide precursor **13**, with a pentyne moiety, was synthesized with Rink amide resin preloaded with *N*-Fmoc-ε-(pent-4-ynamido)-L-lysine[44] under standard solid-phase peptide synthesis (SPPS) conditions as described above. It has been pointed out that such alkynylated peptides bound to a solid support are unable to undergo the Glaser coupling.[45]

Reference peptide **14** was synthesized on solid support using copper(I) iodide, DIPEA, and AFP (**5**) followed by incubation with BIS after click reaction. Radiolabeling of precursor peptide **13** with [^{18}F]AFP (Scheme 5 and Figure 4) was carried out under optimized conditions: peptide/TBTA/$CuSO_4$/sodium ascorbate (1:8:7:10 *v/v*) at high concentrations (10 mg resin **13** in 270–300 μL solution). TBTA at a minimum of 1.1 equiv relative to $CuSO_4$ was crucial for a high RCY, as the RCY decreases significantly if the amount of TBTA is <1 equiv. Thus, the RCY of [^{18}F]**14** was significantly higher and ranged from 46 to 164 MBq (8–13%, d.c.) with RCP $>98\%$ (after purification using only semi-preparative HPLC) and an A_S value of ~5 GBq μmol^{-1}.

Biological investigations of SNEW peptide [^{18}F]14

The stability of [^{18}F]**14** was investigated in vitro by incubation with rat plasma and rat blood for 1 h at 37 °C (samples were taken after 60 min). After this procedure, $>80\%$ of the activity remained as the original compound as indicated by radio-HPLC analyses. Additional hydrophilic metabolites were observed, but were not determined to be [^{18}F]fluoride by means of radio-HPLC analyses. This result indicates that no radiodefluorination of the ^{18}F-propyl moiety occurs in vitro.



Scheme 5. Synthetic pathway to the ^{18}F-labeled SNEW peptide [^{18}F]**14** using [^{18}F]AFP [^{18}F]**5** and reference peptide **14**. *Reagents and conditions*: a) THF, DIPEA, CuI, RT, 3 days; *radiolabeling*: b) DMF/Sørensen phosphate buffer, TBTA, sodium ascorbate, $CuSO_4{\cdot}5H_2O$, 50 W, 1 h.

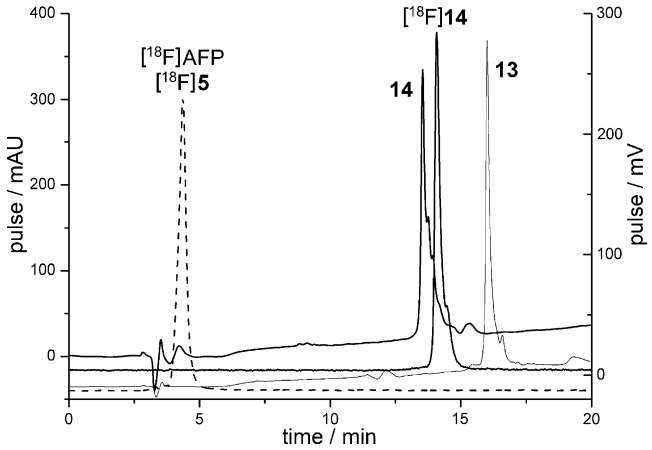


Figure 4. HPLC traces of starting peptide **13**, reference peptide **14**, [^{18}F]AFP [^{18}F]**5**, and purified [^{18}F]**14**.

The in vivo behavior of [^{18}F]**14** in Wistar rats was characterized by fast blood clearance and substantial renal elimination. However, the rapid elimination was combined with degradation of the peptide. [^{18}F]**14** was metabolized with a biological half-life of 4.3 min. At 5 min post-injection, only 17% of the intact peptide was found in blood after a single intravenous injection of [^{18}F]**14**. After 1 h, no activity was detectable in the blood. Three main metabolites ($t_R = 5.4$, 7.8, and 10.2 min) were found in the urine after 1 h, but not identified structurally (Figure 5). This finding can be explained by partial proteolysis of the peptide at the position of arginine.[46] In addition, the radiometabolite at $t_R = 5.4$ min seems to be a very small-molecular-weight ^{18}F-labeled compound, but not [^{18}F]fluoride, as no bone accumulation was observed.

Most of the activity was found in kidneys and bladder (44 and 15% ID, respectively) 90 min after injection. This is consistent with the assumed metabolism of peptide [^{18}F]**14**. This finding is also consistent with the common observation that the kidneys frequently accumulate peptides in the cortical tubuli after glomerular filtration. The PET experiments also revealed very rapid blood clearance accompanied by continuous accumulation in the kidneys, followed by elimination into the urine. On the other hand, the activity concentration in the liver was low (Figures 6 and 7). These results unambiguously indicate that [^{18}F]**14** is unsuitable as a radiotracer for imaging of the EphB2 receptor at this stage. Nevertheless, this new approach using SNEW peptides in fluorine-18 chemistry gives access to labeled peptides that hold promise as radiolabeled Eph ligands. Further improvement of the in vivo stability of the peptide backbone is required, for example, through introduction of D-amino acids or methylation of peptide bonds.

Conclusions

Herein we present a successful radiofluorination approach toward modified SNEW peptides that are potentially suitable for noninvasive PET imaging of the EphB2 receptor. For this purpose, L-cysteine-modified SNEW peptide **2** was first radiofluorinated using the conventional and well-known building block [^{18}F]FBAM. Unfortunately, ^{18}F-labeled peptide [^{18}F]**3** as well as the reference [^{19}F]**3** led to rapid decomposition due to the reversibility of the Michael addition. Thus, [^{18}F]BFP and [^{18}F]AFP were applied as novel and bioorthogonal radiolabeling building blocks that have the beneficial properties of bioorthogonality, hydrophilicity (log *D*), in vitro stability, molecular weight, and the irreversibility of ligation. These building blocks allow the conjugation of azide- and alkyne-functionalized peptides via Cu^I-mediated Huisgen [3+2] cycloaddition (click chemistry approach). Unfortunately, strong complexes were formed between all SNEW peptides and copper, which is required for click labeling. Thus, an alternative synthesis based on radiolabeling with resin-bound peptides and subsequent removal of the copper species by treatment with BIS as a strong chelating agent was evaluated. As a result, azide-containing peptides **6**, **8**, and **10** were successfully radiolabeled with [^{18}F]BFP, but with low RCYs. In this regard, a byproduct was found which was mainly formed in the labeling step from [^{18}F]BFP following the mechanism of Glaser coupling. [^{18}F]AFP has characteristics similar to those of [^{18}F]BFP, but was unable to undergo this side reaction. Hence, the alkyne-containing peptide **13** was successfully prepared and radiolabeled by the click chemistry approach in sufficient radiochemical yield and

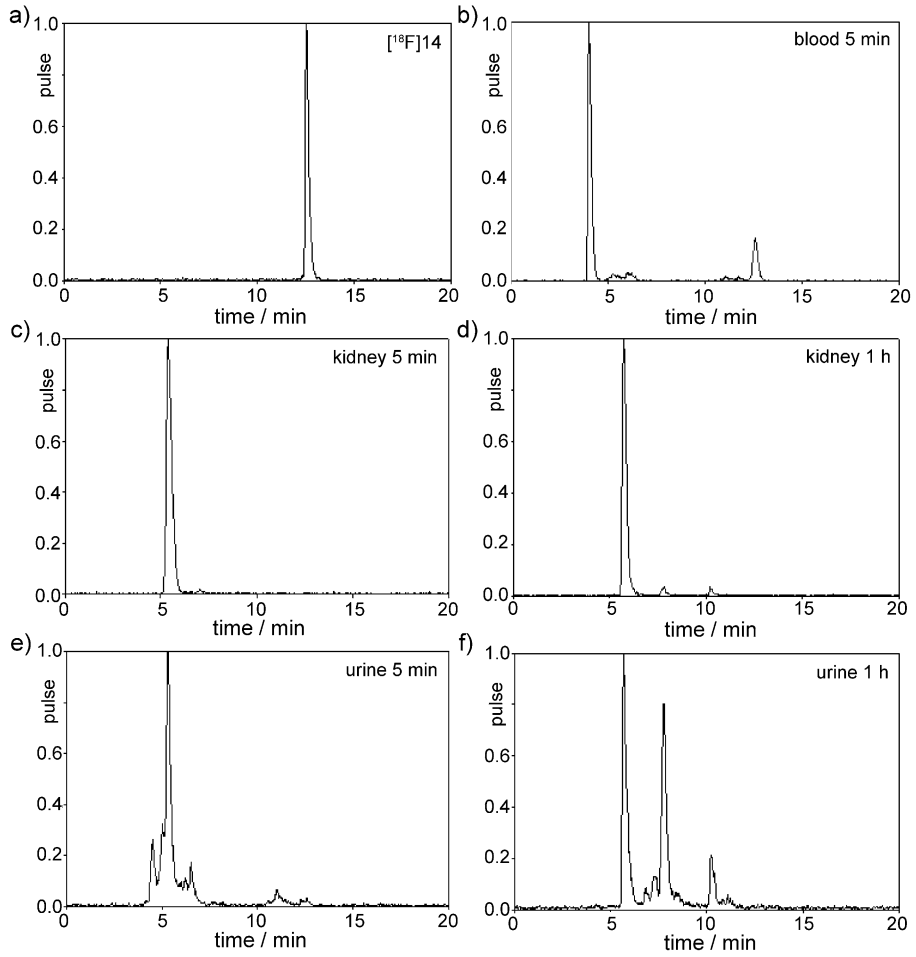


Figure 5. Representative HPLC traces of a) peptide [^{18}F]**14** and determination of radioactive metabolites in b) arterial blood plasma; c, d) kidneys; and e, f) urine following single intravenous injection into a Wistar rat, determined 5 min and 1 h post-injection, as indicated.

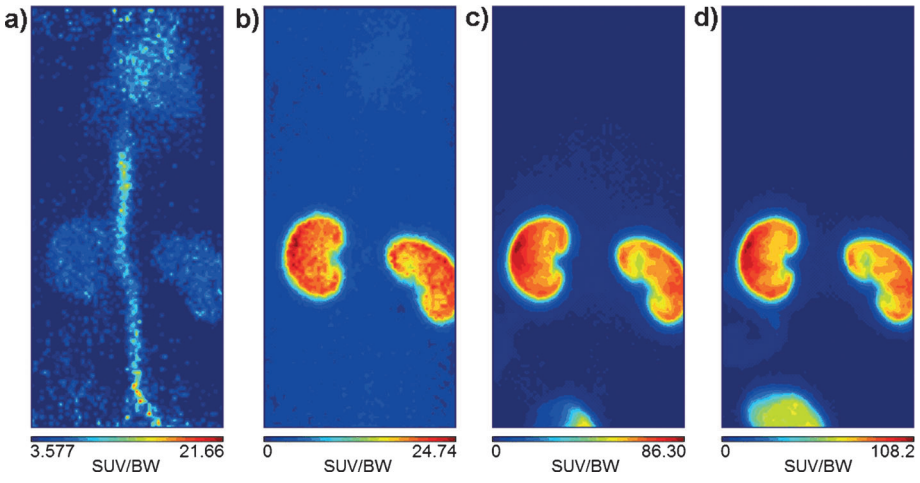


Figure 6. Maximum intensity projections of a representative PET experiment at after single intravenous injection of [^{18}F]**14** into a male Wistar rat a) 1 min, b) 5 min, c) 60 min, d) 120 min.

purity. To remove the copper species for biological investigations, peptide [^{18}F]**14** was synthesized and radiolabeled on resin. Subsequent incubation with BIS was necessary. As a result of the hydrophilicity of [^{18}F]AFP, a more polar ligation product was obtained, and due to the molecular weight, separation from the precursor peptide was possible by SEC or HPLC. Biological evaluations in vitro demonstrated adequate stability of the labeled peptide [^{18}F]**14** in rat plasma in contrast to the FBAM-labeled peptide [^{18}F]**3**. However, small-animal PET experiments revealed a very brief blood retention, accompanied by metabolism and rapid renal elimination of [^{18}F]**14** in rats. At this stage, [^{18}F]**14** is not applicable as a radiotracer for imaging purposes. Based on these results, novel peptides with increased in vivo stability must be evaluated.

Experimental Section

Instrumentation and chemicals. All reagents and solvents were purchased from commercial suppliers and were used without further purification unless otherwise specified. NMR spectra were recorded on a Varian Unity 400 MHz spectrometer. Chemical shifts (δ) are given in ppm and are referenced to the residual solvent resonance signals relative to $(CH_3)_4Si$ (^{1}H, ^{13}C) and $CFCl_3$ (^{19}F). Mass spectra were obtained on a Quattro/LC mass spectrometer (Micromass) by electrospray ionization. Preparative column chromatography was performed on Merck silica gel. Reactions were monitored by thin-layer chromatography (TLC) on Merck silica gel F_{254} aluminum plates, with visualization under UV ($\lambda =$ 254 nm) or by evaluation using ninhydrin and heating. ICP-MS data were obtained on an Elan 9000 Quadrupole instrument (PerkinElmer) using a Merck IV matrix.

General procedure for peptide syntheses. SNEW peptides **2**, **6**, **8**, and **13** were synthesized by an Fmoc SPPS strategy on an automated multiple peptide synthesizer (CEM Microwave Peptide Synthesizer). Fmoc-L-amino acid derivatives used in peptide syntheses were purchased from MultiSynTech GmbH. The reactive side chains of the Fmoc amino acids were protected as follows: amine: fluore-

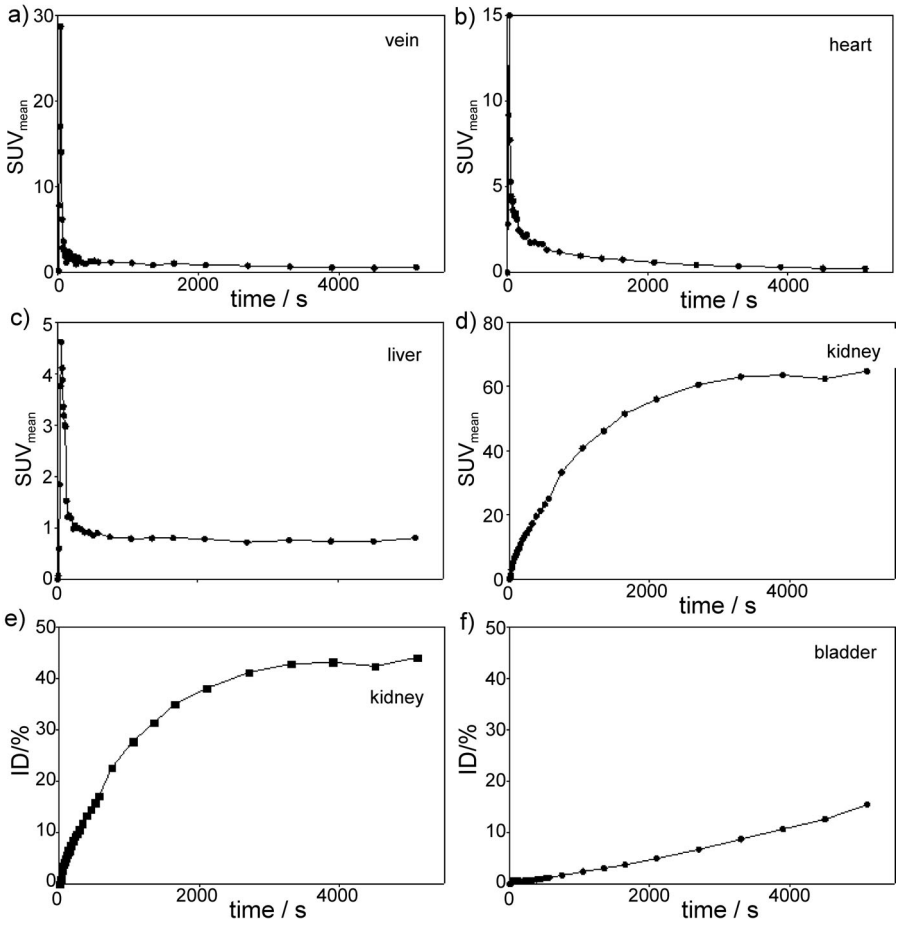


Figure 7. Time–activity curves in a) blood, b) heart, c) liver, and d) kidney given as SUV_{mean}, as well as in e) kidney and f) urine given as percent ID in a male Wistar rat after single intravenous injection of [^{18}F]**14** and followed up to 90 min post-injection.

nylmethyloxycarbonyl (Fmoc); Arg: 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf); Trp: *tert*-butyloxycarbonyl (Boc); Glu and Ser: *O*-*tert*-butyl (*O*-*t*Bu); Asn, Cys, Gln, and His: triphenylmethyl (Trt). Resin was purchased from Bachem. *N*,*N*-Diisopropylethylamine (DIPEA), trifluoroacetic acid (TFA), *N*,*N*-dimethylformamide (DMF), piperidine, and *N*-methyl-2-pyrrolidone (NMP) were purchased from Biosolve Ltd. HBTU (2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) and HOBt (*N*-hydroxybenzotriazole) were purchased from Calbiochem–Novabiochem. All peptides were prepared on Rink amide MBHA resin (extent of labeling: 0.6 mmol g^{-1} loading). Syntheses were performed on a 0.05–0.10 mM scale. The Fmoc protecting group was removed with 20% piperidine in DMF for 15 min. The carboxyl group of the incoming amino acid was activated with HBTU and HOBt. Fmoc-amino acid (5 equiv), HBTU (5 equiv), HOBt (5 equiv), and DIPEA (10 equiv) were dissolved in DMF and NMP, respectively, and added to the resin. The coupling time was 2 h, and the coupling was carried out twice for each residue. All peptides were deprotected and cleaved from the solid support with TFA/TIS/H_2O (95:2.5:2.5 *v*/*v*/*v*) at ambient temperature for 4 h.[47] Afterward, the resin was filtered, the crude peptide was precipitated by adding cold Et_2O and washed with ice-cold Et_2O. Residual ether was removed by evaporation, and the peptides were purified by preparative HPLC onto a Varian Dynamax Microsorb 60-8 C_{18} column (250×21.4 mm) at a flow rate of 20 mL min^{-1} with gradients of H_2O and CH_3CN with 0.1% TFA. Analytical HPLC was performed on a VWR Hitachi unit: System A: Nucleosil Nautilus C_{18} column (250× 4.6 mm, 5 μm; eluent: CH_3CN/H_2O + 0.1% TFA; gradient: 20–50% CH_3CN + 0.1% TFA: 0→10 min, 50–80% CH_3CN + 0.1% TFA: 10→11 min). System B: Phenomenex Aqua C_{18} column (250× 4.6 mm, 5 μm, 100 Å; eluent: CH_3CN/H_2O + 0.1% TFA; flow rate: 1.0 mL min^{-1}; gradient: 10–45% CH_3CN + 0.1% TFA: 0→17 min, 45–95% CH_3CN + 0.1% TFA: 17→18 min).

Peptide syntheses

SNEWILPRLPQHC (2). SNEW peptide **2** was prepared according to the general procedure using 83 mg of unloaded resin and was obtained as a colorless powder after purification by preparative HPLC (20–50% CH_3CN + 0.1% TFA within 10 min, t_R = 7.1 min) and lyophilization. Analytical HPLC (System A): t_R = 7.4 min; yield: 17.3 mg (22%); MS (ESI+): *m*/*z* calcd: 1591 $[M]^+$, found: 1593 $[M+2H]^+$, 808 $[M+H+Na]^{2+}$, 797 $[M+2H]^{2+}$.

SNEWILPRLPQHC-FBAM (3). A solution of FBAM (**1**; 1.50 mg, 4.71 μmol) in CH_3CN (100 μL) was added to a solution of SNEWILPRLPQHC (**2**; 4.70 mg, 2.95 μmol) in Sørensen phosphate buffer (100 μL, pH 7.2, 47.9 mM Na_2HPO_4, 18.1 mM NaH_2PO_4) into a vial and was stirred at 60 °C for 2 h. SNEW peptide **3** was obtained as a colorless powder after purification by preparative HPLC (10–40% CH_3CN + 0.1% TFA within 12 min, t_R = 11.1 min) and lyophilization. Analytical HPLC (System A): t_R = 11.1 min; yield: 4.05 mg (72%); MS (ESI+): *m*/*z* calcd: 1910 $[M]^+$, found: 1911 $[M+H]^+$, 956 $[M+2H]^{2+}$.

SNEWILPRLPQH-Azp (6). SNEW peptide **6** was prepared according to the general procedure for peptides using 275 mg of resin preloaded with *N*-Fmoc-(*S*)-4-azido-L-proline. SNEW peptide **6** was obtained as a colorless powder after purification by preparative HPLC (10–40% CH_3CN + 0.1% TFA within 12 min, t_R = 10.7 min) and lyophilization. Analytical HPLC (System B): t_R = 16.3 min; yield: 14.3 mg (9%); MS (ESI+): *m*/*z* calcd: 1626 $[M]^+$, found: 814 $[M+2H]^{2+}$.

SNEWILPRLPQH-triazolyl-FP (7). BFP **4** (164 μg, 0.83 μmol) was added to a suspension of SNEWILPRLPQH-Azp (**6**) bound to resin (900 μg, 0.55 μmol) in DMF (500 μL) into a frit syringe. Sodium ascorbate (50 μL, 0.6 M, 30 μmol) and $CuSO_4 \cdot 5H_2O$ (50 μL, 0.4 M, 20 μmol) were added, and the solution was stirred at 40 °C for 16 h. The resin was washed with DMF (5 mL), CH_3OH (5 mL) and incubated with bispidine (BIS; 24.5 mg, 40 μmol) in DMF/CH_3OH (1 mL, 1:1 *v*/*v*) at 40 °C for an additional 30 min. Afterward, the peptide was cleaved using the standard procedure described above. SNEW peptide **7** was obtained as a colorless powder after purification by preparative HPLC (10–45% CH_3CN + 0.1% TFA

within 17 min, $t_R = 14.1$ min) and lyophilization. Analytical HPLC (System B): $t_R = 15.0$ min; yield: 1.02 mg (98%); MS (ESI+): *m/z* calcd: 1824 $[M]^+$, found: 1825 $[M+H]^+$, 913 $[M+2H]^{2+}$, 609 $[M+3H]^{3+}$.

SNEWILPRLPQH-Anl (8). SNEW peptide **8** was prepared according to the general procedure for peptides using 86 mg of resin preloaded with *N*-Fmoc-ε-azido-L-norleucine. Afterward, the peptide was cleaved using the standard procedure described above. SNEW peptide **8** was obtained as a colorless powder after purification by preparative HPLC (10–45% CH_3CN + 0.1% TFA within 17 min, t_R = 15.4 min) and lyophilization. Analytical HPLC (System B): t_R = 17,2 min; yield: 6.5 mg (26%); MS (ESI+): *m/z* calcd: 1642 $[M]^+$, found: 1643 $[M+H]^+$, 822 $[M+2H]^{2+}$, 549 $[M+3H]^+$.

SNEWILPRLPQH-ε-(4-(2-(4-(3-fluoropropyl)piperazin-1-yl)ethyl)-1*H*-1,2,3-triazol-1-yl)Nle (9). BFP **4** (9.57 mg, 48.27 μmol) was added to a suspension of SNEWILPRLPQH-Anl (**8**) bound to resin (100 mg, 9.65 μmol) in DMF (1 mL) into a frit syringe. Then, TBTA (12.8 mg, 24.13 μmol), $CuSO_4 \cdot 5H_2O$ (12.1 mg, 48.27 μmol) in H_2O (500 μL), and sodium ascorbate (8.8 mg, 96.50 μmol) in H_2O (500 μL) were added in this order, and the reaction was stirred at 50 °C for 3 days. The resin was washed with DMF (5 mL), CH_3OH (5 mL), and incubated with BIS (41.41 mg, 67.55 μmol) in DMF/CH_3OH/H_2O (6 mL, 1:1:1 *v/v/v*) at 40 °C for an additional 30 min. The resulting blue solution was removed, and the resin was washed with DMF (5 mL), CH_3OH (5 mL), and Et_2O (5 mL). Afterward, the peptide was cleaved using the standard procedure described above. SNEW peptide **9** was obtained as a colorless powder after purification by preparative HPLC (10–45% CH_3CN + 0.1% TFA within 17 min, t_R = 13.3 min) and lyophilization. Analytical HPLC (System B): t_R = 15.2 min; yield: 5.4 mg (86%); MS (ESI+): *m/z* calcd: 1840 $[M]^+$, found: 1841 $[M+H]^+$, 921 $[M+2H]^{2+}$.

***N*-(5-Azidopentanamido)-SNEWILPRLPQH (10).** Succinimidyl 5-azidopentanoate (24 mg, 100 μmol) was added to a suspension of SNEWILPRLPQH bound to resin (112 mg, 25 μmol) in DMF (1 mL) and was stirred at 40 °C for 3 days. Afterward, the peptide was cleaved using the standard procedure described above. SNEW peptide **10** was obtained as a colorless powder after purification by preparative HPLC (10–45% CH_3CN + 0.1% TFA within 17 min, t_R = 16.4 min) and lyophilization. Analytical HPLC (System B): t_R = 18,4 min; yield: 3.6 mg (90%); MS (ESI+): *m/z* calcd: 1613 $[M]^+$, found: 1614 $[M+H]^+$, 808 $[M+2H]^{2+}$, 546 $[M+3H]^+$.

***N*-(5-(4-(2-(4-(3-Fluoropropyl)piperazin-1-yl)ethyl)-1*H*-1,2,3-triazol-1-yl)pentanamido)-SNEWILPRLPQH (11).** BFP **4** (16.6 mg, 84.1 μmol) was added to a suspension of *N*-(5-azidopentanamido)-SNEWILPRLPQH (**10**) bound to resin (23.6 mg, 8.41 μmol) in DMF (1 mL) into a frit syringe. Then, TBTA (4.5 mg, 8.41 μmol), $CuSO_4 \cdot 5H_2O$ (2.1 mg, 8.41 μmol) in H_2O (200 μL), and sodium ascorbate (16.6 mg, 84.10 μmol) in H_2O (200 μL) were added in this order, and the reaction was stirred at 50 °C for 3 days. The resin was washed with DMF (5 mL), CH_3OH (5 mL), and incubated with BIS (10.6 mg, 16.82 μmol) in DMF/CH_3OH/H_2O (6 mL, 1:1:1 *v/v/v*) at 40 °C for an additional 30 min. The resulting blue solution was removed, and the resin was washed with DMF (5 mL), CH_3OH (5 mL), and Et_2O (5 mL). Afterward, the peptide was cleaved using the standard procedure described above. SNEW peptide **11** was obtained as a colorless powder after purification by preparative HPLC (10–45% CH_3CN + 0.1% TFA within 17 min, t_R = 13.3 min) and lyophilization. Analytical HPLC (System B): t_R = 15.4 min; yield: 5.4 mg (77%); MS (ESI+): *m/z* calcd: 1812 $[M]^+$, found: 1813 $[M+H]^+$, 907 $[M+2H]^{2+}$, 605 $[M+3H]^+$.

1,8-Bis(4-(3-fluoropropyl)piperazin-1-yl)octa-3,5-diyne (12). $CuSO_4 \cdot 5H_2O$ (6 mg, 20 μmol) was added to a solution of 1-(but-3-ynyl)-4-(3-fluoropropyl)piperazine (**4**; 45 mg, 230 μmol) and DMF (4 mL), and was stirred at 40 °C for 3 days. The solvent was removed in vacuo, and the crude product was purified by column chromatography (EtOAc→EtOAc/CH_3OH 5:1) to yield compound **12** as an orange solid (10 mg, 11%): R_f = 0.27 (CH_3OH); mp: 68–70 °C; ^1H NMR (400 MHz, $CDCl_3$): δ = 4.48 (dt, $^3J_{H,F}$ = 47.2 Hz, ^3J = 5.9 Hz, 4H, FCH_2), 2.61–2.41 (m, 24H, Pip-H/$FCH_2CH_2CH_2$/NCH_2), 1.95–1.82 (m, 4H, NCH_2CH_2), 1.47–1.44 ppm (m, 4H, FCH_2CH_2); ^{13}C NMR ($CDCl_3$, 101 MHz): δ = 82.7 (d, $^1J_{C,F}$ = 164.3 Hz, FCH_2), 76.0 ($CH_2C\equiv C$), 66.1 ($CH_2C\equiv C$), 56.8 (NCH_2), 54.4 (d, $^3J_{C,F}$ = 5.5 Hz, $FCH_2CH_2CH_2$), 53.2 (Pip-C-2,6), 52.9 (Pip-C-3,5), 28.1 (d, $^2J_{C,F}$ = 19.7 Hz, FCH_2CH_2), 16.9 ppm (NCH_2CH_2); ^{19}F NMR ($CDCl_3$, 376 MHz): δ = −220.6 ppm; MS (ESI+): *m/z* 395 $[M+H]^+$, 198 $[M+2H]^{2+}$.

SNEWILPRLPQH-ε-(pent-4-ynamido)Lys (13). SNEW peptide **13** was prepared according to the general procedure for peptides using 430 mg of resin preloaded with *N*-Fmoc-ε-(pent-4-ynamido)-L-lysine. Afterward, the peptide was cleaved using the standard procedure described above. SNEW peptide **13** was obtained as a colorless powder after purification by preparative HPLC (10–45% CH_3CN + 0.1% TFA within 17 min, t_R = 15.6 min) and lyophilization. Analytical HPLC (System B): t_R = 15.2 min; yield: 3.5 mg (11%); MS (ESI+): *m/z* calcd: 1697 $[M]^+$, found: 1698 $[M+H]^+$, 850 $[M+2H]^{2+}$, 566 $[M+3H]^+$.

SNEWILPRLPQH-ε-(3-(1-(3-fluoropropyl)-1*H*-1,2,3-triazol-4-yl)propanamido)Lys (14). AFP **5** (5.2 mg, 22.8 μmol) was added to a suspension of SNEWILPRLPQH-ε-(pent-4-ynamido)Lys (**13**) bound to resin (33 mg, 11.4 μmol) in THF (1.2 mL) into a frit syringe. Then, DIPEA (99 μg, 570 μmol) and CuI (4.3 mg, 22.8 μmol) were added, and the reaction was stirred at ambient temperature for 3 days. The resin was washed with DMF (5 mL), CH_3OH (5 mL), and incubated with BIS **7** (27.9 mg, 45.6 μmol) in DMF/CH_3OH/H_2O (3 mL, 1:1:1 *v/v/v*) at 40 °C for an additional 30 min. The resulting blue solution was removed, and the resin was washed with DMF (5 mL), CH_3OH (5 mL), and Et_2O (5 mL). Afterward, the peptide was cleaved using the standard procedure described above. SNEW peptide **14** was obtained as a colorless powder after purification by preparative HPLC (10–45% CH_3CN + 0.1% TFA within 17 min, t_R = 14.6 min) and lyophilization. Analytical HPLC (System B): t_R = 13.9 min; yield: 5.4 mg (77%); MS (ESI+): *m/z* calcd: 1926 $[M]^+$, found: 1927 $[M+H]^+$, 964 $[M+2H]^{2+}$, 643 $[M+3H]^+$.

Radiochemical syntheses

No-carrier-added aqueous [^{18}F]fluoride was produced using an IBA Cyclone 18/9 cyclotron by irradiation of [^{18}O]H_2O via the $^{18}O(p,n)^{18}F$ nuclear reaction. Synthesis of [^{18}F]FBAM ([^{18}F]**1**) was performed in a remotely controlled synthesis module (Nuclear Interface, Münster, Germany) as previously described.[31] Analytical radio-HPLC was performed with either System A or B as described above. All products were monitored by a UV detector at λ = 220 nm and by γ detection with a scintillation detector GABI (Raytest). A difference of 0.2 min between UV and γ-trace occurs as a function of the HPLC setup. Semi-preparative HPLC was performed on a Jasco unit with a Nucleosil® Standard C_{18} column (250×16 mm, 7 μm, 100 Å; eluent: CH_3CN/H_2O + 0.1% TFA; flow rate: 4.0 mL min^{-1}; gradient: 10–45% CH_3CN + 0.1% TFA: 0→17 min, 45–95% CH_3CN + 0.1% TFA: 17→18 min). Size-exclusion chromatography (SEC) was performed on an ÄKTA Prime Plus unit using a HighTrap desalting column (2.5×1.6 cm, 5 mL) with a calcium buffer (150 mM NaCl, 20 mM HEPES, 1.2 mM $MgCl_2$, 1.3 mM $CaCl_2$, pH 7.5) as eluent, at

a flow rate of 0.5 mL min^{-1}. Radio-TLC analyses of coupling reactions with [^{18}F]BFP, [^{18}F]AFP, and [^{18}F]FBAM were performed with Merck RP18 F_{254} aluminum plates.

SNEWILPRLPQHC-[^{18}F]FBAM ([^{18}F]3). A solution of [^{18}F]FBAM ([^{18}F]**1**; 200–350 MBq) in CH_3CN (100 μL) was added to a solution of SNEWILPRLPQHC **2** (470 μg, 295 nmol) in Sørensen phosphate buffer (100 μL, pH 7.2, 47.9 mM Na_2HPO_4, 18.1 mM NaH_2PO_4) in a vial and was stirred at 60 °C for 1 h. The rate of conversion of [^{18}F]**1** into [^{18}F]**3** was >94% within 60 min. Analytical radio-HPLC (System A): t_R = 11.2 min; RCY: 30–52 MBq (15%, d.c.); RCP: >94%; A_S: ~2.6 GBq μmol^{-1}.

SNEWILPRLPQH-triazolyl-[^{18}F]FP ([^{18}F]7). TBTA (0.51 mg, 0.96 μmol) was added to a suspension of SNEWILPRLPQH-Azp (**6**) bound to resin (5 mg, 0.31 μmol) in DMF (100 μL) into a frit syringe. Then, $CuSO_4 \cdot 5H_2O$ (0.48 mg, 1.92 μmol) and sodium ascorbate (5.61 mg, 28.3 μmol) in Tris·HCl buffer (100 μL, pH 8.0) were added. Subsequently, [^{18}F]BFP ([^{18}F]**4**; ~770 MBq) in DMF/H_2O (150 μL, 1:1 *v/v*) was added, and the solution was microwave irradiated at 50 W for 1 h. The resin was washed with DMF (2 mL), H_2O (2 mL), and incubated with BIS (2.4 mg, 4 μmol) in DMF/CH_3OH/H_2O (150 μL, 1:1:0.1 *v/v/v*) at 30 W for an additional 20 min. The resin was washed with DMF (2 mL), H_2O (2 mL), and the peptide was cleaved at 30 W for 60 min using TFA/TIS (400 μL, 95:5 *v/v*). SNEW peptide [^{18}F]**7** was obtained after purification by semi-preparative HPLC (t_R = 20.5 min) and removal of the solvent under reduced pressure at 50 °C. Analytical radio-HPLC (System B): t_R = 15.1 min; RCY: 1.4 MBq (0.4%, d.c.); RCP: >98%; A_S: ~1.0 GBq μmol^{-1}.

SNEWILPRLPQH-ε-(4-(2-(4-(3-[^{18}F]fluoropropyl)piperazin-1-yl)ethyl)-1*H*-1,2,3-triazol-1-yl)Nle ([^{18}F]9). TBTA (1.1 mg, 2 μmol) was added to a suspension of SNEWILPRLPQH-Anl (**8**) bound to resin (12.8 mg, 0.79 μmol) in DMF (200 μL) into a frit syringe. Then, $CuSO_4 \cdot 5H_2O$ (0.13 mg, 0.5 μmol) in H_2O (25 μL) and sodium ascorbate (1.98 mg, 10 μmol) in H_2O (37 μL) were added. Subsequently, [^{18}F]BFP (460–960 MBq) in Tris·HCl buffer (100 μL, pH 8.0) was added, and the solution was stirred at 90 °C for 1 h. The resin was washed with DMF (2 mL), H_2O (2 mL), and incubated with BIS **7** (3.06 mg, 5 μmol) in DMF/CH_3OH/H_2O (300 μL, 1:1:0.1 *v/v*) at 30 W for an additional 20 min. The resin was washed with DMF (2 mL), H_2O (2 mL), and the peptide was cleaved at 30 W for 20 min using TFA/TIS (600 μL, 95:5 *v/v*). SNEW peptide [^{18}F]**9** was obtained after purification by semi-preparative HPLC (t_R = 20.5 min) and removal of the solvent under reduced pressure at 50 °C. Analytical radio-HPLC (System B): t_R = 14.8 min; RCY: 1.3–4.0 MBq (0.5–1.4%, d.c.); RCP: >98%; A_S: 1.2 GBq μmol^{-1}.

***N*-(5-(4-(2-(4-(3-[^{18}F]Fluoropropyl)piperazin-1-yl)ethyl)-1*H*-1,2,3-triazol-1-yl)pentanamido)-SNEWILPRLPQH ([^{18}F]11).** TBTA (1.7 mg, 3.2 μmol) was added to a suspension of *N*-(5-azidopentanamido)-SNEWILPRLPQH (**10**) bound to resin (5 mg, 0.31 μmol) in DMF (80 μL) into a frit syringe. Then, $CuSO_4 \cdot 5H_2O$ (0.2 mg, 0.8 μmol) and sodium ascorbate (1.6 mg, 8 μmol) were added. Subsequently, [^{18}F]BFP (~1.76 GBq) in Tris·HCl buffer (100 μL, pH 8.0) was added, and the solution was irradiated at 50 W for 1.5 h. The resin was washed with DMF (2 mL), H_2O (2 mL), and incubated with BIS **7** (7.8 mg, 10 μmol) in DMF/CH_3OH/H_2O (1 mL, 1:1:0.1 *v/v*) at 30 W for an additional 20 min. The resin was washed with DMF (2 mL), H_2O (2 mL), and the peptide was cleaved at 30 W for 30 min using TFA/TIS (600 μL, 95:5 *v/v*). SNEW peptide [^{18}F]**11** was obtained after purification by semi-preparative HPLC (t_R = 20.5 min) and removal of the solvent under reduced pressure at 50 °C. Analytical radio-HPLC (System B): t_R = 15.6 min; RCY: 12.5 MBq (1.8%, d.c.); RCP: >98%; A_S: ~5 GBq μmol^{-1}.

SNEWILPRLPQH-ε-(3-(1-(3-[^{18}F]fluoropropyl)-1*H*-1,2,3-triazol-4-yl)propanamido)Lys ([^{18}F]14). TBTA (2.9 mg, 5.7 μmol) was added to a suspension of SNEWILPRLPQH-ε-(pent-4-ynamido)Lys (**13**) bound to resin (12 mg, 0.71 μmol) in DMF (150 μL) into a frit syringe. Then, $CuSO_4 \cdot 5H_2O$ (1.2 mg, 4.95 μmol) in H_2O (10 μL) and sodium ascorbate (1.4 mg, 7.07 μmol) in H_2O (10 μL) were added. Subsequently, [^{18}F]AFP ([^{18}F]**5**; 2.30–3.34 GBq) in Tris·HCl buffer (100 μL, pH 8.0) was added, and the solution was irradiated at 50 W for 1 h. The resin was washed with DMF (2 mL), H_2O (2 mL), and incubated with BIS **7** (7.8 mg, 10 μmol) in DMF/CH_3OH/H_2O (1 mL, 1:1:0.1 *v/v*) at 30 W for an additional 20 min. The resin was washed with DMF (2 mL), H_2O (2 mL), and the peptide was cleaved under microwave irradiation (30 W) for 20 min using TFA/TIS (700 μL, 95:5 *v/v*). SNEW peptide [^{18}F]**14** was obtained after purification by semi-preparative HPLC (t_R = 20.5 min) and removal of the solvent under reduced pressure at 50 °C. Analytical radio-HPLC (System B): t_R = 14.1 min; RCY: 46–164 MBq (8–13%, d.c.); RCP: >98%; A_S: ~5 GBq μmol^{-1}.

Radiopharmacological studies

Animal experiments were carried out according to the guidelines of the German Regulations for Animal Welfare. The protocol was approved by the local Ethical Committee for Animal Experiments. To investigate in vitro stability, either blood or plasma (400 μL) obtained from male Wistar rats (aged 5–8 weeks) was added to a solution of all purified radiolabeled peptides [^{18}F]**3**, [^{18}F]**7**, and [^{18}F]**14** (dissolved in 100 μL PBS, pH 7.5). The rat blood was diluted 1:1 with Ficoll Paque buffer (pH 7.5). The solutions were stirred at 37 °C for 1 h. Both in vivo stability studies and small-animal PET experiments were also performed in male Wistar rats. Therefore, rats were positioned and immobilized prone with their medial axis parallel to the axial axis of the scanner (microPET P4, Siemens Medical Solutions). The radiotracer was administered intravenously into one tail vein (34 MBq, in 30% albumin/E-153 solution, 1 mL) under desflurane (oxygen/air 7:30%) anesthesia. Transmission scans and PET acquisition were performed according to a protocol reported by us in detail previously.[48] The standardized uptake values (SUV) were calculated over the ROI as the ratio of activity concentration at time *t* and injected dose at the time of injection divided by body weight. The in vivo stability of the radiofluorinated peptide [^{18}F]**14** was determined using arterial blood samples obtained from rats at various time points during the PET scan. Therefore, blood samples (400 μL) were withdrawn via catheter from the right femoral artery at 3, 5, 10, 20, 30, 60, and 90 min post-injection. Plasma was separated by centrifugation (3 min, 16200 *g*, 4 °C) followed by precipitation of the plasma proteins with ice-cold CH_3OH (1.5 parts per 1 part plasma) and centrifugation (3 min, 16200 *g*, 4 °C). The supernatants were analyzed by radio-HPLC on a Zorbax 300SB-C_{18} (250×9.4 mm; 5 μm) column with H_2O/0.05% TFA (A) and CH_3CN/0.04% TFA (B) as eluents at a flow rate of 2 mL min^{-1}. The following gradient was used: 0 min 20% B, at 15 min 50% B, at 16–20 min 90% B.

Acknowledgements

The authors are grateful to Waldemar Herzog, Stephan Preusche, and Tilow Krauss for their excellent technical assistance. We are very thankful to Peggy Wecke for the preparation of N-Fmoc-(S)-

4-azido-L-proline, Uta Lenkeit for the synthesis of [^{18}F]FBAM, Silke Fähnemann for the synthesis and supply of bispidine, Andrea Suhr and Regina Herrlich for in vivo experiments, and Aline Ritter for ICP-MS measurements. This work was supported in part by a grant (to C.M.) from the Fonds der Chemischen Industrie (FCI). This work is part of a research initiative within the Helmholtz-Portfoliothema "Technologie und Medizin—Multimodale Bildgebung zur Aufklärung des In-vivo-Verhaltens von polymeren Biomaterialien".

Keywords: click chemistry · EphB2 · imaging agents · kinases · peptides · receptors

[1] H. Surawska, P. C. Ma, R. Salgia, *Cytokine Growth Factor Rev.* **2004**, *15*, 419 – 433.
[2] R. Noberini, I. Lamberto, E. B. Pasquale, *Semin. Cell Dev. Biol.* **2012**, *23*, 51 – 57.
[3] S. Wang, W. J. Placzek, J. L. Stebbins, S. Mitra, R. Noberini, M. Koolpe, Z. Zhang, R. Dahl, E. B. Pasquale, M. Pellecchia, *J. Med. Chem.* **2012**, *55*, 2427 – 2436.
[4] E. B. Pasquale, *Nat. Rev. Cancer* **2010**, *10*, 165 – 180.
[5] B. Mosch, B. Reissenweber, C. Neuber, J. Pietzsch, *J. Oncol.* **2010**, 135285.
[6] V. Davalos, H. Dopeso, S. Velho, A. M. Ferreira, L. Cirnes, N. Díaz-Chico, C. Bilbao, R. Ramírez, G. Rodríguez, O. Falcón, L. León, R. C. Niessen, G. Keller, G. Dallenbach-Hellweg, E. Espín, M. Armengol, A. Plaja, M. Perucho, K. Imai, H. Yamamoto, J. F. Gebert, J. C. Díaz-Chico, R. M. Hofstra, S. M. Woerner, R. Seruca, S. Schwartz, Jr., D. Arango, *Oncogene* **2007**, *26*, 308 – 311.
[7] E. Batlle, J. T. Henderson, H. Beghtel, M. M. W. van den Born, E. Sancho, G. Huls, J. Meeldijk, J. Robertson, M. van de Wetering, T. Pawson, H. Clevers, *Cell* **2002**, *111*, 251 – 263.
[8] Q. Wu, Z. Suo, G. B. Kristensen, M. Baekelandt, J. M. Nesland, *Gynecol. Oncol.* **2006**, *102*, 15 – 21.
[9] Q. Wu, Z. Suo, B. Risberg, M. G. Karlsson, K. Villman, J. M. Nesland, *Pathol. Oncol. Res.* **2004**, *10*, 26 – 33.
[10] J. T. Dong, *J. Cell. Biochem.* **2006**, *97*, 433 – 447.
[11] M. Nakada, J. A. Niska, H. Miyamori, W. S. McDonough, J. Wu, H. Sato, M. E. Berens, *Cancer Res.* **2004**, *64*, 3179 – 3185.
[12] M. Kandouz, K. Haidara, J. Zhao, M. L. Brisson, G. Batist, *Cell Cycle* **2010**, *9*, 398 – 407.
[13] C. Bardelle, T. Coleman, D. Cross, S. Davenport, J. G. Kettle, E. J. Ko, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5717 – 5721.
[14] R. Noberini, M. Koolpe, S. Peddibhotla, R. Dahl, Y. Su, N. D. P. Cosford, G. P. Roth, E. B. Pasquale, *J. Biol. Chem.* **2008**, *283*, 29461 – 29472.
[15] K. Lafleur, D. Huang, T. Zhou, A. Caflisch, C. Nevado, *J. Med. Chem.* **2009**, *52*, 6433 – 6446.
[16] C. Mamat, B. Mosch, C. Neuber, M. Köckerling, R. Bergmann, J. Pietzsch, *ChemMedChem* **2012**, *7*, 1991 – 2003.
[17] C. Giorgio, I. H. Mohamed, L. Flammini, E. Barocelli, M. Incerti, A. Lodola, M. Tognolini, *PLoS One* **2011**, *6*, e18128.
[18] M. Tognolini, M. Incerti, I. Hassan-Mohamed, C. Giorgio, S. Russo, R. Bruni, B. Lelli, L. Bracci, R. Noberini, E. B. Pasquale, E. Barocelli, P. Vicini, M. Mor, A. Lodola, *ChemMedChem* **2012**, *7*, 1071 – 1083.
[19] C. Xiong, M. Huang, R. Zhang, S. Song, W. Lu, L. Flores II, J. Gelovani, C. Li, *J. Nucl. Med.* **2011**, *52*, 241 – 248.
[20] B. Wu, Z. Zhang, R. Noberini, E. Barile, M. Giulianotti, C. Pinilla, R. A. Houghten, E. B. Pasquale, M. Pellecchia, *Chem. Biol.* **2013**, *20*, 19 – 33.
[21] J. E. Chrencik, A. Brooun, M. I. Recht, G. Nicola, L. K. Davis, R. Abagyan, H. Widmer, E. B. Pasquale, P. Kuhn, *J. Biol. Chem.* **2007**, *282*, 36505 – 36513.
[22] M. Berndt, J. Pietzsch, F. Wuest, *Nucl. Med. Biol.* **2007**, *34*, 5 – 15.
[23] F. Wuest, L. Koehler, M. Berndt, J. Pietzsch, *Amino Acids* **2009**, *36*, 283 – 295.
[24] J. Kapty, T. Kniess, F. Wuest, J. R. Mercer, *Appl. Radiat. Isot.* **2011**, *69*, 1218 – 1225.
[25] M. Pretze, F. Wuest, T. Peppel, M. Köckerling, C. Mamat, *Tetrahedron Lett.* **2010**, *51*, 6410 – 6414.
[26] C. Mamat, M. Franke, T. Peppel, M. Köckerling, J. Steinbach, *Tetrahedron* **2011**, *67*, 4521 – 4529.
[27] E. J. Keliher, T. Reiner, G. M. Thurber, R. Upadhyay, R. Weissleder, *Chem. Open* **2012**, *1*, 177 – 183.
[28] T. Ramenda, T. Knieß, R. Bergmann, J. Steinbach, F. Wüst, *Chem. Commun.* **2009**, 7521 – 7523.
[29] M. Glaser, M. Solbakken, D. R. Turton, R. Pettitt, J. Barnett, J. Arukwe, H. Karlsen, A. Cuthbertson, S. K. Luthra, E. Årstad, *Amino Acids* **2009**, *37*, 717 – 724.
[30] T. Ramenda, J. Steinbach, F. Wuest, *Amino Acids* **2013**, *44*, 1167 – 1180.
[31] J. Marik, J. L. Sutcliffe, *Tetrahedron Lett.* **2006**, *47*, 6681 – 6684.
[32] M. Pretze, C. Mamat, J. Steinbach, *J. Labelled Compd. Radiopharm.* **2011**, *54*, S460.
[33] M. Pretze, C. Mamat, *J. Fluorine. Chem.* **2013**, DOI: 10.1016/j.jfluchem.2013.02.028.
[34] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem.* **2002**, *114*, 2708 – 2711; *Angew. Chem. Int. Ed.* **2002**, *41*, 2596 – 2599.
[35] T. Kniess, M. Kuchar, J. Pietzsch, *Appl. Radiat. Isot.* **2011**, *69*, 1226 – 1230.
[36] B. Shi, M. F. Greaney, *Chem. Commun.* **2005**, 886 – 888.
[37] P. Grosse-Gehling, F. R. Wuest, T. Peppel, M. Köckerling, C. Mamat, *Radiochim. Acta* **2011**, *99*, 365 – 373.
[38] U. Sirion, H. J. Kim, J. H. Lee, J. W. Seo, B. S. Lee, S. J. Lee, S. J. Oh, D. Y. Chia, *Tetrahedron Lett.* **2007**, *48*, 3953 – 3957.
[39] M. Chikira, *J. Inorg. Biochem.* **2008**, *102*, 1016 – 1024.
[40] S. Juran, M. Walther, H. Stephan, R. Bergmann, J. Steinbach, W. Kraus, F. Emmerling, P. Comba, *Bioconjugate Chem.* **2009**, *20*, 347 – 359.
[41] J. M. Collins, N. E. Leadbeater, *Org. Biomol. Chem.* **2007**, *5*, 1141 – 1150.
[42] C. Glaser, *Ber. Dtsch. Chem. Ges.* **1869**, *2*, 422 – 424.
[43] HPLC conditions: Phenomenex Aqua C_{18} column (250 × 4.6 mm, 5 μm, 100 Å; eluent: CH_3CN/H_2O (60:40 isocratic); flow rate: 1.0 mL min^{-1}.
[44] M. Galibert, P. Dumy, D. Boturyn, *Angew. Chem.* **2009**, *121*, 2614 – 2617; *Angew. Chem. Int. Ed.* **2009**, *48*, 2576 – 2579.
[45] C. W. Tornøe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057 – 3064.
[46] R. K. Jain, *J. Controlled Release* **1998**, *53*, 49 – 67.
[47] D. S. King, C. G. Fields, G. B. Fields, *Int. J. Peptide Protein Res.* **1990**, *36*, 255 – 266.
[48] T. Kniess, M. Laube, R. Bergmann, F. Sehn, F. Graf, J. Steinbach, F. Wuest, J. Pietzsch, *Bioorg. Med. Chem.* **2012**, *20*, 3410 – 3421.

Received: February 4, 2013
Published online on April 4, 2013