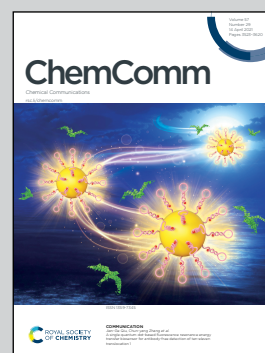


Showcasing research from the group of Professor Bernd Neumaier at the Forschungszentrum Jülich, INM-5, Germany

Rapid ^{18}F -labeling via Pd-catalyzed S-arylation in aqueous medium

This work describes radiolabeling of thiol-containing substrates via Pd-catalyzed S-arylation with readily accessible 2- ^{18}F -fluoro-5-iodopyridine. The novel procedure was applied for the convenient preparation of a novel PSMA-specific PET-tracer as well as for the fast labeling of peptides and proteins.

As featured in:



See Bernd Neumaier *et al.*,
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Rapid ¹⁸F-labeling via Pd-catalyzed S-arylation in aqueous medium†

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We report radiolabeling of thiol-containing substrates via Pd-catalyzed S-arylation with 2-[¹⁸F]fluoro-5-iodopyridine, which is readily accessible using the “minimalist” radiofluorination method. The practicality of the procedure was confirmed by preparation of a novel PSMA-specific PET-tracer as well as labeling of glutathione, Aβ oligomer-binding RD2 peptide, bovine serum albumin and PSMA I&S.

Since its introduction in the 1970s, positron emission tomography (PET) and hybrid methods like PET/CT and PET/MRI have become indispensable tools for high precision medicine and drug development.^{1,2} This imaging technique utilizes probes labeled with positron-emitting radionuclides (PET-tracers), which interact specifically with a molecular target or biochemical process of interest. Fluorine-18 remains the most popular PET radionuclide owing to its favorable decay properties and good accessibility in the form of [¹⁸F]fluoride. However, further PET developments necessitate easy access to emerging and established PET-tracers. A plethora of ¹⁸F-labeling techniques have been established.^{3,4} In particular, recently developed late-stage radiofluorination methods such as transition metal-mediated ¹⁸F-fluorination have significantly simplified the accessibility of numerous PET probes.^{5,6} Emerging approaches based on Al-F, B-F and Si-F chemistry enabled in many cases direct access to ¹⁸F-fluorinated

peptides.⁷ The [¹⁸F]AlF protocol was also used for direct radiolabeling of proteins.⁸ In contrast, the conditions required for other radiofluorination methods are incompatible with sensitive compounds and biomolecules like peptides, proteins and nucleic acids. Furthermore, synthesis of radiolabeling precursors can be challenging, so that direct ¹⁸F-labeling is not always well suited for high throughput production of libraries of radiolabeled compounds. In this case, a modular (indirect) approach that starts with the preparation of a radiolabeled building block bearing a reactive group is more preferable.⁹ In a second step, this prosthetic group is coupled to an appropriate functionality of the molecule to be labeled. Whereas amino and thiol reactive building blocks are conventionally used for ¹⁸F-fluorination of peptides and proteins, tryptophan or tyrosine residues have recently been targeted as well.¹⁰ While most biopolymers contain numerous amino groups (*e.g.* ω-NH₂ of Lys residues), the number of SH-groups (*e.g.* Cys moieties) seldom exceeds 1–2 per molecule, enabling conjugation with higher site selectivity. As thiol-selective prosthetic groups, ¹⁸F-labeled maleimides, vinyl sulfones, phenyloxadiazolyl methylsulfones, and the Umemoto reagent have been used.^{11–16} Among them, S-conjugation of maleimides affords radiolabeled thioethers as mixtures of two stereoisomers, which are not always sufficiently stable under physiological conditions.¹⁷ Vinyl sulfones¹⁸ lack complete selectivity towards S- vs. N-nucleophiles and are less reactive than maleimides. Furthermore, the preparation of these prosthetic groups usually involves multiple steps and subsequent HPLC isolation, increasing synthesis time, diminishing radiochemical yields (RCYs) and preventing automation. Recently, Al Shuaeeb *et al.* disclosed a fast Pd-catalysed S-arylation of Cys-containing molecules with various (hetero)aryl halides in aqueous medium at ambient temperature.¹⁹ The aim of the present study was to transfer this approach into PET-chemistry.

4-[¹⁸F]Fluoriodobenzene ([¹⁸F]FIB) was developed for indirect radiolabeling via Pd-catalyzed cross coupling reactions.²⁰ However, [¹⁸F]FIB prepared from respective aryl-λ₃-iodane²¹ or sulfonium precursors²² must be purified by HPLC to remove aryl iodide side products. Furthermore, in our hands, RCYs of [¹⁸F]FIB did not

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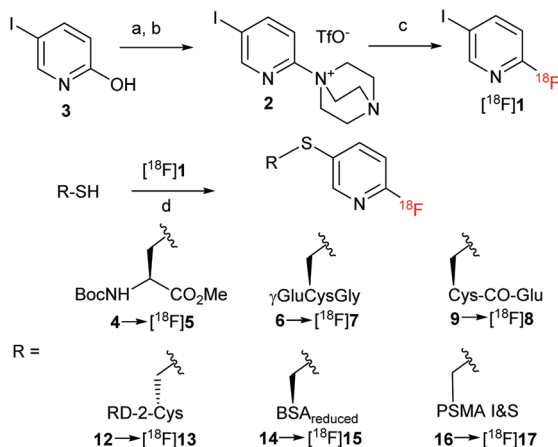
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Scheme 1 Synthesis of 5-iodo-2- ^{18}F fluoropyridine (^{18}F 1) and its conjugation with thiols **4**, **6**, **9**, **12**, **14** and **16**. Conditions: (a) TiF_4 , Py, 0°C to r.t., 16 h; (b) DABCO, THF, 0°C , 5 days, 70–81% over two steps; (c) (i) elution of ^{18}F 1 (0.03–5 GBq) with **2** in MeOH, (ii) evaporation of MeOH, 80°C , 10 min, (iii) DMSO, 100°C , 15 min followed by RP SPE, 77 \pm 7%; (d) XantPhos Pd G3, r.t. – 37°C , 1–6 min. RD-2-Cys: all-D-tridecapeptide: H-p-t-l-h-t-h-n-f₅-c-NH₂. BSA_{reduced}: bovine serum albumin partially reduced by TCEP. PSMA I&S: MA-s₃-y-nal-k[Sub-K-CO-E]-OH. TCEP: tris(2-carboxyethyl)phosphine; MA: mercaptoacetyl; nal: (R)-(2-naphthyl)alanyl; Sub: suberoyl (1,8-octanedioyl); K-CO-E: Lys-ureido-Glu.

exceed 30%. In search of a more practical alternative to ^{18}F FIB, we turned our attention to the hitherto unknown building block 5-iodo-2- ^{18}F fluoropyridine (^{18}F 1).

^{18}F 1 was produced from easily accessible DABCO-substituted precursor **2**, which was in turn prepared in two steps from the commercially available 5-iodo-2-hydroxypyridine (**3**) according to the procedure of Richard *et al.*²³ (Scheme 1 and ESI[†]), using the “minimalist” protocol.^{21,24} To this end, ^{18}F fluoride was eluted from an anion exchange resin with a solution of **2** in MeOH. After evaporation of MeOH, the residue was taken up in DMSO and the reaction mixture was heated at 100°C for 15 min.²⁵ ^{18}F 1 could be isolated by solid phase extraction (SPE) in RCYs of 77 \pm 7% ($n > 10$) with excellent radiochemical purity (>99%) and reasonable molar activity (29 GBq μmol^{-1} for 1.37 GBq of ^{18}F 1) within 25–30 min.

The applicability of ^{18}F 1 for radiolabeling *via* S-arylation using the protocol of Al-Shuaeeb *et al.*¹⁹ was initially assessed using Boc-Cys-OMe (**4**) as a model thiol (Scheme 1). Short incubation (6 min) of ^{18}F 1 with the substrate (2–6 μmol) in THF (0.24 mL) using XantPhos Pd G3 catalyst (2–24 mol%) and Et_3N (3 equiv.) as base at ambient temperature afforded the ^{18}F -labeled protected amino acid ^{18}F 5 in RCYs of >90% (Table S1, ESI[†]).

Next, application of the procedure for peptide labeling was evaluated. Accordingly, reduced L-glutathione ($\text{H}_2\text{N}-\gamma\text{-Glu-Cys-Gly-CO}_2\text{H}$; GSH; **6**) was conjugated with ^{18}F 1 in 50% THF at ambient temperature using XantPhos Pd G3 (2–8 mol%) and Et_3N (3 equiv.), furnishing ^{18}F 7 in >90% RCYs (Table S1, ESI[†]). In these experiments, 3 μmol of thiol **6** was applied, which is a substrate amount suitable for labeling of small molecules including short peptides. However, it is unacceptably high in the case of larger peptides and proteins including

antibody fragments and constructs thereof, as separation of labeled products from remaining precursors can be difficult or even impossible. High precursor content in PET-tracers can affect PET image quality and cause adverse effects in patients. Consequently, optimization of the different parameters of the conjugation step with the aim to achieve reasonable RCYs at lower thiol loading was carried out, first, using aliquots of ^{18}F 1 (Table S1, ESI[†]). Whereas with 2 μmol GSH almost quantitative labeling was achieved, further reduction of precursor amount led to a high variability of RCYs and at GSH amounts below 500 nmol to a sharp decline of RCYs. We hypothesized that under the basic reaction conditions, the thiol precursor could be rapidly consumed by oxidation to the corresponding disulphide (oxidized glutathione).²⁶ In order to prevent oxidation, the conjugation reaction was carried out under nearly neutral conditions using 0.1 M Na phosphate buffer (PB; pH = 6.7–7.8) instead of Et_3N . Furthermore, different organic solvents like MeOH, MeCN and DMF were evaluated (Table S1, ESI[†]). Under optimized reaction conditions >70% RCYs were obtained with only 33 nmol **6**. With 167 nmol **6** almost quantitative formation of ^{18}F 7 in 50% MeCN was observed already after 1 min reaction time.

Having optimized the novel protocol in small-scale experiments, we turned to the study of its applicability for PET tracer production on a practical scale. Accordingly, S-arylation of **6** with ^{18}F 1 was reevaluated using whole batches of the SPE purified radiolabeled building block (Fig. 1 and Table S2, ESI[†]). In this case, a reciprocal influence of precursor **2** amounts on RCYs of ^{18}F 1 and ^{18}F 7 was observed. While higher precursor loading afforded ^{18}F 1 in higher RCYs, it also increased the amount of nonradioactive impurities, presumably mainly 2-[4-(5-iodopyridin-2-yl)piperazin-1-yl]ethanol, which could not be removed by SPE and interfered with the consecutive conjugation step in the case of low GSH amounts (see Fig. 1B). Nevertheless under optimized conjugation conditions [Pd cat. (0.5 mg), 50% MeCN in PB (pH = 7.3; 0.6 mL), r.t., 6 min] and

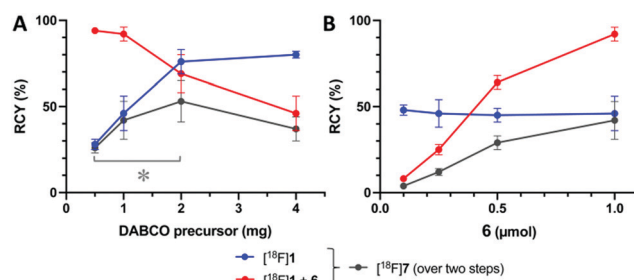
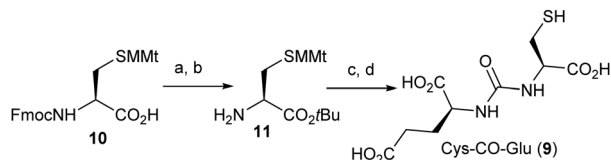


Fig. 1 (A) Dependency of RCYs of ^{18}F 1 (blue) and ^{18}F 7 (over two steps, black) as well as RCYs of the Pd-catalyzed S-arylation of GSH (**6**) with ^{18}F 1 (red) on the amount of **2** used for preparation of ^{18}F 1. (B) Dependency of RCY of ^{18}F 7 (over two steps, black) and RCYs of the Pd-catalyzed S-arylation of GSH (**6**) with ^{18}F 1 (RCY only for the conjugation step, red) on the amount of **6** used for the S-arylation step. RCY of ^{18}F 1 (blue) is also shown. Conditions: experiments were carried out according to GP2 (see Chapter 3.2.2. in ESI[†]) using 1 μmol **6** for the conjugation step (A) or 1 mg **2** for the preparation of ^{18}F 1 (B). All experiments were carried out at least in triplicate.





Scheme 2 Preparation of precursor **9**. Conditions: (a) $\text{CCl}_3(\text{C}=\text{NH})\text{OtBu}$, $\text{BF}_3 \cdot \text{Et}_2\text{O}$, $\text{CH}_2\text{Cl}_2/\text{cyclohexane}$ (1 : 3), 3 h, 63%; (b) Et_2NH , CH_2Cl_2 , 3 h, 89%; (c) **11**, $\text{PhO}(\text{CO})\text{-Glu}(\text{OtBu})_2$ [prepared from $\text{HCl}\cdot\text{H-Glu}(\text{OtBu})_2$; $\text{PhO}(\text{CO})\text{Cl}$, Py , THF , 0°C –r.t., 2 h], DMSO , 60°C , 16 h, 66%; (d) $\text{TFA}/\text{H}_2\text{O}/\text{TIS}$ (90 : 5 : 5), 2 h, 70%.

$^{[18\text{F}]}$ **1** prepared from 1.0 mg DABCO salt **2**, well reproducible conjugation RCYs of 80–90% and 20–30% were obtained using 1 μmol or 250 nmol **6**, respectively (Fig. 1B and Table S2, ESI[†]). Further reduction of the GSH amount was possible if $^{[18\text{F}]}$ **1** isolated by HPLC [RCY = $69 \pm 4\%$ ($n > 10$)] was used.

Being interested in the development of novel PET probes for prostate carcinoma (PCa) imaging,^{27,28} we applied the procedure for the preparation of a prostate specific membrane antigen (PSMA) specific tracer $^{[18\text{F}]}$ **8**. The corresponding thiol precursor **9** was synthesized from Fmoc-Cys(MMt)-OH (**10**) as follows (Scheme 2 and ESI[†]): Fmoc-Cys(MMt)-OH was esterified using *tert*-butyl 2,2,2-trichloroacetamide followed by *N*-Fmoc-deprotection with Et_2NH in CH_2Cl_2 to afford H-Cys(MMt)-OtBu (**11**), which was subsequently allowed to react with crude $\text{PhO}(\text{CO})\text{-Glu}(\text{OtBu})_2$ prepared from phenyl chloroformate and $\text{HCl}\cdot\text{H-Glu}(\text{OtBu})_2$ to give the corresponding protected urea. Finally, deprotection furnished **9** in 26% over four steps. The conjugation reaction between SPE-purified $^{[18\text{F}]}$ **1** and **9** (20 μmol), performed in 50% MeCN in PB (pH 7.3; 0.6 mL) using XantPhos Pd G3 (0.5 mg) and Et_3N to adjust the pH of the reaction mixture to ca. 8.0, afforded after HPLC purification and formulation $^{[18\text{F}]}$ **8** as a ready to use solution in RCYs of $55 \pm 3\%$ ($n = 6$) over two steps and within 90–100 min (Table 1, entry 1). Molar activity of $^{[18\text{F}]}$ **8** was determined to be 11–78 $\text{GBq } \mu\text{mol}^{-1}$ (for 0.015–1.84 GBq ; $n = 4$). Pd content in the final tracer solution amounted to 6.4 ng/batch (ICP-MS) and was well below any level of concern.²⁹ $^{[18\text{F}]}$ **8** was examined by

Table 1 Conjugation of $^{[18\text{F}]}$ **1** with thiol-containing substrates

Entry	Substrate	Amount	Product	RCY \pm SD ^{e,f} [%] (n)
1 ^{a,b,c,d}	Cys-CO-Glu (9)	20 μmol	$^{[18\text{F}]}$ 8	55 ± 3 (6)
2 ^{a,b,c,d}	RD2c (10)	300 nmol	$^{[18\text{F}]}$ 11	40 ± 7 (7)
3 ^{a,b,c,d}	RD2c (12)	3 μmol	$^{[18\text{F}]}$ 13	41 ± 5 (3)
4 ^{a,b,c,d}	BSA (14) ^g	6 nmol	$^{[18\text{F}]}$ 15	40 ± 9 (3)
5 ^{a,b,c,d}	PSMA I&S (16)	0.5 μmol	$^{[18\text{F}]}$ 17	10 ± 3 (3)

^a Reaction solvent: entry 1: 50% MeCN in PB (600 μL ; pH adjusted by Et_3N to 8); entries 2 and 3: 50% MeCN in PB (440 μL); entry 4: 25% MeCN in BB (400 μL); 50% MeCN in PB (600 μL). ^b XantPhos Pd G3; entries 1–3, 5: 0.5 mg; entry 4: 75 μg . ^c T (t): entries 1 and 5: r.t. (6 min); entries 2 and 3: r.t. (12 min); entry 4: 37°C (20 min). ^d Entries 1, 3, 4, 5: SPE purified $^{[18\text{F}]}$ **1**; entry 2: HPLC-purified $^{[18\text{F}]}$ **1**. ^e Isolated yields after HPLC (entries 1–3, 5) or GPC (entry 4). ^f Number of runs. ^g Reduced with TCEP. PB: 0.1 M Na phosphate buffer (pH 7.3); BB: 0.1 M Na borate buffer (pH 8.0). RCY: radiochemical yield over two steps. SD: standard deviation. TCEP: tris(2-carboxethyl)phosphine.

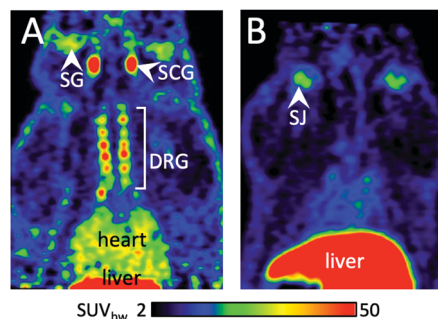


Fig. 2 Biodistribution of $^{[18\text{F}]}$ **8** in a healthy rat determined by μPET . (A): Horizontal view of a summed image taken 60–120 min p.i. (B): Same as A, only with the PSMA blocking agent 2-PMPA (23 mg kg^{-1}). Note that preferential enrichment of $^{[18\text{F}]}$ **8** is observed in the PSMA-positive ganglia with a signal-to-noise ratio of 25 in the SCG (1.5 with 2-PMPA). Liver uptake had a SUV_{bw} of 143 (155 with 2-PMPA). Abbreviations: DRG: dorsal root ganglia; SCG: superior cervical ganglion; SG: salivary gland; SJ: shoulder joint.

μPET imaging in healthy rats, using PSMA-expressing peripheral ganglia as surrogates for small PSMA-positive tumor lesions (Fig. 2).^{27,30}

$^{[18\text{F}]}$ **8** enabled the delineation of the superior cervical and dorsal root ganglia with excellent signal-to-noise ratio. The PSMA specificity of $^{[18\text{F}]}$ **8** accumulation in ganglia was confirmed by inhibition experiments (Fig. 2). Low bone radioactivity uptake indicated high stability of the tracer to *in vivo* defluorination.

Next, we applied the novel protocol for the preparation of a radiolabeled analog of the brain-penetrating all-D arginine-rich dodecapeptide RD2 (H-ptlhthnr₅-NH₂).³¹ RD2 binds specifically to amyloid beta ($\text{A}\beta$) and was developed for the treatment of Alzheimer's disease through elimination of toxic $\text{A}\beta_{42}$ oligomers. Consequently, radiofluorinated RD2 analogs could potentially be applied for $\text{A}\beta$ -PET-imaging. An additional D-Cys residue was introduced at the C-terminus of RD2 and the resulting peptide **12** (300 nmol) was conjugated with HPLC purified $^{[18\text{F}]}$ **1** affording after HPLC isolation and formulation the radiolabeled RD2 conjugate $^{[18\text{F}]}$ **13** in $40 \pm 7\%$ ($n = 7$) RCY over two steps (Table 1, entry 2). $^{[18\text{F}]}$ **13** was also prepared in $41 \pm 5\%$ RCY ($n = 3$) using the SPE purified $^{[18\text{F}]}$ **1** and higher amounts of **12** (3 μmol) (Table 1, entry 3) with a molar activity amounting to 16–100 $\text{GBq } \mu\text{mol}^{-1}$ (for 0.21–1.29 GBq ; $n = 4$).

Next, the protocol was applied for $^{18\text{F}}$ -fluorination of small proteins using bovine serum albumin (BSA, **14**) as a model substrate. This structurally well characterized protein contains one Cys residue and 17 disulfide bonds. Native BSA (6 nmol) was partially reduced using TCEP-HCl (10 equiv.) at 37°C for 1 h and directly conjugated to $^{[18\text{F}]}$ **1**. After 20 min incubation at 37°C , radiolabeled BSA $^{[18\text{F}]}$ **15** was isolated by gel permeation chromatography (GPC) in RCYs of $40 \pm 9\%$ ($n = 3$) (Table 1, entry 4). No formation of $^{[18\text{F}]}$ **15** was observed in the absence of the Pd catalyst. If unreduced BSA was used, $^{[18\text{F}]}$ **15** was isolated in $12 \pm 2\%$ RCY ($n = 3$) (Table S3, ESI[†]).

Planar scintigraphy and single photon emission tomography (SPECT) using $^{99\text{m}}\text{Tc}$ -labeled tracers is still an important



application in nuclear medicine with more than 25 million scans worldwide per year.^{32,33} Several approaches can be used for ^{99m}Tc labeling of peptides. One of them consists of chelation with conjugates containing *N*-terminal thioacetyl or a suitably positioned Cys residue.³⁴ Consequently, *S*-arylation should enable direct transformation of SPECT radiolabeling precursors into PET-tracers, so to speak a “^{99m}Tc/¹⁸F switch”. In order to examine the practicability of this approach, we ¹⁸F-fluorinated PSMA I&S (**16**), a precursor of the ^{99m}Tc-labeled PSMA-targeting ligand used for radioguided surgery of prostate tumors.^{35,36} [¹⁸F]**17** was successfully prepared using [¹⁸F]**1** and 500 nmol PSMA I&S in a RCY of 10 ± 3% (*n* = 3) over two steps (Table S2, ESI† entry 5).

In summary, Pd-catalyzed *S*-arylation with easily accessible 5-iodo-2-[¹⁸F]fluoropyridine is a convenient method for thiol-selective radiolabeling. Rapid kinetics under mild aqueous conditions makes it well-suited for Cys-specific ¹⁸F-labeling of peptides and proteins. The method could also be applied for the preparation of PET-tracers from conjugates originally designed for ^{99m}Tc labeling. Noteworthy, PSMA-specific [¹⁸F]**8** prepared by this novel protocol turned out to be a promising candidate for prostate carcinoma imaging.

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Conflicts of interest

There are no conflicts to declare.

Notes and references

§ Determined by HPLC analysis of the crude reaction mixture after addition of H₂O (radioactivity in solution ≥ 95%).

- Z. Brady, M. L. Taylor, M. Haynes, M. Whitaker, A. Mullen, L. Clews, M. Partridge, R. J. Hicks and J. V. Trapp, *Australas. Phys. Eng. Sci. Med.*, 2008, **31**, 90–109.
- P. M. Matthews, E. A. Rabiner, J. Passchier and R. N. Gunn, *Br. J. Clin. Pharmacol.*, 2012, **73**, 175–186.
- S. M. Ametamey, M. Honer and P. A. Schubiger, *Chem. Rev.*, 2008, **108**, 1501–1516.
- O. Jacobson, D. O. Kiesewetter and X. Chen, *Bioconjugate Chem.*, 2015, **26**, 1–18.
- M. Zhang, S. Li, H. Zhang and H. Xu, *Eur. J. Med. Chem.*, 2020, **205**, 112629.
- J. Ermert, M. Benešová, V. Hugenberg, V. Gupta, I. Spahn, H.-J. Pietzsch, C. Liolios and K. Kopka, *Clinical Nuclear Medicine*, Springer International Publishing, Cham, 2020, pp. 49–191.
- V. Bernard-Gauthier, M. L. Lepage, B. Waengler, J. J. Bailey, S. H. Liang, D. M. Perrin, N. Vasdev and R. Schirmacher, *J. Nucl. Med.*, 2018, **59**, 568–572.
- F. Cleeren, J. Lecina, M. Ahamed, G. Raes, N. Devoogdt, V. Cavaliere, P. McQuade, D. J. Rubins, W. Li, A. Verbruggen, C. Xavier and G. Bormans, *Theranostics*, 2017, **7**, 2924–2939.
- R. Schirmacher, B. Wängler, J. Bailey, V. Bernard-Gauthier, E. Schirmacher and C. Wängler, *Semin. Nucl. Med.*, 2017, **47**, 474–492.
- C. W. Kee, O. Tack, F. Guibbal, T. C. Wilson, P. G. Isenegger, M. Imiolek, S. Verhoog, M. Tilby, G. Boscutti, S. Ashworth, J. Chupin, R. Kashani, A. W. J. Poh, J. K. Sosabowski, S. Macholl, C. Plisson, B. Cornelissen, M. C. Willis, J. Passchier, B. G. Davis and V. Gouverneur, *J. Am. Chem. Soc.*, 2020, **142**, 1180–1185.
- W. Cai, X. Zhang, Y. Wu and X. Chen, *J. Nucl. Med.*, 2006, **47**, 1172–1180.
- D. O. Kiesewetter, O. Jacobson, L. Lang and X. Chen, *Appl. Radiat. Isot.*, 2011, **69**, 410–414.
- B. de Bruin, B. Kuhnast, F. Hinnen, L. Yaouancq, M. Amessou, L. Johannes, A. Samson, R. Boisgard, B. Tavitian and F. Dollé, *Bioconjugate Chem.*, 2005, **16**, 406–420.
- M. Cavani, D. Bier, M. Holschbach and H. H. Coenen, *J. Labelled Compd. Radiopharm.*, 2017, **60**, 87–92.
- A. Chiotellis, F. Sladojevich, L. Mu, A. Müller Herde, I. E. Valverde, V. Tolmachev, R. Schibli, S. M. Ametamey and T. L. Mindt, *Chem. Commun.*, 2016, **52**, 6083–6086.
- S. Verhoog, C. W. Kee, Y. Wang, T. Khotavivattana, T. C. Wilson, J. Kersemans, S. Smart, M. Tredwell, B. G. Davis and V. Gouverneur, *J. Am. Chem. Soc.*, 2018, **140**, 1572–1575.
- A. D. Baldwin and K. L. Kiick, *Bioconjugate Chem.*, 2011, **22**, 1946–1953.
- T. Zhang, J. Cai, H. Wang, M. Wang, H. Yuan, Z. Wu, X. Ma and Z. Li, *Bioconjugate Chem.*, 2020, **31**, 2482–2487.
- R. A. A. Al-Shuaieeb, S. Kolodych, O. Koniev, S. Delacroix, S. Erb, S. Nicolay, J.-C. Cintrat, J.-D. Brion, S. Cianféran, M. Alami, A. Wagner and S. Messaoudi, *Chem. – A Eur. J.*, 2016, **22**, 11365–11370.
- J. Way, V. Bouvet and F. Wuest, *Curr. Org. Chem.*, 2013, **17**, 2138–2152.
- F. Kügler, J. Ermert, P. Kaufholz and H. Coenen, *Molecules*, 2014, **20**, 470–486.
- J. D. Way and F. Wuest, *J. Labelled Compd. Radiopharm.*, 2014, **57**, 104–109.
- M. Richard, S. Specklin, M. Roche, F. Hinnen and B. Kuhnast, *Chem. Commun.*, 2020, **56**, 2507–2510.
- B. D. Zlatopolskiy, J. Zischler, P. Krapf, R. Richarz, K. Lauchner and B. Neumaier, *J. Labelled Compd. Radiopharm.*, 2019, **62**, 404–410.
- R. Richarz, P. Krapf, F. Zarrad, E. A. Urusova, B. Neumaier and B. D. Zlatopolskiy, *Org. Biomol. Chem.*, 2014, **12**, 8094–8099.
- R. Stevens, L. Stevens and N. Price, *Biochem. Educ.*, 1983, **11**, 70.
- B. D. Zlatopolskiy, H. Endepols, P. Krapf, M. Guliyev, E. A. Urusova, R. Richarz, M. Hohberg, M. Dietlein, A. Drzezga and B. Neumaier, *J. Nucl. Med.*, 2019, **60**, 817–823.
- A. Morgenroth, E. A. Urusova, C. Dinger, E. Al-Momani, T. Kull, G. Glatting, H. Frauendorf, O. Jahn, F. M. Mottaghy, S. N. Reske and B. D. Zlatopolskiy, *Chem. – A Eur. J.*, 2011, **17**, 10144–10150.
- ICH guideline Q3D (R1) on elemental impurities, 2021, https://www.ema.europa.eu/en/documents/scientific-guideline/international-conference-harmonisation-technical-requirements-registration-pharmaceuticals-human-use_en-32.pdf.
- H. Endepols, A. Morgenroth, B. D. Zlatopolskiy, P. Krapf, J. Zischler, R. Richarz, S. Muñoz Vásquez, B. Neumaier and F. M. Mottaghy, *BMC Cancer*, 2019, **19**, 633.
- T. van Groen, S. Schemmert, O. Brener, L. Gremer, T. Ziehm, M. Tusche, L. Nagel-Steger, I. Kadish, E. Schartmann, A. Elfgen, D. Jürgens, A. Willuweit, J. Kutzsche and D. Willbold, *Sci. Rep.*, 2017, **7**, 16275.
- National Research Council, *Medical Isotope Production Without Highly Enriched Uranium*, ed. C. Whipple and S. Larson, National Academies Press, Washington, D.C., 2009, DOI: 10.17226/12569.
- I. Amato, *Chem. Eng. News*, 2009, **87**, 58–64.
- D. Papagiannopoulou, *J. Labelled Compd. Radiopharm.*, 2017, **60**, 502–520.
- P. Werner, C. Neumann, M. Eiber, H. J. Wester and M. Schottelius, *EJNMMI Res.*, 2020, **10**, 45.
- S. Robu, M. Schottelius, M. Eiber, T. Maurer, J. Gschwend, M. Schwaiger and H.-J. Wester, *J. Nucl. Med.*, 2017, **58**, 235–242.

