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Isotopic Labeling

Synthesis of ^{18}F-Labelled β-Lactams by Using the Kinugasa Reaction

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Abstract: Owing to their broad spectrum of biological activities and low toxicity, β-lactams are attractive lead structures for the design of novel molecular probes. However, the synthesis of positron emission tomography (PET)-isotope-labelled β-lactams has not yet been reported. Herein, we describe the simple preparation of radiofluorinated β-lactams by using the fast Kinugasa reaction between ^{18}F-labelled nitrone [^{18}F]-**1** and alkynes of different reactivity. Additionally, ^{18}F-labelled fused β-lactams were obtained through the reaction of a cyclic nitrone **7** with radiofluorinated alkynes [^{18}F]-**6a**,**b**. Radiochemical yields of the Kinugasa reaction products could be significantly increased by the use of different Cu^{I} ligands, which additionally allowed a reduction in the amount of precursor and/or reaction time. Model radiofluorinated β-lactam-peptide and protein conjugates ([^{18}F]-**10** and ^{18}F-labelled BSA conjugate) were efficiently obtained in high yield under mild conditions (aq. MeCN, ambient temperature) within a short reaction time, demonstrating the suitability of the developed method for radiolabelling of sensitive molecules such as biopolymers.

Introduction

Amongst the available imaging technologies, positron emission tomography (PET) plays a very important role due to its outstanding potential to visualize physiological processes at the molecular level in real time. PET is therefore essential in clinical diagnostics and has gained major significance in drug development. Beside technical improvements, PET benefits from innovations in the field of tracer development, comprising both progress in labelling strategies and an intelligent design of selective molecular probes with the capability to visualize molecular targets involved in physiological and pathophysiological processes. A prerequisite for the latter is a detailed understanding of the biology underlying normal or diseased states at the molecular level. Molecular probes for PET-imaging must be labelled with suitable β^+-emitting nuclides. Among the spectrum of easily available radionuclides ^{18}F-fluorine is still the nuclide with the highest impact in PET research. This is mainly due to the excellent nuclear properties of ^{18}F in comparison to other cyclotron-produced nuclides. Decay characteristics of ^{18}F [$E(\beta^+) = 630$ keV, abundance: 97%; $t_{1/2} =$ 109.8 min] make it an ideal PET-isotope with respect to half-life and resolution. However, although much effort has been spent on the development of novel methods for incorporation of ^{18}F into molecules of interest, radiofluorination methods are still rather rare in comparison to fluorination methods used in conventional organic chemistry.[1] This is due mainly to the tiny amount of no-carrier-added (n.c.a.) $^{18}F^-$ (subnanomolar range) as well as to time restrictions and radiation safety measures. Hence, even promising modern fluorination techniques cannot easily be transferred from synthetic organic chemistry to radiochemistry.[2] Despite this, there are several successful examples of such translations, including the azide–alkyne "click" reactions[3] and metal-catalyzed fluorination methods, which enable easy access to otherwise inaccessible novel radiotracers.[4,5] Furthermore, a simple and efficient metal-free preparation of ^{18}F-labelled compounds through (3+2) cycloaddition reactions of radiofluorinated 1,3-dipoles other than azide to double or triple C–C bonds has recently been reported.[6]

β-Lactam antibiotics are amongst the most successful therapeutic agents developed to date. They exert their activity by inhibition of bacterial cell wall biosynthesis. The mechanism of action involves an irreversible inactivation of penicillin binding proteins (PBPs), which are serine proteases with transglycosy-

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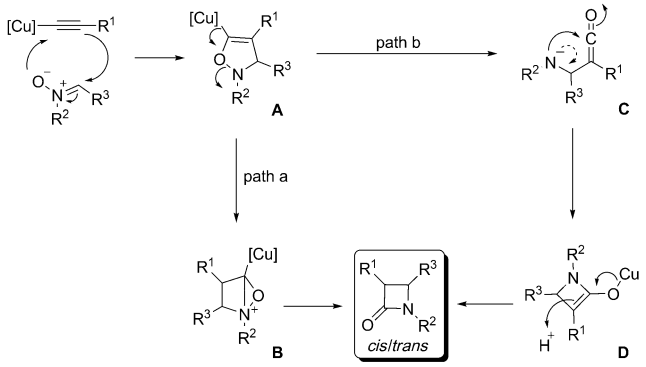
lase, DD-transpeptidase and DD-carboxypeptidase activities, that act through acylation of the nucleophilic serine residue at the enzyme active site.[7] Beside β-lactam antibiotics, numerous β-lactams with nonantibiotic activities have been discovered.[8] They exert their effects by inhibition of further serine proteinases such as thrombin, elastases, prostate specific antigen (PSA), human cytomegalovirus (HCV) protease as well as matrix metalloproteinases, cysteine proteases, and tubulin polymerization. Consequently, radiolabelled β-lactams can potentially be used for PET-imaging of bacterial and viral infections as well as thrombosis, emphysema, and tumors. However, no PET-tracers with a β-lactam structural motif have been published to date.

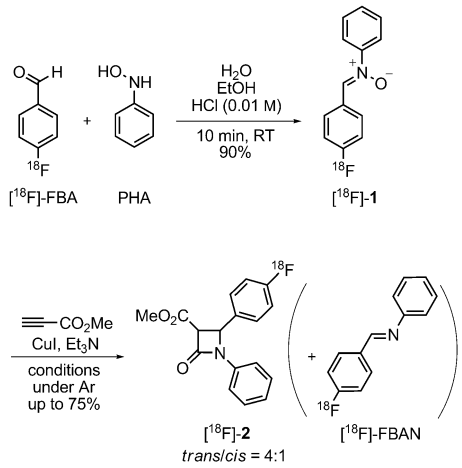
The Kinugasa reaction[9] (Scheme 1) is a valuable tool in preparative organic chemistry, offering easy access to β-lactams through copper(I)-catalyzed reaction between nitrones and terminal alkynes. Two plausible mechanisms of the Kinugasa reaction have been proposed. According to both, the transformation begins with the formation of a copper acetylide in situ (in the initial report[9a] preformed copper acetylides were used) (Scheme 1). Subsequent 1,3-cycloaddition of the latter to the nitrone gives 5-isoxazolinyl cuprate **A**. According to Ding and Irwing (path a),[9b,10a,b] the initially formed cuprate **A** rearranges into the highly strained, extremely unstable intermediate bicyclic oxaziridinium salt **B**, which undergoes spontaneous opening of the oxaziridinium ring to give (after protonation) the corresponding β-lactam as a mixture of *cis*/*trans* isomers. In an alternative mechanism, proposed by Ye et al.[10c] (path b), diastereomeric 2-azetidinones are obtained from cuprate **A** through ring-opening fragmentation followed by recyclization and protonation. Although the Kinugasa reaction usually gives β-lactams as mixtures of racemic diastereoisomers, numerous stereoselective variants of this transformation using the appropriate chiral Cu^{I} ligands or chiral auxiliaries have been reported.[10d–f] Application of the Kinugasa reaction to radiochemistry could allow simple access to radiolabelled 2-azetidinones. Herein, we describe for the first time a method for the preparation of radiofluorinated monocyclic β-lactams through the Kinugasa reaction between the easily accessible ^{18}F-labelled *C*-4-fluorophenyl-*N*-phenyl nitrone ([^{18}F]-**1**)[6b] and a range of terminal alkynes. Furthermore, a model cyclic nitrone **7** was reacted with radiolabelled fluorophenylacetylenes [^{18}F]-**6a**,**b** under Kinugasa conditions to give the corresponding labelled bicyclic β-lactams [^{18}F]-**8a**,**b**. Finally, a study was carried out on the applicability of the radio-Kinugasa reaction for labelling of peptides and biomolecules, using H-βAla-Phe-OMe and bovine serum albumin (BSA) as model peptide and protein, respectively.



Scheme 1. Proposed mechanisms of the Kinugasa reaction. Path a: via oxaziridinium intermediate **B**;[9b,10b] path b: via β-aminoketene intermediate **C**.[10c]

Results and Discussion

[^{18}F]-**1** was prepared by acid-catalyzed condensation of 4-[^{18}F]-fluorobenzaldehyde ([^{18}F]-FBA) with *N*-phenylhydroxylamine, as described earlier,[6b] in a radiochemical yield of 89–92% (Scheme 2). The ^{18}F-labelled synthon was allowed to react with methyl propiolate in the presence of CuI and Et_3N in MeCN at ambient temperature. The reaction was carried out under Ar to avoid the consumption of alkyne through Glaser coupling. The corresponding radiolabelled β-lactam [^{18}F]-**2** was formed after only 10 min reaction time in a radiochemical yield (RCY) of 65%, as a mixture of diastereomers. The thermodynamically favored *trans*-isomer was the predominant isomer obtained (*trans*/*cis* = 4:1). A prolonged reaction time of 20 min resulted in a slightly improved radiochemical yield of 67%. The main impurity (up to 25%) was identified as *N*-4-[^{18}F]-fluorobenzylidene aniline ([^{18}F]-FBAN).



Scheme 2. Preparation of ^{18}F-labelled β-lactam [^{18}F]-**2** under an inert atmosphere.

These promising initial results prompted us to optimize the Kinugasa reaction with respect to precursor amount, reaction time, solvent, Cu^{I}-stabilizing ligands and temperature.

In initial experiments, 10 μmol methyl propiolate precursor was used. Normally, this is sufficiently low for labelling of small molecules. However, this precursor amount is unacceptably high, for example in the case of peptides and proteins for

which precursors are generally difficult to separate from the labelled products. High precursor content can impair the quality of PET-images and/or even cause undesirable effects in patients, impeding the clinical application of the tracer.

To optimize the Kinugasa reaction with respect to precursor amount, nitrone [^{18}F]-**1** was allowed to react with different amounts of methyl propiolate [0.05–10 μmol at ambient temperature and 10 or 20 min reaction time (Figure 1)]. Reducing

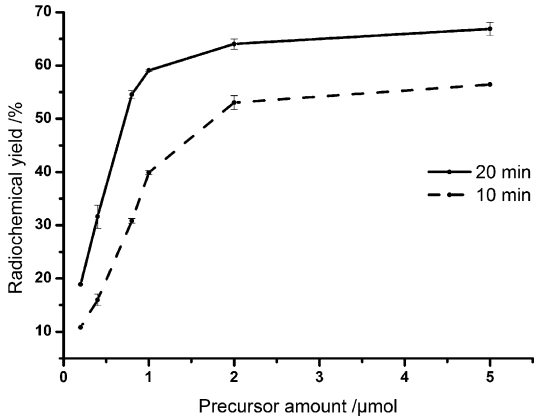


Figure 1. Radiochemical yield of [^{18}F]-**2** as a function of precursor amount.

the amount of the alkyne precursor from 10 to 0.8 μmol caused a gradual decrease of RCYs from 65 and 67% to 31 and 55% after 10 and 20 min, respectively. By further reducing the precursor amount to 0.2 μmol, the RCYs of [^{18}F]-**2** decreased to 11 and 19% after 10 and 20 min, respectively. All further optimization experiments were carried out with 0.8 μmol methyl propiolate, at a reaction time of 10 min at ambient temperature.

Formation of ^{18}F-labelled β-lactam [^{18}F]-**2** was dependent on temperature (Figure 2). Increasing the temperature from 25 to 60 °C improved the yield of the product from 31 to 68% within 10 min. Simultaneously, competitive formation of the side product [^{18}F]-FBAN (up to 30%) was observed. Further elevation of temperature did not increase RCY.

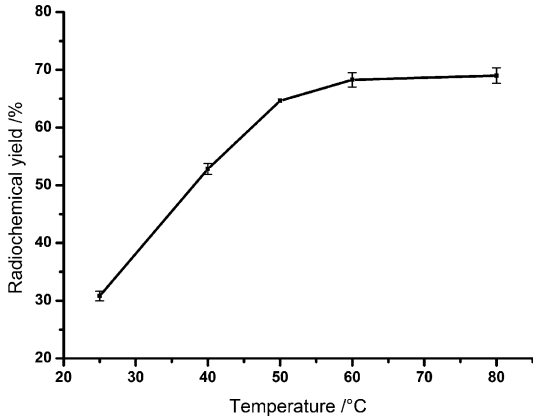


Figure 2. Dependence of radiochemical yield of [^{18}F]-**2** on temperature.

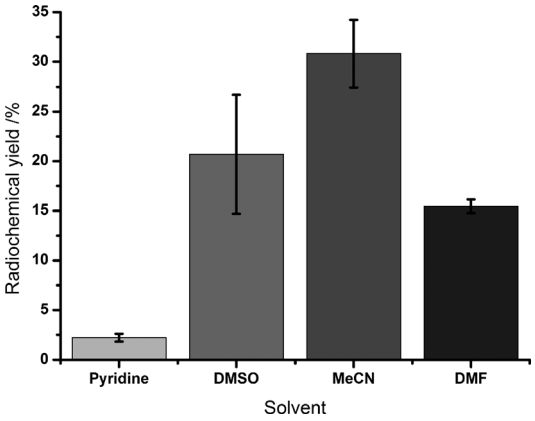


Figure 3. Radiochemical yield of [^{18}F]-**2** in different solvents.

The Kinugasa reaction between [^{18}F]-**1** and methyl propiolate was tested in different solvents (Figure 3). In dimethyl sulfoxide (DMSO) and *N*,*N*-dimethylformamide (DMF) formation of [^{18}F]-**2** was significantly lower than that observed in MeCN (21 and 16% vs. 31%, respectively). When the radiosynthesis was carried out in pyridine only traces of the product were formed (ca. 2%), presumably due to almost instantaneous consumption of methyl propiolate in a Michael reaction with pyridine.[11]

Miura et al.[10b] found that application of particular nitrogen ligands accelerated the Kinugasa reaction due to efficient stabilization of the reactive monomeric copper acetylide, preventing Cu^{I} oxidation and/or disproportionation to Cu^{0} and Cu^{II}.[12] Accordingly, in the presence of pyridine, a moderate increase of radiochemical yield (from 31 to 43%) was observed (Figure 4). A more efficient ligand was 1,10-phenanthroline

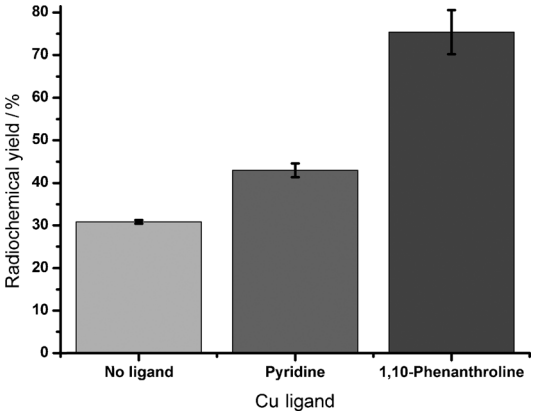
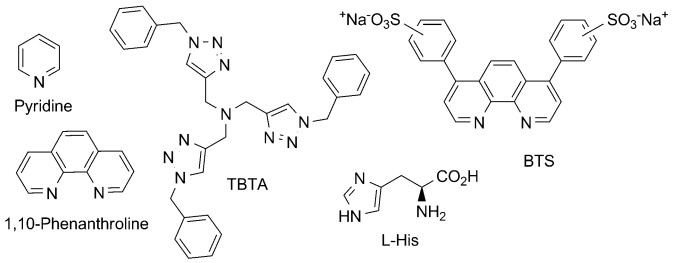


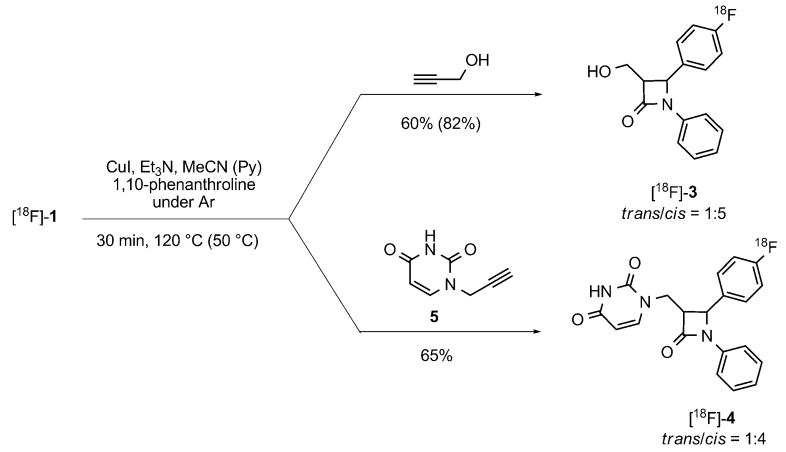
Figure 4. Influence of Cu^{I}-stabilizing ligands on the radiochemical yield of [^{18}F]-**2**.

(Scheme 3); in the presence of this ligand a maximum radiochemical yield of 75% was observed for [^{18}F]-**2**.

Having established the optimized reaction conditions for the radio-Kinugasa reaction with highly reactive alkynes, attention was focused on less reactive alkynes. Propargyl alcohol was chosen as a representative less reactive alkyne (Scheme 4). Preliminary experiments had shown that higher reaction tempera-



Scheme 3. Cu^{I}-stabilizing ligands used in this work.

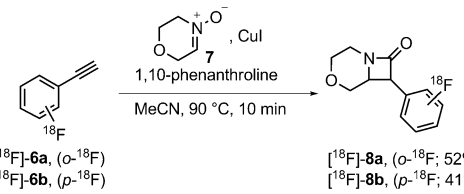


Scheme 4. Synthesis of labelled β-lactam alcohol [^{18}F]-**3** and β-lactam-nucleobase chimera [^{18}F]-**4**.

tures were mandatory to obtain [^{18}F]-**3** in reasonable radiochemical yields within a time-frame that was compatible with the half-life of ^{18}F. Reaction of [^{18}F]-**1** with 10 μmol propargyl alcohol in the presence of 1,10-phenanthroline at 120 °C for 30 min in MeCN produced [^{18}F]-**3** in a radiochemical yield of 60%. Similar results were obtained by using tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA),[12] which is widely used for ligand-promoted acceleration of the copper-catalyzed azide-alkyne cycloaddition reactions. In contrast, a radiochemical yield of 82% of the desired product was obtained at 50 °C in pyridine without ligand. In this case, the preferential formation of the kinetically favored *cis*-isomer (*trans*/*cis* ratio = 1:5) was observed. The main side product detected in the reaction mixture was [^{18}F]-FBA, originating from decomposition of [^{18}F]-**1**. In MeCN solvent, the radiochemical yields decreased rapidly with decreasing alkyne amount. When 1 μmol precursor was used, only 10% [^{18}F]-**3** was formed in the reaction mixture. In contrast, using 1 μmol precursor in pyridine, radiofluorinated β-lactam was obtained in a satisfactory yield of 50%.

With optimized conditions for the Kinugasa reaction between radiofluorinated nitrone [^{18}F]-**1** and moderately activated alkynes to hand, we tried to prepare ^{18}F-labelled β-lactam-nucleobase chimera [^{18}F]-**4** (Scheme 4). Radiofluorinated compounds of this type are of significant potential for the imaging of bacterial infections. To this end, [^{18}F]-**1** was allowed to react with 1-propargyl uracyl (**5**)[13] in MeCN to give the uracyl conjugate [^{18}F]-**4** as a mixture of *cis*/*trans* isomers (4:1) in a radiochemical yield of 65%. In contrast, only traces of [^{18}F]-**4** could be detected when pyridine was used. Instead of the expected product, two hydrophobic ^{18}F-labelled byproducts were observed in the reaction mixture. This unexpected result can probably be attributed to elimination of the nucleobase from the nucleotide analogue [^{18}F]-**4** to give the corresponding 3-methylene-substituted β-lactam.[14] The latter could react further with pyridine or 1-propargyluracil under formation of the respective *aza*-Michael adducts.

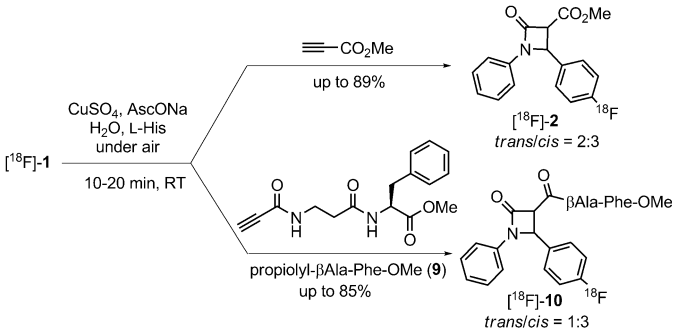
Having developed a method for the preparation of radiofluorinated monocyclic β-lactams through the radio-Kinugasa reaction, the application of the Kinugasa reaction in the synthesis of ^{18}F-labelled fused β-lactams was studied. In this case, easily accessible *o*- and *p*-[^{18}F]-fluorophenyl acetylenes ([^{18}F]-**6a** and [^{18}F]-**6b**, respectively) were used as radiolabelled building blocks (Scheme 5). In the presence of 1,10-phenanthroline these compounds reacted with the model nitrone 3,6-dihydro-2*H*-1,4-oxazine-4-oxide (**7**)[9b] to give the corresponding radiofluorinated bicyclic β-lactams [^{18}F]-**8a** and [^{18}F]-**8b** in radiochemical yields of 52 and 41%, respectively, within 10 min.



Scheme 5. Preparation of the labelled bicyclic β-lactams [^{18}F]-**8a** and [^{18}F]-**8b**.

Given the rapid kinetics of the Kinugasa reaction between C,N-diaryl nitrones and activated alkynes at ambient temperature, we investigated whether this approach might also be suitable for the radiofluorination of peptides and biopolymers. The original Kinugasa reaction should be carried out under an inert atmosphere in anhydrous organic solvents. This is a significant drawback with respect to labelling of the unprotected peptides and proteins because they are often not readily soluble in organic solvents such as acetonitrile. Basak et al.[15] exploited Cu^{I} generation in situ through reduction of $CuSO_4$ with sodium ascorbate as is usual for the azide-alkyne click reaction.[3] They were able to prepare several β-lactams in moderate yields in aqueous MeCN (or DMF) under an inert atmosphere by using Et_3N as a base.

In preliminary experiments we tested the "cold" Kinugasa reaction between **1** and methyl propiolate under "Basak" condi-



Scheme 6. Conjugation of nitrone [^{18}F]-**1** with methyl propiolate and the model dipeptide **9** under click conditions.

tions at ambient temperature and were able to detect only traces of β-lactam **2** in the reaction mixture. In contrast to the published results, we observed formation of the desired product in suitable yields within 10 min *without* base addition. Moreover, it should be pointed out that this modification of the Kinugasa reaction works equally well under air. Nevertheless, reasonable yields of [^{18}F]-**2** from [^{18}F]-**1** could only be achieved with alkyne amounts of more than 50 μmol (Scheme 6). To reduce the precursor amount, we tested the influence of the Cu^{I}-stabilizing ligands TBTA,[12] bathophenanthroline disodium disulfonate (BTS)[16] and L-histidine[17] (Scheme 3), which are known from click chemistry, on the yields of the radio-Kinugasa reaction. In the presence of each of the three ligands only 0.4 μmol methyl propiolate was necessary to obtain [^{18}F]-**2** in 80% radiochemical yields (Figure 5). Nontoxic and inexpensive

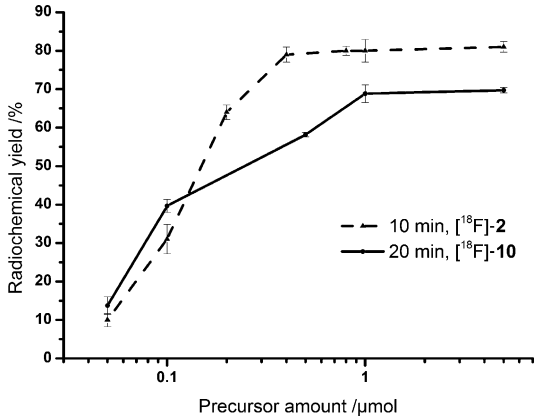


Figure 5. Dependence of radiochemical yields of [^{18}F]-**2** and [^{18}F]-**10** on the amount of alkyne precursors (methyl propiolate and dipeptide **9**, respectively) under click conditions.

L-histidine was chosen for further experiments. In contrast to the preferential formation of the thermodynamically more stable *trans*-isomer under basic "classical" Kinugasa reaction conditions (see above), the kinetically favored *cis*-isomer (*cis*/*trans* = 3:2) was preferentially formed under weak acidic click conditions. In this case, [^{18}F]-FBA (up to 20%), formed through hydrolysis of [^{18}F]-**1**, was identified as the main side product.

We next studied the labelling of propiolyl-substituted βAla-Phe-OMe **9** through conjugation with [^{18}F]-**1** (Scheme 6). Radiofluorinated depsipeptide [^{18}F]-**10** (*cis*/*trans* = 3:1) was obtained in a radiochemical yield of 58% within 10 min. A prolonged reaction time of 20 min was necessary to obtain the maximal radiochemical yields of 68–70%.

The dependency of radiochemical yields of [^{18}F]-**2** and [^{18}F]-**10** on the amount of alkyne precursor was studied under click conditions after 10 and 20 min reaction time, respectively (Figure 5). The maximum radiochemical yield of [^{18}F]-**2** (80%) was already achieved at 0.4 μmol, whereas for [^{18}F]-**10** (69%) the use of 1 μmol precursor was necessary. Minimization of the amount of alkyne to 100 nmol still produced the radiolabelled β-lactams [^{18}F]-**2** and [^{18}F]-**10** in moderate radiochemical yields of 31 and 40%, respectively.

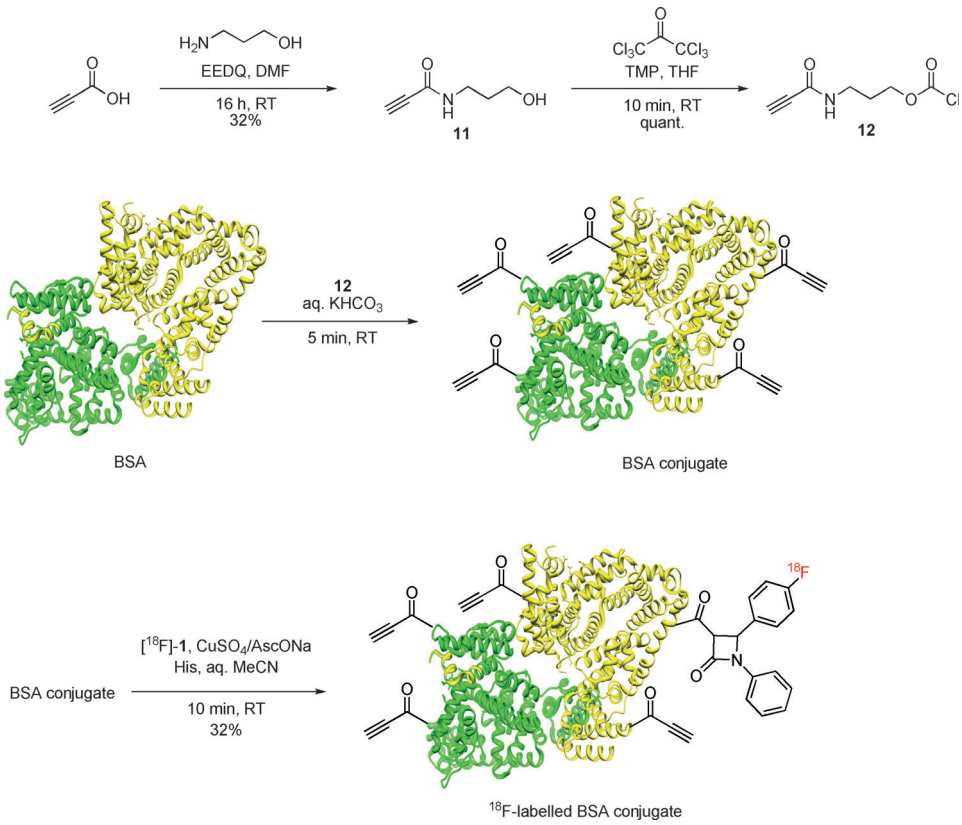
Once the protocol for the radio-Kinugasa reaction under click conditions had been established, we turned to the development of a simplified variant of this radiosynthesis. After reaction of [^{18}F]-FBA with *N*-phenylhydroxylamine at ambient temperature for 10 min in the presence of traces of HCl and subsequent addition of the reaction mixture to an aqueous solution containing the alkyne precursor (1 μmol) and the other reagents ($CuSO_4 \cdot 5H_2O$, AscONa, L-histidine), [^{18}F]-**2** and [^{18}F]-**10** were obtained in excellent radiochemical yields of 89 and 85%, respectively (Scheme 6).

The hydrolytic and serum stability of [^{18}F]-**10** was briefly studied. No decomposition of [^{18}F]-**10** was observed within a pH range from 1 to 9 or in human blood serum at 37 °C for at least 3 h.

The results detailed above raised the question of whether the radio-Kinugasa reaction might be suitable for the radiolabelling of propiolated proteins. To this end, bovine serum albumin (BSA; M_r = 66.5 kDa), chosen as a prototypical substrate, was acylated with chloroformate **12** in 0.15 M $KHCO_3$ for 5 min at ambient temperature (Scheme 7). Compound **12** was prepared as follows: propiolic acid was coupled with 3-aminopropanol-1 by using 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) to give 3-propionamidopropanol-1 (**11**) in moderate yield. The latter was treated with triphosgene in the presence of 2,4,6-trimethylpyridine (TMP) in tetrahydrofuran (THF) to give acylating agent **12**, which was immediately used for protein modification. The crude functionalized protein was purified by ultrafiltration. Propiolated BSA (19 nmol) was labelled with [^{18}F]-**1** (400–500 MBq) under click conditions in aqueous MeCN for 10 min at ambient temperature. Finally, the radiofluorinated BSA conjugate was isolated by simple filtration through a PD10 desalting cartridge to give the desired product in 32% radiochemical yield and in excellent radiochemical and chemical purity (Figure 6). In a control experiment, native BSA was treated with [^{18}F]-**1**. In this case radiolabelling of protein did not take place.

Conclusion

We have demonstrated that the Kinugasa reaction is an efficient tool that can be used for the simple and high-yielding preparation of ^{18}F-labelled β-lactams. The rapid kinetics of the



Scheme 7. Preparation of propiolated BSA and its ^{18}F-labelling through the radio-Kinugasa reaction. EEDQ: 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline, TMP: 2,4,6-trimethylpyridine.

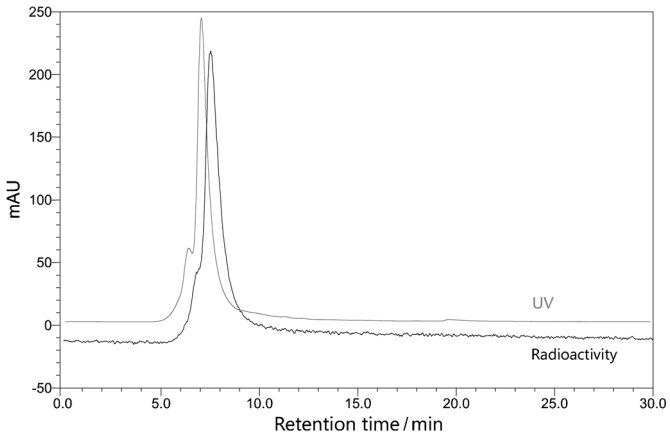


Figure 6. Analytical HPLC analysis of ^{18}F-labelled BSA conjugate: UV ($\lambda = 254$ nm; gray) and radioactivity (black) traces.

radio-Kinugasa reaction with activated alkynes under mild click conditions make it well-suited for the labelling of proteins, peptides and other biopolymers.

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Keywords: click chemistry · imaging agents · isotopic labelling · β-lactams · radiopharmaceuticals

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