Access to ^{18}F-labelled isoxazoles by Ruthenium-promoted 1,3-dipolar cycloaddition of 4-[^{18}F]fluoro-*N*-hydroxybenzimidoyl chloride with alkynes

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Abstract

4-[^{18}F]Fluoro-*N*-hydroxybenzimidoyl chloride (**^{18}FBIC**) an ^{18}F-labelled aromatic nitrile oxide was developed as building block for Ru-promoted 1,3-dipolar cycloaddition with alkynes. **^{18}FBIC** is obtained in a one-pot synthesis in up to 84% radiochemical yield starting from [^{18}F]fluoride with 4-[^{18}F]fluorobenzaldehyde (^{18}FBA) and 4-[^{18}F]fluorobenzaldehyde oxime (^{18}FBAO) as intermediates, by reaction of ^{18}FBAO with *N*-chlorosuccinimide (NCS). **^{18}FBIC** was found to be a suitable and stable synthon to give access to ^{18}F-labelled 3,4-diarylsubstituted isoxazoles by [Cp*RuCl(cod)]-catalyzed 1,3-dipolar cycloaddition with various alkynes. So, the radiosynthesis of a fluorine-18 labelled COX-2 inhibitor [^{18}F]**1b**, a close derivative of valdecoxib, was performed with **^{18}FBIC** and 1-ethynyl-4-(methylsulfonyl)benzene, providing **[^{18}F]1b** in up to 40% RCY after purification in 85 min. The application of **^{18}FBIC** as a building block in the synthesis of ^{18}F-labelled heterocycles will generally extend the portfolio of available PET radiotracers.

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1. Introduction

Positron emission tomography (PET) is a modern imaging technique used for various applications in nuclear medicine, including diagnosis of disease, monitoring of treatment and post-operative medical care of the patient. In pre-clinical research PET has an important impact on the determination of the pharmacokinetics and pharmacodynamics of radiolabelled drugs and on the development and evaluation of new potential radiotracers. Fluorine-18 is produced by cyclotrons and is the most widely and routinely used positron-emitting radionuclide for PET, possessing a very suitable positron energy (0.633 MeV, 97% β^+) and a high spatial resolution (approx. 1mm) in PET images.[1] At 109 min, the half-life of fluorine-18 is adequate for performing multi-step radiolabelling reactions and is sufficient to enable further transport of ^{18}F-labelled radiopharmaceuticals to the application site.

Over the last decades, a broad, ever-widening portfolio of methods has been developed for the synthesis of ^{18}F-labelled compounds (radiotracers) and ^{18}F-labelled medicinal drugs (radiopharmaceuticals). This covers both the direct substitution of a leaving group at the target molecule by the radionuclide (direct approach) as well as the application of so-called building blocks for radiolabelling (indirect approach). The latter makes use of small reactive ^{18}F-labelled molecules which are coupled with the (bio)molecule of interest, forming the final radiotracer. Both approaches have advantages and limitations displayed by such parameters as reaction time and conditions, yield, regioselectivity, requirement of protective groups and metabolic stability of the product, what has been discussed recently in excellent reviews.[2,3]

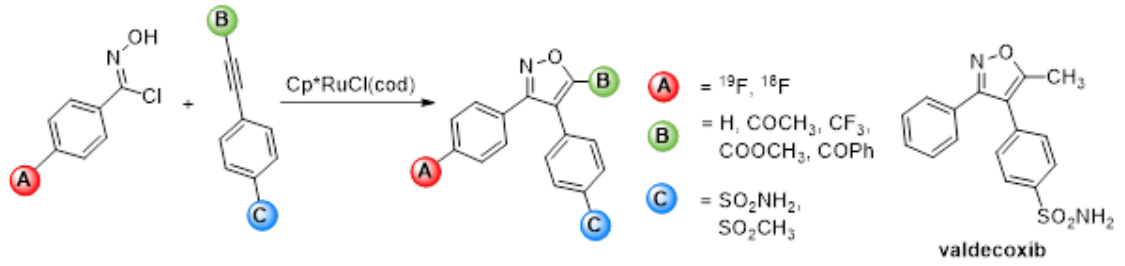
Within the indirect radiolabelling approach, copper-catalysed Huisgen cycloaddition between an azide and a terminal alkyne to form 1,4-disubstituted 1,2,3-triazoles (“click-chemistry”) has emerged as a powerful method of building radiotracers for PET and Single Photon Emission Computed Tomography (SPECT). [3,4,5]

When 1,3-dipolar cycloaddition is performed between nitrile oxides and carbon-carbon double or triple bonds, 2-isoxazolines and isoxazoles can be obtained, respectively. These reactions, although well-known in organic chemistry[6] have, with one exception, not been further exploited for radiotracer synthesis. Zlatopolskiy et al. were the first to describe the generation of an ^{18}F-labelled nitrile oxide *in situ* and its application in 1,3-dipolar cycloaddition with alkenes and alkynes to form ^{18}F-labelled 2-isoxazolines and 3,5-disubstituted or 3,4,5-trisubstituted isoxazoles.[7]

On the basis of our ongoing interest in the imaging of functional expression of cyclooxygenase-2 (COX-2) by PET, we aimed to develop novel ^{18}F-radiolabelled COX-2 inhibitors as radioactive probes for the characterization of inflammatory and tumorigenic lesions.[8] Valdecoxib, a 3,4-diaryl-substituted isoxazole (Scheme 1) with high potency and selectivity to COX-2 (IC_{50} = 5 nmol) was

withdrawn from the market owing to cardiovascular side effects. Nevertheless, valdecoxib might serve as a lead structure for PET radiotracer design because for a PET investigation only a few nanomoles of the drug are applied to the patient. In the past, radiolabelled valdecoxib derivatives have been developed; however, these radiotracers suffered from some limitations as *in vivo* de[^{18}F]fluorination or short half-life of the used radioisotope .[9,10]

Ruthenium-catalysed cycloaddition of nitrile oxides and alkynes was published by Grecian and Fokin as a practicable reaction for building 3,4-diarylsubstituted isoxazoles.[11] We recently found that new fluorine-containing isoxazoles on the basis of a valdecoxib lead structure can be easily formed in one step by ruthenium-promoted 1,3-dipolar cycloaddition of 4-fluoro-substituted nitrile oxides with alkynes.[12] 4-Fluoro-*N*-hydroxybenzimidoyl chloride turned out to be an ideal synthon whereas the ruthenium catalyst steers the reaction to only one isomer, the vicinal diaryl-substitution pattern (Scheme 1). A number of the isoxazoles obtained in this manner exhibited low nanomolar affinity and high selectivity towards COX-2; remarkably, through the introduction of a fluorine substituent the high affinity was preserved.[12] Altogether, these findings make this reaction an interesting method for the development of ^{18}F-labelled PET tracers targeting COX-2.



Scheme 1. $^{18/19}$F-labelled isoxazoles by Ru-promoted 1,3-dipolar cycloaddition of $^{18/19}$F-labelled nitrile oxides with alkynes.

To explore this reaction in fluorine-18 chemistry we set up a study to evaluate Ru-promoted 1,3-dipolar cycloaddition with an ^{18}F-labelled aromatic nitrile oxide in analogy to non-radioactive synthesis. The focus of interest is the establishment of a reliable and robust radiosynthesis of an ^{18}F-labelled nitrile oxide as a building block and the evaluation of the reaction conditions for 1,3-diplolar cycloaddition with alkynes (solvent, temperature, catalyst) in order to build ^{18}F-labelled isoxazoles.

2. Experimental Section

2.1. General

All commercially available chemicals and solvents purchased were used without further purification. The non-radioactive isoxazoles **1a-f** served as reference compounds and the corresponding methyl- and aminosulfonyl-substituted aryl alkynes were synthesized as described recently.[12] 4-formyl-N,N,N,-trimethylanilinium triflate was synthesized according to a published procedure.[13] The non-radioactive 4-fluoro-*N*-hydroxybenzimidoyl chloride (**FBIC**) was prepared as previously reported.[14] No-carrier-added aqueous [^{18}F]fluoride was produced in a CYCLONE 18/9® cyclotron by irradiation of [^{18}O]H_2O via the $^{18}O(p,n)^{18}F$ nuclear reaction. Radio-thin layer chromatography was performed on silica gel F-254 aluminium plates, chromatograms were assessed using a Fuji BAS 2000® scanner system. Radiochemical yields (RCY) are decay corrected. For SPE-based purification the following cartridges were used; Sep-Pak light Accell Plus QMA® (130 mg, Waters, Part. Nr. WAT023525), Sep-Pak Silica Plus Long cartridge (960 mg, Waters, Part. Nr. WAT 020520), Sep-Pak tC18 (400 mg, Waters, Part. Nr. WAT036810); and Sep-Pak Dry Sodium Sulfate (2.85 g, Waters, Part. Nr. WAT054265). Analytical HPLC was performed on a C18 column (Luna, Phenomenex, 5 µm 250 x 4.6 mm) using Agilent 1200 HPLC: pump G1311A, auto sampler G1329A, column oven G1316A, degasser G1322A, UV detector G1315D, gamma-detector Gabi Star®; isocratic eluent: MeCN/H_2O + 0.1% TFA 55/45 (v/v), 1 mL/min flow rate. The products were monitored at λ = 254 nm using a reference wavelength λ ref = 360 nm unless otherwise specified. The shift in the retention time of the signal of UV- and γ-detector originates by the distance between both detectors.

Semi-preparative HPLC and analytical HPLC were performed with a JASCO®-system consisting of a manual injector, a PU2080 degasser gradient pump and a 2075 UV-detector with 254 nm detection, controlled by an LC-Net II ADC interface and integrated gamma-detector GABI-Star under the following conditions: analytical (column Kinetex C18 Phenomenex, 5 µm, 250 x 4.5 mm, isocratic, eluent MeCN / H_2O + 0.1%TFA 50/50 (v/v), 1 mL/min flow rate); semi-preparative (column Discovery C18, Supelco, 5 µm, 250 x 10 mm, isocratic, MeCN / H_2O + 0.1%TFA 50/50 (v/v), 5 mL/min and 4 mL/min flow rate).

*2.2. Radiosynthesis and purification of 4-[^{18}F]fluorobenzaldehyde oxime (**^{18}FBAO**)*

Cyclotron-produced aqueous [^{18}F]fluoride solution was loaded on a QMA cartridge, which had been previously activated by elution with 10 mL of H_2O, 5mL of 1M $NaHCO_3$ and 10 mL H_2O. The cartridge was rinsed with 1 mL of anhydrous methanol to remove water, then the [^{18}F]fluoride was eluted in opposite to the normal elution direction with 0.7 mL of dry methanol containing 8 mg 4-formyl-

N,N,N,-trimethylanilinium triflate into a vial. The methanol was removed by evaporation in a nitrogen stream under reduced pressure at 60°C. Afterwards, 0.5 mL anhydrous DMF was added and the sealed vial was heated for 10 min at 90°C whilst being stirred. Then 14 µL 1 M NaOH was added to a solution of 3 mg $NH_2OH{\cdot}HCl$ in 0.2 mL DMF and the released base was added to the crude 4-[^{18}F]fluorobenzaldehyde (^{18}FBA). The sealed vial was stirred at 45°C for an additional 5 min to yield crude **^{18}FBAO** as used for reactions described in 2.3.

For purification, the solution containing crude **^{18}FBAO** was diluted with 8 mL of hexane and eluted over a Sep-Pak Silica Plus cartridge which had been preconditioned with 20 mL of hexane. The cartridge was dried with air and the **^{18}FBAO** was recovered by elution with 1.5 mL dichloromethane. A typical experiment starting with 520 MBq [^{18}F]fluoride provided 134 MBq of purified **^{18}FBAO** after 40 min (34% RCY).

*2.3. Radiosynthesis and purification of 4-[^{18}F]fluoro-N-hydroxybenzimidoyl chloride (**^{18}FBIC**)*

To a vial containing the crude **^{18}FBAO**, synthesized as described at 2.2., was added a solution of 13 mg *N*-chlorosuccinimide in 200 µL of DMF. The vial was sealed and stirred at 45°C for 5 min. After dilution with 8 mL of water the mixture was passed through a tC18 cartridge, which has been preconditioned with 5 mL ethanol and 20 mL water. The cartridge was dried by flushing with 20 mL of air yielding **^{18}FBIC** adsorbed on the tC18 cartridge suitable for further reactions as described in 2.4.

For analytical purposes and for the synthesis of **[^{18}F]1b**, **^{18}FBIC** was recovered from the tC18 cartridge by elution with 1.5 mL $CHCl_3$ and passed through a Sep-Pak sodium sulfate drying cartridge. In a typical experiment, 440 MBq of purified **^{18}FBIC** was obtained from 773 MBq of [^{18}F]fluoride starting activity after 50 min (79% RCY).

*2.4. Optimization of Ru-promoted 1,3-dipolar [3+2]cycloaddition of **^{18}FBIC** with 4-ethynylbenzene sulfonamide*

^{18}FBIC, adsorbed on a tC18 cartridge as described in 2.3., was eluted with 1.5 mL of solvent as indicated in Table 1. In the case of entry 16 Table 1 only, the solution was passed through a Sep-Pak sodium sulfate drying cartridge. In all experiments, the solution was then added to a vial containing 22 µmol of 4-ethynylbenzene sulfonamide and a magnetic stirrer. 6.5 µmol (2.5 mg) of [Cp*RuCl(cod)] dissolved in 0.3 ml of the corresponding solvent. The closed vial was stirred for 10 min at the temperature given in Table 1. The radiochemical yield of the ^{18}F-labelled cycloaddition products [^{18}F]**1a-f**, the content of unreacted **^{18}FBIC** and of ^{18}F-labelled by-products was determined by analytical radio-HPLC.

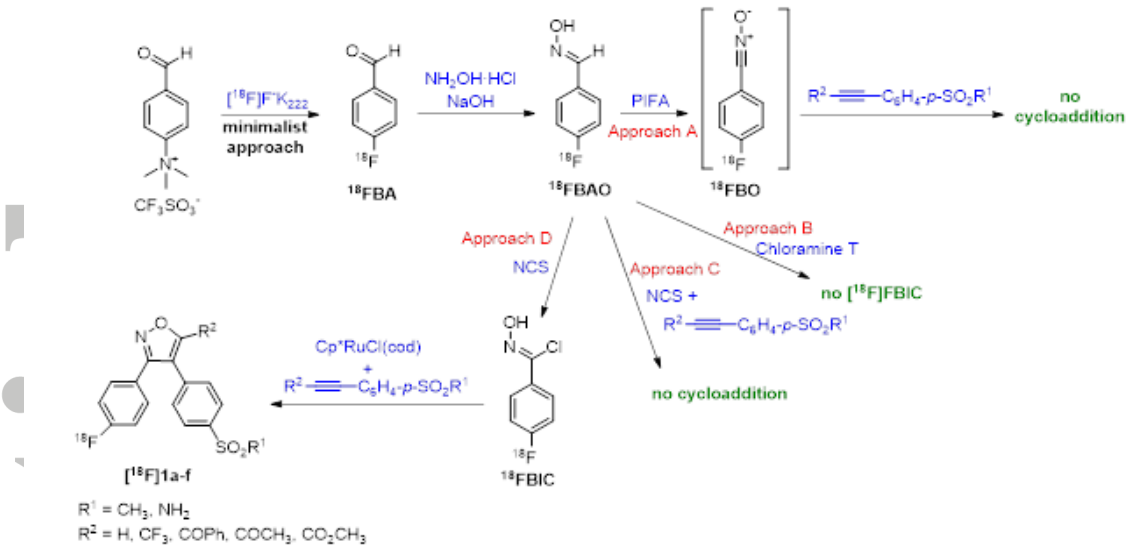
2.5. Radiosynthesis and purification of 3-(4-[^{18}F]fluorophenyl)-4-(4-(methylsulfonyl)phenyl)-isoxazole **[^{18}F]1b**

^{18}FBIC, adsorbed on a tC18 cartridge as described in 2.3., was eluted with 1.5 mL $CHCl_3$ and passed through a sodium sulfate drying cartridge into a vial containing 22 µmol (4 mg) 1-ethynyl-4-(methylsulfonyl)benzene. Cycloaddition was initiated by addition of 6.5 µmol (2.5 mg) of [Cp*RuCl(cod)] dissolved in 0.3 ml $CHCl_3$ and stirred at 45°C for 10 min. Then the $CHCl_3$ was evaporated under reduced pressure and a stream of N_2. The residue was dissolved in 0.6 mL of HPLC eluent (MeCN/H_2O + 0.1%TFA 50/50 (v/v)), passed through a syringe filter and injected into semi-preparative HPLC (MeCN/H_2O + 0.1%TFA 50/50 (v/v), isocratic flow, 4 mL/min). The product fraction of **[^{18}F]1b**, eluting from 9.4 and 10.2 min, was collected and analyzed by radio-HPLC. In a typical experiment 140 MBq of **[^{18}F]1b** were isolated from 595 MBq of [^{18}F]fluoride starting activity within 85 min total synthesis time (40% RCY).

3. Results and discussion

*3.1 Radiosynthesis of 4-[^{18}F]fluorobenzaldehyde oxime (**^{18}FBAO**) and 4-[^{18}F]fluoro-N-hydroxybenzimidoyl chloride (**^{18}FBIC**)*

A reliable and reproducible radiosynthesis of an ^{18}F-labelled nitrile oxide building block is an essential precondition for performing Ru-catalyzed 1,3-dipolar cycloaddition with alkynes. 4-[^{18}F]Fluorobenzaldehyde oxime (**^{18}FBAO**) seems to be an ideal candidate since its radiosynthesis was described recently.[7] We applied the reported synthesis sequence starting with the synthesis of 4-[^{18}F]fluorobenzaldehyde (**^{18}FBA**) as displayed in Scheme 2. In contrast to the majority of published radiosyntheses of **^{18}FBA**, where azeotropic drying of [^{18}F]fluoride is mandatory, we applied so-called "minimalist approach" which was recently published by Neumaier and coworkers which obviates the need of azeotropic drying.[15] Following the "minimalist approach", by elution of the [^{18}F]fluoride from the QMA cartridge with methanol containing 4-formyl-N,N,N,-trimethylanilinium triflate precursor, evaporation of the solvent and subsequent ^{18}F-fluorination for 10 min at 90°C in DMF, we were able to perform **^{18}FBA** synthesis within 25 min with 72-84 % (n=20) yield as determined by radio thin layer chromatography (radio-TLC). DMF was the solvent of our choice since it was found to be the most suitable solvent for the subsequent steps.



Scheme 2. Approaches for ^{18}FBIC synthesis and Ru-promoted 1,3-dipolar cycloaddition

The synthesis of **^{18}FBAO** was performed at 45°C in DMF by reacting the crude **^{18}FBA** with hydroxylamine hydrochloride and 1 M NaOH solution as described by Zlatopolskiy et al.[7], providing the crude **^{18}FBAO** in 59-69% yield (n=3) after two steps as determined by radio-TLC. Initially we used a solvent mixture of MeOH/DMF for **^{18}FBAO** synthesis to enable the subsequent reaction with phenyl iodine bis(trifluoroacetate) (PIFA) followed by cycloaddition with the alkyne as reported.[7,16] The key step of this reaction sequence is the instant oxidation of **^{18}FBAO** with PIFA to 4-[^{18}F]fluorobenzonitrile oxide (**^{18}FBO**), which is unstable and can only be obtained *in situ*. (Scheme 2, Approach A)

Since our first attempts at PIFA-supported cycloaddition of alkyne with the crude **^{18}FBAO** failed, we focused on an intermediate purification of **^{18}FBAO** via SPE-based methods. By testing several types of SPE-cartridges based on reverse-phase (C18) as well as on normal phase (silica), we found that all methods were accompanied with considerable losses of the ^{18}F-labelled oxime. Finally, it was found that **^{18}FBAO** could be purified successfully with a silica gel-based SPE cartridge, providing the compound in 21-34% RCY (n=5). Purified **^{18}FBAO** was applied to a series of Ru-promoted cycloaddition reactions in the presence of PIFA, alkyne and several solvents (methanol/water, DMF, dichloromethane, THF and dichloroethane). In summary, and contrary to our expectations, we were not able to detect any cycloaddition product by radio-HPLC in these PIFA-supported reactions. (Scheme 2, Approach A)

^{18}FBIC was described as a stable precursor for nitrile oxide formation, used for cycloaddition with alkenes and alkynes[7] and also for labelling of biomolecules.[17,18] Hence, we focused on the radiosynthesis of this building block which can be generated by reaction of **^{18}FBAO** with *N*-chloro tosylamide sodium salt (Chloramine T), (Scheme 2, Approach B).[7] However, after reaction of purified

^{18}FBAO with Chloramine T in aqueous methanol we were unable to detect **^{18}FBIC** by means of radio-HPLC. To investigate if Chloramine T-produced **^{18}FBIC** was at least generated *in situ* and hence principally available for further reactions, alkyne and Ru-catalyst were added to the crude reaction mixture, but this likewise did not result in the formation of a cycloaddition product.

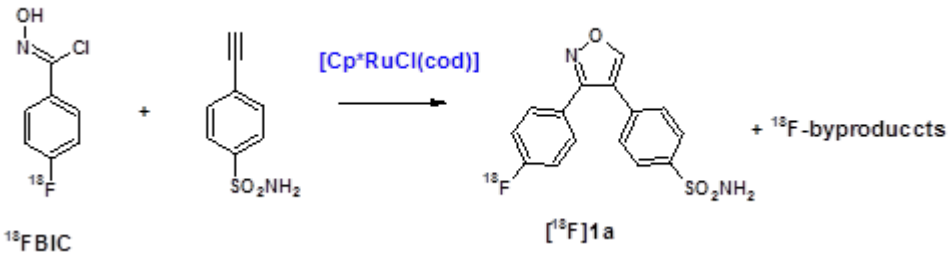
Since the non-radioactive **FBIC** can be prepared easily by reaction of 4-fluorobenzaldehyde oxime with *N*-chlorosuccinimide (NCS)[14], we adapted this route for the synthesis of **^{18}FBIC**. To the best of our knowledge, this approach has not been practised in fluorine-18 chemistry yet and we were very pleased to find by radio-HPLC that after reaction of crude **^{18}FBAO** with NCS in DMF for 5 min at 45°C the **^{18}FBIC** was quantitatively formed. (Scheme 2, Approach D)) Because a subsequent cycloaddition of crude **^{18}FBIC** with alkynes was unsuccessful (Scheme 2, Approach C), we performed a purification of the synthon by SPE with a tC18 cartridge. Notably, the strategy of **^{18}FBIC** purification offered two major advantages; in contrast to **^{18}FBAO** purification, we observed a significantly higher yield of isolated purified product and the synthon obtained was of high purity, suitable for subsequent cycloaddition in a solvent of choice.

In summary, **^{18}FBIC** is accessible from 4-formyl-N,N,N,-trimethylanilinium triflate via three steps covering *i)* nucleophilic substitution with [^{18}F]fluoride using the 'minimalist' approach to form **^{18}FBA**, *ii)* reaction with $NH_2OH{\cdot}HCl$ to form **^{18}FBAO**, *iii)* chlorination with NCS followed by SPE purification. This three-step procedure performed in one pot provides **^{18}FBIC** in 55-84% isolated RCY (n>15) within 50 min synthesis time starting from [^{18}F]fluoride.

3.2. *Ru-promoted 1,3-dipolar [3+2]cycloaddition of **^{18}FBIC** with alkynes*

After having developed a reliable radiosynthesis of **^{18}FBIC**, this synthon was evaluated in a series of experiments (Table 1) to find the most suitable solvent and best reaction temperature for the Ru-promoted 1,3-diploar cycloadditon with alkynes. For that purpose, purified **^{18}FBIC** was eluted from the SPE cartridge with the solvent as indicated (THF, DMF, EtOH, MeCN, DCE, DCM, CCl_4, $CHCl_3$) into a reaction vial containing the alkyne (4-ethynylbenzene sulfonamide) and 10 mol% of [Cp*RuCl(cod)]. Triethylamine (TEA) was added in specific cases and the mixture was stirred for the stated time and temperature. The conversion of **^{18}FBIC** to cycloaddition product 4-(3-(4-[^{18}F]fluorophenyl)isoxazol-4-yl)benzenesulfonamide **[^{18}F]1a** and the content of ^{18}F-radiolabelled by-products was determined by means of radio-HPLC; the results are displayed in Table 1.

Table 1. Impact of solvent and reaction temperature on Ru-promoted 1,3-dipolar cycloaddition of **^{18}FBIC** with 4-ethynylbenzene sulfonamide



^{18}FBIC [^{18}F]1a

#	solvent	additive	reaction temperature	reaction time	ratio of compounds **^{18}FBIC**	**[^{18}F]1a**	[^{18}F]by-products
1	THF	-	r.t.	10 min	92 %	7 %	-
2	THF	-	60°C	10 min	7 %	19 %	73 %
3	THF	TEA	r.t.	10 min	94 %	-	5 %
4	DMF	-	r.t.	10 min	77 %	8 %	14 %
5	DMF	-	80°C	10 min	33 %	57 %	9 %
6	DMF	TEA	r.t.	10 min	53 %	15 %	31 %
7	EtOH	-	r.t.	10 min	47 %	17 %	35 %
8	MeCN		r.t.	10 min	81 %	-	18 %
9	DCE		r.t.	10 min	84 %	15 %	-
10	DCE		60°C	10 min	62 %	21 %	16 %
11	DCM		r.t.	10 min	32 %	57 %	10 %
12	CCl_4	-	r.t.	10 min	98 %	-	2 %
13	$CHCl_3$		r.t.	10 min	12 %	69 %	18 %
14	$CHCl_3$	TEA	r.t.	5 min	-	-	99 %
15	$CHCl_3$	-	r.t.	30 min	13 %	73 %	13 %
16	**$CHCl_3$ (n=3)**	**#** **-**	**r.t.**	**10 min**	**-**	**96-99 %**	**-**

^{18}FBIC, adsorbed on a tC18 cartridge as described in 2.3., was eluted with 1.5 mL $CHCl_3$ and passed through sodium sulfate drying cartridge into the vial for cycloaddition.

As demonstrated in Table 1, the solvent had a significant impact on the Ru-promoted 1,3-dipolar cycloaddition of **^{18}FBIC** with an alkyne. When using THF at r.t., initially only 7% cycloaddition product **[^{18}F]1a** was formed, leaving 92% of **^{18}FBIC** unreacted (entry 1). Increased reaction temperature (60°C) resulted, on the one hand, in higher conversion to **[^{18}F]1a** (19%) and nearly complete consumption of **^{18}FBIC**, but at the same time to increased formation of unidentified ^{18}F-labelled by-products (73%). At room temperature, DMF showed cycloaddition yields comparable to THF, but the yield of **[^{18}F]1a** increased to 57% when the temperature was raised to 80°C (entries 4 and 5).

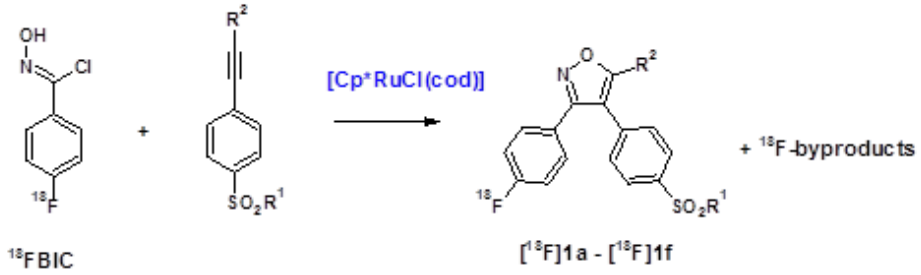
As described in the literature, 4-fluorobenzonitrile oxide (FBO) is the active reagent for 1,3-dipolar cycloaddition, and usually is generated *in situ* from the stable FBIC by addition of TEA. [11,12,19]

Consequently, we added TEA to the cycloaddition reactions in order to increase the radiochemical yields (entries 3, 6). However, we noticed that addition of TEA did not lead to an improved yield of **[^{18}F]1a** but instead to the growing formation of ^{18}F-labelled side products; one of them might correspond to **^{18}FBO**. We therefore performed further cycloadditions without addition of TEA. This is notable since in the majority of cases in non-radioactive chemistry addition of TEA is essential to form the nitrile oxide moiety. It is supposed that the [Cp*RuCl(cod)] complex acts as a transition metal Lewis acid, catalyzing the cycloaddition. The same effect was observed for an acetone-cyclopentadienyl-BIPHOP based ruthenium complex which supported the [3+2] dipolar cycloaddition reaction between nitrile oxides and α,β-unsaturated aldehydes.[20]

After having tested ethanol and acetonitrile as solvents, albeit with minor success, we proceeded with the halogen-containing solvents 1,2-dichloroethane (DCE), dichloromethane (DCM), and carbon tetrachloride. These halogen-containing solvents, especially DCE, are commonly used for Ru-catalyzed 1,3-dipolar cycloaddition[11] but show limited reactivity when containing traces of water. Specifically, DCE gave only low radiochemical yields of **[^{18}F]1a** when reacted at r.t. and at 60°C (entries 9 and 10). In DCM the yield of cycloaddition product was significantly higher (57%) at room temperature; this could not be further increased due to the solvent's low boiling point (entry 11). Carbon tetrachloride turned out to be inappropriate as a solvent, since it left the **^{18}FBIC** unreacted (entry 12). Finally, we performed the cycloaddition in chloroform and achieved 53-69% yield of **[^{18}F]1a** at r.t. The yield was increased to 73% by extending the reaction time to 30 min; the content of ^{18}F-labelled by-products was low (13-18%) (entries 13 and 15). For comparison, an experiment with addition of TEA in $CHCl_3$ was performed, but in line with former findings we observed complete decomposition of **^{18}FBIC** and detected no cycloaddition product (entry 14). For further optimization we performed a drying step by passing the **^{18}FBIC**/$CHCl_3$ solution through a Na_2SO_4 cartridge. This increased the yield of cycloaddition to 96-99% without formation of side products (entry 16).

After having found the best reaction conditions for Ru-promoted 1,3-dipolar cycloaddition of **^{18}FBIC** with 4-ethynylbenzene sulfonamide, we were able to evaluate a series of other alkynes in terms of reactivity; the results are displayed in Table 2. For all cycloadditions, $CHCl_3$ was used as a solvent and the **^{18}FBIC** solution was passed through the Na_2SO_4 drying cartridge; the reaction time was 10 min, the ratio of ^{18}F-labelled cycloaddition product and of non-consumed **^{18}FBIC** was determined by means of radio-HPLC.

Table 2. Results on Ru-promoted 1,3-dipolar cycloaddition of **^{18}FBIC** with various alkynes



^{18}FBIC

[^{18}F]1a - [^{18}F]1f

#	alkyne		reaction temperature	ratio of compounds			
	R^1	R^2		^{18}F-isoxazole		**^{18}FBIC**	^{18}F-byproducts
1	NH_2	H	r.t.	**[^{18}F]1a**	97 %	–	–
2	CH_3	H	r.t.	**[^{18}F]1b**	75 %	14 %	–
3	CH_3	H	45°C	**[^{18}F]1b**	94 %	–	5 %
4	NH_2	CH_3	r.t.	–	–	98 %	–
5	CH_3	Cl	r.t.	–	–	78 %	22 %
6	CH_3	Cl	45°C	–	–	64 %	35 %
7	CH_3	$COCH_3$	r.t.	**[^{18}F]1c**	12 %	74 %	13 %
8	CH_3	$COCH_3$	45°C	**[^{18}F]1c**	30 %	39 %	30 %
9	CH_3	CF_3	45°C	**[^{18}F]1d**	35 %	–	64 %
10	CH_3	$COOCH_3$	45°C	**[^{18}F]1e**	50 %	26 %	23 %
11.	CH_3	COPh	45°C	**[^{18}F]1f**	10 %	2 %	87 %

Three analytical HPLC chromatograms are displayed as examples. Figure 1 shows the γ-HPLC trace of **^{18}FBIC** (t_R = 6.10 min) recorded after cartridge purification. Figure 2 displays the γ-HPLC trace of the reaction mixture of 1,3-dipolar cycloaddition with methyl 3-(4-(methylsulfonyl)phenyl)prop-2-ynoate after 10 min at 45°C to form **[^{18}F]1e** (see Table 2, entry 10). It is obvious that the majority of **^{18}FBIC** has been consumed and the ^{18}F-labelled isoxazole **[^{18}F]1e** (t_R = 8.24 min) was formed as a cycloaddition product in 50% yield. Besides this, two ^{18}F-radiolabelled byproducts were detected (t_R = 3.34 min t_R = 9.69 min). The HPLC chromatogram of the non-radioactive reference compound **1e** (methyl 3-(4-fluorophenyl)-4-(4-(methylsulfonyl)phenyl)isoxazole-5-carboxylate), recorded at 254 nm (t_R = 7.70 min) is shown in Figure 3.

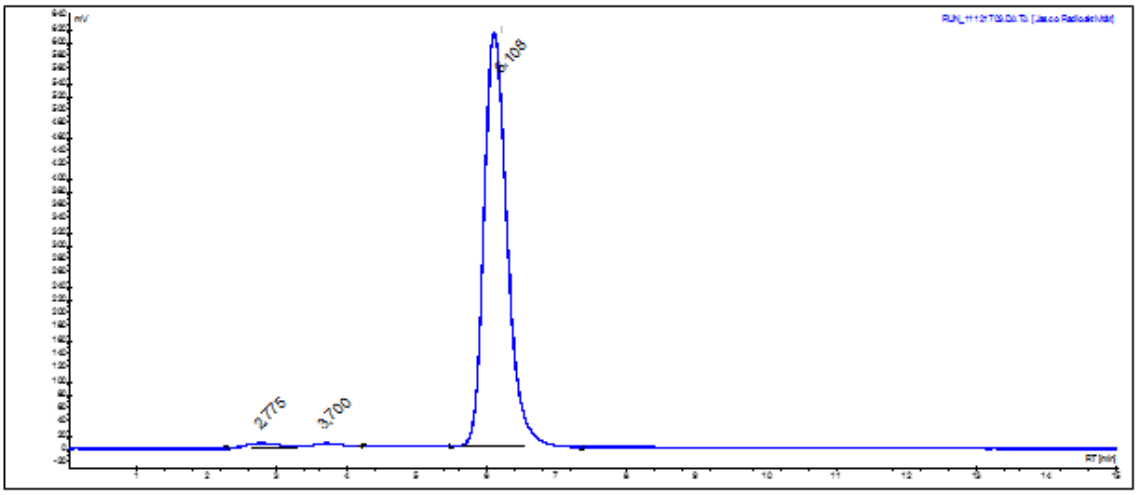


Figure 1. Radio-HPLC chromatogram of purified **^{18}FBIC**; semi-preparative column, 50% MeCN/50% 0.1% TFA (v/v), flow 5 mL/min

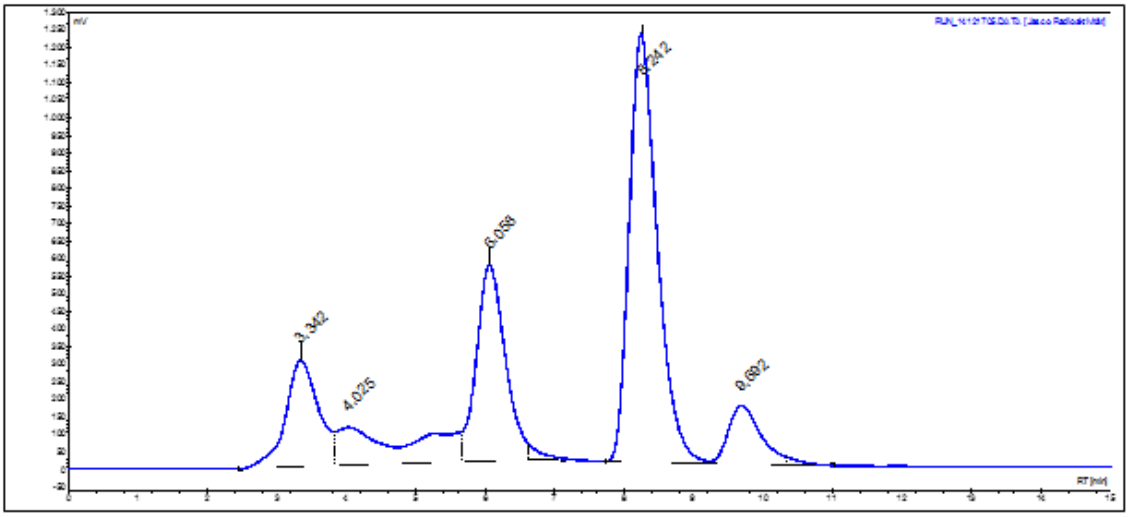


Figure 2. Radio HPLC chromatogram of reaction mixture of Ru-promoted 1,3-dipolar cycloaddition of **^{18}FBIC** with methyl 3-(4-(methylsulfonyl)phenyl)prop-2-ynoate, 10 min, 45°C; semi-preparative column, 50% MeCN/50% 0.1% TFA (v/v), flow 5 mL/min

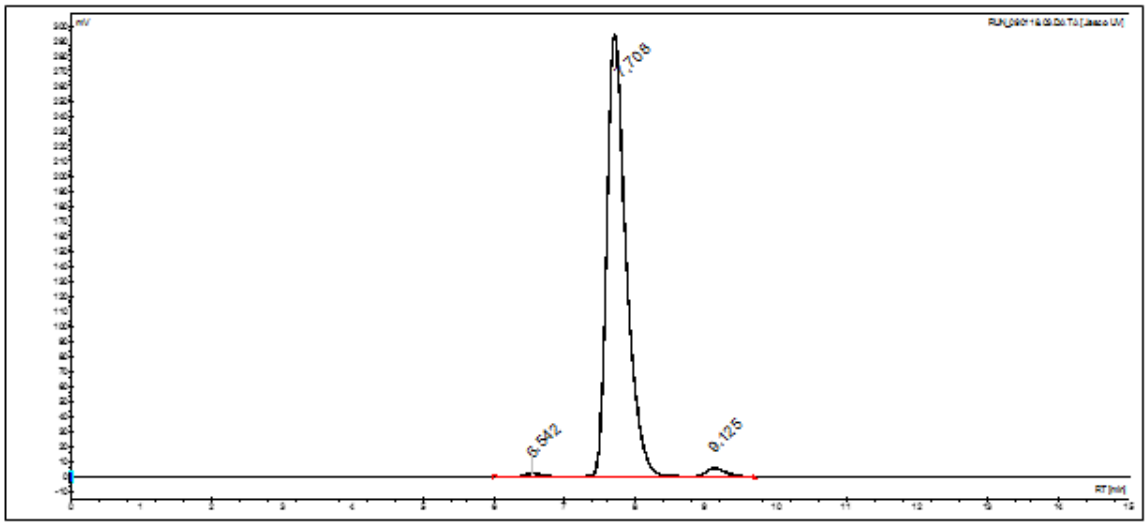


Figure 3. HPLC chromatogram (UV-signal, 254 nm) of reference compound **1e**; semi-preparative column, 50% MeCN/50% 0.1% TFA (v/v), flow 5 mL/min

To summarise the results of Ru-promoted 1,3-cycloaddition of **^{18}FBIC** with various alkynes (Table 2), we found that the unsubstituted alkynes are the most reactive partners, showing nearly quantitative cycloaddition within 10 min at r.t. or 45°C, respectively (entries 1-3). With non-activated alkynes, such as methyl- and chloro-substituted alkynes, no cycloaddition products were formed at r.t.; application of higher reaction temperature resulted in decomposition of **^{18}FBIC** (entry 4-6). This is consistent with our findings from non-radioactive chemistry where we were also unable to synthesize the corresponding methyl- and chloro-substituted isoxazoles via this route.[12] Alkynes carrying a more bulky substituent underwent 1,3-dipolar cycloaddition in moderate yields (10-50%); however, elevated temperatures (45°C) were required, resulting also in higher amounts of ^{18}F-labelled byproducts (entries 7-11).

*3.3. Radiosynthesis of an ^{18}F-labelled valdecoxib derivative **[^{18}F]1b** by Ru-promoted 1,3-dipolar cycloaddition with **^{18}FBIC***

The successful application of **^{18}FBIC** in PET radiotracer synthesis is demonstrated by the cycloaddition-based formation of 3-(4-[^{18}F]fluorophenyl)-4-(4-(methylsulfonyl)phenyl)isoxazole **[^{18}F]1b**. The non-radioactive reference compound **1b** is structurally strongly related to valdecoxib and shows a comparably high affinity and selectivity towards COX-2 (IC_{50} = 0.06 µM).[12] In brief, purified **^{18}FBIC** was reacted with 1-ethynyl-4-(methylsulfonyl)benzene and [Cp*RuCl(cod)] in $CHCl_3$ at 45°C for 10 min, the solvent was evaporated and the mixture was redissolved in eluent. Purification of the crude product by semi-preparative HPLC provided the isoxazole **[^{18}F]1b** in up to 40% RCY, calculated from the starting activity of [^{18}F]fluoride. The radiochemical purity of **[^{18}F]1b** was

determined by analytical γ-HPLC and was found to be > 99 % as shown in Figure 4. The identity of the radiotracer **[^{18}F]1b** with the non-radioactive compound was demonstrated by the retention time of **1b** monitored by the UV-signal at 254 nm. (Figure 5).

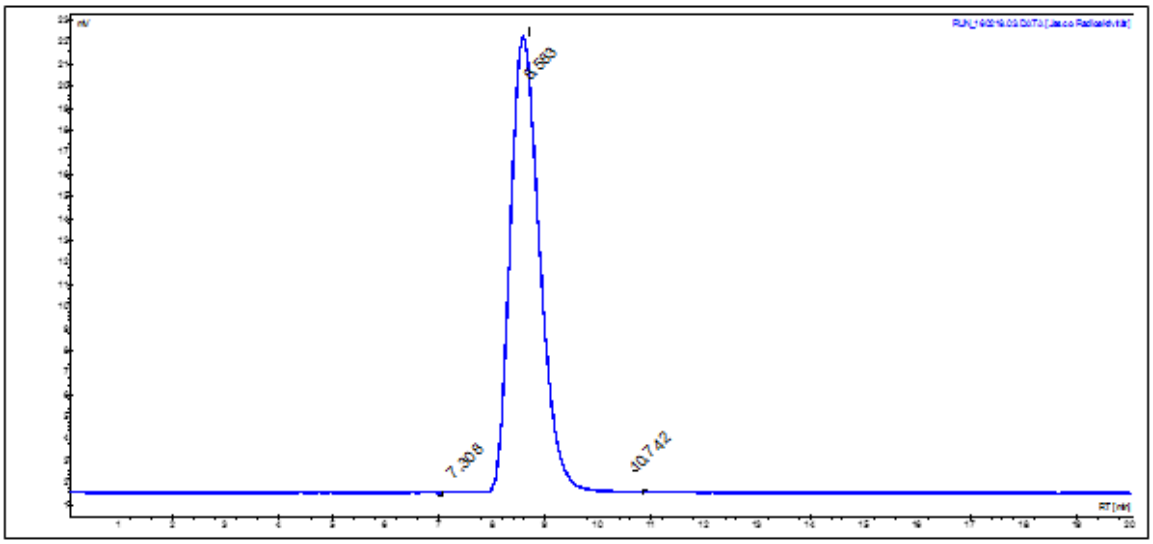


Figure 4. Radio HPLC chromatogram of purified **[^{18}F]1b**; analytical column, 50% MeCN/50% 0.1% TFA (v/v), flow 1 mL/min

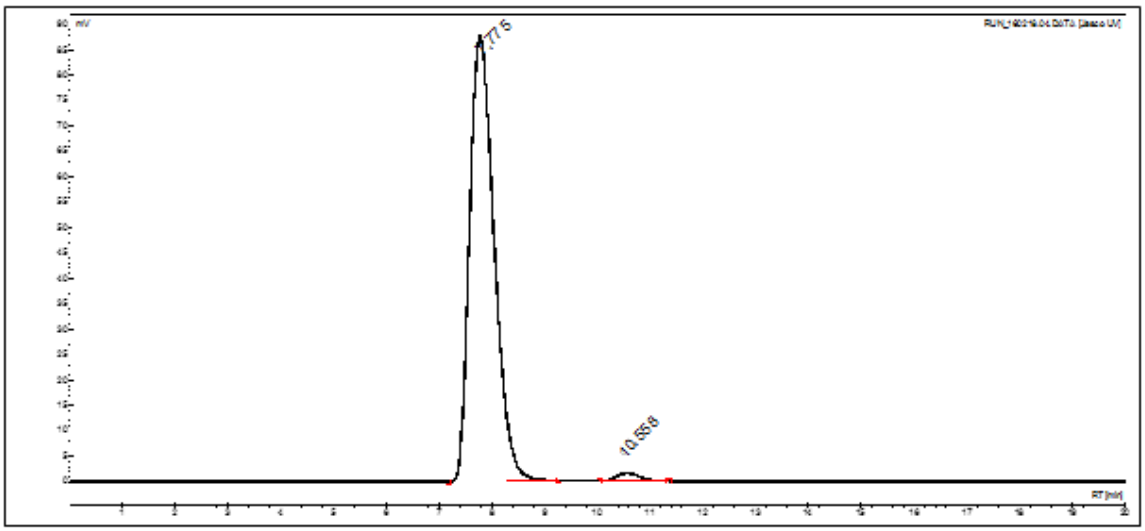


Figure 5. HPLC chromatogram (UV-signal, 254 nm) of reference compound **1b**; analytical column, 50% MeCN/50% 0.1% TFA (v/v), flow 1 mL/min

4. Conclusion

We found recently that Ru-catalyzed 1,3-dipolar cycloaddition of 4-fluoro-substituted nitrile oxides with alkynes gives access to fluorine-substituted isoxazoles, i.e. to novel derivatives of valdecoxib.[12] We hypothesized that by application of this method to ^{18}F-labelled nitrile oxides and corresponding alkynes ^{18}F-labelled isoxazole derivatives would be available and might serve as potential PET probes for visualization of COX-2. Consequently, we have focused on the development of a reliable high-yielding synthesis of an ^{18}F-labelled nitrile oxide as a suitable building block.

We have developed the one-pot radiosynthesis of a stable nitrile oxide precursor: 4-[^{18}F]fluoro-*N*-hydroxybenzimidoyl chloride (**^{18}FBIC**) by optimizing the approach of Zlatopolskiy et al.[7] We performed the synthesis and purification of the compound in a three-step procedure covering the synthesis of 4-[^{18}F]fluorobenzaldehyde and subsequent reaction with hydroxylamine and NCS, and this is characterised by high radiochemical yields and simple SPE-based purification. The **^{18}FBIC** synthesized and purified in this manner was obtained in 55-84% RCY starting from [^{18}F]fluoride within 50 min in a non-automated experimental set-up.

The reactivity of **^{18}FBIC** was explored in Ru-promoted 1,3-dipolar cycloaddition with alkynes in various solvents and at different temperatures, and the highest yield of cycloaddition products was observed in chloroform. Monosubstituted alkynes proved to be the best partners in 1,3-dipolar cycloaddition.

Finally, the potential of **^{18}FBIC** in PET radiotracer synthesis was successfully demonstrated in the four-step radiosynthesis of the valdecoxib derivative **[^{18}F]1b**. In a typical non-automated procedure, the ^{18}F-labelled isoxazole **[^{18}F]1b** was isolated in up to 40% RCY starting from [^{18}F]fluoride after semi-preparative purification within 85 min synthesis time.

In summary, we were able to show that **^{18}FBIC** is a suitable and stable building block in fluorine-18 chemistry. ^{18}F-labelled isoxazoles can be easily formed via Ru-promoted 1,3-dipolar cycloaddition of this synthon with various alkynes. The radiosynthesis provides **^{18}FBIC** in high yields and might be further improved by transformation of the process into an automated system, covering a three-step reaction in one pot in a single solvent (DMF). The SPE-based purification of **^{18}FBIC** might likewise be easily integrated into conventional automated synthesizers.

The extension of the chemistry of **^{18}FBIC** for the synthesis of other ^{18}F-labelled heterocycles, e.g. by reaction with alkenes, nitriles and other activated aromatic systems, is large, as can be reasoned from non-radioactive chemistry. This potential has to be further evaluated and should extend the portfolio regarding the design of ^{18}F-radiotracers.

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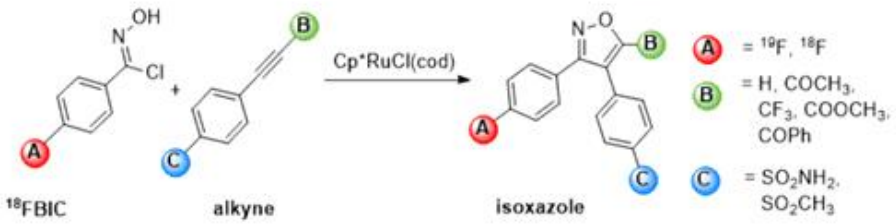
Graphical Abstract

Access to ^{18}F-labelled isoxazoles by Ruthenium-promoted 1,3-dipolar cycloaddition of 4-[^{18}F]fluoro-*N*-hydroxybenzimidoyl chloride with alkynes

Silvia Roscales and Torsten Kniess



4-[^{18}F]fluoro-N-hydroxybenzimidoyl chloride (**^{18}FBIC**) was developed as an ^{18}F-labelled aromatic nitrile oxide building block. **^{18}FBIC** was found to be a suitable and stable synthon to give access to ^{18}F-labelled 3,4-diarylsubstituted isoxazoles by Ru-catalysed 1,3-dipolar cycloaddition in one step.