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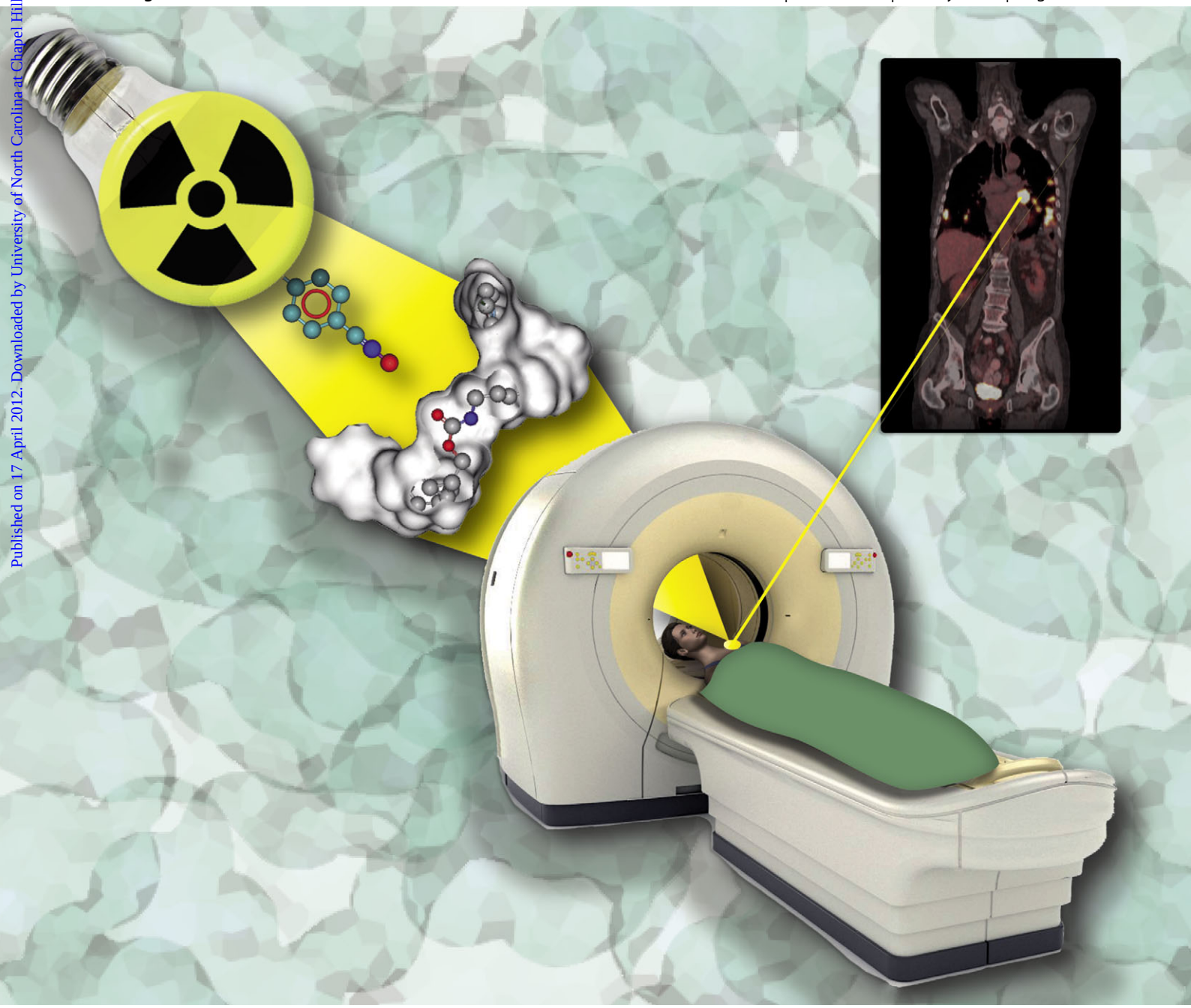

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COMMUNICATION

Beyond azide–alkyne click reaction: easy access to ^{18}F-labelled compounds *via* nitrile oxide cycloadditions†

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Radiofluorinated 4-fluorobenzonitrile oxide and *N*-hydroxy-4-fluorobenzimidoyl chloride rapidly react with different alkenes and alkynes under mild conditions. These cycloadditions are suitable for the preparation of low-molecular weight radiopharmaceuticals and, in a strain-promoted variant, can enable easy labelling of sensitive biopolymers.

Positron emission tomography (PET) is a powerful non-invasive diagnostic molecular imaging tool for quantitative 3D-visualization of physiological and pathological processes *in vivo* using biologically active molecules labelled with β^+-emitting nuclides (PET-tracers).‡ Fluorine-18 (^{18}F) is one of the most widely used radioisotopes for PET. ^{18}F (in the form of $^{18}F^-$) is easily accessible from $H_2{}^{18}O$ *via* $^{18}O(p,n)^{18}F$ nuclear reaction. It decays almost completely by low-energetic β^+-emission [$E(\beta^+)$ = 630 keV, 97%] and, consequently, gives 3D emission scans with a high intrinsic resolution. Furthermore, taking into account logistical considerations ^{18}F has a sufficient half-life of 109.8 min enabling transportation of ^{18}F-labelled radiopharmaceuticals to other clinical sites.

A broad spectrum of direct and indirect methods for ^{18}F-labelling of small molecules and for indirect radiofluorination of biopolymers (*e.g.* proteins and nucleic acids) was developed.[1] Each of them have their own advantages and limitations. An important contribution to this area was the implementation of Cu(I)-catalyzed [3 + 2]-azide–alkyne Huisgen cycloaddition (azide–alkyne click reaction)[2] for radiolabelling. Since Marik and Sutcliffe prepared ^{18}F-labelled peptides by the click reaction between [^{18}F]fluoroalkynes and peptides bearing an azide moiety[3] numerous ^{18}F-labelled tracers were prepared using this approach.[4] Among a variety of advantages of this approach are: regioselectivity, non-requirement of protection groups, mild reaction conditions, acceptable yields under different reaction conditions and metabolic stability of the formed 1,2,3-triazoles. Recently, Feringa *et al.* published the preparation of an ^{18}F-labelled bombesin analogue *via* copper-free strain-promoted cycloaddition between ^{18}F-labelled azides and aza-dibenzocyclooctyne-modified peptides in DMF.[5] This methodology for radiolabelling allows to overcome problems associated with the application of copper catalysts such as cytotoxicity, copper-promoted degradation of peptides and proteins or formation of insoluble copper-acetylide.[4c]

Until quite recently only azide–alkyne coupling was exploited for radiolabelling although numerous other [3 + 2]-dipolar cycloadditions are known.[6] Some of them require only readily available simple starting materials and afford metabolically and chemically stable cycloadducts. This year, we have reported on the first example of a successful application of nitrone–alkene cycloaddition for radiolabelling.[7] ^{18}F-labelled isoxazolidines were obtained in high radiochemical yields (rcy) by rapid reaction of *C*-(4-[^{18}F]fluorophenyl)-*N*-phenyl nitrone ([^{18}F]**1**) with model maleimides. However, due to relatively high reaction temperatures (80–110 °C) this approach is unsuitable for radiolabelling of more sensitive substrates like proteins. Therefore, we turned our attention to other [3 + 2]-dipolar cycloadditions such as fast metal-free cycloaddition reactions between nitrile oxides and carbon–carbon multiple bonds leading to metabolically and chemically stable 2-isoxazolines or isoxazoles. In this communication we describe the *in situ* generation of 4-[^{18}F]fluorobenzonitrile oxide ([^{18}F]**2**) and its subsequent reaction with unsaturated compounds. To demonstrate the potential of this novel approach, the preparation of radiofluorinated COX-2 specific tracers (inflammation markers) is presented. Finally, the application of the proposed methodology for radiolabelling of model dipeptides is described.

Preparation of no-carrier-added [^{18}F]**2** started with the synthesis of 4-[^{18}F]fluorobenzaldehyde ([^{18}F]FB–CHO) according to Haka *et al.*[8] (Scheme 1). [^{18}F]FB–CHO was prepared in 30–50% rcy (4–10 GBq, no decay corrected) within 50 min. 4-[^{18}F]Fluorobenzaldoxime ([^{18}F]**3**) was prepared by reacting [^{18}F]FB–CHO with hydroxylamine in 92.1 ± 2.4% yield (n = 23) and >90% radiochemical purity after 10 min incubation in aqueous MeOH at 40 °C. Phenyl iodine bis(trifluoroacetate) (PIFA) was added to crude oxime [^{18}F]**3**§ to generate nitrile oxide [^{18}F]**2**.[9] Afterwards, [^{18}F]**2** was allowed to react with the appropriate dipolarophile (Scheme 1 and Table 1).

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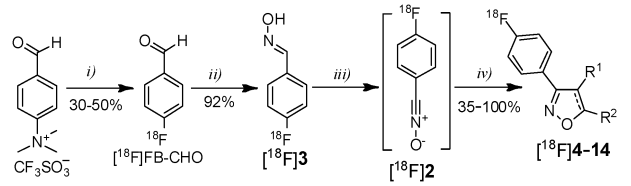
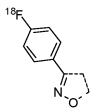
[b] *Max Planck Institute for Neurological Research, Gleueler Str. 50, 50931 Cologne, Germany. E-mail: bernd.neumaier@nf.mpg.de; Fax: +49-221-4726-298; Tel: +49-221-4726-270*
[c] *Department of Nuclear Medicine, Maastricht University Medical Center, P. Debyelaan 25, 6229HX Maastricht, The Netherlands*

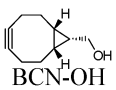
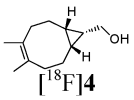
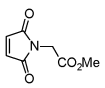
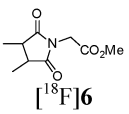
† Electronic supplementary information (ESI) available: Details of the synthesis of the reference compounds and precursors as well as of labelling experiments; NMR-spectra and HPLC traces. See DOI: 10.1039/c2cc31335a



Scheme 1 Preparation of [^{18}F]**2** and its reaction with different dipolarophiles. (i) [K⊂2.2.2]$^{+}$[^{18}F]$^{-}$, DMSO, 85 °C, 10 min; (ii) $NH_2OH{\cdot}HCl$, 1 M NaOH, MeOH, 40 °C, 10 min; (iii) PIFA, 5–10 s; (iv) dipolarophile.

Table 1 Cycloaddition of [^{18}F]**2** to different dipolarophiles[a,b]



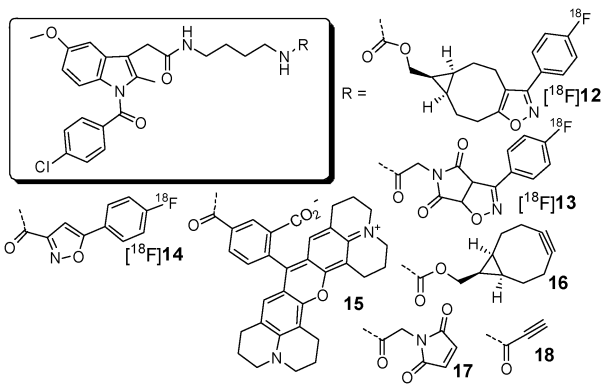
Entry	Dipolarophile	Product	Rcy ± SD (%) [*T*/°C]
1	 BCN-OH	 [^{18}F]**4**	95.1 ± 4.7 (rt)
2		 [^{18}F]**5**	> 95 (rt)
3		 [^{18}F]**6**	> 95 (40)
4	Methyl acrylate	 [^{18}F]**7**	91.3 ± 3.9 (80)
5	Styrene	 [^{18}F]**8**	78.3 ± 3.4 (80)
6	DMAD	 [^{18}F]**9**	69.5 ± 5.0 (40)
7	Methyl propiolate	 [^{18}F]**10**	57.7 ± 1.6 (80)
8	4-Pentyn-1-ol	 [^{18}F]**11**	57.0 ± 3.6 (80)
9[c]	**16**	[^{18}F]**12**	81.4 ± 2.0 (rt)
10[c]	**17**	[^{18}F]**13**	54.8 ± 1.3 (40)
11[c,d,e]	**18**	[^{18}F]**14**	35.3 ± 4.7 (60)

[a] Conditions: a solution of [^{18}F]**3** (100–150 MBq) and the corresponding dipolarophile (10 μmol) in 80% MeOH (450 μL) was treated with PIFA (3.45 mg, 8.23 μmol) in MeOH (50 μL) at rt and the reaction mixture was stirred at the given temperature for 10 min. [b] Each experiment was carried out in triplicate. [c] 1.2–2.5 GBq [^{18}F]**3** was used. [d] A solution of [^{18}F]**2** and oxidant in MeOH (30 μL) was added to a preheated solution of **18** in DMF (35 μL). [e] BAIB (8 μmol) was used instead of PIFA. rcy = radiochemical yield; SD = standard deviation; rt = room temperature.

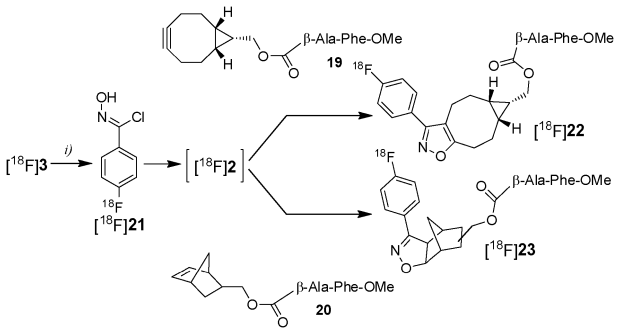
In all cases the respective ^{18}F-labelled 2-isoxazolines/isoxazoles ([^{18}F]**4–11**)† were obtained in good to excellent yields with complete regioselectivity already after 10 min of reaction time.

Surprisingly, even with the less reactive dipolarophiles such as styrene and 4-pentyn-1-ol (entries 5 and 8, respectively) high yields of cycloaddition products were achieved. Highly strained (1*R*,8*S*,9*s*)-9-hydroxymethylbicyclo[6.1.0]nonyne (BCN-OH)[10] reacted with [^{18}F]**2** to give isoxazole [^{18}F]**4** in virtually quantitative yield at room temperature (entry 1). Noteworthily, quantitative cycloaddition of [^{18}F]**2** to much less strained norbornene at room temperature (entry 2) and to moderately activated methyl *N*-maleimidoacetate at 40 °C (entry 3) was also observed. Alkynes other than BCN-OH (entries 6–8) afforded besides isoxazoles noticeable amounts of by-products (10–35%).

To explore the new radiofluorination method for tracer development under "field conditions" we prepared three ^{18}F-labelled indomethacin derivatives ([^{18}F]**12–14**). These indomethacin derivatives were developed to target cyclooxygenase 2 (COX-2) and potentially enable visualization of COX-2 expression in inflammation and cancer (Scheme 2 and Table 1, entries 9–11). Indomethacin diamides with a sufficiently long spacer retain a high affinity to COX-2 even in the case of a bulky second acyl moiety. For example, compound **15** containing a large 5-carboxy-X-rhodamine residue was the first tracer suitable for optical imaging of COX-2 expression *in vivo*.[11] The ^{18}F-labelled conjugates [^{18}F]**12** and [^{18}F]**13** were prepared from **16** and **17**, respectively, in high radiochemical yields using PIFA as an oxidant. In contrast, the rcy of [^{18}F]**14** was quite low due to the relatively low dipolarophilic reactivity and extremely low solubility of precursor **18**. We assumed that in the case of low cycloaddition rates once formed [^{18}F]**2** will be subjected to acid-promoted decomposition, solvolysis or react with minor contaminants in the reaction medium. Therefore, labelling yield will be improved by slow generation of [^{18}F]**2**. Consequently, [bis(acetoxy)iodo]benzene (BAIB) which is a weaker oxidant as PIFA and produces nitrile oxides much slower as PIFA[9,12] was used. Furthermore, during oxidation by BAIB weak acetic acid instead of strong trifluoroacetic acid is liberated. With this milder oxidant the desired conjugate [^{18}F]**14** was obtained in 35% rcy. The conjugation products [^{18}F]**12–14** were purified by HPLC and formulated in 20% ethanol.



Scheme 2 Indomethacin conjugates: [^{18}F]**12–14** prepared *via* [3 + 2]-nitrile oxide–alkene/alkyne cycloaddition; **15**—first COX-2 specific ligand for optical imaging *in vivo*; **16–18**—precursors for radiosynthesis.



Scheme 3 Labelling of model dipeptides **19** and **20** using the radiofluorinated *N*-hydroxyimidoyl chloride [^{18}F]**21**. Conditions: (i) Chloramine T, aq. EtOH, rt, 4 min, then **19** or **20**, rt, 10 min. [^{18}F]**23** was obtained as a mixture of two regioisomers (*ca.* 1.5 : 1).

Table 2 Labelling of model dipeptides **19** and **20** using [^{18}F]**2** and the radiofluorinated *N*-hydroxyimidoyl chloride [^{18}F]**21**. Dependence on precursor amount[a]

Precursor amount/nmol	Rcy of [^{18}F]**22** (%)	Rcy of [^{18}F]**23** (%)
10^{4}[b]	88.3 ± 1.0	52.6 ± 1.6
10^{3}[b]	36.7 ± 3.0	7.6 ± 2.3
10^{3}[c]	83.4 ± 2.6	82.3 ± 6.5
500[c]	83.7 ± 1.4	71.6 ± 2.7
100[c]	76.7 ± 1.8	55.9 ± 5.3
50[c]	70.1 ± 12.2	46.5 ± 2.1
25[c]	64.3 ± 12.6	26.1 ± 8.5
10[c]	—	13.4 ± 3.3
5[c]	55.8 ± 2.3	5.9 ± 0.5
1[c]	2.8 ± 1.4	—

[a] Each experiment was carried out in triplicate. [b] With PIFA as an oxidant, see caption of Table 1. [c] With chloramine T as a chlorination agent: [^{18}F]**3** (25–150 MBq) in EtOH (10 μL) was treated at rt with a solution of chloramine T (0.23 mg, 1 μmol) in H_2O (20 μL) followed after 4 min by the dipolarophile in EtOH (5–100 μL) and the reaction mixture was stirred at rt for 10 min.

A biological study of [^{18}F]**12–14** is in progress and will be reported in due course.

The amounts of dipolarophiles used (10 μmol, Table 1) are acceptably low for labelling of small molecules. However, these amounts are too high, *e.g.* in the case of peptides and proteins where precursors are expensive and generally difficult to separate from the labelled products. Furthermore, high precursor content can impair the quality of PET-images and potentially cause adverse effects in patients. Consequently, the dependency of the labelling yield on the precursor amount was studied. BCN- and norbornene-substituted β-Ala-Phe-OMe (**19** and **20**, respectively) were chosen as model dipolarophiles (Scheme 3 and Table 2). If [^{18}F]**2** was generated by the oxidation by PIFA or BAIB acceptable rcy of cycloaddition products could be obtained only with micromolar amounts of dipolarophiles. Therefore, radiofluorinated *N*-hydroxy-4-fluorobenzimidoyl chloride ([^{18}F]**21**), a more stable synthon of [^{18}F]**2**, was used in these experiments. It was prepared *in situ* by chlorination of [^{18}F]**3** with chloramine T (Scheme 3). This modification allowed us to minimize the amount of the BCN-substituted dipolarophile **19** to 5 nmol (56% rcy of [^{18}F]**22**) and of the norbornene-substituted precursor **20** to 50 nmol (47% rcy of [^{18}F]**23**) without significant drop in yields.

In summary, we demonstrated for the first time that [3 + 2]-nitrile oxide–alkene/alkyne cycloaddition can be applied for [^{18}F]-radiolabelling. Novel labelling precursors, nitrile oxide [^{18}F]**2** and imidoyl chloride [^{18}F]**21**, can be efficiently generated *in situ* from easily accessible oxime [^{18}F]**3** using different oxidants. [^{18}F]**2** and [^{18}F]**21** are valuable building blocks for rapid and high-yielding preparation of ^{18}F-labelled compounds under mild reaction conditions (10 min, ≤80 °C, aqueous media, no catalyst necessary). The proposed method is a possible alternative to radiofluorination *via* copper-catalyzed/copper-free azide–alkyne "click" reaction. Additionally, radiofluorination *via* fast strain-promoted [3 + 2]-nitrile oxide–alkyne cycloaddition can be carried out in aqueous media at room temperature with low amounts of dipolarophile. Therefore, it should be well suited for fast and simple preparation of radiolabelled biopolymers.

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Notes and references

‡ An important exception is blood flow imaging, for which labelled small penetrant molecules and ions such as [^{13}N]NH_3, [^{15}O]H_2O or $^{82}Rb^+$ are used.

§ The crude [^{18}F]**3** can be purified, *e.g.* by RP-SPE extraction. However, in majority of cases, the purification step did not improve yields of the following cycloaddition reactions.

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