Synthesis, Radiosynthesis and *in vitro* Studies on Novel Hypoxia PET Tracers Incorporating [^{18}F]FDR

Manuele Musolino,[a] Ian N. Fleming,[a] Lutz F. Schweiger,[a] David O'Hagan,[b] Sergio Dall'Angelo,*[a] Matteo Zanda,*[a,c]

Dedicated to Professor Franco Cozzi on the occasion of his 70th birthday

[a] Dr M. Musolino, Dr I. N. Fleming, Dr L.F. Schweiger, Dr S. Dall'Angelo, Prof Dr M. Zanda
Institute of Medical Sciences and Aberdeen Biomedical Imaging Centre
University of Aberdeen
AB25 2ZD Aberdeen (Scotland, United Kingdom)
E-mail: s.dallangelo@abdn.ac.uk;

[b] Prof Dr D. O'Hagan
School of Chemistry and Centre for Biomolecular Sciences
University of St. Andrews
KY16 9ST North Haugh, St Andrews, Fife (Scotland, United Kingdom)

[c] Prof Dr M. Zanda
Istituto di Scienze e Tecnologie Chimiche "G. Natta" (SCITEC)
via Mancinelli 7
20131 Milan (Italy)

E-mail: matteo.zanda@cnr.it

Abstract: We report the synthesis of five radiotracers incorporating different oxyamine spacers between the hypoxia-reactive 2-nitroimidazole moiety and the 5-[^{18}F]-fluorodeoxyribose ([^{18}F]FDR, **12**) prosthetic group: three linear alkyl chains with 3, 5, 7 carbon atoms (**15a-c**), a cyclopropyl ring (**15d**) and a 1,4-disubstituted-1,2,3-triazole (**15e**). Experiments in hypoxic cells showed that **15d** displays superior uptake kinetics – and similar selectivity for hypoxic cells – relative to the gold standard hypoxia tracers [^{18}F]fluoroazomycin arabinoside ([^{18}F]FAZA) and [^{18}F]fluoromisonidazole ([^{18}F]FMISO). Lipophilicity and structural rigidity have strong influence on the selectivity of tracers **15** towards hypoxic cells: the lead tracer **15d** displays a logP = 0.38 and the most rigid spacer. A sixth radiotracer (**15f**), with a 2-*H*-imidazole replacing the 2-nitroimidazole moiety of **15d**, was used to demonstrate that the cyclopropyl group does not play a meaningful role in the sensitivity towards hypoxia.

Introduction

Hypoxia occurs in cells and tissues when oxygen demand exceeds supply.[1,2] In many solid tumors irregular vasculature does not sufficiently support the cellular oxygen demand, leading to the development and progression of heterogeneous regions that are poorly responsive to chemo and radio therapies. The decrease of oxygen level below pO_2 = 10 mmHg activates the overexpression of HIF1 (*hypoxia-inducible factor*), which in turn triggers the transcription of many target genes encoding proteins, which modulate angiogenesis, cell proliferation, erythropoiesis, anaerobic glycolysis and glucose transport, thus causing the development of a more malignant and resistant cell phenotype.[3–5] In recent years tumor hypoxia has been extensively investigated by means of non-invasive techniques, such as MRI and PET imaging in addition to more invasive techniques such as polarographic O_2 sensors (Eppendorf Electrode) and immunohistochemical techniques, which remain the most used to measure pO_2 in tumors.[6] Owing to its high sensitivity, PET imaging plays an important role in the characterization and discrimination of different hypoxic areas, which is achieved by employing suitable radiotracers. An efficient hypoxia radiotracer must

reflect sensitivity to oxygen level changes and recognize distinct microenvironments. A suitable lipophilicity level contributes to ensure both a uniform distribution of the tracer in tumors and a rapid clearance from tissues. Furthermore, a practical and rapid synthesis, low toxicity and cost-effectiveness are also important features for a PET tracer.[5,7,8]

In the last two decades several PET tracers for hypoxia have been described, but none of them ticks all the boxes for imaging all the facets of tumor hypoxia. The tracers employed to image hypoxia can be classified in nitroimidazole derivatives – such as [^{18}F]FMISO, [^{18}F]FAZA, [^{18}F]HX4 – and non-nitroimidazole derivatives – such as Cu(II) complexes and [^{18}F]FDG (2-[^{18}F]fluorodeoxyglucose, Figure 1).[5,9,10] Under hypoxic conditions the 2-nitroimidazole function acts as a biomarker, namely it is reduced to form radical intermediates by action of reductase enzymes and is selectively retained by hypoxic cells by formation of a covalent bond with bio-macromolecules such as proteins and DNA.[11]

The radiolabelling of nitroimidazole derivatives gives access to potential radiotracers specific for hypoxia imaging.[12,13] Fluorine-18 is the most employed isotope for radiolabelling nitroimidazole derivatives mainly due to its relatively long half-life (109.5 min).[14,15] The recently developed [^{18}F]FDR (5-[^{18}F]fluorodeoxyribose) tracer (**12**, Scheme 3) represents a promising prosthetic group for fast indirect radiolabelling of bioactive molecules via oxime bond formation.[16–19] As a standalone cancer PET tracer, [^{18}F]FDR was found to be less efficient than the widely used [^{18}F]FDG, which is able to image glucose metabolism in cells.[20] [^{18}F]FDG is phosphorylated by hexokinase enzymes and trapped in the cells as [^{18}F]FDG-6-phosphate until the decay of ^{18}F to ^{18}O allows glycolysis to continue. In contrast, [^{18}F]FDR lacks the terminal OH group and cannot be phosphorylated, which is probably the cause of its more rapid cellular efflux and reduced performance for imaging cancer cells in vivo. However, [^{18}F]FDR is very efficient when used as a prosthetic group for indirect radiofluorination. In fact, under mildly acidic conditions [^{18}F]FDR displays a much higher reactivity than [^{18}F]FDG towards the formation of an oxime bond in the presence of a substrate carrying an oxyamine function. This occurs due to the greater ring opening propensity of the 5-membered ring to the free aldehyde form, a process that is accelerated by fluorine at C5 in [^{18}F]FDR, whereas the fluorine at C2 of [^{18}F]FDG further suppresses ring opening of the 6-membered ring.[17] Thus, [^{18}F]FDG requires harsher conditions to form an oxime bond, leading to significantly lower process yields, as well as longer reaction and purification times, often not compatible with [^{18}F]fluorine decay.[21,22] For these reasons [^{18}F]FDR can be considered a very promising and versatile prosthetic group.[23]

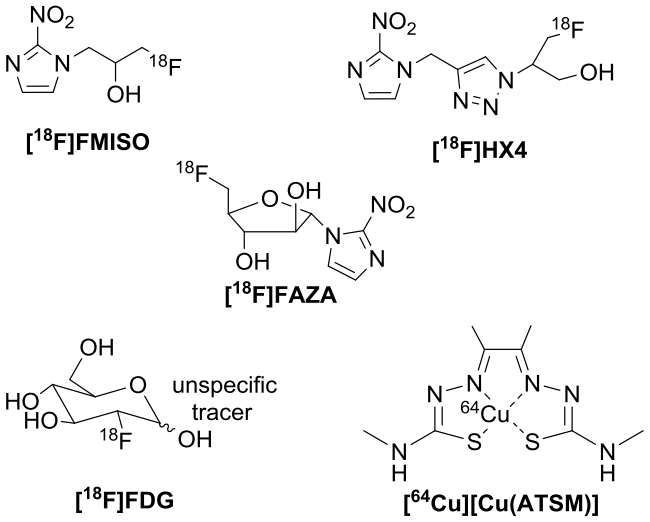


Figure 1. Hypoxia tracers.

In this paper, we report the synthesis of a new class of [^{18}F]FDR radiolabelled 2-nitroimidazole derivatives (**15**) for imaging hypoxia. To identify the most promising candidate for future *in vivo* imaging studies (e.g. **15d**), these compounds were tested *in vitro* on cell lines under hypoxic and normoxic conditions and the

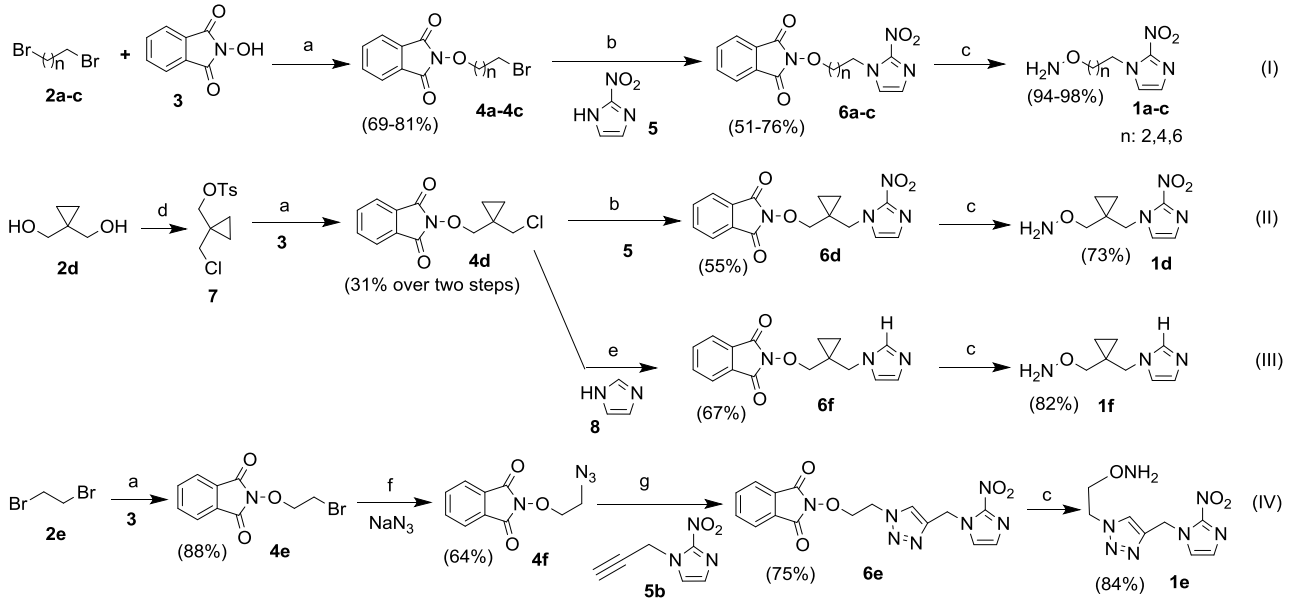
uptake values obtained were compared with the uptake values of [^{18}F]FAZA (specific hypoxia tracer) and [^{18}F]FDG (unspecific hypoxia tracer).

Results and Discussion

The new [^{18}F]fluorine-labelled tracers (**15a-f**, Figure 2) were obtained by oxime bond formation between 2-nitroimidazole alkoxyamine derivatives **1a-f** and [^{18}F]FDR (**12**). The compounds were first synthesized in non-radioactive form, **13a-f**, Scheme 2) using [^{19}F]FDR (**9**) to set and optimize the experimental conditions for the radio-synthesis using [^{18}F]Fluorine. By adapting and improving protocols reported in the literature,[24,25] the syntheses of 2-nitroimidazole alkoxy amines with linear alkyl chains (**1a-1c**) were achieved starting from commercial 1,3-dibromopropane (**2a**), or 1,5-dibromopentane (**2b**) or 1,7-di-bromoheptane (**2c**), respectively (Scheme 1, Eq. I). An excess of di-bromoalkanes **2a-c** was reacted with *N*-hydroxy-phthalimide **3** to limit the formation of di-substituted by-products. Compound **4a** was isolated by crystallization in ethanol. Compounds **4b-4c** were isolated after filtration through a silica pad by elution with toluene. Alkoxyamines **1a-1c** were obtained by nucleophilic substitution of **4a-c** with 2-nitroimidazole **5** affording phthalimides **6a-6c** in good yields, followed by quantitative cleavage of the phthalimido group using hydrazine monohydrate in EtOH at 80 ˚C. The general synthesis above was slightly modified in order to obtain the cyclopropyl derivative **1d** (Scheme 1, Eq. II). Treatment of commercial 1,1-bis(hydroxymethyl)cyclopropane (**2d**) with tosyl chloride afforded the intermediate 1-tosyl 3-chlorine derivative (**7**). Selective displacement of the tosylate function in **7** by *N*-hydroxyphthalimide **3** upon heating to 60 ˚C afforded **4d** in good yield. Subsequent halogen substitution with 2-nitroimidazole **5** and alkoxyamine deprotection from the resulting **6d** was performed in milder conditions than those employed for compounds **1a-1c**, affording the target **1d**.

The series of 2-nitroimidazole derivatives was completed by **1e,** which was obtained in good overall yield (Scheme 1, Eq. IV). The introduction of a 1,2,3 triazole function was achieved by copper (I) catalyzed 1,3-dipolar azide-alkyne cycloaddition (Huisgen cycloaddition).[26] Thus, compound **4e**, obtained by the general procedures already described above for compound **4a-4c**, was reacted with sodium azide affording **4f**. The reaction of azide **4f** with the alkyne **5b** using $CuSO_4$ as catalyst and sodium ascorbate as oxidant afforded exclusively and in good yield the 1,4 disubstituted regioisomer **6e,** that upon treatment with hydrazine gave the target derivative **1e**.

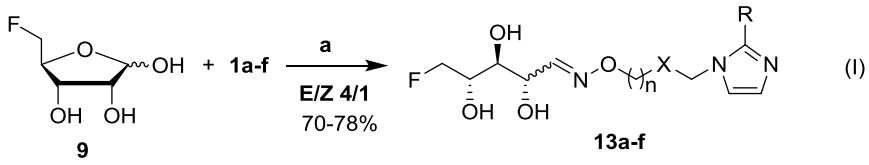
The 2-*H*-imidazole derivative **1f** (Scheme 1, Eq. III) was obtained via halogen displacement of **4d** by imidazole **8** followed by deprotection of the resulting **6f** with hydrazine monohydrate. Unexpectedly, both cyclopropyl alkoxyamines **1d** and **1f** were found to be rather unstable at 4 ˚C and could only be stored at -18˚C for short periods of time (e.g. a few days), so they were generally used for the next step – the oxime bond formation with FDR – soon after preparation from **6d,f**.



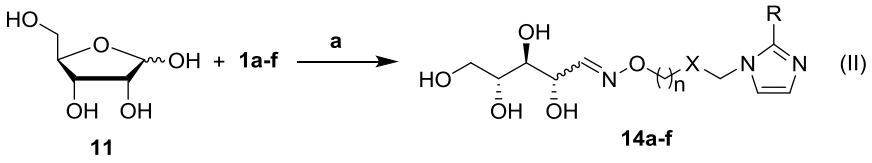
Scheme 1. Synthesis of alkoxyamines **1a-f**. Reagents and conditions: (a) TEA, DMF, r.t. overnight; (b) K_2CO_3,DMF, 80 - 115°C, 4.5 - 6 h; (c) $NH_2NH_2*H_2O$, EtOH, 25 - 80°C , 1 - 4 h; (d) TsCl, TEA, CH_2Cl_2, r.t., 48 h; (e) DMF, 120°C, 52 h; (f) DMF, 120°C, 4 h; (g) $CuSO_4$, sodium ascorbate, *t*-BuOH/H_2O, 20 h.

[^{19}F]FDR (**9**) (Scheme 2) and tosylated d-ribose **10** (Scheme 3), which is the precursor for the radio-synthesis of [^{18}F]FDR **12**, were both synthesised from commercial d-ribose **11** (Scheme 2) using the procedure reported by Li *et al*.[16] and were isolated as mixtures of α- and β- anomers.

The non-radioactive tracers **13a-f** were synthesized via oxime bond formation between **1a-f** and [^{19}F]FDR (**9**) using a solution of acetate buffer at pH 4.6 as reaction medium (Scheme 2, Eq. I). [16,18,27] The coupling reactions went to completion in 15 minutes and the oximes **13a-f** were purified by RP-HPLC and isolated (see yields in Table 1) as mixtures of *E*- and *Z*-diastereoisomers in ratio 4/1, as estimated by ^{1}H-NMR (400 MHz) in CD_3OD. The oxime C*H*=N protons doublets of *E* isomers resonate at higher fields (from 7.46 to 7.30 ppm) with J_E constants ranging from 7.01 to 7.15 Hz, whereas the same protons of *Z* isomers resonate at lower fields (from 6.70 to 6.84 ppm) with J_Z constants ranging from 5.84 to 6.22 Hz.



13a n:1, X: CH_2, R: NO_2
13b n:3, X: CH_2, R: NO_2
13c n:5, X: CH_2, R: NO_2
13d n:1, X: $C(CH_2CH_2)$, R: NO_2
13e n:1, X: 1,2,3 triazole-1,4-disustituted, R: NO_2
13f n:1, X: $C(CH_2CH_2)$, R: H



14a n:1, X: CH_2, R: NO_2
14b n:3, X: CH_2, R: NO_2
14c n:5, X: CH_2, R: NO_2
14d n:1, X: $C(CH_2CH_2)$, R: NO_2
14e n:2, X: 1,2,3 triazole-1,4-disustituted, R: NO_2
14f n:1, X: $C(CH_2CH_2)$, R: H

Scheme 2. Oxime bond formation reactions. Reagents and conditions: (a) 0.1 M acetate buffer CH_3COOH/CH_3COONa pH 4.6, 15 min r.t..

The mixtures of oxime E/Z isomers **13a-f** were not separated, as this was not deemed necessary for pursuing the aims of this work and were stored at -18 ˚C to avoid any degradation.

Non-fluorinated oximes **14a-f** (Scheme 2, Eq. II) were also synthesized as control molecules, as these compounds can be formed by a side reaction consisting in the condensation between **1a-f** and d-ribose **11**, which is the main by-product in the radiosynthesis of [^{18}F]FDR (see Scheme 3), as d-ribose **11** is not trapped by the Chromabond IV cartridge used for the purification of [^{18}F]FDR. By using the previously optimised HPLC conditions it was observed that non-fluorinated oximes **14a-f** are much less retained by a C18 stationary phase than fluoro-oximes **13a-f** indicating that the radiotracer purification step would not be affected by the presence of these nonradioactive compounds and therefore the final Fluorine-18 radiolabelled tracers **15a-f** should not contain the corresponding non-radioactive d-ribose oximes **14a-f** (see also Supporting Information).

Partition coefficients (*P*) of non-radioactive compounds **13a-f** were determined using the RP-HPLC method[28,29] (mobile phase: H_2O/*i*PrOH 90/10; flow rate: 1 mL min^{-1}; T= 27.9 - 28.7˚C) and log *P* values were calculated (Table 1). The log *P* values of AZA (-0.28), FAZA (0.04), FMISO (0.4), IAZA (0.70) and BnOH (1.10), reported in literature,[28,30,31] were used as reference values to extrapolate log *P* values of **13a-f.**

Compound (n)	X	R	Yield (%)	log *P* (±SEM)*
13a (1)	CH_2	NO_2	71.0	0.042 (±0.012)
13b (3)	CH_2	NO_2	72.5	0.57 (±0.02)
13c (5)	CH_2	NO_2	77.7	0.93 (±0.01)

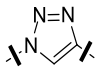
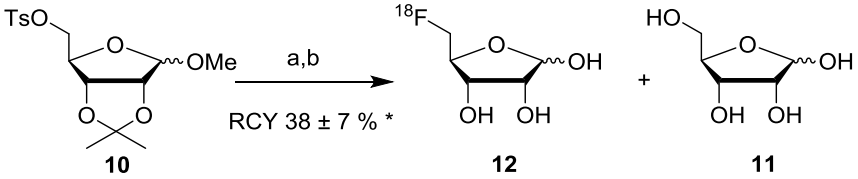
13d (1)		NO_2	74.5	0.38 (±0.04)
13e (2)		NO_2	70.3	-0.21 (±0.02)
13f (1)		H	69.8	0.33 (±0.04)

Table 1. Yields and log *P values* of compounds **13a-f**. *Each log *P* value was obtained by RP-HPLC method on average of three experiments.

The radiosynthesis of the target fluorinated tracers [^{18}F]-**15a-f** (Figure 2) was addressed next. The production of [^{18}F]FDR (**12**) (Scheme 3) was fully optimized and routinely performed following the previously published protocol.[16,18,27] The tosylate precursor **10** was converted into **12** by a two-step automated synthesis using a modular reactor, which took 1 h from [^{18}F]fluoride delivery from the cyclotron to isolation. The decay corrected radiochemical yield (RCY) was 38.0 ± 7% (average of five production runs) and after verification of purity by analytical RP-HPLC and radio TLC, a 10% v/v water solution of a 1 M acetate buffer at pH 4.6 was added to the aqueous solution of [^{18}F]FDR and 2-nitroimidazole *N*-alkoxyamine. The final radiotracers **15a-15f** were collected in good RCY after purification by RP-HPLC using the conditions optimized on **13a-f**.



Scheme 3. Synthesis of **12.** Reagents and conditions :(a) ^{18}F in $H_2{}^{18}O$, K_2CO_3, Chromafix cartridge; K_{222}, ACN, 100°C, 15 min; (b) HCl 1 M, 100°C 15 min, Chromabond IV cartridge. *RCY reported with decay correction and obtained on average of five production runs.

Co-injection of non-radioactive fluorinated reference **13a-f** and corresponding [^{18}F]FDR-oxime **15a-f** was performed for each tracer: the superimposition of the peak detected by photodiode array (PDA) detector and the peak detected by radio detector at the same retention time confirmed the tracer identity and radiochemical purity.(Figure 3 for compound **15d**, see SI for the others)

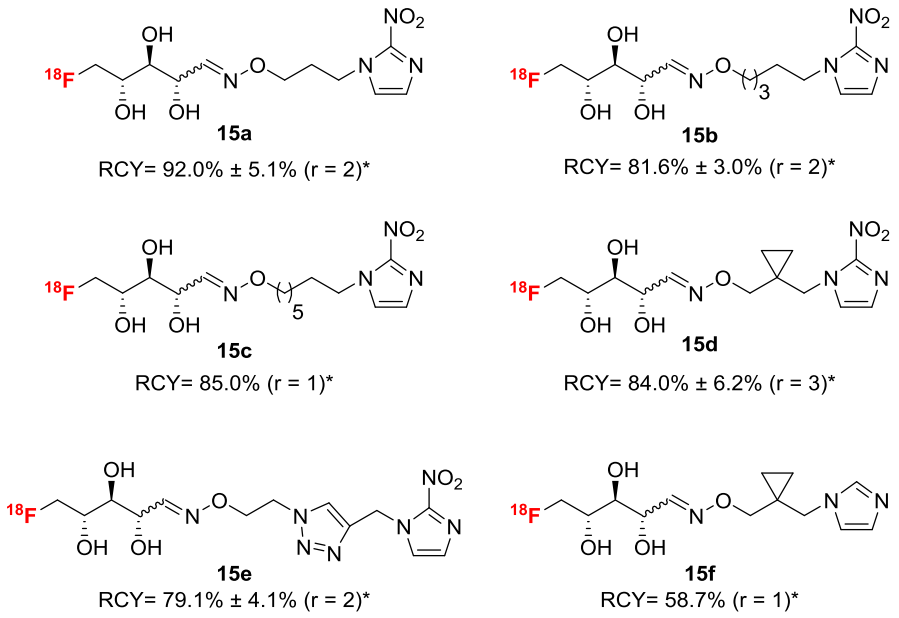


Figure 2. 15a-f radiolabelled using [^{18}F]FDR **12**. *RCY reported with decay correction considering activity of [^{18}F]FDR as starting activity; r = number of experiments.

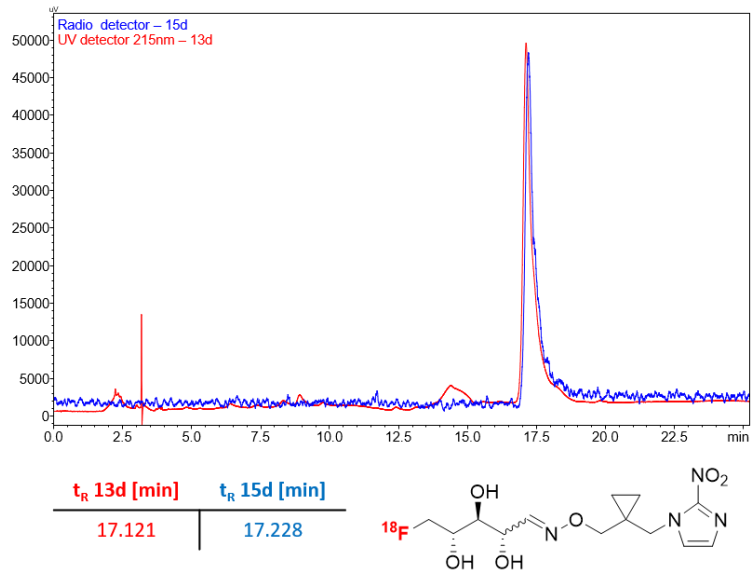


Figure 3. RP-HPLC traces of **13d** (red trace, PDA detector, 215 nm) and **15d** (blue trace, radioactivity detector)**.**

*Biological evaluation of radiotracers **15a-f**.*

Radiotracer uptake experiments were performed in MCF7 breast cancer cells, under normoxic (21% O_2) and hypoxic (1% O_2) conditions, using our optimized radiotracer uptake assay.[32] [^{18}F]FAZA and [^{18}F]FMISO were included in the experiments as gold standard hypoxia tracers, as they exhibit relatively rapid uptake kinetics in tumors, which facilitates relatively fast hypoxia clinical imaging protocols, and produce good hypoxia:normoxia (H:N) uptake ratios, which enables detection of hypoxic regions.[5,9]

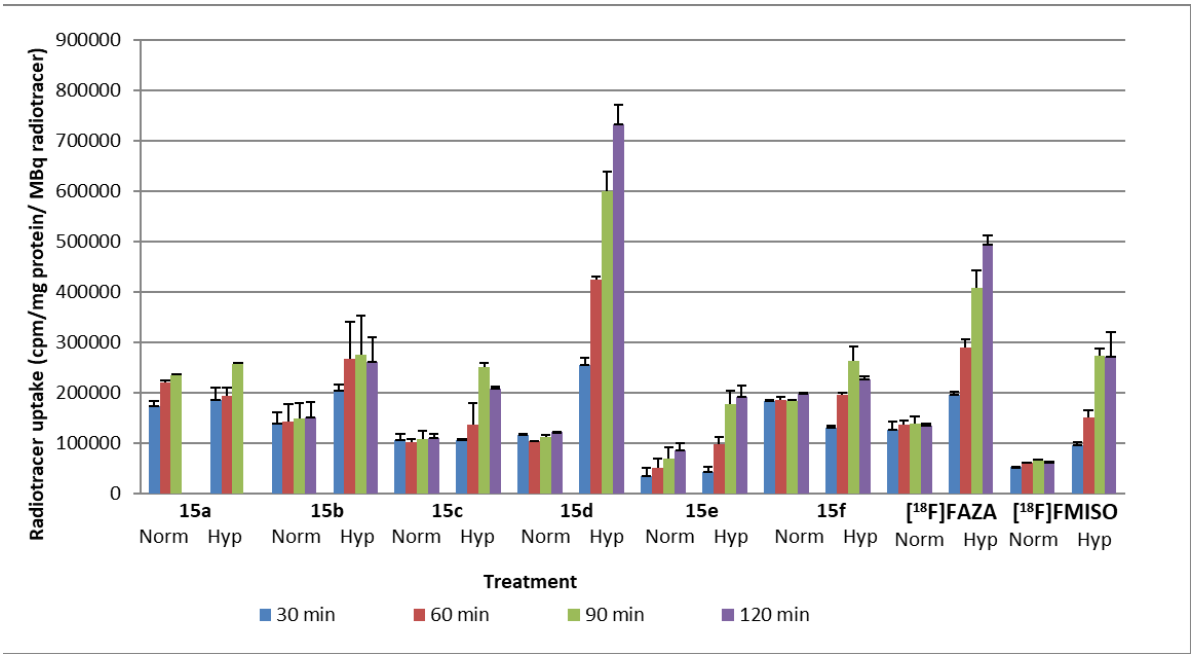


Figure 4. Uptake of [^{18}F]FAZA and tracers **15a-f** in hypoxic and normoxic MCF7 cells. MCF7 cells were pre-equilibrated at 1% O_2 for 1 h in a Coys hypoxia chamber. Each radiotracer was then injected and cells incubated with radiotracer at 1% O_2 for the indicated times. ^{18}F cellular uptake was then determined as described[32]. Each datapoint is the average ± standard deviation of at least 3 independent samples

In agreement with previous publications,[33] we observed selective [^{18}F]FAZA and [^{18}F]FMISO uptake in hypoxic cells compared to normoxic cells (Figure 4). Cellular uptake of both radiotracers was time-dependent and constant over the duration of the experiment. Under the conditions used (1% O_2), [^{18}F]FAZA and [^{18}F]FMISO generated a hypoxia:normoxia (H:N) ratio of approximately 4 and 5, respectively, confirming that they accumulate selectively in hypoxic cells.

As predicted, [^{18}F]FDR did not accumulate in hypoxic cells, confirming that it is not a hypoxia sensor (data not shown). Similarly, compound **15a** exhibited minimal uptake in cells (Figure 4), and the observed accumulation was not hypoxia-specific, indicating that **15a** is unable to detect hypoxic cells. Compounds **15b** and **15c** both displayed hypoxia-specific cellular uptake. Uptake kinetics of both compounds were superior to that of [^{18}F]FAZA and comparable to that of [^{18}F]FMISO, as cellular uptake was completed after 60 min and 90 min, respectively. However, the H:N ratio of these compounds was only approximately 2, likely indicating that it would be challenging to selectively detect hypoxic cells with these compounds *in vivo*.

Compound **15d** showed rapid hypoxia-specific uptake in cells and minimal uptake in normoxic cells, indicating that tracer accumulation was entirely hypoxia-specific. Radiotracer uptake kinetics compared favorably with [^{18}F]FAZA and [^{18}F]FMISO, as the initial uptake rate was more rapid, and uptake was starting to plateau by 120 min. Compound **15d** produced an H:N ratio ~ 6, confirming that it accumulates selectively in hypoxic cells with a comparable – if not slightly superior – H:N ratio relative to both [^{18}F]FAZA and [^{18}F]FMISO.

Compound **15e** demonstrated clear time-dependent uptake in MCF7 cells. Unfortunately, its uptake was detected in both normoxic and hypoxic cells, indicating that both hypoxia-specific and non-specific mechanisms were operating in cells. Hypoxia-specific uptake was relatively rapid, as this was largely complete after 90 min. However, the non-specific uptake mechanism limited the H:N ratio to approximately 2. The limited H:N ratio, combined with the hypoxia-independent uptake mechanism, limit the prospects for compound **5e** as a hypoxia sensor.

There was no specific accumulation of compound **15f** in hypoxic cells, indicating that it is not suitable for detecting hypoxic cells and confirming that the 2-nitroimidazole moiety is essential for hypoxia-targeted reactivity.

In order to perform a more extensive comparison of **15d** with the most advanced 2-nitroimidazole hypoxia radiotracers, uptake experiments were expanded to a panel of diverse cancer cell lines (Figure 5). The results indicate that in MCF7 cells **15d** and [^{18}F]FMISO produced significantly higher H:N ratios than [^{18}F]FAZA. Whereas in OE33 cells, **15d** and [^{18}F]FAZA displayed significantly higher uptake than [^{18}F]FMISO. In contrast, in PC3 cells [^{18}F]FAZA had higher uptake than both of the other tracers, whilst [^{18}F]FAZA was superior to [^{18}F]FMISO in U87MG cells. The radiotracers did not show any significant differences in cellular uptake in AGS cells.

These data indicate that the uptake values of these three tracers are fairly similar across a panel of diverse cell lines. However, there are subtle, but significant, differences between these radiotracers within the cell line panel, which suggest that **15d**, [^{18}F]FAZA or [^{18}F]FMISO may be superior in specific cell types. It seems likely that the subtly different uptake profiles of these tracers within the cell line panel are a function of the lipophilicity of each tracer. The comparable H:N ratios of these two tracers suggest that **15d** and [^{18}F]FAZA/[^{18}F]FMISO are likely to produce similar maximum uptake values in hypoxic tumors *in vivo*. However, the superior uptake kinetics of **15d** compared to [^{18}F]FAZA/[^{18}F]FMISO suggest that it may require a shorter time between radiotracer injection and patient imaging in order to achieve its maximum uptake value.

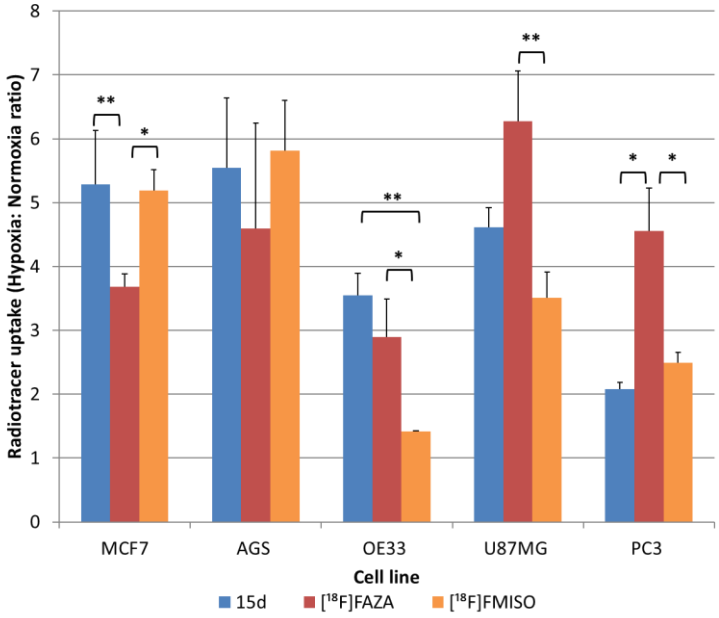


Figure 5. Hypoxia:normoxia uptake ratios of **15d,** [^{18}F]FAZA and [^{18}F]FMISO in a panel of cell lines. Each cell line was incubated with radiotracer for 2 h at 1% O_2. Radiotracers cellular uptake was then determined as described[32] . In each cell line the Hypoxia:Normoxia ratio was calculated for each radiotracer by dividing uptake in hypoxic cells by uptake in normoxic cells (an H:N ratio of 1 means no hypoxia-selective cellular uptake was detected). Each datapoint is the average ± standard deviation of at least 3 independent samples. Statistical analysis: 1-way ANOVA performed in SPSS, * ($p < 0.05$) or ** ($p < 0.01$)

Conclusion

We have designed and synthesized five radiotracers **15a-e** incorporating different spacers between the hypoxia-reactive 2-nitro-imidazole moiety and the [^{18}F]FDR prosthetic group linked through an oxyamine function. Experiments in hypoxic cells showed that tracer **15d**, featuring a cyclopropyl ring spacer, displays

superior uptake kinetics, and similar selectivity for hypoxic cells, relative to the gold standard hypoxia tracers [^{18}F]FAZA and [^{18}F]FMISO. Structure-activity-relationship studies showed that lipophilicity and structural rigidity have a strong influence on the selectivity of these [^{18}F]FDR-tracers towards hypoxic cells, the lead tracer **15d** displaying a logP = 0.38 and the most rigid spacer. Pre-clinical imaging studies will be necessary to determine whether [^{18}F]**15d** can successfully detect hypoxic regions *in vivo*.

Experimental Section

General methods: All reagents and solvents were of highest grade from commercial sources, unless otherwise specified. Reactions were monitored by thin-layer chromatography (TLC), unless otherwise noted. TLCs were performed on Merck silica gel glass plates (60 F_{254}). Visualisation was accomplished by irradiation with a UV lamp and/or staining with a ceric ammonium molybdate or $KMnO_4$ solution. Flash chromatography was performed using Silica gel (60 Å, particle size 40-63 µm) purchased from Merck. ^{1}H-NMR, ^{19}F-NMR and ^{13}C-NMR spectra were recorded on a Bruker Advance AVIII 400 spectrometer at 298 K and calibrated using residual undeuterated solvent as internal reference. Chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (*J*) are given in Hertz (Hz). The following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet-doublet, dt = doublet-triplet, m = multiplet, br = broad. When necessary, resonances were assigned using two-dimensional experiments (COSY, TOCSY, HMBC and HSQC). Mass Analyses (MS) were performed using Agilent 1200 HPLC system coupled to Agilent G6120 single quadrupole detector equipped with an electrospray ionization (ESI) source in direct infusion modality. ESI-MS spectra were recorded in positive mode, unless otherwise noted. HRMS were performed at the EPSCR National Mass Spectrometry Service Centre (Swansea). RP (reverse phase)-HPLC-MS analyses were performed with an Agilent 1200 HPLC system equipped with a DAD and an ESI-MS detector. HPLC semi-preparative purifications were performed using a Agilent 1260 HPLC system with reverse phase column as indicated in individual experiment. HPLC analyses of radioactive compounds were performed using a Shimadzu HPLC system equipped with a SPD-M20A Prominence DAD UV detector and NaI radio-detector (Berthold Technologies). Semi-prep HPLC purification of radioactive compounds were performed using a lead shielded Shimadzu semiprep HPLC system equipped with a SPD-M20A Prominence DAD UV detector and NaI radio-detector (Berthold Technologies). RadioTLC were performed using a Raytest miniGITA RadioTLC scanner. The dose calibrators used to measure doses were CAPINTEC CRC 15R and CAPINTEC CRC 15PET.

***O*-(3-(2-nitro-1*H*-imidazol-1-yl)propyl)hydroxylamine (1a)**: To a solution of **6a** (142 mg, 0.45 mmol) in EtOH (1.5 mL) hydrazine monohydrate (45 µL, 0.90 mmol) was added and the reaction mixture was refluxed for 3 h. After cooling to 0°C the resultant white precipitate was filtered and the filtrate was concentrated under vacuum. The residue was treated with Et_2O (1 mL), the resultant white precipitate was filtered and the filtrate was concentrated under vacuum to afford **1a** as a yellow oil (79 mg, 94.3%), which was used in the next step without any further purification. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.14 (d, *J* = 1.0 Hz, 1H), 7.12 (d, *J* = 1.0 Hz, 1H), 5.38 (br, 2H, -N*H*$_2$), 4.52 (t, *J* = 7.0 Hz, 2H), 3.67 (t, *J* = 5.7 Hz, 2H), 2.17-2.10 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ(ppm) 128.3, 126.3, 71.5, 47.4, 29.2. MS (ESI *m/z)* calc. for $C_6H_1N_4O_3$: 187.1 $[M+H]^+$, 209.1 $[M+Na]^+$; found: 187.1 $[M+H]^+$, 209.0 $[M+Na]^+$.

***O*-(5-(2-nitro-1*H*-imidazol-1-yl)pentyl)hydroxylamine (1b):** To a solution of **6b** (737 mg, 2.14 mmol) in EtOH (5 mL) hydrazine monohydrate (208 µL, 4.28 mmol) was added and the reaction mixture was refluxed for 3 h. After cooling to 0°C the resultant white precipitate was filtered and the filtrate was concentrated under vacuum. The residue was dissolved with EtOAc (2 mL) and the resultant white precipitate was filtered and the filtrate was concentrated under vacuum to afford **1b** as yellow oil (432 mg, 94.2%), which was used in the next step without any further purification. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.13 (d, *J* = 1.0 Hz, 1H), 7.08 (d, *J* = 1.0 Hz, 1H), 5.33 (br, 2H, -N*H*$_2$), 4.41 (t, *J* = 7.4 Hz, 2H), 3.65 (t, *J* = 6.3 Hz, 2H), 1.92-1.78 (m, 2H), 1.66-1.56 (m, 2H), 1.46-1.34 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 128.5, 125.9, 75.5, 50.4, 30.5, 27.9, 23.2. MS (ESI *m/z)* calc. for $C_8H_{14}N_4O_3$: 215.1 $[M+H]^+$, 237.1 $[M+Na]^+$; found: 215.1 $[M+H]^+$, 237.1 $[M+Na]^+$.

***O*-(7-(2-nitro-1*H*-imidazol-1-yl)heptyl)hydroxylamine (1c):** To a solution of **6c** (700 mg, 1.88 mmol) in EtOH (4 mL) hydrazine monohydrate (183 µL, 3.76 mmol) was added and the reaction mixture was refluxed for 3 h. After cooling to 0°C the resultant white precipitate was filtered and the filtrate was concentrated under vacuum. The residue was dissolved with EtOAc (2 mL) and the resultant white precipitate was filtered and the filtrate was concentrated under vacuum to afford **1c** as a yellow oil (448 mg, 98.4%), which was used in the next step without any further purification. ^{1}H NMR ($CDCl_3$, 400MHz) δ (ppm) 7.12 (d, *J* = 1.0 Hz, 1H), 7.07 (d, *J* = 1.0 Hz, 1H),), 5.30 (bs, 2H,-NH_2), 4.39 (t, *J* = 7.4 Hz, 2H), 3.63 (t, *J* = 6.6 Hz, 2H), 1.89-1.78 (m, 2H), 1.60-1.50 (m, 2H), 1.40-1.28 (m, 6H). ^{13}C NMR ($CDCl_3$, 100MHz) δ (ppm) 128.4, 125.9, 76.0, 50.4, 30.6, 29.0, 28.3, 26.4, 25.9. MS (ESI *m/z)* calc. for $C_{10}H_{18}N_4O_3$: 243.1 $[M+H]^+$, 265.1 $[M+Na]^+$ 281.2 $[M+K]^+$; found: 243.2 $[M+H]^+$, 265.1 $[M+Na]^+$, 281.0 $[M+K]^+$.

***O*-((1-((2-nitro-1*H*-imidazol-1-yl)methyl)cyclopropyl)methyl)hydroxylamine (1d):** To a solution of **6d** (125 mg, 0.37 mmol) in EtOH (7 mL) hydrazine monohydrate (36 µL, 0.73 mmol) was added and the reaction mixture was stirred at rt. for 2 h. After cooling to 0°C the resultant white precipitate was filtered and the filtrate was concentrated under vacuum. The residue was dissolved with *i*-PrO_2 (3mL) and the resultant white precipitate was filtered and the filtrate was concentrated under vacuum to afford **1d** as a yellow oil (57 mg, 72.6%), which was used in the next step without any further purification. ^{1}H NMR (CD_3OD, 400MHz) δ (ppm) 7.56 (d, *J* = 1.1 Hz, 1H), 7.15 (d, *J* = 1.1 Hz, 1H), 4.59 (s, 2H), 3.43 (s, 2H), 0.81-0.76 (m, 2H), 0.69-0.64 (m, 2H). ^{13}C NMR (CD_3OD 100MHz) δ (ppm) 128.2 (2C), 81.1, 54.8, 21.9, 10.3 (2C). MS (ESI *m/z)* calc. for $C_8H_{12}N_4O_3$: 213.1 $[M+H]^+$, 235.1 $[M+Na]^+$; found: 213.1 $[M+H]^+$, 235.1 $[M+Na]^+$.

***O*-(2-(4-((2-nitro-1*H*-imidazol-1-yl)methyl)-1*H*-1,2,3-triazol-1-yl)ethyl)hydroxylamine (1e):** To a solution of **6e** (60 mg, 0.16 mmol) in EtOH (2 mL) hydrazine monohydrate (16 µL, 0.32 mmol) was added and the reaction mixture was heated to 60°C for 2 h. After cooling to 0°C the resultant white precipitate was filtered and the filtrate was concentrated under vacuum to give **1e** as a yellow oil (34 mg, 83.9%), which was used in the next step without any further purification. ^{1}H NMR ($CDCl_3$, 400MHz) δ (ppm) 7.77 (s, 1H), 7.37 (d, *J* = 1.1 Hz, 1H), 7.14 (d, *J* = 1.1 Hz, 1H), 5.70 (s, 2H), 5.49 (br, 2H), 4.62-4.58 (m, 2H), 4.02-3.98 (m, 2H). ^{13}C NMR ($CDCl_3$, 100MHz) δ (ppm) 141.3, 128.7, 126.6, 124.2, 73.1, 50.0, 44.8. MS (ESI *m/z)* calc. for $C_8H_{11}N_7O_3$: 254.1 $[M+H]^+$, 276.1 $[M+Na]^+$; found: 254.1 $[M+H]^+$, 276.0 $[M+Na]^+$.

***O*-((1-((1*H*-imidazol-1-yl)methyl)cyclopropyl)methyl)hydroxylamine (1f):** To a solution of **6f** (50 mg, 0.17 mmol) in ethanol (1 mL) hydrazine monohydrate (16 µL, 0.33 mmol) was added and the reaction mixture was heated to 70°C for 1 h. After cooling to 0°C the resultant white precipitate was filtered and the filtrate was concentrated under vacuum to give a crude oil. This crude oil was dissolved with EtOAc (0.5 mL) and the resultant white precipitate was filtered and the filtrate was concentrated under vacuum to afford **1f** as a yellow oil (23 mg, 81.9%), which was used in the next step without any further purification. ^{1}H NMR (CD_3OD, 400MHz) δ (ppm) 9.04 (s, 1H), 7.72 (s, 1H), 7.62 (s, 1H), 4.31 (s, 2H), 3.79 (s, 2H), 1.03-0.96 (m, 2H), 0.90-0.83 (m, 2H). ^{13}C NMR (CD_3OD 100MHz) δ (ppm) 136.8, 123.8, 121.2, 79.8, 54.7, 20.9, 10.7 (2C). MS (ESI *m/z)* calc. for $C_8H_{13}N_3O$: 168.1 $[M+H]^+$; found: 168.1 $[M+H]^+$.

2-(3-bromopropoxy) isoindoline-1,3-dione (4a): To a solution of *N*-hydroxy-phthalimide (**3**) (1.0 g, 6.13 mmol) in DMF (2.5 mL) 1,3-dibromopropane (**2a**) (1.25 mL, 12.26 mmol) and TEA (0.85 mL, 6.13 mmol) were added at rt. The reaction mixture was stirred for 18 h at rt and then the red precipitate formed was filtered and the filtrate was diluted with ice cold water (60 mL). The resultant white precipitate was filtered, washed with cold water (3 x 5 mL) and recrystallized from EtOH to afford (**4a**) as a white solid (1.20 g, 69.2%). mp 64-66 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ(ppm) 7.86-7.79 (m, 2H), 7.78-7.72 (m, 2H), 4.35 (t, *J* = 5.8 Hz, 2H), 3.69 (t, *J* = 6.5 Hz, 2H), 2.33-2.25 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ(ppm) 163.7 (2C), 134.7 (2C), 129.0 (2C), 123.7 (2C), 76.2, 31.6, 29.4. NMR data of the product match literature information.[34] MS (ESI *m/z)* calc. for $C_{11}H_{10}BrNO_3$: 285.0 $[M+H]^+$, 287.0 $[M+2+H]^+$, 307.0 $[M+Na]^+$, 309.0 $[M+2+Na]^+$; found: 285.0 $[M+H]^+$, 287.0 $[M+2+H]^+$, 307.0 $[M+Na]^+$, 309.0 $[M+2+Na]^+$.

2-((5-bromopentyl)oxy)isoindoline-1,3-dione (4b): To a solution of *N*-hydroxy-phthalimide (**3**) (2.0 g, 12.3 mmol) in DMF (5 mL) 1,5-dibromopentane (**2b**) (3.34 mL, 24.5 mmol) and TEA (1.71 mL, 12.3 mmol) were added at rt. The reaction mixture was stirred for 18 h at rt and then the red precipitate formed was filtered and the filtrate was diluted with ice cold water (100 mL). The resultant white precipitate was filtered, washed with cold water (3 x 5 mL), dissolved in toluene and passed through a silica pad. Solvent was removed under vacuum to afford (**4b**) as a white solid (2.8 g, 72.9%). mp 72-74°C. ^{1}H NMR (DMSO d_6, 400 MHz) δ (ppm) 7.85 (s, 4H), 4.14 (t, *J* = 6.4 Hz, 2H), 3.55 (t, *J* = 6.7 Hz, 2H), 1.91-1.82 (m, 2H), 1.75-1.66 (m, 2H), 1.61-1.52 (m, 2H). ^{13}C NMR (DMSO d_6, 100 MHz) δ(ppm) 163.3 (2C), 134.7 (2C), 128.6 (2C), 123.2 (2C), 77.5, 34.9, 31.9, 26.8, 23.9. NMR data of the product match literature information.[35] MS (ESI *m/z)* calc. for $C_{13}H_{14}BrNO_3$: 311.0 $[M+H]^+$, 313.0 $[M+2+H]^+$, 329.0 $[M+H_3O]^+$, 331.0 $[M+2+H_3O]^+$, 334.0 $[M+ Na]^+$ 336.0 $[M+2+Na]^+$; found: 311.0 $[M+H]^+$, 313.0 $[M+2+H]^+$, 329.0 $[M+H_3O]^+$, 331.0 $[M+2+H_3O]^+$, 334.0 $[M+Na]^+$, 336.0 $[M+2+Na]^+$.

2-((7-bromoheptyl)oxy)isoindoline-1,3-dione (4c): To a solution of *N*-hydroxy-phthalimide (**3**) (2.0 g, 12.3 mmol) in DMF (5 mL) 1,7-dibromoheptane (**2c**) (4.2 mL, 24.5 mmol) and TEA (1.71 mL, 12.3 mmol) were added at rt. The reaction mixture was stirred for 24 h at rt and then the red precipitate formed was filtered and the filtrate was diluted with ice cold water (100 mL). The resultant white precipitate was filtered, washed with cold water (3 x 5 mL), dissolved in toluene and passed through a silica pad. Solvent was removed *in vacuo* to afford (**4c**) as a white wax (3.4 g, 81.3%).^{1}H NMR ($CDCl_3$, 400MHz) δ (ppm) 7.87-7.80 (m, 2H), 7.77-7.71 (m, 2H), 4.19 (t, *J* = 6.6 Hz, 2H), 3.40 (t, *J* = 6.8 Hz, 2H), 1.91-1.83 (m, 2H), 1.83-1.74 (m, 2H), 1.75-1.66 (m, 2H), 1.56-1.34 (m, 6H). ^{13}C NMR ($CDCl_3$, 100MHz) δ (ppm) 163.8 (2C), 134.6 (2C), 129.1 (2C), 123.6 (2C), 78.6, 34.2, 32.8, 28.5, 28.2, 28.1, 25.5. MS (ESI *m/z)* calc. for $C_{15}H_{18}BrNO_3$: 339.0 $[M+H]^+$, 341.0 $[M+2+H]^+$, 361.0 $[M+Na]^+$ 363.0 $[M+2+Na]^+$; found: 339.1 $[M+H]^+$, 341.1 $[M+2+H]^+$, 361.0 $[M+Na]^+$, 363.0 $[M+2+Na]^+$.

2-((1-(chloromethyl)cyclopropyl)methoxy)isoindoline-1,3-dione (4d): To a solution of N-hydroxy-phthalimide (**3**) (208 mg, 1.28 mmol) and DIPEA (0.635 mL, 3.84 mmol) in DMF (3 mL) 7 (500 mg, 1.82 mmol) dissolved in DMF (5mL) was added at rt. The reaction mixture was stirred at 60°C for 48 h and then cooled down to rt and diluted with water (50 mL). The resultant mixture was extracted with EtOAc (3 x 40mL) and the organic phases were combined, washed with water (30 mL) and brine (30 mL), dried over Na_2SO_4, filtered and evaporated under vacuum. Purification by FC on silica gel eluting with EtOAc/Hex (8/92) gave **4d** as a white wax (191 mg, 56.2%). ^{1}H NMR ($CDCl_3$, 400MHz) δ(ppm) 7.86-7.80 (m, 2H), 7.78-7.72 (m, 2H), 4.19 (s, 2H), 3.83 (s, 2H), 0.77-0.72 (m, 4H). ^{13}C NMR ($CDCl_3$, 100MHz) δ (ppm) 163.6 (2C), 134.6 (2C), 128.9 (2C), 123.6 (2C), 81.1, 50.2, 22.3, 11.6 (2C). MS (ESI *m/z)* calc. for $C_{13}H_{12}ClNO_3$: 266.1 $[M+H]^+$, 268.1 $[M+2+H]^+$, 288.1 $[M+Na]^+$ 290.1 $[M+2+Na]^+$; found: 266.1 $[M+H]^+$, 268.1 $[M+2+H]^+$, 288.0 $[M+Na]^+$, 290.0 $[M+2+Na]^+$.

2-(2-bromoethoxy)isoindoline-1,3-dione (4e): To a solution of *N*-hydroxy-phthalimide (**3**) (1.0 g, 6.13 mmol) in DMF (2.5 mL) 1,2-dibromoethane (**2e**) (1.06 mL, 12.26 mmol) and TEA (0.854 mL, 6.13 mmol) were added at rt. The reaction mixture was stirred at rt for 18 h and then the red precipitate formed was filtered and the filtrate was diluted with ice cold water (60 mL). The resultant white precipitate was filtered and washed with cold water (3 x 5 mL) and recrystallized by EtOH to afford (**4e**) as a white solid (1.45 g, 87.6%). mp 91-93°C. ^{1}H NMR ($CDCl_3$, 400MHz) δ (ppm) 7.87-7.81 (m, 2H), 7.79-7.73 (m, 2H), 4.49-4.43 (m, 2H), 3.66-3.60 (m, 2H). ^{13}C NMR ($CDCl_3$, 100MHz) δ (ppm) 163.4 (2C), 134.7 (2C), 128.7 (2C), 123.7 (2C), 77.2, 26.6. NMR data of the product match literature information.[35] MS (ESI *m/z)* calc. for $C_{10}H_8BrNO_3$: 270.0 $[M+H]^+$, 272.0 $[M+2+H]^+$, 292.0 $[M+Na]^+$, 294.0 $[M+2+Na]^+$; found: 270.0 $[M+H]^+$, 272.0 $[M+2+H]^+$, 291.9 $[M+Na]^+$, 293.9 $[M+2+Na]^+$.

(2-(2-azidoethoxy)isoindoline-1,3-dione (4f): To a solution of **4e** (260 mg, 0.97 mmol) in DMF (2.5 mL) NaN_3 (138mg, 2.13 mmol) was added under nitrogen atmosphere. The reaction mixture was heated to

reflux for 4 h, then it was cooled to rt and diluted with water (25 mL). The aqueous mixture was extracted with DCM (3 x 10 mL) and the organic phases were combined, washed with brine, dried over Na_2SO_4 and concentrated under vacuum. The residue was washed with Et_2O (5mL) and filtered to afford **4f** as a hygroscopic solid (142 mg, 63.5%). The product 4f was used without any further purification. ^{1}H NMR ($CDCl_3$, 400MHz) δ (ppm) 7.89-7.83 (m, 2H), 7.80-7.73 (m, 2H), 4.35 (t, J = 5.1 Hz, 2H), 3.66 (t, J = 5.1 Hz, 2H). ^{13}C NMR ($CDCl_3$, 400MHz) δ (ppm) 163.5 (2C), 134.8 (2C), 129.0 (2C), 123.9 (2C), 76.9, 49.6. . NMR data of the product match literature information.[36] MS (ESI *m/z)* calc. for $C_{10}H_8N_4O_3$: 233.1 $[M+H]^+$, 250.1 $[M+H_2O]^+$, 255.1 $[M+Na]^+$; found: 233.1 $[M+H]^+$, 250.1 $[M+H_2O]^+$, 255.0 $[M+Na]^+$.

2-nitro-1-(prop-2-yn-1-yl)-1*H*-imidazole (5b): To a solution of propargyl bromide (80% in toluene, 200 µl, 3.6 mmol) in acetone (2.5 mL), 2-nitroimidazole (**5**) (170 mg 1.50 mmol) and K_2CO_3 (436 mg, 3.16 mmol) were added and the mixture was allowed to react at rt overnight. Then the reaction mixture was dilute with EtOAc (5mL) and filtered through a small pad of silica gel. Purification by FC on silica gel eluting with EtOAc/Hex (1/1) gave **5b** as a brown oil (171 mg, 75.4%).NMR data of the product correspond to literature information[26] ^{1}H NMR ($CDCl_3$, 400MHz) δ (ppm) 7.43 (s, 1H), 7.19 (s, 1H), 5.24 (d, *J* =2.5 Hz 2H), 2.63 (t, J = 2.5 Hz, 1H). ^{13}C NMR ($CDCl_3$, 400MHz) δ (ppm) 128.4, 125.1, 76.7, 74.9, 39.9. MS (ESI *m/z)* calc. for $C_6H_5N_3O_2$: 152.0 $[M+H]^+$, 174.0 $[M+Na]^+$; found: 152.1 $[M+H]^+$, 174.1 $[M+Na]^+$.

2-(3-(2-nitro-1*H*-imidazol-1-yl)propoxy)isoindoline-1,3-dione (6a): To a solution of **4a** (350 mg, 1.23 mmol) and K_2CO_3 (170 mg, 1.23 mmol) in dry DMF (5 mL) a solution of 2-nitroimidazole (**5**) (139 mg, 1.23 mmol) in dry DMF (1 mL) was added under nitrogen atmosphere. The reaction mixture was heated to 80°C for 4h, then cooled to rt, diluted with water (30 mL) and extracted with EtOAc (3 x 20 mL). The organic phases were combined, dried over Na_2SO_4, filtered and concentrated under vacuum to give a yellow oil. The oil was dissolved in Et_2O (2 mL) and the solution was kept at -10°C under stirrer for 30 min. The resultant yellow precipitate was filtered, washed with Et_2O (3 x 2 mL) and dried to afford **6a** as a yellow solid (295 mg, 75.8%). mp 178-179°C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ(ppm) 7.88-7.84 (m, 2H), 7.81-7.76 (m, 2H), 7.60 (d, *J* = 1.0 Hz, 1H), 7.19 (d, *J* = 1.0 Hz, 1H), 4.80 (t, *J* = 6.6 Hz, 2H), 4.24-4.19 (m, 2H), 2.35-2. 29 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ(ppm) 163.8 (2C), 135.0 (2C), 128.9 (2C), 128.6, 127.8, 123.9 (2C), 74.0, 46.6, 29.1. MS (ESI *m/z)* calc. for $C_{14}H_{12}N_4O_5$: 317.1 $[M+H]^+$, 339.1 $[M+Na]^+$; found: 317.1 $[M+H]^+$, 339.0 $[M+Na]^+$.

2-((5-(2-nitro-1*H*-imidazol-1-yl)pentyl)oxy)isoindoline-1,3-dione (6b): To a solution of **4b** (1 g, 3.20 mmol) and K_2CO_3 (443 mg, 3.20 mmol) in dry DMF (6 mL) a solution of 2-nitroimidazole (**5**) (362 mg, 3.20 mmol) in dry DMF (2 mL) was added under nitrogen atmosphere. The reaction mixture was heated to 80°C for 4 h, then cooled to rt, diluted with water (40 mL) and extracted with EtOAc (3 x 30 mL). The organic phases were combined, dried over Na_2SO_4, filtered and concentrated under vacuum. The residue was washed with Et_2O (2 x 3 mL) and dried to afford **6b** as a yellow wax (772 mg, 70.1%). ^{1}H NMR (DMSO d_6, 400 MHz) δ (ppm) 7.86 (s, 4H), 7.71 (d, *J* = 1.0 Hz, 1H), 7.18 (d, *J* = 1.0 Hz, 1H), 4.41 (t, *J* = 7.3 Hz, 2H), 4.12 (t, *J* = 6.4 Hz, 2H), 1.89-1.80 (m, 2H), 1.76-1.67 (m, 2H), 1.51-1.41 (m, 2H). ^{13}C NMR (DMSO d_6, 100MHz) δ(ppm) 163.3 (2C), 134.8 (2C), 128.6 (2C), 127.9, 127.8, 123.2 (2C), 77.5, 49.3, 29.4, 27.2, 22.1. MS (ESI *m/z)* calc. for $C_{16}H_{16}N_4O_5$: 345.1 $[M+H]^+$, 367.1 $[M+Na]^+$; found: 345.1 $[M+H]^+$, 367.1 $[M+Na]^+$.

2-((7-(2-nitro-1*H*-imidazol-1-yl)heptyl)oxy)isoindoline-1,3-dione (6c): To a solution of **4c** (1.6 g, 4.70 mmol) and K_2CO_3 (650 mg, 4.70 mmol) in dry DMF (7 mL) a solution of 2-nitroimidazole (**5**) (638 mg, 5.64 mmol) in dry DMF (3 mL) was added under nitrogen atmosphere. The reaction mixture was heated to 80°C for 6 h, then cooled to rt, diluted with water (50 mL) and extracted with EtOAc (3 x 30 mL). The organic phases were combined, dried over Na_2SO_4, filtered and concentrated under vacuum. Purification by FC on silica gel eluting with EtOAc/Hex (1/1) gives **6c** as a yellow oil (885 mg, 50.6%). ^{1}H NMR ($CDCl_3$, 400MHz) δ (ppm) 7.85-7.79 (m, 2H), 7.76-7.71 (m, 2H), 7.12 (d, *J* = 1.0 Hz, 1H), 7.11 (d, *J* = 1.0 Hz, 1H) 4.41 (t, *J* = 7.2 Hz, 2H), 4.19 (t, *J* = 6.3 Hz, 2H), 1.92-1.82 (m, 2H), 1.82-1.72 (m, 2H), 1.58-1.48 (m, 2H), 1.48-1.33 (m, 4H). ^{13}C NMR ($CDCl_3$, 100MHz) δ (ppm) 163.8 (2C), 134.6 (2C), 129.1 (2C),128.5 (2C), 126.0 (2C) 123.6 (2C), 78.4, 50.4,

30.4, 28.6, 28.1, 26.3, 25.5. MS (ESI *m/z)* calc. for $C_{18}H_{20}N_4O_5$: 373.1 $[M+H]^+$, 395.1 $[M+Na]^+$; found: 373.1 $[M+H]^+$, 395.1 $[M+Na]^+$.

2-((1-((2-nitro-1*H*-imidazol-1-yl)methyl)cyclopropyl)methoxy)isoindoline-1,3-dione (6d): To a solution of **4d** (330 mg, 1.24 mmol) and K_2CO_3 (206 mg, 1.49 mmol) in dry DMF (3 mL) a solution of 2-nitroimidazole (**5**) (197 mg, 1.74 mmol) in dry DMF (1 mL) was added under nitrogen atmosphere. The reaction mixture was heated to 80°C for 6h, then cooled to rt, diluted with water (40 mL) and extracted with EtOAc (3 x 20 mL). The organic phases were combined, dried over Na_2SO_4, filtered and evaporated under vacuum. The residue was washed with a solution Et_2O/MeOH 20/1 (3 x 3 mL) and dried under vacuum to give **6c** as a gummy solid (233 mg, 54.9%). ^{1}H NMR ($CDCl_3$, 400MHz) δ (ppm) 7.88-7.82 (m, 2H), 7.81-7.76 (m, 2H), 7.76 (d, *J* = 1.0 Hz, 1H), 7.20 (d, *J* = 1.0 Hz, 1H), 4.82 (s, 2H), 3.92 (s, 2H), 0.94-0.87 (m, 2H), 0.77-0.70 (m, 2H). ^{13}C NMR ($CDCl_3$, 100MHz) δ (ppm) 163.6 (2C), 134.9 (2C), 128.9 (2C), 128.6, 127.5, 123.9 (2C), 81.4, 52.2, 20.9, 10.0 (2C). MS (ESI *m/z)* calc. for $C_{16}H_{14}N_4O_5$: 343.1 $[M+H]^+$, 365.1 $[M+Na]^+$; found: 343.1 $[M+H]^+$, 365.1 $[M+Na]^+$.

2-(2-(4-((2-nitro-1*H*-imidazol-1-yl)methyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)isoindoline-1,3-dione (6e): To a solution of **4f** (80.0 mg, 0.35 mmol) in *t*-BuOH/H_2O (1/1 v/v, 2mL) **5b** (44.0 mg 0.29 mmol), $CuSO_4$ (4.6 mg, 0.029 mmol), sodium ascorbate (11.5 mg, 0.58mmol) were added and the mixture was stirred at rt for 18 h. Then DCM (6 mL) was added to the mixture and the organic layer was separated, washed with water (2 mL), dried over Na_2SO_4 and concentrated under vacuum. Purification by FC on silica gel eluting with EtOAc/Hex (2/1) gave **6e** as a yellow oil (82 mg, 73.8%). ^{1}H NMR ($CDCl_3$, 400MHz) δ (ppm) 8.36 (s, 1H), 7.87-7.82 (m, 2H), 7.81-7.75 (m, 2H), 7.37 (d, *J* = 1.1 Hz, 1H), 7.15 (d, *J* = 1.1 Hz, 1H), 5.77 (s, 1H), 4.79-4.73 (m, 2H), 4.60-4.55 (m, 2H). ^{13}C NMR ($CDCl_3$, 400MHz) δ(ppm) 163.6 (2C), 141.4, 135.7 (2C), 128.7 (2C), 126.4, 125.4, 124.0 (2C), 77.4, 76.6, 49.2, 44.9. MS (ESI *m/z)* calc. for $C_{16}H_{13}N_7O_5$: 384.1 $[M+H]^+$, 406.1 $[M+Na]^+$; found: 384.1 $[M+H]^+$, 406.0 $[M+Na]^+$.

2-((1-((1*H*-imidazol-1-yl)methyl)cyclopropyl)methoxy)isoindoline-1,3-dione (6f): To a solution of **4d** (100 mg, 0.38 mmol) in dry DMF (1 mL) imidazole (**8**) (51.2 mg, 0.75 mmol) in dry DMF (1 mL) was added under nitrogen atmosphere. The reaction mixture was heated to 120°C for 52h, then cooled to rt, diluted with water (40 mL) and extracted with EtOAc (3 x 20 mL). The organic phases were combined, washed with brine and dried over Na_2SO_4, filtered and evaporated under vacuum to give **6f** (75 mg, 66.5%). The product was used without any further purification. ^{1}H NMR ($CDCl_3$, 400MHz) δ (ppm) 7.87-7.82 (m, 2H), 7.79-7.73 (m, 3H), 7.18 (br, 1H), 7.10 (br, 1H), 4.20 (s, 2H), 3.87 (s, 2H), 0.79-0.74 (m, 4H). ^{13}C NMR ($CDCl_3$, 100MHz) δ (ppm) 163.6 (2C), 138.0, 134.8 (2C), 129.4, 128.9 (2C), 123.8 (2C), 119.9, 81.1, 50.5, 21.1, 9.9 (2C). MS (ESI *m/z)* calc. for $C_{16}H_{15}N_3O_3$: 298.1 $[M+H]^+$, 330.1 $[M+Na]^+$; found: 298.1 $[M+H]^+$, 330.1 $[M+Na]^+$.

(1-(chloromethyl)cyclopropyl)methyl 4-methylbenzenesulfonate (7): To a solution of 1,1 bis(hydroxymethyl)cyclopropane (**2d**) (1.50 g, 14.69 mmol) and pyridine (7.09 mL, 88.12 mmol) in anhydrous THF (30 mL) tosyl chloride (8.12 g, 33.00 mmol) was added portionwise over 20 min at 0 °C under stirring and then the mixture was allowed to react for 48h at rt. Then the white precipitate formed was filtered and the mixture was poured into water (50 mL) and then extracted with Et_2O (3 x 30 mL). The organic phases were combined washed with water (25 mL) and brine (25 mL) dried over Na_2SO_4, filtered and evaporated under vacuum. Purification by FC on silica gel eluting with EtOAc/Hex (5/95) gave **7** as a hygroscopic solid (2.22 g, 55.0%). ^{1}H NMR ($CDCl_3$, 400MHz) δ (ppm) 7.83-7.74 (m, 2H), 7.37-7.29 (m, 2H), 4.00 (s, 2H), 3.44 (s, 2H), 2.45 (s, 3H), 0.73-0.69 (m, 4H). ^{13}C NMR ($CDCl_3$, 100MHz) δ (ppm) 145.0, 133.0, 130.0 (2C), 128.0 (2C), 73.6, 49.5, 30.4, 22.5, 21.7, 12.3 (2C). MS (ESI *m/z)* calc. for $C_{12}H_{15}ClO_3$: 275.0 $[M+H]^+$, 277.0 $[M+2+H]^+$, 292.0 $[M+H_2O]^+$, 294.0 $[M+2+H_2O]^+$, 295.0 $[M+Na]^+$, 297.0 $[M+2+Na]^+$, 313.1 $[M+K]^+$, 314.1 $[M+2+K]^+$; found: 275.0 $[M+H]^+$, 277.0 $[M+2+H]^+$, 292.0 $[M+H_2O]^+$, 294.0 $[M+2+H_2O]^+$, 297.0 $[M+Na]^+$, 299.0 $[M+2+Na]^+$, 313.0 $[M+K]^+$, 314.0 $[M+2+K]^+$.

***(3S,4S)-(E/Z)*-5-fluoro-2,3,4-trihydroxypentanal *O*-(3-(2-nitro-1*H*-imidazol-1-yl)propyl)oxime (13a):** A mixture of **1a** (7 mg, 0.038 mmol) and FDR (**9**) (6 mg, 0.039 mmol) in sodium acetate buffer (1 mL, 0.1 M, pH 4.6) was allowed to react at rt for 15 min, then it was diluted with ACN/H_2O (1/1, 4 mL) and purified by

RP-HPLC (Column: Phenomenex Luna C18(2) 250×10.00 mm, 5µm, 100 Å); mobile phase: A (H_2O), B (ACN); gradient: from 5% B to 30% B in 15 min; flow: 5 mL min^{-1}; t_R *Z* diastereoisomer: 11.6 min; t_R *E* diastereoisomer: 12.0 min). Pure fractions were combined and freeze dried to give compound **13a** as a mixture of *E*/*Z* isomers (4/1), as a yellow oil (8.6 mg, 71.0% yield). ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 7.52 (d, *J* = 1.0 Hz, 1 H_Z), 7.50 (d, *J* = 1.0 Hz, 1 H_E), 7.45 (d, *J* = 7.0 Hz, 1 H_E), 7.15-7.13 (m, 1H_E, 1 H_Z), 6.84 (d, *J* = 6.1 Hz, 1 H_Z), 5.02-4.98 (m, 1 H_Z), 4.67-4.56 (m, 3 H_E, 3 H_Z), 4.54-4.46 (m, 1H_E, 1 H_z), 4.37 (dd, J_1 = 7.0 Hz , J_2= 6.9, 1 H_E), 4.13-4.06 (m, 2H_E, 2 H_Z), 3.82-3.69 (m, 2H_E, 2 H_Z), 2.31-2.17(m, 2H_E, 2 H_Z). ^{13}C NMR (CD_3OD, 100MHz) δ (ppm) *E* diastereoisomer 152.0, 128.7, 128.5, 85.9 (d, J_{CF} = 169 Hz), 74.1 (d, J_{CF} = 7 Hz), 72.2 (d, J_{CF} = 19 Hz), 71.4, 71.2, 48.5, 30.9. *Z* diastereoisomer 153.7, 128.7, 128.5, 86.2 (d, J_{CF} = 169 Hz), 73.4 (d, J_{CF} = 7 Hz), 71.4 (d, J_{CF} = 19 Hz), 71.4, 71.3, 67.9, 48.5, 30.9. ^{19}F NMR (376 MHz, CD_3OD) δ (ppm) -235.5 (dt, *J* = 48.0 Hz, *J* = 22.7 Hz, *E* isomer), -236.0 (dt, J_1 = 48.4 Hz, J_2 = 23.3 Hz, *Z* isomer).

. MS (ESI *m/z)* calc. for $C_{11}H_{17}FN_4O_6$: 321.1 $[M+H]^+$, 343.1 $[M+Na]^+$; found: 321.1 $[M+H]^+$, 343.1 $[M+Na]^+$. HRMS: m/z $[M+H]^+$ calc. for $C_{11}H_{18}FN_4O_6$: 321.1204; found 321.1207.

***(3S,4S)-(E/Z)*-5-fluoro-2,3,4-trihydroxypentanal *O*-(5-(2-nitro-1*H*-imidazol-1-yl)pentyl) oxime (13b):** A mixture of **1b** (8.2 mg, 0.038 mmol) and FDR (**9**) (6 mg, 0.039 mmol) in sodium acetate buffer (1 mL, 0.1 M, pH 4.6) was allowed to react at rt for 15 min and then it was diluted with a solution of ACN/H_2O (3/1, 4 mL) and purified RP-HPLC (Column: Phenomenex Luna C18(2) 250×10.00 mm, 5µm, 100 Å); mobile phase: A (H_2O), B (ACN); gradient: from 10% B to 30% B in 15 min; flow: 5 mL min^{-1}; t_R *Z* diastereoisomer: 14.6 min; t_R *E* diastereoisomer: 15.0 min).Pure fractions were combined and freeze dried to give compound **13b** as a yellow oil (9.6 mg, 72.5% yield) as a mixture of *E*/*Z* isomers (4/1). ^{1}H NMR (CD_3OD, 400MHz) δ (ppm) 7.52-7.49 (m, 1 H_E, 1 H_Z), 7.45 (d, *J* = 7.1 Hz,1 H_E), 7.16-7.13 (m 1 H_E, 1 H_Z), 6.80 (d, *J* = 5.8 Hz, 1 H_Z), 4.99-4.95 (m, 1 H_Z), 4.69-4.56 (m, 1H_E, 1 H_Z), 4.55-4.44 (m, 3 H_E, 3 H_Z), 4.37 (dd, J_1 = 7.2 Hz, J_2= 7.1, 1 H_E), 4.13-4.02 (m, 2 H_E, 2 H_Z), 3.83-3.66 (m, 2 H_E, 2 H_Z), 1.96-1.84 (m, 2 H_E, 2 H_Z), 1.78-1.66 (m, 2 H_E, 2 H_Z), 1.52-1.40 (m, 2 H_E, 2 H_Z). ^{13}C NMR (CD_3OD, 100MHz) δ (ppm) *E* diastereoisomer 151.1, 128.5, 128.3, 85.8 (d, J_{CF}= 168 Hz), 74.3, 74.1 (d, J_{CF}= 7 Hz), 72.2 (d, J_{CF}= 19 Hz), 71.5, 51.1, 31.1, 29.5, 23.9. *Z* diastereoisomer 153.2, 128.5, 128.3, 85.8 (d, J_{CF}= 168 Hz), 74.6, 73.3 (d, J_{CF}= 7 Hz), 71.3 (d, J_{CF}= 19 Hz) , 68.3, 51.1, 31.1, 29.5, 23.9. ^{19}F NMR (376 MHz, CD_3OD), δ (ppm) -235.5 (dt, J_1 = 48.0 Hz, J_2 = 22.6 Hz, *E* isomer), -236.0 (dt, J_1 = 48.3 Hz, J_2 = 23.3 Hz, *Z* isomer).

MS (ESI *m/z)* calc. for $C_{13}H_{21}FN_4O_6$: 349.1 $[M+H]^+$, 371.1 $[M+Na]^+$, 387.2 $[M+K]^+$; found: 349.1 $[M+H]^+$, 371.1 $[M+Na]^+$, 387.1 $[M+K]^+$. HRMS: m/z $[M+H]^+$ calc. for $C_{13}H_{22}FN_4O_6$: 349.1523; found 349.1519.

(3S,4S)-(E/Z)-5-fluoro-2,3,4-trihydroxypentanal *O*-(7-(2-nitro-1*H*-imidazol-1-yl)heptyl) oxime (13c): A mixture of **1c** (9.2 mg, 0.038 mmol) and FDR (**9**) (6 mg, 0.039 mmol) in sodium acetate buffer (1 mL, 0.1 M, pH 4.6) was allowed to react at rt for 15 min and then it was diluted with ACN (4 mL) and purified by RP-HPLC (Column: Phenomenex Luna C18(2) 250×10.00 mm, 5µm, 100 Å); mobile phase: A (H_2O), B (ACN); gradient: from 20% B to 23% B in 37 min; flow: 5 mL min^{-1}; t_R *Z* diastereoisomer: 34.0 min; t_R *E* diastereoisomer: 37.0 min). Pure fractions were combined and freeze dried to give compound **13c** as a yellow oil (11.1 mg, 77.7% yield), as a mixture of *E*/*Z* isomers (4/1). ^{1}H NMR (CD_3CN, 400MHz) δ (ppm) 7.37 (d, *J* = 6.4 Hz, 1 H_E), 7.32-7.30 (m, 1 H_E, 1 H_Z), 7.09-7.07 (m, 1 H_E, 1 H_Z), 6.74 (d, *J* = 6.0 Hz, 1 H_Z), 4.88-4.82 (m, 1 H_Z), 4.63-4.51 (m, 1H_E, 1 H_Z), 4.51-4.40 (m, 1 H_E, 1 H_Z), 4.40-4.33 (m, 2 H_E, 2 H_Z), 4.29-4.23 (m, 1H_E), 4.10-3.95 (m, 2 H_E, 2 H_Z), 3.81-3.66 (m, 1 H_E, 1 H_Z), 3.66-3.55 (m, 2 H_E, 2 H_Z), 3.46 (br, 1 -*O*H_E, 1 -*O*H_Z), 3.36 (br, 2 -*O*H_E, 2 -*O*H_Z), 1.87-1.77 (m, 2 H_E, 2 H_z), 1.66-1.56 (m, 2 H_E, 2 H_Z), 1.40-1.25 (m, 6 H_E, 6 H_Z). ^{13}C NMR (CD_3OD, 100MHz) δ (ppm) *E* diastereoisomer 149.9, 134.9, 121.8, 118.9, 83.9 (d, J_{CF}= 169 Hz), 76.1, 72.1 (d, J_{CF}= 7 Hz), 70.3 (d, J_{CF}= 18 Hz), 69.3, 53.3, 20.0, 8.5 (2C). *Z* diastereoisomer 151.6, 134.9, 121.8, 118.9, 84.2 (d, J_{CF} = 167 Hz), 75.9, 71.2 (d, J_{CF}= 7 Hz), 69.6 (d, J_{CF}= 18 Hz), 65.6, 53.0, 20.0, 8.5 (2C). ^{19}F NMR (376 MHz, CD_3OD) −235.3 (td, J_1 = 47.9 Hz, J_2 = 23.4 Hz, *E* isomer), -235.7 (td, J_1 = 47.9 Hz, J_2 = 23.4 Hz, *Z* isomer), -76.9 (s, CF_3)^{19}F NMR (376 MHz, CD_3CN) δ (ppm) -233.9 (td, J_1 = 48.0 Hz, J_2 = 23.4 Hz, *E* isomer), -234.2 (td, J_1 =

48.0 Hz, J_2 = 23.4 Hz, *Z* isomer). MS (ESI *m/z)* calc. for $C_{15}H_{25}FN_4O_6$: 377.1 $[M+H]^+$, 399.1 $[M + Na]^+$; found: 377.1 $[M+H]^+$, 399.1 $[M + Na]^+$. HRMS: m/z $[M+H]^+$ calc. for $C_{15}H_{26}FN_4O_6$: 377.1836; found 347.1830.

***(3S,4S)-(E/Z)*-5-fluoro-2,3,4-trihydroxypentanal *O*-((1-((2-nitro-1*H*-imidazol-1yl)methyl)cyclopropyl) methyl)oxime (13d):** A mixture of **1d** (9.1 mg, 0.043 mmol) and FDR (**9**) (5 mg, 0.033 mmol) in sodium acetate buffer (1 mL, 0.1M, pH 4.6) was allowed to react at rt for 15 min, then it was diluted with ACN/H_2O (1/1, 4 mL) and purified by RP-HPLC (Column: Phenomenex Luna C18(2) 250×10.00 mm, 5µm, 100 Å); mobile phase: A (H_2O), B (ACN); gradient: from 10% B to 20% B in 20 min; flow: 5 mL min^{-1}; t_R E/Z mixture: 18.8 min).Pure fractions were combined and freeze dried to give compound **13d** as a mixture of *E*/*Z* isomers (4/1), as a yellow oil (8.5 mg, 74.5% yield). ^{1}H NMR (CD_3OD, 400MHz) δ (ppm) 7.61 (d, *J* = 1.0 Hz, 1 H_Z), 7.56 (d, *J* = 1.0 Hz, 1 H_E), 7.29 (d, *J* = 7.0 Hz, 1 H_E), 7.16 (d, *J* = 1.1 Hz, 1 H_Z), 7.13 (d, *J* = 1.1 Hz, 1 H_E), 6.76 (d, *J* = 6.1 Hz, 1 H_Z), 4.92-4.89 (m, 1 H_Z), 4.67-4.44 (m, 4 H_E, 4 H_Z), 4.29 (dd, J_1 = 7.0 Hz , J_2= 7.0 Hz, 1 H_E), 3.86 (s, 2 H_E,), 3.85 (s, 2 H_Z), 3.80-3.65 (m, 2 H_E, 2 H_Z), 0.83-0.77 (m, 2 H_E, 2 H_Z), 0.77-0.69 (m, 2 H_E, 2 H_Z). ^{13}C NMR (CD_3OD, 100MHz) δ (ppm) *E* diastereoisomer 151.8, 128.3 (2C), 85.9 (d, J_{CF}= 169 Hz), 79.3, 74.1 (d, J_{CF}= 7 Hz), 72.1 (d, J_{CF} = 18 Hz), 71.4, 55.1, 22.4, 10.5 (2C). *Z* diastereoisomer 153.5, 128.3 (2C), 85.7 (d, J_{CF} = 169 Hz), 79.0, 73.3 (d, J_{CF} = 7 Hz), 71.5, 67.9, 54.5, 22.4, 10.3 (2C). ^{19}F NMR (376 MHz, CD_3OD), δ (ppm) -235.6 (td, J_1 = 48.1 Hz, J_2 = 22.6 Hz, *E* isomer), -235.9 (td, J_1 = 48.4 Hz, J_2 = 23.5 Hz, *Z* isomer). MS (ESI *m/z)* calc. for $C_{13}H_{19}FN_4O_6$: 347.1 $[M+H]^+$,369.1 $[M+Na]^+$; found: 347.1 $[M+H]^+$, 369.1 $[M+Na]^+$. HRMS: m/z $[M+H]^+$ calc. for $C_{13}H_{20}FN_4O_6$: 347.1367; found 347.1363.

***(3S,4S)-(E/Z)*-5-fluoro-2,3,4-trihydroxypentanal *O*-(2-(4-((2-nitro-1*H*-imidazol-1-yl)methyl)-1*H*-1,2,3-triazol-1yl) ethyl) oxime (13e):** A mixture of **1e** (9.9 mg, 0.039 mmol) and FDR (**9**) (6 mg, 0.039 mmol) in sodium acetate buffer (1 mL, 0.1 M, pH 4.6) was allowed to react at rt for 15 min, then it was diluted with a solution of H_2O/ACN (7/1, 4 mL) and purified by RP-HPLC (Column: Phenomenex Luna C18(2) 250×10.00 mm, 5µm, 100 Å); mobile phase: A (H_2O), B (ACN); gradient: from 5% B to 8% B in 16 min and then from 8% B to 25% B in 11 min; flow: 5 mL min^{-1}; t_R E/Z mixture: 24.1 min). Pure fractions were combined and freeze dried to give compound **13e** as a yellow oil (10.6 mg, 70.3% yield), as a mixture of E/Z isomers (4/1). ^{1}H NMR (CD_3OD, 400MHz) δ (ppm) 8.10 (s, 1 H_Z), 8.05 (s, 1 H_E), 7.56 (d, *J* = 1.1 Hz, 1 H_E), 7.53 (d, *J* = 1.1 Hz, 1 H_Z), 7.46 (d, *J* = 7.0 Hz, 1 H_E), 7.18-7.15 (m, 1Hz, 1H_E), 6.83 (d, *J* = 6.0 Hz, 1 H_Z), 5.77 (s, 2 H_E, 2 H_Z), 4.89-4.86 (m, 1 H_Z), 4.73-4.56 (m, 3 H_E, 3 H_Z), 4.56-4.31 (m, 4 H_E, 3 H_Z), 3.85-3.63 (m, *2* H_E, 2 H_Z). ^{13}C NMR (CD_3OD, 100MHz) δ (ppm) *E* diastereoisomer 153.1, 143.0, 128.8, 128.3, 126.0, 85.9 (d, J_{CF}= 168 Hz), 74.0 (d, J_{CF}= 7 Hz), 72.6, 72.1 (d, J_{CF}= 18 Hz), 71.3, 50.8, 45.6. Z diastereoisomer 154.7, 143.0, 141.6, 128.8, 128.2, 126.3, 86.3 (d, J_{CF} = 169 Hz), 73.2, 73.1 (d, J_{CF} = 7 Hz), 71.1 (d, J_{CF}= 18 Hz), 68.2, 45.6. ^{19}F NMR (376 MHz, CD_3OD) δ (ppm) -235.4 (td, J_1 = 48.1 Hz, J_2 = 22.6 Hz, *E* isomer),-236.0 (td, J_1 = 48.4 Hz, J_2 = 23.5 Hz, *Z* isomer). MS (ESI *m/z)* calc. for $C_{13}H_{18}FN_7O_6$: 410.1 $[M+Na]^+$; found: 410.1 $[M+Na]^+$. HRMS: m/z $[M+H]^+$ calc. for $C_{13}H_{19}FN_7O_6$: 388.1381; found 388.1376.

***1-((1-(((((3S,4S)-(E/Z)*-5-fluoro-2,3,4-trihydroxypentylidene)amino)oxy)methyl)cyclopropyl)methyl)-1H-imidazol-1-ium 2,2,2-trifluoroacetate (13f):** A mixture of **1f** (5.9 mg, 0.035 mmol)and FDR (**9**) (5 mg, 0.033 mmol) in sodium acetate buffer (1 mL, 0.1M, pH 4.6) was allowed to react at rt for 15 min, then it was diluted with solution of H_2O/ACN (7/1, 4 mL) and purified by RP-HPLC (Column: Phenomenex Luna C18(2) 250×10.00 mm, 5µm, 100 Å; mobile phase: A (H_2O + 0.1% TFA), B (ACN + 0.1% TFA); gradient: from 5% B to 10% B in 18 min; flow: 5 mL min^{-1}; t_R E/Z mixture: 13.5 min). Pure fractions were combined and freeze dried to give compound **13f** as a clear oil (9.6 mg, 69.9% yield), as a mixture of E/Z isomers (4/ 1). ^{1}H NMR (CD_3OD, 400MHz) δ (ppm) 8.91 (s, 1 H_E), 8.86 (s, 1 H_Z), 7.58 (s, 1 H_E, 1 H_Z), 7.45 (b, 1 H_E, 1 H_Z), 7.30 (d, *J* = 6.9 Hz, 1 H_E), 6.70 (d, *J* = 6.2 Hz, 1 H_Z), 4.87-4.94 (m, 1 H_Z), 4.58-4.45 (m, 1 H_E, 1 H_Z), 4.45-4.33 (m, 1 H_E, 1 H_Z), 4.26 (dd, J_1 = 6.8 Hz, J_2= 6.7 Hz, 1H_E), 4.24-4.05 (m, 2H_E, 2 H_Z), 3.78-3.56 (m, 3 H_E, 3 H_Z), 0.82-0.73 (m, 2 H_E, 2 H_Z), 0.71-0.64 (m, 2H_E, 2 H_Z). ^{13}C NMR (CD_3OD, 100MHz) δ (ppm) *E* diastereoisomer 149.9, 134.9, 121.8, 118.9, 83.9 (d, J_{CF}= 169 Hz), 76.1, 7, 72.1 (d, J_{CF}= 7 Hz), 70.3 (d, J_{CF}= 18 Hz), 69.3, 53.3, 20.0, 8.5 (2C). *Z*

diastereoisomer 151.6, 134.9, 121.8, 118.9, 84.2 (d, J_{CF} = 167 Hz), 75.9, 71.2 (d, J_{CF}= 7 Hz), 69.6 (d, J_{CF}= 18 Hz), 65.6, 53.0, 20.0, 8.5 (2C). ^{19}F NMR (376 MHz, CD_3OD) −235.3 (td, J_1 = 47.9 Hz, J_2 = 23.4 Hz, *E* isomer), -235.7 (td, J_1 = 47.9 Hz, J_2 = 23.4 Hz, *Z* isomer), -76.9 (s, CF_3). MS (ESI *m/z)* calc. for $C_{13}H_{20}FN_3O_4$: 302.1 $[M+H]^+$, 324.1 [M+Na]; found: 302.1 $[M+H]^+$, 324.1 $[M+Na]^+$. HRMS: m/z $[M+H]^+$ calc. for $C_{13}H_{21}FN_3O_4$: 302.1561; found 302.1510.

5-deoxy-5-[^{18}F]fluoro-α/β,d-ribose ([^{18}F]FDR) (12): The automated radiosynthesis of [^{18}F]FDR (**12**) was accomplished and optimised using Eckert & Ziegler Eurotope Modular-Lab. After the EOB, [^{18}F]fluoride dissolved in heavy water ([^{18}O]H_2O) was driven by a flow of helium gas to the synthesis module contained in a lead shielded hot cell. Subsequently [^{18}F]fluoride solution passing through a CHROMAFIX anion exchange cartridge (Macherey Nagel, Germany) was retained by and the heavy water was collected in a proper vial. Then [^{18}F]fluoride was eluted into the reaction vessel with K_2CO_3 (0.5 mL of a 6 mg/mL water solution, 21.7 µmol) and a solution of Kriptofix 2.2.2 (16.2 mg, 43.0 µmol) in 1 mL ACN dry was added. The azeotropic mixture was evaporated heating to 90°C using a stream of helium to form the dried complex [K/K_{222}]^{18}F (the drying process was performed twice with 1 mL of ACN). Precursor **10** dissolved in 1 mL of ACN dry was added and the reaction mixture was heated at 100°C for 15 min. ACN was removed with a stream of helium, and the fluorinated intermediate compound formed was hydrolysed with 1 M HCl (0.9 mL) heating to 100°C for 10 min to give [^{18}F]FDR **12**. After cooling the solution was transferred onto a CHROMABOND IV purification cartridge (Macherey Nagel, Germany) and the cartridge was washed with water (3 mL). The [^{18}F]FDR **12** was obtained from a further elution of the cartridge with sterile water (10 mL) and collected into a vial placed in a second lead shielded hot cell. The radiochemical purity of **12** was > 90%, estimated by Radio TLC (mini-Gita) and Radio HPLC analysis (Column: Phenomenex Luna C18(2) 250×4.6 mm, 5µm, 100 Å; mobile phase: A (H_2O), B (ACN); gradient 0% B for 4 min and then from 0% to 60% in 10 min; flow: 1 mL min^{-1}; t_R: 4.0 min). The radiochemical purity was affected by the activity of the [^{18}F]fluoride unreacted and not retained by CHROMABOND IV purification cartridge. The radiochemical yield for **12** was 38.3% ± 7.0 decay corrected and calculated taking in consideration the percentage of activity due to the [^{18}F]fluoride contained in the mixture analysed (the value was obtained on average of five production runs). The bio-conjugation reactions via oxime bond formation were not affected by the presence of [^{18}F]fluoride, which was completely eliminated by RP-HPLC during the radiotracers purification. The amount of ribose potentially formed during the radiosynthesis, was not estimable by RP-HPLC analysis; eventual ribose conjugate formed was easily removed during RP-HPLC purification.

*General procedure for the synthesis of **15a-f:*** To a solution of **1a-f** (2.5 mg, 9.8-14.5 µmol) and [^{18}F]FDR (56-207 MBq) in 0.5-1.0 mL of H_2O) was added 10% (v/v) of a sodium acetate buffer solution (1 M, pH = 4.6). After 15-20 min the mixture was purified by RP-HPLC (Column: Phenomenex Luna C18(2) 250×10.00 mm, 5µm, 100 Å) using the condition optimised in cold. The collected fraction was diluted with water (5 times the volume collected) and loaded into a Waters Oasis® HLB Cartridge (conditioning 2 mL EtOH, 4 mL water). The cartridge was washed with 20 mL of water (x2) and the desired product was collected eluting with 1-2 mL of EtOH. Decay corrected radiochemical yield calculated using the amount of [^{18}F]FDR as starting activity. This non-optimised procedure allowed the recovery of > 80% of the loaded activity. Radio HPLC analysis of the formulated product showed >98% radiochemical and chemical purity. Identity of the products was confirmed by co-injection with the non-radioactive reference (Supporting information).

Acknowledgements

M.M. thanks SULSA for a PhD studentship. We gratefully acknowledge financial support from the EPSRC (grant EP/I034793/1).

Keywords: Hypoxia • Radiochemistry • Positron Emission Tomography • FDR • Cancer

[1] J. M. Brown, W. R. Wilson, *Nat. Rev. Cancer* **2004**, *4*, 437–447.
[2] P. Lee, N. S. Chandel, M. C. Simon, *Nat. Rev. Mol. Cell Biol.* **2020**, *21*, 268–283.

[3] Q. Ke, M. Costa, *Mol. Pharmacol.* **2006**, *70*, 1469–1480.
[4] H. Harada, *J. Radiat. Res.* **2011**, *52*, 545–556.
[5] I. N. Fleming, R. Manavaki, P. J. Blower, C. West, K. J. Williams, A. L. Harris, J. Domarkas, S. Lord, C. Baldry, F. J. Gilbert, *Br J Cancer* **2015**, *112*, 238–250.
[6] H. M. Swartz, A. B. Flood, P. E. Schaner, H. Halpern, B. B. Williams, B. W. Pogue, B. Gallez, P. Vaupel, *Physiol. Rep.* **2020**, *8*, e14541.
[7] K. A. Krohn, J. M. Link, R. P. Mason, *J. Nucl. Med.* **2008**, *49*, 129S – 148.
[8] O. J. Kelada, D. J. Carlson, *Radiat. Res.* **2014**, *181*, 335–349.
[9] G. Smith, L. Carroll, E. O. Aboagye, *Mol. Imaging. Biol.* **2012**, *14*, 653–666.
[10] E. Lopci, I. Grassi, A. Chiti, C. Nanni, G. Cicoria, L. Toschi, C. Fonti, F. Lodi, S. Mattioli, S. Fanti, *Am. J. Nucl. Med. Mol. Imaging* **2014**, *4*, 365–384.
[11] H. Nagasawa, Y. Uto, K. L. Kirk, H. Hori, *Biol. Pharm. Bull.* **2006**, *29*, 2335–2342.
[12] G. Mees, R. Dierckx, C. Vangestel, C. Wiele, *Eur. J. Nucl. Med. Mol. Imaging* **2009**, *36*, 1674–1686.
[13] S. Carlin, J. L. Humm, *J. Nucl. Med.* **2012**, *53*, 1171–1174.
[14] P. W. Miller, N. J. Long, R. Vilar, A. D. Gee, *Angew. Chem., Int. Ed.* **2008**, *47*, 8998–9033.
[15] X. Deng, J. Rong, L. Wang, N. Vasdev, L. Zhang, L. Josephson, S. H. Liang, *Angew. Chem., Int. Ed.* **2019**, *58*, 2580–2605.
[16] X.-G. Li, S. Dall'Angelo, L. F. Schweiger, M. Zanda, D. O'Hagan, *Chem. Commun.* **2012**, *48*, 5247–5249.
[17] X.-G. Li, M. Haaparanta, O. Solin, *J. Fluorine Chem.* **2012**, *143*, 49–56.
[18] S. Dall'Angelo, Q. Zhang, I. N. Fleming, M. Piras, L. F. Schweiger, D. O'Hagan, M. Zanda, *Org. Biomol. Chem.* **2013**, *11*, 4551–4558.
[19] X.-G. Li, K. Helariutta, A. Roivainen, S. Jalkanen, J. Knuuti, A. J. Airaksinen, *Nat. Protoc.* **2013**, *9*, 138–145.
[20] S. Dall'Angelo, N. Bandaranayaka, A. D. Windhorst, D. J. Vugts, D. van der Born, M. Onega, L. F. Schweiger, M. Zanda, D. O'Hagan, *Nucl. Med. Biol.* **2013**, *40*, 464–470.
[21] M. Namavari, Z. Cheng, R. Zhang, A. De, J. Levi, J. K. Hoerner, S. S. Yaghoubi, F. A. Syud, S. S. Gambhir, *Bioconjugate Chem.* **2009**, *20*, 432–436.
[22] O. Keinänen, D. Partelová, O. Alanen, M. Antopolsky, M. Sarparanta, A. J. Airaksinen, *Nucl. Med. Biol.* **2018**, *67*, 27–35.
[23] R. Haubner, S. Maschauer, O. Prante, *BioMed Res. Int.* **2014**, *2014*, 1–16.
[24] L. Bauer, K. S. Suresh, *J. Org. Chem.* **1963**, *28*, 1604–1608.
[25] R. J. Abdel-Jalil, M. Aqarbeh, D. Löffler, B. Shen, S. A. Orabi, W. Voelter, H.-J. Machulla, *J. Radioanal. Nucl. Chem.* **2010**, *283*, 239–243.
[26] R. Bejot, L. Carroll, K. Bhakoo, J. Declerck, V. Gouverneur, *Bioorg. Med. Chem.* **2012**, *20*, 324–329.
[27] M. Musolino, S. Dall'Angelo, M. Zanda, *SynOpen* **2017**, *01*, 0173–0179.
[28] P. Kumar, D. Stypinski, H. Xia, A. J. B. McEwan, H. Machulla, L. I. Wiebe, *J. Labelled Compd. Radiopharm.* **1999**, *42*, 3–16.
[29] S. Altomonte, G. L. Baillie, R. A. Ross, J. Riley, M. Zanda, *RSC Adv.* **2014**, *4*, 20164–20176.
[30] G. E. Adams, M. S. Cooke, *Int. J. Radiat. Biol. Relat. Stud. Phys., Chem. Med.* **1969**, *15*, 457–471.
[31] P. Kumar, S. Emami, Z. Kresolek, J. Yang, A. J. B. McEwan, L. I. Wiebe, *Med. Chem.* **2009**, *5*, 118–129.
[32] T. A. D. Smith, M. Zanda, I. N. Fleming, *Nucl. Med. Biol.* **2013**, *40*, 858–864.
[33] M. Busk, M. R. Horsman, S. Jakobsen, J. Bussink, A. van der Kogel, J. Overgaard, *Eur. J. Nucl. Med. Mol. Imaging* **2008**, *35*, 2294–2303.
[34] F. Ito, S. Ando, M. Iuchi, T. Ukari, M. Takasaki, K. Yamaguchi, *Tetrahedron* **2011**, *67*, 8009–8013.
[35] R. Yoda, Y. Matsushima, *Chem. Pharm. Bull.* **1994**, *42*, 1935–1937.
[36] F. Tang, Y. Yang, Y. Tang, S. Tang, L. Yang, B. Sun, B. Jiang, J. Dong, H. Liu, M. Huang, M.-Y. Geng, W. Huang, *Org. Biomol. Chem.* **2016**, *14*, 9501–9518.