Synthesis and Radiosynthesis of Prospective 2-Nitroimidazole Hypoxia PET Tracers via Thiazolidine Ligation with 5-Fluorodeoxy-ribose (FDR)

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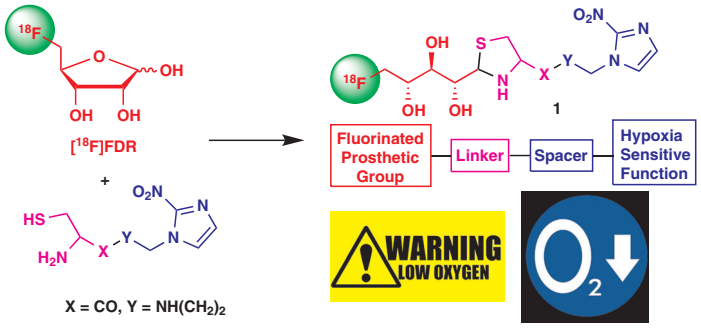

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Abstract The first prospective fluorinated PET tracers for imaging hypoxia obtained via thiazolidine-ligation are reported. Three 1,2-thiol-amine linkers were combined with four different 2-nitroimidazole spacers via amide or urea bond formation. The resulting compounds were submitted to thiazolidine-ring-forming ligation reaction with the fluorinated carbohydrate L-5-fluoro-5-deoxy-ribose (FDR), affording the desired candidate PET tracers in variable yields. The same ligation reactions performed on L-ribose – a by-product of [^{18}F]FDR radiosynthesis – under conditions mimicking a radiochemical production showed that the fluorinated adducts can be efficiently purified and isolated by HPLC. Finally, one of the prospective hypoxia tracers was successfully produced in radiolabelled form in 29.2% radiochemical yield from [^{18}F]FDR.

Key words hypoxia, radiofluorination, thiazolidines, nitroimidazole, bio-orthogonal ligation

Hypoxia occurs in cells and tissues when oxygen demand exceeds supply.[1,2] The irregular vasculature typical of solid tumours does not sufficiently support cellular oxygen demand, leading to the development and progression of heterogeneous hypoxic cancer areas, which are generally poorly responsive to chemo- and radio-therapies.[3,4] Accurate imaging of hypoxic regions could allow clinicians to stratify patients and develop more efficient treatment strategies to improve therapeutic outcomes.[5,6] Identification and quantification of hypoxic areas in tumours – and in other pathologies – is therefore important for planning the most appropriate and personalised therapeutic approach.[7,8] Owing to its high sensitivity and non-invasive nature, PET imaging is emerging as the method of choice for in vivo identification, characterization and discrimination of hypoxic areas.[6,9] In the last two decades, several PET tracers for hypoxia have been described, but all of them are affected by significant drawbacks, such as low signal-to-noise ratios, slow accumulation in hypoxic regions and poor brain uptake, therefore the development of new hypoxia-targeted PET tracers remains a very active area of research.[10,11] L-5-Fluoro-5-deoxy-ribose ([^{18}F]FDR) **2** (Figure 1) has recently emerged as a promising prosthetic group for rapid, indirect radiolabelling of bioactive molecules via oxime bond formation.[12–15]

As an alternative to the oxime bond, thiazolidine ring formation could be used as a site-specific ligation method via reaction of a 1,2-thiol-amine function with a carbonyl group – including masked carbonyls of carbohydrates and hemiacetals – in mildly acidic or basic conditions (pH 4 to 8).[16–18] Importantly, the thiazolidine ring is generally stable in a wide pH range (from 4 to 10), thus representing an attractive linkage option. In order to further investigate the efficiency of [^{18}F]FDR as a radiolabelling agent and expand the library of prospective PET tracers for hypoxia imaging, we designed a novel class of candidate tracers [^{18}F]**1** (Figure 1) taking advantage of the last-step formation of a thiazolidine ring linkage between [^{18}F]FDR **2** and terminal 2-amino-thiols **3** carrying a hypoxia-reactive 2-nitroimidazole group.

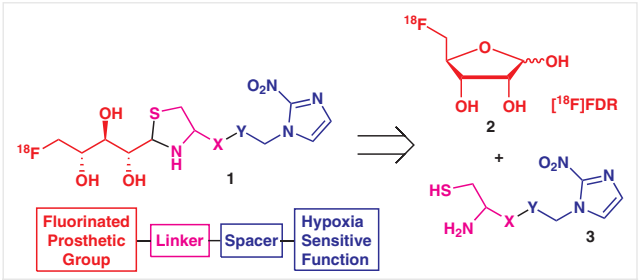
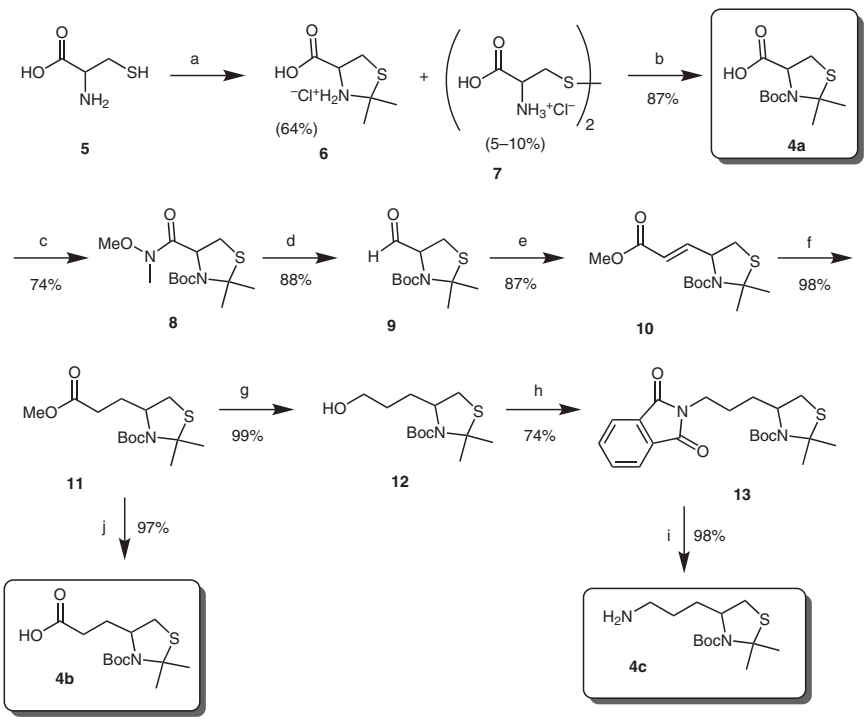


Figure 1 Novel hypoxia PET tracers via thiazolidine ligation with [^{18}F]FDR

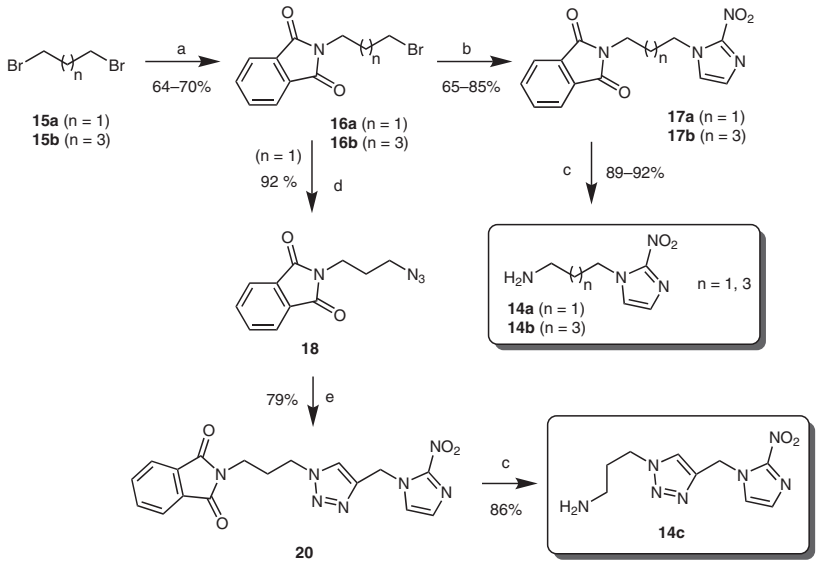
Three different 2-aminoethanethiol linkers **4a–c** (Scheme 1) were selected to modulate the steric constraints and lipophilicity of the final candidate tracers. The synthesis was based on the conditions described by Duthaler et al.[19] A mixture of racemic cysteine **5** and conc. HCl in acetone was heated at reflux for 6 h, affording the thiazolidine intermediate **6**. Samples of **6** were invariably found (by ^{1}H NMR spectroscopy) to contain 5–10% of cysteine hydrochloride **7**. The mixture of **6** and **7** was allowed to react with $(Boc)_2O$ in pyridine for 3 days to give the *N*-Boc-derivative **4a**.[19] NMR spectroscopy showed that this compound exists as a mixture of rotamers, the signals of which did not show coalescence at 60 °C either in $CDCl_3$ or in CD_3OD. Compound **4a** was converted into the Weinreb amide **8** by reaction with HATU and DIPEA, followed by addition of *N*,*O*-dimethylhydroxylamine hydrochloride. Reduction of **8** using $LiAlH_4$ at 0 °C provided in good yield the aldehyde **9**, which was submitted to Wittig reaction with the phosphonium ylide $Ph_3P{=}CHCO_2Me$ to give exclusively the *trans* isomer of the α,β-unsaturated ester **10**.[20] Hydrogenation reaction of **10** using H_2 over Pd/C catalyst gave in quantitative yield the saturated intermediate **11**, which afforded the free carboxylic acid **4b**[21] by basic hydrolysis of the ester function. The carbinol **12** was obtained upon treatment of **11** with $LiAlH_4$ at 0 °C, whereas the amine derivative **4c**[22] was obtained via Mitsunobu reaction of phthalimide with **12** to give compound **13**, followed by phthalimide-ring cleavage with hydrazine monohydrate.

The 2-nitro-imidazole spacers **14a–d** (Scheme 2 and Scheme 3) were selected with the aim of introducing structural diversity within the series. The structure of the spacer was expected to have an important effect on lipophilicity, metabolic stability and ultimately on the imaging potential of the candidate tracers **1**.

Amines **14a**,**b** were synthesised via Gabriel reaction (Scheme 2) starting respectively from commercial 1,3-dibromopropane (**15a**) and 1,5-dibromopentane (**15b**). The resulting phthalimides **16a**,**b**[23] were reacted with 2-nitroimidazole and K_2CO_3 in DMF upon heating to 115 °C to give compounds **17a**,**b**[24] in good yields. The desired amines **14a**,**b** were obtained by quantitative cleavage of the phthalimido group with hydrazine monohydrate. The 1,2,3-triazole-amine **14c** was prepared via Huisgen cycloaddition reaction between the azide **18**, which was obtained by bromine displacement reaction of **16a** with sodium azide,[25] and 1-propargyl-2-nitroimidazole **19**, which was prepared according to the literature,[26] to afford phthalimide derivative **20**. Removal of the phthalimido group with hydrazine gave compound **14c** in good overall yield.

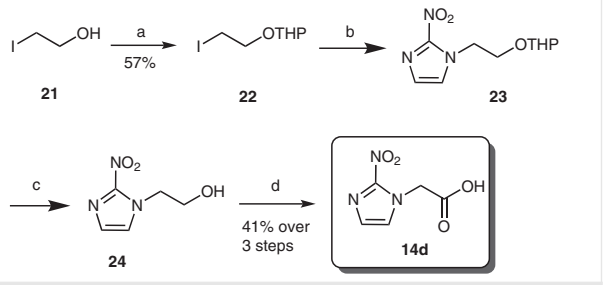


Scheme 1 Synthesis of thiazolidine linkers **4a–c**. *Reagents and conditions*: (a) acetone, conc. HCl, reflux, 6 h; (b) Boc_2O, pyridine, N_2 atm., r.t., 72 h; (c) *N*,*O*-Dimethylhydroxylamine hydrochloride, DIPEA, CH_2Cl_2, HATU, from 0 °C to r.t., 18 h; (d) $LiAlH_4$, Et_2O, N_2 atm., 0 °C, 15 min; (e) $Ph_3P{=}CHCO_2Me$, THF, reflux, 18 h; (f) Pd/C 10wt. %, H_2 atm., MeOH, r.t., 24 h; (g) $LiAlH_4$, THF, N_2 atm., 0 °C, 1 h; (h) Phthalimide, PPh_3, diisopropyl azodicarboxylate (DIAD), THF, r.t., 16 h; (i) $NH_2NH_2{\cdot}H_2O$, reflux, 3 h; (j) LiOH, THF, r.t., 18 h.



Scheme 2 Synthesis of 2-nitroimidazole spacers **14a–c**. *Reagents and conditions*: (a) Phthalimide, TEA, DMF, r.t., 48 h; (b) 2-Nitroimidazole, K_2CO_3, DMF, 110–120 °C, 3–5 h; (c) $NH_2NH_2{\cdot}H_2O$, EtOH, 60–100 °C, 1–4 h; (d) NaN_3, DMF, 120 °C, 4 h; (e) 1-Propargyl-2-nitroimidazole **19**, $CuSO_4$, sodium ascorbate, *t*-BuOH/H_2O, r.t., 20 h.

2-Nitro-imidazolyl-acetic acid **14d**[27] (Scheme 3) was prepared in four steps starting from **21**, which provided compound **22** after protection of the hydroxy group as tetrahydropyranyl acetal (THP) followed by introduction of the 2-nitroimidazole function in K_2CO_3 and DMF upon heating to 115 °C. The resulting intermediate **23** was then dissolved in a 6 M aq. HCl solution in MeOH to cleave the THP group, followed by treatment of the resulting carbinol **24** with Jones reagent (CrO_3/H_2SO_4/acetone) in acetone to give the desired compound **14d** in 41% yield over the three steps.



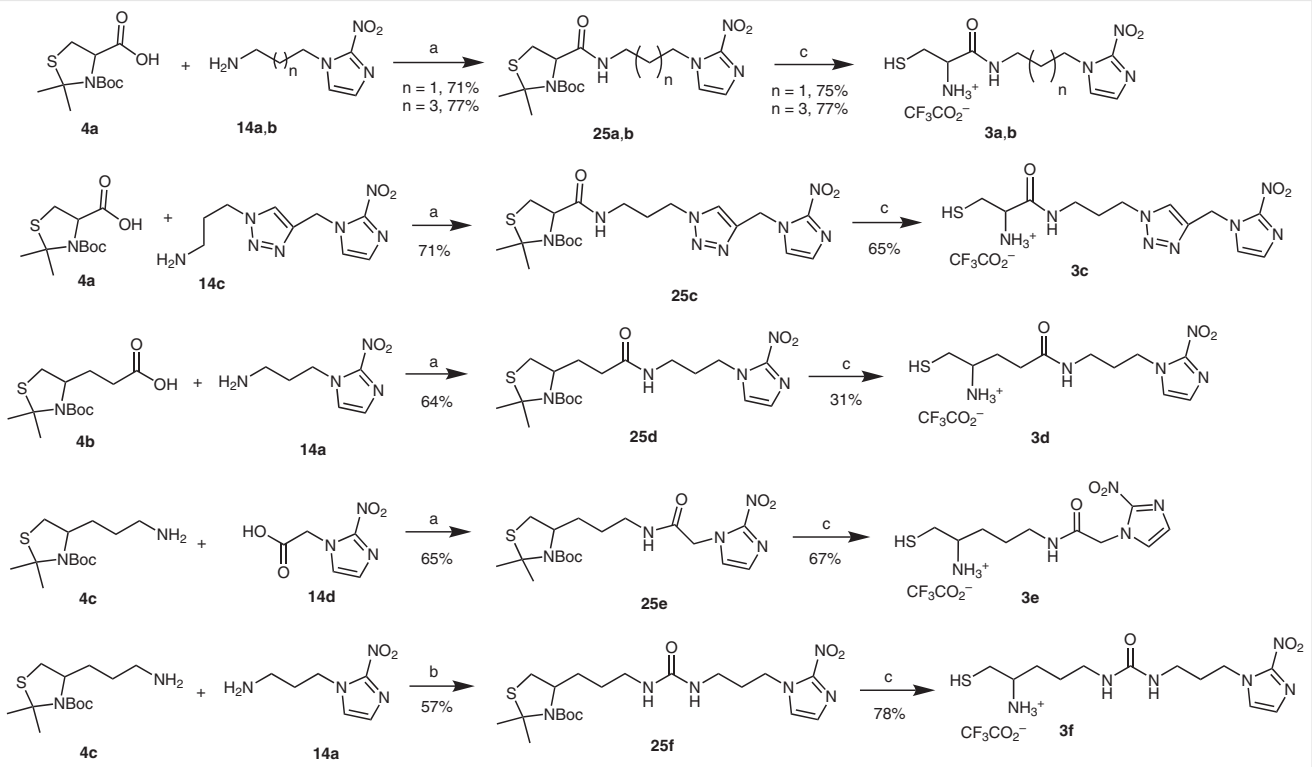
Scheme 3 Synthesis of 2-nitroimidazole spacer **14d**. *Reagents and conditions*: (a) DHP, PPTS, CH_2Cl_2, r.t., 18 h; (b) 2-Nitroimidazole, K_2CO_3, DMF, 115 °C, 5 h; (c) 6 M HCl, MeOH, r.t., 18 h; (d) CrO_3/H_2SO_4/acetone, r.t., 12 h.

Assembling of linkers **4a–c** and spacers **14a–d** to give the tracers' precursors **3a–f** is shown in Scheme 4. Treatment of carboxylic acid derivatives **4a–c** with HATU and DIPEA gave the corresponding activated esters, which were reacted *in situ* with the amines **14a–d** to afford the amides **25a–e**.[28] Different conditions were used to prepare the urea derivative **25f**.[29] In this case, the amine **4c** was added dropwise to a solution of carbonyldiimidazole (CDI) in CH_2Cl_2 at 0 °C to give the intermediate imidazocarboxyamide, which gave the desired urea **25f** upon *in situ* treatment with the amine **14a**.

The final unprotected 2-aminoethanethiol derivatives **3a–f**[30] (Scheme 4) were obtained by treatment of **25a–f** with a TFA/H_2O/MeOH 3:2:1 mixture upon heating to 65 °C for 2–4 h, followed by solvents removal under reduced pressure at 60 °C. Then the crude compounds were dissolved in ethanol (except compound **3c**, which is only soluble in aqueous solutions) and eluted through a Silia*Bond*® carbonate pad (silica bound equivalent of tetramethylammonium carbonate), which trapped residual TFA, acid by-products and free-based 2-nitroimidazolium trifluoroacetate salts formed during the thiazolidine hydrolysis.

In all cases, variable amounts of disulphide dimers were obtained in mixture with the desired thiol monomers **3a–f**, as evidenced by both HPLC/MS analysis and NMR spectroscopy. However, we did not attempt to purify further the samples, as the disulphide dimers could be readily reduced back to the monomeric thiols by treatment with 1,4-dithiothreitol (DTT) before the following ligation reaction with FDR **2** (Scheme 5).

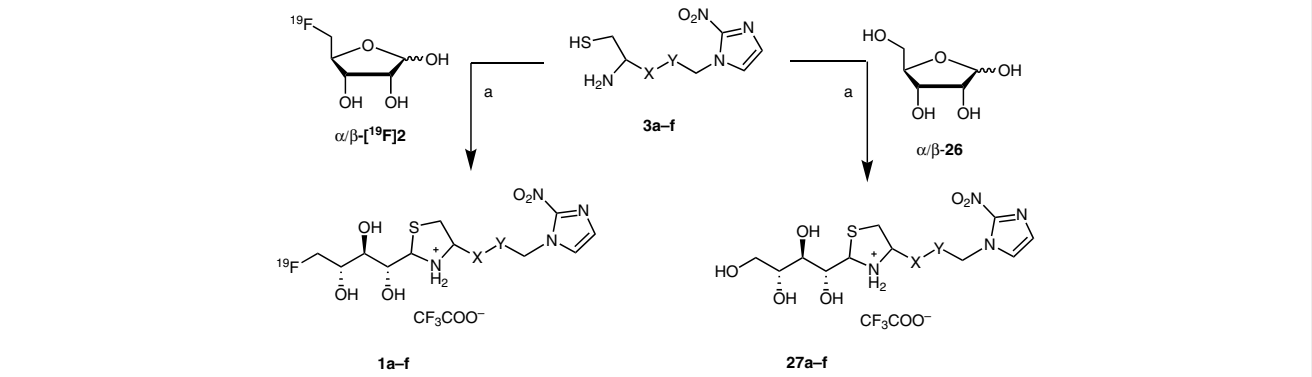
The thiazolidine ring formation was performed by reaction of **3a–f** with cold [^{19}F]FDR **2** using 1 M acetate buffer as reaction medium in the presence of DTT. Acetate buffers with different molarity (from 0.1 to 4.0) and pH (from 3 to 6) were tested at different temperatures (from r.t. to 50 °C) in order to optimise the thiazolidine ring formation rate. The optimised conditions were 2.5 equiv of **3a–f** reacted with 1 equiv of [^{19}F]FDR(**2**) in the presence of 2.5 equiv of DTT, using 1 M acetate buffer at pH 4.5 as reaction medium,



Scheme 4 Synthesis of the 2-amino-thiol tracer precursors **3a–f**. *Reagents and conditions*: (a) HATU, DIPEA, CH_2Cl_2, r.t., 18 h; (b) CDI, CH_2Cl_2, from 0 °C to r.t., 18 h; (c) TFA/H_2O/MeOH 3:2:1, from r.t. to 65 °C, 2–4 h.

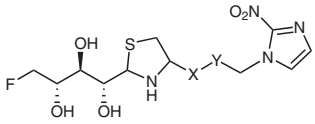
for 20 min at 30 °C. The purification step was performed by gradient RP-HPLC using a mixture of H_2O/ACN + 0.05% (v/v) of TFA as eluent. 1,2-Aminothiol derivatives **3a–f** showed markedly different reactivity towards [^{19}F]FDR **2**, showing that the spacers' structure plays an important role in the cyclisation reaction (see Table 1 for yields). Only the 1,2-thiol-amine derivative **3c**, incorporating a triazole ring, failed to react under all the conditions explored, affording in very low yields (<5%) the corresponding thiazolidine **1c**, which could not be isolated in pure form by RP-HPLC purification.

The radiosynthesis of [^{18}F]FDR **2** is known to produce an excess of L-ribose **26** as by-product,[12] which, although less reactive than **2**, will compete with it in the thiazolidine ring formation, affording the corresponding non-fluorinated thiazolidines **27a–f** (Scheme 5) and decreasing the chemical purity of the tracer.



Scheme 5 (a) 1 M acetate buffer CH_3COOH/CH_3COONa, pH 4.5, r.t., 20 min

Table 1 Synthesis of Cold Tracers [^{19}F]**1a–f**



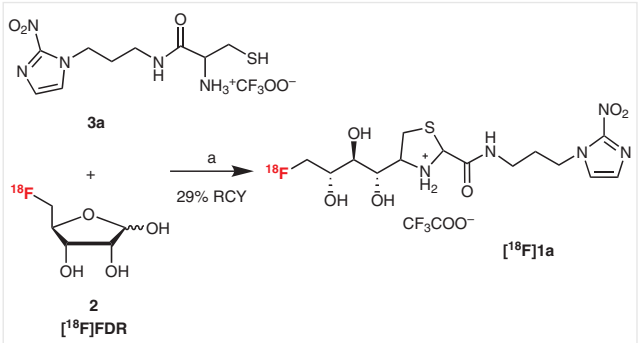
Compound	X	Y	Yield (%)	t_{R-F} (min)	t_{R-OH} (min)[a]	log *P* (±SED)
1a	CO	$NH(CH_2)_2$	61.3	12.5	8.3	0.33 (±0.04)
1b	CO	$NH(CH_2)_4$	67.9	27.3, 28.4	20.0	0.64 (±0.04)
1d	$(CH_2)_2CO$	$NH(CH_2)_2$	11.2	22.4–23.4	20.3	0.68 (±0.04)
1e	$(CH_2)_3NH$	CO	31.8	15.6–18.4	10.0–11.5	0.26 (±0.04)
1f	$(CH_2)_3NH$	$CONH(CH_2)_3$	42.3	27.7–29.4	22.0–23.5	0.50 (±0.02)

[a] Retention time of the corresponding L-ribose analogue **26**.

To simulate the radiosynthesis conditions, the thiazolidine ring formation reaction was carried out in the presence of 10 equiv of **26** along with 1 equiv of [^{19}F]FDR **2** and 1,2-aminothiol derivatives **3a–f**. This experiment was performed with the aim of assessing the formation of the desired FDR thiazolidines in the presence of L-ribose **26** and the possibility of performing an HPLC purification for separating the [^{18}F]FDR-derived tracers **1a–f** from the non-radioactive L-ribose-derived thiazolidines **27a–f**. As shown in Table 1, as well as in the HPLC profiles (see the Supporting Information), the retention times of the target FDR-thiazolidines **1a–f** are indeed significantly different to those of the ribose-derived thiazolidines **27a–f**.

Therefore, the final cold tracers [^{19}F]**1a–f** could be isolated and characterised by LC-MS. Their Log *P* values were determined by RP-HPLC (isocratic phase H_2O/EtOH 90:10). Considering that the gold standard hypoxia tracer [^{18}F]FMISO has a Log *P* = 0.42, candidate tracers **1** appear to have suitable lipophilicity for use in vivo. Thiazolidines **1a–f** presented very complex NMR spectra owing to the presence of four diastereomers, originated by the two (*R*/*S*) thiazolidine stereogenic centres, plus different rotamers and trifluoroacetate salts. Only compound **1a**[31] was isolated in sufficient quantity for being satisfactorily characterised by NMR spectroscopy, after treatment with Silia*Bond*® carbonate in order to freebase the trifluoroacetate salts.

Radiolabelling tests for producing [^{18}F]**1a**[32] were conducted on the 1,2-aminothiol derivative **3a**, which was treated with [^{18}F]FDR (**2**)[12,13] using a sodium acetate buffer solution (Scheme 6). Also in this case, different reaction conditions were tested with the aim of achieving the maximum radiochemical conversion within 40 minutes (see Table 1S, Supporting Information).

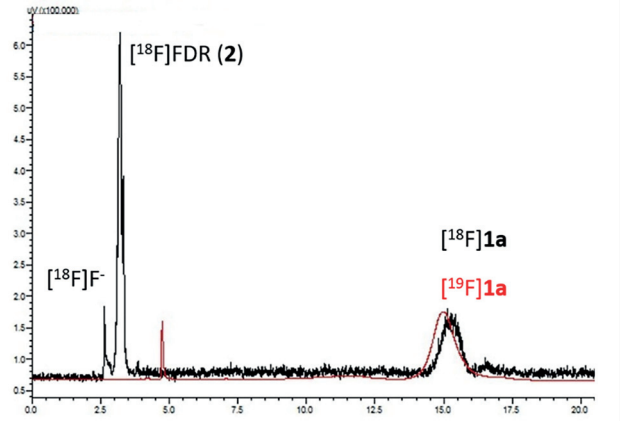


Scheme 6 Radiosynthesis of [^{18}F]**1a**. *Reagents and conditions*: (a) 6 M acetate buffer, pH 4.5, r.t., 30 min.

Eventually, we found that the use of a 6 M acetate buffer solution (70% v/v concentration) in the reaction mixture containing [^{18}F]FDR (**2**), **3a** and DTT (1:1) (in the range 2–4 M) at pH 4.5, provided the highest RCY (29.2%, decay corrected). No further improvements could be achieved by changing buffer concentration, pH or extending further the reaction time.

The tracer identity was confirmed by superimposition of the UV-HPLC profile of the cold reference [^{19}F]**1a** with the semi-preparative RP-HPLC radio-chromatogram of [^{18}F]**1a**, acquired before purification of the radiotracer (Figure 2).

In conclusion, we have designed and synthesised the first candidate PET tracers (**1**) for hypoxia imaging based on the use of [^{18}F]FDR **2** as radiolabelling agent. The synthesis is based on the formation of a thiazolidine-ring-linkage between [^{18}F]FDR **2** and 1,2-thiol-amines **3**, which occurs with moderate to good efficiency depending on the structure of spacer and linker featured in **3**. The method was successfully tested for the radiosynthesis of [^{18}F]**1a**, which was produced in 29.2% radiochemical yield and successfully purified by RP-HPLC.



Compounds	Peak Rt [min]	Area [%]
[18F]FDR (**2**)	3.194	51.0
[18F]**1a**	15.138, 16.522	37.9; 6.2
[19F]**1a**	14.877	

Figure 2 In black: semi-prep RP-HPLC Radio-analysis of radiotracer [^{18}F]**1a** formation using 70% v/v of a 6 M acetate buffer solution in the aqueous solution of [^{18}F]FDR (**2**). In red: superimposed UV chromatogram of the cold reference [^{19}F]**1a** obtained using the same RP-HPLC conditions.

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Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/s-0036-1591863.

References and Notes

(1) Brown, J. M.; Wilson, W. R. *Nat. Rev. Cancer* **2004**, *4*, 437.
(2) Höckel, M.; Vaupel, P. *JNCI J. Natl. Cancer Inst.* **2001**, *93*, 266.
(3) Ke, Q.; Costa, M. *Mol. Pharmacol.* **2006**, *70*, 1469.
(4) Muz, B.; de la Puente, P.; Azab, F.; Azab, A. K. *Hypoxia* **2015**, 83.
(5) Harada, H. *J. Radiat. Res. (Tokyo)* **2011**, *52*, 545.
(6) Padhani, A. R.; Krohn, K. A.; Lewis, J. S.; Alber, M. *Eur. Radiol.* **2007**, *17*, 861.
(7) Wigerup, C.; Påhlman, S.; Bexell, D. *Pharmacol. Ther. (Supplement C)* **2016**, *164*, 152.
(8) Horsman, M. R.; Mortensen, L. S.; Petersen, J. B.; Busk, M.; Overgaard, J. *Nat. Rev. Clin. Oncol.* **2012**, *9*, 674.
(9) Carlin, S.; Humm, J. L. *J. Nucl. Med.* **2012**, *53*, 1171.
(10) Lopci, E.; Grassi, I.; Chiti, A.; Nanni, C.; Cicoria, G.; Toschi, L.; Fonti, C.; Lodi, F.; Mattioli, S.; Fanti, S. *Am. J. Nucl. Med. Mol. Imaging* **2014**, *4*, 365.
(11) Peeters, S. G. J. A.; Zegers, C. M. L.; Lieuwes, N. G.; van Elmpt, W.; Eriksson, J.; van Dongen, G. A. M. S.; Dubois, L.; Lambin, P. *Int. J. Radiat. Oncol.* **2015**, *91*, 351.
(12) Li, X.-G.; Dall'Angelo, S.; Schweiger, L. F.; Zanda, M.; O'Hagan, D. *Chem. Commun.* **2012**, *48*, 5247.
(13) Dall'Angelo, S.; Zhang, Q.; Fleming, I. N.; Piras, M.; Schweiger, L. F.; O'Hagan, D.; Zanda, M. *Org. Biomol. Chem.* **2013**, *11*, 4551.
(14) Keinänen, O.; Li, X.-G.; Chenna, N. K.; Lumen, D.; Ott, J.; Molthoff, C. F.; Sarparanta, M.; Helariutta, K.; Vuorinen, T.; Windhorst, A. D.; Airaksinen, A. J. *ACS Med. Chem. Lett.* **2015**, *7*, 62.
(15) Li, X.-G.; Helariutta, K.; Roivainen, A.; Jalkanen, S.; Knuuti, J.; Airaksinen, A. J. *Nat. Protoc.* **2013**, *9*, 138.
(16) Forget, D.; Boturyn, D.; Defrancq, E.; Lhomme, J.; Dumy, P. *Chem. Eur. J.* **2001**, *7*, 3976.
(17) Zhang, L.; Tam, J. P. *Anal. Biochem.* **1996**, *233*, 87.
(18) Liu, C.-F.; Tam, J. P. *J. Am. Chem. Soc.* **1994**, *116*, 4149.
(19) Duthaler, R. O.; Wyss, B. *Eur. J. Org. Chem.* **2011**, *24*, 7419.
(20) O'Connell, C. E.; Ackermann, K.; Rowell, C. A.; Garcia, A. M.; Lewis, M. D.; Schwartz, C. E. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2095.
(21) **Synthesis of 4b**: An aqueous 1 M LiOH solution (1.64 mL, 1.64 mmol) was added at r.t. to a solution of **11** (200 mg, 0.66 mmol) in THF (1.7 mL). The reaction mixture was stirred for 18 h at r.t. and then neutralised with a 1 M aq. HCl solution, then extracted with EtOAc (3 × 2 mL), dried and concentrated under reduced pressure to give **4b** (186 mg, 97.4%) as an oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 9.07 (br, 1 H), 4.35 (br, 1 H), 3.12 (dd, *J* = 11.9, 5.9 Hz, 1 H), 2.58 (d, *J* = 11.9 Hz, 1 H), 2.39–2.19 (m, 2 H), 2.12–1.93 (m, 2 H), 1.72 (s, 6 H), 1.45 (s, 9 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 178.9, 152.7, 80.7, 69.6, 63.7, 51.2, 32.3, 31.3, 29.6 (2C), 28.4 (3C) MS (ESI): *m*/*z* calcd for $C_{13}H_{23}NO_4S$: 290.2 $[M+H]^+$, 312.1 $[M+Na]^+$; found: 290.2 $[M+H]^+$, 312.1 $[M+Na]^+$
(22) **Synthesis of 4c**: Hydrazine monohydrate (124 µL, 2.52 mmol) was added to a solution of **13** (340 mg, 0.84 mmol) in EtOH (5 mL) and the reaction mixture was heated at reflux for 3 h. After cooling to 0 °C the resulting white precipitate was filtered off and the filtrate was concentrated under reduced pressure. The residue was dissolved with Et_2O (5 mL) and the resulting white precipitate was filtered, then the filtrate was concentrated under reduced pressure to afford **4c** (448 mg, 98.4%) as a yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.16 (br, 1 H), 3.00 (dd, *J* = 11.6, 6.1 Hz, 1 H), 2.65–2.54 (m, 2 H), 2.47 (d, *J* = 11.6 Hz, 1 H), 1.73–1.49 (m, 4 H), 1.60 (s, 6 H), 1.44–1.41 (m, 2 H), 1.33 (s, 9 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 152.3, 79.8, 69.4, 64.1, 41.8, 31.3, 30.9, 30.2, 29.6 (2C), 28.4 (3C). MS (ESI): *m*/*z* calcd for $C_{13}H_{26}N_2O_2S$: 275.1 $[M+H]^+$, 297.2 $[M+Na]^+$ 303.2 $[M+K]^+$; found: 275.2 $[M+H]^+$, 297.2 $[M+Na]^+$, 303.1 $[M+K]^+$
(23) Böhmer, V.; Dozol, J.-F.; Grüttner, C.; Liger, K.; Matthews, S. E.; Rudershausen, S.; Saadioui, M.; Wang, P. *Org. Biomol. Chem.* **2004**, *2*, 2327.
(24) Hay, M. P.; Wilson, W. R.; Moselen, J. W.; Palmer, B. D.; Denny, W. A. *J. Med. Chem.* **1994**, *37*, 381.
(25) Ebran, J.-P.; Dendane, N.; Melnyk, O. *Org. Lett.* **2011**, *13*, 4336.
(26) Bejot, R.; Carroll, L.; Bhakoo, K.; Declerck, J.; Gouverneur, V. *Bioorg. Med. Chem.* **2012**, *20*, 324.
(27) A shorter synthesis of **14d** has been reported, see: Joyard Y., Azzouz R., Bischoff L., Papamicaël C., Labar D., Bol A., Bol V., Vera P., Grégoire V., Levacher V., Bohn P.; *Bioorg. Med. Chem.*; **2013**,

21: 3680; and references therein. However, imidazolyl carbinol **24** was already being used in our labs for a related project, therefore it was used as an intermediate for **14d**

(28) **Synthesis of 25a**: DIPEA (156 µL, 0.92 mmol) and HATU (350 mg, 0.92 mmol) were added to a solution of **4a** (200 mg, 0.77 mmol) in anhydrous CH_2Cl_2 (10 mL) at 0 °C and the mixture was allowed to react at r.t. for 1 h. Then the amino derivative **14a** (260 mg, 1.53 mmol) dissolved in CH_2Cl_2 (2 mL) was added to the mixture. After 16 h under stirring, the mixture was washed with a 0.5 M aq. NaOH solution (3 × 6 mL) and then with a 0.1 M aq. HCl solution (3 × 6 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was purified by flash chromatography (Hex/EtOAc, from 8:2 to 7:3) to afford **25a** (229 mg, 72.3%) as a yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz, mixture of rotamers): δ = 7.32 (s, 1 H), 7.08 (s, 1 H), 6.55 (br, 1 H), 4.72 (br, 1 H), 3.42–3.11 (m, 4 H), 1.82 (s, 3 H), 1.73 (s, 3 H), 1.42 (s, 9 H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.7, 153.3, 144.7, 128.4, 127.0, 81.8, 71.4, 67.5, 47.4, 36.0, 31.0, 29.3, 28.9, 28.4 (3C). MS (ESI): *m*/*z* calcd for $C_{17}H_{27}N_5O_5S$: 436.2 $[M+Na]^+$, 452.0 $[M+K]^+$; found: 436.1 $[M+Na]^+$, 452.0 $[M+K]^+$

(29) **Synthesis of 25f**: A solution of **4c** (152 mg, 0.56 mmol) in CH_2Cl_2 (2 mL) was added dropwise to a solution of CDI (90 mg, 0.56 mmol) in anhydrous CH_2Cl_2 (3 mL), at 0 °C under N_2 atmosphere, then the mixture was allowed to react at r.t. for 1 h. After 16 h under stirring, the mixture was added via syringe to a solution of **14a** (226 mg, 1.33 mmol) in CH_2Cl_2 (3 mL) under N_2 atmosphere. After 16 h under stirring the mixture was concentrated under recued pressure. Purification by FC on silica gel (Hex/EtOAc, from 3:7 to 7:3) gave **25f** (148 mg, 56.7%) as a yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz, 2 rotamers): δ = 7.35 (br, 1 H), 7.02 (br, 1 H), 5.55 (br, 2 H), 4.40 (t, *J* = 6.9 Hz, 2 H), 4.19 (br, 1 H), 3.24–2.94 (m, 5 H), 2.49 (d, *J* = 11.8 Hz, 1 H), 2.05–1.86 (m, 2 H), 1.81–1.66 (m, 2 H), 1.63 (s, 3 H), 1.61 (s, 3 H), 1.48–1.26 (m, 11 H). ^{13}C NMR ($CDCl_3$, 100 MHz, 2 rotamers): δ = 159.0, 152.7, 144.6, 128.2, 127.1, 80.3, 69.4, 64.0, 47.8, 39.8, 36.6, 31.6, 31.4, 30.6, 30.1, 29.6, 28.4 (3C), 27.3. MS (ESI): *m*/*z* calcd for $C_{20}H_{34}N_6O_5S$: 471.2 $[M+H]^+$, 493.2 $[M+Na]^+$; found: 471.2 $[M+H]^+$, 493.2 $[M+Na]^+$

(30) **Synthesis of 3a**: Compound **25a** was dissolved in a TFA/H_2O/MeOH 3:2:1 mixture and heated to 65 °C for 2 h. Solvents were then concentrated under reduced pressure at 60 °C, then the residue was dissolved in ethanol and passed through a Silia*Bond*® carbonate pad to give the crude **3a** as trifluoroacetate salt (55.7 mg, 74.8%). The compound was used in the next reaction without any further purification. ^{1}H NMR (CD_3OD, 400 MHz, in mixture with the dimer): δ = 7.62–7.54 (m, 1 H), 7.20–7.15 (m, 1 H), 4.60–4.42 (m, 2 H), 4.31–4.19 (m, 1 H), 3.52–3.33 (m, 2 H), 3.24–3.09 (m, 2 H), 2.19–2.00 (m, 2 H). ^{13}C NMR (CD_3OD, 100 MHz, in mixture with the dimer): δ = 167.4, 144.6, 127.4, 127.2, 51.7, 47.4, 37.8, 36.3, 29.7. MS (ESI): *m*/*z* calcd for $C_9H_{15}N_5O_3S$: 274.1 $[M+H]^+$, 296.1 $[M+Na]^+$; found: 274.0 $[M+H]^+$, 296.0 $[M+Na]^+$

(31) **Synthesis of 1a**: [^{19}F]FDR ([^{19}F]**2**) (5 mg, 0.033 mmol) was added to a solution of **3a** (32.0 mg, 0.083 mmol) and DTT (12.8 mg, 0.083 mmol) in a 1 M sodium acetate buffer solution (pH 4.5), then the mixture was allowed to react at 30 °C for 20 min. Purification by RP-HPLC (Column: Phenomenex Luna C18 250 × 10.00 mm, 5 µm; mobile phase: A (H_2O + 0.05% TFA), B (ACN + 0.05% TFA); gradient: from 5% B to 6% B in 15 min; flow: 5 mL min^{-1}; t_R: 12.5 min) gave **1a** as trifluoroacetate salt (10.6 mg, 61.3 %). NMR analyses were performed after treatment of **1a** with Silia*Bond*® carbonate (10% w/w) in EtOH, under gentle stirring for 1 h in order to freebase trifluoroacetate salt. ^{1}H NMR (CD_3OD, 400 MHz, – four diastereoisomers – two major isomer in ~3:2 ratio were identified): δ = 7.55 (d, *J* = 1.2 Hz, 1 H), 7.16 (d, *J* = 1.2 Hz, 1 H), 4.89–4.83 (m, 1 H), 4.62–4.42 (m, 4 H), 4.25 (dd, *J* = 7.0, 6.8 Hz, 1 H), 4.09–4.04 (m, 1 H), 3.91 (dd, *J* = 7.4, 4.6 Hz, 1 H), 3.66 (dd, *J* = 7.4, 5.8 Hz, 1 H), 3.39–3.23 (m, 3 H), 3.02–2.91 (m, 1 H), 2.16–2.05 (m, 2 H); δ (second isomer) = 7.57 (d, *J* = 1.2 Hz, 1 H), 7.17 (d, *J* = 1.2 Hz, 1 H), 4.92 (d, *J* = 2.6 Hz, 1 H), 4.67 (dd, *J* = 9.8, 3.0 Hz, 1 H), 4.63–4.42 (m, 3 H), 4.25 (dd, *J* = 7.0, 6.8 Hz, 1 H), 4.04–3.95 (m, 1 H), 3.85–3.75 (m, 2 H), 3.39–3.14 (m, 3 H), 3.02–2.91 (m, 1 H), 2.16–2.05 (m, 2 H). ^{13}C NMR (CD_3OD, 100 MHz, – four diastereoisomers – two major isomers in ~3:2 ratio were identified): δ (first isomer) = 172.5, 144.7, 127.2, 127.1, 84.1 (d, J_{CF} = 167 Hz), 73.6 (d, J_{CF} = 7 Hz), 72.3, 72.1, 71.8 (d, J_{CF} = 18 Hz), 71.4, 65.8, 35.8, 34.9, 30.0; δ (second isomer) = 172.4, 144.7, 127.2, 127.1, 84.3 (d, J_{CF} = 167 Hz), 73.6 (d, J_{CF} = 7 Hz), 72.3, 72.0, 71.9, 71.9 (d, J_{CF} = 18 Hz), 70.2, 66.2, 36.6, 34.9. 29.9. ^{19}F NMR (376 MHz, CD_3OD): δ (first isomer) = –233.0 (dt, *J* = 48.0, 22.7 Hz); δ (second isomer) = –233.6 (dt, J_1 = 48.0 Hz, J_2 = 22.3 Hz); MS (ESI): *m*/*z* calcd for: $C_{14}H_{22}FN_5O_6S$: 408.1 $[M+H]^+$, 430.1 $[M+Na]^+$; found: 408.0 $[M+H]^+$, 430.0 $[M+Na]^+$

(32) **Optimised radiosynthesis of [^{18}F]1a**: A solution of sodium acetate buffer (6 M, pH 4.5) was added to a solution of **3a** (2.5 mg, 6.4 µmol), DTT (1 mg, 6.4 µmol) and [^{18}F]FDR ([^{18}F]**2**) (2.5–10 MBq) in 0.5–1.0 mL of H_2O to form a 70% v/v sodium acetate buffer solution (final concentration 4.2 M). After ~20 min the mixture was purified by RP-HPLC (Column: Phenomenex Luna C18 250 × 10.00 mm, 5 µm) to give [^{18}F]**1a** in 29% RCY (decay corrected)