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Improved synthesis of the hypoxia probe 5-deutero-5-fluoro-5-deoxy-azomycin arabinoside (FAZA) as a model process for tritium radiolabeling

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ABSTRACT

Tritium-labelled fluoroazomycin arabinoside, [^{3}H]-FAZA, is a useful probe for the investigation of hypoxia, furthermore it is safer and easier to handle than the PET tracer [^{18}F]-FAZA when used in cell based assays. The only known synthesis of deuterium- and tritium-labelled FAZA was re-investigated and optimized. Then, a new and improved synthesis of [^{2}H]-FAZA was developed as a model process for tritium radiolabelling. This novel synthesis is expected to greatly facilitate access to [^{3}H]-FAZA.

1. Introduction

Most tumour types feature hypoxic (low oxygen) regions, and in some neoplastic pathology hypoxia may be present in up to 60% of patients [1]. Generally, tumours with high hypoxic volumes have a poor prognosis because they are associated with an aggressive phenotype and increased risk of metastasis [2]. Furthermore these tumours tend to respond poorly to radiotherapy and/or chemotherapy [3]. Accordingly, tumour hypoxia is a key driver of tumour growth, proliferation, maintenance and resistance to therapy. For the reasons above, a robust and reliable method to identify hypoxia in tumours would have significant value as a predictive biomarker to identify patients and tumours likely to be non-responsive to chemotherapy and radiotherapy. PET imaging is a particularly attractive option to study hypoxia, as it is non-invasive, quantitative, does not require biopsy tissue, and can provide information across the entire tumour [4]. In the last decades, several PET and SPECT hypoxia markers have been developed, many of which are based on the use of nitroimidazole derivatives as first proposed by Chapman in 1979 [5]. Nitroimidazole tracers are able to detect tumour hypoxia since they accumulate in hypoxic tissues by a bio reductive linkage mechanism. The nitroso and hydroxylamino-imidazole metabolites produced in hypoxic tissues covalently bind to cellular molecules therefore trace quantities of labelled compounds are chemically bound to hypoxic cells [6].

Among the fluorinated hypoxia PET tracers, [^{18}F]-FAZA, (fluoroazomycin arabinoside **1**, Fig. 1) developed by Kumar in 1998 [7], was found to be useful for imaging hypoxia in various tumours such as glioblastoma, with a remarkably high tumour-to-background and it can be considered the "gold standard" for the measurement of tissue hypoxia [8].

Although ^{18}F-FAZA is nowadays produced in many clinical PET facilities and the ^{18}F radiosynthesis has been widely investigated, optimized, and automated [9], the deuterium (**2**) and tritium labelled (**3**) parent compounds are not commercially available. [^{3}H]-FAZA is an attractive molecular tool for *in vitro* studies and comparison of different and novel hypoxia tracers. Moreover it is safer and much easier to handle than [^{18}F]-FAZA when used in cell based assays. Last but not least, [^{3}H]-FAZA can be conveniently stored for many years (tritium half-life is 12.32 years).

Here we report our studies towards the synthesis of the deuterium labelled [^{2}H]-FAZA **2**, which was obtained by two different synthetic strategies, as a model process for [^{3}H]-FAZA synthesis. The first route is based on a modification of the Kumar method [7], the only one reported to date for the synthesis of **2** and **3**, which unfortunately in our hands could not be reproduced as originally described by the authors. The second is an optimized alternative route to **2** based on the use of different protective

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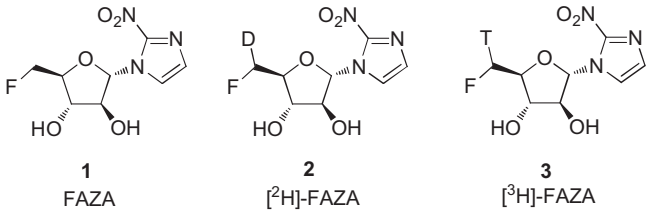


Fig. 1. FAZA and its labelled derivatives.

groups, which should represent a more efficient access to [^{3}H]-FAZA **3**.

2. Results and discussion

2.1. Synthesis of FAZA *1*

Our synthesis of FAZA (**1**) is based on the coupling of 2-nitro-1-(triethylsilyl)imidazole **4** with the protected arabinose derivative **5** (Scheme 1), according to the strategy published by Schweifer and Hammerschmidt [10]. The key intermediate **6** was then converted into FAZA (**1**) following the pathway proposed by Kumar and co-workers [7].

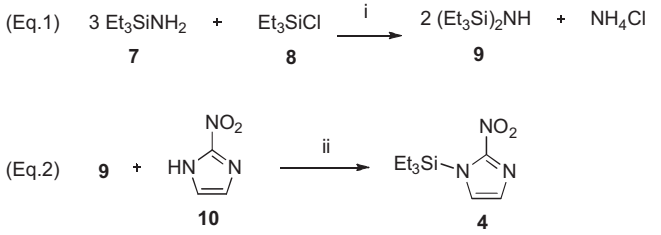
Both starting substrates **4** (Scheme 2) and **5** (Scheme 3) had to be prepared. Hexaethyldisilazane **9** (Scheme 2, Eq. 1) was prepared from (triethylsilyl)amine **7** and chlorotriethylsilane **8** in the presence of a catalytic amount of triethylsilyl triflate in dry toluene. A refluxing mixture of 2-nitroimidazole (**10**) and hexaethyldisilazane **9** in pyridine afforded quantitatively the desired substrate **4** (Scheme 2, Eq. 2) [10].

The synthesis of FAZA (**1**) (Scheme 3) started from D-arabinose (**11**) which was fully protected obtaining a mixture of anomeric acetates **5** (α:β = 60:40) [10]. The coupling reaction between 2-nitro-1-(triethylsilyl)imidazole **4** and the anomers **5** afforded the nitroimidazole D-arabinofuranose derivative **6** that was subjected to selective deprotection of the primary hydroxyl group in order to obtain the compound **12** [10]. The subsequent deoxyfluorination was achieved using diethylaminosulfur trifluoride (DAST) in DCM, which gave the protected fluorinated precursor **13** that was then fully deprotected with ammonia in methanol giving FAZA (**1**) [7].

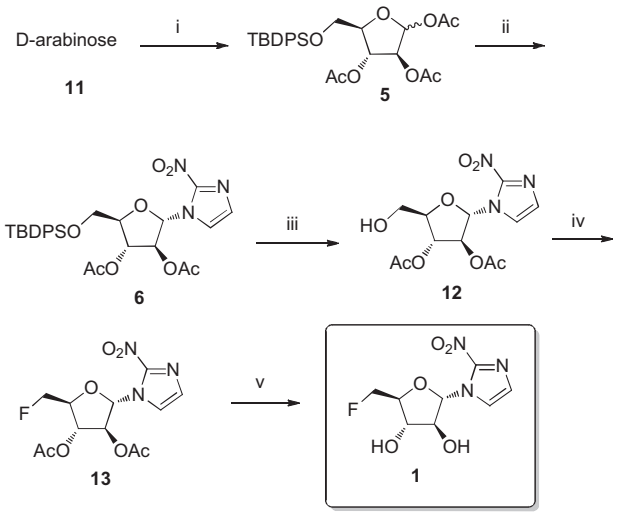
2.2. Synthesis of [^{2}H]-FAZA *2*

Once the synthesis of FAZA **1** was optimized, we switched to the synthesis of the deuterated analogue **2**, initially following the procedure reported by Kumar and co-workers [7]. The retro-synthetic approach (Scheme 4) involved the oxidation of the alcohol **12** to the corresponding aldehyde **14a**, followed by reduction with a deuterating agent to provide the deuterated alcohol **15a**. Subsequently, using the same reactions used for the synthesis of FAZA, we planned to obtain our target molecule **2**.

However, in our hands the oxidation step from **12** to **14a** (Scheme 5) could not be reproduced following the conditions (Pfitzner–Moffatt oxidation) described by Kumar et al. [7]. It is worth noting that the Kumar's protocol involved the treatment of **12** "dissolved in anhydrous dimethyl sulfoxide" with DCC followed



Scheme 2. *Reagent and conditions*: (i) TfOTES, toluene (79%) and (ii) Pyridine, reflux (quantit.).



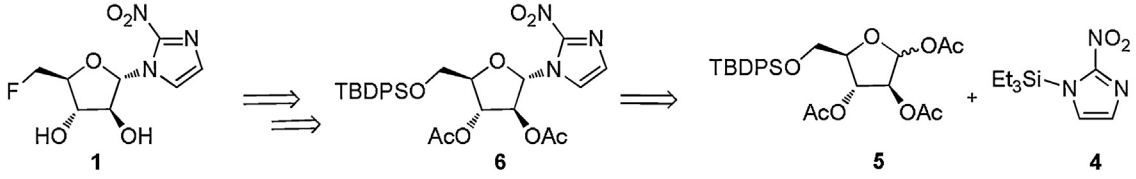
Scheme 3. Synthesis of FAZA. *Reagent and conditions*: (i) (1) TBDPSCl, Py, −20 °C; (2) Ac_2O, rt (71% over 2 steps); (ii) 4, TfOTES, CH_3CN, −8 °C (72%); (iii) KF, benzoic acid, CH_3CN, reflux (80%); and (iv) DAST, DCM, rt (50%); v: 2 N NH_3 in MeOH, 4 °C (60%).

by cooling on an ice bath, which not surprisingly in our hands resulted in a solid mixture and no reaction. Upon heating until melting of the mixture, we observed the formation of a complex mixture of unidentified products, in which we could not identify the desired aldehyde **14a**.

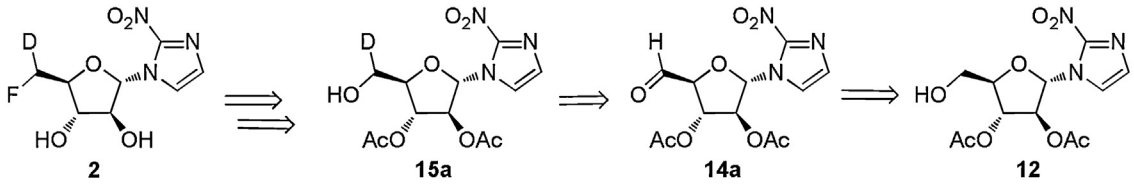
Stimulated by the need of a more effective and reliable procedure, we first modified the conditions of the Pfitzner–Moffatt oxidation as shown in Scheme 6 and Table 1, entry 1. We obtained the desired aldehyde **14a** but also a consistent quantity of the by-product **14b**, arising from elimination of the acetate in position 3 of the arabinose ring. Unfortunately these two aldehydes could be separated neither by flash chromatography nor by HPLC.

At that point we investigated different oxidation conditions with the aim of suppressing the formation of the by-product **14b** (Table 1, entries 2–6). Eventually, the best conditions we were able to identify afforded a more acceptable 30% of the undesired aldehyde **14b** (entry 3).

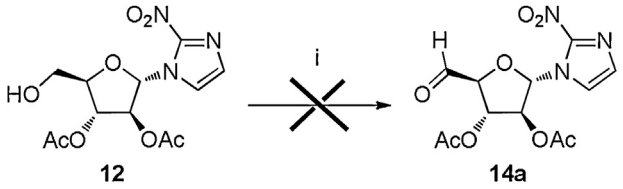
The inseparable mixture of the two aldehydes **14a** and **14b** (Scheme 7) was then reduced with deuterated sodium cyanoborohydride ($NaBD_3CN$) affording the two deuterated alcohols **15a** and



Scheme 1. Retro-synthesis of FAZA **1**.



Scheme 4. Retro-synthesis of [^{2}H]-FAZA **2**.



Scheme 5. Attempted oxidation of **12–14a** under "Kumar conditions." *Reagent and conditions*: (i) DMSO, DCC, Cl_2CHCO_2H, oxalic acid, DCM.

15b. An HPLC separation provided **15a** that was deoxyfluorinated with DAST and deprotected with NH_3/MeOH giving [^{2}H]-FAZA **2**.

The preparation of 3-deoxy-3,4-didehydro-5-aldehydes such as **14b** is a known transformation in carbohydrate chemistry, but it generally involves the treatment of 5-formyl-furanoses having an *anti*-stereochemistry at carbons 2,3 (ribose and arabinose derivatives) with organic bases such as TEA or DABCO [11], and does not usually occur in the absence of any basic treatment.

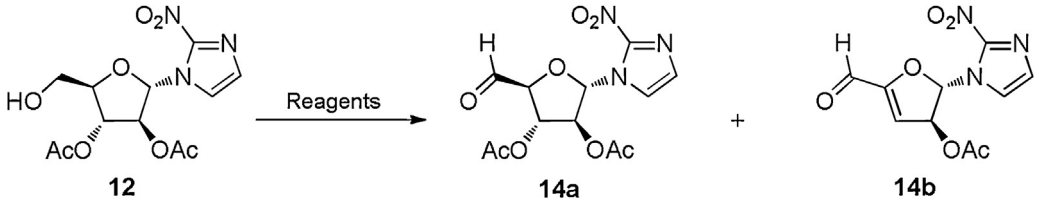
In order to better understand the mechanism involved in the spontaneous formation of the elimination by-product, we performed an NMR study (Fig. 2) monitoring the Pfitzner–Moffatt oxidation reaction (DMSO-d_6, EDC, Cl_2CHCO_2H in DMSO-d_6/benzene-d_6 1:1). We recorded a series of NMR spectra starting from 0 °C and progressively increasing the temperature to 25 °C. In order to monitor the formation of the two aldehydes **14a,b** we focussed our attention on the corresponding CHO protons, which have diagnostic low field chemical shifts (9.87 ppm for **14a** and 9.81 ppm for **14b** in DMSO-d_6).

At 0 °C, 5 °C, 10 °C, 15° and 20 °C the reaction did not proceed as it can be seen by the absence of any CHO signal in spectra 1–5, Fig. 2. When the temperature was increased to 25 °C the aldehyde proton of **14a** (signal at 9.87 ppm) started to become visible, meaning that just the desired product was forming (spectra 6–12, Fig. 2). After 3 h the reaction was quenched by addition of deuterated water. The NMR spectrum was recorded immediately afterwards (spectra 13 Fig. 2), showing the presence of two aldehyde signals (4:6 ratio), the new signal corresponding to the **14b** aldehyde proton at 9.81 ppm. This NMR study therefore demonstrates that the elimination by-product **14b** is formed exclusively during the reaction quenching.

Based on the evidence above, we investigated the reaction quenching by testing a number of different conditions after performing the oxidation under the optimized conditions of Table 1, entry 3. Direct evaporation at reduced pressure without any quenching unexpectedly afforded **14b** as the sole product. Filtration on silica gel or Celite® without quenching afforded a **14a**:**14b** mixture in 1:9 ratio. Addition of NH_4Cl instead of water afforded **14a**:**14b** in 7:3 ratio, whereas the use of even mildly basic water solutions produced partial hydrolysis of the acetyl groups. Finally, changing the organic solvent used for the extraction from EtOAc to Et_2O or DCM had no tangible effect.

The synthesis above is affected by a number of drawbacks, therefore we decided to investigate an alternative and more convenient route to [^{2}H]-FAZA **2**. To overcome the elimination of the 3-β-acetoxy group, which is a good leaving group, we focussed our attention on the synthesis of the 2,3 *O*-PMB protected arabinoside. In fact, we reasoned that elimination of a benzyloxy group should be considerably less favoured than that of an acetoxy group, therefore the elimination side-reaction could be suppressed or limited. Unfortunately attempts to install the *O*-PMB protection on deacetylated **6** (Scheme 3) failed when NaH and PMBCl or *p*-methoxybenzyl trichloroacetimidate in combination with PPTS, PTSA or BF_3 were used.

A different strategy based on a 3-PMB protected acetate donor was therefore investigated (Scheme 8). Starting from the readily

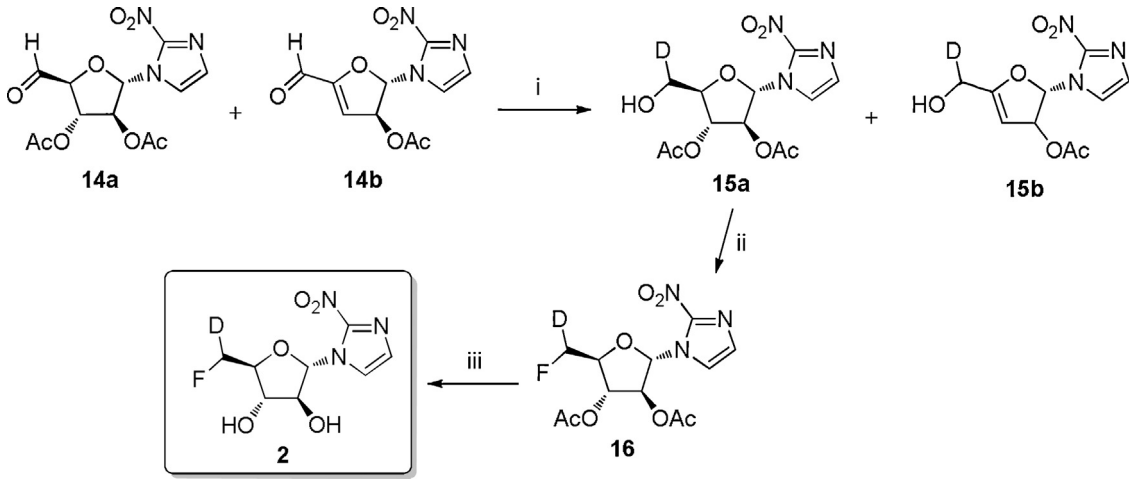


Scheme 6. Screening of conditions for the Pfitzner-Moffatt oxidation of **12–14a,b**.

Table 1
Screening of conditions for the Pfitzner-Moffatt oxidation of **12–14a,b**.

Entry	Reagents	Solvents and conditions[a]	Ratio **14a:14b**	Yields
1	DMSO, EDC, Cl_2CHCO_2H	DMSO/benzene (1:1), 0–25 °C	40:60	70
2	DMSO, EDC, Py·TFA	DMSO/benzene (1:1), 0 °C	No product	None
3	DMSO, EDC, Py·TFA	DMSO/benzene (1:1), 0–25 °C	70:30	75
4	DMSO, $(COCl)_2$, Et_3N	DCM, −78 °C	0:100	80
5	TEMPO, TCC	DCM, 0 °C to rt	No product	None
6	DMP, Py	DCM, 0 °C to rt	No product	None
7	TPAP, NMO	DCM rt	No product	None

[a] Reactions quenched with water. Key: EDC = N-(3-dimethylaminopropyl)-N-ethylcarbodiimide; TEMPO = 2,2,6,6-Tetramethyl-1-piperidinyloxy; TCC = Trichloroisocyanuric Acid; DMP = Dess-Martin periodinane.



Scheme 7. Completion of the "Kumar-inspired" synthesis of [^{2}H]-FAZA **2**. *Reagent and conditions*: (i) $NaBD_3CN$, EtOH (70%); (ii) DAST, DCM, rt (45%); and (iii) 2 N NH_3 in MeOH, 4 °C (55%).

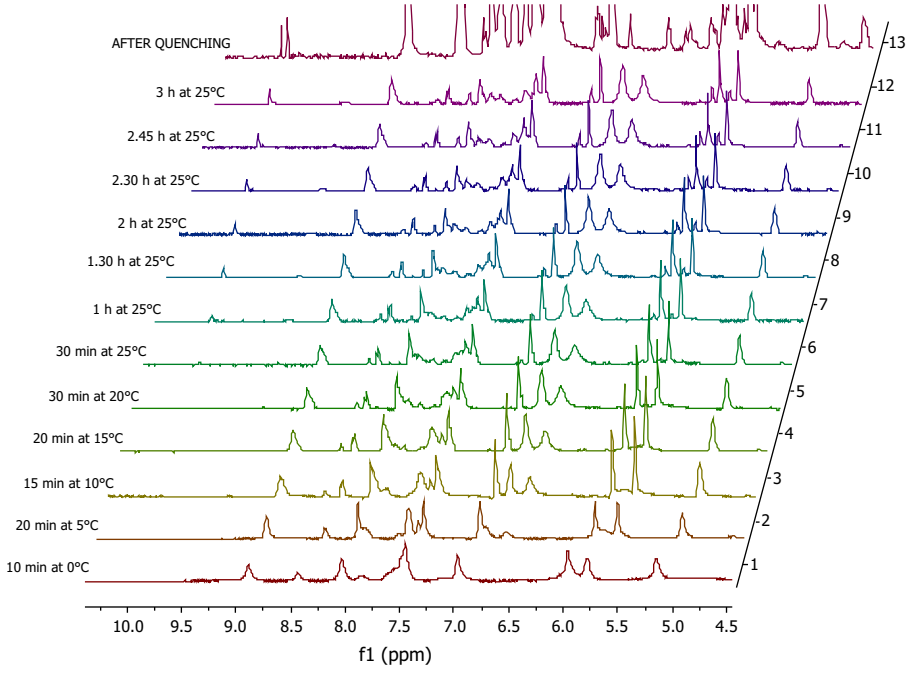
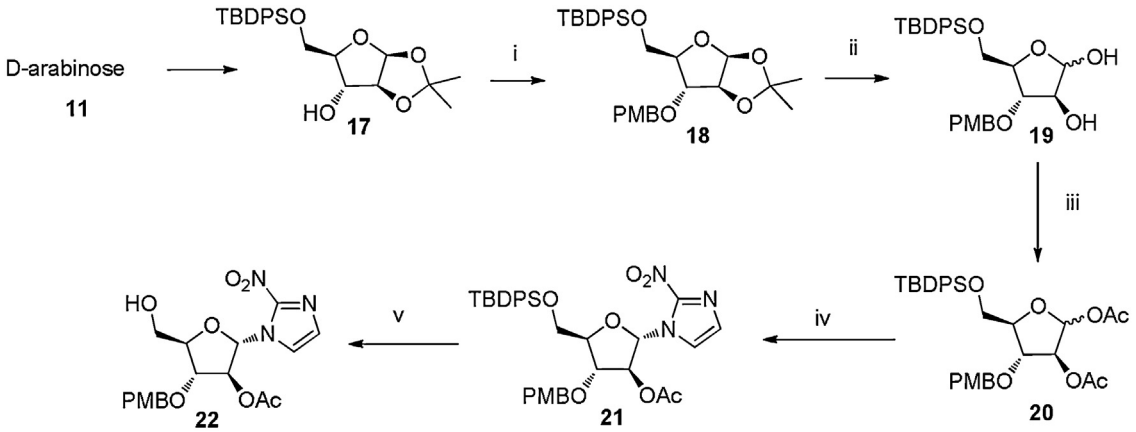
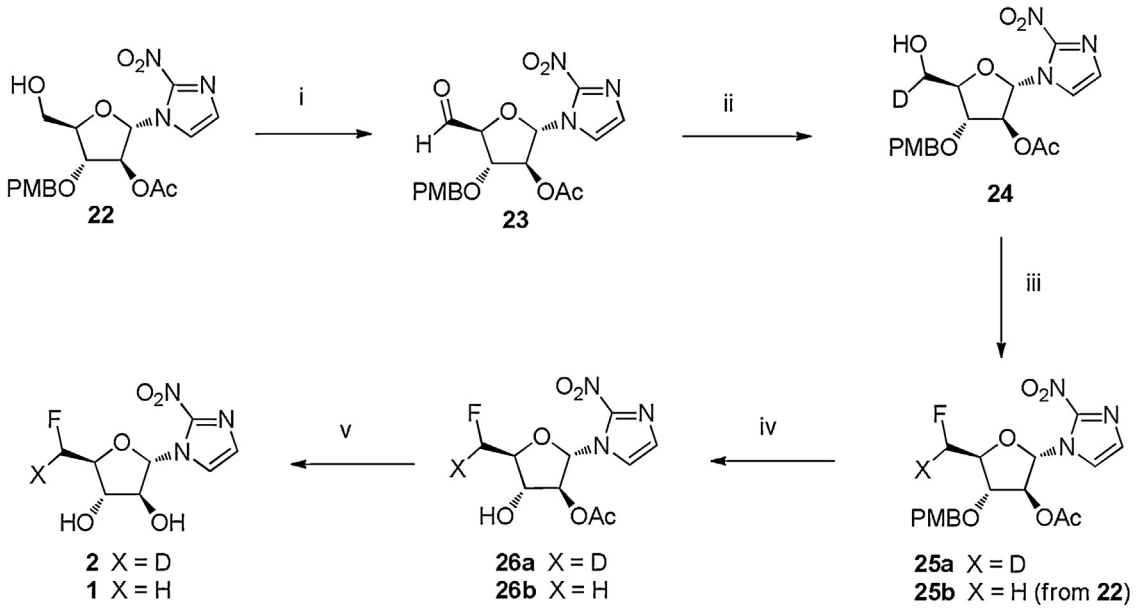


Fig. 2. NMR study of Pfitzner–Moffatt oxidation.



Scheme 8. *Reagent and conditions*: (i) PMBCl, NaH, TBAI, DMF, 0° to rt (72%); (ii) aqueous TFA 50%, DCM (52%), rt; (iii) Ac_2O, Py, rt (100%); (iv) 1-TES-2-nitroimidazole (**4**), TfOTES, MeCN, −20 °C to −8 °C (76%); and (v) KF, PhCOOH, MeCN, 75 °C (95%).



Scheme 9. *Reagents and conditions*: (i) EDC-HCl, Py-TFA, DMSO/benzene 1:1 (100%), rt; (ii) $NaBD_3CN$ or $NaBH_3CN$, EtOH rt (75%); (iii) DAST, DCM, 0 °C to rt (35%); (iv) CAN, acetone/H_2O 4:1, rt (93%); and (v) NH_3 2 M in MeOH, 4 °C (100%).

available arabino-acetonide **17**, prepared in two steps from D-arabinose **11** [12], the fully protected 3-PMB sugar **18** was obtained in good yield using NaH, PMBCl and TBAI in anhydrous DMF. The hydrolytic acetonide deprotection required extensive optimization, because of the instability of the TBDPS and PMB groups in aqueous hydrochloric and concentrate trifluoroacetic acid respectively. Optimal conditions were found using aqueous TFA (50%) in DCM as solvent. The lactol **19**, obtained as a 1:1 mixture of anomers, was then diacetylated with acetic anhydride in pyridine, affording the diacetate glycosyl donor **20**, again as an inseparable anomeric mixture in quantitative yields. The glycosylation step was accomplished by treating **20** with 1-triethylsilyl-2-nitroimidazole and a catalytic amount of triethylsilyl triflate in anhydrous acetonitrile at −8 °C affording the 2-nitromidazole glycoside **21** in 76% yield as a single α-anomer, thanks to the anchimeric participation of the 2-β-acetate in the stabilization of the intermediate oxocarbenium ion. The compound **21** was then *O*-TBDPS deprotected with KF and the key alcohol intermediate **22** was therefore obtained.

As in the previous approach to **2** (see Scheme 7), the alcohol **22** was oxidized to the aldehyde **23** using EDC and DMSO (Scheme 9). Gratifyingly, no elimination products were observed in this case. The reduction with $NaBD_3CN$ gave the deuterated alcohol **24**, that was deoxyfluorinated with DAST to **25a** in a rather modest 35% yield, which could not be optimized further. The fluoride **25a** was then oxidatively deprotected with CAN to the secondary alcohol **26a** which was subjected to the final deacetylation to provide the target [^{2}H]-FAZA **2** in satisfactory overall yields. The corresponding non-deuterated compounds **25,26b** were also obtained directly from **22**, eventually leading to FAZA **1**.

3. Conclusions

We have conducted a detailed study on the synthesis of one of the most important fluorine-containing compounds in the realm of molecular imaging, FAZA (**1**). An optimized approach to deuterium-labelled FAZA (**2**) was developed as a model process which is expected to greatly facilitate the synthesis of tritium-labelled FAZA (**3**), a potentially very useful molecular probe for the biological investigation of tumour hypoxia, whose use has been hitherto hampered by its extremely limited availability and difficult preparation. Tritium-labelled FAZA had previously been obtained by means of the same reducing agent used here albeit in tritium-labelled form, i.e. NaB^3H_3CN [7], therefore we envisage that our synthesis will be actually used for the preparation of [^{3}H]-FAZA.

4. Experimental

4.1. General Information

^{1}H (400.13 MHz), ^{13}C (100.58 MHz) and ^{19}F (376.45 MHz) NMR spectra were recorded on a Bruker ADVANCE III spectrometer. ^{1}H NMR chemical shifts are reported relative to TMS, and the solvent resonance was employed as the internal standard ($CDCl_3$, δ = 7.26). ^{13}C NMR spectra were recorded with complete proton decoupling, and the chemical shifts are reported relative to TMS with the solvent resonance as the internal standard ($CDCl_3$, δ = 77.0). ^{19}F NMR spectra were recorded with complete proton decoupling. The following abbreviations are used to describe spin multiplicity: s = singlet, d = doublet, t = triplet, m = multiplet, dd = doublet–doublet, dt = doublet–triplet, ddd = doublet–doublet–doublet, and br = broad signal. All chemical shifts (δ) are expressed in parts per million and coupling constant (J) are given in Hertz. LC–MS experiments were performed on an Agilent Technologies 1200 Series HPLC system equipped with a DAD and a 6120 MS detector composed by a ESI ionization source and a Single Quadrupole mass selective detector using a Analytical C18 RP column (Phenomenex Luna, C18, 250 mm × 4.60 mm, 5 μ, 100 Å). HPLC purifications were performed on the Agilent 1200 system using a semi preparative C18 RP column (Phenomenex Luna, 250 mm × 10.00 mm, 5 μ, 100 Å). All reactions were carried out in oven- or flame-dried glassware under nitrogen atmosphere, unless stated otherwise. All commercially available reagents were used as received. Reactions were magnetically stirred and monitored by TLC on silica gel (60 F254 pre-coated glass plates, 0.25 mm thickness). Visualization was accomplished by irradiation with a UV lamp and/or staining with a ceric ammonium molybdate or $KMnO_4$ solution. Flash chromatography was performed on silica gel (60 Å, particle size 0.040–0.062 mm). Yields refer to chromatographically and spectroscopically pure compounds, unless stated otherwise. Abbreviations used: DMSO for dimethylsulfoxide, DCM for

dichloromethane, THF for tetrahydrofurane, EtOAc for ethyl acetate, DMF for dimethylformamide, Et_2O for diethyl ether, Py for pyridine, DMP for Dess-Martin periodinane, TFA for trifluoroacetic acid, TESOTf for triethylsilyl trifluoromethanesulfonate, EDC for *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide, DAST for (diethylamino)sulfur trifluoride, CAN for ammonium cerium(IV) nitrate, and TBAI for tetrabuthylammonium iodide. Compounds **5**, **6, 7, 9** and **12** were synthesized as previously described [10]. The synthesis of compound **13** and its conversion to **1** were performed as previously described [7].

4.2. (2S,3S,4S)-4-(acetyloxy)-5-formyl-2-(2-nitro-1H-imidazol-1-yl)oxolan-3-yl acetate and (2S,3S)-5-formyl-2-(2-nitro-1H-imidazol-1-yl)-2,3-dihydrofuran-3-yl acetate (***14a*** *and* ***14b***)

The alcohol **12** (150 mg, 0.15 mmol) was dissolved in a mixture of DMSO/benzene 1:1 (4.5 mL) and cooled to 0 °C. Pyridinium trifluoroacetate (14.5 mg, 0.075 mmol) and EDC HCl (77.6 mg, 0.40 mmol) were added and the reaction mixture was allowed to warm to r.t. After 2 h the reaction was quenched with water and extracted with EtOAc (3 × 15 mL). The organic layers were washed with brine (3 × 5 mL), dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. An inseparable mixture of the two aldheydes **14a** and **14b** was obtained (140 mg, ratio 14a:14b = 7:3) as a pale yellow oil and it was subjected to the next step without further purification. R*f* 0.60 (EtOAc 100%); ^{1}H NMR (400 MHz, $CDCl_3$, **14b** signals are marked by *) δ: 2.01 (s, 3H), 2.15 (s, 3H), 2.19 (s, 3H)*, 4.99 (s, 1H), 5.39 (d, *J* = 0.8 Hz, 1H), 5.44 (s, 1H), 5.59 (t, *J* = 2.6 Hz, 1H)*, 6.29 (d, *J* = 2.8 Hz, 1H)*, 6.75 (s, 1H), 7.10 (d, *J* = 2.3 Hz, 1H)*, 7.14 (s, 1H)*, 7.22 (s, 1H)*, 7.24 (d, *J* = 1.1 Hz, 1H), 7.44 (d, *J* = 1.1 Hz, 1H), 9.66 (s, 1H)*, 9.77 (s, 1H).

4.3. (2S,3S,4R,5R)-4-(acetyloxy)-5-[hydroxy(2H$_1$)methyl]-2-(2-nitro-1H-imidazol-1-yl)oxolan-3-yl acetate (***15a***)

The inseparable crude mixture of the two aldehydes **14a** and **14b** was dissolved in ethanol (1.5 mL), cooled at 0 °C and $NaBD_3CN$ (9.3 mg, 0.15 mmol) was added. The pH was adjusted to 4.5 adding one or two drops of acetic acid and the mixture was allowed to warm to r.t. and stirred overnight. The solvent was then removed under reduced pressure and the residue was dissolved in EtOAc (22 mL). The organic layer was washed with water (5 mL), brine (5 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. A mixture of two alcohols **15a** and **15b** was separated by HPLC (Semi-preparative C18 Luna column, Eluent: A: H_2O, Eluent B: CH_3CN; in isocratic condition Eluent A: 80% and Eluent B: 20%; retention time: 10.8 min). The collected fractions were concentrated under vacuum to give compound **15a** (15.9 mg, 32% over two steps) as a white foam. R*f* 0.42 (EtOAc 100%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.03 (s, 3H), 2.20 (s, 3H), 3.91 (m, 1H), 4.54 (m, 1H), 5.16 (m, 1H), 5.47 (t, *J* = 1.7 Hz, 1H), 6.67 (d, *J* = 1.4 Hz, 1H), 7.24 (d, *J* = 1.0 Hz, 1H), 7.40 (d, *J* = 1.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.6, 61.7 (t, *J* = 22 Hz, D), 76.5, 81.5, 88.1, 92.8, 122.0, 128.6, 169.0, 169.7; MS (ESI), calculated m/z $C_{12}H_{14}DN_3O_8$ 330 $[M]^+$, found m/z (relative intensity) 353 $[M+Na]^+$ (75), 331 $[M+H]^+$ (100).

4.4. (2S,3S)-5-[hydroxy(2H$_1$)methyl]-2-(2-nitro-1H-imidazol-1-yl)-2,3-dihydrofuran-3-yl acetate (***15b***)

Synthesized from the mixture of two aldehydes **14a** and **14b** as **15a** (see above). Purified by HPLC (Semi-preparative C18 Luna column, Eluent: A: H_2O, Eluent B: CH_3CN; in isocratic condition Eluent A: 80% and Eluent B: 20%; retention time: 12.0 min). The collected fractions were concentrated under vacuum to give compound **15b** (5.2 mg, 13% over two steps) as a white foam. *Rf* 0.42 (EtOAc 100%); ^{1}H NMR (400 MHz, CDC_{l3}) δ: 2.06 (s, 3H), 4.39 (m, 1H), 5.38 (m, 1H), 5.74 (m, 1H), 7.02 (d, *J* = 1.5 Hz, 1H), 7.20 (d, *J* = 1.2 Hz, 1H), 7.32 (d, *J* = 1.1 Hz, 1H); MS (ESI), calculated m/z $C_{10}H_{10}DN_3O_6$ 270 $[M]^+$, found m/z (relative intensity) 293 $[M+Na]^+$ (45), 271 $[M+H]^+$ (100).

4.5. (2S,3S,4S,5S)-4-(acetyloxy)-5-[fluoro(2H$_1$)methyl]-2-(2-nitro-1H-imidazol-1-yl)oxolan-3-yl acetate (***16***)

To an ice cooled solution of **15a** (15 mg, 0.05 mmol) in anhydrous DCM (0.2 mL), DAST (6.5 μL, 0.048 mmol) was added drop wise under stirring. After 10 min the reaction was slowly warmed to r.t. and stirred for additional 30 min. The mixture was then cooled to 0 °C and MeOH (0.06 mL) was added to quench the reaction. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography (Hexane/EtOAc 1:9) to give compound **16** (7.5 mg, 45%) as a pale yellow oil. R*f* 0.70 (EtOAc 100%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.05 (s, 3H), 2.21 (s, 3H), 4.63 (m, 2H), 4.68 (m, 1H), 4.75 (m, 1H), 5.21 (m, 1H), 5.47 (t, *J* = 1.3 Hz, 1H), 6.70 (d, *J* = 1.7 Hz, 1H), 7.25 (d, *J* = 1.2 Hz, 1H), 7.38 (d, *J* = 1.1 Hz, 1H); ^{19}F NMR (376.45 MHz, $CDCl_3$) δ: −228.9; ^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.4, 76.1, 81.2 (dt, *J* = 23.4, 75.2 Hz), 86.1 (d, *J* = 19.9 Hz), 86.2, 90.1, 121.8, 128.6, 169.0, 169.5; MS (ESI), calculated m/z $C_{12}H_{13}DFN_3O_7$ 332 $[M]^+$, found m/z (relative intensity) 355 $[M+Na]^+$ (50), 333 $[M+H]^+$ (100).

4.6. (2S,3S,4S,5S)-2-[fluoro(2H$_1$)methyl]-5-(2-nitro-1H-imidazol-1-yl)oxolane-3,4-diol (***^{2}H-FAZA***, ***2***)

To neat compound **16** (3.8 mg, 0.013 mmol) cooled at 0 °C was added a solution of anhydrous ammonia in MeOH 2 M (1 mL). The reaction was left at 4 °C overnight. The solvent was removed under reduced pressure and the crude product was purified by HPLC (Semi-preparative C18 Luna column, Eluent: A: H_2O, Eluent B: CH_3CN; in isocratic condition Eluent A: 90% and Eluent B: 10%; retention time: 11 min). The collected fractions were concentrated under vacuum to give compound **2** (1.7 mg, 55%) as a white solid. R*f* 0.56 (EtOAc 100%); ^{1}H NMR (400 MHz, CD_3OD) δ: 4.12 (bs, 1H), 4.32 (s, 1H), 4.54 (m, 1H), 4.66 (m, 1H), 6.47 (bs, 1H), 7.16 (bs, 1H), 7.70 (bs, 1H); ^{19}F NMR (376.45 MHz, CD_3OD) δ: −227.4; ^{13}C NMR (100 MHz, CD_3OD) δ: 75.8, 82.2, 82.8 (dt, *J* = 22.9, 76.2 Hz), 87.7 (d, *J* = 21 Hz), 95.5, 124.0 126.9; MS (ESI), calculated m/z $C_8H_9DFN_3O_5$ 248 $[M]^+$, found m/z (relative intensity) 249 $[M+H]^+$ (100).

4.7. {[(3aS,5R,6R,6aS)-6-[(4-methoxyphenyl)methoxy]-2,2-dimethyl-tetrahydro-2H-furo[2,3-d][1,3]dioxol-5-yl]methoxy}(tert-butyl)diphenylsilane (***18***)

To a solution of **17** (synthesized as previously reported [12], 2.6 g, 6.0 mmol) and *p*-methoxybenzyl chloride (1.2 mL, 9.0 mmol) in anhydrous DMF (35 mL) cooled at 0 °C, TBAI (222 mg, 0.6 mmol) was added followed by the addition of NaH (60% in mineral oil, 480 mg, 12 mmol) under vigorous stirring. After 10 min the ice bath was removed and the reaction was allowed to warm to r.t. and stirred for additional 6 h. The reaction mixture was then cooled to 0 °C and the excess of NaH was carefully quenched with water. The mixture was acidified (pH = 2) adding 1 M HCl and extracted with EtOAc (3 × 30 mL); the combined organic phases were washed with brine (10 mL) dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude was purified by flash chromatography (Hexane/Et_2O 9:1) to give the desired product **18** (2.37 g, 72%) as a pale yellow oil. R*f* 0.82 (Hexane/EtOAc 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.07 (s, 9H), 1.32 (s, 3H), 1.37 (s, 3H), 3.82 (m, 4H), 4.57 (d, *J* = 2.9 Hz, 2H), 4.67 (d, *J* = 4.0 Hz, 1H), 5.90 (d, *J* = 4.0 Hz, 1H), 6.89 (d, *J* = 8.6 Hz, 2H), 7.28 (d, *J* = 8.6 Hz, 2H), 7.42 (m, 6 H), 7.67 (d, *J* = 6.8 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 19.3,

26.3, 27.0, 27.1, 55.3, 63.6, 71.4, 82.5, 85.3, 85.3, 105.8, 112.5, 114.0, 129.6, 133.2, 133.4, 135.7, 135.7, 159.5; MS (ESI), calculated m/z $C_{32}H_{40}O_6Si$ 548 $[M]^+$, found m/z (relative intensity) 549 $[M+H]^+$ (100).

4.8. (3S,4S,5R)-5-{[(tert-butyldiphenylsilyl)oxy]methyl}-4-[(4-methoxyphenyl)methoxy]oxolane-2,3-diol (**19**)

Compound **18** (1.0 g, 1.8 mmol) was dissolved in DCM (50 mL) and the solution was cooled to 0 °C. TFA (50% in water, 20 mL) was added under vigorous stirring and the reaction mixture was allowed to warm to r.t. After 3 h the mixture was quenched by careful addition of small portions of solid $NaHCO_3$. Water was added and the mixture was extracted with EtOAc (3 × 30 mL). The organic layer was washed with brine, dried over $MgSO_4$, filtered and the solvent evaporated under reduced pressure. The crude product was purified by flash chromatography (Hexane/EtOAc 1:1) to give a mixture of two anomers (481 mg, 52%) as a colourless oil. R*f* 0.71 (Hexane/EtOAc 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.06 (s, 4.5H), 1.08 (s, 4.5H), 3.58 (ddd, *J* = 11.2, 3.9, 1.9 Hz, 1H), 3.81 (s, 1.5H), 3.83 (s, 1.5H), 3.85 (m, 1H), 4.09 (m, 2.5H), 4.32 (bs, 0.5H), 4.51 (m, 1H), 4.64 (m, 1H), 5.34 (bs, 1H), 6.91 (m, 2H), 7.27 (m, 2H), 7.46 (m, 6H), 7.64 (m, 2H), 7.69 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 26.7, 26.8, 55.3, 64.2, 64.7, 71.6, 72.0, 76.2, 76.3, 82.6, 82.7, 84.0, 84.3, 97.7, 103.9, 113.9, 114.1, 128.0, 128.7, 128.8, 129.5, 129.7, 130.1, 130.2, 131.6, 132.0, 135.6, 135.7, 136.7, 159.4, 159.6; MS (ESI), calculated m/z $C_{29}H_{36}O_6Si$ 508 $[M]^+$, found m/z (relative intensity) 531 $[M+Na]^+$ (100).

4.9. (3S,4R,5R)-2-(acetyloxy)-5-{[(tert-butyldiphenylsilyl)oxy]methyl}-4-[(4-methoxyphenyl)methoxy]oxolan-3-yl acetate (**20**)

To a solution of **19** (480 mg, 0.94 mmol) in anhydrous pyridine (3 mL), acetic anhydride (0.50 mL, 5.20 mmol) was added drop wise and the mixture was stirred overnight. The volatile components were evaporated under reduced pressure, EtOAc (20 mL) was added and the mixture was washed with 0.5 M HCl (10 mL), saturated $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$, filtered and the solvent evaporated under reduced pressure. The mixture of two anomers **20** was obtained (557 mg, 100%) as a pale yellow oil and was used without further purification. R*f* 0.4 (Hexane/EtOAc 6:4); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.04 (s, 4.5H), 1.09 (s, 4.5H), 1.90 (s, 1.5H), 2.04 (s, 1.5H), 2.08 (s, 1.5H), 2.13 (s, 1.5H), 3.80 (m, 2H), 3.81 (s, 1.5H), 3.82 (s, 1.5H), 4.14 (m, 1H), 4.29 (m, 0.5H), 4.40 (m, 0.5H), 4.54 (d, *J* = 4.7 Hz, 1H), 4.57 (d, *J* = 2 Hz, 2H), 4.69 (d, *J* = 11.6 Hz, 1H), 5.27 (m, 1H), 6.21 (s, 0.5H), 6.38 (d, *J* = 4.7 Hz, 0.5H), 6.87 (m, 2H), 7.24 (m, 2H), 7.42 (m, 6H), 7.66 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.6, 20.8, 21.0, 21.2, 26.7, 26.8, 55.2, 55.3, 62.6, 64.0, 72.0, 72.2, 77.2, 77.4, 79.3, 81.3, 82.0, 82.3, 85.7, 93.8, 100.2, 113.9, 125.3, 127.7, 127.8, 127.9, 129.1, 129.3, 129.5, 129.7, 129.8, 129.9, 133.0, 133.1, 133.2, 133.3, 135.5, 135.6, 135.7, 159.4, 169.4, 169.7; MS (ESI), calculated m/z $C_{33}H_{40}O_8Si$ 592 $[M]^+$, found m/z (relative intensity) $[M+Na]^+$ 615 $[M+Na]^+$ (40), 593 $[M+H]^+$ (100).

4.10. (2S,3S,4R,5R)-5-{[(tert-butyldiphenylsilyl)oxy]methyl}-4-[(4-methoxyphenyl)methoxy]-2-(2-nitro-1H-imidazol-1-yl)oxolan-3-yl acetate (**21**)

2-Nitroimidazole (85 mg, 0.75 mmol) and bis(triethylsilyl)amine (580 mg, 1.50 mmol) were refluxed in anhydrous pyridine (1.5 mL) for 30 min. The volatiles were removed under high vacuum distillation. The remaining solid was dissolved in anhydrous CH_3CN (3 mL) and a solution of **20** (345 mg, 0.58 mmol) in anhydrous CH_3CN (2 mL) was added. The mixture was cooled to −20 °C and TESOTf 1 M in DCM (0.6 mL, 0.60 mmol) was added drop wise under stirring. The reaction was warmed to −8 °C and stirred at this temperature for 3 h. A saturated solution of $NaHCO_3$ (2 mL) was added and the mixture was extracted with EtOAc (3 × 20 mL). The organic phase was washed with brine (5 mL), dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (Hexane/EtOAc 7:3) to give compound **21** (295 g, 76%) as a white waxy solid. R*f* 0.23 (Hexane/EtOAc 7:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.97 (s, 9H), 1.91 (s, 3H), 3.60 (dd, *J* = 6.9, 10.8 Hz, 1H) 3.70 (s, 3H), 3.71 (m, 1H), 4.05 (d, *J* = 1.3 Hz, 1H), 4.42 (dd, *J* = 27.3, 11.7 Hz, 2H), 4.52 (ddd, *J* = 2.1, 5.4, 7.2 Hz, 1H), 5.29 (s, 1H), 6.45 (d, *J* = 8.7 Hz, 2H), 7.00 (d, *J* = 8.7 Hz, 2H), 7.10 (d, *J* = 1.2 Hz, 1H), 7.35 (m, 7H), 7.54 (dt, *J* = 1.2, 8,0 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 19.2, 20.7, 26.8, 55.3, 63.3, 71.7, 77.2, 81.4, 81.8, 88.5, 93.2, 113.9, 123.1, 127.8, 128.3, 128.8, 129.4, 130.0, 132.8, 133.0, 135.5, 135.6, 159.5, 169.2; MS (ESI), calculated m/z $C_{34}H_{39}N_3O_8Si$ 645 $[M]^+$, found m/z (relative intensity) 668 $[M+Na]^+$ (30), 646 $[M+H]^+$ (100).

4.11. (2S,3S,4R,5R)-5-(hydroxymethyl)-4-[(4-methoxyphenyl)methoxy]-2-(2-nitro-1H-imidazol-1-yl)oxolan-3-yl acetate (**22**)

Potassium fluoride (183 mg, 3.15 mmol) and benzoic acid (385 mg, 3.15 mmol) were added to a solution of **21** (295 mg, 0.46 mmol) in anhydrous CH_3CN (15 mL). The mixture was refluxed overnight under vigorous stirring. After cooling the mixture at −20 °C the solid was filtered over celite, and the pad was washed with cold CH_3CN. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography (EtOAc 100%) to give compound **22** (178 mg, 95%) as a pale yellow wax. R*f* 0.42 (EtOAc 100%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.85 (s, 3H), 3.76 (m, 2H), 3.85 (s, 3H), 4.07 (d, *J* = 1.9 Hz, 1H), 4.56 (m, 3H), 5.41 (s, 1H), 6.64 (s, 1H), 6.84 (d, *J* = 8.6 Hz, 2H), 7.08 (d, *J* = 8.6 Hz, 2H), 7.17 (d, *J* = 1.0 Hz, 1H), 7.47 (d, *J* = 1.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.6, 55.3, 62.3, 72.1, 81.4, 81.8, 88.9, 93.1, 114.0, 123.1, 128.3, 129.4, 159.6, 169.4; MS (ESI), calculated m/z $C_{18}H_{21}N_3O_8$ 407 $[M]^+$, found m/z (relative intensity) 430 $[M+Na]^+$ (40), 408 $[M+H]^+$ (100).

4.12. (2S,3S,4S)-5-formyl-4-[(4-methoxyphenyl)methoxy]-2-(2-nitro-1H-imidazol-1-yl)oxolan-3-yl acetate (**23**)

The alcohol **22** (41 mg, 0.10 mmol) was dissolved in a DMSO/benzene 1:1 mixture (3 mL) cooled to 0 °C. Pyridinium trifluoroacetate (8.7 mg, 0.05 mmol) and EDC HCl (51.8 mg, 0.27 mmol) were added and the reaction mixture was allowed to warm to r.t. After 2 h the reaction was quenched with water and extracted with EtOAc (3 × 150 mL). The organic layers were washed with brine (3 × 5 mL), dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude aldehyde **23** (40 mg) was obtained as a pale yellow oil and was used directly without further purification. R*f* 0.50 (EtOAc 100%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.82 (s, 3H), 4.23 (s, 1H), 4.50 (m, 3H), 4.92 (s, 1H), 6.71 (s, 1H), 6.84 (d, *J* = 8.7 Hz, 1H), 7.03 (d, *J* = 8.7 Hz, 1H), 7.16 (d, *J* = 1.2 Hz, 1H), 7.45 (d, *J* = 1.2 Hz, 1H), 9.71 (s, 1H).

4.13. (2S,3S,4R,5R)-5-[hydroxy(2H_1)methyl]-4-[(4-methoxyphenyl)methoxy]-2-(2-nitro-1H-imidazol-1-yl)oxolan-3-yl acetate (**24**)

The crude aldehyde **23** was dissolved in ethanol (1 mL), cooled at 0 °C and $NaBD_3CN$ (5.6 mg, 0.09 mmol) was added. The pH was adjusted to 4.5 by adding one or two drops of acetic acid and the mixture was allowed to warm to r.t. and stirred overnight. The

solvent was then removed under reduced pressure and the residue was dissolved in EtOAc (20 mL). The organic layer was washed with water (5 mL), brine (5 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (EtOAc 100%) to give compound **24** (30.6 mg, 75% over two steps) as a white foam. R*f* 0.42 (EtOAc 100%); ^{1}H NMR (400 MHz, $CDCl_3$): δ: 2.19 (s, 3H), 3.78 (m, 4H), 4.06 (d, *J* = 2.1 Hz, 1H), 4.53 (m, 3H), 5.41 (s, 1H), 6.65 (s, 1H), 6.85 (d, *J* = 8.6 Hz, 2H), 7.09 (d, *J* = 8.6 Hz, 2H), 7.18 (d, *J* = 1.0 Hz, 1H), 7.48 (d, *J* = 1.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ: 20.6, 55.3, 62.0 (t, *J* = 23.2 Hz, D), 72.1, 81.4, 81.8, 88.8, 93.1, 114.0, 123.1, 128.4, 129.4, 159.6, 169.3; MS (ESI), calculated m/z $C_{18}H_{20}DN_3O_8$ 408 $[M]^+$, found m/z (relative intensity) 431 $[M+Na]^+$ (35), 409 $[M+H]^+$ (100).

*4.14. (2S,3S,4S,5S)-5-[fluoro(2H$_1$)methyl]-4-[(4-methoxyphenyl)methoxy]-2-(2-nitro-1H-imidazol-1-yl)oxolan-3-yl acetate (**25a**)*

To an ice cooled solution of **24** (15 mg, 0.04 mmol) in anhydrous DCM (0.2 mL), DAST (6.5 μL, 0.048 mmol) was added drop wise under stirring. After 10 min the reaction was slowly warmed to r.t. and stirred for additional 30 min. The mixture was then cooled to 0 °C and MeOH (0.06 mL) was added to quench the reaction. The solvent was removed under reduced pressure and the crude product was filtered over silica (Hexane/EtOAc 1:9) to give compound **25a** (5.8 mg, 35%) as a pale yellow oil. R*f* 0.73 (EtOAc 100%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.17 (s, 3H), 3.80 (s, 3H), 4.51 (m, 3H), 4.58 (m, 4H), 5.39 (s, 1H), 6.63 (s, 1H), 6.83 (d, *J* = 8.7 Hz, 2H), 7.05 (d, *J* = 8.7 Hz, 2H), 7.15 (d, *J* = 1.1 Hz, 1H); 7.45 (d, *J* = 0.9 Hz, 1H); ^{19}F NMR (376.45 MHz, $CDCl_3$) δ: −227.8; ^{13}C NMR (100 MHz, $CDCl_3$): 20.7, 55.3, 72.1, 81.0, 81.6, 86.2, 87.6, 93.1, 114.0, 122.9, 123.1, 128.4, 129.5, 159.6, 169.3; MS (ESI), calculated m/z $C_{18}H_{19}D_FN_3O_7$ 410 $[M]^+$, found m/z (relative intensity) 433 $[M+Na]^+$ (33), 411 $[M+H]^+$ (100).

*4.15. (2S,3S,4S,5S)-5-[fluoro(2H$_1$)methyl]-4-hydroxy-2-(2-nitro-1H-imidazol-1-yl)oxolan-3-yl acetate (**26a**)*

Compound **25a** (5.8 mg, 0.014 mmol) was dissolved in an acetone/water 4:1 mixture (0.5 mL) then a solution of CAN (15.5 mg, 0.028 mmol) in water (0.2 mL) was added drop wise under stirring. After 30 min another portion of CAN (15.5 mg, 0.028 mmol) in water (0.2 mL) was added. After additional 30 min a saturated solution of $NaHCO_3$ (1 mL) was added and the mixture was extracted with EtOAc (3 × 10 mL). The organic layers were washed with brine (3 × 2 mL), dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (Hexane/EtOAc 2:8) to give compound **26a** (3.8 mg, 93%) as a waxy solid. R*f* 0.62 (EtOAc 100%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.20 (s, 3H), 4.39 (m, 1H), 4.56 (m, 1H), 4.63 (m, 1H), 5.19 (t, *J* = 1.0 Hz, 1H), 6.70 (d, *J* = 2.1 Hz, 1H), 7.20 (s, 1H), 7.47 (s, 1H); ^{19}F NMR (376.45 MHz, $CDCl_3$) δ: -229.57; ^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.7, 75.5 (d, *J* = 6.0 Hz), 85.7, 85.7, 85.9 (d, *J* = 20.3 Hz), 92.2, 122.4, 128.6, 170.7; MS (ESI), calculated m/z $C_{10}H_{11}DFN_3O_6$ 290 $[M]^+$, found m/z (relative intensity) 313 $[M+Na]^+$ (40), 291 $[M+H]^+$ (100).

*4.16. (2S,3S,4S,5S)-5-(fluoromethyl)-4-[(4-methoxyphenyl)methoxy]-2-(2-nitro-1H-imidazol-1-yl)oxolan-3-yl acetate (**25b**)*

Synthesized following the same procedure for obtaining **25a**. Starting from **22**, compound **25b** (28 mg, 35%) was obtained as a pale yellow oil. R*f* 0.73 (EtOAc 100%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.16 (s, 3H), 3.79 (s, 3H), 4.05 (m, 1H), 4.41 (m, 1H), 4.51 (m, 3H), 4.68 (m, 1H), 5.38 (s, 1H), 6.63 (s, 1H), 6.82 (d, *J* = 8.7 Hz, 2H), 7.05 (d, *J* = 8.7 Hz, 2H), 7.14 (d, *J* = 1.0 Hz, 1H), 7.41 (d, *J* = 1.0 Hz); ^{19}F NMR (376.45 MHz, $CDCl_3$) δ: −228.36; ^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.5, 55.2, 71.8, 80.8, 81.5, 86.5, 88.7, 93.2, 114.0, 122.8, 128.4, 129.5; MS (ESI), calculated m/z $C_{18}H_{20}FN_3O_7$ 409 $[M]^+$, found m/z (relative intensity) $[M+Na]^+$ 432 (55), 410 $[M+H]^+$ (100).

*4.17. (2S,3S,4S,5S)-5-(fluoromethyl)-4-hydroxy-2-(2-nitro-1H-imidazol-1-yl)oxolan-3-yl acetate (**26b**)*

Synthesized following the same procedure for obtaining **26a**. Starting from **25b**, compound **26b** was obtained (18 mg, 94%) as a pale yellow wax. R*f* 0.62 (EtOAc 100%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.19 (s, 3H), 4.38 (m, 1H), 4.60 (m, 3H), 5.19 (t, *J* = 1.8 Hz, 1H), 6.72 (d, *J* = 1.8 Hz, 1H), 7.18 (s, 1H), 7.48 (s, 1H); ^{19}F NMR (376.45 MHz, $CDCl_3$) δ: −229.24; ^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.7, 75.4 (d, *J* = 5.8 Hz), 81.4 (d, *J* = 175 Hz), 85.3, 86.3 (d, *J* = 19.9 Hz), 93.4, 122.6, 128.5, 170.7; MS (ESI), calculated m/z $C_{10}H_{12}FN_3O_6$ 291 $[M]^+$, found m/z (relative intensity) 314 $[M+Na]^+$ (40), 292 $[M+H]^+$ (100).

*4.18. (2S,3S,4S,5S)-2-(fluoromethyl)-5-(2-nitro-1H-imidazol-1-yl)oxolane-3,4-diol (**FAZA**, **1**)*

Synthesized following the same procedure for obtaining **2**. Starting from **26b**, compound **1** was obtained (14 mg, 95%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ: 4.13 (t, *J* = 2.1 Hz, 1H), 4.33 (s, 1H), 4.56 (m, 1H), 4.66 (m, 2H), 6.48 (d, *J* = 1.0 Hz, 1H), 7.16 (d, *J* = 1.0 Hz, 1H), 7.69 (d, *J* = 1.0 Hz, 1H); ^{19}F NMR (376.45 MHz, CD_3OD) δ: −227.2; ^{13}C NMR (100 MHz, CD_3OD) δ: 75.8 (d, *J* = 5.6), 82.2 (d, *J* = 170 Hz), 82.2, 87.7 (d, *J* = 20.6 Hz), 95.5, 123.8, 126.9; MS (ESI), calculated m/z $C_8H_{10}FN_3O_5$ 247 $[M]^+$, found m/z (relative intensity) $[M+Na]^+$ 270 (100).

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