

## Solid-supported cyanoborohydride cartridges for automating reductive amination radiochemistry

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### Supporting Information

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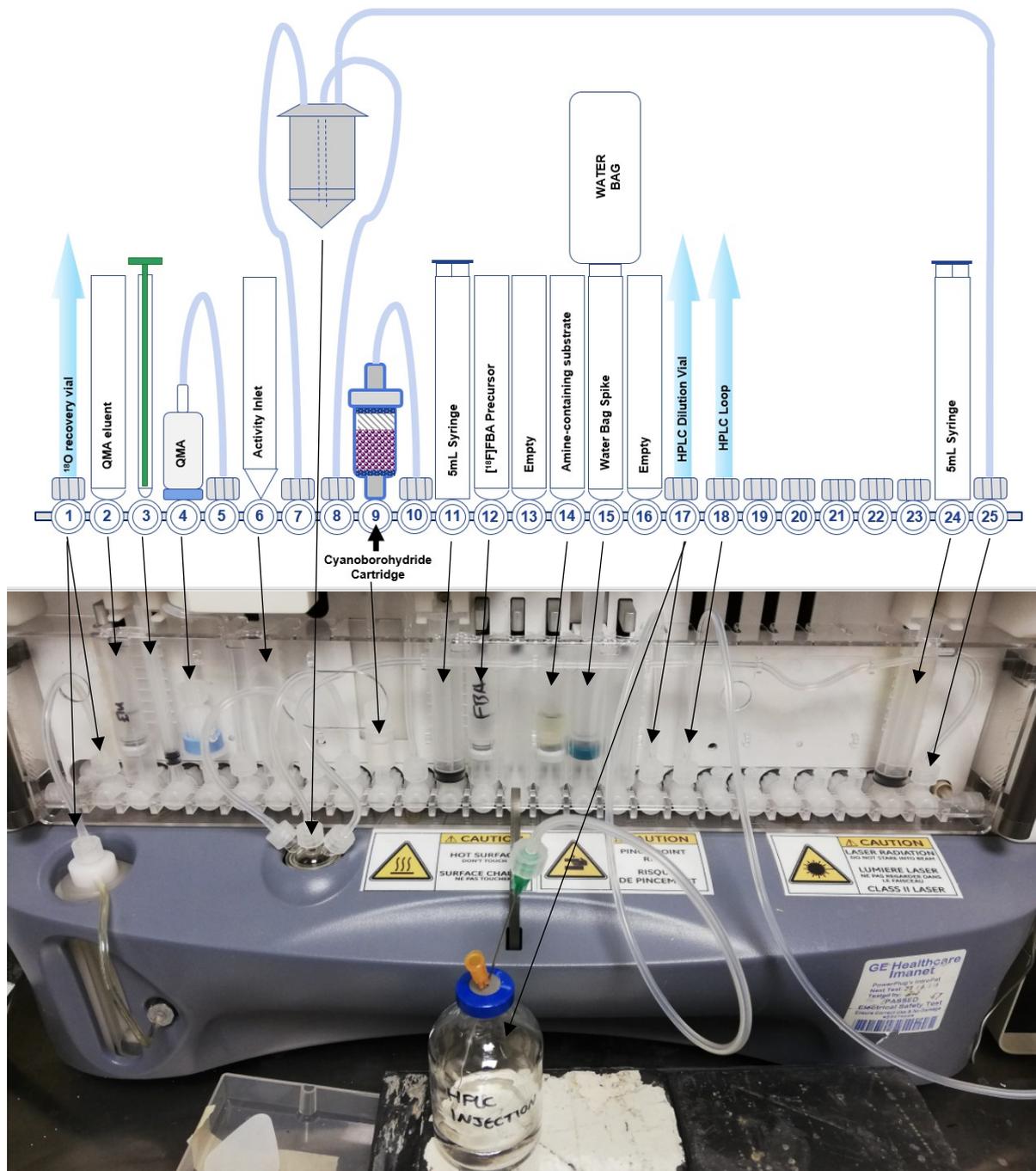
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## 1.0 Materials and methods

All reagents and solvents were purchased from commercial sources and were used without further purification unless otherwise stated. Compound **2** (1,3,4,6-tetra-O-acetyl- $\beta$ -D-glucosamine HCl) was purchased from Carbosynth (Newbury, Berkshire, UK). HPLC grade acetonitrile, dichloromethane (DCM), ethyl acetate (EtOAc), ethanol (EtOH), methanol (MeOH), toluene, potassium carbonate ( $K_2CO_3$ ), 4-fluorobenzaldehyde (FBA), solid-supported cyanoborohydride (526304-5G), Cyanide test kit (1.10044 – Cyanide Test) empty 1 mL SPE cartridges (57607-U), and hexane were purchased from Sigma Aldrich (Gillingham, Dorset, UK). [ $^{18}F$ ]Fluoride was produced by a GE PETtrace cyclotron by 16 MeV irradiation of enriched [ $^{18}O$ ]H $_2$ O target, supplied by Alliance Medical Radiopharmacy Ltd (Warwick, UK). Automated radiosyntheses were performed using the GE FASTlab<sup>™</sup> automated synthesis module (GE Healthcare Life Sciences, Amersham, UK). Solid phase extraction (SPE) cartridges were purchased from Waters (Elstree, Hertfordshire, UK) and used according to the manufacturers recommended guidelines. 4-Formyl-N,N,N-trimethylanilinium trifluoromethanesulfonate ([ $^{18}F$ ]FBA precursor) was synthesised following a literature procedure.<sup>1</sup>  $^1H$ ,  $^{13}C$  and  $^{19}F$  NMR spectra were obtained using a Bruker 400 MHz spectrometer operating at room temperature. Chemical shifts ( $\delta$ ) are reported in parts per million (ppm) and residual solvent peaks have been used as an internal reference. Peak multiplicities have been abbreviated as follows: s (singlet), d (doublet), dd (double-doublet), m (multiplet). NMR spectra were analysed using MestReNova v11 (Santiago de Compostela, Spain). Reaction efficiency and radioactive product identity was determined by RP-HPLC using an Agilent 1200 series instrument connected to a flow-ram detector (Lablogic, Sheffield, UK). The system was equipped with a Phenomenex Gemini 5 $\mu$  C18 110 Å (150  $\times$  4.6 mm) column; the mobile phase was A: H $_2$ O (0.1% TFA) and B: MeCN. The gradient was: 0 – 1 min, 95% A. 1 – 22 min, 5% A. 22 – 24 min, 95% A at 1 mL/min. Elution profiles were analysed using Laura software (Lablogic, Sheffield, UK). Semi-preparative RP-HPLC was performed using a Shimadzu LC20-AT pump attached to a custom-built system, equipped with an Agilent Eclipse XDB-C18, 5 $\mu$  (250  $\times$  9.4 mm) column. The mobile phase was 30:70 MeCN / H $_2$ O / 0.1% TFA (v/v).

## 2.0 GE FASTLab™ cassette setup

The FASTlab™ cassette was assembled as shown in figure 1. The radiolabelling method for [ $^{18}\text{F}$ ]FBA, [ $^{18}\text{F}$ ]2 and [ $^{18}\text{F}$ ]4 is described below.



**Figure 1.** Diagram and photograph of the GE FASTLab™ cassette. A detailed description of the reagent setup is given in table 1.

**Table 1.** Setup of the GE FASTlab™ cassette and radiochemistry method.

Position	Content	Function
1	Tubing to <sup>18</sup> O recovery vial	<sup>18</sup> O recovery from QMA
2	K <sub>222</sub> (6.0 mg/mL, 800 μL MeCN) KHCO <sub>3</sub> (3.5 mg/mL, 200 μL H <sub>2</sub> O)	Eluent for QMA
3	Syringe (1 mL)	Eluting QMA cartridge
4 - 5	QMA Carbonate SPE cartridge (Waters)	Trapping [ <sup>18</sup> F]F <sup>-</sup> for drying
6	[ <sup>18</sup> F]Fluoride	Activity inlet to synthesiser
7 – 8 & 25	Reactor vessel	A single reactor vessel is used through the radiosynthesis
9-10	Solid-supported NaCNBH <sub>3</sub> cartridge (200 mg)	Reducing agent for reductive amination
11	Syringe (5 mL)	Liquid movement around the cassette
12	4-formyl-N,N,N-trimethylanilinium trifluoromethanesulfonate (2 mg) in MeCN (1000 μL)	Precursor for the synthesis of [ <sup>18</sup> F]FBA (70% added to reactor) <sup>a</sup>
14	Amine substrate (100 μmol) in MeCN (1500 μL)	Substrate for radiolabelling with [ <sup>18</sup> F]FBA <i>via</i> reductive amination (50% added to reactor) <sup>a</sup>
15	Water for injection	Rinsing the reactor for RP-HPLC purification
17	Tubing to off-board vial (100 mL) containing H <sub>2</sub> O + 0.1% TFA (v/v) (5 mL)	Reservoir for diluting the reaction mixture for semi-preparative RP-HPLC purification
18	Tubing to preparative HPLC loop	Direct connection between GE FASTLab™ and off-board semi-preparative RP-HPLC

<sup>a</sup> **Note:** Quantities of reagents shown above represent amounts added to the reagent vials; dead-volume in the vials prevents all reagent from being added to the reactor.

### Radiolabelling method:

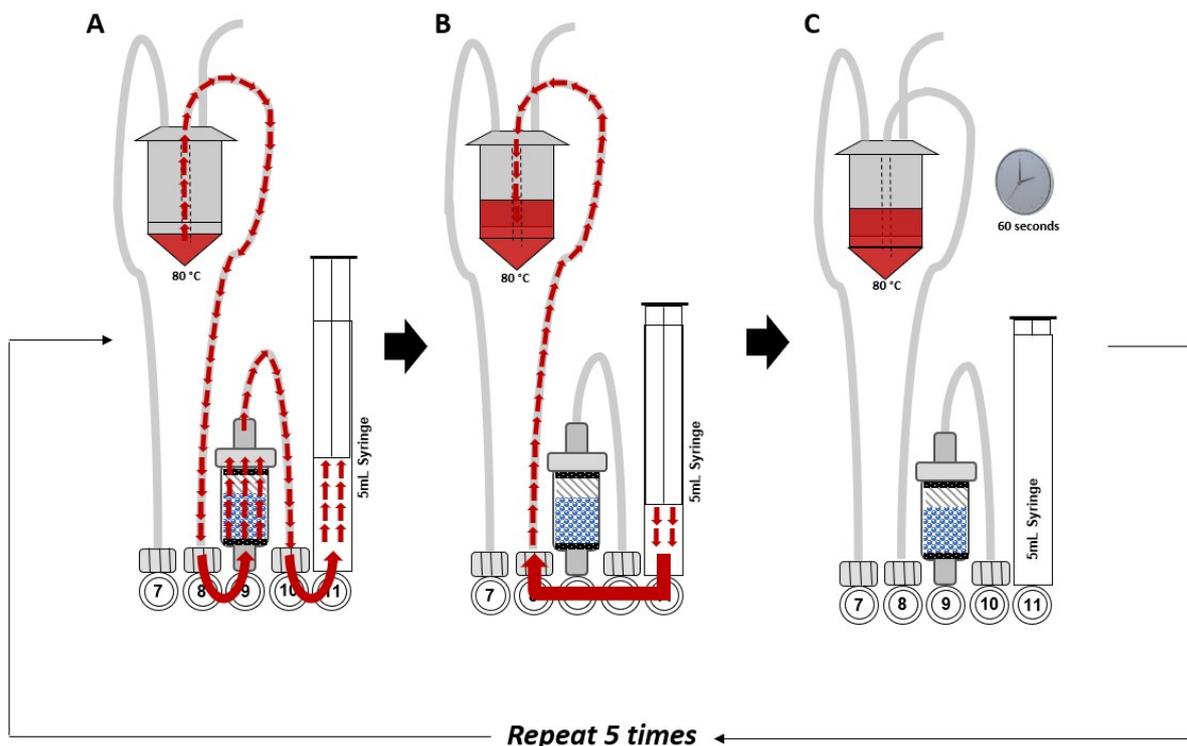
**Fluoride drying:** Aqueous [<sup>18</sup>F]fluoride (ca. 1 – 2 GBq) in oxygen-18 water (ca. 2 mL) was pulled onto the cassette from an external vial *via* the activity inlet (position 6) under negative pressure. The [<sup>18</sup>F]fluoride solution was pulled through the QMA cartridge (position 4 – 5) under negative pressure to trap the [<sup>18</sup>F]fluoride on the solid phase and the oxygen-18 water was collected in the receiver vial (position 1). The QMA cartridge was dried under vacuum and nitrogen flow for 5 min.

The trapped [ $^{18}\text{F}$ ]fluoride was eluted from the QMA using the prepared eluent (position 2,  $\text{K}_{222}$  [6.0 mg/mL, 800  $\mu\text{L}$  MeCN] and  $\text{KHCO}_3$  [3.5 mg/mL, 200  $\mu\text{L}$   $\text{H}_2\text{O}$ ]) using the 1 mL syringe (position 3) directly into the reactor vial (position 7) which was vented through position 25. The [ $^{18}\text{F}$ ]fluoride was azeotropically dried under a flow of nitrogen and vacuum at 90 °C for 6 min, after which the reactor was cooled to ca 45 °C (5 min).

[ $^{18}\text{F}$ ]FBA radiolabelling: a solution of 4-formyl-N,N,N-trimethylanilinium trifluoromethanesulfonate precursor (position 12, 2 mg in 1 mL MeCN) was pressurised with nitrogen and released into the reactor under negative pressure *via* position 8. The reactor was sealed and heated to 90 °C for 6 min, after which the reactor was cooled to ca 45 °C (5 min). The radiochemical yield (determined by HPLC) of the [ $^{18}\text{F}$ ]FBA was determined at this step by sampling from the reactor. The [ $^{18}\text{F}$ ]FBA was used without further purification.

Reductive amination radiochemistry: To the reactor containing [ $^{18}\text{F}$ ]FBA (ca. 1 mL MeCN) was added the amine-containing model compound (position 14) by pressurising the vial with nitrogen and releasing the contents into the reactor under negative pressure *via* position 8. The reactor was sealed and heated to 65 °C for 15 min to form the imine intermediate. Using the syringe (5 mL, position 11) the reaction mixture was pulled through the cyanoborohydride cartridge and pushed back into the reactor (position 9 – 10) 5 times over 5 min while heating the reactor vessel to 80°C to maintain an elevated reaction temperature while the crude reaction mixture flowed through the cartridge. After this, the reaction mixture was returned to the reactor vessel for HPLC purification. This procedure is represented graphically in Scheme S1.

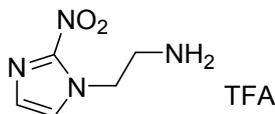
HPLC purification: The total volume (1.5 - 2 mL) of crude reaction mixture was transferred *via* nitrogen flow (1 min) into an external dilution vial (position 17) containing  $\text{H}_2\text{O}$  + 0.1% TFA (5 mL), the cyanoborohydride cartridge was washed with water (2 x 2 mL) into the dilution vial and nitrogen bubbled through to mix. Using the syringe (5 mL, position 11) in two passes, the mixture was carefully loaded onto a 10 mL loop on an external preparative HPLC system (see Materials & Methods for conditions) and injected once complete. The desired radioactive peak was cut and collected into an external vial. The molar activity of the final radioactive compounds [ $^{18}\text{F}$ ]2 and [ $^{18}\text{F}$ ]4 was ca. 1 – 2 GBq/ $\mu\text{mol}$  determined by HPLC analysis, where the UV region under the radioactive compound was integrated and the area referenced to authentic non-radioactive standards.



**Scheme 1.** Visual representation of how the solid-supported cyanoborohydride cartridges were used to perform reductive amination radiochemistry. **A)** crude reaction mixture is flowed through the solid-supported reducing agent cartridge; **B)** crude reaction mixture is pushed back into the reactor vessel; **C)** the reaction mixture is kept in the reactor at 80 °C for 60 seconds. The cycle is repeated 5 times. Steps **A** and **B** are performed over ca 1.5 min.

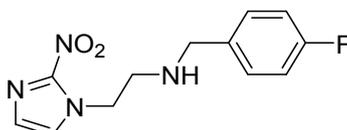
### 3.0 Synthesis

#### 3.2 2-(2-nitro-1H-imidazol-1-yl)ethan-1-amine (1)



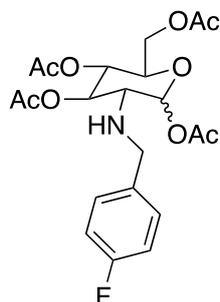
2-Nitroimidazole (500 mg, 4.42 mmol) and  $K_2CO_3$  (916 mg, 6.63 mmol) were stirred in DMF (10 mL). 2-(Boc-amino)ethyl bromide (891 mg, 3.98 mmol) dissolved in DMF (5 mL) was added dropwise. The reaction was stirred at 60 °C for 16 h after which, the reaction was cooled and filtered. The filtrate was evaporated, dissolved in EtOAc (25 mL) and washed with brine (2 × 20 mL). The organic layer was separated, dried over  $MgSO_4$  and evaporated *in vacuo*. The crude was purified by column chromatography (EtOAc, silica). After the evaporation, the product was dissolved in DCM (2 mL) and trifluoroacetic acid (2 mL) and stirred for 2 h at room temperature. The solvent was evaporated *in vacuo* and used without further purification (361 mg, 30% yield).  $^1H$ -NMR (400 MHz,  $DMSO-d_6$ )  $\delta$ : 3.33 (t, 2H,  $J = 6.0$  Hz), 4.64 (t, 2H,  $J = 6.1$  Hz), 7.24 (s, 1H), 7.64 (s, 1H), 8.07 (s, 3H).  $^{13}C$ -NMR (101 MHz,  $DMSO-d_6$ )  $\delta$ : 39.19, 47.22, 128.46, 145.48, 158.47. ESI-MS ( $m/z$ ):  $[M+H]^+$  calcd for  $C_5H_9N_4O_2$ , 157.0720; found, 157.0728.

#### 3.3 N-(4-fluorobenzyl)-2-(2-nitro-1H-imidazol-1-yl)ethan-1-amine (2)



Compound **1** (100 mg, 0.37 mmol) was dissolved in a mixture of MeCN (6 mL) and water (2 mL). To the stirred solution, 4-fluorobenzaldehyde (35  $\mu$ L, 0.32) was added and allowed to stir at room temperature for 30 min.  $NaCNBH_3$  (117 mg, 1.86 mmol) was added and the reaction stirred for 16 h. The reaction mixture was evaporated *in vacuo*, dissolved in EtOAc (20 mL) and washed with brine (2 × 20 mL). The organic layer was separated and dried over  $MgSO_4$ , evaporated *in vacuo* and purified by column chromatography (EtOAc/Hexane 3:2, silica) to give the desired product (26 mg, 27% yield).  $^1H$ -NMR (400 MHz, Chloroform-*d*)  $\delta$ : 7.14 – 7.07 (m, 2H), 7.06 (s, 2H), 6.96 – 6.86 (m, 2H), 4.44 (t,  $J = 5.9$  Hz, 2H), 3.66 (s, 2H), 2.96 (t,  $J = 5.9$  Hz, 2H).  $^{13}C$ -NMR (101 MHz, Chloroform-*d*)  $\delta$ : 48.5, 50.1, 52.8, 115.2, 115.4, 126.4, 129.5, 135.2, 160.8, 163.2.  $^{19}F$ -NMR (376 MHz, Chloroform-*d*)  $\delta$ : -115.5. ESI-MS ( $m/z$ ):  $[M+H]^+$  calcd for  $C_{12}H_{14}FN_4O_2$ , 265.1095; found, 265.1110.

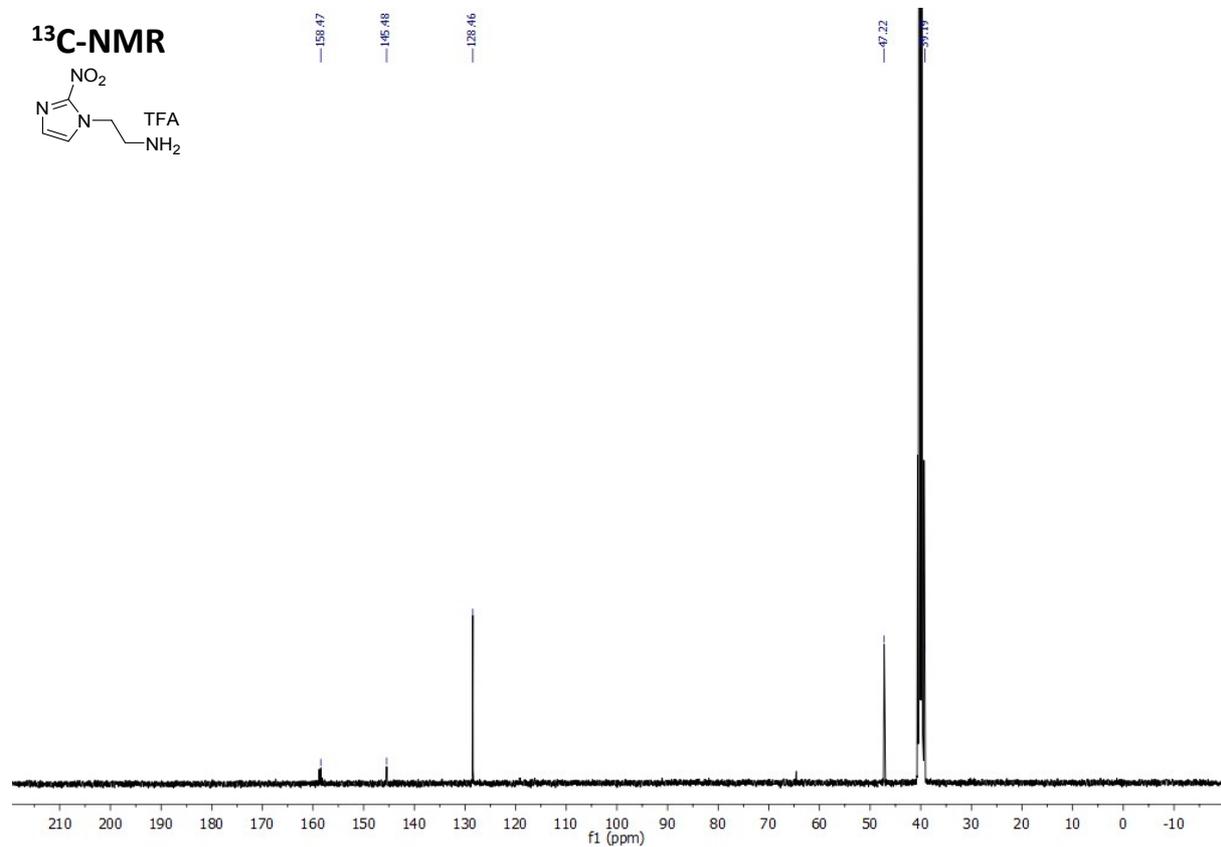
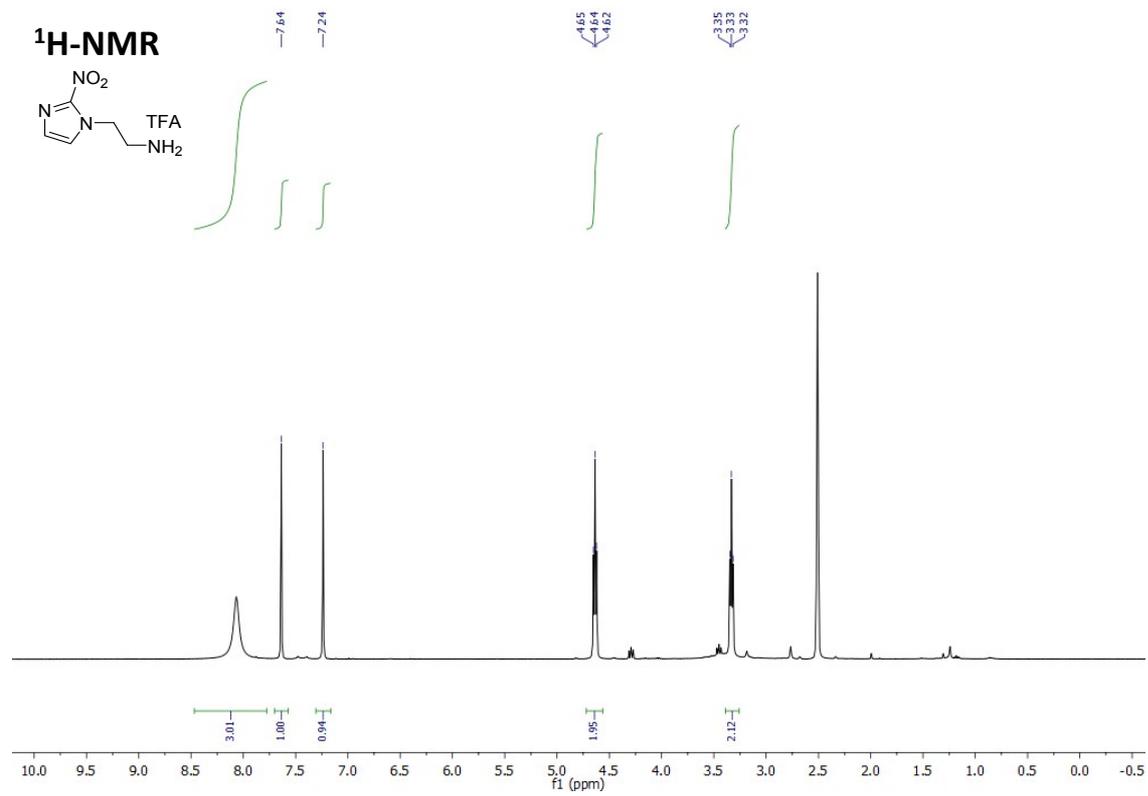
3.4. (3*R*,4*R*,5*S*,6*R*)-6-(acetoxymethyl)-3-((4-fluorobenzyl)amino)tetrahydro-2*H*-pyran-2,4,5-triyl triacetate (**4**)



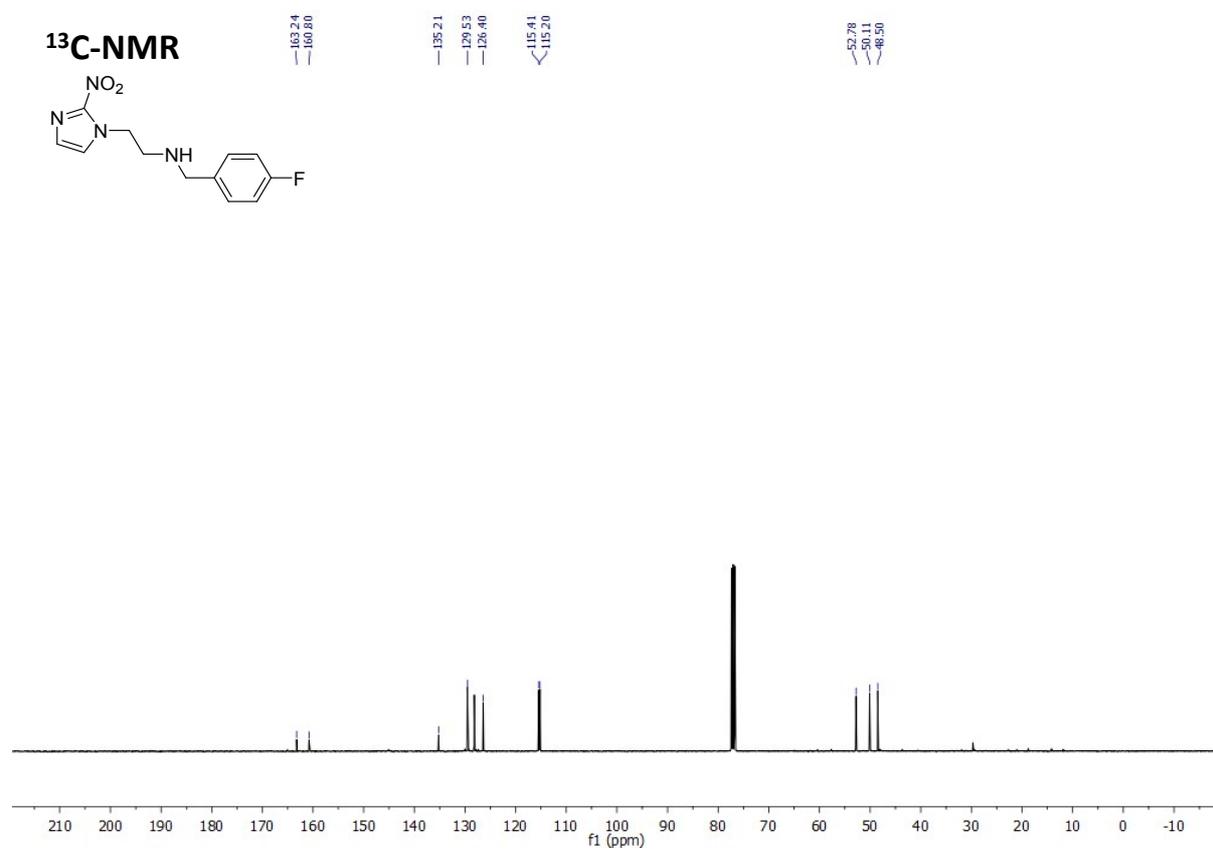
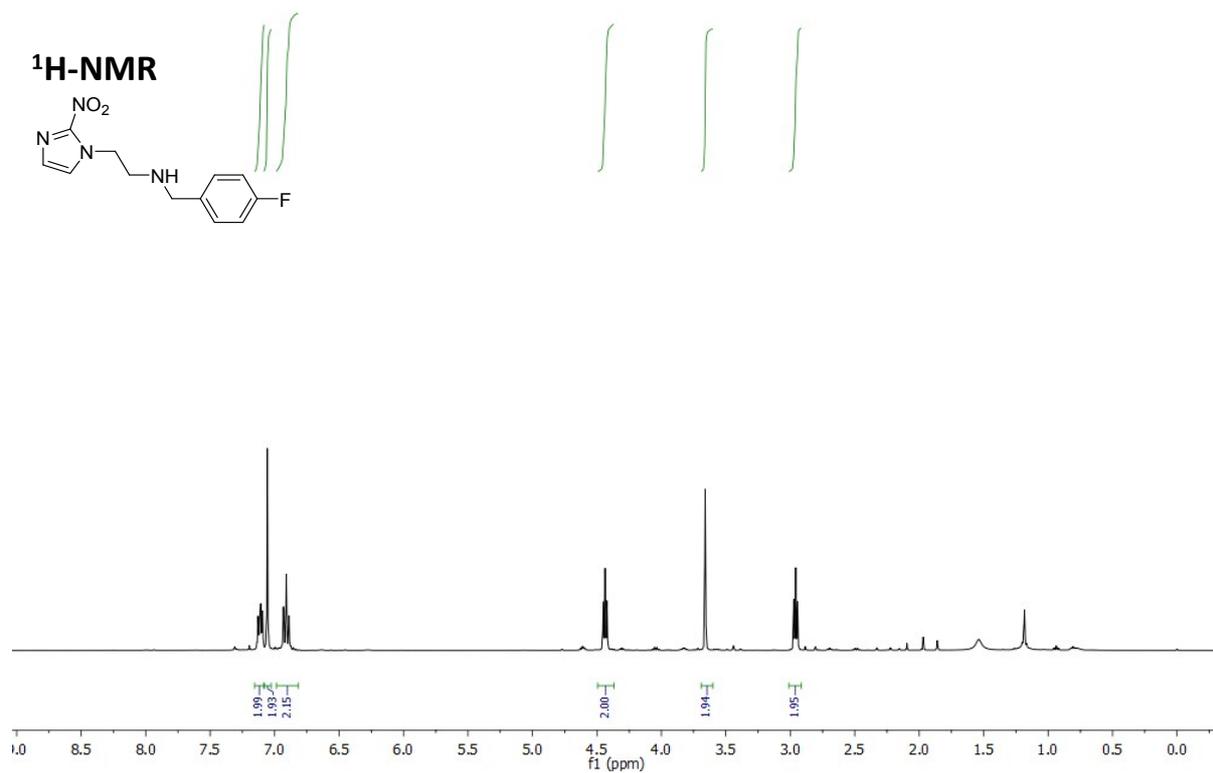
To a flask was added 1,3,4,6-Tetra-O-acetyl-2-amino-2-deoxy- $\beta$ -D-glucopyranose hydrochloride (100 mg, 0.26 mmol) and 4-fluorobenzaldehyde (55  $\mu$ L, 0.52 mmol) in MeCN/H<sub>2</sub>O (8 mL, 3:1 v/v). The reaction was stirred at RT for 1 h, followed by the addition of NaCNBH<sub>3</sub> (47 mg, 0.72 mmol). The reaction was stirred overnight and monitored using TLC (toluene/EtOAc, 1:1 v/v; R<sub>f</sub> = 0.42). Solvent was removed *in vacuo* and the residue was purified by column chromatography (toluene:EtOAc, 1:1 v/v) to give the desired product (77 mg, 65% yield). <sup>1</sup>H-NMR (400 MHz, MeCN-*d*<sub>3</sub>)  $\delta$ : 1.99 (s, 3H), 2.01 (s, 6H), 2.12 (s, 3H), 2.82 (dd, 1H, *J*= 10.4, 8.6 Hz), 3.78 (m, 1H), 3.85 – 3.89 (m, 2H), 4.02 (dd, 1H, *J*= 12.5, 2.3 Hz), 4.21 (dd, 1H, *J*= 12.4, 4.7 Hz), 4.94 (dd, 1H, *J*= 10.1, 9.4 Hz), 5.15 (dd, 1H, *J*= 10.4, 9.4 Hz), 5.67 (d, 1H, *J*= 8.6 Hz), 7.05 – 7.12 (m, 2H), 7.29 – 7.34 (m, 2H). <sup>13</sup>C-NMR (101 MHz, MeCN-*d*<sub>3</sub>)  $\delta$ : 20.4, 20.5, 20.7, 20.9, 51.0, 60.4, 62.3, 69.1, 72.6, 73.9, 95.2, 115.2, 117.9, 130.4, 137.6, 161.1, 163.5, 169.7, 170.2, 170.8, 171.0. <sup>19</sup>F-NMR (376 MHz, MeCN-*d*<sub>3</sub>)  $\delta$ : -117.9. ESI-MS (*m/z*): [M+H]<sup>+</sup> calcd for C<sub>21</sub>H<sub>26</sub>NO<sub>9</sub>F, 456.1664; found, 456.1676.

## 4.0 NMR Spectra

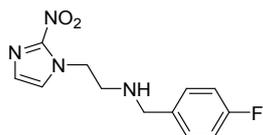
### 4.1 2-(2-nitro-1H-imidazol-1-yl)ethan-1-amine (1)



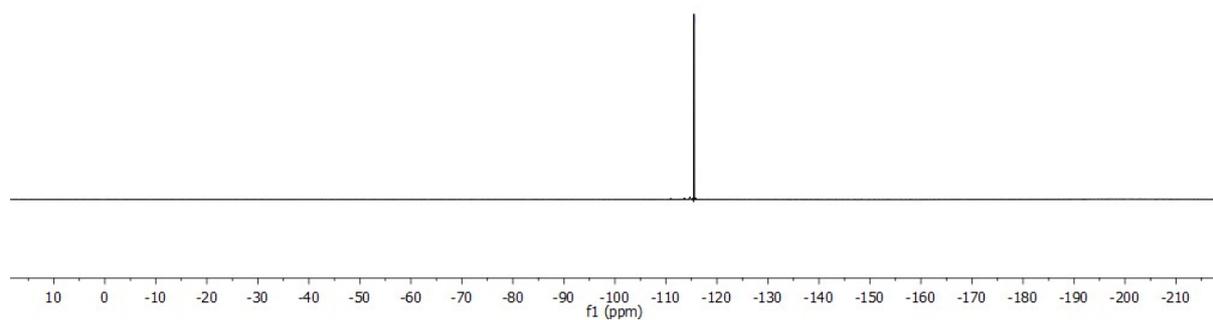
#### 4.2 N-(4-fluorobenzyl)-2-(2-nitro-1H-imidazol-1-yl)ethan-1-amine (2)



### <sup>19</sup>F-NMR

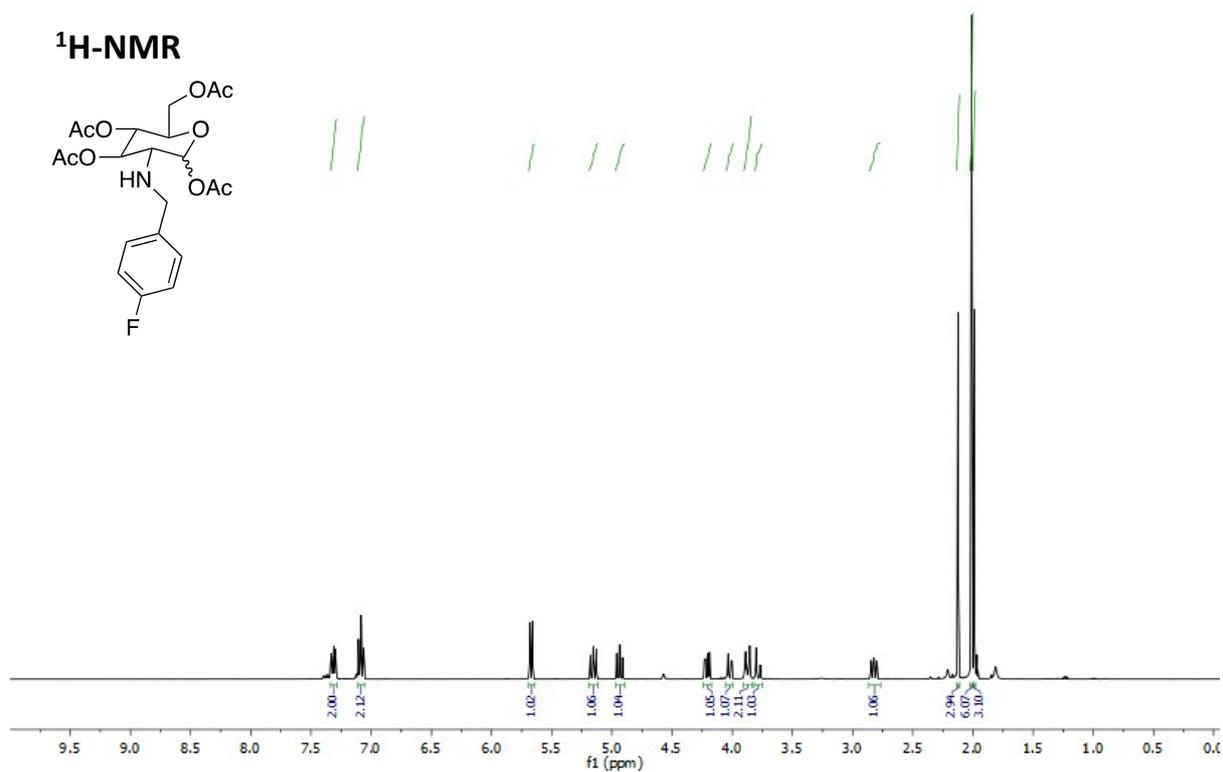
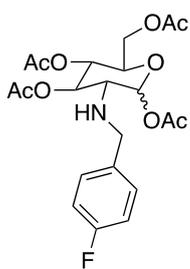


-115.49

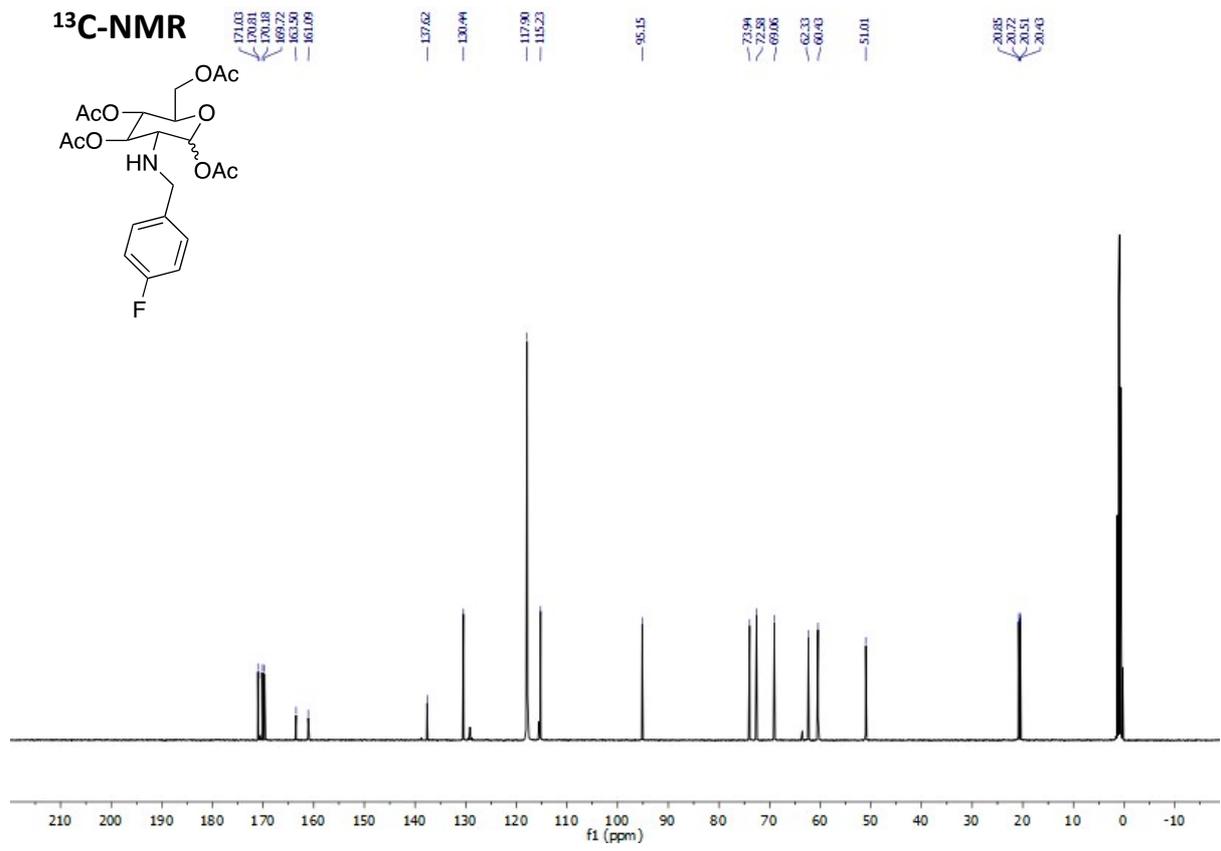
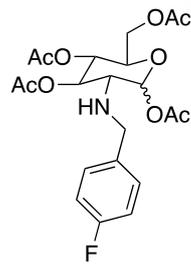


### 4.3. (3R,4R,5S,6R)-6-(acetoxymethyl)-3-((4-fluorobenzyl)amino)tetrahydro-2H-pyran-2,4,5-triyl triacetate (**4**)

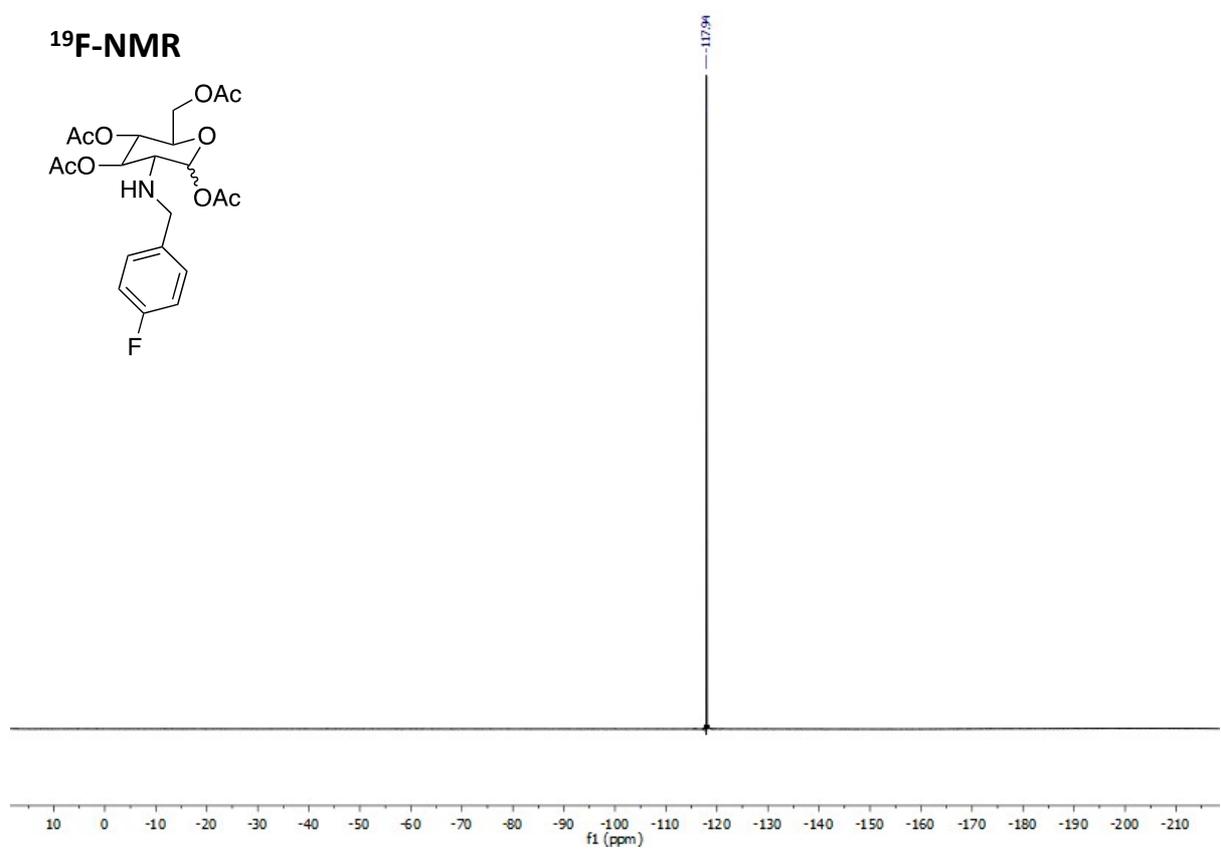
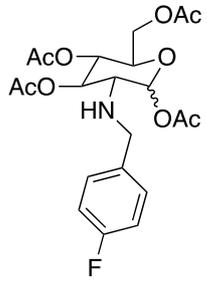
### <sup>1</sup>H-NMR



### <sup>13</sup>C-NMR

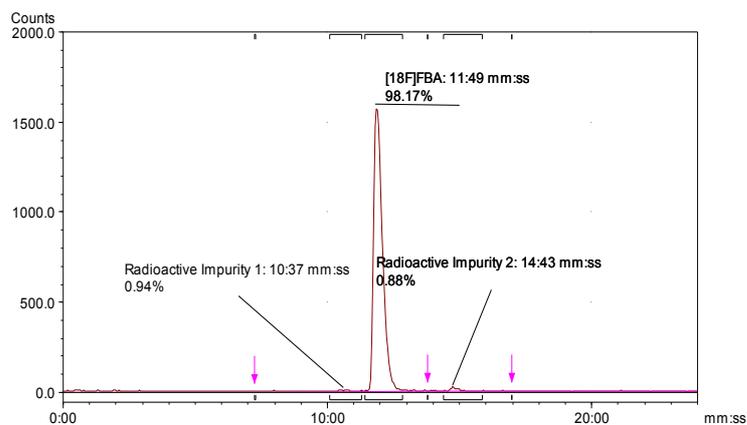


### <sup>19</sup>F-NMR

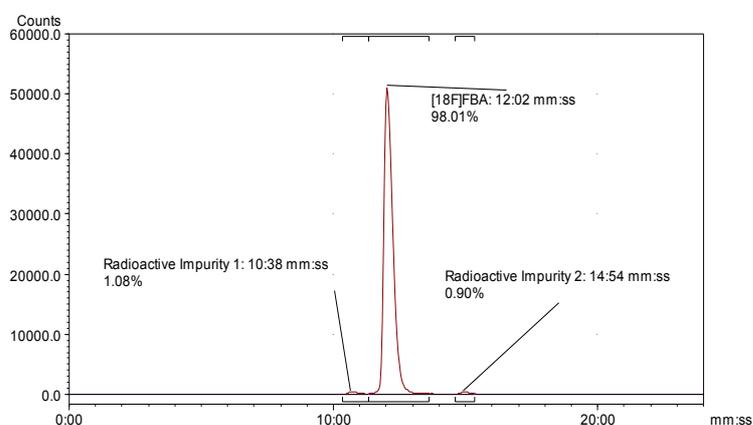


## 5.0 HPLC Chromatograms

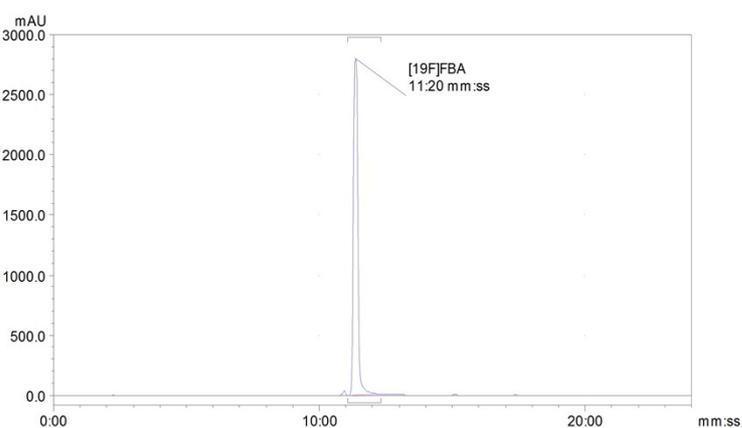
**A**



**B**

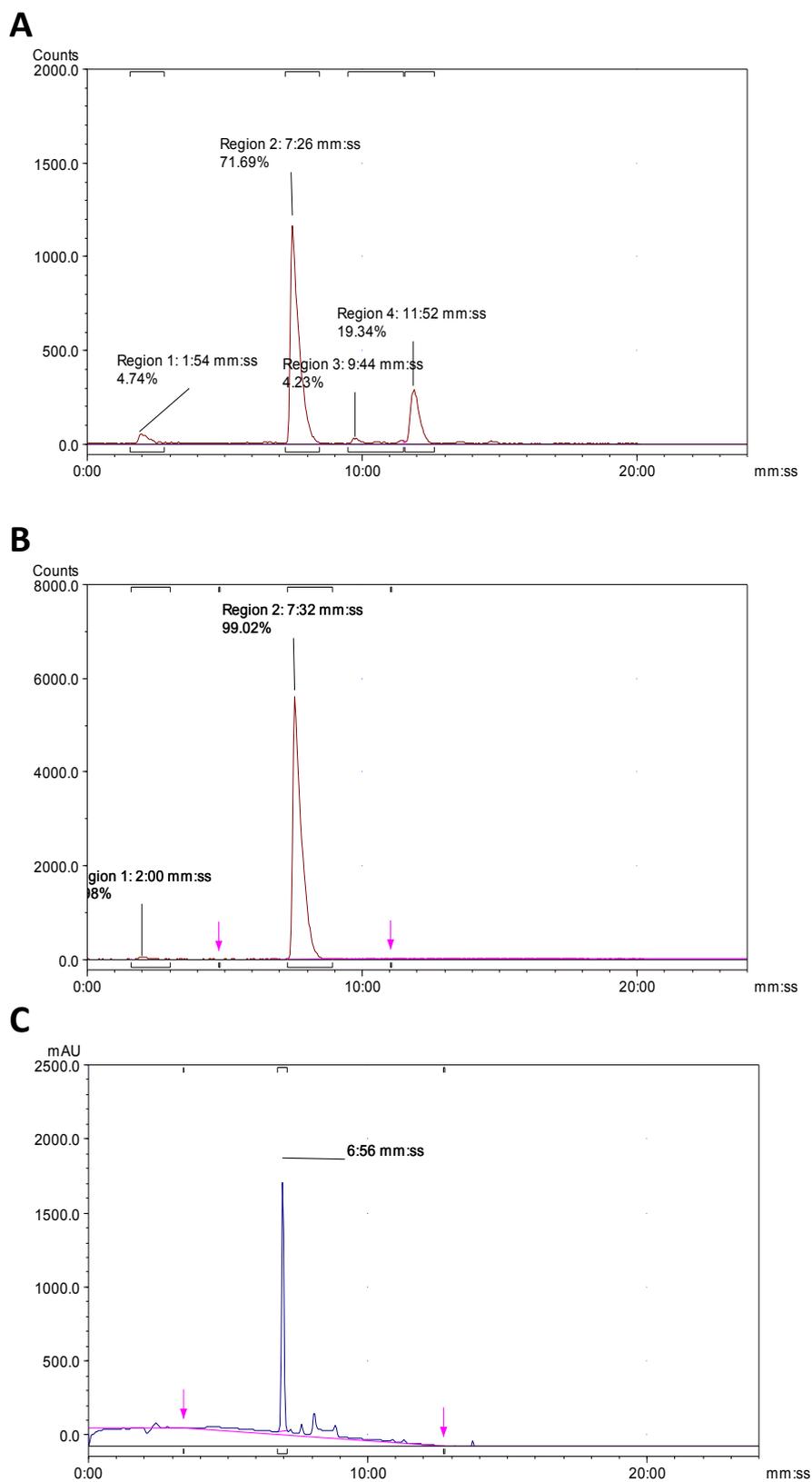


**C**

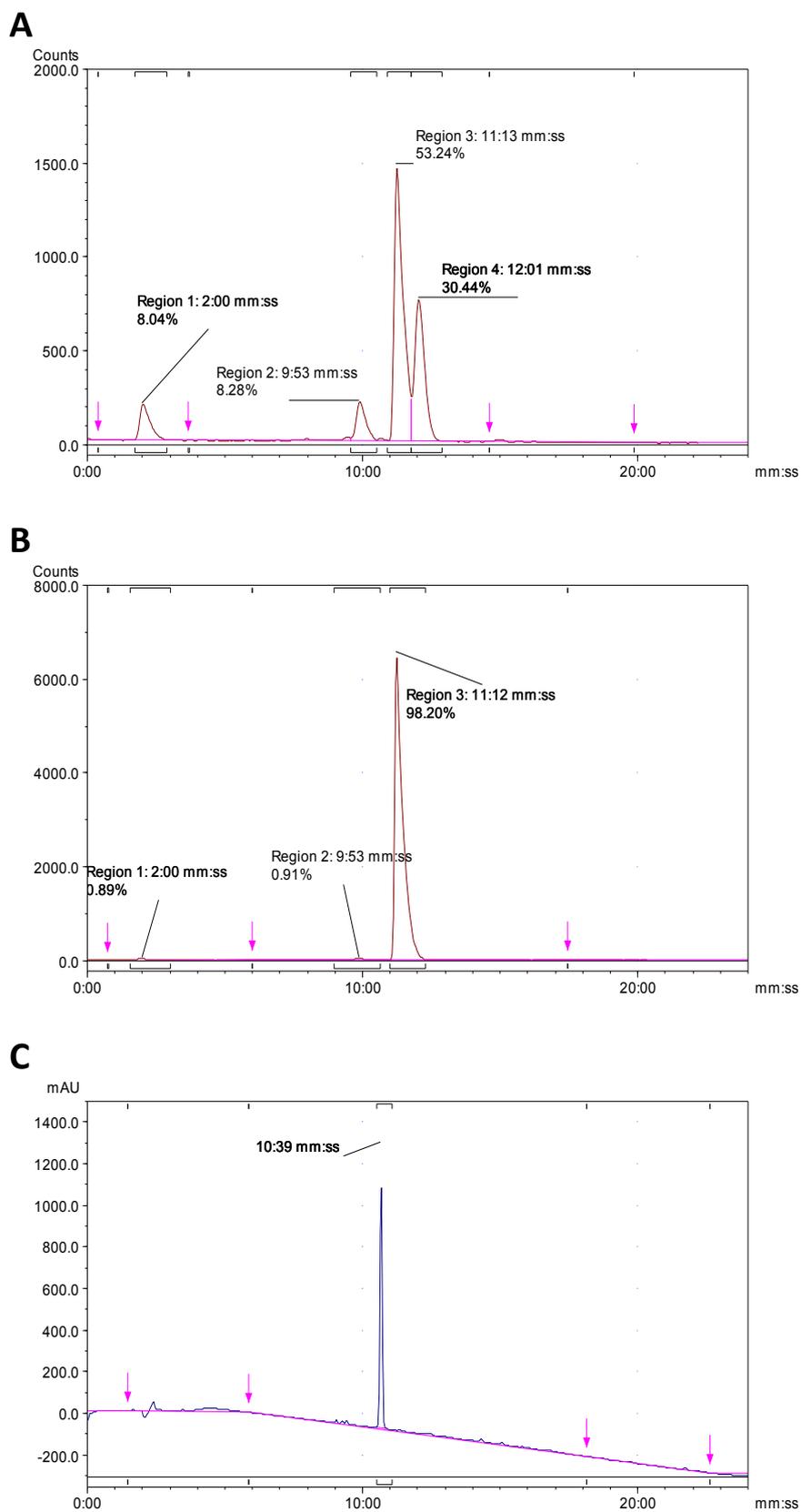


**Figure 2.** Radio-HPLC chromatogram of **A)** [<sup>18</sup>F]FBA crude reaction mixture in acidic mobile phase (H<sub>2</sub>O + 0.1% TFA / MeCN) and **B)** [<sup>18</sup>F]FBA crude reaction mixture in neutral mobile phase (H<sub>2</sub>O / MeCN). Radiochemical purity >98% indicating low residual [<sup>18</sup>F]fluoride after the radiolabelling reaction. **C)** non-radioactive [<sup>19</sup>F]FBA reference (t<sub>R</sub> = 11:20).

Ory et al. report the effect of [ $^{18}\text{F}$ ]fluoride retention when using an acidic HPLC mobile phase with C18 reverse-phase HPLC columns, which may artificially inflate radiochemical yields.<sup>2</sup> To ensure our reported radiochemical yields (determined by HPLC) were accurate, we investigated this effect on the radiosynthesis of [ $^{18}\text{F}$ ]FBA. No residual [ $^{18}\text{F}$ ]fluoride was found when using neutral pH mobile phase (Figure 2).



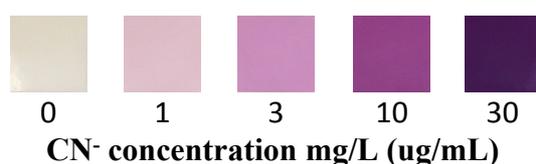
**Figure 3.** Representative radio-HPLC chromatograms showing: **A)** reaction efficiency between **1** and [<sup>18</sup>F]FBA ( $t_R = 11:52$ ) to synthesise [<sup>18</sup>F]**2** ( $t_R = 7:26$ ); **B)** purified [<sup>18</sup>F]**2** ( $t_R = 7:32$ ); **C)** non-radioactive reference standard ( $t_R = 6:56$ ).



**Figure 4.** Representative radio-HPLC chromatograms showing: **A)** reaction efficiency between **3** and [ $^{18}\text{F}$ ]FBA ( $t_{\text{R}} = 12:01$ ) to synthesise [ $^{18}\text{F}$ ]**4** ( $t_{\text{R}} = 11:13$ ); **B)** purified [ $^{18}\text{F}$ ]**4** ( $t_{\text{R}} = 11:12$ ); **C)** non-radioactive reference standard ( $t_{\text{R}} = 10:39$ ).

## 6.0 Colorimetric Free Cyanide Test

A colorimetric cyanide test kit was purchased from Sigma Aldrich (1.10044 – Cyanide Test) and used according to the manufacturer's instructions. The limit of detection of the test strips was 1 mg/L (1 µg/mL) which is sufficient for our application.



	Test Strip	Test Sample	CN <sup>-</sup> Concentration
<b>A</b>		Analysis of cyanoborohydride beads in test solution	10 – 30 mg/L (ug/mL)
<b>B</b>		Analysis of CN <sup>-</sup> leaching from beads from 5 mL elution with distilled water	1 – 3 mg/L (ug/mL)
<b>C</b>		Analysis of CN <sup>-</sup> leaching after 10 mL elution with distilled water	1 – 3 mg/L (ug/mL)
<b>D</b>		Analysis of distilled water	0 mg/L (ug/mL)
<b>E</b>		Analysis of CN <sup>-</sup> concentration in final radiotracer formulation	0 mg/L (ug/mL)

**Table 2.** Semi-quantitative analysis of free cyanide by-products from the solid-supported cyanoborohydride cartridges: **A**, analysis of the solid-supported cyanoborohydride beads directly in the test kit solution (positive control); **B** & **C**, sample of eluent from the beads, simulating CN<sup>-</sup> content in the reaction vessel; **D**, analysis of distilled water (negative control); **E**, analysis of the radiotracer after HPLC purification.

## 7.0 References

- 1 F. Basuli, X. Zhang, E. M. Jagoda, P. L. Choyke and R. E. Swenson, *J. Label. Compd. Radiopharm.*, , DOI:10.1002/jlcr.3623.
- 2 D. Ory, J. Van den Brande, T. de Groot, K. Serdons, M. Bex, L. Declercq, F. Cleeren, M. Ooms, K. Van Laere, A. Verbruggen and G. Bormans, *J. Pharm. Biomed. Anal.*, , DOI:10.1016/j.jpba.2015.04.009.