Supplementary Information for:

Radiosynthesis and in vivo evaluation of ^{18}F-labelled glycosylated duramycin peptides for the imaging of phosphatidylethanolamine during apoptosis

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ABBREVIATIONS

TLC	thin layer chromatography
n.d.c.	non decay corrected
[^{18}F]NFP	4-nitrophenyl 2-[^{18}F]fluoropropionate
DCM	dichloromethane
MeCN	acetonitrile
tBuOH	*tert*-butanol
DMSO	dimethylsulfoxide
DMF	dimethylformamide
TBA-HCO_3	tetrabutylammonium bicarbonate
K_{222}	kryptofix

EXPERIMENTAL

General. All reagents were purchased from commercially available sources and were used as received. Duramycin **1** (≥ 90% purity) was obtained from Sigma Aldrich (MO, USA). N-succinimidyl 4-fluorobenzoate was obtained from Advanced Biomedical Compounds (ABX; Radberg, Germany). No-carrier-added [^{18}F]fluoride ion was obtained from a PETtrace 16.5 MeV cyclotron incorporating a high pressure niobium target (GE Healthcare, USA and Cyclotek, Vic., Australia) via the ^{18}O(p,n)^{18}F nuclear reaction. [^{18}F]Fluoride ion-separation cartridges (Waters Accell Plus QMA Sep-Pak Light, MA, USA) were pre-conditioned with the base employed in the radiofluorination step (K_2CO_3 or $KHCO_3$). Reversed phase SPE cartridges (Phenomenex, Strata X 33 μ polymeric reversed phase (30 mg/mL), CA, USA) were pre-conditioned with ethanol and water before use. Radio-HPLC analyses were performed using a Shimadzu HPLC (SCL-10AVP system controller, SIL-10ADVP auto injector, LC-10ATVP solvent delivery unit, CV-10AL control valve, DGU-14A degasser, and SPD-10AVPV detector, Kyoto, Japan) coupled to a scintillation detector (Ortec 276 Photomultiplier Base with Preamplifier, Ortec 925-SCINT ACE mate Preamplifier, Amplifier, BIAS supply and SCA, and a Bicron 1 M 11/2 Photomultiplier Tube). Manual radio-HPLC purifications were achieved using a similar system fitted with a manual injector. Semi-preparative HPLC purification of non-radioactive materials was performed using an Agilent 1100 series HPLC system. Analytical HPLC was performed on an Agilent 1100 series HPLC system. The following columns, solvents and flow rates were used for the purification and analysis of both radioactive and non-radioactive peptides:

Method A: Luna 5μ C18(2) 100Å. 150 × 21.2 mm, (0.1% TFA in 10–80% MeCN:H_2O over 85 min), 4 mL/min. These conditions were used for the semi-preparative HPLC purification of non-radioactive peptides.

Method B: Luna 5 μ C18(2) 100 Å, 250 × 10.0 mm, 5μ, (0.1% TFA in 10–80% MeCN:H_2O over 40 min) 4 mL/min. These conditions were used for the semi-preparative HPLC purification of radioactive peptides.

Method C: Phenomenex Jupiter 5u Proteo 90 Å, 250 × 4.6 mm, (0.1% TFA in 2–80% MeCN:H_2O over 40 min), 1 mL/min. These conditions were used for the QC analysis of radioactive peptides.

MS data was obtained using Agilent 6510 Q-TOF LC/MS mass spectrometer (CA, USA) equipped with an Agilent 1100 LC system.

Radiosynthesis of [^{18}F]NFP **3** was conducted as reported by Haskali et. al.[1] The radiosynthesis of [^{18}F]SFB **4** was conducted as reported by Scott and Shao.[2] Fmoc-aminomethylgalacturonic acid (Fmoc-Gal*-OH **6)** was synthesized as reported.[1,3]

LogD Measurment. A portion of the corresponding isolated radiolabelled peptide (about 37 MBq) was isolated on a C18 cartridge and eluted with MeCN (1 ml). MeCN was evaporated by a stream of nitrogen and the radiolabelled peptide was reconstituted in PBS buffer (1 ml, pH: 7.4, 1 M) and aliquots (300 μL) were dispensed in ependorf tubes. n-Octanol (300 μL) was added and the mixture was vortexed for 5 minutes. The mixture was then centrifuged at 1100 rpm for 5 min. 100 μL of the n-octanol and PBS layers were dispensed into separate tubes and diluted to 1 ml with the corresponding solvent. The activity in each solvent layer was measured using a well counter (187-950-A100 MCA; Biomedex Medical Systems attached to amulti channel analyzer interfaced with Atomlabs 950 software). Log D at pH 7.4 was then calculated by taking the log of the ratio of activity in PBS/n-octanol.

FB-Duramycin 7. To duramycin **1** (2 mg, 0.9 μmole) in 1:1 DMF:phosphate buffer (1 ml, pH 8, 0.1 M) was added *N*-hydroxysuccinimidyl 4-fluorobenzoate (0.3 mg, 1.08 μmole). The mixture was left for 1 hr at room temperature, then water (5ml) was added and the mixture was purified by HPLC (Method A).

Fractions collected were lyophilised to afford the title compound **7** as a white foam (≥99% purity). ESI-MS; *m/z* 2136 $[M+H]^+$, 1068 $[M+2H]^{2+}$, 711 $[M+3H]^{3+}$.

[^{18}F]FB-Duramycin 8. *N*-Hydroxysuccinimidyl 4-[^{18}F]fluorobenzoate ([^{18}F]SFB) **4** isolated on a C18 cartridge was eluted with DMF (0.3 mL) into a small vial charged with duramycin **1** (0.5 mg, 0.2 μmol) in a lead pot. Phosphate buffer (0.3 mL, pH 8, 0.1 M) was added and the vial was heated at 40 °C for 30 min in an aluminium block. The mixture was diluted with aqueous 0.1 % TFA (3 ml) and purified by HPLC (Method B). The HPLC fraction containing the product was diluted with sterile water (20 ml) and isolated on a C18 cartridge. The cartridge was washed with PBS (5 ml) and eluted with ethanol (0.6 ml). The eluent was diluted with PBS (7 ml) and filtered (Millex GV, 0.22 μm, 33 mm) into a sterile vial, to obtain fully sterile [^{18}F]FB-Duramycin **8** (20 mCi, 25 % radiochemical yield from [^{18}F]SFB n.d.c., specific activity, 2.5 Ci/μmol). Log $D_{7.4}$: 0.22 ± 0.05 (n = 3). Retention time of the analytical sample matched that of the cold FB-duramycin **7** reference standard.

Galacto-duramycin 9. To Fmoc-Gal*-OH **6 (**0.7 mg, 1.6 μmol) in THF (1 ml) was added DCC (0.33 mg, 1.6 μmol) and *N*-hydroxysuccinimide (0.19 mg, 1.6 μmol). After 1 hr, duramycin **1** (3 mg, 1.4 μmol) in DMF (300 μL) was added to the reaction mixture. After standing for 1 hr, DBU (65 μL, 5% v/v) was added and the mixture left for 5 min at rt. Water (5 ml) was added and the mixture was purified by HPLC (Method A). Fractions collected were lyophilised to afford the title compound **9** as a white foam (≥99% purity). ESI-MS; *m/z* 2203 $[M+H]^+$, 1101 $[M+2H]^{2+}$, 734 $[M+3H]^{3+}$.

FP-Galacto-duramycin 10. To a solution of NFP **3** (0.24 mg, 0.56 μmol) and galacto-duramycin **9** (1 mg, 0.45 μmol) in DMF (0.3 ml) was added Et_3N (5 μL, 3.59 μmol). The reaction was stirred for 1 hr then was diluted with water (2.5 ml) and purified by HPLC (Method A). Fractions collected were

lyophilised to afford the title compound **10** as a white foam (≥99% purity). ESI-MS; *m/z* 2276 $[M+H]^+$, 1138 $[M+2H]^{2+}$, 759 $[M+3H]^{3+}$.

[^{18}F]FP-Galacto-duramycin 11. To galacto-duramycin **9** (0.50 mg, 0.23 μmol) was added [^{18}F]NFP **3** (80 mCi, 1.08 μmol) in DMSO (0.3 μl). Et_3N (10 μL of 2% solution in DMSO) was added and the reaction was left for 5 min then water (1.5 ml) was added and the mixture was purified by HPLC (Method B) to afford the title compound **11** in a clear solution (36 mCi, 45 % yield from [^{18}F]NFP **3**, n.d.c.) . The peptide was formulated according to the general procedure. Retention time of the analytical sample matched that of the cold FP-galacto-duramycin **10** reference standard.

Digalacto-duramycin 12. A solution of Fmoc-Gal*-OH **6** (2.5 mg, 6.0 μmol), HATU (2.3 mg, 6.1 μmol) and HOAt (0.8 mg, 6.1 μmol) in DMF (0.5 ml) was added to duramycin **1** (0.33 mg, 1.6 μmol). After 15 min, DBU (25μL, 5% v/v) was added and the mixture was stirred for 5 min. Water (5 ml) was added and the mixture was purified by HPLC (Method A). Fractions collected were lyophilised to afford the title compound as a white foam (≥99% purity). ESI-MS; *m/z* 2391 $[M+H]^+$, 1196 $[M+2H]^{2+}$, 797 $[M+3H]^{3+}$.

FP-Digalacto-duramycin 13. To a solution of 2-fluoropropionic acid (2.1 μL of 4 μL/200 μL DMF, 0.54 μmol), HATU (10 μL of 4.2 mg/200 μL DMF, 0.54 μmol) and HOAt (3.80 μL of 4 mg/200μL DMF, 0.54 μmol) in DMF (0.2 ml) was added digalacto-duramycin **12** (1 mg, 0.45 μmole). After 15 min, DBU (10.8 μL, 5% v/v) was added and the mixture was stirred for 5 min. Water (5 ml) was added then the mixture was purified by HPLC (Method A). Fractions collected were lyophilised to afford the title compound **13** as a white foam (≥99% purity). ESI-MS; *m/z* 2203 $[M+H]^+$, 1101 $[M+2H]^{2+}$, 734 $[M+3H]^{3+}$.

[^{18}F]FP-Digalacto-duramycin 14. To digalacto-duramycin **13** (1.00 mg, 0.45 μmol), a solution of [^{18}F]NFP **3** (80 mCi, 1.08 μmol) in DMSO (0.3 ml) was added at 50 °C. Et_3N (20.0 μL) was added and the reaction was left for 5 min at 50 °C. Water (1.5 ml) was added and the mixture was purified by HPLC (Method B). The HPLC fraction containing the product was diluted with sterile water (20 ml) and isolated on a C18 cartridge. The cartridge was washed with PBS (5 ml) and eluted with ethanol (0.6 ml). The eluent was diluted with PBS (7 ml) and filtered into a sterile vial, to obtain fully sterile [^{18}F]FP-digalacto-duramycin **14** (10.4 mCi, 13 % radiochemical yield from [^{18}F]NFP n.d.c., specific activity, 3.0 Ci/μmol). Log D: –2.6 ± 0.04 (n = 3). Retention time of the analytical sample matched that of the cold reference compound **13**.

Small-Animal PET

All procedures involving mice were performed in accordance with the National Health and Medical Research Council animal ethics guidelines and were approved by the Peter MacCallum Cancer Centre Ethics committee (approval number E445).

DAB/2J mice were injected via the lateral tail vein with ~20 MBq of radiotracer in 100 μL of saline. After 110 min post-injection, mice were anesthetized by inhalation of 2.5% isoflurane/50% O2 in air delivered at a flow rate of 200 mL/min and scanned on a Mosaic small-animal PET device (Philips Medical Systems) as previously described.[4] The resolution of this system was 2.7 mm at the center of the field of view (FOV).[5] The energy window was 450–700 keV, and the coincidence-timing window was 6 ns. Data were acquired in 3-dimensional mode and corrected for decay and randoms. Acquisition time was 10 min per bed position. Reconstruction was performed with the 3-dimensional RAMLA algorithm.[6,7] Scatter and attenuation corrections were not applied.

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