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General experimental

Air and moisture sensitive reactions were carried out under an atmosphere of argon in oven-dried glassware. Room temperature (rt) refers to 18-25 °C. All evaporations and concentrations were performed under reduced pressure (*in vacuo*). All reagents use for compound preparation (from Sigma Aldrich UK, Fluka UK, Alfa Aesar UK, Acros UK or Fisher UK) were of synthetic grade and were used without further purification, unless stated otherwise. When necessary, reagents were dried or purified prior to use according to standard methods.[1] Anhydrous solvents (DCM, THF, Et_2O) were obtained from MBraun MB SPS-800 solvent purification system by passage through two drying columns and dispensed under an argon atmosphere. Anhydrous MeOH and MeCN were distilled from calcium hydride in a recycling still.[1]

The course of reactions was followed by thin-layer chromatography (TLC) using aluminium plates coated with silica gel ($60F_{245}$ Merck). TLC plates were examined under UV light (254 nm and 266 nm) before being visualised with ammonium heptamolybdate, anisaldehyde-sulfuric acid, alkaline potassium permanganate or ceric sulfate-sulfuric acid and developed by heating. Column chromatography was performed using silica gel 60 (40–63 μm) under a positive pressure of compressed air, eluting with solvents (reported as v/v) as supplied. Reverse phase column chromatography was performed using Thermo Scientific HyperSep(tm) C18 cartridges.

NMR spectra were recorded on Bruker Advance 300, 400 or 500 instruments. ^{1}H spectra were recorded using deuterated solvent as the lock and the residual solvent signal as the internal standard and for ^{13}C NMR spectra signal of deuterated solvent was used as the internal standard. ^{19}F NMR spectra were referenced to $CFCl_3$ as an external standard. Chemical shifts are reported in parts per million (ppm) and coupling constants (J) are given in Hertz (Hz). The abbreviations for the multiplicity of the proton, carbon and fluorine signals are as follows: s singlet, d doublet, dd doublet of doublets, ddd doublet of doublet of doublets, t triplet, dt doublet of triplets, q quartet, m multiplet, br s broad singlet. When necessary, resonances were assigned using two-dimensional experiments (COSY, HSQC, HMBC, TOCSY). Compounds are numbered according to customary purine numbering.

High resolution electrospray ionisation mass spectra were obtained on a Micromass LCT or ThermoFisher Excalibur Orbitrap spectrometers operating in positive or negative mode, from solutions in MeOH, MeCN or water by the Mass Spectrometry Service at the University of St Andrews. HPLC analyses/semi-preparations for non-radioactive experiments were performed using a Shimadzu Prominence (SIL-20A HT autosampler, CL-20AT ternary pump, DGU-20A3R solvent degasser, SPD 20A UV detector and CBM-20A controller module) with reverse phase column as indicated in individual experiment. Samples were freeze dried from frozen solutions in water or tBuOH in water (20% v/v) in a Christ Alpha 1-2 LO Plus freeze drier.

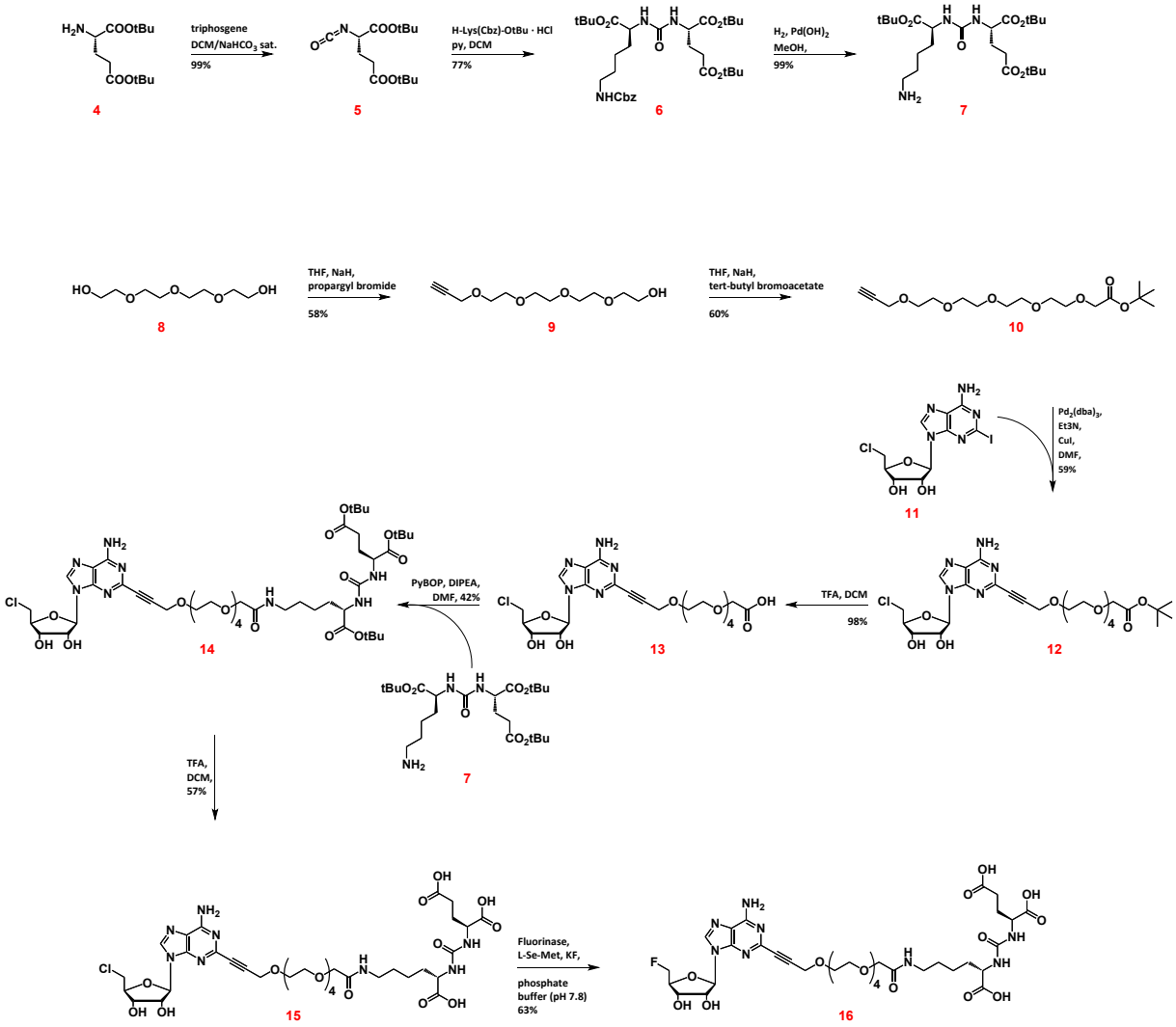
$[^{18}F]$Fluoride was produced on a cyclotron by proton bombardment of 97% enriched $[^{18}O]H_2O$ (Cambridge Isotope Laboratories, Inc.) by the $^{18}O(p,n)^{18}F$ nuclear reaction. The silver target (1.1ml) was pressurised to 600psi and irradiated with 11 MeV protons produced by the CTI/SIEMENS RDS-111 cyclotron at the John Mallard Scottish PET Centre in Aberdeen. At the end of bombardment (EOB) the target was unloaded within 5 min using argon gas.

HPLC analyses of radioactive compounds were performed using a Shimadzu HPLC system equipped with a SPD-M20A Prominence DAD UV detector and NaI radio-detector (Berthold Technologies). Semi-

prep HPLC purification of radioactive compounds were performed using a lead shielded Shimadzu semiprep HPLC system equipped with a SPD-M20A Prominence DAD UV detector and NaI radio-detector (Berthold Technologies). The dose calibrators used to measure doses were CAPINTEC CRC 15R and CAPINTEC CRC 15PET.

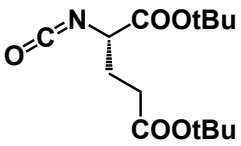
Protein concentrations were measured on a NanoDrop 1000 spectrophotometer at 280 nm using an extinction coefficient of 10.42 as calculated by ExPASy ProtParam tool.[2] SDS-PAGE was run using NuPAGE 4–12% BisTris gels (Novex) in MOPS SDS running buffer. Proteins were visualised using Instant Blue Coomassie-based gel stain (Expedion).

Compound preparation



Scheme S1. Synthesis of (*t*-butyl protected)GUL 7, ClDA-PEG-GUL 15 and FDA-PEG-GUL 16.

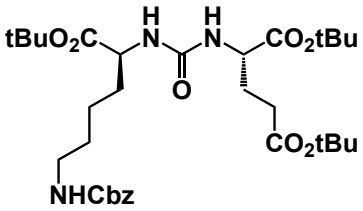
di-tert-butyl (S)-2-isocyanatopentanedioate (5)



H-Glu(*Ot*BU)-O*t*Bu hydrochloride salt **4** (600 mg, 2.02 mmol) was suspended in a mixture of DCM (10 mL) and saturated $NaHCO_3$ (20 mL). The mixture was cooled to 0 °C and then triphosgene (300 mg, 1.01 mmol) was added in one portion. The reaction mixture was vigorously stirred at 0 °C for 20 minutes, then warmed to room temperature, diluted with DCM (10 mL) and washed with brine. The organic layers were collected, dried over Na_2SO_4 and concentrated in vacuo, affording the isocyanate

intermediate (570 mg) as a colourless oil, which was used in the following step without further purifications.

tri-tert-butyl (9S,13S)-3,11-dioxo-1-phenyl-2-oxa-4,10,12-triazapentadecane-9,13,15-tricarboxylate (6)



A solution of the isocyanate intermediate **5** (200 mg, 0.70 mmol) in dry DCM (1mL) was added dropwise to a mixture of H-Lys(Cbz)-O*t*Bu hydrochloride salt (279 mg, 0.75 mmol) and dry pyridine (61 µL, 0.75 mmol) in dry DCM (7 mL). The reaction was stirred at room temperature overnight, diluted with DCM and then extracted with HCl 0.1 M and brine. The organic layers were collected, dried over Na_2SO_4 and concentrated in vacuo. The crude material was purified by FCC (*n*Hex/EtOAc gradient from 8/2 to 4/6) affording the desired product as a colourless oil (335 mg, 77% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38-7.31 (m, 5H), 5.30- 5.25 (m, 3H), 5.15-5.07 (m, 2H), 4.39-4.31 (m, 2H), 3.24-3.14 (m, 2H), 2.37-2.23 (m, 2H), 2.11-2.03 (m, 1H), 1.89-1.73 (m, 3H), 1.67-1.27 (m, 31H); ^{13}C NMR (101 MHz, , $CDCl_3$) δ 172.51, 172.48, 172.42, 156.94, 156.63, 136.73, 128.47, 128.06, 128.0, 82.13, 81.72, 80.50, 66.53, 53.28, 52.98, 40.66, 32.64, 31.59, 29.35, 28.35, 28.08, 28.02, 28.00, 22.27. ESI-MS (*m/z*): calcd for $C_{32}H_{52}N_3O_9$ [M + H]$^+$ 622.36, found 622.4. Data are in agreement with the literature.[3]

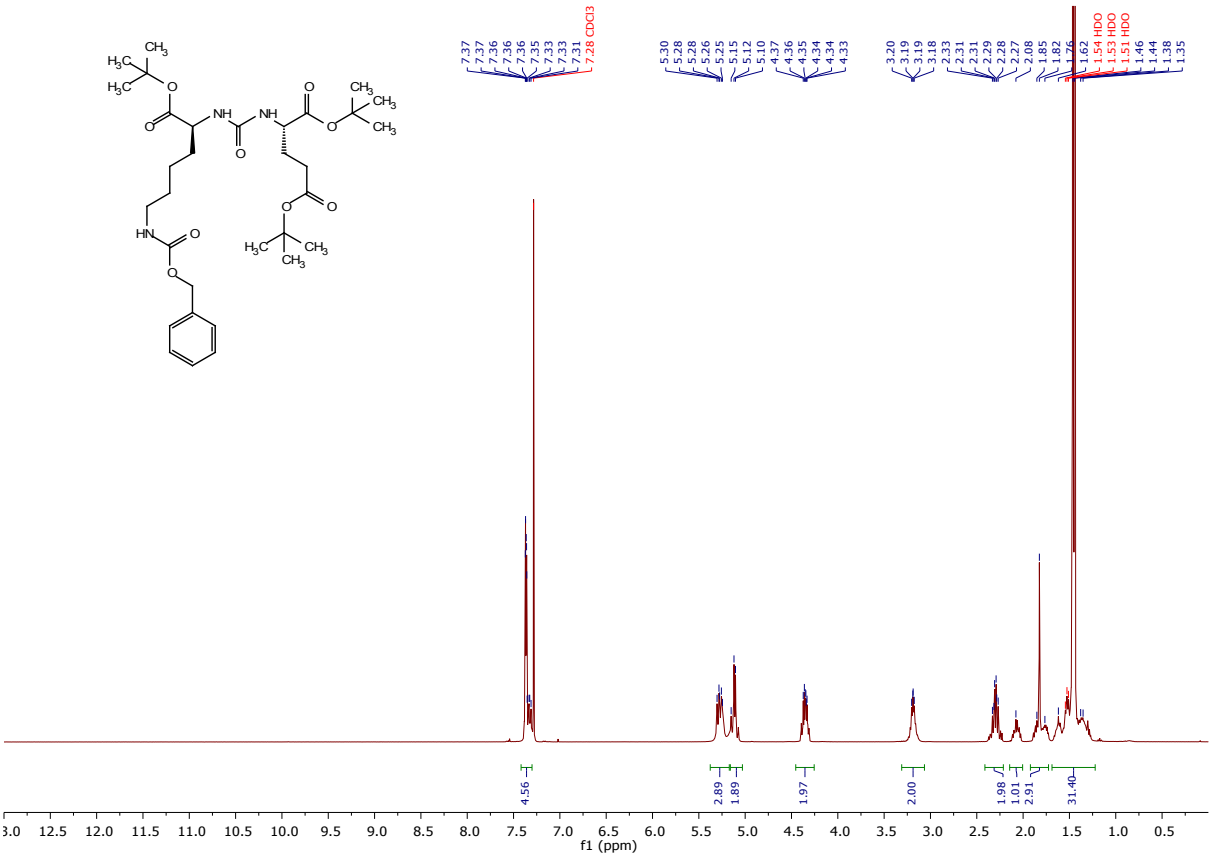


Figure S1. ^{1}H NMR spectrum (500.1 MHz, *d*$_4$-MeOD) of **6**.

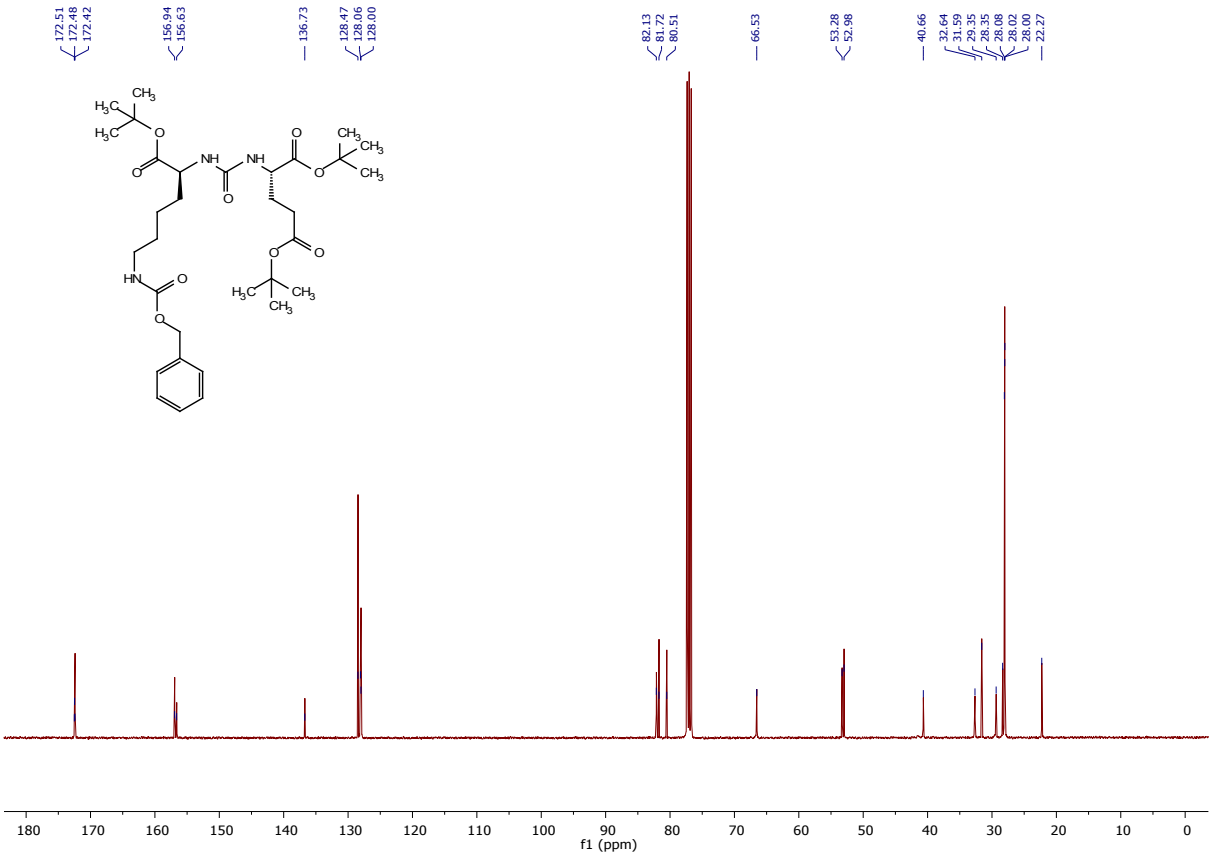
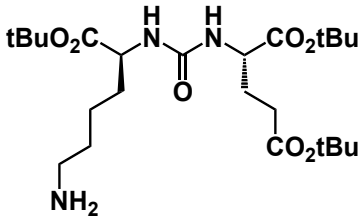


Figure S2. ^{13}C NMR spectrum (101 MHz, $CDCl_3$) of **6**.

di-tert-butyl (((S)-6-amino-1-(tert-butoxy)-1-oxohexan-2-yl)carbamoyl)-L-glutamate (7)



The fully protected peptidomimetic **5** (250 mg, 0.4 mmol) was dissolved in methanol and a catalytic amount of $Pd(OH)_2$ was added. The reaction was stirred overnight at room temperature under H_2 (1 atm), then filtered on a Celite pad and the solvent evaporated under reduced pressure to afford the desired product (194 mg) as a colourless oil without further purification. ^1H NMR (400 MHz, $CDCl_3$) δ 5.15-5.12 (m, 2H), 4.38-4.32 (m, 2H), 2.69 (t, *J* = 6.8 Hz, 2H), 2.40-2.25 (m, 2H), 2.13-2.04 (m, 1H), 1.91-1.75 (m, 2H), 1.68-1.58 (m, 1H), 1.50-1.29 (m, 31 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.54, 172.46, 172.16, 156.78, 82.04, 81.71, 80.52, 53.47, 53.03, 41.91, 33.36, 33.04, 31.60, 28.42, 28.08, 28.03, 28.01, 22.37; ESI-MS (*m/z*): calcd for $C_{24}H_{46}N_3O_7$ $[M + H]^+$ 488.33, found 488.3. Data are in agreement with the literature.[3]

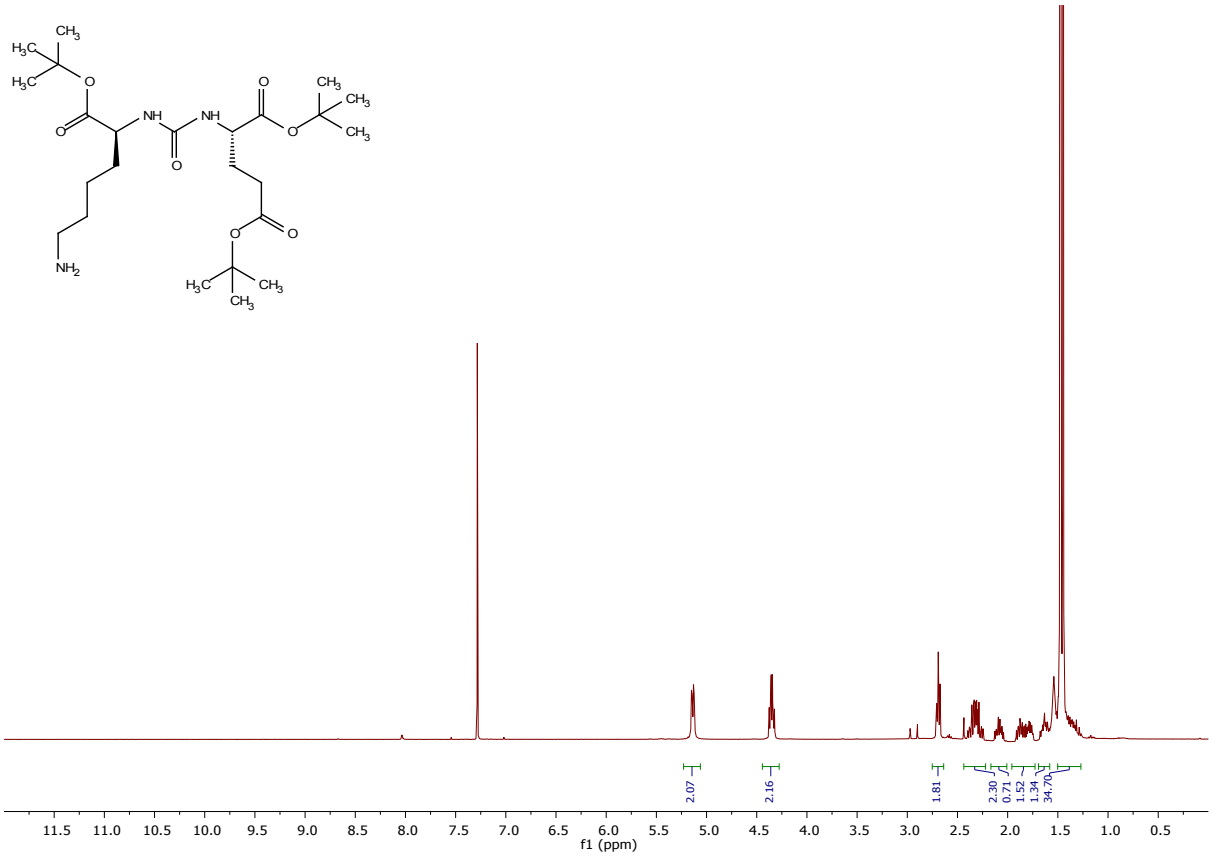


Figure S3. ^{13}C NMR spectrum (101 MHz, $CDCl_3$) of **7**.

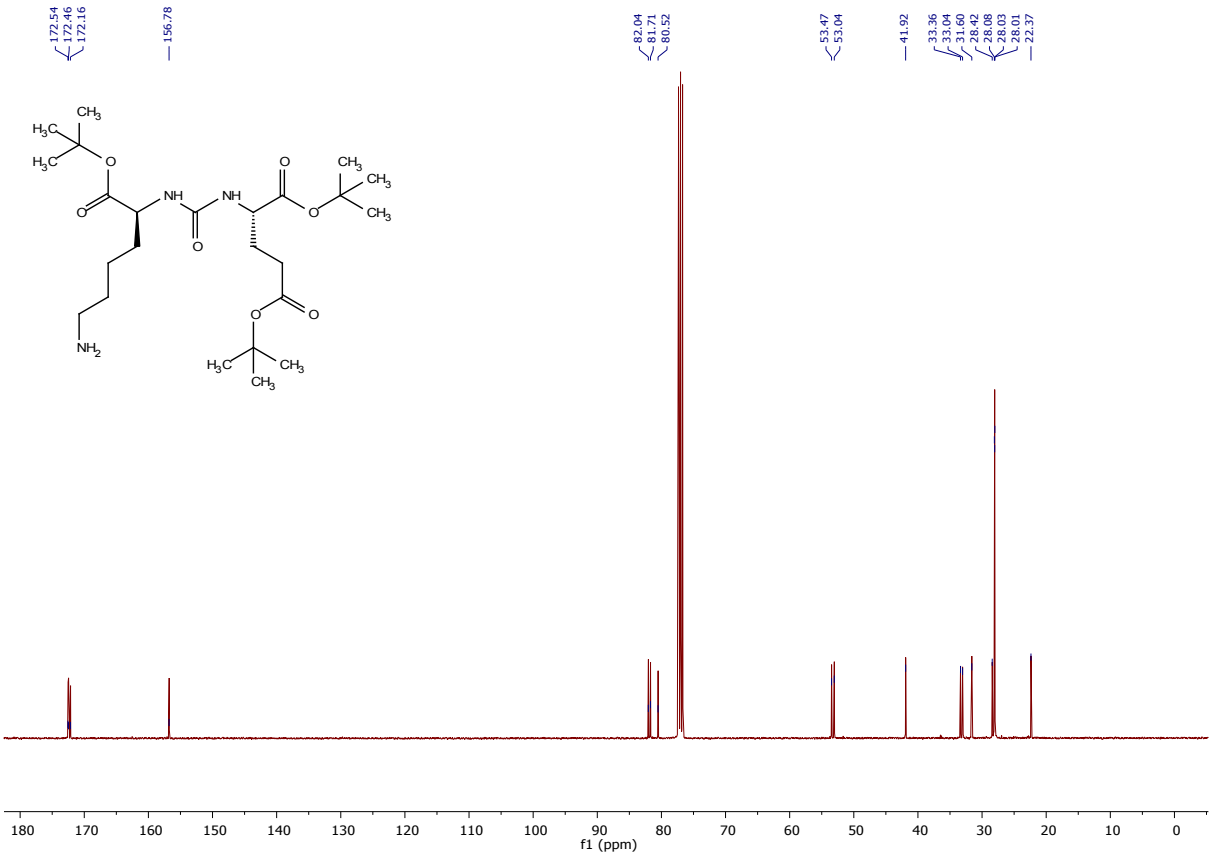
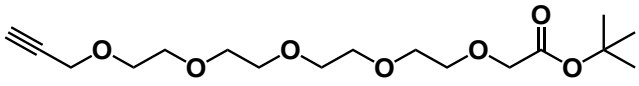


Figure S4. ^{13}C NMR spectrum (101 MHz, $CDCl_3$) of **7**.

tert-Butyl 3,6,9,12,15-pentaoxaoctadec-17-ynoate (10)



tert-Butyl 3,6,9,12,15-pentaoxaoctadec-17-ynoate (**10**) was synthesised *via* a 2 step procedure, using previously reported methods[4]; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 4.23 (d, *J* = 2.4 Hz, 2H, CH_2C≡CH), 4.04 (s, 2H, OC$\underline{H}_2$(C=O)), 3.79 – 3.66 (m, 16 H, PEG CH_2), 2.45 (t, *J* = 2.4 Hz, 1H, CH_2C≡C*H*), 1.50 (s, 9H, Boc CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.7 (Boc *C*=O), 81.6 ($(CH_3)_3$*C*), 79.7 ($CH_2\underline{C}$≡C), 74.5 (CH_2C≡*C*H), 70.7, 70.63, 70.60, 70.4, 69.13 (PEG $\underline{C}H_2$), 69.05 (*C*H_2C(C=O)), 58.4 (*C*H_2C≡CH), 28.1 (Boc *C*H_3); HRMS (ESI$^+$) calculated for $C_{17}H_{30}O_7Na$ [M+Na]$^+$ 369.1889, found 369.1882. Data are in agreement with the literature.[4]

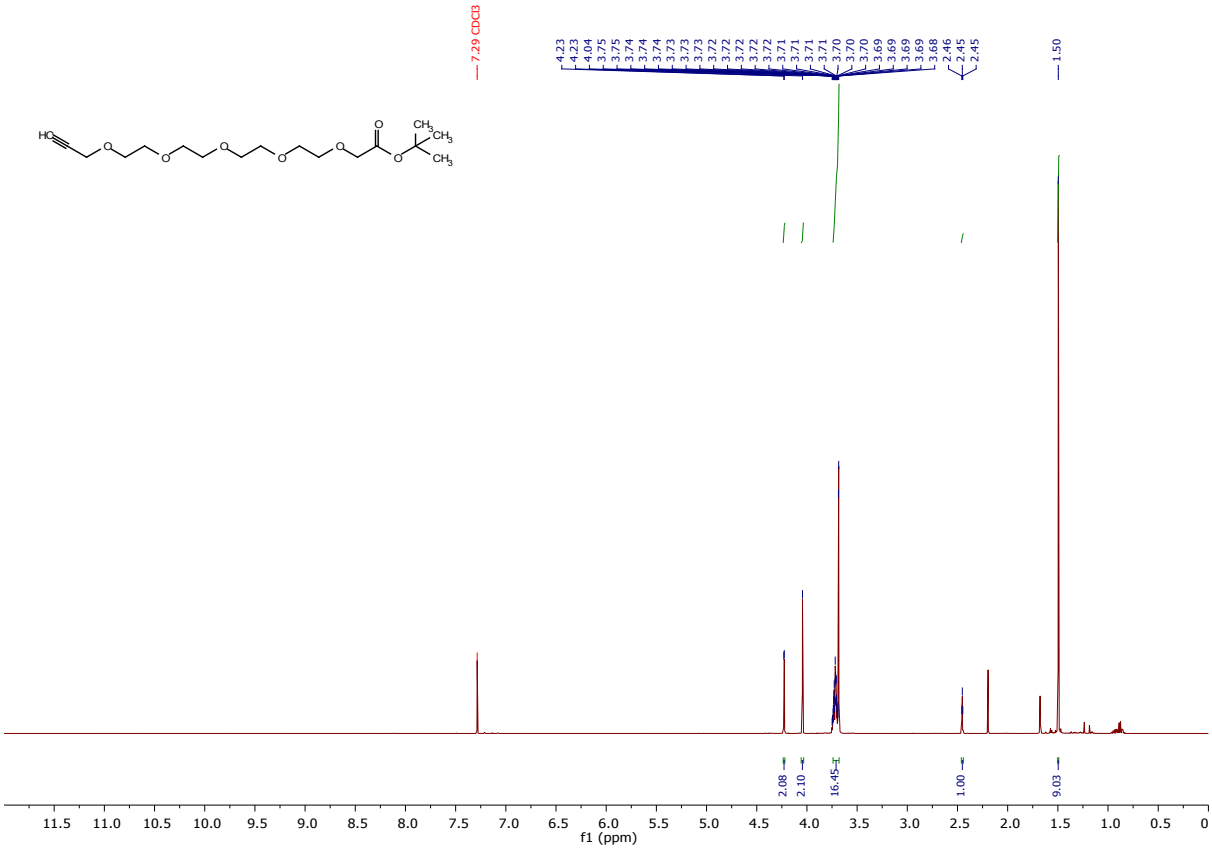


Figure S5. ^{1}H NMR spectrum (400.1 MHz, $CDCl_3$) of tert-Butyl 3,6,9,12,15-pentaoxaoctadec-17-ynoate **10**.[4]

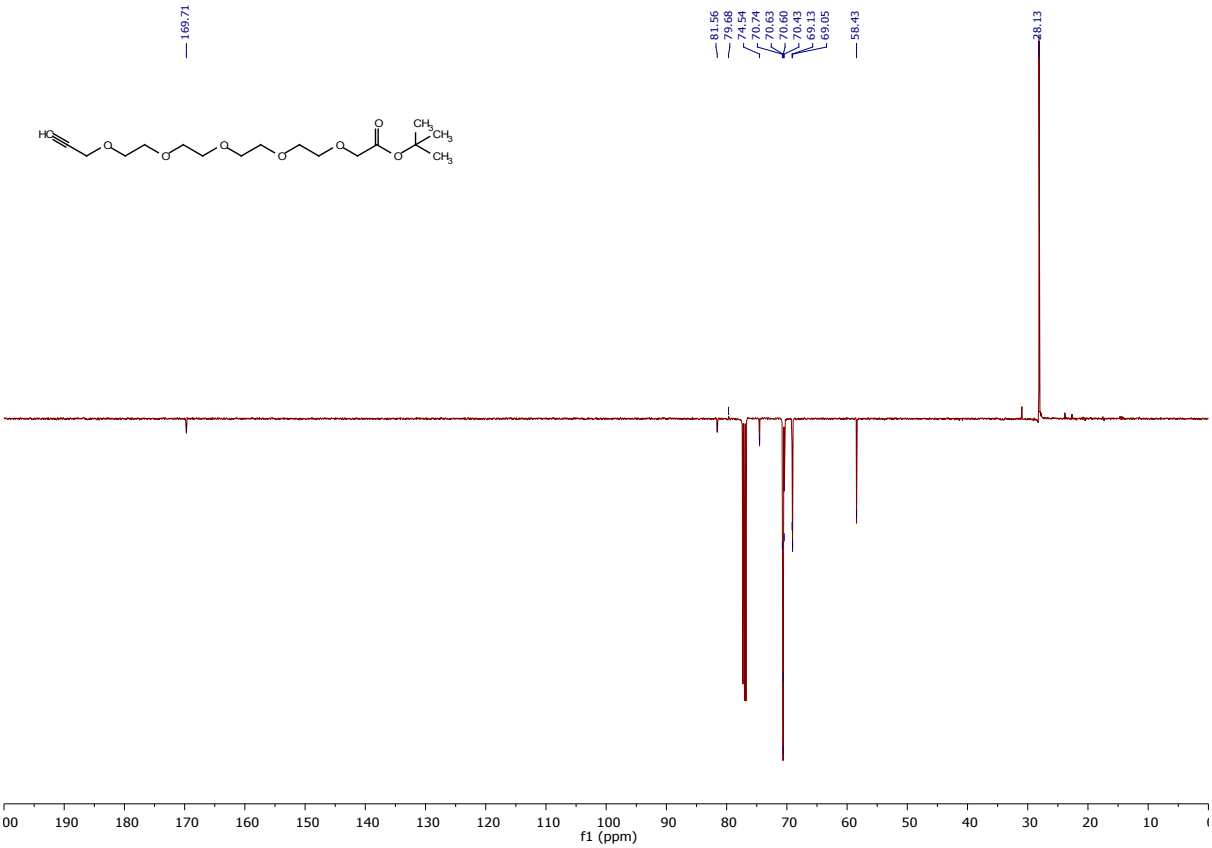
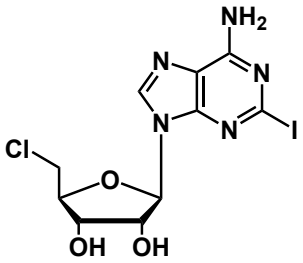


Figure S6. ^{13}C NMR spectrum (126 MHz, $CDCl_3$) of tert-Butyl 3,6,9,12,15-pentaoxaoctadec-17-ynoate **10**.[4]

5′-Chloro-5′-deoxy-2-iodoadenosine (11)



5′-Chloro-5′-deoxy-2-iodoadenosine **11** was synthesised using a 5 step procedure, using previously reported methods[5]; ^{1}H NMR (500.1 MHz, *d4*-MeOD) δ 8.17 (s, 1H, Ade H-8), 5.97 (d, *J* = 5.1 Hz, 1H, ribose H-1′), 4.79 (dd, *J* = 5.2, 5.1 Hz, ribose H-2′), 4.41 (dd, *J* = 5.2, 4.2 Hz, ribose H-3′), 4.30 – 4.27 (m, 1H, ribose H-4′), 3.97 (dd, *J* = 11.8, 5.3 Hz, 1H, ribose H-5′a), 3.87 (dd, *J* = 11.8, 5.3 Hz, 1H, ribose H-5′b); ^{13}C NMR (126 MHz, *d4*-MeOD) δ 155.7 (C-6), 149.5 (C-4), 139.8 (C-8), 119.3 (C-5), 119.1 (C-2), 89.1 (C-1′), 84.1 (C-4′), 73.4 (C-2′), 71.4 (C-3′), 43.6 (C-5′); m/z (ES^{+}) 412 $[M+H]^{+}$, 434 $[M+Na]^{+}$; HRMS calculated for $C_{10}H_{12}ClIN_5O_3$ $[M+H]^{+}$ 411.9673, found 411.9668. Data are in agreement with the literature.[5]

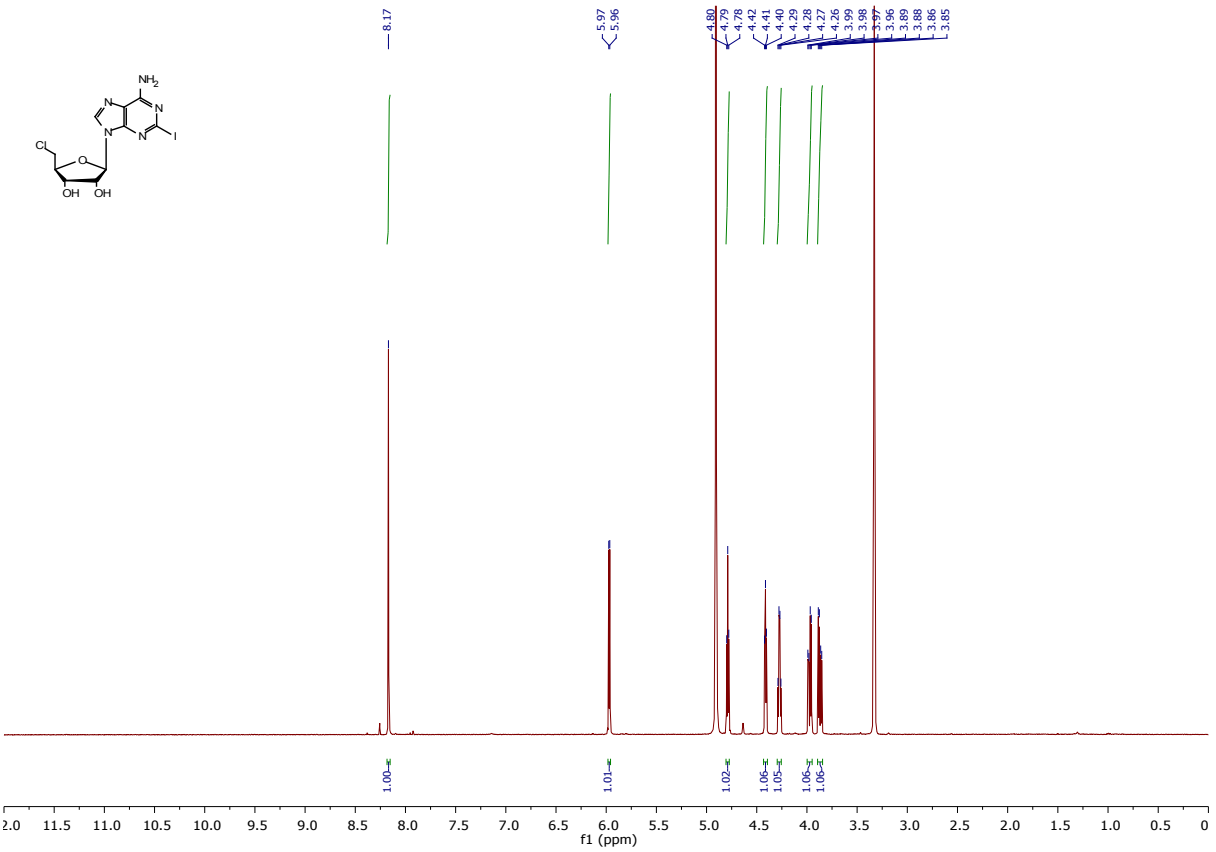


Figure S7. ^{1}H NMR spectrum (400.1 MHz, *d4*-MeOD) of 5′-Chloro-5′-deoxy-2-iodoadenosine **11**.[4]

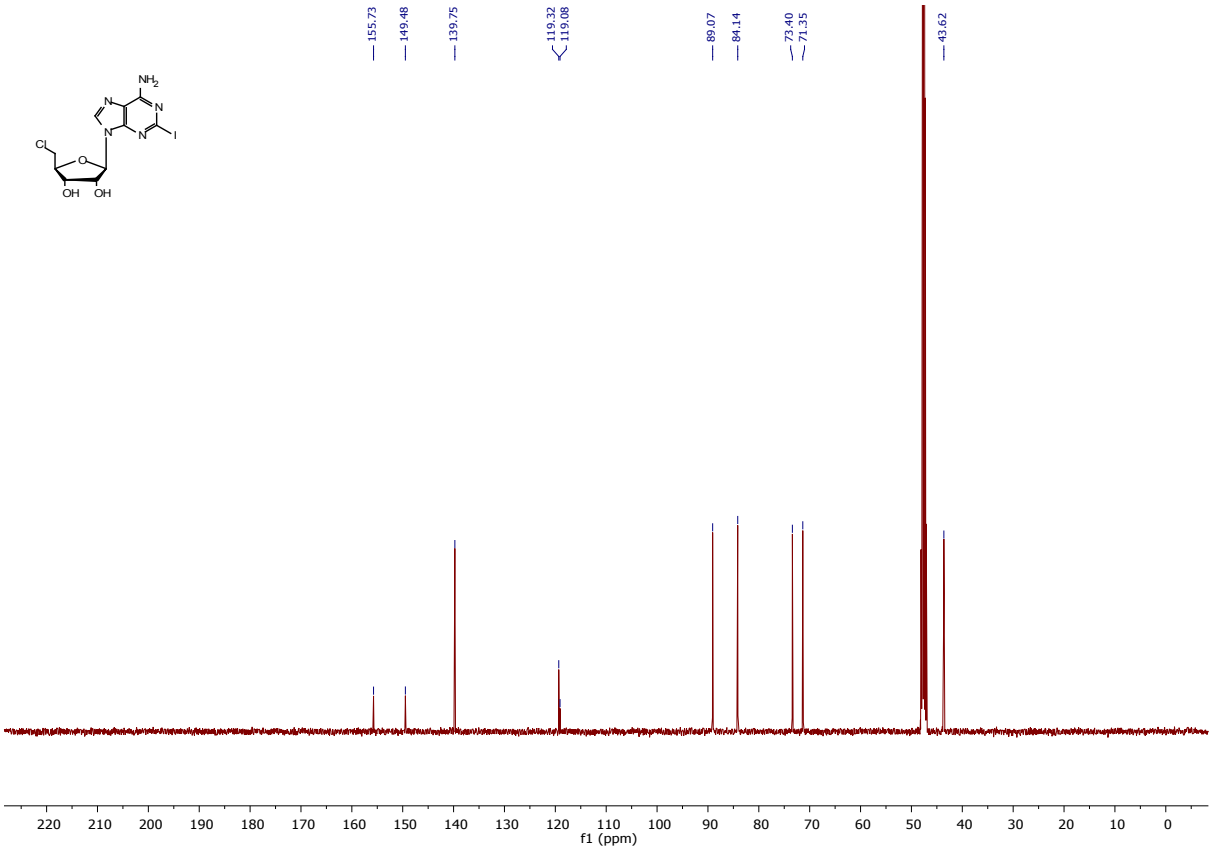
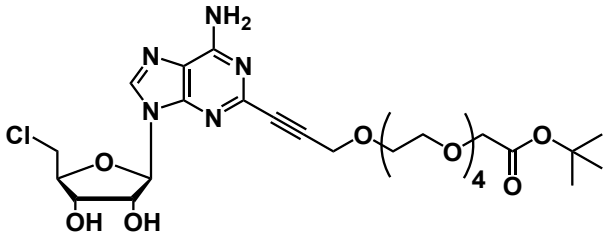


Figure S8. ^{13}C NMR spectrum (126 MHz, *d4*-MeOD) of 5′-Chloro-5′-deoxy-2-iodoadenosine **11**.[4]

ClDA-PEG-ester (12)



ClDA-PEG-ester (**12**) was synthesised from **10** and **11** using previously reported methods[4]; ^{1}H NMR (500.1 MHz, *d4*-MeOD) δ 8.41 (s, 1H, Ade H-8), 6.05 (d, *J* = 5.0 Hz, 1H, ribose H-1′), 4.76 (dd, *J* = 5.1, 5.1 Hz, 1H, ribose H-2′), 4.51 (s, 2H, PEG OC*H*$_2$C≡C), 4.41 (dd, *J* = 5.2, 4.3 Hz, 1H, ribose H-3′), 4.30 (m, 1H, ribose H-4′), 4.05 (s, 2H, OC*H*$_2$(C=O)), 4.00 (dd, *J* = 11.9, 5.0 Hz, 1H, H-5′a), 3.89 (dd, *J* = 11.9, 5.0 Hz, 1H, H-5′a), 3.82 –3.65 (m, 16H, PEG C*H*$_2$), 1.49 (s, 9H, Boc C*H*$_3$); ^{13}C NMR (126 MHz, *d4*-MeOD) δ 170.3 (C=O), 155.8 (C-6), 149.2 (C-2), 145.7 (C-4), 140.6 (C-8), 118.8 (C-5), 88.9 (C-1′), 84.9 (C≡*C*CH$_2$), 83.9 (C-4′), 81.4 ((CH$_3$)$_3$*C*), 81.1 (*C*≡CCH$_2$), 73.6 (C-2′), 71.2 (C-3′), 70.3, 70.14, 70.12, 69.1 (PEG *C*H$_2$), 68.4 (*C*H$_2$C(C=O)), 58.0 (C≡C*C*H$_2$), 43.8 (C-5′), 27.0 (Boc *C*H$_3$); HRMS (ESI$^+$) calculated for $C_{27}H_{40}ClN_5O_{10}Na$ [M+Na]$^+$ 652.2361, found 652.2349. Data are in agreement with the literature.[4]

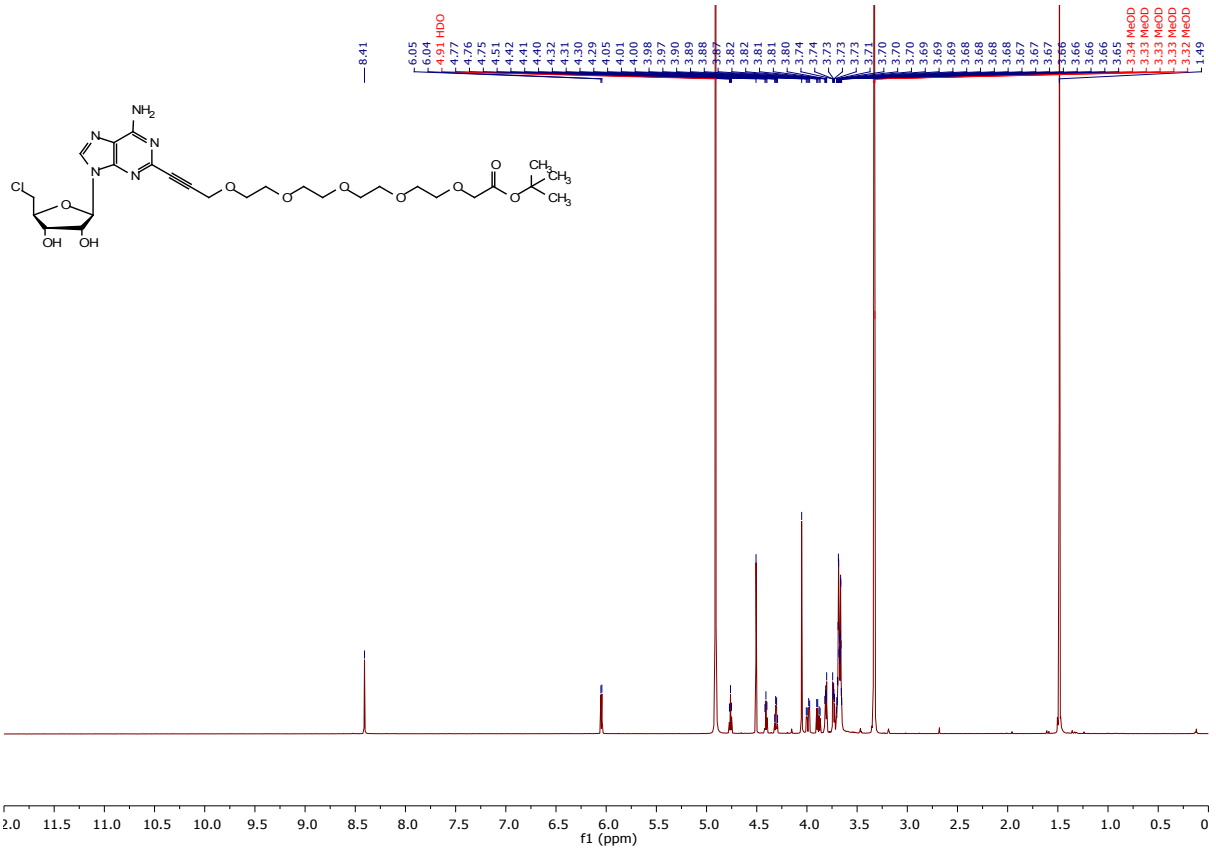


Figure S9. ^{1}H NMR spectrum (500.1 MHz, *d4*-MeOD) of ClDA-PEG-ester **12**.[4]

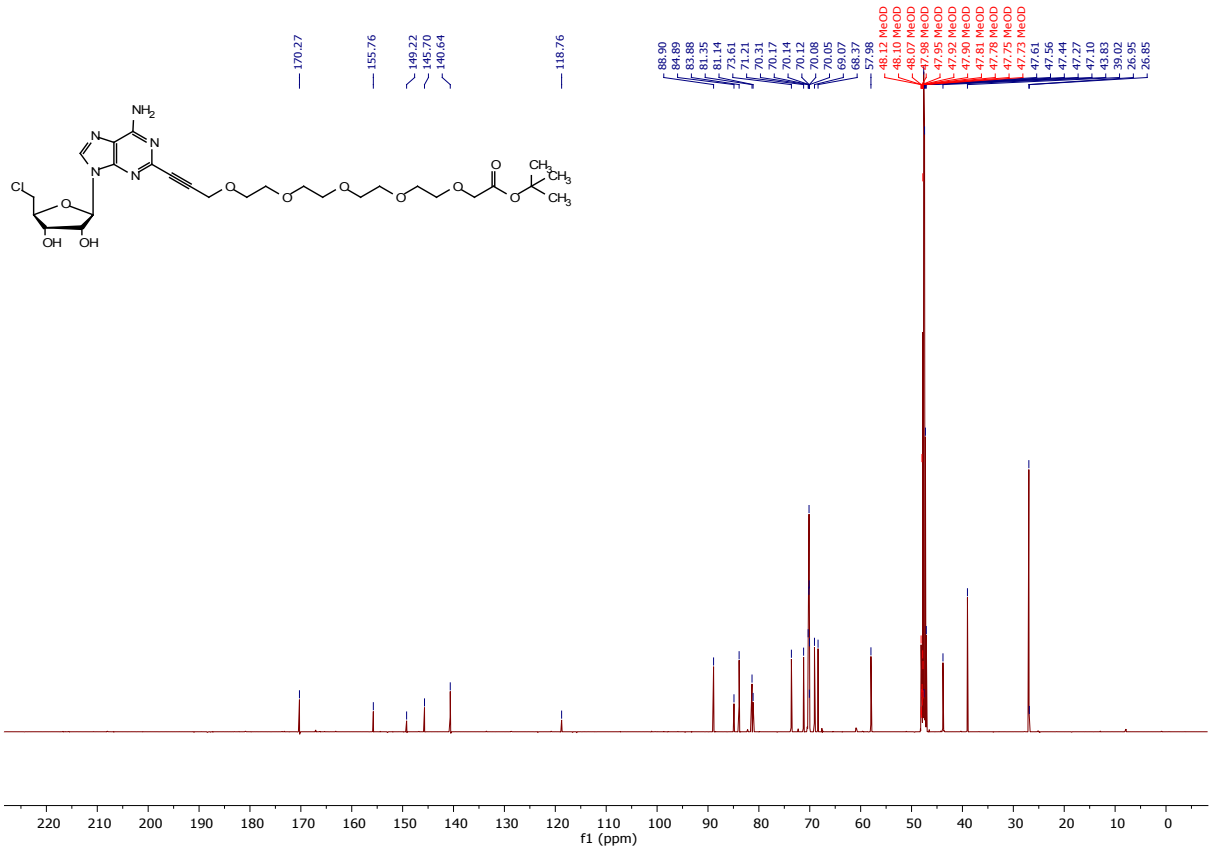
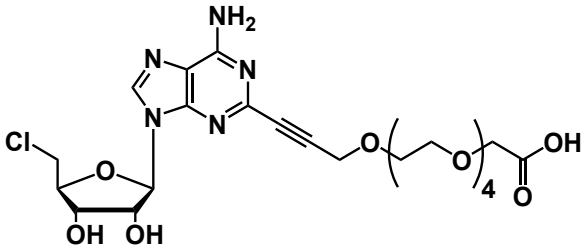


Figure S10. ^{13}C NMR spectrum (126 MHz, *d4*-MeOD) of ClDA-PEG-ester **12**.[4]

CLDA-PEG-acid (13)



ClDA-PEG-acid (**13**) was synthesised from **12** using previously reported methods[4]; ^{1}H NMR (500.1 MHz, *d4*-MeOD) δ 8.43 (s, 1H, Ade H-8), 6.05 (d, *J* = 5.0 Hz, 1H, ribose H-1'), 4.75 (dd, *J* = 5.1 Hz, 5.1 Hz, 1H, ribose H-2'), 4.52 (s, 2H, PEG OC*H*$_2$C≡C), 4.41 (dd, *J* = 5.1, 4.3 Hz, 1H, ribose H-3'), 4.32 (m, 1H, ribose H-4'), 4.15 (s, 2H, OC*H*$_2$(C=O)), 3.99 (dd, *J* = 11.9, 4.8 Hz, 1H, H-5'a), 3.89 (dd, *J* = 11.9, 4.8 Hz, 1H, H-5'b), 3.83 -3.65 (m, 16H, PEG C*H*$_2$); ^{13}C NMR (126 MHz, *d4*-MeOD) δ$_C$ 172.8 (C=O), 153.9 (C-6), 148.9 (C-2), 142.3 (C-4), 141.6 (C-8),118.8 (C-5), 89.0 (C-1'), 84.9 (C≡*C*CH$_2$), 84.0 (C-4'), 82.2 (*C*≡CCH$_2$), 73.8 (C-2'), 71.1 (C-3'), 70.3, 70.10, 70.07, 69.2 (PEG *C*H$_2$), 67.6 (*C*H$_2$C(C=O)), 57.9 (C≡C*C*H$_2$), 43.8 (C-5'); HRMS (ESI$^-$) calculated for $C_{23}H_{31}ClN_5O_{10}$ [M-H]$^-$ 572.1765, found 572.1764. Data are in agreement with the literature.[4]

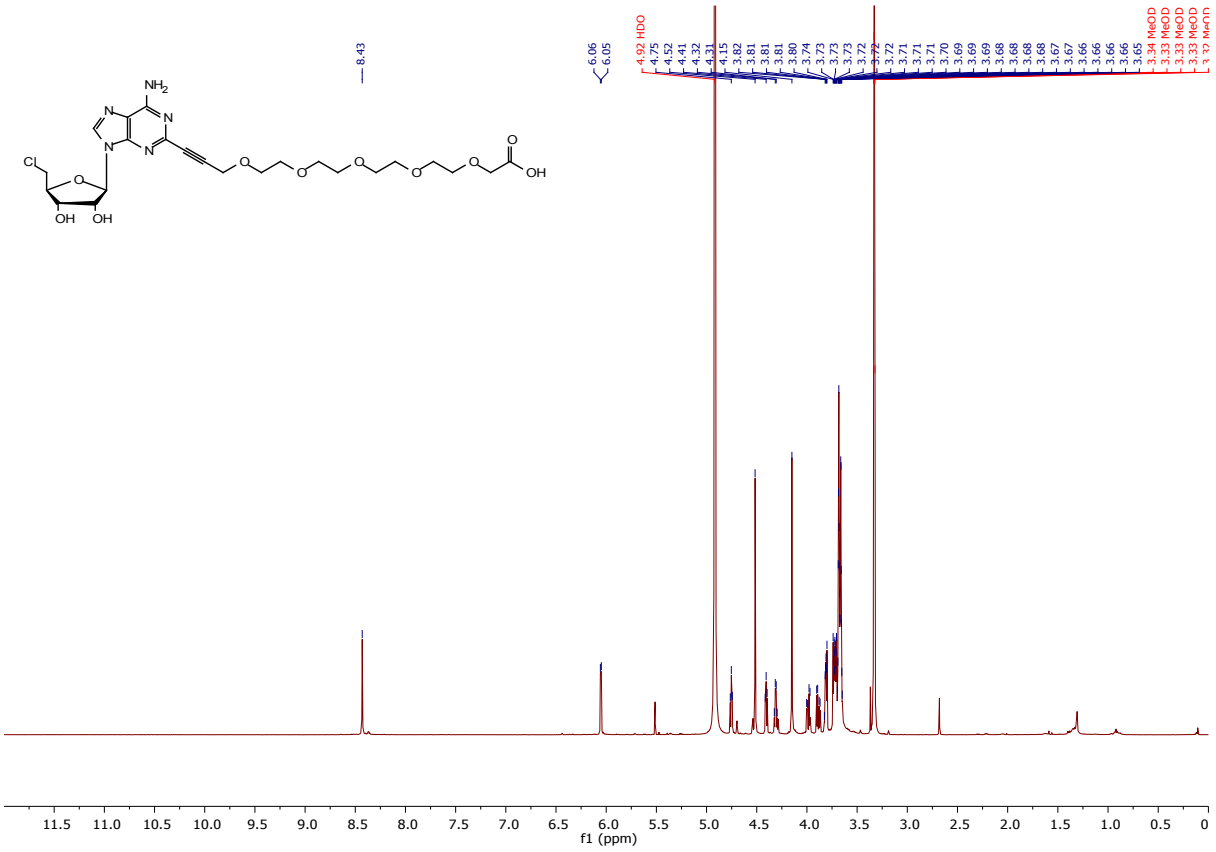


Figure S11. ^{1}H NMR spectrum (500.1 MHz, *d*$_4$-MeOD) of ClDA-PEG-acid **13**.[4]

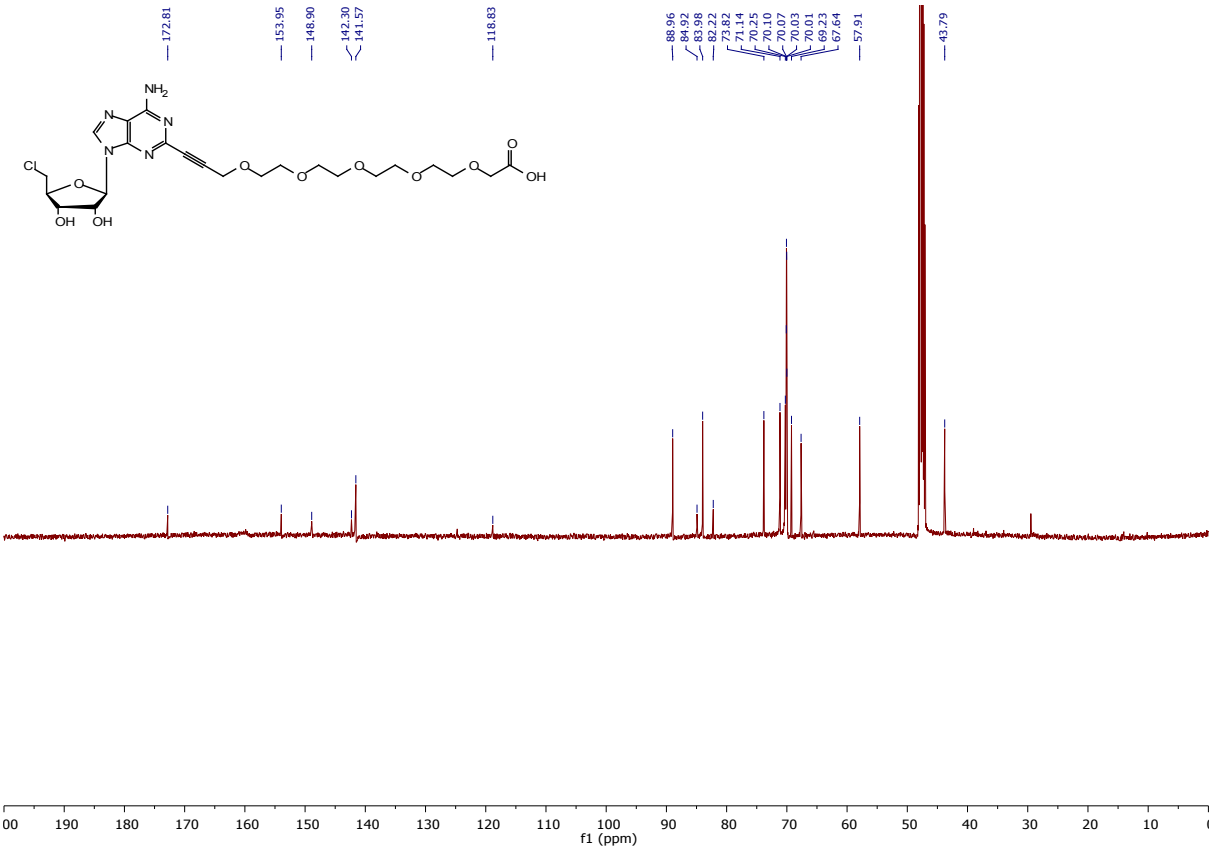
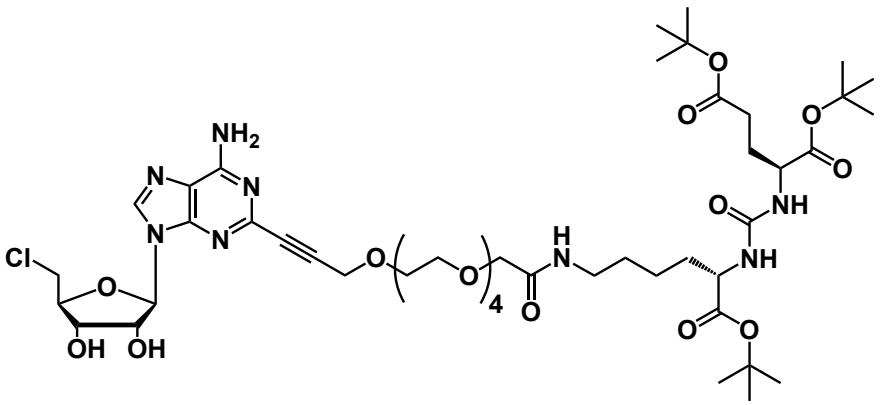


Figure S12. ^{13}C NMR spectrum (126 MHz, *d4*-MeOD) of ClDA-PEG-acid **13**.[4]

ClDA-PEG-GUL(protected) (14)



A solution of ClDA-PEG-Acid·TFA **13** (10 mg, 14.6 µmol, 1 eq.), **7** (10.62 mg, 21.8, µmol, 1.5 eq.) and PyBOP (12 mg, 23.0 µmol, 1.6 eq.) in DMF (1 mL) was prepared and cooled to 0 °C. DIPEA (5 µL, 27.0 µmol, 1.9 eq.) was slowly added over a period of 20 min, the reaction was then allowed to warm to rt and stirred for 36 h. The reaction mixture was then concentrated *in vacuo*, and the residue dissolved in DMSO:H_2O (50:50, 8 mL) and loaded onto a reverse phase cartridge (1000 mg Thermo Scientific HyperSep(tm) preconditioned with water), washed with water (10 mL), and eluted with water: MeCN (50:50, 2 × 10 mL). The fraction/s containing **14** were pooled, concentrated and further purified by semi-preparative HPLC on a Shimadzu Prominence system using a Phenomenex Luna C18 column (250 x 10.00 mm, 5µm) and a guard cartridge; Mobile phase: 0.05% TFA in water (solvent A) and 0.05% TFA

in MeCN (solvent B); linear gradient: 15% solvent B to 60% solvent B over 14 min, 60% solvent B to 95% solvent B over 3 min, 95% B for 5 min, and back to 15% B for 10 min to re-equilibrate the column. Flow rate: 2.5 mL/min $^{-1}$; detection: 254 nm. The relevant fractions were collected (t_R = 21.0 min), concentrated and lyophilized affording the product **14** (6.5 mg, 42 %) as a white solid. ^{1}H NMR (500.1 MHz, *d4*-MeOD) δ 8.34 (s, 1H, Ade H-8), 6.04 (d, *J* = 4.9 Hz, 1H, ribose H-1'), 4.78 (dd, *J* = 5.2 Hz, 5.0 Hz, 1H, ribose H-2'), 4.48 (s, 2H, PEG OC*H*$_2$C≡C), 4.41 (dd, *J* = 4.9, 4.8 Hz, 1H, ribose H-3'), 4.29 – 4.31 (m, 1H, ribose H-4'), 4.13 – 4.23 (m, 1H, α-H), 4.13 – 4.15 (m, 1H, α-H), 3.99 (dd, *J* = 11.9, 5.0 Hz, 1H, ribose H-5'a), 3.99 (s, 2H, PEG OC*H*$_2$C≡C), 3.89 (dd, *J* = 11.9, 5.0 Hz, 1H, ribose H-5'b), 3.82 -3.65 (m, 16H, PEG C*H*$_2$), 3.25 – 3.28 (m, 2H, C*H*$_2$), 2.28 – 3.39 (m, 2H, C*H*$_2$), 1.40 – 2.09 (m, 8H, 4xC*H*$_2$), 1.49 (s, 9H, Boc C*H*$_3$), 1.48 (s, 9H, Boc C*H*$_3$), 1.46 (s, 9H, Boc C*H*$_3$); HRMS (ESI$^+$) calculated for $C_{47}H_{75}ClN_8NaO_{16}$ [M+Na]$^+$ 1065.4887, found 1065.4882.

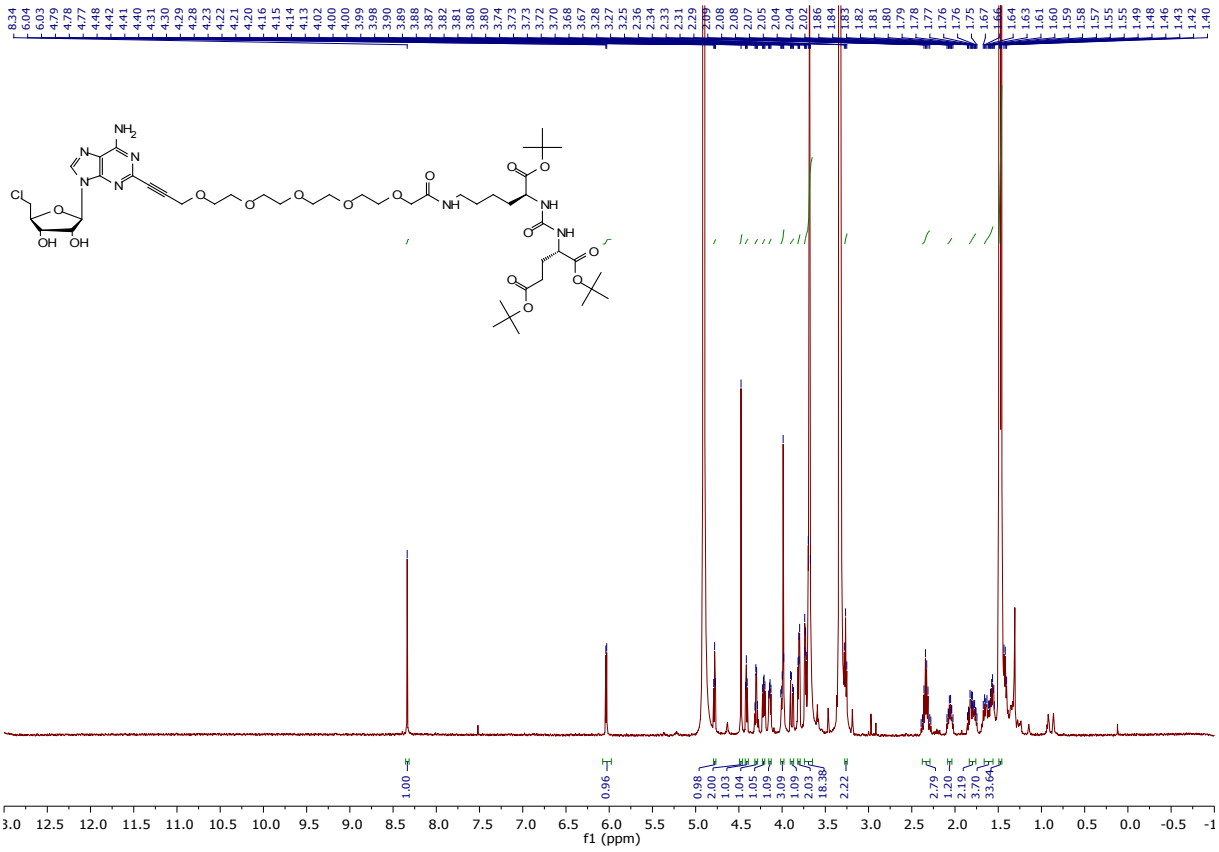


Figure S13. ^{1}H NMR spectrum (500.1 MHz, *d*$_4$-MeOD) of ClDA-PEG-(protected)GUL **14**.

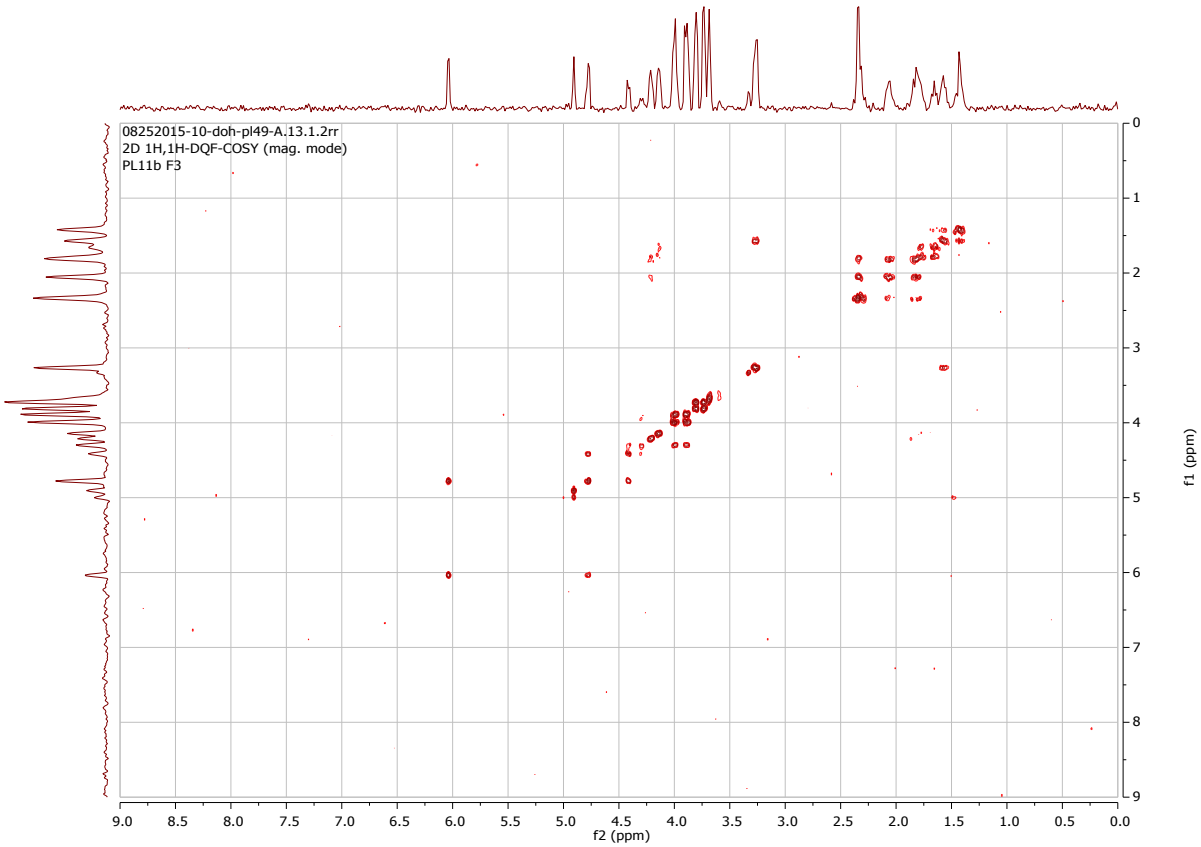


Figure S14. COSY NMR spectrum (500.1 MHz, *d4*-MeOD) of ClDA-PEG-(protected)GUL **14**.

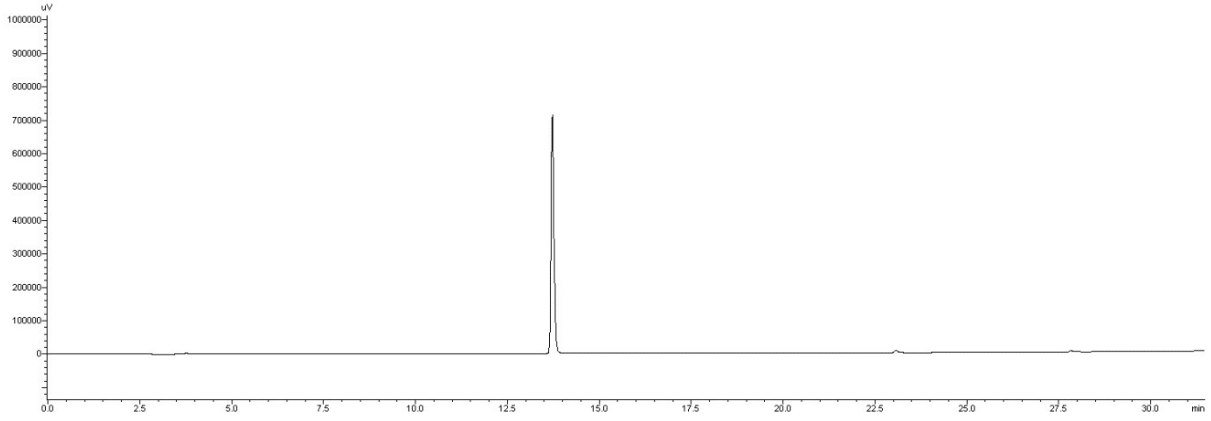
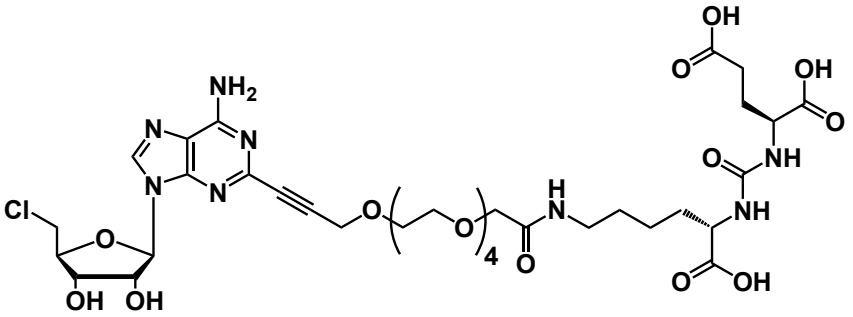


Figure S15. Analytical HPLC trace of **14** after semi prep HPLC purification. HPLC analysis was performed on a Shimadzu Prominence system using a Kinetex 5µm XB-C18 100A (150 mm × 4.6 mm) column and a guard cartridge. Mobile phase: 0.05% TFA in water (solvent A) and 0.05% TFA in MeCN (solvent B); linear Gradient: 15% solvent B to 95% solvent B over 25 min, 95% for 5 min, and back to 15% B for 10 min to re-equilibrate the column. Flow rate: 1 mL/min^{-1}; detection: 254 nm. For additional information on HPLC set up for non-radioactive experiments see general experimental.

ClDA-PEG-GUL (15)



Trifluoroacetic acid (0.4 mL) was added dropwise to a solution of **14** (5 mg, 0.028 mmol) in DCM (2 mL) at 0 °C, the reaction was monitored by analytical HPLC to prevent formation of by-products (most prominently arising from the cleavage of the 5′-chloro-5′-deoxy-ribose sugar). Upon 70-80% conversion to **15**, approximately 1.5 - 2 h, the reaction mixture was then concentrated *in vacuo* and residual TFA removed by co-evaporated with ether. The crude reaction mixture purified by semi-preparative HPLC on a Shimadzu Prominence system using a Phenomenex Luna C18 column (250 x 10.00 mm, 5µm) and a guard cartridge; Mobile phase: 0.05% TFA in water (solvent A) and 0.05% TFA in MeCN (solvent B); linear gradient: 25% solvent B to 95% solvent B over 17 min, 95% B for 5 min, and back to 25% B for 10 min to re-equilibrate the column. Flow rate: 2.5 mL/min $^{-1}$; detection: 254 nm. The relevant fractions were collected (t_R = 13.4 min), concentrated and lyophilized affording the product **15** (1.4 mg, 57%) as a white solid; ^{1}H NMR (500.1 MHz, *d4*-MeOD) δ 8.35 (s, 1H, Ade H-8), 6.04 (d, *J* = 4.9 Hz, 1H, ribose H-1′), 4.78 (dd, *J* = 5.1 Hz, 5.0 Hz, 1H, ribose H-2′), 4.48 (s, 2H, PEG OC*H*$_2$C≡C), 4.41 (dd, *J* = 4.8, 4.8 Hz, 1H, ribose H-3′), 4.32 – 4.35 (m, 1H, α-H), 4.29 – 4.32 (m, 1H, ribose H-4′), 4.26 – 4.29 (m, 1H, α-H), 3.99 (dd, *J* = 11.9, 5.0 Hz, 1H, ribose H-5′a), 3.99 (s, 2H, PEG OC*H*$_2$C≡C), 3.89 (dd, *J* = 11.9, 5.0 Hz, 1H, ribose H-5′b), 3.82 -3.66 (m, 16H, PEG C*H*$_2$), 3.25 – 3.28 (m, 2H, C*H*$_2$), 2.47 – 2.39 (m, 2H, C*H*$_2$), 1.40 – 2.20 (m, 8H, 4xC*H*$_2$); HRMS (ESI$^+$) calculated for $C_{35}H_{51}ClN_8NaO_{16}$ $[M+Na]^+$ 897.3009, found 897.3004.

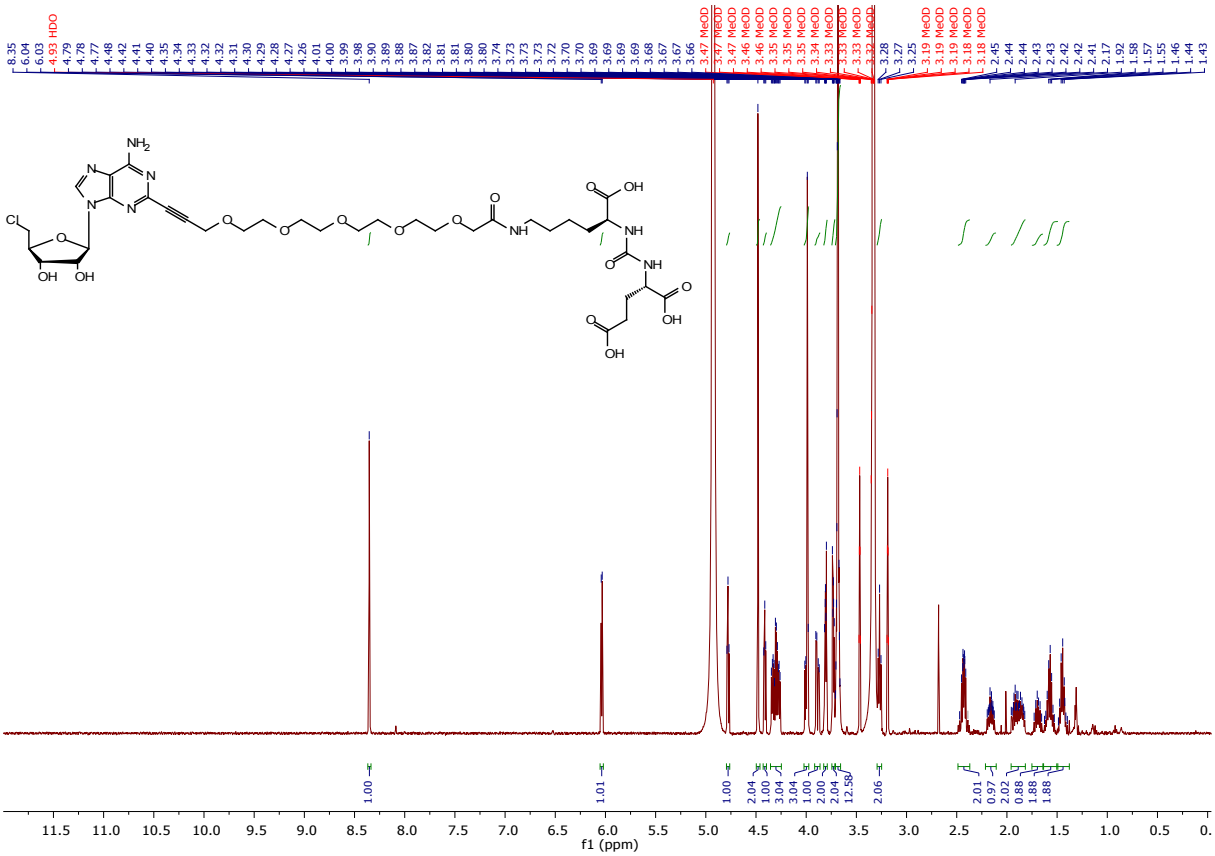


Figure S16. ^{1}H NMR spectrum (500.1 MHz, *d4*-MeOD) of ClDA-PEG-GUL **15**.

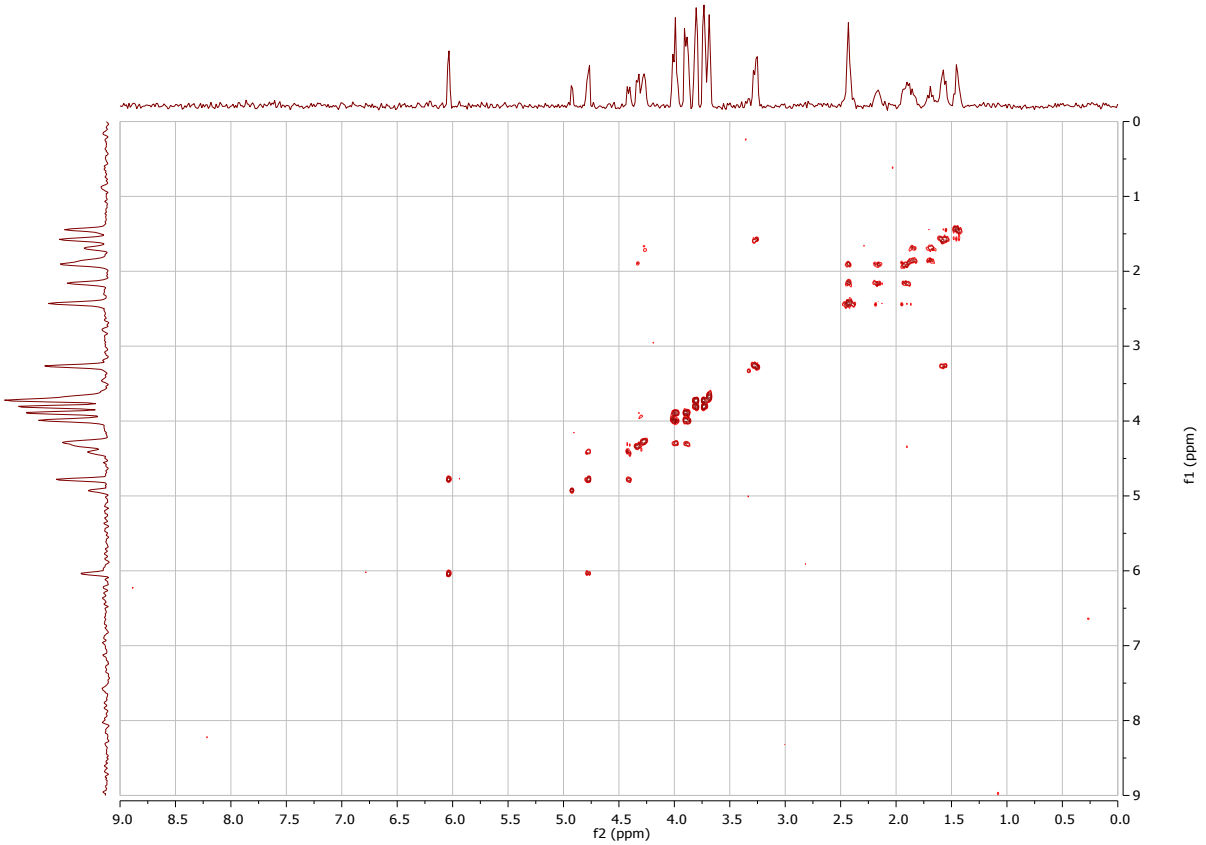


Figure S17. COSY NMR spectrum (500.1 MHz, *d4*-MeOD) of ClDA-PEG-GUL **15**.

Figure S18. TOCSY NMR spectrum (500.1 MHz, *d4*-MeOD) of ClDA-PEG-GUL **15**.

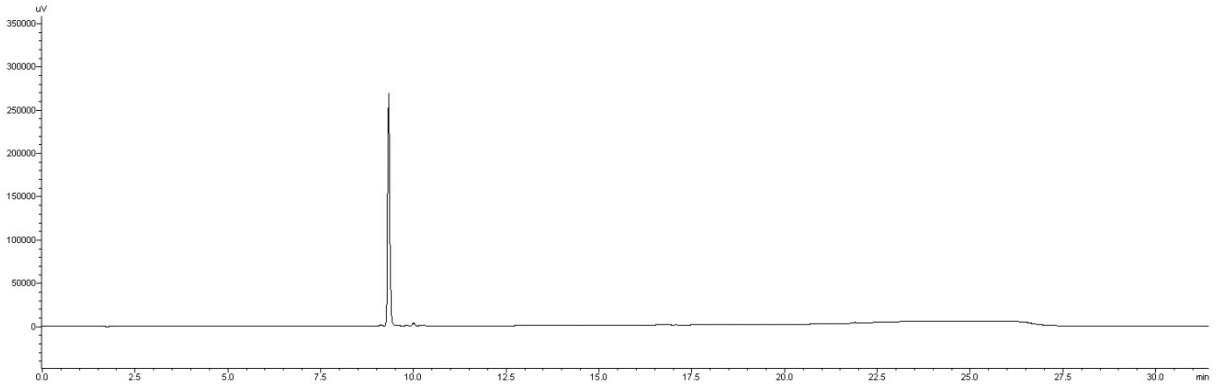
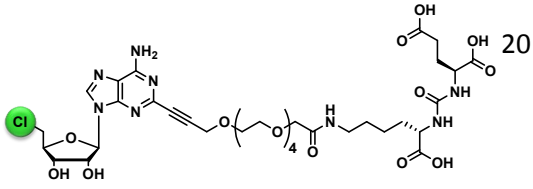


Figure S19. Analytical HPLC trace of **15** after semi prep HPLC purification. HPLC analysis was performed on a Shimadzu Prominence system using a Kinetex 5µm XB-C18 100A (150 mm × 4.6 mm) column and a guard cartridge. Mobile phase: 0.05% TFA in water (solvent A) and 0.05% TFA in MeCN (solvent B); linear Gradient: 15% solvent B to 95% solvent B over 25 min, 95% for 5 min, and back to 15% B for 10 min to re-equilibrate the column. Flow rate: 1 mL/min^{-1}; detection: 254 nm. For additional information on HPLC set up for non-radioactive experiments see general experimental.

Fluorinase over-expression in *E. coli* and purification

E. coli BL21 (DE3) Gold cells bearing the pET-28a(+)-flA vector[6] were grown while shaking (180 rpm) in autoinduction medium[7] (4 × 400 mL in 2 L flasks) containing kanamycin (100 µg/mL) at 37 °C for 48 h. Cells were then harvested by centrifugation (6500 rpm, 15 min), and the cell pellet resuspended in buffer (100 mL, 150 mM NaCl, 20 mM TRIS pH8, 20 mM imidazole), before protease inhibitor cocktail (1 × Complete Mini, EDTA-free, Roche) and bovine pancreatic DNAse (Sigma, 5 mg) were added. The cells then were lysed using a cell disruptor (Constant Systems) at 30 kPSI, the lysate was centrifuged (20 000 rpm, 30 min) and the pellet discarded before being filtered (0.45 µm). The recombinant protein was purified from the lysate by passing the supernatant though a Ni^{2+} affinity column (Ni Sepharose 6 Fast Flow, GE, 5-7 mL bed volume) which was pre-equilibrated with the lysis buffer. The bound protein was washed (3 × 15 mL, 20 mM TRIS pH 8, 20 mM imidazole, 500 mM NaCl) before being eluted (3 × 15 mL, 20 mM TRIS pH 8, 400 mM imidazole, 500 mM NaCl). After SDS-PAGE analysis, fractions containing the desired protein were pooled, adenosine deaminase (ADA) suspension (50 µL calf spleen ADA, Sigma Aldrich) was added, and the mixture dialysed against buffer (20 mM TRIS pH 8, 7 L or 20 mM phosphate buffer pH 7.8) overnight. Adenosine removal was monitored by HPLC of samples of fluorinase which had been adjusted to the same protein concentration, denatured by heating at 95 °C for 5 min, before being clarified by centrifugation (13 000 rpm, 10 min). Samples of the supernatant (60 µL) were removed for analysis by HPLC (performed on a Shimadzu Prominence system using a Kinetex 5µm XB-C18 100A (150 mm × 4.6 mm) column and a guard cartridge. Mobile phase: 0.05% TFA in water (solvent A) and 0.05% TFA in MeCN (solvent B); linear Gradient: 15% solvent B to 95% solvent B over 25 min, 95% for 5 min, and back to 15% B for 10 min to re-equilibrate the column. Flow rate: 1 mL/min^{-1}; detection: 254 nm). The non-His-tagged ADA enzyme was then separated from the fluorinase by passing the supernatant though another Ni^{2+} affinity column (Ni Sepharose 6 Fast Flow, GE, 5-7 mL bed volume, using the same procedure, the resultant eluate was dialysed against buffer (20 mM TRIS pH 8, 7 L or 20 mM phosphate buffer pH 7.8) overnight. The solution was concentrated by centrifugation (Amicon Ultra-15, 10 000 kDa cutoff) to give recombinant fluorinase (5-20 $mg.mL^{-1}$). The enzyme solution was then aliquotted into portions (5 mg or 10 mg, based on fluorinase), before being lyophilised and stored at −78 °C until needed.

Analytical 15 to 16 Transhalogenation Assay



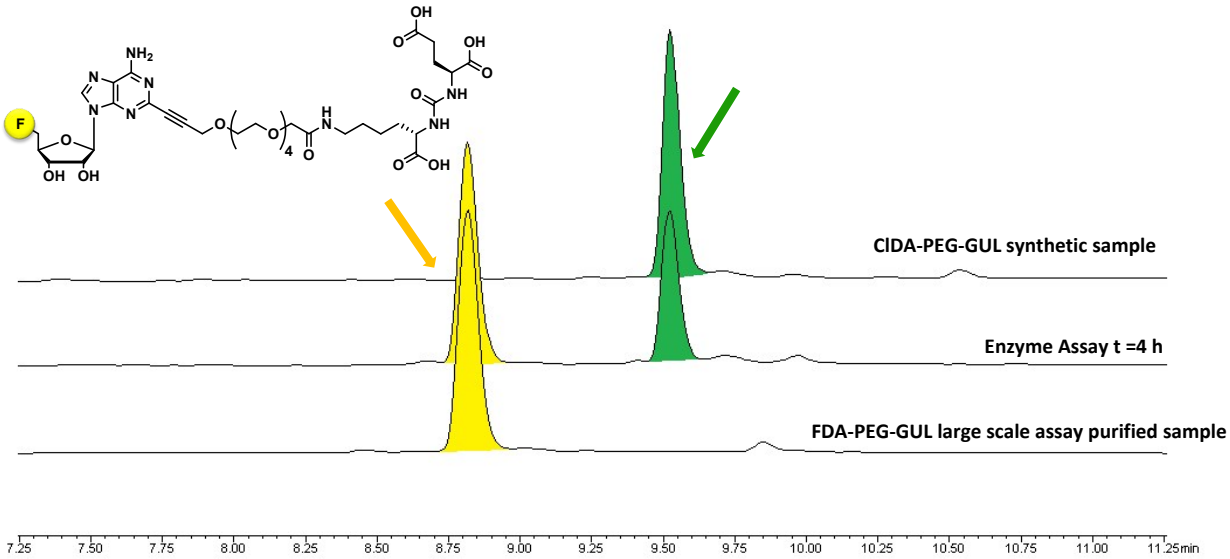
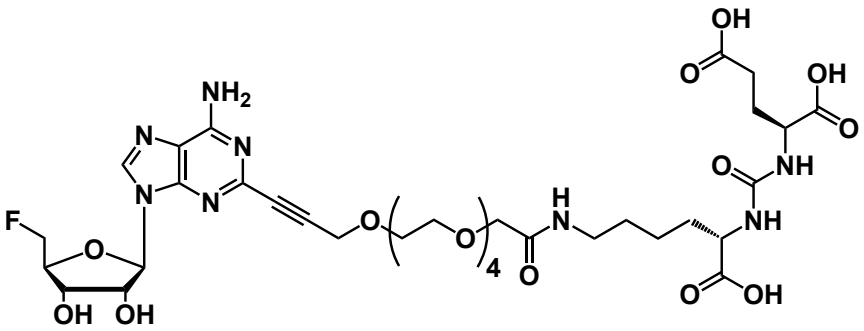


Figure S20. Analytical HPLC (UV, 254 nm) of fluorinase catalysed conversion of **15** to **16** after 4 h, with references. Reaction and HPLC conditions: See Below. For additional information on HPLC set up for non-radioactive experiments see general experimental.

In a total reaction volume of 1000 µL (in 50 mM phosphate buffer, at pH 7.8), recombinant fluorinase (0.7 mg/mL^{-1}) was incubated with **15** (0.1 mM), L-SeMet (0.075 mM) and KF (50 mM) at 37 °C. Samples (50 µL) were periodically removed, the protein precipitated by heating at 95 °C for 5 min, before being clarified by centrifugation (13 000 rpm, 10 min). Samples of the supernatant (40 µL) were removed for analysis by HPLC, and the reaction was monitored until completion. HPLC analysis was performed on a Shimadzu Prominence system using a Kinetex 5µm XB-C18 100A (150 mm × 4.6 mm) column and a guard cartridge. Mobile phase: 0.05% TFA in water (solvent A) and 0.05% TFA in MeCN (solvent B); linear Gradient: 15% solvent B to 95% solvent B over 25 min, 95% for 5 min, and back to 15% B for 10 min to re-equilibrate the column. Flow rate: 1 mL/min^{-1}; detection: 254 nm; injection volume: 40 µL.

Preparative enzymatic synthesis of 16.



Large scale transhalogenation reactions were performed in a total reaction volume of 4000 µL (in 50 mM phosphate buffer, at pH 7.8), recombinant fluorinase (3 mg/mL^{-1}) was incubated with **15** (2.5 mg), L-SeMet (0.075 mM) and KF (100 mM) at 37 °C. Samples (20 µL) were periodically removed, the protein precipitated by heating at 95 °C for 5 min, diluted (40 µL H_2O) before being clarified by centrifugation (13 000 rpm, 10 min) and analysed by HPLC. HPLC analysis was performed on a Shimadzu Prominence system using a Kinetex 5µm XB-C18 100A (150 mm × 4.6 mm) column and a guard cartridge. HPLC on a Shimadzu Prominence system using a Phenomenex Luna C18 column (250 x 10.00 mm, 5µm) and a guard cartridge; Mobile phase: 0.05% TFA in water (solvent A) and 0.05% TFA in MeCN (solvent B); linear Gradient: 15% solvent B to 95% solvent B over 25 min, 95% for 5 min, and back to 15% B for 10 min to re-equilibrate the column. Flow rate: 1 mL/min^{-1}; detection: 254 nm; injection volume: 40 µL. The reaction was periodically monitored until conversion of **15** to **16** had exceeded 90%, at which point the reaction was stopped and the protein removed by heating at 95 °C for 5 min, before being clarified by centrifugation (13 000 rpm, 10 min). Semi-preparative HPLC purification was performed on a Shimadzu Prominence system using a Phenomenex Kingsorb C18 (250 × 10.00 mm, 5µ) column and a guard cartridge. Mobile phase: 0.05% TFA in water (solvent A) and 0.05% TFA in MeCN (solvent B); linear gradient: 25% solvent B to 95% solvent B over 17 min, 95% B for 5 min, and back to 25% B for 10 min to re-equilibrate the column. Flow rate: 2.5 mL/min $^{-1}$; detection: 254 nm. The relevant fractions were collected (t_R = 13.4 min), concentrated and lyophilized affording the product **16** (1.7 mg,70%) as a white solid. ^{1}H NMR (500.1 MHz, *d4*-MeOD) δ 8.30 (s, 1H, Ade H-8), 6.09 (d, *J* = 4.0 Hz, 1H, ribose H-1'), 4.79 (ddd, *J* = 48.3, 10.7, 2.5 Hz, 1H, ribose H-5'a), 4.70 (ddd, *J* = 46.4, 10.6, 2.6 Hz, 1H, ribose H-5'b), 4.57 – 4.59 (m, 1H, ribose H-2'), 4.49 (s, 2H, PEG OCH_2C≡C), 4.41 (dd, *J* = 5.2, 5.2 Hz, 1H, ribose H-3'), 4.32 – 4.35 (m, 1H, α-H), 4.23 – 4.31 (m, 1H, ribose H-4'), 4.26 – 4.29 (m, 1H, α-H), 3.99 (s, 2H, PEG OCH_2C≡C), 3.82 -3.68 (m, 16H, PEG CH_2), 3.26 – 3.28 (m, 2H, CH_2), 2.46 – 2.41 (m, 2H, CH_2), 1.42 – 2.20 (m, 8H, 4xCH_2); ^{19}F NMR (471 MHz, *d4*-MeOD) δ -232.56 (ddd, *J* = 47.7, 47.7, 27.9 Hz, 1F, CH_2F); HRMS (ESI$^+$) calculated for $C_{35}H_{51}FN_8NaO_{16}$ $[M+Na]^+$ 881.3304, found 897.3299.

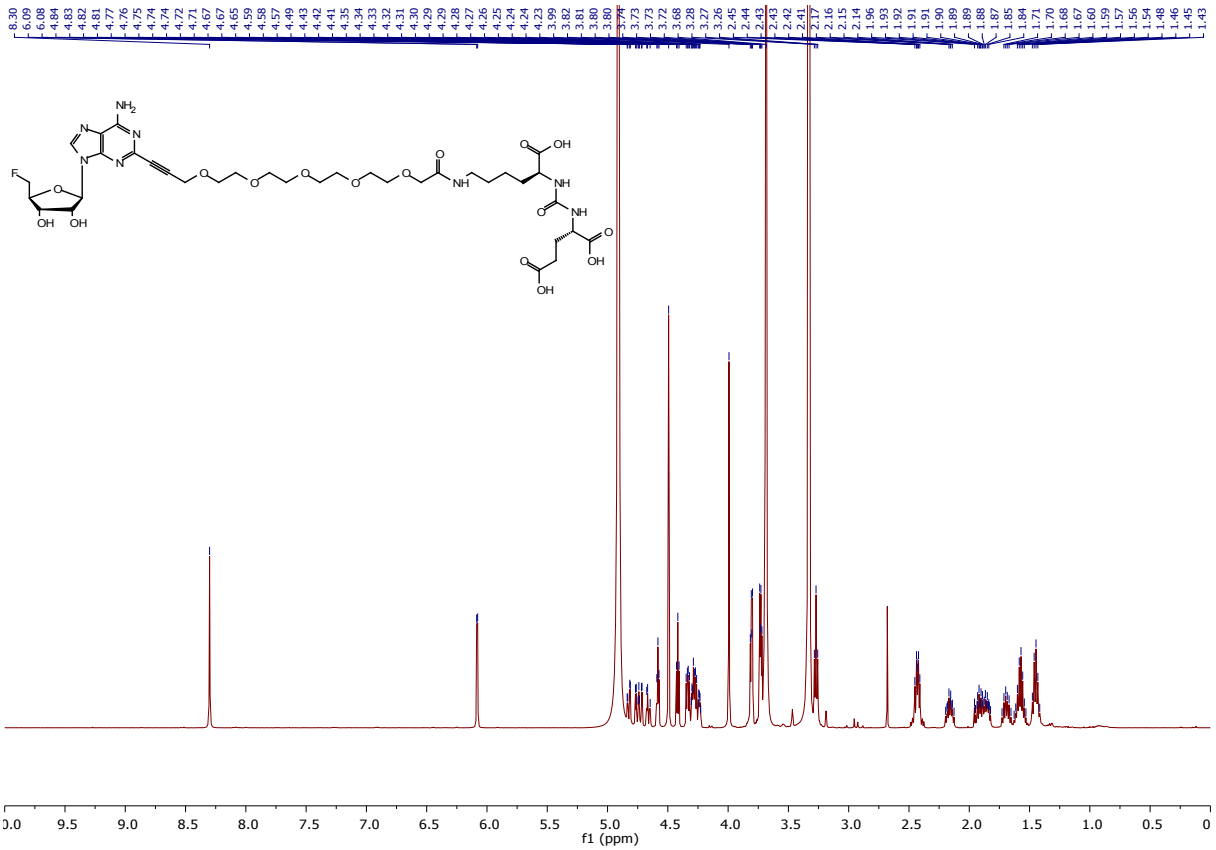


Figure S21. ^{1}H NMR spectrum (500.1 MHz, *d*$_4$-MeOD) of FDA-PEG-GUL **16**.

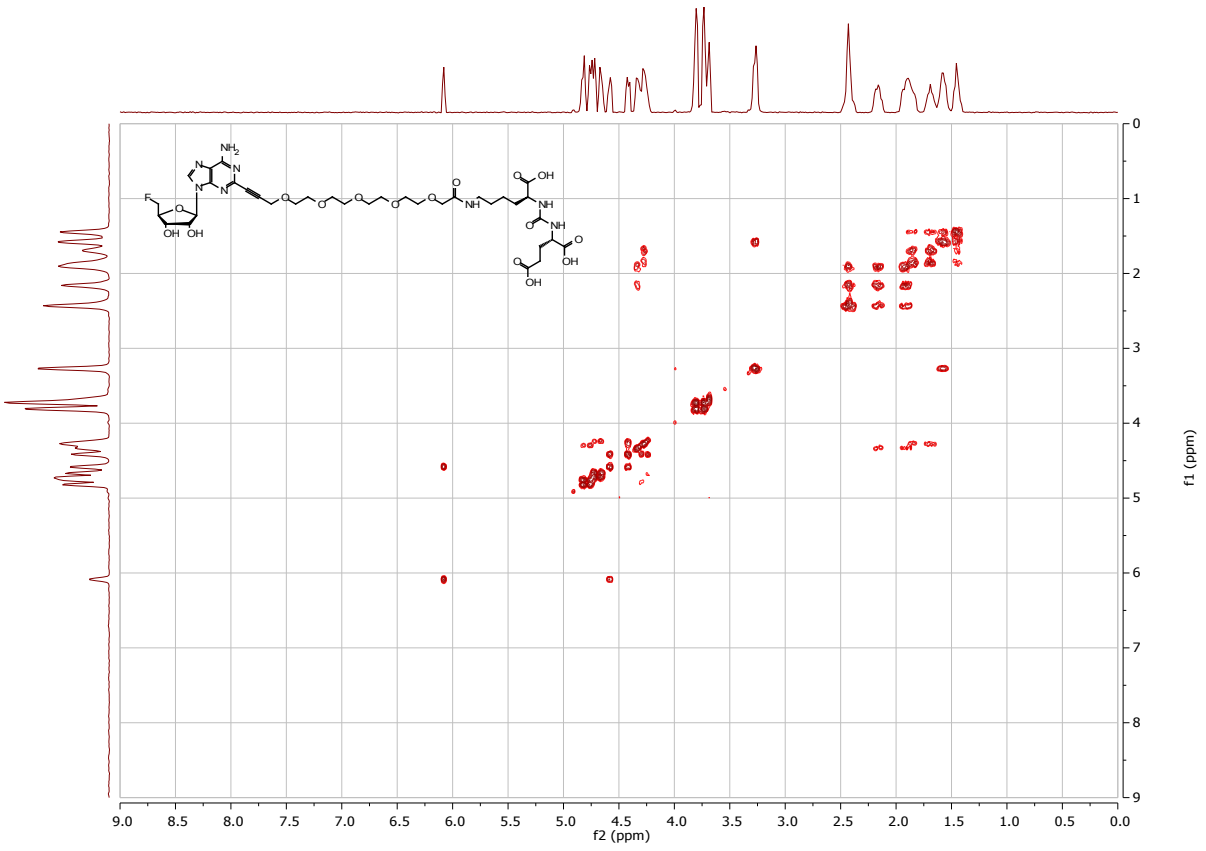


Figure S22. COSY NMR spectrum (500.1 MHz, *d*$_4$-MeOD) of FDA-PEG-GUL **16**.

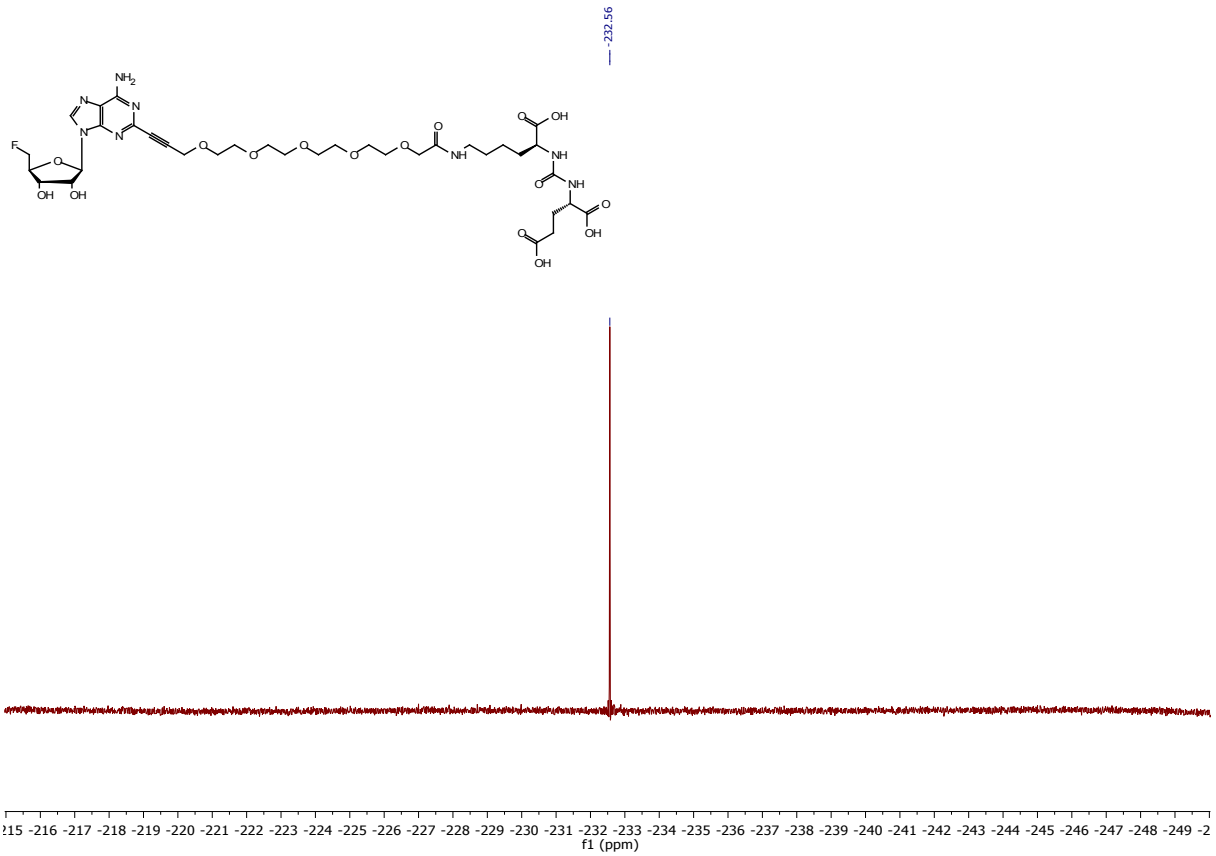


Figure S23. ^{19}F NMR spectrum (471 MHz, *d*$_4$-MeOD) of FDA-PEG-GUL **16**.

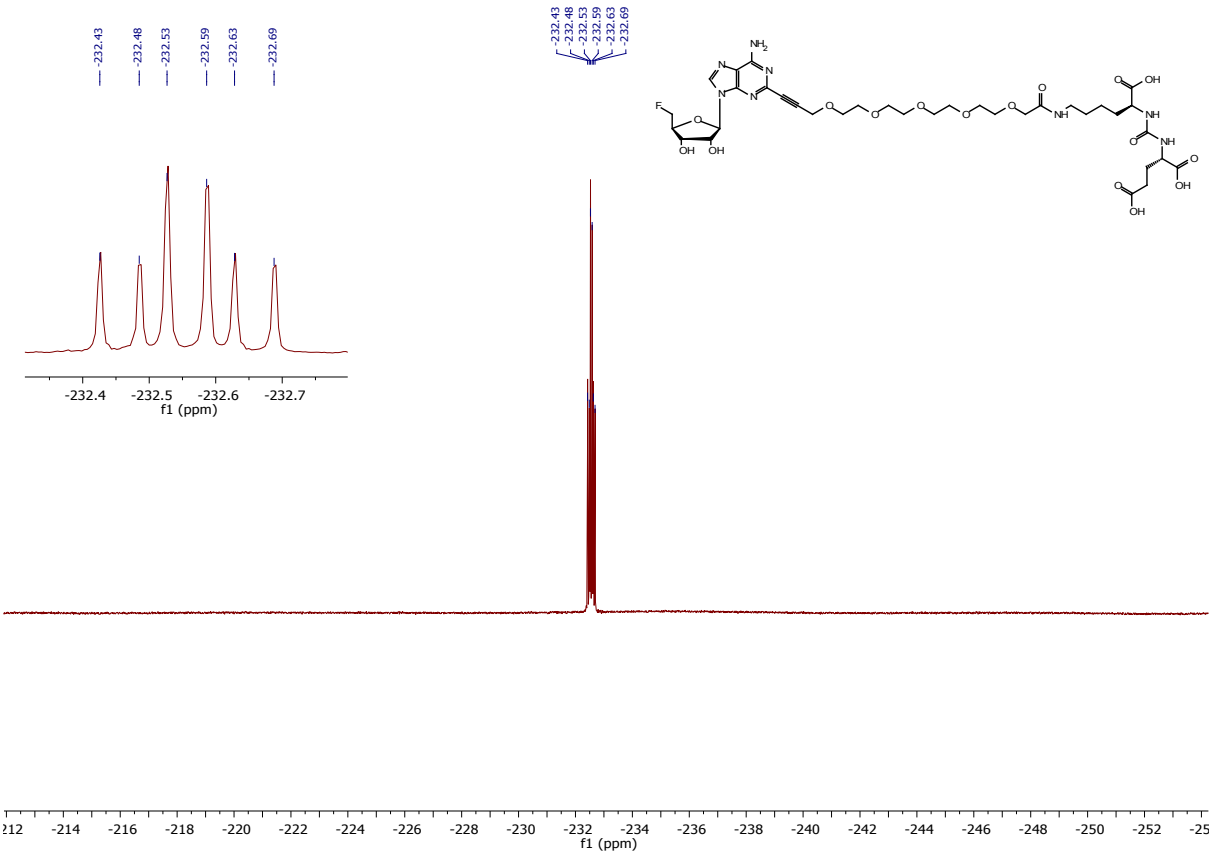


Figure S24. ^{19}F NMR spectrum (471 MHz, *d*$_4$-MeOD) of FDA-PEG-GUL **16**.

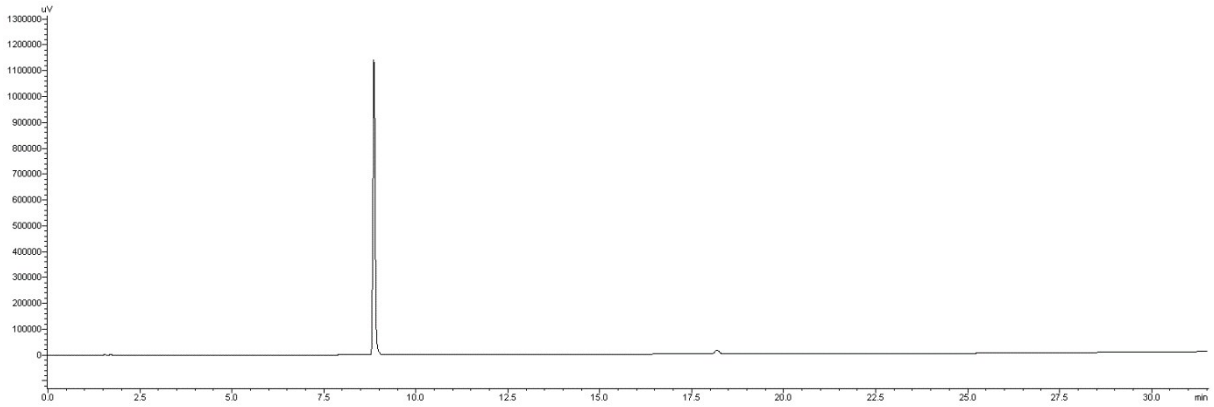


Figure S25. Analytical HPLC trace of **15** after semi prep HPLC purification. HPLC analysis was performed on a Shimadzu Prominence system using a Kinetex 5µm XB-C18 100A (150 mm × 4.6 mm) column and a guard cartridge. Mobile phase: 0.05% TFA in water (solvent A) and 0.05% TFA in MeCN (solvent B); linear Gradient: 15% solvent B to 95% solvent B over 25 min, 95% for 5 min, and back to 15% B for 10 min to re-equilibrate the column. Flow rate: 1 mL/min^{-1}; detection: 254 nm. For additional information on HPLC set up for non-radioactive experiments see general experimental.

PSMA inhibition assays

PSMA inhibition assays were performed as previously reported.[8] In initial experiments, purified glutamate was used to establish that there is a linear relationship between the amount of free glutamate present and fluorescence in the assay. Thereafter a glutamate standard curve was included in each experiment to act as a positive control. Purified recombinant human PMSA protein was incubated with each test compound in the presence of 20µM N-acetylaspartylglutamate substrate at 37 °C for 60 min. The reaction was then stopped by heating at 95 °C for 5 min. The resulting solution was incubated with a 15 mM solution of ortho-phthaldialdehyde (OPA) in OPA buffer (0.2M NaOH and 0.1% (v/v) β-mercaptoethanol) for 10 min at room temperature. 100µl aliquots were then assessed for fluorescence in F16 black maxisorp plates in a microplate reader using an excitation wavelength of 330nm and an absorption wavelength of 450 nM. The binding affinity of each test compound to purified recombinant human PMSA was expressed as its 50% inhibitory concentration (IC_{50} value) in the assay. IC_{50} values were calculated using GraphPad Prism 5.

Radiochemical transhalogenation assay 15 to [^{18}F]16

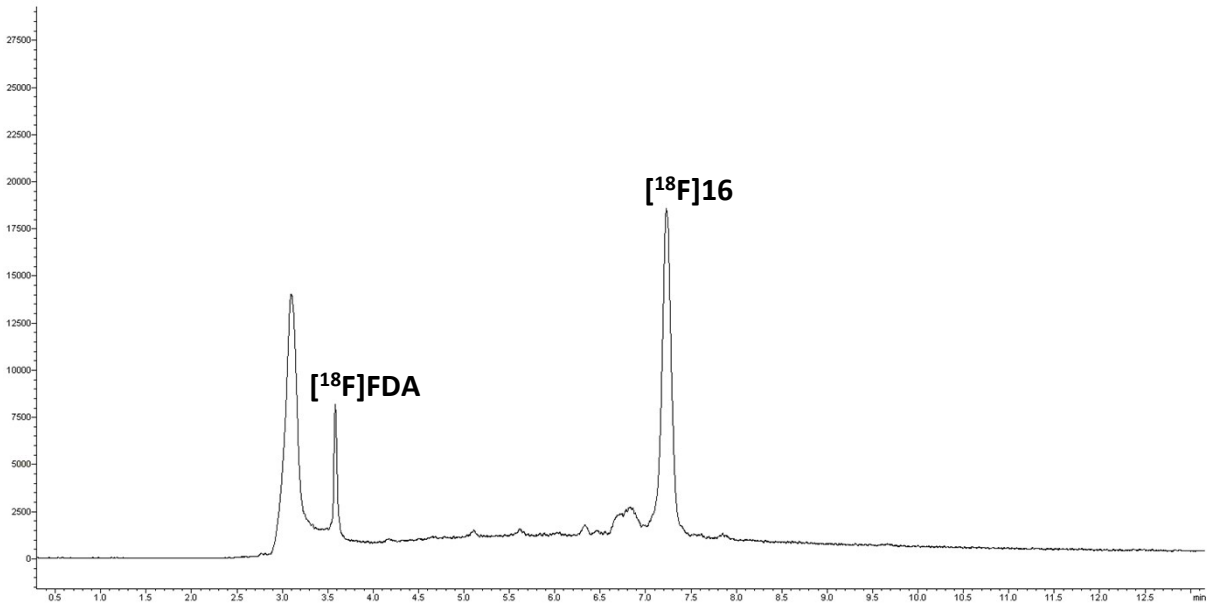


Figure S26. HPLC radio trace of fluorinase catalysed conversion of **15** to **[^{18}F]16**. Reaction conditions: Fluorinase 20 mg/mL, **15** (0.2 mg), L-Se-Met (40 µL of a 2mM solution in water), $^{18}F^-$ in [^{18}O]H_2O (233 MBq, 50 µL), in phosphate buffer (25 mM, pH 7.8, made up to a total reaction volume of 240 µL), at 37 °C, 60 min; HPLC condition: Phenomenex Kingsorb C18 (250 × 10.00 mm, 5µ) column and a guard cartridge. Mobile phase: 0.05% TFA in water (solvent A) and 0.05% TFA in MeCN (solvent B); linear Gradient: 15% solvent B to 38% solvent B over 16 min, 95% for 5 min, and back to 15% B for 10 min to re-equilibrate the column. Flow rate: 2.5 mL/min^{-1}. Collected peak for [^{18}F]**16** (t_R = 7.2 min) is annotated, as is [^{18}F]FDA (t_R = 3.6 min)– a common side product generated by the fluorinase from residual SAM in this reactions. For additional information on HPLC set up for radioactive experiments see general experimental.

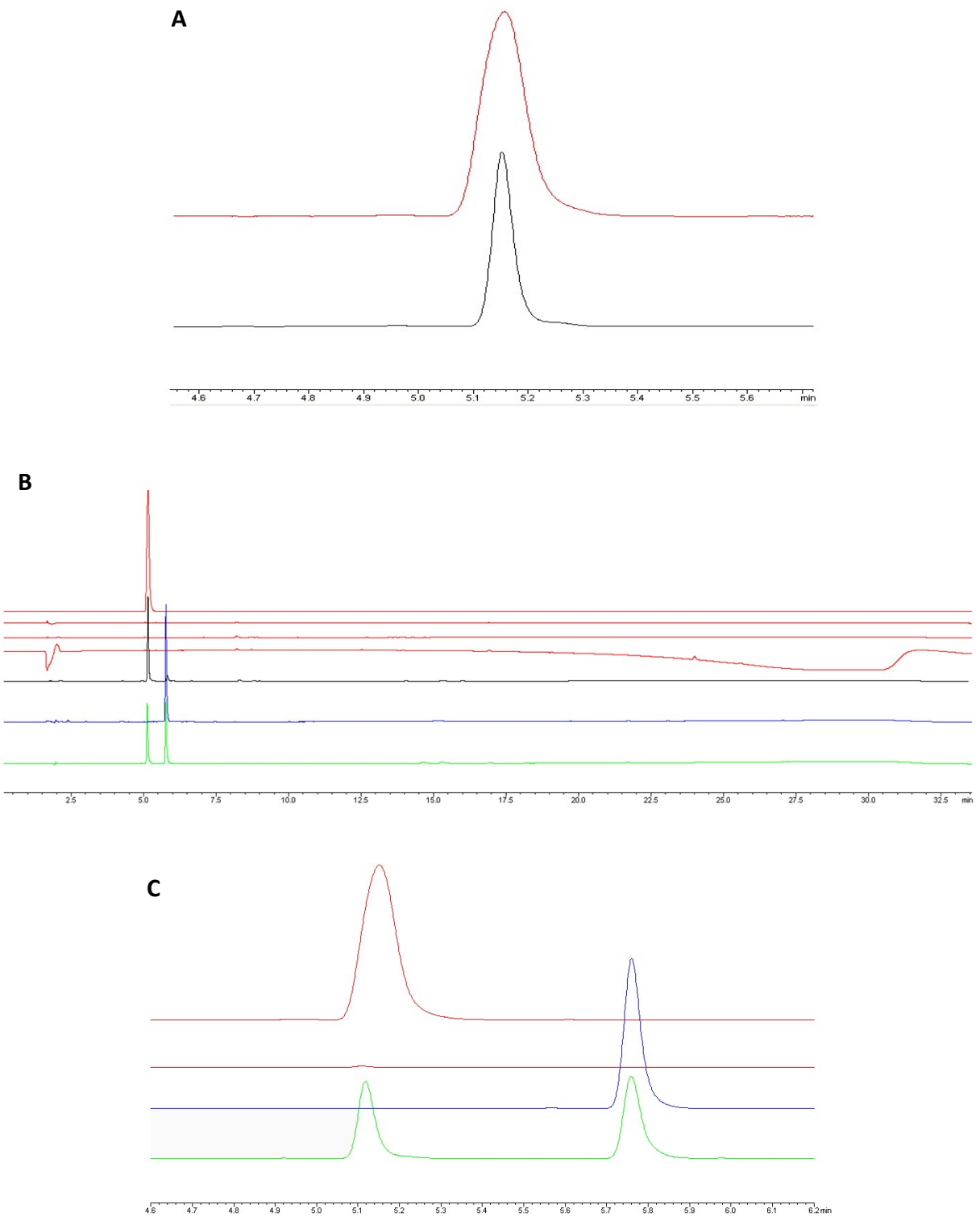


Figure S27. A) Red - Analytical HPLC radio trace of purified **[^{18}F]16**, Black - HPLC (UV, 254 nm) trace of purified **[^{18}F]16** spiked with **16**, (t_R = 5.1 min). **B)** Red - Analytical HPLC radio trace of and UV traces (220 nm top, 254 nm middle, 280 botton) of purified **[^{18}F]16**, Black - HPLC (UV, 254 nm) trace of purified **[^{18}F]16** spiked with **16**. UV HPLC reference trace (254 nm) of **15**, Co-injection of UV HPLC reference traces (254 nm) of **15** and **16**. Expanded HPLC traces for: **C)** Red - Analytical HPLC radio trace of and UV trace (254 nm) of purified **[^{18}F]16**, UV HPLC reference trace (254 nm) of **15**, Co-injection of UV HPLC reference traces (254 nm) of **15** and **16**. For additional information on HPLC set up for radioactive experiments see general experimental.

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