Supplementary Information for:

Enzymatic transhalogenation of dendritic RGD peptide constructs with the fluorinase

Stephen Thompson[a], Ian N. Fleming[b], David O'Hagan[a]*.

*do1@st-andrews.ac.uk

[a]*School of Chemistry and Biomedical Sciences Research Centre, University of St Andrews, North Haugh, St Andrews KY16 9ST, United Kingdom.*
[b]*Aberdeen Biomedical Imaging Centre, School of Medicine and Dentistry, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, UK*

Contents

1. General experimental

Air and moisture sensitive reactions were carried out under an atmosphere of argon in flame-dried glassware. Room temperature (rt) refers to 18-25 °C. All evaporations and concentrations were performed under reduced pressure (*in vacuo*). All reagents (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. c(RGDfK[N_3]) **29**was purchased from Peptides International, USA. 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 on Merck Geduran silica gel 60 (250-400 mesh) under a positive pressure of compressed air eluting with solvents (reported as *v/v*) as supplied. Reverse phase column chromatography was performed using Extract Clean C_{18}-HC prepacked cartridges.

NMR spectra were recorded at 298 K on Bruker Avance 300, Avance II 400, or Avance 500. Avance III 500, or Avance III HD 500 instruments. ^{1}H and ^{13}C NMR spectra were recorded using deuterated solvent as the lock and the residual solvent signal 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. Compounds are numbered according to customary purine numbering. When necessary, resonances were assigned using two-dimensional experiments (COSY, HSQC, HMBC, TOCSY).

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. MALDI MS was acquired using a 4800 MALDI TOF/TOF Analyser (ABSciex) equipped

with a Nd:YAG 355 nm laser and calibrated using a mixture of peptides by Mass Spectrometry Service at the University of St Andrews.

Melting points were recorded on an Electrothermal IA9100 melting point apparatus, or on a Griffin MPA 350.BM2.5 melting point apparatus and are uncorrected.

HPLC analyses/semi-preparations were performed using either a Shimadzu Prominence (SIL-20A HT autosampler, CL-20AT ternary pump, DGU-20A3R solvent degasser, SPD 20A UV detector and CBM-20A controller module) or a Varian Prostar (Varian 400 autosampler, Varian Prostar 230 solvent delivery system, Varian Prostar 235 UV-Vis detector) or a Dionex UltiMate 3000 system (autosampler, pump, column oven, diode array detector, and Berthold Flowstar LB513 radioactivity detector) with reverse phase column as indicated in individual experiment.

LC-MS analysis was performed on a Waters 2795 HPLC coupled in parallel to a Waters 2996 photodiode array detector and Micromass LCT TOF mass spectrometer in ESI in positive mode using the column indicated in the individual experiment.

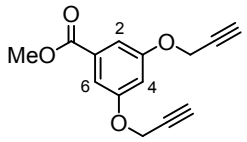
Activity of fluorine-18 containing samples was measured using a Capintech Amersham radioisotope calibrator ARC-120.

Samples were freeze dried from frozen solutions in water or ^{t}BuOH in water (20% *v/v*) in a Christ Alpha 1-2 LO Plus freeze drier.

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).

2. Compound preparation

2.1. Methyl 3,5-bis(prop-2-yn-1-yloxy)benzoate 9



Propargyl bromide (80% *w/w* in toluene, 9.9 mL, 89.2 mmol, 3 eq.) was added to a suspension of methyl 3,5-dihydroxybenzoate (5.00 g, 29.7 mmol, 1 eq.) and K_2CO_3 (12.3 g, 89.2 mmol, 3 eq.) in acetone (50 mL), and the mixture heated under reflux for 48 h. The mixture was cooled before being filtered *in vacuo*, and the residue washed with acetone (3 × 20 mL). The combined filtrates were concentrated, and the resultant solid was dissolved in DCM and passed through a short plug of silica, eluting with DCM, and the fractions containing product concentrated *in vacuo*. The crude product was recrystallised from of MeOH to give **9** as pale yellow crystals (6.32 g, 87%): R_f = 0.61 (DCM); mp 104–107 °C (MeOH); δ_H (500.1 MHz, $CDCl_3$): 7.29 (2 H, d, *J* 2.4, *H*-2, *H*-6), 6.81 (1 H, t, *J* 2.4, *H*-4), 4.72 (4 H, d, *J* 2.4, CH_2), 3.91 (3 H, s, OCH_3), 2.54 (2 H, t, *J* 2.4, C≡C-*H*); δ_C (125.8 MHz, $CDCl_3$): 166.6 (*C*=O), 158.6 (*C*-3, *C*-5), 132.3 (*C*-1), 109.0 (*C*-2, *C*-6), 107.5 (*C*-4), 78.1 (*C*≡C-H), 76.1 (C≡*C*-H), 56.3 (CH_2), 52.5 (OCH_3); *m/z* (ES^+) 267 ($[M+Na]^+$, 100%), 245 ($[M+H]^+$, 10); HRMS (ES^+) calc. for $C_{14}H_{12}O_4Na$ $[M+Na]^+$ 267.0628, found 267.0623. Data are in agreement with the literature.[3]

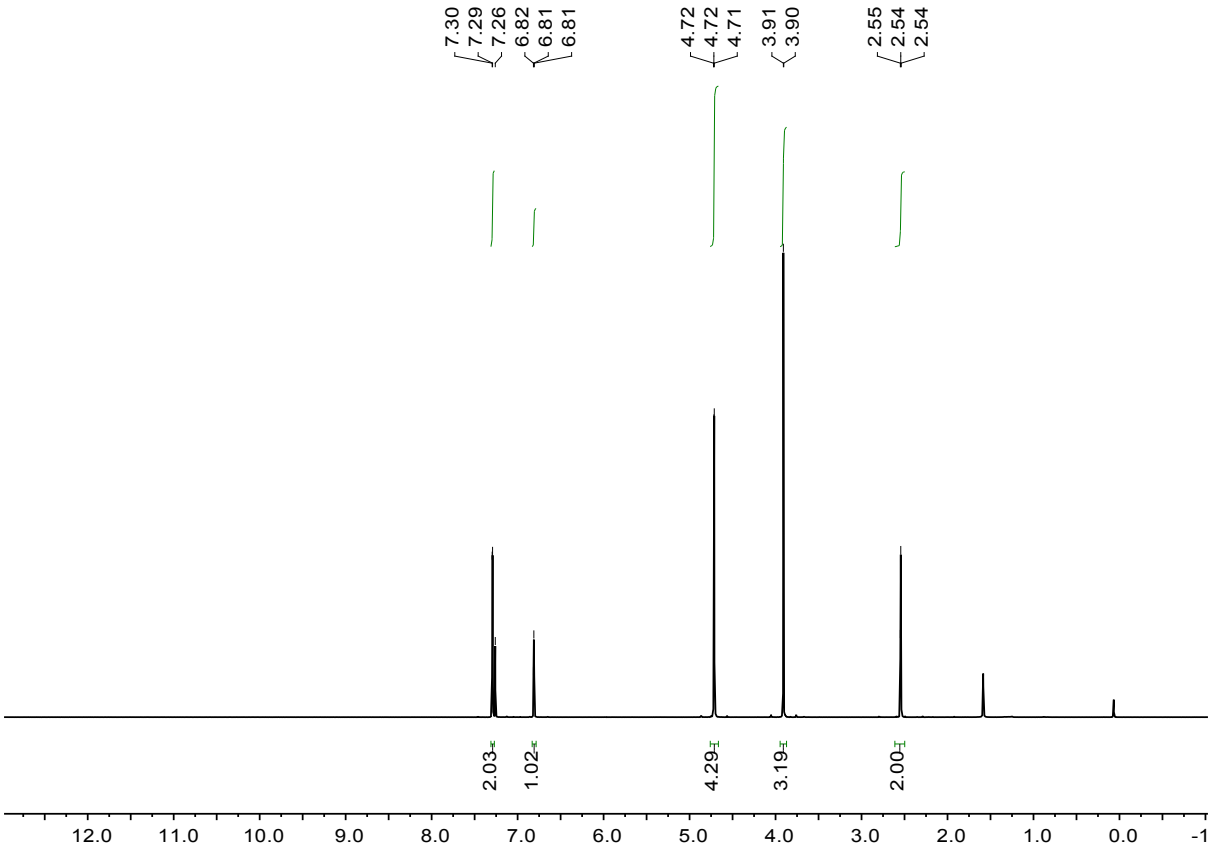


Figure S1. ^1H NMR spectrum (500.1 MHz, $CDCl_3$) of **9**.

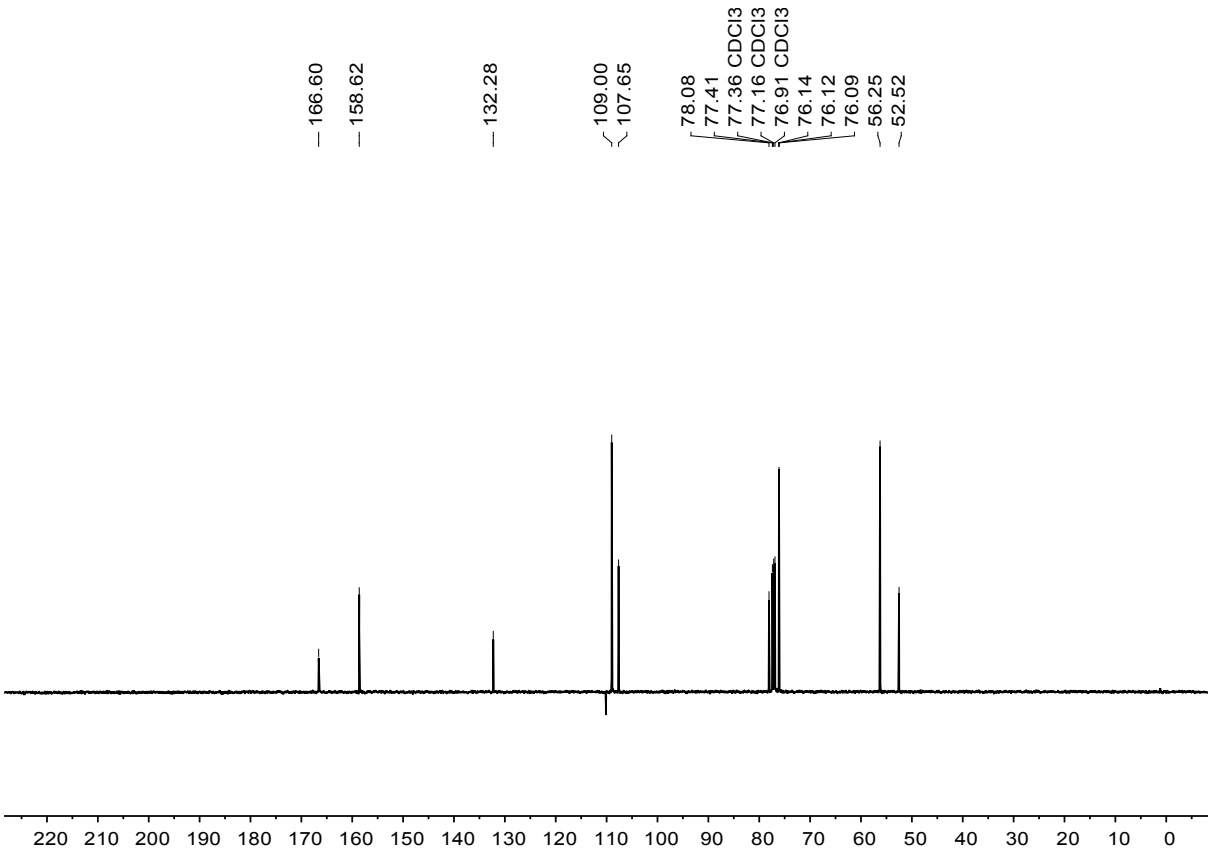
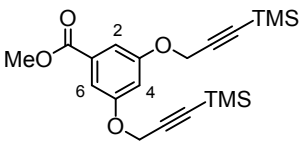


Figure S2. ^{13}C NMR spectrum (125.8 MHz, $CDCl_3$) of **9**.

2.2. Methyl 3,5-bis((3-(trimethylsilyl)prop-2-yn-1-yl)oxy)benzoate 10



n-BuLi (2.5 M in hexanes, 21.7 mL, 54.3 mmol, 2.1 eq.) was added dropwise to a solution of diisopropylamine (8.41 mL, 59.6 mmol, 2.3 eq.) in THF (20 mL) at −78 °C. This solution was stirred for 20 min before dialkyne **9** in THF (40 mL) was added dropwise and this mixture stirred for 30 min before TMSCl (7.56 mL, 59.6 mmol, 2.3 eq.) was added slowly. This mixture was stirred for 10 min, before being allowed to warm to rt and stir for 16 h. The reaction was quenched by addition of sat. aqueous NH_4Cl (50 mL) before volatile components were removed under reduced pressure. The mixture was diluted with DCM (100 mL) and the phases separated and the aqueous phase extracted with DCM (3 × 50 mL). The combined organic phases were washed with sat. aqueous $NaHCO_3$ (50 mL) and brine (50 mL) and dried over Na_2SO_4 before being filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (petroleum ether to 90:10 petroleum ether: Et_2O to 80:20 petroleum ether: Et_2O) to give **10** as a colourless oil (8.41 g, 84%): R_f = 0.49 (80:20 petroleum ether: Et_2O; δ_H (499.9 MHz, $CDCl_3$): 7.30 (2 H, d, *J* 2.4, *H*-2, *H*-6), 6.79 (1 H, t, *J* 2.4, *H*-4), 4.69 (4 H, s, CH_2), 3.90 (3 H, s, OCH_3), 0.17 (18 H, s, Si(CH_3)$_3$); δ_C (125.7 MHz, $CDCl_3$): 166.7 (*C*=O), 158.8 (*C*-3, *C*-5), 132.1 (*C*-1), 109.1 (*C*-2, *C*-6), 107.9 (*C*-4), 99.6 (*C*≡CTMS), 93.5 (C≡*C*TMS), 57.2 (*C*H$_2$), 52.4 (O*C*H$_3$), −0.18 (Si(*C*H$_3$)$_3$); *m/z* (ES$^+$) 799 ([2M+Na$^+$, 15%), 411 ([M+Na]$^+$, 100), 389 ([M+H]$^+$, 7); HRMS (ES$^+$) calc. for $C_{20}H_{28}O_4Si_2Na$ [M+Na]$^+$ 411.1418, found 411.1409.

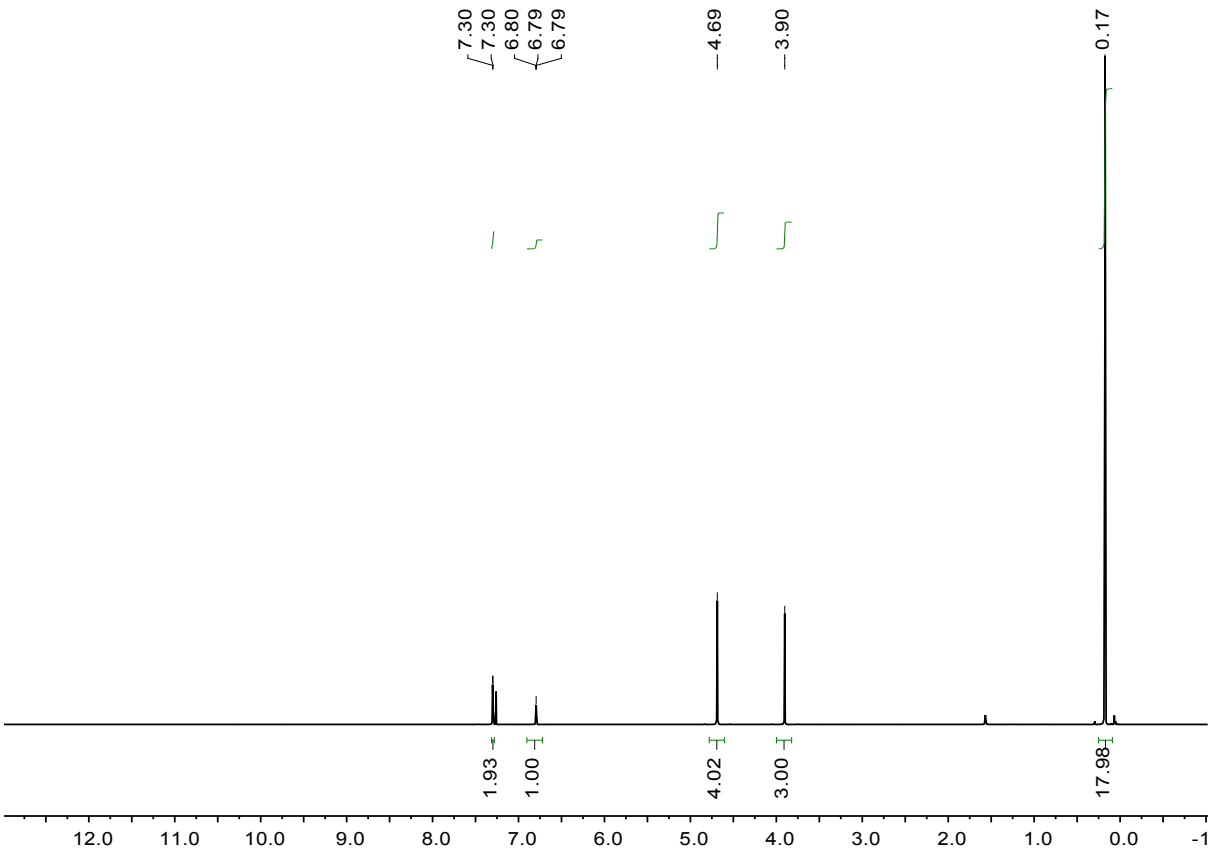


Figure S3. ^{1}H NMR spectrum (499.9 MHz, $CDCl_3$) of **10**.

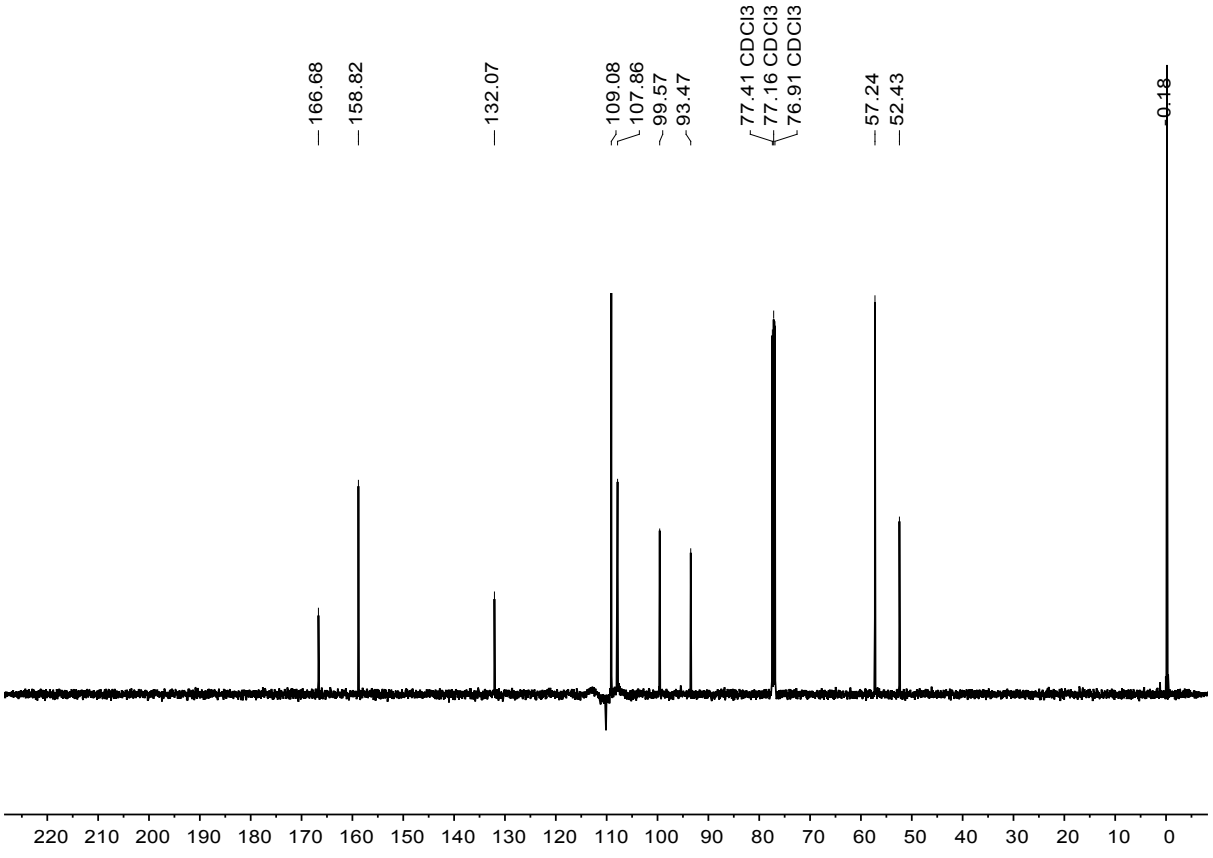
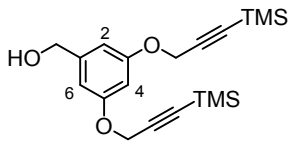


Figure S4. ^{13}C NMR spectrum (125.7 MHz, $CDCl_3$) of **10**.

2.3. 3,5-Bis((3-(trimethylsilyl)prop-2-yn-1-yl)oxy)benzyl alcohol 34



Lithium aluminium hydride (3.70 g, 97.4 mmol, 4.5 eq.) was added portion-wise to a solution of ester **10** (8.41 g, 21.6 mmol, 1 eq.) in Et_2O (100 mL) at 0 °C, and the suspension allowed to warm to rt and stirred for 3 h. The reaction was quenched after cooling the mixture to 0 °C, by the slow addition of water (3.7 mL), NaOH (2 M, 3.7 mL), and additional water (11.1 mL). The mixture stirred for 15 min before addition of $MgSO_4$, and further stirring for 15 min before the solids were removed by filtration, and the solvents removed *in vacuo*. The crude product was purified by column chromatography (67:33 petroleum ether: Et_2O) to give **34** as a colourless oil (5.06 g, 65%): R_f = 0.32 (50:50 petroleum ether: Et_2O); δ_H (500.1 MHz, $CDCl_3$): 6.59 (2 H, d, *J* 2.3, *H*-2, *H*-6), 6.51 (1 H, t, *J* 2.3, *H*-4), 4.63 (4 H, s, ArOCH_2), 4.60 (2 H, s, HOCH_2Ar), 1.95 (1 H, br s, O*H*), 0.17 (18 H, s, Si(CH_3)$_3$); δ_C (125.8 MHz, $CDCl_3$): 159.1 (*C*-3, *C*-5), 143.5 (*C*-1), 106.3 (*C*-2, *C*-6), 101.6 (*C*-4), 100.0 (*C*≡CTMS), 93.0 (C≡*C*TMS), 65.2 (HO*C*H_2Ar), 57.0 (ArO*C*H_2), −0.2 (Si(*C*H_3)$_3$); *m/z* (ES$^+$) 383 ([M+Na]$^+$, 100%), 367 ([M+H]$^+$, 31); HRMS (ES$^+$) calc. for $C_{19}H_{28}O_3Si_2Na$ [M+Na]$^+$ 383.1469, found 383.1458. Data are in agreement with the literature.[4]

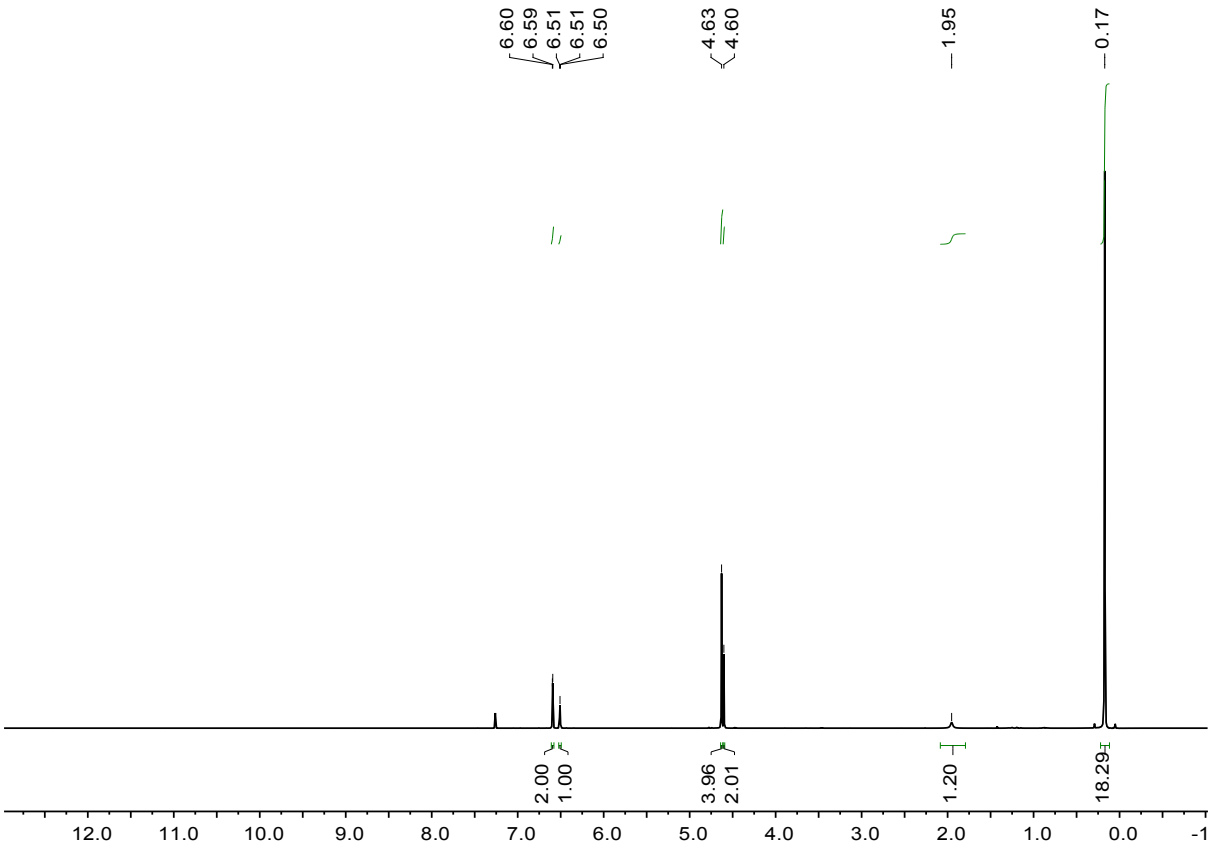


Figure S5. ^{1}H NMR spectrum (500.1 MHz, $CDCl_3$) of **34**.

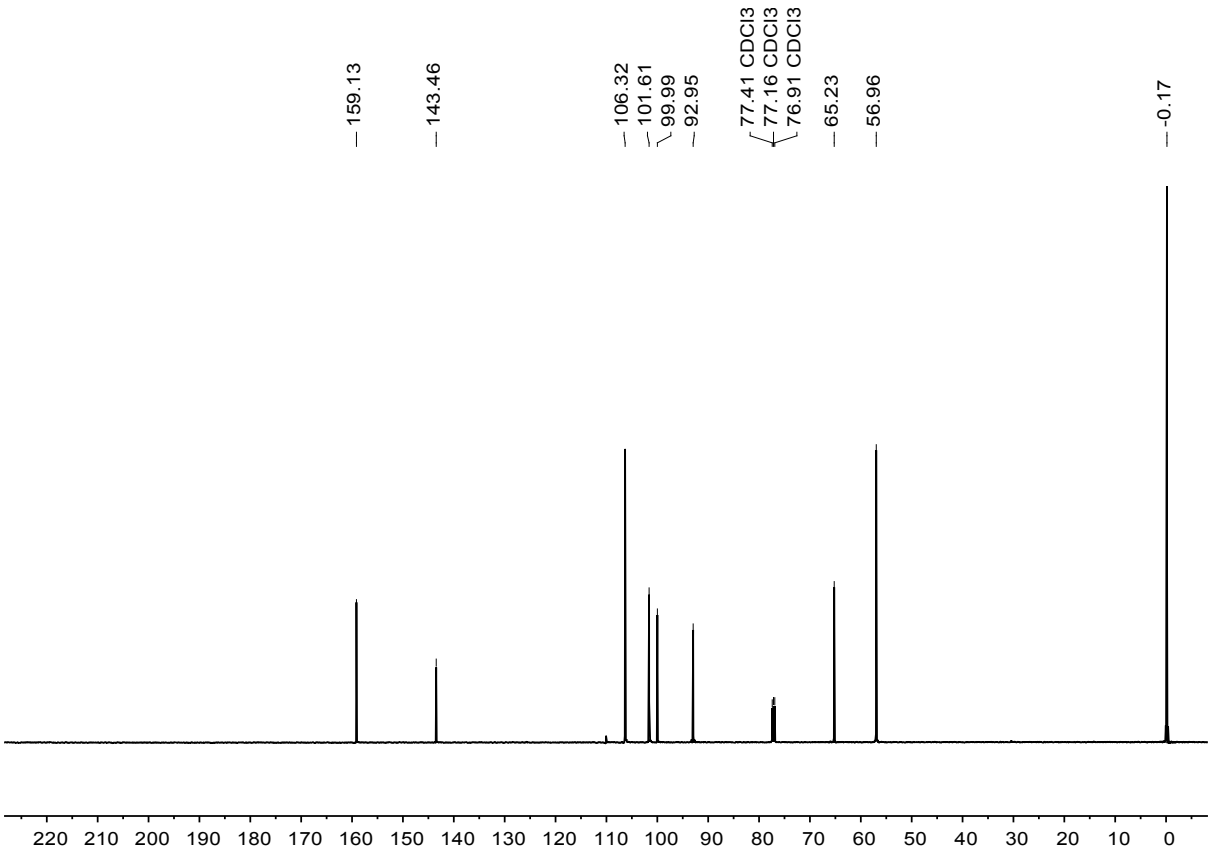
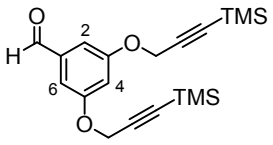


Figure S6. ^{13}C NMR spectrum (125.8 MHz, $CDCl_3$) of **34**.

2.4. 3,5-Bis((3-(trimethylsilyl)prop-2-yn-1-yl)oxy)benzaldehyde 11



Dess-Martin periodinane (5.50 g, 13.0 mmol, 1.1 eq.) was added to a solution of alcohol **34** (4.25 g, 11.8 mmol, 1 eq.) in DCM (100 mL), and the solution stirred at rt for 1 h. The reaction was quenched by the addition of sat. aqueous $NaHCO_3$ (100 mL), followed by Et_2O (200 mL) and $Na_2S_2O_3$ (11.0 g) and the biphasic mixture stirred vigorously for 1 h. The phases were separated and the aqueous phase extracted with Et_2O (3 × 100 mL). The combined organic phases were dried over Na_2SO_4 before being filtered and concentrated *in vacuo* to give to give aldehyde **11** as a colourless oil without further purification (4.30 g, quant.): R_f = 0.86 (50:50 petroleum ether: Et_2O); δ_H (500.1 MHz, $CDCl_3$): 9.91 (1 H, s, ArC*H*O), 7.12 (2 H, d, *J* 2.4, *H*-2), 6.86 (1 H, t, *J* 2.4, *H*-4), 4.71 (4 H, s, CH_2), 0.17 (18 H, s, Si(CH_3)$_3$); δ_C (125.8 MHz, $CDCl_3$): 191.7 (Ar*C*HO), 159.4 (*C*-3, *C*-5), 138.4 (*C*-1), 109.2 (*C*-4), 108.9 (*C*-2, *C*-6), 99.3 (C≡*C*TMS), 93.8 (*C*≡CTMS), 57.2 (*C*H_2), −0.2 (Si(*C*H_3)$_3$); *m/z* (ES$^+$) 413 ([M+MeOH+Na]$^+$, 100%), 381 ([M+Na]$^+$, 30), 359 ([M+H]$^+$, 5); HRMS (ES$^+$) calc. for $C_{19}H_{27}O_3Si_2$ [M+H]$^+$ 359.1493, found 359.1452.

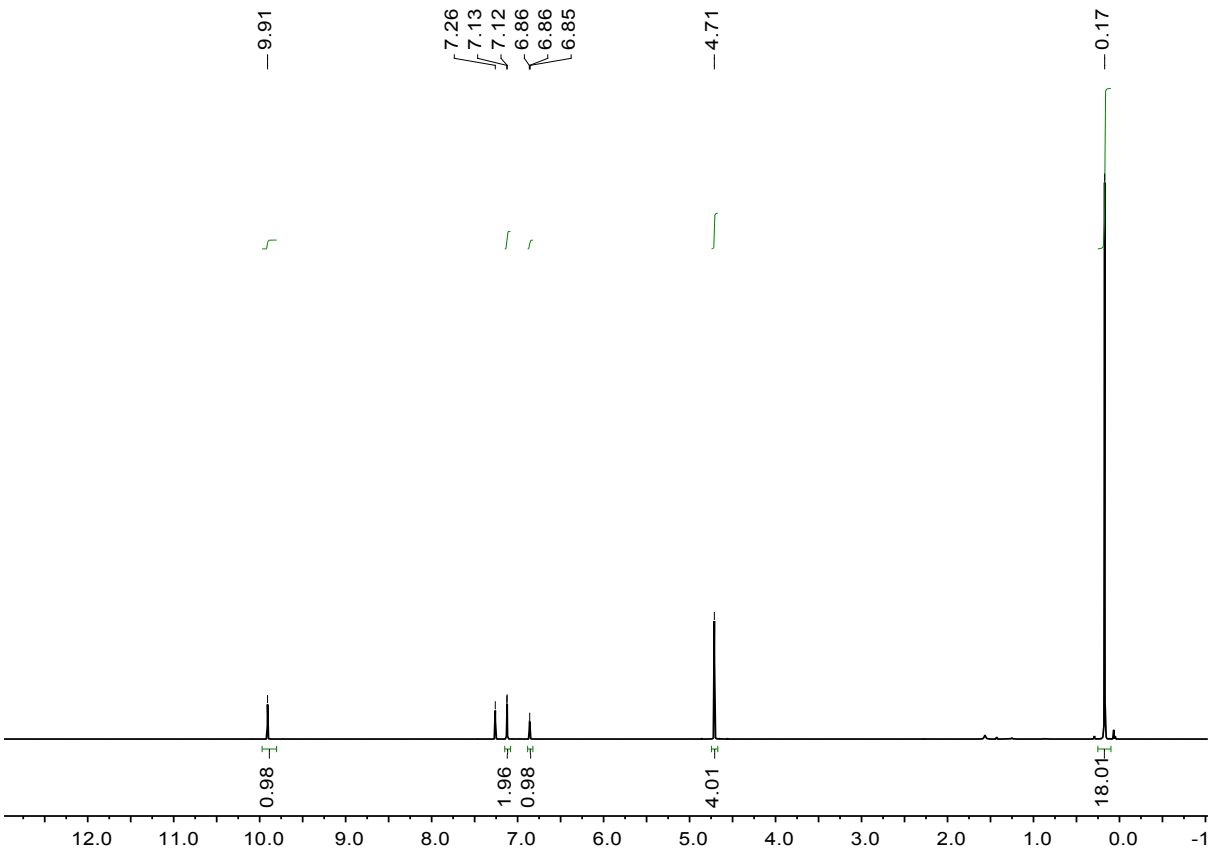


Figure S7. ^{1}H NMR spectrum (500.1 MHz, $CDCl_3$) of **11**.

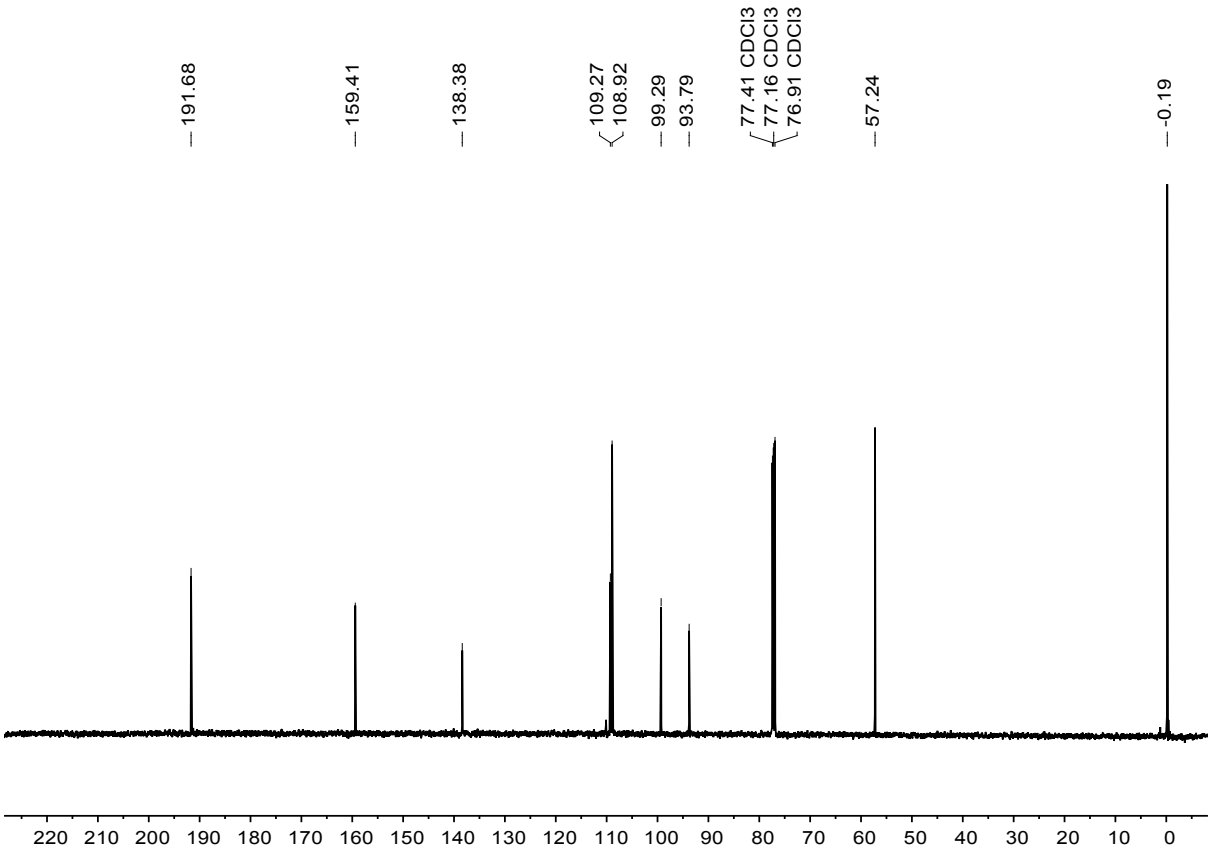
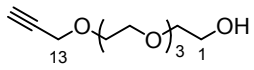


Figure S8. ^{13}C NMR spectrum (125.8 MHz, $CDCl_3$) of **11**.

2.5. 3,6,9,12-Tetraoxapentadec-14-yn-1-ol 35



NaH (60% dispersion in mineral oil, 0.61 g, 15.3 mmol, 1.1 eq.) was added to a solution of tetraethyleneglycol (5.00 g, 25.7 mmol, 1.9 eq.) in THF (50 mL) at 0 °C. The suspension was allowed to warm to rt and stirred for 60 min, before being re-cooled to 0 °C. Propargyl chloride (0.98 mL, 14 mmol, 1.0 eq.) was added dropwise over 20 min, before the mixture was warmed to rt and stirred for 2 h. The reaction was quenched by addition of sat. aqueous NH_4Cl (10 mL), and volatile components removed *in vacuo*. The residue was diluted with DCM (100 mL) and water (30 mL) and the phases separated. The aqueous phase was extracted with DCM (3 × 50 mL). The combined organic phases were dried over Na_2SO_4 before being filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (50:50 petroleum ether: acetone) to give **35** as a colourless oil (1.49 g, 47%): R_f = 0.26 (50:50 petroleum ether: acetone); δ_H (500.1 MHz, $CDCl_3$): 4.20 (2 H, d, *J* 2.4, C*H*$_2$C≡CH), 3.73–3.60 (16 H, m, PEG C*H*$_2$), 2.43 (1 H, t, *J* 2.4, $CH_2C{\equiv}CH$), 2.23 (1 H, br s, CH_2OH); δ_C (125.8 MHz, $CDCl_3$): 79.7 ($CH_2C{\equiv}CH$), 74.7 ($CH_2C{\equiv}CH$), 72.7 (CH_2OH), 70.8, 70.7, 70.7, 70.5, 70.5, 69.2, 61.9 (PEG CH_2), 58.5 ($CH_2C{\equiv}CH$); *m/z* (ES^+) 255 ($[M+Na]^+$, 65); HRMS (ES^+) calc. for $C_{11}H_{20}O_5Na$ $[M+Na]^+$ 255.1203, found 255.1195. Data are in agreement with the literature.[5]

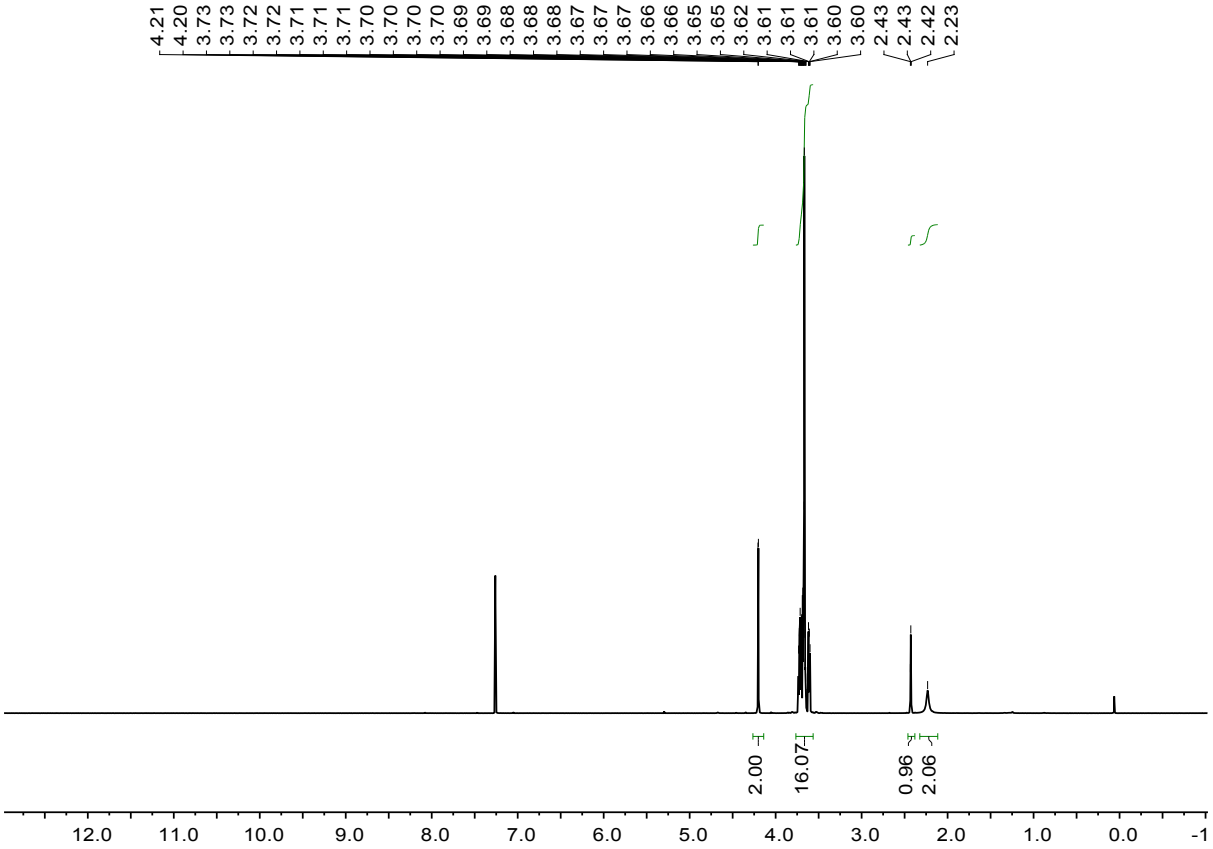


Figure S9. ^1H NMR spectrum (500.1 MHz, $CDCl_3$) of **35**.

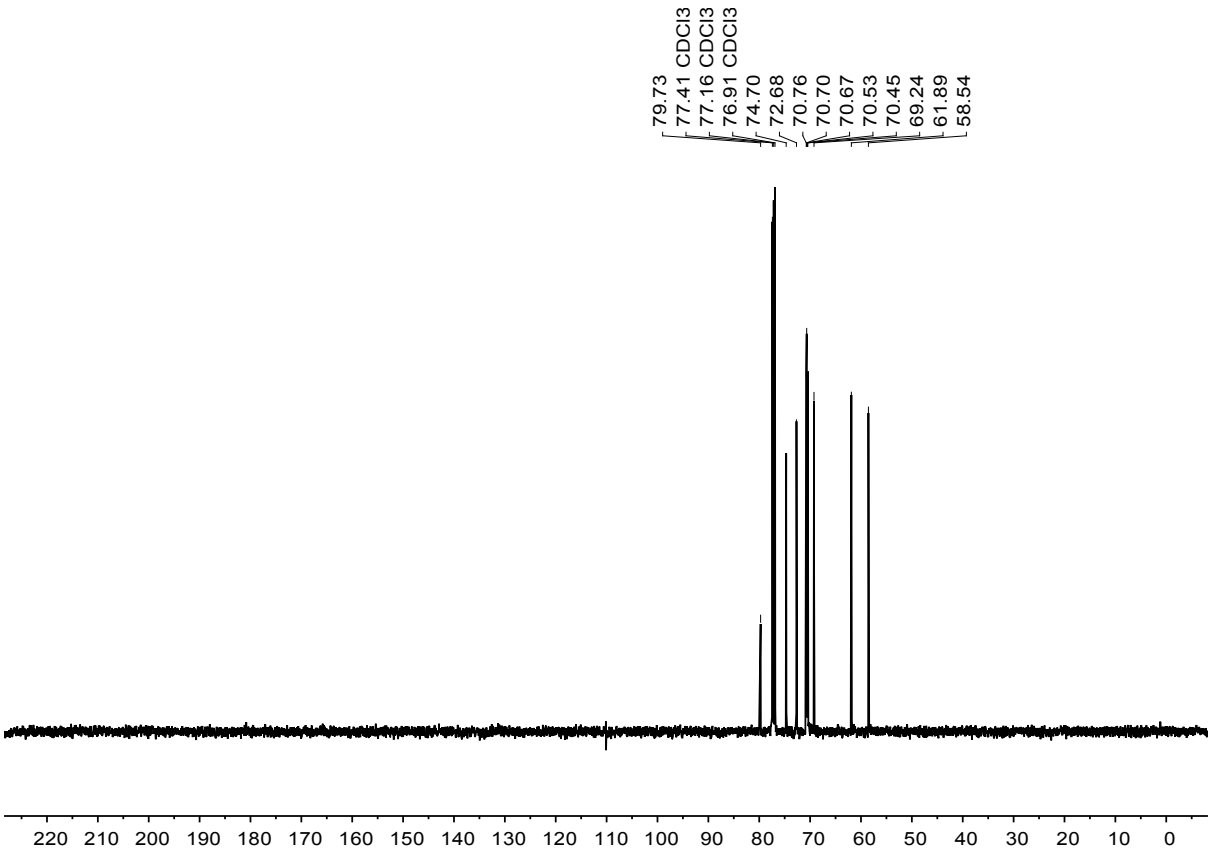
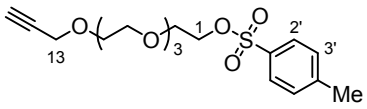


Figure S10. ^{13}C NMR spectrum (125.8 MHz, $CDCl_3$) of **35**.

2.6. 3,6,9,12-Tetraoxapentadec-14-yn-1-yl tosylate 13



Tosyl chloride (1.46 g, 7.65 mmol, 1.2 eq.) was added to a solution of alcohol **35** (1.48 g, 6.41 mmol, 1 eq.) and Et_3N (2.66 mL, 19.1 mmol, 3 eq.) in DCM (20 mL) at 0 °C, and the solution stirred at rt for 18 h. The reaction was quenched by addition of sat. aqueous NH_4Cl (50 mL) and DCM (50 mL), the phases separated and the aqueous phase extracted with DCM (4 × 50 mL). The combined organic phases were washed with aqueous HCl solution (1 M, 50 mL), followed by brine (50mL), and dried over Na_2SO_4 before being filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (DCM to 98:2 DCM: MeOH to 95:5 DCM: MeOH) to give **13** as a pale yellow oil (2.22 g, 90%): R_f = 0.71 (95:5 DCM: MeOH); δ_H (500.1 MHz, $CDCl_3$): 7.81–7.78 (2 H, m, *H*-2′, *H*-6′), 7.35–7.26 (2 H, m, *H*-3′, *H*-5′), 4.19 (2 H, d, *J* 2.4, CH_2C≡CH), 4.17–4.14 (2 H, m, PEG CH_2), 3.70–3.61 (10 H, m, PEG CH_2), 3.59–3.57 (4 H, m, PEG CH_2), 2.45 (3 H, s, C4′-CH_3), 2.42 (1 H, t, *J* 2.4, CH_2C≡C*H*); δ_C (125.8 MHz, $CDCl_3$): 144.9 (*C*-1′), 133.1 (*C*-4′), 130.0 (*C*-3′, *C*-5′), 128.1 (*C*-2′, *C*-6′), 79.8 (CH_2*C*≡CH), 74.7 (CH_2C≡*C*H), 70.9, 70.7, 70.7, 70.7, 70.5, 70.5, 69.4, 69.3, 68.1 (PEG *C*H_2), 58.4 (*C*H_2C≡CH), 21.8 (C4′-*C*H_3); *m/z* (ES^+) 409 ($[M+Na]^+$, 83%), 425 ($[M+Na]^+$, 100). Data are in agreement with the literature.[5]

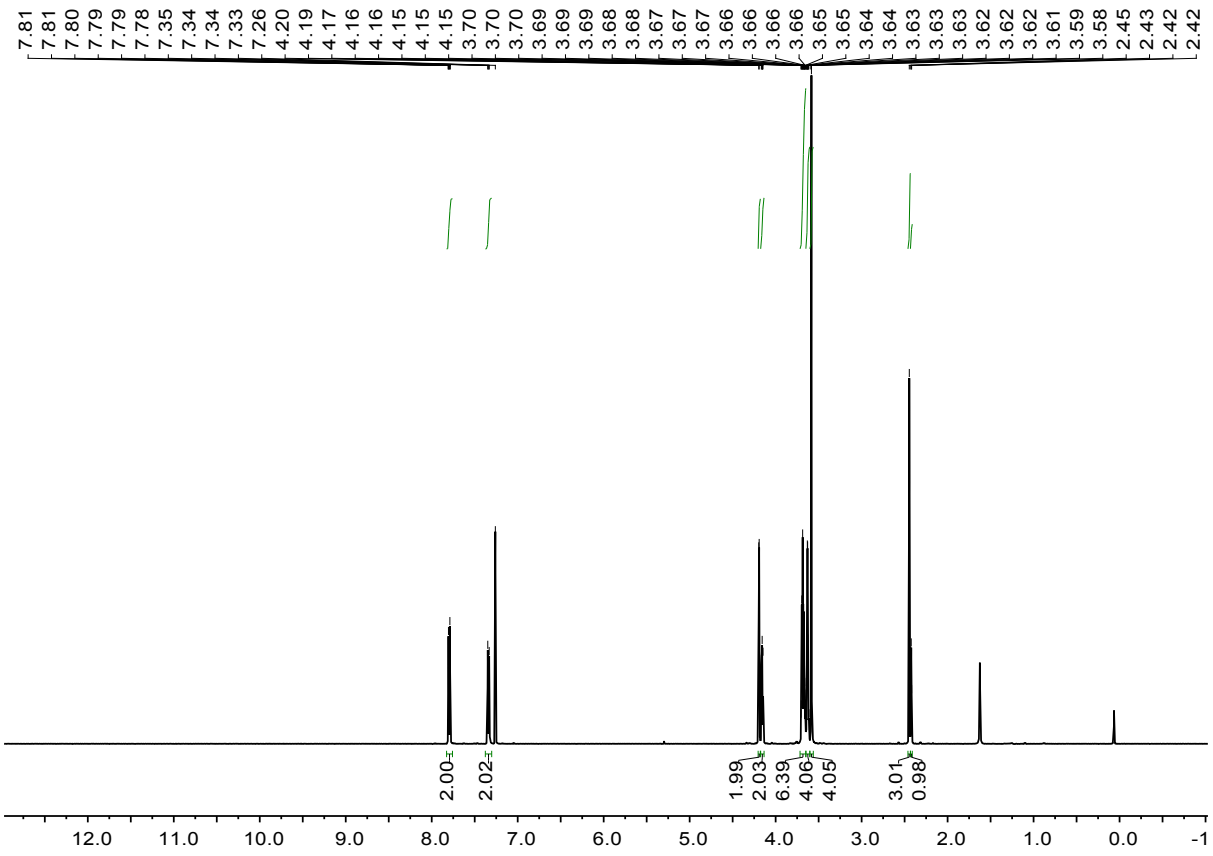


Figure S11. ^{1}H NMR spectrum (500.1 MHz, $CDCl_3$) of **13**.

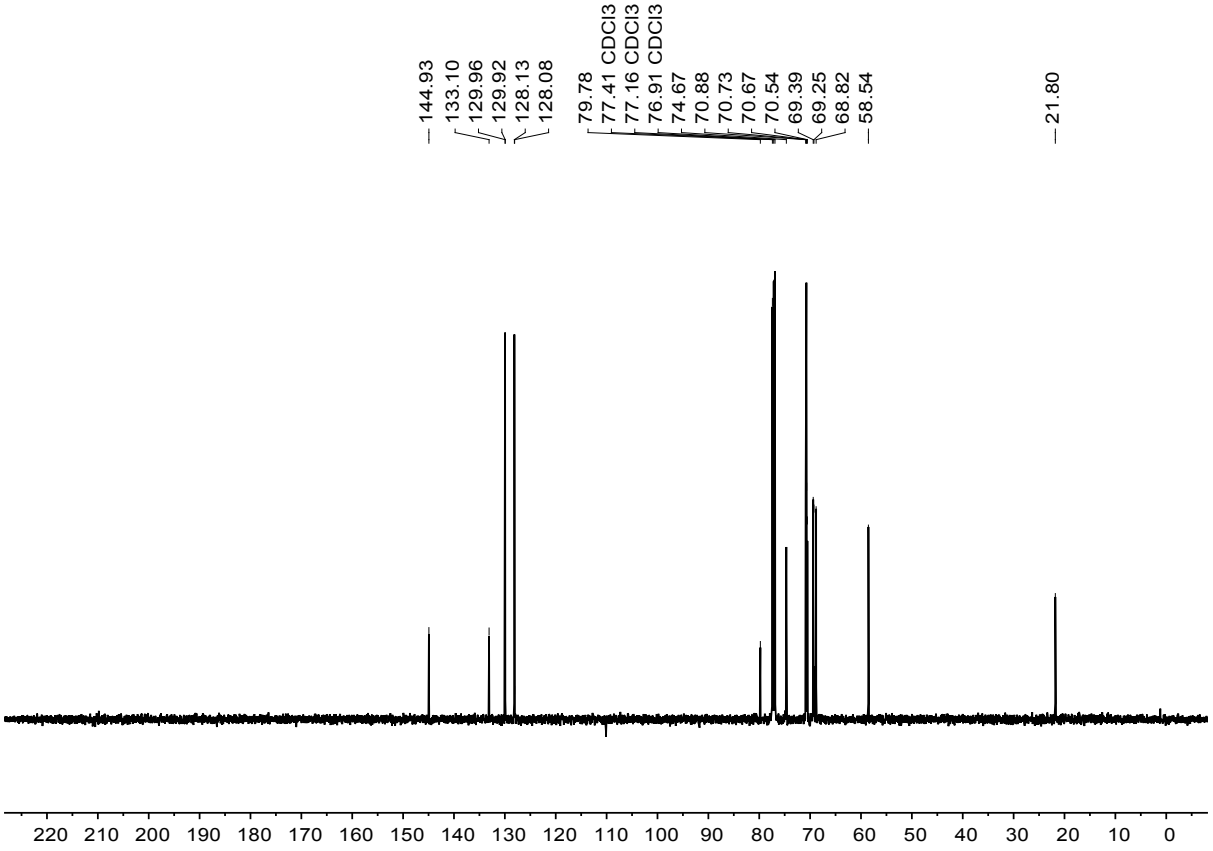
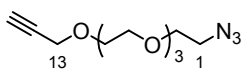


Figure S12. ^{13}C NMR spectrum (125.8 MHz, $CDCl_3$) of **13**.

2.7. 1-Azido-3,6,9,12-tetraoxapentadec-14-yne 36



NaN_3 (0.441 g, 7.87 mmol, 2.5 eq.) was added to a solution of tosylate **13** (1.00 g, 2.71 mmol, 1 eq.) in DMF (5 mL), and the solution stirred at 50 °C for 18 h. DMF was removed *in vacuo*, and the residue was purified by column chromatography (50:50 petroleum ether: Et_2O) to give the azide **36** as a colourless oil (552 mg, 79%): R_f = 0.13 (50:50 petroleum ether: Et_2O); δ_H (500.1 MHz, $CDCl_3$): 4.20 (2 H, d, *J* 2.4, CH_2C≡CH), 3.73–3.60 (14 H, m, PEG CH_2), 3.41–3.36 (2 H, m, CH_2N_3), 2.42 (1 H, t, *J* 2.4, CH_2C≡C*H*); δ_C (125.8 MHz, $CDCl_3$): 79.8 (CH_2*C*≡CH), 74.6 (CH_2C≡*C*H), 70.8, 70.8, 70.8, 70.8, 70.6, 70.2, 69.3 (PEG *C*H_2), 58.6 (*C*H_2C≡CH), 50.8 (*C*H_2N_3); *m/z* (ES^+) 537 ([2M+Na]$^+$, 52%), 280 ([M+Na]$^+$, 27), 258 ([M+H]$^+$, 100); HRMS (ES^+) calc. for $C_{11}H_{20}N_3O_4$ [M+H]$^+$ 258.1448, found 258.1452. Data are in agreement with the literature.[6]

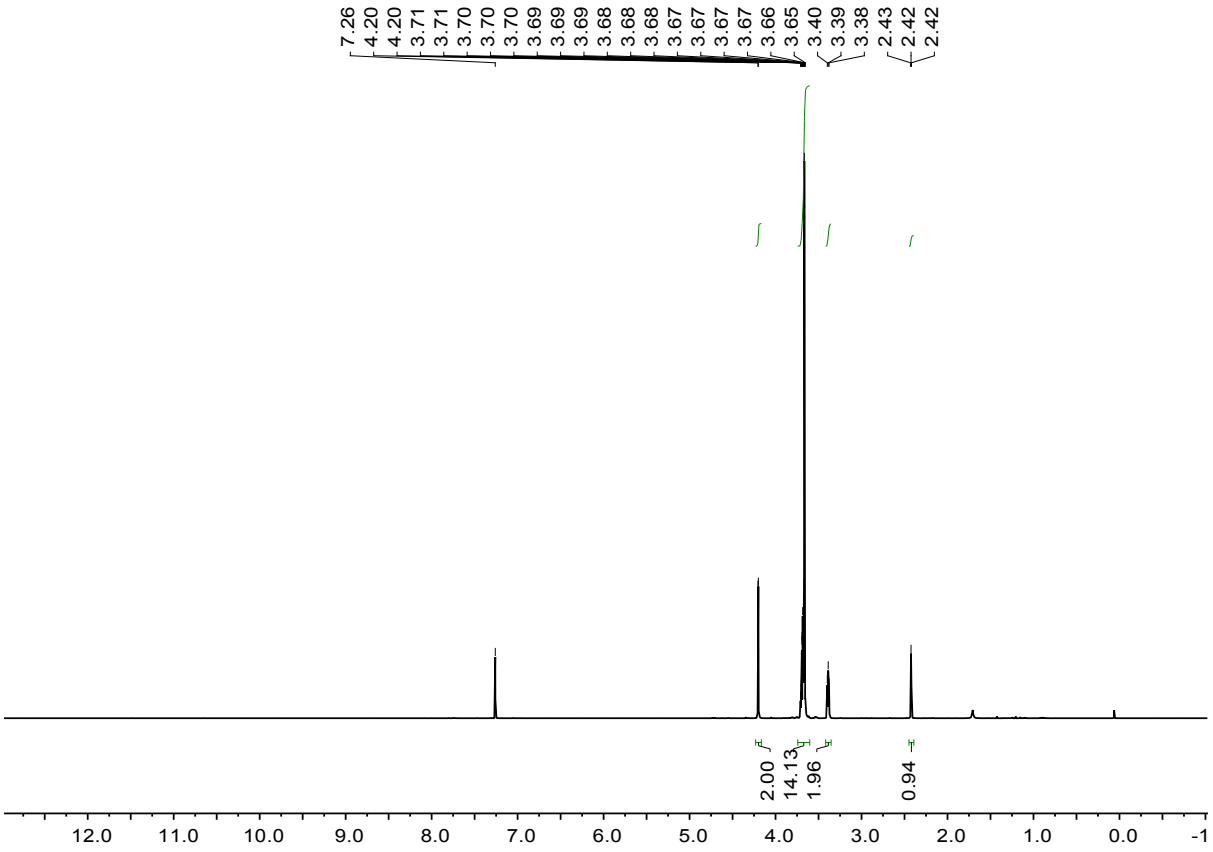


Figure S13. ^{1}H NMR spectrum (500.1 MHz, $CDCl_3$) of **36**.

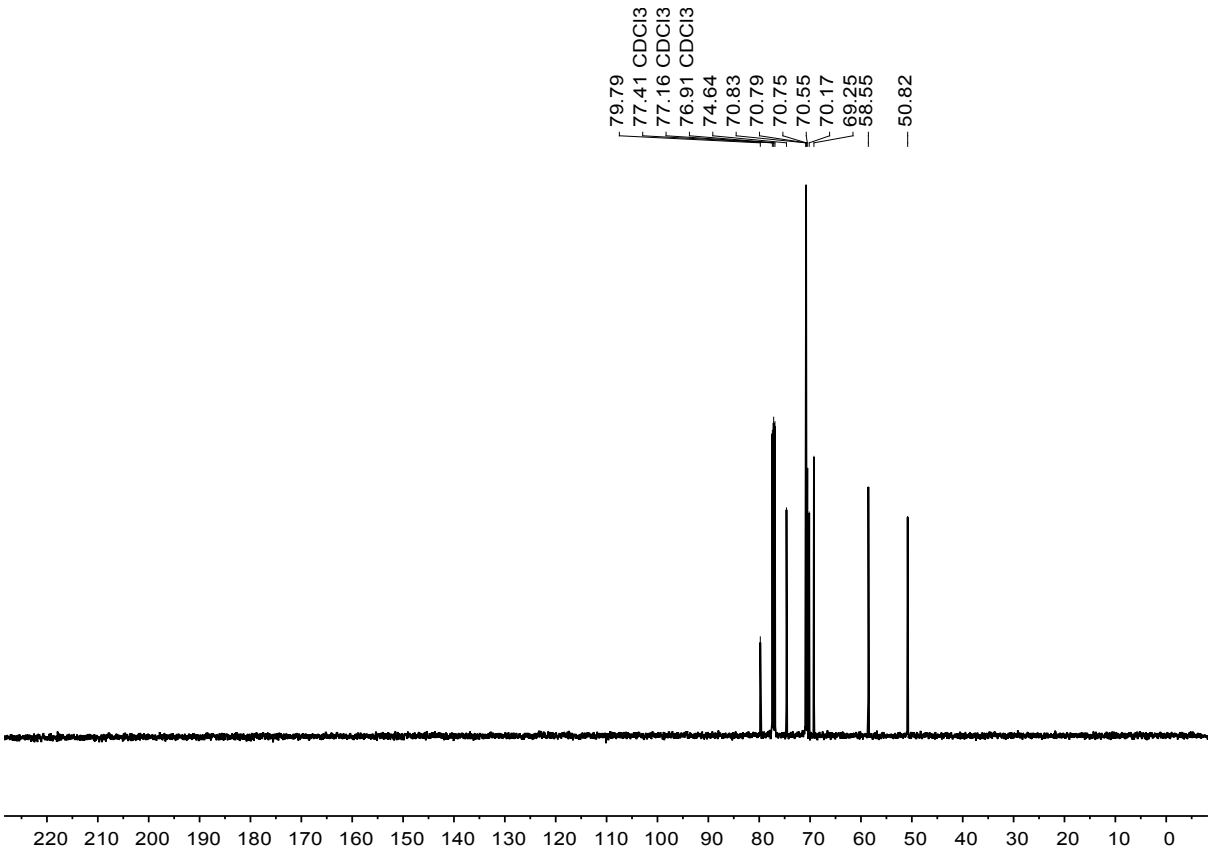
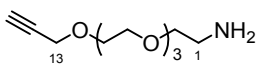


Figure S14. ^{13}C NMR spectrum (125.8 MHz, $CDCl_3$) of **36**.

2.8. 1-Amino-3,6,9,12-tetraoxapentadec-14-yne 14



PPh_3 (2.13 g, 8.12 mmol, 1.2 eq.) and water (0.12 mL, 6.76 mmol, 1 eq.) were added to a solution of azide **36** (1.74 g, 6.76 mmol, 1 eq.) in THF (15 mL), and the solution stirred at rt for 72 h. All volatiles were removed *in vacuo*, and the residue was purified by column chromatography (DCM to 95:5 DCM: MeOH to 90:10 DCM: MeOH to 89:10:1 DCM: MeOH: Et_3N) to give the amine **14** as a colourless oil (1.36 g, 87%): R_f = 0.13 (89:10:1 DCM: MeOH: Et_3N); δ_H (500.1 MHz, $CDCl_3$): 4.19 (2 H, d, *J* 2.4, C*H*$_2$C≡CH), 3.71–3.61 (12 H, m, PEG C*H*$_2$), 3.51 (2 H, t, *J* 5.3, C*H*$_2$CH$_2$NH$_2$), 2.86 (2 H, t, *J* 5.3, CH$_2$C*H*$_2$NH$_2$), 2.42 (1 H, t, *J* 2.4, CH$_2$C≡C*H*), 1.78 (2 H, br s, N*H*$_2$); δ_C (125.8 MHz, $CDCl_3$): 79.6 (CH$_2$*C*≡CH), 74.6 (CH$_2$C≡*C*H), 73.2, 70.6, 70.6, 70.6, 70.4, 70.3, 69.1 (PEG *C*H$_2$), 58.4 (*C*H$_2$C≡CH), 41.7 (*C*H$_2$NH$_2$); *m/z* (ES$^+$) 254 ([M+Na]$^+$, 62%), 232 ([M+H]$^+$, 100). Data are in agreement with the literature.[6]

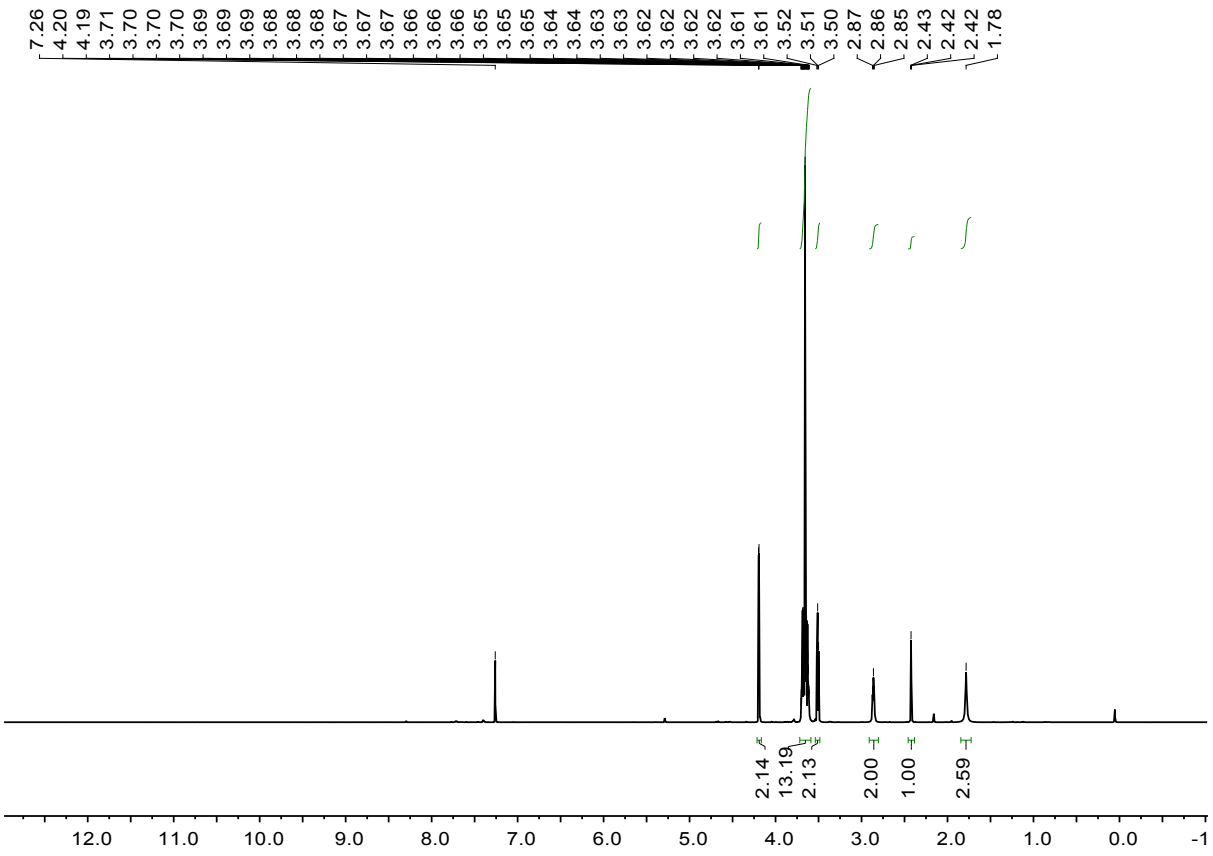


Figure S15. ^{1}H NMR spectrum (500.1 MHz, $CDCl_3$) of **14**.

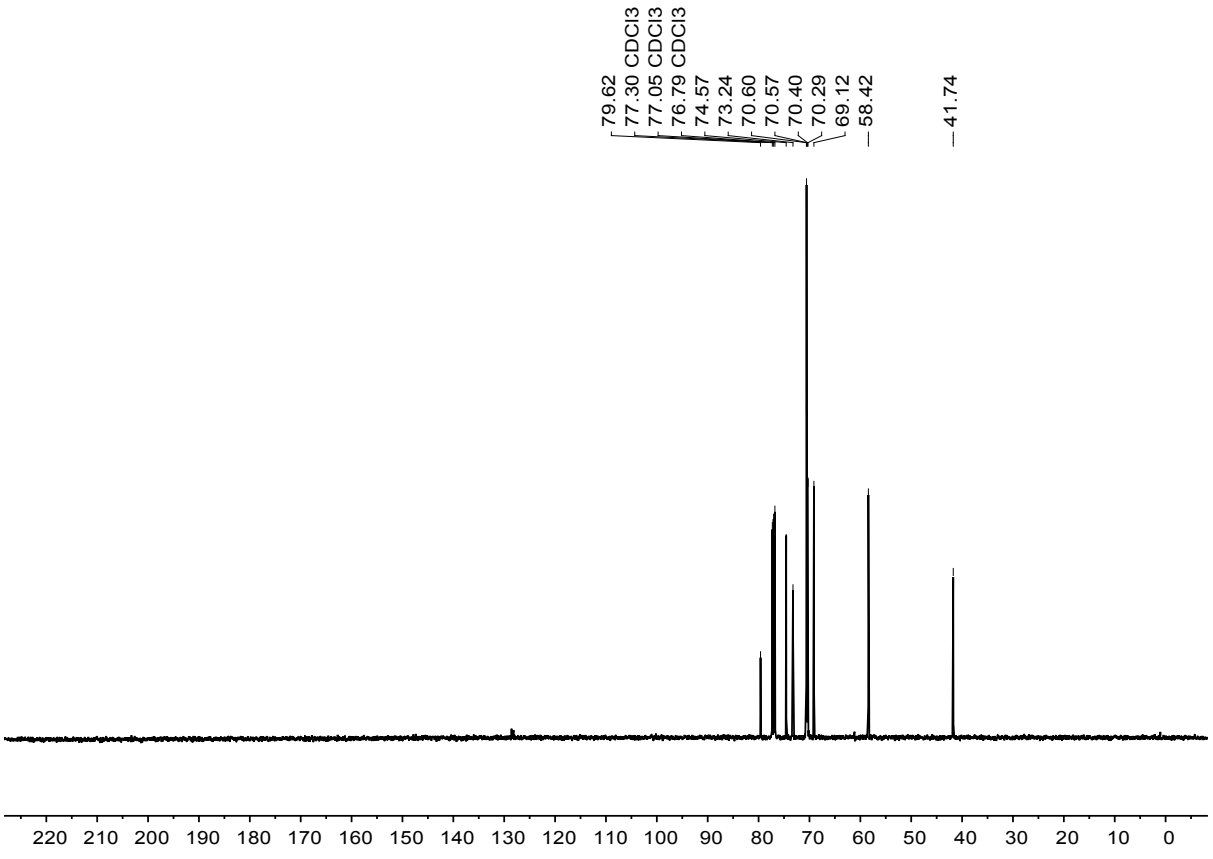
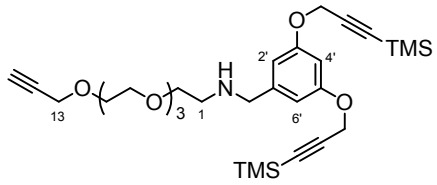


Figure S16. ^{13}C NMR spectrum (125.8 MHz, $CDCl_3$) of **14**.

2.9. *N*-(3',5'-bis((3-(trimethylsilyl)prop-2-yn-1-yl)oxy)benzyl)-3,6,9,12-tetraoxapentadec-14-yn-1-amine 15



Acetic acid (0.60 mL, 10.6 mmol, 2 eq.) and $NaB(OAc)_3H$ (4.50 g, 21.22 mmol, 4 eq.) were slowly added to a solution of aldehyde **11** (1.90 g, 5.31 mmol, 1 eq.) and amine **14** (1.35 g, 5.84 mmol, 1.1 eq.) in THF (10 mL), and the mixture stirred at rt for 18 h. The mixture was diluted with Et_2O (50 mL) and quenched by careful addition of sat. aqueous $NaHCO_3$ (50 mL), before the phases were separated and the aqueous phase extracted with Et_2O (3 × 50 mL). The combined organic phases were dried over Na_2SO_4 before being filtered and concentrated *in vacuo* The crude product was purified by column chromatography (DCM to 97:3 DCM: MeOH to 95:5 DCM: MeOH) to give **15** as a colourless oil (1.09 g, 35%): R_f = 0.44 (90:10 DCM: MeOH); δ_H (400.3 MHz, $CDCl_3$): 6.58 (2 H, d, *J* 2.3, *H*-2′, *H*-6′), 6.49 (1 H, t, *J* 2.3, *H*-4′), 4.64 (4 H, s, $CH_2C{\equiv}CSi$), 4.20 (2 H, d, *J* 2.4 $CH_2C{\equiv}CH$), 3.75 (2 H, bs, $NHCH_2Ar$), 3.70–3.58 (14 H, m, PEG CH_2), 2.79 (2 H, t, *J* 5.2, CH_2NHCH_2Ar), 2.42 (1 H, t, *J* 2.4, $CH_2C{\equiv}CH$), 0.18 (18 H, s, $Si(CH_3)_3$); δ_C (125.7 MHz, $CDCl_3$): 159.0 (*C*-3′, *C*-5′), 142.7 (*C*-1′), 107.7 (*C*-2′, *C*-6′), 101.0 (*C*-4′), 100.1 (2 × $CH_2C{\equiv}CSi$), 92.7 (2 × $CH_2C{\equiv}CSi$), 79.7 ($CH_2C{\equiv}CH$), 74.7 ($CH_2C{\equiv}CH$), 70.7, 70.7, 70.6, 70.6, 70.5, 70.4, 69.2 (PEG CH_2), 58.5 ($CH_2C{\equiv}CH$), 56.9 (2 × $CH_2C{\equiv}CSi$), 58.8 ($NHCH_2Ar$), 48.6 (CH_2NHCH_2Ar), −0.17 ($Si(CH_3)_3$); *m/z* (ESI^+) 574 ($[M+H]^+$, 100%); HRMS (ESI^+) calc. for $C_{30}H_{48}NO_6Si_2$ $[M+H]^+$ 574.3015, found 574.3003.

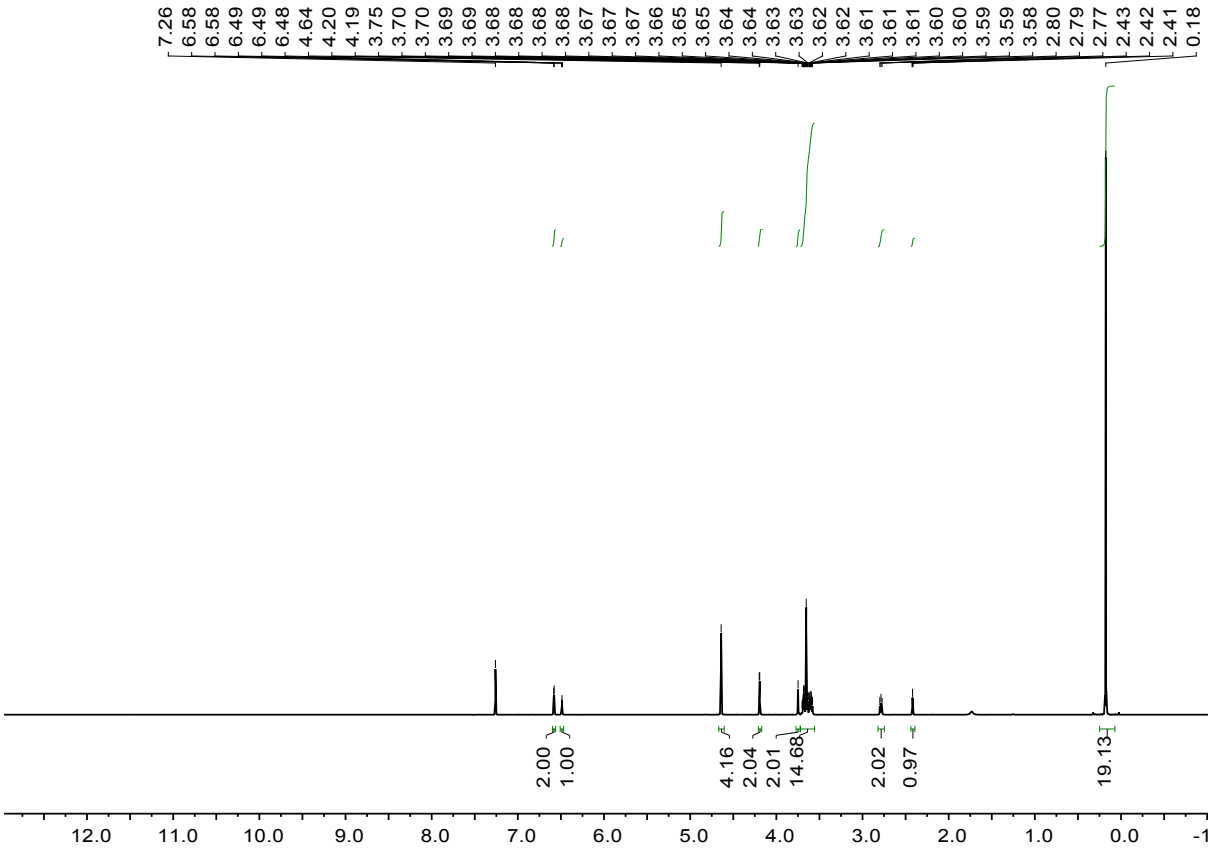


Figure S17. ^1H NMR spectrum (400.3 MHz, $CDCl_3$) of **15**.

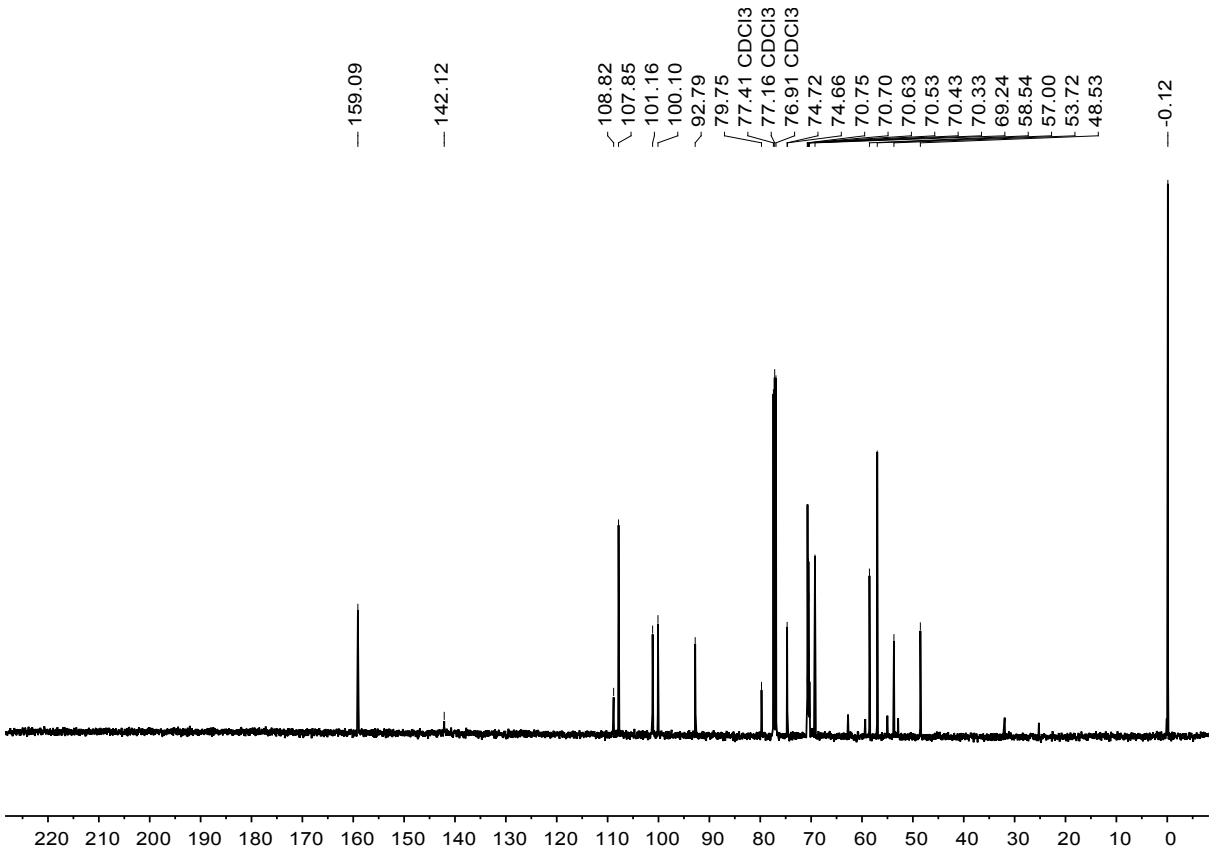
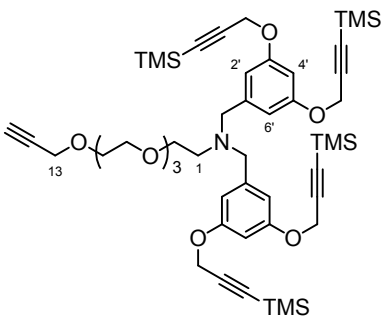


Figure S18. ^{13}C NMR spectrum (125.8 MHz, $CDCl_3$) of **15**.

2.10. *N*,*N*-bis(3',5'-bis((3-(trimethylsilyl)prop-2-yn-1-yl)oxy)benzyl)-3,6,9,12-tetraoxapentadec-14-yn-1-amine 16



Acetic acid (0.60 mL, 10.6 mmol, 2 eq.) and $NaB(OAc)_3H$ (4.50 g, 21.22 mmol, 4 eq.) were slowly added to a solution of aldehyde **11** (1.90 g, 5.31 mmol, 1 eq.) and amine **14** (1.35 g, 5.84 mmol, 1.1 eq.) in THF (10 mL), and the mixture was stirred at rt for 18 h. The mixture was diluted with Et_2O (50 mL) and quenched by careful addition of sat. aqueous $NaHCO_3$ (50 mL), before the phases were separated and the aqueous phase extracted with Et_2O (3 × 50 mL). The combined organic phases were dried over Na_2SO_4 before being filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (DCM to 97:3 DCM: MeOH to 95:5 DCM: MeOH) to give **16** as a colourless oil (500 mg, 21%): R_f = 0.85 (90:10 DCM: MeOH); δ_H (300.1 MHz, $CDCl_3$): 6.62 (4 H, d, *J* 2.3, *H*-2′, *H*-6′), 6.47 (2 H, t, *J* 2.4, *H*-4′), 4.63 (8 H, s, CH_2C≡CSi), 4.18 (2 H, d, *J* 2.4 CH_2C≡CH), 3.69–3.52 (18 H, m, PEG CH_2, $CH_2N(CH_2Ar)_2$), 2.67 (2 H, t, *J* 6.1, C$H_2N(CH_2Ar)_2$), 2.41 (1 H, t, *J* 2.4, $CH_2C{\equiv}CH$), 0.17 (36 H, s, $Si(CH_3)_3$); δ_C (75.5 MHz, $CDCl_3$): 158.9 (*C*-3′, *C*-5′), 142.4 (*C*-1′), 108.3 (*C*-2′, *C*-6′), 100.8 (*C*-4′), 100.2 (4 × $CH_2C{\equiv}CSi$), 92.7 (4 × $CH_2C{\equiv}CSi$), 79.8 ($CH_2C{\equiv}CH$), 74.7 ($CH_2C{\equiv}CH$), 70.7, 70.7, 70.7, 70.5, 70.5, 70.1, 69.2 (PEG CH_2), 59.1 ($CH_2N(CH_2Ar)_2$), 58.2 ($CH_2C{\equiv}CH$), 57.0 (4 × $CH_2C{\equiv}CSi$), 52.9 ($CH_2N(CH_2Ar)_2$), −0.11 ($Si(CH_3)_3$); *m/z* (ES^+) calc. isotope pattern for $C_{49}H_{74}NO_8Si_4$ $[M+H]^+$ 916.4 (100%), 917.4 (53), 918.4 (13), 919.4 (10), found 916.5 (100%), 917.5 (53), 918.5 (14), 919.5 (8).

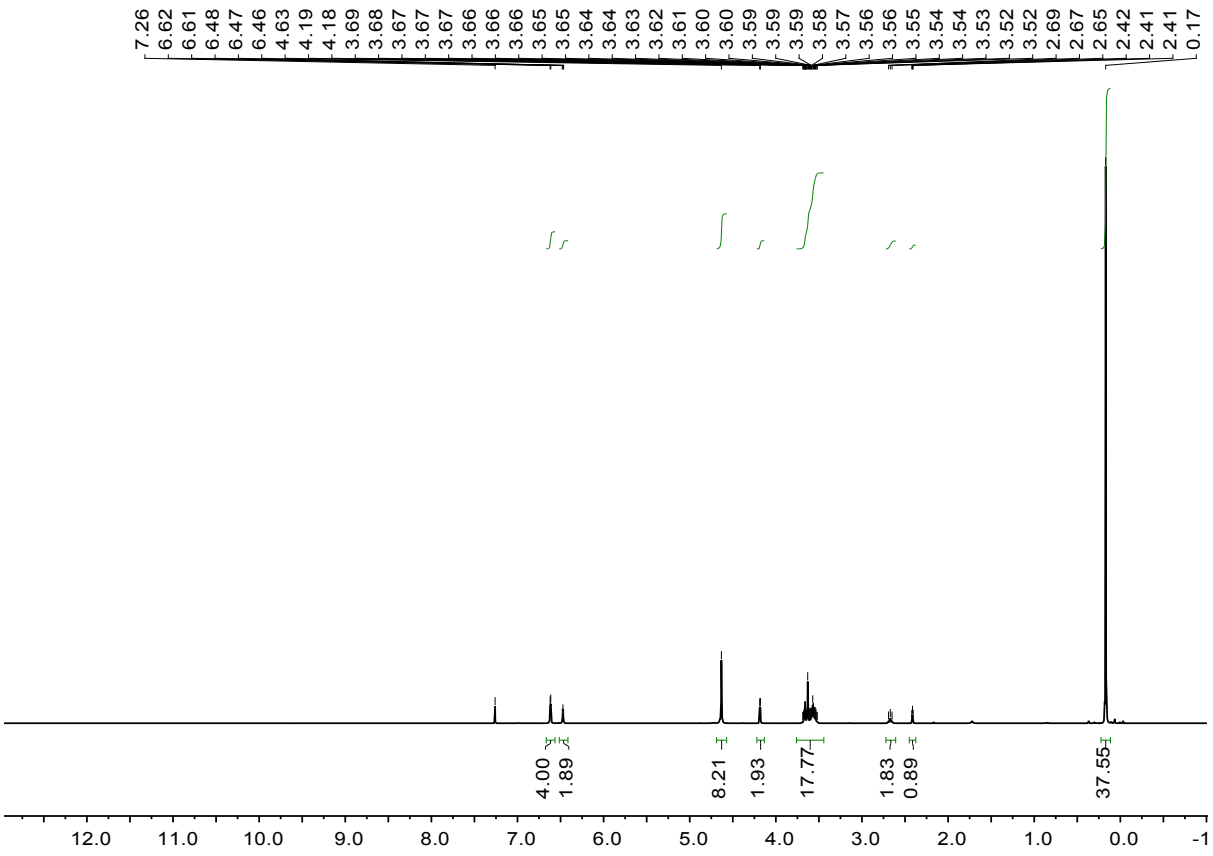


Figure S19. ^{1}H NMR spectrum (300.1 MHz, $CDCl_3$) of **16**.

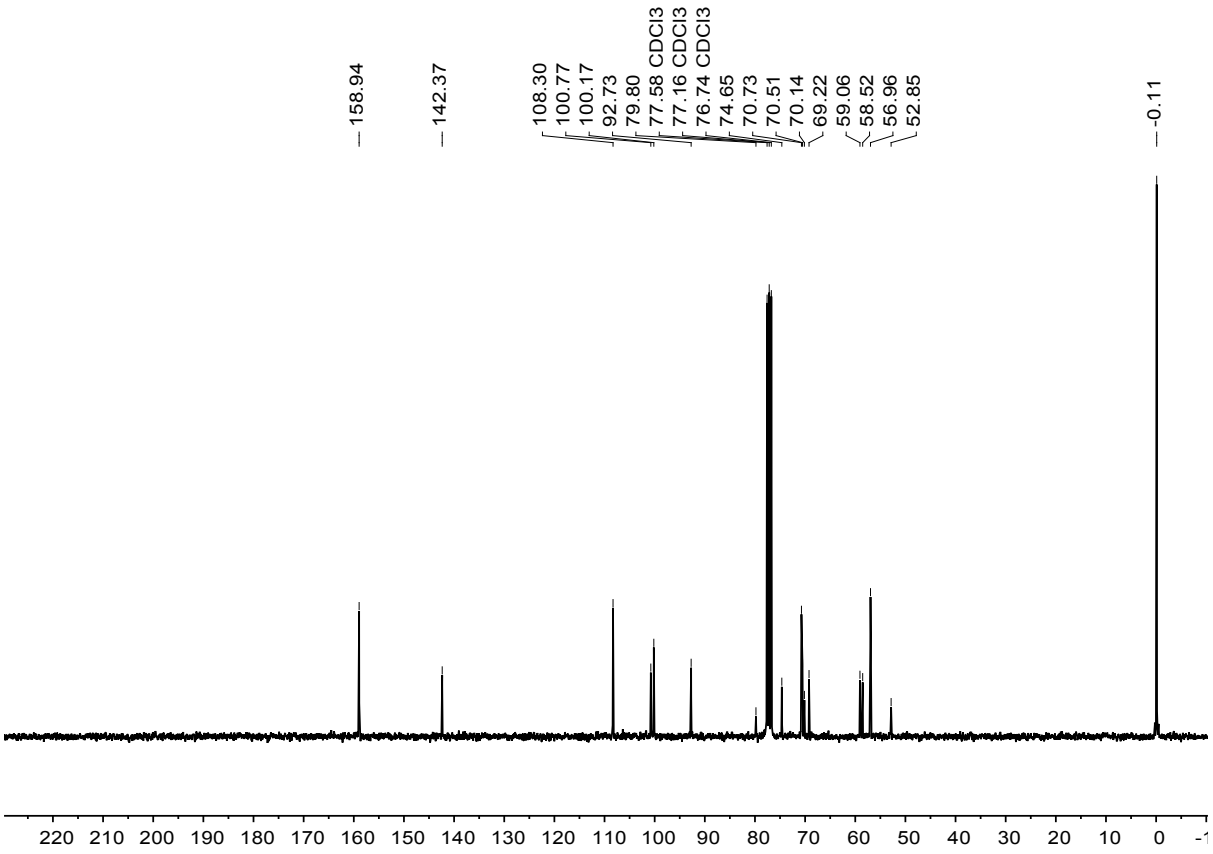
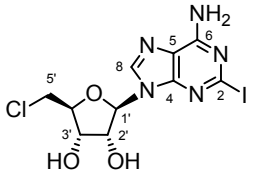


Figure S20. ^{13}C NMR spectrum (75.5 MHz, $CDCl_3$) of **16**.

2.11. 5′-Chloro-5′-deoxy-2-iodoadenosine 18



Pyridine (103 µL, 1.27 mmol, 2.0 eq.) and thionyl chloride (103 µL, 1.91 mmol, 3.0 eq.) were slowly added to a suspension of 2-iodoadenosine[7] (250 mg, 0.363 mmol, 1.0 eq.) in MeCN (12 mL) at 0 °C. The mixture was stirred at 0 °C for 4 h, warmed to rt and stirred for a further 18 h. The volatile components were removed, and the residue was diluted with MeOH:water:aqueous ammonia solution (35% *v/v*) (10:2:1, 10 mL) and the solution stirred for 2 h. After concentration, the residue was purified by column chromatography (95:5 DCM:MeOH to 90:10 DCM:MeOH) to afford the product **18** as a white foam (167 mg, 64%): R_f = 0.47 (20:80 petroleum ether:acetone); mp 127–130 °C (DCM); δ_H (500.1 MHz, d_4-MeOD): 8.15 (1 H, s, *H*-8), 5.95 (1 H, d, *J* 5.1, *H*-1′), 4.77 (1 H, dd, *J* 5.2, 5.1, *H*-2′), 4.39 (1 H, dd, *J* 5.2, 4.2, *H*-3′), 4.27-4.24 (1 H, m, *H*-4′), 3.95 (1 H, dd, 11.8, 5.3, *H*-5′a), 3.85 (1 H, dd, 11.8, 5.2, *H*-5′b); δ_C (125.8 MHz, d_4-MeOD): 155.7 (*C*-6), 149.6 (*C*-4), 139.7 (*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^+) 434 ($[M+Na]^+$ 60%), 412 ($[M+H]^+$, 24); HRMS (ES^+) calc. for $C_{10}H_{11}{}^{35}ClN_5O_3INa$ $[M+Na]^+$ 433.9487, found 433.9477.

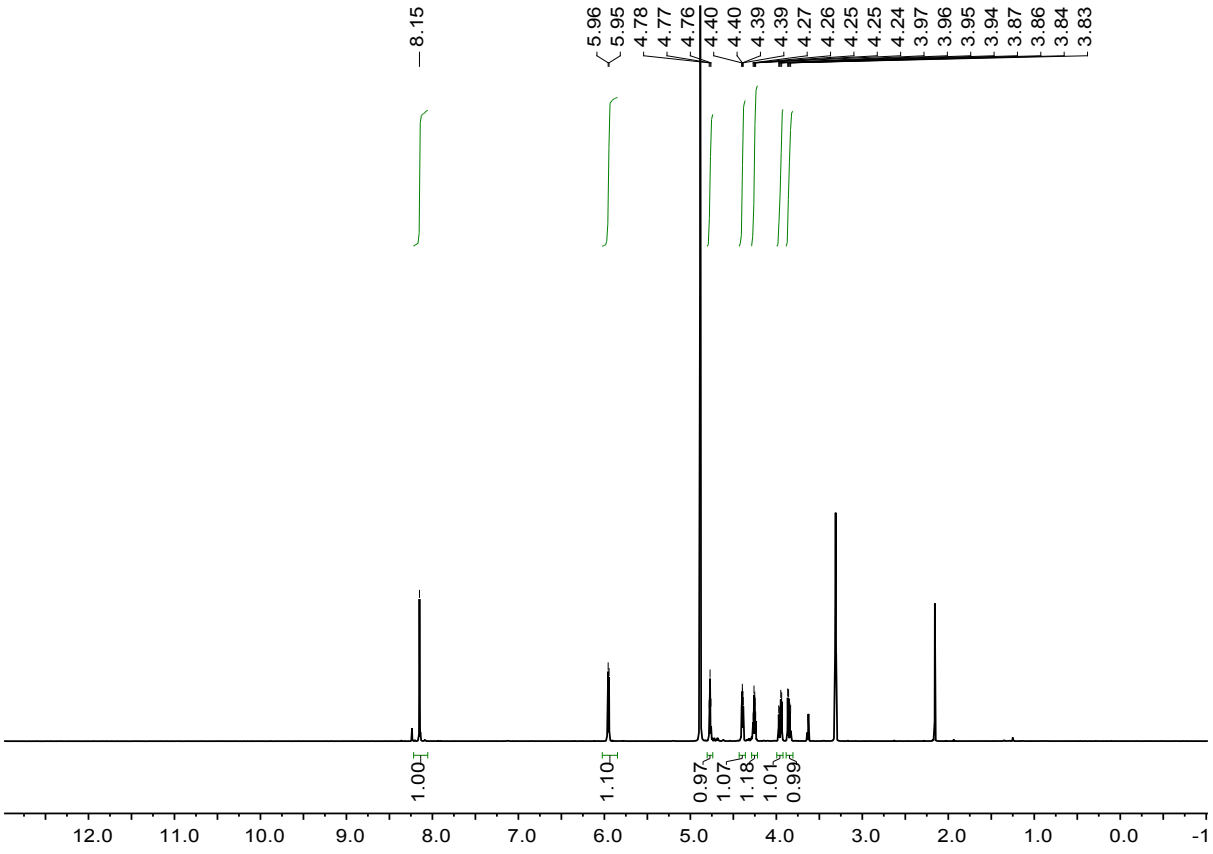


Figure S21. ^{1}H NMR spectrum (500.1 MHz, d_4-MeOD) of **18**.

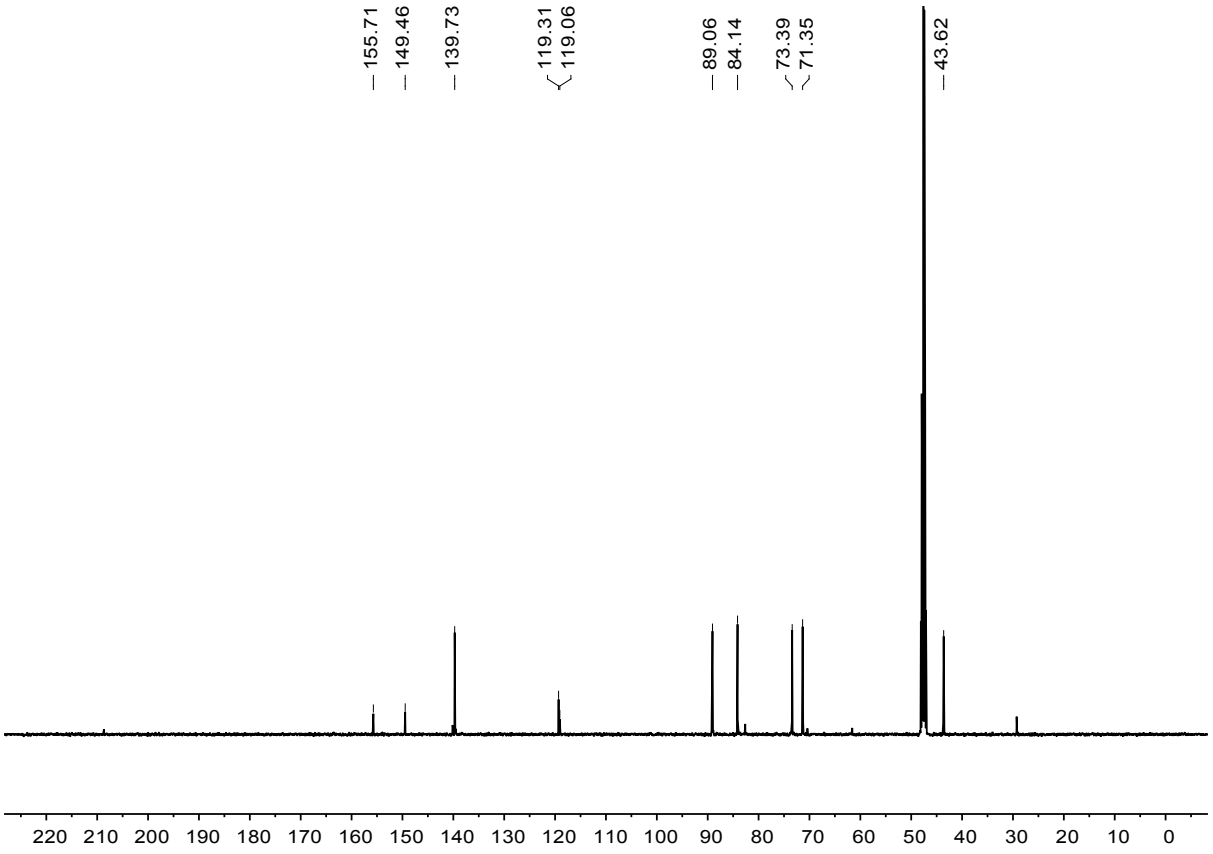
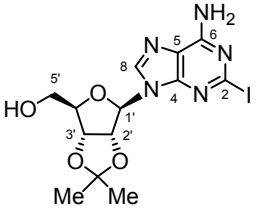


Figure S22. ^{13}C NMR spectrum (125.8 MHz, d_4-MeOD) of **18**.

2.12. 2′,3′-*O*-Isopropylidene-2-iodoadenosine 19



2,2-Dimethoxypropane (1.55 mL, 12.7 mmol, 10 eq.) and perchloric acid (70% *v/v* in water, 75 μL, 0.839 mmol, 0.67 eq.) were slowly added to a suspension of 2-iodoadenosine (500 mg, 1.27 mmol, 1.0 eq.) in acetone (15 mL) at 0 °C. The mixture was allowed to slowly warm to rt, and stirred for a further 18 h. The reaction was quenched by addition of sat. aqueous $NaHCO_3$ solution (1 mL) and concentrated *in vacuo*. The residue was adsorbed onto silica and purified by column chromatography (20:80 petroleum ether:acetone) to give the product **19** as a white foam (550 mg, quant.): R_f = 0.56 (20:80 petroleum ether:acetone); mp 131–135 °C (petroleum ether:acetone); δ_H (500.1 MHz, $CDCl_3$): 7.73 (1 H, s, *H*-8), 6.08 (2 H, br s, NH_2), 5.79 (1 H, d, *J* 5.3, *H*-1′), 5.36 (1 H, dd, *J* 11.8, 1.9, C-5′ O*H*), 5.18 (1 H, dd, *J* 5.7, 5.3, *H*-2′), 5.11 (1 H, dd, *J* 5.7, 1.4, *H*-2′), 4.52 (1 H, ddd, *J* 1.8, 1.7, 1.4, *H*-4′), 3.99 (1 H, m, *H*-5′a), 3.82 (1 H, m, *H*-5′b), 1.63 (3 H, s, C(CH_3)$_2$), 1.38 (3 H, s, C(CH_3)$_2$); δ_C (125.8 MHz, $CDCl_3$): 155.5 (*C*-6), 149.2 (*C*-4), 140.3 (*C*-8), 121.0 (*C*-5), 119.4 (*C*-2), 114.3 (*C*(CH$_3$)$_2$), 94.2 (*C*-1′), 86.1 (*C*-4′), 82.8 (*C*-2′), 81.7 (*C*-3′), 63.5 (*C*-5′), 27.8 (C(*C*H$_3$)$_2$), 25.4 (C(*C*H$_3$)$_2$); *m/z* (ES$^+$) 456 ([M+Na]$^+$ 100%), 434 ([M+H]$^+$, 23); HRMS (ES$^+$) calc. for $C_{13}H_{16}N_5O_4INa$ [M+Na]$^+$ 456.1039, found 456.1031.

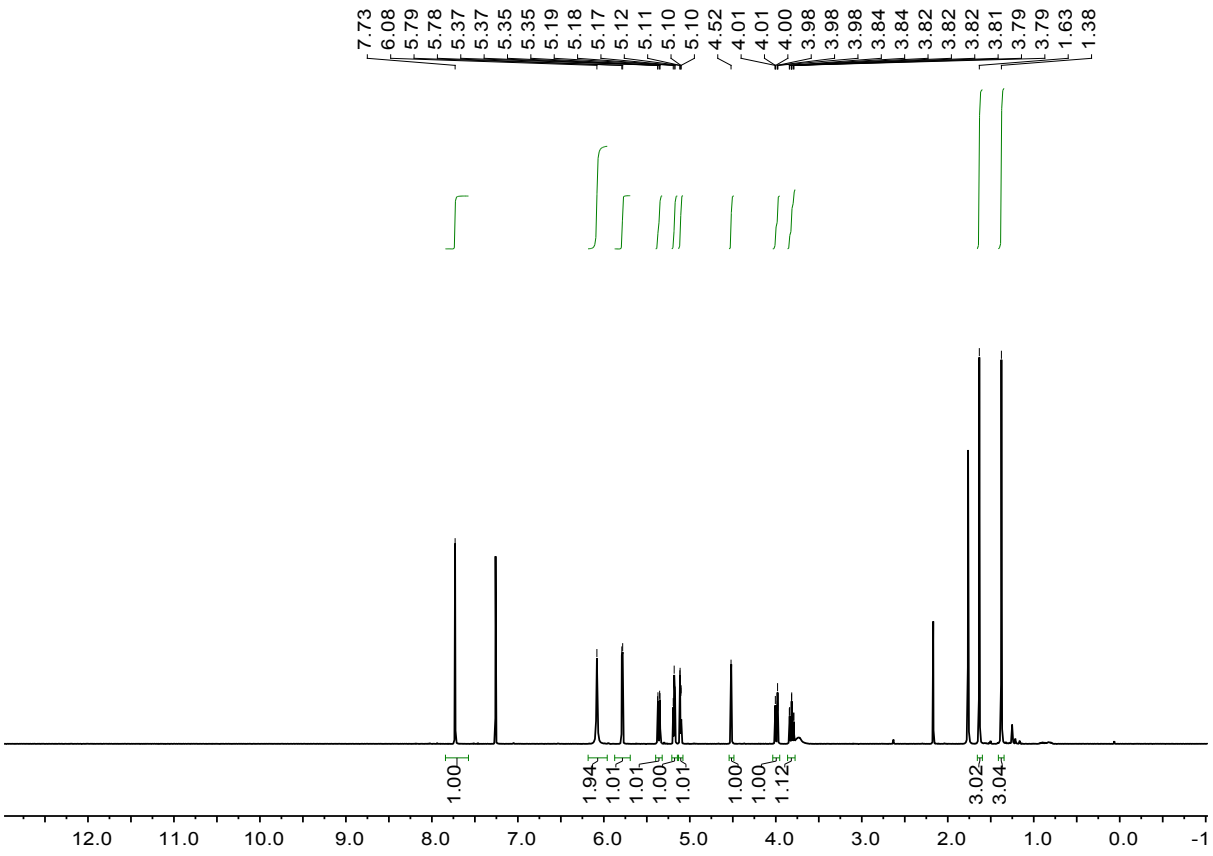


Figure S23. ^{1}H NMR spectrum (500.1 MHz, $CDCl_3$) of **19**.

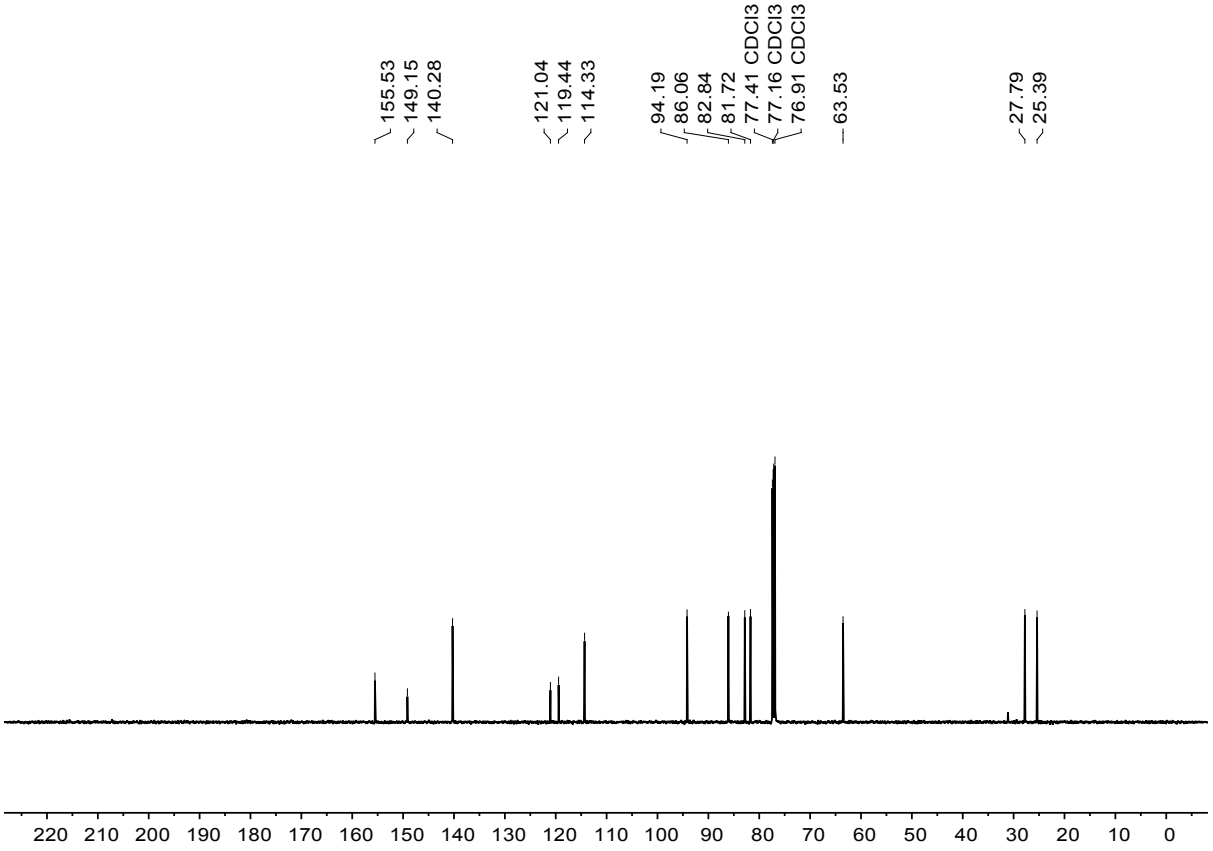
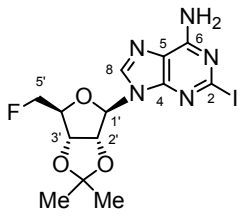


Figure S24. ^{13}C NMR spectrum (125.8 MHz, $CDCl_3$) of **19**.

2.13. 5′-Fluoro-5′-deoxy-2′,3′-*O*-isopropylidene-2-iodoadenosine 37



TsF (442 mg, 2.54 mmol, 2.0 eq.) and TBAF (1 M in THF, 5.08 mL, 5.08 mmol, 4.0 eq.) were added to a solution of **19**(550 mg, 1.27 mmol, 1.0 eq.) in THF (16 mL). The mixture was heated to 60 °C for 18 h, and cooled to rt. The volatile components were removed and the orange residue was purified by column chromatography (80:20 petroleum ether:acetone to 70:30 petroleum ether:acetone to 60:40 petroleum ether:acetone) to give the product **37** as a white foam (270 mg, 49%): R_f = 0.79 (50:50 petroleum ether:acetone); mp 88–90 °C (petroleum ether:acetone); δ_H (500.1 MHz, $CDCl_3$): 7.81 (1 H, s, *H*-8), 6.14 (1 H, d, *J* 2.1, *H*-1′), 6.05 (2 H, br s, NH_2), 5.25-5.23 (1 H, m, *H*-2′), 5.07 (1 H, dd, *J* 6.2, 3.5, *H*-3′), 4.68 (1 H, ddd, *J* 46.9, 10.3, 3.6, *H*-5′a), 4.63 (1 H, ddd, *J* 47.0, 10.3, 5.2, *H*-5′b), 4.53-4.46 (1 H, m, *H*-4'), 1.63 (3 H, s, C(CH_3)$_2$), 1.40 (3 H, s, C(CH_3)$_2$); δ_C (125.8 MHz, $CDCl_3$): 155.2 (*C*-6), 149.6 (*C*-4), 139.2 (*C*-8, d, *J* 3.5), 119.8 (*C*-5, *C*-2), 114.8 (*C*(CH$_3$)$_2$), 90.5 (*C*-1′), 85.9 (*C*-4′, d, *J* 19.3), 84.6 (*C*-2′), 82.9 (*C*-5′, d, *J* 171.6), 80.7 (*C*-3′, d, *J* 7.0), 27.2 (C(*C*H$_3$)$_2$), 25.4 (C(*C*H$_3$)$_2$); δ_F (470.3 MHz, $CDCl_3$): −227.93 (ddd, *J* 47.0, 46.9, 22.7, CH_2F); *m/z* (ES$^+$) 458 ([M+Na]$^+$ 100%), 436 ([M+H]$^+$ 37); HRMS (ES$^+$) calc. for $C_{13}H_{15}N_5O_3FINa$ [M+Na]$^+$ 458.0096, found 458.0086.

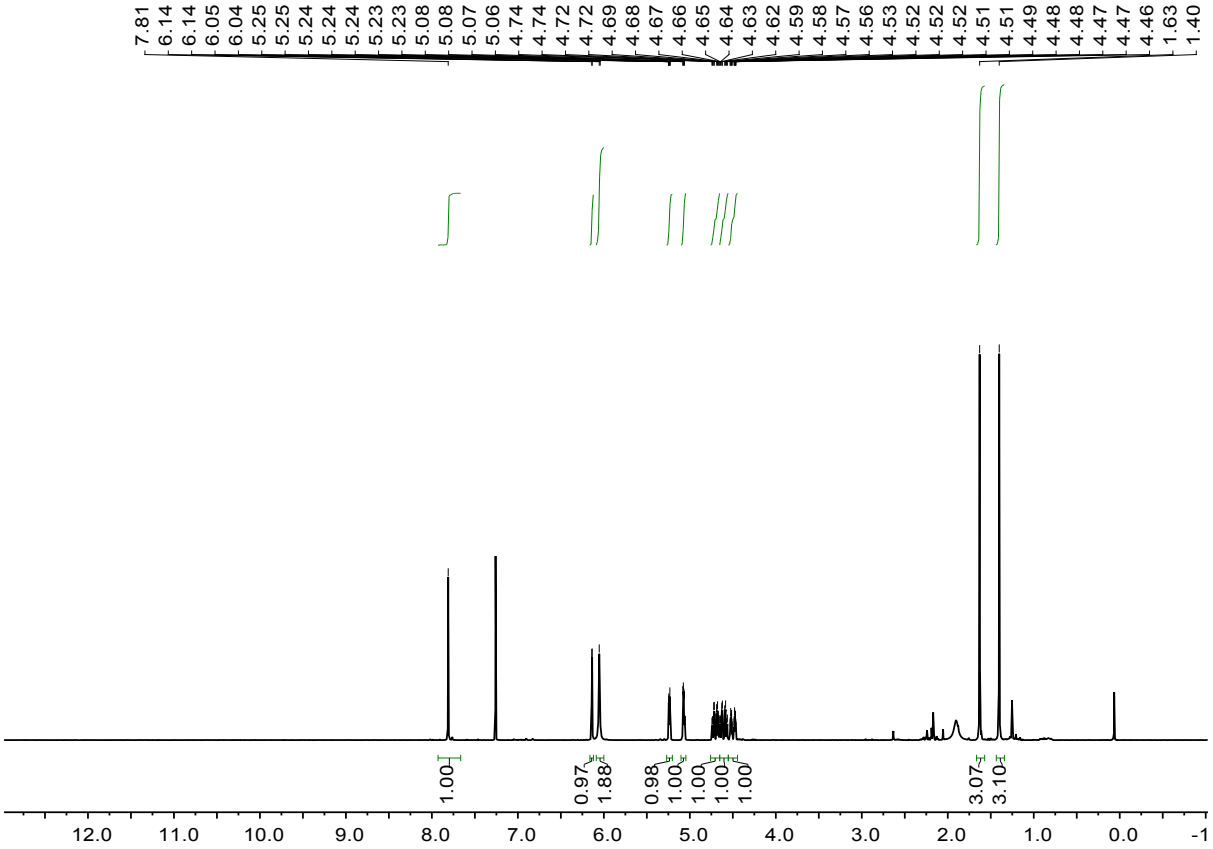


Figure S25. ^{1}H NMR spectrum (500.1 MHz, $CDCl_3$) of **37**.

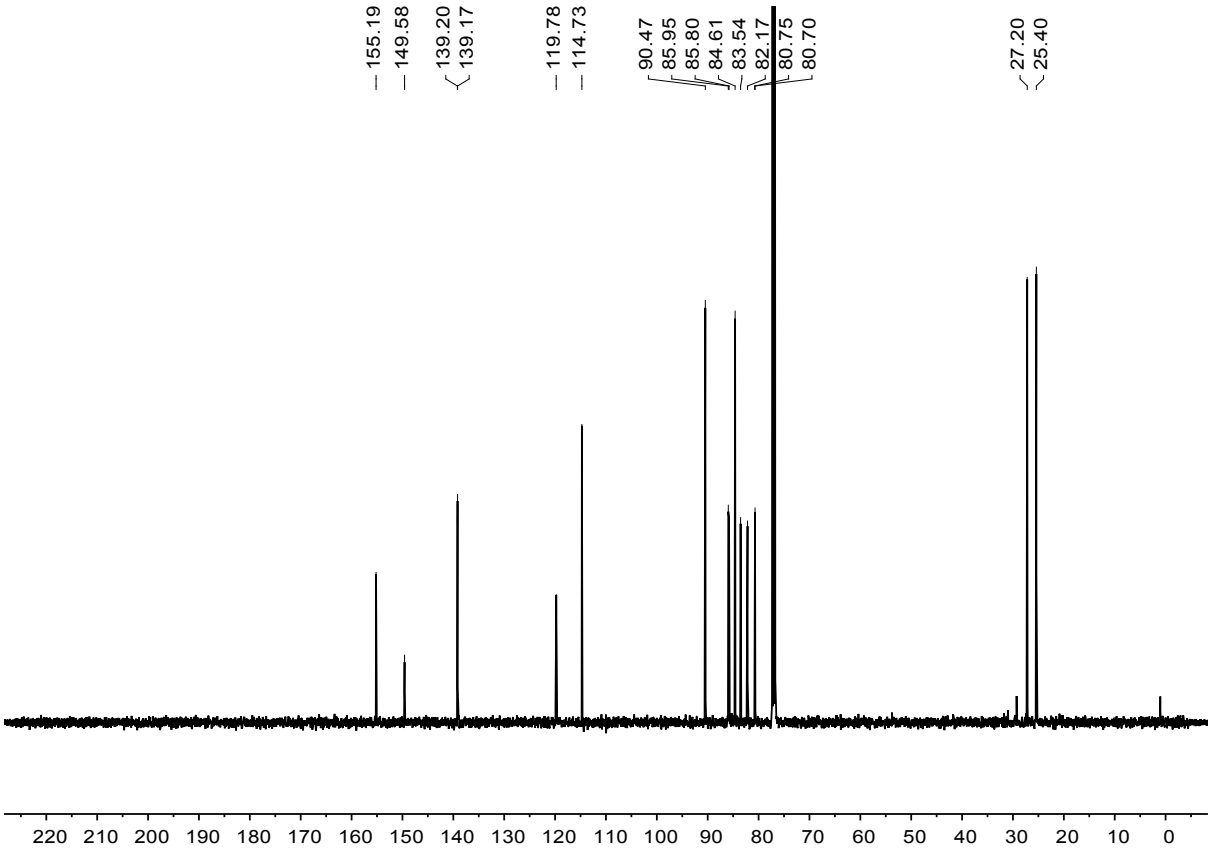


Figure S26. ^{13}C NMR spectrum (125.8 MHz, $CDCl_3$) of **37**.

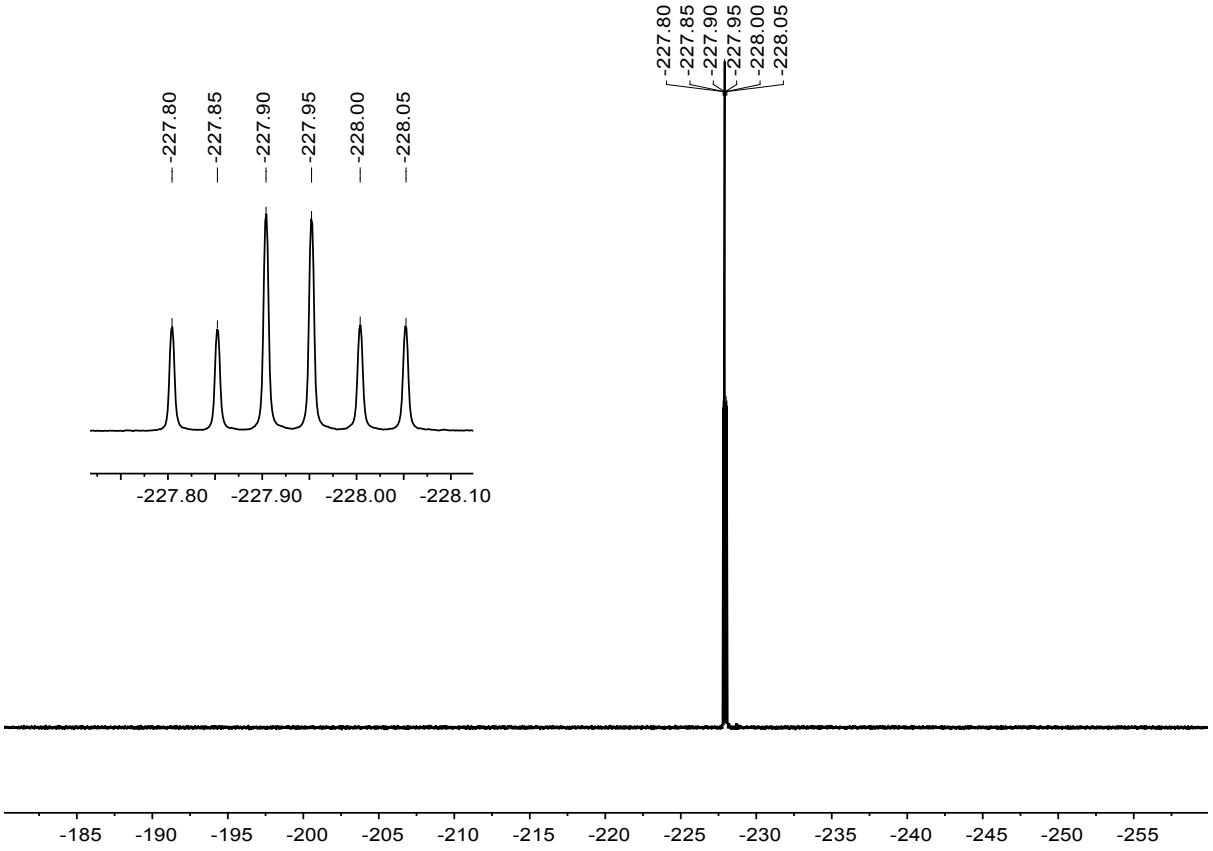
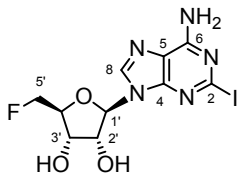


Figure S27. ^{19}F NMR spectrum (470.1 MHz, $CDCl_3$) of **37**.

2.14. 5′-Fluoro-5′-deoxy-2-iodoadenosine 20



Anhydrous TsOH (16.0 mg, 0.0930 mmol, 0.15 eq.) was added to a solution of **37** (270 mg, 0.620 mmol) in MeOH (10 mL), and the mixture was heated to reflux for 48 h, before being concentrated The residue was adsorbed onto silica and purified by column chromatography (50:50 petroleum ether:acetone) to give the product **20** as a white powder (245 mg, 71%): R_f = 0.0.23 (50:50 petroleum ether:acetone); mp 210 °C (decomp.) (petroleum ether:acetone);. δ_H (500.1 MHz, d_4-MeOD): 8.09 (1 H, s, *H*-8), 8.00 (2 H, br s, NH_2), 5.99 (1 H, d, *J* 4.2, *H*-1′), 4.74 (1 H, ddd, *J* 48.0, 10.6, 2.8, *H*-5′a), 4.68 (1 H, ddd, *J* 47.4, 10.6, 3.8, *H*-5′b), 4.57-4.55 (1 H, m, *H*-2′), 4.39 (1 H, dd, *J* 5.2, 5.2, *H*-3′), 4.27-4.17 (1 H, m, *H*-4'); δ_C (125.8 MHz, d_4-MeOD): 155.7 (*C*-6), 149.5 (*C*-2), 138.9 (*C*-8, d, *J* 4.5), 119.4 (*C*-5), 119.2 (*C*-2), 89.0 (*C*-1′), 83.1 (*C*-4′, d, *J* 18.7), 82.1 (*C*-5′, d, *J*, 170.5), 74.2 (*C*-2′), 69.6 (*C*-3′, d, *J* 5.3); δ_F (470.3 MHz, d_4-MeOD): −232.1 (ddd, *J* 48.0, 47.4, 26.8, CH_2F); *m/z* (ES$^+$) 418 ([M+Na]$^+$ 100%), 396 ([M+H]$^+$ 12); HRMS (ES$^+$) calc. for $C_{10}H_{11}FIN_5O_3Na$ [M+Na]$^+$ 417.9784, found 417.9774.

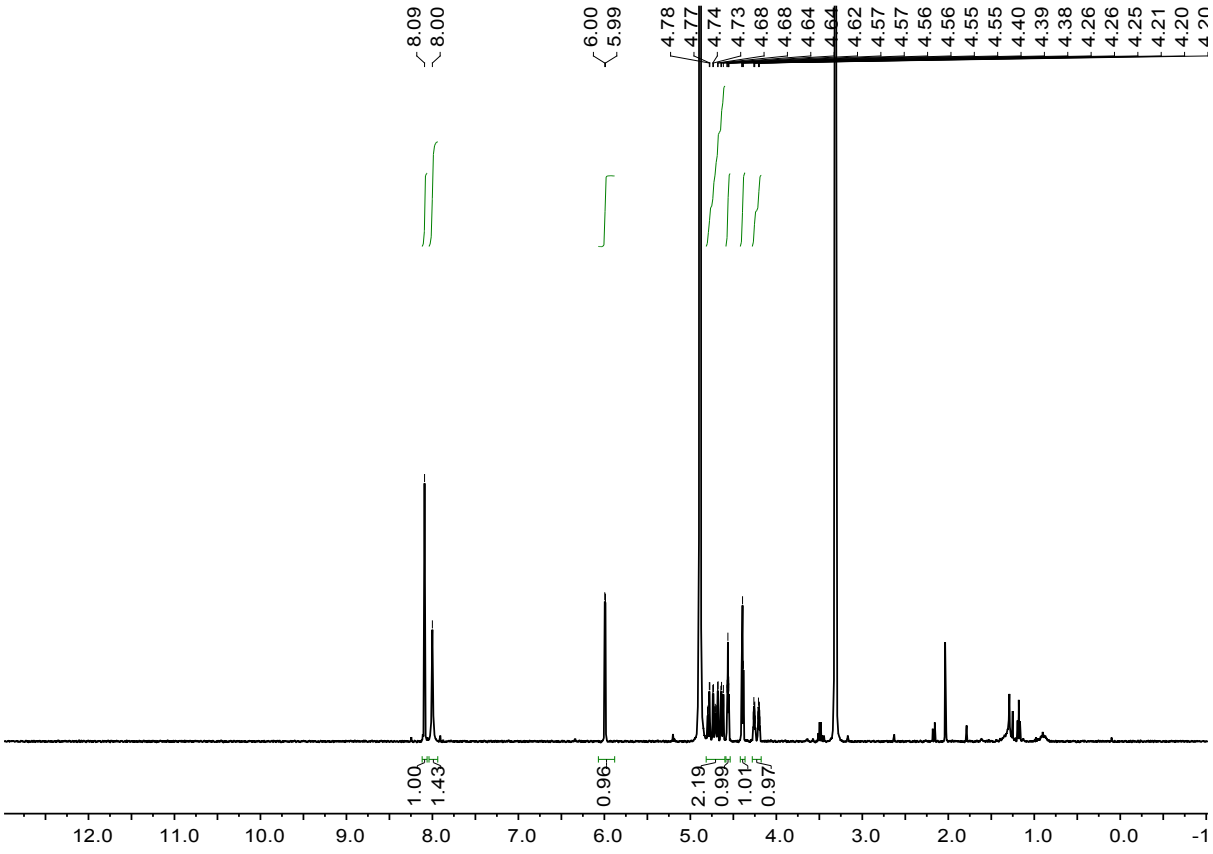


Figure S28. ^{1}H NMR spectrum (500.1 MHz, d_4-MeOD) of **20**.

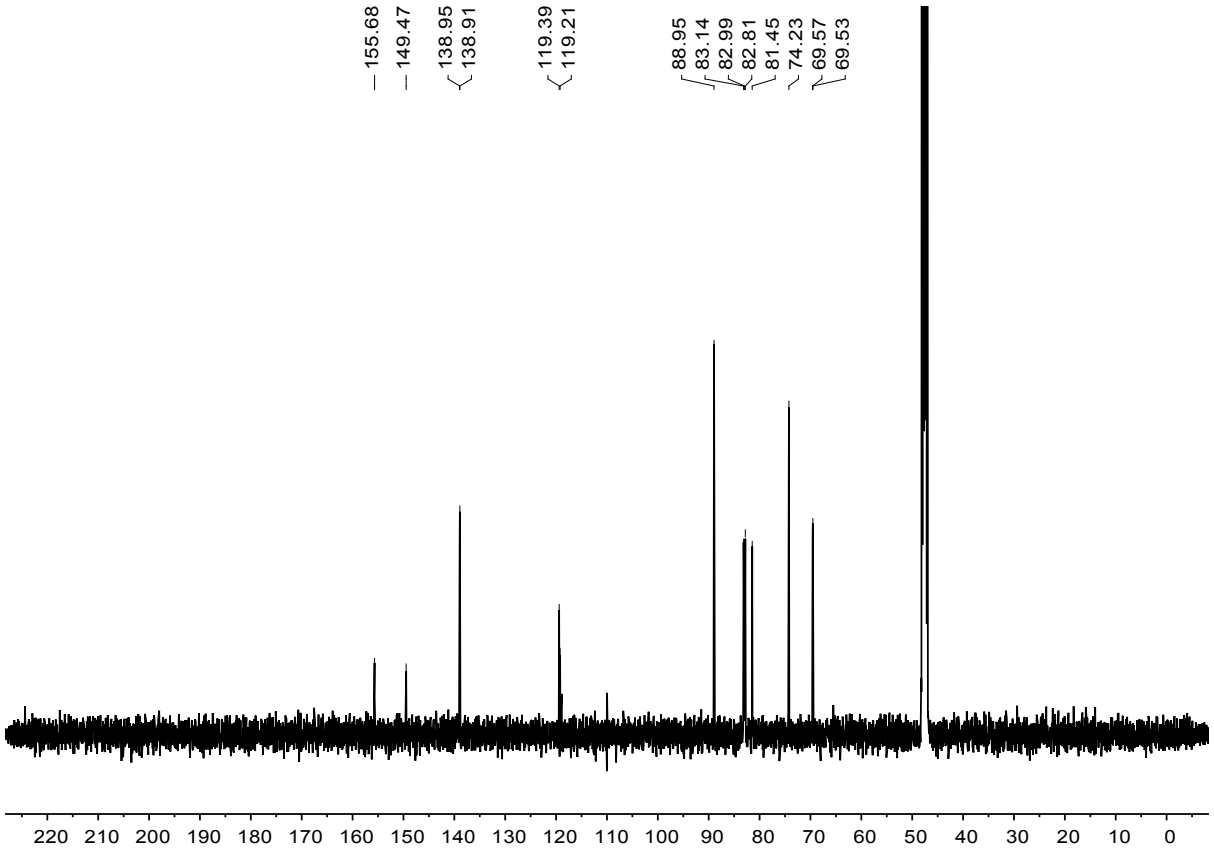


Figure S29. ^{13}C NMR spectrum (125.8 MHz, d_4-MeOD) of **20**.

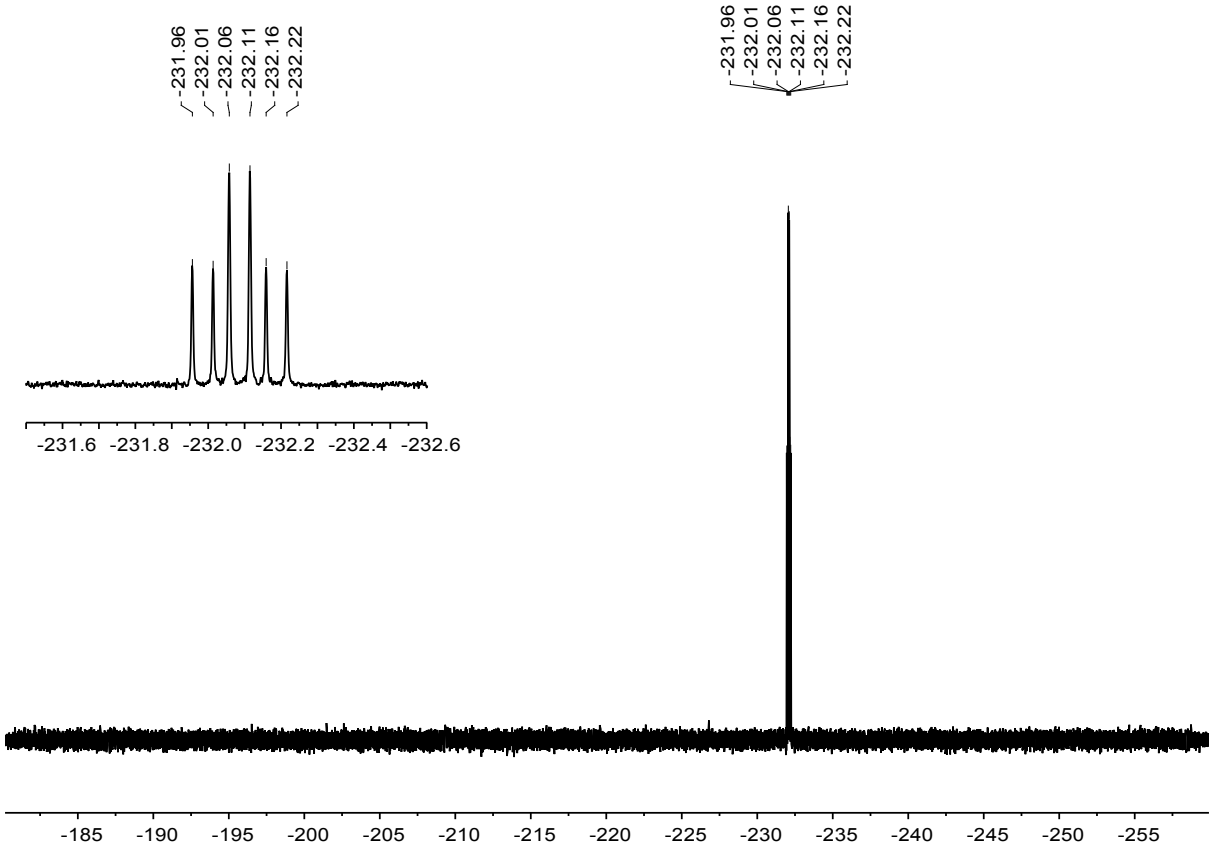
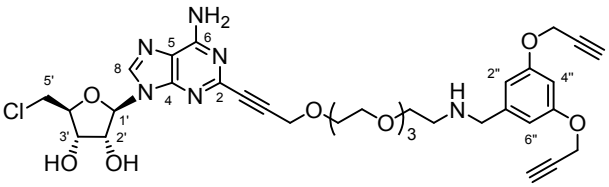


Figure S30. ^{19}F NMR spectrum (470.1 MHz, d_4-MeOD) of **20**.

2.15. ClDEA-PEG-(C≡CH)$_2$ 22



$Pd(PPh_3)_2Cl_2$ (6.8 mg, 0.0097 mmol, 0.1 eq.), copper(I) iodide (1.9 mg, 0.0097 mmol, 0.1 eq.) and triethylamine (68 µL, 0.49 mmol, 5 eq.) were added to a solution of iodo compound **18** (40 mg, 0.097 mmol, 1.0 eq.) and alkyne **15** (112 mg, 0.194 mmol, 2.0 eq.) in degassed DMF (5 mL). The mixture was heated to 80 °C for 48 h, after which time the volatile components were removed *in vacuo*. The residue was partly purified by column chromatography (50:50 petroleum ether:acetone to 40:60 petroleum ether:acetone to 80:20 petroleum ether:acetone to 80:20 EtOAc:acetone to 10:90 MeOH:acetone to 10:89:1 MeOH:acetone:1% aqueous ammonia (30%)) to afford fractions containing the coupled product (identified by ESMS). After concentration, the fractions were further purified by column chromatography (acetone to 5:95 MeOH:acetone to 10:90 MeOH:acetone) to afford the crude coupled product **21** (20 mg) which was used directly, without further purification.

The crude product **21** (20 mg) was dissolved in MeOH (10 mL) and 3HF.Et_3N (200 µL) was added and the mixture stirred for 18 h. The reaction was quenched by addition of sat. aqueous $NaHCO_3$ (2 mL) and the mixture concentrated under reduced pressure. The mixture was partly purified by column chromatography (99:1 acetone:aqueous ammonia (30%) to 5:94:1 MeOH:acetone:aqueous ammonia (30%) to 10:89:1 MeOH:acetone: aqueous ammonia (30%)). Fractions containing product were combined and concentrated. The residue was dissolved in MeCN: water (20:80) and purified by semi-preparative HPLC on the Shimadzu system (Phenomenex Kingsorb C_{18} (250 × 10.00 mm, 5µ); mobile Phase: A (H_2O + 0.05% TFA), B (MeCN + 0.05% TFA); linear gradient: 20% B at 0 min to 70% B at 18 min, to 95% B at 19 min, to 20% B at 24 min, to 20% B at 30 min; flow rate: 2.5 mL.min^{-1}; detection 220 nm. Fractions containing product (t_R = 16.7 min) were concentrated under a stream of compressed air, and lyophilised from a solution of ^{t}BuOH in water (20% *v/v*) affording **22** as a white solid (3.4 mg, 4.2% over two steps): δ_H (500.1 MHz, 1:1 D_2O:d_3-MeCN): 8.78 (1 H, s, *H*-8), 7.15 (2 H, d, *J* 2.2, *H*-2''), 7.08 (1 H, t, *J* 2.2, *H*-4"), 6.43 (1 H, d, *J* 4.9, *H*-1′), 5.20 (4 H, d, *J* 2.3, OC*H*$_2$C≡CH), 5.11 (1 H, dd, *J* 5.0, 4.9, H-2'), 4.87 (2 H, s, ArC≡CC*H*$_2$PEG), 5.84 (1 H, dd, *J* 5.0, 5.0, *H*-3'), 4.77 (1 H, *H*-4', under HDO peak, observed in 2D), 4.61 (2 H, s, PEGCH$_2$NHC*H*$_2$Ar), 4.40 (1 H, dd, *J* 12.2, 3.9, *H*-5'a), 4.34 (1 H, dd, *J* 12.2, 5.0, *H*-5'b), 4.22–4.08 (14 H, m, PEG-C*H*$_2$), 3.63 (2 H, t, *J* 5.0, PEGC*H*$_2$NHCH$_2$Ar), 3.44 (2 H, t, *J* 2.3 CH$_2$C≡C*H*); δ_C (125.8 MHz, 1:1 D_2O:d_3-MeCN): 158.4 (*C*-3", *C*-5"), 154.4 (*C*-6), 148.7 (*C*-4), 143.8 (*C*-2), 140.5 (*C*-8), 132.8 (*C*-1"), 118.3 (*C*-5, observed in 2D), 109.1 (*C*-2", *C*-6"), 102.1 (*C*-4"), 87.6 (*C*-1′), 83.4 (Ar*C*≡CCH$_2$PEG), 83.0 (*C*-4′), 82.9 (2 × C≡*C*H), 78.0 (2 × C≡*C*H), 76.5 (*C*-2′), 73.4 (*C*-3′), 70.5, 69.5, 69.4, 69.3, 69.2, 69.2, 68.9, 64.8 (PEG-*C*H$_2$), 68.7 (ArC≡*C*CH$_2$PEG), 57.8 (ArC≡C*C*H$_2$PEG), 55.6 (2 × O*C*H$_2$C≡CH), 50.0 (PEG*C*H$_2$NHCH$_2$Ar), 46.0 (PEGCH$_2$NH*C*H$_2$Ar), 44.7 (*C*-5′); *m/z* (ES$^+$) calc. isotope pattern for $C_{34}H_{43}ClN_6O_9$ $[M+H]^+$ 713.3 (100), 714.3 (36), 715.3 (32), 716.3 (12), found 713.3 (40), 714.3 (100), 715.3 (87), 716.3 (28); HRMS (ES$^+$) calc. for $C_{34}H_{43}{}^{35}ClN_6O_9$ $[M+H]^+$ 714.2755, found 714.2749.

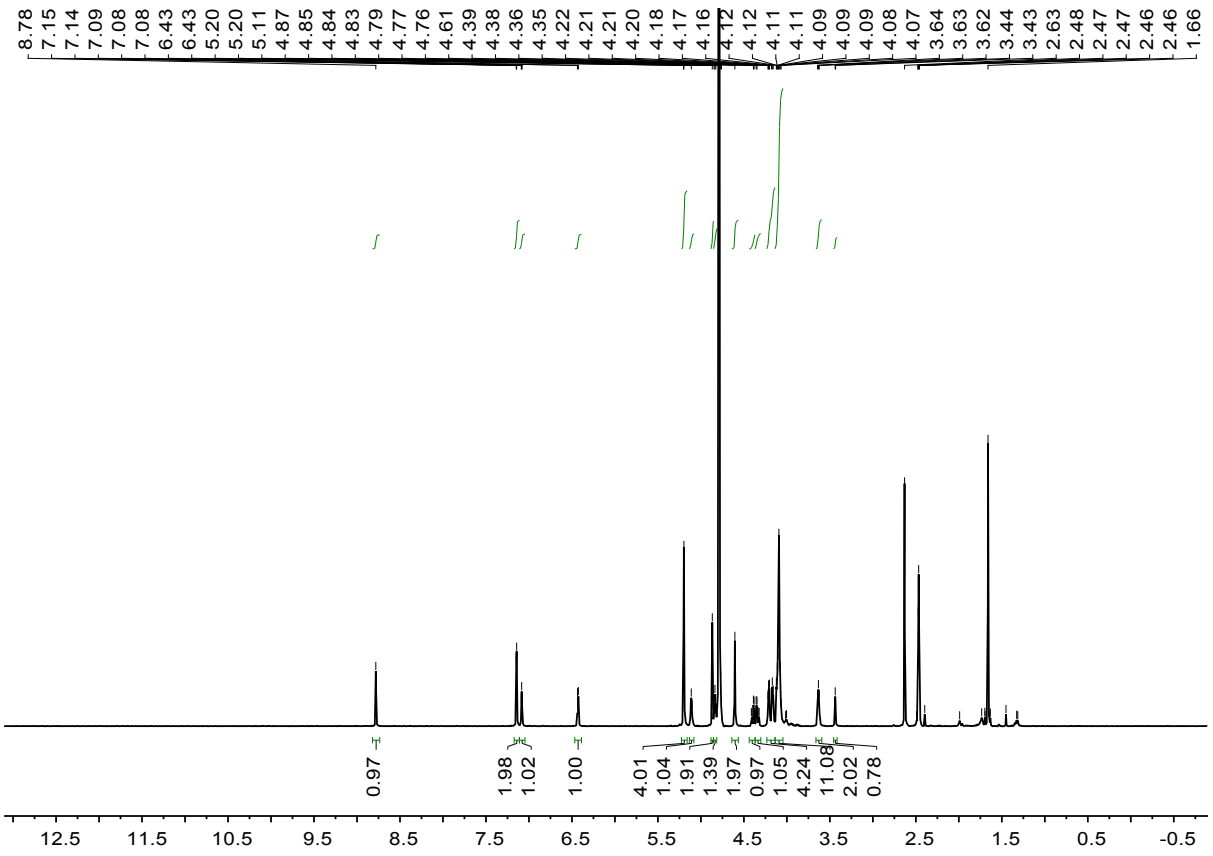


Figure S31. ^{1}H NMR spectrum (500.1 MHz, 1:1 D_2O:d_3-MeCN) of **22**. The large peak at 1.6 ppm is residual ^{t}BuOH from the freeze drying process.

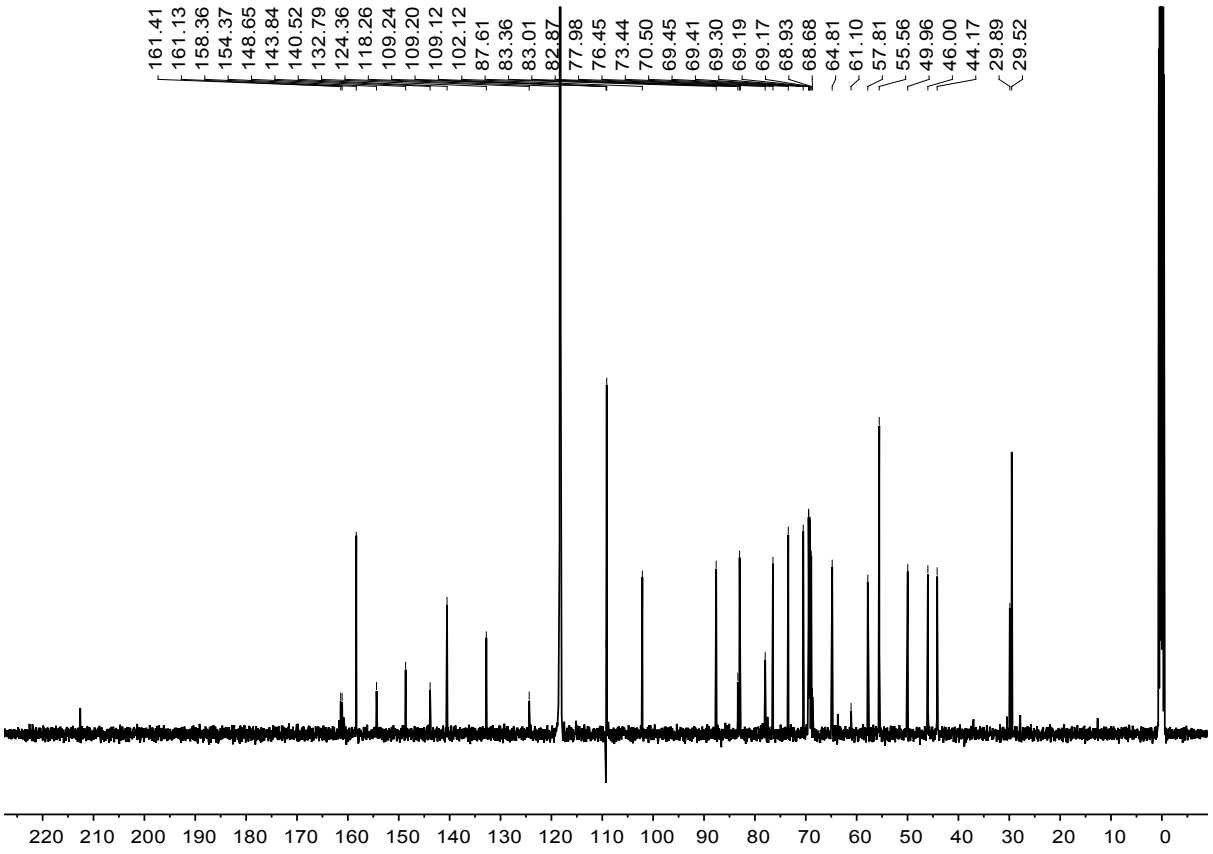


Figure S32. ^{13}C NMR spectrum (125.8 MHz, 1:1 D_2O:d_3-MeCN) of **22**.

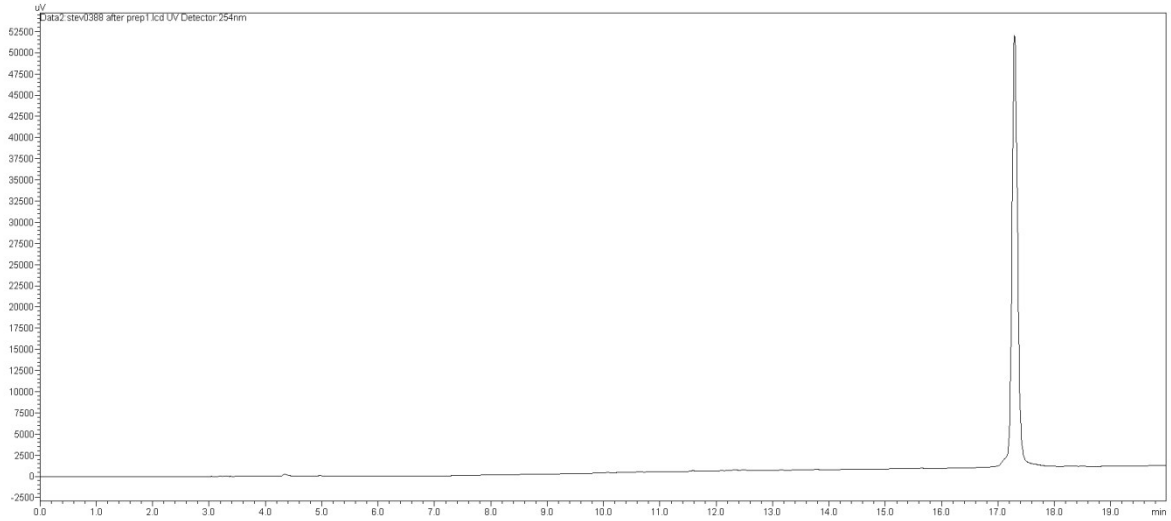
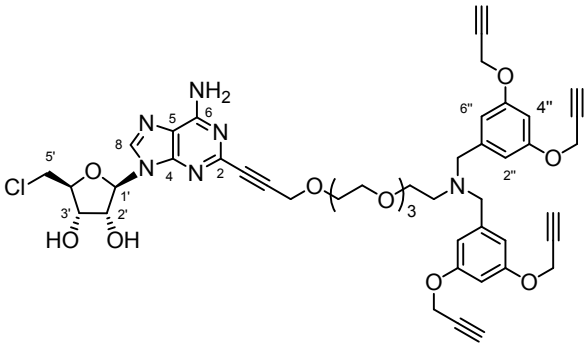


Figure S33. HPLC trace (254 nm) of **22** after purification.

2.16. ClDEA-PEG-(C≡CH)$_4$ 26



$Pd(PPh_3)_2Cl_2$ (13 mg, 0.018 mmol, 0.1 eq.), copper(I) iodide (3.5 mg, 0.018 mmol, 0.1 eq.) and triethylamine (129 µL, 0.923 mmol, 5 eq.) were added to a solution of iodo compound **18** (76 mg, 0.185 mmol, 1.0 eq.), alkyne **16** (338 mg, 0.369 mmol, 2.0 eq.) and PPh_3 (24 mg, 0.092 mmol, 0.5 eq.) in degassed DMF (5 mL). The mixture was heated to 57 °C for 48 h, after which time the volatile components were removed *in vacuo*. The residue was partly purified by column chromatography (Et_2O to 70:30 Et_2O:acetone) to afford fractions containing the coupled product (identified by ESMS). The compound was taken up into toluene (50 mL) and NaS_2O_7 solution (20% *w/v*, 50 mL) and the mixture heated to 60 °C for 1 h. After cooling, the phases were separated and the aqueous phase extracted with toluene (3 × 20 mL), and the organic phases combined, dried over Na_2SO_4 and concentrated. The product was further purified by column chromatography (90:10 petroleum ether:acetone to 70:30 petroleum ether:acetone to 60:40 petroleum ether:acetone) to afford the crude coupled product **25** (40 mg), which was used directly in the next step, without further purification.

The crude product **25** (40 mg) was dissolved in MeOH (15 mL) and 3HF.Et_3N (250 µL) was added and the mixture stirred for 20 h. The reaction was quenched by addition of sat. aqueous $NaHCO_3$ (4 mL) and the mixture concentrated under reduced pressure. The mixture was partly purified by column chromatography (DCM to 92.5:7.5 DCM:MeOH). Fractions containing product were combined and concentrated. The residue was dissolved in MeCN: water (20:80) and purified by semi-preparative HPLC on the Shimadzu system (Phenomenex Kingsorb C_{18} (250 × 10.00 mm, 5µ); mobile Phase: A (H_2O + 0.05% TFA), B (MeCN + 0.05% TFA); linear gradient: 20% B at 0 min to 70% B at 20 min, to 95% B at 21 min; to 95% B at 25 min, B to 5% B at 26 min, to 5% B at 35 min; flow rate: 2.5 mL.min^{-1}; detection 220 nm. Fractions containing product (t_R = 18.7 min) were concentrated under a stream of compressed air, and lyophilised from a solution of ^{t}BuOH in water (20% *v/v*) affording **26** as a white solid (5.3 mg, 2.8% over two steps): δ_H (499.9 MHz, d_4-MeOD): 8.31 (1 H, s, *H*-8), 6.69 (4

H, d, *J* 2.3, *H*-2'', *H*-6''), 6.47 (2 H, t, *J* 2.3, *H*-4''), 6.00 (1 H, d, *J* 4.9, *H*-1′), 4.74 (1 H, dd, *J* 5.0, 4.9, *H*-2'), 4.70 (8 H, d, *J* 2.4, OCH_2C≡CH), 4.42 (2 H, s, ArC≡CCH_2PEG), 4.39 (1 H, dd, *J* 5.0, 4.9, *H*-3'), 4.27 (1 H, m, *H*-4'), 3.97 (1 H, dd, *J* 11.9, 4.9, *H*-5'a), 3.86 (1 H, dd, *J* 11.9, 4.9, *H*-5'b), 3.75-3.55 (18 H, m, PEG-CH_2, PEGCH_2N(CH_2Ar)$_2$), 2.95 (4 H, t, *J* 2.4, CH_2C≡C*H*), 2.66 (2 H, t, *J* 5.8, PEGCH_2N(CH_2Ar)$_2$); δ_C (125.7 MHz, MeOD): 158.7 (2 × *C*-3'', 2 × *C*-5''), 152.0 (*C*-6), 150.1 (*C*-4, observed in 2D), 146.2 (*C*-2), 142.2 (2 × *C*-1''), 140.5 (*C*-8), 119.8 (*C*-5, observed in 2D), 107.9 (2 × *C*-2'', 2 × *C*-6''), 100.6 (2 × *C*-4''), 86.0 (Ar*C*≡CCH_2PEG, observed in 2D), 82.3 (ArC≡*C*CH_2PEG, observed in 2D), 88.9 (*C*-1′), 83.8 (*C*-4′), 79.3 (4 × *C*≡CH, observed in 2D), 78.5 (*C*-2′), 75.5 (4 × C≡*C*H), 73.6 (*C*-3′), 71.2, 70.3, 70.2, 70.2, 70.0, 70.0, 69.5, 69.1 (PEG-CH_2), 58.6 (2 × PEGCH_2N(*C*H_2Ar)$_2$), 58.0 (ArC≡C*C*H_2PEG), 55.6 (4 × O*C*H_2C≡CH), 52.6 (PEG*C*H_2N(CH_2Ar)$_2$), 43.8 (*C*-5′); *m/z* (ES$^+$) calc. isotope pattern for $C_{47}H_{52}ClN_6O_{11}$ $[M+H]^+$ 911.3 (100), 912.3 (51), 913.3 (32), 914.3 (16), 915.3 (4); found 911.3 (100), 912.3 (56), 913.3 (38), 914.3 (17), 915.3 (5). HRMS (ES$^+$) calc. for $C_{47}H_{52}{}^{35}ClN_6O_{11}$ $[M+H]^+$ 911.3377, found 911.3358.

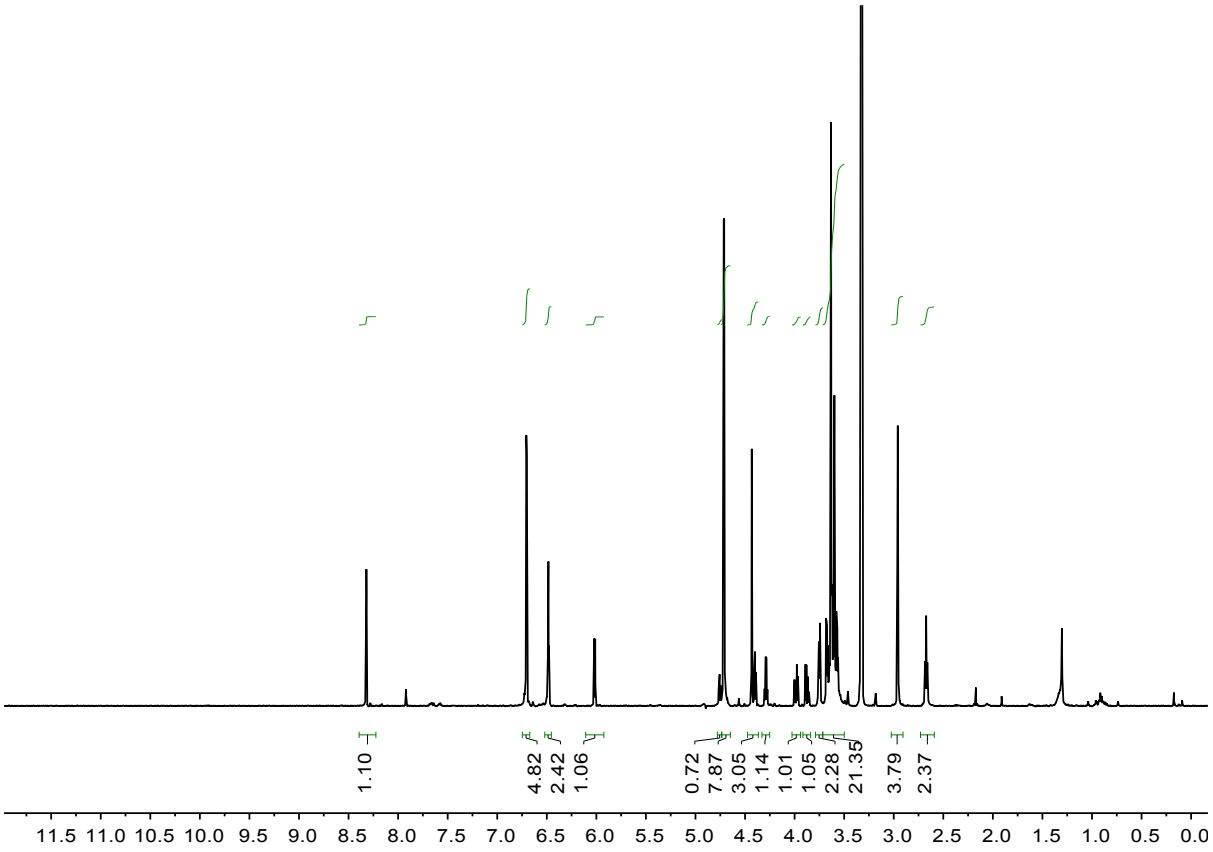


Figure S34. ^{1}H NMR spectrum (499.9 MHz, d_4-MeOD) of **26**.

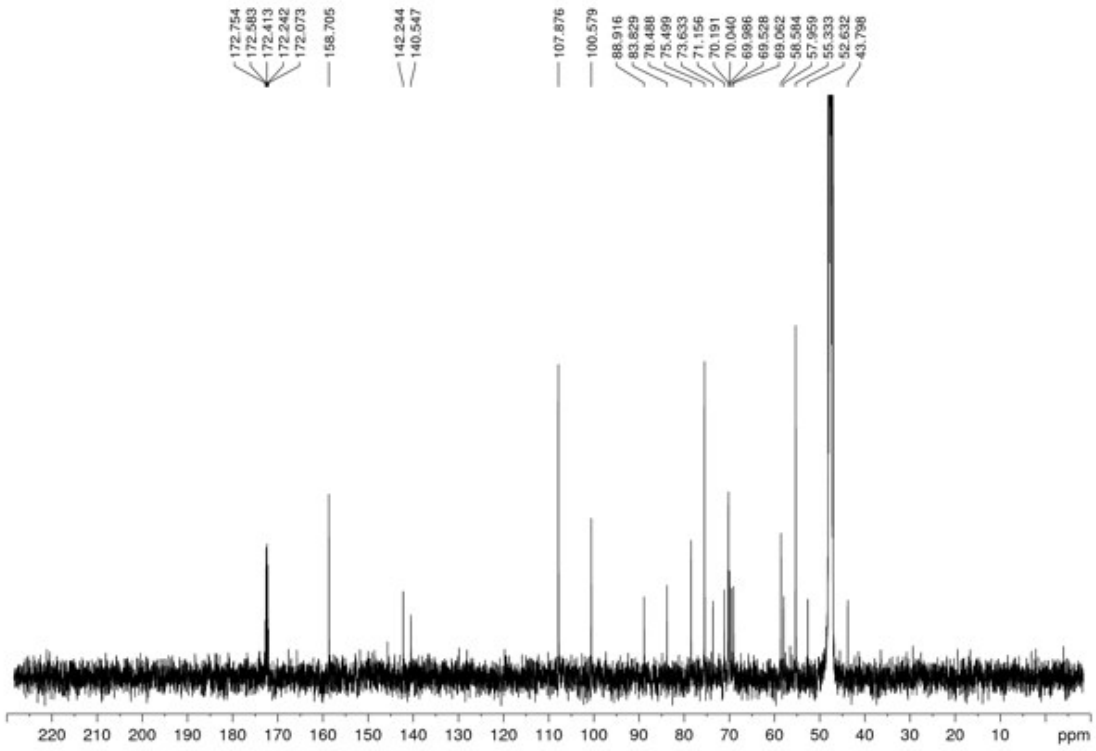


Figure S35. ^{13}C NMR spectrum (125.7 MHz, d_4-MeOD) of **26**.

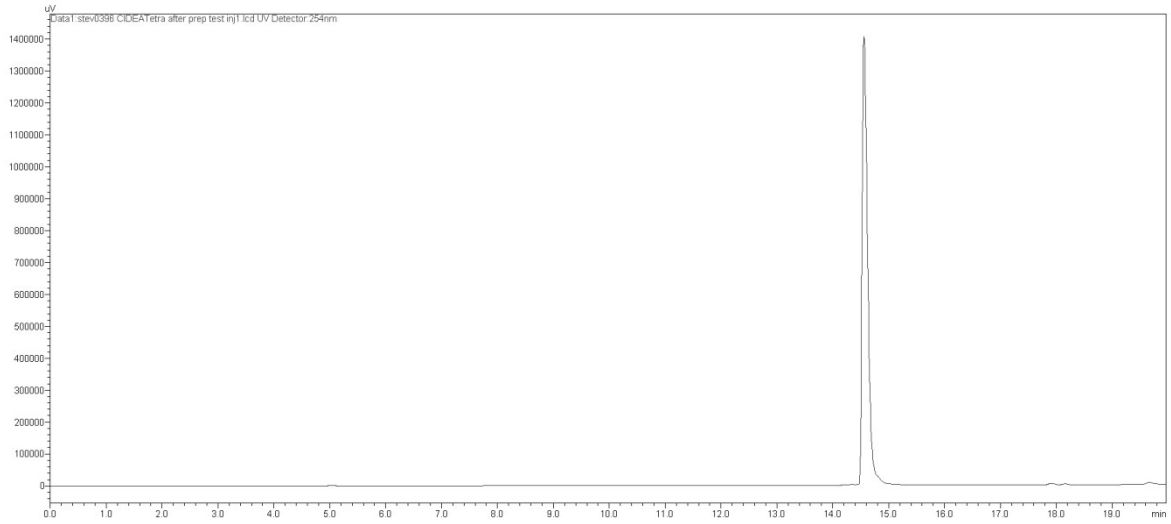
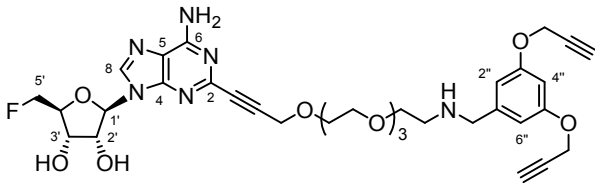


Figure S36. HPLC trace (254 nm) of **26** after purification.

2.17. FDEA-PEG-(C≡CH)$_2$ 24



$Pd(PPh_3)_2Cl_2$ (15.4 mg, 0.022 mmol, 0.1 eq.), copper(I) iodide (4.2 mg, 0.022 mmol, 0.1 eq.) and triethylamine (150 µL, 1.10 mmol, 5 eq.) were added to a solution of iodo compound **20** (87 mg, 0.22 mmol, 1.0 eq.) and alkyne **15** (101 mg, 0.176 mmol, 0.8 eq.) in degassed DMF (4 mL). The mixture was heated to 60 °C for 48 h, after which time the volatile components were removed *in vacuo*. The resulting residue was partly purified by column chromatography (50:49:1 Et_2O:acetone:Et_3N to 20:79:1 Et_2O:acetone:Et_3N to 99:1 acetone:Et_3N to 70:20:10 EtOAc:isopropanol:water) to afford fractions containing the coupled product **23** (identified by MS), which were combined and concentrated.

The crude product **23** was dissolved in MeOH (20 mL) and 3HF.Et_3N (800 µL) was added and the mixture stirred overnight. The reaction was quenched by addition of sat. aqueous $NaHCO_3$ (20 mL) and the mixture concentrated under reduced pressure. The aqueous mixture was loaded onto a reverse phase cartridge (1000 mg Extract Clean C_{18}-HC, preconditioned with water), washed with water (10 mL), and the product eluted with water: MeCN (50:50) (2 × 10 mL). The eluted fractions were combined and concentrated. The residue was dissolved in MeCN: water (20:80) and purified by semi-preparitive HPLC on the Shimadzu system (Phenomenex Luna C_{18} (250 × 10.00 mm, 5µ); mobile Phase: A (H_2O + 0.05% TFA), B (MeCN + 0.05% TFA); linear gradient: 20% B at 0 min to 56% B at 13 min, to 95% B at 14 min; to 95% B at 19 min, B to 20% B at 20 min, to 20% B at 30 min; flow rate: 2.5 mL.min^{-1}; detection 220 nm. Fractions containing product (t_R = 13.8 min) were concentrated under a stream of compressed air, and lyophilised from a solution of ^{t}BuOH in water (20% *v/v*) affording **24** as a white solid (5.3 mg, 2.5% over two steps): δ_H (500.1 MHz, d_4-MeOD): 8.26 (1 H, s, *H*-8), 6.73 (2 H, d, *J* 2.3, *H*-2'', *H*-6''), 6.69 (1 H, t, *J* 2.3, *H*-4''), 6.04 (1 H, d, *J* 4.4, *H*-1′), 4.82-4.61 (6 H, m, OCH_2C≡CH, H-5'), 4.54 (1 H, m, H-2'), 4.43 (2 H, s, ArC≡CCH_2PEG), 4.39 (1 H, dd, *J* 5.2, 5.2, *H*-3'), 4.29-4.17 (3 H, m, PEGCH_2NHCH_2Ar, *H*-4'), 3.83-3,57 (14 H, m, PEG-CH_2), 3.28 (2 H, t, *J* 5.1, PEGCH_2NHCH_2Ar), 3.00 (2 H, t, *J* 2.4 CH_2C≡C*H*); δ_C (125.8 MHz, d_4-MeOD): 160.7 (*C*-3'', *C*-5''), 156.9 (*C*-6), 150.6 (*C*-4), 146.6 (*C*-2), 141.3 (d, *J* 4.9, *C*-8), 134.5 (*C*-1''), 120.0 (*C*-5), 110.6 (*C*-2''), 103.8 (*C*-4''), 90.0 (*C*-1′), 85.9 (Ar*C*≡CCH$_2$PEG), 84.6 (d, *J* 18.2, *C*-4′), 83.6 (d, *J* 163.8, *C*-5′), 82.9 (ArC≡*C*CH$_2$PEG), 79.4 (*C*≡CH), 77.4 (C≡*C*H), 75.9 (*C*-2′), 71.6, 71.5, 71.4, 71.3, 71.1 (PEG-*C*H$_2$), 73.4 (d, *J* 4.9, *C*-3′), 70.6, 66.6 (PEG-*C*H$_2$), 59.4 (ArC≡C*C*H$_2$PEG), 56.9 (O*C*H$_2$C≡CH), 51.9 (PEGCH$_2$NH*C*H$_2$Ar), 47.9 (PEG*C*H$_2$NHCH$_2$Ar); δ_F (470.5 MHz, d_4-MeOD): −232.48 (1F, ddd, *J* 47.8, 47.8, 28.4, CH$_2$*F*); *m/z* (ES$^+$) 697 ([M+H]$^+$ 100%); HRMS (ES$^+$) calc. for $C_{34}H_{42}FN_6O_9$ [M+H]$^+$ 697.2992, found 697.2984.

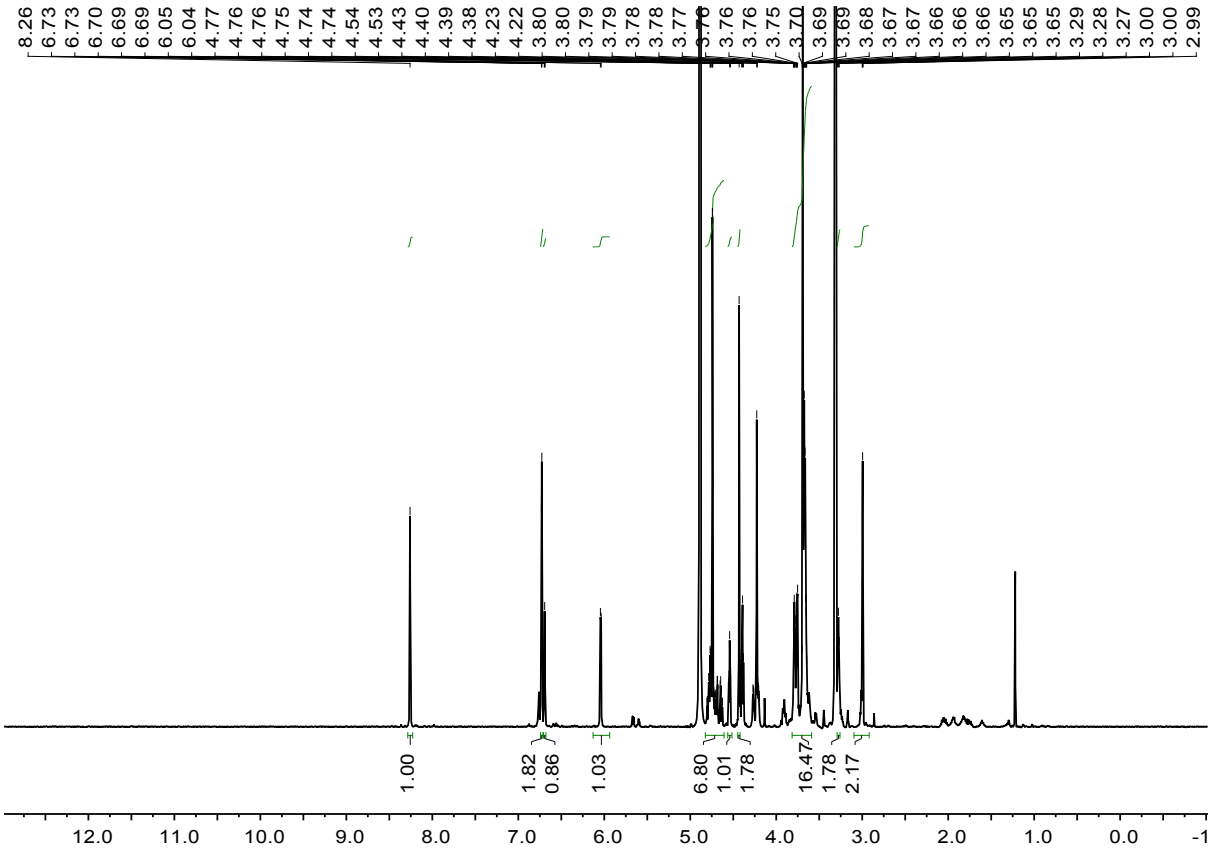


Figure S37. ^{1}H NMR spectrum (500.1 MHz, d_4-MeOD) of **24**. The peak at 1.2 ppm is residual ^{t}BuOH from the freeze drying process.

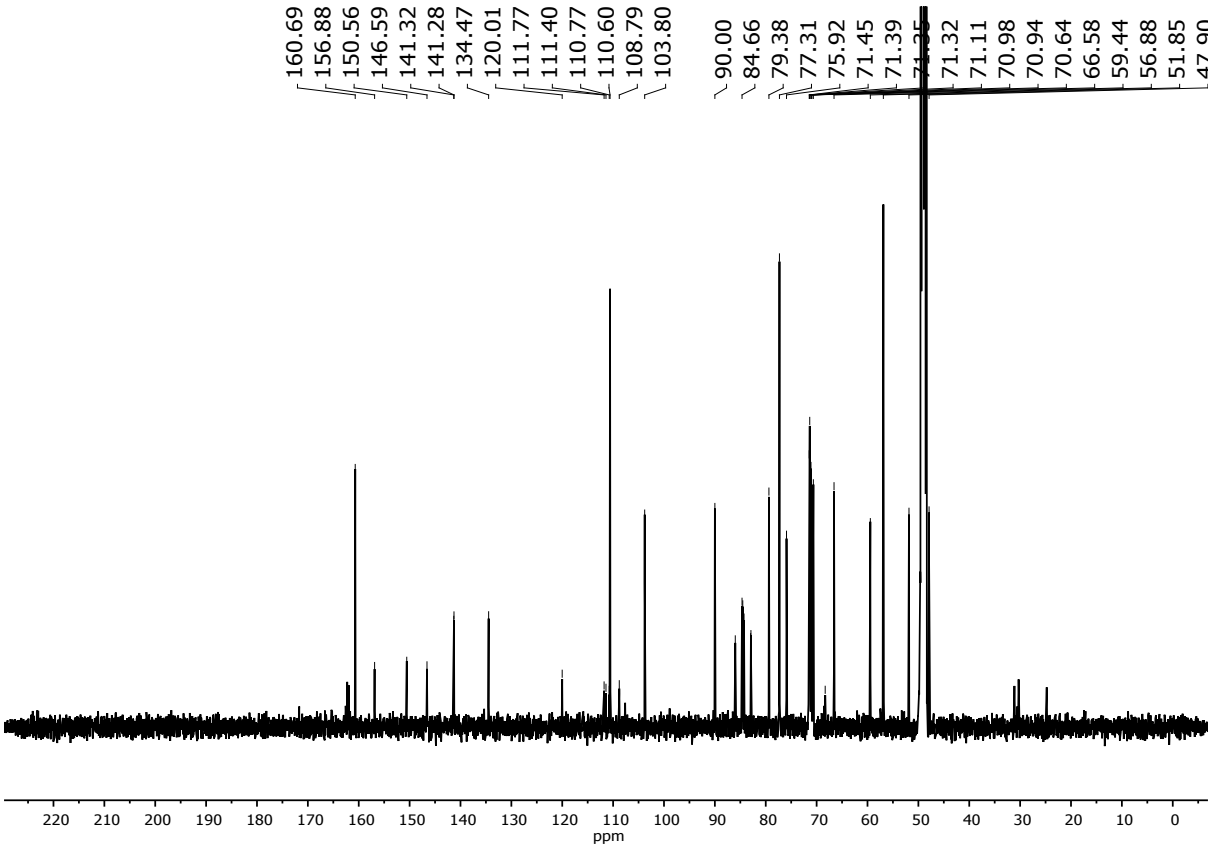


Figure S38. ^{13}C NMR spectrum (125.7 MHz, d_4-MeOD) of **24**.

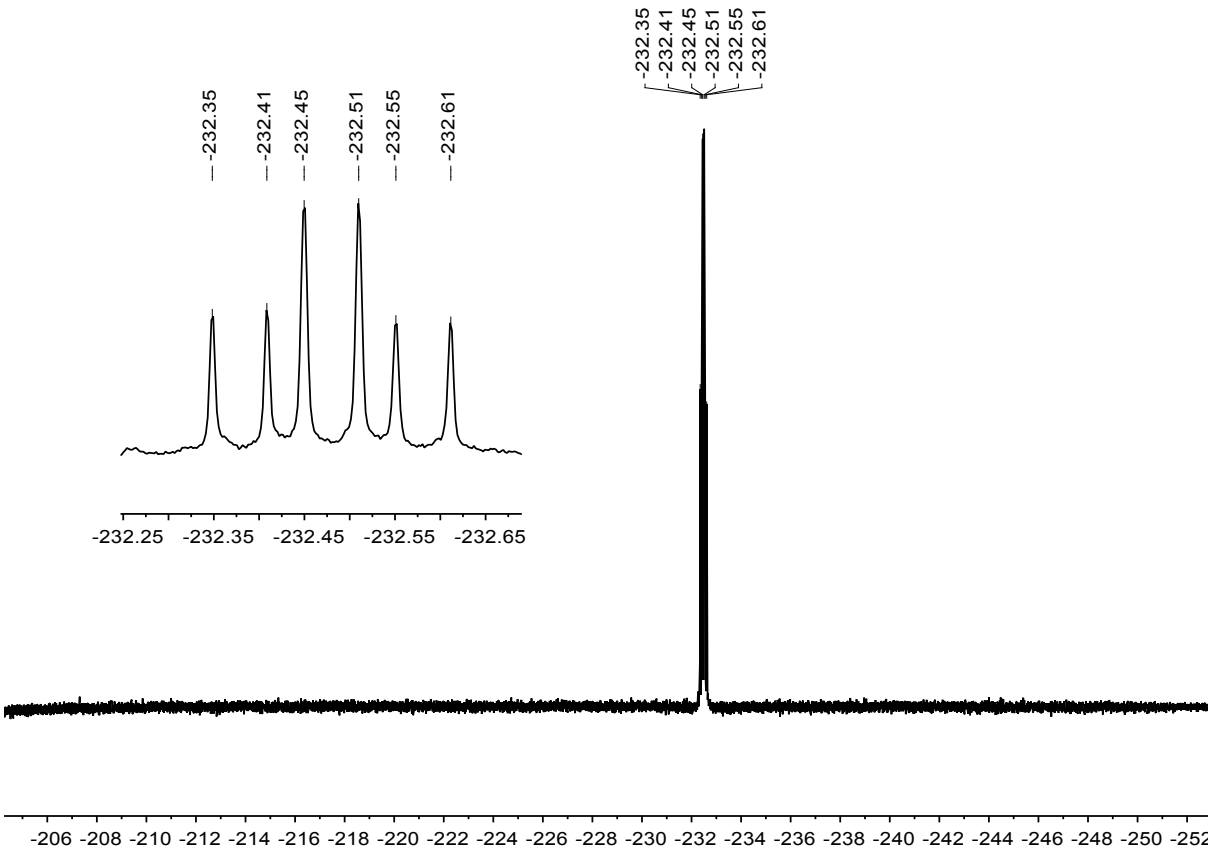


Figure S39. ^{19}F NMR spectrum (470.5 MHz, d_4-MeOD) of **24**.

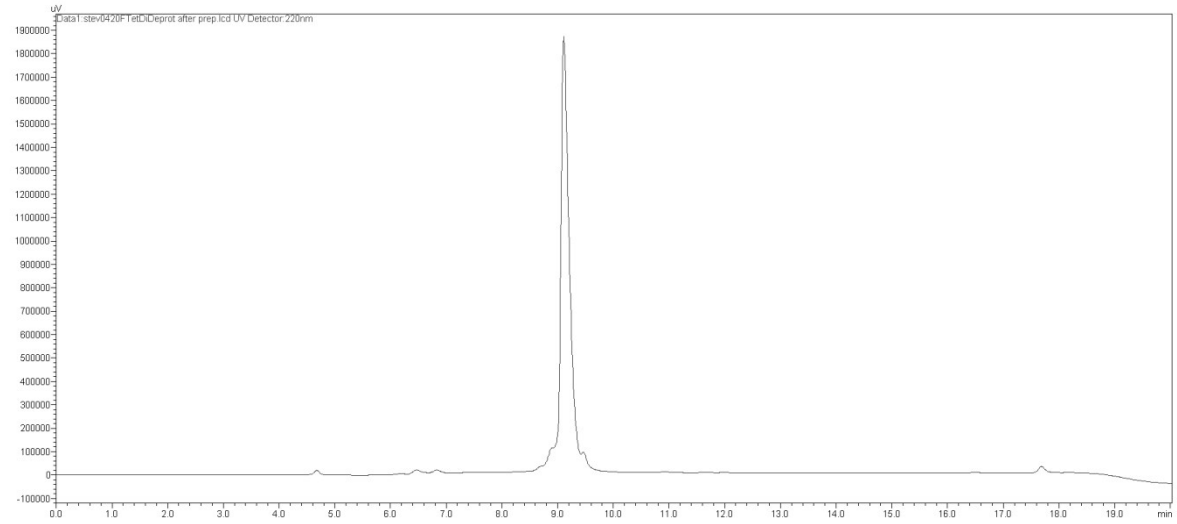
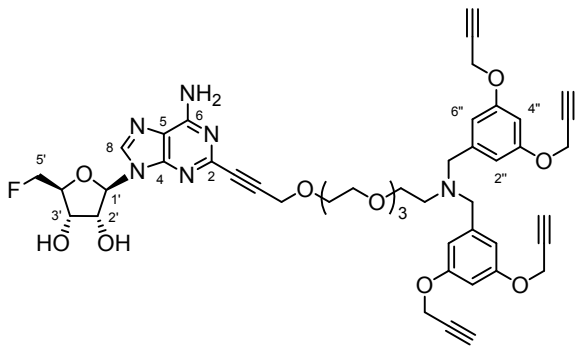


Figure S40. HPLC trace (220 nm)of **24** after purification.

2.18. FDEA-PEG-(C≡CH)$_4$ 28



$Pd(PPh_3)_2Cl_2$ (15.4 mg, 0.022 mmol, 0.1 eq.), copper(I) iodide (4.2 mg, 0.022 mmol, 0.1 eq.) and triethylamine (150 µL, 1.10 mmol, 5 eq.) were added to a solution of iodo compound **20** (87 mg, 0.22 mmol, 1.0 eq.) and alkyne **16** (403 mg, 0.440 mmol, 2 eq.) in degassed DMF (4 mL). The mixture was heated to 60 °C for 48 h, after which time the volatile components were removed *in vacuo*. The residue was partly purified by column chromatography (50:49:1 Et_2O:acetone:Et_3N to 99:1 acetone:Et_3N) to afford fractions containing the coupled product **27** (identified by ESMS), which were combined and concentrated.

The crude product **27** was dissolved in MeOH (20 mL) and 3HF.Et_3N (800 µL) was added and the mixture stirred for 18 h. The reaction was quenched by addition of sat. aqueous $NaHCO_3$ (20 mL) and the mixture concentrated under reduced pressure. The aqueous mixture was loaded onto a reverse phase cartridge (1000 mg Extract Clean C_{18}-HC, preconditioned with water), washed with water (10 mL), and the product eluted with water: MeCN (50:50) (2 × 10 mL) and MeCN (2 × 10 mL). The water:MeCN and MeCN fractions were combined and concentrated. The residue was dissolved in MeCN: water (33:66) and purified by semi-preparative HPLC on the Shimadzu system (Phenomenex Luna C_{18} (250 × 10.00 mm, 5µ); mobile Phase: A (H_2O + 0.05% TFA), B (MeCN + 0.05% TFA); linear gradient: 33% B at 0 min to 70% B at 13 min, to 95% B at 14 min; to 95% B at 95 min, B to 33% B at 20 min, to 33% B at 30 min; flow rate: 2.5 mL.min^{-1}; detection 220 nm. Fractions containing product (t_R = 13.3 min) were concentrated under a stream of compressed air, and lyophilised from a solution of ^{t}BuOH in water (20% *v/v*) affording **28** as a white solid (5.4 mg, 2.3% over two steps): δ_H (500.1 MHz, d_4-MeOD): 8.23 (1 H, s, *H*-8), 6.95–6.57 (6 H, m, *H*-2'', *H*-4''), 6.02 (1 H, d, *J* 4.2, *H*-1′), 4.80-4.62 (14 H, m, OCH_2C≡CH, PEGCH_2N(CH_2Ar)$_2$, H-5'), 4.53 (1 H, dd, *J* 4.6, 4.6 *H*-2'), 4.39-4.37 (3 H, m, ArC≡CCH_2PEG, *H*-3'), 4.26-4.11 (1 H, m, *H*-4'), 3.76-3.62 (14 H, m, PEG-CH_2), 3.31 (2 H, under solvent peak, PEGCH_2N(CH$_2$Ar)$_2$), 2.99 (4 H, t, *J* 2.3 CH$_2$C≡C*H*); δ_F (470.3 MHz, d_4-MeOD): −232.61 (1 F, ddd, *J* 47.6, 47.6, 27.9 Hz), CH$_2$*F*); *m/z* (ES$^+$) calc. isotope pattern for $C_{47}H_{52}FN_6O_{11}$ $[M+H]^+$ 895.4 (100), 896.4 (53), 897.4 (16), 898.4 (2); found 895.4 (100), 896.4 (52), 897.4 (13), 898.4 (2); HRMS (ES$^+$) calc. for $C_{47}H_{52}FN_6O_{11}$ $[M+H]^+$ 895.3673, found 895.3660.

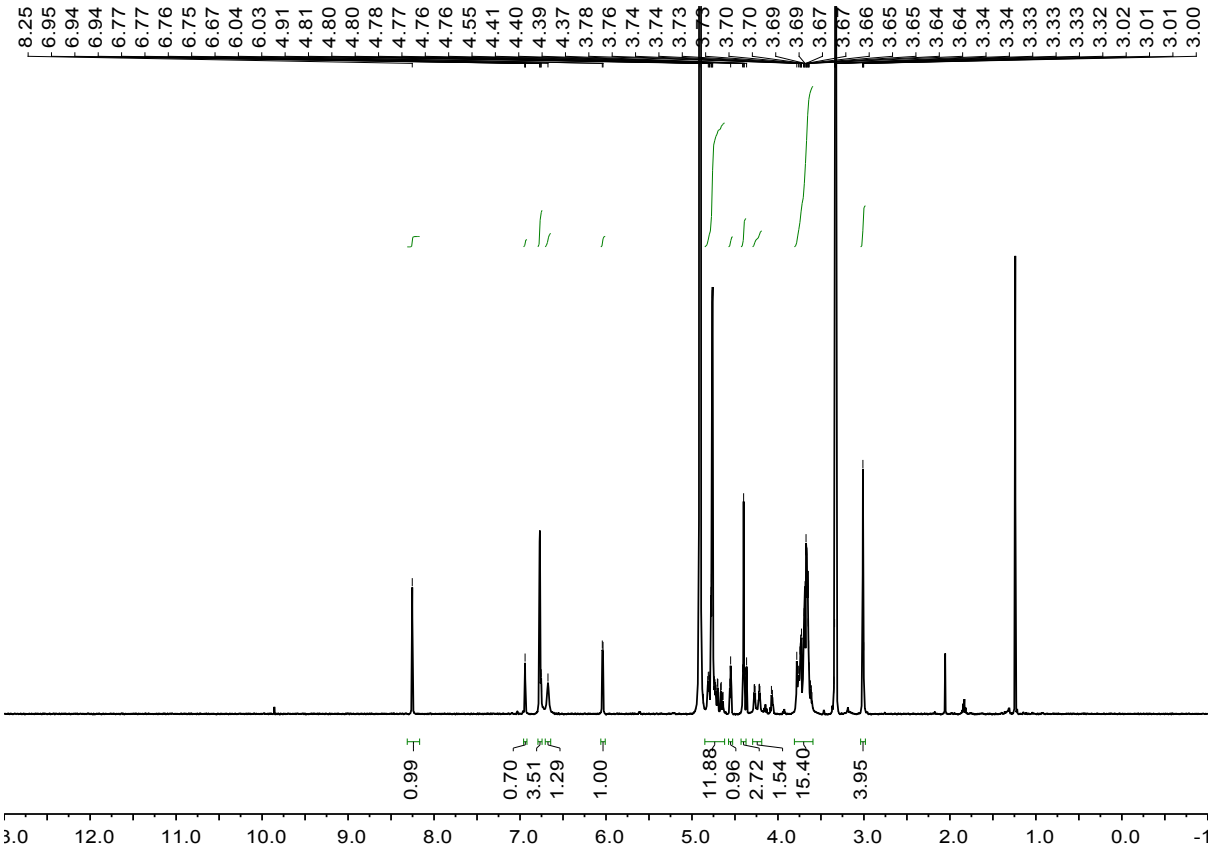


Figure S41. ^{1}H NMR spectrum (500.1 MHz, d_4-MeOD) of **28**. The large peak at 1.2 ppm is residual ^{t}BuOH from the freeze drying process.

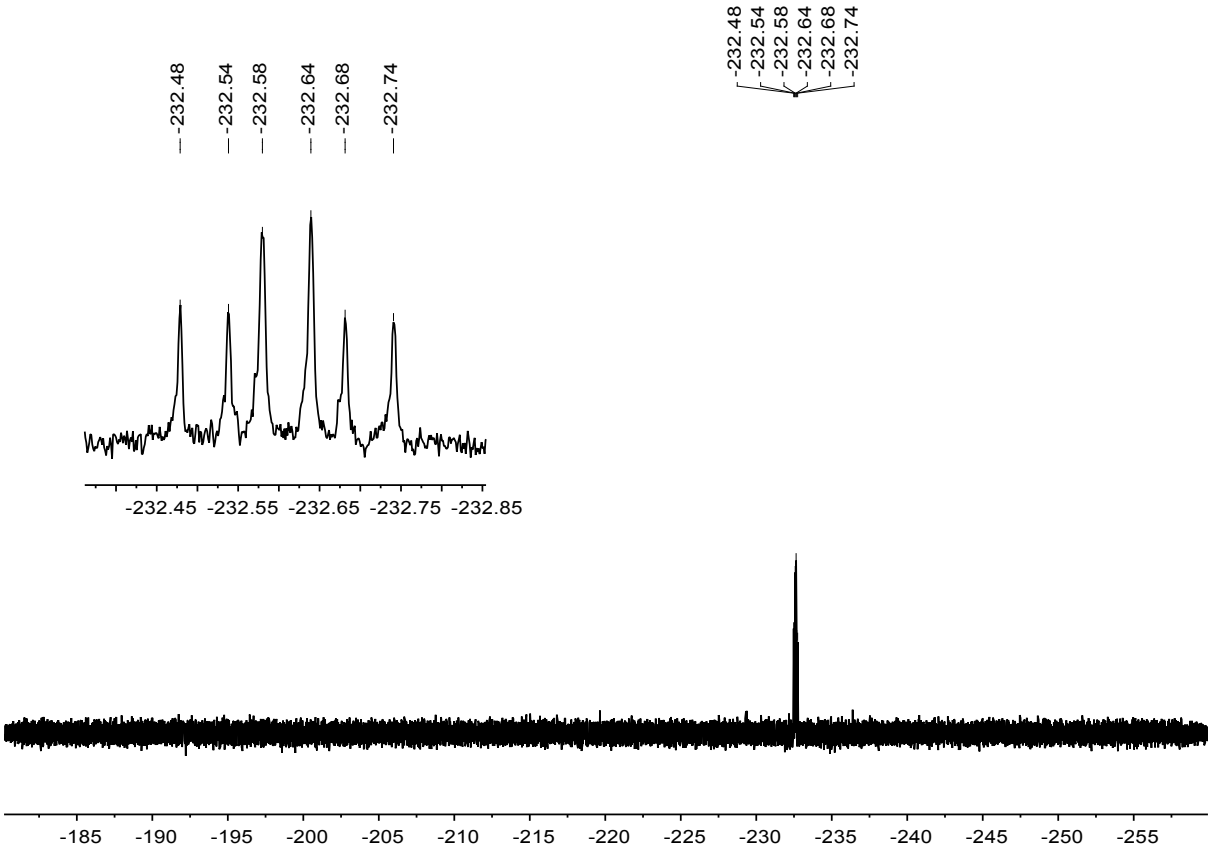


Figure S42. ^{19}F NMR spectrum (470.3 MHz, d_4-MeOD) of **28**.

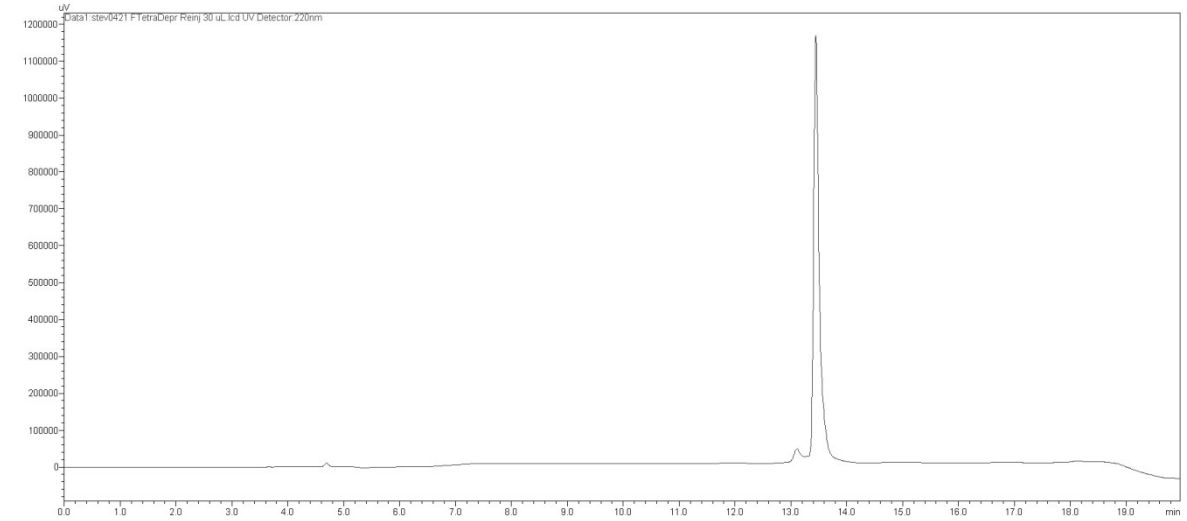
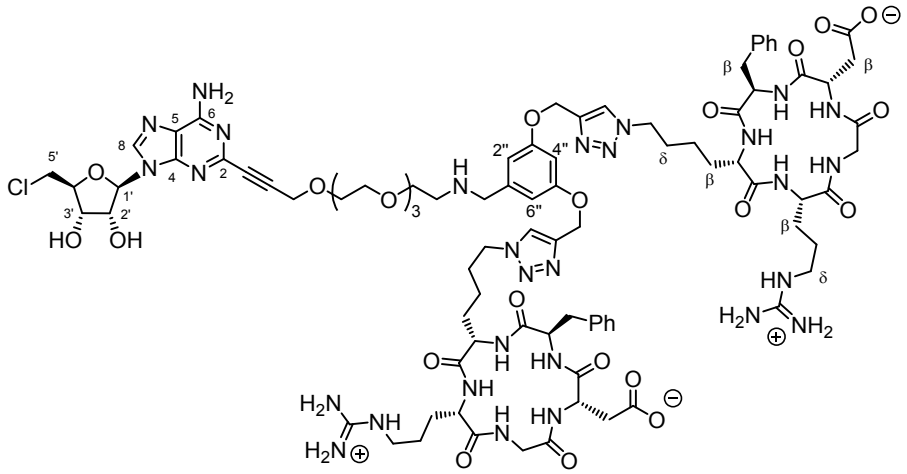


Figure S43. HPLC trace (220 nm) of **28** after purification.

2.19. ClDEA-PEG-(RGD)$_2$ 30



A suspension of ClDEA-PEG-(C≡CH)$_2$ **22** (0.72 mg, 1.0 μmol, 1 eq.) and c(RGDfK[N_3]) **29** (1.60 mg, 2.53 μmol, 2.5 eq.) in water (6.05 mL) was sonicated until homogenous. To this solution, aqueous sodium ascorbate (507 μL, 20 mM, 10 eq.), followed by $CuSO_4$-TBTA complex (203 μL, 10 mM in 55:45 DMSO: water) were added, and the reaction allowed to proceed for 75 min at rt, when HPLC analysis showed reaction to be complete. The reaction mixture was loaded onto a reverse phase cartridge (1000 mg Extract Clean C_{18}-HC, preconditioned with water), washed with water (5 mL), and the product eluted with water: MeCN (50:50) (2 × 5 mL). The eluted fractions were combined and solvents removed under reduced pressure. The residue was dissolved in MeCN: water (20:80) and purified by semi-preparative HPLC on the Shimadzu system (Phenomenex Kingsorb C_{18} (250 × 10.00 mm, 5μ); mobile Phase: A (H_2O + 0.05% TFA), B (MeCN + 0.05% TFA); linear gradient: 20% B at 0 min to 70% B at 20 min, to 95% B at 21 min; to 95% B at 25 min, B to 5% B at 26 min, to 5% B at 35 min; flow rate: 2.5 mL.min^{-1}; detection 220 nm, Fractions containing product (t_R = 10.4 min) were concentrated under a stream of compressed air, and lyophilised from a solution of ^{t}BuOH in water (20% *v/v*) affording **30** as a white solid (1.8 mg, 78%): δ_H (500.1 MHz, D_2O): 8.23 (1 H, s, *H*-8), 7.88 (2 H, s, triazole-*H*), 7.02–6.92 (10 H, m, D-Phe Ar*H*), 6.63 (2 H, br s, *H*-2″, *H*-6″), 6.57 (1 H, br s, *H*-4″), 5.93 (1 H, d, *J* 4.7, *H*-1′), 5.15 (4 H, br s, ArO-CH_2-triazole), 4.68–4.65 (3 H, m, Asp α-*H*, *H*-2'), 4.47–4.40 (3 H, m, D-Phe α-*H, H*-3'), 4.35–4.31 (5 H, m, *H*-4', Arg α-*H*, AdoC≡CCH_2O), 4.28–4.20

(4 H, m, Lys ε-CH_2), 4.18–4.13 (4 H, m, CH_2NHCH_2Ar, Gly α-*H*a), 3.72–3.43 (16 H, m, Lys α-*H*, PEG CH_2), 3.44 (2 H, d, *J* 14.7, Gly α-*H*b), 3.18–3.09 (6 H, m, CH_2NHCH_2Ar, Arg δ-CH_2), 2.96 (2 H, dd, *J* 13.0, 5.8, D-Phe β-*H*a), 2.78 (2 H, dd, *J* 13.0, 11.0, D-Phe β-*H*b), 2.69–2.61 (2 H, m, Asp β-*H*a), 2.56–2.47 (2 H, m, Asp β-*H*b), 1.84–1.77 (2 H, m, Arg β-*H*a), 1.67–1.57 (6 H, m, Lys δ-CH_2, Arg β-*H*b), 1.50–1.37 (8 H, m, Lys β-CH_2, Arg δ-*H*b), 0.69–0.57 (4 H, m, Lys γ-CH_2); *m/z* (MALDI) calc. for $C_{88}H_{120}{}^{35}ClN_{28}O_{23}$ $[M+H]^+$: 1971.9, found 1971.9.

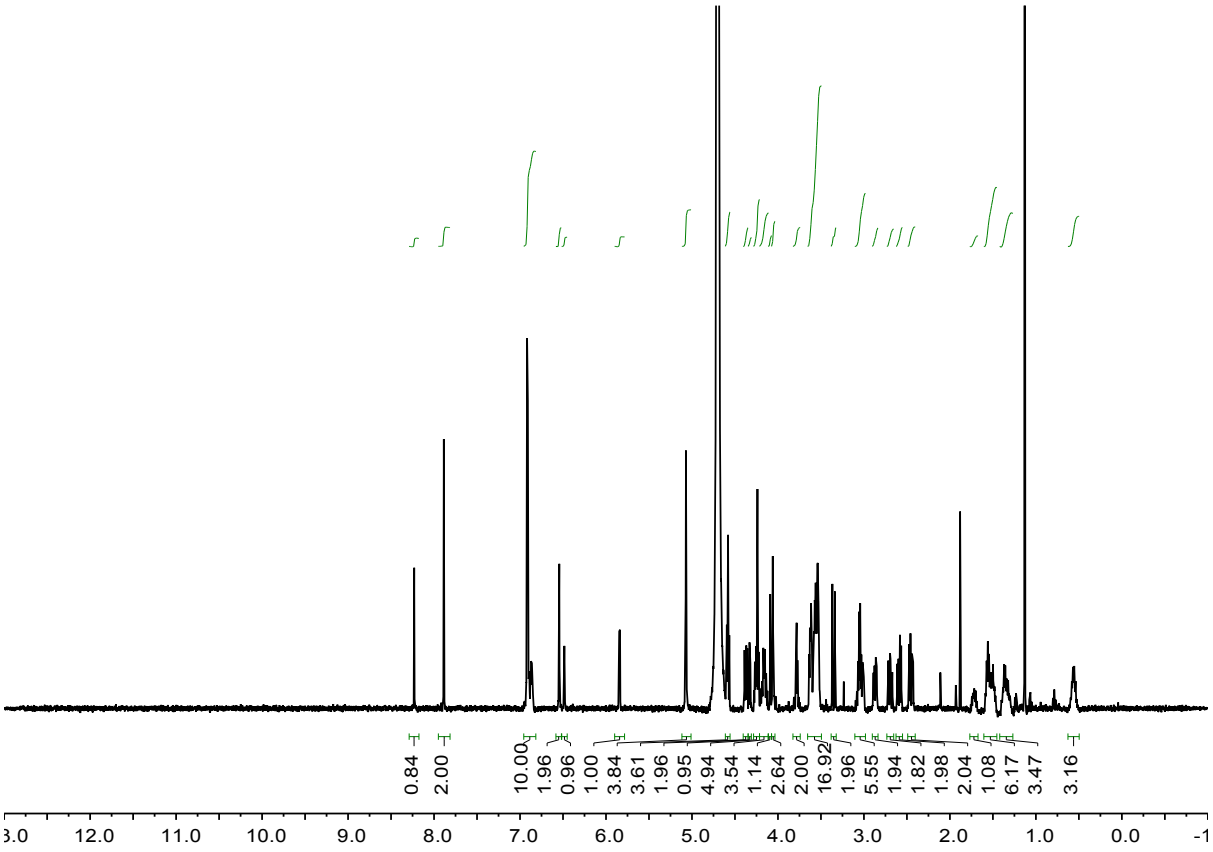


Figure S44. ^{1}H NMR spectrum (500.1 MHz, D_2O) of **30**. The large peak at 1.2 ppm is residual ^{t}BuOH from the freeze drying process.

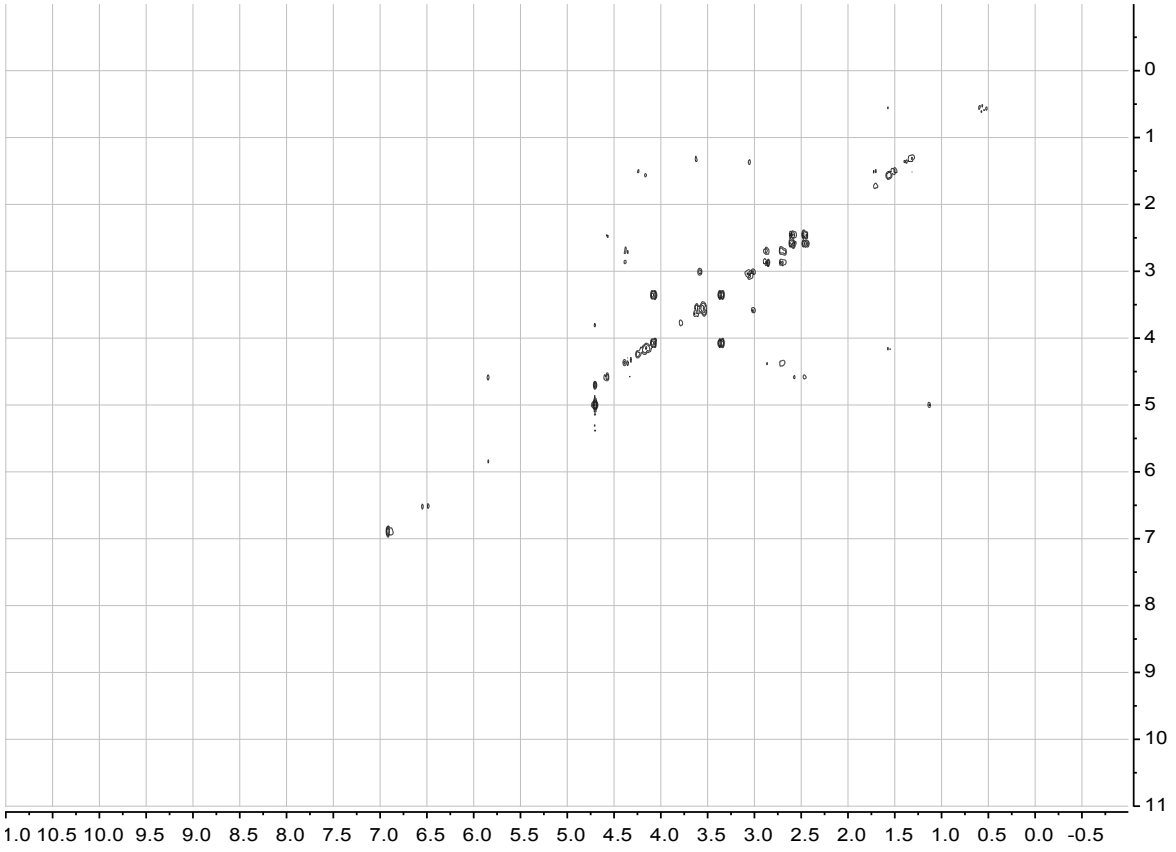


Figure S45. COSY NMR spectrum (500.1 MHz, D_2O) of **30**.

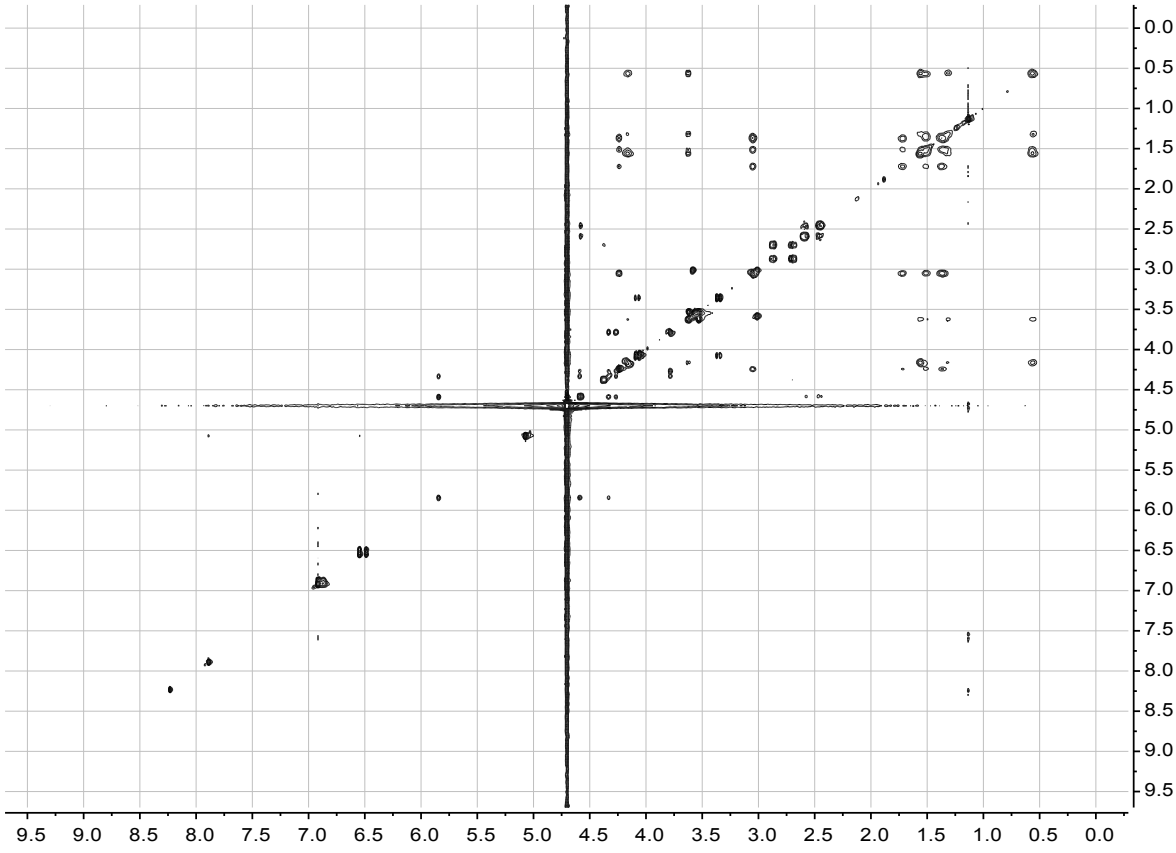


Figure S46. TOCSY NMR spectrum (500.1 MHz, D_2O, 90ms mixing time) of **30**.

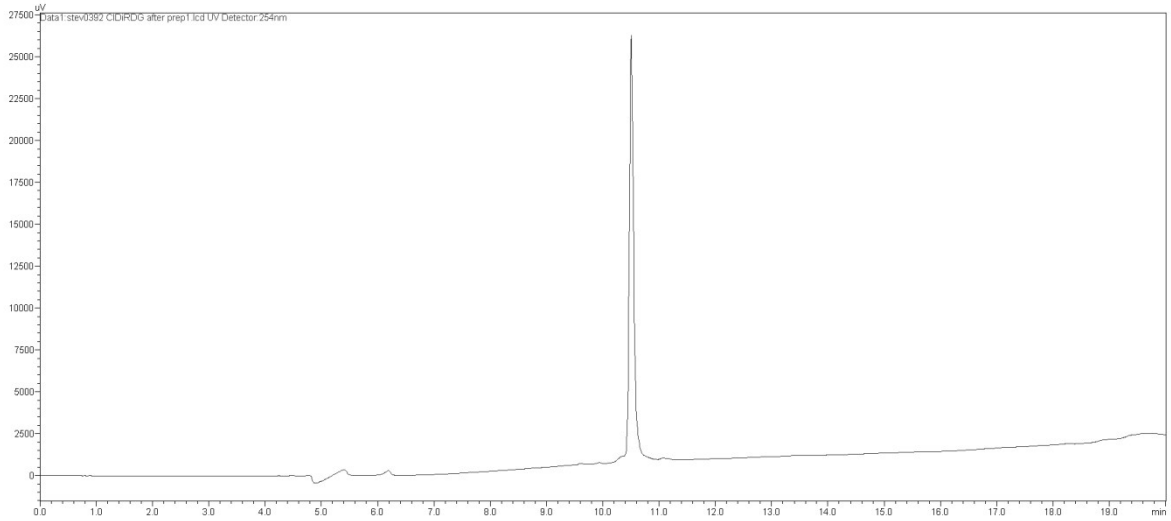


Figure S47. HPLC trace (254 nm) of **30** after purification.

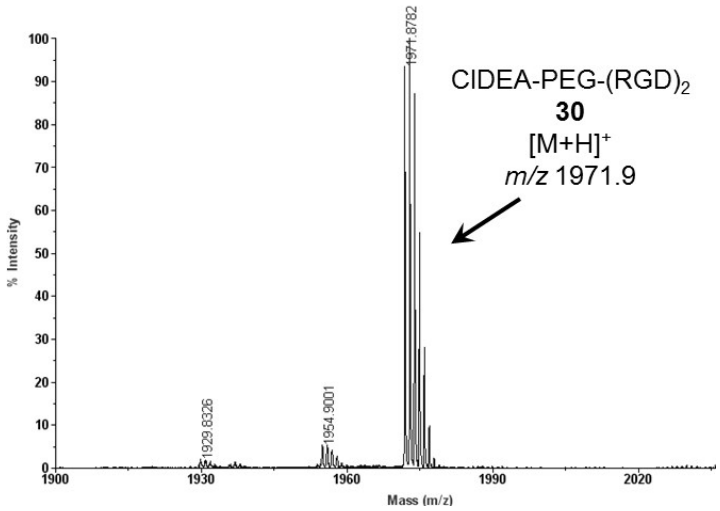
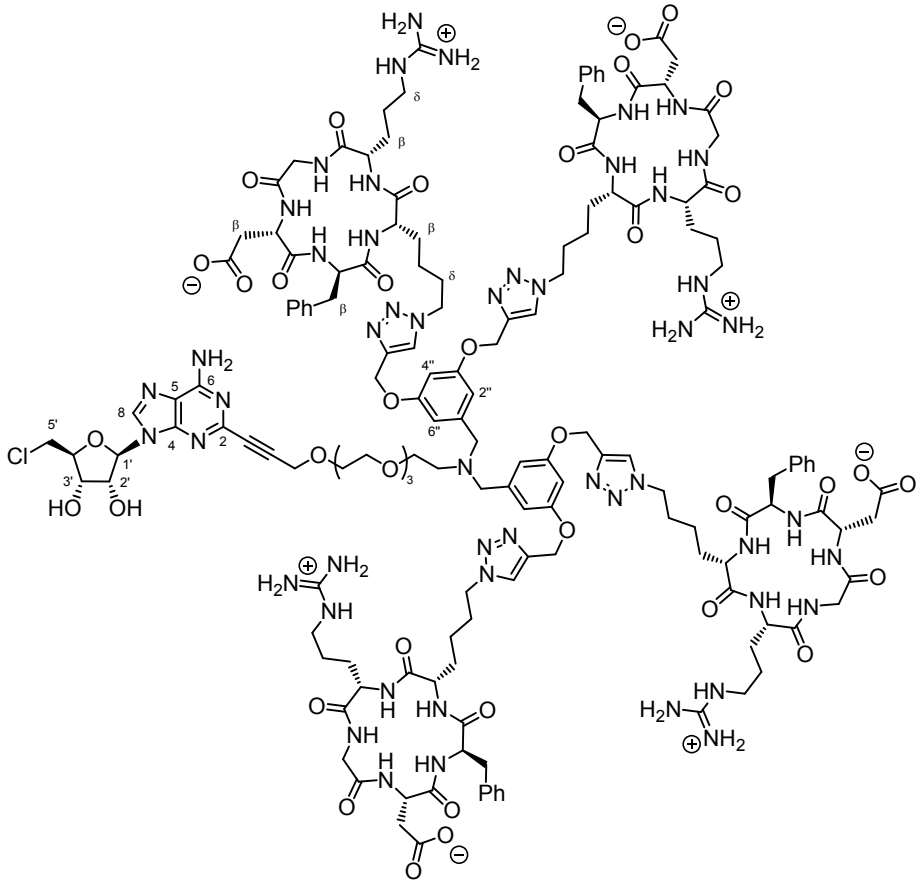


Figure S48. MALDI-TOF MS spectrum of **30** after purification, showing the $[M+H]^+$ ion at *m/z* = 1971.9. MALDI-TOF MS acquired by the University of St Andrews Mass Spectrometry Service.

2.20. ClDEA-PEG-$(RGD)_4$ 32



Fresh aqueous sodium ascorbate (291 µL, 20 mM, 10 eq.), followed by $CuSO_4$-TBTA complex (117 µL, 10 mM in 55:45 DMSO: water) were added to a solution of ClDEA-PEG-$(C{\equiv}CH)_4$ **26** (0.67 mg, 0.58 µmol, 1 eq.) in DMSO: water (75:25, 1.5 mL) and a solution of c(RGDfK[N_3]) **29** (1.83 mg, 2.91 µmol, 5 eq.) in DMSO: water (75:25, 1.5 mL). The reaction was allowed to proceed for 70 min at rt, when HPLC analysis showed reaction to be complete. The reaction mixture was diluted with water (4 mL) and loaded onto a reverse phase cartridge (1000 mg Extract Clean C_{18}-HC, preconditioned with water), washed with water (10 mL), and the product eluted with water: MeCN (50:50) (2 × 5 mL) and water: MeCN (25:75) (1 × 5 mL). The eluted fractions were combined and excess MeCN removed under a stream of compressed air to give a cloudy, aqueous solution. MeCN (200 µL) was added until the solution became clear and this mixture purified by semi-preparative HPLC on the Shimadzu system (Phenomenex Kingsorb C_{18} (250 × 10.00 mm, 5µ); mobile Phase: A (H_2O + 0.05% TFA), B (MeCN + 0.05% TFA); linear gradient: 5% B to 50% B at 10 min, to 95% B at 11 min; to 95% B at 14 min, to 5% B at 15 min, to 5% B at 25 min; flow rate: 2.5 mL.min^{-1}; detection 220 nm, t_R = 12.1–12.6 min). Fractions containing product (t_R = 10.2 min) were concentrated under a stream of compressed air, and lyophilised from a solution of ^{t}BuOH in water (20% *v/v*) affording **32** as a white solid (1.4 mg, 70%): δ_H (500.1 MHz, 10% d_3-MeCN in D_2O): 8.44 (1 H, s, *H*-8), 8.13 (4 H, s, triazole-*H*), 7.26–7.15 (20 H, m, D-Phe Ar*H*), 6.82 (6 H, br s, *H*-2″, *H*-4″, *H*-6″), 6.06 (1 H, d, *J* 4.6, *H*-1′), 5.31 (8 H, br s, ArO-CH_2-triazole), 4.85 (4 H, t, *J* 7.4, Asp α-*H*), 4.79 (1 H, *H*-2', overlapped by HOD, observed in 2D), 4.77 (4 H, dd, *J* 10.0, 6.1, D-Phe α-*H*), 4.53 (1 H, t, *J* 5.1, *H*-3'), 4.47–4.38 (19 H, m, *H*-4', Lys ε-CH_2, Arg α-*H*, $CH_2N(CH_2Ar)_2$, AdoC≡CCH_2O), 4.32 (4 H, d, *J* 15.0, Gly α-*H*a), 4.02 (1 H, dd, *J* 12.2, 3.6, *H*-5'a), 3.98 (1 H, dd, *J* 12.2, 4.9, *H*-5'b), 3.94 (4 H, dd, *J* 11.0, 4.0 Lys α-*H*), 3.82–3.66 (14 H, m, PEG CH_2), 3.60 (4 H, d, *J* 15.0, Gly α-*H*b), 3.33–3.25 (10 H, m, CH_2N$(CH_2Ar)_2$, Arg δ-CH_2), 3.09 (4 H, dd, *J* 13.2, 6.1, D-Phe β-*H*a), 2.98 (4 H, dd, *J* 13.2, 10.0, D-Phe β-*H*b), 2.93 (4 H, dd, *J* 16.6, 7.4, Asp β-*H*a), 2.77 (4 H, dd, *J* 16.6, 7.4, Asp β-*H*b), 2.01–1.94 (4

H, m, Arg β-*H*a), 1.85–1.72 (16 H, m, Lys β-CH_2, Arg β-*H*b, Arg γ-*H*a), 1.66–1.56 (12 H, m, Lys δ-CH_2, Arg γ-*H*b), 0.97–0.92 (8 H, m, Lys γ-CH_2); *m/z* (MALDI) calc. for $C_{155}H_{208}{}^{35}ClN_{50}O_{39}$ $[M+H]^+$: 3428.6, found 3428.5.

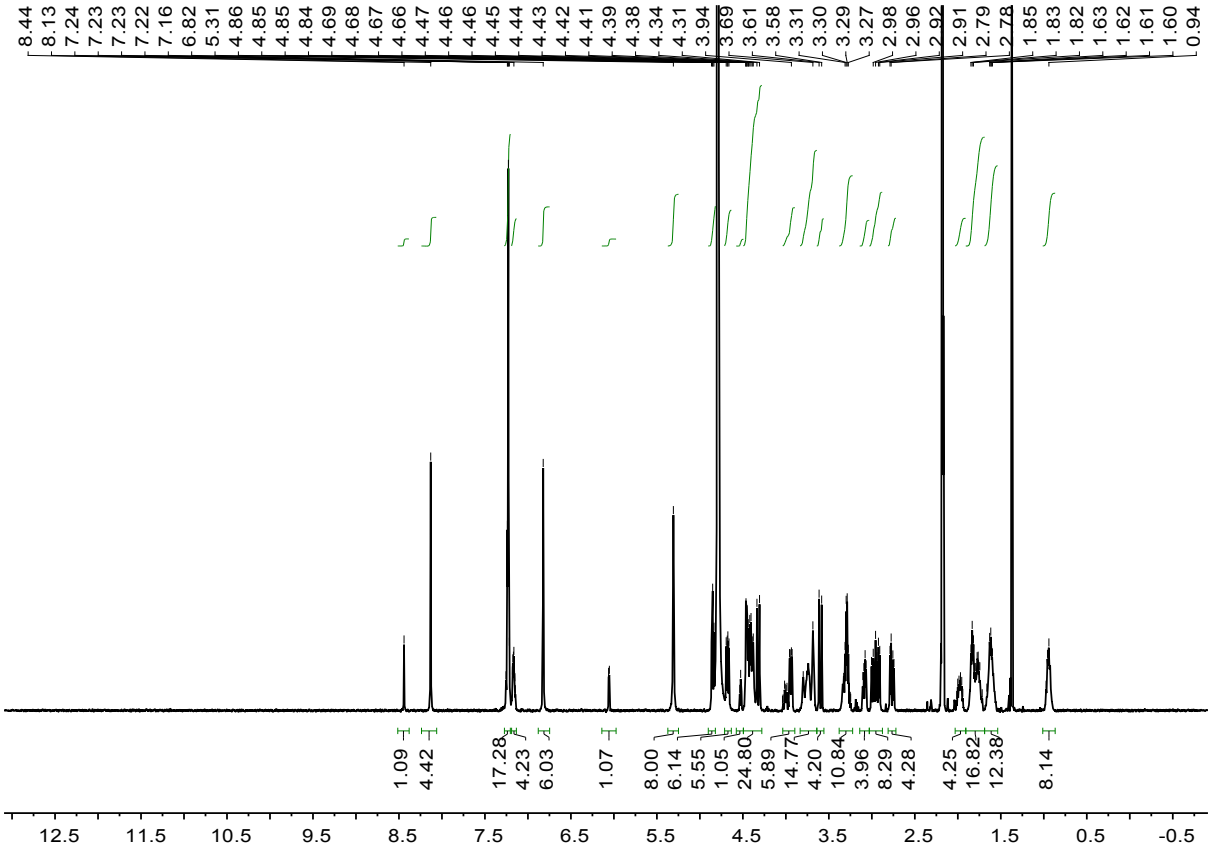


Figure S49. ^{1}H NMR spectrum (500.1 MHz, 10% d_3-MeCN in D_2O) of **32**. The large peak at 1.4 ppm is residual ^{t}BuOH from the freeze drying process.

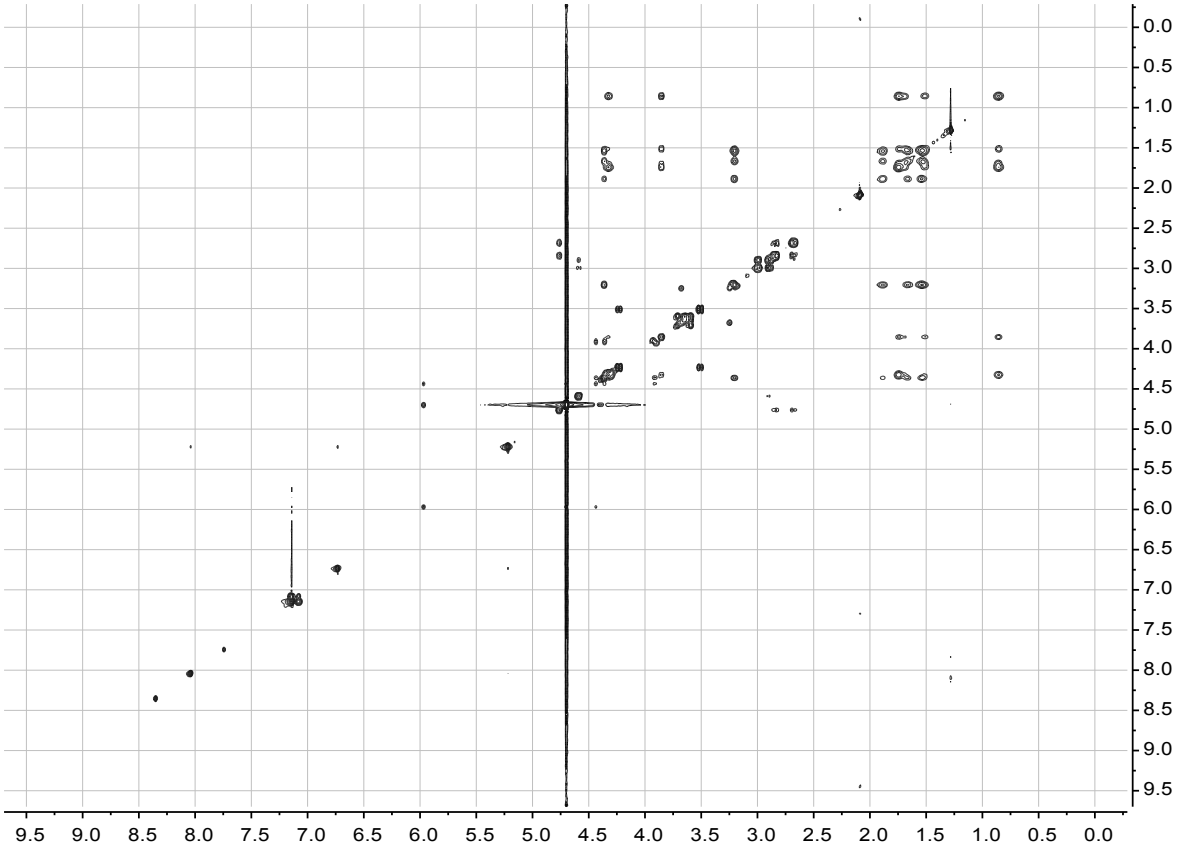


Figure S50. COSY NMR spectrum (500.1 MHz, 10% d_3-MeCN in D_2O) of **32**.

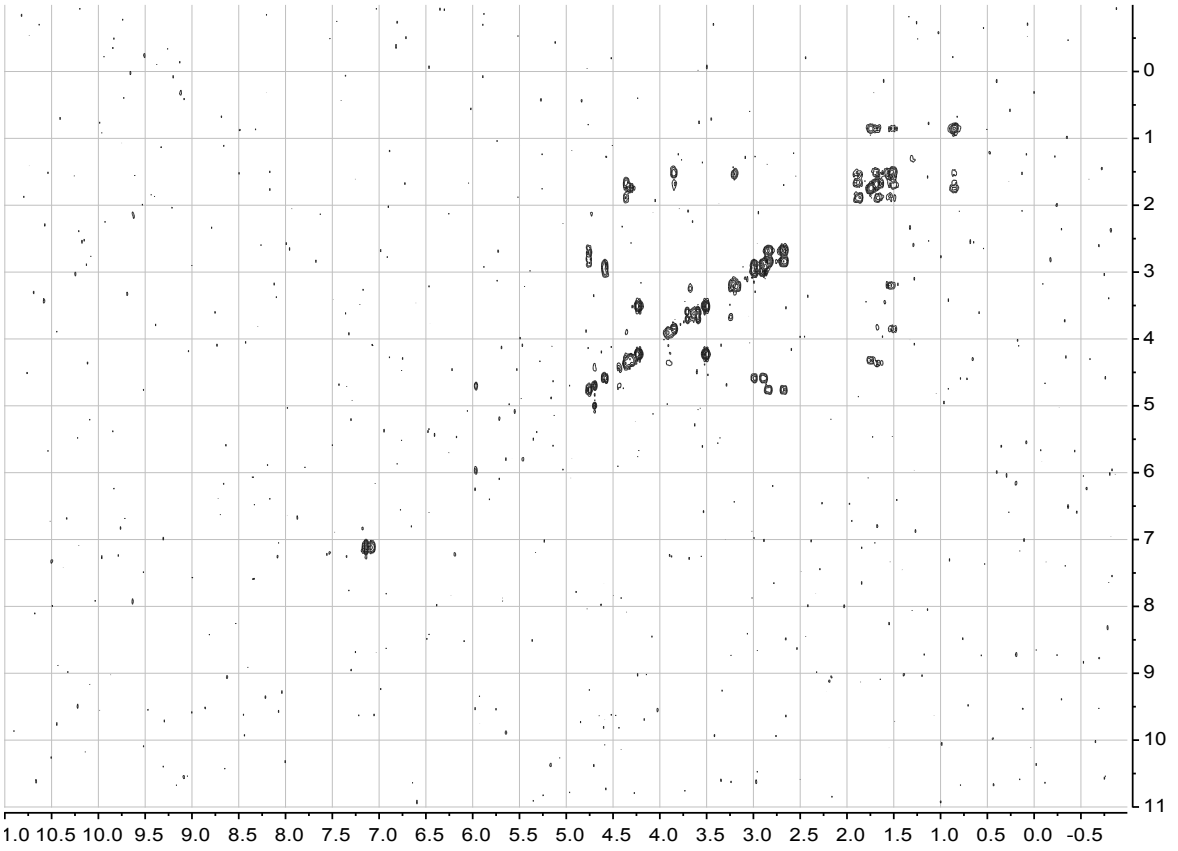


Figure S51. TOCSY NMR spectrum (500.1 MHz, 10% d_3-MeCN in D_2O, 90ms mixing time) of **32**.

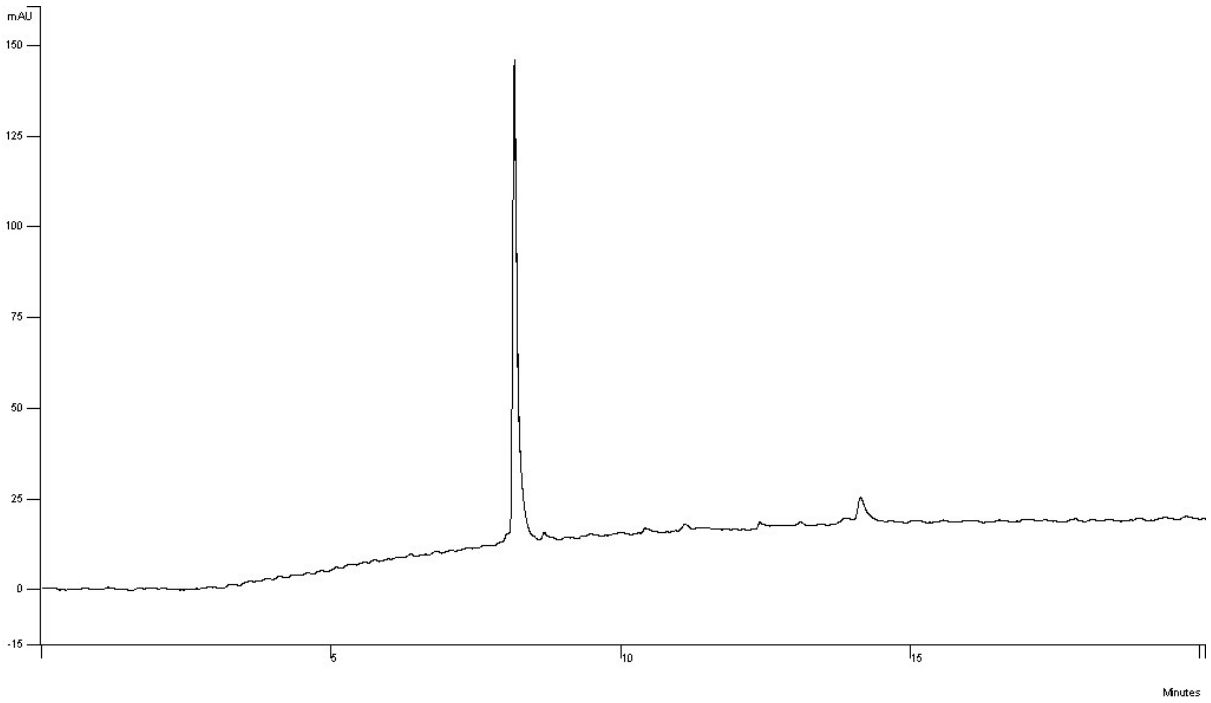


Figure S52. HPLC trace (220 nm) of **32** after purification.

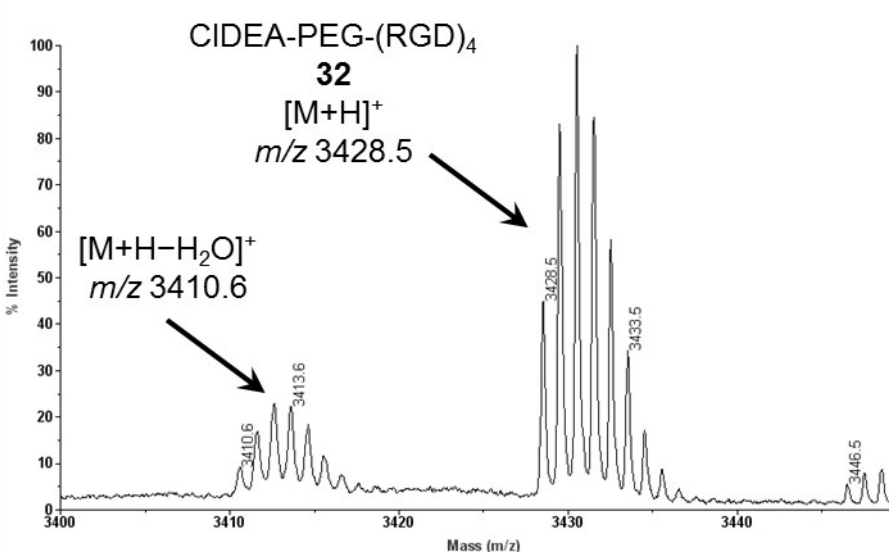
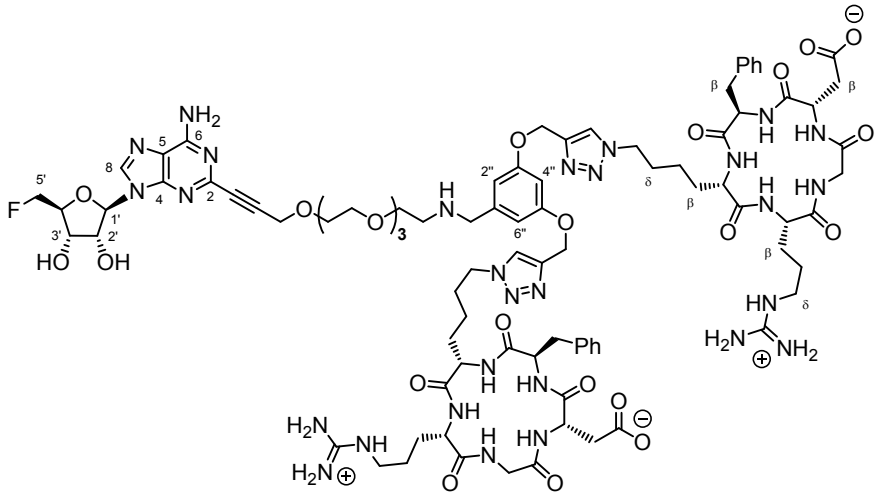


Figure S53. MALDI-TOF MS spectrum of **32** after purification, showing the [M+H]+ ion at m/z = 3428.5. MALDI-TOF MS acquired by the University of St Andrews Mass Spectrometry Service.

2.21. FDEA-PEG-$(RGD)_2$ 31



Fresh aqueous sodium ascorbate (541 µL, 20mM, 10 eq.), followed by $CuSO_4$-TBTA complex (216 µL, 10 mM in 55:45 DMSO: water) were added to a solution of FDEA-PEG-$(C{\equiv}CH)_2$ **24** (1.0 mg, 1.08 µmol, 1 eq.) and c(RGDfK[N_3]) **29** (1.7 mg, 7.70 µmol, 2.5 eq.) in DMSO: water (50:50, 6.5 mL). The reaction was allowed to proceed for 70 min at rt, when HPLC analysis showed reaction to be complete. The reaction mixture was loaded onto a reverse phase cartridge (1000 mg Extract Clean C_{18}-HC, preconditioned with water), washed with water (10 mL), and the product eluted with water: MeCN (50:50) (2 × 10 mL) and MeCN (1 × 10 mL). The water: MeCN fractions were dried under a stream of compressed air. The residue was suspended in MeCN:H_2O (20:80) and this mixture purified by semi-preparative HPLC on the Shimadzu system (Phenomenex Kingsorb C_{18} (250 × 10.00 mm, 5µ); mobile Phase: A (H_2O + 0.05% TFA), B (MeCN + 0.05% TFA); linear gradient: 20% B to 42% B at 8 min, 42% B to 95% B at 9 min; 95% B at 14 min, 95% B to 20% B at 15 min, 20% B at 25 min; flow rate: 2.5 mL.min^{-1}; detection 220 nm. Fractions containing product (t_R = 10.0 min) were concentrated under a stream of compressed air, and lyophilised from a solution of ^{t}BuOH in water (20% *v/v*) affording **31** as a white solid (0.67 mg, 27%): *m/z* (MALDI) calc. for $C_{88}H_{120}FN_{23}O_{23}$ $[M+H]^+$: 1955.9, found 1955.9.

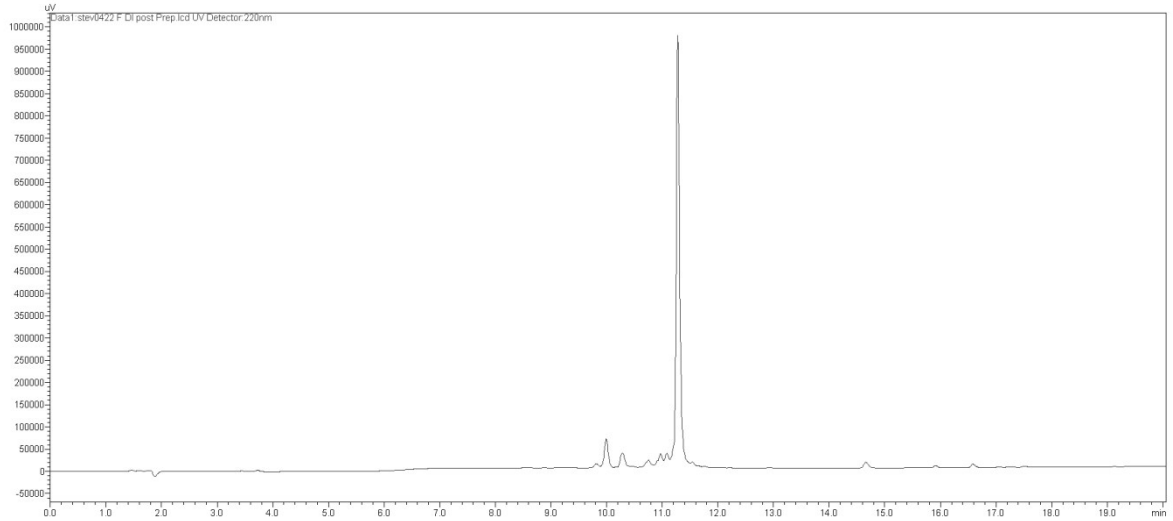


Figure S54. HPLC trace (220 nm) of **31**after purification.

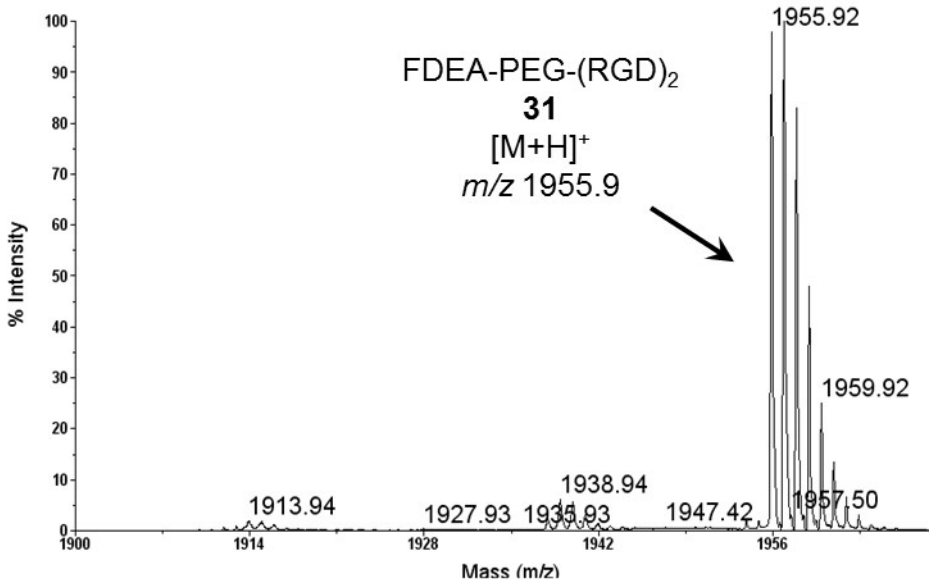
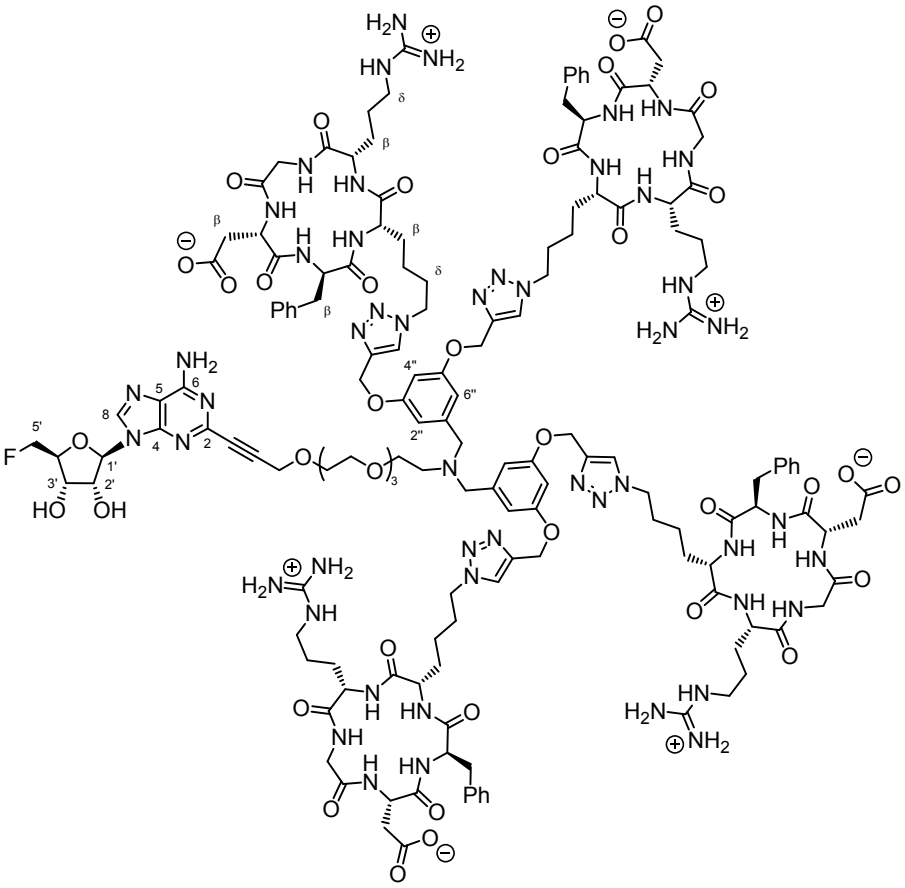


Figure S55. MALDI-TOF MS spectrum of **31** after purification, showing the [M+H]+ ion at m/z = 1955.9. MALDI-TOF MS acquired by the University of St Andrews Mass Spectrometry Service.

2.22. FDEA-PEG-(RGD)$_4$ 33



Fresh aqueous sodium ascorbate (262 µL, 20mM, 10 eq.), followed by $CuSO_4$-TBTA complex (145 µL, 10 mM in 55:45 DMSO:water) were added to a solution of FDEA-PEG-(C≡CH)$_4$ **28** (0.75 mg, 0.72 µmol, 1 eq.) and c(RGDfK[N_3]) **29** (2.28 mg, 3.62 µmol, 5 eq.) in DMSO: water (50:50, 4.3 mL). The reaction was allowed to proceed for 90 min at rt, when HPLC analysis showed reaction to

be complete. The reaction mixture was loaded onto a reverse phase cartridge (1000 mg Extract Clean C_{18}-HC, preconditioned with water), washed with water (10 mL), and the product eluted with water: MeCN (50:50) (2 × 10 mL) and MeCN (1 × 10 mL). The water: MeCN and MeCN fractions were dried a stream of compressed air. The residue was suspended in MeCN:H_2O (20:80) and this mixture purified by semi-preparative HPLC on the Shimadzu system (Phenomenex Kingsorb C_{18} (250 × 10.00 mm, 5μ); mobile Phase: A (H_2O + 0.05% TFA), B (MeCN + 0.05% TFA); linear gradient: 20% B to 42% B at 18 min, 42% B to 95% B at 19 min; 95% B at 24 min, 95% B to 20% B at 25 min, 20% B at 35 min; flow rate: 2.5 mL.min^{-1}; detection 220 nm. Fractions containing product (t_R = 13.3 min) were concentrated under a stream of compressed air, and lyophilised from a solution of ^{t}BuOH in water (20% *v/v*) affording **33** as a white solid (0.66 mg, 27%): $C_{155}H_{208}FN_{50}O_{39}$ $[M+H]^+$: 3412.6, found 3412.6.

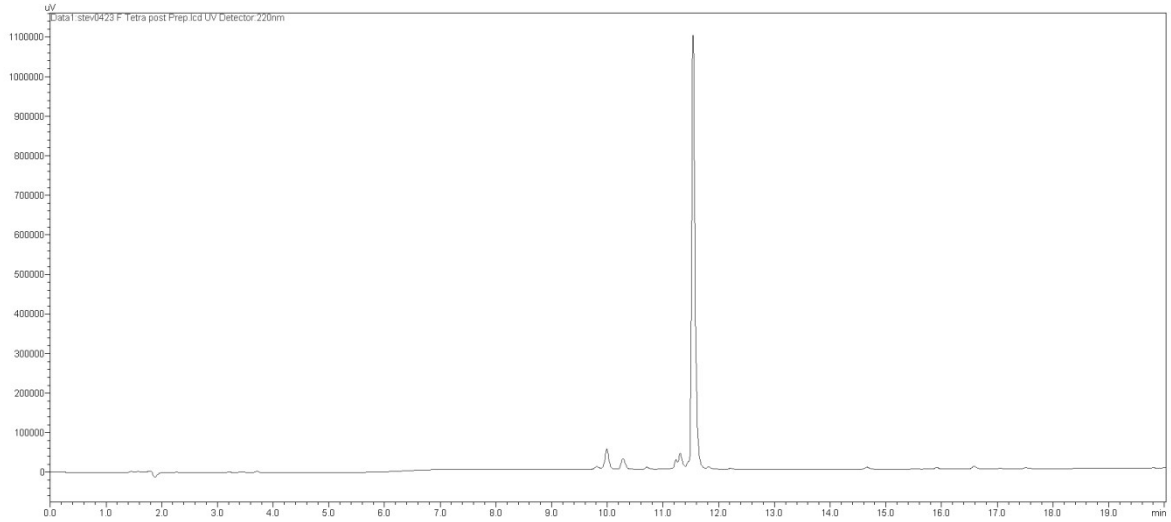


Figure S56. HPLC trace (220 nm) of **33** after purification.

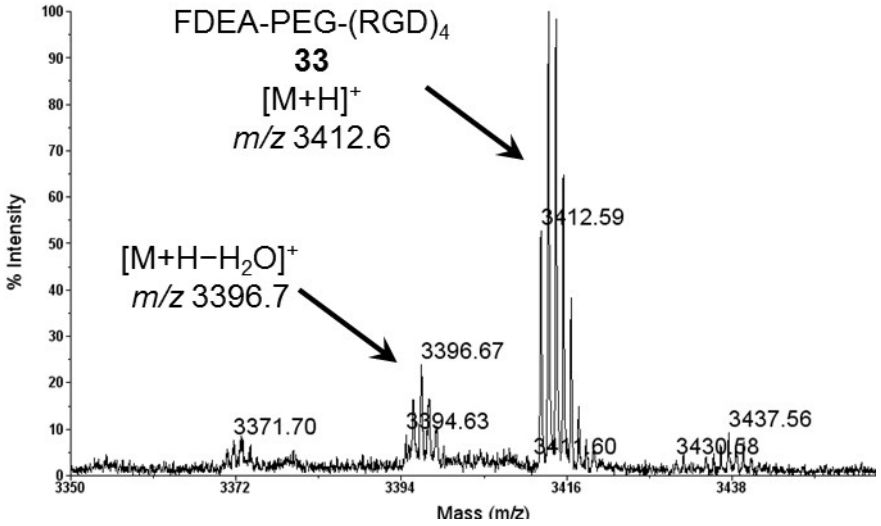


Figure S57. MALDI-TOF MS spectrum of **33** after purification, showing the $[M+H]^+$ ion at *m/z* = 3412.6. MALDI-TOF MS acquired by the University of St Andrews Mass Spectrometry Service.

3. Enzymatic Assays

3.1. Preparation of fluorinase

The fluorinase enzyme used in these investigations was prepared according to the procedure outlined in Thompson *et al*.[8]

3.2. ClDEA-PEG-(RGD)$_2$ 30 to FDEA-PEG-(RGD)$_2$ 31

In a total reaction volume of 1000 µL, recombinant fluorinase (1 mg.mL^{-1}) was incubated with ClDEA-PEG-(RGD)$_2$ **30** (0.04 mM), L-SeMet (0.075 mM) and KF (50 mM) at 37 °C. Samples (100 µL) were periodically removed, denatured by heating at 95°C for 3 min, before being clarified by centrifugation (13 000 rpm, 10 min). Samples of the supernatant (100 µL) were removed for analysis by HPLC. Control experiments without enzyme, L-SeMet and fluoride were also performed and showed no formation of FDEA-PEG-(RGD)$_2$ **31**. In the control without fluoride, conversion of ClDEA-PEG-(RGD)$_2$ **30** to SeEAM-PEG-(RGD)$_2$ was observed.

HPLC analysis was performed on a Shimadzu Prominence system using a Kinetix 5µm XB-C$_{18}$ 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); Isocratic: 22% solvent B for 20 min, then from 22% to 95% of solvent B in 1 min, hold at 95% B for 2 min, and back to 22% B for 7 min to re-equilibrate the column. Flow rate: 1 mL.min^{-1}; Detection: 220 nm; Injection volume: 20 µL.

3.3. ClDEA-PEG-(RGD)$_4$ 32 to FDEA-PEG-(RGD)$_4$ 33

In a total reaction volume of 1000 µL, recombinant fluorinase (1 mg.mL^{-1}) was incubated with ClDEA-PEG-(RGD)$_4$ **32** (0.02 mM), L-SeMet (0.075 mM) and KF (50 mM) at 37 °C. Samples (100 µL) were periodically removed, denatured by heating at 95°C for 3 min, before being clarified by centrifugation (13 000 rpm, 10 min). Samples of the supernatant (100 µL) were removed for analysis by HPLC. Control experiments without enzyme, L-SeMet and fluoride were also performed and showed no formation of FDEA-PEG-(RGD)$_4$ **33**. In the control without fluoride, conversion of ClDEA-PEG-(RGD)$_4$ **32** to SeEAM-PEG-(RGD)$_4$ was observed.

HPLC analysis was performed on a Shimadzu Prominence system using a Kinetix 5µm XB-C$_{18}$ 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); Isocratic: 24% solvent B for 20 min, then from 22% to 95% of solvent B in 1 min, hold at 95% B for 2 min, and back to 24% B for 7 min to re-equilibrate the column. Flow rate: 1 mL.min^{-1}; Detection: 220 nm; Injection volume: 20 µL.

3.4. Optimisation of transhalogenation of ClDA 4 to [^{18}F]FDA [^{18}F]-2

A standard experiment for the synthesis of [^{18}F]FDA [^{18}F]-**2** is described below. In experiments to determine optimal incorporation conditions, each factor was changed as described in **Table S1**. Concentrations shown are final concentrations.

To a solution of ClDA **4** (0.04 mM, 20 µL), L-SeMet (0.075 mM, 38 µL) and fluorinase (5 mg, freeze dried), a solution of [^{18}F]fluoride in water (200 µL, 56.1 MBq) was added and the final volume made up to 500 µL with water. The mixture was incubated at 37 °C for 30 min before the enzyme was denatured by heating the sample to 100 °C for 5 min. The sample was centrifuged (3000-4000 rpm) for 5 min before a sample of the supernatant was analysed using the Dionex HPLC system.

Table S1. Conditions investigated for optimization of incorporation of [^{18}F]fluoride to [^{18}F]FDA [^{18}F]-**2**. Conditions changed are shown in bold. All reactions performed in a total volume of 500 µL.

Component final concentration

ClDA **4**/mM	fluorinase/mg.mL^{-1}	L-SeMet/mM
0.04	10	0.125
0.04	**10** (alt. batch)	0.125
0.04	**2.8** (stoichiometric)	0.125
0.04	**0.7** (catalytic)	0.125
0.04	10	**0.020**
0.3	**0.7**	0.125
0.3	10	0.125
0.3	**20**	0.125
0.6	**20**	0.125

3.5. ClDEA-PEG-(RGD)$_2$ 30 to [^{18}F]FDEA-PEG-(RGD)$_2$ [^{18}F]-31

A standard experiment for the synthesis of [^{18}F]-**31** is described below. Final concentrations of each component were used according to the optimised conditions described above, were the substrate was used at 0.3 mM, L-SeMet at 0.08 mM and fluorinase at 20 mg.mL^{-1}.

To ClDEA-PEG-(RGD)$_2$ **30** (0.1 mg) and fluorinase (2.88 mg), L-SeMet (6 µL, 2 mM) was added, followed by a solution of [^{18}F]fluoride in water (138 µL, 42.4 MBq). The mixture was incubated at 37 °C for 30 min before the enzyme was denatured by heating the sample to 100 °C for 5 min. The sample was centrifuged (3000-4000 rpm) for 5 min before a sample of the supernatant was analysed by HPLC.

Analysis was performed on a Dionex system using a Kinetix 5µm XB-C$_{18}$ 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); Isocratic: 22% solvent B for 20 min, then from 22% to 95% of solvent B in 1 min, hold at 95% B for 2 min, and back to 22% B for 7 min to re-equilibrate the column. An alternate gradient was also used in some experiments: Linear gradient: 5% solvent B to 95% solvent B over 20 min, 95% solvent B for 4 min then to 5% solvent B in 1 min and hold for 10 min to re-equilibrate the column; Flow rate: 1 mL.min^{-1}; Detection: 220 nm; Injection volume: 10-50 µL.

Retention time comparison between the peaks observed in radio HPLC for [^{18}F]FDEA-PEG-(RGD)$_2$ **[^{18}F]-31** and that observed in UV for [^{19}F]FDEA-PEG-(RGD)$_2$ **31** is shown below in **Figure S58.**

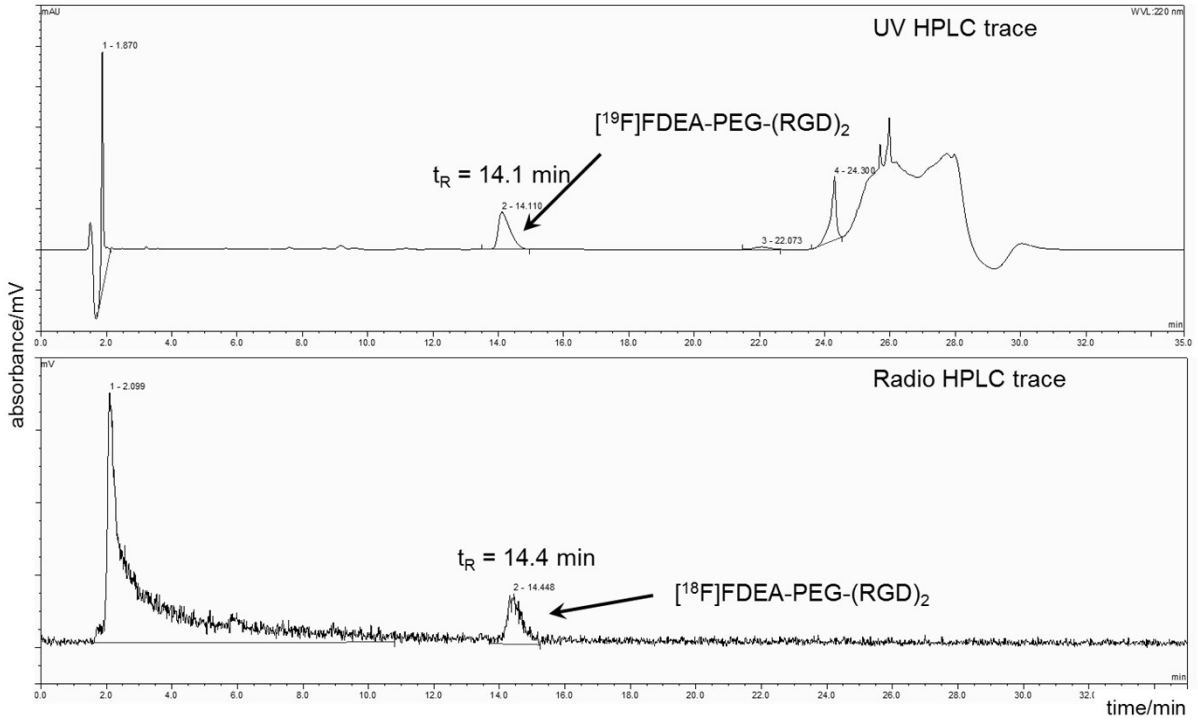


Figure S58. Comparison of retention times for [^{19}F]FDEA-PEG-(RGD)$_2$ **31** by UV HPLC (top) and [^{18}F]FDEA-PEG-(RGD)$_2$ **[^{18}F]-31** by radio HPLC (bottom). Retention time in the radio HPLC is slightly delayed due to the detectors being arranged in series.

[^{18}F]FDA [^{18}F]-**2** is also observed in radiolabelling samples with mentioned in **Figure 3**. An HPLC trace of a enzymatic radiolabelling experiment run under gradient conditions, showing the presence of peaks for [^{18}F]fluoride, [^{18}F]FDA [^{18}F]-**2** and [^{18}F]FDEA-PEG-(RGD)$_2$ [^{18}F]-**31** is shown below in **Figure S59**.

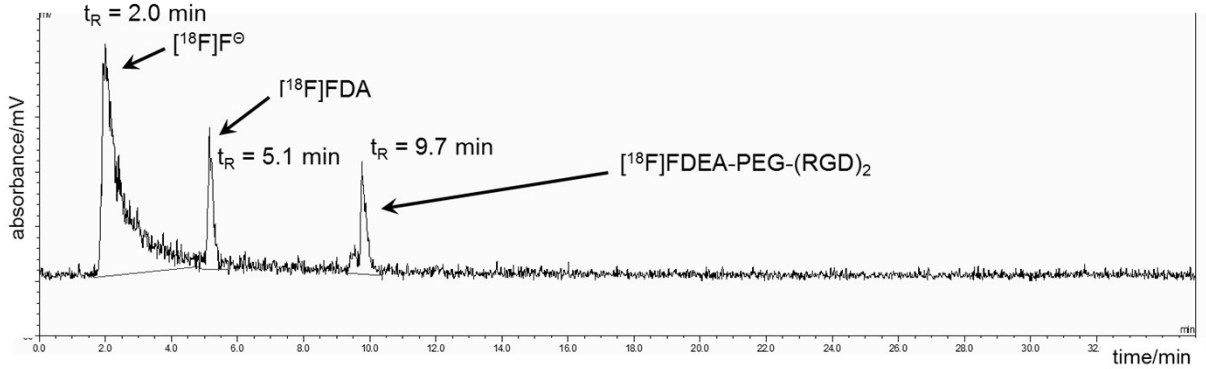


Figure S59. HPLC radioactivity trace (gradient solvent conditions) of experiment where ClDEA-PEG-(RGD)$_2$ **30** (0.3 mM) with the fluorinase (20 mg.mL^{-1}). [^{18}F]FDEA-PEG-(RGD)$_2$ [^{18}F]-**31** was observed at t_R = 9.7 min. An additional peak was also observed at t_R = 5.1 min, identified as [^{18}F]FDA [^{18}F]-**2**, produced due to the presence of residual SAM **1** co-purified with the fluorinase

3.6. ClDEA-PEG-(RGD)$_4$ 32 to [^{18}F]FDEA-PEG-(RGD)$_4$ [^{18}F]-33

A standard experiment for the synthesis of [^{18}F]-**33** is described below. Final concentrations of each component were used according to the optimised conditions described above, were the substrate was used at 0.3 mM, L-SeMet at 0.08 mM and fluorinase at 20 mg.mL^{-1}.

To ClDEA-PEG-$(RGD)_4$ **32** (0.2 mg) and fluorinase (3.34 mg), L-SeMet (7 µL, 2 mM) was added, followed by a solution of [^{18}F]fluoride in water (160 µL, 34.4 MBq). The mixture was incubated at 37 °C for 30 min before the enzyme was denatured by heating the sample to 100 °C for 5 min. The sample was centrifuged (3000-4000 rpm) for 5 min before a sample of the supernatant was analysed by HPLC.

Analysis was performed on a Dionex system using a Kinetix 5µm XB-C_{18} 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: 5% solvent B to 95% solvent B over 20 min, 95% solvent B for 4 min then to 5% solvent B in 1 min and hold for 10 min to re-equilibrate the column; Flow rate: 1 $mL.min^{-1}$; Detection: 220 nm; Injection volume: 10-50 µL.

Retention time comparison between the peaks observed in radio HPLC for [^{18}F]FDEA-PEG-$(RGD)_4$ **[^{18}F]-33** and that observed in UV for [^{19}F]FDEA-PEG-$(RGD)_4$ **33** is shown below in **Figure S60.**

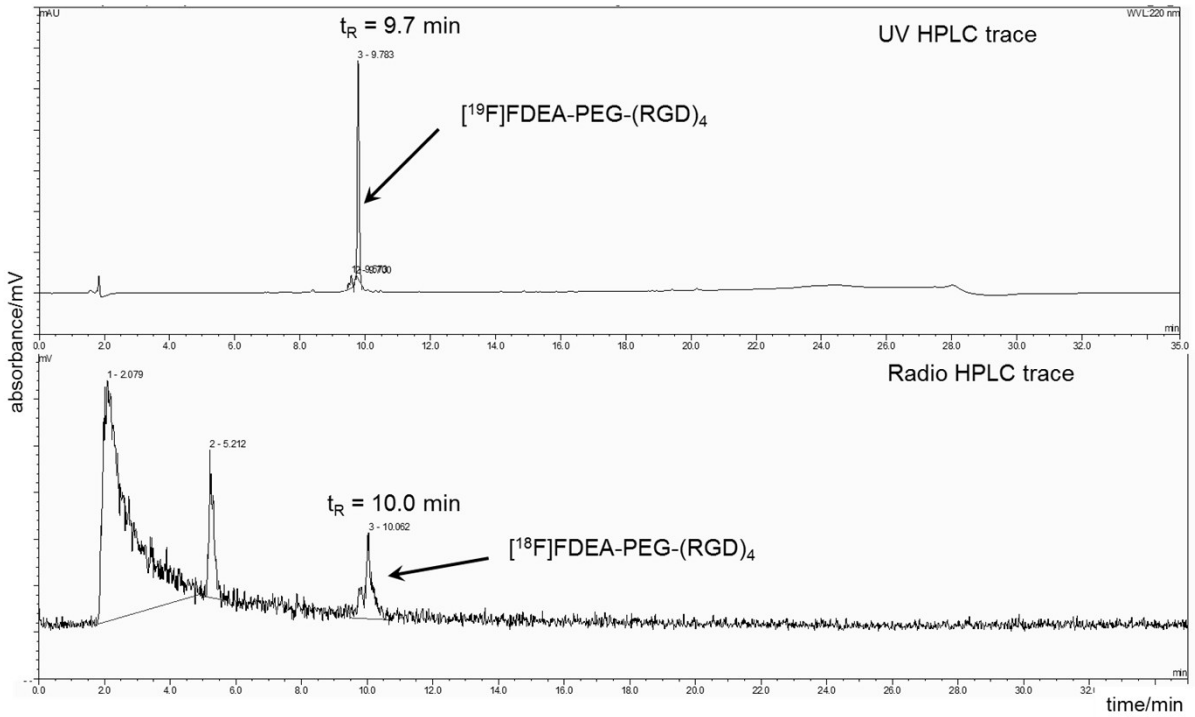


Figure S60. Comparison of retention times for [^{19}F]FDEA-PEG-$(RGD)_4$ **33** by UV HPLC (top) and [^{18}F]FDEA-PEG-$(RGD)_4$ **[^{18}F]-33** by radio HPLC (bottom). Retention time in the radio HPLC is slightly delayed due to the detectors being arranged in series.

3.7. Control experiment, no substrate addition

In a control experiment, a solution of [^{18}F]fluoride ion was added to the fluorinase enzyme and L-SeMet, *without* the addition of any nucleoside substrate. After 30 min incubation at 37 °C and subsequent heat precipitation of the enzyme, the supernatant was analysed by HPLC using the same gradient conditions. The resultant radio-chromatogram (**Figure S61**) revealed the presence of a radiolabelled compound, eluting at t_R = 5.2 min. Spiking of this sample with a solution of [^{19}F]FDA **2** revealed that the peaks co-elute, suggesting that the radioactive compound was indeed [^{18}F]FDA [^{18}F]-**2**.

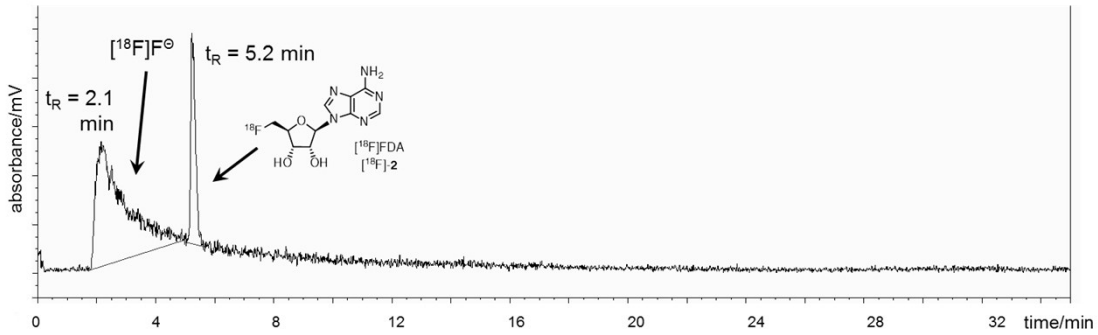


Figure S61. HPLC radioactivity trace of the control reaction where the fluorinase (20 mg.mL^{-1}) was incubated with only [^{18}F]fluoride for 30 min. [^{18}F]FDA [^{18}F]-**2** was observed at t_R = 5.2 min. Run with under gradient conditions.

The conversion of approx. 20% of [^{18}F]fluoride to [^{18}F]FDA [^{18}F]-**2** under these conditions suggested that traces of *S*-adenosylmethionine **1** (SAM) were present in the reaction mixture. The native fluorinase enzyme from *S. cattleya* was known to co-purify with the substrate, SAM **1**, bound to the active site.[9]

Upon incubation with [^{18}F]fluoride, the low levels of residual SAM **1** would be converted, under the action of the enzyme, to [^{18}F]FDA [^{18}F]-**2**. During previous radiolabelling experiments involving the fluorinase, with either ClDEA **5** or ClDEA-TEG-RGD **7**, the use of high substrate concentrations (0.3 mM) is thought to result in the substrates effectively out-competed any residual SAM **1**. This may be why significant conversion to [^{18}F]FDA [^{18}F]-**8** was not observed in these experiments.

4. ELISA Affinity Assays

Binding affinity of cold bioconjugates **31** and **33** to purified $\alpha_v\beta_3$ integrin was determined by ELISA as described previously.[10]

5. References

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