Supporting Information:

Synthesis of organometallic PNA oligomers by click chemistry

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I. Experimental Section

a) Materials. All chemicals were of reagent grade quality or better, obtained from commercial suppliers and used without further purification. Solvents were used as received or dried over 4 Å molecular sieves.

b) Instrumentation and methods. *^{1}H and ^{13}C NMR spectra* were recorded in deuterated solvents on a Bruker DRX 400 spectrometer at 30°C. The chemical shifts, δ, are reported in ppm (parts per million). The residual solvent peaks have been used as an internal reference. The abbreviations for the peak multiplicities are as follows: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet) and br (broad). *Infrared spectra* were recorded on a ATR unit using a Bruker Tensor 27 FTIR spectrophotometer at 4 cm^{-1} resolution. Signal intensity is abbreviated br (broad). *ESI mass spectra* were recorded on a Bruker Esquire 6000. The *matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF) mass spectra* were measured on a Bruker Daltonics Autoflex. The experiments were performed in linear mode with positive polarity using sinapinic acid as the matrix.

c) Peptide nucleic acid synthesis. The PNAs were synthesised using an automated Expedite 8909 nucleic acid synthesiser (Applied Biosystems) adapted for PNA synthesis. Synthesis was performed on a 2-μmol scale with Tentagel R Ram-Lys(Boc)Fmoc resin (0.20 mmol/g) from Rapp Polymer using Fmoc/Bhoc-protected monomers from commercial suppliers (ASM Research Chemicals and Link Technologies). Synthon **Fmoc-1-OH**, dissolved in *N*-methylpyrrolidone (NMP), was inserted in one of the free positions of the synthesizer and a double coupling was applied in order to ensure a full coupling. Before cleavage, the resin was

shrunk with methanol and dried. The non-ferrocene containing PNAs were then cleaved using a mixture of trifluoroacetic acid:water:triisopropylsilane 95:2.5:2.5 while the ferrocene containing PNAs were cleaved using a mixture of trifluoroacetic acid:phenol:triisopropylsilane 85:10:5 [3 x 400 μl (1h30 each)]. The resulting solutions were first evaporated to dryness before being precipitated with ice-cold ether. The solids were centrifuged, washed with ice-cold ether and finally air dried. The crude products were analysed via MALDI-TOF MS.

d) Synthesis, characterisation and NMR spectra of compounds Fmoc-1-O^{t}Bu, Fmoc-1-OH, Fmoc-2-O^{t}Bu and 3.

***tert*-Butyl 2-(*N*-(2-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)ethyl)pent-4-ynamido)acetate (Fmoc-1-O^{t}Bu).** *tert*-Butyl *N*-[2-(*N*-9-fluorenylmethoxycarbonyl)aminoethyl)] glycinate hydrochloride[1] (1.50 g, 3.45 mmol) was dissolved in CH_2Cl_2 (210 mL) and then washed with an aqueous solution of $NaHCO_{3\,(sat)}$ (120 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated to dryness. The resulting oil was dissolved in DMF (13 mL). This solution was added to a solution of 4-pentynoic acid (0.38 g, 3.81 mmol), *N*-methylmorpholine (0.60 g, 5.88 mmol), hydroxybenzotriazole (0.81 g, 5.88 mmol) and 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate (1.44 g, 3.81 mmol) in DMF (5 mL), which had been stirred for 20 minutes at room temperature. The reaction mixture was stirred for 66h at room temperature. The mixture was evaporated to dryness and the residual oil purified by column chromatography on silica with ethyl acetate:hexane 1:1 as the eluent (Rf = 0.53) to give *tert*-butyl 2-(*N*-(2-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)ethyl)pent-4-ynamido)acetate (**Fmoc-1-O^{t}Bu**) as a pale yellow oil. Yield. 1.28 g (78 %).

NB: In the event that the purification by column chromatography affords a mixture of 4-pentynoic acid and the desired product, **Fmoc-1-O^{t}Bu** can be easily obtained by washing a dichloromethane solution of these two compounds with an aqueous solution of $NaHCO_{3\,(sat)}$.

Characterisation Data of Fmoc-1-O^{t}Bu. IR bands (ν, cm^{-1}): 3302 w, 3066 w, 2977 w, 2938 w, 1717 s, 1646 m, 1516 m, 1449 m, 1394 w, 1367 m, 1233 s, 1150 s, 1103 m, 1055 w, 1009 m, 944 w, 913 w, 847 m, 759 s, 738 s, 643 m, 620 m. ^{1}H NMR Spectrum ($CDCl_3$): δ 1.49 (br s, 9H, O-(C*H*$_3$)$_3$), 1.72 (min) and 1.88 (maj) (rotamers, s, 1H, *H*C≡C), 2.42-2.62 (m, 4H, HC≡C-C*H*$_2$ and CH$_2$-C*H*$_2$CO), 3.37 (m, 2H, NH-C*H*$_2$-CH$_2$), 3.52 (m, 2H, CH$_2$-C*H*$_2$-N), 3.92 (maj) and 3.96 (min) (rotamers, s, 2H, N-C*H*$_2$-COOC(CH$_3$)$_3$), 4.22 (m, 1H, C*H* Fmoc-CH$_2$O), 4.38 (m, 2H, CH Fmoc-C*H*$_2$O), 5.48 (min) and 5.90 (maj) (rotamers, br s, 1H, CH$_2$-N*H*-COO), 7.30 (m, 2H, C*H* Fmoc arom), 7.40 (m, 2H, C*H* Fmoc arom), 7.59 (m, 2H, C*H* Fmoc arom), 7.75 (m, 2H, C*H* Fmoc arom). ^{13}C NMR Spectrum ($CDCl_3$): δ 14.36 (maj) and 14.43 (min) (rotamers, HC≡C-*C*H$_2$), 27.99 (min) and 28.01 (maj) (rotamers, OC(*C*H$_3$)$_3$), 31.86 (min) and 32.17 (maj) (rotamers, CH$_2$-*C*H$_2$-CO), 39.40 (maj) and 39.48 (min) (NH-*C*H$_2$-CH$_2$), 47.19 (maj) and 47.24 (min) (rotamers, *C*H Fmoc-CH$_2$O), 48.09 (min) and 49.28 (maj) (CH$_2$-*C*H$_2$-N), 49.85 (maj) and 51.47 (min) (rotamers, N-*C*H$_2$-COOC(CH$_3$)$_3$), 66.68 (min) and 66.99 (maj) (rotamers, CH Fmoc-C*H*$_2$O), 68.77 (maj) and 68.82 (min) (rotamers, H*C*≡C), 83.09 (maj) and 83.15 (min) (rotamers, O-(*C*H$_3$)$_3$), 119.90 (maj) and 119.94 (min) (rotamers, *C*H Fmoc arom), 125.00 (maj) and 125.08 (min) (rotamers, *C*H Fmoc arom), 127.00 and 127.04 (rotamers, *C*H Fmoc arom), 127.62 (min) and 127.68 (maj) (rotamers, *C*H Fmoc arom), 141.25 (rotamers, br, *C* Fmoc arom), 143.84 (maj) and 143.94 (min) (rotamers, *C* Fmoc arom), 157.11 (NH*C*OO), 168.52 (maj) and 169.50 (min) (rotamers, *C*OOC(CH$_3$)$_3$, 171.52 (maj) and 172.30 (min) (rotamers, CH$_2$-*C*ON). ESI-MS (m/z): 499.2 $[M+Na]^+$ (100%).

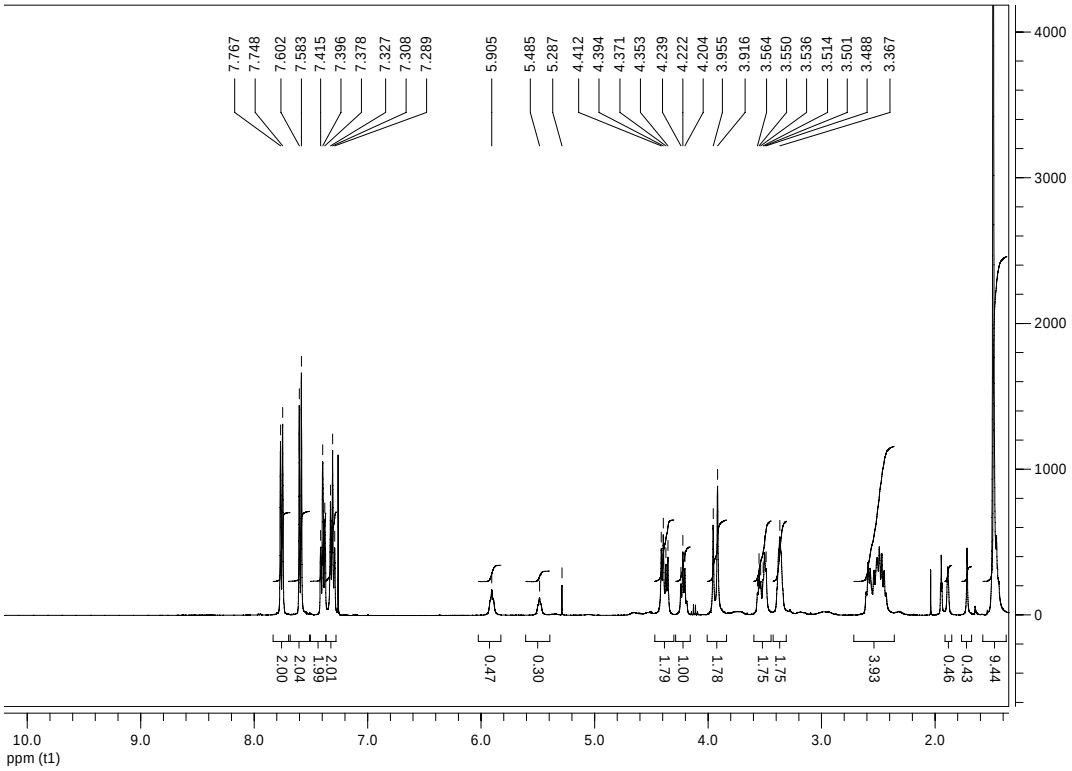

Figure 1: ^{1}H NMR of **Fmoc-1-O^{t}Bu**.

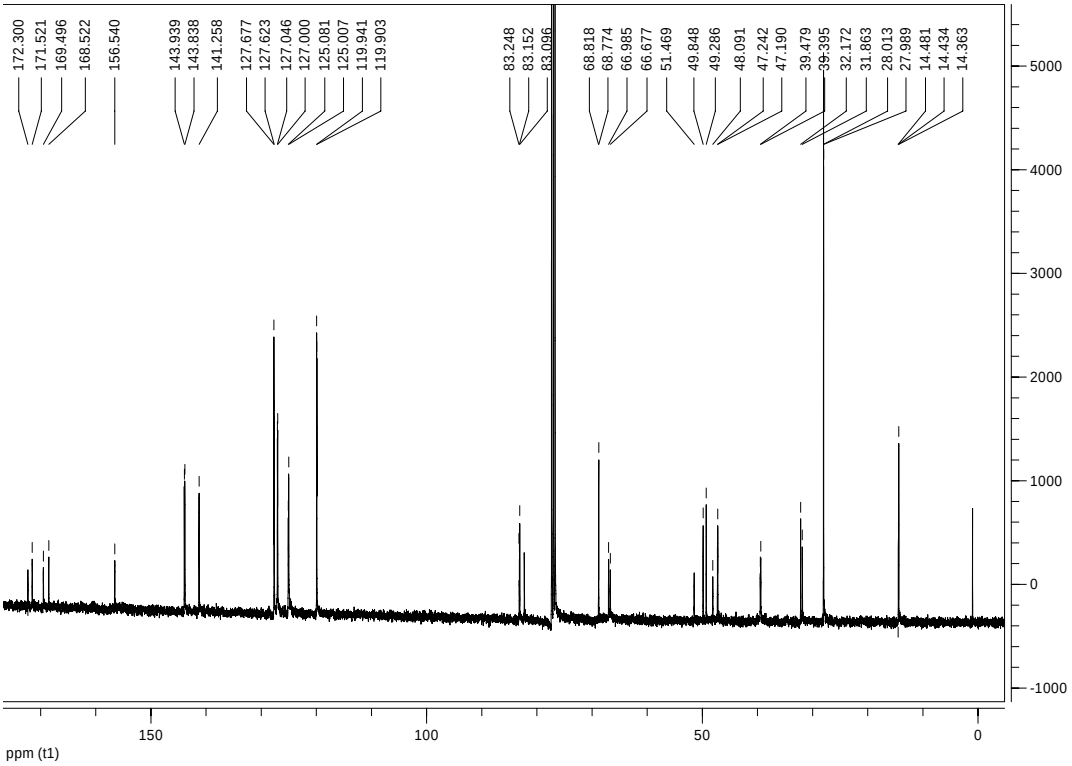

Figure 2: ^{13}C NMR of **Fmoc-1-O^{t}Bu**.

2-(*N*-(2-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)ethyl)pent-4-ynamido)acetic acid (Fmoc-1-OH). To a cold (0°C), stirring suspension of **Fmoc-1-O^{t}Bu** (0.63 g, 1.32 mmol) in CH_2Cl_2 (11.2 mL) was cautiously added TFA (5.46 mL) and triethylsilane (2.17 mL). After 30 minutes, the mixture was allowed to warm to room temperature and stirred for another 4h. The solvent was then removed under reduced pressure to give a colourless oil which was purified by column chromatography on silica with ethyl acetate:methanol 10:3 as the eluent (Rf = 0.18). The combined fractions were concentrated to approximately one hundredth of the original volume and cold hexane was added to the colourless oil to precipitate the acid. The resulting solids were collected by filtration and dried in vacuo to give 2-(*N*-(2-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)ethyl)pent-4-ynamido)acetic acid (**Fmoc-1-OH**) as an amorphous white solid. Yield. 0.55 g (89 %).

Characterisation Data of Fmoc-1-OH. IR bands (ν, cm^{-1}): 3297 w, 2944 w, 1700 m, 1628 m, 1525 m, 1477 m, 1448 m, 1245 m, 1186 s, 1140 s, 1104 m, 1007 m, 836 m, 798 m, 759 s, 740 s, 622 s. ^{1}H NMR Spectrum (*d*6-Acetone): δ 2.29 (rotamers, m, 1H, *H*C≡C), 2.38-2.70 (m, 4H, HC≡C-C*H*$_2$ and CH$_2$-C*H*$_2$CO), 3.28-3.44 (m, 2H, NH-C*H*$_2$-CH$_2$), 3.50-3.62 (m, 2H, CH$_2$-C*H*$_2$-N), 4.11 (min) and 4.28 (maj) (rotamers, s, 2H, N-C*H*$_2$-COOH), 4.23 (m, 1H, C*H* Fmoc-CH$_2$O), 4.33 (min) and 4.36 (maj) (rotamers, m, 2H, CH Fmoc-C*H*$_2$O), 6.30 (min) and 6.50 (maj) (rotamers, br s, 1H, CH$_2$-N*H*-COO), 7.33 (m, 2H, C*H* Fmoc arom), 7.40 (m, 2H, C*H* Fmoc arom), 7.67 (m, 2H, C*H* Fmoc arom), 7.85 (m, 2H, C*H* Fmoc arom). ^{13}C NMR Spectrum (*d*6-Acetone): δ 15.72 (min) and 15.88 (maj) (rotamers, HC≡C-*C*H$_2$), 33.46 (maj) and 33.80 (min) (rotamers, CH$_2$-*C*H$_2$-CO), 39.57 (min) and 40.74 (maj) (NH-*C*H$_2$-CH$_2$), 49.03 (maj) and 49.79 (min) (rotamers, CH$_2$-*C*H$_2$-N), 49.38 (maj) and 51.44 (min) (rotamers, N-*C*H$_2$-COOH), 67.98 (min) and 68.01 (maj) (rotamers, CH Fmoc-C*H*$_2$O), 67.98 (min) and 71.01 (maj) (rotamers, H*C*≡C), 85.20 (min) and 85.27 maj) (rotamers, HC≡*C*-CH$_2$), 121.79 (br, rotamers, *C*H Fmoc arom), 126.99 (maj) and 127.08 (min) (rotamers, *C*H Fmoc arom),

128.93 (br, rotamers, *C*H Fmoc arom), 129.50 (br, rotamers, *C*H Fmoc arom), 143.10 (rotamers, br, *C* Fmoc arom), 146.14 (maj) and 146.23 (min) (rotamers, *C* Fmoc arom), 158.20 (min) and 158.28 (maj) (rotamers, NH*C*OO), 172.47 (maj) and 172.61 (min) (rotamers, CH_2-*C*ON), 173.02 (*C*OOH). ESI-MS (m/z): 419.1 $[M-H]^-$ (59 %), 533.1 $[M+TFA+H]^-$ (100 %).

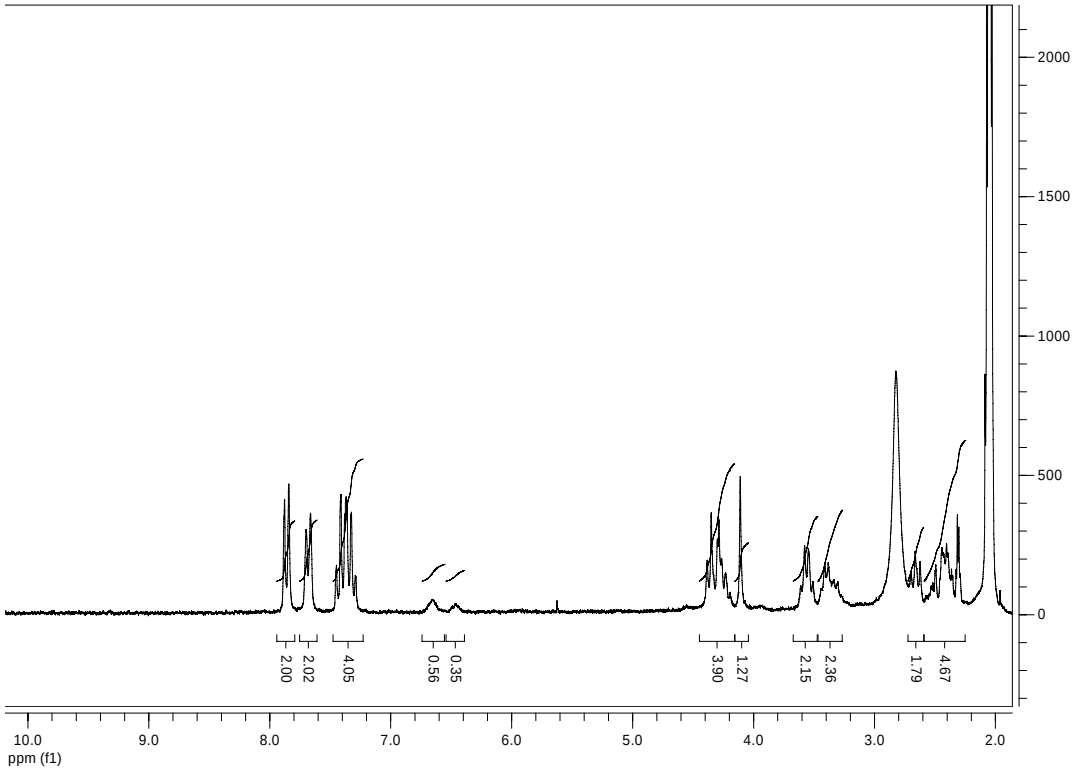

Figure 3: ^{1}H NMR of **Fmoc-1-OH**.

***tert*-butyl 2-(*N*-(2-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)ethyl)-3-(1-ferrocenyl-1*H*-1,2,3-triazol-4-al)propanimido)acetate (Fmoc-2-O^{t}Bu).** To a solution of azidoferrocene (25.0 mg, 0.11 mmol) and **Fmoc-1-O^{t}Bu** (52.4 mg, 0.11 mmol) in acetone (880 μL) and H_2O (440 μL) was added $CuSO_4{\cdot}5H_2O$ (10 μL of a 0.11 M solution in water) and sodium ascorbate (11 μL of a freshly prepared 1M solution in water). The mixture was stirred for 1 day at room temperature before the solvents were evaporated. CH_2Cl_2 (20 mL) and H_2O (10 mL) were then added to the residual oil and the organic phase extracted, washed with H_2O (10 mL),

dried over Na_2SO_4 and evaporated to dryness to give a pale yellow oil. Purification by column chromatography on silica with ethyl acetate:hexane 3:2 as the eluent was performed to give *tert*-butyl 2-(*N*-(2-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)ethyl)-3-(1-ferrocenyl-1*H*-1,2,3-triazol-4-al)propanimido)acetate (**Fmoc-2-O^{t}Bu**) as an orange solid (Rf=0.13). Yield. 44 mg (57 %).

Characterisation Data of Fmoc-2-O^{t}Bu. IR bands (ν, cm^{-1}): 2962 w, 2928 w, 1717 br m, 1647 m, 1515 m, 1448 m, 1367 m, 1259 s, 1246 s, 1229 s, 1152 s, 1104 s, 1041 s, 1019 s, 877 m, 801 s, 759 s,, 741 s, 667 m, 656 m, 619 s. ^{1}H NMR Spectrum ($CDCl_3$): δ 1.47 (maj) and 1.50 (min) (rotamers, s, 9H, O-C(C*H*$_3$)$_3$), 2.67 (min) and 2.80 (maj) (rotamers, m, 2H, C*H*$_2$-CH$_2$-triazol ring), 3.09 (m, 2H, CH$_2$-C*H*$_2$-triazol ring), 3.37 (m, 2H, NH-C*H*$_2$-CH$_2$), 3.53 (m, 2H, CH$_2$-C*H*$_2$-N), 3.93 (maj) and 3.99 (min) (rotamers, s, 2H, N-C*H*$_2$-COOC(CH$_3$)$_3$), 4.10-4.23 (m, 8H, 7 C*H* Fc and C*H* Fmoc), 4.33 (CH Fmoc-C*H*$_2$O), 4.72 (maj) and 4.77 (min) (rotamers, m, 2H, C*H* Fc), 5.55 (min) and 5.95 (maj) (rotamers, br s, 1H, CH$_2$-N*H*-COO), 7.30 (m, 2H, C*H* Fmoc arom), 7.39 (m, 2H, C*H* Fmoc arom), 7.45 (maj) and 7.59 (min) (rotamers, s, 1H, C*H* triazol ring), 7.59 (m, 2H, C*H* Fmoc arom), 7.75 (m, 2H, C*H* Fmoc arom). ^{13}C NMR Spectrum ($CDCl_3$): δ 20.95 (min) and 20.99 (maj) (rotamers, CH$_2$-*C*H$_2$-triazol ring), 27.99 (min) and 28.05 (maj) (rotamers, OC(*C*H$_3$)$_3$), 32.13 (maj) and 32.26 (min) (rotamers, *C*H$_2$-CH$_2$-triazol ring), 39.42 (maj) and 39.53 (min) (rotamers, NH-*C*H$_2$-CH$_2$), 46.95 (maj) and 47.07 (min) (rotamers, *C*H Fmoc-CH$_2$O), 48.36 (min) and 49.34 (maj) (CH$_2$-*C*H$_2$-N), 49.79 (maj) and 51.45 (min) (rotamers, N-*C*H$_2$-COOC(CH$_3$)$_3$, 61.81 (maj) and 61.92 (rotamers, C*H* Fc), 66.39 (maj) and 66.48 (min) (rotamers, C*H* Fc), 66.73 (min) and 67.08 (maj) (rotamers, CH Fmoc-*C*H$_2$O), 70.01 (min) and 70.05 (maj) (rotamers, C*H* Fc), 82.22 (min) and 83.09 (maj) (rotamers, O-(*C*H$_3$)$_3$), 93.83, 119.69 (min) and 119.91 (maj) (rotamers, *C*H Fmoc arom), 120.96 (maj) and 121.17 (min) (rotamers, *C*H triazol ring), 125.16 (rotamers, br, *C*H Fmoc arom), 127.01 (maj) and 127.09 (min) (rotamers, *C*H Fmoc arom),

127.61 (min) and 127.67 (maj) (rotamers, *C*H Fmoc arom), 141.21 (maj) and 141.24 (min) (rotamers, *C* Fmoc arom), 143.88 (min) and 143.97 (min) (rotamers, *C* Fmoc arom), 146.70 (*C* triazol ring), 156.49 (maj) and 156.56 (min) (rotamers, NH*C*OO), 168.84 (maj) and 169.54 (min) (rotamers, *C*OOC$(CH_3)_3$), 172.50 (maj) and 173.34 (min) (rotamers, CH_2-*C*ON). ESI-MS (m/z): 703.2 $[M]^+$ (77 %), 726.2 $[M+Na]^+$ (100 %).

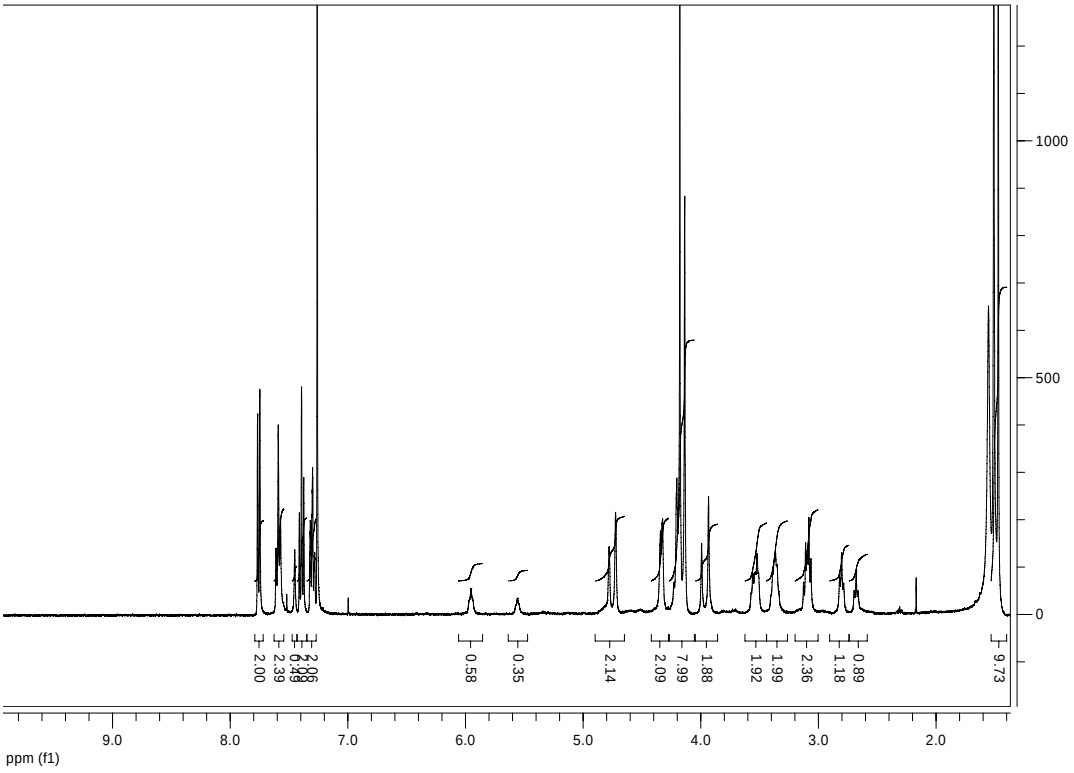

Figure 4: ^{1}H NMR of **Fmoc-2-O^{t}Bu**.

Azidoferrocene (3). Azidoferrocene was synthesised as previously reported.[2] Surprisingly, no NMR or IR data are available to the best of our knowledge. Therefore, they are presented herein.

Characterisation Data of 3. IR bands (ν, cm^{-1}): 3095 w, 2925 w, 2105 s, 1457 s, 1409 m, 1374 w, 1285 s, 1163 w, 1105 s, 1022 m, 1000 s, 917 m, 813 s, 741 m. ^{1}H NMR Spectrum ($CDCl_3$): δ 4.09 (m, 2H, C*H* Fc), 4.30 (s, 5H, C*H* Fc), 4.33 (m, 2H, C*H* Fc). ^{13}C NMR Spectrum ($CDCl_3$): δ 62.36 (*C*H Fc), 67.19 (*C*H Fc), 70.80 (*C*H Fc), 100.19 (*C* Fc).

e) Characterisation of PNA1-4.

Table 1. Characterisation of the PNA sequences synthesised in this study.

PNA Code	$[M+H]^+$ found[a]	$[M+H]^+$ calculated
PNA1	3125.1	3123.3
PNA2	3126.1	3123.3
PNA3	3126.9	3123.3
PNA4	3305.3	3303.4

[a] measured by MALDI-TOF MS

f) Synthesis and characterisation of Fc-PNA1-3 and Fc_2-PNA4.

General procedure for the Click Chemistry between PNA and azidoferrocene (Fc-PNA1-3 and Fc_2PNA4).

The synthon **1** containing PNA resin was shrunk with methanol, dried over vacuum and then transferred into a fritted syringe. The resin was then swollen with DMF for 1h. Azidoferrocene (1.5 eq.) and CuI (2.5 eq.) was then introduced into the syringe (from the top). Afterwards, a mixture of ethyldiisopropylamine (150 eq.) and DMF (100 µl per µmol of resin) were aspired up the syringe and the mixture was shaken for 2 days at room temperature in the absence of light and under an argon atmosphere. The resin was then washed with DMF (5x), CH_2Cl_2 (5x) and DMF (5x) successively. See above (section I.c) for the details of the subsequent cleavage of the resin.

Note that the quantities stated above were logically doubled for the synthesis of **Fc_2-PNA4**.

Table 2. Characterisation of the PNA "clicked" sequences.

PNA Code	$[M+H]^+$ found[a]	$[M+H]^+$ calculated
Fc-PNA1	3350.8	3350.3
Fc-PNA2	3352.7	3350.3
Fc-PNA3	3351.3	3350.3
Fc_2-PNA4	3762.3	3757.4

[a] measured by MALDI-TOF MS

II. MALDI-TOF mass spectra

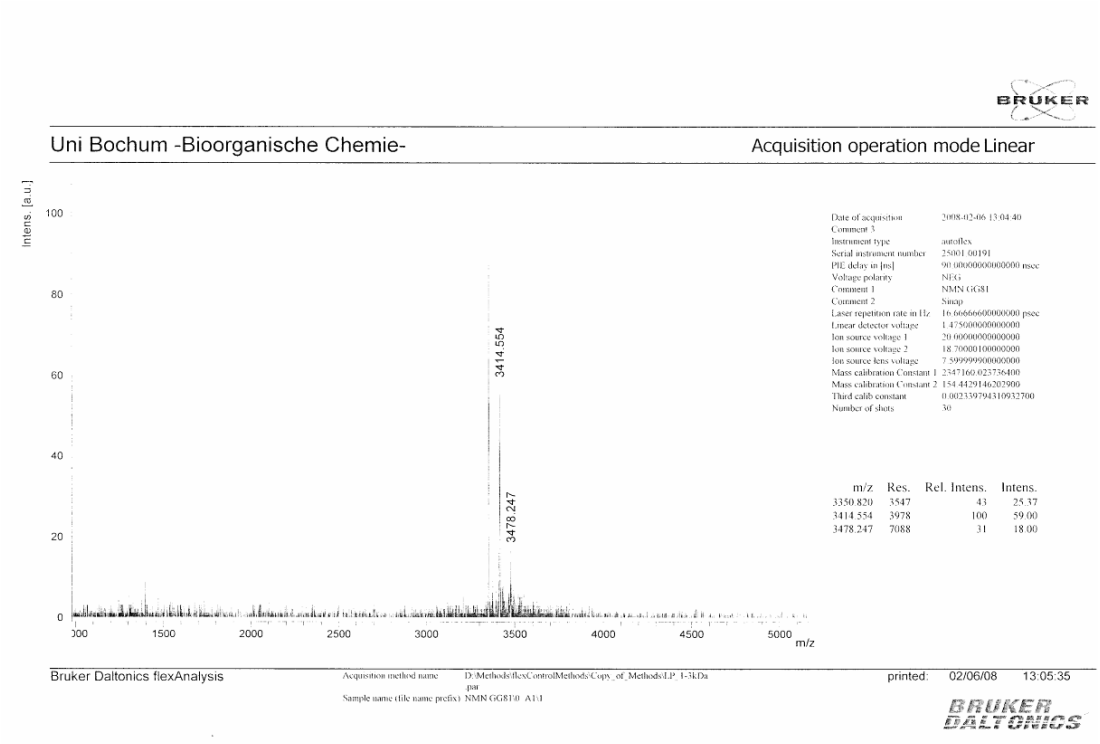

Figure 5: MALDI-TOF mass spectrum of Fc-PNA1.

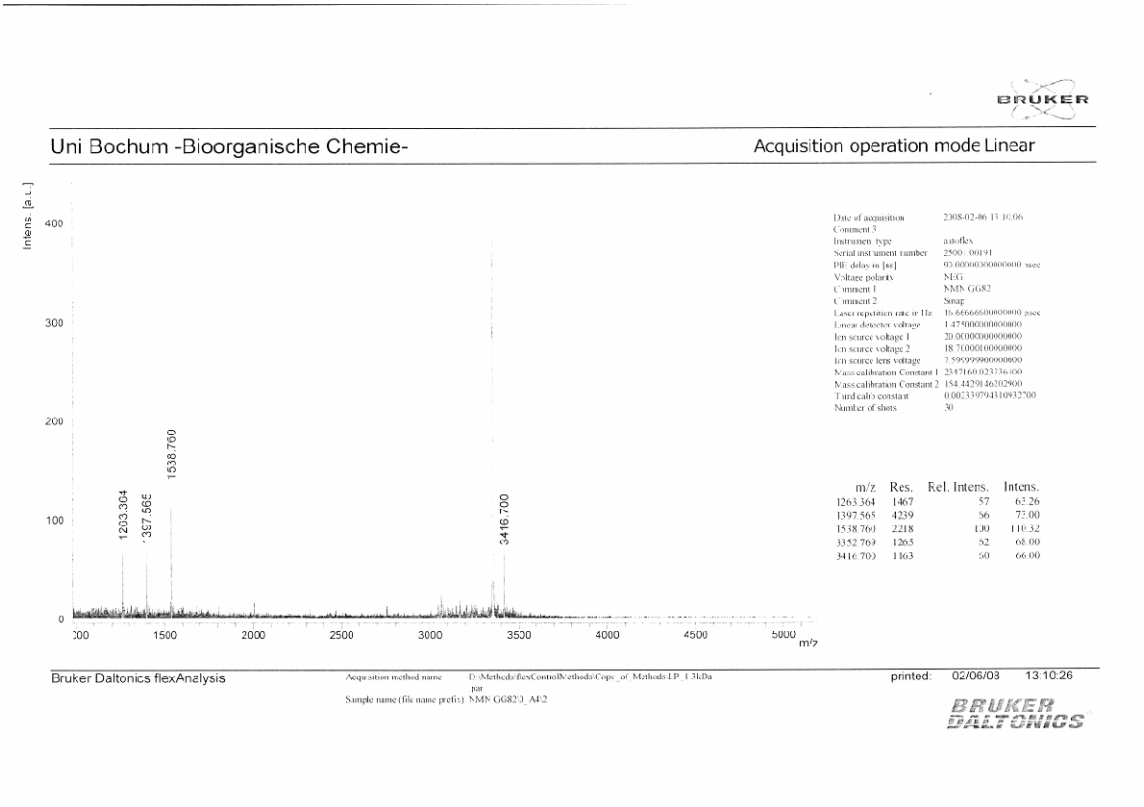

Figure 6: MALDI-TOF mass spectrum of Fc-PNA2.

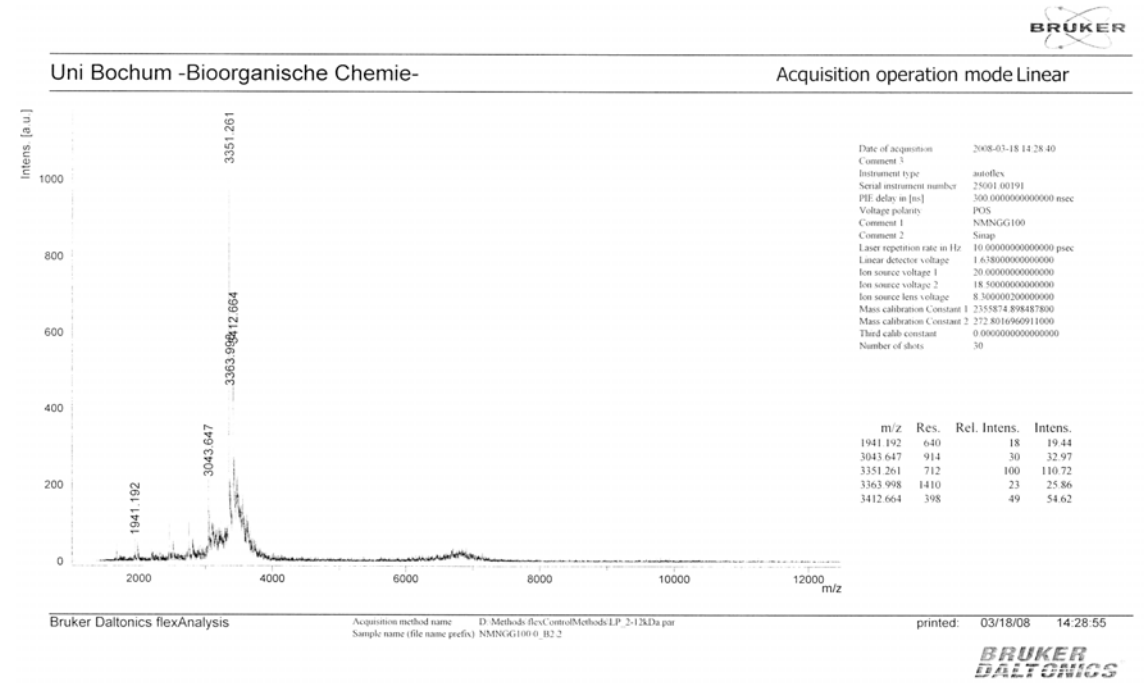

Figure 7: MALDI-TOF mass spectrum of Fc-PNA3.

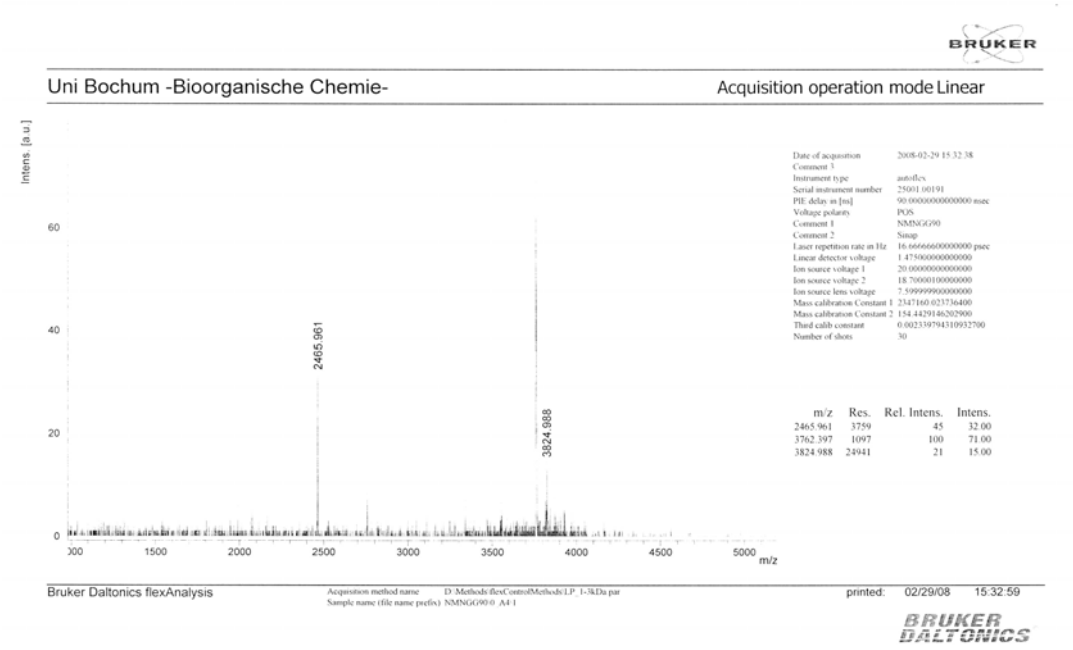

Figure 8: MALDI-TOF mass spectrum of Fc_2-PNA4.

III. References

1. Thomson, S. A.; Josey, J. A.; Cadilla, R.; Gaul, M. D.; Hassman, C. F.; Luzzi, M. J.; Pipe, A. J.; Reed, K. L.; Ricca, D. J.; Wiethe, R. W.; Noble, S. A., *Tetrahedron* **1995,** 51, (22), 6179-6194.

2. Sheinker, Y. N.; Senyavina, L. B.; Zheltova, V. N. D. A. N. S., 160(6), 1339-42., *Dokl. Akad. Nauk SSSR* **1965,** 160, (6), 1339-1342.