Supplementary Information:

Dual end-functionalisation of poly(beta-amino ester) gene delivery vectors using multicomponent chemistry

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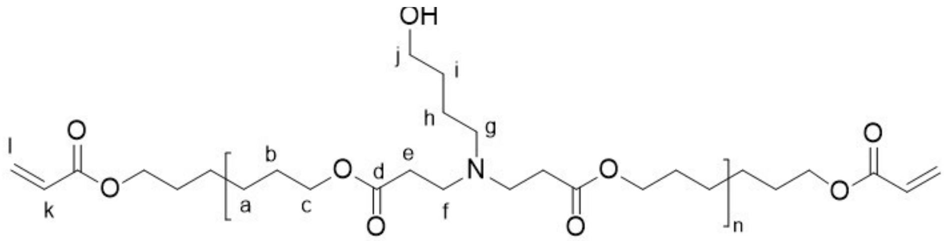
List of Figures:

Polymer Characterisation

pHDD-4AB

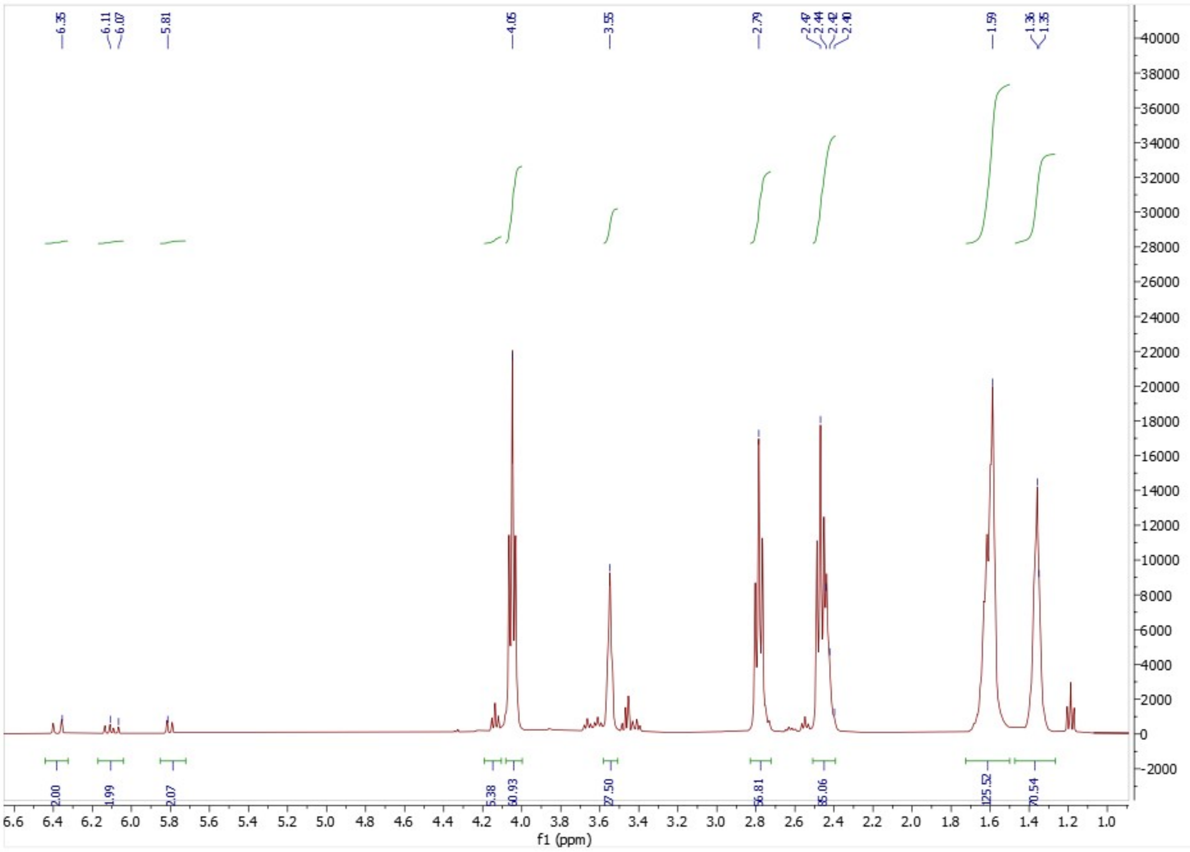


^{1}H NMR (400 MHz, $CDCl_3$) δ/ppm: δ 6.36 (d, J = 17.83 Hz, 2H, $CH_{k,\,l}$), 6.11-6.07 (dd, J = 16.54, 8.64 Hz, 2H, $CH_{k,\,l}$), 5.81 (d, J = 9.93 Hz, 2H, $CH_{k,\,l}$), 4.05 (t, J = 6.69 Hz, 60H, CH_c), 3.55 (m, 30H, CH_j), 2.79 (t, J = 7.39 Hz, 60H, CH_e), 2.44 (m, 90H, $CH_{f,\,g}$), 1.59 (m, 120H, CH_b, $CH_{h,\,i}$), 1.35 (m, 60H, CH_a)

^{13}C NMR (101 MHz, $CDCl_3$) δ/ppm: δ 172.43 (d); 130.57, 128.56 (k, l); 64.50, 62.57 (c, j); 53.99, 48.87 (f, g); 31.79 (e); 28.48, 25.59, 24.98, 15.27 (a, b, h, i)

ν_{max}/cm^{-1}: 3445 (OH alcohol), 1725 (C=O)

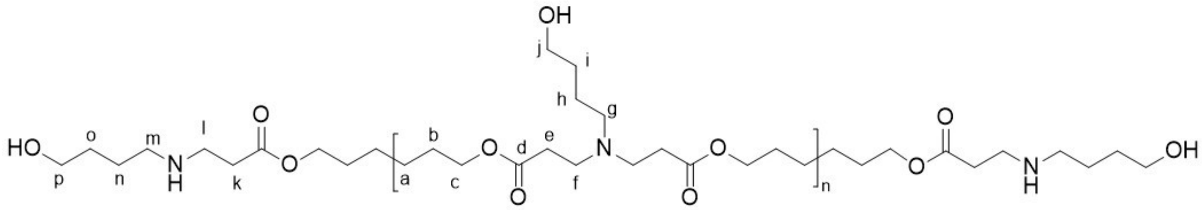
M_n, SEC: *Mn* 10900, *Đ* = 1.22



Spectrum S1: ^{1}H NMR (400 MHz, $CDCl_3$-d1) of pHDD-4AB

Exemplar spectra are included.

OH-(pHDD-4AB)-OH

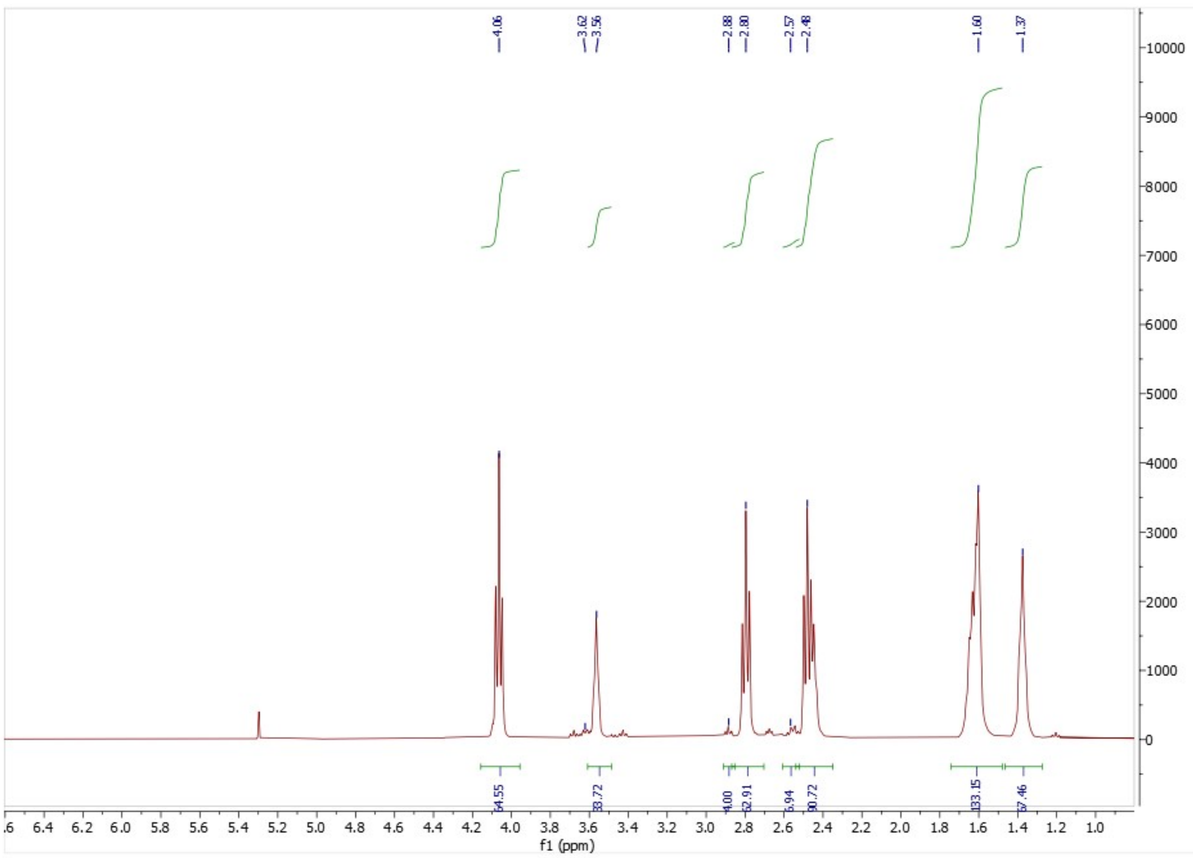


^{1}H NMR (400 MHz, $CDCl_3$) δ/ppm: δ 4.06 (t, J = 6.61 Hz, 60H, CH_c), 3.62 (m, 4H, CH_p), 3.56 (m, 30H, CH_j), 2.88 (t, J = 6.20 Hz, 4H, CH_k), 2.80 (t, J = 7.30 Hz, 60H, CH_e), 2.57 (m, 8H, $CH_{l,\,m}$), 2.48 (m, 90H, $CH_{f,\,g}$), 1.60 (m, 128H, $CH_{b,\,h,\,i,\,n,\,o}$), 1.37 (m, 60H, CH_a)

^{13}C NMR (101 MHz, $CDCl_3$) δ/ppm: δ 172.42 (d); 64.30, 62.12 (c, j, p); 53.68, 48.83 (f, g, I, m); 31.94, 31.10 (e, k); 28.34, 25.45, 24.36 (a, b, h, i, n, o)

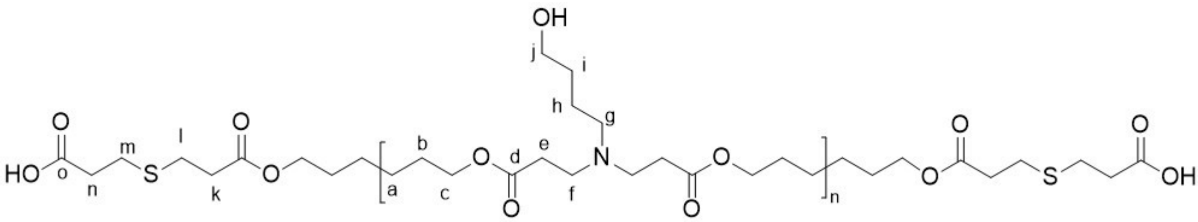
ν_{max}/cm^{-1}: 3443 (OH, alcohol), 1729 (C=O, ester)

M_n, SEC: Mn 12900, $Đ$ = 1.24



Spectrum S2: ^{1}H NMR (400 MHz, $CDCl_3$-d1) of OH-(pHDD-4AB)-OH

COOH-(pHDD-4AB)-COOH

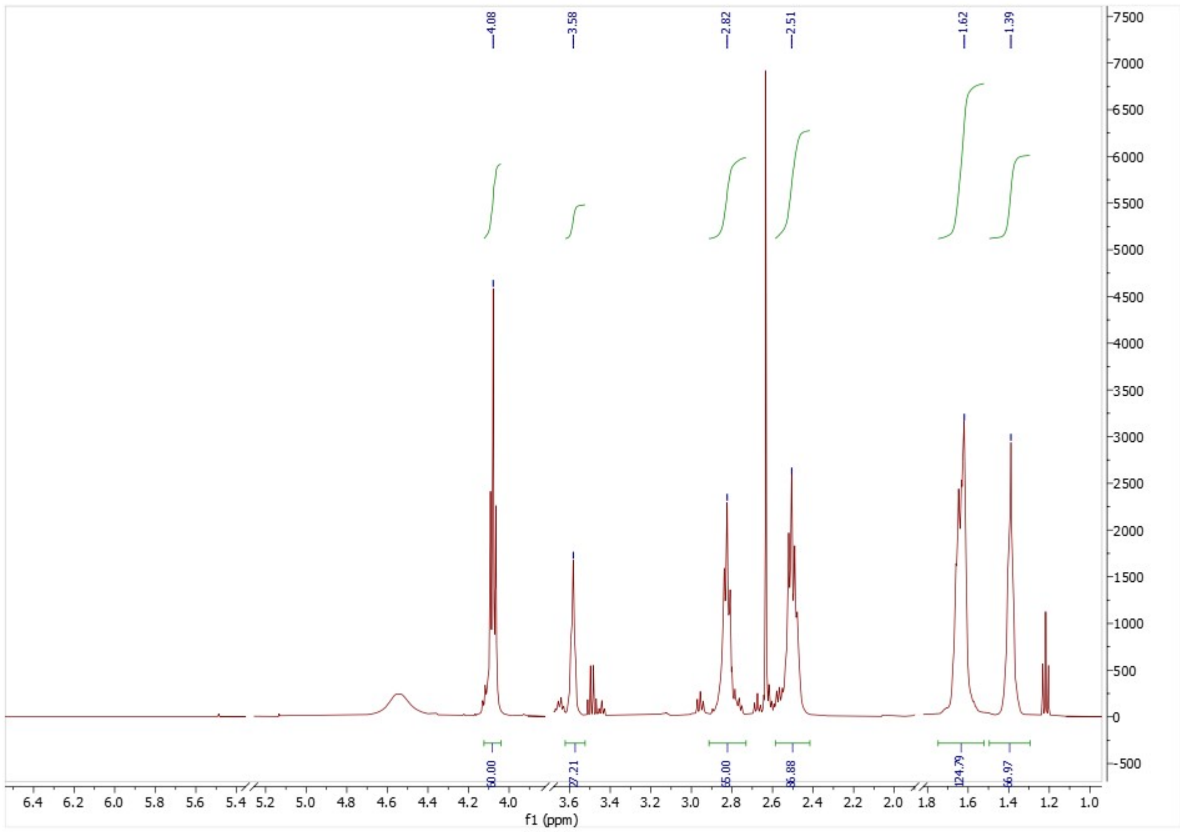


^{1}H NMR (400 MHz, $CDCl_3$) δ/ppm: δ 4.08 (t, J = 6.57 Hz, 60H, CH_c), 3.58 (m, 30H, CH_j), 2.82 (m, 72H, $CH_{e, k, m, n}$), 2.51 (m, 94H, $CH_{f, g, l}$), 1.62 (m, 120H, $CH_{b, h, i}$), 1.39 (m, 60H, CH_a)

^{13}C NMR (101 MHz, $CDCl_3$) δ/ppm: δ 199.83 (o); 172.42 (d); 77.24 (k, n); 70.98 (l, m); 64.58, 62.45 (c, j); 53.87 (g); 48.79 (f); 34.96 (e); 28.47 (b); 25.72, 25.58, 25.35 (a, h, i)

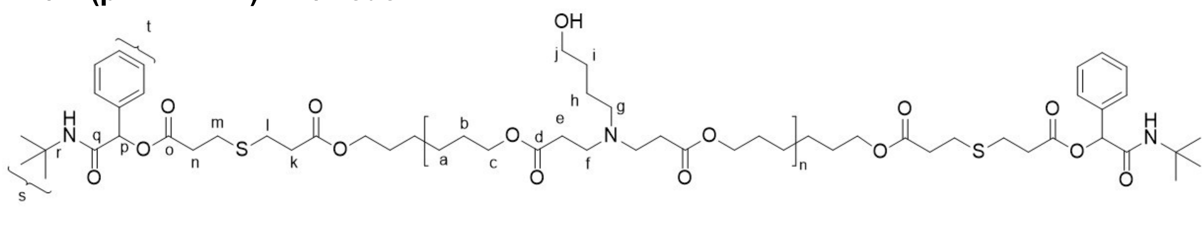
ν_{max}/cm^{-1}: 2934 (COOH), 2861 weak (OH alcohol), 1727 (C=O), 1179 (C-N, amine)

M_n, SEC: *Mn* 12000, *Đ* = 1.26



Spectrum S3: ^{1}H NMR (400 MHz, $CDCl_3$-d1) of COOH-(pHDD-4AB)-COOH

AI1/I1-(pHDD-4AB)-AI1/I1cdcl1



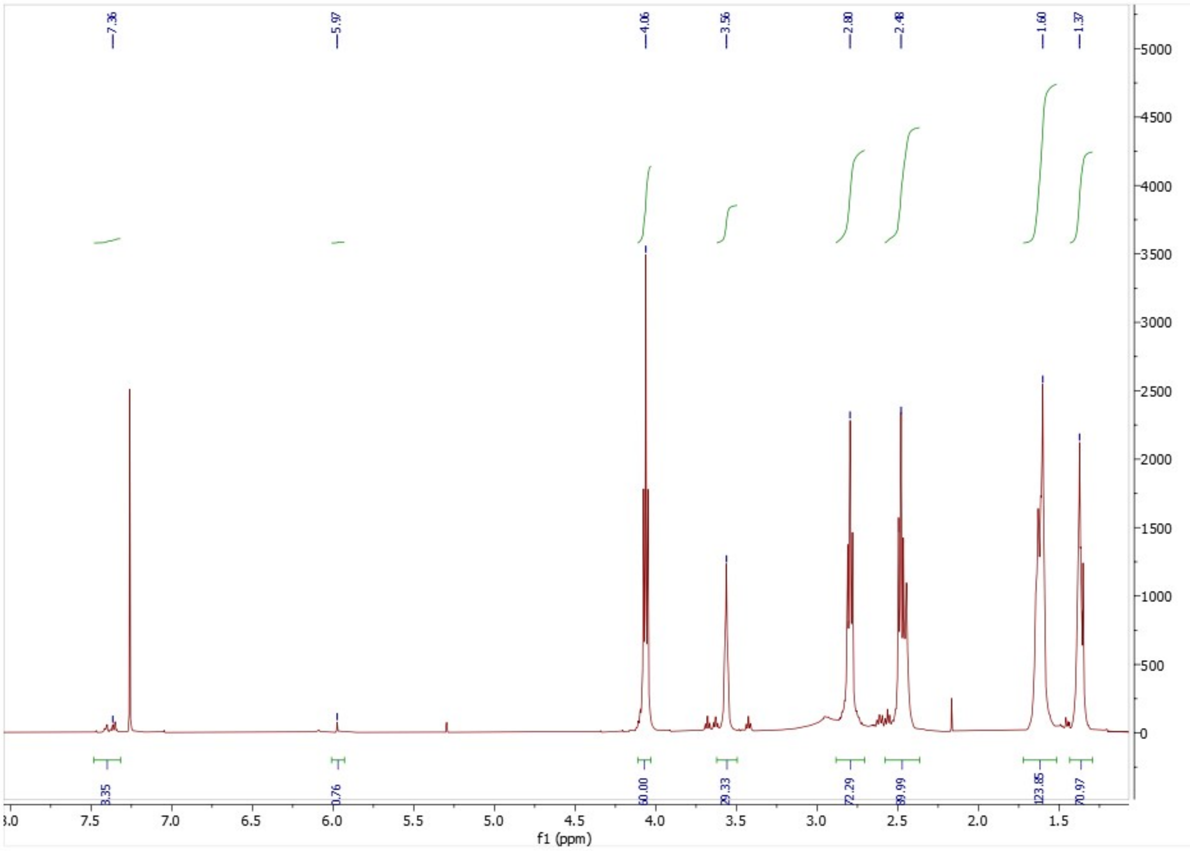
^{1}H NMR (400 MHz, $CDCl_3$) δ/ppm: δ 7.36 (m, 3H, CH_t), 5.97 (m, 0.76H, CH_p), 4.06 (t, J = 6.23 Hz, 60H, CH_c), 3.56 (m, 30H, CH_j), 2.80 (t, J = 7.04 Hz, 72H, $CH_{e, k, m, n}$), 2.48 (m, 94H, $CH_{f, g, l}$), 1.60 (m, 120H, $CH_{b, h, i}$), 1.37 (m, 71H, $CH_{a, s}$)

^{13}C NMR (101 MHz, $CDCl_3$) δ/ppm: δ 172.32 (d, o, q); 128.02, 122.97, 121,25 (t); 111.07 (p); 64.57 (c, r); 62.78 (j); 53.71 (g); 48.83 (f); 34.78, 32.52, 31.58 (e, k, n); 28.48 (b, l, m); 25.73, 25.58, 25.32 (a, h, i, s)

ν_{max}/cm^{-1}: 3366 (OH, alcohol), 2835 (NH, amine salt), 1726 (C=O, ester)

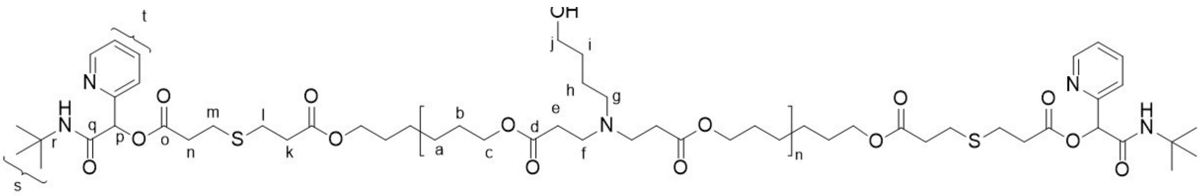
M_n, SEC: Mn 10200, $Đ$ = 1.23

% P3CR conversion as determined by proton NMR: 38%



Spectrum S4: ^{1}H NMR (400 MHz, $CDCl_1$-d1) of AI1/I1-(pHDD-4AB)-AI1/I1

AI2/I1-(pHDD-4AB)-AI2/I1



^{1}H NMR (400 MHz, $CDCl_3$) δ/ppm: δ 7.75-7.54 (m, 4H, CH_t), 6.04 (m, 0.96H, CH_p), 4.06 (t, J = 6.67 Hz, 60H, CH_c), 3.56 (m, 30H, CH_j), 2.79 (t, J = 7.15 Hz, 72H, $CH_{e, k, m, n}$), 2.48 (m, 94H, $CH_{f, g, l}$), 1.60 (m, 120H, $CH_{b, h, i}$), 1.37 (m, 60H, CH_a), 1.32 (s, 8.72H, CH_s)

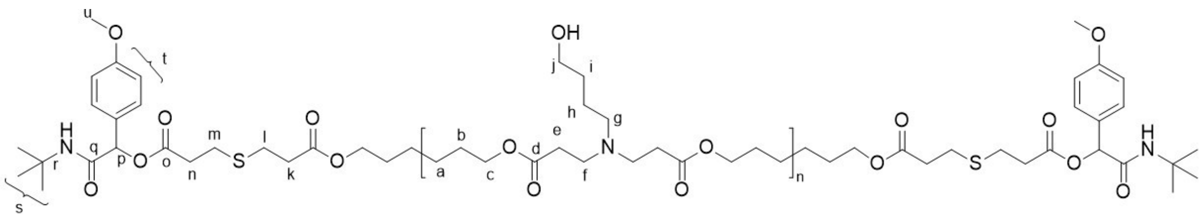
^{13}C NMR (101 MHz, $CDCl_3$) δ/ppm: δ 172.18, 167.24 (d, o, q); 129.30, 127.84 (t); 102.96 (p); 64.63 (c, r); 62.42 (j); 53.38 (g); 48.82 (f); 34.92, 31.34 (e, k, n); 28.48, 28.47 (b, l, m); 26.26, 25.57 (a, h, i, s)

ν_{max}/cm^{-1}: 3340 (OH, alcohol), 2837 (NH, amine salt), 1728 (C=O, ester)

M_n, SEC: *Mn* 11800, *Đ* = 1.27

% P3CR conversion as determined by proton NMR: 48%

AI3/I1-(pHDD-4AB)-AI3/I1



^1H NMR (400 MHz, $CDCl_3$) δ/ppm: δ 7.31, 6.89 (m, 3H, CH_t), 5.93 (m, 0.72H, CH_p), 4.06 (t, J = 6.49 Hz, 60H, CH_c), 3.79 (s, 2.21H, CH_u), 3.56 (m, 30H, CH_j), 2.80 (t, J = 7.19 Hz, 72H, $CH_{e, k, m, n}$), 2.48 (m, 94H, $CH_{f, g, l}$), 1.60 (m, 120H, $CH_{b, h, i}$), 1.37 (m, 67H, $CH_{a, s}$)

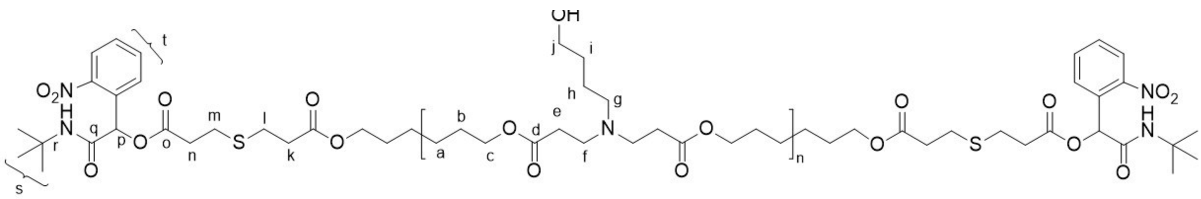
^{13}C NMR (101 MHz, $CDCl_3$) δ/ppm: δ 172.19, 166.89 (d, o, q); 120.81, 118.26 (t); 93.88 (p); 64.54 (c, r); 62.26 (j); 53.70 (g, u); 48.80 (f); 39.04, 33.98, 31.50 (e, k, n); 28.40 (b, l, m); 25.71, 25.51 (a, h, i, s)

ν_{max}/cm^{-1}: 3397 (OH, alcohol), 2937 (NH, amine salt), 1726 (C=O, ester)

M_n, SEC: *Mn* 11200, *Đ* = 1.37

% P3CR conversion as determined by proton NMR: 36%

AI4/I1-(pHDD-4AB)-AI4/I1



^1H NMR (400 MHz, $CDCl_3$) δ/ppm: δ 7.99, 7.68, 7.51 (m, 6H, CH_t), 6.37 (m, 1.59H, CH_p), 4.06 (t, J = 6.39 Hz, 60H, CH_c), 3.56 (m, 30H, CH_j), 2.80 (t, J = 7.15 Hz, 72H, $CH_{e, k, m, n}$), 2.48 (m, 94H, $CH_{f, g, l}$), 1.60 (m, 120H, $CH_{b, h, i}$), 1.37 (m, 74H, $CH_{a, s}$)

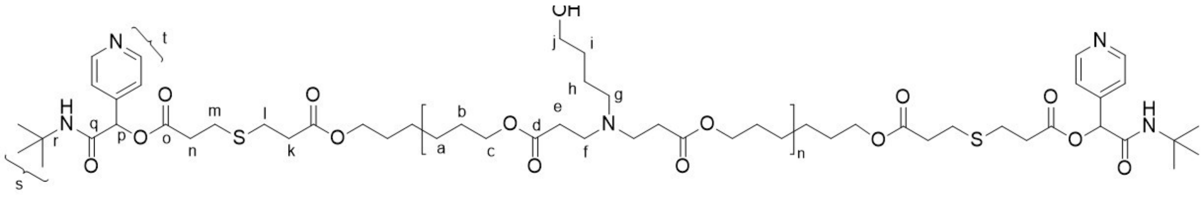
^{13}C NMR (101 MHz, $CDCl_3$) δ/ppm: δ 172.14, 169.48 (d, o, q); 134.06, 129.43, 124.50 (t); 109.00 (p); 68.30 (r); 64.56 (c); 62.65 (j); 53.98 (g); 48.85 (f); 32.69, 31.71 (e, k, n); 28.58, 28.49 (b, l, m); 26.45, 25.59 (a, h, i, s)

ν_{max}/cm^{-1}: 3398 (OH, alcohol), 2937 (NH, amine salt), 1726 (C=O, ester)

M_n, SEC: *Mn* 12700, *Đ* = 1.45

% P3CR conversion as determined by proton NMR: 80%

AI5/I1-(pHDD-4AB)-AI5/I1



^{1}H NMR (400 MHz, $CDCl_3$) δ/ppm: δ 8.62, 7.36 (m, 5H, CH_t), 5.96 (m, 1.22H, CH_p), 4.06 (t, J = 6.53 Hz, 60H, CH_c), 3.56 (m, 30H, CH_j), 2.80 (t, J = 7.23 Hz, 72H, $CH_{e, k, m, n}$), 2.48 (m, 94H, $CH_{f, g, l}$), 1.60 (m, 120H, $CH_{b, h, i}$), 1.37 (m, 71H, $CH_{a, s}$)

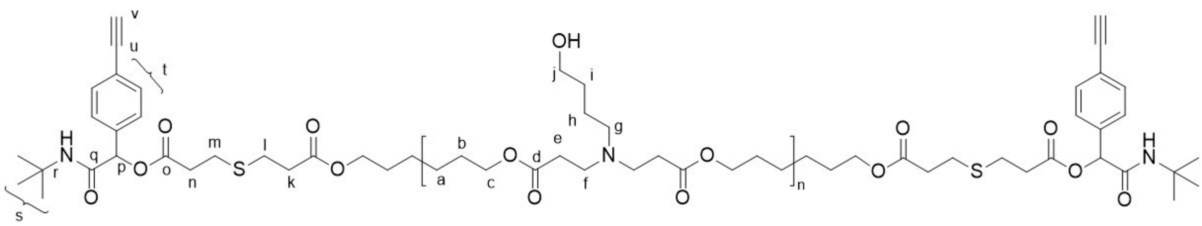
^{13}C NMR (101 MHz, $CDCl_3$) δ/ppm: δ 172.29 (d, o, q); 149.81, 121.62 (t); 90.17 (p); 74.31 (r); 64.41 (c); 62.08 (j); 53.67 (g); 48.80 (f); 39.10, 31.71, 31.02 (e, k, n); 28.50, 28.35 (b, l, m); 25.46, 24.16 (a, h, i, s)

ν_{max}/cm^{-1}: 3407 (OH, alcohol), 2863 (NH, amine salt), 1278 (C=O, ester)

M_n, SEC: *Mn* 8900, *Đ* = 1.48

% P3CR conversion as determined by proton NMR: 61%

AI6/I1-(pHDD-4AB)-AI6/I1



^{1}H NMR (400 MHz, $CDCl_3$) δ/ppm: δ 7.49, 7.39 (m, 3H, CH_t), 5.96 (m, 0.69H, CH_p), 4.06 (t, J = 6.60 Hz, 60H, CH_c), 3.56 (m, 31H, $CH_{j, v}$), 2.80 (t, J = 7.14 Hz, 72H, $CH_{e, k, m, n}$), 2.48 (m, 94H, $CH_{f, g, l}$), 1.60 (m, 120H, $CH_{b, h, i}$), 1.37 (m, 66H, $CH_{a, s}$)

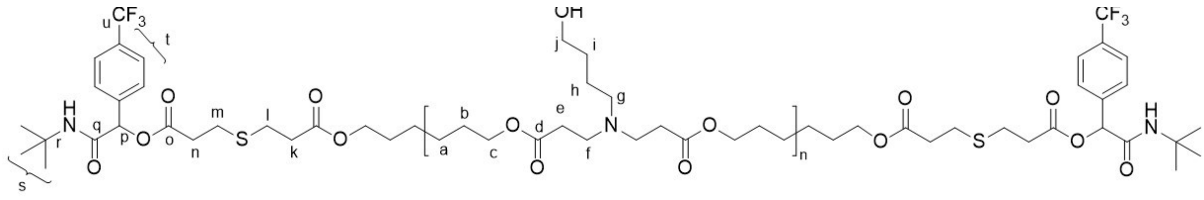
^{13}C NMR (101 MHz, $CDCl_3$) δ/ppm: δ 175.40 (d, o, q); 149.13, 143.36, 135.91, 130.85 (t); 102.25 (p); 85.82 (u, v); 74.66 (r); 65.42 (c); 62.92 (j); 56.33 (g); 48.76 (f); 39.13, 32.69 (e, k, n); 28.41 (b, l, m); 25.54 (a, h, i, s)

ν_{max}/cm^{-1}: 3265 (OH, alcohol), 2861 (NH, amine salt), 1726 (C=O, ester)

M_n, SEC: *Mn* 11100, *Đ* = 1.23

% P3CR conversion as determined by proton NMR: 35%

AI7/I1-(pHDD-4AB)-AI7/I1



^{1}H NMR (400 MHz, $CDCl_3$) δ/ppm: δ 7.64-7.52 (m br, 6H, CH_t), 6.02 (m, 1.41H, CH_p), 4.06 (t, J = 6.55 Hz, 60H, CH_c), 3.56 (m, 30H, CH_j), 2.80 (t, J = 7.23 Hz, 72H, $CH_{e, k, m, n}$), 2.48 (m, 94H, $CH_{f, g, l}$), 1.61 (m, 120H, $CH_{b, h, i}$), 1.38 (m, 73H, $CH_{a, s}$)

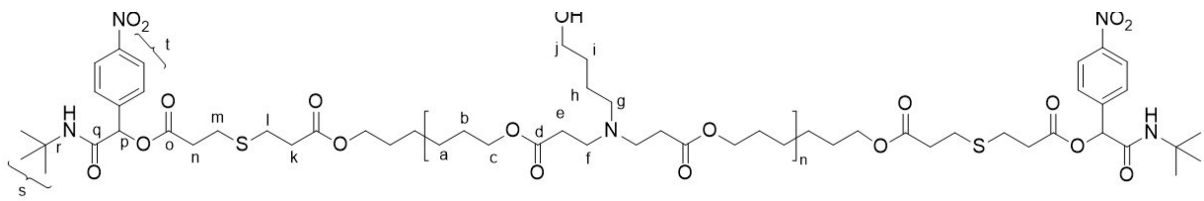
^{13}C NMR (101 MHz, $CDCl_3$) δ/ppm: δ 175.69, 173.66 (d, o, q); 154.48, 145.71, 131.25, 123.36 (t, u); 84.96 (p); 68.81 (r); 63.71 (c); 58.64 (j); 57.10 (g); 46.23 (f); 40.99, 37.00, 35.74 (e, k, n); 27.96 (b, l, m); 25.29 (a, h, i, s)

ν_{max}/cm^{-1}: 3405 (OH, alcohol), 2937 (NH, amine salt), 1726 (C=O, ester)

M_n, SEC: *Mn* 12100, *Đ* = 1.17

% P3CR conversion as determined by proton NMR: 71%

AI8/I1-(pHDD-4AB)-AI8/I1



^{1}H NMR (400 MHz, $CDCl_3$) δ/ppm: δ 8.24, 7.63 (m, 8H, CH_t), 6.06 (m, 1.90H, CH_p), 4.07 (t, J = 6.53 Hz, 60H, CH_c), 3.57 (m, 30H, CH_j), 2.80 (t, J = 7.40 Hz, 72H, $CH_{e, k, m, n}$), 2.48 (m, 94H, $CH_{f, g, l}$), 1.61 (m, 120H, $CH_{b, h, i}$), 1.36 (m, 78H, $CH_{a, s}$)

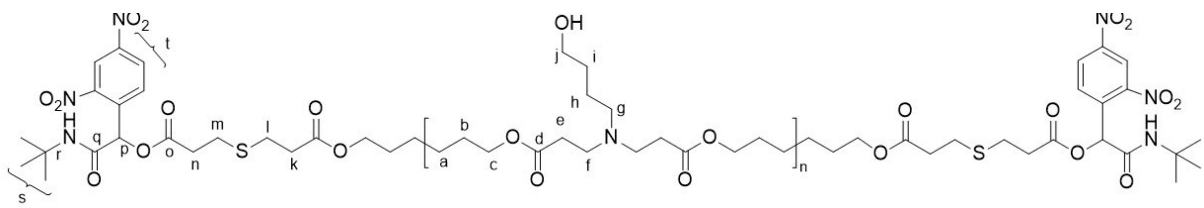
^{13}C NMR (101 MHz, $CDCl_3$) δ/ppm: δ 172.95, 171.89 (d, o, q); 148.06, 127.99, 123.43 (t); 104.26 (p); 70.54 (r); 64.36 (c); 61.55 (j); 53.40 (g); 48.67 (f); 38.43, 33.86, 31.25 (e, k, n); 28.37, 28.21 (b, l, m); 25.32 (a, h, i, s)

ν_{max}/cm^{-1}: 3408 (OH, alcohol), 2937 (NH, amine salt), 1726 (C=O, ester)

M_n, SEC: *Mn* 9000, *Đ* = 1.65

% P3CR conversion as determined by proton NMR: 95%

AI9/I1-(pHDD-4AB)-AI9/I1



^{1}H NMR (400 MHz, $CDCl_3$) δ/ppm: δ 8.64-7.98 (m br, 3H, CH_t), 5.89 (m, 1.04H, CH_p), 4.07 (m, 60H, CH_c), 3.57 (m, 30H, CH_j), 2.84 (m, 72H, $CH_{e, k, m, n}$), 2.53 (m, 94H, $CH_{f, g, l}$), 1.63 (m, 120H, $CH_{b, h, i}$), 1.36 (m, 69H, $CH_{a, s}$)

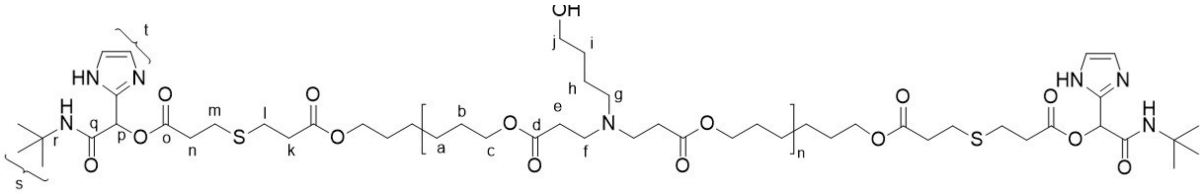
^{13}C NMR (101 MHz, $CDCl_3$) δ/ppm: δ 180.33 (d, o, q); 149.65, 133.39, 130.06, 126.27 (t); 101.02 (p); 69.13 (r); 65.23 (c); 59.73 (j); 53.05 (g); 48.56 (f); 35.89 (e, k, n); 28.23 (b, l, m); 25.51 (a, h, i, s)

ν_{max}/cm^{-1}: 3347 (OH, alcohol), 2937 (NH, amine salt), 1688 (C=O, ester)

M_n, SEC: *Mn* 7500, *Đ* = 1.84

% P3CR conversion as determined by proton NMR: 52%

AI10/I1-(pHDD-4AB)-AI10/I1



^1H NMR (400 MHz, $CDCl_3$) δ/ppm: δ 9.76 (s, 0.47H, NH), 7.34 (s, 0.48H, CH_t), 7.01 (s, 0.49H, CH_t), 4.07 (t, J = 6.68 Hz, 60H, CH_c), 3.56 (m, 30H, CH_j), 2.80 (t, J = 7.38 Hz, 72H, $CH_{e, k, m, n}$), 2.48 (m, 94H, $CH_{f, g, l}$), 1.61 (m, 120H, $CH_{b, h, i}$), 1.37 (m, 64H, $CH_{a, s}$)

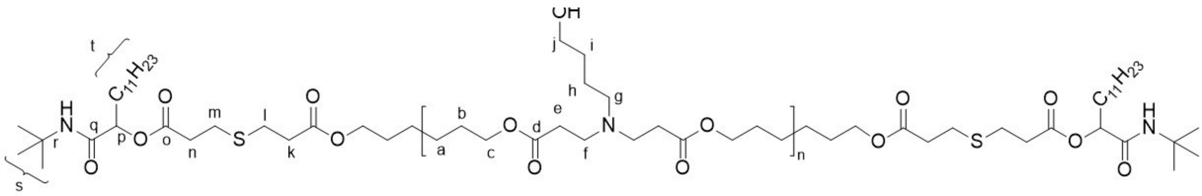
^{13}C NMR (101 MHz, $CDCl_3$) δ/ppm: δ 175.81 (d, o, q); 154.89, 119.08 (t); 97.77 (p); 75.94 (r); 64.59 (c); 62.82 (j); 54.03 (g); 48.82 (f); 32.67, 32.52 (e, k, n); 28.48, 28.30 (b, l, m); 25.71, 25.58, 25.53 (a, h, i, s)

ν_{max}/cm^{-1}: 3356 (OH, alcohol), 2935 (NH, amine salt), 1726 (C=O, ester)

M_n, SEC: *Mn* 11600, *Đ* = 1.32

% P3CR conversion as determined by proton NMR: 24%

AI11/I1-(pHDD-4AB)-AI11/I1



^1H NMR (400 MHz, $CDCl_3$) δ/ppm: δ 5.07 (m, 1.08H, CH_p), 4.07 (t, J = 6.70 Hz, 60H, CH_c), 3.56 (m, 30H, CH_j), 2.80 (t, J = 7.34 Hz, 72H, $CH_{e, k, m, n}$), 2.48 (m, 94H, $CH_{f, g, l}$), 1.60 (m, 120H, $CH_{b, h, i}$), 1.38 (m, 60H, CH_a), 1.35 (s, 10H, CH_s), 1.25 (m, 26H, CH_t)

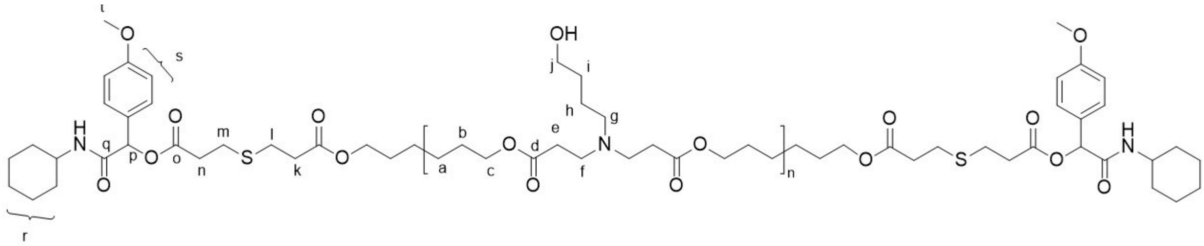
^{13}C NMR (101 MHz, $CDCl_3$) δ/ppm: δ 169.85 (d, o, q); 92.42 (p); 70.33 (r); 63.66 (c); 62.38 (j); 54.88 (g); 48.75 (f); 31.84, 30.67 (e, k, n); 28.33 (b, l, m); 25.49, 23.34 (a, h, i, s, t)

ν_{max}/cm^{-1}: 3433 (OH, alcohol), 2935 (NH, amine salt), 1728 (C=O, ester)

M_n, SEC: *Mn* 16300, *Đ* = 1.13

% P3CR conversion as determined by proton NMR: 54%

AI3/I2-(pHDD-4AB)-AI3/I2



^{1}H NMR (400 MHz, $CDCl_3$) δ/ppm: δ 7.34, 6.87 (m, 4H, CH_s), 6.01 (m, 1.10H, CH_p), 4.07 (m, 60H, CH_c), 3.80 (s, 3H, CH_t), 3.57 (m, 32H, $CH_{j, r}$), 2.82 (t, J = 7.19 Hz, 72H, $CH_{e, k, m, n}$), 2.52 (m, 94H, $CH_{f, g, l}$), 1.62 (m, 123H, $CH_{b, h, i, r}$), 1.37 (m, 67H, $CH_{a, r}$)

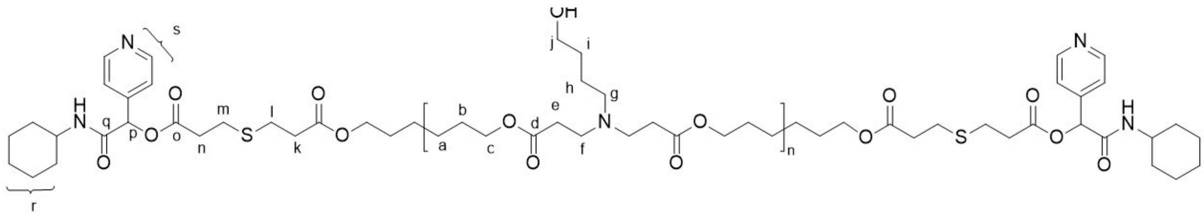
^{13}C NMR (101 MHz, $CDCl_3$) δ/ppm: δ 172.06, 170.44 (d, o, q); 148.82, 138.73, 123.53 (s); 92.82 (p); 74.30 (r); 64.70 (c); 62.37 (j); 53.84 (g, t); 48.75 (f); 40.95, 35.18, 31.08 (e, k, n); 28.45, 27.34 (b, l, m); 25.58, 25.56 (a, h, i, r)

ν_{max}/cm^{-1}: 3446 (OH, alcohol), 2935 (NH, amine salt), 1728 (C=O, ester)

M_n, SEC: *Mn* 4700, *Đ* = 1.43

% P3CR conversion as determined by proton NMR: 55%

AI5/I2-(pHDD-4AB)-AI5/I2



^{1}H NMR (400 MHz, $CDCl_3$) δ/ppm: δ 8.61, 7.37 (m, 8H, CH_s), 6.03 (m, 1.88H, CH_p), 4.06 (t, J = 6.25 Hz, 60H, CH_c), 3.56 (m, 32H, $CH_{j, r}$), 2.80 (t, J = 7.19 Hz, 72H, $CH_{e, k, m, n}$), 2.50 (m, 94H, $CH_{f, g, l}$), 1.61 (m, 128H, $CH_{b, h, i, r}$), 1.37 (m, 72H, $CH_{a, r}$)

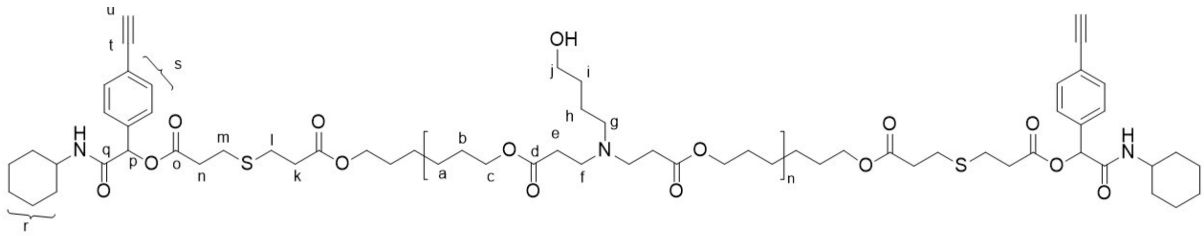
^{13}C NMR (101 MHz, $CDCl_3$) δ/ppm: δ 172.36 (d, o, q); 150.07, 138.78, 121.66 (s); 109.67 (p); 74.59 (r); 64.56 (c); 62.44 (j); 53.73 (g); 48.85 (f); 38.58, 32.90, 31.61 (e, k, n); 28.47, 27.11 (b, l, m); 25.58, 25.38, 24.83 (a, h, i, r)

ν_{max}/cm^{-1}: 3263 (OH, alcohol), 2933 (NH, amine salt), 1726 (C=O, ester)

M_n, SEC: *Mn* 6100, *Đ* = 1.94

% P3CR conversion as determined by proton NMR: 94%

AI6/I2-(pHDD-4AB)-AI6/I2



^{1}H NMR (400 MHz, $CDCl_3$) δ/ppm: δ 7.46, 7.39 (m, 7H, CH_s), 6.04 (m, 1.76H, CH_p), 4.06 (t, J = 6.38 Hz, 60H, CH_c), 3.56 (m, 34H, $CH_{j, r, u}$), 2.80 (t, J = 7.19 Hz, 72H, $CH_{e, k, m, n}$), 2.48 (m, 94H, $CH_{f, g, l}$), 1.60 (m, 128H, $CH_{b, h, i, r}$), 1.37 (m, 72H, $CH_{a, r}$)

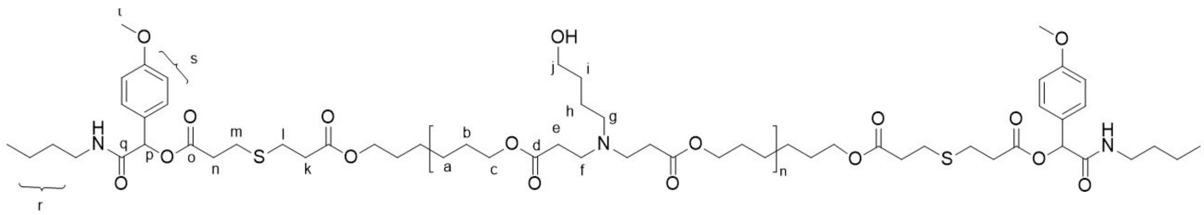
^{13}C NMR (101 MHz, $CDCl_3$) δ/ppm: δ 172.36 (d, o, q); 132.42, 127.38 (s); 104.03 (p); 82.82, 77.25 (t, u); 75.54 (r); 64.56 (c); 62.51 (j); 53.98 (g); 48.84 (f); 41.00, 34.71, 31.66 (e, k, n); 28.48, 27.02 (b, l, m); 25.58, 24.83 (a, h, i, r)

ν_{max}/cm^{-1}: 3476 (OH, alcohol), 2933 (NH, amine salt), 1726 (C=O, ester)

M_n, SEC: *Mn* 7900, *Đ* = 1.76

% P3CR conversion as determined by proton NMR: 88%

AI3/I3-(pHDD-4AB)-AI3/I3



^{1}H NMR (400 MHz, $CDCl_3$) δ/ppm: δ 7.32, 6.87 (m, 2H, CH_s), 6.03 (m, 0.43H, CH_p), 4.06 (m, 60H, CH_c), 3.79 (s, 2H, CH_t), 3.56 (m, 30H, CH_j), 2.80 (t, J = 7.19 Hz, 72H, $CH_{e, k, m, n}$), 2.49 (m, 94H, $CH_{f, g, l}$), 1.61 (m, 120H, $CH_{b, h, i}$), 1.37 (m, 64H, $CH_{a, r}$)

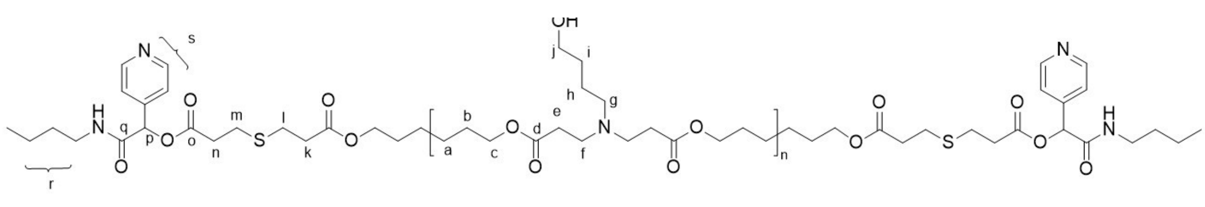
^{13}C NMR (101 MHz, $CDCl_3$) δ/ppm: δ 171.98 (d, o, q); 131.74, 118.43 (s); 102.02 (p); 86.18 (r); 64.64 (c); 62.29 (j); 53.96 (g, t); 48.75 (f); 40.95, 31.44 (e, k, n); 28.46 (b, l, m); 25.57 (a, h, i, r)

ν_{max}/cm^{-1}: 3446 (OH, alcohol), 2935 (NH, amine salt), 1726 (C=O, ester)

M_n, SEC: *Mn* 3700, *Đ* = 1.53

% P3CR conversion as determined by proton NMR: 21%

AI5/I3-(pHDD-4AB)-AI5/I3



^{1}H NMR (400 MHz, $CDCl_3$) δ/ppm: δ 8.59, 7.37 (m, 8H, CH_s), 6.04 (m, 1.94H, CH_p), 4.04 (t, J

= 6.23 Hz, 60H, CH_c), 3.54 (m, 30H, CH_j), 2.78 (t, J = 7.43 Hz, 72H, $CH_{e, k, m, n}$), 2.46 (m, 94H, $CH_{f, g, l}$), 1.58 (m, 120H, $CH_{b, h, i}$), 1.36 (m, 78H, $CH_{a, r}$)

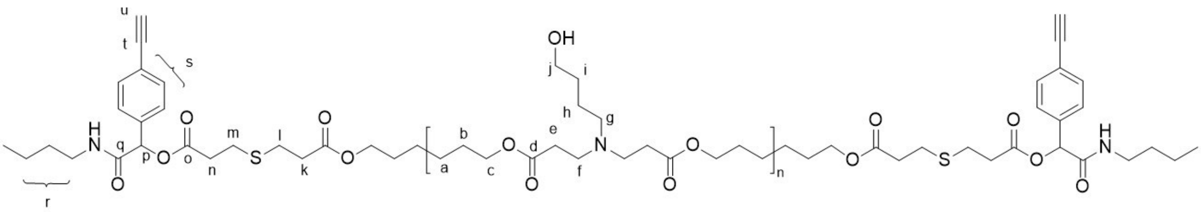
^{13}C NMR (101 MHz, $CDCl_3$) δ/ppm: δ 172.37, 171.65 (d, o, q); 150.02, 121.66 (s); 100.69 (p); 74.31 (r); 64.53 (c); 62.49 (j); 53.94 (g, t); 48.85 (f); 40.97, 39.30, 34.67 (e, k, n); 31.70, 31.44, 28.47 (b, l, m); 25.57 (a, h, i, r)

ν_{max}/cm^{-1}: 3372 (OH, alcohol), 2935 (NH, amine salt), 1726 (C=O, ester)

M_n, SEC: *Mn* 5200, *Đ* = 1.80

% P3CR conversion as determined by proton NMR: 97%

AI6/I3-(pHDD-4AB)-AI6/I3



^{1}H NMR (400 MHz, $CDCl_3$) δ/ppm: δ 7.46, 7.40 (m, 7H, CH_s), 6.07 (m, 1.85H, CH_p), 4.06 (t, J = 6.52 Hz, 60H, CH_c), 3.56 (m, 32H, $CH_{j, u}$), 2.80 (m, 72H, $CH_{e, k, m, n}$), 2.48 (m, 94H, $CH_{f, g, l}$), 1.61 (m, 120H, $CH_{b, h, i}$), 1.37 (m, 78H, $CH_{a, r}$)

^{13}C NMR (101 MHz, $CDCl_3$) δ/ppm: δ 172.13 (d, o, q); 132.42, 127.35 (s); 106.53 (p); 84.46, 77.25 (t, u); 72.44 (r); 64.64 (c); 62.51 (j); 53.68 (g); 48.81 (f); 40.99, 36.63, 31.43 (e, k, n); 28.45, 27.23 (b, l, m); 25.56, 21.94 (a, h, i, r)

ν_{max}/cm^{-1}: 3286 (OH, alcohol), 2935 (NH, amine salt), 1726 (C=O, ester)

M_n, SEC: *Mn* 5200, *Đ* = 1.68

% P3CR conversion as determined by proton NMR: 93%

TEM Imaging:

Widefield imaging of the polyplexes detailed in the paper are included below:

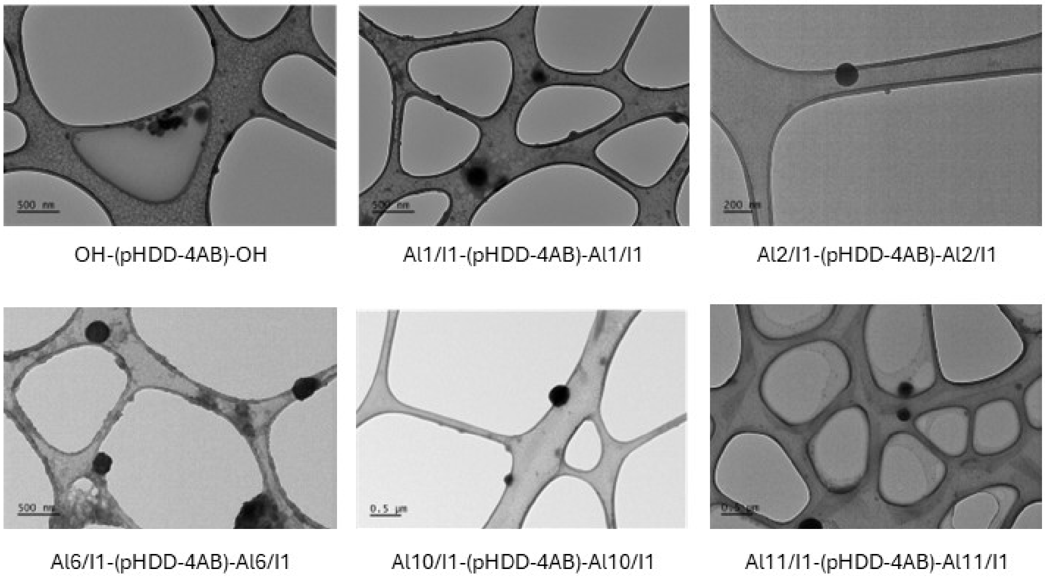


Figure S 1: TEM images collected from a Tecnai T12 BioTWIN Transmission Electron Microscope, showing polyplexes formed at NP 64 between PolyA and pHDD-4AB derived PBAEs. Polyplexes are stained with uranyl acetate and appear black.

Dynamic Light Scattering:

PDI of the polyplexes measured and detailed in Figure 4A are shown in Figure S 2 below. Figure S 3 shows particle diameter and PDI on the same graph.

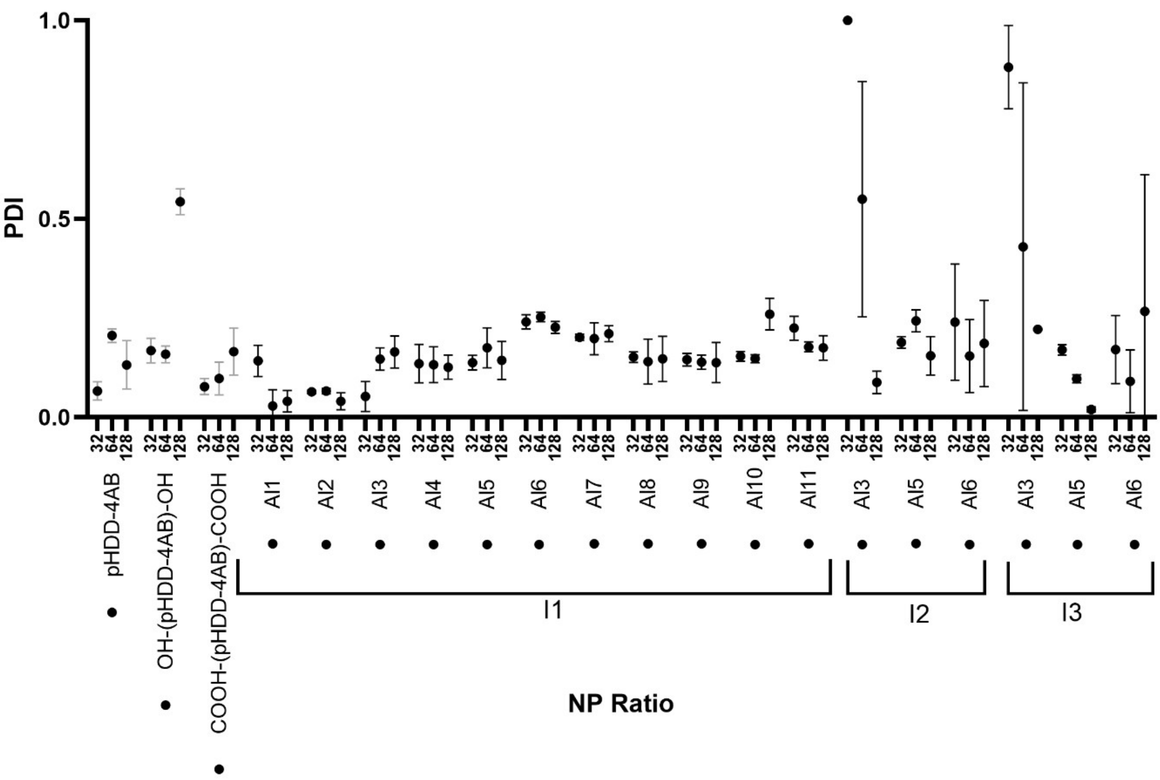


Figure S 2: Polyplex PDI as measured by dynamic light scattering. Each point is the mean of three measurements.

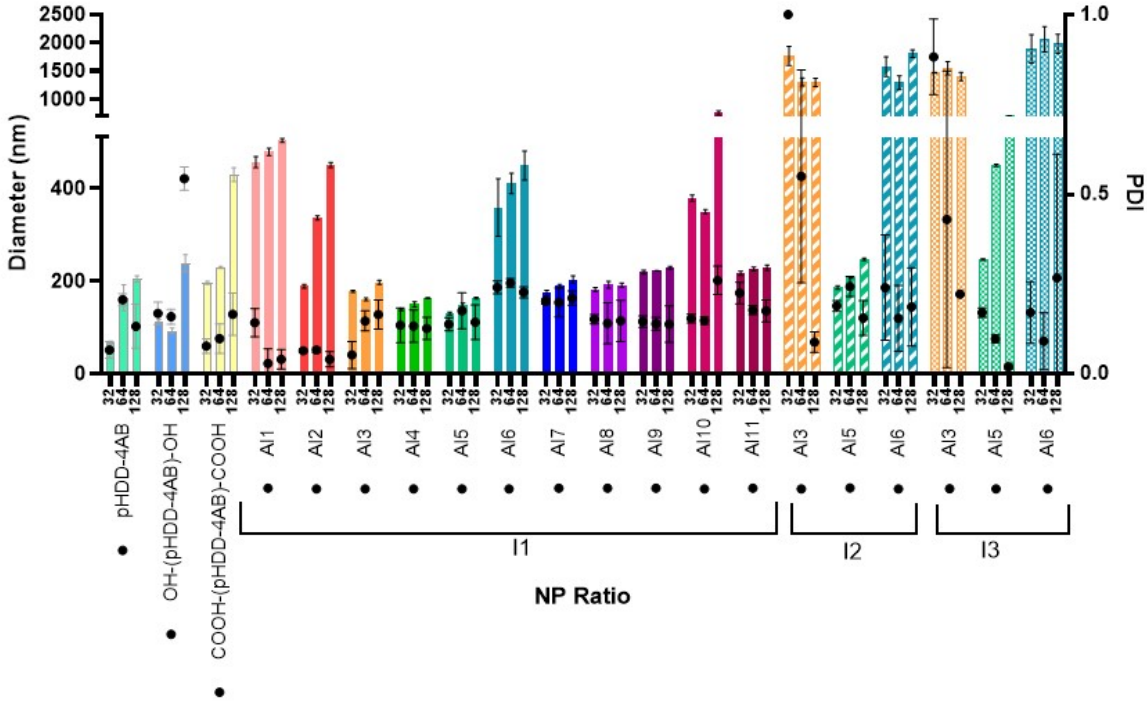
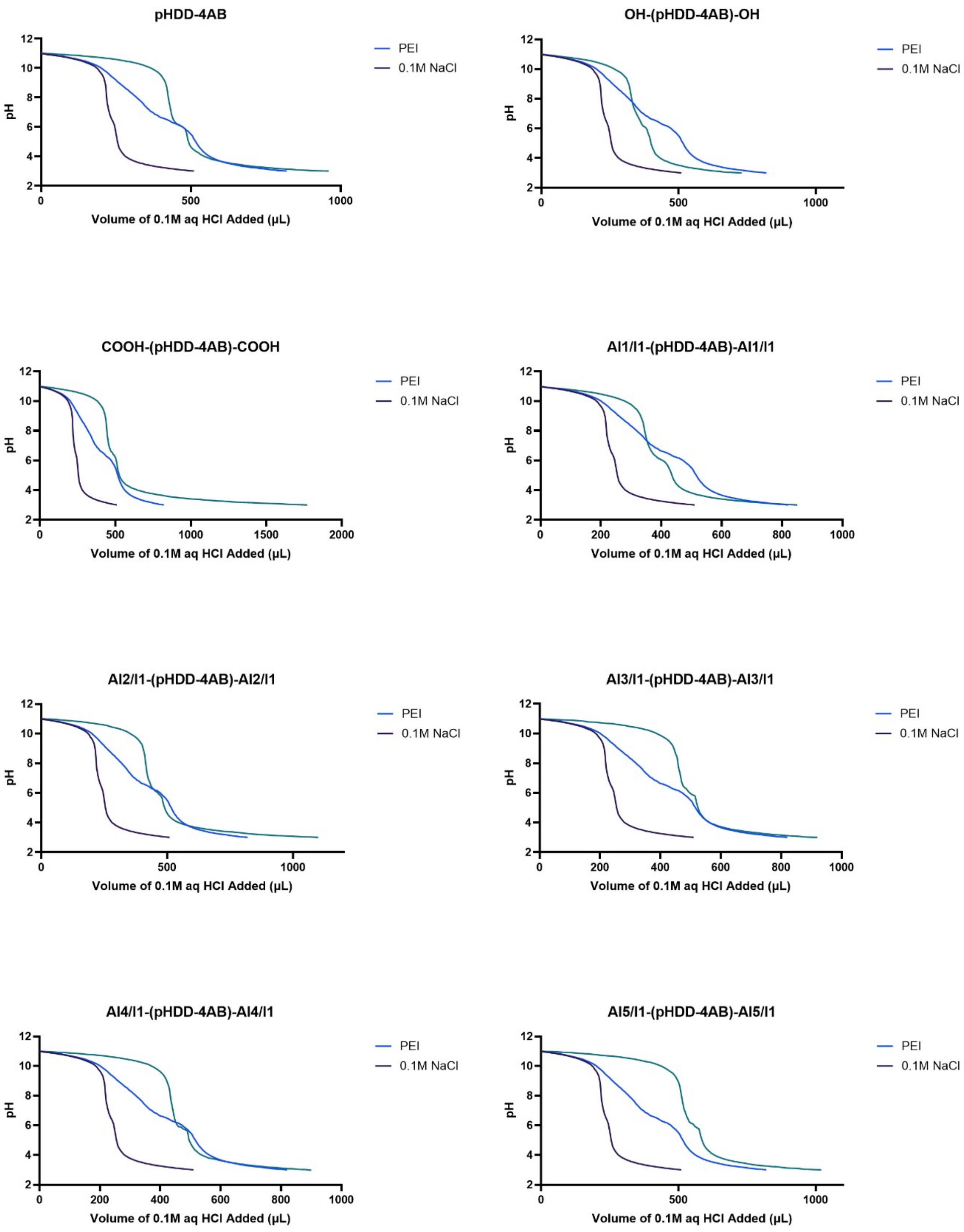


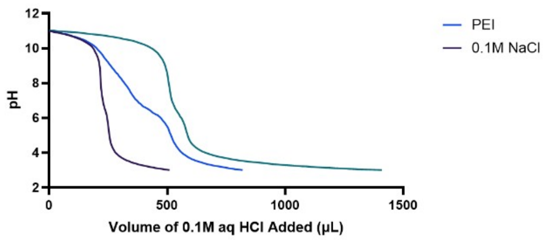
Figure S 3: Polyplex diameter (as reported in Figure 4A) and PDI as measured by dynamic light scattering. Each point is the mean of three measurements.

Polymer pH Titration Curves:

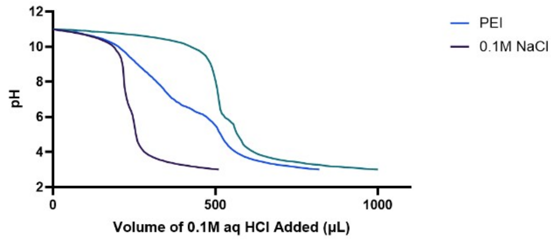
Full pH curves for HCl titration of the polymers are shown below. Each polymer is compared to 0.1M NaCl and 2mg PEI as controls.


pHDD-4AB
OH-(pHDD-4AB)-OH
COOH-(pHDD-4AB)-COOH
AI1/I1-(pHDD-4AB)-AI1/I1
AI2/I1-(pHDD-4AB)-AI2/I1
AI3/I1-(pHDD-4AB)-AI3/I1
AI4/I1-(pHDD-4AB)-AI4/I1
AI5/I1-(pHDD-4AB)-AI5/I1
PEI
0.1M NaCl
pH
Volume of 0.1M aq HCl Added (μL)

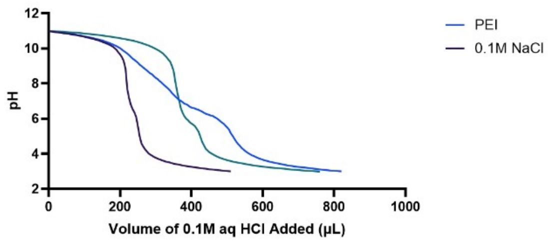
Al6/I1-(pHDD-4AB)-Al6/I1



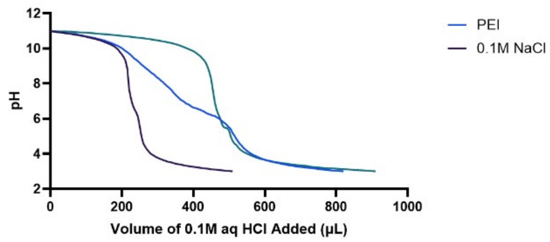
Al7/I1-(pHDD-4AB)-Al7/I1



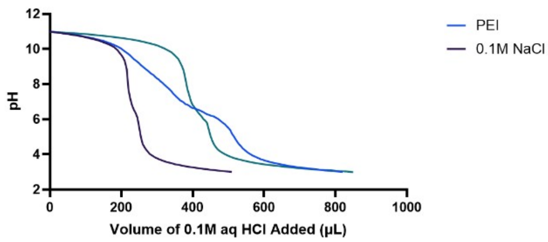
Al8/I1-(pHDD-4AB)-Al8/I1



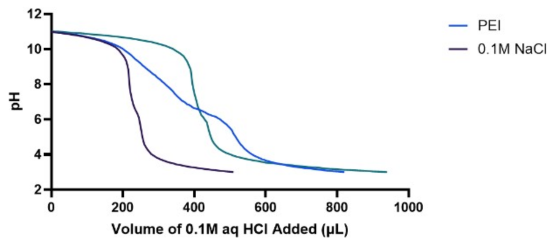
Al9/I1-(pHDD-4AB)-Al9/I1



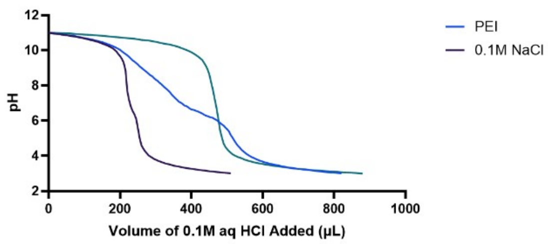
Al10/I1-(pHDD-4AB)-Al10/I1



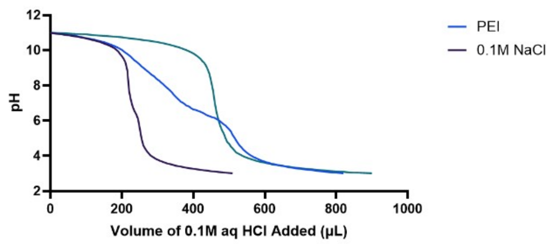
Al11/I1-(pHDD-4AB)-Al11/I1



Al3/I2-(pHDD-4AB)-Al3/I2



Al5/I2-(pHDD-4AB)-Al5/I2



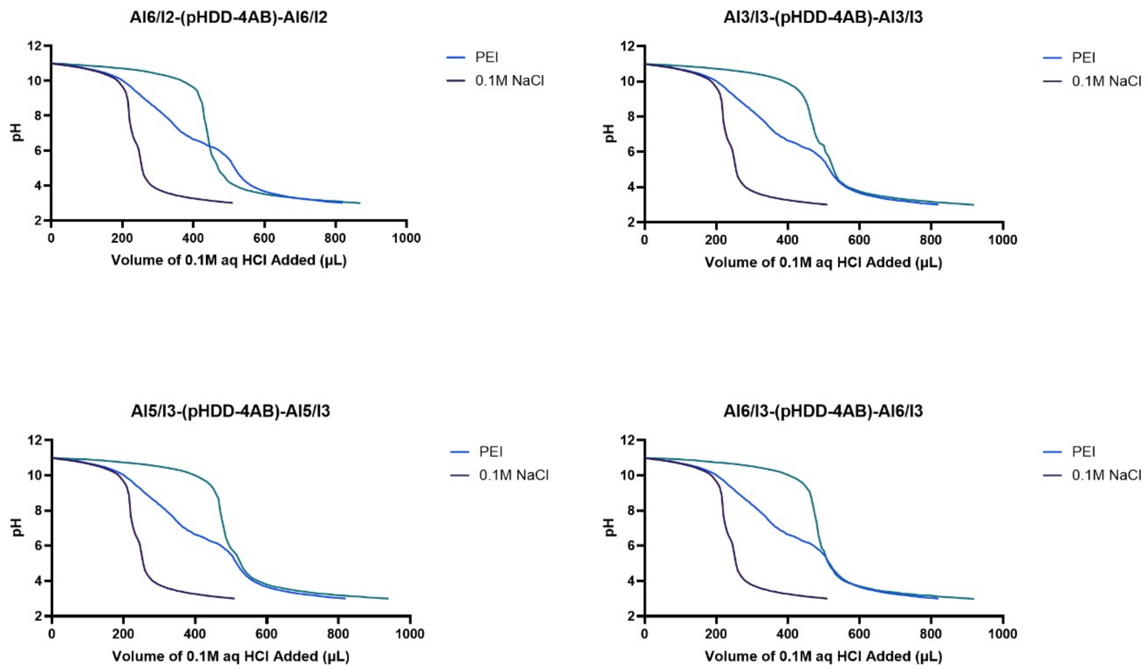


Figure S 4: pH curves of the polymers in this study, following titration from pH 11 to pH 3 with 0.1M HCl.

Calculated Buffering Capacity:

Table S1: *Buffering capacities of the polymers calculated by titration against 0.1M HCl, and normalised against both mass of polymer, and moles of protonatable amine. PEI is used as a control.*

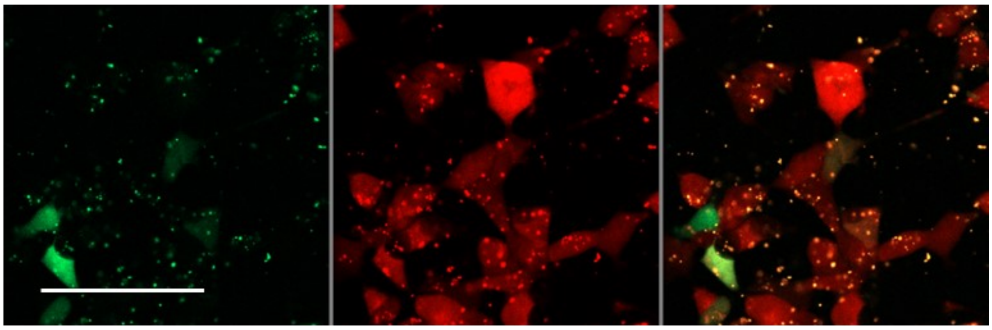
Polymer	Buffering Capacity normalised by mass of polymer (moles/g)	Buffering Capacity normalised by moles of protonatable amine (no units)
pHDD-4AB	0.00285	0.8989
OH-(pHDD-4AB)-OH	0.00105	0.3312
COOH-(pHDD-4AB)-COOH	0.00255	0.8043
AI1/I1-(pHDD-4AB)-AI1/I1	0.00365	1.1512
AI2/I1-(pHDD-4AB)-AI2/I1	0.00315	0.9935
AI3/I1-(pHDD-4AB)-AI3/I1	0.00255	0.8043
AI4/I1-(pHDD-4AB)-AI4/I1	0.00265	0.8358
AI5/I1-(pHDD-4AB)-AI5/I1	0.00310	0.9778
AI6/I1-(pHDD-4AB)-AI6/I1	0.00310	0.9778
AI7/I1-(pHDD-4AB)-AI7/I1	0.00310	0.9778
AI8/I1-(pHDD-4AB)-AI8/I1	0.00260	0.8201
AI9/I1-(pHDD-4AB)-AI9/I1	0.00160	0.5047
AI10/I1-(pHDD-4AB)-AI10/I1	0.00270	0.8516
AI11/I1-(pHDD-4AB)-AI11/I1	0.00180	0.5677
AI3/I2-(pHDD-4AB)-AI3/I2	0.00080	0.2523
AI5/I2-(pHDD-4AB)-AI5/I2	0.00110	0.3470
AI6/I2-(pHDD-4AB)-AI6/I2	0.00095	0.2996

Al3/I3-(pHDD-4AB)-Al3/I3	0.00225	0.7097
Al5/I3-(pHDD-4AB)-Al5/I3	0.00195	0.6150
Al6/I3-(pHDD-4AB)-Al6/I3	0.00120	0.3785
PEI	0.00765	0.3293

Microscopy Studies of Cellular Transfection:

A large number of the puncta observed in the mCherry channel colocalise exactly with those observed in the GFP channel. The effect could also be seen to some degree in the Hoechst channel when brightness is sufficiently increased, but the signal is weak and so in the original images is typically obscured by emissions from the Hoechst dye applied to the cells.

A



B

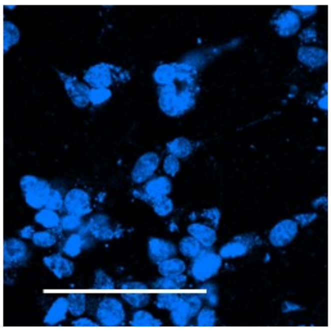


Figure S 5: Fluorescence imaging of mCherry-Gal9 expressing HEK293T cells treated with Al6/I1-(pHDD-4AB)-Al6/I1/GFP-mRNA polyplexes. Cells imaged 23 hours after treatment. Scale bar is 100 μM. A. GFP channel (left), mCherry channel (middle) and merged image (right