

Supporting Information

Modifications at the C-Terminus to Improve Py/Im Polyamide Activity in Cell Culture

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| | | | | | |
|----------|------------|--------|----------|-----------|--------|
| Compound | MALDI-ToF: | Purity | Compound | MALDI-ToF | Purity |
|----------|------------|--------|----------|-----------|--------|

| Number | Expected (M+H ⁺); Observed (M+H ⁺) | (HPLC, 310 nm) | Number | Expected (M+H): Observed (M+H): | (HPLC, 310 nm) |
|-----------|---|-------------------|-----------|------------------------------------|-------------------|
| 1 | 1274.49; 1274.51 | 99.3 | 23 | 1237.59; 1237.48 | 95.0 |
| 2 | 1246.50; 1246.81 | 99.3 | 24 | 1209.59; 1209.89 | 95.6 |
| 3 | 1231.49; 1231.77 | 99.7 | 25 | 1194.58; 1194.53 | 100.0 |
| 4 | 1175.43; 1175.61 | 98.7 | 26 | 1138.52; 1138.50 | 99.7 |
| 5 | 1191.42; 1191.38 | 97.2 | 27 | 1154.51; 1154.62 | 96.8 |
| 6 | 1394.52; 1394.46 | 98.0 | 28 | 1357.61; 1357.59 | 98.8 |
| 7 | 1379.50; 1379.72 | 99.3 | 29 | 1342.60; 1342.55 | 99.6 |
| 8 | 1323.44; 1323.52 | 99.9 | 30 | 1286.64; 1286.59 | 99.0 |
| 9 | 1323.44; 1323.44 | 95.6 | 31 | 1286.54; 1286.56 | 96.1 |
| 10 | 1297.44; 1297.48 | 95.9 | 32 | 1260.54; 1260.63 | 95.3 |
| 11 | 1319.42; 1319.52 ^a | 97.2 | 33 | 1260.54; 1260.51 | 96.4 |
| 12 | 1238.58; 1238.51 | 96.5 | 34 | 1238.58; 1238.56 | 99.0 |
| 13 | 1210.59; 1210.76 | 98.1 | 35 | 1210.59; 1210.59 | 98.0 |
| 14 | 1195.58; 1195.57 | 98.7 | 36 | 1195.58; 1195.56 | 99.3 |
| 15 | 1139.51; 1139.49 | 98.8 | 37 | 1139.51; 1139.77 | 95.1 |
| 16 | 1155.51; 1155.83 | 98.4 | 38 | 1155.51; 1155.40 | 97.4 |
| 17 | 1358.60; 1358.66 | 97.8 | 39 | 1358.60; 1358.70 | 95.4 |
| 18 | 1343.59; 1343.76 | 99.9 | 40 | 1343.59; 1343.52 | 100.0 |
| 19 | 1287.53; 1287.56 | 99.4 | 41 | 1287.53; 1287.60 | 98.4 |
| 20 | 1287.53; 1287.73 | 97.6 | 42 | 1287.53; 1287.53 | 95.9 |
| 21 | 1261.53; 1261.87 | 97.3 | 43 | 1261.53; 1261.56 | 98.9 |
| 22 | 1261.53; 1261.51 | 98.8 | 44 | 1261.53; 1261.51 | 96.2 |

Table SI 1: MALDI-ToF and purity data for compounds **1-44**. MALDI-ToF data gives calculated exact mass for (M+H⁺) and observed mass for (M+H⁺) for polyamides **1-10** and **12-44**. ^a MALDI-ToF data for **11** corresponds to the (M+Na⁺) peak. Purity was measured by analytical HPLC at 310 nm; purity for oxime-linked compounds **9-11**, **20-22**, **31-33** and **42-44** included both E and Z isomers.

| Compound | T _m (°C) | ΔT _m (°C) | Compound | T _m (°C) | ΔT _m (°C) |
|----------|---------------------|----------------------|----------|---------------------|----------------------|
|----------|---------------------|----------------------|----------|---------------------|----------------------|

| Number | | | Number | | |
|-----------------|------------|------|-----------------|------------|------|
| DNA Only | 64.7 ± 0.2 | | DNA Only | 60.8 ± 0.2 | |
| 1 | 77.1 ± 0.2 | 12.4 | 23 | 75.1 ± 0.3 | 14.3 |
| 2 | 79.5 ± 0.2 | 14.8 | 24 | 78.1 ± 0.2 | 17.3 |
| 3 | 77.4 ± 0.2 | 12.7 | 25 | 75.1 ± 0.2 | 14.3 |
| 4 | 78.2 ± 0.2 | 13.5 | 26 | 77.0 ± 0.2 | 16.2 |
| 5 | 75.3 ± 0.1 | 10.6 | 27 | 72.0 ± 0.3 | 11.2 |
| 6 | 76.4 ± 0.2 | 11.7 | 28 | 75.0 ± 0.3 | 14.2 |
| 7 | 73.7 ± 0.0 | 9.0 | 29 | 71.2 ± 0.2 | 10.4 |
| 8 | 74.2 ± 0.2 | 9.5 | 30 | 71.9 ± 0.2 | 11.1 |
| 9 | 76.1 ± 0.2 | 11.4 | 31 | 72.3 ± 0.2 | 11.5 |
| 10 | 74.1 ± 0.2 | 9.4 | 32 | 73.3 ± 0.1 | 12.5 |
| 11 | 75.2 ± 0.2 | 10.5 | 33 | 73.9 ± 0.3 | 13.1 |
| 12 | 66.3 ± 0.2 | 1.6 | 34 | 64.4 ± 0.2 | 3.6 |
| 13 | 67.8 ± 0.2 | 3.1 | 35 | 67.2 ± 0.1 | 6.4 |
| 14 | 66.8 ± 0.2 | 2.1 | 36 | 59.9 ± 0.2 | -0.9 |
| 15 | 68.9 ± 0.0 | 4.1 | 37 | 66.1 ± 0.2 | 5.3 |
| 16 | 64.8 ± 0.2 | 0.1 | 38 | 62.8 ± 0.2 | 2.0 |
| 17 | 65.6 ± 0.2 | 0.9 | 39 | 64.8 ± 0.2 | 4.0 |
| 18 | 65.4 ± 0.2 | 0.7 | 40 | 63.4 ± 0.2 | 2.6 |
| 19 | 65.2 ± 0.2 | 0.5 | 41 | 62.3 ± 0.2 | 1.5 |
| 20 | 65.6 ± 0.3 | 0.8 | 42 | 63.4 ± 0.0 | 2.6 |
| 21 | 65.7 ± 0.2 | 0.9 | 43 | 64.8 ± 0.1 | 4.0 |
| 22 | 65.5 ± 0.2 | 0.8 | 44 | 64.2 ± 0.3 | 3.4 |

Table SI 2: Melting temperature and ΔT_m values for **1-44** on 14-mer oligonucleotides (oligonucleotide sequences provided in Materials and Methods). All values reported are averages of at least three melting temperature experiments (standard deviations indicated in parentheses.) $\Delta T_m = T_m(\text{DNA+polyamide}) - T_m(\text{DNA only})$.

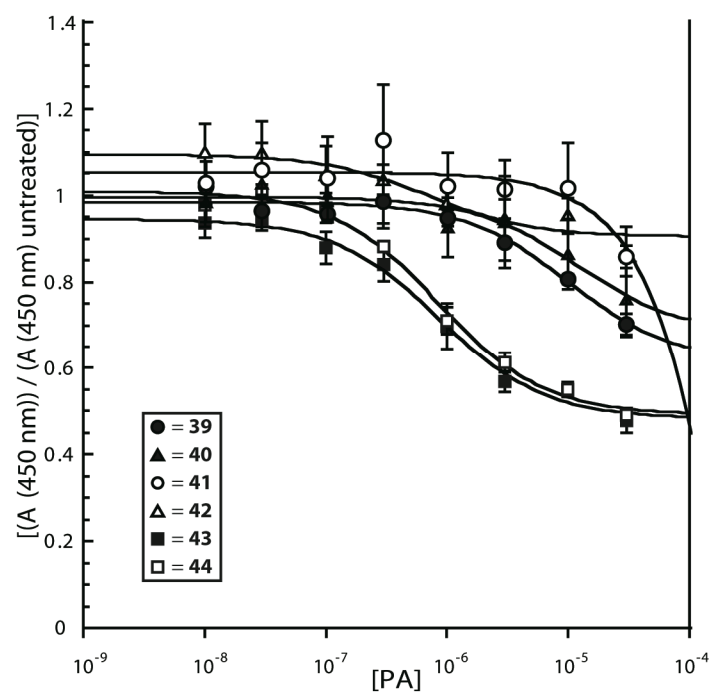


Figure SI 1: Cell viability data for **39-44** in LNCaP cells at 30, 10, 3, 1, 0.3, 0.1, 0.03 and 0.01 μM , performed according to the protocol provided in main text.

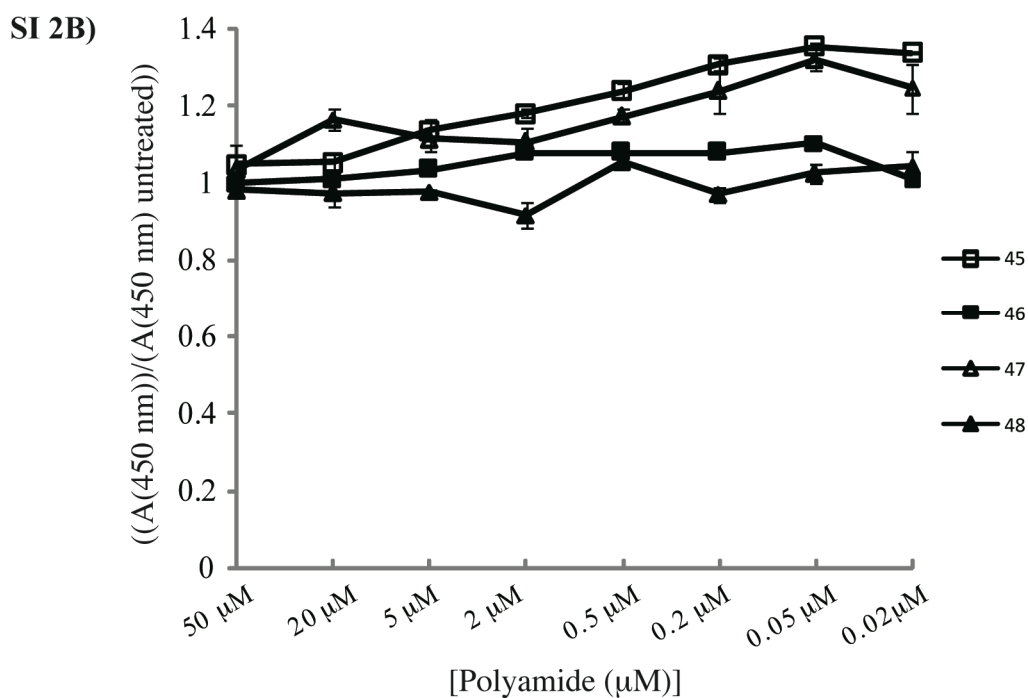
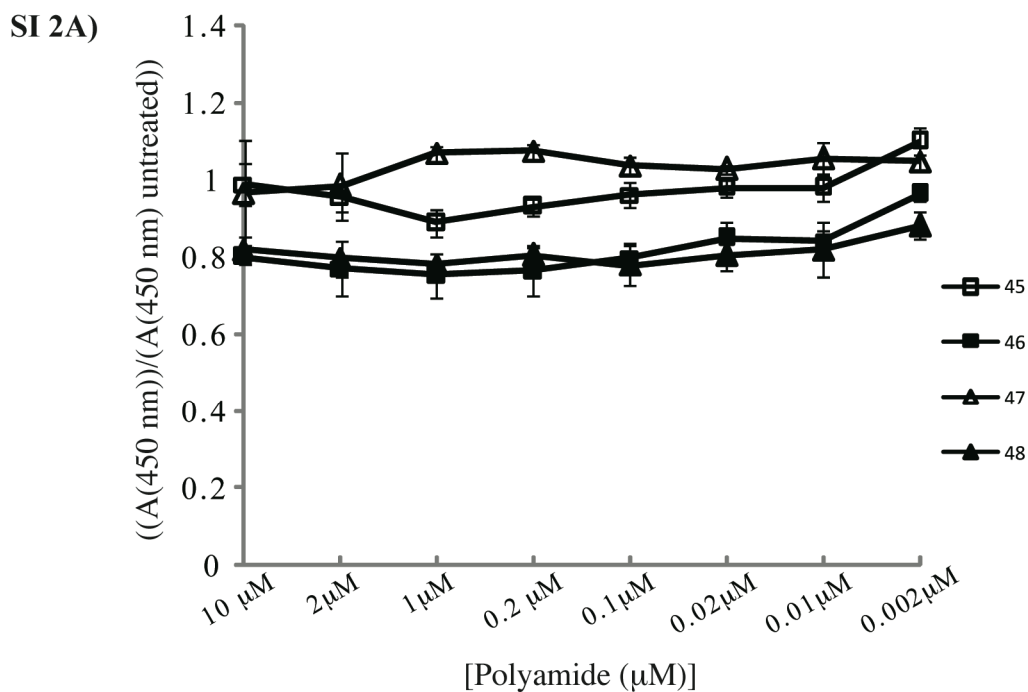


Figure SI 2: Cell viability data for **45-48** in U251 and LNCaP cells, performed according to the protocol provided in main text. A) Cell viability data for **45-48** in U251 cells at 10, 2, 1, 0.2, 0.1, 0.02, 0.01 and 0.002 μM . B) Cell viability data for **45-48** in LNCaP cells at 50, 20, 5, 2, 0.5, 0.2, 0.05 and 0.02 μM .