A Click Chemistry Approach to Targeted DNA Crosslinking with \textit{cis-}\textit{Platinum(II)-Modified Triplex-Forming Oligonucleotides}

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S-1: General Information – Materials and Methods

Chemicals, reagents and HPLC grade solvents were sourced from Sigma-Aldrich (Ireland) Ltd., unless otherwise stated, and were used without any further purification. Dichloromethane (DCM) was distilled from calcium hydride and stored under argon. All other solvents were used as supplied.

**Note:** Organic azides are hazardous materials and are known to be heat- and shock-sensitive. Explosive decomposition can potentially occur with very little energy input. To ensure safe manipulation and non-explosiveness for organic azides, the rule is that the number of nitrogen atoms must not exceed that of carbon, where \((N_C + N_O)/N_N \geq 3\). (N = number of atoms).[^2] 4 is a potentially hazardous material, however all relevant safety precautions were undertaken while working with the material.

S-1.1: General Synthesis

\(^1\)H-NMR (600 MHz), \(^{13}\)C-NMR (151 MHz) and \(^{195}\)Pt-NMR (129 MHz) spectra were obtained on a Bruker AC 600 MHz NMR Spectrometer. All experiments were performed at room temperature in either CDCl\(_3\), DMSO-d\(_6\) or DMF-d\(_7\) where indicated. Chemical shift signals (δ) are given in parts per million (ppm) using the residual proton signals in the indicated solvents as internal standards. Coupling constants (J) are quoted in hertz (Hz). Signal peak multiplicities are assigned with the following: singlet (s), doublet (d), doublet of doublets (dd), doublet of doublet of doublets (ddd), triplet (t), doublet of triplets (dt), quartet (q), quintuplet (qn) and multiplet (m). Experimental spectra were analysed using MestReNova software (v. 14.2.0-26256, Mestrelab Research S.L.) FT-IR spectra were obtained from neat solids on a Perkin Elmer Spectrum Two Spectrometer. Melting points were obtained on a Stanford Research Systems MPA100 Optimelt apparatus. ESI-MS analysis was performed on a MaXis HD ESI-QTOF mass spectrometer (Bruker Daltonik GmbH) with data processing performed using Compass Data Analysis software (v 4.3, Bruker Daltonik GmbH).

S-1.2: Oligonucleotide Synthesis

Solid supports, standard DNA phosphoramidites and all other reagents used in the synthesis were purchased from Sigma Aldrich. Modified phosphoramidites were purchased from Glen Research. Oligonucleotides (ODNs) were synthesised on a K&A Laborgeräte H-8-SE LNA, DNA/RNA synthesiser

Figure S-1. Current crosslinking hybrid constructs.[^1]
using the standard 1.0 µmol phosphoramidite cycle. Coupling efficiencies were monitored by the trityl cation conductivity monitoring facility and was >98% for all oligonucleotides. Standard monomers (A, G, C and T) were coupled for 35 s and non-standard monomers were coupled for 360 s. ODNs were deprotected and cleaved from the solid support using a concentrated ammonia solution for 1 h at r.t., followed by heating in sealed vials for 5 h at 55 °C. ODNs were purified using reverse-phase HPLC on a Gilson HPLC system using a Luna 10 µm C8 100 Å 250 x 10 mm column. For standard alkyne-modified ODNs, the gradient was 10-45 % buffer B over 20 min with flow rate of 4 mL/min (buffer A: 0.1 M triethylammonium acetate (TEAA), buffer B: 0.1 M TEAA with 50% MeCN). Fraction volumes were reduced by rotary evaporation prior to redissolution in water and desalted using NAP-10 gel filtration columns purchased from GE Healthcare. All ODNs were characterised by negative-mode HPLC-mass spectrometry using a Waters Xevo G2-XS QT mass spectrometer with an Acquity UPLC system equipped with an Acquity UPLC oligonucleotide BEH C18 column (particle size: 1.7 µm; pore size 130 Å; column dimensions: 2.1 x 50 mm). Data were deconvoluted and analysed using Waters Mass Lynx software.

Oligonucleotide synthesis with modified nucleobases was performed as described above with some minor deviations. Standard monomers (A, G, C, and T) were coupled for 35 s and non-standard monomers were coupled for 360 s. ODNs with incorporated pdU additions and those that were labelled with thiazole orange (TOB6) or fluorescein/cyanine-5 dyes were purified with ammonium acetate (NH₄OAc) with a gradient of 15-45% buffer B over 25 min, flow rate of 4mL/min (buffer A: 0.1 M NH₄OAc, buffer B: 0.1 M NH₄OAc 50% MeCN).

S-1.3: Thiazole orange (TO) TFO:TO-NHS ester labelling
In a total volume of approx. 350 µL, 2000 nmol (10 eq.) of TOB6 NHS ester in DMF was added to 200 nmol of oligonucleotide dissolved in carbonate buffer (NaHCO₃/Na₂CO₃, 0.5 M, pH 8.74). The solution was shaken at 20 °C for 2 h prior to desalting by NAP column and purified by reverse-phase HPLC utilising a C8 column with a gradient of 0.1 M NH₄OAc + MeCN (50%) (Buffer B) in 0.1 M NH₄OAc (Buffer A). ESI-MS analysis, as previously described, was used to determine the purity of the labelled oligonucleotide. For ODNs that contained two labelling sites, 20 eq. of TOB6 NHS ester was used. For TOTFOs that were clicked to N₂-platinum(II) complexes, no purification of the TO labelled TFO was performed. Purification was only performed after click conjugation to ensure optimum yield.

S-1.4: Platinum(II)-TFO hybrid generation using click chemistry
In a total volume of 500 µL (50:50 H₂O:DMSO or DMF, complex dependent) 500 nmol of Pt(II)-azide complex was added to 50 nmol of the alkyne-modified TFO. The solutions were degassed with argon and 500 nmol of Cu-TBTA complex in 50:50 H₂O:DMSO was added prior to final addition of 1000 nmol of Na-L-ascorbate. The click solutions were further degassed and stirred at 25 °C for 3-6 h prior to desalting by elution through a NAP-10 column. For click reactions concerning the 5'-BCN(CEP II)-dC modified TFO, the reaction was performed as above without the use of Cu-TBTA or Na-L-ascorbate. The volume was reduced and the residue re-dissolved in H₂O before purification by gradient reverse-phase HPLC utilising a C8 column. The Pt(II)-TFO hybrid purity was analysed by HPLC-ESI-MS.
S-1.5: UV-Thermal Melting Studies
Oligonucleotides were quantified on a NanoDropND-1000 UV-Vis Spectrophotometer. Thermal melting studies were performed on a Varian Cary 100 UV-Visible Spectrophotometer equipped with a 6 x 6 Peltier multicell system with temperature controller in Starna Scientific black-walled quartz cuvettes of 10 mm path length and 100 µL sample volume. Experimental measurements were monitored at 260 nm using Cary WinUV thermal application software. The TFO/Pt(II)-TFO hybrid and duplex samples were combined in a 2.5:1 µM ratio and dissolved in triplex buffer - 10 mM phosphate, 150 mM NaCl and 2 mM MgCl₂ (pH 6). Pt-N₃-Complex/duplex melting samples were prepared similarly. Thermal melting was recorded between 12-90 ºC (0.5 ºC/min with 2 min hold). In total, 3 heating ramps were performed. Tₘ was calculated as an average of the first derivative of sigmoidal non-linear regression analysis of the triplex melting curve using GraphPad Prism 8 software.

S-1.6: Fluorescent thermal melting
Cisplatin and duplex samples were combined in a 2.5:1 µM ratio and dissolved in triplex buffer - 10 mM phosphate, 150 mM NaCl and 2 mM MgCl₂ (pH 6) prior to incubation at 37 ºC for 48 hours. SYBR green I (1 mL, Roche) was added to each sample and the melting profile for the triplex was analysed on a LightCycler® 480 II (Roche). Samples were heated to 99 ºC with 10 fluorescence measurements recorded per ºC. A plot of sample fluorescence versus temperature is obtained, normalised and the first negative derivative of the sample was calculated. Samples were analysed in triplicate and Tₘ was calculated as an average of the first negative derivative of the melting curve. Melting curves were analysed and graphed using GraphPad Prism 8 software.

S-1.7: Triplex formation
In a total volume of 5 µL triplex buffer, duplex target (40 bp, 1 pmol) was treated with increasing concentrations of alkyne-modified TFO (2.5-50 eq.). Samples were incubated at 37 ºC for 0-48 h prior to addition of 6X loading dye (Thermo Scientific) and loaded onto a 20% polyacrylamide gel (50 mM Tris acetate, 150 mM NaCl, 2 mM MgCl₂, pH 6). Electrophoresis was performed at 70 V for 240 mins in triplex running buffer (50 mM Tris acetate, pH 6). Polyacrylamide gels were post-stained with SybrGold and visualised and imaged on a Syngene G:Box Mini 9 gel documentation system.

S-1.8: Triplex-mediated crosslink activity
In a total volume of 5 µL triplex buffer, target duplex (57 bp, 1 pmol) and off-target sequence (40 bp, 1 pmol) were treated with increasing concentrations of platinum(II)-TFO hybrid (2.5-50 eq.) and incubated at 37 ºC for up to 48 h. Electrophoresis and visualisation was performed as previously reported.
S-1.9: Reversal of triplex formation by sodium cyanide
In a total volume of 5 µL triplex buffer, fluorescently labelled duplex target (40 bp, 1 pmol) was treated with alkyne-modified TFO (50 eq.) and separately with platinum(II)-TFO hybrid (50 eq.). Samples were incubated at 37 ºC for 48 h prior to addition of increasing concentrations of NaCN solution (1,000-300,000 eq.). Combined samples were incubated at r.t. for 18 h and quenched with 30% glycerol solution. Electrophoresis was performed as before. Gel visualisation and imaging was performed on a Syngene G:Box Mini 9 gel documentation system with integral Cy5 and 6-FAM filters.

S-1.10: Crosslink investigation by denaturing PAGE
In a total volume of 5 µL triplex buffer, fluorescently labelled duplex sequences (21/17 bp, 21 bp, 1 pmol) were treated with platinum(II)-TFO hybrid (TFO-17A – C, 50 eq.) Samples were incubated at 37 ºC for a minimum of 48 h prior to quenching with 2X denaturing loading dye. Samples were loaded onto 20% denaturing PAGE (1X TBE, 7.4 M urea, pH 8.2) and subjected to electrophoresis at 300 V for 60 mins in 1X TBE running buffer.
S-2: Synthesis of azide-functionalised ligands and platinum(II) complexes

Route 1: Synthesis of cis-[Pt(2-azidopropane-1,3-diamine)Cl₂]

Synthesis of Di-tert-boc-2-hydroxy-1,3-diaminopropane (1)\(^{[3]}\)

Reaction protocol was adapted from the literature method as reported by Kane et al.\(^{[3]}\) To a solution of 1,3-diamino-2-propanol (2.000 g, 22.19 mmol, 1 eq.) and triethylamine (4.49 g, 44.38 mmol, 2 eq.) in a 1:1 THF/H₂O mix (80 ml) stirring at 0 °C was added di-tert-butyl dicarbonate (12.11 g, 55.48 mmol, 2.5 eq.) in THF (40 ml). The resulting solution was stirred at r.t. overnight (approx. 18 h). THF was removed in vacuo and approx. 40 ml of distilled H₂O was added to the solution before extraction with EtOAc (3 x 50 ml). Organic layer was dried over MgSO₄ and solvent removed in vacuo to yield the desired product as a clear pale oil, which subsequently solidified to white solid. Yield: 5.617 g, 87%. \(^1\)H NMR (600 MHz, CDCl₃): 5.14 (s, 2H, NH), 3.78 – 3.73 (m, 1H, CH), 3.31 – 3.14 (m, 4H, (CH₂)₂), 1.46 (s, 18H, Boc). \(^1^3\)C NMR (151 MHz, CDCl₃): 157.27 (C, carbonyl), 79.81 (C, Boc), 71.14 (CH), 43.57 (CH₂), 28.36 (CH₃, Boc). IR (ATR, cm⁻¹): 3490, 3369, 3313, 2983, 2933, 1681, 1659, 1524, 1274, 1253, 1164, 1084, 970, 865, 581. mp 91-93 °C. Anal. Calcd for C₁₅H₂₆N₂O₅: C, 53.78; H, 9.03; N, 9.65. Found: C, 53.88; H, 9.19; N, 9.59.

Synthesis of Di-tert-boc-2-methanesulfonyl-1,3-diaminopropane (2)\(^{[4]}\)

2 was synthesised, with slight adaptation, according to a procedure reported by Abd Karim et al.\(^{[4]}\) Di-tert-boc-2-hydroxy-1,3-diaminopropane (5.000 g, 17.22 mmol, 1 eq.) and triethylamine (5.23 g, 51.66 mmol, 3 eq.) were dissolved in 80 ml anhydrous DCM and purged with argon for 20 mins. Methanesulfonyl chloride (3.95 g, 34.44 mmol, 2 eq.) was dissolved in approx. 8 ml anhydrous DCM and added dropwise to the degassed solution with stirring over 30 minutes at 0 °C. The mixture was allowed to gradually warm to r.t. and stirred overnight (approx. 18 h). 70 ml DI water was added to the mixture to react with excess MsCl and stirred for 3 h. Organic layer was separated and washed sequentially with 100 ml 10% NaHCO₃ and 100 ml brine (x2). Organic layer was dried over MgSO₄ and solvent removed in vacuo to afford a pale yellow/beige solid and recrystallised using hexane, to yield white solid. Yield: 4.755 g, 75%. \(^1\)H NMR (600 MHz, CDCl₃): 5.18 (t, J = 6.6 Hz, 2H, NH), 4.66 (p, J = 5.2 Hz, 1H, CH), 3.49 (ddd, J = 14.8, 7.5, 4.5 Hz, 2H, CH₂), 3.30 (dt, J = 14.9, 5.8 Hz, 2H, CH₂), 3.09 (s, 3H, CH₃), 1.44 (s, 18H, Boc). \(^1^3\)C NMR (151 MHz, CDCl₃): 156.47 (C, carbonyl), 80.19 (C, Boc), 79.20 (CH), 40.96 (CH₂), 38.31(CH₃, OMs), 28.46 (CH₃, Boc). IR (ATR, cm⁻¹): 3448, 3379, 2976, 2937, 1704, 1516, 1345, 1251, 1172, 1116, 1045, 950, 917, 800, 529, 460. mp 138-140 °C. Anal. Calcd for C₁₅H₂₆N₂O₅S: C, 54.64; H, 7.66; N, 7.60. Found: C, 54.44; H, 7.42; N, 7.31.

Synthesis of Di-tert-boc-2-azido-1,3-diaminopropane (3)\(^{[5]}\)

3 was synthesised, with slight adaptation, according to a procedure reported by Veerendhar et al.\(^{[5]}\) To a solution of di-tert-boc-2-methanesulfonyl-1,3-diaminopropane (2.000 g, 5.43 mmol, 1 eq.) in 30 ml anhydrous DMF, NaN₃ (1.412 g, 21.72 mmol, 4 eq.) was added and the mixture stirred at 80 °C for 18 h. The mixture was allowed to cool to ambient temperature, poured into approx. 50 ml DI water and
further cooled at to 0 °C prior to filtering to obtain white solid product. Yield: 1.38 g, 81%. 1H NMR (600 MHz, CDCl3): 5.06 (s, 2H, NH), 3.64 (s, 1H, CH), 3.43 – 3.31 (m, 2H, CH2), 3.18 – 3.07 (m, 2H, CH2), 1.44 (s, 18H, Boc). 13C NMR (151 MHz, CDCl3): 156.20 (C, carbonyl), 79.91 (C, Boc), 60.92 (CH), 40.80 (CH2), 28.35 (CH3, Boc). IR (ATR, cm⁻¹): 3340, 2966, 2936, 2110

**Synthesis of 2-azidopropane-1,3-diamine dihydrochloride (4)**

4 was synthesised according to the literature method previously reported by Urankar et al. To a solution of di-tert-boc-2-azido-1,3-diaminopropane (0.400 g, 1.27 mmol, 1 eq.) in EtOAc (3 ml) was added aqueous 6 M HCl (approx. 1.5 ml) dropwise with stirring. The reaction mixture was stirred at 0 °C for 30 mins followed by stirring for approximately 18 h at r.t. before cooling at 5 °C overnight. The resulting white precipitate was filtered and washed several times with EtOAc to afford the product as a white crystalline solid. Yield: 0.221 g, 93 %. 1H NMR (600 MHz, DMSO-d6): 8.38 (s, 6H, (NH)2), 4.23 (tt, J = 8.6, 4.0 Hz, 1H, CH), 3.15 (dd, J = 13.5, 4.0 Hz, 2H, CH2), 2.90 (dd, J = 13.4, 8.9 Hz, 2H, CH2). 13C-NMR (151 MHz, DMSO-d6): 57.70 (CH), 40.49 (CH2). IR (ATR, cm⁻¹): 2946, 2851, 2127 (N3), 1503, 1462, 1361, 1279, 1203, 1081, 957, 933, 606. mp 227-233 °C.

**Synthesis of cis-[Pt(2-azidopropane-1,3-diamine)Cl2] (5, Pt-N3-Cis)**

Reaction protocol was adjusted from the literature method as previously reported by Urankar et al. 1,8-Diazabicyclo[5.4.0]jundec-7-ene (DBU) (0.081 g, 0.53 mmol, 2 eq.) was added to a solution of 2-azidopropane-1,3-diamine dihydrochloride (0.050 g, 0.265 mmol, 1 eq.) in 0.7 ml anhydrous DMF with stirring. To this solution was added cis-[Pt(DMSO)2Cl2] (0.112 g, 0.265 mmol, 1 eq.) and the solution was stirred at r.t. for 3 days. Approximately 2 ml DI H2O was added and the mixture was cooled at 5 °C overnight. A grey precipitate was collected by filtration, and the product washed with DI H2O (2 x 2 ml). Yield: 0.027 g, 27 %. 1H NMR (600 MHz, DMF-d7) δ 5.26-5.18 (d, J = 45.3 Hz, 4H, NH2), 4.19 (m, 1H, CH), 2.93 (m, 2H, CH2), 2.85 (ddq, J = 13.1, 6.7, 3.9 Hz, 2H, CH2). 13C NMR (151 MHz, DMF-d7) δ 59.88 (CH), 46.54 (CH2). 195Pt NMR (129 MHz, DMF-d7) δ -2272. IR (ATR, cm⁻¹): 3324, 3182, 3118, 2108 (N3), 1586, 1258, 1172, 1027, 821. ESI-MS: m/z. 403.9 [M+Na]+, 784.9 [2M+Na]+. C30H32Cl2N8Pt[C+Na]⁺: 402.9781. Found: 403.9758.

**Route 2: Synthesis of cis-[Pt(2-azidopropane-1,3-diamine)(CBDCA)] and cis-[Pt(2-azidopropane-1,3-diamine)(Oxalate)]**

**cis-[Pt(2-azidopropane-1,3-diamine)Cl2] (6)**

To a solution of K2PtCl4 (0.300 g, 0.723 mmol, 1 eq.) in approx. 8 ml DI H2O was added KI (1.20 g, 7.23 mmol, 10 eq.) and the mixture was stirred for 2 h. A solution of 2-azidopropane-1,3-diamine dihydrochloride (0.0.136 g, 0.723 mmol, 1 eq.) in 3 ml aqueous NaOH (0.058 g, 1.45 mmol, 2 eq.) was then added dropwise to the stirring mixture. Subsequently, the entire mixture was then heated at 60 °C for 10 mins, cooled to 0 °C and filtered to obtain brown/tan solid product. Sequential washes with DI H2O, MeOH and Et2O (5 x 5 ml) followed by drying over vacuum afforded cis-[Pt(2-azidopropane-1,3-diamine)Cl2] as a brown solid. Yield: 0.352 g, 86% (from K2PtCl4).
cis-[Pt(2-azidopropane-1,3-diamine)(CBDCA)] (7, Pt-N₃-Carbo)⁶

7 was synthesised, with slight adaptation, according to the literature method previously reported by Urankar et al.⁶ cis-[Pt(2-azidopropane-1,3-diamine)]₂ (0.352 g, 0.624 mmol, 1 eq.) was suspended in approx. 30 ml DI H₂O and a 5 ml aqueous solution of AgNO₃ (0.212 g, 1.25 mmol, 2 eq.) was then added dropwise with stirring. The solution was stirred for 3 h before filtering through a 0.22 µm Millipore pad to remove AgI. To the filtrate was added 3 ml of an aqueous solution of cyclobutane-1,1-dicarboxylic acid (CBCDA) (0.090 g, 0.624 mmol, 1 eq.) and NaOH (0.050 g, 1.25 mmol, 2 eq.). The solution was stirred for approx. 18 h in the dark and filtered through a 0.22 µm Millipore pad prior to the removal of solvent to yield a crude grey residue. 5 ml DI H₂O was added to the residue and cooled at 5 °C before vacuum filtration, washed with 5x5 ml H₂O, MeOH and Et₂O and finally dried to obtain an off-white solid.

Yield: 0.118 g, 36 % (from K₂PtCl₄). ¹H NMR (600 MHz, DMSO-d₆) δ 5.45 (s, 2H, NH₂), 5.28 (s, 2H, NH₂), 3.91 (s, 1H, CH), 2.65 (q, 4H, (CH₂)₂), 2.53 (m, 2H, CH₂), 2.48 (m, 2H, CH₂), 1.64 (q, 2H, CH₂). ¹³C NMR (151 MHz, DMSO-d₆): 177.89 (C, carbonyl), 59.16 (CH, N₃), 56.06 (C, CBDCA), 45.86 (CH₂, N₃), 31.04 (CH₂, CBDCA), 30.71 (CH₂, CBDCA), 15.45 (CH₂, CBDCA). ¹⁹⁵Pt NMR (129 MHz, DMSO-d₆) δ -1942. IR (ATR, cm⁻¹): 3217, 3092, 2938, 2120 (N₃), 1622, 1576, 1342, 1268, 1106, 1024, 900, 768. ESI-MS: m/z. 453.1 [M+H]+, 475.1 [M+Na]+. ESI-MS calc. for C₉H₁₆N₅O₄₁⁹⁵Pt+ [M+H]+: 453.0850. Found: 453.0846.

cis-[Pt(2-azidopropane-1,3-diamine)(Oxalate)] (8, Pt-N₃-Oxali)

Reaction protocol was performed as reported in (7) with only minor changes. After the removal of AgI, an aqueous solution (4 ml) of sodium oxalate (0.188 g, 1.40 mmol, 1 eq.) was added to the retained filtrate and the entire solution was stirred for approx. 18 h in the dark. The solution was filtered through a 0.45 µm Millipore pad to obtain a grey solid. Yield: 0.405 g, 72% (from K₂PtCl₄). ¹H NMR (600 MHz, DMSO-d₆) δ 5.76 (dt, J = 10.5, 4.5 Hz, 2H, NH₂), 5.46 (dt, J = 15.3, 5.1 Hz, 2H, NH₂), 3.99 (m, 1H, CH), 2.63-2.56 (m, 2H, CH₂), 2.55-2.51 (m, 2H, CH₂). ¹³C NMR (151 MHz, DMSO-d₆): 166.57 (C, carbonyl), 58.89 (CH), 45.48 (CH₂). ¹⁹⁵Pt NMR (129 MHz, DMSO-d₆) δ -1968. IR (ATR, cm⁻¹): 3177, 3092, 2938, 2120 (N₃), 1622, 1576, 1342, 1268, 1106, 1024, 900, 768. ESI-MS: m/z. 453.1 [M+H]+, 475.1 [M+Na]+. ESI-MS calc. for C₇H₁₀N₅O₄₁⁹⁵Pt+ [M+H]+: 453.0850. Found: 453.0846.
S-3: NMR spectroscopy of azide-functionalised ligands and platinum(II) complexes

Figure S-2. 600 MHz (CDCl₃) $^1$H NMR for Di-tert-boc-2-hydroxy-1,3-diaminopropane (1).

Figure S-3. 151 MHz (CDCl₃) $^{13}$C NMR for Di-tert-boc-2-hydroxy-1,3-diaminopropane (1).
Figure S-4. 600 MHz (CDCl₃) ¹H NMR for Di-tert-boc-2-methanesulfonyl-1,3-diaminopropane (2).

Figure S-5. 151 MHz (CDCl₃) ¹³C NMR for Di-tert-boc-2-methanesulfonyl-1,3-diaminopropane (2).
Figure S-6. 600 MHz (CDCl₃) $^1$H NMR for Di-tert-boc-2-azido-1,3-diaminopropane (3).

Figure S-7. 151 MHz (CDCl₃) $^{13}$C NMR for Di-tert-boc-2-azido-1,3-diaminopropane (3).
Figure S-8. 600 MHz (DMSO-$d_6$) $^1$H NMR for 2-azidopane-1,3-diamine dihydrochloride (4).

Figure S-9. 151 MHz (DMSO-$d_6$) $^{13}$C NMR for 2-azidopane-1,3-diamine dihydrochloride (4).
Figure S-10. 600 MHz (DMF-$d_7$) $^1$H NMR for cis-[Pt(2-azidopropane-1,3-diamine)Cl$_2$] (5).

Figure S-11. 151 MHz (DMF-$d_7$) $^{13}$C NMR for cis-[Pt(2-azidopropane-1,3-diamine)Cl$_2$] (5).
Figure S-12. 129 MHz (DMF-d₇) $^{195}$Pt NMR for cis-[Pt(2-azidopropane-1,3-diamine)Cl₂] (5).

Figure S-13. ESI-MS for cis-[Pt(2-azidopropane-1,3-diamine)Cl₂] (5).
Figure S-14. 600 MHz (DMSO-d$_6$) $^1$H NMR for cis-[Pt(2-azidopropane-1,3-diamine)CBDCA] (7).

Figure S-15. 151 MHz (DMSO-d$_6$) $^{13}$C NMR for cis-[Pt(2-azidopropane-1,3-diamine)CBDCA] (7).
**Figure S-16.** 129 MHz (DMSO-de) $^{195}$Pt NMR for cis-[Pt(2-azidopropane-1,3-diamine)CBDCA] (7).

**Figure S-17.** ESI-MS for cis-[Pt(2-azidopropane-1,3-diamine)CBDCA] (7).
Figure S-18. 600 MHz (DMSO-d6) $^1$H NMR for cis-[Pt(2-azidopropane-1,3-diamine)Oxalate] (8).

Figure S-19. 151 MHz (DMSO-d6) $^{13}$C NMR for cis-[Pt(2-azidopropane-1,3-diamine)Oxalate] (8).
Figure S-20. 129 MHz (DMSO-d$_6$) $^{195}$Pt NMR for cis-[Pt(2-azidopropane-1,3-diamine)Oxalate] (8).

Figure S-21. ESI-MS for cis-[Pt(2-azidopropane-1,3-diamine)Oxalate] (8).
S-4: Mass spectroscopy data of oligonucleotides

Table S-4. Oligonucleotide, Pt(II)-hybrid oligonucleotide sequences and modifications. Calculated and observed m/z: (a) = -71 m/z caused by loss of the chloride ligands during ionisation process; (b) = -88 m/z caused by loss of oxalate ligand during ionisation process. 5’-dC = 5’-(5)octadiynyl-deoxycytidine; int-dU = internal-octadiynyl-deoxyuridine; 5’-BCN-dC = 5’-BCN(CEP II)-deoxycytidine; \( N_2\)-Pt(II)-Cis = cis-[Pt(2-azidopropane-1,3-diamine)Cl]; \( N_2\)-Pt(II)-Carbo = cis-[Pt(2-azidopropane-1,3-diamine)(CBDCA)]; \( N_2\)-Pt(II)-Oxali = cis-[Pt(2-azidopropane-1,3-diamine)(Oxalate)]; DA tgt. = Duplex A polypurine target strand; DA comp. = Duplex A poly pyrimidine complementary strand. Duplex A is the target sequence for oligos TFO1-TFO3. DB and DC = Duplex B and Duplex C. Duplex B is the target sequence for oligos TFO4-TFO5. Duplex C is the target sequence for TFO6.

| Oligo   | Sequence                                                                 | Modification (X)(C)(U) | Calc. Mass | Obs. Mass |
|---------|---------------------------------------------------------------------------|------------------------|------------|-----------|
| TFO1    | 5’- CTC TTT CCT TCC TTT CTT CTT TCG TCT TCC TC -3’                      | 5’-dC                  | 8694.1     | 8694.3    |
| TFO1-Cis| 5’ - CTC TTT CCT TCC CTT CTT TCG TCT TCC TC -3’                         | 5’-dC-N\(_2\)-Pt(II)-Cis | 9075       | 9001.7\(\^a\) |
| TFO1-Carbo | 5’ - CTC TTT CCT TCC CTT CTT TCG TCT TCC TC -3’                         | 5’-dC-N\(_2\)-Pt(II)-Carbo | 9146       | 9146      |
| TFO1-Oxali | 5’ - CTC TTT CCT TCC CTT CTT TCG TCT TCC TC -3’                         | 5’-dC-N\(_2\)-Pt(II)-Oxali | 9091.2     | 9002\(\^b\) |
| TFO1-pdU | 5’- CTC TTT CUC TCC CTT CTT TCG TCT TCC TC -3’                         | 5’-dC, pdU             | 8733       | 8733.1    |
| TFO2-pdU | 5’- CTC TTT CUC TCC CTU CTT TCG TCT TCC TC -3’                         | 5’-dC, pdU (x2)        | 8772       | 8772      |
| TOTFO1  | 5’- CTC TTT CUC TCC CTT CTT TCG TCT TCC TC -3’                         | 5’-dC, pdU-TO          | 9120       | 9119.7    |
| TOTFO1-Carbo | 5’ - CTC TTT CUC TCC CTT CTT TCG TCT TCC TC -3’                         | 5’-dC-N\(_2\)-Pt(II)-Carbo, pdU-TO | 9572       | 9571.7    |
| TOTFO2  | 5’- CTC TTT CUC TCC CTT CTT TCG TCT TCC TC -3’                         | 5’-dC, pdU-TO (x2)    | 9546       | 9545      |
| TOTFO2-Carbo | 5’ - CTC TTT CUC TCC CTT CTT TCG TCT TCC TC -3’                         | 5’-dC-N\(_2\)-Pt(II)-Carbo, pdU-TO (x2) | 9998       | 9997.2    |
| TFO2    | 5’ - XTC TTT CCT TCC CTT CTT TCG TCT TCC TC -3’                         | 5’-BCN-dC              | 8932       | 8933.6    |
| TFO2-Cis| 5’ - XTC TTT CCT TCC CTT CTT TCG TCT TCC TC -3’                         | 5’-BCN-dC-N\(_2\)-Pt(II)-Cis | 9313       | 9240\(\^a\) |
| TFO2-Carbo | 5’ - XTC TTT CCT TCC CTT CTT TCG TCT TCC TC -3’                         | 5’-BCN-dC-N\(_2\)-Pt(II)-Carbo | 9384       | 9385.1    |
| TFO2-Oxali | 5’ - XTC TTT CCT TCC CTT CTT TCG TCT TCC TC -3’                         | 5’-BCN-dC-N\(_2\)-Pt(II)-Oxali | 9330       | 9240.8\(\^b\) |
| TFO3    | 5’ - CTC TTT CCT TCC CTT CTT TCG TCT UCC TC -3’                         | Int-dU                 | 8680       | 8680.5    |
| TFO3-Cis| 5’ - CTC TTT CCT TCC CTT CTT TCG TCT UCC TC -3’                         | Int-dU-N\(_2\)-Pt(II)-Cis | 9061       | 9087.8\(\^a\) |
| TFO3-Carbo | 5’ - CTC TTT CCT TCC CTT CTT TCG TCT UCC TC -3’                         | Int-dU-N\(_2\)-Pt(II)-Carbo | 9132       | 9132      |
| TFO3-Oxali | 5’ - CTC TTT CCT TCC CTT CTT TCG TCT UCC TC -3’                         | Int-dU-N\(_2\)-Pt(II)-Oxali | 9078       | 8987.8\(\^b\) |
|                  | 5’- |                  | 3’- |                  |
|------------------|-----|------------------|-----|------------------|
| **DA tgt.**      | GAA GGG AAG AAA GCG AAA | 5’- ACC GTC GCG AGA AAG | 3’- TGG CAC CGC TCT TTC |
|                  | GGA GCG G -3’ | | |
| **DA comp.**     | CTT CCC TCT TTT GCG TTT | | CTT CCC C -5’ |
|                  | - | | |
| **TF04**         | 5’- | CTT CCC TCT TCT CGC | 5’-dC | 6315 | 6315.7 |
|                  | CTG TCC -3’ | | |
| **TF04-Cis**     | 5’- | CTT CCC TCT TCT CGC | 5’-dC-N$_2$-Pt(II)-Cis | 6696 | 6623 |
|                  | CTG TCC -3’ | | |
| **TF04-Carbo**   | 5’- | CTT CCC TCT TCT CGC | 5’-dC-N$_2$-Pt(II)-Carbo | 6767 | 6767 |
|                  | CTG TCC -3’ | | |
| **TF04-Oxali**   | 5’- | CTT CCC TCT TCT CGC | 5’-dC-N$_2$-Pt(II)-Oxali | 6713 | 6623.2 |
|                  | CTG TCC -3’ | | |
| **TF05**         | 5’- | CTT CCC TCT TUC CGC | Int-dU | 6301 | 6301.7 |
|                  | CTG TCC -3’ | | |
| **TF05-Cis**     | 5’- | CTT CCC TCT TUC CGC | Int-dU-N$_2$-Pt(II)-Cis | 6682 | 6608.9 |
|                  | CTG TCC -3’ | | |
| **TF05-Carbo**   | 5’- | CTT CCC TCT TUC CGC | Int-dU-N$_2$-Pt(II)-Carbo | 6753 | 6753 |
|                  | CTG TCC -3’ | | |
| **TF05-Oxali**   | 5’- | CTT CCC TCT TUC CGC | Int-dU-N$_2$-Pt(II)-Oxali | 6699 | 6608.9 |
|                  | CTG TCC -3’ | | |
| **DB tgt.**      | GAA AGG CGG ACA GGT AT | 5’- CGC TTC CGG AAG GGA | 3’- GCG AAG GGC TTC CCT |
|                  | - | | |
| **DB comp.**     | CTT TCC GCC TGT CTA TA | | 5’- |
|                  | - | | |
| **TF06**         | 5’- | UAA GGG AAA GAA GGG | 5’-dU | 7696 | 7696.7 |
|                  | AAG GAA AGA -3’ | | |
| **DC tgt.**      | AAG GGA AGA AAG CGA AAG | 5’-CCG TGG CGA GAA AGG | GA-3’ |
|                  | - | | |
| **DC comp.**     | TTC CCT TCT TCT GCT TTC | | CT-5’ |
|                  | - | | |
| **57 bp tgt.**   | 5’- | AAG CGG CGG AAC GTG | 5’- AAG CGG AGG GAA GAG | 3’- GCG AGG AAA GGA CGC GGC |
|                  | GGC AGG AAG GAA GGG AGG | | GCT AGG -3’ |
|                  | - | | |
| **57 bp comp.**  | 3’- | TTC GGC CCC TGC CAC | 3’- TTC GGC CCC TGC CAC | |
|                  | TTT GGC TCT TCT CCC TTC | | |
|                  | - | | |
| **D3 tgt.**      | AAG GAA GGG AAG AAA GCG | 5’-Cy5- ACC GTC GCG AGA | 5’-Cyanine 5 label | 13157.9 | 13156.9 |
|                  | AAA GGA GCG G -3’ | | |
| **D3 comp.**     | CTT CCC TCT TTT GCG TTT | | 5’-6-FAM label | 12507.2 | 12505.8 |
|                  | CCT GCC C -6FAM-5’ | | |
| **40 bp off tgt.** | 5’- | TGA CTC CCC GTC GTG | 5’- | 12336.9 | |
Figure S-22. A. ESI-MS analysis of alkyne-modified TFO3. B. TFO3-Carbo platinum(II)-TFO hybrid.
S-5: Platinum(II)-TFO hybrid click chemistry yields

Table S-5. 5'-dC = 5'-octadiynyl-deoxycytidine; int-dU = internal-octadiynyl-deoxyuridine; 5'-BCN-dC = 5'-BCN(CEP II)-deoxycytidine; pdU = 5-(1-propargylamino)-deoxyuridine; pdU-TO = pdU with TO (thiazole orange) coupled; \(N_3\)-Pt(II)-Cis = \(cis\)-[Pt(2-azidopropane-1,3-diamine)Cl2]; \(N_3\)-Pt(II)-Carbo = \(cis\)-[Pt(2-azidopropane-1,3-diamine)(CBDCA)]; \(N_3\)-Pt(II)-Oxali = \(cis\)-[Pt(2-azidopropane-1,3-diamine)(Oxalate)]. Platinum(II)-TFO hybrid yields were calculated using obtained absorbance values after quantification on a NanoDrop ND-1000 UV-Vis Spectrophotometer.

| Oligo    | Modification                  | Yield (%) |
|----------|-------------------------------|-----------|
| TFO1-Cis | 5'-dC-N3-Pt(II)-Cis           | 18.7      |
| TFO1-Carbo | 5'-dC-N3-Pt(II)-Carbo         | 28.3      |
| TFO1-Oxali | 5'-dC-N3-Pt(II)-Oxali       | 48.2      |
| TFO2-Cis | 5'-BCN-dC-N3-Pt(II)-Cis      | n/a       |
| TFO2-Carbo | 5'-BCN-dC-N3-Pt(II)-Carbo  | 63.5      |
| TFO2-Oxali | 5'-BCN-dC-N3-Pt(II)-Oxali  | 57.1      |
| TFO3-Cis | Int-dU-N3-Pt(II)-Cis           | 34.4      |
| TFO3-Carbo | Int-dU-N3-Pt(II)-Carbo         | 35.7      |
| TFO3-Oxali | Int-dU-N3-Pt(II)-Oxali      | 49.1      |
| TFO4-Cis | 5'-dC-N3-Pt(II)-Cis           | 21.0      |
| TFO4-Carbo | 5'-dC-N3-Pt(II)-Carbo         | 18.1      |
| TFO4-Oxali | 5'-dC-N3-Pt(II)-Oxali       | 49.5      |
| TFO5-Cis | Int-dU-N3-Pt(II)-Cis           | 34.1      |
| TFO5-Carbo | Int-dU-N3-Pt(II)-Carbo         | 37.9      |
| TFO5-Oxali | Int-dU-N3-Pt(II)-Oxali      | 41.3      |
| TOTFO1-Carbo | 5'-dC-N3-Pt(II)-Carbo       | 43.4      |
| TOTFO2-Carbo | 5'-dC-N3-Pt(II)-Carbo       | 32.7      |
| TFO-17A-Carbo | 5'-dU-N3-Pt(II)-Carbo    | 36.3      |
| TFO-17B-Carbo | 3'-dU-N3-Pt(II)-Carbo       | 30.1      |
| TFO-17C-Carbo | Int-dU-N3-Pt(II)-Carbo     | 32.4      |
S-6: UV Thermal Melting Analysis

Table S-6. T_M values were recorded in 10 mM phosphate (PO_4^3-), 150 mM NaCl, 2 mM MgCl_2, pH 6 buffer. Samples were incubated for 48 h prior to melting analysis. T_M temperatures were calculated by the first derivative of a non-linear sigmoidal regression curve using GraphPad Prism 8.0. Final T_M values are an average of 3 melting cycles. ΔT_m values for Pt(II)TFOs are relative to the unclicked alkyne TFO precursor (e.g. TFO1 and TFO1-Carbo). 5'-dC = 5'-(5)octadiynyl-deoxycytidine; int-dU = internal-octadiynyl-deoxuridine; 5'-BCN-dC = 5'-BCN(CEP II)-deoxycytidine; pdU = 5-(1-propargylamino)-deoxuridine; pdU-TO = pdU with TO (thiazole orange) coupled; N_3-Pt(II)-Cis = cis-[Pt(2-azidopropane-1,3-diamine)Cl]; N_3-Pt(II)-Carbo = cis-[Pt(2-azidopropane-1,3-diamine)(CBDCA)]; N_3-Pt(II)-Oxali = cis-[Pt(2-azidopropane-1,3-diamine)(Oxalate)]; n.t.o. = no triplex observed; n.d. = not determined due to T_M < 12 ºC.

| Oligo   | Modification | T_M (ºC) - 24 h | ΔT_M - 24 h | T_M (ºC) - 48 h | ΔT_M - 48 h |
|---------|--------------|----------------|-------------|----------------|-------------|
| TFO1    | 5'-dC        | 45.6 ± 0.2     | -           | 49.6 ± 0.3     | -           |
| TFO1-Cis| 5'-dC-N_3-Pt(II)-Cis | n.t.o.         | -           | n.t.o.         | -           |
| TFO1-Carbo| 5'-dC-N_2-Pt(II)-Carbo | 49.2 ± 1.3    | 3.7         | 49.2 ± 0.7     | -0.4        |
| TFO1-Oxali| 5'-dC-N_2-Pt(II)-Oxali | 45.9 ± 0.3    | 0.3         | 43.5 ± 0.9     | -6.1        |
| TOTFO1-Carbo | 5'-dC-N_2-Pt(II)-Carbo | pdU-TO (x1)  | 55.6 ± 0.3  | 10.0          | 57.2 ± 0.7  | 11.6        |
| TOTFO2-Carbo | 5'-dC-N_2-Pt(II)-Carbo | pdU-TO (x2)  | > 65.0      | -             | > 65.0      | -           |
| TFO2    | 5'-BCN-dC    | 48.3 ± 0.2     | -           | 51.4 ± 0.5     | -           |
| TFO2-Cis| 5'-BCN-dC-N_3-Pt(II)-Cis | n.t.o.         | -           | n.t.o.         | -           |
| TFO2-Carbo| 5'-BCN-dC-N_2-Pt(II)-Carbo | 47.2 ± 0.7    | -1.1        | 46.8 ± 0.1     | -4.6        |
| TFO2-Oxali| 5'-BCN-dC-N_2-Pt(II)-Oxali | 44.7 ± 0.5    | -3.5        | n.t.o.         | -           |
| TFO3    | Int-dU       | 48.6 ± 0.2     | -           | 52.0 ± 0.1     | -           |
| TFO3-Cis| Int-dU-N_3-Pt(II)-Cis | 42.7 ± 1.4    | -5.9        | 42.6 ± 0.5     | -9.4        |
| TFO3-Carbo| Int-dU-N_2-Pt(II)-Carbo | 46.3 ± 0.3    | -2.3        | 45.5 ± 0.1     | -6.6        |
| TFO3-Oxali| Int-dU-N_2-Pt(II)-Oxali | 44.0 ± 0.4    | -4.6        | 44.1 ± 0.6     | -7.9        |
| TFO4    | 5'-dC        | n.d.           | -           | n.d.           | -           |
| TFO4-Cis| 5'-dC-N_3-Pt(II)-Cis | n.t.o.         | -           | n.t.o.         | -           |
| TFO4-Carbo| 5'-dC-N_2-Pt(II)-Carbo | n.t.o.         | -           | n.t.o.         | -           |
| TFO4-Oxali| 5'-dC-N_2-Pt(II)-Oxali | n.t.o.         | -           | n.t.o.         | -           |
| TFO5    | Int-dU       | 19.8 ± 0.2     | -           | 22.9 ± 0.2     | -           |
| TFO5-Cis| Int-dU-N_3-Pt(II)-Cis | n.d.           | -           | n.d.           | -           |
| TFO5-Carbo| Int-dU-N_2-Pt(II)-Carbo | 23.0 ± 0.2    | 3.2         | 22.1 ± 0.3     | -0.9        |
| TFO5-Oxali| Int-dU-N_2-Pt(II)-Oxali | n.d.           | -           | 20.7 ± 1.0     | -2.2        |
Table S-7. $T_M$ values were recorded in triplicate in 10 mM phosphate ($\text{PO}_4^{3-}$), 150 mM NaCl, 2 mM MgCl$_2$, pH 6 buffer. Samples were incubated at 37 °C for 48 h prior to melting analysis. $T_M$ temperatures were calculated by the first negative derivative of the melting curve using GraphPad Prism 8.0 software. Final $T_M$ values are an average of 3 melting cycles.

| Oligo | $T_M$ (°C) | $T_M$ (°C) - + Cis | $\Delta T_M$ (°C) - +Cis |
|-------|------------|---------------------|--------------------------|
| Target A | 82.46 ± 0.01 | 56.40 ± 2.34 | -26.06 |
| Target B | 79.97 ± 0.03 | 69.19 ± 1.10 | -10.78 |
| Target C | 79.80 ± 0.06 | 75.07 ± 0.19 | -4.73 |

Figure S-23. A. High resolution fluorescent melting of Duplex A-C controls with and without cisplatin treatment. Melting curves are normalised with control melts (black) plotted in comparison to duplex melts after 48h cisplatin incubation (blue). B. First negative derivative of normalised fluorescence of duplex melting curves.
S-8: PAGE analysis of triplex formation

**Figure S-24.** A. Triplex formation for TFO2. Target duplex (57 bp, 1 pmol) and off-target (40 bp, 1 pmol) treated with increasing concentrations of TFO2 (2.5-50 eq., lanes 4-8) in triplex buffer. No off-target selectivity observed during triplex formation. B. Triplex formation for TFO3. PAGE analysis for both TFO probes was performed in TA triplex buffer (10 mM phosphate 150 mM NaCl 2 mM MgCl₂, pH 6) at 70 V for 240 mins.

**Figure S-25.** A. Triplex formation for TOTFO1-3 Carbo (50 eq., Lanes 7-9). TOTFO2 displays enhanced triplex formation compared with TOTFO1-Carbo and TOTFO3-Carbo. Gel is post-stained with SYBRGold stain. B. Non-stained visualisation of the same gel. TOTFO2-Carbo displays enhanced fluorescent emission. PAGE analysis was performed in TA triplex buffer (10 mM phosphate 150 mM NaCl 2 mM MgCl₂, pH 6) at 70 V for 240 mins. C. Propargylamino-dU nucleobase modification with coupled TO₈₆.
Figure S-26. Optimisation of triplex reversal with sodium cyanide. Triplex formation for TFO3 (50 eq.) with fluorescently labelled Duplex 3 (1 eq., 1 pmol) (Lane 3). Introduction of increasing concentrations of NaCN solution (1000-300000 pmol, Lanes 4-13). PAGE performed in TA triplex buffer (10 mM phosphate 150 mM NaCl 2 mM MgCl₂, pH 6) at 70 V for 240 mins. Gel visualised and imaged using Cy5 filter. A portion of this figure is duplicated in Figure 7B (Cy5) within the main manuscript.
S-9: Raw PAGE Images

Table S-9A. Raw PAGE analysis images for manuscript Figures 6 & 7C. Images were visualised using a Syngene G:Box Mini 9 gel documentation system. Raw PAGE images for: A. Manuscript Figure 6B. B. Manuscript Figure 6C. C. Manuscript Figure 6D. D. Manuscript Figure 7C (Cy5). E. Manuscript Figure 7C (FAM). F. Manuscript Figure 7C (Multiplex).
Table S-9B. Raw PAGE analysis images for manuscript Figure 8. Images were visualised on a Syngene G:Box Mini 9 gel documentation system. Raw PAGE images for: A. Manuscript Figure 8D (Multiplex). B. Manuscript Figure 8D (Cy5). C. Manuscript Figure 8D (FAM).
S-10. General summary regarding CuAAC and SPAAC strategies

Overall, we investigated both CuAAC and SPAAC click chemistry strategies to generate platinum(II) hybrids with the latter displaying higher reaction yields when quantified using UV absorption (Table S-5). The use of an ascorbate reductant (required for the CuAAC reaction) may be responsible for lower yields due to unwanted side reaction with both the platinum(II) complex and the TFO probe. The use of strain-promoted azide-alkyne cycloaddition (SPAAC) may therefore offer advantages in the design of future probes offering potential assembly in living systems.[7] Several platinum(II)-TFO hybrids incorporating Pt-N3-Cis did not form effective DNA triplexes which may result from the promiscuity of the cisplatin-type moiety and the plethora of binding opportunities present within the triplex recognition site, thereby overriding Hoogsteen interactions derived from the TFO. Of the platinum(II)-TFOs that formed triplexes (i.e. Pt-N3-Carbo, Pt-N3-Oxali, and several Pt-N3-Cis hybrids), all displayed destabilising effects associated with Pt(II) crosslinking. However, no off-target effects were observed in the presence of GG/AG rich sequences lacking the triplex recognition site. Importantly, the introduction of thiazole orange (TO) into the TFO probe reverses triplex destabilisation with the inclusion of two TO units pushing triplex stability towards that of the underlying duplex target.

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