Click chemistry-based biomimetic ligands efficiently capture G-quadruplexes in vitro and help localize them at DNA damage sites in human cells

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I. Chemistry

Ia. Generalities. TSTU was purchased from Iris Biotech GmbH. Solvents were purchased from VWR or Carlo Erba. All other chemicals were from Sigma Aldrich, Acros organics, or Fisher Scientific. Cyclen was a gift from Chematech (Dijon, France). Media and supplements for cell culture were bought from Dutcher SAS. Unless noted otherwise, all commercially available reagents and solvents were used without further purification. Dicalite was purchased from Carlo Erba. TLC were carried out on Merck DC Kieselgel 60 F-254 aluminum sheets. The spots were visualized via staining with ninhydrin or through illumination with a UV lamp (λ = 254 nm). Column chromatography purifications were performed manually on silica gel (40-63 μm) from Sigma-Aldrich (technical grade). Dry CH₂Cl₂ (HPLC-grade) was dried over alumina cartridges using a solvent purification system PureSolv PS-MD-5 model from Innovative Technology. HPLC-gradient grade CH₃CN used for HPLC-MS analyses was obtained from Carlo Erba. CH₃CN used in semi-preparative RP-HPLC purifications was obtained from VWR (technical, >99% but distilled prior to use). All aq. mobile-phases for HPLC were prepared using water purified with a PURELAB Ultra system from ELGA (purified to 18.2 MΩ.cm). Yields were calculated based on isolation of the compounds.
Ib. Instruments and methods. Lyophilization was performed with a Christ Alpha 2-4 LD plus. \(^1\)H-, and \(^13\)C- NMR spectra were recorded on Bruker spectrometers, an Avance Neo 500 MHz equipped with a 5 mm BBOF iProbe and Avance III HD. Chemical shifts are expressed in parts per million (ppm) from the residual non-deuterated solvent signal summarized in 2010 by Fulmer et al. (*Organometallics* 2010, 29, 2176-2179) \(J\) values are expressed in Hz. Coupling constants \((J)\) are reported in hertz (Hz). Standard abbreviations indicating multiplicity were used as follows: \(s = \) singlet, \(d = \) doublet, \(t = \) triplet, \(q = \) quadruplet, \(m = \) multiplet, \(br = \) broad. High-resolution mass spectrometry analyses were recorded on a LTQ Orbitrap XL mass spectrometer (Thermo Scientific) equipped with an electrospray ionization source (HESI 2). The following source parameters were used if no further specification is mentioned: Heater Temperature: 50°C, Gas Flow: Sheath 15 / Aux 10 / Sweep 0, Spray Voltage: 4 kV, Capillary Temperature: 275°C, Capillary Voltage: 22 V, Resolution \((m/z = 400): 60\,000.\) HPLC-MS analyses were performed on a Thermo-Dionex Ultimate 3000 instrument (pump + autosampler at 20 °C + column oven at 25 °C) equipped with a diode array detector (Thermo-Dionex DAD 3000-RS) and a MSQ Plus single quadrupole mass spectrometer. The corresponding low-resolution mass spectra (LRMS) were recorded with this latter mass spectrometer, with an electrospray (ESI) source (HPLC-MS coupling mode). HPLC systems were equipped with a Phenomenex Kinetex C18 column, 2.6μm, 2.1 × 50 mm or a Jupiter Proteo 4 μm 90Å column, 250 x 4.6 mm. Two analytical methods: Method A: from 5% to 100% MeCN/H\(_2\)O+0.1% formic acid (FA) in 5 min; Method B: from 5% to 15% MeCN/H\(_2\)O+0.1% FA in 5 min, from 15% to 70% MeCN/ H\(_2\)O+0.1% FA in 20 min, from 70% to 100% MeCN/ H\(_2\)O+0.1% FA in 3 min. Final compounds (TriazoTASQ and BiotriazoTASQ) were analysed with a ACCUCORE column, method: from 0% to 20% MeCN/H\(_2\)O+0.1% formic acid (FA) in 2.4 min and from 20% to 100% MeCN/H\(_2\)O+0.1% formic acid (FA) in 2.6 min. Purifications by semi-preparative HPLC were performed on a Thermo-Dionex Ultimate 3000 instrument equipped with a RS Variable Detector (four distinct wavelengths). HPLC system was equipped with a Jupiter Proteo 4 μm 90Å column (250 x 21.2 mm, AXIA packed).

Ic. Synthesis and characterizations.

5-azidopentyl methanesulfonate: To a solution of 5-aminopentanol (1.0 g, 9.6 mmol, 1 equiv.) and 1H-imidazole-1-sulfonyl azide·H\(_2\)SO\(_4\) (2.6 g, 9.6 mmol, 1 equiv.) in MeOH (100 mL) was added triethylamine (6.6 mL, 48.2 mmol, 5 equiv.). The reaction was stirred for 1 h, after which 2 additional equiv. of triethylamine were added in order to restor basic pH. The reaction was stirred overnight and completion was assessed by TLC (CH\(_2\)Cl\(_2\)/MeOH 9:1). The solvent was evaporated and 50 mL of EtOAc and 100 mL of an aq. HCl 1M solution were then added to the
mixture. The aqueous phase was extracted with EtOAc (3 x 30 mL). The organic phases were pooled together, dried over MgSO₄, filtered and dried under vacuum. The crude product was dissolved in a mixture of THF (100 mL) and triethylamine (6.6 mL, 48.2 mmol, 5 equiv.), and MsCl (1.5 mL, 19.3 mmol, 2 equiv.) was added slowly at 0 °C. After 2 hours, TLC analysis (9.5/0.5, CH₂Cl₂/MeOH) indicated a partial conversion of the starting material only. In order to reach completion, additional equiv. of triethylamine (3 mL) and pyridine (10 mL) were added to the mixture, stirred at RT, and the reaction followed by TLC. The solvent was evaporated, the mixture was then added 50 mL of EtOAc and 100 mL of an aqueous solution of HCl 1M. The aqueous phase was extracted 3 times with EtOAc (30 mL each); the organic phases were pooled together, dried over MgSO₄, filtered and dried under vacuum. The residue was purified by silica gel column (CH₂Cl₂/MeOH 0% to 4%) to afford 5-azidopentyl methanesulfonate (836.1 mg, 4.1 mmol, 42 % yield over two steps). ¹H NMR (500 MHz, Chloroform-d) δ 4.19 (t, J = 6.5 Hz, 2H), 3.26 (t, J = 6.5 Hz, 2H), 2.97 (s, 3H), 1.75 (dt, J = 14.0, 6.5 Hz, 2H), 1.60 (dt, J = 14.0, 6.5 Hz, 2H), 1.47 (dt, J = 8.0, 6.5 Hz, 2H). ¹³C NMR (126 MHz, Chloroform-d) δ 69.7, 51.1, 37.3, 28.6, 28.2, 22.7. IR 2092.10 cm⁻¹ (azide), 1168.46 cm⁻¹ (sulfonyl).

**Compound 1:** To a solution of cyclen (72 mg, 0.4 mmol, 1 equiv.) in acetonitrile (6 mL) were added 5-azidopentyl methanesulfonate (499 mg, 2.4 mmol, 6 equiv.) and K₂CO₃ (452 mg, 3.3 mmol, 8 equiv.). The reaction was heated at 50 °C overnight and complete conversion was assessed by HPLC-MS. The mixture was filtered and the solvent evaporated under vacuum. The crude material was purified by RP-HPLC (H₂O/MeCN + 0.1% TFA, gradient from 5% to 60% over 45 minutes). The solvent was removed by freeze-drying to provide compound 1 (65 mg, 0.1 mmol, 25% yield). ¹H NMR (500 MHz, Chloroform-d) δ 3.24 (t, J = 6.5 Hz, 8H), 3.11 – 2.86 (m, 24H), 1.75 – 1.53 (m, 16H), 1.48 – 1.30 (m, 8H). ¹³C NMR (126 MHz, Chloroform-d) δ 53.2, 51.1, 48.5, 28.4, 24.2, 21.6. ESI-HRMS [M+H]+ m/z = 617.49366 (calcd. for C₂₈H₂₇N₁₆: 617.49466). HPLC-MS (Method A): retention time = 3.76 min; purity: >94% at 201 nm; (Method B): retention time = 7.94 min; purity: >98% at 201 nm

**Compound 2:** Boc-G₁₈NA-OH (199 mg, 0.5 mmol, 1 equiv.), 2-propyn-amine (67 μL, 1.0 mmol, 2 equiv.) and HBTU (384 mg, 1.0 mmol, 2 equiv.) were dissolved in anhydrous DMF (5 mL). DIPEA was added (250 μL, 1.5 mmol, 3 equiv.) and the solution stirred for 1 hour at RT (the complete conversion was assessed by HPLC-MS). The solution was then concentrated under vacuum, and compound 2 purified by RP-HPLC in a H₂O/MeCN + 0.1% TFA mixture (gradient from 2% to 35% over 45 minutes), obtained as a white solid after lyophilization (102 mg, 0.2 mmol, 47 % yield). ¹H NMR (500 MHz, Methanol-d₄) Major cis/trans isomer: δ 8.43 (s, 1H), 5.24 (s, 2H), 4.09 (s, 2H), 3.98 (d, J = 2.5 Hz, 1H),
3.60 (t, J = 6.5 Hz, 1H), 3.38 (t, J = 6.5 Hz, 2H), 2.58 (t, J = 2.5 Hz, 1H), 1.42 (s, 9H). Minor isomer δ 8.47 (s, 1H), 5.10 (s, 2H), 4.27 (s, 2H), 4.07 (s, 2H), 3.46 (t, J = 6.4 Hz, 1H), 3.19 (t, J = 6.5 Hz, 2H), 2.67 (t, J = 2.5 Hz, 1H), 1.48 (s, 9H). ^13C NMR (101 MHz, Methanol-d4) δ 167.6, 166.9, 79.1, 78.9, 71.2, 71.0, 49.6, 48.3, 48.1, 47.8, 47.6, 47.4, 47.2, 47.0, 44.6, 38.4, 28.2, 27.5, 27.4. ESI-HRMS [M+Na]^+ m/z = 469.19193 (calcd. for C_{19}H_{26}N_{8}NaO_{5}: 469.19184). HPLC (Method A): retention time = 2.943 min; purity: >94% at 214 nm, >99% at 280 nm; (Method B): retention time = 4.203 min; purity: >99% at both 214 and 280 nm.

(Boc)_4TriazoTASQ·Cu(II) : In anhydrous DMF (0.7 mL) were mixed compound 1 (14.5 mg, 31 µmol, 4 equiv.), compound 2 (8.3 mg, 7.7 µmol, 1 equiv.) and tris-(benzyltriazolylmethyl)amine TBTA (2.1 mg, 3.8 µmol, 0.5 equiv.). The catalyst (MeCN)_4Cu·PF_6 (4.3 mg, 11.6 µmol, 1.5 equiv.) was added to the mixture which was stirred at RT for 3 hours (conversion assessed by HPLC-MS). The solvent was evaporated under vacuum and the crude product purified by RP-HPLC in a H_2O/MeCN + 0.1% TFA mixture (gradient of 5 to 60% over 45 minutes). After lyophilization of the solvents, (Boc)_4TriazoTASQ·Cu(II) was obtained (4.34 mg, 1.76 µmol, 23 % yield). The copper content (1.91 %) was assessed by ICP-MS. ESI-HRMS [M]^2+ m/z = 1233.11523 (calcd. for C_{104}Cu_{160}N_{48}O_{20}: 1232.11318), HPLC-MS (Method A): retention time = 3.80 min; purity: >98% at 280 nm.

Study of the conversion of cyclen to (Boc)_4TriazoTASQ·Cu(II)

The conversion of compound 1 to (Boc)_4TriazoTASQ·Cu(II) depending on the source of Cu(I) was studied with three sources of copper: in situ generated Cu(I) (obtained by mixing 1 equiv. of Cu(II) and sodium ascorbate), (MeCN)_4Cu·PF_6, and (MeCN)_4Cu·PF_6 and TBTA (as a stabilizing ligand of Cu(I) and accelerator of CuAAC). The kinetics of conversion (Fig S1) show that the conversion reached a plateau at 40 % after 150 min with conversion in situ generated copper (I) and addition of catalyst was required to complete the reaction. (MeCN)_4Cu·PF_6 provided the desired compound in approx. 6h, while the addition of TBTA strongly accelerated the conversion since half of the starting material reacted in 30 min vs. 60 for (MeCN)_4Cu·PF_6 alone.
Figure S1. HPLC monitoring of the formation of the metalated, protected TriazoTASQ as a function of the Cu source

Figure S2. HPLC profile of metallated, protected TriazoTASQ

Figure S3. HRMS profile profile of metallated, protected TriazoTASQ

\((\text{Boc})_4\text{TriazoTASQ} : (\text{Boc})_4\text{TriazoTASQ-Cu(II)}\) (4.34 mg, 1.76 \(\mu\)mol, 1 equiv.) was dissolved in MeCN (200 \(\mu\)L) and Na\(_2\)S (0.5 mg, 7 \(\mu\)mol, 4 equiv.) in H\(_2\)O (200 \(\mu\)L) was added. After 2 minutes, centrifugation was performed (4000 RPM, 15 min), the
supernatant removed and (Boc)_4TriazoTASQ isolated after lyophilization (4.24 mg, 1.76 µmol). Demetallation was monitored by HPLC and copper content (0.02 %) assessed by ICP-MS. Maldi-TOF [M+H]^+ m/z = 2403.38 (calcd. for C_{104}H_{161}N_{48}O_{20}: 2403.74) HPLC-MS (Method A): retention time = 3.580 min; purity: >93% at 214 nm; (Method B): retention time = 6.347 min; purity: >94% at 280 nm.

TriazoTASQ: (Boc)_4TriazoTASQ (1 mg, 0.4 µmol) was stirred in TFA (50 µL) at 25 °C for 1 hour. Complete conversion was assessed by HPLC and the mixture was diluted in water (100 µL). TriazoTASQ (1.86 mg, 0.756 µmol) was obtained after lyophilization. ESI-HRMS [M+2H]^2+ m/z = 1001.54657 (calcd. for C_{84}H_{130}N_{48}O_{12}: 1001.55134). HPLC-MS (Method B): retention time = 0.303 min; purity: >95% at 214 nm, >99% at 280 nm.
Biotin-AMC (described in S. Y. Yang, et al. Nat. Commun. 2018, 9, 4730): To the hydrochloride salt of AMC (349 mg, 0.9 mmol, 1 equiv.) was added an aqueous solution of NaOH 16 M (10 mL) then liquid-liquid extraction was performed with CHCl₃ (3 x 15 mL) to isolate the free base. The organic phase was dried with MgSO₄, filtered and the solvent was removed under
vacuum. Separately, to a solution of biotin (227 mg, 1 mmol, 1 equiv.) in anhydrous DMF (2 mL) was added TSTU (317 mg, 1 mmol, 1.1 equiv.) and DIPEA (321 µL, 1.85 mmol, 2 equiv.), and the mixture was stirred until a complete activation of the carboxylic acid monitored by HPLC-MS (3 h). The AMC was diluted in DMF (60 mL) and the solution of N-hydroxysuccimide ester of the biotin was added to the mixture dropwise (1 mL/hour) until the completion of the reaction (assessed by HPLC-MS). Solvents were evaporated under vacuum and the crude was then purified by preparative RP-HPLC in a H₂O/MeCN + 0.1% TFA mixture (gradient of 2 to 50 % over 45 minutes). After evaporation of the solvents, biotin-AMC (256.68 mg, 0.2906 mmol) was obtained in 32% yield. 

**Compound 3**: Biotin-AMC (100 mg, 0.1 mmol, 1 equiv.) was mixed with 5-azidopentyl methanesulfonate (140.8 mg, 0.7 mmol, 6 equiv.) in MeCN (3 mL). Then, K₂CO₃ (127.3 mg, 0.9 mmol, 8 equiv.) was added and the mixture was stirred under reflux at 50 °C for 72 hours (conversion assessed by HPLC-MS). The mixture was filtered, the solvent evaporated under vacuum and the crude purified by RP-HPLC in a H₂O/MeCN + 0.1% TFA mixture (gradient of 2 to 50 % over 45 minutes). After evaporation of the solvents, compound 3 was obtained as a white solid (75.42 mg, 0.086 mmol, 37 % yield). 

**H NMR** (500 MHz, D₂O) δ 4.61 (dd, J = 8.0, 4.5 Hz, 1H), 4.41 (dd, J = 8.0, 4.5 Hz, 1H), 3.36 – 2.60 (m, 37H), 2.30 (t, J = 10.5, 10.5 Hz, 2H), 1.75 – 1.54 (m, 19H), 1.50 – 1.40 (m, 6H), 1.38 – 1.27 (m, 4H). 

**C NMR** (126 MHz, D₂O) δ 161.1, 160.5, 160.2, 77.2, 76.9, 76.6, 60.8, 51, 50.8, 50.8, 50.8, 40.2, 34.8, 28.4, 28.3, 28.3, 28.1, 25, 24.2, 23.8, 23.7, 23.7, 1.8. 

MALDI-TOF [M+H]⁺ m/z = 872.51 (calcd. for C₃₉H₇₄N₁₉O₅S: 873.20). HPLC-MS (Method A): retention time = 3.493 min; purity: >94% at 201 nm.
(Boc)₄BioTriazoTASQ·Cu(II): In anhydrous DMF (1.8 mL) were mixed compound 2 (27.6 mg, 61.9 µmol, 4.2 equiv.), compound 3 (19.1 mg, 1.5 µmol, 1 equiv.) and TBTA (3.9 mg, 7.4 µmol, 0.5 equiv.). (MeCN)₄Cu·PF₆ (8.2 mg, 22.1 µmol, 1.5 equiv.) was added to the mixture which was stirred at RT till reaction completion (6 h, assessed by HPLC-MS). The solvent was evaporated under vacuum and the crude mixture purified by RP-HPLC in a H₂O/MeCN + 0.1% TFA mixture (gradient of 5 to 60% over 45 minutes). After lyophilization of the solvents, (Boc)₄BioTriazoTASQ·Cu(II) was obtained (9.17 mg, 3.37 µmol, 23 % yield) and subsequently demetallated after dissoluation in MeCN (200 µL) and addition of Na₂S (1.1 mg, 13.6 µmol, 4 equiv.) in H₂O (200 µL). After 2 min, centrifugation was performed (4000 RPM, 15 min), the supernatant removed and (Boc)₄BioTriazoTASQ (9.7 mg, 3.45 µmol) isolated after lyophilization (the copper content (0.19 %) was assessed by ICP-MS). ESI-HRMS [M+H+Na]⁺ m/z = 1340.20428 (calcd. for C₁₁₅H₁₇₈N₅₁NaO₂₂S: 1340.19925). HPLC-MS (Method A): retention time = 3.540 min; purity: >93% at 214 nm, >94% at 280 nm; (Method B): retention time = 6.887 min; purity: >92% at 280 nm.

![Chromatogram](image)

**Figure S8.** HPLC profile of protected BioTriazoTASQ (method A)
Figure S9. HPLC profile of protected BioTriazoTASQ (method B)

**BioTriazoTASQ**: (Boc)_4BioTriazoTASQ (2.87 mg, 1.08 µmol) was mixed in TFA (50 µL) at RT for 1 hour. After the completion of the reaction (assessed by HPLC), and BioTriazoTASQ (2.38 mg, 1.05 µmol) was isolated by lyophilization.

**ESI-HRMS** [M+2H]^2+ m/z = 1129.10726 (calcd. for C_{95}H_{147}N_{31}O_{14}S: 1129.10342). HPLC-MS (*Method A*): retention time = 0.360 min; purity: >98% at 280 nm.
**Figure S11.** HPLC profile of BioTriazoTASQ

**Figure S12.** HRMS profile of BioTriazoTASQ
II. FRET-melting assays. FRET-melting experiments were performed in a 96-well format using a Mx3000P qPCR machine (Agilent) equipped with FAM filters ($\lambda_{ex} = 492$ nm; $\lambda_{em} = 516$ nm) in 100 μL (final volume) of 10 mM lithium cacodylate buffer (pH 7.2) plus 10 mM KCl/90 mM LiCl (F-duplex-T) or plus 1 mM KCl/99 mM LiCl (F-Myc-T and F-Terra-T) with 0.2 μM of labelled oligonucleotide and 1 μM of TASQ. Competitive experiments (figure S15) were carried out with labelled oligonucleotide (0.2 μM), 1 μM TASQ and 50 mol. equiv. of the unlabelled competitor ds17. After an initial equilibration step (25°C, 30 s), a stepwise increase of 1°C every 30s for 65 cycles to reach 90°C was performed, and measurements were made after each cycle. Final data were analyzed with Excel (Microsoft Corp.) and OriginPro®9.1 (OriginLab Corp.). The emission of FAM was normalized (0 to 1), and $T_{1/2}$ was defined as the temperature for which the normalized emission is 0.5; $\Delta T_{1/2}$ values are means of 3 triplicates.

![Figure S13. AccuCore HPLC-MS profile of BioTrioTASQ](image)

![Figure S14. Additional competitive FRET-melting results (n=3)](image)
III. Pull-down assays.

IIIa. Fluorescence-based pull-down assay. The streptavidin MagneSphere® beads (Promega) were washed 3 times with TrisHCl buffer containing 1 mM KCl, 99 mM LiCl and 10 mM MgCl₂. Either BioCyTASQ or BioTriazoTASQ (10 µM) was mixed with 5’-labelled oligonucleotides (F-ON, 1 µM: F-22AG, F-Myc, F-duplex, F-NRAS and F-TRF2), MagneSphere® beads (32 µg) in the same TrisHCl buffer (320 µL final volume) and stirred for 1 h at 25 °C. The beads were immobilized (fast centrifugation (< 2 s), magnet) and the supernatant removed. The solid residue was resuspended in 320 µL of TBS 1X buffer, heated for 8 min at 90 °C (gentle stirring 800 r.p.m.) and then centrifuged for 2 min at 8900 rpm. The supernatant was taken up for analysis (magnet immobilization), after being distributed in 3 wells (100 µL each) of a 96-well plate, using a ClarioStar® machine (BMG Labtech) equipped with FAM filters (λex = 492 nm; λem = 516 nm). Competitive experiments (figure S16) were carried out with labelled oligonucleotide (1 µM), 10 µM TASQ and in the presence of the calf thymus DNA (CT-DNA) competitor (25 mol. equiv.). Data were analyzed with Excel (Microsoft Corp.) and OriginPro®9.1 (OriginLab Corp.); normalized FAM emission values are means of 3 triplicates; each analysis comprises: a/ 3 control wells with F-ON and beads only, in order to quantify the non-specific F-ON/bead binding, the FAM emission of the solution was normalized to 1; and b/ 3 wells comprising solutions that resulted from experiments performed with F-ON, BioCyTASQ/BioTriazoTASQ and beads, in order to quantify the actual BioTASQ/BioCyTASQ capture capability when compared to the control experiments.

Figure S15. Additional pull-down and competitive pull-down results (n=3)
IIb. qPCR pull-down assay. The pull-down experiments were performed as above, with the following modifications: a/ the buffer was replaced here by the so-called G4RP buffer (25 mM Tris pH 7.4 plus 150 mM KCl, 5 mM EDTA, 0.5 mM DTT, 0.5% NP40); b/ the incubation time was changed for 2 h at 25 °C; c/ the output was changed for qPCR analyses: polymerase reactions were carried out in triplicate in 96-well format using a Mx3005P qPCR machine (Agilent) equipped with FAM filters ($\lambda_{ex} = 492$ nm; $\lambda_{em} = 516$ nm) in 20 µL (final volume) of G4-1R primer (1 µL, 300 nM), TASQ/ODN mixture (3.7 µL) in 10 µL iTaq™ Universal SYBR® Green Supermix (Bio-Rad) + KCl (5.3 µL, 100 mM). After a first denaturation step (95 °C, 5 min), a two-step qPCR comprising a hybridization step (85 °C, 10 s) and an elongation step (60 °C, 15 s) for 33 cycles was performed, and measurements were made after each cycle. Final data were analyzed with OriginPro®9.1 (OriginLab Corp.). The starting emission (first qPCR cycle) of SYBR Green (FI) was set to 2200 fluorescence intensity and the FI at the 33th cycle was used for calculation. Three biological triplicates ($n = 3$) were used.

IV. Molecular dynamics.

The 3D structures of the ligands were manually constructed with UCSF Chimera, placing the four guanines in a plan with an average distance between the heavy atoms forming the hydrogen bonds of 3.5 Å. After a geometry optimization procedure, the RESP charges were computed and the GAFF parameters obtained using a combination of antechamber and acype programs. Water was described with TIP3P. Bonds containing an hydrogen were constrained with LINCS. A value of 8 Å was used for the non-bonding interactions cutoffs (standard value for the Amber force fields). The ligand structures were submitted to NPT molecular dynamics simulations in cubic boxes of 43 Å length. Velocities were generated at 200K and the systems were heated up to 350 K in 100 ps and were then kept at that temperature for 100 ps (velocity-rescale thermostat, 1 fs timestep). We then ran production simulations with a 2 fs timestep (velocity-rescale thermostat); for each ligand, 3 independent simulations of 500 ns were performed with Gromacs2021, during which the positions of the guanine atoms were restrained with a smooth potential of 1000 kJ/mol/nm² to their initial position in order to force the guanine tetrad to be formed. Post-analysis (including SASA calculations) were done using Gromacs tools and the Plumed library.

Regarding the synthesis G-quartet accessibility: The structure of CyTASQ was found to be more compact than the structure of TriazoTASQ, with a distance between the cyclen ring (defined as the four nitrogen atoms) and the guanines (defined by the oxygen atoms) of 6.0Å for CyTASQ and 7.0Å.
for TriazoTASQ. Moreover, TriazoTASQ was found to be much more flexible with a number of
cluster found of 943 vs 50 for CyTASQ (computed with the gromos method with a cutoff of 0.20
nm). The accessibilities of the two tetrades were assessed in the following way: (1) we defined a
virtual atom positioned 3 Å above the center of the four oxygen of the guanines (“above” meaning
on the opposite direction as the cyclen ring), (2) we computed the distance between this virtual
atom and the oxygens from the amides closest to the guanine, (3) we computed the probability
that this distance is below a given value d₀, i.e. the probability that the arm could form a barrier.
For d₀ = 8 Å, we found that such a barrier exists 4.03% of the simulation for CyTASQ and 3.87% for
TriazoTASQ: thus, TriazoTASQ is more accessible than CyTASQ. For other values of d₀ (7 or 9 Å),
similar trends are observed.

![Minimized structures of CyTASQ and TriazoTASQ, highlighting the difference of accessibility to the TASQ synthetic G-quartet (see the text for a more detailed analysis)](image)

**Figure S16.** Minimized structures of CyTASQ and TriazoTASQ, highlighting the difference of accessibility to the TASQ synthetic G-quartet (see the text for a more detailed analysis)

V. **Cell culture and imaging.**

V.a. **Material and Methods.** MCF7 cells cultured as described in the main text.

V.b. **Pre-targeted G4 imaging.** See the main text.

Images were processed with ImageJ ([https://fiji.sc](https://fiji.sc)); the automated *foci* quantification was made according to home-made FIJI add-ons, which is provided as an accompanying file (.ijm file) and can be download (free of charge) at [https://github.com/ICMUB/TASQ/blob/main/macro-eng_quantif-foci-int_TASQ-gH2AX.ijm](https://github.com/ICMUB/TASQ/blob/main/macro-eng_quantif-foci-int_TASQ-gH2AX.ijm)
V.c. PDS pre-treatment. Round coverslips (12 mm) were sterilised with 70% ethanol before cell seeding. MCF7 cells were seeded at a density of 6.10^4 cells per coverslip on chambered coverslips (4 well-plate) and allowed to recover overnight. Cells were incubated with either PDS (5 µM, 6 h, 0.5% DMSO) or supplemented DMEM-DMSO solution (6 h, 0.5% DMSO; untreated) at 37 °C, washed once with PBS 1X (L0615 Dutscher), fixed and permeabilized with cold MeOH for 10 min at 4 °C, washed with PBS 1X (3 x 5 min), then incubated with: 1/ the blocking buffer (PBS 1X/BSA 1%/Triton X-100 0.1%), 10 min at RT; 2/ BioTriazoTASQ (10 µM) or no TASQ (control) with anti-γH2AX antibody
(Merck Millipore 05-636, mouse monoclonal Ab, 1/1000), overnight in dark chamber, at 4 °C; 3/ PBS 1X/Triton X-100 0.1% (3 x 5 min); 4/ Streptavidin-Cy3 (ThermoFisher Scientific 434315, 1 μg/mL) with anti-mouse Ig-AF647 antibody (donkey, 1/500), 45 min in dark chamber, at RT; 5/ PBS 1X/Triton X-100 0.1% (3 x 5 min); 6/ DAPI (1 μg/mL), 5 min in dark chamber; and 7/ PBS 1X (3 x 5 min). Cells were mounted (Fluoromount-G) and imaged with a Leica TCS SP8 confocal laser-scanning microscope with a 63X oil objective, collected through the following channels: DAPI (emission 448-475 nm), AF647 (emission 644-696 nm) and Cy3 (emission: 558-575 nm). Images were processed with ImageJ (https://fiji.sc) and foci quantification made according to home-made FIJI add-ons. One biological triplicate (n= 3) were used. For statistical hypothesis tests Student’s t-test and Welch’s unequal variances t-test were used depending on variances equality.

Figure S19. Additional analysis of TASQ-detected G4 in cells (all data refered to Figure 3E). Automated quantification (vide supra) of γH2AX foci/cell, of TASQ foci/cell and of TASQ foci overlapping with γH2AX foci per cell in γH2AX-positive cells (assigned as coloc. foci), with or without PDS treatment (6hrs) in live MCF7 cells and incubated with or without TASQ as a probe after fixation. Raw values are represented as (A) mean value of each independent experiment (n≥2 without TASQ incubation; n=3 with TASQ incubation) or (B) for each cells analyzed for quantification over the same independent experiments combined (n cells≥ 433). Ratio of coloc. foci to all γH2AX foci calculated for each independent experiment is represented (C) normalized to TASQ-incubated untreated value or (D) as raw values. Error bars represent standard deviation. P values were calculated using an unpaired multiple Student’s t-test (ns: p>0.05; *: p<0.05; **: p<0.01).

Figure S20. Pre-targeted optical images without or with PDS pre-treatment (5 µM, 6 h) and without or with BioTriazoTASQ, used post-fixation (10 µM, ON) (scale bar = 10 μm).