Aza-macrocyclic Triphenylamine Ligands for G-Quadruplex Recognition.

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This article is dedicated to Juan Faus Professor of Inorganic Chemistry at the University of Valencia honoring his career as an inspiring scientist for many generations of undergraduate and post graduate students.

**Abstract:** A new series of triphenylamine-based ligands with one (**TPA1PY**), two (**TPA2PY**) or three pending aza-macrocycle(s) (**TPA3PY**) have been synthesised and studied by means of pH-metric titrations, UV/Vis spectroscopy and fluorescence experiments. The affinity of these ligands for G-quadruplex (G4) DNA and its selectivity over duplex DNA were investigated by FRET melting assays, fluorimetric titrations and circular dichroism (CD) spectroscopy. Interestingly, the interaction of the bi- and specially the tri-branched ligand with G4 leads to a very intense red-shifted fluorescence emission band which may be associated with intermolecular aggregation between the molecule and the DNA. This light-up effect allows the application of the ligands as fluorescence probes to selectivity detect G4.

Introduction

Besides the canonical double stranded DNA structure, a variety of other alternative nucleic acid structures are known, for example triplexes, i-motifs, three-way junctions, Holliday junctions and G-quadruplexes (G4s).[1] The latter is formed from stacks of two or more planar guanine tetrads that arise from a hydrogen bond network of four guanines assembled and stabilised by alkali metal cations (Figure 1).[2]

**Figure 1.** G-quadruplex schematic representation.

A large number of putative G-quadruplex forming sequences (over 700000) have been identified *in vitro* in the human genome.[3] These G4 DNA-forming sequences can be found at telomeres, promoter regions of various oncogenes, introns and immunoglobulin switch regions.[4] There is mounting evidence suggesting that these non-canonical DNA secondary structures play a pivotal role in key biological processes.[5] Consequently, G4s have been identified as potential targets for therapeutic intervention using small molecules.[6]

The design of G4 DNA binders largely relies on the presence of π-delocalized extended rings (which can display strong π-π interactions with the G-tetrads) and positively charged substituents, which increase DNA affinity via interactions with the negatively charged phosphate backbone and the nucleobases.[7]

The most studied G4 DNA structures to date are those found in the human telomere[8] and in the promoter regions of various oncogenes such as *c-kit*,[9] *Bcl-2*,[10] *k-RAS*[11] and VEGF.[12] More recently, there has been significant interest in G4s formed in RNAs such as TERRA.[13] Although all these sequences form G4s, they show a high structural diversity since they vary in strand orientation, base composition, nature of sugar ring, conformation of glycosidic bonds and size/shape of loops/grooves formed.[14] This variety of G4 polymorphisms has not yet been extensively explored and there are still relatively few molecules which show high selectivity towards a specific G-quadruplex topology over others.[15]

In this scenario, we envisaged that ligands based on the triphenylamine (TPA) unit with polyamine pending arms might strongly bind to G4 sequences. Our interest in the TPA scaffold arises from the fitting of its structural features with other potential G4 binders. It is well-known that TPA adopts a propeller star-shape structure with a D**3** symmetry in which the three phenyl rings are symmetrically twisted from the plane and this conformation is tightly controlled by two different and opposite forces, the π-aromatic conjugation of the phenyl rings and the steric repulsion of the inner-ring hydrogens.[16]

Previously, Teulade-Fichou *et al.* developed several series of triphenylamine derivatives. Some of them showed interesting photophysical properties in the presence of duplex DNA that has allowed their application in cellular imaging.[17] However, to the best of our knowledge, there are no studies reporting the interaction G4s with TPA derivatives with protonable amine groups as side chains.

Herein, we report a novel series of ligands which include a TPA core functionalized by one (**TPA1PY**), two (**TPA2PY**) and three (**TPA3PY**) polyamine macrocyclic arms (Figure 2). We study their acid-base behaviour by using potentiometric, absorption and fluorescence emission methods. In addition, we explore their interaction and selectivity towards a panel of G-quadruplex structures by means of fluorescence, circular dichroism and FRET melting assays.

**Figure 2.** Molecular structures of **TPA-PY** derivatives under study.

Results and Discussion

***Synthesis of TPA-PY derivatives.***

The synthetic pathway used for the preparation of the **TPA-PY** derivatives is shown in Figure 3. Macrocycle **1** was obtained using a protocol previously reported by some of us. [18]

The corresponding mono- (**2**), di- (**3**) or tri-aldehyde (**4**) of triphenylamine was reacted in dry ethanol with 1, 2 or 3 equivalents of **1** to obtain the imines of **TPA1PY**, **TPA2PY** and **TPA3PY**, respectively. The ligands were obtained as hydrochloride salts after imine reduction with NaBH4 and precipitation with HCl. All the compounds were fully characterized by NMR spectroscopy (1H and 13C), mass spectrometry and elemental analysis (see Experimental Details).

**Figure 3.** Synthetic route for the preparation of the ligand series of **TPA-PY**.

***Acid-base behaviour of TPA-PY ligands.***

****Since the protonation state of the ligands is a key point to understand their interaction with negatively charged oligonucleotides, the acid-base behaviour of the **TPA-PY** ligands was studied by potentiometry, UV/Vis and fluorescence emission spectroscopies.

The stepwise protonation constants obtained by means of potentiometric titrations are shown in Table 1. **TPA1PY** displays three, **TPA2PY** six and **TPA3PY** nine measurable protonation steps in the studied 2.5-10.5 pH range, which correspond to the number of secondary amine groups in the compounds. As it is well known, the low basicity of the triphenylamine moiety prevents the determination of its protonation constant in the pH range used in the titrations.[19] Above pH 10.5, some precipitation was observed and this part of the titration was not amenable to analysis. The net positive charge at pH 7.4, at which the interaction studies with DNA were done, is 2.1, 5.0 and 7.1 for **TPA1PY**, **TPA2PY** and **TPA3PY**, respectively.

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| **Table 1.** Logarithms of the stepwise protonation constants of the three **TPA-PY** ligands determined by potentiometric titrations. | | | | |
| **Reaction a** | | **TPA1PY** | **TPA2PY** | **TPA3PY** |
| H + L ⇄ HL | | 8.74(3)b | 9.87(5) | 10.38(6) |
| H + HL ⇄ H2L | | 8.59(1) | 9.49(6) | 9.55(9) |
| H + H2L ⇄ H3L | 6.80(3) | | 8.82(6) | 9.5(1) |
| H + H3L ⇄ H4L |  | | 8.19(6) | 8.98(9) |
| H + H4L ⇄ H5L |  | | 7.51(6) | 8.51(9) |
| H + H5L ⇄ H6L |  | | 6.69(8) | 8.13(8) |
| H + H6L ⇄ H7L |  | |  | 7.50(7) |
| H + H7L ⇄ H8L |  | |  | 7.24(5) |
| H + H8L ⇄ H9L |  | |  | 6.21(5) |
| Log ßc | 24.13(3) | | 50.57(6) | 76.04(6) |
| [a]Charges omitted for clarity; [b]Experiments were carried out in H2O 0.15 M NaCl at 298.1 ± 1 K; [c]Values in parentheses are standard deviation in the last significant figure. | | | | |

The UV/Vis spectra of the **TPA-PY** ligands exhibit two absorption bands at 260 – 270 nm and 285 – 330 nm. The band at 260 – 270 nm is associated with the pyridine ring of the pendant arms whilst that at 285 – 330 nm can be assigned to a π-π\* transition of the TPA moiety.[20] Apart from these bands, there is a longer wavelength band centred above 375 nm, which only emerges with **TPA3PY** (Figures 4, S1 and S2).

While deprotonation of the **TPA-PY** ligands yields relatively small changes in the UV/Vis spectra, significant changes are observed in the emission spectra. Indeed, upon decreasing the pH, there is a remarkable quenching of the fluorescence in addition to a red shift of the emission maximum. These features, which are particularly noticeable for the ligand with the three arms (**TPA3PY**), may be associated with the well-known behaviour of the TPA moiety, in terms of both Twisted Intramolecular Charge Transfer (TICT) and Aggregation-Induced Emission (AIE).[21]

**Figure 4.** a) UV/Vis spectra of **TPA3PY** versus pH; b) Normalized fluorescence emission of **TPA3PY** versus pH and c) Molar fraction distribution diagram for protonated species of **TPA3PY** superposed to its normalized emission at 375 nm (λex = 314 nm) (**●**) and the absorbance at 300 nm (**●**). The photophysical experiments were recorded in H2O; *T* = 298.1 ± 1 K; *l* = 1 cm; I = 0.15 M NaCl.

As mentioned above, the high charge positive of the ligands in acidic solution inhibits the formation of intermolecular interactions; furthermore, the intramolecular rotations of the phenyl rings open up the non-radiative pathway enabling the dissipation of the excited-state energy. Consequently, the emission at low pH is partially quenched which may be associated with the TICT emission. As the pH increases, deprotonation of the **TPA-PY** ligands is likely to allow for the formation of supramolecular interactions, such as H-bonding or π-π stacking. In this scenario, molecules would aggregate leading to a Restriction of Intramolecular Rotations (RIR). The RIR effect blocks the non-radiative pathway and as a result, aggregates of molecules exhibit a remarkable emission (AIE effect).[22]

To corroborate the aggregation in basic medium, the absorption and emission spectra of **TPA3PY** were recorded upon increasing its concentration in solution at pH 12.

**Figure 5.** a) UV/Vis spectra upon increasing **TPA3PY** concentration at pH = 12; b) Absorbance at 270, 300 and 375 nm versus **TPA3PY** concentration.

In Figure 5 it can be observed that the absorbance reaches a plateau as the concentration is increased. Indeed, when plotting three different maxima of the spectra versus **TPA3PY** concentration, a non-linear trend emerges from 100 μM solution. In addition to this, levelled-off tails in the visible region of the spectra can be observed, which indicate the formation of aggregates at nanoscale sizes.[23]

The emission spectra upon increasing the concentration of **TPA3PY** were recorded firstly using 314 nm as excitation wavelength (Figure 6a). An initial increase of the fluorescence was observed, followed by a remarkable red-shift to 480 nm. The emission at longer wavelengths is typically associated with AIE effect.[24] Thus, the spectra were recorded once again using 375 nm as excitation wavelength since it was the optimal excitation for the emission at 480 nm (Figure 6b). Whilst at low ligand concentrations, when the aggregates rarely form, there was no emission, at higher ligand concentrations a clear fluorescence switch on effect was observed and a band centred around 480 nm emerges, which may be associated with the aggregated state.[25]

**Figure 6.** Emission upon increasing **TPA3PY** concentration at pH = 12. a) λex = 314 nm and b) λex = 375 nm.

The aggregation process at basic pH has been checked by dynamic light scattering (DLS) measurements (pH = 10 and 12) which show particle sizes ranging from 782 nm to 1.6 μm (Figure S3a and S3 b).

***Photophysical properties of TPA-PY ligands.***

In order to get more insight into the AIE effect of the **TPA-PY** ligands, their photophysical properties were explored in solvents of different polarity. For solvents such as water, the molecules are well-dispersed in solution and their low fluorescence may be associated with the TICT state owing to the rotational movement of phenyl groups. However, in solvents such as THF that favour intermolecular interactions, molecules tend to gather together and aggregate enhancing the RIR effect and consequently, showing a remarkable emission due to the AIE effect.

The emission spectra of the mono-branched **TPA1PY** ligand does not show significant changes in the emission band in different solvents upon excitation at the absorption maxima of 314 nm (Figure S4a). However, the bi-branched **TPA2PY** and, particularly, the tri-branched **TPA3PY** ligands show important modifications (Figure 7 and S4b); upon excitation at 314 nm the emission of both ligands is red-shifted in non-polar solvents (THF, DCM) with respect to the polar ones (water, CH3CN). Non-protic, low-polarity solvents enhance the supramolecular interactions between the molecules with a concomitant formation of aggregates. These aggregates lead to a RIR effect producing an enhancement and a red-shift of the emission.[26]

**Figure 7.** Normalized emission fluorescence of **TPA3PY** in different solvents, λex = 314 nm.

In order to confirm this aggregation process in non-polar solvents, we recorded the emission spectra in different H2O:THF solvent mixtures. All the **TPA-PY** ligands exhibited a high enhancement of the emission at 480 nm (λex = 314 nm) with increasing percentage of THF. Moreover, upon excitation at 375 nm, both **TPA2PY** and **TPA3PY** show a very large increase of fluorescence intensity at 480 nm, which corresponds to the formation of the aggregate state. (Figure 8, S5 and S6).[27]



**Figure 8.** Fluorescence emission of **TPA3PY** of different H2O:THF solutions. a) λex = 314 nm and b) λex = 375 nm.

***Spectrofluorimetric studies with DNA.***

A recently described approach for specifically sensing DNA takes advantage of molecules that, upon binding to a particular DNA topology, form aggregates giving rise to very intense fluorescence emission.[28]

Following from these previous studies, we investigated the **TPA-PY** aggregation induced emission with a range of DNA structures. We hypothesised that the ligands would become more rigid upon interacting with G4 structures and hence, an increase in fluorescence intensity would be expected.

We recorded the emission spectra of the three ligands upon addition of DNA (Figure 9, Figures S7-S17).

**TPA3PY** shows an emission band at 390 nm upon excitation at 314 nm, whereas no emission is observed when exciting at 375 nm. Upon the addition of G-quadruplex DNA there is a blue shift of the emission at 390 nm (λex = 314 nm) with a concomitant appearance of an emission band at around 480 nm, which is the only band observed using 375 nm as excitation wavelength (Figure 9b). Interestingly, the titration of **TPA3PY** with duplex ds26, does not produce any significant emission enhancement at 480 nm (Figures 9b and 9d).

Consequently, **TPA3PY** may be considered as a selective fluorescence probe to distinguish G-quadruplex from duplex DNA structures. For example **TPA3PY** shows at 480 nm 1.5x106-fold greater fluorescence enhancement for G4 *B-cl2* than for duplex ds26 (Figure 9e). In contrast to the behaviour displayed by **TPA3PY**, bi-branched and mono-branched compounds, **TPA2PY** and **TPA1PY**, show only moderate changes. (Figures S7 to S17).

**Figure 9.** Fluorimetric titration of **TPA3PY** with *Bcl-2*: a) λex = 314 nm and b) λex = 375 nm. Fluorimetric titration of **TPA3PY** with ds26: c) λex = 314 nm and d) λex = 375 nm; e) Emission change experienced upon the addition of 2 equivalents of different DNA sequences to a **TPA3PY** solution (λex = 375nm).

Comparing these results with those obtained aggregation studies of the free ligands, it can be proposed that the interaction between **TPA3PY** and **G4s** leads to the formation of **TPA3PY-G4** aggregates. This would restrict the intramolecular rotation of **TPA3PY** in solution, with the consequent appearance of the band centred over 480 nm.

The fluorescence titrations were fitted to a 1:1 binding model and the affinity constants were determined (Table 2, S1, and S2; Figures 10 and S18 to S20).[29] The values of the constants for **TPA3PY** are three orders of magnitude higher than those for **TPA2PY**. The mono-branched **TPA1PY** ligand has even lower values. Taking into account the stability constants of TPA3PY, we can conclude that the ligand has high affinity towards G4 structures, in particular towards c-kit2, which has a constant two orders of magnitude higher than duplex ds26.

Figure 10. Plot of (F0-F)/F0 *vs* DNA Concentration for TPA3PY titrations with HTelo22-Na and ds26 showing the corresponding fitted curves.



**Figure 11**. Representation of FRET melting values for the interaction between **TPA-PY** ligands and various DNA sequences. The concentration of DNA was 0.2 μM and the DNA:ligand ratios were 1:2, 1:5 and 1:10. Errors denote the standard deviation of at least three independent experiments.

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| **Table 2.** Logarithms of affinity constants (log *Ka*) calculated from the fluorimetric titrations for the system **TPA3PY**-DNA. | |
| **DNA Sequence** | **log *Ka*** |
| HTelo22-K | 6.7(4) |
| HTelo22-Na | 6.6(6) |
| 22CTA | 6.5(2) |
| *Bcl-2* | 7.2(1) |
| CEB25 | 7.4(3) |
| *c-Myc* | 6.3(4) |
| *c-kit1* | 7.0(2) |
| *c-kit2* | 8.0(6) |
| ds26 | 6.2(3) |
| [a]Number in parenthesis are standard deviations in the last significant figures. | |

***FRET melting studies.***

To complement the spectrofluorimetric titrations presented in the previous section, we studied the DNA stabilisation induced by the **TPA-PY** ligands using FRET melting assays.[30]

As expected, **TPA3PY** shows the strongest stabilisation effect for all the G4 structures while **TPA2PY** has a moderate stabilisation effect and **TPA1PY** practically does not induce any stabilisation (Figure 11). These results are in agreement with the values of the affinity constants obtained. As the number of substituents increases, an increase in stabilisation is observed. This can be rationalised by considering that the number of macrocyclic substituents and positive charges play a key role in the supramolecular interactions between the ligand and the G4s.

At low molar ratios (*r* ≤ 5), none of the ligands displays any significant stabilisation with duplex DNA (ds26). Only the highly charged ligand **TPA3PY** shows a stabilisation towards ds26 DNA at very high ratios (Δ*T*m = ~ 16 oC, *r* = 10).

A more detailed analysis of the values of Δ*T*m showed the highest stabilization for the antiparallel telomeric G4 HTelo21-Na and the parallel G4 *c-Myc* for both **TPA3PY** and **TPA2PY** ligands (Figure 11).

In order to establish more accurately the G-quadruplex *vs.* duplex selectivity, FRET competition assays were carried out with **TPA3PY**, since this was the ligand inducing the strongest G4 DNA stabilisation. The addition of an excess of duplex DNA (up to 100 equivalents) did not yield any significant decrease of the Δ*T*m values for HTelo21-Na, 22CTA and *Bcl-2* whereas it caused a slight decrease of the Δ*T*m values of HTelo21-K and *c-Myc* (Figure 12). Therefore, it can be highlighted the selectivity of **TPA3PY** for G4 over duplex sequences, which corroborates the previously commented results.



**Figure 12**. Representation of the FRET competition assays data for **TPA3PY** (1 µM) with different labelled G4 sequences (0.2 μM) with increasing concentration of non-labelled ds26 competitor. Errors denote the standard deviation of at least three independent experiments.

***Circular Dichroism Spectroscopic Studies.***

To gain a better understanding of the potential changes in G4 DNA topology upon binding to **TPA-PY** ligands, we carried out circular dichroism (CD) studies with the following G4 structures: HTelo22-K (hybrid topology), 22CTA (antiparallel) and *c-Myc* (parallel).[31]

**Figure 13**. CD titration of a) HTelo22-K b) *c-Myc* and c) 22CTA with **TPA3PY**.

The 22CTA sequence shows the characteristic bands of an antiparallel conformation: a positive band centred at 295 nm and a negative one at 265 nm. While addition of the **TPA-PY** ligands gives a decrease in the intensity of the original bands, the overall shape of the spectrum is retained, indicating that the G4 largely maintains its conformation upon binding to **TPA-PY** ligands (Figure 13c, S21c and S22c). More interestingly, a noticeable induced band at 360 nm appeared suggesting that the molecules acquire a chiral conformation from either binding into the grooves or stacking onto the top of the G-quartets. The changes in the CD spectra are more pronounced for the ligands with higher number of macrocycles, i.e., **TPA3PY > TPA2PY > TPA1PY** (Figure 13c, S21c and S22c).

Regarding the hybrid G-quadruplex HTelo22-K, we observed a similar effect. Addition of the TPA derivatives yielded a decrease of the positive band at 295 nm and the appearance of an ICD band in the 320 – 340 nm range, all maintaining the overall shape (Figure 13a, S21a and S22a).

For the parallel G4 *c-Myc,* the modifications in the spectra upon the addition of ligands are analogous to the previously described G4 structure; however, the ICD band is less intense (Figure 13b, S21b and S22b).

These results suggest not only that the ligands strongly bind to the studied G4 (due to the appearance of ICD bands), but also that the G4 structure is not disrupted upon the interaction since the overall shape of the spectrum is retained.

Conclusions

Three new triphenylamine-based ligands with one, two and three pendant aza-macrocycle(s) arms (**TPA1PY**, **TPA2PY** and **TPA3PY**) were synthesised and characterised.

The ligands behave as polyprotic bases with the same number of protonation steps than the number of secondary amine groups.

The fluorescence emission is enhanced upon deprotonation of the ligands at basic pH values due to an aggregation process (AIE).

The ligands show different affinity for G-quadruplex DNA depending on the number of amine groups, being the strongest interaction the one of the tri-branched **TPA3PY** ligand (*Ka* (G4) ~ 106 M-1 and Δ*T*m (G4) ~ 20ºC). All of ligands presented high-to-moderate selectivity for G4s over duplex structures. Particularly, **TPA3PY** exhibits a remarkable selectivity since the Δ*T*m values for G4 do not show any decrease until the addition of 100 equivalents of duplex competitor. In addition to this, there is a light-up effect of the **TPA3PY**’s emission in the presence of G4, whereas no significant changes in fluorescence are observed upon the addition of ds26, which may allow for the selective sensing of these G4 structures.

Experimental Section

All reagents were obtained from commercial sources. 1H NMR and 13C NMR spectra were recorded on either a Bruker Advance 300 spectrometer operating at 300 MHz for 1H and at 75.4 MHz for 13C. Mass spectrometric analysis was performed under either ESI+ condition on a LCT Premier mass spectrometer. The UV/Vis spectrophotometer used was Cary 300. The emission spectrums were recorded at a PTI-Fluorimeter equipped with a GL-3300 nitrogen laser, a GL-302 dye laser and a photomultiplier detection system

All the oligonucleotides were purchase from Eurogentec. The sequences for the unlabelled oligonucleotides were HTelo22 (5’-AGGGTTAGGGTTAGGGTTAGGG-3’), *c-Myc* (5’-TGAGGGTGGGTAGGGTGGGTAA-3’), 22CTA (5’-AGGGCTAGGGCTAGGGCTAGGG-3’), *c-kit1* (5’-AGGGAGGGCGCTGGGAGGAGGG-3’), *c-kit2* (5’-CGGGCGGGCGCGAGGGAGGGG-3’), CEB25 (5’-AAGGGTGGGTGTAAGTGTGGGTGGGT-3’), *Bcl-2* (5’-GGGCGCGGGAGGAATTGGGCGGG-3’) and ds26 (5’-CAATCGGATCGAATTCGATCCGATTG-3’). The labelled oligonucleotides used correspond to the same described sequences but including 5’-FAM and 3’-TAMRA. For labelled HTelo21 the sequence was (5’-GGGTTAGGGTTAGGGTTAGGG-3’). Ligands were dissolved in miliQ water to give 2 mM stock solutions. All solutions were stored at -20 ºC. Before using, they were defrosted and diluted to yield the appropriate concentrations.

**Synthesis of 4-(((2-(3,6,9-triaza-1(2,6)-pyridinacyclodecaphane-6-yl)ethyl)amino)methyl)-N,N-diphenylaniline (TPA1PY).** A solution of **1** (0.43 g, 1.7 mmol) in 100 ml anhydrous ethanol was added dropwise to a solution of **2** (0.42 g, 1.55 mmol) in anhydrous ethanol (120 ml) during 1 h and the mixture stirred for 12 h at room temperature. NaBH4 (0.65 g, 17 mmol) was added and the mixture stirred for 2 h The solvent was removed under reduced pressure and the resulting residue was treated with H2O (25 mL) and extracted with DCM (3 x 30 mL). The organic phase was dried over Na2SO4 and evaporated to afford a yellow oil that was dissolved in anhydrous ethanol and precipitated in order to obtain the product as hydrochloride salt of **TPA1PY** in 23% yield. 1H NMR (300 MHz, D2O): *δ* = 7.92 – 7.87 (t, *J* = 8 Hz, 1H), 7.40 – 7.37 (d, *J* = 8 Hz, 2H), 7.23 – 7.20 (d, *J* = 8 Hz, 2H), 7.02 – 6.97 (t, *J* = 15 Hz, 4H), 6.84 – 6.81 (d, 8H), 4.57 (s, 2H), 4.08 (s, 2H), 3-29 – 3.15 (m, 6H), 3.04 – 2.96 (m, 2 H), 2.88 – 2.78 (m, 4H); 13C NMR (75.4 MHz, D2O): *δ* = 149.20, 147.47, 140.17, 131.64, 129.92, 125.14, 124.08, 123.06, 122.56, 51.39, 50.83, 49.87, 46.29, 30.63; MS: *m/z* (%): 507.3 ([M+H]+); elemental analysis calcd. (%) for C32H38N6·3HCl·2.5H2O: C 58.17; H 7.02; N 12.72; found: C 57.97, H 7.53; N 12.66.

**Synthesis of 4-(((2-(3,6,9-triaza-1(2,6)-pyridinacyclodecaphane-6-yl)ethyl)amino)methyl)-N-(4-(((2-(3,6,9-triaza-1(2,6)-pyridinacyclodecaphane-6-yl)ethyl)amino)methyl)phenyl)-N-phenylaniline (TPA2PY).** A solution of **1** (0.32 g, 1.27 mmol) in 100 ml anhydrous ethanol was added dropwise to a solution of **3** (0.19 g, 0.60 mmol) in anhydrous ethanol (120 ml) during 1 h and the mixture stirred for 12 h at room temperature. NaBH4 (0.48 g, 12.7 mmol) was added and the mixture stirred for 2 h The solvent was removed under reduced pressure and the resulting residue was treated with H2O (25 mL) and extracted with DCM (3 x 30 mL). The organic phase was dried over Na2SO4 and evaporated to afford a yellow oil that was dissolved in anhydrous ethanol and precipitated in order to obtain the product as hydrochloride salt of **TPA2PY** in 47% yield. 1H NMR (300 MHz, D2O): *δ* = 8.03 – 7.98 (t, *J* = 8 Hz , 2 H), 7.51 – 7.40 (m, 8 H), 7.27 – 7.19 (m, 7 H) 4.68 (s, 8 H), 4.29 (s, 4 H), 3.41 – 3.30 (m, 12 H), 3.16 – 3.10 (m, 4 H), 2.99 – 2.95 (m, 8 H); 13C NMR (75.4 MHz, D2O): *δ* = 151.79, 151.46, 149.75, 142.69, 134.15, 132.79, 128.61, 127.52, 126.63, 125.10, 54.00, 53.81, 53.41, 52.42, 48.84, 45.46; MS: *m/z* (%): 384.03 ([M+2H]2+). ]+); elemental analysis calcd (%) for C46H61N11·5HCl·6.7H2O: C 51.58; H 7.47; N 14.39; found: C 51.86; H 7.76; N 14.30.

**Synthesis of tris(4-(((2-(3,6,9-triaza-1(2,6)-pyridinacyclodecaphane-6-yl)ethyl)amino)methyl)phenyl)amine (TPA3PY).** A solution of **1** (0.70 g, 2.8 mmol) in 50 ml anhydrous ethanol was added to a solution of **4** (0.31 g, 0.93 mmol) in 100 mL anhydrous ethanol and the mixture was stirred for 12 h at room temperature. NaBH4 (1.1 g, 28 mmol) was then added and the resulting solution stirred for 1 h at room temperature. The ethanol was removed under reduced pressure. The resulting residue was treated with H2O (25 mL) and extracted with DCM (3 x 30 mL). The organic phase was removed at reduced pressure and the resulting residue was dissolved in dry dichloromethane and precipitated as hydrochloride salt of **TPA3PY** in 22 % yield. 1H RMN (300 MHz, D2O): *δ* = 7.98 (t, *J* = 8 Hz, 3H), 7.46 (m, 12H), 7.22 (d, *J* = 9 Hz, 6H), 4.66 (s, 12H), 4.28 (s, 6H), 3.39 (m, 6H), 3.30 (m, 12H), 3.12 (m, 6H), 2.96 (t, *J* = 5 Hz, 12H); 13C RMN (75.4 MHz, D2O): δ = 149.49, 148.76, 140.41, 131.94, 126.07, 125.21, 122.81, 51.73, 51.51, 51.12, 50.11, 46.54, 43.29; elemental analysis calc (%) for C60H84N16·9HCl·11H2O: C 46.32, H 7.44, N 14.40; found: C 46.82; H 7.83; N, 14.01.

**EMF measurements:** The potentiometric titrations were carried out at 298.1 ± 0.1 K using NaCl 0.15 M as supporting electrolyte. The experimental procedure (burette, potentiometer, cell, stirrer, microcomputer etc.) has been fully described elsewhere. The acquisition of the EMF data was performed with the computer program PASAT. The reference electrode was an Ag/AgCl electrode in saturated KCl solution. The glass electrode was calibrated as a hydrogen ion concentration probe by titration of previously standardized amounts of HCl with CO2-free NaOH solutions and the equivalent point determined by the Gran’s method, which gives the standard potential, E0’, and the ionic product of water (pKw = 13.73(1)). The computer program HYPERQUAD was used to calculate the protonation and stability constants. The HYSS program was used to obtain the distribution diagrams. The pH range investigated was 2.5-11.0.[32]

**DLS Measurements:** 1 mM solutions of **TPA3PY** were prepared by solving the compound in milli-Q water and then the pH was adjusted to 10 and 12 by adding NaOH. The DLS measurements were performed in triplicate using a Malvern Mastersizer 2000 equipment equilibrating the sample at 25 °C.

**FRET melting assays:**. Labelled DNA was dissolved as a 20 µM stock solution in milliQ water, then they were further diluted to 0.4 μM using the appropriate buffer and annealed at 95 ºC for 5 min. Finally, they were allowed to slowly cool down to room temperature overnight. The buffer used for the antiparallel G4 HTelo21-Na was 10 mM NaCl, 90 mM LiCl, 10 mM LiCac, for the G4 *c-Myc* was 1 mM KCl, 99 mM LiCl, 10 mM LiCac, for the G4 *B-cl2* was 100 mM KCl, 10 mM LiCac and for the rest of DNA was 10 mM KCl, 90 mM LiCl, 10 mM LiCac. Ligands were diluted from stock solutions (see above) yielding specific final concentrations in the same buffer as the tested sequence. Each well of a 96- well plate (Applied Biosystem) was prepared with 60 mL, with a final 200 nM DNA concentration and increasing concentration of the ligands (0–4 µM). Measurements were performed on a PCR Stratagene Mx3005P (Agilent Technologies) with excitation at 450–495 nm and detection at 515–545 nm. Readings were taken from 25 ºC to 95 ºC (interval of 0.5 ºC). Each measurement was done in triplicate. The normalised fluorescence signal was plotted against the compound concentration and the Δ*T*m values were determined (Figures S28 – S36).

**FRET competition assays:** Labelled oligonucleotides were annealed as a 400 nM concentration as previously described. Ligands were diluted from stock solutions to final concentrations in the same buffer as the DNA. Each well of a 96- well plate (MJ Research, Waltham, MA) was prepared with a final 200 nM oligo concentration, 1 µM ligand concentration, and the ds26 concentration to test. Measurements were performed under the same conditions as the FRET melting assay.

**Emission titrations:** The DNA was dissolved in potassium or sodium cacodylate buffer (above described) and annealed at 95 ºC for 5 min before cooling to room temperature overnight. The concentration of DNA was checked using their molar extinction coefficients. Annealing concentrations were approximately 1 mM. For emission titrations, ligands (10 µM) in buffer were titrated with the corresponding DNA until saturation of fluorescence. The emission spectra were recorded between 330 and 590 nm with an excitation wavelength of 314 nm or 390 and 650 nm with an excitation wavelength of 375 nm in 1 cm path-length quartz cuvettes. The emission spectra were recorded on a Varian Cary Eclipse Spectrometer. Spectra were smoothed using the Savitzky–Golay algorithm and emission maxima were fitted to 1:1 binding model using the Levenberg– Marquardt algorithm and equations reported previously.[29]

**CD titrations:** The oligonucleotides were dissolved in milliQ. water to yield a 1 mM stock solution. They were then diluted using 10 mM Tris-HCl and 50 mM KCl (pH 7.0) buffer to 5 μM. Prior to use in the CD assay, the DNA solution was annealed by heating the solution to 95 °C for 5 min and then cooling to room temperature overnight. The CD spectra were measured in a 1 cm path-length cuvette. The CD spectra were measured in the spectral range of 200-600 nm of annealed DNA with **TPA-PY** ligands.

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**Keywords:** G-quadruplex • fluorescent probes • triphenylamine polyamines• DNA • aggregation-induced emission

1. a) M. Duca, P. Vekhoff, K. Oussedik, L. Halby, P. B. Arimondo, *Nucleic Acids Res.* **2008**, *36*, 5123–5138; b)H. A. Day, P. Pavlou, Z. A. E. Waller, *Bioorganic Med. Chem.* **2014**, *22*, 4407–4418; c) D. R. Duckett, D. M. Lilley, *EMBO J.* **1990**, *9*, 1659–64; d)Y. Liu, S. West, *Nat. Rev. Mol. Cell Biol.* **2004**, *5*, 933–937.
2. a) N. H. Campbell, G. N. Parkinson, *Methods* **2007**, *43*, 252–263; b) S. Burge, G. N. Parkinson, P. Hazel, A. K. Todd, S. Neidle, *Nucleic Acids Res.* **2006**, *34*, 5402–5415.
3. a) V. S. Chambers, G. Marsico, J. M. Boutell, M. Di Antonio, G. P. Smith, S. Balasubramanian, *Nat. Biotechnol.* **2015**, *33*, 877–881; b) J. L. Huppert, S. Balasubramanian, *Nucleic Acids Res.* **2005**, *33*, 2908–2916; A. K. Todd, M. Johnston, S. Neidle, *Nucleic Acids Res.* **2005**, *33*, 2901–2907.
4. a) E. Blackburn, *Nature* **1991**, *350*, 569–573; b) S. Balasubramanian, L. H. Hurley, S. Neidle, *Nat. Rev. Drug Discov.* **2011**, *10*, 261–275; c) J. Eddy, N. Maizels, *Nucleic Acids Res.* **2008**, *36*, 1321–1333; d) D. Sen, W. Gilbert, *Nature* **1988**, *334*, 364–366.
5. a) H. J. Lipps, D. Rhodes, *Trends Cell Biol.* **2009**, *19*, 414–422; b) A. L. Valton, M. N. Prioleau, *Trends Genet.* **2016**, *32*, 697–706; c) N. Maizels, *Nat. Struct. Mol. Biol.* **2006**, *13*, 1055–1059; d) D. Rhodes, H. J. Lipps, *Nucleic Acids Res.* **2015**, *43*, 8627–8637.
6. a) S. Neidle, *J. Med. Chem.* **2016**, *59*, 5987–6011; b) S. Balasubramanian, S. Neidle, *Curr. Opin. Chem. Biol.* **2009**, *13*, 345–353; c) R. Rigo, M. Palumbo, C. Sissi, *Biochim. Biophys. Acta - Gen. Subj.* **2017**, *1861*, 1399–1413; d) H. Han, L. H. Hurley, *Trends Pharmacol. Sci.* **2000**, *21*, 136–142.
7. a) A. Arola, R. Vilar, *Curr. Top. Med. Chem.* **2008**, 1405–1415; b) B. R. Vummidi, J. Alzeer, N. W. Luedtke, *ChemBioChem* **2013**, *14*, 540–558; c) J.-H. Tan, L.-Q. Gu, J.-Y. Wu, *Mini-reviews Med. Chem.* **2008**, *8*, 1163–1178; d) T. M. Ou, Y. J. Lu, J. H. Tan, Z. S. Huang, K. Y. Wong, L. Q. Gu, *ChemMedChem* **2008**, *3*, 690–713; e) D. Monchaud, M.-P. Teulade-Fichou, *Org. Biomol. Chem.* **2008**, *6*, 627–636.
8. G. N. Parkinson, M. P. H. Lee, S. Neidle, *Nature* **2002**, *417*, 876–880.
9. a) S. Rankin, A. P. Reszka, J. Huppert, M. Zloh, G. N. Parkinson, A. K. Todd, S. Ladame, S. Balasubramanian, S. Neidle, *J. Am. Chem. Soc.* **2005**, *127*, 10584–10589; b) A. K. Todd, S. M. Haider, G. N. Parkinson, S. Neidle, *Nucleic Acids Res.* **2007**, *35*, 5799–5808.
10. a) T. S. Dexheimer, D. Sun, L. H. Hurley, *J. Am. Chem. Soc.* **2006**, *128*, 5404–5415; b) J. Dai, T. S. Dexheimer, D. Chen, M. Carver, A. Ambrus, R. A. Jones, D. Yang, *J. Am. Chem. Soc.* **2006**, *128*, 1096–1098.
11. a) R. K. Morgan, H. Batra, V. C. Gaerig, J. Hockings, T. A. Brooks, *Biochim. Biophys. Acta - Gene Regul. Mech.* **2016**, *1859*, 235–245; b) S. Cogoi, L. E. Xodo, *Nucleic Acids Res.* **2006**, *34*, 2536–2549.
12. D. Sun, K. Guo, J. J. Rusche, L. H. Hurley, *Nucleic Acids Res.* **2005**, *33*, 6070–6080.
13. G. W. Collie, S. M. Haider, S. Neidle, G. N. Parkinson, *Nucleic Acids Res.* **2010**, *38*, 5569–5580.
14. D. J. Patel, A. T. Phan, V. Kuryavyi, *Nucleic Acids Res.* **2007**, *35*, 7429–7455.
15. a) J. Seenisamy, S. Bashyam, V. Gokhale, H. Vankayalapati, D. Sun, A. Siddiqui-Jain, N. Streiner, K. Shin-ya, E. White, W. D. Wilson, et al., *J. Am. Chem. Soc.* **2005**, *127*, 2944–2959; b) I. M. Dixon, F. Lopez, A. M. Tejera, J. P. Estève, M. A. Blasco, G. Pratviel, B. Meunier, *J. Am. Chem. Soc.* **2007**, *129*, 1502–1503; c) N. H. Campbell, M. Patel, A. B. Tofa, R. Ghosh, G. N. Parkinson, S. Neidle, *Biochemistry* **2009**, *48*, 1675–1680; d) T. P. Garner, H. E. L. Williams, K. I. Gluszyk, S. Roe, N. J. Oldham, M. F. G. Stevens, J. E. Moses, M. S. Searle, *Org. Biomol. Chem.* **2009**, *7*, 4194.
16. a) G. Meijer, G. Berden, W. L. Meerts, H. E. Hunziker, *Chem Phys* **1992**, *163*, 209–222; b) H. B. Lueck, J. L. McHale, W. D. Edwards, *J. Am. Chem. Soc.* **1992**, *114*, 2342–2348; c) M. U. Munshi, G. Berden, J. Martens, J. Oomens, *Phys. Chem. Chem. Phys.* **2017**, *19*, 19881–19889; d) I. Reva, L. Lapinski, N. Chattopadhyay, R. Fausto, *Phys. Chem. Chem. Phys.* **2003**, *5*, 3844–3850.
17. a) R. Chennoufi, H. Bougherara, N. Gagey-Eilstein, B. Dumat, E. Henry, F. Subra, S. Bury-Moné, F. Mahuteau-Betzer, P. Tauc, M. P. Teulade-Fichou, et al., *Sci. Rep.* **2016**, *6*, 1–12; b) R. Lartia, C. Allain, G. Bordeau, F. Schmidt, C. Fiorini-Debuisschert, F. Charra, M. P. Teulade-Fichou, *J. Org. Chem.* **2008**, *73*, 1732–1744; c) G. Bordeau, R. Lartia, G. Metge, C. Fiorini-Debuisschert, F. Charra, M. P. Teulade-Fichou, *J. Am. Chem. Soc.* **2008**, *130*, 16836–16837; d) B. Dumat, G. Bordeau, E. Faurel-Paul, F. Mahuteau-Betzer, N. Saettel, G. Metge, C. Fiorini-Debuisschert, F. Charra, M. P. Teulade-Fichou, *J. Am. Chem. Soc.* **2013**, *135*, 12697–12706; e) B. Dumat, E. Faurel-Paul, P. Fornarelli, N. Saettel, G. Metgé, C. Fiorini-Debuisschert, F. Charra, F. Mahuteau-Betzer, M.-P. Teulade-Fichou, *Org. Biomol. Chem.* **2016**, *14*, 358–370; f) R. Chennoufi, H. Bougherara, N. Gagey-Eilstein, B. Dumat, E. Henry, F. Subra, F. Mahuteau-Betzer, P. Tauc, M.-P. Teulade-Fichou, E. Deprez, *Chem. Commun.* **2015**, *51*, 14881–14884; g) B. Dumat, G. Bordeau, A. I. Aranda, F. Mahuteau-Betzer, Y. El Harfouch, G. Metgé, F. Charra, C. Fiorini-Debuisschert, M.-P. Teulade-Fichou, *Org. Biomol. Chem.* **2012**, *10*, 6054.
18. B. Verdejo, A. Ferrer, S. Blasco, C. E. Castillo, J. González, J. Latorre, M. A. Máñez, M. G. Basallote, C. Soriano, E. García-España, *Inorg. Chem.* **2007**, *46*, 5707–5719.
19. a) C. Quinton, V. Alain-Rizzo, C. Dumas-Verdes, F. Miomandre, G. Clavier, P. Audebert, *Chem. - A Eur. J.* **2015**, *21*, 2230–2240; b) T. Manifar, S. Rohani, *Can. J. Chem. Eng.* **2004**, *82*, 323–334; c) J. Luo, O. Conrad, I. F. J. Vankelecom, *J. Mater. Chem.* **2012**, *22*, 20574; d) A. J. Hoefnagel, M. A. Hoefnagel, B. M. Wepster, *J. Org. Chem.* **1981**, *46*, 4209–4211; e) I. Kaljurand, R. Lilleorg, A. Murumaa, M. Mishima, P. Burk, I. Koppel, I. A. Koppel, I. Leito, *J. Phys. Org. Chem.* **2013**, *26*, 171–181.
20. a) B. Xu, J. He, Y. Liu, B. Xu, Q. Zhu, M. Xie, Z. Zheng, Z. Chi, W. Tian, C. Jin, et al., *J. Mater. Chem. C* **2014**, *2*, 3416; b) M. Aydemir, G. Haykır, F. Türksoy, S. Gümüş, F. B. Dias, A. P. Monkman, *Phys. Chem. Chem. Phys.* **2015**, *17*, 25572–25582.
21. a) J. Mei, N. L. C. Leung, R. T. K. Kwok, J. W. Y. Lam, B. Z. Tang, *Chem. Rev.* **2015**, *115*, 11718–11940; b) S. Sasaki, G. P. C. Drummen, G. Konishi, *J. Mater. Chem. C* **2016**, *4*, 2731–2743; c) R. Hu, E. Lager, J. Liu, J. W. Y. Lam, H. H. Y. Sung, I. D. Williams, Y. Zhong, K. S. Wong, *J. Phys. Chem. C* **2009**, *113*, 15845–15853.
22. a) M. Zhang, W. Yang, T. Gong, W. Zhou, R. Xue, *Phys. Chem. Chem. Phys.* **2017**, *19*, 21672–21682; b) W. Yang, C. Li, M. Zhang, W. Zhou, R. Xue, H. Liu, Y. Li, *Phys. Chem. Chem. Phys.* **2016**, *18*, 28052–28060.
23. C. Li, W. Yang, W. Zhou, M. Zhang, R. Xue, M. Li, Z. Cheng, *New J. Chem.* **2016**, *40*, 8837–8845.
24. S.-Y. Kim, Y.-J. Cho, G. F. Jin, W.-S. Han, H.-J. Son, D. W. Cho, S. O. Kang, *Phys. Chem. Chem. Phys.* **2015**, *17*, 15679–15682.
25. C. X. Yuan, X. T. Tao, L. Wang, J. X. Yang, M. H. Jiang, *J. Phys. Chem. C* **2009**, *113*, 6809–6814.
26. M. Yang, D. Xu, W. Xi, L. Wang, J. Zheng, J. Huang, J. Zhang, H. Zhou, J. Wu, Y. Tian, *J. Org. Chem.* **2013**, *78*, 10344–10359.
27. a) M. T. Gabr, F. C. Pigge, *Rsc Adv.* **2015**, *5*, 90226–90234; b) T. Ishi-I, K. Ikeda, Y. Kichise, M. Ogawa, *Chem. - An Asian J.* **2012**, *7*, 1553–1557.
28. a) H. Wang, K. Ma, B. Xu, W. Tian, *Small* **2016**, *12*, 6613–6622; b) A. Fürstenberg, T. G. Deligeorgiev, N. I. Gadjev, A. A. Vasilev, E. Vauthey, *Chem. - A Eur. J.* **2007**, *13*, 8600–8609; c) Y. Chen, K. Ma, T. Hu, B. Jiang, B. Xu, W. Tian, J. Z. Sun, W. Zhang, *Nanoscale* **2015**, *7*, 8939–8945; d) J. Liang, B. Z. Tang, B. Liu, *Chem. Soc. Rev.* **2015**, *44*, 2798–2811; e) S. Li, S. M. Langenegger, R. Häner, *Chem. Commun.* **2013**, *49*, 5835; f) Y. Hong, J. W. Y. Lam, B. Z. Tang, *Chem. Commun.* **2009**, 4332; g) H. Tong, Y. Hong, Y. Dong, M. Häußler, J. W. Y. Lam, Z. Li, Z. Guo, Z. Guo, B. Z. Tang, *Chem. Commun.* **2006**, 3705–3707; h) D. Ding, K. Li, B. Liu, B. Z. Tang, *Acc. Chem. Res.* **2013**, *46*, 2441–2453; i) X. Li, K. Ma, S. Zhu, S. Yao, Z. Liu, B. Xu, B. Yang, W. Tian, *Anal. Chem.* **2014**, *86*, 298–303; j) X. Xu, S. Yan, Y. Zhou, R. Huang, Y. Chen, J. Wang, X. Weng, X. Zhou, *Bioorganic Med. Chem. Lett.* **2014**, *24*, 1654–1656.
29. a) F. H. Stootman, D. M. Fisher, A. Rodger, J. R. Aldrich-Wright, *Analyst* **2006**, *131*, 1145; b) P. Thordarson, *Chem. Soc. Rev.* **2011**, *40*, 1305–1323.
30. a) A. De Rache, J. L. Mergny, *Biochimie* **2015**, *115*, 194–202; b) A. De Cian, L. Guittat, M. Kaiser, B. Saccà, S. Amrane, A. Bourdoncle, P. Alberti, M. P. Teulade-Fichou, L. Lacroix, J. L. Mergny, *Methods* **2007**, *42*, 183–195.
31. a) A. Randazzo, G. Piero Spada, M. Webba da Silva, *Quadruplex Nucleic Acids* **2012**, 67–86; b) J. Carvalho, J. A. Queiroz, C. Cruz, *J. Chem. Educ.* **2017**, *94*, 1547–1551.
32. a) Alderighi, P. Gans, A. Ienco, D. Peters, A. Sabatini, A. Vacca, *Coord. Chem. Rev.* **1999**, *184*, 311–318; b) A. Sabatini, A. Vacca, P. Gans, *Coord. Chem. Rev.* **1992**, *120*, 389–405; c) P. Gans, A. Sabatini, A. Vacca, *Talanta* **1996**, *43*, 1739–1753; d) H. Rossotti, F. J. C. Rossotti, *J. Chem. Educ.* **1965**, *42*, 375–378; e) G. Gran, H. Dahlenborg, S. Laurell, M. Rottenberg, *Acta Chem. Scand.* **1950**, *4*, 559–577; f) E. Garcia-espafia, M. Ballester, F. Lloret, J. M. Moratal, J. Faus, A. Bianchi, *J. Chem. Soc. Dalt. Trans.* **1988**, 101–104.

**Entry for the Table of Contents** (Please choose one layout)

Layout 1:

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| FULL PAPER | | | | |
| This work comprises the synthesis and the study of novel triphenylamine derivatives as a selective fluorescence probes towards G-quadruplex (G4) DNA. To get insight into the interaction between the molecules and several DNA structures, we have performed different studies by means of fluorescence spectroscopy, FRET-melting assays and circular dichroism.The TPA3PY ligand shows high affinity towards G4 DNA and a remarkably light-up effect due to an aggregation-induced emission effect. |  |  |  | Isabel Pont, Jorge González-García,\* Mario Inclán, Matthew Reynolds, Estefanía Delgado-Pinar, M. Teresa Albelda, Ramon Vilar,\* Enrique García-España.\*  Page No. – Page No.  Aza-macrocyclic Triphenylamine Ligands for G-Quadruplex Recognition. |
|  |  |  |