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Vinylnaphthalene-bearing hexaoxazole as a fluorescence turn-on type G-quadruplex ligand

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Oxazole-type fluorophores show an increase of fluorescence intensity upon interaction with nucleic acids, and therefore can be used as tools for nucleic acid-sensing and fluorescence imaging. Here, we developed a novel stilbene-type fluorophore, MO-VN (**1**), consisting of a mono oxazole bearing a vinyl naphthalene moiety. This compound (**1**) was embedded in a trioxazole **2** and a cyclic hexaoxazole **3a**. The fluorescence properties of **1**, **2**, and **3a** were evaluated in the presence of various nucleic acid sequences. Compound **3** showed significant fluorescent enhancement upon interacting with G-quadruplex (G4) structure, which plays critical roles in various biological phenomena. Further structural development focusing on the vinyl naphthalene moiety of **3a** afforded a turn-on type G4 ligand **3e** that shows G4-specific fluorescence. Measurement of the fluorescence of **3e** during titration of a telomeric DNA, telo24, with its C-rich complementary sequence, which unwinds the G4 structure, allowed us to monitor the dynamics of G4.

Introduction

Some intercalating dyes, such as oxazole yellow, thiazole orange, and ethidium bromide, show increased fluorescence upon interaction with nucleic acids.[1] Among them, com-pounds with an oxazole skeleton, such as YOYO-1 and YOPRO-1, have been employed as fluorescent imaging markers for cell death.[2] In particular, a series of oxazole-type fluorophores of YOYO (YOYO-1) exhibits a greater increment of fluorescence intensity in the presence of nucleic acids than other types of dyes having a thiazole (TOTO) or ethidium backbone (EthD).[3] Furthermore, stilbene-type fluorophores consisting of oxazole and benzene have a long-wavelength emission maximum at around 700 nm due to their wide π-conjugation, and their Stokes shift reaches ca. 200 nm.[4] Therefore, fluorochromes containing oxazole are useful as sensor molecules for nucleic acids, offering high signal-to-noise ratios between bound and unbound forms with nucleic acids.

Among nucleic acid structures, the G-quadruplex (G4), which forms in guanine-rich regions of single-stranded DNA, has received much attention. The G4 higher-order structure exists widely in the genome,[5] especially in telomeres and promoter regions of oncogenes,[6] and it is thought to be formed dynamically during various critical biological events, such as replication,[7] transcription,[8] and epigenetic modification.[9] Thus, fluorescent G4 probes, especially turn-on fluorescent probes that show fluorescence only in the presence of G4,[10,11] would be extremely useful as tools to monitor the dynamics of G4, and thus to elucidate G4 functions.

In this study, we focused on mono oxazole bearing vinyl naphthalene (MO-VN) as a novel stilbene-type fluorophore for nucleic acids. We then embedded MO-VN in a cyclic hexaoxazole framework, 6OTD,[12] which we previously developed as a G4 ligand. Specifically, we synthesized MO-VN (**1**), and then embedded it in a linear trioxazole vinylnaphthalene TO-VN (**2**), and a cyclic hexaoxazole OTD-VN (**3a**) in order to vary the p-conjugation structure (Figure 1). We evaluated the fluorescence properties of these compounds, and then further modified **3a** to obtain a fluorescence turn-on probe that selectively interacts with G4, generating strong fluorescence. We show that this probe can monitor G4 dynamics in a telomeric sequence, telo24.

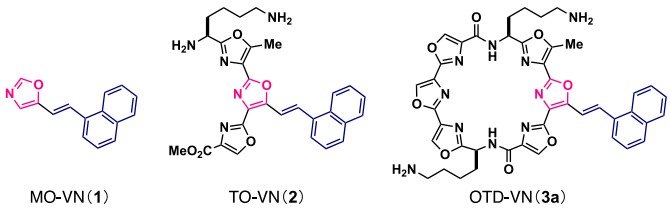

Figure 1. Structures of MO-VN (**1**), and MO-VN (**1**)-embedded trioxazole TO-VN (**2**) and cyclic hexaoxazole OTD-VN (**3a**).

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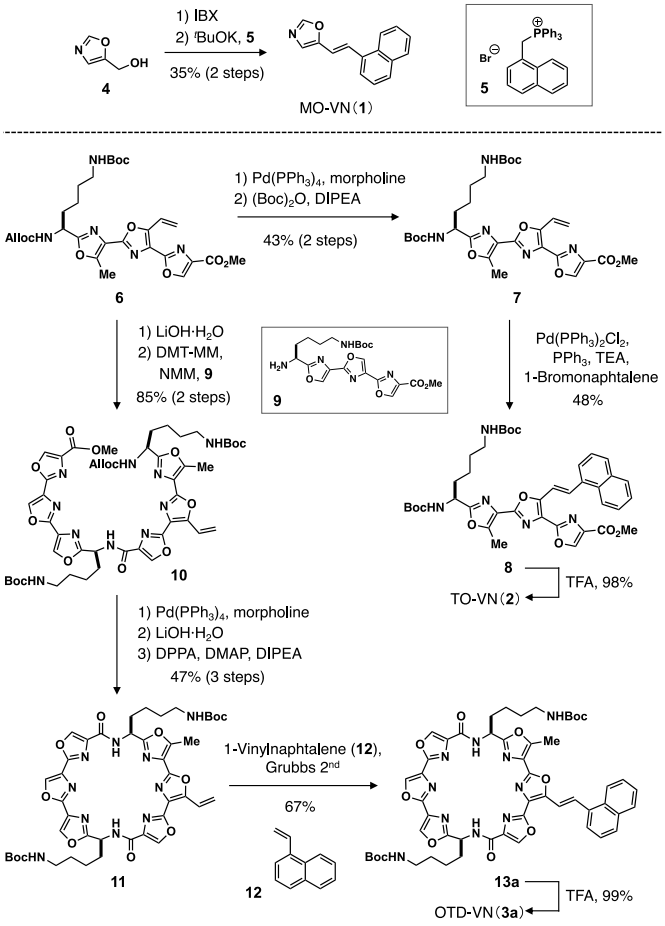
Results and discussion

Synthesis of compounds 1, 2 and 3a

Synthesis of oxazoles **1**, **2** and **3a** with vinyl naphthalene group was summarized in Scheme 1. Oxidation of primary alcohol in **4**[13] with IBX followed by Wittig olefination with **5** in the presence of ^{t}BuOK gave MO-VN (**1**) in 35% yield. TO-VN (**2**) was synthesized from a trioxazole **6**, which was synthesized according to our previously reported procedure.[14] The Alloc group in **6** was deprotected in the presence of Pd(0) catalyst, and the resulting amine was reacted with Boc anhydride to give trioxazole **7** in 43% yield. Introduction of the naphthalene group in **7** was carried out by the olefin metathesis reaction, but the reaction did not complete in this case. Therefore, we investigated alternative reactions for this substrate and found that Pd-mediated Heck-reaction with 1-bromonaphthalene gave **8**, which was subsequently subjected to the deprotection of Boc group with TFA to give TO-VN (**2**) in 47% yield. Synthesis of OTD-VN (**3a**) was carried out as follows. The ester group in trioxazole **6** was hydrolyzed with LiOH, and the resulting carboxylic acid was coupled with amine **9** in the presence of DMT-MM to give hexaoxazole **10**. After deprotection of Alloc group with $Pd(PPh_3)_4$ followed by hydrolysis of the methyl ester group, the resulting amino acid was subjected to macrolactamization with diphenylphosphoryl azide (DPPA) in the presence of DMAP and DIPEA to give cyclic hexaoxazole **11** in 47% yield from **10**. Then, olefin metathesis reaction between **11** and 1-vinylnaphthalene **12** was conducted in the presence of Grubbs 2nd generation catalyst to give bis-Boc-protected **13a**, which was subjected to the deprotection of Boc groups with TFA to give OTD-VN (**3a**) in 66% from **11**.



Scheme 1. Synthesis of MO-VN (**1**), TO-VN (**2**), and OTD-VN (**3a**).

Fluorescence titration of synthetic compounds 1, 2 and 3a in the presence of nucleic acids.

Compounds **1**, **2**, and **3a** were synthesized, and their fluorescence properties in the presence of nucleic acids were investigated.[15] The fluorescence properties of **1**, **2**, and **3a** in the absence and presence of telo24 or double-stranded DNA (dsDNA), which are G4-forming and non-G4-forming nucleic acids, respectively, are summarized in Table 1. The maximum emission wavelength (Em) of compound **1** did not show significant change in the presence of telo24. On the other hand, the Em of compound **2** and **3a** showed slightly redshift and blueshift change, respectively. All compounds did not show the significant fluorescence change in the presence of dsDNA. Notably, the quantum yield (Φ_F) of compound **3a** showed to increase ca. 10-fold by the addition of telo24 compare to the dsDNA. Moreover, the fluorescence titration spectra and the changes of fluorescence intensity of **1**, **2**, and **3a** in the presence of various concentrations of telo24 or dsDNA recorded at 482 nm are shown in Figure 2. In the case of MO-VN (**1**), no significant change in fluorescence intensity was observed upon addition of telo24 or dsDNA, whereas a concentration-dependent increment of the fluorescence intensity was observed upon addition of telo24 or dsDNA to **2**, which has a wider π-conjugation plane than **1**. Notably, in the case of compound **3a**, an increase in fluorescence intensity was observed selectively only when telo24 was added.

Next, the G4-stabilizing ability of **1**, **2**, and **3a** was evaluated. CD melting analysis of **1**, **2**, and **3a** was conducted with telo24 in the presence of K^+, with monitoring at 290 nm, and the ΔT_m values were found to be 1.3, 3.0. and 16.2 °C, respectively (Figure S1). Thus, compound **3a** is both a selective fluorescence probe and a selective stabilizer for G4.

Table 1. Optical data of **1**, **2**, and **3a** (Ex: Excitation length, Em: Emission length, Φ_F: Quantum yield, Φ_F ratio: $\Phi_{F\,telo}/\Phi_{F\,dsDNA}$)

		Compounds		
		1	**2**	**3a**
without DNA	Ex (nm)	322	364	377
	Em (nm)	390	448	485
	Φ_F	0.0070	0.0028	0.011
with telo24	Ex (nm)	322	386	387
	Em (nm)	390	467	472
	Φ_F	0.0087	0.0081	0.064
with	Ex (nm)	322	375	383

dsDNA	Em (nm)	390	452	479
	Φ_F	0.0082	0.0063	0.0063
Φ_F ratio		1.06	1.29	10.2

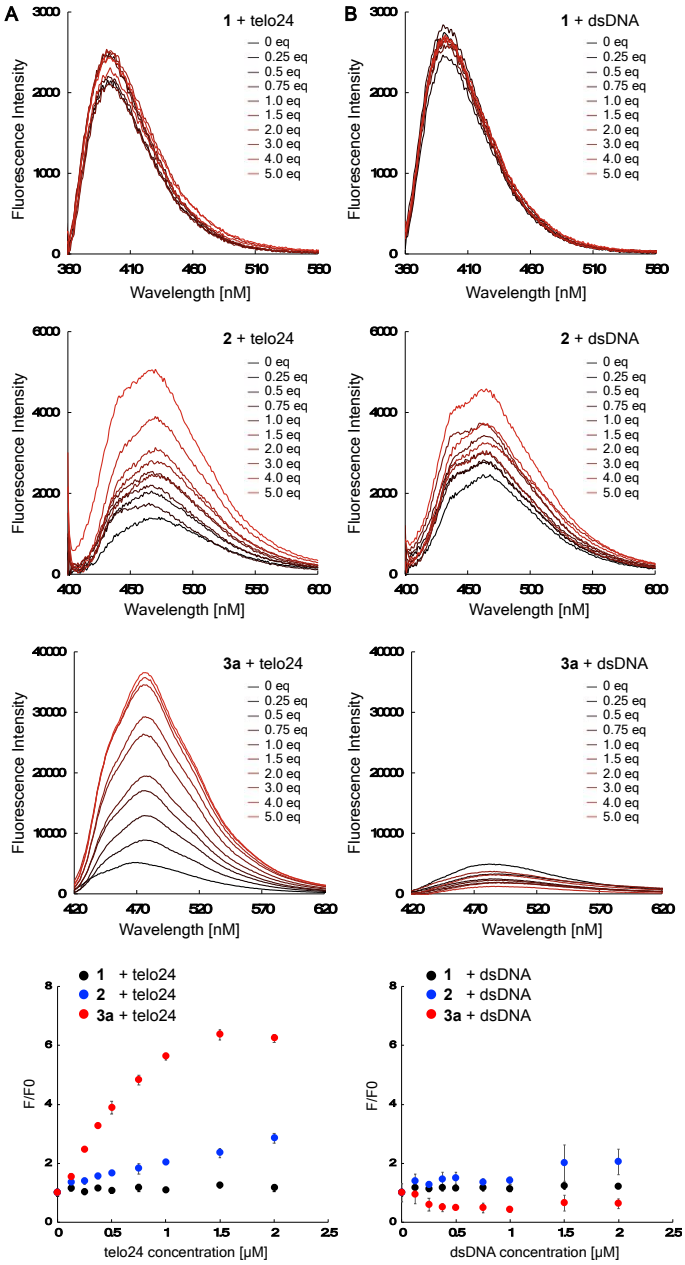


Figure 2. Fluorescence titration spectra and Fluorescence intensity enhancement of **1**, **2**, **3a** (0.5 μM) in the presence of A) telo24 and B) dsDNA.

Development of turn-on type G4 ligand 3e

Building on these results, we carried out structural development of **3a**, focusing on the electronic nature of the naphthalene moiety, based upon Hammett's rule (Figure S2).[16] Specifically, four new derivatives **3b**-**3e** were synthesized by introducing a methanesulfonyloxy (OTD-VN-OMs), methoxy (OTD-VN-OMe), hydroxy (OTD-VN-OH), and dimethylamino group (OTD-VN-NMe_2), respectively, at the C4 position of the naphthyl group (Figure 3A). The fluorescence properties of **3b**-**3e** in the absence and presence of telo24 or dsDNA, respectively, are summarized in Table 2. The maximum emission wavelength of **3b** and **3c** showed the blueshift in the presence of telo24. On the other hand, the change of maximum emission wavelength of **3d** and **3e** was not observed. All compounds did not show a significant fluorescence change in the presence of dsDNA likewise **3a**. In particular, the ligand **3e** was found to have a fluorescence peak of 602 nm, and it shows a large Stokes shift of 200 nm (Table 2).

Table 2. Optical data of **3b**-**3e** (Ex: Excitation length, Em: Emission length, Φ_F: Quantum yield, Φ_F ratio: $\Phi_{F\ telo}/\Phi_{F\ dsDNA}$)

		Compounds			
		3b	**3c**	**3d**	**3e**
without DNA	Ex (nm)	373	399	404	392
	Em (nm)	482	533	528	602
	Φ_F	0.0052	0.0046	0.00043	0.00034
with telo24	Ex (nm)	391	404	412	413
	Em (nm)	472	512	527	602
	Φ_F	0.032	0.028	0.016	0.010
with dsDNA	Ex (nm)	376	399	422	426
	Em (nm)	483	530	530	601
	Φ_F	0.0063	0.0069	0.00042	0.00035
Φ_F ratio		5.08	4.06	38.1	28.6

As expected, the fluorescence intensity of each of **3b**-**3e** increased in a concentration-dependent manner with respect to telo24, but not dsDNA, in a similar manner to that observed for ligand **3a** (Figure S3). The Kd values for telo24 and dsDNA are summarised in Table S2 and Figure 3D. The binding affinity of vinylnaphthalene-bearing hexaoxazole **3a**-**3e** for telo24 showed the hundreds nanomolar level (Kd = 127 to 219 nM), whereas the comparable concentrations for dsDNA were not able to observe the fluorescence enhancement, and higher concentrations of dsDNA (> 2500 nM) were required to obtain the corresponding Kd values for dsDNA. Since the ΔT_m values of **3b**-**3e** in CD melting analysis were found to be 9.4-12.9 °C, these ligands are G4-selective fluorescent probes (Figure S4). Among these derivatives, **3d** and **3e** showed fluorescence only in the presence of G4, not in its absence, and thus are G4-selective turn-on type ligands. Irradiation with UV light at 365 nm in the presence of telo24 resulted in strong yellow and orange fluorescence of **3d** and **3e**, respectively, which was clearly visible to the naked eye (Figure 3B, Figure S5) (these colours are consistent with the wavelengths shown in Figure S3). We compared the fluoresce properties of ligand **3e** with Thioflavin T (ThT)[17] and Thiazole orange (TO),[18] which are known as fluorescent probes against G4 (Table S3). The ratio of quantum yield with telo24 and dsDNA was 3.5 to15-folds higher

than ThT and TO, respectively. Thus, ligand **3e** showed fluorescent more selective to telo24 than ThT or TO.

Next, the fluorescence spectra of **3e** were investigated with G4 forming sequences (telo24, telo22, *c-myc*, *K-ras*, *c-kit1* and *bcl2*) and non-G4 forming sequences (dsDNA, telo24 mutant sequence,[19] and C-telo24, which forms an i-motif under acidic conditions[20]). In the case of mut-telo24 and C-telo24, no enhancement of the fluorescence was observed, as in the case of dsDNA. On the other hand, increased fluorescence intensity of **3e** was observed in the presence of the seven G4-forming sequences in a concentration-dependent manner. The Kd values of **3e** for G4 forming sequences were found to be 127 to 909 nM, which are approximately 2.5-20 times lower than the Kd values of **3e** for non-G4 forming sequences (Kd = >2500 nM). These results indicate that **3e** shows characteristic fluorescence in the presence of G4 structure in general (Figure 3C and 3D).

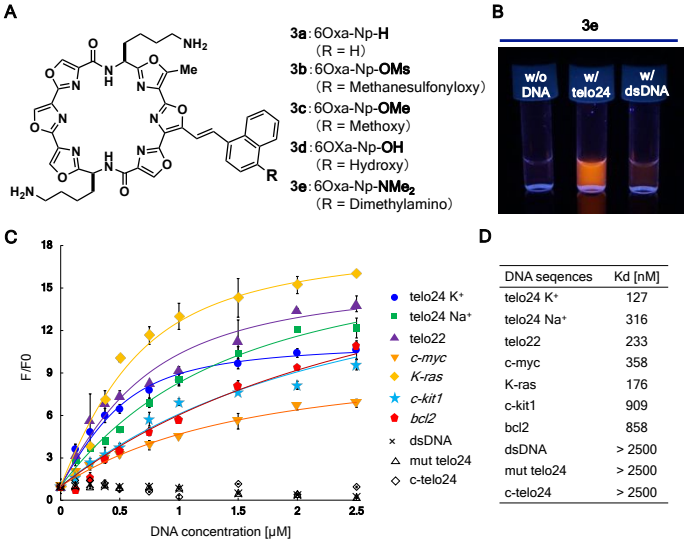


Figure 3. A) Structures of OTD-VN derivatives **3a**-**3d**; B) Fluorescence images of **3e** without DNA (w/o) or with (w/) telo24 or dsDNA upon illumination with a UV lamp (365 nm); C) Fluorescence intensity enhancement of **3e** (0.5 μM) at 602 nm in the presence of various DNAs (0-2.5 μM).

Since compound **3e** only shows fluorescence in the presence of G4, we next examined the fluorescence turn-on mechanism. In general, stilbene-type fluorophores with two aromatic groups connected with an olefin show weak fluorescence in low-viscosity solvents, and this is attributed to free rotation of the bond between the olefin and the aromatic compound.[21] The fluorescence increases in high-viscosity solvents such as glycerol due to the restriction of the rotation.[22] Thus, we examined the fluorescence spectra of **3e** in the presence of various concentrations of glycerol, i.e., solutions with similar polarity yet drastically different viscosity, to elucidate the effect of restriction of the bond rotation on the fluorescence properties of **3e**. Indeed, the intensity of fluorescence of **3e** increased with increasing concentration of glycerol (Figure 4A). These results suggest that **3e** alone does not show fluorescence due to free rotation of the bond between the oxazole and naphthyl groups, but restriction of the free rotation upon interaction with G4 increases the planarity of **3e**, resulting in strong fluorescence. Since the cyclic polyoxazole skeleton in **3e** is known to interact with the G-quartet of G4 through π-π stacking,[23] this idea seems reasonable (Figure 4B). Indeed, docking studies support the interaction of telomeric G4 with **3e** in a planar form by calculation of the dihedral angle, as shown in Figure S6.

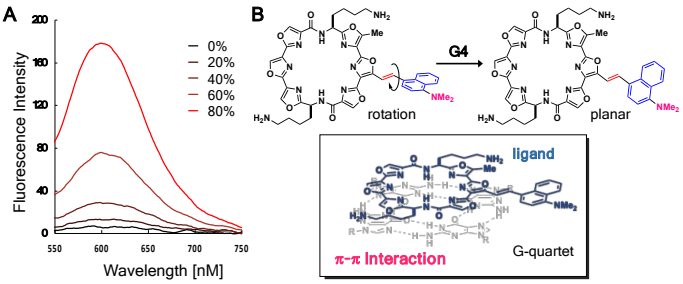


Figure 4. A) Fluorescence titration of **3e** (10 μM) with stepwise addition of glycerol (0-40 %) in 50 mM Tris-HCl buffer (with 100 mM KCl, pH 7.4); B) Schematic model of the interaction of **3e** with G4.

Monitoring the dynamic unwinding process of telomeric G4 in the titration of its complementary sequence

The formation of G4 is in equilibrium with the corresponding single-strand structure, and is regulated by cations and other external factors including ligands. Thus, we examined the fluorescence intensity change of the complex of the ligand **3e** and telo24 upon addition of the complementary sequence of telo24, C-telo24. Addition of C-telo24 to a mixture of **3e** and telo24 decreased the fluorescence intensity in a concentration-dependent manner. Then, CD spectra analysis was carried out to evaluate the interaction between the ligand **3e** and C-telo24 or dsDNA formed by the addition with C-telo24 (Figure S7). In these cases, no significant spectral change was observed in the presence or absence of **3e**. Thus, it was suggested that **3e** does not interact with C-telo24 or dsDNA. This means that **3e**-derived fluorescence was observed only when G4 was formed in the presence of **3e**, and was lost when the G4 structure was disrupted (Figure 5).

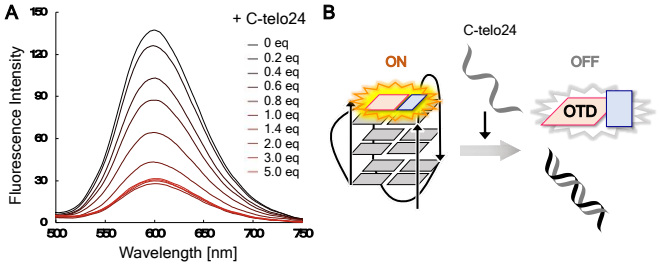


Figure 5. A) Fluorescence titration of the complex of **3e** (0.5 μM) and telo24 (1.5 μM) by stepwise addition of C-telo24 at 25 ˚C in 50 mM Tris-HCl buffer (with 50 mM KCl, pH 7.4); B) Schematic diagram of the detection of G4 dynamics.

Conclusions

In conclusion, we have developed new fluorophores incorporating oxazole bearing a vinyl-naphthyl group. In particular, cyclic hexaoxazole OTD-VN (**3a**) bearing a vinyl-naphthyl group shows fluorescence selectively upon interaction with telomeric G4. Structural modification of the naphthyl group in **3a** led to a turn-on type G4 ligand, OTD-VN-NMe_2 (**3e**), which shows fluorescence only when inter-acting with telo24 or other G4-forming sequences. The fluorescence derived from the complex of **3e** and telo24 was decreased by the addition of the C-rich complementary sequence of telo24, C-telo24, which breaks the G4 structure of telo24. Ligand **3e** is expected to be a useful tool to follow the dynamics of G4 in terms of fluorescence change *in vitro*.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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Notes and references

1 A. S. Biebricher, I. Heller, R. F. Roijmans, T. P. Hoekstra, E. J. Peterman and G. J. Wuite, *Nat. Commun.*, 2015, **6**, 7304; V. Karunakaran, J. L. Pérez Lustres, L. Zhao, N. P. Ernsting and O. Seitz, *J. Am. Chem. Soc.*, 2006, **128**, 2954-2962; F. Koeppel, J. F. Riou, A. Laoui, P. Mailliet, P. B. Arimondo, D. Labit, O. Petitgenet, C. Hélène and J. L. Mergny, *Nucl. Acids Res.*, 2001, **29**, 1087-1096; A. Larsson, C. Carlsson, M. Jonsson and B. Albinsson, *J. Am. Chem. Soc.*, 1994, **116**, 8459-8465; J. B. LePecq and C. Paoletti, *J. Mol. Biol.*, 1967, **27**, 87-106; T. L. Netzel, K. Nafisi, M. Zhao, J. R. Lenhard and I. Johnson, *J. Phys. Chem.*, 1995, **99**, 17936-17947; J. Nygren, N. Svanvik and M. Kubista, *Biopolymers*, 1998, **46**, 39-51; J. Ren and J. B. Chaires, *Biochemistry*, 1999, **38**, 16067-16075; S. Schramm and D. Weiß, in *Advances in Heterocyclic Chemistry*, eds. E. F. V. Scriven and C. A. Ramsden, Academic Press, 2019, vol. 128, pp. 103-179.

2 S. Fujisawa, Y. Romin, A. Barlas, L. M. Petrovic, M. Turkekul, N. Fan, K. Xu, A. R. Garcia, S. Monette, D. S. Klimstra, J. P. Erinjeri, S. B. Solomon, K. Manova-Todorova and C. T. Sofocleous, *Cytotechnology*, 2014, **66**, 259-273; D. Marie, D. Vaulot and F. Partensky, *Appl. Environ. Microbiol.*, 1996, **62**, 1649-1655.

3 H. S. Rye, S. Yue, D. E. Wemmer, M. A. Quesada, R. P. Haugland, R. A. Mathies and A. N. Glazer, *Nucl. Acids Res.*, 1992, **20**, 2803-2812.

4 N. I. Gadjev, T. G. Deligeorgiev, I. Timcheva and V. Maximova, *Dyes Pigm.*, 2003, **57**, 161-164.

5 V. S. Chambers, G. Marsico, J. M. Boutell, M. Di Antonio, G. P. Smith and S. Balasubramanian, *Nat. Biotechnol.*, 2015, **33**, 877-881; J. L. Huppert and S. Balasubramanian, *Nucl. Acids Res.*, 2005, **33**, 2908-2916; A. K. Todd, M. Johnston and S. Neidle, *Nucl. Acids Res.*, 2005, **33**, 2901-2907; W. Yoshida, H. Saikyo, K. Nakabayashi, H. Yoshioka, D. H. Bay, K. Iida, T. Kawai, K. Hata, K. Ikebukuro, K. Nagasawa and I. Karube, *Sci. Rep.*, 2018, **8**, 3116.

6 S. Balasubramanian and S. Neidle, *Curr. Opin. Chem. Biol.*, 2009, **13**, 345-353; H. Han and L. H. Hurley, *Trends Pharmacol. Sci.*, 2000, **21**, 136-142; L. Oganesian and T. M. Bryan, *Bioessays*, 2007, **29**, 155-165; H. Seimiya, *Cancer Sci.*, 2020, **111**, 3089-3099; Q. Wang, J. Q. Liu, Z. Chen, K. W. Zheng, C. Y. Chen, Y. H. Hao and Z. Tan, *Nucl. Acids Res.*, 2011, **39**, 6229-6237.

7 Y. Kanoh, S. Matsumoto, R. Fukatsu, N. Kakusho, N. Kono, C. Renard-Guillet, K. Masuda, K. Iida, K. Nagasawa, K. Shirahige and H. Masai, *Nat. Struct. Mol. Biol.*, 2015, **22**, 889-897; K. Paeschke, J. A. Capra and V. A. Zakian, *Cell*, 2011, **145**, 678-691.

8 A. Siddiqui-Jain, C. L. Grand, D. J. Bearss and L. H. Hurley, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 11593-11598; R. Chaudhuri, S Bhattacharya, J Dash and S. Bhattacharya, *J. Med. Chem.*, 2021, **64**, 42-70.

9 S. Q. Mao, A. T. Ghanbarian, J. Spiegel, S. Martínez Cuesta, D. Beraldi, M. Di Antonio, G. Marsico, R. Hänsel-Hertsch, D. Tannahill and S. Balasubramanian, *Nat. Struct. Mol. Biol.*, 2018, **25**, 951-957; P. Sarkies, P. Murat, L. G. Phillips, K. J. Patel, S. Balasubramanian and J. E. Sale, *Nucl. Acids Res.*, 2012, **40**, 1485-1498.

10 For recent reviews see; E. Largy, A. Granzhan, F. Hamon, D. Verga and M. P. Teulade-Fichou, *Top. Curr. Chem.*, 2013, **330**, 111-177; J. H. Yuan, W. Shao, S. B. Chen, Z. S. Huang and J. H. Tan, *Biochem. Biophys. Res. Commun.*, 2020, **531**, 18-24.

11 For selected examples of turn-on fluorescent probes, see; X. C. Chen, S. B. Chen, J. Dai, J. H. Yuan, T. M. Ou, Z. S. Huang and J. H. Tan, *Angew. Chem. Int. Ed. Engl.*, 2018, **57**, 4702-4706; V. Grande, F. Doria, M. Freccero and F. Würthner, *Angew. Chem. Int. Ed. Engl.*, 2017, **56**, 7520-7524; W. C. Huang, T. Y. Tseng, Y. T. Chen, C. C. Chang, Z. F. Wang, C. L. Wang, T. N. Hsu, P. T. Li, C. T. Chen, J. J. Lin, P. J. Lou and T. C. Chang, *Nucl. Acids Res.*, 2015, **43**, 10102-10113; A. Laguerre, L. Stefan, M. Larrouy, D. Genest, J. Novotna, M. Pirrotta and D. Monchaud, *J. Am. Chem. Soc.*, 2014, **136**, 12406-12414; B. Maji, K. Kumar, M. Kaulage, K. Muniyappa and S. Bhattacharya, *J. Med. Chem.*, 2014, **57**, 6973-6988; J. Mohanty, N. Barooah, V. Dhamodharan, S. Harikrishna, P. I. Pradeepkumar and A. C. Bhasikuttan, *J. Am. Chem. Soc.*, 2013, **135**, 367-376; A. Shivalingam, M. A. Izquierdo, A. L. Marois, A. Vyšniauskas, K. Suhling, M. K. Kuimova and R. Vilar, *Nat. Commun.*, 2015, **6**, 8178; M. Tera, T. Hirokawa, S. Okabe, K. Sugahara, H. Seimiya and K. Shimamoto, *Chem. Eur. J.*, 2015, **21**, 14519-14528; Q. Zhai, C. Gao, J. Ding, Y. Zhang, B. Islam, W. Lan, H. Hou, H. Deng, J. Li, Z. Hu, H. I. Mohamed, S. Xu, C. Cao, S. M. Haider and D. Wei, *Nucl. Acids Res.*, 2019, **47**, 2190-2204.

12 Y. Ma, K. Iida, S. Sasaki, T. Hirokawa, B. Heddi, A. T. Phan and K. Nagasawa, *Molecules*, 2019, **24**, 263; Y. Ma, Y. Tsushima, M. Sakuma, S. Sasaki, K. Iida, S. Okabe, H. Seimiya, T. Hirokawa and K. Nagasawa, *Org. Biomol. Chem.*, 2018, **16**, 7375-7382; M. Tera, H. Ishizuka, M. Takagi, M. Suganuma, K. Shin-ya and K. Nagasawa, *Angew. Chem. Int. Ed. Engl.*, 2008, **47**, 5557-5560.

13 B. K. Senapati, L. Gao, S. I. Lee, G.-S. Hwang and D. H. Ryu, *Org. Lett.*, 2010, **12**, 5088-5091.

14 K. Iida, S. Majima, T. Ohtake, M. Tera, K. Shin-ya and K. Nagasawa, *Heterocycles*, **2012**, 84, 401-411.

15 Fluorescence properties of **1**-**3** in the presence or absence of nucleic acids are described in the supporting information.
16 L. P. Hammett, *J. Am. Chem. Soc.*, 1937, **59**, 96-103; C. Hansch, A. Leo and R. W. Taft, *Chem. Rev.*, 1991, **91**, 165-195.
17 J. Mohanty, N. Barooah, V. Dhamodharan, S. Harikrishna, P. I. Pradeepkumar and A. C. Bhasikuttan, *J. Am. Chem. Soc.*, 2013, **135**, 367-376.
18 I. Lubitz, D. Zikich and A. Kotlyar, *Biochemistry*, 2010, **49**, 3567-3574.
19 T The G4 forming sequences are known to form three kinds of topologies, i.e., hybrid in the presence of K^+ (telo24 and *bcl2*), anti-parallel in the presence of Na^+ (telo24) and in the presence of K^+ (telo22), and parallel in the presence of K^+ (*c-myc* and *K-ras*), respectively.
20 H. A. Day, P. Pavlou and Z. A. Waller, *Bioorg. Med. Chem.*, 2014, **22**, 4407-4418; K. Gehring, J. L. Leroy and M. Guéron, *Nature*, 1993, **363**, 561-565.
21 F. Liu, T. Wu, J. Cao, S. Cui, Z. Yang, X. Qiang, S. Sun, F. Song, J. Fan, J. Wang and X. Peng, *Chem. Eur. J.*, 2013, **19**, 1548-1553.
22 X. Wang, F. Song and X. Peng, *Dyes Pigm.*, 2016, **125**, 89-94.
23 W. J. Chung, B. Heddi, M. Tera, K. Iida, K. Nagasawa and A. T. Phan, *J. Am. Chem. Soc.*, 2013, **135**, 13495-13501.