ChemComm

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2014, **50**, 1573

Received 30th October 2013,
Accepted 4th December 2013

DOI: 10.1039/c3cc48297a

www.rsc.org/chemcomm

Highly emissive deoxyguanosine analogue capable of direct visualization of B–Z transition†‡

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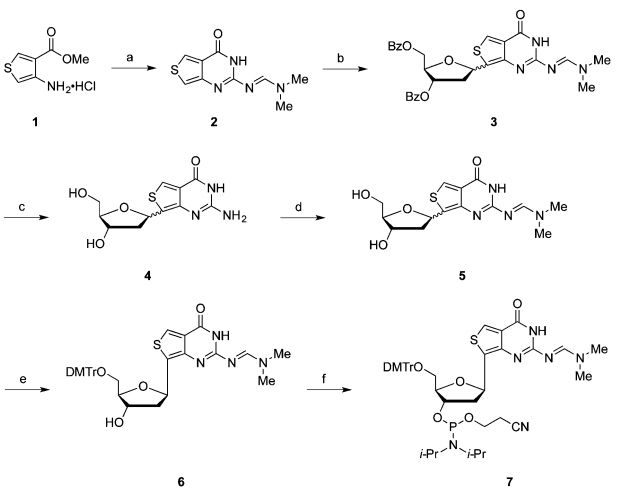
A 2-aminothieno[3,4-*d*]pyrimidine G-mimic deoxyribonucleoside, thdG, was synthesized and incorporated readily into oligonucleotides as a versatile fluorescent guanine analogue. We demonstrate that thdG enables the visual detection of Z-DNA successfully based on different π-stacking of B- and Z-DNA.

Fluorescent probes are powerful and indispensable tools to detect biomolecules and monitor their functions. Their development is opening up new fields of research, and many biological phenomena have been understood by tracking fluorescent signals in living systems.[1] The development of fluorescent probes for nucleic acids is essential because nucleic acids are not fluorescent. In addition, a fluorescent base analogue has great significance for the expansion of an artificial genetic alphabet with diverse functionality.[2–5] Many artificial fluorescent nucleobase analogues have been developed to date and researchers are continuing to design and synthesize new forms that fulfill both isomorphicity such as native Watson–Crick base pairing and practical photophysical properties.[6–11] Recently, Tor and coworkers have developed isomorphic fluorescent RNA nucleosides derived from thieno[3,4-*d*]-pyrimidine, which have very significant photophysical features including visible light emission and a high quantum yield.[12,13] This suggested the potential of using such nucleobase analogues with a thienopyrimidine heterocycle and led us to exploit the thieno[3,4-*d*]-pyrimidine DNA nucleoside. Here, we focus on guanine as it plays an important role in the structural dynamics of DNA such as the formation of quadruplexes and the transition between B-DNA and Z-DNA. We describe the synthesis, photophysical properties and DNA incorporation of a fluorescent base analogue, 2-aminothieno[3,4-*d*]pyrimidine G-mimic deoxyribonucleoside, th**dG**. In addition, we have achieved successfully the direct visualization of B–Z transition using th**dG** based on different π-stacking of B- and Z-DNA.

The synthesis of th**dG** was performed by following published procedures for generating RNA nucleosides (Scheme 1).[12] The parent heterocycle with a thiophene group was synthesized from the commercially available methyl 4-aminothiophene-3-carboxylate hydrochloride (**1**) by reaction with chloroformamidine hydrochloride in dimethylsulfone at 125 °C. The amino group on 2-amino-thieno[3,4-*d*]pyrimidine-4(3*H*)-one was protected as the *N*,*N*-dimethylformamidine form. The obtained thienoguanine (**2**) could be converted into the protected deoxyribonucleoside (**3**) through Friedel–Crafts C-glycosylation with an acylated sugar derivative. This coupling afforded a mixture of β- and α-anomers with a



Scheme 1 Synthesis of th**dG**. *Reagents and conditions*: (a) (i) chloroformamidine hydrochloride, $DMSO_2$, 125 °C; (ii) dimethylformamide dimethyl acetal, DMF, 98%; (b) β-D-deoxyribofuranose 1-acetate 4,5-dibenzoate, $SnCl_4$, $MeNO_2$, 0 °C to RT, 54%; (c) NH_3–MeOH, 65 °C, 71%; (d) dimethylformamide dimethyl acetal, DMF–MeOH, 76%; (e) DMTrCl, Py, 51%; (f) 2-cyanoethyl *N*,*N*-diisopropylchloro phosphoramidite, iPr_2NEt, DCM–MeCN, 0 °C to RT.

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† This communication is dedicated to Professor Andrew D. Hamilton on the occasion of his 60th birthday.

‡ Electronic supplementary information (ESI) available. See DOI: 10.1039/c3cc48297a

Table 1 Photophysical data of thdG

Solvent	λ_{abs}/nm (ε/10^3 M^{-1} cm^{-1})	λ_{em}/nm (ϕ)	$\phi\varepsilon$	τ/ns	Stokes shift/cm^{-1}
Water	319 (4.26)	455 (0.58)	2471	20.5	9370
Dioxane	329 (5.37)	429 (0.85)	4565	14.0	7085
MeOH	327 (3.80)	460 (0.47)	1786	13.7	8842

52% yield at a β : α proportion of 3 : 1. The benzoyl protecting groups were removed in a methanolic ammonia and the *N*,*N*-dimethylformamidine group was reintroduced for protection of the purine amino group. Subsequently, the 5′-hydroxyl group was protected as the dimethoxytrityl ether (DMTr) and the desired β-isomer could be isolated at a yield of 51%. The configuration at the C-1 carbons of anomers was confirmed by 1D and 2D (NOESY) ^{1}H NMR experiments (see ESI‡).

The fundamental photophysical properties of the thdG monomer were investigated and are shown in Table 1. The fluorescence of the thdG deoxyribonucleoside (**4**) shows absorption at 319 nm and visible emission at 455 nm with a high quantum yield under neutral conditions in water, dioxane, and MeOH. These results are similar to those of thG RNA nucleoside reported by the Tor group.[12] In addition, the thdG deoxyribonucleoside displays a relatively high quantum yield under dioxane (0.85) and a longer excited state lifetime in water (20.5 ns) compared with a RNA nucleoside thG. To evaluate oligonucleotides including the isomorphic dG surrogate, the phosphoramidite of thdG was synthesized and incorporated into the center of 18-mer DNA oligonucleotides of 5′–d(CGTCCGTC**X**TACGCACGC)–3′, where **X** = thdG, by automated solid-phase synthesis and phosphoramidite chemistry. The complementary strands of ODN1 containing matched or mismatched bases and the corresponding natural DNA duplexes with guanine were also prepared. As shown in Fig. 1, the thdG–C base pair afforded almost identical thermal stability (T_m = 72.3 °C) compared with natural duplex DNA with a G–C base pair (T_m = 72.1 °C). A very similar tendency to decrease T_m was observed for thdG (ODN1) and G (ODN2) using the complementary strands including mismatched bases (ODN3 ~ ODN7). The thermodynamic stability and base pairing selectivity indicate that thdG could replace a G base in the strand without causing structural disruption. Furthermore, the fluorescent properties of ODN1 were evaluated upon hybridization with complementary strands containing matched or mismatched bases (Fig. S6, ESI‡). Interestingly, significant changes in the fluorescence intensity (match < T < G < A) were observed, indicating that the fluorescence of thdG is sensitive to the local environment of DNA.[14]

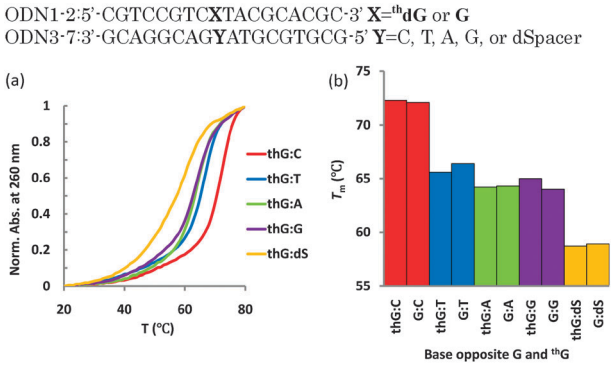

Fig. 1 Thermal stability and selectivity of base pairing. (a) Thermal melts of thG containing DNA. ODN1:5′-CGTCCGTCthdGTACGCACGC-3′ paired with ODN3-7:5′-GCGTGCGTA**Y**GACGGACG-3′ **Y** = C, T, A, G, or dSpacer. (b) Comparison of T_m values. ODN1,2:5′ CGTCCGTC**X** TACGCACGC-3′ **X** = thdG or G paired with ODN3-7. All samples contained 5 μM of each oligonucleotide strand, 20 mM Na cacodylate (pH 7.0) and 100 mM NaCl. 5′-*O*-Dimethoxytrityl-1′,2′-dideoxyribose-3′-[(2-cyanoethyl)-(*N*,*N*-diisopropyl)]-phosphoramidite was used as dSpacer (dS).

The isomorphicity and the photophysical properties of thdG prompted us to exploit this new G-mimic deoxyribonucleoside. We intended to monitor the conformational changes of DNA through changes in fluorescence intensity of thdG.[15] DNA has a remarkable conformational flexibility. Among them, the most typical example is the structural transition between right-handed B-DNA and left-handed Z-DNA.[16] In particular, the dynamic structural change from the continuous π-stacks in B-DNA to the discrete four-base π-stacks in Z-DNA is a very attractive option for constructing DNA-based nanodevices.[17,18] We have demonstrated that the B–Z transition could be detected by measuring the fluorescence intensity of 2-aminopurine.[18] Based on previous studies, we expected that thdG fluorescence could be applied to monitor the change of electronic properties between B- and Z-DNA. To test this possibility thdG-containing the duplex decamer, 5′–d(CGCGC**X**CGCG)–3′ (ODN8), where **X** = thdG, was prepared and investigated. However CD spectroscopy indicated that the B–Z transition became more difficult when thdG was incorporated as a replacement for a G nucleotide. Indeed, the duplex ODN8 produced an almost B-conformation even in 5 M NaCl. This might be explained because thdG favors the anti-conformation and stabilizes B-form DNA when it is located in DNA oligonucleotide strands. The theoretical calculations for the *syn*–*anti* conformation of thdG were performed at the ground state with the density functional B3LYP and the 6-31* basis set. This optimized the lowest energy conformations, suggesting that the anti-conformation is more stable than the *syn* conformation (Fig. S10, ESI‡). We therefore decided on the introduction of a Z-stabilizing unit into DNA sequences and 8-methylguanine (m^8G) was chosen corresponding to this objective. Previously, we demonstrated that the incorporation of a methyl group at the guanine C8 position (m^8G) markedly stabilizes the Z conformation at low salt conditions. The self-complementary dodecamer d(CGC**X**C**Y**CGCG)$_2$ (ODN9), where **X** = thdG and **Y** = m^8G, was synthesized and fluorescence intensity was observed at various salt concentrations. To our delight, ODN9 exhibited a dramatic change of fluorescence intensity in response to the B–Z transition. ODN9 showed the typical CD spectrum of Z-DNA, a negative cotton effect around 295 nm and a positive cotton effect around 260 nm, at high salt concentrations. As shown in Fig. 2, a very strong fluorescence enhancement was observed in Z-DNA, whereas the fluorescence was weak in B-DNA. The intense fluorescence in Z-DNA can be explained by disruption of charge transfer attributable to its four-base π-stacks (Fig. S11, ESI‡).[18]

The fluorescence of ODN9 increased proportionally when we increased the ratio of the Z-conformation by adjusting the $NaClO_4$ concentration. Furthermore, as shown in Fig. 2c, robust brightness of ODN9 in the visible region enabled visualization of the B–Z transition. This inspired us with visual detection of DNA–protein interactions.[19] The DNA-binding domain of double-stranded RNA adenosine deaminase (ADAR1), called Zα domain, specifically binds

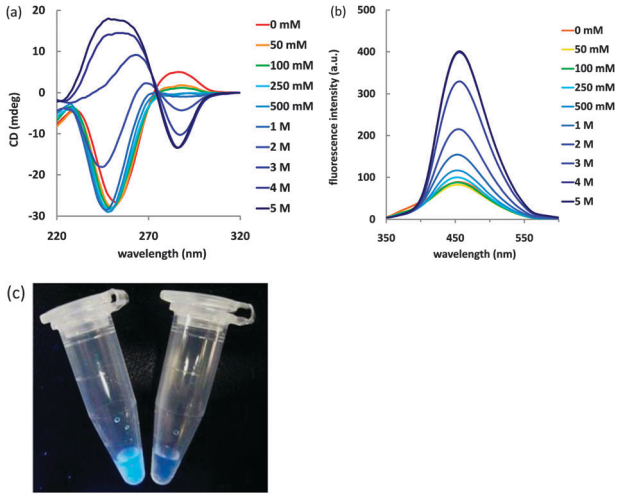

Fig. 2 Observation of the conformational changes from B-DNA to Z-DNA through changes in fluorescence intensity (a) observation of the B–Z transition by CD spectroscopy. (b) Change of fluorescence of $NaClO_4$ at 5 °C. (c) Visual detection of B–Z transition. Samples contained 5 μM of ODN9 in 20 mM sodium cacodylate buffer (pH 7.0). 5 M (left) or 50 mM (right) of $NaClO_4$ at *ca.* 5 °C. The photo was taken under UV irradiation.

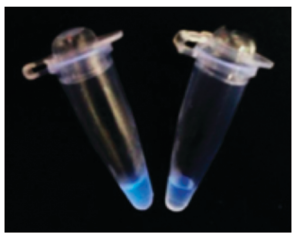

Fig. 3 Visual detection of B–Z transition by Zαβ interaction. 4 eq. (left) or 0 eq. (right) of Zαβ was added to 1.3 μM of ODN9 and 100 mM of NaCl in 20 mM Tris-HCl buffer (pH 7.5). After incubation at 37 °C for 30 min, the photo was taken under UV irradiation.

to Z-DNA.[20,21] Crystal structure studies revealed that two Zα domains form a stable complex with a Z-forming CG repeat sequence.[21] Z-DNA-specific binding proteins have received attention with relevance to the influence of Z-DNA formation on transcriptional activity. We employed a Zαβ protein containing an extra Zβ domain, a second Z-binding domain of ADAR1. If Zαβ is titrated into DNA solution, the fluorescence of oligonucleotides containing th**dG** would be strongly increased in real time as B–Z transition occurs. Consequently, when we added Zαβ into the solution containing ODN9, ODN9 afforded the distinguishable bluish emission compared with the pretreated solution (Fig. 3). This indicates that oligonucleotides containing th**dG** can be a useful tool in the development of visual detection methodologies for DNA.

In conclusion, th**dG** has been synthesized as a versatile fluorescent guanine analogue. It could be incorporated readily into oligonucleotides using phosphoramidite chemistry and was successfully substituted for G bases with an almost identical thermodynamic stability and base pairing selectivity. Furthermore, its remarkable photophysical properties including the strong visible emission enabled th**dG** to be applied admirably as an efficient fluorescent probe for the detection of the conformational changes in DNA. This study raises our hope that the present fluorescent nucleobase, th**dG**, could be superior to 2-Ap which is quenched in DNA and emits in the UV region.[6,22] We believe that th**dG** will expand the repertoire of fluorescent base analogues. We are currently exploring the application of th**dG** further, including its charge transfer properties and incorporation into living cells.

We express our sincere thanks for the CREST grant from the Japan Science and Technology Corporation (JST), grants from the WPI program (iCeMS, Kyoto University), and for the global COE program from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

Notes and references

1 (*a*) G. Mayer and A. Heckel, *Angew. Chem., Int. Ed.*, 2006, **45**, 4900–4921; (*b*) R. W. Sinkeldam, N. J. Greco and Y. Tor, *Chem. Rev.*, 2010, **110**, 2579–2619.
2 A. A. Henry and F. E. Romesberg, *Curr. Opin. Chem. Biol.*, 2003, **7**, 727–733.
3 S. A. Benner, *Acc. Chem. Res.*, 2004, **37**, 784–797.
4 A. T. Krueger and E. T. Kool, *Curr. Opin. Chem. Biol.*, 2007, **11**, 588–594.
5 I. Hirao, M. Kimoto and R. Yamashige, *Acc. Chem. Res.*, 2012, **45**, 2055–2065.
6 D. C. Ward, E. Reich and L. Stryer, *J. Biol. Chem.*, 1969, **244**, 1228–1237.
7 N. B. Gaied, N. Glasser, N. Ramalanjaona, H. Beltz, P. Wolff, R. Marquet, A. Burger and Y. Mély, *Nucleic Acids Res.*, 2005, **33**, 1031–1039.
8 H. Kimura, T. Katoh, T. Kajimoto, M. Node, M. Hisaki, Y. Sugimoto, T. Majima, Y. Uehara and T. Yamori, *Anticancer Res.*, 2006, **26**, 91–97.
9 F. Seela, E. Schweinberger, K. Xu, V. R. Sirivolu, H. Rosemeyer and E.-M. Becker, *Tetrahedron*, 2007, **63**, 3471–3482.
10 Q. Xiao, R. T. Ranasinhe, A. M. P. Tang and T. Brown, *Tetrahedron*, 2007, **63**, 3483–3490.
11 M. Weinberger, F. Berndt, R. Mahrwald, N. P. Ernsting and H.-A. Wagenknecht, *J. Org. Chem.*, 2013, **78**, 2589–2599.
12 D. Shin, R. W. Sinkeldam and Y. Tor, *J. Am. Chem. Soc.*, 2011, **133**, 14912–14915.
13 (*a*) Y. Tor, S. D. Valle, D. Jaramillo, S. G. Srivatsan, A. Rios and H. Weizman, *Tetrahedron*, 2007, **63**, 3608–3614; (*b*) N. J. Greco and Y. Tor, *Tetrahedron*, 2007, **63**, 3515–3527.
14 (*a*) K. D. Kourentzi, G. E. Fox and R. C. Willson, *Anal. Biochem.*, 2003, **322**, 124–126; (*b*) A. A. Marti, S. Jockusch, Z. Li, J. Ju and N. J. Turro, *Nucleic Acids Res.*, 2006, **34**, 6, e50.
15 (*a*) M. Menger, F. Eckstein and D. Porschke, *Biochemistry*, 2000, **39**, 4500–4507; (*b*) T. Kimura, K. Kawai, M. Fujitsuka and T. Majima, *Chem. Commun.*, 2004, 1438–1439; (*c*) J. Li, J. J. Correia, L. Wang, J. O. Trent and J. B. Chaires, *Nucleic Acids Res.*, 2005, **33**, 4649–4659; (*d*) R. D. Gray, L. Petraccone, R. Buscaglia and J. B. Chaires, *Mol. Biol.*, 2010, **608**, 121–136; (*e*) J. Johnson, R. Okyere, A. Joseph, K. Musier-Forsyth and B. Kankia, *Nucleic Acids Res.*, 2013, **41**, 220–228.
16 (*a*) A. Rich, A. Nordheim and A. H.-J. Wang, *Annu. Rev. Biochem.*, 1984, **53**, 791–846; (*b*) T. M. Jovin, D. M. Soumpasis and L. P. McIntosh, *Annu. Rev. Phys. Chem.*, 1987, **38**, 521–560.
17 (*a*) C. Mao, W. Sun, Z. Shen and N. C. Seeman, *Nature*, 1991, **397**, 144–146; (*b*) L. Feng, A. Zhao, J. Ren and X. Qu, *Nucleic Acids Res.*, 2013, **41**, 7987–7996.
18 (*a*) R. Tashiro and H. Sugiyama, *Angew. Chem., Int. Ed.*, 2003, **42**, 6018–6020; (*b*) R. Tashiro and H. Sugiyama, *J. Am. Chem. Soc.*, 2005, **127**, 2094–2097.
19 (*a*) T. D. Bradrick and J. P. Marino, *RNA*, 2004, **10**, 1459–1468; (*b*) G. Tamulaitis, M. Zaremba, R. H. Szczepanowski, M. Bochtler and V. Siksnys, *Nucleic Acids Res.*, 2007, **35**, 4792–4799; (*c*) L. J. Reha-Krantz, *Mol. Biol.*, 2009, **521**, 381–396; (*d*) L. J. Reha-Krantz, C. Hariharan, U. Subuddhi, S. Xia, C. Zhao, J. Beckman, T. Christian and W. Konigsberg, *Biochemistry*, 2011, **50**, 10136–10149.
20 T. Schwaltz, M. A. Rould, K. Lowenhaupt, A. Herbert and A. Rich, *Science*, 1999, **284**, 1841–1845.
21 S. Bae, D. Kim, K. K. Kim, Y. Kim and S. Hohng, *J. Am. Chem. Soc.*, 2011, **133**, 668–671.
22 (*a*) J. M. Jean and K. B. Hall, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 37–41; (*b*) N. P. Johnson, W. A. Baase and P. H. Hippel, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 3426–3431.