

Single Site Discrimination of Cytosine, 5-Methylcytosine and 5-Hydroxymethylcytosine in Target DNA using Anthracene-Tagged Fluorescent Probes

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Single Site Discrimination of Cytosine, 5-Methylcytosine and 5-Hydroxymethylcytosine in Target DNA using Anthracene-Tagged Fluorescent Probes

Jean-Louis H. A. Duprey,[§] Gemma A. Bullen,[§] Zheng-yun Zhao,[§] Dario M. Bassani,[†] Anna F. A. Peacock,[§] John Wilkie,[§] and James H. R. Tucker^{*,§}

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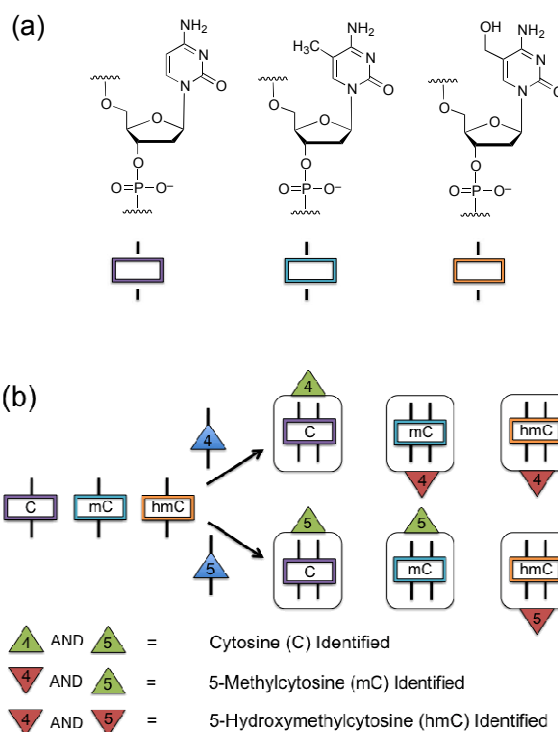
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Supporting Information Placeholder

ABSTRACT: The ability to discriminate between epigenetic variants in DNA is a necessary tool if we are to increase our understanding of the roles that they play in various biological processes and medical conditions. Herein it is demonstrated how a simple two-step fluorescent probe assay can be used to differentiate all three major epigenetic variants of cytosine at a single locus site in a target strand of DNA.

The roles that modified nucleobases in DNA may play in cellular processes, gene expression and evolutionary development show the importance that nature places on structural diversity.¹ Cytosine modification is a crucial epigenetic process² and methylation at the C5 position is well documented.³ However the recent discovery of 5-hydroxymethylcytosine (hmC) in mammalian tissue DNA⁴ suggests rather subtle but important biological roles with potential medical significance for the cohort of cytosine variants (Figure 1a).^{5,6} It also highlights and reinforces the importance of identifying and discriminating between various cytosine modifications in DNA, that may range from large hyper- or hypo-methylated regions to single-site loci.⁷ Bisulfite sequencing can successfully discriminate between cytosine and methylcytosine (mC) residues but until very recently,⁸ this widely-used method could not discriminate between methyl- and hydroxymethyl modifications, as both prevented bisulfite mediated oxidation of cytosine to uracil.^{9,10} A number of other approaches have been used in an attempt to differentiate between various cytosine variants, including single-molecule real time sequencing (SMRT),¹¹ chemical labeling,¹² liquid chromatography/mass spectrometry (LC/MS-MS),¹³ antibodies,¹⁴ peptides,¹⁵ nanopores^{16,17} and electrochemical sequencing.¹⁸ However, to date relatively few strategies have attempted to use a simple and direct fluorescent readout to accomplish discrimination between these epigenetic markers (vide infra). This is in contrast to the wide range of fluorescence-based strategies used to identify canonical base variations in DNA (i.e. single nucleotide polymorphisms or SNPs) using so-

called base discriminating fluorophores (BDFs).^{19,20} There are some examples of related approaches to sense modifications in particular DNA bases, e.g. oxo-G from G,^{21,22} but the similarity in size and structure of modified nucleobases tend to make the identification of such changes a challenging exercise.



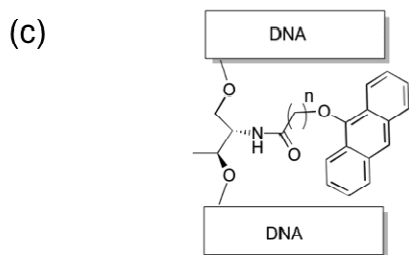


Figure 1 Sensing epigenetic changes in DNA. (a) Target nucleobases cytosine (C), 5-methylcytosine (mC) and 5-hydroxymethylcytosine (hmC) within a DNA strand; (b) Schematic representation of the fluorescent sensing assay where the colored boxes denote the different bases to be probed, the blue triangles represent the anthracene tagged probes **P-4** and **P-5**, and the red and green triangles denote increased and decreased fluorescence emission upon hybridization; (c) Structure of the anthracene tag in the DNA probe strands **P-n**, where *n* is the number of CH₂ groups.

We have previously reported on the use of anthracene tagged oligonucleotide probes to detect SNPs in target DNA strands^{23,24} via a modulation of the fluorescent output from the excited anthracene chromophore upon hybridization, which was found to increase or decrease depending on the identity of the base at a single locus in the target strand. As with other BDF approaches, this method takes advantage of the simplicity of monitoring fluorescence changes at room temperatures as opposed to the need to precisely control the extent of hybridization via temperature variation, as is used in a number of existing commercial assays.²⁵ Recently we extended the strategy to demonstrate the successful discrimination between C and mC bases at the centre of a target DNA strand.²⁶ Herein we report a further extension and optimization of this technique in which we not only report for the first time the sensing of hmC through a direct fluorescence sensing method but also the ability to discern all three epigenetic cytosine variants in one coupled sensing assay (Figure 1b) using two closely related probes.

The anthracene oligonucleotide probes **P-n** containing various alkyl linker lengths, where *n* represents the number of methylene groups in the linker (Figure 1c), were synthesized via standard solid-phase DNA synthesis using a previously described synthetic procedure.²⁶ The use of an enantiopure non-nucleosidic threoninol backbone, as used before by us and others,²⁷ offered both a more straightforward synthesis of the tag and the assurance of forming only one diastereoisomer of the sensing strand. The tags (see Supporting Information) were incorporated into the centre of a 15-mer sequence **P-n** that allowed a CpG sequence to be targeted directly opposite the anthracene position, this being the most common site for methylation. The anthracene modified strands, the unmodified control **P-G** and the three target strands **S-C**, **S-mC** and **S-hmC** were all purified and then characterized

by analytical RP-HPLC and ES mass spectrometry (see Supporting Information).

(a)

Strand	Sequence
P-G	5'-TGGACTCGCTCAATG-3'
P-n (n = 3-7)	5'-TGGACTCnCTCAATG-3'
S-C	3'-ACCTGAGCGAGTTAC-5'
S-mC	3'-ACCTGAGmCGAGTTAC-5'
S-hmC	3'-ACCTGAGhmCGAGTTAC-5'

(b)

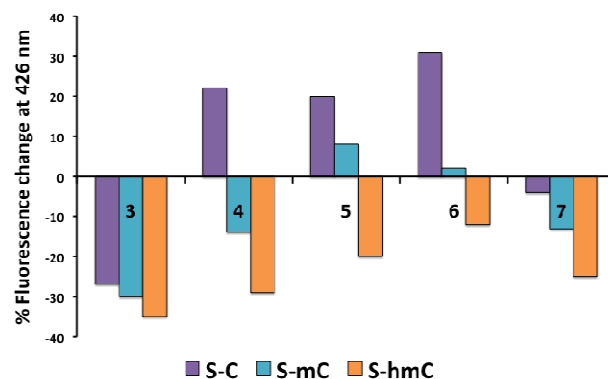


Figure 2 DNA strands and fluorescent response. (a) Table of the DNA probe and target strands synthesized; (b) Bar chart showing the change in fluorescence upon hybridization between probes **P-n** (where *n* = 3-7) and the targets **S-C**, **S-mC** and **S-hmC** at 426 nm, [DNA] = 1 μ M, λ_{ex} = 350 nm.

The quantum yields arising from emission from the photo-excited anthracene tag were found to differ to some extent among the single stranded probes **P-n** (e.g. ϕ = 0.029 and 0.039 for **P-4** and **P-5** respectively, see Supporting Information). This suggests that changing the alkyl linker length has an effect on the anthracene environment upon its incorporation into DNA. Next, hybridization of each probe (concentration 1 μ M) with one molar equivalent of a matching strand containing either cytosine, mC or hmC directly opposite the anthracene tag was undertaken at RT. Emission spectra were then run and the variation in fluorescence output at 426 nm recorded as a percentage change of the emission intensity from that of the anthracene of the unbound probe at that wavelength (Figure 2a).

The data clearly indicates the fluorescence sensing of the DNA target strands, with percentage changes varying from +32 to -34%. Most notably, a consistent trend in emission intensity was observed for duplexes formed with each probe as follows: **S-C** > **S-mC** > **S-hmC**. Furthermore the emission intensities were found to increase upon duplex formation with **S-C** targets for three probes, whereas the emission intensities decreased for all the duplexes formed with **S-hmC**. These results illustrate the highly sensitive nature of the sensing system, with small

changes in both linker length and cytosine methylation status having a remarkable effect on the emissivity of the anthracene tag upon duplex formation.

We then set about identifying a method through which the identity of all three targets (if unknown) could be identified through a simple read-out. The **P-4** probe distinguishes between **S-C** and **S-mC** via a positive and negative change in emission intensity respectively.²⁶ Hybridization with **S-hmC** gives a larger decrease in signal which, although distinguishable, was not considered ideal for simple ON-OFF sensing. However, the **P-5** probe gives slightly more emissive duplexes, with now only the **S-hmC** strand resulting in a decrease in emission. Consequently, by splitting the sample and running two hybridization assays, one with **P-4** and the other with **P-5**, we were able to distinguish identify the methylation status (i.e. cytosine, mC or hmC) at a single locus using basic instrumentation and a simple read-out methodology (Figure 1b).

It was important to confirm that the fluorescence changes were a result of 1:1 duplex formation in each case rather than the extent to which hybridization had occurred. This was done in two ways; firstly through titrations and the addition of an excess amount of target strand, which gave no further changes to the spectra. Secondly, variable temperature UV spectroscopy measurements were undertaken on the duplexes to determine their melting points (T_m values, Table 1). As expected, these values were considerably higher than RT (293 K), indicating the full formation of duplexes at the sensing study temperature. The values also gave some insight into the sensing results. First of all, very small differences in the T_m values for different modifications of cytosine were observed for the unmodified duplexes formed with **P-G**; this trend (albeit not outside of experimental error for the **S-C** system) is the same as that found in previous studies.²⁸ Furthermore, replacing the G base in **P-G** with the anthracene tag (to give **P-n**) gave little or no change in melting temperature despite there being one less C-G H-bonding base pair. This suggests that the anthracene moiety can intercalate into the DNA duplex, causing duplex stabilization via aromatic stacking interactions. CD spectroscopy also supports this hypothesis (see Supporting Information); as expected, the anthracene-modified DNA adopts a B-DNA structure. However, an extra band is observed at ca. 260 nm, which can be ascribed to an induced CD signal that is indicative of strong interactions between the anthracene unit and the DNA duplex.

Table 1. UV melting points (T_m values) for **P-G**, **P-4** and **P-5** with **S-C**, **S-mC** and **S-hmC**. Recorded at 260 nm, [DNA] = 5 μ M, 10 mM pH 7 phosphate buffer, 100 mM NaCl.

Strand ^[a]	P-G	P-4	P-5
S-C	52	51	51
S-mC	53	52	49
S-hmC	51	50	50

^aAverage of 3 runs after annealing. Confidence limit is ± 0.5 °C

In order to further rationalize the sensing mechanism, fluorescent lifetime and molecular dynamic modelling studies were undertaken (see Supporting Information). The former show the existence of three different decay pathways in the single strands with approximately equal weightings. These have been tentatively identified as corresponding to decay from base-contact static quenching (τ_1), water dynamic quenching (τ_2) and fluorescence emission from a solvent-shielded environment (τ_3). Hybridisation leads to changes in the weightings of the different lifetimes and, in some instances, a disappearance of the third decay pathway. These observations give a further indication of how sensitive the anthracene tag is to structural modifications to the linker length and to the base opposite, which in turn must affect its position in its immediate local environment. In support of this, the molecular dynamics studies also identify a number of different environments in which the anthracene can find itself in one duplex system, including fully intercalated, partially intercalated and minor groove binding configurations.

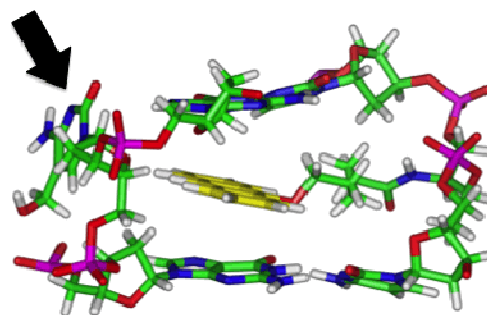


Figure 3 Molecular model of the **P-5:S-hmC** duplex showing the three base-pair core with the anthracene tag (yellow) intercalated and the 5-hydroxymethylcytosine base opposite it (black arrow) flipped out of the base-pair stack.

It has been shown through molecular dynamics studies that modifications to cytosine affect the dynamics of base pairing and duplex stability.¹⁶ Interestingly, the same studies identified the ability of the hydroxyl group in hmC to increase its solvation within the major groove by forming polar cavities that capture water molecules within the first solvation shell. As water could be one of the fluorescence quenching pathways, it may partially explain the lower emission intensity of the **S-hmC** system relative to the other modifications, with the τ_2 pathway indeed showing a marginally greater contribution and shorter lifetime for some duplexes (see Supporting Information). Another factor that may influence the preponderance of one anthracene position over another (and therefore emission intensity) is the degree to which the cytosine base opposite it can “flip out” from the base-pair stack, a process that is in fact integral to the enzymatic conversion of C to mC in nature.²⁹ Our models indicate that this process can occur for all three epigenetic variants when

1 the anthracene is fully intercalated (e.g. Figure 3). Overall
2 these studies indicate that both the cytosine base and the
3 anthracene moiety are highly dynamic, with the overall
4 distribution of the anthracene across these environments
5 resulting in the observed steady-state spectra in the sens-
6 ing assay.

7 Conclusion

8 We have conceived a simple fluorescence-based assay for
9 discriminating between the three major epigenetic struc-
10 tural variants within DNA bases: cytosine, 5-
11 methylcytosine and 5-hydroxymethylcytosine. The sys-
12 tem requires only two modified oligonucleotide probe
13 strands that can hybridise around the base of interest,
14 which can then be unambiguously identified by a simple
15 read-out (UP or DOWN) of two fluorescence readings
16 upon duplex formation. Given the important role that
17 these epigenetic variations appear to play in a number of
18 biological and medically-related processes, the need to
19 accurately and rapidly identify site-specific loci is likely
20 to become even more pressing in the future. This new
21 sensing system provides a potential avenue towards the
22 goal of simple, low cost and rapid base-specific sensors
23 that should be attractive to many researchers interested
24 in the role of specific genetic and epigenetic changes in
25 DNA.

26 Methods

27 The synthesis of the anthracene phosphoramidites was carried
28 out as reported previously.²⁶ Automated DNA synthesis of probe
29 sequences was performed on an Applied Biosystems ABI 394
30 synthesizer using standard phosphoramidites and reagents. The
31 dMeC and dHOMeC phosphoramidites used in the target
32 strands were obtained from Link and were deprotected accord-
33 ing to the manufacturer's instructions. Millipore pure H₂O was
34 used in all syntheses and studies of oligonucleotides. HPLC
35 purification was carried out using a Dionex system with Summit
36 P580 pump and Summit UVD 170s UV/VIS Multi-Channel
37 Detector with prep flow cell. Electrospray Mass spectra were
38 measured by a Waters micromass LCT Time of flight mass
39 spectrometer.

40 UV/Vis spectra were recorded at the University of Birmingham
41 using a Varian Cary 5000 or Varian Cary 50 spectrometer. DNA
42 melting temperatures were determined on a Varian Cary 5000
43 with a peltier heating accessory on a range of 15 to 85 °C with
44 a heating rate of 0.5 °C/min. The value of the T_m was calculated
45 from the first derivative of the melting curve using Varian soft-
46 ware. All samples were monitored at 260 nm. Circular Dichro-
47 ism spectra were taken on a Jasco J-810 spectropolarimeter
48 scanning at a rate of 100nm/min, medium sensitivity.

49 Quantum yields and fluorescence titrations were carried out on a
50 Shimadzu RF-5301 PC spectrofluorimeter. Fluorescence decay
51 profiles and lifetimes were taken using a Houriba Jobin Yvan
52 Fluorolog 211 using a 371 nm pulsed LED excitation source
53 operated at 1 MHz. The detection was accomplished with a
54 cooled Hammamatsu 6158 single photon counting accessory.
55 The decay profiles were fitted using a multiexponential decay
56 function. Different exponential fitting functions were applied
57 and the best fitting one was chosen by consideration of the χ^2
58 parameter and Durbin-Watson test statistic.

Molecular modeling using the AMBER modeling program. The
anthracene modifications were built into a B-form duplex (con-
sistent with CD data), replacing a pre-existing guanine nucleo-
side. Molecular dynamics modeling was then carried out on the
University of Birmingham Bluebear computer cluster for a total
simulation time of 10 ns.

ASSOCIATED CONTENT

Supporting Information Available: Oligomer characterization
(HPLC, MS), fluorescence spectra, CD spectra and modeling
data. This material is available free of charge via the Internet
at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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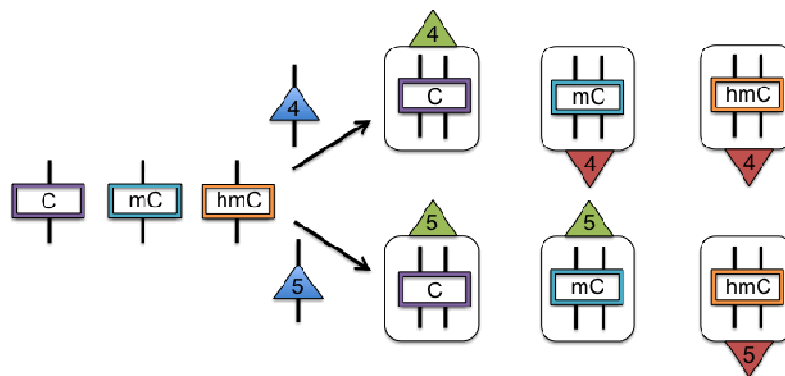
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REFERENCES

- (1) Carell, T., Brandmayr, C., Hienzsch, A., Müller, M., Pearson, D., Reiter, V., Thoma, I., Thumbs, P., and Wagner, M. (2012) Structure and function of noncanonical nucleobases. *Angew. Chem. Int. Ed.* 51, 7110–7131.
- (2) Nabel, C. S., Manning, S. A., and Kohli, R. M. (2011) The curious chemical biology of cytosine: deamination, methylation, and oxidation as modulators of genomic potential. *ACS Chem. Biol.* 7, 20–30.
- (3) Jones, P. A. (2012) Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat. Rev. Genet.* 13, 484–492.
- (4) Kriaucionis, S., and Heintz, N. (2009) The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. *Science* 324, 929–930.
- (5) Klose, R. J., and Bird, A. P. (2006) Genomic DNA methylation: the mark and its mediators. *Trends Biochem. Sci.* 31, 89–97.
- (6) Lercher, L., McDonough, M. A., El-Sagheer, A. H., Thalhammer, A., Kriaucionis, S., Brown, T., and Schofield, C. J. (2014) Structural insights into how 5-hydroxymethylation influences transcription factor binding. *Chem. Commun.* 50, 1794–1796.
- (7) Kristensen, L. S., and Hansen, L. L. (2009) PCR-based methods for detecting single-locus DNA methylation biomarkers in cancer diagnostics, prognostics, and response to treatment. *Clin. Chem.* 55, 1471–1483.
- (8) Booth, M. J., Branco, M. R., Ficiz, G., Oxley, D., Krueger, F., Reik, W., and Balasubramanian, S. (2012) Quantitative sequencing of 5-methylcytosine and 5-hydroxymethylcytosine at single-base resolution. *Science* 336, 934–937.
- (9) Huang, Y., Pastor, W. A., Shen, Y., Tahiliani, M., Liu, D. R., and Rao, A. (2010) The behaviour of 5-Hydroxymethylcytosine in Bisulfite Sequencing. *PLoS ONE* 5, e8888.
- (10) Jin, S.-G., Kadam, S., and Pfeifer, G. P. (2010) Examination of the specificity of DNA methylation profiling techniques towards 5-methylcytosine and 5-hydroxymethylcytosine. *Nucleic Acids Res.* 38, e125

- (11) Flusberg, B. A., Webster, D. R., Lee, J. H., Travers, K. J., Olivares, E. C., Clark, T. A.; Korlach, J., and Turner, S. W. (2010) Direct detection of DNA methylation during single-molecule, real-time sequencing. *Nat. Methods* 7, 461–465.
- (12) Szwagierczak, A., Bultmann, S., Schmidt, C. S., Spada, F., and Leonhardt, H. (2010) Sensitive enzymatic quantification of 5-hydroxymethylcytosine in genomic DNA. *Nucleic Acids Res.* 38, e181
- (13) Le, T., Kim, K.-P., Fan, G., and Faull, K. F. (2011) A sensitive mass spectrometry method for simultaneous quantification of DNA methylation and hydroxymethylation levels in biological samples. *Anal. Biochem.* 412, 203–209.
- (14) Ito, S., D'Alessio, A. C., Taranova, O. V., Hong, K., Sowers, L. C., and Zhang, Y. (2010) Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. *Nature* 466, 1129–1133.
- (15) Nomura, A., Sugizaki, K., Yanagisawa, H., and Okamoto, A. (2011) Discrimination between 5-hydroxymethylcytosine and 5-methylcytosine by a chemically designed peptide. *Chem. Commun.* 47, 8277–8279.
- (16) Wanunu, M., Cohen-Karni, D., Johnson, R. R., Fields, L., Benner, J., Peterman, N., Zheng, Y., Klein, M. L., and Drndic, M. (2010) Discrimination of methylcytosine from hydroxymethylcytosine in DNA molecules. *J. Am. Chem. Soc.* 133, 486–492.
- (17) Li, W.-W., Gong, L., and Bayley, H. (2013) Single-Molecule Detection of 5-Hydroxymethylcytosine in DNA through Chemical Modification and Nanopore Analysis. *Angew. Chem. Int. Ed.* 52, 4350–4355.
- (18) Yang, Z., Jiang, W., Liu, F., Zhou, Y., Yin, H., and Ai, S. (2015) A novel electrochemical immunosensor for the quantitative detection of 5-hydroxymethylcytosine in genomic DNA of breast cancer tissue. *Chem. Commun.* 51, 14671–14673.
- (19) Sigurdsson, S. T., Gardarsson, H., and Cekan, P. (2008) Single base interrogation by a fluorescent nucleotide: Each of the four DNA bases identified by fluorescence spectroscopy. *Chem. Commun.* 3393–3395.
- (20) Yoshida, Y., Niwa, K., Yamada, K., Tokeshi, M., Baba, Y., Saito, Y., Okamoto, A., and Saito, I. (2010) A Probe Containing Two Base-discriminating Fluorescent (BDF) Nucleosides for SNP Typing. *Chem. Lett.* 39, 116–117.
- (21) Nasr, T., Li, Z., Nakagawa, O., Taniguchi, Y., Ono, S., and Sasaki, S. (2009) Selective fluorescence quenching of the 8-oxoG-clamp by 8-oxodeoxyguanosine in ODN. *Bioorg. Med. Chem. Lett.* 19, 727–730.
- (22) Greco, N. J., Sinkeldam, R. W., and Tor, Y. (2009) An emissive C analog distinguishes between G, 8-oxoG, and T. *Org. Lett.* 11, 1115–1118.
- (23) Moran, N., Bassani, D. M., Desvergne, J.-P., Keiper, S., Lowden, P. A. S., Vyle, J. S., and Tucker, J. H. R. (2006) Detection of a single DNA base-pair mismatch using an anthracene-tagged fluorescent probe. *Chem. Commun.* 48, 5003–5005.
- (24) Zhao, Z., San, M., Duprey, J.-L. H. A., Arrand, J. R., Vyle, J. S., and Tucker, J. H. R. (2012) Detection of single nucleotide polymorphisms within a sequence of a gene associated with prostate cancer using a fluorophore-tagged DNA probe. *Bioorg. Med. Chem. Lett.* 22, 129–132.
- (25) McGuigan, F. E.; and Ralston, S. H. (2002) Single nucleotide polymorphism detection: allelic discrimination using TaqMan. *Psychiatr. Genet.* 12, 133–136.
- (26) Duprey, J.-L. H. A., Zhao, Z., Bassani, D. M., Manchester, J., Vyle, J. S., and Tucker, J. H. R. (2011) Detection of DNA base variation and cytosine methylation at a single nucleotide site using a highly sensitive fluorescent probe. *Chem. Commun.* 47, 6629–6631.
- (27) Kashida, H., Liang, X., and Asanuma, H. (2009) Rational design of functional DNA with a non-ribose acyclic scaffold. *Curr. Org. Chem.* 13, 1065–1084.
- (28) Rodriguez Lopez, C. M., Lloyd, A. J., Leonard, K., and Wilkinson, M. J. (2012) Differential effect of three base modifications on DNA thermostability revealed by high resolution melting. *Anal. Chem.* 84, 7336–7342.
- (29) Klimasauskas, S., Kumar, S., Roberts, R. J., and Cheng, X. (1994) HhaI methyltransferase flips its target base out of the DNA helix. *Cell* 76, 357–369.

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