Synthesis of 1*H*-pyrrolo[3,2-h]quinoline-8-amine derivatives that target CTG trinucleotide repeats

Jun Matsumoto, Jinxing Li, Chikara Dohno, Kazuhiko Nakatani

PII: S0960-894X(16)30561-3
DOI: http://dx.doi.org/10.1016/j.bmcl.2016.05.062
Reference: BMCL 23921

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 23 April 2016
Revised Date: 16 May 2016
Accepted Date: 21 May 2016

Please cite this article as: Matsumoto, J., Li, J., Dohno, C., Nakatani, K., Synthesis of 1*H*-pyrrolo[3,2-h]quinoline-8-amine derivatives that target CTG trinucleotide repeats, *Bioorganic & Medicinal Chemistry Letters* (2016), doi: http://dx.doi.org/10.1016/j.bmcl.2016.05.062

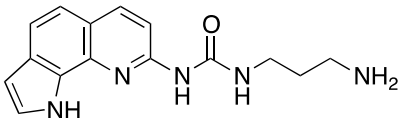
Graphical Abstract

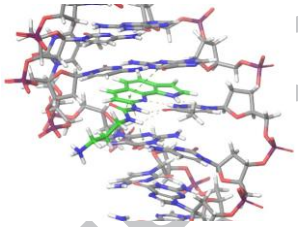
To create your abstract, type over the instructions in the template box below.
Fonts or abstract dimensions should not be changed or altered.

Synthesis of 1*H*-pyrrolo[3,2-h]quinoline-8-amine derivatives that target CTG trinucleotide repeats

Leave this area blank for abstract info.

Jun Matsumoto, Jinxing Li, Chikara Dohno, and Kazuhiko Nakatani





Bioorganic & Medicinal Chemistry Letters
journal homepage: www.elsevier.com

Synthesis of 1*H*-pyrrolo[3,2-h]quinoline-8-amine derivatives that target CTG trinucleotide repeats

Jun Matsumoto[a], Jinxing Li[a], Chikara Dohno[a], and Kazuhiko Nakatani[a]*

[a]*The Institute of Scientific and Industrial Research, Osaka University, 8-1 Mihogaoka, Ibaraki 567-0047, Japan*

ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

Keywords:
Small molecule ligand
Mismatch base pair
CTG repeat

ABSTRACT

We describe a new molecular design, synthesis, and investigation of small molecules that bind to CTG trinucleotide repeats in DNA. 1*H*-pyrrolo[3,2-h]quinoline-8-amine (**PQA**) has a tricyclic aromatic system with unique non-linear hydrogen-bonding surface complementary to thymine. We have synthesized a series of **PQA** derivatives with different alkylamino linkers. These **PQA**s showed binding to pyrimidine bulge DNAs and CNG (N = T and C) repeats depending on the linker structure, while quinoline derivatives lacking the pyrrole ring showed much lower binding affinity. **PQA** is a useful molecular unit for both CTG and CCG repeat binding.

Myotonic dystrophy type 1 (DM1), the most common form of muscular dystrophy presenting 1 in 8000 people, is one of more than 30 inheritable disorders classified as trinucleotide repeats (TNR) disorders that are caused by aberrant expansion of triplet repeats of specific genes.[1, 2] DM1 is caused by the anomalous expansion of CTG trinucleotide repeats in 3'-untranslated region of dystrophia myotonica protein kinase (DMPK) gene. Normal individuals have <37 CTG repeats, while DM1 patients carry between 50 and many thousands of repeats sequence. The expanded CTG is transcribed to CUG repeats, which cause toxic gain of functions.[3-8] Targeting the toxic RNA by oligonucleotides and small molecular ligands has been a promising strategy to alleviate the pathologic features.[8-14] Targeting CTG repeat DNA could be another approach for the diseases, which could affect on transcription step and repeat stability related to repeat expansion and contraction.[15-17] Here, we report a series of new molecules consisting of 1*H*-pyrrolo[3,2-h]quinoline-8-amine (**PQA**) skeleton as a CTG repeat binding ligand. Binding assays including thermal melting temperature, surface plasmon resonance (SPR) and isothermal titration calorimetry (ITC) measurement showed that the **PQA** derivatives bound to CTG and CCG repeats but not to CAG and CGG repeats.

CTG repeats sequence was suggested to form hairpin structure consisting of a tandem array of non-canonical T–T mismatch base pair flanked by G–C base pairs.[18] We have developed a series of synthetic ligands for mismatched base pairs in DNA.[19-22] The T–T mismatch could be a target in order to develop small ligands for CTG repeats because T–T mismatch specifically formed in the CTG repeats hairpin structure. To our knowledge, there is few known small molecule that selectively and strongly binds to T–T mismatch.[9, 23-25] The difficulty can be

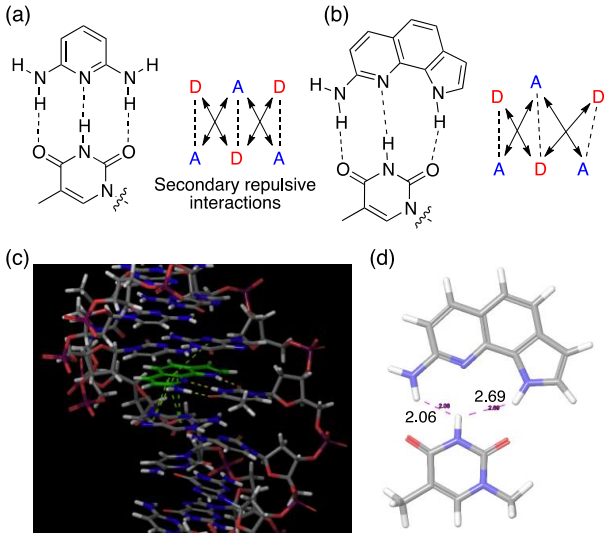
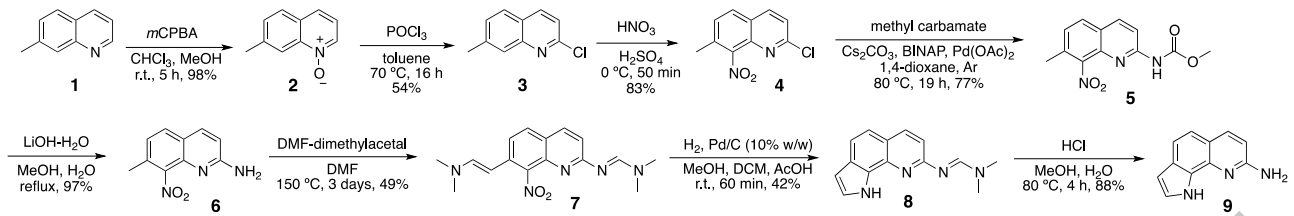


Figure 1. Molecular design of a new ligand targeting thymine. (a) Hydrogen bonding between 2,6-diaminopyridine and thymine. Hydrogen bond donor and acceptor are represented as D and A, respectively. Secondary repulsive and attractive interactions are represented by black and red arrows, respectively. (b) Structure of **PQA** and possible hydrogen bonding motif with thymine. (c) Simulated structure of the complex between **PQA (green)** and DNA duplex containing T–T mismatch. The simulated structure shows that **PQA** interacts with thymine and neighboring guanines. The hydrogen-bondings and stacking interaction are represented by yellow and green dashed line, respectively. (d) Energy-minimized structure of **PQA**–thymine. The distances (Å) between two positively polarized donor hydrogens are shown.

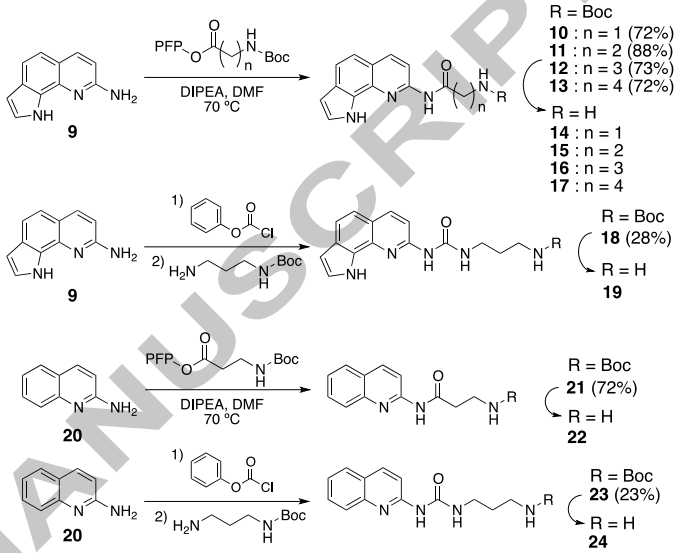


Scheme 1. Synthesis of **PQA**

attributed to the alternating hydrogen-bonding motif of acceptor-donor-acceptor (A-D-A) in thymine, which is known to form weak base pair, because of the repulsive secondary interactions between the positively polarized donor hydrogens and between the negatively polarized atoms.[26-28] For example, association constant (K_A) between thymine and 2,6-diaminopyridine having A-D-A/D-A-D motif was reported to be 100 M^{-1}, which is 100 to 1000-fold lower than that of G–C base pair having A-D-D/D-A-A motif (Figure 1a). This can be rationally explained by the presence of secondary repulsive force in the hydrogen-bonded pair: 2,6-diaminopyridine–thymine have four repulsive interactions, while G–C has two repulsive and two attractive interactions..The limitation in A-D-A/D-A-D motif underscores the need to develop a new motif for thymine recognition. We here designed 1*H*-pyrrolo[3,2-h]quinoline-8-amine (**PQA**) as a thymine recognition unit (Figure 1b). **PQA** is a tricyclic aromatic heterocycle having a complementary hydrogen-bonding surface to thymine. Non-linear arrangement of hydrogen bond donor and acceptor in **PQA** will reduce the secondary repulsive interaction by the longer distance, and the extended aromatic system will increase the stacking interaction with the neighboring bases. We carried out the molecular modeling simulations using MacroModel in Maestro (Schrödinger). In this simulation, we used DNA duplex containing a T–T mismatch as a host structure in which one of thymine base is flipped out. The simulated complex structure and the **PQA**–thymine pair in the complex are shown in Figure 1c and d, respectively. The distance between hydrogens of pyrrole N-H and thymine N3-H was 2.69 Å (Figure 1d) and is longer than that in 2,6-diaminopyridine–thymine pair (2.34 Å, Figure 1a). Tricyclic system of **PQA** was well stacked with neighboring G–C base pair in this binding pocket (Figure 1c).

We adopted Leimgruber–Batcho indole synthesis for construction of a key 1*H*-pyrrolo[3,2-h]quinoline structure (Scheme 1).[29] Starting from commercially available 7-methylquinoline **1**, corresponding N-oxide **2** was obtained by oxidation of **1** with 3-chloroperbenzoic acid. Chlorination of **2** with phosphoryl chloride provided chloroquinoline **3**. **3** was nitrated with nitric acid at 8 position to give **4**. Buchwald–Hartwig cross coupling of **4** with methylcarbamate followed by hydrolysis of the carbamate with lithium hydroxide gave aminoquinoline **6**. **6** was reacted with N,N-dimethylformamide dimethyl acetal to give a precursor of Leimgruber–Batcho indole synthesis **7**. The cyclization of **7** followed by deprotection with hydrogen chloride to give **9** (**PQA**). In order to enhance interaction of the **PQA** ligands with anionic DNA and solubility in water, cationic alkylamino linker was attached on amino group of **PQA** at position 8 (Scheme 2). **PQA** derivatives **14**-**17** have an alkylamino liker via amide linkage with different number of intervening methylenes, while an alkylamino linker was introduced via carbamide linkage in **19**. In order to investigate the effect of pyrrole ring in **PQA**, control compounds **22** and **24** having a quinoline instead of **PQA** were synthesized.

Binding ability of synthetic ligands to thymine bulge was investigated by measuring a melting temperature (T_m) of DNA duplex containing a thymine bulge (5'-d(A GGT CTC GTT G)-3'/3'-d(T CCA G_G CAA C)-5'). The effects of ligands were calculated by the difference of the T_m (ΔT_m) in the absence and presence of each ligand (Table 1). The increase of T_m was observed for all **PQA** derivatives **14**–**19** and indicated the stabilization of DNA duplex by the binding of the ligands. Especially, ligand **15** and **19** provided the highest ΔT_m of +4.7 ºC and +5.2 ºC among the tested ligands (Table 1). The simulation studies indicated that the linker in the ligand **14** is too short to interact with the neighboring bases and phosphate groups. On the other hand, terminal amino group in ligands **15**–**17** can reach to the neighboring bases and phosphate groups, indicating the linkers could contribute to the stabilization of the ligand binding. The high ΔT_m for ligand **15** may be explained by the shortest linker among the ligand **15**–**17** that provides the minimum loss of conformational freedom upon the binding. The carbamide linkage in the ligand **19** is favorable for the binding, which was further verified by the SPR experiments described later. In contrast, control quinoline derivatives lacking the pyrrole ring (**22** and **24**) gave little T_m increase, indicating that the third pyrrole ring in **PQA** has significant effect on the binding to the T-bulge DNA.



Scheme 2. Synthesis of **PQA** derivatives with alkylamino linker.

To know the bulge base selectivity, we have conducted T_m measurement of DNA duplex containing the other bulged base in the presence of **15** or **19** (Table 2). C-bulge DNA exhibited ΔT_m of +3.5 ºC and +5.8 ºC for **15** and **19**, respectively, which are comparable to T-bulge DNA. Since little ΔT_m was observed in A- or G-bulge DNA and full-complementary DNA (Table 2), **PQA** ligands apparently showed a selectively toward pyrimidine base bulge.

Table 1. ΔT_m (ºC) values for DNA duplex containing a T-bulge in the presence of ligands.[a]

DNA	Ligand	$Tm_{(-)}$[b]	$Tm_{(+)}$[c]	ΔTm
dT bulge	**14**	30.3 (0.6)	33.2 (0.7)	2.9 (0.7)
	15	30.3 (0.6)	35.0 (0.9)	4.7 (0.9)
	16	30.3 (0.6)	33.9 (0.5)	3.6 (0.5)
	17	30.3 (0.6)	32.9 (0.9)	2.6 (0.9)
	19	30.3 (0.6)	35.5 (0.3)	5.2 (0.3)
	22	30.3 (0.6)	30.6 (0.8)	0.3 (0.8)
	24	30.3 (0.6)	31.6 (0.7)	1.3 (0.7)

[a]T_m values of duplexes (5'-d(A GGT CTC GTT G)-3'/3'-d(T CCA G_G CAA C)-5') (5.0 μM) in buffer (pH 7.0) containing 100 mM NaCl. All measurements were taken three times, and standard deviations are shown in parentheses. [b]T_m values of duplex. [c]T_m values in the presence of ligand (50 μM).

Table 2. ΔT_m (ºC) values for DNA duplexes containing a A, G, C-bulge or full-complementary duplex.[a]

DNA	Ligand	$Tm_{(-)}$[b]	$Tm_{(+)}$[c]	ΔTm
dA bulge	**15**	26.9 (1.2)	28.6 (2.6)	1.7 (2.6)
	19	26.9 (1.2)	28.3 (1.0)	1.4 (1.0)
dG bulge	**15**	33.6 (0.6)	35.3 (0.9)	1.7 (0.9)
	19	33.6 (0.6)	34.6 (0.8)	1.0 (0.8)
dC bulge	**15**	34.0 (0.3)	37.5 (1.6)	3.5 (1.6)
	19	34.0 (0.3)	39.8 (0.3)	5.8 (0.3)
Full Match	**15**	47.5 (0.2)	48.4 (0.8)	0.9 (0.8)
	19	47.5 (0.2)	48.3 (0.9)	0.8 (0.9)

[a]T_m values of duplexes (5'-d(A GGT CNC GTT G)-3'/3'-d(T CCA G_G CAA C)-5') (5.0 μM) in buffer (pH 7.0) containing 100 mM NaCl. N is either A, G, or C. Full-matched duplex is a 10-mer lacking X. All measurements were taken three times, and standard deviations are shown in parentheses. [b]T_m values of duplexes. [c]T_m values in the presence of ligand (50 μM).

Investigations of the ligand-binding to CTG repeat sequence were carried out by surface plasmon resonance (SPR) assay (Figure 2 and S2). 5'-Biotinylated DNA consisting of 9 CTG repeats (5'-biotin-d$(CTG)_9$-3') was immobilized to streptavidin-coated (SA) sensor chip. Binding of the ligands to d$(CTG)_9$ repeat was analyzed by single cycle kinetics assay, where ligands of different concentrations were injected stepwise to the sensor chip without regeneration step of the surface. Sensorgrams for binding of **19** and **15** are shown in Figure 2a and b. Ligand **19** binds to the d$(CTG)_9$ repeat with an apparent dissociation constant (K_D) of 20 μM. Among the tested ligands, ligand **19** showed the highest response units and the lowest apparent K_D value. We also conducted isothermal titration calorimetry (ITC) measurement for the binding of **19** to d$(CTG)_9$ repeat (Figure 3). The binding was analyzed with estimation of 4:1 stoichiometry of ligand to d$(CTG)_9$ repeat, since possible hairpin structure of d$(CTG)_9$ consists of four CTG/CTG sites. Fitting the isotherm with a single set of identical binding sites model gave K_D of 17 μM, which is in good agreement with the SPR analysis.

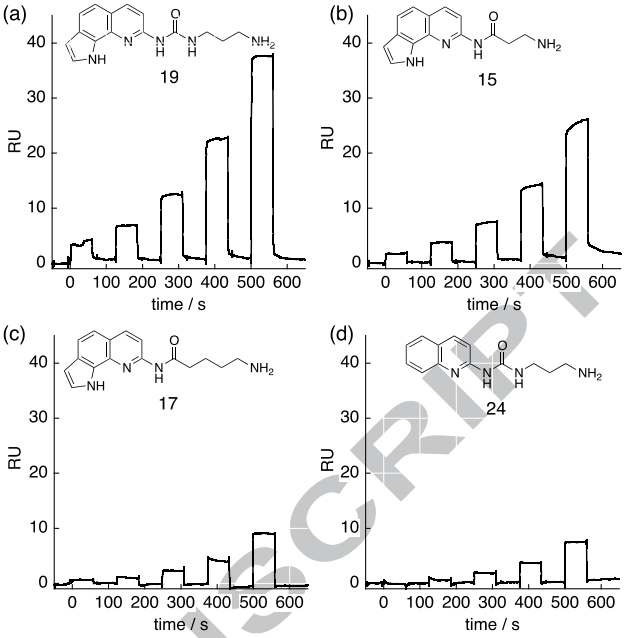


Figure 2. SPR single cycle kinetic analysis of ligand binding to the d$(CTG)_9$. (a) **19**. (b) **15**. (c) **17**. (d) **24**. Ligand was added stepwise at concentrations of 0.63 μM, 1.3 μM, 2.5 μM, 5.0 μM, and 10 μM.

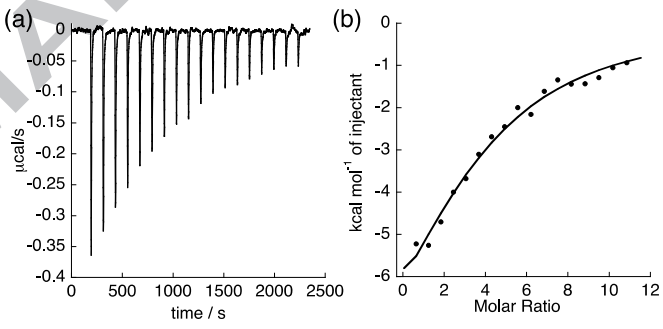


Figure 3. (a) Isothermal titration calorimetry (ITC) for the binding of **19** to d$(CTG)_9$ at 25 ºC. Titration was conducted by adding 2 μM of **19** (300 μM) every 2 min into a buffer solution (10 mM sodium cacodylate pH7.0, 100 mM NaCl) containing d$(CTG)_9$ (5 μM). (b) The best fit curve for a single set of identical binding sites model.

The effects of linker and additional pyrrole ring on the binding to d$(CTG)_9$ repeat were studied by SPR assay. Figure 2c showed SPR sensorgrams for ligand **17**. Higher binding ability of **19** than that of **17** can be attributable to the carbamide linkage, because the alkylamino linker in **17** consists of the same number of atoms to the linker in **19** but is connected by an amide linkage instead of carbamide linkage. The rigid linker enhance the binding likely due to the extended hydrogen bonding surface and possible increase of stacking interaction. Comparison between **19** and **24** clearly showed the importance of the **PQA** skeleton for the binding (Figure 2d). In order to examine the stacking interaction between the **PQA** chromophore and neighboring bases, UV titration experiments of the ligand **19** with d$(CTG)_9$ repeat were performed (Figure S1). The ligand **19** exhibits an absorption band at 349 nm, which was red-shifted by 8 nm upon titrating with the d$(CTG)_9$ repeat, indicating stacking interaction of the **PQA** with neighboring base pairs (Figure S1a). In contrast, 2,6-diaminopyridine did not show any apparent spectral changes under the same condition (Figure S1c). 2,6-Diaminopyridine is capable of hydrogen-bonding to thymine, but is unfavorable for binding to the repeat DNA. The extended tricyclic π-system and/or additional hydrogen bond donor in the pyrrole ring contributed to the enhanced binding ability of **19**.

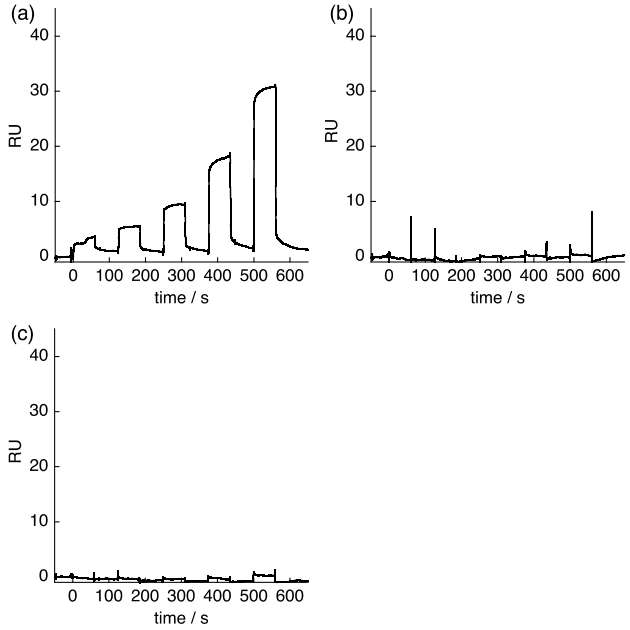


Figure 4. SPR single cycle kinetic analysis of **19** binding to the $d(CNG)_9$ repeats. (a) $d(CCG)_9$, (b) $d(CAG)_9$, and (c) $d(CGG)_9$. Ligand **19** was added stepwise at concentrations of 0.63 μM, 1.3 μM, 2.5 μM, 5.0 μM, and 10 μM.

We carried out SPR single cycle kinetics assay for the other CNG repeats (Figure 4a–c). Ligand **19** binds to the $d(CCG)_9$ repeat with an apparent K_D of 33 μM which is comparable to that for $d(CTG)_9$ (K_D = 20 μM). In contrast, the binding to $d(CAG)_9$ and $d(CGG)_9$ repeats are undetectable under the same conditions. These results indicated that ligand **19** bound selectively to CNG repeats containing pyrimidine-pyrimidine mismatch base pairs. These data are consistent with the T_m data showing preferable binding to T and C-bulges (Table 2). **PQA** derivatives bound to the pyrimidine bulges and mismatches most likely because the tricyclic system fit to the space opposite the pyrimidine base in terms of size and shape. On the other hand, hydrogen-bonding recognition by **PQA** is not high enough to distinguish between thymine and cytosine bases. **PQA** was designed to form three hydrogen bonds to thymine, but two or three hydrogen bonds can be involved in the binding to the unpaired cytosine (Figure S3). To append higher selectivity between CTG and CCG repeats, further optimization in hydrogen bonding recognition is required.

In conclusion, we have designed 1*H*-pyrrolo[3,2-h]quinoline (**PQA**) as a recognition unit for thymine and synthesized a series of **PQA** derivatives targeting CTG repeat. Among the tested ligands, **PQA** ligand containing carbamide linkage showed the highest binding ability to T-bulge DNA and CTG repeat sequence. Tricyclic system in **PQA** is a useful molecular unit for both CTG and CCG repeat binding, but further optimization is required to distinguish between CTG and CCG repeat.

Acknowledgments

This work was supported by JSPS KAKENHI Grant-in-Aid for Specially Promoted Research (26000007) for KN.

Supplementary Material

Supplementary data associated with this article can be found, in the online version, at XXX.

References and notes

1. McMurray, C. T. *Nat. Rev. Genet.* **2010**, *11*, 786–799.
2. Mirkin, S. M. *Nature* **2007**, *447*, 932–940.
3. Tian, B.; White, R. J.; Xia, T.; Welle, S.; Turner, D. H.; Mathews, M. B.; Thornton, C. A. *RNA* **2000**, *6*, 79–87.
4. Teplova, M.; Patel, D. J. *Nat. Struct. Mol. Biol.* **2008**, *15*, 1343–1351.
5. de Haro, M.; Al-Ramahi, I.; De Gouyon, B.; Ukani, L.; Rosa, A.; Faustino, N. A.; Ashizawa, T.; Cooper, T. A.; Botas, J. *Hum. Mol. Genet.* **2006**, *15*, 2138–2145.
6. Fernandez-Costa, J. M.; Garcia-Lopez, A.; Zuñiga, S.; Fernandez-Pedrosa, V.; Felipo-Benavent, A.; Mata, M.; Jaka, O.; Aiastui, A.; Hernandez-Torres, F.; Aguado, B.; Perez-Alonso, M.; Vilchez, J. J.; Lopez de Munain, A.; Artero, R. D. *Hum. Mol. Genet.* **2013**, *22*, 704–716.
7. Pettersson, O. J.; Aagaard, L.; Jensen, T. G.; Damgaard, C. K. *Nucleic Acids Res.* **2015**, *43*, 2433–2441.
8. Chau, A.; Kalsotra, A. *Dev. Dyn.* **2015**, *244*, 377–390.
9. Arambula, J. F.; Ramisetty, S. R.; Baranger, A. M.; Zimmerman, S. C. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 16068–16073.
10. Jahromi, A. H.; Nguyen, L.; Fu, Y.; Miller, K. A.; Baranger, A. M.; Zimmerman, S. C. *ACS Chem. Biol.* **2013**, *8*, 1037–1043.
11. Wong, C. H.; Nguyen, L.; Peh, J.; Luu, L. M.; Sanchez, J. S.; Richardson, S. L.; Tuccinardi, T.; Tsoi, H.; Chan, W. Y.; Chan, H. Y. E.; Baranger, A. M.; Hergenrother, P. J.; Zimmerman, S. C. *J. Am. Chem. Soc.* **2014**, *136*, 6355–6361.
12. Parkesh, R.; Childs-Disney, J. L.; Nakamori, M.; Kumar, A.; Wang, E.; Wang, T.; Hoskins, J.; Tran, T.; Housman, D.; Thornton, C. A.; Disney, M. D. *J. Am. Chem. Soc.* **2012**, *134*, 4731–4742.
13. Warf, M. B.; Nakamori, M.; Matthys, C. M.; Thornton, C. A; Berglund, J. A. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 18551–18556.
14. Siboni, R. B.; Bodner, M. J.; Khalifa, M. M.; Docter, A. G.; Choi, J. Y.; Nakamori, M.; Haley, M. M.; Berglund, J. A. *J. Med. Chem.* **2015**, *58*, 5770–5780.
15. Hagihara, M.; He, H.; Nakatani, K. *ChemBioChem* **2011**, *12*, 1686–1689.
16. Nakamori, M.; Gourdon, G.; Thornton, C. A. *Mol. Ther.* **2011**, *19*, 2222–2227.
17. Pearson, C. E.; Nichol Edamura, K.; Cleary, J. D. *Nat. Rev. Genet.* **2005**, *6*, 729–742.
18. Petruska, J.; Arnheim, N.; Goodman, M. F. *Nucleic Acids Res.* **1996**, *24*, 1992–1998.
19. Nakatani, K.; Hagihara, S.; Goto, Y.; Kobori, A.; Hagihara, M.; Hayashi, G.; Kyo, M.; Nomura, M.; Mishima, M.; Kojima, C. *Nat. Chem. Biol.* **2005**, *1*, 39–43.
20. Peng, T.; Dohno, C.; Nakatani, K. *Angew. Chem. Int. Ed.* **2006**, *45*, 5623–5626.
21. Kobori, A.; Horie, S.; Suda, H.; Saito, I.; Nakatani, K. *J. Am. Chem. Soc.* **2004**, *126*, 557–562.
22. Kobori, A.; Nakatani, K. *Bioorg. Med. Chem.* **2008**, *16*, 10338–10344.
23. Lian, C.; Robinson, H.; Wang, A. H.-J. *J. Am. Chem. Soc.* **1996**, *118*, 8791–8801.
24. David, A.; Bleimling, N.; Beuck, C.; Lehn, J. M.; Weinhold, E.; Teulade-Fichou, M. P. *ChemBioChem* **2003**, *4*, 1326–1331.
25. Zhao, C.; Rajendran, A.; Dai, Q.; Nishizawa, S.; Teramae, N. *Anal. Sci.* **2008**, *24*, 693–695.
26. Murray, T. J.; Zimmerman, S. C. *J. Am. Chem. Soc.* **1992**, *114*, 4010–4011.
27. Quinn, J. R.; Zimmerman, S. C.; Del Bene, J. E.; Shavitt, I. *J. Am. Chem. Soc.* **2007**, *129*, 934–941.
28. Ong, H. C.; Arambula, J. F.; Rao Ramisetty, S.; Baranger, A. M.; Zimmerman, S. C. *Chem. Commun.* **2009**, 668–670.
29. Siu, J.; Baxendale, I. R.; Ley, S. V. *Org. Biomol. Chem.* **2004**, *2*, 160–167.