



A Journal of the Gesellschaft Deutscher Chemiker

Angewandte Chemie

GDCh

International Edition

www.angewandte.org

Accepted Article

Title: 2,6-Dimercuriphenol as a Bifacial Dinuclear Organometallic Nucleobase

Authors: Dattatraya Uttam Ukale and Tuomas Lönnberg

This manuscript has been accepted after peer review and appears as an Accepted Article online prior to editing, proofing, and formal publication of the final Version of Record (VoR). This work is currently citable by using the Digital Object Identifier (DOI) given below. The VoR will be published online in Early View as soon as possible and may be different to this Accepted Article as a result of editing. Readers should obtain the VoR from the journal website shown below when it is published to ensure accuracy of information. The authors are responsible for the content of this Accepted Article.

To be cited as: *Angew. Chem. Int. Ed.* 10.1002/anie.201809398
Angew. Chem. 10.1002/ange.201809398

Link to VoR: <http://dx.doi.org/10.1002/anie.201809398>
<http://dx.doi.org/10.1002/ange.201809398>

2,6-Dimercuriphenol as a Bifacial Dinuclear Organometallic Nucleobase

Dattatraya Uttam Ukale and Tuomas Lönnberg

Department of Chemistry, University of Turku, Vatselankatu 2, 20014 Turku, Finland

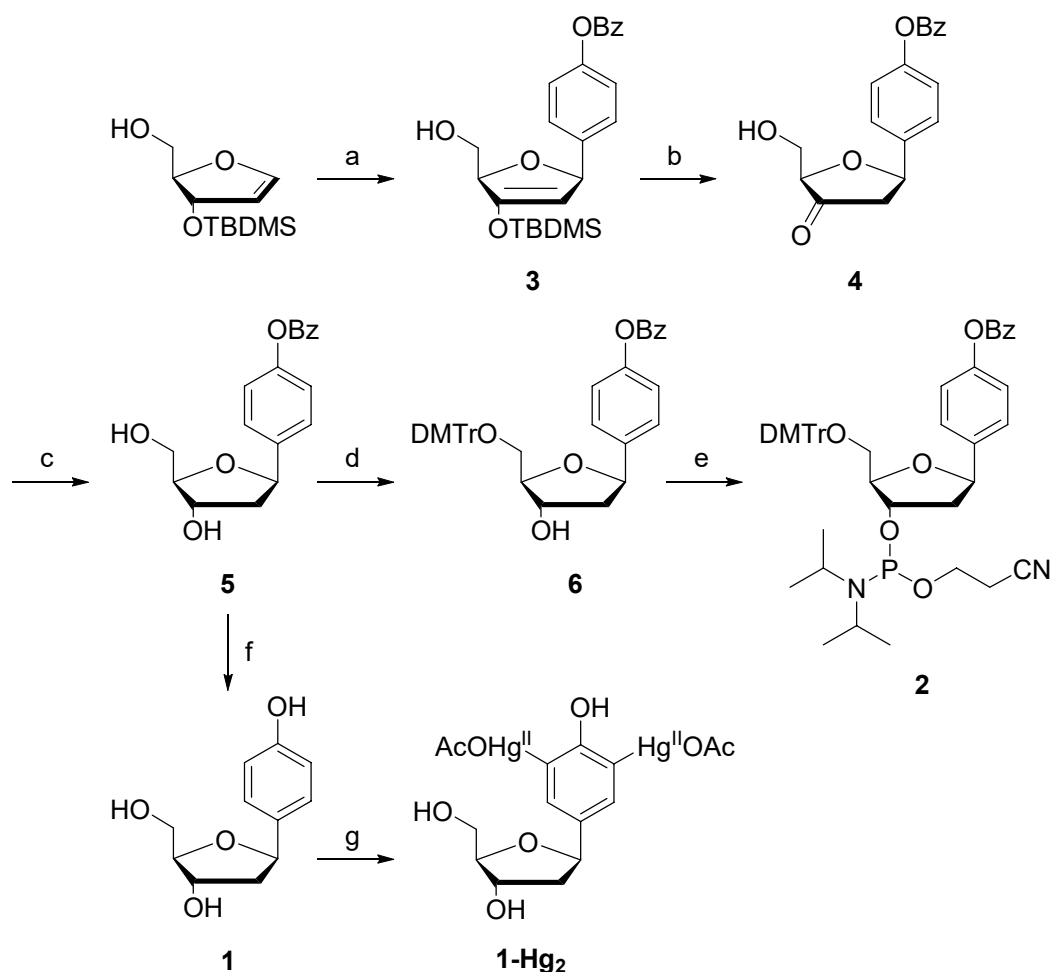
Email: tuanlo@utu.fi

A C-nucleoside having 2,6-dimercuriphenol as the base moiety has been synthesized and incorporated into an oligonucleotide. NMR and UV melting experiments revealed the ability of this bifacial organometallic nucleobase surrogate to form stable dinuclear Hg(II)-mediated base triples with adenine, cytosine and thymine (or uracil) in solution as well as within a triple-helical oligonucleotide. A single Hg(II)-mediated base triple between 2,6-dimercuriphenol and two thymines increased both Hoogsteen and Watson-Crick melting temperatures of a 15-mer pyrimidine•purine*pyrimidine triple helix by more than 10 °C relative to an unmodified triple helix of the same length. This novel binding mode could be exploited in targeting certain pathogenic nucleic acids as well as in DNA nanotechnology.

Keywords: base triple; hybridization; oligonucleotide; mercury; organometallic

Metal-mediated base pairing can substantially promote hybridization of oligonucleotides.^[1] Harnessing the high stability of metal-mediated base pairs under the metal-deficient conditions of the intracellular medium can, in principle, be achieved with either coordination complexes of kinetically inert metal ions, such as Pt(II)^[2] or Ru(II)^[3] or organometallic complexes of more labile metal ions, such as Hg(II) or Pd(II). We have recently become interested in the latter approach and reported on metal-mediated base pairing by covalently mercurated^[4,5] and palladated^[6] nucleobase surrogates. As could be expected, dinuclear metal-mediated base pairs can increase the stability of a double helix even more than their mononuclear counterparts.^[7] In these structures the two bridging metal ions are both coordinated on the “Watson-Crick” face of an appropriately modified nucleobase and converge on a single nucleobase on the complementary strand. On the other hand, bridging metal ions on both the “Watson-Crick” and the “Hoogsteen” face of a nucleobase should allow formation of a dinuclear metal-mediated base triple and thus promote both Watson-Crick and Hoogsteen hybridization within a triple helix. The viral transcript polyadenylated nuclear (PAN) RNA^[8] offers an example of a naturally occurring pathogenic nucleic acid that could be targeted utilizing such a binding mode. In the present article we describe the synthesis and base pairing properties of a 2,6-dimercuriphenol C-nucleoside, as well as a correspondingly modified oligonucleotide. To the best of our knowledge, this is the first example of a dinuclear covalently metalated nucleobase surrogate to be incorporated into an oligonucleotide.

Synthesis of the phenol C-nucleoside 1 and the respective phosphoramidite building block 2 is described in Scheme 1. First, compound 3 was prepared Heck coupling between {(2R,3S)-3-[(tert-butyl)dimethylsilyl]oxy]-2,3-dihydrofuran-2-yl}methanol and 4-iodophenyl benzoate. Desilylation of 3 gave the ketone intermediate 4 and subsequent reduction the benzoyl-protected C-nucleoside 5. Finally, compound 5 was either debenzoylated to give the unprotected C-nucleoside 1 or 5'-tritylated and 3'-phosphitylated to give the phosphoramidite 2. Compound 5 has been prepared previously by organolithium chemistry^[9] but the method presented herein is significantly more β -stereoselective.



Scheme 1. Synthesis of the phenol C-nucleoside **1**, its dimercurated derivative **1-Hg₂** and phosphoramidite building block **2**. Reagents and conditions: a) $\text{Pd}(\text{OAc})_2$, $\text{P}(\text{C}_6\text{F}_5)_3$, 4-iodophenyl benzoate, Ag_2CO_3 , CHCl_3 , Ar atmosphere, 70 °C, 12 h; b) $\text{Et}_3\text{N}\cdot 3\text{HF}$, THF, Ar atmosphere, 25 °C, 15 min; c) $\text{NaBH}(\text{OAc})_3$, AcOH, MeCN, Ar atmosphere, 0 °C, 15 min; d) DMTrCl, pyridine, 25 °C, 16 h; e) 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite, Et_3N , CH_2Cl_2 , N_2 atmosphere, 25 °C, 3 h; f) NH_3 , MeOH, H_2O , 25 °C, 16 h; g) $\text{Hg}(\text{OAc})_2$, MeOH, 25 °C, 16 h.

Dimercurated of the phenol C-nucleoside **1** was carried out by overnight treatment with $\text{Hg}(\text{OAc})_2$ in MeOH at 25 °C (Scheme 1). ^1H NMR spectrum of the product showed loss of H2 and H6, consistent with exchange of these protons for Hg. Formation of the desired dimercurated product **1-Hg₂** was further verified by ^{199}Hg NMR and HRMS. For reference, 2'-deoxyadenosine was subjected to the same conditions and found to be unreactive.

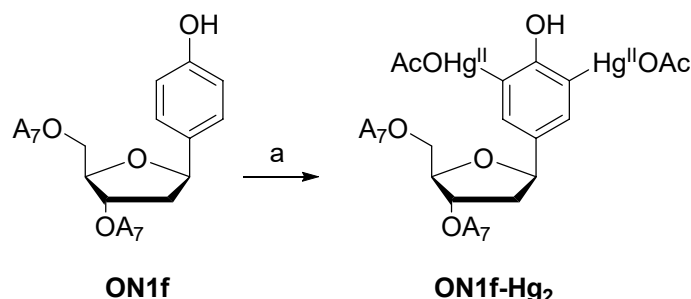
Sequences of the oligonucleotides used in the present study are summarized in Table 1. Homoadenine and homothymine sequences were chosen to allow pyrimidine•purine*pyrimidine triple helix formation under physiological conditions. The modified oligonucleotide ON1f, bearing a single phenol residue in the middle of the chain, was assembled on an automated DNA synthesizer by the conventional phosphoramidite strategy, employing an extended time for coupling of building block **2**. Dimercurated of ON1f was accomplished by overnight treatment with $\text{Hg}(\text{OAc})_2$ in aqueous

NaOAc at 70 °C (Scheme 2). The product ON1f-Hg₂ was purified by sequential IE- and RP-HPLC. Both ON1f and ON1f-Hg₂ were characterized mass spectrometrically and quantified UV spectrophotometrically. Finally, the site of mercuration in ON1f-Hg₂ was verified by mass spectrometric analysis of the product mixture after digestion by P1 nuclease.

Table 1. Oligonucleotides used in the present study.

Oligonucleotide	Sequence ^[a]
ON1f	5'-AAA AAA <u>A</u> FA AAA AAA -3'
ON1f-Hg ₂	5'-AAA AAA <u>A</u> F ^{Hg₂} A AAA AAA -3'
ON1a	5'-AAA AAA <u>AAA</u> AAA AAA -3'
ON2a	5'-TTT TTT <u>TAT</u> TTT TTT -3'
ON2c	5'-TTT TTT <u>TCT</u> TTT TTT -3'
ON2g	5'-TTT TTT <u>TGT</u> TTT TTT -3'
ON2t	5'-TTT TTT <u>TTT</u> TTT TTT -3'
ON2s	5'-TTT TTT <u>TST</u> TTT TTT -3'

[a] F refers to phenol, F^{Hg₂} to 2,6-dimercuriphenol and S to an abasic site (2-(hydroxymethyl)tetrahydrofuran-3-ol spacer). In each sequence, the residue varied in the hybridization studies has been underlined.



Scheme 2. Dimercuration of oligonucleotide ON1f. Reagents and conditions: Hg(OAc)₂, NaOAc, H₂O, 70 °C, 16 h.

Binding affinity of the 2,6-bis(acetoxymethyl)phenol C-nucleoside 1-Hg₂ for the canonical ribonucleoside-5'-monophosphates was quantified NMR spectrometrically at 25 °C and pH 7.2. 5'-monophosphates were used instead of their constituent nucleosides to overcome the low solubility of 1-Hg₂ in water. For the same reason, a mixture of D₂O and DMSO (1:1, v/v) was used as the solvent. Samples containing a 4 mM concentration of 1-Hg₂ and an 8 mM concentration of either 5'-AMP, 5'-CMP, 5'-GMP or 5'-UMP were prepared and gradually diluted, maintaining a constant pH and ionic strength. A control experiment in the absence of any nucleoside monophosphate was also carried out. At each concentration, the chemical shift of the H3 and H5 protons of 1-Hg₂ was recorded (Figure 1). Owing to the kinetic lability of Hg(II) complexes, this signal represents the average of all species in equilibrium with each other.

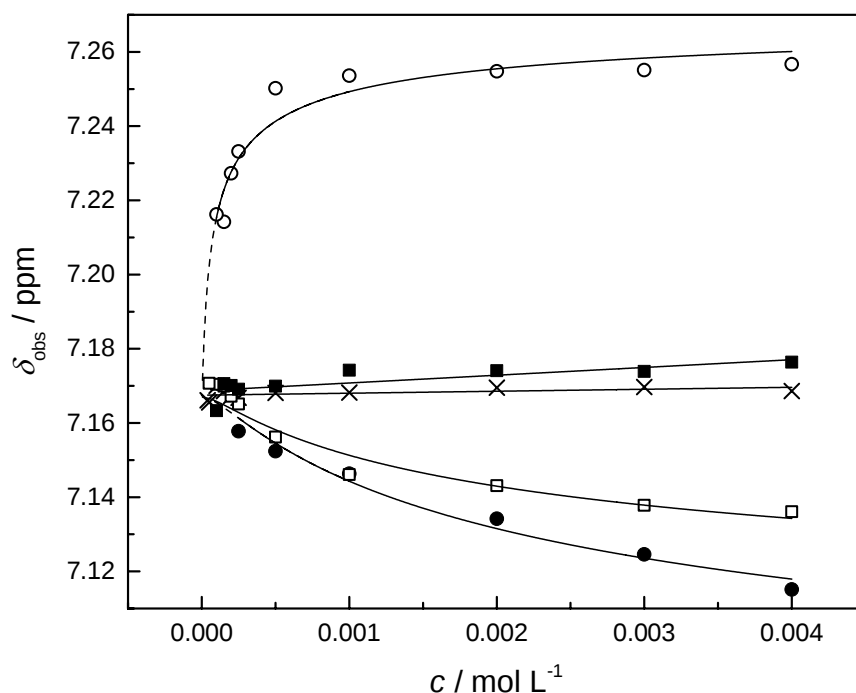


Figure 1. Chemical shift of the H3 and H5 protons of 1-Hg₂ as a function of concentration in a 1:2 mixture with 5'-AMP (●), 5'-CMP (□), 5'-GMP (■) and 5'-UMP (○), as well as in the absence of any nucleoside monophosphate (×); T = 25 °C; pH = 7.2 (120 mM phosphate buffer in a mixture of D₂O and DMSO (1:1, v/v)).

A very small linear downfield shift of the H3 and H5 signal was observed on increasing concentration of 1-Hg₂ in the absence of any nucleoside monophosphate, indicating negligible intermolecular interactions. Somewhat unexpectedly, a similar plot was obtained in the presence of 5'-GMP, known to be a good ligand for Hg(II).^[10] It should be noted, however, that the main signal of H3 and H5 was accompanied with a number of minor signals of similar chemical shifts so formation of an intractable higher-order structure cannot be ruled out. Guanine can simultaneously coordinate Hg(II) on both N1 and N7 and is unique among the canonical nucleobases in its ability to self-assemble into complex structures.^[11] The other nucleoside monophosphates induced more marked shifts, either downfield (5'-UMP) or upfield (5'-AMP and 5'-CMP), with saturation at high concentration. Assuming formation a ternary 1:2 complexes between 1-Hg₂ and the nucleoside monophosphates, with equal equilibrium constants for the two coordination steps (justification for this simplification comes from the fact that the plots exhibit no biphasicity), the observed chemical shifts, δ_{obs} , may be expressed by Equation (1):^[12]

$$\delta_{obs} = \delta_0 + (\delta_{\infty} - \delta_0) \frac{\left(K_d + 2c - \sqrt{K_d^2 + 4K_d c}\right)^2}{4c^2} \quad (1)$$

where δ_0 and δ_∞ are the H3 and H5 chemical shifts at zero and infinite concentration, respectively, K_d is the common dissociation constant for binary and ternary complexes between 1-Hg₂ and the nucleoside monophosphate and c is the concentration of 1-Hg₂. The K_d values obtained with 5'-AMP, 5'-CMP and 5'-UMP by nonlinear least-squares fitting of the experimental data to Equation (1) were 700 ± 200 , 600 ± 300 and 15 ± 4 μ M, respectively. In other words, complexation with 5'-UMP is strongly favored, consistent with the ability of uracil to serve as an anionic ligand under the experimental conditions.^[10, 13] Similarly, Hg(II)-mediated base pairs are preferentially formed between two thymine or uracil bases, with concomitant N3 deprotonation of both partners.^[14]

The ability of the 2,6-dimercuriphenol-modified oligonucleotide ON1f-Hg₂ to form triple helices with unmodified oligonucleotides was studied by UV melting temperature measurements at pH 7.4 (20 mM cacodylate buffer) and ionic strength of 0.10 M (adjusted with sodium perchlorate). For reference, similar experiments were also performed with the unmercurated counterpart ON1f, as well as the natural oligonucleotide ON1a, bearing an adenine residue in place of the phenol residue. In each experiment, the homoadenine strand (ON1a, ON1f or ON1f-Hg₂, 1.0 μ M) was hybridized with two equivalents of the homothymine strand (ON2a, ON2c, ON2g, ON2t or ON2s, 2.0 μ M) and only the central base triple was varied.

Melting profiles for ON2t•ON1a•ON2t, ON2t•ON1f•ON2t and ON2t•ON1f-Hg₂•ON2t are presented in Figure 2A (respective plots for other combinations can be found in the Supporting Information). With ON1a and ON1f, monophasic sigmoidal curves were obtained, with melting temperatures of 34.8 ± 0.8 and 21.5 ± 0.9 °C, respectively (Figure 3A, all T_m values are tabulated in the Supporting Information). The melting profile of ON2t•ON1f-Hg₂•ON2t, in turn, was biphasic, with melting temperatures of 19.8 ± 0.9 and 45.7 ± 0.7 °C. Evidently a triple helix is formed only with ON1f-Hg₂ under the experimental conditions, suggesting more than a 10 °C increase in the Hoogsteen T_m compared to ON1a (stable triplexes with the latter were, however, obtained at 10 mM Mg(II), melting profiles presented in the Supporting Information). Increase of the Watson-Crick T_m was also considerable, 11 °C compared to ON1a and 24 °C compared to ON1f. Even greater stabilizations were observed with ON2a or ON2c as the complementary strand although the absolute Watson-Crick T_m values still remained 7 – 9 °C degrees lower than with ON2t. In contrast, with ON2s, placing an abasic site opposite to the variable residue, ON1a formed a more stable duplex than either ON1f or ON1f-Hg₂. Finally, no sigmoidal melting curve could be obtained with ON2g•ON1f-Hg₂•ON2g. The results are, hence, in good agreement with those of the NMR studies and consistent with formation of Hg(II)-mediated base triplets between 2,6-dimercuriphenol and adenine, cytosine and, especially, thymine (Figure 4). It also seems likely that the anomalous results with guanine in both the NMR and UV melting studies share a common origin.

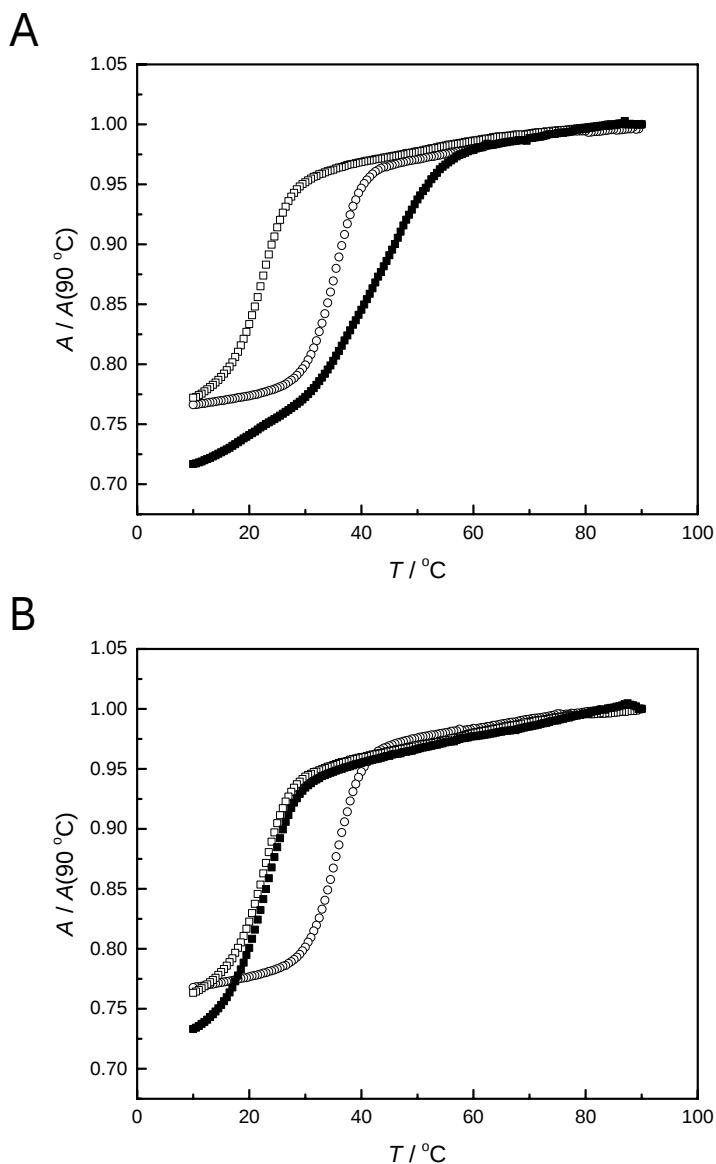


Figure 2. UV melting profiles of ON2t•ON1a*ON2t (○), ON2t•ON1f*ON2t (□) and ON2t•ON1f-Hg₂*ON2t (■) in the A) absence and B) presence of 2-mercaptoethanol; pH = 7.4 (20 mM cacodylate buffer); [ON1a] = [ON1f] = [ON1f-Hg₂] = 1.0 μM; [ON2t] = 2.0 μM; [2-mercaptoethanol] = 0 / 100 μM; I(NaClO₄) = 0.10 M.

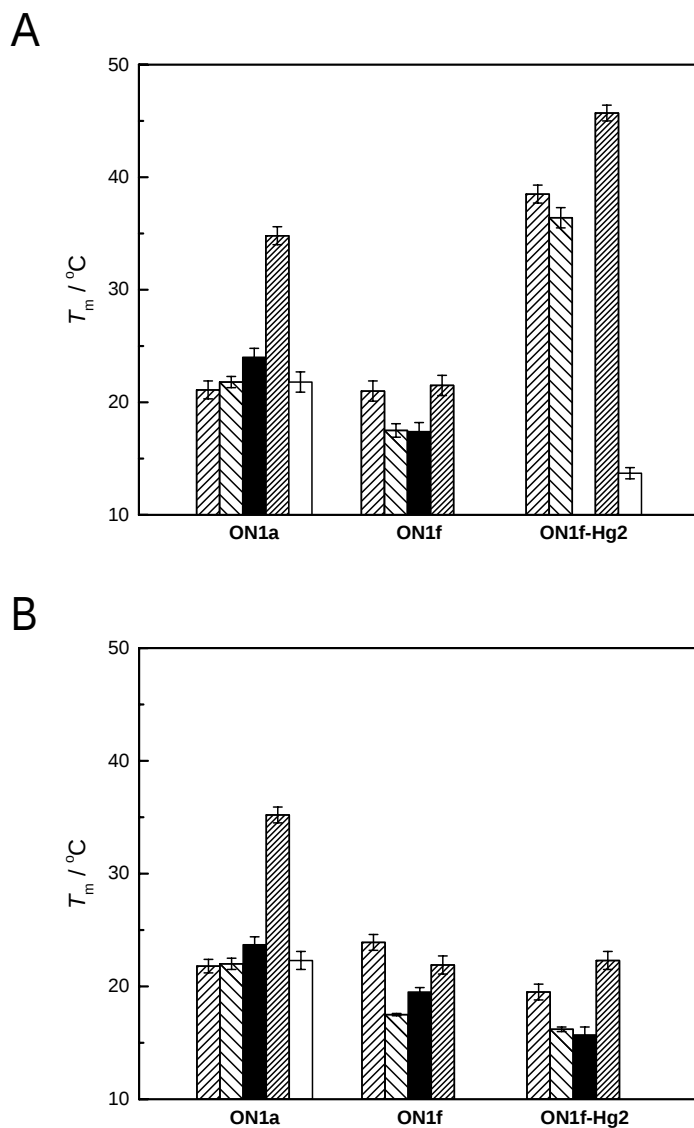


Figure 3. Watson-Crick melting temperatures of triplexes formed by ON1a, ON1f and ON1f-Hg₂ with ON2a (medium hash), ON2c (sparse hash), ON2g (black), ON2t (dense hash) and ON2s (white) in the A) absence and B) presence of 2-mercaptoethanol; pH = 7.4 (20 mM cacodylate buffer); [ON1a] = [ON1f] = [ON1f-Hg₂] = 1.0 μM ; [ON2t] = 2.0 μM ; [2-mercaptoethanol] = 0 / 100 μM ; I(NaClO₄) = 0.10 M.

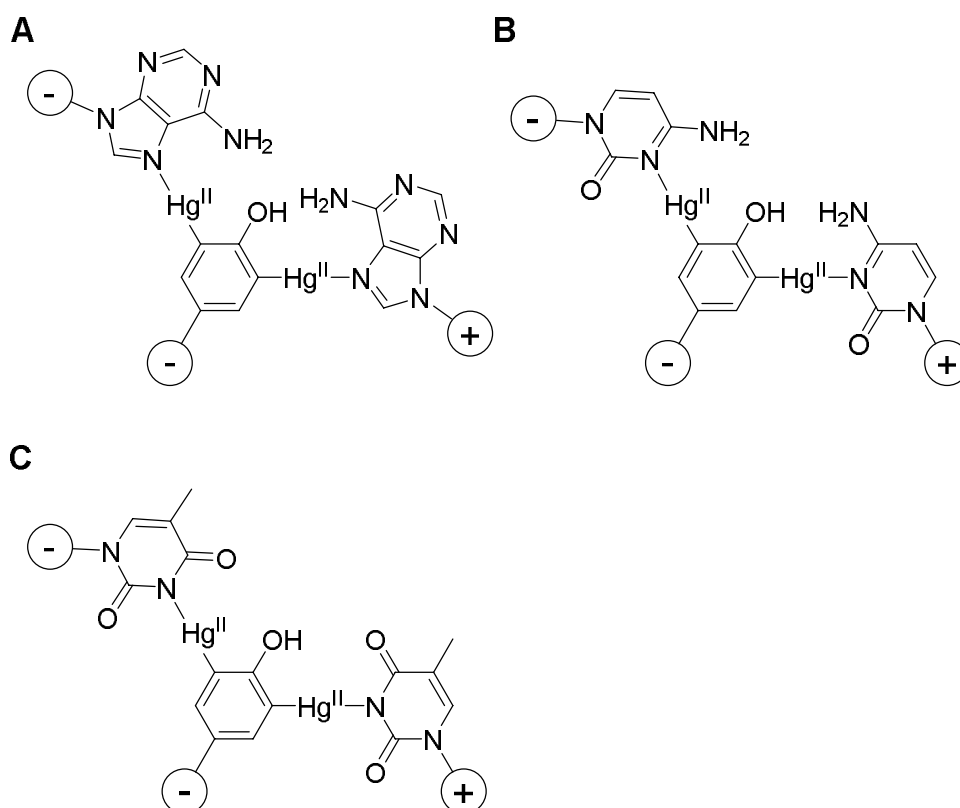


Figure 4. Proposed structures of base triples formed between 2,6-dimercuriphenol and A) adenine, B) cytosine and C) thymine. Relative polarities of the three strands are indicated by (+) and (-) signs. The possibility of hydrogen bonding between the phenolic hydroxy function and exocyclic amino and oxo functions of the canonical nucleobases cannot be verified or excluded based on the data at hand. In fact, dimercuriation may substantially acidify the phenolic OH function so even its protonation state cannot be firmly established. In the case of cytosine and adenine, coordination to the exocyclic amino group is also a viable alternative.^[15]

The UV melting experiments described above were repeated in the presence of 100 μM 2-mercaptoethanol, a strong competing ligand for Hg(II) ,^[16] to verify that the enhanced hybridization of ON1f-Hg_2 is indeed attributable to Hg(II) -mediated base pairing. As expected, melting temperatures of the unmercurated triplexes were largely unaffected (Figure 2B and 3B). In contrast, melting temperatures of the triplexes formed by ON1f-Hg_2 dropped to the levels observed with the unmercurated counterpart ON1f. Mass spectrometric analysis of the samples after several heating and cooling cycles in the presence of 2-mercaptoethanol revealed considerable demercuration of ON1f-Hg_2 to ON1f (a representative mass spectrum is presented in the Supporting Information).

While Hoogsteen and Watson-Crick melting partially overlap in the UV melting profiles of the dimercurated triplexes, precluding detailed thermodynamic analysis, it is evident that the thermal denaturation is much more gradual than with the unmercurated triplexes. This observation is in line with previous reports on similar systems^[4, 17] and can be taken as a final piece of evidence for Hg(II) -mediated base pairing. The gradual thermal denaturation translates to a relatively low entropic

penalty of hybridization which, in turn, has been attributed to the dehydration of Hg(II) on formation of a Hg(II)-mediated base pair.^[18]

Folding of the dimercurated triplexes to the expected pyrimidine•purine•pyrimidine secondary structure was verified by CD spectropolarimetric analysis. The measurements were carried out at 5 °C intervals over a temperature range of 10 – 90 °C under otherwise the same conditions as those used for the UV melting experiments. At 10 °C, the spectra of ON2a•ON1f-Hg₂*ON2a, ON2c•ON1f-Hg₂*ON2c and ON2t•ON1f-Hg₂*ON2t were characteristic of pyrimidine•purine•pyrimidine triple helices,^[19] with minima at 248 nm and maxima at 260 and 284 nm (spectra presented in the Supporting Information). As could be expected, folding into a triplex was most evident with ON2t•ON1f-Hg₂*ON2t. The spectra of ON2g•ON1f-Hg₂*ON2g and ON2s•ON1f-Hg₂*ON2s, in turn, resembled that of a B-type double helix. In all cases, diminution of the minima and maxima on increasing temperature was observed, consistent with thermal denaturation.

In summary, formation of stable dinuclear Hg(II)-mediated base triples between 2,6-dimercuriphenol and adenine, cytosine and thymine (or uracil) has been demonstrated in solution as well as within a triple-helical oligonucleotide. In the latter case, increases of more than 10 °C in both Hoogsteen and Watson-Crick melting temperatures and a good selectivity for thymine were observed. This novel binding mode could be used for targeting certain pathogenic nucleic acids, such as the viral PAN RNA, as well for construction of new types of metalated DNA nanostructures.

Acknowledgements: We thank the Academy of Finland (decision #286478) and the Finnish National Agency for Education (decision #TM-17-10348) for financial support of this work.

References

- [1] P. Scharf, J. Müller, *ChemPlusChem* 2013, 78, 20-34; Y. Takezawa, M. Shionoya, *Acc. Chem. Res.* 2012, 45, 2066-2076; G. H. Clever, C. Kaul, T. Carell, *Angew. Chem. Int. Ed.* 2007, 46, 6226-6236; G. H. Clever, M. Shionoya, *Coord. Chem. Rev.* 2010, 254, 2391-2402; J. Müller, *Nature* 2006, 444, 698-698; J. Müller, *Eur. J. Inorg. Chem.* 2008, 2008, 3749-3763; S. Mandal, J. Müller, *Curr. Opin. Chem. Biol.* 2017, 37, 71-79; Y. Takezawa, J. Müller, M. Shionoya, *Chem. Lett.* 2016, 46, 622-633; B. Lippert, P. J. Sanz Miguel, *Acc. Chem. Res.* 2016, 49, 1537-1545; S. Taherpour, O. Golubev, T. Lönnberg, *Inorg. Chim. Acta* 2016, 452, 43-49; B. Jash, J. Müller, *Chem. Eur. J.* 2017, 23, 17166-17178.
- [2] C. Colombier, B. Lippert, M. Leng, *Nucleic Acids Res.* 1996, 24, 4519-4524; K. S. Schmidt, M. Boudvillain, A. Schwartz, G. A. van der Marel, J. H. van Boom, J. Reedijk, B. Lippert, *Chem. Eur. J.* 2002, 8, 5566-5570; M. K. Graham, T. R. Brown, P. S. Miller, *Biochemistry* 2015, 54, 2270-2282; R. Manchanda, S. U. Dunham, S. J. Lippard, *J. Am. Chem. Soc.* 1996, 118, 5144-5145; D. Cech, J. Schliepe, U. Berghoff, B. Lippert, *Angew. Chem. Int. Ed.* 1996, 35, 646-648.
- [3] M. Hibino, Y. Aiba, Y. Watanabe, O. Shoji, *ChemBioChem* 2018, 0; J. C. Verheijen, G. A. van der Marel, J. H. van Boom, N. Metzler-Nolte, *Bioconjugate Chem.* 2000, 11, 741-743; S. Le Gac, M. Foucart, P. Gerbaux, E. Defrancq, C. Moucheron, A. Kirsch - De Mesmaeker, *Dalton Trans.* 2010, 39, 9672-9683; M. Röthlingshöfer, K. Gorska, N. Winssinger, *Org. Lett.* 2012, 14, 482-485; T. Joshi, G. J. Barbante, P. S. Francis, C. F. Hogan, A. M. Bond, G. Gasser, L. Spiccia, *Inorg. Chem.* 2012, 51, 3302-3315.
- [4] D. Ukale, V. S. Shinde, T. Lönnberg, *Chem. Eur. J.* 2016, 22, 7917-7923.
- [5] D. U. Ukale, T. Lönnberg, *ChemBioChem* 2018, 19, 1096-1101.
- [6] S. K. Maity, T. Lönnberg, *Chem. Eur. J.* 2018, 24, 1274-1277; M. Hande, S. Maity, T. Lönnberg, *Int. J. Mol. Sci.* 2018, 19, 1588.

- [7] S. Mandal, A. Hepp, J. Müller, Dalton Trans. 2015, 44, 3540-3543; S. Mandal, M. Hebenbrock, J. Müller, Angew. Chem. Int. Ed. 2016, 55, 15520-15523; S. Mandal, M. Hebenbrock, J. Müller, Inorg. Chim. Acta 2018, 472, 229-233; H. Yang, H. Mei, F. Seela, Chem. Eur. J. 2015, 21, 10207-10219; S. K. Jana, X. Guo, H. Mei, F. Seela, Chem. Commun. 2015, 51, 17301-17304; G.-J. Cao, Sci. Rep. 2017, 7, 14896.
- [8] R. M. Mitton-Fry, S. J. DeGregorio, J. Wang, T. A. Steitz, J. A. Steitz, Science 2010, 330, 1244; N. K. Conrad, Virus Res. 2016, 212, 53-63; N. K. Conrad, S. Mili, E. L. Marshall, M.-D. Shu, J. A. Steitz, Mol. Cell 2006, 24, 943-953.
- [9] Y. Hari, S. Kashima, H. Inohara, S. Ijitsu, T. Imanishi, S. Obika, Tetrahedron 2013, 69, 6381-6391.
- [10] R. B. Simpson, J. Am. Chem. Soc. 1964, 86, 2059-2065.
- [11] J. T. Davis, G. P. Spada, Chem. Soc. Rev. 2007, 36, 296-313.
- [12] E. F. Douglass, C. J. Miller, G. Sparer, H. Shapiro, D. A. Spiegel, J. Am. Chem. Soc. 2013, 135, 6092-6099.
- [13] R. B. Martin, Acc. Chem. Res. 1985, 18, 32-38.
- [14] Y. Miyake, H. Togashi, M. Tashiro, H. Yamaguchi, S. Oda, M. Kudo, Y. Tanaka, Y. Kondo, R. Sawa, T. Fujimoto, T. Machinami, A. Ono, J. Am. Chem. Soc. 2006, 128, 2172-2173; Y. Tanaka, S. Oda, H. Yamaguchi, Y. Kondo, C. Kojima, A. Ono, J. Am. Chem. Soc. 2007, 129, 244-245.
- [15] F. Zamora, M. Sabat, B. Lippert, Inorg. Chem. 1996, 35, 4858-4864.
- [16] G. Schwarzenbach, M. Schellenberg, Helv. Chim. Acta 1965, 48, 28-46.
- [17] H. Torigoe, A. Ono, T. Kozasa, Chem. Eur. J. 2010, 16, 13218-13225.
- [18] J. Šebera, J. Burda, M. Straka, A. Ono, C. Kojima, Y. Tanaka, V. Sychrovský, Chem. Eur. J. 2013, 19, 9884-9894; H. Yamaguchi, J. Šebera, J. Kondo, S. Oda, T. Komuro, T. Kawamura, T. Dairaku, Y. Kondo, I. Okamoto, A. Ono, J. V. Burda, C. Kojima, V. Sychrovský, Y. Tanaka, Nucleic Acids Res. 2014, 42, 4094-4099.
- [19] P. M. Gannett, E. Darian, J. Powell, E. M. Johnson, 2nd, C. Mundoma, N. L. Greenbaum, C. M. Ramsey, N. S. Dalal, D. E. Budil, Nucleic Acids Res. 2002, 30, 5328-5337; J. E. Herrera, J. B. Chaires, Biochemistry 1989, 28, 1993-2000.