Supramolecular Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713649759

Toward Nano-assembly of Metals Through Engineered DNAs

Kentaro Tanaka[a]; Motoyuki Tasaka[b]; Honghua Cao[b]; Mitsuhiko Shionoya[a]
[a] Department of Chemistry, Graduate School of Science, The University of Tokyo, Tokyo, Japan [b] The Graduate University for Advanced Studies, Okazaki, Japan

Online publication date: 29 October 2010

To cite this Article Tanaka, Kentaro , Tasaka, Motoyuki , Cao, Honghua and Shionoya, Mitsuhiko(2002) 'Toward Nano-assembly of Metals Through Engineered DNAs', Supramolecular Chemistry, 14: 2, 255 — 261
To link to this Article: DOI: 10.1080/10610270290026167
URL: http://dx.doi.org/10.1080/10610270290026167

Supramolecular Chemistry, 2002 Vol. 14 (2-3), pp. 255–261

Taylor & Francis
Taylor & Francis Group

Toward Nano-assembly of Metals Through Engineered DNAs

KENTARO TANAKA[a], MOTOYUKI TASAKA[b], HONGHUA CAO[b] and MITSUHIKO SHIONOYA[a,*]

[a]*Department of Chemistry, Graduate School of Science, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*
[b]*The Graduate University for Advanced Studies, Myodaiji, Okazaki 444-8585, Japan*

(Received 3 December 2001)

INTRODUCTION

Worldwide research into the generation of novel, bio-inspired molecular architectures has been growing exponentially for more than a quarter of a century. Motivation in this research field is based on the belief that a "bottom–up" approach from the atomic or molecular level to control or to renew basic building blocks that have been embodied by Nature can lead to an enormous range of possible structures and functions within what are rationally designed molecular structures. The self-assembly hierarchy, which is natural in origin, has long been recognized as a conceptually important, nonbiological approach to the generation of self-assembled, nano-structured molecules or materials, and a number of elegant examples are now known [1]. However, the bio-related aspects of molecular architecture as applied in this way, although full of promise, are not as well developed as various abiological ones. Although biological systems contain only a limited number of fundamental building blocks, such as nucleosides, amino acids, lipids, and carbohydrates, these molecules are chemically diverse and can be polymerized or assembled in an almost infinite number of ways. Further, owing to recent advances in chemical synthesis and biotechnology, these biomolecular building blocks can be arranged so as to produce a whole range of bio-inspired products that has not previously been possible to conceive. In this regard, the incorporation of metal complexes into biomolecules is recognized as being a key motif in the design and synthesis of functionalized biopolymers [2–18].

Among a variety of biomolecules, DNA molecules with a variety of structures (e.g. single or double helix, triplex, quadruplex, hairpin, circle, junction, etc.) and the highly controlled functions have long attracted many synthetic researchers. DNA is a biopolymer consisting of four kinds of monomeric nucleoside units with a distinct nucleobase (adenine (A), thymine (T), guanine (G), or cytosine (C)), and these building blocks are linked by phosphodiester bonds in a specific order that reflects their ability to encode genetic information. Despite the complexity of the genetic code, the base pairing process between two complementary DNA or RNA strands is rather uncomplicated and predictable. Hydrogen-bonding and aromatic stacking interactions between nucleobases are the main non-covalent forces that stabilize the complementary DNA strands. In particular, hydrogen bonding is a key principle in highly specific interstrand recognition.

So far, a number of strategies have been employed for modifying the periphery of DNA with metal complexes [19–22]. However, little has been done to explore the DNA core. We have recently envisioned that the natural hydrogen-bonded base pairs present in natural DNA could be replaced by alternative base pairing modes. Such strategy would lead not only to an expansion of the genetic alphabet but also to novel DNA structures and functions based on the controlled and periodic spacing of the building blocks along the helix axis. On the basis of this concept, we and others have recently initiated the study on the placement of charged or uncharged metal complexes as the building blocks in the interior of the DNA helix, thereby introducing a novel

*Corresponding author. Tel./Fax: +81-3-5841-8061. E-mail: shionoya@chem.s.u-tokyo.ac.jp.

ISSN 1061-0278 print/ISSN 1029-0478 online © 2002 Taylor & Francis Ltd
DOI: 10.1080/10610270290026167

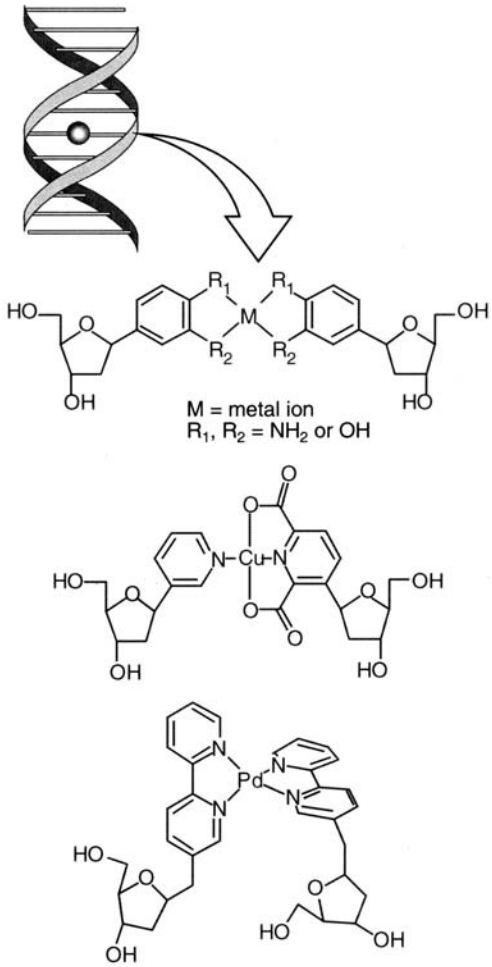
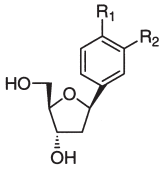


CHART 1

binding motif into duplex DNA (Chart 1) [23–31]. A key principle of our approach is the direct modification of a DNA base itself, turning it into a metal-chelating nucleobase wherein two nucleobases are paired through metal coordination.

As mentioned in the introduction, this paper provides a summary of our recent progress devoted to the introduction of metal-assisted base pairs into the interior of the DNA helix. In particular, the syntheses of artificial β-*C*-nucleosides bearing a chelator nucleobase (*o*-phenylenediamine (**1**), catechol (**2**), or 2-aminophenol (**3**)) (Chart 2), their metal coordination properties with metal ions, and the incorporation of these building blocks into DNA oligomers are summarized. In the course of these efforts, several novel synthetic strategies for the



	R_1	R_2
1	NH_2	NH_2
2	OH	OH
3	OH	NH_2

CHART 2

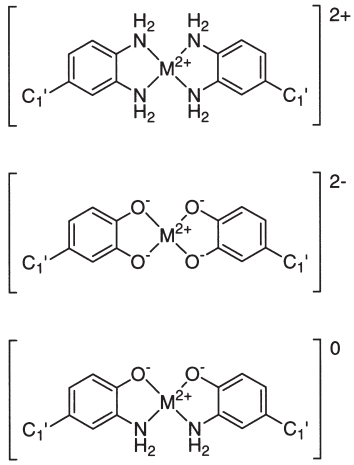


CHART 3

reconstruction of nucleic acids using metal complexes have been developed. These results raise the appealing possibility that this approach could provide in due course a new approach to the nanoassembly of metals.

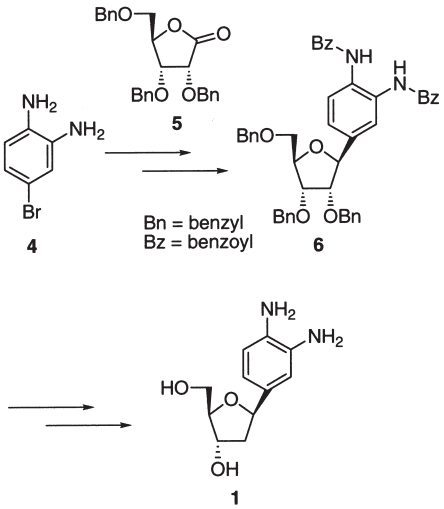
METAL-ASSISTED BASE PAIRING OF ARTIFICIAL NUCLEOSIDES

The molecules we have synthesized in this study are β-*C*-nucleosides having an *o*-phenylenediamine (**1**), catechol (**2**), or 2-aminophenol (**3**), as a metal-chelating site. These were predicted to form potentially a 2:1 square-planar or tetrahedral, or a 3:1 octahedral complex with a transition metal ion. It should be noted that, when these nucleosides form a 2:1 complex with a divalent metal ion, the complexes of **1**, **2**, and **3** have +2, −2, and 0 charges, respectively. Therefore, these base pairs could be incorporated into oligonucleotides at positions adjacent to each other (Chart 3). In addition, these nucleosides are designed so as to have geometrical analogy with natural base pairs.

Synthesis and Metal-assisted Base Pairing Of *o*-Phenylenediamine-bearing Nucleosides

The synthesis of the deoxy-β-*C*-nucleoside **1** started from 4-bromo-*o*-phenylenediamine **4** and 2,3,5-tri-*O*-benzyl-D-1,4-ribonolactone **5** and established a route in *C*-glycoside synthesis (Scheme 1) [23]. Lithiation of *N*-protected **4** followed by addition of **5** resulted in the intermediate formation of the corresponding hemiacetals, which upon reduction with Et_3SiH afforded selectively the β-isomeric *C*-nucleoside **6**.

After a series of typical transformations for the selective removal of the 2′-hydroxy group [32,33], *C*-nucloside **1** was obtained. The anomeric



SCHEME 1 A synthetic route for the deoxy-β-*C*-nucleoside bearing *o*-phenylenediamine as the nucleobase, **1**.

configuration of **1** was determined by ^{1}H NOE experiments of the intermediate **6** and by examining the coupling constants between H-1′ and H-2′ resonances.

The formation of a 2:1 complex between nucleoside **1** and Pd^{2+} ion was confirmed by ^{1}H NMR spectroscopy in D_2O and ESI-TOF mass spectrometry (Figs. 1 and 2) [23]. Proton resonances in the aromatic region were found to shift to lower field almost in proportion to increasing concentrations of Pd^{2+} ion, and full complexation was achieved when the concentration of the Pd^{2+} ion reached half that of **1**. This observation indicates that **1** and Pd^{2+} ion form a stable 2:1 complex with a high binding constant. Although there are two possible structures (*cis* and *trans*) for the Pd^{2+} complex **7**, we observed only one set of signals in the NMR spectra. The mass spectrum also provided clear evidence for the 2:1 complexation.

Synthesis and Metal-assisted Base Pairing of Catechol-bearing Nucleosides

In the synthesis of catechol-bearing nucleoside **2** (Scheme 2) [26], a key step is the Friedel–Crafts coupling reaction between *O*-protected catechol **8** and 1-*O*-methyl-3,5-protected 2-deoxy-D-ribofuranose **9** in the presence of $SnCl_4$ as a Lewis acid promoter. The anomeric configuration for the main product **10** was determined to be β by the ^{1}H NMR coupling constants between H-1′ and H-2′ resonances. The removal of ethoxycarbonyl and *t*-butyldimethylsilyl groups afforded nucleoside **2** in high yield. Its phosphoramidite derivative **11** was also synthesized with the aim of incorporating it into DNA oligomers using a standard automated DNA synthesizer [25].

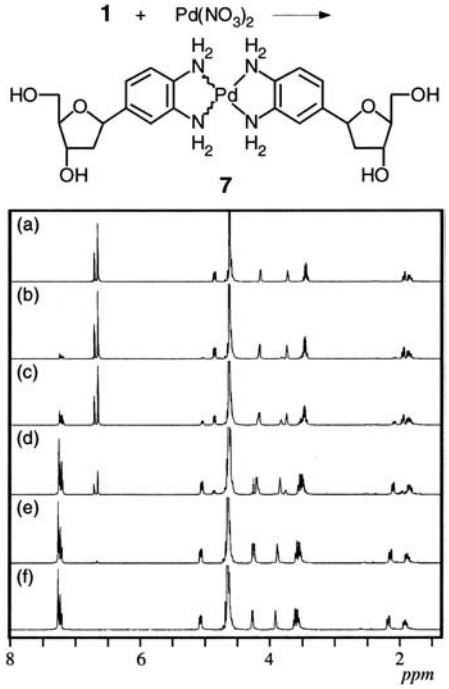


FIGURE 1 ^{1}H NMR spectra of **1** with increasing amount of Pd^{2+} ion. [**1**] = 26 mM in D_2O. [Pd^{2+}]/[**1**] = (a) 0.00, (b) 0.08, (c) 0.19, (d) 0.38, (e) 0.49, and (f) 0.57.

The catechol-bearing β-*C*-nucleoside **2** was treated with half amount of trimethyl borate in the presence of trimethylamine in DMSO-d_6 at room temperature to obtain a 2:1 complex **12** between nucleoside **2** and a boron ion (Scheme 2) [25]. ^{1}H NMR resonances of the aromatic protons of the complex **12** were shifted upfield from those of the nucleoside **2** (Fig. 3). The signals for catechol protons disappeared upon complexation with a boron ion, whereas those for the ribose moiety remained unchanged. The isotopically resolved ESI-TOF mass spectrum of this solution in the negative mode provided clear evidence for the complex **12** (Fig. 4). The enlarged plot of the signal at m/z 459.15 [**12**-Et_3NH^+]$^-$ was in good agreement with the theoretical isotopic distribution, indicating the boron-assisted base pairing with **2** in the

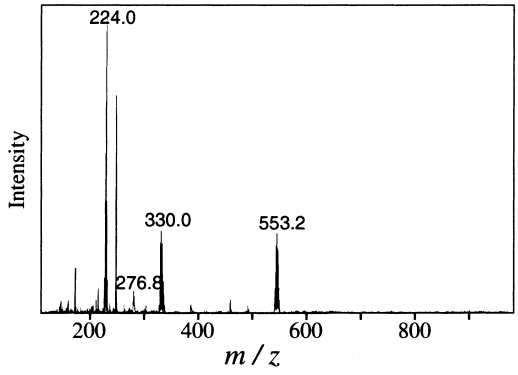
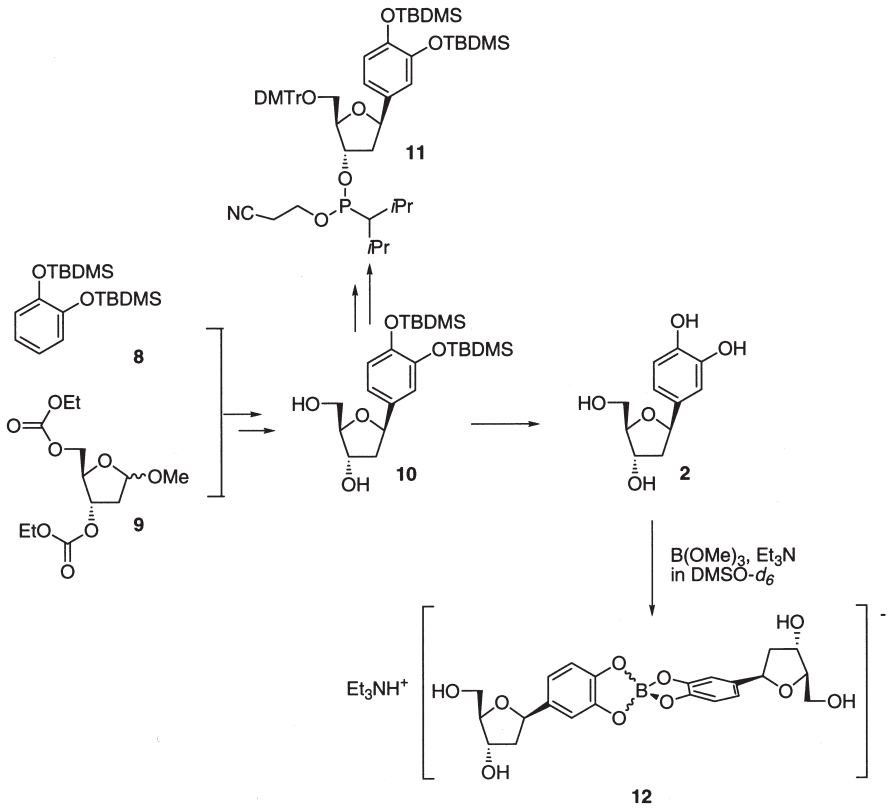


FIGURE 2 ESI mass spectrum of the Pd^{2+} complex **7**: 553.2 ([ML_2-H]$^+$; calcd. 553.13), 330.0 ([ML]$^+$; calcd. 330.02), 276.8 ([ML_2]$^{2+}$; calcd. 277.07), 224.0 ([L]$^+$; calcd. 224.12), where M = Pd^{2+}, L = nucleoside **1**.



SCHEME 2 A schematic representation of a synthetic route for catechol-nucleoside **2** and its borane complex **12**.

deprotonated form. These results also indicate that the boron complex **12** exists as a stable negatively charged complex in solution. Although there are two possible diastereomeric structures for the complex **12** arising from chiral centers on both the D-ribose skeleton and the boron center, we observed only one set of proton signals for the complex **12** in the spectrum. The boron complex is assumed to adopt a tetrahedral geometry. Although we tried using other transition metal ions to affect the metal-induced base pairing of **2**, complex formation was often accompanied by redox reactions that afforded unidentified products.

Synthesis and Metal-assisted Base Pairing of 2-aminophenol-bearing Nucleosides

Scheme 3 depicts a schematic representation of a synthetic route for the β-*C*-nucleoside which has a 2-aminophenol as the nucleobase [26]. A Friedel–Crafts approach, proceeding via electrophilic aromatic substitution, was used to build up the carbon skeleton of the nucleoside **3**. The reaction of *O*-benzyloxy-trifluroacetanilide **13** with 1-*O*-methyl-3,5-*O*-ditoluoyl-2-deoxy-D-ribofuranose **14** in CH_2Cl_2 in the presence of $SnCl_4$ produced β-*C*-nucleoside **15** with high selectivity (α-**16** : β − **15** = 1 : 10). The β configuration of the epimer **15** was clearly determined by X-ray analysis and the coupling constant trend between H-1′ and H-2′ in its ^{1}H NMR spectrum in $CDCl_3$ [26,27]. ^{1}H NOE differentiation experiments also provided clear evidence for the anomeric configuration of **15**. Removal of the protecting groups present in **15** in three steps afforded the desired β-*C*-nucleoside **3**.

Pd^{2+} ion-assisted base pairing with **3** was examined by ^{1}H NMR spectroscopy. Proton resonances of **3** in the presence of an equimolar amount of $NaHCO_3$ were compared with those of a mixture of **3** and K_2PdCl_4 at a ratio of 2:1 in D_2O–CD_3OD (4:1). As shown in Fig. 5, significant changes in the chemical shifts were observed in the aromatic region upon complexation with Pd^{2+} ion. Two sets of signals for H-3 and H-5 appeared at significantly lower field with an increased intensity in proportion to increas-

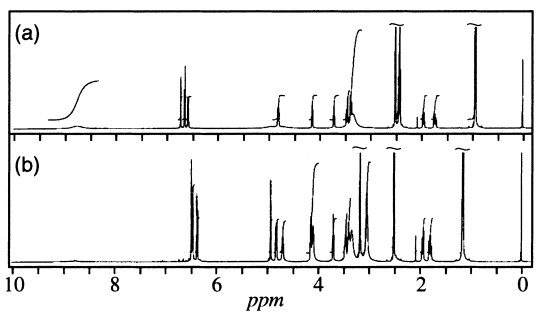


FIGURE 3 ^{1}H NMR spectra of (a) nucleoside **2** and (b) **2**-borate (2:1) complex, **12**, in DMSO-d_6. [**2**] = 44 mM. [$B(OMe)_3$] = (a) 0 and (b) 22 mM. [Et_3N] = 22 mM.

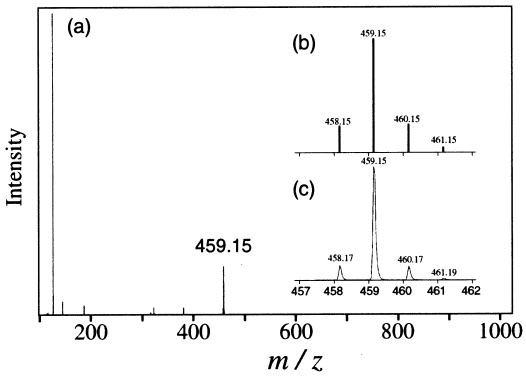
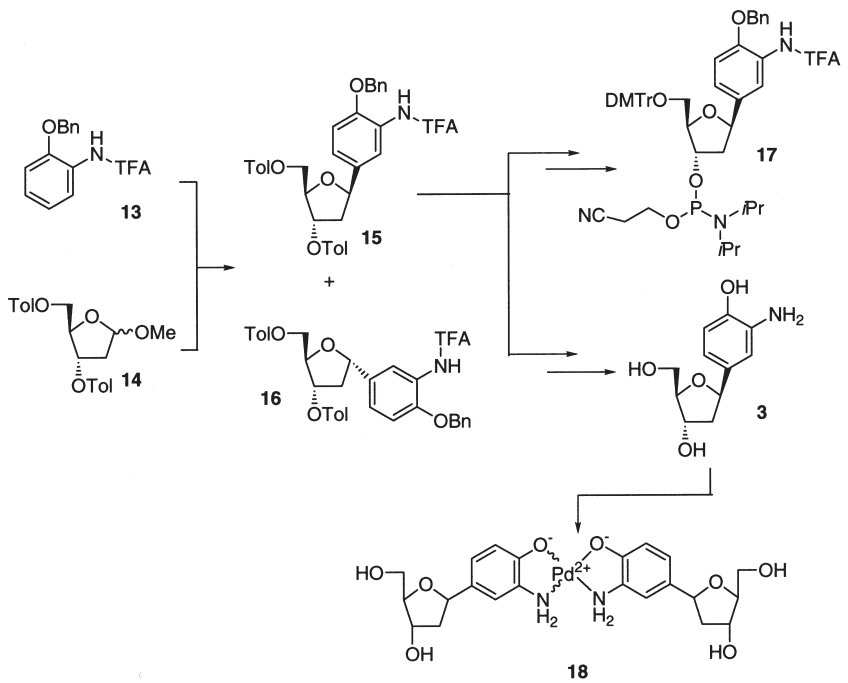


FIGURE 4 ESI-TOF mass spectrum of complex **12** in the negative mode: (a) m/z 100–1000, (b) the theoretical isotopic distribution and (c) the experimental isotopic distribution at m/z 459.15 ([**12**-Et_3NH]$^-$ calcd. 459.15).

ing concentration of Pd^{2+} ion, whereas the two sets of signals for H-6 appeared at higher field, compared with those for the nucleoside **3**. Complexation was deemed complete when the concentration of Pd^{2+} ion reached almost half the concentration of **3** (Fig. 5(b)). Although there are two possible structures, *cis* or *trans*, for the square-planar Pd^{2+} complex, in light of the *trans*-effect that should occur between the two phenolates bound to the Pd^{2+} center at the trans positions, the set of H-6 signals appearing at higher field (6.62 ppm) are assigned to the H-6 protons of the *trans*-complex with higher electron density. The ratio of *cis* to *trans* was approximately 1:1. In addition to the result of the ^1H NMR titration supporting the formation of a 2:1 complex between **3** and Pd^{2+}, the ESI-TOF mass spectrum of the complex in the positive mode provided clearer evidence for the ligand-metal ratio as shown in Fig. 6. The signals centered at m/z 555.09 (Fig. 6(a)) correspond to the +1 charged cationic species, $[Pd(\mathbf{3}\text{-}H)_2 + H]^+$, which is in good agreement with the theoretical isotopic distribution (Fig. 6(b)). These results establish that the nucleoside **3** forms a stable 2:1 complex **18** with Pd^{2+} ion with concomitant deprotonation of the phenolic proton originally present in **18**. Nucleoside **3** also forms 2:1 complexes with a Zn^{2+} or Cd^{2+} ion in a manner similar to complex **18**, and this was evidenced by ESI-TOF mass spectrometry (Fig. 7).

The protected β-C-nucleoside-**15** was converted to phosphoramidite **17** so as to allow its incorporation into oligonucleotides by automated solid-phase methods (Scheme 3). This was done using standard coupling chemistry to give 21-mer sequences (Chart 4). After cleavage from the resin and subsequent deprotection, the structural integrity of these oligomers was analyzed by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectroscopy.

To examine the effect of Cu^{2+} ion on the thermal stability of the base pair of *O*-Bn-2-aminophenol-bearing nucleotides (**X**) in DNA, the melting temperature (T_m) was determined by thermal denaturation monitored on UV absorption at 260 nm (Fig. 8). In the absence of Cu^{2+} ion, a 1:1 mixture of oligonucleotides, containing **X**, $(dA)_{10}\mathbf{X}(dA)_{10}$ and $(dT)_{10}\mathbf{X}(dT)_{10}$, melts as 42.5°C (Fig. 8(a)). For comparison, the duplex where the artificial base pair is replaced by $dA - dT$ pair melts at 46.5°C (Fig. 8(b)). Therefore, incorporation of the **X** base pair destabilizes the duplex structure, because it



SCHEME 3 A synthetic route for thedeoxy-β-*C*-nucleoside bearing 2-aminophenol as the nucleobase, **3**.

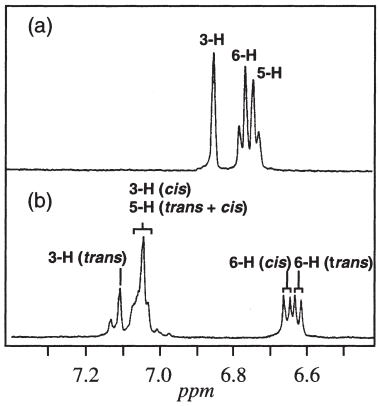


FIGURE 5 ^{1}H NMR spectra of nucleoside **3** in the absence or presence of K_2PdCl_4; [**3**] = 2.0 mM, [Pd^{2+}] = (a) 0 and (b) 1.0 mM, [$NaHCO_3$] = 2.0 mM in D_2O–CD_3OD (4:1) at room temperature.

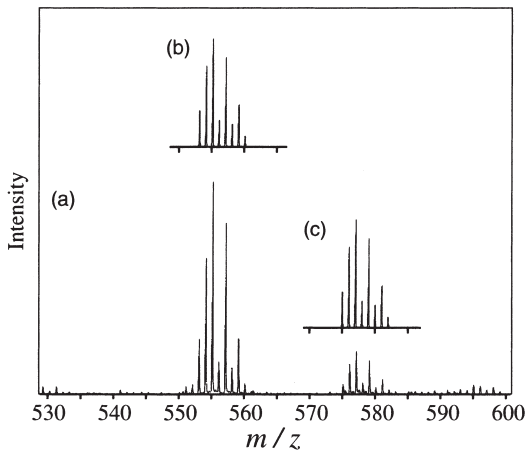


FIGURE 6 ESI-TOF mass spectrum in the positive mode for the 2:1 complex between **3** and Pd^{2+} ion (m/z 530–600); (a) the experimental isotopic distribution; (b) theoretical isotopic distribution for $[M + H]^+$, and (c) theoretical isotopic distribution for $[M + Na]^+$, where M = Pd(**3**-H)$_2$.

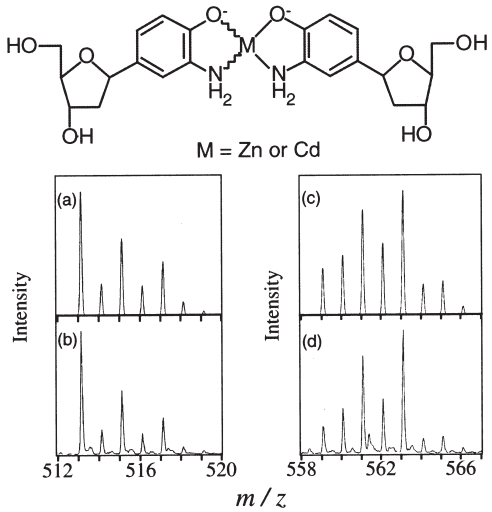


FIGURE 7 ESI-TOF mass spectra in the positive mode for the 2:1 complex between **3** and a Zn^{2+} or a Cd^{2+} ion; (a) the theoretical isotopic distribution for $[Zn(\mathbf{3}\text{-H})_2 + H]^+$ (b) the experimental isotopic distribution for the Zn^{2+} complex, (c) the theoretical isotopic distribution for $[Cd(\mathbf{3}\text{-H})_2 + H]^+$, and (d) the experimental isotopic distribution for the Cd^{2+} complex.

$(dA)_{21}$ AAAAAAAAAAAAAAAAAAAAA
$(dT)_{21}$ TTTTTTTTTTTTTTTTTTTTT
$(dA)_{10}X(dA)_{10}$ AAAAAAAAAA**X**AAAAAAAAAA
$(dT)_{10}X(dT)_{10}$ TTTTTTTTTT**X**TTTTTTTTTT

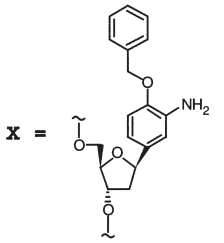


CHART 4

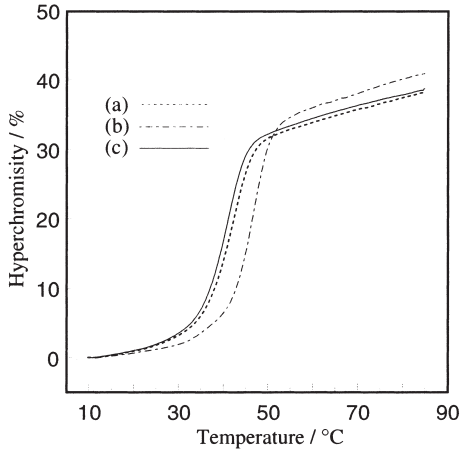


FIGURE 8 Normalized UV-melting curves at 260 nm ([duplex] = 1.19 μM in 10 mM Mops, 0.1 M NaCl (pH 7.0)). (a) $(dA)_{10}\mathbf{X}(dT)_{10}(dT)_{10}\mathbf{X}(dT)_{10}$, and (b) $(dA)_{21}\mathbf{X}(dT)_{21}$, and (c) $(dA)_{10}\mathbf{X}(dT)_{10}(dT)_{10}\mathbf{X}(dT)_{10}$, with Cu^{2+} ion (1.19 μM).

behaves as a mismatch pair in the absence of Cu^{2+} ion. Addition of 1 equivalent of Cu^{2+} ion slightly lowered the T_m for the duplex $(dA)_{10}\mathbf{X}(dT)_{10}$ $(dT)_{10}\mathbf{X}(dT)_{10}$ (Fig. 8(c)). Studies on artificial oligonucleotides including several types of other metal-assisted base pairs are now underway and the results will be reported elsewhere.

SUMMARY

This paper described the synthesis of three types of artificial β-*C*-nucleosides with a metal coordination site as new letters in an extended genetic alphabet. The structure, thermodynamically, and the kinetic behavior of double-stranded DNAs including these nucleosides could be controlled by the sequence and size of each strand, the coordination geometry, oxidation state, and ligand exchange rate of the metal ions lying at the center of base pairs. The methods developed in this study should lead to novel DNA or RNA molecules with unique binding and catalytic

properties not only for artificial gene control but also for DNA-based nano-technology. The site-specific incorporation of other artificial nucleotides into oligonucleotides will be reported elsewhere.

Acknowledgements

This work was supported by Grant-in-Aids for Scientific Research (No. 12440185, 12740357, 13031018, and 13554024) from the Ministry of Education, Science, Sports, and Culture, Japan.

References

[1] Lehn, J.-M. (1995) Supramolecular Chemistry: Concepts and Perspectives (VCH, Weinheim).
[2] Dreyer, G.R. and Dervan, P.B. (1985), *Proc. Natl Acad. Sci. USA* **82**, 968.
[3] Chen, C.-H.B. and Sigman, D.S. (1988), *J. Am. Chem. Soc.* **110**, 6570.
[4] Telser, J., Cruickshank, K.A., Schanze, K.S. and Netzel, T.L. (1989), *J. Am. Chem. Soc.* **111**, 7221.
[5] Bannwarth, W., Pfleiderer, W. and Müller, F. (1991), *Helv. Chim. Acta.* **71**, 1991, and references cited therein.
[6] Murphy, C.J., Arkin, M.R., Jenkins, Y., Ghatlia, N.D., Bossmann, S.H., Turro, N.J. and Barton, J.K. (1993), *Science* **262**, 1029.
[7] Bashkin, J.K., Frolova, E.I. and Sampath, U. (1994), *J. Am. Chem. Soc.* **116**, 5981.
[8] Matsumura, K., Endo, M. and Komiyama, M. (1994), *J. Chem. Soc., Chem. Commun.*, 2019.
[9] Magda, D., Miller, R.A., Sessler, J.L. and Iverson, B.L. (1994), *J. Am. Chem. Soc.* **116**, 7439.
[10] Meade, T.J. and Kayyem, J.F. (1995), *Angew. Chem., Int. Ed. Engl.* **34**, 352.
[11] Hall, J., Hüsken, D. and Häner, R. (1996), *Nucleic Acids Res.* **24**, 3522.
[12] Manchanda, R., Dunham, S.U. and Lippard, S.J. (1996), *J. Am. Chem. Soc.* **118**, 5144.
[13] Schliepe, J., Berghoff, U., Lippert, B. and Cech, D. (1996), *Angew. Chem., Int. Ed. Engl.* **35**, 646.
[14] Mucic, R.C., Herrlien, M.K., Mirkin, C.A. and Letsinger, R.L. (1996), *Chem. Commun.*, 555.
[15] Dandliker, P.J., Holmin, R.H. and Barton, J.K. (1997), *Science* **275**, 1465.
[16] Meggers, E., Kusch, D. and Giese, B. (1997), *Helv. Chim. Acta* **80**, 640.
[17] Ihara, T., Nakayama, M., Murata, M., Nakano, K. and Maeda, M. (1997), *Chem. Commun.*, 1609.
[18] Magda, D., Crofts, S., Lin, A., Miles, D., Wright, M. and Sessler, J.L. (1997), *J. Am. Chem. Soc.* **119**, 2293.
[19] Hurley, D.J. and Tor, Y. (1998), *J. Am. Chem. Soc.* **120**, 2194.
[20] Lewis, F.D., Helvoigt, S.A. and Lestinger, R.L. (1999), *Chem. Commun.*, 327.
[21] Kahn, S.I., Beilstein, A.E. and Grinstaff, M.W. (1999), *Inorg. Chem.* **38**, 418.
[22] Rack, J.J., Krider, E.S. and Meade, T.J. (2000), *J. Am. Chem. Soc.* **122**, 6287.
[23] Tanaka, K. and Shionoya, M. (1999), *J. Org. Chem.* **64**, 5002.
[24] Shionoya, M. and Tanaka, K. (2000), *Bull. Chem. Soc. Jpn* **73**, 1945.
[25] Cao, H., Tanaka, K. and Shionoya, M. (2000), *Chem. Pharm. Bull.* **48**, 1745.
[26] Tanaka, K., Tasaka, M., Cao, H. and Shionoya, M. (2001), *Eur. J. Pharm. Sci.* **13**, 77.
[27] Tasaka, M., Tanaka, K., Shiro, M. and Shionoya, M. (2001), *Supramol. Chem.* **13**, 671.
[28] Meggers, E., Holland, P.L., Tolman, W.B., Romesberg, F.E. and Schultz, P.G. (2000), *J. Am. Chem. Soc.* **122**, 10714.
[29] Atwell, S., Meggers, E., Spraggon, G. and Schultz, P.G. (2001), *J. Am. Chem. Soc.*, **123**, 12364.
[30] Weizman, H. and Tor, Y. (2001), *Chem. Commun.*, 453.
[31] Weizman, H. and Tor, Y. (2001), *J. Am. Chem. Soc.* **123**, 3375.
[32] Hildbrand, S. and Leumann, C. (1996), *Angew. Chem., Int. Ed. Engl.* **35**, 1968.
[33] Hildbrand, S., Blaset, A., Parelin, S.P. and Leumann, C. (1997), *J. Am. Chem. Soc.* **119**, 5499.