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Soft Metal-Mediated Base Pairing with Novel Synthetic Nucleosides Possessing an *O*,*S*-Donor Ligand

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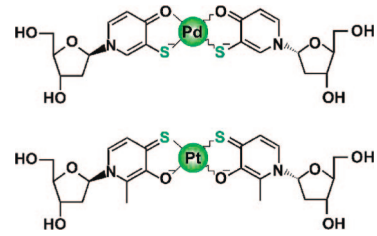
Received March 14, 2008



Metal-mediated base pairing with artificial ligand-bearing nucleosides allows site-selective metal incorporation inside DNA duplexes. In particular, this strategy has provided a general way of discrete, heterogeneous metal arrays in a programmable manner. To increase the kind of metallo-building blocks, we have newly synthesized two artificial nucleosides which have an *O*,*S*-donor ligand as the nucleobase moiety, mercaptopyridone (**M**) and hydroxypyridinethione (**S**). These nucleosides were found to efficiently form metal-mediated base pairs with soft transition metal ions such as Pd^{2+} and Pt^{2+}.

Introduction

Incorporation of artificial base pairs into DNA double helices has shown promise not only for expansion of genetic information[1] but also for construction of functional molecules.[2] So far, a number of excellent examples have been reported on artificial base pairs through alternative hydrogen-bonding,[3] hydrophobic packing interactions,[4] and metal-coordination.[5–18] In particular, because metal complexes have unique chemical and physical properties such as redox-, optical-, magnetic-functions and Lewis acidity, metallo-base pairs have been expected to provide novel structures and functions for artificial DNAs.

Since we reported Pd^{2+}-mediated base pairing[6] between two *o*-phenylenediamine-bearing nucleosides, we[6–9] and others[10–15] have demonstrated artificial ligand-type nucleosides that form

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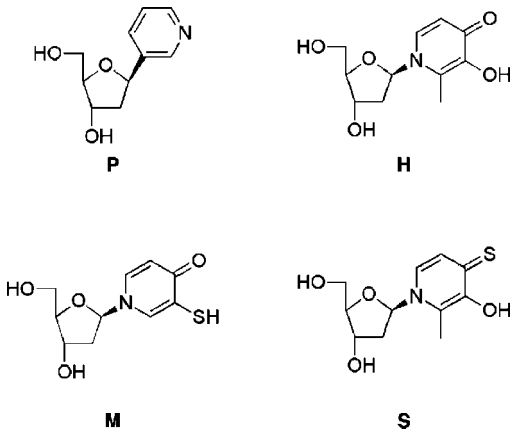
10.1021/jo800587d CCC: $40.75 © 2008 American Chemical Society
Published on Web 07/17/2008

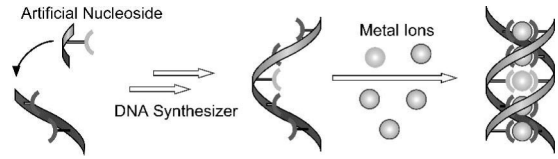
CHART 1. Examples of Artificial Ligand-Type Nucleosides



metal-mediated base pairs with Pd^{2+}, Cu^{2+}, Ag^{+}, Ni^{2+}, Mn^{3+}, and so on. Some of them were successfully incorporated into oligonucleotides in a programmable manner, and then provided metal-mediated artificial base pairs mainly in duplexes. For instance, we reported a pyridine-bearing nucleoside (**P**),[8] which forms a 2:1 linear complex with Ag^{+} or Hg^{2+}, and a hydroxypyridone-bearing nucleoside (**H**),[9] which forms a 2:1 square-planar complex with Cu^{2+} (Chart 1). Both nucleosides were individually incorporated into DNA double strands and they stabilized the duplexes through **P**−Ag^{+}/Hg^{2+}−**P** or **H**−Cu^{2+}−**H** base pairing. Moreover, the multiple incorporation of nucleoside **H** allowed one-dimensional discrete assembly of Cu^{2+} ions through **H**−Cu^{2+}−**H** base pairing to form $n$$Cu^{2+}$•d(5′-G**H**$_n$C-3′)$_2$ (n = 1−5) inside the artificial DNA duplexes. The Cu^{2+} ions stacked on top of each other were coupled ferromagnetically with one another through unpaired *d* electrons.[16] Heterogeneous metal arrays of Cu^{2+} and Hg^{2+} were also established by means of two nucleosides, **P** and **H**,[17] in which quantitative formation of Cu^{2+}−Hg^{2+}−Cu^{2+} and Cu^{2+}−Cu^{2+}−Hg^{2+}−Cu^{2+}−Cu^{2+} were achieved using predesigned artificial DNA strands, d(5′-G**HPH**C-3′) and d(5′-G**HHPHH**C-3′), respectively (Chart 2).

The principal advantage of DNA synthesis is its "bottom-up" protocol and straightforward preparation by automated DNA

CHART 2. Template-Directed Heterogeneous Metal Arrays in Artificial DNA



synthesizer. Because oligonucleotides with any desired sequence and length up to about 100 can be easily obtained by the consecutive condensation of monomers, artificial nucleosides can be similarly incorporated into DNA strands at the desired positions in a designed sequence. In view of their possible permutations, development of novel metallo-base pairs would allow highly diverse metal arrays inside DNA, leading to the functional fine-tuning of the metal complexes by predetermining the sequence of ligand-type bases.

Toward this end it is essential to increase the kind of nucleobase ligands that are specific to some metal ion(s). To obtain well-defined metal-assembled complexes within DNA, artificial metallo-base pairs should be designed so that their size and shape go well with those of natural base pairs that are all flat and stacked on top of each other. In light of the preferred geometry of metallo-base pairs, linear-coordinating Ag^{+} and Hg^{2+} and square-planar-coordinating Cu^{2+}, low-spin Ni^{2+}, Pd^{2+}, and Pt^{2+} were first chosen from a series of transition metals. In addition, the metal binding sites were placed at positions corresponding to those of hydrogen bond donors or acceptors of the natural base pairs. Both monodentate[8,10] and bidentate ligand-type nucleosides[9,11] formed homogeneous metallo-base pairs as two-coordinate linear and four-coordinate square-planar complexes, respectively. Furthermore, heterogeneous base pairing with [1 + 3]-coordination[12,13] has been exploited using a tridentate and a monodentate ligands. A salen-type nucleoside also provided a base pair that is mediated by both metal coordination and a reversible covalent cross-linking with ethylenediamine.[14]

The selectivity in metal complexation is closely related to the property of donor atoms of ligands as well as the kind of metals. In this regard, the "hard and soft acids and bases" (HSAB) rule, that hard (soft) ligands tend to bind to hard (soft) metal ions, is a useful standpoint when we chose a proper combination of donor atoms and metals.[19] Artificial nucleosides developed in this study were designed based on this rule, and directed toward the site-selective, heterogeneous incorporation of metals into DNA. Herein we describe the syntheses of novel nucleosides, mercaptopyridone-bearing nucleoside (**M**) and hydroxypyridinethione-bearing nucleoside (**S**), possessing *O*,*S*-donor atoms as a bidentate ligand. These novel nucleosides were found to form novel base pairs with soft metal ions such as Pd^{2+} and Pt^{2+}. Comparison of these nucleosides with hydroxypyridone-bearing nucleoside (**H**) is also described.

Results and Discussion

Molecular Design of Novel Nucleosides. We designed mercaptopyridone-bearing nucleoside (**M**) and hydroxypyridinethione-bearing nucleoside (**S**) having a thiol group and a thiocarbonyl group, respectively, as a soft donor atom (Chart 1). These nucleosides were expected to form [2 + 2]-type base

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(18) It is well-known that two thymine bases in the deprotonated form coordinate to an Hg^{2+} ion to form T−Hg^{2+}−T metallo-base pair. See: (a) Kukleynik, Z.; Marzilli, L. G. *Inorg. Chem.* **1996**, *35*, 5654–5662. (b) Miyake, Y.; Togashi, H.; Tashiro, M.; Yamaguchi, H.; Oda, S.; Kudo, M.; Tanaka, Y.; Kondo, Y.; Sawa, R.; Fujimoto, T.; Machinami, T.; Ono, A. *J. Am. Chem. Soc.* **2006**, *128*, 2172–2173.

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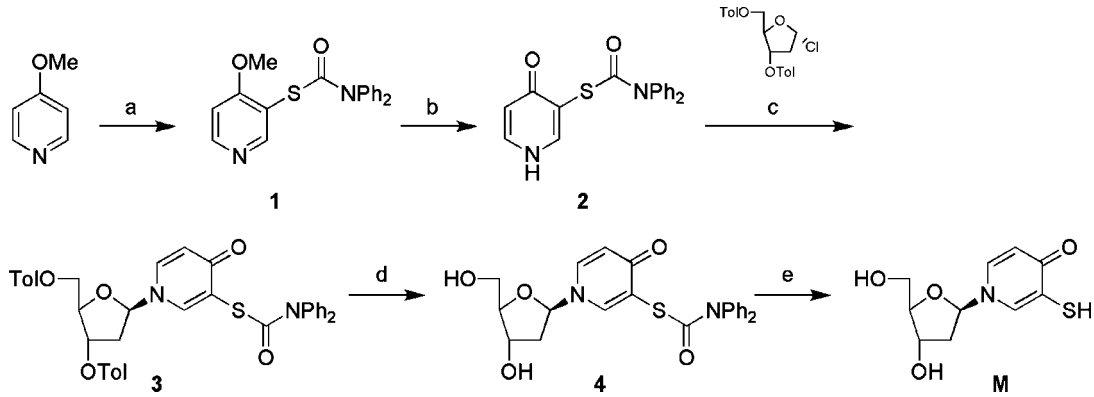
SCHEME 1. Synthetic Route for Mercaptopyridone-Bearing Nucleoside (M)[a]



[a] Reagents and conditions: (a) (i) PhLi, THF, −78 °C → 0 °C; (ii) S_8, THF, −40 °C → −20 °C; (iii) Ph_2NCOCl, THF, −78 °C → 0 °C, 55%; (b) TMSI, CH_3CN, reflux, quant.; (c) 1′-α-chloro-3′,5′-di-*O*-toluoyl-2′-deoxyribose, iPr_2EtN, CH_2Cl_2, rt, 23%; (d) K_2CO_3, CH_2Cl_2/CH_3OH, rt, 87%; (e) saturated aqueous NH_4OH, DTT, 90 °C, 31%.

pairs with softer transition metals in contrast to Cu^{2+}-mediated base pairing with hydroxypyridone-bearing nucleoside (**H**).[9] Several examples of metal complexation of *O*,*S*-donor ligands have been reported so far, such as *N*-substituted 3-hydroxy-2-methyl-4-pyridinethione[20,21] and 3-hydroxy-2-methyl-4-thiopyrone (thiomaltol).[21,22] Lewis et al. reported that thiomaltol forms a 2:1 complex with Ni^{2+} having a *cis* square-planar geometry, and thereby concluded that the ligand exhibits a strong *trans* influence.[22] This tendency indicates that nucleosides possessing *O*,*S*-donor ligands are likely to form square-planar metallo-base pairs with a *cis* geometry that could fit well to DNA duplexes. In addition, when these nucleosides, **M** and **S**, form a 2:1 base pair with a divalent metal ion, metallo-base pairs become neutral due to spontaneous deprotonation of the ligand upon metal coordination. Therefore, these artificial base pairs were expected to be rather stable due to hydrophobic effects within DNA helices.

Synthesis of Mercaptopyridone-Bearing Nucleoside (M). Mercaptopyridone-bearing nucleoside (**M**) was synthesized according to Scheme 1. The introduction of a protected thiol functionality into the nucleobase framework was carried out by regioselective lithiation[23] at the C3 position of 4-methoxypyridine[24] followed by thiolation and protection with a diphenylcarbamoyl group. The structure of the resulting **1** was determined by X-ray diffraction analysis (Figure S1) as well as ^1H NMR spectroscopy. Subsequent demethylation with trimethylsilyl iodide afforded a 4-carbonyl compound **2**. During the demethylation, nitrogen bubbling was essential to prevent the formation of an *N*-methyl substituted byproduct.

The coupling reaction between the protected nucleobase **2** and α-chloro-2-deoxyribose[25] furnished the nucleside **3** as a mixtrure of α- and β-anomers (α/β = 9:4) in 73% yield. The

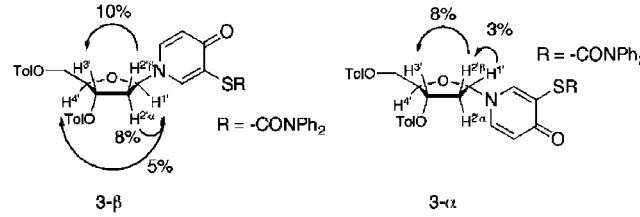


FIGURE 1. Illustrations of nuclear Overhauser enhancements for two anomeric isomers of **3**.

anomeric mixtures were separated by silica gel column chromatography to afford the desired β-nucleoside in 23%. Although there was a possibility that not only desired *N*-nucleosides but also *O*-nucleosides were obtained in the coupling reaction, the formation of *N*-nucleoside was confirmed by its ^{13}C NMR spectrum that showed a signal of a carbonyl carbon at the 4 position of pyridone ring. The anomeric configuration of **3** was determined by proton nuclear Overhauser effects (NOE) (Figure 1). When the 2′α proton resonances around 2.72 ppm were separately irradiated, a nuclear Overhauser enhancement of 8% was observed at the 1′ proton. The irradiation of the 2′β proton gave a 10% enhancement at the 3′ proton, whereas only 1% enhancement was observed at the 1′ proton. Furthermore, the irradiation of the 1′ proton gave 5% enhancement at the 2′α proton and 5% at the 4′ proton. These NOE trends provide a clear evidence for β-anomer **3-*β***. The NOE measurement of α-anomer **3-α** was also carried out and gave reasonable results.

Deprotection of the toluoyl groups at the 3′ and 5′ positions of nucleoside **3** afforded nucleoside **4**. To avoid spontaneous generation of the corresponding disulfide, the deprotection of the diphenylcarbamoyl group of **4** was performed in saturated ammonia solution in the presence of 1,4-dithiothreitol (DTT) at 90 °C for 24 h, and the desired thiol-functional nucleoside **M** was successfully obtained.

Synthesis of Hydroxypyridinethione-Bearing Nucleoside (S). Hydroxypyridinethione-bearing nucleoside (**S**) was synthesized from the precursor of hydroxypyridone-bearing nucleoside (**H**)[9] (Scheme 2). Conversion of the carbonyl group of hydroxypyridone **5** to a thiocarbonyl group by phosphorus pentasulfide (P_4S_{10})[26] afforded pyridinethione **6**. The transformation of the

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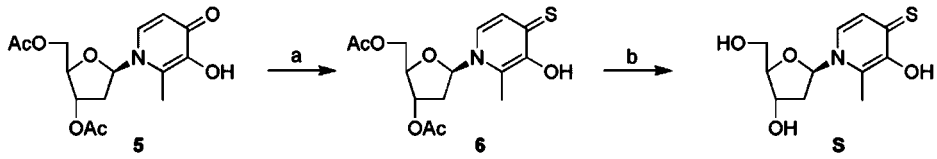
(27) In the IR spectrum of **6**, no absorption peak was observed around 1620 cm^{-1} for the carbonyl group of hydroxypyridone (Figure S3, Supporting Information).

SCHEME 2. Synthetic Route for Hydroxypyridinethione-Bearing Nucleoside (S)[a]



[a] Reagents and conditions: (a) P_4S_{10}, iPr_2EtN, CH_3CN, rt, 75%; (b) K_2CO_3, CH_3OH, rt, 94%.

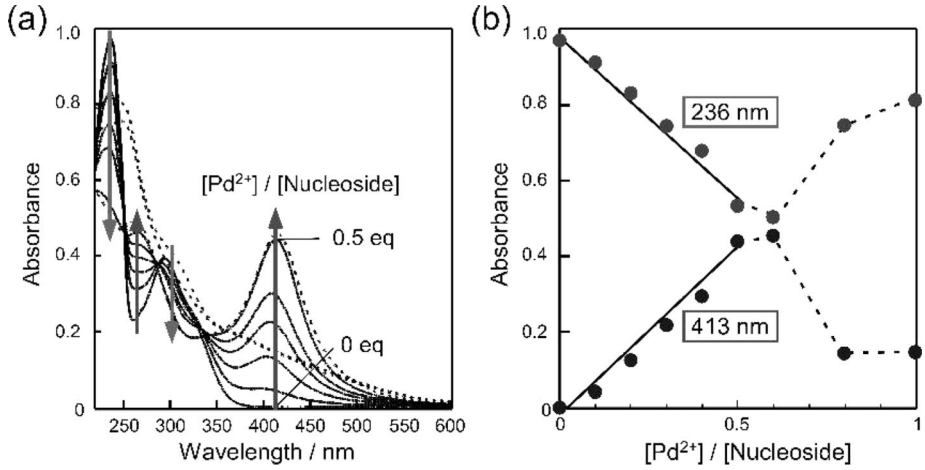


FIGURE 2. (a) UV absorption changes of nucleoside **M** at various concentrations of K_2PdCl_4. (b) Plots of absorbance at 236 and 413 nm against the ratio of Pd^{2+} to **M**. [**M**] = 50 μM in 25 mM MOPS (pH = 7.0) at 25 °C, l = 1.0 cm.

functional group was confirmed by IR[27] and electrospray ionization-time-of-flight (ESI-TOF) mass spectra (found: m/z = 364.07 (z = 1), calculated for $C_{15}H_{19}NO_6SNa$ $[M + Na]^+$: 364.08). The following deacetylation of **6** gave the desired nucleoside **S**. The X-ray diffraction analysis of its single crystals confirmed the regioselective introduction of the sulfur atom and ensured that the anomeric configuration was conserved (Figure S2).

Metal-Mediated Base Pairing of the Nucleosides. Metal-mediated base pairing of mercaptopyridone-bearing nucleoside **M** was examined by the photometric titration study in a 3-(*N*-morpholino)propanesulfonate (MOPS) buffer (pH 7.0) at 25 °C. Complexation of **M** with Pd^{2+} took about 30 min, and with an increase in Pd^{2+} concentrations, a new absorption peak at 413 nm appeared and the spectra changed linearly until the ratio of $[Pd^{2+}]/[\mathbf{M}]$ reached 0.5 (Figure 2). An ESI-TOF mass spectrum of a 2:1 mixture of **M** and Pd^{2+} in H_2O-CH_3CN (1:1) showed a signal at m/z 589.80 which is in good agreement with the theoretical data for $[\mathbf{M}_2Pd]^+$ complexes (Figure 3; found: 589.80, calculated for $[bp - e]^+$: 590.00). This result indicates that a metal-mediated base pair, **M**−Pd^{2+}−**M**, was stoichiometrically formed.

In the titration of the nucleoside **M** with Ni^{2+}, complexation was rapidly completed and a shoulder peak around 375 nm appeared upon addition of Ni^{2+} (Figure 4). The spectra changed linearly in the range of $[Ni^{2+}]/[\mathbf{M}]$ from 0 to 0.3. In the ^1H NMR spectrum in the case of the $[Ni^{2+}]/[\mathbf{M}]$ ratio of 0.33:1 in D_2O, the signals of the nucleobase moiety were extremely broadened (Figure 5). These results indicate the quantitative formation of the octahedral 3:1 Ni^{2+} complex, and therefore the nucleoside **M** would provide a metal-mediated base triplet motif in the triple-stranded DNA. Further addition of Ni^{2+} resulted in the appearance of new peaks around the aromatic region. The spectra converged when the ratio of $[Ni^{2+}]/[\mathbf{M}]$ reached 1:2, which suggests that an **M**−Ni^{2+}−**M** base pair with a diamagnetic square-planar structure with low-spin Ni^{2+} was

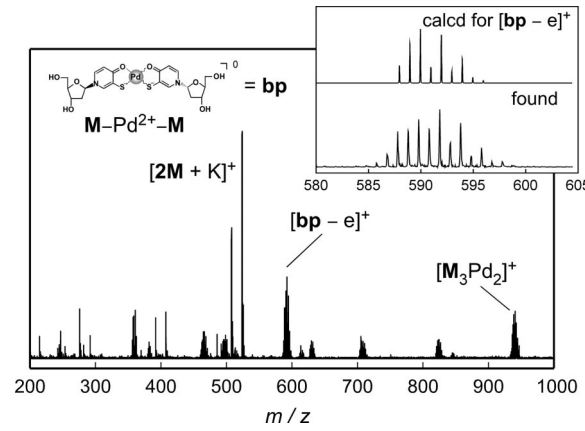


FIGURE 3. ESI-TOF mass spectrum of a 2:2:1 mixture of **M**, $NaHCO_3$, and K_2PdCl_4 in H_2O−CH_3CN (1:1) (bp: metallo-base pair, 2**M**: corresponding disulfide).

quantitatively formed. Its ESI-TOF mass spectrum also confirmed the Ni^{2+}-mediated base pairing (Figure 6; found: 542.04, calculated for $[bp - e]^+$: 542.03).

The ability of hydroxypyridinethione-bearing nucleoside **S** to form a metal-mediated base pair was then examined with square-planar-coordinating metal ions. Complexation of **S** with Pt^{2+} reached to its equilibrium within 24 h. With an increase in Pt^{2+} concentrations, the absorption at 338 nm arising from the pyridinethione moiety gradually decreased and a new peak at 413 nm spontaneously appeared with two isosbestic points (Figure 7). The spectra continued to change linearly until the ratio of $[Pt^{2+}]/[\mathbf{S}]$ reached 0.5. This result indicates the stoichiometric formation of the metallo-base pair, **S**−Pt^{2+}−**S**. Its ESI-TOF mass spectrum also provided evidence for the complexation in the ratio of $[Pt^{2+}]/[\mathbf{S}]$ = 1:2 (Figure 8; found: 730.13, calculated for $[bp - e]^+$: 730.08).

The metal-mediated base pairing properties of other combination of the ligand-type nucleosides and metal ions were also

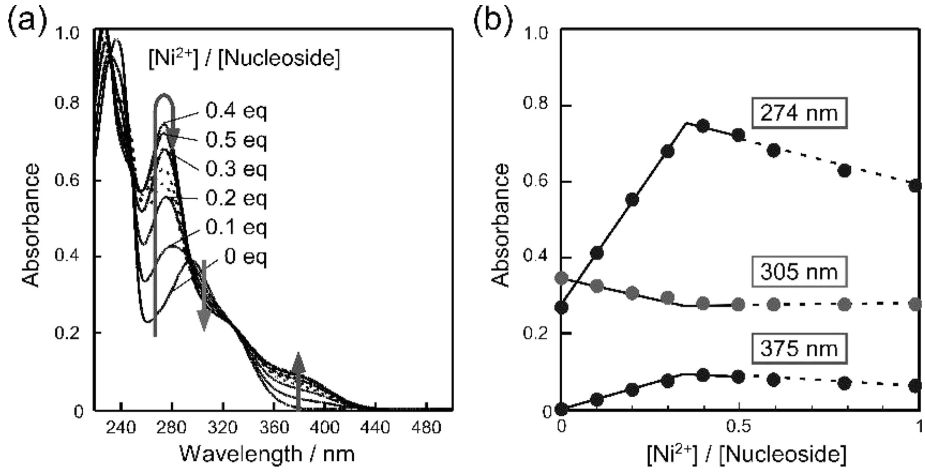


FIGURE 4. (a) UV absorption changes of nucleoside **M** at various concentrations of $NiCl_2$. (b) Plots of absorbance at 274, 305, and 375 nm against the ratio of Ni^{2+} to **M**. [**M**] = 50 μM in 25 mM MOPS (pH = 7.0) at 25 °C, l = 1.0 cm.

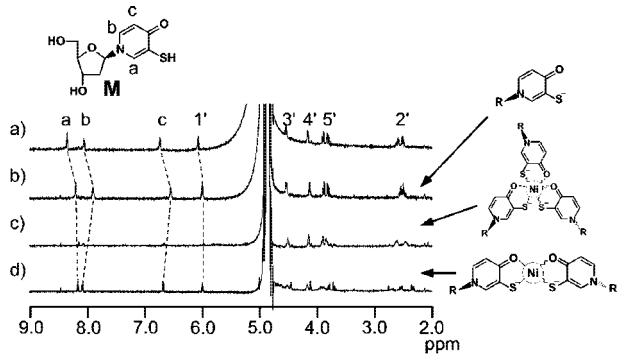


FIGURE 5. ^{1}H NMR spectra of (a) **M**, (b) **M** + 1.0 equiv of $NaHCO_3$, (c) (b) + 0.33 equiv of $NiCl_2$, and (d) (b) + 0.50 equiv of $NiCl_2$. [**M**] = 2 mM in D_2O at 300 K.

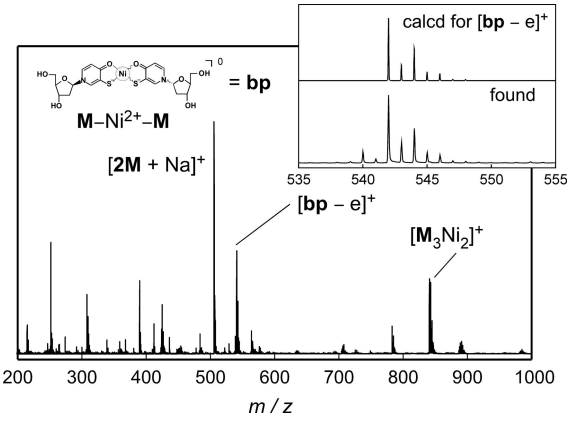


FIGURE 6. ESI-TOF mass spectrum of a 2:2:1 mixture of **M**, $NaHCO_3$, and $NiCl_2$ in H_2O−CH_3CN (1:1) (bp: metallo-base pair, 2**M**: corresponding disulfide).

estimated qualitatively by titration experiments (see Supporting Information). The results are summarized with the absorption maxima of metallo-base pairs as shown in Table 1. While both **M** and **S** formed base pairs efficiently with relatively soft metal ions such as Pd^{2+}, Ni^{2+}, and Pt^{2+}, hydroxypyridone-bearing nucleoside (**H**) did not quantitatively form a base pair with such softer ions under the same condition. This result suggests that the incorporation of both **H** and the sulfur-containing nucleoside, **M** or **S**, into artificial oligonucleotides may allow heterogeneous metal arrays in DNA duplexes. Moreover, metallo-base pairs with **M** and **S** exhibited their absorption over 340 nm, whereas the absorption peak of metallo-base pairs with **H** appeared around 300 nm. Thus, UV−vis absorption measurement would be an excellent way to identify site-selective metal complexation in artificial metallo-DNA.

Conclusion

In this paper, we described the efficient syntheses of two artificial nucleosides, **M** and **S**, possessing an *O*,*S*-donor ligand that coordinate to soft transition metals. The photometric titration experiments with metal ions showed that both nucleosides form metal-mediated base pairs efficiently with square-planar relatively soft metals such as Pd^{2+}, Ni^{2+}, and Pt^{2+}. Thus, both **M** and **S** have great potential to form soft metal-mediated base pairs within DNA oligomers and to align d^8 transition metal ions in a controllable way. Furthermore, these nucleosides would assemble a variety of metals heterogeneously in DNA duplexes in combination with a Cu^{2+}-selective **H** nucleoside in a programmable fashion. Further investigation on the incorporation of the nucleosides into DNA strands is currently under way.

Experimental Section

4-Methoxy-3-thiodiphenylcarbamoylpyridine (1). 4-Methoxypyridine (2.35 g, 21.6 mmol) was dissolved in THF (100 mL) and cooled to −78 °C. To the solution was added dropwise phenyllithium (1.1 M in cyclohexane-diethyl ether, 40.0 mL). After stirring at 0 °C for 2 h, the mixture was kept at −40 °C and sulfur powder (1.30 g, 40.5 mmol) was then added. The suspended solution was stirred vigorously at −20 °C for 20 h to prepare thiolate. The reaction mixture was cooled again to −78 °C followed by the addition of a precooled THF solution (50 mL) of diphenylcarbamoyl chloride (10.2 g, 44.0 mmol) and gradually allowed to warm up to room temperature. After stirring for 20 h, the reaction mixture was quenched with H_2O (200 mL) and extracted with $CHCl_3$ (200 mL × 3). The combined organic phase was dried over anhydrous $MgSO_4$ and then concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$:CH_3OH = 50:1) to afford **1** (4.52 g, 13.4 mmol, 62.1%) as a pale brown oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.61 (br, 1H), 8.56 (s, 1H), 7.47−7.34 (m, 10H), 7.17 (br, 1H), 4.10 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.0, 165.9, 156.8, 152.9, 141.3, 129.3, 127.5, 115.3, 106.9. 56.0. FT-IR (ATR): 1675, 1574, 1491, 1481, 1277, 1145, 1029, 1015, 823, 815, 695, 615, 575 cm^{-1}. HRMS (ESI) *m/z*: [M + Na]$^+$ calcd for $C_{19}H_{16}N_2O_2SNa$ 359.0830, found 359.0833.

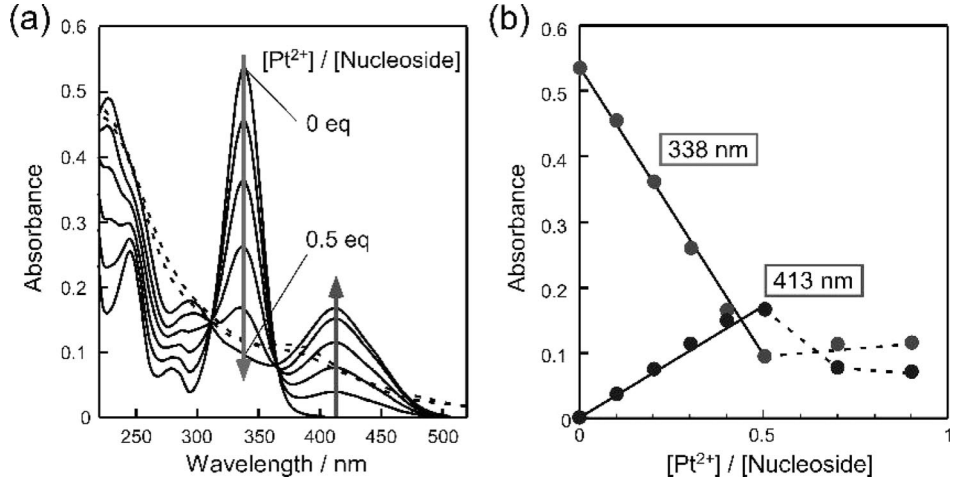


FIGURE 7. (a) UV absorption changes of nucleoside **S** at various concentrations of K_2PtCl_4. (b) Plots of absorbance at 338 and 413 nm against the ratio of Pt^{2+} to **S**. [**S**] = 250 μM in 25 mM MOPS (pH = 7.0) at 25 °C, l = 0.1 cm.

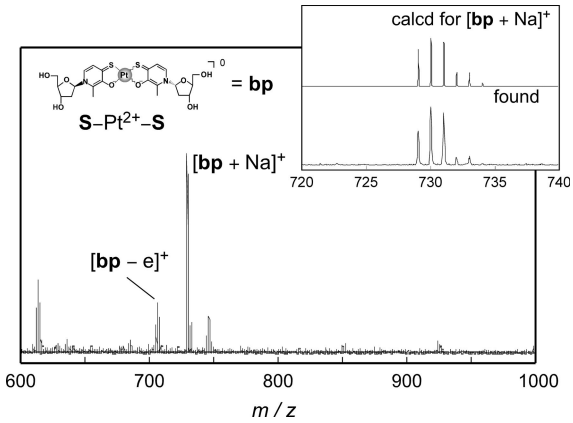


FIGURE 8. ESI-TOF mass spectrum of a 2:2:1 mixture of **S**, $NaHCO_3$, and K_2PtCl_4 in H_2O-CH_3OH (1:1) (bp: metallo-base pair).

TABLE 1. Metal-Mediated Base Pairing Properties of Nucleosides with Mercaptopyridone (M), Hydroxypyridinethione (S), and Hydroxypyridone (H)

	base-pairing property with metal ions[a] (λ_{max} of metallo-base pair)			
nucleoside	Cu^{2+}	Pd^{2+}	Ni^{2+}	Pt^{2+}
M	—[b,c] (325 nm)	Quantitatively[c] (413 nm)	—[d] (375 nm[e])	Partially[c] (340 nm[e])
S	—[b,f] (400 nm[e])	Quantitatively[f] (397 nm)	Quantitatively[f] (400 nm[e])	Quantitatively[f] (413 nm)
H	Quantitatively[c,g] (303 nm)	Partially[f] (316 nm)	Partially[f] (308 nm)	Not formed[f]

[a] In 25 mM MOPS (pH = 7.0) at 25 °C. [b] The formation of several species was speculated because of their complicated spectral changes with no isosbestic point. [c] [Nucleoside] = 50 μM. [d] Quantitative formation of the 2:1 complex could not be confirmed by photometric titration experiments. [e] Shoulder peaks. [f] [Nucleoside] = 250 μM. [g] Reference 9.

3-Thiodiphenylcarbamoyl-4-pyridone (2). A solution of **1** (453 mg, 1.35 mmol) and trimethylsilyl iodide (210 μL, 1.48 mmol) in acetonitrile (30 mL) was heated at reflux for 20 h. To remove methyl iodide generated, N_2 bubbling was continued during the reaction. The reaction was quenched by addition of CH_3OH (5 mL) and the solvent was evaporated. Silica gel column chromatography ($CHCl_3$:CH_3OH = 100:1 − 10:1) of the residue afforded **2** (444 mg, 1.38 mmol) quantitatively as a pale yellow foam. ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.68 (br, 1H), 7.95 (s, 1H), 7.64 (d, J = 7.2 Hz, 1H), 7.45−7.38 (m, 10H), 6.15 (d, J = 7.1 Hz, 1H), 4.10 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 175.4, 165.9, 143.8, 141.5, 137.5, 129.4, 129.1, 128.0, 127.6, 117.5, 116.7. FT-IR (ATR): 1672, 1627, 1489, 1271, 1141, 851, 756, 699, 613, 580, 533 cm^{-1}. HRMS (ESI) m/z: [M + Na]$^+$ calcd for $C_{18}H_{14}N_2O_2SNa$ 345.0674, found 345.0692.

Fully Protected Mercaptopyridone-Bearing Nucleoside 3. To a solution of **2** (170 mg, 0.526 mmol) in CH_2Cl_2 (2.0 mL) were added diisopropylethylamine (94 μL, 0.540 mmol) and 1′-α-chrolo-3′,5′-di-*O*-toluoyl-2′-deoxyribose (243 mg, 0.626 mmol). As stirring at room temperature, the suspended solution gradually turned to a yellow clear solution. After 3.5 h, the reaction mixture was washed with H_2O (50 mL) and the aqueous layer was extracted with $CHCl_3$ (50 mL × 2). The combined organic phase was dried over anhydrous $MgSO_4$ and then concentrated. Silica gel column chromatography ($CHCl_3$:CH_3OH = 1:0 − 50:1) of the residue afforded β-anomer (**3**) (59.6 mg, 0.0735 mmol, 14%) as a colorless foam, while α-anomer (144 mg) and the mixture of both anomers (65.9 mg) were also obtained. The mixture was purified by further column chromatography to obtain β-anomer in 23% total yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.99 (d, J = 2.4 Hz, 1H), 7.92 (d, J = 8.2 Hz, 2H), 7.90 (d, J = 8.1 Hz, 2H), 7.53 (dd, J = 2.4, 7.8 Hz, 1H), 7.36−7.25 (m, 12H), 7.23 (d, J = 8.1 Hz, 2H), 6.39 (d, J = 7.8 Hz, 1H), 5.80 (dd, J = 5.4, 8.7 Hz, 1H), 5.62 (d, J = 6.1 Hz, 1H), 4.75 (dd, J = 3.3, 12.3 Hz, 1H), 4.66 (dd, J = 3.0, 12.3 Hz, 1H), 4.58 (dd, J = 3.0, 4.8 Hz, 1H), 2.72 (ddd, J = 1.3, 5.4, 14.3 Hz, 1H), 2.50 (ddd, J = 6.1, 8.7, 14.3 Hz, 1H), 2.44 (s, 3H), 2.36 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 176.8, 166.4, 166.1, 165.8, 144.8, 144.6, 142.3, 135.4, 130.1, 129.8, 129.5, 129.4, 129.2, 129.0, 127.3, 126.3, 126.1, 120.6, 118.4, 93.5, 83.5, 74.8, 64.1, 39.8, 21.8, 21.7. FT-IR (ATR): 1715, 1681, 1633, 1591, 1489, 1265, 1176, 1093, 830, 750, 694, 613 cm^{-1}. HRMS (ESI) m/z: [M + Na]$^+$ calcd for $C_{39}H_{34}N_2O_7SNa$ 697.1984, found 697.2009.

Protected Mercaptopyridone-Bearing Nucleoside 4. A solution of **3** (567 mg, 0.825 mmol) and K_2CO_3 (252 mg, 1.82 mmol) in a mixture of CH_2Cl_2 (5.0 mL) and CH_3OH (5.0 mL) was stirred at room temperature for 2 h. The reaction mixture was concentrated and then the residue was purified by silica gel chromatography ($CHCl_3$:CH_3OH = 50:1 − 5:1) to obtain **4** (313 mg, 0.715 mmol, 87%) as a colorless foam. ^{1}H NMR (500 MHz, CD_3OD): δ 8.42 (d, J = 2.3 Hz, 1H), 8.09 (dd, J = 2.3, 7.6 Hz, 1H), 7.46−7.33 (m, 10H), 6.50 (d, J = 7.6 Hz, 1H), 5.90 (dd, J = 6.5, 6.5 Hz, 1H), 4.47−4.44 (m, 1H), 4.00 (dd, J = 3.3, 6.4 Hz, 1H), 3.82 (dd, J = 3.2, 12.1 Hz, 1H), 3.75 (dd, J = 3.2, 12.1 Hz, 1H), 2.45−2.40 (m, 1H), 2.38−2.33 (m, 1H). ^{13}C NMR (125 MHz, CD_3OD): δ 179.8, 168.3, 146.4, 139.8, 130.4, 129.3, 129.1, 120.0, 118.4, 95.4, 89.9, 72.0, 62.6, 43.2. FT-IR (ATR): 1678, 1630, 1561, 1489, 1268, 1180, 1145, 1095, 1056, 1002, 958, 830, 761, 698, 615 cm^{-1}.

HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{23}H_{22}N_2O_5SNa$ 461.1147, found 461.1133.

Mercaptopyridone-Bearing Nucleoside (M). Compound **4** (46.7 mg, 0.106 mmol) and 1,4-dithio-D,L-threitol (DTT, 59.6 mg, 0.386 mmol) were dissolved in saturated aqueous ammonia solution (8.0 mL) and heated in a screw-capped tube at 90 °C for 24 h. The reaction mixture was concentrated and ammonia was removed in vacuo. The residue was purified by reverse-phase silica gel column chromatography (Wakogel 50C18, H_2O). The fraction containing the product was washed with AcOEt to remove DTT. The aqueous layer was lyophilized to afford **M** (8.3 mg, 0.034 mmol, 32%) and stored under nitrogen. Nucleoside **M** was stable under the condition of the titration experiments without degassing the solvents, while it was easily oxidized when heated. ^{1}H NMR (500 MHz, CD_3OD): δ 8.37 (d, J = 2.2 Hz, 1H), 8.07 (dd, J = 2.2, 7.2 Hz, 1H), 6.58 (d, J = 7.2 Hz, 1H), 5.94 (dd, J = 6.5, 6.5 Hz, 1H), 4.45 (m, 1H), 4.03 (m, 1H), 3.83 (dd, J = 3.2, 12.1 Hz, 1H), 3.76 (dd, J = 3.5, 12.1 Hz, 1H), 2.45−2.43 (m, 1H), 2.37−2.31 (m, 1H). ^{13}C NMR (125 MHz, CD_3OD): δ 175.0, 137.0, 136.0, 132.1, 111.9, 85.9, 90.1, 72.1, 62.7, 43.5. Anal. Calcd for $C_{10}H_{13}NO_4S \cdot H_2O$: C, 45.97; H, 5.79; N, 5.36. Found: C, 46.16; H, 5.66; N, 5.30.

Protected Hydroxypyridinethione-Bearing Nucleoside 6. Hydroxypyridone nucleoside **5** (2.87 g, 8.82 mmol) and P_4S_{10} (2.01 g, 4.52 mmol) were dissolved in CH_3CN (30 mL). To the suspension was added dropwise a solution of diisopropylethylamine (6.3 mL, 35 mmol) in CH_3CN (40 mL) keeping it cold. The resulting mixture was stirred for 4.5 h at room temperature followed by addition of H_2O (5 mL) with trapping generated H_2S gas. After evaporation of the solvent, the products were extracted with $CHCl_3$ (100 mL × 3) from H_2O (200 mL). The combined organic layer was dried over $MgSO_4$ and then concentrated in vacuo. Silica gel column chromatography ($CHCl_3$:CH_3OH = 100:1 − 10:1) afforded the title compound (2.31 g, 6.77 mmol, 77%) as a brown-yellow powder. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.73 (s, 1H), 7.63 (d, J = 7.1 Hz, 1H), 7.54 (d, J = 7.1 Hz, 1H), 6.10 (dd, J = 5.7, 7.7 Hz, 1H), 5.28 (ddd, J = 2.4, 2.5, 6.5 Hz, 1H), 4.42 (m, 1H), 4.40 (m, 2H), 2.63 (ddd, J = 2.5, 5.7, 14.3 Hz, 1H), 2.54 (s, 3H), 2.28 (m, 1H), 2.15 (s, 3H), 2.10 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.3, 170.2, 170.1, 153.5, 126.1, 124.9, 124.7, 89.4, 83.4, 73.8, 63.3, 39.9, 20.9, 20.8, 12.5. FT-IR (ATR): 1735, 1601, 1447, 1429, 1382, 1363, 1337, 1234, 1199, 1107, 1032, 954, 878, 856, 653, 621 cm^{-1}. HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{15}H_{19}NO_6SNa$ 364.0831, found: 364.0824.

Hydroxypyridinethione-Bearing Nucleoside (S). Compound **6** (0.16 g, 0.50 mmol) and K_2CO_3 (0.18 g, 1.3 mmol) were dissolved in CH_3OH (20 mL) and stirred at room temperature for 3 h. After removal of the solvent, silica gel column chromatography ($CHCl_3$:CH_3OH = 10:1 − 1:1) afforded the desired nucleoside **S** (0.12 g, 0.46 mmol, 94%) as a yellow powder. ^{1}H NMR (500 MHz, CD_3OD): δ 8.16 (d, J = 7.0 Hz, 1H), 7.44 (d, J = 7.0 Hz, 1H), 6.28 (dd, J = 6.1, 6.1 Hz, 1H), 4.44 (m, 1H), 4.04 (m, 1H), 3.87 (dd, J = 3.1, 12.2 Hz, 1H), 3.78 (dd, J = 3.7, 12.2 Hz, 1H), 2.56−2.53 (m, 1H), 2.54 (s, 3H), 2.28 (m, 1H). ^{13}C NMR (125 MHz, CD_3OD): δ 171.1, 154.3, 128.5, 128.1, 126.8, 91.5, 89.9, 71.3, 62.2, 43.2, 12.3. MS *m/z* calcd for $C_{11}H_{15}NO_4S$ ($[M + H]^+$): 258.08; found: 258.07. Anal. Calcd for $C_{11}H_{15}NO_4S$: C, 51.35; H, 5.88; N, 5.44. Found: C, 51.12; H, 5.94; N, 5.24.

Photometric Titration Experiments. The UV−vis spectra were recorded at 25 °C. Solutions for titrations contained 50 μM of the nucleoside **M** or 250 μM of **S**, and metal ions in 25 mM MOPS-Na buffer (pH = 7.0). Prior to the measurements, the samples containing Pd^{2+} or Pt^{2+} ions were allowed to stand at room temperature until the equilibrium was reached: approximately 30 min for Pd^{2+} and 24 h for Pt^{2+}.

ESI-TOF MS Measurements. The ESI-TOF mass spectra were recorded on positive modes. The sample solutions were prepared as follows: nucleoside (100 μM), K_2PdCl_4, $NiCl_2 \cdot 6H_2O$ or K_2PtCl_4 (50 μM), and $NaHCO_3$ (100 μM) in H_2O-CH_3CN (1:1) or H_2O-CH_3OH (1:1).

Acknowledgment. This work is supported by a Grant-in-Aid for Exploratory Research to K.T. (No. 17655058), Scientific Research (S) to M.S. (No. 16105001), and the Global COE Program for Chemistry Innovation from the Ministry of Education, Culture, Sports, Science and Technology (Japan), and Grant-in-Aid for JSPS Fellows to Y.T. (No. 11921) from Japan Society for the Promotion of Science (Japan).

Supporting Information Available: Characterization data including ^{1}H, ^{13}C NMR spectra, NOE data, crystallographic data, CIF files for **1** and **S**, UV absorption spectra of titration with other combination of nucleosides and metal ions, and comparative spectral data for competitive metal complexation. This material is available free of charge via the Internet at http://pubs.acs.org.

JO800587D