Synthesis of a Novel Nucleoside for Alternative DNA Base Pairing through Metal Complexation

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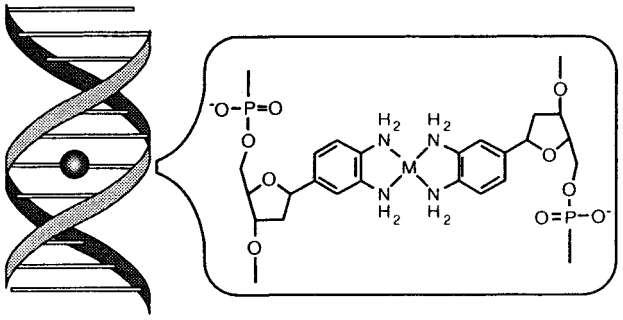
Chemical modification of nucleic acid constituents has gained more and more attention from the viewpoints of medicinal chemistry as well as material sciences. So far, a large number of nonnatural analogues of DNA nucleosides have been synthesized,[1] and in particular, it has been generally accepted that the incorporation of metal complexes into oligonucleotides is a key design target for the functionalization of DNA.[2] In recent years, a variety of oligonucleotides containing photoactive and redox-active metal complexes have been constructed for the development of energy and electron-transfer systems through DNA, DNA hybridization probes and sensors, and site-specific DNA cleavage.

In the DNA duplex formation, hydrogen bonding is one of the most principal driving forces as well as the main factor of sequence specificity in the hybridization. An alternative approach we have used for the incorporation of metal complexes into oligonucleotides is the more direct changing of a DNA base itself into a chelator-containing nucleobase. In this strategy, hydrogen-bonded base pairing is replaced by metal-assisted base pairing, thereby creating a novel binding motif in duplex DNA.[3] Such an approach would provide a wide range of applications based on its use as the third base pair along with the other two natural base pairs, AT and GC, and on the metal alignment through DNA duplex formation. The molecule we have chosen to synthesize and study is a β-C-nucleoside[4] having a phenylenediamine as the chelator-type base, **9**, which was expected to form a 2:1 square-planar complex with a metal ion such as Pd^{2+}, Pt^{2+}, Cu^{2+}, and Ni^{2+} (Chart 1). In addition, the metal-assisted base pair would have geometrical analogy to a natural base pair.

Chart 1



A scheme for the synthesis of the phenylenediamine β-C-nucleoside **9** is shown in Scheme 1. We first tried the coupling reaction of organocadmium species of the protected phenylenediamine derivatives with the well-known α-chlorosugar of Hoffer (2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride).[5] However, the preparation of organomagnesium species before transmetalation was not successful. Then as starting material for the synthesis of **9** the readily available ribonolactone, 2,3,5-tri-*O*-benzyl-D-ribonic γ-lactone,[6] and the STABASE (*N*-1,1,4,4-tetramethyldisililazacyclopentane) adduct of 4-bromo-*o*-phenylenediamine[7] **1** were used. The coupling and the conversion to deoxyribonucleoside followed the synthesis of C-nucleosides by Leumann et al.[8] It is well-known that this coupling reaction is not applicable to the 2′-deoxy analogue of ribonolactone due to enolization at C(2) under the conditions.[8b] Lithiation-substitution methodology was applicable to the stabase adduct of **1**. Treatment of this adduct with *n*BuLi at −78 °C and in situ reaction with ribonolactone and the subsequent benzoyl protecting of amino groups furnished a mixture of hemiacetal **2** in 36% yield in two steps. The reduction of **2** with excess of $Et_3SiH/BF_3{\cdot}Et_2O$ provided *only* the naturally configured β-epimer **3**. The anomeric configuration of **3** was determined in a close connection with the structural assignment for **8** as mentioned afterward. Debenzylation of **3** with BBr_3 provided the *N*-protected ribo-C-nucleoside **4** in 85% yield. Selective protection of the 3′- and 5′-hydroxyl groups with $TIPDSCl_2$ in pyridine gave **5** in 82% yield. Treatment of **5** with *p*-tolyl chlorothionoformate followed by homolytic reductive cleavage of the C−O bond with AIBN and nBu_3SnH afforded 2′-deoxy derivative **7** quantitatively. Desilylation of **7** with tetra-*n*-butylammonium fluoride provided the *N*-protected phenylenediamine 2′-deoxy-C-nucleoside **8**, which was then converted into the free C-nucleoside **9** by treatment with aqueous NaOH.[9]

The anomeric configuration for **8** was determined by ^1H NOE experiments and by examination of coupling constants

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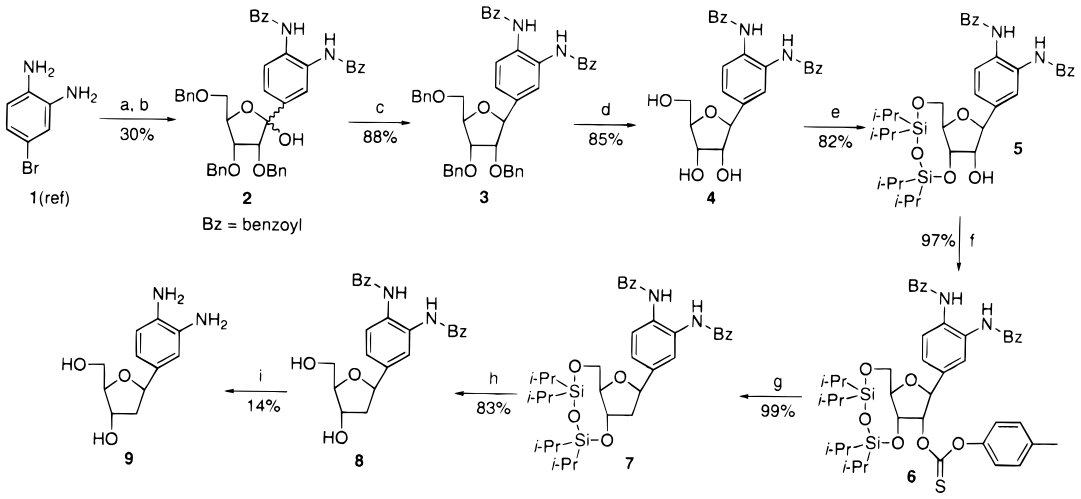
Scheme 1[a]



[a] Reagents and conditions: (a) 1,2-bis(chlorodimethylsilyl)ethane, DBU, DMF, 120 °C, 82%; (b) (i) *n*BuLi, 2,3,5-tri-*O*-benzyl-D-ribonic γ-lactone, THF, −78 °C, 0 °C; (ii) benzoyl chloride, triethylamine, CH_2Cl_2, 0 °C, rt, 36% (two steps); (c) triethylsilane, $BF_3{\cdot}OEt_2$, CH_2Cl_2, −78 °C, rt, 88%; (d) BBr_3, CH_2Cl_2, −78 °C, rt, 85%; (e) $TIPDSCl_2$, pyridine, 0 °C, rt, 82%; (f) *O*-*p*-tolyl chlorothionoformate, DMAP, MeCN, rt, 97%; (g) AIBN, nBu_3SnH, toluene, 80 °C, 99%; (h) nBu_4NF, THF, rt, 83%; (i) NaOH, H_2O, 80 °C, 14%.

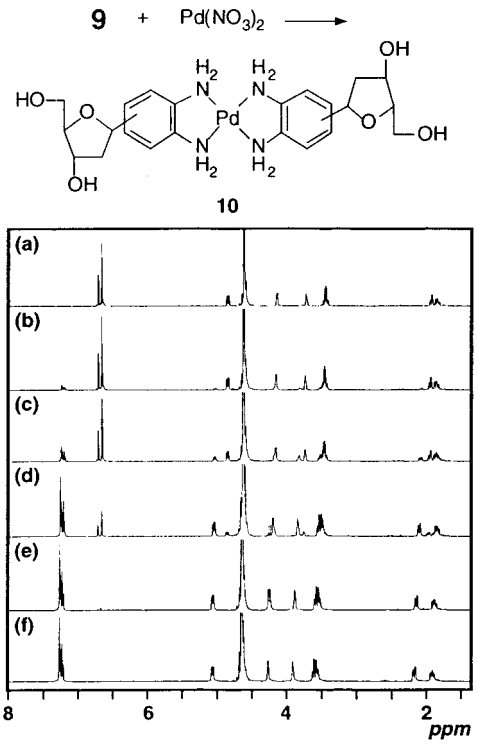


Figure 1. 500 MHz ^{1}H NMR spectra of nucleoside **9** with increasing amounts of Pd^{2+}. [**1**] = 26 mM in D_2O. [Pd^{2+}]/[**9**] = (a) 0.00, (b) 0.08, (c) 0.19, (d) 0.38, (e) 0.49, (f) 0.57.

for 1′- and 2′-protons as done by Kool et al.[1f] In β-anomers, the 2′α-proton is only near the 1′-proton. When the 1′-proton was irradiated, we observed a 4% enhancement at the 2′α-proton. The epimer **8** had a 1′-resonance which appeared as a nearly evenly spaced doublet of doublets (J = 6.4 and 10.7 Hz). This 1′−2′ coupling constant trend is consistent with similar coupling constants reported for related β-C-nucleosides.[1f,10]

Complex formation between nucleoside **9** and Pd^{2+} in D_2O was followed by ^{1}H NMR spectroscopy (Figure 1). Proton

(10) The product should be carefully handled due to its instability in solution under aerobic conditions. Indeed, nucleoside **9** was used directly for the subsequent metal complexation study after rapid purification.

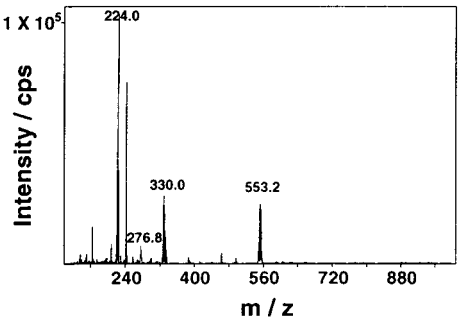


Figure 2. ESI mass spectrum of Pd^{2+} complex **10**: 553.2 ([ML_2 − H^+]$^+$ calcd 553.13), 330.0 ([ML]$^+$ calcd 330.02), 276.8 ([ML_2]$^{2+}$ calcd 277.07), 224.0 ([L]$^+$ calcd 224.12), where M = Pd^{2+}, L = nucleoside **9**.

resonances in the aromatic region as well as those in the ribose moiety shifted to lower field almost in proportion to increasing concentration of Pd^{2+}, and when the concentration of Pd^{2+} reached half the concentration for **9** the complexation was completed. This result shows that **9** and Pd^{2+} form a stable 2:1 complex **10** with a high binding constant. Although there are two possible structures (cis and trans) for complex **10**, we observed only one species in the NMR spectra. The electrospray ionization (ESI) mass spectrum also provided clear evidence for the 2:1 complexation (Figure 2).

The present work demonstrates a convenient synthesis of a β-C-nucleoside-containing phenylenediamine as a "chelator-base" moiety, providing an alternative DNA base pairing through metal complexation. The site-specific incorporation of the newly synthesized nucleoside into oligonucleotides is now underway.

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Supporting Information Available: Synthetic procedures and analytical data for selected new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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