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An approach to metal-assisted DNA base pairing: novel β-*C*-nucleosides with a 2-aminophenol or a catechol as the nucleobase

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Abstract

The metal-chelating β-*C*-nucleoside having a phenylenediamine moiety as the nucleobase was previously found to form a stable 2:1 complex with a Pd^{2+} ion in aqueous media, where hydrogen bonding is replaced by metal coordination in the base pairing, thereby creating a novel hybridization motif in duplex DNA. In this regard, we have further designed two types of artificial β-*C*-nucleosides possessing a metal-chelating site (a 2-aminophenol or a catechol) as the nucleobase moiety. These artificial nucleosides are directed toward controlling the net charges of the metal-assisted base pairs. This paper describes convenient syntheses of the artificial nucleosides bearing a 2-aminophenol or a catechol moiety. Each nucleoside was directly synthesized through 2′-deoxy derivative via a Friedel–Crafts coupling reaction as the key step between the aromatic ring and ribose moiety, whereas the nucleoside having a phenylenediamine moiety was prepared in rather longer steps through an RNA type intermediate followed by the removal of 2′-hydroxyl group.

Keywords: Artificial DNA; Metal-chelating nucleoside; Nucleoside synthesis

1. Introduction

Hydrogen-bonding plays central roles in the high selective recognition in the DNA base pair identification. Geometrical modification of the Watson–Crick pairing mode will lead not only to a great understanding of the molecular programming language developed by nature, but also to new functional molecular architectures in the fields of biosciences and material sciences. In recent years, a large number of nonnatural DNA nucleosides have been synthesized (Lesser et al., 1990; Kornberg and Baker, 1992; SantaLucia et al., 1992; Smith et al., 1994; Schweizer and Kool, 1995; Ren et al., 1996; Hildbrand et al., 1997), and it has been generally accepted that the incorporation of metal complexes into DNA is a useful tool for the functionalization of DNA (Dreyer and Dervan, 1985; Chen and Sigman, 1988; Telser et al., 1989; Bannwarth et al., 1991; Murphy et al., 1993; Bashkin et al., 1994; Matsumura et al., 1994; Magda et al., 1994; Meade and Kayyem, 1995; Manchanda et al., 1996; Schliepe et al., 1996; Mucic et al., 1996; Dandliker et al., 1997; Meggers et al., 1997; Ihara et al., 1997; Magda et al., 1997; Hurley and Tor, 1998). Thus far, a variety of oligonucleotides containing photo- and redox-active transition metal complexes have been developed for energy and electron-transfer systems through DNA, probing systems for DNA hybridization, and site-specific DNA cleavage. An alternative approach we have used for the incorporation of metal ions into DNA is the more direct modification of a DNA base itself into a metal-chelating nucleobase. In this artificial DNA, hydrogen-bonded base pairing is replaced by metal-induced base pairing thereby creating a novel hybridization motif in double-stranded DNA (Fig. 1). Such an approach would provide a wide range of applications based on its use as the alternative base pairs along with the canonical base pairs, AT and GC.

We have previously found that the metal-chelating nucleoside **1** having a phenylenediamine moiety forms a stable 2:1 square-planar complex with a Pd^{2+} ion in aqueous media, where hydrogen bonding is replaced by metal coordination in the base pairing (Scheme 1) (Tanaka et al., 1998; Tanaka and Shionoya, 1999). In this regard, we have further designed two types of artificial β-*C*-nucleosides, **2** and **3**, possessing a metal-chelating site (a 2-aminophenol and a catechol, respectively) as the nu-

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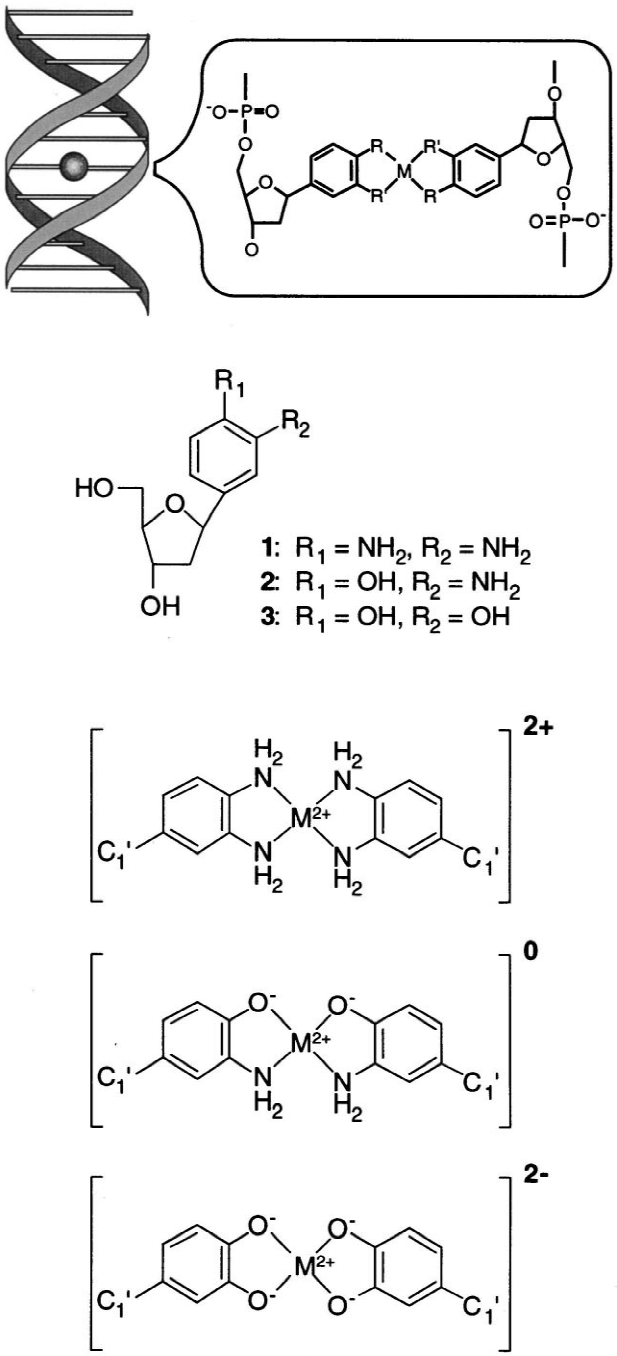
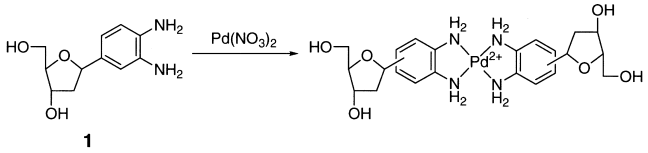


Fig. 1.

cleobase moiety. These artificial nucleosides are directed toward controlling the net charges of the metal-assisted base pairs (Fig. 1). When these nucleosides form a 2:1 complex with a divalent metal ion, the complexes of **2** and **3** have no charge and negative double charge, respectively, and therefore could possibly be incorporated into oligonucleotides at the adjacent positions. These nucleosides are expected to form metal-assisted base pairs in DNA and thereby to create novel structures and functions of DNA. Herein we present convenient synthetic routes for **2** and **3**.



Scheme 1.

2. Materials and methods

2.1. General

Unless otherwise noted, all reactions were carried out in oven dried glassware under an argon atmosphere with commercial dehydrated solvents (Wako). 1-*O*-Methyl-2-deoxy-D-ribofuranose and 1-*O*-methyl-3,5-*O*-ditoluoyl-2-deoxy-D-ribofuranose were prepared according to previously reported procedures (Hoffer, 1960). 2-Aminophenol and trifluoroacetic anhydride were obtained from TCI and *tert*-butyldimethylsilyl chloride was obtained from Shin-Etsu. nBu$_4$NF in THF (1 M) was purchased from Aldrich. All the other reagents were purchased from Wako and were used without further purification. Column chromatography was performed using Wakogel C-300 silica gel (Wako). ^{1}H and ^{13}C NMR spectra were recorded on a JEOL Lambda 500 or a JEOL Alpha 500 spectrometer (500 MHz for ^{1}H; 125.65 MHz for ^{13}C). The spectra are referenced to tetramethylsilane. Chemical shifts (δ) are reported in ppm; multiplicities are indicated by: s (singlet), d (doublet), dd (doublet of doublets), ddd (doublet of doublets of doublets), q (quartet), m (multiplet), and br (broad). Coupling constants, *J*, are reported in Hz. Electron ionization mass spectra were obtained with a Shimadzu QP1000EX spectrometer (low resolution, 70 eV), and fast atom bombardment (FAB) mass and elemental analyses were carried out at the Research Center for Molecular Materials, the Institute for Molecular Science. Electrospray ionization (ESI) mass spectra were recorded on a PE SCIEX API-300 spectrometer.

2.2. 2-Trifluoroacetamidephenol (*4*)

To a solution of 2-aminophenol (17.6 g, 161 mmol) and dry pyridine (13.7 ml, 169 mmol) in CH_2Cl_2 (350 ml) was added a solution of trifluoroacetic anhydride (22.6 ml, 164 mmol) in CH_2Cl_2 (90 ml) over 10 min at 0°C. The reaction mixture was stirred for 100 min at 0°C. After the solvent was evaporated, the residue was dissolved in a 1% HCl aqueous solution (250 ml), which was extracted with three portions of AcOEt. The combined organic layer was washed with brine. The aqueous layer was further extracted with two portions of AcOEt. The combined organic phase was dried over anhydrous $MgSO_4$, followed by concentration. The resulting solid was recrystallized from toluene to give 25.1 g (76%) of **4** as colorless thin plates,

which were sublimed at 80°C. ^{1}H NMR (DMSO-d_6): δ 6.83 (ddd, 1H, J=7.6, 7.6, 1.4 Hz), 6.94 (dd, 1H, J=8.1, 1.2 Hz), 7.14 (ddd, 1H, J=8.1, 7.5, 1.6 Hz), 7.32 (dd, 1H, J=7.8, 1.5 Hz), 9.97 (br, 1H, D_2O exchangeable), 10.42 (br, 1H, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 116.0 (q, J_{C-F}=288 Hz, CF_3), 116.0, 118.9, 122.1, 126.3, 127.9, 151.2, 154.9 (q, J^2_{C-F}=36.2 Hz, CO). LRMS (70 eV, EI) m/z (relative intensity, proposed ion): 205 (71.4, M^+), 136 (100.0, M^+-CF_3), 108 (50.4, M^+-CF_3CO).

2.3. O-Benzyl-2-trifluoroacetamidephenol (5)

To a solution of 2-trifluoroacetamidephenol **4** (47.7 g, 233 mmol) and iPr$_2$EtN (60 ml, 350 mmol) in dry 1,2-dichloroethane (470 ml) was added benzyl chloride (40 ml, 348 mmol) at room temperature. The reaction mixture was stirred for 30 min at 55°C, and then heated at reflux for 15.5 h. After cooling down to room temperature, iPr$_2$EtN (60 ml) and benzyl chloride (40 ml) were added to the solution. After refluxing for 4 h, the solution was poured into a 2% HCl aqueous solution (1.23 l). The organic layer was separated, and then the aqueous layer was extracted with CH_2Cl_2. The organic layer was washed with brine, and then the aqueous layer separated was further extracted with two portions of CH_2Cl_2. The combined organic extracts were dried over anhydrous $MgSO_4$ and the solvent was concentrated. The residue was purified by silica gel column chromatography (9ϕ×24 cm) with AcOEt-n-hexane (3:97), followed by recrystallization from n-hexane to afford 33.9 g (49%) of **5** as colorless needles, mp 76.0–76.5°C. ^{1}H NMR ($CDCl_3$): δ 5.17 (s, 2H), 7.01 (dd, 1H, J=8.3, 1.5 Hz), 7.03 (ddd, 1H, J=7.8, 7.8, 1.5 Hz), 7.15 (ddd, 1H, J=7.9, 7.9, 1.7 Hz), 7.36–7.43 (m, 5H), 8.32 (dd, 1H, J=8.3, 1.8 Hz), 8.64 (br, 1H, D_2O exchangeable). ^{13}C NMR ($CDCl_3$): δ 71.3, 112.2, 115.7 (q, J_{C-F}=288 Hz, CF_3), 120.3, 121.7, 125.5, 126.0, 127.3, 128.5, 128.9, 135.8, 147.5, 154.3 (q, J^2_{C-F}=37.2 Hz, CO). LRMS (70 eV, EI) m/z (relative intensity, proposed ion): 295 (100.0, M^+), 204 (9.7, $M^+-CH_2C_6H_5$), 135 (48.4, $M^+-CH_2C_6H_5-CF_3$).

2.4. O-Benzyl-2-trifluoroacetamide-4-(1,2-dideoxy-3,5-O-ditoluoyl-β-D-ribofuranos-1-yl)-phenol (6)

A solution of 1-*O*-methyl-3,5-*O*-ditoluoyl-2-deoxy-D-ribofuranose (520 mg, 1.35 mmol) and *O*-benzyl-2-trifluoroacetamidephenol **5** (403 mg, 1.36 mmol) in CH_2Cl_2 (2 ml) in the presence of $SnCl_4$ (310 μl, 2.65 mmol) was stirred for 30 min at 0°C. The reaction mixture was quenched by saturated $NaHCO_3$ aqueous solution (10 ml) and the resulting precipitates were removed through a thin pad of Celite. The organic filtrate was washed with brine, dried over anhydrous $MgSO_4$, and then concentrated. The residue was chromatographed (2.4ϕ×14 cm) on silica gel with AcOEt-n-hexane (1:9) to give 134 mg (15%) of **6** and 13 mg (1.5%) of **7**. β-anomer **6**: colorless solid, mp 127.5–128.0°C. ^{1}H NMR ($CDCl_3$): δ 2.21 (ddd, 1H, J=13.8, 11.0, 6.3 Hz), 2.40 (s, 3H), 2.44 (s, 3H), 2.53 (ddd, 1H, J=13.9, 5.1, 0.6 Hz), 4.51 (ddd, 1H, J=4.0, 4.0, 2.0 Hz), 4.63 (dd, 1H, J=11.8, 3.8 Hz), 4.66 (dd, 1H, J=12.0, 4.0 Hz), 5.15 (s, 2H), 5.22 (dd, 1H, J=10.5, 5.0 Hz), 5.59 (dd, 1H, J=5.3, 1.3 Hz), 6.96 (d, 1H, J=8.5 Hz), 7.21 (d, 2H, J=7.5 Hz), 7.24–7.29 (m, overlapped with residual $CHCl_3$) 7.35–7.43 (m, 5H), 7.94 (d, 2H, J=8.5 Hz), 7.98 (d, 2H, J=8.0 Hz), 8.36 (d, 1H, J=2.0 Hz), 8.61 (br, 1H, D_2O exchangeable). ^{13}C NMR ($CDCl_3$): δ 21.7, 21.7, 41.6, 65.0, 71.4, 77.1, 80.3, 83.0, 112.4, 115.6 (q, J_{C-F}=288 Hz, CF_3), 118.4, 123.2, 125.3, 126.9, 127.1, 127.3, 128.6, 128.9, 129.1, 129.2, 129.8, 134.1, 135.7, 143.7, 144.1, 147.1, 154.3 (q, J^2_{C-F}=37.2 Hz, CO), 166.1, 166.4. FAB mass (positive): m/z, proposed ion: 648, $[M+H]^+$. α-anomer **7**: colorless solid, mp 127.0–128.5°C. ^{1}H NMR ($CDCl_3$): δ 2.29 (ddd, 1H, J=13.7, 6.4, 4.2 Hz), 2.39 (s, 3H), 2.41 (s, 3H), 2.95 (ddd, 1H, J=14.0, 7.0, 7.0 Hz), 4.54 (dd, 1H, J=11.8, 4.4 Hz), 4.58 (dd, 1H, J=11.7, 4.9 Hz), 4.70 (br, 1H), 5.16 (s, 2H), 5.31 (dd, 1H, J=6.8, 6.8 Hz), 5.59 (ddd, 1H, J=6.9, 4.0, 3.1 Hz), 6.68 (d, 1H, J=8.5 Hz), 7.17 (d, 2H, J=7.9 Hz), 7.22–7.25 (m, 3H), 7.35–7.43 (m, 5H), 7.79 (d, 2H, J=8.1 Hz), 7.96 (d, 2H, J=8.1 Hz), 8.42 (d, 1H, J=2.2 Hz), 8.62 (br, 1H, D_2O exchangeable).

2.5. O-Benzyl-2-trifluoroacetamide-4-(1,2-dideoxy-β-D-ribofuranos-1-yl)-phenol (8)

To a solution of **6** (2.00 g, 3.09 mmol) in dry MeOH (47 ml) was added in one portion a solution of 28% MeONa in MeOH (1.65 ml, 8.55 mmol) at room temperature. The reaction mixture was stirred for 2.2 h at room temperature. After adding Dowex 50W×8 (pyridinium form) (31.7 g), the mixture was further stirred for 2 min. After removing the resin, the filtrates were combined and then concentrated. The resulting oil was purified by silica gel column chromatography (3ϕ×10.5 cm) with MeOH–CH_2Cl_2 (5:95) to afford 1.21 g (95%) of **8** as colorless solid, mp 126.5°C. ^{1}H NMR ($CDCl_3$): δ 1.85 (d, 1H, J=4.2 Hz, D_2O exchangeable), 1.98 (dd, 1H, J=6.2, 6.2 Hz, D_2O exchangeable), 2.06 (ddd, 1H, J=13.3, 10.0, 6.2 Hz), 2.26 (ddd, 1H, J=13.3, 5.7, 2.1 Hz), 3.77 (ddd, 1H, J=11.6, 6.6, 4.9 Hz), 3.86 (ddd, 1H, J=11.8, 5.7, 4.2 Hz), 4.02 (ddd, 1H, J=4.3, 4.3, 3.1 Hz), 4.47 (ddd, 1H, J=8.9, 3.4, 2.7 Hz), 5.16 (dd, 1H, J=10.3, 5.9 Hz), 5.17 (s, 2H), 6.97 (d, 1H, J=8.3 Hz), 7.14 (dd, 1H, J=8.5, 2.2 Hz), 7.37–7.42 (m, 5H), 8.38 (d, 1H, J=2.0 Hz), 8.64 (s, 1H, D_2O exchangeable).

2.6. 2-Amino-4-(1,2-dideoxy-β-D-ribofuranos-1-yl)-phenol (2)

A solution of **8** (132 mg, 0.321 mmol) in 40% (w/v) $MeNH_2$–MeOH (0.64 ml, 8.24 mmol) was stirred for 11 h.

The solvent was evaporated in vacuo to give 105 mg of colorless solid. Without further purification,[1] a solution of the solid (25 mg) in MeOH (8 ml) in the presence of 10% Pd–C (34 mg) was stirred for 2 h at room temperature under a hydrogen atmosphere. The Pd–C was filtered off, and washed with MeOH repeatedly. The filtrate was evaporated in vacuo to give colorless syrup, which was then chromatographed on silica gel (1.6ϕ×10 cm) with MeOH–CH_2Cl_2 (16:84) to give 2.8 mg (16% from **8**) of **2** as a colorless oil. ^{1}H NMR (DMSO-d_6): δ 1.72 (ddd, 1H, J=12.7, 10.6, 5.7 Hz), 1.92 (ddd, 1H, J=12.8, 5.3, 1.3 Hz), 3.45 (m, 1H), 3.69 (ddd, 1H, J=6.1, 5.4, 2.2 Hz), 4.12 (m, 1H), 4.44 (br, 2H, D_2O exchangeable), 4.70 (dd, 1H, J=5.6, 5.6 Hz, D_2O exchangeable), 4.76 (dd, 1H, J=10.5, 5.1 Hz), 4.97 (d, 1H, J=3.9 Hz, D_2O exchangeable), 6.37 (dd, 1H, J=8.1, 2.2 Hz), 6.55 (d, 1H, J=8.1 Hz), 6.57 (d, 1H, J=2.2 Hz), 8.90 (br, 1H, D_2O exchangeable). The signal of 5′-H overlapped with that of H_2O in DMSO-d_6. ESI mass (MeOH, positive): m/z, proposed ion: 226.2, $[M+H]^+$.

2.7. O,O′-Bis(tert-butyldimethylsilyl)catechol (**10**)

To a solution of *tert*-butyldimethylsilyl chloride (9.7 g, 64 mmol) in pyridine (10 ml) was added catechol (3.54 g, 32.2 mmol). After stirring for 2 days at 80°C, the reaction mixture was poured into ice-cold water (500 ml). The solution was extracted with three portions of AcOEt, and the combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography with *n*-hexane-triethylamine (100:1) to give 9.11 g (84%) of **10** as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 0.19 (s, 12H), 0.99 (s, 18H), 6.81 (m, 4H).

2.8. O,O′-Bis(tert-butyldimethylsilyl)-4-[1,2-dideoxy-3,5-O,O′-bis(ethoxycarbonyl)-α- and -β-D-ribofuranos-1-yl]catechol (**12**: β, 13: α)

To a solution of 1-*O*-methyl-2-deoxy-D-ribofuranose (Hoffer, 1960) (9.6 g, 65 mmol) and ethyl chloroformate (12.4 ml, 130 mmol) in dry CH_2Cl_2 (70 ml) was added pyridine (10.5 ml, 130 mmol) at room temperature. After stirring for 1.5 h, ethyl chloroformate (12.4 ml, 130 mmol) and pyridine (10.5 ml 129.8 mmol) were added to the reaction mixture, which was further stirred for 40 min at room temperature. The reaction mixture was diluted with CH_2Cl_2 (200 ml) and washed with three portions of 2% H_2SO_4 aqueous solution and brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography with *n*-hexane–diethyl ether (5:2) to afford 16.3 g (78%) of **11** as a colorless oil.

1-α-,β-*O*-Methyl-2-deoxy-3,5-di-*O*-(ethoxycarbonyl)-D-ribofuranose (**11**) (20.0 g, 68.4 mmol) and *O,O′*-bis(*tert*-butyldimethylsilyl)catechol (**10**) (20.6 g, 60.8 mmol) were dissolved in dry CH_2Cl_2 (80 ml), and $SnCl_4$ (13.7 ml, 127.0 mmol) in CH_2Cl_2 (130 ml) was added dropwise to the solution within 10 min at 0°C. After stirring for 15 min at 0°C, the reaction was quenched by saturated $NaHCO_3$ aqueous solution (1 l), and the solution was extracted with four portions of CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography with *n*-hexane–diethyl ether (8:1) to afford 13.2 g (36%) of **12** (β-anomer) as a pale yellow oil and 3.0 g (8.2%) of **13** (α-anomer) as a pale yellow oil. ^{1}H NMR ($CDCl_3$): δ β-anomer **12**: 0.18 (br, 12H), 0.98 (s, 18H), 1.31 (t, 3H, J=7.2 Hz), 1.34 (t, 3H, J=7.2 Hz), 2.05 (ddd, 1H, J=14.0, 11.2, 6.1 Hz), 2.32–2.35 (m, 1H), 4.18–4.28 (m, 5H), 4.36 (dd, 1H, J=11.4, 3.8 Hz), 4.40 (dd, 1H, J=11.5, 4.2 Hz), 4.98 (dd, 1H, J=11.2, 4.6 Hz), 5.16–5.18 (m, 1H), 6.78 (d, 1H, J=8.1 Hz), 6.80 (dd, 1H, J=8.2, 1.8 Hz), 6.84 (d, 1H, J=1.7 Hz); α-anomer: δ 0.18 (s, 3H), 0.18 (s, 3H), 0.19 (s, 3H), 0.19 (s, 3 H), 0.97 (s, 9 H), 0.98 (s, 9 H), 1.30 (t, 3H, J=7.5 Hz), 1.32 (t, 3H, J=7.3 Hz), 2.10 (ddd, 1H, J=13.5, 8.0, 5.3 Hz), 2.79 (ddd, 1H, J=13.8, 7.0, 7.0 Hz), 4.19 (q, 2H, J=7.2 Hz), 4.22 (q, 2H, J=7.2 Hz), 4.32 (dd, 1H, J=11.5, 5.2 Hz), 4.35 (dd, 1H, J=11.3, 3.7 Hz), 4.40–4.43 (m, 1H), 5.04 (dd, 1H, J=7.5, 7.5 Hz), 5.18 (ddd, 1H, J=7.4, 5.3, 3.8 Hz), 6.78 (d, 1H, J=8.3 Hz), 6.81 (dd, 1H, J=8.3, 2.0 Hz), 6.85 (d, 1H, J=2.0 Hz).

2.9. O,O′-Bis(tert-butyldimethylsilyl)-4-(1,2-dideoxy-β-D-ribofuranos-1-yl)catechol (**14**)

O,O′-Bis(*tert*-butyldimethylsilyl)-4-[1,2-dideoxy-3,5-*O,O′*-bis(ethoxycarbonyl)-β-D-ribofuranos-1-yl]catechol (**12**) (2.57 g 4.30 mmol) was treated with 1% (w/v) K_2CO_3 in MeOH (25 ml) for 50 min at room temperature. The mixture was neutralized with aqueous acetic acid solution and then concentrated. The residue was dissolved in CH_2Cl_2 (200 ml) and washed with water, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography with $CHCl_3$–MeOH (20:1) to afford 1.64 g (84%) of **14** as a pale yellow oil. ^{1}H NMR ($CDCl_3$): δ 0.18–0.19 (m, 12H), 0.98 (s, 9H), 0.98 (s, 9H), 1.88 (d, 1H, J=3.7 Hz), 1.91 (d, 1H, J=6.2 Hz), 2.01 (ddd, 1H, J=13.3, 10.3, 6.2 Hz), 2.20 (ddd, 1H, J=13.4, 5.6, 2.0 Hz), 3.72 (ddd, 1H, J=11.6, 5.7, 5.7 Hz), 3.81 (ddd, 1H, J=11.4, 4.8, 4.8 Hz), 3.99 (ddd, 1H, J=4.5, 4.5, 3.0 Hz), 4.42–4.43 (br, 1H), 5.06 (dd, 1H, J=10.3, 5.6 Hz), 6.82–6.79 (m, 3H).

[1] After purification by silica gel column chromatography (1.8ϕ×8.5 cm) with MeOH–CH_2Cl_2 (1:9), **9** was obtained as colorless solid in 90% yield, mp 121.5–123.5°C. ^{1}H NMR ($CDCl_3$): δ 1.82 (d, 1H, J=4.0 Hz, D_2O exchangeable), 1.92 (dd, 1H, J=6.0, 6.0 Hz, D_2O exchangeable), 2.05 (ddd, 1H, J=13.4, 10.1, 6.4 Hz), 2.19 (ddd, 1H, J=13.3, 5.5, 2.3 Hz), 3.73 (ddd, 1H, J=11.5, 6.5, 5.3 Hz), 3.83 (ddd, 1H, J=11.8, 4.5, 4.5 Hz), 3.87 (br, 2H, D_2O exchangeable), 3.98 (ddd, 1H, J=4.8, 4.0, 3.3 Hz), 4.43 (br, 1H), 5.06 (dd, 1H, J=10.0, 5.5 Hz), 5.08 (s, 2H), 6.68 (dd, 1H, J=8.5, 2.0 Hz), 6.74 (d, 1H, J=2.0 Hz), 6.82 (d, 1H, J=8.0 Hz), 7.33 (m, 1H), 7.38 (m, 2H), 7.43 (m, 2H).

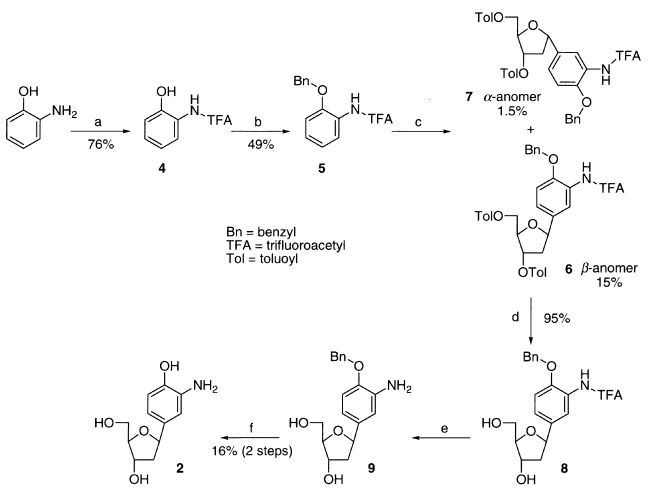
2.10. 4-(1,2-Dideoxy-β-D-ribofuranos-1-yl)catechol (3)

nBu_4NF in THF (1 M, 1.14 ml, 1.14 mmol) was added dropwise to a solution of *O,O′*-bis(*tert*-butyldimethylsilyl)-4-(1,2-dideoxy-β-D-ribofuranos-1-yl)catechol (**14**) (0.26 g, 0.57 mmol) in THF (6 ml). After stirring for 10 min, a 5% $NaHCO_3$ aqueous solution (2.6 ml) was added to the reaction mixture, which was then concentrated. The residue was purified by silica gel column chromatography with $CHCl_3$–MeOH (4:1) to give 0.13 g (99%) of **3** as colorless form. After recrystallization from acetonitrile, colorless needles were obtained, mp 182.0–184.0°C (dec.). ^{1}H NMR (CD_3OD): δ 1.93 (ddd, 1H, J=13.2, 10.6, 6.0 Hz), 2.08 (ddd, 1H, J=13.2, 5.3, 1.5 Hz), 3.61 (dd, 1H, J=11.7, 5.4 Hz), 3.65 (dd, 1H, J=11.7, 5.1 Hz), 3.88 (ddd, 1H, J=5.3, 5.3, 2.3 Hz), 4.29 (ddd, 1H, J=5.7, 1.8, 1.8 Hz), 4.96 (dd, 1H, J=10.5, 5.4 Hz), 6.69 (dd, 1H, J=8.1, 1.7 Hz), 6.71 (d, 1H, J=8.1 Hz), 6.81 (d, 1H, J=1.7 Hz).

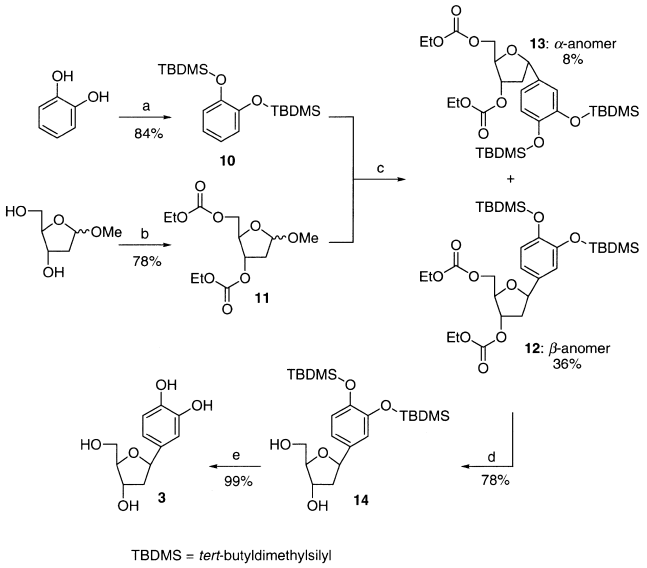
3. Results and discussion

Synthetic routes for the 2-aminophenol β-*C*-nucleoside **2** and the catechol β-*C*-nucleoside **3** are shown in Schemes 2 and 3, respectively. Each nucleoside was efficiently synthesized via a Friedel–Crafts coupling reaction as the key step between the aromatic ring and ribose moiety, whereas the phenylenediamine β-*C*-nucleoside **1** was prepared in rather longer steps through an RNA type intermediate followed by the removal of 2′-hydroxyl group (Tanaka and Shionoya, 1999).

The most common method for carbon–carbon bond formation at the anomeric carbon involves nucleophilic attack on this naturally electrophilic center (for reviews of



Scheme 2. Reagents and conditions: (a) trifluoroacetic anhydride, pyridine, CH_2Cl_2, 0°C, 76%; (b) benzyl chloride, *i*Pr_2EtN, 1,2-dichloroethane, reflux, 49%; (c) 1-*O*-methyl-3,5-*O*-ditoluoyl-2-deoxy-D-ribofuranose, $SnCl_4$, CH_2Cl_2, 0°C, 15% (β-anomer), 1.5% (α-anomer); (d) MeONa, MeOH, rt, 95%; (e) $MeNH_2$, MeOH, rt; (f) H_2, Pd–C, MeOH, rt, 16% (two steps).



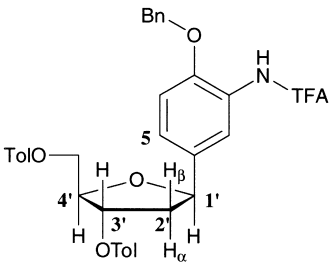
Scheme 3. Reagents and conditions: (a) *tert*-butyldimethylsilyl chloride, pyridine, 80°C, 84%; (b) ethyl chloroformate, pyridine, CH_2Cl_2, rt, 78%; (c) $SnCl_4$, CH_2Cl_2, 0°C, 36% (β-anomer), 8% (α-anomer); (d) K_2CO_3, MeOH, rt, 78%; (e) *n*Bu_4NF, THF, rt, 99%.

C-Glycoside synthesis, see Levy and Tang, 1995; Postema, 1995; Jaramillo and Knapp, 1994). The Friedel–Crafts approach proceeding via electrophilic aromatic substitution was used to build up the carbon skeletons of the nucleosides **2** and **3**, where $SnCl_4$ was used as the Lewis acid promotor. In the synthesis of nucleoside **2**, *O*-benzyl-2-trifluoroacetamidephenol **5** was used as the aromatic nucleophile, which was prepared from 2-aminophenol in two steps. The reaction of **5** with 3,5-protected 2-deoxy-D-ribofuranose bearing a methoxy group at the anomeric position was examined at 0°C in CH_2Cl_2 in the presence of $SnCl_4$, and the β-*C*-nucleoside **6** was found to be produced in good selectivity (α-**7**:β-**6**=1:10), albeit in low yield. Stereochemistry of these isomers was determined by the comparison of their ^{1}H NMR spectra. The anomeric configuration for **6** was determined by ^{1}H NOE differentiation experiments (Table 1) and by examination of coupling constants for 1′- and 2′-protons as done by Kool et al. (1996). In β-anomers, the 2′Hα is only near the 1′-proton. When the 1′Hα and 2′Hα were separately irradiated, we observed 4.2 and 10% enhancements at the 2′α- and 1′-protons, respectively. The epimer **6** had a 1′-resonance which appeared as nearly evenly spaced doublet of doublets (J=10.5, 5.0 Hz). This 1′–2′ coupling constant trend is consistent with similar coupling constants reported for related β-*C*-nucleosides (Ren et al., 1996). These results clearly demonstrated that the aryl nucleoside **6** is a β-isomer. Removal of toluoyl groups of **6** with MeONa in MeOH provided **8** quantitatively, which was then converted into the free *C*-nucleoside **2** by treatment with $MeNH_2$ in MeOH and subsequently Pd–C under a hydrogen atmosphere.

As for the synthesis of the catechol nucleoside **3**, silyl

Table 1
Proton NOE differentiation data for a β-isomer of aryl nucleoside **6** in $CDCl_3$

	Irradiation at					
	H1′	H2′α	H2′β	H3′	H4′	H5
NOE observed:						
H1′	–	10	0	0	2.0	4.1
H2′α	4.2	–	–	0	0	0
H2′β	0	–	–	3.0	0	2.0
H3′	0	0	8.0	–	3.2	0
H4′	2.5	0	0	3.8	–	0
H5	5.1	0	2.9	0	0	–



6

protected catechol **10** was employed for the aromatic moiety in the Friedel–Crafts coupling reaction with 1-*O*-methyl-3,5-protected 2-deoxy-D-ribofuranose **11** to afford fully protected nucleosides **12** (36%) and **13** (8%). The anomeric configurations for these compounds, **12** and **13**, were assigned to be β and α, respectively, from the proton NMR coupling constants between H-1′ and H-2′ positions. Epimer **12** showed two distinct H-1′ to H-2′ coupling constants (11.2 and 4.6 Hz), compared with epimer **13** (8.0 and 7.0 Hz). Ethylcarbonate groups of **12** were removed by treatment with K_2CO_3 in MeOH in 78% yield, followed by deprotection of *tert*-butyldimethylsilyl groups with nBu$_4$NF to afford nucleoside **3** quantitatively.

The present work demonstrates convenient syntheses of β-*C*-nucleosides containing a 2-aminophenol or a catechol as the 'chelator-base' moiety, which would provide alternative DNA base pairs through metal complexation. Metal complex formation behaviors with these nucleosides and their site-specific incorporation into oligo-DNA sequences are under investigation.

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References

Bannwarth, W., Pfleiderer, W., Müller, F., 1991. Energy transfer within oligonucleotides from a lumazine (=pteridine-2,4(1H,3H)-dione) chromophore to bathophenanthroline-ruthenium(II) complexes. Helv. Chim. Acta 74, 1991–1999.

Bashkin, J.K., Frolova, E.I., Sampath, U., 1994. Sequence-specific cleavage of HIV mRNA by a ribozyme mimic. J. Am. Chem. Soc. 116, 5981–5982.

Chen, C.-H.B., Sigman, D.S., 1988. Sequence-specific scission of RNA by 1,10-phenanthroline-copper linked to deoxyoligonucleotides. J. Am. Chem. Soc. 110, 6570–6572.

Dandliker, P.J., Holmlin, R.E., Barton, J.K., 1997. Oxidative thymine dimer repair in the DNA helix. Science 275, 1465–1468.

Dreyer, G.R., Dervan, P.B., 1985. Sequence-specific cleavage of single-stranded DNA: Oligodeoxynucleotide-EDTA·Fe (II). Proc. Natl. Acad. Sci. USA 82, 968–972.

Hildbrand, S., Blaser, A., Parel, P., Leumann, C.J., 1997. 5-Substituted 2-aminopyridine *C*-nucleosides as protonated cytidine equivalents: increasing efficiency and selectivity in DNA triple-helix formation. J. Am. Chem. Soc. 119, 5499–5511.

Hoffer, M., 1960. α-Thymidine. Chem. Ber. 93, 2777–2781.

Hurley, D.J., Tor, Y., 1998. Metal-containing oligonucleotides: solid-phase synthesis and luminescence properties. J. Am. Chem. Soc. 120, 2194–2195.

Ihara, T., Nakayama, M., Murata, M., Nakano, K., Maeda, M., 1997. Gene sensor using ferrocenyl oligonucleotide. Chem. Commun. 1609–1610.

Jaramillo, C., Knapp, S., 1994. Synthesis of C-aryl glycosides. Synthesis 1–20.

Kornberg, A., Baker, T.A., 1992. DNA Replication. 2nd Edition. Freeman, New York.

Lesser, D.R., Kurpiewski, M.R., Jen-Jacobson, L., 1990. The energetic basis of specificity in the Eco RI endonuclease-DNA interaction. Science. 250, 776–786.

Levy, D.E., Tang, C., 1995. In: The Chemistry of C-Glycosides. Pergamon, Oxford.

Magda, D., Miller, R.A., Sessler, J.L., Iverson, B.L., 1994. Site-specific hydrolysis of RNA by europium(III) texaphyrin conjugated to a synthetic oligodeoxyribonucleotide. J. Am. Chem. Soc. 116, 7439–7440.

Magda, D., Crofts, S., Lin, A., Miles, D., Wright, M., Sessler, J.L., 1997. Synthesis and kinetic properties of ribozyme analogues prepared using phosphoramidite derivatives of dysprosium(III) texaphyrin. J. Am. Chem. Soc. 119, 2293–2294.

Manchanda, R., Dunham, S.U., Lippard, S.J., 1996. Automated solid-

phase synthesis of site-specifically platinated oligodeoxyribonucleotides. J. Am. Chem. Soc. 118, 5144–5147.

Matsumura, K., Endo, M., Komiyama, M., 1994. Lanthanide complex-oligo-DNA hybrid for sequence-selective hydrolysis of RNA. J. Chem. Soc., Chem. Commun. 2019–2020.

Meade, T.J., Kayyem, J.F., 1995. Electron transfer through DNA: site-specific modification of duplex DNA with ruthenium donors and acceptors. Angew. Chem. Int. Ed. Engl. 34, 352–354.

Meggers, E., Kusch, D., Giese, B., 1997. An efficient synthesis of enantiomerically pure Δ-and Λ-ruthenium(II)-labelled oligonucleotides. Helv. Chim. Acta 80, 640–652.

Mucic, R.C., Herrlien, M.K., Mirkin, C.A., Letsinger, R.L., 1996. Synthesis and characterization of DNA with ferrocenyl groups attached to their 5′-termini: electrochemical characterization of a redox-active nucleotide monolayer. Chem. Commun. 555–557.

Murphy, C.J., Arkin, M.R., Jenkins, Y., Ghatlia, N.D., Bossmann, S.H., Turro, N.J., Barton, J.K., 1993. Long-range photoinduced electron transfer through a DNA helix. Science 262, 1025–1029.

Postema, M.H.D., 1995. In: C-Glycoside Synthesis. CRC Press, Boca Raton, FL.

Ren, R.X.-F., Chaudhuri, N.C., Paris, P.L., Rumney IV, S., Kool, E.T., 1996. Naphthalene, phenanthrene, and pyrene as DNA base analogues: synthesis, structure, and fluorescence in DNA. J. Am. Chem. Soc. 118, 7671–7678.

SantaLucia, J., Kierzek, R., Turner, D.H., 1992. Context dependence of hydrogen bond-free energy revealed by substitutions in an RNA hairpin. Science 256, 217–219.

Schliepe, J., Berghoff, U., Lippert, B., Cech, D., 1996. Automated solid phase synthesis of platinated oligonucleotides via nucleoside phosphonates. Angew. Chem. Int. Ed. Engl. 35, 646–648.

Schweizer, B.A., Kool, E.T., 1995. Hydrophobic, non-hydrogen-bonding bases and base pairs in DNA. J. Am. Chem. Soc. 117, 1863–1872.

Smith, S.A., Rajur, S.B., McLaughlin, L.W., 1994. Specific purine N^7-nitrogens are critical for high affinity binding by the Trp repressor. Nat. Struct. Biol. 1, 198–200.

Tanaka, K., Shionoya, M., 1999. Synthesis of a novel nucleoside for alternative DNA base pairing through metal complexation. J. Org. Chem. 64, 5002–5003.

Tanaka, K., Cao, H., Shionoya, M., 1998. Double strands formation of artificial DNAs induced by metal complexation. Nucleic Acids Symp. Ser. 39, 171–172.

Telser, J., Cruickshank, K.A., Schanze, K.S., Netzel, T.L., 1989. DNA oligomers and duplexes containing a covalently attached derivative of tris(2,2′-bipyridine) ruthenium(II): synthesis and characterization by thermodynamic and optical spectroscopic measurements. J. Am. Chem. Soc. 111, 7221–7226.