An Alternative Base-Pairing of Catechol-Bearing Nucleosides by Borate Formation

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A chelator-type β-C-nucleoside having a catechol ligand as the nucleobase was found to form a stable 2:1 complex with trimethyl borate, which was characterized by 1H-NMR and electrospray ionization time-of-flight (ESI-TOF) mass spectroscopies. Both phosphoramidite and phosphotriester derivatives of this nucleoside were also prepared as synthetic precursors for DNA oligomer syntheses.

Key words DNA; base-pairing; boron complex; C-nucleoside

In recent years, a large number of nonnatural analogues of DNA nucleosides have been synthesized as probes or regulators of DNA as well as drugs.1) Especially, most studies in this field have been focused on modification of hydrogen bonding patterns, because hydrogen bonding plays a central role in the high selective recognition in DNA base pair identification.

Recently,2) we have reported an alternative base pairing of artificial nucleosides in which hydrogen-bonded base pairing is replaced by metal-induced base pairing, thereby creating a novel hybridization motif in DNA duplexes (Chart 1). This approach has been expected to provide a wide range of applications to functionalized molecules based on its use as an alternative base pair along with the naturally occurring base pairs, AT and GC. Natural nucleobases form pairs with slightly propeller twisted planar motifs. In contrast, our strategy based on coordination geometry would provide not only a planar nucleoside but also structurally unique characters that could be introduced into a DNA duplex or triplex motif. In contrast, our strategy based on coordination geometry would provide not only a planar nucleoside but also structurally unique characters that could be introduced into a DNA duplex or triplex motif. Our recent report on a palladium(II)-assisted base pair of o-phenylenediamine-bearing β-C-nucleoside 1 showed its planar coordination geometry.3) Herein we present an alternative base pairing of catechol-bearing β-C-nucleoside 3 mediated by borate formation where two catechol moieties may possibly be at right angles to each other on the tetrahedral boron(III) center.3,4) Incorporation of this base pair into DNA strands could induce structural change of a DNA double strand (bending of a duplex or hydrogen bond scission of neighboring base pairs) and subsequent biological events. Such a geometrical control of DNA base pairing would be applicable to molecular biology, medicinal chemistry, and supramolecular chemistry.

Catechol-bearing β-C-nucleoside 3, which was synthesized from a 2’-deoxyribose derivative 4 and O-protected catechol 5 via Friedel–Crafts coupling reaction and subsequent deprotection,5) was treated with half amount of trimethyl borate and triethylamine in DMSO-d6 at room temperature to obtain a 2:1 complex 7 between nucleoside 3 and a boron ion (Chart 2). The isotopically resolved ESI-TOF mass spectrum of this solution in the negative mode provided clear evidence for the 2:1 complexation between 3 and a boron ion. The enlarged plot of the signal at m/z 459.15 [7–Et3NH+] gave excellent agreement with the theoretical isotopic distribution, indicating the boron-assisted base pairing with 3 (Fig. 1). 500 MHz 1H-NMR resonances of the aromatic protons of the complex 7 in DMSO-d6 were upfield-shifted from those of the nucleoside 3. The signals for hydroxy protons of the catechol moiety disappeared upon complexation whereas those for the ribose moiety remained unchanged (Fig. 2). These results indicate that the phenoxy groups coordinated to the boron(III) center in the deprotonated form. Although there are two possible diastereomeric structures for the complex 7 arising from chiral centers on both the o-ribose skeleton and the boron ion, we observed only one set of proton signals for the complex 7 in the spectrum. These results establish that the boron complex 7 is stable in solution.

Chart 3 shows the synthetic routes for compounds 11 and 12 to incorporate a catechol-type β-C-nucleoside as a “chelator-nucleoside” building block into a DNA strand by means of a phosphotriester or a phosphoramidite approach. The hydroxy group at the 5’-position of 6 was tritylated (4,4’-dimethoxytrityl) and then the TBDMS protecting groups at the phenoxy groups of the catechol moiety were replaced by acetyl groups to obtain 10 as a common intermediate for both approaches. These groups were expected to be readily removed by treatment with aqueous ammonium hydroxide solution after DNA oligomer synthesis.5) Compound 11 was synthesized from 10 in 90% yield by the phosphotriester approach. Compound 10 was also converted into cyanoethyl...
phosphoramidite 12 in 47% yield, which can be used in a standard automated DNA synthesizer.

The present work demonstrates a novel base pairing mode assisted by borate formation, providing an alternative DNA base pair. Site-specific incorporation of this novel base pair into oligo-DNA is now underway.

Experimental

General Information
All reactions were carried out in oven dried glassware under argon atmosphere with commercial dehydrated solvents (Wako). 4-(1,2-Dideoxy-β-D-ribofuranos-1-yl)catechol was prepared according to previously published procedures.\(^4\) 4,4'-Dimethoxytrityl chloride (DMTr-Cl), 4-chlorophenyl dichlorophosphate, N-methylimidazol, and 3-hydroxypropionitrile were purchased from TCI. 1 M n-Bu4NF in THF and 2-cyanoethyl N,N-diisopropylchlorophosphoramidite were purchased from Aldrich. All other reagents were purchased from Wako and were used without further purification. Column chromatography was performed using Wakogel C-300 gel (Wako).

\(^1\)H-NMR spectra referenced to TMS were recorded on a JEOL lambda 500 (500 MHz) or a Bruker DRX500 (500 MHz) spectrometer. \(^31\)P-NMR spectrum was recorded on a JEOL lambda 500 (202.35 MHz) spectrometer. The spectrum was referenced to external 10 mM phosphoric acid in D\(_2\)O. Chemical shifts (\(\delta\)) are reported in ppm; multiplicities are indicated by: s (singlet), d (doublet), dd (doublet of doublets), ddd (doublet of doublets of doublets), m (multiplet), br (broad). Coupling constants, \(J\), are reported in Hz. Electrospray ionization time-of-flight (ESI-TOF) mass spectra were recorded on a Micromass LCT spectrometer.

Synthesis of Bis-O,O'-tert-butyldimethylsilyl-4-[1,2-dideoxy-5-O-(4,4'-dimethoxytrityl)-β-D-ribofuranos-1-yl)catechol (8)

To a solution of bis-O,O'-tert-butyldimethylsilyl-4-(1,2-dideoxy-β-D-ribofuranos-1-yl)catechol, 6, (0.43 g, 0.95 mmol) in dry pyridine (4 ml) was added DMTr-Cl (0.39 g, 0.78 mmol) in 6 portions at 0 °C and stirred for 2 h at room temperature. The reaction was quenched with methanol (2 ml), the mixture was poured into ice-water (100 ml), and extracted with AcOEt three times. The combined organic layer was dried over anhydrous MgSO\(_4\), and concentrated. Column chromatography was performed using Wakogel C-300 gel (Wako).

\(^1\)H-NMR spectra referenced to TMS were recorded on a JEOL lambda 500 (500 MHz) or a Bruker DRX500 (500 MHz) spectrometer. 1746 Vol. 48, No. 11

Fig. 1. ESI-TOF Mass Spectrum of Complex 7 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 ([\(\text{[C}_2\text{H}_9\text{N}_2\text{Et}_3\text{NH}]^+\)] Calcd 459.15).

Fig. 2. 500 MHz \(^1\)H-NMR Spectra of (a) C-Nucleoside 3 and (b) 3-Borate (2:1) Complex, 7, in DMSO-d\(_6\).

[3]=44 ms. [B(OMe)\(_3\)]\(^-\)(a) 0 and (b) 22 ms. [Et\(_3\)N]=22 ms.

Chart 2. A Schematic Representation of a Synthetic Route for Catechol-nucleoside 3 and Its Borane Complex

Synthesis of 4-[1,2-Dideoxy-5-O-(4,4’-dimethoxytrityl)-β-D-ribofuranos-1-yl)catechol (9)
To a solution of 8 (0.59 g, 0.78 mmol) in 14 ml dry
THF was added dropwise 1 mL n-Bu,NF in THF (1.59 mL, 1.59 mmol). After 10 min, the reaction was quenched by an aqueous solution of 10% NaHCO₃ (1.3 mL). The mixture was concentrated and purified by silica gel column chromatography with CHCl₃–CH₃OH (20 : 1) to obtain 0.38 g of a colorless solid. ¹H-NMR (CDCl₃): δ 7.45—7.47 (2H, m), 7.33—7.37 (4H, m), 7.20—7.30 (7H, m, including residual CHCl₃), 7.08—7.16 (3H, m), 5.05 (1H, dd, J = 5.6, 10.0 Hz), 4.42 (1H, m), 4.03 (1H, ddd, J = 2.7, 4.8, 4.8 Hz), 3.79 (6H, s), 3.35 (1H, dd, J = 5.4, 9.9 Hz), 2.72—2.76 (7H, m), 2.64 (1H, ddd, J = 5.4, 9.9 Hz), 2.22 (3H, m), 2.12—2.17 (1H, m).

Synthesis of Di-O,O′-acetylated-[1,2-dideoxy-5-O-(4,4′-dimethoxytrityl)-β-D-ribofuranos-1-yl]catechol (10)  To a solution of 4-(1,2-dideoxy-5-O-(4,4′-dimethoxytrityl)-β-D-ribofuranos-1-yl)catechol (0.56 g, 0.92 mmol) in dry CH₃CN (2.7 mL) was added potassium tert-butoxide (50 mg, 0.45 mmol) and a THF solution (0.4 mL) of acetic anhydride (42 μL, 0.45 mmol). Thirty minutes later, additional potassium tert-butoxide (50 mg, 0.45 mmol) and a THF solution (0.4 mL) of acetic anhydride (42 μL, 0.45 mmol) were added and stirred for 30 min. The reaction mixture was evaporated and the resulting residue was chromatographed on silica gel with CHCl₃–CH₃OH (40 : 1) to obtain 0.18 g (67%) of 10 as an oil. ¹H-NMR (CDCl₃): δ 7.44—7.46 (2H, m), 7.31—7.36 (4H, m), 7.19—7.29 (6.4H, m, including residual CHCl₃), 7.12—7.13 (1H, m), 6.81—6.84 (4H, m), 5.15 (1H, dd, J = 5.6, 10.0 Hz), 4.40 (1H, m), 4.04 (1H, ddd, J = 2.7, 4.8, 4.8 Hz), 3.79 (6H, s), 3.33 (1H, m), 2.19 (1H, ddd, J = 4.5, 9.9 Hz), 3.26 (1H, dd, J = 5.2, 9.9 Hz), 2.22—2.27 (7H, m), 0.93 (7H, t, J = 13.1 Hz), 1.83 (1H, d, J = 3.2 Hz, D₂O exchangeable).

Synthesis of Di-O,O′-acetyl-4-[1,2-dideoxy-3-O-(4-chlorophenoxy)cyanoethoxy]phosphinyl]-5-O-(4,4′-dimethoxytrityl)-β-borofuranos-1-yl]catechol (11)  4-Chlorophenyl dichlorophosphate (0.31 mL, 1.99 mmol) was dissolved in 0.6 mL dry CH₂CN and N-methylimidazole (0.19 mL, 2.4 mmol) was added dropwise into the solution at −37°C. Then the mixture was added dropwise 3-hydroxypropionitrile (0.16 mL, 2.3 mmol) in 1 mL dry CH₂CN at −25°C, and the reaction mixture was stirred for 1.5 h at −9°C (solution A).

To the solution of di-O,O′-acetyl-4-[1,2-dideoxy-5-O-(4,4′-dimethoxytrityl)-β-D-ribofuranos-1-yl]catechol (0.56 g, 0.92 mmol) in dry CH₂CN (2.7 mL) was added N-methylimidazole (0.27 mL, 3.4 mmol) and the mixture was stirred for 30 min at −25°C. To the reaction mixture was added dropwise solution A and stirred for 1 h at −4°C. Then the mixture was poured into ice water (100 mL), extracted with AcOEt (100 mL×3), and dried over anhydrous MgSO₄. The residue was purified by silica gel column chromatography with CHCl₃ to obtain 0.71 g (91%) of 11 as a colorless foam consisting of a 1 : 1 mixture of diastereomers. ¹H-NMR (CDCl₃): δ 7.41—7.43 (2H, m), 7.20—7.33 (17H, m, including residual CHCl₃), 7.08—7.16 (3H, m), 6.80—6.83 (4H, m), 5.06 (0.5H, dd, J = 4.8, 10.9 Hz), 4.24—4.31 (3H, m), 3.78 (3H, s), 3.78 (3H, s), 2.84—3.35 (2H, m), 2.69 (1H, t, J = 6.2 Hz), 2.64 (1H, t, J = 6.2 Hz), 2.54 (0.5H, dd, J = not detected), 5.0, 13.9Hz), 2.45 (0.5H, dd, J = not detected), 5.6, 13.9 Hz), 2.28 (3H, m), 2.22 (3H, m), 2.12—2.17 (1H, m).

Chart 3

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(a) DMT-CI, pyridine, r.t., 73%; (b) TBAF, THF, r.t., 92%; (c) Ac₂O, tert-ButOK, THF, r.t., 68%; (d) p-chlorophenyl 2-cyanoethyl chlorophosphate, N-methylimidazole, MeCN, −4°C, 90%; (e) 2-cyanoethyl disopropylchlorophosphonamide, p-Pr₂EN, CH₂Cl₂, r.t., 47%.
References and a Note


5) The acetyl groups of the catechol moiety could be removed quantitatively in aqueous ammonium hydroxide solution at room temperature to obtain 3.