Nucleic Acids Research, Vol. 20, No. 13 3325–3332

Synthesis and properties of mirror-image DNA

Hidehito Urata, Emiko Ogura, Keiko Shinohara, Yoshiaki Ueda and Masao Akagi*
Osaka University of Pharmaceutical Sciences, 2-10-65 Kawai, Matsubara, Osaka 580, Japan

Received April 23, 1992; Revised and Accepted June 4, 1992

ABSTRACT

We have investigated the conformations of the hexadeoxyribonucleotide, L-d(CGCGCG) composed of L-deoxyribose, the mirror image molecule of natural D-deoxyribose. In this paper, we report the synthesis of four L-deoxynucleosides and the L-oligonucleotide-ethidium bromide interactions. The L-deoxyribose synthon 9 was synthesized from L-arabinose with an over all yield of 28.5% *via* the Barton–McCombie reaction. The L-deoxynucleosides were obtained by a glycosylation of appropriate nucleobase derivatives with the 1-chloro sugar 9. After derivatization to nucleoside phosphoramidites, L-deoxycytidine and L-deoxyguanosine were incorporated into a hexadeoxynucleotide, L-d(CGCGCG) by a solid-phase β-cyanoethylphosphoramidite method. This L-hexanucleotide was resistant to digestion with nuclease P1. The conformations of L-d(CGCGCG) were an exact mirror image of that of the corresponding natural one as described previously, and the conformations of the L-d(CGCGCG)-ethidium bromide complex were also the mirror images of those of the D-d(CGCGCG)-ethidium bromide complex under both low and high salt conditions. These results suggest that ethidium bromide prefers not a right-handed helical sense, but the base-base stacking geometry of the B-form rather than that of the Z-form. Thus, L-DNA would be a useful tool for studying DNA-drug interactions.

INTRODUCTION

Genetic informations of nucleic acids are constituted by only four major nucleobases (A, G, C and T). The replication and transcription are regulated by proteins recognizing nucleic acids. Not only the base sequence but also the local higher-order structures of DNA would be important for DNA-protein interaction (recognition). Since the chirality of the repeating units of polymers is essential factor for the formation of the higher-order structure and its function (1,2), deoxyribose is one of the most valuable molecule for phenomena of life. Although enantiomers have the same chemical and physical properties, organisms on the earth utilize only the D-sugar. This would be closely related to origin and evolution of life. Furthermore, since oligonucleotides composed of L-deoxyribose show resistance to digestion by certain nucleases, several investigators try to apply these molecules to an antisense oligonucleotide due to their stability to the intracellular metabolism (3–7). Thus, chemical, biochemical and physico-chemical properties of nucleic acids composed of L-sugar are an interesting problem. Recently, we reported the conformational properties of L-d(CGCGCG) which has the exact mirror image conformations of the natural D-isomer under both low and high salt conditions (8). In this paper, we describe the synthesis of the four L-deoxynucleosides and some other properties of L-d(CGCGCG).

Although several reports for the synthesis of L-deoxynucleosides are available (9–11), the method for L-deoxyguanosine gives a very poor yield (12). However, we improved this point and succeeded in the incorporation of the L-deoxyguanosine residues into the L-oligodeoxynucleotide.

RESULTS AND DISCUSSION

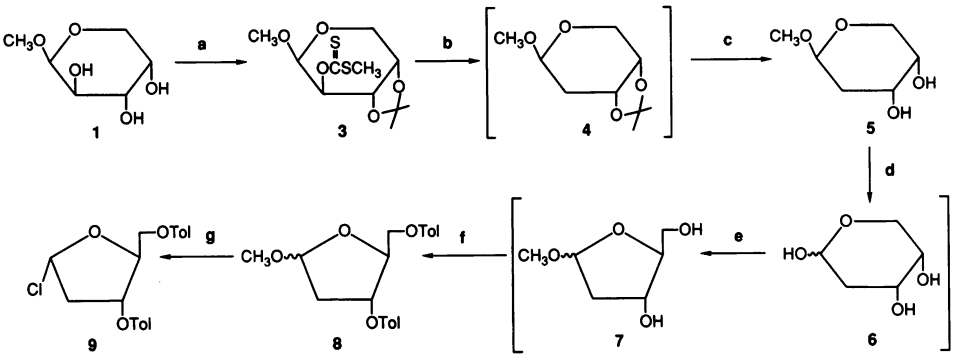
Synthesis of the L-deoxyribose unit

There are some reports for the synthesis of the L-nucleosides (9–11,13), the most of them, however, are limited methods which are useful only for the synthesis of the pyrimidine nucleosides (9) or the ribonucleosides (10,11). In order to enable us to synthesize the four deoxynucleosides, we chose the enantiomer **9** of the Hoffer's chloro sugar as an important intermediate for the L-deoxynucleoside synthesis, and employed the method (Scheme I) of Chattopadyaya and coworkers (14,15), which was developed for the synthesis of D-nucleosides derivatives deuteriated at the sugar moiety, with some modifications. This method has considerable improvements on the overall yield with respect to the glycal method (13).

Isomerically pure methyl β-L-arabinopyranoside **1** (16) easily prepared from L-arabinose was treated with 2,2-dimethoxypropane in the presence of Amberlyst 15 (H^+ form) instead of *p*-TsOH. After filtration of the resin and subsequent evaporation of the solvent, the residue was applied to the next reaction without further purification. Compound **2** was converted to the corresponding (methylthio)-thiocarbonate **3** in 90.4% yield from **1** as a pale yellow crystal. The reduction of **3** to **4** was performed with tri-*n*-butyltin hydride-AIBN (17). Although Pathak *et al.* failed to isolate the corresponding 2-deoxy-compound (14), we successfully isolated the 2-deoxyribose derivative with 60.3% yield. Because it, however, has difficulties to isolate this compound at this stage, the deoxygenated crude mixture was subjected to *in situ* acid treatment with 80% AcOH. The resulting

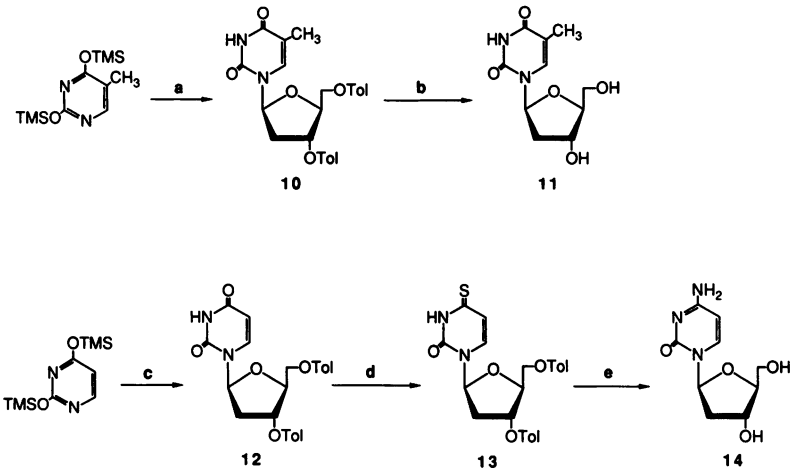
* To whom correspondence should be addressed

Scheme I[a]



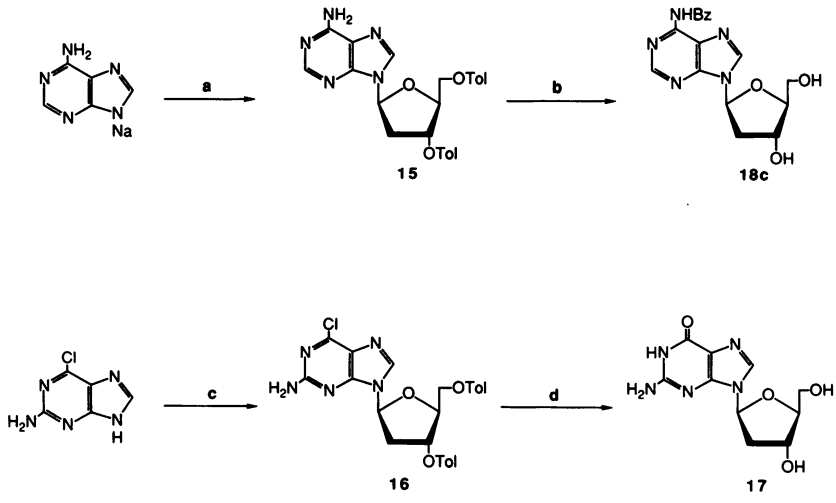
[a](a) 1. Dimethoxypropane, Amberlyst 15(H^+), DMF, 2. NaH, CS_2, CH_3I; (b) *n*-Bu_3SnH, AIBN; (c) 80% AcOH; (d) 1. 0.8 M HCl; (e) 0.1% HCl, MeOH; (f) *p*-Toluoyl-Cl, pyridine; (g) HCl gas, ether

Scheme II[a]



[a](a) **9**, $CHCl_3$; (b) NH_3, MeOH; (c) **9**, $CHCl_3$; (d) P_2S_5, pyridine; (e) NH_3, MeOH

Scheme III[a]



[a](a) **9**, acetone; (b) 1. Bz-Cl, pyridine, 2. 2 M NaOH; (c) **9**, NaH, DMF; (d) $HOCH_2CH_2SH$, NaOMe

mixture contained water soluble compounds **5**, **2** (mechanistic aspects of production of **2**, see ref. 17) and some other compounds derived from the reagents which can be removed easily by the extraction with organic solvents. The separation of compounds **5** and **2** by silica gel column chromatography afforded pure **5**. L-Deoxyribose **6** was obtained by the treatment with **5** with 0.8 M HCl and was transformed into the chloro sugar **9** according to the literature procedure (18,19).

Synthesis of the L-nucleosides

L-Thymidine **11** was readily synthesized by condensation of chloro sugar **9** with bissilylated thymine (20) and subsequent deprotection by treatment with methanolic ammonia (Scheme II).

For the synthesis of L-deoxycytidine, a direct glycosylation of silylated cytosine with chloro sugar **9** is accompanied by the production of a considerable amount of the undesired α-anomer which decreases the yield of the β-anomer, and the separation of both anomers is difficult (14,15). Thus, we chose the uracil to cytosine conversion pathway (9,21). The glycosylation of silylated uracil with **9** in chloroform is highly stereoselective and gives a high yield. The obtained L-deoxyuridine derivative **12** was converted to the 4-thio derivative **13** as reported previously. Compound **13** was then converted to L-deoxycytidine **14** by treatment with methanolic ammonia (Scheme II).

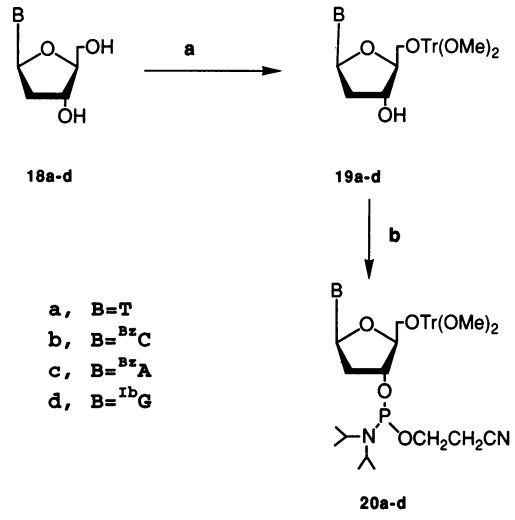
To synthesize the L-deoxyadenosine unit, we employed the method (22) which uses the sodium salt of adenine as an aglycone, instead of the more conventional 6-chloropurine because the amination step includes more drastic conditions. Thus, the reaction of the sodium salt of adenine with chloro sugar **9** in acetone afforded 3', 5'-di-O-*p*-toluoyl-L-deoxyadenosine **15** in 31% yield (Scheme III). This compound was easily converted to the N^6-benzoyl derivative **18c** which was extensively characterized.

There have been few reports on the synthesis of not only L-deoxyguanosine but also D-deoxyguanosine by a glycosylation. The synthetic yield of L-deoxyguanosine by the glycosylation method in the only one report (12) is very poor (3.9%). Thus, we employed a direct glycosylation method for the synthesis of L-deoxyguanosine **17** as described by Robins *et al.* (23) for the synthesis of D-deoxyguanosine (Scheme III). The slight excess of the sodium salt of 2-amino-6-chloropurine (prepared from *in situ* treatment of 2-amino-6-chloropurine with NaH) in DMF was glycosylated with chloro sugar **9**. The reaction showed the complexity of the products which contained several regio- and anomeric isomers. The desired 9-isomer was separated by silica gel column chromatography, however, it was still a mixture containing the undesired α-isomer (α:β=1:4). The recrystallization of the mixture from MeOH afforded the pure β-L-6-chloro-nucleoside **16** in 31.7% yield. The refluxing of a mixture of **16** and 2-mercaptoethanol and sodium methoxide in MeOH gave the L-deoxyguanosine sodium salt (24). The treatment of this salt with the Dowex 50w cation exchange resin (pyridinium form) afforded pure L-deoxyguanosine **17** with 67.4%.

Synthesis of building blocks for solid-phase phosphoramidite assembly

For the protection of the base moiety, the benzoyl and *iso*-butyryl group were used for **14**, **15** and **17**, respectively and the protection of the 5'-hydroxyl group was performed by using the dimethoxytrityl group with a standard procedure (25). The 5'-O-DMTr protected nucleosides (**19a–d**) were phosphitylated with 2-cyanoethyl N,N,N',N'-tetraisopropylphosphorodiamidite in the presence

Scheme IV[a]



[a](a) $(MeO)_2$Tr-Cl, pyridine; (b) 2-cyanoethyl N,N,N',N'-tetraisopropylphosphordiamidite, diisopropylammonium tetrazolide

of diisopropylammonium tetrazolide (26). After silica gel column chromatography, the nucleoside phosphoramidites (**20a–d**) of sufficient purity (checked by ^{31}P NMR) for nucleotide assembly on the solid-support were obtained (Scheme IV).

Synthesis of L-d(CGCGCG)

The controlled pore glass (CPG) support carrying the L-deoxyguanosine **19d** unit was prepared according to the literature procedure (27). This is a useful procedure for precious modified nucleosides compared to the conventional one. The protected phosphoramidite nucleotides were assembled on this CPG support as reported for the synthesis of D-oligonucleotides (28).

After deblocking and purification, the D- and L-hexamers were subjected to nuclease P1 digestion as described previously (29) to confirm the structures and to check the ability as a substrate for the enzyme. The D-hexamer (Fig. 1a) was completely degraded to the expected nucleotides and 5'-end nucleoside in the expected ratios (Fig. 1b). Although the retention time of the L-hexamer was consistent with that of the D-hexamer, the L-hexamer could not be digested at all (Fig. 1c) as expected.

Ethidium bromide interactions

Ethidium bromide binds to DNA with a intercalation mode at pyrimidine (3'-5') purine sites preferentially (30,31). We compared the interaction mode of ethidium bromide to D- and L-d(CGCGCG) with CD spectra. Recently, we reported that the conformation of L-d(CGCGCG) in solution is the exact mirror image of the corresponding natural D-hexamer under both low and high salt conditions (8). The CD spectrum of the D-d(CGCGCG)-ethidium bromide complex at low salt concentration (Fig. 2a) is similar to that of the poly (dG-dC)·poly (dG-dC)-ethidium bromide complex (30). On the other hand, the CD profile of the L-d(CGCGCG)-ethidium bromide complex (Fig. 2b) is completely inverted one of Fig. 2a. These results are clearly shown to be the same DNA-drug interaction mode between D- and L-DNA with an achiral drug such as ethidium

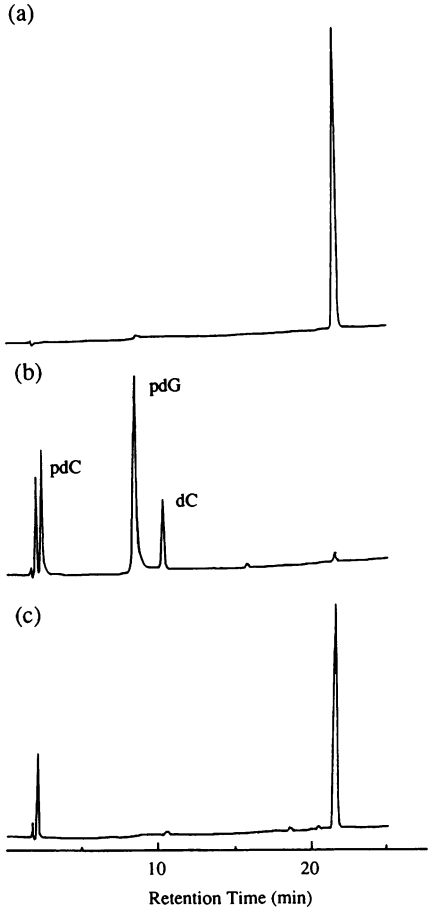


Figure 1. HPLC analysis of enzymatic digestion of D- and L-d(CGCGCG) with nuclease P1; (a) D-d(CGCGCG), (b) the digestion mixture of D-d(CGCGCG) and (c) the digestion mixture of L-d(CGCGCG). Elution was performed with a linear gradient of CH_3CN (0−10%) in 50 mM ammonium acetate during 20 min with UV detection at 254 nm.

bromide. Fig. 2c, d show the CD spectra of the D- and L-d(CGCGCG)-ethidium bromide complexes under high salt conditions. Pohl *et al.* reported that the CD spectrum of the poly (dG-dC)·poly (dG-dC)-ethidium bromide complex at high salt conditions is similar to that at low salt conditions (30), and they concluded that ethidium bromide inhibits B-to-Z transition. Similarly, the CD spectrum of the L-hexamer-ethidium bromide complex at high salt conditions is identical to that at low salt conditions. Thus, the intercalation mode of ethidium bromide to D- and L-DNA is same except for chirality and ethidium bromide prefers not the right-handed helical sense, but the base-base stacking geometry of the B-form rather than that of the Z-form. These results also indicate that non-chiral molecules such as ethidium cannot discriminate the chirality of DNA. L-DNA would be a useful tool for discrimination between specific interaction and nonspecific association on specific DNA-drug or DNA-protein interactions.

EXPERIMENTAL SECTION

Materials and Methods

L-Arabinose, 2-amino-6-chloropurine were purchased from Aldrich. Nuclease P1 was obtained from Yamasa Shoyu Co., LTD. Melting points were measured on a Yanagimoto apparatus and are uncorrected. Thin layer chromatography (TLC) was performed on Kieselgel 60 F_{254} plates (Merck) with a chloroform-methanol system. CD spectra and specific rotations were measured by a JASCO J-500 spectropolarimeter and JASCO DIP-181 digital polarimeter, respectively. ^1H NMR spectra were obtained by a Varian gemini-200 spectrometer and ^{31}P NMR spectra were obtained by a Varian XL-300 spectrometer. Chemical shifts were measured relative to internal tetramethylsilane ($CDCl_3$) or internal *t*-butyl alcohol (D_2O) for ^1H NMR or external trimethylphosphate for ^{31}P NMR. HPLC was performed by a Shimadzu LC-6A system on a column (ϕ3.9×150 mm) of μBondasphere C18 100Å. Methyl β-L-arabinopyranoside **1** was synthesized by the literature procedure (16) for the corresponding D-isomer.

Methyl 3,4-isopropylidene-2-O-[(methylthio)thiocarbonyl]-β-L-arabino-pyranoside (3)

To the mixture of methyl β-L-arabinopyranoside **1** (16.4 g, 100 mmol) and dimethoxypropane (38.6 mL, 300 mmol) in dry DMF (130 mL) was added 1 g of Amberlyst 15 (H^+ form) and stirred at ambient temperature during 18 h. After the filtration of the resin, the filtrate was concentrated to give sufficiently pure **2** (23.04 g, quant.). ^1H NMR ($CDCl_3$) δ 4.71 (d, H1, 1), 4.25−4.15 (m, H3, H4, 2), 3.93 (s, H5, H5′, 2), 3.78 (m, H2, 1), 3.44 (s, OCH_3, 3), 2.43 (d, 2-OH, 1), 1.53 and 1.36 (2s, isopropylidene, 3 each)

To the solution of the above residue in THF (160 mL) was added 50% NaH (11.5 g, 239.5 mmol) at 0°C and then, refluxed for 2 h. After cooling in an ice-bath, 59.6 mL (990 mmol) of carbon disulfide was added and stirred at room temperature for 2 h. Subsequently, methyl iodide (14.8 mL, 238 mmol) was added at 0°C and then, stirred at room temperature over night. The mixture was poured into ice-water and extracted with ethyl acetate. The organic layer was washed by brine, dried (Na_2SO_4) and evaporated under reduced pressure. The residue was crystallized from benzene-*n*-hexane to give pale yellow prisms of **3**, 26.6 g (90.4% from **1**), mp 127−130°C. ^1H NMR ($CDCl_3$): δ 5.78 (dd, H2, 1), 4.98 (d, H1, 1), 4.50 (dd, H3, 1), 4.30 (m, H4, 1), 4.01 (m, H5, H5′, 2), 3.40 (d, OCH_3, 3), 2.60 (s, SCH_3, 3), 1.55 and 1.39 (2s, isopropylidene, 3 each).

Methyl 2-deoxy-β-L-*erythro*-pentopyranoside (5)

Compound **3** (2.94 g, 10 mmol) was dissolved in dry xylene (100 mL). The solution was refluxed and degassed with nitrogen bubbling for 20 min. Tri-*n*-butyltin hydride (4.3 mL, 16 mmol) and AIBN (320 mg) were added. The mixture was refluxed for an additional 2 h. Volatile matters were evaporated and the residue was treated with 80% AcOH (40 mL) for 15 h. The solvent was evaporated and the residue was partitioned in water-ether. The aqueous layer was washed with ether, concentrated and coevaporated successively with EtOH, benzene and chloroform. The residue was purified by silica gel chromatography to give pure **5** (1.1 g, 81.9%) as a crystalline powder, mp 75−78°C. ^1H NMR ($CDCl_3$): δ 4.79 (t, H1, 1), 4.03 (m, H3, 1), 3.88−3.69 (m, H4, H5 and H5′, 3), 3.35 (s, OCH_3, 3), 2.47 (d, OH, 1), 2.30 (d, OH, 1), 1.89 (dd, H2 and H2′, 2).

Methyl 2-deoxy-3,5-di-O-*p*-toluoyl-L-*erythro*-pentose (8)

5.99 g (40.4 mmol) of **5** was treated with 0.8 M HCl (200 mL) for 40 h. The reaction mixture was neutralized by Dowex 1-X2 (OH^- form). After the resin was removed by filtration, the

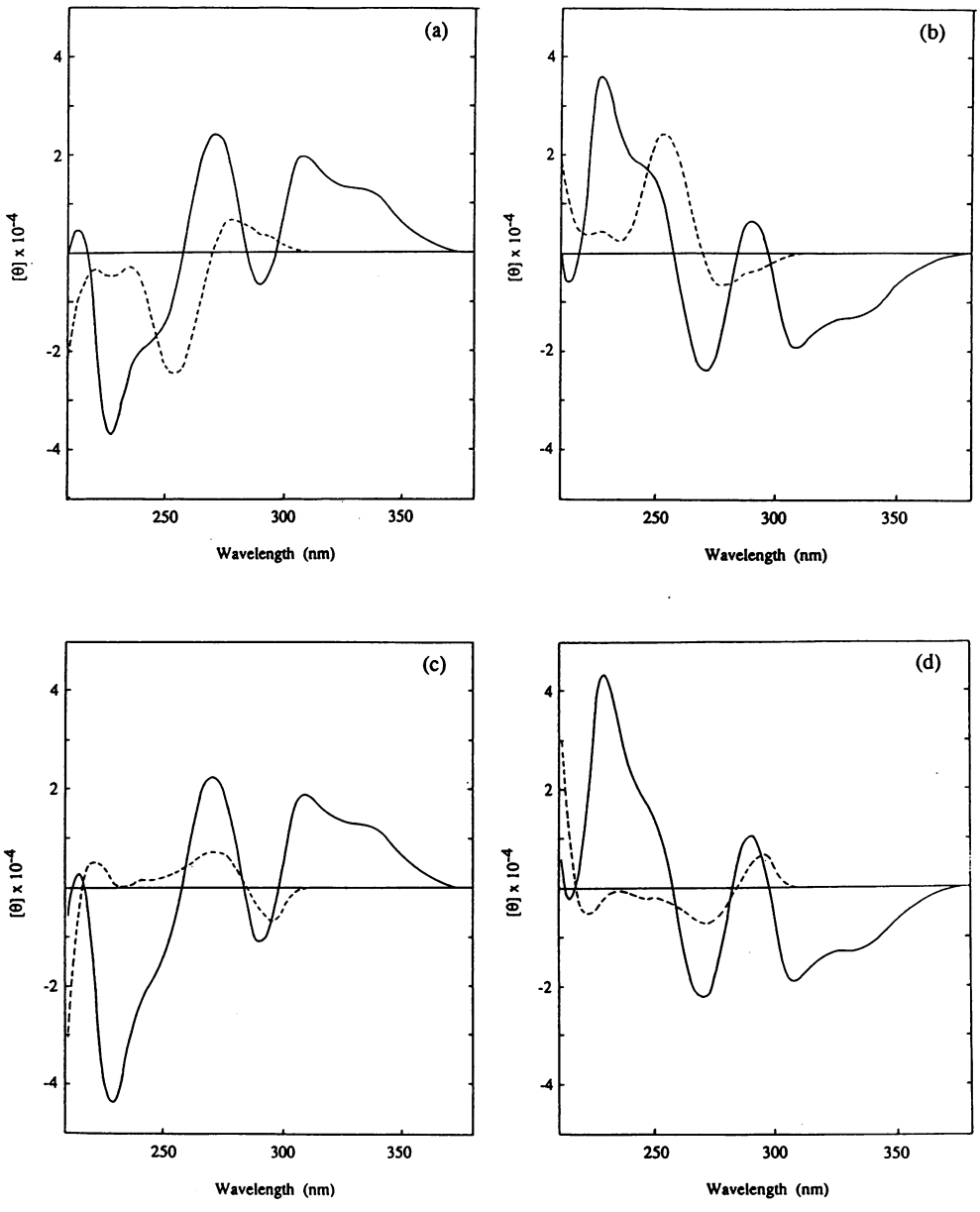


Figure 2. CD spectra of D- or L-d(CGCGCG) and their ethidium bromide complexes at 0.1 M NaCl (a, b) and at 4 M NaCl (c, d) at 0°C. Samples were contained the hexamer of 5 μM duplex concentration and 0 (dashed line) or 30 (solid line) μM ethidium bromide in 10 mM sodium phosphate, pH 7.0.

solvent was evaporated to give a colorless syrup of **6**, 4.68g. ^{1}H NMR spectrum was identical to that of 2-deoxy-D-ribose from commercial source. To the residue dissolved in MeOH (100 mL) was added 10.4 mL of 1% HCl/MeOH. After 2 h, the solution was neutralized by dry pyridine, then volatile matters were evaporated. The residue was loaded on a silica gel column to remove the pyrano-sugar which is the minor by-product and the product was eluted with 3−4% methanol in chloroform. The concentration of the eluents afforded 4.33 g (83.8%) of the anomeric mixture of **7**. The residue was dissolved in dry pyridine (90 mL) to which was added 16 mL (120 mmol) of *p*-toluoyl chloride at 0°C. After stirring 1 h at 0°C, stirring was continued overnight at ambient temperature. The mixture was poured into crushed ice and extracted with ether. The organic layer was washed with saturated sodium hydrogen carbonate and dried by Na_2SO_4. After removal of the solvent, the residue was purified by silica gel column chromatography to give 10.06 g (63.5% from **5**) of **8**. ^{1}H NMR ($CDCl_3$): δ 7.18−8.02 (m, aromatic), 5.59 (m, H3 of β-anomer), 5.41 (m, H3 of α-anomer), 5.21 (dd, H1 of β-anomer), 5.19 (d, H1 of α-anomer), 4.6−4.5 (m, H4 and H5 of both anomers), 3.41 (s, OCH_3 of α-anomer), 3.35 (s, OCH_3 of β-anomer), 2.40 (2s, aromatic CH_3 each).

2-Deoxy-3,5-di-O-*p*-toluoyl-α-L-*erythro*-pentofuranosyl chloride (9)

10.9 g (28.3 mmol) of **8** was dissolved in dry ether. Dry hydrogen chloride gas was bubbled into the solution at 0°C. After several minutes, the product started crystallizing out and the mixture was cooled in an ice-bath for 30 min. The crystals were filtered off and washed with cold dry ether to give 7.93 g (72.1%) of **9**,

mp 118−121°C. ^{1}H NMR ($CDCl_3$): δ 7.95 (2d, aromatic, 4), 7.25 (2d, aromatic, 4), 6.48 (d, H1, 1), 5.57 (m, H3, 1), 4.86 (q, H4, 1), 4.65 (m, H5, H5′. 2), 2.82 (m, H2, H2′, 2), 2.39 (2s, aromatic CH_3, 6). Anal. Calcd. for $C_{21}H_{21}O_5Cl$: C, 64.86%; H, 5.44%; Found: C, 64.64%; H, 5.37%.

3′,5′-Di-O-*p*-toluoyl-β-L-thymidine (10)

To a clear solution of 2,4-di-O-(trimethylsilyl)thymine (2.16 g, 8 mmol) (20) in pure chloroform (20 mL), 1.55 g (4 mmol) of chloro sugar **9** was added under nitrogen atmosphere. After stirring for 12 h at room temperature, chloroform was added and the solution was washed with water. The organic layer was dried by Na_2SO_4 and the solvent was removed *in vacuo*. The residue was purified by silica gel column chromatography to give a mixture of α- and β-isomers. The recrystallization of the mixture from MeOH afforded crystals of the pure β-isomer **10**, 1.49 g (77.8%) mp 197−200°C. ^{1}H NMR ($CDCl_3$): δ 8.88 (br, H3, 1), 7.2−8.0 (m, aromatic, 9), 6.47 (dd, H1′, 1), 5.63 (d, H3′, 1), 4.72 (m, H5′, H5″, 2), 4.52 (q, H4′, 1), 2.71 (dd, H2″, 1), 2.43 (s, aromatic CH_3, 6), 2.31 (m, H2′, 1), 1.62 (s, 5-CH_3, 3).

L-Thymidine (11)

Ammonia gas was bubbled into a suspension of 1.77 g (3.7 mmol) of **10** in MeOH at 0°C. After saturation of ammonia, the reaction vessel was sealed and heated at 60°C for 12 h. The mixture was concentrated and dissolved in water. The solution was washed with CH_2Cl_2 and ether. The aqueous layer was concentrated, and the residue was recrystallized from EtOH to give 784 mg (87.7%) of **11**, mp 189−192°C. ^{1}H NMR (D_2O): δ 7.68 (s, H6, 1), 6.31 (t, H1′, 1), 4.49 (q, H3′, 1), 4.05 (q, H4′, 1), 3.82 (m, H5′, H5″, 2), 2.40 (dd, H2′, H2″, 2), 1.91 (s, 5-CH_3, 3). $[\alpha]^{23}_D$ = −23.84° (c= 0.192, H_2O); lit.(32) $[\alpha]^{23}_D$ = −20.3° (c= 0.192, H_2O). Anal. Calcd. for $C_{10}H_{14}N_2O_5$ 1/16H_2O: C, 49.35%; H, 5.85%; N, 11.50%; Found: C, 49.10%; H, 5.80%; N, 11.24%.

3′,5′-Di-O-*p*-toluoyl-β-L-2′-deoxyuridine (12)

A suspension of uracil (672 mg, 6 mmol) in a mixture of hexamethyldisilazane (18 mL) and trimethylchlorosilane (1.8 mL) was refluxed. When the mixture turned into a clear solution, volatile matters were evaporated and coevaporated with dry xylene. The residue was dissolved in pure chloroform (15 mL) and 1167 mg (3 mmol) of chloro sugar **9** was added. After stirring for 18 h at room temperature, chloroform was added and the solution was washed with water. The organic layer was dried over Na_2SO_4, filtered and evaporated to dryness *in vacuo* and the residue was purified by silica gel column chromatography to give a mixture of α- and β-isomers. Recrystallization from MeOH afforded crystals of the pure β-isomer **12**, 1146 mg (76%), mp 219−222°C. ^{1}H NMR ($CDCl_3$): δ 8.43 (br, H3, 1), 7.93 (2d, aromatic, 4), 7.54 (d, H6, 1), 7.27 (2d, aromatic, 4), 6.42 (dd, H1′, 1), 5.61 (m, H5 and H3′, 2), 4.71 (2d, H5′, H5″, 2), 4.54 (q, H4′, 1), 2.75 (m, H2″,1), 2.43 (s, aromatic CH_3, 6), 2.31 (m, H2′, 1).

3′,5′-Di-O-*p*-toluoyl-2′-deoxy-4-thio-L-uridine (13)

To 1.5 g (3.24 mmol) of **12** dissolved in pyridine (100 mL) was added H_2O (64 μL) and 3.6 g (16.2 mmol) of phosphorus pentasulfide. The mixture was refluxed for 6 h and concentrated *in vacuo*. The residue was treated with water (200 mL) and was stirred for 1 h. Insoluble materials were filtered off and dissolved in chloroform (100 mL). The solution was washed with water (50 mL×2) and dried by Na_2SO_4, then concentrated. Recrystallization from EtOH afforded yellow crystals of **13**, 1.26 g (80.8%), mp 189−191°C. ^{1}H NMR ($CDCl_3$): δ 9.68 (br, H3, 1), 7.90 (2d, aromatic, 4), 7.39 (d, H6, 1), 7.28 (2d, aromatic, 4), 6.34 (dd, H1′, 1), 6.28 (dd, H5, 1), 5.61 (m, H3′, 1), 4.71 (2d, H5′, H5″, 2), 4.58 (q, H4′, 1), 2.80 (m, H2″, 1), 2.42 (s, aromatic CH_3, 6), 2.32 (m, H2′, 1).

2′-Deoxy-L-cytidine hydrochloride (14)

Ammonia gas was bubbled into a suspension of 1.25 g (2.6 mmol) of **13** in MeOH (80 mL) at 0°C. When ammonia was saturated, the autoclave was sealed. The mixture was heated at 100°C for 10 h. After cooling carefully, the solvent was removed *in vacuo* and the residue was dissolved in water (70 mL) and then, was washed with ether three times. The aqueous layer was concentrated and the residue was dissolved in EtOH and 2 mL of 2 M HCl was added. After concentration, the crystalline residue was recrystallized from 90% EtOH to give 576 mg (84.1%) of **14**, mp 172−180°C. ^{1}H NMR (D_2O): δ 8.12 (d, H6, 1), 6.22 (m, H5 and H1′, 2), 4.44 (m, H3′, 1), 4.10 (q, H4′, 1), 3.79 (m, H5′, H5″, 2), 2.41(m, H2″, H2′, 2). $[\alpha]^{23}_D$ = −56.9° (c= 1.0, H_2O) and the corresponding D-isomer from commercial source; $[\alpha]^{23}_D$ = +55.8° (c= 1.0, H_2O). Anal. Calcd for $C_9H_{13}N_3O_4$ HCl: C, 41.00%; H, 5.35%; N, 15.94%; Found: C, 41.06%; H, 5.23%; N, 15.88%.

2′-Deoxy-3′,5′-di-O-*p*-toluoyl-L-adenosine (15)

To a suspension of the sodium salt of adenine (1.51 g, 9.6 mmol) in dry acetone (100 mL), 1.94 g (5 mmol) of chloro sugar **9** was added. After stirring for 19 h at room temperature, chloroform was added to the mixture and washed with brine. After the organic layer was dried by Na_2SO_4 and concentrated, the residue was purified with silica gel column chromatography. The fractions containing **15** were concentrated *in vacuo* and the residue was recrystallized from ethyl acetate to give 755 mg (31%) of **15**, mp 163−167°C. ^{1}H NMR ($CDCl_3$): δ 8.33 (s. H8, 1), 7.2−8.5 (m, aromatic, 9), 6.55 (dd, H1′, 1), 5.87 (br.s, NH_2, 2), 5.82 (m, H3′, 1), 4.6−4.8 (m, H4′ and H5′, H5″, 3), 3.10 (m, H2″, 1), 2.84 (m, H2′, 1), 2.40 and 2.44 (s, aromatic CH_3, 3 each).

N^6-Benzoyl-2′-deoxy-L-adenosine (18c)

1.51 g (3.1 mmol) of **15** was treated with benzoyl chloride (0.74 mL, 6.4 mmol) in dry pyridine (16 mL). After stirring for 4 h at room temperature, the solution was poured into a mixture of crushed ice-$NaHCO_3$-chloroform (15 g−1 g−20 mL) and stirred vigorously for 15 min. Chloroform was added to the mixture and the organic layer was washed by saturated aq. $NaHCO_3$ three times, then dried by Na_2SO_4 and the solvent was removed *in vacuo*. The residue was dissolved in pyridine-THF (12 mL−8 mL) and treated with 32 mL of 2M NaOH-EtOH (1:1) at 0°C. After stirring for 15 min, the solution was neutralized by Dowex 50w (pyridinium form, 35 mL). The resin was removed by filtration and the resulting clear solution was concentrated to about 1/2 volume. Ether was added to the solution and vigorously shaken followed by standing at 4°C to give 896 mg (81.5%) of **18c**, mp 116−119°C. ^{1}H NMR (DMSO-d_6): δ 8.75 (s, H8, 1), 8.69 (s, H2, 1), 8.07 (d, aromatic, 2), 7.60 (t, aromatic, 1), 7.55 (t, aromatic, 2), 6.51 (t, H1′, 1), 4.49 (m, H3′, 1), 3.93 (q, H4′, 1), 3.61 (m, H5′, H5″, 2), 2.80 (m, H2″, 1), 2.40 (m, H2′, 1). $[\alpha]^{23}_D$ = +13.92° (c= 0.305, MeOH);

the corresponding D-isomer, $[\alpha]^{23}_D$ = −13.81° (c=0.308, MeOH). Anal. Calcd. for $C_{17}H_{17}N_5O_4 \cdot 1/2H_2O$: C, 56.0%; H, 4.98%; N, 19.21%; Found: C, 56.11%; H, 4.88%; N, 19.33%.

2-Amino-6-chloro-9-(2′-deoxy-3′,5′-di-O-*p*-toluoyl-β-L-*erythro*-pentofuranosyl)-purine (16)

To a solution of 2-amino-6-chloropurine (551 mg, 3.15 mmol) in dry DMF was added 180 mg (3.75 mmol) of 50% NaH under a nitrogen atomosphere and stirred for 30 min. Compound **9** was added in portions with stirring in 30 min and then the stirring was continued for a further period of 20 h. The mixture was concentrated *in vacuo* and was purified by silica gel column chromatography (mixture of the α, β-isomers, 59%). Recrystallization from MeOH afforded the pure β-isomer **16**, 1.99 g (31.7%), mp 181−185°C. ^{1}H NMR ($CDCl_3$): δ 7.91 (m, aromatic, 5), 7.22 (2d, aromatic, 4), 6.38 (dd, H1′, 1), 5.81 (m, H3′, 1), 5.21 (br. s, NH_2, 2), 4.4−4.9 (m, H4′ and H5′, H5″, 3), 3.16 (m, H2″, 1), 2.76 (m, H2′, 1), 2.43 and 2.40 (s, aromatic CH_3, 3 each).

2′-Deoxy-L-guanosine (17)

To a suspension of compound **16** (1.7 g, 3.24 mmol) in MeOH (26 mL) was added 0.82 mL (11.66 mmol) of 2-mercaptoethanol in 1 M NaOMe (11.34 mL) and H_2O (97 mL) and the mixture was refluxed for 5 h under the nitrogen atomosphere. After cooling, the precipitated Na^+ salt of **17** was filtrated and was dissolved in 30% pyridine/water. The solution was neutralized by Dowex 50w (pyridinium form) and was concentrated after removal of the resin. The residue was crystallized from water to give 584 mg (67.4%) of **17**, mp > 300°C. ^{1}H NMR (D_2O): δ 7.98 (s, H8, 1), 6.29 (t, H1′ 1), 4.60 (m, H3′, 1), 4.11 (q, H4′, 1), 3.78 (2d, H5′, H5″, 2), 2.75 and 2.50 (2m, H2″ and H2′, 1 each). $[\alpha]^{23}_D$ = +23.5° (c= 1.0, DMF), lit. (12) $[\alpha]^{23}_D$ = +20.5° (c= 1.0, DMF). Anal. Calcd. for $C_{10}H_{13}N_5O_4 \cdot 1/2H_2O$: C, 43.47%; H, 5.11%; N, 25.35%; Found: C, 43.60%; H, 5.35%; N, 25.33%.

General procedure for the synthesis of L-deoxynucleoside phosphoramidites (20a−d)

To the protected nucleoside **19a−d** (benzoyl for L-dC, L-dA, isobutyryl for L-dG, and dimethoxytrityl for 5′-hydroxyl) (1 mmol) dissolved in dry CH_2Cl_2 (5 mL) was added 86 mg (0.5 mmol) of diisopropylammonium tetrazolide and 381 μL (1.3 mmol) of 2-cyanoethyl N,N,N′,N′-tetraisopropylphosphorodiamidite and stand for 2 h. After addition of saturated sodium hydrogen carbonate, the mixture was extracted by CH_2Cl_2. The organic layer was washed by saturated sodium hydrogen carbonate three times and was dried with anhydrous $MgSO_4$, then concentrated. The residue was purified by silica gel column chromatography with a benzene-methanol system containing 1% triethylamine and concentrated to give a colorless foam, 68−91%. ^{31}P NMR ($CDCl_3$): **20a**, B= T, δ= 145.69, 146.11; **20b**, B= ^{bz}C, δ= 145.97, 146.55; **20c**, B= ^{bz}A, δ= 145.96, 146.11; **20d**, B= ^{ib}G, δ= 145.04, 145.80. R_f value ($CHCl_3$: MeOH= 10:1): **20a**, B= T, 0.59, 0.55; **20b**, B= ^{bz}C, 0.68, 0.63; **20c**, B=^{bz}A, 0.73, 0.67; **20d**, B= ^{ib}G, 0.55.

Synthesis of L-d(CGCGCG)

The controlled pore glass (CPG) support carrying the L-deoxyguanosine unit was prepared according to the literature procedure (27). The protected nucleoside phosphoramidites were assembled on this CPG support as reported for the synthesis of D-oligonucleotides (28). After the detachment from the CPG and the deprotection with concentrated ammonia, the product was purified by C18 open column chromatography. The fractions containing the product having the dimethoxytrityl group were collected and treated with 80% AcOH to deprotect the trityl group. The product was purified by the C18 open column again and desalted by a DEAE-cellulose column by eluting with triethylammonium bicarbonate buffer (pH 7.5). Finally, the product was passed through a Dowex 50w (Na^+ form) column and obtained as a sodium salt.

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid for Encouragement of Young Scientists from the Ministry of Education, Science, and Culture, Japan.

REFERENCES

1. Blout, E. R., Doty, P., and Yang, J. T. (1957) *J. Am. Chem. Soc.* **79**, 749−750.
2. Oida, F., Ota, N., Mino, Y., Nomoto, K.and Sugiura, Y. (1989) *J. Am. Chem. Soc.* **111**, 3436−3437.
3. Anderson, D. J., Reischer, R. J., Taylor, A. J.and Wechter, W. J. (1984) *Nucleosides Nucleotides* **3**, 499−512.
4. Fujimori, S., Shudo, K. and Hashimoto, Y. (1990) *J. Am. Chem. Soc.* **112**, 7436−7438.
5. Morvan, F., Génu, C., Rayner, B., Gosselin, G. and Imbach, J.-L. (1990) *Biochem. Biophys. Res. Commun.* **172**, 537−543.
6. Damha, M. J., Giannaris, P. A., Marfey, P. and Reid, L. S. (1991) *Terahedron Lett.* **32**, 2573−2576.
7. Asseline, U., Hau, J.-F., Czernecki, S., Diguarher, T. L., Perlat, M.-C., Valery, J.-M. and Thuong, N. T. (1991) *Nucleic Acids Res.* **19**, 4067−4074.
8. Urata, H., Shinohara, K., Ogura, E., Ueda, Y. and Akagi, M. (1991) *J. Am. Chem. Soc.* **113**, 8174−8175.
9. Holý, A. (1972) *Collection Czechoslov. Chem. Commun.* **37**, 4072−4087.
10. Holý, A. and Šorm, F. (1969) *Collection Czechoslov. Chem. Commun.* **34**, 3383−3401.
11. Acton, E. M., Ryan, K. J. and Goodman, L. (1964) *J. Am. Chem. Soc.* **86**, 5352−5354.
12. Robins, M. J., Khwaja, T. A. and Robins, R. K. (1970) *J. Org. Chem.* **35**, 636−639.
13. Deriaz, R. E., Overend, W. G., Stacey, M., Teece, E. G. and Wiggins, L. F. (1949) *J. Chem. Soc.* 1879−1883.
14. Pathak, T. and Chattopadhyaya, J. (1987) *Tetrahedron* **43**, 4227−4234.
15. Wu, J.-C., Bazin, H. and Chattopadhyaya, J. (1987) *Tetrahedron* **43**, 2355−2368.
16. Pratt, J. W., Richtmyer, N. K. and Hudson, C. S. (1952) *J. Am. Chem. Soc.* **74**, 2200−2205.
17. Crich, D. and Quintero, L. (1989) *Chem. Rev.* **89**, 1413−1432.
18. Hoffer, M. (1960) *Chem. Ber.* **93**, 2777−2781.
19. Hoffer, M., Dushinsky, R. and Fox, J. J. (1959) *J. Am. Chem Soc.* **81**, 4112−4113.
20. Nishimura, T. and Iwai, I. (1964) *Chem. Pharm. Bull.* **12**, 352−356.
21. Fox, J. J., Praag, D. V., Wempen, I., Doerr, I. L., Cheong, L., Knoll, J. E., Eidinoff, M. L., Bendich, A. and Brown, G. B. (1959) *J. Am. Chem. Soc.* **81**, 178−187.
22. Kawakami, H., Matsushita, H., Naoi, Y., Itoh, K. and Yoshikoshi, H. (1989) *Chem. Lett.* 235−238.
23. Kazimierczuk, Z., Cottam, H. B., Revankar, G. R. and Robins, R. K. (1984) *J. Am. Chem. Soc.* **106**, 6379−6382.
24. Lee, W. W., Martinez, A. P., Goodman, L. and Henry, D. W. (1972) *J. Org. Chem.* **37**, 2923−2927.
25. Ikehara, M., Ohtsuka, E., Uesugi, M. and Tanaka, T. (1988) In Townsend, L. B., (ed.), Chemistry of Nucleosides and Nucleotides. Plenum, New York, Vol. I, pp283−367.
26. Schulhof, J. C., Molko, D. and Teoule, R. (1987) *Nucleic Acids Res.* **15**, 397−416.
27. Damha, M. J., Giannaris, P. A. and Zabarylo, S. V. (1990) *Nucleic Acids, Res.* **18**, 3813−3821.

28. Sinha, N. D., Biernat, J., McManus, J. and Köster, H. (1984) *Nucleic Acids Res.* **12**, 4539–4557.
29. Urata, H., Ueno, M., Yamasaki, F. and Akagi, M. (1991) *Biochem. Biophys. Res. Commun.* **175**, 537–542.
30. Pohl, F. M., Jovin, T. M., Baehr, W. and Holbrook, J. J. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 3805–3809.
31. Patel, D. J. and Canuel, L. L. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 3343–3347.
32. Šmejkal, J. and Šorm, F. (1964) *Collection Czechoslov. Chem. Commun.* **29**, 2809–2813.