Synthesis of Wobble Pairing Oligoribonucleotides[1)]

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Hexaribonucleotides containing inosine at a pairing position adjacent to the other four major nucleosides (GGINCC, N = A, C, G, U) were synthesized in solution by the phosphotriester method using 2′-*O*-tetrahydrofuranyl protecting groups. 5′-*O*-Dimethoxytrityl-2′-*O*-tetrahydropyranylinosine 3′-*O*-(methyl-*N*,*N*-diisopropyl)phosphoramidite was used for solid-phase syntheses of decaribonucleotides (CGNGAUCICG, N = A, C, G, U). Thermal stabilities and circular dichroism spectra for these self-complementary duplexes were measured. The stabilizing effects of wobble base pairs in these duplexes were estimated as IC > IA > IU > IG.

Keywords inosine; oligoribonucleotide; wobble base pair; thermal stability; CD

Synthetic approaches are essential for structural studies of oligonucleotides, especially when modified nucleosides are involved. Inosine (I) is known to be located at a wobble position in codon–anticodon interaction during protein biosynthesis.[2)] Oligodeoxyribonucleotides containing deoxyinosine (dI) have been used for hybridization with genes for proteins containing amino acids with degenerate codons.[3,4)] Nuclear magnetic resonance (NMR)[5)] and X-ray crystallography[6)] studies on the structure of base pairs at dIdA in short deoxyribonucleic acid (DNA) have demonstrated the presence of hydrogen bonds between the two bases. Syntheses and thermal stabilities for hexa[7)]- and dodeca[8)]-deoxyribonucleotides have also been reported. Thermodynamic properties of short DNA duplexes containing deoxyinosine have been reported[9,10)] and those of self-complementary oligoribonucleotides having inosine at terminal positions have also been investigated.[11)] Syntheses of short ribonucleic acid (RNA) containing wobble base pairs are necessary in order to compare the stabilizing effects of wobble base pairs in RNA. In this paper we report syntheses of hexaribonucleotides (GGINCC) and decaribonucleotides (CGNGAUCICG, N = A, C, G and U). Two approaches were employed for these syntheses, and inosine was derivatized appropriately. As suggested in structural studies involving dAdG[12)] and dAdI base pairs,[5,6)] the local sequence contributes to the restriction of hydrogen bond formation. It may be important to investigate local structures for wobble base pairs in several oligonucleotides with different base sequences. In the present report, temperature–ultraviolet (UV) absorption profiles and circular dichroism (CD) spectra for two series of oligoribonucleotides are compared.

Materials and Methods

General Methods Thin layer chromatography (TLC) was performed on precoated Silica gel 60 F_{254} (Merck) and silanized silica gel (Merck). For column chromatography, Wako gel C-300 (Wako Pure Chemicals) and C-18 silica gel (55—105 μm, Waters) were used. Temperature–UV profiles were recorded with a Beckman DU-8B spectrophotometer and CD spectra were measured with a JASCO J-500A spectropolarimeter.

2′-*O*-Tetrahydrofuranylnucleotides were prepared as described previously[13)] and 2′-*O*-tetrahydropyranylnucleosides[14)] were also prepared by methods which were essentially the same as those described for the tetrahydrofuranyl derivatives. Acyl and dimethoxytrityl derivatives of nucleosides were synthesized as described.[15)] *o*-Chlorophenyl phosphoroditriazolide[16)] was used for phosphorylation in the phosphotriester approach and protected oligonucleotides were synthesized as described.[15)] Phosphitylation was performed with chloro-*N*,*N*-diisopropylaminomethoxyphosphine[17)] and decaribonucleotides were synthesized using a DNA synthesizer[18)] (Applied Biosystems 380 A).

3′-5′-*O*-(Tetraisopropyldisiloxan-1,3-diyl)-inosine (1) Inosine (8.05 g, 30 mmol) was dried by evaporation of pyridine, and then 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (11.4 ml, 36 mmol) was added dropwise at 0 °C. The mixture was heated at 35 °C under stirring for 3 h and treated with water (20 ml). The product was extracted with chloroform, and the extract was washed with water, dried with sodium sulfate, and concentrated by evaporation. The residue was purified on silica gel (270 g) using chloroform (4% methanol). The yield was 12.5 g (81%). mp 210—211 °C (ethanol). UV: λ_{max}^{MeOH} nm: 245, 248, (H^+) 249, (OH^-) 252. ^{1}H-NMR (DMSO-d_6) ppm: 12.38 (br s, 1H, NH), 8.12 (s, 1H, H-8), 7.96 (s, 1H, H-2), 5.84 (d, 1H, H-1′), 5.7—5.6 (br s, 1H, OH), 4.7—4.4 (m, 2H, H-2′, 3′), 4.2—3.8 (m, 3H, H-4′,5′), 1.4—0.7 (m, 28H, isopropyl).

2′-*O*-Tetrahydrofuranylinosine (2) The 3′,5′-protected inosine (1) (8.58 g, 16.7 mmol) was dissolved in dichloromethane (20 ml) and 2,3-dihydrofuran (2.53 ml, 32.4 mmol) was added dropwise in the presence of pyridinium *p*-toluenesulfonate (PPTS) (0.84 g, 3.2 mmol). The mixture was stirred for 40 h, and the product was extracted with dichloromethane (150 ml). The extract was washed with water, dried with sodium sulfate and concentrated. The residue was dissolved in tetrahydrofuran (THF) (8.5 ml) and stirred with 1 M tetrabutylammonium fluoride (8.5 ml) for 1 h. A mixture of pyridine–methanol–water (3 : 1 : 1, v/v) (150 ml) was added and the mixture was applied to a column (25 ml) of Dowex 50W × 8 (pyridinium form). The eluate and washings were concentrated and the residue was precipitated with hexane (100 ml) from its solution in chloroform (10 ml). The precipitate was purified by silica gel (120 g) column chromatography. Two diastereomers were separated by elution with 7% and 9% methanol in chloroform, respectively. The yield was 50.2%. "High" isomer with a higher *Rf* on TLC, 1.58 g (mp 163—165 °C); "low" isomer, 1.26 g (mp 166—168 °C). UVλ_{max} nm: 244,248, (H^+) 250, (OH^-) 252.

High Isomer: ^{1}H-NMR (DMSO-d_6) ppm: 12.39 (br s, 1H, NH), 8.35 (s, 1H, H-8), 8.05 (s, 1H, H-2), 5.97 (d, 1H, H-1′, J = 6.1 Hz), 5.2—5.0 (m, 2H, OH, Thf[–CH–]), 4.9 (br s, 1H, OH), 4.6 (t, 1H, H-2′), 4.4—4.1 (m., 1H, H-3′), 4.0—3.9 (m, 1H, H-4′), 3.8—3.5 (m, 4H, H-5′, Thf[–CH_2–]), 1.8 (m, 4H, Thf[–CH_2–CH_2–]).

Low Isomer: ^{1}H-NMR (DMSO-d_6) ppm: 12.39 (br s, 1H, NH), 8.35 (s, 1H, H-8), 8.04 (s, 1H, H-2), 5.91 (d, 1H, H-1′, J = 6.4 Hz), 5.3—5.0 (m, 3H, OH × 2, Thf [–CH–]), 4.6 (t, 1H, H-2′), 4.3 (m, 1H, H-3′), 3.95 (m, 1H, H-4′), 3.7—3.2 (m, 4H, H-5′, Thf[–CH_2–]), 1.8 (m, 4H, Thf[–CH_2–CH_2–]). *Anal.* Calcd for $C_{14}N_{18}O_6N_4$: C, 49.70; H, 5.36; N, 16.56. Found: C, 49.74; H, 5.36; N, 16.56.

5′-*O*-Dimethoxytrityl-2′-*O*-tetrahydrofuranylinosine 2′-*O*-Tetrahydrofuranylinosine (high isomer, 1.52 g, 4.5 mmol) was dried by evaporation of pyridine and treated with dimethoxytrityl chloride (1.83 g, 5.4 mmol) in pyridine–*N*,*N*-dimethylformamide (DMF) (4 : 1, 25 ml) for 4 h. The reaction was stopped by addition of methanol and chloroform. The organic layer was washed with water and concentrated by evaporation. The product was isolated by chromatography on silica gel (150 g) using chloroform–2% methanol, and purified by precipitation with hexane. The yield was 2.50 g (86.9%). ^{1}H-NMR ($CDCl_3$) ppm: 12.20 (br s, 1H, NH), 7.91 (s, 1H, H-8), 7.77 (s, 1H, H-2), 7.5—7.1 (m, 9H, H-arom), 6.8—6.7 (m, 4H, H-arom), 6.08 (d, 1H, H-1′, J = 5.9 Hz), 4.85 (t, 1H, H-2′), 4.5—4.4 (m, 2H, H-3′, OH), 4.3—4.2 (m, 1H, H-4′), 3.76 (s, 6H, –OCH_3 × 2), 3.7—3.4 (m, 2H, H-5′), 3.33 (m, 2H, Thf [–CH_2–]), 1.9 (m, 4H, Thf [–CH_2CH_2–]).

2′-*O*-Tetrahydropyranylinosine (3) The 3′,5′-protected inosine (**1**) (1.70 g, 3.3 mmol) was dissolved in dichloromethane (20 ml) and reacted with 3,4-dihydro-2*H*-pyran (0.61 ml, 6.6 mmol) in the presence of *p*-toluenesulfonic acid (0.13 g, 0.66 mmol) for 40 h. Dichloromethane (30 ml) and saturated sodium bicarbonate were added to the reaction mixture. The organic layer was washed with water, dried by filtration through a phase separation filter (1PS) and concentrated. The residue was dissolved in tetrahydrofuran (30 ml) and treated with 1 M tetrabutylammonium fluoride (3 ml) for 1 h. A mixture of pyridine–methanol–water (3 : 1 : 1, v/v) (30 ml) was added and the mixture was passed through a column (10 ml) of Dowex 50W × 8 (pyridinium form). The filtrate and washings were concentrated and the residue was precipitated with hexane (100 ml) from its solution in chloroform. The precipitate was applied to a column of silica gel (100 g) and two isomers of **3** were eluted with 8% and 9% methanol in chloroform. The yield was 66%. High isomer, 0.42 g, mp 163—165 °C (ethyl acetate); low isomer, 0.35 g, mp 166—168 °C (ethanol). UV: λ_{max} nm: 244, 248, (H^+) 250, (OH^-) 252.

High Isomer: ^{1}H-NMR (DMSO-d_6) ppm: 12.39 (br s, 1H, NH), 8.34 (s, 1H, H-8), 8.05 (s, 1H, H-2), 6.00 (d, 1H, H-1′, J = 5.5 Hz), 5.2—5.0 (m, 2H, OH × 2), 4.7—4.5 (m, 2H, H-2′, Thp [–CH–]), 4.4—4.2 (m, 1H, H-3′), 4.1—3.8 (m, 1H, H-4′), 3.7—3.5 (m, 24H, H-5′), 3.4—3.3 (m, 2H, Thp [–CH_2–]), 1.6—1.3 (m, 6H, Thp [–CH_2–CH_2–CH_2–]).

Low Isomer: ^{1}H-NMR (DMSO-d_6) ppm: 12.39 (br s, 1H, NH), 8.31 (s, 1H, H-8), 8.04 (s, 1H, H-2), 6.02 (d, 1H, H-1′, J = 6.3 Hz), 5.3—5.0 (m, 2H, OH × 2), 4.8—4.6 (m, 2H, H-2′, Thp [–CH–]), 4.4—4.2 (m, 1H, H-3′), 4.1—3.9 (m, 1H, H-4′), 3.7—3.5 (m, 2H, H-5′), 3.2—3.0 (m, 2H, Thp[–CH_2–]), 1.9—1.4 (m, 6H, Thp[–CH_2–CH_2–CH_2–]).

5′-*O*-Dimethoxytrityl-2′-*O*-tetrahydropyranylinosine 2′-*O*-Tetrahydropyranylinosine (high isomer, 0.39 g, 1.1 mmol) was dried by evaporation with pyridine, and treated with dimethoxytrityl chloride (0.45 g, 1.3 mmol) for 2 h. Methanol and chloroform were added to the mixture. The organic layer was washed with water and concentrated, and the residue was applied to a column of silica gel. The product was eluted with 1% methanol in chloroform purified by precipitation with hexane. The yield was 0.55 g, 76.4%. ^{1}H-NMR ($CDCl_3$) ppm: 12.98 (br s, 1H, NH), 8.04 (s, 1H, H-8), 7.97 (s, 1H, H-2), 7.5—7.2 (m, 9H, H-arom), 6.9—6.6 (m, 4H, H-arom), 6.09 (d, 1H, H-1′, J = 5.1 Hz), 4.80 (t, 1H, H-2′), 4.7—4.5 (m, 1H, Thp[–CH–]), 4.5—4.2 (m, 2H, H-3′, OH), 4.1—3.8 (m, 1H, H-4′), 3.76 (s, 6H, –OCH_3 × 2), 3.6—3.4 (m, 2H, H-5′), 3.29 (m, 2H, Thp [–CH_2–]), 1.9—1.4 (m, 6H, Thf[–CH_2CH_2–CH_2–]).

5′-*O*-Dimethoxytrityl-2′-*O*-tetrahydrofuranyl-*N*-isobutyrylguanosine-3′-*O*-(*o*-chlorophenyl)phosphoro(3′—5′)2′-*O*-tetrahydrofuranyl-*N*-isobutyrylguanosine-3′-*O*-(*o*-chlorophenyl)phosphate 5′-*O*-Dimethoxytrityl-2′-*O*-tetrahydrofuranyl-*N*-isobutyrylguanosine (1.45 g, 2.0 mmol) was dried by evaporation of pyridine and stirred with a solution of *o*-chlorophenyl phosphoroditriazolide in dioxane (8.87 ml, 2.66 mmol) for 1 h. Aqueous pyridine (30%) was added and the product was extracted with chloroform. The organic layer was washed with 0.1 M triethylammonium bicarbonate, dried by evaporation of pyridine and condensed with *N*-2′-protected guanosine using 1-(2-mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole (MSNT) (1.10 g, 4.0 mmol) for 30 min. Aqueous pyridine (30%) was added. The product was extracted with chloroform, and the extract was washed with 0.1 M triethylammonium bicarbonate, dried by filtration through 1PS, and concentrated by evaporation. The desired dimer was separated from the 3′–3′ linked by-product by chromatography on silica gel (60 g) and purified by precipitation with hexane (100 ml). The yield of the protected dinucleoside monophosphate was 1.08 g (51%). An aliquot of the product (0.66 g, 0.50 mmol) was phosphorylated by the procedure described above using a solution of *o*-chlorophenyl phosphoroditriazolide (0.67 mmol) to yield the protected dinucleotide 0.74 g (92%).

GGICCC The protected GGp (0.12 g, 0.071 mmol) and ICCC (0.12 g, 0.053 mmol) were dried by evaporation of added pyridine and treated with MSNT (50 mg, 0.16 mmol) in pyridine (1.5 ml) for 1.5 h. Aqueous pyridine was added to the mixture. The product was extracted with chloroform, and the extract was washed with 0.1 M triethylammonium bicarbonate, dried by filtration through 1PS and concentrated by evaporation. The protected hexamer was applied to a column of silica gel (40 g), eluted with 5% methanol in chloroform and precipitated with a mixture of hexane–ether (5 : 1). The yield was 0.15 g (74%).

The protected hexamer (50 mg) was dissolved in 1 M 1,1,3,3-tetramethylguanidinium 2-pyridinealdoximate (TMG-PAO) in dioxane (4.4 ml) and diluted with water (4.4 ml). The mixture was kept at 30 °C for 17 h and concentrated by evaporation. The residue was dissolved in pyridine (2 ml), then aqueous ammonia (25%, 15 ml), was added, and the mixture was heated at 60 °C for 5 h and concentrated. The residue was subjected to reversed-phase chromatography on C-18 silica gel and treated with 0.01 N hydrochloric acid (pH 2.0) at room temperature for 16 h. The product was desalted by gel filtration on Sephadex G-25 and purified as described in the legends to Fig. 3 and Fig. 4a.

Pentachlorophenyl 3′(2′)-[2′(3′)-*O*-Benzoyl-5′-*O*-dimethoxytrityl-N^2-isobutyrylguanosyl]succinate 3′(2′)-[2′(3′)-*O*-Benzoyl-5′-*O*-dimethoxytrityl-N^2-isobutyrylguanosyl]succinic acid (1.96 g, 2.50 mmol), which was prepared from 2′(3′)-*O*-benzoyl-5′-*O*-dimethoxytrityl-N^2-isobutyrylguanosine (2.41 g, 3.50 mmol) by treatment with succinic anhydride (0.53 g, 5.25 mmol) in dichloromethane (12 ml) in the presence of dimethylaminopyridine (0.65 g, 5.25 mmol),[19] was dissolved in DMF (19 ml) and the solution was stirred with pentachlorophenol (0.69 g, 3.75 mmol) at room temperature for 17 h using dicyclohexylcarbodiimide (DCC) (3.75 mmol) as the condensing reagent. Precipitated material was removed and the filtrate was concentrated. The product was dissolved in benzene and purified by precipitation with pentane. The yield was 2.43 g (91%).

Guanosine Resin Controlled pore glass (CPG) (Fluka, 550A, 120—200 mesh, aminopropylated 0.114 mmol/g) (1 g) was shaken with the activated succinate obtained above (176 mg, 0.171 mmol) in DMF (10 ml) in the presence of triethylamine (25 μl) for 17 h and washed with DMF then with pyridine. The resin was treated with a mixture of 0.1 M dimethylaminopyridine in pyridine (9 ml) and acetic anhydride (1 ml) for 10 min with shaking, then washed with pyridine and dichloromethane. The resin contained 0.049 mmol of the nucleoside.

5′-*O*-Dimethoxytrityl-2′-*O*-tetrahydropyranylinosine 3′-*O*-(Methyl-*N*,*N*-diisopropyl)phosphoramidite 5′-*O*-Dimethoxytrityl-2′-*O*-tetrahydropyranylinosine (0.25 g, 0.38 mmol) was dried by evaporation of pyridine, toluene and dichloromethane, successively. The nucleoside was dissolved in dichloromethane (2 ml) and treated with chloro-*N*,*N*-diisopropylaminomethoxyphosphine (0.11 ml, 0.57 mmol) and diisopropylethylamine (0.20 ml, 1.1 mmol) by addition with a syringe for 30 min under an argon atmosphere. The mixture was concentrated and the residue was dissolved in ethyl acetate (15 ml). This solution was washed with saturated sodium bicarbonate and water. The solution was dried by filtration through 1PS and concentrated. The product in ethyl acetate was applied to a column of silica gel (10 g) and eluted with ethyl acetate. The product was obtained as a foam (0.32 g, 0.43 mmol).

Decaribonucleotides Decamers were synthesized using 1 μmol of the guanosine resin and a DNA synthesizer with 50 steps. The product was cleaved from the support and purified by reversed phase and anion-exchange chromatography.

Results

Protection of the 2′-Hydroxyl Group of Inosine 2′-*O*-Tetrahydrofuranylinosine (**2**) and 2′-*O*-tetrahydropyranylinosine (**3**) were prepared *via* 3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)inosine (**1**) as shown in Fig. 1. Two kinds of protecting groups for the 2′-hydroxyl group were used for the present synthesis, since the easily removable tetrahydrofuranyl group derivative is compatible with solution-phase synthesis but is too labile under the acid conditions used to remove the 5′-dimethoxytrityl group in the synthesis on a polymer support. For the solid-phase synthesis of decaribonucleotides, the tetrahydropyranyl derivative (**3**) was used in combination with 5′-*O*-dimethoxytrityl and 3′-*O*-(methyl-*N*,*N*-diisopropyl)phosphoramidite under conditions described previously.

Synthesis of GGINCC (N = A, C, G, and U) Self-complementary hexamers containing two wobble base pairs at the middle of the duplexes were synthesized on a large scale in order to investigate their physical properties. The phosphotriester method in solution was employed for this synthesis, as illustrated in Fig. 2. Thus 2′-*O*-tetrahydrofuranylinosine (**2**) was converted to the 5′-*O*-dimethoxytrityl derivative and phosphorylated using *o*-chlorophenyl phosphoroditriazolide[16] to yield the diester (**4**). The phosphorylated inosine derivative (**4**) was condensed with 3′,5′-unprotected nucleosides to yield dimers (**7**, N = A, C, G and U), which were phosphorylated and elon-

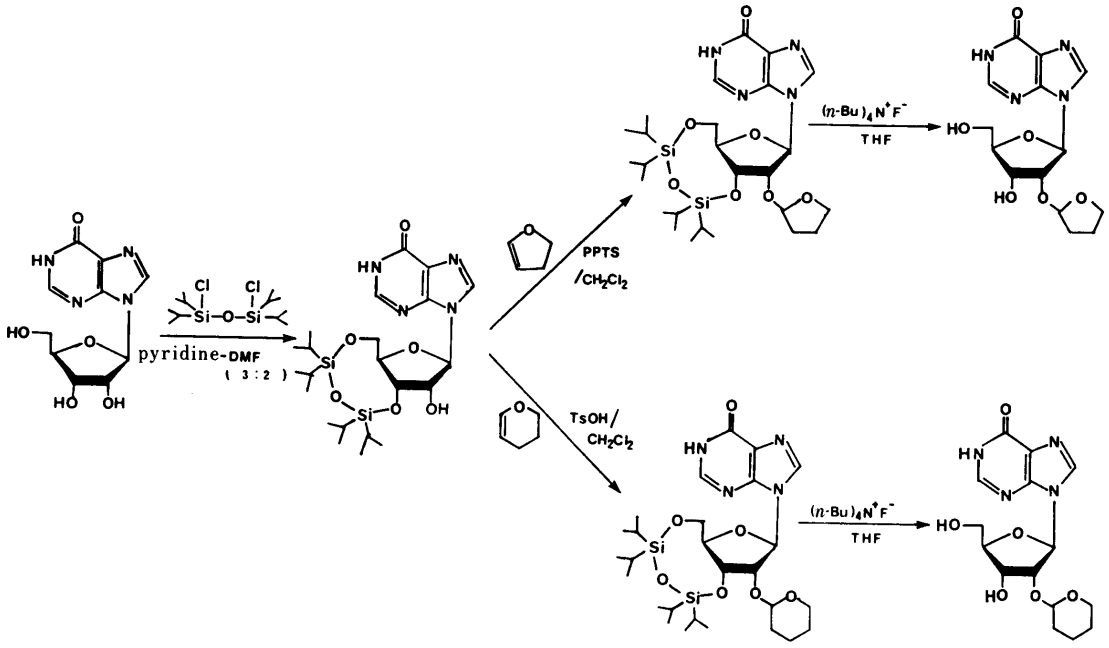

Fig. 1. Protection of the 2′-Hydroxyl Group of Inosine

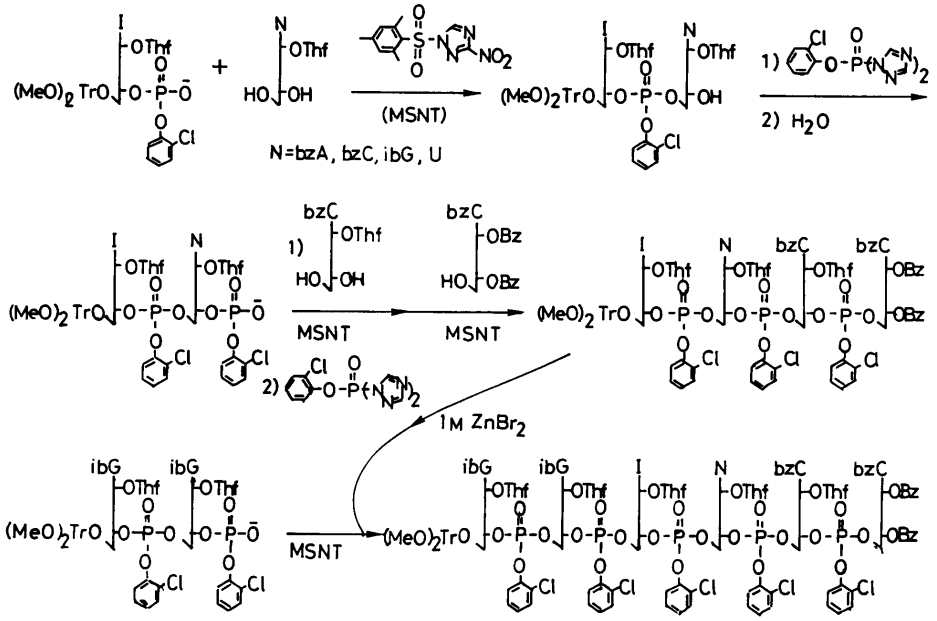

Fig. 2. Synthesis of Hexaribonucleotides

N is *N*-benzoylcytosin-1-yl (bzC), uracil-1-yl (U), *N*-benzoyladenin-9-yl (bzA) or *N*-isobutyrylguanin-9-yl (ibG); Thf, tetrahydrofuranyl; Bz, benzoyl.

gated in the 3′-direction by the same procedure to give the tetramers (**9**). The protected hexamers (**11**) were synthesized by condensation of a dinucleotide (**10**) with the 5′-deblocked tetramer, which was obtained from **9** by treatment with 1 M zinc bromide. The product was then deblocked by successive treatments with TMG-PAO and concentrated ammonium hydroxide. The partially deblocked hexamer was isolated by reversed-phase chromatography and treated with diluted hydrochloric acid to give the unprotected hexamer, which was desalted by gel filtration on Sephadex G-25. The product was purified by reversed-phase chromatography. The elution profile for GGICCC is shown in Fig. 3. The main fraction of the second peak was subjected to rechromatography on the same C-18 silica gel column as illustrated in Fig. 4a and analyzed by ion-exchange high performance liquid chromatography (HPLC) (Fig. 4b).

Synthesis of Decaribonucleotides Containing Inosine Decaribonucleotides (CGNGAUCICG, N=A,C,G and U) were synthesized by the phosphoramidite method using 2′-tetrahydropyranyl protection. The scheme for the synthesis is shown in Fig. 5. 5′-*O*-Dimethoxytrityl-2′-*O*-benzoyl-3′-*O*-succinylguanosine was linked to CPG and this nucleoside resin was used as the starting unit. The chain was elongated in the 5′-direction using a DNA synthesizer by removing the 5′-*O*-dimethoxytrityl group with dichloroacetic acid. The protecting groups of the internucleotidic phosphates were removed by treatment

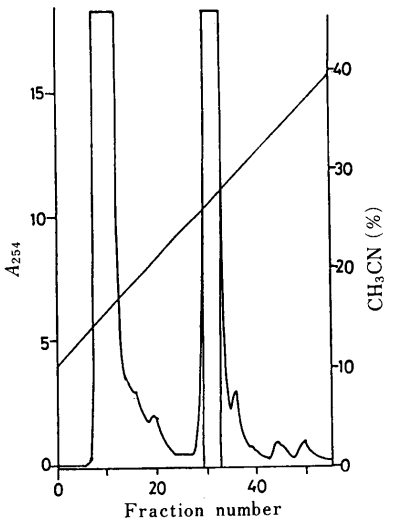

Fig. 3. Reversed-Phase Chromatography of GGICCC on a Column (15 mm × 17 cm) of C-18 Silica Gel (Waters, 55—105 μm)

Elution was performed with a gradient of acetonitrile (10—40%) in 50 mM triethylammonium bicarbonate (total 400 ml).

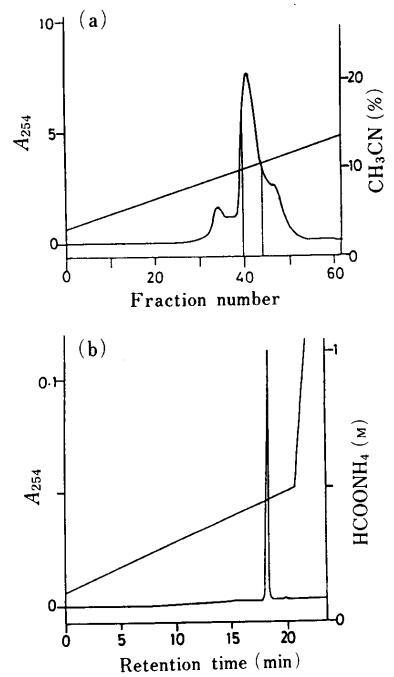

Fig. 4. Results of Chromatography

a) Rechromatography of GGICCC using the conditions described in Fig. 3 with a shallower gradient (3—13%). b) Analysis of GGICCC by anion-exchange HPLC on a column (6 × 250 mm) of TSK-gel DEAE-2SW (Tosoh Co.). A gradient (B: 5—25%) was formed from solvent A (20% aqueous acetonitrile) and solvent B (20% aqueous acetonitrile, 2 M ammonium formate).

with thiophenol and the decamer was cleaved from the support with ammonium hydroxide. The product was completely deblocked as shown in Fig. 5 and subjected to gel filtration on Sephadex G-25. The decamer was purified by reversed-phase and ion-exchange HPLC. The purity of each decamer was checked by HPLC and each decamer (*ca.* $2A_{260}$ units) was desalted by gel filtration.

Thermal Stabilities and CD Spectra of GGINCC and CGNGAUCICG Two series of self-complementary oligoribonucleotides containing an inosine residue were obtained and the thermal stabilities of these wobble duplexes were measured by observing the temperature–UV absorption profiles in 0.01 M cacodylate and 0.1 M sodium chloride (Table I). The hexaribonucleotides containing IC or IA pairs showed cooperative melting. The T_m values for IC and IA hexamers were 36.7 °C and 22.0 °C, respectively. However, IU and IG hexamers did not show reproducible melting curves. The IC hydrogen bonds in the hexaribonucleotide stabilized the duplex to a larger extent than those in the deoxyribohexamer, which showed much lower T_m (18 °C).

Decaribonucleotide duplexes containing two IN pairs at the third position from the ends revealed reasonable stabilities (Table I). The IU decamer showed a higher T_m (34.6 °C) than IA decamer (31.0 °C). The order was reversed in the hexamer. The hydrogen bonds in the IU pair seem to have greater stabilizing effects than those in the IA pair, if they exist between Watson–Crick type base pairs as in this decaribonucleotide. The stabilizing effects of these wobble base pairs may be significantly dependent on base sequences.

CD spectra for the IN hexamers were measured at the same strand and ionic concentrations, as shown in Fig. 6. The hexamers all showed non-conservative spectra, which could suggest a structure of RNA-A form. IC and IA hexamers had almost the same profile as each other and had a more distinct peak near 260 nm compared to those of the deoxyisomers. From a preliminary NMR study on the IA hexaribonucleotide, a helical nature different from that of

Table I. Melting Temperatures

	T_m (°C)	
	GGINCC	CGNGAUCICG
N=C	36.7	49.0
N=A	22.0	31.0
N=U	<5	34.6
N=G	—	—

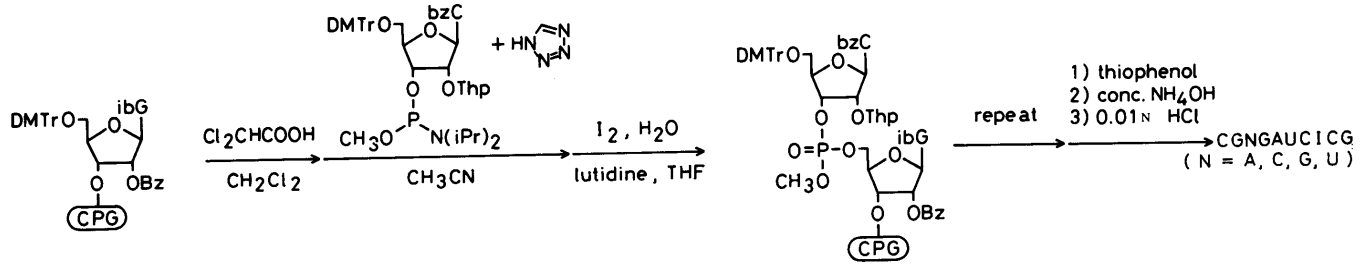

Fig. 5. Synthesis of Decaribonucleotides on CPG

Thp, tetrahydropyranyl.

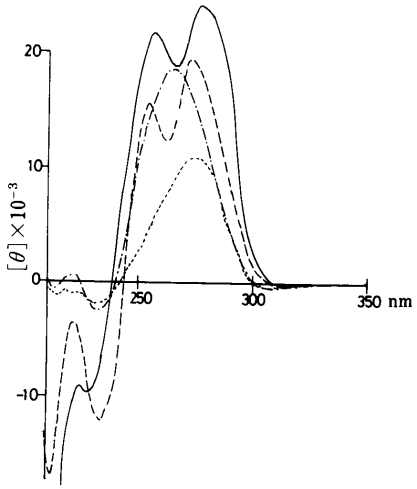

Fig. 6. CD Spectra for CCINGG

——, N=C; – – –, N=A; —·—, N=G; ------, N=U.

the deoxyisomer was suggested by the wider line width of the signal of the imino proton of inosine.

Discussion

A large scale synthesis of hexaribonucleotides containing inosine was performed using 2′-*O*-tetrahydrofuranyl derivatives of nucleosides. 5′-Dimethoxytrityl protection is compatible with the 2′-*O*-tetrahydrofuranyl group for preparation of oligonucleotides in solution. The approach using $ZnBr_2$ for elongation of the chain is suitable for short RNA fragments. However, the automated phosphoramidite method involving acid treatments is preferred for the synthesis of longer oligonucleotides. In this study the slightly more stable 2′-*O*-tetrahydropyranylinosine derivative was prepared and applied to the synthesis of decaribonucleotides. A combination of 5′-*O*-dimethoxytrityl and 2′-*O*-tetrahydropyranyl groups has already been used for small-scale syntheses of RNA fragments.[18] In order to obtain longer oligoribonucleotides on a large scale, other combinations of 2′- and 5′- protecting groups may have to be introduced. Temperature–UV absorption profiles of the present oligoribonucleotides indicated distinct differences from oligodeoxyribonucleotides. In this paper, two series of oligoribonucleotides containing wobble base pairs were prepared and the stabilizing effects of IC and IA base pairs in short RNA duplexes were compared. The effects were shown to be dfferent in the hexamers compared to the decamers, probably due to the sequence difference. The stabilizing effect of IU hydrogen bonds in the CGUGAUCICG duplex was larger than that of IG in the CGGGAUCICG duplex. In the deoxy series, the pairing of dIdG stabilized the dodecamer more than dIdT. Hydrogen bonds between guanine and uracil are also assumed to involve N^1 of G and O^2 of U.[10]

So far no direct experimental evidence has been reported on the relative of stabilities of RNA duplexes containing wobble base pairs. In the present study the order of the stabilizing effect was estimated to be IC > IA > IU > IG in the self-complementary hexamer. This is the first evidence to support the proposal that inosine in the first position of the anticodon can stabilize the duplex by forming so-called wobble base pairing with A, C and U in messenger RNA (mRNA), probably by the formation of hydrogen bonds. The effects of modified bases at the first position of the anticodon on efficient translation of codons in protein biosynthesis have been described.[20] NMR studies of the imino protons of the inosine residues in oligoribonucleotides are required in order to elucidate the nature of the hydrogen bonds in wobble base pairs.

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References and Notes

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