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# Studies on Transfer Ribonucleic Acids and Related Compounds. XLIII.[1] Synthesis of Oligoribonucleotides by using 5′-Selective Phosphorylation of 2′-*O*-Tetrahydrofuranyl Nucleosides

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2′-*O*-Tetrahydrofuranyl nucleosides have been synthesized from 2,3-dihydrofuran *via* 3′,5′-bis-*tert*-butyldimethylsilylnucleosides. These nucleosides were used as intermediates for oligonucleotide syntheses by the phosphotriester method. Trimers C–C–A and C–C–U were synthesized by the stepwise addition of monomers. U–U–U–U–U–U, A–U–G–A–U–G and A–U–G–A–U–G–A–U–G were obtained by the block condensation of trimers in which the 5′-hydroxyl group had been selectively phosphorylated.

**Keywords**——2′-*O*-tetrahydrofuranyl nucleoside; 5′-*O*-phosphorylation; *o*-chlorophenyl phosphate; phosphoro-*p*-anisidate; ribooligonucleotide

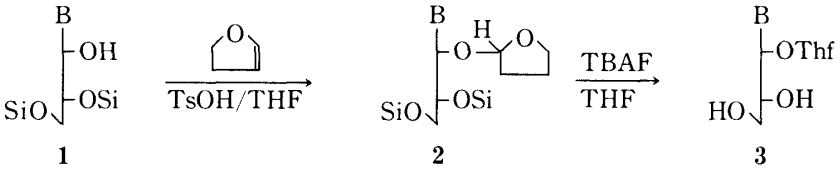
For the synthesis of ribooligonucleotides, protection of the 2′-hydroxyl group is an important procedure. We have previously reported the usage of a photolabile *o*-nitrobenzyl group for the protection of the 2′-hydroxyl function.[2] Acid labile 2′-*O*-tetrahydropyranyl and methoxytetrahydropyranyl groups have also been used in combination with acid stable 5′-*O*-protecting groups.[3] In this paper we describe the use of the easily removable tetrahydrofuranyl group[4] as a protection for the 2′-hydroxyl function of ribonucleosides in the synthesis of oligonucleotides. C–C–A and C–C–U were synthesized by stepwise addition of monomers to elongate the chain in the 3′-direction. U–U–U–U–U–U, A–U–G–A–U–G and A–U–G–A–U–G–A–U–G were synthesized by block condensation of trinucleotides obtained by selective 5′-phosphorylation with *o*-chlorophenyl *p*-anisidophosphorochoridate.[5] A preliminary account of the use of the tetrahydrofuranyl group as protection for the 3′-hydroxyl function in the synthesis of (2′–5′)oligoadenylates has appeared.[6]

## Preparation of 2′-*O*-Tetrahydrofuranylnucleosides

2′-*O*-Tetrahydrofuranyl (Thf) derivatives of uridine, *N*-benzoylcytidine,[7] *N*-benzoyladenosine[8] and *N*-isobutyrylguanosine[9] (**3**) were prepared *via* 3′,5′-bis-*tert*-butyldimethylsilylnucleosides (**1**), which were obtained by a slight modification of the published procedure.[10] As shown in Chart 1, 3′,5′-substituted nucleosides (**1**) were treated with dihydrofuran in the presence of *p*-toluenesulfonic acid to give intermediates (**2**) which were then treated with tetra-*n*-butylammonium fluoride (TBAF) to remove the *tert*-butyldimethylsilyl (TBDMS) groups. The products (**3**) contained two diastereoisomers. The yields and properties of the isomers of each nucleoside are summarized in Table I. The location of the tetrahydrofuranyl group was confirmed by decoupling experiments with the di-*O*-acetyl derivatives (**4**). $^1$H-Nuclear magnetic resonance (NMR) data for these compounds are listed in Table II. Large low-field shifts of the $C_{3'}$–H signal were observed in each series.[2b]

## Synthesis of C–C–U and C–C–A by Stepwise Addition of Nucleosides

CpCpU was synthesized by elongation of the chain in the 3′-direction as shown in Chart 2. The cytidine derivative (**3b**-1) was 5′-protected with monomethoxytrityl chloride (MMTrCl) to give **5**, which was phosphorylated with *o*-chlorophenyl phosphoroditriazolidate.[11] After treatment with aqueous pyridine the diester was condensed with protected cytidine (**3b**) using mesitylenesulfonyl tetrazolide (MSTe). The dimer (**6**) thus obtained was separated from the




1 2 3

**a** : B=U
**b** : B=bzC
**c** : B=bzA
**d** : B=ibG

TBAF=tetra-*n*-butylammonium fluoride
Si=TBDMS=*tert*-butyldimethylsilyl
Thf=tetrahydrofuranyl

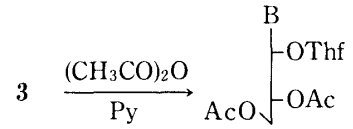



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Chart 1

TABLE I. Yield, mp and *Rf* values of **3**

| Compd. | Yield (%) | mp (°C) | *Rf*[a] |
|---|---|---|---|
| **3a**-l | 42 | 153—156 | 0.48 |
| **3a**-h | 35 | 80—82 | 0.56 |
| **3b**-l | 24 | 210—212 | 0.56 |
| **3b**-h | 33 | 175—177 | 0.59 |
| **3c**-l | 44 | | 0.49 |
| **3c**-h | 44 | | 0.53 |
| **3d**-l | 33 | | 0.58 |
| **3d**-h | 42 | | 0.66 |

a) $CHCl_3$ : MeOH 5 : 1, l : low *Rf* isomer, h : high *Rf* isomer.

TABLE II. $^1$H-NMR Spectra of 2′-*O*-Tetrahydrofuranylnucleosides and Its Diacetyl Derivatives

| Compd. | DMSO-$d_6$ ppm | | | | | |
|---|---|---|---|---|---|---|
| | H1′ | $J_{1',2'}$ (Hz) | H2′ | H3′ | H4′ | H5′ |
| **3a**-l | 5.86 | 6 | 4.3—4.0 | | 3.9 | — |
| **3a**-h | 5.86 | 5 | 4.2—4.0 | | 4.0—3.8 | |
| **3b**-l | 5.97 | 4 | 4.3—4.0 | | 3.9 | — |
| **3b**-h | 5.86 | 1 | —— 4.3 —— | | | |
| **3c**-l | 6.11 | 6 | 4.8 | 4.4 | 4.0 | 3.9 |
| **3c**-h | 6.17 | 6 | 4.8 | 4.4 | — | — |
| **3d**-l | 5.89 | 7 | 4.6 | 4.3 | — | — |
| **3d**-h | 5.91 | 7 | 4.6 | 4.3 | — | — |
| **4a**-l | 5.81 | 6 | 4.47 | 5.0 | 4.2 | |
| **4a**-h | 5.74 | 7 | 4.47 | 5.0 | 4.2 | |
| **4b**-l | 5.91 | 4 | 4.52 | 5.1 | 4.3 | |
| **4b**-h | 5.85 | 5 | 4.57 | 5.2 | 4.3 | |
| **4c**-l | 6.07 | 6 | 5.13 | 5.4 | 4.4 | |
| **4c**-h | 6.18 | 6 | 5.30 | 5.5 | 4.3 | |
| **4d**-l | 5.82 | 7 | 4.87 | 5.4 | 4.3 | |
| **4d**-h | 5.92 | 8 | 4.99 | 5.3 | 4.3 | |

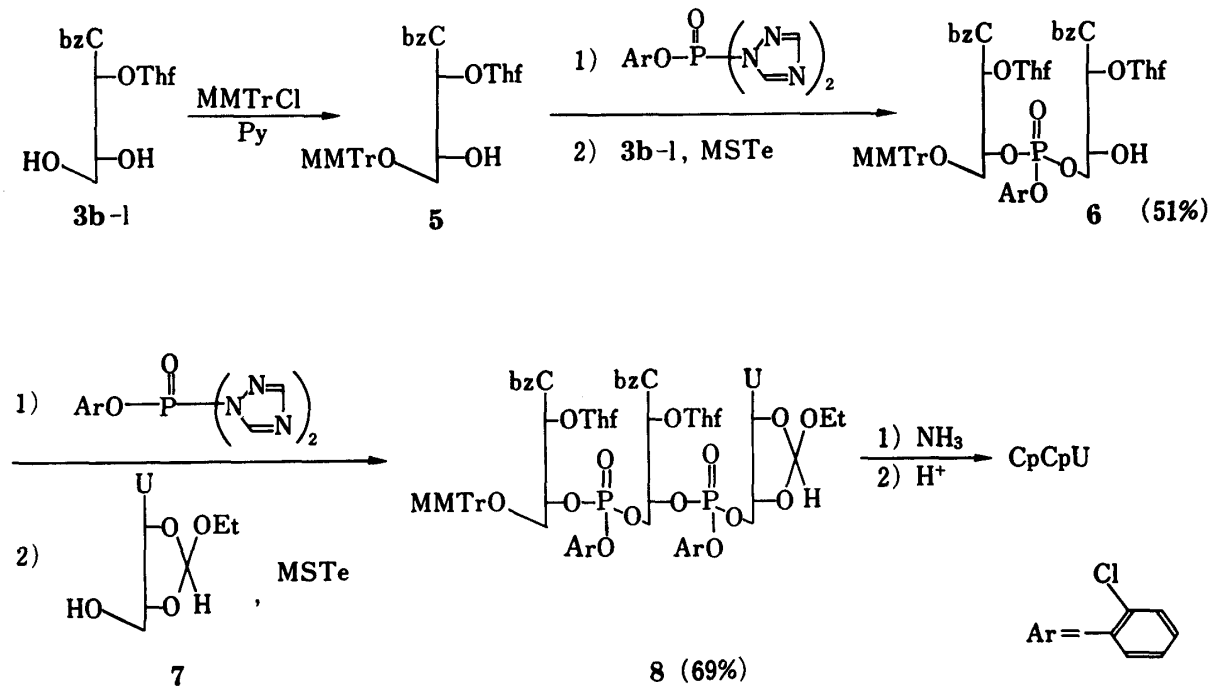


Chart 2

undesired 3′–3′ linked compound by reverse-phase chromatography on $C_{18}$ silica gel in a yield of 51%. Formation of the 3′–3′ linked side product could have been reduced by using an excess of nucleoside (**3d**) as shown in the synthesis of C–C–A (see "Experimental"). The dimer (**6**) was then phosphorylated as above and condensed with 2′,3′-ethoxymethylideneuridine[12] to give the trimer (**8**) in a yield of 69%. The trimer was deblocked by treatment with concentrated ammonia and with acid. Dilute hydrochloric acid or acetic was used. The absence of migration of internucleotidic linkages was confirmed by hydrolysis of C–C–U with RNase T2. The ratio of Cp to U was found to be as expected. The purity of the trimer was also confirmed by high pressure liquid chromatograpy (HPLC) on $C_{18}$ silica gel.

The other trimer C–C–A was synthesized by the same method. The other diastereoisomer (with higher *Rf* value) of **3b** was used to obtain the protected dimer (**6**). The yield was 65%. The fully protected C–C–A was obtained by condensation with 2′,3′-ethoxymethylidene *N*-benzoyladenosine[13] in a yield of 71%. The deblocked product C–C–A was characterized by hydrolysis with RNase T2.

**Selective Phosphorylation of 2′-*O*-Tetrahydrofuranyl Nucleosides**

In order to prepare protected oligonucleotide blocks suitable for condensation, the 5′-hydroxyl group of the nucleosides (**3**) was phosphorylated with *o*-chlorophenyl *p*-anisidophosphorochloridate (**9**)[14] (Chart 3).

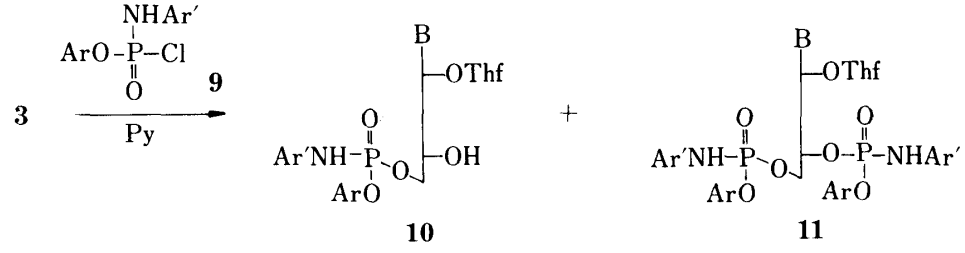


Chart 3

The yields of the 5′-phosphorylated compounds (**10**) and bisphosphorylated side products (**11**) are summarized in Table III together with the conditions used for phosphorylation. The desired products (**10**) were obtained in yields of 80 to 30%. Compound **10d**-l was obtained in a yield of 50% from **3d**-l by using methylimidazole (1.2 eq) in acetonitrile. The nucleotides (**10**) were used as key intermediates for preparing oligonucleotide blocks as described below.

TABLE III. Summary of Phosphorylation

| Compd. | **9** (eq) | Time (h) | Yield (%) **10** | **11** |
|---|---|---|---|---|
| **3a**-l | 1.0+0.3 | 15 | 61 | 13 |
| **3a**-h | 1.0 | 9 | 52 | 10 |
| **3b**-l | 1.0+0.3+0.3 | 26 | 66 | 28 |
| **3b**-h | 1.0+0.5+0.5 | 24 | 80 | 16 |
| **3c**-l | 1.0 | 5 | 52 | 6 |
| **3c**-h | 1.0 | 7 | 47 | 6 |
| **3d**-l | 1.0+0.2 | 8 | 29 | 20 |
| **3d**-h | 1.0+0.2 | 8 | 32 | 26 |

## Synthesis of pU–U–U–U–U–U, A–U–G–A–U–G and A–U–G–A–U–G–A–U–G

Chart 4 illustrates the synthesis of the hexanucleotide pU–U–U–U–U–U (**18**) by condensation of two trimers (**13** and **16**). Compound **13** was prepared from **10a** (lower isomer) by elongation in the 3′-direction using the same phosphorylation and condensation methods as described for the synthesis of **8**. The trinucleotide **16** was obtained from 2′,3′-ethoxymethylideneuridine 5′-(*o*-chlorophenyl)phosphate by condensation with **10a** (lower isomer). Elongation of the chain in the 5′-direction was performed by removing the anisidate group with isoamyl nitrite. Yields of protected oligonucleotides ranged from 50—60%. Due to the poor lipophilicity of uridine derivatives, a part of the product was not extracted with organic solvents.

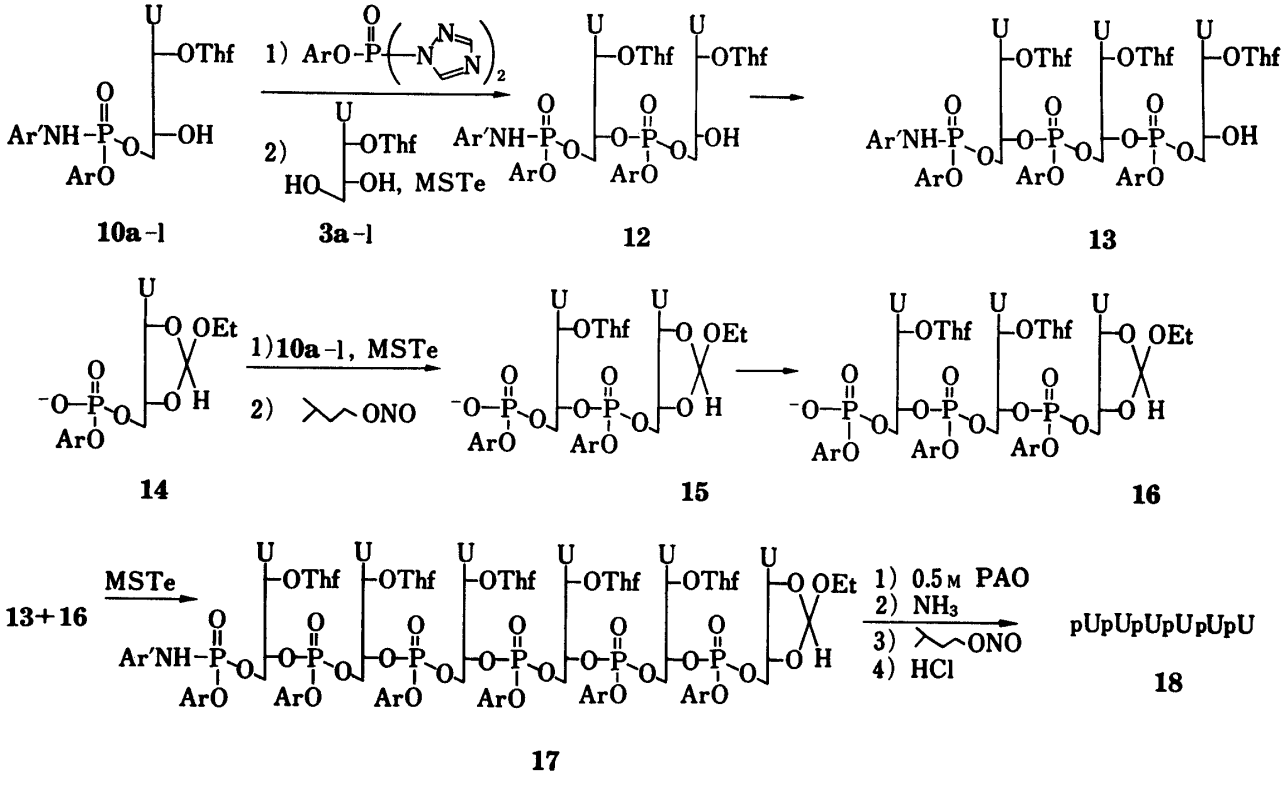


Chart 4

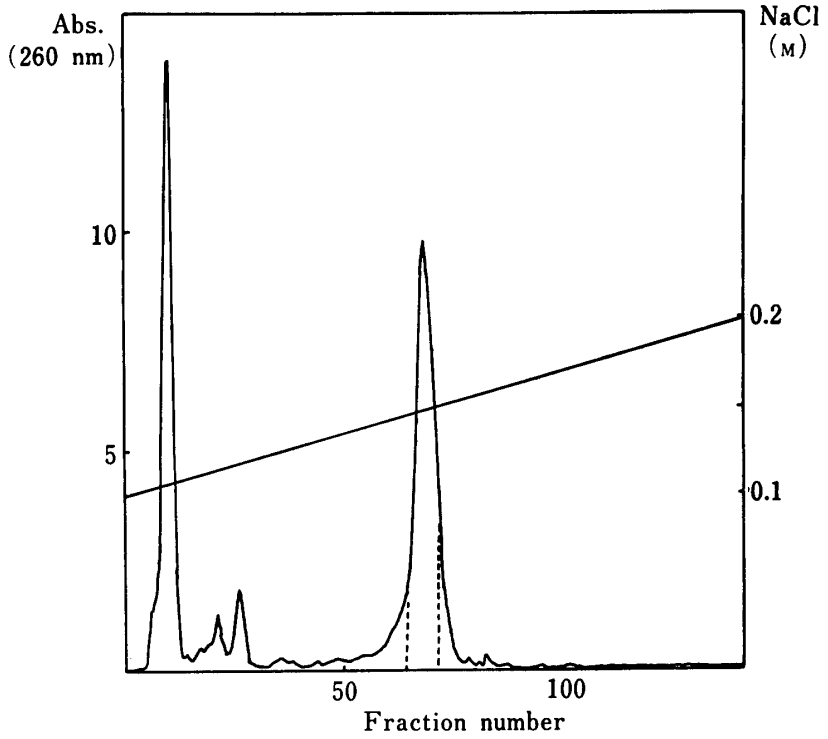


Fig. 1. Chromatography of A–U–G–A–U–G on a Column (1.6 × 40 cm) of DEAE-cellulose

Elution was performed with a linear gradient of sodium chloride (0.1—0.3 M, total 2000 ml) in 7 M urea and 0.02 M Tris–HCl (pH 7.5).

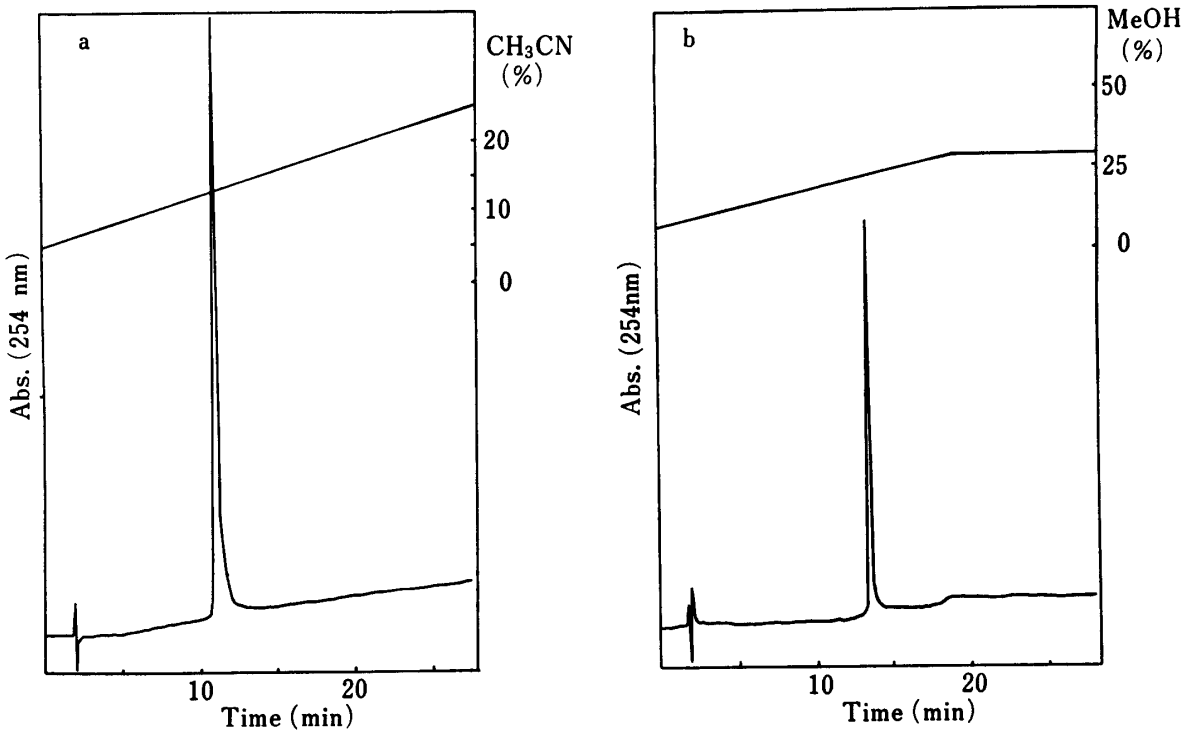


Fig. 2. Reversed Phase HPLC of A–U–G–A–U–G (a) and A–U–G–A–U–G–A–U–G (b)

$C_{18}$ silica gel ($\mu$-Bondapak, 5 $\mu$) was used with a linear gradient of acetonitrile in triethylammonium acetate (0.1 M, pH 7.0).

The fully protected hexanucleotide (**17**) was deblocked by treatment with 1,1,3,3-tetramethyl guanidinium 2-pyridinaldoxamate,[15] followed by concentrated ammonia, and then isoamyl nitrite. The furanyl protected hexanucleotide was separated by gel filtration and treated with 0.01 N hydrochloric acid. The hexanucleotide (**18**) was purified by anion-exchange chromatography and then by HPLC on $C_{18}$ silica gel.

For the synthesis of the hexamer (**23**) and nonamer (**28**) containing the methionine codon AUG, three trimer blocks (**19**, **21** and **24**) (Chart 5) were prepared as described for the synthesis

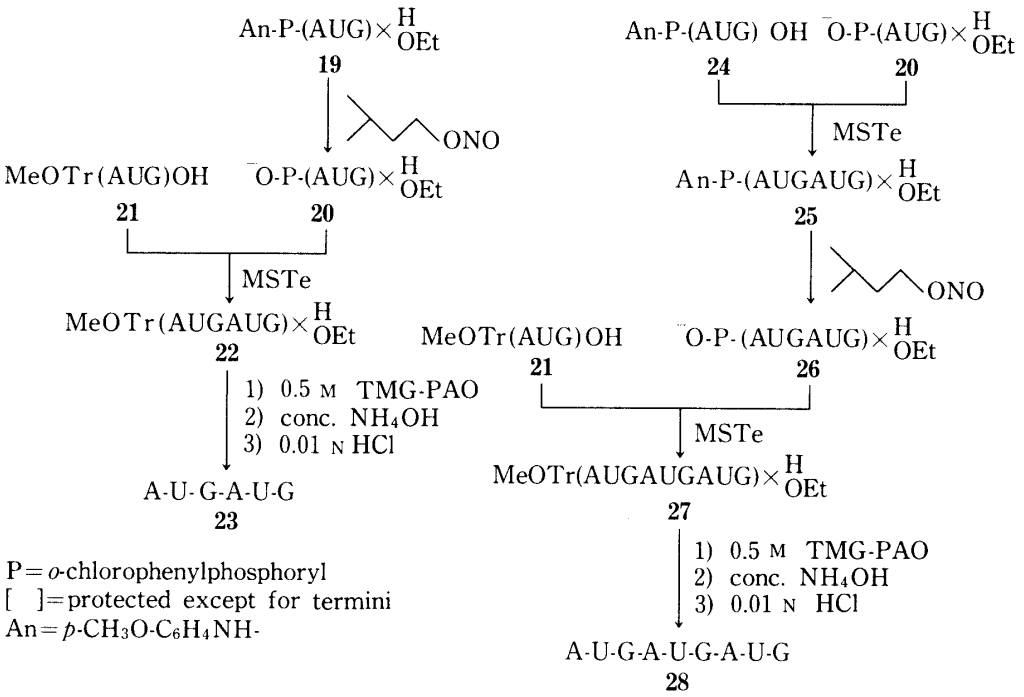


Chart 5

of **13** by elongation in the 3′-direction starting from **10c** in the cases of **19** and **24** or from the 5′-monomethoxytrityl-2′-tetrahydrofuranyladenosine (for **21**). The protected oligomers were isolated by chromatography on $C_{18}$ silica gel in yields of 62 to 83%. The hexamer was obtained by converting the 3′-terminal block (**19**) to the diester (**20**) with isoamyl nitrite followed by condensation with **21**, in a yield of 25%. Deblocking was performed as described for **18**. The deprotected hexamer A–U–G–A–U–G (**23**) was isolated by anion-exchange chromatography (Fig. 1) and analyzed by HPLC on $C_{18}$ silica gel (Fig. 2). The yield of the deblocking step was 45%. The nonamer A–U–G–A–U–G–A–U–G (**28**) was synthesized by two condensations as shown in Chart 5. The hexamer intermediate (**25**) was obtained by condensation of **20** with **24** in a yield of 33%. The 5′-phosphoro-*p*-anisidate of **25** was reacted with isoamyl nitrite and the phosphodiester (**26**) was condensed with the 5′-terminal block (**21**). The nonamer (**27**), which was obtained in a yield of 19%, was deblocked and purified as above. The hexamer (**23**) and nonamer (**28**) were characterized by analyses of base ratio, 5′-nucleotide and mobility shift in two dimensional homochromatography.

## Conclusion

Elongation of chains in the 3′-direction can be performed by phosphorylation of the 3′-hydroxyl group of 5′-protected **3** with *o*-chlorophenyl phosphate followed by selective condensation with the 5′-hydroxyl group of *N*,2′-protected nucleosides (**3**). This approach was applied to the synthesis of trimers C–C–U and C–C–A. Monomer addition and activation of elongating

chains are not advantageous for the synthesis of longer oligonucleotides. For the preparation of protected oligonucleotides suitable for block condensations, the 5′-hydroxyl group of **3** was phosphorylated selectively with *o*-chlorophenyl *p*-anisidophosphorochloridate to give **10**. The 5′-phosphoro-*p*-anisidate was converted to the phosphodiesters after preparation of oligonucleotide blocks and reacted with the 3′-hydroxyl group of the protected oligonucleotides. Uridine hexanucleotide (**18**), A–U–G–A–U–G (**23**) and AUG nonamer (**28**) were synthesized by this method. The condensation involving the 3′-hydroxyl of uridine trimer (**13**) proceeded satisfactorily. However, those involving the guanosine 3′-hydroxyl group did not go to completion under the conditions tested. The synthesis of 2′–5′ linked oligoadenylates by condensation of the 2′-hydroxyl and 5′-phosphodiester was achieved in a satisfactory yield.[6] Conformational factors for the 3′-hydroxyl group of nucleosides may affect the yields in condensations with the 5′-phosphodiesters. Condensations involving the 3′-hydroxyl group of four nucleotides will be reported elsewhere.

## Experimental

Paper chromatography (PPC) was performed by the descending technique using the following solvent system: isopropyl alcohol–conc.ammonia–water (7: 1: 2, v/v). Paper electrophoresis (PEP) was performed with 0.05 M triethylammonium bicarbonate (TEAB) (pH 7.5) at 900 V/40 cm. Thin-layer chromatography (TLC) was performed on plates of silica gel (Kieselgel $HF_{254}$, Merck) a mixture of chloroform–methanol. For reversed phase thin layer chromatography (RTLC), silanized silica gel, HPTLC RP-2 or RP-8 $F_{254}$ (Merck) was used with a mixture of acetone–water. For columns, silica gel (Merck, type 60 or H) was packed with the aid of chloroform and compounds were applied as concentrated solution in chloroform. Elution was performed with a mixture of chloroform–methanol, unless otherwise specified. For reversed phase column chromatography, alkylated silica gel ($C_{18}$ silica gel, 35—105 μ, Waters) was used with aqueous acetone. HPLC was carried out on an Altex 332 MP apparatus.

Other general methods for the characterization of ribooligonucleotides were as described previously.[14,16,17]

**3′,5′-Bis-*tert*-butyldimethylsilylnucleosides** (**1**)——**1a—c** were prepared by the published procedure[7] with a slight modification (increasing the amount of imidazole in dimethylformamide (DMF)). Uridine (49.0 mmol) in DMF (100 ml) was treated with imidazole (219 mmol) and *tert*-butyldimethylsilyl chloride (TBDMS-Cl) (110 mmol) at room temperature for 100 min. The yield of **1a** was 7.57 g (33%). Compound **1b** was similarly obtained in a yield of 50%, and **1c** was prepared in a yield of 43% by the same procedure as described for **1a** except that a larger excess of TBDMS-Cl (190 mmol) was used. The guanosine derivative (**1d**) was obtained by treatment of *N*-isobutyrylguanosine (29.3 mmol) in DMF (30 ml) with imidazole (178 mmol) and TBDMS-Cl (92 mmol) at room temperature for 1 h. The mixture was treated for another 1 h with TBDMS-Cl (30 mmol) and then ice water was added. The product was extracted with chloroform, and the extract was washed with sat. sodium bicarbonate then with water and sat. sodium chloride. The organic layer was concentrated and applied to a column (6.5 × 23 cm) of Silica gel 60 (360 g). The compound was eluted with benzene–ethyl acetate (25—50%); yield, 2.73 g (16%). mp 128—134°C. Compound **1d** was characterized from the $^1H$ NMR chemical shifts of the 2′ and 3′-hydroxyl groups.

**2′-*O*-Tetrahydrofuranyluridine** (**3a**)——**1a** (10 mmol), *p*-toluenesulfonic acid (3.5 mmol) and tetrahydrofuran (THF) (50 ml) were stirred with 2,3-dihydrofuran (100 mmol) in an ice bath. After 2 h, **1a** was detected on TLC (10: 1) and the mixture was treated with 2,3-dihydrofuran (42 mmol) for 2.5 h. The whole mixture was then neutralized with conc. ammonia (0.44 ml) and concentrated. The product was extracted with chloroform (250 ml). The solution was washed twice with sat. sodium bicarbonate, once with water, and once with sat. sodium chloride, then concentrated to dryness. The residue was stirred with TBAF (36 mmol) in THF (100 ml) for 2 h, then the THF was evaporated off. The residue was precipitated with hexane–ether (200—40 ml) from its solution in chloroform (15 ml) and the syrup was reprecipitated as above. The decanted syrup was applied to a column (3.6 × 27 cm) of Silica gel 60 (120 g) and eluted with chloroform containing increasing amounts of methanol (up to 10%). The yield and properties are shown in Table I and II.

**2′-*O*-Tetrahydrofuranyl-*N*-benzoylcytidine** (**3b**)——**1b** (10 mmol) was treated with 2,3-dihydrofuran (94 mmol) as above and isolated by chromatography on Silica gel 60 (80 g) using chloroform containing up to 5% methanol.

**2′-*O*-Tetrahydrofuranyl-*N*-benzoyladenosine** (**3c**) **and *N*-Isobutyrylguanosine** (**3d**)——The compounds were prepared as described for **3a**. Further treatment with 2,3-dihydrofuran and *p*-toluenesulfonic acid was necessary if any starting material remained. **3d** was extracted with chloroform–pyridine (200: 60) after treatment with TBAF.

**3′,5′-*O*-Diacetyl-2′-*O*-tetrahydrofuranylnucleosides** (**4**)——For example, **3a—h** (33 mg, 0.11 mmol) was treated with acetic anhydride (1 ml) in pyridine (2 ml) at room temperature for 18 h. Methanol (2 ml)

was added and the mixture was concentrated. The residue was dried by coevaporation of ethanol and water.

**C-C-U**——**3d**-l (0.6 mmol) was dried by addition and evaporation of pyridine and treated with monomethoxytrityl chloride (0.81 mmol) in pyridine (3 ml) at room temperature for 18 h. Water (2 ml) was added with cooling, and then sat. sodium bicarbonate (20 ml) was added. The product (**5**) was extracted with chloroform (30 ml), concentrated and applied to a column (3.2×2.2 cm) of Silica gel H, (7 g). Elution was performed with chloroform-methanol. The yield of **5** was 362 mg, 88%. The 5′-protected nucleoside (**5**) (0.6 mmol) in pyridine (1 mmol) was phosphorylated with *o*-chlorophenyl phosphoroditriazolide (obtained by mixing *o*-chlorophenyl phosphorodichloridate (0.99 mmol) with triazole (2.7 mmol) and triethylamine (2.1 mmol) in dioxane (3 ml) at 0°C, and stirring the mixture for 1 h at room temperature). Aqueous pyridine (5 ml) and 0.1 M TEAB (20 ml) were added with cooling. The product was extracted with chloroform (30 ml), washed twice with 0.1 M TEAB (20 ml) and concentrated. The residue was dried by addition and evaporation of pyridine three times and mixed with cytidine derivative (**3d**-l, 0.38 mmol). The mixture was dried by addition and evaporation of pyridine three times, then treated with MSTe (1.15 mmol) in pyridine (1 ml) at 30°C for 25 min. Aqueous pyridine (50%, 3 ml) and 0.1 M TEAB (20 ml) were added with cooling. The dimer (**6**) was extracted with chloroform (30 ml) washed twice with 0.1 M TEAB, applied to a column (3×13 cm) of $C_{18}$ silica gel and eluted with acetone–20 mM TEAB (65: 35). The yield was 251 mg (51%). For the synthesis of the trimer **8**, protected C–C (**6**, 232 mg, 0.81 mmol) was phosphorylated as above by using *o*-chlorophenyl phosphoroditriazolide (9.36 mmol) and condensed with 2′,3′-ethoxymethylideneuridine (**7**) (0.15 mmol) in the presence of MSTe (0.40 mmol) in pyridine (1 ml) at 30°C for 30 min. The mixture was worked up as described for **6**, and **8** was isolated by chromatography on $C_{18}$ silica gel as above. Elution was performed with acetone(70—75%)–20 mM TEAB. **8** was precipitated with pentane in a yield of 180 mg (69%). An aliquot (19 mg, 0.011 mmol) was dissolved in pyridine (2 ml), heated with conc. ammonia (10 ml) at 55°C for 5 h, and concentrated. The residue was dissolved in pyridine (4 ml) and one half was concentrated to remove pyridine. The residue was dried three times by coevaporation with toluene, then treated with 80% acetic acid (2 ml) for 4.5 h. One quarter of the solution was concentrated. The residue was dissolved in 0.1 M TEAB (5 ml), and the solution was washed with ethyl acetate and subjected to PPC. C–C–U ($22A_{260}$ units, *ca.* 77%) was eluted with water and an aliquot ($2A_{260}$ units) was hydrolyzed with RNase T2 (1 μunit) in ammonium acetate (pH 4.5, 5 μl) at 37°C for 2 h. The mixture was subjected to PEP (pH 7.5) and absorbance of the products (uridine, Rm 0.18, Cp, Rm 0.97) was measured in 0.01 M HCl (2 ml) to obtain 0.51 $A_{262}$ and 1.33 $A_{279}$. The trimer was synthesized by essentially the same procedure as described for C–C–U. Another isomer (**3b**-h, 0.60 mmol) was used as the starting material and 0.48 mmol of the monomethoxytritylated compound was phosphorylated with *o*-chlorophenyl phosphorodichloridate (1.0 mmol). The protected Cp was condensed with an excess of **3b**-h (0.53 mmol), using MSTe (1.1 mmol). The yield of the protected dimer was 393 mg (64%). C–C–A was prepared by condensing the dimer (0.15 mmol) and 2′,3′-ethoxymethylidene-*N*-benzoyladenosine (0.14 mmol). After deblocking, C–C–A was isolated by PPC in a yield of 77% and characterized by hydrolysis with RNase T2 to yield adenosine (0.57 $A_{257}$ in 0.01 N HCl) and Cp (1.06 $A_{279}$ in 0.01 N HCl) in a ratio of 1.00: 2.08.

**Preparation of 5′-Phosphorylated Nucleosides (10) and the Bisphosphorylated Nucleosides (11)**——2′-*O* Tetrahydrofuranyluridine (**3a**-l) (1.01 mmol) was added to *o*-chlorophenyl-*p*-anisidophosphorochloridate (**9**, 1.01 mmol) in pyridine (3.6 ml) at 0°C, and the mixture was stirred at room temperature for 9 h. Aqueous pyridine (3 ml) and sat. sodium bicarbonate (45 ml) were added with cooling. The product was extracted with chloroform, washed with sat. sodium bicarbonate, concentrated, and applied to a column (3×3.3 cm) of Silica gel 60 (10 g). The bisphosphorylated product (**11a**) was eluted with 6% methanol in chloroform (118 mg, 13%) then the 5′-phosphorylated compound (**10a**) was eluted. The yield of **10a** was 377 mg (61%).

**The trinucleotide (13)**——**10a** (0.2 mmol) was phosphorylated with *o*-chlorophenyl phosphoroditriazolide (0.3 mmol) and elongated in the 3′-direction by the procedure described for the preparation of C–C–U. The uridine 3′-phosphodiester was precipitated with ether–pentane (1: 1.75 ml) from its solution in chloroform to yield 151 mg (0.17 mmol, 84%) of product, which was then condensed with **3a** (0.14 mmol) using MSTe (0.44 mmol) in pyridine (1 ml) at 30°C for 20 min. The dimer **12** was isolated by preparative TLC on silica gel (8: 1) in a yield of 77 mg (50%). **12** (0.070 mmol) was phosphorylated with *o*-chlorophenyl phosphoroditriazolide (0.100 mmol) and condensed with **3a** (0.064 mmol) by using MSTe (0.170 mmol) for 35 min. The trimer (**13**) was isolated by preparative TLC (8: 1) in a yield of 55 mg (55%).

**The Trinucleotide (16)**——2′,3′-Ethoxymethylideneuridine was prepared by treatment of uridine (2 mmol) with ethylorthoformate (2.5 ml) in the presence of *p*-toluenesulfonic acid (0.13 equivalent with respect to uridine) and isolated by chromatography on Silica gel 60 (10 g). The nucleoside (0.57 mmol) was phosphorylated with *o*-chlorophenyl phosphoroditriazolides (0.75 mmol) at room temperature for 1 h, then the reaction was stopped by addition of aqueous pyridine (3 ml) with cooling. The nucleotide (**14**) was extracted with pyridine–butanol (1: 2, 30 ml) and the organic layer was washed twice with 0.1 M TEAB. **14** (170 mg, 50%) was precipitated with ether–pentane (1: 1) and 0.13 mmol of **14** was condensed with **10a** (0.1 mmol) by using MSTe (0.31 mmol) in pyridine (1 ml) at 30°C for 1 h. The fully protected dinucleotide was isolated by preparative TLC (10: 1) and the band was eluted with chloroform–methanol (5: 1). The yield was 69 mg (64%). A part (61 mg, 0.060 ml) of the product was treated with isoamyl nitrite (2.4 mmol) in pyridine–acetic acid (5: 4, 1.6 ml) at 30°C for 3 h to yield **15**. Pyridine (10 ml) and 0.2 M TEAB were added

with cooling. TEAB (0.3 M, 10 ml) was added and the mixture was washed with ether–pentane (1: 1). **15** was extracted with chloroform, washed with 0.3 M TEAB and precipitated with ether–pentane (1: 1, 50 ml) from its solution in chloroform (5 ml). The yield of **15** was 50 mg (77%). **15** (50 mg, 0.046 mmol) was then condensed with **10a** (0.044 mmol) as above and the fully protected trimer was isolated in a yield of 61% (42 mg); it was converted to the 5′-phosphomonoester (**16**) by treatment with isoamyl nitrite.

**pU-U-U-U-U-U (18)**——**16** (0.016 mmol) and **13** (0.014 mmol) were treated with MSTe (0.079 mmol) in pyridine (*ca.* 1 ml) at 30°C for 1 h. Aqueous pyridine (2 ml) was added with cooling. Pyridine (10 ml) and 0.1 M TEAB (20 ml) were added to the mixture, then the product (**17**) was extracted with chloroform (30 ml). The organic layer was washed with 0.1 M TEAB and subjected to preparative TLC (7: 1). The band containing **17** was eluted with 20% methanol in chloroform and **17** (27 mg, 64%) was precipitated with pentane (30 ml). **17** (12.7 mg, 4 μmol) was deblocked first by treatment with 0.5 M tetramethylguanidinium pyridine-2-aldoxamate (0.96 ml) at room temperature for 24 h. The mixture was diluted with aqueous pyridine (30%, 10 ml), and extracted twice with ethylacetate, then the organic phase was back extracted with aqueous pyridine. The aqueous pyridine was passed through a column (0.4 × 3.5 cm) of Dowex 50 W × 2 (pyridinium form) and concentrated. The residue was dissolved in pyridine (0.1 ml) and treated with conc. ammonia (0.5 ml) at 55°C for 4 h. The volatile materials were removed by evaporation and the residue was applied to a column (0.7 × 147 cm) of Sephadex G-50 equilibrated with 0.1 M TEAB. Compounds eluted in the first peak (30 $A_{260}$) were treated with 0.01 N HCl (2 ml) at room temperature for 2 h and neutralized with 0.01 N ammonia. The product (**18**) was isolated by ion-exchange chromatography on a column (1 × 20 cm) of diethyl aminoethyl (DEAE)-cellulose. Elution was performed with a linear gradient of TEAB (0.2—0.7 M, total 300 ml). **18** (34 $A_{260}$) was eluted at 0.4 M salt and further purified by HPLC on $C_{18}$ Silica gel (Hypersil ODS). The yield of **18** was 4 $A_{260}$ units (2.6% from **17**).

**The Trinucleotide (20)**——**10c** (1.55 mmol) was phosphorylated with *o*-chlorophenyl phosphoroditriazolide (2.4 mmol) and condensed with **3a** according to the procedure described for **13**. The protected pAU (1.038 mmol) thus obtained was phosphorylated and condensed with 2′,3′-ethoxymethylidene-*N*-isobutyryl-guanosine (1.09 mmol) using MSTe (2.09 mmol) at 30°C for 25 min. The trimer (**19**) was separated on a column (3.2 × 15 cm) of $C_{18}$ silica gel with acetone–0.2% aqueous pyridine (50—70% acetone) in a yield of 83% (1.568 g, 0.868 mmol). **19** (0.5 mmol) was treated with isoamyl nitrite (20 mmol) in pyridine–acetic acid (5: 4, 13 ml) at 30°C for 3 h, then the mixture was diluted with pyridine–0.2 M TEAB (1: 2, 60 ml). The mixture was washed three times with ether–pentane (1: 1, 100 ml) and extracted first with chloroform (70 ml) then twice with chloroform–pyridine (2: 1, 15 ml). The solution was concentrated and the residue was dried by coevaporation with pyridine and toluene. **20** was precipitated with ether–pentane in a yield of 90% (813 mg, 0.448 mmol).

**The Trimer (21)**——5′-*O*-Monomethoxytrityl-2′-*O*-tetrahydrofuranyl-*N*-benzoyladenosine (0.850 mmol) was phosphorylated and condensed with **3a** (1.03 mmol) as described above to yield the dinucleotide (690 mg, 0.575 mmol, 68%), which was phosphorylated with *o*-chlorophenyl phosphoroditriazolide (0.860 mmol) and condensed with **3d**-h (0.749 mmol using MSTe (1.20 mmol) at 30°C for 22 min. The trimer (**21**) was isolated by silica gel chromatography in a yield of 62%, 641 mg, 0.357 mmol.

**The Hexamer, A-U-G-A-U-G (23)**——**20** (0.082 mmol) and **21** (0.081 mmol) were dried by coevaporation with pyridine and treated with MSTe (0.23 mmol) at 30°C for 1 h. The extent of reaction was checked by TLC and RTLC. Further addition of MSTe (0.21 mmol) was carried out, and after 1 h the reaction was stopped by addition of aqueous pyridine. The mixture was applied to a column (3 × 11 cm) of $C_{18}$ silica gel in acetone–0.2% aq. pyridine (50—80% acetone). The protected hexamer (**22**) was precipitated with pentane in a yield of 25% (70 mg, 0.020 mmol). **22** (35 mg, *ca.* 10 μmol) was deblocked by treatment with oxamate and conc.ammonia as described for (**18**). Acid-labile protecting groups were removed by dissolving the product in 0.01 N HCl and adjusting the solution to pH 2 with 0.1 N HCl. The deblocked product (**23**) was isolated by chromatography on DEAE-cellulose. The main part of the peak in Fig. 1 (400 $A_{260}$) was purified by HPLC (Fig. 2) in a yield of 70%. A–U–G–A–U–G (**23**) was characterized by mobility shift analysis (Fig. 3a) and base composition analysis by anion-exchange HPLC after complete digestion with nuclease P1. The ratio of pA: pU: pG was 1: 2.28: 2.25.

**The Hexamer (26)**——The trimer (**24**) was prepared by essentially the same method as described for the synthesis of **19** and **20** except that **3d** was used in the last condensation. The yields of the dimer and trimer (**24**) were 72 and 62%, respectively. **24** (0.110 mmol) and **20** (0.101 mmol) were treated with MSTe (0.983 mmol) at 25°C for 80 min. The mixture was applied to a column (3 × 11 cm) of $C_{18}$ silica gel (acetone–0.2% pyridine, 50—80% acetone). The trimer (**24**) (81 mg) was recovered and the product (**25**) was obtained in a yield of 33% (116 mg). **25** (163 mg, 0.047 mmol) was treated with isoamyl nitrite to yield **26** (153 mg, 0.044 mmol, 93%) by the same procedure as described for the preparation of **20**.

**The Nonamer A-U-G-A-U-G-A-U-G (28)**——The hexamer (**26**) (65 mg, 0.036 mmol) and **21** (104 mg, 0.030 mmol) were dried by evaporation with pyridine and treated with MSTe (0.29 mmol) in pyridine (0.3 ml) at 30°C for 3 h. Aqueous pyridine (0.3 ml) was added and the mixture was applied to a column (1.8 × 5.5 cm) of $C_{18}$ silica gel (acetone–0.2% aq. pyridine, 60—86% acetone). The trimer (**21**) (21 mg) was recovered and the product (**28**) was obtained in a yield of 19% (36 mg, 6.9 μmol). **28** was deblocked as described for the hexamer (**23**) and chromatographed on DEAE-cellulose in 7 M urea, 0.02 M Tris-HCl, pH 7.5. Elution

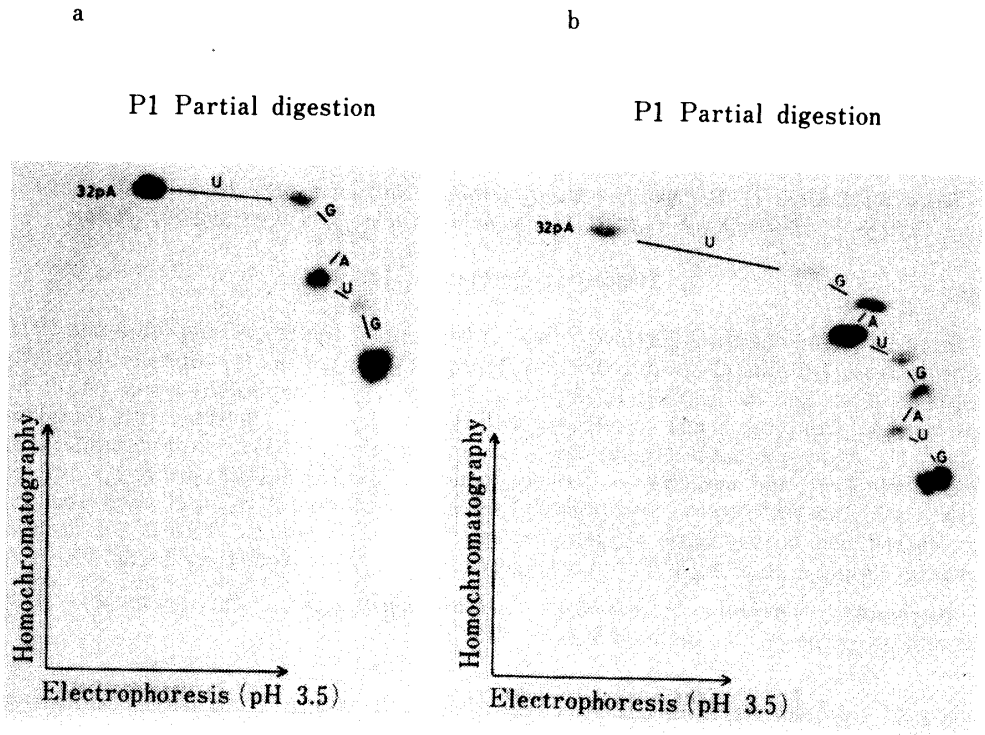


Fig. 3. Two Dimentional Homochromatography of A–U–G–A–U–G (a) and A–U–G–A–U–G–A–U–G(b)

was performed with a linear gradient of sodium chloride and the nonamer (**28**) was eluted with 0.2 M salt. The product was further purified by HPLC (μ-Bondapak C-18) using 50% methanol and increasing amounts of 1/15 M phosphate (pH 7.5). The fractionated **28** (32 $A_{260}$) was checked by HPLC and analyzed by the same procedure as described for **23**. The result of mobility shift analysis is shown in Fig. 3b.

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