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Studies on Transfer Ribonucleic Acids and Related Compounds. XXXVIII.[1] A Rapid Method for the Synthesis of Ribooligonucleotides by using 3′,5′-Unsubstituted Nucleosides. Synthesis of a Hexanucleotide containing Anticodon Triplet of *E. coli* $tRNA_f^{Met}$

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N-Protected 2′-*O*-(*o*-nitrobenzyl)nucleosides have been used as condensing units with protected nucleoside 3′-phosphates. After formation of the 3′—5′ linkage, the 3′-hydroxyl group of the dinucleoside monophosphate was phosphorylated by treatment with ether *p*-chlorophenyl phosphate plus DCC or *p*-chlorophenyl phosphoroditriazolide. The dinucleotide was further elongated in the 3′-direction by condensation with the *N*- and 2′-*O*-protected nucleosides followed by phosphorylation. Protected C–Gp and C–A–Up were prepared by this procedure. The trimer was used in the synthesis of hexamer C–A–U–A–A–C by condensation with the 3′-*O*-(*o*-nitrobenzyl)-containing trimer block. This hexamer corresponds to the bases 35 to 40 of *E. coli* $tRNA_f^{Met}$, which are located in the anticodon loop. A trimer containing uridine in the middle position has also been synthesized by this method in combination with an extraction procedure for the intermediate to permit rapid isolation.

Keywords——tRNA fragments; 2′-O-(*o*-nitrobenzyl)nucleosides; 3′,5′-unsubstituted ribonucleosides; phosphorylation; nucleotide condensation; 3′-*O*-(*o*-nitrobenzyl)nucleosides; extraction

We have been synthesizing ribooligonucleotides corresponding to fragments of $tRNA_f^{Met}$ from *E. coli* by using 2′-*O*-(*o*-nitrobenzyl)nucleosides as intermediates.[2] These 2′-(nBzl)-nucleosides[3] were prepared by treatment with either *o*-nitrobenzyl bromide[2a,4] or *o*-nitrophenyldiazomethane.[1] The 3′-isomers were also obtained as a by-product, especially during preparations using *o*-nitrophenyl-diazomethane. The 3′(nBzl)adenosine was a useful starting material for the synthesis of (2′-5′) oligoadenylates.[5] The 3′-(nBzl)nucleosides can be suitable 3′-terminal units in the synthesis of oligonucleotides by the triester method, since the alkali-stable 3′-substituent should prevent undesired attack of the 3′-hydroxyl group on internucleotidic phosphates during removal of aryl groups with alkali. In the present paper we report the use of 3′-*O*-(nBzl)nucleosides as the terminal units during the synthesis of the hexanucleotide C–A–U–A–A–C, which has a sequence corresponding to the anticodon loop of *E. coli*. For this synthesis we employed a rapid method for the preparation of di- and trinucleotide blocks from 3′,5′-unsubstituted nucleosides. An extraction procedure is described for the synthesis of trimers containing uridine in the middle position.

Synthesis of Oligonucleotides by using 3′,5′-Unsubstituted Nucleosides

Suitably protected ribooligonucleotide blocks, especially trimers, are useful intermediates for syntheses of polynucleotides. We have previously reported a general method for the preparation of ribotriester blocks with the 3′-phosphodiester end by using *p*-chlorophenyl-N phenylchlorophosphoroamidate,[6] and these blocks were used in syntheses of ribopolynucleotides.[2d–f] If the 5′-hydroxyl group of *N*-,2′-protected ribonucleosides can be condensed with the 3′-phosphodiester of the growing chain without formation of 3′-3′ linkages, preparation of nucleotide blocks can be simplified. Neilson used *N*-acyl-2′O-tetrahydropyranylibonucleosides in the synthesis of protected oligonucleotides and did not detect 3′-3′ linked compounds.[7] Cashion *et al.*[8] and Agarwal *et al.*[9] reported syntheses of deoxyribooligonucleotides by condens-

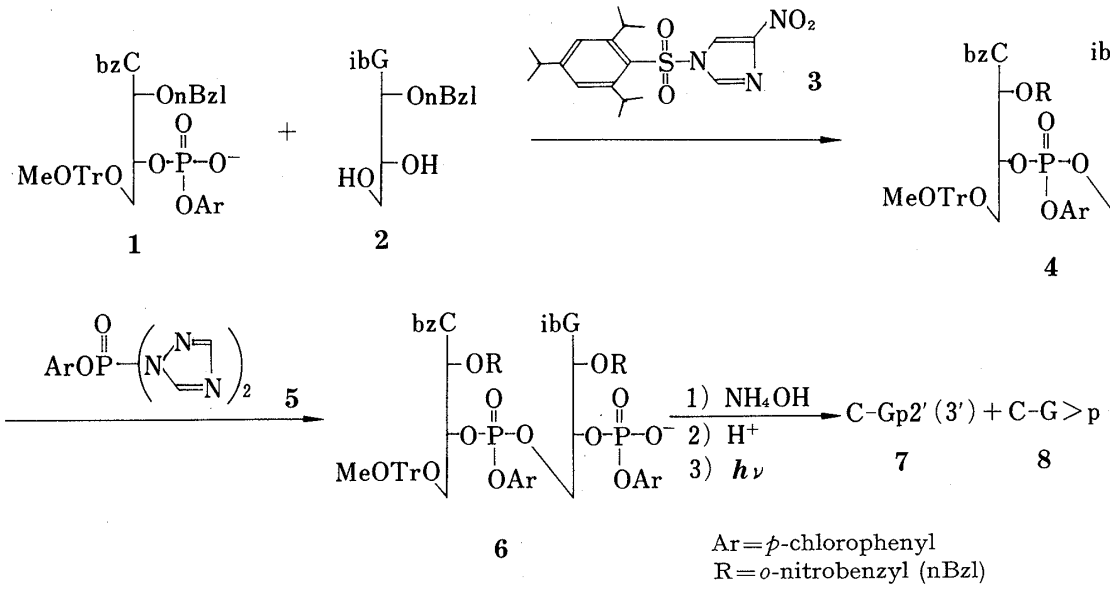


Chart 1

ing 3',5'-unprotected nucleosides. However, in the case of deoxyribonucleosides, the 3'-hydroxyl group seemed to be less hindered compared to ribonucleosides. We have isolated 3'-3' linked deoxyribodinucleosides monophosphates when *N*,5'-protected nucleoside 3'-*p*-chlorophenylphosphates were condensed with 3'-unsubstituted deoxyribonucleosides with a variety of yields, depending upon the sequences.[10] Chattopadhyaya and Reese reported a similar result.[11] It was considered that the 3'-hydroxyl group of 2'-substituted ribonucleosides must be less reactive than in the deoxy series and the formation of 3'-3' linked compounds may not disturb the synthesis if a small amount of the by-product could be removed by chromatography on silica gel. We investigated the possibility of formation of 3'-3' linked dinucleoside monophosphates by condensation of a 3'-phosphodiester with N,2'-protected guanosine, as shown in Chart 1. A bulky condensing reagent 2,4,6-triisopropylbenzenesulfonyl 3-nitroimidazolide[12] (TPS–NI, **3**) was used as the condensing reagent, since the active intermediate was supposed to be a mixed anhydride[13] of a phosphate and a sulfonic acid, or a phosphoronitroimidazolide. The phosphodiester **1** was treated with the reagent **3** for 30 min and then reacted with 3',5'-unprotected nucleoside (**2**) at 30° for 2 days. Thin layer chromatography (TLC) showed two new spots besides the starting material (**2**), These monomethoxytrityl-containing products were isolated by silica gel chromatography in a ratio of 1 : 1, and the yield was 88%. To determine whether these were diastereoisomers of **4** or whether one of them was the 3'-3' linked dinucleoside, the compounds were phosphorylated by treatment with *p*-chlorophenyl phosphoroditriazolide (**5**).[14] The 3'-5' linked compound (**14**) should give the dinucleotide (**6**) which can be deblocked by successive treatments with concentrated ammonia, 80% acetic acid and UV light at a wavelength longer than 280 nm to give **7** and **8**. The 3'-3' linked by-product would give C3'p3'-G5'p(p-ClC_6H_4) which could be distinguished from **7** and **8** only by 5'-phosphorylation using polynucleotide kinase[15] and [γ-^{32}P]ATP. The result of the phosphorylation showed that both of the fractions gave *pC–Gp and *pC–G>p. Thus it was proved that 3'-3' linked compounds were not formed in the reaction shown in Chart 1, and the products were two diasteroisomers of **4**. The deblocked products **7** and **8** were also characterized by digestion with RNase T1 and venom phosphodiesterase to exclude the possibility of contamination by by-products.

This approach was then applied to the synthesis of a protected trinucleotide (**11**, Chart 2) corresponding to the anticodon of *E. coli* $tRNA_f^{Met}$, which could be condensed with a trimer having the free 5'-hydroxyl group. The nucleotide (**1**) was condensed with 1.4 equivalents of

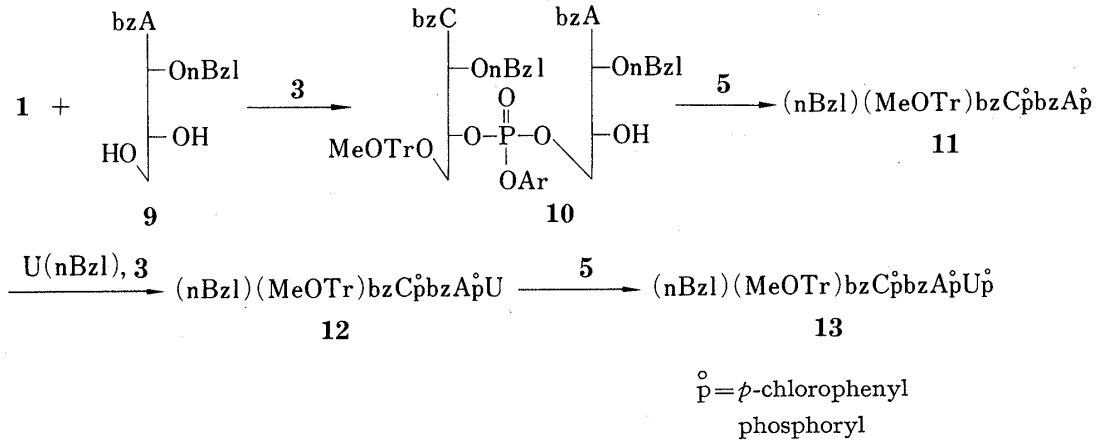


Chart 2

the 3′,5′-unprotected adenosine (**9**) to yield the dinucleoside monophosphate (**10**) in a yield of 77%, and phosphorylated with a three-fold excess of the phosphorylating reagent (**5**) to yield **11**, which was characterized by digestion with venom phosphodiesterase and RNase T2 after deblocking as described for the synthesis of **4**. The dinucleotide (**11**) was allowed to react with U (nBzl) using the same condensing reagent (**3**), and **12** was separated from the nucleoside by silica gel chromatography. *Rf* values for the diastereoisomers of the product (**12**) were much higher (0.50, 0.53) than that of U(nBzl) (0.24). **12** was then phosphorylated with **5** to give **13** in a yield of 88%. The trimer was characterized by the method described for the dimer (**6**).

Synthesis of a Hexanucleotide containing the Anticodon of $tRNA_f^{Met}$ by using 3′-*O*-(*o*-Nitrobenzyl)cytidine as the Terminal Unit

The trinucleotide block (**13**) obtained above served as a key intermediate in the synthesis of a hexanucleotide corresponding to the bases 35 to 40 of the $tRNA_f^{Met}$ by condensation with protected A–A–C (**18**), as shown in Chart 3. 2′-*O*-(Benzoyl)-3′-*O*-(*o*-nitrobenzyl)N-benzoylcytidine (**14**) which had been prepared by monomethoxytritylation of bzC3′ (nBzl)[1] followed by 2′-benzoylation and 5′-demonomethoxytritylation, was reacted with a 1.2-fold excess of **15** in the presence of triisopropylbenzenesulfonyl tetrazolide (TPS–Te).[16] The condensed product was isolated by chromatography on silica gel and acidic treatment afforded the dimer **16** in a yield of 66%. Condensation was repeated with a 1.2-fold excess of **15** with respect to **16**, and the trimer (**17**) was isolated by chromatography on silica gel in a yield of 68%. An

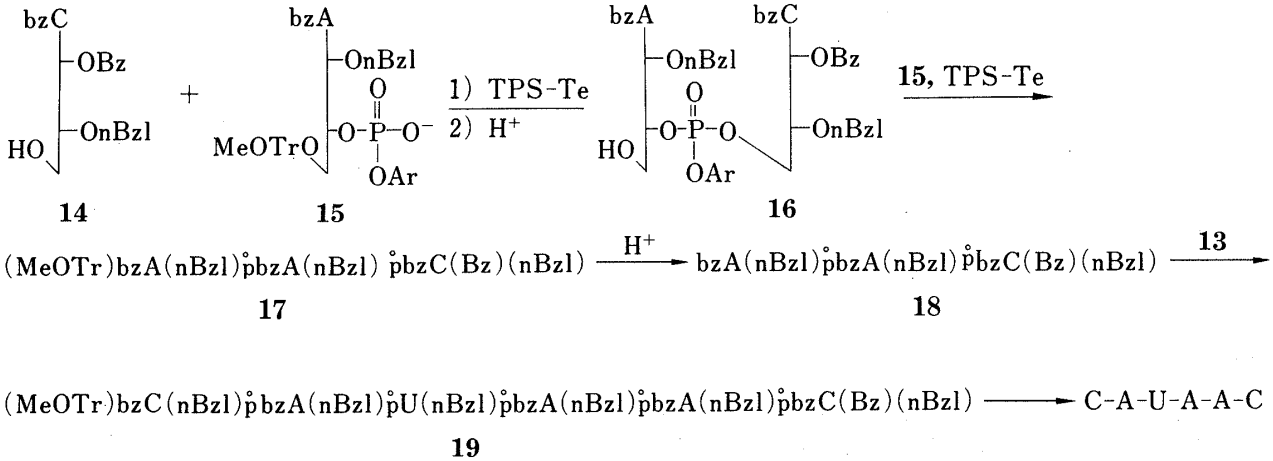
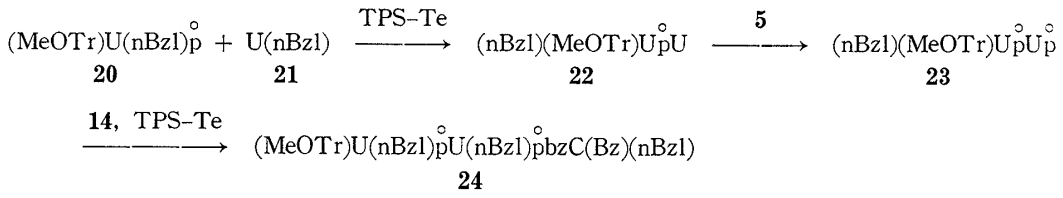


Chart 3

aliquot of **17** was deblocked as described for **6** and A–A–C was characterized by digestion with venom phosphodiesterase. The deblocked trimer was further characterized by 5′-labelling with [γ-^{32}P]ATP and polynucleotide kinase to confirm the absence of phosphodiester linkages involving the 2′-hydroxy group of cytidine. The labelled product was subjected to homochromatography[17] to confirm the structure.

The trimer **17** was demonomethoxytritylated by treatment with 80% acetic acid and **18** was reacted with **13** by treatment with TPS–Te. After 2 days, TLC showed no starting materials and the mixture was applied to a column of silica gel. The hexamer (**19**) was isolated and deblocked by treatment with ammonia, and then with 80% acetic acid. The *o*-nitrobenzylated hexamer was applied to a column of DEAF-cellulose and eluted with bicarbonate buffer containing 20% ethanol without the delay which was observed in the separation of *o*-nitrobenzyl-containing oligonucleotides (probably due to interaction of the *o*-nitrobenzyl groups with cellulose in the absence of ethanol). The partially protected hexamer was deblocked by irradiation with UV light and purified by paper chromatography and paper electrophoresis. Acidic electrophoresis resolved a slower travelling compound which was tentatively assigned as C–A–C–A–A–C formed *via* a 4-substituted pyrimidine by upon prolonged treatment with TPS–Te, as suggested by Ubasawa and Reese.[18] The product C–A–U–A–A–C was characterized by mobility shift analysis[19] using electrophoresis and homochromatography after labelling the 5′-end with ^{32}P.



20 **21** **22** **23** **24**

Chart 4

A Rapid Method for the Synthesis of Trimers containing Uridine in the Middle Position

By essentially the same method as described for the synthesis of **13**, a protected trimer (**24**, Chart 4) was prepared by extraction of the intermediate (**22**) with organic solvent. The starting mononucleotide (**20**) was reacted completely by condensing a twofold excess of U (nBzl) (**21**) with TPS–Te overnight and **21** was removed with triethylammonium bicarbonate from the mixture in chloroform. The dimer (**22**) was then phosphorylated with **5** and **23** was reacted with **14** using TPS–Te. The trimer (**24**) was isolated by chromatography on silica gel in an overall yield of 52%. An aliquot was deblocked for characterization. By-products caused by attack of the 2′-hydroxyl group of cytidine during ammonia treatment were not detected in homochromatography after labelling. The product U–U–C was hydrolyzed by treatment with RNase A to yield Up and C in a correct ratio. This method is applicable to syntheses of trinucleotides having the 3′-phosphate if suitably protected nucleoside 3′-phosphates are used instead of **14**. Only U(nBzl) can be removed under the present conditions, since other *o*-nitrobenzyl derivatives of *N*-acylnucleosides are insoluble in water. If suitable conditions are found to dissolve these derivatives during extraction from solutions of reaction mixtures in organic solvents, trinucleotides can be prepared with the use of chromatography only at the last steps.

Experimental

Paper chromatography was performed by the descending technique with the following solvent systems: A, isopropanol–concentrated ammonia–water (7 : 1 : 2, v/v); B, *n*-propanol–concentrated ammonia–water (55 : 10 : 35, v/v). Paper electrophoresis was performed using 0.05 M triethylammonium bicarbonate (pH 7.5) or 0.2 M morpholinium acetate (pH 3.5) at 900 v/40 cm. *Rf* values and mobilities in paper electrophoresis

Table I

Compound	Paper chromatography *Rf* Solvent A	Paper electrophoresis Relative mobilities pH 7.5 R(C–Cp)
C	0.54	0.00
C>p	0.46	0.60
Cp	0.14	1.00
Gp	0.05	
G>p	0.32	
G	0.26	
U	0.54	
Up	0.15	
U(nBzl)p–OAr		0.38
C(nBzl)p–OAr		0.38
G(nBzl)p–OAr		0.50
U(nBzl)–C(nBzl)p–OAr	0.65	
U–C>p		0.75
G(nBzl)–U(nBzl)p–OAr	0.58	
G–U>p	0.14	0.78
C(nBzl)–G(nBzl)p–OAr	0.61	
C–G>p		0.71
(nBzl)A–A–C	0.52	
A–A–C		0.56
(nBzl)C–A–U–A–A–C	0.62	
C–A–U>p		0.93
C–A–U–A–A–C		0.78

are shown in Table I. Thin-layer chromatography was performed on plates of silica gel (Merck, Kieselgel 60 F_{254}) with a mixture of chloroform–ethanol. For columns, silica gel (Merck, type G) was used. Other general methods for the characterization of oligonucleotides and removal of the *o*-nitrobenzyl group were as described previously.[2a,c]

(MeOTr)bzC(nBzl)p̊ ibG(nBzl) (**4**)——The diester (**1**) (821.5 mg, 31900 A_{260}, 0.8 mmol) which was prepared by phosphorylation either with *p*-chlorophenyl phosphate plus DCC[2c] or with *p*-chlorophenyl phosphoroditriazolide[9] was dried by addition of pyridine and its removal by evaporation. The dried diester was treated with TPS-NI (**3**) (456 mg, 1.2 ml) in pyridine for 30 min and the nucleoside (**2**, 488.5 mg, 1.0 mmol) was added. After 2 days, TPS-NI (0.4 mmol) was added and the mixture was kept for another 2 days to complete the reaction. Aqueous pyridine (30 ml) was added and the product was extracted with chloroform (50 ml × 2) after 1 hr. The extract was washed with 0.1 M triethylammonium bicarbonate (30 ml × 2) and water (20 ml × 2). The organic layer was concentrated, and the residue was evaporated with pyridine then with toluene, dissolved in a small amount of chloroform and applied to a column of silica gel. The column was washed with chloroform and the product was eluted with 30: 1 chloroform-ethanol. The two isomers were precipitated with hexane from the concentrated eluates: higher isomer, 464.4 mg, 41%; lower isomer, 532.3 mg, 47%.

The Dinucleotide (**6**)——*p*-Chlorophenyl phosphorodichloridate (0.495 mmol) was added with a mixture of 1,2,4-triazole (1.09 mmol) and triethylamine (1.09 mmol) in anhydrous dioxane (10 ml) at 0°. The mixture was stirred at 0° for 10 min and at room temperature for 1 hr. The filtered solution of (**5**) was mixed with **4** (464 mg, 0.33 mmol) in anhydrous pyridine (4 ml). The mixture was concentrated to 8 ml and kept at 30° overnight. After confirmation of the disappearance of **4**, aqueous pyridine (50%, 10 ml) was added and the product was extracted with chloroform (20 ml × 2). The chloroform layer was washed with 0.1 M triethylammonium bicarbonate (20 ml × 3), and then with water (20 ml × 3), concentrated, and coevaporated with pyridine. The product **6** was precipitated with hexane from its solution in chloroform. The yield was 562.1 mg. An aliquot was treated successively with 4: 1 concentrated ammonia-pyridine at 50° for 2 hr, and 80% acetic acid at room temperature for 2 hr. After UV irradiation, it was subjected to paper chromatography in solvent A. C–G>p was phosphorylated with [γ-^{32}P]ATP and polynucleotide kinase. Venom phosphodiesterase digestion of C–G>p gave C (0.44 A_{278}) and pG>p (0.39 A_{254}) as measured in 0.01 N HCl. The ratio was 1.05 to 1.00.

The Dinucleotide (**11**)——The dinucleoside monophosphate (**10**, 0.565 mmol) was prepared from the mononucleotide (**1**, 0.73 mmol) and **9** (1.0 mmol) as described for the synthesis of **4**. **10** (519 mg, 0.36 mmol) was phosphorylated with **5** (1.08 mmol) by the method described for the synthesis of **6**. The yield was 621 gm, 14570 A_{263}, 80%. An aliquot was deblocked and characterized by enzymatic digestion with venom

phosphodiesterase and RNase T2, to yield 1.02: 1.00 C (2.47 A_{270})-pA>p (4.11 A_{260}) and 1.00: 1.06 Cp (0.63 A_{260}, pH 2)-Ap (0.77 A_{260}).

The Trinucleotide (13)——**11** (598.3 mg, 14035 A_{263}, 0.28 mmol) was dried by addition of pyridine and its removal by evaporation. The dried compound was treated with TPS-NI (**3**, 159.4 mg, 0.42 mmol) at room temperature for 30 min. The mixture was reacted with U (nBzl) (159.3 mg, 0.42 mmol) at 30° for 2 days and kept for another 24 hr with additional **3** (0.28 mmol). Aqueous pyridine (50%, 10 ml) was added and the product was applied to a column of silica gel (30 g) after work-up as described for **4**. **12** was eluted with 40: 1 chloroform-ethanol and phosphorylated with **5** (0.84 mmol) by the method as described for the synthesis of **6**. After 2 days, the mixture was treated with aqueous pyridine (5 ml). The product (**13**) was isolated by chromatography on a column (2.5×9.5 cm) of silica gel (20 g) by elution with 20: 1 chloroform-ethanol after removal of **12** by elution with 20: 1 chloroform–ethanol. The yield was 494.9 mg, 12.4×10^3 A_{259}, 0.19 mmol. UV: λ_{max} 260, 310 (sh) nm in 95% ethanol. **12** (5217 mg, 0.025 mmol) was recovered. The overall yield of **13** from **11** was 68%. An aliquot was deblocked and C–A–U>p was digested with venom phosphodiesterase and RNase T2 to give 0.92: 1.09: 1.00 C (1.47 A_{270})-pA (2.94 A_{260})-pU>p (1.76 A_{260}) and 1.00: 0.98: 1.03 Cp (0.852 A_{280}, pH 2)-Ap (0.971 A_{257}, pH 2)-Up (0.675 A_{261}, pH 2).

***2′-O*-Benzoyl-3′-*O*-(*o*-nitrobenzyl)-N-benzoylcytidine (14)**——bzC3′ (nBzl) 927.6 mg, 1.92 mmol) was treated with monomethoxytrityl chloride (770.8 mmol, 2.5 mmol) in pyridine (15 ml) at room temperature for 3 days. The reaction mixture was extracted with chloroform (50 ml×2) after treatment with 95% ethanol (4 ml) for 45 min followed by treatment with ice-water (100 ml). The chloroform layer was washed with 5% sodium bicarbonate (50 ml×2), water (50 ml) and evaporated with pyridine. The product was precipitated with hexane (200 ml) from its solution in benzene, and benzoylated with benzoyl chloride (0.8 ml, 6 mmol) at 0° for 1 hr and at room temperature for 2 hr. The mixture was poured to ice-water (100 ml) and extracted with chloroform (60 ml×2). The extract was washed with 5% sodium bicarbonate (40 ml×2), then with water (40 ml×2) and concentrated. The residue was treated with 80% acetic acid (50 ml) at room temperature overnight. The reaction mixture was concentrated and coevaporated with pyridine, and the residue was applied to a column of silica gel (40 g) after evaporation with toluene. The product was eluted with 40: 1 chloroform–ethanol and precipitated with hexane from its concentrated solution in chloroform. The yield was 592.7 mg (52.6%). mp 64—66°. UV: λ_{max} 225 (sh), 261, 300 (sh) nm. NMR (DMSO-d_6) 4.65 (m, 1H, C_3'–H); 5.87 (m, 1H, C_2'–H); 6.20 (d, 1H, C_1'–H).

The Trimer (18)——The 3′-(nBzl)nucleoside (**14**, 322.6 mg, 0.55 mmol) and the nucleoside (**15**, 816.9 mg, 1644 A_{279}, 0.65 mmol) were treated with TPS-Te (627.9 mg, 1.95 mmol) at 30° overnight. An aqueous pyridine (50%, 20 ml) was added and the whole was kept at room temperature for 1 hr then extracted with chloroform. The chloroform layer was washed with 0.1 M triethylammonium bicarbonate (15 ml×2) and with water, and then concentrated with added pyridine. The residue was coevaporated with toluene and applied to a column of silica gel (60 g). The product (**17**) was eluted with 45: 1 chloroform–ethanol and an aliquot was characterized by digestion with venom phosphodiesterase after deblocking. The monomethoxytrityl was removed by treatment with 80% acetic acid (20 ml) at 30° overnight and the dimer was precipitated with hexane. The yield was 479 mg (68%). **16** (479 mg, 0.37 mmol) was condensed with **15** (553.8 mg, 1140 A_{279}, 0.44 mmol) by using TPS-Te (1.32 mmol) as described above. The product (**18**) was eluted from a column of silica gel (30 g) with 45: 1 chloroform–ethanol. The yield was 551 mg (66%). UV: λ_{max} 275, 265, 235 (sh) nm in 99% ethanol. An aliquot was deblocked and A–A–C was hydrolyzed with venom phosphodiesterase to give 0.93: 1.02: 1.00 A (1.64 A_{258})-pA (1.77 A_{258})-pC (1.50 A_{280}) at pH 2. Digestion with RNase T2 of A–A–C yield 2.10: 1.00 Ap–C.

The Hexanucleotide (19) and C-A-U-A-A-C——**13** (243.5 mg, 6112 A_{259}, 0.093 mmol) and **18** (119.3 mg, 0.06 mmol) were reacted in the presence of TPS–Te (0.279 mmol) at 30° overnight. The mixture was treated with aqueous pyridine (50%, 2 ml) for 1 hr, extracted with chloroform (10 ml×2) and washed with 0.1 M triethylammonium bicarbonate (8 ml×2) and water (8 ml×2). Solvents were removed and the residue was coevaporated with pyridine and applied to a column of silica gel (20 g). The hexamer (**19**) was eluted with 45: 1 chloroform–ethanol, concentrated and precipitated with hexane (100 ml). The yield was 183.7 mg, 72.5%. An aliquot (84.4 mg, 0.02 mmol) was treated with 1: 4 pyridine–concentrated ammonia (25 ml) at 50° for 3 hr, then concentrated. The residue was dissolved in acetic acid (16 ml), water (4 ml) was added, and the whole was kept at 30° overnight. The volatile materials were removed by coevaporation with toluene and the residue was dissolved in 0.1 M triethylammonium bicarbonate Monomethoxytritanol was removed by extraction with ether, and the aqueous solution was concentrated to remove ether, diluted to 30 ml (20% ethanol) and applied to a column (1.2×13 cm) of DEAE-cellulose. The column was washed with 0.1 M triethylammonium bicarbonate in 20% ethanol and elution was performed with a linear gradient of the same salt in 20% ethanol (0.1—0.8 M, total 3 l). The hexamer was eluted with salt concentration of 0.33—0.38 M and the yield was 2250 A_{260} units. The *o*-nitrobenzyl group was removed by UV irradiation in a solution of 4 A_{260}/ml for 2.5 hr and C–A–U–A–A–C was isolated by paper chromatography in solvent A. The product was further purified by paper electrophoresis at pH 3.5 to remove a contaminating by-product (C–A–C–A–A–C) which may well be formed during ammonia treatment of a base-modified U formed by prolonged treatment with TPS–Te.

Protected U-U-C (24)——**20**[2c] (237.6 mg, 2820 A_{260}, 0.2 mmol) was treated with TPS-Te (0.6 mmol) for

30 min in pyridine (2 ml) then **2** (151.7 mg, 0.4 mmol) was added. The mixture was kept at 30° overnight and treated with 50% aqueous pyridine for 1 hr. The dimer (**22**) was extracted with chloroform (15 ml×2) and the extract was washed with 0.1 M triethylammonium bicarbonate (15 ml×2) and then with water (10 ml×2). Solvents were removed by coevaporation with toluene and the residue was dissolved in chloroform (30 ml). The organic layer was washed with water (30 ml×3) to remove the nucleoside (**21**). **22** was phosphorylated with *p*-chlorophenyl phosphoroditriazolide (**5**) prepared from *p*-chlorophenyl phosphorodichloridate (0.6 mmol) as described for the synthesis of **6**. **23** was condensed with *N*,2′-*O*-dibenzoyl-3′-*O*-(*o*-nitrobenzyl)cytidine (**14**, 176 mg, 0.3 mmol) by using TPS-Te (0.6 mmol) in pyridine (3 ml). After 10 hr, aqueous pyridine (50%, 5 ml) was added and the mixture was allowed to stand for 1 hr. The product (**24**) was extracted with chloroform and washed as above, then applied to a column of silica gel (25 g). Elution was performed with 45: 1 chloroform-ethanol and the trimer **24** was precipitated with hexane (100 ml). The yield was 233.4 mg, 52%. An aliquot was deblocked and labelled with ^{32}P by using polynucleotide kinase. Homochromatography showed no by-product having extra phosphate end groups. RNase A digestion yielded 2.13: 1.00 Up ($0.285\ A_{260}$)-C ($0.123\ A_{270}$).

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