Synthesis of the 3′-Terminal Half of Yeast Alanine Transfer Ribonucleic Acid ($tRNA^{Ala}$) by the Phosphotriester Approach in Solution. Part 2

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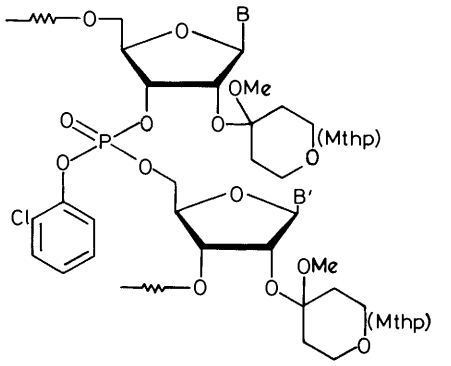
The preparation, by the phosphotriester approach in solution, of the 3′-terminal decaribonucleoside nonaphosphate [UpCpGpUpCpCpApCpCpA (**48**)], nonadecaribonucleoside octadecaphosphate [ApUpUpCpCpGpGpApCpUpCpGpUpCpCpApCpCpA (**49**)], and heptatriacontaribonucleoside hexatriacontaphosphate [GpGpApGpApGpGpUpCpUpCpCpGpGpTpψpCpGpApUpUpCpCpGpGpApCpUpCpGpUpCpCpApCpCpA (**50**)] sequences of yeast alanine transfer ribonucleic acid are described. With the exception of the 3′-terminal adenosine residue which was protected as its 2′,3′-*O*-methoxymethylene [Mm] derivative, the 2′-hydroxy functions were protected with 4-methoxytetrahydropyran-4-yl [Mthp] groups. The 5′-hydroxy functions of intermediate building blocks were protected with 2-(dibromomethyl)benzoyl [Dbmb (**2**)], 2-(isopropylthiomethoxymethyl)benzoyl [Ptmt (**3**)] or 9-phenylxanthen-9-yl [Px (**4**)] groups. The base residue of the adenosine, cytidine, guanosine, uridine, pseudouridine and 5-methyluridine building blocks were protected as in (**5**), (**6**), (**7**), (**8**), (**9**), and (**10**), respectively. Internucleotide linkages were protected with the 2-chlorophenyl group, and the 2,4-dinitrobenzyl (Dnb) group was used for the temporary protection of 3′-phosphodiester functions. The first phosphorylation step (leading to 3′-phosphodiester intermediates) was carried out by treatment with 2-chlorophenyl bis(1,2,4-triazoyl)phosphate (**11**) in the presence of 1-methylimidazole in tetrahydrofuran followed by triethylamine and water. 1-(Mesitylene-2-sulphonyl)-3-nitro-1,2,4-triazole [MSNT (**15**)] was used as the condensing agent in the second phosphorylation step. The final unblocking procedure involved treatment with (i) N^1,N^1,N^3,N^3-tetramethylguanidinium *E*-2-nitrobenzaldehyde oxime in dioxane–acetonitrile–water, (ii) concentrated aqueous ammonia, and (iii) 0.01 M hydrochloric acid.

For a number of years, we have been particularly concerned with the chemical synthesis of oligo- and poly-ribonucleotides in solution.[1–4] We were unable to make significant progress with this project prior to the development of the phosphotriester approach,[2] with aryl[5] [especially 2-chlorophenyl,[1] as in (**1**)] protecting groups for the internucleotide linkages. Four other studies have also been of crucial importance to our work in this area. The first of these studies involved the development of the achiral 4-methoxytetrahydropyran-4-yl [Mthp, as in (**1**)] acetal group[6] for the protection of the 2′-hydroxy functions of the ribose moieties. The second study was concerned with the development of a novel class of 'protected' protecting groups, including 2-(dibromomethyl)benzoyl[7] [Dbmb (**2**)] and 2-(isopropylthiomethoxymethyl)benzoyl[8,9] [Ptmt (**3**)] for the temporary blocking of the 5′-hydroxy functions. The third study was concerned with the development of suitable groups for the protection of guanine and uracil residues.[4,9,10] The final study was concerned with a search for a suitable group for the temporary blocking of 3′-terminal 2-chlorophenyl phosphate functions, and led to the introduction of the 2,4-dinitrobenzyl (Dnb) group[11] (see below). These and other aspects of oligo- and poly-ribonucleotide synthesis in solution have just been reviewed by one of us,[12] and the whole field of polyribonucleotide synthesis has also been reviewed recently.[13]



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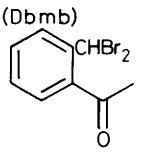

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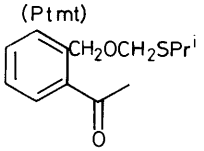

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In recent years, we have illustrated the improvements in our synthetic methodology by undertaking the preparation[3,4] of 3′-terminal sequences of yeast alanine transfer ribonucleic acid ($tRNA^{Ala}$) of increasing molecular weight. In this paper, we describe the synthesis of the 3′-terminal heptatriacontaribonucleoside hexatriacontaphosphate sequence (GpGpApGpApGpGpUpCpUpCpCpGpGpTpψpCpGpApUpUpCpCpGpGpApCpUpCpGpUpCpCpApCpCpA) of yeast $tRNA^{Ala}$. The latter sequence (Figure 1) contains thirty-seven nucleoside residues, that is one nucleoside residue less than one-half of the tRNA molecule. The details relating to the preparation of all the nucleoside building blocks required and a discussion about the blocking groups used to protect their base-residues and appropriate hydroxy functions are contained in the preceding paper.[9] While the Dbmb group (**2**) was used most frequently for

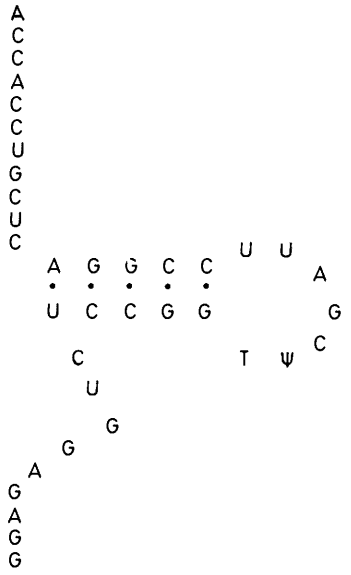
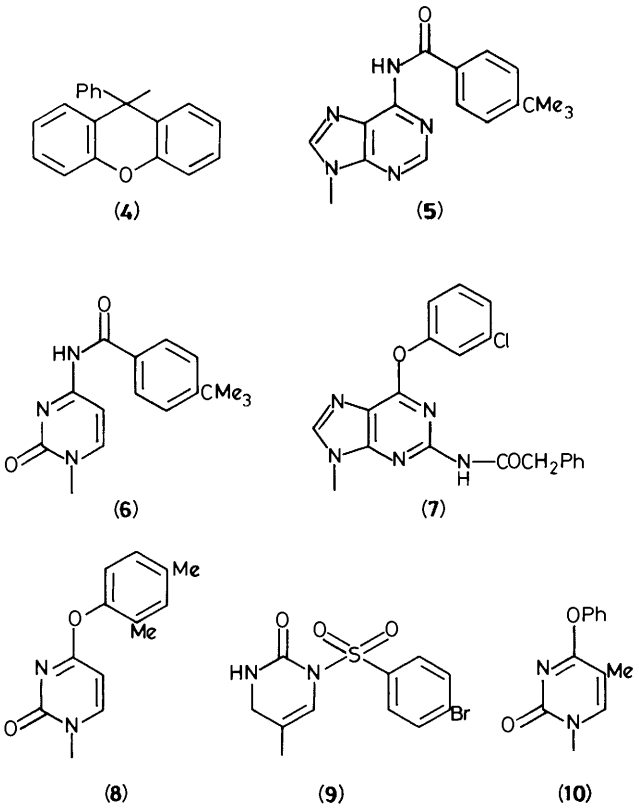


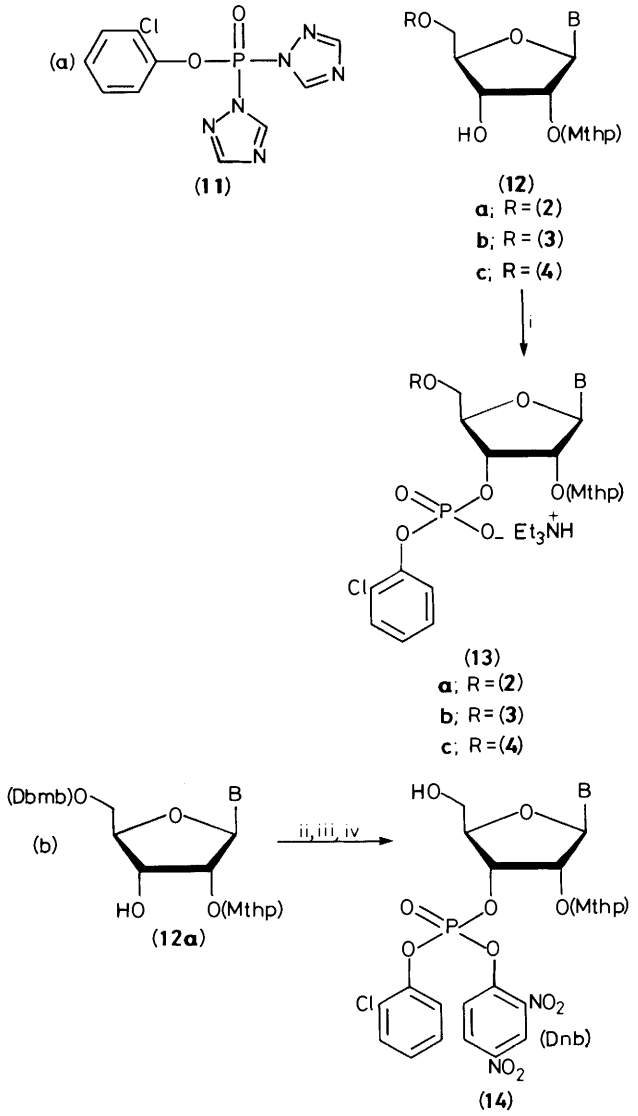
Figure 1. The 3′-terminal heptatriacontanucleoside hexatriacontaphosphate (37-mer) sequence of yeast alanine transfer ribonucleic acid ($tRNA^{Ala}$)



(4) (5) (6) (7) (8) (9) (10)

the protection of the 5′-hydroxy functions, the Ptmt protecting group (**3**) was used, when appropriate (see below), for the same purpose, and the 9-phenylxanthen-9-yl [Px(**4**)] group[14] was used to protect the 5′-hydroxy function of the building block destined for the 5′-terminus of the heptatriacontamer sequence. The base-residues of the adenosine, cytidine, guanosine, uridine, pseudouridine and 5-methyluridine building blocks were protected[9] as in (**5**), (**6**), (**7**), (**8**), (**9**), and (**10**), respectively.

Two different types of mononucleotide building blocks are

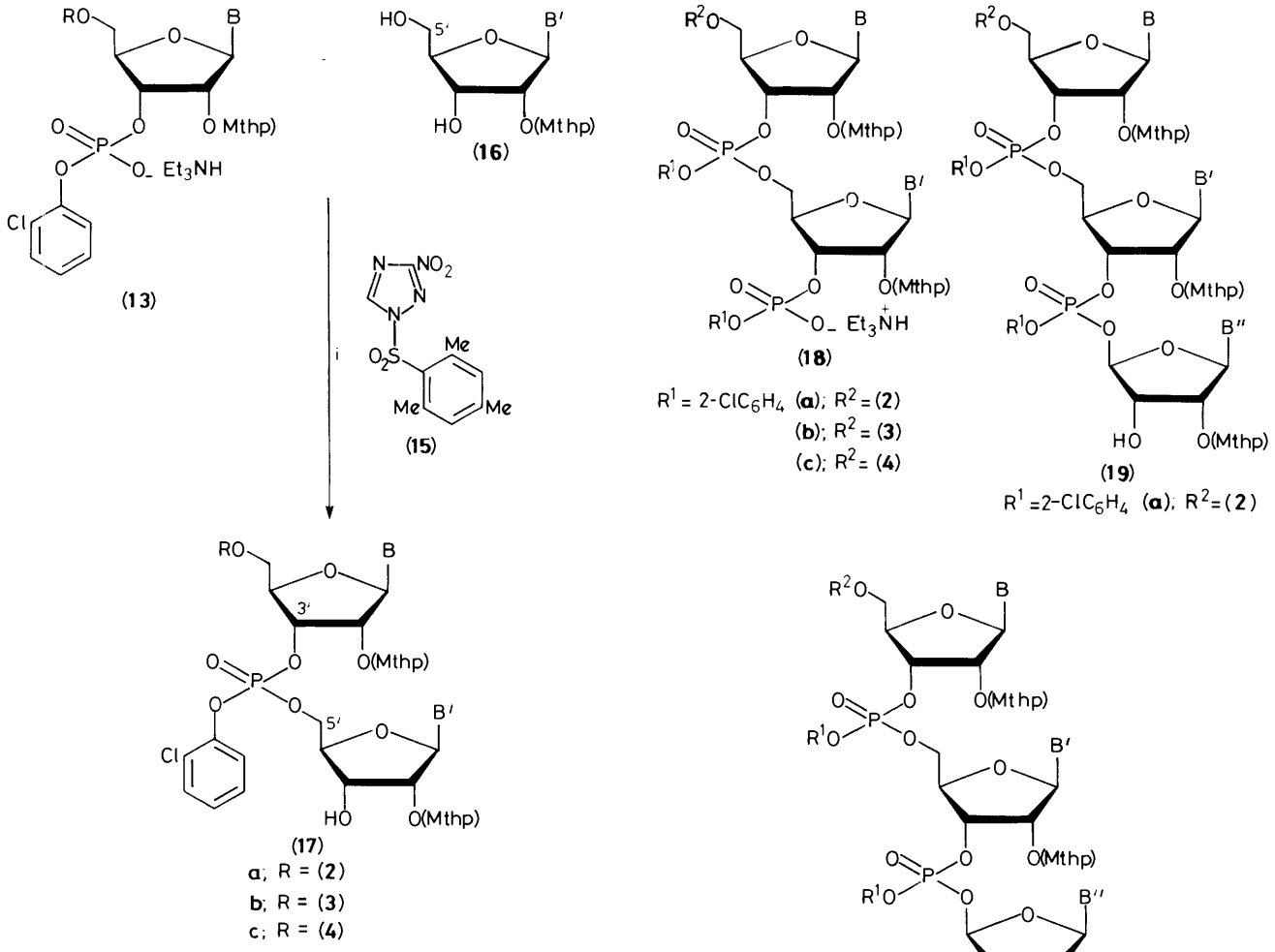


(11) (12) (13) (12a) (14)

Scheme 1. *Reagents:* i, (*a*) (**11**), 1-methylimidazole, THF, 15 min, r.t., (*b*) NEt_3–H_2O; ii, reagent obtained by allowing $Cl_2P(O)OC_6H_4Cl$, (1.5 mol. equiv.), 1-hydroxybenzotriazole[16] (3.0 mol. equiv.) and NEt_3 (3.0 mol equiv.) to react together in THF at 0 °C for 20 min; iii, 2,4-$(O_2N)_2C_6H_3CH_2OH$ (2.0 mol equiv.), pyridine; iv, (*a*) $AgClO_4$, 2,4,6-collidine, THF–H_2O (98:2), (*b*) morpholine

needed in our approach to the synthesis of oligo- and polyribonucleotides in solution. The first type is the triethylammonium salt of a protected ribonucleoside 3′-(2-chlorophenyl) phosphate (**13**); this is the standard mononucleotide building block which we have used in our previously reported work on the synthesis of the 3′-terminal decaribonucleoside nonaphosphate[3] and nonadecaribonucleoside octadecaphosphate[4] sequences of yeast $tRNA^{Ala}$. The latter building blocks (**13**) may readily be prepared[3] (Scheme 1a) in very high yields by treating the corresponding ribonucleoside derivatives (**12**)[9] with an excess of 2-chlorophenyl bis(1,2,4-triazolyl)phosphate (**11**)[15] in the presence of 1-methylimidazole in tetrahydrofuran (THF). We required eight such mononucleotide derivatives [five 5′-*O*-Dbmb derivatives (**13a**; B = **5**—**9**), two 5′-*O*-Ptmt derivatives (**13b**; B = **5** and **8**), and one 5′-*O*-Px derivative (**13c**; B = **7**)] in the synthesis of the heptatriacontamer, and they were obtained in isolated yields of between 91 and 97% (see Experimental).

The second type of mononucleotide building block [(**14**),



Scheme 2: *Reagent:* i, MSNT (**15**), pyridine, 20—30 min, r.t.

Scheme 1b] required is a ribonucleoside 3′-(2-chlorophenyl) phosphate in which the phosphodiester is protected with a 2,4-dinitrobenzyl (Dnb)[11] group. The latter derivatives (**14**) may be obtained in three steps (Figure 1b and Experimental) from the corresponding 2′-*O*-Mthp-5′-*O*-Dbmb ribonucleoside building blocks[9] (**12a**). 2,4-Dinitrobenzyl alcohol, which was prepared from benzyl chloride in 48% overall yield by a modification (see Experimental) of a literature procedure,[17] is required in the preparation of these Dnb-protected mononucleotide units (**14**). We needed four such Dnb-protected mononucleotides (**14**; B = **5**—**8**) in the synthesis of the heptatriacontamer sequence, and they were obtained in isolated overall yields of between 51 and 67% (see Experimental).

When the first type of mononucleotide building block (**13**) is allowed to react with a 2′-*O*-Mthp ribonucleoside derivative[9] (**16**) and *ca.* 2.5 mol equiv. of 1-(mesitylene-2-sulphonyl)-3-nitro-1,2,4-triazole [MSNT, (**15**)][3,18] in anhydrous pyridine solution at room temperature for 20—30 min, partially-protected dinucleoside phosphates (**17**) are obtained, usually in yields of *ca.* 80%. The 2′-*O*-Mthp ribonucleoside derivatives (**16**) appear to undergo virtually regiospecific phosphorylation on their 5′-hydroxy functions[3] and the products (**17**) obtained appear to be uncontaminated with isomeric (3′ ⟶ 3′)-dinucleoside phosphates. Ten such dinucleoside phosphates (**17**) (see Table, entries nos. 1—10; see below for an explanation of the abbreviations) were needed in the synthesis of the heptatriacontamer sequence.

Our approach to the synthesis of oligo- and poly-ribonucleotides in solution is based essentially on two types of trinucleotide blocks. The first type (type I) of trinucleotide block (**20a**) was prepared as follows. Phosphorylation of a partially-protected dinucleoside phosphate (**17a**) with 2-chlorophenyl bis(1,2,4-triazolyl)phosphate (**11**), according to the procedure indicated in Scheme 1a, gave the corresponding dinucleotide (**18a**), usually in yields of *ca.* 95%. When such dinucleotides (**18a**) were allowed to react with 2′-*O*-Mthp ribonucleosides (**16**) and MSNT (**15**) in anhydrous pyridine, according to the procedure indicated in Scheme 2, the corresponding partially-protected trinucleoside diphosphates (**19a**) were obtained, usually in yields of *ca.* 80%. Again, phosphorylation appears to occur regiospecifically[3] on the 5′-hydroxy function of the 2′-*O*-Mthp ribonucleoside derivative. Five such trinucleoside diphosphates (**19a**) (Table, entries nos. 11—15) are needed in the synthesis of the heptatriacontamer sequence. When the latter trinucleoside diphosphates (**19a**) were treated with 2-chlorophenyl bis(1,2,4-triazolyl)phosphate (**11**), according to the procedure indicated in Scheme 1a, the corresponding trinucleotides (**20a**) were obtained in high (95—97%) yields.

Table. Preparation of dinucleoside phosphate (**17**), trinucleoside diphosphate (**18**), and trinucleotide (**21**) blocks

Entry no.	3′-Phosphodiester component (mmol)	5′-Hydroxy component (mmol)	MSNT (15) (mmol)	Reaction time (min)	Product (% isolated yield)	R_F[a]
1	Dbmb-*A′p* (0.89)	HO-*C′*-OH (0.75)	2.24	20	Dbmb-*A′pC′*-OH (76)	0.65
2	Dbmb-*C′p* (1.10)	HO-*C′*-OH (0.96)	2.87	20	Dbmb-*C′pC′*-OH (79)	0.73
3	Dbmb-*G′p* (1.50)	HO-*A′*-OH (1.25)	3.75	20	Dbmb-*G′pA′*-OH (80)	0.71
4	Dbmb-*G′p* (0.80)	HO-*G′*-OH (0.67)	2.00	25	Dbmb-*G′pG′*-OH (79)	0.64
5	Dbmb-*G′p* (1.00)	HO-*U′*-OH (0.83)	2.49	20	Dbmb-*G′pU′*-OH (82)	0.70
6	Dbmb-*U′p* (0.90)	HO-*C′*-OH (0.75)	2.25	20	Dbmb-*U′pC′*-OH (78)	0.72
7	Dbmb-*ψ′p* (1.00)	HO-*C′*-OH (0.83)	2.49	20	Dbmb-*ψ′pC′*-OH (73)	0.73
8	Ptmt-*A′p* (0.57)	HO-*U′*-OH (0.48)	1.43	25	Ptmt-*A′pU′*-OH (79)	0.66
9	Ptmt-*U′p* (1.20)	HO-*C′*-OH (1.00)	3.00	30	Ptmt-*U′pC′*-OH (66)	0.69
10	Px-*G′p* (0.69)	HO-*G′*-OH (0.58)	1.73	20	Px-*G′pG′*-OH (84)	0.71
11	Dbmb-*A′pC′p* (0.54)	HO-*C′*-OH (0.45)	1.62	20	Dbmb-*A′pC′pC′*-OH (84)	0.64
12	Dbmb-*C′pC′p* (0.75)	HO-*G′*-OH (0.62)	1.87	20	Dbmb-*C′pC′pG′*-OH (84)	0.66
13	Dbmb-*G′pA′p* (0.60)	HO-*G′*-OH (0.50)	1.50	20	Dbmb-*G′pA′pG′*-OH (68)	0.71
14	Dbmb-*G′pG′p* (0.53)	HO-*T′*-OH (0.44)	1.33	25	Dbmb-*G′pG′pT′*-OH (76)	0.69
15	Dbmb-*U′pC′p* (0.58)	HO-*C′*-OH (0.49)	1.45	20	Dbmb-*U′pC′pC′*-OH (78)	0.75
16	Dbmb-*G′pA′p* (0.74)	HO-*C′p*-Dnb (0.62)	1.85	30	Dbmb-*G′pA′pC′p*-Dnb (93)	0.77
17	Dbmb-*G′pU′p* (0.65)	HO-*C′p*-Dnb (0.54)	1.62	30	Dbmb-*G′pU′pC′p*-Dnb (64)	0.83
18	Dbmb-*ψ′pC′p* (0.58)	HO-*G′p*-Dnb (0.48)	1.45	25	Dbmb-*ψ′pC′pG′p*-Dnb (79)	0.81
19	Ptmt-*A′pU′p* (0.36)	HO-*U′p*-Dnb (0.30)	1.20	30	Ptmt-*A′pU′pU′p*-Dnb (87)	0.89
20	Ptmt-*U′pC′p* (0.35)	HO-*C′p*-Dnb (0.29)	1.16	45	Ptmt-*U′pC′pC′p*-Dnb (82)	0.81
21	Ptmt-*U′pC′p* (0.30)	HO-*G′p*-Dnb (0.25)	1.00	40	Ptmt-*U′pC′pG′p*-Dnb (88)	0.78
22	Px-*G′pG′p* (0.48)	HO-*A′p*-Dnb (0.40)	1.20	30	Px-*G′pG′pA′p*-Dnb (89)	0.85

[a] T.l.c. plates were developed in solvent system A [$CHCl_3$–MeOH (9:1)].

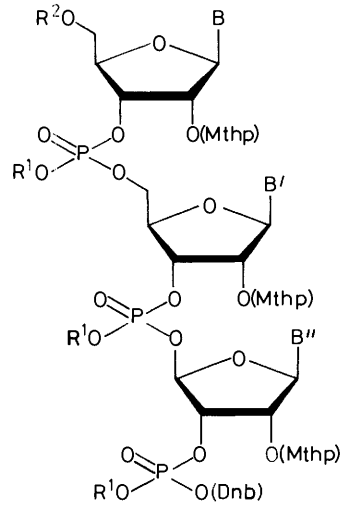
The second type (type II) of trinucleotide block (**21**) was prepared by allowing one of the above dinucleotides (**18**) to react with a Dnb-protected mononucleotide (**14**) and MSNT (**15**) in anhydrous pyridine solution. Seven such fully-protected trinucleotide blocks (**21**) (Table, entries nos. 16—22) are needed in the synthesis of the heptatriacontamer sequence and yields of 80% or more were usually obtained. The 5′-*O*-Ptmt and 5′-*O*-Px protected type II trinucleotide blocks [(**21b**) and (**21c**), respectively] were rapidly converted by treatment with 2-thiocresol* and triethylamine in acetonitrile solution at room temperature into the corresponding type **I** trinucleotide blocks [(**20b**) and (**20c**), respectively]. The removal of the Dnb protecting group was generally complete within *ca.* 1 min, and the reactions were quenched by the addition of an excess of allyl chloride after 5 min. Good (77—95%) yields of the type **I** blocks [(**20b**) and (**20c**)] were obtained. The 5′-*O*-Dbmb group could be removed from type II trinucleotide blocks (**21a**) by the standard two step procedure[7] (Scheme 1b; reagents iv) to give the corresponding blocks (**21**; R = H) with free 5′-hydroxy functions. Only rather modest isolated yields (average *ca.* 50%; see Experimental) of the desired products (**21**; R = H) were obtained.

With all the required monomeric and indeed trimeric building blocks available, we first repeated the synthesis of the 3′-terminal decaribonucleoside nonaphosphate sequence (UpCpGpUpCpCpApCpCpA) of yeast $tRNA^{Ala}$, essentially following a previously reported[3] strategy. First, the type I trinucleotide (**20a**; B = **5**, B′ = B″ = **6**) was allowed to react with 2′,3′-*O*-methoxymethylene-6-*N*-(4-t-butylbenzoyl)adenosine[9] (**22**) and an excess of MSNT (**15**) in anhydrous pyridine solution for 25 min at room temperature to give the fully-protected tetraribonucleoside triphosphate (**23**; R = **2**, B = **5**, B′ = B″ = **6**) in 95% yield. Removal of the 5′-*O*-Dbmb protecting group by the standard two step procedure (Scheme 1b; reagents iv) gave (**23**; R = H, B = **5**, B′ = B″ = **6**) in 86% yield.

* 2-Thiocresol has been used in preference to 4-thiocresol[11] in order to minimize the possibility of thiolate ion attack at C-6 and C-4, respectively, of protected guanine and uracil residues [(**7**) and (**8**)].

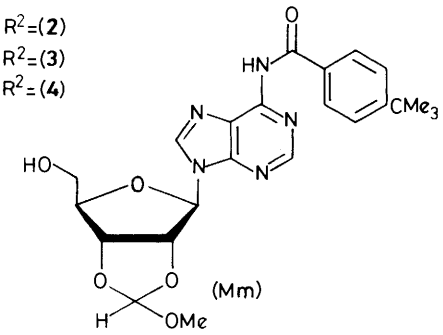
At this stage it is necessary to use the system of abbreviations for fully- and partially-protected oligo- and poly-ribonucleotides that we have described previously.[4] In this system, base-protected nucleoside residues [with the protecting groups indicated in (**5**), (**6**), (**7**), (**8**), (**9**), and (**10**)] and phosphate groups esterified with 2-chlorophenol are indicated simply by the appropriate italicized letters (*i.e. A*, *C*, *G*, *U*, *ψ*, *T* and *p*, respectively). Nucleoside residues containing unprotected base moieties, and phosphate groups that are not esterified with 2-chlorophenol, are indicated by the same letters, but they are not italicized. If a prime is placed after an abbreviated nucleoside residue (*e.g. C′*), this indicates that the 2′-hydroxy function of the ribose moiety of the nucleoside residue is protected with a Mthp group. In general, until the final unblocking step (see below) at the end of a synthesis, the 2′-hydroxy functions of all the nucleoside residues except that at the 3′-terminus are protected with Mthp groups, and the latter residue is usually protected with a 2′,3′-*O*-methoxymethylene (Mm) group. The standard conventon of placing the 5′-group on the left-hand and the 3′-group on the right-hand side of a particular nucleoside residue is observed. The 3′- and 5′-terminal hydroxy functions, phosphate (*e.g.* 2-chlorophenyl phosphate, *p*) groups and protecting groups [Dbmb (**2**), Ptmt (**3**), Px (**4**), Dnb (see **14**), and Mm (see **22**)] are always indicated. Thus the two type I trinucleotide blocks [(**20a**; B = **8**, B′ = B″ = **6**) and (**20b**; B = **8**, B′ = **6**, B″ = **7**)] required in the synthesis of the fully-protected 3′-terminal decaribonucleoside nonaphosphate sequence (**28**) (Scheme 3) of yeast $tRNA^{Ala}$ are abbreviated to Dbmb-*U′pC′pC′p* (**24**) and Ptmt-*U′pC′pG′p* (**27**), respectively, and the partially-protected tetraribonucleoside triphosphate (**23**; R = H, B = **5**, B′ = B″ = **6**) required is abbreviated to HO-*A′pC′pC′pA*-Mm (**25**).

The strategy that we have adopted in the synthesis of the 3′-terminal heptatriacontamer sequence (Figure 1) of yeast $tRNA^{Ala}$ has been first to prepare 9—10-mer blocks from three smaller units [usually type I and type II trinucleotide blocks (**20** and **21**, respectively)], then to link two such blocks together to give 18–19-mers, and finally to join the appropriate 18-mer and 19-mer blocks together to give the desired fully-protected heptatriacontamer sequence (see below). In the development of



(21)

R^1 = 2-ClC_6H_4 (a); R^2=(2)
(b); R^2=(3)
(c); R^2=(4)



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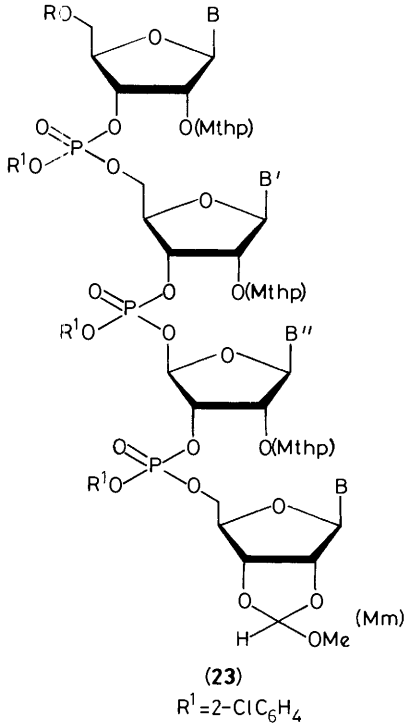

(23)

R^1=2-ClC_6H_4

our strategy, we have paid particular regard to purification considerations. We first carried out the preparation of the fully-protected 3′-terminal decaribonucleoside nonaphosphate (**28**). The first step [Scheme 3(a)] involved the reaction between the type I trinucleotide block (**24**), the partially-protected tetraribonucleoside triphosphate (**25**) and an excess of MSNT (**15**) in anhydrous pyridine solution for 60 min at room temperature to give the corresponding fully-protected heptaribonucleoside hexaphosphate in 84% isolated yield. The Dbmb protecting group (**2**) was removed by the standard procedure to give the partially-protected heptaribonucleoside hexaphosphate (**26**) in 69% overall yield, based on (**25**). The latter heptamer (**26**), a slight excess of a second type I trinucleotide block (**27**) [prepared in 87% isolated yield by the action of 2-thiocresol and triethylamine on the corresponding type II trinucleotide (Ptmt-*U′pC′pG′p*-Dnb) in acetonitrile solution] and an excess of MSNT (**15**) were allowed to react together [Scheme 3(b)] in anhydrous pyridine solution for 2.5 h at room temperature to give the fully-protected decaribonucleoside nonaphosphate (**28**) in 80% isolated yield.

The fully-protected nonaribonucleotide block (**33**) required next in the synthesis of the 3′-terminal heptatriacontamer sequence of yeast $tRNA^{Ala}$ was prepared according to the procedure outlined in Scheme 4. The Dbmb protecting group was removed from the corresponding type II trinucleotide block (**21a**; B = **7**, B′ = **5**, B″ = **6**) to give (**30**) in 61% isolated yield. Reaction between the latter product (**30**), a slight excess of the type I trinucleotide block (**29**) and an excess of MSNT (**15**) in anhydrous pyridine solution [Scheme 4(a)] for 45 min at room temperature gave the fully-protected hexanucleotide (Dbmb-*C′pC′pG′pG′pA′pC′p*-Dnb) in 79% yield. Following the removal of the Dbmb protecting group, the partially-protected hexanucleotide (**31**) was isolated in 53% overall yield, based on (**30**). The latter hexamer (**31**), a slight excess of the type I trinucleotide block (**32**) [prepared in good yield from the corresponding type II trinucleotide block (Ptmt-*A′pU′pU′p*-Dnb)] and an excess of MSNT (**15**) were allowed to react together [Scheme 4(b)] in anhydrous pyridine for 60 min at room temperature to give the fully-protected nonanucleotide (**33**) in 72% isolated yield.

We were now in a position to undertake the preparation of the fully-protected 3′-terminal nonadecaribonucleotide octadecaphosphate sequence (**36**). The 5′-*O*-Ptmt protecting group was removed from the fully-protected decaribonucleoside nonaphosphate (**28**) by the standard procedure[8,9] [Scheme 5(a) and Experimental] to give (**34**) in 72% isolated yield, and the 3′-Dnb protecting group was removed from the fully-protected nonaribonucleotide (**33**) by treatment with toluene-2-thiolate ions [Scheme 5(b)] to give (**35**) in 78% isolated yield. The fully-protected nonadecamer (**36**) was obtained [Scheme 5(c)] when the partially-protected decamer (**34**), a slight excess of the nonanucleotide (**35**) and an excess of MSNT (**15**) were allowed to react together in anhydrous pyridine solution for 3.5 h at room temperature; following work-up and chromatography of the products on Sephadex LH 60, (**36**) was isolated in *ca.* 50% yield, based on (**34**). The actual quantity of pure nonadecaribonucleoside octadecaphosphate (**36**) isolated was 0.378 g (*ca.* 0.028 mmol). As the chain-lengths of fully-protected oligo- and poly-nucleotides increase, it becomes more difficult to purify them by short column chromatography[19] on silica gel, the technique that has been used widely and successfully for the purification of oligonucleotides of moderate chain-length, such as the fully-protected decaribonucleoside nonaphosphate (**28**) and the fully-protected nonanucleotide (**33**). For this reason, the relatively high molecular weight nonadecamer (**36**) was purified[20] by gel filtration on Sephadex LH 60. For the latter technique to be fully effective, it is essential that all the other components in the mixture should be of much lower molecular weight. It was for this reason that the nonadecamer (**36**) was prepared from oligoribonucleotide blocks [(**34**) and (**35**)] with only *ca.* one-half of its molecular weight.

As indicated above, the final step of our strategy for the assembly of the fully-protected heptatriacontamer sequence involves the linking together of the appropriate nonadecamer and octadecamer blocks. The required fully-protected

(a) Dbmb-U′*pC′pC′p* + HO-*A′pC′pC′pA*-Mm $\xrightarrow{i,\ ii}$ HO-*U′pC′pC′pA′pC′pC′pA*-Mm
(**24**) (**25**) (**26**)

(b) Ptmt-*U′pC′pG′p* + (**26**) $\xrightarrow{i}$ Ptmt-*U′pC′pG′pU′pC′pC′pA′pC′pC′pA*-Mm
(**27**) (**28**)

Scheme 3. *Reagents:* i, MSNT (**15**), pyridine; ii, (*a*) $AgClO_4$, 2,4,6-collidine, THF–H_2O (98:2), (*b*) morpholine

(a) Dbmb-*C′pC′pG′p* + HO-*G′pA′pC′p*-Dnb $\xrightarrow{i,\ ii}$ HO-*C′pC′pG′pG′pA′pC′p*-Dnb
(**29**) (**30**) (**31**)

(b) Ptmt-*A′pU′pU′p* + (**31**) $\xrightarrow{i}$ Ptmt-*A′pU′pU′pC′pC′pG′pG′pA′pC′p*-Dnb
(**32**) (**33**)

Scheme 4. *Reagents:* i, MSNT (**15**), pyridine; ii (*a*) $AgClO_4$, 2,4,6-collidine, THF–H_2O (98:2), (*b*) morpholine

(a) Ptmt-*U′pC′pG′pU′pC′pC′pA′pC′pC′pA*-Mm $\xrightarrow{i}$ HO-*U′pC′pG′pU′pC′pC′pA′pC′pC′pA*-Mm
(**28**) (**34**)

(b) Ptmt-*A′pU′pU′pC′pC′pG′pG′pA′pC′p*-Dnb $\xrightarrow{ii}$ Ptmt-*A′pU′pU′pC′pC′pG′pG′pA′pC′p*
(**33**) (**35**)

(c) (**34**) + (**35**) $\xrightarrow{iii}$ Ptmt-*A′pU′pU′pC′pC′pG′pG′pA′pC′pU′pC′pG′pU′pC′pC′pA′pC′pC′pA*-Mm
(**36**)

Scheme 5. *Reagents:* i, (a) $Hg(ClO_4)_2$, 2,4,6-collidine, THF–H_2O (98:2), (b) 0.2M NEt_3/THF-H_2O (2:1); ii, toluene-2-thiol, NEt_3, CH_3CN; iii, MSNT (**15**), pyridine

(a) Ptmt-*U′pC′pC′pG′pG′pT′pψ′pC′pG′p*-Dnb $\xrightarrow{i}$ HO-*U′pC′pC′pG′pG′pT′pψ′pC′pG′p*-Dnb
(**37**) (**38**)

(b) Px-*G′pG′pA′pG′pA′pG′pG′pU′pC′p*-Dnb $\xrightarrow{ii}$ Px-*G′pG′pA′pG′pA′pG′pG′pU′pC′p*
(**39**) (**40**)

(c) (**38**) + (**40**) $\xrightarrow{iii}$ Px-*G′pG′pA′pG′pA′pG′pG′pU′pC′pU′pC′pC′pG′pG′pT′pψ′pC′pG′p*-Dnb
(**41**)

Scheme 6. *Reagents:* i, (a) $Hg(ClO_4)_2$, 2,4,6-collidine, THF–H_2O (98:2), (b) 0.2M NEt_3/THF–H_2O (2:1); ii, toluene-2-thiol, NEt_3, CH_3CN; iii, MSNT (**15**), pyridine

octadecaribonucleotide (**41**) (Scheme 6) was prepared from two fully-protected nonaribonucleotides [(**37**) and (**39**)] which were, in turn, both prepared (see Experimental) from three triribonucleotide blocks (Ptmt-*U′pC′pC′p*, Dbmb-*G′pG′pT′p*, HO-*ψ′pC′pG′p*-Dnb, and Px-*G′pG′pA′p*, Dbmb-*G′pA′pG′p*, HO-*G′pU′pC′p*-Dnb, respectively), following the procedure used above in the preparation of Ptmt-*A′pU′pU′pC′pC′pG′pG′pA′pC′p*-Dnb (**33**) (Scheme 4). The yields obtained in all steps except those involving the removal of 5′-*O*-Dbmb protecting groups from type II trinucleotide blocks [(**21**); *i.e.* Dbmb-*ψ′pC′pG′p*-Dnb and Dbmb-*G′pU′pC′p*-Dnb; see above and Experimental] were very satisfactory. The 5′-*O*-Ptmt protecting group was removed from the fully-protected nonaribonucleotide derivative (**37**) by the standard procedure [Scheme 6(a)] to give (**38**) in 81% isolated yield, and the 3′-Dnb protecting group was removed from the other fully-protected nonaribonucleotide (**39**) to give (**40**) [Scheme 6(b)] in 66% isolated yield. The fully-protected octadecaribonucleotide (**41**) was obtained when (**38**), a slight excess of (**40**) and an excess of MSNT (**15**) were allowed to react together [Scheme 6(c)] in anhydrous pyridine solution for 3.5 h; following work-up and chromatography of the products on Sephadex LH 60, (**41**) was isolated in *ca.* 34% yield, based on (**38**). The actual quantity of pure fully-protected octadecaribonucleotide (**41**) obtained was 0.169 g (0.012 mmol).

The 5′-*O*-Ptmt protecting group was then removed from the fully-protected nonadecaribonucleoside octadecaphosphate (**36**) in the usual way [Scheme 7(a)] to give (**42**) in 87% isolated yield, and the 3′-*O*-Dnb protecting group was removed from the fully-protected octadecanucleotide (**41**) by the standard procedure [Scheme 7(b)] to give (**43**) in what was estimated to be only *ca.* 60% yield. The relatively high yield of (**42**) obtained was very encouraging indeed and emphasizes the value of the Ptmt protecting group in the synthesis of high molecular weight oligo- and poly-ribonucleotides in solution. On the other hand, the yield of (**43**) was disappointingly low, and it appeared that *ca.* 40% of the putative fully-protected octadecaribonucleotide (**41**) was resistant to attack by toluene-2-thiolate ions. The possibility that the thiolate-resistant material did not contain a 3′-terminal Dnb group, and therefore that the latter protecting group was not completely stable under all the reaction conditions (including treatment with base) involved in the assembly of (**41**), could not be ruled out. So far, we have not succeeded in identifying the cause of this problem. Due to the relatively low yield of (**43**), the latter partially-protected octadecaribonucleotide (*ca.* 5 μmol) was allowed to react with a slight excess (*ca.* 6.9 μmol) of the partially-protected nonadecaribonucleoside octadecaphosphate (**42**) in the presence of a large excess (0.34 mmol) of MSNT (**15**) in anhydrous pyridine (*ca.* 0.5 ml) solution at room temperature for 24 h. Following work-up and chromatography of the products on Sephadex LH 60, the fully-protected heptatriacontaribonucleoside hexatriacontaphosphate (**44**), was obtained in 26% yield, based on (**42**). The actual quantity of purified fully-protected heptatriacontamer (**44**) obtained was 0.047 g (1.8 μmol).

(a) (**36**) $\xrightarrow{i}$ HO-*A'pU'pU'pC'pC'pG'pG'pA'pC'pU'pC'pG'pU'pC'pC'pA'pC'pC'pA*-Mm (**42**)

(b) (**41**) $\xrightarrow{ii}$ Px-*G'pG'pA'pG'pA'pG'pG'pU'pC'p U'pC'pC'pG'pG'pT'pψ'pC'pG'p* (**43**)

(c) (**42**) + (**43**) $\xrightarrow{iii}$ Px-*G'pG'pA'pG'pA'pG'pG'pU'pC'pU'pC'pC'pG'pG'pT'pψ'pC'pG'pA'pU'pU'pC'pC'pG'pG'pA'pC'pU'pC'pG'pU'pC'-pC'pA'pC'pC'pA*-Mm (**44**)

Scheme 7. *Reagents:* i, (a) $Hg(ClO_4)_2$, 2,4,6-collidine, THF–H_2O (98:2), (b) 0.2M NEt_3/THF–H_2O (2:1); ii, toluene-2-thiol, NEt_3, CH_3CN; iii, MSNT (**15**), pyridine

(a) Ptmt-*U'pC'pG'pU'pC'pC'pA'pC'pC'pA*-Mm (**28**) $\xrightarrow{i,ii}$ HO-U'pC'pG'pU'pC'pC'pA'pC'pC'pA-Mm (**45**)

(b) Ptmt-*A'pU'pU'pC'pC'pG'pG'pA'pC'pU'pC'pG'pU'pC'pC'pA'pC'pC'pA*-Mm (**36**) $\xrightarrow{i,ii}$ HO-A'pU'pU'pC'pC'pG'pG'pA'pC'pU'pC'pG'pU'pC'pC'pA'pC'pC'pA-Mm (**46**)

(c) Px-*G'pG'pA'pG'pA'pG'pG'pU'pC'pU'pC'pC'pG'pG'pT'pψ'pC'pG'pA'pU'pU'pC'pC'pG'pG'pA'pC'pU'pC'pG'pU'pC'pC'pA'pC'-pC'pA*-Mm (**44**) $\xrightarrow{i,ii}$ Px-G'pG'pA'pG'pA'pG'pG'pU'pC'pU'pC'pC'pG'pG'pT'pψ'pC'pG'pA'pU'pU'pC'pC'pG'pG'pA'pC'pU'pC'pG'pU'pC'-pC'pA'pC'pC'pA-Mm (**47**)

Scheme 8. *Reagents:* i, N^1,N^1,N^3,N^3-Tetramethylguanidinium *E*-2-nitrobenzaldehyde oximate/dioxane–CH_3CN–H_2O (5:4:1); ii, conc. aq. NH_3 (*d* 0.88)

The rather low isolated yield of (**44**) is somewhat exaggerated by the fact that it is based on the partially-protected nonadecaribonucleoside octadecaphosphate (**42**), which was used in excess. In all the other MSNT-promoted condensation reactions described above (including the preparation of the fully-protected decaribonucleoside nonaphosphate [(**33**), Scheme 4; isolated yield, 80%], the fully-protected nonadecaribonucleoside octadecaphosphate [(**36**), Scheme 5; isolated yield, *ca.* 50%], and the fully-protected octadecaribonucleotide [(**41**), Scheme 6; isolated yield, *ca.* 34%], a slight deficiency (0.7—0.8 mol equiv.) of the 5′-hydroxy component [(**31**), (**34**), and (**38**), respectively] was used. It is, in any case, clear that the isolated yields of MSNT-promoted condensation reactions appear to decrease with increasing chain length. We believe that there are at least three principal reasons for this. First, the rates of the condensation reactions not unsurprisingly seem to fall as the chain length increases. As, in order to maintain anhydrous reaction conditions, a relatively large excess of MSNT (**15**) is required when reactions are carried out on a small scale (say, 10 μmol or less of each oligoribonucleotide component), there is then an increasing danger of arenesulphonylation competing with the phosphorylation of the component with the 5′-hydroxy function. Secondly, despite the effectiveness of Sephadex LH 60 chromatography, fractionation of fully-protected oligo- and poly-ribonucleotides becomes more difficult and probably less efficient with increasing molecular weight. Thirdly, as suggested above, the Dnb protecting group does not appear to be completely stable under the whole range of reaction conditons involved in the assembly of relatively large oligoribonucleotide blocks, such as the fully-protected octadecaribonucleotide (**41**). We nevertheless regard the fact that we were able to synthesize the fully-protected heptatriacontamer (**44**), which has a molecular weight of more than 26,000 daltons from two components [(**42**) and (**43**), Scheme 7] each with a molecular weight of *ca.* 13,000 daltons on a *ca.* 5 μmolar scale as being very encouraging, and believe that certainly if the above problem relating to the Dnb protecting group could be solved, a whole tRNA molecule could be assembled by the above approach.

The final stages of an oligo- or poly-ribonucleotide synthesis are concerned with the removal of all of the protecting groups. In our synthetic approach, the unblocking process consists[3,4] of three separate steps. In the first step (Scheme 8, reagent i), the fully-protected material is treated with a large excess of *E*-2-nitrobenzaldehyde oximate[4,21] and almost as large an excess of N^1,N^1,N^3,N^3-tetramethylguanidine in anhydrous dioxane solution at room temperature. After further periods of time, acetonitrile[9,22] and water are added (Experimental) in quantities such that the relative proportions of dioxane, acetonitrile and water are 5:4:1, and a homogeneous solution is maintained throughout the unblocking process. This step, which is normally complete within 24 h, leads to the removal of the 2-chlorophenyl protecting groups from the internucleotide linkages and also to the removal of the 3-chlorophenyl, 2,4-dimethylphenyl, phenyl, and 4-bromobenzenesulphonyl protecting groups from the guanosine, uridine, 5-methyluridine, and pseudouridine residues, respectively. The second step (Scheme 8, reaction ii) involves treatment with concentrated aqueous ammonia at room temperature for periods of up to 72 h. This leads to the removal of the *N*-acyl protecting groups from the base residues, and also to the removal of the 5′-terminal Ptmt protecting group when present. Following these two unblocking steps (Scheme 8), only the acid-labile protecting groups [*i.e.* Mthp, Mm, and Px (if present)] remain. The latter protecting groups are readily removable, in the third unblocking step (see below), under mild conditions of acidic hydrolysis. However, we believe that it is advisable to isolate and store synthetic oligo- and poly-ribonucleotides with their 2′-hydroxy functions protected, and to delay the acidic hydrolysis step until the fully-unblocked RNA sequence is required. This ensures that the synthetic products are not digested by any contaminating endonucleases that may be present. The partially-protected decaribonucleoside nonaphosphate (**45**), nonadecaribonucleoside octadecaphosphate (**46**), and heptatriacontaribonucleoside hexatriacontaphosphate (**47**) were therefore isolated as their triethylammonium salts after they had been purified by chromatography on DEAE Sephadex A25, Sephadex G50 and Sephadex G75, respectively. The chromatographic elution profiles obtained are illustrated in Figure 2: 225 A_{260} units of (**45**) [Figure 2(a)] were obtained from 0.028 g (4.0 μmol) of (**28**), 110 A_{260} units of (**46**) [Figure 2(b)] were obtained from 0.020 g (1.5 μmol) of (**36**), and 105 A_{260} units of (**47**) [Figure 2(c)] were obtained from 0.016 g (0.6 μmol) of (**44**).

In the third and final unblocking step, the acid-labile protecting groups were removed from the partially-protected 3′-terminal decaribonucleoside nonaphosphate (**45**),

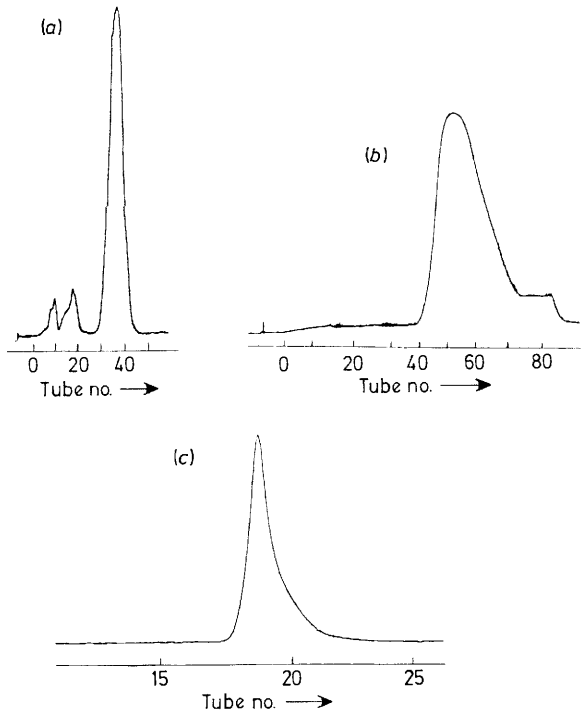

Figure 2. Elution profiles obtained from chromatography of (*a*) the partially-protected decaribonucleoside nonaphosphate [HO-U′pC′-pG′pU′pC′pC′pA′pC′pC′pA-Mm (**45**)] on DEAE Sephadex A25 with a linear gradient (0.01—1.00M over 1 000 ml) of triethylammonium hydrogen carbonate buffer (pH 7.5); (*b*) the partially-protected nonadecaribonucleoside octadecaphosphate [HO-A′pU′pU′pC′pC′pG′-pG′pA′pC′pU′pC′pG′pU′pC′pC′pA′pC′pC′pA-Mm (**46**)] on Sephadex G50 with 0.05M triethylammonium hydrogen carbonate buffer (pH 7.5) as eluting agent; (*c*) the partially-protected heptatriacontaribonucleoside hexatriacontaphosphate [Px-G′pG′pA′pG′pA′-pG′pG′pU′pC′pU′pC′pC′pG′pG′pT′pψ′pC′pG′pA′pU′pU′pC′pC′-pG′pG′pA′pC′pU′pC′pG′pU′pC′pC′pA′pC′pC′pA-Mm (**47**)] on Sephadex G75 with 0.01M triethylammonium hydrogen carbonate (pH 7.5) as the eluting solvent

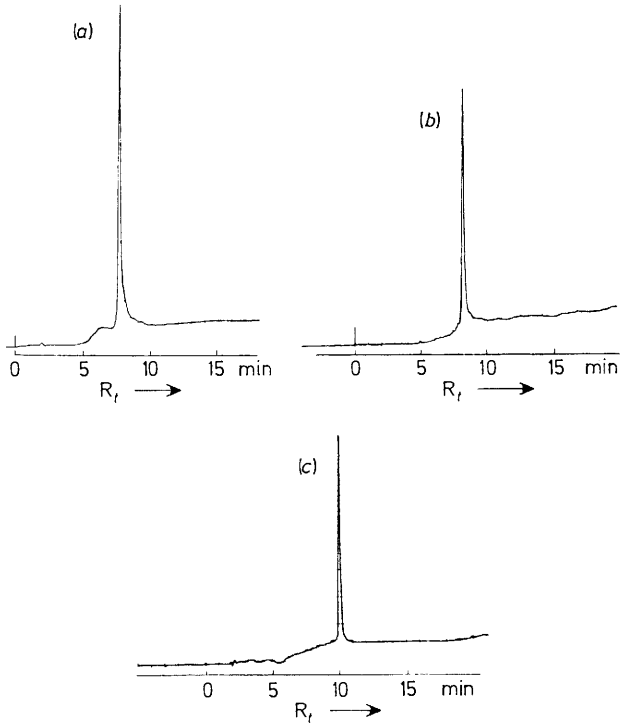

Figure 3. Liquid chromatography profiles (Jones APEX ODS 5 μ column, eluted with 0.1M triethylammonium acetate (pH 7.0)/acetonitrile) of (*a*) UpCpGpUpCpCpApCpCpA (**48**); (*b*) ApUpUpCpCpGpGpApCpUpCpGpUpCpCpApCpCpA (**49**); and (*c*) GpGpApGpApGpGpUpCpUpCpCpGpGpTpψpCpGpApUpUpCpCpGpGpApCpUpCpGpUpCpCpApCpCpA (**50**)

nonadecaribonucleoside octadecaphosphate (**46**) and heptatriacontaribonucleoside hexatriacontaphosphate (**47**) sequences of yeast $tRNA^{Ala}$ by treatment with dilute hydrochloric acid (0.01M, pH 2)[3,4] at room temperature for 6, 14, and 24 h, respectively. In this way, the completely unprotected 3′-terminal yeast $tRNA^{Ala}$ sequences UpCpGpUpCpCpApCpCpA (**48**), ApUpUpCpCpGpGpApCpUpCpGpUpCpCpApCpCpA (**49**) and GpGpApGpApGpGpUpCpUpCpCpGpGpTpψpCpGp ApUpUpCpCpGpGpApCpUpCpGpUpCpCpApCpCpA (**50**) were obtained. The chemical synthesis of the 5′-tritriacontamer (33-mer) sequence of *E. coli* $tRNA^{Gly}$ has previously been reported,[23] and Chinese workers have described[24] the total synthesis of yeast $tRNA^{Ala}$ by a combination of chemical and enzymatic methods.

Our synthetic yeast $tRNA^{Ala}$ sequences were found to be homogeneous by reverse phase liquid chromatography (Figure 3) and by polyacrylamide gel electrophoresis (Figure 4). The three unblocked tRNA sequences were treated in turn with a phosphodiesterase [Crotalus altrox venom phosphodiesterase in the cases of (**48**) and (**49**), and ribonuclease T2 in the case of (**50**)] and bacterial alkaline phosphatase; in this way, they underwent complete digestion to give their constituent ribonucleosides. The latter were separated by liquid chromatography and, in the cases of all three sequences [(**48**), (**49**), and (**50**)], their proportions were found, within the limits of experimental error, to be as expected.

The unblocking of all the fully-protected decaribonucleoside nonaphosphate (**28**) or nonadecaribonucleoside octadecaphosphate (**36**) obtained in this study would have yielded a really substantial quantity of the corresponding unprotected RNA sequence [(**45**) or (**46**)] and, assuming that the removal of the acid-labile protecting groups would not affect u.v. absorbance significantly, the unblocking of all the fully-protected heptatriacontaribonucleoside hexatriacontaphosphate (**44**) obtained (*i.e.* 0.047 g) would have yielded *ca.* 300 A_{260} units of the pure free half-tRNA molecule (**47**). Despite the recent progress in the development of the solid phase synthesis of oligo- and poly-ribonucleotides,[25] it is therefore reasonable to conclude that, if relatively large quantities of high molecular weight RNA sequences of high quality are required, the phosphotriester approach in solution, as described above, would appear still to be the synthetic method of choice.

Experimental

^1H N.m.r. spectra were measured, unless otherwise stated, at 250 MHz with a Bruker WM 250 spectrometer; tetramethylsilane was used as an internal standard. U.v. absorption spectra were measured either with a Perkin-Elmer 402 or a Cary 17 recording spectrometer. Merck silica gel 60 F_{254} pre-coated plates (Art 5715 and Art 5642) which, unless otherwise stated, were developed in solvent system A [$CHCl_3$–MeOH (9:1)], were used for thin layer chromatography (t.l.c.). Liquid chromatography (l.c.) was carried out on a Jones Apex Octadecyl 5 μ column which was eluted with 0.1M triethylammonium acetate/-acetonitrile mixtures. Polyacrylamide gel electrophoresis was carried out in a Raven Vertical Slab Gel Apparatus connected to an LKB Bromma 2197 power supply. Merck Kieselgel H (Art 7736 and Art 9385) was used for short column chromatography. Gel filtration was carried out on Sephadex G10, Sephadex G50,

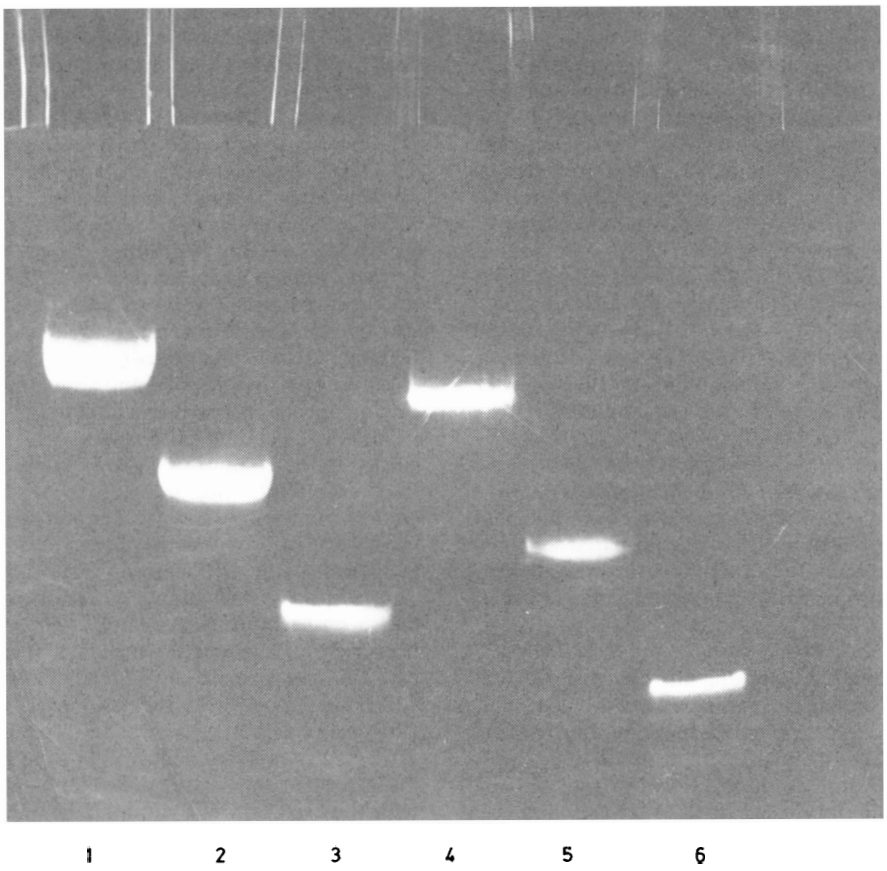

Figure 4. Polyacrylamide gel electrophoretogram (visualized by ultraviolet light) of Px-G'pG'pA'pG'pA'pG'pG'pU'pC'pU'pC'pC'pG'pG'-pT'pψ'pC'pG'pA'pU'pU'pC'pC'pG'pG'pA'pC'pU'pC'pG'pU'pC'pC'pA'pC'pC'pA-Mm [(**47**), lane 1]; HO-A'pU'pU'pC'p C'pG'pG'pA'pC'pU'-pC'pG'pU'pC'pC'pA'pC'pC'pA-Mm [(**46**)], lane 2]; HO-U'pC'pG'pU'pC'pC'pA'pC'pC'pA-Mm ((**45**), lane 3]; GpGpApGpApGpGpUpC-pUpCpCpGpGpTpψpCpGpApUpUpCpCpGpGpApCpUpCpGpUpCpCpApCpCpA [(**50**), lane 4]; ApUpUpCpCpGpGpApCpUpCpGpUpCpC-pApCpCpA [(**49**), lane 5]; UpCpGpUpCpCpApCpCpA [(**48**), lane 6]

Sephadex G75 and Sephadex LH 60 supports. Anion-exchange chromatography on DEAE Sephadex A-25 was carried out with linear gradients of triethylammonium hydrogen carbonate buffer (pH 7.5). Acetonitrile, dioxane, pyridine, tetrahydrofuran (THF) and triethylamine were dried by heating, under reflux, with calcium hydride for 3—5 h, and pyridine was additionally heated, under reflux, with mesitylene-2-sulphonyl chloride; these solvents were then distilled at atmospheric pressure and stored over molecular sieves (no. 4A). Phosphorolytic enzymes were purchased from the Sigma Chemical Co.

Triethylammonium salt of 5'-O-[2-(*Dibromomethyl*)*benzoyl*]-2'-O-(4-*methoxytetrahydropyran*-4-*yl*)-6-N-(4-*t*-*butylbenzoyl*)-*adenosine* 3'-(2-*chlorophenyl*)*phosphate* (**13a**; *B* = **5**).—2-Chlorophenyl phosphorodichloridate (0.88 g, 3.58 mmol), 1,2,4-triazole (0.545 g, 7.89 mmol), triethylamine (1.00 ml, 7.17 mmol) and THF (30.8 ml) were stirred together at room temperature. After 20 min, a solution of 5'-*O*-[2-(dibromomethyl)benzoyl]-2'-*O*-(4-methoxytetrahydropyran-4-yl)-6-*N*-(4-t-butylbenzoyl)-adenosine[9] (0.977 g, 1.19 mmol) and 1-methylimidazole (0.38 ml, 4.77 mmol) in THF (5 ml) were added, and the resulting mixture was stirred for 20 min. Triethylamine (3.00 ml, 21.5 mmol) and enough water (*ca.* 5 ml) to give a clear solution were then added, and the resulting solution was stirred for 10 min and then concentrated under reduced pressure. The residue was dissolved in chloroform (150 ml), and the solution was extracted first with saturated aqueous sodium hydrogen carbonate (2 × 120 ml) and then with water (2 × 120 ml). The aqueous layers were back-extracted with chloroform (3 × 120 ml). The organic layers were combined, dried ($MgSO_4$), and concentrated under reduced pressure. A solution of the residue in chloroform (10 ml) was added dropwise with stirring to light petroleum (b.p. 30—40 °C) (500 ml) to give the *title compound* as a colourless precipitate (1.285 g, 97%).

Triethylammonium salt of 5'-O-[2-(*dibromomethyl*)*benzoyl*]-2'-O-(4-*methoxytetrahydropyran*-4-*yl*)-4-N-(4-*t*-*butylbenzoyl*)-*cytidine* 3'-(2-*chlorophenyl*) *phosphate* (**13a**; *B* = **6**). The *title compound* (1.248 g, 96%) was prepared as above from 5'-*O*-[2-(dibromomethyl)benzoyl)-2'-*O*-(4-methoxytetrahydropyran-4-yl)-4-*N*-(4-t-butylbenzoyl)cytidine[9] (0.952 g, 1.20 mmol).

Triethylammonium salt of 5'-O-[2-(*dibromomethyl*)*benzoyl*]-2'-O-(4-*methoxytetrahydropyran*-4-*yl*)-6-O-(3-*chlorophenyl*)-2-N-*phenylacetylguanosine* 3'-(2-*chlorophenyl*) *phosphate* (**13a**; *B* = **7**). The *title compound* (2.98 g, 95%) was prepared as above from 5'-*O*-[2-(dibromomethyl)benzoyl]-2'-*O*-(4-methoxytetrahydropyran-4-yl)-6-*O*-(3-chlorophenyl)-2-*N*-phenylacetylguanosine[9] (2.37 g, 2.63 mmol).

Triethylammonium salt of 5'-O-[2-(*dibromomethyl*)*benzoyl*]-2'-O-(4-*methoxytetrahydropyran*-4-*yl*)-4-O-(2,4-*dimethylphenyl*)*uridine* 3'-(2-*chlorophenyl*) *phosphate* (**13a**; *B* = **8**). The *title compound* (0.925 g, 95%) was prepared as above from 5'-*O*-[2-(dibromomethyl)benzoyl]-2'-*O*-(4-methoxytetrahydropyran-4-yl)-4-*O*-(2,4-dimethylphenyl)uridine[9] (0.697 g, 0.94 mmol).

Triethylammonium salt of 1-N-(4-*bromobenzenesulphonyl*)-5'- O-[2-(*dibromomethyl*)*benzoyl*]-2'-O-(4-*methoxytetrahydropyran*-4-*yl*)-5-β-D-*ribofuranosyluracil* 3'-(2-*chlorophenyl*) *phosphate* (**13a**; *B* = **9**). The *title compound* (1.142 g, 91%) was

prepared as above from 1-*N*-(4-bromobenzenesulphonyl)-5′-*O*-[2-(dibromomethyl)benzoyl]-2′-*O*-(4-methoxytetrahydropyran-4-yl)-5-β-D-ribofuranosyluracil[9] (0.939 g, 1.10 mmol).

Triethylammonium salt of 5′-O-[2-(*isopropylthiomethoxymethyl*)*benzoyl*]-2′-O-(4-*methoxytetrahydropyran*-4-*yl*)-6-N-(4-*t-butylbenzoyl*)*adenosine* 3′-(2-*chlorophenyl*) *phosphate* (**13b**; B = **5**). The *title compound* (0.604 g, 94%) was prepared as above from 5′-*O*-[2-(isopropylthiomethoxymethyl)benzoyl]-2′-*O*-(4-methoxytetrahydropyran-4-yl)-6-*N*-(4-t-butylbenzoyl)-adenosine[9] (0.467 g, 0.61 mmol).

Triethylammonium salt of 5′-O-[2-(*isopropylthiomethoxymethyl*)*benzoyl*]-2′-O-(4-*methoxytetrahydropyran*-4-*yl*)-4-O-(2,4-*dimethylphenyl*)*uridine* 3′-(2-*chlorophenyl*) *phosphate* (**13b**; B = **8**). The *title compound* (1.172 g, 95%) was prepared as above from 5′-*O*-[2-(isopropylthiomethoxymethyl)benzoyl]-2′-*O*-(4-methoxytetrahydropyran-4-yl)-4-*O*-(2,4-dimethylphenyl)uridine[9] (0.862 g, 1.26 mmol).

Triethylammonium salt of 5′-O-(9-*phenylxanthen*-9-*yl*)-2′-O-(4-*methoxytetrahydropyran*-4-*yl*)-6-O-(3-*chlorophenyl*)-2-N-*phenylacetylguanosine* 3′-(2-*chlorophenyl*) *phosphate* (**13c**; B = **7**). The *title compound* (0.816 g, 95%) was prepared as above from 5′-*O*-(9-phenylxanthen-9-yl)-2′-*O*-(4-methoxytetrahydropyran-4-yl)-6-*O*-(3-chlorophenyl)-2-*N*-phenylacetylguanosine[9] (0.643 g, 0.73 mmol).

2,4-*Dinitrobenzyl alcohol.*[17]—Fuming nitric acid (*d.* 1.5) (180 ml) was added with stirring to cooled (ice-salt bath), concentrated sulphuric acid (98%; 250 ml). Benzyl chloride (90.2 ml, 0.784 mol) was then added dropwise over a period of *ca.* 1 h to this stirred, cooled nitration medium at such a rate that the reaction temperature did not exceed 20 °C. After they had been allowed to stand at room temperature for a further period of 1 h, the products were carefully poured into a mixture of ice (2 000 g) and water (2 000 ml). The resulting mixture was extracted with dichloromethane (3 × 1 000 ml). Evaporation of the dried ($MgSO_4$) dichloromethane solution gave a pale yellow oil (161 g).

Potassium acetate (90.67 g, 0.924 mol) was added to a stirred solution of the latter oil (100 g) in glacial acetic acid (500 ml) and the reaction mixture was heated at 120 °C for 18 h. The products were then cooled, poured into dichloromethane (1 000 ml) and the resulting mixture was washed with water (2 × 2 000 ml). Evaporation of the dried ($MgSO_4$) dichloromethane solution gave a yellow solid (108.3 g). A portion of the latter product (55 g), contained in a 500 ml flask, was agitated with methanol (50 ml) until a fine suspension was obtained. 50% Sulphuric acid (obtained by diluting concentrated sulphuric acid with an equal volume of water) was then added to the vigorously stirred suspension in such a way as to ensure that it was finely dispersed. The flask was then fitted with a reflux condenser and the reactants were heated at 100 °C for 20 min. After the products had been allowed to cool to room temperature, they were further cooled (ice-water bath), and then poured into water (1 000 ml). The resulting mixture was extracted with ether (500 ml + 300 ml), the extracts were washed with water (1 000 ml) and the aqueous washings were back-extracted with ether (200 ml). The dried ($MgSO_4$), combined ether extracts were evaporated to give a solid which was recrystallized from a mixture of chloroform (450 ml) and carbon tetrachloride (150 ml) to give the *title compound* as yellow crystals (23.37 g, 48% for the 3 steps starting from benzyl alcohol), m.p. 113—115 °C (lit.[17] 114—115 °C); $\delta_H[(CD_3)_2SO]$ 4.94 (2 H, d, J 5.0 Hz), 5.88 (1 H, t, J 5.3 Hz), 8.13 (1 H, d, J 8.7 Hz), 8.59 (1 H, dd, J 2.3, 8.7 Hz), and 5.76 (1 H, d, J 2.3 Hz).

2′-O-(4-*Methoxytetrahydropyran*-4-*yl*)-6-O-(3-*chlorophenyl*)-2-N-*phenylacetylguanosine* 3′-(2-*chlorophenyl*) (2,4-*dinitrobenzyl*) *phosphate* (**14**; B = **7**).—1-Hydroxybenzotriazole (0.405 g, 3.0 mmol) and triethylamine (0.42 ml, 3.0 mmol) were added to a stirred solution of 2-chlorophenyl phosphorodichloridate (0.365 g, 1.49 mmol) in anhydrous THF (10 ml), contained in a flask fitted with a rubber septum, at 0 °C (ice-bath). The ice-bath was removed and, after 20 min, a solution of 5′-*O*-[2-(dibromomethyl)benzoyl]-2′-*O*-(4-methoxytetrahydropyran-4-yl)-6-*O*-(3-chlorophenyl)-2-*N*-phenylacetylguanosine[9] (0.902 g, 1.0 mmol) in pyridine (5 ml) was introduced by means of a syringe. After 20 min, a solution of 2,4-dinitrobenzyl alcohol (0.396 g, 2.0 mmol) in pyridine (2 ml) was introduced in the same way and, after an additional period of 2 h, half-saturated aqueous sodium hydrogen carbonate (1 ml) was added. After 10 min, chloroform (30 ml) was added and the resulting mixture was extracted with saturated aqueous sodium hydrogen carbonate (80 ml). The aqueous layer was back-extracted with chloroform (2 × 100 ml), and the combined, dried ($MgSO_4$) organic layers were concentrated under reduced pressure. The residue was fractionated by short column chromatography on silica gel: the column was eluted in turn with light petroleum (b.p. 60—80 °C)–acetone (95:5—90:10), chloroform, and chloroform–ethanol (98:2). The desired product {assumed to be 5′-*O*-[2-(dibromomethyl)benzoyl]-2′-*O*-(4-methoxytetrahydropyran-4-yl)-6-*O*-(3-chlorophenyl)-2-*N*-phenylacetyl guanosine 3′-(2-chlorophenyl)(2,4-dinitrobenzyl) phosphate} was eluted with chloroform–ethanol (98:2): the appropriate fractions were combined and evaporated under reduced pressure to give a t.l.c. homogeneous (R_F 0.60) glass; yield, 0.954 g.

2,4,6-Collidine (1.13 ml, 8.5 mmol) and silver perchlorate (3.65 g, 17.6 mmol) were added to a solution of the above fully-protected mononucleotide (0.954 g) in THF–water (98:2) (51 ml) at room temperature. The reactants were stirred together, with the exclusion of light, for 2 h. Lithium chloride (1.305 g, 30.8 mmol) was then added and, after 10 min, the products were filtered through celite. The celite bed was thoroughly washed with THF–pyridine (4:1) (*ca.* 6 × 5 ml). Morpholine (0.52 ml, 6.0 mmol) was then added to the filtrate and washings and, after 10 min, 1.5M aqueous potassium dihydrogen phosphate (4.0 ml, 6.0 mmol) was added. The resulting mixture was partitioned between chloroform (30 ml) and saturated aqueous sodium hydrogen carbonate (50 ml). The aqueous layer was back-extracted with chloroform (2 × 100 ml) and the combined, dried ($MgSO_4$) organic layers were evaporated under reduced pressure. The residual glass was purified by short column chromatography on silica gel: the appropriate fractions, eluted with chloroform–ethanol (98:2) were combined and evaporated under reduced pressure. When a solution of the residual glass in chloroform (2 ml) was added dropwise to light petroleum (b.p. 30—40 °C) (50 ml), the *title substance* was obtained as a colourless precipitate; yield, 0.507 g (51%); R_F 0.46.

2′-*O*-(4-*Methoxytetrahydropyran*-4-*yl*)-6-N-(4-*t-butylbenzoyl*)*adenosine* 3′-(2-*chlorophenyl*) (2,4-*dinitrobenzyl*) *phosphate* (**14**: B = **5**). The *title compound* (0.499 g, 54%) was prepared as above from 5′-*O*-[2-(dibromomethyl)benzoyl]-2′-*O*-(4-methoxytetrahydropyran-4-yl)-6-*N*-(4-t-butylbenzoyl)adenosine[9] (0.817 g, 1.00 mmol); R_F 0.46.

2′-O-(4-*Methoxytetrahydropyran*-4-*yl*)-4-N-(*t-butylbenzoyl*)*cytidine* 3′-(2-*chlorophenyl*) (2,4-*dinitrobenzyl*) *phosphate* (**14**; B = **6**). The *title compound* (0.502 g, 56%) was prepared as above from 5′-*O*-[2-(dibromomethyl)benzoyl]-2′-*O*-(4-methoxytetrahydropyran-4-yl)-4-*N*-(4-t-butylbenzoyl)cytidine[9] (0.793 g, 1.00 mmol); R_F 0.50.

2′-O-(4-*Methoxytetrahydropyran*-4-*yl*)-4-O-(2,4-*dimethylphenyl*)*uridine* 3′-(2-*chlorophenyl*) (2,4-*dinitrobenzyl*) *phosphate* (**14**; B = **8**). The *title compound* (0.564 g, 67%) was prepared as above from 5′-*O*-[2-(dibromomethyl)benzoyl]-2′-*O*-(4-methoxytetrahydropyran-4-yl)-4-*O*-(2,4-dimethylphenyl)uridine[9] (0.738 g, 1.00 mmol); R_F 0.41.

Preparation of Partially-protected Dinucleoside Phosphates

Exemplified by Dbmb-A′pC′-OH (**17a**; B = **5**, B'' = **6**).—The triethylammonium salt of 5′-*O*-[2-(dibromomethyl)benzoyl]-2′-*O*-(4-methoxytetrahydropyran-4-yl)-6-*N*-(4-t-butylbenzoyl)-adenosine 3′-(2-chlorophenyl) phosphate (0.993 g, 0.89 mmol) and 2′-*O*-(4-methoxytetrahydropyran-4-yl)-4-*N*-(t-butylbenzoyl)cytidine[9] (0.386 g, 0.75 mmol) were dissolved in anhydrous pyridine (*ca.* 25 ml) and the resulting solution was evaporated under reduced pressure (oil-pump) with gentle warming. When the solution ceased to be mobile, more pyridine (to give a total volume of *ca.* 18 ml) was added and the solution was again concentrated under reduced pressure to *ca.* one-half volume. 1-(Mesitylene-2-sulphonyl)-3-nitro-1,2,4-triazole[3] (MSNT, 0.663 g, 2.24 mmol) was then added, with magnetic stirring, to the resulting pyridine solution at room temperature. After 20 min, saturated aqueous sodium hydrogen carbonate (1 ml) was added to the stirred solution and, after a further period of 5 min, the products were concentrated under reduced pressure and the residue was partitioned between chloroform (100 ml) and saturated aqueous sodium hydrogen carbonate (200 ml). The aqueous layer was back-extracted with chloroform (2 × 100 ml), and the combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was fractionated by short column chromatography on silica gel: the appropriate fractions, which were eluted with chloroform–ethanol (95:5), were combined and concentrated under reduced pressure. When a solution of the residue in chloroform (10 ml) was added dropwise with magnetic stirring to light petroleum (b.p. 30—40 °C) (500 ml), the *title substance* was precipitated as a colourless solid (0.857 g, 76%); R_F 0.65.

The nine other partially-protected dinucleoside phosphates required in the synthesis of the 3′-terminal heptatriacontamer sequence were prepared in the same way. The results obtained are summarized in the Table (entries nos. 2—10).

Preparation of Partially-protected Dinucleotides Exemplified by the Triethylammonium Salt of Dbmb-A′pC′p (**18a**; B = **5**, B' = **6**).—2-Chlorophenyl phosphorodichloridate (0.393 g, 1.60 mmol), 1,2,4-triazole (0.259 g, 3.75 mmol), triethylamine (0.48 ml, 3.44 mmol) and THF (12 ml) were stirred together at room temperature. After 10 min, a solution of Dbmb-*A′pC′*-OH (0.857 g, 0.57 mmol) in THF (5 ml) and 1-methylimidazole (0.19 ml, 2.38 mmol) were added, and the resulting mixture was stirred for 30 min at room temperature. Triethylamine (1.43 ml, 10.3 mmol) and water (4 ml) were added, and the resulting solution was stirred for 5 min and then concentrated under reduced pressure. The residue was dissolved in chloroform (200 ml), and the solution was extracted first with saturated aqueous sodium hydrogen carbonate (200 ml) and then with water (200 ml). The aqueous layers were both back-extracted with chloroform (3 × 200 ml), and the combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure. A solution of the residue in chloroform (10 ml) was added dropwise with stirring to light petroleum (b.p. 30—40 °C) (500 ml) to give the *title substance* as a colourless precipitate (0.974 g, 95%).

The triethylammonium salts of the nine other dinucleotide blocks [Dbmb-*C′pC′p*, Dbmb-*G′pA′p*, Dbmb-*G′pG′p*, Dbmb-*G′pU′p*, Dbmb-*U′pC′p*, Dbmb-*ψ′pC′p*, Ptmt-*A′pU′p*, Ptmt-*U′pC′p*, and Px-*G′pG′p*] required in the synthesis of the 3′-terminal heptatriacontamer sequence were prepared in the same way, and were isolated in yields ranging from 93% to almost quantitative.

Preparation of Partially-protected Trinucleotide Diphosphates Exemplified by Dbmb-A′pC′pC′-*OH* (**19a**; B = **5**; B' = B'' = **6**).—The triethylammonium salt of Dbmb-*A′pC′p* (0.974 g, 0.54 mmol) and 2′-*O*-(4-methoxytetrahydropyran-4-yl)-4-*N*-(t-butylbenzoyl)cytidine[9] (0.233 g, 0.45 mmol) were dissolved in anhydrous pyridine (*ca.* 16 ml) and the resulting solution was evaporated under reduced pressure (oil-pump) with gentle warming. When the solution ceased to be mobile, more pyridine (to give a total volume of *ca.* 11 ml) was added and the solution was again concentrated under reduced pressure to *ca.* one-half volume. MSNT[3] (0.48 g, 1.62 mmol) was then added, with magnetic stirring, to the resulting pyridine solution at room temperature. After 20 min, saturated aqueous sodium hydrogen carbonate (1 ml) was added to the stirred solution and, after a further period of 5 min, the products were concentrated under reduced pressure and the residue was partitioned between chloroform (100 ml) and saturated aqueous sodium hydrogen carbonate (200 ml). The aqueous layer was back-extracted with chloroform (2 × 100 ml), and the combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was fractionated by short column chromatography on silica gel: the appropriate fractions, which were eluted with chloroform–ethanol (95:5), were combined and concentrated under reduced pressure. When a solution of the residue in chloroform (10 ml) was added dropwise with magnetic stirring to light petroleum (b.p. 30—40 °C) (500 ml), the *title substance* was precipitated as a colourless solid (0.833 g, 84%); R_F 0.64.

The four other partially-protected trinucleoside diphosphates required in the synthesis of the 3′-terminal heptatriacontamer sequence were prepared in the same way. The results obtained are summarized in the Table (entries nos. 12—15).

Preparation of Fully-protected Trinucleotide 3′-(2,4-*Dinitrobenzyl*) *Esters Exemplified by Ptmt*-U′pC′pG′p-*Dnb* (**21b**; B = **8**, B' = **6**, B'' = **7**).—The triethylammonium salt of Ptmt-*U′pC′p* (0.500 g, 0.30 mmol) and 2′-*O*-(4-methoxytetrahydropyran-4-yl)-6-*O*-(3-chlorophenyl)-2-*N*-phenylacetylguanosine 3′-(2-chlorophenyl) (2,4-dinitrobenzyl) phosphate (0.249 g, 0.25 mmol) were dissolved in anhydrous pyridine (*ca.* 9 ml) and the resulting solution was evaporated under reduced pressure (oil-pump) with gentle warming. When the solution ceased to be mobile, more pyridine (to give a total volume of *ca.* 6 ml) was added and the solution was again concentrated under reduced pressure to *ca.* one-half volume. MSNT[3] (0.296 g, 1.00 mmol) was then added, with magnetic stirring, to the resulting pyridine solution at room temperature. After 40 min, saturated aqueous sodium hydrogen carbonate (*ca.* 0.5 ml) was added to the stirred solution and, after a further period of 5 min, the products were concentrated under reduced pressure and the residue was partitioned between chloroform (60 ml) and saturated aqueous aqueous sodium hydrogen carbonate (120 ml). The aqueous layer was back-extracted with chloroform (2 × 60 ml), and the combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was allowed to react with 2-chlorophenyl bis(1,2,4-triazolyl)phosphate[15] [prepared from 2-chlorophenyl phosphorodichloridate (0.245 g, 1.0 mmol), 1,2,4-triazole (0.15 g, 2.2 mmol), 1-methylimidazole (0.11 ml, 1.4 mmol) and triethylamine (0.28 ml, 2.0 mmol)] in THF (10 ml) at room temperature for 1 min. Triethylamine (0.83 ml, 6.0 mmol) and water (1 ml) were then added and, after a further period of 1 min, the products were concentrated under reduced pressure. The residue was partitioned between saturated aqueous sodium hydrogen carbonate (300 ml) and chloroform (5 × 200 ml). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure, and the residue was fractionated by short column chromatography on silica gel: the appropriate fractions, which were eluted with chloroform–ethanol (95:5), were combined and concentrated under reduced pressure. When a solution of the residue in chloroform (5 ml) was added dropwise with magnetic stirring to light petroleum (b.p. 30—40 °C) (250 ml), the *title substance* was precipitated as a colourless solid (0.563 g, 88%); R_F 0.78.

The six other fully-protected trinucleotide 3′-(2,4-dinitrobenzyl) esters required in the synthesis of the 3′-heptatriacontamer sequence were prepared in the same way. The results

obtained are summarized in the Table (entries nos. 16—20 and 22).

Preparation of Partially-protected Trinucleotides—(a) *Exemplified by the triethylammonium salt of Dbmb*-A′pC′pC′p (**20a**; $B = \mathbf{5}$, $B' = B'' = \mathbf{6}$). 2-Chlorophenyl phosphorodichloridate (0.263 g, 1.07 mmol), 1,2,4-triazole (0.173 g, 2.50 mmol), triethylamine (0.317 ml, 2.27 mmol) and THF (8 ml) were stirred together at room temperature. After 10 min, a solution of Dbmb-*A′pC′pC′*-OH (0.833 g, 0.38 mmol) in THF (3.5 ml) and 1-methylimidazole (0.125 ml, 1.57 mmol) were added, and the resulting mixture was stirred for 30 min at room temperature. Triethylamine (0.95 ml, 6.8 mmol) and water (3 ml) were added, and the resulting solution was stirred for 5 min and then concentrated under reduced pressure. The residue was dissolved in chloroform (200 ml), and the solution was extracted first with saturated aqueous sodium hydrogen carbonate (200 ml) and then with water (200 ml). The aqueous layers were back-extracted with chloroform (3 × 200 ml), and the combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure. A solution of the residue in chloroform (10 ml) was added dropwise with stirring to light petroleum (b.p. 30—40 °C) (500 ml) to give the *title substance* as a colourless precipitate (0.913 g, 96%). The triethylammonium salts of four of the other trinucleotide blocks (Dbmb-*C′pC′pG′p*, Dbmb-*G′pA′pG′p*, Dbmb-*G′pG′pT′p*, and Dbmb-*U′pC′pC′p*) required in the synthesis of the 3′-terminal heptatriacontamer sequence were prepared in the same way, and were isolated in yields ranging from 95—97%.

(b) *Exemplified by the triethylammonium salt of Ptmt*-U′pC′pG′p (**20b**; $B = \mathbf{8}$, $B' = \mathbf{6}$, $B'' = \mathbf{7}$). Triethylamine (0.10 ml, 0.72 mmol) was added to a stirred solution of Ptmt-*U′pC′pG′p*-Dnb (0.534 g, 0.21 mmol) and 2-thiocresol (0.104 g, 0.84 mmol) in acetonitrile (4.2 ml) at room temperature. After 5 min, allyl chloride (0.086 ml, 1.06 mmol) was added and, after a further period of 5 min, the products were evaporated under reduced pressure. The residue was partitioned between saturated aqueous sodium hydrogen carbonate (200 ml) and chloroform (4 × 200 ml). The combined organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was fractionated by short column chromatography on silica gel: the appropriate fractions, which were eluted with chloroform–ethanol (80:20), were combined and concentrated under reduced pressure. When a solution of the residue obtained in chloroform (10 ml) was added dropwise with magnetic stirring to light petroleum (b.p. 30—40 °C) (500 ml), the *title substance* was obtained as a colourless solid precipitate (0.454 g, 87%). The triethylammonium salts of four of the other trinucleotide blocks (Ptmt-*A′pU′pU′p*, Ptmt-*U′pC′pC′p*, Dbmb-*ψ′pC′pG′p*, and Px-*G′pG′pA′p*) required in the synthesis of the 3′-terminal heptatriacontamer sequence were prepared in the same way, and were isolated in yields ranging from 77—95%.

Preparation of Dbmb-A′pC′pC′pA-*Mm* (**23**; $R = \mathbf{2}$; $B = \mathbf{5}$, $B' = B'' = \mathbf{6}$).—The triethylammonium salt of Dbmb-*A′pC′pC′p* (0.913 g, 0.367 mmol) and 2′,3′-*O*-methoxymethylene-6-*N*-(4-t-butylbenzoyl)adenosine [9] (0.150 g, 0.32 mmol) were dissolved in anhydrous pyridine (*ca.* 12 ml) and the resulting solution was evaporated under reduced pressure (oil-pump) with gentle warming. When the solution ceased to be mobile, more pyridine (to give a total volume of *ca.* 8 ml) was added and the resulting solution was again concentrated under reduced pressure to *ca.* one-half volume. MSNT [3] (0.378 g, 1.28 mmol) was then added, with magnetic stirring, to the resulting pyridine solution at room temperature. After 25 min, saturated aqueous sodium hydrogen carbonate (0.5 ml) was added to the stirred solution and, after a further period of 5 min, the products were concentrated under reduced pressure and the residue was partitioned between chloroform (100 ml) and saturated aqueous sodium hydrogen carbonate (200 ml). The aqueous layer was back-extracted with chloroform (2 × 100 ml), and the combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was fractionated by short column chromatography on silica gel: the appropriate fractions, which were eluted with chloroform–ethanol (95:5), were combined and concentrated under reduced pressure. When a solution of the residue in chloroform (10 ml) was added dropwise with magnetic stirring to light petroleum (b.p. 30—40 °C) (500 ml), the *title substance* was precipitated as a colourless solid (0.858 g, 94%); R_F 0.78.

Procedure for the Removal of the 2-(*Dibromomethyl*)*benzoyl* (*Dbmb*) *Protecting Group from Fully-protected Oligoribonucleotides.*—(a) *Preparation of HO*-A′pC′pC′A-*Mm* (**23**; $R = H$, $B = \mathbf{5}$, $B' = B'' = \mathbf{6}$). Silver perchlorate (0.60 g, 2.9 mmol) was added, with the exclusion of light, to a magnetically-stirred solution of Dbmb-*A′pC′pC′pA*-Mm (0.823 g, 0.29 mmol) and 2,4,6-collidine (0.287 ml, 2.17 mmol) in THF–water (98:2) (14.2 ml) at room temperature. After 2.5 h, lithium chloride (0.156 g, 3.7 mmol) was added and, after a further period of 5 min, the products were centrifuged. The supernatant was decanted and the residue was washed with THF (4 × 5 ml). The combined washings were evaporated under reduced pressure, redissolved in THF (2 ml) and the solution added to the original supernatant. Morpholine (0.50 ml, 5.7 mmol) was added and the solution was stirred for 15 min. The products were then poured into saturated aqueous sodium hydrogen carbonate (500 ml), and the resulting mixture was extracted with chloroform (6 × 200 ml). The combined organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was fractionated by short column chromatography on silica gel: the appropriate fractions, which were eluted with chloroform–ethanol (94:6), were combined and concentrated under reduced pressure. When a solution of the residue in chloroform (10 ml) was added dropwise with magnetic stirring to light petroleum (b.p. 30—40 °C) (500 ml), the *title substance* was precipitated as a colourless solid (0.644 g, 86%); R_F 0.67.

(b) *Preparation of partially-protected trinucleotide* 3′-(2,4-*dinitrobenzyl*) *esters exemplified by HO*-G′pA′pC′p-*Dnb* (**21**; $R = H$, $B = \mathbf{7}$, $B' = \mathbf{5}$, $B'' = \mathbf{6}$). The 5′-*O*-Dbmb protecting group was removed from Dbmb-*G′pA′pC′p*-Dnb by a slight modification of the above procedure. The substrate (1.338 g, 0.50 mmol) was treated with silver perchlorate (1.037 g, 5.0 mmol) and 2,4,6-collidine (0.50 ml, 3.8 mmol) in THF–water (98:2) (25 ml) at room temperature for 135 min. The products were treated, as above, with lithium chloride (0.233 g, 5.5 mmol) and then with morpholine (0.245 ml, 2.8 mmol). Aqueous potassium dihydrogen phosphate (1.5M; 2.8 ml, 4.2 mmol) was added, and the products were then worked-up and chromatographed. The *title substance* was obtained as a colourless precipitated solid (0.732 g, 61%); R_F 0.61. In the same way, HO-*ψ′pC′pG′p*-Dnb (R_F 0.71) and HO-*G′pU′pC′p*-Dnb (R_F 0.68) were prepared from Dbmb-*ψ′pC′pG′p*-Dnb and Dbmb-*G′pU′pC′p*-Dnb in 37 and 57% yields, respectively.

Preparation of Fully-protected 3′-*Terminal Decaribonucleoside Nonaphosphate Sequence, Ptmt*-U′pC′pG′pU′pC′pC′pA′-pC′pC′pA-*Mm* (**28**).—The triethylammonium salt of Dbmb-*U′pC′pC′p* (0.787 g, 0.33 mmol) and HO-*A′pC′pC′pA*-Mm (0.644 g, 0.25 mmol) were dissolved in anhydrous pyridine (*ca.* 11 ml) and the resulting solution was evaporated under reduced pressure (oil-pump) with gentle warming. When the solution ceased to be mobile, more pyridine (to give a total volume of *ca.* 7 ml) was added and the solution was again concentrated under reduced pressure to *ca.* one-half volume. MSNT [3] (0.296 g, 1.00 mmol) was then added, with magnetic stirring, to the resulting pyridine solution at room temperature. After 60 min, saturated aqueous sodium hydrogen carbonate

(0.5 ml) was added to the stirred solution and, after a further period of 5 min, the products were concentrated under reduced pressure and the residue was partitioned between chloroform (100 ml) and saturated aqueous sodium hydrogen carbonate (200 ml). The aqueous layer was back-extracted with chloroform (2 × 100 ml), and the combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was allowed to react with 2-chlorophenyl bis(1,2,4-triazolyl)-phosphate[15] [prepared from 2-chlorophenyl phosphorodichloridate (0.245 g, 1.0 mmol), 1,2,4-triazole (0.15 g, 2.2 mmol), 1-methylimidazole (0.11 ml, 1.4 mmol) and triethylamine (0.28 ml, 2.0 mmol)] in THF (10 ml) at room temperature for 1 min. Triethylamine (0.83 ml, 6.0 mmol) and water (1 ml) were then added and, after a further period of 1 min, the products were concentrated under reduced pressure. The residue was partitioned between saturated aqueous sodium hydrogen carbonate (300 ml) and chloroform (6 × 200 ml). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure, and the residue was fractionated by short column chromatography on silica gel: the appropriate fractions, which were eluted with chloroform–ethanol (92:8), were combined and concentrated under reduced pressure. When a solution of the residue in chloroform (10 ml) was added dropwise with magnetic stirring to light petroleum (b.p. 30—40 °C) (500 ml), the putative fully-protected heptamer, Dbmb-*U'pC'pC'pA'-pC'pC'pA*-Mm was obtained as a colourless solid precipitate (1.03 g, 84%); R_F 0.75.

The 5'-*O*-Dbmb protecting group was removed from the latter material by the procedure described above in the 5'-unblocking of Dbmb-*A'pC'pC'pA*-Mm. The substrate (1.00 g, 0.206 mmol) was treated with silver perchlorate (0.427 g, 2.06 mmol) and 2,4,6-collidine (0.206 ml, 1.56 mmol) in THF–water (98:2) (10 ml) at room temperature for 3 h. The products were then treated as above with lithium chloride (0.096 g, 2.26 mmol), followed by morpholine (0.105 ml, 1.20 mmol). After work-up and short column chromatography [elution with chloroform–ethanol (92:8)], the putative 5'-unblocked heptamer, HO-*U'pC'pC'pA'pC'pC'pA*-Mm was precipitated as a colourless solid (0.77 g, 81%; 69% for the two steps starting from HO-*A'pC'pC'pA*-Mm); R_F 0.69.

The triethylammonium salt of Ptmt-*U'pC'pG'p* (0.520 g, 0.21 mmol) and HO-*U'pC'pC'pA'pC'pC'pA*-Mm (0.77 g, 0.168 mmol) were dissolved in anhydrous pyridine (*ca.* 7 ml) and the resulting solution was evaporated under reduced pressure (oil-pump) with gentle warming. When the solution ceased to be mobile, more pyridine (to give a total volume of *ca.* 3 ml) was added and the solution was again concentrated under reduced pressure to *ca.* one-half volume. MSNT[3] (0.20 g, 0.67 mmol) was then added, with magnetic stirring, to the resulting pyridine solution at room temperature. After 60 min, an additional quantity of MSNT (0.10 g, 0.34 mmol) was added and, after a further period of 90 min, saturated aqueous sodium hydrogen carbonate (*ca.* 0.5 ml) was added to the stirred solution. After 5 min, the products were concentrated under reduced pressure and the residue was partitioned between chloroform (100 ml) and saturated aqueous sodium hydrogen carbonate (20 ml). The aqueous layer was back-extracted with chloroform (3 × 100 ml), and the combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was treated with 2-chlorophenyl bis(1,2,4-triazolyl)phosphate[15] [prepared from 2-chlorophenyl phosphorodichloridate (0.245 g, 1.0 mmol), 1,2,4-triazole (0.15 g, 2.2 mmol), 1-methylimidazole (0.11 ml, 1.4 mmol) and triethylamine (0.28 ml, 2.0 mmol)] in THF (10 ml) at room temperature for 1 min, and the products were worked-up and chromatographed [the appropriate fractions were eluted with $CHCl_3$–EtOH (94:6)] as above. The *title substance* was obtained as a colourless solid precipitate (0.938 g, 80%; 55.5% for the three steps starting from HO-*A'pC'pC'pA*-Mm); R_F 0.78.

Preparation of Partially-protected Hexanucleotide 3'-(2,4-*Dinitrobenzyl*) *Esters Exemplified by HO*-C'pC'pG'pG'pA'-pC'p-*Dnb* (**31**).—The triethylammonium salt of Dbmb-*C'pC'-pG'p* (1.02 g, 0.396 mmol) and HO-*G'pA'pC'p*-Dnb (0.732 g, 0.308 mmol) were dissolved in anhydrous pyridine (*ca.* 12 ml) and the resulting solution was evaporated under reduced pressure (oil-pump) with gentle warming. When the solution ceased to be mobile, more pyridine (to give a total volume of *ca.* 8 ml) was added and the solution was again concentrated under reduced pressure to *ca.* one-half volume. MSNT[3] (0.362 g, 1.22 mmol) was then added, with magnetic stirring, to the resulting pyridine solution at room temperature. After 45 min, saturated aqueous sodium hydrogen carbonate (*ca.* 0.75 ml) was added to the stirred solution and, after a further period of 5 min, the products were concentrated under reduced pressure and the residue was partitioned between chloroform (100 ml) and saturated aqueous sodium hydrogen carbonate (200 ml). The aqueous layer was back-extracted with chloroform (3 × 100 ml), and the combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was treated with 2-chlorophenyl bis(1,2,4-triazolyl)phosphate[15] [prepared from 2-chlorophenyl phosphorodichloridate (0.245 g, 1.0 mmol), 1,2,4-triazole (0.15 g, 2.2 mmol), 1-methylimidazole (0.11 ml, 1.4 mmol) and triethylamine (0.28 ml, 2.0 mmol)] in THF (10 ml) at room temperature for 1 min, and the products were worked-up and chromatographed [the appropriate fractions were eluted with $CHCl_3$–EtOH (94:6)] as above. The putative fully-protected hexanucleotide, Dbmb-*C'pC'pG'pG'-pA'pC'p*-Dnb was obtained as a colourless solid precipitate (1.174 g, 79%); R_F 0.79.

The 5'-*O*-Dbmb protecting group was removed from the latter material by the procedure described above in the 5'-unblocking of Dbmb-*G'pA'pC'p*-Dnb. The substrate (1.17 g, 0.24 mmol) was treated with silver perchlorate (0.476 g, 2.3 mmol) and 2,4,6-collidine (0.23 ml, 1.74 mmol) in THF–water (98:2) (12 ml) at room temperature for 135 min. The products were then treated with lithium chloride (0.11 g, 2.6 mmol), followed by morpholine (0.122 ml, 1.4 mmol) and aqueous potassium dihydrogen phosphate (1.5M; 1.4 ml, 2.1 mmol). After work-up and short column chromatography [elution with chloroform–ethanol (94:6)], the *title compound* was precipitated as a colourless solid (0.745 g, 67%; 53% for the two steps starting from HO-*G'pA'pC'p*-Dnb); R_F 0.76. In the same way, the two other partially-protected hexanucleotides [HO-*G'pG'pT'pψ'-pC'pG'p*-Dnb (R_F 0.78) and HO-*G'pA'pG'pG'pU'pC'p*-Dnb (R_F 0.71)] required in the synthesis of the 3'-terminal heptatriacontamer sequence were prepared from HO-*ψ'pC'pG'p*-Dnb and HO-*G'pU'pC'p*-Dnb in 39 and 38% overall yields, respectively.

Preparation of Fully-protected Nonanucleotide 3'-(2,4-*Dinitrobenzyl*) *Esters Exemplified by Ptmt*-A'pU'pU'pC'pC'-pG'pG'pA'pC'p-Dnb (**33**).—The triethylammonium salt of Ptmt-*A'pU'pU'p* (0.484 g, 0.208 mmol) and HO-*C'pC'pG'-pG'pA'pC'p*-Dnb (0.733 g, 0.161 mmol) was dissolved in anhydrous pyridine (*ca.* 10 ml) and the resulting solution was evaporated under reduced pressure (oil-pump) with gentle warming. When the solution ceased to be mobile, more pyridine (to give a total volume of *ca.* 4 ml) was added and the solution was again concentrated under reduced pressure to *ca.* one-half volume. MSNT[3] (0.237 g, 0.80 mmol) was then added, with magnetic stirring, to the resulting pyridine solution at room temperature. After 60 min, saturated aqueous sodium hydrogen carbonate (*ca.* 0.5 ml) was added to the stirred solution and, after a further period of 5 min, the products were concentrated under reduced pressure and the residue was partitioned between chloroform (100 ml) and saturated aqueous sodium hydrogen carbonate (200 ml). The aqueous layer was back-extracted with

chloroform (3 × 100 ml), and the combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was treated with 2-chlorophenyl bis(1,2,4-triazolyl)-phosphate [*prepared* from 2-chlorophenyl phosphorodichloridate (0.245 g, 1.0 mmol), 1,2,4-triazole (0.15 g, 2.2 mmol), 1-methylimidazole (0.11 ml, 1.4 mmol) and triethylamine (0.28 ml, 2.0 mmol)] in THF (10 ml) at room temperature for 1 min, and the products were worked-up and chromatographed [the appropriate fractions were eluted with $CHCl_3$–EtOH (95:5)] as above. The *title substance* was isolated as a colourless solid precipitate (0.787 g, 72%); R_F 0.78. In the same way, the two other fully-protected nonanucleotides [Ptmt-*U'pC'pC'pG'-pG'pT'pψ'pC'pG'p*-Dnb (R_F 0.80) and Px-*G'pG'pA'pG'pA'-pG'pG'pU'pC'p*-Dnb (R_F 0.74)] required in the synthesis of the 3'-terminal heptatriacontamer sequence were prepared from (i) Ptmt-*U'pC'pC'p* and HO-*G'pG'pT'p'ψpC'pG'p*-Dnb and (ii) Px-*G'pG'pA'p* and HO-*G'pA'pG'pG'pU'pC'p*-Dnb, and were obtained in 80 and 90% yields, respectively.

Procedure for the Removal of the 2-(*Isopropylthiomethoxymethyl*)*benzoyl* (*Ptmt*) *Protecting Group from Fully-protected Oligoribonucleotides.*—(a) *Preparation of HO*-U'pC'pG'pU'-pC'pC'pA'pC'pC'pA-*Mm* (**34**). A solution of mercury(II) perchlorate (0.2M, 12.5 ml, 2.5 mmol) in THF–water (98:2) was added to a magnetically-stirred solution of Ptmt-*U'pC'pG'-pU'pC'pC'pA'pC'pC'pA*-Mm (0.693 g, 0.10 mmol) and 2,4,6-collidine (0.66 ml, 5.0 mmol) in THF-water (98:2) (12.5 ml) at room temperature. After 15 min, aqueous thioacetamide (0.67M; 3.75 ml, 2.5 mmol) and triethylamine (0.70 ml, 5.0 mmol) were added. After a further period of 5 min, the products were centrifuged, and the supernatant was decanted and retained. The residue was washed with THF (5 × 5 ml), and the washings were decanted, combined and evaporated under reduced pressure. The residue was redissolved in THF (2 ml). Aqueous triethylamine (0.6M; 15 ml, 9.0 mmol) was added to a mixture of the latter solution and the original supernatant at room temperature. After 5 min, aqueous potassium dihydrogen phosphate (0.75M; 15 ml) was added, the products were poured into saturated aqueous sodium hydrogen carbonate (200 ml), and the mixture obtained was extracted with chloroform (6 × 200 ml). The combined organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was fractionated by short column chromatography on silica gel: the appropriate fractions, which were eluted with chloroform–ethanol (93:7) were combined and concentrated under reduced pressure. When a solution of the residue in chloroform (5 ml) was added dropwise with magnetic stirring to light petroleum (b.p. 30—40 °C) (500 ml), the *title substance* was precipitated as a colourless solid (0.484 g, 72%); R_F 0.76.

(b) *Preparation of HO*-U'pC'pC'pG'pG'pT'pψ'pC'pG'p-*Dnb* (**38**). A solution of mercury(II) perchlorate (0.2M, 5.06 ml, 1.01 mmol) in THF–water (98:2) was added to a magnetically-stirred solution of Ptmt-*U'pC'pC'pG'pG'pT'pψ'pC'pG'p*-Dnb (0.302 g, 0.045 mmol) and 2,4,6-collidine (0.27 ml, 2.0 mmol) in THF–water (98:2) (5.06 ml) at room temperature. After 15 min, the products were treated with a THF–water (98:2) solution of 2,4,6-collidine (0.4M; 1 ml, 0.4 mmol), aqueous thioacetamide (0.67M; 1.52 ml, 1.0 mmol) and triethylamine (0.28 ml, 2.0 mmol) for 5 min, and finally with triethylamine in THF–water. The products were then worked-up as above and chromatographed [the appropriate fractions were eluted with chloroform–ethanol (93:7)]. The *title substance* was obtained as a colourless precipitated solid (0.238 g, 81%); R_F 0.76.

Procedure for the Removal of the 2,4-*Dinitrobenzyl* (*Dnb*) *Protecting Group from Fully-protected Nonanucleotides.*—Triethylamine (0.057 ml, 0.41 mmol) was added to a stirred solution of Ptmt-*A'pU'pU'pC'pC'pG'pG'pA'pC'p*-Dnb (0.774 g, 0.114 mmol) and 2-thiocresol (0.057 g, 0.46 mmol) in pyridine (2.3 ml) at room temperature. After 10 min, allyl chloride (0.05 ml, 0.61 mmol) was added and, after a further period of 5 min, the products were evaporated under reduced pressure. The residue was partitioned between saturated aqueous sodium hydrogen carbonate (300 ml) and chloroform (4 × 200 ml). The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The residue was fractionated by short column chromatography on silica gel: the appropriate fractions, which were eluted with chloroform–ethanol (80:20), were combined and concentrated under reduced pressure. When a solution of the residue in chloroform (5 ml) was added dropwise with magnetic stirring to light petroleum (b.p. 30—40 °C) (250 ml), Ptmt-*A'pU'pU'pC'pC'pG'pG'pA'pC'p* was obtained as a colourless solid precipitate (0.596 g, 78%). The triethylammonium salt of Px-*G'pG'pA'pG'pA'pG'pG'pU'pC'p* (0.357 g, 66%) was similarly prepared from Px-*G'pG'pA'pG'pA'-pG'pG'pU'pC'p*-Dnb.

Preparation of the Fully-protected 3'-*Terminal Nonadecaribonucleoside Octadecaphosphate Sequence, Ptmt*-A'pU'pU'pC'-pC'pG'pG'pA'pC'pU'pC'pG'pU'pC'pC'pA'pC'pC'pA-*Mm* (**36**).—The triethylammonium salt of Ptmt-*A'pU'pU'pC'pC'-pG'pG'pA'pC'p* (0.535 g, 0.080 mmol) and HO-*U'pC'pG'pU'-pC'pC'pA'pC'pC'pA*-Mm (0.382 g, 0.057 mmol) were dissolved in anhydrous pyridine (*ca.* 4 ml) and the resulting solution was evaporated under reduced pressure (oil-pump) with gentle warming. When the solution ceased to be mobile, more pyridine (to give a total volume of *ca.* 2 ml) was added and the solution was again concentrated under reduced pressure to *ca.* one-half volume. MSNT[3] (0.101 g, 0.34 mmol) was then added, with magnetic stirring, to the resulting pyridine solution and, after 1 h, an additional quantity of MSNT (0.101 g, 0.34 mmol) was added. After a further period of 2.5 h, saturated aqueous sodium hydrogen carbonate (*ca.* 1.5 ml) was added to the stirred solution and, after 5 min, the products were concentrated under reduced pressure, and the residue was partitioned between chloroform (50 ml) and saturated aqueous sodium hydrogen carbonate (100 ml). The aqueous layer was back-extracted with chloroform (3 × 50 ml), and the combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was fractionated by short column chromatography on silica gel: the appropriate fractions, which were eluted with chloroform–ethanol (93:7), were combined and concentrated under reduced pressure. When a solution of the residue in chloroform (5 ml) was added dropwise with magnetic stirring to light petroleum (b.p. 30—40 °C) (250 ml), the crude *title substance* was obtained as a colourless solid precipitate (0.506 g). A column (75 cm × 3 cm diameter) was prepared from a slurry of Sephadex LH 60 in THF–methanol (95:5). A solution of the latter crude nonadecamer (0.021 g) in THF–methanol (95:5) was applied to the column which was then eluted with the same solvent system. A flow rate of *ca.* 130 ml/h was maintained, and 10 ml fractions were collected. The appropriate fractions (nos. 14—20) were combined and concentrated under reduced pressure. A further quantity of the crude nonadecamer (0.021 g) was chromatographed in the same way, and the combined purified material obtained from the two fractionations was rechromatographed on the Sephadex LH 60 column. The appropriate fractions (nos. 15—19) were combined and evaporated under reduced pressure. The purified *title substance* [0.029 g, 46%, based on HO-*U'pC'pG'pU'pC'pC'pA'pC'pC'-pA*-Mm (**34**)] was obtained in the usual way as a colourless precipitated solid. The remaining crude material (0.464 g) was similarly fractionated in two equal batches on the same Sephadex LH 60 column; yield, 0.349 g [50%, based on (**34**)].

Preparation of the Fully-protected Octadecanucleotide

Sequence, Px-G'pG'pA'pG'pA'pG'pG'pU'pC'pU'pC'pC'pG'-pG'pT'pψ'pC'pG'p-*Dnb* (**41**).—The triethylammonium salt of Px-*G'pG'pA'pG'pA'pG'pG'pU'pC'p* (0.338 g, 0.047 mmol) and HO-*U'pC'pC'pG'pG'pT'pψ'pC'pG'p*-Dnb (0.238 g, 0.036 mmol) were dissolved in pyridine (4 ml) and the resulting solution was evaporated under reduced pressure (oil-pump) with gentle warming. When the solution ceased to be mobile, more pyridine (to give a total volume of *ca.* 1 ml) was added and the solution was again concentrated under reduced pressure to *ca.* one-half volume. MSNT[3] (0.054 g, 0.18 mmol) was then added, with magnetic stirring, to the resulting pyridine solution and, after 1 h and 3 h, additional quantities of MSNT (2 × 0.054 g, 0.36 mmol; total amount, 0.162 g, 0.54 mmol) were added. After a total reaction time of 7 h, saturated aqueous sodium hydrogen carbonate (0.5 ml) was added to the stirred solution and, after a further period of 5 min, the products were partitioned between chloroform (50 ml) and saturated aqueous sodium hydrogen carbonate (100 ml). The aqueous layer was back-extracted with chloroform (3 × 50 ml), and the combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was fractionated by short column chromatography on silica gel: the appropriate fractions, which were eluted with chloroform–ethanol (92:8), were combined and concentrated under reduced pressure. The crude *title substance* was obtained in the usual way as a colourless precipitated solid (0.359 g). The latter material was chromatographed, as above, in three batches (*ca.* 0.015, 0.172, and 0.172 g) on the Sephadex LH 60 column used in the purification of the fully-protected 3'-terminal nonadecaribonucleoside octadecaphosphate (**36**). The appropriate 10 ml fractions (nos. 20—24, 20—24, and 19—24, respectively) were combined and evaporated under reduced pressure. The purified *title substance* (0.169 g, 34%, based on HO-*U'pC'pC'pG'pG'pT'pψ'pC'pG'p*-Dnb) was obtained in the same way as a colourless precipitated solid.

Preparation of the Fully-protected 3'-Terminal Heptatriacontanucleoside Hexatriacontaphosphate Sequence, Px-G'pG'-pA'pG'pA'pG'pG'pU'pC'pU'pC'pC'pG'pG'pT'pψ'pC'pG'-pA'pU'pU'pC'pC'pG'pG'pA'pC'pU'pC'pG'pU'pC'pC'pA'-pC'pC'pA-*Mm* (**44**).—A solution of mercury(II) perchlorate (0.2M; 2.37 ml, 0.47 mmol) in THF–water (98:2) was added to a magnetically-stirred solution of fully-protected 3'-terminal nonadecaribonucleoside octadecaphosphate [(**36**), 0.133 g, 0.010 mmol] and 2,4,6-collidine (0.125 ml, 0.95 mmol) in THF–water (98:2) (2.37 ml) at room temperature. After 15 min, the products were treated with a THF–water (98:2) solution of 2,4,6-collidine (0.4M; 0.5 ml, 0.2 mmol), aqueous thioacetamide (0.67M; 0.71 ml, 0.47 mmol) and triethylamine (0.13 ml, 0.93 mmol) for 5 min, and finally, as in the unblocking (see above) of the corresponding 3'-terminal decaribonucleoside nonaphosphate (**28**), with triethylamine in THF–water. The products were then worked-up as above and chromatographed [the appropriate fractions were eluted with chloroform–ethanol (92:8)]. The putative 5'-unblocked nonadecamer (**24**) was obtained as a colourless precipitated solid (0.115 g, 87%).

Triethylamine (0.013 ml, 0.09 mmol) was added to a stirred solution of the fully-protected octadecanucleotide [(**41**), 0.169 g, 0.012 mmol] and 2-thiocresol (0.013 g, 0.10 mmol) in pyridine (0.25 ml) at room temperature. After 10 min, allyl chloride (0.01 ml, 0.12 mmol) was added and, after a further period of 5 min, the products were evaporated under reduced pressure. The residue was partitioned between saturated aqueous sodium hydrogen carbonate (50 ml) and chloroform (6 × 50 ml). The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. When a solution of the residue in chloroform (3 ml) was added dropwise with magnetic stirring to light petroleum (b.p. 30—40 °C) (250 ml), the putative crude triethylammonium salt of Px-*G'pG'pA'pG'pA'pG'pG'pU'pC'-pU'pC'pC'pG'pG'pT'pψ'pC'pG'p* (**43**) was obtained as a solid precipitate (0.167 g).*

The latter material [(**43**), 0.123 g, *ca.* 0.005 mmol] and HO-*A'pU'pU'pC'pC'pG'pG'pA'pC'pU'pC'pG'pU'pC'pC'pA'pC'-pC'pA*-Mm [(**42**), 0.09 g, 0.0069 mmol] were dissolved in anhydrous pyridine (2 × 2 ml) and the resulting solution was evaporated under reduced pressure (oil-pump) with gentle warming. When the solution ceased to be mobile, more pyridine (to give a total volume of *ca.* 1 ml) was added and the solution was again concentrated under reduced pressure to *ca.* one-half volume. MSNT[3] (0.03 g, 0.10 mmol) was then added, with magnetic stirring, to the resulting pyridine solution and, after 1 h, 3 h, and 7 h, additional quantities of MSNT (0.03 g, 0.03 g, and 0.01 g; total amount, 0.10 g, 0.34 mmol) were added. After a total reaction time of 24 h, saturated aqueous sodium hydrogen carbonate (0.2 ml) was added to the stirred solution and, after a further period of 5 min, the products were partitioned between chloroform (50 ml) and saturated aqueous sodium hydrogen carbonate (100 ml). The aqueous layer was back-extracted with chloroform (4 × 50 ml), and the combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was fractionated by short column chromatography on silica gel: the appropriate fractions, which were eluted with chloroform–ethanol (93:7), were combined and concentrated under reduced pressure. The crude *title substance* was obtained in the usual way as a colourless precipitated solid (0.162 g). The latter material was chromatographed, as above, in two batches (*ca.* 0.015 g and *ca.* 0.147 g) on the Sephadex LH 60 column used in the purification of the fully-protected 3'-terminal nonadecaribonucleoside octadecaphosphate (**36**). The appropriate 10 ml fractions (nos. 15—19 and 14—19 respectively) were combined and evaporated under reduced pressure. The purified *title substance* [0.047 g, 26% based on the 5'-unblocked nonadecaribonucleoside octadecaphosphate (**42**)] was obtained in the same way as a colourless precipitated solid.

Two-Step Partial Unblocking Procedure of the Fully-protected tRNA Sequences.—(a) *Ptmt*-U'pC'pG'pU'pC'pC'pA'pC'pC'-pA-*Mm* (**28**). A dioxane solution of N^1,N^1,N^3,N^3-tetramethylguanidine (TMG, 1.2M, 0.36 ml, 0.43 mmol) was added to a magnetically-stirred solution of substrate [(**28**), 0.028 g, 0.004 mmol] and *E*-2-nitrobenzaldehyde oxime (0.08 g, 0.48 mmol) in dioxane (0.36 ml) at room temperature. After 4 h, acetonitrile (0.58 ml) was added and, after a further period of 2 h, water (0.14 ml) was added to the stirred solution. After a total reaction time of 22 h, the products were evaporated under reduced pressure and concentrated aqueous ammonia (*d* 0.88, 5 ml) was added to the residue. After the reaction solution had been stirred at room temperature for 60 h, it was evaporated under reduced pressure. Water (10 ml) was added and the resulting mixture was extracted with dichloromethane (5 × 10 ml) and ether (10 ml). 0.1M Hydrochloric acid was then added to the aqueous layer until the pH fell to 7.5, and it was then extracted with chloroform (5 × 10 ml), and concentrated under reduced pressure. The residue was dissolved in 0.01M triethylammonium bicarbonate buffer (pH 7.5, *ca.* 1 ml), and the solution was applied to a column (20 cm × 2 cm diameter) of Sephadex G10. The column was eluted with the same buffer, and the fractions containing the first-eluted ultraviolet-absorbing component were combined and concentrated under reduced pressure. The residue was redissolved in the same buffer (*ca.* 1 ml), and the solution was applied to a column (20 cm × 2 cm diameter) of DEAE Sephadex A25, also suspended in 0.01M triethylammonium bicarbonate buffer. The column was eluted with

* It was clear by t.l.c. (system A) that only *ca.* 60% of this material was the desired 3'-phosphodiester (**43**).

triethylammonium bicarbonate buffer (pH 7.5, linear gradient from 0.01—1.00M over 1 000 ml), and fractions of 10 ml were collected. Fractions 69—85 were combined and evaporated under reduced pressure to give HO-U′pC′pG′pU′pC′pC′-pA′pC′pC′pA-Mm (225 A_{260} units).

(b) *Ptmt*-A′pU′pU′pC′pC′pG′pG′pA′pC′pU′pC′pG′pU′-pC′pC′pA′pC′pC′pA-*Mm* (**36**). A solution of TMG (0.042 ml, 0.33 mmol) in dioxane (0.1 ml) was added to a magnetically-stirred solution of the substrate [(**36**), 0.020 g, 0.0015 mmol] and *E*-2-nitrobenzaldehyde oxime (0.062 g, 0.37 mmol) in dioxane (0.4 ml). After 4 h, acetonitrile (0.6 ml) was added to the stirred solution and, after 14 h more, water (0.1 ml) was added. After a further period of 24 h, the products were concentrated under reduced pressure and concentrated aqueous ammonia (*d* 0.88, 5 ml) was added to the residue. After 72 h, the products were worked-up in the way described under heading (a) above. After chromatography on Sephadex G10, the material obtained was fractionated on a column (50 cm × 2 cm diameter) of Sephadex G-50 which was eluted with 0.05M triethylammonium bicarbonate buffer. The appropriate fractions were combined and evaporated under reduced pressure to give HO-A′pU′pU′pC′-pC′pG′pG′pA′pC′pU′pC′pG′pU′pC′pC′pA′pC′pC′pA-Mm (110 A_{260} units).

(c) *Px*-G′pG′pA′pG′pA′pG′pG′pU′pC′pU′pC′pC′pG′pG′-pT′pψ′pC′pG′pA′pU′pU′pC′pC′pG′pG′pA′pC′pU′pC′pG′-pU′pC′pC′pA′pC′pC′pA-*Mm* (**44**). A dioxane solution of TMG (1.2M, 0.25 ml, 0.30 mmol) was added to a magnetically-stirred solution of the substrate [(**44**), 0.016 g, 0.0006 mmol] and *E*-2-nitrobenzaldehyde oxime (0.055 g, 0.33 mmol) in dioxane (0.25 ml) at room temperature. After 2 h, acetonitrile (0.40 ml) and, after a further period of 6 h, water (0.10 ml) were added to the stirred solution. After a total reaction time of 24 h, the products were evaporated under reduced pressure and concentrated aqueous ammonia (*d* 0.88, 5 ml) was added to the residue. After 72 h, the products were worked-up in the way described under heading (a) above. After chromatography on Sephadex G10, the material obtained was fractionated on a column (75 cm × 3 cm diameter) of Sephadex G75 which was eluted with 0.01M triethylammonium bicarbonate (pH 7.5) buffer. Fractions of 10 ml were collected: fractions 18—20 were combined and evaporated under reduced pressure to give Px-G′pG′pA′pG′pA′-pG′pG′pU′pC′pU′pC′pC′pG′pG′pT′pψ′pC′pG′pA′pU′pU′-pC′pC′ pG′pG′pA′pC′pU′pC′pG′pU′pC′pC′pA′pC′pC′pA-Mm (105 A_{260} units).

The Complete Unblocking and Enzymatic Digestion of the Synthetic tRNA Sequences.—(a) *Decaribonucleoside Nonaphosphate* (**48**). The partially-protected decamer [(**45**), see paragraph (a) above; 5 A_{260} units] was dissolved in 0.01M hydrochloric acid (7 ml) at room temperature and the pH was adjusted to 2.0 by the careful addition of 0.1M hydrochloric acid. After 6 h, the products were neutralized with dilute aqueous ammonia and concentrated under reduced pressure. The material obtained was found [l.c. and polyacrylamide gel electrophoresis (see Figures 3 and 4)] to be homogeneous UpCpGpUpCpCpApCp-CpA (**48**). A solution of phosphodiesterase I (from *Crotalus altrox* venom; 10 μg) in 0.1M tris hydrochloride buffer (pH 8.3, 0.01 ml) was diluted with the same buffer (0.10 ml; 0.01M with respect to magnesium chloride), and the resulting solution was added to a solution of (**48**) (*ca.* 2 A_{260} units) in water (0.01 ml). The reactants were maintained at 37 °C for 16 h, and then a solution (in 2.6M aqueous ammonium sulphate) of bacterial alkaline phosphatase (3.6 units in 0.04 ml) was added. After 3 h, the hydrolysate was examined by l.c. and found to consist solely of adenosine, cytidine, guanosine, and uridine in the estimated relative molar proportions of 2.0:5.0:1.23:2.07.

(b) *Nonadecaribonucleoside Octadecaphosphate* (**49**). The partially-protected nonadecamer [(**46**), see paragraph (b) above; 5 A_{260} units] was treated with hydrochloric acid (pH 2) at room temperature as above for 14 h. The material obtained after neutralization and concentration was found [l.c. and polyacrylamide gel electrophoresis (see Figures 3 and 4)] to be homogeneous ApUpUpCpCpGpGpApCpUpCpGpUpCp-CpApCpCpA. The latter material (*ca.* 2 A_{260} units) was digested as above, first with phosphodiesterase I at 37 °C for 24 h and then with alkaline phosphatase at 37 °C for 6 h. Examination of the hydrolysate by l.c. revealed adenosine, cytidine, guanosine, and uridine in the estimated relative molar proportions of 4.0:8.11:2.94:4.17.

(c) *Heptatriacontaribonucleoside Hexatriacontaphosphate* (**50**). The partially-protected heptatriacontamer [(**47**), see paragraph (c) above; 2 A_{260} units] was treated with hydrochloric acid (pH 2) at room temperature as above for 24 h. The material obtained after neutralization and concentration was found [l.c. and polyacrylamide gel electrophoresis (see Figures 3 and 4)] to be be homogeneous GpGpApGpApGpGp-UpCpUpCpCpGpGpTpψpCpGpApUpUpCpCpGpGpApCp-UpCpGpUpCpCpApCpCpA. The latter material (*ca.* 1 A_{260} unit) was digested first with ribonuclease T-2 [7 units in 0.1M ammonium acetate (pH 4.5, 0.1 ml)] at 37 °C for 48 h, and then, as above, with alkaline phosphatase at 37 °C for 2 h. Examination of the hydrolysate by l.c. revealed adenosine, cytidine, guanosine + 5-methyluridine, uridine and pseudouridine in the estimated relative molar proportions of 6.0:12.12:12.27:6.48:1.12.

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