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Chemical Synthesis of a Pentaribonucleoside Tetraphosphate Constituting the 3′-Acceptor Stem Sequence of *E. coli* tRNA^{Ile} Using 2′-*O*-(3-Methoxy-1,5-dicarbomethoxypentan-3-yl)-ribonucleoside Building Blocks. Application of a New Achiral and Acid-labile 2′-Hydroxyl Protecting Group in tRNA Synthesis

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A synthesis of a pentaribonucleotide fragment constituting the residues 59–63 of 3′-terminus of *E. coli* tRNA^{Ile}, 5′-ApGpUpCpC-3′, has been carried out using a new, easily accessible and achiral 2′-ketal protecting group. The new 2′-ketal group has an additional advantage in that it is easily functionalized to the diamide with aqueous ammonia in the penultimate step of deblocking of fully protected oligoribonucleotides.

Such a functionalization of the 2′-ketal group at the penultimate step of deblocking of the fully protected tRNA molecule enhances its relative rate of removal under an acidic condition with a minimum of damage of the target tRNA molecule.

Regioselective chemical operation to introduce a 3′→5′ phosphodiester linkage in the synthesis of an oligoribonucleotide warrants the design of a set of complementary protecting groups;[1] however, it is the 2′-protecting group which often dictates the choice of all other protecting groups in an effective strategy.[1] The use of 9-phenyl-9*H*-xanthen-9-yl-(pixyl),[2] 7-chlorophenyl-9*H*-thioxanthen-9-yl- (CAT)[3,4] as 2′-protecting groups in olgoribonucleotide synthesis has been earlier demonstrated. Recently, a novel strategy[5] has also been developed in this laboratory for oligoribonucleotide synthesis with two complementary acid-labile groups, 5′-pixyl-[6] and 2′-*O*-(4-methoxtetrahydropyranyl)- (MTHP)[7] groups as shown in *1*, which culminated into the synthesis of an undecaribonucleotide sequence of tRNA.^{Phe} The sole basis of this strategy rests on finding[5,8,9] that a 2′-acid-labile group with an adjacent 3′-phosphotriester, as in *2*, is stabilized 240 fold over a corresponding ribonucleoside block *1*; the conversion of the phosphotriester to a diester, as in a naturally occurring RNA molecule *3*, makes the acid-labile group three times more labile[10] than in a corresponding ribonucleoside block *1*. This enhanced rate of removal of the 2′-acid-labile group causes a minimum of damage to the rest of the molecule giving *4* selectively. We

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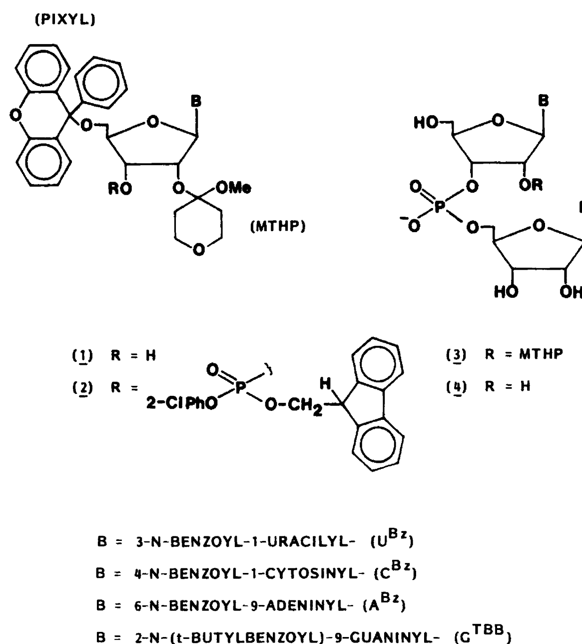
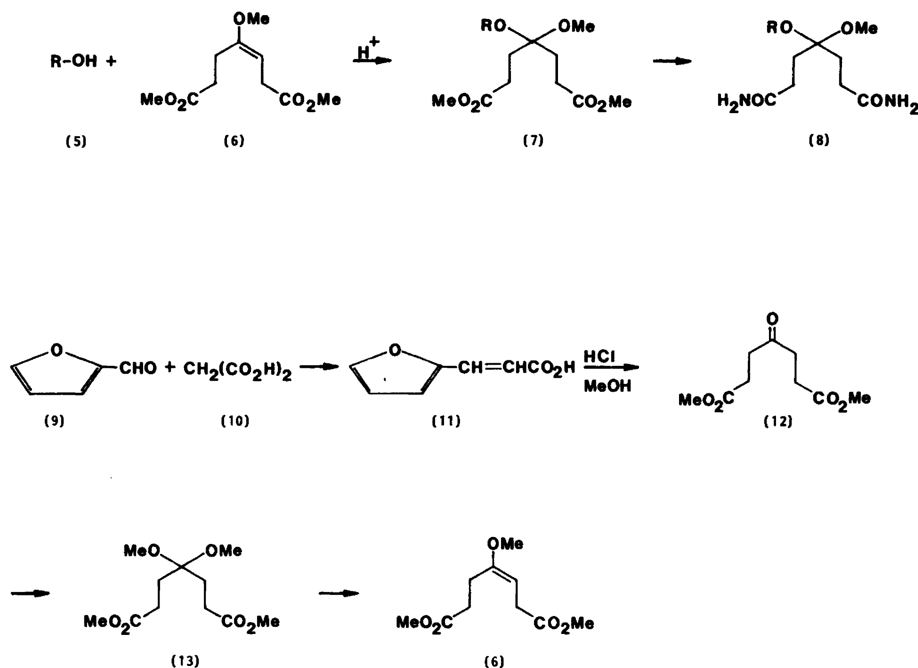


herein report a new 2′-acid-labile group, as shown in 7, that is completely stable during the removal of the 5′-pixyl group from a fully protected phosphotriester to give pure 5′-hydroxy blocks in a high yield for further chain elongation for polyribonucleotide synthesis; yet, at the end of the synthesis of the target molecule, this 2′-group is readily convertible to another functionality that reduces its acid-stability considerably. The latter criteria is considered important in view of the acid promoted degradation of a RNA molecule at the phosphate linkage during the final removal of the 2′-acid-labile group.[11] The design of the new achiral, readily available and inexpensive 2′-ketal group, as shown in 7, has been based upon a comparison of pK_a[12] of ethylamine (10.79) with 3-aminopropionic acid methylester (9.1) and 3-aminopropionic acid (10.39). Such a comparison[7] of pK_a indicates that a β-substituent indeed destabilizes an ammonium ion depending on the nature of the substituent. We reasoned[13] that a substituent placed two-carbon unit away from a carbenium ion would have a corresponding influence on its stability. Its was then envisaged that the creation of a ketal functionality on the C-3 carbon that is flanked by two two-carbon units with a appropriate β-electron-withdrawing substituent, as shown in the general formula: 7 would produce a cumulative effect on the destabilization of the carbenium ion and would also result into a symmetrical hydroxyl protecting group. During these considerations, it was clear that the actual choice of an electron-withdrawing substituent on the β-carbon would rest entirely firstly, on its relative stability under normal conditions of synthetic manipulations; and, secondly, on the feasibility of its selective chemical conversion. This conversion takes place at the penultimate step of deblocking of the the fully protected tRNA molecule, to a group which would reverse or neutralize the effect and, consequently, enhance its relative rate of removal under a mild acidic condition.

Appropriately protected 2′-*O*-(3-methoxy-1,5-dicarbomethoxypentanyl)- (MDMP) derivatives of ribonucleosides, *19–22* were thus prepared and it was found that the MDMP group in these derivatives had an adequate acid-stability under standard conditions of



Scheme 1.

chemical manipulations.[5] The half-lives of removal of the MDMP group from different nucleoside blocks, *19–22*, were 6, 4, 18 and 15 min, respectively, in 80 % aqueous acetic acid (v/v) at room temperature while the half-lives of removal of the MTHP group from the corresponding MTHP derivatives were 13, 6, 26 and 22 min under an identical condition. The MDMP group, upon its conversion to the diamide *8* by the treatment of aq. NH_3 (*d* 0.9) at 20 °C cleaved off at a 17 fold faster rate under the latter acidic condition. It should be noted that in the current synthetic methodologies in the phophotriester approach,[1] a treatment of aqueous ammonia is normally used in the penultimate step of deblocking of a fully protected oligoribonucleotide; while 80 % acetic acid is used as the final deblocking agent of the 2′-ketal group.[5,11]

The reagent, 3-methoxy-1,5-dicarbomethoxy-2-pentene (*6*) may be conveniently prepared in 200 g scale in four steps starting from inexpensive, non-corrosive and non-volatile starting materials[14] like furfural *9* and malonic acid *10* as outlined in Scheme 1. Pentane-3-one-1,5-dicarboxylic acid dimethyl ester *12* was prepared through furylacrylic acid *11* following a literature procedure as for the corresponding diethylester.[15] The ketone *12* was then converted[16] to the ketal *13* in 73 % yield; The ketal *13* was subsequently converted[16] to the desired enol ether *6* in 74 % yield (*ca.* 95 % pure). Subsequently the new MDMP group was employed in the chemical synthesis of a pentaribonucleotide fragment, 5′ ApGpUpCpC 3′, (*48*) as an actual example of usefulness of the MDMP group in oligoribonucleotide synthesis.

Preparation of appropriately protected ribonucleoside blocks and the mononucleotide blocks 19–38. The fully protected pentaribonucleotide block, 5′ Px-ApGpUpCpC-ac$_2$ 3′ (*46*) was synthesized starting from appropriately protected 2′-*O*-(3-methoxy-1,5-dicar-

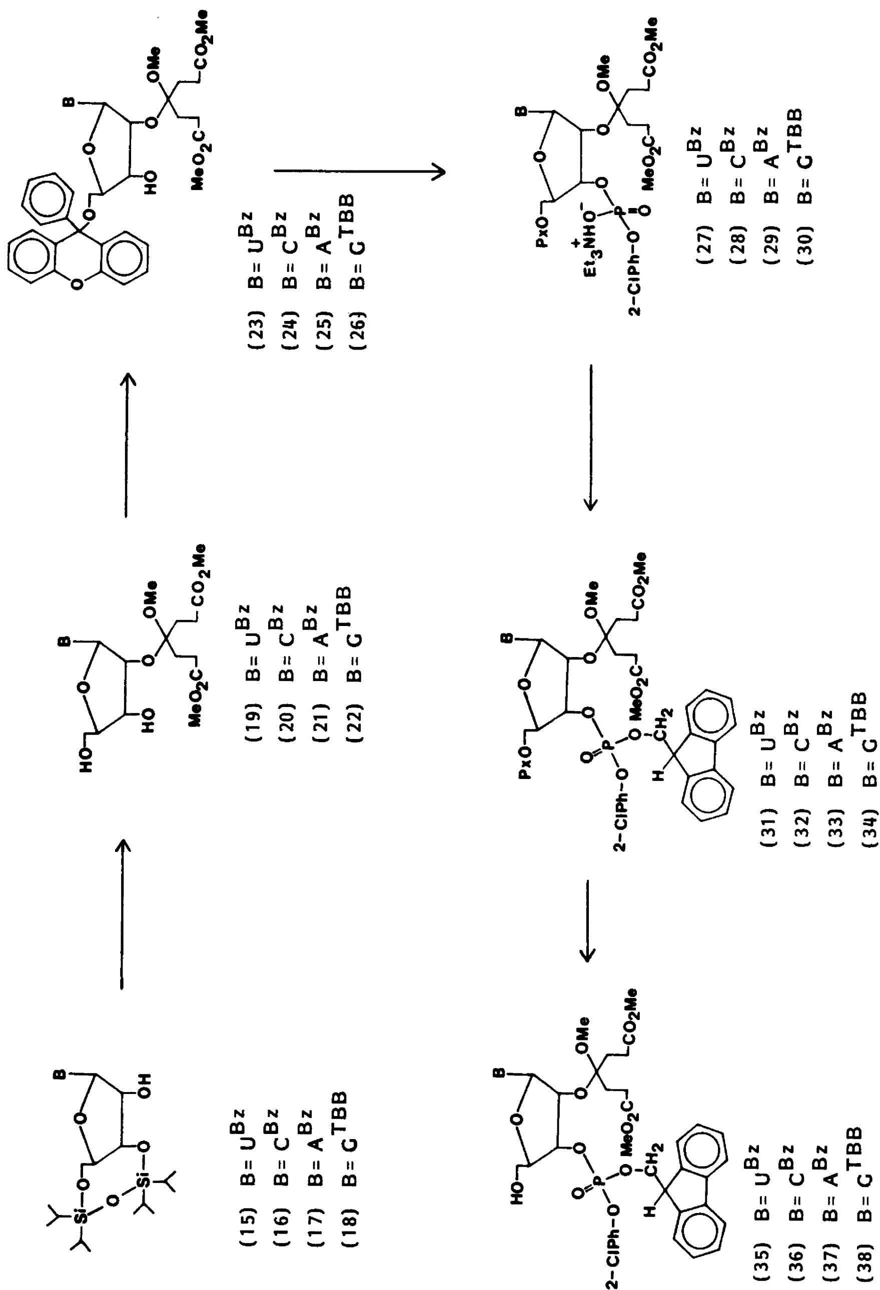

(15) B= U^Bz
(16) B= C^Bz
(17) B= A^Bz
(18) B= G^TBB
(19) B= U^Bz
(20) B= C^Bz
(21) B= A^Bz
(22) B= G^TBB
(23) B= U^Bz
(24) B= C^Bz
(25) B= A^Bz
(26) B= G^TBB
(27) B= U^Bz
(28) B= C^Bz
(29) B= A^Bz
(30) B= G^TBB
(31) B= U^Bz
(32) B= C^Bz
(33) B= A^Bz
(34) B= G^TBB
(35) B= U^Bz
(36) B= C^Bz
(37) B= A^Bz
(38) B= G^TBB

Table 1. Yield (%) for the preparation of building blocks *19* to *34* and their acid hydrolytic properties.

Compound	% Yield	Relative rates of hydrolysis of 5′-pixyl and 2′-MDMP groups (in min)											
		$TsOH.H_2O$ (25 eq.) in CH_2Cl_2–MeOH (7:3 v/v) at 0 °C, (0.10 M solution)				$ZnBr_2$ (25 eq.) in $MeNO_2$ (0.10 M solution) at 0 °C				Cl_3CCOOH (10 eq.) in 2 % EtOH–$CHCl_3$ at 0 °C (0.055 M solution)			
		Px		MDMP		Px		MDMP		Px		MDMP	
		$t_{\frac{1}{2}}$	t_∞	$t_{\frac{1}{2}}$	t_∞	$t_{\frac{1}{2}}$	t_∞	$t_{\frac{1}{2}}$	t_∞	$t_{\frac{1}{2}}$	t_∞	$t_{\frac{1}{2}}$	t_∞
19	82												
20	76												
21	63												
22	69												
23	94		0.5	1	12								
24	87		1	1	12								
25	94		0.5	1.5	12								
26	91		0.5	1.5	12								
27	93												
28	100	0.25	2	15	45								
29	98	0.25	2	20	65								
30	–												
31	91	3	15	70		0.25	4		95[b]	60	300		300[c]
32	88	2	15	90		0.5	8		60[b]	45	200		200[c]
33	87	1	4	135			0.5		310[d]	30	180		180[d]
34	73[a]		1	15	60	0.25	8	40	80	0.5	10		10[e]

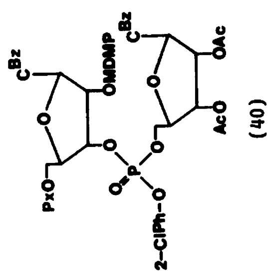
[a] Yield based on compound *26*. [b] 100 % baseline material at time given in the table. [c] Less than 10 % loss of MDMP group at time given. [d] Less than 10 % baseline material at time given. [e] Including baseline material less than 5 % at time given.

bomethoxypentanyl)-ribonucleoside blocks *19–22*. These building blocks were obtained as powders in a "one-pot" procedure starting from 3',5'-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxyl) ribonucleoside blocks[5,17] in 82, 76, 76 and 70 % yields, respectively.

Subsequently, the 5'-hydroxy function of these building blocks have been protected by the acid-labile 9-phenylxanthen-9-yl- group;[6] the resulting 2',5'-*bis*-protected derivatives *23–26* have been obtained in 95, 87, 92 and 84 % yields, respectively. The 3'-*O*-(o-chlorophenyl)phosphodiester blocks *27–30* were prepared in almost quantitative yields by the reaction of 2',5'-*bis*-protected blocks *23–26* with o-chlorophenylphosphoro-bis-(1,2,4-triazolide) followed by a hydrolysis step.[18] These phosphodiesters were then protected in the form of their triesters with 9-fluorenylmethyl-(FM) group using a methodology that we have earlier introduced in ribonucleic acid chemistry;[5] fully protected phosphotriesters *31–34* were thus obtained in 91, 88, 87 and 74 % yields, respectively.

Table 1 records the study on the relative rates of acid hydrolysis of the 5'-pixyl and 2'-MDMP groups from a variety of substrates, *19–34*, under different acidic conditions. A perusal of these data in Table 1 clearly points to the following conclusions: (*a*) the relative rates of hydrolysis of the MDMP group in 80 % acetic acid at room temperature from blocks *19–34* are dependent upon the nature of aglycons; (*b*) a 3'-triethylammonium-2-chlorophenyl phosphate function, as in *27–30*, stabilizes the MDMP group 3–4 fold as opposed to the destabilizing effect by the internucleotide phosphodiester function as reported in the literature;[10] (*c*) a 3'-phosphotriester function, as in *31–34*, stabilizes the MDMP and the pixyl groups variedly depending upon the nature of aglycons; (*d*) an appropriate acidic condition for a selective removal of the pixyl group should be carefuly chosen; thus it is clear from Table 1 that a 2 % ethanol–chloroform solution (0.055 M) of trichloroacetic acid (10 equiv.) at 0 °C seems to be a better reagent for selective removal of the pixyl group from the phosphotriesters *31–34*. Using such a condition, we have isolated the corresponding 5'-hydroxy blocks, *35–38*, in 74, 74, 71 and 70 % yields, respectively.

Preparation of fully protected oligoribonucleotide blocks 40–46. Fully protected dimer 5' Px-CpC-ac_2 3' (*40*), as a 3'-anchored block, have been prepared in 80 % yield as a powder by a condensation reaction[5] of the 5'-protected block *28* and the 5'-hydroxy block *39* in dry pyridine solution in presence of 1-(mesitylenesulfonyl)-3-nitro-1,2,4-triazole (MS–NT).[19] The second fully protected dimer block with a 3'-terminal phosphotriester *43* was prepared in 87 % yield by a condensation reaction of *29* and *38*, using a procedure which was identical to that of the preparation of *40*. This was converted to the phosphodiester salt *44* in 94 % yield as a powder by removal of the 9-fluorenylmethyl group[5] with triethylamine (20 eq.) in dry pyridine solution at 20 °C. The 5'-pixyl group was subsequently removed from the dimer *40*, using zinc bromide (20 eq.) in dry nitromethane at *ca.* 0 °C for 2 h to obtain the 5'-hydroxy dimer block *41* in 79 % yield as a powder. The fully protected trimer 5' Px-UpCpC-ac_2 3' *42* was then synthesized by the condensation of *27* and *41*, under a usual condition,[5] in 77 % yield. The 5'-pixyl group from this trimer was removed for a final condensation reaction to give the 5'-hydroxy trimer block *45* in 77 % yield, using a condition that was devised for the preparation of *41*. Finally, the 5'-protected dimer block *44* and the 5'-hydroxy block *45* were coupled to give the fully protected pentaribonucleotide *46* in 49 % yield. Compounds *42* and *46* were then deprotected and purified using literature procedures.[5] Fully deprotected oligoribonucleotides *47* and *48* were thus obtained in 80 and 81 % yields, respectively. The purities of these oligoribonucleotides are shown in Figs. 2 and 4. They were characterized in a usual way[5] by digestions with Snake venom phosphodiesterase, Spleen phosphodiesterase and 0.2 M sodium hydroxide (Experimental).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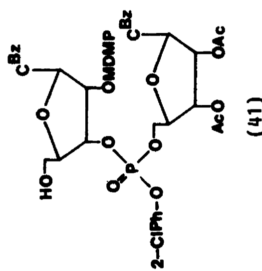
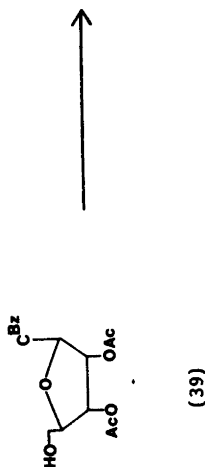
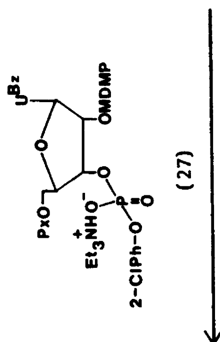
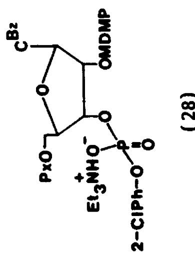
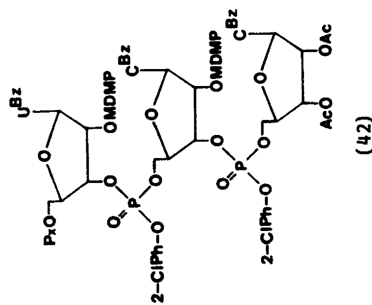
2-ClPh-O
AcO
OAc
(40)

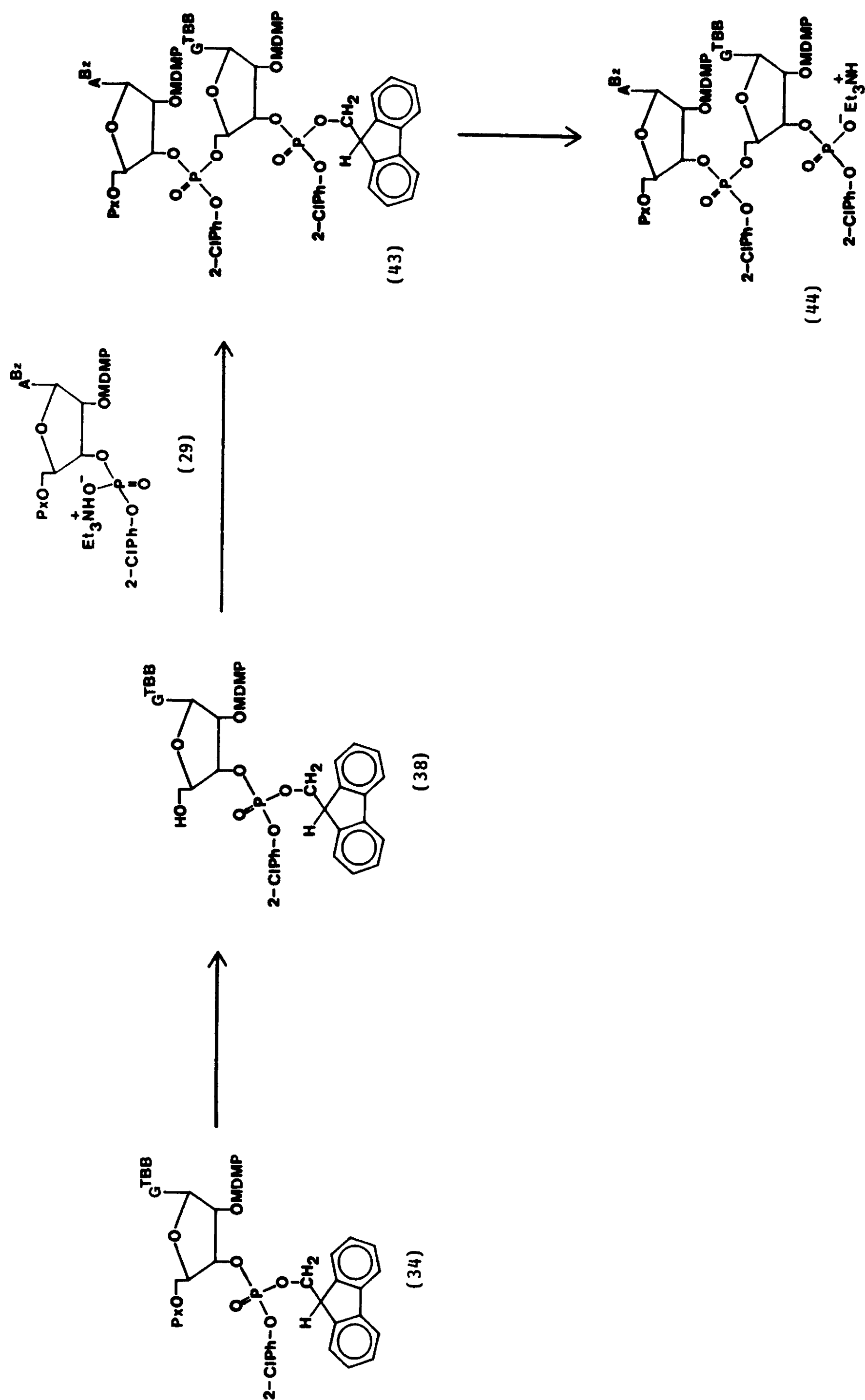

HO
OMDMP
2-ClPh-O
AcO
OAc
(41)

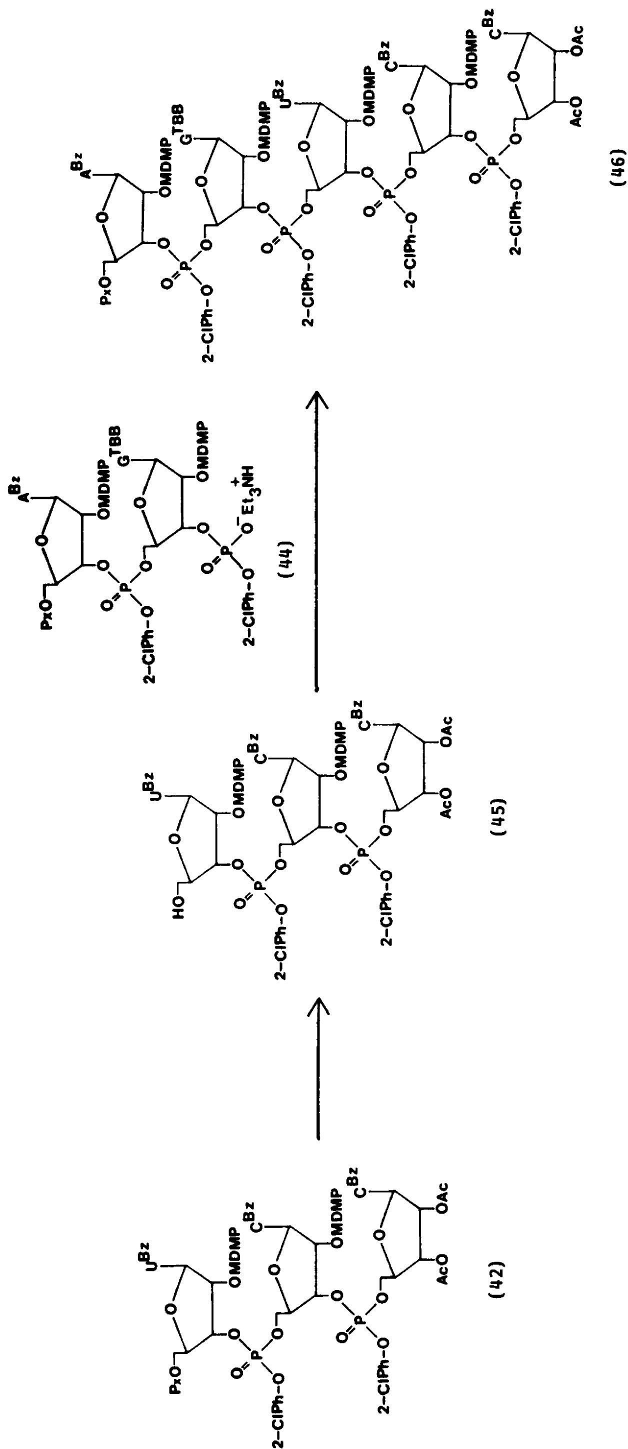



PxO
OMDMP
2-ClPh-O
(27)


PxO
OMDMP
2-ClPh-O
(28)


PxO
OMDMP
OMDMP
2-ClPh-O
2-ClPh-O
AcO
OAc
(42)


(34)
(38)
(29)
(43)
(44)


(42)
(45)
(44)
(46)

EXPERIMENTAL

^{1}H NMR spectra were measured at 60 MHz with a Perkin-Elmer R 600 and at 90 MHz with a Jeol FX 90Q spectrometer using tetramethylsilane as an internal standard. ^{31}P NMR spectra were recorded at 36 MHz in the same solvent mixture as for ^{1}H NMR using phosphoric acid as an external standard. UV absorption spectra were recorded with a Cary 2200 spectrometer. Reactions were monitored by using Merck pre-coated silica gel 60 F_{254} plates using the following solvent systems:

(A) 10 % methanol–chloroform (v/v)
(B) 20 % methanol–chloroform (v/v)
(C) 30 % methanol–chloroform (v/v)

High performance liquid chromatography (HPLC) was performed[21] with the help of LDC equipments, model III pumps, UV III monitor and a gradient master; Merck Kieselgel G was used for short column chromatography.[20] Dried solvents were prepared using our literature procedures.[5]

The key reagents 1,1,3,3-tetraisopropyl-1,3-dichloro-1,3-disiloxane,[17] 9-chloro-9-phenylxanthene (Px–Cl),[6] 1-(mesitylenesulfonyl)-3-nitro-1,2,4-triazole (MS–NT),[19] *o*-chlorophenylphosphorobis-(1,2,4-triazolide)[18] were prepared using literature procedures.

Preparation of 2-furylacrylic acid[14] *(11).* A mixture of malonic acid (*10*) (208 g, 2.0 mol), furfural (*9*) (192.6 g, 2.0 mol) and pyridine (95 ml) was refluxed (100–105 °C) for 2.5 h under dry condition. This mixture was then cooled in an ice bath and water (200 ml) was added. An 18 % aqueous hydrochloric acid solution (1250 ml) is slowly added. The furylacrylic acid was then collected by filtration and washed several times with water (400 ml). The furylacrylic acid thus obtained was then taken to the next step after drying *in vacuo*. ^{1}H NMR (DMSO-d_6+$CDCl_3$): δ 7.75 (*m*, 1 H); 7.28 (*s*, 1 H); 6.85 (*m*, 1 H); 6.59 (*m*, 1 H); 6.30 (*s*, 1 H); 6.02 (*s*, 1 H).

Preparation of 1,5-dicarbomethoxypentane-3-one[15] *(12).* Crude furylacrylic acid from the last step was dissolved in methanol (1 l). The solution was heated to boiling and anhydrous hydrogen chloride was introduced during 3 h. The reaction vessel was then stoppered and left at room temperature over night. Volatile matters were removed on a rotavapor and sodium bicarbonate was added to the residue. The reaction mixture was filtered and the filtrate was extracted with chloroform (4 x 250 ml). The combined chloroform layers were concentrated on a rotavapor and the residue was distilled under reduced pressure to give the title compound. Yield: 159.9 g (0.79 mol; 39 % from malonic acid); b.p.=116–125 °C/0.15 mbar. ^{1}H NMR ($CDCl_3$): δ 3.68 (*s*, 6 H) methyl esters; 3.0–2.4 (*m*, 8 H) methylenes.

Preparation of 3,3-dimethoxy-1,5-dicarbomethoxypentane[16] *(13).* To a refluxing solution of compound (*12*) (276 g, 1.36 mol) in absolute methanol (250 ml) and trimethylorthoformate (291 g, 2.74 mol) was added benzenesulphonic acid in approx. 0.5 g portions (2.75 g, 0.017 mol). After 3 h the reaction mixture was neutralized with sodium methoxide. The pure title compound was obtained after distillation at reduced pressure. Yield: 247 g, 72.9 %; b.p.=163–165 °C/14 Torr. ^{1}H NMR ($CDCl_3$): δ 3.68 (*s*, 6 H) methyl esters; 3.17 (*s*, 6 H) methoxyls; 2.5–1.7 (*m*, 8 H) methylenes.

Preparation of 1,5-dicarbomethoxy-3-methoxy-2-pentene (6). A solution of compound (*13*) (245 g, 0.99 mol) in trimethylorthoformate (50 ml) was placed in a 250 ml distillation unit (with a 15 cm vigreux column and a Liebig condenser). The reaction mixture was heated in an oil bath (oil temp. 168 °C). To this magnetically stirred solution benzenesulfonic acid (560 mg, 0.004 mol) was added (in 100 mg portions). After 10 h the reaction mixture was allowed to cool and was neutralized with dry triethylamine. Reduced pressure distillation yielded 157.8 g (73.8 %) of the title compound with a purity of *ca.* 95 %; b.p.=150–154 °C/14 Torr.

^{1}H NMR ($CDCl_3$): δ 4.56 (*m*, 1 H) H-2; 3.66 (*s*, 8 H) methyl esters and methylene; 3.52 (*s*, 2 H) methylene; 3.12 (*d*, 7.2, 2 H) methylene; 2.44 (*s*, 3 H) methoxyl.

^{13}C NMR ($CDCl_3$): δ 25.7, 31.6, 32.0, 51.4, 54.3, 89.7, 101.6, 157.5, 172.8, 173.2.

2'-O-(3-Methoxy-1,5-dicarbomethoxypentanyl)- (MDMP)-6-N-benzoyladenosine (21). A general procedure. To a dry dioxane solution (10 ml) of (*17*) (5.5 g, 8.96 mmol) at room

temperature, was added 3-methoxy-1,5-dicarbomethoxy-2-pentene (*6*) (20 ml, 92.5 mmol) and benzenesulfonic acid (420 mg, 2.66 mmol) under stirring in an argon atmosphere. After 1 h, dry pyridine (10 ml) was added and the mixture was poured into a separating funnel containing saturated sodium bicarbonate solution (150 ml). The mixture was extracted with chloroform (4×100 ml). The organic layers were pooled and coevaporated with toluene (3×30 ml). The residue was dissolved in dry tetrahydrofuran (150 ml) and tetrabutylammonium fluoride (TBAF) (19.7 ml; 1 M solution in tetrahydrofuran) was added. After 20 min of stirring the solvent was evaporated and the residue was coevaporated with toluene, then triturated with light petroleum. The light petroleum was decanted and the oily residue was purified by silica gel chromatography [20] using 4 % ethanol–chloroform as eluent to give (*21*) (3.99 g, 75.8 %).

Compounds *19*, *20* and *22* were similarly prepared in 82, 76 and 70 % yields starting from *15*, *16* and *18*, respectively.

Compound 19. R_f=0.64 (solvent B). ^{1}H NMR ($CDCl_3$+CD_3OD): δ 8.12 (*d*, 1 H) H-6, 8.0–7.1 (*m*, 5 H) benzoyl; 6.00 (*d*, 1 H) H-1′; 5.87 (*d*, 1 H) H-5′; 4.53 (*t*, 1 H) H-2′; 4.15 (*m*, 2 H) H-3′ and H-4′; 3.80 (*s*, 2 H) H-5; 3.64 and 3.65 (two *s*, 6 H) MDMP-methyl esters; 3.19 (*s*, 3 H) MDMP-methoxyl; 2.5–1.6 (*m*, 8 H) MDMP-methylenes.

Compound 20. R_f=0.65 (solvent B). ^{1}H NMR ($CDCl_3$+D_2O): δ 8.17 (*d*, 10.8, 1 H) H-6; 8.1–7.3 (*m*, 6 H) H-5 & benzoyl; 5.75 (*d*, 4.8, 1 H) H-1′; 4.87 (*t*, 4.8, 1 H) H-2′; 4.30 (*m*, 2 H) H-3′ and H-4′; 3.89 (*s*, 2 H) H-5′; 3.68 (*s*, 6 H) MDMP-methyl esters; 3.11 (*s*, 3 H) MDMP-methoxyl; 2.9–1.6 (*m*, 8 H) MDMP-methylenes.

Compound 21. R_f=0.65 (solvent B). ^{1}H NMR ($CDCl_3$+CD_3OD): δ 8.80 (*s*, 1 H) H-8; 8.28 (s, 1H) H-2; 8.2–7.4 (*m*, 5 H) benzoyl; 5.98 (*d*, 8.0, 1 H) H-1′; 5.11 (*dd*, 8.0, 4.8, 1 H) H-2′; 4.40 (*d*, 4.8, 1 H) H-3′; 4.32 (*s*, 1 H) H-4′; 3.91 (*m*, 2 H) H-5′; 3.68 (*s*, 3 H) MDMP-methylester; 3.60 (*s*, 3 H) MDMP-methylester; 2.74 (*s*, 3 H) MDMP-methoxyl; 2.5–1.4 (*m*, 8 H) MDMP-methylenes.

Compound 22. R_f=0.56 (solvent B). ^{1}H NMR ($CDCl_3$+CD_3OD): δ 8.30 (*s*, 1 H) H-8; 8.1–7.4 (*m*, 4 H) *t*-butylbenzoyl (TBB); 5.92 (*d*, 3.5, 1 H) H-1′; 4.75 (*t*, 1 H) H-2′; 4.46 (*t*, 1 H) H-3′; 4.19 (*s*, 1 H) H-4′; 3.97 (*s*, 2 H) H-5′; 3.68 (*s*, 3 H) MDMP-methylester; 3.58 (*s*, 3 H) MDMP-methyl ester; 3.08 (*s*, 3 H) MDMP-methoxyl; 2.5–1.7 (*m*, 8H) MDMP-methylenes; 1.35 (*s*, 9 H) TBB-methyls.

*2′-*O*-MDMP-5′-*O*-(9-phenylxanthen-9-yl)-6-*N*-benzoyladenosine (25) A general procedure.* To a dry pyridine solution (20 ml) of compound *21* (1.46 g, 2.48 mmol) was added 9-chloro-9-phenylxanthene (1.13 g, 3.88 mmol). This mixture was stirred for 80 min at room temperature under argon. The reaction mixture was then poured into a separating funnel containing saturated sodium bicarbonate solution (150 ml). This was extracted with chloroform (4×100 ml) and the pooled organic layers were co evaporated with toluene (3 X *ca.* 40 ml). The residue was then purified by short silica gel chromatography using 3 % ethanol–chlororm as eluent to give *25*. The title compound was collected and precipitated from light petroleum; 1.99 g (94.9 %).

Compounds *23*, *24* and *26* were similarly prepared in 95, 87 and 84 % yields, respectively.

Compound 23. R_f=0.78 (solvent A). ^{1}H NMR ($CDCl_3$+CD_3OD): δ 8.2–7.0 (*m*, 19 H) H-6, benzoyl, 9-phenylxanthen-9-yl (pixyl); 6.09 (*d*, 5.4, 1 H) H-1′; 5.64 (*d*, 7.8, 1 H) H-5; 4.69 (*m*, 1 H) H-2′; 4.11 (*m*, 2 H) H-3′ and H-4′; 3.65 and 3.62 (two *s*, 6 H) MDMP-methylesters; 3.20 (*m*, 5 H) H-5′ and MDMP-methoxyl; 2.8–1.5 (*m*, 8 H) MDMP-methylenes.

Compound 24. R_f=0.60 (solvent A). ^{1}H NMR ($CDCl_3$+CD_3OD): δ 8.32 (*d*, 9.0, 1 H) H-6; 8.1–7.0 (*m*, 19 H) pixyl, benzoyl & H-5; 6.19 (*d*, 4.7, 1 H) H-1′; 4.58 (*m*, 1 H) H-2′; 4.10 (*m*, 2 H) H-3′ & H-4′; 3.64 and 3.60 (two *s*, 6 H) MDMP-methylesters; 3.32 (*m*, 2 H) H-5′; 3.15 (*s*, 3 H) MDMP-methoxyl; 2.5–1.7 (*m*, 8 H) MDMP-methylenes.

Compound 25. R_f=0.62 (solvent A). ^{1}H NMR ($CDCl_3$+CD_3OD): δ 8.73 (*s*, 1 H) H-8; 8.33 (*s*, 1 H) H-2; 8.2–7.0 (*m*, 18 H) pixyl & benzoyl; 6.18 (*d*, 6.6, 1 H) H-1′; 5.18 (*dd*, 6.6, 4.8, 1 H) H-2′; 4.25 (*m*, 2 H) H-3′ & H-4′; 3.64 (*s*, 6 H) MDMP-methyl esters; 3.26 (*m*, 2 H) H-5′; 2.83 (*s*, 3 H) MDMP-methoxyl; 2.6–1.6 (*m*, 8 H) MDMP-methylenes.

Compound 26. R_f=0.59 (solvent A). ^{1}H NMR ($CDCl_3$+CD_3OD): δ 8.1–6.8 (*m*, 18 H) pixyl, TBB & H-8; 5.98 (*d*, 4.2, 1 H) H-1′; 4.83 (*m*, 1 H) H-2′; 4.25 (*m*, 2 H) H-3′ & H-4′; 3.62 (*s*, 3 H) MDMP-methyl ester; 3.56 (*s*, 3 H) MDMP-methyl ester; 3.30 (*m*, 2 H) H-5′;

3.03 (*s*, 3 H) MDMP-methoxyl; 2.5−1.9 (*m*, 8 H) MDMP-methylenes; 1.35 (*s*, 9H) TBB-methyls.

Triethylammonium salt of 2'-O-MDMP-5'-O-pixyl-3'-O-(o-chlorophenyl)-phosphate-6-N-benzoyladenosine (29). A general procedure. To a dry pyridine solution (12 ml) of compound *25* (1.64 g, 1.94 mmol) was added *o*-chlorophenylphosphorobis(1,2,4-triazolide) (0.25 M solution in acetonitrile; 16 ml, 4 mmol) at 20 °C. This solution was stirred for 110 min and then triethylamine (1.2 ml, 8.63 mmol) and water (0.6 ml) was added and stirring continued for another 30 min. The reaction mixture was poured into a separating funnel containing saturated sodium bicarbonate solution (150 ml) and the mixture was extracted with chloroform (3×100 ml). The pooled organic layers were washed with saturated sodium bicarbonate solution (100 ml) and coevaporated with toluene (3 x *ca.* 40 ml). The residue was subsequently dried on vacuum pump (0.1 mbar/20 °C) to give (*29*) (2.16 g, 98 %).

Compounds *27* and *28* were similarly prepared in 93 and 91 % yields, respectively. Compound *30* was directly coevaporated with dry pyridine and taken to the next step.

Compound 27. R_f=0.58 (solvent C). ^{1}H NMR ($CDCl_3$+DABCO): δ 8.08 (*d*, 8.1, 1 H) H-6; 8.0−6.6 (*m*, 22 H) pixyl, benzoyl and *o*-chlorophenyl-; 6.25 (*d*, 5.4, 1 H) H-1'; 5.59 (*d*, 8.1, 1 H) H-5; 4.88 (*m*, 2 H) H-2' and H-3'; 4.54 (*m*, 1 H) H-4'; 3.61 (*s*, 3 H) MDMP-methyl ester; 3.53 (*s*, 3 H) MDMP-methyl ester; 3.30 (*s*, 3 H) MDMP-methoxyl; 3.23 (*m*, 2 H) H-5'; 2.90 (*q*, 6 H) triethylammoniummethylenes; 2.5−1.6 (*m*, 8 H) MDMP-methylanes; 1.17 (*t*, 9 H) triethylammonium-methyls. ^{31}P NMR: δ −6.24.

Compound 28. R_f=0.52 (solvent C). ^{1}H NMR ($CDCl_3$+CD_3OD+pyridine-d_5): δ 8.28 (*d*, 7.2, 1 H) H-6; 8.1−6.6 (*m*, 23 H) benzoyl, pixyl, *o*-chlorophenyl- & H-5; 6.44 (*d*, 5.4, 1 H) H-1'; 4.85 (*m*, 2 H) H-2' & H-3'; 4.51 (*m*, 1 H) H-4'; 3.62 and 3.58 (two *s*, 6 H) MDMP-methyl esters; 3.23 (*s*, 2 H) H-5'; 3.14 (*s*, 3 H) MDMP-methoxyl; 3.06 (*q*, 7.3, 6 H) triethylammonium-methylenes; 2.3−1.5 (*m*, 8 H) MDMP-methylenes; 1.26(*t*, 7.3, 9 H) triethylammonium-methyls. ^{31}*P-NMR:* δ −6.1.

Compound 29. R_f=0.51 (solvent C). ^{1}H NMR ($CDCl_3$+CD_3OD): 8.75 (*s*, 1 H) H-8; 8.7−7.0 (*m*, 23 H) pixyl, benzoyl, H-2 and *o*-chlorophenyl; 6.35 (*d*, 7.0, 1 H) H-1'; 5.30 (*m*, 1 H) H-2'; 5.00 (*m*, 1 H) H-3'; 4.62 (*m*, 1 H) H-4'; 3.56 (*s*, 6 H) MDMP-methyl esters; 3.30 (*m*, 2 H) H-5'; 2.95 (*q*, 7.2, 6 H) triethylammonium-methylenes; 2.76 (*s*, 3 H) MDMP-methoxyl; 2.5−1.8 (*m*, 8 H) MDMP-methylenes; 1.23 (*t*, 7.2, 9 H) triethylammonium-methyls. ^{31}P NMR: δ −5.9.

Compound 30. R_f=0.57 (solvent C). ^{1}H NMR ($CDCl_3$+CD_3OD): δ 8.1−6.7 (*m*, 22 H) pixyl, *o*-chlorophenyl-, TBB & H-2; 6.00 (*d*, 4.2, 1 H) H-1'; 5.03 (*m*, 2 H); 4.52 (*m*, 1 H); 3.50 and 3.43 (two *s*, 6 H) MDMP-methylesters; 3.4−2.5 (*m*, 11 H) H-5', MDMP-methoxyl, triethylammonium-methylenes; 2.5−1.6 (*m*, 8 H) MDMP-methylenes; 1.27 (*s*, 9 H) TBB-methyls; 1.11 (*t*, 6.6, 9H) triethylammonium-methyls.

Preparation of fully protected nucleotide (33). A general procedure: A mixture of compound (*29*) (2.16 g, 1.90 mmol) and 9-fluorenylmethanol (810 mg, 4.1 mmol) was coevaporated with dry pyridine (2 x *ca.* 10 ml) and redissolved in dry pyridine (19 ml) and 1-(mesitylenesulfonyl)-3-nitro-1,2,4-triazole (MS−NT) (1.28 g, 4.32 mmol) was added. The reaction mixture was stirred at room temperature under argon for 65 min and then poured into a separating funnel containing saturated sodium bicarbonate solution (150 ml). The mixture was extracted with chloroform (4×150 ml) and the combined organic layers were coevaporated twice with toluene. The residue was then purified on a short silica gel column using 3 % ethanol-chloroform as eluent. The desired fractions were collected, filtered and, after concentration on a rotavapor, was precipitated and reprecipitated from light petroleum to give (*33*). Yield: 2.02 g (87.4 %).

Compounds *31*, *32* and *34* were similarly prepared in 91, 88 and 74 % yields. Yield of compound *34* is based on compound *26*.

Compound 31. R_f=0.83 (solvent A). ^{1}H NMR. ($CDCl_3$): δ 8.01 (*d*, 8.1, 1 H) H-6; 7.9−7.1 (*m*, 30 H) *o*-chlorophenyl, benzoyl, pixyl, fluoren-9-methyl (FM); 6.25 (*d*, 6.1, 1 H) H-1'; 5.67 (*d*, 8.1, 1 H) H-5; 4.91 (*m*, 2 H); 4.52 (*m*, 2 H); 4.11 (*m*, 2 H); 3.59, 3.55, 3.51 and 3.47 (four *s*, 6 H) MDMP-methylesters; 3.29 and 3.26 (two *s*, 3H) MDMP-methoxyl; 3.1−2.9 (*m*, 2H); 2.4−1.7 (*m*, 8 H) MDMP-methylenes. ^{31}P NMR: δ −7.45.

Compound 32. R_f=0.76 (solvent A). ^{1}H NMR ($CDCl_3$): δ 8.28 (*d*, 7.7, 1 H) H-6; 8.1−7.0 (*m*, 31 H) H-5, benzoyl, pixyl, FM & *o*-chlorophenyl-; 6.48 (*m*, 1 H) H-1'; 4.96 (*m*, 2 H); 4.55 (*m*, 2 H); 4.16 (*m*, 2 H); 3.53, 3.50, 3.48 and 3.34 (four *s*, 6 H) MDMP-methyl

esters; 3.15 and 3.12 (two *s*, 3 H) MDMP-methoxyls; 3.10 (*m*, 2 H); 2.5−1.5 (*m*, 8 H) MDMP-methylenes. ^{31}P NMR: δ −7.32; −7.42.

Compound 33. R_f=0.76 (solvent A). ^{1}H NMR ($CDCl_3$): δ 8.71 (*s*, 1 H) H-8; 8.30 (*s*, 1 H) H-2; 8.2−6.5 (*m*, 30 H) benzoyl, pixyl, *o*-chlorophenyl & FM; 6.18 (*d*, 7.1, 1 H) H-1′; 5.54 (*m*, 1 H) H-2′; 5.14 (*m*, 1 H); 4.40 (*m*, 4 H); 3.47 (*s*, 6 H) MDMP methyl esters; 3.2(*m*, 2 H); 2.68 (*s*, 3 H) MDMP-methoxyl; 2.4−1.3 (*m*, 8 H) MDMP-methylenes. ^{31}P NMR: δ −7.10; −7.23.

Compound 34. R_f=0.62 (solvent A). ^{1}H NMR ($CDCl_3$+CD_3OD): δ 8.1−6.4 (*m*, 30 H) pixyl, TBB, H-8, *o*-chlorophenyl & FM; 5.94 (*d*, 5.8, 1 H) H-1′; 5.30 (*m*, 2 H); 4.40 (*m*, 4 H); 3.52 and 3.47 (two *s*, 6 H) MDMP-methylesters; 3.20 (*m*, 2 H); 2.91 and 2.85 (two *s*, 3 H) MDMP-methoxyls; 2.4−1.5 (*m*, 8 H) MDMP-methylenes; 1.37 (*s*, 9 H) TBB-methyls. ^{31}P NMR: δ −6.66; −7.15.

Preparation of 5′-hydroxy block (37). A general procedure. To a solution of compound *33* (255 mg, 0.21 mmol) in chloroform (28 ml) was added 2,2,2-trichloroacetic acid in chloroform (10.4 ml, 0.202 M, 10 eq) and the mixture was stirred at 0 °C for 165 min. The reaction mixture was then poured into a separating funnel containing saturated sodium bicarbonate solution (100 ml) and the mixture extracted with chloroform (4×100 ml). The combined organic layers were coevaporated with toluene and purified on short silica gel column using 3 % ethanol−chloroform as eluent. The title compound was collected, filtered, concentrated on rotavapor and precipitated from light petroleum (143,3 mg; 71.3 %).

Compounds *35*, *36* and *38* were similarly prepared in 74, 74 and 70 % yields, respectively.

Compound 35. R_f=0.61 (solvent A). ^{1}H NMR ($CDCl_3$+CD_3OD): δ 8.3−6.9 (*m*, 18 H) *o*-chlorophenyl, FM, benzoyl & H-6; 6.14 (*d*, 6.3, 1 H) H-1′; 5.42 (*d*, 8.1, 1 H) H-5; 4.96 (*m*, 1 H); 4.63 (*m*, 3 H); 4.22 (*m*, 2 H); 3.67 (*m*, 2 H); 3.55 (*s*, 6 H) MDMP-methyl esters; 3.12 (*s*, 3 H) MDMP-methoxyl; 2.4−1.6 (*m*, 8 H) MDMP-methylenes. ^{31}P NMR: δ −7.29.

Compound 36. R_f=0.57 (solvent A). ^{1}H NMR ($CDCl_3$+CD_3OD): δ 8.4−6.8 (*m*, 19 H) *o*-chlorophenyl, FM, benzoyl, H-6 & H-5; 6.12 (*d*, 7.2, 1 H) H-1′; 4.93 (*m*, 1 H) H-2′; 4.8−3.1 (*m*, 5 H); 3.70 (*m*, 2 H) H-5′; 3.56 (*s*, 6 H) MDMP-methyl esters; 2.97 (*s*, 3 H) MDMP-methoxyl; 2.6−1.5 (*m*, 8 H) MDMP-methylenes. ^{31}P NMR: δ −6.9.

Compound 37. R_f=0.57 (solvent A). ^{1}H NMR ($CDCl_3$+CD_3OD): δ 8.80 (*s*, 1 H) H-8; 8.26 (*s*, 1 H) H-2; 8.2−7.0 (*m*, 17 H) benzoyl, FM & *o*-chlorophenyl; 5.95 (*d*, 8.2, 1 H) H-1′; 5.25 (*m*, 2 H); 4.60 (*m*, 2 H); 4.32 (*m*, 2 H); 3.99 (*s*, 1 H); 3.74 (*s*, 1 H); 3.54 and 3.50 (two *s*, 6 H) MDMP-methyl esters; 2.58 (*s*, 3 H) MDMP-methoxyl; 2.5−1.7 (*m*, 8 H) MDMP-methylenes. ^{31}P NMR: δ −7.15; −7.22.

Compound 38. R_f=0.45 (solvent A). ^{1}H NMR ($CDCl_3$+CD_3OD): δ 8.1−7.1 (*m*, 17 H) TBB, *o*- chlorophenyl-, FM & H-8; 5.87 (*m*, 1 H) H-1′; 5.17 (*m*, 2 H); 4.63 (*m*, 2 H) 4.34 (*m*, 2 H); 3.81 (*m*, 2 H); 3.54, 3.52 and 3.51 (three *s*, 6 H) MDMP-methylester; 2.79 and 2.76 (two *s*, 3H) MDMP-methoxyl; 2.3−1.5 (*m*, 8 H) MDMP-methylenes; 1.35 (*s*, 9 H) TBB-methyls. ^{31}P NMR: δ −7.05; −7.22.

Preparation of fully protected dimer CpC (40). A mixture of (*28*) (900, mg, 0.81 mmol) and 2′,3′-*O*-di-acetyl-4-*N*-benzoylcytidine (*39*) (317 mg, 0.736 mmol) was coevaporated twice with dry pyridine, and then redissolved in dry pyridine (10 ml) and MS−NT was added (1.09 g, 3.68 mmol). The reaction mixture was stirred at 20 °C for 45 min. Then *o*-chlorophenylphosphorobis(1,2,4-triazolide) (5 ml, 1.25 mmol) was added and stirring was continued for another 20 min. The reaction mixture was poured into a separating funnel containing saturated sodium bicarbonate solution (200 ml) and the mixture was extracted with chloroform (4×100 ml). The pooled organic layers were co-evaporated with toluene and the residue was purified by silica gel chromatography using 4 % ethanolic chloroform. The desired fractions were pooled and concentrated on a rotavapor and precipitated from light petroleum to give *40* (850 mg, 80.3 %). R_f=0.51 (solvent A).

Removal of the 5′-pixyl group from fully protected CpC (40). The fully protected dimer CpC (850 mg, 0.591 mmol) was dissolved in cooled (0 °C) nitromethane (30 ml) and zinc bromide (6.65 g, 29.5 mmol) in nitromethane (60 ml) solution was added. The mixture was stirred at 0 °C for 2 h which was followed by a usual[5] work up procedure. The reaction mixture was purified on a short silica gel column using 5 % ethanol-chloroform as eluent. Appropriate fractions were collected and concentrated on a rotavapor and finally

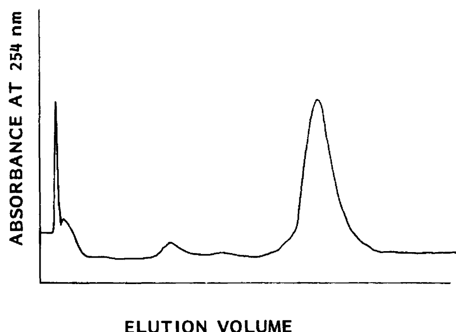


Fig. 1. DEAE Sephadex A 25 elution profile of fully deprotected trimer, 5'-UpCpC-3' *42*, using triethylammonium bicarbonate buffer (pH 7.3; linear gradient: 0.0075 to 0.45 M, 400 ml each).

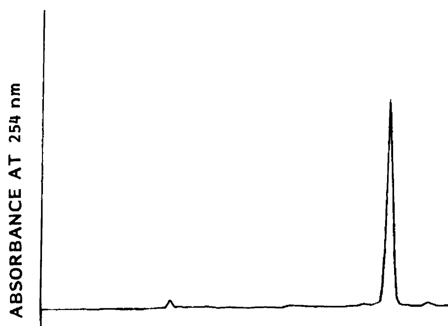


Fig. 2. HPLC elution profile of the main peak in Fig. 1 using 0.0005 M aqueous tetrapentylammonium phosphate in 0 to 20 % acetonitrile in a linear gradient mode for 30 min in the mobile phase (R_t=22.17 min for the main peak).

precipitated from light petroleum to give *41* as a powder (550 mg, 79 %). R_f=0.47 and 0.43 (solvent A).

Preparation of the fully protected trimer UpCpC (42). A mixture of compound *27* (734 mg, 0.659 mmol) and the 5'-hydroxy dimer *41* (600 mg, 0.5 mmol) was co-evaporated with dry pyridine (2 x *ca.* 3 ml) and redissolved in dry pyridine (8 ml). To this solution was added MS–NT (740 mg, 2.5 mmol). After 60 min stirring at 20 °C *o*-chlorophenylphosphorobis-(1,2,4-triazolide) (4 ml, 0.25 M in dry acetonitrile, 1 mmol) was added, and stirring was continued for an additional 15 min. After the usual work up procedure[5] the mixture was purified on a short silica gel column using 4 % ethanol–chloroform as eluent. Appropriate fractions were collected, concentrated on a rotavapor and precipitated from light petroleum. Yield: 854.6 mg, 77.4 %. R_f=0.53 (solvent A).

Preparation of the 5'-hydroxy trimer block of UpCpC (45). The fully protected trimer UpCpC (*42*) (550 mg, 0.2527 mmol) was dissolved in cold (0 °C) dichloromethane (15 ml). To this solution was added a cold (0 °C) solution of 4-toluenesulfonic acid monohydrate (1.088 g, 6.316 mmol) in chloroform-methanol mixture (7/3 v/v, 54 ml). The mixture was stirred at 0 °C for 20 min. After the usual work up procedure[5] and silica gel column

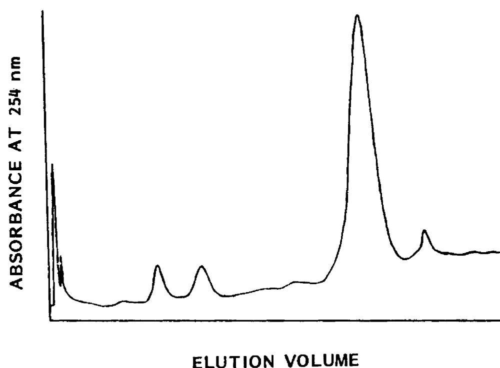


Fig. 3. DEAE Sephadex A 25 elution profile of fully deprotected pentamer, 5'-ApGpUpCpC-3' *46*, using triethylammonium bicarbonate buffer (linear gradient: 0.0075 to 0.65 M, 450 ml each).

chromatography (4 % ethanol–chloroform as eluent) the title compound was precipitated from light petroleum. Yield: 374.2 mg, 77.1 %. R_f=0.42 and 0.47 (solvent A).

Preparation of fully protected dimer ApGp (43). A mixture of compound *38* (450 mg, 0.438 mmol) and compound (*29*) (597 mg, 0.525 mmol) was co-evaporated with dry pyridine (2 x *ca.* 3 ml), redissolved in dry pyridine (5 ml) and MS–NT (518.6 mg, 1.75 mmol) was added. The reaction mixture was stirred at 20 °C for 45 min followed by a usual work up procedure as for *40*. The reaction mixture was subsequently purified by using short silica gel chromatography with 4 % ethanol–chloroform as eluent. Appropriate fractions were collected, concentrated on a rotavapor and precipitated from light petroleum. Yield: 800.9 mg (89.5 %). R_f=0.58 (solvent A).

Conversion of fully protected ApGp 43 to the corresponding phosphodiester 44. The fully protected dimer ApGp (780 mg, 0.3815 mmol) was coevaporated with dry pyridine (2 x *ca.* 5 ml) and redissolved in dry pyridine (5 ml). To this solution, dry triethylamine (1.06 ml, 7.6 mmol) was added. The reaction mixture was then stirred at 20 °C for 60 min. The reaction mixture was co-evaporated several times with toluene and then precipitated from light petroleum to give *44* as a powder. Yield: 720 mg, 97 %. R_f=0.57 (solvent C).

Preparation of fully protected pentamer ApGpUpCpC (46). A mixture of ApGp *44* (80.3 mg, 40.82 μmol and 5′-hydroxy-UpCpC (*45*) (50.52 mg, 26.53 μmol) was co-evaporated with dry pyridine (3 x *ca.* 4 ml) and then redissolved in dry pyridine (2 ml). To this solution, MS–NT (214 mg, 722 μmol) was added. After stirring for 110 min at 20 °C saturated sodium bicarbonate solution (5 ml) was added and stirring was continued for another 15 min; then the mixture was worked up according to a standard procedure.[5] Purification was carried out by two short silica gel columns using 3.6 % ethanol–chloroform as eluent and two preparative TLC with 6 % ethanol–chloroform mixture as the mobile phase. Appropriate fractions and bands were respectively collected and concentrated to obtain a residue; after co-evaporation of this residue with toluene the title compound was precipitated from light petroleum. Yield: 49.1 mg, 49.3 %. R_f=0.51 (solvent A).

Deblocking of fully protected trimer (42). a solution of fully protected trimer (*42*) (20 mg, 0.0078 mmol), *syn*-4-nitrobenzaldoxime (30.5 mg, 0.184 mmol) and N^1,N^1,N^3,N^3-tetramethylguanidine (19.04 mg, 0.166 mmol) in dioxane–water (1:0.3 v/v, 1.3 ml) was stirred at room temperature. After 24 h aqueous ammonia (30 %; 5 ml) was added. After 3 d the solution was evaporated *in vacuo* and the residue was dissolved in a water-diethyl ether mixture (40 ml, 1:1 v/v). The aqueous layer was washed with diethyl ether (2×20 ml) and concentrated on a rotavapor. The residue was dissolved in 80 % acetic acid (20 ml) and the solution was stirred at 20 °C for 6 h. The mixture was then coevaporated several times with water, redissolved in water (20 ml) and washed with dichloromethane (3 x 20 ml). The aqueous layer was then concentrated on a rotavapor. The residue was then purified on a Sephadex DEAE A25 (150 mm×20 mm) column using triethylammonium bicarbonate aqueous buffer (pH 7.3, 0.0075 to 0.45 M; linear gradient) as eluent as shown in the elution profile in Fig. 1. The purity of the deblocked trimer *47* was checked by HPLC[21] (spherisorb ODS 10μ column, 0.0005 M tetrapentylammonium phosphate (TPeAm phosphate in 0 to 20 % acetonitrile, linear gradient in 30 min, flow 2.0 ml/min) (Fig. 2). R_f=22.17 min for (*47*). Yield: 133 o.d. units at A_{260} (80 %); UV: λ_{max}=264.5 nm.

Deblocking of fully protected pentamer ApGpUpCpC (46). The same procedure as for the trimer *42*, was used for the deblocking of the pentamer *46* (15.2 mg, 0.00405 mmol). Purification was carried out in the same way as above but a linear gradient 0.0075 to 0.65 M triethylammonium bicarbonate was used. The elution profile is shown in Fig. 3. Purity of the deblocked pentamer *48* was checked on HPLC[21] as for the trimer *47* but with 0.0075 M tetrabutylammonium sodiumsulfate in an acetonitrile-water gradient (15–33 % acetonitrile, linear gradient in 30 min.) as shown in Fig. 4. R_t=30 min for *48*. Yield: 127 o.d. units at A_{260} (81 %). UV: λ_{max} 259.1 nm (pH 6.18) and at 264.8 nm (pH 2).

Digestion of deprotected oligonucleotides

(A) *With calf spleen phosphodiesterase.* (a) *Trimer UpCpC (47).* To a solution of the unprotected trimer UpCpC (3 o.d. units) in water (20 μl) was added calf spleen phosphodiesterase (20 μg) in ammonium acetate buffer (100 μl, 0.002 M, pH 7, 0.002 M

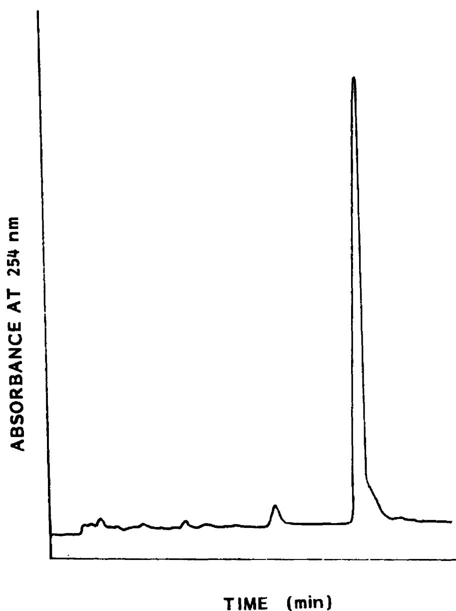


Fig. 4. HPLC elution profile of the main peak in Fig. 3 using 0.0075 M aqueous tetrabutylammonium sodiumsulfate in 15 to 35 % acetonitrile in a linear gradient mode for 30 min in the mobile phase (R_t=30 min for the main peak).

Table 2. HPLC quantitation of oligoribonucleotides, *47* and *48* after they were fully digested with calf spleen phosphodiesterase.

	(a) UpCpC *47*				(b) ApGpUpCpC *48*		
		Ratios				Ratios	
	R_t (min)	Obs.	Calc.		R_t (min)	Obs.	Calc.
Cytidine	3.49 }	1.9	2	Cytidine	3.08	0.85	1
3′-CMP	14.61 }			3′-CMP	16.17	0.90	1
3′-UMP	18.27	1.09	1	3′-UMP	18.56	0.93	1
				3′-GMP	19.52	0.99	1
				3′-AMP	22.56	1.0	1

Table 3. HPLC quantitation of oligoribonucleoties, *47* and *48*, after they were fully digested with *Crotalus Adamanteus* snake venom phosphidiesterase and monoesterase mixture.

	(a) UpCpC *47*				(b) ApGpUpCpC *48*		
		Ratios				Ratios	
	R_t (min)	Obs.	Calc.		R_t (min)	Obs.	Calc.
Cytidine	2.21	2.01	2	Cytidine	2.91	2.01	2
Uridine	2.51	0.99	1	Uridine	3.52	1.05	1
				Guanosine	5.83	1.09	1
				Adenosine	8.66	1.00	1

Table 4. Alkaline hydrolyses of oligoribonucleotides, *47* and *48* with 0.2 M sodium hydroxide and HPLC quantitation of the reaction mixture.

	(a) UpCpC *47*				(b) ApGpUpCpC *48*		
		Ratios				Ratios	
	R_t (min)	Obs.	Calc.		R_t (min)	Obs.	Calc.
Cytidine	2.27	0.90	1	Cytidine	2.65	0.80	1
2′(3′)-CMP	13.84, 15.19	1.03	1	2′(3′)-CMP	9.04, 9.93	1.07	1
2′(3′)-UMP	19.45, 20.10	1.05	1	2′(3′)-UMP	12.31, 13.48	1.07	1
				2′(3′)-GMP	13.48, 15.97	1.07	1
				2′(3′)-AMP	18.48, 21.14	1.0	1

EDTA and containing 0.05 % tween 40). The resulting solution was kept at 37 °C for 48 h.

(b) *Pentamer ApGpUpCpC (48).* The same procedure as above was used but 40 μg enzyme was added.

(B) *With crotalus adamanteus snake venom phosphodiesterase.* (a) *Trimer UpCpC (47).* A solution of snake venom phosphodiesterase (20 μg) in Tris-hydrochloride buffer (0.1 M, pH 9, 0.01 M $MgCl_2$, 70 μl) was added to a solution of trimer (3 o.d. units) in water (20 μl). The resulting solution was kept at 37 °C for 18 h.

(b) *Pentamer ApGpUpCpC (48).* The same procedure as above was used.

(C) *With 0.2 M sodium hydroxide.* (a) *Trimer UpCpC (47).* To a solution of the trimer (3 o.d. units) in water (20 μl) was added NaOH (0.2 M, 150 μl). The solution was kept at 37 °C for 48 h.

(b) *Pentamer ApGpUpCpC (48).* The same procedure as above was used.

HPLC analyses of six digestions. All hydrolysates were analysed by HPLC[21] using a spherisorb ODS 10 μ column with 0.0005 M TPeAM phosphate in water (solvent A) and 0.0005 M TPeAm phosphate in 20 % acetonitrile-water (solvent B) on a linear gradient mode 0−100 % of B for 30 min; flow rate 2.0 ml/min.

The calculated and the observed ratios between the different mononucleotides and nucleosides are shown in Tables 2 to 4.

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