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The 9-(4-Octadecyloxyphenylxanthen)-9-yl- Group. A new Acid-labile Hydroxyl Protective Group and Its Application in the Preparative Reserve-phase Chromatographic Separation of Oligoribonucleotides

M. KWIATKOWSKI and J. CHATTOPADHYAYA *

Bioorganic Chemistry Laboratory, Department of Microbiology, Box 581, Biomedical Center, Uppsala University, S-752 13 Uppsala, Sweden

A new 5′-hydroxyl protecting group, 9-(4-octadecyloxyphenylxanthen)-9-yl-(C_{18}Px), and the properties of its ribonucleoside and the ribonucleotide derivatives, have been described. The preparation of a simple reverse-phase silica gel support, which is inexpensive and easily accessible, has also been devised for the routine preparative use in the ribonucleotide synthesis in conjunction with the C_{18}Px group. The present work also constitutes the first report of the 2-phenylsulfonylethyl- as a 3′-terminal phosphodiester protecting group in the ribonucleotide chemistry. Finally, the application of these new observations has been shown by the actual chemical syntheses of two ribonucleotide fragments.

The success in polyribonucleotide synthesis depends upon the proper choice of a set of complementary protecting groups, phosphorylation procedures inasmuch as the efficient separation techniques to purify oligoribonucleotide blocks.[1] The latter problem has been somewhat circumvented in the case of chemical synthesis of DNA fragments due to the progress that has been made in the solid phase synthesis.[2] The concept of employing highly lipophilic protecting groups[3] on both the 5′ [3c,3d], and the phosphate residues of oligodeoxyribonucleotides, have previously been used by several groups of workers for rapid solvent extractions and both for reverse-phase analytical and preparative high performance liquid chromatography (HPLC). However, the use of commercially available reverse-phase support for routine preparative HPLC work is expensive! We have been, therefore, interested for a long time to devise procedures that should use a lipophilic protecting group at the 5′-end and employ a reverse-phase support, that is easily accessible and relatively inexpensive, for the routine preparative work in the laboratory.

For this purpose, we have sought to derivatize the *para* position of the phenyl group of 9-phenylxanthen-9-yl-(pixyl),[4] which is an acidlabile protective group, that has been widely used by us both in DNA[4,5] and RNA[6] chemistry and also in the synthesis of 2′→5′ oligoisoadenylates.[7]

We have thus prepared 9-(4-octadecyloxyphenylxanthen)-9-yl (C_{18}Px) and 9-anisylxanthen-9-yl (C_1Px) derivatives, *3* and *4*, respectively, of 2′,3′-di-*O*-acetyl ridine (*1*) by following a procedure that have been earlier employed for the preparation of 5′-*O*-(9-phenylxanthen-9-yl(px))-2′,3′-di-*O*-acetyl uridine (*2*).[6] Figs. 1, 2 and 3, thus, show the results of an apparently specific hydrophobic interaction between the alkyl chains of the sorbent and sorbates, like commercially available RP-2, RP-8 and RP-18 plates, which are reflected in the R_f values of compounds *1* to *4*. It thus appeared quite likely

* Author to whom correspondence should be addressed.

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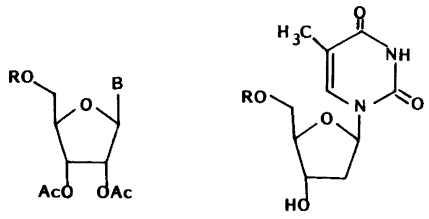
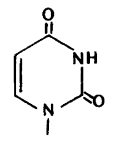
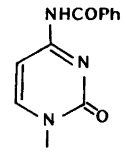




(U)



(bzC)

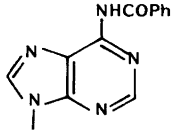
1, R = H; B = U (Uracil-1-yl-)

2. R = Px ; B = U

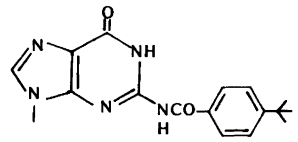
3. R = C_{18}Px ; B = U

4, R = C_1Px ; B = U

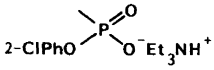
5, R = C_{18}Px



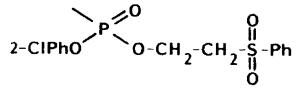
(bzA)



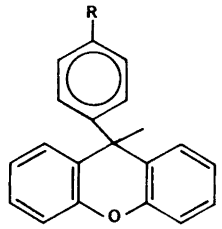
(t-bbzG)



phosphodiester



phosphotriester



(g); R = H (Px)

(h); R = -OCH_3 (C_1Px)

(i); R = -O-CH_2-$(CH_2)_{16}$-CH_3 (C_{18}Px)

that a chemically modified silica gel with the hydrophobic property, that is intermediary between RP-2 and RP-8 grade silica gel, might assist in an optimum resolution of the reactants and products during the preparative separations of oligoribonucleotides containing C_{18}Px group at the 5'-end. This has led us to silanise commercially available Kieselgel G, in suspension in methylene chloride containing dry pyridine (20 mmol/g of the silica), with trimethylsilyl chloride (10 mmol/g of the silica) for 24 h at room temperature. The silica gel, thus obtained after usual steps of filtration, washing and drying at 150 °C for 13 h (*ca.* 20 mmHg), has been used as thin layer chromatographic support material to prepare plates, from a slurry containing 4 g of the above silica gel in aqueous acetone (1:1 v/v). The silica gel plates were then dried in an oven (100 °C; *ca.* 20 mmHg) for an hour and then the chromatographic properties of the compounds *3* and *4* were first examined on these plates and were then compared with those of the commercially available RP-2 and RP-8 plates. The R_f values of *3* and *4* on this silanized support were

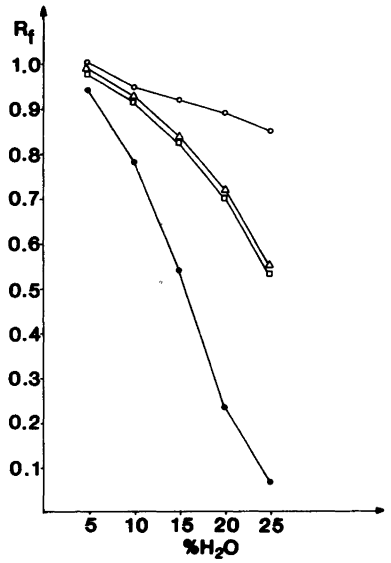


Fig. 1. Demonstration of the measure of the hydrophobic interactions on RP-2 plates depending upon the nature of the protective group at the 5′-end. ○=*1*; △=*4*; □=*2*; ●=*3*.

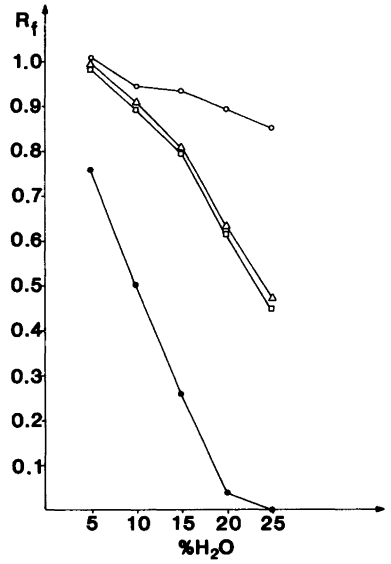


Fig. 2. Demonstration of the measure of the hydrophobic interactions on RP-8 plates depending upon the nature of the protective group at the 5′-end. ○=*1*; △=*4*; □=*2*; ●=*3*.

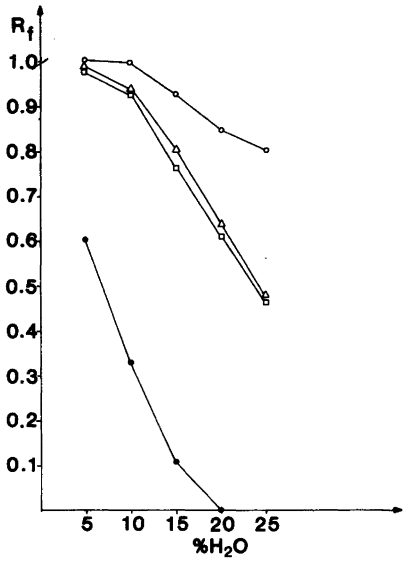


Fig. 3. Demonstration of the nature of the hydrophobic interactions on RP-18 plates depending upon the nature of the protective group at the 5′-end. ○=*1*; △=*4*; □=*2*; ●=*3*.

respectively 0.19 and 0.72; Figs. 1 and 2 show that the R_f values of compounds *3* and *4* were respectively 0.23 and 0.72 on RP-2 and 0.03 and 0.63 on RP-8 plates when they were run in mobile phase containing a water–acetone mixture (2:8 v/v). Thus, the hydrophobic property of the new silanized silica gel seem to lie somewhere between RP-2 and RP-8 supports. We have then proceeded to examine the hydrolytic properties of *3* and *4* with respect to 5′-*O*-(9-phenylxenthen-9-yl) thymidine (*5*)[4a] in a mixture of 80 % acetic acid containing dioxane (4:1 v/v) at room temperature. We observed that, despite the presence of the alkoxy substituents, as in *3* and *4*, in the *para* position of the Px-group, as in *5*, the relative acid labilities of C_{18}Px-, C_1Px- and Px-group are comparable ($t_{½}$ *ca.* 4 min and t_{∞} *ca.* 50 min). This observation is important to our present strategy in view of the fact that a protective group should be stable under the standard conditions of manipulations that are normally required in a multistep chemical synthesis.

These encouraging results have subsequently led us to prepare the partially protected building blocks, *10* to *13*, with free 3′-hydroxyl functions, in 67, 65, 72, 71 % yields, respectively using a

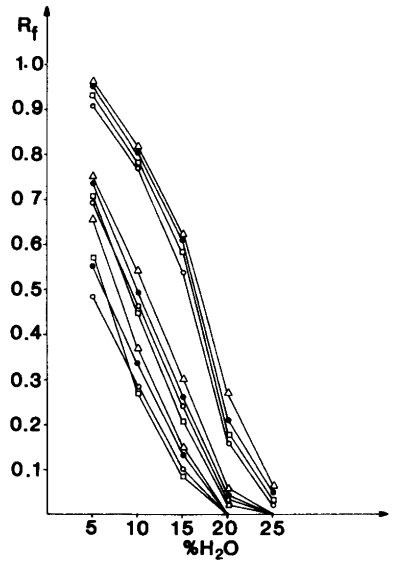


Fig. 4. Hydrophobic interactions of the 5′-C_{18}Px-containing ribonucleoside building blocks on: (A) RP-2; (B) RP-8; (C) RP-18 plates. △=*10*; ●=*12*; □=*13*; ○=*11*.

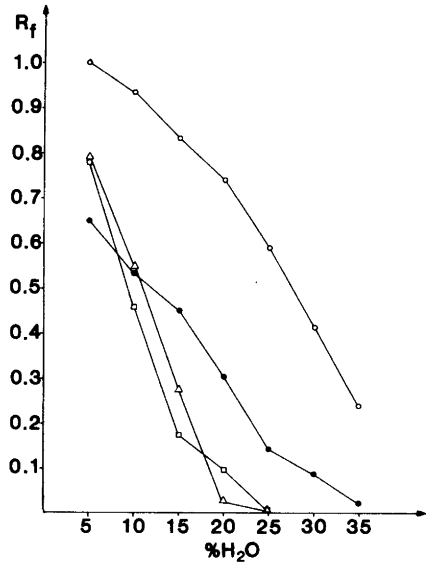


Fig. 6. Demonstration of the nature of the hydrophobic interactions of the 5′-C_{18}Px-containing ribonucleotide building blocks on RP-8 plates. ○=*23*; ●=*15*; △=*20*; □=*26*.

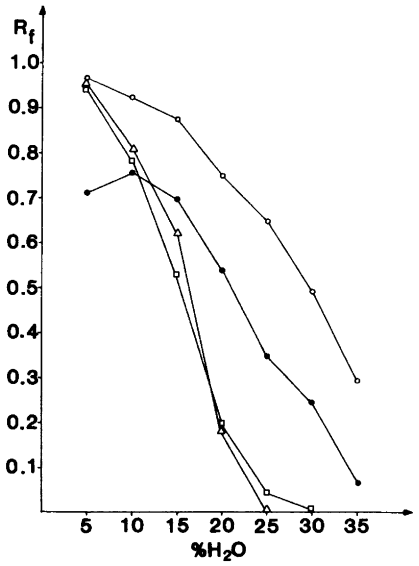


Fig. 5. Demonstration of the nature of the hydrophobic interactions of the 5′-C_{18}Px-containing ribonucleotide building blocks on RP-2 plates. ○=*23*; ●=*15*; △=*20*; □=*26*.

literature procedure.[6] The specific interactions, in terms of the R_f values, between the alkyl chain of the C_{18}Px group of compounds *10* to *13*, the essential building blocks for the RNA synthesis, with the adsorbates of RP-2, RP-8 and RP-18 plates are shown in the Fig. 4.

We have then explored the reverse-phase chromatographic properties of a phosphodiester, a triester and a 5′-hydroxy phosphotriester building block. This is relevant because in any condensation reaction using the phosphotriester approach, that leads to the formation of a (3′→5′)-internucleotidic linkage, as in *26*, or a fully protected 3′-terminal phosphotriester block, as in *19*, the reactants are generally a 5′-protected phosphodiester block, as in *15*, a hydroxy block, as in *23*, and an activating agent. Figs. 5 and 6 thus record the R_f values of two such reactants and two fully protected nucleotides on RP-2 and RP-8 TLC plates after they have been run with the mixtures of solvents containing different proportions of water and acetone.

Fig. 7, then, shows two elution profiles of two artificial mixtures containing: (a) *15*, *19* and *23*; and (b) *15*, *19* and *26* on the new reverse-phase silica gel support employing a preparative short

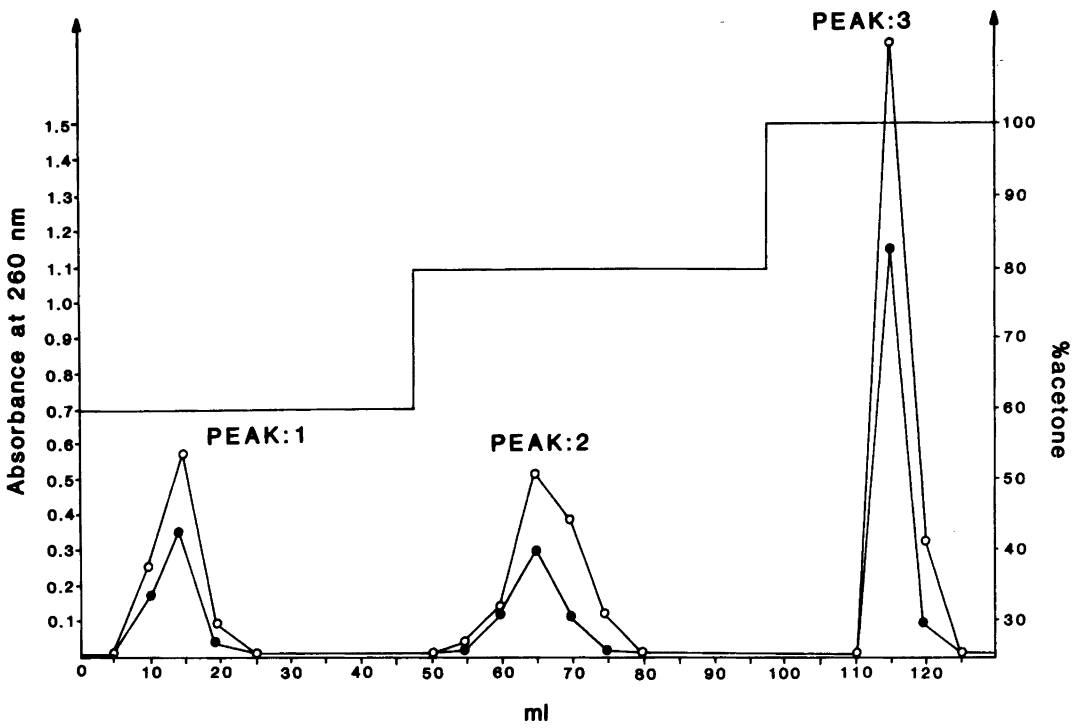
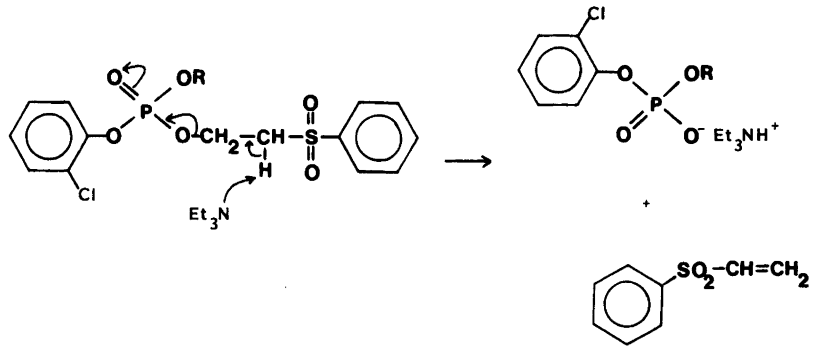


Fig. 7. Elution profiles of two artifical mixtures: ○=*23*: peak 1; *15*: peak 2; *19*: peak 3 ●=*23*; peak 1: *15*: peak 2; *26*: peak 3.

column chromatographic mode;[8] they clearly show that a combination of a simple modified reverse-phase support and a 5′-C_{18}Px protective group, that we report in this communication, may indeed assist to resolve a mixture of product and reactants to a desired degree with a greater facility.

These observations have subsequently led us to employ them in the chemical syntheses of a tri- and tetra-ribonucleotide sequence; (1) CCC and (2) AUAA, as two actual examples. The preparation of appropriately protected 2′-*O*-(4-methoxytetrahydropyranyl) (MTHP) blocks, *6* to *9*, that are required for the syntheses of the target compounds, have already been reported.[6] The preparation of 5′-*O*-C_{18}Px building blocks, with a free 3′-hydroxyl function, as in *10* to *13*, have been carried out with the help of C_{18}Px–Cl using a procedure that we have reported recently[6] for the preparation of 5′-*O*-Px building blocks and were obtained in 67, 72, 71 and 65 % yields respectively. These blocks have then been converted into their 3′-*O*-2-chlorophenylphosphate salts *14* to *17* quantitatively using 2-chlorophenylphosphorobis-(1,2,4-triazolide) and then hydrolysis.[9] One needs[1] to convert these phosphodiesters into their triesters to carry out the preparations of the 5′-hydroxy blocks which should be eventually required to construct a molecule of fully protected dinucleoside-(3′→5′)-phosphate as in *26*. Thus the chemical construction of a triester, as in *18* to *21*, should be such as that it is regiospecifically and quantitatively covertable to a diester functionality in a dinucleotide block, as exemplified by the conversion of *30* to *31*. Earlier we have introduced 2-phenylsulfonylethyl-(PSE)[10], in DNA chemistry for such a protection of a phosphodiester block at the level of a triester. During these studies it has been observed that the PSE group is stable during normal chemical operations which are usually required in DNA chemistry using the phosphotriester approach;[1] the PSE group, however, is easily removed using triethylamine in



Scheme 1.

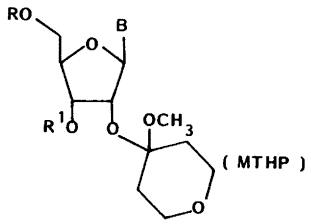
dry pyridine solution at room temperature within 30 min. The chemistry of its deprotection centers on the acidic nature of the proton on the β-carbon atom, and hence its abstraction by base; the PSE group fragment *via* β-elimination liberating the phosphodiester as shown in Scheme 1.

Thus, through this present work, the application of the PSE group has also been explored, for

Table 1. ^{1}H and ^{31}P NMR absorptions of the intermediates.

Compounds	Solvent	^{1}H and ^{31}P NMR (Chemical shifts in δ)
4-Octadecyloxy-bromobenzene	$CDCl_3$	7.35 (*d*, 9.6 Hz, 2 H); 6.75 (*d*, 9.6 Hz, 2 H); 3.91 (*t*, 6 Hz, 2 H); 1.27 (*m*, 32 H); 0.85 (*t*, 3 H).
9-Hydroxy-9-(4-octadecyloxy-phenylxanthen)	$CDCl_3$	7.46–6.70 (*m*, 12 H); 3.88 (*t*, 2 H); 1.23 (*m*, 32 H); 0.82 (*t*, 3 H)
9-Hydroxy-9-anisylxanthen	$CDCl_3$	7.1–6.71 (*m*, 12 H); 3.73 (*s*, 3 H).
3	$CDCl_3$+ CD_3OD	7.87 (*d*, 8 Hz, IH); 7.24 (*m* 12 H); 6.18 (*d*, 7.2 Hz, IH); 5.56 (*d*, 8 Hz, IH); 5.42 (*m*, IH); 4.15 (*m*, IH); 3.93 (*t*, 6 Hz, 2 H); 3.17 (*m*, 2 H); 2.11 (*s*, 3 H); 2.05 (*s*, 3 H); 1.25 (*m*, 35 H).
4	$CDCl_3$+ CD_3OD	7.88 (*d*, 8 Hz, IH); 7.25 (*m*, 12 H); 6.20 (*d*, 7.2 Hz, IH); 5.60 (*d*, 8 Hz, IH); 5.52 (*m*, IH); 4.19 (*m*, IH); 3.78 (*s*, 3 H); 2.11 (*s*, 3 H); 2.05 (*s*, 3 H).
10	$CDCl_3$+ CD_3OD+ DABCO	7.90 (*d*, 8 Hz, 1 H); 7.25 (*m*, 12 H); 6.20 (*d*, 7.2 Hz, 1 H); 5.62 (*d*, 8 Hz, 1 H); 4.64 (*m*, 1 H); 4.15 (*m*, 1 H); 3.95 (*t*, 2 H); 3.76 (*m*, 4 H) 2.87 (*s*, 3 H); 1.86 (*m*, 4 H); 1.26 (*m*, 32 H); 0.88 (*t*, 3 H).
11	$CDCl_3$+ CD_3OD	8.38 (*d*, 8.4 Hz, 1 H); 7.94 (*m*, 2 H); 7.30 (*m*, 16 H); 6.24 (*d*, 6 Hz, 1 H); 4.56 (*m*, 1 H); 4.11 (*m*, 1 H); 3.97 (*m*, 2 H): 3.68 (*m*, 4 H); 3.12 (*s*, 3 H); 1.86 (*m*, 4 H); 1.80 to 0.8 (*m*, 35 H).
12	$CDCl_3$+ CD_3OD+ DABCO	8.71 (*s*, 1 H); 8.26 (*s*, 1 H); 8.10–7.9 (*m*, 2 H); 7.6–6.6 (*m*, 15 H); 6.19 (*d*, 7.2 Hz, 1 H); 5.16 (*m*, 1 H); 4.23 (*m*, 1 H); 3.88 (*t*, 2 H); 3.43 (*m*, 4 H); 2.77 (*s*, 3 H); 1.86 (*m*, 4 H); 1.80–0.8 (*m*, 35 H).
13	$CDCl_3$+ CD_3OD+ DABCO	7.94 (*s*, 1 H); 7.83 (*d*, 12 Hz, 2 H); 7.44 (*d*, 12 Hz, 2 H); 7.20 (*m*, 12 H); 5.96 (*d*, 7.2 Hz, 1 H) 5.03 (*m*, 1 H); 4.19 (*m*, 1 H); 2.87 (*s*, 3 H); 1.80 to 1.3 (*m*, 44 H).

14	$CDCl_3$+ Pyridine-d_5	8.55–6.61 (*m*, 17 H); 6.36 (*d*, 7 Hz, 1 H); 5.64 (*d*, 8 Hz, 1 H); 4.90 (*m*, 1 H); 4.55 (*m*, 1 H); 4.20–3.16 (*m*, 9 H); 3.05 (*s*, 3 H); 1.77 (*m*, 4 H); 1.5–0.8 (*m*, 50 H). ^{31}P NMR: −6.22
15	$CDCl_3$+ Pyridine-d_5	8.1–6.5 (*m*, 23 H); 6.39 (*d*, 7 Hz, 1 H); 4.75 (*m*, 2 H); 4.5–3.0 (*m*, 9 H) 2.93 (*s*, 3 H); 1.8–0.8 (*m*, 54 H). ^{31}P NMR: −6.42
16	$CDCl_3$+ Pyridine-d_5	8.75 (*s*, 1 H); 8.59 (*m*, 2 H); 8.33 (*s*, 1 H); 8.1 to 7.8 (*m*, 2 H) 7.8–6.7 (*m*, 17 H); 6.35 (*d*, 8 Hz, 1 H); 5.34 (*m*, 1 H); 4.9 (*m*, 1 H); 2.77 (*s*, 3 H); 1.9 to 0.8 (*m*, 54 H). ^{31}P NMR: −6.25
17	$CDCl_3$+ CD_3OD+ DABCO	8.53 (*s*, 1 H); 8.12–6.7 (*m*, 20 H); 6.06 (*d*, 6 Hz, 1 H); 5.20 (*m*, 2 H); 3.54 (*m*, 4 H) 2.79 (*s*, 3 H); 2.0–0.6 (*m*, 63 H). ^{31}P NMR: −6.04
18	$CDCl_3$+ CD_3OD+ Pyridine-d_5	8.66 (*d*, 5 HZ, 1 H); 8.12–6.97 (*m*, 21 H); 6.26 (*d*, 5 Hz, 1 H); 6.20 (*d*, 5 Hz, 1 H); 5.64 (*d*, 6 Hz, 1 H); 5.02–4.44 (*m*, 2 H); 4.5–3.36 (*m*, 11 H); 3.21 & 3.19 (two *s*, 3 H); 1.75 (*m*, 4 H); 1.27 (*m*, 32 H); 0.86 (*t*, 3 H). ^{31}P NMR: −7.86 and −8.1
19	$CDCl_3$	8.1–6.6 (*m*, 28 H); 6.39 (*d*, 7 Hz, 1 H); 4.85 (*m*, 2 H); 4.7–3.35 (*m*, 13 H); 3.11 (2 *s*, 3 H); 1.8–0.7 (*m*, 39 H). ^{31}P NMR: −7.88
20	$CDCl_3$	8.75 (*s*, 1 H); 8.27 (*s*, 1 H); 8.1 to 6.6 (*m*, 26 H); 6.19 (*d*, 8 Hz, 1 H); 5.46 (*m*, 1 H); 5.05 (*m*, 1 H); 2.64 (*s*, 3 H); 1.7–0.8 (*m*, 39 H). ^{31}P NMR: −7.82
21	$CDCl_3$	8.2–6.5 (*m*, 27 H); 6.21–5.94 (*dd* J=7.8 Hz and 8.4 Hz); 5.28 (*m*, 2 H); 4.85–2.9 (*m*, 13 H); 2.70 (two *s*, 3 H); 1.9–0.8 (*m*, 48 H). ^{31}P NMR: −7.81 and −8.48
22	$CDCl_3$+ CD_3OD	8.4–7.00 (*m*, 10 H); 5.87 (*d*, 1 H); 5.73 (*d*, 1 H); 5.01 (*m*, 1 H); 4.60 (*m*, 1 H); 4.30–3.30 (*m*, 11 H); 3.20 (*s*, 3 H); 1.78 (*t*, 4 H). ^{31}P NMR: −7.87
23	$CDCl_3$+ CD_3OD	8.3–6.9 (*m*, 16 H); 6.01 (*d*, 7 Hz, 1 H); 5.0 (*m*, 2 H); 4.7–3.1 (*m*, 11 H); 2.95 (2 *s*, 3 H); 1.78 (*m*, 4 H). ^{31}P NMR: −7.9
24	$CDCl_3$+ CD_3OD	8.83 (*s*, 1 H); 8.4–7.15 (*m*, 15 H); 6.08 (*d*, 8 Hz, 1 H); 5.51 (*m*, 1 H); 5.20 (*m*, 1 H); 4.92 (*m*, 11 H); 2.54 (2 *s*, 3 H); 1.74 (*m*, 4 H). ^{31}P NMR: −7.81
25	$CDCl_3$+ CD_3OD	8.2–7.1 (*m*, 14 H); 6.10–5.88 (*dd*, 8 Hz and 7 Hz); 5.35–5.14 (*m*, 2 H); 4.75 to 3.50 (*m*, 11 H); 2.66 (*d*, 3 H); 1.76 (*m*, 4 H); 1.31 (*s*, 9 H). ^{31}P NMR: −7.98



6, B=U; R=R^1=H
7, B=bzC; R=R^1=H
8, B=bzA; R=R^1=H
9, B=t-bbzG; R=R^1=H
10, B=U; R=C_{18}Px; R^1=H
11, B=bzC; R=C_{18}Px; R^1=H
12, B=bzA; R=C_{18}Px; R^1=H
13, B=t-bbzG; R=C_{18}PX; R^1=H
14, B=U; R=C_{18}Px; R^1=phosphodiester
15, B=bzC; R=C_{18}Px; R^1=phosphodiester
16, B=bzA; R=C_{18}Px; R^1=phosphodiester
17, B=t-bbzG; R=C_{18}Px; R^1=phosphodiester
18, B=U; R=C_{18}Px; R^1=phosphotriester
19, B=bzC; R=C_{18}Px; R^1=phosphotriester
20, B=bzA; R=C_{18}Px; R^1=phosphotriester
21, B=t-bbzG; R=C_{18}Px; R^1=phosphotriester
22, B=U; R=H; R^1=phosphotriester
23, B=bzC; R=H; R^1=phosphotriester
24, B=bzA; R=H; R^1=phosphotriester
25, B=t-bbzG; R=H; R^1=phosphotriester

the first time, in RNA chemistry. We have, therefore, converted the phosphodiesters, *(14* to *17)*, to their PSE-phosphotriesters *(18* to *21)*, respectively using an identical procedure that have been previously used in the DNA chemistry.[10] The PSE-phosphotriesters, *(18* to *21)*, have been thus obtained in 97, 95, 93 and 91 % yields respectively as powders. The ^{1}H and ^{31}P NMR data have clearly corroborated their structures (Table 1).

The phosphotriesters, *18* to *21*, are easily convertable to their parent phosphodiesters using triethylamine (20 equiv.) in dry pyridine (10 ml/mmol) at room temperature within 30 min yet they have been stable under the conditions of normal manipulations which are required in the RNA chemistry[6] using the phosphotriester approach.[1] The 5′-C_{18}Px groups from these fully protected mononucleotide blocks have then been removed, to prepare the corresponding 5′-hydroxyl blocks, with the help of a 2 % solution of 4-toluenesulfonic acid monohydrate (25 equiv.) in 30 % methanol-methylene chloride mixture at *ca.* 0 °C for 2½ min.[6] The 5′-hydroxyl blocks, *(22*

Table 2. Preparation and properties of fully protected oligoribonucleotides.

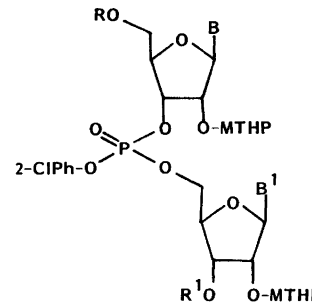
Experiment Number	5′protected component (mmol)	5′-hydroxy component (mmol)	Pyridine (ml)	MS–NT (mmol)	Reaction time	Fully protected RNA fragments	R_f[a] A	B	C	Yield %
1	*(15)* (0.73)	*(23)* (0.61)	7.0	2.4	45 min	(26)	0.49	0.18	0.27	95
2	*(16)* (0.482)	*(22)* (0.402)	5.0	2.0	45 min	(30)	0.48	0.10	0.3	69
3	*(16)* (0.482)	*(24)* (0.402)	5.0	3.0	45 min	(28)	0.51	0.09	0.26	76
4	*(15)* (0.459)	*(27)* (0.383)	5.0	1.15	45 min	(32)	0.45	0.07	0.21	65
5	*(31)* (0.355)	*(29)* (0.296)	10.0	2.1	30 min	(33)	0.47	0.12	0.33	70.5

[a] The TLC properties have been examined on the following supports: (A) Merck silica gel 60 F_{254} using 10 % MeOH–$CHCl_3$, v/v; (B) Merck RP-2 plates using 20 % water-acetone, v/v as the mobile phase; (C) Merck RP-8 plates using 15 % water-acetone, v/v as the mobile phase.

to *25)*, have been thus obtained, after usual purification steps, in 82, 79, 89 and 62 % yield, respectively as powders.

Subsequently, appropriate 5′-protected component and the 5′-hydroxy component have been coupled, in the usual manner,[6] to obtain the fully protected dimer blocks, *26*, *28* and *30* in 95, 76 and 69 % yields, respectively (Table 2), upon the purification of the crude reaction mixtures with the help of short column chromatography[8] using our modified reverse-phase silica gel as support (Experimental). It should be pointed out that, during such a separation, step gradients have been found to be most successful. Thus, all non-C_{18}Px-containing compounds have been eluted out with 70 % acetone–water mixture (100 ml, v/v); 5′-C_{18}-Px-phosphodiester component has been eluted out with 80 % acetone–water mixture (100 ml, v/v) and finally, the full protected phosphotriester component is eluted with 100 % acetone. It should be mentioned that no mixed fractions have been obtained using such a system and also have observed no loss of C_{18}Px-containing product, during chromatography, due to any irreversible adsorption on the new support.

The C_{18}Px-group has been subsequently removed from the fully protected dimers, *26* and *28* using the usual acid hydrolysis step,[6] as described in this paper, to obtain the 5′-hydroxy dimer blocks, (*27* and *29*), in 72 and 78 % yields respectively. The 2-phenylsulfonylethyl-, the 3′-terminal phosphodiester protecting group, have also been removed from *30* with the help of triethylamine (20 equiv.) in dry pyridine solution (20 ml/mmol) to obtain the 5′-protected phosphodiester component *31* quantitatively. Two further condensations (Table 2): (*15*+*27*) and (*29*+*31*), in dry pyridine solutions in presence of 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole (MS–NT),[11] have finally given the fully protected trimeric (*32*) and tetrameric (*33*) components, in 65 and 70.5 % yield, respectively, upon the purification of the reaction mixtures on our reverse-phase silica gel support as has been



26, B=B¹=bzC; R=C_{18}Px; R¹=phosphotriester
27, B=B¹=bzC; R=H; R¹=phosphotriester
28, B=B¹=bzA; R=C_{18}Px; R¹=phosphotriester
29, B=B¹=bzA; R=H; R¹=phosphotriester
30, B=bzA; B¹=U; R=C_{18}Px; R¹=phosphotriester
31, B=bzA; B¹=U; R=C_{18}Px; R¹=phosphodiester

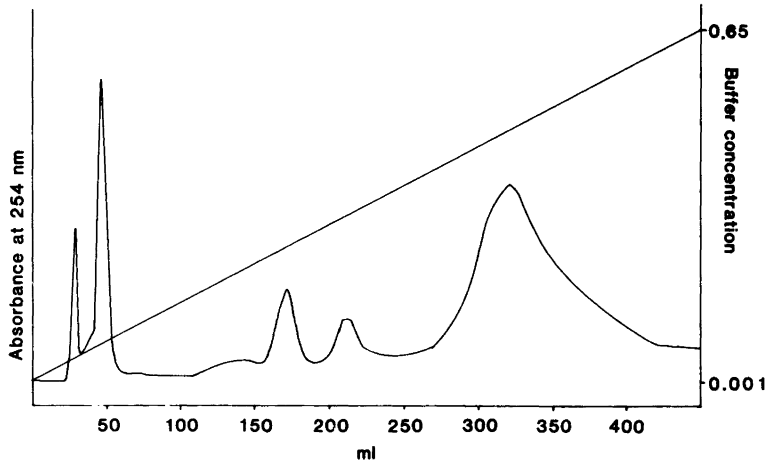


Fig. 8. Purification of CCC *34* using a DEAE Sephadex A 25 column using triethylammonium bicarbonate in the mobile phase.

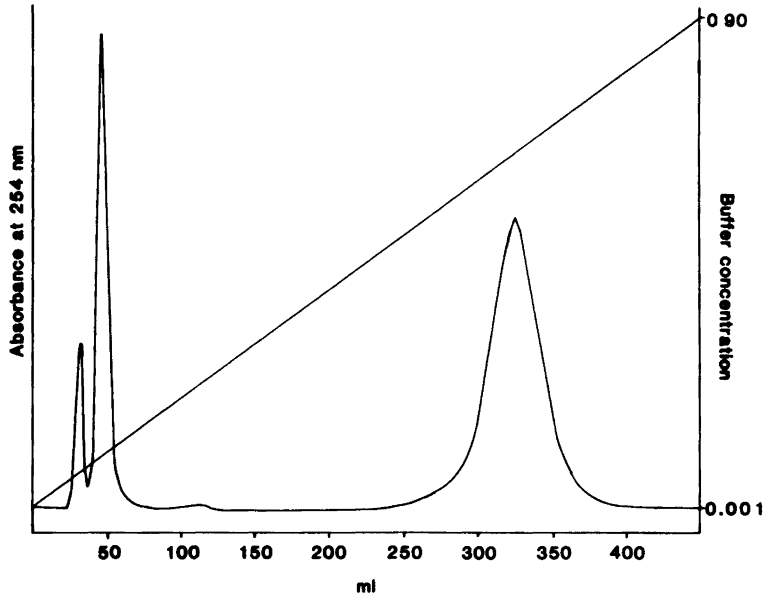
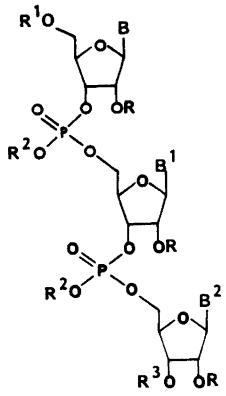
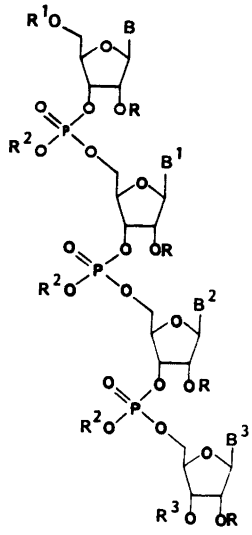


Fig. 9. Purification of AUAA *35* using a DEAE Sephadex A 25 column using a triethylammonium bicarbonate buffer in the mobile phase.



32, B=B^1=B^2=bzC
R^1=C_{18}Px; R=MTHP
R^2=2-ClPh;
R^3=phosphotriester



33, B=bzA; B^1=U; B^2=B^3=bzA
R^1=C_{18}Px; R=MTHP
R^2=2-ClPh
R^3=phosphotriester

34, B=B^1=Cytosin-1-yl-
R=R^1=R^2=H
R^3=phosphodiester

35, B=B^2=B^3=Adenin-9-yl-
B^1=Uracil-1-yl-
R=R^1=R^2=H
R^3=phosphodiester

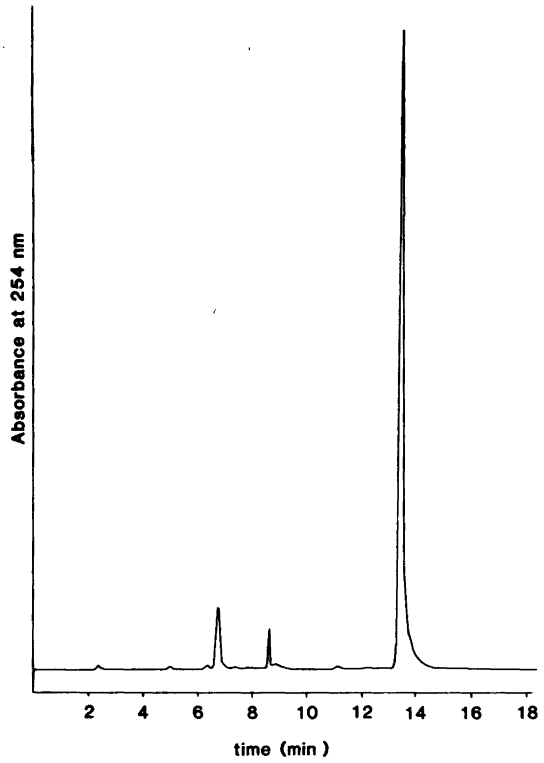


Fig. 10. HPLC purity of CCC *34*; R_t=13.4 min.

described in this paper. These fully protected oligoribonucleotides have been subsequently deprotected in the following order: (1) 4-nitrobenzaldoximate ions for 18 h at 20 °C; (2) aq. NH_3 (d 0.9) for 50 h at 20 °C; (3) evaporation of all volatile matters and then a treatment with 80 % acetic acid (6 h) at 20 °C; and (4) again evaporation of all volatile matters and coevaporation with toluene to remove last traces of acetic acid. The resultant reaction mixture has then been chromatographed using a DEAE-Sephadex A25 column using triethylammonium-bicarbonate (pH 7.3); linear gradient: 0.001 to 0.6 M (200 ml each). The main peaks in the elution profiles, as shown in Figs. 8 and 9, were collected and found to contain 80 and 95 % of total UV absorbing material (in terms of A_{260} o.d. units). Figs. 10 and 11 show the HPLC purity of these collected peaks. The structures of the trimer (*34*) and the tetramer (*44*) were then confirmed [6] by complete digestion to monomeric components by calf spleen phosphodiesterase and also by degradation with aq. NaOH followed by quantitation using HPLC.

EXPERIMENTAL

UV absorption spectra were measured with a cecil CE 545 double beam scanning spectrophotometer; ^{1}H NMR spectra were measured at 60 MHz with a Perkin-Elmer R 600 spectrometer and at 90 MHz with a Jeol FX 90Q spectrometer; trimethylsilane and phosphoric acid were used as internal standards. IR spectra were measured with a Perkin-Elmer 298 spectrometer. High performance liquid chromatography (HPLC) was performed either with the help of LDC equipment, Constametric III pumps, UV III monitor and a gradient master; or with Waters equipment, M6000A pumps, model 440 absorbance detector and model 660 solvent programmer.

Merck silica gel 60 F_{254} pre-coated plates or Merck or HPTLC-fertigplatten RP2 F_{254}s or Merck HPTLC-fertigplatten RP8 F_{254}s were used for monitoring reaction mixtures.

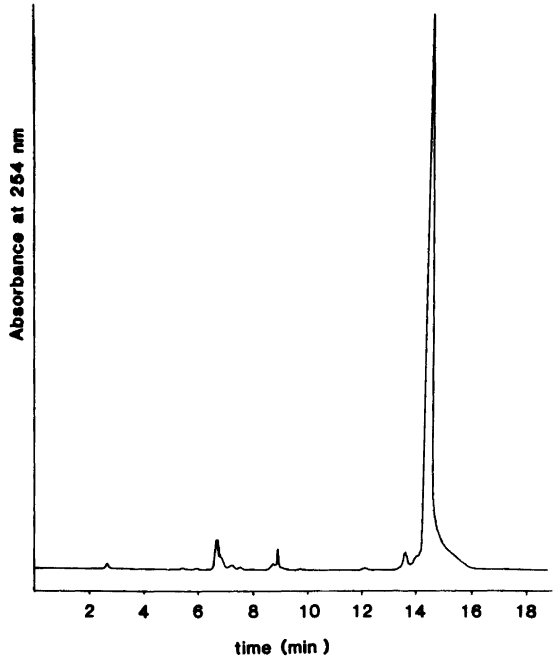


Fig. 11. HPLC purity of AUCC *35*; R_t=14.5 min.

Dioxan, pyridine and acetonitrile were dried by heating, under reflux, with CaH_2 for *ca.* 3 h; these solvents were then distilled at atmospheric pressure and stored over molecular sieves (4Å) in dark bottles. The key reagents: 1,1,3,3-tetraisopropyl-1,3-dichlorosiloxane (TIPDSiCl$_2$)[12], 5,6-dihydro-4-methoxy-2*H*-pyran,[13] 9-chloro-9-phenylxanthen (Px–Cl),[4] 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole (MS–NT)[11] and 2-chlorophenylphosphorobis(1,2,4-triazolide)[9] were prepared using literature procedures.

4-Octadecyloxybromobenzene. This has been prepared for the first time using a modified literature procedure.[15]

In a three-necked flask equipped with a condenser and a dropping funnel, absolute ethanol (200 ml) was placed; sodium (6.56 g, 0.285 g atom) was added to it in portions. After the reaction was complete a solution of 4-bromophenol (49.3 g, 0.285 mol) in absolute ethanol (50 ml) was added dropwise for a period of 15 min. To this reaction mixture, a solution of octadecylbromide (100 g, 0.30 mol) in absolute ethanol (100 ml) was added within a period of 30 min. The reaction mixture was then refluxed for 3 h and subsequently cooled. The volatile matters were then removed using a rotavapor. The residual liquid was dissolved in diethylether (300 ml) and was subsequently washed with water (3×100 ml), 2.5 M sodium hydroxide (2×100 ml) and water (4×100 ml). The ethereal layer was dried ($MgSO_4$), filtered, concentrated under a reduced pressure and distilled. b.p. 210–215 °C (0.05 mmHg); mp 53°C; Yield: 66.5 g (55 %).

9-Hydroxy-9-(4-octadecyloxyphenylxanthen). 4-Octadecyloxybromobenzene (65 g 0.155 mol), magnesium (4.52 g, 0.186 mol) were placed in a three-necked flask (250 ml); dry tetrahydrofuran was added and the mixture was refluxed for 16 h. Xanthone (22.77 g, 0.116 mol) was then added to the latter mixture in small portions and the suspension was refluxed for an additional 1.5 h. The reaction mixture was then concentrated under a reduced pressure and the residue was dissolved in concentrated hydrochloric acid (400 ml). The solution was filtered and the filtrate was poured into water (4 l) to obtain crystals which were filtered and washed with water (3×200 ml) and dried. Yield: 80 g; m.p. 74–75°C (ethanol). (Found: C, 81.6; H, 9.1; $C_{37}H_{50}O_3$ requires: C, 81.92; H, 9.23 %).

9-Chloro-9-(4-octadecyloxyphenylxanthen); (C_{18}Px–Cl). This has been prepared using a literature condition that was used for the preparation of 9-chloro-9-phenylxanthen.

2′-*O*-Methoxytetrahydropyranyl uridine (*6*),

2′-*O*-methoxytetrahydropyranyl-4-*N*-benzoylcytidine (*7*), 2′-*O*-methoxytetrahydropyranyl-6-*N*-benzoyladenosine (*8*) and 2′-*O*-methoxytetrahydropyranyl-*N*-2-(4-butylbenzoyl) guanosine (*9*) have been prepared using literature procedures.[6,14]

5′-O-9-(4-Octadecyloxyphenylxanthen)-2′, 3′-di-O-acetyl uridine (3). 2′,2′-di-*O*-acetyluridine (125 mg, 0.38 mmol) (*1*) was coevaporated with dry pyridine (2×20 ml). The residue was then redissolved in dry pyridine (5 ml) and C_{18}Px−Cl was added. The reaction was complete within 15 min at room temperature. The reaction mixture was poured into a separating funnel containing saturated sodium bicarbonate solution (50 ml). The aqueous phase was extracted with chloroform (3×30 ml) and the extracts were pooled. The volatile matters were removed under a reduced pressure. The residual gum was then chromatographed on a short Merck silica gel G column using an eluent mixture containing chloroform and methylene chloride (1:1 v/v). Appropriate fractions were collected and volatile matters were removed to obtain a gummy product which was precipitated from light petroleum (30−50°C). The precipitate was dried to obtain 281 mg (87 %) of the title product. ^{1}H NMR (Table 1) confirmed the structure.

9-Hydroxy-9-anisylxanthen. The title compound was prepared in 95 % yield following a literature procedure[4] that is reported for the preparation of the parent compound: 9-hydroxy-9-phenylxanthen. m.p. 146−147 °C (diethylether); (Found: C, 78.7; H, 5.1; $C_{20}H_{16}O_3$ requires: C, 78.95; H, 5.26). ^{1}H NMR (Table 1) further confirmed the structure.

9-Chloro-9-anisylxanthen. This was prepared using a procedure that was used for the preparation of 9-chloro-9-phenylxanthen.[4]

5′-O-9-(Anisylxanthen)-2′3′-di-O-acetyl uridine (4). The title compound has been prepared in 87 % yield using an identical condition that is reported for the preparation of *3*. ^{1}H NMR data (Table 1) confirmed the structure.

Procedure for the silanization of Merck Kieselgel G. Commercially available Merck Kieselgel G (100 g) was dried *in vacuo* (*ca.* 20 mmHg) at 200 °C for 24 h. The silica gel was cooled *in vacuo* and then it was suspended in a mixture of dry methylenechloride and pyridine (162 ml; 1:1 v/v; 20 mmol of pyridine/g of silica). To this magnetically stirred suspension trimethylsilylchloride (126 ml, 10 mmol/g of silica) was added and the mixture was stirred for 24 h at room temperature. The reaction mixture was filtered and washed on a filtration funnel with light petroleum (30−50°) (2×200 ml); acetone (2×200 ml) and finally with methanol (2×200 ml). The product was then dried in vacuo at 150° for 18 h.

General method of preparation of 5′-O-9-(4-octadecylphenylxanthen-2′-O-(4-methoxytetrahydropyranyl)-N-protected ribonucleosides: (10 to 13). Appropriately base protected 2′-*O*-methoxytetrahydropyranylated ribonucleosides blocks, (*6* to *9*), have been prepared using literature conditions.[6,14]

These 2′,5′-diprotected ribonucleoside blocks have been prepared from the 2′-*O*-(4-methoxytetrahydropyranyl)-ribonucleoside blocks using our literature condition[6] that we used for the preparation of the corresponding 5′-*O*-(9-phenylxanthen-9-yl) derivatives.

The chemical structure of these C_{18}Px-derivatives was confirmed by detailed ^{1}H NMR data (Table 1). These compounds failed to crystallize despite our repeated attempts. They (*10* to *13*) were thus precipitated from light petroleum 30−50°C and were obtained in 67, 65, 72 and 71 % yields, respectively as powders from their precursors: (*6* to *9*).

General method for the preparation of the phosphodiester salts (14 to 17). They have been prepared following the conditions that are reported in our published procedure.[6] ^{1}H and ^{31}P NMR data (Table 1) were used to substantiate their structures and purities.

General method of preparation of the fully protected phosphotriester blocks: (18 to 21). Phosphodiester salt (1 mmol) was co-evaporated twice with dry pyridine under a reduced pressure. The residue was then dissolved in dry pyridine (10 ml/mmol) and 2-phenylsulfonylethanol (2 mmol) was then added. To this stirred reaction mixture, 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole (MS−NT)[11] (5 mmol) was added. The reaction mixture was stirred for 1 h at room temperature. Saturated sodiumbicarbonate solution (0.5 ml) was added to destroy the excess of MS−NT. After 10 min, the reaction mixture was poured in a separating funnel containing saturated sodium bicarbonate solution (80 ml). The aqueous phase was extracted with chloroform (3×100 ml) and the extracts were pooled and concentrated to obtain a glass. This was purified by short column chromatography on silica gel using 2 % ethanol−chloroform mixture in the mobile phase or by employing the new reverse-phase support with the help of acetone−water mixture. Appropriate fractions were pooled and volatile matters were removed under a reduced pressure. The residue was isolated as powder after precipitation from light petroleum (30−50 °C). The compounds *18* to *21*, were thus obtained in 97, 95, 93 and 91 % yields, respectively. ^{1}H and ^{31}P NMR (Table 1) have subsequently

confirmed their structures.

General method of the selective removal of the C_{18}Px-group from the 5'-end of fully protected monomers, (18 to 21), and dimers (26 and 28). Fully protected building block was co-evaporated with toluene to remove the last traces of pyridine and then the residue was dissolved in a mixture of methylenechloride and methanol (7:3 v/v) (20 ml/mmol)[6] and cooled to 0 °C. Now, a cooled solution of 4-toluenesulfonic acid monohydrate (25 equiv.) in the latter solvent mixture was added to the substrate solution. The reaction mixture was stirred at 0 °C for 2.5 min and was quenched by pouring into the separating funnel containing saturated sodium bicarbonate solution (50 ml). The aqueous phase was extracted with chloroform (3×100 ml), in the usual way, and the extracts were pooled. The volatile matters were removed under a reduced pressure and the residue was then chromatographed on a short silica gel column. Appropriate fractions were collected and concentrated. The residues were then subsequently isolated as powders, in the usual way, after precipitation from light petroleum (30–50°C). Thus, the 5'-hydroxymononucleotide blocks, (*22*) to (*25*), were isolated in 82, 79, 89 and 62 % yields, respectively. They were subsequently characterized by ^{1}H NMR spectroscopy (Table 1).

Similarly, the 5'-hydroxy dinucleotide blocks, *27* and *29*, were isolated in 72.5 and 78 % yields as powders.

General procedure for the condensation reaction (Table 2). The general method for 1+1 and 2+2 condensations that are used in the present work is basically similar to the condition that we have reported in the literature.[6] Table 2 summerizes the stoichiometry of the condensation reactions which have been used to synthesize the fully protected target molecules (*32* and *33*), however, the general procedure for the separation of the fully protected oligoribonucleotide from the reaction mixture is as follows:

The crude reaction mixture was worked-up following the usual procedure[6] and was then dissolved in acetone (13 ml/mmol). To this solution, silanized silica gel was added in portions with stirring, which finally formed a dry powder. To this, pyridine (1 ml) and water (6 ml) were added. The resultant "slurry" was loaded on top of a silanized silica gel column (4 cm high; 1 cm ∅) that was made of a mobile phase containing a mixture of acetone–pyridine–water (65:5:30 v/v/v). The mobile phases, in a three-step gradient, that were used to separate 1 mmol of the reaction product from the reaction mixture were as follows: (1) acetone-water (7:3 v/v; 100 ml) which eluted out all non-C_{18}Px containing by-products; (2) acetone-water (8:2 v/v; 100 ml) eluted out *only* C_{18}Px containing phosphodiester; (3) finally 100 % acetone which eluted out C_{18}Px containing phosphotriesters. A typical elution pattern is shown in Fig. 7.

Unblocking of a fully protected CpCpCp 32 and ApUpApAp 33. This has been carried out essentially using the method that has already been described by us in the literature.[6] The deprotected materials, containing 3'-*O*-2-chlorophenylphosphate residues *34* and *35*, respectively were purified using DEAE Sephadex A_{25} columns using triethylammonium bicarbonate (pH 7.3) as the eluting buffer (linear gradient: 0.001 to 0.6 M (200 ml each). Figs. 8 and 9 are the elution profiles from the DEAE Sephadex column. Thus, the unprotected tri- and tetramer, (*34* and *35*), were isolated in 80 and 95 % yields, respectively. The purity of the isolated products were finally examined by HPLC (Spherisorb C_{18} reverse-phase column; mobile phase: linear gradient from 0.001 M tetrabutylammonium sodium sulfate (pH 6.9) to 0.001 M tetrabutylammonium sulfate containing 50 % acetonitrile; 20 min gradient; flow rate: 2 ml/min) as shown in Figs. 10 and 11.

Characterization of the deblocked trimer 34 and the tetramer 35.

(1) *Digestion with spleen phosphodiesterase.* This was carried out according to a published procedure.[6,14] *34* was thus completely digested to 3'-cytidylic acid and the tetramer *35* gave a mixture of 3'-adenylic acid and 3'-uridylic acid (observed ratio: 2.93: 1.11; calculated: 3:1).

(2) *Digestion with 0.1 M NaOH.* This was again performed using a literature procedure.[6,14] The trimer *34* gave a mixture of 2'- and 3'-cytidylic acids (R_T are respectively 22.96 and 23.63 min) in a ratio of 1:4 respectively; whereas the tetramer *35* gave a mixture of 2'- and 3'-adenylic (R_T=30.98 and 32.53 min respectively) and 2'- and 3'-uridylic acid (R_T=26.13 min) (observed ratio: 2.97: 1.04; calculated: 3:1). The above quantitation was carried out by HPLC (Nucleosil RP-18 number of plates: 14.000; 30 cm column; Flow rate: 1.5 ml/min; mobile phase: NaH_2PO_4 buffer (0.06 M, pH 7) containing tetrapentylammonium phosphate (6.25×10^{-4} M) (linear gradient: 0–20 % CH_3CN) in 30 min and a Hewlett-Packard integrator.

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