Synthesis of an mRNA Fragment of Alanyl-tRNA Synthetase Gene in *Escherichia coli* Using the 6-Methyl-3-pyridyl Group for Protection of the Imide Functions of Uridine and Guanosine

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The synthesis of 5′-GpGpUpGpU-3′ is reported to demonstrate the synthetic use of the 6-methyl-3-pyridyl group for the protection of the O-4 and O-6 imide functions of uridine and guanosine, respectively. The 2′- and 5′-hydroxyl functions of the key intermediates were protected with two acid-labile groups: 3-methoxy-1,5-dicarbomethoxypentane-3-yl (MDMP) and 9-(4-octadecyloxyphenyl)xanthen-9-yl (C_{18}Px), respectively. The internucleotide phosphotriesters were protected with 2-chlorophenyl and the 9-fluorenylmethyl group was employed for 3′-terminal phosphate protection.

It is clear from the literature that a considerable number of by-products are formed[1-11] if the imide functions of uridine and guanosine are not suitably protected during the synthesis of a tRNA or an mRNA fragment. We have recently shown[21] that the pyridyl groups at the O-6 position of guanine and the O-4 of uracil in compounds *1–10* can undergo displacement reactions by primary and secondary amines, thiolates and oximate ions. Such facile nucleophilic displacement reactions are useful for site-specific modifications during deprotection of a fully protected oligoribonucleotide. We herein report that these pyridyl groups at the O-4 of uracil and at the O-6 of an N-2 protected guanine residue in compounfs *1–10* do also fulfil all criteria of an imide protecting group, as demonstrated by the synthesis of a pentaribonucleotide fragment, 5′-GpGpUpGpU-3′, of alanyl-tRNA synthetase gene in *Escherichia coli*. The synthesis of such a complex target molecule, containing only guanine and uracil bases, was considered important for two reasons: first, it was the purpose of the present work to show that the use of the 6-methyl-3-pyridyl group could prevent the formation of by-products due to side reactions with these bases during phosphorylation reactions and, secondly, the results from such an exercise could be useful to design better synthetic and separation methodologies for making *larger* and *purer* oligoribonucleotides.

The pyridyl groups in compounds *1–10* were found to be stable under various acidic[14,15] and basic[16,17] conditions and also to treatment of fluoride ion.[18] We subsequently established that the pyridyl groups from the target RNA molecule are removable in the final deprotection step using 4-nitrobenzaldoxime[5] (10 eq.) and N^1,N^1,N^3,N^3-tetramethylguanidine (10 eq.) in dioxane/water: 1:1, v/v at 20 °C. This study showed that the removal of different pyridyl groups from *1, 3, 4* and *5* were virtually complete within 3 min while the complete removal of the 6-methyl-2-pyridyl group from *2* took 35 min ($t_{1/2}$ ca. 5 min). On the other hand, it turned out that the 3-pyridyl in *6* and 6-methyl-3-pyridyl in *7* had half-lives of 45 and 60 min, respectively, while the 2-thiopyridyl

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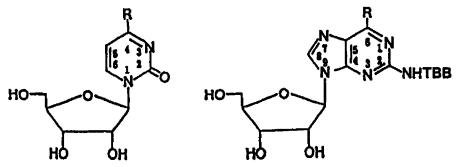
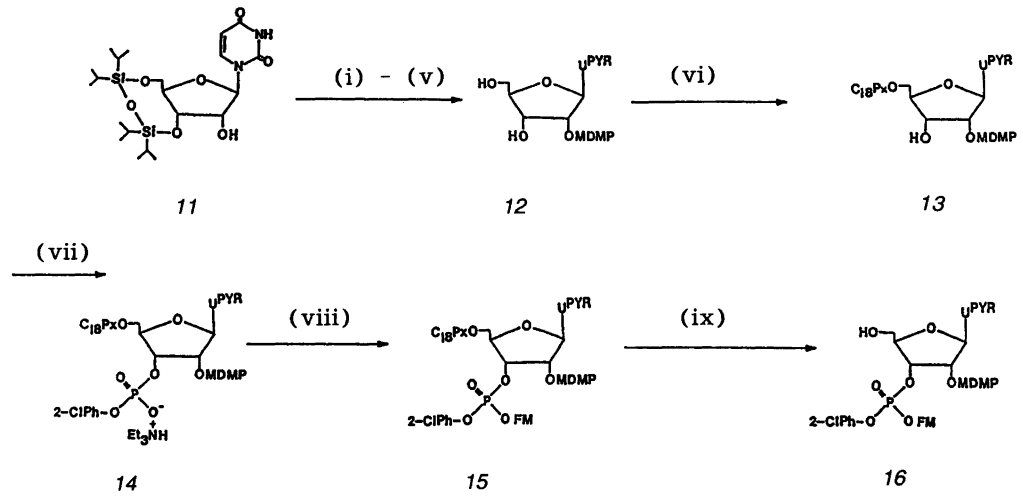


1; R = 2-pyridyloxy
2; R = 6-methyl-2-pyridyloxy
3; R = 3-pyridyloxy
4; R = 6-methyl-3-pyridyloxy
5; R = pyridylthio
6; R = 3-pyridyloxy
7; R = 6-methyl-3-pyridyloxy
8; R = 4-pyridyloxy
9; R = 4-pyridone-1-yl
10; R = 2-pyridylthio

TBB = 4-(*t*-butyl)benzoyl



11 *12* *13*

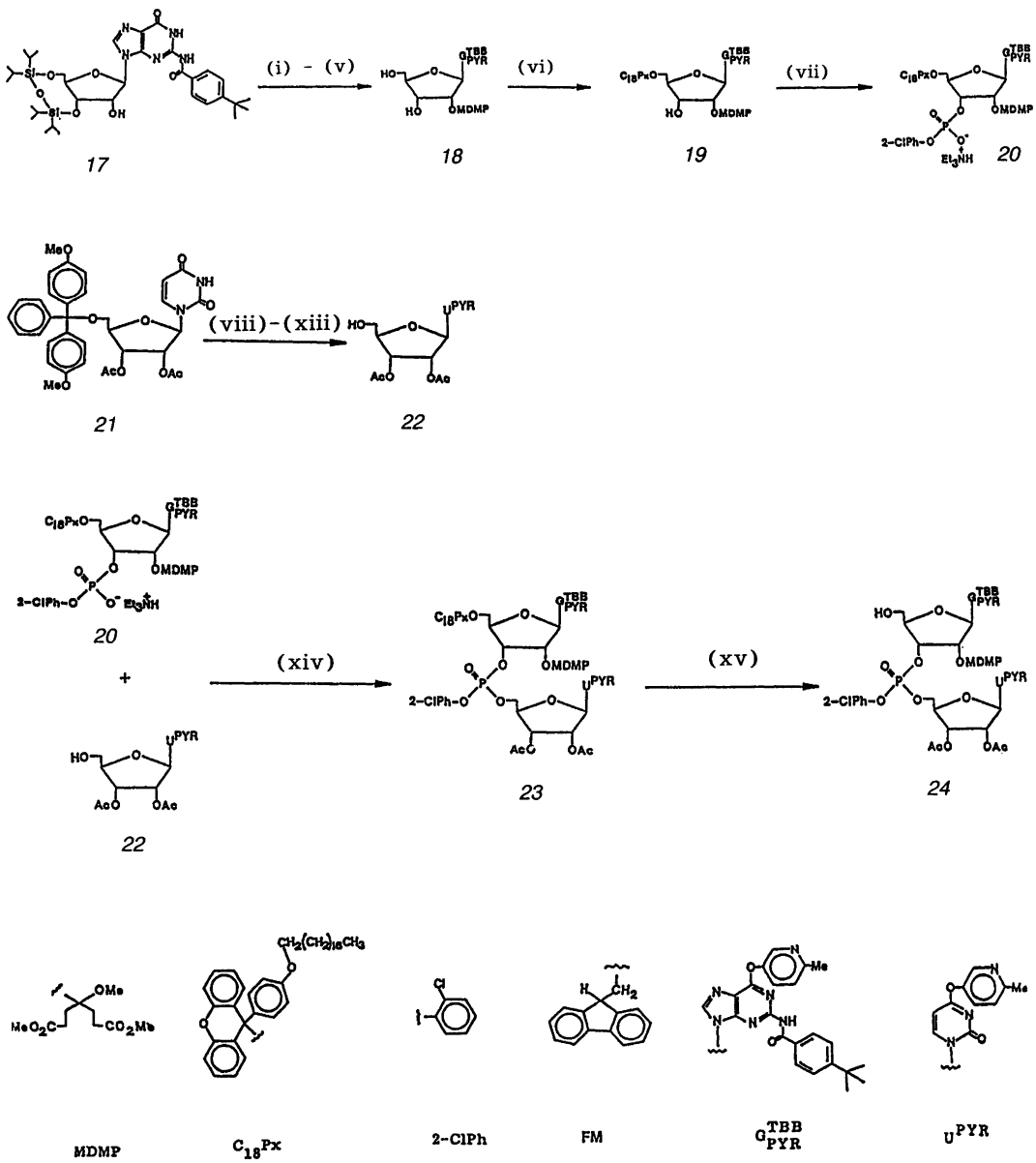
14 *15* *16*

Scheme 1. (i) 3-Methoxy-1,5-dicarbomethoxy-2-pentene (10 eq.), benzenesulfonic acid (0.1 eq.) in dioxane, 5 min. (ii) 2-Mesitylenesulfonyl chloride (3 eq.), triethylamine (5 eq.), DMAP (0.2 equiv) in dichloromethane, 1 h. (iii) Triethylamine (5 eq.) in dichloromethane, 10 min. (iv) 6-Methyl-3-hydroxypyridine (5 eq.) in dichloromethane, 1 h. (v) Tetrabutylammonium fluoride (2 eq.) in tetrahydrofuran, 2 min. (vi) 9-Chloro-9-(4-octadecyloxyphenyl)xanthene (1.5 eq.) in dry pyridine, 40 min. (vii) 2-Chlorophenylphosphoro-bis-1,2,4-triazolide (2 eq.) in dry pyridine, 20 min.(viii) 9-Fluorenylmethanol (1.2 eq.), 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole (5 eq.) in dry pyridine, 15 min. (ix) Trichloroacetic acid (4 eq.) in ethanol/chloroform (7:3 v/v), 5 min at 0 °C.

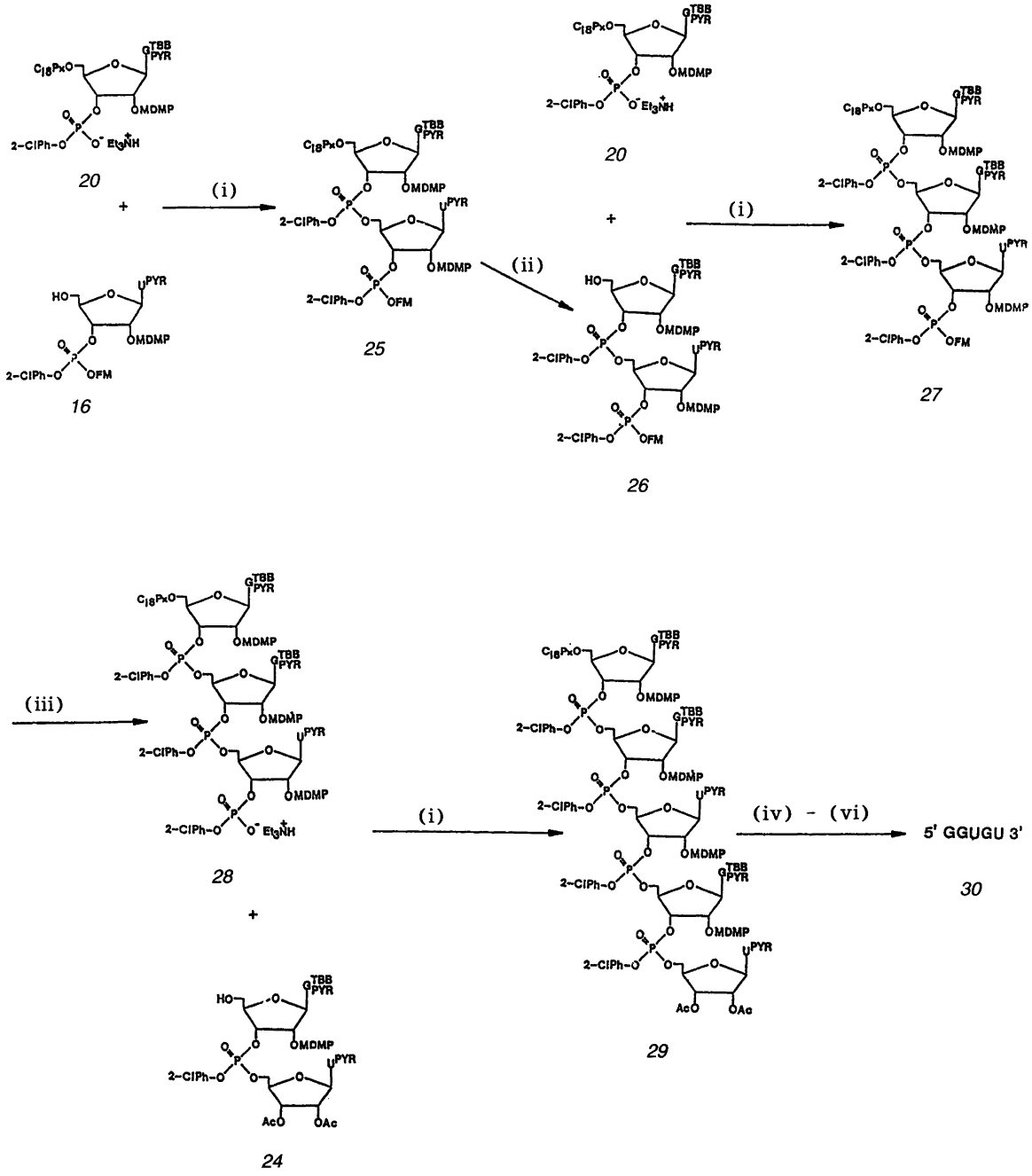
in *10* was too stable ($t_{1/2} \sim 6$ h) for any practical use in oligoribonucleotide synthesis.[14,15] Finally, the 6-methyl-3-pyridyl group was chosen for the O-4 and the O-6 protection of appropriately protected uridine and guanosine blocks, *16* (Scheme 1) and *20* (Scheme 2) respectively, to synthesize the pentaribonucleotide, 5′-GpGpUpGpU-3′ (*30*), in order to firmly establish its synthesis use in nucleic acid chemistry in the phosphotriester approach.[12,14]

Preparation of monomer, dimer, trimer and pentamer blocks

The key building blocks *15* and *20*, which were protected at the 2′ and at the 5′ ends by 3-methoxy-1,5-dicarbomethoxypentane-3-yl[15] (MDMP) and 9-(4-octadecycloxophenyl)-xanthen-9-yl (C_{18}Px)[19] were prepared (Schemes 1 and 2).[14,15] The 3′ terminal block *22* was prepared in 84 % overall yield.[14] The fully protected dinucleotides *23* and *25* were prepared in high yields by two condensation reactions with a slight excess of an appropriate phosphodiester block, *20*, with *22* and with *16*, respectively, in the presence of an large excess off 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole (MS-NT) in dry pyridine solution at 20 °C (Table 1 and Experimental).[14,15] Compounds *23* and *25* were selectively deprotected[14,15] at the 5′ end by the removal of the C_{18}Px group with trichloroacetic acid in acetonitrile solution at 4 °C to give *24* and *26* in 92 and 88 % yields, respectively. A slight excess of *20* was then condensed with *26* in an identical way as



Scheme 2. (i) 3-Methoxy-1,5-dicarbomethoxy-2-pentene (14 eq.), benzenesulfonic acid (0.3 eq.) in dioxane, 25 min. (ii) 2-Mesitylenesulfonyl chloride (3 eq.), triethylamine (3 eq.), DMAP (0.2 eq.) in dichloromethane, 2.5 h. (iii) Trimethylamine (10 eq.), 6-methyl-3-hydroxypyridine (5 eq.) in dichloromethane, 20 min. (iv) DABCO (1 eq.), 1 h. (v) Tetrabutylammonium fluoride (2 eq.) in tetrahydrofuran. (vi) 9-Chloro-9-(4-octadecyloxyphenyl)xanthene (2 eq.) in dry pyridine, 15 min. (vii) 2-Chlorophenylphosphoro-bis-1,2,4-triazolide (2 eq.) in dry pyridine, 20 min. (viii) Dimethoxytrityl chloride in dry pyridine. (ix) Acetic anhydride in dry pyridine. (x) 2-Mesitylenesulfonyl chloride (3 eq.), triethylamine (9 eq.), DMAP (0.2 eq.) in dichloromethane. (xi) 6-Methyl-3-hydroxypyridine (5 eq.), triethylamine (5 eq.). (xii) DABCO (0.2 eq.). (xiii) 4-Toluenesulfonic acid monohydrate (2 %) in 30 % methanolic chloroform. (xiv) 1-Mesitylenesulfonyl-3-nitro-1,2,4-triazole (MS-NT, 2.75 eq.) in dry pyridine, 30 min. (xv) Trichloroacetic acid (10 eq.) in ethanol/chloroform (7:3, v/v).



Scheme 3. (i) MS-NT in dry pyridine, 20 min. (ii) Trichloroacetic acid (10 eq.) in ethanol/chloroform (7:3 v/v). (iii) Triethylamine (20 eq.) in dry pyridine. (iv) 4-Nitrobenzaldoxime (90 eq.), N^1,N^1,N^3,N^3-tetramethylguanidine (80 eq.) in dioxane/water (5:1 v/v). (v) Concentrated aq. ammonia ($d = 0.9$). (vi) 80 % Aq. acetic acid for 6 h.

Table 1. Condensation reactions of phosphodiesters with alcohols in dry pyridine solution (10 ml/mmol) at 20 °C.

Phosphodiesters/mmol		Alcohols/mmol		MS-NT[b]/ mmol	Time/ min	Yield/ %	Product
14	0.4	FM-OH[a]	0.5	2.0	20	90	*15*
20	0.31	*16*	0.26	1.3	20	88	*25*
20	0.6	*22*	0.55	2.75	30	81	*23*
20	0.19	*26*	0.14	0.7	20	75	*27*
28	0.042	*24*	0.039	0.42	25	90	*29*

[a]9-Fluorenylmethanol. [b]1-Mesitylenesulfonyl-3-nitro-1,2,4-triazole.

for the preparation of *23* or *25* to give the fully protected trimer *27* (75 %). The 9-fluorenylmethyl (FM) group from the 3′-phosphotriester end of *27* was selectively removed with teiethylamine in dry pyridine solution,[14,15] to give *28* (81 %). Finally, a slight excess of *28* was coupled to *24*[14,17] to give *29* (90 %) (Table 1). The fully protected pentamer *29* was deprotected by employing the following series of reactions: (i) treatment with 4-nitrobenzxaldoximate ion[5,14,17] for 48 h at 20 °C, (ii) with aqueous ammonia for 72 h at 20 °C, (iii) with 80 % aqueous acetic acid[14,17] for 6 h at 20 °C (see Experimental). The deprotected pentamer *30* was then purified by a DEAE-Sephadex A25 column using a gradient of 7 M urea, 0.01 M tris-HCl (pH 7.0) to 7 M urea, 0.01 M tris-HCl and 0.5 M NaCl (pH 7.0). The purified pentamer *30* (80 %) was then characterized as the title compound by its digestion to the monomeric components by calf spleen and snake venom phosphodiesterases and 0.1 M sodium hydroxide, followed by their quantitation by HPLC[17] (Table 2).

Experimental

A Jeol FX90 Q spectrometer was used at 89.5 MHz to record the ^{1}H NMR spectra, in δ scale, TMS being used as internal standard. The ^{31}P NMR spectra were recorded at 36 MHz in the same solvent as for ^{1}H NMR using phosphoric acid as external standard (δ scale). UV spectra were measured using a Cary/Varian 2200 spectrometer. TLC was carried out using precoated silica gel F_{254} plates in the following solvent systems: (A) ethanol/chloroform 9:1, v/v; (B) ethanol/chloroform 8.5:1.5, v/v; (C) ethanol/chloroform 7:3, v/v; (D) ethanol/chloroform 8:2, v/v. The short column chromatographic separations

Table 2. Digestion of 5′-GpGpUpGpU-3′ (*30*) to monomeric components and their quantitations by HPLC.

Mode of digestion	Fragments obtained after digestion	Ratios	
		Calculated	Observed
Snake venom[a] Phosphodiesterase:	Guanosine	1.0	1.0
	Uridine	0.67	0.65
Calf spleen phosphodiesterase:	3′-Guanylic acid	1.0	1.0
	3′-Uridylic acid	0.33	0.31
	Uridine	0.33	0.34
0.1 *M* aq. NaOH	2′(3′)-Guanylic acid	1.0	1.0
	2′(3′)-Uridylic acid	0.33	0.31
	Uridine	0.33	0.25

[a]Contains a substantial amount of phosphomonoesterase.

were carried out using Merck G60 silica gel in the usual way.[22]

2'-O-(3-Methoxy-1,5-dicarbomethoxypentane-3-yl-4-O-(6-methylpyridine-3-yl)uridine (12). Compound *11* (1.0 g, 2.0 mmol) was coevaporated with toluene, then dissolved in dry dioxane (1.2 ml) and 3-methoxy-1,5-dicarbomethoxy-2-pentene (MDMO-enol ether) (4.3 g, 20 mmol) added followed by anhydrous benzenesulfonic acid (0.1 g, 0.2 mmol) in a small volume of dioxane/toluene (5 ml, 1:1, v/v). After 5 min, dry pyridine was added and the mixture stirred for 10 min before it was worked up by pouring into concentrated sodium hydrogen carbonate solution (100 ml) and extracting with dichloromethane (2×50 ml). The organic layers were combined and evaporated to dryness. The crude product was used for the next step without purification. The residue thus obtained was then co-evaporated with toluene dissolved in dichloromethane. Triethylamine (2.0 g, 20 mmol) was added followed by 2-mesitylenesulfonyl chloride (0.84 g, 6.0 mmol) and 4-dimethylaminopyridine (0.06 g, 0.4 mmol). The reaction was allowed to proceed for 90 min then 6-methyl-3-hydroxypyridine (1.2 g, 10 mmol) and Dabco (40 mg, 0.04 mmol) added. The reaction mixture was stirred for 30 min then worked up.[22] Finally, the trimethylsilyl group was removed by a standard treatment.[22] Yield: 0.82 g (76%). ^{1}H NMR ($CDCl_3$): 8.32 (*d*, 2.2 Hz 1H) H-2 of pyridyl; 8.29 (*d*, 7.3 Hz, 1H) H-6; 7.49 (*dd*, 2.8 and 8.3 Hz, 1H) H-4 of pyridyl; 7.18 (*d*, 8.5 Hz, 1H) H-5 of pyridyl; 6.19 (*d*, 7.3 Hz, 1H) H-5; 5.75 (*d*, 5.1 Hz, 1H) H-1'; 4.73 (*t*, 4.7 Hz, 1H) H-2'; 4.29 (*m*, 1H) H-3'; 4.15 (*m*, 1H) H-4'; 3.87 (*m*, 2H) H-5'; 3.67 (2×*s*, 6H) CH_3 esters of MDMP; 3.16 (*s*, 3H) methoxyl of MDMP; 2.55 (*s*, 3H) CH_3 of pyridyl; 2.31 (*m*, 4H) and 1.99 (*m*, 4H) CH_2 of MDMP.

5'-(9-(4-Octadecyloxyphenyl)xanthene-9-yl)-2'-O-(3-methoxy-1,5-dicarbomethoxypentane-3-yl)-4-O-(6-methylpyridine-3-yl)uridine (13). Compound *12* (0.45 g, 0.82 mmol) was dissolved in dry pyridine, evaporated to dryness then redissolved in 8 ml of pyridine. 9-Chloro-9-(4-octadecyloxyphenyl)xanthene (3.95 ml, 0.25 M solution in dichloromethane) was added, and the reaction mixture stirred for 1 h. The reaction was then found to be complete by TLC (A). The mixture was worked up and purified.[22] Yield: 0.75 g (83%). ^{1}H NMR ($CDCl_3$): 8.44 (*d*, 7.6 Hz, 1H) H-6; 8.32 (*d*, 2.2 Hz, 1H) H-2 of pyridyl; 7.53–6.77 (*m*, 14H) H-4 and -5 of pyridyl and aromatic protons of pixyl; 6.14 (*d*, 4.4 Hz, 1H) H-1'; 5.95 (*d*, 7.3 Hz, 1H) H-5; 4.60 (*m*, 1H) H-2'; 4.30 (*m*, 1H) H-3'; 4.14 (*m*, 3H) H-4' and CH_2 of octadecyl; 3.65 and 3.61 (2×*s*, 6H) CH_3 esters of MDMP; 3.16 (*m*, 2H) H-5'; 3.11 (*s*, 3H) CH_3 of MDMP; 2.57 (*s*, 3H) CH_3 of pyridyl; 2.30 (*m*, 4H) and 1.95 (*m*, 4H) CH_2 of MDMP; 1.75 (*m*, 2H) 1.25 (*m*, 30H) and 0.89 (*m*, 3H) octadecyl group.

4-O-(6-Methylpyridine-3-yl)-2'-O-(MDMP)-5'-O-(9-(4-octadecyloxyphenyl) xanthene-9-yl) uridine-3'-O-(2-chlorophenyl)phosphate triethylammonium salt (14). Compound *13* (0.45 g, 0.41 mmol) was coevaporated with dry pyridine then dissolved in 5 ml of pyridine. To this solution, was added 2-chlorophenylphosphoro-bis-1,2,4-triazole (3.3 ml, 0.25 m). After 30 min, TLC (A) showed consumption of all of the higher R_f material and formation of a product on the baseline. The mixture was worked up.[22] The product was precipitated from hexane at −78°C, dried *in vacuo* and found to be homogeneous on TLC (C). The compound was stored at −20°C in the absence of any acid. ^{1}H NMR ($CDCl_3$): 8.32 (*m*, 2H) H-3 of pyridyl, H-6; 7.68–6.76 (*m*, 18H) H-4 and -5 of pyridyl, 2-chlorophenyl and pixyl aromatic protons; 6.43 (*d*, 7.3 Hz, 1H) H-1'; 5.99 (*d*, 7.3 Hz, 1H) H-5; 4.90 (*m*, 1H) H-3'; 4.84 (*m*, 1H) H-2'; 4.56 (*m*, 1H) H-4'; 3.91 (*m*, 2H) CH_2 of octadecyl; 3.61 and 3.56 (2×*s*, 6H) CH_3 ester of MDMP; 3.23 (*s*, 3H) CH_3 of MDMP; 3.13 (*m*, 2H) H-5'; 3.0 (*q*, 6H) CH_2 of triethylammonium; 2.54 (*s*, 3H) CH_3 of pyridyl; 2.34 (*m*, 4H) and 2.13 (*m*, 4H) CH_2 of MDMP; 1.72 (*m*, 2H), 1.25 (*m*, 39H) and 0.87 (*m*, 3H) octadecyl group and CH_3 of triethylammonium ion. ^{31}P NMR ($CDCl_3$): −6.3.

Compound 15. Compound *14* and 9-fluorenylmethanol were condensed using 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole[14,15] for condensation reactions (Table 1). ^{1}H NMR ($CDCl_3$): 8.34 (*m*, 2H) H-6 and H-2 of pyridyl; 7.67–6.76 (*m*, 23H) aromatic protons of fluorenylmethyl, pixyl, 2-chlorophenyl and 6-methyl pyridyl; 6.44 (*m*, 1H) H-1'; 6.03 (*d*, 7.1 Hz, 1H) H-5; 4.87 (*m*, 2H) H-3' and 2'; 4.54 (*m*, 2H) H-4' and H-9 of fluorenyl; 4.23 (*m*, 2H) CH_2 of fluorenyl; 3.91 (*m*,

2H) CH_2 of C_{18}Pixyl; 3.60, 3.56, 3.53 and 3.47 (4×*s*, 6H) CH_3 ester of MDMP; 3.23 and 3.18 (2×*s*, 3H) CH_3 of MDMP; 3.16 (*m*, 2H) H-5′; 2.54 (*s*, 3H) CH_3 of pyridyl; 1.73, 1.25, 0.86 (3×*m*, 35H) octadecyl group. ^{31}P NMR ($CDCl_3$): −7.4.

4-O-(6-Methylpyridine-3-yl)-1-(2′3′-di-O-acetyl-β-D-ribofuranosyl)uracil (22). Compound *21* (2.2 g, 3.4 mmol) was dissolved in dichloromethane (50 ml) containing triethylamine (3.5 g, 34 mmol). 1-mesitylenesulfonyl chloride (1.5 g, 11.2 mmol) was added followed by 4-*N*,*N*-dimethylaminopyridine, and the mixture stirred for 1 h. When all starting material was consumed [TLC in (A)], 6-methyl-3-hydroxypyridine was added, followed by a further 5 eq. of triethylamine and Dabco (0.3 g). The mixture was stirred for a further 1 h then worked up.[20] The combined organic layers were evaporated to dryness, dissolved in a 2% 4-toluenesulfonic acid monohydrate solution in 30% methanolic chloroform, stirred for 5 min at 20 °C, worked up and purified.[22] Yield: 1.47 g (84% based on *21*). ^{1}H NMR: 8.32 (*d*, 3.2 Hz, 1H) H-2 of pyridyl; 8.26 (*d*, 7.3 Hz, 1H) H-6; 7.49 (*dd*, 2.5 and 8.4 Hz, 1H) H-4 of pyridyl; 7.19 (*d*, 8.3 Hz, 1H) H-5 of pyridyl; 6.19 (*d*, 7.3 Hz, 1H) H-5; 6.14 (*d*, 4.7 Hz, 1H) H-1′; 5.48 (*m*, 2H) H-2′ and -3′; 4.23 (*m*, 1H) H-4′; 3.88 (*m*, 2H) H-5′; 2.56 (*s*, 3H) CH_3 of pyridyl; 2.11, 2.08 (2×*s*, 6H) acetyl groups.

*6-O-(6-Methylpyridine-3-yl)-2-*N*-t-butylbenzoyl-2′-O-(3-methoxy-1,5 dicarbomethoxy-3-yl)guanosine* (18). Compound *17* (2.15 g, 3 mmol) was dissolved in dioxane (3 ml) and 3-methoxy-1,5-dicarbomethoxy-2-pentene (9 ml, 42 mmol) added, followed by benzenesulfonic acid (142 mg, 0.9 mmol). The reaction was allowed to proceed for 25 min, pyridine (1 ml) was then added, stirred for 5 min and the reaction mixture subsequently worked up.[22] The residue obtained was used directly, treated sequentially with 2-mesitylenesulfonyl chloride, triethylamine, 6-methyl-3-hydroxypyridine and finally tetrabutylammonium fluoride.[22] The product isolated after chromatography on silica gel was precipitated from petroleum ether and characterised. Yield: 1.61 g (70.2%). ^{1}H NMR ($CDCl_3$): 8.58–7.18 (*m*, 8H) arom.; 5.93 (*d*, 6.1 Hz, 1H) H-1′; 5.20 (*dd*, 5.9 Hz, 1H) H-2′; 4.55 (*dd*, 3.3 Hz, 1H) H-3′; 4.29 (*dd*, 3.4 Hz, 1H) H-4′; 3.93 (*m*, 2H) H-5′; 3.66, 3.58 (2×*s*, 6H) MDMP; 2.93 (*s*, 3H) MDMP; 2.58 (*s*, 3H) pyr.; 2.25–1.70 (*m*, 8H) MDMP; 1.31 (*s*, 9H) tBB.

*5′-O-C_{18}Px-2′-O-MDMP-6-O-(6-methyl-3-pyridyl)-2-*N*-TBB guanosine* (19). Compound *18* (1.16 g, 1.52 mmol) was treated with 0.25 M solution of 9-chloro-9-(4-octadecyloxyphenyl)xanthene (12 ml, 3.0 mmol) using the same conditions for the preparation of *13*. Yield: 1.51 g (73%). ^{1}H NMR ($CDCl_3$ + pyridine-d_5): 8.61–6.74 (*m*, 20H) arom.; 6.13 (*d*, 5.3 Hz, 1H) H-1′; 5.10 (*t*, 2.7 Hz, 1H) H-2′; 4.38 (*t*, 4.1 Hz, 1H) H-3′; 4.19 (*m*, 1H) H-4′; 3.86 (*m*, 2H) CH_2 of octadecyl; 3.60, 3.58 (2×*s*, 6H) MDMP; 3.3 (*m*, 2H) H-5′; 2.99 (*s*, 3H) MDMP; 2.60 (*s*, 3H) CH_3 of pyr.; 2.39–1.72 (*m*, 8H) MDMP; 1.34 (*s*, 9H) tBB; 1.25–0.85 (*m*, 37H) octadecyl.

*5′-O-C_{18}Px-2′-O-MDMP-6-O-(6-methyl-3-pyridyl)-2-*N*-TBB-3′-(2-chlorphenyl)phosphate* (20). Compound *19* (803 mg, 0.6 mmol) was treated with an excess of 2-chlorophenylphosphoro-bis-1,2,4-triazolide (1.2 mmol) using the same conditions as for the preparation of *14*. The product (93%) was precipitated from hexane at −78 °C and used in the next step directly.^{1}H NMR: ($CDCl_3$ + pyridine-d_5): 8.83–6.70 (*m*, 24H) arom.; 6.32 (*d*, 7.1 Hz, 1H) H-1′; 5.31 (*m*, 1H) H-2′; 4.84 (*m*, 1H) H-3′; 4.61 (*m*, 1H) H-4′; 3.84 (*m*, 2H) C_{18}px; 3.53, 3.45 (2×*s*, 6H) MDMP; 3.26 (*m*, 2H) H5′; 2.94 (*s*, 3H) MDMP; 2.59 (*s*, 3H) Pyr.; 2.30–1.65 (*m*, *8H) MDMP; 1.33 (s*, 9H) tBB; 1.25–0.85 (*m*, 37H) C_{18}px. ^{31}P NMR: −6.0.

Compound 23. The alcohol (*22*) and the phosphodiester (*20*) were reacted together using the general condensation procedure (Table 1). ^{1}H NMR ($CDCl_3$): 3.55 and 3.46 (2×*s*, 6H) CH_3 esters of MDMP; 3.18 and 3.00 (2×*s*, 3H) methoxyl of MDMP; 2.62 (*s*, 3H) CH_3 of pyridyl on guanosine; 2.54 (*s*, 3H) CH_3 of pyridyl on uridine; 2.06 (*s*, 6H) 2 acetate groups; 1.30 (*s*, 9H) t-butyl; 1.75, 1.25 and 0.88 (35H) octadecyl. ^{31}P NMR: −7.4 and −7.7.

Compound 24. Compound *23* was treated with trichloroacetic acid (10 eq.).[15] ^{1}H NMR ($CDCl_3$): 3.57 (*m*, 6H) CH_3 esters of MDMP; 2.93 and 2.79 (2×*s*, 3H) methoxyl of MDMP; 2.93 (*s*, 3H) CH_3 of pyridyl of guanosine; 2.54 (*s*, 3H) CH_3 of pyri-

dyl of uridine; 2.16, 2.14, 2.11 and 2.09 (4×*s*, 6H) 2 acetate groups; 2.24 and 1.90 (*m*, 8H) CH_2 of MDMP; 1.32 (*s*, 9H) t-butyl group.^{31}P NMR ($CDCl_3$): −8.2, −6.5.

Compound 16. Compound *15* was subjected to trichloroacetic acid treatment (6 eq.) to give *16* in 96 % yield. ^{1}H NMR ($CDCl_3$): 8.33 (*m*, 1H) H-2 of pyridyl; 8.07 (*d*, 7.3 Hz, 1H) H-6; 7.79–7.11 (*m*, 1H) arom.; 6.20 (*d*, 7.3 Hz, 1H) H-5; 5.75 (*d*, 3.2 Hz, 1H) H-1′; 4.96 (*m*, 2H) H-2′ and -3′; 4.61 (*m*, 2H) H-4′ and 9 of fluorenyl; 4.16 (*m*, 4H) H-5′ and CH_2 of fluorenyl; 3.58, 3.56, 3.55 and 3.53 (4×*s*, 6H) CH_3 esters of MDMP; 3.03 and 3.01 (2×*s*, 3H) methoxy of MDMP; 2.54 (*s*, 3H) CH_3 of pyridyl; 2.28 (*m*, 4H) and 1.84 (*m*, 4H) CH_2 of MDMP. ^{31}P NMR ($CDCl_3$): −6.6 and −6.8.

Compound 25. The general conditions for condensation reactions were used to couple *20* with *16* (Table 1). ^{1}H NMR ($CDCl_3$): 3.52–3.44 (*m*, 12H) CH_3 esters of MDMP; 2.99 and 2.83 (2×*s*, 6H) methoxyl of MDMP; 2.61 (*s*, 3H) CH_3 of pyridyl of guanosine; 2.53 (*s*, 3H) CH_3 of pyridyl of uridine; 1.31 (*s*, 9H) t-butyl group; 1.78, 1.25 and 0.87 (35H) octadecyl group. ^{31}P NMR ($CDCl_3$): −7.0, −7.2, −7.3, −7.6, −7.7.

Compound 26. The standard depixylation treatment to *25* using trichloacetic acid (10 eq.) gave *26* in 88 % yield. ^{31}P NMR ($CDCl_3$): −6.2, −6.7, −6.8, −7.0, −8.0, −8.2.

Compound 27. The phosphodiester *20* and the dimer *26* were condensed using the general procedure (Table 1). ^{31}P NMR ($CDCl_3$): −6.5, −6.55, −6.6, −6.65, −6.8, −6.85, −7.0, −7.45, −7.5, −7.6, −7.7, −7.8, −7.9, −8.0.

Compound 28. To a solution of *27* (0.27 g, 0.08 mmol) in dry pyridine, was added triethylamine (0.22 ml, 1.6 mmol) and the solution stirred. After 2.5 h, the reaction was complete; the solvent and volatile matter were removed *in vacuo*, the residue coevaporated with toluene then precipitated from hexane at −78 °C. This powder was used without further purification. Yield: 0.19 g (81 %). ^{31}P NMR ($CDCl_3$): −5.3, −6.5, −8.0.

Compound 29. Compounds *24* and *28* were coupled using the general condensation procedure (Table 1).

Deprotection of fully protected pentamer 5′-GpGpUpGpU-3′. Compound *29* (20 mg) was dissolved in dioxane (1 ml), 4-nitrobenzaldoxime (65.7 mg, 90 equiv.) added and the mixture stirred. Tetramethylguanidine (47.1 µl, 85 equiv.) dissolved in water (0.5 ml) was added slowly. The mixture was stirred for 48 h, aqueous ammonia ($d = 0.9$, 2 ml) was added and stirred for a further 72 h, then all solvents were removed. The residue was co-evaporated with water (5×1 ml) and dissolved in 80 % acetic acid (2 ml). After 6 h, the acetic acid was evaporated in vacuo and the residue co-evaporated three times with water, then dissolved in water (20 ml) and extracted (5×20 ml) with ethyl acetate. The aqueous layer was concentrated, and the deprotected pentamer (*30*) purified in the following way: a column (1×25 cm) was charged with Sephadex A25 prepared in 7.0 M urea, 0.01 M Tris HCl (pH 7.0). The crude deprotected pentamer was dissolved in the same buffer and loaded onto the column. The chromatography was carried out using the same buffer and a gradient of sodium chloride (0–0.5 M). Elution of the products was followed by observing the absorption at 260 nm. Fractions of approximately 3 ml were collected. The appropriate fractions (43–65) were pooled and diluted to 4× the total volume with the 7.0 M urea buffer. A second column (1×1 cm) of Sephadex A25 was prepared and the solutions containing the pentamer passed through it. During this process, the absorbance was continuously monitored; no UV-absorbing material was observed to be eluted. After all of the solution had been passed through the column, the pentamer was eluted using 1.0 M sodium chloride in the buffer. The urea was then removed by passing the solution through a column (2×25 cm) of Sephadex G10. Appropriate fractions were pooled (80.9 %; o.d. at 260 nm), water removed *in vacuo*, and lyophilised to yield a white powder. This was finally digested by snake venom and spleen phosphoriesterase and 0.1 M NaOH according to the literature procedure.[14,15] The monomeric blocks thus generated were quantitated by HPLC[20] (Table 1).

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