Pyridyl Groups for Protection of the Imide Functions of Uridine and Guanosine. Exploration of Their Displacement Reactions for Site-specific Modifications of Uracil and Guanine Bases

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For the protection of the O-4 function of uridine and the O-6 of guanosine, 2-, 3- and 4-hydroxopyridines, 2-pyridinethiol, 6-methyl-2-hydroxy- and 6-methyl-3-hydroxypyridines have been employed. These substituted pyridines gave pyridyl-*N*- and/or pyridyl-*O*-substituted derivatives, depending *both* upon the position of the hydroxyl and methyl groups in the pyridine ring, at the C-4 and the C-6 of the uracil and guanine residues, respectively. These groups were found to be good leaving groups for nucleophilic substitution reactions by amines, thiolates and oximate. If needed, the rate of these substitution reactions could be conveniently increased by almost 1000-fold by conversion of the pyridyl moiety to its methiodide.

The protection of the O-4/N-3 urethane function of uridine and O-6/N-1 lactam function of guanosine is necessaary in the synthesis of nucleic acids in order to avoid the formation of by-products during phosphorylation reactions.[1-11] Several protecting groups have been proposed.[8-23] These groups protect the desired functions and are simply removed at the end of the synthesis; however, they cannot be used as leaving groups for site-specific modifications. The only exceptions are triazolyl and tetrazolyl groups[24-27] which have been employed for site-specific modifications at C-4 and C-6 of uracil (thymine) and guanine residues, respectively.

We herein report on substituted pyridyl groups for the protection of the O-4 of uracil and the O-6 of N-2 protected guanine residues of ribonucleosides which are stable and can be stored indefinitely at room temperature. These pyridyl groups are found to be good leaving groups for nucleophilic substitution reactions by amines, thiolates and oximate; the rate of these substitution reactions could be increased by almost 1000-fold by conversion of the pyridyl moiety to its methiodide.

As suitable candidates[28] for our studies, 2-pyridone (pK_a ca. 11 and 5.23), 6-methyl-2-pyridone, 4-pyridone (pK_a 11.09 and 3.07), 2-pyridinethiol (pK_a 9.97 and 5.27), 3-hydroxypyridine (pK_a 8.72 and 4.86) and 6-methyl-3-hydroxypyridine were considered. It was envisaged that the actual distribution of products after a nucleophilic substitution reaction, due to the attack by pyridone oxygen versus pyridone nitrogen, would be controlled by the delicate shift of the tautomeric equilibrium (oxo $\rightleftharpoons$ enol) of a particular substituted or unsubstituted 2- or 4-hydroxy- or 2- or 4-mercaptopyridine under the reaction condition;[29-30] while the 3-hydroxypyridine and its derivatives[31,32] should react entirely through their conjugate bases which may be considered as the equivalent of phenolate ion, particularly in a basic reaction medium.

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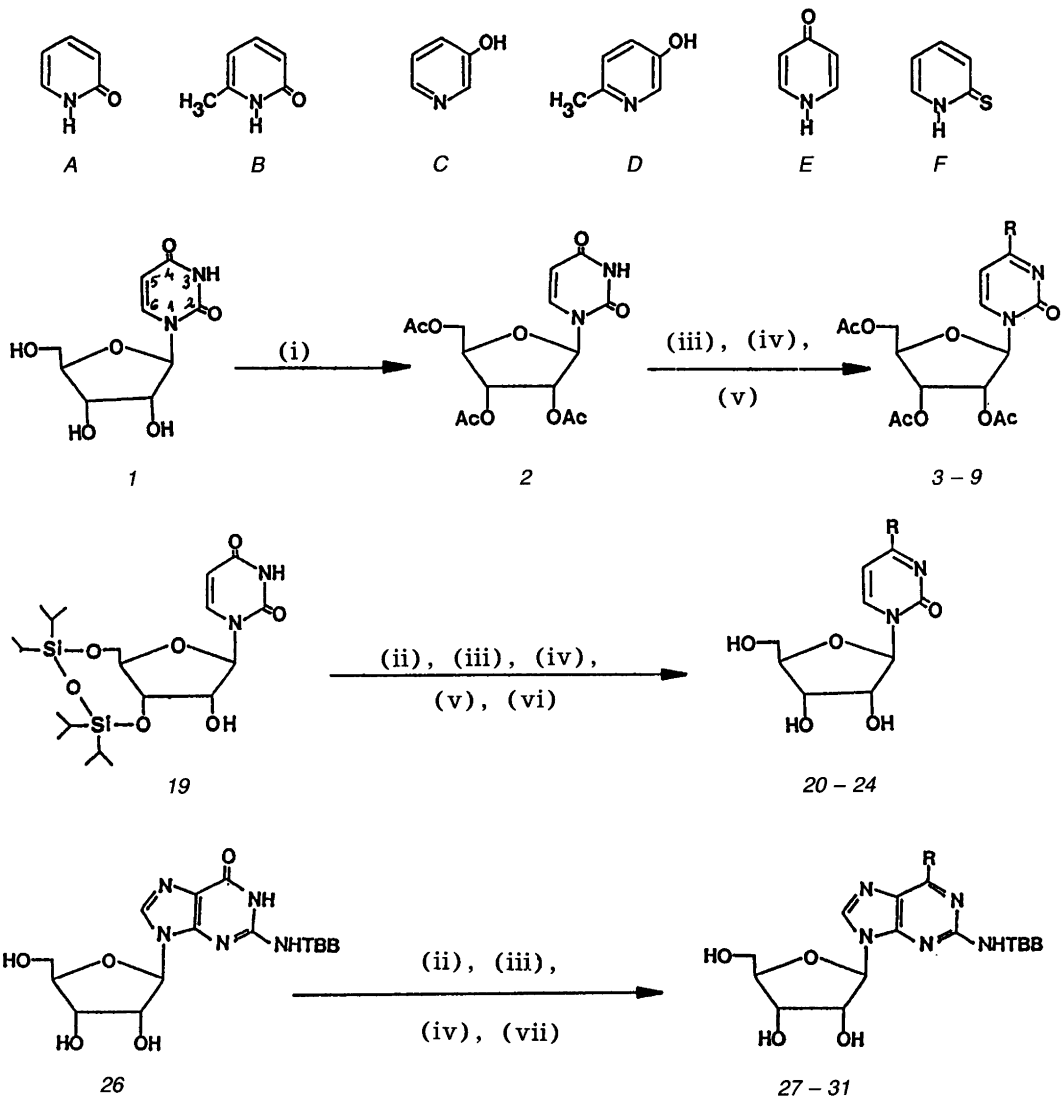


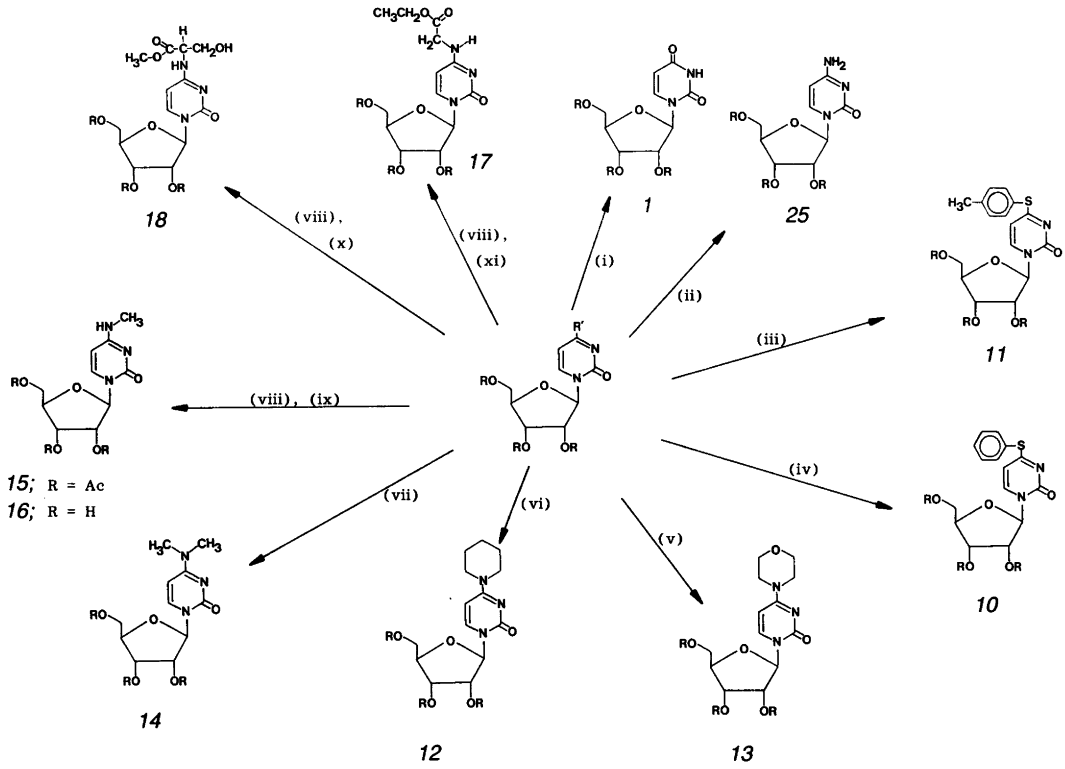
TBB = 4-(t-butyl)benzoyl

Compounds	Substituents
3, :	R = 2-pyridyloxy
4, 20, :	R = 2-pyridone-1-yl
5, 21, :	R = 6-methyl-2-pyridyloxy
6, 22, 27:	R = 3-pyridyloxy
7, 23, 28:	R = 6-methyl-3-pyridyloxy
29 :	R = 4-pyridyloxy
8, 30 :	R = 4-pyridone-1-yl
9, 24, 31:	R = 2-pyridylthio

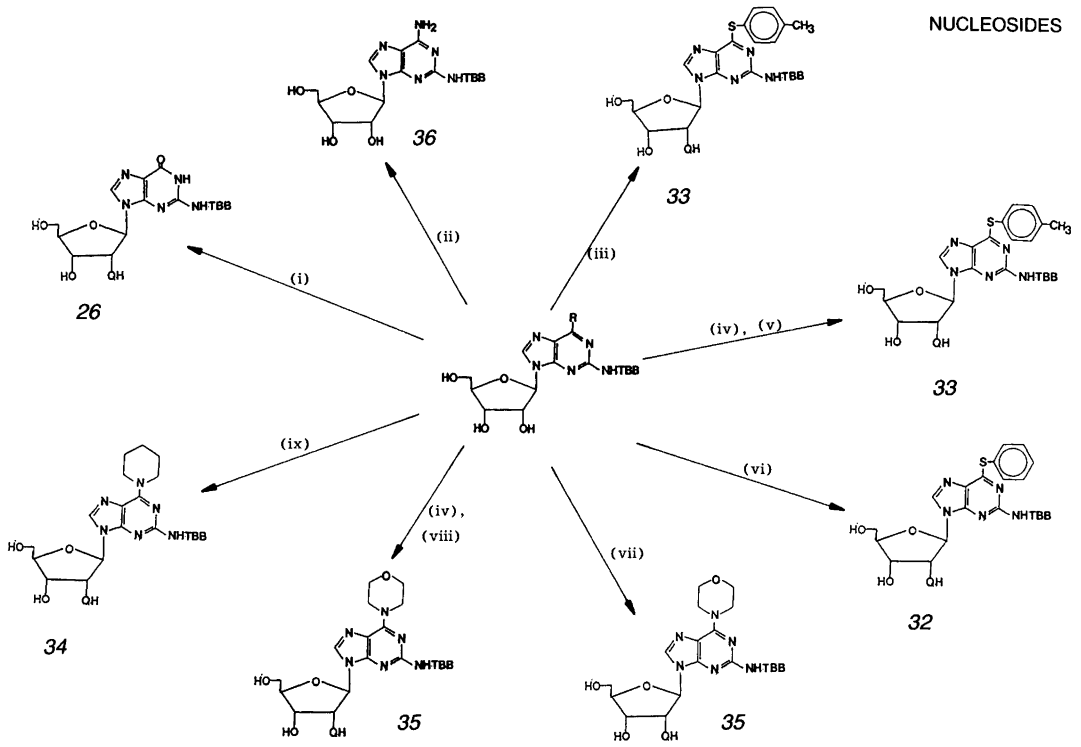
Scheme 1. (i) Acetic anhydride (10 eq.) in dry pyridine at 20 °C, 1 h. (ii) Trimethylchlorosilane (3 eq.) in dichloromethane, 20 min at 20 °C. (iii) 2-Mesitylenesulfonyl chloride (3 eq.), trimethylamine (5 eq.), DMAP (0.2 eq.) in dichloromethane, 1 h at 20 °C. (iv) Triethylamine (10 eq.) in dichloromethane, 10 min at 20 °C. (v) *A–F* (5 eq.), DABCO (0.1 eq.) in dichloromethane, 1 h at 20 °C. (vi) Tetrabutylammonium fluoride (4 eq.) in tetrahydrofuran, 2 min at 20 °C. (vii) *p*-Toluenesulfonic acid monohydrate (0.1 M) in dichloromethane/ methanol (7:3, v/v) at 20 °C.

Preparation of pyridyl derivatives of uridine and guanosine

The *C*-4-triethylammonium derivative of *2*,[20,21] was reacted with a substituted pyridine, (*A*) to (*F*), to give the corresponding *C*-4-pyridyl uridines (*3*) to (*9*) (Scheme 1). The 4-pyridone (*E*), as expected,[32] gave the *N*-substituted product *8* in 91 % yield. The 2-pyridone (*A*) gave a mixture of *O*- and *N*-substituted products *3* and *4* in 1:2 ratio while the 6-methyl-2-pyridone (*B*) and 2-pyridinethiol (*F*) gave almost quantitatively the *O*- and *S*-substituted products *5* and *9*, respectively. On the other hand, 3-hydroxy- and 6-methyl-3-hydroxy-pyridines, (*C*) and (*D*), gave respectively the expected[31,32] *O*-substituted products *6* and *7* in 89 and 80 % yields. Subsequently, a general one-pot procedure was devised for the preparation of *O*-4 protected pyridyl derivatives of uridine (*20–24*) and the *O*-6 protected pyridyl derivatives of guanosine (*27-31*),[36] (Scheme 1), with *free* sugar hydroxyls, in order to explore their utility in oligoribonucleotide synthesis[33,34] (following paper). Compounds *19* and *26* were thus subjected to a



Scheme 2. (i) 4-Nitrobenzaldoxime (10 eq.), *N,N,N′,N′*-tetramethylguanidine (9 eq.) in dioxane/water (1:1, v/v) for 3 min at 20 °C; R = H; substrates = *20–24*. (ii) Ammonia (50 eq.) in dry methanol for 5 h at 20 °C; R = H; substrates = *20–24*. (iii) Triethylammonium 4-methylbenzenethiolate (1.5 eq.) in dioxane, 1 min at 20 °C; R = Ac; substrates = *3* and *5–9*. (iv) Triethylammonium benzenethiolate (1.5 eq.) in dioxane, 1 min at 20 °C; R = Ac; substrates = *3* and *5–9*. (v) Morpholine (5 eq.) in tetrahydrofuran, 1–7 h at 20 °C; R = Ac; substrates = *3–9*. (vi) Piperidine (5 eq.) in tetrahydrofuran, 15 min–1 h at 20 °C; R = Ac; substrates = *3–9*. (vii) Dimethylamine hydrochloride (3 eq.), triethylamine (3 eq.) in dry ethanol, 1.0 min at 20 °C; R = Ac; substrate = *5*. (viii) Iodomethane (10 eq.) in acetonitrile, 8 h at 20 °C. (ix) Methylamine hydrochloride (3 eq.), triethylamine (6 eq.) in dry ethanol, 10 min at 20 °C; R = Ac: substrate = *6* or *7*. (x) L-Serine methyl ester hydrochloride (2 eq.), triethylamine (4 eq.), DMAP (1 eq.) in acetonitrile, 20 min at 20 °C; R = Ac; substrate = *6* or *7*. (xi) Glycine methyl ester hydrochloride (2 eq.), triethylamine (4 eq.), DMAP (1 eq.) in acetonitrile, 20 min at 20 °C; R = Ac; substrate = *6* or *7*.



Scheme 3. Substrates = compounds *27* to *31*: (i) 4-Nitrobenzaldoxime (10 eq.), *N,N,N',N'*-tetramethylguanidine (10 eq.) in dioxane/water (10:2, v/v), 16 h, 20 °C. (ii) Liquid ammonia in dioxane, 72 h, 20 °C. (iii) Trimethylammonium 4-methylbenzenethiolate (50 eq.) in dioxane, 18 h, 20 °C. (iv) Iodomethane (3 eq.) in DMF at 20 °C. (v) Triethylammonium 4-methylbenzenethiolate (10 eq.) in DMF, 20 min, 20 °C. (vi) Triethylammonium benzenethiolate (50 eq.) in dioxane at 20 °C. (vii) Morpholine (20 eq.) in tetrahydrofuran, 40 h, 20 °C. (viii) Morpholine (20 eq.) in tetrahydrofuran, 20 min, 20 °C. (ix) Piperidine (20 eq.) in tetrahydrofuran, 8.5 h, 20 °C.

five-step one-pot procedure (monitored by TLC; see Experimental): (i) trimethylsilylation of the hydroxyl functions; (ii) treatment of the resulting silyl derivative with 2-mesitylenesulfonyl chloride under basic conditions to give *O*-4 and *O*-6-mesitylenesulfonate of the silyl derivatives of uridine and guanosine, respectively; (iii) conversion of these sulfonates to the corresponding trialkylammonium derivatives;[20] (iv) nucleophilic substitutions of trialkylammonium group by substituted pyridines; and finally, (v) removal of the silyl groups either by fluoride ion (for uridine derivatives) or by a brief treatment with an acid (for guanosine derivatives).

Nucleophilic substitution reactions of pyridyl derivatives of uridine and guanosine

It is well established[37] that the mutagenic and carcinogenic modifications such as alkylation, occur at the O-4 site of pyrimidine and the O-6 site of guanine residues of nucleic acids. A survey of pertinent pyridine chemistry[29,31] made it clear that the pyridyl group and especially its methiodide would constitute a good leaving group in a nucleophilic substitution reaction. Such conversions, if possible in these systems, would establish these pyridyl groups both as protecting groups for uracil and guanine residues and also serve as useful building blocks for any site-specific modifications.[24,27] With these justifications, compounds *3–9* and *27–31* were subjected to nucleophilic substitution reactions using a variety of thiolates and amines as nucleophiles (Schemes 2 and 3, respectively). The reaction of benzenethiolate and 4-methylbenzethiolate ion on the latter substrates gave *10, 11, 32* and *33*, in 71–93 % yields, while the reaction with piperidine, morpholine and dimethylamine gave *12, 13, 14, 34* and *35* in 87–96 % yields. A comparison of half-

lives in the latter reactions, along with the reactions with different primary amines are shown in Table 4. These data clearly revealed that the nucleophilic substitution reactions of *3* to *9* and *27* to *31* with primary amines were several-fold slower than with secondary amines, as one would expect from a comparison of their respective pK_as.[28] However, compounds *16, 17* and *18* were easily obtained in 64–93 % when the *methiodides* of *6* and *7* were subjected to the similar reactions ($t_{1/2}$ ca. 10 min) with methylamine, glycine ethyl ester and serine methyl ester.

The yields, chromatographic properties, UV, ^{1}H and ^{13}C NMR data for all new compounds are presented in Tables 1–3 in support of their structures. These results clearly show that an efficient protecting group for the lactam functions of uridine and guanosine should be able not only to protect the aglycones from any subsequent electrophilic attack during phosphorylation and condensation reactions but also act as a good leaving group for substitution reactions with appropriate nucleophiles for the purpose of site-specific modifications. Work is in progress in this laboratory to show[35] that such site-specific modifications (e.g. uracil to cytosine or *N*-methylcytosine and

Table 1. Yields, chromatographic properties and UV data for substituted nucleosides. λ_{max}/nm

Compound	Yield/%	R_f	pH 2	pH 7	pH 13	ε at pH 7
4	50[a]	0.62[e]	313	313	315	8200
5	92[a]	0.62[e]	271 (284)	271 (284)	273 (285)	7200
6	89[a]	0.54[e]	270 (284)	270 (284)	260, 289	7300
7	90[a]	0.49[e]	278	276 (270)	276	8550
8	91[a]	0.22[e]	318 (328)	318 (228)	270 (241)	32600
9	78[a]	0.48[e]	287	287	273, 310	13000
10	94[b]	0.65[e]	301	301	309, 272	12400
11	92[b]	0.61[e]	302 (275)	302 (275)	307 (273)	13050
12	93[b]	0.56[e]	287	278	284	14700
13	91[b]	0.47[e]	285	278	284	12850
14	96[b]	0.59[e]	285	275	282	11750
15	79[c]	0.56[e]	261, 299	260, 299	274	11100
16	64[c]	0.28[f]	262, 298	261, 300	274	10500
17	89[c]	0.59[e]	279	264	275	9600
18	93[c]	0.32[e]	280	268	276 (248)	9350
20	60[d]	0.57[f]	314	314	314	7600
21	50[d]	0.62[f]	270 (282)	270 (282)	272 (284)	7900
22	55[d]	0.48[f]	277	270, 280	284	7800, 8300
23	*59*[d]	0.51[f]	281	278	278	8600
24	88[d]	0.56[f]	263, 312	306 (277)	307 (275)	11800
27	71[h]	0.54[g]	276, 244	274, 241	272, 235	25700, 20700
28	79[h]	0.57[g]	277, 243	277, 243	275, 238	26400, 17700
29	30[h]	0.49[g]	278, 253	277, 242	280, 238	25300, 20700
30	16[h]	0.38[g]	320, 273	320, 285	297	11500, 17700
31	72[h]	0.62[g]	330, 274	300	297	21400
			254	252	248	25300
32	85[i]	0.55[g]	292	296	296	22100
			258	256	250	26100
33	71[i]	0.57[g]	290, 260	296, 256	298, 245	25300, 27200
34	93[i]	0.63[g]	270	280	280	20800
				254	255	28600
35	87[i]	0.55[g]	270	280(s)	280	20800
				254	255	28000
36	84[i]	0.24[g]	291, 253	280, 248	280, 248	19700, 21000

[a]Based on *2*; [b]based on *5, 6* or *7*; [c]based on *6* and *7* via methiodide formation; [d]based on 19; [e]solvent system: (A); [f]solvent system: (C); [g]solvent system: (D); [h]based on *26*; [i]based on *27* and *28*.

Table 2. ^{1}H NMR of substituted nucleosides.

Compound	H-1′	H-2′	H-3′	H-4′	H-5′	H-5	H-6	H-8	Acetyl	Methyl	Pyridyl	Other
2	6.0 (*d*, 2.4)	5.38 (*m*)	5.38 (*m*)	4.36 (*m*)	4.36 (*m*)	5.80 (*d*, 8.2)	7.41 (*d*, 8.2)		2.11 2.09	–	–	–
3	6.18 (*d*, 3.9)	5.36 (*m*)	5.36 (*m*)	4.46 (*m*)	4.46 (*m*)	6.24 (*d*, 7.5)	7.90 (*d*, 7.4)		2.16 2.10	–	8.46 (1H), 7.82 (2H) 7.32 (1H)	–
4	6.18 (*d*, 3.4)	5.36 (*m*)	5.36 (*m*)	4.46 (*m*)	4.46 (*m*)	6.58 (*d*, 9.0)	8.24 (*d*, 8.0)		2.14 2.10	–	8.08 (1H), 7.47 (2H) 6.34 (1H)	–
5	6.11 (*d*, 3.9)	5.36 (*m*)	5.36 (*m*)	4.38 (*m*)	4.38 (*m*)	6.22 (*d*, 7.3)	7.89 (*d*, 7.2)		2.15 2.10	2.53	7.70 (1H) 7.03 (2H)	
6	6.07 (*d*, 3.7)	5.40 (*m*)	5.40 (*m*)	4.41 (*m*)	4.41 (*m*)	6.28 (*d*, 7.4)	7.95 (*d*, 7.2)		2.17 2.12	–	8.02 (1H) 7.63–7.34 (3H)	– –
7	6.07 (*d*, 3.6)	5.38 (*m*)	5.38 (*m*)	4.38 (*m*)	4.38 (*m*)	6.19 (*d*, 7.4)	7.95 (*d*, 7.3)		2.14 2.09	2.54	8.34 (1H) 7.46 (1H) 7.29 (1H)	–
8	6.13 (*d*, 3.7)	5.39 (*m*)	5.39 (*m*)	4.43 (*m*)	4.43 (*m*)	6.53 (*d*, 6.9)	8.19 (*d*, 7.0)		2.16 2.14 2.10	–	8.37 (2H) 6.45 (2H)	–
9	6.05 (*d*, 3.7)	5.36 (*m*)	5.36 (*m*)	4.37 (*m*)	4.37 (*m*)	6.31 (*d*, 7.1)	7.68 (*d*, 7.1)		2.15 2.12 2.10	–	8.68 (1H) 7.84–7.27 (2H)	–
10	6.06 (*d*, 3.4)	5.33 (*m*)	5.33 (*m*)	4.35 (*m*)	4.35 (*m*)	5.84 (*d*, 8.0)	7.60 (*d*, 7.6)		2.09 2.11	–	–	7.50 (*m*)
11	6.04 (*d*, 3.7)	5.35 (*m*)	5.35 (*m*)	4.35 (*m*)	4.35 (*m*)	5.92 (*d*, 7.3)	7.60 (*d*, 7.1)		2.07 2.09	2.38	–	7.40 (*m*)
12	6.23 (*d*, 3.4)	5.36 (*m*)	5.36 (*m*)	4.35 (*m*)	4.35 (*m*)	5.95 (*d*, 7.8)	7.45 (*d*, 7.6)		2.14 2.11 2.09	–	–	3.66 (4H) 1.66 (6H)
13	6.21 (*d*, 4.4)	5.36 (*m*)	5.36 (*m*)	4.36 (*m*)	4.36 (*m*)	5.91 (*d*, 7.1)	7.54 (*d*, 7.0)		2.14 2.11 2.10	–	–	3.74 (8H)
14	6.26 (*d*, 2.0)	5.35 (*m*)	5.35 (*m*)	4.36 (*m*)	4.36 (*m*)	5.90 (*d*, 6.7)	7.49 (*d*, 6.7)		2.15 2.11 2.09	–	–	3.22 3.10
15	6.04 (*d*, 2.4)	5.34 (*m*)	5.34 (*m*)	4.36 (*m*)	4.36 (*m*)	5.80 (*d*, 8.1)	7.40 (*d*, 8.3)		2.15 2.13 2.11	2.64	–	–
16	5.79 (*s*)	3.93 (*m*)	3.93 (*m*)	3.40 (*m*)	3.63 (*m*)	6.17 (*d*, 6.9)	7.70 (*d*, 6.8)			2.75		
17	5.98 (*d*, 3.4)	5.26 (*m*)	5.26 (*m*)	4.27 (*m*)	4.27 (*m*)	5.88 (*d*, 5.4)	7.40 (*d*, 7.1)		2.15 2.10	–	–	4.13 (*q*) 1.20 (*t*) 4.14 (*s*)

cont.

Compound	H-1′	H-2′	H-3′	H-4′	H-5′	H-5	H-6	H-8	Acetyl	Methyl	Pyridyl	Other
18	6.03 (*d*, 3.2)	5.35 (*m*)	5.35 (*m*)	4.35 (*m*)	4.35 (*m*)	5.92 (*d*, 7.1)	7.41 (*d*, 7.1)		2.13 2.10	–	–	4.96 4.03 3.77
20	5.81 (*d*, 2.1)	4.05 (*m*)	3.98 (*m*)	3.34 (*m*)	3.73 (*m*)	7.1 (*d*, 7.2)	8.73 (*d*, 7.4)			–	8.00, 6.39 7.53 6.52	–
21	5.764 (*s*)	3.96 (*m*)	3.96 (*m*)	3.41 (*m*)	3.70 (*m*)	6.33 (*d*, 7.3)	8.58 (*d*, 7.1)			2.45	7.86 (1H), 7.24 (1H) 7.05 (1H)	
22	5.74 (*s*)	3.94 (*m*)	3.94 (*m*)	3.20 (*m*)	3.70 (*m*)	6.39 (*d*, 7.3)	8.59 (*d*, 7.5)				8.49 (1H) 7.7–7.4 (3H)	
23	5.79 (*s*)	3.94 (*m*)	3.94 (*m*)	3.35 (*m*)	3.65 (*m*)	6.36 (*d*, 7.0)	8.55 (*d*, 7.1)			2.54	8.35 (1H), 7.60 (1H) 7.42 (1H)	
24	5.72 (*d*, 2.5)	3.94 (*m*)	3.94 (*m*)	3.39 (*m*)	3.69 (*m*)	6.35 (*d*, 7.1)	8.40 (*d*, 7.1)			–	8.63 (1H) 7.86 (2H) 7.49 (1H)	–
27	6.03 (*d*, 5.8)	4.68 (*t*, 5.6)	4.26 (*q*, 4.0)	4.02 (*m*)	3.68 (*m*)			8.50		–	8.68–7.45 (*m*)	1.31
28	6.07 (*d*, 5.4)	4.73 (*t*, 5.1)	4.31 (*m*)	4.06 (*m*)	3.70 (*m*)			8.67		2.50	8.53–8.31 (*m*)	1.31
29	6.03 (*d*, 5.9)	4.68 (*t*, 5.8)	4.32–3.91 (*m*)	4.32–3.91 (*m*)	3.70 (*m*)			8.65		–	8.71–8.46	1.31
30	6.09 (*d*, 5.9)	4.68 (*t*, 4.7)	4.34–3.93 (*m*)	4.34–3.93 (*m*)	3.70 (*m*)			8.84		–	9.37, 9.23 6.57, 6.58	8.04–7.50 1.33
31	5.99 (*d*, 5.8)	4.66 (*t*, 5.9)	4.30–3.89 (*m*)	4.30–3.89 (*m*)	3.66 (*m*)			8.63		–	8.59–7.26	1.31
32	5.95 (*d*, 5.9)	4.64 (*t*, 5.9)	4.29–3.88 (*m*)	4.29–3.88 (*m*)	3.64 (*m*)			8.61		–	7.94–7.40	1.29
33	6.03 (*d*, 5.6)	4.70 (*t*, 5.4)	4.30 (*t*, 3.8)	4.00 (*m*)	3.71 (*m*)			8.66				7.88–7.21 2.31 1.29
34	5.90 (*d*, 5.3)	4.58 (*t*, 5.1)	4.21 (*m*)	3.94 (*m*)	3.58 (*m*)			8.27		–	–	7.91–7.45 4.21, 1.61, 1.32
35	5.90 (*d*, 6.0)	5.03 (*t*, 5.5)	4.57 (*m*)	3.93 (*m*)	3.63 (*m*)			8.32		–	–	7.80–7.48, 4.16 (4H) 3.68 (4H)
36	5.89 (*d*, 5.9)	4.60 (*t*, 5.6)	4.20 (*m*)	3.90 (*m*)	3.62 (*m*)			8.31				7.84–7.52, 1.32

Table 3. ^{13}C NMR of substituted nucleosides.

Compound	C-1′	C-2′	C-3′	C-4′	C-5′	C-2	C-4	C-5	C-6	C-8	Others
2	87.7	70.4	73.0	80.1	63.4	150.7	163.7	103.5	139.9	–	21.0, 20,7, 20.6, 170.5, 169.9
3	89.0	69.3	73.5	79.8	62.3	154.3	169.2	95.8	144.1	–	148.2, 139.5, 122.0, 170.7, 162.2, 116.3, 169.8, 20.5, 20.2
4	89.4	69.5	73.9	80.1	62.6	154.6	162.7	101.2	143.7	–	170.7, 169.8, 164.0, 141.1, 133.7, 122.8, 107.2, 20.9, 20.5
5	89.1	69.6	73.4	79.6	62.6	154.6	169.3	96.0	143.9	–	170.8. 169.9, 169.2, 139.6, 157.3, 157.8, 121.5, 113.0, 29.4, 23.7, 20.5, 20.2
6	89.3	69.5	73.4	79.5	62.5	154.4	169.2	95.5	143.0	–	170.7, 169.8, 146.7, 144.3, 129.4, 123.8, 20.5, 20.2
7	89.3	69.7	73.5	79.7	62.5	154.5	169.3	95.6	144.1	–	171.0, 169.9, 155.7, 146.0, 142.0, 129.6, 123.4, 23.6, 20.6, 20.3
8	89.9	69.7	73.6	80.1	62.6	153.6	160.7	93.2	146.3	–	170.0, 169.5, 180.1, 134.3, 119.2, 20.7, 20.1
9	89.5	69.6	73.6	79.8	62.7	152.9	178.2	103.1	140.6	–	170.1, 169.4, 150.9, 150.5, 137.6, 130.2, 123.9, 20.7, 20.5
10	89.3	69.7	73.6	79.7	62.8	152.8	181.0	101.9	140.5	–	170.1, 169.4, 135.6, 130.3, 129.7, 127.1, 20.7, 20.5
11	89.3	69.7	73.6	79.7	62.8	152.8	181.5	101.7	140.5	–	170.1, 169.4, 135.6, 130.3, 129.7, 127.1, 20.7, 20.5
12	87.7	70.1	73.1	79.1	63.1	155.1	161.6	92.1	140.0	–	170.2, 169.7, 38.1, 37.6, 20.9, 20.6
13	88.0	70.1	73.3	79.4	63.2	155.0	162.5	91.9	140.7	–	170.1, 169.6, 66.4, 44.6, 20.7, 20.4
14	87.8	70.3	73.3	79.4	63.3	155.0	163.2	92.5	139.9	–	170.2, 169.7, 38.1, 37.6, 20.9, 20.6
15	88.8	69.8	73.7	79.9	–	154.9	164.9	99.9	141.6	–	170.1, 169.5, 26.0, 20.8, 20.5
16	89.4	69.5	74.2	84.2	60.6	155.8	163.9	94.7	140.2	–	26.8
17	88.5	70.2	73.4	79.5	63.3	155.3	163.5	91.2	140.0	–	170.1, 169.7, 170.4, 61.7, 42.6, 20.9, 20.6, 14.2
18	89.2	70.2	73.4	79.3	63.2	156.1	163.6	96.9	140.1	–	170.5, 169.8, 62.5, 52.6, 20.8, 20.6, 14.1
20	91.1	68.1	75.0	84.4	59.3	154.6	163.4	100.7	145.8	–	172.5, 161.6, 141.3, 134.1, 121.7
21	90.2	68.6	74.6	84.2	59.7	154.5	170.6	94.3	146.2	–	157.8, 157.3, 140.3, 121.6, 113.4, 23.5
22	90.5	68.6	74.7	84.4	59.8	154.6	170.8	94.3	146.4	–	148.6, 147.0, 143.7, 130.1, 124.6
23	90.5	68.6	74.7	84.4	59.8	154.6	170.9	94.4	146.5	–	155.4, 146.3, 142.5, 130.3, 122.8, 23.4
24	90.3	68.4	74.5	84.1	59.5	152.6	176.1	101.9	142.8	–	150.6, 138.0, 130.3

cont.

Compound	C-1′	C-2′	C-3′	C-4′	C-5′	C-2	C-4	C-5	C-6	C-8	Others
27	87.5	73.7	70.5	85.8	61.4	152.0	154.1	118.1	149.0	142.9	165.5, 158.9, 154.9, 146.3, 142.9, 131.4, 128.1, 125.0, 124.3, 34.6, 30.8
28	87.5	73.8	70.5	85.9	61.4	152.0	154.0	118.1	147.0	142.9	165.5, 158.6, 154.8, 154.6, 141.8, 131.4, 129.5, 128.1, 124.9, 123.5, 57.5, 34.6, 30.8
29	87.4	73.6	70.4	85.8	61.3	152.0	154.4	118.4	157.3	143.3	165.5, 159.1, 154.9, 151.2, 131.4, 128.1, 125.0, 116.1, 34.6, 30.8
30	87.5	73.7	70.3	85.8	61.2	152.1	154.8	118.1	146.1	143.9	178.7, 165.4, 155.0, 136.4, 134.4, 129.9, 128.0, 125.1, 34.7, 30.8
31	87.3	73.6	70.4	85.8	61.3	150.6	152.2	121.1	152.8	143.5	165.4, 162.6, 154.8, 157.1, 149.6, 137.3, 131.3, 128.8, 128.0, 125.0, 34.6, 30.8
32	87.2	73.6	70.4	85.7	61.3	150.1	152.2	127.2	158.6	143.1	165.5, 154.6, 134.1, 131.4, 129.0, 128.6, 128.8, 127.8, 124.9, 34.6, 30.8
33	88.0	74.0	70.7	86.1	61.6	150.0	152.5	128.1	160.0	143.5	161.1, 155.5, 131.5, 135.6, 139.6, 130.4, 128.3, 125.5, 123.3, 35.0, 31.2
34	88.1	74.3	70.9	86.2	61.9	151.8	153.5	117.6	152.7	138.2	166.5, 155.3, 132.3, 128.2, 125.6, 46.1, 35.1, 30.3, 26.3, 24.5
35	87.0	73.6	70.4	85.6	61.4	151.7	152.9	116.6	152.9	138.1	165.4, 154.3, 132.0, 127.4, 124.8, 66.1, 45.0, 34.5, 30.8
36	88.0	74.0	70.9	86.2	61.9	150.5	153.1	117.0	155.5	140.2	165.9, 155.7, 131.9, 128.2, 125.7, 35.1, 31.3

guanine to 2,6-diaminopurine etc.) are indeed possible after the construction of the fully protected oligoribonucleotide with appropriate protecting groups.

Experimental

The ^{1}H and ^{13}C NMR spectra were recorded in δ scale using a Jeol FX90-Q spectrometer at 89.5 and 23.5 MHz, respectively, TMS being used as internal standard for ^{1}H NMR and $CDCl_3$ (= 77.14 ppm) or DMSO (= 39.38 ppm) as references for ^{13}C NMR in $CDCl_3$ and DMSO-d_6, respectively. The ^{1}H resonances were assigned by homodecoupling, and ^{13}C by selective decoupling. UV spectra were measured using a Cary/Varian 2200 spectrometer. High resolution mass spectral data which supported out structural assignments, including the elemental composition of the molecular ions of all new compounds *3–36*, were obtained with a Jeol DX 303 mass spectrometer equipped with a Jeol DA 5000 data system by fast atom bombardment spectroscopy using argon or xenon as collision gas. TLC was car-

ried 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 were carried out using Merck G60 silica gel.

*Preparation of C-4 substituted 1-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)pyrimidine-2-ones, (3)–(9): a general procedure.* Compound *2* (0.37 g, 1 mmol) was dissolved in dichloromethane (10 ml) and triethylamine (1.0 g, 10 mmol) added followed by 2-mesitylenesulfonyl chloride (0.42 g, 3 eq.) and 4-*N*,*N*-dimethylaminopyridine (DMAP) (0.024 g, 0.2 mmol). After 1 h, TLC (A) showed the consumption of all starting material. The substituted pyridine *A* to *F* (5 mmol) was then added followed by 1,4-diazabicyclo[2,2,2]octane (Dabco, 0.5 eq.) and a further 5 eq. of triethylamine. The reaction mixture was stirred for a further 1–2 h. The reaction mixture was worked up and purified in the usual way.[36]

Preparation of N*-methylcytidine* 16 *and its triacetate* 15. Compound *6* (0.23 g, 0.5 mmol) was dissolved in acetonitrile (5 ml), iodomethane (0.71 g, 5 mmol) added and the mixture stirred for 8 h. All of the volatile material was then removed. The residue was redissolved in ethanol (5 ml); methylamine hydrochloride (0.1 g, 1.5 mmol) and triethylamine (0.15 g, 1.5 mmol) were added. After 10 min, the reaction was worked up and the dichloromethane extracts collected and evaporated to dryness. Examination of the mixture by TLC (A) showed 2 compounds to be present. NMR analysis of these components showed them to be the 2',3',5'-tri-*O*-acetyl-4-*N*-methylcytidine (*15*) and a deacetylated product. Treatment of the entire mixture with acetic anhydride in pyridine yielded *15* as the sole product, while treatment with aqueous ammonia (5 ml) gave 4-methylcytidine (*16*).

*1-(2',3',5'-Tri-*O*-acetyl-β-D-ribofuranosyl)-4(glycyl ethyl ester) 2-pyrimidinone* (17). To a solution of *6* or *7* (0.5 mmol) in acetonitrile (5 ml) was added iodomethane (0.71 g, 5 mmol) and the mixture stirred for 8 h. The volatile matter was then removed *in vacuo* and the residue redissolved in acetonitrile (10 ml). Glycine ethyl ester hydrochloride (0.14 g, 1 mmol) was added followed by triethylamine (0.2 g, 2 mmol) and DMAP (0.06 g, 0.5 mmol). After 20 min, the reaction was worked up, purified and characterized.[36] Compound *18* was prepared and characterized in a similar manner.

Preparation of C-4 substituted 1(β-D-ribofuranosyl)-2-pyrimidones, (20)–(24): a general procedure. To a stirred solution of *19*[34,36] (0.49 g, 0.5 mmol) in dichloromethane (10 ml), triethylamine

Table 4. Studies of nucleophiic substitution reactions ($t_{\frac{1}{2}}$ in min at 20 °C) of substituted uridines and guanosines.

Starting compound	PhS^-	4-$TolS^-$	Morpholine	Piperidine	$(CH_3)_2NH$	NH_2CH_2COOEt	$HOCH_2CH(NH_2)COOMe$	H_2NMe
3	[a]	[a]	[a]	[a]	[b]	[b]	[b]	[b]
4	[c]	[c]	20	[a]	[b]	[b]	[b]	[b]
5	[a]	1	1	[a]	[a]	[b]	[b]	[b]
6	[a]	[a]	20	1	[a]	2160	2160	1440
7	[a]	[a]	60	3	[a]	2160	2160	1440
8	1	1	1	[a]	[b]	[b]	[b]	[b]
9	1	1	1	1	[b]	[b]	[b]	[b]
27	720	660	210	32				
28	1470	1170	300	44				
29	600	450	180	18				
30	150	150	90	12				
31	30	17	1680	52				

[a]Too short to estimate. [b]No data available. [c]Substrate stable for 12 h at 20 °C.

(0.15 g, 3 eq.) and trimethylchlorosilane (0.33 g, 3 eq.) were added. After 10 min, the reaction mixture was worked up.[36] The residue was redissolved in dichloromethane. Triethylamine (1.0 ml, 10 mmol), 2-mesitylenesulfonyl chloride (0.42 g, 3 mmol) and DMAP were then added. Upon completion of the reaction as judged by TLC (A), the appropriate substituted pyridine was added, followed by Dabco and the reaction mixture stirred for a further 1 h. The mixture was then poured into saturated sodium hydrogen carbonate solution and extracted with dichloromethane. The combined organic layers were evaporated to dryness. The resulting gum was dissolved in tetrahydrofuran (15 ml) and tetrabutylammonium fluoride solution (2 ml, 1.0 M) added. The solution was stirred for 5 min, worked up and characterized.[36]

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