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AN ACETAL GROUP SUITABLE FOR THE PROTECTION OF 2'-HYDROXY FUNCTIONS IN RAPID OLIGORIBONUCLEOTIDE SYNTHESIS

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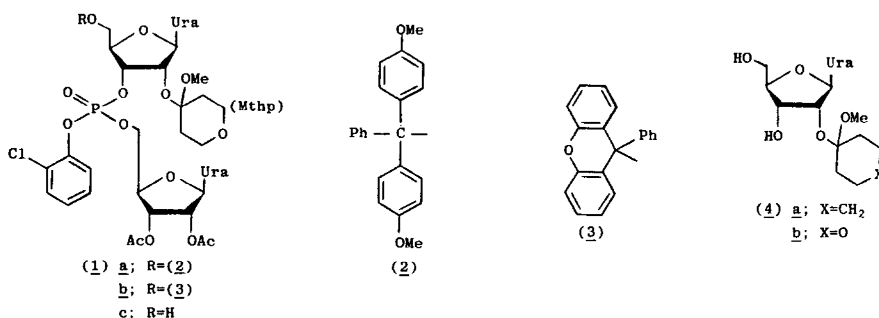
Summary: The 1-[(2-chloro-4-methyl)phenyl]-4-methoxypiperidin-4-yl [Ctmp, as in (14a)] has an acid lability similar to that of the 4-methoxytetrahydropyran-4-yl (Mthp) protecting group under mild hydrolytic conditions [pH 2-3]; however, under the relatively more drastic conditions required for the complete removal of a 9-phenylxanthen-9-yl (Px) group, the Ctmp protecting group remains virtually intact.

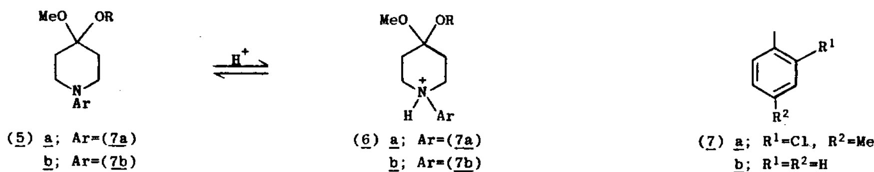
The 4-methoxytetrahydropyran-4-yl [Mthp, as in (1)] group[1] has proved[2,3] to be particularly suitable for the protection of 2'-hydroxy functions in oligoribonucleotide synthesis in solution[4,5]. However, we are now directing our attention towards the development of methods for the rapid synthesis of oligo- and poly-ribonucleotides both on solid supports and in solution and, for reasons of practical convenience, we believe[6] that it is advisable to use a modified trityl group [such as 4,4'-dimethoxytrityl (2) or 9-phenylxanthen-9-yl (3)] to protect terminal 5'-hydroxy functions [as in (1a) or (1b)]. As some recent studies have shown[6] that the relatively drastic protic acid conditions required for the removal of a 5'-O-(9-phenylxanthen-9-yl) group from a fully-protected dinucleoside phosphate (1b) lead to appreciable concomitant removal of the 2'-O-Mthp group, we have sought an alternative to the latter protecting group.



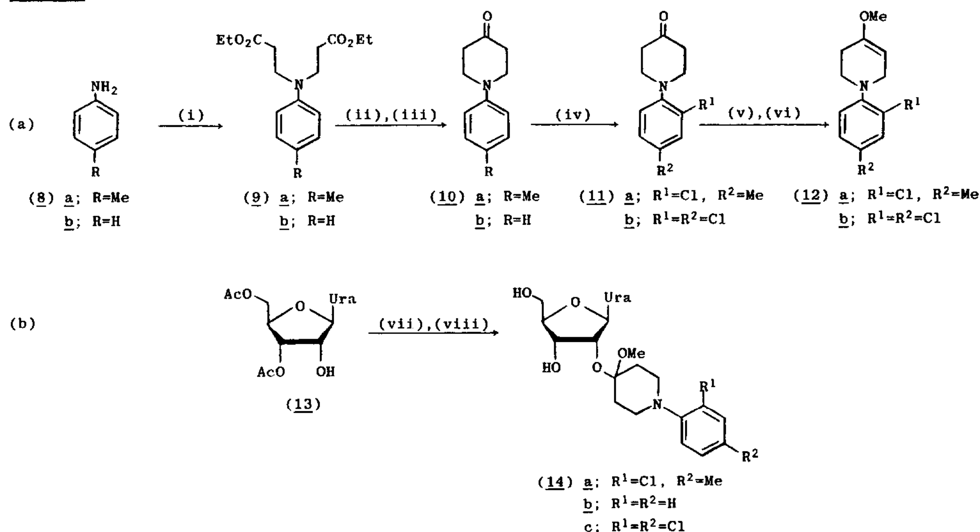
The 1-methoxycyclohex-1-yl group [as in (4a)] was found[1] to be much too acid-labile to be of use in oligoribonucleotide synthesis. However, acetals can be stabilized to acidic hydrolysis by the introduction of electron-withdrawing groups[7]. Thus, 2'-O-(4-methoxytetrahydropyran-4-yl)uridine [(4b); $t_{\frac{1}{2}}$ = 20.5 min, pH 2, 25°C; Table, entry no. 2] undergoes acid-catalyzed hydrolysis at a rate that is more than 2 orders of magnitude slower than that of (4a)[1]. Some later studies[8] on the hydrolysis of 5'-protected thymidine derivatives

suggested that the replacement of O by S [as in (4; X = S)] would increase the rate of acetal hydrolysis by a factor of ca. 5 whereas the replacement of oxygen by a sulphone group [as in (4; X = SO_2)] would decrease the rate of hydrolysis by a factor of more than 400.

Scheme 1



For reasons that we have discussed in outline previously[2,6], we believe that it is desirable to retain an acid-labile group for the protection of the 2'-hydroxy functions. It occurred to us that it should be possible to design a weakly basic 1-N-aryl-4-methoxypiperidin-4-yl group which, at pH 2 - 2.5, would be largely unprotonated on N-1 [as in (5), Scheme 1] and thus as labile as the Mthp group[1] but which, under the more strongly acidic conditions required[6] for the removal of the 9-phenylxanthen-9-yl protecting group, would be largely protonated [as in (6)] and perhaps therefore as stable to acidic hydrolysis as the sulphone system[8] [as in (4; X = SO_2)] referred to above. We now report that the 1-[(2-chloro-4-methyl)phenyl]-4-methoxypiperidin-4-yl [Ctmp; as in (5a)] group appears to have essentially the required properties.



Reagents: (i) $CH_2{=}CHCO_2Et$, CuCl, AcOH, reflux; (ii) NaH, benzene, reflux; (iii) concentrated hydrochloric acid–water (1:4, v/v), reflux; (iv) N-chlorosuccinimide; (v) $(MeO)_3CH$, TsOH, MeOH, reflux; (vi) TsOH, heat; (vii) (12), CF_3CO_2H, dioxane; (viii) NH_3, MeOH.

1-(p-Tolyl)piperidin-4-one (10a) was prepared (Scheme 2a) in ca. 54% overall yield by a three step procedure[9]. When (10a) was heated, under reflux, with a slight excess of N-chlorosuccinimide in dichloromethane solution for 3 hr, 1-[(2-chloro-4-methyl)phenyl]-piperidin-4-one (11a), m.p. 65-66°C, was obtained in 69% yield. The latter ketone (11a) was converted (Scheme 2a) into the corresponding enol ether (12a) in 67% overall yield. The reaction

between (12a) and 3',5'-di-O-acetyluridine[10] (13) and the conversion of the product obtained into (14a) [m.p. 185-186°C, 85% overall yield] is indicated in outline in Scheme 2b.

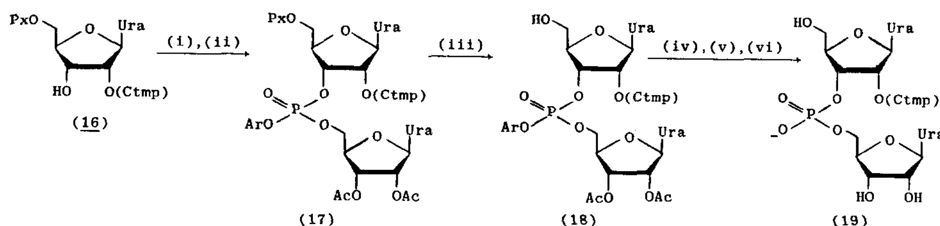
TABLE. Removal of 2'-Protecting Groups by Acidic Hydrolysis[a] at 25°C.

Entry No.	Substrate	pH	$t_{\frac{1}{2}}$(min)[b]	$t_{0.99}$[c]	Entry No.	Substrate	pH	$t_{\frac{1}{2}}$(min)[b]	$t_{0.99}$[c]
1	(4b)	1.0	0.9	6 min	9	(14a)	3.0	80	532 min
2	(4b)	2.0	20.5	136 min	10	(14a)	3.5	168	18.6 hr
3	(4b)	3.0	126	837 min	11	(14b)	1.0	498	55 hr
4	(14a)	0.5	33.5	223 min	12	(14b)	2.0	660	73 hr
5	(14a)	1.0	35.5	236 min	13	(14c)	1.0	3.9	26 min
6	(14a)	1.5	35	233 min	14	(14c)	2.0	9.7	64 min
7	(14a)	2.0	41	272 min	15	(19)[d]	2.0	21.5	143 min
8	(14a)	2.5	52	345 min	16	(19)	2.5	23.5	156 min

[a]Substrates (ca. 0.5 - 1.0 mg) were dissolved in 3.0 ml of hydrochloric acid or 0.2 M-glycine hydrochloride buffer at the specified pH. Aliquots (0.2 ml) of the reaction solutions were removed after suitable intervals of time, neutralized with triethylammonium bicarbonate and analyzed by h.p.l.c. (Jones APEX ODS column). [b]Pseudo first order kinetics were observed for all reactions ($t_{\frac{1}{2}}$ = half-time): straight lines were obtained when logarithms of the percentages of substrates remaining were plotted against time. [c]$t_{0.99}$ represents the calculated time for 99% removal of the 2'-protecting group. [d]For the preparation of (19), see Scheme 3.

It can be seen from the Table that, as expected[1], the rate of hydrolysis of the 2'-O-Mthp derivative of uridine [(4b), entries nos. 1-3] increases sharply with decreasing pH: thus it is 140 times faster at pH 1.0 [entry no. 1] than it is at pH 3.0 [entry no. 3]. On the other hand, the rate of hydrolysis of (14a) is virtually unchanged between pH 0.5 and 1.5 [entries nos. 4-6] and is only ca. 2.25 times faster at pH 1.0 [entry no. 5] than it is at pH 3.0 [entry no. 9]. It is also apparent that the rates of removal of the Ctmp [as in (14a)] and the Mthp [as in (4b)] protecting groups do not differ appreciably in the pH range 2.0 - 3.0 [entries nos. 2, 3, 7, 8 and 9]. It can further be seen from the Table [entries nos. 11-14] that the 1-phenyl-4-methoxypiperidin-4-yl [as in (14b)][12] and 1-(2,4-dichlorophenyl)-4-methoxypiperidin-4-yl [as in (14c)][12] protecting groups are, respectively, too stable [i.e. too basic; see Scheme 1 and above] and insufficiently stable [i.e. too weakly basic] for the present purpose. Finally, it can be seen [entries nos. 7, 8, 15, 16] that the vicinal phosphotriester group in the partially-protected dinucleoside phosphate (19) facilitates[13] the acid-catalyzed hydrolysis of the Ctmp protecting group.

Scheme 3



Px = 9-phenylxanthen-9-yl; Ar = 2-chlorophenyl; Ctmp = 1-[(2-chloro-4-methyl)phenyl]-4-methoxypiperidin-4-yl

Reagents: (i)(a) 2-chlorophenyl phosphorodi-(1,2,4-triazolide), 1-methylimidazole, tetrahydrofuran, (b) Et_3N, H_2O, tetrahydrofuran; (ii) 2',3'-di-O-acetyluridine, 1-(mesitylene-2-sulphonyl)-3-nitro-1,2,4-triazole, C_5H_5N; (iii) CF_3CO_2H, pyrrole, CH_2Cl_2; (iv) Ac_2O, C_5H_5N; (v) E-2-nitrobenzaldoxime, N^1,N^1,N^3,N^3-tetramethylguanidine, dioxane; (vi) aqueous NH_3 (d 0.88).

The 2'-O-Ctmp derivative of uridine (14a) was converted[15] into (16) in 91% yield. The latter compound was then converted, by the standard two-step procedure[2] [Scheme 3], into the

fully-protected dinucleoside phosphate (17) in ca. 83% overall yield. When a 0.025 M - solution of (17) in dichloromethane was treated [Scheme 3] with 5.5 mol. equiv. of trifluoroacetic acid and 16.5 mol. equiv. of pyrrole[16] for 30 seconds at room temperature, no starting material remained and the partially-protected dinucleoside phosphate (18)[17] was isolated from the products in 95.5% yield. When the acid treatment of (17) was extended to 30 minutes under the same conditions, (18) was isolated in 85% yield. From these results it can be estimated that less than 0.2% concomitant removal of the 2'-O-Ctmp group occurs in the time required for the complete removal of the 5'-O-(9-phenylxanthen-9-yl) protecting group. It is reasonable to conclude from the data presented here that the Ctmp protecting group is likely to be suitable for the rapid synthesis of oligo- and poly-ribonucleotides both on solid supports and in solution.

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[11] Satisfactory microanalytical data were obtained for the three new 2'-protected uridine derivatives [(14a), (14b), and (14c)] described.

[12] The procedures used for the preparation of the enol ethers [(12; $R^1 = R^2 = H$) and (12b)] and the corresponding 2'-protected uridine derivatives [(14b) and (14c), respectively] are indicated in Scheme 2.

[13] A similar effect has been observed previously in the acid-catalyzed hydrolysis both of the tetrahydropyranyl[14] and the Mthp[5] protecting groups.

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[16] We recommend that pyrrole should also be used in the removal of 9-phenylxanthen-9-yl [and presumably 4,4'-dimethoxytrityl] protecting groups in oligodeoxyribonucleotide synthesis. The 9-phenylxanthen-9-yl residue is then quantitatively and irreversibly transferred to pyrrole [to give what is believed to be 2-(9-phenylxanthen-9-yl)-pyrrole].

[17] The procedure for the conversion of (18) into (19) is indicated in Scheme 3. The uridylyl-(3'→5')-uridine, obtained by the acidic hydrolysis of (19) [Table], underwent complete digestion to give the expected monomeric products in the presence both of calf spleen and snake venom phosphodiesterases.

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