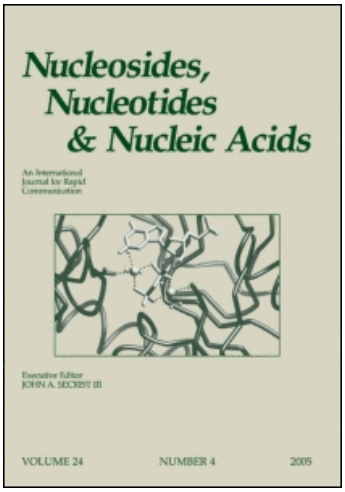

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The Protection of The 2'-Hydroxyl Function in Oligoribonucleotide Synthesis

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THE PROTECTION OF THE 2'-HYDROXYL FUNCTION IN OLIGORIBONUCLEOTIDE SYNTHESIS

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Summary:
A new, easily accessible and achiral 2'-ketal protective group has been designed for the use in the chemical synthesis of oligoribonucleotides; the proposed 2'-ketal group(1) has the additional advantage that it could be easily functionalized to the diamide (6)with aq. ammonia at the penultimate step of deblocking of oligoribonucleotides which makes it more acid-labile than the parent 2'-ketal group during the final acid-promoted deprotection step.

It is the choice of 2'-protecting group which dictates the choice of all other groups in any successfull strategy for the chemical synthesis of oligoribonucleotides.[1] It had been earlier shown[2] that the acetal and ketal systems could be stabilized to the acidic hydrolysis by the introduction of electron withdrawing groups. Subsequently, Reese and his coworkers[3] proposed 4-methoxytetrahydropyranyl- (MTHP) group for the protection of the 2'-hydroxyl function basing their arguments on the fact that the inductive effect of a β-oxygen atom, in a constrained ring system, was sufficient to destabilize a substituted ammonium ion as was apparent from a comparison of pK_a of morpholine (8.7) and piperidine (11.12). Thus these workers showed that the half-lives of removal of 2'-O-(1-methoxycyclohexyl-) and 2'-O-(4-methoxytetrahydropyranyl-) groups were 10 min(pH 4) and 24 min(pH 2) respectively. We herein report a new achiral 2'-ketal protective group, 3-methoxy-1,5-dicarbomethoxypentanyl- (MDMP), as in (1), which is readily accessible in a large scale involving non-corrosive, non-lachrymatory, non-hygroscopic and inexpensive reagents or intermediates. Thus, a comparison[4] of the pK_as of ethylamine (10.79), 2-chloroethylamine (5.44), 2-fluoroethylamine (8.79), 3-aminopropionic acid methyl ester (9.1) and 3-amino propionic acid (10.39) indicate that a β-substituent indeed destabilizes an ammonium ion depending on the nature of the substituent and, similarly, would have a corresponding influence on the stability of a carbenium ion. It was then envisaged that a creation of a ketal functionality on the C-3 carbon that is

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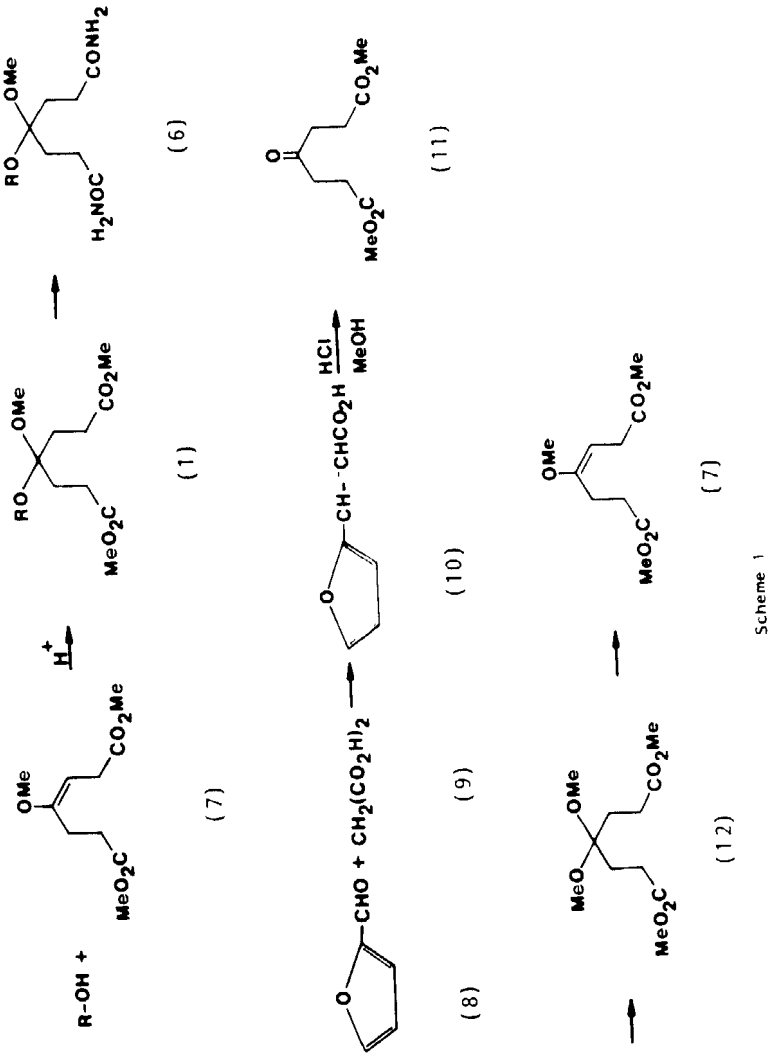
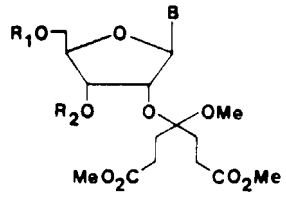


Scheme 1



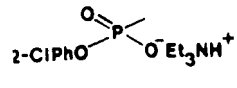
(2a) $R_1 = R_2 = H$; B = U
(2b) $R_1 = R_2 = H$; $B = U^{Bz}$
(3) $R_1 = R_2 = H$; $B = C^{Bz}$
(4) $R_1 = R_2 = H$; $B = A^{Bz}$
(5) $R_1 = R_2 = H$; $B = G^{TBB}$

(13) R_1 = Px ; $R_2 = H$; $B = U^{Bz}$
(14) R_1 = Px ; $R_2 = H$; $B = C^{Bz}$
(15) R_1 = Px ; $R_2 = H$; $B = A^{Bz}$
(16) R_1 = Px ; $R_2 = H$; $B = G^{TBB}$

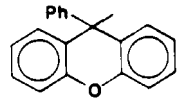
(17) R_1 = Px ; R_2 = Phosphodiester ; $B = U^{Bz}$
(18) R_1 = Px ; R_2 = Phosphodiester ; $B = C^{Bz}$
(19) R_1 = Px ; R_2 = Phosphodiester ; $B = A^{Bz}$
(20) R_1 = Px ; R_2 = Phosphodiester ; $B = G^{TBB}$

(21) R_1 = Px ; R_2 = Phosphotriester ; $B = U^{Bz}$
(22) R_1 = Px ; R_2 = Phosphotriester ; $B = C^{Bz}$
(23) R_1 = Px ; R_2 = Phosphotriester ; $B = A^{Bz}$
(24) R_1 = Px ; R_2 = Phosphotriester ; $B = G^{TBB}$

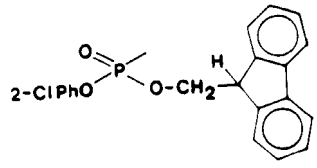
(25) $R_1 = H$; R_2 = Phosphotriester ; $B = U^{Bz}$
(26) $R_1 = H$; R_2 = Phosphotriester ; $B = C^{Bz}$
(27) $R_1 = H$; R_2 = Phosphotriester ; $B = A^{Bz}$
(28) $R_1 = H$; R_2 = Phosphotriester ; $B = G^{TBB}$



Phosphodiester



Pixyl (Px)



Phosphotriester

flanked by two two-carbon units with an appropriate β-electron-withdrawing substituent would produce a cumulative effect on the stabilization of the resultant 2'-ketal protective group which would be symmetrical in nature. It was clear to us during these considerations that the actual choice of an electron-withdrawing substituent on the β-carbon would entirely rest, firstly, on its relative stability under conditions of synthetic manipulations and secondly, on the feasibilty of its selective chemical conversion, at the last step of the synthesis, to a group which would neutralize or reverse the effect and, consequently, facilitate its removal under a mild acidic condition. We have thus prepared the 2'-MDMP derivatives of appropriately protected nucleosides, (2) to (5), in 79, 82, 76, 63 and 69% yields respectively. The half-lives of the removal of the MDMP group from different nucleoside blocks, (2) to (5), are 6, 6, 4, 18 and 15 min. respectively in 80% acetic acid (v/v) at 22° C, while the half-lives of removal of the MTHP group from the corresponding MTHP derivatives are 9, 13, 6, 26, and 22 min under an identical condition. The MDMP group upon its conversion to the diamide (6) by the treatment of aq. ammonia (d 0.9) is cleaved off at a faster rate (ca. 17 fold) under the above acidic condition.

The reagent, 3-methoxy-1,5-dicarbomethoxy-2,3-pentene (7) may be conveniently prepared in 200g scale in four steps involving inexpensive, non-corrosive and non-volatile starting materials like furfural (8) and malonic acid (9) as outlined in scheme 1: the pentane-3-one-1,5-dimethylcarboxylate (11) was prepared in 65% overall yield starting from (8) and (9) using a literature procedure[5,6] that has been described for the corresponding diethyl ester- (the intermediate crude furfurylacrylic acid (10) was directly used in the next step). The dimethyl ester (11) was then converted to the ketal (12) in 96% yield (b.p 148°C/ 0.8 mbar) which gave the desired enol-ether (7) in 85% yield (130°C/0.1 mbar, ca. 95% pure, NMR)[7]. We have subsequently used this 2'-MDMP group in conjunction with the 5'-O-(9-phenylxanthen-9-yl-) (pixyl) group in the chemical synthesis of oligoribonucleotides using building blocks like (13) to (24). The 5'-hydroxy-3'-phosphotriester-2'-MDMP derivatives, (25) to (28),were obtained in 74, 74, 71 and 91% yields respectively by selective removal of the pixyl group from the corresponding parent compounds, (21) to (24), with the help of a 2% ethanolic chloroform solution (0.055M) of trichloroacetic acid (10 equiv.) at 2°C (±1°). Condensation reactions with appropriate building blocks have finally led to the synthesis of a fully protected dimer (CpC) and a trimer (UpCpC) which have been characterized ,after a usual deblocking procedure [8] , by enzymatic and alkaline digestions. Details of the experimental procedures of these syntheses will be published in a full paper. Acknowledgements: Financial supports from Swedish Board for Technical Development and Natural Science Research Council are gratefully acknowledged.

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