Alternatives to the 4,4′-dimethoxytrityl (DMTr) protecting group

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Abstract—The 9-phenyl- and the 9-(p-tolyl)-xanthen-9-yl groups 2a and 2b are recommended as alternatives to the 4,4′-dimethoxytrityl group 1 for the protection of the 5′-hydroxy functions in oligonucleotide synthesis.

Forty years ago, Khorana and his co-workers introduced 1 the acid-sensitive 4,4′-dimethoxytrityl (DMTr) group for the protection of 5′-hydroxy functions in oligonucleotide synthesis. This protecting group has since been used very widely indeed, especially in the solid phase synthesis of oligonucleotides and their analogues. 2 Twenty-five years ago, we introduced 3 the 9-phenylxanthen-9-yl (Px) group 2a as an alternative to the DMTr protecting group 1. Unlike the corresponding 5′-O-DMTr derivatives (as in 3a), 5′-O-Px derivatives (as in 3b) of the commonly used N-acyl-2′-deoxyribonucleosides crystallise readily. 3 Another advantage of the Px group that was apparent from our original study 3 was that it is marginally (ca. 33%) more labile than the DMTr group in acetic acid–water (4:1 v/v) solution. This is advantageous because the glycosidic linkages of purine (especially 6-N-acyladenine) deoxyribonucleosides are readily cleaved under acidic conditions. Nevertheless, the Px group 2a has so far been used much less widely in the solid phase synthesis both of DNA and RNA sequences than the DMTr protecting group 1. One possible reason for this is that the required monomeric building blocks are not commercially available.

In a later study, we demonstrated 4 that the acid-lability of the Px protecting group could either be increased or decreased by the introduction of electron-donating or electron-withdrawing substituents. Of particular significance in the context of oligonucleotide synthesis was the observation that the introduction of a para-methyl substituent in the 9-phenyl residue (as in 2b) of the Px group increased its acid-lability without compromising its robustness. Thus 5′-O-[9-(p-tolyl)xanthen-9-yl]thymidine (5′-O-Tx-thymidine) 3c was converted 4 into thymidine (3; R = H) by treatment with trifluoroacetic acid (TFA) in dichloromethane–ethanol (95:5 v/v) solution at 23 °C ca. 2.5 times more rapidly than the corresponding 5′-O-Px derivative 3b. We now report a more detailed study of the comparative unblocking rates of the DMTr, Px and Tx protecting groups (1, 2a and 2b, respectively). In the deoxy-series, we chose the 5′-protected 3′-O-acetylthymidine derivatives 5a–c as model substrates and dichloroacetic acid as the unblocking agent. In order to measure the relative unblocking rates in solution, it is necessary to add a reagent that irreversibly scavenges trityl cations (i.e., DMTr+ and Px+). We have found pyrrole 6 to be particularly useful for this purpose and have demonstrated its efficacy as a rapid scavenger for Px+ and DMTr+ cations. 6,7 The pKₐ of pyrrole (∼0.27) is such that it is only partially protonated by dichloroacetic acid (pKₐ 1.25) and is incompletely protonated both by trichloro- and trifluoro-acetic acids (pKₐ 0.66 and 0.23, respectively). 8 Although ‘trityl’ scavengers may also be

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The 5'-O-DMT protecting group has also been used widely in the solid phase synthesis of RNA sequences. As the glycosidic linkages of ribonucleoside derivatives are much more resistant to acidic hydrolysis than those of the corresponding 2'-deoxyribonucleoside derivatives, concomitant depurination should not be a problem during the removal of the 5'-O-DMT group. A crucial aspect of oligonucleotide synthesis is the choice of the protecting group for the 2' hydroxy functions. The tert-butyldimethyl-silyl (TBDMS) 5, 1-(2-fluorophenyl)-4-methoxy-piperidin-4-yl (Fpmp) 6 and (tri-isopropyl-silyloxy)methyl (TOM) 7 groups are among the 2'-protecting groups that have commonly been used in combination with the 5'-O-DMT group in solid phase oligonucleotide synthesis. We have favoured the use of the Fpmp and related 1-aryl-4-alkoxy-piperidin-4-yl protecting groups. Recently, the 1-(4-chlorophenyl)-4-ethoxypiperidin-4-yl (Cpep) group 8, which has improved hydrolysis properties (i.e., greater stability at low and greater lability at high pH), has been developed as an alternative to the Fpmp group 6. A unique and most important property of the Fpmp, Cpep and related protecting groups is that their use allows chemically- and ribonuclease-stable 2'-protected oligonucleotides to be isolated in a pure state and then converted into fully-unblocked RNA sequences under mild conditions of acidic hydrolysis, such that cleavage and migration of the internucleotide linkages can occur only to a negligible extent. Although the Fpmp and Cpep groups have also been designed to resist hydrolytic cleavage under relatively strong acidic conditions, it is nevertheless desirable that the 5'-protecting group should be removable as rapidly as possible. We have therefore carried out a comparative unblocking study on a group of these 5'-protected (i.e., 5'-O-DMT, 5'-O-Px and 5'-O-Tx)-2'-O-Cpep-ribonucleoside derivatives 9a-c.

The ribonucleoside substrates used in this part of the study were the 5'-O-DMT-, 5'-O-Px- and 5'-O-Tx-derivatives (9a-c, respectively) of 4-N-benzyol-2'-O-[1-(4-chlorophenyl-4-ethoxypiperidin-4-yl)]-3'-O-levulinylcytidine 10. As the rate of cleavage of the Cpep protecting group 8 is virtually constant in the pH range 0.5–2.5, it seemed sensible to use a stronger acid than dichloroacetic acid in the unblocking reactions. These reactions (Scheme 1) were carried out by treating 0.025 M solutions of the substrates 9a-c with 6.0 mol equiv of trifluoroacetic acid and 15 mol equiv of

![Scheme 1](image-url)

**Scheme 1.** Reagents and conditions: (i) CF<sub>3</sub>CO<sub>2</sub>H, pyrrole, CH<sub>2</sub>Cl<sub>2</sub>, 0°C.
In conclusion, we recommend that the 5'-O-DMTr group 1 should be replaced either by the 5'-O-Px or by the 5'-O-Tx protecting group in the solid phase synthesis both of DNA and RNA sequences. In reaching this conclusion, it should be borne in mind that, if solid phase synthesis is to be carried out with 5'-O-DMTr- or 5'-O-Tx-protected phosphoramidites, it may be advisable to use a less acidic activating agent for the removal of the 5'-hydroxy functions with solid phase oligonucleotide synthesis. Indeed, in our original study involving the use of the 2'-O-Fpmmp protecting group in solid phase synthesis, the 5'-hydroxy functions were protected with the Px group. All subsequent work was carried out with 5'-O-DMTr-2'-O-Fpmmp-protected monomers as they were commercially available. As in the case of DMTr-protected building blocks, coupling yields obtained with Px (and presumably also with Tx)-protected building blocks can be assayed spectrophotometrically.

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References and notes

5. 5'-Protected 3'-O-acetylimidazolium derivatives 4a–e were chosen as model substrates rather than the corresponding 5'-protected thymidine derivatives 3a–e for two reasons. First, in solid phase oligonucleotide synthesis, the terminal nucleoside residues are always acetylated on their 3'-hydroxy functions and this would be expected to affect the 5'-unblocking rates. Secondly, HPLC analysis of the partially-unblocked mixtures was facilitated by using acetylated substrates. The three substrates 4a–e were all prepared in greater than 90% yields by treating the corresponding 5'-protected thymidine derivatives 3a–e with acetic anhydride in pyridine solution. Their 1H NMR spectra (360 MHz, in (CD$_3$)$_2$SO) are as follows: 4a [1.43 (3H, s), 2.04 (3H, s), 2.32 (1H, m), 2.44 (1H, m), 3.22 (1H, dd, J 3.9 and 10.4), 3.74 (6H, s), 4.07 (1H, m), 5.30 (1H, m), 6.22 (1H, dd, J 6.0 and 8.5), 6.09 (4H, d, J 7.9), 7.12–7.40 (9H, m), 7.53 (1H, s), 11.40 (1H, br s)] 4b [1.46 (3H, s), 2.03 (3H, s), 3.36 (1H, m), 2.50 (1H, m), 3.15 (1H, dd, J 3.6 and 10.3), 3.20 (1H, dd, J 3.0 and 10.5), 4.05 (1H, m), 5.29 (1H, m), 6.20 (1H, dd, J 6.1 and 8.3), 7.08–7.45 (13H, m), 7.58 (1H, s), 11.40 (1H, br s)] 4c [1.47 (3H, s), 2.02 (3H, s), 2.22 (3H, s), 2.34 (1H, m), 2.45 (1H, m), 3.13 (1H, dd, J 3.6 and 10.3), 3.18 (1H, dd, J 3.8 and 10.3), 4.04 (1H, m), 5.26 (1H, m), 6.19 (1H, dd, J 6.1 and 8.3), 7.12 (7H, m), 7.25–7.44 (5H, m), 7.58 (1H, s), 11.40 (1H, br s)].
9. The rates of the 5'-unblocking reactions were determined as follows. Pyrrole (0.10–0.21 mL, 6.5–3.0 mmol) was added to a solution of substrate 4a or 4c (0.10 mmol) and 2',3',5'-tri-O-acetyluridine (0.037 g, 0.10 mmol) in dichloromethane (2.0 mL). The stirred solution was cooled to 0°C and a precooled (to 0°C) 0.30–1.50 M solution of dichloroacetic acid in dichloromethane (2.0 mL) was added. After appropriate intervals of time, aliquots (0.1 mL) of the reaction solution were removed and basified with 0.7 M methanolic triethylamine. The samples were analysed by HPLC on a Jones C18 reversed phase column. Straight lines were obtained by plotting log$_{10}$ [% substrate remaining] against time. The times required for 50% unblocking ($t_{1/2}$) are indicated in Table 1.
18. Ribonucleoside substrates 9a–c were prepared in 86–90% yield from 4-N-benzoyl-2'-O-[1-(4-chlorophenyl)-4-ethoxy-piperidin-4-yl]-3'-O-levulinucleotide$^{10}$ and DMTr–Cl, Px–Cl and Tx–Cl, respectively. Their 1H NMR spectra (360 MHz, in (CD$_3$)$_2$SO) are as follows: 9a [0.96 (3H, t, J 7.0), 1.60 (1H, m), 1.83 (3H, m), 2.10 (3H, s), 2.56 (2H, m), 2.72 (2H, m), 2.89 (1H, m), 3.04 (1H, m), 3.13 (1H, m), 3.25 (3H, m), 3.56 (1H, m), 3.47 (1H, dd, J 4.1 and 10.7), 3.75 (6H, s), 4.20 (1H, m), 4.86 (1H, m), 5.27 (1H, m), 6.20 (1H, d, J 7.4), 6.92 (6H, m), 7.16–7.42 (12H, m), 7.52 (2H, m), 7.64 (1H, m), 8.01 (2H, m), 8.15 (1H, m), 11.40 (1H, br s); 9b [0.97 (3H, t, J 6.9), 1.64 (1H, m), 1.83 (3H, m), 2.10 (3H, s), 2.53 (2H, m), 2.70 (2H, m), 2.91 (1H, m), 3.03 (1H, m), 3.10–3.42 (6H, m), 4.18 (1H, m), 4.80 (1H, m), 5.18 (1H, m), 6.17 (1H, d, J 7.2), 6.93 (2H, d, J 9.1), 7.20 (2H, d, J 8.7)].
7.14–7.47 (16H, m), 7.53 (2H, m), 7.64 (1H, m), 8.01 (2H, m), 8.16 (1H, m), 11.38 (1H, br)]; 9c [0.93 (3H, t, J 7.0), 1.65 (1H, m), 1.83 (3H, m), 2.10 (3H, s), 2.26 (3H, s), 2.52 (2H, m), 2.70 (2H, m), 2.92 (1H, m), 3.04–3.42 (7H, m), 4.18 (1H, m), 4.79 (1H, m), 5.17 (1H, m), 6.17 (1H, d, J 7.2), 6.93 (2H, d, J 9.1), 7.11–7.47 (15H, m), 7.53 (2H, m), 7.64 (1H, m), 8.02 (2H, d, J 7.3), 8.17 (1H, m), 11.38 (1H, br)].

20. The experimental procedure used for the determination of the rates of 5'-deprotection of the ribonucleoside substrates 9a–c was essentially the same as that used above9 in the 5'-deprotection of the thymidine derivatives 4a–c.