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Synthesis of pentathymidylate using a 4-monomethoxytritylthio (MMTrS) group as a 5′-hydroxyl protecting group: toward oligonucleotide synthesis without acid treatment

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Abstract—A phosphoramidite unit having 4-monomethoxytritylthio as a new 5′-hydroxyl protecting group was prepared and employed in oligonucleotide synthesis. The new phosphoramidite enabled the synthesis of oligonucleotides without the use of acids such as TFA or DCA.

Several alkyl- or arylthio groups have been used for sulfenamide-type protection of various amino compounds.[1] In particular, protection of nucleic acid bases by the tritylthio (TrS)[2] group has been studied widely because it can be removed by treatment with mild neutral reagents such as tributyltin hydride[2a] and iodine.[2b,c] Although the property of the TrS group as an amino protecting group has been well studied, little is known about this protecting group as a hydroxyl protecting group in oligonulcleotide chemistry. For example, Bazin et al. reported the introduction of a TrS group to the 5′-hydroxyl group of an adenosine derivative, but the application to the oligonucleotide synthesis was not described.[3] In this paper we report 4-monomethoxytritylthio (MMTrS) as a new hydroxyl protecting group in oligonucleotide synthesis. The MMTrS group has an electron-donating methoxy group which is expected to accelerate the deprotection as compared to the conventional TrS group.

4-Monomethoxytritylsulfenyl chloride (**1**: MMTrSCl) used in this study was synthesized in 76% yield by treatment of 4-monomethoxytriphenylmethanethiol (MMTrSH)[4] with 1,3-dichloro-5,5-dimethylhydantoin (0.5 equiv.) in 1,4-dioxane at room temperature for 30 min.[5] Sulfuryl chloride used in the TrSCl synthesis[6] was not effective probably because use of the acidic chlorinating agent led to decomposition of the acid-labile MMTrSH. The MMTrS group was successfully introduced to the 5′-hydroxyl group of 3′-*O*-*tert*-butyldimethylsilylthymidine (**2**) in 76% yield by the selective sulfenylation of a 5′-alkoxy species generated by lithium hexamethyldisilazide (2.2 equiv.) with MMTrSCl (1.6 equiv.). Interestingly, the use of 1.1 equiv. of hexamethyldisilazane lithium in this reaction gave 3-*N*-MMTrS-3′-*O*-*tert*-butyldimethylsilylthymidine in 26% as a single product even after the prolonged reaction (15 h). The TBDMS group of **3**[7] thus obtained was removed by TBAF·H_2O and then the resulting 3′-hydroxyl group of **4**[8] was phosphitylated in 82% yield by treatment with chloro(2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphine[9] (1.5 equiv.) in the presence of 2.3 equiv. of *N*,*N*-diisopropylethylamine (Scheme 1).

Previously, Christodoulou et al. reported that 2,4-dinitrobenzenesulfenyl (DNBS) esters were not compatible with phosphoramidite functional groups because of the intramolecular nucleophilic reaction between the sulfur and the tervalent phosphorus atom.[10] However, it was found that the phophoramidite (**5**) was quite stable during the isolation process and in storage for months regardless of the presence of both the sulfenyl ester and the tervalent phosphorus functions. The stability of the MMTrS ester must be attributed to the steric bulk and the lower electron-withdrawing property of the MMTr moiety both of which reduce the electrophilicity of the sulfur atom of MMTrS as compared to that of the corresponding DNBS ester. The stability of the MMTrS group of **4** was evaluated under various conditions. The MMTrS group was stable in both aq NH_3–EtOH (3:1, v/v, 24 h, rt) and 1 M *tert*-BuOOH/CH_3CN

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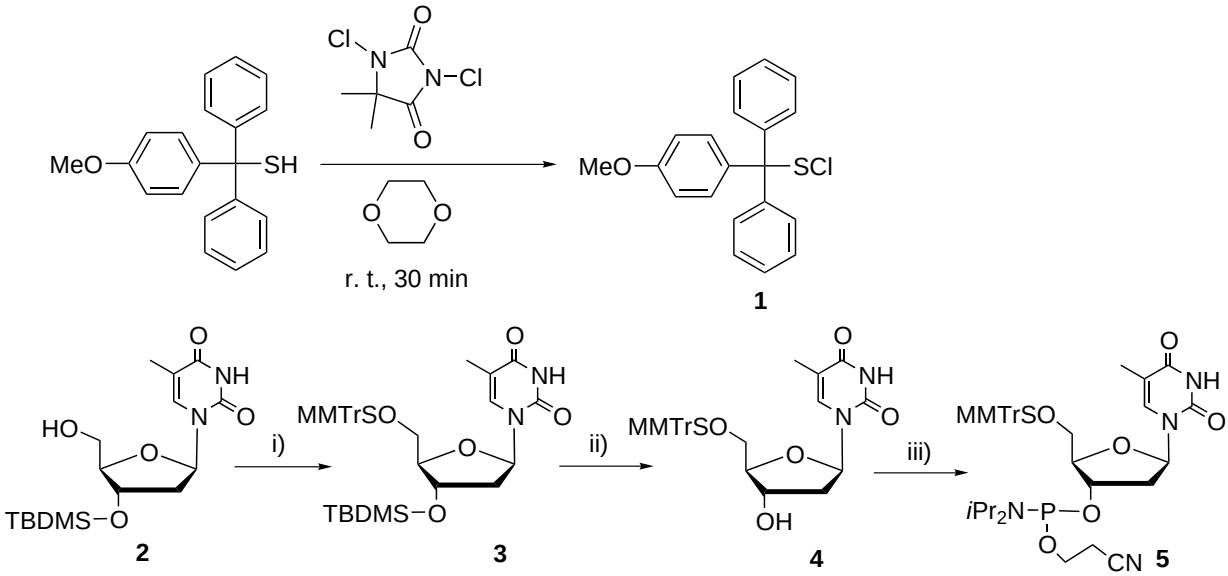
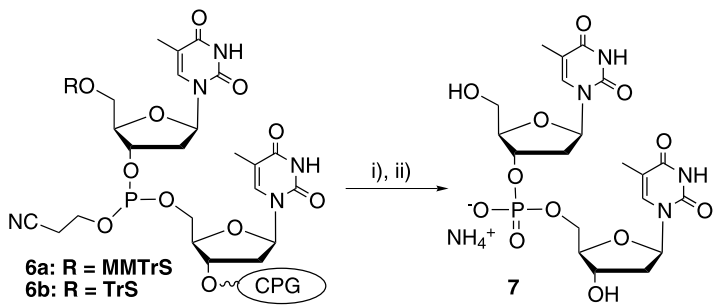


Scheme 1. *Reagents and conditions*: (i) lithium hexamethyldisilazide (2.2 equiv.), THF, rt, 25 min then **1** (1.6 equiv.), THF, rt, 1 h; (ii) TBAF·H_2O (1.2 equiv.), THF, rt, 1 h; (iii) *N*,*N*-diisopropylethylamine (2.3 equiv.), chloro(2-cyanoethoxy)-(diisopropylamino)phosphine (1.5 equiv.), CH_2Cl_2, rt, 1 h.

(20 min, rt), while it was removed rapidly to give thymidine by treatment with 0.1 M I_2/CH_3CN–pyridine–H_2O (10:9:1, v/v/v, $T_{comp}=1$ min).

The deprotection using iodine was further examined in the solid-phase oligonucleotide synthesis. The protected thymdine dimer **6a** was prepared on solid support as shown in Scheme 2 using commercially available 5′-DMTr-thymidine CPG support having a long chain alkylamino (LCAA) linker. The TrS-protected dimer **6b** was also prepared according to the procedure almost identical to that of **6a**. The dimers **6a** and **6b** were treated with 0.1 M I_2/CH_3CN–pyridine–H_2O (10:9:1, v/v/v) for 2 min and then with aqueous ammonia for 30 min. As shown in Fig. 1a, it turned out that the MMTrS group could be removed completely to give thymidylyl(3′–5′)thymidine (TpT) in 76% yield after reversed-phase HPLC purification in the case of **6a**. On the other hand, the TrS group of **6b** was not removed completely under the conditions examined here (Fig.



Scheme 2. *Reagents*: (i) 0.1 M I_2/CH_3CN–pyridine–H_2O (9:10:1, v/v/v); (ii) aq. NH_3.

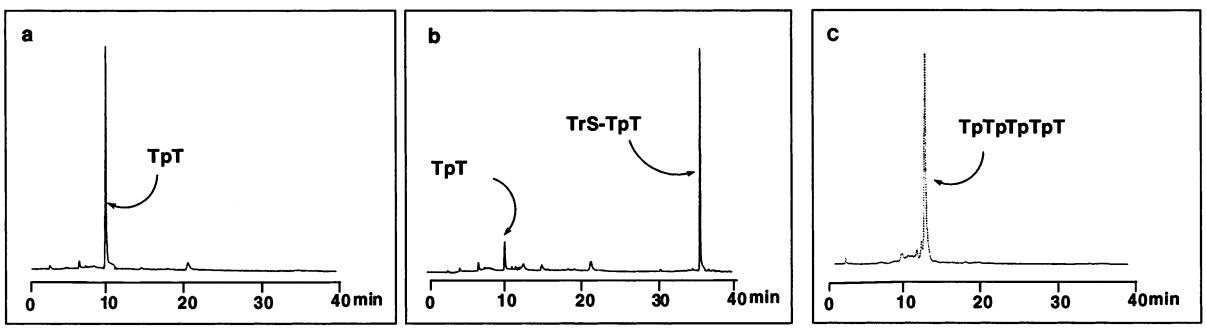
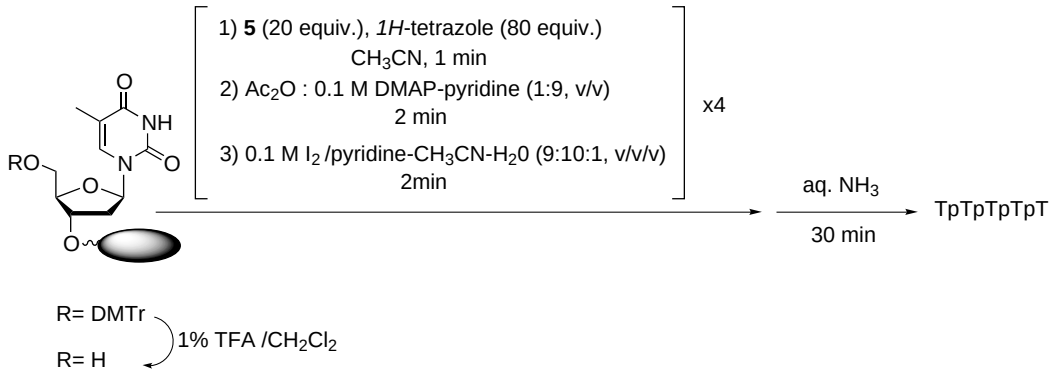


Figure 1. Reversed-phase HPLC profile of the deprotection of (a) **6a**, and (b) **6b** (Scheme 2); (c) reversed-phase HPLC profile of the pentathymidylate synthesized according to the procedure depicted in Scheme 3.



Scheme 3. Pentathymidylate synthesis by acid free chain elongation using phosphramidite **5**.

1b). Because the order of the reaction rate observed here is in good correlation with that of the stability of the corresponding trityl cations, we can hypothesize that the deprotection reaction proceeded via the trityl cation intermediate. While the direct detection of the trityl cation and reaction intermediates is necessary to confirm this reaction mechanism, it was difficult because the deprotection was carried out in aqueous media. Other kinetic study and model reactions in organic solvents are now under way to clarify the detailed reaction mechanism.

It should be noted that in the oligonucleotide synthesis depicted in Scheme 2, the deprotection of the MMTrS group and the oxidation of the phosphite intermediate could be carried out simultaneously. This simultaneous deprotection–oxidation reduced the reaction steps required for the correct oligonucleotide synthesis in addition to avoiding any acid treatment which may cause unfavorable side reactions during longer oligoDNA and RNA synthesis. The usefulness of phosphoramidite **5** was further evaluated by the pentathymidylate synthesis according to our acid-free procedure (Scheme 3). The reversed-phase HPLC profile of the pentathymidylate (Fig. 1c) clearly shows that the MMTrS-protected phosphoramidite **5** is applicable to oligonucleotide synthesis. The pentathymidylate was obtained in 48% yield after reversed-phase HPLC purification, and the structure was confirmed by MALDI-TOF mass spectroscopy.[11] An application of the MMTrS group to synthesize oligoDNA containing all four common nucleotides is now under way and will be reported in due course.

Acknowledgements

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References

1. (a) Craine, L.; Raban, M. *Chem. Rev.* **1989**, *89*, 689–712 and references cited therein; (b) Netscher, T.; Wellar, T. *Tetrahedron* **1991**, *47*, 8145–8154; (c) Ueki, M.; Honda, M.; Kazama, Y.; Katoh, T. *Synthesis* **1994**, 21–22.
2. (a) Seio, K.; Sekine, M. *J. Chem. Soc., Perkin Trans. 1* **1993**, 3087–3093; (b) Takaku, H.; Imai, K.; Nagai, M. *Chem. Lett.* **1988**, 857–860; (c) Alvarez, K.; Tworkowski, I.; Vasseur, J.-J.; Imbach, J.-L.; Rayner, B. *Nucleosides Nucleotides* **1998**, *17*, 365–378.
3. Bazin, H.; Heikkilä, J.; Chattopadhyaya, J. *Acta Chem. Scand. B* **1985**, *39*, 391–400.
4. MMTrSH was synthesized as follows: A suspension of 4-monomethoxytrityl alcohol (124 g, 0.43 mol) in toluene (2 L) was added Lawesson's reagent (104 g, 0.24 mol), and the resulting suspension was heated to give a dark-colored solution (ca. 75°C). The solution was cooled to room temperature and the precipitation was removed by filtration. The filtrate was washed with water (1 L) and the organic layer was dried with magnesium sulfate, filtered and evaporated under reduced pressure. The residue was chromatographed on silica gel column with hexane and the fraction was concentrated under reduced pressure. The residue was added to isopropyl ether and the resulting precipitation was filtered to give MMTrSH (80 g, 57%). ^{1}H NMR (270 MHz, $CDCl_3$): δ 3.06 (1H, s), 3.78 (3H, s), 6.80 (2H, d, $J=7.6$ Hz), 7.16 (2H, d, $J=7.6$ Hz), 7.26 (10H, m); ^{13}C NMR (67.8 MHz, $CDCl_3$): δ 55.27, 62.46, 112.95, 126.67, 127.67, 129.15, 130.39, 139.16, 147.31, 158.11.
5. The detailed synthetic procedure of MMTrSCl (**1**) is as follows: A solution of MMTrSH (10 g, 32.5 mmol) in dioxane (100 mL) was added 1,3-dichloro-5, 5-dimethylhydantoin (3.2 g, 16 mmol). The solution was stirred for 30 min, diluted with diethyl ether (200 mL), washed with water (100 mL) and then washed three times with saturated sodium bicarbonate (100 mL each). The organic layer was dried over magnesium sulfate, filtered, and evaporated under reduced pressure. The residue was treated with ethyl acetate, and the precipitate was collected by filtration to give **1** (7.2 g, 65%). ^{1}H NMR (270 MHz, $CDCl_3$): δ 3.81 (3H, s), 6.85 (2H, d, $J=8.9$ Hz), 7.23 (2H, d, $J=10.2$ Hz), 7.31–7.35 (10H, m); ^{13}C NMR (67.8 MHz, $CDCl_3$): δ 55.29, 71.82, 113.43, 127.70, 128.03, 129.74, 131.08, 133.24, 142.02, 159.05.

6. (a) Vorlander, D.; Mittag, E. *Chem. Ber. B* **1919**, *52*, 413–423; (b) Woulfe, S. R.; Miller, M. J. *J. Org. Chem.* **1986**, *51*, 3133–3139.
7. Spectral and analytical data for **3**: ^{1}H NMR (270 MHz, $CDCl_3$): δ 0.01 (6H, s), 0.83 (9H, s), 1.91 (4H, m, CH_3), 2.14 (1H, m), 3.40 (1H, d, $J=11.7$ Hz), 3.52 (1H, d, $J=11.7$ Hz), 3.69 (1H, m), 3.81 (3H, s), 4.08 (1H, m), 6.25 (1H, t, $J=8.2$ Hz), 6.83 (2H, d, $J=8.2$ Hz), 7.23–7.35 (12H, m), 7.48 (1H, s), 8.33 (1H, br); ^{13}C NMR (67.8 MHz, $CDCl_3$): δ –4.74, –4.62, 12.72, 17.93, 25.73, 41.09, 55.21, 71.83, 71.93, 84.81, 86.36, 110.88, 113.27, 127.33, 127.90, 129.69, 129.74, 130.94, 133.69, 135.43, 142.67, 142.70, 150.21, 158.66, 163.78. Anal. calcd for $C_{36}H_{44}N_2O_6SSi\cdot 1/4H_2O$: C, 64.98; H, 6.74; N, 4.21; S, 4.82. Found: C, 64.75; H, 6.15; N, 3.96; S, 4.16.
8. Spectral and analytical data for **4**: ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.95 (3H, s), 2.00 (1H, m), 2.25 (1H, m), 3.42 (1H, d, $J=11.7$ Hz), 3.62 (1H, dd, $J=11.7$ Hz, $J=2.3$ Hz), 3.73 (1H, m), 3.82 (3H, s), 4.07 (1H, m), 6.26 (1H, t, $J=6.6$ Hz), 6.85 (2H, d, $J=8.6$ Hz), 7.24–7.35 (12H, m), 7.51 (1H, s,), 8.33 (1H, br); ^{13}C NMR (67.8 MHz, $CDCl_3$): δ 12.79, 40.77, 55.33, 71.47, 72.11, 84.69, 85.76, 110.93, 113.37, 127.45, 128.00, 129.81, 130.97, 133.69, 135.42, 142.62, 142.71, 149.93, 158.79. Anal. calcd for $C_{30}H_{30}N_2O_6S\cdot 1/4H_2O$: C, 65.37; H, 5.58; N, 5.08; S, 5.82. Found: C, 65.29; H, 5.17; N, 4.70; S, 5.08.
9. Sinha, N. D.; Biernat, J.; McManus, J.; Köster, H. *Nucleic Acids Res.* **1984**, *12*, 4539–4557.
10. (a) Christodoulou, C.; Agrawal, S.; Gait, M. J. *Nucleosides Nucleotides* **1987**, *6*, 341–344; (b) Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1992**, *48*, 2223–2311.
11. The structure of the pentathymidylate was confirmed by MALDI-TOF mass spectroscopy. MALDI-MS $[M-H]^-$ calcd for $C_{50}H_{65}N_{10}O_{33}P_4$ 1457.27, found 1457.98.