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PHOSPHOTRIESTER APPROACH TO OLIGOTHYMIDYLATE SYNTHESIS UTILIZING A SIMPLE THYMIDINE UNIT AND A NEW TYPE OF CONDENSING AGENTS

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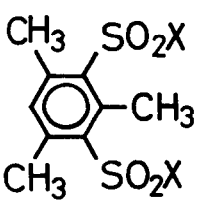
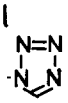
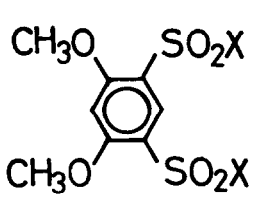
Summary: DMTrTp(SPh)$_2$ was proposed as a simplest "nucleotide unit" for the synthesis of olygothymidylates. One phenylthio group was removed selectively and rapidly from dithiol-esters by 1 M pyridinium hypophosphonate. Two kinds of arenedisulfonyl chlorides were newly prepared and utilized as promising condensing agents.

Oligonucleotide synthesis by the modern phosphotriester approach involves the preparation of the four common nucleoside derivatives bearing a phosphate group protected with two different protecting groups.[1] A wide variety of phosphate protecting groups have been explored in a lot of laboratories. However, the phosphotriester approach has still a crucial problem, i.e., sulfonylation of the 5'-hydroxyl of an oligonucleotide building block, which causes separation of the product from the sulfonylated byproduct much difficult.[2]

In this paper, we report a novel and convenient method for the synthesis of oligothymidylateds utilizing a thymidine unit bearing a 3'-phosphate group protected with the same two phenylthio groups and also describe a new type of condensing agents. In this study, we desighed initially that a condensing agent should have multiple roles of not only condensation but also an additional function that makes it easy to isolate the desired product from the byproducts and the condensing agent (or its hydrolyzed sulfonic acid derivative). Consequently, two new condensing agent, mesitylenedisulfonyl chloride (MDS)[3] and 4,6-dimethoxybenzene-1,3-disulfonyl chloride (DMS)[4], were found to be very effective for phosphorylation of nucleoside and internucleotidic bond formation and also to fulfill the above requirements.



MDS: X = Cl
MDSTe: X = 

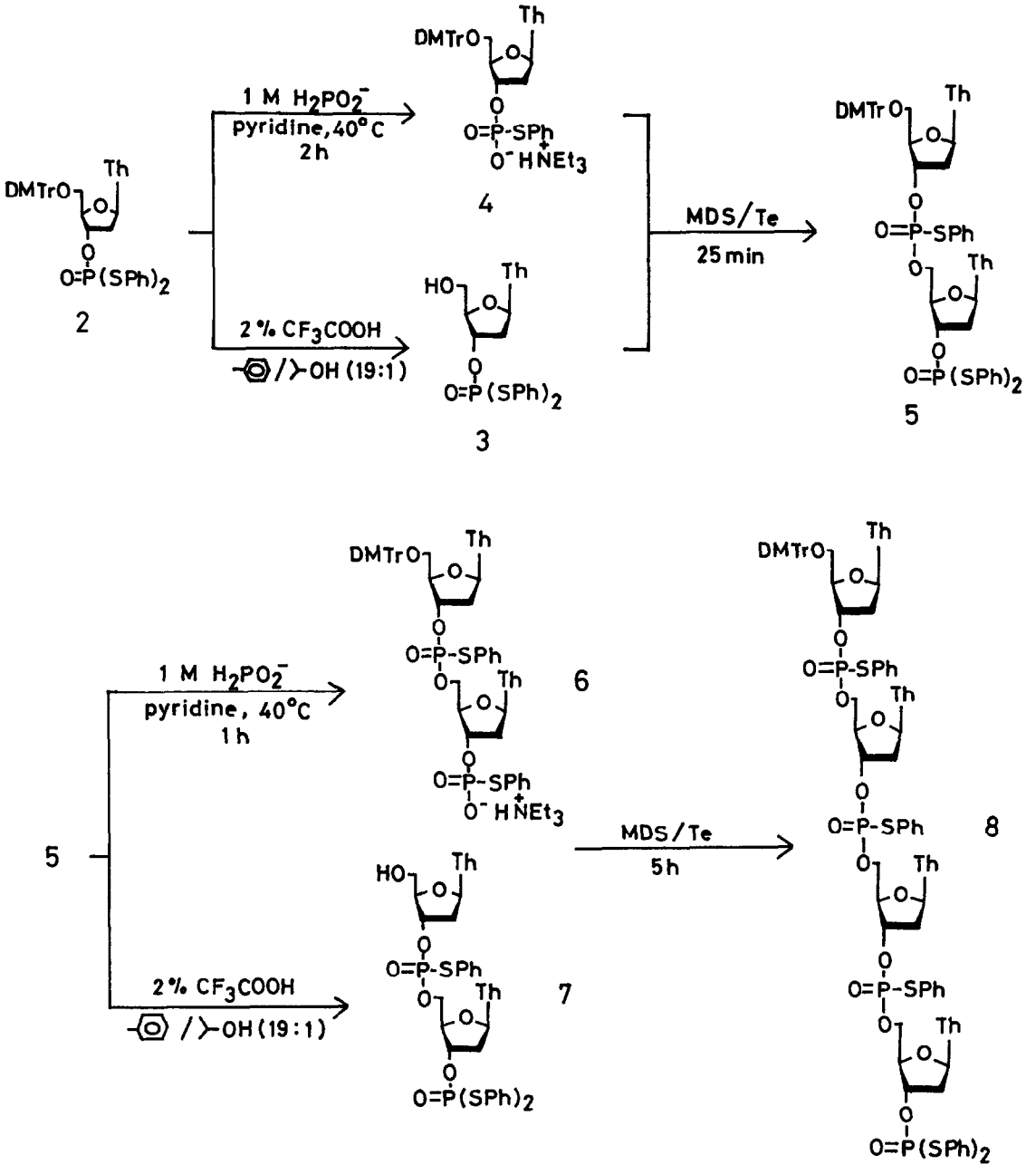


DMS: X = Cl
DMSTe: X = 

5'-O-Dimethoxytritylthymidine (2.72 g, 5 mmol) was allowed to react with cyclohexylammonium S,S-diphenyl phosphorodithioate (1)[5] (2.01 g, 5.5 mmol) in the presence of DMS (2.13 g, 6 mmol) in dry pyridine. The reaction was completed after 6 h and then quenched with ice (1 g). After stirring for 5 min, the mixture was extracted with CH_2Cl_2 (4 X 20 ml). The combined organic extracts were dried over Na_2SO_4, concentrated, coevaporated with toluene twice, and chromatographed on silica gel (CH_2Cl_2-MeOH) to afford a thymidine unit (2) in 87% yield. During the workup, the excess DMS was hydrolyzed to the dipyridinium sulfonate derivative which came clearly into the aqueous layer after the extraction so that the DMTr group remained intact because of the absence of acidic substances in the CH_2Cl_2 extracts. The analytically pure product was easily obtained within 2 h. Therefore, a relatively large scale synthesis of the unit might be possible. The DMTr group could be removed from 2 conveniently by treatment with 2% CF_3COOH in toluene-iPrOH (19:1, v/v) at room temperature for 10 min followed by removal of the solvents and CF_3COOH (bp 72°C) by rapid evaporation whereupon the latter was removed faster than toluene. Therefore, the extraction procedure can be eliminated and the detritylated product (3) could be obtained in 96% yield by flash column chromatography[6] or simply by precipitation from the CH_2Cl_2 solution into hexane-ether.

In a previous paper,[5] we reported the mild and selective removal of one phenylthio group from appropriately protected thymidine S,S-diphenyl phosphorodithioates by use of pyridinium phosphonate. It is now found that pyridinium hypophosphonate (PHP) is much more effective for removal of phenylthio group than pyridinium phosphonate.[7] The use of PHP in pyridine shortened dramatically the time for removal of the phenylthio group as compared with the latter. Thus, the selective removal of one phenylthio group from 2 could be achieved by using 1 M PHP in pyridine at 40°C for 2 h to give the diester (4) as triethylammonium salt in 92% yield. *It is noteworthy that under the conditions both the DMTr group and the internucleotidic phenylthio group were stable.*

These findings led us to study a new phosphotriester approach using 2 as the simplest unit among those previously reported.[2] In our preliminary studies, the condensation of 4 with 3 was carried out by using the corresponding di-tetrazolide derivatives (DMSTe) and (MDSTe). However, purification and drying of these reagents *often led to explosions.* Jay[2d] has recently reported that a combined use of arenesulfonyl chloride and tetrazole was superior to arenesulfonyltetrazole itself in points of coupling yield and supression of 5'-sulfonylation. Therefore, we used a combined use of DMS/tetrazole or MDS/tetrazole for coupling reactions. Both combinations were effective but MDS/tetrazole resulted in the optimum yield (86%) of the dimer (5) when 4 (98 mg, 0.12 mmol) was coupled with 3 (51 mg, 0.1 mmol) by using MDS (84 mg, 0.3 mmol) and tetrazole (42 mg, 0.6 mmol). The reaction proceeded rapidly and was completed within 25 min. In this reaction, the dimer 5



was isolated quite easily as pure material since a spot corresponding to the 5'-sulfonylated byproduct might be converted to more polar pyridinium sulfonate derivative by hydrolysis of the remaining chlorosulfonyl group and/or tetrazoylsulfonyl group during the workup. In a simlar manner, the selective deprotection of phenylthio group or DMTr group from the dimer 5 was perfomed.

Thus, the dimer brocks (6) and (7) were obtained in 97 and 94% yields, respectively. The condensation of 6 with 7 by using MDS/tetrazole for 5 h gave the tetramer (8) in 80% yield. Deprotection of all the protecting groups from 8 was performed rapidly as follows: 1) 0.2 M NaOH-dioxane (1:1, v/v) at room temperature for 5 min followed by neutralization with Dowex 50W X 2 (PyH^+ form); 2) I_2 (30 equiv.) in pyridine-H_2O (2:1, v/v) at room temperature for 15 min followed by removal of excess iodine by extraction with ether; 3) 80% CH_3COOH at room temperature for 5 min. Thus, TpTpTpTp was isolated in 80% yield after chromatography

$$8 \xrightarrow[5\ \text{min}]{OH^-} \text{DMTrTpTpTpTp(SPh)} \xrightarrow[15\ \text{min}]{I_2} \text{DMTrTpTpTpTp} \xrightarrow[5\ \text{min}]{H^+} \text{TpTpTpTp}$$

using Whatman 3 MM paper (nPrOH-conc.NH_4OH-H_2O, 55:10:35, v/v/v). The tetramer was completely degraded by spleen phosphodiesterase to give a single spot of Tp.

Further extension of this work is now in progress.

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4) DMS was prepared as follows: The reaction of 1,3-dimethoxybenzene (0.2 mol) with chlorosulfonic acid (0.56 mol) in CH_2Cl_2 at -5°C to room temperature for 2 h gave 4,6-dimethoxybenzene-1,3-disulfonic acid in 98% yield. After the acid was converted to dipyridinium salt by addition of pyridine, the salt (50 mmol) was heated with phosphorus pentachloride (120 mmol) at 60°C for 4 h to give DMS (98%): mp 175-178°C (decomp). Calcd for $C_8H_8Cl_2O_6S_2$: C, 28.67; H, 2.40; Cl, 21.16%. Found: C, 28.90; H, 2.37; Cl, 21.11%.

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6) Isolation of all the products described in this paper was carried out by using a modified method of "medium pressure column chromatography" reported by W. C. Still, M. Kahn, and A. Mitra, J. Org. Chem., 43, 2923 (1978).

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