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AN EFFICIENT APPROACH TOWARD THE SYNTHESIS OF PHOSPHOROTHIOATE DIESTERS *VIA* THE SCHÖNBERG REACTION

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ABSTRACT: Easily accessible phenacetyl or benzoyl disulfide proved to be very convenient reagents for a rapid P-sulfurization of phosphite-triesters and H-phosphonate diesters, respectively.

Phosphorothioate (PS) analogues of nucleotides are useful probes to study phosphoryl and nucleotidyl transferring enzymes[1,2]. Further, PS analogues of nucleic acids are of great interest because of their potential use as anti-sense *inhibitors*[3].

The introduction of an internucleotide PS diester can be realized in two ways: i.e., *via* a phospho- or a phosphite-triester approach. In the former case the PS function is either introduced in one step *via* phosphorothioylating reagents (e.g., 2,5-dichlorophenyl phosphorodichloridothioate[4] or its analogue **14**[5]), or a two-step process involving phosphorylation with an O-aryl-N-phenylamidophosphorochloridate[6,7] followed by a PN→PS conversion. In the second approach, thioylation is effected by treating a phosphite-triester with elemental sulfur[8,9]. This process is however, due to the insolubility of S_8 in most organic solvents, not completely satisfactory. The latter is especially disadvantageous[10] in an automated solid-support-directed synthesis of DNA in which one or more phosphite-triester(s), purposely devised[11] for the rapid formation of internucleotide phosphodiester bonds, have to be converted into PS functions.

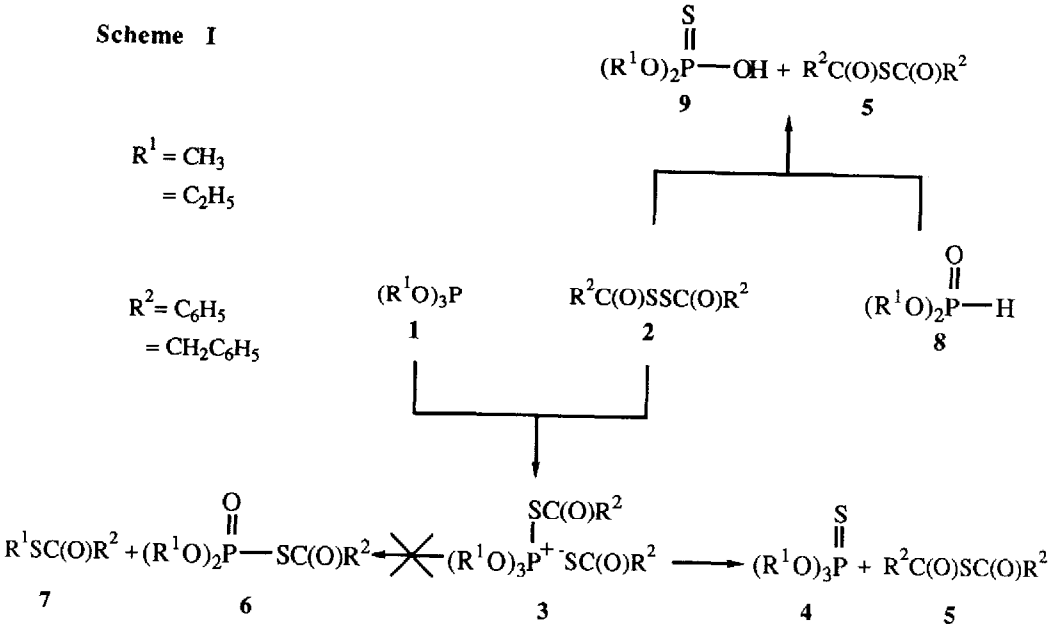
We now report that phosphite-triesters or H-phosphonate-diesters can be rapidly and easily sulfurized with acyl disulfides.

In 1935, Schönberg reported[12] in a note that triphenylphosphine reacted smoothly with benzoyl disulfide to give triphenylphosphonothioate and benzoyl sulfide. We expected that the Schönberg reaction could be an attractive alternative for the sulfurization of phosphite-triesters with S_8. In order to extend the scope of the Schönberg reaction, we first treated (Scheme I) triethyl phosphite (**1**, R^1=Et) with a solution of phenacetyl disulfide[13] (**2**, R^2=$CH_2\Phi$, 1.1 eq.) in 1,2-dichloroethane (DCE) at 20°C. Monitoring of the sulfurization by ^{31}P-NMR revealed rapid (5 min) and exclusive formation of triethylphosphorothioate **4** (R^1=Et, δp 67.8 ppm). Further, analysis of the crude reaction mixture by ^{1}H- and ^{13}C-NMR confirmed the exclusive formation[14] of **4** (R^1=Et) and phenylacetyl sulfide **5**. Work-up of the reaction mixture gave, after distillation, homogeneous **4** (R^1=Et) in 70% yield. We also established, following the same experimental and analytical procedure, that trimethyl phosphite (**1**, R^1=Me) was quantitatively converted into **4** (R^1=Me, δp 73.3 ppm) and **5** (R^2=$CH_2\Phi$). The outcome of the latter experiment clearly indicates that sulfurization of **1** (R^1=Me) is not accompanied by an Arbusov reaction which would afford the products **6** (R^1=Me, R^2=$CH_2\Phi$) and **7**. Further, the above obtained results also indicate that the formation of intermediate **3**, as proposed by Schönberg, is the rate determining step of the reaction.

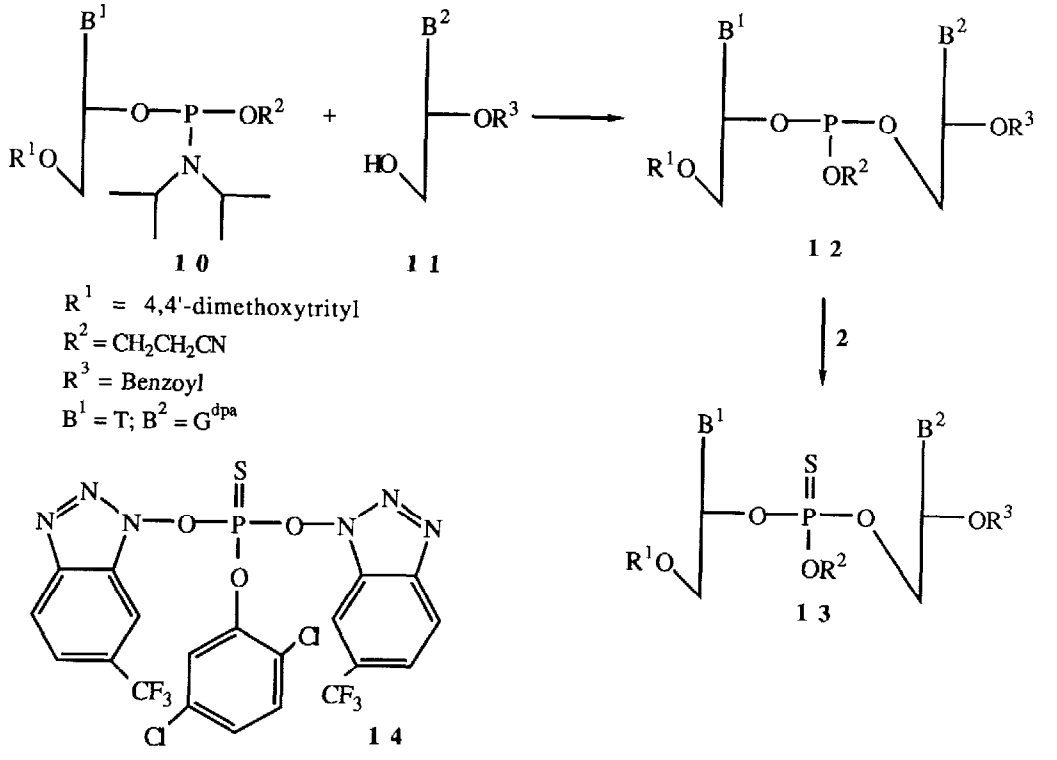
The applicability of the thioylation reaction was further illustrated (Scheme I) by the conversion of dimethyl phosphite **8** into the PS derivative **9**. Sulfurization of H-phosphonate diesters of nucleic acids using S_8 has been reported earlier[15,16] and requires the presence of a weak nonnucleophilic base. Treatment however of **8** (R^1=Me) with **2** (R^2=$CH_2\Phi$) in the presence of diisopropylethylamine (DIPEA) gave rise to an intense colouring of the reaction mixture. The latter could be overcome by using benzoyl disulfide[13] as the sulfurization reagent. Thus to a solution of **8** (R^1=Me, 0.12 mmol) in DCE (1.8 ml) was added **2** (R^2=C_6H_5, 0.36 mmol) and DIPEA (0.1 ml). Work-up of the colourless solution, after 30 min at 20°C, afforded homogeneous **9**[14] (Na^+- salt, δp 59.1 ppm, $^2J_{PH}$=12Hz) in a yield of 95%.

The use of the sulfurization reaction was further demonstrated by the preparation in solution and on a solid-support

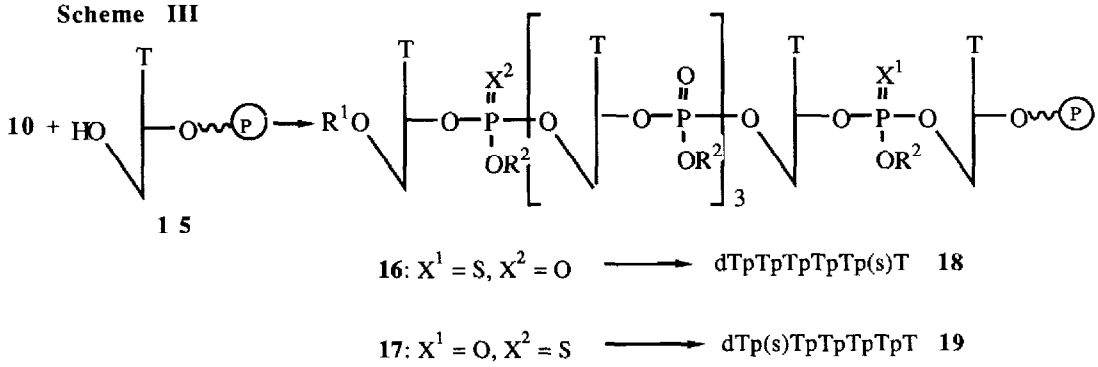
Scheme I



Scheme II



Scheme III



of PS containing DNA fragments. For instance, sulfurization of phosphite-triester **12** (0.44 mmol), which was prepared by 1H-tetrazole-mediated coupling of phosphoramidite **10** with **11** according to Köster et al.[17], with **2** (R^2=$CH_2\Phi$, 0.16 mmol) in DCE/2,4,6-collidine, for 5 min at 20°C, furnished fully-protected dimer **13** (δp 67.6 and 67.4 ppm) in 90% yield. Dimer **13** was completely deblocked by ammonolysis (aq. 25% NH_3, 50 h, 60°C) followed by acidic hydrolysis (80% HOAc, 30 min, 20°C) to afford, after purification (Sephadex A-25), dimer d-Tp(s)G. The ^{1}H- and ^{31}P- NMR spectra[14] of dimer thus isolated were identical with those of the same product prepared by using the phosphorothioylating reagent **14**. Finally, the PS containing hexamers **18** and **19** were assembled using an automated Gene Assembler (Pharmacia) in which stepwise elongation of immobilized (succinyl linkage) deoxythymidine **15** (Scheme III) was accomplished[17] by 1H-tetrazole-catalyzed coupling of the incoming phosphoramidite **10** followed by oxidation (I_2-H_2O) of intermediate phosphite-triester, and subsequent acidolysis of the 4,4'-dimethoxytrityl (R^1) group. Sulfurization in stead of oxidation was *in each case executed by treating the appropriate phosphite-triester intermediate with a solution (5%) of* **2** (R^2=$CH_2\Phi$) *in* DCE/2,4,6-collidine for 5 min at 20°C. According to this protocol immobilized and fully-protected hexamers **16** and **17** were obtained. Complete deblocking of **16** and **17**, as mentioned earlier for the preparation of dimer dTp(s)G, furnished, after purification (Sephadex G-50), hexamers **18**[14] and **19**[14], respectively. Analysis of crude **18** and **19** by FPLC revealed in each case the absence[18] of the non-PS containing hexamer: thus indicating the high efficiency of the sulfurization step. Further, ^{1}H-, and ^{31}P-NMR data of purified **18** and **19** were in excellent agreement with those recorded for the same hexamers prepared in solution using reagent **14** for the introduction of the PS functions.

In conclusion, the sulfurization procedure reported here will be of great value for the preparation of PS analogues of nucleic acids and other naturally occurring phosphate-diester(s) containing compounds.

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10. For example thioylation of an internucleotide methyl phosphite-triester present in an immobilized DNA fragment could be accomplished [B.A. Connolly et al., Biochemistry, **23**, 3443 (1984)] with a suspension of elemental sulfur in pyridine and gently shaking of the mixture for 2 h at 20°C, followed by washing the solid support extensively with CS_2/pyridine. On the other hand, in a similar solid-support-directed synthesis of DNA the thioylation step could be driven to completion in 15 min at 60°C [W.J. Stec et al., J. Am. Chem. Soc., **106**, 6077 (1984)], or in 9 min at 20°C [C.A. Stein et al., Nucl. Acids Res., **16**, 3209 (1988)] by using S_8 in 2,6-lutidine or a solution of S_8 in pyridine and obnoxious CS_2, respectively.

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14. Relevant NMR data in ppm of compounds: **4**(R^1=Et) ^{1}H-NMR($CDCl_3$) δ 4.13 (dq, 6H, CH_2, $^2J_{PC}$=9.6 Hz), 1.33 (t, 9H, CH_3); ^{13}C-NMR ($CDCl_3$) δ 63.3 (d, CH_2, $^1J_{PC}$=4 Hz), 15.2 (d, CH_3, $^3J_{PC}$=7 Hz). **5** (R^2=$CH_2\Phi$) ^{1}H-NMR $CDCl_3$) δ 7.3 (m, 5H, C_6H_5), 3.99 (s, 2H, CH_2); ^{13}C-NMR ($CDCl_3$) δ 191.3 (CO), 131.6 (C^1-Ar), 129 (C^2-Ar), 128.1 (C^3-Ar), 127.0 (C^4-Ar), 51.3 (CH_2). **9** (R^1=Me) ^{1}H-NMR (D_2O) δ 3.61 (d, 6H, CH_3, $^2J_{PC}$=16 Hz); ^{13}C-NMR (D_2O) δ 53.7 (d, CH_3, $^1J_{PC}$=6 Hz). dTp(s)G (mixture of diastereoisomers) ^{1}H-NMR (D_2O) δ 8.10 and 8.07 (2xs, 2x1H, H^8-G), 7.46 and 7.43 (2xs, 2x1H, H^6-T); ^{31}P-NMR (D_2O) δ 55.9 and 55.2. Hexamer **19** (Na^+- salt), ^{31}P-NMR (D_2O) δ - 0.5 (PO) and + 56.0 (PS) in a ratio of 4:1, respectively. Hexamer **18** (Na^+- salt), ^{31}P-NMR (D_2O) δ - 0.3 (PO) and 55.8 + 56.1 (PS) in a ratio of 4:1, respectively.

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18. Fast protein liquid chromatography (FPLC-Pharmacia; column MONO Q HR 5/5; eluens, buffer A 0.01M NaOH (pH 12) and buffer B 1.2 M NaCl in buffer A; flow rate 2 ml/min; UV-detection at 254 nm) analysis showed a retention time of 10.7 min for hexamers **17-18**, and 8.9 min for $dTp(Tp)_4T$.

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