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# THE APPLICATION OF 2-(4-CHLOROPHENYL)-SULFONYLETHOXYCARBONYL (CPSEC) GROUP IN THE SYNTHESIS OF A DNA SEGMENT USING THE PHOSPHOTRIESTER APPROACH

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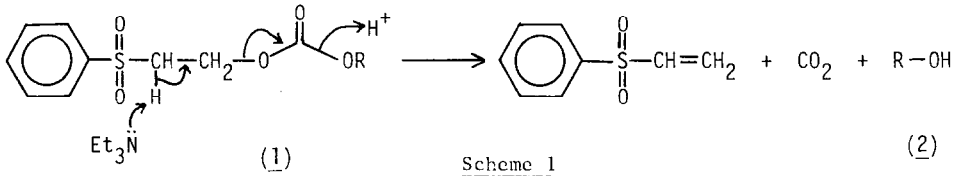
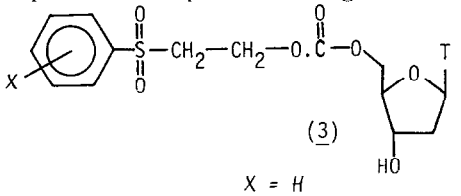
Abstract: The new base labile CPSEC group has been successfully employed for the protection of the 5'-hydroxyl function to synthesize a "universal Stop codon" DNA sequence: $^{5'}$d(TCAATCAATCA)$^{3'}$.

We have recently introduced the 2-Phenylsulfonylethoxycarbonyl(PSEC) group[1] for the protection of the hydroxyl function and demonstrated its use in presence of a variety of acid and base labile protecting groups. The structural feature of the PSEC-group permitted us to deblock the PSEC-derivatives, as in (1), to generate the free alcohol general formula (2); Scheme 1) with the help of $Et_3N$ (15 eq., in dry pyridine soln., 15 ml/mmol, 20 h. at 20°C), a volatile non-nucleophilic tertiary base. It was conceived at the outset of the present work that it should be possible to introduce electron-withdrawing or electron-donating substituent in the benzene ring of the PSEC-group to control the acidity of the proton adjacent to the sulfonyl function which would, in turn, steer



(1) Scheme 1 (2)

"the stability versus the lability" of a protecting group in question. Thus the Table 1 clearly illustrate the realization of this viewpoint through the relative rates of deprotections, following the mechanism depicted in the Scheme 1, with a series of substituted 2-arylsulfonylethylcarbonate derivatives of thymidine (3)[4]. The choice of using any one of the potential protecting groups of the Table 1 in the oligonucleotide synthesis, following the phosphotriester approach with the o-chlorophenyl group for the internucleotide phosphate protection[8], would clearly depend upon the fulfilment of the following conditions: (1) the stability of the fully protected oligonucleotide with the new base labile 5'-O-protecting group, during the ordinary manipulations, work-up procdures and purification process through standard silica gel chromatography, should permit isolation



(3)

*Deprotection condition:* $Et_3N$ *(15 eq.) in dry pyridine (15 ml/ mmol) at 20°C.*

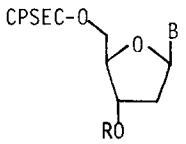
| | $t_{1/2}$ (min.) | $t_{\infty}$ (min.) |
|---|---|---|
| X = H | 180 | 1200 |
| X = 4-Methyl- | 1140 | - |
| X = 4-Chloro- | 60 | 300 |
| X = 4-Nitro- | 10 | 60 |

Table 1

of the intermediates in high yields; (2). The removal condition of the new base labile 5'-O-protecting group should not contribute to the degradation of any other base labile protecting groups, like o-chlorophenyl in the internucleotide junctions and N-acyl protecting groups on exocyclic amino functions of adenine, guanine and cytosine residues, in the protected oligonucleotide fragments. However, it is the latter condition[11] which is much more difficult to fulfil especially when the chain length of the fully protected oligonucleotide becomes longer.

Thus, after a careful evaluation of the preliminary results of our studies with the substituted 2-arylsulfonylethylcarbonate derivatives in the Table 1, we realized that the 2-(4-chlorophenyl)-

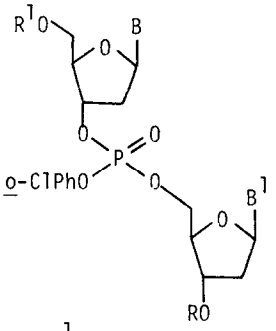
sulfonylethylcarbonate (CPSEC) group at the 5'-position would indeed satisfy the above requirements. We would, therefore, like to demonstrate the application of the new CPSEC-group in the synthesis of an undecamer long DNA segment, $^{5'}$d(TCAATCAATCA)$^{3'}$, through the phosphotriester approach using a strategy originally developed by Chattopadhyaya and Reese[2]. The dimer blocks (7) and (8) were synthesized in 66.7 and 61.0% yields (powders) through a "One pot" synthesis using an excess of 1-methylimidazole (16 eq.)[1,3,7]. The 5'-O-CPSEC-2'-O-deoxyribonucleoside blocks[4] (3) to (5) were prepared using a standard procedure available in the literature[2] for the selective acylation at the 5'-position of 2'-deoxyribonucleosides. The 5'-hydroxy-3'-O-pixyl-6-N-m-ClBz-2'-deoxyadenosine[4,5] which was used as a 3'-terminal block was prepared in 87.5% yield after the removal of the CPSEC-group from (6)[4] by the treatment of $Et_3N$ (15 eq.) in dry pyridine (15 ml/mmol) soln. at 20°C for 5 h. The phosphodiester functions in (9) and (10) were introduced by reacting (7) and (8) (for expts. 1-4 in the Table 3) with o-chlorophenylphosphorobis-(1,2,4-triazolide) followed by $Et_3N$-$H_2O$ treatment[2,6,7]. The 5'-protected hexamer phosphodiester salt (expt. 5 in Table 3)was prepared,

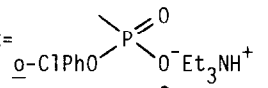
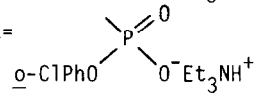



(3) R=H, B=T
(4) R=H, B=C
(5) R=H, B=A
(6) R=Px,B=A

Px=Pixyl(9-phenylxanthen-9-yl)
T =Thymin-1-yl
A =6-N-m-chlorobenzoyladenin-9-yl
C =4-N-benzoylcytosin-1-yl

CPSEC= 2-(4-Chlorophenyl)-sulfonyl-ethoxycarbonyl- (CPSEC) group




(7) $R^1$=CPSEC, R=H, B=T, $B^1$=C
(8) $R^1$=CPSEC, R=H, B=$B^1$=A
(9) is same as (7) except R= 
(10) is same as (8) except R= 

after the removal of the pixyl group[5], in an usual way[2,6,7].The 5'-hydroxy components(for the expts 1-5 in Table 3) were prepared by two separate methods (Table 2). The first method involves the action of $Et_3N$ (15 eq.) in dry pyridine (10 ml/mmol) at 20°C for 5 h and the second method involves the treatment of 0.04 M $K_2CO_3$ in dioxan: pyridine: water; 1:1:2 v/v/v at 20°C. The Table 2 records the yields of the actual isolation of the pure 5'-hydroxy compounds in the form of powders

Table 2: *Deprotection of 5'-CPSEC-group to prepare the 5'-hydroxy blocks*

| *Fully protected DNA segments*[a] | *% yields of the 5'-hydroxy compounds (powders)* $ET_3N$-*Pyridine condition, 5h.* | 0.04M $K_2CO_3$ *condition:* | *Reaction time*[b] |
|---|---|---|---|
| 1. d(CPSEC-A-O-Px) | 87.5 | 84.2 | 1 min. |
| 2. d(CPSEC-TpC-O-Px) | 83.5 | 82.0 | 1 min. |
| 3. d(CPSEC-TpCpA-O-Px) | 85.4 | 77.5 | 1 min. |
| 4. d(CPSEC-ApApTpC-O-Px) | 79.0 | 74.0 | 2 min. |
| 5. d(CPSEC-ApApTpCpA-O-Px) | 84.5 | 71.0 | 2 min. |
| 6. d(CPSEC-TpCpApApTpCpApApTpCpA-O-Px) | 78.0 | 66.0 | 3 min. |

[a] Dry precipitated powder was used for each set of deprotection study
[b] These are the optimized reaction period at 20°C.

at the end of the deprotection procedures and, thus, clearly illustrate the advantage of $Et_3N$-pyridine deblocking condition over the alkaline hydrolytic condition. It is also noteworthy in the Table 2 that the reaction period for 0.04 M $K_2CO_3$ promoted deblocking condition has been remarkably shortened due to the increased lability of the CPSEC-group over the PSEC-[1] (Table 1 for the direct comparison of the deprotection rates). This shorter reaction period for deblocking the CPSEC-group under the alkaline hydrolytic condition has indeed reduced the degradation of the fully protected oligomer due to the removal of o-chlorophenyl groups from the internucleotide junctions and thus has resulted in improved yields of 5'-hydroxy blocks. This point has been well clarified through the preparations of 5'-hydroxy dimer, tetramer and octamer components respectively from the 5'-O-PSEC protected di-, tetra-, and octa-thymidylic acids in 82.4, 64.3 and 47.7% yields by the treatment of 0.04 M $K_2CO_3$ in aq. dioxan (1:1 v/v) treatments (6 min. for $T_2$ and $T_4$ and 8 min. for $T_8$ at 20°C)[1]. The Table 3 records the reaction conditions for all block condensations in dry pyridine solution in presence of 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole[2,7,8] and the composition of EtOH in the $CHCl_3$ phase to purify the fully protected DNA blocks by column chromatography on silica gel. It is also clearly evident from the isolated yields of the fully protected DNA segments in Table 3 that the CPSEC-group is indeed remarkably stable during the standard work-up[2,6,7]

Table 3: *Condensation reactions[b] leading to fully protected DNA fragments.*

| Expt. no. | 5'-Protected components (mmol)[a,c] | 5'-Hydroxy components (mmol)[a,c] | Fully protected DNA segments[a] ($R_f$)[d],(% yield)[c] | %EtOH-$CHCl_3$ to elute[e] |
|---|---|---|---|---|
| 1. | d(CPSEC-TpCp) (0.17) | d(HO-A-O-Px) (0.14) | d(CPSEC-TpCpA-O-Px) (0.40),(78.6%) | 5.0 |
| 2. | d(CPSEC-ApAp) (0.10) | d(HO-TpCpA-O-Px) (0.085) | d(CPSEC-ApApTpCpA-O-Px) (0.31),(87.3%) | 5.0 |
| 3. | d(CPSEC-ApAp) (0.12) | d(HO-TpC-O-Px) (0.10) | d(CPSEC-ApApTpC-O-Px) (0.36),(90.0%) | 5.0 |
| 4. | d(CPSEC-TpCp) (0.10) | d(HO-ApApTpC-O-Px) (0.070) | d(CPSEC-TpCpApApTpC-O-Px) (0.30),(72.4%) | 8.0 |
| 5. | d(CPSEC-TpCpApApTpCp) (0.012) | d(HO-ApApTpCpA-O-Px) (0.011) | d(CPSEC-TpCpApApTpCpApApTpCpA-O-Px) (0.20),(63.6%) | 11.0 |

[a] 9-Phenylxanthen-9-yl[5]is abbreviated to Px(pixyl), 6-N-(m-chlorobenzoyl)-2'-deoxyadenosine, 2-N-(p-t-butylbenzoyl)-2'-deoxyguanosine and 4-N-benzoyl-2'-deoxycytidine are represented as A,G and C respectively. Internucleotide phosphotriester is protected with o-chlorophenyl group. 3'-terminal p indicate o-chlorophenylphosphodiester triethylammonium salt.

[b] Dry pyridine (10 ml/mmol) and 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole (5 eq. for 1+1, 10 eq. for 2+1, 2+2, 2+3 and 20 eq. for 5+6 condensations) were respectively used as solvent and the condensing agent (see ref. 2 and 7 for details of the reaction conditions).

[c] Isolated as powders after precipitating a $CHCl_3$ soln.(1-2ml.) of the compound from pet.ether

[d] 10% MeOH-$CHCl_3$ was used for TLC on pre-coated Merck silica gel 60 $F_{254}$ plates.

[e] 2cm. X 2cm. silica gel column (merck Kiesel gel H) was used for short column chromatography (B.J. Hunt and W. Rigby. Chem. & Ind. 1868 (1967)).

(satd. $NaHCO_3$, pH ca. 8.3, extractions etc.) and purification procedure. Finally the fully protected undecamer (expt. 5 in the Table 3) was deprotected in the following order: (1) replacement with the benzoyl groups at the 3'- and the 5'-ends after the removal of the pixyl[5]- and CPSEC-group respectively; (2) treatment with syn-4-nitrobenzaldoximate ions[8]; (3) aq. $NH_3$ (d0.9) for 70h. at 20°C. The volatile matters were then removed and the residue was dissolved in pure water (5 ml) and partitioned with $CH_2Cl_2$ (10x5ml). The aqueous phase was then concentrated and examined by HPLC (Permaphase AAX at 65°C, linear gradient: 0.01 M $KH_2PO_4$, 0.0 M KCl to 0.05 M $KH_2PO_4$ and 0.4 M KCl, pH 4.45) and the main peak in the elution profile (Fig. 1) contained 82.0% of $A_{260}$ O.D. units. An aliquot of this material was $^{32}P$-labelled at the 5'-end with $^{32}P$-$\gamma$-ATP

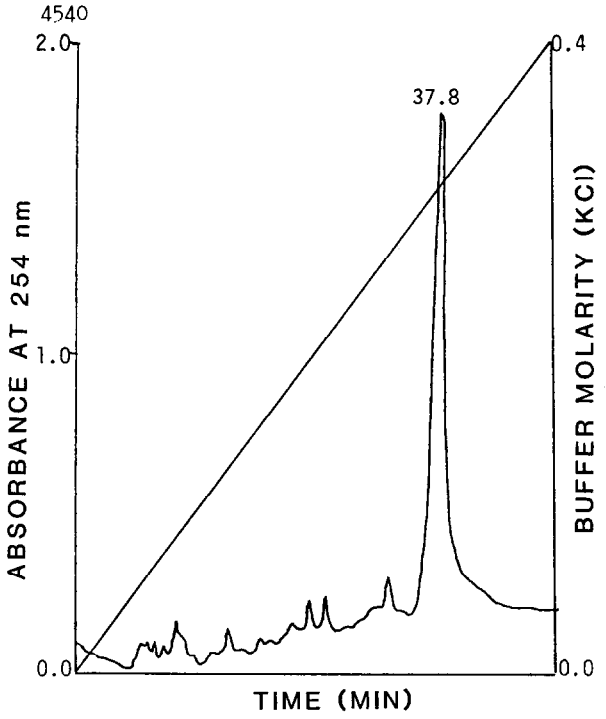



Fig. 1

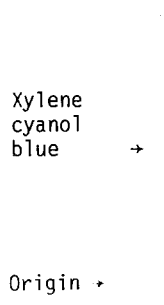



Fig. 2

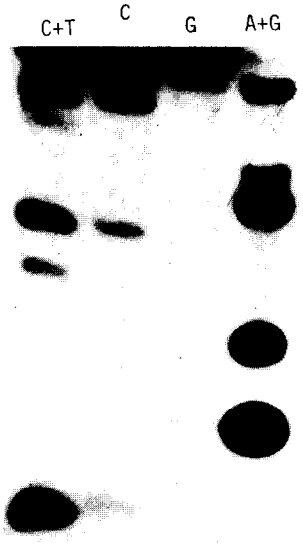



Fig. 3

and kinase[9] and electrophoresed on a 20% polyacrylamide gel. An autordiography revealed the presence of a single compound of the expected mobility (Fig. 2). The oligonucleotide was fully digested with Crotalus adamanteus snake venom phosphodiesterase confirming the exclusive presence of 3'→5' linkages in the final synthetic DNA segment. The 5'-$^{32}$P-labelled DNA was then sequenced by Maxam-Gilbert's procedure[10] (Fig. 3) which unambiguously confirmed the structure of the undecamer long DNA sequence: $^{5'}$d(TCAATCAACTA)$^{3'}$.

In conclusion, we believe that the CPSEC-group for the hydroxyl protection fulfils all important criteria which are required of an ideal and useful protecting group[12]. We further believe that the present report of an actual example of a multistep chemical synthesis leading to a functional DNA molecule of high purity would stimulate chemists to employ the CPSEC-group in other areas of the synthetic organic chemistry.

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