Use of 2-Phenylsulfonylethyl as a Phosphate Protecting Group in DNA Synthesis Using the Phosphite-Triester Approach

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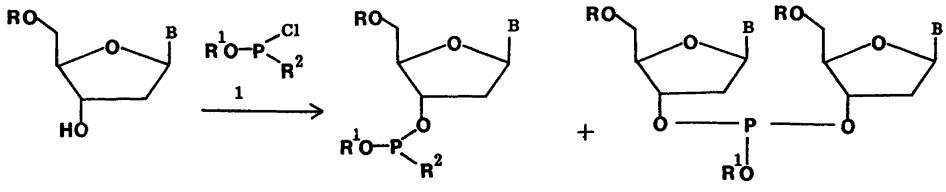
The phosphite-triester approach was first introduced by Letsinger and his coworkers employing *o*-chlorophenyl[1] and 2,2,2-trichloroethyl[2] phosphorodichloridite as a means of introducing a phosphodiester function into the target DNA molecule. Such a reactive P(III) phosphitylating agent resulted in the formation of a large amount of 3′→3′ symmetrical by-products. It was Caruthers[3] and Ogilvie[4] who independently demonstrated the usefulness of the 5′-*O*,*N*-protected phosphoromonochloridite blocks in solid phase synthesis of defined DNA sequences using silica gel as the support. Since then both Caruthers[5] and Adams[6] have studied the effects of different dialkylamino groups on the stability and reactivity of *1* and *2*. It has emerged during these studies that both *2c* and *2d* are relatively



2 3

a. R = DMTr; R^1 = Me; R^2 = Cl

b. R = DMTr; R^1 = Me; R^2 = NMe_2

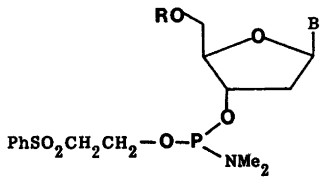
c. R = DMTr; R^1 = Me; R^2 = $N(CHMe_2)_2$

d. R = DMTr; R^1 = Me; R^2 = N-morpholinyl

e. R = DMTr; R^1 = Cl_3CCMe_2 ; R^2 = Cl

f. R^1 = $PhSO_2CH_2CH_2$; R^2 = Cl

g. R = DMTr; R^1 = $PhSO_2CH_2CH_2$; R^2 = NMe_2



4. B = 1-thyminyl

5. B = 4-N-benzoyl-1-cytosinyl

6. B = 2-N-(t-butylbenzoyl)-9-guaninyl

7. B = 6-N-(m-chlorobenzoyl)-9-adeninyl

DMTr = 4,4′-Dimethoxytriphenylmethyl

Scheme 1.

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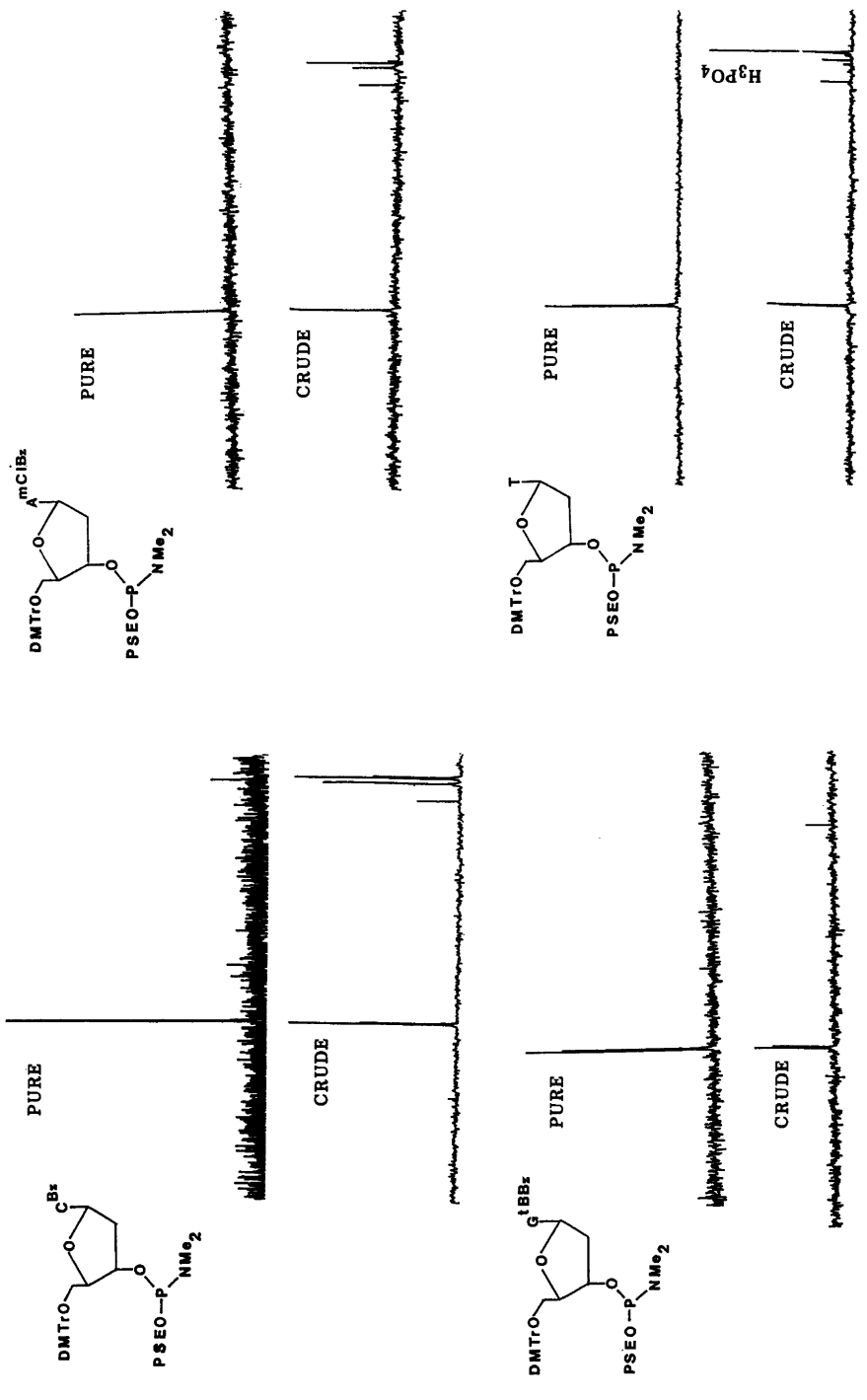

Fig. 1. A ^{31}P NMR comparison of the *crude* and the *chromatographed* (pure) specimens of 5′-*O*-(4,4-dimethoxytriphenylmethyl)-3′-*O*-(*N*,*N*-dimethyl 2-phenylsulfonylethyl phosphoramidites *4*–*7*.

stable and may be purified by careful column chromatography.[5,7] These blocks could then be used in the synthesis of DNA on a solid support. At the end of the synthesis, the internucleotide methyl group is removed by treatment of the support either with a mixture of thiophenol and triethylamine in dioxane[8] or with *t*-butylamine in methanol.[9] The support is then washed and treated with aqueous ammonia at 50 °C overnight to give the unprotected DNA molecule. Other authors have also studied the effect of different internucleotide protecting groups. Fourrey[10] has employed *o*-chlorophenyl group, in conjunction with morpholino, to prepare stable phosphoramidites and found them useful in DNA synthesis. Letsinger[11] has demonstrated that use of 2,2,2-trichlorodimethylethyl phosphorodichloridite *1e* selectively produces *2e* at −78 °C. Recently, Köster,[12] van Boom[13] and Pfleiderer[14] have proposed 2-cyanoethyl, 2-methylsulfonylethyl and 4-nitrophenylethyl, respectively, as internucleotide protecting groups. None of these reports, except the work by van Boom and coworkers,[13] actually state if they purified their phosphoramidite building blocks chromatographically.

We have previously demonstrated that the 2-phenylsulfonylethyl- (PSE) group[15] could be used successfully, in the phosphotriester approach,[16] for the protection of the 3′-terminal phosphodiester residue. We now wish to report that (1) the 2-phenylsulfonylethyl *N,N*-dimethylphosphoramidite blocks *2g* have higher solubility in acetonitrile and a reactivity comparable to that of their methyl analogues *2b*, but enhanced stability, allowing them to be purified by ordinary column chromatography[7] and stored as purified blocks; (2) that the 2-phenylsulfonylethyl group can be cleaved conveniently together with other base-labile protecting groups in a single step using aqueous ammonia at 50 °C overnight.

We prepared pure phosphitylating agent *1f* in 60 % yield (experimental section). *1f* was then reacted, in the presence of excess triazole in dry tetrahydyrofuran solution, with appropriately protected nucleosides to give the corresponding crude nucleoside phosphoramidites *4*–*7*. They were subsequently purified by silica gel column chromatography using a mixture of ethyl acetate, dichloromethane and triethylamine (2:2:1; v/v/v)[7] to give pure 5′-*O*-(4,4-dimethoxytriphenylmethyl)-3′-*O*-(*N,N*-dimethyl 2-phenylsulfonylethyl phosphor-

Table 1. Steps involved in one complete elongation cycle.[a]

Step	Solvents and reagents	Time (s)	Flow (ml/min)
1.	Benzenesulfonic acid (1 %) in acetonitrile	60	3
2.	Acetonitrile	180	3
3.	Recycle	6	6
4.	Acetonitrile	30	3
5.	Compound *2g* in acetonitrile(3.33 equiv.; 0.03 M)	6	3
6.	Tetrazole in acetonitrile (25 equiv.; 0.2 M)	6	3
7.	Compound *2g* in acetonitrile (3.33 equiv.)	6	3
8.	Tetrazole in acetonitrile (25 equiv.)	6	3
9.	Compound *2g* in acetonitrile (3.33 equiv.)	6	3
10.	Acetonitrile	12	3
11.	Recycle	600	6
12.	Acetonitrile	30	3
13.	Iodine (0.1 M in tetrahydrofuran-collidine-water; 10:1:4.8, v/v/v	45	3
14.	Acetonitrile	30	3
15.	Acetic acid anhydride (42.3 mmol)+4-(*N,N*-dimethylamino)pyridine (19.6 mmol)+collidine (30.3 mmol) in tetrahydrofuran (40 ml)	45	3
16.	Acetonitrile	180	3

[a] Fractosil 500 has been used as the solid support, loading 60–100 μmol of 4,4′-dimethoxytrityl-2′-deoxynucleoside per gram of the support. For every run 3 μmol of bound nucleoside has been used.

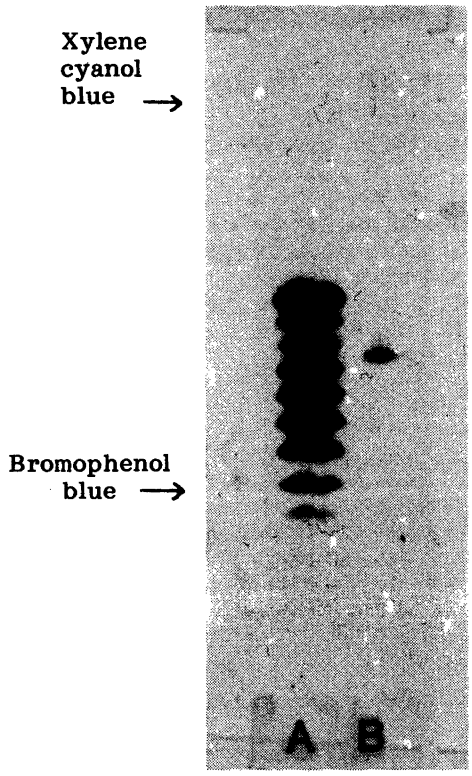

Fig. 2. 20 % polyacrylamide gel electrophoresis of ^{32}P-labelled oligodeoxyribonucleotides. Chanel A: a mixture of oligothymidylic acids upto dCT_{15}. Chanel B: crude 5′-d(T–G–C–G–A–C–C–C–A–G–A–C–T–C)3′.

amidite)nucleoside building blocks, *4–7*, in 95, 83, 91 and 92 % yields respectively. A ^{31}P NMR comparison of the *crude* and the *chromatographed* *4–7* is shown in Fig. 1. Furthermore, a comparison of the solubility properties between *2c* and *2g* (B=2-*N*-(*t*-butylbenzoyl)-9-guaninyl) clearly established that the latter is *ca.* 3 fold more soluble, despite the fact that the former has a *N,N*-diisopropylamino phosphine function. In order to demonstrate the feasibility of application of *4–7* in DNA synthesis, after activations by tetrazole, we have synthesized a tetradecanucleotide, 5′d(T–G–C–G–A–C–C–C–A–G–A–C–T–C)3′ using Fractosil 500 as support (experimental section). The reaction cycle is shown in the Table 1. It may be noted that only a ten-fold excess of amidites, *4–7*, was used in the latter reaction cycle, together with a fifty-fold excess of tetrazole. The average yield of each condensation is around 90 % per cycle as estimated by the release of the 4,4′-dimethoxytriphenylmethyl (DMTr) colour upon the deprotection of the 5′-end (Table 1). At the end of the synthesis, the support was treated with acid to remove the DMTr group, capped by acetylation, and treated with aqueous ammonia to release the mixture of oligonucleotides. The crude mixture was then directly labelled with γ–^{32}P–ATP and kinase and was subsequently checked on a 20 % polyacrylamide gel, which mainly showed a single component (Fig. 2). The crude mixture was purified using our published procedure.[18] The DNA sequence of the target molecule was finally confirmed by Maxam-Gilbert sequence analysis.[19]

In conclusion, the PSE group could be conveniently introduced using an easily accessible phosphitylating agent *1f*; it was found to stabilize the amidite building blocks, *4–7* and to enhance their solubilities in a lipophilic medium; it was also found to be compatible with the *N*-protecting groups, which are all removable under uniform alkaline condition.

Experimental. ^{1}H NMR spectra were measured at 90 MHz with a Jeol FX 90Q spectrometer in deuterochloroform using tetramethylsilane as an internal standard; ^{31}P

NMR spectra were recorded at 36 MHz in the same solvent as for ^{1}H NMR, using phosphoric acid as an external standard (δ scale). UV absorption spectra were recorded by a Cary 2200 spectrometer. Reactions were monitored by using Merck pre-coated silica gel 60 F_{254} plates with the following solvent system: (A) dichloromethane-ethyl acetate-triethylamine (2:2:1, v/v/v).

Programmed synthesis was carried out with an automated DNA synthesizer (NUCSYN) supplied by AB Analysteknik, Sweden. High performance liquid chromatography (HPLC) was performed[18] with the help of LDC equipment, model III pumps, UV III monitor and a gradient master. Fractosil 500 has been purchased from Merck.

Preparation of 2-phenylsulfonylphosphorodichloridite 1f.[13] To freshly distilled phosphorus trichloride (0.7 mol) in dry acetonitrile (40 ml) was added 2-phenylsulfonylethanol (0.1 mol) in dry acetonitrile (20 ml) dropwise for 30 min, and the mixture was stirred for 4 h at 20 °C. The excess of phosphorus trichloride and acetonitrile was then distilled off. The residue was then distilled, b.p. 200 °C at 0.007 mm Hg, to give the title compound, yield 17.2g. (60 %). ^{31}P NMR: 178.9.

*Preparation of 5'-O-(4,4'-dimethoxytriphenylmethyl)-3'-O-(*N,N*-dimethyl-2-phenylsulfonylphosphoramidite) 4–7.* General procedure:[17] To a mixture of 2-phenylsulfonylphosphorodichloridite (2 mmol) and triazole (7 mmol) in dry tetrahydrofuran (5 ml), chilled to −20 °C, was added dry diisopropylethylamine (7 mmol), and the mixture was stirred for 10 min. The 5'-*O*,*N*-protected-2'-deoxynucleoside block (1 mmol) in dry tetrahydrofuran (5 ml) was then added dropwise. The mixture was then stirred for 30 min at −20 °C and trimethylsilyl-*N*,*N*-dimethylamine (7 mmol) was added; it was subsequently stirred for 10 min and allowed to warm up to room temperature. The mixture was then poured into saturated sodium chloride solution and extracted with ethyl acetate (3×50 ml). The ethyl acetate layers were combined and washed again with saturated sodium chloride solution (2×40 ml), dried over anhydrous magnesium sulfate, filtered and evaporated. The residue was then purified on a short silica gel column using a mixture of ethyl acetate, dichloromethane and triethylamine.[7] The desired fractions were collected, co-evaporated with toluene and precipitated from chilled (−20 °C) hexane, filtered and dried.

Compound 4. Yield: 760 mg (95 %); R_f 0.6. (system A), ^{31}P NMR: 146.6, 146.8.
Compound 5. Yield: 740 mg (83 %); R_f 0.62 (system A), ^{31}P NMR: 147.0, 146.4.
Compound 6. Yield: 900 mg (91 %); R_f 0.1 (system A), ^{31}P NMR: 146.9, 146.0.
Compound 7. Yield: 740 mg (92 %); R_f 0.62. (system A), ^{31}P NMR: 147.1, 146.5.

Acknowledgements. The authors are grateful to the Swedish Board for Technical Development for generous financial assistance.

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Received June 14, 1985.