Chemical synthesis of a tetradecadeoxyribonucleoside tridecaphosphate using 2-phenylsulfonylethyl as a phosphate protecting group

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ABSTRACT

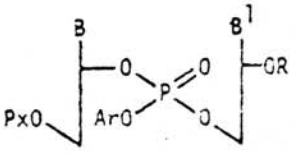
The 2-phenylsulfonylethyl group has been conveniently employed to protect the 3'-terminal phosphodiester in the synthesis of a tetradecamer, $^{5'}$d(CTGGCGCGTTTCAT)$^{3'}$ using the phosphotriester approach.

RESULTS AND DISCUSSION

In the phosphotriester approach[1] it is desirable to synthesize the dimeric building blocks with o-chlorophenyl group[2] for the protection of internucleotide linkages. These building blocks should be appropriately protected at the 5'-end by a suitable protecting group and the 3'-end with a phosphotriester function. Such a fully protected dideoxyribonucleoside diphosphate could then be conveniently used for chain extensions either at the 3'- or at the 5'-end. This strategy was originally proposed by Catlin and Cramer[3] in 1973. Narang and his co-workers[4] have widely exploited this approach for the synthesis of several biologically important DNA fragments using 4,4'-dimethoxytrityl group at the 5'-end and p-chlorophenyl 2-cyanoethyl phosphotriester at the 3'-end. van Boom et al.[5] and several other groups[6] have also used a similar strategy. We have also used such an approach[7] to synthesize several oligodeoxyribonucleotide fragments using 9-phenylxanthen-9-yl (pixyl)[8] for the protection of the 5'-hydroxyl function and o-chlorophenyl 2,2,2-tribromoethyl phsophotriester[7] for the 3'-end.

In our attempts to develop a suitable protecting group to protect a 3'-terminal phosphodiester to the triester level, we have recently introduced[9] 2-phenylsulfonylethyl as a new phosphate protecting group which is removable with 2 equiv. of Et_3N under 3 h at 20°C. We have also clearly demonstrated[9] its application in the synthesis of dodecathymidine undecaphosphate in high overall yield. This fully protected dodecamer, after deprotection[2,7,12], gave dodecathymidylic acid in over 95% yield. We now report

the application of 2-phenylsulfonylethyl (PSE) as a phosphate protecting group in the synthesis of a tetradecamer, $^{5'}$d(CTGGCGCGTTTCAT)$^{3'}$, using the Catlin-Crammer strategy[3] as adopted by us[7]. Thus, we synthesized five 5'-O-pixyl dideoxyribonucleoside monophosphates[7] (1) to (5) following a modified one step procedure[10], using an excess of 4-dimethylaminopyridine, in 55,73,48 and 62% yields respectively. The phophodiester function was then introduced at 3'-positions of (1) to (5) by reacting with a slight excess of o-chlorophenylphosphorobis-(1,2,4-triazolide)[6,11,12,13] to obtain (6) and (9) respectively in over 95% yields. The fully protected dideoxynucleoside diphosphates (10) and (11), with their 5'- and 3'-ends blocked by pixyl[7,8,14] and o-clorophenyl 2-phenylsufonylethyl phosphate[9] respectively, were obtained in 93 and 76% yields (powder) (R_f^{9a}=0.55 in 10% EtOH-$CHCl_3$). They were easily prepared by treating the corresponding o-chlorophenylphosphoromono-1,2,4-triazolide[11,14] of (2) and (5) with an excess of 2-phenylsulfonylethanol[9] (2 equiv.) for 2 h at 20°C followed by work-up and chromatography[7]. The d(HO-ApTBz) was prepared following our literature procedure[7]. The 2-phenylsulfonylethyl group from the fully protected tetramer (expt. 2, table) and the hexamer (expt. 4, table) could be conveniently removed with 4 equiv. of Et_3N under 3 h at 20°C. The phosphodiester triethylammonium salts (expt. 5 & 6, table), thus regenerated, were isolated in the pure form over 95% yields as powders following an usual work-up[9] (R_f^{9a}=0.34 & 0.56 respectively in 30% EtOH-$CHCl_3$). The pixyl group was deprotected[7,9,12,14] in an usual fashion using p-toluenesulfonic acid. H_2O (2 equiv.) in 2% EtOH-$CHCl_3$ at 20°C to give the 5'-hydroxy components (table). The fully protected hexamer (expt. 4, table) and octamer (expt. 5, table) blocks for the fully protected tetradecamer synthesis (expt. 6, table) were assembled by the block condensation of the appropriate 3'- or 5'-partially protected oligodeoxyribonucleotide blocks (cf. table for the de-



(1);B=T,B^1=C,R=H (5);B=T,B^1=T,R=H
(2);B=C,B^1=G,R=H (6);B & B^1 as in (1),R=Arylphosphodiester salt
(3);B=G,B^1=G,R=H (7);B & B^1 as in (2),R=Arylphosphodiester salt
(4);B=C,B^1=T,R=H (8);B & B^1 as in (3),R=Arylphosphodiester salt
(9);B & B^1 as in (4),R=Arylphosphodiester salt,(10),B & B^1 as in (2),R=Aryl PSE-phosphate
(11);B & B^1 as in (5),R=Aryl PSE-phosphate.

T = thymine-1-yl; A = adenine-9-yl; Ar = o-chlorophenyl (aryl)
C = cytosine-1-yl; G = guanine-9-yl; PSE = 2-phenylsulfonylethyl ($PhSO_2$-CH_2-CH_2-);
Px = 9-phenylxanthen-9-yl (pixyl).

TABLE: Condensation reactions[b] leading to higher deoxyoligoribonucleotides with MS-NT in pyridine solution at 20°C

Expt. No.	5'-protected component (mmol)[a,c]	5'-hydroxy component (mmol)[a,c]	Product[a,c]	% yield[c]	R_f[d]	% Et-OH in $CHCl_3$[f]
1	d[Px-TpCp](0.16)	d[HO-ApTBz] (0.137)	d[Px-TpCpApTBz]	74.8	0.54	5.0
2	d[Px-CpGp](0.08)	d[HO-TpTp-PSE](0.062)	d[Px-CpGpTpTp-PSE]	72.6	0.36	5.0
3	d[Px-GpGp](0.075)	d[HO-CpGp-PSE](0.07)	d[Px-GpGpCpGp-PSE]	64.2	0.35[e]	7.0
4	d[Px-CpTp](0.05)	d[HO-GpGpCpGp-PSE](0.04)	d[Px-CpTpGpGpCpGp-PSE]	65.0	0.42[e]	9.0
5	d[Px-CpGpTpTp](0.035)	d[HO-TpCpApTBz](0.032)	d[Px-CpGpTpTpTpCpApTBz]	75.0	0.46	8.0
6	d[Px-CpTpGpGpCpGp](0.025)	d[HO-CpGpTpTpTpCpApTBZ](0.02)	d[Px-CpTpGpGpCpGpCpGpTpTpTpCpApTBz]	65.0	0.53[e]	12.0

[a] 9-phenylxanthen-9-yl is abbreviated to Px(pixyl); 6-N-(m-chlorobenzoyl)-2'-deoxyadenosine, 2-N-(p-t-butylbenzoyl)-2'-deoxyguanosine, 4-N-benozyl-2'-deoxycytidine are represented by A,G and C respectively. Internucleotide phosphotriester is protected with o-chlorophenyl group. 3'-terminal p and p-PSE indicate o-chlorophenylphosphodiestertriethylammonium salt and o-chlorophenyl2-phenylsulfonylethyl phosphotriester respectively.

[b] Dry pyridine (15 ml/mmol), 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole (MS-NT) (10 equiv. for 2+2, 15 equiv. for 2+4 & 4+4 and 20 equiv. for 6+8 condensations) were used as solvent and condensing agents respectively.

[c] Isolated as powder after precipitating a $CHCl_3$ soln. (1-2 ml) of the compound from petrolium ether (30-50°C)

[d] 10% EtOH-$CHCl_3$ was used for TLC (Merck silica gel 60 F_{254})

[e] 15% EtOH-$CHCl_3$ was used for TLC (Merck silica gel 60 F_{254})

[f] a 2 cm x 2 cm silica gel column (Merck Kieselgel H) was used for short column chromatography (Rigby et al. Chem.&Ind.(1976) 1868.)

tails of the reaction conditions leading to the condensed products, % yields etc).

The fully protected tetradecamer, thus obtained, was deprotected[2,7] and purified by Hplc[7,9] (fig. 1 for elution pattern; permaphase AAX). The deoxyoligoribonucleotide which eluted under the main peak was collected in four

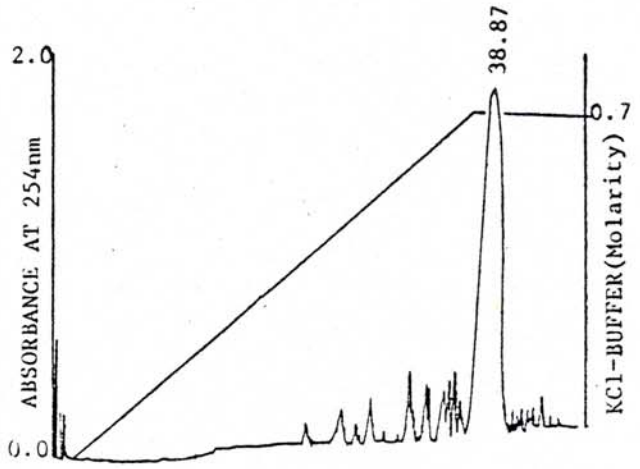


FIGURE 1

Autoradiography after electrophoresis on 20% polyacrylamide gel.

FIGURE 2

FIGURE 3

fractions in 71% yield. They were then ^{32}P-labelled along with the crude deprotected material (slot no. 5 in fig.2) by γ-^{32}P-ATP and kinase[15]. These ^{32}P-labelled products were then electrophoresed on a 20% polyacrylamide gel and autoradiographed which demonstrated their purity (four fractions are respectively in slot 1 to 4 in fig.2). The fraction 4 (slot no. 4 in figure 2) contained one pure species. This was sequenced by the Maxam-Gilbert procedure[16] as shown in fig. 3 which corresponds to the tetradecanulceotide sequence: $^{5'}$d(CTGGCGCGTTTCAT)$^{3'}$.

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