A new condensing reagent, 1-(2,4,6-triisopropylbenzenesulfonyl)-5-(pyridin-2-yl)tetrazolide and its use in the synthesis of λ cro binding heptadecanucleotide on a polymer support

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ABSTRACT

A new condensing reagent 1-(2,4,6-triisoprophylbenzenesulfonyl)-5-(pyridin-2-yl)tetrazolide (TPSPy) was found to give one diastereoisomer of dinucleoside monophosphate aryl esters. Several oligodeoxynucleotide blocks were prepared using this reagent. A heptadecanucleotide,dTATCCCTTGCGGTGATA, which had the same sequence as the λ cro binding DNA sequence was synthesized by condensing mono-, tri- and dodecanucleotide blocks using this reagent on a polystyrene support.

INTRODUCTION

Arenesulfonyl chlorides were used as condensing reagents for phosphomonoesters in the synthesis of oligonucleotides[1] and found to activate phosphomonoesters much faster than phosphodiesters. Arenesulfonyl azolides were introduced as activating reagents for phosphodiesters.[2] We have previously reported briefly the synthesis of arenesulfonyl 5-(pyridin-2-yl)tetrazolides and their use in the stereospecific synthesis of phosphotriesters[3]. It is assumed that the stereospecific synthesis of phosphotriester blocks is advantageous in preparation of protected oligonucleotides since separation of products become easier. In this paper we wish to report a use of this reagent for a synthesis of a trinucleotide and its condensation on a polymer for the synthesis of a heptadecamer dTATCCCTTGCGGTGATA which corresponds to one of strands of the λ cro binding site (O_R3)[4]. Synthesis of this DNA duplex by the solution triester method will be reported elsewhere.[5]

RESULTS

Synthesis of arenesulfonyl 5-substituted tetrazolides

Two 5-substituted tetrazoles were prepared from 2-cyanopyridine and cyanobenzene by treatment with sodium azide with modifications of published methods.[6] 5-(Pyridin-2-yl)tetrazole and 5-phenyltetrazole were condensed

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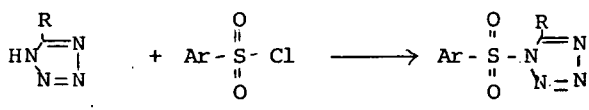
with mesitylenesulfonyl chloride (MS) or 2,4,6-tri-isopropyl-benzenesulfonyl chloride (TPS) as described for the preparations of TPS-tetrazolide [7] to give corresponding arenesulfonyl 5-(pyridin-2-yl)tetrazolides (MSPy and TPSPy) or arenesulfonyl 5-phenyltetrazolides (MSPh and TPSPh) (Chart 1). As reported previously,[3] MSPy and TPSPy gave stereospecific synthesis of phosphotriesters in condensation of N,5'-protected deoxynucleoside o-chlorophenylphosphates with the 5'-hydroxyl group of protected nucleosides or nucleotides but phenyl substituted reagents (MSPh and TPSPh) showed diastereoisomers as observed in products obtained by using mesitylenesulfonyl nitrotriazolide (MSNT)[8]. This may mean that the specificity does not arise from a bulkyness of the reagents but from the presence of the nitrogen atom of pyridine. TPSPy was found to be active in methylene chloride if even a trace of pyridine was present. Since methylene chloride is a suitable solvent for swelling of polystyrene, the reagent may be useful for solid phase oligonucleotide syntheses.

Chart 1



TPSPy (Ar=2,4,6-tripropyl benzene
R = pyridin-2-yl)

Preparation of protected trinucleotide block

Protected dTGAp was prepared by condensation of N-benzoyldeoxy-adenosine 3'-(p-anisido)-o-chlorophenyl phosphate with 5'-O-dimethoxytrityl-isobutyryldeoxyguanosine 3'-o-chlorophenyl phosphate using TPSPy followed by 5'-deblocking and condensation with protected Tp (Chart 2). The reaction conditions and results are summarized in Table I. Since the anisidate was a mixture of two diastereoisomers, the fully protected dimer gave two spots in high performence thin layer chromatography (HPTLC) of silica gel. In reversed phase thin layer chromatography (RTLC) the dimer traveled homogeneously. The fully protected dinucleotide and trinucleotide were isolated by reverse phase column chromatography in yields of 71 and 76 %, respectively.

Synthesis of the heptadecamer on polysterene support

The heptadecanucleotide, dTATCACCGCAAGGGATA was synthesized on 1% polystyrene containing benzylamine by linking 5'-O-dimethoxytrityl-N-benzoyldeoxyadenosine 3'-succinyl group as pentachlorophenyl ester.[9] Since the 3'-terminal benzoyladenosine was known to be depurinated easily by acid treatment, $ZnBr_2$ in methylene chloride and

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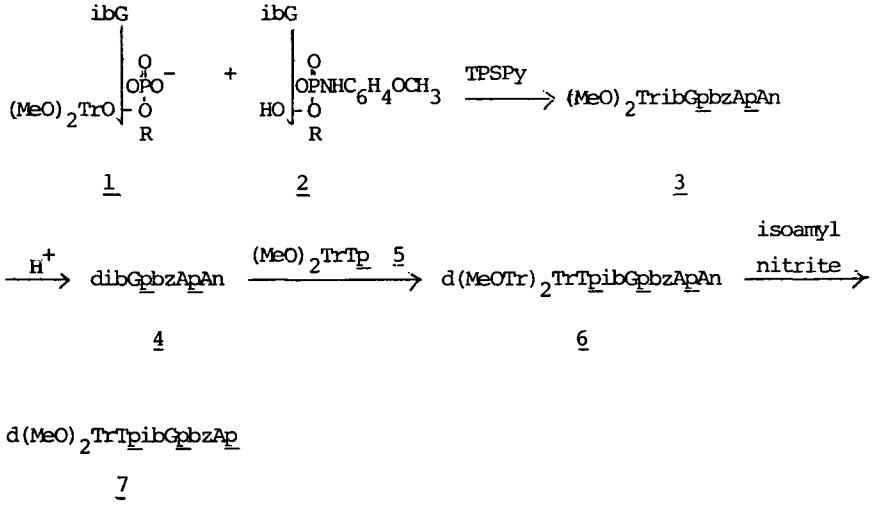
Chart 2



isopropanol[10] was used to remove the 5'-dimethoxytrityl group. A mononucleotide dimethoxytritylthymidine-3'-(o-chlorophenyl)phosphate, the protected trinucleotide dTGAp and a protected dodecanucleotide dTATCCCTTGCGGp[5] which was prepared by the liquid phase method were condensed successively with TPSPy(Chart 3). Using excesses of 7, 23 and 16 fold of incoming nucleotides, yields of the di-, penta- and heptadeca-nucleotides were 24, 67 and 63 %, respectively. Thus the large oligonucleotide block could be condensed on a solid support in yields comparable to the one obtained for trimer blocks. If protected oligonucleotides are available, oligonucleotide condensations are advantageous to reduce the step for removal of the 5'-dimethoxytrityl group on a support.

Table I

3'-P Component	5'-OH Component	TPSPy (mmol)	Solvent (ml)	Product (mmol)	Yield (%)
$d(MeO)_2TribGp$ (0.29 mmol)	dbzApAn (0.29 mmol)	243 mg (0.58)	pyridine, 1 CH_2Cl_2, 2	$d(MeO)_2TribGpbzApAn$ 304 mg (0.20)	71
$(MeO)_2TrTp$ (0.5 mmol)	dibGpbzApAn (0.41 mmol)	338 mg (0.81)	pyridine, 1 CH_2Cl_2, 2	$d(MeO)_2TrTpibGpbzApAn$ 582 mg (0.38)	76

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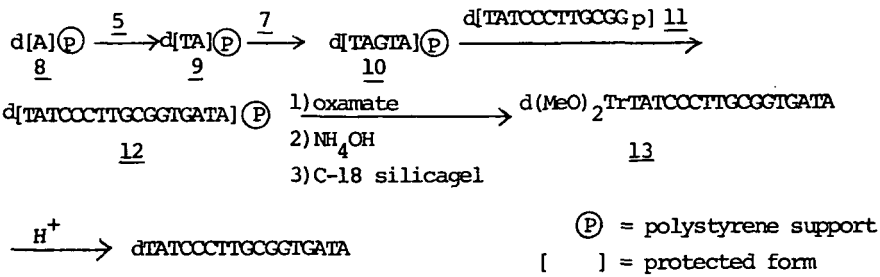
Chart 3



EXPERIMENTAL

Thin layer chromatography (TLC) was performed on plates of silica gel (Kieselgel HF_{254}, Merck and HPTLC glass plates for nano-TLC, Merck) using a mixture of chloroform and methanol. For reverse phase TLC, silanized silicagel, HPTLC RP-2 or RP-18 F_{254} (Merck) were used with a mixture of acetone-water. For columns, silica gel (60 or 60H, Merck) and alkylated silica gel (C-18, 35-105 u, Waters) were used in a mixture of chloroform-ethanol and acetone-water, respectively.

Preparation of deoxynucleoside 3'-p-anisido-o-chlorophenyl phosphates, removal of the anisidate and other general methods were as described previously.[11]

Polystyrene (aminomethylated, 0.13 mmol/g) was obtained from Peptide Institute, Inc.

5-(Pyridin-2-yl-tetrazole)

To a solution of 2-cyanopyridine (19.7 g, 189 mmol) in 50 ml DMF were added sodium azide (24.6 g, 378 mmol) and ammonium chloride (20.2 g, 378 mmol). The mixture was stirred at 90° for 6 hr and poured into 300 ml ice-water. The solution was adjusted to pH 3.0 with conc. HCl. Precipitates were collected by filtration, dried over P_2O_5 under vacuum and recrystallized from ethanol to give 20.8 g (75%). mp. 223-224.4 Anal. Calcd for $C_6H_5N_5$: C, 48.98; H, 3.43; N, 47.60. Found: C, 49.17, H, 3.43; , 47.23.

5-Phenyltetrazole

To a solution of cyanobenzene (5.15 g, 50 mmol) in 20 ml DMF were added sodium azide (6.5 g, 100 ml) and ammonium chloride (5.35 g, 100 mol). The mixture was stirred at 90° for 7 hr and poured into 100 ml ice-water. The solution was adjusted to pH 3.0 with conc HCl. Precipitates were collected by filtration, dried over P_2O_5 under vacuum and recrystallized

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from ethanol to give 6.3 g (86 %). mp. 225-226.5°C Anal. Calcd for $C_7H_6N_4$: C, 57.53; H, 4.14; N, 38.34. Found: C, 57.73; H, 4.09; N, 38.06.

1-(Mesitylenesulfonyl)-5-(pyridin-2-yl)tetrazolide (MSPy)

To an ice-cooled solution of mesitylenesulfonyl chloride (1.44 g, 6.6 mmol) in dioxane (25 ml) were added 5-(pyridin-2-yl)tetrazole (973 mg, 6.6 mmol) and triethylamine (0.920 ml). The mixture was stirred at ambient temperature for 2 hr and filtered to remove triethylammonium chloride. The solution was concentrated and the residue was dissolved in methylene chloride. The organic solution was washed with water, dried over Na_2SO_4 and evaporated under reduced pressure. MSPy was crystallized from small amount of ether to give 1.44 g (66 %). mp. 146.5-147.5°C. NMR(in $CDCl_3$) δ :2.36(s, 3H, methyl), 2.52(s, 6H, methyl) 6.97(s, 2H, benzene ring), 7.07-8.77(m, 4H, pyridine ring).

1-(2,4,6-Triisopropylbenzenesulfonyl)-5-(pyridin-2-yl)tetrazolide (TPSPy)

TPSPy was prepared as described for the synthesis of MSPy. mp 137-139°C. NMR (in $CDCl_3$) δ: 1.20 (d, 12H, methyl) 1.23 (d, 6H, methyl), 3.9-4.5 (m, 3H, methyl), 7.03 (s, 2H, benzene ring), 7.2-8.8 (m, 4H, pyridine ring).

Synthesis of $(MeO)_2$TrbzAp(o-ClC_6H_4)bzA(bz) with TPSPy in methylene chloride

5'-O-Dimethoxytrityl-N-benzoyldeoxyadenosine 3'-(o-chlorophenyl)phosphate (0.05 mmol), N,3'-O-dibenzoyldeoxyadenosine (0.04 mmol) were dried twice by evaporation of pyridine and coevaporated twice with toluene. The mixture was treated with TPSPy (0.09 mmol) in methylene chloride (1 ml) for 1 hr. Unchanged starting materials were remained even after treatment of additional TPSPy (0.09 mmol) for 1 hr. The reaction was completed after 10 min by addition of a mixture of pyridine-methylene chloride (1:20, 0.2 ml). Insoluble materials were removed and the solution was washed with 5% sodium bicarbonate and back extracted with methylene chloride. The product was isolated by chromatography on silica gel H with a stepwise elution of chloroform and 1 to 3 % methanol. The product was precipitated with pentane from its solution in chloroform. The yield was 56 mg, quantitative. The product traveled together with the lower isomer of the protected dApA in HPTLC ($CHCl_3$:MeOH, 15:1), which was obtained by the ditriazolide method[12] and separated from the higher isomer by elution with chloroform containing 1 % methanol from silicagel 60.

Preparation of the protected dTGAp (7)

The protected dGp(1) and dAp(2) were treated with TPSPy for 1 hr under the conditions shown in Table I. The mixture was added with aqueous

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pyridine (30 %), concentrated and the residue was dissolved in acetone. The mixture was applied to C-18 silica gel column with addition of water until slight turbidity. The fully protected dinucleotide (3) was eluted with acetone-water (7:3). The fractions were concentrated and the product was extracted with chloroform. The organic layer was washed with water. The protected dGpAp(3) was precipitated with n-pentane from its solution in chloroform to give 304 mg (71 %). 3 was dedimethoxytritylated with benzenesulfonic acid and condensed with 5 as summarized in Table I. After 20 min the product (6) was isolated by chromatography on C-18 silica gel as above and converted to the diester (7) by treatment with isoamyl nitrite.[11]

Synthesis and purification of the heptadecanucleotide

The polymer linked 5'-O-dimethoxytrityl-3'-O-succinyl-N-benzoyldeoxyadenosine (8)(356 mg, 38 μmol) was treaded twice with 1 M $ZnBr_2$[10] (5 ml) for 20 min, washed 3 times with isopropanol-methylene chloride (15:85, 5 ml) with shaking for 1 min and washed with 1 M ammonium acetate (10 ml), then dried twice with evaporation of pyridine. The compound was reacted with 5'-O-dimethoxytrityl-thymidine 3'-(o-chlorophenyl)phosphate (0.270 mmol) and TPSPy (0.75 mmol) in methylene chloride-pyridine(2:1, 4.5 ml) for 2 hr. The resin was washed twice with pyridine, acetylated for 30 min with acetic anhydride in pyridine (1:9, 10 ml) in the presence of 4-dimethylaminopyridine, washed twice with pyridine, then five times with CH_2Cl_2. The 5'-deblocking was repeated as above. The estimated amount of the dimethoxytritanol was 6.5 μmol (24 %). The trinucleotide (8) (0.206 mmol) was then condensed to the chain using TPSPy (0.272 mmol) as above. The yield was estimated by removing dimethoxytrityl group to find 4.3 μmol (67 %). The protected dodecamer (11) (591 mg, 96 μmol) was reacted with the linked nucleotide using TPSPy (180 ul) for 2 hr. The resin was washed and deblocked by successive treatments with 0.5 M tetramethylguanidium pyridine aldoxamate in 50 % dioxane (10 ml) for 2 days followed by passage through a column of Dowex 50 x 2 (pyridinium form, 20 ml). The solution and washings (30% pyridine) were concentrated, dissolved in pyridine (3 ml) treated with conc. ammonia (20 ml) at 50° for 6 hr, evaporated and the residue in water (10 ml) was washed with ethyl acetate (10 ml, 5 ml x 2). The aqueous layer was concetrated and applied to a column of C-18 silica gel. Elution was performed with a gradient of acetonitrile (5-40 %) in 0.1 M triethylammonium acetate[13]. The trityl containing heptadecamer (13) was eluted with 32 %. acetonitrile. The dimethoxytrityl group was removed with 80% acetic acid (5 ml) at room

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temperature for 20 min and the mixture was washed with ethyl acetate. The product (14) was isolated by chromatography on C-18 silica gel using a gradient of acetonitrile (5-25 %) (67 A_{260} units, 0.57 μmol) and analyzed by high pressure liquid chromatography on u-Bondapk C-18 silica gel to find a single peak, which had the identical retention time as the heptadecamer obtained by the solution phase synthesis.[5]

REFERENCES

1) Khorana, H.G., (1968) Pure Appl. Chem. 17, 344-381.
2) Ikehara, M., Ohtsuka, E. and Markham, A.F. (1979) Adv. Carb. Chem. Biochem.36, 135-213.
3) Ohtsuka, E., Tozuka, Z. and Ikehara, M. (1981) Tetrahedron Lett. 22, 4483-4486.
4) Ptashne, M., Jeffrey, A., Johnson, A.D., Maner, R., Meyer, B.J., Pabo, C.O., Roberts, T.M. and Sauer, R.T. (1980) Cell 19, 1-11.
5) Ohtsuka, E., Tozuka, Z., Ohta, A., Kitano, K. and Ikehara, M. paper in preparation.
6) Brauer-van Straaten, B., Solinger, D., van de Westeringh, C. and Veldstrc, H (1958) Rec. Trav. Chim. 77, 1129-1135
7) Stawinski, J., Hozumi, T., Narang, S.A., Bahl, C.B. and Wu, R. (1977) Nucleic Acids Res. 4, 353-371.
8) Reese, C.B., Titmas, R. and Yau, L. (1978) Tetrahedron Lett. 2727-2730.
9) Miyoshi, K, Miyake, T., Hozumi, T. and Itakura, K. (1980) Nucleic Acids Res. 8, 5473-5489.
10) Ito, H, Ike, Y., Ikuta, S. and Itakura, K. (1982) Nucleic Acids Res., 10, 1755-1769.
11) Ohtsuka, E., Taniyama, Y., Marumoto, R., Sato, H., Hirosaki, H. and Ikehara, M. (1982) Nucleic Acids Res., 10, 2597-2608.
12) Broka, C., Hozumi, T., Arenzene, R. and Itakura, K. (1980) Nucleic Acids Res., 8, 5461-5471.
13) Fritz, H.J.,Belagaje, R., Brown, E.L., Fritz, R.H., Lees, R.G., and Khorana, H.G. (1977) Biochemistry 17, 1257-1267.

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