Tetrahedron Letters,Vol.24,No.9,pp 877-880,1983 0040-4039/83/090877-04$03.00/0
Printed in Great Britain

A NEW SYNTHESIS OF OLIGODEOXYNUCLEOSIDE METHYLPHOSPHONATES ON CONTROL PORE GLASS POLYMER SUPPORT USING PHOSPHITE APPROACH

N.D. Sinha, V. Großbruchhaus and H. Köster

Institute of Organic Chemistry and Biochemistry
University of Hamburg, Martin-Luther-King-Platz 6
D-2000 Hamburg 13, FRG

ABSTRACT

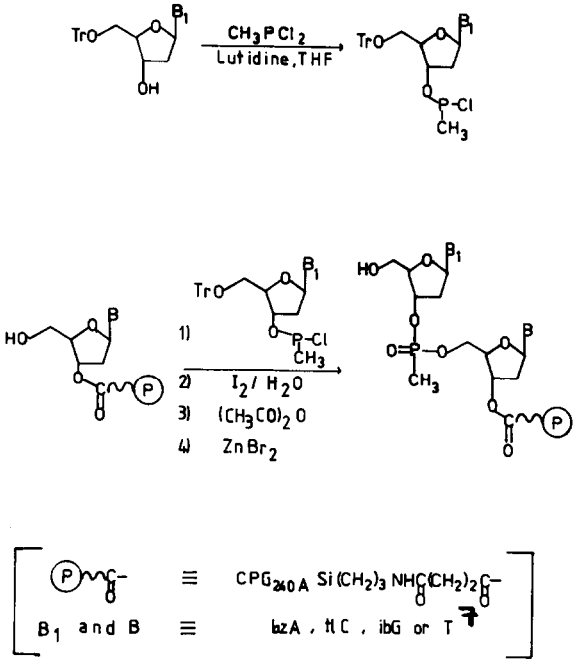
Dimer, trimer and tetramer blocks of deoxynucleoside methylphosphonodiesters d(AC), d(AT), d(ATG) and d(ATTT) were synthesized on CPG polymer support using deoxynucleoside methylphosphonomonochloridite. Only dinucleotide methylphosphonates were phosphorylated at 5'-OH with T4-polynucleotide kinase. These phosphorylated dimers hydrolized quantitatively with NaOH (0.5 M) in 15 minutes at room temperature.

Nucleic acid analogues possessing modification either at sugar or internucleotide bonds have been synthesized and used to study physico-chemical properties and to understand interactions of nucleic acids in biological processes. The nucleotide analogues used for the above purposes are nucleoside phosphites[1], nucleoside phosphonates[2], nucleoside thiophosphates[3]. Recently deoxynucleoside methylphosphonate diesters have been synthesized by T'so et al.[4] and PMR and CD have been studied by this group. Phenyl and methylphosphonodiesters of deoxynucleoside have also been synthesized by Agarwal and Reftina[5] using their modified triester method in solution phase, where longer condensation time and extensive purification were needed to isolate the desired product in pure form (D. Rothermund, unpublished observation). In this communication we would like to report the first synthesis of oligodeoxynucleoside methylphosphonodiesters using polymer support synthesis and intermediates with trivalent phosphorus.

Recently we have introduced controlled pore glass (CPG) as polymer support for rapid synthesis of oligonucleotides[6] in reasonably large yields. CPG has now been used in the synthesis of deoxynucleoside methylphosphonodiesters using phosphite triester approach to give pure products in large yield in very short time. The basis of this strategy is outlined in scheme 1.

The general procedure of the synthesis is as follows: CPG bound N-protected deoxynucleoside was prepared according to the report from our laboratory[6]. N-acyl-5'-O-trityldeoxynucleoside (1 mmol) dissolved in dry THF (1 ml) was added to a mixture of 2,6-lutidine (5 mmol) and methyldichlorophosphine (1 mmol) in THF (1 ml) at -78° C under Argon atmosphere over 15 - 20 minutes and after 30 minutes allowed to warm up slowly to room temperature. Lutidine hydrochloride was filtered and the filtrate was concentrated under reduced

Scheme



pressure. After the second evaporation to foam, with toluene/THF-mixture (10 ml, 1:1), it was dissolved in THF (5 ml) containing 2,6-lutidine (5 mmol) to give 0.2 M solution. Active nucleoside methylphosphonomonochloridite solution (1 ml) was added by syringe to a septum-capped column containing nucleoside (10-20 μmol) bound to CPG beads (100-200 mg). After 15 minutes reagent was removed by Argon pressure, the system was evacuated and was charged again with the activated nucleoside (1 ml) for 10 minutes. The yield of the condensation was determined by measuring the trityl cation concentration. When more than 95% condensation was achieved the phosphite diester was oxidized with 0.1 M iodine in THF/pyridine/water (40:20:1), oxidation was complete in 3-5 minutes. Excess I_2 was removed by washing with pyridine (5-10 ml) until no colour remained, followed by THF washing (20-30 ml). A mixture (2 ml) of DMAP/THF/Ac_2O/lutidine (0.6 g, 10 ml, 1.0 g, 1.1 g) was passed through the column followed by washing with pyridine (5 ml), and then THF (30-50 ml) to remove any remaining pyridine/lutidine. The column was dried under high vacuum for 10 minutes. A saturated solution of $ZnBr_2$ in either CH_2Cl_2/CH_3OH (7:3) or nitromethane/water (100:1) was passed through the beads until the detritylation was complete. Finally washing with nBuOH/lutidine/THF mixture (4/1/5) (5 ml) and then THF alone (30-50 ml) was performed. The next nucleoside unit was then introduced by repeating the above process with the appropriate nucleoside methylphosphonomonochloridite. The time period for one complete cycle was 45 minutes to 1 hour.

The nucleoside methylphosphonodiester chain was removed from the support

Table

Oligomer	yield (%)	λ_{max} (nm)[d]	λ_{min} (nm)[d]	R_f[a]	R_f[b]
d(AC)	93	265	245	0.12[c]	0.04[c]
d(AT)	94	264	234	0.26[c]	0.05[c]
d(ATG)	85	271	243	0.21	0
d(ATTT)	76	268	243	0.32	0

Solvent systems: a) CH_3CN/H_2O = 8:2 (v/v); b) $CHCl_3/CH_3OH$ = 7:3 (v/v); c) Two diastereomers were visible but did not separate as distinct spots; d) Measured in 10 mM Tris·HCl (pH 7.5), 1 mM EDTA.

with NH_3 (25%, 2 ml) at 35° C for 15 hours, the supernatant liquid was removed and the beads were rinsed with bidistilled water (2-3 ml). The combined liquid was concentrated under reduced pressure and purified on tlc silica gel plates (acetonitrile/water: 80:20) for removal of non-nucleotidic material. The nucleotide band on the plate was eluted with $CHCl_3/CH_3OH$ (7:3). The product was concentrated and lyophilized with bidistilled water.

We have used purine as well as pyrimidine deoxynucleoside methylphosphonomonochloridites for condensation with CPG bound purine and pyrimidine deoxynucleosides. To demonstrate the utility of this method d(AC), d(AT), d(ATG) and d(ATTT) methylphosphonodiesters were synthesized. The overall yield, UV-absorption and R_f-values are given in the table.

We attempted to phosphorylate the dimers, trimer and tetramer obtained above at 5'-OH with (γ-P^{32})ATP and T4 polynucleotide-kinase under usual conditions. Only d(AC) and d(AT) were quantitatively phosphorylated. Even under drastic conditions such as 12 hour-incubation with T4-polynucleotide kinase d(ATTT) was phosphorylated in traces where as d(ATG) could not be phosphorylated at all.

Phosphorylated d(AT) and d(AC) were found to hydrolize completely with 0.5 M NaOH solution in 15 minutes at room temperature as demonstrated in figure 2.

Work is in progress to achieve characterization of degradation products and sequence analysis of oligodeoxynucleoside methylphosphonates.

Acknowledgment

This work was supported by the Deutsche Forschungsgemeinschaft and Bundesminister für Forschung und Technologie.

References

1) A. Holy and F. Sorm, Collect Czech. Chem. Comm. 32, 1562 (1966).
2) D.H. Rammler, L. Yenogoyan, A.V. Paul and P.C. Bax, Biochemistry 6, 1828 (1967).
3) M.E. Wolff and A. Berger, J. Amer. Chem. Soc. 90, 5337 (1957).

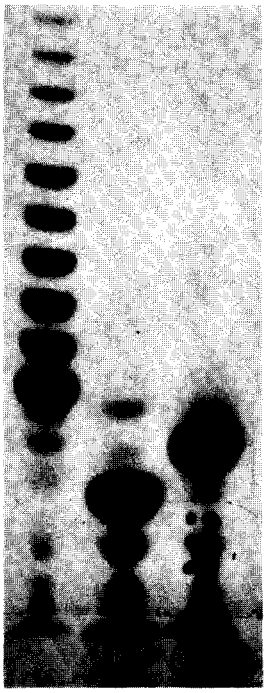


Figure 1: Electrophoresis was carried out with polyacrylamide gel (20%). Lane 1, 2 and 3 contained oligo dT chain length standard, d(AC) and d(AT) respectively. Mobility for d(AT) phosphonate diester was in the range of d(T-T) phosphodiester, whereas d(AC) moving faster than d(AT) phosphonate diester according to less retention of C compared to T[8].

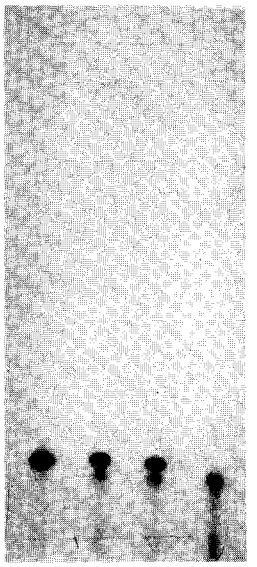


Figure 2: The phosphorylated d(AT) and d(AC) phosphonates after elution from polyacrylamide gel, were taken up in bidistilled water (100 µl) and treated with 1 M NaOH soln. (100 µl). A sample of 10 µl was taken out from this and the hydrolysis was stopped with dried Dowex 50 WX8 resin (pyridinium form) at different time interval. The samples were spotted on silica gel plate, developed in CH_3CN/H_2O : 8/2 mixture, and exposed to X-ray film. Lane 1, 2, 3 and 4 represent reaction times of 0, 2, 5 and 10 minutes, respectively.

4) P.S. Miller, J.E. Yano, C. Caroll, K. Jayaraman and P.O.P. T'so, Biochemistry 18, 5134 (1979).
5) K.L. Agarwal and P. Reftina, Nucleic Acids Res. 6, 3009 (1979).
6) H. Köster, A. Stumpe and A. Wolter, Tetrahedron Let., submitted for publication.
7) bzA = benzoyldeoxyadenosine, tlC = 2-toluyldeoxycytosine, ibG = isobutyryldeoxyguanosine, T = deoxythymidine.
8) R. Frank and H. Köster, Nucleic Acids Res. 6, 2069 (1979).

(Received in Germany 16 November 1982)