Tetrahedron Letters, Vol.22, No.35, pp 3367 - 3370, 1981
Printed in Great Britain

0040-4039/81/353367-04$02.00/0

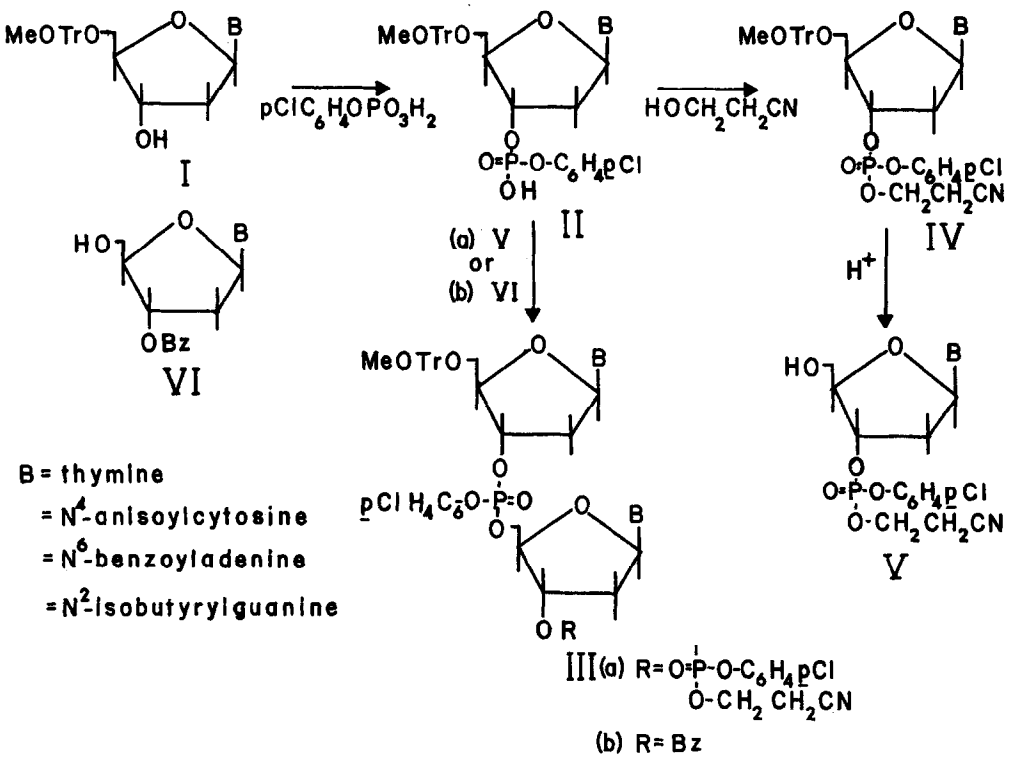
A SIMPLIFIED STRATEGY FOR THE SYNTHESIS OF DIDEOXYRIBONUCLEOTIDE BLOCKS

K.L. Sadana[+], F.E. Hruska[#] and P.C. Loewen[+*]
Department of Microbiology[+] and Department of Chemistry[#]
University of Manitoba, Winnipeg, Manitoba, Canada, R3T 2N2

The rapid synthesis of dideoxyribonucleotide blocks in 60-85% isolated yields has been achieved by combining the phosphorylation and condensation steps in a sequential reaction series which also allows the recovery of unreacted nucleotides.

The modified triester procedure for the synthesis of oligodeoxyribonucleotides (1) has involved the synthesis and isolation of fully protected mononucleotides IV which were then converted to the 3'-phosphodiesters II for condensation with a free 5'-hydroxyl group on a suitably protected nucleoside VI or nucleotide V. Efforts to speed the process have centered on the elimination of various isolation steps by using 1.1-1.2 equivalents of arylphosphoditriazolides (2,3) or by hydrolyzing excess phosphorylating reagent when 1.4 equivalents were used (4). In this report we present an alternate strategy for the rapid and economical preparation of dideoxyribonucleotide blocks by the sequential phosphorylation of a 3'-hydroxyl group and condensation with a 5'-hydroxyl group in a 'one flask reaction' which bypasses the isolation of any intermediates.

The 5'-monomethoxytrityl (MeOTr-) protected nucleosides were phosphorylated in four hours using 1-1.05 equivalents of p-chlorophenylphosphate and 2.25 equivalents of triisopropylbenzenesulfonyl chloride (TPS) (5). Only in the case of deoxyguanosine were undesired side products formed and these did not interfere with the subsequent condensation. The p-chlorophenylphosphate was prepared by the hydrolysis of p-chlorophenylphosphodichloridate as described for the ortho isomer (6). The procedure used to prepare 2,2,2-trichloroethylphosphodichloridate (7) was adapted for the rapid (less than 2 hours) preparation of p-chlorophenylphosphodichloridate from $POCl_3$ and p-chlorophenol (b.p. 105°C at 1.6 mm of mercury; literature b.p., 135°C at 9 mm (8)). The condensing reagent for the second stage of the 'one flask' procedure, mesitylenesulfonyltetrazolide (9) was prepared in ethyl acetate (10) and was stable when stored at -78°C for up to 9 months as determined by silica gel t.l.c. in ether-hexane (1:1, v/v). Included in the second stage of the reaction to convert I into III were 0.5-2.0 equivalents of the 5'hydroxyl component, either V or VI and either 2.25 equivalents of mesitylenesulfonyltetrazolide or 0.75 equivalents of TPS and 9.0 equivalents of tetrazole. The dideoxyribonucleotide III was produced within one hour. The condensation reaction was sufficiently fast that no sulfonation of the 5'-hydroxyl component occurred, a fact also noted by Seth and Jay (11) when TPS and tetrazole were used as condensing reagents. Purifications were carried out on silica gel plates developed in the solvents listed



SCHEME

in the Table and the isolated yields of dinucleotide including those containing dG were in the 60-80% range with no difference between reactions using either V or VI.

The one flask reaction was also used to produce nucleotide V required for chain extension in the 3'-direction. The fully protected phosphotriesters IV were prepared as described in the scheme with 2-3 equivalents of 2-cyanoethanol as the hydroxyl component. Following quenching and solvent removal, but without further purification, the monomethoxytrityl group was removed by treatment with 80% acetic acid for 4 hours or with 2% benzenesulphonic acid (12). After removal of the excess reagent, the monomethoxytritanol was removed in a hexane or ether-hexane wash and the nucleotides were purified on silica gel plates from which they were eluted by stirring in chloroform-ethanol (2:1, v/v) for 2 or more hours. If any decyanoethylation occurred during this elution, simple extraction with ether, ether-hexane or ether-methylene chloride separated the desired product both at the mononucleotide (V) stage and the dinucleotide (IIIa) stage. Isolated yields of V were 69% when B was N^2-isobutyrylguanine, 81% when B was N^4-anisoylcytosine, 83% when B was N^6-benzoyladenine and 81% when B was thymine.

Of importance in large scale synthesis is the ability to recover unreacted material and this was accomplished in cases where an excess of the phosphodiester II was used by the addition of 2 equivalents of 2-cyanoethanol without any further condensing reagent. The unreacted phosphodiester II was converted to phosphotriester IV within 30 minutes and the subsequent

Table. Summary of the yields, purification conditions, and chromatographic properties of synthesized dideoxyribonucleotide blocks.

5'-Protected[a] nucleoside mmol	5'-Hydroxyl component mmol	Yield[b] %	Number of Silica gel plates	Solvent Chloroform:ethanol (v/v)	Rf[d]
[MeOTr]danC 2.0	dT-OBz 4.0	62	12	12:1	0.41
[MeOTr]danC 0.4	dT± 0.2	67	2	10:1	0.24
[MeOTr]danC 0.5	danC± 0.9	83	4	13:1	0.29
[MeOTr]disobG 0.6	dT-OBz 0.9	59	4	10:1	0.38,0.44
[MeOTr]disobG 0.2	dT± 0.4	65	2	10:1	0.22
[MeOTr]dbzA 4.0	dT-OBz 6.0	69	12	12:1	0.62
[MeOTr]dbzA 5.0	dT± 2.5	71	12	14:1	0.30
[MeOTr]dbzA 0.6	danC-OBz 0.45	70	4	12:1	0.66,0.72
[MeOTr]dbzA 0.6	danC± 0.33	70	4	11:1	0.29
[MeOTr]dT 0.2	disobG± 0.4	63	2	8:1	0.16
[MeOTr]d6MeU 0.7	dT-OBz 1.4	78	4	16:1	0.36,0.41

a. Abbreviations are as suggested in (13) and a phosphotriester is represented by ±.
b. Yields based on the lesser component.
c. 20 x 20 cm plates were coated with 20 g of silica gel (MN).
d. Rf value were determined on precoated thin layer silica gel plates (MN) developed in 12:1 chloroform-ethanol. Two values indicate separated diastereomers.

separation of the monodeoxyribonucleotide and dideoxyribonucleotide blocks was achieved on silica gel plates. In one example, 77% of the excess II, where B was N^6-benzoyladenine, was recovered.

All of the deoxyribonucleotides were characterized by enzymatic degradation after complete deprotection with concentrated NH_4OH and pyridine (4:1, v/v) for 6 hours at 60-70°C followed by 80% acetic acid at room temperature for four hours. The monomethoxytritanol was removed in an ether wash and the products were purified by paper chromatography.

The combination of the phosphorylation and condensation steps in a sequential reaction series without intermediate purification has reduced the time required for the preparation of dinucleotide blocks with no adverse effects on yields. The ability to recover excess mononucleotides from the final condensation during a single purification step has also improved the economy of the dinucleotide synthesis. The nucleotide products could be tailored to allow subsequent elongation in either the 3'- or the 5'-directions and the procedure was easily adapted to the synthesis of longer oligodeoxyribonucleotides. For example, [MeOTr]danC was condensed with danC±anC±T-OBz to produce a tetranucleotide in 79% yield.

Acknowledgement

We gratefully acknowledge the financial support from the Natural Sciences and Engineering Research Council Canada in grant A9600.

References

1. K. Itakura, N. Katagiri and S.A. Narang (1974) Can. J. Chem., 52, 3689-3693.
2. P. Cashion, K. Porter, T. Cadger, G. Sathe, T. Tranquilla, H. Notman and E. Jay (1976) Tetrahedron Lett. 3769-3772.
3. K.L. Agarwal and F. Riftina (1978) Nucl. Acids Res. 5, 2809-2823.
4. C. Broka, T. Hozumi, R. Arentzen and K. Itakura (1980) Nucl. Acids Res. 8, 5461-5471.
5. J.H. van Boom, P.M.J. Burgers, G.R. Owen, C.B. Reese and R. Saffhill (1971) Chem. Commun. 869-871.
6. J.H. van Boom, J.F.M. de Rooy and C.B. Reese (1973) J. Chem. Soc., Perkin I 2513-2517.
7. F. Eckstein and I. Rizk (1969) Chem. Ber. 102, 2362-2377.
8. N. Bojars (1968) Chem. Abs. 68, 4952.
9. J. Stawinski, T. Hozumi and S.A. Narang (1976) Can. J. Chem. 54, 670-672.
10. K.L. Sadana and P.C. Loewen (1978) Tetrahedron Lett., 5095-5098.
11. A.K. Seth and E. Jay (1980) Nucl. Acids Res. 8, 5445-5460.
12. J. Stawinski, T. Hozumi, S.A. Narang, C.P. Bahl and R. Wu (1977) Nucl. Acids Res. 4, 353-371.
13. IUPAC-IUB (1970) Biochemistry 9, 4022-4027.

(Received in USA 5 May 1981)