Tetrahedron Letters No. 17, pp 1445 - 1448, 1975. Pergamon Press. Printed in Great Britain.

THE USE OF A LIGHT-SENSITIVE PHOSPHATE PROTECTING GROUP FOR SOME MONONUCLEOTIDE SYNTHESES

M. Rubinstein, B. Amit and A. Patchornik

Department of Organic Chemistry, The Weizmann Institute of Science, Rehovot, Israel

(Received in UK 3 March 1975; accepted for publication 19 March 1975)

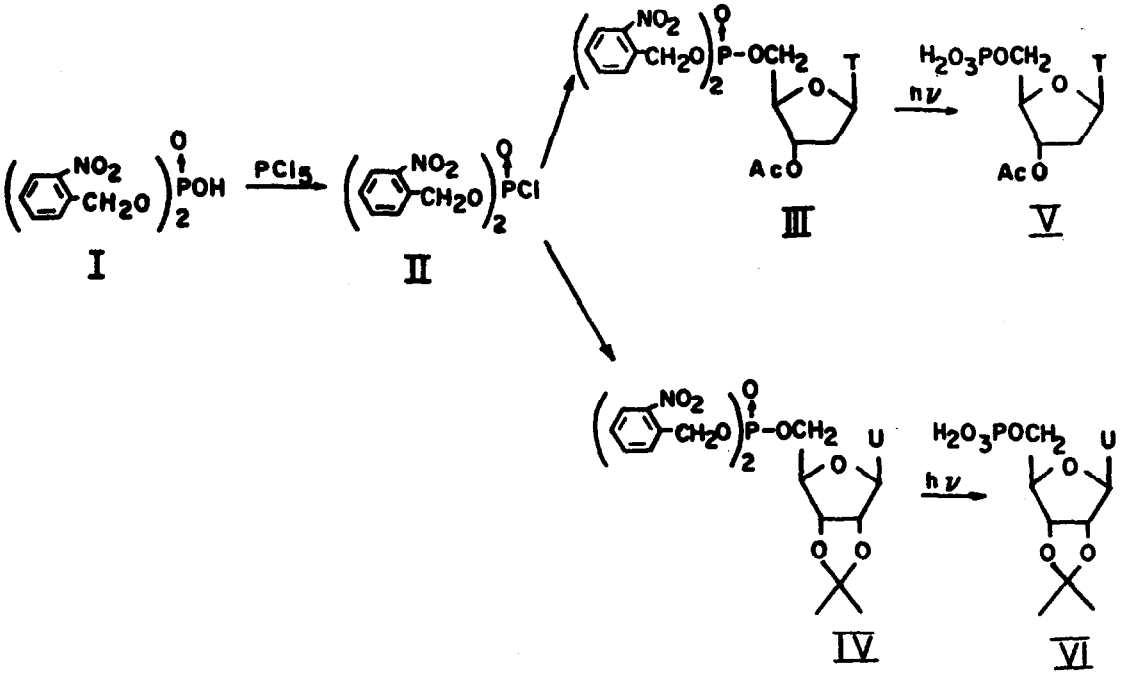
In nucleotide synthesis, the groups which are generally used to protect the phosphate function are removed by various methods, such as oxidation, reduction, β-elimination and acidic or basic hydrolysis. Since oligonucleotides are not fully stable under these chemical processes, it is useful to search for other masking procedures with milder deprotection conditions. Recently, there has been a growing interest in the development of light-sensitive protecting groups for that purpose. The first reported photochemical reaction which hinted at a possible protecting group for the phosphate function was the photohydrolysis of m-nitrophenyl phosphate to produce m-nitrophenol and phosphoric acid.[1] Later on, the m-nitrophenyl and related groups were indeed used by other researchers to protect phosphoric acid derivatives, followed by light-induced demasking.[2-4] Kirby and Varvoglis,[3] for example, used the light-sensitive 3,5-dinitrophenyl protecting group in the synthesis of adenosine-5' phosphate. The photoinduced internal redox reaction of nitroaromatics having a benzylic hydrogen ortho to the nitro group[5] has been utilized for the design of protecting groups for various functional groups such as amino, carboxyl, thiol and hydroxyl.[6]

In this communication we describe the use of the o-nitrobenzyl protecting group for the phosphate function in some mononucleotide syntheses. In preliminary

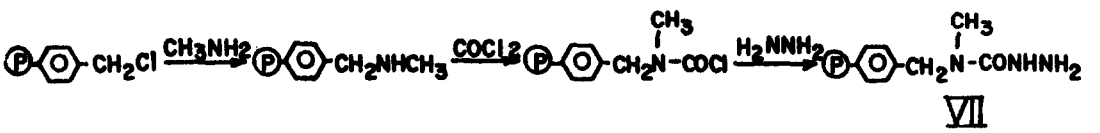
experimentation, di-o-nitrobenzyl phosphate (I)[7] was irradiated in ethanolic solution at wavelengths greater than 305 nm. Free phosphoric acid was obtained in high yield (>95%); this was determined by inorganic phosphate analysis (phosphomolybdic acid method). Mononucleotide synthesis, using the o-nitrobenzyl protecting group, was then undertaken. For this purpose ester I was reacted with phosphorus pentachloride in chloroform, to yield di-o-nitrobenzyl chlorophosphate (II) (mp 83-84°, 90% yield). The molecular weight (theoretical: 387. found: 393) of II was determined by the titration (using sodium methoxide and thymol blue[8]) of aniline hydrochloride formed by briefly heating II with aniline. Compound II was reacted with 3'-O-acetylthymidine (16 hr in pyridine at -20°) to yield 3'-O-acetylthymidine di-o-nitrobenzyl phosphate (III) (Anal: calcd. for $C_{26}H_{27}N_4O_{13}P$: C 49.21, H 4.29, N 8.83, P 4.88; found: C 49.10,H 4.42, N 8.87, P 4.88). 2',3'O-isopropylideneuridine-5' di-o-nitrobenzyl phosphate (IV) was similarly obtained by reacting II with 2',3'O-isopropylideneuridine (Anal: calcd. for $C_{26}H_{27}N_4O_{13}P$: C 49.21, H 4.29, N 8.83, P 4.88; found: C 49.02, H 4.60, N 8.76, P 5.09).

The removal of the o-nitrobenzyl groups from III to yield 3'-O-acetylthymidine-5' phosphate (V) was effected by irradiation ($\lambda > 305$ nm) in aqueous tert-butanol. Thin layer chromatography on cellulose plates using isopropanol - concentrated ammonia-water (7:1:2) (solvent A) afforded V in 77% yield, as determined spectrophotometrically. Similarly, 2',3'-O-isopropylideneuridine-5' phosphate (VI) was obtained in 98% yield upon irradiation of IV in aqueous ethanol.

Initial attempts to isolate nucleotides V and VI on a preparative scale were unsuccessful due to the presence of a large amount of dark-colored photo by-products. These impurities could not be completely separated from V and VI either by chromatography on Whatmann 3MM paper using solvent A, or by high voltage electrophoresis at pH 6.5. These by-products originate from the o-nitrobenzyl protecting group, probably via carbonyl-containing intermediates such as o-nitrosobenzaldehyde. In order to overcome this difficulty we introduced an insoluble polymeric carrier of carbonyl reagents capable of covalently binding carbonyl compounds.



4-Methyl semicarbazide-4-methylated polystyrene cross-linked with 2% divinylbenzene (VII) was prepared by subsequently reacting cross-linked chloromethylated polystyrene with methylamine, phosgene and hydrazine. When irradiations of protected nucleotides III and IV were performed in the presence of polymer VII, formation of the soluble, colored photo by-products was negligible and the irradiated solution remained almost colorless.



By this method, the isolation of nucleotides V and VI from their irradiated solution on a preparative scale could be carried out. Thus, for example, polymer VII (1 g, 1.4 mmol of semicarbazide residues) was suspended in a solution of IV (96 mg, 0.15 mmol) in chloroform (100 ml) and the suspension irradiated for 48 hr with stirring at room temperature. After filtration, the ionically bound nucleotide VI was selectively eluted from the colored polymer with a mixture of triethylamine (10%) in aqueous dioxan (1:2). The combined filtrate and washings

were concentrated and pure nucleotide VI was obtained chromatographically on Whatmann 3MM paper using solvent A (R_f = 0.35, the same as an authentic sample of VI). The yield was 91%, determined spectrophotometrically. Nucleotide V was similarly obtained in 70% yield.

Acknowledgments: This work has been supported in part by Grant No. AM 5098 from the National Institutes of Health and in part by a Grant from the United States-Israel Binational Science Foundation.

References

1. E. Havinga, R.O. De Jongh and W. Dorst, Rec.Trav.Chim.Pays-Bas 75 378 (1956)
2. J. Kirby and A.G. Varvoglis, Chem.Comm. 405 (1967)
3. J. Kirby and A.C. Varvoglis ibid 406 (1967)
4. D.L. Miller and T. Ukena, J.Amer.Chem.Soc. 91, 3050 (1969)
5. H.A. Morrison in: The Chemistry of the Nitro and Nitroso Groups, H. Fever ed. John Wiley & Sons, N.Y. 1970, Part I, pp.185-191
6. B. Amit, U. Zehavi and A. Patchornik, Israel J.Chem. 12, 103 (1974)
7. M. Rubinstein and A. Patchornik (in preparation)
8. A. Patchornik and S. Ehrlich-Rogosinski, Anal.Chem. 31 985 (1959)