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USE OF THE NAPHTHALENE RADICAL ION IN DEBLOCKING O-METHOXYTRITYL NUCLEOTIDE DERIVATIVES[1]

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The mono-p-methoxytrityl group has found extensive use in protecting hydroxyl functions in nucleosides and nucleotides[2]. Resistant to common phosphorylating agents and to alkaline conditions employed to deprotect acyl nucleotides, the methoxytrityl ethers are readily cleaved under mild acidic conditions. In addition, they serve as convenient markers on chromatograms since they afford a characteristic yellow color with aqueous perchloric acid, and as lipophilic substances, they provide a means for separating the protected from the non-protected nucleotidic material[3].

In connection with a study of phosphoramidate analogs of oligonucleotides[4], a need developed for a method for deblocking methoxytritylthymidyl under non-acidic conditions. Trityl ethers have been cleaved by catalytic hydrogenolysis[5]; however, in our hands attempts to convert 3'-O-mono-p-methoxytritylthymidine to thymidine by hydrogenolysis (palladium or platinum catalyst) in neutral solution (ethanol) were unsuccessful. Trityl ethers have also been cleaved by sodium or potassium[6] suspended in ether and by lithium in a mixture of tetrahydrofuran and liquid ammonia at -70°[7]. These reagents are unattractive for use with nucleotide derivatives, however, since the thymine ring is reduced by sodium in liquid ammonia[8].

We have found that methoxytrityl ether derivatives can be deblocked conveniently and effectively by reaction with the naphthalene radical anion in hexamethylphosphoric triamide. With this reagent reduction of the thymine ring is insignificant, if it occurs at all. For example, reaction of 3'-O-mono-p-methoxytritylthymidine (13 mg; 280 O. D.$_{266}$ units) with excess naphthalene radical anion (from 7 mg of lithium and 160 mg of naphthalene) in hexamethylphosphoric triamide (4 ml) for one hour, followed by addition of water, neutralization, and extraction of naphthalene and the triamide solvent with chloroform, afforded 220 O. D.$_{266}$ units of thymidine

(92%). In a duplicate experiment in which naphthalene was omitted, reduction of the thymine ring was extensive; only 5 O. D.$_{266}$ units (2%, as thymidine) of uv-absorbing material was found.

Results of the application of this technique in deblocking a series of phosphoramidate oligo nucleotides protected at the terminal 3'-oxygen.with the mono-p-methoxytrityl group are given in table 1. To facilitate dissolution, the higher oligomers were dissolved in pyridine prior to addition to hexamethylphosphoric triamide. The desired phosphoramidate oligonucleotides were isolated in > 50% yield. Apparently some degradation occurred during the reaction or work-up procedure; however, the thymine rings remained intact. It may be noted that the reductive cleavage is very rapid (exp. for n = 4) and that the oligomers survive exposure to the reagents for an extended period of time (exp. for n = 1). Recent work to be reported elsewhere indicates that this technique will also be useful in deblocking methoxytrityl derivatives of oligonucleotides possessing all four common bases and natural 3'-5'-phosphodiester bonds[9].

Table I. d-(NH_2)T[p(NH)T]$_n$OMTr $\longrightarrow$ d-(NH_2)T[p(NH)T]$_n$

n	Metal	Time min.	Product[(a)] %	R_m^{dpT}	R_f^A	R_f^F	Acid Hydrol.[b] d-(NH_2)Tp/d-$(NH)_2$T
1	Li[(c)]	60	92	0	0.21	0.56	1.1
1	Na	60	54				1.1
3	Na	15	65	0.42	0.02	0.33	3.2
4	Na	3	53	0.58	0.008	0.27	4.5

(a) R^{dpT} refers to the electrophoretic mobility at pH 7.2 (phosphate buffer) on paper relative to dpT. R_f^A is the R_f value for chromatography on paper with solvent A (experimental section). (b) Products determined on Varian LC-1000. (c) Exp. with Li carried out with 105 O. D.$_{266}$ units of nucleotide. For quantities with Na, see exp. section.

General Procedure. A solution of the naphthalene radical anion was prepared by adding naphthalene (1.2 mmol) to a solution of sodium (1 mmol) in hexamethylphosphoric triamide (5 ml). The nucleotidic material (50 O. D.$_{266}$ units) in anhydrous pyridine (0.3 ml) was added to the dark green solution and the mixture was stirred for the indicated time. After addition of several drops of water to react with the excess reducing agents, water (40 ml) was added, the

pH was adjusted to pH 8-9 with carbon dioxide, and the solution was extracted with chloroform (4x30 ml). The aqueous layer was concentrated under reduced pressure and applied to a DEAE-Sephadex column (0.8x17 cm) equilibrated with 0.01 M ammonium bicarbonate (pH 8.5). Following elution of pyridine and low molecular weight products with the 0.01 M buffer, the oligomers were eluted with higher concentrations of ammonium bicarbonate (gradient elution to 0.075 M for the dinucleotide and to 0.35 M for the pentanucleotide). Products were isolated from the appropriate fractions by repeated lyophilization to remove the ammonium bicarbonate and were characterized by electrophoresis (2000 v, phosphate buffer at pH 7.2), paper chromatography, and hydrolysis. The solvents for paper chromatography were: A, 2-propanol--ammonium hydroxide--water, 7:1:2, v/v; and F, 1-propanol--ammonium hydroxide--water, 11:2:7, v/v. Yields were determined from the optical density at 266 nm. All products gave positive ninhydrin tests and negative tests for trityl groups (perchloric acid).

Hydrolytic Degradation. A portion of the phosphoramidate oligonucelotide (4 O.D.$_{266}$ units) was hydrolyzed by warming with 0.2 M hydrochloric acid for 10 minutes at 100°. The products were characterized by chromatography on paper in solvent A, by electrophoresis, and, for quantitative ratios, by chromatography with a Varian LCS-1000 chromatograph (PC 37 cation column, 0.05 M ammonium phosphate buffer at pH 6.5). These tests revealed two major components as expected, both ninhydrin positive: d-(NH_2)T (R_m^{dpT} -0.6; R_f^A 0.45) and d-(NH_2)-Tp (R_m^{dpT} + 0.45; R_f^A 0.06). In addition one or two very minor components were generally found in the hydrolytic products (R_m^{dpT} -0.1; R_f^A 0.16 or 0). They were observed as very faint spots in the ninhydrin test even when in too low a concentration to observe on paper under ultraviolet light. Although not identified, it is plausible that they are pyrophosphates formed during the acid hydrolysis.

Hydrolyses with snake venom phosphodiesterase[10] were carried out on 2-3 O.D.$_{266}$ units of the deblocked oligonucleotides. Quantitative analysis on the LCS-1000 chromatograph showed the major product to be d-(NH_2)T in each case. Thus, the products from the pentanucleotide analog consisted of d-(NH_2)T (81% of the total O.D.; elution time 8 min; R_m^{dpT} -0.6; R_f^A 0.45) and unidentified material which was eluted at the void volume (3.7 min; 13% of total O.D.; R_f^A

0.05) and at 5.3 min. (6%; R_f^A 0.0). Similar analysis of the tetranucleotide analog gave d-(NH_2)-T (90%) and a material eluting at the void volume (10%).

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