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ANTHRANILIC ACID: A NEW COREAGENT FOR THE DEBLOCKING OF 2,2,2-TRICHLOROETHYLPHOSPHATE PROTECTING GROUPS

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Summary: Deblocking of 2,2,2-trichloroethyl groups from 3'-terminal phosphodiesters has been achieved by treatment with activated zinc powder using the novel coreagent anthranilic acid. This deblocking procedure has been used in the construction of the guanosine-rich octadeoxyribonucleotide d(GGGGGATC).

The current phosphotriester approach in polymer supported synthesis of oligodeoxynucleotides requires the preparation of very pure, suitably protected mono- and dinucleotide blocks as building units (for high molecular weight oligomers). During preparation of these intermediates we use fully protected nucleotides bearing a 2,2,2-trichloroethyl group as a temporary phosphate protecting group.

Despite the favourable properties of a) facile introduction by the aid of the monofunctional phosphorylating agent 2-chlorophenyl-2,2,2-trichloroethylphosphorochloridate[1], b) high stability during condensation and purification steps, c) specific cleavage under non alkaline and non acidic conditions by O-alkyl-cleavage, we observed some difficulties during deblocking of this group. Deprotection with activated zinc powder in pyridine and the coreagents reported to date did not meet the necessary requirements when applied to our substrates on dimer level:
(I) Use of triisopropylbenzenesulphonic acid[2] required unacceptably high reaction times to deprotect the dimer block (1) although mononucleotide deprotection was fast (table I, row a). After 60 minutes reaction time, only 50% of phosphotriester was converted to phosphodiester, and a considerable amount of unidentified by-products was detected. The ability of triisopropylbenzenesulphonic acid to serve as a coreagent may be due mainly to the formation of pyridinium ion from the strong sulphonic acid (pka$\sim$0.7) in the pyridine solution. This is demonstrated by the use of pyridiniumhydrobromide under the same reaction conditions (table I, row d).
(II) Acetylacetone as a coreagent[3] gave very good results in the deprotection reaction itself even when applied to higher oligomers[4] (table I, row b), but created several difficulties in later condensation reactions, e.g. excessive sulphonylation of incoming OH-components and very slow condensation

Table I: Removal of 2,2,2-trichloroethyl group using zinc powder in the presence of coreagents

	Nucleotidic model-compound[1]	Amount of substance (mMol)	Cleavage variant[2]	Solvent	Coreagent	Time (min)	Conversion to phosphodiester[3]
a.	(1)	0.015	B	Py	TPSOH	60	50
						120	90
b.	(2)	0.250	A	Py	Acetylacetone	5	100
c.	(2)	0.125	A	MeOH	Acetylacetone	14	100
d.	(2)	0.125	B	Py	Py·HBr	10	98
						40	100
e.	(2)	0.500	A	Py	Pyridine-3-sulphonic acid	10	70
						120	90
f.	(2)	0.250	A	Py	Anthranilic acid	10	98
						17	90
g.	(1)	0.015	A	Py	Anthranilic acid	35	95
h.	(2)	0.125	A	MeOH	Anthranilic acid	20	3
i.	(2)	0.025	A	Py	DTT	14	90
						60	100
j.	(3)	0.015	A	Py	DTE	35	95

1) (1) = (Tr)G_d^{ib}pA$_d^{bz}$p(te); (2) = (Tr)T_dp(te); (3) = (Tr)G_d^{ib}pG_d^{ib}p(te); te = OCH_2CCl_3; p = -$_i$P(O)(2-ClPh)

2) A: 5 equiv. coreagent; 0.05 M phosphotriester solution
B: 0.2 equiv. coreagent; 0.2 M phosphotriester solution

3) Estimated by tlc

reaction rates. This is mainly due to a slight zinc-acetylacetonate contamination which sticks to the oligonucleotide chain. It is known that zinc ions chelated by acetylacetone are able to form complexes with N-substituted amides and amines (e.g. DMF-complex)[5].

A suitable coreagent should not only serve as an acidic catalyst, but should also be able to form stable complexes with the zinc ions in order to remove them from the reaction equilibrium and therefore drive the reaction to completion by regenerating the zinc surface. The formed complexes should be easily removable by having either, a negligible solubility in the work up solvents or a much greater solubility in water than in chloroform, which allows application of filtration or extraction procedures, respectively.

Among various reagents, we tested pyridine-3-sulphonic acid, threo- and erythro-1,4-dimercapto-2,3-butanediol (DTT, DTE) and anthranilic acid. The results are summarized in table I. The most promising results were obtained with DTT or DTE and anthranilic acid. Of these, anthranilic acid gave the best match to our criteria for selection of coreagents: acceptable reaction times on dimer blocks, formation of insoluble zinc anthranilate in nearly all solvents (except pyridine), easy detection of coreagent and coreagent-zinc-complex by strong bright fluorescence on silica gel tlc.

In some of the deblocking mixtures, 2% - 4% of an unpolar by-product was observed, which was isolated after silica-gel-chromatography and could not be cleaved by repeating the zinc-anthranilic acid procedure. In these cases we subjected the phosphodiesters to flush-chromatography on very short layers of silica gel 60 H, eluting with a stepwise gradient of methanole in chloroform

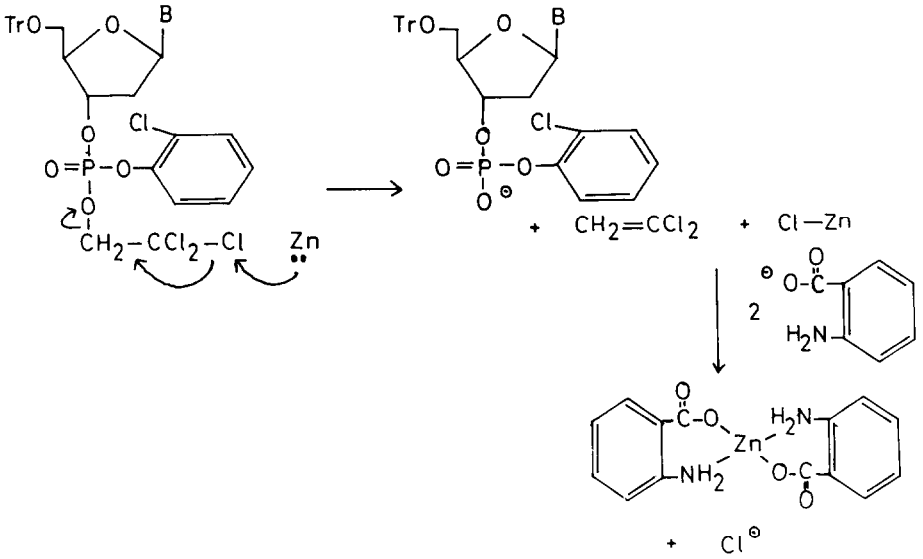

Figure 1: Cleavage of 2,2,2-trichloroethylphosphate protecting groups by means of zinc powder/anthranilic acid.

(3,6,15%).

The ability of the zinc-anthranilic acid procedure to give phosphodiesters which were able to serve as phosphate components in further condensation reactions was demonstrated by the synthesis of a guanine-rich octanucleotide in solution following the CatlinCramer approach[7]. Starting from the fully protected dinucleotide blocks (Tr)G_d^{ib}pG$_d^{ib}$p(te) (3) and (Tr)G_d^{ib}pA_d^{bz}p(te) (1), the corresponding phosphodiester components were obtained in 96% and 94% yield, respectively. These were condensed with the dimeric OH-components G_d^{ib}pG$_d^{ib}$p(te) and T_dpC_d^{tl}(Bb), by the aid of MSNT to give 32% and 74 % yield of fully protected tetramers (Tr)G_d^{ib}pG_d^{ib}pG_d^{ib}pG_d^{ib}p(te) (4) and (Tr)G_d^{ib}pA_d^{bz}pT_dpC_d^{tl}(Bb) (5). In the same reaction time as for the dimer blocks, even the most inconvenient tetramer block (4) could be converted to its corresponding phosphodiester (95% conversion after 30 minutes as estimated on tlc; 68% isolated material after chromatography on RP-18 silica gel). Condensation with the detritylated tetramer (5) (by means of $ZnBr_2$ in methanole solution) afforded the fully protected octanucleotide d(GGGGGATC) in a yield of 10%, as expected for a condensation of this complexity. After removal of protecting groups by a) $ZnBr_2$, b) oximate and c) NH_3, the octamer was chromatographed on DEAE-cellulose and further purified by gel electrophoresis. The sequence was confirmed by the mobility shift method.

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6) General procedure: To a heavily stirred solution of 1 mmole 3'-phosphotriester and 5 mmole anthranilic acid (686 mg) in 20 ml pyridine were added 10 mmole activated zinc dust. After 15 minutes a mixture of 5 mmole triethylamine (634 µl), 0.3 ml water and 0.5 ml pyridine was added and the reaction mixture stirred for another 5 minutes. Excess zinc was removed by filtration and washed with pyridine. The filtrate was worked up by extraction of the phosphodiester with chloroform from saturated sodium bicarbonate solution followed by two times coevaporation with toluene/chloroform 3/7 and precipitation in 100 ml of diethylether/hexane 1/1 from 4 ml chloroform.
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