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A NEW STRATEGY FOR THE PROTECTION OF DEOXYGUANOSINE DURING OLIGONUCLEOTIDE SYNTHESIS

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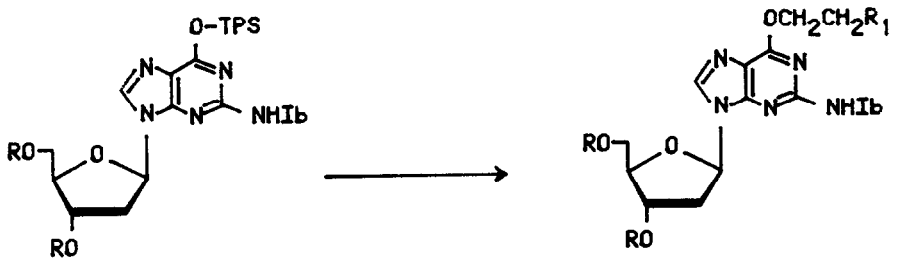
Abstract: The protection of the 6-oxo group of deoxyguanosine with the 2-trimethylsilylethyl (2), phenylthioethyl (3), 4-nitrophenylthioethyl (4), 4-nitrophenethyl (5), and cyanoethyl (6) groups is described. Each protecting group is introduced in good yield and is cleaved under mild conditions. Compatibility with the various approaches to oligonucleotide synthesis is discussed.

The long-standing problem of side-reactions of guanine residues during oligonucleotide synthesis continues to be cited,[1-6] despite the major advances in synthetic methodology reported in recent years. The source of the problem seems to lie in the susceptibility of the guanine 6-oxygen to sulfonylation by condensing agents[7-9] and to phosphorylation by activated nucleotides.[10] Although some of the derivatives formed may be converted back to the parent compound,[3,10] in many instances they degrade to polar, fluorescent materials. The problems with guanine degradation are, of course, most severe with longer oligomers, where repeated exposure of the guanine residues to possible sulfonylation or phosphorylation leads to constant decreases in yield with increasing chain length. Use of very short reaction times for the condensation reactions may minimize, but cannot eliminate this type of degradation, and may lead to incomplete reaction.

We sought to eliminate this degradation by developing a new protecting group strategy for guanine that would include O-6 protection.[12] For the protecting group itself we chose to investigate the trimethylsilylethyl group, cleaved under neutral conditions with fluoride ion,[13-17] and substituted ethyl groups which are cleaved by β-elimination reactions. Since many of the latter have been developed (as phosphate protecting groups),[1,18] groups with a wide range of stabilities are immediately available from which to choose the one most suitable to complement a particular synthetic strategy.

Synthesis of 2c, 3c, and 4c[19] was done from 1a as reported in the preceeding paper. Compounds 5c and 6c[19] were prepared from 1b, as they would not survive the basic conditions employed for hydrolysis of 3' and 5' isobutyryl groups. In fact, the conditions normally used for desilylation - tetra-n-butylammonium fluoride (TBAF) in THF - also cleave the 6-O-cyanoethyl and 4-nitrophenethyl moieties. However, this loss can be avoided by using a buffered desilylating reagent consisting of a mixture of TBAF and pyridine HF.[20] With 2M HF/1M TBAF in pyridine (1 eq TBAF) desilylation is complete in 24 hours, with no loss of the guanine protecting group. The reagent is conveniently prepared by adding the required



1a,b

2a-c - 6a-c

a R=Me_2CHCO

b R=Me_3CSiMe_2

c R=H

2 R_1=$SiMe_3$
3 R_1=SC_6H_5
4 R_1=$SC_6H_4NO_2$
5 R_1=$C_6H_4NO_2$
6 R_1=CN

amount of TBAF/THF and aqueous HF to excess pyridine and drying by concentration on a rotary evaporator.

The properties of these five protecting groups are summarized in the Table below. To be a useful addition to synthetic strategy a protecting group must meet stringent criteria. These would include ease of introduction, high yield cleavage under mild conditions, and compatibility with other protecting groups and procedures employed during the synthesis. While each protecting group may be introduced in good yield, and cleaved under mild conditions, it is clear that not each of them is suitable for all approaches to oligonucleotide synthesis. The trimethylsilylethyl (TSE) group, for example, was found to be extraordinarily sensitive to acid, so much so that even the mild conditions commonly employed for cleavage of the 5'-dimethoxytrityl group also remove the TSE group. It is, however, stable during removal of an alternative 5' protecting group, the 2-dibromomethylbenzoyl (DBMB) group.[21] The phenylthioethyl and 4-nitrophenylthioethyl groups, on the other hand, are quite stable, until oxidized to the sulfoxide, and are compatible with all current synthetic methods. As expected, 4c is more readily cleaved after oxidation than is 3c, but is much more resistant to oxidation and thus appears to offer no advantage over the latter. The nitrophenethyl group also appears to be generally applicable, and it has the advantage that no oxidation step is required before cleavage by DBU. In terms of ease of removal, the cyanoethyl group appears to be the most promising, since it is cleaved simply with aqueous ammonia. Although it is cleaved in aqueous ammonia and with aqueous triethylamine it is relatively stable to triethylamine under anhydrous conditions. For example, in the 1:1 mixture of triethylamine:acetonitrile used for β-elimination of the 3' cyanoethyl phosphate function 6b shows only 50% cleavage after 20 hours.

Compound	Yield	Removal	Compatibility[a]
4c	65%	5eq TBAF/THF, 5 min or $ZnBr_2/CH_3NO_2$, 10 min	DBMB CNEP
5c	81%	1) $NaIO_4$, 6 hrs 2) DBU, 18 hrs or 2) NH_4OH, 50°, 24 hrs	DMT DBMB CNEP
6c	75%	1) $NaIO_4$, 24 hrs 2) DBU, 1 hr or 2) NH_4OH, 50°, 2 hrs	DMT DBMB CNEP
7c	75%	DBU, 2 hrs	DMT DBMB CNEP
8c	70%	DBU, 10 min or NH_4OH, 1 hr	DMT CNEP

Reagents: TBAF/THF is a 1M solution of tetra-n-butylammonium fluoride in tetrahydrofuran, DBU is 1,8-diazabicyclo[5.4.0] undec-7-ene, DMT is the 5'-O-dimethoxytrityl group, DBMB is the 5'O-dibromomethylbenzoyl group, CNEP is the 3'-O-(chlorophenyl)cyanoethylphosphate group.

[a]Compatibility with DBMB means that no loss of the protecting group was detectable after 5 hours with Ag^+ and 5 hours 0.2 M Na_2CO_3 in dioxane:water; with CNEP no loss after 2 hours with 1:1 triethylamine:acetonitrile; with DMT no loss after 1 hour with $ZnBr_2$ in nitromethane, or ½ hour with 2% toluenesulfonic acid.

Finally, we treated samples of 2a, 3a, 4a, 5b, and 6b with 5 eq of each of the condensing agents 1-(mesitylene-2-sulfonyl)-4-nitroimidazole, 1-(mesitylene-2-sulfonyl)-tetrazole, triisopropylbenzenesulfonyl tetrazole, and triisopropylbenzenesulfonyl chloride plus tetrazole in pyridine for 24 hours. In each case there was no degradation detectable by tlc. Under these conditions triisobutyryl deoxyguanosine itself is extensively degraded. Thus each group does indeed protect the guanine ring. As expected, condensation reactions involving these protected deoxyguanosine derivatives proceed in higher yield and without the dark colors and side products otherwise evident in such reactions.[22] In addition to successfully protecting the guanine residues during condensation reactions, each protecting group also improves the solubility and chromatographic properties of deoxyguanosine and oligomers containing it. Full details describing use of some of these derivatives in oligonucleotide synthesis will be reported shortly.

Each of the protected deoxyguanosine derivatives reported herein is readily prepared and each is cleaved under mild conditions in high yield. Moreover, the wide range of stabilities available in this series allows maximum flexibility in design of synthetic strategy. In fact, the examples that we have reported undoubtedly will be only the first representatives of this promising class of guanine protecting groups. Certainly many others will be developed, now that a practical synthetic route is available, to meet particular synthetic requirements.

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