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USE OF 2-F-dI TO PRODUCE N2-MODIFIED dG DERIVATIVES

Introduction

To our list of Convertible Nucleosides[1], we are adding 2-F-dI-CE Phosphoramidite (1). 2-Fluoro-2'-deoxylnosine (Figure 1) can be converted to 2-substituted dG derivatives by reaction with a primary amine, which displaces the fluorine atom.[2-5] The timing of the conversion step is a little tricky because small alkyl primary amines are capable of doing the conversion while also cleaving and deprotecting the oligonucleotide. For example, reaction with ethylamine would convert 2-F-dI to N2-ethyl-dG but would simultaneously cleave and deprotect the oligonucleotide. Although that may be interesting in its own right, we have chosen to focus on larger primary amines in our development work. For example, treatment of the oligonucleotide (while still fully protected on the synthesis column) with dansyl cadaverine converts the nucleoside to an N2-dansyl-dG derivative, as shown in Figure 2. Following removal of the O6-protecting group with DBU, further conventional deprotection of the oligonucleotide leads to the final product. The product oligonucleotide now has a fluorescent tag which, when hybridized to the target strand, will project into the minor groove of the double-stranded duplex. In a further example repeating work originally described by the Verdine group[2,3], we used cystamine to convert the 2-F-dI to a product containing an alkyl thiol group at the N2 position (Figure 2). Once this converted oligo is hybridized to the target, the thiol is available for cross-linking to, for example, a protein binding to the minor groove. The thiol can also form a disulfide crosslink with a similarly modified G on the complementary strand.[2,3]

As with all convertible nucleosides, we caution that these reactions are not trivial and should be undertaken by researchers with a good background in chemistry and access to appropriate analytical techniques.

References

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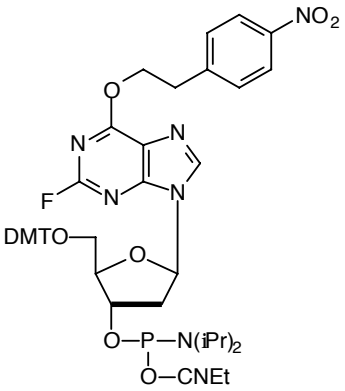
(2) D. Erlanson, L. Chen, and G.L. Verdine, J. *Amer. Chem. Soc.*, 1993, **115**, 12583-12584.
(3) D.A. Erlanson, J.N.M. Glover, and G.L. Verdine, *J. Amer. Chem. Soc.*, 1997, **119**, 6927-6928.
(4) L.V. Nechev, C.M. Harris, and T.M. Harris, *Chem Res Toxicol*, 2000, **13**, 421-429.
(5) A.R. Diaz, R. Eritja, and R.G. Garcia, *Nucleos Nucleot Nucleic Acids*, 2000, **19**, 703.

FIGURE 1: **CONVERSION OF 2-F-dI TO 2-AMINO DERIVATIVES**



(1) 2-F-dI-CE Phosphoramidite

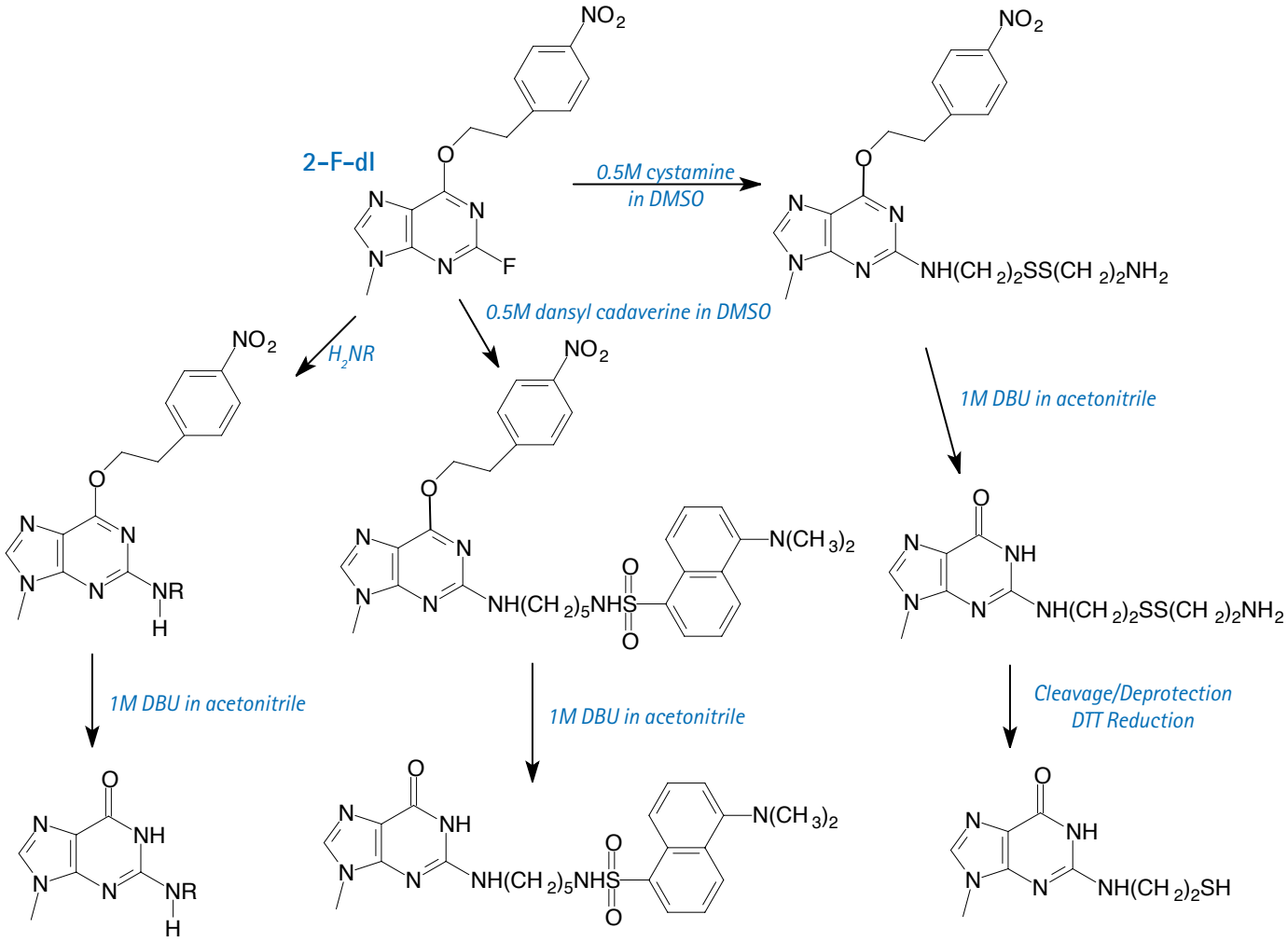
2-F-dI Conversion

2-F-dI is a convertible nucleoside that allows synthesis of oligos containing an N2 substituted deoxy-guanosine. 2-F-dI is unique in that the conversion reaction must be performed prior to cleavage and deprotection of the other nucleoside bases.

Protocol:

1. Oligo Synthesis:
   Incorporate 2-F-dI at the desired position using standard synthesis conditions.
2. Nucleoside Conversion:
   At the conclusion of oligonucleotide synthesis, rinse the synthesis column with acetonitrile and roughly dry the support with argon. Dissolve desired primary amine in 1-2 mL DMSO (other organic solvent can be substituted depending on solubility of amine) at a concentration of 0.5 M. Treat the support in the column with the amine solution using two 1 mL disposable syringes. Incubate for 18-24 hours at RT to effect conversion. An alternate approach is to transfer the support to a Sarstedt tube, add the amine solution and incubate as above.
3. Preliminary O6 Deprotection:
   Wash the support 2 times with DMSO then 3 times with acetonitrile and roughly dry the support with

FIGURE 2: **CONVERSION OF 2-F-dI TO 2-AMINO DERIVATIVES**



argon. Using two disposable syringes as above, treat the support two times with 1 mL each 1 M DBU in acetonitrile one hour each time. Rinse the support with 1 mL each of Methanol x 2, and Acetonitrile x 3.
Roughly dry the support with argon.

4. Oligo Deprotection:
Deprotect the oligo with ammonium hydroxide as normal.

Experimental Results

1. We use the conversion of 2-F-dI with dansyl cadaverine to illustrate the technique and to optimize the reaction. A simple oligo-dT containing a single insertion of 2-F-dI was used. Each sample was from a 0.2 µmole synthesis and the reaction volume was 0.2 mL. The reactions were carried out as shown in Table 1. The oligo was cleaved with ammonium hydroxide for 45 minutes at room temperature and the product was analyzed by RP HPLC. The dansyl modified oligonucleotide was retained longer by HPLC and the percent conversion was estimated.
2. 2-F-dI conversion with cystamine. Each sample was from a 0.2 µmole synthesis with a reaction volume of 0.2 mL. The results are shown in Table 2.

Conclusion

The fluorine in 2-F-dI is efficiently displaced by a primary amine in DMSO. The reaction is concentration dependent, probably related to molar ratio of amine. The reaction is performed in organic (DMSO) solvent.We recommend using 1 mL of at least 0.1 M amine in DMSO 18 hrs. at RT.

TABLE 1: **REACTION OF 2-F-dI WITH DANSYL CADAVERINE**

Dansyl Cad. Conc.	Incubation Time (Hours)	Incubation Temp.(°C)	Conversion(%)
10 mM	18	RT	29
10 mM	60	RT	41
50 mM	18	RT	87
50 mM	18	37	92

TABLE 2: **REACTION OF 2-F-dI WITH CYSTAMINE**

Cystamine Conc.	Solvent	Incubation Time (Hours)	Incubation Temp.(°C)	Conversion (%)
1.0M	0.1M Na phos. pH 8.4	88	RT	≈100*
0.5M	DMSO	88	RT	100

* Two substitution products formed, the second with a longer retention time and a pronounced red shift in the spectrum from 262 nm to 312 nm. The later eluting peak is probably not DNA and is most likely a cystamine related product formed in aqueous media.