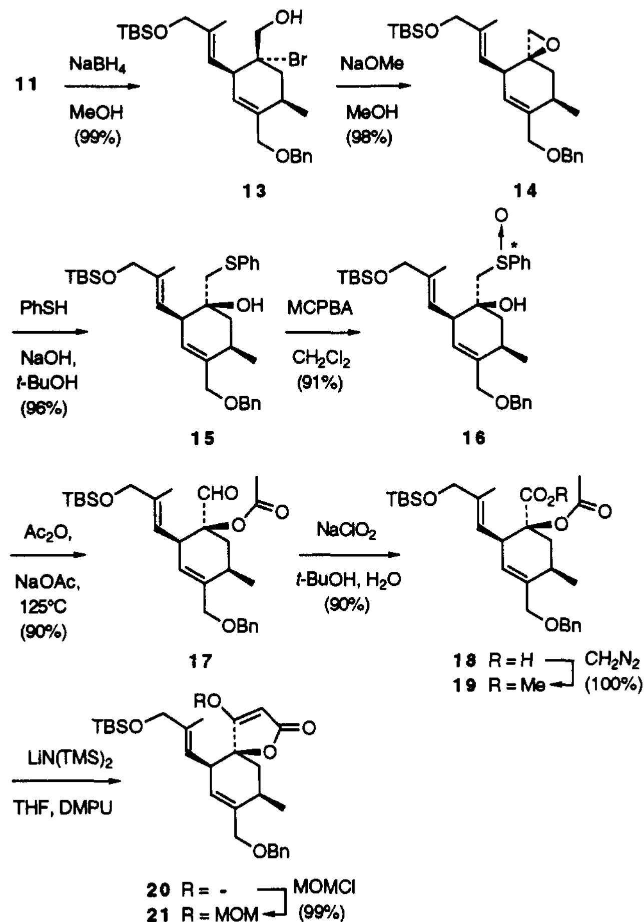
near-quantitative yield. Oxidation of the derived sulfide 15 with MCPBA afforded a separable 3:1 mixture of diastereomeric sulfoxides. Each was converted to the same acetoxy aldehyde 17 upon heating in Ac_2O and NaOAc.[10]



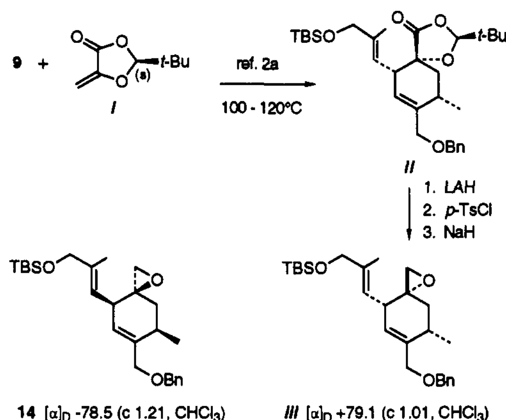
Completion of the synthesis was effected through the cyclization methodology of Ireland and Thompson, but substituting DMPU for HMPA as the cosolvent.[2f] Addition of MOMCl to the basic solution gave the spirotetronate 21, $[\alpha]_D$-28.0(c1.70, $CHCl_3$), in 99% yield for the two steps. The ^1H NMR spectrum of this product was in excellent agreement with that of a close analogue (DPS in place of OBn) prepared by Roush and Brown.[2a]

Acknowledgment. We are indebted to Dr. Kevin Pinney for assistance with ^1H NMR analyses. We thank Dr. Michael Walla and Dr. William Cotham for their valuable assistance with mass spectra interpretation. Special thanks to Professor Roush for ^1H NMR comparisons of spirotetronates. Support for this work was provided by Research Grant CA34247 from the National Cancer Institute of the NIH.

Supplementary Material Available: Experimental procedures and ^1H NMR spectra for all key intermediates (43 pages). This material is contained in many libraries on microfiche, immediately follows this article in the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(8) For previous applications of this methodology, see: Marshall, J. A.; Welmaker, G. S.; Gung, B. W. *J. Am. Chem. Soc.* 1991, *113*, 647. In the present case by making reasonable assumptions regarding the preferred conformation of the *O*-methyl mandelate in the relatively crowded environment of the carbinyl center, we can apply the method to the primary alcohol 13. As a rule only secondary alcohols can be reliably assigned by this method: Trost, B. M.; Belletire, J. L.; Godleski, S.; McDougal, P. G.; Balkovec, J. M.; Baldwin, J. J.; Christy, M. E.; Ponticello, G. S.; Varga, S. L.; Springer, J. P. *J. Org. Chem.* 1986, *51*, 2370.

(9) We also carried out a chemical correlation as follows:



(10) Cf. Lee, A. W.; Martin, V. S.; Masamune, S.; Sharpless, K. B.; Walker, F. J. *J. Am. Chem. Soc.* 1982, *104*, 3515.

Synthesis of an Oligonucleotide Suicide Substrate for DNA Methyltransferases

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Summary: The large-scale chemical synthesis of an oligodeoxynucleotide containing 5-fluoro-2′-deoxycytidine (FdC) and its characterization are described. The FdC residue is introduced via the corresponding 4-*O*-(2,4,6-trimethylphenyl)-2′-deoxyuridine derivative, which undergoes clean conversion to FdC during removal of the oligonucleotide protecting groups with ammonia. A double-stranded oligodeoxynucleotide containing FdC inactivated the DNA methyltransferase enzyme M.*Hae* III by irreversible formation of a covalent protein–DNA complex.

DNA (cytosine-5)-methyltransferases (DCMtases) catalyze the methylation of DNA by the cofactor *S*-adenosyl-L-methionine (SAM). This reaction has aroused widespread interest not only for its profound effects on genomic structure and function[1] but also for its unusual character, involving electrophilic substitution at a formally unactivated vinyl carbon in neutral aqueous solution (Figure 1, X = H). DCMtase-catalyzed methylations are believed to involve the intermediacy of a covalent pro-

(1) For general references on enzymatic DNA methylation, see: Razin, A., Cedar, H., Riggs, A. D., Eds. *DNA Methylation: Biochemistry and Biological Significance*; Springer-Verlag: Berlin, 1984. Adams, R. L. P.; Burdon, R. H. *Molecular Biology of DNA Methylation*; Springer-Verlag: New York, 1985.

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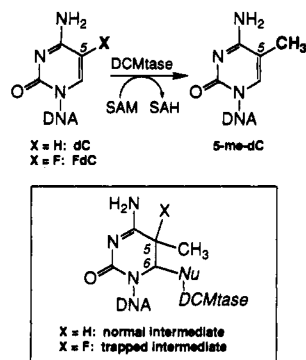

Figure 1. Top: DNA methylation reaction catalyzed by DNA cytosine methyltransferase; bottom (box): putative catalytic intermediate. Nu denotes a nucleophilic amino acid residue of the protein: SAM, *S*-adenosyl-L-methionine; SAH, *S*-adenosyl-L-homocysteine.

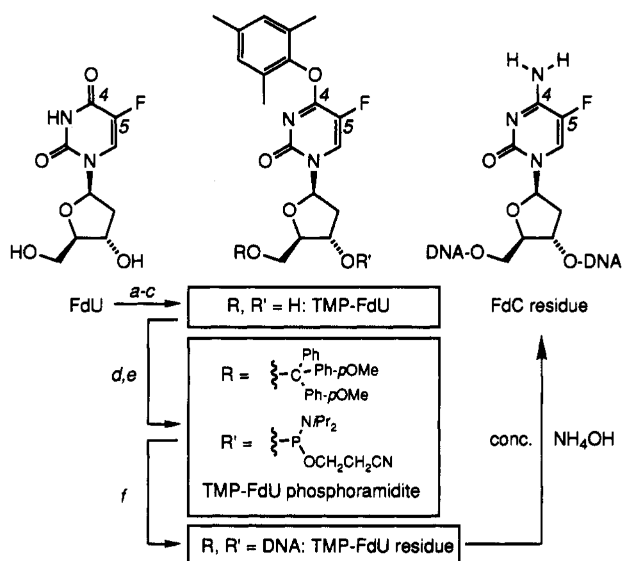

Figure 2. Synthesis of FdU-TMP phosphoramidite and FdC containing DNA: (a) Ac_2O, pyr, DMAP; (b) 1,2,4-triazole, $P(O)Cl_3$, 2,4,6-trimethylphenol, pyr; (c) NH_3, MeOH; (d) 4,4'-dimethoxytrityl chloride, pyr, DMAP; (e) 2-cyanoethyl (*N,N'*-diisopropylamino)chlorophosphoramidite, diisopropylethylamine, THF; (f) automated DNA synthesis.

tein–DNA complex,[2] which can be trapped in stable form by the substitution of fluorine for hydrogen at cytosine C-5 (Figure 1, X = F).[3] Although such a trapped protein–DNA complex could afford an unprecedented view of catalysis on the surface of DNA, this opportunity has not been realized since it has thus far proven difficult to incorporate the suicide substrate, 5-fluoro-2'-deoxycytidine (FdC), into DNA site-specifically.[3] Here we report the chemical synthesis and characterization of DNA containing FdC at a single, predetermined site.

Attempts to incorporate FdC into DNA during solid-phase synthesis have been frustrated by the failure of conventional amide protecting group strategies.[3,4] We reasoned that these problems might be circumvented by using a surrogate for the amine group during automated DNA synthesis, converting it to the amine only during the final ammonia deprotection step; previous work[5–7] had shown that aryl ethers were effective as such surrogates. The convertible nucleoside[6,7] 4-*O*-(2,4,6-trimethylphenyl)-5-fluoro-2'-deoxyuridine (TMP-FdU)—prepared from 5-fluoro-2'-deoxyuridine (FdU, Figure 2)—was shown to withstand the acidic and oxidative conditions of automated DNA synthesis and yet undergo rapid, quantitative conversion to FdC upon treatment with ammonia.[8,10] TMP-FdU was therefore converted to the corresponding phosphoramidite reagent (Figure 2) required for DNA synthesis.[11]

The TMP-FdU phosphoramidite along with the four conventional (A, C, G, T) phosphoramidites was employed in the 10 μmol-scale synthesis[12] of the fully protected 16-mer 5'-d(CGCATAGG(**TMP-FdU**)CATGACG). Following treatment with concentrated aqueous ammonia (55 °C, 14 h; Figure 2), the crude oligonucleotide was purified by reversed-phase HPLC (Beckman Ultrasphere) and detritylated to yield 1.7 μmol of 5'-d(CGCATAGG(**FdC**)CATGACG).[13] The size of the purified 16-mer was confirmed by polyacrylamide gel electrophoresis (not shown), and its composition was examined by enzymatic digestion followed by HPLC analysis of the constituent nucleosides (Figure 3). In addition to the four native nucleosides, a single extra peak was observed, for which the retention time (8.0 min) and UV spectrum (inset) were identical to authentic FdC; the ratio of FdC to dA, dC, dG, and T (after correction for differences in extinction coefficients at 254 nm) was as predicted from the sequence. Notably absent in the digest were the products of incomplete conversion (TMP-FdU, 29.0 min) and hydrolysis (FdU, 8.7 min). Thus, the convertible nucleoside approach offers a clean and efficient route for the site-specific incorporation of FdC into DNA.

The effect of FdC on the stability of duplex DNA structure was addressed through thermal denaturation

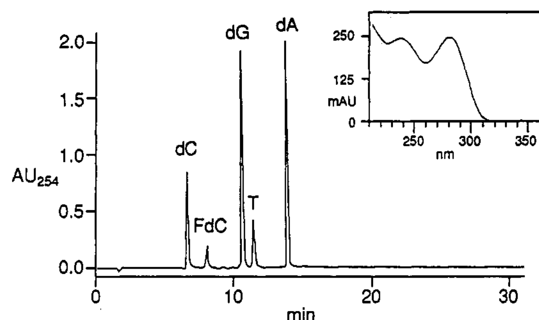

Figure 3. Nucleoside composition analysis of 5'-d(CGCATAGG(**FdC**)CATGACG). Inset: on-line diode array UV spectrum of the peak at 8.0 min.

(2) Santi, D. V.; Garrett, C. E.; Barr, P. J. *Cell* **1983**, *33*, 9. Wu, J. C.; Santi, D. V. *J. Biol. Chem.* **1987**, *262*, 4778.
(3) Osterman, D. G.; DePillis, G. D.; Wu, J. C.; Matsuda, A.; Santi, D. V. *Biochemistry* **1988**, *27*, 5204.
(4) MacMillan, A. M.; Verdine, G. L. Unpublished results.
(5) Zhou, X.-X.; Chattopadhyaya, J. *Tetrahedron* **1986**, *42*, 5149.
(6) MacMillan, A. M.; Verdine, G. L. *J. Org. Chem.* **1990**, *55*, 5931. MacMillan, A. M.; Verdine, G. L. *Tetrahedron* **1991**, *47*, 2603.
(7) Ferentz, A. E.; Verdine, G. L. *J. Am. Chem. Soc.* **1991**, *113*, 4000.
(8) TMP-FdU was synthesized via the 4-triazolyl derivative.[9] Like TMP-FdU, the triazolide could be converted to FdC upon treatment with ammonia. However, the triazolide was found to be exceedingly unstable toward acid and was therefore deemed unsuitable for automated DNA synthesis.
(9) Cf.: Sung, W. L. *J. Chem. Soc., Chem. Commun.* **1981**, 1089. Sung, W. L. *J. Org. Chem.* **1982**, *47*, 3623.
(10) See supplementary material for full details; these materials may also be obtained directly from the authors by FAX [(617) 495-8755].
(11) Tritylation: Schaller, H.; Weimann, G.; Lerch, B.; Khorana, H. G. *J. Am. Chem. Soc.* **1963**, *85*, 3821. Phosphitylation: Sinha, N. D.; Biernat, J.; McManus, J.; Köster, H. *Nucleic Acids Res.* **1984**, *12*, 4539.
(12) Gait, M. J., Ed. *Oligonucleotide Synthesis: A Practical Approach*; IRL Press: Oxford, 1984.
(13) A control 16-mer, synthesized at the same time with only commercial phosphoramidites, yielded the same amount of purified oligonucleotide. Oligonucleotides were synthesized on an Applied Biosystems 381A instrument using the manufacturer's coupling cycle.

experiments. The FdC-containing 16-mer, base-paired with its complementary strand, 5′-d(CGTCATGG(5-me-dC)CTATGCG),[14] exhibited a highly cooperative melting half-transition at 68.0 °C, as compared to 67.6 °C for a control oligonucleotide that contained dC in place of FdC.[16] The ^{19}F-NMR spectrum of the duplex 16-mer showed a single resonance at −89.4 ppm with respect to external trifluoroacetic acid (compared with −89.7 ppm for the free nucleoside), and the ^{31}P-NMR of the duplex molecule exhibited a dispersion envelope from −3.8 to −4.7 ppm vs external trimethyl phosphate, typical of small oligonucleotides. These data, taken together, indicate that the FdC residue does not significantly perturb either the structure or the stability of duplex DNA.

Incubation of the FdC-containing duplex 16-mer with the overproduced DCMtase enzyme M.*Hae* III, in the presence of the cofactor SAM, has been shown to result in the formation of an irreversible covalent protein–DNA complex. The purification and preliminary biochemical characterization of this complex have been reported elsewhere.[17]

Access to large quantities of a homogeneous FdC-containing oligonucleotide lead directly to high-resolution structural studies of a protein–DNA complex frozen in the midst of a catalytic event, a structure for which no precedent currently exists.[18] Given the mechanism by which FdC causes inactivation and the isosteric nature of the fluorine–hydrogen substitution, it is likely that the structure of the complex within the active site will resemble closely the native intermediate in catalysis.[20]

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Supplementary Material Available: Complete experimental details for the synthesis and characterization of the TMP-FdU phosphoramidite and FdC-containing 16-mer (4 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(14) The sequence of the duplex suicide substrate is

5′-d(CGCATA**GGFC**ATGACG)

3′-d(GCGTAT**CMGG**TACTGC)

in which **F** = FdC and **M** = 5-me-dC. The sequence in boldface type corresponds to the recognition site for the DCMtase M.*Hae* III [5′-d-(GGCC)].[15] M.*Hae* III ordinarily methylates both of the inner, symmetry-related cytosines in its recognition site; the duplex suicide substrate mimics a native site in which the C on one strand is already methylated.

(15) Slatko, B. E.; Croft, R.; Moran, L. S.; Wilson, G. G. *Gene* **1988**, *74*, 45.

(16) Samples were prepared to an OD_{260} of 1.08 in 1 M NaCl, 10 mM KH_2PO_4, pH 7.5. T_m's were obtained from first derivative plots of absorbance vs temperature curves. Data were collected on a Perkin-Elmer Lambda 3B spectrophotometer equipped with an immersible temperature probe and digital temperature controller interfaced to an IBM-XT personal computer.

(17) Chen, L.; MacMillan, A. M.; Chang, W.; Ezaz-Nikpay, K.; Lane, W. S.; Verdine, G. L. *Biochemistry* **1991**, *30*, 11018.

(18) Although the X-ray structures of two catalytic DNA binding proteins complexed with unmodified oligonucleotides have been reported,[19] in these cases the active sites were not assembled sufficiently to gain detailed information regarding catalysis.

(19) *Eco*R I endonuclease: Kim, Y.; Grable, J. C.; Love, R.; Greene, P. J.; Rosenberg, J. M. *Science (Washington, D.C.)* **1990**, *249*, 1307. DNase I: Suck, D.; Lahm, A.; Oefner, C. *Nature (London)* **1988**, *332*, 464.

(20) See, for example: Matthews, D. A.; Appelt, K.; Oatley, S. J.; Xuong, Ng. H. *J. Mol. Biol.* **1990**, *214*, 923.