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Synthesis and Characterization of Oligodeoxynucleotides Containing Formamidopyrimidine Lesions and Nonhydrolyzable Analogues

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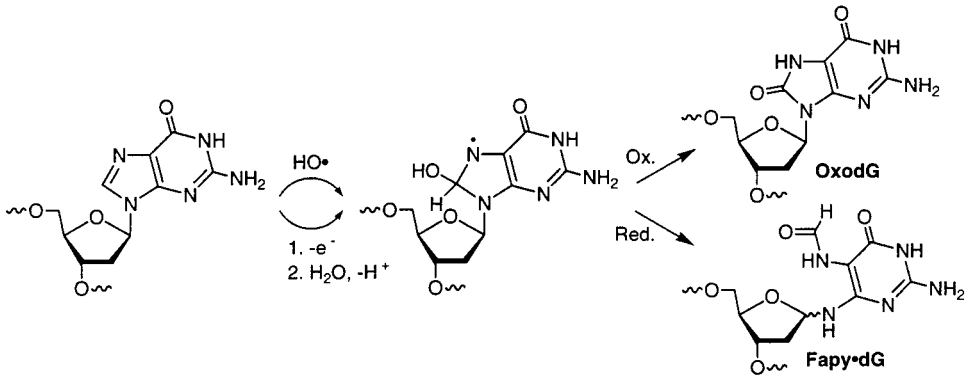
Abstract: Oligodeoxynucleotides containing formamidopyrimidine lesions and C-nucleoside analogues at defined sites were prepared by solid-phase synthesis and in some cases enzymatic ligation. Formamidopyrimidine lesions were introduced as dinucleotides to prevent rearrangement to their pyranose isomers. Oligodeoxynucleotides containing single diastereomers of C-nucleoside analogues of Fapy·dA were introduced by using the respective phosphoramidites. The formamidopyrimidine lesions reduce the T_M of dodecamers relative to their unmodified nucleotide counterparts when opposite the nucleotide proper base-pairing partner. However, duplexes containing Fapy·dG-dA mispairs melt significantly higher than those comprised of dG-dA. All duplexes containing Fapy·dA-dX or its C-nucleoside analogue melt lower than the respective complexes containing dA-dX. Studies of the alkaline lability of oligodeoxynucleotides containing formamidopyrimidine lesions indicate that Fapy·dA is readily identified as an alkali-labile lesion with use of piperidine (1.0 M, 90 °C, 20 min), but Fapy·dG is less easily identified in this manner.

DNA undergoes a variety of chemically induced structural changes when exposed to reactive oxygen species (e.g. hydroxyl radical) and other chemical agents.[1,2] Many of the lesions produced in DNA result from attack by radicals and other electrophiles on the nucleobases and are involved in aging and diseases such as cancer.[3] The effects of these lesions on polymerase enzyme activity and their recognition by DNA repair enzymes are critical in determining their biological role.[4] These interactions are a manifestation of the lesions' structures and their effect on the shape and stability of the DNA duplex as a whole. Synthesis of a biopolymer containing a specific lesion at a defined site provides a valuable tool for elucidating the structural and functional properties of damaged DNA. Although synthesis of unmodified nucleic acids is routine, preparation of DNA containing damaged nucleotides can be challenging due to the decreased stability of a lesion to synthesis and deprotection conditions.[5,6]

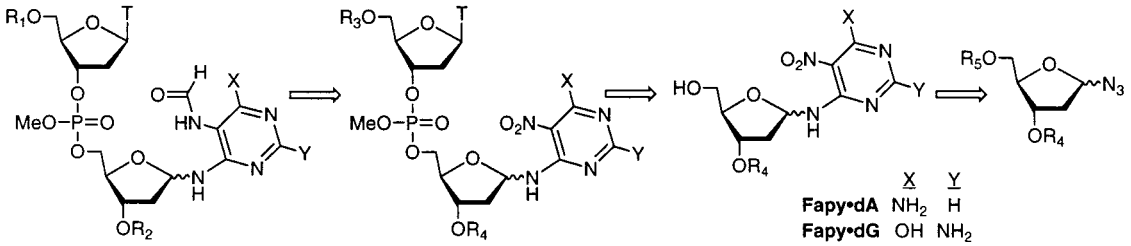
The formamidopyrimidines are examples of DNA lesions that are of biological and chemical interest. These lesions are recognized by a variety of DNA repair enzymes.[7] γ-Radiolysis produces Fapy·dG in greater amounts in vivo than the respective 8-oxopurine lesion (OxodG), which is formed from a common intermediate (Scheme 1).[8] Fapy·dA and 8-oxodeoxyadenosine (OxodA) are formed via an analogous mechanism. The effects of formamidopyrimidines on polymerase enzymes are uncertain, but evidence suggests that both are premutagenic lesions.[9] Cleavage of the purine imidazole ring in deoxyadenosine and deoxyguanosine introduces additional functional groups and a greater number of degrees of freedom in the formamidopyrimidine lesions than are present in the native nucleotides or the 8-oxopurines. This expands the number of possible hydrogen bonding motifs that can interact with enzymes, but also increases the synthetic challenge. Synthesis of oligodeoxynucleotides containing Fapy·dA and Fapy·dG are complicated by the fact that the monomeric species readily isomerize to their pyranose congeners.[10] We developed an approach that overcomes this and other obstacles and enables one to synthesize an oligode-

(1) (a) von Sonntag, C. *The Chemical Basis of Radiation Biology*; Taylor and Francis: London, 1987. (b) Greenberg, M. M. *Chem. Res. Toxicol.* **1998**, *11*, 1235. (c) Greenberg, M. M. In *Comprehensive Natural Products Chemistry, Vol. 7, DNA and Aspects of Molecular Biology*; Kool, E. T., Ed.; Pergamon: Oxford, 1999; pp 371−426.
(2) Gates, K. S. In *Comprehensive Natural Products Chemistry, Vol. 7, DNA and Aspects of Molecular Biology*; Kool, E. T., Ed.; Pergamon: Oxford, 1999; pp 491−552.
(3) (a) Hoeijmakers, J. H. *Nature* **2001**, *411*, 366. (b) DePinho, R. A. *Nature* **2000**, *408*, 248.
(4) (a) Lindahl, T.; Wood, R. D. *Science* **1999**, *286*, 1897. (b) Laval, J.; Jurado, J.; Saparbaev, M.; Sidokina, O. *Mutation Res.* **1998**, *402*, 93. (c) Delaney, J. C.; Essigmann, J. M. *Chem. Biol.* **1999**, *6*, 743. (d) Wang, D.; Kreutzer, D. A.; Essigmann, J. M. *Mutat. Res.* **1998**, *400*, 99.
(5) Iyer, R. P.; Beaucage, S. L. In *Comprehensive Natural Products Chemistry*; Kool, E. T., Ed.; Pergamon: Amsterdam, 1999; Vol. 7, pp 105−152.
(6) (a) For a recent review see: Butenandt, J.; Burgdorf, L. T.; Carrell, T. *Synthesis* **1999**, 1085. (b) Taylor, J.-S.; O'Day, C. L. *J. Am. Chem. Soc.* **1989**, *111*, 401. (c) Shibutani, S.; Takeshita, M.; Grollman, A. P. *Nature* **1991**, *349*, 431. (d) Iwai, S.; Shimizu, M.; Kamiya, H.; Ohtsuka, E. *J. Am. Chem. Soc.* **1996**, *118*, 7642. (e) Kobertz, W. R.; Essigmann, J. M. *J. Am. Chem. Soc.* **1997**, *119*, 5960. (f) Sambandam, A.; Greenberg, M. M. *Nucleic Acids Res.* **1999**, *27*, 3597. (g) Iwai, S. *Angew. Chem., Int. Ed.* **2000**, *39*, 3874.
(7) (a) Boiteux, S.; Gajewski, E.; Laval, J.; Dizdaroglu, M. *Biochemistry* **1992**, *31*, 106. (b) Krakaya, A.; Jaruga, P.; Bohr, V. A.; Grollman, A. P.; Dizdaroglu, M. *Nucleic Acids Res.* **1997**, *25*, 474. (c) Dizdaroglu, M.; Bauche, C.; Rodriguez, H.; Laval, J. *Biochemistry* **2000**, *39*, 5586.
(8) Pouget, J.-P.; Douki, T.; Richard, M.-J.; Cadet, J. *Chem. Res. Toxicol.* **2000**, *13*, 541.
(9) Graziewicz, M.-A.; Zastawny, T. H.; Oli'nski, R.; Tudek, B. *Mutation Res.* **1999**, *434*, 41.
(10) (a) Raoul, S.; Bardet, M.; Cadet, J. *Chem. Res. Toxicol.* **1995**, *8*, 924. (b) Berger, M.; Cadet, J. *Z. Naturforsch.* **1985**, *40b*, 1519.

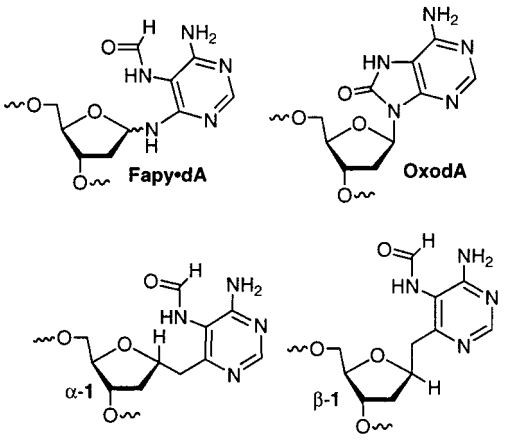
Scheme 1



Scheme 2



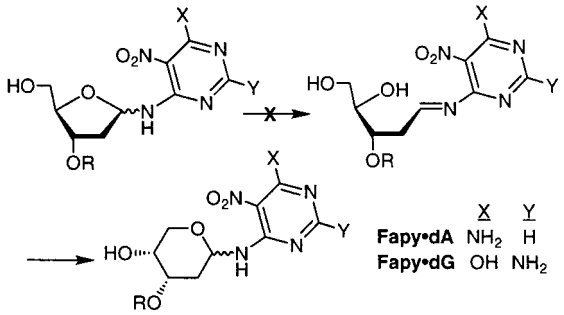
oxynucleotide containing Fapy·dA or Fapy·dG at a defined site.[11] The ability to prepare oligodeoxynucleotides containing DNA lesions at defined sites greatly facilitates examination of biological, chemical, and structural issues. Nucleoside analogues also play useful roles in such studies and we have prepared configurationally stable C-nucleosides of Fapy·dA (α,*β*-**1**) for this purpose.[12,13]



Results and Discussion

Synthesis of Dinucleotide Phosphoramidites Containing Formamidopyrimidine Lesions. We recently reported the synthesis of oligodeoxynucleotides containing Fapy·dG at defined sites.[11] The successful approach involved a strategy that respected the incompatibility of the formamidopyrimidine and primary hydroxyl group. Radiolysis experiments on nucleosides

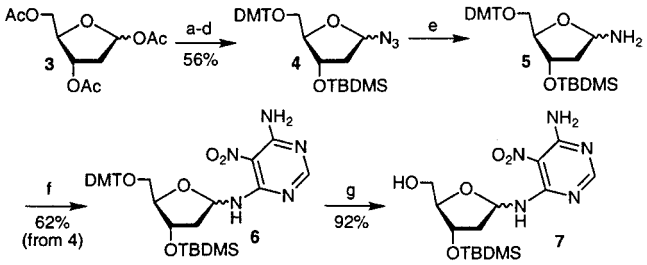
Scheme 3



indicated that formamidopyrimidine nucleosides exist predominantly as the α,*β*-pyranosyl isomers and minor amounts of the respective α,*β*-furanosyl species.[10] We reasoned this apparently facile rearrangement would prohibit a traditional oligodeoxynucleotide synthesis approach in which monomers are added one nucleotide at a time in the 3′ → 5′ direction. In principle, the formamidopyrimidine lesions could also be incorporated into growing chains as the mononucleotide by using reverse phosphoramidites in which the dimethoxytrityl group (DMT) protects the 3′-OH and the phosphoramidite is appended to the primary alcohol. However, this strategy would require carrying the labile phosphoramidite functionality through several steps of its synthesis and was not deemed likely to be successful. We avoided enabling rearrangement of the formamidopyrimidine nucleosides to their pyranose forms by introducing the lesions as the 3′-component of a dinucleotide. Our retrosynthetic analysis (Scheme 2) relied upon the assumption that a 5-nitro substituent would destabilize the acyclic imine intermediate necessary for isomerization and that we could transform this functional group into a formamide following formation of the dinucleotide phosphate triester (Scheme 3). This strategy would not prevent epimerization of later synthetic intermediates containing formamidopyrimidine groups. However, stereocontrol at the anomeric center was considered unnecessary because the anomers of Fapy lesions readily epimerize in water. For instance, we determined that **2**, which cannot rearrange to a pyranose

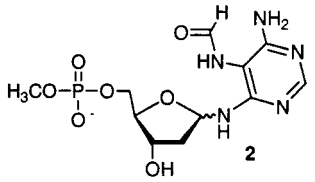
(11) Haraguchi, K.; Greenberg, M. M. *J. Am. Chem. Soc.* **2001**, *123*, 8636.
(12) For some recent examples see: (a) Kool, E. T. *Annu. Rev. Biophys. Biomol. Struct.* **2001**, *30*, 1. (b) Sun, L.; Wang, M.; Kool, E. T.; Taylor, J.-S. *Biochemistry* **2000**, *39*, 14603. (c) Morales, J. C.; Kool, E. T. *J. Am. Chem. Soc.* **2000**, *122*, 1001. (d) Tae, E. L.; Wu, Y.; Xia, G.; Schultz, P. G.; Romesberg, F. E. *J. Am. Chem. Soc.* **2001**, *123*, 7439.
(13) (a) Zharkov, D. O.; Rosenquist, T. A.; Gerchman, S. E.; Grollman, A. P. *J. Biol. Chem.* **2000**, *275*, 28607. (b) Chepanoske, C. L.; Porello, S. L.; Fujiwara, T.; Sugiyama, H.; David, S. S. *Nucleic Acids Res.* **1999**, *27*, 3197.

Scheme 4[a]

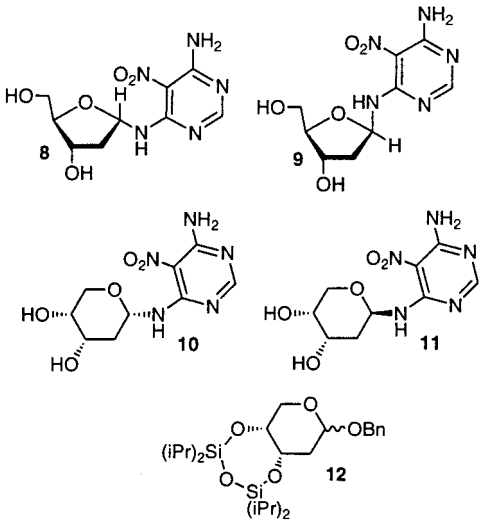


[a] Key: (a) $TMSN_3$, TMSOTf, CH_2Cl_2. (b) NaOMe, MeOH. (c) DMTCl, pyridine. (d) TBDMSCl, imidazole, pyridine. (e) H_2, $Pd/CaCO_3$, EtOH. (f) 6-Amino-4-chloro-5-nitropyrimidine, Et_3N, dioxane. (g) $AcOH/H_2O$ (4:1), CH_2Cl_2.

isomer, reaches equilibrium between its α- and β-anomers in less than 7 h at room temperature (10 mM phosphate buffer, pH 7.5).[14]



The stability of nitropyrimidine **7** was crucial to the success of this approach for the synthesis of an appropriate phosphoramidite. This intermediate was prepared via nucleophilic aromatic substitution of the crude amine obtained upon selective reduction of the azide in **4**. There was no evidence for detritylation during the hydrogenation of **4**, which was prepared in 7 steps from deoxyribose via the known triacetate (**3**, Scheme 4).[15] The azide (**4**) was obtained in 38% yield overall and this method was superior in our hands to a slightly shorter one proceeding through 2-deoxyribonolactone that was not amenable to producing the necessary azide in suitable quantities.[16] The tritylated glycosylation product (**6**) was obtained as a mixture of anomers (3:1) that were separable upon deprotection to the configurationally stable primary alcohols (**7**). The α-product was found to be the major isomer. Stereochemical assignments were based upon chemical shifts of the carbohydrate protons in **7**α,β, **8**, and **9**, as well as NOE experiments on the diols (**8**, **9**). ^{1}H NMR analysis of analogous compound **2** revealed that the cis orientation of the exocyclic heteroatom substituents at C1′ and C3′ resulted in a significant chemical shift difference of the diastereotopic C2′ protons in the α-isomer.[14] In contrast, the effects of the heteroatom substituents at these positions opposed one another in the β-anomer, giving rise to a pair of protons whose chemical shifts were unresolved. These trends were upheld in **7**−**9**.[17] Furthermore, NOE experiments on **8** and **9** affirmed the assigned stereochemistry. Irradiation of the C1′ proton in **9** gave rise to an enhancement at C4′. In contrast, irradiation of the C1′ proton in **8** resulted in an enhancement of the more deshielded C2′ proton but no effect on the respective C4′ proton was observed.

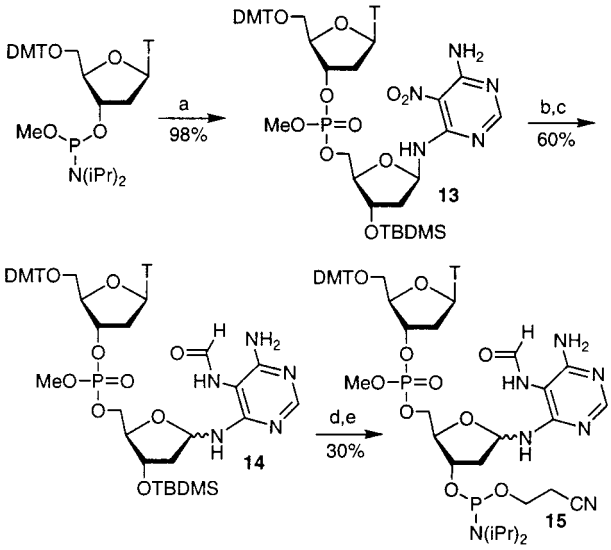


The desired isomers (**8**, **9**) were also readily distinguished by using NMR from the pyranose rearrangement products (**10**, **11**). The pyranose isomers (**10**, **11**) were independently synthesized from the benzyl acetal (**12**) by a similar route and were easily distinguished from **8** and **9** by examination of the ^{1}H NMR chemical shifts.[18] The C1′ protons in the furanose species were significantly more deshielded than the analogous protons in **10** and **11**.[19] This trend is consistent with that observed for deoxyglucopyranosyl containing analogues of DNA.[20] The pyranose isomers were also distinguishable from **8** and **9** qualitatively by reacting each with $NaIO_4$. The compounds identified by NMR to contain furanose rings failed to react under conditions that rapidly consumed **10** and **11**. Importantly, these experiments enabled us to determine rearrangement had not occurred during detritylation of **6**. This is in contrast to the analogous Fapy·dG species, which are moderately reactive under acidic conditions (see below).

All attempts to acylate the N4 and/or N6 amino groups in **6** were unsuccessful. Presumably, the combination of the 5-nitro substituent and electron-poor pyrimidine ring accounted for the poor nucleophilicity of these amines. Consequently, we elected to proceed by using the major isomer (**7**α) with the amines unprotected (Scheme 5). It was necessary to carry out dinucleotide formation with **7** using the *O*-methyl phosphoramidite of thymidine because the β-cyanoethyl group was unstable to the subsequent hydrogenation conditions. Excellent yields of the phosphate triester (**13**) were obtained when *tert*-butylhydroperoxide was used as oxidant. There was no evidence for epimerization during this reaction, but isomerization did occur during the next step in which the nitro group is reduced. Reduction was carried out using large amounts Lindlar's catalyst to complete reduction overnight. The crude amine was then formylated in the presence of excess acetic formic anhydride. No attempts were made to separate the mixture of anomers of **14**, because we established previously that the formamidopyrimidine anomers rapidly equilibrate in water.[14] Formylation

(14) Greenberg, M. M.; Hantosi, Z.; Wiederholt, C. J.; Rithner, C. D. *Biochemistry* **2001**, *40*, 15856.
(15) Gold, A.; Sangaiah, R. *Nucleosides Nucleotides* **1990**, *9*, 907.
(16) Wichai, U.; Woski, S. A. *Org. Lett.* **1999**, *1*, 1173.
(17) The following $H_{2'}$ chemical shifts were recorded at 300 MHz: **7**α ($CDCl_3$): *pro-S*, 2.30−2.46, *pro-R*, 2.03; **7**β ($CDCl_3$): *pro-S* and *pro-R*, 2.15−2.34; **8** (CD_3OD): *pro-S*, 2.33−2.44, *pro-R*, 1.96−2.04; **9** (CD_3OD): *pro-S* and *pro-R*, 2.20−2.32.
(18) See Supporting Information.
(19) The following $H_{1'}$ chemical shifts were recorded at 300 MHz in CD_3OD: **8**, 6.42; **9**, 6.37; **10**, 5.90; **11**, 5.83.
(20) (a) Otting, G.; Billeter, M.; Wütrich, K.; Roth, H.-J.; Leumann, C.; Eschenmoser, A. *Helv. Chim. Acta* **1993**, *76*, 2701. (b) Böhringe, M.; Roth, H.-J.; Hunziker, J.; Göbel, M.; Krishnan, R.; Giger, A.; Schweizer, B.; Schreiber, J.; Leumann, C.; Eschenmoser, A. *Helv. Chim. Acta* **1992**, *75*, 1416.

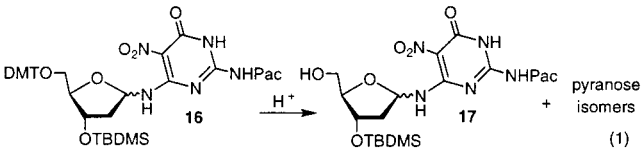
Scheme 5[a]



[a] Key: (a) α-**7**, tetrazole, then *t*-BuOOH. (b) H_2, Pd/$CaCO_3$, MeOH, THF. (c) HC(O)OAc, pyridine, THF. (d) Bu_4N^+ F^-, AcOH, THF. (e) 2-Cyanoethyl phosphoramidic chloride, diisopropylethylamine, CH_2Cl_2.

occurred exclusively at the N5-position, reflecting the effect of the pyrimdine ring nitrogen atoms, which are ortho and para to the N4 and N6 amines, but meta with respect to the position where reaction occurred. Attempts to protect the free amines in the presence of the formamide group were again unsuccessful. Under forcing conditions deformylation and/or transacylation at N5 were observed. We reasoned that if the free amines were so unreactive with acylating agents then there was no need to protect them during oligodeoxynucleotide synthesis. Consequently, **14** was carried on to the requisite dinucleotide phosphoramidite (**15**) by desilylating with buffered TBAF and phosphitylating with standard conditions.[5]

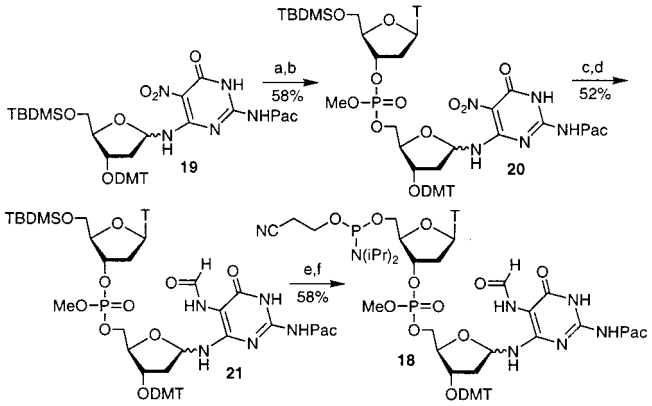
A similar overall approach was reported in preliminary form for the synthesis of a dinucleotide phosphoramidite suitable for preparing oligodeoxynucleotides containing Fapy·dG.[11] However, the properties of the Fapy·dG nucleoside were sufficiently different from those of the adenine analogue so as to require significant modifications in the synthesis described above. Despite extensive investigation, cleavage of the 5′-dimethoxytrityl group in **16** under acidic conditions yielded the desired product (**17**) accompanied by varying amounts of pyranosyl isomers (eq 1). After considering several alternative protecting



group ensembles that would enable us to unmask the primary hydroxyl group under nonacidic conditions, we proceeded with the 5′-silyloxy, 3′-dimethoxytrityl combination.[11] Although this approach committed us to synthesize oligodeoxynucleotides containing Fapy·dG in the 5′ → 3′ direction, it enabled us to prepare the requisite phosphoramidite (**18**) with the minimum number of protecting group manipulations.[21] Many of the

(21) Rippe, K.; Jovin, T. M. *Methods in Enzymology*; Lilley, D. M. J., Dahlberg, J. E., Eds.; Academic Press: San Diego, CA, 1992; Vol. 211, pp 199−231.

Scheme 6[a]



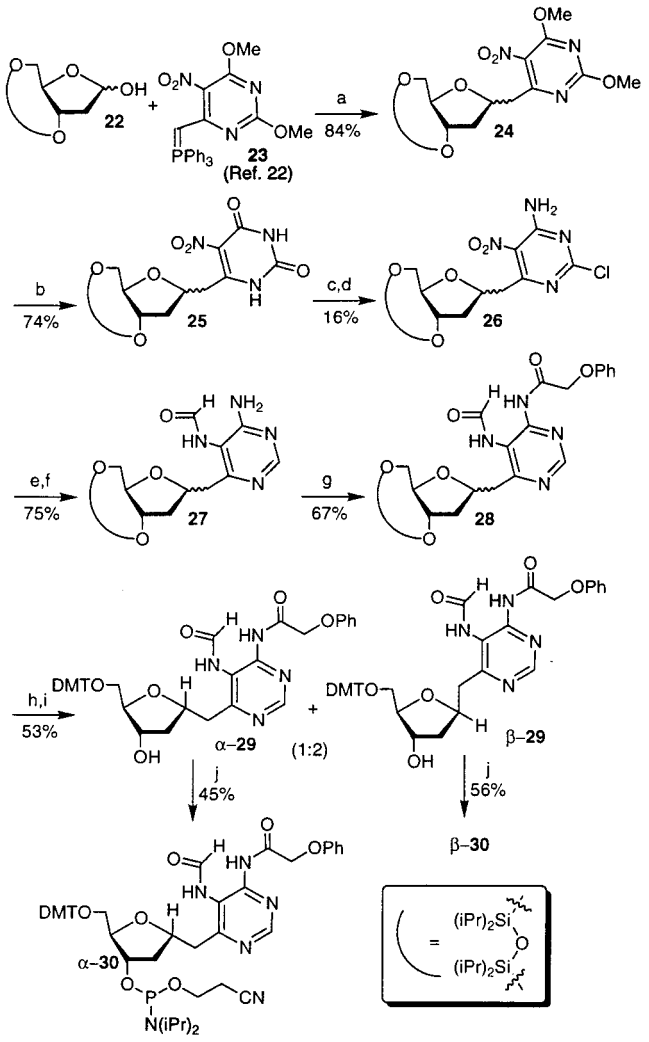
[a] Key: (a) Bu_4N^+ F^-, AcOH, THF. (b) 5′-Silyl thymidine phosphoramidite, then *t*-BuOOH. (c) H_2, 10% Pd/C, MeOH, THF. (d) HC(O)OAc, pyridine, THF. (e) Bu_4N^+ F^-, AcOH, THF. (f) 2-Cyanoethyl phosphoramidic chloride, diisopropylethylamine, CH_2Cl_2.

comparable transformations were carried out in a similar manner as described for the synthesis of **15** (Scheme 6). However, some specific steps deserve mention. For instance, it was necessary to carry out the desilylation of **19** and **21** with buffered TBAF to prevent cleavage of the phenoxyacetyl group. The lability of this group was also problematic during the reduction of the nitro group in **20** and it was necessary to carry out the reduction with the more reactive catalyst (10% Pd/C) to minimize exposure of the phenoxyacetamide to the C5-amino group.

Synthesis of a C-Nucleoside Analogue of Fapy·dA. The issue of furanose pyranose equilibration was replaced by other concerns in the synthesis of a nonhydrolyzable analogue of Fapy·dA (α,*β*-**1**). The core structure was assembled as a mixture of epimers via a Wittig reaction and subsequent recyclization (Scheme 7).[22] The lactol (**22**) was prepared from the lactone via reduction with DIBAL-H.[16] Following demethylation the dicarbonyl compound (**25**) was converted into **26**. The overall yield of the dichlorination/ammonolysis procedure was low. This was not due to the nucleophilic substitution step, which proceeded with high selectivity for reaction at C4 versus C2 (∼6:1). A considerable amount of material was lost due to desilylation under the chlorination conditions. Despite extensive experimentation with alternative reagents and variation of reaction conditions, the yield of the dichlorination step could not be improved significantly and we elected to move forward. The remaining chloride was removed and the nitro group reduced in one pot. The crude diamine was formylated with use of acetic formic anhydride. Selective formylation occurred at the N5-position to yield **27**, but care had to be taken to prevent formyl migration to the N4-amino group. Regioisomeric formamides were separable from one another and distinguishable by ^1H NMR. The desired N5-formamides exhibited a significant NOE between the formyl and benzylic like methylene groups, as well as a 0.2−0.3 ppm upfield shift of the C2 vinyl protons relative to the N4-formylation products. The desired formamide was obtained as the exclusive formylated product in 75% yield from **26** by using a modest excess of pyridine at 0 °C. Acyl migration indicated that the N4-amine needed to be protected during oligodeoxynucleotide synthesis. This was accomplished in the absence of a nucleophilic base by the in situ formation of phenoxyacetic anhydride. Following desilylation and subse-

(22) Cupps, T. L.; Wise, D. S.; Townsend, L. B. *J. Org. Chem.* **1986**, *51*, 1086.

Scheme 7[a]



[a] Key: (a) i) Toluene, Δ; (ii) NaOMe, MeOH. (b) NaI, TMSCl, $CHCl_3$. (c) $POCl_3$, diethylamine, toluene. (d) NH_3, THF. (e) H_2, Na_2CO_3, Pd/C, EtOH. (f) HC(O)OAc, pyridine, THF. (g) Phenoxyacetic anhydride, CH_2Cl_2. (h) $Et_3N{\cdot}3HF$, THF. (i) DMTCl, DMAP (cat.), pyridine. (j) 2-Cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropyl phosphane, diisopropylamino tetrazolide, CH_2Cl_2.

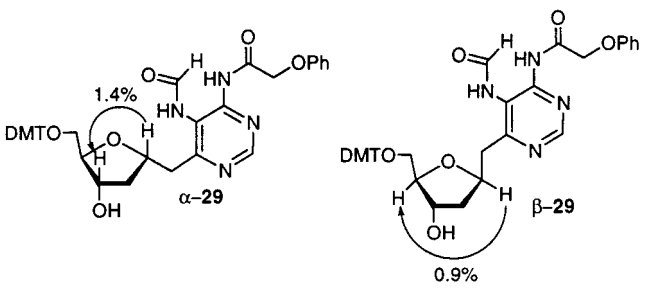
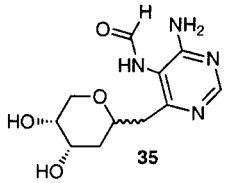


Figure 1. Determination of stereochemistry in **29** by NOE experiments.

quent dimethoxytritylation, the epimeric mixture of products was separated by column chromatography and the stereochemistry at the nominal anomeric center was determined by NOE experiments (Figure 1). Irradiation of the C1′ proton results in an enhancement at C4′ in *β*-**29** but not the α-anomer. In contrast, an interaction is observed between C1′ and C3′ in the α-isomer but not in *β*-**29**. Phosphoramidite synthesis was completed by using the tetrazolide that was generated in situ.

Oligodeoxynucleotide Synthesis and Characterization. Prior to carrying out solid-phase oligodeoxynucleotide synthesis the substrates were tested for their stability to the appropriate synthesis and deprotection (anhydrous K_2CO_3, MeOH) reagents. All of the lesions were stable to K_2CO_3 (0.05 M) in anhydrous MeOH for 3 h at 25 °C. This was particularly important for the C-nucleoside analogues given the above-described acyl migration. The instability of the formamidopyrimidine nucleosides to acid was accounted for by their incorporation as dinucleotides. The substrates underwent transacylation to varying degrees in the presence of the standard capping conditions (Ac_2O, lutidine, *N*-methylimidazole). Dodecameric (**31**, **32**) oligodeoxynucleotides containing α,*β*-**1** were prepared with isobutyryl anhydride in place of acetic anhydride after the modified nucleotide is incorporated. In addition, the coupling time of **30** was extended to 5 min and a second batch of activated phosphoramidite was reacted ("double-coupling") prior to proceeding onto the capping step of the synthesis cycle. In each instance the overall yield, as measured by the trityl response, of the synthesis was ∼2-fold greater when *β*-**30** was coupled. Isolated yields of oligodeoxynucleotides following deprotection (0.05 M K_2CO_3/MeOH) and gel purification were consistent with the measured trityl responses. Analysis by ESI-MS suggested a minor impurity that corresponded to dehydration product, presumably resulting from cyclization between the formyl group and N4. Further examination revealed that dehydration was occurring in the spectrometer. The cone voltage of the probe affected the relative amounts of dehydrated product and uncyclized material. Furthermore, dehydrated material is evident in the ESI-MS of monomeric material (**35**), for which ^1H NMR shows no evidence for cyclized product. There was no evidence for transacylated product in any of the dodecamers.

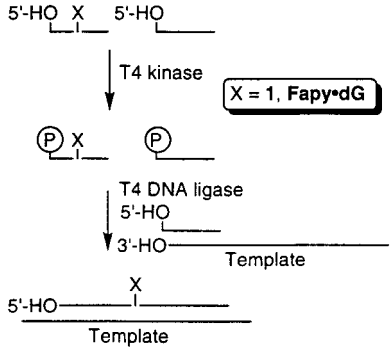
5′-d(GCT CTC **X**CT CGT)
31a,**b**
5′-d(GCT CTG **X**GT CGT)
32a,**b**
5′-d(AGG CGT TCA ACG GCT CTC **X**CT CGT ACG TCC CAT GGT)
33a,**b**
5′-d(AGG CGT TCA ACG GCT CTG **X**GT CGT ACG TCC CAT GGT)
34a,**b**
a, X = α-**1**; **b**, X = β-**1**



Longer oligodeoxynucleotides (**33**, **34**) containing **1** (36mers) were prepared by enzymatic ligation of the above dodecamers (Scheme 8).[23] After phosphorylating the dodecamer containing the modification (e.g. **31**, **32**) and the 3′-terminal oligodeoxynucleotide, these oligodeoxynucleotides and the 5′-HO-terminal fragment were hybridized to a DNA template (42mer) and reacted with T4 DNA Ligase. The desired products were obtained following gel purification in yields as high as 98%, but were typically between 53% and 66%. As previously described, a comparable approach was taken for the synthesis of oligodeoxynucleotides containing Fapy·dG.[11] Dodecamers (**36**, **37**) were synthesized by solid-phase synthesis and **37** was

(23) (a) Ordoukhanian, P.; Taylor, J.-S. *Nucleic Acids Res.* **1997**, *25*, 3783. (b) Smith, C. A.; Taylor, J.-S. *J. Biol. Chem.* **1993**, *268*, 11143.

Scheme 8



used to prepare **38**. However, oligodeoxynucleotides (**39**−**41**) containing Fapy·dA as long as 30 nucleotides (**41**) were successfully prepared via solid-phase synthesis. The coupling yields of **15** were greater than 90% with use of a 15 min double-coupling procedure. We have no evidence to explain why **15** coupled so much more efficiently than **18**, but it is tempting to speculate that the use of a reverse dinucleotide phosphoramidite during the preparation of Fapy·dG containing oligodeoxynucleotides is a factor. To avoid transacylation capping was carried out with pivalic anhydride and lutidine in the absence of *N*-methyl imidazole activating agent after the dinucleotide was coupled. To help make up for the less reactive acylation conditions, capping reaction times were extended to 30 s (from 5 s). There was no evidence for transacylation in the ESI-MS of any of the formamidopyrimidine containing oligodeoxynucleotides.[18]

5'-d(TGC AGT **X**TC AGC)
36 X = **Fapy•dG**
5'-d(TGC AGT **X**AC AGC)
37 X = **Fapy•dG**
5'-d(AGG CGT TCA ACG TGC AGT **X**AC AGC ACG TCC CAT GGT)
38 X = **Fapy•dG**
5'-d(TGC ACT **X**TC AGC)
39 X = **Fapy•dA**
5'-d(TGC ACT **X**AC AGC)
40 X = **Fapy•dA**
5'-d(CGT TCA ACG TGC ACT **X**AC AGC ACG TCC CAT)
41 X = **Fapy•dA**

Alkaline Lability of Oligodeoxynucleotides Containing Formamidopyrimidine Lesions. Alkali lability of DNA lesions can serve as a useful initial probe for their presence. For instance, the formation of alkali labile lesions at deoxyguanosine is useful for the quantitative analysis of electron hole migration in DNA.[24] Similarly, the formation of adducts of a DNA cleavage product serves as a fingerprint for the presence of the 2-deoxyribonolactone lesion.[25] We used 5′-^{32}P-**41** and 5′-^{32}P-**38** to determine the alkaline lability of Fapy·dA and Fapy·dG (Table 1). None of the alkaline conditions completely cleaved DNA at the Fapy·dG site in 20 min. Also, unlike OxodG, the presence of thiol reducing agent had no effect on cleavage of oligodeoxynucleotides containing Fapy·dA or Fapy·dG.[26] The relative amounts of cleavage of the two lesions under various conditions parallel the greater thermal stability of Fapy·dG and are consistent with the intermediate formation of an abasic site.[14] The formation of two products containing different 3′-end termini in varying ratios depending upon the degree of harshness of the cleavage conditions is also consistent with this interpretation. Treatment with 1 M piperidine at 90 °C produced a faster moving product consistent with a 3′-phosphate terminus as the major product. By analogy to previous studies on abasic sites, the minor slower moving product, which becomes the major one under milder cleavage conditions (e.g. *N*,*N*′-dimethylethylenediamine), is believed to be the intermediate β-elimination product.[27,28]

Table 1. Alkaline Lability of Fapy·dA and Fapy·dG[a]

		% cleavage	
treatment[a]	temp (°C)	Fapy·dA	Fapy·dG
piperidine (1.0 M)	90	99.0 ± 1.5	39.4 ± 3.4
piperidine (1.0 M) + BME (0.25 M)	90	96.5 ± 1.1	54.9 ± 9.9
piperidine (1.0 M)	37	34.9 ± 3.7	6.15 ± 0.1
N,*N*′-dimethylethylenediamine (0.1 M)	37	19.6 ± 3.3	7.8 ± 1.8
NaOH (1.0 M)	55	8.9 ± 0.7	2.2 ± 0.03
NaOH (1.0 M)	90	73.5 ± 2.5	52.5 ± 5.5
NaOH (0.1 M)	55	3.5 ± 0.7	1.3 ± 0.1
NaOH (0.1 M)	90	12.2 ± 1.4	16.5 ± 0.2

[a] All cleavage reactions were carried out for 20 min.

Table 2. UV-melting Temperature of Duplex DNA Containing Fapy·dG

5'-d(T G C A G T **Y** T C A G C)
(A C G T C A **X** A G T C G)

Y = dG, Fapy·dG

	T_M (°C)	
X	dG·X	Fapy·dG·X
dC	56.6 ± 0.4	53.7 ± 0.4
dA	43.5 ± 0.4	52.1 ± 0.4
dG	47.1 ± 0.5	43.8 ± 0.1
T	46.8 ± 0.3	44.0 ± 0.3

Scheme 9

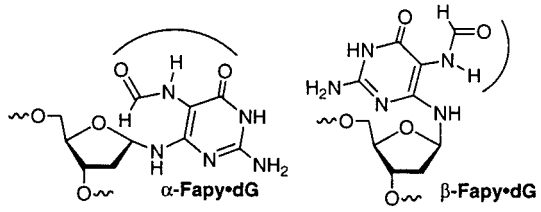


Table 3. UV-Melting Temperature of Duplex DNA Containing Fapy·dA

5'-d(T G C A C T **Y** A C A G C)
(A C G T G A **X** T G T C G)

Y = dA, Fapy·dA

	T_M (°C)	
X	dA·X	Fapy·dA·X
T	54.1 ± 0.2	47.0 ± 0.3
dA	41.8 ± 0.4	40.7 ± 0.3
dG	45.5 ± 0.3	41.9 ± 0.6
dC	40.7 ± 0.5	38.6 ± 0.2

UV-Melting Studies on Duplex DNA Containing Formamidopyrimidines and C-Nucleoside Analogues. Melting tem-

(24) (a) Schuster, G. B. *Acc. Chem. Res.* **2000**, *33*, 253. (b) Giese, B. *Acc. Chem. Res.* **2000**, *33*, 631. (c) Williams, T. T.; Odom, D. T.; Barton, J. K. *J. Am. Chem. Soc.* **2000**, *122*, 9048.
(25) Hwang, J. T.; Tallman, K. T.; Greenberg, M. M. *Nucleic Acids Res.* **1999**, *27*, 3805.
(26) (a) Bodepudi, V.; Shibutani, S.; Johnson, F. *Chem. Res. Toxicol.* **1992**, *5*, 608. (b) Cullis, P. M.; Malone, M. E.; Merson-Davies, L. A. *J. Am. Chem. Soc.* **1996**, *118*, 2775.

Table 4. UV-Melting Temperature of Duplex DNA Containing **1**

5'-d(G C T C T G **Y** G T C G T)
(C G A G A C **X** C A T C A)

Y = dA, α-**1**, β-**1**

	T_M (°C)		
X	dA·X	α-**1**·X	*β*-**1**·X
T	56.6 ± 0.5	46.8 ± 0.1	46.8 ± 0.1
dA	46.7 ± 0.5	46.6 ± 0.5	46.4 ± 0.5
dG	48.3 ± 0.6	41.8 ± 0.1	42.6 ± 0.5
dC	44.7 ± 0.5	46.4 ± 0.6	45.6 ± 0.9

peratures of duplexes containing the lesions were compared to the respective DNA dodecamers containing native nucleotides. All melting temperatures were measured at 2.2 micromolar duplex. Substitution of the purine in a native base pair by any of the corresponding lesions depressed the duplex T_M. The decrease in T_M was smallest for the dodecamer containing Fapy·dG (Table 2). Moreover, placing dA opposite Fapy·dG produced a duplex that melted almost as high as that containing a Fapy·dG·dC base pair and significantly higher than the dodecamer containing dA opposite dG. Although it could be fortuitous, it is interesting to note that a similar preference for base pairing with dA was observed for OxodG.[29] Given the ability of the formamidopyrimidines to epimerize one can envision several possible base pairing schemes. Two hypothetical conformations of Fapy·dG that enable it to present a thymidine like hydrogen bonding pattern are presented for illustrative purposes (Scheme 9). These simple pictures do not take into account possible distortions of the DNA duplex required to accommodate the hydrogen bonding patterns shown, and further studies are needed.

In contrast, melting temperatures of duplexes containing Fapy·dA (Table 3) or its C-nucleoside analogues (Table 4) do not indicate as obvious a preference for base pairing to a base other than thymine than does Fapy·dG. All three compounds depress the T_M compared to the native nucleotide (dA). It may be significant to note that the duplex containing a Fapy·dA·dA mispair melts relatively higher compared to the appropriate duplex containing dA than do duplexes containing other Fapy·dA mispairs. It is also worth noting that the qualitative effects of α,*β*-**1** on duplex T_M are similar to those of Fapy·dA, suggesting that the C-nucleoside analogues are good models of the lesion.

Acknowledgment. Financial support of this work from the National Institutes of Health (CA-74954) is greatly appreciated.

Supporting Information Available: Detailed experimental procedures, ESI-MS, and MALDI-TOF MS of **31**−**34** and **36**−**41** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA012135Q

(27) Bailly, V.; Verly, W. G. *Biochem. J.* **1988**, *253*, 553.
(28) McHugh, P. J.; Knowland, J. *Nucleic Acids Res.* **1995**, *23*, 1664.
(29) Plum, G. E.; Grollman, A. P.; Johnson, F.; Breslauer, K. J. *Biochemistry* **1995**, *34*, 16148.