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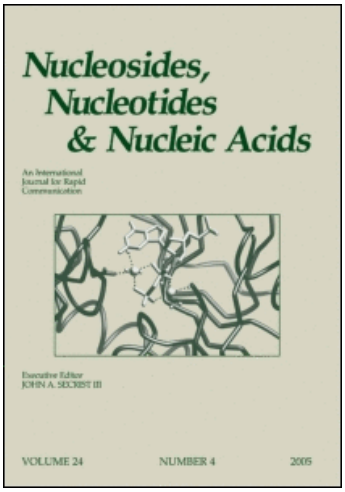

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Chemical Synthesis of Oligonucleotides Containing Damaged Bases for Biological Studies

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CHEMICAL SYNTHESIS OF OLIGONUCLEOTIDES CONTAINING DAMAGED BASES FOR BIOLOGICAL STUDIES

Shigenori Iwai □ *Division of Chemistry, Graduate School of Engineering Science, Osaka University, Toyonaka, Osaka, Japan*

□ *Since nucleic acids are organic molecules, even DNA, which carries genetic information, is subjected to various chemical reactions in cells. Alterations of the chemical structure of DNA, which are referred to as DNA damage or DNA lesions, induce mutations in the DNA sequences, which lead to carcinogenesis and cell death, unless they are restored by the repair systems in each organism. Formerly, DNA from bacteria and bacteriophages and DNA fragments treated with UV or γ radiation, alkylating or crosslinking agents, and other carcinogens were used as damaged DNA for biochemical studies. With these materials, however, it is difficult to understand the detailed mechanisms of mutagenesis and DNA repair. Recent progress in the chemical synthesis of oligonucleotides has enabled us to incorporate a specific lesion at a defined position within any sequence context. This method is especially important for studies on mutagenesis and translesion synthesis, which require highly pure templates, and for the structural biology of repair enzymes, which necessitates large amounts of substrate DNA as well as modified substrate analogs. In this review, the various phosphoramidite building blocks for the synthesis of lesion-containing oligodeoxyribonucleotides are described, and some examples of their applications to molecular and structural biology are presented.*

Keywords DNA damage; DNA repair; Molecular recognition; Oligonucleotides; Structural biology

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INTRODUCTION

DNA is the genetic material. The information is stored as the sequence of four nucleobases, i.e., adenine, guanine, cytosine, and thymine, and is transmitted to daughter cells and to the next generation by replication, primarily depending on the correct base-pair formation between adenine and thymine and between guanine and cytosine. The genetic integrity is secured under conditions in which the DNA is absolutely stable and the DNA polymerase, the enzyme that synthesizes DNA *in vivo*, replicates DNA with perfect fidelity. The error rates of replicative DNA polymerases are less than 10^{-5} per

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This article is dedicated to Professor Eiko Ohtsuka on the occasion of her 70th birthday.
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nucleotide,[1] and the mismatch repair system,[2] which corrects replication errors, reduces the mutation frequency to a great extent. However, DNA is inherently unstable and is subjected to various chemical reactions in cells, and alterations of the chemical structure of DNA, which are referred to as DNA damage or DNA lesions, increase the mutation frequency. The amino group of the cytosine base is hydrolyzed more easily than those of adenine and guanine,[3] and the product, uracil, forms a base pair with adenine, which results in a C·G → T·A transition mutation. Glycosidic bonds, especially those of the purine bases, are hydrolyzed in acidic solutions, and such hydrolysis occurs even under physiological conditions, although the reaction rate is much lower.[4] This "depurination" produces abasic sites, which are non-instructional lesions, and induces chain cleavage by the β-elimination mechanism. Another spontaneous damage to DNA is the oxidation of nucleobases with reactive oxygen species, especially hydroxyl radicals (·OH), which are generated during aerobic metabolism.[5] The reactive oxygen species are also generated by exposure to ionizing radiation, and the oxidized bases are unstable and easily degraded. In addition, ionizing radiation produces other types of DNA lesions, including cyclic nucleosides and strand breaks. Exposure to UV radiation also generates DNA lesions, and the major types are covalently linked pyrimidine dimers.[6] There are many chemical agents that damage DNA and, among them, several polycyclic aromatic hydrocarbons are known as carcinogens.[7] These compounds do not react with DNA in themselves, but are activated to become electrophiles in the metabolic process.

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Previously, DNA from bacteria and bacteriophages[8,9] and DNA fragments[10] treated with DNA-damaging agents were used as damaged DNA in biochemical studies. Since the mid-1980s, when the solid-phase synthesis of oligonucleotides by the phosphoramidite approach[11] was established, oligonucleotides containing a specific lesion in a defined sequence have been synthesized chemically. Figure 1 shows a typical scheme for the oligonucleotide synthesis. Chain assembly is usually performed on DNA synthesizers in the 3′ → 5′ direction, starting with the 3′-end nucleoside attached to a solid support. The key compound is 5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxynucleoside 3′-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite, which is a building block for the oligonucleotide synthesis. This compound is activated with tetrazole and is used for chain elongation. If a building block containing a damaged base is prepared successfully, then it can be incorporated into oligonucleotides. However, in addition to the difficulty in preparing the building blocks, another problem is that some of the DNA lesions are labile, especially under alkaline conditions. After the chain assembly, cleavage from the support and removal of the protecting groups are required to obtain the oligonucleotides, and aqueous ammonia is usually used in both of these processes. Since the 3′-end nucleoside is attached to the support

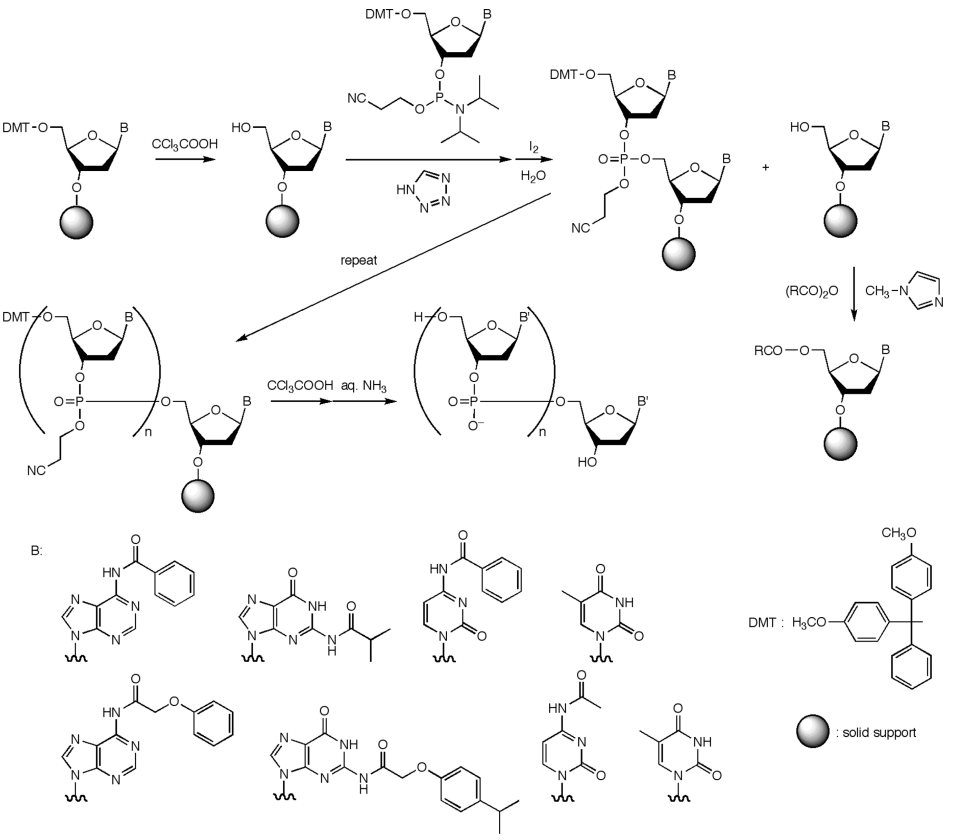

FIGURE 1 Solid-phase synthesis of oligodeoxyribonucleotides by the phosphoramidite approach.

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via an ester linkage, the oligonucleotides can be cleaved by an ammonia treatment at room temperature, and the 2-cyanoethyl group protecting the internucleotide phosphates is removed simultaneously by the β-elimination mechanism. However, the resulting ammoniac solutions must be heated to remove the conventional protecting groups (the upper bases in Figure 1) from the exocyclic amino functions of the base moieties. For some alkali-labile lesions, it is advantageous to use nucleoside phosphoramidites of the normal bases protected with more labile acyl groups (the lower bases in Figure 1), which can be removed by a brief treatment with aqueous ammonia at room temperature.

In this review, I describe the various phosphoramidite building blocks for the synthesis of damage-containing oligonucleotides that have been reported in the literature. Butenandt et al. published a related review in 1999,[12] which focused on the synthetic methods for the building blocks. The major aim of this review is to introduce this field of chemistry to biologists, as well as to update the previous review.

Abasic Lesions

Even under physiological conditions, the glycosidic bonds of the purine bases are subjected to hydrolysis, which leaves 2-deoxyribose in DNA. The same abasic site is formed when a monofunctional base excision repair enzyme removes a damaged base.[13] Since both purine and pyrimidine bases are removed in the latter case, the product is designated as an apurinic/apyrimidinic (AP) site. Although the AP site is an important type of DNA damage, it is extremely alkali-labile. Since 2-deoxyribose exists in an equilibrium between the cyclic hemiacetal and open aldehyde forms and the phosphate is a good leaving group, chain cleavage by the β-elimination mechanism occurs for the aldehyde form, and this instability makes the AP site very difficult to handle. Therefore, a tetrahydrofuran derivative, which lacks a hydroxyl function at the C1 position, has been used as a stable AP site analog. The first phosphoramidite building block of this AP site analog (**1a**) had a methyl group for the protection of the phosphoramidite,[14] and a 2-cyanoethyl-protected phosphoramidite (**1b**) was subsequently reported (Figure 2).[15] The latter compound is commercially available now. The tetrahydrofuran-type AP site analog was incorporated into oligonucleotides in the same way as the normal nucleosides and was used for biological studies. For example, the crystal structures of repair enzymes, endonuclease IV[16] and APE1,[17] and DNA polymerases[18–20] complexed with the AP site analog-containing DNA have been solved. In the complex with *Escherichia coli* endonuclease IV, the DNA helix was bent by about 90°, and both the AP site nucleotide and the opposite nucleotide were flipped out of the helix.[16] In the human APE1 case, the DNA was bent by about 35°, the helical axis was kinked, and only the AP site was flipped out.[17] The crystal structures of the bacteriophage RB69 replicative DNA polymerase[18,19] and *Sulfolobus*

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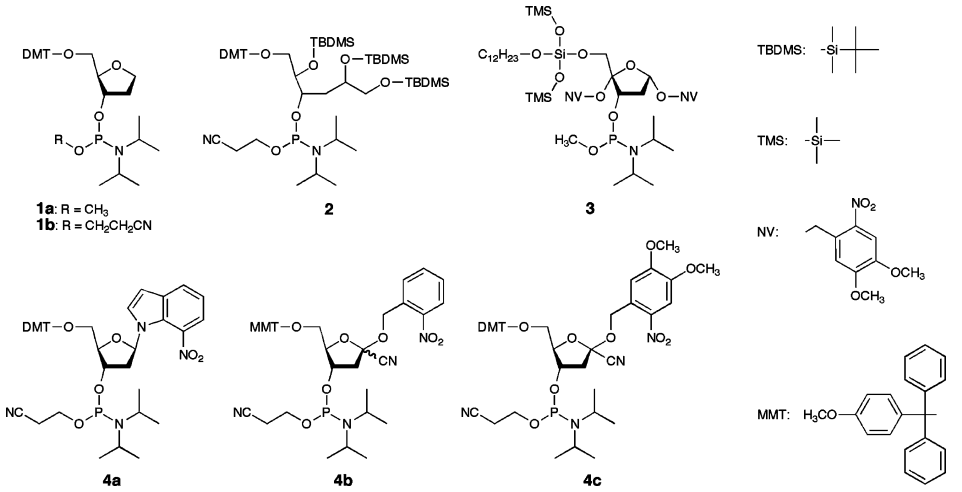

FIGURE 2 Building blocks for the incorporation of abasic lesions.

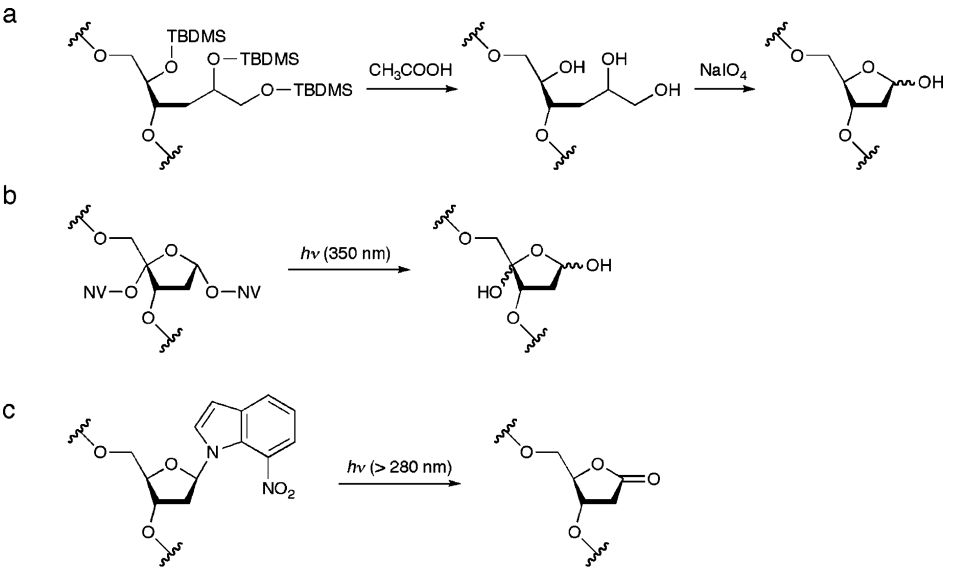

FIGURE 3 Generation of the "true" AP site (a), a C4′-oxidized abasic site (b), and 2-deoxyribonolactone (c) after oligonucleotide synthesis using **2**, **3**, and **4a**, respectively.

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solfataricus DNA polymerase IV[20] with the AP DNA provided insights into the mechanisms of the replication block and the translesion synthesis, respectively.

Shishkina and Johnson developed a method for the incorporation of the "true" AP site.[21] For this purpose, a phosphoramidite building block of *tert*-butyldimethylsilyl-protected 3-deoxyhexitol (**2**) was used, and after chain assembly and deprotection of the oligonucleotide, the 2-deoxyribose moiety was generated by mild oxidation of the diol with sodium periodate (Figure 3a). Other lesions in this category include a C4′-oxidized abasic site (**3**),[22] which is generated by bleomycin, and 2-deoxyribonolactone (**4a–c**),[23–25] which is produced by exogenous toxic agents and endogenous lipid peroxidation (Figure 3, b, and c). In the latter case, "photocaged" nucleoside analogs were incorporated into oligonucleotides, and 2-deoxyribonolactone was generated by photolysis.

Oxidized Purine Bases and Related Degradation Products

A major type of lesion found in DNA exposed to reactive oxygen species is 7,8-dihydro-8-oxoguanine (8-oxoG). This oxidized base adopts a *syn* conformation and can form a Hoogsteen base pair with adenine, which results in a G·C → T·A transversion mutation. Johnson and coworkers reported the phosphoramidite building blocks for the incorporation of 8-oxoG and 7,8-dihydro-8-oxoadenine into oligonucleotides (**5a** and **6**, respectively, in Figure 4).[26] These oxidized bases were prepared by the conversion of

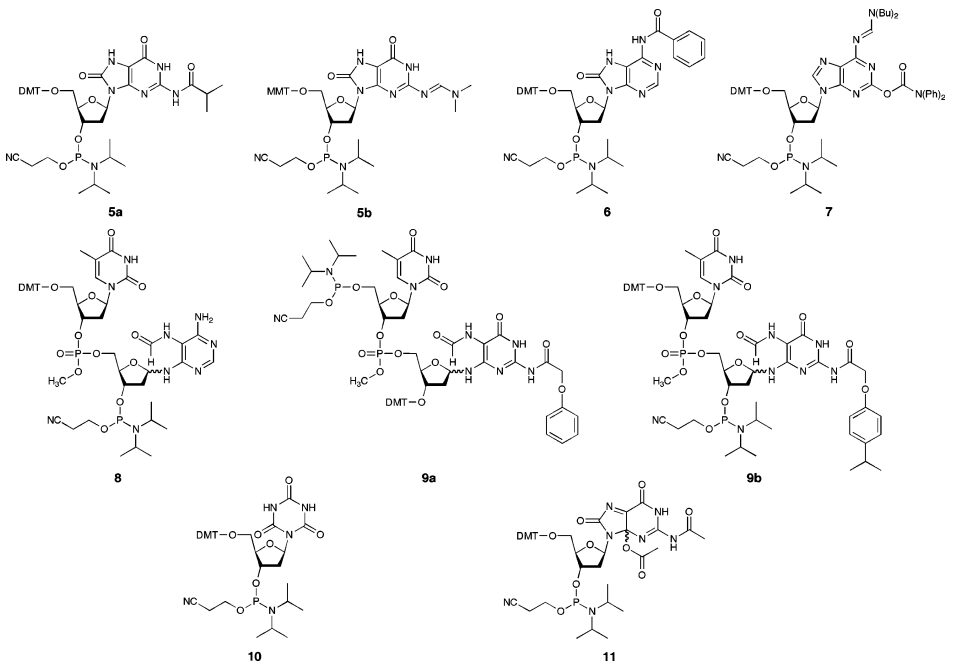

FIGURE 4 Building blocks for the incorporation of oxidized purine bases and their degradation products.

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8-bromopurine nucleosides to the corresponding 8-benzyloxypurine nucleosides, followed by catalytic hydrogenation (Figure 5, upper scheme). Koizume et al. also reported the synthesis of an 8-oxoG building block (**5b**) using 8-bromo-2′-deoxyguanosine as the starting material, but their intermediate was 8-methoxyguanine, which was successfully converted to 8-oxoG by treatment with thiophenol (Figure 5, lower scheme).[27] Although chain assembly was performed with these building blocks by the ordinary method, 2-mercaptoethanol had to be added, at a concentration of 0.1–0.25 M, for the deprotection using aqueous ammonia at 55°C. With the 8-oxoG-containing oligonucleotides synthesized by this method, the mechanisms of damage recognition by the repair enzymes and the mutations caused by this oxidative lesion were elucidated by X-ray crystallography. In the protein–DNA complex of human 8-oxoguanine DNA glycosylase 1 (hOGG1), the DNA helix was bent by about 70°, and the 8-oxoG residue was fully extruded from the helix and inserted into an active site cleft of the enzyme.[28] The helix bending and the base flipping seem to be common features in the damage recognition by repair enzymes. In the crystal structure of the DNA-bound form of MutY, which initiates the repair of the adenine·8-oxoG base pair by hydrolyzing the glycosidic bond of adenine, 8-oxoG in an *anti* glycosidic bond conformation was recognized by an extensive network of hydrogen bonds, and the adenine base was extrahelically extruded.[29] The crystal structures of human DNA polymerase β[30] *Bacillus stearothermophilus* DNA polymerase I[31]

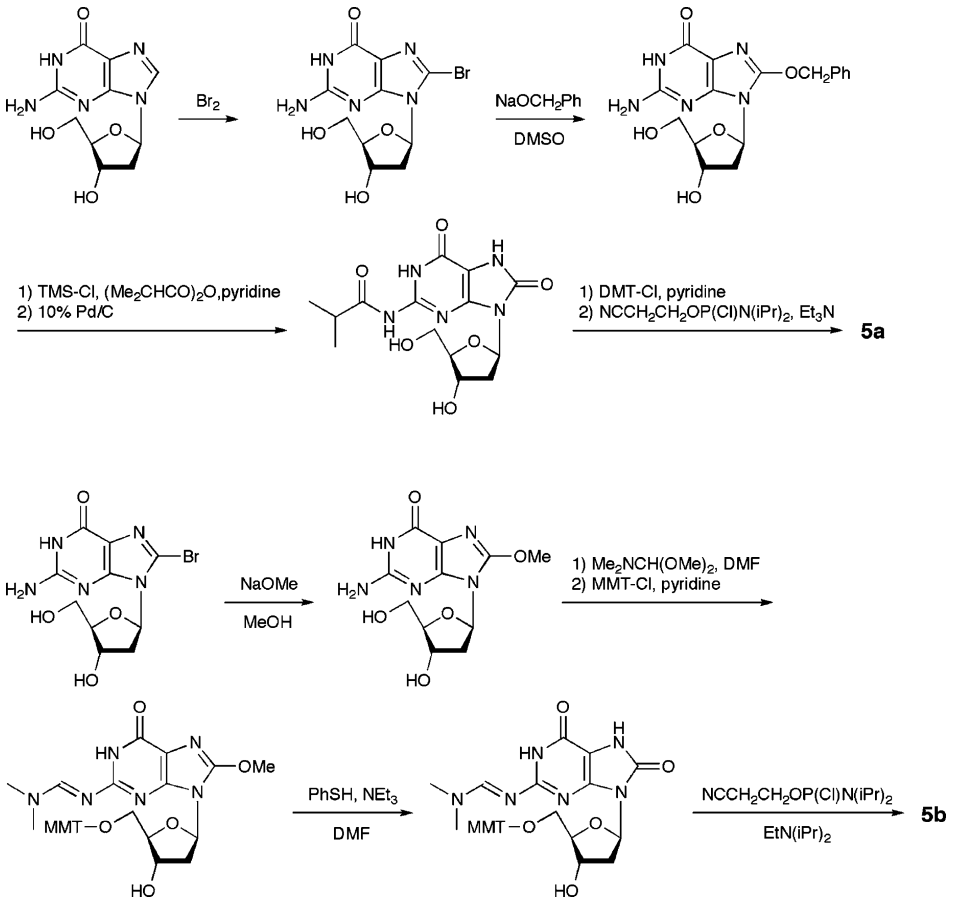

FIGURE 5 Synthesis of the building blocks of 8-oxoG.

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and T7 DNA polymerase[32] in complex with 8-oxoG-containing DNA have provided the structural basis for the dual coding potential of this oxidized base.

Ionizing radiation produces 2-hydroxyadenine, which exists in an equilibrium with 1,2-dihydro-2-oxoadenine. This oxidized base is also called isoguanine, and it forms a stable base pair with isocytosine. Its building block for the incorporation into oligonucleotides (**7**) was synthesized to study the isoguanine·isocytosine base pair.[33]

The 8-oxoG lesion is produced by the attack of a hydroxyl radical on the C8 position of the guanine base, and from the same intermediate, another product, formamidopyrimidine (Fapy), is also formed. The only difference in the chemical structure between Fapy and 8-oxopurine is that the C8–N9 bond is cleaved in the former case, but Fapy has the unique properties of fast epimerization between the α- and β-anomers and rearrangement to pyranose isomers. To avoid the isomerization to pyranose during the oligonucleotide

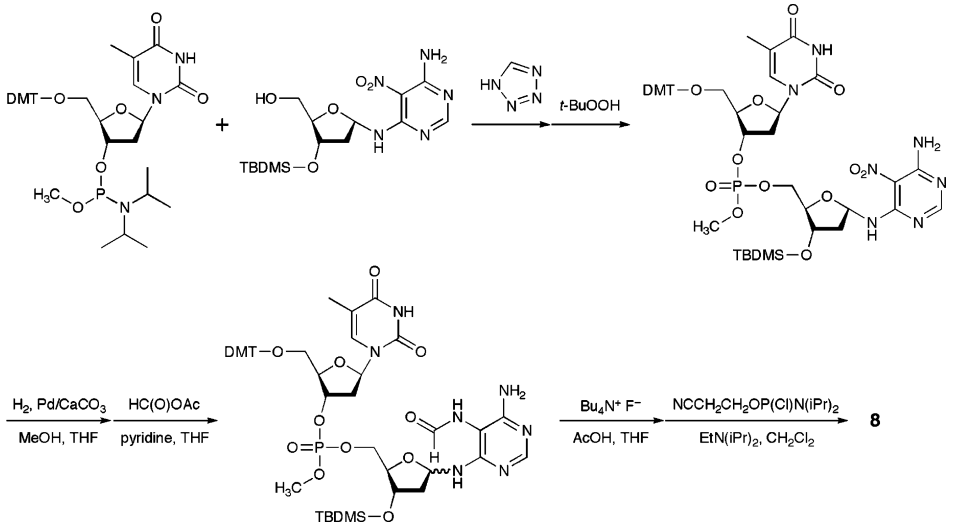

FIGURE 6 Synthesis of the building block for the incorporation of Fapy-dA.

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synthesis, Greenberg and coworkers synthesized dinucleotide-type building blocks. For Fapy-dA, a 3′-phosphoramidite with 5′-DMT protection (**8**) was synthesized, as shown in Figure 6,[34] but because the nitropyrimidine intermediate for Fapy-dG was rearranged to the pyranose isomer when the 5′-DMT group was removed with acid, a 5′-phosphoramidite of the dinucleoside monophosphate for the reverse synthesis in the 5′ → 3′ direction (**9a**) was prepared in the Fapy-dG case.[34,35] Using a mild Lewis acid, a 3′-phosphoramidite building block for the incorporation of Fapy-dG (**9b**) was subsequently synthesized.[36]

Cadet and coworkers reported phosphoramidite building blocks for the incorporation of two other lesions related to purine oxidation. One is a cyanuric acid nucleoside (**10**),[37] and the other is 4,8-dihydro-4-hydroxy-8-oxo-2′-deoxyguanosine (**11**).[38] Both of them are produced by singlet oxygen oxidation of 8-oxoG.

Oxidized Pyrimidine Bases and Related Degradation Products

Thymine glycol (5,6-dihydro-5,6-dihydroxythymine) is a major type of oxidation product of the pyrimidine bases. This lesion is produced by the reaction of the thymine base with a hydroxyl radical generated by ionizing radiation or as a consequence of aerobic metabolism. There are four diastereoisomers of thymine glycol, (5*R*,6*S*), (5*R*,6*R*), (5*S*,6*R*), and (5*S*,6*S*), but due to epimerization at the C6 position in solution, thymine glycol exists as either the 5*R* or 5*S* *cis–trans* pair. I synthesized the phosphoramidite building blocks of the (5*R*,6*S*)- and (5*S*,6*R*)-thymine glycols (**12a** and **13**, respectively, in Figure 7), as shown in Figure 8.[39,40] The two *cis* isomers were separated by

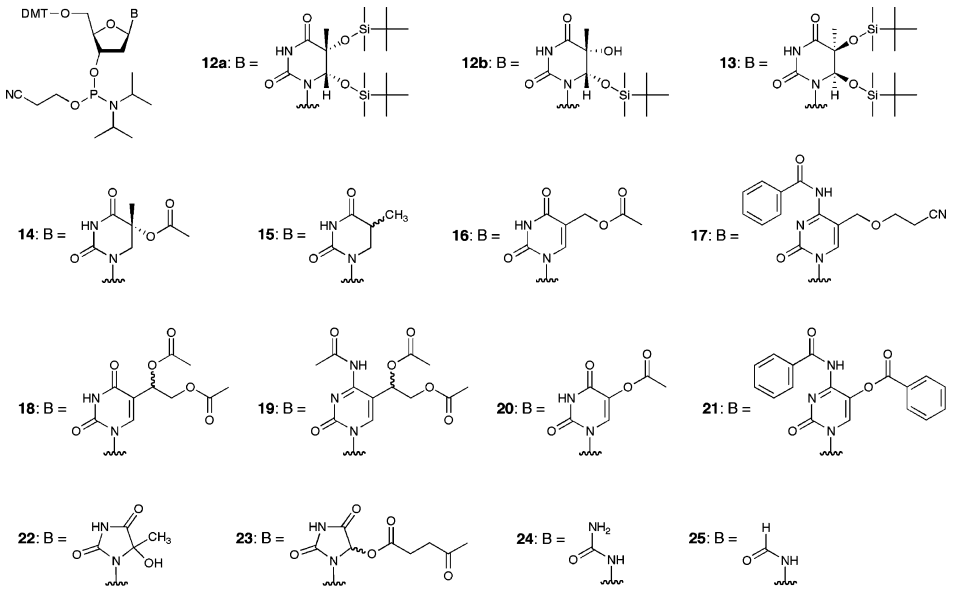

FIGURE 7 Building blocks for the incorporation of oxidized pyrimidine bases and their degradation products.

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chromatography on a silica gel column after OsO_4 oxidation of the protected thymidine, but because the ratio of the (5*R*,6*S*)- and (5*S*,6*R*)-thymine glycols formed in this reaction was 6:1, the synthesis of oligonucleotides containing the latter isomer was not practical. Preferential formation of the (5*S*,6*R*)-thymine glycol was achieved by the Sharpless asymmetric dihydroxylation reaction, using an ionic liquid as a co-solvent.[41] The *tert*-butyldimethylsilyl group was employed to protect the two hydroxyl functions of thymine glycol. As described below, however, the protection of the tertiary hydroxyl function was not necessary, and a building block without the 5-OH protection (**12b**) was also used successfully for the oligonucleotide synthesis.[40] Synthetic oligonucleotides separately containing 5*R*- and 5*S*-thymine glycols

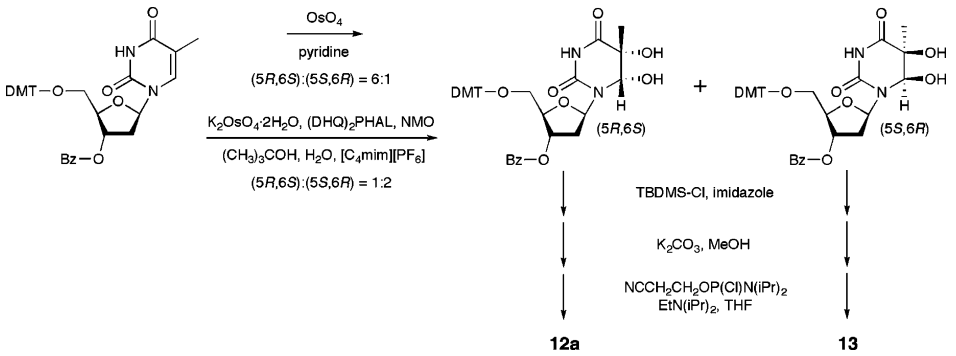

FIGURE 8 Synthesis of the building blocks of the two stereoisomers of thymine glycol.

were applied to biochemical studies on translesion replication and base excision repair. Human DNA polymerase η, which is responsible for translesion synthesis, catalyzed replication past 5*R*-thymine glycol more efficiently than past the 5*S* isomer.[42] Human NTH1 and NEIL1 had higher activities for 5*R*-thymine glycol, whereas *Escherichia coli* endonuclease III preferred the 5*S* isomer.[43]

Other types of 5,6-saturated thymine bases are also formed when DNA is exposed to damaging agents. Phosphoramidite building blocks of 5*R*-5,6-dihydro-5-hydroxythymine (**14**)[44] and 5,6-dihydrothymine (**15**)[45] have been synthesized and incorporated into oligonucleotides. These base moieties, as well as thymine glycol, are labile under alkaline conditions. Therefore, it is necessary, in the oligonucleotide synthesis, to use phosphoramidites of the normal nucleosides in which the exocyclic amino functions are protected by easily removable acyl groups.

When a hydroxyl radical attacks the methyl group of thymine or 5-methylcytosine, a naturally occurring modified base, 5-hydroxymethylpyrimidine is formed, and γ-irradiation of these methyl-containing bases also produces 5-formylpyrimidine. Phosphoramidite building blocks for the incorporation of 5-hydroxymethyluracil (**16**),[46] 5-hydroxymethylcytosine (**17**),[47] 5-formyluracil (**18**),[48] and 5-formylcytosine (**19**)[49] have been synthesized. The formyl residue was generated by the oxidation of the 1,2-dihydroxyethyl group in **18** and **19** with sodium periodate after the oligonucleotide synthesis (Figure 9, a and b). Another type of oxidation damage is 5-hydroxypyrimidine. Building blocks of 5-hydroxyuracil (**20**)[50,51] and 5-hydroxycytosine (**21**)[51] have also been prepared. The initial oxidation product in DNA is 5-hydroxycytosine, and 5-hydroxyuracil is its deamination product.

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Ionizing radiation causes degradation of the pyrimidine bases, and one of the major products is hydantoin. The phosphoramidite building blocks of 5-hydroxy-5-methylhydantoin (**22**)[52,53] and 5-hydroxyhydantoin (**23**),[54] which are formed by oxidation of thymine and cytosine, respectively, have been synthesized. Since epimerization at the C5 position occurred during the ammonia treatment for the oligonucleotide deprotection, the 5*R* and 5*S* isomers were not separated after the formation of the hydantoins. Other degradation products include 2′-deoxyribosylurea and 2′-deoxyribosylformamine. These residues have also been incorporated into oligonucleotides, using the corresponding building blocks (**24**, **25**).[55,56]

Radiation-Induced Cyclonucleosides

A unique class of DNA lesions formed by ionizing radiation is the cyclonucleosides, in which the C5′ atom is covalently linked to the base moiety (Figure 10). Cadet and coworkers reported the synthesis of phosphoramidite building blocks of 5′*S*-5′,8-cyclo-2′-deoxyadenosine

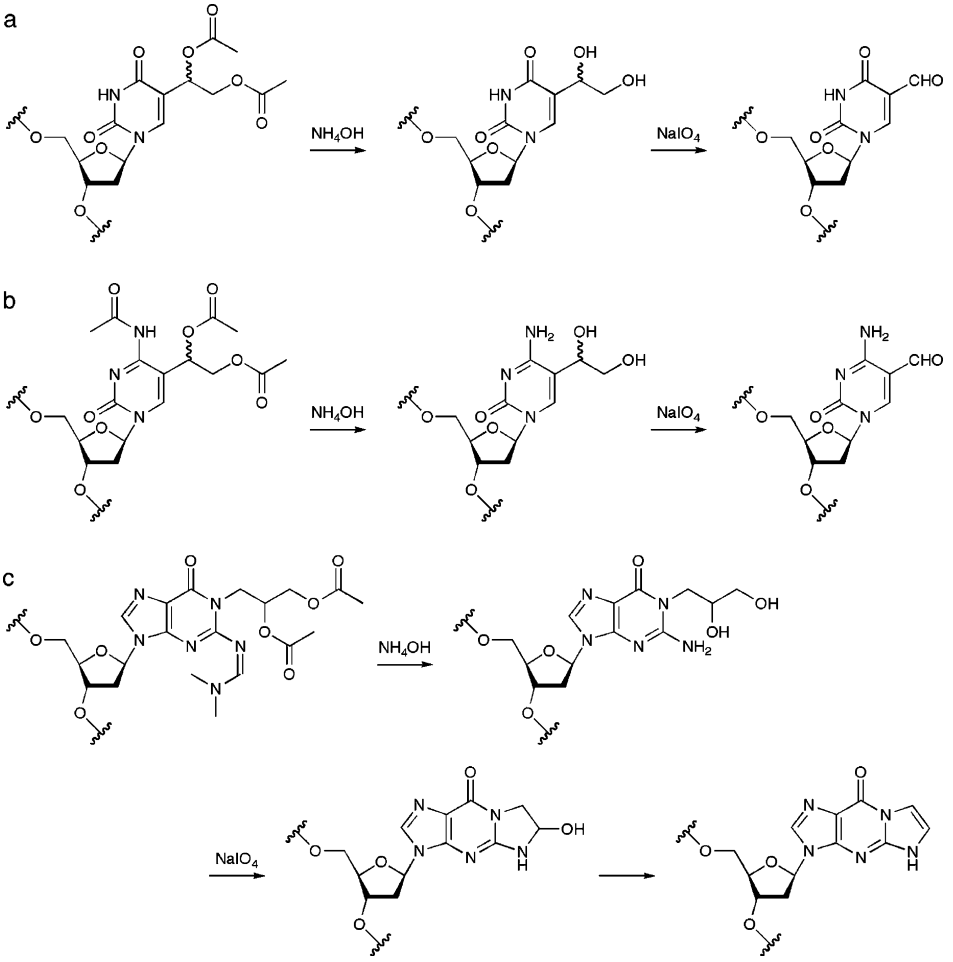

FIGURE 9 Generation of 5-formyluracil (a), 5-formylcytosine (b), and 1,N^2-ethenoguanine (c) after oligonucleotide synthesis using **18**, **19**, and **43**, respectively.

(**26**),[57] (5′*S*,6*S*)-5′,6-cyclo-5,6-dihydrothymidine (**27**),[58] (5′*S*,6*S*)-5′,6-cyclo-5,6-dihydro-2′-deoxyuridine (**28**),[59] and (5′*S*,5*S*,6*S*)-5′,6-cyclo-5-hydroxy-5,6-dihydro-2′-deoxyuridine (**29**)[60] and their incorporation into oligonucleotides. It was shown that the phosphodiester linkages on both the 5′ and 3′ sides of the cyclonucleosides were not hydrolyzed by nucleases or phosphodiesterases.

UV-Induced Pyrimidine Dimers

Ultraviolet light induces photochemical reactions between two adjacent pyrimidine bases, and the major products are the *cis–syn* cyclobutane pyrimidine dimer (CPD) and the pyrimidine(6–4)pyrimidone photoproduct ((6–4) photoproduct). The latter is produced by ring opening of an oxetane

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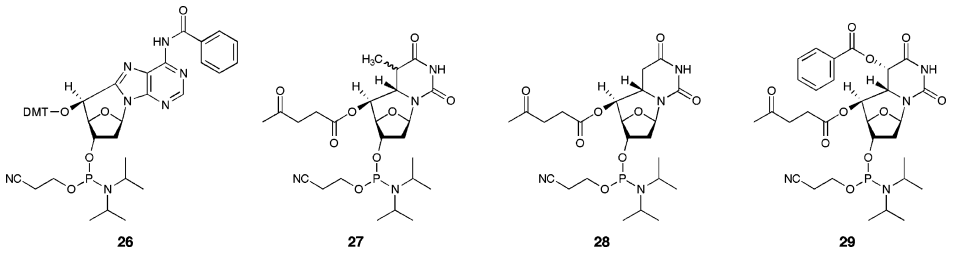

FIGURE 10 Building blocks for the incorporation of radiation-induced cyclonucleosides.

or azetidine intermediate formed by $2\pi + 2\pi$ photocycloaddition similar to the CPD case. Both of these UV lesions efficiently block DNA replication. Although the effect of the CPD formation at TT sites upon mutation is small, the accelerated deamination of cytosine-containing CPDs results in a C·G → T·A transition mutation, and the (6–4) photoproduct induces various types of mutations when it is replicated. Photolyases, which restore the UV lesions to the original pyrimidine bases using near-UV to blue light, have been discovered for both types of photoproducts, although only some species possess this repair system. In mammalian cells, the (6–4) photoproduct is efficiently repaired by the nucleotide excision repair pathway. CPD repair by this pathway is slow, but the CPD formed at a TT site can be bypassed accurately by a translesion DNA polymerase, such as human DNA polymerase η.

Taylor et al. reported the first synthesis of a building block for the incorporation of the *cis–syn* CPD formed at a TT site into oligonucleotides (**30**) (in Figure 11) in 1987.[61] They also reported a building block of the *trans–syn* CPD (**31**).[62] Since the protecting group for the phosphate was not 2-cyanoethyl, which is generally used for the current oligonucleotide synthesis, we improved the *cis–syn* CPD building block (**32**).[63] Afterwards, we reported a building block of the thymine–uracil-type CPD (**33**), which is the deaminated form

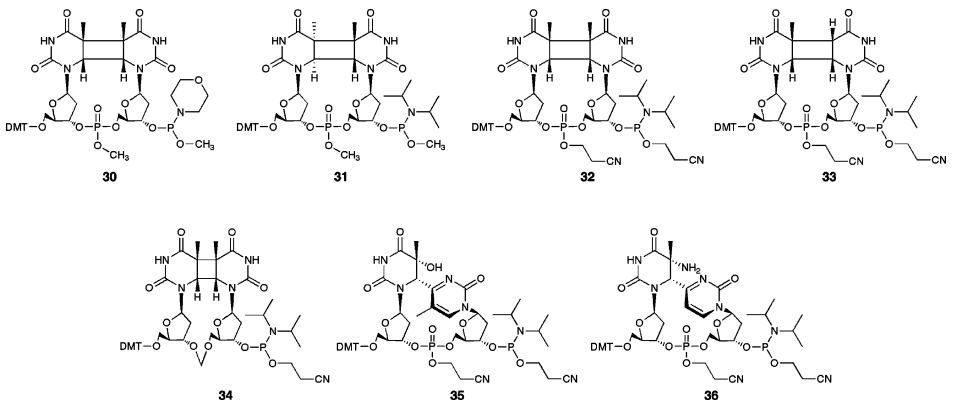

FIGURE 11 Building blocks for the incorporation of UV-induced lesions.

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of the thymine–cytosine CPD.[64] The CPD-containing oligonucleotides synthesized by these methods were used for several important biological studies. In structural biology, Morikawa's group, in collaboration with ours, solved the crystal structure of bacteriophage T4 endonuclease V in complex with a CPD-containing DNA duplex.[65] This was the first tertiary structure of a repair enzyme–DNA complex, which revealed the flipping of the opposite adenine base, together with the sharp helix kink. Recently, the crystal structures of the protein–DNA complexes of DNA polymerases[66,67] and a photolyase[68] have been reported. In the latter case, the phosphodiester linkage at the CPD site was replaced with a formacetal moiety using a modified building block (**34**).[69] In molecular biology, an enzyme designated as human DNA polymerase η, which catalyzes accurate replication past the *cis–syn* CPD formed at a TT site, was discovered and characterized using oligonucleotides synthesized by our method.[70–72] One of the CPD-containing oligonucleotides was used by many research groups to study translesion synthesis by various DNA polymerases.[73–77]

While the CPD can be obtained in a good yield by triplet-sensitized photolysis, the (6–4) photoproduct is formed only in a low yield, and this lesion is labile under the alkaline conditions used for the oligonucleotide deprotection. Therefore, we may have been reckless when we undertook the chemical synthesis of oligonucleotides containing the (6–4) photoproduct. However, we obtained the photoproduct in a practical yield by UV irradiation of partially protected thymidylyl(3′–5′)thymidine, and successfully converted it to a phosphoramidite building block (**35**), as shown in Figure 12.[78] The tertiary hydroxyl function in the (6–4) photoproduct was left unprotected, because treatment of the base moiety with acetic anhydride in the presence of a catalyst resulted in acetylation only at the N3 position of the 5′ component. Oligonucleotides containing the (6–4) photoproduct were synthesized using the phosphoramidites of normal nucleosides bearing protecting groups removable under mild conditions, and this success led me to the

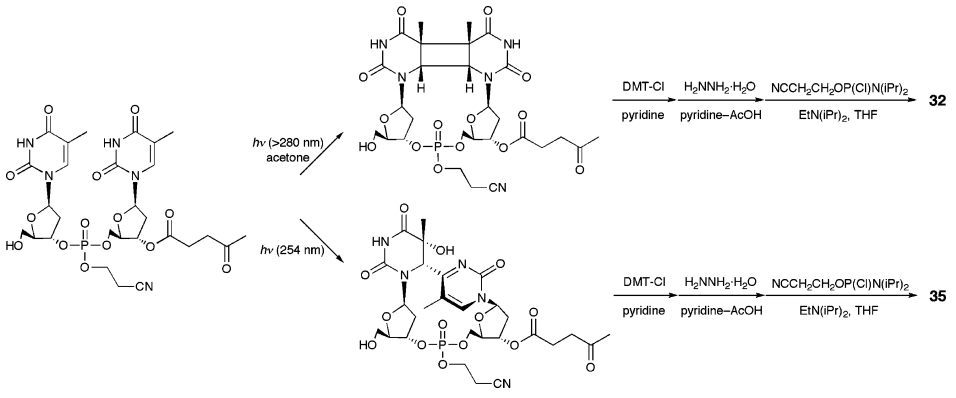

FIGURE 12 Synthesis of the building blocks of the UV lesions formed at TT.

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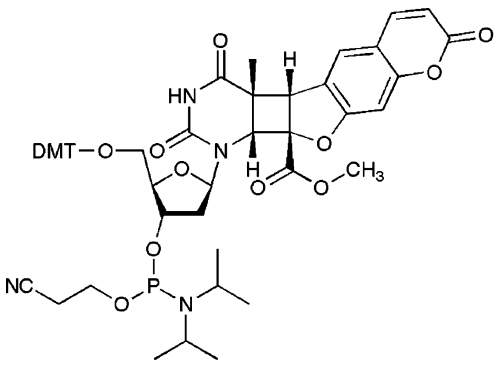
synthesis of the aforementioned building blocks of thymine glycol, which resembles the 5′ component of the (6–4) photoproduct. A building block of the (6–4) photoproduct formed at a TC site (**36**) was synthesized in a similar manner, and was successfully incorporated into oligonucleotides, although several side reactions occurred.[79] A problem in the synthesis of the (6–4) photoproduct-containing oligonucleotides was a side reaction that was assumed to be the coupling of nucleotides to the N3 position of the 5′ component of the photoproduct, but the by-product formation was prevented by using benzimidazolium triflate as an activator.[80] The (6–4) photoproduct-containing oligonucleotides synthesized using **35** were used in biochemical studies on (6–4) photolyase[81,82] and proteins involved in nucleotide excision repair.[83–85]

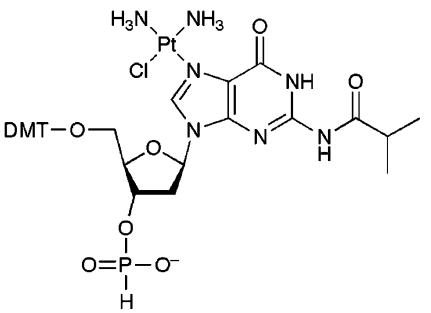
Drug Cross-Links

Psoralen, a linear furocoumarin used for the treatment of skin diseases and cutaneous T-cell lymphoma, forms an interstrand cross-link at TA·TA sites upon exposure to long-wavelength UV light. The product contains *cis–syn* cyclobutane rings, like the CPD, on both sides of this compound. A phosphoramidite building block of its furan-side monoadduct (**37**) (in Figure 13) was synthesized and incorporated into oligonucleotides.[86] Site-specific formation of a psoralen cross-link was achieved by irradiation with 366 nm light after hybridization to the complementary strand.

Cisplatin (*cis*-diamminedichloroplatinum(II)), an anticancer drug, also forms intrastrand and interstrand cross-links by substitution of the two chlorine atoms with the N7 atoms of purine bases. A building block of the monofunctional cisplatin adduct (**38**) was synthesized.[87] The H-phosphonate approach was used to incorporate this adduct, because platination of a protected 2′-deoxyguanosine 3′-phosphoramidite was not successful.



37



38

FIGURE 13 Building blocks for the formation of drug cross-links.

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Alkylated Bases

Methylating agents, such as *N*-nitroso-*N*-methylurea and methyl methanesulfonate, are known as carcinogens. Methylation occurs at the N7 position of guanine and the N3 position of adenine, but the glycosidic bonds of these positively charged bases are too weak to be handled. The carbonyl group of the guanine base is also methylated, and the product, O^6-methylguanine, can form a stable base pair with thymine and induces a G·C → A·T transition mutation. Building blocks of O^6-methylguanine (**39**) (in Figure 14)[88] and O^4-methylthymine (**40**)[89] have been synthesized and incorporated into oligonucleotides. Since the treatment of these methylated oligonucleotides with aqueous ammonia results in the substitution of the methoxy group with the amino group, deprotection should be performed using 1,8-diazabicyclo[5.4.0]undec-7-ene in methanol.

Metabolites derived from vinyl chloride act as alkylating agents and form exocyclic etheno derivatives by reacting with nucleophilic groups in DNA. Phosphoramidite building blocks for the incorporation of $1,N^6$-ethenoadenine (**41**),[90] $3,N^4$-ethenocytosine (**42**),[90] and $1,N^2$-ethenoguanine (**43**)[91] have been synthesized. After the oligonucleotide synthesis using **43**, the guanine adduct was generated via a 6-hydroxyethano intermediate by oxidation with sodium periodate, followed by facile dehydration (Figure 9c).[91] Building blocks of pyrimido[1,2-*a*]purin-10(3*H*)-one (**44**),[92,93] which is the major adduct of malondialdehyde generated during membrane lipid peroxidation, and 8-hydroxymethyl-$3,N^4$-ethenocytosine (**45**),[94] which is an alkylation product of cytosine with glycidaldehyde, have also been reported. Furthermore, building blocks for the incorporation of adenine and cytosine adducts with *p*-benzoquinone, which is the major metabolite of benzene, have been synthesized (**46** and **47**, respectively).[95]

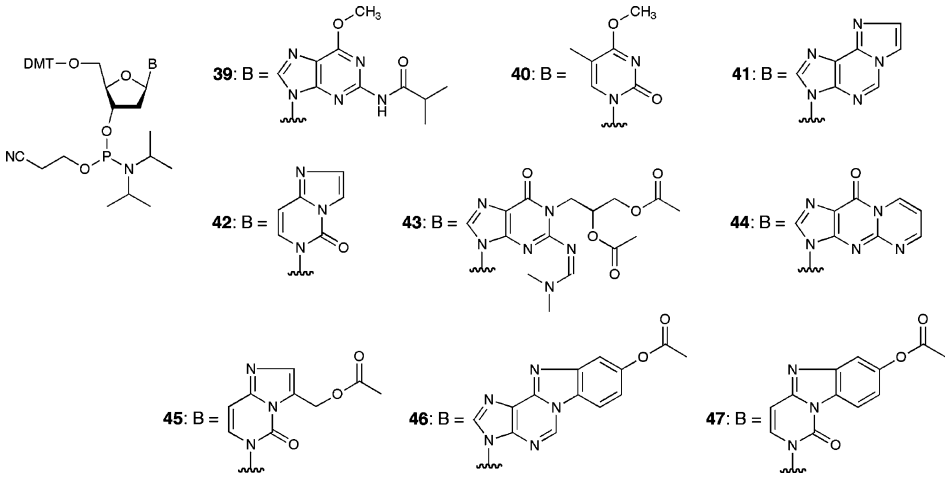

FIGURE 14 Building blocks for the incorporation of alkylated bases.

An oligonucleotide duplex containing $1,N^6$-ethenoadenine was used for co-crystallization of a base excision repair enzyme, human alkyladenine DNA glycosylase (AAG).[96] As in the other base excision repair enzymes, the DNA was bent in the complex with AAG, and the damaged base was flipped out of the helix. The alkylated base was recognized by the aromatic side chains in the enzyme active site. The same research group reported another crystal structure of a related enzyme, *Escherichia coli* AlkA, in complex with DNA.[97] In the AlkA case, they used a substrate analog containing 1-azaribose, which mimics the carbocation in the transition state. A phosphoramidite building block of a similar transition state analog, (2*R*,3*S*)-2-hydroxymethyl-3-hydroxypyrrolidine, has also been synthesized.[98]

Adducts with Bulky Carcinogens

Benzo[*a*]pyrene is a polycyclic aromatic hydrocarbon that was demonstrated to be a carcinogen more than 70 years ago. Even now, it is known as the most highly carcinogenic compound, and it exists in automobile exhaust fumes and cigarette smoke. Although this compound is not carcinogenic in itself, one of its metabolites, 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene, forms an adduct with the amino group of the purine base. Two groups have reported the synthesis of phosphoramidite building blocks of benzo[*a*]pyrene adducts (**48a**, **48b**, **49a**, and **49b**) (in Figure 15).[99–101] Among the stereoisomers caused by the four chiral carbons, 7*R*,8*S*-dihydroxy-9*S*,10*R*-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene ((+)-*anti*-BPDE) is formed in great excess by the metabolism of benzo[*a*]pyrene, and this compound affords an adduct with the (7*R*,8*S*,9*R*,10*S*) configuration.[102] In the synthesis of the building blocks, a racemic mixture of *anti*-BPDE or its derivative was used, and after

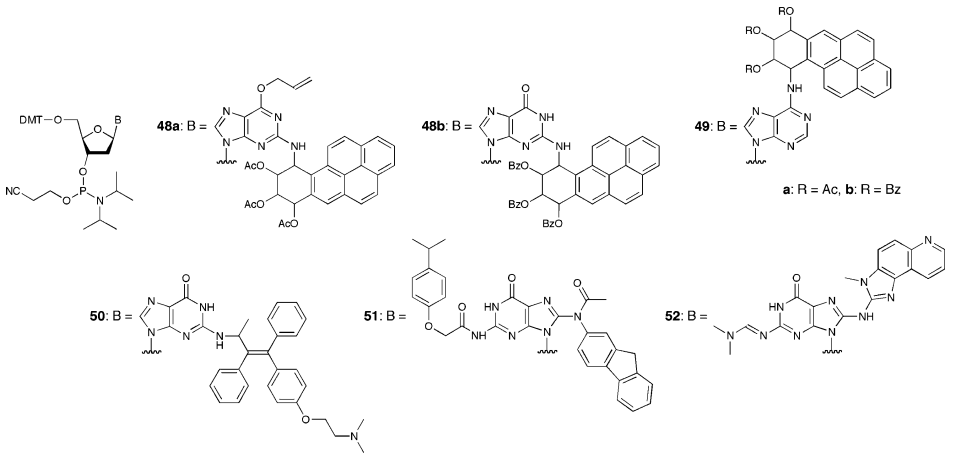

FIGURE 15 Building blocks for the incorporation of carcinogen adducts.

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the chain assembly, oligonucleotides containing each BPDE enantiomer were separated at the final HPLC purification step. Using **49a**, the crystal structure of a complex between a DNA polymerase and a DNA duplex containing the BPDE adduct was solved.[101] In this case, the 10*R* and 10*S* isomers of the *cis*-opened adduct were separated at the nucleoside level, and only the 10*R* phosphoramidite was used for the oligonucleotide synthesis and the following crystallization.

Other carcinogenic adducts have also been incorporated into oligonucleotides via a phosphoramidite building block. Tamoxifen ((*E*-1-(4-(2-(dimethylamino)ethyl)phenyl)-1,2-diphenyl)butene) (**50**),[103] which is used for breast cancer treatment, forms an adduct with the amino function of guanine. In addition, 2-acetylaminofluorene (**51**)[104] forms an adduct at the C8 position of guanine and is used as reference in mutagenesis studies, and 2-amino-3-methylimidazo[4,5-*f*]quinoline (**52**),[105] which is produced in the process of cooking, also forms an adduct at the C8 position of guanine.

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Perspectives

Organic chemists have synthesized the phosphoramidite building blocks of various damaged bases, and have successfully incorporated them into oligonucleotides. Some of the oligonucleotides synthesized by their methods have been used for biochemical and structural studies on mutagenesis, translesion replication, and DNA repair. Among the building blocks presented in this review, several compounds, e.g., those of the tetrahydrofuran-type AP site analog, 8-oxoG, thymine glycol, and even the CPD, are commercially available now. Biologists can obtain DNA fragments containing these lesions simply by ordering them from a custom oligonucleotide synthesis service. However, many of the building blocks have to be synthesized by organic chemists, and some of them can be synthesized only by the research group that developed the synthetic method for the particular compound. This means that many types of lesion-containing oligonucleotides are not widely available for biologists, even though such materials provide a deeper understanding of the molecular recognition in living organisms. For the elucidation of various biological processes and their interactions, collaborations between organic chemists and biologists will be increasingly important.

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