Accounts

Design of DNA-Cleaving Agents

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The review focuses on the molecular design of artificial sequence-specific DNA-cleavers which have recently been developed in our laboratories. These include: DNA cleavers mimicking natural antitumor antibiotic like enediyne antibiotics, bleomycins, and kapurimycin A_3. The design of photochemical DNA-cleaving molecules is also described. These are classified as: i) nonselective DNA cleavers, ii) thymine selective DNA cleavers, iii) photo-Fenton reagents, iv) photoinducible DNA alkylating agents, and v) DNA cleavers via photoinduced electrontransfer. The scope and limitation of these DNA-cleaving agents are described with a special emphasis on the concept and strategy for designing novel sequence-specific DNA cleavers, as well as on the mechanistic aspects of their DNA cleavage.

The design and investigation of artificial DNA-cleaving molecules is an area of exciting research that has numerous important biochemical and biomedical applications.[1] Synthetic DNA-cleaving molecules, i.e., DNA cleavers, can be used as a useful tool in molecular biology, especially for studying DNA conformation[2] and DNA protein interactions.[1,2] In addition to the exploration of novel mechanistic strategies for DNA cleavage,[3] new DNA-cleaving agents are of practical interest as potential antitumor agents, as prosthetic groups for antisense oligonucleotides,[4] as artificial nucleases[5] or photonucleases,[6] as well as for designing new gene-selective drugs.[1] For more than a decade, it has been a dream for organic chemists to design sequence-specific DNA cleavers that bind to DNA at any desired sequence and cleave DNA efficiently at the binding site.

Naturally occurring antitumor antibiotics such as bleomycin, calicheamicin, and neocarzinostatin have evolved over millions of years. Such antibiotics are capable of high-yielding sequence-specific reactions at a single atom of specific sequence along duplex DNA.[7] The design of artificial sequence-specific DNA-cleavers including natural antitumor antibiotic models requires the integration into a single molecule of two separate functions: molecular recognition and DNA cleavage. Such nonnatural DNA-cleavers should be equipped with a chemical or photochemical tag capable of modifying or cleaving DNA at the site of binding. In most cases the DNA-cleaving principle of these DNA cleavers involves i) oxidative destruction of DNA deoxyribose backbone by hydrogen-abstraction reactions or ii) alkylation of DNA bases, and in fewer cases, iii) metal-activated hydrolytic cleavage of phosphodiester linkage of DNA.[3] Within the last decade, a wide variety of synthetic DNA-cleaving agents have been reported.[1,3]

For the last decade, our laboratory has focused upon exploitation of new strategies for sequence-specific DNA cleavage and the design of DNA-cleaving molecules, including DNA cleavers, which can mimick natural antitumor antibiotics.[6b] In this account, special emphasis is devoted to the concept and strategy for designing sequence-specific DNA cleavers which have recently been developed in our laboratory. We also present the mechanistic aspect of their DNA cleavage, since several reviews on DNA cleavage have appeared recently.[1,3,7]

DNA Cleavers Mimicking Natural Antitumor Antibiotics

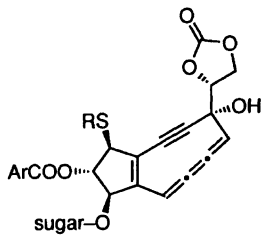
Various types of DNA-cleaving or modifying antitumor antibiotics are known in nature.[7] Such drugs bind to DNA with remarkable sequence selectivity and cleave DNA at the site of binding. A considerable number of such natural DNA-cleaving antitumor antibiotics contain carbohydrate residues as DNA recognition elements. Elucidation of the mechanisms of DNA cleavage by these naturally occurring antitumor antibiotics is a topic of much current interest.[7] The rational structural modification of natural DNA-cleaving molecules or the synthesis of their model compounds can establish the importance of the structural, electronic or conformational factors necessary for a better understanding of natural drug-DNA molecular recognition and their DNA-cleaving processes. Such antitumor antibiotic models, eventually equipped with a chemical tag capable of modifying or cleaving DNA at the site of binding, should have great potential as chemotherapeutic drugs or as molecular probes for DNA polymorphism as well. The scope of this section

is not to cover all studies in this area, but to focus mainly on the design of DNA cleavers which can mimick natural DNA cleavers, which have recently been developed in our laboratory.

1) Enediyne Antitumor Antibiotic Models.[8] Antitumor antibiotics having a characteristic cyclic structure consisting a conjugated ene-diyne or diene-diyne system have been recently discovered.[7,8] Such enediyne antibiotics like neocarzinostatin (NCS), calicheamicin-esperamicin, and dynemicin attracted much attention due to the extremely potent cytotoxicity and the novel mode of actions for DNA cleavage (Chart 1). NCS-chromophore (NCS-Chr) **1**, an essential core of NCS for DNA cleavage, can be activated by thiol addition, giving ene-yne-cumulene intermediate **2** (Chart 2), which spontaneously undergoes cyclization (so-called Myers type cyclization) to produce σ-sp^2 benzenoid diradical, as typically represented in Eq. 1. Three other antibiotics can also produce benzenoid diradical by spontaneous cyclization of their cyclic enediyne systems (Masamune-Bergman cyclization) by a chemical triggering (Eq. 2). DNA cleavage induced by these antibiotics was initiated by hydrogen abstraction by the reactive benzenoid diradical from DNA deoxyribose backbone. For a better understanding of the diradical-generating system and for designing novel DNA cleavers, we have prepared several artificial model systems having ene-yne-allene and ene-yne-ketene systems for mimicking the model of action of these natural ene-diyne antibiotics.

(1)

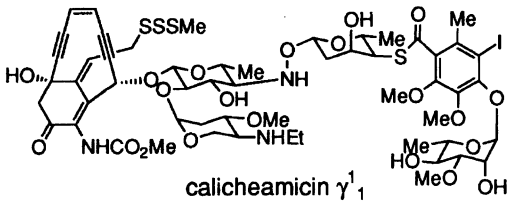
(2)



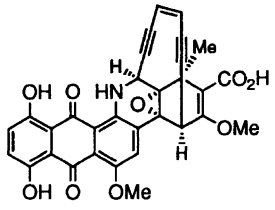
2

Chart 2.

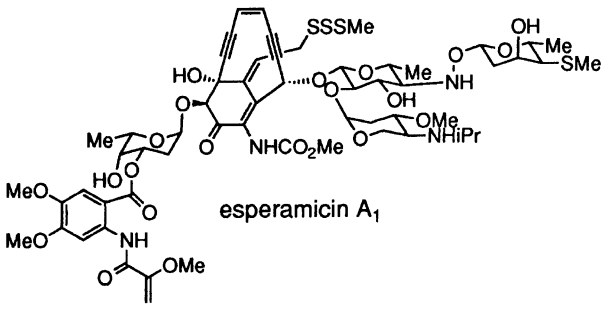
At first, we studied the cyclization of ene-yne-allene as a model for these antibiotics, based on the idea that the replacement of one of the acetylenes in acyclic enediyne by allene may shorten the distance between the two sp-hybridized carbons. This is a critical factor for producing benzenoid radicals via spontaneous Masamune–Bergman cyclization at mild physiological temperatures (Eq. 3).[9] Ene-yne-allene **3** was prepared from the reaction of enediyne alcohol with chloro diphenylphosphine via [2,3]-sigmatropic rearrangement, which was stable enough to be isolated by silica-gel chromatography at ambient temperature. Heating ene-yne-allene **3** at 37 °C in benzene in the presence of 1,4-cyclohexadiene as H donor produced aromatized products **4** and **5** and dimeric compound **6**. More than 90% of the deuterium was incorporated into C6 and C6′ positions of the dimeric products when thermal decomposition of **3** was carried out in the presence of THF-d_8–H_2O (5 : 1) at 60 °C, but this was not the case in D_2O–THF solution. The results obtained in our laboratories by Nagata et al. in 1989 were the first to demonstrate that thermal cyclization of ene-yne-allene systems like **3** can actually generate biradical intermediate **7** via Myers–Saito type cyclization.[9,10] Half lives for various ene-yne-allenes **3**, **8**, and **9** at 50 °C and **10** at 60 °C (Chart 3)



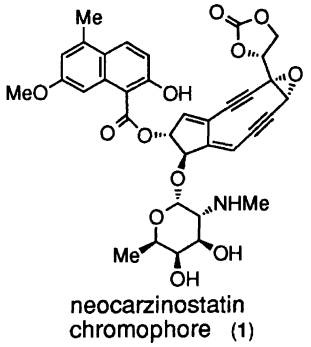
calicheamicin γ^1_1



dynemicin A



esperamicin A_1



neocarzinostatin chromophore (1)

Chart 1.

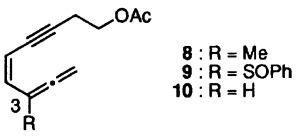
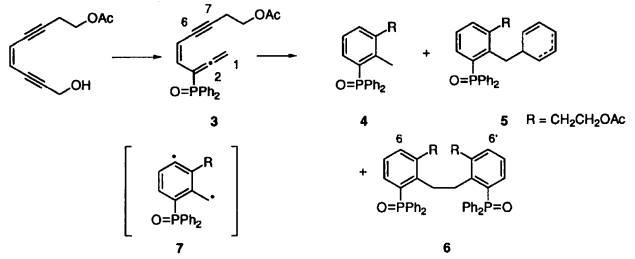


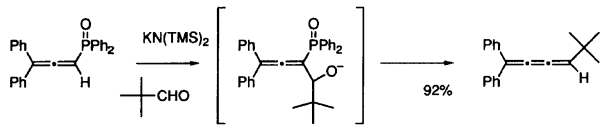
Chart 3.

were 24.6, 34.0, 6.5, and 383 min respectively, showing that the substituent at C3 position is critical for the efficient cyclization. A bulky substituent at C3 position can control the ene-yne-allene system as *s-cis* conformation.[10] After our work was published, a number of model systems including enediyne, ene-yne-allene, ene-yne-cumulene, and ene-yne-ketene systems have been demonstrated.[8]

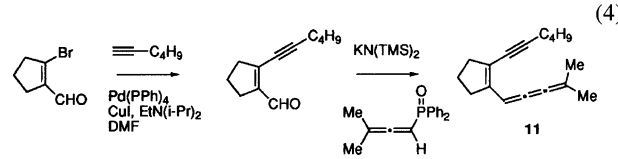


(3)

DNA-cleaving activity of these ene-yne-allene systems was examined by relaxation assay using supercoiled plasmid DNA. DNA cleavage by the ene-yne-allene system occurred at a physiological temperature, while their efficiencies were not always very high.[10] Extension of ene-yne-allene to ene-yne-cumulene system is needed to explore novel methods for the synthesis of unstable, highly conjugated and partially substituted cumulene under very mild conditions. The Horner–Emmons type reaction of allenyldiphenylphosphine oxides with aldehydes or ketones was found to be very effective for this purpose (Eq. 4 and Eq. 5).[11] The ene-yne-cumulene **11** prepared by this method was relatively stable in a dilute solution under nitrogen at room temperature and showed a weak DNA-cleaving activity. Nicolaou et al. have also reported the radical cyclization and the DNA cleavage of closely related ene-yne-allene system (Eq. 6).[12]



(4)



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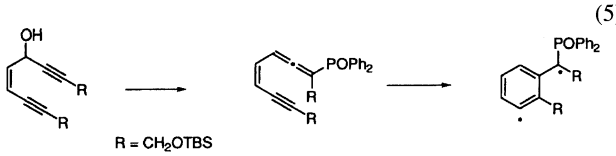
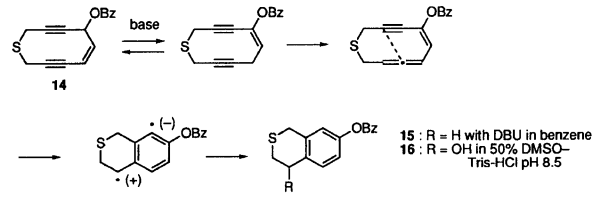


(6)

The synthesis and reactivities of enediyne antitumor antibiotic models have been extensively studied in the last decade.[8] Described below are some selected recent examples, particularly the mimicking systems developed in this country. Hirama and co-workers have synthesized various types of 10-membered cyclic models (e.g., **12** and **13**) for NCS-Chr and studied extensively the thermal chemistry of these model systems (Chart 4).[13] Analysis of DNA cleavage by **12** using ^{32}P-end-labeled DNA fragment indicated a specific cleavage at purine bases (G>A), indicative of DNA alkylation by this reagent.[13a] Takahashi and co-workers also reported the synthesis of 9-membered cyclic models for NCS-Chr.[14]

Toshima et al. reported the decomposition of cyclic enediyne **14** in nonaqueous and aqueous solutions to produce 3,4-dihydro-1*H*-2-benzothiopyranes **15** and **16** (Eq. 7).[15] In benzene the reaction seems to proceed via a radical mechanism, while the formation of hydroxy-substituted isothiochromane **16** showed an ionic mechanism in an aqueous solution. Analysis of DNA cleavage using labeled DNA fragments indicated an intense cleavage at guanine residue, implying again an alkylation mechanism for the DNA cleavage by **14**.[15]

Recently, Shibuya et al. employed a novel allene-forming reaction for the synthesis of enediyne models leading to the radical cyclization to give aromatized products (Eq. 8).[16] Some zwitterionic mechanism was suggested by labeling experiments. Myers and co-worker elegantly synthesized cyclic dienediyne **17**, which eventually produced cyclic diene-yne-cumulene intermediate **18** on addition of methyl thioglycolate.[17] Two benzenoid diradicals **19** (path a) and **20** (path b) were produced by cyclization of **18** in a ratio of ca 3 : 1 (Eq. 9).



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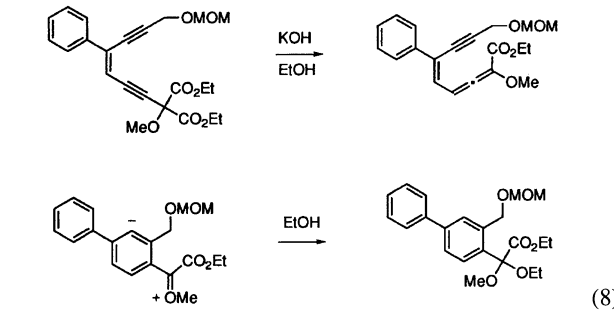

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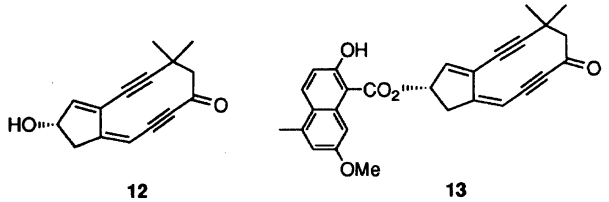
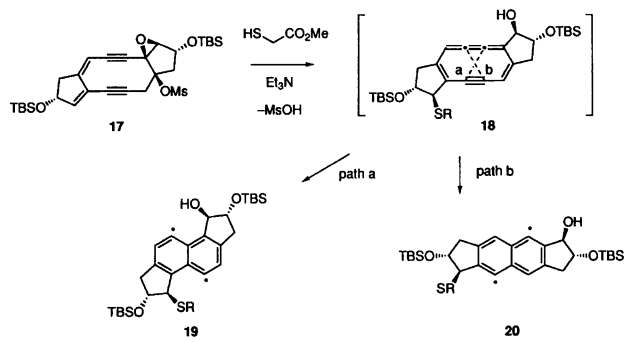
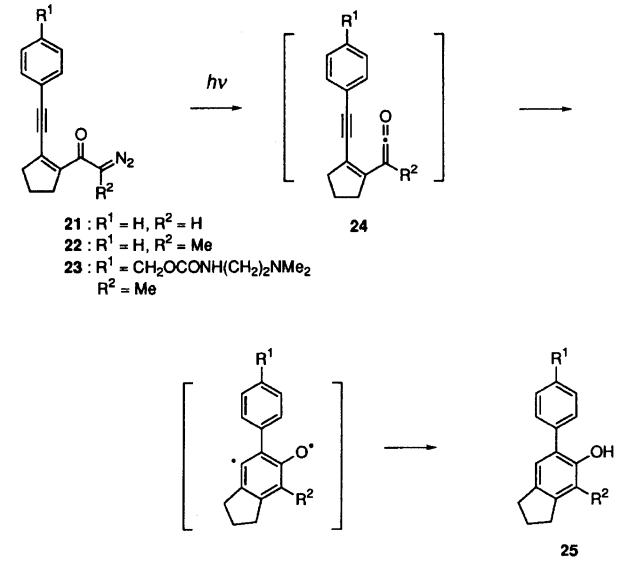


Chart 4.



(9)

Since the ene-yne-ketene system was known to undergo efficient radical cyclization,[18] replacement of allene moiety of the ene-yne-allene system with ketene may provide further advantages in producing benzenoid diradicals by photochemical activation. We have designed α-diazo ketones **21**, **22**, and **23** possessing ene-yne functionality. These can produce an ene-yne-ketene system by photochemical Wolff rearrangement of the diazoketone functionality, eventually leading to indanols via radical cyclization. Photoirradiation of **22** in toluene in the presence of 1,4-cyclohexadiene produced indanol **25** (R^1=H, R^2=H) (Eq. 10).[19] The reaction was very inefficient in the absence of 1,4-cyclohexadiene, suggesting that **25** is produced by radical cyclization of ene-yne-ketene **24**.



(10)

DNA relaxation assays for **22**, **23**, and **26** revealed an efficient DNA-cleaving activity of these α-diazo ketones under photoirradiation conditions (Chart 5)).[20] Since anthracene has an intense absorption over 400 nm, **26** was shown to cleave DNA by photoirradiation with 425 nm light. The photoreaction of these diazoketones in aqueous solvents under the conditions where a DNA-cleaving experiment was actually conducted did not produce a significant amount of radical cyclization product, indanols. When a 50% aqueous acetonitrile solution of **22** was irradiated with 366 nm light for 2 h under nitrogen, two isolable products were obtained, along with the recovery of the starting material **22** (42%)

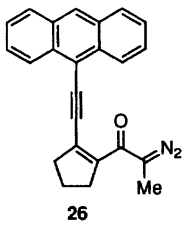
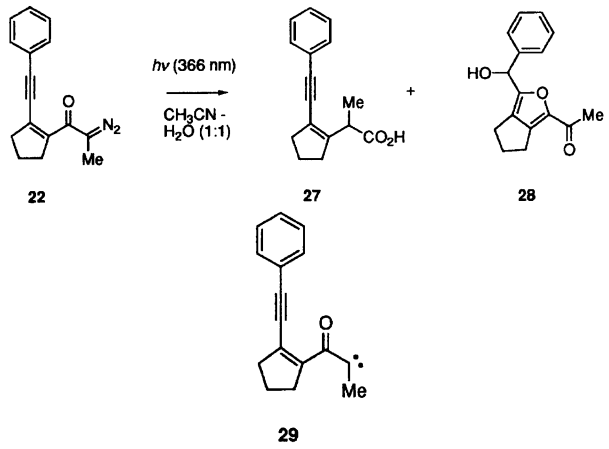


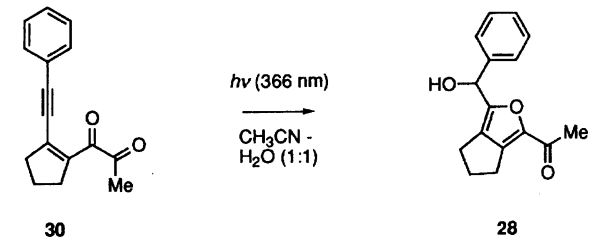
Chart 5.

(Eq. 11). While one product was carboxylic acid **27** (39%) resulting from water addition to the ene-yne-ketene intermediate, the other product was assigned as **28** (8%). The formation of **28** and carboxylic acid **27** from **1** under DNA-cleaving conditions was confirmed by HPLC analysis. While the precise mechanism of the formation of **28** was not clear at this moment, the trapping of initially formed α-keto carbene **29** with molecular oxygen was suggested in the photoreaction of **22** under oxygen atmosphere. Thus, under the aerobic conditions, the isolated yield of **28** increased up to 24%.



(11)

In order to determine whether **28** is formed from **22** via diketone **30**, we independently synthesized **30** and examined the photoreaction. Irradiation of **30** in aqueous acetonitrile under aerobic conditions gave **28** in 44% yield (Eq. 12).



(12)

The detailed mechanism and the scope and limitation of the this novel photoreaction are now under investigation. These results suggest that the photoreaction of **22** in an aqueous solution produces α-keto carbene **29**, which is immediately trapped with molecular oxygen, giving diketone **30**. It was also confirmed by DNA-cleaving experiments that the DNA-cleaving activity of **30** was substantially lower than that of **22** under 366 nm photoirradiation. These results taken together imply that photochemically generated α-keto carbene **29** or

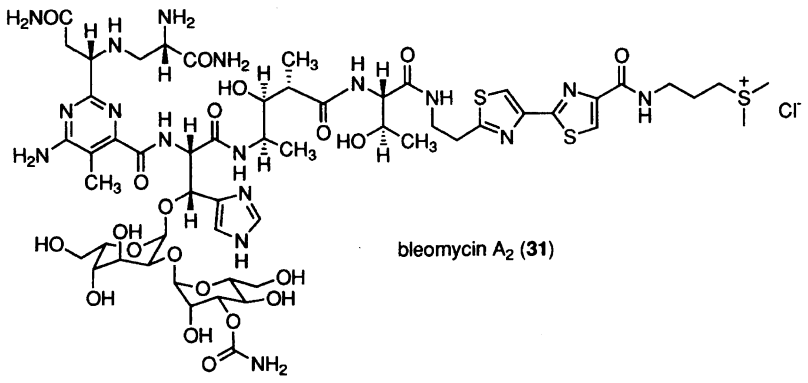


Chart 6.

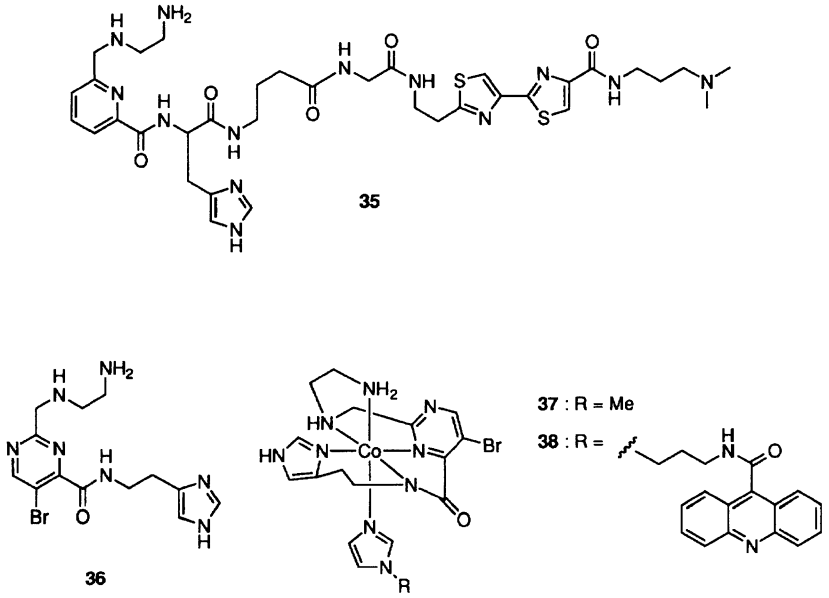
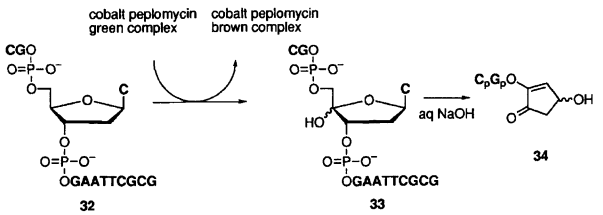


Chart 7.

its oxygenated species would be a reactive species that is responsible for the observed DNA cleavage.

2) Cobalt-Bleomycin Models. Bleomycin A_2 (**31**) is a member of glycopeptide-derived antibiotics bleomycins (BLMs),[7a,7b] which have been used clinically against non-Hodgkin lymphomas, squamous cell carcinomas, and testicular tumors (Chart 6). The cytotoxic activity of BLMs against mammalian cells is probably related to their DNA-cleaving activities. It has been shown that DNA strand scission by BLMs requires the presence of a transition metal which binds to the pyrimidine, β-alanine, and imidazole. The positively charged bithiazole moiety in the structure of BLMs is important for DNA binding and DNA cleavage at GC sites. In order to gain further insight into the structure-activity relationship, a number of BLM analogs have already been synthesized and their DNA-cleaving activity as well as dioxygen activation capability upon complexation with various transition metals were extensively studied.[7a,7b] In contrast to iron(II) complexes of BLMs possessing the most strong and spontaneous DNA cleavage activity, the cobalt complex of bleomycin is chemically inert but mediates DNA strand scission only when photoirradiated at 330—450 nm. Two stable cobalt-bleomycin complexes (green and brown complexes) were obtained by mixing peplomycin, a derivative of BLM, with $CoCl_2$ under air. The green hydroperoxy complex was shown to cleave DNA upon photoirradiation.[21] We also examined the reaction of cobalt peplomycin green complex with self-complementary dodecanucleotide d(CGCGAATTCGCG) (**32**) (Eq. 13).[21]



(13)

Accompanied by the formation of the brown complex, dinucleotide **34** was obtained as one of major products by alkaline treatment of the initially formed alkali-labile product, which was therefore identified as **33**. These results indicate that the photochemical conversion of the green hydroperoxy complex to the brown aqua complex may be responsible for the

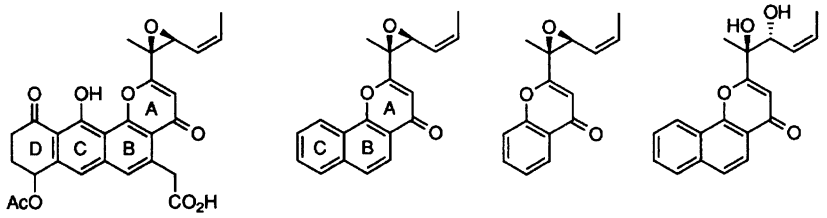


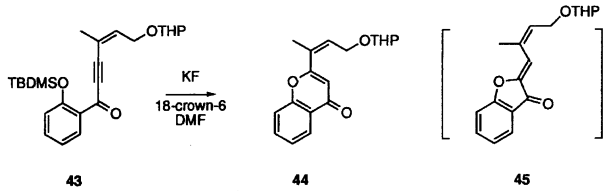
Chart 8.

ribose C-4′ hydroxylation. The detailed mechanism of DNA cleavage by cobalt green complex is now being investigated in our laboratories.

We have synthesized a cobalt complex of simple bleomycin model **35** possessing AMPHIS, *N*-[6-[(2-aminoethylamino)methyl]pyridin-2-ylcarbonyl]histidine, as a ligand.[22] The cobalt complex was shown to serve as a photochemical DNA cleaver. Thereafter, Mascharak and co-workers have developed a smilar cobalt complex of designed ligand PMA **36** (Chart 7).[23] While the DNA-cleaving activity of **37** was not so high, they demonstrated that acridine-tethered cobalt complex **38** induced very effective DNA cleavage upon photoirradiation.

3) Kapurimycin A_3 Model. Kapurimycin A_3 (**39**) is an antitumor antibiotic possessing a novel 4*H*-anthra-[1,2-*b*]pyran-4-one ring system with 1,2-epoxy-3-pentenyl side chain at C2 position.[24] This epoxide ring is shown to be the site of nucleophilic attack by guanine N_7 in duplex DNA, resulting in an effective DNA-alkylation.[25] To get insight into the extraordinarily high reactivity of the epoxide ring of **39**, we have examined the DNA-cleaving activity of truncated kapurimycin models **40**, **41**, and **42** (Chart 8).

To achieve an efficient and general synthesis of the 2-substituted 4*H*-chromen-4-one ring system contained in **39** and its model, we have developed 6-endo-digonal cyclization of *o*-hydroxyphenyl ethynyl ketones, giving predominantly the 4*H*-chromen-4-one ring system.[26] Thus, the reaction of ketone **43** with spray dried KF and 18-crown-6 in anhydrous DMF proceeded smoothly at room temperature to give chromenone **44** in 97% isolated yield (Eq. 14). No benzofuranone **45** was detected in ^{1}H NMR of the crude mixture. Addition of a small amount of methanol as a proton source to the same reaction system dramatically changed the product ratio, with the major product now being benzofuranone **45**. The presence of a phenolic hydrogen also resulted in a substantial loss of the selectivity. These results strongly suggest that the 5-*exo*-digonal process is reversible. The facile synthesis of truncated kapurimycin **40** demonstrated the usefulness of the 6-*endo*-digonal cyclization (Eq. 15).



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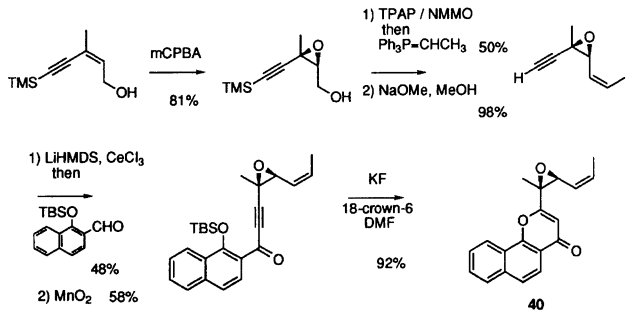


(15)

DNA-cleaving activities of these model compounds **40**—**42** were assessed by relaxation of supercoiled plasmid DNA, showing that **40** undergoes very effective DNA cleavage, whereas this was not the case for **41** and diol **42**. Therefore, the ABC ring system is essential for the efficient DNA cleavage.[27] The DNA cleavage analyzed by using ^{32}P-5′-end-labeled DNA fragment indicated that both **39** and **40** cleaved DNA specifically at guanine residue upon hot piperidine treatment.

Photochemical DNA Cleavers

There has been much current interest in the design of artificial DNA-cleaving molecules that are chemically stable and activatable by photoirradiation, particularly by a pulse of light, since such photoactivatable DNA-cleaving molecules can be used to probe nucleic acid structure, as designed "photonucleases", as photo-footprinting agents, and as a potent photodrug for photochemotherapy. Most non-photochemical DNA-cleaving molecules including natural antitumor antibiotics require a co-reagent, conditions hardly compatible with in vivo applications, whereas advantage might be found in photochemical DNA-cleaving molecules which are not toxic in the dark, but are activatable by light. Furthermore, their action would be controllable within space and time by the choice of proper irradiation methods. Such a photochemical approach has been actually realized in photochemotherapy in the treatment of tumors and in virus photoinactivation in blood products.[28]

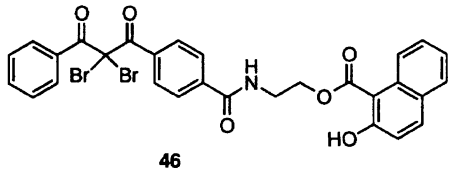
Various types of DNA-binding molecules have so far been reported to induce DNA cleavage upon photoirradiation.[6b] Such photochemical DNA-cleaving molecules can function by a variety of mechanisms, such as those involving generation of active oxygen species[29] or photoreactive metal center,[2,30] an electron transfer from DNA nucleobases,[31] a hydrogen abstraction from DNA sugar backbone by photogenerated radicals[6b,32] and a photoinduced DNA alkyl-

ation.[33] Within the last decade, our laboratory has focused upon exploitation of new methodologies for selective DNA-cleavage by photoirradiation. Minimum requirements for designing practically useful photochemical DNA cleavers are; i) high absorptivity in the UV A region (320—400 nm) and for phototherapy purposes in the red-absorbing region (ca. 600 nm) for penetration of light within tissues or blood; ii) efficient generation of nondiffusible reactive species capable of reacting with nucleic acid base or DNA deoxyribose backbone; and iii) accessibility for tethering to DNA binding molecules. Described below are recent examples of site or base selective DNA cleavage upon photoirradiation in our laboratories.

1) Nonselective Photochemical DNA Cleavers. Footprinting method using enzymes such as DNase I and II is a technique widely used for determining binding site for drugs and proteins. Chemical footprinting agents such as methidium–EDTA–iron(II) and 1,10-phenanthroline copper(I) are well-known. Recently, photofootprinting agents have also been investigated in several groups. These agents have an advantage over enzymatic methods or non-photochemical footprinting agents in that DNA cleavage is controllable by photoirradiation.

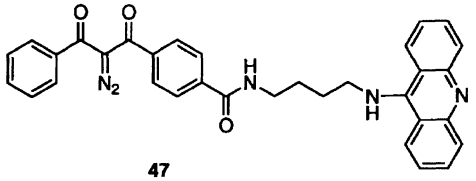
We have so far developed two types of photofootprinting reagents. The first, reagent **46**, has a brominated dibenzoylmethane structure (Chart 9), which undergoes homolytic cleavage of C–Br bond by 366 nm irradiation to give a nondiffusible carbon-centered radical.[34] Analysis of DNA cleavage pattern using ^{32}P-5′-end-labeled DNA indicated that this compound induces a frank strand cleavage at all bases together with a guanine specific cleavage after piperidine treatment. The former cleavage seemed to be ascribable to hydrogen abstraction from DNA sugar backbone by the radical derived from **46**, whereas the latter cleavage was due to the oxidation of guanine base by singlet oxygen.

The second, reagent **47**, consists of dibenzoyldiazomethane (DBDM) unit and a well-known DNA intercalater, acridine (Chart 10).[35] While direct excitation of DBDM resulted in formation of benzoylketene (see next section), the formation of dibenzoylmethane via H abstraction by triplet carbene



46

Chart 9.



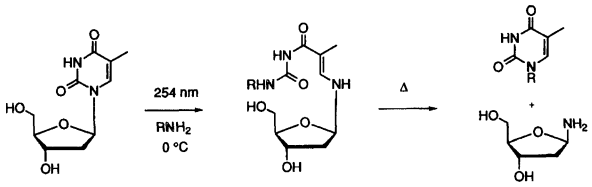
47

Chart 10.

was the main reaction under triplet photosensitization conditions using acridine as a sensitizer. This reagent is able to cleave DNA by photoirradiation at 400 nm without any base selectivity, regardless of hot piperidine treatment, indicating that DNA cleavage proceeds via H abstraction by the resulting triplet carbene from DNA sugar backbone.

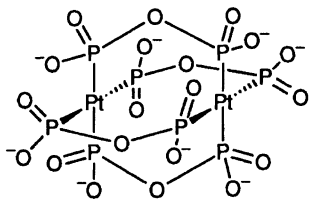
Nielsen et al. have studied the DNA photocleavage by uranyl(VI) salt.[36] The estimated affinity constant of uranyl(VI) ion to DNA is $10^{10}\,M^{-1}$ ($M = mol\,dm^{-3}$), which is considerably stronger than the binding of either Mg^{2+} or Na^{+}. Photocleavage by uranyl (VI) ion does not seem to involve diffusible species such as hydroxyl radicals and occurred proximal to the phosphate to which the uranyl ion is bound without any sequence selectivity. More recently, Thorp and co-workers have reported the anionic diplatinum agent $[Pt_2(pop)_4]^{4-}$ **48** ($pop = P_2O_5H_2^{2-}$) as a photofootprinting agents (Chart 11).[37] The photoreaction of $[Pt_2(pop)_4]^{4-}$ with ^{32}P 5′-end-labeled duplex 25-*mer* produced ladder phosphate terminated bands. The mechanism of DNA cleavage by **48** seems to involve C–H activation at the C4′ and C5′, where the tetraanionic complex collides, followed by oxidative degradation of deoxyribose. This unusual reaction of **48** has been used for footprinting of λ repressor bound to the O_{R1} sequence.

2) Thymine (T)-Selective DNA Cleavage. More than ten years ago, we found a novel photochemical ring-opening reaction of thymidine in the presence of primary amines upon 254 nm irradiation (Eq. 16).[38] This photoreaction can be successfully applied to a T-specific cleavage of DNA fragments. For example, irradiation of ^{32}P-end labeled DNA fragment in the presence of 1 M spermine at 254 nm at 0 °C resulted in a T-specific DNA cleavage. This method has been used for determining T residues in DNA sequencing, in combination with the standard Maxam–Gilbert C+T reaction to provide independent confirmatory reading.[39]



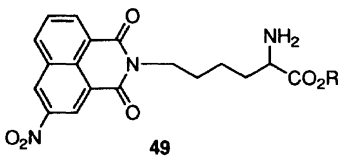
(16)

In an effort to devise practically useful DNA-cleaving amino acids, we have synthesized nitro-substituted naphthalimide **49** (Chart 12).[40] Upon irradiation of **49** in the presence



48

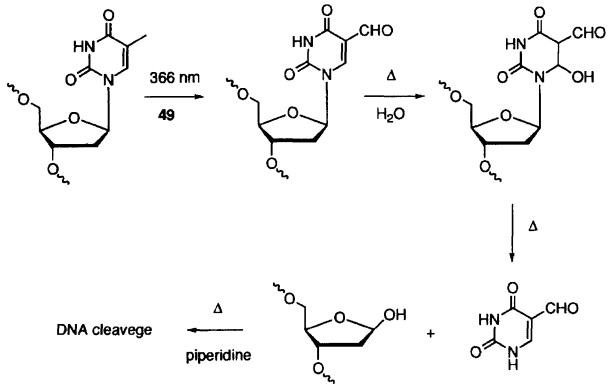
Chart 11.



49

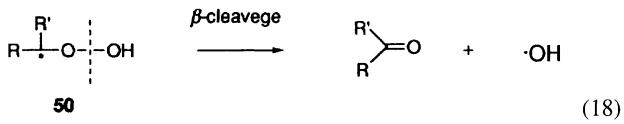
Chart 12.

of ^{32}P-end labeled double stranded DNA fragments, followed by piperidine treatment, T-specific DNA cleavage has been observed with 366 nm light. It was demonstrated that the first step of the T-specific cleavage of double-stranded DNA by **49** is an oxidative transformation of the T methyl group into a formyl group initiated by the hydrogen abstraction from the T methyl group by the photoexcited nitro group. Heating 5-formyluracil-containing sites in DNA in the presence of piperidine resulted in a DNA strand scission via the mechanism shown in Eq. 17.



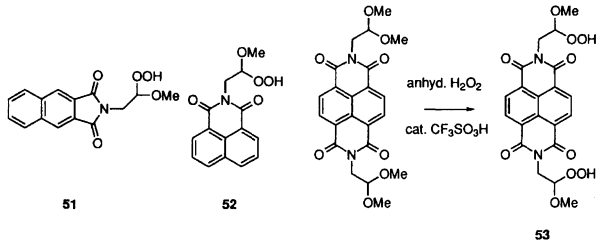
(17)

3) Photo-Fenton Reagents. In view of the high level of interest in the hydroxyl radical (·OH) in biological and other systems, approaches toward the development of efficient methods for ·OH generation without using transition metal ions and hydrogen peroxide have been investigated in our laboratories. Our objective is to design an efficient organic precursor that generates ·OH by low-energy irradiation, such as long-wavelength UV light (>350 nm) or more preferably by visible light irradiation. Such molecules, referred to as "photo-Fenton reagents", are particularly attractive as a controllable and mechanistically less complicated ·OH sources for applications in a number of biologically important reactions such as cross-linking of biopolymers and cleavage of DNA or proteins. The design criteria include the ease of synthesis, stability at ambient temperature, solubility in aqueous solvents and ability to produce ·OH by irradiation with long-wavelength light. Our strategy for the generation of ·OH is based on the well-known photochemical γ-hydrogen abstraction of phthalimide carbonyl and the earlier finding that hydroperoxyalkyl radicals such as **50** undergo extremely facile β-cleavage of the labile O–O bond, giving ·OH (Eq. 18).



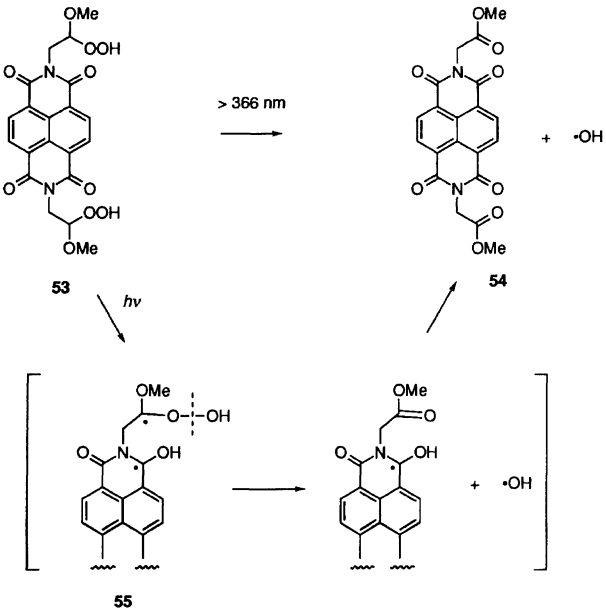
50 (18)

We have prepared hydroperoxides **51**, **52**, and **53** from the corresponding dimethyl ketals by treatment with ethereal hydrogen peroxide in the presence of triflic acid in dichloromethane (Eq. 19).[41,42] Particularly, *N*,*N*′-bis(2-hydroperoxy-2-methoxyethyl-1,8 : 4,5-naphthalenebis(dicarboximide) **53** was designed in order to improve the efficiency of ·OH generation per molecule and to enhance the absorption at longer wavelengths. The thermally stable hydroperoxide **53** was soluble in aqueous organic solvent (up to 2.5 mM in acetonitrile–water 8 : 92) and has a very strong absorption at 377 nm (log ε=4.45).



51 **52** **53** (19)

Photolysis of **53** in acetonitrile at 366 nm proceeded rapidly to give ester **54** quantitatively with a quantum yield of ϕ=0.18 (Eq. 20). In the presence of adamantane,



53 **54** **55** (20)

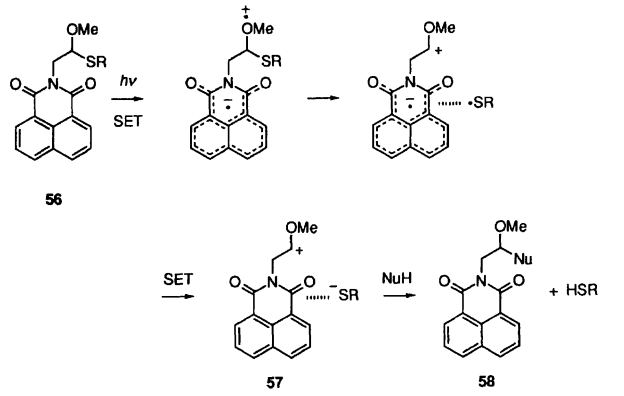
1-adamantanol, 2-adamantanol, and adamantanone were obtained, and their formation was inhibited by addition of an ·OH scavenger such as dimethyl sulfoxide.[42] Generation of ·OH was also confirmed by an ESR spin-trapping method using DMPO as a spin-trapping reagent. Upon brief exposure of a solution of **53** and DMPO in sodium cacodylate buffer (pH 7.0) to 366 nm light, intense ESR signals characteristic of an ·OH-DMPO adduct were detected. Exclusive formation

of ester **54** accompanied by ·OH generation is consistent with a mechanism involving γ-hydrogen abstraction by the carbonyl in the diimide moiety, followed by cleavage of the labile O–O bond of the resulting biradical **55**.

We have examined the DNA-cleaving activity of **53** upon exposure to 366 nm light by using supercoiled circular ϕX174 RFI DNA (form I).[42] Single-strand breaks and a small number of double-strand breaks at higher conversion were observed, as evidenced by the production of form II and form III DNA, respectively, by means of gel electrophoresis. Addition of sodium benzoate as ·OH scavenger to the reaction system inhibited the DNA cleavage. The base and sequence specificity of DNA cleavage was analyzed by using ^{32}P-end-labeled DNA fragments. Hydroperoxide **53**-induced DNA strand cleavage preferentially at the 5′ side of 5′-GG-3′ sequences after piperidine treatment. No cleavage was observed at other sites including single G residues. This is in marked contrast to the photocleavage of DNA mediated by singlet oxygen, where the cleavage occurs equally at each G residue after piperidine treatment.[29] The –GG–specific cleavage after piperidine treatment most probably proceeded via electron-transfer mechanism. This method of ·OH generation is very efficient and could be quite attractive for the use in applications requiring ·OH in a number of other systems.[43] The reagent **53** adsorbed on silica gel was also used for the photocrosslinking of eye lens proteins and for enzyme inactivation.[44]

4) Photoinducible DNA-Alkylating Agents. Methods generating highly electrophilic species in aqueous solutions under physiological conditions have drawn considerable attention due to their inherent possibility of modifying biomolecules such as peptides, enzymes and DNA. Photo-triggered generation of such super electrophiles under mild conditions at the target site in a biopolymer is particularly attractive. While a few reports have described the photoinduced DNA alkylation,[33] the direct evidence for DNA alkylation has not been obtained. One of the difficulties in studying photoinduced DNA alkylation was that the G selective cleavage induced by guanine alkylation after hot piperidine treatment was not easily distinguishable from the G specific cleavage by singlet oxygen. Identification of alkylated purine bases is primarily important.

We have developed two types of photoinducible DNA-alkylating agents. The first strategy for designing photoinducible DNA-alkylating agents lies in an electron-transfer-initiated fragmentation of monothioacetals (Eq. 21).[45] The photoreaction of **56** in the presence of nucleophiles such as ethanol and *t*-butylamine produced the corresponding trapping product **58** and thiol, implying an overall ionic dissociation of the C–S bond to give cationic species **57**. Photoreaction of *N*-benzoyl-2′-deoxyadenosine **59** in the presence of **56** (R=Me) followed by deprotection of benzoyl group produced stable N7-alkylated adenine adduct **60** (Eq. 22). The formation of **60** in the photoreaction of **56** with calf thymus DNA was also confirmed by HPLC analysis. Based on these results, DNA cleavage by **56** (R=Me) under photoirradiation conditions was concluded to proceed via alkylating of DNA with photogenerated cationic species **57**.



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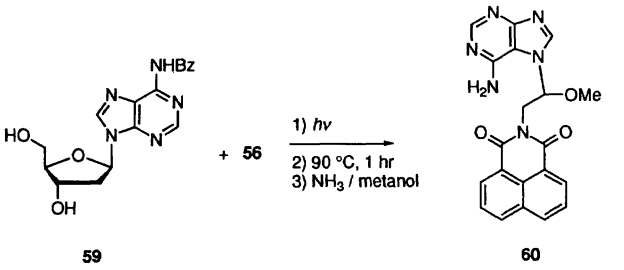
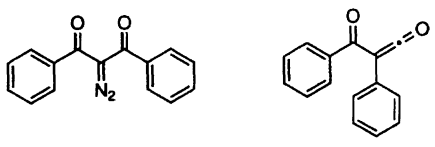


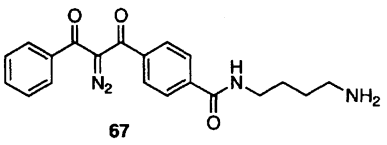
(22)

The second type of DNA-alkylating reagent utilizes Wolff rearrangement (WR) of dibenzoyldiazomethane (DBDM) **61** and its derivatives, giving highly electrophilic benzoylketene **62** upon photoirradiation (Chart 13), which can be trapped by nucleophilies in aqueous solvents.[46] When **61** was irradiated with transilluminator (366 nm) in 50% aqueous acetonitrile, benzyl phenyl ketone **64** was obtained in 71% as a single product with a quantum efficiency of 0.037 (Eq. 23). The formation of **64** indicates the addition of water to the highly electrophilic ketene **62** produced from **61** by WR, followed by decarboxylation of the resulting β-keto acid **63**. The trapping of **62** with amines, especially amino acid derivatives, also proceeded quite effectively in competition with water addition. Thus, photoirradiation of **61** in the presence of two molar amounts of glycine ethyl ester (Gly·OEt) produced amide **65** in 77% with the formation of **64** (12%). Likewise, photoirradiation of **61** with N^{α}-Boc-L-Lys–OMe (2 equiv) in 50% aqueous acetonitrile gave amide **66** in 55%, indicating an efficient trapping of **62** with nucleophiles in aqueous solvents.



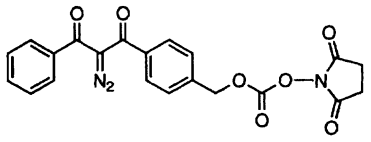
61 (DBDM) **62**

Chart 13.



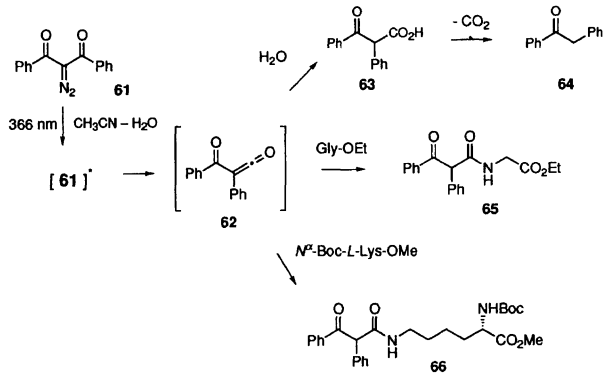
67

Chart 14.



68 (DBDM-OSu)

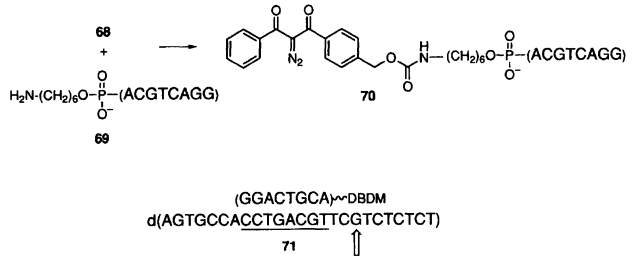
Chart 15.



(23)

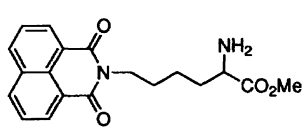
DBDM derivative **67** having aminoalkyl side chain can very efficiently cleave supercoiled pBR322 DNA upon photoirradiation (Chart 14). Cleavage of ^{32}P-5′-end-labeled-DNA by **67** occurred at guanine residues with modest preference over other nucleobases even in the presence of a large excess of singlet oxygen quencher, sodium azide. In order to achieve photoinduced DNA cleavage at specific guanine sites, dibenzoyldiazomethane-oligonucleotide conjugates were synthesized. For this purpose we have developed a new reagent DBDM-OSu **68** which can facilitate connections of DBDM unit to DNA binders such as DNA oligomers containing aminoalkyl linker and peptides (Chart 15).[47]

Reaction of DBDM-OSu **68** with DNA oligomer **69** having aminoalkyl linker at 5′-end effectively produced DBDM-ODN conjugate **70** (Eq. 24). The photoreaction of **70** was carried out in the presence of 25-mer **71**, containing both complementary sequence and targeted guanine (indicated by arrow). Upon photoirradiation, crosslinking of both oligomers was observed as evidenced by gel electrophoresis. Heating of the crosslinked oligomers with hot piperidine induced a single-site cleavage at the targeted guanine residue, clearly indicating that the targeted guanine was alkylated by photogenerated ketene from **70**. DBDM-oligonucleotide conjugate **70** is a very important class of photonucleases, which is capable of cleaving DNA at targeted guanine site.

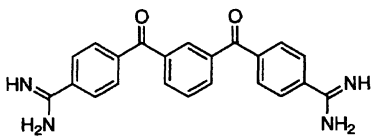


(24)

5) DNA Cleavage via Photochemical Electron Transfer. For many years there has been great interest in one-electron oxidations of DNA, in connection with DNA damage caused by ionizing radiation, oxidizing agents, and photooxidation with endogenous photosensitizers. As is well-known, guanine is the most readily oxidizable base; it has the lowest ionization potential among the DNA nucleobases.[31a] The electron loss center created in DNA duplex may ultimately end up at guanine residues via hole migration.[31a,48] While certain photoinduced DNA cleavage reactions were suggested to proceed via one-electron-transfer process,[31] there had been no direct evidence for the electron-transfer process. Recently, our laboratories have devised a new class of DNA-cleaving agent, photocleaving amino acid (PCA) **72** (Chart 16), and have demonstrated that **72** generates alkaline labile sites at the 5′-guanine (G) of 5′-GG-3′ sequence with a lower frequency at the 5′-G of 5′-GA-3′ sequence upon 366 nm photoirradiation.[40] Such GG-selective DNA photocleavage was already observed with other photosensitizers including **53**,[42] riboflavin,[31d] $[Co(NH_3)_6]^{3+}$,[49,50] 3-nitroanisole,[50] 4-nitroveratrole,[50] and water-soluble benzophenone derivative **73**.[51] We recently reported the first direct evidence for one-electron transfer from a guanine base in GG-containing duplex deoxyoligonucleotides to triplet excited **72** by detecting $\mathbf{72}^{-\bullet}$ by means of excimer laser flash photolysis (Eq. 25). We demonstrated that the most readily oxidizable sites in B form DNA are the 5′-G of 5′-GG-3′ sequence, due to the π-stacking interaction of the two guanine bases.[52] Recently, Schuster et al. have also observed similar GG-selective photodamage of duplex DNA via electron transfer in the presence of anthraquinone derivatives.[31f,53]

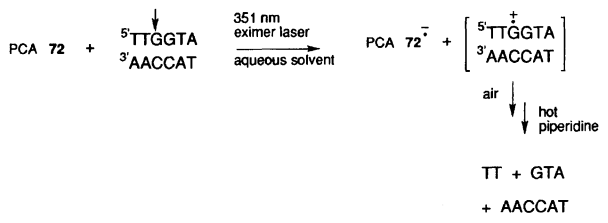


PCA (72)



73

Chart 16.



(25)

On the basis of experimental data obtained from one-electron oxidations of various contiguous guanine (G_n)-containing oligomers and ab initio calculations[54] of stacked nucleobases, we propose a very important and general rule for predicting the most electron-donating sites, i.e., the most readily oxidizable sites in B form DNA.[51] This rule, referred to as the "guanine-guanine (G–G) stacking rule", shows the DNA sites which are most susceptible to one-electron oxidations such as those in ionizing radiation and various types of chemical and photochemical oxidations.[55] Recent results by Melvin et al.[56] on the GG-selective DNA cleavage by direct photoionization using 193 nm excimer laser can reasonably be explained by the GG-stacking rule.[57] Furthermore, this rule is also very important in understanding HOMO–LUMO interactions associated with B form DNA. In such a HOMO–LUMO or a charge-transfer type interaction with electron-accepting,molecules, the 5′-G of contiguous guanine (–G_n–) in B form DNA is the most electron-releasing and therefore most strongly interacting site.[54] Detail of the GG-stacking rule will be published elsewhere.

Conclusion and Prospects

To date our knowledge of the sequence-specific DNA recognition, particularly recognition of major groove, is still insufficient. New principles of sequence-specific DNA recognition are being discovered from the ongoing studies of DNA-binding proteins and natural drugs; these are becoming available for synthetic design and ingenuity. It is therefore too early to propose definite guidelines for the design of artificial sequence-specific DNA binders. Many studies, many compounds, and hence much time will be necessary before we can perfectly control the targeting to define DNA sequences by the use of designed synthetic ligands. Most importantly, new concepts based on more sophisticated chemistry is crucially important to create practically useful sequence-specific DNA-binding and -cleaving molecules. Design of new chemical or photochemical tags capable of cleaving or modifying DNA is also indispensable. Furthermore, precise understanding of the DNA-ligand interactions by means of NMR techniques is also critically important. The design of new DNA-cleaving agents is a real challenge that is still far from being solved completely. However, this approach might lead to the discovery of a number of new methodologies for molecular biology as well as for the development of new tumor-active drugs and photodrugs.

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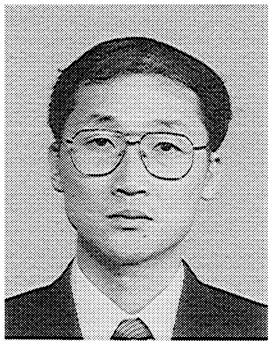

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