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Design, Synthesis and Evaluation of Novel DNA Alkylating Agents Based on the Chemistry of Antibiotic Kapurimycin A₃

Akimitsu Okamoto

1998
Preface

The study presented in this thesis has been carried out under the direction of Professor Isao Saito at the Department of Synthetic Chemistry and Biological Chemistry of Kyoto University during April, 1993 to March, 1998. The study is concerned with design, synthesis and evaluation of novel DNA alkylating agents based on the chemistry of antibiotic kapurimycin A₃.

The author wishes to express his sincere gratitude to Professor Isao Saito for his kind guidance, valuable suggestions, and encouragement throughout this work. The author is deeply grateful to Associate Professor Kazuhiko Nakatani for his constant advice, valuable discussions, and encouragement during the course of this study. The author is also indebted to Professor Hiroshi Sugiyama for their helpful suggestions. The author is also indebted to Dr. Yoshikatsu Ito and Dr. Kenzo Fujimoto for their helpful suggestions.

The author wishes to thank Mr. Yoichi Uosaki and Mr. Mitsunobu Hara, Kyowa Hakko Kogyo Co., Ltd. for providing natural kapurimycin A₃ and its structural information for comparative analysis. The author is grateful to Mr. Haruo Fujita and Mr. Tadao Kobatake for the measurements of NMR spectra and mass spectra, respectively. The author is thankful to Professor Jun-ichi Yoshida, Professor Yoshihiko Ito and Professor Junzo Sunamoto for the measurements of cyclic voltammetry, optical rotation and circular dichroism, respectively. The author wishes to Messrs. Mikito Yamanuki, Kazuhito Tanabe, Shinsuke Sando, Satoshi Okuda, Takahiro Matsuno and Shinya Hagihara for their collaboration. The author is also indebted to Mr. Junya Shirai,
Mr. Takashi Nakamura and Dr. Masami Takayama for their collaboration.

The author is also grateful to Messrs. Satoshi Maekawa, Tomonori Sakurai, Hideki Okita, Yasuki Komeda, Kiyohiko Kawai, Nobuhiro Higashida, Kazuhiko Fujisawa, Taisuke Iwanami, Hiroshi Miyazaki, Yohei Ozeki, Chikara Dohno, Yusuke Nomura, Kaoru Adachi and Mitsuhiro Iwasaki for their helpful suggestions and hearty encouragement. The author also thanks to other members of Prof. Saito's research group.

The author thanks Japan Society for the Promotion of Science for financial support (Fellowship for Japanese Junior Scientists).

Finally, the author expresses his deep appreciation to his parents, Mr. Katsumi Okamoto and Mrs. Etsuko Okamoto for their constant assistance and affectionate encouragement.

Akimitsu Okamoto

January, 1998
DNA sequence selectivity is achieved by both proteins and small molecules. DNA-binding proteins have evolved to recognize the sequence dependent features of DNA in order to participate in a variety of genetic events such as control of gene expression and replication of DNA. While overall sequence selectivity is generally lower, small molecules such as DNA reactive drugs and carcinogens may also exhibit DNA sequence recognition. These drugs and carcinogens may share common DNA recognition motifs with proteins and because of their relatively small molecular weight and corresponding reduced structural complexity, they may serve as useful models for more complex protein-DNA recognition mechanisms.

DNA basically has the nature to undergo electrophilic modification by alkylating agents. Nitrogen atom of guanine N7 position in DNA is one of the most nucleophilic position in DNA. A large number of guanine N7 alkylating agents are known at present such as aflatoxin B1 oxide, pluramycin A, hedamycin as naturally occurred molecules, as well as dimethyl sulfate, nitrogen mustard and bromoacetate derivatives. These DNA guanine N7 alkylating agents separates to mainly two groups, the molecules which alkylate all guanine equally (dimethyl sulfate and bromoacetate derivative are typical) and the molecules which recognize sequence of some base including guanine base and alkylate guanine N7 such as aflatoxin and pluramycin. The latter type of guanine N7 alkylating agents were shown in Figure 1. Each molecule in Figure 1 has a unique sequence selectivity for their alkylation.
Aflatoxin B₁ is a DNA-reactive natural carcinogen. Humayun et al.,⁴ and Loechler et al.,⁴c described extensive and carefully executed experiments revealing the sequence selectivity of activated aflatoxin B₁ reaction with DNA. The statistical treatment of the data gave trinucleotide priority to 5'-GG*G, followed closely by 5'-GG*T. They invoked a sequence-dependent noncovalent binding site for aflatoxin B₁ oxide in DNA major groove. The importance of binding interactions was also supported by other lines of evidence which they listed.

The sequence selectivity of DNA alkylation by recently isolated pluramycin antibiotics has been also investigated by Hurley et al.,⁶c,lo and Wickham et al.,⁶d They have divided these pluramycins into three groups of sequence selectivity. The first group represented by pluramycin A and hedamycin showed 5'-CG* > 5'-TG* >> 5'-AG* = 5'-GG* sequence selectivity. Altromycin B preferred 5'-AG* sequence and altromycin H showed the greatest reactivity with 5'-AG* and 5'-TG* sequences.¹⁰a,¹¹

These agents which become alkylated to DNA in a sequence-selective fashion may recognize the sequence by either noncovalent binding or by alkylation mechanisms, or perhaps more likely by a combination of both mechanisms. The sequence selective alkylation agents in the Figure 1 possess the common structural feature involving aromatic rings for DNA binding and epoxide ring for DNA alkylation. However, it has been unknown how their structures work as a factor for their sequence selective alkylation. In order to get considerable insights into the molecular basis of its sequence selective reaction with DNA, it is very important to do experiments designed to maximize sequence selectivity information.

In 1990, the novel antitumor antibiotic, kapurimycin A₁, has been found in the culture of Streptomyces sp. DO-115 by Kyowa Hakko Kogyo group.¹²,¹³ It exhibited strong antibacterial and cytotoxic
activities and showed a potent activity against murine leukemia P388 in vivo. It consists of the tetrahydroanthra-γ-pyranone skeleton and the β,γ-unsaturated δ-keto carboxylic acid structure. In addition, at C2 position of ring skeleton it has the side chain with propenyl substituted epoxide in Z-configuration.

Kyowa Hakko Kogyo group investigated covalent modification and strand scission of DNA by kapurimycin A3 in 1990. They described that it caused single strand cleavage of supercoiled pBR322 DNA and binded to calf thymus DNA and the thermal treatment of this adduct resulted in a release of a guanine attached to C16 of kapurimycin A3 through one of its nitrogen atoms. Previously, our group also investigated DNA cleavage reaction of kapurimycin A3 with deoxytetranucleotide d(CGCG)2. This study provided evidence that kapurimycin A3 alkylated DNA at N7 of guanine to produce a thermolabile adduct which could undergo depurination to produce a more stable kapurimycin-guanine adduct, together with the formation of its abasic site-containing oligomer. However, the relationship between the structural characteristics of kapurimycin A3 and the guanine selectivity in its DNA alkylation remains unsolved.

In spite of a large number of the studies on the reactivity and sequence selectivity of natural compounds that alkylate guanine N7 in DNA, it is not enough to discuss the general structural requirements for both their reactivities to guanine and their sequence selectivities for DNA alkylation. The synthetic analogs of reduced structural complexity for the natural products would serve as useful models for the more complex natural products–DNA recognition and alkylation mechanisms. For exemplary purposes, we designed the kapurimycin analogs with a very simple structure which just consists of aromatic rings for DNA binding and epoxide side chain for DNA alkylation. Subsequently, these analogs have been synthesized and compared in DNA alkylation in order to understand the molecular basis for the DNA sequence selectivity of DNA alkylation.

This thesis consists of five chapters on the design, synthesis and evaluation of novel DNA alkylating agents based on the chemistry of antibiotic kapurimycin A3.

In chapter 1, novel 4-pyranone ring formation for the synthesis of kapurimycin analogs is reported. The 6-endo-digonal selective cyclization method of o-silyloxyphenyl ethynyl ketones under mild basic conditions was developed.

In chapter 2, essential structure for efficient DNA alkylation by kapurimycin A3 is discussed. It was shown that ABC-ring analog of kapurimycin A3 effectively alkylated DNA guanine bases and the sequence selectivity of DNA alkylation was closely similar to that of natural kapurimycin A3.

In chapter 3, guanine-guanine sequence selectivity for DNA alkylation by kapurimycin analogs is described. By comparison of DNA alkylation by kapurimycin analogs involving different number of aromatic rings, the mechanism of guanine-guanine sequence selective DNA alkylation by kapurimycin analogs was elucidated.
In chapter 4, the effect of absolute configuration of epoxy subunit on guanine-guanine sequence selective alkylation is discussed. By comparison of DNA alkylation by both enantiomers of kapurimycin analogs, it was shown that the stereochemical orientation of epoxide in the complex with DNA is considerably important for sequence selective alkylation by kapurimycin analogs.

In chapter 5, the sequence selective alkylation of continuous guanine sequences by DNA intercalators possessing epoxy side chain is discussed. It was shown that the interaction of DNA HOMO with LUMO of DNA alkylating agents played a critical role in the selectivity toward continuous guanine sequences such as GGG.

References


(13) The absolute configuration of 1 has been determined to be 8S, 14S and 16S. Uosaki, Y.; Saito, H. Abstract paper p 1013, 69th annual meeting of the Chemical Society of Japan, Kyoto (1995).


CHAPTER 1

Novel 4-Pyranone Ring Formation for the Synthesis of Kapurimycin Analogs

Abstract: The cyclization of o-hydroxyphenyl ethynyl ketones was examined from theoretical and experimental standpoints in order to develop efficient synthetic methods for the construction of 2-substituted pyranones of significant biological activities. *Ab initio* studies at HF/6-31G* level on the cyclization indicated that both 6-endo-digonal and 5-exo-digonal cyclizations giving benzopyranones and benzofuranones, respectively, were endothermic and reversible in aprotic media, and the irreversible protonation of the resulting anions would be critical for the product formation. We generated phenoxide ion under aprotic conditions *in situ* by desilylation of o-silyloxyphenyl ethynyl ketones with potassium fluoride and 18-crown-6 in anhydrous DMF. Under these conditions the cyclization of variety o-hydroxyphenyl ethynyl ketones proceeded smoothly to produce benzopyranone derivatives with exceedingly high selectivity. Theoretical and experimental results strongly suggested that the presence of a small amount of proton donor effecting the protonation of the resulting benzopyranone anion was essential for the high 6-endo-digonal selectivity.
**Introduction**

Kapurimycin A, (1) is an antitumor antibiotic possessing a novel anthra-γ-pyrene ring system with a vinyl epoxide side chain at the C2 position.\(^1\) The structure of 1 is closely resembled to those of pluramycin family antibiotics,\(^2\) which have a common 4H-anthra[1,2-b]pyrane ring system and characteristic functionalities attached to the C2 position as well as deoxyamino sugars at C8 and C10 positions. Pluramycin A (2),\(^4\) hedamycin (3)\(^4\) and 1 all having epoxide functionalities on the side chain attached to the C2 position are known to covalently bound to DNA by a nucleophilic ring opening of the epoxide with guanine N7 in DNA.\(^5\)\(^8\)

In spite of the significant biological features and unique reactivity toward DNA, the structure–activity relationships on these antibiotics was not examined primarily due to the difficulty in the synthesis of 2-substituted benzopyranone ring system.\(^9\)\(^10\) For example, this problem has been addressed by the synthesis of O-methylkidamycinone (5) (eq 1),\(^9\) showing that conventional synthetic scheme for benzopyranone systems using acid-catalyzed cyclization of 1-(o-hydroxyphenyl)-1,3-diketones\(^11\)\(^12\) (e.g., 4) is not applicable to the synthesis of these antibiotics because of the competitive formation of undesired dihydropyranone (e.g., 6).

To investigate structure–activity relationship of these antibiotics we focused our attention on developing efficient synthetic methods for 2-substituted benzopyranone ring systems from readily available precursors. As a candidate for such a process, the 6-endo-digonal cyclization of o-hydroxyphenyl ethynyl ketones was first examined (eq 2), because the starting phenyl ethynyl ketones could be readily synthesized from salicylic aldehyde and acetylenic compounds.
According to the Baldwin’s rule the 6-endo-digonal cyclization is a favorable process, although the cyclization of \( \alpha \)-hydroxyphenyl ethynyl ketones under basic conditions are reported to produce not only benzopyranone via the 6-endo-digonal cyclization but also benzofuranone by a simultaneous 5-exo-digonal cyclization, with the product ratio being highly dependent on the reaction conditions. To get insight into the factors governing the 6-endo-digonal and the 5-exo-digonal cyclization of \( \alpha \)-hydroxyphenyl ethynyl ketones, we have investigated this reaction from theoretical and experimental viewpoints. We herein describe experimental results in combination with theoretical calculations indicating that both 6-endo-digonal and 5-exo-digonal cyclizations of \( \alpha \)-hydroxyphenyl ethynyl ketones are reversible in aprotic media, and that the irreversible protonation of the resulting vinyl anion gives rise to the benzopyranone formation with exceedingly high selectivity.

\[ \text{For calculation studies R denotes Me} \]

Results and Discussion

Theoretical Calculations for 6-Endo-digonal and 5-Exo-digonal Cyclizations.

To discuss the cyclization of \( \alpha \)-hydroxyphenyl ethynyl ketone in detail, \textit{ab initio} molecular orbital calculations of phenoxide ion 10, vinyl anions 11 and 12 (where R denotes Me), and two transition states TS-6 and TS-5 for the 6-endo-digonal and 5-exo-digonal cyclizations, respectively, were carried out. While in our preliminary communication we reported the theoretical calculations at the HF/3-21G(*) level, more accurate calculations at higher HF/6-31G* level was performed at this time for precise discussions. For these calculations the initial structures for 10, 11, and 12 were surveyed at the semiempirical PM3 level. Two stable s-trans and s-cis conformers were found for 10, with the former being more stable than the latter by 1.80 kcal/mol. Therefore, the s-trans conformer of 10 shown in Figure 1 was used for further calculations. While the reaction of nucleophiles to the carbon–carbon triple bond may proceed via either \textit{syn} or \textit{anti} addition, the \textit{E}-configuration for the exocyclic alkene in 12 supported by previous theoretical studies was used for the calculation. We could not develop a reasonable transition state model for the \textit{syn} addition of the phenoxide ion in 10 to the carbon–carbon triple bond via the 5-exo-digonal cyclization. These structures obtained by the PM3 calculations were optimized at the HF/3-21G(*) and then at the HF/6-31G* level. Two transition states TS-6 and TS-5 were initially generated empirically using the transition structure module incorporated in Spartan and finally calculated at the HF/6-31G* level. Frequency analyses for TS-6 and TS-5 showed the only one imaginary vibrational frequency at \(-524.62 \text{ and } -525.25 \text{ cm}^{-1} \), respectively (Figure 1). In both structures the approaching angle of the phenoxide ion to the carbon–carbon triple bond was 115.4° and 118.9°, respectively. The potential energy diagram for the reaction of 10 to 11 and 12 was shown in Figure 2. The potential energies of two transition states TS-6 and TS-5 were very close in each other, therefore there was no obvious difference in the activation energies for the two cyclization
TS-5 (-532.399409)  
O - C8 1.840 Å  
∠O-C8-C9 = 118.9°

TS-6 (-532.400397)  
O - C9 1.898 Å  
∠O-C9-C8 = 115.4°

10 (-532.438009)  
11 (-532.421144)  
12 (-532.409586)

**Figure 1.** Optimized structures for 10, 11, 12, TS-6, and TS-5 at the HF/6-31G* level. Numbers in parenthesis indicated the total energy in hartree.

**Figure 2.** Potential energy diagram for the reaction of 10 to 11 and 12. Numbers in parenthesis indicated the relative potential energy from 10 in kcal/mol.

processes (23.60 kcal/mol for $\Delta G^\ddagger_{10 \rightarrow 11}$ and 24.22 kcal/mol for $\Delta G^\ddagger_{10 \rightarrow 12}$). On the other hand, the produced vinyl anion 11 was more stable than anion 12 by 7.26 kcal/mol. As a result the activation energy for the ring-opening reaction of 12 to 10 (6.38 kcal/mol) was substantially smaller than that for the conversion of 11 to 10 (13.02 kcal/mol). Since both cyclization reactions were shown to be endothermic processes, the irreversible protonation of the resulting anions 11 and 12 would be critical for the product formation. These theoretical results led to the following speculations for the cyclization of 10. 1) Under kinetically controlled conditions the selectivity for the 6-endo-digonal cyclization would not be so high. 2) Under thermodynamic conditions the product formation is favorable for the 6-endo-digonal process, if all three anions were equilibrated and the selective protonation of 11 proceeded irreversibly.

**Synthesis of o-Silyloxyphenyl Ethynyl Ketones.**

To achieve thermodynamically controlled reaction conditions for the selective 6-endodi-gonal cyclization, we examined *in situ* generation of the phenoxide in an aprotic medium by desilylation of o-silyloxyphenyl ethynyl ketone with fluoride. The o-silyloxyphenyl ethynyl ketone 16 used for the cyclization studies was synthesized from the silyl-protected salicylic aldehyde 13 and readily available 14 (Scheme 1). Addition of bromomagnesium salt 14 to 13 gave benzyl alcohol 15. Oxidation of 15 with manganese dioxide ($\text{MnO}_2$) cleanly produced ethynyl ketone 16. Desilylation of 15 followed by oxidation with $\text{MnO}_2$ produced phenol 18.
Effects of Reaction Conditions for The 6-Endo-digonal Cyclization.

Various reaction conditions for the 6-endo-digonal cyclization were tested by using 16. Considering that the protonation of the vinyl anion is essential for the product formation, we first examined the commercially available THF solution of tetra-n-butylammonium fluoride (TBAF) containing approximately 5% (v/v) of water as a fluoride ion source. The reaction of 16 with TBAF in THF at 0 °C for 1.5 h produced both 19 and 20 in 90% yield with very low selectivity (19:20 = 47:53) (eq 3). In the early stage of the reaction the phenol 18 was detected on TLC indicating the existence of phenoxide ion under the conditions, which slowly underwent cyclization to 19 and 20. The structure of 19 was unambiguously confirmed by the HMBC spectrum indicating the hydrogen–carbon connectivities as shown in Figure 3. The stereochemistry of the exocyclic alkene in 20 was not confirmed by spectroscopic methods, but transition state for the 5-exo-digonal cyclization (TS–5) may suggest the preferential formation of Z-isomer.

To reduce the concentration of proton donor (e.g., water) in the reaction system, spray-dried potassium fluoride (KF) in the presence of 18-crown-6 was used for the fluoride source. The reaction of 16 with spray dried KF–18-crown-6 in anhydrous DMF proceeded smoothly giving 19 as a sole product in a quantitative yield (97%). The formation of 20 was not detected by 1H NMR analysis of the crude mixture.

Figure 3. The selected hydrogen–carbon connectivities observed in the HMBC spectrum of 19.
anion by moisture already contaminated in the reaction system. The observation that the cyclization of 16 became exceedingly slow when the reaction was carried out in the presence of activated molecular sieves 4A, may support in situ protonation of the resulting anion. On the other hand, the addition of a large excess of methanol (20% v/v) to the reaction mixture dramatically changed the reaction course giving 20 in 66% yield accompanied by the minor formation of 19 (13%). The formation of the substantial amount of 20 (9%) along with 19 (83%) by the cyclization of phenol 18 under KF–18-crown-6–DMF conditions revealed that even the phenolic hydrogen could be effective as a proton donor in the cyclization to result in a decrease of the selectivity for the formation of 19. These results indicated that the presence of only a small amount of proton donor like moisture in the reaction system plays a critical role not only in governing the selectivity but also in the smooth product formation. Evidence for the vinyl anion formation in the cyclization of 16 was obtained when the cyclization was carried out in DMF–CH$_3$OD (99 atom % D) (4:1) solution (eq 4). Both $d_1$-19 and $d_1$-20 formed in a ratio of 1:5 contained deuterium at the exocyclic olefinic position with the deuterium incorporation efficiency being more than 97% in both cases.

![Figure 4](image)

Figure 4. Selected $^1$H NMR spectra (5.5 – 8.5 ppm) of the crude mixture for the reaction of 16 under the KF–18-crown-6–DMF conditions after aqueous work-up (aq. NH$_4$Cl) at the indicated reaction time. The compounds were identified by the triplet-like signals of the olefinic hydrogen observed at 6.11 ppm for 16, 6.21 ppm for 18, 6.06 ppm for 19, and 5.90 ppm for 20. line a; 0 min, 16, line b; 10 min at -20 °C, line c: 30 min at -20 °C, line d: 1 h at -20 °C, line e; warming up to 0 °C after 1 h at -20 °C. * DMF

The time-course of the cyclization of 16 was monitored by $^1$H NMR spectroscopy. The reaction mixtures of 16 under KF–18-crown-6–DMF conditions at -20 °C were subjected to aqueous work-up at an indicated time interval and the $^1$H NMR of each of the crude mixture was recorded (Figure 4). The starting material 16 was no more detected after 10 min reaction, with phenol 18, benzopyranone 19, and benzofuranone 20 being observed in a ratio of 29:52:19 (line b). While after 30 min the ratio of three compounds reached to 9:54:37 (line c), upon prolonged reaction (1 h) phenol 18 was completely consumed,
with the ratio of 19 and 20 being 81:19 (line d). Warming the reaction mixture to 0 °C followed by aqueous work-up resulted in an almost exclusive formation of 19 (line e). The fact that the amount of the initially formed benzofuranone 20 decreased on a prolonged reaction with increase of benzopyranone 19 suggested that there was an equilibrium among either 18, 19, and 20 or their anions.

To identify the stage for the equilibration, we examined the interconversion between 19 and 20 under KF-18-crown-6-DMF conditions and found that both compounds were absolutely inert at ambient temperature under the conditions. It was also confirmed that pentacoordinate silicate having strong Lewis acidity formed in situ in the reaction did not induce the ring opening of 20. Thus, the reaction of a mixture of 16 and 20 (approximately 1:1) under KF-18-crown-6-DMF conditions afforded 19 with a complete recovery of 20 (eq 5). These experiments clearly indicated that protonation of benzopyranone and benzofuranone anions (e.g., 11 and 12, respectively) was an irreversible process under the conditions, and the equilibration should, therefore, exist at anion states. When the reaction of 16 under the KF-18-crown-6-DMF conditions was quenched with D2O before the equilibration is completed (e.g., after 15 min at -20 °C), it was confirmed that deuterium is efficiently incorporated into the exocyclic alkenic position of benzofuranone, while it was not the case for the benzopyranone (Figure 5). Thus, under these conditions protonation of the benzofuranone anion by a small amount of proton donor existing in the reaction system was much less efficient than that of benzopyranone anion.

![Figure 5](image-url)

Considering theoretical calculations and the experimental results obtained above, we can rationalize the cyclization reaction of o-hydroxyphenyl ethynyl ketones under basic conditions as illustrated in Scheme 2. Under the conditions where there is a sufficient amount of proton donor, the reaction produces varying amounts of both benzopyranone and benzofuranone anions 11 and 12, which are protonated to give 8 and 9, respectively. On the other hand, under the conditions where only a limited amount of the proton donor was available, all three anions 10, 11 and 12 are equilibrated. The most stable phenoxide anion 10 would be expected to be preferentially...
protonated to give phenol 7. However, under the basic reaction conditions, 7, if formed, would be equilibrated with 10 immediately. While the protonation of the benzofuranone anion 12 was relatively slow under the conditions, the benzopyranone anion 11 was immediately and irreversibly protonated. As a result of the equilibrium among these three anions and of the irreversible protonation of 11, highly selective formation of benzopyranone 8 was attained. However, at this moment we do not know the reason why the protonation of 12 is relatively slow compared with that for 11.

Scheme 2.

Synthesis of Various 2-Substituted Benzopyranones.

With an efficient synthetic method for the selective formation of benzopyranones in hand, we examined the reaction of phenyl ethynyl ketones 21, 22, 23, and 24 under KF-18-crown-6-DMF conditions. The epoxy-substituted ketones 22 and 23 were synthesized using epoxy alkynes prepared from commercially available (Z)- and (E)-3-methyl-2-penten-4-yn-1-ol, respectively, as detailed in experimental section. As expected benzopyranones 25, 26, 27, and 28 were selectively obtained in good to excellent yields as indicated in the parenthesis. The hydrolysis of THP group of 28 to the known 2-hydroxymethyl-4H-chromen-4-one gave further evidence for the structure. Under these conditions the stereochemical integrity for the carbon–carbon double bond and the epoxide moiety was completely retained.

To examine the feasibility of this synthetic method for kapurimycin A₁ synthesis, we investigated the construction of tricyclic ring system as a simple kapurimycin model. Ethynyl ketone 29 was prepared from 1-silyloxy-2-naphthaldehyde according to the procedure described for the synthesis of 16. The cyclization of 29 under KF-18-crown-6-DMF conditions at ambient temperature proceeded smoothly giving the tricyclic compound 30 in 81% yield (Scheme 3). These results clearly indicated that our method has a high potential for the synthesis of 1 and
its congeners to study the structure-reactivity relationship of kapurimycin A.

Scheme 3.

Experimental Section

General Procedures. Theoretical calculations were performed on SGI INDY (R4000SC personal workstation) with Spartan molecular modeling software (version 3.1) and Gaussian 92 program. $^1$H NMR spectra were measured with Varian GEMINI 200 (200 MHz), JEOL JNM $\alpha$-400 (400 MHz) and JEOL JNM $\alpha$-500 (500 MHz) spectrometers. Coupling constants ($J$ values) are reported in Hz. $^{13}$C NMR spectra were measured with Varian GEMINI 200 (50 MHz), JEOL JNM $\alpha$-400 (100 MHz) and JEOL JNM $\alpha$-500 (125 MHz) spectrometers. The chemical shifts are expressed in ppm downfield from tetramethylsilane, using residual chloroform ($\delta = 7.24$ in $^1$H NMR, $\delta = 77.0$ in $^{13}$C NMR) and dimethylsulfoxide ($\delta = 2.49$ in $^1$H NMR, $\delta = 39.5$ in $^{13}$C NMR) as an internal standard. The following abbreviations were used for the description of the signal multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. IR spectra were recorded on a JASCO FT/IR-5M spectrophotometer. Melting points were obtained on a Yanagimoto Seisakusho micro melting point apparatus and are uncorrected. Electron impact mass spectra (MS) and high-resolution mass spectra (HRMS) were recorded on JEOl JMS-DX 300 or JEOl JMS-SX 102A. Precoated TLC plates Merck silica gel 60 F$_{254}$ was used for monitoring the reactions and also for preparative TLC. Wako gel (C-200, particle size 75-150 $\mu$m, Wako) was used for silica gel flash chromatography. Anhydrous reactions were performed under N$_2$ atmosphere. Ether and tetrahydrofuran (THF) were distilled under N$_2$ from sodium/benzophenone ketyl prior to use. Yields refer to chromatographically and spectroscopically ($^1$H NMR) homogeneous materials, unless otherwise stated.
2-(t-Butyldimethylsilyloxy)benzaldehyde (13). To a solution of salicylaldehyde (1.21 g, 10.8 mmol) and 2,6-lutidine (1.75 mL, 15.0 mmol) in dichloromethane (30 mL) was added t-butyldimethylsilyl trifluoromethanesulfonate (3.50 mL, 15.2 mmol) at -78 °C, and the mixture was stirred at -78 °C for 2 h. After diluted with sat. aq. NaHCO₃, the reaction mixture was warmed to ambient temperature and extracted with ethyl acetate. The organic phase was washed with brine, dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (SiO₂, 2% ethyl acetate/hexane) to give 13 (2.26 g, 89%) as a yellow oil: ¹H NMR (400 MHz, CDCl₃) δ 10.45 (d, 1H, J = 1.0 Hz), 7.79 (dd, 1H, J = 1.9, 7.7 Hz), 7.44 (dd, 1H, J = 2.0, 7.3, 8.4 Hz), 7.02 (m, 1H), 6.87 (dd, 1H, J = 1.0, 8.3 Hz), 1.00 (s, 9H), 0.26 (s, 6H); IR (CHCl₃) 3016, 2957, 2932, 2860, 1684, 1600, 1478, 1256, 1217 cm⁻¹; MS m/e (%) 179 [(M-13ut) (56), 57 (100)]; HRMS calcld for C₉H₁₁O₂Si [M-13ut] [M-13ut] 179.0528; found, 179.0506.

(Z)-1-[2-(t-Butyldimethylsilyloxy)phenyl]-4-methyl-6-(2-tetrahydropranyloxy)-4-hexen-2-yn-1-ol (15). To a solution of (Z)-3-methyl-2-penten-4-yn-1-ol (5.30 g, 55.1 mmol) and 3,4-dihydro-2H-pyran (15.0 mL, 164 mmol) in dichloromethane (60 mL) was added a catalytic amount of pyridinium p-toluenesulfonate (PPTS) at 0 °C, and the mixture was stirred for 3 h. The reaction mixture was diluted with sat. aq. NaHCO₃, and extracted with ethyl acetate. The organic phase was washed with brine, dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (SiO₂, 10% ethyl acetate/hexane) to give (Z)-3-methyl-1-(2-tetrahydropranyloxy)-2-penten-4-yn (9.78 g, 98%) as a yellow oil: ¹H NMR (500 MHz, CDCl₃) δ 5.90 (m, 1H), 4.63 (dd, 1H, J = 3.1, 4.1 Hz), 4.38 (ddq, 1H, J = 1.4, 6.2, 12.6 Hz), 4.22 (ddq, 1H, J = 1.0, 7.2, 12.5 Hz), 3.87 (m, 1H), 3.50 (m, 1H), 3.13 (s, 1H), 1.88 (q, 3H, J = 1.3 Hz), 1.81 (m, 1H), 1.70 (m, 1H), 1.60-1.49 (4H); IR (CHCl₃) 3305, 3010, 2948, 2855, 1442, 1202, 1118, 1023 cm⁻¹; MS m/e (%) 180 (M⁺) (0.8), 149 (4), 85 (100), 79 (57); Anal. Calcld for C₁₁H₁₆O₂: C, 73.30; H, 8.95. Found: C, 73.02; H, 8.95. To a solution of ethylmagnesium bromide (0.47 mL, 1.41 mmol) in THF (5 mL) was added a solution of the above acetylene compound (0.25 g, 1.40 mmol) in THF (2 mL) at 0 °C and the mixture was stirred at 50 °C for 1.5 h to give bromomagnesium salt 14. A solution of 13 (0.34 g, 1.44 mmol) in THF (2 mL) was added to the solution of 14 at ambient temperature and the whole mixture was stirred for 1 h. The reaction mixture was diluted with sat. aq. NH₄Cl and extracted with ethyl acetate. The organic phase was washed with brine, dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (SiO₂, 15% ethyl acetate/hexane) to give 15 (0.37 g, 64%) as a colorless oil: ¹H NMR (500 MHz, CDCl₃) δ 5.90 (m, 1H), 4.63 (dd, 1H, J = 3.1, 4.1 Hz), 4.38 (ddq, 1H, J = 1.4, 6.2, 12.6 Hz), 4.22 (ddq, 1H, J = 1.0, 7.2, 12.5 Hz), 3.87 (m, 1H), 3.50 (m, 1H), 3.13 (s, 1H), 1.88 (q, 3H, J = 1.3 Hz), 1.81 (m, 1H), 1.70 (m, 1H), 1.60-1.49 (4H); IR (CHCl₃) 3305, 3010, 2948, 2855, 1442, 1202, 1118, 1023 cm⁻¹; MS m/e (%) 415 [(M-H)⁺] (2), 398 [(M-H₂O)⁺] (3), 314 (46), 257 (98), 85 (66), 75 (100); Anal. Calcld for C₂₄H₃₆O₄Si: C, 69.19; H, 8.71. Found: C, 69.05; H, 8.64.
(Z)-1-(2-(t-Butyldimethylsilyloxy)pheny1)-4-methyl-6-(2-tetrahydropyranloxy)-4-hexen-2-yn-1-one (16). To a solution of 15 (0.92 g, 2.20 mmol) in dichloromethane (30 mL) was added manganese dioxide (2.0 g) and the mixture was stirred for 3 h at ambient temperature. The reaction mixture was diluted with ethyl ether, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (SiO₂, 15% ethyl acetate/hexane) to give 11 (0.89 g, 97%) as a yellow oil:

**1H NMR** (500 MHz, CDCl₃) δ 7.93 (dd, 1H, J = 1.7, 7.8 Hz), 7.38 (ddd, 1H, J = 1.8, 7.3, 8.3 Hz), 7.00 (m, 1H), 6.87 (dd, 1H, J = 0.8, 8.3 Hz), 6.11 (m, 1H), 4.62 (dd, 1H, J = 3.1, 4.2 Hz), 4.43 (ddq, 1H, J = 1.3, 6.2, 12.9 Hz), 4.28 (ddd, 1H, J = 1.0, 7.3, 12.8 Hz), 3.83 (m, 1H), 3.46 (m, 1H), 1.97 (d, 3H, J = 1.2 Hz), 1.79 (m, 1H), 1.69 (m, 1H), 1.57–1.50 (4H), 0.99 (s, 9H), 0.21 (s, 6H); **IR** (CHCl₃) 3012, 2952, 2860, 2189, 1643, 1478, 1447, 1257, 1233, 1022, 914, 841, 772, 759, 748 cm⁻¹; **MS** m/e (%) 357 [(M-13BuO)] (26), 273 (100), 235 (98). 85 (74).

(Z)-1-(2-Hydroxyphenyl)-4-methyl-6-(2-tetrahydropyranloxy)-4-hexen-2-yn-1-ol (17). To a solution of 15 (33.5 mg, 80.4 pmol) in THF (1 mL) was added tetrabutylammonium fluoride (80 µL, 1.0 M in THF, 80.0 pmol) at 0 °C and the mixture was stirred at ambient temperature for 20 min. The reaction mixture was diluted with sat. aq. NH₄Cl and extracted with ethyl acetate. The organic phase was washed with brine, dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (SiO₂, 15% ethyl acetate/hexane) to give 16 (23.0 mg, 95%) as a yellow oil: **1H NMR** (500 MHz, CDCl₃) δ 11.67 (s, 1H), 8.00 (dd, 1H, J = 1.7, 8.0 Hz), 7.49 (ddd, 1H, J = 1.7, 7.1, 8.5 Hz), 6.97 (m, 1H), 6.93 (ddd, 1H, J = 1.1, 7.3, 8.0 Hz), 6.21 (m, 1H), 4.72 (t, 2H, J = 3.2 Hz), 4.35–4.21 (2H), 3.86 (m, 1H), 3.51 (m, 1H), 2.03 (q, 3H, J = 1.3 Hz), 1.81 (m, 1H), 1.72 (m, 1H), 1.62–1.49 (4H); **IR** (CHCl₃) 2949, 2191, 1597, 1243, 1022 cm⁻¹; **MS** m/e (%) 284 [(M-H₂O)] (7), 200 (97), 171 (38), 84 (91), 55 (100).

(Z)-2-[1-(2-Tetrahydropyranloxy)buten-3-yl]-4H-chromen-4-one (19). To a solution of 16 (20.3 mg, 49.0 µmol) and 18-crown-6 (26.2 mg, 99.1 µmol) in N,N-dimethylformamide (1 mL) was added spray dried potassium fluoride (5.7 mg, 98.1 µmol) at 0 °C and the mixture was stirred at ambient temperature for 2 h. The reaction mixture was diluted with sat. aq. NH₄Cl and extracted with ethyl acetate. The organic phase was washed with brine, dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (SiO₂, 15% ethyl acetate/hexane) to
give 19 (14.2 mg, 97 %) as a yellow oil: 1H NMR (500 MHz, CDCl3) δ 8.17 (ddd, 1H, J = 0.5, 1.7, 7.9 Hz), 7.65 (ddd, 1H, J = 1.7, 7.2, 8.4 Hz), 7.43 (ddd, 1H, J = 0.5, 1.1, 8.4 Hz), 7.38 (dd, 1H, J = 1.0, 7.0, 8.0 Hz), 6.28 (s, 1H), 6.06 (m, 1H), 4.65 (m, 1H), 4.62 (ddq, 1H, J = 1.7, 5.7, 14.5 Hz), 4.41 (ddq, 1H, J = 1.4, 6.2, 14.3 Hz), 3.85 (m, 1H), 3.49 (m, 1H), 2.09 (q, 3H, J = 1.5 Hz), 1.81 (m, 1H), 1.72 (m, 1H), 1.60–1.51 (4H); 13C NMR (50 MHz, CDCl3) δ 178.7, 164.1, 156.3, 135.4, 133.9, 129.2, 125.8, 125.3, 123.9, 118.1, 110.7, 98.7, 98.5, 65.0, 62.4, 30.5, 25.2, 21.0, 19.3; IR (CHCl3) 3012, 2949, 2873, 2855, 1649, 1642, 1567, 1444, 1383, 1212, 1132, 1024 cm⁻¹; MS m/e (%): 300 (M⁺), 216 (100) [(M-THP+H⁺)], 202 (79), 199 (75), 187 (63), 121 (83), 85 (79); HRMS calcd for C₁₃H₁₂O₃ [(M-THP+H⁺)], 216.0787; found, 216.0806.

2-[2-Methyl-4-(2-tetrahydropyranyloxy)-2-butenylidenyl]benzofuran-3-one (20). To a solution of 16 (14.4 mg, 34.7 pmol) in tetrahydrofuran (1 mL) was added a tetrahydrofuran solution of tetra-n-butylammonium fluoride (35 μL, 1 M in tetrahydrofuran, 35 pmol) at 0 °C and the mixture was stirred for 90 min at that temperature. The reaction mixture was diluted with sat. aq. NH₄Cl and extracted with ethyl acetate. The organic phase was washed with brine, dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (SiO₂, 25% ethyl acetate/hexane) to give 20 (5.0 mg, 48%) as a colorless oil accompanied with 19 (4.4 mg, 42%). 20: 1H NMR (400 MHz, CDCl₃) δ 7.75 (dd, 1H, J = 0.6, 1.4, 7.7 Hz), 7.61 (dd, 1H, J = 1.4, 7.3, 8.5 Hz), 7.21 (dt, 1H, J = 0.7, 8.3 Hz), 7.17 (dt, 1H, J = 0.7, 8.4 Hz), 6.83 (d, 1H, J = 0.9 Hz), 5.90 (m, 1H), 4.66 (dd, 1H, J = 3.1, 4.3 Hz), 4.46 (ddq, 1H, J = 1.4, 6.4, 13.3 Hz), 4.26 (ddq, 1H, J = 1.1, 7.3, 13.3 Hz), 3.89 (m, 1H), 3.54 (m, 1H), 2.24 (q, 3H, J = 1.3 Hz), 1.82 (m, 1H), 1.72 (m, 1H), 1.64–1.48 (4H); 13C NMR (50 MHz, CDCl₃) δ 185.14, 166.28, 147.29, 136.99, 135.70, 132.46, 124.71, 123.36, 121.66, 112.96, 109.62, 98.38, 63.44, 62.31, 30.42, 25.23, 22.51, 19.29; IR (CHCl₃) 3020, 2947, 1717, 1606, 1462, 1301 cm⁻¹; MS m/e (%): 216 [M-THP+H⁺]²⁄³ (23), 185 (37), 134 (100).

Internal quenching with DMF–CH₃OD. The reaction of 16 (33.3 mg, 0.08 mmol) with potassium fluoride (9.3 mg, 0.15 mmol), 18-crown-6 (44.6 mg, 0.17 mmol) in N,N-dimethylformamide (2 mL) and methanol-d (0.5 mL, 99 atm% D) was carried out for 10 min at ambient temperature and worked-up as usual. Integration of the signal at 6.83 ppm for 20 in 1H NMR showed the deuterium content of the produced 20 was 97%. More than 97% deuterium content for 19 was determined by the disappearance of the olefine hydrogen in 1H NMR. Chromatographic separation afforded d₁-19 (2.7 mg, 11%) and d₁-20 (11.6 mg, 48%). d₁-19: MS m/e (%): 301 (1) (M⁺), 217 (100) [(M-THP+H⁺)], 202 (79), 201 (80), 188 (56); HRMS calcd for C₁₃H₁₁O₃D [M-THP+H⁺], 217.0849; found, 217.0769. d₁-20: MS m/e (%): 301 (1) (M⁺), 217 (28) [(M-THP+H⁺)], 186 (41); HRMS calcd for C₁₃H₁₁O₃D [(M-THP+H⁺)], 217.0849; found, 217.0779.

Time-course of the cyclization of 16. Four reactions of 16 under the standard KF–18-crown-6–DMF conditions (see the procedure for preparation of 19) were carried out at -20 °C, and three of four reactions were quenched by adding sat. aq. NH₄Cl after 10, 30, and 60 min at that temperature. The remaining reaction was warmed up to 0 °C after 60 min at -20 °C and quenched as previous. Each reaction mixture was extracted as for the preparation of 19 to give a crude.
mixture, which was analyzed by $^1$H NMR in CDC$_1$.$_3$. The result was shown in Figure 4.

**Cyclization of 16 in the presence of 21.** In a NMR tube a solution of DMF-d$_7$ (1 mL) containing 16 (5.0 mg, 0.012 mmol), 21 (3.6 mg, 0.012 mmol), and 18-crown-6 (12.8 mg, 0.048 mmol) was prepared and $^1$H NMR of the starting mixture was recorded. To the solution was added potassium fluoride (2.8 mg, 0.048 mmol) at room temperature and the mixture was sonicated for 10 min. $^1$H NMR spectrum of the resulting dark brown solution was then recorded.

D$_2$O quenching before the equilibration is completed. The reaction of 16 described for the time-course experiment was quenched by adding D$_2$O-ND$_4$Cl solution after 15 min at -20 °C. The resulting mixture was worked-up as usual and $^1$H NMR spectrum of the crude product was recorded in CDC$_1$.$_3$. The result was shown in Figure 5.

(E)-1-[2-(t-Butyldimethylsilyloxy)phenyl]-4-methyl-6-(2-tetrahydropranyloxy)-4-hexen-4-en-1-ol (21). According to the method described in the synthesis of 15 the reaction of (E)-3-methyl-2-penten-4-yn-1-ol (5.29 g, 55.0 mmol) gave (E)-3-methyl-1-(2-tetrahydropyranyloxy)-4-pentyn-2-ene (7.87 g, 79%) as a colorless oil: $^1$H NMR (400 MHz, CDC$_1$.$_3$) $\delta$ 6.04 (m, 1H), 4.61 (m, 1H), 4.26 (dd, 1H, $J = 6.2, 12.5$ Hz), 4.10 (dd, 1H, $J = 7.1, 13.2$ Hz), 3.84 (m, 1H), 3.50 (m, 1H), 2.71 (d, 1H, $J = 5.6$ Hz), 1.83 (d, 3H, $J = 1.5$ Hz), 1.81-1.49 (m, 6H), 1.02 (s, 9H), 0.29 and 0.26 (s×2, total 6H); IR (CHCl$_3$) 3592, 3015, 2954, 2860, 1488, 1454, 1258, 1023, 912, 840, 745 cm$^{-1}$; MS m/e (%) 359 [(M-'Bu]+) (3), 315 (12), 275 (19), 257 (35), 179 (100). Oxidation of this alcohol (1.16 g, 2.79 mmol) with manganese dioxide (ca. 2 g) produced 21 (1.05 g, 91%) as a yellow oil: $^1$H NMR (400 MHz, CDC$_1$.$_3$) $\delta$ 7.90 (dd, 1H, $J = 1.9, 7.8$ Hz), 7.38 (dd, 1H, $J = 1.8, 7.2, 8.1$ Hz), 7.00 (dd, 1H, $J = 1.1, 7.3, 7.8$ Hz), 6.86 (dd, 1H, $J = 1.1, 8.2$ Hz), 6.29 (m, 1H), 4.62 (t, 1H, $J = 3.4$ Hz), 4.32 (ddq, 1H, $J = 1.1, 0.9, 6.1, 14.0$ Hz), 4.15 (dd, 1H, $J = 0.9, 7.0, 13.9$ Hz), 3.84 (dd, 1H, $J = 3.2, 8.3, 11.6$ Hz), 3.51 (m, 1H), 1.90 (d, 3H, $J = 1.2$ Hz), 1.80 (m, 1H), 1.70 (m, 1H), 1.62-1.49 (4H), 0.99 (s, 9H), 0.21 (s, 6H); IR (CHCl$_3$) 3016, 2953, 2860, 2190, 1642, 1478, 1448, 1256, 910, 840, 761 cm$^{-1}$; MS m/e (%) 357 [(M-'Bu]+) (12), 273 (8), 245 (46), 203 (24), 179 (17), 149 (18), 85 (100).

(4R*, 5R*)-1-[2-(t-Butyldimethylsilyloxy)phenyl]-4,5-epoxy-6-(1-ethoxyethyloxy)-4-methyl-2-hexyn-1-one (22). To a solution of (Z)-3-methyl-2-penten-4-yn-1-ol (5.21 g, 26.2 mmol) and disodium hydrogenphosphate (9.06 g, 63.8 mmol) in dichloromethane
(100 mL) was added 3-chloroperbenzoic acid (9.08 g, 52.6 mmol) at 0 °C and the mixture was stirred at ambient temperature for 20 h. The mixture was diluted with sat. aq. Na₂SO₃ and sat. aq. NaHCO₃, and extracted with ethyl acetate. The organic phase was washed with brine, dried over anhydrous Na₂SO₃, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (SiO₂, 30% ethyl acetate/hexane) to give (2S*, 3R*)-2,3-epoxy-3-methyl-4-pentyne-1-ol (2.45 g, 84%) as a colorless needle: ¹H NMR (500 MHz, CDCl₃) δ 3.91 (dd, 1H, J = 4.6, 12.3 Hz), 3.82 (dd, 1H, J = 6.2, 12.4 Hz), 3.08 (dd, 1H, J = 4.7, 6.1 Hz), 2.38 (s, 1H), 1.76 (br, 1H), 1.57 (s, 3H), 1.56, 1H, 1.19 (t, 1H, J = 7.1 Hz); IR (CHCl₃) 3305, 3015, 1440, 1377, 1326, 1225 cm⁻¹; MS m/e (%) 318 (M⁺, (5), 95 (13), 73 (100); Anal. Caled for C₁₀H₁₆O₃: C, 64.27; H, 7.19. Found C, 65.02; H, 8.75. To a solution of this compound (0.92 g, 5.01 mmol) in tetrahydrofuran (5 mL) was added n-butyl lithium (3.10 mL, 1.62 M in hexane, 5.02 mmol) at −78 °C and the mixture was stirred at −78 °C for 15 min. After addition of a solution of 13 (1.18 g, 5.01 mmol) in tetrahydrofuran (5 mL) was added at −78 °C the mixture was stirred for 2 h. The mixture was diluted with sat. aq. NH₄Cl and extracted with ethyl acetate. The organic phase was washed with brine, dried over anhydrous Na₂SO₃, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (SiO₂, 15–50% ethyl acetate/hexane) to give (4R*, 5R*)-1-[2-(t-butyldimethylsilyloxy)phenyl]-4,5-epoxy-6-(1-ethoxyethoxy)-4-methyl-2-hexyn-1-ol (77% as a mixture of four diastereomeric isomers) as a colorless oil: ¹H NMR (400 MHz, CDCl₃) δ 7.51 (m, 1H), 7.18 (m, 1H), 6.95 (m, 1H), 6.80 (m, 1H), 5.72 (m, 1H), 4.73 (m, 1H), 3.85–3.58 (3H), 3.45 (m, 1H), 3.08 (m, 1H), 2.86–2.66 (1H), 1.56 (m, 3H), 1.28 (m, 3H), 1.16 (m, 3H), 1.01 (s, 9H), 0.27 (m, 6H); IR (CHCl₃) 2955, 1488, 1259 cm⁻¹; MS m/e (%) 347 (42), 327 (9), 307 (25), 273 (100), 243 (88), 179 (100); Anal. Caled for C₁₅H₂₄O₄Si: C, 65.68; H, 8.63. Found C, 65.77; H, 8.73. Oxidation of this alcohol (41.0 mg, 97.5 µmol) with manganese dioxide gave 22 (30.7 mg, 75%) as a yellow oil: ¹H NMR (400 MHz, CDCl₃) δ 7.90 (dd, 1H, J = 1.8, 7.9 Hz), 7.40 (ddd, 1H, J = 1.8, 7.3, 8.3 Hz), 7.00 (m, 1H), 6.87 (ddd, 1H, J = 0.4, 1.1, 8.3 Hz), 4.74 (q, 1H, J = 5.3 Hz), 3.88–3.59 (3H), 3.46 (m, 1H), 3.19 (m, 1H), 1.64 (s, 3H), 1.30 (d, 3H, J = 5.3 Hz), 1.15 and 1.14 (t×2, total 3H, J = 7.1 Hz), 1.00 (s, 9H), 0.22 (s, 6H); IR (CHCl₃) 2932, 1650, 1478, 1256 cm⁻¹; MS m/e (%) 418 (M⁺) (0.3), 289 (41), 235 (48), 152 (72), 143 (87), 121 (100).

(4S*, 5R*)-1-[2-(t-Butyldimethylsilyloxy)phenyl]-4,5-epoxy-6-(1-ethoxyethoxy)-4-methyl-2-hexyn-1-one (23). According to the method described in the synthesis of 22 the reaction of (E)-3-methyl-2-penten-4-yn-1-ol (2.51 g, 26.1 mmol) gave (2R*, 3R*)-2,3-
epoxy-3-methyl-4-pentyn-1-ol (2.16 g, 74%) as a colorless oil: \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 3.83 (dd, 1H, \(J = 4.4, 12.4\) Hz), 3.69 (dd, 1H, \(J = 6.2, 12.4\) Hz), 3.36 (ddd, 1H, \(J = 4.5, 6.2\) Hz), 2.31 (s, 1H), 1.78 (br, 1H), 1.54 (s, 3H); IR (CHCl\(_3\)) 7.17. The alcohol group to give pentyne (2.46 g, 88%) as a colorless oil:

\(\begin{align*}
4.75 (q, 1H, \(J = 5.4\) Hz), 3.68-3.44 (4H), 3.34 (t, 1H, \(J = 1.8, 7.3, 8.2\) Hz), 0.99 (s, 9H), 0.28 and 0.26 (sx2, total 3H), 0.21 (s, 6 H); IR (CHCl\(_3\)) 2933, 2860, 1480, 1258, 1221, 1025, 733 cm\(^{-1}\); MS \(m/e (\%)\) 403 [(M-Me)\(^+\)] (2), 361 (67), 289 (31), 259 (25), 201 (100), 179 (54).
\end{align*}\)

1-[2-(t-Butyldimethylsilyloxy)phenyl]-4-(2-tetrahydropyranyloxy)-2-butyn-1-one (24). To a solution of 1-(2-tetrahydropyranyloxy)-2-propyne (0.33 g, 2.35 mmol) in THF (15 mL) was added n-butyl lithium (1.5 mL, 1.62 M in hexane, 2.43 mmol) at -78 °C and the mixture was stirred at -78 °C for 15 min. To the resulting alkynyllithium solution, tetrahydrofuran (4 mL) solution of \(\text{13} (0.55 g, 2.35\text{ mmol})\) was added at -78 °C and the mixture was stirred for 2 h. The reaction mixture was quenched with sat. aq. NH\(_4\)Cl and extracted with ethyl acetate. The organic phase was washed with brine, dried over anhydrous Na\(_2\)SO\(_4\), filtered, and concentrated in vacuo.

The crude product was purified by flash chromatography (SiO\(_2\), 15-50% ethyl acetate/hexane) to give 1-[2-(t-butyldimethylsilyloxy)phenyl]-4-(2-tetrahydropyranyloxy)-2-butyn-1-ol (0.60 g, 68%) as a colorless oil: \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.54 (dd, 1H, \(J = 1.6, 7.5\) Hz), 7.18 (dt, 1H, \(J = 1.8, 7.8\) Hz), 6.96 (dt, 1H, \(J = 1.1, 7.5\) Hz), 6.81 (m, 1H), 5.73 (m, 1H), 4.79 (t, 1H, \(J = 3.3\) Hz), 4.38-4.25 (2H), 3.81 (m, 1H), 3.49 (m, 1H), 2.73 (d, 1H, \(J = 5.8\) Hz), 1.85–1.46 (m, 6H), 1.01 (s, 9H), 0.28 and 0.26 (sx2, total 3H); IR (CHCl\(_3\)) 3010, 2951, 2860, 1480, 1258, 1221, 1025, 919, 840, 753 cm\(^{-1}\); MS \(m/e (\%)\) 275 (2), 236 (9), 217 (13), 179 (39), 85 (100); Anal. Calcd. for C\(_{21}\)H\(_{32}\)O\(_4\)Si: C, 66.98; H, 8.57. Found C, 66.71; H, 8.71. Oxidation of this alcohol (0.42 g, 1.12 mmol) with manganese dioxide produced 24 (0.29 g, 69%) as a yellow oil: \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.90 (dd, 1H, \(J = 1.9, 7.9\) Hz), 7.38 (ddd, 1H, \(J = 1.9, 7.3, 9.1\) Hz), 7.00 (m, 1H), 6.86 (dd, 1H, \(J = 0.9, 8.3\) Hz), 4.75 and 4.74 (q\(\times2\), total 1H, \(J = 5.3\) Hz), 3.71–3.57 (3H), 3.48 (m, 1H), 3.45 (t, 1H, \(J = 5.4\) Hz), 1.60 (s, 3H), 1.31 (dx2, total 3H, \(J = 5.4\) Hz), 1.19 (t, 3H, \(J = 7.0\) Hz), 0.99 (s, 9H), 0.21 (s, 6 H); IR (CHCl\(_3\)) 2933, 2860, 1480, 1258, 1221, 1025, 919, 840, 753 cm\(^{-1}\); MS \(m/e (\%)\) 391 [(M-Me)\(^+\)] (2), 361 (67), 289 (31), 259 (25), 201 (100), 179 (54).
The same procedure described for the synthesis of 19 was applied for 22 (11.7 mg, 84%) as a yellow oil; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.15 (dd, 1H, J = 1.6, 8.0 Hz), 7.65 (dd, 1H, J = 1.7, 7.2, 8.7 Hz), 7.43 (m, 1H), 7.38 (m, 1H), 6.41 (s, 1H), 4.79, 4.78 (q×2, total 1H, J = 5.4 Hz), 3.97–3.62 (3H), 3.50 (m, 1H), 3.37 (dd, 1H, J = 5.1, 5.5 Hz), 1.71 and 1.68 (sx2, total 3H), 1.34 (d, 3H, J = 5.4 Hz), 1.19 (t, 3H, J = 7.1 Hz); IR (CHCl\(_3\)) 3011, 2990, 1609, 1466, 1383, 1131 cm\(^{-1}\); MS m/e (%) 259 [(M–OEt)\(^+\)] (5), 232 (16), 214 (100), 189 (54), 171 (50).

2-[(2S*, 3R*)-2,3-epoxy-1-(1-ethoxyethyloxy)butane-3-yl]-4H-chromen-4-one (27). The same procedure described for the synthesis of 19 was applied for 23 (53.5 mg, 0.13 mmol) to afford 27 (28.0 mg, 72%) as a yellow oil; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.15 (dd, 1H, J = 1.6, 8.0 Hz), 7.65 (dd, 1H, J = 1.7, 7.2, 8.7 Hz), 7.43 (m, 1H), 7.38 (m, 1H), 6.41 (s, 1H), 4.79, 4.78 (q×2, total 1H, J = 5.4 Hz), 3.97–3.62 (3H), 3.50 (m, 1H), 3.37 (dd, 1H, J = 5.1, 5.5 Hz), 1.71 and 1.68 (sx2, total 3H), 1.34 (d, 3H, J = 5.4 Hz), 1.19 (t, 3H, J = 7.1 Hz); IR (CHCl\(_3\)) 3011, 2990, 1609, 1466, 1383, 1131 cm\(^{-1}\); MS m/e (%) 259 [(M–OEt)\(^+\)] (5), 232 (16), 214 (100), 189 (54), 171 (50).

2-[(2-tetrahydropyranloxy)methyl]-4H-chromen-4-one (28). The same procedure described for the synthesis of 19 was applied for 24 (40.7 mg, 0.11 mmol) to afford 28 (20.6 mg, 73%) as a yellow oil; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.17 (dd, 1H, J = 1.7, 7.9 Hz), 7.65 (dd, 1H, J = 1.7, 7.2, 8.7 Hz), 7.42 (dd, 1H, J = 0.6, 8.5 Hz), 7.38 (dd, 1H, J = 0.8, 7.1, 8.1 Hz), 6.46 (t, 1H, J = 0.9 Hz), 4.78 (t, 1H, J = 3.3 Hz), 4.62 (dd, 1H, J = 1.0, 15.0 Hz), 4.44 (dd, 1H, J = 0.8, 14.9 Hz), 3.85 (m, 1H), 3.55 (m, 1H), 1.90–1.51 (6H); \(^1\)C NMR (50 MHz, CDCl\(_3\)) \(\delta\) 178.5, 165.9, 156.5, 133.8, 125.9, 125.2, 124.2, 118.1, 109.4, 98.3, 64.6, 62.0, 30.0, 25.1, 18.6; IR (CHCl\(_3\)) 3020, 3008, 2949, 2885, 1651, 1467, 1215, 1122, 1036, 745 cm\(^{-1}\); MS m/e (%) 205 (6), 176 [(M–THP+H)\(^+\)] (37), 160 (100), 85 (79); Anal. Calcd. for C\(_{10}\)H\(_8\)O\(_3\); C, 69.22; H, 6.20. Found: C, 69.09; H, 6.19; HRMS calcd for C\(_{10}\)H\(_8\)O\(_3\) [(M–THP+H)\(^+\)], 176.0474; found, 176.0485. Hydrolysis of 28 (10.5 mg, 40.3 \(\mu\)mol) in refluxing acetone (0.6 mL) and water (0.2 mL) containing a catalytic amount of pyridinium p-toluenesulfonate gave 2-hydroxymethyl-4H-chromen-4-one (6.0 mg, 84%) as a white powder; \(^1\)H NMR (200 MHz, DMSO-\(d_6\)) \(\delta\) 8.02 (dd, 1H, J = 1.8, 8.2 Hz), 7.79
(dd, 1H, J = 1.7, 7.0, 8.5 Hz). 7.60 (dd, 1H, J = 1.0, 8.5 Hz). 7.47 (dd, 1H, J = 1.1, 7.1, 8.1 Hz). 6.33 (s, 1H). 5.79 (br, 1H). 4.44 (s, 2H); 'C NMR (50 MHz, DMSO-d6) δ 177.14. 170.09. 155.97. 144.40. 125.54. 125.13. 123.62. 118.38. 107.44. 59.81. These data were identical with those reported.

1-[2-(1-Butyldimethylsiloxy)naphthyl]-4-(2-tetrahydropranyloxy)-2-butyn-1-one (29). To a solution of ethylmagnesium bromide (3 M in ethyl ether, 0.26 ml, 0.78 mmol) in THF (4 mL) was added a solution of 1-(2-tetrahydropyranyloxy)-2-propyne (98.8 mg, 0.70 mmol) in THF (1 mL) at 0°C and the mixture was stirred at 50°C for 1 h. After the reaction mixture was cooled to ambient temperature, a solution of 2-(1-t-butyldimethylsiloxy)naphthaldehyde (0.22 g, 0.77 mmol) in THF (1 mL) was added and the whole mixture was stirred at ambient temperature for 20 min. The mixture was diluted with sat. aq. NH4Cl and extracted with ethyl acetate. The organic phase was washed with brine, dried over anhydrous Na2SO4, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (SiO2, 15% ethyl acetate/hexane) to give 29 (46.8 mg, 73%) as a yellow oil: 'H NMR (400 MHz, CDCl3) δ 8.24 (m, 1H). 7.90 (d, 1H, J = 8.8 Hz). 7.78 (m, 1H). 7.56 (dd, 1H, J = 1.3, 6.9, 8.1 Hz). 7.49 (dd, 1H, J = 1.5, 8.4 Hz). 7.48 (m, 1H). 4.85 (t, 1H, J = 3.2 Hz). 4.50 (s, 2H). 3.84 (dd, 1H, J = 3.2, 9.3, 12.1 Hz). 3.53 (m, 1H). 1.84-1.50 (m, 6H). 1.12 (s, 9H). 0.10 (s×2, 6H, J = 0.7 Hz); IR (CHCl3) 3021, 2952, 2932, 1649, 1619, 1395, 1231, 1122, 827, 725 cm⁻¹; MS m/e (%) 426 [(M-Me)⁺] (7), 367 (99), 283 (97), 267 (99), 239 (100); HRMS calcd for C23H29O4Si [(M-Me)⁺] 409.1835; found, 409.1826.

2-[2-(tetrahydropranyloxy)methyl]-4H-naphtho[1,2-b]pyran (30). To a solution of 29 (89.5 mg, 0.21 mmol) and 18-crown-6 (137 mg, 0.52 mmol) in N,N-dimethylformamide (3 mL) was added a potassium fluoride (24.6 mg, 0.52 mmol) and the mixture was stirred for 36 h at ambient temperature. The reaction mixture was diluted with ethyl ether, filtered and concentrated in vacuo. The crude product was purified by flash chromatography (SiO2, 15% ethyl acetate/hexane) to give 30 (53.3 mg, 81%) as a yellow oil: 'H NMR (400 MHz, CDCl3) δ 8.45 (m, 1H). 8.13 (d, 1H, J = 8.6 Hz). 7.92 (m, 1H). 7.76 (d, 1H, J = 8.6 Hz). 7.69 (m, 1H). 7.65 (m, 1H). 6.62 (s, 1H). 4.85 (t, 1H, J = 3.2 Hz). 4.78 (dd, 1H, J = 0.8, 14.7 Hz). 4.61 (d, 1H, J = 14.7 Hz). 3.90 (dd, 1H, J = 3.1, 9.3, 12.4 Hz). 3.59 (m, 1H). 1.91-1.53 (6H); IR (CHCl3) 3021, 2358,
1652, 1212, 774 cm$^{-1}$; MS m/e (%) 310 (M$^+$) (100), 254 (38), 226 (61), 210 (98), 181 (85); HRMS calcd for C$_{19}$H$_{18}$O$_4$ (M$^+$) 310.1203; found, 310.1183.

References and Notes


(18) All semiempirical and *ab initio* calculations were carried out using Spartan molecular modeling software (version 3.1).
(20) The energy gap between the two conformers calculated at the 3-21G(*) level became large (4.72 kcal/mol), with the *s*-trans conformer being more stable.
(22) Analyses of the vibrational frequencies were carried out using Gaussian 92 program. Frisch, M. J.; Trucks, G. W.; Head-Gordon, M.; Gill, P. M. W.; Wong, M. W.; Foresman, J. B.; Johnson, B. G.; Schlegel, H. B.; Robb, M. A.; Repogle, E. S.; Gomperts, R.; Andres, J. L.; Raghavachari, K.; Binkley, J. S.; Gonzalez, C.; Martin, R. L.; Fox, D. J.; Defrees, D. J.; Baker, J.; Stewart, J. J. P.; Pople, J. A. Gaussian. Inc. Pittsburgh PA, 1992.
CHAPTER 2

Essential Structure for Efficient DNA Alkylation by Kapurimycin A₃

Abstract: An antibiotic kapurimycin A₃ (1) is well-known to efficiently alkylate DNA guanine bases. In order to know the origin of effective guanine alkylation, we have investigated the relationship between the structure of 1 and the activity for DNA guanine alkylation by 1. One of the kapurimycin A₃ analogs, ABC-ring analog 2, was prepared through the coupling of BC-ring fragment with optically active side chain fragment synthesized by the use of Sharpless asymmetric dihydroxylation followed by 6-endo-dig selective cyclization of o-silyloxyphenyl ethynyl ketone and intramolecular Mitsunobu reaction for epoxide formation. Based on the results of DNA cleavage by ABC-ring analog 2, diol 15, AB-ring analog 16 and epoxyalcohol 17, we found that 2 has a minimum structure required for effective DNA alkylation. Besides, the reactivity of 2 toward DNA was about one tenth of that for 1. The enzymatic digestion of DNA modified by 2 showed that DNA modification by 2 selectively occurred at guanine bases. We also isolated and identified the 2-guanine adduct by NMR and MS spectroscopy. In the cleaving assay of ³²P-5'-end-labeled DNA, ABC-ring analog 2 showed a very similar sequence selectivity to that for natural kapurimycin A₃ (G*G > G*A > G*T > G*C, G* denotes alkylated guanine).
Introduction

Kapurimycin A, \(1\) is an antitumor antibiotic isolated by Kyowa Hakko Kogyo group in 1990. Kapurimycin A consists of a tetrahydroanthrapyranone ring and a vinyl epoxide side chain attached to C2-position of the pyranone ring system. By our group and others, a guanine-kapurimycin A adduct, obtained from the reaction of DNA with \(1\), was isolated and identified, and it was shown kapurimycin A alkylates guanine base in DNA duplex.

Antibiotics which modify guanine base in DNA duplex are typically represented by aflatoxin B\(_1\) oxide and altromycin B. In several studies on these DNA alkylating antibiotics, the binding structure of DNA-drug complex was investigated by NMR spectroscopy. All of these antibiotics intercalate into DNA in their aromatic rings and subsequently generate guanine-adduct with nucleophilic attack of guanine N7 to the reactive epoxide group of the drugs. Kapurimycin A, \(1\) has an aromatic ring system and an epoxy subunit like other drugs and alkylates guanine base.

We were interested in the structure of DNA natural alkylating agents and investigated a minimal structure required for efficient alkylation of guanine base. In this study, we focused our attention on kapurimycin A, \(1\) and have synthesized a diverse type of kapurimycin analogs. Herein we report that ABC ring analog 2 effectively alkylates DNA guanine base with a very similar sequence selectivity to that of natural kapurimycin A, \(1\).

Results and Discussion

The synthetic route for ABC-ring analog \(2\) is outlined in Scheme 1. Asymmetric dihydroxylation of p-methoxybenzyl ether \(4\) obtained from a commercially available alcohol \(3\) with AD-mix-\(\beta\) produced diol \(5\) which was converted to alcohol \(8\). Oxidation of \(8\) and Wittig olefination provided the alkene \(10\) \((Z:E = 5:1)\) which was then desilylated to \(11\). Addition of 1-tert-butyldimethylsilyloxy-2-naphthaldehyde to lithiated \(11\) provided the corresponding coupling product \(12\), which was subsequently oxidized with manganese dioxide to ketone \(13\). Selective formation of pyranone ring was successfully achieved by treating \(13\) with KF and 18-crown-6 in anhydrous DMF as reported earlier to give tricyclic compound \(14\). Hydrolysis of the acetal produced diol \(15\) which was subjected to intramolecular Mitsunobu reaction to furnish \((S, S)-2\). Other kapurimycin analogs \(16\) and \(17\) were synthesized via a similar route.

We determined the absolute configuration of diol \(15\) by analysis of NOESY spectrum of \(14\) and NMR analysis by the modified Mosher’s method. In NOESY spectrum of \(14\), we observed the correlation between H12 and H14. Besides, it was determined that the chirality of C13 was \((R)\) using MTPA esterification of diol 15 and NMR analysis of MTPA esters by the modified Mosher’s method. Thus, the absolute configuration of diol \(15\) is \((11S, 13R)\).
Next, we determined the absolute configuration of ABC analog 2 by use of solvolysis of 2 and comparison of the structural data of solvolysis product with those of 15. Hydrolysis of 2 in Tris-HCl buffer (pH 7.6) gave 11,13-dihydroxy compound (53%) and 11,15-dihydroxy compound (7%) (eq. 1). It was indicated from the observation of 'H NMR spectrum and optical rotation that the given 11,13-dihydroxy compound was identified with 15 (15: [α]D25 = -197.3, c 0.15, MeOH; the hydrolysis product: [α]D25 = -135.7, c 0.07, MeOH). Methanolysis of 2 also gave 11-hydroxy-13-methoxy compound (50%) and 11-hydroxy-15-methoxy compound (15%) which relative structure was confirmed by HSQC, HMBC and MS (eq. 2). This result shows that solvolysis of the epoxide occurs at C13. Furthermore, in the NOESY spectrum of 2 the correlation between H12 and H13 was observed. As clear from above, the absolute configuration of ABC analog 2 is (11S, 13S). Intramolecular Mitsunobu reaction from (11S, 13R)-diol 15 served (11S, 13S)-ABC analog 2 through SN2 inversion at C13 and hydrolysis of 2 mainly generated again (11S, 13R)-diol 15 through SN2 inversion at C13.

Figure 1. NMR experiment (400 MHz) for structural identification. (a) NOESY observation of compound 14; (b) modified Mosher's method (The numbers in figure are the difference of chemical shift between (S)-MTPA ester and (R)-MTPA ester of 15); (c) NOESY observation of ABC-ring analog 2.
The DNA cleavage activities of 2, 15, 16 and 17 were demonstrated by relaxation assay of pBR322 supercoiled DNA cleaving assay (Figure 2). We investigated 10 μM of each drug with 40 μM of DNA in Tris-HCl (pH 7.6) at 37 °C for 5 h. After ethanol precipitation for removal of unreacted and hydrolyzed drugs, a half of the recovered DNA was incubated again in water at 37 °C for 24 h. The different forms of DNA were separated on agarose gel. Only the incubation with 2 clearly converted supercoiled DNA (form I) into nicked-circular (form II) and linear (form III) DNAs. The DNAs treated with 15 and 16 showed no cleavage. Weak DNA cleavage was observed for DNA treated with 17. This result suggests that epoxide, ABC-ring system and alkenyl group attached to epoxide are the units essential for efficiently reaction with DNA. The thermal treatment of DNA reacted with 2 resulted in distinct formation of form II DNA. This result indicates that DNA cleavage by 2 proceeded through alkylation of DNA nucleobase.

We have investigated hydrolysis rates of epoxides of 1, 2, 16 and 17 to estimate the nucleophilicity of epoxide in reaction buffer (Figure 3). We incubated 100 μM of each drug in Tris-HCl (pH 7.6). The hydrolysis reaction was monitored by HPLC at each time. The half lifetimes of 1, 2 and 16 are 3.5 h, 5.6 h and 5.9 h, respectively, and the hydrolysis of 17 was found to be very slow (t_{1/2} > 150 h). The compounds containing a double bond directly attached to epoxide like 1, 2 and 16 completely decomposed in 48 h, whereas the compound without double bond like 17 showed a very little hydrolysis rate. The difference of DNA alkylation rate of 2 and 17 in DNA cleavage assay arises from the nucleophilicity of their epoxides.
Next, we investigated the reaction of 2 with calf thymus DNA to know the nucleobase selectivity of the alkylation by 2. Calf thymus DNA was incubated with 2 in Tris-HCl (pH 7.6) at 37 °C and digested with snake venom phosphodiesterase and alkaline phosphatase. Decreases of given nucleosides were monitored with HPLC (Figure 5). The content of dG decreased to 73% of that in intact DNA in 24 h, while other nucleobases remained unchanged. This result shows that 2 is a good guanine alkylating agent.

Sequence selectivity for DNA alkylation by 2 was examined by using $^{32}$P-5'-end-labeled EcoRI/Rsal fragment of pBR322 DNA. The labeled DNA was incubated in presence of drug at 37 °C for 24 h and recovered by ethanol precipitation. Subsequently, a half of each DNA sample was heated in water at 90 °C for 30 min and another half was treated with hot piperidine. The result of the assay is shown in Figure 6. ABC analog 2 exhibited guanine selective DNA cleavage like kapurimycin A₃ (1) by treatment with hot piperidine. In the case of the heat-treated DNAs, the cleavage bands did not comigrate with those of Maxam-Gilbert guanine bands, and slightly shifted to 3'-side of Maxam-Gilbert.
guanine bands. This result suggests that ABC analog 2 cleaves DNA via guanine alkylation mechanism. Furthermore, we found that 2 cleaved DNA in sequence selective manner. The selectivity of GN sequence decreased in the order, G*G > G*A > G*T > G*C (G* denotes alkylated guanine). In continuous guanine sequences, 5'-side guanine was more reactive than 3'-side guanine. The sequence selectivity of 2 was very similar to that of 1.

ABC analog 2 was incubated with herring sperm DNA at 37 °C for 5 h in order to get 2-guanine adduct (Scheme 2). After incubation and ethanol precipitation, we heated the recovered DNA in water at 90 °C for 30 min and obtained the adduct by butanol extraction followed by column purification. The data obtained from 1H-NMR spectra in methanol-d₄ and mass spectra were shown in Table 1. The mass spectrum shows a peak at m/e 444 ([M+H]+). In the NMR, the signal of H13 of ABC analog 2 considerably shifted to downfield, compared with the other signal of 2. The chemical shift of the signals of 2-guanine adduct is closely similar to those of reported 1-guanine adduct. These data implies that 2-guanine adduct is produced from the nucleophilic attack of guanine N7 to C13 of 2.

Figure 6. Cleavage assay of 32P-5'-end-labeled DNA fragment (513 bp EcoRI-Rsal fragment of pBR322 DNA) by 1 and 2. DNA was incubated in the presence of 1 µM of 1 (lanes 2 and 3) and 10 µM of 2 (lanes 4 and 5) for 24 h at 37 °C in Tris-HCl buffer (pH 7.6). Recovered DNA by ethanol precipitation was heated at 90 °C for 30 min in the absence (lanes 2 and 4), and the presence (lanes 3 and 5) of 10% piperidine, and analyzed by electrophoresis on 8% denatured polyacrylamide gel. lane 1, Maxam-Gilbert G+A; lane 2, 1 with heating; lane 3, 1 with piperidine treatment; lane 4, 2 with heating; lane 5, 2 with piperidine treatment.
Kapurimycin A₃ was cytotoxic at μM level against HeLa S₃ and T24 cells in vitro. We investigated the cytotoxicities of synthetic analogs 2 and 16 against HeLa cell (Table 2). While 16 didn’t show significant cytotoxicity in 10 μM dose, 2 showed one tenth of IC₅₀ for 1 (IC₅₀ = 2.7 μM). This result is consistent with DNA cleaving experiment.

### Conclusion

We investigated the structure-activity relationship for DNA alkylation by antitumor antibiotic kapurimycin A₃ (1) and its simplified analogs. It was shown that the epoxide moiety for DNA alkylation, the double bond for activation of epoxide and the tricyclic structure for DNA binding were all required for the efficient alkylation of DNA. Kapurimycin ABC-ring analog 2 fulfills these requirements and efficiently alkylates DNA with a similar sequence selectivity as that for kapurimycin A₃ (G*G > G*A > G*T > G*C, G* denotes alkylated guanine).

### Table 1. The ¹H NMR (400 MHz) chemical shift assignments for the guanine adducts in methanol-d₄

<table>
<thead>
<tr>
<th>2-guanine adduct</th>
<th>1-guanine adduct</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ (Δδ)</td>
<td>δ (Δδ)</td>
</tr>
<tr>
<td>H3 6.47 (-0.09)</td>
<td>H3 6.28 (-0.10)</td>
</tr>
<tr>
<td>H5 1.81 (-0.06)</td>
<td>H15 1.81 (-0.10)</td>
</tr>
<tr>
<td>H13 6.59 (+2.69)</td>
<td>H16 6.44 (+2.43)</td>
</tr>
<tr>
<td>H14 6.15 (+1.01)</td>
<td>H17 6.15 (+1.00)</td>
</tr>
<tr>
<td>H15 6.05 (+0.25)</td>
<td>H18 6.02 (+0.20)</td>
</tr>
<tr>
<td>H16 1.94 (+0.12)</td>
<td>H19 1.90 (+0.08)</td>
</tr>
<tr>
<td>G-J1 8.17</td>
<td>G-J8 8.08</td>
</tr>
</tbody>
</table>

a Δδ = (chemical shift of 2-guanine adduct) – (chemical shift of 2)
b Δδ = (chemical shift of 1-guanine adduct) – (chemical shift of 1)

### Table 2. In vitro cytotoxic activities of 1, 2 and 16 against HeLa cell

<table>
<thead>
<tr>
<th>HeLa S₃</th>
<th>1</th>
<th>2</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC₅₀ ⁵</td>
<td>0.28 μM</td>
<td>2.7 μM</td>
<td>&gt; 10 μM ⁶</td>
</tr>
</tbody>
</table>

| ⁵Drug concentration causing 50% inhibition of cell growth with 72 h drug incubation |
| ⁶Highest dose tested |
Experimental Section

General Techniques. $^1$H NMR spectra were measured with JEOL JNM a-400 (400 MHz) spectrometers. Coupling constants ($J$ values) are reported in Hz. $^{13}$C NMR spectra were measured with Varian GEMINI 200 (50 MHz) spectrometers. The chemical shifts are expressed in ppm downfield from tetramethylsilane, using residual chloroform ($\delta = 7.24$ in $^1$H NMR, $\delta = 77.0$ in $^{13}$C NMR) as an internal standard. The following abbreviations were used to explain the multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. IR spectra were recorded on JASCO FT/IR-5M spectrophotometers. Melting points were obtained on a Yanagimoto Seisakusho micro melting point apparatus and are uncorrected. Optical rotations were recorded using a Perkin-Elmer 243 polarimeter. Electron impact mass spectra (MS) and high-resolution mass spectra (HRMS) were recorded on JEOL JMS-DX 300 or JEOL JMS-SX 102A. Microanalyses were performed by Kyoto University Microanalytical Center.

All reactions were monitored by thin layer chromatography carried out on 0.25-mm E. Merck silica gel plates (60F-254) using UV light, 5% ethanolic phosphomolybdic acid, or $p$-anisaldehyde solution and heat as developing agent. Wako gel (C-200, particle size 75-150 $\mu$m, Wako) was used for column chromatography. Plasmid pBR322 DNA was purchased from Wako. Tetrahydrofuran and ethyl ether were distilled over sodium-benzophenone. Dichloromethane, toluene and N,N-dimethylformamide was distilled over calcium hydride. All reagents were purchased at highest commercial quality and used without further purification unless otherwise stated.

All reactions were carried out under nitrogen atmosphere with anhydrous solvents under anhydrous conditions, unless otherwise noted.

Yields refer to chromatographically and spectroscopically ($^1$H NMR) homogeneous materials, unless otherwise stated.

$(E)$-1-(4-Methoxybenzyloxy)-3-methyl-2-penten-4-yne (4). To a suspension of sodium hydride (60 %, 4.5 g, 113.3 mmol) in N,N-dimethylformamide (10 mL) was added 3 (10.01 g, 104.1 mmol) at 0 °C, and the reaction mixture was stirred for 10 min. To this mixture was added $p$-methoxybenzyl chloride (15.5 mL, 114.3 mmol) at 0 °C, and the reaction mixture was stirred for 30 min. The mixture was diluted with sat. $aq.$ NH$_4$Cl and extracted with ethyl acetate. The crude product was purified by column chromatography on silica gel, eluting with 5 % ethyl acetate in hexane to give 4 (20.6 g, 102.0 mmol, 98 %) as a yellow oil: $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 7.24 (d, 2 H, $J = 8.8$ Hz), 6.86 (d, 2 H, $J = 8.7$ Hz), 6.07 (dtq, 1 H, $J = 0.6$, 1.5, 6.6 Hz), 4.42 (s, 2 H), 4.05 (dt, 2 H, $J = 0.7$, 6.6 Hz), 3.79 (s, 3 H), 2.81 (s, 1 H); IR (CHCl$_3$) 3304, 3021, 3009, 1612, 1513, 1249, 1217, 1173, 1035, 773, 769 cm$^{-1}$.

$(2R,3R)$-1-(4-Methoxybenzyloxy)-3-methyl-4-pentyn-2,3-diol (5). The mixture of AD-mix-$\beta$ (Aldrich, 12.0 g) and methanesulfonamide (0.82 g, 8.62 mmol) in $t$-butanol (40 mL) and water (40 mL) was stirred at 0 °C for 30 min. To this mixture was added 4 (1.73 g, 8.54 mmol) at 0 °C, and the reaction mixture was stirred for 2 days. To the mixture was added sodium sulfite (15 g) and the reaction mixture was stirred for 2 h. This mixture was extracted with ethyl acetate, and then the combined organic layer extracted with 1 N sodium hydroxide. The crude product was purified by column chromatography on silica gel, eluting with 20 %-100 % ethyl acetate in hexane to give 5 (1.63 g, 6.49 mmol, 76 %) as a colorless oil: $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 7.24 (d, 2 H, 63
H, J = 8.7 Hz), 6.86 (d, 2 H, J = 8.7 Hz), 4.48 (d×2, 2 H, J = 11.4, 13.2 Hz), 3.79 (s, 3 H), 3.74 (d×2, 2 H, J = 4.7, 9.2 Hz), 3.59 (dd, 1 H, J = 6.3, 9.2 Hz), 3.26 (s, 1 H), 2.53 (d, 1 H, J = 5.0 Hz), 2.46 (s, 1 H), 1.47 (s, 3 H); IR (CHCl₃) 3562, 3306, 3011, 2937, 1514, 1250, 1174, 1087, 1036, 734 cm⁻¹; MS (El) m/e (%): 250 (M⁺, 21), 137 (100), 121 (100); HRMS (El) calcd for C₁₄H₈O₄ 250.1204 (M⁺), found 250.1201.

(4R,5R)-4-Ethynyl-5-(4-methoxybenzyl)oxymethyl)-2,2,4-trimethyl-1,3-dioxolane (6). To a solution of 5 (1.97 g, 7.87 mmol), 2,2-dimethoxypropane (4.9 mL, 39.85 mmol) and anhydrous copper sulfate (6.29 g, 39.41 mmol) in acetone (50 mL) was added dl-camphorsulfonic acid (92.4 mg, 0.40 mmol) at 0 °C, and the reaction mixture was stirred for 3 h. The mixture was filtered, diluted with sat. aq. NaHCO₃, concentrated and extracted with ethyl acetate. The crude product was purified by column chromatography on silica gel, eluting with 15 % ethyl acetate in hexane to give 6 (2.17 g, 7.48 mmol, 95%) as a colorless oil: 1H NMR (CDCl₃, 400 MHz) δ 7.26 (d, 2 H, J = 8.8 Hz), 6.86 (d, 2 H, J = 8.7 Hz), 4.58 (d, 1 H, J = 11.7 Hz), 4.46 (d, 1 H, J = 11.7 Hz), 4.39 (dd, 1 H, J = 5.0 Hz), 3.79 (s, 3 H), 3.555 (d, 1 H, J = 5.0 Hz), 3.551 (d, 1 H, J = 6.4 Hz), 2.48 (s, 1 H), 1.46 (d, 3 H, J = 0.5 Hz), 1.42 (d, 3 H, J = 0.5 Hz), 1.34 (s, 3 H); IR (CHCl₃) 3020, 1513, 1375, 1249, 1088, 780 cm⁻¹; MS (El) m/e (%) 290 (M⁺, 13), 275 (47), 232 (81), 137 (97), 121 (100); HRMS (El) calcd for C₁₇H₂₂O₄ (M⁺) 290.1518, found 290.1524.

(4R,5R)-5-Hydroxymethyl-2,2,4-trimethyl-4-(2'-trimethylsilyl)ethynyl)-1,3-dioxolane (8). To a solution of 7 (0.31 g, 0.85 mmol) in dichloromethane (18 mL) and water (1 mL) was added dichlorodicyanoquinone (0.23 g, 1.01 mmol) at 0 °C, and the reaction mixture was stirred at ambient temperature for 4 h. The mixture was diluted with sat. aq. NaHCO₃, and extracted with ethyl acetate. The crude product was purified by column chromatography on silica gel, eluting with 60 % toluene in hexane to give 8 (0.18 g, 0.84 mmol, 99%) as a colorless needle: mp. 45 °C; 1H NMR (CDCl₃, 400 MHz) δ 4.31 (dd, 1 H, J = 3.9, 7.4 Hz), 3.73 (2 H), 1.73 (m, 1 H), 1.49 (s, 3 H), 1.43 (s, 3 H), 1.37 (s, 3 H), 0.14 (s, 9 H); IR (CHCl₃) 3020, 1217, 847, 754 cm⁻¹; Anal. Calcd for C₁₂H₂₅O₃Si: C, 59.46; H, 9.15. Found: C, 59.20; H, 9.30.
(4R,5S)-5-Formyl-2,2,4-trimethyl-4-(2'-trimethylsilyl-ethynyl)-1,3-dioxolane (9). To a solution of 8 (0.81 g, 3.34 mmol) in dichloromethane (30 mL) was added Dess-Martin periodinane (1.7 g, 4.01 mmol) at ambient temperature, and the reaction mixture was stirred at ambient temperature for 1 h. The mixture was diluted with sat. aq. Na₂S₂O₅ and sat. aq. NaHCO₃, and then extracted with ethyl acetate. The crude product was purified by column chromatography on silica gel, eluting with 25% ethyl acetate in hexane to give 9 (d, 2 H, 235 [(M-Me)+, 10], 4R,5R,1'Z)-5-{3-[1-(t-Butyldimethylsilyloxy)-2-naphthyl]-3-hydroxy-1-propynyl}-5-(1'-propenyl)-2,2,4-trimethyl-1,3-dioxolane (11). To a solution of 10 (0.21 g, 0.82 mmol) in methanol (10 mL) was added a drop of sodium methoxide methanol solution at 0 °C, and the reaction mixture was stirred at ambient temperature for 2 h. The mixture was diluted with sat. aq. NH₄Cl and extracted with pentane, ether and water. After its concentration, 11 (0.12 g, 0.66 mmol, 80%) was afforded as a colorless oil: 1H NMR (CDCl₃, 400 MHz) δ 5.84 (ddq, 1 H, J = 1.2, 7.0, 11.1 Hz), 5.40 (ddq, 1 H, J = 1.8, 8.6, 11.1 Hz), 5.08 (dd, 1 H, J = 1.1, 8.6 Hz), 2.47 (s, 1 H), 1.78 (dd, 3 H, J = 1.8, 7.0 Hz), 1.48 (s, 3 H), 1.44 (s, 3 H), 1.34 (s, 3 H); IR (CDCl₃) 3017, 1730, 1250, 1077, 788 cm⁻¹.
naphthalene), 7.68 (1 H, naphthalene), 7.52 (1 H, naphthalene), 7.44 (2 H, naphthalene), 5.99 (1 H, J = 4.5 Hz, naph–CH), 5.78 (1 H, CH₃–CH=CH), 5.38 (1 H, CH₃–CH=CH–CH=CH), 5.04 (1 H, CH₂–CH=CH–CH–CH), 2.12 (1 H, OH), 1.70, 1.61 (3 H, J = 1.8, 7.0 Hz, CH₃–CH=CH), 1.47-1.41 (6 H, acetonide), 1.36 (s×2, total 3 H, 4-CH₃), 1.12 (s×2, total 9 H, Si–Bu), 0.18 (6H, Si–CH₃); IR (CHCl₃) 3017, 2932, 1721, 1291, 1222, 1077, 737 cm⁻¹; MS m/e (%) 466 (M⁺, 7), 281 (32); HRMS (EI) calcd for C₂₈H₃₈O₄Si [(M⁺) 466.2540, found 466.2533.

(4R,5R,1'Z)-4-[3-[1-(t-Butyldimethylsilyloxy)-2-naphthyl]-3-oxo-1-propynyl]-5-(1'-propenyl)-2,2,4-trimethyl-1,3-dioxolane (13). To a solution of 12 (30.2 mg, 64.7 μmol) in dichloromethane (2 mL) was added manganese (IV) oxide (11.3 mg), and the reaction mixture was stirred for 24 h at ambient temperature. The mixture was diluted with ethyl ether, filtered, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel, eluting with 15 % ethyl acetate in hexane to give 13 (12.1 mg, 34.5 μmol, 80 %) as a yellow oil: ¹H NMR (CDCl₃, 400 MHz) δ 8.28 (m, 1 H), 7.84 (d, 1 H, J = 8.0 Hz), 7.56 (dd, 1 H, J = 1.2, 6.8, 8.1 Hz), 7.51-7.45 (2 H), 5.87 (ddq, 1 H, J = 1.2, 7.1, 11.0 Hz), 5.44 (ddq, 1 H, J = 1.8, 8.7, 10.8 Hz), 5.18 (dd, 1 H, J = 1.1, 8.7 Hz), 1.78 (dd, 3 H, J = 1.8, 7.1 Hz), 1.49 (s, 3 H), 1.45 (s, 3 H), 1.10 (s, 9 H), 0.11 (s, 6 H); IR (CHCl₃) 3021, 1649, 1390, 1230, 1109, 1015, 796 cm⁻¹; MS (EI) m/e (%) 407 [(M–Me)⁺, 6], 293 (52), 280 (100), 238 (73), 222 (80), 149 (94); HRMS (EI) calcd for C₂₆H₃₉O₂Si [(M–Me)⁺] 407.1679, found 407.1652.

(4S,5R,1'Z)-4-[3H-naphtho[1,2-b]pyran-2-yl]-5-(1'-propenyl)-2,2,4-trimethyl-1,3-dioxolane (14). To a solution of 13 (20.1 mg, 43.3 μmol) and 18-crown-6 (23.2 mg, 87.8 μmol) in N,N-dimethylformamide (1 mL) was added a potassium fluoride (5.2 mg, 89.6 μmol) at 0 °C, and the reaction mixture was stirred at ambient temperature for 2 h. The mixture was diluted with sat. aq. NH₄Cl, and extracted with ethyl acetate. The crude product was purified by column chromatography on silica gel, eluting with 15 % ethyl acetate in hexane to give 14 (12.1 mg, 34.5 μmol, 80 %) as a yellow oil: ¹H NMR (CDCl₃, 400 MHz) δ 8.92 (d, 1 H, J = 11.4 Hz), 7.76 (d, 1 H, J = 8.6 Hz), 7.69 (ddd, 1 H, J = 1.4, 7.0, 8.2 Hz), 7.63 (ddd, 1 H, J = 1.4, 6.9, 8.3 Hz), 6.81 (s, 1 H), 5.99 (ddq, 1 H, J = 1.0, 7.1, 11.1 Hz), 5.72 (ddq, 1 H, J = 1.8, 9.0, 10.9 Hz), 5.02 (dd, 1 H, J = 1.0, 9.0 Hz), 1.61 (s, 3 H), 1.60 (s, 3 H), 1.53 (s, 3 H), 1.40 (dd, 3 H, J = 1.8, 7.1 Hz); IR (CHCl₃) 3021, 1649, 1390, 1230, 1109, 1015, 796 cm⁻¹; MS (EI) m/e (%) 350 (M⁺, 5), 335 [(M–Me)⁺, 11], 293 (46), 280 (77), 239 (100); HRMS (EI) calcd for C₂₆H₂₅O₃Si [(M–Me)⁺] 335.1283, found 335.1297.

2-[(2S,3R,4Z)-2,3-Dihydroxy-4-hexen-2-yl]-4H-naphtho[1,2-b]pyran (15). To a solution of 14 (7.0 mg, 20.0 μmol) in tetrahydrofuran (0.5 mL) and acetic acid (0.5 mL) was added hydrochloric acid (0.05 mL) at 0 °C, and the reaction mixture was stirred at ambient temperature for 5 days. The mixture was poured onto sat. aq. NaHCO₃ and ethyl acetate, and extracted. The crude product was purified by column chromatography on silica gel, eluting with 50 % ethyl acetate in hexane to give 15 (6.1 mg, 19.7 μmol, 98 %) as a white solid: ¹H NMR (CDCl₃, 400 MHz) δ 8.37 (m, 1 H), 7.93 (d, 1 H, J = 8.8 Hz), 7.87 (m, 1 H), 7.68 (2 H), 7.60 (d, 1 H, J = 8.6 Hz), 6.78 (s, 1 H), 5.86 (ddq, 1 H, J = 0.9, 7.0, 11.0 Hz), 5.67 (ddq, 1 H, J = 1.8, 9.3, 11.0 Hz), 5.04 (d, 1 H, J = 9.1 Hz), 3.40 (s, 1 H), 2.70 (s, 1 H),
1.76 (dd, 3 H, J = 1.7, 7.0 Hz), 1.57 (s, 3 H); IR (CHCl₃) 3020, 1647, 1630, 1391, 1217, 795 cm⁻¹; MS (EI) m/e (%) 310 (M⁺, 4), 240 (100), 197 (82); HRMS (EI) calcd for C₁₅H₁₄O₄ (M⁺) 310.1205, found 310.1204.

2-[(2S*,3S*,4Z)-2,3-Epoxy-4-hexen-2-yl]-4H-naphtho[1,2-b]pyran (2). To a solution of 15 (39.7 mg, 0.13 mmol) and triphenylphosphine (50.5 mg, 0.19 mmol) in toluene (2 mL) was added diethyl azadicarboxylate (0.060 mL, 0.38 mmol) at 0 °C, and the reaction mixture was stirred at ambient temperature for 3 h. The mixture was filtered and concentrated in vacuo. The crude product was purified by column chromatography on silica gel, eluting with 20 % toluene in hexane to give optically active 2 (28.6 mg, 0.098 mmol, 76 %) as a white solid: mp. 118.0–119.2 °C; [α]D = −61.5° (c 0.26, MeOH); ¹H NMR (CDCl₃, 400 MHz) δ 8.46 (1 H, J = 6.7, 15.4 Hz), 5.17 (ddq, Z-1 H, J = 1.9, 8.7, 11.0 Hz), 3.82 (d, Z-1 H, J = 8.6 Hz), 3.52 (d, E-1 H, J = 8.6 Hz), 1.80 (dd, Z-3 H, J = 1.8, 7.1 Hz), 1.76 (s, Z-3 H), 1.72 (s, E-3 H), 1.65 (dd, E-3 H, J = 1.7, 6.6 Hz); IR (CHCl₃) 3020, 1649, 1608, 1252, 849 cm⁻¹; MS (EI) m/e (%) 242 (M⁺, 13), 227 (15), 211 (24), 200 (92), 185 (73), 121 (100); HRMS (EI) calcd for C₁₅H₁₄O₃ (M⁺) 242.0942, found 242.0920.

2-[(2S*,3S*)-2,3-Epoxy-4-hydroxy-2-buty1]-4H-naphtho[1,2-b]pyran (17). mp. 175 °C; ¹H NMR (CDCl₃, 400 MHz) δ 8.45 (1 H, H), 8.11 (d, 1 H, J = 8.8 Hz), 7.92 (m, 1 H), 7.76 (d, 1 H, J = 8.6 Hz), 6.69 (2 H), 6.53 (s, 1 H), 3.72 (2 H), 3.44 (dd, 1 H, J = 4.9, 6.3 Hz), 1.86 (s, 3 H), 1.72 (br, 1 H); IR (CHCl₃) 3022, 1650, 1441, 1391 cm⁻¹; MS (EI) m/e (%) 282 (M⁺, 39), 212 (40), 170 (100), 114 (68); HRMS calcd for C₁₇H₁₄O₄ (M⁺) 282.0892, found 282.0875.

Hydrolysis of 2. A solution of 2 (29.3 mg, 0.10 μmol) in 10% (v/v) acetonitrile/50 mM Tris-HCl buffer (10 mL, pH 7.6) was stirred at 37 °C for 5 days. After concentration, the mixture was extracted with ethyl acetate and water. The crude product was purified by column chromatography on silica gel, eluting with 20-60% ethyl acetate in hexane to give 9 (16.5 mg, 53.2 μmol, 53%) as a white solid, 12 (2.2 mg, 7.1 μmol, 7%) and recovered 2 (11.0 mg, 37.6 μmol, 38%).

Methanolation of 2. To 9 mL of methanol solution of Tris-HCl salt, which was obtained by concentration of 0.5 M Tris-HCl buffer (9 mL, pH 7.6) in vacuo, was added 2 (14.6 mg, 49.9 μmol) in acetonitrile (1 mL). After stirred at reflux for 5 days, the mixture was concentrated and extracted with ethyl acetate and water. The crude product was purified by column chromatography on silica gel, eluting with 20%
ethyl acetate in hexane to give 13 (8.1 mg, 25.0 μmol, 50%) as a white solid, 14 (2.4 mg, 7.4 μmol, 15%) and recovered 2 (7.7 mg, 12.7 μmol, 25%).

Cleavage of pBR322 Supercoiled DNA by 2, 9, 10 and 11. Kapurimycin analogs 2, 9, 10 or 11 (10 μM) was incubated with 40 μM pBR322 supercoiled DNA (Nippon Gene) in 10% (v/v) acetonitrile/50 mM Tris-HCl buffer (10 μL, pH 7.5) at 37 °C for 5 h. The samples which require the thermal treatment were successively precipitated in ethanol to remove the drug and the recovered DNA pellets were dried and incubated again in 10 μL of 50 mM Tris-HCl buffer at 37 °C for 24 h. To both thermal-treated and no treated samples was added the 10 μL of loading buffer containing 0.05% (w/v) bromophenol blue and 6% (v/v) glycerol for electrophoresis. The different form of DNA were separated at ambient temperature on a 1% (w/v) agarose gel involving ethidium bromide (0.5 μg/mL). The gels were placed on a UV transilluminator (313 nm) and photographed with Polaroid 665 film. The result was shown in Figure 2.

Observation of Hydrolysis Rates of Drugs. The 100 μM of 1, 2, 10 or 11 was incubated in 10% (v/v) acetonitrile/50 mM Tris-HCl buffer (200 μL, pH 7.5) at 37 °C. The amount of drug in the reaction buffer was monitored by HPLC analysis on a COSMOSIL 5C18-AR column (4.6×150 mm, elution with a solvent mixture (1:1) of 0.05 M ammonium formate and acetonitrile at a flow rate of 1.0 mL/min for 20 min). The amount of drug was determined by ratio of the peak areas of original drug to all peak areas. The result was shown in Figure 3.

Cleavage of pBR322 Supercoiled DNA by 1 and 2. Kapurimycin A3 1 or its analog 2 (10 μM or 1 μM) was incubated with 40 μM pBR322 supercoiled DNA (Nippon Gene) in 10% (v/v) acetonitrile/50 mM Tris-HCl buffer (10 μL, pH 7.5) at 37 °C for 5 h. The samples were successively precipitated in ethanol to remove the drug and the recovered DNA pellets were dried and incubated again in 10 μL of 50 mM Tris-HCl buffer at 37 °C for 24 h. To thermal-treated samples was added the 10 μL of loading buffer containing 0.05% (w/v) bromophenol blue and 6% (v/v) glycerol for electrophoresis. The different form of DNA were separated at ambient temperature on a 1% (w/v) agarose gel involving ethidium bromide (0.5 μg/mL). The gels were placed on a UV transilluminator (313 nm) and photographed with Polaroid 665 film. The result was shown in Figure 4.

HPLC Analysis of Base-Selectivity of 2. The 50 μM of 2 was incubated with 200 μM of calf thymus DNA in 10% (v/v) acetonitrile/50 mM Tris-HCl buffer (100 μL, pH 7.5) at 37 °C for 24 h. The resulting DNA was digested with snake venom phosphodiesterase and alkaline phosphatase. The concentrations of the produced four nucleotides were confirmed by HPLC analysis on a COSMOSIL 5C18-AR column (4.6×150 mm, elution with a solvent mixture of 0.05 M ammonium formate, linear gradient over 20 min from 2% to 5% acetonitrile at a flow rate of 1.0 mL/min), and determined by comparison of the peak areas of dG, dT and dA with that of dC. The result was shown in Figure 5.

Preparation of 32P-5'-End-Labeled DNA Fragments of pBR322 DNA. Digestion of supercoiled pBR322 plasmid DNA (Nippon Gene) with EcoRI restriction endonuclease followed by
treatment with alkaline phosphatase gave a linearized pBR322 with hydroxyl termini at its 5'-ends. Labeling at the 5'-end of the linearized DNA was achieved by treatment with [γ-^32P]ATP and T4 polynucleotide kinase. The labeled DNA was further digested with Rsal restriction endonuclease to yield two 5'-end-labeled DNA fragments (167 and 513 base pair) which purified on 6% nondenaturing polyacrylamide gel. The labeled DNA was recovered from the gel by a crush and soak method.\(^\text{11}\)

**Cleavage of ^32P-5'-End-Labeled Fragment of pBR322 DNA.**

The 1 µM of 1 or 10 µM of 2 was incubated with 10 µM of calf thymus DNA and ca. 1.0×10^6 cpm ^32P-5'-end-labeled DNA fragment in 20 mM Tris-HCl buffer (100 µL, pH 7.6) at 37 °C for 24 h. The reaction mixture was precipitated with ethanol and dried. The recovered DNA was dissolved in 100 µL of water or 10% (v/v) piperidine and heated at 90 °C for 30 min. The mixture was concentrated in vacuo and resuspended in 10 µL of 80% formamide loading buffer (80% formamide, 1 mM EDTA, 0.1% xylene cyanole and 0.1% bromophenol blue). The samples (1 µL) were loaded onto 8% polyacrylamide and 7 M urea sequence gel and electrophoresed at 1900 V for ca. 2 h. The gel was dried and exposed to X-ray film with intensifying sheet at −70 °C. The result was shown in Figure 6.

**2-Guanine Adduct.** A solution of herring sperm DNA (10 mg) in 10% (v/v) acetonitrile/50 mM Tris-HCl buffer (2 mL, pH 7.5) was stirred with 2 (2.9 mg, 0.01 mmol) at 37 °C for 5 h. The reaction mixture was successively precipitated in ethanol to remove the drug and the recovered DNA pellets were dried and incubated again in 50 mM Tris-HCl buffer (2 mL) at 90 °C for 30 min. The mixture was extracted with n-butanol and water and the organic layer was concentrated in vacuo. The crude product was purified by reversed column chromatography on ODS (Wakogel LP-40C18, 20–40 µm) eluting with 30% water in acetonitrile to give 2-guanine adduct (0.8 mg, 1.8 µmol, 18%) as white solid.
References and Notes


(2) The absolute configuration of 1 has been determined to be 8S, 14S and 16S. Uosaki, Y.; Saito, H. Abstract paper p 1013, 69th annual meeting of the Chemical Society of Japan, Kyoto (1995).


(7) E- and Z-mixtures are not separable throughout the synthesis of 2.


CHAPTER 3

Guanine-Guanine Sequence Selectivity for DNA Alkylation by Kapurimycin Analogs

Abstract: In nature there has been known several guanine selective DNA alkylating antitumor antibiotics. In order to estimate the contribution of the DNA binding ability to the guanine-guanine (GG) selective cleavage, we synthesized kapurimycin A, analogs (S, S)-ABC 2 and (S, S)-ABCD'-ring analogs 3, and compared their sequence selectivity for DNA cleavage. We found that 3 exceeded 2 in DNA cleaving activity and in sequence selectivity of DNA cleavage (GG > GA > GT > GC) as judged from the cleaving assay of 32P-5'-end-labeled oligodeoxynucleotides. The tetracyclic aromatic ring system of 3 intercalates into DNA duplex more efficiently than 2. Besides, in the cleaving assay of oligonucleotides having bulge sites, it was shown that ABCD'-ring analog 3 selectively alkylated 5'-side guanine of its intercalation site. The above results suggest the selective alkylation of 5'-side guanine of GG sequence by ABCD'-ring analog 3 was originated by the intercalation of 3 into two guanines of GG sequence. We speculate the reaction mechanism of GG selective DNA alkylation by natural antibiotics that is the closely related to the mechanism for the model system.
Introduction

In nature, there are several types of antitumor antibiotics that can alkylate guanine base of duplex DNA. Aflatoxin B1 oxide, psorospermin and kapurimycin A3 are typical such DNA alkylating agents of natural origin. These antibiotics commonly possess an aromatic ring system for DNA binding and an epoxy subunit for DNA alkylation. The mechanism of guanine alkylation by these compounds involves two steps, a DNA binding step and a guanine alkylation step as shown in eq 1. The DNA binding step is most important for the efficiency and the selectivity for guanine alkylation.

We investigated the mechanism of GG selective DNA alkylation in order to know the correlation of their DNA binding ability with the sequence selective alkylation. We prepared kapurimycin ABCD'-ring analog 3 containing four aromatic rings, and compared the sequence selectivity for DNA alkylation with that of 2. Herein, we report that the intercalation of the aromatic ring into DNA is very important for GG selective alkylation.

Results and Discussion

The synthetic route for ABCD’-ring analog 3 is outlined in Schemes 1 and 2. The BCD'-ring unit was prepared starting from commercially available 1-hydroxyanthraquinone (4). Anthracene 6 was obtained by reduction of anthraquinone 5 which was then formylated to 7. The protective group of phenol 7 was converted from methoxymethyl group to tert-butyldimethylsilyl group to give 9. The side chain moiety 10 was prepared as shown in a previous paper. Addition of 9 to lithiated 10 provided coupling product 11. The oxidation of 11 with manganese dioxide produced the corresponding ketone which was subsequently treated with KF and 18-crown-6 in DMF as reported earlier to give tetracyclic compound 12. Hydrolysis of the acetal produced diol which
was subjected to intramolecular Mitsunobu reaction to furnish (13S, 15S)-3.7

Scheme 1.a

\[
\begin{array}{cccc}
4: R_1^1 = H & b & c & 7: R_2^2 = \text{MOM} \\
5: R_1^1 = \text{MOM} & & & 8: R_1^2 = H \\
& d & e & 9: R_1^2 = \text{TBDMS}
\end{array}
\]

Reagents and Conditions: a) MOMCl, 96%; b) NaBH₄, 87%; c) n-BuLi then DMF, 80%; d) HCl, 93%; e) TBDMSOTf, crude product.

Scheme 2.a

\[\text{Reagents and Conditions: a) LHMDS, CeCl₃ then } 9, 35\%; \text{ b) MnO}_2, 69\%; \text{ c) KF, 18-crown-6, DMF, 75\%; d) HCl, 91\%; e) DEAD, PPh₃, 63\%.}\]

The DNA cleaving activity of 2 and 3 was demonstrated by monitoring the conversion of supercoiled pBR322 DNA (form I) to nicked circular (form II) and linear duplex (form III) DNAs. We incubated 10 μM or 1 μM of 2 and 3 with 40 μM of DNA in Tris-HCl (pH 7.5) at 37 °C for 5 h. After ethanol precipitation for the removal of unreacted and hydrolyzed drug, the DNA was incubated again in water at 37 °C for 24 h. The different forms of DNA were separated on agarose gel. The incubation of 3 with DNA converted form I DNA to form II and form III DNAs in a dose-dependent fashion. ABCD'-ring analog 3 cleaved DNA more efficiently than ABC-ring analog 2.

In order to know the relationship between ring system of kapurimycin A₃ (1) and its analogs 2 and 3 and the sequence selectivity for DNA cleavage, the reactions of DNA with 1, 2 and 3 were examined by using 32P-5'-end-labeled oligodeoxynucleotides and analyzed by electrophoresis on denatured polyacrylamide gel (Figure 2). The 32P-5'-end-labeled oligodeoxynucleotide duplex was incubated with these drugs at 37 °C for 24 h and heated in 10% piperidine at 90 °C for 30 min to induce strand breakage. DNA cleavage was observed at all guanine sites in every case. The time required to reduce the intact DNA to 60% is 30 min for 1, 7 h for 2 and 5 h for 3. Of special interest is that cleavage by 3 was highly sequence selective at 5'-side guanine of GG sequence. The 5'-side G selectivity for 5'-GN sequence decreased in the order of 5'-G*G > G*A > G*T > G*C. It is worthwhile to note that 3 well exceeded natural kapurimycin A₃ (1) in terms of GG sequence selectivity. As a result of this experiment, it was confirmed that both DNA cleaving activity and the sequence selectivity considerably increased with increasing the number of the aromatic rings of their synthetic drugs.
Figure 2. Strand breakage assays depicting reactive sites for 1, 2 and 3 on the oligodeoxynucleotide duplex. 32P-S-labeled oligodeoxynucleotide was treated with 1, 2 or 3 (50 μM) in a reaction buffer (20 mM Tris-HCl, pH 7.6 and 10 μM calf thymus DNA) at 37 °C. After ethanol precipitation, the residue was heated in 10% piperidine at 90 °C for 30 min. The resulting DNAs were analyzed by electrophoresis on 15% denatured polyacrylamide gel. The height of bars in this histogram shows the percentage of strand breakage at a given site relative to the total strand breakage. The cleaving sites of DNA treated with 1 for 30 min is shown by white bars; 2 for 7 h is shown by gray bars; 3 for 5 h is shown by black bars.

We examined the DNA cleaving assay for 1 and 3 by the use of oligonucleotide containing 7-deazaguanine in order to confirm whether 1 or 3 modifies guanine N7-position. Oligodeoxynucleotides having 7-deazaguanine were prepared by primer elongation method. The primer was elongated by exo'-Klenow fragment in presence of dATP, dCTP, dTTP and deaza-dGTP. The result obtained from the cleaving assay of DNA involving 7-deazaguanine bases was shown in Figure 3. As clear from figures, the DNA cleavage never occurred at deaza G site. The result of figures clearly shows that the cleavage bands were generated
by the alkylation of guanine N7. Drug 3 binds to DNA and efficiently alkylate guanine N7 in the major groove.

We next examined the DNA binding ability of the aromatic ring of kapurimycin analogs 13, 14 and 15. We prepared compounds 13, 14 and 15 which are lacking in epoxide side chain, and examined unwinding assay of supercoiled DNA using topoisomerase I. Topoisomerase I is allowed to adjust DNA linking number in the presence of an intercalator that influences the DNA unwinding angle, resulting in the shift of the gaussian distribution of topoisomers. A solution of pBR322 plasmid DNA with each drug was incubated in the presence of human topoisomerase I at 37 ℃ and the resulting DNAs were analyzed by agarose gel electrophoresis (Figure 4). As clear from Figure 4, the DNA treated with 15 was more strongly unwound than that treated with 14. Only little unwinding was observed for DNA treated with 13. This result clearly indicates that the aromatic moiety of kapurimycin analog 3 intercalates into DNA and that the binding ability of these drugs increases with increasing the number of aromatic ring.

In order to gain insight into the mechanism of GG selective DNA alkylation by ABCD'-ring analog 3, we have examined the guanine cleavage reaction of duplex oligodeoxynucleotide (ODN) having a bulge site opposite to guanine doublet and guanine triplet of the target strand (Figure 5). In DNA, the stabilization of bulge structures by intercalating agents has been suggested as a mutagenesis mechanism, and direct measurements of ethidium binding to synthetic DNAs support this conclusion. Specific strand scission near a bulge can be monitored to determine if intercalation is the mode of binding of any drug that cleaves DNA. As clear from the Figure 5, the most effective cleavage site of ODN1 is highly dependent on the location of the bulge site, with considerably increased alkylation selectivity at 5'-G of the guanine doublet, i.e., 5'-GG-3'/3'-CTC-5'. Since bulge site is well known to be the favorable site for intercalation, it is highly likely that ABCD'-ring
analog 3 intercalates two guanine bases of GG doublet and alkylates selectively the 5'-side G.

**Figure 5.** Determination of alkylation site of the target 35-mer (ODN 1) by 3 in the presence of complementary strand ODN 2, 3, 4 and 5. Upon duplex formation with ODN 1, ODN 3, 4 and 5 produce the bulge structure at the complementary sequence of G17G18, G21G22 and G22G23 of ODN 1. 32P-5'-end-labeled ODN 1 annealed with a complementary ODN was treated with 3 (50 μM). lane 1, G+A sequencing reaction; lane 2, ODN 2; lane 3, ODN 3; lane 4, ODN 4; lane 5, ODN 5.

**Conclusion**

We have focused on the contribution of the aromatic ring of DNA alkylating antibiotics to the sequence selectivity for guanine alkylation, and compared the DNA cleavage by kapurimycin A3, ABC-ring analog 2 and ABCD'-ring analog 3. ABCD'-ring analog 3 exceeded ABC-ring analog 2 in both the reactivity and the sequence selectivity of DNA cleavage (G*G > G*A > G*T > G*C). It was elucidated by the experiments shown above that the intercalation of aromatic ring system of 3 into DNA is a very important factor for effective GG selective DNA alkylation. Investigation of the mechanism of DNA cleavage by synthetic analog 3 enables us to explain why the GG selective DNA alkylation occurs with naturally occurred antibiotics.
Experimental Section

General Techniques. $^1$H NMR spectra were measured with JEOL JNM a-400 (400 MHz) spectrometers. Coupling constants ($J$ values) are reported in Hz. The chemical shifts are expressed in ppm downfield from tetramethylsilane, using residual chloroform ($\delta = 7.24$ in $^1$H NMR) as an internal standard. The following abbreviations were used to explain the multiplicities: $s$, singlet; $d$, doublet; $t$, triplet; $q$, quartet; $m$, multiplet; $br$, broad. IR spectra were recorded on JASCO FTIR-5M spectrophotometers. Melting points were obtained on a Yanagimoto Seisakusho micro melting point apparatus and uncorrected. Optical rotations were recorded using a Perkin-Elmer 243 polarimeter.

Electron impact mass spectra (MS) and high-resolution mass spectra (HRMS) were recorded on JEOL JMS-DX 300 or JEOL JMS-SX 102A. Microanalyses were performed at Kyoto University Microanalytical Center.

All reactions were monitored by thin layer chromatography carried out on 0.25-mm E. Merck silica gel plates (60F-254) using UV light, 5% ethanolic phosphomolybdic acid, or $p$-anisaldehyde solution and heat as developing agent. Wako gel (C-200, particle size 75-150 $\mu$m, Wako) was used for column chromatography. Plasmid pBR322 DNA was purchased from Wako. Tetrahydrofuran and ethyl ether were distilled over sodium-benzophenone. Dichloromethane, toluene and $N,N$-dimethylformamide was distilled over calcium hydride. All reagents were purchased at highest commercial quality and used without further purification unless otherwise stated.

All reactions were carried out under nitrogen atmosphere with anhydrous solvents under anhydrous conditions unless otherwise noted. Yields refer to chromatographically and spectroscopically ($^1$H NMR) homogeneous materials unless otherwise stated.

1-Methoxymethoxy-9,10-anthraquinone (5). A suspension of 4 (6.0 g, 26.7 mmol) in chloroform (80 mL) was treated with $N,N$-dissopropylethylamine (41.9 mL, 240 mmol) and chloromethyl methyl ether (12.2 mL, 160 mmol) at ambient temperature and subsequently heated to reflux for 1 h. The mixture was allowed to cool to ambient temperature and washed with 1N aqueous sodium hydroxide for 1 h and extracted with chloroform. The organic phase was concentrated and then the resulting solid was washed successively with 1N sodium hydroxide, water and then ethanol. Two recrystallizations from ethyl acetate produced 5 (6.87 g, 25.6 mmol, 96%) as a yellow solid: $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 8.26-8.22 (2H), 8.02 (dd, 1H, $J = 7.7, 1.1$ Hz), 7.79-7.70 (2H), 7.68 (dd, 1H, $J = 8.4, 7.7$ Hz), 7.56 (dd, 1H, $J = 8.4, 1.1$ Hz), 5.39 (s, 2H), 3.56 (s, 3H); IR (CHCl$_3$) 1673, 1586, 1211, 769 cm$^{-1}$; MS (EI) $m/e$ (%) 268 (M$^+$, 49), 237 (87), 208 (100), 180 (46), 151 (38), 139 (61); HRMS (EI) calcd for C$_{16}$H$_{12}$O$_4$ (M$^+$) 268.0735, found 268.0722; Anal. Calcd for C$_{16}$H$_{12}$O$_4$: C, 71.64; H, 4.51. Found: C, 71.62; H, 4.63.

1-Methoxymethoxyanthracene (6). To a suspension of 5 (1.67 g, 6.2 mmol) in 2-propanol (30 mL) was added sodium borohydride (0.34 g, 9.1 mmol) at 0 °C. The reaction mixture was heated to reflux for 18 h, and treated with 2N hydrochloric acid at 0 °C until the pH of the mixture became 4–6. The mixture was extracted with ethyl acetate. The crude product was purified by column chromatography on silica gel, eluting with toluene to give 6 (1.29 g, 5.4 mmol, 87%) as a yellow solid: $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 8.83 (s, 1H), 8.36 (s, 1H), 8.04–
7.96 (2H), 7.63 (d, 1H, J = 8.6 Hz), 7.47-7.42 (2H), 7.34 (dd, 1H, J = 8.6, 7.5 Hz), 7.00 (d, 1H, J = 8.4 Hz), 5.46 (s, 2H), 3.58 (s, 3H); IR (CHCl₃) 3056, 1221, 1141, 1045 cm⁻¹; MS (EI) m/e (%) 238 (M⁺, 100), 208 (53), 193 (26), 175 (35), 165 (100), 163 (21); HRMS (EI) calcd for C₁₆H₁₄O₂ (M⁺) 238.0994, found 238.0971; Anal. Calcd for C₁₆H₁₄O₂: C, 80.65; H, 5.84. Found: C, 80.55; H, 5.92.

1-Methoxymethoxy-2-anthraldehyde (7). To a solution of 6 (1.99 g, 8.35 mmol) in tetrahydrofuran (30 mL) was added n-butyl lithium (1.6M solution in hexane, 10.5 mL, 16.8 mmol) at 0 °C, and the mixture was stirred for 1 h at 0 °C. To the mixture was added N,N-dimethylformamide (1.94 mL, 25.0 mmol) at 0 °C and stirred at 0 °C for 1 h. The mixture was diluted with sat. aq. NH₄Cl at 0 °C, and extracted with ethyl acetate. The crude product was purified by column chromatography on silica gel, eluting with 10% ethyl acetate in hexane to give 7 (1.78 g, 6.70 mmol, 80%) as a yellow solid: mp. 108-110 °C; 1H NMR (CDCl₃, 400 MHz) δ 10.56 (s, 1H), 8.81 (s, 1H), 8.42 (s, 1H), 8.08-8.00 (2H), 7.82-7.80 (2H), 7.58-7.51 (2H), 5.38 (s, 2H), 3.7 (s, 3H); IR (CHCl₃) 1675, 1621, 1235, 923, 890 cm⁻¹; MS (EI) m/e (%) 266 (M⁺, 60), 236 (20), 220 (100), 206 (39), 199 (25), 165 (80), 164 (40); HRMS (EI) calcd for C₁₇H₁₄O₃ (M⁺) 266.0943, found 266.0952; Anal. Calcd for C₁₇H₁₄O₃: C, 76.68; H, 5.30. Found: C, 76.21; H, 5.38.

1-Hydroxy-2-anthraldehyde (8). To a solution of 7 (0.70 g, 2.6 mmol) in methanol (40 mL) was added conc. hydrochloric acid (0.50 mL) at ambient temperature, and the reaction mixture was stirred for 4 h. The mixture was diluted with sat. aq. NaHCO₃ and extracted with ethyl acetate. The crude product was purified by column chromatography on silica gel, eluting with 5% ethyl acetate in hexane to give 8 (0.54 g, 2.4 mmol, 93%) as a yellow solid: mp. 138-140 °C; 1H NMR (CDCl₃, 400 MHz) δ 13.29 (s, 1H), 9.95 (s, 1H), 9.07 (s, 1H), 8.30 (s, 1H), 8.08 (d, 1H, J = 8.4 Hz), 7.98 (d, 1H, J = 8.2 Hz), 7.59-7.48 (3H), 7.41 (d, 1H, J = 9.0 Hz); IR (CHCl₃) 3550-3100, 1621, 1210, 735, 667 cm⁻¹; MS (EI) m/e (%) 222 (M⁺, 100), 221 (20), 165 (50), 164 (20); HRMS (EI) calcd for C₁₅H₁₀O₂ (M⁺) 222.0681, found 222.0688; Anal. Calcd for C₁₅H₁₀O₂: C, 81.06; H, 4.53. Found: C, 80.76; H, 4.278.08.

1-(tert-Butyldimethylsilyloxy)-2-anthraldehyde (9). To a solution of 8 (432 mg, 1.94 mmol) and 2,6-lutidine (0.45 mL, 3.88 mmol) in dichloromethane (15 mL) was added tert-butyldimethylsilyl trifluoromethanesulfonate (0.89 mL, 3.88 mmol) at -78 °C, and the reaction mixture was stirred at -78 °C for 2 h. After diluted with sat. aq. NaHCO₃ at -78 °C, the mixture was warmed to ambient temperature and extracted with ethyl acetate. Short column chromatography on silica gel with 5% ethyl acetate in hexane gave crude product 9 (0.57 g) as an orange solid. This crude product was used for next reaction without purification.

(4R,5R,1'Z)-4-{3-[1-(tert-Butyldimethylsilyloxy)-2-anthryl]-3-hydroxy-1-propynyl}-5-(1'-propenyl)-2,2,4-trimethyl-1,3-dioxorane (11). To a solution of 10 (99.2 mg, 0.55 mmol) and anhydrous cerium chloride (411 mg, 1.7 mmol) in tetrahydrofuran (5 mL) being stirred at ambient temperature for 10 min was added lithium hexadisilazide (1.0 M in tetrahydrofuran, 1.1 mL, 1.1 mmol) at -78 °C, and the reaction mixture was stirred at -78 °C for 15 min. After addition of a solution of 9 (187 mg) in tetrahydrofuran (1 mL) at -78 °C, the reaction mixture was stirred at -78 °C for 15 min. It was
diluted with *sat. aq.* NH₄Cl at -78 °C, and extracted with ethyl acetate. The crude product was purified by column chromatography on silica gel, eluting with 35% toluene in hexane to give 11 (98.8 mg, 0.19 mmol, 35%) as a yellow oil: ¹H NMR (CDCl₃, 400 MHz) δ 8.67 (s, 1H), 8.34 (s, 1H), 7.99, 7.96 (sx₂, total 2H), 7.73–7.63 (2H), 7.50–7.43 (2H), 6.06–6.04 (1H), 5.85–5.75 (1H), 5.42–5.36 (1H), 5.07, 5.03 (ddx₂, total 1H, J = 8.6, 1.1 Hz), 2.16–2.12 (1H), 1.70, 1.60 (ddx₂, total 3H, J = 7.0, 1.7 Hz), 1.48–1.43 (6H), 1.37, 1.35 (sx₂, total 3H), 1.20, 1.19 (sx₂, total 9H), 0.24–0.22 (6H); IR (CHCl₃) 3571, 1650, 1386, 1105 cm⁻¹; MS (EI) m/e (%) 457.1840. To solution of ketone (29.8 mg, 57.9 μmol) and triphenylphosphine (7.0 mg, 26.7 μmol) in toluene (1 mL) was added diethyl azodicarboxilic acid (6 μL, 37.9 μmol) at 0 °C, and the reaction mixture was stirred at ambient temperature for 3 days. The mixture was poured onto *sat. aq.* NaHCO₃ and ethyl acetate, and extracted. The crude product was purified by column chromatography on silica gel, eluting with 50% ethyl acetate in hexane to give the corresponding diol (6.9 mg, 19.1 μmol, 91%) as a white solid: ¹H NMR (CDCl₃, 400 MHz) δ 8.92 (s, 1H), 8.39 (s, 1H), 8.12–8.04 (2H), 7.90 (d, 1H, J = 9.0 Hz), 7.72 (d, 1H, J = 9.0 Hz), 7.64–7.57 (2H), 6.82 (s, 1H), 5.93–5.85 (m, 1H), 5.74–5.67 (m, 1H), 5.13 (d, 1H, J = 9.3 Hz), 3.34 (br, 1H), 2.53 (br, 1H), 1.80 (dd, 3H, J = 7.0, 1.8 Hz), 1.64 (s, 3H); MS (FAB) (NBA) m/e 361 [(M+H)+]; HRMS (FAB) calcd for C₂₃H₂₄O₄ [(M+H)+] 361.1440, found 361.1444. To a solution of the diol (6.9 mg, 19.1 μmol) and triphenylphosphine (7.0 mg, 26.7 μmol) in toluene (1 mL) was added diethyl azadicarboxilic acid (6 μL, 37.9 μmol) at 0 °C, and the reaction mixture was stirred at ambient temperature for 24 h. The mixture was concentrated in vacuo and purified by column chromatography on silica gel, eluting with 13% toluene in hexane to give 3 (4.1 mg, 12.0 μmol, 63%) as a white solid: ¹H NMR (CDCl₃, 400 MHz) δ 9.00 (s, 1H), 8.47 (s, 1H), 8.14 (m, 1H), 8.08–8.04 (2H), 7.88 (d, 1H, J = 9.0 Hz), 7.63–7.56 (2H), 6.85 (s, 1H), 6.12–6.04 (m, 1H), 5.85–5.78 (m, 1H), 5.10 (d, 1H, J = 10.0 Hz), 1.68 (s, 3H), 1.63 (s, 3H), 1.56 (s, 3H), 1.43 (dd, 3H, J = 7.0, 1.8 Hz); IR (CHCl₃) 3263, 2995, 1650, 1386, 1105 cm⁻¹; MS (EI) m/e (%) 400 (M⁺, 59), 289 (100), 288 (75), 149 (43); HRMS (EI) calcd for C₂₆H₂₄O₄ (M⁺) 400.1674, found 400.1658.

(4S,5R,1'Z)-4-[(4H -anthra[1,2-b]pyran-2-yl)-4H-anthra[1,2-b]-2,2,4-trimethyl-1,3-dioxorane (12). To a solution of 11 (56.1 mg, 109 μmol) in dichloromethane (3 mL) was added manganese dioxide (50 mg), and the reaction mixture was stirred for 3 h at ambient temperature. The mixture was diluted with ethyl ether, filtered, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel, eluting with 13% ethyl acetate in hexane to give the corresponding ketone (38.7 mg, 75.2 μmol, 69%) as a yellow oil: MS (EI) m/e (%) 457 [(M-Bu'⁺), 10], 368 (18), 294 (37), 149 (100); HRMS (EI) calcd for C₂₂H₂₀O₂Si [(M-Bu'⁺) 457.1836, found 457.1840. To solution of ketone (29.8 mg, 57.9 μmol) and 18-crown-6 (30.7 mg, 116 μmol) in N,N-dimethylformamide (3 mL) was added potassium fluoride (6.8 mg, 117 μmol) at 0 °C, and the reaction mixture was stirred at ambient temperature for 1 h. The mixture was diluted with *sat. aq.* NH₄Cl, and extracted with ethyl acetate. The crude product was purified by column chromatography on silica gel, eluting with 13% ethyl acetate in hexane to give 12 (17.3 mg, 43.2 μmol, 75%) as a yellow oil: ¹H NMR (CDCl₃, 400 MHz) δ 8.84 (s, 1H), 8.46 (s, 1H), 8.08–8.04 (3H), 7.87 (d, 1H, J = 9.0 Hz), 7.63–7.56 (2H), 6.85 (s, 1H), 6.12–6.04 (m, 1H), 5.85–5.78 (m, 1H), 5.10 (d, 1H, J = 10.0 Hz), 1.68 (s, 3H), 1.63 (s, 3H), 1.56 (s, 3H), 1.43 (dd, 3H, J = 7.0, 1.8 Hz); IR (CHCl₃) 3263, 2995, 1650, 1386, 1105 cm⁻¹; MS (EI) m/e (%) 400 (M⁺, 59), 289 (100), 288 (75), 149 (43); HRMS (EI) calcd for C₂₆H₂₄O₄ (M⁺) 400.1674, found 400.1658.
5.81 (ddd, 1H, J = 11.2, 7.1, 1.1 Hz), 5.18 (dq, 1H, J = 8.6, 1.7 Hz), 3.95 (d, 1H, J = 8.6 Hz), 1.96 (s, 3H), 1.85 (dd, 3H, J = 7.1, 1.7 Hz); IR (CHCl₃) 3129, 1651, 1223, 909 cm⁻¹; MS (EI) m/e (%): 342 (M⁺, 100), 300 (33), 220 (76); HRMS (EI) calcd for C₂₃H₁₈O₃ (M⁺) 342.1256, found 342.1245.

Cleavage of pBR322 Supercoiled DNA by 2 and 3. Kapurimycin ABC analog 2 or ABCD’ analog 3 (10 μM or 1 μM) was incubated with 40 μM pBR322 supercoiled DNA (Nippon Gene) in 10% (v/v) acetonitrile/50 mM Tris-HCl buffer (10 μL, pH 7.5) at 37 °C for 5 h. The samples were successively precipitated with ethanol to remove the drug and the recovered DNA pellets were dried and incubated again in 10 μL of 50 mM Tris-HCl buffer at 37 °C for 24 h. To heat treated samples was added 10 μL of loading buffer containing 0.05% (w/v) bromophenol blue and 6% (v/v) glycerol for electrophoresis. Different forms of DNA were separated at ambient temperature on a 1% (w/v) agarose gel involving ethidium bromide (0.5 pg/mL). The gels were placed on a UV transilluminator (313 nm) and photographed with Polaroid 665 film. The result was shown in Figure 1.

Preparation of ³²P-5'-End-Labeled Oligodeoxynucleotide

Duplex. 400 pmol of single-strand oligodeoxynucleotide, purchased from Greiner Japan Co. Ltd. was 5'-end-labeled by phosphorylation with 4 μL of [γ³²P]ATP (Amersham, 370 MBq/μL) and 4 μL T4 polynucleotide kinase (Takara, 10 units/μL) using standard procedures. The 5'-end-labeled DNA was recovered by ethanol precipitation and further purified by 15% nondenatured gel electrophoresis and isolated by the crush and soak method. The isolated DNA was incubated with equimolar of complementary DNA in 100 μL of water at 90 °C for 5 min and cooled slowly to ambient temperature for forming the duplex.

Cleavage of ³²P-5'-End-Labeled Oligodeoxynucleotide. Single-stranded 43-mer DNA oligomers 5'-d(TTTTGTGTTAGTGTTTGGTGATTGTTTTT)-3' and the corresponding complementary oligomer were purchased from Greiner Japan Co. Ltd. The ³²P-5'-end-labeled ODN duplex was prepared as shown above. 50 μM of 1, 2 or 3 was incubated with 10 μM of calf thymus DNA and ca. 1.0x10⁶ cpm ³²P-5'-end-labeled ODN duplex in 20 mM Tris-HCl buffer (100 μL, pH 7.6) at 37 °C. At each time, the sample (10 μL) was separated from the reaction mixture, and precipitated with methanol. The recovered DNA was dissolved in 100 μL of 10% (v/v) piperidine and heated at 90 °C for 30 min. The mixture was concentrated in vacuo and resuspended in 10 μL of 80% formamide loading buffer (80% formamide, 1 mM EDTA, 0.1% xylene cyanole and 0.1% bromophenol blue). The samples (1 μL) were loaded onto 15% polyacrylamide and 7 M urea sequence gel and electrophoresed at 1900 V for ca. 2 h. The gel was dried and exposed to X-ray film with intensifying sheet at -70 °C. The result was shown in Figure 2.

Preparation of Oligodeoxynucleotide with 7-Deazaguanine.

Both 16-mer DNA oligomer 5'-d(TTTTGCTGATTGGTGT)-3' as primer for DNA elongation and the 32-mer DNA oligomer 5'-d(AAAAGCATCAACACACACAATCGAAGC)-3' as a template for DNA elongation were purchased from Greiner Japan Co. Ltd. Before oligomer elongation, the primer was ³²P-end-labeled as shown above and annealed with template oligomer. To a solution of the annealed oligomer complex in reaction buffer (10 mM Tris-HCl, pH ___
7.3, 10 mM MgCl₂, 1 mM DTT) was added dATP, dCTP, dTTP (1 mM, Takara), 7-deaza-dGTP (1 mM, Boehringer Mannheim) and exo-Klenow Fragment (5 units, Ambion). The reaction mixture was incubated at 37 °C for 2 h. The reaction mixture was precipitated with ethanol and purified by 20% non-denatured gel electrophoresis and isolated by the crush and soak method.9

Cleavage of ³²P-5'-End-Labeled Oligodeoxynucleotide with 7-Deazaguanine Sites. ³²P-5'-End-labeled ODN duplex was prepared as shown above. Kapurimycin A₃ (1) (5 μM) or its analog 3 (50 μM) was incubated with 10 μM of calf thymus DNA and ca. 1.0x10⁶ cpm ³²P-5'-end-labeled ODN duplex in 20 mM Tris-HCl buffer (100 μL, pH 7.6) at 37 °C for 24 h. The sample was precipitated with methanol and dried. The recovered DNA was dissolved in 100 μL of 10% (v/v) piperidine and heated at 90 °C for 30 min. The mixture was concentrated in vacuo and resuspended in 10 μL of 80% formamide loading buffer (80% formamide, 1 mM EDTA, 0.1% xylene cyanole and 0.1% bromophenol blue). The samples (1 μL) were loaded onto 15% polyacrylamide and 7 M urea sequence gel and electrophoresed at 1900 V for ca. 2 h. The gel was dried and exposed to X-ray film with intensifying sheet at −70 °C. The result was shown in Figure 3.

Supercoiled DNA Unwinding Assay. To a solution of pBR 322 plasmid DNA (250 ng, Nippon Gene) in topo I reaction buffer (10 mM Tris-HCl, pH 7.9, 1 mM EDTA, 150 mM NaCl, 0.1 mM spermidine, 5% glycerol, 0.1% BSA) was added human topoisomerase I (TopoGEN, 4 units). The reaction mixture was incubated for 30 min at 37°C. After the drug (100 μM) was added, the incubation was continued for another 30 min at 37°C. The reaction was terminated by addition of SDS to 1%.

After proteinase K was added to 50 μg/mL, the mixture was digested for 20 min at 56°C. Addition of 0.1 vol. of 10× gel loading buffer was followed by chloroform extraction. Different forms of DNA were separated at room temperature on a 1% agarose gel. The gel was stained for 30 min with ethidium bromide (0.5 μM/mL) and destained for 20 min in water. It was placed on a UV transilluminator (313 nm) and photographed with Polaroid 665 film. The result was shown in Figure 4.

Cleavage of ³²P-5'-End-Labeled Oligodeoxynucleotide Duplex with Bulge Sites. Each of single-stranded DNA oligomers in Figure 8 (ODNs 1–5) were purchased from Greiner Japan Co. Ltd. The ³²P-5'-end-labeled ODN duplex was prepared as shown above. 50 μM of 3 was incubated with 10 μM of calf thymus DNA and ca. 1.0x10⁶ cpm ³²P-5'-end-labeled ODN duplex with or without the bulge site in 20 mM Tris-HCl buffer (100 μL, pH 7.6) at 37 °C for 5 h. The sample was precipitated with methanol and dried. The recovered DNA was dissolved in 100 μL of 10% (v/v) piperidine and heated at 90 °C for 30 min. The mixture was concentrated in vacuo and resuspended in 10 μL of 80% formamide loading buffer (80% formamide, 1 mM EDTA, 0.1% xylene cyanole and 0.1% bromophenol blue). The samples (1 μL) were loaded onto 15% polyacrylamide and 7 M urea sequence gel and electrophoresed at 1900 V for ca. 2 h. The gel was dried and exposed to X-ray film with intensifying sheet at −70 °C. The result was shown in Figure 5.
References


(5) Refer to the previous chapter.


CHAPTER 4

Effect of Absolute Configuration of Epoxy Subunit on Guanine-Guanine Sequence Selective Alkylation

Abstract: We prepared two kapurimycin analogs, (S, S)-ABCD' 2 and enantiomeric (R, R)-ABCD' ent-2, and compared their sequence selectivity for DNA alkylation. (S, S)-Analog 2 exhibited a high sequence selectivity (G*G > G*A > G*T > G*C), whereas (R, R)-analog ent-2 showed a lower reactivity toward DNA than (S, S)-isomer 2 and equally reacted at all guanine sites without sequence selectivity of its alkylation. DNA unwinding assay demonstrated that both analogs have very similar binding ability. The model of drug-DNA complex showed that the stereochemical orientation of the epoxy subunit considerably affects the reactivity toward DNA as well as the sequence selectivity for DNA alkylation.
Introduction

The study of interactions of DNA with ligands of differing chiralities provides a rich ground for applications to mechanisms of molecular recognition, probing of polymorphism of DNA, and structure–biological activity correlations. Comparing the DNA-interaction chemistry between individual members of enantiomeric or diastereomeric pairs of chiral metal complexes, antitumor agents, and carcinogens has provided sharp insights into such phenomena. Recent notable examples are the alkylation of DNA by the stereoisomers of the carcinogenic benzo[a]pyrene epoxides (BPDE), the natural and unnatural enantiomers of CC-1065, and enantiomeric pairs of CC-1065 functional derivatives and of duocarmycin SA, antibiotics mitomycin C and staurosporin as well as the enantiospecific recognition of DNA for cleavage by bleomycin. NMR studies of the DNA adducts of the BPDE enantiomers (+)- and (-)-anti-BPDE revealed an extraordinary relationship among the stereochemistry of the drug-DNA linkage, adduct orientation in the minor groove, and tumorigenicity. The two enantiomers of CC-1065 were shown to be potent cytotoxins, which however displayed distinct DNA sequence selectivities and opposite groove orientation of the adducts.

An antitumor antibiotic kapurimycin A₃ (1) is known to selectively alkylate guanine N7 of guanine-guanine (GG) sequence. In the previous study, using the analog of 1, 2, we investigated the relationship between the aromatic ring system and the sequence selectivity for DNA alkylation and elucidated that the intercalation of the planar aromatic portion into two guanine bases of GG sequence was very important for their GG selective alkylation. The binding of the aromatic ring system to DNA facilitates the approach of the epoxy subunit close to guanine N7. Thus, the sequence selectivity of DNA binding directly reflects the sequence selective DNA alkylation. The guanine N7 alkylation step by the epoxy group also seems to be important for the sequence selective alkylation.

The use of the enantiomer of the DNA binding antibiotic was the promising methods to get insight into the action mechanism of antibiotics with DNA and provided very useful information on the structure of drug-DNA complex and the subsequent alkylation. In order to understand the mechanism of sequence selective guanine alkylation, we prepared the (S, S)-ABCD' analog 2 having the same absolute configuration of the epoxy subunit as that of natural kapurimycin A₃, and the enantiomer (R, R)-ABCD' analog ent-2, and compared their sequence selectivities for guanine alkylation. Herein, we report the relationship between the absolute configuration of the epoxy subunit and the sequence selectivity for guanine alkylation.

Results and Discussion

(R, R)-ABCD' analog ent-2 with an opposite configuration to (S, S)-ABCD' analog 2 was prepared from optically active side chain unit 3 as described previously. Asymmetric dihydroxylation of p-
methoxybenzyl ether 4 obtains from a commercially available (E)-3-methyl-2-penten-4-yn-1-ol with AD-mix-$\alpha$ produced optically active diol 5 which was converted to alcohol 3 (91% e.e.). We optically purified alcohol 3 by means of optical resolution. In the purification of 3, we condensed 3 with (−)-(S)-2-methoxy-2-trifluoromethylphenylacetic (MTPA) acid and removed the undesired isomer by use of HPLC with chiral column (Scheme 1 and Figure 1). Reduction of collected MTPA ester by DIBAL gave optically pure 3 again (>99% e.e.). (R, R)-ABCD' analog ent-2 was synthesized from optically pure 3 according to the synthetic route of 2.

\[ \text{Scheme 1.} \]

\[
\begin{align*}
\text{OPMB} & \xrightarrow{\text{AD-mix-$\alpha$}} \text{OPMB} & \quad \text{ref. 4} \\
\text{4} & \quad & \text{5} \\
& \quad & \text{ref. 4} \\
& \quad & \text{TMS} \\
\end{align*}
\]

\[ \begin{align*}
(\text{S})-\text{MTPA} & \quad \text{DCC} / \text{DMAP} \\
\text{3} & \quad \text{2} \text{Separation} \\
& \quad \text{(Figure 1)} \\
\end{align*}\]

\[
\begin{align*}
\text{O-(S)-MTPA} & \quad \text{ref. 4} \\
\text{TMS} & \quad \text{TMS} \\
\text{3} & \quad \text{ent-2} \\
\end{align*}
\]

In order to know the relationship between the epoxide absolute configuration and the sequence selectivity, the reaction of DNA with 2 and ent-2 was examined using $^{32}$P-5'-end-labeled oligodeoxynucleotides and analyzed by electrophoresis on denatured polyacrylamide gel (Figure 2). The DNA cleavage by treatment with 2 or ent-2 followed by hot piperidine treatment was observed at all guanine sites. The DNA cleavage experiment indicated that (S, S)-ABCD' analog 2 alkylated DNA much more effectively than (R, R)-isomer ent-2. Of special interest is that the alkylation by 2 was highly selective for 5'-side guanine of 5'-GG sequences (G*G > G*A > G*T > G*C), but ent-2 alkylated at guanine with no sequence selectivity.
Figure 2. Strand breakage assays depicting reactive sites for 2 and ent-2 on the oligodeoxynucleotide duplex. 32P-5'-end-labeled oligodeoxynucleotide previously prepared was treated with 2 or ent-2 (50 μM) in a reaction buffer (20 mM Tris-Cl, pH 7.6 and 10 μM calf thymus DNA) at 37 °C. After ethanol precipitation, the residue was heated in 10% piperidine at 90 °C for 30 min. The resulting DNAs were analyzed by electrophoresis on 15% denatured polyacrylamide gel. The height of bars in this histogram showed the percentage of strand breakage at a given site relative to the total strand breakage. The cleaving sites of DNA treated with 2 for 5 h is shown by black bars; ent-2 for 24 h is shown by white bars.

Next, we examined the binding ability of kapurimycin analogs 2 and ent-2 to DNA by means of DNA unwinding assay using topoisoeromerase I. The solution of pBR322 supercoiled DNA treated with 2 or ent-2 was incubated with topoisoeromerase I at 37 °C for 30 min (Figure 3). The DNAs treated with ent-2 exhibited closely similar mobility to those with 2. It is indicated that ABCD' analog ent-2 of (R, R)-configuration had almost same intercalative DNA binding ability as (S, S)-isomer 2. Thus, the lack of reactivity and sequence selectivity observed for ent-2 is completely different from the DNA binding by its planar aromatic portion.

Figure 3. Unwinding of supercoiled pBR322 DNA by topoisoeromerase I in the presence of kapurimycin analogs 2 and ent-2. Supercoiled pBR322 DNA (250 ng) was first treated with topoisoeromerase I (topo I) for 30 min in a reaction buffer (10 mM Tris-Cl, pH 7.9, 1 mM EDTA, 150 mM NaCl, 0.1 mM spermidine, 5% glycerol, 0.1% BSA), and further incubated for 30 min in the absence (lane 1) and presence of 2 and ent-2 (10 μM) (lanes 3 and 5, respectively). The resulting DNAs were analyzed by electrophoresis on 1% native agarose gel at 1.3 V/cm for 18 h. DNAs in lanes 2, 4 and 6 were treated as for lanes 1, 3 and 5, respectively, in the absence of topo I. lane 1, topo I; lane 2, intact DNA; lane 3, 2 with topo I; lane 4, 2; lane 5, ent-2 with topo I; lane 6, ent-2.

Figure 4. DNA-drug binding models. (a) exo-aflatoxin B1 oxide-GG/CC complex; (b) endo-aflatoxin B1 oxide-GG/CC complex; (c) (S, S)-ABCD 2-GG/CC complex. These models were obtained from optimization of drug-5'-d(TGTT)-3'S'-d(ACCA)-3' complex by Amber* force field in water by mean of Macromodel. DNA backbone and A/C base pairs were removed and guanine N9 and cytosine N1 was substituted by methyl group, respectively.
The (S, S)-analog 2 reacts efficiently with DNA, whereas (R, R)-analog ent-2 has only little reactivity toward DNA in spite of its effective DNA binding. A rational reason for the different efficiency of their DNA alkylation was proposed in the DNA alkylation by aflatoxin B$_1$ oxide. Carcinogen aflatoxin B$_1$ is converted to biologically active form by chemical oxidation with dimethyldioxirane or by enzymatically by cytochrome P450 mixed-function oxidases. Both processes gave rise to a mixture of the exo- and endo-8,9-epoxides of aflatoxin B$_1$. While exo-aflatoxin B$_1$ oxide is a potent mutagen, endo isomer fails to react with DNA. Harris et al. have previously proposed that the reaction of the exo epoxide of aflatoxin B$_1$ with DNA involves an intercalated transition state. They have obtained a number of lines of evidence which indicate that aflatoxin B$_1$ and many of its derivatives strongly associate with DNA and that the association involves intercalation of the planar portion of the aflatoxin moiety into the DNA. According to their proposal, we built a binding model of aflatoxin B$_1$ oxide with DNA in order to rationalize the remarkable difference in the reactivity of the aflatoxin B$_1$ oxide stereoisomers with DNA (Figure 4). Whereas the intercalation of the planar portion of exo-aflatoxin epoxide suitably oriented for the oxirane moiety for SN$_2$ attack to the epoxy group (Figure 4a), a similar intercalation of the endo epoxide places the epoxide ring inaccessible for SN$_2$ attack (Figure 4b). The binding model of kapurimycin (S, S)-ABCD' analog 2 with DNA also showed that the orientation of 2 is highly suitable for backside attack of guanine N7 on the epoxide (Figure 4c). Furthermore, the distance from epoxide carbon of 2 to 5'-side guanine N7 of the intercalation site is only ca. 2.9 Å and close enough for S$_8$2 reaction. Thus, when (S, S)-ABCD' analog 2 intercalates into DNA, the epoxide is oriented at the favorable site for S$_8$2 attack by guanine N7. However, (R, R)-isomer ent-2 was less reactive and less sequence selective than (S, S)-isomer 2. According to these models, the lack of the reactivity and the sequence selectivity in ent-2 would indicate that the intercalation mode of the aromatic planar portion of ent-2 is not optimally suited for S$_8$2 attack of guanine N7 to the epoxide.

Conclusion

We have synthesized kapurimycin analogs, (S, S)-ABCD' 2 and its enantiomeric (R, R)-ABCD' ent-2 and compared their sequence selectivities for DNA alkylation. While (S, S)-analog 2 showed a higher reactivity and the sequence selectivity for the guanine alkylation, (R, R)-isomer ent-2 reacted with DNA more slowly with no sequence selectivity. When (S, S)-ABCD' analog 2 intercalates into DNA, the orientation of (S, S)-epoxide is optimally suited for the S$_8$2 attack from the back side of the epoxide by 5'-side guanine N7 at its intercalation site. The (S, S) of epoxide showed 5'-side guanine selectivity in the alkylation of GG sequence. The mechanistic study of sequence selective DNA alkylation using (S, S)-ABCD' analog 2 would be very useful for explaining the mechanism of the GG sequence selective alkylation by an antibiotic kapurimycin A$_3$ as well as 3'-side guanine selectivity in GG selective alkylation by aflatoxin B$_1$ oxide and psorospermin.
Experimental Section

General Techniques. ¹H NMR spectra were measured with JEOL JNM a-400 (400 MHz) spectrometers. Coupling constants (J values) are reported in Hz. The chemical shifts are expressed in ppm downfield from tetramethylsilane, using residual chloroform (δ = 7.24 in ¹H NMR) as an internal standard. The following abbreviations were used to explain the multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. IR spectra were recorded on JASCO FT/IR-5M spectrophotometers. Melting points were obtained on a Yanagimoto Seisakusho micro melting point apparatus and are uncorrected. Optical rotations were recorded using a Perkin-Elmer 243 polarimeter. Electron impact mass spectra (MS) and high-resolution mass spectra (HRMS) were recorded on JEOL JMS-DX 300 or JEOL JMS-SX 102A. Microanalyses were performed by Kyoto University Microanalytical Center.

All reactions were monitored by thin layer chromatography carried out on 0.25-mm E. Merck silica gel plates (60F-254) using UV light, 5% ethanolic phosphomolybdic acid, or p-anisaldehyde solution and heat as developing agent. Wako gel (C-200, particle size 75-150 μm, Wako) was used for column chromatography. Plasmid pBR322 DNA was purchased from Wako. Tetrahydrofuran and ethyl ether were distilled over sodium-benzophenone. Dichloromethane, toluene and N,N-dimethylformamide was distilled over calcium hydride. All reagents were purchased at highest commercial quality and used without further purification unless otherwise stated.

All reactions were carried out under nitrogen atmosphere with anhydrous solvents under anhydrous conditions, unless otherwise noted.

Yields refer to chromatographically and spectroscopically (¹H NMR) homogeneous materials, unless otherwise stated.

Purification of Alcohol 3 for the Synthesis of (R, R)-ABCD′-Analog ent-2. To a solution of alcohol 3 (196 mg, 0.92 mmol) in dichloromethane (5 mL) was added (-)-(S)-2-methoxy-2-trifluoromethylphenylacetic (MTPA) acid (215 mg, 0.92 mmol), dicyclohexylcarbodiimide (191 mg, 0.92 mmol) and N,N-dimethylaminopyridine (12.0 mg, 0.098 mmol) at ambient temperature, and the resulting solution mixture was stirred at ambient temperature for 1 h. The mixture was diluted with ethyl acetate, filtered and concentrated in vacuo. The crude product was purified by column chromatography on silica gel, eluting with toluene to give diastereomeric mixture of MTPA ester (354 mg). The mixture was separated by HPLC on Daicel CHIRALCEL OJ (10x250 mm, elution with 2% hexane in 2-propanol at a flow rate of 1.0 mL/min). The solution of given optically pure MTPA ester in elution solvent was concentrated in vacuo. ¹H NMR (CDCl₃, 400 MHz) δ 7.55–7.53 (2 H), 7.42–7.37 (3 H), 4.47–4.37 (3 H), 3.55 (d, 3 H, J = 1.1 Hz), 1.44 (s, 3 H), 1.40 (s, 3 H), 1.36 (s, 3 H), 0.13 (s, 3 H). Subsequently, to the solution of optically pure MTPA ester (101 mg, 0.22 mmol) in dichloromethane (2 mL) was added diisobutylaluminum hydride (1.0 M in toluene, 0.5 mL, 0.5 mmol) at −78 °C, and the solution was stirred at −78 °C for 15 min and at 0 °C for 15 min. The mixture was diluted with methanol (10 mL) and stirred at ambient temperature for 10 min, and further diluted with each 5 mL of ethyl acetate and sat. aq. Rochelle salt and stirred at ambient temperature for 30 min. The mixture was extracted with ethyl acetate. The crude product was purified by column chromatography on silica gel, eluting with 30% ethyl acetate in hexane to give diastereomeric mixture of
MTPA ester (41.0 mg, 0.19 mmol): [α]_D^25 = -19.17 (c 0.120, MeOH);
1H NMR (CDCl₃, 400 MHz) δ 4.31 (dd, 1 H, J = 3.9, 7.4 Hz), 3.73 (2 H), 1.73 (m, 1 H), 1.49 (s, 3 H), 1.43 (s, 3 H), 1.37 (s, 3 H), 0.14 (s, 9 H); IR (CHCl₃) 3020, 1217, 847, 754 cm⁻¹; Anal. Calcd for C₁₂H₂₂O₃Si: C, 59.46; H, 9.15. Found: C, 59.20; H, 9.30.

Preparation of ³²P-5'-End-Labeled Oligodeoxynucleotide Duplex. The 400 pmol of single-stranded oligodeoxynucleotide, purchased from Greiner Japan Co. Ltd., was 5'-end-labeled by phosphorylation with 4 μL of [γ-³²P]ATP (Amersham, 370 MBq/μL) and 4 μL T4 polynucleotide kinase (Takara, 10 units/μL) using standard procedures. The 5'-end-labeled DNA was recovered by ethanol precipitation and further purified by 15% nondenatured gel electrophoresis and isolated by crush and soak method.¹ The isolated DNA was incubated with equimolar of the complementary strand in 100 μL of water at 90 °C for 5 min and cooled slowly to ambient temperature for forming duplex.

Cleavage of ³²P-5'-End-Labeled Oligodeoxynucleotide. Single-stranded 43-mer DNA oligomers 5'-d(TTTTTGTTTGTTAGTT-CGTTTGGCTTGGATTGTTTGGTTT)-3' and the corresponding complementary oligomer were purchased from Greiner Japan Co. Ltd. The ³²P-5'-end-labeled ODN duplex was prepared as shown above. 50 μM of 1, 2 or 3 was incubated with 10 μM of calf thymus DNA and ca. 1.0×10⁶ cpm ³²P-5'-end-labeled ODN duplex in 20 mM Tris-HCl buffer (100 μL, pH 7.6) at 37 °C. At each time, the sample (10 μL) was separated from the reaction mixture and precipitated with methanol. The recovered DNA was dissolved in 100 μL of 10% (v/v) piperidine and heated at 90 °C for 30 min. The mixture was concentrated in vacuo and resuspended in 10 μL of 80% formamide loading buffer (80% formamide, 1 mM EDTA, 0.1% xylene cyanole and 0.1% bromophenol blue). The samples (1 μL) were loaded onto 15% polyacrylamide and 7 M urea sequence gel and electrophoresed at 1900 V for ca. 2 h. The gel was dried and exposed to X-ray film with intensifying sheet at -70 °C. The result was shown in Figure 2.

Supercoiled DNA Unwinding Assay. To a solution of pBR 322 plasmid DNA (250 ng, Nippon Gene) in topo I reaction buffer (10 mM Tris-HCl, pH 7.9, 1 mM EDTA, 150 mM NaCl, 0.1 mM spermidine, 5% glycerol, 0.1% BSA) was added human topoisomerase I (4 units, TopoGEN). The reaction mixture was incubated for 30 min at 37 °C. After the test drug (100 μM) was added, the mixture was incubated for another 30 min at 37 °C. The reaction was terminated by addition of SDS to 1%. After proteinase K was added to 50 μg/mL, the mixture was digested for 20 min at 56 °C. Addition of 0.1 vol. of 10× gel loading buffer was followed by chloroform extraction. Different forms of DNA were separated at room temperature on a 1% agarose gel. The gel was stained for 30 min with ethidium bromide (0.5 μM/mL) and destained for 20 min in water. It was placed on a UV transilluminator (313 nm) and photographed with Polaroid 665 film. The result was shown in Figure 3.
References and Notes

(9) Refer to chapter 3.
(12) We performed energy minimizations starting from idealized B-form DNA helices to which kapurimycin ABCD'-ring analog had been intercalated. We used the AMBER* set of force field parameters on

(13) The mean value of the results obtained from three calculations of drug-DNA complex.

CHAPTER 5

Sequence Selective Alkylation of Continuous Guanine Sequences by DNA Intercalators Possessing Epoxy Side Chain

Abstract: In order to elucidate the origin of sequence selectivity for the intercalation into DNA by aromatic ring of DNA alkylating agents, we synthesized DNA alkylating agents with various intercalators such as anthraquinone 4, anthracene 5 and methoxyanthracene 6. In the cleavage assay using $^{32}$P-5'-end-labeled DNA, the anthraquinone 4 having the most electron-poor ring system among these three synthetic intercalators, showed the highest DNA cleavage activity. The most reactive site of DNA cleavage in this experiment was 5'-side guanine of GG sequence. From DNA unwinding assay and CD titration, anthraquinone 4 intercalated into DNA and its intercalation ability was the highest among three synthetic intercalators. The ab initio calculation of LUMO energy of anthraquinone, anthracene and methoxyanthracene provided that anthraquinone has a lowest LUMO energy. Further, anthraquinone has the lowest reduction potential among these intercalators. We investigated the alkylation of GGG sequence whose HOMO have already been calculated and showed that anthraquinone 4 intercalated between 5'-side guanine and central guanine of GGG sequence. This result indicates that the sequence selective intercalation is created by interaction of HOMO localized at 5'-side guanine in GGG sequence with LUMO of the intercalator.
Introduction

In several previous papers it has so far been reported to modelize the interaction between DNA and DNA interacting agents, such as ethidium bromide, sulfur mustard and nitrogen mustard with a goal to access the interaction mechanism.\(^1\) Their calculations suggest that the intercalated complexes of drugs with DNA are stabilized by frontier orbital interactions between the lowest unoccupied molecular orbital (LUMO) of drug and the highest occupied molecular orbital (HOMO) of DNA. Our group has also examined the most reactive sites in DNA toward one electron photooxidation by the use of \textit{ab initio} calculation of the stacked base pair systems.\(^2\) Comparing HOMO energies obtained from \textit{ab initio} 6-31G* calculations, we found that the GG/CC system had the highest HOMO among the seven possible guanine-containing base pairs and that the susceptibility of GN sequences is in the following order, GG > GA > GT > GC. When two guanines are stacked each other in a B-form geometry, the HOMO is localized only on the 5'-side guanine.

An antitumor antibiotic kapurimycin A\(_1\) (1)\(^3\) intercalates into DNA and alkylate guanine N7 as well as aflatoxin B\(_1\) oxide (2)\(^4\) and psorospermin (3)\(^5\) do. In our study using kapurimycin analogs,\(^6\) we found that DNA cleavage by these analogs was highly sequence selective at 5'-side guanine of GG sequence. The 5'-side G selectivity for 5'-GN sequence decreased in the order of 5'-GG > GA > GT > GC.

The order of sequence selectivity for guanine alkylation by kapurimycin analogs is in good agreement with the results of our calculation. We assumed that the key to solve the reason for GG sequence selective alkylation was the interaction of guanine with HOMO on the guanine and LUMO of intercalated subunit of DNA alkylating agents. In order to elucidate the contribution of the LUMO energy of intercalators in such HOMO-LUMO interactions, we synthesized DNA alkylating agents possessing various intercalators such as 4, 5 and 6, and compared their reactivity toward DNA and their sequence selectivity for DNA alkylation. Herein, we report the relationship between the LUMO energy of intercalators and the sequence selectivity of guanine alkylation.
Results and Discussion

We synthesized DNA alkylating agent 4 from 1-hydroxyanthraquinone which was coupled with (2S)-(+)glycidyl 3-nitrobenzenesulfate as shown in Scheme 1. The other compounds 5 and 6 were also prepared from the corresponding 1-hydroxyanthracene derivatives in a similar fashion.

Scheme 1.

First, we examined the DNA cleaving assay by the use of the synthetic alkylating agents to know the relationship between the ring system of intercalators and their sequence selective DNA cleavage activity. The reaction of DNA with 4, 5 and 6 was examined using 32P-5'-end-labeled oligodeoxynucleotide and analyzed by electrophoresis on denatured polyacrylamide gel. The labeled oligodeoxynucleotide duplex was incubated with agents at 37 °C for 24 h and heated in 10% piperidine at 90 °C for 30 min to induce strand breakage. The result of the assay was shown in Figure 1. DNA cleavage was observed at all guanine sites in all these three cases. The reaction rates increased in the order of 4 > 5 > 6 and the DNA cleaving ability of 6 was very weak. Of special interest is that the cleavage by 4 was sequence selective at 5'-side guanine of GG sequence.

In order to know the correlation between the DNA binding modes and sequence selectivity, we examined DNA unwinding assay using topoisomerase I. Topoisomerase I is allowed to adjust DNA linking number in the presence of intercalator that influences the DNA unwinding angle, resulting in the shift of the gaussian distribution of topoisomers. The solution of pBR322 plasmid DNA and each drug was incubated with human topoisomerase I at 37 °C and the resulting DNAs

Figure 1. Strand breakage assays depicting reactive sites of oligodeoxynucleotide duplex for 4, 5 and 6. 32P-5'-end-labeled oligodeoxynucleotide previously prepared was treated with 4, 5 or 6 (500 μM) in a reaction buffer (20 mM Tris-HCl, pH 7.6 and 10 μM calf thymus DNA) at 37 °C for 24 h. After ethanol precipitation, the residue was heated in 10% piperidine at 90 °C for 30 min. The resulting DNAs were analyzed by electrophoresis on 15% denatured polyacrylamide gel. (a) Result of autoradiography. (b) DNA cleavage sites by 4. The height of bars in the histogram showed the percentage of strand breakage at a given site relative to the total strand breakage.
were analyzed by agarose gel electrophoresis. As clear from Figure 2, anthraquinone 4 intercalated into DNA much more strongly than 5 and 6 did. Furthermore, we observed the structural change of duplex DNA by addition of anthraquinone 4 by means of CD spectroscopy. The structure of calf thymus DNA (0.1 mM) in the presence of 4 (0.05 mM) was compared with that of calf thymus DNA in the absence of 4 in a buffer (10 mM Tris-HCl, pH 7.3, 10% (v/v) acetonitrile). The addition of 4 to a DNA solution led to the decrease of the characteristic Cotton effects ascribed to B-form DNA, the positive CD at 275 nm and the negative CD at 245 nm, together with the increase of the negative induced CD at 450 nm derived from the formation of 4-DNA complex. This result shows the structural change of DNA induced by intercalation of 4 to DNA duplex.

![Open-circular vs. Supercoiled DNA](image)

**Figure 2.** Unwinding of supercoiled pBR322 DNA by topoisomerase I in the presence of kapurimycin analogs 4, 5, and 6. Supercoiled pBR322 DNA (250 ng) was first treated with topoisomerase I (topo I) for 30 min in a reaction buffer (10 mM Tris-HCl, pH 7.9, 1 mM EDTA, 150 mM NaCl, 0.1 mM spermidine, 5% glycerol, 0.1% BSA), and further incubated for 30 min in the absence (lane 2) and presence of 4, 5, and 6 (10 μM) (lanes 4, 6, and 8, respectively). The resulting DNAs were analyzed by electrophoresis on 1% native agarose gel at 1.3 V/cm for 18 h. DNAs in lanes 1, 3, 5, and 7 were treated as for lanes 2, 4, 6, and 8, respectively, in the absence of topo I. lane 1, intact DNA; lane 2, topo I; lane 3, 6; lane 4, 6 with topo I; lane 5, 5; lane 6, 5 with topo I; lane 7, 4; lane 8, 4 with topo I.

Next, in order to know the reason for the different binding abilities of the intercalators, we investigated the electronic structure of intercalator by means of ab initio calculations. In this study, we performed the MO calculation of the molecules in which oxiranylmethoxy group was replaced with methyl group. The calculated LUMO energies of intercalators were shown in Table 1. The LUMO energy of anthraquinone was the lowest among the three intercalators and the order of the heights of LUMO energies of aromatic compounds was comparable to the order of the reactivities of the corresponding alkylating agents to DNA. Then, the reduction potentials of 4, 5, and 6 were measured by cyclic voltammetry. The reduction potentials were shown in Table 1. The reduction potential of 4 was the smallest among the three intercalators and the order of their potentials was in agreement with LUMO calculation. These results suggest that there is a correlation between the LUMO energy of the intercalators and the observed sequence selectivity for DNA alkylation.
Figure 4. LUMO of anthraquinone 4. MO calculation was performed at the HF/6-31G* level utilizing Spartan (version 4.0.2 GL).

Table 1. Reduction Potentials and LUMO Energies of Alkoxy-substituted Anthraquinone and Anthracene.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Reduction Potential (V)</th>
<th>LUMO Energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-(methoxymethoxy)anthraquinone</td>
<td>-0.70</td>
<td>0.0951^c</td>
</tr>
<tr>
<td>1-(methoxymethoxy)anthracene</td>
<td>-1.99</td>
<td>0.2118^d</td>
</tr>
<tr>
<td>1,5-bis(methoxymethoxy)anthracene</td>
<td>-2.02</td>
<td>0.2307^e</td>
</tr>
</tbody>
</table>

^a Reduction potential given from the cyclic voltammograms of DNA intercalators in 0.1 M LiClO₄ solution in acetonitrile. Scan rate: 100 mV/s. Working electrode: Pt wire. Reference electrode: Ag/AgCl (1.0 M KCl). Counter electrode: glassy carbon.

^b LUMO energies was calculated by ab initio (HF/6-31G*).

^c 1-Methoxyanthraquinone was calculated.

^d 1-Methoxyanthracene was calculated.

^e 1,5-Dimethoxyanthracene was calculated.

Next, we have examined the cleaving assay of the DNA containing GGG sequence by the use of 4 to elucidate that the interaction between DNA HOMO and intercalator LUMO. Our previous calculations have indicated that stacking of three guanine bases significantly lowered the HOMO energy and that the HOMO of the stacked 5'-GGG-3' was localized mainly on the 5'-side guanine in a B-form DNA duplex (Figure 5). If there is a HOMO-LUMO interaction between GGG sequence and intercalators, intercalators would not equally insert into two intercalation sites, i.e. the site between 5'-side guanine and central guanine (Figure 6, site A) and the site between central guanine and 3'-side guanine (Figure 6, site B). When the HOMO-LUMO interaction prevails, then the intercalators predominantly insert into site A.

Each drug was incubated with ³²P-5' end-labeled oligodeoxynucleotide as shown in Figure 7 and the DNA sample was subsequently separated from the reaction mixture at each time and treated with 10% piperidine solution at 90 °C for 30 min after ethanol precipitation. The result analyzed by polyacrylamide gel electrophoresis was shown in Figure 7. Anthraquinone 4 cleaved DNA preferentially at 5'-side guanine site of continuous guanine sequence. The ratio of cleaving intensity at each guanine in guanine triplet (G₁G₂G₃) by 4 in 5 h was about 6:2:1 and the ratio in guanine doublet G₁G₂ sequence was about 10:1. The selective alkylation of 5'-side guanine in GG sequence shows that 4 selectively alkylates 5'-side guanine at its intercalation site. Thus, judging from the efficiency of DNA cleavage at 5'-side guanine and central guanine in GGG sequence, 4 predominantly intercalates into site A and alkylates 5'-side guanine of the intercalation site. This result shows that the interaction between the HOMO on 5'-side guanine of GGG sequence and the LUMO of intercalators determines the sequence selectivity for the intercalation into DNA. In other words, this result suggests that the 5'-side guanine of continuous guanines is extremely important in HOMO–LUMO interactions of B-form DNA with electron-deficient intercalators.
Figure 5. HOMO of 5'-d(TGGGT)-3'/5'-d(ACCCA)-3'. MO calculation was performed at the HF/6-31G* level utilizing Spartan (version 4.0.2 GL).

Figure 6. Possible intercalation sites in GGG sequence. (a) Intercalation of 4 between G1 and G2 (site A). (b) Intercalation of 4 between G2 and G3 (site B). The length of arrow shows the probabilities of G alkylation by 4 intercalated into GG doublet being estimated on the basis of the result of Figure 1.

Figure 7. Strand breakage assays depicting reactive sites for 4 on the oligodeoxynucleotide duplex. 32P-5'-end-labeled oligodeoxynucleotide previously prepared was treated with 4 (500 μM) in a reaction buffer (20 mM Tris-HCl, pH 7.6 and 10 μM calf thymus DNA) at 37 °C. After ethanol precipitation, the residue was heated in 10% piperidine at 90 °C for 30 min. The resulting DNAs were analyzed by electrophoresis on 15% denatured polyacrylamide gel. (a) The result of autoradiography. (b) DNA cleavage sites by 4 in 5 h. The height of bars in the histogram shows the percentage of strand breakage at a given site relative to the total strand breakage.

Conclusion

We synthesized DNA alkylating agents possessing various intercalators such as anthraquinone 4, anthracene 5 and methoxy-anthracene 6 to investigate the sequence selectivity of their intercalation. It was shown that the ability of sequence selective DNA intercalation increased in the order of 4 > 5 > 6, which were reverse to the order of their LUMO energies. In 5'-G1G2G3 sequence, anthraquinone 4 having the lowest LUMO energy selectively intercalated between G1 and G2. This result shows that the sequence selectivity for intercalative DNA
Experimental Section

General Techniques. ¹H NMR spectra were measured with Varian Mercury (400 MHz) spectrometers. Coupling constants (J values) are reported in Hz. ¹³C NMR spectra were measured with Varian Mercury (100 MHz) spectrometers. The chemical shifts are expressed in ppm downfield from tetramethylsilane, using residual chloroform (δ = 7.24 in ¹H NMR, δ = 77.0 in ¹³C NMR) as an internal standard. The following abbreviations were used to explain the multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. IR spectra were recorded on JASCO FT/IR-5M spectrophotometers. UV-visible spectra were recorded on JASCO V-550 UV/Vis spectrophotometers. Melting points were obtained on a Yanagimoto Seisakusho micro melting point apparatus and are uncorrected. Optical rotations were recorded using a Perkin-Elmer 243 polarimeter. Electron impact mass spectra (MS) and high-resolution mass spectra (HRMS) were recorded on JEOL JMS-DX 300 or JEOL JMS-SX 102A. Cyclic voltammetry was performed using BAS 100B/W electrochemical analyzer. Circular dichromism spectra were recorded on JASCO J-700 spectrophotometer. Microanalyses were performed by Kyoto University Microanalytical Center.

All reactions were monitored by thin layer chromatography carried out on 0.25-mm E. Merck silica gel plates (60F-254) using UV light, 5% ethanolic phosphomolybdic acid, or p-anisaldehyde solution and heat as developing agent. Wako gel (C-200, particle size 75-150 μm, Wako) was used for column chromatography. Tetrahydrofuran and ethyl ether were distilled over sodium-benzophenone. Dichloromethane, toluene and N,N-dimethylformamide were distilled over calcium hydride. All
reagents were purchased at highest commercial quality and used without further purification unless otherwise stated.

All reactions were carried out under nitrogen atmosphere with anhydrous solvents under anhydrous conditions, unless otherwise noted. Yields refer to chromatographically and spectroscopically (1H NMR) homogeneous materials, unless otherwise stated.

1-(2-Oxiranylmethoxy)anthracene-9,10-dione (4). To a suspension of sodium hydride (60%, 20.2 mg, 0.51 mmol) in N,N-dimethylformamide (2 mL) was added 1-hydroxyanthraquinone (99.5 mg, 0.44 mmol) at 0 °C, and the reaction mixture was stirred at 80 °C for 30 min. After cooling down to ambient temperature, to this mixture was added a solution of (2S)-(+)-glycidyl 3-nitrobenzenesulfate (15.5 mL, 114.3 mmol) in N,N-dimethylformamide (1 mL) at ambient temperature, and the reaction mixture was stirred at 100 °C for 30 min. After cooling down to ambient temperature, the mixture was diluted with sat. aq. NH₄Cl and extracted with ethyl acetate. The crude product was purified by column chromatography on silica gel, eluting with 30% ethyl acetate in hexane to give 4 (31.0 mg, 0.11 mmol, 25%) as a yellow solid and the recovered 1-hydroxyanthraquinone (72.4 mg, 0.32 mmol, 73%): mp. 174-175°C; 1H NMR (CDCl₃, 400 MHz) δ 8.27-8.21 (2H), 7.99 (dd, 1H, J = 7.7, 1.1 Hz), 7.77-7.67 (3H), 7.36 (dd, 1H, J = 8.5, 1.1 Hz), 4.48 (dd, 1H, J = 11.3, 2.7 Hz), 4.23 (dd, 1H, J = 11.2, 4.6 Hz), 3.50-3.48 (m, 1H), 3.13 (dd, 1H, J = 5.1, 2.7 Hz), 2.98 (dd, 1H, J = 5.1, 4.0 Hz); MS (EI) m/e (%): 280 (M⁺, 100), 237 (31), 224 (100), 139 (54); HRMS (EI) calcd for C₁₇H₁₂O₄ (M⁺) 280.0736, found 280.0733; Anal. Calcd for C₁₇H₁₂O₄: C, 72.85; H, 4.32. Found: C, 72.56; H, 4.45.

1-(2-Oxiranylmethoxy)anthracene-9,10-dione (5). The same procedure described for the synthesis of 4 was applied for 1-hydroxyanthraquinone (143.6 mg, 0.74 mmol) to afford 5 (143.9 mg, 77%) as a pale yellow solid: 1H NMR (CDCl₃, 400 MHz) δ 8.86 (s, 1H), 8.36 (s, 1H), 8.06-8.03 (m, 1H), 7.98-7.96 (m, 1H), 7.59 (d, 1H, J = 8.6 Hz), 7.48-7.44 (2H), 7.33 (t, 1H, J = 8.5 Hz), 6.71 (d, 1H, J = 7.5 Hz), 4.47 (dd, 1H, J = 11.0, 3.1 Hz), 4.19 (dd, 1H, J = 11.1, 6.7 Hz), 3.59-3.57 (m, 1H), 3.02 (t, 1H, J = 4.5 Hz), 2.90 (dd, 1H, J = 4.9, 2.6 Hz); MS (EI) m/e (%): 250 (M⁺, 100), 208 (27), 193 (37), 165 (96); HRMS (EI) calcd for C₁₇H₁₄O₂ (M⁺) 250.0994, found 250.0999.

1-Methoxy-5-(2-oxiranylmethoxy)anthracene (6). The same procedure described for the synthesis of 4 was applied for 1-hydroxy-5-methoxyanthracene (10.6 mg, 47.3 µmol) to afford 6 (6.1 mg, 46%) as a yellow solid: 1H NMR (CDCl₃, 400 MHz) δ 8.80 (s, 1H), 8.76 (s, 1H), 7.64-7.61 (2H), 7.37-7.28 (2H), 6.75-6.71 (2H), 4.20 (dd, 1H, J = 11.0, 5.7 Hz), 4.06 (s, 3H), 3.50-3.48 (m, 1H), 3.00 (t, 1H, J = 4.5 Hz), 2.89 (dd, 1H, J = 4.8, 2.6 Hz); MS (EI) m/e (%): 280 (M⁺, 100), 223 (60), 195 (35), 152 (34); HRMS (EI) calcd for C₁₇H₁₆O₃ (M⁺) 280.1100, found 280.1093.

Preparation of 32P-5'-End-Labeled Oligodeoxynucleotide Duplex. 400 pmol of single-strand oligodeoxynucleotide, purchased from Greiner Japan Co. Ltd., was 5'-end-labeled by phosphorylation with 4 µL of [γ-³²P]ATP (Amersham, 370 MBq/µL) and 4 µL T4 polynucleotide kinase (Takara, 10 units/µL) using standard procedure. The 5'-end-labeled DNA was recovered by ethanol precipitation and further purified by 15% non-denatured gel electrophoresis and isolated by crush and soak method. The isolated DNA was incubated with
equimolar of the complementary DNA in 100 µL of water at 90 °C for 5 min and cooled slowly to ambient temperature for forming duplex.

**Cleavage of 32P-5'-End-Labeled Oligodeoxynucleotide.** Single-stranded 43-mer DNA oligomers 5'-d(TTTTTGTTTGTTAGTTCG-TTTGCTTGTTGTTGATTGTTTTT)-3' and the corresponding complementary oligomer were purchased from Greiner Japan Co. Ltd. 32P-5'-end-labeled ODN duplex was prepared as shown above. A solution of 4, 5 or 6 (each 500 µM) was incubated with 10 µM of calf thymus DNA and ca. 1.0×10⁶ cpm 32P-5'-end-labeled ODN duplex in 20 mM Tris-HCl buffer (100 µL, pH 7.6) at 37 °C for 24 h. The samples were precipitated with methanol, and the recovered DNA was dissolved in 100 µL of 10% (v/v) piperidine and heated at 90 °C for 30 min. The mixture was concentrated in vacuo and resuspended in 10 µL of 80% formamide loading buffer (80% formamide, 1 mM EDTA, 0.1% xylene cyanole and 0.1% bromophenol blue). The samples (1 µL) were loaded onto 15% polyacrylamide and 7 M urea sequence gel and electrophoresed at 1900 V for ca. 2 h. The gel was dried and exposed to X-ray film with intensifying sheet at −70 °C. The result was shown in Figure 1.

**Cleavage of 32P-5'-End-Labeled Oligodeoxynucleotide.** Single-stranded 33-mer DNA oligomers 5'-d(CGTTATCATGTTGTTGATTGTTTTT)-3' and the corresponding complementary oligomer were purchased from Greiner Japan Co. Ltd. The 32P-5'-end-labeled ODN duplex was prepared as shown above. A solution of 4 (500 µM) was incubated with 10 µM of calf thymus DNA and ca. 1.0×10⁶ cpm 32P-5'-end-labeled ODN duplex in 20 mM Tris-HCl buffer (100 µL, pH 7.6) at 37 °C. At each time, the sample (30 µL) was separated from the reaction mixture and precipitated with methanol. The recovered DNA was dissolved in 100 µL of 10% (v/v) piperidine and heated at 90 °C for 30 min. The mixture was concentrated in vacuo and resuspended in 10 µL of 80% formamide loading buffer (80% formamide, 1 mM EDTA, 0.1% xylene cyanole and 0.1% bromophenol blue). The samples (1 µL) were loaded onto 15% polyacrylamide and 7 M urea sequence gel and electrophoresed at 1900 V for ca. 2 h. The gel was dried and exposed to X-ray film with intensifying sheet at −70 °C. The result was shown in Figure 1.
References and Notes


(6) Refer to the previous chapter.


List of Publications

Chapter 1
Highly Efficient Synthesis of 2-Substituted 4H-Chromen-4-ones by means of F-Induced 6-Endo-Digonal Cyclization of o-(Silyloxy)phenyl Ethynyl Ketone Derivatives.

6-Endo- and 5-Exo-Digonal Cyclizations of o-Hydroxyphenyl Ethynyl Ketones: A Key Step for Highly Selective Benzopyranone Formation.

Chapter 2
Synthesis of ABC Ring Analog of Kapurimycin A3 as an Effective DNA Alkylating Agent.

Truncated Analogs of Kapurimycin A3 and their DNA Alkylation Mechanism.

Chapter 3
Studies on the Mechanism of DNA Sequence Selective Alkylation by Kapurimycin A3 Analogs.

Chapter 4
Highly Selective Alkylation at 5'-G of 5'GG3' Sequence by an Aglycon Model of Pluramycin Antibiotics through Preferential Intercalation into GG Step.

Chapter 5
Okamoto, A.; Nakatani, K.; Saito, I. To be submitted.
List of Oral Presentations

1. "Synthetic Studies on Kapurimycin A, and its Analogs: Indispensable Subunit for Efficient DNA Alkylation"

2. "Synthetic Studies on Kapurimycin A, and its Analogs"

3. "Synthesis of Kapurimycin A, Analogs and their Reaction with DNA"


5. "Studies on DNA Alkylation by Analogs of Antitumor Antibiotic Kapurimycin A,"

6. "DNA Alkylation Mechanism of ABCD-ring Analogs of Kapurimycin A,"

7. "Mechanism of Guanine-Guanine Sequence-Selective DNA Alkylation by Kapurimycin A, Analogs"

8. "Studies on the Mechanism of DNA Sequence Selective Alkylation by Kapurimycin A, Analogs"

Other Oral Presentation

1. "Intramolecular Silylstannylation to Acetylene"