Synthesis of Glucopyranosyl *Schiff* Base Zinc(II) Complexes Capable of Interacting with Mononucleotides, and Their DNA-Cleavage Activities

by **Misaki Nakai***[a]), **Hironobu Fukuda**[a]), **Shigenobu Yano**[b])[c]), and **Yasuo Nakabayashi**[a])

[a]) Department of Chemistry and Materials Engineering, Faculty of Chemistry, Materials and Bioengineering, Kansai University, 3-3-35, Yamatecho, Suita-shi, Osaka, 564-8680, Japan (phone: +81-6-6368-1111-5908, e-mail: nakai@kansai-u.ac.jp)

[b]) Office of Society-Academia Collaboration for Innovation, Kyoto University, Kyoto University Katsura, Nishikyo-ku, Kyoto-daigaku Katsura, Kyoto 615-8520, Japan

[c]) Graduate School of Material Science, Nara Institute of Science and Technology, 8916-54, Takayama-cho, Ikoma, Nara, 630-0192, Japan

New glucopyranosyl *Schiff* base zinc complexes, [Zn(GlcSal)$_2$] (**1**; GlcSalH = *N*-(2-deoxy-β-D-glucopyranos-2-yl-salicylaldimine) and [Zn(AcOGlcSal)$_2$] (**2**; AcOGlcSalH = *N*-(2-deoxy-β-D-1,3,4,6-tetraacetylglucopyranos-2-yl-salicylaldimine) were synthesized, and characterized by spectral and analytical methods. The interaction between the Zn complexes and mononucleotides was investigated by ^{1}H-NMR, ^{31}P-NMR and UV/VIS spectroscopies. Mononucleotides, cytidine 5′-monophosphate (CMP) and uridyl 5′-monophosphate (UMP), interacted with these complexes to form a 1:1 complex with **1** and a 1:2 complex with **2**, depending on the presence of the OH group of glucopyranosyl substituents. The DNA-cleavage activities of **1** and **2** were studied using plasmid DNA (pBR322) in a medium of 5 mM *Tris*·HCl/50 mM NaCl buffer in the presence of H_2O_2. The DNA-cleavage activity decreased in the order of **2** > **1** > Zn(OAc)$_2$, indicating the significant promoting effect of the glucopyranosyl *Schiff* base ligand and the participation of the glucopyranosyl OH groups in the cleavage mechanism. The mechanism of the DNA cleavage by **1** and **2** was investigated by evaluation of the effect of a HO$^\bullet$ radical scavenger and a singlet-oxygen ($^1O_2$) quencher under aerobic conditions. The former exhibited little effect, excluding the HO$^\bullet$ radical as an active species and supporting the hydrolysis mechanism for the main process of the DNA cleavage. The latter quencher somewhat hindered the cleavage, indicating the partial participation of a $^1O_2$ as a competitive active species in the present system.

Introdution. – Cisplatin, which is in widespread clinical use, is very efficient and often used for chemotherapy of cancer treatment. However, cisplatin has serious side effects such as nephrotoxicity, neurotoxicity, *etc.* [1]. As cisplatin-resistant cells were also reported in recent years [2], it is very important to develop new metal complexes which can act as antitumor drugs. Therefore, several metal complexes with antitumor activity, such as palladium [3–6], ruthenium [7–11], copper [12–14], zinc, and vanadium [15–17], were synthesized and investigated, expecting significant interaction with DNA and high DNA-cleavage activity. In this report, we have focused on Zn complexes, since Zn is one of the most important metals in the biological system and plays the role of the active site of more than 200 enzymes [18][19]. Some Zn-containing enzymes are known to act in the DNA and RNA reactions [20]. Further, as reported by *Arunakaran* and co-workers, Zn inhibits proliferation of prostate cancer, and acts as a potential chemopreventive agent in targeting the prostate cancer [21]. Therefore, Zn can be expected as an antitumor or a tumor-inhibiting agent.

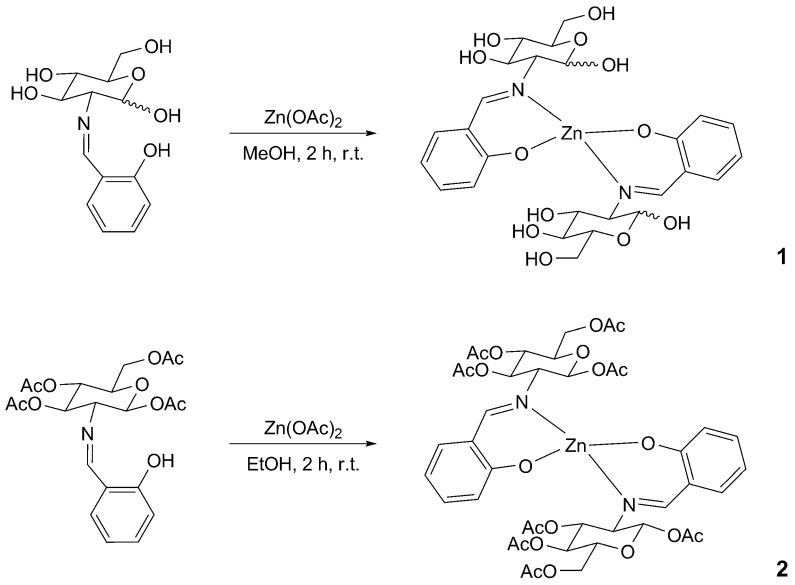
Recently, some *Schiff* base Zn complexes were synthesized and investigated for their DNA-cleavage and antitumor activities [22–25]. The major mechanism of the DNA cleavage with these Zn complexes has been explained by hydrolysis of the phosphate ester [26]. The Zn complex binds to DNA through an O-atom in the phosphodiester backbone of DNA, resulting in activation of the P–O bond to be cleaved. An adjacent coordinated H_2O molecule attacks the P-atom of the phosphodiester to form a pentacoordinated intermediate, followed by the cleavage of one of the P–O ester bonds *via* the intramolecular charge transfer to accomplish the DNA cleavage [26].

On the other hand, carbohydrate is one of the components of cell walls and used as an energy source in biological system [27]. Because a cancer cell incorporates glucose much more efficiently than that of a normal tissue, glucose derivatives have been used as a diagnostic drug for, *e.g.*, positron emission tomography (PET) [28]. In addition, glucosyl conjugation of metal complexes was found useful for antitumor drugs and photodynamic-therapy agents [6][29][30]. It has been reported that introduction of carbohydrate into an antitumor drug is efficient for uptake by cancer cell and antitumor activities [31]. From this viewpoint of the effective glucosyl conjugation, we here synthesized new Zn^{II} complexes attached to the glucosamine derivatives. Although some *Schiff* base Zn complexes conjugated with glucosamine have been synthesized [32–34], there are a few reports on their potential for interaction with mononucleotides or for the DNA-cleavage activity. Since it is assumed that a H-bond between a metal complex and DNA plays an important role for the DNA-binding affinity of the metal complex [11][35][36], the present study was undertaken to clarify how the glucopyranosyl conjugation to the *Schiff* base Zn complex affects the interaction with DNA and the DNA-cleavage activity. The acetylglucopyranosyl analog was also studied to understand the effect of the OH groups.

Results and Discussion. – *Syntheses and Characterization.* The ligands were synthesized from D-glucosamine, and characterized by using ^{1}H-NMR. The Zn complex [Zn(GlcSal)$_2$] (**1**; GlcSalH = *N*-(2-deoxy-β-D-glucopyranos-2-yl)salicylaldimine) was prepared by the reaction of *N*-(2-deoxy-β-D-glucopyranos-2-yl)salicylaldimine with Zn(OAc)$_2$ according to the procedure reported in [33]. The Zn complex **2** was synthesized in the analogous way: the reaction of *N*-(2-deoxy-β-D-1,3,4,6-tetraacetylglucopyranos-2-yl)salicylaldimine (AcOGlcSalH) with Zn(OAc)$_2$ was performed in EtOH to produce [Zn(AcOGlcSal)$_2$] in the 30% yield after reprecipitation from Et_2O (*Scheme*). HR-ESI-MS of **1** and **2** recorded in the positive-ion mode exhibited peaks at *m*/*z* 651.1071 ($[M+Na]^+$) for **1** and 965.1933 (M^+) for **2**, respectively, indicating formation of the expected complexes. In the IR spectrum of **1**, a broad band at *ca.* 3400 cm^{-1} was assigned to the stretching vibration of the H-bonded OH groups of the glucosamine moiety. On the other hand, **2** exhibited a strong band at 1750 cm^{-1}, which was ascribed to the C=O stretching vibration of the Ac group. The bands attributed to the C=N stretching vibration of the imino group were observed at 1630 cm^{-1} [23].

In the ^{1}H-NMR spectra, two sets of signals were observed for the H-atoms of azomethine and glucose groups of GlcSalH and **1** (in DMSO/D_2O), and for the phenol OH group of GlcSalH (in DMSO), indicating the presence of two isomers. In case of

Scheme. *Syntheses of Zn Complexes* **1** (α- and β-anomers) *and* **2** (β-anomer)



GlcSalH, the coupling constants of the clear two *doublet*s of anomeric H-atoms were 3.4 and 7.9 Hz, which correspond to the typical *gauche* and *trans* vicinal H,H coupling values, respectively [27]. Therefore, these two sets of peaks were attributed to the α- and β-anomers of glucosamine moieties. The ratio α/β anomers of GlcSalH and **1** was both *ca*, 4 : 6. On the other hand, in case of AcOGlcSalH and **2**, only one set of signals was observed for the corresponding H-atoms, indicating that the compounds are not mixtures of isomers. This is compatible with the steric effect of the Ac group in the glucosamine moiety, and the plausible isomer is the β-anomer. *Fig. 1* shows the UV/VIS spectra of the Zn complexes in DMSO. Two peaks were observed, at *ca.* 260 and at 320–380 nm, which were attributed to the π–π^* transition of the salicyl group and the n–π^* transition of the imino group, respectively [37].

Interaction between Zn Complexes and Mononucleotides. Interactions between the Zn complexes **1** and **2**, and mononucleotides, 5′-cytidylic acid (CMP) and 5′-uridylic acid (UMP), as monomers of DNA and RNA, respectively, were investigated by using ^{1}H-NMR and ^{31}P-NMR in DMSO/D_2O 4 : 1 (*v*/*v*). Since it was reported that Zn complexes with an imino group were often hydrolyzed in H_2O [38], the ^{1}H-NMR spectral changes of **1** and **2** in DMSO/D_2O 4 : 1 (*v*/*v*) were determined after 0 h and 2 h. As depicted in *Fig. 2*, no spectral change was observed, indicating that decomposition of **1** and **2** by hydrolysis is negligible.

Fig. 3 shows ^{1}H-NMR spectra of **1** and **2** in the presence of CMP. After addition of mononucleotides, peaks ascribed to free ligands, GlcSalH and AcOGlcSalH, were observed, suggesting that the CMP coordination to Zn was accompanied by the partial

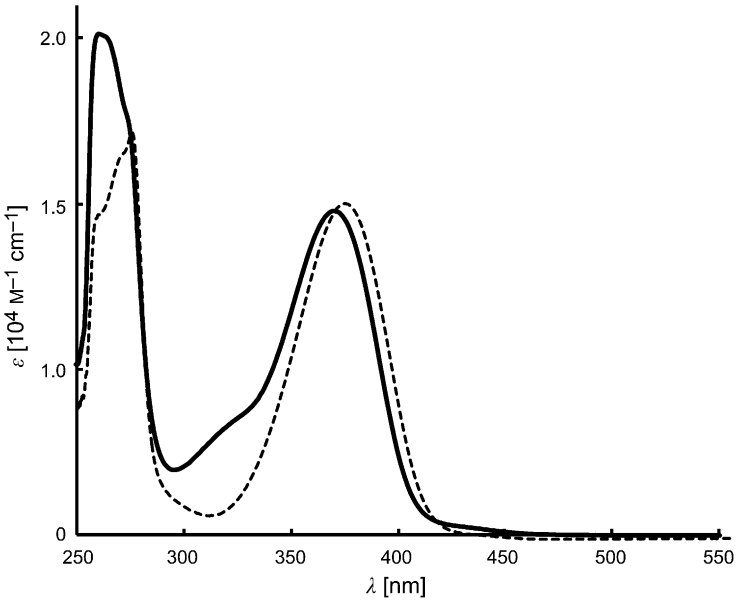


Fig. 1. *UV/VIS Spectra of complexes* **1** *and* **2** *in DMSO solutions.* —: **1**, - - -: **2**.

dissociation of the *Schiff* base ligands. The signals of CMP were shifted and broadened after coordination to **1** and **2**, as observed previously with other Zn complexes [39]. In case of **2**, the signals of CMP coordinated to **2** were not clear in a [CMP]/[**2**] ratio of 1 : 1, but broad peaks of CMP bonded to **2** were clearly observed at [CMP]/[**2**] 2 : 1. This indicates the coordination of two molecules of CMP to Zn, forming [Zn(AcOGlcSal)$_2$(CMP)$_2$] or [Zn(AcOGlcSal)(CMP)$_2$]. As shown in *Fig. 3*, the chemical shifts ($\Delta\delta$) of CMP H-atoms (H–C(1), H–C(5), CH_2(6); marked with ○) after coordination to **1** and **2** were less than 0.2 ppm. Since the chemical-shift values of CMP are reported to be within only 0.2 ppm after coordination to Ru *via* phosphate anions [40], the chemical-shift values of CMP and UMP with **1** and **2** suggested the coordination of mononucleotides to **1** and **2** *via* phosphate anions.

Since the fairly broad ^{1}H-NMR peaks of CMP and UMP after coordination to **1** and **2** were not suitable to further investigate the coordination mode, the coordination of CMP or UMP to Zn was examined by ^{31}P-NMR. In the ^{31}P-NMR spectra, the ^{31}P resonances of CMP and UMP were also shifted to the lower fields and broadened after addition to the solutions of **1** and **2** (*Fig. 4*). As seen in the *Table*, these shifts are more remarkable than those observed with ^{1}H-NMR and ascribed to the coordination of CMP and UMP with phosphate anions to **1** and **2**, as observed previously with other *Schiff* base Zn complexes [25]. In addition, the chemical-shift values of CMP and UMP are greater after ligation to **2** than with **1**, suggesting the stronger binding of **2** than **1** [24].

The interactions of **1** and **2** with mononucleotides were also investigated by UV/VIS spectroscopy. *Fig. 5* shows UV/VIS spectral changes of **1** and **2** after addition of CMP in DMSO/H_2O 4 : 1 (*v*/*v*). In the 300–500-nm regions, where the nucleotide absorption is

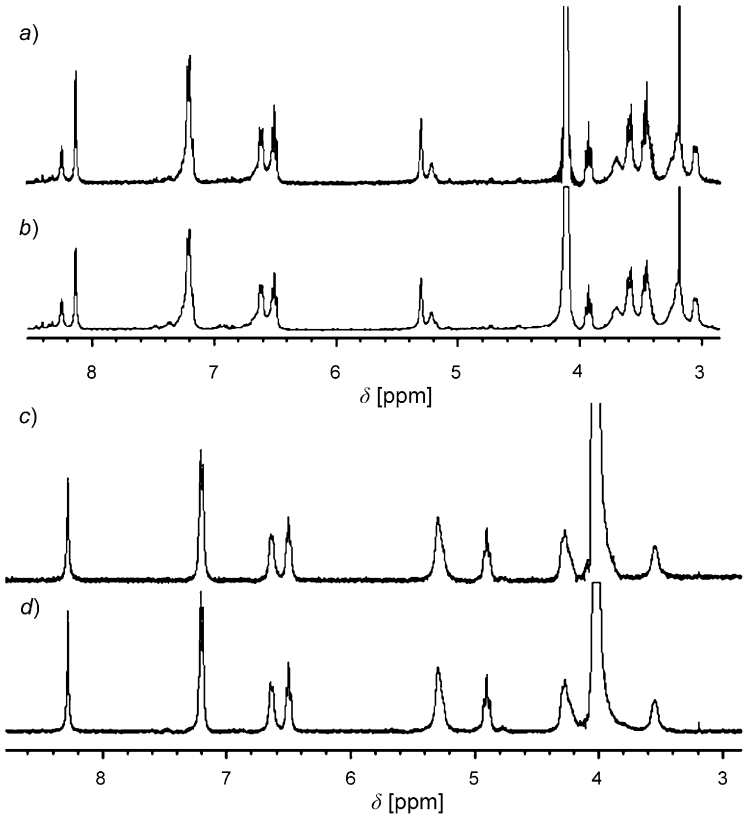


Fig. 2. *^{1}H-NMR Spectra of complex* **1** *after 0 h* (*a*) *and 2 h* (*b*); of complex **2** *after 0 h* (*c*) *and 2 h in DMSO/D_2O* 4:1 *(v/v)* (*d*)

negligible, the intensity of the low-energy band at *ca.* 380 nm of **1** and **2** decreased, accompanied with the increase in the new bands at *ca.* 323 nm, indicating that nucleotides bind to **1** and **2** as observed with another *Schiff* base Zn complex [25]. The spectral change ceased when the concentration of CMP became equivalent to that of **1**. On the other hand, in case of **2**, the spectral change continued until the [CMP]/[**2**] ratio became 2. The intrinsic-binding-constant (K_b) values were estimated according to *Eqn. 1* [41].

$$\frac{[\mathrm{CMP}]}{|\varepsilon_a - \varepsilon_b|} = \frac{[\mathrm{CMP}]}{|\varepsilon_b - \varepsilon_f|} + \frac{[\mathrm{CMP}]}{K_b|\varepsilon_b - \varepsilon_f|} \tag{1}$$

where [CMP] represents the concentration of CMP, and ε_a, ε_f, and ε_b are the apparent extinction coefficient (Aobs/[M]), the extinction coefficient for free metal complex (M), and the extinction coefficient for the free metal complex (M) in the fully bound form, respectively. As shown in *Table*, the K_b value of **2** is greater than that of **1**, supporting the stronger binding affinity of **2** to CMP than **1** as indicated by ^{31}P-NMR.

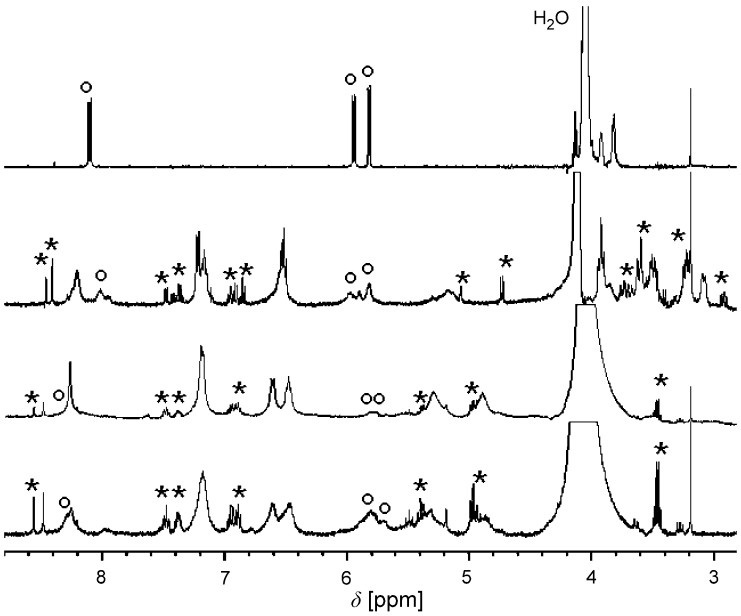


Fig. 3. *^{1}H-NMR Spectra of* a) *CMP,* b) *CMP*/**1** *1:1,* c) *CMP*/**2** *1:1,* d) *CMP*/**2** *2:1 in DMSO*/D_2O 4:1 *(v/v)*. *: Peaks of the free ligands; ○: peaks of mononucleotide.

Orvig and co-workers have reported the formation of Re^I tricarbonyl complexes of *N*-(2′-hydroxybenzyl)-2-amino-2-deoxy-D-glucose [42]. Tridentate coordination of the glucosamine ligand with C(3)–OH(glucose), NH(amino), and O(phenolato) suggests the similar tridentate coordination of GlcSal to Zn with C(1) or C(3)–OH(glucose), N(imino), and O(phenolato) in **1**. In case of **2**, the absence of the glucose OH groups after acetylation suggests the bidentate coordination of AcOGlcSal to Zn, leaving open or solvent coordination sites at the Zn center. Thus, in case of **1**, the mono-coordination of nucleotide to Zn may form a tetracoordinate complex after dissociation of one of two GlcSal ligands such as [Zn(GlcSal)(CMP)]. Similar dissociation of one of two tridentate ligands was also reported by *Mitra et al.*, in the reaction of phosphate anions with the hexacoordinate Zn complex of 1-[2′-deoxy-2′-(iminomethyl)-D-glucopyranosyl]-2-hydroxynaphthalene forming a tetrahedral Zn complex, in which case ligations of three dihydrogen phosphate and one *Schiff* base ligand in the monodentate form have been proposed [34]. Since coordination of one molecule of CMP was indicated in case of **1**, the GlcSal ligand seems to keep the tridentate coordination mode. In case of **2**, two nucleotide molecules may coordinate to Zn after dissociation of one of two AcOGlcSal ligands to form a tetracoordinate complex such as [Zn(AcOGlcSal)$(CMP)_2$], but the probability of coordinatiton without dissociation of AcOGlcSal to form a hexacoordinate complex, *e.g.*, [Zn$(AcOGlcSal)_2(CMP)_2$], may not be excluded.

Plasmid DNA-Cleavage Activity. The chemical nuclease activities of **1** and **2**, together with $Zn(OAc)_2$ and ligands, GlcSalH and AcOGlcSalH, as reference, were

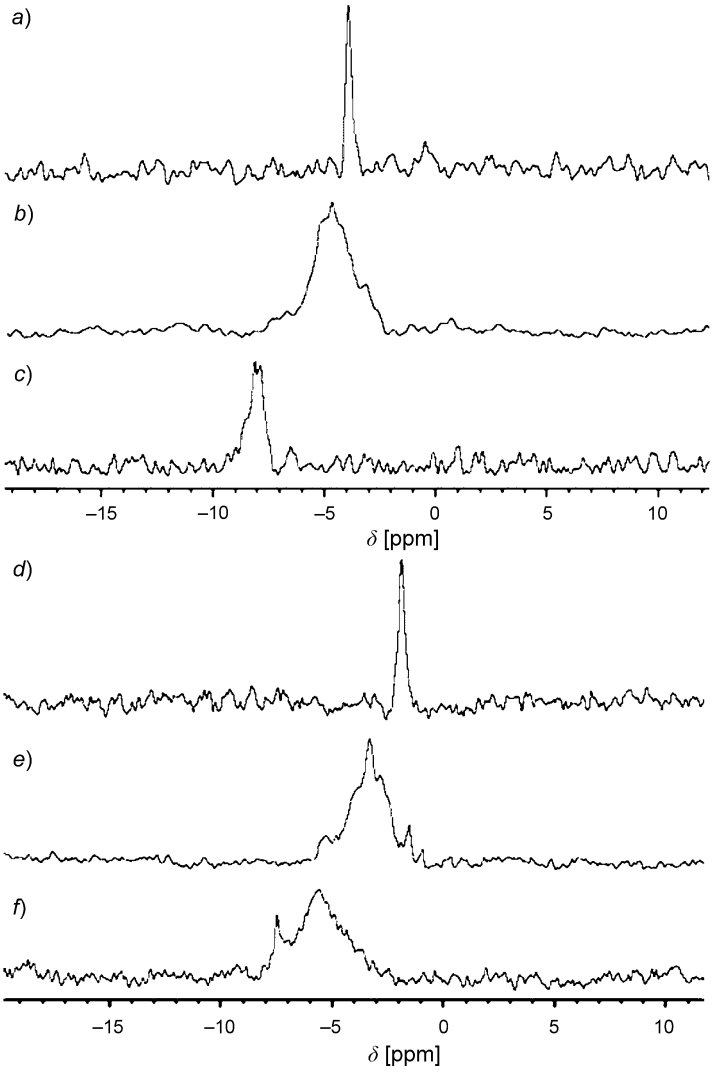


Fig. 4. *^{31}P-NMR Spectra of* a) *CMP, CMP in the presence of* b) **1** *or* c) **2**; *^{31}P-NMR spectra of* d) *UMP; UMP in the presence of* e) **1** or f) **2** *in DMSO/D_2O* 4:1 (*v*/*v*) (mononucleotide/Zn complex 1:1). All spectra were recorded with H_3PO_4 in DMSO/D_2O 4:1 (*v*/*v*) as an external reference.

Table. *^{31}P-NMR Chemical Shifts* ($\Delta\delta$) *Observed for Mononucleotide with Complex* **1** *and* **2**, *and the Binding-Constant* (K_b) *Values of* **1** *and* **2** *for CMP in DMSO/D_2O* 4:1 (*v*/*v*)

Complex	$\Delta\delta$ [ppm] (CMP)	$\Delta\delta$ [ppm] (UMP)	K_b [M^{-1}]
1	0.98	1.06	7.13×10^3
2	4.22	3.62	8.46×10^3

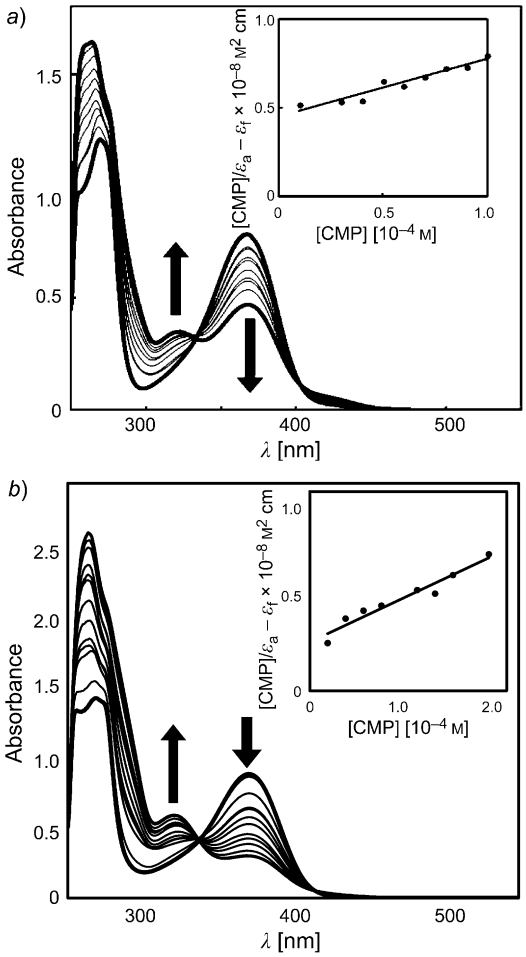


Fig. 5. *UV/VIS Spectral change of* a) **1** *and* b) **2** *with increasing concentration of CMP in DMSO/H_2O 4 : 1* (*v*/*v*). *Insets:* plots of [DNA]/$\varepsilon_a - \varepsilon_f$ *vs.* [DNA] for the titration of CT-DNA with complexes at 323 nm. [Complex] = 1.0×10^{-4} M, [CMP] = 0 a) *ca.* 1.0×10^{-4} M, and b) 0–2.0×10^{-4} M.

studied using plasmid DNA (pBR322) in a medium of 5 mM *Tris*·HCl/50 mM NaCl buffer (pH 7.5) at 37° and for 8 h incubation in the presence of H_2O_2. Substrates were dissolved in 5 mM *Tris*·HCl/50 mM NaCl buffer containing 1% DMSO solutions adjusted to 100 μM/l. Results are shown in *Fig. 6*.

As shown in *Fig. 6*, *Lane 1*, the control experiment only with plasmid in the absence of H_2O_2 indicates the mobility of both the supercoiled form (form I) as a major component and the circular form (form II) as a minor component. The other control experiments under the conditions using only H_2O_2 (*Fig. 6*, *Lane 2*), AcOGlcSalH with H_2O_2 (*Fig. 6*, *Lane 4*), GlcSalH with H_2O_2 (*Fig. 6*, *Lane 5*), **1** without H_2O_2 (*Fig. 6*, *Lane 8*), and **2** without H_2O_2 (*Fig. 6*, *Lane 9*), did not show any DNA-cleavage activity. However, in the presence of H_2O_2, $Zn(OAc)_2$ (*Fig. 6*, *Lane 3*), **1** (*Fig. 6*, *Lane 6*), and **2**

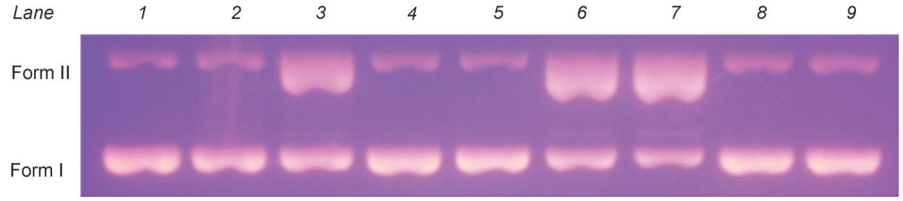


Fig. 6. *The cleavage patterns of the agarose gel electrophoresis for pBR322 plasmid DNA in 5 mM* Tris·*HCl/50 mM NaCl* (pH 7.5) *solutions. Lane 1:* DNA control; *Lane 2:* DNA with H_2O_2 (1.0 mM); *Lane 3:* DNA with H_2O_2 and $Zn(OAc)_2$ (100 μM); *Lane 4:* DNA with H_2O_2 and AcOGlcSalH (100 μM); *Lane 5:* DNA with H_2O_2 and GlcSalH (100 μM); *Lane 6:* DNA with H_2O_2 and **1** (100 μM); *Lane 7:* DNA with H_2O_2 and **2** (100 μM); *Lane 8:* DNA with **1** (without H_2O_2); *Lane 9:* DNA with **2** (without H_2O_2).

(*Fig. 6*, *Lane 7*) showed the component formed by the single-strand breaks. Especially, the complex **2** exhibited the highest DNA-cleavage activity, in the order of **2** > **1** > $Zn(OAc)_2$. The different activities, *i.e.*, **2** > **1**, pallalel the different binding constants of these complexes with nucleotide, CMP, suggesting that the DNA-cleavage activity depends on the binding affinity of these complexes with DNA. The different activities, *i.e.*, **1** > $Zn(OAc)_2$, indicate that the glucosamine ligand enhances the DNA-cleavage activity, probably because the glucosamine OH groups assist the proximity of **1** to DNA by the H-bond formation.

The DNA-cleavage mechanism by glucosamine Zn complexes was investigated by evaluation of the effect of a HO$^{\bullet}$ radical scavenger (PhCOONa) and a singlet-oxygen ($^1O_2$) quencher (NaN_3) on the cleavage activity [43][44]. *Fig. 7* shows the cleavage patterns of the agarose gel electrophoresis for pBR322 plasmid by the complex **2** under different conditions. As evident from the patterns of *Fig. 7*, *Lane 2* (without any scavenger), and *Fig. 7*, *Lane 3* (with PhCOONa), any significant effect of PhCOONa was not observed. This result rules out the probability of the DNA cleavage by the HO$^{\bullet}$ radical. Another finding that the DNA cleavage proceeds in the solution containing 1% DMSO (*Fig. 7*, *Lane 2*) supports the non-HO$^{\bullet}$ radical mechanism, because DMSO is known as a HO$^{\bullet}$ radical scavenger. The addition of NaN_3 led to a pattern shown in *Fig. 7*, *Lane 4*, and clearly decreased the extent of form II relative to form I in comparison with the pattern in *Fig. 7*, *Lane 2*. This indicates that $^1O_2$ oxygen participates in the DNA cleavage as an active species, though the $^1O_2$ process is not a main process. To ascertain the participation of $^1O_2$ under the conditions for *Fig. 7*, *Lane 2*, the experiment was performed in the dark (*Fig. 7*, *Lane 5*). Similarly to the effect of the NaN_3 addition, the slight inhibition of conversion to form II was observed,

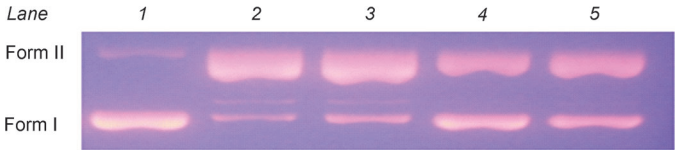


Fig. 7. *The cleavage patterns of the agarose gel electrophoresis for pBR322 plasmid by* **2**. *Lane 1:* DNA control; *Lane 2:* DNA with H_2O_2 and **2** (100 μM) alone; *Lane 3:* DNA with **2** and PhCOONa (50 mM); *Lane 4:* DNA with **2** and NaN_3 (50 mM); *Lane 5:* DNA with H_2O_2 and **2** (100 μM) under dark.

indicating the light-dependent generation of $^1O_2$. Probability of the DNA cleavage by $^1O_2$ was also reported by *Chang* and co-workers using Zn complexes [44]. Salen-type Zn complexes were reported to exhibit fluorescence which originates from the singlet ligand-centered excited state [37][45]. Since this energy gap is enough to generate $^1O_2$, it is very probable that the Zn complexes **1** and **2** generate $^1O_2$ with the light irradiation.

The present results using **1** and **2** indicated that the $^1O_2$ process is minor in comparison with the process which is not sensitive to either scavenger, *i.e.*, the HO$^\bullet$ radical or $^1O_2$. Alternatively, a hydrolytic mechanism is the most probable major process as proposed for cleavage by various Zn complexes [26][44][46]. Formation of Zn–OH has been proposed as an active species in the hydrolytic mechanism [46][47], but the present results indicated the participation of H_2O_2 in the formation of the active species. Since the addition of H_2O_2 is effective to generate the DNA cleavage activities of **1** and **2** (*Fig. 6*, *Lanes 6–9*), it is probable that the peroxide ion coordinated to Zn^{II} works as an active species for the DNA cleavage. Zn–OOH is the probable peroxide species, and its enhanced nucleophilicity and depressed electrophilicity seem to be responsible for the present enhanced DNA cleavage activity [48]. The peroxide species may be formed *via* Zn-$(H_2O_2)_2$ in H_2O [49], but it is rather difficult to accumulate direct evidences for these active species.

Conclusions. – Glucopyranosyl-conjugated *Schiff* base Zn complexes were synthesized. The ^{31}P-NMR and UV/VIS spectroscopic studies indicated that the complex **2** was bound more tightly to mononucleotides compared with the complex **1**. The DNA-leavage activity was in the order $\mathbf{2} > \mathbf{1} > Zn(OAc)_2$, depending on the interaction affinity with DNA. The cleavage is assumed to proceed with the hydrolytic mechanism, in which H_2O_2 plays an important role for formation of an active species. For application of the present complexes to the antitumor-drug design, further research *in vivo* and *in vitro* is necessary, since the antitumor activity of the metal complex may depend on factors other than interaction with DNA, *e.g.*, cell uptake [50].

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Experimental Part

General. All chemicals and reagents were purchased from commercial sources and were used without further purification. *N*-(2-Deoxy-β-D-glucopyranos-2-yl)salicylaldimine (GlcSalH), [Zn(GlcSal)2] [33], and 1,3,4,6-tetra-*O*-acetyl-β-D-glucosamine hydrochloride [51] were prepared according to the methods reported previously. UV/VIS Spectra: *JASCO V-670* spectrophotometer equipped with a temp. controller. IR Spectra: *Varian 660*-IR, as KBr disks. ^{1}H-NMR Spectra: in (D_6)DMSO or (D_6)DMSO/D_2O 4:1 (*v*/*v*); *JEOL GX 400* MHz spectrometer; all chemical shifts (δ) rel. to TMS. ^{31}P-NMR Spectra: *JEOL JNM-ECA600*; recorded with H_3PO_4 in DMSO/D_2O 4:1 (*v*/*v*) as external reference (NAIST). HR-ESI-MS: *JEOL AccuTOFJMS-T100LC* (NAIST).

Synthesis of [Zn(GlcSal)$_2$] (**1**). A MeOH soln. (20 ml) of $Zn(OAc)_2$ (0.19 g, 1.0 mmol) was added to a MeOH soln. (20 ml) of GlcSalH (0.28 g, 0.4 mmol). After 2 h of vigorous stirring, the solvent was evaporated *in vacuo*. The light-yellow precipitate that appeared after the addition of Et_2O was filtered and washed with Et_2O. The complex was dried overnight *in vacuo*. Yield: 0.11 g (38%). UV/VIS: 366 (9.2×10^3), 270 (1.15×10^4). IR (KBr): 3464 (OH), 1627 (N=C), 1448 (C=C), 1084 (C–O), 1043 (C–O).

^{1}H-NMR (D_2O/(D_6)DMSO 4:1 (*v*/*v*)): 8.25 (*s*, azometine); 8.12 (*s*, azometine); 7.22–7.18 (*m*, salicyl); 6.63–6.61 (*d*, *J* = 8.7, salicyl); 6.53 (*t*, *J* = 7.1, salicyl); 5.31–5.22 (*m*, H–C(1) of Glc); 3.91 (*dd*, *J* = 9.3, H–C(3) of Glc); 3.59–3.70 (*m*, H–C(4), H–C(5), 1 H of CH_2(6) of Glc); 3.50–3.42 (*m*, H–C(2), 1 H of CH_2(6) of Glc). HR-ESI-MS: 651.1071 ($[M+Na]^+$, $C_{26}H_{32}N_2NaO_{12}Zn^+$; calc. 651.1144).

Synthesis of N-*(1,3,4,6-Tetra*-O-*acetyl-2-deoxy-β-*D-*glucopyranos-2-ylsalicylaldimine* (AcOGlcSalH). 1,3,4,6-Tetra-*O*-acetyl-β-D-glucosamine hydrochloride (0.38 g, 1.0 mmol) and $NaHCO_3$ (84 mg, 1.0 mmol) were dissolved in 30 ml of H_2O/MeOH 1:2 (*v*/*v*), and salicylaldehyde (126 µl) was added. The mixture was stirred vigorously at r.t. and, after 2 h, afforded a yellow precipitate. The yellow precipitate was filtered, washed with cold H_2O, and dried *in vacuo*. Yield: 0.32 g (70%). UV/VIS: 319 (6.5×10^3), 260 (1.75×10^4). ^{1}H-NMR ((D_6)DMSO): 12.24 (br., OH of phenyl); 8.59 (*s*, 1 H of azometine); 7.51–7.48 (*m*, 1 H of salicyl); 7.38–7.32 (*m*, 1 H of salicyl); 6.93–6.85 (*m*, 2 H of salicyl); 6.14 (*d*, *J*(1,2) = 8.3, H–C(1) of Glc); 5.58 (*dd*, *J*(2,3) = 9.5, *J*(3,4) = 9.6, H–C(3) of Glc); 4.98 (*dd*, *J*(3,4) = 9.6, *J*(4,5) = 9.8, H–C(4) of Glc); 4.31–4.18 (*m*, H–C(5), 1 H of CH_2(6) of Glc); 4.01 (*dd*, *J*(6,6) = 1.8, *J*(5,6) = 12.2, 1 H of CH_2(6) of Glc); 3.56 (*dd*, *J*(1,2) = 8.3, *J*(2,3) = 9.5, H–C(2) of Glc); 2.01 (*s*, AcO); 2.00 (*s*, AcO); 1.97 (*s*, AcO); 1.85 (*s*, AcO). ESI-MS: 474.1346 ($[M+Na]^+$, $C_{21}H_{25}NNaO_{10}^+$; calc. 474.1376).

Synthesis of [Zn(AcOGlcSal)$_2$]. A EtOH soln. (20 ml) of $Zn(OAc)_2$ (55 mg, 0.3 mmol) was added to a EtOH soln. (20 ml) of AcOGlcSalH (113 mg, 0.3 mmol). After 2 h of vigorous stirring, the solvent was evaporated *in vacuo*. The light-yellow precipitate that appeared after the addition of Et_2O was filtered and washed with Et_2O. The complex was dried overnight *in vacuo*. Yield: 73 mg (30%). UV/VIS: 375 (9.5×10^3), 279 (1.09×10^4). IR (KBr): 1750 (C=O), 1629 (N=C), 1448 (C=C), 1151 (C–O), 1033 (C–O). ^{1}H-NMR ((D_6)DMSO): 8.20 (*s*, 1 H of azometine); 7.10–7.04 (*m*, 2 H of salicyl); 6.50 (*d*, *J* = 9.1, 1 H of salicyl); 6.34 (*t*, *J* = 7.1, 1 H of salicyl); 5.43–5.06 (*m*, H–C(3), H–C(4) of Glc); 4.87–4.80 (*m*, H–C(5) of Glc); 4.36–4.19 (*m*, H–C(1), 1 H of CH_2(6) of Glc); 3.95–3.89 (*m*, 1 H of CH_2(6) of Glc); 3.43–3.40 (*m*, H–C(2) of Glc); 1.97 (*s*, AcO); 1.90 (*s*, AcO); 1.85 (*s*, AcO); 1.82 (*s*, AcO). HR-ESI-MS: 965.1933 (M^+, $C_{42}H_{48}N_2O_{20}Zn^+$; calc. 695.2170).

Absorption-Spectral Studies [41]. The interactions between CMP and Zn complexes were evaluated in DMSO/D_2O 4:1 (*v*/*v*) by using UV/VIS spectroscopy. The intrinsic binding constant K_b of the complex to CMP was determined from *Eqn. 1*, through a plot of [CMP]/$\varepsilon_a - \varepsilon_f$ *vs.* [CMP], where [CMP] represents the concentration of CMP, and ε_a, ε_f, and ε_b, the apparent extinction coefficient (A_{obs}/[M]), the extinction coefficient for free metal complex (M), and the extinction coefficient for the free metal complex (M) in the fully bound form, resp. In plots of [CMP]/$\varepsilon_a - \varepsilon_f$ *vs.* [CMP], K_b is given by the ratio slope/intercept. These absorption-spectral studies were performed on *JASCO V-670* spectrophotometer. Absorption-spectral titration experiments were performed by maintaining a constant concentration of the complex and varying mononucletides.

DNA-Cleavage Activities. The cleavage experiments of supercoiled pBR322 DNA by Zn complexes or ligands (100 µM), and H_2O_2 (1 mM) in 5 mM *Tris*·HCl 50 mM NaCl buffer at pH 7.5 were performed by agarose gel electrophoresis. All compounds were dissolved in 5 mM *Tris*·HCl 50 mM NaCl buffer containing 1% DMSO solns. The samples were incubated for 8 h at 37°. DNA Restriction fragments were loaded on 1% (*w*/*v*) agarose gel containing 0.5 µg ml^{-1} EtBr in TBE buffer (89 mM *Tris*·borate/2 mM EDTA) and after running the gel at 100 V for 1 h. The bands were visualized by photographing the fluorescence of intercalated EtBr under a UV (312 nm) illuminator. The DNA cleavages with added inhibitors were monitored as in case of cleavage experiment without added inhibitors using agarose gel electrophoresis. Reactions using pBR322 DNA in 5 mM *Tris*·HCl/50 mM NaCl buffer at pH 7.5 was treated with complex **2** (100 µM), and PhCOONa (50 mM) or NaN_3 (50 mM). The samples were incubated for 8 h at 37°, and analyzed for cleaved products using gel electrophoresis as described above.

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