Supporting Information

Novel Cu(II) Complexes as DNA-destabilizing Agents and Their DNA Nuclease Activity

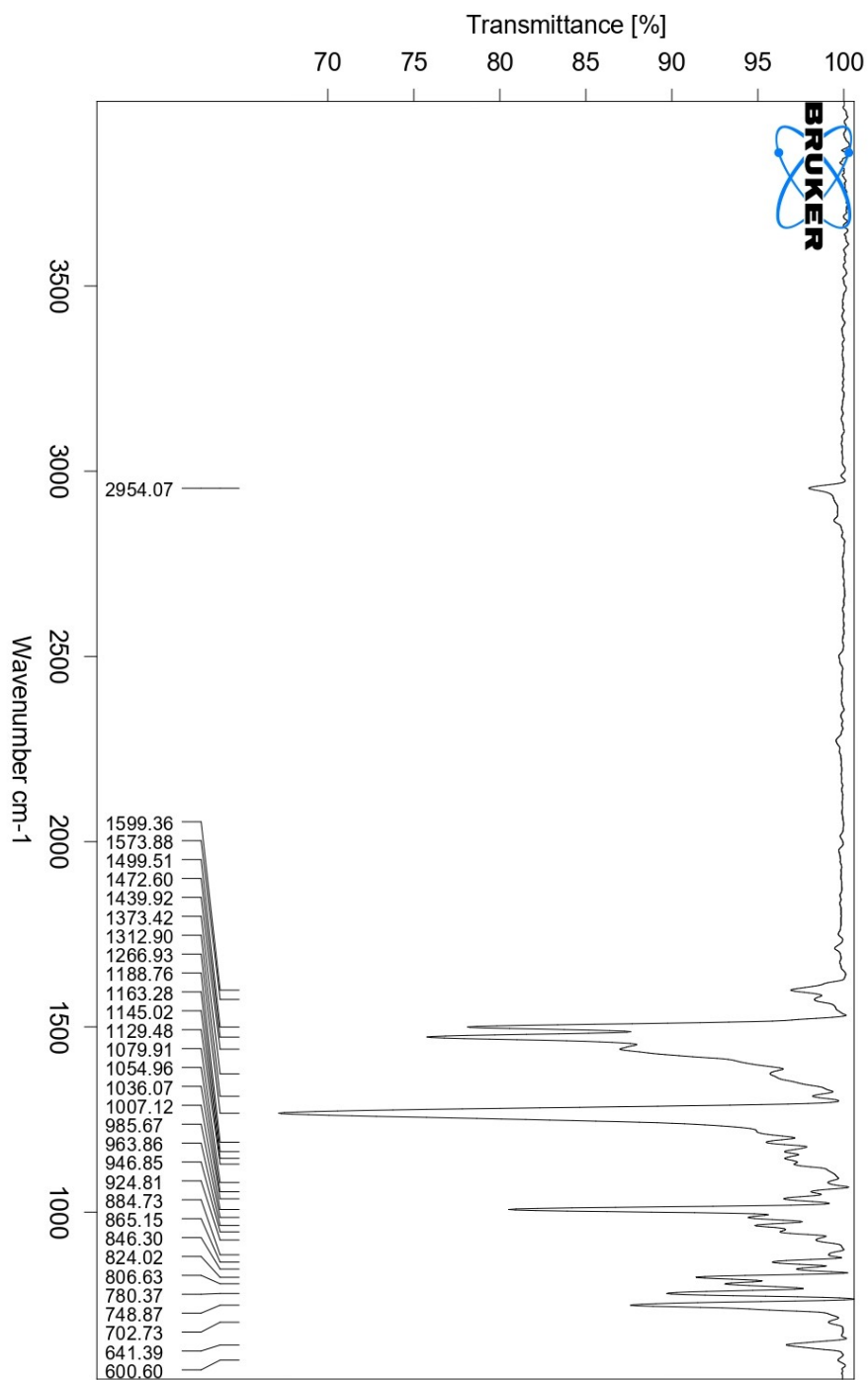Hee Chang Kwon,[a] Da Hyun Lee,[a] Minyoung Yoon,[b] Saira Nayab,[b,c] Hyosun Lee,[b,]* and Ji Hoon Han[a,]*

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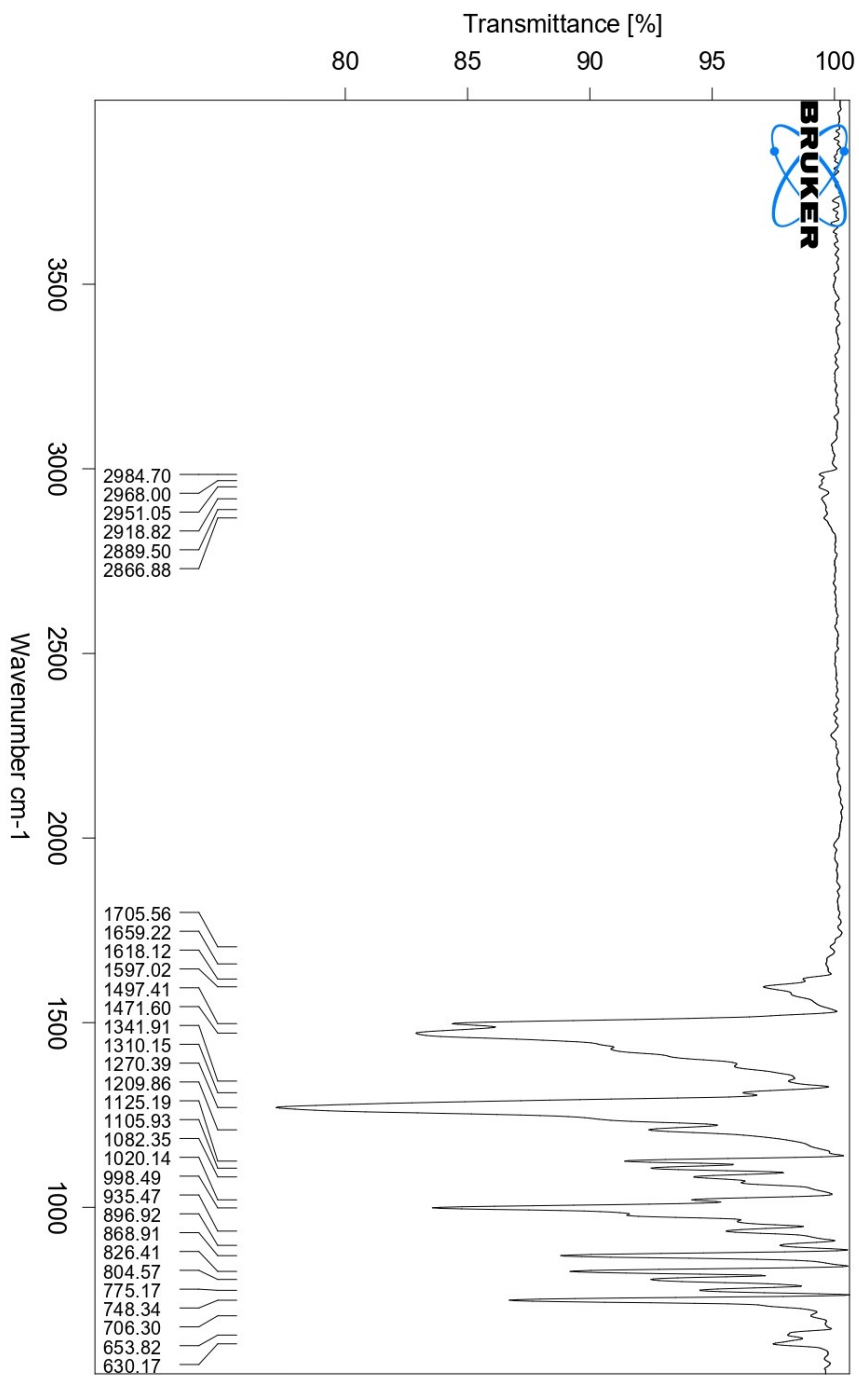
Contents

FIGURE S1.

Transmittance [%]

100 95 90 85 80 75 70

BRUKER

2954.07

1599.36
1573.88
1499.51
1472.60
1439.92
1373.42
1312.90
1266.93
1188.76
1163.28
1145.02
1129.48
1079.91
1054.96
1036.07
1007.12
985.67
963.86
946.85
924.81
884.73
865.15
846.30
824.02
806.63
780.37
748.87
702.73
641.39
600.60

3500 3000 2500 2000 1500 1000

Wavenumber cm-1

D:\LUG\연구실\22.10.07_lab_com_backup\JG(PhD_courses)\@@@연구데이터+관련자료\@@DATA\2. IR\20220905_IR\JG-7-60.0   JG-7-60   In 2022-09-05

Page 1/1

Figure S2. FT

Transmittance [%]

Wavenumber cm-1

2984.70
2968.00
2951.05
2918.82
2889.50
2866.88

1705.56
1659.22
1618.12
1597.02
1497.41
1471.60
1341.91
1310.15
1270.39
1209.86
1125.19
1105.93
1082.35
1020.14
998.49
935.47
896.92
868.91
826.41
804.57
775.17
748.34
706.30
653.82
630.17

D:\LJG연구실\22.10.07_lab_com_backup\JG(PhD_courses)\@@@연구데이터+관련자료\@@DATA\2. IR\20220905_IR\JG-7-61_1.0   JG-7-61   2022-09-05

Figure S3. FTIR spectra of [L_CCu(µ-Cl)Cl]₂ (Cu4).

E:\2. IR\CuKJG-4-20_CuK36_PEAKS.0   JG-4-20_CuK36   Instrument type and / or accessory   2021-08-30

Page 1/1

Figure S4. ORTEP drawing of **[L$_A$Cu(NO$_3$)$_2$] (Cu1)** with thermal ellipsoids at 30% probability. All hydrogen atoms are omitted for clarity.

Figure S5. ORTEP drawing of **[L$_B$Cu(NO$_3$)$_2$] (Cu2)** with thermal ellipsoids at 50% probability. All hydrogen atoms are omitted for clarity.

Figure S6. ORTEP drawing of **[L_cCu(μ-Cl)Cl]₂ (Cu4)** with thermal ellipsoids at 50% probability. All hydrogen atoms are omitted for clarity.
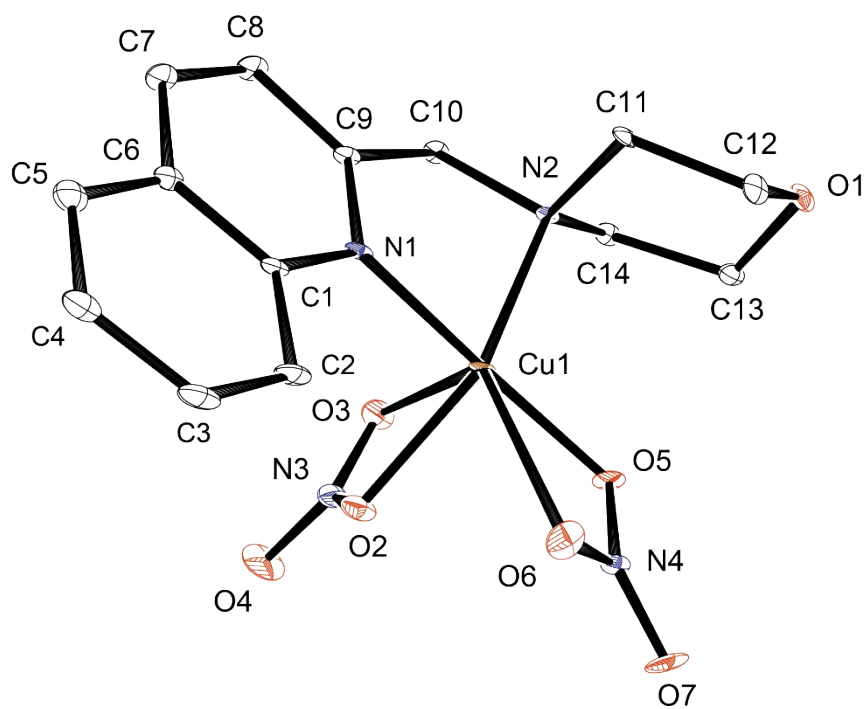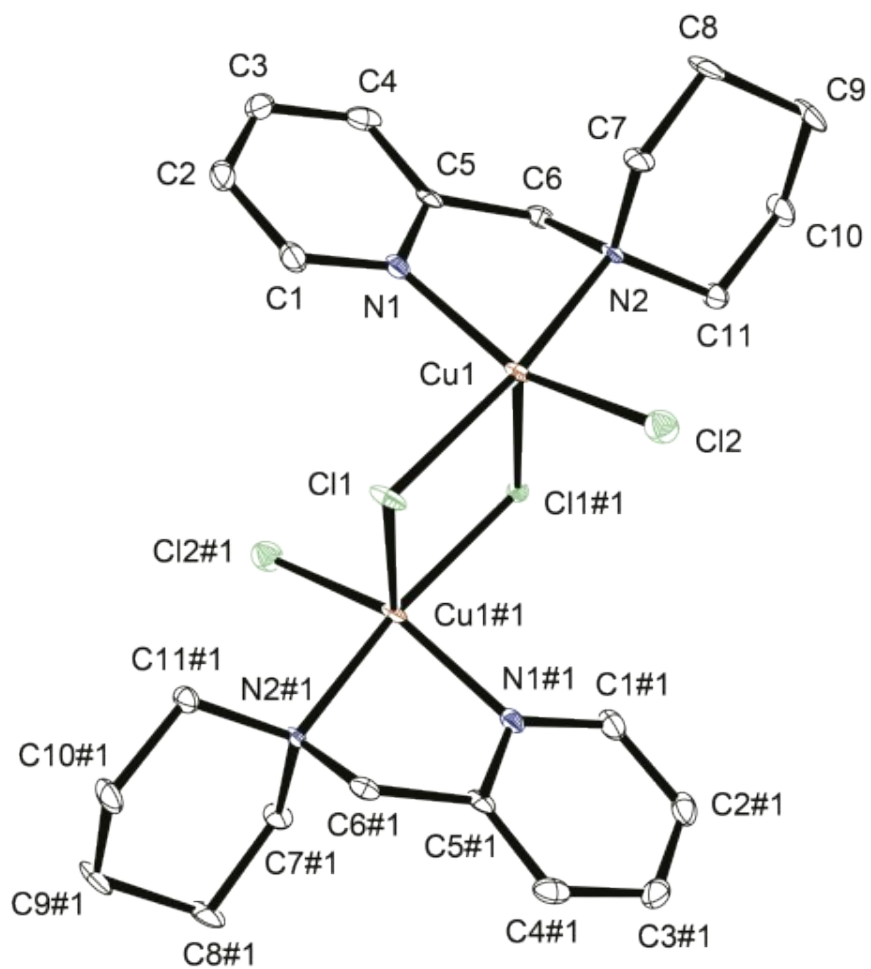
Cu1

$V_{Bur} \% = 44.6$

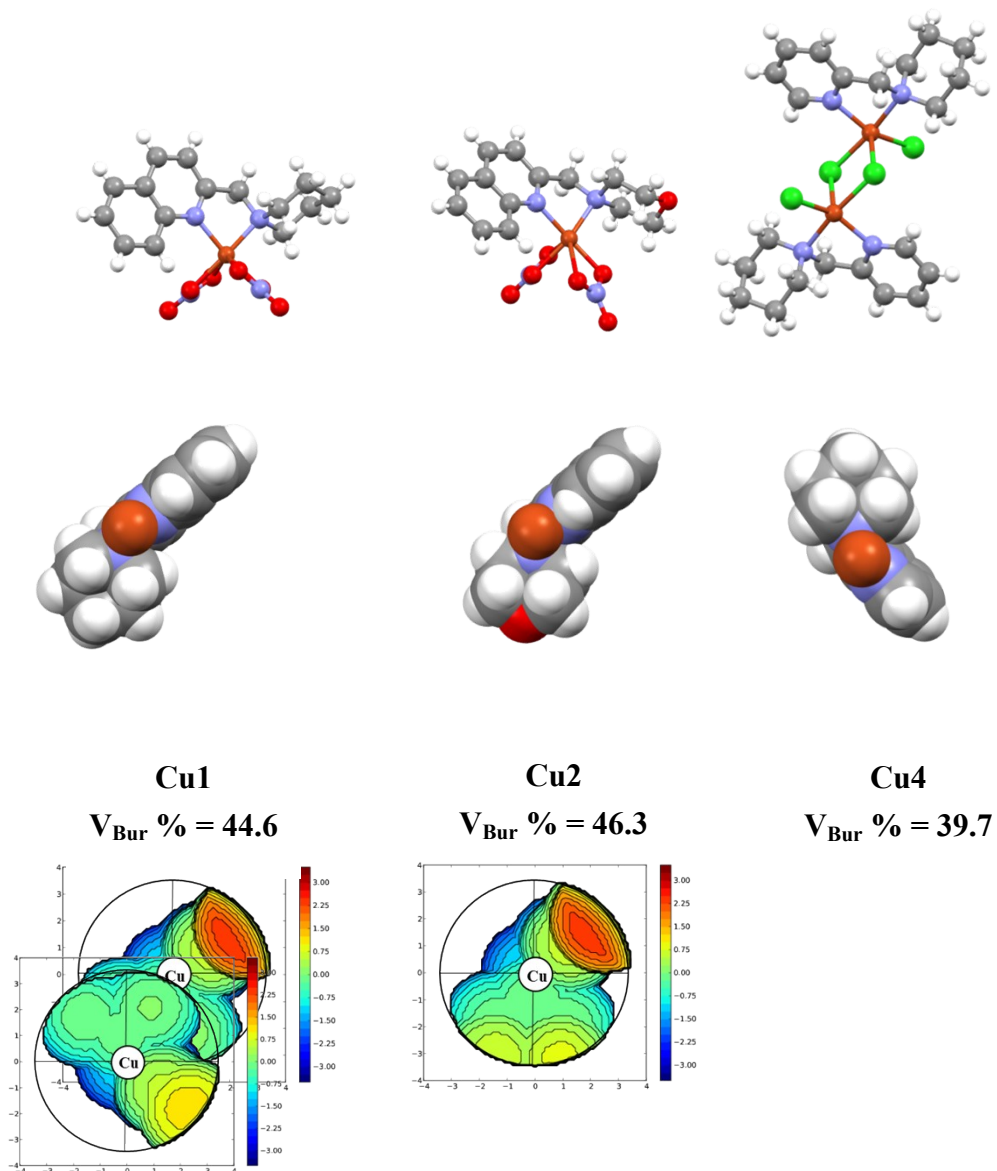**Cu2**

$V_{Bur} \% = 46.3$

**Cu4**

$V_{Bur} \% = 39.7$

Figure S7. Ball and stick models, space-filling models, and topographic steric maps of complexes

Figure S8. Elemental analysis of the synthesized complexes.

```
                    Eager 300 Summarize Results

                        Date : 2022-09-08 at 08:49:35
                    Method Name : NCHS
                Method Filename : EA-A_20220907.mth


Group No : 1        Element %
Sample Name         Nitrogen%        Carbon%         Hydrogen%        Sulphur%
---------------- --------------- --------------- --------------- ---------------
JG-7-60             13.27818871      43.98248672     4.334647655       0    [L_ACu(NO_3)_2]
JG-7-60             13.66265583      43.95064926     4.317540169       0

            2 Sample(s) in Group No : 1
Component Name Average
--------------- ---------------
Nitrogen%      13.47042227
Carbon%        43.96656799
Hydrogen%      4.326093912
Sulphur%       0


                    EagerSmart Summarize Results

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Group No : 1        Element %
Sample Name         Nitrogen         Carbon          Hydrogen          Sulphur
---------------- --------------- --------------- --------------- ---------------
JG-7-61             13.89069843      40.90753937     3.900032043     0
JG-7-61             13.78213024      40.63606262     3.858319521     0     [L_BCu(NO_3)_2]

            2 Sample(s) in Group No : 1
Component Name Average
-------------- --------------
Nitrogen       13.83641434
Carbon         40.77180099
Hydrogen       3.879175782
Sulphur        0


                    Eager 300 Summarize Results

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                Method Filename : EA-A_20220128.mth


Group No : 1        Element %
Sample Name         Nitrogen%        Carbon%         Hydrogen%        Sulphur%
---------------- --------------- --------------- --------------- ---------------
JG-5-27             8.92653656       42.37840271     5.389563084     0
JG-5-27             8.866337776      42.49068832     5.387815952     0     [L_CCu(μ-Cl)Cl]_2

            2 Sample(s) in Group No : 1
Component Name Average
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Carbon%        42.43454552
Hydrogen%      5.388689518
Sulphur%       0
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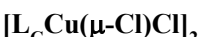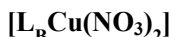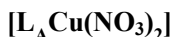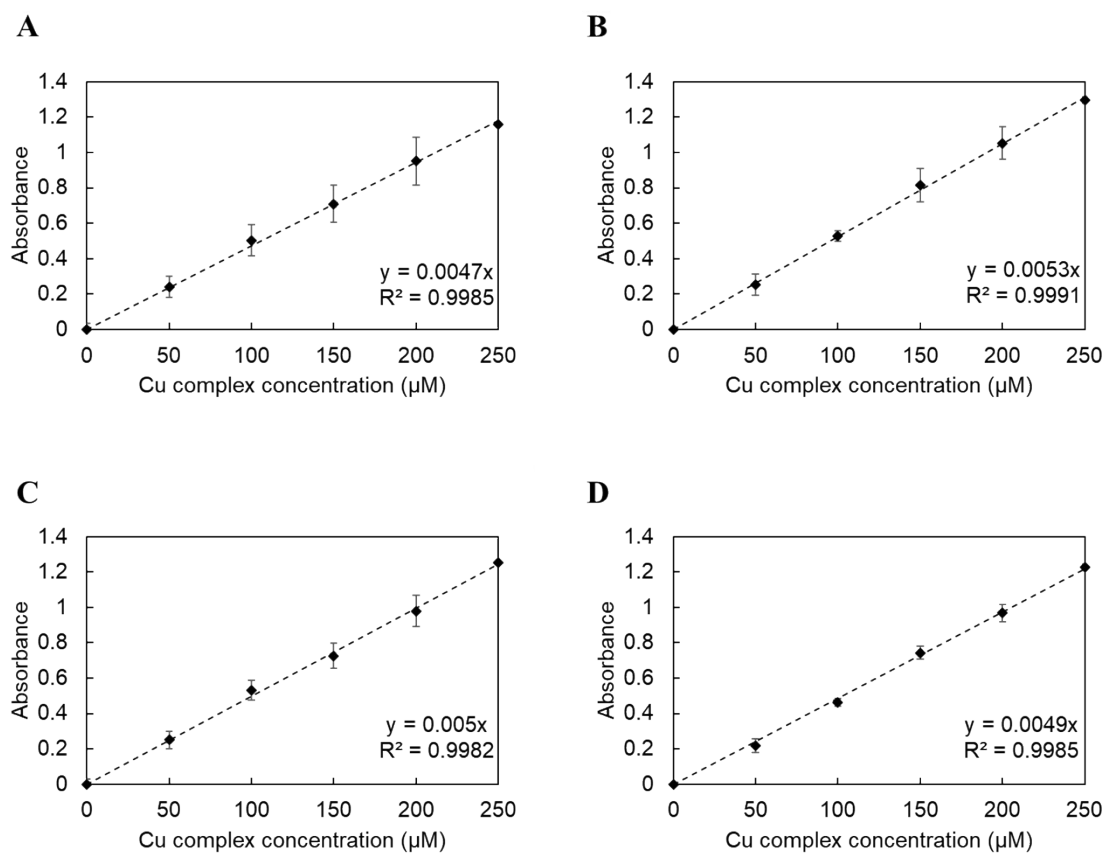
Figure S9. Determination of the extinction coefficient for Cu complexes: Cu1(A), Cu2(B), Cu3(C), and Cu4(D).

Figure S10. CD spectra of CT-DNA in the absence and presence of the various Cu complexes under high salt conditions: Cu1 (A), Cu2 (B), Cu3 (C), and Cu4 (D), respectively. [CT-DNA] = 100 μM and [Cu complex] = 0 (black dashed line), 50 (green line), 100 (blue line), and 150 μM (red line). All samples contain 5 mM cacodylate buffer (pH 7.0) and 100 mM NaCl.
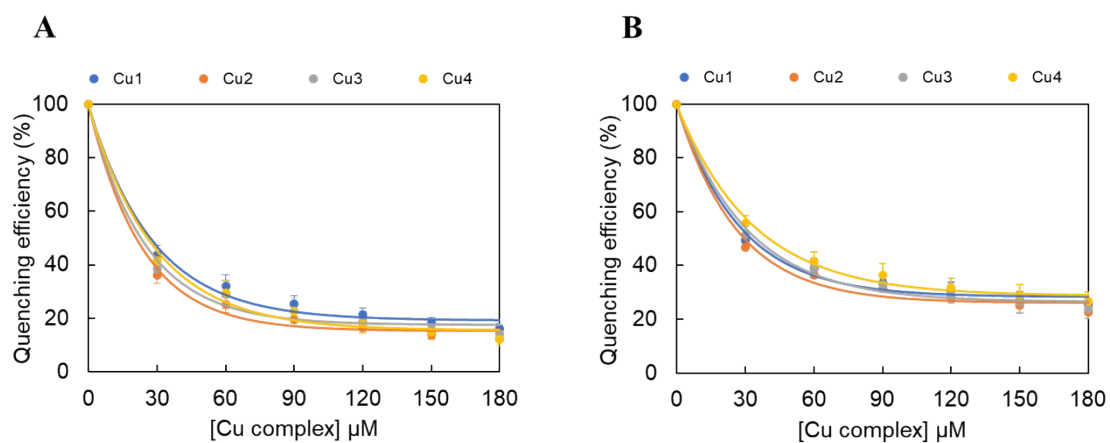
Figure S11. Comparative analysis for fluorescence quenching of limited bound the various DNA binder, EtBr (A) and Hoe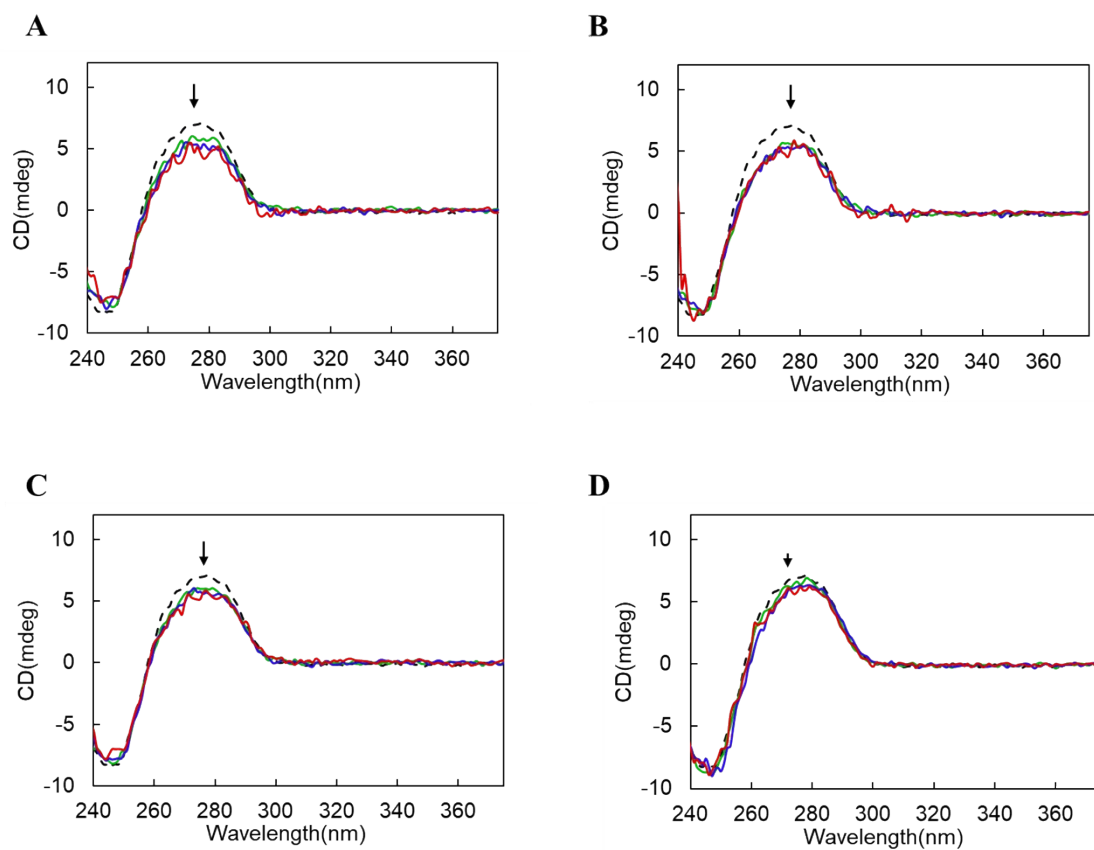chst 33258 (B), at ratio = 0.1, by the addition of Cu1 (blue dot), Cu2 (orange dot), Cu3 (gray dot), and Cu4 (yellow dot). All data show the average value with an error bar after the experiment in duplicate. All samples contain 5 mM cacodylate buffer (pH 7.0) and 10 mM NaCl.
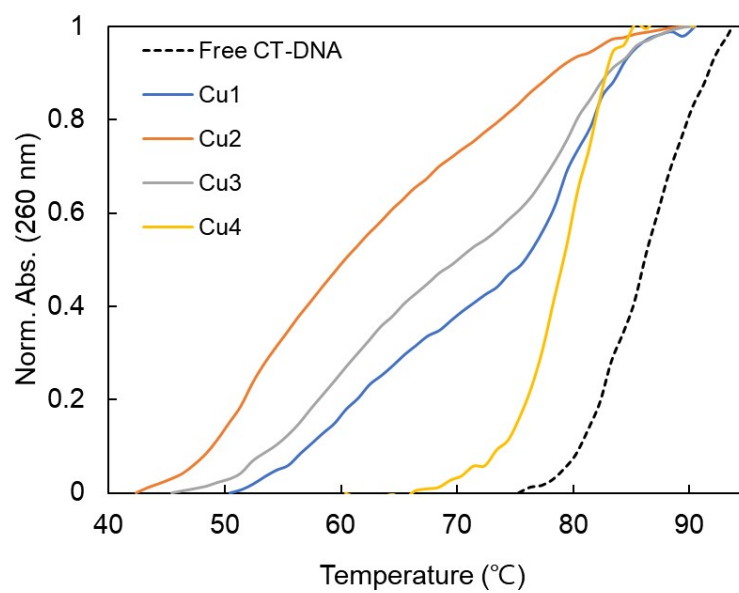
Figure S12. Thermal denaturation curves of CT-DNA in the absence and presence of Cu complexes, Cu1(blue line), Cu2(orange line), Cu3(gray line) and Cu4 (yellow line). [CT-DNA] = 50 μM and [Cu complex] = 100 μM. All samples contain 5 mM cacodylate buffer (pH 7.0) and 100 mM NaCl.
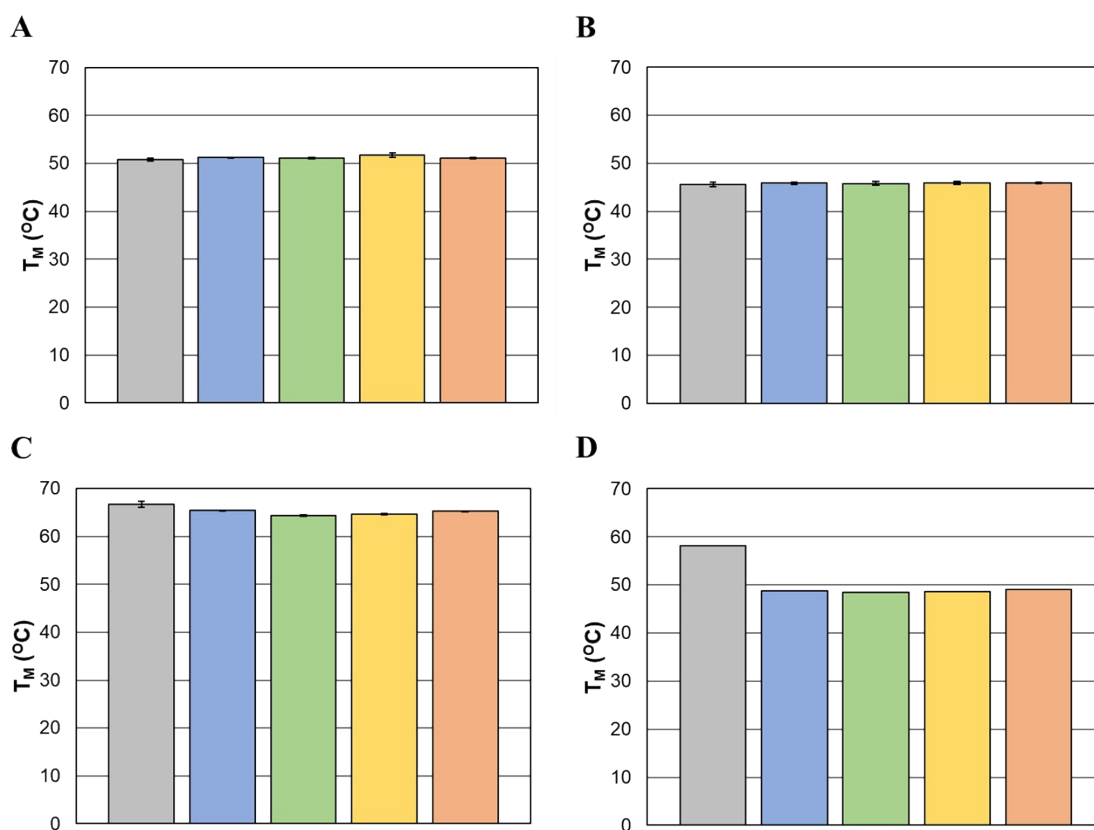
Figure S13. Histogram of melting temperature of homogenous and alternative A-T and G-C oligomers in the absence (gray) and presence of various Cu complexes, Cu1 (blue), Cu2 (green), Cu3 (yellow), and Cu4 (orange). 24mer of alternative A-T (A), 20mer of homogenous A-T (B), 15mer of alternative G-C (C), 15mer of homogenous G-C (D), were used. The ratio of [Cu complex] / [Oligomer] was 10.

Table S1. Crystal data and structure refinement for Cu(II) complexes.

|	$[L_ACu(NO_3)_2]$	$[L_BCu(NO_3)_2]$	$[L_CCu(\mu\text{-}Cl)Cl]_2$
Empirical formula	$C_{15}H_{18}CuN_4O_6$	$C_{14}H_{16}CuN_4O_7$	$C_{22}H_{32}Cl_4Cu_2N_4$
Formula weight	413.87	415.85	621.39
Temperature (K)	100(2)	100(2)	150(2)
Wavelength (Å)	0.610	0.610	0.630
Crystal system	Monoclinic	Triclinic	Monoclinic
Space group	$P2_1/n$	$P\text{-}1$	$P2_1/c$
Unit cell dimensions			
a (Å)	9.968(2)	7.1870(1)	10.682(2)
b (Å)	7.9070(2)	8.2690(2)	7.1300(1)
c (Å)	21.463(4)	14.574(3)	16.493(3)
α (°)	90	74.02(3)	90
β (°)	101.99(3)	89.47(3)	100.03(3)
γ (°)	99	69.01(3)	90
Volume (Å³), Z	1654.7(6), 4	773.6(3), 2	1236.9(4), 2
Density (calculated) (Mg/m³)	1.661	1.785	1.668
Absorption coefficient (mm⁻¹)	0.901	0.967	1.553
F(000)			
Crystal size (mm³)	852	426	636
Theta range for data collection	0.080 × 0.075 × 0.050	0.200 × 0.080 × 0.070	0.075 × 0.035 × 0.015
Index ranges	1.665 to 33.665°	2.333 to 33.652°	1.716 to 25.999°

	-16 ≤ h ≤ 16,	-12 ≤ h ≤ 12,	-14 ≤ h ≤ 14,
Reflections collected	-12 ≤ k ≤ 12,	-13 ≤ k ≤ 13,	-9 ≤ k ≤ 9,
Independent reflections	-35 ≤ l ≤ 35	-22 ≤ l ≤ 23	-22 ≤ l ≤ 22
Completeness to theta	27826	11409	12134
Absorption correction	8692 [R(int) = 0.0625]	7198 [R(int) = 0.0272]	3481 [R(int) = 0.0996]
Refinement method	99.4 % (21.469°)	95.1 % (21.469°)	100.0 % (22.210°)
	Empirical	Empirical	Empirical
Data / restraints / parameters	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Goodness-of-fit on F^2	8692 / 0 / 307	7198 / 0 / 235	3481 / 0 / 145
Final R indices [I>2sigma(I)]			
R indices (all data)	1.150	1.050	0.967
	R_1 = 0.0476	R_1 = 0.0396	R_1 = 0.0415
Largest diff. peak and hole (e.Å^{-3})	$w R_2$ = 0.1382	$w R_2$ = 0.1178	$w R_2$ = 0.0975
	R_1 = 0.0586	R_1 = 0.0417	R_1 = 0.0576
	$w R_2$ = 0.1433	$w R_2$ = 0.1191	$w R_2$ = 0.1016
	1.042 and -1.847	0.977 and -1.236	1.012 and -1.030

Table S2. Relative amounts (%) of Forms (I), (II), and (III) in the presence of various concentrations (30 and 50 µM) of Cu(II) complexes with ascorbate

	Concentration (µM)	Form I (%)	Form II (%)	Form III (%)
sc DNA		83.5 ± 3.7	16.5 ± 3.7	0
Cu1	30	70.9 ± 9.0	29.1 ± 9.0	0
	50	10.3 ± 9.0	56.4 ± 48.9	33.3 ± 57.7
Cu2	30	10.8 ± 9.3	55.9 ± 48.4	33.3 ± 57.7
	50	0	0	100
Cu3	30	25.9 ± 22.8	40.7 ± 35.5	33.3 ± 57.7
	50	0	24.9 ± 43.2	75.1 ± 43.2
Cu4	30	0	0	100
	50	0	0	100

Table S3. Effect of various ROS scavengers on *sc*DNA cleavage[a]

	Scavengers	Form I (%)	Form II (%)	Form III (%)
	sc DNA + Cu1	21.9±26.6	78.1±26.6	0
	Tiron	51.1±3.4	48.9±3.4	0
Cu1	Sodium azide	40.6±13.2	59.4±13.2	0
	Catalase	59.6±1.5	40.4±1.5	0
	DMSO	51.4±11.3	48.6±11.3	0
	sc DNA + Cu2	0	0	100
	Tiron	2.2±1.0	91.6±11.4	6.2±10.7
Cu2	Sodium azide	0	86.1±12.0	13.9±12.0
	Catalase	33.8±7.5	59.2±10.9	7.0±3.9
	DMSO	0	0	100
	sc DNA + Cu3	0	73.9±6.3	26.1±6.3
	Tiron	17.5±14.4	82.5±14.4	0
Cu3	Sodium azide	0	83.4±3.8	16.6±3.8
	Catalase	41.6±2.9	52.2±3.4	6.2±2.0
	DMSO	0	81.0±7.4	19.0±7.4
	sc DNA + Cu4	0	0	100
	Tiron	0	89.2±10.3	10.8±10.3
Cu4	Sodium azide	0	92.3±7.1	7.7±7.1
	Catalase	7.0±6.6	88.8±8.6	4.2±3.8
	DMSO	0	0	100

[a]Data were obtained from Figure 6 for the Cu(II) complex. [*sc*DNA] = 100 μM, [ascorbate] = 100 μM, and [Cu(II) complex] = 50 μM