Electronic Supplementary Information

Unprecedented dinuclear silver(I)-mediated base pair involving the DNA lesion 1,N^6-ethenoadenine

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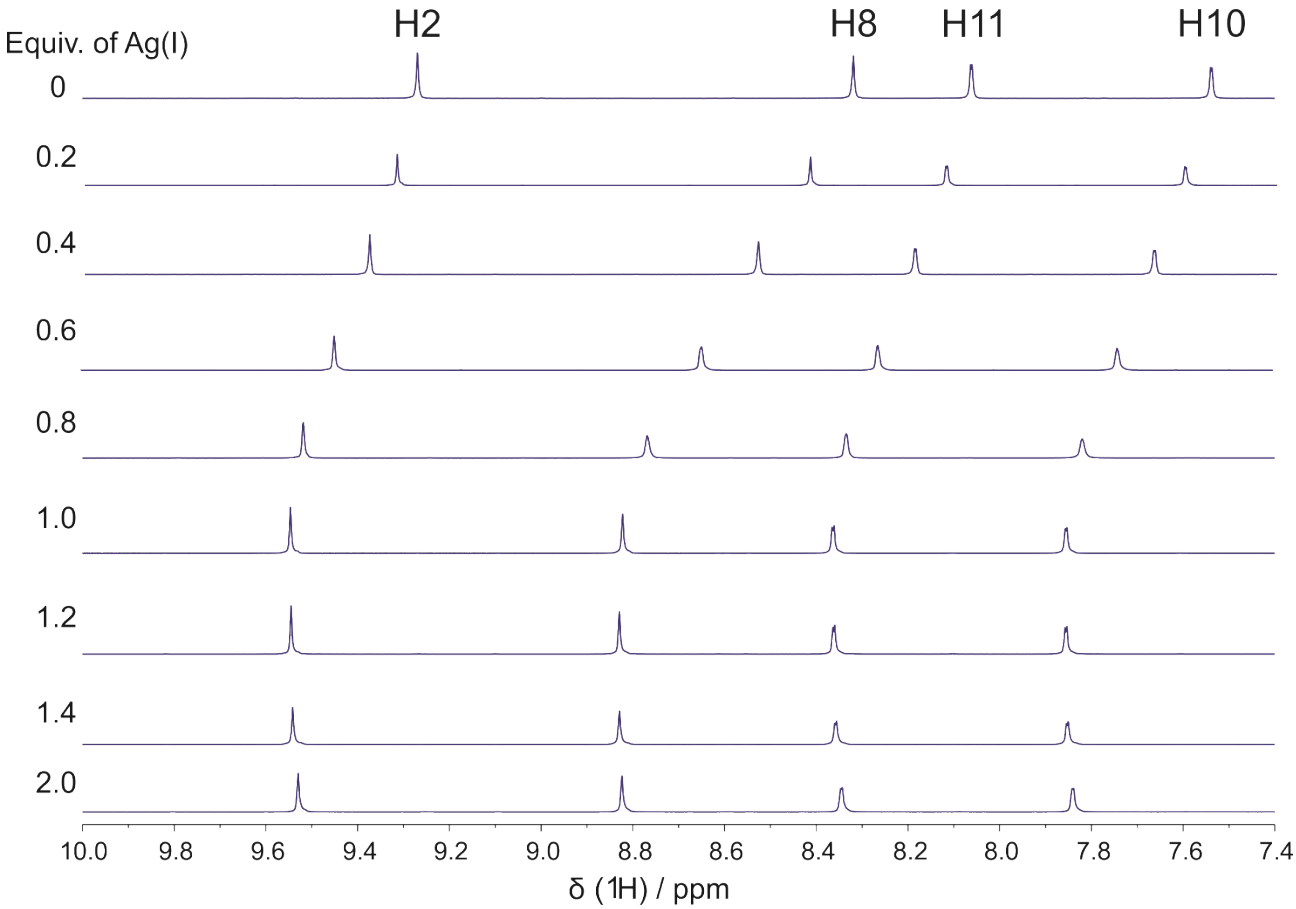
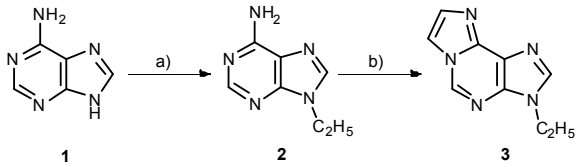


Figure S1: Stack plot of the ^{1}H NMR spectra (aromatic region only) of the titration of model nucleobase 9-ethyl-1,N6-ethenoadenine **3** with increasing amounts of Ag(I) ions. A plot of the chemical shifts *versus* the equivalents of added Ag(I) can be found in Figure 3a.

Experimental Details

1,N^6-Ethenodeoxyadenosine (X) phosphoramidite and all other phosphoramidites required for the synthesis of the investigated oligonucleotide sequences were purchased from Glen Research. DNA syntheses were performed on a K&A Laborgeräte H8 DNA/RNA synthesizer under DMT-off mode by following standard protocols (except for using ultramild Cap Mix A: THF/pyridine/phenoxyacetic anhydride was used instead of THF/pyridine/acetic anhydride). Post synthesis, the oligonucleotides were cleaved from the solid support and deprotected by treating them with 0.05 M K_2CO_3 in methanol (4 hours, r.t.). Thereafter, they were purified by denaturing urea polyacrylamide gel electrophoresis (gel solution: 7 M urea, 1 M TBE buffer; 18% polyacrylamide–bisacrylamide (29 : 1); loading buffer: 11.8 M urea; 42 mM Tris/HCl, pH 7.5; 0.83 mM EDTA, pH 8.0; 8% sucrose; 0.08% dye (xylene cyanol, bromophenol blue)). After purification, the oligonucleotides were desalted by using NAP 10 columns. The desalted oligonucleotides were characterized by MALDI-TOF mass spectrometry (5′-d(GAG GGA XAG AAA G)-3′: calcd. for $[M+H]^+$: 4130 Da, found: 4129 Da; 3′-d(CTC CCT YTC TTT C)-5′: calcd. for $[M+H]^+$: 3836 Da, found: 3835 Da). MALDI-TOF mass spectra were recorded on a Bruker Reflex IV instrument using a 3-hydroxypicolinic acid/ammonium citrate matrix. During the quantification of the oligonucleotides, a molar extinction coefficient ε_{260} of 5.0 cm^2 μmol^{-1} was used for 1,N^6-ethenodeoxyadenosine. NMR spectra were recorded using Bruker Avance(I) 400 and Bruker Avance(III) 400 spectrometers at 300 K. Chemical shifts were recorded with reference to residual DMSO-d_5 (DMSO-d_6, δ = 2.50 ppm) or TSP (D_2O, δ = 0 ppm). ^1H and ^{13}C resonances were assigned based on gHSQC and qHMBC experiments. ^{15}N resonances were assigned based on gHMBC experiments.The ^1H NMR-spectroscopy-based titration of 9-ethyl-1,N^6-ethenoadenine **3** (11 mg, 58 µmol) against varying amount of $AgClO_4$ in DMSO-d_6 showed distinct changes of the chemical shifts (Figure S1). Hence, the chemical shift values of the aromatic protons were plotted against the equivalents of Ag(I) to obtain the stoichiometry of the adduct formed (Figure 3a).
Similarly, the ^{15}N NMR-spectroscopy-based titration of 9-ethyl-1,N^6-ethenoadenine **3** (8.6 mg, 45 µmol) against varying amount of $AgClO_4$ in DMSO-d_6 showed distinct changes of the chemical shifts (Figure 3b).
The UV melting experiments were carried out on a UV spectrometer CARY 100 Bio instrument. Measurements were done in a 1 cm quartz cuvette. The UV melting profiles were measured at 260 nm in buffer (3 µM oligonucleotide duplex, 150 mM $NaClO_4$, 5mM MOPS, pH 6.8) either in absence or in presence of $AgNO_3$, at a heating rate of 1 °C min^{-1} with data being recorded at an interval of 1 °C. Prior to each measurement, the sample was equilibrated by heating to 70 °C followed by cooling to 5 °C at a rate of 1 °C min^{-1}. Melting temperatures were determined from the maxima of the first derivatives of the melting curves. CD spectra was recorded at 5 °C measured with a J-815 spectropolarimeter (JASCO) in buffer (3 µM oligonucleotide duplex, 150 mM $NaClO_4$, 5 mM MOPS, pH 6.8) either in the absence or presence of $AgNO_3$. A 1 cm quartz cuvette was used.



1 2 3

Scheme S1: Synthesis of 9-ethyl-1,N^6-ethenoadenine;.a.) C_2H_5I, Cs_2CO_3, DMF, 55℃, 16 h; b.) $ClCH_2CHO$, H_2O, pH ~ 4-4.5, r.t., 72 h

Synthesis of 9-ethyladenine (2)

The synthesis was carried out using a modified literature procedure.[1] Adenine **1** (2.05 g, 15 mmol) were dissolved in 15 mL dry DMF and stirred for 30 min at room temperature. Thereafter, cesium carbonate Cs_2CO_3 (6.27 g, 19.2 mmol) was added to the reaction mixture and stirred for 45 min at room temperature. Then, ethyl iodide (1.2 mL, 15.1 mmol) was added drop wise, and the resultant reaction mixture was stirred for 16 h at 55 °C. The mixture was dried in vacuum, and the product was extracted from the solid residue in $CHCl_3$, dried ($MgSO_4$) and finally evaporated to dryness. The crude product was purified by column chromatography (SiO_2, dichloromethane-methanol eluent system) yielding **2** as a white solid.

Yield: 2.13 g (86%).

^{1}H NMR (400 MHz, D_2O, pD 7.7): δ(ppm) = 8.00 (s, 1H, H2), 7.89 (s, 1H, H8), 4.11 (q, J = 7.3 Hz, 2H, CH_2), 1.41 (t, J = 7.3 Hz, 3H, CH_3).

^{13}C NMR (100 MHz, D_2O, pD 7.7): δ(ppm) = 154.6 (C6), 151.3 (C2), 147.7 (C4), 141.1 (C8), 117.7 (C5), 39.0 (CH$_2$), 14.2 (CH$_3$).

ESI-MS m/z: 164.0931 $(M+H)^+$ (calcd. 164.0936).

Elemental Analysis (%): found: C 51.4, H 5.5, N 42.8; calcd. for $C_7H_9N_5$: C 51.5, H 5.6, N 42.9.

Synthesis of 9-ethyl-1,N^6-ethenoadenine (3)

The synthesis was carried out using a modified literature procedure.[2] 9-Ethyladenine **2** (0.92 g, 5.6 mmol) was dissolved in 4 mL buffer solution (2 M NaOAc-HOAc), and the solution was stirred for 10 min at room temperature. Chloroacetaldehyde (7.12 mL, 56.4 mmol) was added dropwise, and the final reaction mixture was stirred for further 72 h at room temperature. The mixture was then evaporated to dryness, and the residue was dissolved in $CHCl_3$ and filtered. The resulting solution was treated with $NaHCO_3$ and finally dried ($MgSO_4$). The final filtrate was evaporated to dryness and the crude product was purified by column chromatography (SiO_2, dichloromethane-ethylacetate-methanol eluent system).

Yield: 0.83 g (79%).

^{1}H NMR (400 MHz, D_2O, pD 7.2): δ(ppm) = 8.36 (s, 1H, H2), 7.73 (s, 1H, H8), 7.46 (d, J = 1.7 Hz, 1H, H11), 7.16 (d, J = 1.7 Hz, 1H, H10), 3.91 (q, J = 7.6 Hz, 2H, CH_2), 1.34 (t, J = 7.4 Hz, 3H, CH_3).

^{13}C NMR (100 MHz, D_2O, pD 7.2): δ(ppm) = 140.5 (C8), 139.9 (C6), 137.3 (C4), 135.9 (C2), 131.5 (C10), 120.2 (C5), 111.9 (C11), 39.5 (CH$_2$), 14.3 (CH$_3$).

^{1}H NMR (400 MHz, DMSO-d_6): δ(ppm) = 9.27 (s, 1H, H2, $^1J_{HC}$ = 215 Hz), 8.32 (s, 1H, H8, $^1J_{HC}$ = 203 Hz), 8.06 (d, 1H, H11, $^1J_{HC}$ = 199 Hz), 7.53 (d, 1H, H10, $^1J_{HC}$ = 189 Hz), 3.91 (q, 2H, CH_2), 1.32 (t, 3H, CH_3).

^{13}C NMR (100 MHz, DMSO-d_6): δ(ppm) = 140.7 (C6), 140.6 (C8), 138.5 (C4), 136.5 (C2), 132.4 (C10), 122.6 (C5), 111.8 (C11), 39.5 (CH$_2$), 14.3 (CH$_3$).

^{15}N NMR (40 MHz, DMSO-d_6): δ(ppm) = 241 (N7), 231 (N6), 230 (N3), 202 (N1), 169 (N9).

ESI-MS m/z: 188.0936 $(M+H)^+$ (calcd. 188.0936).

Elemental Analysis (%): found: C 57.6, H 4.8, N 37.1; calcd. for $C_9H_9N_5$: C 57.7, H 4.8, N 37.4.

Synthesis of the dinuclear model complex:
The dinuclear complex of 9-ethyl-1,N^6-ethenoadenine **3** and $AgClO_4$ has been synthesized *in situ* in an NMR tube by titrating a solution $AgClO_4$ to a solution of the ligand (8.6 mg, 45 μmol) in DMSO-d_6.
^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 9.55 (s, 1H, H2, $^1J_{HC}$ = 220 Hz), 8.81 (s, 1H, H8, $^1J_{HC}$ = 219 Hz), 8.37 (d, 1H, H11, $^1J_{HC}$ = 201 Hz), 7.85 (d, 1H, H10, $^1J_{HC}$ = 197 Hz), 4,54 (q, 2H, CH_2), 1.56 (t, 3H, CH_3).
^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) = 143.6 (C8), 139.5 (C6), 139.2 (C4), 138.4 (C2), 132.4 (C10), 119.5 (C5), 113.9 (C11), 40.0 (CH$_2$), 15.2 (CH$_3$).
^{15}N NMR (40 MHz, DMSO-d_6): δ (ppm) = 233 (N3), 201 (N1), 197 (N7), 189 (N6), 175 (N9).

References

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2. T. Saito, M. Murakami, T. Inada, H. Hayashibara, T. Fujii, *Chem. Pharm. Bull.,* 1993**, 41**, 453-457.