Supplementary Material (ESI) for Organic and Biomolecular Chemistry

Synthesis and characterization of the 5-methyl-2'-deoxycytidine glycol–dioxoosmium–bipyridine ternary complex in DNA

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Electronic Supplementary Information

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Experimental information

Preparation of DNA-Os-Bpy complexes and its digestion procedure

Oligonucleotide (5 μM) was treated with 2,2'-bipyridine (100 mM), potassium osmate (5 mM) and potassium hexacyanoferrate(III) (100 mM) in a buffer solution [100 mM Tris-HCl buffer (pH 7.7)–acetonitrile, 9:1(v/v)] for 1 h at 50 °C. The excess reactants were removed with Micro Bio-Spin®. The eluate was fully digested with calf intestine alkaline phosphatase (50 U/mL), snake venom phosphodiesterase (0.15 U/mL), and P1 nuclease (50 U/mL) at 37 °C for 3 h. The digested product was analyzed by RP-HPLC on tandem 5-ODS-H columns (10 × 150 mm × 2) with an eluent [0.1 M triethylammonium acetate (TEAA, pH 7.0)–acetonitrile, 93:7 (v/v)] at a flow rate 3.0 mL/min. This condition was used for all RP-HPLC experiments.

Synthesis of M-Os-Bpy. An aqueous solution of 2'-deoxy-5-methylcytidine (100 μL, 1 mM), a solution of 2,2'-bipyridine in acetonitrile (100 μL, 1 M), an aqueous solution of potassium osmate (200 μL, 25 mM), a freshly prepared aqueous solution of potassium hexacyanoferrate(III) (100 μL, 1 M), and 1 M Tris-HCl buffer (100 μL, pH 7.7) were mixed and measured up to 1 mL with water. After 1 h at 50 °C, the reaction mixture was analyzed by RP-HPLC. Two diastereomers were purified using RP-HPLC, and then the elution buffer was removed by repeating lyophilization.

(5*R*,6*S*)-M-Os-Bpy (major isomer). ^{1}H NMR (400 MHz, D_2O) δ_H 9.18 (1H, d, J = 4.5, H−C(3-bpy)), 8.95 (1H, d, J = 4.4 H−C(3'-bpy)), 8.52 (2H, m, H−C(6,6'-bpy)), 8.36−8.30 (2H, m, H−C(4,4'-bpy)), 7.87−7.77 (2H, m, H−C(5,5'-bpy)), 6.32 (1H, dd, J = 8.8, 5.9, H−C(1')) 5.37 (1H, s, H−C(6)), 4.28−4.25 (1H, m, H−C(3')), 3.82−3.79 (1H, m, H−C(4')), 3.58−3.49 (2H, m, H−C(5')), 2.50−2.42, 2.22−2.16 (2H, m, H−C(2',2''), 1.62 (3H, s, H_3C(5)); FAB-HRMS (*m*/*z*): found 654.1236 ($[M + H]^+$, $C_{20}H_{24}N_5O_8Os$ calc. 654.1240).

(5*S*,6*R*)-M-Os-Bpy (minor isomer). ^{1}H NMR (400 MHz, D_2O) δ_H 9.19 (1H, d J = 4.4, H−C(3-bpy)), 9.10 (1H, d J = 4.4, H−C(3'-bpy)), 8.58 (2H, m, H−C(6,6'-bpy)), 8.41−8.34 (2H, m, H−C(4,4'-bpy)), 7.88−7.80 (2H, m, H−C(5,5'-bpy)), 6.22 (1H, q, J = 6.9, H−C(1')), 5.27 (1H, s, H−C(6)), 4.57−4.31 (1H, m, H−C(3')), 3.87−3.86 (1H, m, H−C(4')), 3.69−3.65, 3.57−3.53 (2H, m, H_2C(5')), 2.37−2.21 (2H, m, H−C(2',2'')),

1.65(3H, s, $H_3C(5)$); FAB-HRMS (*m*/*z*): found 654.1235 ($[M + H]^+$, $C_{20}H_{24}N_5O_8Os$ calc. 654.1240).

(5*R*,6*S*)-T-Os-Bpy (major isomer). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 10.23 (1H, br s, H−N(3)), 9.16 (1H, d, *J* = 4.4, H−C(3-bpy)), 9.05 (1H, d, *J* = 4.4 H−C(3'-bpy)), 8.96−8.93 (2H, m, H−C(6,6'-bpy)), 8.57−8.51 (2H, m, H−C(4,4'-bpy)), 8.04−8.02 (1H, m, H−C(5-bpy)), 7.98−7.96 (1H, m, H−C(5'-bpy)), 6.23 (1H, dd, *J* = 8.1, 5.9, H−C(1')), 5.25 (1H, s, H−C(6)), 5.14 (1H, br d, *J* = 3.7, H−O(3')), 4.72−4.70 (1H, m, H−O(5')), 4.16−4.15 (1H, m, H−C(3')), 3.67−3.64 (1H, m, H−C(4')), 3.41−3.35 (2H, m, $H_2C(5')$), 2.45−2.40, 2.09−2.05 (2H, m, $H_2C(2',2'')$), 1.53 (3H, s, $H_3C(5)$); FAB-HRMS (*m*/*z*): found 655.1078 ($[M + H]^+$, $C_{20}H_{23}N_4O_9Os$ calc. 655.1080).

(5*S*,6*R*)-T-Os-Bpy (minor isomer). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 10.25 (1H, br s, H−N(3)), 9.17 (1H, d *J* = 5.0, H−C(3-bpy)), 9.12 (1H, d, *J* = 5.0, H−C(3'-bpy)), 8.96−8.93 (2H, m, H−C(6,6'-bpy)), 8.57−8.51 (2H, m, (4,4'-bpy)), 8.05−8.03 (1H, m, H−C(5-bpy)), 7.98−7.96 (1H, m, H−C(5'-bpy)), 6.09 (1H, q, *J* = 6.6, H−C(1')), 5.22 (1H, s, H−C(6)), 5.15 (1H, d, *J* = 4.6, H−O(3')), 4.62−4.60 (1H, m, H−O(5')), 4.21−4.18 (1H, m, H−C(3')), 3.73−3.71 (1H, m, H−C(4')), 3.54−3.47 (2H, m, H−C(5')), 2.33−2.29, 2.17−2.12 (2H, m, H−C(2',2'')), 1.56 (3H, s, $H_3C(5)$); FAB-HRMS (*m*/*z*): found 655.1084 ($[M + H]^+$, $C_{20}H_{23}N_4O_9Os$ calc. 655.1080).

Monitoring the deamination of 5-methylcytosine glycol-dioxoosmium-2,2'-bipyridine complexes. A 1 mM solution of M-Os-Bpy in water was incubated at 50 °C. The progress of deamination was monitored using RP-HPLC analysis.

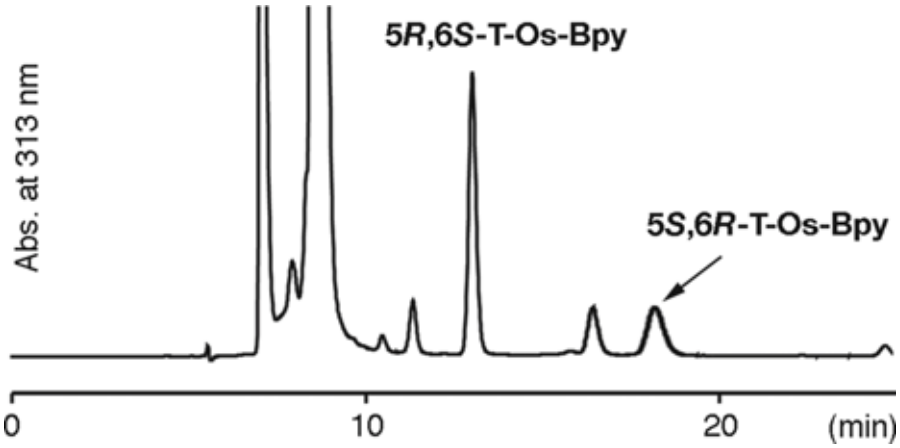

Fig. S1 HPLC analysis of the crude mixture of chemically synthesized T-Os-Bpy.

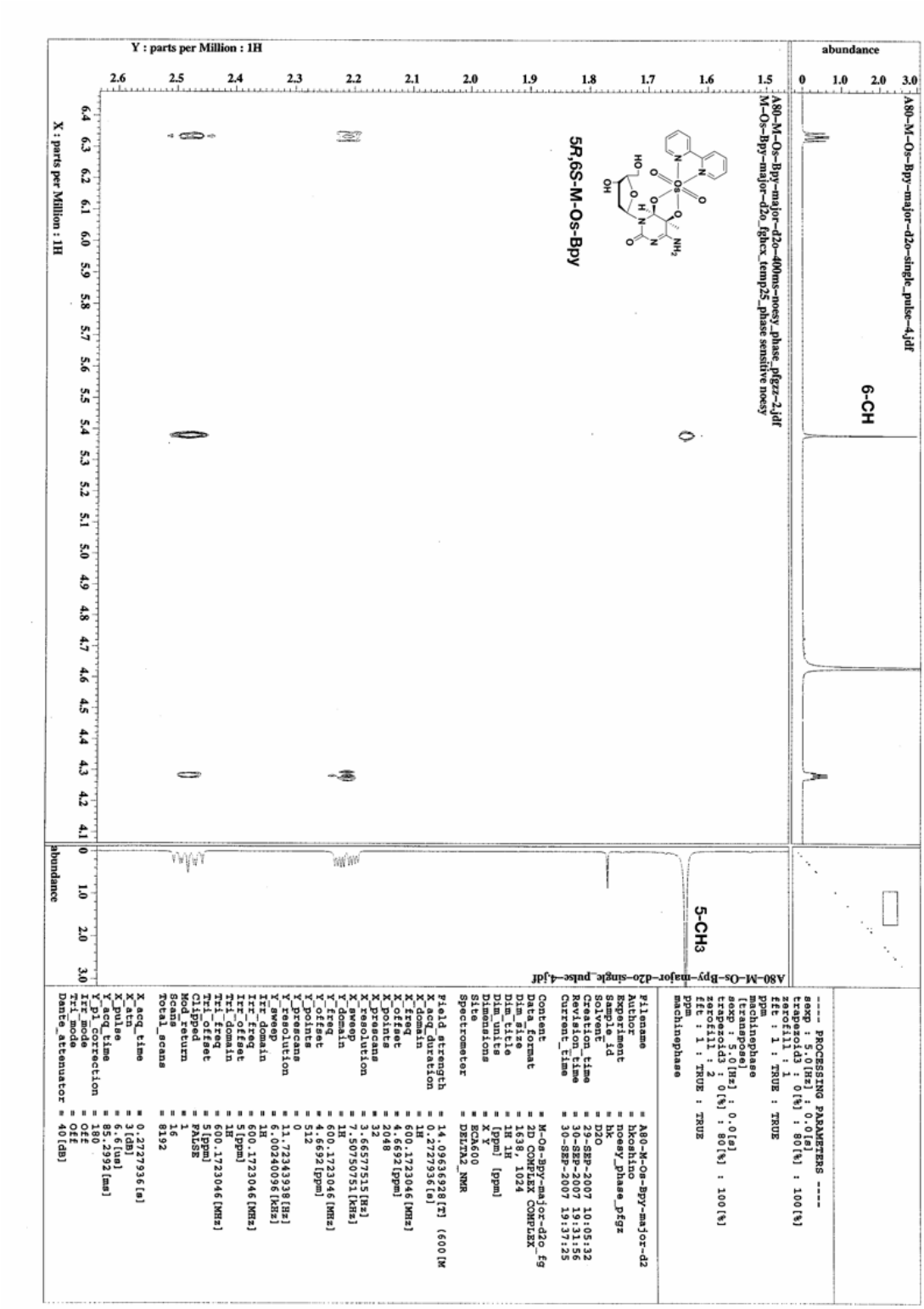

Fig. S2 NOESY profile of (5*R*,6*S*)-5-methyl-2'-deoxycytidine glycol–dioxo-osmium(VI)–2,2'-bipyridine ternary complex.

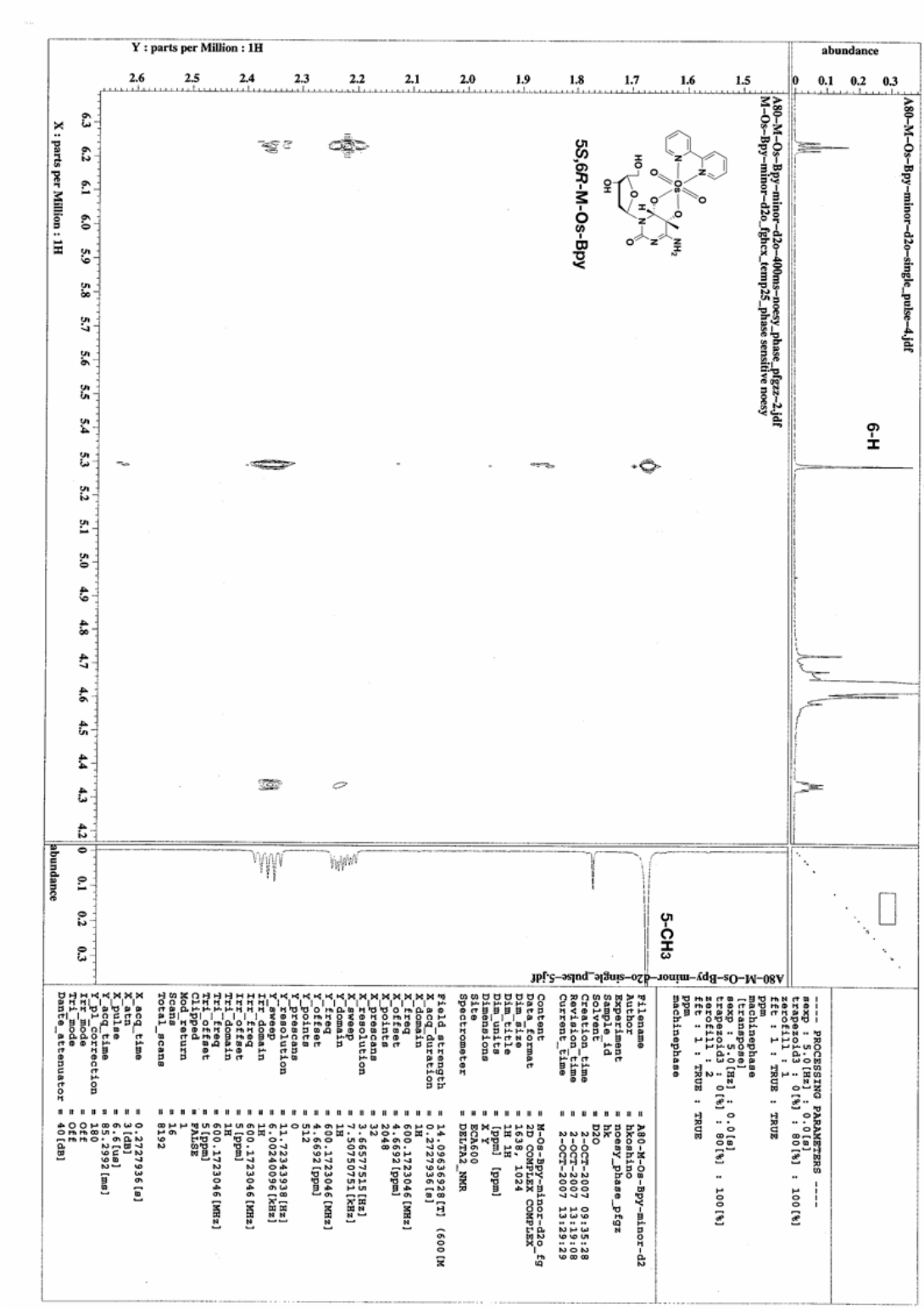

Fig. S3 NOESY profile of (5*S*,6*R*)-5-methyl-2'-deoxycytidine glycol–dioxo-osmium(VI)–2,2'-bipyridine ternary complex.