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Induced-fit recognition of DNA by organometallic complexes with dynamic stereogenic centers

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Organometallic chemistry offers novel concepts in structural diversity and molecular recognition that can be used in drug design. Here, we consider DNA recognition by \( \eta^6 \)-arene Ru(II) anticancer complexes by an induced-fit mechanism. The stereochemistry of the dinuclear complex \([([\eta^6\text{-biphenyl}]\text{RuCl}(\text{en}))_2\]-(CH\(_2\)_6\(\text{N}^+\text{PF}_6^-\)). \(\text{Ru}\) configurations are more stable than \(\text{Ru}^+\text{N}^+\text{PF}_6^-\) (73:27). X-ray and NMR studies showed that reactions of 2 and 3 with 9-ethylguanine gave rise selectively to \(\text{Ru}^+\text{N}^+\text{PF}_6^-\) diastereomers. Dynamic chiral recognition of guanine can lead to high diastereoselectivity of DNA binding. The dinuclear complex 3 induced a large unwinding (31°) of plasmid DNA, twice that of mononuclear 2 (14°), and effectively inhibited DNA-directed RNA synthesis \(\text{in vitro}\). This dinuclear complex gave rise to interstrand cross-links on a 213-bp plasmid fragment with efficiency similar to bifunctional cisplatin, and to 1,3-GG interstrand and 1,2-GG and 1,3-GTG intrastrand cross-links on site-specifically ruthenated plasmid DNA, twice that of mononuclear 2.

Experimental Procedures

Synthesis. The tetraamine ligand \(N,N',N'^{-}\text{bis}(2\text{-aminoethyl})\text{-1,6-diaminohexane}\) was synthesized stepwise as the tetrahydrochloride salt (Scheme 1), which is published as supporting information on the PNAS web site. This was reacted with \([([\eta^6\text{-Bip}]\text{RuCl}_2)_2\] to form the dinuclear complex \([([\eta^6\text{-Bip}]\text{RuCl(en)}_2\text{[PF}_6^-\text{][Bis(Ru,Bip)]]_3\text{,}}\text{where Bip is biphenyl. The monomeric complex }\[([\eta^6\text{-Bip}]\text{RuCl(Et-en)}_2\text{][PF}_6^-\text{) (2) was prepared by reaction of N-ethyl-en (Et-en) with }\[([\eta^6\text{-Bip}]\text{RuCl}_2]_2\text{. Slow diffusion of ether into a methanol solution of 2 produced crystals suitable for x-ray diffraction. Crystalline 2 consisted of mixture of orange lumps of diastereomer 2(A)\(\text{Ru}^+\text{N}^+\text{PF}_6^-\)) and yellow flakes of diastereomer 2B (\(\text{Ru}^+\text{N}^+\text{PF}_6^-\)), which were separated by hand. The 9-ethylguanine \(9\text{EtG}\) adducts \([([\eta^6\text{-Bip}]\text{Ru(N7-9EtG)(Et-en)}_2\text{[PF}_6^-\text{][Bis(Ru,Bip)]]_3\text{,}((4) and \([([\eta^6\text{-Bip}]\text{Ru(N7-9EtG)(en)}_2\text{[CH}_2\text{]}_3\text{][PF}_6^-\text{] (5) were prepared by reactions of 9EtG with 2 or \([([\eta^6\text{-Bip}]\text{Ru(H}_2\text{O}(\text{en})_2\text{[CH}_2\text{]}_3\text{][PF}_6^-\text{] (3ww} in water (G/Ru = 12:1). Recrystallization of 4 (\text{PF}_6^-\text{ salt from methanol gave yellow crystals suitable for x-ray diffraction. Full details of synthesis and characterization are in the Supporting Text, which is published as supporting information on the PNAS web site.

Kinetic Studies. Reactions of chloro or aqua complexes 1-3 (2.55 mM Ru, except 0.5 mM for 3) with 9EtG in a 1:1 Ru/9EtG molar ratio were carried out in 10% D\(_2\text{O}/90% \text{H}_2\text{O}\) in NMR tubes. Aqua complexes were prepared by treatment of chloro com-

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Abbreviations: Bip, biphenyl; dien, diethylenetriamine; CT, calf thymus; en, ethylenediamine; 9EtG, 9-ethylguanine.

Data deposition: The x-ray crystal structures of complexes 2A, 2B, and 4 have been deposited in the Cambridge Structural Database, Cambridge Crystallographic Data Centre, Cambridge CB2 1EZ, United Kingdom, (CSD reference nos. 223919, 223918, and 223917, respectively).

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plexes with a stoichiometric amount of AgNO₃. Interconversion of diastereomers 2A and 2B (0.5 mM Ru) was studied in D₂O in NMR tubes. When necessary, 180 mM NaCl was used to suppress hydrolysis. Interconversion rate constants (k₋₁, 2A → 2B; k₋₂, 2B → 2A) were obtained by fitting rate equations for first-order reversible reactions by using the program SCIENTIST (version 2.0, MicroMath, St. Louis).

DNA Modification. Calf thymus (CT) DNA and plasmid DNAs were incubated with metal complexes in 10 mM NaClO₄ at 310 K for 48 h in the dark, unless stated otherwise. The molar ratios of bound metal complex to nucleotide phosphate (n₀) were determined by flameless atomic absorption spectrophotometry. DNA transfections of CT DNA measured by 1H NMR chemical shifts were determined by flameless atomic absorption spectrophotometry.

DNA Transcription by RNA Polymerase in Vitro. Transcription of the (NdeI/HpaI) restriction fragment of pSP73KB DNA with SP6 or T7 RNA polymerase and electrophoretic analysis of transcripts were performed according to the protocols recommended by Promega as described (21). Before aliquots containing the transcripts were loaded on the polyacrylamide gel, the radioactivity associated with these samples was adjusted so that equal transcript loads were placed on the gel. 1H NMR chemical shifts were determined by using the general priority rule (27), consistent with those of 72.9:27.1 for 72.9:27.1 for 72.9:27.1. H₂O was studied in D₂O, consistent with each Ru unit to behave independently (Fig. 1). The strategy of linking two or three Pt centers has already been used successfully in drug design (27).

X-Ray Crystallography. Diffraction data for all compounds were collected with Mo-Kα radiation for 2A, at 220 K on a Stoe (Darmstadt, Germany) Stadi-4 diffractometer, for 2B and 4 at 150 K on a Bruker-AXS (Madison, WI) SMART APEX CCD diffractometer; both were equipped with Oxford Cryosystems (Long Hanborough, U.K.) low-temperature devices. Absorption corrections were carried out by using ψ-scans or the multisiscan procedure SADABS (23). All structures were solved by direct methods and refined against F² by using all data [SHELXTL (24) for 2A and 2B; CRYSTALS (25) for 4]. H atoms were placed in calculated positions, and non-H atoms were modeled with anisotropic displacement parameters. WEBLAB VIEWERPRO 4.0 was used for the graphics in Fig. 2.

NMR Spectroscopy. NMR data were acquired on Bruker DMX 500 (1H = 500 MHz) and Avance 600 (1H = 600 MHz) spectrometers. Standard pulse sequences were used for 2D heteronuclear single quantum coherence (HSQC), total correlation spectroscopy, COSY, double quantum filtered COSY, NOESY, etc. (mixing time up to 1,000 ms), and rotating-frame Overhauser effect spectroscopy (mixing time 150 ms). The water resonance was suppressed via the double pulsed-field-gradient spin-echo method (26). 2D [¹H,¹5N] HSQC NMR spectra were acquired for complexes 2-5 with ¹5N in natural abundance. Data processing was carried out by using XWIN-NMR (version 3.0, Bruker Biospin, Karlsruhe, Germany). ¹H NMR chemical shifts were internally referenced to (CD₂F₂)SO (2.50 ppm), (CD₂F₂)CO (2.06 ppm), sodium 3-(trimethylsilyl)-2,2,3,3-d₂-propanoate (0 ppm), or dioxane (3.76 ppm), and ¹5N to 1 M ¹5NH₄Cl in 1.5 M HCl (external) at 0 ppm.

Molecular Modeling. Modeling was carried out by using SYBYL (version 6.3, Tripos Associates, St. Louis) by docking a model of {[(t⁻⁶-Bip)Ru(N⁷-G)(en)]₂(CHO)₂} (based on the x-ray structure of 4, onto B-form duplex DNA 5'-AATGTCTAA-3'/3'-TTACAGATT-5'.

Supporting Information. Full details for experimental procedures (materials, synthesis, acidity constants, DNA experiments, and kinetics), Tables 1–4 (crystallographic data, rate constants, half-lives, equilibrium constants), and Figs. 6–21 ([¹H NMR, 2D COSY, heteronuclear single quantum coherence, NOESY, and rotating-frame Overhauser effect spectroscopy spectra for 2-5; kinetics of 9EtG and CT DNA binding, cross-linking, ethidium fluorescence, modeling) are published as supporting information on the PNAS web site.

Results and Discussion

Steric Conformation and Epimerization Rates. We synthesized the dinuclear complex [{[(t⁻⁶-Bip)RuCl(en)]₂(CHO)₂}²⁺ [bis(RuBip), 3] by linking two {{[(t⁻⁶-Bip)RuCl(en)]²⁺} units with a hexamethylene chain, which is long and flexible enough to allow each Ru unit to behave independently (Fig. 1). The strategy of linking two or three Pt centers has already been used successfully in drug design (27). Complex 3 contains four sterogenic centers (Ru, N, N, Ru), giving rise to 10 possible configurations (Fig. 13). Studies of the dinuclear analogue [{[(t⁻⁶-Bip)RuCl(Et-en)]²⁺} (2), which contains similar sterogenic Ru and N centers, allowed subsequent elucidation of the structure and dynamics of 3.

Synthesis of 2 gave rise to two diastereomers 2A(R₉[R₈]) and 2B(S₉[R₈]) (28) in a 73.7:26.3 mol ratio, as revealed by x-ray crystallography (Figs. 2 and 6) and NMR studies (Figs. 8–11). The 2D [¹H,¹5N] heteronuclear single quantum coherence NMR spectra of 2 and 3 in DMSO-d₆ are almost identical (Fig. 8), suggesting that each Ru unit of 3 is present as either an A(R₉[R₈]) or B(S₉[R₈]) configuration and that each Ru unit has little influence on the other. Complex 3 can be treated as a diastereomeric mixture of A₁(1), A₁B₁(2), and B₁B₂(3) (Figs. 3A, 3B, and 3C) with that of 72.9:27.1 for 72.9:27.1 for 72.9:27.1. The AA/AB/BB ratio was determined to be 67.2:24.0:8.3 by 2D total correlation spectroscopy NMR (Fig. 3B), consistent with that of 72.9:27.1 for A/B from 2D [¹H,¹5N] NMR (Fig. 8ii).

The displacement of Cl by H₂O appears to have negligible...
The rate constant was determined to be 7.57 (±0.01) and 7.35 (±0.01), respectively (Fig. 17). Each Ru unit of 3 (Cl or H2O) can be envisioned to undergo a similar interconversion process in aqueous solution, giving rise to the same observed equilibrium ratio of 72:28 B as for 2. Our studies suggest that for both 2 and 3 (Cl or H2O) the A(RRu(RN)) configurations are thermodynamically preferred, and that each Ru unit is in dynamic equilibrium between configurations A(RRu(RN)) and B(S(RRu(RN))).

### Diastereoselectivity in Guanine N7 Recognition

Reaction of 9EtG with the diastereomeric mixture of 3 (A/B = 72.9:27.1) or 2 (A/B = 73.7:26.3) gave rise to the final products ([η5-Bip]Ru(N7-9EtG)(en))2(CH2)2]+ (5) and [η5-Bip]Ru(N7-9EtG)(Et-en)]+ (4), respectively, with ~90% formation (Fig. 7). N7 coordination for 4, 5, and 3gw [intermediate (9EtG-N7)Ru~H2O] was confirmed by pH titrations, giving associated pKa values: pKa(G NH) 8.03 (4), 7.83 (3gw), 7.86 (5), compared with 9.66(G NH) and 2.4(G N7) for free 9EtG (Figs. 7 and 14).

Analysis of the configurations of 5 was aided by the structural analysis of 4. X-ray crystallography (Figs. 2C and 6n) and NMR studies (Figs. 8 and 15) showed that 4 contains a single (95%) diastereomeric pair B(S(RRu(RN))), with the N2H2 proton pointing down toward the G base. Complex 5 showed one set of en NH crosspeaks in the 2D [1H,15N] heteronuclear sequential quantum correlation NMR spectrum (acetone-d6), almost identical to that for 4 (Fig. 8), indicating that each Ru unit of 5 has the same configuration B(S(RRu(RN))) as for 4. This was confirmed by other 2D NMR data (Figs. 3C and 16).

The B(S(RRu(RN))) configurations are therefore highly favored (95%) for the 9EtG adducts 4 and 5 (Figs. 7 and 8), whereas the A(RRu(RN)) configurations are strongly destabilized by steric interactions between the G and the en alkyl substituent. This finding is in contrast to their parent chloro complexes 2 or 3 and their aqua adducts, for which the A(RRu(RN)) configuration is thermodynamically preferred (72%) and in which the en alkyl substituent points down toward Cl or H2O. Displacement of Cl or H2O by 9EtG forces the alkyl substituent to tilt up and to give the B configuration, which can be stabilized by stereospecific H bonding between en NH and G O6 (Fig. 2). Facile epimerization at Ru or N centers appears to allow dynamic switching between configurations, leading to high diastereoselectivity in the formation of G adducts.11 In a reported study (5), reaction of the diastereomeric mixture of [(η5-C6H5)RuCl(t-ala)] with 9EtG did not change the abundance of Ru6Sc and Sc6Ru (65:35). In this case, the α–C center has a fixed configuration 5C and no epimerization potential; its methyl substituent is distant from the G base and has little influence on the reaction.

Structural and dynamic studies of interactions of 9EtG with 3 and 2 provided insights into the recognition of natural DNA by 3. Substitution of an en NH proton in 1 by the alkyl group to give 2 or 3 (Fig. 1) had little effect on the kinetics of reaction of the chloro complexes with CT DNA (1, t1/2 = 10 min; 3, t1/2 = 15 min), or on reactions of the aqua (1w, 2w, 3ww; t1/2 = ~35 min) or chloro (1, 2, 3; t1/2 = ~55 min) complexes with 9EtG (Table 4). All of these reactions were >80% complete, indicating that the alkyl substituent does not significantly hinder G binding when epimerization is facile.

**Diinuclear BisRu(Bip) Distorts DNA.** The dinuclear complex 3 binds rapidly to CT DNA (Fig. 18). The binding was stable with little loss of bound Ru after extensive dialysis in 0.01 M NaClO4 or

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11NMR data for the 5’ GMP adducts ([η5-Bip]Ru(N7-5’GMP)(en))2+ (H8: 8.73 and 8.82 ppm) and ([η5-Bip]Ru(N7-5’GMP)(en))2+ (H8: 8.72 and 8.83 ppm) are consistent with this interpretation. 1H NMR peaks for the S(Ru) (μ-ο) and S(Ru) (μ-η) diastereomers were present in a 1:1 mol ratio (10% D2O, pH 7.2), suggesting that there may be no enantioselectivity between S(Ru) and S(Ru) for DNA binding.
1.0 M NaCl. An assay of DNA-directed RNA synthesis revealed that each Ru unit of 3 coordinates preferentially to G bases of DNA (Fig. 4). Complex 3 shows termination sequences somewhat different from cisplatin, although the intensity of the bands corresponding to major stop sites was similar (Fig. 4A). Furthermore, adducts of complex 3 inhibit RNA synthesis more effectively than those of mononuclear complexes 1 (14), 2 (Fig. 4A), and 2A (data not shown). The pattern of stop sites for transplatin is different, and no termination is observed for monofunctional [Pt(dien)Cl]2– (data not shown). Previous studies (14) have shown that 1 induces an unwinding angle twice that of [(η6-p-cymene)RuCl(en)]+ (7°) because of the contribution from the Bip ring, possibly by intercalation. Compared with the DNA unwinding induced by the linkage isomers cis-[Pt(NH3)2(N3/N8-ethidium)Cl]2+ [15°/19° (22)], the dinuclear complex [(trans-PtCl(NH3)2)2–H2N(CH2)6NH2]2+ [10° (30)], trinuclear...
Ru and Pt complexes (mononuclear)...ethidium (about two times) more effectively...pendant phenyl rings. Additionally, complex linking of DNA and perturbation of DNA structure by the two unwinding angle (31°/H11001 cymene)RuCl(en)3 modiﬁed...inhibition of RNA synthesis by RNAs). (polymerase, which used the bottom or upper strand of templates.

Chen et al...nated to N7 of G4 and G13 with en NH H-bonding to G O6. The pendant phenyl ring from each Bip is partially intercalated into the DNA base pairs. Modeling therefore suggests that the multifunctional interactions with DNA proposed for 3 are compatible.

In contrast to octahedral metallointercalators such as Tris-(phenanthroline)Ru(II) derivatives (20, 32), [Ru(phenanthroline)2Cl2] (33), and [Ru(bipyridine)2Cl2] (34), which are essentially inert to racemization, have rigid frameworks, and are compatible.

Further evidence for DNA cross-linking by 3 was obtained. The efficiency of interstrand cross-linking on a 213-bp NdeI/EcoRI fragment of pSP73 randomly modified by 3 (~5% frequency) was similar to that for cisplatin (Fig. 5D), and a site-specifically ruthenated 20-mer formed a 1,3-GG (5′ to 5′) interstrand cross-link (20% frequency, Fig. 19). Studies of -TGGT- and -TGTGT- 20-mer duplexes showed that complex 3 is also able to form intrastrand 1,2-GG and 1,3-GTG cross-links. A model for possible 1,3 interstrand cross-linking by 3 on B-form duplex DNA (5′-AATGTCTAA-3′/3′-TTACAGATT-5′) is shown in Fig. 21. Each Ru unit adopts the B(S4′,R5′) configuration, and the two Ru atoms are coordinated to N7 of G4 and G13 with en NH H-bonding to G O6. The pendant phenyl ring from each Bip is partially intercalated...
expand the potential of organometallic (η₆-arene)Ru(II) complexes in medicine.

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