New Antitumor-Active Azole-Bridged Dinuclear Platinum(II) Complexes: Synthesis, Characterization, Crystal Structures, and Cytotoxic Studies

Seiji Komeda,[†] Martin Lutz,[‡] Anthony L. Spek,[‡] Masahiko Chikuma,[§] and Jan Reedijk*,[†]

Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden University, PO Box 9502, 2300 RA Leiden, The Netherlands, Department of Crystal & Structural Chemistry, Bijvoet Center for Biomolecular Research, Utrecht Universuty, Padualaan 8, 3584 CH Utrecht, The Netherlands, and Osaka University of Pharamaceutical Sciences, 4-20-1 Nasahara, Takatsuki 569-1094, Japan

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Three new derivatives of the cytotoxic azole-bridged dinuclear platinum(II) complex [{*cis*-Pt(NH_3)$_2$}$_2$(μ-OH)(μ-pz)][NO_3]$_2$ (**1**) have been prepared and structurally characterized. Their formulas are [{*cis*-Pt(NH_3)$_2$}$_2$(μ-OH)(μ-1,2,3-ta)][NO_3]$_2$ (**2**) (1,2,3-ta = 1,2,3-triazolate), [{Pt(*R*,*R*-dach)}(μ-OH)(μ-pz){Pt(*S*,*S*-dach)}][NO_3]$_2$ (**3**) (dach = 1,2-diaminocyclohexane, pz = pyrazolate), and [{Pt(*R*,*R*-dach)}(μ-1,2,3-ta)$_2$Pt(*S*,*S*-dach)}][NO_3]$_2$ (**4**). The compounds were characterized by ^{1}H, ^{13}C, and ^{195}Pt NMR spectroscopy, and elemental analysis, and their crystal structures were determined. Relevant data for **2**: triclinic, space group $P\bar{1}$, a = 8.5225(15) Å, b = 9.1977(18) Å, c = 9.9771(7) Å, α = 66.988(10)°, β = 75.423(9)°, γ = 67.321(13)°, Z = 2. **3**: orthorhombic, space group $Pca2_1$, a = 17.7653(3) Å, b = 12.4076(3) Å, c = 10.7091(3) Å, Z = 4. **4**: orthorhombic, space group *Pbca*, a = 13.8944(1) Å, b = 17.8668(1) Å, c = 20.7647(2) Å, Z = 8. In the crystal structures of **2**, and **3**, the intramolecular distances between the two Pt atoms are 3.4411(6) and 3.4873(5) Å, and the dihedral angles between the platinum coordination planes are 14.1(3) and 9.3(4)°, respectively. In **2**, an intramolecular hydrogen bond is observed between N9 of the ammine ligand and the noncoordinated nitrogen atom (N3) of the triazole ring (N9···N3: 2.962(10) Å). **4** has a boat-form structure, and the two coordination planes cross at 83.64(10)°. A cytotoxicity assay of these dinuclear platinum(II) compounds on human tumor cell lines was performed. In most of the cell lines, **1** and **2** showed much higher cytotoxicity than those of cisplatin. On the other hand, **3** was found to be moderately active, and **4** was found only marginally cytotoxic. Implications of these findings are discussed in the context of a structure−activity relationship.

Introduction

Since the discovery of cisplatin,[1−3] numerous platinum complexes have been prepared in order to improve the clinical inconvenience such as nephrotoxicity[4,5] and drug resistance.[6,7] Despite large efforts, most of the cisplatin analogues show cross-resistance to cisplatin.[6] It is generally accepted that cisplatin's cytotoxic effect originates from the interaction with DNA. The major DNA adduct of cisplatin is the 1,2-intrastrand GG cross-link,[8,9] which induces distortions of the duplex with a kink of 30−35° toward the major groove.[10] The local conformational changes of the DNA coordinated by platinum complexes seem to be deeply concerned with the DNA-repair systems, as well as with the cytotoxic profiles. It has been reported[11] that the activity of the DNA repair system in the resistant cells is significantly increased to efficiently remove adducts of cisplatin. The adducts of the derivatives, which serve as essentially similar DNA-binding modes to cisplatin, appear to be easy to repair as well, resulting in the cross-resistance.

Therefore, recent studies have focused on platinum complexes with different DNA binding modes to circumvent the cross-resistance[3,10,12] In the 1980s, Kidani[12,13] introduced mononuclear platinum(II) complexes with a chiral diamine. Complexes with 1,2-diaminocyclohexane (dach) were developed, one of which is now in clinical use, to overcome cross-resistance.[14] Additionally, in the past decade, Farrell[15,16] reported successful approaches with bis- or tris(platinum) complexes containing two or three platinum−amine units linked by a variable-length

[†] Leiden University.
[‡] Utrecht University.
[§] Osaka University of Pharmaceutical Sciences.

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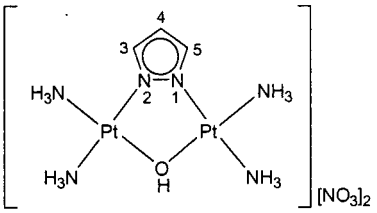
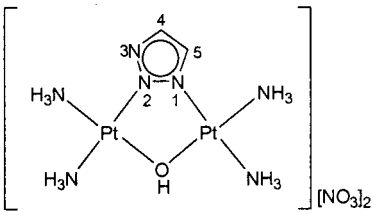
diamine chain, aiming at quite different kinds of intra- and interstrand cross-links compared to those of cisplatin. Other mononuclear and polynuclear Pt compounds have recently also been reported from our laboratory.[17]

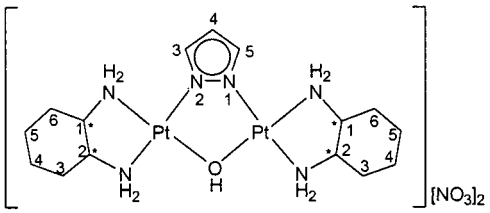
It is of great importance to understand what adducts are critical for killing cancer cells. DNA adducts of transplatin (*trans*-DDP), which cannot form 1,2-intrastrand cross-links but 1,3-intrastrand cross-link, seem to suggest that the extensive DNA distortion enhances DNA repair in cancer cells, resulting in much lower anticancer activity.[18,19] The inactive profile of many of the monofunctional platinum complexes might originate from the inability of serving as cross-link. In other words, 1,2-intrastrand cross-links appear to be necessary to exhibit antitumor activity, but no large DNA distortion is wanted. It is worthwhile designing dinuclear platinum complexes that provide 1,2-intrastrand cross-link with minimal distortion of the DNA. Therefore, some of us have suggested the dinuclear platinum(II) complex [{*cis*-Pt(NH_3)$_2$}$_2$(μ-OH)(μ-pz)][NO_3]$_2$ (**1**) (Figure 1) as a member of a new class, which has been shown to be effective in vitro on a cisplatin-resistant cell line.[20] As a main structural feature, **1** has a leaving hydroxide group, an appropriate Pt···Pt distance, and some flexibility to induce minimal distortion in 1,2-intrastrand adducts. Furthermore, a recent study has validated that **1** does provide 1,2-intrastrand GG cross-links with less local distortions upon binding DNA, compared to cisplatin and its mononuclear derivatives.[21,22] In fact, this kind of 1,2-intrastrand DNA adduct could be a trigger to induce cytotoxic effects and be favorable for escaping from the DNA recognition and repair systems in the cells. For a further study of this hypothesis, and to develop a structure−activity relationship, the group of azole-bridged dinuclear platinum(II) complexes has now been extended with *1H*-1,2,3-triazole (1,2,3-Hta) and *trans*-1,2-diaminocyclohexane (*trans*-dach), as alternative bridging agents and carrier ligands, respectively. This paper describes the synthesis, characterization, and three single-crystal X-ray structures, as well as a preliminary cytotoxic study of these compounds.

Experimental Section

Materials. K_2PtCl_4 was obtained from Johnson & Matthey (Reading, U.K.). Pyrazole (Hpz) and *1H*-1,2,3-triazole (1,2,3-Hta) were obtained from Fisher Scientific Nederland and Aldrich, respectively, and further purification was not carried out. *trans*-1,2-Diaminocyclohexane (*trans*-dach) was purchased as a racemic mixture from Aldrich and distilled under vacuum. The starting materials [*cis*-Pt(NH_3)$_2$(μ-OH)]$_2$[NO_3]$_2$, and [*cis*-{Pt(NH_3)$_2$}$_2$(μ-OH)(μ-pz)][NO_3]$_2$ (**1**) were synthesized according to the published methods.[21,23] Another starting compound, [{Pt(*R,R*-dach)}(μ-OH)$_2$Pt(*S,S*-dach)}][NO_3]$_2$, was synthesized on the basis of the literature[24] and recrystallized from water before further use.







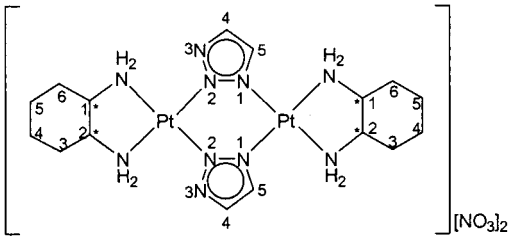


Figure 1. Schematic representation of [{*cis*-Pt(NH_3)$_2$}$_2$(μ-OH)(μ-pz)]-[NO_3]$_2$ (**1**), [{*cis*-Pt(NH_3)$_2$}$_2$(μ-OH)(μ-1,2,3-ta)][NO_3]$_2$ (**2**), [{Pt(*trans*-dach)}$_2$(μ-OH)(μ-pz)][NO_3]$_2$ (**3**), and [Pt(*trans*-dach)(μ-1,2,3-ta)]$_2$[NO_3]$_2$ (**4**).

Preparation of [{*cis*-Pt(NH_3)$_2$}$_2$(μ-OH)(μ-1,2,3-ta)][NO_3]$_2$ (2). A 0.224 g amount of 1,2,3-Hta (3.24 mmol) was added to a solution of 0.5 g of [*cis*-Pt(NH_3)$_2$(μ-OH)]$_2$[NO_3]$_2$ (0.81 mmol) in 50 mL of water. The solution was stirred and heated at 60 °C for 3 h under darkness and was then filtered and evaporated to dryness. The resulting white material was washed with 200 mL of ice-cold MeOH and diethyl ether. Recrystallization was carried out from water. Yield: 0.31 g (57%). Anal. Calcd for $C_2H_{15}N_9O_7Pt_2$: C, 3.60; H, 2.27; N, 18.89. Found: C, 3.65; H, 2.27; N, 18.77%. ^{1}H NMR (D_2O): δ (1,2,3-ta resonance) 7.67 (1H, d), 7.74 (1H, d). ^{195}Pt NMR (D_2O): δ −2101, −2147.

Preparation of [{Pt(*R,R*-dach)}(μ-OH)(μ-pz){Pt(*S,S*-dach)}]-[NO_3]$_2$ (3). A 50 mL volume of a water solution containing 0.224 g of Hpz (3.24 mmol) and 0.62 g of [*cis*-Pt(*trans*-dach)(μ-OH)]$_2$(NO_3)$_2$ (0.81 mmol) was stirred and heated at 60 °C under darkness. After 3 h, the solution was filtered and evaporated to dryness, and the resulting white material was washed with 200 mL of ice-cold EtOH and diethyl ether. A recrystallization was carried out from water. Yield: 0.37 g (54%). Anal. Calcd for $C_{15}H_{32}N_8O_7Pt_2\cdot H_2O$: C, 21.33; H, 4.06; N, 13.27. Found: C, 21.09; H, 4.06; N, 13.13. ^{1}H NMR (D_2O): δ (pz resonance) 7.39, 7.33 (2H, d), 6.30 (1H, t), (cyclohexane resonance) 2.40 (4H, b),

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Table 1. Crystal Data for [*cis*-{$Pt(NH_3)_2$}$_2$(μ-OH)(μ-1,2,3-ta)][NO_3]$_2$ (**2**), [{Pt(*R,R*-dach)}(μ-Oh)(μ-pz){Pt(*S,S*-dach)}][NO_3]$_2$·H_2O (**3a**), and [{Pt(*R,R*-dach)}(μ-1,2,3-ta)$_2$Pt(*S,S*-dach)}][NO_3]$_2$ (**4**)

	2	**3a**	**4**
chem formula	$C_2H_{15}N_9O_7Pt_2$	$C_{15}H_{34}N_8O_8Pt_2$	$C_{16}H_{32}N_{12}O_6Pt_2$
fw	667.41	844.68	878.72
cryst system	triclinic	orthorhombic	orthorhombic
space group	$P\bar{1}$ (No. 2)	$Pca2_1$	Pbca
a (Å)	8.5225(15)	17.7653(3)	13.8944(1)
b (Å)	9.1977(18)	12.4076(3)	17.8668(1)
c (Å)	9.9771(7)	10.7091(3)	20.7647(2)
α (deg)	66.988(10)	90	90
β (deg)	75.423(9)	90	90
γ (deg)	67.321(13)	90	90
V (Å^3)	659.48(18)	2360.55(1)	5154.80(7)
Z	2	4	8
ρ_{calc} (g/cm^3)	3.361	2.377	2.265
μ (mm^{-1})	21.240	11.897	10.900
abs corr	DELABS	DELABS	DELABS
transm	0.23−0.69	0.32−0.75	0.23−0.69
cryst color	colorless	colorless	colorless
cryst size (mm^3)	0.25 × 0.15 × 0.08	0.38 × 0.13 × 0.13	0.38 × 0.30 × 0.20
no. reflcns measd	6424	10 573	26 759
no. unique reflcns	3010	3974	5907
no. params	185	410	362
R1 (all reflcns)	0.0389	0.0294	0.0308
R1 (obs. reflcns)	0.0289	0.0274	0.0272
wR2 (all reflcns)	0.0663	0.0723	0.0610
wR2 (obs. reflcns)	0.0629	0.0707	0.0596

2.07 (4H, b), 1.59 (4H, b), 1.32 (4H, b), 1.17 (4H, b). ^{13}C NMR (D_2O): δ (pz resonance) 138.9, 107.0, 63.2, 63.1, 62.2, 62.1, 32.9, 32.7, 24.7. ^{195}Pt NMR (D_2O): δ −2289.

Preparation of [{Pt(*R,R*-dach)}(μ-1,2,3-ta)$_2$Pt(*S,S*-dach)}][NO_3]$_2$ (4). A 0.224 g amount of Hta (3.24 mmol) was added to a solution of 0.62 g of [*cis*-Pt(*trans*-dach)(μ-OH)]$_2$(NO_3)$_2$ (0.81 mmol) in 50 mL of water. The solution was stirred and heated at 60 °C for 20 h under darkness. The resulting suspension was evaporated to dryness, and the crude material was washed with 200 mL of ice-cold EtOH and ether. A recrystallization was carried out from water. Yield: 0.32 g (35%). Anal. Calcd for $C_{16}H_{32}N_{12}O_6Pt_2$: C, 21.87; H, 3.67; N, 19.13. Found: C, 22.07; H, 3.78; N, 19.36%. ^{1}H NMR (D_2O): δ (1,2,3-ta resonance) 7.84 (1H, d), 7,81 (1H, d), 7.79 (2H, d); δ (cyclohexane resonance) 2.73 (4H, b), 2.14 (4H, b), 1.67 (4H, b), 1.43 (4H, b), 1.32 (4H, b), 1.23 (4H, b). ^{13}C NMR (D_2O): 136.5, 135.8, 62.7, 32.9, 24.7. ^{195}Pt NMR (D_2O): δ −2685.

NMR Measurements. All 1D and 2D NMR spectra were recorded on a 300 MHz Bruker DPX300 spectrometer in D_2O at 298 K. ^{1}H, ^{13}C, and ^{195}Pt NMR chemical shifts were referenced to TSP (sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4), TMS (tetramethylsilane), and Na_2PtCl_6 ($\delta = 0$), respectively. A NOESY spectrum was acquired with 512 increments in t_1, 2048 complex data points in t_2, mixing time of 1 s, acquisition time of 2 s, and 64 scans at a sweep width of 2524 Hz.

X-ray Structural Determination. Single crystals of **2**−**4** were obtained by slow evaporation of their saturated water solutions. X-ray intensities were collected at 150(2) K on an Enraf-Nonius CAD4T diffractometer (compound **2**) and a Nonius KappaCCD diffractometer (compounds **3** and **4**), both equipped with a rotating anode and graphite monochromator (Mo Kα radiation, $\lambda = 0.710\,73$ Å). The structures were solved by automated Patterson techniques (DIRDIF 97[25]) and refined on F^2 by full-matrix least-squares techniques (SHELXL 97[26]). All structural drawings and geometrical calculations were performed with PLATON.[27]

In the crystal structure of compound **2,** the assignment of atoms N3 and C5 of the triazolate moiety was based on the hydrogen-bonding scheme. While N3 accepts an intramolecular hydrogen bond, there is no potential hydrogen bond donor for the site C5. Therefore it is concluded, that N3 is the nitrogen position and C5 the carbon position.

In the crystal structure of compound **3**, one nitrate ion and both cyclohexyl groups were refined with disorder models (see text).

In the crystal structure of compound **4**, one cyclohexyl groups was refined with a disorder model (see text). The assignment of carbons and nitrogens in the triazol moiety was based on intermolecular hydrogen bonding.

Further details of the crystal structure determinations are given in Table 1.

Cytotoxic Studies. In vitro cytotoxicity assays in human tumor cell lines were performed at the Dr. Daniel den Hoed Kliniek (Rotterdam Cancer Institute), Department of Medical Oncology (Rotterdam, The Netherlands). The seven well-characterized cell lines used were MCF7 and EVSA-T (breast cancer), WIDR (colon cancer), IGROV (ovarian cancer), M19 (melanoma), A498 (renal cancer), and H226 (nonsmall cell lung cancer). All cell lines were maintained in a continuous logarithmic culture in RPMI 1640 medium with Hepes and phenol red. The medium was supplemented with 10% FCS, 100 IU/mL penicillin, and 100 μg/mL streptomycin. Cisplatin and compounds **1**−**4** were dissolved in water (1 mg/200 mL) and finally diluted in full medium. After 48 h preincubation of the tumor cells in 96-well flatbottom microtiter plates, the solutions of the test compounds were added. The plates were incubated at 37 °C with 8.5% CO_2 for 120 h. IC_{50} values were determined using the microculture sulforhodamine-B test (SRB).[28]

Results and Discussion

Characterization by Solution NMR Study. [{*cis*-Pt(NH_3)$_2$}$_2$-(μ-OH)(μ-1,2,3-ta)][NO_3]$_2$ (2). As shown in Figure 2, in the case of nonplanarity **2** consists of two diastereoisomers which arise from the asymmetrically bridging 1,2,3-ta. In the ^{1}H NMR spectrum, two doublets assigned to H(4) and H(5) are observed at 7.67 and 7.74 ppm, respectively. As a result, two inequivalent [N_3O_1] platinum environments, with binding to N1 and N2 (Pt1 and Pt2) of the triazole ring, are expected.[29] Indeed ^{195}Pt chemical shifts of Pt1 and Pt2 appear at −2147 and −2101 ppm, respectively.

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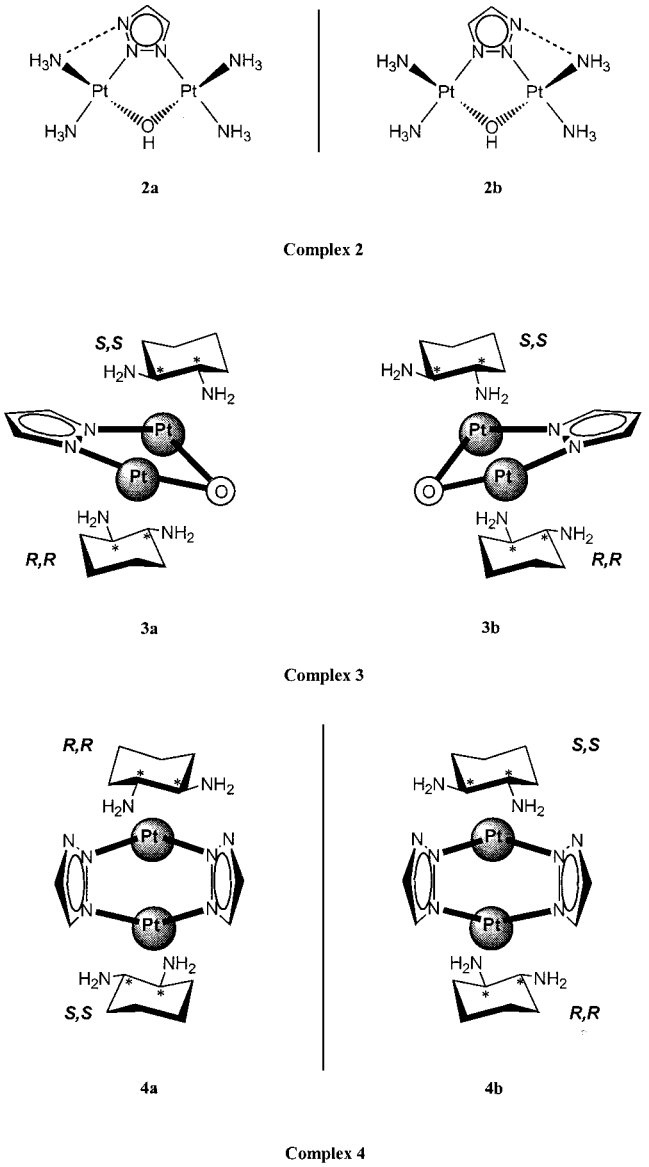

Figure 2. Potential stereoisomers of [{*cis*-Pt(NH_3)$_2$}$_2$(μ-OH)(μ-1,2,3-ta)][NO_3]$_2$ (**2**), [{Pt(*R*,*R*-dach)}(μ-OH)(μ-pz){Pt(*S*,*S*-dach)}][NO_3]$_2$ (**3**), and [{Pt(*R*,*R*-dach)}(μ-1,2,3-ta)$_2$Pt(*S*,*S*-dach)}][NO_3]$_2$ (**4**).

[{Pt(*R*,*R*-dach)}(μ-OH)(μ-pz){Pt(*S*,*S*-dach)}][NO_3]$_2$ (3). The stereoisomers (**3a**,**3b**) also schematically shown in Figure 2 have two different *trans*-dach ligands with *R*,*R* and *S*,*S* configurations in one complex. In the ^{1}H NMR spectrum, one triplet corresponding to H(4) of the pz ring is observed at 6.30 ppm, and two doublets relating to H(3) and H(5) appear at 7.39 and 7.33 ppm (ratio of the integration value is 2:3.). The 2D NOESY experiment does not show a NOE cross-peak between the two doublets but instead between H(4) and both of them (see Figure 3). Accordingly, each of the doublets corresponds to both of H(3) and H(5) in one of the isomers, i.e., **3a** or **3b**. A temperature dependence experiment was performed to clarify whether these isomers are interconvertible in water solution. With measurement of the spectra up to 353 K, the two protons due to pz(3,5) do not merge and remain in a ratio 2:3 (data not shown); accordingly, the interconversion between the isomers is not possible. The isomers must be generated in such a way that the bridging OH in the symmetric starting material, [{Pt(*R*,*R*-dach)}(μ-OH)$_2${Pt(*S*,*S*-dach)}][NO_3]$_2$, is substituted by pyrazolate from slightly different directions with different

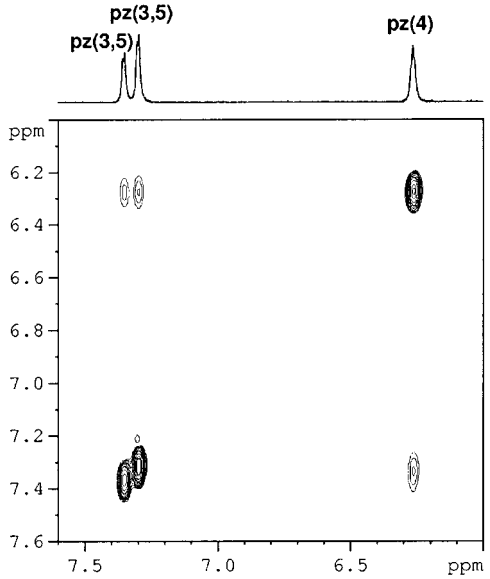

Figure 3. Section of 2D NOESY spectrum of species **3**. Atom numbering of the pyrazole ring accords to the schematic representation in Figure 1.

kinetics. The ^{13}C NMR spectrum confirms the symmetry-induced inequivalence of C(1) and C(2) and C(3) and C(6) in the dach ligands. Additionally, both peaks corresponding to C(1) and C(2) are split into two by a difference as small as 0.1 ppm, which can be interpreted as originating from **3a**,**b**. The ^{13}C chemical shifts of pz(3,5) and pz(4) appear at 138.9 and 107.0 ppm, respectively. The ^{195}Pt signal is observed at −2289 ppm, confirming that both platinum environments are [N_3O_1].[29] No difference for *S*,*S* or *R*,*R* coordination would be expected in this case.

[{Pt(*R*,*R*-dach)}(μ-1,2,3-ta)$_2${Pt(*S*,*S*-dach)}][NO_3]$_2$ (4). Also, compound **4** gives rise to two diastereoisomers (**4a**,**b**), as depicted in Figure 2. The difference between these diastereoisomers is that the corresponding dach ligands are mirrored. Two ^{1}H NMR chemical shifts assigned to H(5) of two of the bridging 1,2,3-ta rings are found at 7.84 and 7.81 ppm, and a doublet observed at 7.74 ppm can be assigned to H(4). ^{13}C NMR chemical shifts of C(5) and C(4) in the 1,2,3-ta appear at 136.5 and 135.8 ppm. At this stage, it is not clear whether the two N1 nitrogen atoms of the 1,2,3-ta rings are pointed in the same direction or not. The single ^{195}Pt NMR chemical shift at −2685 ppm would suggest the N3's are located toward different Pt atoms and characterize [N_4] platinum environments.[29] Similarly, only four peaks assigned to the atom pairs C(1)/C(2), C(3)/C(6), and C(4)/C(5) are found at 62.7, 32.9, and 24.7 ppm, respectively, in ^{13}C. As will be shown below, the solid-state X-ray structure unambiguously proves that both N1 atoms bind to the same Pt.

X-ray Crystal Structures. [{*cis*-Pt(NH_3)$_2$}$_2$(μ-OH)(μ-1,2,3-ta)][NO_3]$_2$ (2). Compound **2** crystallizes in the centrosymmetric space group $P\bar{1}$ with $Z = 4$. Consequently, both diastereomers depicted in Figure 2 are present in equal amounts. Bond length and angles are given in Table 2 and do not show unusual features. The Pt−N(triazolate) bonds are somewhat shorter than the Pt−NH_3 bonds. A PLUTON drawing of the isomer **2a** is redrawn in Figure 4. The Pt complex molecules are nearly planar with the oxygen atom only deviating 0.291(6) Å from the mean molecular plane. In the lattice the flat molecules are stacked on top of each other in the direction of the crystallographic *a*-axis with an intermolecular distance of $a/2 = 4.2613(2)$ Å. The Pt complex is involved in extensive hydrogen bonding with the

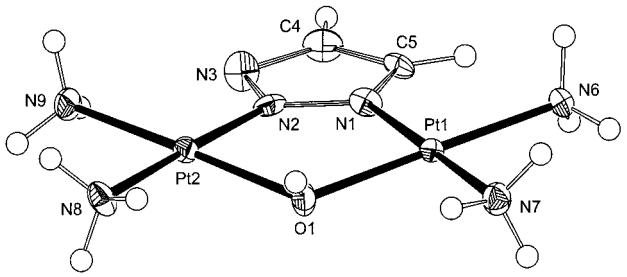

Figure 4. Displacement ellipsoid plot (50% probability) of the cation $[\{cis\text{-}Pt(NH_3)_2\}_2(\mu\text{-}OH)(\mu\text{-}1,2,3\text{-}ta)]^{2+}$ (**2a**) in the crystal structure. H atoms were introduced at calculated positions.

Table 2. Selected Bond Distances (Å) and Angles (deg) in $[\{cis\text{-}Pt(NH_3)_2\}_2(\mu\text{-}OH)\ (\mu\text{-}1,2,3\text{-}ta)][NO_3]_2$ (**2**)

Pt(1)−Pt(2)	3.4411(6)	Pt(2)−N(2)	1.981(7)
Pt(1)−N(1)	1.992(6)	Pt(2)−O(1)	2.022(5)
Pt(1)−O(1)	2.018(5)	Pt(2)−N(9)	2.029(6)
Pt(1)−N(6)	2.030(6)	Pt(2)−N(8)	2.036(6)
Pt(1)−N(7)	2.036(6)		
N(1)−Pt(1)−O(1)	88.9(2)	N(2)−Pt(2)−O(1)	88.3(2)
N(1)−Pt(1)−N(6)	91.5(2)	N(2)−Pt(2)−N(9)	90.7(2)
O(1)−Pt(1)−N(6)	179.4(2)	O(1)−Pt(2)−N(9)	177.8(2)
N(1)−Pt(1)−N(7)	178.9(2)	N(2)−Pt(2)−N(8)	178.3(3)
O(1)−Pt(1)−N(7)	90.2(2)	O(1)−Pt(2)−N(8)	90.2(2)
N(6)−Pt(1)−N(7)	89.4(2)	N(9)−Pt(2)−N(8)	90.7(3)

Table 3. Hydrogen Bonds for **2** (Å and deg)[a]

D−H···A	*d*(D−H)	*d*(H···A)	*d*(D···A)	<(DHA)
	Intermolecular			
O(1)−H(1)···O(13)	0.86	1.93	2.761(8)	160.2
N(6)−H(6A)···O(21)^{i}	0.91	2.12	2.971(9)	155.4
N(6)−H(6B)···O(23)^{ii}	0.91	2.04	2.911(8)	160.7
N(6)−H(6C)···O(11)^{iii}	0.91	2.38	3.156(8)	142.8
N(7)−H(7A)···O(13)	0.91	2.26	3.016(9)	140.4
N(7)−H(7C)···O(12)^{iv}	0.91	2.07	2.941(9)	159.8
N(8)−H(8A)···O(11)	0.91	2.06	2.957(8)	169.5
N(8)−H(8B)···O(21)^{v}	0.91	2.29	3.024(8)	137.7
N(8)−H(8C)···O(22)^{v}	0.91	2.36	2.976(9)	125.2
N(8)−H(8C)···O(11)^{vi}	0.91	2.21	3.007(9)	145.8
N(9)−H(9A)···O(23)^{vii}	0.91	2.49	3.058(9)	120.9
N(9)−H(9B)···O(21)^{v}	0.91	2.39	3.119(9)	137.2
N(9)−H(9B)···O(22)^{vii}	0.91	2.60	3.159(9)	120.7
	Intramolecular			
N(9)−H(9A)···N(3)	0.91	2.43	2.962(10)	117.3

[a] Hydrogens H8C, H9A, and H9B are involved in bifurcated hydrogen bonds. Symmetry transformations: i, $-x+1, -y, -z+1$; ii, $x+1, y, z+1$; iii, $x+1, y-1, z$; iv, $-x+1, -y+1, -z+2$; v, $x, y+1, z$; vi, $-x+1, -y+2, -z+1$; vii, $-x+1, -y+1, -z$.

hydroxo and ammine groups as hydrogen bond donors and the oxygen atoms of the nitrates as acceptors. Additionally a weak intramolecular hydrogen bond is observed with N9 of an ammine ligand as donor and the noncoordinated N3 of the triazolate as acceptor (see Table 3).

The coordination of the Pt atoms is square planar (angle sums 360.0 and 359.9°) with an interplanar angle of 14.1(3)° between the coordination planes resulting in a Pt1···Pt2 distance of 3.4411(6) Å. The Pt−N distances are in the expected range, with the Pt−N(ammine) distances slightly longer (2.029(6)−2.036(6) Å) than the Pt−N(triazolate) distances (1.981(7)−1.992(6) Å), probably a consequence of the negative charge on the triazolate. Similar distances were found in the crystal structure of **1**.[21]

[{Pt(*R,R*-dach)}(μ-OH)(μ-pz){Pt(*S,S*-dach)}][NO$_3$]$_2$·H$_2$O (3). Compound **3** crystallizes in the achiral space group $Pca2_1$ with $Z = 4$. The glide (mirror) planes of this space group necessarily lead to a racemic crystal. More difficult to answer

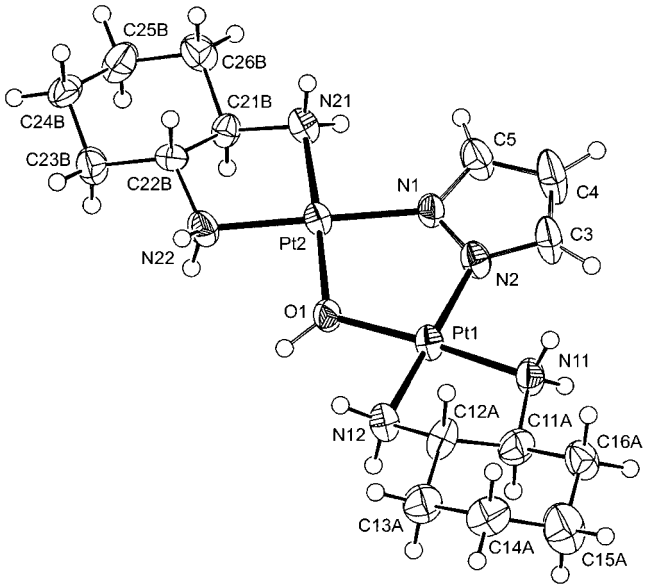

Figure 5. Molecular structure of the $[\{Pt(R,R\text{-dach})\}(\mu\text{-OH})(\mu\text{-pz})\{Pt(S,S\text{-dach})\}]^{2+}$ cation (**3a**). The cyclohexyl rings were refined with a disorder model consisting of interpenetrating rings with *R,R* and S,S configuration, respectively. The *R,R*/*S,S* ratio is 0.34(2):0.66(2) for ring C11−C16 and 0.54(2):0.46(2) for C21−C26. Shown in the displacement ellipsoid plot (50% probability) are the major disorder components.

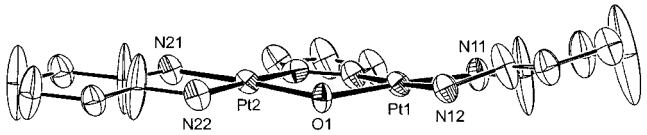

Figure 6. Average structure of compound **3a**. The elongated displacement ellipsoids suggest severe disorder of the cyclohexyl rings.

Table 4. Selected Bond Distances (Å) and Angles (deg) in $[\{Pt(R,R\text{-dach})\}(\mu\text{-Oh})(\mu\text{-pz})\{Pt(S,S\text{-dach})\}][NO_3]_2$ (**3a**)

Pt(1)−Pt(2)	3.4873(5)	Pt(2)−N(1)	2.001(6)
Pt(1)−N(2)	2.004(7)	Pt(2)−N(21)	2.023(7)
Pt(1)−N(12)	2.027(7)	Pt(2)−O(1)	2.025(6)
Pt(1)−O(1)	2.028(6)	Pt(2)−N(22)	2.043(6)
Pt(1)−N(11)	2.029(7)		
N(2)−Pt(1)−N(12)	176.4(3)	N(1)−Pt(2)−N(21)	95.1(3)
N(2)−Pt(1)−O(1)	88.3(3)	N(1)−Pt(2)−O(1)	87.6(3)
N(12)−Pt(1)−O(1)	93.2(3)	N(21)−Pt(2)−O(1)	176.3(3)
N(2)−Pt(1)−N(11)	94.4(3)	N(1)−Pt(2)−N(22)	177.5(3)
N(12)−Pt(1)−N(11)	84.3(3)	N(21)−Pt(2)−N(22)	83.9(3)
O(1)−Pt(1)−N(11)	176.4(3)	O(1)−Pt(2)−N(22)	93.3(3)

is the question whether one single molecule contains two *R,R*-dach ligands or one *R,R* and one *S,S* ligand. Bond length and angles are given in Table 4 and show no unusual features. A projection of isomer **3a** is redrawn in Figure 5.

The preliminary structure solution of **3** resulted in two quite planar cyclohexyl rings with huge displacement ellipsoids perpendicular to these planes and artificially shortened C−C distances (see Figure 6). This situation had been resolved by the refinement of disorder models for the cyclohexyl rings. Each planar ring is split up into two interpenetrating cyclohexyl rings in chair conformation, one of them *R,R* and the other *S,S*. Refinement of the occupancies results in a *R,R*/*S,S* ratio of 0.34(2):0.66(2) for the ring C11−C16 and 0.54(2):0.46(2) for C21−C26. These unequal occupancies suggest that disorder is really observed and are not the consequence of a wrong space group choice.

Table 5. Selected Bond Distances (Å) and Angles (deg) [{Pt(*R*,*R*-dach)}(μ-1,2,3-ta)$_2${Pt(*S*,*S*-dach)}][NO_3]$_2$ (**4**)

Pt(1)−Pt(2)	3.3343(2)	Pt(2)−N(2)	2.000(4)
Pt(1)−N(1)	2.015(4)	Pt(2)−N(12)	2.008(4)
Pt(1)−N(11)	2.019(4)	Pt(2)−N(31)	2.028(4)
Pt(1)−N(22)	2.031(4)	Pt(2)−N(32)	2.042(4)
Pt(1)−N(21)	2.037(4)		
N(1)−Pt(1)−N(11)	89.76(16)	N(2)−Pt(2)−N(12)	87.73(16)
N(1)−Pt(1)−N(22)	176.88(17)	N(2)−Pt(2)−N(31)	93.10(16)
N(11)−Pt(1)−N(22)	93.36(17)	N(12)−Pt(2)−N(31)	174.42(16)
N(1)−Pt(1)−N(21)	92.86(17)	N(2)−Pt(2)−N(32)	174.73(16)
N(11)−Pt(1)−N(21)	176.12(16)	N(12)−Pt(2)−N(32)	95.01(16)
N(22)−Pt(1)−N(21)	84.03(18)	N(31)−Pt(2)−N(32)	83.74(16)

Although the disorder model indicates the presence of at least some amount of two *R*,*R* ligands (or two *S*,*S* ligands, respectively) in the same molecule, the reliability of the model is not high enough to prove/disprove the results from the 2D NOESY NMR study in solution, in which we could show that each molecule contains one *R*,*R* and one *S*,*S* dach ligand.

As already seen with compound **2**, compound **3** also expresses an extended hydrogen-bonding motif, involving the Pt complex, the nitrates, and the noncoordinated hydrate water. Because the NH_2 groups of the dach ligands are disordered just like the nitrate anions, the description of the hydrogen bonding is not straightforward and shall not be given here.

The Pt centers are square planar coordinated. The N−Pt−N angles of the five-membered chelate rings[30] are smaller, and the other N−Pt−N angles somewhat larger than 90°. The interplanar angle of the coordination planes is 9.3(4)°. The distance Pt1···Pt2 is 3.4873(5) Å and thus slightly longer than in **2** as a consequence of the even higher planarity of the central part of **3**.

[{Pt(*R*,*R*-dach)}(μ-1,2,3-ta)$_2${Pt(*S*,*S*-dach)}][NO_3]$_2$ (4). Compound **4** crystallizes as a racemate in the centrosymmetric space group *Pbca* with $Z = 8$. Both of the bridging 1,2,3-ta rings are arranged in the same direction. The whole figure of the cation has a boat form, and two intramolecular platinum coordination planes appear to cross at almost a right angle (interplanar angle 83.64(10)°). The interplanar angle of the two triazolates is 72.9(3)°. Relevant distances and angles are given in Table 5; a projection of the molecule structure is depicted in Figure 7.

By the bending of the molecule in **4**, the Pt centers come significantly closer to each other than in the nearly planar structures of **2** and **3**. Here, the Pt1···Pt2 distance is 3.3343(2) Å.

Again both cyclohexyl groups are disordered with interpenetrating *R*,*R* and *S*,*S* molecules in chair conformation. In the structure refinement only the disorder of the ring C31−C36 could be resolved. The refinement of the occupancies resulted in a ratio *R*,*R*/*S*,*S* of 0.596(14):0.404(14).

The square planar geometries at the Pt centers are clearly shown by the angle sums of 360.0 and 359.6°, in which the N−Pt−N angles of the five-membered chelate ring are smaller and the others larger than 90°.

Cytotoxicity Studies. The cytotoxicity of complexes **1**−**4** and cisplatin for comparison on several human tumor cell lines, MCF7 and EVSA-T (breast cancer), WIDR (colon cancer), IGROV (ovarian cancer), M19 (melanoma), A498 (renal cancer), and H226 (nonsmall cell lung cancer), is summarized in Table 6. Interestingly, **1** and **2** show much higher cytotoxicity than cisplatin on most of the cell lines. For instance, **1** was found to be as 39 times as effective as cisplatin in MCF7 and 37 times more cytotoxic than cisplatin in M19. **2** has exhibited 26 times more effective cytotoxicity than that of cisplatin in MCF7. With comparison of **1** and **2**, **1** appears to be slightly more cytotoxic in all of the cell lines except for IGROV (ovarian cancer). **3** was found to be fairly active. Complexes **1**−**3** show great efficacy especially in MCF7 and M19, and their whole cytotoxicity spectra against these tumor cell lines are comparable. In contrast, cisplatin demonstrates the highest activity in IGROV, and the cytotoxicity spectrum is slightly different from the others. Compound **4** was found only marginally cytotoxic and showed no drug response at drug concentrations >68.3 μM, in agreement with expectation for all-N donor ligands.[30]

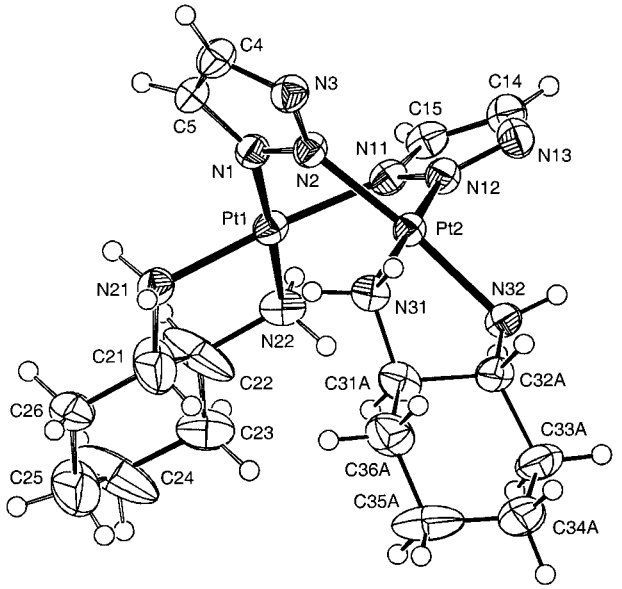

Figure 7. Molecular structure of the [{Pt(*R*,*R*-dach)}(μ-1,2,3-ta)$_2$-{Pt(*S*,*S*-dach)}]$^{2+}$ cation (**4a**). Both cyclohexyl rings were disordered, but the disorder was only resolved for ring C31−C36. Shown in the displacement ellipsoid plot (50% probability) is the major disorder component. The ratio *R*,*R*/*S*,*S* ratio is 0.596(14):0.404(14) for C31−C36.

Table 6. In Vitro Cytotoxicity Assay of the Dinuclear Platinum(II) Complexes and Cisplatin on Human Tumor Cell Lines[a]

test compd	IC_{50} (μM)						
	MCF7	EVSA-T	WIDR	IGROV	M19	A498	H226
1	0.06	0.15	0.12	0.59	0.05	0.53	0.68
2	0.09	0.32	0.40	0.13	0.19	1.24	2.72
3	12.8	20.9	>72.6	25.6	13.2	59.1	>72.6
4	>68.3	>68.3	>68.3	>68.3	>68.3	>68.3	>68.3
cisplatin	2.33	1.41	3.22	0.56	1.86	7.51	10.9

[a] Cell lines: MCF7, breast cancer; EVSA-T, breast cancer; WIDR, colon cancer; IGROV, ovarian cancer; M19, melanoma A498, renal cancer; H226, nonsmall cell lung cancer.

Structure−Activity Relationships. As a result of this study, some extent of structure−activity relationship of the azole-bridged dinuclear platinum(II) complexes has been revealed. First of all it has become clear that 1,2,3-triazole is equally suitable as pyrazole as a bridging agent. Although mononuclear platinum(II) complexes which have dach as a carrier ligand show high anticancer activity,[13] **3** was found to be only moderately cytotoxic. In the crystal structure of the [{*cis*-Pt(NH_3)$_2$(9EtG-κN^7)}$_2$(μ-pz)]$^{3+}$ (9EtG = 9-ethylguanine) cation, a model compound of **1** for a 1,2-intrastrand GG cross-link, two intramolecular hydrogen bonds are observed between ammine ligands and O6 oxygen atoms of the 9EtG.[20] Therefore, tightly formed hydrogen bonds with O6 or with a phosphate group might be required to stabilize DNA adducts. Otherwise, bulky

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cyclohexane rings might have an effect on the kinetics and adduct conformation of **3**.

So far, ammonia appears to be the most appropriate ligand for highly cytotoxic azole-bridged complexes. In addition, some derivatives of **1** with primary and secondary amine ligands such as ethylenediamine and isopropylamine exhibit less cytotoxicity (unpublished data). This tendency agrees with the general structure−activity relationship in mononuclear cisplatin derivatives.[31] As can be seen from the structure of **4**, both Pt coordination spheres are occupied with four nitrogen atoms; in other words, **4** essentially possess no proper leaving group. Consequently, the only marginal cytotoxicity of **4** has confirmed the importance of an appropriate leaving group in a molecule, suggesting that 1,2-intrastrand cross-links or other sort of DNA adducts made by azole-bridged complexes are necessary to inhibit the tumor cell growth. From the different cytotoxic trends for cisplatin and the dinuclear platinum(II) complexes (**1**−**3**), it can be supposed that their binding properties to DNA will differ.

Concluding Remarks

In this paper, the crystal structures and cytotoxicity of three new azole-bridged dinuclear platinum(II) complexes have been reported. Like the structure of **1**,[19] the platinum(II) coordination planes and the azole rings of **2** and **3** are arranged in a nearly planar way. The different diastereomers will form sterically distinct DNA adducts. Compound **4** has a boat form and will therefore not form covalent DNA interactions. The cytotoxicity against cisplatin-resistant cell lines has not been determined. However, the remarkable cytotoxicity of **1** and **2** has opened the field for new platinum anticancer agents. Recent studies have focused on a 1,2-intrastrand cross-link with **1**;[21] nevertheless, other probable binding modes to DNA can be also anticipated and will attract great interest. Ongoing studies deal with the reactions and kinetic profiles of these and other azole-bridged dinuclear platinum(II) complexes with nucleic acids and small DNA fragments.

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Supporting Information Available: Complete listings of crystallographic data (coordinates, displacement parameters, bond lengths, bond angles, and torsion angles) for the structures of **2**, **3a**, and **4** in tabular and CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

IC000273V

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