Analysis of Two Cycloplatinated Compounds Derived from N-(4-Methoxyphenyl)-α-benzoylbenzylidenamine. Comparison of the Activity of These Compounds with Other Isostructural Cyclopalladated Compounds

Carmen Navarro-Ranninger,[*,†] Isabel López-Solera,[†] José M. Pérez,[‡] Jesús Rodríguez,[†] José L. García-Ruano,[†] Paul R. Raithby,[§] José R. Masaguer,[†] and Carlos Alonso[*,‡]

Departamento de Química and Centro de Biología Molecular Severo Ochoa, Facultad de Ciencias, Universidad Autónoma de Madrid, 28049 Madrid, España, and Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, U.K.

In the present paper we report the synthesis, structural characterization, biochemical properties, and antiproliferative activity of two organo-*cis*-platinum cyclometalated compounds of formula $[M(4\text{-}OMeC_6H_4N=C(COC_6H_5)C_6H_4)X]_2$, where M = Pt and X = Cl (4) or OAc (5). The IR and ^1H and ^{13}C NMR data of the chloro-bridged compound 4 showed that it has a planar structure. As indicated by IR and ^1H and ^{13}C NMR, the acetate-bridged compound 5 has an open-book shape structure. This structure was further confirmed by X-ray diffraction. The comparison of the biochemical properties and antiproliferative activity of these compounds relative to the isostructural palladium compounds $[Pd(4\text{-}OMeC_6H_4N=C(COC_6H_5)C_6H_4)X]_2$ [X = AcO (1) and (2) or Cl (3)] indicated that the activity of compounds 4 and 5 is higher than that of the corresponding isostructural compounds 3 and 1-2, respectively, since their ID_{50} are 2-9-fold lower. It seems that there are not differences in the antiproliferative activity of all of these compounds against leukemic HL-60 cells or mammary cancer MDA-MB 468 cells. Compounds 4 and 5 modify also the DNA structure of the oc and ccc forms of plasmid DNA. The acetate-bridged compound 5 showed the highest antiproliferative activity which is even higher than that of *cis*-DPP. Our data indicate that the Pt(II) compounds are more active than those having Pd(II) as the metal center.

Introduction

The study of *cis*-DDP analogues of general formula ML_2X_2 has revealed that several factors influence their antitumour activity: (a) the electrophilic character of the metal (M),[1,2] (b) the nature of the ligand (L) which may modify the electrophilic properties of the metal and the stereochemistry of the complex,[3] and (c) the nature of the salient group (X).[4] These studies have shown, moreover, that among all of the compounds analyzed so far having either Pt, Pd, or Ru as the metal center, the Pt compounds show the highest antitumour activity and that carboxylates and/or diaminocyclohexanes increase the activity of these compounds. Recently,[5] we have studied the biochemical and antitumour properties of acetate-bridged cyclopalladated compounds derived from the N-(4-methoxyphenyl)-α-benzoylbenzylidenamine ligand showing that they destabilize the DNA and that they may have potential antitumour value.

In order to perform a detailed study of the influence of all the factors indicated above on the structure and biochemical properties of isostructural Pt and Pd compounds, we have synthesized a new family of compounds which have an identical ligand with variable metal (Pt or Pd) centers and salient groups (chloro or acetate). In the present paper we report the synthesis, structural characterization, biochemical properties, and antiproliferative activity of such isostructural cyclometalated compounds of formula $[M(4\text{-}OMeC_6H_4N=C(COC_6H_5)C_6H_4)X]_2$, where M = Pt and X = Cl or AcO.

Results and Discussion

Synthesis and Characterization. It has been shown that the reaction of $Pd(OAc)_2$ with the imine 4-OMe-

C₆H₄N=C(COC₆H₅)C₆H₅ in glacial AcOH, under N₂ at 50 °C, leads to the formation of two atropisomers (1 and 2) due to the hindered rotation around the COCN bond which becomes a chiral axis.[6] The atropisomers of formula $[Pd(4\text{-}OMeC_6H_4N=C(COC_6H_5)C_6H_4)OAc]_2$ have an open-book shape. It was also observed that the metathetical reaction of these complexes with NaCl leads to the formation of a single chloro-bridged cyclometalated compound 3.[7] In order to carry out an analysis of the structural characteristics and biochemical activity of the Pd atropisomers 1 and 2 and of the chloro-bridged cyclometalated Pd compound 3 with isostructural cycloplatinated compounds, complexes 4 and 5 were prepared. It was observed that these compounds could not be synthesized using standard methods: K_2PtCl_4 in MeOH or $EtOH/H_2O$[8] or in dioxane/H₂O.[9] If, in these conditions, we attempted to orthoplatinate the ligand, only the coordination compound of 4-methoxyaniline was formed as a result of its hydrolysis in aqueous medium. Presumably the hydrolysis occurs because the benzylidenamines derived from benzyl are readily hydrolyzable ligands as a consequence of the withdrawing effect of the COC_6H_5 group. The orthoplatinated compounds could only be obtained when the starting material was $[Pt(\mu\text{-}Cl)(\eta^3\text{-}C_4H_7)]_2$. This complex can increase its coordinative unsaturation level, and it has an electrophilic character. As has been also previously indicated,[10] this complex may lead to the formation of chloroplatinated compounds due to its η^3-η^1 isomerization or because the η^3-allyl is a π-acceptor. In our experiments, however, cycloplatination of the $4\text{-}OMeC_6H_4N=C(COC_6H_5)C_6H_5$ ligand was not possible when acetone was used as the solvent.[10] Thus, the orthoplatinated compound was only obtained when chloroform was used. The chloro-bridged compound was converted into the acetate-bridged analogue (compound 5) by treatment of compound 4 with silver acetate in
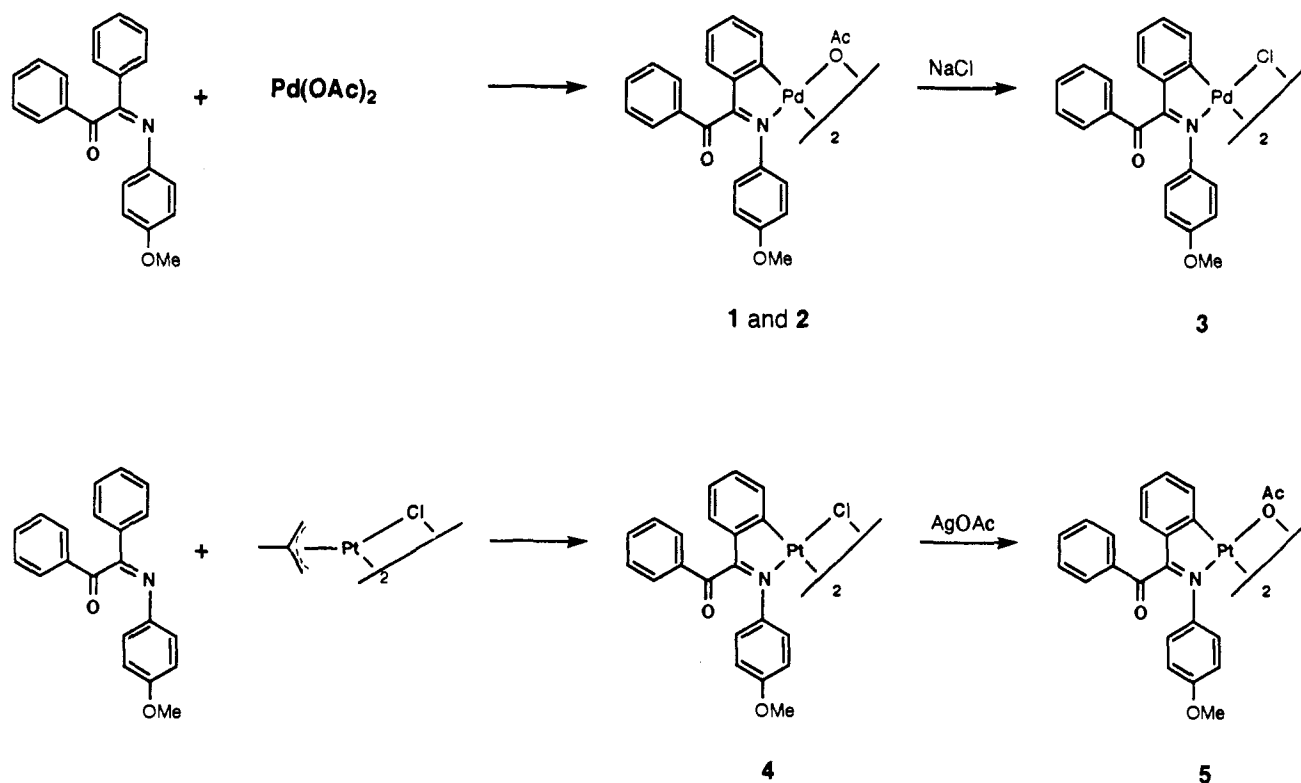
† Departamento de Química.
‡ Centro de Biología Molecular Severo Ochoa.
§ University of Cambridge.

Scheme I

1 and 2

3

4

5

chloroform. All of these complexes are air-stable solids (Scheme I).

The microanalytical data of complexes **4** and **5** are consistent with the empirical formula [Pt(4-OMeC$_6$H$_4$N=C(COC$_6$H$_5$)C$_6$H$_4$)X] where X = Cl in **4** and AcO in **5**. The IR spectrum of compound **4** exhibits two asymmetric stretching absorptions ν(Pt-Cl) at 322 and 307 cm^{-1} which are absent in the acetate-bridged species (compound **5**). However the IR spectrum of compound **5** exhibits the asymmetric and symmetric stretching modes of the acetate groups with strong absorptions at 1587 and 1421 cm^{-1}. The separation between these two absorption bands is consistent with acetate bridging.[11] The shift of the ν(C=O) vibration toward higher wavenumbers and of the ν(C=N) vibration toward lower frequency indicates that the platinum atom is bonded to the nitrogen atom of the C=N imine group.[12]

The ^{1}H NMR spectra were assigned on the basis of the chemical shift and the spin–spin coupling information and were unambiguously confirmed by selective proton decoupling. The spectra clearly demonstrate the formation of a Pt–C bond as shown by the deshielding of all protons resulting from the metalation of the ligand. The deshielding is very drastic in the H11 which appear as a doublet at 8.30 ppm (J(^{1}H–^{1}H) = 7.1 Hz; J(^{195}Pt–^{1}H) = 40.9 Hz). The deshielding is higher than that detected in the palladium complexes occurring at 7.87 ppm,[6] d (J(^{1}H–^{1}H) = 8.1 Hz). We think that it could be due to the proximity of the metal.

The ^{1}H NMR signals of complex **5** are broadened in the 7.54–6.40 ppm region, even at 80 °C in DMSO-d_6. There is only one signal for the acetate methyl group at 1.88 ppm (which suggests a *trans* disposition of ligands[13]). This chemical shift can be considered to have a normal value for an acetate-bridged compound and it represents an

Table I. ^{13}C RMN Parameters (δ, ppm) of the Complexes **4** and **5**

| | | X |
carbon	compd **4**^{a}	compd **5**^{b}
C1	185.9 (93.4)	182.2 (36.3)
C2	191.9 (52.8)	192.4
C3	133.0	132.4
C4,4'	129.2	129.0
C5,5'	129.4	129.0
C6	135.6	134.6
C7	144.5 (56.1)	145.1 (92.1)
C8	129.8 (37.4)	128.5
C9	124.6	126.0
C10	133.8	131.7 (40.4)
C11	133.7	134.6 (48.3)
C12	147.0 (968.2)	140.3 (1168.0)
C13	137.6 (22.2)	138.1 (20.4)
C14,14'	126.0	122.9
C15,15'	112.6	112.8
C16	157.9	158.6
C17	55.0	55.3
C18		n.o.
C19		23.4

^{a} DMSO-d_6. ^{b} CDCl$_3$. The numbers in parentheses corresponding to J(^{195}Pt–^{13}C) in hertz. n.o. = not observed.

upfield shift relative to that of [Pd(OAc)$_2$]$_3$ (δ = 2.05 ppm) and with respect to analogous palladium complexes **1** and **2**.

The ^{13}C NMR parameters of the ligand and of the compounds **4** and **5** are indicated in Table I. The assignments shown were confirmed by heteronuclear correlation two-dimensional NMR spectroscopy[14] and

Table II. Selected Bond Distances and Angles for Compound 5

Distances (Å)			
Pt1–N101	2.02(2)	Pt2–N201	2.01(2)
Pt1–C108	1.96(3)	Pt2–C208	2.00(2)
Pt1–O109	2.03(2)	Pt2–O209	2.03(2)
Pt1–O211	2.12(2)	Pt2–O111	2.14(2)
N101–C102	1.28(3)	N201–C202	1.26(3)
C102–C103	1.47(5)	C202–C203	1.47(5)
C102–C121	1.50(4)	C202–C221	1.53(3)
C103–C108	1.40(4)	C203–C208	1.42(3)
O109–C110	1.33(3)	O209–C210	1.24(2)
C110–O111	1.21(4)	C210–O211	1.29(4)
C110–C112	1.48(4)	C210–C212	1.51(4)
C121–O122	1.19(2)	C221–O222	1.23(3)
C121–C123	1.52(4)	C221–C223	1.51(5)
Pt1–Pt2	3.062(2)		

Angles (deg)			
O109–Pt1–O211	87.4(7)	O209–Pt2–O111	89.7(8)
C108–Pt1–O109	94(1)	C208–Pt2–O209	92.5(9)
N101–Pt1–O211	97.1(8)	N201–Pt2–O111	95.1(8)
N101–Pt1–C108	81(1)	N201–Pt2–C208	82(1)
Pt1–N101–C102	116(2)	Pt2–N201–C202	116(2)
N101–C102–C103	116(3)	N201–C202–C203	117(2)
C102–C103–C108	113(2)	C202–C203–C208	114(2)
Pt1–C108–C103	115(2)	Pt2–C208–C203	111(2)

Figure 1. Molecular structure of **5** showing the atom numbering scheme. H atoms have been omitted for clarity.

quaternary carbon atoms by heteronuclear NOE effect.[15] The chemical shifts are similar to the palladium cyclometalated compounds, indicating that there is a high structural analogy between the palladium and the platinum compounds. The most important difference between both compounds is the level of the C12 displacement which is linked directly to the platinum atom. The lower C12 displacement of the platinum compound relative to that of the palladium compounds is probably due to anisotropic local terms derived from the different nature of the metal.

To determine the Pt–C and Pt–N bond distances and chelate bond angles and other significant structural features of the cyclometalated palladium and platinum compounds, the single-crystal X-ray structure of complex 5 was determined. It seems that complex 5 is a diacetate-bridged dimer without any element of symmetry. Each platinum atom is bonded to four atoms: (a) the nitrogen, (b) the *ortho* carbon of phenyl ring supporting the iminic carbon, and (c) the oxygen atoms from each of the two bridging acetates. The geometry of platinum in complex 5 is approximately square-planar distorted toward a tetrahedral geometry. The chelation angles in the Pt environment are lower than those expected (90°) for a square planar arrangement (N101–Pt1–C108 = 81° and N201–Pt2–C208 = 82°). These values are similar to those observed in the Pd(II) analogues[6] (range 84.9–81.0°). The observed 3.062(2)-Å Pt–Pt distance is similar to that of the Pd analogues (2.927 Å in 1 and 3.046 Å in 2)[6] but larger than those detected in compounds containing a Pt–Pt bond (2.495–2.557 Å).[16,17] In the cyclometalated platinum compound 5 the Pt–Pt distance is slightly shorter (0.05 Å) than the Pt–Pt distance of the cyclometalated β-diesters (3.1179 Å)[18] and of the dimers of bis((phenylazo)acetaldoximato)platinum(II) (3.15 Å).[19] The most significant bond distances and angles of compound 5 are given in Table II. An ORTEP drawing is shown in Figure 1.

The Pt–N bond distances (Pt1–N101 = 2.02(2), Pt2–N201 = 2.01(2) Å) are also similar to those of the Pd(II) analogues[6] (1.96 and 2.02 Å in 1 and 2.030 and 2.027 Å in 2) and of the same order as the Pt–N bonds (2.02 Å) in the Pt(bpy)$_2^{2+}$ and Pt(phen)$_2^{2+}$ compounds.[20,21] However, the Pt–C bond distances of the platinum complexes are slightly longer (1.96(3) and 2.00(2) Å) than the Pd–C
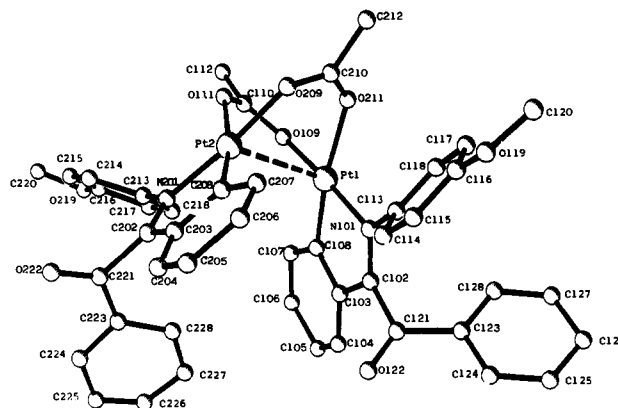
distances of the palladium analogues (1.94 Å in 1 and 1.958 and 1.944 Å in 2).[6] These distances are short when compared with other Pt(II)–carbon compounds which tend to have Pt–C bond distances in the order of 2.05–2.18 Å.[22] The shortening in Pt–C bond distances have been also observed in a complex with platinum–chlorine bond *trans* to the ligand (1.99 Å)[23] and in Pt–homoleptic compounds (1.99 Å).[24] The existence of C103–C108 and C203–C208 ligand bond distances close to the idealized value for an aromatic bond (1.39 Å) indicates that there is a significant amount of conjugation between the two fused rings (the aromatic and the cyclometalated). The N101–C102–C121–O122 and N201–C202–C221–O222 torsion angles with values of 115.0 and 114.0°, respectively, are similar to those of the atropisomer 1 and different from those of the atropisomer 2.[6] The dihedral angle between planes PdOONC has a value of 43.7°, similar to that of compound 1. This similarity is to be expected from the similar M–M distance in both complexes and because the interligand repulsions should also be similar. It should be noted that this is the first time in which an X-ray diffraction analysis of a cyclometalated compound derived from a benzylidenamine, in which the synthesis was controlled, has been determined.[18]

Antiproliferative Studies. The ID$_{50}$ values (μM) obtained against MDA-MB 468 (breast carcinoma) and HL-60 (leukemia) human cancer cell lines after incubation with the Pt(II)–*N*-(4-methoxyphenyl)-α-benzoylbenzylidenamine cyclometalated compounds and the isoestructural palladium complexes are shown in Figure 2. No differences were detected between the antiproliferative activity of these compounds against the HL-60 cells and the one observed against MDA-MB 468 cells. The data suggest that the chirality and the nature of the metal (Pt or Pd) and of the salient group may play some role in the activity of these compounds since the atropisomers 1 and 2, identical in all structural parameters with the exception of the specific orientation of the benzoyl group with respect to the chiral CO–CN bond, differ in their antiproliferative activity. It seems, also, that the platinum cyclometalated complexes are more active than the analogous palladium compounds. Differences in the antiproliferative activity of these compounds against other types of cancer cells have been also reported.[5] It seems that compounds 1, 4, and 5 may be regarded as having potential antitumor properties since they have ID$_{50}$ values of a similar order of magnitude as that of *cis*-DDP.[25] Among those, compound 5, however, seems to be the most promising one seems it has an ID$_{50}$ value of 2.0 μM against MDA-MB 468
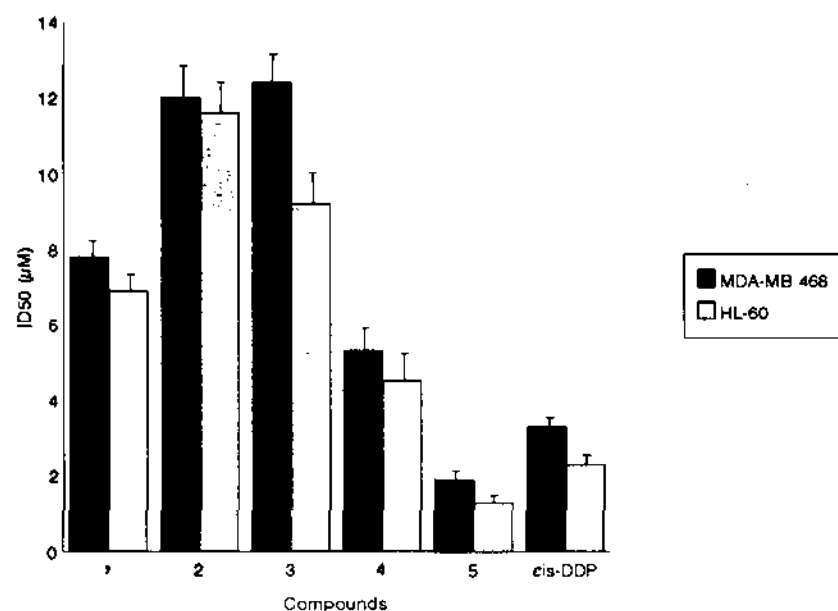
Figure 2. ID_{50} (μM) values obtained for Pd(II)- and Pt(II)-cyclometalated complexes against the tumor cell lines MDA-MB 468 and HL-60 *in vitro*, respectively.
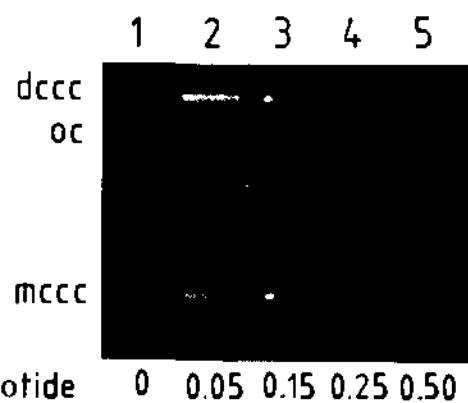

Figure 3. Changes in the electrophoretic mobility of the dccc (dimeric covalently closed circular), oc (open circular), and mccc (monomeric covalently closed circular) forms of pUC8 plasmid DNA after incubation with compound **3**. Control (lane **1**), compound **3** (lanes **2-5**).

cells and 1.3 μM against HL-60 cells which is even lower than that of *cis*-DDP (3.3 and 2.3 μM, respectively).

Electrophoretic Data of Drug–DNA Complexes. In an attempt to analyze the effect of the interaction of the Pd and Pt isostructural compounds with the DNA, we have determined the alteration in mobility on agarose gels of the ccc (covalently closed circular) and oc (open circular) forms of the pUC8 plasmid DNA upon binding of these compounds. Figures 3 and 4 show that while complex **3** does not alter the mobility of the ccc and oc forms of the DNA, binding of complexes **4** and **5** to plasmid DNA leads to significative changes in the structure of the DNA since the mobility of the ccc and oc forms was drastically altered particularly at high Pt/nucleotide ratios. The alteration in mobility of the ccc forms induced by compound **5** at r_i = 0.5 is similar to that of the Pd[5] analogous but smaller than the changes induced by *cis*-DDP. In agreement with the data reported for the interaction of Pt compounds with plasmid DNA[26,27] which increase the mobility of the oc forms of the DNA, binding of compounds **4** and **5** seems to affect also the mobility of the oc forms particularly at high r_i. The low mobility of the ccc forms of the DNA incubated with compounds **4** and **5** relative to control DNA may be due to the uncoiling of the helix since, as indicated by several authors, the Pt compounds induce the formation of DNA microloops.[26,28] It was also observed that compound **5** alters the mobility of the ccc and oc forms of the DNA more than compound **4**. Interestingly, as indicated above, the ID_{50} of compound **5** against the tumor cell lines tested is higher than that of compound **4**. Whether the higher antiproliferative activity of compound **5** relative to
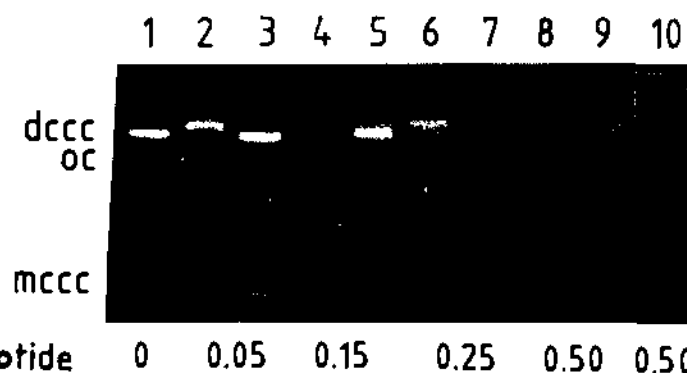

Figure 4. Changes in the electrophoretic mobility of the dccc (dimeric covalently closed circular), oc (open circular), and mccc (monomeric covalently closed circular) forms of pUC8 plasmid DNA after incubation with compounds **4** and **5**. Control (lane **1**); compounds: **4** (lanes **2, 4, 7** and **8**), **5** (lanes **3, 5, 6** and **9**), and *cis*-DDP (lane **10**).

Table III. T_m (°C) of DNA Incubated with the Complexes[a]

compd	15 min	1 h	5 h	16 h	24 h
1	53.5	55.5	55.5	57.5	57.5
2	55.5	56.5	57.0	57.5	57.5
3	67.0	67.0	65.0	62.0	62.0
4	61.0	61.0	63.0	63.0	63.0
5	61.0	61.0	63.0	65.0	65.0
cis-DDP	57.0	56.5	56.5	56.0	56.0

[a] T_m(DNA control) = 58.5 °C.

Table IV. DC of the Complexes **1–5** [θ (deg·cm²·dmol⁻¹ × 10³) λ (nm)]

compd	θ_{max}	$\lambda_{\theta max}$	θ_{min}	$\lambda_{\theta min}$
DNA[a]	5.69	276	−7.37	244
1–DNA[b]	3.91	275	−5.95	245
1–DNA[c]	4.35	277	−6.46	245
2–DNA[b]	3.00	274	−5.88	245
2–DNA[c]	2.09	277	−3.75	246
3–DNA[b]	2.80	276	−7.85	245
3–DNA[c]	2.62	278	−3.65	245
4–DNA[b]	5.94	275	−6.59	246
4–DNA[c]	7.78	276	−9.33	244
5–DNA[b]	6.28	275	−8.35	245
5–DNA[c]	6.26	276	−7.13	246

[a] DNA control. [b] r_i = 0.01. [c] r_i = 0.1.

compound **4** can be correlated with the differences in alteration in secondary structure cannot be assessed at present.

Spectrophotometric Studies of the DNA upon Binding of Compounds 3–5. Table III shows that in contrast to *cis*-DDP,[29] the binding to DNA of the Pt compounds **4** and **5** leads to a strong stabilization of the double helix. In fact, while the T_m of native DNA was 58.5 °C, the T_m of the complexes formed by incubation of compounds **4** and **5** with the DNA after 15 min of incubation was, for both DNA–compound complexes, 61.0 °C. In both complexes the increase in T_m is directly proportional to the period of complex formation. It is likely that the formation of stabilizing Pt–DNA adducts reaches completion after 16 h of incubation of the DNA with the drugs since the maximum value of the T_m reaches a plateau at that time. We have observed that the stabilizing effect on the double helix induced by these compounds is independent on whether they are acetate or chloro-bridged. Table III also shows that the chloro-bridged Pd cyclometalated complex induced a strong stabilization of the DNA to heat denaturation. Since, however, the behavior of the chloro-bridged compound **3** is different from that of the acetate-bridged Pd compounds,[5] we think that the bridge linking both cyclometalated structures plays a substantial role in the
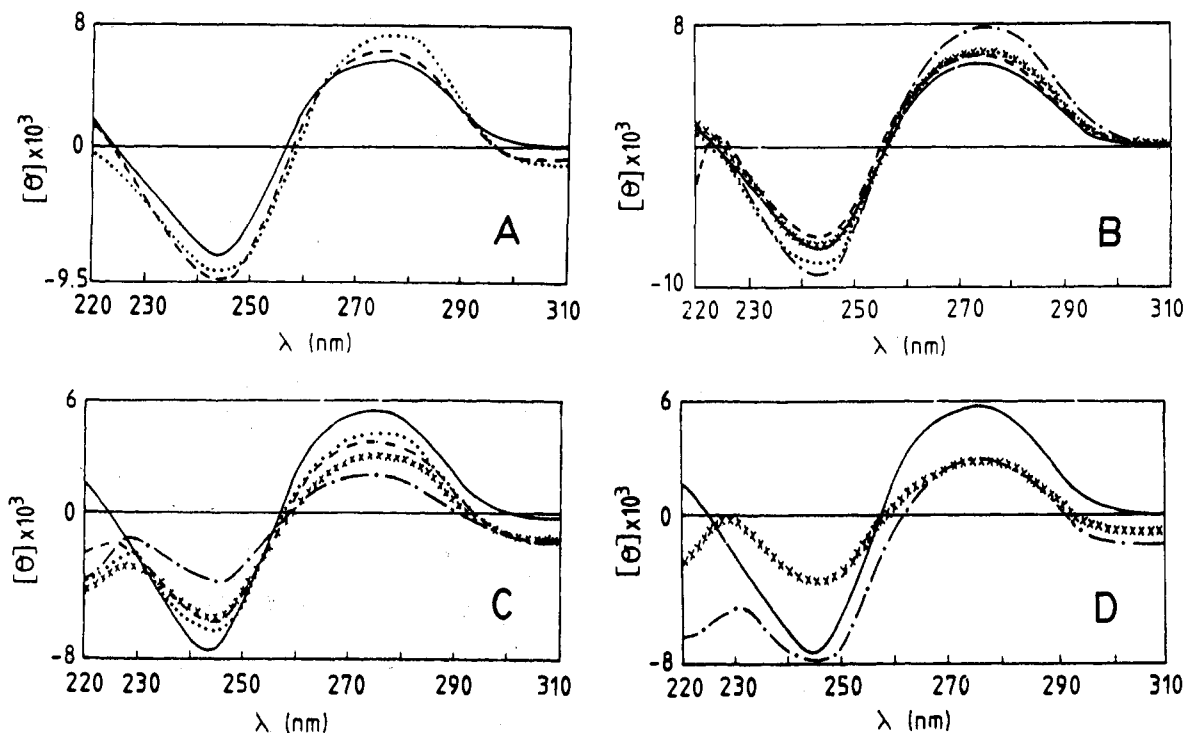
Figure 5. CD spectra of DNA after incubation with complexes 1–5 and *cis*-DDP. (A) (—) DNA; (- - -) *cis*-DDP ($r_i = 0.01$); (⋯) *cis*-DDP ($r_i = 0.1$). (B) (—) DNA; (- - -) 4 ($r_i = 0.01$). (—·—) 4 ($r_i = 0.1$); (⋯) 5 ($r_i = 0.01$); (×××) 5 ($r_i = 0.1$). (C) (—) DNA; (- - -) 1 ($r_i = 0.01$); (⋯) 1 ($r_i = 0.1$); (×××) 2 ($r_i = 0.01$); (—·—) 2 ($r_i = 0.1$). (D) (—) DNA; (—·—) 3 ($r_i = 0.01$); (×××) 3 ($r_i = 0.1$).

definition of the properties of the compound. Remarkably, in contrast with the stabilizing effect induced by the Pt compounds 4 and 5 on the DNA, the effect induced by the Pd compound 3 is inversely proportional to the time of complex formation since the T_m decreases 5 °C after 16 h relative to 15 min. As previously indicated,[4] it is likely that the lowering in T_m observed after a long period of incubation of the DNA with the drugs is due to the lability of the Pd–DNA adducts.

CD Spectra of Drug–DNA Complexes. The CD spectra and the wavelength at which the maximum and minimum values of ellipticity occurs in native DNA and in DNA incubated with *cis*-DDP and compounds 1–5 at $r_i = 0.01$ and 0.1 are shown in Table IV and Figure 5. In agreement with the melting data indicated above, it seems that all the drugs modify the secondary structure of the DNA since they induce changes in the maximum value of ellipticity of the positive band and in the minimum value of ellipticity of the negative band and also in the area under these bands. The binding to the DNA of the cyclopalladated compounds 1, 2, and 3 (Figure 5C,D) causes a decrease in both the θ_{max} and the θ_{min} relative to the values of native DNA. While in compound 2–DNA and compound 3–DNA complexes the value of θ_{max} decreases to 2.09 at 277 nm and to 2.62 units at 278 nm at $r_i = 0.1$, respectively, in the compound 1–DNA complex, on the other hand, the maximum decrease in θ_{max} and in θ_{min} occurs at $r_i = 0.01$ (3.91 at 275 nm and −5.95 at 245 nm). In all cases there is also a change in the wavelength of θ_{max} relative to the r_i. In contrast, the cycloplatinated compounds 4 and 5 increase the value of θ_{max} as it does the *cis*-DDP compound. In the compound 4–DNA complex the increase is higher at $r_i = 0.1$ than at $r_i = 0.01$, while in the compound 5–DNA complex the value of θ_{max} is similar at both r_i. As in the case of *cis*-DDP, compounds 4 and 5 increase the θ_{min}, but the change is r_i dependent. The CD data presented also suggest that the conforma-

tional changes induced on DNA by compounds 1–5 are probably correlated with opening and rotation of the stacked bases since they not only alter the conservative nature of the spectrum of native DNA but they also cause a displacement of the curve.[30] However, the modification due to Pd binding should be different than that due to Pt as also shown by the electrophoretic data.

Conclusions

The reaction of compound $[Pt(\mu\text{-}Cl)(\eta^3\text{-}C_4H_7)]_2$ with the 4-$OMeC_6H_4N$=$C(COC_6H_5)C_6H_5$ ligand led to the formation of the chloro-bridged Pt(II) cyclometalated compound 4. The acetate-bridged Pt(II) cyclometalated compound 5 resulted from the metathetical reaction of the chloro-bridged compound with silver acetate. As demonstrated by IR and ¹H and ¹³C NMR analysis, compounds 1, 2, and 3[6] and compounds 4 and 5 are isostructural. The crystal structure determination given by X-ray diffraction of compound 5 confirms the structure proposed for this compound by IR and ¹H and ¹³C NMR.

The degree of activity of the synthesized isostructural compounds, having the same ligand in common, appears to be a function of the chirality, the leaving group, and the nature of the metal (Pd or Pt). It was observed that the Pt cyclometalated compounds drastically modify the oc and ccc forms of plasmid DNA and the linear form of CTDNA. The Pd compounds also modify the linear form of CTDNA. The data indicate that these isostructural complexes do not exhibit specificity in their activity against leukemic cells (HL-60) or against mammary human cancer cells (MDA-MB 468). The lowest ID_{50} value of the synthesized compounds corresponded to compound 5 with a mean value of 2.0 μM for MDA-MB 468 cells and 1.3 μM for HL-60 cells, close to the ID_{50} values of *cis*-DDP (means of 3.3 and 2.3 μM, respectively). It was observed, moreover, that the platinum compounds are more active than those with palladium.

Experimental Section

General Procedures. The infrared spectra were recorded as Nujol mulls and KBr pellets in the 4000-200-cm⁻¹ range using a Perkin-Elmer Model 283 spectrophotometer. NMR spectra were recorded on a Bruker WP-200-SY (200 MHz) spectrometer in CDCl₃ with TMS as internal standard and in DMSO-d_6. The C, H, and N analyses were carried out in a Perkin-Elmer 240B microanalyzer. All solvents were purified, prior to use, by a standard methods.[31] K₂PtCl₄ was purchased from Aldrich. The ligand and complexes 1, 2, and 3 were synthesized as previously described.[6,7,32]

Synthesis of Compounds 4 and 5. Synthesis of Compound [LPtCl]₂ (4). To a solution of 1 equiv of di-μ-chloro-bis(η^3-2-methylallylplatinum) in chloroform 2 equiv of the imine-4-OMe-C₆H₄N=C(COC₆H₅)C₆H₅ were added. The mixture was heated under reflux until a precipitate was formed. The precipitate was filtered off, washed with chloroform and ether, and dried *in vacuo*. Anal. (C₂₁H₁₈NO₂ClPt) C, N; H: calcd, 2.89; found 2.94. Mp: >300 °C dec (yield 73%). ¹H NMR: δ(ppm) 8.30 (^3J(¹H–¹H) = 7.1 Hz (d), J(¹⁹⁵Pt–¹H) = 40.9 Hz, 1H, H11), 7.77 (m, 2H, H4), 7.69 (m, 1H, H6), 7.51 (m, 2H, H5), 7.32 (^3J(¹H–¹H) = 7.1 Hz, ^4J(¹H–¹H) = 1.8 Hz (dt), 1H, H9), 7.10 (^3J(¹H–¹H) = 7.1 Hz (t), 1H, H10), 6.69 (^3J(¹H–¹H) = 7.1 Hz, ^4J(¹H–¹H) = 1.8 Hz (dd), 1H, H8), 6.97 and 6.70 (AA'BB', 4H), 3.65 (s, 3H). IR: ν_{max} 1673, 1603, 322, 307 cm⁻¹.

Synthesis of Compound [LPtOAc]₂ (5). To a suspension of compound 4 in chloroform 2 equiv of AgOAc were added. After 10 min the whitish solution changed to red. Afterward the solution was filtered and concentrated. When methanol was added, the product immediately precipitated out as a deep red solid. The solid was, then, recrystallized in chloroform/methanol. Anal. (C₂₃H₁₉NO₄Pt) C, H; N: calcd, 2.44; found, 2.46. Mp: 170–171 °C (yield 82%). ¹H NMR: δ(ppm) 7.54–6.40 (m, 13H), 3.72 (s, 3H), 1.88 (s, 3H). IR: ν_{max} 1671, 1605, 1587, 1421 cm⁻¹.

Structural Determination and Refinement of Compound 5. Intensity data were recorded on a Stoe four-circle diffractometer using graphite monochromated Mo Kα (λ = 0.710 73 Å) radiation. The data, the details of the data collection, and the structural analyses are summarized in Table V. Three standard reflections were measured every 30 min. There was no evidence of crystal decomposition. The data were corrected for absorption. They were averaged to give 4338 unique observed reflections with $F > 4\sigma(F)$. The structure was solved by a combination of Patterson and Fourier difference techniques and refined by a full-matrix least-squares methodology to R = 0.086 with all non-H atoms anisotropic; H atoms were placed in idealized positions and allowed to ride on the relevant C atom, C–H 0.96 Å. A CHCl₃ solvate was located.

Isolation of pUC8 Plasmid DNA. pUC8 plasmid DNA was obtained from the JM83 strain of *E. coli* according to the alkaline lysis method.[33] Scanning of pUC8 samples, after they were electrophoresed in 1.5% agarose gels, showed that 80% of the plasmid DNA was in supercoiled monomer or dimer (mccc or dccc) forms and 20% was in open circular (oc) form.

Formation of Drug–pUC8 Complexes. Compounds 1–5 were dissolved in an aqueous solution of 2.5% DMSO. The solutions were prepared immediately before use. Aliquots of these compounds were added to the DNA in a buffer solution containing 50 mM NaCl, 10 mM Tris·HCl (pH 7.4) 0.1 mM EDTA. The amount of each drug added to the DNA solution was expressed as r_i (input molar ratio of Pd or Pt to nucleotides). The compounds and the DNA were incubated at 37 °C for 24 h.

Determination of the ID₅₀ Value. MDA-MB 468 cells were cultured in DMEM (Dulbeaco Modified Eagle's Medium) with glucose (4.5 g/L) and without sodium pyruvate supplemented with 10% FCS (fetal calf serum), 10 μg/mL insulin, and 1% of an antibiotic–antimycotic solution. The replication period of MDA-MB 468 cells was 24 h when cultured in this medium at 37 °C in an atmosphere of 90% air and 10% CO₂, reaching the logarithmic growth phase after 72 h. HL-60 cells were cultured in RPMI 1640 medium free of serum and supplemented with human transferrin (10 μg/mL) and 1% of an antibiotic-antimycotic solution. The replication period of the HL-60 cells was 16 h when cultured in this medium and in an atmosphere of 95% air and 5% CO₂. The cells reached the logarithmic growth

Table V. Crystal Analysis Parameters of Compound 5

Crystal Data	
formula	C₄₈H₃₈N₂O₈Pt₂·¹/₂CHCl₃
symmetry	triclinic, $P\bar{1}$
unit cell determination	least-squares fit from 25 reflections
unit cell dimensions: a, Å	12.793(4)
b, Å	14.262(4)
c, Å	15.213(3)
α, deg	66.24(1)
β, deg	65.40(1)
γ, deg	89.19(1)
packing: V (Å³), Z	2270(1), 2
D_c (g·cm⁻³), M, $F(0,0,0)$	1.750, 1196.1, 1153
μ (cm⁻¹)	63.0
Experimental Data	
technique	stoe four circle diffractometer bisecting geometry graphite oriented monochromator Mo Kα (λ = 0.710 73 Å) scan, ω/θ
temperature (K)	290
number of reflections:	
measured	5949
observed	4338 (4σ(F) criterion)
range of hkl	–12 13, –13 15, 0 16
value of R_{int} (%)	0.0
standard reflections	3 rflns every 30 min, no variation
Solution and Refinement	
solution	Patterson
refinement	least-squares on F_o
H atoms	geometric calculations
ω scheme	$\omega^{-1} = \sigma^2(F) + 0.0045F^2$
final ΔF_o peaks (e Å⁻³)	2.67
final R and R_w	0.086, 0.110
programs	a
scattering f actors	b
anomalous dispersion	b

^{a} SHELXTL PLUS, Program version 4.0, Siemens Analytical Instruments, Madison, WI, 1990. ^{b} *International Tables of X-ray Crystallography*; Kynoch Press: Birmingham, 1974; Vol. IV, pp 99–100 and 149.

phase after 24 h. In order to calculate the concentration of the Pd(II)– and Pt(II)–imine compounds which produce 50% inhibition of cell growth (ID₅₀), 200 μL of the cell suspension (2.5 × 10⁵ cells/mL) were exposed to every compound at concentrations ranging from 0 to 100 μM. After incubation periods of 72 h (for MDA-MB 468 cells) and 24 h (for HL-60 cells) the cell density was determined both in controls and in drug-treated cultures. The ID₅₀ value represents the mean of six experiments.

Melting of Drug–CTDNA Complexes. Aliquots of each of the compounds at a concentration of 10⁻⁴ M were added to the DNA (calf thymus DNA, Sigma) in 0.02 SSPE buffer (SSDE = 180 mM NaCl, 10 mM NaH₂PO₄, 1 mM EDTA, pH = 7.0). The amount of each compound added to the DNA solution was expressed as r_i = 0.1 (the input molar ratio of Pd to nucleotides). The drug–DNA complexes were formed by incubation of the DNA (20 μg/mL) with each of the compounds for 15 min, 1 h, 5 h, 16 h, and 24 h at 37 °C in the dark. Melting profiles were recorded at 260 nm by differential spectrophotometry and at an increase rate of 1 °C/min from 45 °C to 95 °C in a Beckman Acta C III attached to a temperature programmer.

Gel Electrophoresis of Pd(II) Cyclometalated Compound-pUC8 Complexes. DNA aliquots of pUC8 (100 μg/mL) were incubated in the presence of the drugs in a buffer solution containing 50 mM NaCl, 10 mM Tris·HCl, pH 7.4, and 0.1 mM EDTA at several input Pd or Pt/nucleotide molar ratios (r_i). Incubations were carried out in the dark at 37 °C for 24 h. Aliquots of 20 μL of drug-DNA complexes containing 1 μg of DNA were subjected to 1.5% agarose gel electrophoresis for 16 h at 1 V/cm in Tris-acetate buffer (40 mM and EDTA 2 mM, pH 8.0). After electrophoresis, ethidium bromide (0.5 μg/mL) was used as the staining agent. The gels were photographed in a MP-4 Polaroid camera using a 665 Polaroid film and an orange filter.

Circular Dichroism Studies. The CD spectra of drug-CTDNA complexes (DNA concentration = 15 μg/mL, r_i = 0.01

and 0.1) formed in 24 h were recorded on a 1-cm rectangular quartz cell in a JASCO J-600 spectrophotometer at room temperature using a computer for spectral substraction and noise reduction. Each sample was scanned in a range of wavelengths between 220 and 310 nm. The CD spectra of the drugs were substracted from the CD spectra of each of the complexes by computer software. Each CD spectrum represents the mean of three scans. The data are expressed as mean residue molecular ellipticity (θ).

Supplementary Material Available: Listings of anisotropic thermal parameters for non-hydrogen atoms, positional and isotropic thermal parameters for hydrogen atoms, and all bond distances and angles for 5 (8 pages); observed and calculated structure factors for 5 (14 pages). Ordering information is given on any current masthead page.

References

(1) Cleare, M. J. *Coord. Chem. Rev.* **1974**, *12*, 349.
(2) Sadler, P. J. *Chem. Br.* **1982**, *18*, 182.
(3) Farrell, N. In *Transition Metal Complexes as Drugs and Chemotherapeutic Agents*; Ugo, C. R., James, B. R., Eds.; Kluwer Academic Publishers: Boston, 1989; p 142–167.
(4) Graham, R. D.; Williams, D. R. *J. Inorg. Nucl. Chem.* **1979**, *41*, 1249.
(5) Navarro-Ranninger, C.; López-Solera, I.; Pérez, J. M.; Masaguer, J. R.; Alonso, C. *Appl. Organomet. Chem.* **1993**, *7*, 57.
(6) García-Ruano, J. L.; López-Solera, I.; Masaguer, J. R.; Navarro-Ranninger, C.; Rodríguez, J. H.; Martínez-Carrera, S. *Organometallics* **1992**, *11*, 3013.
(7) Navarro-Ranninger, M. C.; Alvarez-Valdés, A.; Camazón, M. J.; Román, J.; Lozano, R. *J. Organomet. Chem.* **1987**, *331*, 107.
(8) Onue, H.; Minami, K.; Nakagawa, K. *Bull. Chem. Soc. Jpn.* **1970**, *43*, 3480.
(9) Cope, A. C.; Siekman, R. W. *J. Am. Chem. Soc.* **1965**, 3272.
(10) Pregosin, P. S.; Wombacher, F.; Albinati, A.; Lianza, F. *J. Organomet. Chem.* **1991**, *418*, 249.
(11) Nakamoto, K. *IR and Raman spectra of Inorganic and Coordination Compounds*; 4th ed.; Wiley Interscience: New York, 1986.
(12) Omae, I. *Chem. Rev.* **1979**, *28*, 97.
(13) Two singlets should appear to a *cis*-disposition.
(14) Bax, A.; Morris, G. A. *J. Magn. Reson.* **1981**, *42*, 501.
(15) Sánchez-Farrando, F. *Magn. Reson. Chem.* **1985**, *23*, 185.
(16) (a) Carrondo, M. A. A. F. de C. T.; Skapski, A. C. *Acta Crystallogr.* **1978**, *B34*, 1857. (b) Carrondo, M. A. A. F. de C. T.; Skapski, A. C. *Acta Crystallogr.* **1978**, *B34*, 3576.
(17) Schagen, J. D.; Overbeek, A. R.; Schenk, H. *Inorg. Chem.* **1978**, *17*, 1938.
(18) Newkome, G. R.; Theriot, K. J.; Fronczek, F. R.; Villar, B. *Organometallics* **1989**, *8*, 2513.
(19) Bandyopadhyay, D.; Bandyopadhyay, P.; Chakravorty, A.; Cotton, F.; Falvello, L. R. *Inorg. Chem.* **1983**, *22*, 1315.
(20) (a) Dong, V.; Endres, H.; Keller, H. J.; Moroni, W.; Nöthe, D. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1977**, *B33*, 2428. (B) Endres, H.; Keller, H. J.; Moroni, W.; Nöthe, D.; Dong, V. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1978**, *B34*, 1823.
(21) Hazell, A.; Mukhopadhyay, A. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1980**, *B36*, 1647.
(22) (a) Bardi, R.; del Pral, A.; Piazzesi, A. M.; Trozzi, M. *Cryst. Struct. Commun.* **1981**, *10*, 301. (b) Koten, G. van; Timmer, K.; Noltes, J. G.; Spek, J. G. *J. Chem. Soc., Chem. Commun.* **1978**, 250.
(23) Elder, R. C.; Cruea, R. D.; Morrison, R. F. *Inorg. Chem.* **1976**, *15*, 1623.
(24) Chassot, L.; Muller, E.; Zelewsky, A. von. *Inorg. Chem.* **1984**, *23*, 4249.
(25) Farrell, N. *J. Med. Chem.* **1989**, *32*, 2240.
(26) Herman, T. S.; Teicher, B. A.; Chan, V.; Collins, L. S.; Kaufmann, M. E.; Loh, C. L. *Cancer Res.* **1988**, *48*, 2335.
(27) Hydes, D. C. *Platinum coordination complexes in cancer chemotherapy*; Hacker, M. P., Douple, E. B., Krakoff, I. H., Eds.; Martinus Nifhoff Publishing: Boston, 1984; p 216.
(28) Cohen, G. L.; Lippard, S. J. *Science* **1979**, *203*, 1014.
(29) Eastman, A. *Biochemistry* **1983**, *22*, 3927.
(30) Perrin, D. D.; Armarego, W. L. F.; Perrin, D. R. *Purification of Laboratory Chemicals*, 2nd ed.; Pergamon Press: Oxford, 1980.
(31) Alcaide, B.; León-Santiago, M. A.; Pérez-Ossorio, R.; Plumet, J.; Sierra, M. A.; De la Torre, M. *Synthesis* **1982**, 989.
(32) Birboin, H. C.; Doly, J. *Nucl. Acids Res.* **1969**, *7*, 1513.