DALTON
FULL PAPER

Anti-tumour platinum acylthiourea complexes and their interactions with DNA

Alison Rodger,*[a] Kirti K. Patel,[a] Karen J. Sanders,[a] Michael Datt,[b] Cheryl Sacht *[b] and Michael J. Hannon *[a]

[a] *Department of Chemistry, University of Warwick, Coventry, UK CV4 7AL. E-mail: a.rodger@warwick.ac.uk; m.j.hannon@warwick.ac.uk*
[b] *Department of Chemistry, Rhodes University, PO Box 94, Grahamstown, 6140, South Africa*

Received 24th May 2002, Accepted 23rd August 2002
First published as an Advance Article on the web 10th September 2002

The interactions of a series of anti-tumour platinum acylthiourea complexes of formula [Pt(L)Cl(DMSO)] (HL = R′C(O)NHC(S)NR_2; R′ = aryl, NR_2 = amine) with natural and synthetic DNAs and with nucleotides were investigated. Circular dichroism experiments confirmed binding and linear dichroism showed that the binding is specific (rather than random), leading to orientation of the complexes on the DNA and resulting in bending of the DNA. Mass spectrometry confirmed coordination of the platinum to the nitrogen-containing base of the nucleic acids with the displacement of chloride. No evidence was obtained for bis-substitution involving concomitant loss of the chloride and DMSO. The mode of binding of the platinum to DNA is affected by the nature of the substituents on the acylthiourea, but this behaviour does not correlate with the biological activity observed.

Introduction

Metals and metal complexes have been used for centuries in the treatment of ailments and diseases[1,2] and indeed cisplatin: *cis*-[$PtCl_2(NH_3)_2$] (Fig. 1), is an important drug used clinically

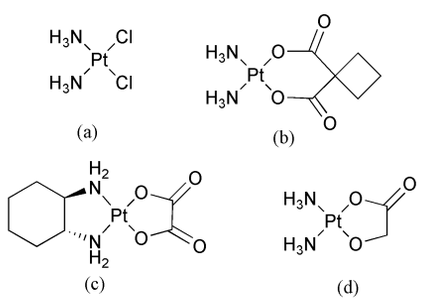


Fig. 1 (a) *Cis*-diamminedichloroplatinum(II), commonly known as cisplatin; (b) carboplatin, (c) oxaliplatin and (d) nedaplatin.

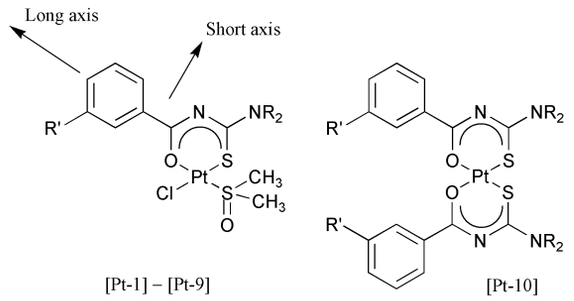
in the treatment of cancers.[3–11] The anti-tumour properties of cisplatin were first recognised in the 1960s[3] and it has been shown to be very effective against testicular and ovarian cancers, and active against a variety of cancers including head, neck and cervical cancers and useful against melanomas. The discovery of the anticancer activity of cisplatin has led to an increase in interest in platinum coordination chemistry and the interactions between platinum metal complexes and biomolecules.[1–14] Cisplatin's target is DNA, where coordination of the platinum to N7 of guanine bases and (to a lesser extent) adenine bases prevents replication and/or transcription, thereby halting cancer cell proliferation.[4–6] The major adduct formed involves intrastrand crosslinkage of adjacent guanines.[4–6,13] This cross-linking causes DNA kinking and the kinked DNA is recognised by nuclear proteins of the HMG class.[14]

A range of cisplatin analogues such as carboplatin, oxaliplatin and nedaplatin (Fig. 1) have also entered clinical use and appear to have similar molecular level actions.[5] However, challenges remain to be overcome including side-effects, toxicity, targeting, delivery, acquired resistance and cancer specificity. To circumvent such problems, drugs with different molecular-level actions are required and a variety of alternate agents such as poly-nuclear platinum species, *trans*-platinum species, cationic platinum species, complexes with sterically bulky ligands, platinum(IV) species and ruthenium compounds are under investigation to try to address some or all of the issues.[15–23] Like the first and second generation platinum drugs currently used in the clinic, these new agents also focus predominantly on covalent binding to DNA.

In this context, non-classical platinum complexes containing acylthiourea ligand systems have recently been described by Sacht *et al.*[12] The acylthiourea ligand systems are extremely versatile in that small structural changes can be readily made that lead to very different chemical and physical properties. Indeed cytotoxicity studies using HeLa cancer cell lines have demonstrated that some of these platinum acylthioureas do show cytotoxic behaviour, with the antiproliferative effects being dependent on the nature/type of the substituent on the acylthiourea ligand.[12] To probe this effect further, we have investigated the DNA binding of complexes of this class to investigate whether the differences in cytotoxicity arise from their molecular-level interactions with the DNA or from other intra- or extra-cellular events. Herein we report the interaction of a set of ten such compounds, most of general formula [Pt(L)Cl(DMSO)] (where HL refers to an acylthiourea ligand system R′C(O)NHC(S)NR_2, R′ = aryl and NR_2 = amine, (O) and (S) denote points of attachment of the ligand to platinum(II)). From the initial DNA screen, six of the compounds were selected for more detailed analysis and their interactions with nucleotides, calf thymus (ct), poly[d(A–$T)_2$] (AT) and poly[d(G–$C)_2$] (GC) DNAs are reported.

The compounds, denoted [Pt-1] to [Pt-10], are illustrated in Fig. 2. [Pt-1]–[Pt-9] are similar in structure differing only in the substituents attached to the basic acylthiourea ligand. They each contain a square-planar platinum(II) centre bound to one bidentate acylthiourea with the remaining two *cis* coordination sites being occupied by a chloride (*trans* to the acylthiourea S) and a S-bonded DMSO molecule (*trans* to the acylthiourea O). The variations of the amine attached to the thiocarbonyl functionality (NR_2), are (i) diethylamine (NR_2 = $N(CH_2CH_3)_2$); (ii) morpholine (NR_2 = $N(CH_2CH_2)_2O$); or (iii) diethanolamine

 DOI: 10.1039/b205056k



	R′=H	R′=NO_2	R′=OCH_3
NR_2=N(CH_2CH_3)$_2$	[Pt-1], [Pt-10]	[Pt-2]	[Pt-3]
NR_2=N O	[Pt-4]	[Pt-5]	[Pt-6]
NR_2=N(CH_2CH_2OH)$_2$	[Pt-7]	[Pt-8]	[Pt-9]

Fig. 2 General structure of [Pt-*X*], *X* = 1–9 and [Pt-10]. The axis system used for the transition moment polarisation determination is also illustrated.

(NR_2 = N(CH_2CH_2OH)$_2$). The substituents on the aryl ring (R′) are (a) hydrogen (R′ = H), (b) nitro (R′ = NO_2) or (c) methoxy (R′ = OCH_3) affording substituents with different electron withdrawing/donating properties. Compounds [Pt-2] (NR_2 = N(CH_2CH_3)$_2$; R′ = NO_2), [Pt-5] (NR_2 = N(CH_2CH_2)$_2$O; R′ = NO_2), and [Pt-6] (NR_2 = N(CH_2CH_2)$_2$O; R′ = OMe) exhibit biological activity against HeLa and MCF-7 cell lines.[12] [Pt-10] is a control compound, having only the bulky acylthiourea ligand system that is common to all the [Pt-*X*] complexes.

Variation of the aryl substituents R′ influences the electronic properties of the molecule[12] and the rate of substitution of the chloro–platinum ligand (Pt–Cl) bond (which is *cis* rather than *trans* to the aryl group) is also a function of the aryl susbstituent (NO_2 > Cl > OCH_3 ≈ H ≈ CH_3).[12] Previous studies on azide displacement have found no evidence for DMSO substitution.[12]

Materials and methods

[Pt-*X*] complexes

The platinum complexes were prepared and characterised as previously described.[12]

Nucleic acids

Duplex ct-DNA, sodium salt, 'highly polymerised' Type 1 was purchased from Sigma; synthetic DNAs, poly[d(A–T)]$_2$ and poly[d(G–C)]$_2$ (AT and GC DNA respectively) for *in vitro* use were obtained from Pharmacia Biotech. DNA concentrations were determined spectroscopically using ε_{258} = 6600 mol^{-1} dm^3 cm^{-1} for ct-DNA, ε_{254} = 8400 mol^{-1} dm^3 cm^{-1} for poly[d(G–C)]$_2$, and ε_{262} = 6600 mol^{-1} dm^3 cm^{-1} for poly[d(A–T)]$_2$. 9-Methylguanine (MG) and the di-sodium salts of the 5′-monophosphates were purchased from Sigma-Aldrich and used without further purification.

ct-DNA was initially dissolved in water; its double stranded behaviour in all solvent systems was verified by performing melting curves and basic spectroscopic studies. Sodium chloride salt and a constant concentration of sodium cacodylate buffer (1 mM) were used to maintain AT and GC DNA structure in the solvent systems used (see below). AT was used with a minimum of 30 mM NaCl, 1 mM sodium cacodylate buffer, pH 6.92. GC was used with a minimum of 10 mM NaCl, 1 mM sodium cacodylate buffer, pH 6.92. These concentrations were shown to be sufficient to retain their duplex structures.

Other materials

Polyvinyl alcohol (PVA) (Type III low molecular weight (hot water soluble)) was purchased from Sigma. HPLC grade solvents acetonitrile, DMSO and DMF were used to dissolve the Pt compounds, followed by addition of water (18.2 MΩ).

Spectroscopy

The spectroscopic techniques used to determine whether interactions are taking place in these [Pt-*X*] : DNA systems were: UV-visible absorbance spectroscopy, circular dichroism (CD), flow linear dichroism (LD) and also film LD to determine transition polarisations. CD and UV-visible absorbance spectroscopy was performed in a 1 cm pathlength quartz cuvette. Absorbance spectra were measured using either a Jasco V-550 or a Cary IE spectrometer. Flow LD measurements were made using a 1 mm pathlength quartz couette flow cell.[24] For film LD, both absorbance and film LD measurements were taken both before and after stretching to ×2 original film length (using a mechanical stretching device and heat). CD and LD spectra were measured using a Jasco J-715 spectropolarimeter. The films for a baseline (no [Pt-*X*] compound) and with [Pt-*X*] were made using a published procedure.[25]

For all experiments, fresh samples of [Pt-*X*] complexes were prepared in minimum acetonitrile to ensure their complete dissolution and used immediately. The DNA conformation (as shown by its CD spectrum, data not shown) was unchanged up to 60% v/v acetonitrile–water. Titrations were set up where the DNA concentration was kept constant as the [Pt-*X*] concentration was varied. Mixing ratios, *R*, are stated in the order DNA base : [Pt-*X*]. The order of addition was first the minimum amount of acetonitrile required to dissolve the platinum complex followed by water, buffer and then the DNA solution.

By keeping the DNA concentration constant the solution viscosity remains approximately constant. The presence of acetonitrile in the solution leads to problems due to evaporation, low viscosity (hence low orientation parameter), and increased turbulence (acetonitrile is necessary due to the low aqueous solubility of these neutral complexes). Therefore, the cell was sealed with parafilm between sample additions and a slower than usual couette motor speed was adopted to reduce turbulence effects. The lower orientation parameter meant that more spectra (in this case eight with a response time of 1 s) needed to be averaged over to get acceptable signal : noise

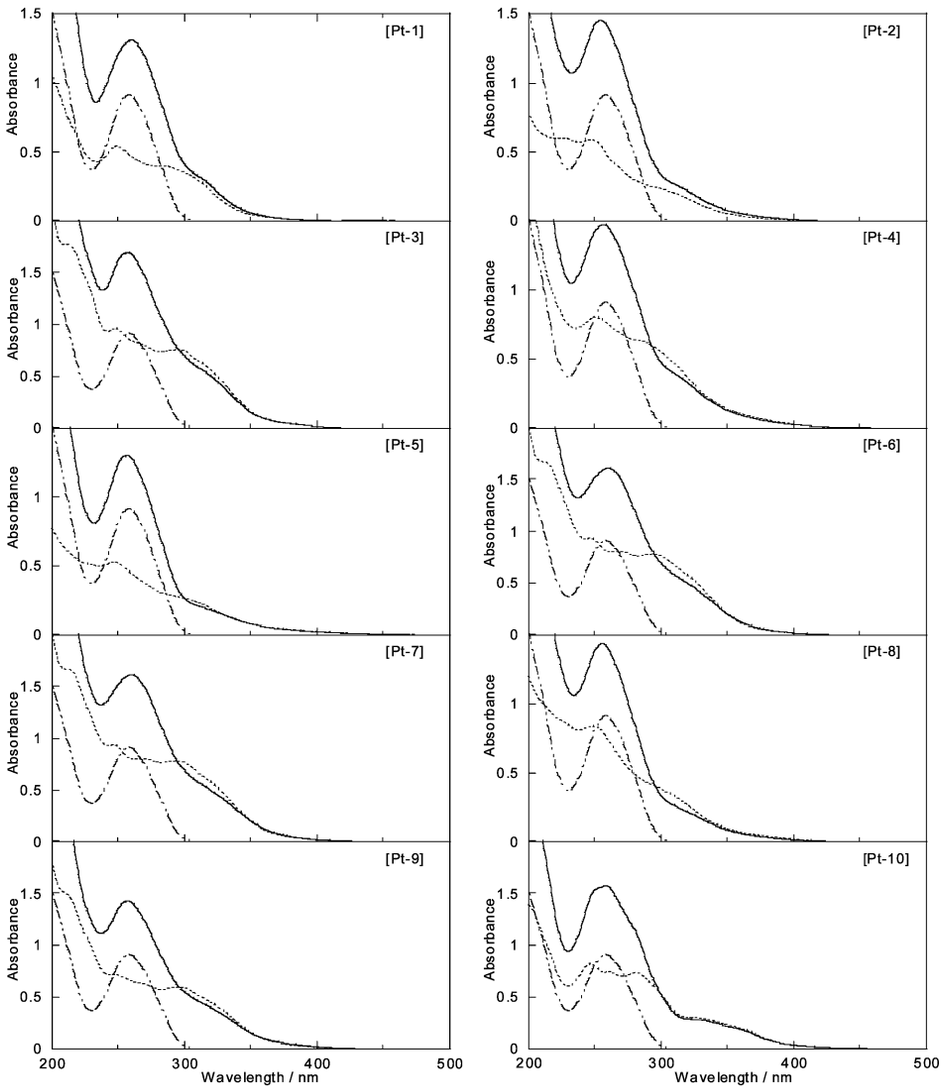


Fig. 3 Absorbance spectra for ct-DNA (125 μM, - - -); [Pt-*X*], *X* = 1–10 (· · ·); and [Pt-*X*] added to the ct-DNA solution (solid line). [Pt-1] 33 μM in 24% MeCN; [Pt-2] 30 μM in 54% MeCN; [Pt-3] 60 μM in 35% MeCN; [Pt-4] 50 μM in 10% MeCN; [Pt-5] 36 μM in 25% MeCN; [Pt-6] 57 μM in 26% MeCN; [Pt-7] 50 μM in 18% MeCN; [Pt-8] 50 μM in 15% MeCN; [Pt-9] 50 μM in 40% MeCN; [Pt-10] 40 μM in 50% MeCN.

ratios. It proved impossible to collect reproducible LD data for AT DNA as the orientation was too low.

Electrospray mass spectra were recorded on a Bruker BioTOF instrument in positive ion mode using 50% aqueous MeCN.

Results

Preliminary spectroscopic study

In order to determine whether there were any interactions with ct-DNA, preliminary UV-Visible absorbance and CD studies were conducted on [Pt-1]–[Pt-10] using [Pt-*X*] solution concentrations of between 30 and 60 μM [Pt-*X*] complex and a constant 125 μM ct-DNA solution to give a ct-DNA : [Pt-*X*] mixing ratio of between 6 : 1 and 2 : 1. Spectra are shown in Figs. 3 and 4 respectively. In these preliminary studies the addition of ct-DNA to a known concentration of [Pt-*X*] results in a slight dilution of [Pt-*X*]. All the complexes except [Pt-10] do exhibit a change in shape and intensity confirming binding to the DNA.

Any CD signal above 300 nm can be attributed to the interaction of the complexes with DNA, since [Pt-*X*] is achiral. Below 300 nm any change from the DNA spectrum is due either to the DNA induced CD (ICD) of the metal complex or the metal complex induced perturbation of the DNA spectrum. The CD spectra of the DNA–[Pt-*X*] mixtures, except DNA–[Pt-10], confirm that they are binding to the DNA. The hydrogen set (R′ = H), [Pt-1], [Pt-4], [Pt-7], and the methoxy set (R′ = MeO), [Pt-3], [Pt-6], [Pt-9], exhibit two similar ICD signals of varying magnitude above 300 nm (outside the DNA region): the first a positive shoulder (which becomes a more pronounced band for the methoxy set) decreasing in magnitude from 300 nm to ~340 nm and a small negative signal centred at ~375 nm. The nitro set (R′ = NO_2), [Pt-2], [Pt-5], [Pt-8], do not exhibit much CD intensity at 300 nm and have a more distinct positive CD band centred at ~325 nm and a small negative CD transition centred at 375 nm. These data suggest that the H and methoxy compounds have similar DNA binding interactions. Thus the methoxy and nitro sets were selected for a more detailed study.

The [Pt-10]–DNA spectrum exactly overlays the DNA CD spectrum once the small dilution effect is accounted for. A further absorbance and CD titration of [Pt-10] with a constant DNA concentration was undertaken over a range of mixing ratios, this also showed no interaction. Thus it can be concluded that [Pt-10] does not interact with DNA. It is almost certainly the presence of two bulky acylthiourea ligands that is responsible for this lack of interaction with DNA.[26] The DNA interactions of complexes 1–9 may therefore be concluded to be primarily *via* the Cl and DMSO sites, rather than the acylthiourea ligand.

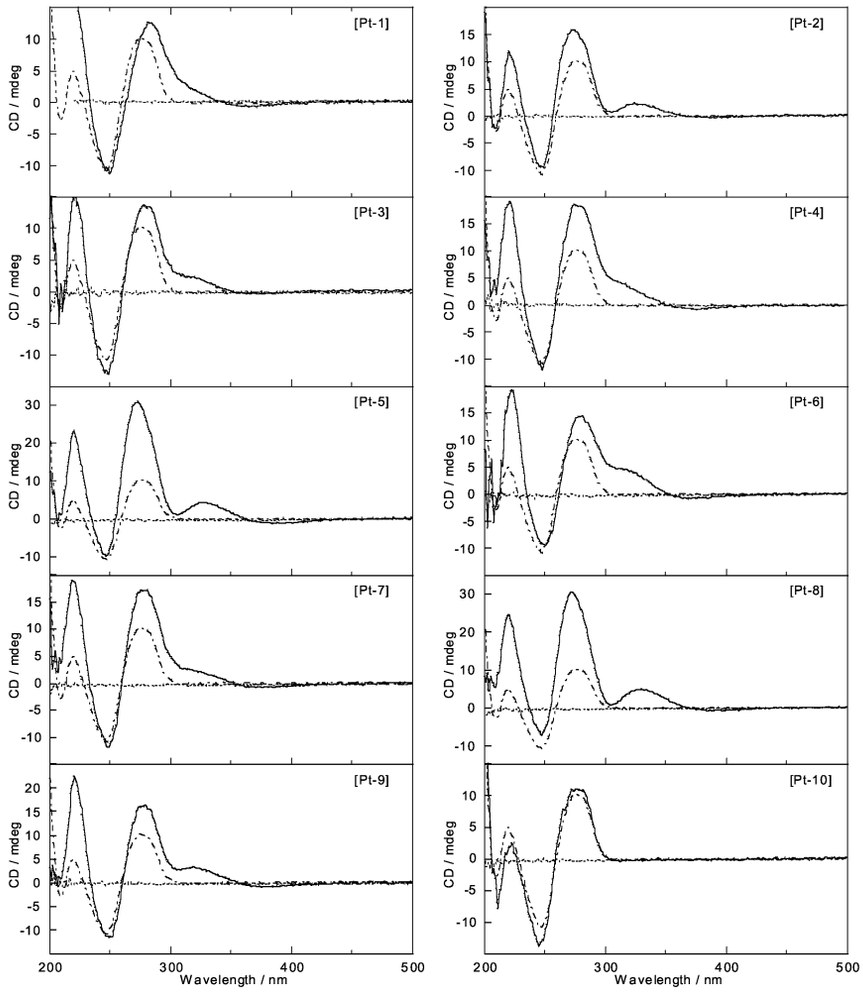


Fig. 4 CD spectra for ct-DNA (125 μM, - - -); [Pt-*X*], *X* = 1–10 (• • •); and [Pt-*X*] added to the ct-DNA solution (solid line). Concentrations are as for Fig. 3.

[Pt-*X*] transition polarisation determination

Flow linear dichroism is a powerful technique for probing orientation of chromophores bound to DNA. However, for a more detailed analysis of the spectroscopy of the DNA–[Pt-*X*] complexes, the transition polarisations are required. When a molecule is absorbed into a stretched film, it is assumed that the long axis of the molecule will orient along the direction of orientation (stretch), whilst the short axis will lie at right angles to the stretch direction. The molecular axis system used is shown in Fig. 2. [Pt-2] was used as a model compound for the whole series. The film absorbance (*A*), film LD and film LD^r = LD/*A* of [Pt-2] are shown in Fig. 5. The film absorbance spectrum of [Pt-2] resembles that of the solution spectrum (Fig. 3) but with a sloping baseline. The LD^r spectrum consists of a positive LD^r maximum centred at 250 nm and positive LD^r signals above 400 nm in the MLCT region (suggesting predominately *z*-polarised transitions) with negative LD^r maxima bands at 210, 290, and ~330–340 nm (suggesting predominantly *y*-polarised transitions). 310 nm is probably a negative minimum, so *z*-polarised. The sloping LD^r signal from negative to positive 350–400 nm is assumed to be due to an overlap of short and long axis polarised transitions.

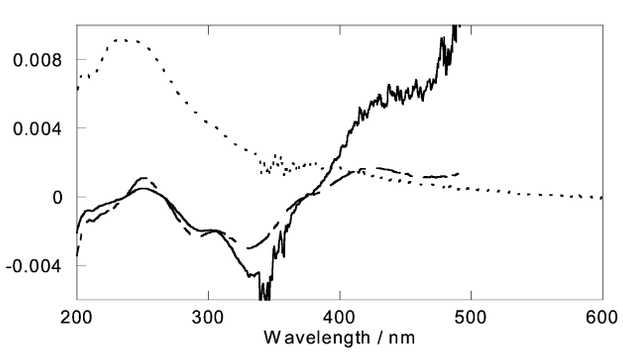


Fig. 5 [Pt-2] film absorbance divided by 5 (• • •); LD (- - -); and LD^r divided by 50 (——).

For compounds such as those of Fig. 2, a range of metal to ligand charge transfer transitions (MLCT) are expected: (i) the Pt–acylthiourea ligand system (made up of the Pt–benzoyl and Pt–thiocarbonyl amine portions of the ligand) denoted Pt–L, (ii) Pt–Cl; and (iii) Pt–DMSO bands. In addition, Pt–OH, $Pt–OH_2$ and Pt–purine MLCT bands might be expected if appropriate coordination species are formed. The 350–400 nm region almost certainly involves the acylthiourea ligand system, since [Pt-10] only has this ligand and has a larger absorbance band in this region than the other complexes (Fig. 3). The absorption band between 300 – 350 nm, by way of contrast, is not as significant for [Pt-10] as for the other complexes, therefore it is predominantly Pt–Cl and Pt–DMSO transitions. Using these conclusions and the literature on similarly assigned Pt–Cl or –OH, –OMe transitions,[27–33] the deductions to be made from the film LD are summarised in Table 1. In addition, the methoxy and nitro substituted aryl rings are expected to exhibit transitions at ~217 and ~269 nm respectively.[34] Thus, the transitions at ~217 and ~269 nm wavelengths can be attributed to these chromophores.

Table 1 Summary of literature MLCT wavelength assignments and LD transition polarisation assignments for the [Pt–X] (X = Cl, DMSO, L, OH or OMe) complexes

Transition	Wavelength/nm	Short axis (*y*)/nm	Long axis (*z*)/nm
Pt–Cl [27,28,31,32]	305, 317, 330, 345	330–340	
Pt–DMSO [30,31]		290–310	310
Pt–L [32,34]		210, 300–400	250, 350–400
Pt–OH [30,31]	308, 325, 332/339		
Pt–OMe [31]	310, 327, 339		

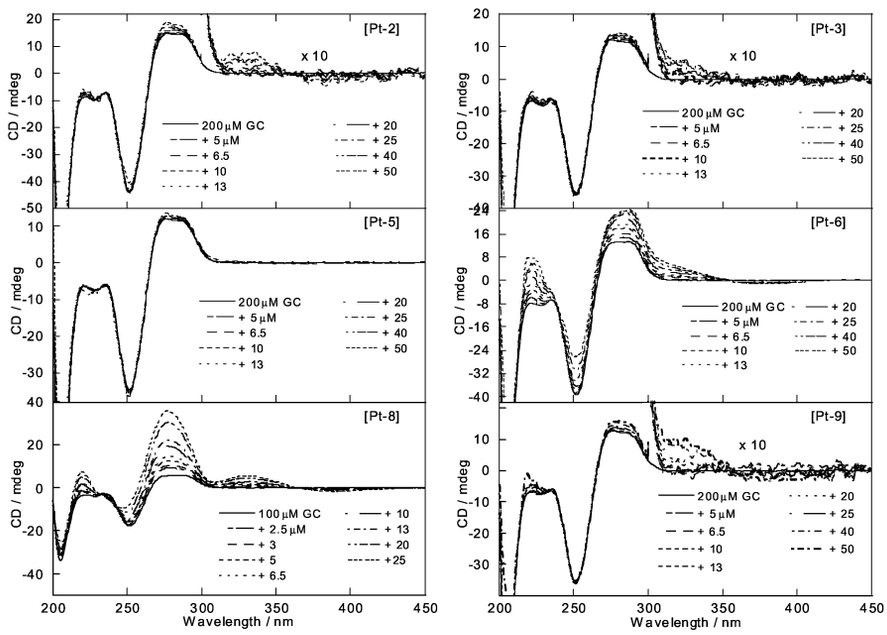


Fig. 6 CD titration series for the nitro and methoxy subsets of compounds with GC DNA (200 μM, 50 mM NaCl, except for [Pt-2] where it is 20 mM NaCl and [Pt-8] where it is 100 μM DNA, 10 mM NaCl). DNA base : [Pt-*X*] ratios are in increasing order of CD at 320 nm as indicated on the figures. % MeCN is as in Fig. 3.

Spectroscopic titration studies to investigate DNA–[Pt-*X*] interactions

Absorbance, CD and flow LD with ct-, AT and GC DNA were measured for the nitro and methoxy subsets. With ct-DNA, a constant 100 μM DNA concentration was used over a mixing ratio, *R*, of [20 : 1–1 : 1] DNA base : [Pt-*X*]. With GC and AT DNA, a constant 200 μM DNA concentration was used (unless otherwise stated) and interactions over *R* = [40 : 1–2 : 1] DNA base : [Pt-*X*] investigated. The DNA concentration was maintained by adding concentrated DNA aliquots with every [Pt-*X*] increment.

The general trends in UV-Visible absorbance for all complexes with all three DNAs is summarised by the data in Fig. 3.

Figs. 6 and 7 show the GC and AT CD data. The titration series for ct-DNA does not show any features not given by the preliminary experiments (Fig. 4) and so additional spectra are not presented. The following observations may be made.

(i) All six complexes interact with ct-DNA as evidenced by their induced [Pt-*X*] CD signal above 300 nm. The signature is a positive ICD from 300–350 nm and a much smaller negative ICD band above 350 nm. The nitro subset 325 nm band is more distinct than the methoxy one.

(ii) All six complexes exhibit a zero isosbestic point between the two ICD bands with ct-DNA, indicating a single binding mode. For the nitro subset with ct-DNA the wavelengths are: [Pt-2] (370 nm), [Pt-5] (365 nm), [Pt-8] (~365 nm). For the methoxy subset the wavelengths are: [Pt-3] (~340 nm), [Pt-6] (350 nm), [Pt-9] (350 nm).

(iii) Despite the significant absorbance intensity (with and without DNA) at 300 nm, the nitro series have small CD signals at that wavelength. This suggests a negative nitro ICD at 300 nm. A similar effect is apparent with the methoxy compounds, though it does not dominate the spectrum. The most likely candidate for this signal is Pt–DMSO charge transfer transitions, suggesting that this group is not displaced to bind to the DNA.

(iv) Only [Pt-6] and [Pt-8] show dramatic ICD signals with GC DNA. The spectra of all complexes except [Pt-5] (which gives little indication of an interaction especially at low drug load) are similar in shape to those of ct-DNA but the 325 nm region of the methoxy series has even less of a distinct band than with ct-DNA. This may correlate with less interaction between the acylthiourea ligand and GC than with AT. It is reasonable to assume the ICD relates to binding constant, however, it should be noted that the NaCl concentration is higher in these experiments than with ct-DNA.

(v) All the complexes interact with AT DNA, [Pt-2] and [Pt-5] showing the least interaction. The other compounds (the methoxy series and the alcohol derivative of the nitro series, [Pt-8]) all show a negative band just below 300 nm in contrast to the GC and ct-DNA behaviours.

Flow linear dichroism titration series

If [Pt-*X*] is oriented in solution it will have an LD signal for its transitions. As the [Pt-*X*] can only be flow oriented upon binding to DNA, the presence of an induced LD at wavelengths that correspond to platinum transitions confirms that an interaction has taken place between the platinum complex and the DNA and that the interaction is not random but leads to binding of the complex to the DNA in specific orientation(s).

The flow LD spectra of the [Pt-*X*]–DNA complexes with ct-DNA (Fig. 8) show similar spectra with unstructured negative ligand signals above 300 nm of varying magnitudes and differing extents of DNA bending (as indicated by an LD magnitude decrease at 260 nm). The transitions being probed

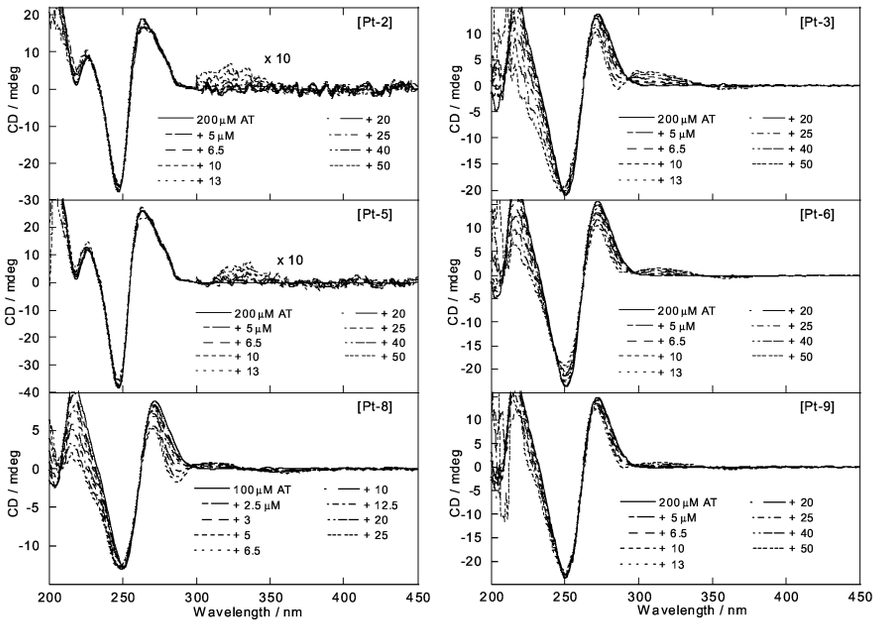


Fig. 7 CD titration series for the nitro and methoxy subsets of compounds with AT DNA (200 μM, except for [Pt-8] where it is 100 μM DNA). [Pt-2], [Pt-5] and [Pt-9] are 40 mM NaCl). [Pt-3], [Pt-6] and [Pt-8] are 30 mM NaCl. DNA base : [Pt-*X*] concentrations are in increasing order of CD at 265 nm as indicated on the figures. % MeCN is as in Fig. 3.

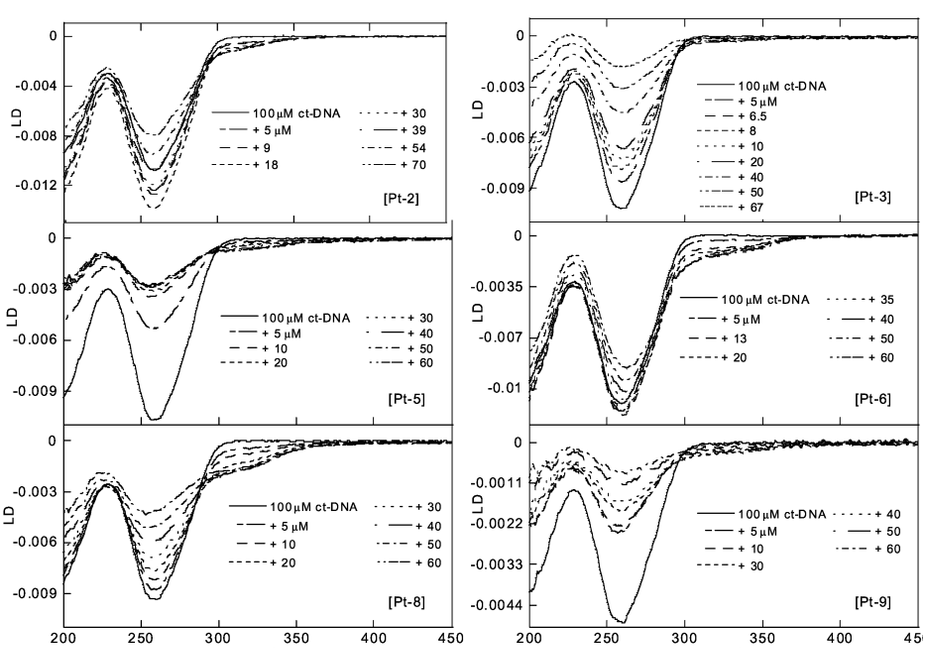


Fig. 8 LD titration series for the nitro and methoxy subsets of compounds with ct-DNA (100 μM). DNA base : [Pt-*X*] ratios are in decreasing order of LD magnitude at 260 nm as indicated on the figures. % MeCN is as in Fig. 3.

are thus oriented more parallel to the DNA bases than to the DNA helix axis. On the assumption that the MLCT bands are predominantly in the plane of the complex this indicates an orientation with the plane of the Pt complex located approximately parallel to the bases. The orientation of [Pt-3], [Pt-5], [Pt-8] and [Pt-9] at low drug load is small and the binding bends the DNA significantly. The observation of DNA bending precludes a true intercalative mode of binding, though it is possible that the edge of the metal complex inserts between two base pairs to induce the observed bending. It may relate to the bending induced into DNA by cisplatin.[35]

With GC (Fig. 9), above 300 nm the spectra are even less structured than with ct-DNA. [Pt-2], [Pt-5] and [Pt-6] show no Pt orientation for the first few titration points. [Pt-3] also has a small signal.

It is tempting to conclude that the [Pt-*X*] are binding *via* a covalent bond to G-N7 by analogy with other platinum complexes. However, the spectroscopic data provided no evidence either to support or contradict this. In order to test this hypothesis some simple experiments with the 5′-chiral di-sodium salts of the mono-nucleotides: guanosine 5′-monophosphate (GMP), adenosine 5′-monophosphate (AMP), thymidine 5′-monophosphate (TMP) and cytidine 5′-monophosphate (CMP) were undertaken. Each nucleotide was mixed individually with the [Pt-*X*] complexes of the nitro and methoxy sets and the products probed using CD. 5 : 1 ratios of NMP : [Pt-*X*] were used. The ICD observed for the complexes (data not shown) shows interaction with GMP and AMP and also a small signal with CMP (except for [Pt-8]). There is no evidence of binding to TMP, except for a small signal with [Pt-8]. This is in accord with a covalent interaction since the common covalent bonding metal binding sites on the nucleotides are

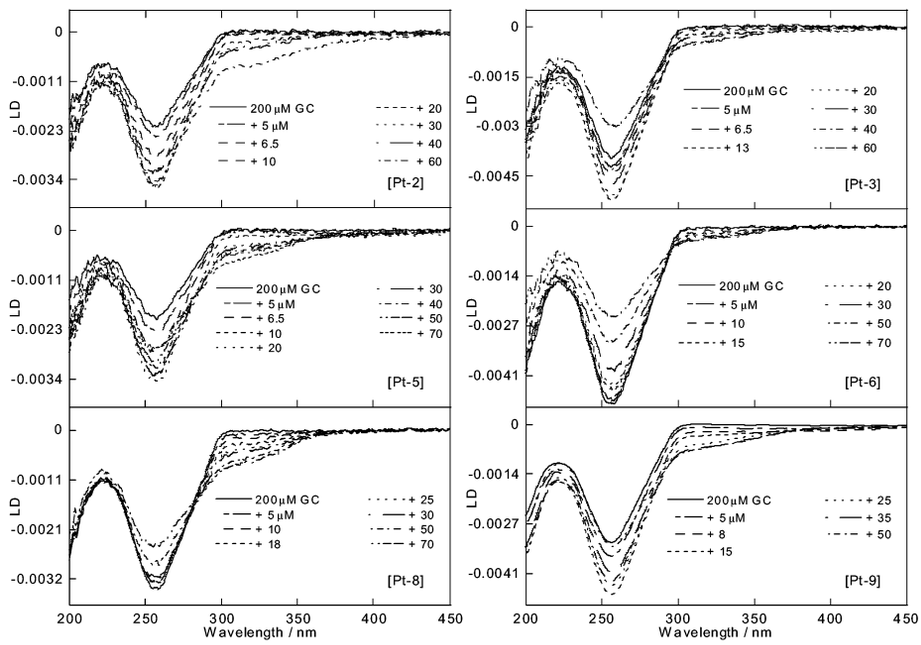


Fig. 9 LD titration series for the nitro and methoxy subsets of compounds with GC-DNA (100 μM). DNA base : [Pt-*X*] ratios are in decreasing order of LD magnitude at 260 nm as indicated on the figure. % MeCN is as in Fig. 3.

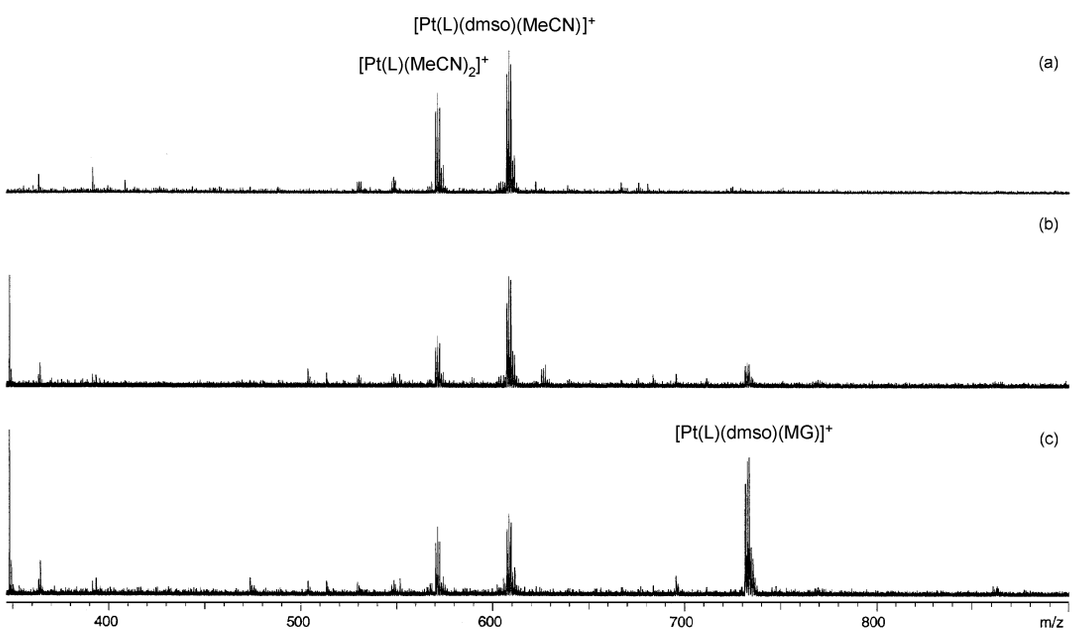


Fig. 10 Electrospray mass spectrum of [Pt-5] (a) in MeCN–H_2O; (b) immediately after addition of 9-methylguanine (MG); and (c) 35 mintues after addition of MG.

GN7, AN7 and CN3.[3,8] However, only GN7 and AN7 are accessible from solvent in double stranded DNA,[3] suggesting that the [Pt-*X*] binding might be to these nucleotides (which are also the targets of cisplatin).

To further investigate whether the binding was through metal coordination or other non-covalent interactions, some simple electrospray MS experiments (Fig. 10) were undertaken with the nitro series and 9-methylguanine (MG). The mass spectra of each of the [Pt-*X*] complexes alone were dominated by peaks corresponding to the presence of adducts corresponding to substitution of Cl or both Cl and DMSO by the solvent acetonitrile as illustrated by the spectra of Fig. 10a. (The parent compound is neutral and not observed. Similarly, if DMSO alone were replaced, the resulting adduct would be neutral, so not observed.) MG was found to bind coordinatively to [Pt-*X*] with displacement of Cl^-. No bis adducts involving displacement of both Cl and DMSO by MG were observed. This does not totally preclude the possibility of bis adduct formation with DNA, since the chelate effect might enhance the subsitution of the DMSO following binding *via* the Cl site. However, it is difficult to envisage how such a binding mode could be consistent with the plane of the Pt complex being approximately parallel to the DNA bases (seen in the LD experiments).

Conclusion

All the complexes studied bind to ct-DNA over the full mixing ratio range. The LD signals suggest that all the MLCT transitions are oriented more parallel to the DNA bases than perpendicular to them, thus indicating the plane of the platinum metal complexes are more or less parallel to the DNA bases (without actually intercalating). The most likely binding

involves a mono-adduct following displacement of the Cl ligand. The binding to the alternating homopolymers is, however, more variable with [Pt-5] showing no interaction with GC and little with AT. The other biologically active nitro compound, [Pt-2], has a small interaction with GC and AT. The biologically active methoxy compound, [Pt-6], interacts with all three DNAs, but has the smallest negative (except for [Pt-5] and [Pt-2]) AT CD band at 280 nm.

[Pt-10] which has two bulky acylthiourea ligands does not bind to DNA so the interacting face of the other complexes must involve the Cl and/or DMSO groups. The mononucleotide work might lead us to propose that the complexes bind covalently to DNA *via* the loss of the Cl on the platinum. However, the variation in spectral signatures between the compounds implies that this cannot be true for all compounds. In particular the biologically active nitro compounds have little interaction with the alternating homopolymers and least with GC, where covalent binding is most expected. By way of contrast the biologically active methoxy compound, [Pt-6], has the largest GC interactions of all the compounds. If B-DNA is indeed the target for these molecules, we must conclude that the mode of action of the active nitro and methoxy compounds differ since their DNA binding differs. It is possible that they target different DNA geometries such as tetraplexes or hairpins.

The major conclusion of this work therefore is that DNA binding does not necessarily equal biological activity for these platinum metal complexes. Molecular structure is related to biological activity, but all aspects of this must be considered. It is interesting to note that the biologically active nitro compounds also have the least water solubility, suggesting that their membrane transport would be facilitated. The active methoxy compound is, however, slightly more soluble than the others.

References and notes

1 Z. Guo and P. J. Sadler, *Angew. Chem., Int. Ed.*, 1999, **38**, 1512.
2 Z. Guo and P. J. Sadler, *Med. Inorg. Chem.*, 2000, **49**, 183.
3 (*a*) B. Rosenberg, L. Van Camp and T. Krigas, *Nature*, 1965, **205**, 698; (*b*) B. Rosenberg and L. Van Camp, *Cancer Res.*, 1970, **30**, 1799.
4 J. Reedijk, *Chem. Commun.*, 1996, 801.
5 B. Lippert, ed., *Cisplatin: Chemistry and biochemistry of a leading anticancer drug*, Wiley-VCH, Weinheim, 1999.
6 E. R. Jamieson and S. J. Lippard, *Chem. Rev.*, 1999, **99**, 2467.
7 S. E. Sherman and S. J. Lippard, *Chem. Rev.*, 1987, **87**, 1553.
8 B. Lippert, *Coord. Chem. Rev.*, 1999, **182**, 263.
9 J. Reedijk, *Chem Rev.*, 1999, **19**, 2499.
10 E. Wong and C. M. Gianomenico, *Chem. Rev.*, 1999, **99**, 2451.
11 T. W. Hambley, *Coord. Chem. Rev.*, 1997, **166**, 181.
12 (*a*) C. Sacht, M. S. Datt, S. Otto and A. Roodt, *J. Chem. Soc., Dalton Trans.*, 2000, 727; (*b*) C. Sacht, M. S. Datt, S. Otto and A. Roodt, *J. Chem. Soc., Dalton Trans.*, 2000, 4579; (*c*) C. Sacht and M. S. Datt, *Polyhedron*, 2000, **19**, 1347.
13 (*a*) P. M. Takahara, A. C. Rosenzweig, C. A. Frederick and S. J. Lippard, *Nature*, 1995, **377**, 649; (*b*) P. M. Takahara, C. A. Frederick and S. J. Lippard, *J. Am. Chem. Soc.*, 1996, **118**, 12309.
14 U.-M. Ohndorf, M. A. Rould, Q. He, C. O. Pabo and S. J. Lippard, *Nature*, 1999, **399**, 708.
15 M. J. Clarke, F. Zhu and D. R. Frasca, *Chem. Rev.*, 1999, **99**, 2511.
16 (*a*) J. D. Roberts, J. Peroutka and N. Farrell, *J. Inorg. Biochem.*, 1999, **77**, 51; (*b*) V. Brabec, J. Kasparkova, O. Vrana, O. Novakova, J. W. Cox, Y. Qu and N. Farrell, *Biochemistry*, 1999, **38**, 6781; (*c*) J. W. Cox, S. Berners-Price, M. S. Davies, Y. Qu and N. Farrell, *J. Am. Chem. Soc.*, 2001, **123**, 1316.
17 N. Farrell, Y. Qu, U. Bierbach, M. Valsecchi and E. Menta, in *Cisplatin, Chemistry and Biochemistry of a leading anti-cancer drug*, B. Lippert, ed., Wiley-VCH, Weinheim, 1999, p. 479.
18 M. Coluccia, A. Nassi, A. Boccarelli, D. Giordano, N. Cardellichio, F. P. Intini, G. Natile, A. Barletta and A. Paradiso, *Int. J. Oncol.*, 1999, **15**, 1039.
19 Y. Liu, C. Pacifico, G. Natile and E. Sletten, *Angew. Chem., Int. Ed.*, 2001, **40**, 1226.
20 G. Sava, R. Gagliardi, A. Bergam, E. Alessio and G. Mestroni, *Anticancer Res.*, 1999, **19**, 969.
21 W. Peti, T. Pieper, M. Sommer, B. K. Keppler and G. Geister, *Eur. J. Inorg. Chem.*, 1999, 1551.
22 R. E. Morris, R. E. Aird, P. D. Murdoch, H. M. Chen, J. Cummings, N. D. Hughes, S. Parsons, A. Parkin, G. Boyd, D. I. Jodrell and P. J. Sadler, *J. Med. Chem.*, 2001, **44**, 3616.
23 Y. Chen, Z. J. Guo, S. Parsons and P. J. Sadler, *Chem. Eur. J.*, 1998, **4**, 672.
24 A. Rodger and B. Nordén, *Circular Dichroism and Linear Dichroism*, Oxford University Press, Oxford, 1997.
25 M. A. Ismail, K. J. Sanders, G. C. Fennel, H. C. Latham, P. Wormell and A. Rodger, *Biopolymers*, 1998, **46**, 127.
26 This is unsurprising; displacement of a chelate ligand would be required to permit coordination of platinum to the nucleic bases and the complex is neutral so there is no electrostatic driving force for binding (neutral intercalators bind only very weakly).
27 X. Y. Zhou and N. M. Kostic, *Polyhedron*, 1990, **9**, 1975.
28 J. A. Bailey, M. G. Hill, R. E. Marsh, V. M. Miskowski, W. P. Schaefer and H. B. Gray, *Inorg. Chem.*, 1995, **34**, 4591.
29 K. W. Jennette, J. T. Gill, J. A. Sadownick and S. J. Lippard, *J. Am. Chem. Soc.*, 1976, 6159.
30 C. S. Peyratout, T. K. Aldridge, D. K. Crites and D. R. McMillin, *Inorg. Chem*, 1995, **34**, 4484.
31 T. K. Aldridge, E. M. Stacy and D. R. McMillin, *Inorg. Chem.*, 1994, **33**, 722.
32 E. M. A. Ratilla, B. K. Scott, M. S. Moxness and N. M. Kostic, *Inorg. Chem.*, 1990, **29**, 918.
33 E. M. A. Ratilla, H. M. Brothers II and N. M. Kostic, *J. Am. Chem. Soc.*, 1987, **109**, 4592.
34 D. H. Williams and I. Fleming, *Spectroscopic methods in Organic chemistry*, McGraw Hill, London, 1995, 5th edn.
35 F. Coste, J. M. Malinge, L. Serre, W. Shepard, M. Roth, M. Leng and C. Zelwer, *Nucleic Acids Res.*, 1999, **27**, 1937–1846.