Synthesis and DNA-binding of Metalloccyclic Architectures

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A thesis submitted to the University of Sydney in partial fulfilment of the requirements for the degree of Doctor of Philosophy

School of Chemistry
The University of Sydney
February 2009
“After my own decision, they confused me so
My love said never question your will at all, in the end you’ve got to go
Look before you leap, and don’t you hesitate at all – no no”

–Yes, Owner of a Lonely Heart
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Acknowledgements

Getting to the stage where I can submit this Thesis has been an almighty struggle. But what doesn’t kill me makes me stronger and one thing’s for sure – it would most certainly have been a much greater struggle had it not been for the assistance of several people, many of whom I count among my good friends.

To my very approachable supervisor A/Prof. Lou Rendina: thanks for having me. I really owe you for all the support and pearls of wisdom I have received, although I can’t say I would ever want to go through four years like that again.

I’d also like to say a big thank you to Prof. Trevor Hambley and E/Prof. Len Lindoy for stepping into the breach and mentoring me when Lou was nowhere to be found. It is quite admirable how these two eminent researchers can be such great gentlemen and have so much consideration for the welfare of their students.

An interdisciplinary project, such as that described in this Thesis, can only be made possible with the most helpful of collaborators. Indeed, I wish to thank both Prof. Margaret Harding (UNSW) and Dr Jenny Beck (UOW) for sharing just a tiny bit of their expertise with me. Many thanks also go to Dr Thitima Urathamakul (UOW) for her patience in teaching me the finer points of mass spectrometry.

Thank you to Dr Ian Luck for not laughing at my stupid NMR questions and Dr Keith Fisher for letting me use the mass spectrometer even after I had probably broken it on more than one occasion. Cheers to Dr Paul Jensen as well, for taking time out to show me the ways of the diffractometer and for being an all-round great dude. At this point I must also pay tribute to Fernando Barasoain, Lee Mears and Janette Thant, to whom I am indebted for their technical support. Needless to say, their tireless work does not go unnoticed.

Throughout my time in the Chemistry building I have felt privileged to be part of a most generous and friendly posse of colleagues who have made coming into Uni every day (OK, maybe not every day!) less of a slog. There is not room enough for me to list you all here, so I hope it suffices for me to say: thank you so much to everyone who has acknowledged my existence, whether it be giving me advice, a chemical, or a friendly greeting in the corridor. Particular thanks go to Katie Cergol, Cindy Aquino, Rebecca Lesic and Paul Saines for their
comradery. I also would like to express my gratitude to the members of the Hambley group, not only for adopting me into their fraternity, but also for allowing me to bounce my far-fetched ideas off them. To Natsuho Yamamoto, Jenny Zhang and Joan Doan: I can’t thank you enough for your support and friendship.

Well now, I must also put in a word of thanks to each and every inhabitant, both past and present, of the self-proclaimed ‘Centre for More Advanced Catalysis and Better Sustainability’ (room 410). I’m not sure how much catalysis goes on there, but it’s certainly a fun place to work in (sometimes). Come to think of it, I can’t remember life before joining 410. Thanks to Alexandra Yeung for being the unofficial secretary of 410 and officially one of the nicest members of said office. And with respect to Dr David Bray, well I’m yet to meet a more selfless human being than this young man. Thanks for being a great role model and for making all of us laugh (even at your own expense). I’d like thank Dr Jack Clegg for also being with me from the start and for giving me crystal structures the same day I give him crystals. It is necessary here for me to give thanks to the members of the Rendina research group. I would probably have handed this thing in a year ago had I not joined in with your many methods of procrastination – but I sure would have regretted it! Thanks to Ellen Crossley for being an entertaining, irrepressible personality who somehow gets given all the jobs nobody wants to do. Thank you as well to Erin Sheridan, from whom a witty repartee is never too far away and to Joseph Ioppolo, for putting up with my ridiculous accents and sharing many a joke (and one fumehood) with me. And to your partner in crime, Vincent Ching – cheers for making me feel good about myself – you always seem to know the right thing to do/say in any situation. Thanks to Daniel Morrison who gave me high fives for even my most trivial of ‘achievements,’ and to Simin Hosseini for being the kind person least responsible for the noise that pervades 410.

I must give thanks to my other (i.e. non-chemist) friends for keeping me (relatively) sane throughout my studies, despite me being absorbed in my PhD (or rather, my PhD absorbing me). Thanks for updating me about happenings in the real world, and for nodding/smiling when I tell you about what it is I actually do.

To a certain someone whose name (at their request) I will not mention here: thanks a million. I am so fortunate to have met such a caring individual who understands me better
than anyone else. And lastly, thank you to Mum, Geni and Edgar (and Maja). You have been there for me no matter what and I hope I can make you proud one day.

David Schilter ☺

February 2009
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>1-D</td>
<td>one-dimensional</td>
</tr>
<tr>
<td>2-D</td>
<td>two-dimensional</td>
</tr>
<tr>
<td>A</td>
<td>adenine</td>
</tr>
<tr>
<td>Ac</td>
<td>acetyl</td>
</tr>
<tr>
<td>apym</td>
<td>2-aminopyrimidine</td>
</tr>
<tr>
<td>apyz</td>
<td>aminopyrazine</td>
</tr>
<tr>
<td>a.u.</td>
<td>arbitrary units</td>
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<tr>
<td>bipy</td>
<td>bipyridine</td>
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<tr>
<td>Bn</td>
<td>benzyl</td>
</tr>
<tr>
<td>br</td>
<td>broad</td>
</tr>
<tr>
<td>C</td>
<td>cytosine</td>
</tr>
<tr>
<td>CT</td>
<td>calf-thymus</td>
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<tr>
<td>COSY</td>
<td>correlation spectroscopy</td>
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<tr>
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<td>doublet</td>
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<tr>
<td>dd</td>
<td>doublet of doublets</td>
</tr>
<tr>
<td>D</td>
<td>deuterium</td>
</tr>
<tr>
<td>δ</td>
<td>chemical shift</td>
</tr>
<tr>
<td>DMF</td>
<td>$N,N$-dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>dppp</td>
<td>1,3-bis(diphenylphosphino)propane</td>
</tr>
<tr>
<td>ds</td>
<td>double-stranded</td>
</tr>
<tr>
<td>ε</td>
<td>molar absorptivity</td>
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<tr>
<td>en</td>
<td>1,2-diaminoethane</td>
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<td>electrospray ionisation</td>
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<td>ethyl</td>
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<tr>
<td>FT-ICR</td>
<td>Fourier transform ion cyclotron resonance</td>
</tr>
<tr>
<td>G</td>
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</tr>
<tr>
<td>HMBC</td>
<td>heteronuclear multiple bond connectivity</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>HSQC</td>
<td>heteronuclear single quantum coherence</td>
</tr>
<tr>
<td>λ</td>
<td>wavelength</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>m/z</td>
<td>mass-to-charge ratio</td>
</tr>
<tr>
<td>m</td>
<td>multiplet</td>
</tr>
<tr>
<td>Me</td>
<td>methyl</td>
</tr>
<tr>
<td>MMLCT</td>
<td>metal-metal-to-ligand charge transfer</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>[^{n}J_{ij}]</td>
<td>n bond coupling between nuclei i and j</td>
</tr>
<tr>
<td>ORTEP</td>
<td>Oak Ridge thermal ellipsoid plot</td>
</tr>
<tr>
<td>PEGda</td>
<td>(O,O')-bis(2-aminoethyl)octadeca(ethylene glycol)</td>
</tr>
<tr>
<td>Ph</td>
<td>phenyl</td>
</tr>
<tr>
<td>phen</td>
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</tr>
<tr>
<td>qu</td>
<td>quintet</td>
</tr>
<tr>
<td>ss</td>
<td>single-stranded</td>
</tr>
<tr>
<td>T</td>
<td>thymine</td>
</tr>
<tr>
<td>(T_m)</td>
<td>DNA melting temperature</td>
</tr>
<tr>
<td>Tf</td>
<td>trifluoromethylsulfonyl</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>tmeda</td>
<td>(N,N,N',N')-tetramethyl-1,2-diaminoethane</td>
</tr>
<tr>
<td>TOF</td>
<td>time-of-flight</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
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<td>visible</td>
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Abstract

A new family of cationic N-heterocyclic ligand derivatives was prepared and characterised. Among these compounds are halide salts of the dications \([Y(spacer)Y]^{2+}\), each of which comprise two \(N\)-heterocyclic donor groups \((Y = 4,4'-bipy, pyz, apyz, apym)\) linked by a conformationally flexible spacer such as \((\text{CH}_2)_n\), \(\alpha,\alpha'-\text{xylylene}\), 2,6-lutidylene or thiabicyclo[3.3.1]nonane-2,6-diyl. The diquaternary halide salts were converted to \(\text{NO}_3^-\) and \(\text{PF}_6^-\) salts, and interaction of these bridging ligands with labile palladium(II) and platinum(II) precursors afforded several multinuclear complexes. Bis(4,4'-bipyridinium) dications were incorporated into the dinuclear macrocycles \([M_2(2,2'-\text{bipy})_2\{4,4'-\text{bipy}(\text{CH}_2)_n4,4'-\text{bipy}\}]^8+\) \((M = \text{Pd, Pt}; n = 4, 6)\), \(\text{cis-}[\text{Pd}_2\text{Cl}_4\{4,4'-\text{bipy}(\text{CH}_2)_34,4'-\text{bipy}\}]^{4+}\), \([\text{Pt}_2(\text{dppp})_2\{4,4'-\text{bipy}(1,2-\text{xylylene})4,4'-\text{bipy}\}]^8+\) and \(\text{cis-}[\text{Pt}_2\text{Cl}_4\{4,4'-\text{bipy}(1,2-\text{xylylene})4,4'-\text{bipy}\}]^4+\). While bis(pyrazinium) analogues were unreactive towards the palladium(II) and platinum(II) precursors, the doubly deprotonated bis(3-aminopyrazinium) and bis(2-aminopyrimidinium) derivatives served as charge-neutral quadruply-bridging ligands in the complexes \([\text{Pt}_4(2,2'-\text{bipy})_4\{\text{apyz(}\text{CH}_2)\text{apyz}–2\text{H}\}]^8+\) and \([\text{Pt}_4(2,2'-\text{bipy})_4\{\text{apym(}\text{CH}_2)\text{apym}–2\text{H}\}]^8+\), both of which feature Pt(II)···Pt(II) interactions. Larger species formed when the diamine \(O,O'-\text{bis(2-aminoethyl)}\)octadeca(ethylene glycol) (PEGda) was treated with \(\text{cis-}\)-dinitratopalladium(II) and platinum(II) precursors. The resulting complexes \([M(N,N)(\text{PEGda})]^2+\) \((M = \text{Pd, Pt};N,N = 2,2'-\text{bipy}, \text{en, tmeda})\) possessed great size (62-membered chelate rings) and aqueous solubility.

DNA-binding studies were conducted with selected complexes in order to investigate the types of interactions these species might participate in (see figure).

Equimolar mixtures containing either the 16mer duplex DNA \(\textbf{D2}\) or the single strand \(\textbf{D2a}\) and palladium(II)/platinum(II) complexes were prepared and analysed by negative-ion ESI-MS. Studies of \(\textbf{D2}/\text{Pd(II)}\) mixtures suggested extensive fragmentation was occuring, and the use of \([\text{Pd(tmeda)}(\text{PEGda})]^2+\) and \([\text{Pd}_2(2,2'-\text{bipy})_2\{4,4'-\text{bipy}(\text{CH}_2)_44,4'-\text{bipy}\}]^8+\) resulted in \(\textbf{D2}\) adducts of \([\text{Pd(tmeda)}]^2+\) and \([4,4'-\text{bipy}(\text{CH}_2)_44,4'-\text{bipy}]^2+\), respectively.
Decomposition also occurred when D2a was used, although 1 : 1 adducts were observed with [Pd(tmeda)(PEGda)]^{2+}, [Pd(2,2′-bipy)(PEGda)]^{2+} and [Pd_{2}(2,2′-bipy)_{2}{4,4′-bipy(CH_{2})_{4}4,4′-bipy}]^{8+}. The low intensities of these adducts indicated that they are unstable towards ESI-MS.

Analogous ESI-MS experiments using platinum(II) derivatives were performed and, in contrast to those with palladium(II), indicated that the complexes remained largely intact. ESI-MS analysis of D2/Pt(II) mixtures allowed for the detection of 1 : 1 D2 adducts of [Pt(en)(PEGda)]^{2+}, [Pt(tmeda)(PEGda)]^{2+} and [Pt_{2}(2,2′-bipy)_{2}{4,4′-bipy(CH_{2})_{4}4,4′-bipy}]^{8+}. Intensities of the adduct ions suggested the greater charge and aryl surface area allow the dinuclear species to bind D2 most strongly. Both [Pt(2,2′-bipy)(Mebipy)]^{4+} and [Pt(2,2′-bipy)(NH_{3})_{2}]^{2+} gave rise to 1 : 2 adducts of D2, although the latter was found to be a weaker binder, perhaps owing to its lower charge. Data obtained using 1 : 5 (D2 : complex) mixtures were consistent with the results above and suggested that D2 can bind more molecules of daunomycin than any of the platinum(II) species. Analyses of D2a/Pt(II) mixtures gave results similar to those obtained with D2, although fragmentation was more pronounced, indicating that the nucleobases in D2a play more significant roles in mediating decomposition than those in D2, in which they are paired in a complementary manner.

Investigations into the effects of selected platinum(II) complexes on the thermal denaturation of calf-thymus DNA (CT-DNA) in solution were conducted. Both [Pt_{2}(2,2′-bipy)_{2}{4,4′-bipy(CH_{2})_{6}4,4′-bipy}]^{8+} and [Pt(2,2′-bipy)(Mebipy)]^{4+} greatly stabilised CT-DNA, most likely by intercalation. In contrast, [Pt(tmeda)(PEGda)]^{2+} and [Pt(en)(PEGda)]^{2+} (as well as PEGda) caused negligible changes in melting temperature (ΔT_m), suggesting that these interact weakly with CT-DNA. Data for [Pt(2,2′-bipy)(PEGda)]^{2+} and [Pt(2,2′-bipy)(NH_{3})_{2}]^{2+} indicated that these species perhaps intercalate CT-DNA, with similar ΔT_m values for both complexes implying that PEGda does not play a major role in binding. While findings from ESI-MS experiments were similar to those from the thermal denaturation experiments, discrepancies between results from the two methods could be found. In particular, fragmentation of cyclic species during ESI-MS caused the binding strength of the species to be underestimated when this method was employed.
Chapter One: Introduction

1.1 Supramolecular chemistry

Large molecular assemblies are ubiquitous in biochemical systems. Nature has constructed innumerable molecular architectures held together by weak interactions, namely H-bonding, \(\pi-\pi\) interactions, hydrophobic interactions, electrostatic interactions and van der Waals forces.\(^1\) Such assemblies include proteins – Nature’s building blocks – whose structure and ability to ‘fold’ and ‘unfold’ are reliant upon H-bonding.\(^2\) Supramolecular chemistry, the study of (predominantly synthetic) molecular assemblies comprising weakly bound components, has been described by Lehn as “the designed chemistry of the intermolecular bond.”\(^3\) Lehn, along with Cram\(^4\) and Pedersen\(^5\) shared the 1987 Nobel Prize in Chemistry “for their development and use of molecules with structure-specific interactions of high selectivity.”\(^6\) Their efforts founded an area of chemistry, largely concerned with non-covalently bound molecular assemblies, which is one of today’s fastest growing areas of research and encompasses aspects of many fields spanning biology to materials science.\(^7\)

1.2 DNA – a natural supramolecule

Perhaps the most elegant of all supramolecular architectures, either natural or synthetic, are those adopted by deoxyribonucleic acid (DNA),\(^8\) the biomolecule responsible for the storage and transfer of genetic information in biological systems. This entity comprises a large number of deoxyribonucleotides, which in turn each consist of an aromatic nucleobase (\textit{vide infra}), a 2-deoxy-D-ribose sugar and a phosphate group.\(^9\) While the nucleobases serve to carry genetic information, the sugar and phosphate groups perform a structural role, existing as the scaffold (or ‘backbone’) of the very long, threadlike molecule. In addition, at physiological pH (7.4) DNA molecules bear a large negative charge, owing to the acidity of the phosphate groups (pK\(_a\) < 1).\(^10\)

The information present in each single strand of DNA (ssDNA) is inherent in the sequence of nucleobases appended to each sugar group in the backbone. The four different nucleobases present in DNA are: the purines adenine (A) and guanine (G) and the pyrimidines thymine (T) and cytosine (C). Each of the nucleobases contains H-bond donor and acceptor groups, with stable H-bonded base-pairs forming between adenine and thymine, as well as guanine and cytosine (Figure 1.1, right). This is termed Watson-Crick
complementary base-pairing, after the researchers who deduced a structure of DNA in 1953.\textsuperscript{8}

Figure 1.1: The tertiary structure of B-DNA (left) and Watson-Crick H-bonding between nucleobases (right). Donor (blue) and acceptor (red) pairs are shown. AT and GC base-pairs are stabilised by two and three primary H-bonding interactions, respectively.

Three conformations are commonly adopted by DNA: A-, B- and Z-DNA, of which B-DNA predominates under physiological conditions. It comprises two antiparallel DNA strands connected by H-bonded bases, with an overall right-handed double helical structure of diameter \(~23.7\ \text{Å}\). As a result of the complementary H-bonding, the two strands are also complementary and, in general, non-equivalent. This causes the DNA double helix to have two different grooves – the major and minor grooves.\textsuperscript{11,12} These are of similar depths\textsuperscript{11} and are formed by base-pairs which sit directly on the helical axis (Figure 1.1, left).

From a supramolecular chemistry viewpoint, the stability of double-stranded DNA (dsDNA) arises from three forms of weak interactions: H-bonding, π–π stacking, and hydrophobic effects. In particular, the H-bonded bases attached to the DNA backbone are arranged such
that $\pi-\pi$ interactions are promoted between adjacent bases (whose mean planes are ~3.4 Å apart) in each strand. Additionally, in aqueous solution hydrophobic effects aid the formation of a double helix, in which the relatively hydrophobic core comprising aromatic moieties is surrounded by the more hydrophilic sugar-phosphate scaffold. Indeed, this scaffold allows for DNA to be strongly hydrated. While the hydrophobic effect may be considered less directional than H-bonding and $\pi-\pi$ interactions, it is clear that it may have a marked influence on solution structures. What also becomes evident is that the presence of several weak interactions within a supramolecular system may cooperatively aid the formation of elaborate structures of remarkable stability.

The findings of Watson and Crick, in particular that the two strands are of a complementary nature, immediately suggested that each strand templates the formation of the other in DNA replication. In other words, the two strands are supramolecular receptors for one another, making the double helix an exquisite example of molecular recognition.

### 1.3 Host-guest systems

In order for genetic information to be expressed in biological systems, it is essential that proteins are able to form stable supramolecular complexes with DNA. To this end, proteins must be able to recognise DNA by means of H-bonding, hydrophobic effects and electrostatic interactions. These influences form the basis of three general modes of protein binding to DNA, typically characterised according to their interaction ‘footprints,’ which may be single-headed, double-headed or enveloping in nature. The former two modes entail binding to the major and/or minor grooves of DNA, with the protein adopting either a $\alpha$-helical or $\beta$-sheet secondary structure in order to maximise interactions with base-pairs and backbone components. In contrast, the enveloping mode is characterised by positively charged amino acid residues interacting electrostatically with the DNA backbone. When binding to DNA in such a fashion, proteins incorporate a cleft shape into their overall structure. These proteins, which act as ‘hosts’ for DNA ‘guests’, are referred to as being toroidal or ring-shaped and are central to several cellular processes. It is no accident that proteins involved in DNA metabolism all share similar structural motifs – indeed it has been suggested that the structures of these proteins have converged (‘evolved’) to toroidal geometries owing to the unique structure of the DNA guest.
Much attention has recently focussed on the toroidal proteins because of their key role in the DNA polymerase holoenzyme complex that catalyses DNA replication, repair and recombination. The enzymatic polymerisation of several hundreds/thousands of nucleotides per second without dissociation from the template DNA is made possible by the mechanical association between DNA and a subunit referred to as the DNA “sliding clamp”. For example, in *Escherichia coli*, the β-subunit dimer of DNA polymerase III holoenzyme performs the role of the sliding clamp. The high processivity of the enzyme results from the unhindered motion of the sliding clamp along duplex DNA, the former holding the catalytic subunits in close proximity to the primed template; such a situation is demonstrated by the X-ray structure of the DNA-protein complex determined by Kong and co-workers (Figure 1.2).

![Figure 1.2: X-ray crystal structure of the β-subunit dimer of DNA polymerase III (E. coli) topologically associated with B-DNA.](image)

The central cavity of the toroidal protein is approximately 35 Å in diameter, allowing for DNA to be accommodated without steric strain. Data from the structure determination, as well as theoretical studies, have indicated the presence of an overall positive charge on the surface of the central cavity. The electrostatic interactions between this positively-charged cavity and the negatively-charged phosphate groups of DNA serve to stabilise the supramolecular complex. From a topological viewpoint, the sliding clamp may be considered a ‘wheel’, and the encircled DNA an ‘axle’, with the overall arrangement being referred to as a pseudorotaxane, namely a [2]pseudorotaxane, as two individual components are involved.
Examples of topologically-interesting supramolecules are not limited to those occurring in natural systems. In particular, the past two decades have seen several reports of synthetic interlocked molecular assemblies.\(^{19}\) An example of such an assembly is given in Scheme 1.1, which depicts the formation of a [2]pseudorotaxane (1) from a cationic axle and a crown ether wheel.\(^{20}\)

![Scheme 1.1: Formation of a [2]pseudorotaxane (1) from a 1,2-bis(4,4′-bipyridinium)ethane ‘axle’ and a dibenzo-24-crown-8 ‘wheel’, and its conversion to a rotaxane (2) with tert-butylbenzyl ‘stoppers’.

The formation of pseudorotaxane 1 is driven by three primary forms of weak interaction. H-bonds between methylene protons of the axle and the ether O atoms (shown in red) serve to stabilise the complex. These ether O atoms also participate in ion-dipole interactions with the quaternised N atoms (shown in blue) of the axle. In addition, aromatic regions within the axle (pyridyl rings) and wheel (phenylene groups) are arranged such that \(\pi–\pi\) stacking can occur. While the collective influences of these interactions result in the thermodynamic stability of the pseudorotaxane structure, sterically demanding groups can be appended onto the ends of the axle in order to confer kinetic stability on the system as well. In such a case the wheel would be unable to leave the axle; interlocked structures such as 2 are known as [2]rotaxanes.
1.4 *Metallosupramolecular chemistry*

The subclass of supramolecular chemistry involving the use of metal ions as structurally defining elements has been termed metallosupramolecular chemistry (despite metal-ligand coordinate bonds often having substantial covalent character). As such, the stereochemical information inherent in individual metal ions, combined with ligand directionality, can often be useful in the predictable synthesis of chosen molecular architectures.

In the mid-1980s the potential of the above strategy for producing supramolecular structures was demonstrated. For example, Sauvage and co-workers were successful in synthesising a pair of interlocked molecular rings – a [2]catenane (3) – by means of two elegant chemical steps involving the metal-templation procedure illustrated in Scheme 1.2.

Their use of copper(I), a metal ion typically exhibiting a tetrahedral coordination geometry, allowed for the preorganisation of the bound phenanthroline precursor derivatives in a mutually perpendicular fashion. The (irreversible) closing of the rings, involving the kinetically-controlled alkylation of the phenolic moieties, represented the second and final step in the synthesis.

![Scheme 1.2: Synthesis of a [2]catenane directed by a tetrahedrally coordinated copper(I) ion.](image)
Recently, the incorporation of metal ions as members of cyclic structures, sometimes referred to as metallocyclic chemistry, has proven to be a particularly useful approach for obtaining new cyclic molecular architectures. This is exemplified in the preparation of the first metallocyclic square (Scheme 1.3) which was reported in 1990 by Fujita and co-workers.\(^{24}\)

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{ONO}_2 \\
\text{Pd} & \quad \text{H}_2\text{N} \\
\text{ONO}_2 & \quad \text{H}_2\text{N}
\end{align*}
\]

Scheme 1.3: A metallosupramolecular square incorporating four angular and four linear components.

This product was found to form by ‘self-assembly’, a process defined by Stang as “the spontaneous assembly of molecules into structured, stable non-covalently joined aggregates”.\(^{25}\) In the above reaction, corner units comprising cis-protected palladium(II) centres (defining 90° angles)\(^{26}\) self-assemble in the presence of complementary linear 4,4´-bipyridine bridging units to give the desired tetranuclear square complex, whose connectivity incorporates four palladium(II) ions. The predicted structure (4), based on the propensity for palladium(II) to adopt a square-planar coordination geometry and the inherent linear directionality of the 4,4´-bipyridine bridging ligand, was obtained in high yield after stirring an aqueous suspension of the precursors for ten minutes at room temperature. While feasible kinetic pathways to acyclic oligomeric structures also exist, entropy considerations (\textit{vide infra}) and the principle of ‘self-correction’\(^ {21}\) apparently make this synthesis (and indeed, more generally, many other syntheses in the field of metallosupramolecular chemistry) viable.

The phenomenon of ‘self-correction’ is proposed to manifest itself once an equilibrium is established between reactants and all possible products. Provided the metal coordination bonds are sufficiently labile, such an equilibrium may, for example, entail the interconversion of oligomeric species into discrete products. It is claimed that discrete products are generally preferred entropically over oligomeric ones on account of their
assembly from fewer components. In the formation of 4, NMR studies showed that a kinetically distributed oligomeric mixture was initially generated, which, by self-correction ultimately gave the entropically favoured discrete square species in high yield. It is clear that the required interconversion is reliant upon rapid metal-ligand bond formation and dissociation. Replacement of palladium(II) with another $d^8$ metal ion, platinum(II), largely resulted, under the same reaction conditions, in a reaction mixture containing more persistent oligomeric species. This was a result of the increased inertness of platinum(II) coordinate bonds acting to inhibit the self-correction process, the formation of Pt–N bonds in analogous systems being essentially irreversible at room temperature. This problem had two straightforward solutions: the use of prolonged reaction time at elevated temperature (28 days under reflux), or the addition of polar media (NaNO$_3$) to the reaction mixture. The latter serves to increase the dielectric constant of the solution, promoting square formation due to the induced lability of the Pt–N bonds, the greater influence of hydrophobic aggregation, as well as the reduced internuclear repulsion under these conditions. This self-assembled system falls into the particular class of ‘strict self-assembly’, which refers to precursor components assembling into structures that represent the thermodynamic product(s) of the system.

Subsequent work demonstrated that in solution a small amount of a triangular analogue also forms in equilibrium with the square. It is argued that while this triangular species may be entropically favoured (as it assembles from fewer components), it is disfavoured enthalpically due to greater angular strain. This, and similar square-triangle equilibria have now been well documented and, with careful design of reactants and conditions, it is possible to isolate either species preferentially.
Since the preparation of the above supramolecular square complexes, many further examples of molecular polygons have been reported, several of which incorporate cis-protected square-planar metal centres. For example, interaction of cis-[Pd(en)(NO$_3$)$_2$] with equimolar 1,4-bis[(4-pyridyl)methyl]benzene under dilute conditions resulted in the quantitative formation of an ellipsoid complex (5, Scheme 1.4), incorporating a hydrophobic cavity.$^{32}$ At higher concentrations, however, a second species was found to be in dynamic equilibrium with the ellipsoid; this species was found to be a catenated dimer of the form 5$_2$. In contrast to the permanently interlocked rings of Sauvage et al.,$^{22}$ this catenane was found to form under thermodynamic control.

![Scheme 1.4: The formation of a [2]catenane (5$_2$) from two identical rings, mediated by $\pi$-$\pi$ interactions.](image)

It was demonstrated that the catenane is stabilised by the presence of $\pi$-$\pi$ stacking interactions between the ligands in each ring, as well as the favourable hydrophobic association of the two units. The latter was exploited to perturb the equilibrium ratio of monomer and dimer by altering the solvent system. This can be rationalised by considering the varying propensities of molecules to solvate the dimer structure, as well as their dielectric properties$^1$ (as each ring bears a 4+ charge). In particular, polar mixtures (such as 1.0 M NaNO$_3$ in D$_2$O) were found to favour the catenane, whereas less polar solvents (such as 1:1 D$_2$O/glycerol) favoured the free metallocycles. Additionally, the presence of (4-methoxyphenyl)acetate (a guest molecule capable of complexation with 5 but not 5$_2$) also favoured the monomer. This is an example of ‘guest-promoted self-assembly’, a phenomenon which arises when the formation of a stabilised host-guest complex represents...
an overwhelmingly favourable state such that species in equilibrium with the host are barely observed.

The syntheses of several metallocyclic polygons including ellipsoids,\(^{33,34}\) triangles,\(^{35}\) squares,\(^{36}\) rectangles,\(^{34,37}\) pentagons\(^ {38}\) and hexagons\(^ {39}\) have recently been reported. Three further examples of two-dimensional supramolecular architectures are given below: a triangle incorporating bis(\(\beta\)-diketonato) ligands (6), an alkynylplatinum(II)-derived square (7) and a hexagon assembled using both coordination and H-bonding (8).\(^ {40}\)

Other systems may often be constructed through the designed variation of one or more structural elements such that required geometries are generated. For example, in trinuclear molecular triangle 6, altering the aryl substitution pattern in the \(\beta\)-diketonato ligands from \textit{para} to \textit{meta} gives rise to a dinuclear ellipsoid incorporating two such ligands.\(^ {33}\) Indeed, “supramolecular libraries”\(^ {41}\) have been proposed which, in almost a combinatorial fashion, summarise the molecular properties required to generate a desired structure.

1.5 Bio-inorganic chemistry of metallosupramolecular assemblies

The last decade of the 20\(^{th}\) century saw a dramatic increase in the research output of metallosupramolecular chemistry, and several polygonal as well as polyhedral species of unprecedented size were reported. At present there is an increasing onus on the functions, not solely the (often aesthetically pleasing) structures, of these systems.\(^ {42}\) Recently, considerable attention has been paid to the study of the bio-inorganic chemistry, in particular the DNA-binding properties, of such architectures. Indeed, their large size and charge have the potential to allow for modes of DNA-binding and biological activity not previously observed with other synthetic molecules. For example, the molecular square [Pt\(_3\)(en)\(_4\)(4,4'-bipy)\(_4\)](NO\(_3\))\(_8\) (the platinum(II) analogue of 4) has the ability to significantly affect the secondary and tertiary structures of DNA (for an overview of DNA-binding, see
In addition, the square complex was found to cause apoptosis and displayed cytotoxicity comparable to that of cis-diaminedichloridoplatinum(II) (cisplatin) against the HL-60 leukaemia cell line. More recently, the species has also been found to effectively bind a G-quadruplex and inhibit telomerase, an enzyme that aids in the protection of chromosomes and is particularly active in cancer cells.

A related complex, the platinum(II) ‘metallalixarene’ 9, has also been the subject of biological investigations and binds mononucleotides, as well as calf thymus DNA (CT-DNA) through non-covalent interactions. At low metallalixarene concentrations, DNA supercoiling is observed, whereas atomic force microscopy imaging suggests the DNA uncoils into long rigid structures when more metal complex is present.

The diiron(II) triple helicate 10 has been shown to bind to natural DNA as well as the palindromic hexanucleotide 5′-d(CGTACG)-3′. In the latter case, X-ray analysis of single crystals obtained from the helicate/oligonucleotide mixture revealed the presence of a three-way DNA junction 11, rather than the expected duplex DNA. Such junctions are observed during DNA replication (the replication fork) but are not well understood. In a beautiful example of molecular recognition and guest-promoted self-assembly, the triple helicate guest was found in the centre of the DNA host, undoubtedly stabilising the Y-junction motif.

As such, the supramolecular chemist is ideally suited to exploit the many opportunities the area presents. According to Hannon, the area of DNA-binding is “affording new opportunities for supramolecular chemistry, where shape, fit and orientation play a central role.” While designing species to interact with the large and chiral structure of DNA can often present challenges, genomic and structural information from biology suggests that supramolecular DNA recognition is an area of substantial promise.
1.6 DNA nanoshuttles

The most intricate synthetic assemblies reported to date remain rather primitive compared to even the simplest of natural supramolecules. Research in supramolecular chemistry has taken many steps towards complex functional molecules. However, while this area of chemistry has been developing for half a century, living systems have had four billion years over which to evolve. Indeed, biology has often served as a source of inspiration in the design of synthetic molecular architectures. For example, catenanes comprising DNA are widespread in prokaryotic and eukaryotic cells, and both double- and single-stranded versions can be prepared stereoselectively. Much attention has also been given to the development of bio-hybrid systems, which incorporate both natural and synthetic molecular components. In terms of the present study, it was of interest to prepare a system incorporating both synthetic and natural components held together by weak interactions (rather than having them covalently bonded to each other). While several examples of purely biomolecular or purely synthetic supramolecular assemblies exist, far fewer hybrid systems have been reported.

The preparation of a macrocycle capable of encircling double-stranded linear DNA represents a particularly attractive synthetic goal. Such a DNA/macrocycle hybrid pseudorotaxane would be unprecedented and perhaps would lead to systems in which the macrocycle could be capable of probing DNA structure and function by ‘shuttling’ along the biomolecule. Consequently, such synthetic analogues of the aforementioned toroidal proteins can be referred to as “DNA nanoshuttles”.

From the definition above, and upon consideration of the properties of a DNA sliding clamp, it becomes evident that candidate molecules must satisfy strict design criteria. The most important of these is that the cyclic structure must possess a cavity with the appropriate dimensions (i.e. 30 – 40 Å) to accommodate double-stranded DNA. In addition, a macrocycle bearing a high positive charge would interact electrostatically with the polyanionic backbone of DNA in a favourable manner. It is likely that this would confer significant aqueous solubility on such a species, which is necessary for study with DNA under biologically relevant conditions. Finally, the macrocycle should maintain its integrity in the presence of DNA, that is, be inert to chemical reaction with any component of the biomacromolecule.
Self-assembled metallocycles, which are often H₂O-soluble and highly charged, were considered as promising options towards the preparation of DNA nanoshuttles. Previous work within the author’s group has resulted in the high-yielding preparation of a metallosupramolecular hexagon (12) bearing a 24+ charge. This designed synthesis involved the reaction of six linear and six angular (120°) components to give the dodecaplatinum(II) complex, which is expected to have a central cavity larger than 30 Å. Given the considerable size and charge of 12, as well as its H₂O solubility, it was considered to satisfy the ‘nanoshuttle’ criteria and DNA-binding experiments were conducted with this hexagon using standard molecular biology techniques. The incubation of linear pBR322 plasmid DNA with the hexagonal complex was undertaken with the aim of forming [n]pseudorotaxanes (linear DNA threaded through macrocycles, Figure 1.3). These were not characterised as such, but were treated with T4 DNA ligase, which served to generate bio-hybrid [n]catenanes, comprising permanently interlocked circular DNA and hexagons.*

* The catenanes that formed may have varying numbers of either ring. Acyclic oligomers or larger macrocycles of DNA are also potential products. Hexagons which are non-mechanically (electrostatically) bound to the DNA are removed by a salting procedure and, in any event, are likely to dissociate during electrophoresis.
Agarose gel electrophoresis of the products indicated reduced mobility when higher concentrations of platinum complex were employed. Such a result is consistent with an increase in mass, as well as charge neutralisation due to the topologically-associated cationic (platinum complex) and anionic (DNA) components – indeed this is strong evidence for nanoshuttle behaviour.

![Diagram of DNA ligase forming a hexagon-DNA hybrid](image)

**Figure 1.3: Formation of interlocked macrocycle-DNA hybrids mediated by DNA ligase.**

However, the preparation of hexagon 12 was found to be unreliable, perhaps owing to the formation of oligomers† and/or hydration of the ketone groups. As one might imagine, the synthesis of large macrocycles (either organic or inorganic) is nontrivial; strategies for their preparation are presented in the following discussion.

† [Pt₆(NH₃)₁₂(4,4′-dipyridylketone)₆]^{12+} and trans-[Pt₆(NH₃)₁₂(4,4′-bipy)₆]^{24+} are more likely to be the thermodynamic products. The precipitation of material (probably oligomeric in nature) after extended heating is consistent with the formation of extended linear species of the latter type. Nevertheless, 12 may predominate under kinetic control.
1.7 **Large macrocyclic species**

It is well known that the preparation of macrocycles becomes increasingly difficult with larger numbers of ring members.\(^{57}\) While high-dilution conditions (which favour intramolecular cyclisation over intermolecular oligomerisation) can be successfully employed in such syntheses, often the entropic cost of bringing two ends of a chain together is too high. In order to increase the probability of cyclisation, large end groups were employed by Fujita and co-workers in their preparation of the palladium(II) complex 13\(_2\).

The overall cyclic structure incorporates over one hundred atoms, causing this to be termed an “ultramacrocycle” that can be considered to border the molecular and nanoscale domains.\(^{58}\) Monomeric 13 in DMSO-\(d_6\) solution converts to the cyclic dimer upon addition of D\(_2\)O, the driving force for this being hydrophobic catenation (see discussion of species 5\(_2\)). In particular, the macrocycle has an appropriate diameter for a DNA nanoshuttle (~3.0 nm). However, the cavity is largely occupied by the polyaromatic catenated ligands and the system lacks the necessary H\(_2\)O-solubility.

Examples of purely organic ultramacrocycles in the chemical literature also exist. Rothe and co-workers reported the cyclooligomerisation of deca-\(\varepsilon\)-aminocaproic acid to give a cyclic oligopeptide mixture containing a 700-membered caprolactam heptamer having a molecular weight of 11,315 Da.\(^{59}\) However, while large macrocycles are accessible, the preparation of pure monodisperse macrocycles with the desired aqueous solubility and size/charge is far from trivial. What is clear is that the assembly of macrocycles under kinetic control, which requires high dilution\(^{60}\) or an effective template,\(^{61}\) is typically not as efficient as the examples of metallosupramolecular synthesis presented thus far, which, in many cases, give a single thermodynamic product in high yield.
1.8 The present study - synthesis and DNA-binding of metallocyclic architectures

If a macrocycle with the required ‘nanoshuttle’ features can be prepared, the crucial step remains the treatment of (either polymeric or oligomeric) DNA with such a species, and the characterisation of any resulting interactions. The two most common strategies for pseudorotaxane formation are ‘clipping’ and ‘threading’, these are are illustrated in Scheme 1.5.

![Scheme 1.5: Pathways for pseudorotaxane formation: ‘clipping’ (top) and ‘threading’ (bottom).](image)

In terms of the target DNA/macrocyle pseudorotaxane, the clipping method would entail the use of DNA as a template for macrocycle formation. Two (or more) molecular components are required to form the cyclic structure, which might involve the selection of the optimal macrocyclic host for the templating DNA guest out of a possible equilibrium mixture of species. Such selection or amplification has been referred to by Lehn as “Supramolecular Darwinism” and may be compared to the evolution of certain proteins into a toroidal motif. However, such a strategy would not be conducive to pseudorotaxane formation if the precursor components were to react with DNA. For example, metal centres with weakly bound ligands (e.g. labile complexes of Pd(II) or Pt(II)) may undergo substitution reactions with guanine and adenine nucleobases instead of forming metallocyclic complexes incorporating the bridging ligands.

The threading strategy, on the other hand, would involve the pre-formation of the cyclic species and subsequent combination of this with DNA. This approach was adopted in the present study, the aims of which included the (i) synthesis of large metallocyclic
architectures using the supramolecular approaches described thus far, and (ii) characterisation of the interactions between these species and DNA (Scheme 1.6).

Scheme 1.6: Interaction of a macrocycle with DNA might result in surface or topological binding.

The preparation of examples of the hitherto unreported class of DNA-macrocycle rotaxanes serves as motivation for the present study. This Thesis documents the chemistry towards these topologically interesting and unique bio-hybrid species.

1.9 References


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Chapter Two: Synthesis of N-heterocyclic ligand derivatives

2.1 Design

As was highlighted in the design aspects described in the previous Chapter, it was of interest to prepare metallocyclic products bearing substantial positive charge. One focus of the present study was to generate a library of cationic bridging ligand derivatives, from which it was hoped that the desired large, multinuclear metal complexes could be synthesised. The organocations prepared included a family of ditopic species, each of which incorporate two positively charged N-heterocycles, linked by a conformationally flexible spacer unit, as outlined in Scheme 2.1.

![Scheme 2.1](image)

Scheme 2.1: Diquaternary halide salts were prepared from bis(alkyl halide) (X–spacer–X) and N-heterocycle (Y) precursors and could be converted to other salts via anion exchange.

Importantly, these compounds represent a family of ligands which can be engineered in terms of their: (i) size – by varying the spacer, (ii) solubility – by varying the counteranion (Cl, Br, NO₃, ClO₄, PF₆ for H₂O-solubility and PF₆ for organo-solubility) and (iii) basicity – by varying the nature of the donor heterocycle. Four heterocyclic precursors were incorporated into dicationic species: 4,4'-bipyridine (4,4'-bipy), pyrazine (pyz), aminopyrazine (apyz) and 2-aminopyrimidine (apym).

2.2 Synthesis

The bridging ligands were conveniently prepared by using adaptations of a procedure described by Attalla and co-workers. Originally developed for the preparation of α,ω-bis(4,4'-bipyridinium)alkane bromide salts, this method can be generalised with respect to the N-heterocyclic species, as well as the alkyl halides employed. Briefly, the N-nucleophiles displace the halide ions from the substrate in an S₆2 manner, a process which is undoubtedly favoured by the use of polar aprotic solvents such as DMF and
MeCN.\textsuperscript{2} For the synthesis of dicationic species, the nucleophile is typically used in excess (~4 equiv.) in order to promote disubstitution (Scheme 2.2). Despite this, after overnight heating and subsequent cooling, the products which had precipitated from solution were usually impure. These could be readily crystallised from hot \( \text{H}_2\text{O} \) or \( \text{EtOH}/\text{H}_2\text{O} \) to give satisfactory (unoptimised) yields of the halide salts as pure, hygroscopic solids. In general, owing to the high aqueous solubility of the halide salts, only small volumes of solvent were required for the crystallisations.

The halide ions could be exchanged for \( \text{NO}_3^- \), \( \text{ClO}_4^- \) and \( \text{PF}_6^- \) in order to generate a suite of new ionic compounds. While, as mentioned above, anion exchange has implications in terms of ligand solubility, it was performed primarily to eliminate the halide ions, which might otherwise compete with the \( N \)-donor atoms for metal coordination sites. Typically, a halide salt was dissolved in \( \text{H}_2\text{O} \) and treated with an aqueous solution of a suitable reagent to furnish the new product. For example, reaction of an aqueous halide salt with \( \text{AgNO}_3 \) solution, followed by removal of \( \text{AgX} \) by filtration, afforded, upon evaporation of the resulting solution, the organic \( \text{NO}_3^- \) salt. The \( \text{ClO}_4^- \) and \( \text{PF}_6^- \) salts were prepared simply by treating an aqueous halide salt with saturated aqueous \( \text{LiClO}_4 \) or \( \text{KF}_6 \), respectively, upon which the desired organic \( \text{ClO}_4^- \) or \( \text{PF}_6^- \) salt precipitated and could be isolated by filtration.

\textit{Scheme 2.2: Alkylation of} \( N \)-heterocycles affords organic dications as their halide salts (\( X = \text{Cl}, \text{Br} \)).
The compounds which were prepared in this work are summarised in Table 2.1; new species were typically characterised using $^1$H and $^{13}$C$^1$H NMR spectroscopy, positive-ion electrospray-ionisation mass spectrometry (ESI-MS) and CHN microanalysis.

Table 2.1: The mono-, di- and triquaternary salts prepared in this work. Note: Bn = benzyl, tbn = thiabicyclo[3.3.1]nonane, mes(CH$_2$)$_3$ = 2,4,6-tris(methylene)mesitylene.

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<th>4,4'-bipy</th>
<th>pyz</th>
<th>apyz</th>
<th>apym</th>
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<tr>
<td>CH$_3$</td>
<td>I (14), NO$_3$ (15)</td>
<td>I (42), NO$_3$ (43), PF$_6$ (44)</td>
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Indeed, a range of organic spacer components could be incorporated into quaternary salts of the four heterocyclic precursors. The spacers shown in Figure 2.1 are ditopic, in that they are each able to bear two $N$-heterocycles. The thiabicyclo[3.3.1]nonane group is a semi-rigid bicyclic scaffold, with essentially a fixed angle between substituents (Y) bonded to the C2
and C6 atoms. The remaining linkers can be attached to N-heterocycles which are then conformationally flexible in terms of the angles between the heterocycles, owing to rotation at the methylene junctions.

![Diagram of Y(spacer)Y^2+](image)

**Figure 2.1:** The dications [Y(spacer)Y]^2+ prepared incorporated combinations of spacer units and heterocyclic species Y.

In addition, monotopic N-methylated and N-benzylated heterocyclic derivatives were also prepared as model compounds, representing “half” of a ditopic species. Lastly, the tritopic 1,3,5-tris(methylene)mesitylene core was also incorporated into new salts. Details, including chemical structures of all the compounds of the above type prepared, can be found in the Experimental Sections 6.3, 6.6 and 6.7.

### 2.3 4,4′-Bipyridinium and pyrazinium derivatives

![Diagram of 4,4′-bipy and pyz](image)

Several ditopic species of the form [4,4′-bipy(CH₂)ₙ4,4′-bipy]^2+ and [pyz(CH₂)ₙpyz]^2+ have previously been reported, with such cations often featuring in electrochemical and supramolecular systems. Their readily-engineered structure and electron-deficient nature allow them to be incorporated into non-covalent charge-transfer complexes with electron-rich partners. Indeed, they have often served as components in interlocked motifs, such as rotaxanes (e.g. 2). In addition, there have also been reports on the coordination chemistry of these heterocyclic compounds; this aspect will be addressed in the next Chapter.
The synthesis of the diquaternary 4,4′-bipyridinium salts was carried out according to a general procedure, whereby a suitable bis(alkyl halide) was combined with an excess of 4,4′-bipyridine (4 equiv.) in DMF solution. While this simple method could be employed to prepare a range of derivatives, typical $^1$H NMR spectroscopic analyses of the crude products indicated that substantial (~10 – 20%) amounts of impurities were present. While four doublets assigned to the 4,4′-bipyridinium groups could be observed, often two further doublets were found in the low field region. These doublets were assigned to the symmetric species $[\text{Br}($spacer$)4,4′$-bipy($\text{spacer}$)\text{Br}]^{2+}$ and $\text{cyclo-}[(4,4′$-bipy$)_2($spacer$)_2]^{4+}$, both of which could be removed by recrystallisation from H$_2$O to afford the bis(4,4′-bipyridinium) salts as pure solids.

One example of a diquaternary species was structurally characterised. Slow evaporation of an aqueous solution containing $[4,4′$-bipy$(\pm$)-tbn-2,6-diyl$)4,4′$-bipy$]\text{Cl}_2$ (30) afforded colourless single crystals suitable for an X-ray structure determination. The salt was found to crystallise as a racemic twin; the molecular structure of the enantiomer $[4,4′$-bipy(tbn-2$S,6S$-diyl$)4,4′$-bipy$]$ is given in Figure 2.2.

![Figure 2.2](image-url)

**Figure 2.2:** ORTEP of 30, with ellipsoids at the 50% probability level. H$_2$O solvate molecules and the Cl$^-$ anions are omitted for clarity.

As expected, the analysis confirmed the substitution at C2 and C6 to afford the chiral bis(4,4′-bipyridinium) dication. Importantly, the reaction was found to proceed with the retention of stereochemistry, due to the anchimeric assistance of the neighbouring nucleophilic S atom.* This results in the mean planes of the two 4,4′-bipy groups being approximately 120° apart. A structure for this dication (as its PF$_6^-$ salt, 32) has previously

* This occurs by intramolecular S$_2$N$_2$ displacement of Cl$^-$ by the thioether group to give a sulfenium intermediate, which is reactive towards the 4,4′-bipy nucleophile.
been reported by Stang and co-workers,\textsuperscript{7*} although 32 was found to crystallise in an achiral (Cmcm) space group owing to the disorder present in the dication.

As with the bis(4,4′-bipyridinium) derivatives mentioned above, bis(pyrazinium) species of type $[\text{pyz(CH}_2\text{)}_n\text{pyz}]^{2+}$ ($n = 4, 5, 6$) could also be prepared as their Br, NO\textsubscript{3} and PF\textsubscript{6} salts, which were characterised using the techniques mentioned above. In particular, $^{13}\text{C}\{	ext{H}\}$ NMR spectra of these species revealed one-bond coupling between adjacent $sp^2$-hybridised $^{14}\text{N}$ and $^{13}\text{C}$ atoms. This results in a 1 : 1 : 1 triplet $^{13}\text{C}$ resonance ($^{1}J_{\text{C,N}} \approx 8$ Hz), which was not observed in the NMR spectra of bis(4,4′-bipyridinium) salts. Owing to the poor donor ability of these ligands towards palladium(II) and platinum(II) (see Chapter Three), only a limited number of analogues were prepared during the course of this study.

### 2.4 3-Aminopyrazinium derivatives

Species of the formula $[\text{apyz(CH}_2\text{)}_n\text{apyz}]^{2+}$ have been reported previously, their syntheses being analogous to those discussed above.\textsuperscript{8} Such dications result from the alkylation of aminopyrazine at N4, which is less sterically hindered than N1.\textsuperscript{†} Comparatively little research has been conducted on these $\alpha,\omega$-bis(3-aminopyrazinium)alkane species, relative to $[4,4′-\text{bipy(CH}_2\text{)}_n4,4′-\text{bipy}]^{2+}$ and $[\text{pyz(CH}_2\text{)}_n\text{pyz}]^{2+}$ derivatives.

Several bis(3-aminopyrazinium) species and one tris derivative were prepared and characterised in this work. $^{1}\text{H}$ NMR spectroscopic analysis of the Br and NO\textsubscript{3} salts in D\textsubscript{2}O revealed that the amino protons readily exchange with the solvent, although these protons can be detected as broad singlets when the PF\textsubscript{6} salts are studied in CD\textsubscript{3}CN. As with the bis(pyrazinium) salts, these species exhibited $^{1}J_{\text{C,N}}$ coupling, with the presence of triplets in their $^{13}\text{C}\{	ext{H}\}$ NMR spectra.\textsuperscript{8}

\begin{footnotesize}
\begin{itemize}
  \item This ligand could be incorporated into large hexagonal dodecaplatinum(II) complexes similar to 12.\textsuperscript{7}
  \item The amino group is less nucleophilic owing to its lone pair being in conjugation with the aromatic $\pi$-system.
\end{itemize}
\end{footnotesize}
The organic salts prepared in this work, in particular the amino derivatives, allowed for the detection of several ions using positive-ion ESI-MS. For example, when the triquaternary species \([\text{mes(CH}_2\text{apyz)}_3](\text{PF}_6)_3\) (55) was subjected to these conditions, a progression of gas phase ions, resulting from the loss of \(\text{PF}_6^-\) and \(\text{H}^+\), was detected (Figure 2.3). The acidity of the amidine groups conjugated to the quaternary centres undoubtedly allows for this progression of resonance-stabilised ions to be observed.

Interestingly, ions corresponding to the loss of apyz groups were also observed, suggesting that fragmentation was occurring. Indeed, MS/MS analysis was performed in this example, confirming the decomposition of \([55 – \text{PF}_6]^+\) to the daughter ions \([55 – \text{apyz} – 2\text{PF}_6^- – \text{H}^+]^+\) and \([55 – \text{apyz} – 3\text{PF}_6^- – 2\text{H}^+]^+\). The fragmentation and resulting loss of heterocycles was also observed when apym derivatives were studied by ESI-MS, although no MS/MS analyses were conducted for these species.

One aminopyrazinium salt, the simple methylated species \([\text{Meapyz}]\text{NO}_3\) (43), was characterised by an X-ray structure determination. Slow evaporation of an aqueous solution containing \([\text{Meapyz}]\text{NO}_3\) afforded colourless single crystals, crystallographic analysis of which resulted in the molecular structure given in Figure 2.4.
Chapter Two: Synthesis $N$-heterocyclic ligand derivatives

Figure 2.4: ORTEP of 44, with ellipsoids at the 50% probability level (left) and a diagram depicting the solid state packing (right).

The structure of [Meapyz]NO$_3$ is largely unremarkable with, as expected, the amino N atom having a trigonal planar geometry ($sp^2$-hybridisation) owing to its conjugation with the aromatic $\pi$-system. Interestingly however, the distances between the aromatic ring and NO$_3^-$ anion (r$_{\text{centroid-O(1)}}$ = 3.09 Å, r$_{\text{N(2)-N(4)}}$ = 3.07 Å) suggest the presence of anion-$\pi$ interactions in the solid state. Interactions of this type have attracted recent attention due to their variable nature and their use in the binding of anions. The NO$_3^-$ anions also participate in H-bonding interactions with the amino groups, which serve to link the [Meapyz]$^+$ species into a 1-D helical polymer along the crystallographic $c$-axis.

2.5 2-Aminopyrimidinium derivatives

2.5.1 General

In contrast to the other three heterocyclic precursors used in the present study, of which there are several reported ditopic (cationic) derivatives, 2-aminopyrimidine has not, to the best of the author’s knowledge, been incorporated into such compounds. Diquaternary halide salts could be readily prepared by heating DMF solutions containing 2-aminopyrimidine and suitable bis(alkyl halide) derivatives, affording the new compounds as pure solids without the need for recrystallisation. Although the amino group of 2-aminopyrimidine sterically hinders access to the more nucleophilic pyrimidine N atoms, this electron-donating substituent forms part of a guanidine moiety which was found to be highly reactive towards the alkyl halides.
employed. Indeed, the yields observed for these reactions were typically higher than those obtained when the other $N$-heterocyclic nucleophiles were used.

As was the case for the apyz derivatives, one monotopic apym derivative $[\text{Bnapym}]\text{PF}_6$ (61) was characterised in the solid state. Colourless single crystals of this compound formed upon slow evaporation of a saturated aqueous $\text{KPF}_6$ solution containing the $\text{Br}^-$ salt. The X-ray structure of this representative 2-aminopyrimidinium derivative is given in Figure 2.5.

As expected, the benzyl group is bound directly to the pyrimidine heterocycle at N(1) rather than through the amino N(2) atom. The latter has a trigonal planar geometry, which was also the case in $[\text{Meapyz}]\text{NO}_3$. Inspection of the crystal packing in 61 (Figure 2.6) reveals the presence of $[\text{Bnapym}]^+$ dimers, each of which is held together by two H-bonds. These dimers are linked by H-bonded $\text{PF}_6^-$ anions to form 1-D chains which propagate along the crystallographic $b$-axis.  

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§ It is possible that these extensive H-bonding interactions are responsible for the lower solubility of some 2-aminopyrimidinium derivatives relative to the other quaternary salts.
In some cases, including when species incorporating aryl (e.g. xylylene) spacer groups were prepared, 2-D NMR techniques, including $^1$H-$^1$H correlation spectroscopy (COSY), were used to aid in the assignment of 1-D NMR spectra. COSY allows for the identification of spin systems of coupled nuclei, which are observed as cross-peaks in the 2-D spectra. This was particularly useful when information regarding $^1$H-$^1$H coupling could not be extracted due to poor spectral resolution. An example, in the case of [apym($m$-xylylene)apym]Br$_2$ (78), is provided in Figure 2.7.

![Figure 2.7: Partial $^1$H NMR spectrum (left) and $^1$H-$^1$H COSY spectrum (right) of [apym($m$-xylylene)apym]$^{2+}$ recorded in D$_2$O at 300 MHz (residual DMF solvate present).](image)

Crosspeaks (circled) between the pyrimidyl protons H4, H5 and H6 allow for these to be correlated and correctly distinguished from the xylylene aryl protons H2', H4' and H5'.

### 2.5.2 Dimroth rearrangement of 2-aminopyrimidinium species

Some organic cations derived from 2-aminopyrimidine exhibited interesting reactivity in aqueous solution. Crude (~80%) 1,2,4,5-tetrakis[1-(2-aminopyrimidinium)methyl]benzene bromide (88) was prepared from 1,2,4,5-tetrakis(bromomethyl)benzene and 2-aminopyrimidine. Recrystallisation of the white solid was effected from warm H$_2$O. The product which was isolated, rather than being the pure target material, was found to be a product of lower symmetry, owing to the greater number of resonances in its $^1$H NMR spectrum. This is most likely a result of two of the 2-aminopyrimidinium groups undergoing Dimroth rearrangement$^{10}$ (Scheme 2.3) to give a mixture of products, including 89.

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$^{10}$ The amino protons of such cations exchange with D$_2$O. Also, the 2-aminopyrimidinium derivatives often contained DMF solvate, in some cases even after crystallisation from H$_2$O.
A similar reaction occurred when [apym(o-xylylene)apym]Br₂ (75) was treated with warm aqueous AgNO₃ with the intention of generating the NO₃⁻ salt. Removal of AgBr by filtration and concentration of the resulting solution afforded colourless single crystals. ¹H NMR spectroscopic analysis of these suggested the product was of a lower symmetry than would be expected for [apym(o-xylylene)apym]²⁺. A suitable crystal was analysed by X-ray diffraction and the molecular structure of the product (90) confirmed (Figure 2.8).

Figure 2.8: ORTEP of 90, with ellipsoids at the 50% probability level. Three H₂O solvate molecules and the NO₃⁻ anion are omitted for clarity.
Indeed, it was found that one of the two apym ‘arms’ had undergone rearrangement to furnish a monocation in which the pyrimidine rings were non-equivalent. Thus, the ortho-xylylene spacer is now attached to the rearranged arm at N(4), which, by virtue of the Dimroth reaction, is a secondary amine. The result can be viewed as being equivalent to ‘half’ of the tetrasubstituted case 89. Further details and a description of the crystal packing can be found in the Appendices (Section A.3).

Surprisingly, the presence of AgNO$_3$ was found to be necessary for the o-xylylene derivative to rearrange, as the Br$^-$ salt does not undergo rearrangement in boiling H$_2$O. This can perhaps be attributed to the presence of trace Ag$_2$O acting as a base and catalysing the reaction. However, the meta- and para-xylylene, as well as 1,3,5-tris(methylene)mesitylene-linked analogues, when subjected to the same conditions, did not undergo rearrangement.$^{††}$ Indeed, this reaction occurs in order to relieve steric strain between the 2-aminopyrimidinium group and a large ortho substituent, and thus does not proceed in neutral H$_2$O for other substitution patterns. The rearrangement of the ortho derivative could not be reproduced and further synthetic attempts resulted only in the formation of [apym(o-xylylene)apym](NO$_3$)$_2$ (76), the intended target of the original procedure.

The Dimroth rearrangement has been known to proceed via a simple hydrolysis and imine condensation route, depicted in Scheme 2.3 above. In the case of 1,2-dihydro-2-imino-1-alkylypyrimidines (which are tautomers of 1-alkyl-2-aminopyrimidines), basic conditions are required,$^{10}$ although no added base was used in the cases above. It is likely that this reaction is promoted by the presence of either acid or base to activate the neutral 1,2-dihydro-2-imino-1-alkylypyrimidine species toward rearrangement. Consequently, the salts prepared in the present study, which are conjugate acids of such species, may spontaneously rearrange when heated in aqueous solution.

### 2.5.3 Rearrangement under basic conditions

Having fortuitously induced a rearrangement in some 2-aminopyrimidinium compounds, it was of interest to effect the conversion in a more efficient and reproducible manner, and also induce rearrangement of all the 2-aminopyrimidinium groups, rather than just one of two ortho substituents. Selected 2-aminopyrimidinium derivatives were subjected to heating under typical Dimroth conditions (1 M NaOH, 60°C) and were found to afford secondary

$^{††}$ [Bnapym]Br also failed to undergo rearrangement in neutral H$_2$O solution.
amine products cleanly. In each case, the precursor salt was suspended in basic solution upon which it immediately became yellow. After heating, the product could be isolated by centrifugation. As expected, the rate of reaction was found to depend on the steric bulk surrounding the 2-aminopyrimidinium centres. Notably, the ortho species [apym(o-xylylene)apym]Br₂ (75) underwent complete rearrangement to 91 within 2 h,‡‡ while in the case of the para analogue [apym(p-xylylene)apym]Br₂ (81) the formation of 92 was incomplete after 2 h (indeed, 24 h was required). A series of secondary amine products 91 – 96 was prepared under the Dimroth conditions.

In contrast to their charged precursors, these solid compounds are largely insoluble in many polar solvents such MeOH and H₂O. While it is likely that these species have the propensity to form metal complexes, these compounds were not investigated further.

### 2.6 Larger ligand derivatives

The simple procedure employed in the synthesis of diquaternary salts can be repeated to generate much larger and more positively charged bidentate ligand species. This is particularly the case for 4,4'-bipyridinium derivatives, for which it is expected that the nucleophilicity of the free N atoms, owing to their remoteness from the quaternised centres,

‡‡ Gondi and co-workers attempted to synthesise this compound directly from 2-aminopyrimidine and 1,2-bis(bromomethyl)benzene using NaH as the base. However, their conditions were found to result in the formation of 2-pyrimidin-2-yl-2,3-dihydro-1H-isoiindole, rather than the intended product. In this context, 1,2-bis(aminomethyl)benzene and 2-chloropyrimidine are probably more logical precursors.
is quite substantial. For example, the diquaternary salt 1,1′-bis(4-bromobutyl)-4,4′-bipyridinium bromide ([Br(CH_2)_44,4′-bipy(CH_2)_4Br]Br_2, 97) could be prepared from 4,4′-bipyridine and 1,4-dibromobutane\(^{11}\) (Scheme 2.4; pentylene and hexylene species were also prepared in this work).

Another iteration of the alkylation procedure, namely the reaction of this species with excess 4,4′-bipyridine, afforded the potential bridging ligand [4,4′-bipy(CH_2)_44,4′-bipy(CH_2)_44,4′-bipy]Br_4 (100), from which the Br\(^-\) ions could be exchanged to furnish the NO_3\(^-\) (102) and PF_6\(^-\) salts (103).

The hexylene analogues (n = 6; 101: Br\(^-\) salt; 104: NO_3\(^-\) salt; 105: PF_6\(^-\) salt) could be prepared in much the same way\(^{12}\) and, in each case, among other analyses, \(^1\)H NMR spectroscopy confirmed the structures of the tetraquaternary products. Most diagnostic was the presence of six low-field doublet resonances in the range \(\delta 9.2 – 7.9\) ppm, each resulting from six unique pyridine/pyridinium \(^1\)H environments. When ESI-MS analysis of the salts was conducted, several cations could be identified in the gas phase. For example, in the case of the tetraquaternary salt [4,4′-bipy(CH_2)_64,4′-bipy(CH_2)_64,4′-bipy](NO_3)_4 (103), the ions [2M – NO_3\(^-\)]\(^+\), [5M – 3NO_3\(^-\)]\(^3+\), [3M – 2NO_3\(^-\)]\(^2+\), [4M – 3NO_3\(^-\)]\(^3+\), [M – NO_3\(^-\)]\(^+\) and [M – 2NO_3\(^-\)]\(^2+\) could all be observed under the ionisation conditions employed. Further details of the six compounds 100 – 105 of type [4,4′-bipy(CH_2)_n4,4′-bipy(CH_2)_n4,4′-bipy]X_4 (n = 4, 6; X = Br, NO_3, PF_6) prepared during the course of this study can be found in Section 6.6.

\(^{12}\) Attempts to reproduce the synthesis of the related (larger) dodecylene bromide salt were unsuccessful.
The simple quaternisation reactions have been employed by Summers and co-workers to generate extremely large structures, such as the decaquaternary salt \( [4,4'\text{-bipy(}\text{CH}_2\text{)}_44,4'\text{-bipy(}\text{CH}_2\text{)}_44,4'\text{-bipy(}\text{CH}_2\text{)}_44,4'\text{-bipy(}\text{CH}_2\text{)}_44,4'\text{-bipy(}\text{ClO}_4\text{)}_{10} ] \) \( (106) \). This compound represents a potentially useful bidentate ligand derivative, and if longer oligo(methylene) spacers could be incorporated into the motif then even larger ligands could be accessed.

The author’s attempts to prepare these salts found little success even when oligo(methylene) spacers of varying lengths were employed. Typically, an intractable mixture of products was generated and a clean separation could not be achieved by repeated recrystallisation. It was noted that the \( \text{ClO}_4^- \) salts which resulted from the syntheses have poor aqueous solubility at room temperature, although anion exchange could possibly afford more soluble products if the initial syntheses could be achieved. Presumably, this lack of solubility limits the number of quaternary centres that can be incorporated into such species.

2.7 Concluding remarks

The approach adopted in the present study has resulted in the preparation of several new flexible, cationic \( N \)-heterocyclic derivatives. Four examples were characterised by X-ray structure determinations. In the case of the amino derivatives, the analyses revealed H-bonding and anion-\( \pi \) interactions in the solid state and, not surprisingly, several compounds closely related to these have been shown to be potent anion-binding agents.\(^{13}\)

2-Aminopyrimidinium salts were investigated in terms of their potential to undergo Dimroth rearrangement. It was found that this can occur in aryl-linked species with two ortho 1-(2-aminopyrimidinium)methyl groups, even in neutral aqueous solution. The reaction was generalised to a wide range of substrates, which, when subjected to basic solution, were all found to undergo complete rearrangement.

Given the family of multidentate \( N \)-donor ligands synthesised, it was envisaged that the interaction of these species with selected metal ions should give rise to a range of multinuclear complexes with varying sizes and solubility properties. Indeed, conformationally flexible heterocyclic bridging ligands have been identified as having the
potential to afford new classes of metal complexes not available from rigid polyaromatic ligands.\textsuperscript{14} The coordination chemistry of the new ligands described above will be discussed in the following Chapter.

### 2.8 References

Chapter Three: Synthesis of palladium(II) and platinum(II) complexes

3.1 Background

In terms of the present study, it was of interest to integrate cationic N-donor bridging ligands into highly-charged multinuclear metal complexes. Ligands possessing conformational flexibility are more likely to afford architectures whose structures are concentration independent, when compared to their rigid counterparts. Despite this, the use of the latter is more widespread in supramolecular chemistry, although employing such ligands can often lead to complicated equilibria in which the free energies of several species (often of the same empirical formula but of varying nuclearity, e.g. square/triangle systems) are similar, owing to the entropic and enthalpic changes having comparable magnitudes.¹

It was anticipated that flexible bridging ligands might reliably form multinuclear complexes, and that these might be of sufficient size to encircle duplex DNA. With respect to metal complexation in aqueous solution, the most relevant of the organic ligands discussed in the previous Chapter are the (H₂O-soluble) diquaternary NO₃⁻ salts. This is because, as mentioned earlier, the poor donor ability of NO₃⁻ ions causes them to be unlikely to compete with the N-donor ligands for metal complexation. In parallel, the coordination chemistry of selected PF₆⁻ salts was also studied in this work, although these compounds are typically only soluble in organic media such as MeCN and Me₂CO.

The metal ions chosen for complexation to the heterocyclic cations, palladium(II) and platinum(II) (d⁸), were selected for their propensity to form stable, well-defined square-planar complexes with N-donor ligands.² This thermodynamic stability was considered necessary to counteract the Coulombic repulsion between the metal ions and cationic bridging ligands. The precursors used typically have two coordination sites occupied by strongly-bound ligand donor atoms, with the remaining sites featuring two more weakly interacting ligands. The latter were to be substituted by the N-donor ligands being studied. Often, the weakly-bound ligands take the form of O-donors such as NO₃⁻ or OTf⁻ (trifluoromethanesulfonate, triflate) anions, which are displaced by pyridine ligands in a highly exothermic reaction, with only a small associated entropic penalty (Scheme 3.1). The associated binding constants, which are in the order of 10⁶ M⁻¹, imply that the complexation is essentially quantitative at typical NMR concentrations (>10 mM).³
Scheme 3.1: Successive binding constants for pyridine ligands and \textit{cis}-ditriflato complexes in CHCl$_3$ solution.$^3$

Just as both aqueous- and organo-soluble ligands were prepared in this work, a variety of labile metal complex precursors were also employed, which exhibited solubility in either of these media (Figure 3.1).

Two complexes depicted in Figure 3.1 bear benzonitrile ligands, which are weakly-bound owing to the poor donor ability of the \textit{sp}$^2$-hybridised N atoms. It was expected that this would cause them to be easily displaced by the \textit{sp}$^2$-hybridised N atoms of the bridging diquaternary \textit{N}-heterocyclic ligands.

Experiments were carried out by using a number of metal precursor/bridging ligand combinations. In each case, characterisation of the product(s) was attempted by using NMR spectroscopy. In particular, for platinum(II) complexes, use was also made of $^{195}$Pt NMR, a technique which can provide valuable information regarding the nature of the coordination

Figure 3.1: The metal complex precursors used in the present study. Labile ligands are highlighted in red (phen = 1,10-phenanthroline; dppp = 1,3-bis(diphenylphosphino)propane).
environment of $^{195}$Pt nuclei (33.8% abundant, $I = \frac{1}{2}$). $^{195}$Pt resonances are found over a wide chemical shift range (15,000 ppm) and consequently each unique $^{195}$Pt environment should give rise to a unique signal.\textsuperscript{4} All reaction products were also subjected to positive-ion ESI-MS and, in some cases, characterised by CHN microanalysis and UV-vis spectroscopy.

### 3.2 Complexes of bis(4,4'-bipyridinium) and bis(pyrazinium) derivatives

In order to assess whether cyclic species might form from bridging ligands of the type [4,4'-bipy(CH$_2$)$_n$4,4'-bipy](NO$_3$)$_2$, the coordination of these to [M(2,2'-bipy)(NO$_3$)$_2$] (M = Pd, Pt) was investigated (Scheme 3.2). These complexes typically exist as the diaqua species [M(2,2'-bipy)(OH$_2$)$_2$]$^{2+}$ in H$_2$O solution\textsuperscript{5} and are generally very reactive towards N-donor ligands.

![Scheme 3.2: The formation of dinuclear [2 + 2] complex species incorporating two [M(2,2'-bipy)]$^{2+}$ fragments and two [4,4'-bipy(CH$_2$)$_n$4,4'-bipy]$^{2+}$ bridging ligands.](image)

Typically, addition of D$_2$O to an equimolar mixture of the alkyl-bridged 4,4'-bipyridinium NO$_3$ salt [4,4'-bipy(CH$_2$)$_n$4,4'-bipy](NO$_3$)$_2$ ($n = 4, 6$) and [M(2,2'-bipy)(NO$_3$)$_2$] was followed by heating at 80°C, which caused dissolution (and reaction) of the dinitrato complex. The reaction mixture was monitored by $^1$H NMR spectroscopy and the spectrum exhibited a single set of resonances after 24 h or 48 h heating (for palladium(II) and platinum(II), respectively). This is consistent with the formation of a single product.

\textsuperscript{*} For example, resonances for platinum(II) ions existing in a N$_4$ coordination environment typically appear between $\delta$ --2200 and --2800 ppm, relative to Na$_2$[PtCl$_4$].
possessing high symmetry. The complexes could be isolated as PF₆⁻ salts; data for [4,4'-bipy(CH₂)₄4,4'-bipy]²⁺ and its (2,2'-bipyridyl)palladium(II) complex are given in Figure 3.2.

The protons α- to the non-quaternary N atoms (H₂' in the free ligand, H₂ in the complex) were found to resonate at lower field upon reaction with palladium(II) (Δδ = 0.48 ppm). This is diagnostic of ligation to the electrophilic metal centres and is consistent with, but not necessarily indicative of, dinuclear complex formation. Indeed, while NMR analysis can often confirm the symmetry of metallosupramolecular species, it provides no evidence regarding nuclearity.⁴ This is often gleaned from ESI-MS data, although ions corresponding to intact dinuclear complexes were not observed when the NO₃⁻ salts were subjected to such analysis, with only smaller cationic fragments being detected.⁴ Samples of each compound were converted to PF₆⁻ salts with saturated aqueous KPF₆ and it was anticipated that MS analyses of these salts would be more successful. Typically, PF₆⁻ ions associate with cationic species more weakly than NO₃⁻ ions, allowing for better characterisation. Additionally, PF₆⁻ salts are soluble in Me₂CO or MeCN solvents, which, owing to their greater volatility, can be more amenable to MS analysis than H₂O or H₂O/MeOH. Despite this, successful analysis could only be achieved in the case of [Pt₂(2,2'-bipy)₂{4,4'-bipy(CH₂)₄4,4'-bipy}₂](PF₆)₈ (the PF₆⁻ salt of 110), for which the intact complex ions [110 – 2PF₆⁻]²⁺ (m/z 1182.153886, calcd 1182.156840) and [110 – 3PF₆⁻]³⁺ (m/z 739.781613, calcd 739.782983) were observed in the ESI-FT-ICR (electrospray ionisation Fourier transform ion cyclotron resonance) mass spectrum. This high-resolution

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⁴ For example, in the case of the NO₃⁻ salt of 110, ESI-MS: m/z 930.70 [Pt(2,2'-bipy){4,4'-bipy(CH₂)₄4,4'-bipy}][NO₃]⁺, 670.47 [Pt₂(2,2'-bipy)₂{4,4'-bipy(CH₂)₄4,4'-bipy}][NO₃]²⁺.
technique can often be used for the unambiguous assignment of multinuclear species and was used in the present study to confirm low-resolution ESI-MS data of metal complexes, when intact species could be observed using this method. It was considered likely that, by analogy, complexes of formula $[M_2(2,2\text{′}-\text{bipy})_2(4,4\text{′}-\text{bipy(CH}_2)_34,4\text{′}-\text{bipy})_2](\text{PF}_6)_8$ (n = 4, 6; M = Pd, Pt), each incorporating two metal ions and two bridging ligands, are the products in the remaining three systems. Further details of 107 – 110, which were isolated and characterised as their PF$_6^-$ salts, can be found in the Experimental section. When the H$_2$O-soluble NO$_3^-$ salts were required (e.g. for DNA-binding; see Chapter Four), aqueous solutions of the dinuclear complexes, instead of being treated with KPF$_6$, were evaporated to dryness to give the desired compounds as glassy yellow solids.

As was mentioned above, the coordination chemistry of the NO$_3^-$ salts was investigated in aqueous solution. In parallel, the organo-soluble PF$_6^-$ salts of the organocations were also combined with complex precursors to see whether related dinuclear species could form. Equimolar mixtures of the bridging ligand and a suitable complex precursor (in this case either [Pt(dppp)(OTf)$_2$], cis-[Pt(PhCN)$_2$Cl$_2$] or [Pd(PhCN)$_2$Cl$_2$]) were heated in CD$_3$CN. The Pd(II)/Pt(II) precursors employed were chosen based on their organo-solubility and it was expected than the phosphine and chlorido ligands might labilise the weakly-bound ligands trans to these (triflato and benzonitrile, respectively), perhaps resulting in rapid formation of the thermodynamic product(s) in each case. The syntheses of three examples of dinuclear metalloccycles $[\text{Pt}_2(\text{dppp})_2(4,4\text{′}-\text{bipy(o-xyllylene)4,4\text{′}-bipy})_2](\text{PF}_6)_4(\text{OTf})_4$ (111), cis-$[\text{Pt}_2\text{Cl}_4(4,4\text{′}-\text{bipy(o-xyllylene)4,4\text{′}-bipy})_2](\text{PF}_6)_4$ (112) and cis-$[\text{Pd}_2\text{Cl}_4(4,4\text{′}-\text{bipy(CH}_2)_34,4\text{′}-\text{bipy})_2](\text{PF}_6)_4$ (113) were attempted from [Pt(dppp)(OTf)$_2$], cis-[Pt(PhCN)$_2$Cl$_2$] and [Pd(PhCN)$_2$Cl$_2$], respectively. While 111 and 113 could be successfully prepared and characterised using $^1$H NMR spectroscopy, ESI-MS and CHN microanalysis, the $^1$H NMR data for 112 suggest that it is contaminated with a side product (~30%) that remains present even after extended heating of a CH$_3$CN solution containing cis-[Pt(PhCN)$_2$Cl$_2$] and [4,4′-bipy(o-xyllylene)4,4′-bipy](PF$_6$)$_2$. The nature of this side product could not be determined, although CHN analytical results for the crude solid isolated suggest that it is likely to have the same empirical formula as 112.
During the course of this work, Quintela and co-workers reported the preparation of the closely-related dinuclear metallocyclic complexes 114 and 115.\textsuperscript{7} It was found that these species could be integrated into catenane topologies, owing to the ability of the metallocycle to bind electron-rich organic macrocycles such as dibenzo-24-crown-8 (DB24C8).
In contrast to the bis(4,4′-bipyridinium) salts, the bis(pyrazinium) analogues were found to be unreactive towards the palladium(II) or platinum(II) complex precursors employed in this work. In each case, the combination of bis(pyrazinium) species and metal complex resulted in only the starting materials being observed by \(^1\)H NMR spectroscopy and ESI-MS. It appears that the [pyz(spacer)pyz]\(^{2+}\) species are substantially deactivated relative to their analogues incorporating 4,4′-bipy, in which the quaternised N atoms are further removed from the Lewis basic N atoms. It is possible that the pyrazinium derivatives are only useful for coordination to a metal ion when the resulting complexes exhibit significant \(\pi\)-backbonding into unfilled ligand-centred \(\pi^*\) orbitals (e.g. pentammineruthenium(II) derivative 116)\(^9\) and/or where electrostatic interactions might play a key role (e.g. dinuclear pentacyanoferrate(II) complex 117).\(^9\)
3.3 Complexes of 3-aminopyrazinium derivatives

It was envisaged that bis(3-aminopyrazinium) derivatives might be incorporated into dinuclear platinum(II) complex species in much the same way as the bis(4,4′-bipyridinium) ligands described above. While the electron-donating amino substituents positioned ortho to the free heterocyclic N atoms undoubtedly increase the basicity of these atoms, it was originally believed that the amino groups would not take part in metal complexation (see the discussion of [Pt(2,2′-bipy)(2-aminopyridine)$_2$](NO$_3$)$_2$ below). Thus, when equimolar amounts of [apyz(spacer)apyz](NO$_3$)$_2$ and [Pt(2,2′-bipy)(NO$_3$)$_2$] were combined in aqueous solution, dinuclear complexes of the type [Pt$_2$(2,2′-bipy)$_2$[apyz(spacer)apyz]$_2$]$^{8+}$ (118) were expected to form (Scheme 3.3).

Scheme 3.3: The bis(3-aminopyrazinium) salt 49 did not lead to the formation of a dinuclear [2 + 2] metallocycle (top) but rather a tetranuclear complex exhibiting Pt(II)···Pt(II) interactions.

Heating a D$_2$O suspension containing equimolar [apyz(CH$_2$)$_6$apyz](NO$_3$)$_2$ and [Pt(2,2′-bipy)(NO$_3$)$_2$] effected dissolution of the starting materials and resulted in a clear orange solution, rather than the pale-yellow one expected. The solution became red upon further heating, with $^1$H NMR analysis suggesting a complex mixture of species, including free [apyz(CH$_2$)$_6$apyz]$^{2+}$, was present. ESI-FT-ICR-MS analysis of a solution treated with saturated aqueous KPF$_6$ was consistent with the presence of a tetranuclear species [Pt$_4$(2,2′-bipy)$_4$[apyzC$_6$H$_{12}$apyz$\cdot$2H]$_2$](PF$_6$)$_8$ incorporating two quadruply-bridging zwitterionic ligands ($m/z$ 891.768992, calcd 891.768257 [M – 3PF$_6$]$^{3+}$). The deprotonation of the bis(3-aminopyrazinium) species allows them to bind four platinum(II) ions, each of
which are equivalent according to $^{195}$Pt NMR spectroscopic data ($\delta$ = -2361 ppm is consistent with an $N_4$ coordination sphere). The binding of each (2,2'-bipyridine)platinum(II) centre to a pyrazine N atom as well as an amido N atom is necessary in order to satisfy the identical coordination spheres of the platinum(II) ions.

Further evidence for the tetradentate nature of the flexible ligands could be obtained from the UV-vis spectrum of this compound, which features an absorption at 428 nm, assigned to metal-metal-to-ligand charge transfer (MMLCT).\(^\text{10}\) The Pt(II)···Pt(II) and $\pi$-$\pi$ interactions present in the [Pt$_2$(2,2'-bipy)$_2$]$^{4+}$ moieties give rise to this absorption (resulting in the deep red colour of this complex) and stabilise the octacationic species.\(^\text{‡}\) Each of the [Pt$_2$(2,2'-bipy)$_2$]$^{4+}$ groups can be referred to as “head-to-tail” dimers because each platinum(II) ion is bound to nonequivalent atoms in the bridging ligands. As the formula of the tetrannuclear complex suggests, it can be prepared using a 2 : 1 (Pt : bridging ligand) molar ratio of the precursors, in which case only one set of hexylene signals is observed for the reaction mixture (i.e. no free ligand is present). A possible structure for the product is pictured above (119) but given the complexity of the $^1$H NMR spectrum (the low field region of which could not be assigned) this could not be confirmed as the identity of the complex.\(^\text{§}\)

Metal-metal interactions of the above type are not uncommon for $d^8$ ions,\(^\text{11}\) and certain arrangements of metal centres, including those involving the stacking of such metallodimers in infinite 1-D chains, can give rise to anisotropic materials with unique optical properties.\(^\text{12}\) Such interactions have been analysed in terms of molecular orbitals and the overlap of filled $d_{z^2}$ orbitals is known to be involved in the interactions.\(^\text{13}\)

The formation of species incorporating [Pt$_2$(2,2'-bipy)$_2$]$^{4+}$ fragments is well-documented, and several reports exist of these bridged structures. For example, Sakai and co-workers found that [Pt(2,2'-bipy)(2-aminopyridine)$_2$][NO$_3$]$_2$ (120) could be prepared from [Pt(2,2'-bipy)(NO$_3$)$_2$] and 2-aminopyridine (Scheme 3.4).\(^\text{14}\) However, this species was shown to decompose in supercritical H$_2$O to afford the Pt(II)···Pt(II) bonded species [Pt$_2$(2,2'-bipy)$_2$(2-aminopyridinato)$_2$][NO$_3$]$_2$ (121) and free 2-aminopyridinium.\(^\text{10}\)

\(^\text{‡}\) No reaction products could be successfully identified when bis(3-aminopyrazinium) salts were treated with [Pd(2,2'-bipy)(NO$_3$)$_2$], perhaps suggesting that strong metal-metal interactions are required to stabilise the reaction products. Similar results were obtained when bis(2-aminopyrimidinium) salts were used (vide infra).

\(^\text{§}\) Another possible structure satisfying the data is a double helix, which differs to that given (119) by the inversion of the chirality at one metal centre (resulting in a ‘twist’ in the bridging ligands).
species, owing to the “head-to-tail” arrangement of ligands, is chiral, and it is expected that a racemic mixture would form under the conditions employed.

![Scheme 3.4: The formation of a head-to-tail diplatinum(II) complex from a simple mononuclear derivative in supercritical H₂O.](image)

In terms of the present study, the increased acidity of the aminopyrazinium groups, relative to 2-aminopyridine, is the likely reason why supercritical conditions are not required when these ligands are employed. As a consequence, the facile formation of 119 from the possible intermediate 118 hinders the spectroscopic detection of the latter species. ** This is in contrast to the example reported by Sakai, in which the intermediate could be isolated.

In order to compare the aqueous reactivity of aminopyrazinium ligands to that of 2-aminopyridine, the chemistry of a simple ligand incorporating one (instead of two) heterocycles was studied. Heating an equimolar suspension of [Pt(2,2'-bipy)(NO₃)₂] and [Meapyz](NO₃) (43) in D₂O gave rise to a red solution in which the dinuclear complex [Pt₂(2,2'-bipy)₂(Meapyz–H)₂](NO₃)₄ was the major product (Scheme 3.5).

![Scheme 3.5: Formation of a diplatinum(II) complex with the bridging amidinato-like Meapyz-H ligand.](image)

Although parent ions of 122 (or its PF₆⁻ salt) could not be observed using ESI-MS, NMR†† and UV-vis spectroscopic as well as CHN microanalytical data are consistent with the

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** Heating of the mixture is necessary in order to dissolve [Pt(2,2'-bipy)(NO₃)₂]. This heating would also cause any 118 which might form to convert rapidly to 118.

†† A very complex ¹H NMR spectrum, similar to that of 119, was obtained. The structure presented for the product (122) represents one of the enantiomers that may form.
expected structure. Of particular interest are the $\lambda_{\text{MMLCT}}$ (Figure 3.3) and $\delta^{(195}\text{Pt})$ values for the di- and tetranuclear complexes, which are almost identical.

![Figure 3.3: UV-vis spectra for the platinum(II) complexes 119 and 122. In addition to ligand absorptions at ~300 nm, MMLCT bands (which, as expected, have $\varepsilon_{\text{tetranuclear}} > \varepsilon_{\text{dinuclear}}$) were observed at ~440 nm.](image)

As with the other ligand types, the coordination chemistry of organo-soluble derivatives was also explored, in order to compare their behaviour to that in aqueous solution. However, it was found that the PF$_6^-$ salts of 3-aminopyrazinium ligands were not reactive towards the palladium(II) and platinum(II) precursor complexes used in the present study. It was thought that the use of a suitable base was necessary in order to induce deprotonation of the amino groups and allow complexation of such species. Despite this, the addition of NEt$_3$ to mixtures containing the 3-aminopyrazinium salts and metal complex precursors failed to promote complex formation.

### 3.4 Complexes of 2-aminopyrimidinium derivatives

Given that “head-to-tail” platinum(II) complexes of guanadinato derivatives have been reported,$^{12}$ it was of interest to prepare complexes of bis(2-aminopyrimidinium) nitrate salts. The 2-aminopyrimidinium fragments, each of which incorporates a guanadinium moiety, were expected to behave in much the same way as the related 3-aminopyrazinium species discussed above.
Heating a D$_2$O suspension of [apym(CH$_2$)$_n$apym](NO$_3$)$_2$ (64) and [Pt(2,2'-bipy)(NO$_3$)$_2$] (2 equiv.) afforded a clear red solution, suggesting that [Pt(2,2'-bipy)(NO$_3$)$_2$] had reacted to possibly form a Pt(II)--Pt(II) bonded species (Scheme 3.6). Despite this, NMR and ESI-MS data did not conclusively support the formation of a tetránuclear species such as 123. Extended heating of the mixtures resulted in the precipitation of an intractable dark-red material.

When the above procedure was repeated using the related salt [apym(CH$_2$)$_n$apym](NO$_3$)$_2$ (67), the reaction product(s) could be identified with greater success. Precipitation of an orange solid (using KPF$_6$) gave a product whose ESI-FT-ICR mass spectrum featured an ion distribution centred at $m$/z 1395.61458, which was assigned to [124 – 2PF$_6$]$^{2+}$. Superimposed on this envelope is another ion distribution centred at $m$/z 1395.11783, corresponding to the dinuclear fragment ion [$\frac{1}{2}$124 – PF$_6$]$^+$. In both cases, the difference between isotopologue $m$/z values for each species (~0.5 and ~1, respectively) is consistent with the proposed formulae. $^1$H NMR spectroscopic analysis suggests the presence of one major product with trace amounts of another species, possibly a diastereomer of the major product, also being present (the dinuclear fragment is not expected to be stable in the solution state). A single $^{195}$Pt NMR resonance at –2254 ppm was observed, which is consistent with a PtN$_4$ core. Despite this, the UV-vis spectrum lacks an absorption at ~450 nm (the reaction mixture was orange, rather than red) which is often diagnostic of Pt(II)--Pt(II) interactions in similar systems. As a consequence, further characterisation of this and related species is required.

The reactivity of selected 2-aminopyrimidinium dications towards labile palladium(II) and platinum(II) complexes was also investigated in organic solvents. For these purposes, the

‡‡ The product might also take the form of a chiral double helicate, as in the case of 119.
organic PF$_6^-$ salts were combined with metal complex precursors. As with the PF$_6^-$ salts of the 3-aminopyrazinium derivatives, the 2-aminopyrimidinium species were found to be poor ligands. The representative species [apym($m$-xylene)apym](PF$_6$)$_2$ (80) was combined with an equimolar amount of [Pt(dppp)(OTf)$_2$] in CD$_3$CN (Scheme 3.7). No evidence of any reaction could be obtained from NMR or ESI-MS analyses.

![Scheme 3.7: 2-Aminopyrimidinium derivatives were found to be unreactive towards the organo-soluble complex precursors employed.](image)

Slow evaporation of the solution afforded colourless single crystals, which were characterised by an X-ray structure determination. The crystals were found to be a mixed OTf/PF$_6^-$ salt of the 1,3-bis(1-methyl-2-aminopyrimidinium)benzene dication (80).§§ As was discussed above for the 3-aminopyrazinium salts, deprotonation of the conjugated amine (in this case, guanidinium) groups was considered as a method to prepare metal complexes. However, the addition of NEt$_3$ to the above mixture failed to induce any coordination.

### 3.5 Larger ligand derivatives

Despite the cationic nature of many of the N-heterocyclic ligands and metal precursor fragments, many new metallocycles could be prepared from combinations of these species. The origin of the stability of charged metallo-supramolecular architectures has been the subject of an excellent review by Piguet,\textsuperscript{15} in which theoretical calculations on dinuclear ruthenium(II) complexes demonstrated that solvation processes shield what would otherwise be strong intramolecular repulsions between positive charges. This shielding is most pronounced in H$_2$O, which is a more effective dielectric medium than MeCN.\textsuperscript{16***}

In general, it was found that all species of type [pyz(spacer)pyz]$^{2+}$ and the PF$_6^-$ salts of 3-aminopyrazinium and 2-aminopyrimidinium derivatives have negligible affinity for the

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§§ The solid state structure of this species is largely unremarkable. An ORTEP representation and crystallographic details can be found in the Appendices (Section A.3).

*** The dielectric constant values $\varepsilon$(H$_2$O) = 80.10 and $\varepsilon$(MeCN) = 38 ($\varepsilon$(vacuum) = 1) suggest that electrostatic forces between ions have approximately twice the strength in MeCN, relative to H$_2$O.
labile palladium(II) or platinum(II) precursors employed in MeCN solution. However, bis(4,4'-bipyridinium) derivatives could be incorporated into new [2 + 2] metallocyclic complexes of palladium(II) and platinum(II). The use of bis(3-aminopyrazinium) and bis(2-aminopyrimidinium) salts instead gave tetranuclear complexes in which each ligand bridges two pairs of platinum(II) ions, each of which is also held together by Pt(II)···Pt(II) interactions. In terms of DNA-binding, the species discussed above are of insufficient size to encircle double-stranded DNA, although single-stranded DNA might be accommodated in the cavities of some of the metallocycles. It appears that the formation of larger species from the present class of ligands can only be successful if other conditions were to be employed, for example, where a suitable templation strategy can be devised.

A simpler and more obvious means of integrating flexible ligands into larger macrocycles would be to increase the size of the spacer unit between the donor atoms. While these longer ligands may afford mononuclear (in which both ends of the flexible ligand coordinate to the same metal ion) instead of dinuclear species, such a compound may be of appropriate size to encircle double-stranded DNA, if a sufficiently large bidentate ligand could be prepared (Figure 3.4).

Figure 3.4: Di- (left) and mononuclear (right) complexes incorporating flexible N,N-bidentate ligands and [Pt(2,2'-bipy)]^{2+} fragments. The use of intermediate spacer lengths may lead to equilibrium mixtures of di- and mononuclear complexes.

Although very large ligands (for example, those containing more than three linked 4,4'-bipyridinium components) were not prepared during the course of this study, a simple, commercially available ligand could be integrated into metal complexes of considerable size; its chemistry is described in the following Section.

††† For example, in the species [Pt_{2}(2,2'-bipy)]_{2}^{2+} there exists a 19 Å separation between the two Pt centres (Spartan '04).
3.6 PEGda complexes of palladium(II) and platinum(II)

The diamine $O,O'$-bis(2-aminoethyl)octadeca(ethylene glycol) (hereafter referred to as PEGda) appeared to have the appropriate size, functionality and solubility for the formation of large metalloccycles in aqueous solution. The reversible metal-complexation process is amenable to macrocycle preparation in that simple $[1 + 1]$ macrocycles could form in preference to (either linear or cyclic)‡‡‡ oligomeric products on account of entropic considerations.

Incorporating PEGda into complexes of palladium(II) and platinum(II) appeared to be a straightforward means towards the formation of large cationic macrocycles. The complexation of PEGda with $cis$-protected metal ions has the potential to furnish $H_2O$-soluble species of the form $[M(N,N)(PEGda)]^{2+}$ ($M = Pd, Pt; N,N\ = \ small \ N,N\text{-chelating ligand}$), featuring impressive 62-membered chelate rings (Scheme 3.8). However, owing to the length of PEGda, complexation might also occur in a bridging, rather than a chelating manner. It was thought the latter mode would be entropically preferred, and that under thermodynamic control, mononuclear species would predominate over oligomeric material. As a consequence, solutions of PEGda and suitable metal complex precursors were heated for extended periods, over which time it was anticipated that any oligomeric intermediates would convert to the desired $[1 + 1]$ macrocycles. Such conditions could also eliminate the need for high dilution conditions to be employed.

The compound $[Pd(2,2'-\text{bipy})(PEGda)](NO_3)_2$ (125) appeared to be an ideal (and disarmingly simple) initial target. Stirring a warm aqueous suspension of $[Pd(2,2'-\text{bipy})(NO_3)_2]$ and PEGda afforded, after 2 h, a clear yellow solution. $^1H$ NMR spectroscopic analysis of this solution suggested a product of high symmetry, such as the desired species, had formed. This was supported by high resolution ESI-FT-ICR-MS

‡‡‡ These include catenated species which, given the length of PEGda, must come into consideration.
characterisation, which confirmed the presence of \([\text{Pd}(2,2′\text{-bipy})(\text{PEGda})]^2+\) ions (m/z 579.27006, calcd 579.27153). Importantly, no evidence could be found for the formation of oligomeric species, which suggests that the moderate lability of Pd–N bonds allowed the thermodynamic product to form quickly. However, this lability was thought to potentially allow for the integrity of the complex to be compromised in the presence of DNA, perhaps by covalent interaction with the nucleobases. It was for this reason that the kinetically inert platinum(II) derivative, which was considered more likely to remain intact in the presence of DNA, was also prepared in this work.

By analogy with the palladium(II) complex above, the synthesis of \([\text{Pt}(2,2′\text{-bipy})(\text{PEGda})][\text{NO}_3]_2\) (126) was attempted by heating an aqueous suspension of \([\text{Pt}(2,2′\text{-bipy})(\text{NO}_3]_2\] and PEGda. Dissolution of the platinum(II) precursor occurred, but over time the solution developed an orange-red colour and upon cooling a red precipitate formed, which was filtered off. Its $^1$H NMR (D$_2$O) spectrum featured resonances exclusively at low field, suggesting that PEGda (either free or coordinated) was not present in the material isolated, which was believed to possibly contain the hydroxo-bridged species \([\text{Pt}_2(2,2′\text{-bipy})_2(\mu\text{-OH})_2][\text{NO}_3]_2\). The use of dilute acid (HNO$_3$, 0.01 M aqueous solution) as the reaction solvent, in order to suppress the formation of any hydroxo complexes, failed to alter this outcome. Instead, it was found that heating a dry DMF solution of the precursors (60°C, 48 h) caused the desired reaction to proceed cleanly. Upon solvent evaporation, $^1$H NMR spectroscopic analysis of the resultant oily yellow solid suggested the desired product had formed exclusively. More conclusive evidence could be obtained from the accompanying $^{195}$Pt NMR spectrum, which exhibited a single resonance at ~2661 ppm, consistent with the presence of a single platinum(II) environment in which the metal centres are each coordinated to four N atoms. This was further supported by ESI-FT-ICR-MS analysis, which confirmed the presence of \([\text{Pt}(2,2′\text{-bipy})(\text{PEGda})]^2+\) ions (m/z 624.29932, calcd 624.30133).

§§§ Heating at temperatures > 70°C caused decomposition, causing the yellow solution to become orange. Note also that, unlike \([\text{Pt}(2,2′\text{-bipy})(\text{NO}_3]_2\], the product is highly H$_2$O-soluble.
It should be noted that the [Pt(2,2′-bipy)]\(^{2+}\) moiety present in [Pt(2,2′-bipy)(PEGda)]\(^{2+}\) has the potential to bind to DNA by intercalation (see Chapter Four),\(^{17}\) a mode which may be undesirable in the context of the present study. Consequently, it was of interest to prepare a related complex for which intercalation cannot complicate binding; [Pt(tmeda)(PEGda)](NO\(_3\))\(_2\) (127, tmeda = N,N,N,N′-tetramethyl-1,2-diaminoethane) is one such species. Its synthesis was attempted via the labile intermediate [Pt(tmeda)(OD\(_2\))]\(^{2+}\), which was generated by stirring a warm suspension of [Pt(tmeda)I\(_2\)] and AgNO\(_3\) in D\(_2\)O (70°C, 48 h). After removal of AgI by filtration, the colourless solution was added to PEGda and the mixture heated for a further 2 days, over which time a small amount of white precipitate had formed, which was removed by filtration. \(^1\)H and \(^{195}\)Pt NMR spectroscopic analyses of the filtrate suggested that a mixture of products had formed, the latter indicating the presence of three distinct \(^{195}\)Pt environments. A signal at –2675 ppm suggested that a platinum(II) product with four N-donor atoms (possibly [Pt(tmeda)(PEGda)]\(^{2+}\)) had formed, but further resonances at –2421 and –2136 ppm indicated that other platinum(II) species, perhaps with N\(_3\)O coordination spheres, were also present.**** The same result was also found to occur when the [Pt(tmeda)(NO\(_3\))]\(^2\) intermediate was isolated and subsequently treated with PEGda.

When DMF was used as the solvent in place of H\(_2\)O, [Pt(tmeda)(PEGda)](NO\(_3\))\(_2\) could be cleanly generated as a sticky solid from [Pt(tmeda)(dmf)]\(^2\)(NO\(_3\))\(_2\) (prepared in situ from [Pt(tmeda)I\(_2\)] and AgNO\(_3\)) and PEGda in DMF solution. As with the 2,2′-bipyridine analogue, it appears that the ability of DMF to dissolve potentially insoluble reaction intermediates and/or the curbing of hydrolysis (which might otherwise occur) allow for the reaction to proceed smoothly. Most diagnostic of this is the single NMR resonance observed at \(\delta^{(195}\)Pt) –2695 ppm, which is consistent with the presence of platinum(II) coordinated to four N atoms. Further data in support of this could be obtained using \(^1\)H NMR spectroscopy and ESI-FT-ICR-MS. The analogue [Pt(en)(PEGda)](NO\(_3\))\(_2\) (128) was prepared and characterised in a similar manner, using [Pt(en)I\(_2\)] as the precursor complex. This analogue, as with the other complexes of PEGda, could be isolated as an oily solid.††††

Relatively few coordination compounds of acyclic diaminopolyethers have been reported to date. The short ligand 129 can bind a variety of transition\(^{18}\) (as well as alkaline earth\(^{19}\)

****ESI-FT-ICR-MS analysis: \(m/z\) calcd for C\(_{46}\)H\(_{101}\)N\(_4\)O\(_{20}\)Pt\(^+\), [Pt(tmeda)(\(\eta_1\)-PEGda)(OH)]\(^+\): 1225.66073. Found: 1225.666247.
†††† This precluded their characterisation by CHN microanalysis.
metal ions through both the amino and ether electron pairs. For example, the complex \([\text{Cu}(129)]^{2+}\) incorporates square-planar, \(\text{N}_2\text{O}_2\)-coordinated copper(II).\(^{20}\) While the larger analogue 130 has been incorporated into dinuclear cobalt(III) complexes,\(^{21}\) there are no reports of any metal-containing species with longer aliphatic dianinopolyethers. The related aromatic derivatives \(O,O'\)-dianilinoethylene glycol (131) and \(O,O'\)-dianilinotetra(ethylene glycol) (132) have been found to bind metal ions to afford \(\text{cis-}[\text{Pd}(131)\text{Cl}_2]\)\(^{22}\) and \([\text{K}(132)]^+\),\(^{23}\) respectively.

The complexes of type \([\text{M}(N,N)(\text{PEGda})]^{2+}\) described above represent a class of very large metallocyclic compounds. Owing to their 62-membered chelate rings, they can be referred to as ‘gigantocycles’, defined by Vögtle\(^{24}\) as macrocycles having between 50 and 100 atoms in their rings. The species prepared in this work obviously bear close resemblance to simple aliphatic crown ethers, of which several large examples, including 81-crown-27 (133), have been prepared. As its name suggests, 81-crown-27 incorporates 27 ethyleneoxy units connected to form an 81-membered ring, and its solid state structure reveals that this flexible gigantocycle is twice folded onto itself.\(^{25}\) It is probable that complexes of PEGda also exist in similarly folded conformations, which are likely favoured over more open ‘extended’ states due to entropic and stereoelectronic effects.\(^{26}\) More conformationally-rigid crown ethers can be prepared by the incorporation of aryl rings into macrocycles. One such species is the 66-membered tetrabenzocrown 134 which could be synthesised in 11% yield, with a smaller, 33-membered species being the major (51%) product.\(^{27}\) This example clearly highlights the limitations of macrocycle synthesis using kinetically-controlled (organic) chemistry, in this case the \([2 + 2]\) cyclisation of a bis(4-toluenesulfonate) precursor with resocinol. In contrast, the palladium(II) and platinum(II) complexes of PEGda prepared for the present study form in quantitative yield (by \(^1\)H NMR spectroscopy).

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3.7 **Concluding remarks**

The diquaternary and PEGda \( N \)-donor ligands were found to be useful precursors for the preparation of metallocyclic compounds. Indeed, these ligands could be incorporated into palladium(II) and platinum(II) complexes of varying size, solubility and nuclearity.

In terms of encircling duplex DNA, many species bearing positive charge and possessing high aqueous solubility were prepared during the present study. The most important prerequisite for ‘DNA nanoshuttle’ behaviour, namely the presence of a large cavity, is best fulfilled by complexes of type \([M(N,N)(\text{PEGda})]^2+\). If the conformationally flexible PEGda ligand were to extend to form a roughly circular chelate ring, it is anticipated that this would be of considerable size (~3 nm).

The DNA-binding behaviour of selected metallocyclic species, as well as simple acyclic analogues, is the central subject of the remainder of this Thesis.

3.8 **References**

Chapter Four: DNA-binding studies

4.1 Anion binding

While the study of cation binding by organic ligands is a well-established discipline, the rational design of agents capable of coordinating anions is a pursuit which has only recently gained much attention. Indeed, for decades the term “coordination chemistry” was used almost exclusively to refer to complexation of metal cations, in particular transition metal ions. However, the significant roles anions play in biology and in the environment serve to motivate the development of this emerging area of chemistry.

Anions have larger radii than their isoelectronic cations, and their more diffuse electron density can cause interactions involving anions to be weak and without directionality. Thus, the use of multiple H-bonding, electrostatic and π–π/hydrophobic interactions is often required in order for a successful anion/receptor complex to form. By carefully exploiting H-bonding interactions, Crabtree and co-workers were able to demonstrate that a simple, non-preorganised (conformationally flexible) diamide (135) can form a stable 1:1 complex with a single Br⁻ ion. The elegant isophthalamide motif, which possesses three H-bond donors groups, has been incorporated into several higher order structures, including catenanes and rotaxanes. Indeed, not only can such species act as anion receptors, but very often the formation of these interlocked architectures is reliant upon anion templation.

A more elaborate (chiral) receptor (136) is able to bind 4-nitrobenzoate by means of H-bonding, as well as electrostatic and π–π interactions. Additionally, the greater affinity of 136 for L-N-acetyltryptophan over D-N-acetyltryptophan saw it become the first synthetic host capable of binding anions enantioselectively.

4.2 DNA-binding

The binding of small molecules to DNA, both in terms of fundamental chemistry as well as drug design, is a topic of intense research activity. The largely non-covalent interactions
involved in the assembly of supramolecular architectures also feature heavily in DNA recognition. The 1960s saw the discovery of five modes by which small molecules bind B-DNA; these are presented in Figure 4.1.

These modes of interaction are:

- **Intercalation** – whereby extended aromatic regions of species insert between stacked base-pairs in a non-covalent manner. The complex \([Ru(2,2'-bipy)_2(dppz)]^{2+}\) (137) incorporates such a region in the form of the dipyrido[3,2-a:2',3'-c]phenazine (dppz) ligand, and binding is promoted by electrostatic and \(\pi-\pi\) interactions.

- **Major groove binding** – which involves molecules recognising the major groove, often by sequence-specific H-bonding and hydrophobic/electrostatic interactions. The delocalised lipophilic cation methyl green (138) is a major groove binder.

- **Minor groove binding** – a common mode of binding for small molecules (typically those containing several aryl groups) in which non-covalent interactions (H-bonding, hydrophobic effects) allow for DNA-drug complex formation. The bis(benzimidazole)
Hoechst-33258 (139), which incorporates H-bonding functionality, binds to AT-rich regions in the minor groove.\textsuperscript{14}

- Sugar-phosphate backbone binding – by which cationic molecules bind to the DNA backbone, in particular, the phosphodiester groups. The complex trication hexamminecobalt(III) (140) binds to the backbone; this is promoted by stabilising electrostatic and N–H···O\textsubscript{phosphate} H-bonding interactions.\textsuperscript{15}

- Covalent binding – whence a drug molecule covalently binds to DNA. Unlike the above binding modes involving non-covalent interactions, covalent binding, owing to its greater strength, is often irreversible in nature. The anticancer drug cisplatin (141) can undergo aquation and subsequently bind in a (coordinate) covalent manner to the N7 atoms of guanine or adenine nucleobases.\textsuperscript{16}

### 4.3 Non-covalent DNA-binding of platinum(II) complexes

While a vast amount of research into platinum(II)-DNA chemistry has focused on covalent binders, such as cisplatin and its many analogues,\textsuperscript{17} square-planar platinum(II) complexes have also been demonstrated to bind DNA in a non-covalent manner. In particular, complexes incorporating polyaromatic chelating ligands are ideally suited to act as metallointercalators. Lippard was the first to investigate the non-covalent interactions between such species and DNA, initially studying complexes of the type \[\text{[Pt(2,2':6',2"-terpyridine)X]}^+ \quad (X = \text{Cl}, \text{S(CH}_2)_2\text{OH}).\textsuperscript{18}\] The labile chlorido complex 142 was found to bind DNA covalently, as it can undergo aquation and subsequent nucleobase coordination. However, the kinetically inert thiolato derivative 143 does not readily aquate and instead intercalates, causing a loss of the regular helical structure of DNA.

Several platinum(II) complexes of \(N,N\)-bidentate aromatic ligands have been reported to interact non-covalently with DNA. In particular, simple bis(chelate) species such as \([\text{Pt(phen)(en)}]^2+ \quad (144),\textsuperscript{19}\) by virtue of the high affinity \([\text{Pt(phen)}]^2+\) fragments have for DNA,\textsuperscript{20} are potent intercalators. Studies on the \textit{in vitro} activity of the related complex
[Pt(phen)(1S,2S-diaminocyclohexane)]^{2+} (145) have shown it to be toxic to several cancer cell lines, with its mode of action believed to involve DNA intercalation.\textsuperscript{21} It was found that the enantiomer [Pt(phen)(1R,2R-diaminocyclohexane)]^{2+} had different biological activity to 145, a fact which is unsurprising given the chiral nature of DNA and the high likelihood of the amino groups participating in H-bonding interactions with the macromolecule.

4.4 Complexes employed for DNA-binding

The study of DNA-binding to metal complexes need not be limited to small mononuclear derivatives. As was highlighted in the Introduction to this Thesis (Section 1.5), interdisciplinary supramolecular and bio-inorganic chemical research has recently garnered several remarkable findings regarding the behaviour of metallosupramolecular species in a biological setting, in particular when DNA is involved. Encouraged by these early results, it was of interest to investigate the interactions the new types of metallocycles discussed in the previous Chapter might have with DNA.

The complexes prepared during the course of this study which were selected for use in DNA-binding experiments are presented below.

As was alluded to in Section 3.6, the PEGda complexes [M(2,2'-bipy)(PEGda)](NO\textsubscript{3})\textsubscript{2} (125: M = Pd, 126: M = Pt), owing to their [M(2,2'-bipy)]^{2+} moieties, are potential
metallointercalators, with the palladium(II) and platinum(II) analogues differing in their lability. The related (non-intercalating) compounds [Pt(tmeda)(PEGda)](NO₃)₂ (127) and [Pt(en)(PEGda)](NO₃)₂ (128), which differ in their H-bonding functionality, were also employed in the studies. In order to probe the influence that macrocyclic structure might have on DNA-binding, the simple acyclic complex [Pt(2,2'-bipy)(NH₃)₂]²⁺ was also prepared. This complex is known to bind nucleic acids²² and was considered a fragment of the complex [Pt(2,2'-bipy)(PEGda)](NO₃)₂ that could potentially intercalate into DNA, but not encircle it. While the Cl⁻ salt [Pt(2,2'-bipy)(NH₃)₂]Cl₂ has been reported previously,²³ for consistency the NO₃⁻ salt [Pt(2,2'-bipy)(NH₃)₂](NO₃)₂ (146) was prepared and used in the present study.

The dinuclear palladium(II) and platinum(II) complexes 107 – 110 were employed and again acyclic analogues of these complexes were also investigated. To this end, two further compounds of the form [M(2,2'-bipy)(Mebipy)₂](NO₃)₄ (147: M = Pd, 148: M = Pt; Mebipy⁺ = 1-methyl-4,4'-bipyridinium) were prepared (from [Mebipy]NO₃ (15) and [M(2,2'-bipy)(NO₃)₂]) and used in the DNA-binding experiments.† These were prepared as model compounds, with each representing roughly one half of the dinuclear derivatives. Comparison of the DNA-binding properties of the mononuclear and dinuclear complexes would perhaps shed light on how the greater positive charge and cyclic structure of 107 – 110 might influence DNA-binding.

The importance of successfully characterising the non-covalent interactions between DNA and small molecules cannot be understated. This can be achieved using a variety of techniques including crystallography, calorimetry, vibrational spectroscopy, NMR spectroscopy, circular dichroism, fluorescence, UV-visible spectroscopy and mass spectrometry.²⁴ Use was made of the last two techniques in the present study; descriptions of these methods and the conclusions drawn from the analyses of DNA mixtures containing selected palladium(II) and platinum(II) complexes are presented in the following Sections of this Chapter.

* This complex was prepared from aqueous NH₃ and [Pt(2,2'-bipy)(NO₃)₂] (see Experimental Section 6.11).
† A third such compound, the 1,10-phenanthroline complex [Pd(phen)(Mebipy)₂](NO₃)₂ (149) was also prepared, although it was not used in any binding experiments. Further details can be found in Section 6.11.
4.5 Mass spectrometric analysis of DNA adducts

4.5.1 Background

The development of macromolecular ESI-MS analysis by Fenn\(^{25}\) has provided scientists with an efficient and sensitive technique for the analysis of biomolecules, including DNA.\(^{26}\) Indeed, ionisation conditions can be sufficiently mild such that non-covalent interactions are often preserved,\(^{24,27}\) allowing for information regarding binding strength and stoichiometry to be obtained.\(^{28}\) The characterisation of non-covalent DNA/metal complex interactions using ESI-MS has recently become an area of great research interest,\(^{29,30}\) no doubt motivated by potential applications which might result from this work, such as the design of agents capable of repairing\(^{31}\) or probing the structure of DNA.\(^{12}\)

Beck and co-workers\(^{32}\) demonstrated that a range of transition metal complexes are able to effectively bind 16 base-pair (16mer) duplex oligonucleotides. Their work showed that these short DNA sequences, as well as their adducts with small molecules, are very much amenable to characterisation using ESI-MS.\(^{30}\) In recent work, the complex \([\text{Pt(phen)(en)}]\)\(^{2+}\), as well as a number of its analogues in which the phen ligands are methylated, were found to have significant affinities for dsDNA, as evidenced by the high abundance of DNA/metal complex adduct ions in the mass spectra of these mixtures. The ESI-MS studies showed that the position and number of methyl groups has subtle effects on the DNA-binding strength of these complexes. In particular, the dimethylated species \([\text{Pt}(4,7-\text{Me}_2\text{phen})(\text{en})]\)\(^{2+}\) was found to bind with higher affinity than \([\text{Pt(phen)(en)}]\)\(^{2+}\), an observation which might be attributed to the greater hydrophobicity of the latter species. Binding experiments were also carried out with intercalators of the type \([\text{M(phen)}_2(\text{dppz})]\)\(^{2+}\) (M = Ni, Ru), with the results suggesting that these octahedral complexes, despite their extended aromatic dppz ligands, represent weaker intercalators than the square-planar platinum(II) complexes. Indeed, while octahedrally coordinated metal ions might bind poorly due to the steric demands of ligands, square-planar complexes are not hindered by the presence of axial ligands and are ideally suited to insert between DNA base-pairs. Stoichiometry also plays a role in binding and, as expected, increasing concentrations of metal complexes resulted in the formation of adducts in which more complex species were bound to each DNA molecule.

\(^{\dagger}\) John B. Fenn, along with Koichi Tanaka and Kurt Wüthrich, shared the 2002 Nobel Prize in Chemistry “for the development of methods for identification and structure analyses of biological macromolecules.”

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The following discussion outlines some considerations with respect to experimental design for the analysis of oligonucleotides by ESI-MS.

### 4.5.2 Experimental Approach

The present study utilised electrospray ionisation time-of-flight mass spectrometry (ESI-TOF-MS) for the analysis of DNA and its metal complex adducts. For these purposes, two 16mer dsDNA sequences, including that used by Beck and co-workers in the work described above (D2), were employed as models for B-DNA. In comparison to the situation with polymeric DNA, such as that of calf thymus, the hypothetical threading of synthetic molecules might proceed more readily with oligonucleotides, as the DNA termini are more accessible than in the polymeric case. Given that such macrocycles might ‘shuttle’ freely along duplex DNA, this could also affect the thermodynamic stability of the bio-hybrids. In particular, the dissociation (unthreading) of a macrocycle from very short oligonucleotides might occur very rapidly, causing observation of the overall interaction to be difficult, owing to its transient and weak nature. It was anticipated that the sequence length used would be long enough to promote an observable interaction with the complexes of interest.

The dsDNA oligonucleotides D2 and D2’ were used in the present study; their sequences are given in Table 4.1. These represent well-studied duplexes each comprising two different (i.e. non-self-complementary) strands of different masses. This latter aspect is important as it allows for more information to be gleaned from MS data than would be the case if the individual ssDNA were of equal mass.

<table>
<thead>
<tr>
<th>dsDNA</th>
<th>ssDNA</th>
<th>sequence (5’-3’)</th>
<th>M / Da</th>
</tr>
</thead>
<tbody>
<tr>
<td>D2</td>
<td>D2a</td>
<td>CCTCATGGCCATGACC</td>
<td>4802.0</td>
</tr>
<tr>
<td></td>
<td>D2b</td>
<td>GGTCATGGCCATGAGG</td>
<td>4962.5</td>
</tr>
<tr>
<td>D2’</td>
<td>D2’a</td>
<td>biotin-CCTCATGGCCATGACC</td>
<td>5373.8</td>
</tr>
<tr>
<td></td>
<td>D2’b</td>
<td>biotin-GGTCATGGCCATGAGG</td>
<td>5533.6</td>
</tr>
</tbody>
</table>

The study of DNA solutions using ESI-MS can be carried out in either positive- or negative-ion modes. While mixtures of nucleic acids, particularly those containing DNA bound to several cationic drug species, can be successfully characterised using positive-ion analysis, this was not attempted in the present study. It was thought that the complexes employed
would bind weakly, and given that they were typically not used in high concentrations, it was not expected that many would bind to a single duplex oligonucleotide. Thus, the detection of gas phase ions from DNA solutions in the presence and absence of metal complexes was carried out in the negative-ion mode.

The present study focused primarily on the interactions of palladium(II) and platinum(II) complexes with D2; the mass spectrometric experiments characterising D2 and its adducts are presented below.

4.5.3 ESI-MS of D2

Equimolar amounts of the single-strands D2a and D2b were carefully annealed to afford the duplex D2. The negative-ion ESI mass spectra of D2 in (a) H2O and (b) 0.1 M NH4OAc are given in Figures 4.2(a) and 4.2(b), respectively.

Figure 4.2: ESI-MS of D2 in (a) H2O and (b) 0.1 M NH4OAc; * [D2 – nH+]n– (n = 4, 5, 6); o [D2a – nH+]n– (n = 3, 4, 5, 6, 8); ob [D2b – nH+]n– (n = 3, 4, 5, 6).
As expected, the analysis of free D2 allowed for its detection in several charge states. Thus, owing to the acidity of the phosphodiester groups (any number of which may be deprotonated on a single D2 molecule) a progression of [D2 – nH+]\(^{\text{n+}}\) ions was observed, with the 6–, 5– and 4– anions being the most abundant. Ions assigned to the single strands [D2\(_a\) – nH+]\(^{\text{n+}}\) and [D2\(_b\) – nH+]\(^{\text{n+}}\) were also detected. The relative intensities of ions of dsDNA and ssDNA, as well as the charge states they exist in, are highly dependent on the conditions employed, both in terms of instrumentation and sample preparation. For example, with respect to the former, high cone voltages are known to favour the detection of more highly charged species.\(^{34}\) The medium in which the oligonucleotides are dissolved is also important. As shown in Figure 4.2(a), the stability of D2 in pure H\(_2\)O is not particularly high, a result evidenced by the high intensities of ions corresponding to D2\(_a\) and D2\(_b\) (relative to those of D2). However, the duplex form of DNA is stabilised in solutions of high ionic strength (i.e. strongly dielectric media)\(^{35}\) and remains largely intact under ESI-MS conditions.\(^{33}\) Indeed, as reported by Beck and co-workers (and shown in Figure 4.2(b)), mass spectra of dsDNA in 0.1 M NH\(_4\)OAc can often be acquired in which only relatively small amounts of ssDNA are detected.\(^{36}\)

Aqueous NH\(_4\)OAc is readily volatilised to NH\(_3\) and HOAc (and H\(_2\)O) during desolvation and ionisation processes, typically allowing for the observation of DNA and its metal complex adducts without interference from other species. This is a necessary detail, as the presence of nonvolatile ionic compounds, such as those containing Na\(^+\), would complicate spectral interpretation owing to the large number of ion clusters that might form.\(^5\) Due to the affinity Na\(^+\) ions have for the DNA sugar-phosphate backbone,\(^37\) the presence of these cations would result in several species of formula [D2 + mNa\(^+\) – nH+]\(^{\text{(m-n)+}}\) to be detected, which may add spectral ambiguity. For example, the major (almost 100% naturally abundant) isotopologue of [D2 – 5H+]\(^{\text{3+}}\) was detected at m/z 1952 with a less intense ‘tail’ corresponding to the sodiated progression [D2 + mNa\(^+\) – nH+]\(^{\text{(m-n)+}}\). Such species were observed in all D2-containing mixtures and the trace amounts of sodiated ions detected were considered to be acceptable.

Mixtures containing selected palladium(II) and platinum(II) complexes and either D2 or D2\(_a\) were prepared. If the metal complexes were to bind these 16mers, it was expected that the adducts would be observed as ions having higher m/z values than free DNA. These

\(^5\) Such cations can also skew the distribution of charge states observed.
preliminary experiments typically involved the analysis of freshly prepared mixtures containing [complex] = [D2 or D2a] = 10 µM (i.e. a DNA : complex ratio of 1 : 1) in 0.01 M NH₄OAc (pH 7.0). The results of the ESI-MS analyses are summarised in the following Sections of this Chapter.

4.5.4 Interactions of palladium(II) complexes with D2

The negative-ion ESI-MS analysis of a D2/[Pd(tmeda)(PEGda)]²⁺ mixture resulted in the spectrum given in Figure 4.3, which is presented with a full assignment of all ions.

![Figure 4.3: ESI-MS of D2 with [Pd(tmeda)(PEGda)]²⁺ (1 : 1 in 0.01 M NH₄OAc); • [D2 – nH⁺]ⁿ⁻ (n = 5, 6); ◦ [D2a – 3H⁺]²⁻; ▪ [D2b – 3H⁺]²⁻; ● [D2 + Pd(tmeda)(PEGda)]²⁺ – nH⁺]ⁿ⁻²⁻ (n = 7, 8); ★ [D2 + PEGda – nH⁺]ⁿ⁻ (n = 5, 6); ★' [D2 + Pd(tmeda)]²⁺ – nH⁺]ⁿ⁻²⁻ (n = 7, 8); ★'★' [D2 + 2Pd(tmeda)]²⁺ – nH⁺]ⁿ⁻⁻²⁻ (n = 9, 10); ★'★'★' [D2 + Pd(tmeda)(PEGda)]²⁺ + Pd(tmeda)²⁺ – 9H⁺]²⁻; ★'★'★'★' [D2 + Pd(tmeda)(PEGda)]²⁺ + 2Pd(tmeda)²⁺ – 12H⁺]²⁻.](image)

It was found that the most intense ions were those of free D2 in the 6⁻ and 5⁻ charge states, with weaker ions, assigned to the single strands D2a and D2b, also being present. The detection of the adduct [D2 + Pd(tmeda)(PEGda)]²⁺ – nH⁺]ⁿ⁻²⁻, which incorporates one D2 molecule bound to one (presumably intact) complex, provides evidence of an interaction between its constituents in the gas phase. Given the low intensity of these 1 : 1 adduct ions, the binding of [Pd(tmeda)(PEGda)]²⁺ is likely to be weak and/or in competition with other processes. In particular, the moderate lability of palladium(II) complexes can result in their decomposition during the ionisation process. If the resulting fragments bind DNA then it is clear that these side reactions prevent some portion of DNA and complex species

**In some cases 0.1 M NH₄OAc (pH 7.4) was used.
†† Notation: • D2; ◦ D2a; ▪ D2b; ● intact complexes; ◊ 4,4'-bipy(CH₂)₄4,4'-bipy²⁺ (n = 4, 6), Mebipy²⁺ or PEGda; ◊' [M(N,N)²⁺ (M = Pd, Pt; N,N = 2,2'-bipy, en, tmeda). In general, circles denote DNA, and squares metal complexes; black symbols denote intact species, and white symbols denote fragments thereof. Juxtapositions of symbols refer to adducts incorporating the relevant components with, in all cases, loss of nH⁺, where n determines the overall charge, e.g. •• [D2 + Pd(tmeda)(PEGda)]²⁺ – nH⁺]ⁿ⁻²⁻ (n = 7, 8) refers to a 1 : 1 adduct present as 6⁻ and 5⁻ ions.
participating in what might already be a very weak mode of interaction. In the present example, ions corresponding to the binding of the individual fragments PEGda and [Pd(tmeda)]^{2+} to D2 were also observed. The diamine PEGda is dibasic (doubly protonated at pH 7.0 or 7.4) and PEGdaH_{2}^{2+} might bind non-covalently to the sugar-phosphate backbone of D2 through H-bonding and electrostatic interactions. The fragment [Pd(tmeda)]^{2+}, on the other hand, is likely to bind D2 in a coordinate covalent manner by virtue of its two reactive coordination sites. This interaction is likely to be moderately strong – indeed, ions assigned to D2 binding two [Pd(tmeda)]^{2+} fragments were detected, as well as D2 adducts of [Pd(tmeda)(PEGda)]^{2+} with up to two additional [Pd(tmeda)]^{2+} fragments.

It should be noted that most of the species observed by ESI-MS were present in two charge states, with the respective ions differing in composition by a single H^+. The detection of ion progressions in multiple charge states undoubtedly aids the unambiguous assignment of ESI mass spectra of oligonucleotides.

For the purposes of comparison, the binding experiment was also performed in the absence of NH_4OAc. Analysis of an aqueous mixture of D2 and [Pd(tmeda)(PEGda)]^{2+} allowed for a similar spectrum to be obtained (see Appendices, Section A.4.1, Figure A.21(b)). As expected, ions corresponding to D2a and D2b had greater intensities (relative to D2) when H_2O was used in place of 0.01 M NH_4OAc. Additionally, 1:1 adducts of these single strands with [Pd(tmeda)]^{2+} were observed. It is clear that, although the use of a weaker dielectric medium would strengthen interactions between dsDNA and complex cations, the stability of the former is markedly reduced under these conditions, and the relative intensities of ions assigned to adducts of dsDNA decrease as a result.

The analysis of a 0.01 M NH_4OAc solution containing D2 and [Pd_2(2,2'-bipy)_2{[4,4'-bipy(CH_2)_44,4'-bipy}]_4]^{8+} was carried out in much the same way as in the previous example. The resulting ESI mass spectrum (Figure 4.4) featured ions assigned to free D2 (and D2a, D2b), along with weaker ions corresponding to several DNA adducts, none of which contained intact octacationic complexes. Ions were observed for D2, D2a and D2b adducts of [4,4'-bipy(CH_2)_44,4'-bipy]^{2+}, a species whose large aromatic surface area and positive charge were thought to allow it to interact with DNA. As was found when using [Pd(tmeda)(PEGda)]^{2+}, palladium(II) chelate fragments, in this case [Pd(2,2'-bipy)]^{2+}, were observed to interact with D2. The binding of [M(2,2'-bipy)]^{2+} fragments (M = Pd, Pt) to
DNA is well understood, and these have been shown to bind by a non-intercalative mode involving reversible coordination to nucleobases.\(^\text{38}\)

![Figure 4.4: ESI-MS of D2 with \([\text{Pd}(2,2'\text{-bipy})_2\{4,4'\text{-bipy(CH}_2)_44,4'\text{-bipy}\}_2]^{6+}\) (1:1 in 0.01 M NH\(_4\)OAc); • \([D2 - \text{nH}^+]^{0+}\) (n = 5, 6); ◦ \([\text{D2a} - 3\text{H}^+]^{3+}\); □ \([\text{D2b} - 3\text{H}^+]^{3+}\); ◆ \([D2 + 4,4'\text{-bipy(CH}_2)_44,4'\text{-bipy}^{2+} - \text{nH}^+]^{(0-2)-}\) (n = 7, 8); ◆\(\ominus\) \([D2 + 2\{4,4'\text{-bipy(CH}_2)_44,4'\text{-bipy}\}^{2+} - \text{nH}^+]^{(0-4)-}\) (n = 9, 10); ◆\(\ominus\) \([D2 + \text{Pd}(2,2'\text{-bipy})^{2+} - \text{nH}^+]^{(0-2)-}\) (n = 7, 8); ◆\(\ominus\) \([D2 + \text{Pd}(2,2'\text{-bipy})\{4,4'\text{-bipy(CH}_2)_44,4'\text{-bipy}\}^{2+} - \text{nH}^+]^{(0-4)-}\) (n = 9, 10); ◆\(\ominus\) \([D2 + \text{Pd}(2,2'\text{-bipy})\{4,4'\text{-bipy(CH}_2)_44,4'\text{-bipy}\}^{2+} - \text{nH}^+]^{(0-6)-}\) (n = 11, 12); ◆\(\ominus\) \([D2 + \text{Pd}(2,2'\text{-bipy})_2\{4,4'\text{-bipy(CH}_2)_44,4'\text{-bipy}\}^{6+} - 12\text{H}^+]^{6+}\); ◆\(\ominus\) \([D2a + 4,4'\text{-bipy(CH}_2)_44,4'\text{-bipy}^{2+} - 5\text{H}^+]^{5+}\); ◆\(\ominus\) \([D2b + 4,4'\text{-bipy(CH}_2)_44,4'\text{-bipy}^{2+} - 5\text{H}^+]^{5+}\).]

The D2 adducts of larger and more positively charged fragments (including \([\text{Pd}(2,2'\text{-bipy})\{4,4'\text{-bipy(CH}_2)_44,4'\text{-bipy}\}_2]^{6+}\)) could also be identified, although such ions were of lower intensity than the adducts of the simpler fragments mentioned above. It was thought that the binding of \([\text{Pd}(2,2'\text{-bipy})\{4,4'\text{-bipy(CH}_2)_44,4'\text{-bipy}\}_2]^{6+}\) might involve intercalation, whereas the related hexacation \([\text{Pd}_2(2,2'\text{-bipy})_2\{4,4'\text{-bipy(CH}_2)_44,4'\text{-bipy}\}]^{8+}\), which was also found to bind D2, has the possibility of interacting covalently through its two vacant coordination sites. Overall, given the cationic nature of the \([\text{Pd}(2,2'\text{-bipy})]^{2+}\) and \([4,4'\text{-bipy(CH}_2)_44,4'\text{-bipy}]^{2+}\) species which make up the dinuclear complex, it is not surprising that it fragments under the ESI-MS conditions and, furthermore, that these fragments bind D2.

Having investigated the reactivity of \([\text{Pd(tmeda)(PEGda)}]^{2+}\) and \([\text{Pd}_2(2,2'\text{-bipy})_2\{4,4'\text{-bipy(CH}_2)_44,4'\text{-bipy}\}_2]^{8+}\) towards D2, it also was of interest to conduct experiments with the ssDNA D2a.
4.5.5 Interactions of palladium(II) complexes with D2a

The experimental procedure described above for D2 was also performed using D2a; its ESI mass spectrum is given in Figure 4.5(a).

![Figure 4.5: ESI-MS of D2a (a) alone and (b) with equimolar [Pd(tmeda)(PEGda)]^{2+} (in 0.01 M NH₄OAc); a [D2a + nH⁺]^{n+} (n = 3, 4, 5); a [D2a + Pd(tmeda)(PEGda)]^{2+} − 6H⁺; a− [D2a + PEGda − 4H⁺]^{5+}; a− [D2a + Pd(tmeda) + nH⁺]^{(n−2)+} (n = 5, 6, 7); a+ [D2a + 2Pd(tmeda) + nH⁺]^{(n−4)+} (n = 8, 9); ♦ unidentified ion.](image)

As was observed with D2, a progression of ions could be detected, with the composition of these differing only in the number of protons bound to the phosphodiester backbone. The most intense ions were assigned to [D2a − 5H⁺]^{5−}, [D2a − 4H⁺]^{6+} and [D2a − 3H⁺]^{7+}. A weak ion was also observed at m/z 1919, although its identity could not be established. It was often observed when mixtures containing D2a were analysed and it is most likely due to the presence of a trace impurity.

A 0.01 M NH₄OAc solution containing equimolar D2a and [Pd(tmeda)(PEGda)]^{2+} was prepared and subjected to ESI-MS. Ions corresponding to D2a were the most intense in the resulting spectrum (Figure 4.5(b)), which was, however, quite different to that of D2a alone. A weak ion detected at m/z 1479 was consistent with the formula for [D2a + Pd(tmeda)(PEGda)]^{2+} − 6H⁺^{6+}, an ion which, assuming the palladium(II) complex is intact, would represent a 1 : 1 species held together by non-covalent interactions. The observation of intense ions for 1 : 1 and 1 : 2 D2a adducts of [Pd(tmeda)]^{2+}, along with an ion corresponding to the binding of PEGdaH₂^{2+}, suggests that [Pd(tmeda)(PEGda)]^{2+} fragments under the conditions employed, as discussed in the previous Section. Given the ‘availability’ of the nucleobases in D2a (relative to those in D2, which would participate in...
Watson–Crick base-pairing) it is not unexpected that such covalent adducts can readily form owing to the greater number of accessible Lewis basic sites.

It is likely that electrostatic and H-bonding interactions between \([\text{D2a} - n\text{H}^+]^{n-}\) and \([\text{Pd(tmeda)(PEGda)}]^2+\) cause these ions to come into close proximity with one another, whence ligand substitution might occur, affording \([\text{Pd(tmeda)}(\eta_2\cdot\text{D2a} - n\text{H}^+)]^{(n-2)-}\).‡‡ A parallel conclusion was drawn by Beck and co-workers in experiments concerning the interactions of nickel(II) complexes with the quadruplex \((\text{TTGGGGGT})_4\) \((\text{Q1})_4\). It was observed that \(\text{Q1}\) formed adducts of \([\text{Ni(phen)}_2(\text{dppz})]^2+\) and \([\text{Ni(phen)}_3]^2+\), however in both cases with the loss of a phen ligand. These results were consistent with the initial formation of non-covalent complexes of \(\text{Q1}\) and the nickel(II) species, followed by ligand dissociation. Thus, the observation of metal complex fragments (rather than intact species) binding DNA does not necessarily suggest that the binding of intact complexes does not occur, but rather that if it does, the resulting DNA adducts are unstable to ESI-MS analysis.

Results similar to those using \([\text{Pd(tmeda)(PEGda)}]^2+\) were obtained when a related PEGda complex, \([\text{Pd}(2,2'\text{-bipy})(\text{PEGda})]^2+\), was employed in the analysis (Figure 4.6). This complex was also found to undergo decomposition, with ions representing \(\text{D2a}\) adducts of \([\text{Pd}(2,2'\text{-bipy})]^2+\) and PEGdaH\(^2+\) (in addition to very weak ions of \([\text{D2a} + \text{Pd(tmeda)(PEGda)}^2+ - 6\text{H}^+]^{4+}\) being observed.

![Figure 4.6: ESI-MS of D2a with [Pd(2,2'-bipy)(PEGda)]^2+ (1 : 1 in 0.01 M NH\(_4\)OAc); ○ [D2a - nH\(^+\)]\(^{n-}\) (n = 3, 4, 5); □ [D2a + Pd(2,2'-bipy)(PEGda)]\(^2+ - 6\text{H}^+\)\(^+\); △ [D2a + PEGda - 4\text{H}^+]\(^+\); □ [D2a + Pd(2,2'-bipy)]\(^2+ - n\text{H}^+]\(^{(n-2)-}\) (n = 5, 6, 7); □ [D2a + 2Pd(2,2'-bipy)]\(^2+ - n\text{H}^+]\(^{(n-4)-}\) (n = 8, 9).](image-url)

The interactions of the metallocycle \([\text{Pd}_2(2,2'-\text{bipy})_2\{4,4'\text{-bipy(CH\(_2\))_4},4,4'\text{-bipy}\}_2]^8+\) with \(\text{D2a}\) were studied in much the same way as those described for \(\text{D2}\). Importantly, while this dinuclear complex was not expected to be of a sufficient size to encircle dsDNA, its cavity might be large enough to host ssDNA. The mass spectrum of a mixture containing \(\text{D2a}\) and \([\text{Pd}_2(2,2'-\text{bipy})_2\{4,4'\text{-bipy(CH\(_2\))_4},4,4'\text{-bipy}\}_2]^8+\) (1 : 1) is given in Figure 4.7.

‡‡ The other possible explanation is that \([\text{Pd(tmeda)(PEGda)}]^2+\) first fragments, and only then binds to \(\text{D2a}\).
The complex \([\text{Pd}_2(2,2^'\text{-bipy})_2\{4,4'^\text{-bipy}(\text{CH}_2)_44,4'^\text{-bipy}\}]_2^{8^+}\) was found to fragment to a large extent when \(\text{D2a}\) was present, this being evidenced by the observation of several combinations of the individual components binding \(\text{D2a}\). Nevertheless, the results are significantly different to those obtained with \(\text{D2}\). The single-stranded \(\text{D2a}\) molecules appear to have a particularly high affinity for \(\text{Pd}(2,2'^\text{-bipy})^{2^+}\) moieties, with the most intense ion in the spectrum being assigned to \([\text{D2a} + \text{Pd}(2,2'^\text{-bipy})]^{4^+} - 6\text{H}^+\)^{4+}. Indeed, ions containing \(\text{D2a}\) and up to four \(\text{Pd}(2,2'^\text{-bipy})^{2^+}\) fragments were observed, highlighting the ability of ssDNA to form covalent adducts with compounds of the present type. While the binding of larger fragments of the dinuclear complexes to \(\text{D2a}\) was detected, it was also found that \(\text{D2a}\) adducts of intact \([\text{Pd}_2(2,2'^\text{-bipy})_2\{4,4'^\text{-bipy}(\text{CH}_2)_44,4'^\text{-bipy}\}]_2^{8^+}\) (1 : 1) were formed. This is in contrast to the situation with \(\text{D2}\), to which no intact metallocycles were found to bind.

The tetracation \([\text{Pd}(2,2'^\text{-bipy})(\text{Mebipy})]^{4^+}\), a mononuclear analogue of the dipalladium(II) complex above, was treated with \(\text{D2a}\) in order to compare its binding with that of the dinuclear complex. ESI-MS analysis confirmed the presence of adducts containing the fragments \(\text{Mebipy}^+\), \([\text{Pd}(2,2'^\text{-bipy})]^{2^+}\) and \([\text{Pd}(2,2'^\text{-bipy})(\text{Mebipy})]^{3^+}\) bound to \(\text{D2a}\).
(Figure 4.8). It is likely that coordination of nucleobases to palladium(II) is involved in the binding of the latter two fragments.

In contrast to the situation with the dinuclear complex, no ions corresponding to **D2a** adducts of intact [Pd(2,2'-bipy)(Mebipy)$_2$]$^{4+}$ could be observed. This might be ascribed to the lower affinity these less cationic complexes have for **D2a**, or perhaps the rapid dissociation of Mebipy$^+$ ligands from the palladium(II) ions during the ionisation process.

### 4.5.6 Interaction of platinum(II) complexes with D2

Owing to the propensity of the palladium(II) complexes to fragment in the presence of **D2** and **D2a**, it was anticipated that similar experiments using analogous platinum(II) species might provide more valuable information regarding the DNA-binding of (intact) metallocycles. The use of the more kinetically inert platinum(II) derivatives would most likely result in DNA-binding, rather than fragmentation, being the dominant process. This Section describes ESI-MS studies of the interactions between the platinum(II) compounds 108, 110, 126 – 128, 146, 148 and **D2**.

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$^\dagger$ As will become clear in the next Section, in the discussion of **D2** binding of [Pt(2,2'-bipy)(Mebipy)$_2$]$^{4+}$, the latter reason appears much more feasible.
The complex \([\text{Pt}(\text{tmeda})(\text{PEGda})]^2+\) was treated with \(\text{D2}\) in anticipation of observing strong ions corresponding to \(\text{D2}\) adducts of intact PEGda macrocycles. The ESI mass spectrum of a solution containing equimolar quantities of the two components is presented in Figure 4.9.

![Figure 4.9: ESI-MS of D2 with [Pt(tmeda)(PEGda)]^2+ (1 : 1 in 0.01 M NH_4OAc). Intensities of ions in the ranges \(m/z\) 1800 – 1850 and 2100 – 2300 are magnified \(\times10\); • \([\text{D2} – n\text{H}^+]^{n–}\) (\(n = 5, 6\)); ◆ \([\text{D2a} – 3\text{H}^+]^{3–}\); ◆ \([\text{D2b} – 3\text{H}^+]^{3–}\); • \([\text{D2} + \text{Pt}(\text{tmeda})(\text{PEGda})^2+ – n\text{H}^+]^{(n–2)–}\) (\(n = 7, 8\)); ◆ ◆ ◆ \([\text{D2} + \text{PEGda} – 5\text{H}^+]^{5–}\); □ ◆ ◆ ◆ ◆ ◆ \([\text{D2} + \text{Pt}(\text{tmeda})(\text{PEGda})^2+ + \text{Pt}(\text{tmeda})^2+ – 9\text{H}^+]^{5–}\).]

In a similar result to that obtained with the palladium(II) analogue, the high intensity of free \(\text{D2}\) (\([\text{D2} – n\text{H}^+]^{n–}\) \(n = 5, 6\)) relative to other ions, indicated that the binding of \(\text{D2}\) to [\(\text{Pt}(\text{tmeda})(\text{PEGda})\)]^2+ is very weak. While the intensities of adduct ions were very low (these regions are expanded in Figure 4.9 to allow for them to be viewed), the species [\(\text{D2} + \text{Pt}(\text{tmeda})(\text{PEGda})^{2+} – n\text{H}^+]^{(n–2)–}\) (\(n = 7, 8\)) could indeed be detected in the gas phase. While decomposition of [\(\text{Pt}(\text{tmeda})(\text{PEGda})^{2+}\) was found to occur (\(\text{D2}\) was observed to bind the fragments [\(\text{Pt}(\text{tmeda})^{2+}\) and PEGdaH_2^{2+}], this complex is much more stable to MS analysis than the palladium(II) analogue, in which ions incorporating the respective fragments and \(\text{D2}\) were of greater intensity, relative to free \(\text{D2}\).

The use of [\(\text{Pt}(\text{en})(\text{PEGda})^{2+}\) in place of [\(\text{Pt}(\text{tmeda})(\text{PEGda})^{2+}\) allowed for a spectrum to be collected (see Appendices, Section A.4.1, Figure A.22) which was comparable to that presented above, a result which was not unexpected, considering the structural similarity of the two complexes. Given the strength with which [\(\text{Pt}(\text{tmeda})^{2+}\) and [\(\text{Pt}(\text{en})^{2+}\) are expected to bind \(\text{D2}\), it is likely that intense ions corresponding to \(\text{D2}\) adducts of these chelates would be observed if the fragments were to be present in significant quantities. This appears not to be the case in the present example, and consequently it can be inferred that [\(\text{Pt}(\text{tmeda})(\text{PEGda})^{2+}\) can survive the ionisation process intact and that its gas phase interactions with \(\text{D2}\) are genuinely very weak. No \(\text{D2}\) adducts of any form could be
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identified when the related complex \([\text{Pt}(2,2\prime\text{-bipy})(\text{PEGda})]^{2+}\) was employed. In this case, the only ions observed (see Appendices, Section A.4.1, Figure A.23) were those of free \(D2\); at present it is not known why the results obtained using \([\text{Pt}(2,2\prime\text{-bipy})(\text{PEGda})]^{2+}\) are different to those for the other PEGda complexes discussed above. Interestingly, the simple diammine complex \([\text{Pt}(2,2\prime\text{-bipy})(\text{NH}_{3})_{2}]^{2+}\), an acyclic analogue of \([\text{Pt}(2,2\prime\text{-bipy})(\text{PEGda})]^{2+}\), was found to have significant affinity for \(D2\). At a 1 : 1 molar ratio, two of these dications could bind each duplex, with up to four complexes interacting with a single \(D2\) when a 1 : 5 molar ratio is used (Figure 4.10).

![Figure 4.10: ESI-MS of D2 with \([\text{Pt}(2,2\prime\text{-bipy})(\text{NH}_{3})_{2}]^{2+}\) (1 : 5 in 0.1 M \(\text{NH}_{4}\text{OAc}\); • \([D2 – n\text{H}^+]^{n–}\) (n = 4, 5, 6); \(\circ\) \([\text{D2a} – 3\text{H}^+]^{3–}\); \(\triangle\) \([\text{D2b} – 3\text{H}^+]^{3–}\); \(\ast\ast\) \([\text{D2} + \text{Pt}(2,2\prime\text{-bipy})(\text{NH}_{3})_{2}]^{2+} – n\text{H}^+\)\(^{(n-2)}\) (n = 6, 7, 8); \(\ast\ast\ast\) \([\text{D2} + 2\text{Pt}(2,2\prime\text{-bipy})(\text{NH}_{3})_{2}]^{2+} – n\text{H}^+\)\(^{(n-4)}\) (n = 8, 9, 10); \(\ast\ast\ast\ast\) \([\text{D2} + 3\text{Pt}(2,2\prime\text{-bipy})(\text{NH}_{3})_{2}]^{2+} – n\text{H}^+\)\(^{(n-6)}\) (n = 11, 12); \(\ast\ast\ast\ast\ast\) \([\text{D2} + 4\text{Pt}(2,2\prime\text{-bipy})(\text{NH}_{3})_{2}]^{2+} – 13\text{H}^+\)\(^{5–}\).

The most intense adduct ions observed are assigned to 1 : 1 species, with ions corresponding to the binding of two, three and four diammine complexes being successively weaker. While both \([\text{Pt}(2,2\prime\text{-bipy})(\text{NH}_{3})_{2}]^{2+}\) and \([\text{Pt}(2,2\prime\text{-bipy})(\text{PEGda})]^{2+}\) can potentially intercalate into \(D2\), it appears that the former has a much higher propensity to form adducts detectable by ESI-MS. This disparity was considered to perhaps arise from the PEGda complex being less stable than its acyclic analogue, \(***\) or the PEGda ligand somehow hindering intercalation (as well as not participating in a topological interaction with DNA).

ESI-MS analysis of a mixture containing equimolar amounts of the diplatinum(II) complex \([\text{Pt}_{2}(2,2\prime\text{-bipy})_{2}[\text{4,4′-bipy(CH}_{2})_{4 \text{4′-bipy}}]_{2}]^{8+}\) and \(D2\) was performed. The results obtained (Figure 4.11), in addition to confirming the presence of ions from free DNA (\(D2, D2a\) and

*** It is unlikely that decomposition, if it is occurring, involves reaction with \(\text{NH}_{4}\text{OAc}\). \([\text{Pt}(2,2\prime\text{-bipy})(\text{NH}_{3})_{2}]^{2+}\) and \([\text{Pt}(2,2\prime\text{-bipy})(\text{OAc})_{2}]\) are likely products of this process, species which would be expected to give detectable adducts of \(D2\).
D2b), also revealed the formation of 1 : 1 D2 adducts of the metallocycle. No ions indicating the binding of D2 to fragments of the octacation were detected, suggesting that the latter is somewhat stable to the ionisation process. This can be contrasted to the situation with the related (but more labile) dipalladium(II) species, in which no D2 adducts of intact species could be detected, although several fragments of the complex were found to bind D2.

![Figure 4.11: ESI-MS of D2 with [Pt₂(2,2'-bipy)₂][4,4'-bipy(CH₂)₄4,4'-bipy]₂⁸⁺ (1 : 1 in 0.01 M NH₄OAc); • [D2 – nH⁺]ⁿ⁻ (n = 5, 6); ◊ [D2a – 3H⁺]³⁺; ◭ [D2b – 3H⁺]⁺; • [D2 + Pt₂(2,2'-bipy)₂][4,4'-bipy(CH₂)₄4,4'-bipy]₂⁴⁺ – 6H⁺]⁻⁻ (n = 13, 14).](image)

Interestingly, when either positive or negative-ion ESI-MS analysis of the dinuclear platinum(II) species alone (as its NO₃⁻ salt) was performed, no ions corresponding to intact complexes could be detected. The fact that D2 could be observed to bind the intact [Pt₂(2,2'-bipy)₂][4,4'-bipy(CH₂)₄4,4'-bipy]₂⁸⁺ metallocycles suggests that the presence of [D2 – nH⁺]ⁿ⁺ polyanions might stabilise the platinum(II) complex. It is possible that the binding of these duplexes to the metallocycle allows for strong electrostatic interactions to hold the complex together. When DNA is absent, however, the species dissociates into its (mutually repulsive) cationic components.

In order to better understand the effects that size and charge of complex species have on DNA-binding, the interactions of D2 were also studied with the mononuclear derivative [Pt(2,2'-bipy)(Mebipy)₂]⁴⁺. This complex, which represents approximately one half of the species [Pt₂(2,2'-bipy)₂][4,4'-bipy(CH₂)₄4,4'-bipy]₂⁸⁺, was combined with equimolar D2 and the resulting mixture subjected to ESI-MS (Figure 4.12). The spectrum features intense 6⁻ ions arising from free D2 and its 1 : 1 adduct with [Pt(2,2'-bipy)(Mebipy)₂]⁴⁺, with ions

††† When 2.5 molar equivalents of the related complex [Pt₃(2,2'-bipy)₂][4,4'-bipy(CH₂)₄4,4'-bipy]₂⁸⁺ were used, ions assigned to two of these binding D2 were detected (see Appendices, Section A.4.2, Figure A.35). However, the higher concentration also allowed for D2 adducts of fragment ions to be observed.
of these species in the 5– charge state also being prominent. Additionally, small amounts of the single strands D2a and D2b were also detected, some of which could be observed to bind [Pt(2,2′-bipy)(Mebipy)$_2$]$^{4+}$.

Figure 4.12: ESI-MS of D2 with [Pt(2,2′-bipy)(Mebipy)$_2$]$^{4+}$ (1 : 1 in 0.01 M NH$_4$OAc); • [D2 – nH$^+$]$^n$ (n = 4, 5, 6); ◦ [D2a – 3H$^+$]$^3$; ◼ [D2b – 3H$^+$]$^3$; ••• [D2 + Pt(2,2′-bipy)(Mebipy)$_2$]$^{4+}$ – nH$^+$]$^{(n-4)}$ (n = 9, 10); •••• [D2 + 2Pt(2,2′-bipy)(Mebipy)$_2$]$^{4+}$ – nH$^+$]$^{(n-8)}$ (n = 13, 14); ◆◆◆ [D2a + Pt(2,2′-bipy)(Mebipy)$_2$]$^{4+}$ – 7H$^+$]$^3$; ◆◆◆◆ [D2b + Pt(2,2′-bipy)(Mebipy)$_2$]$^{4+}$ – 7H$^+$]$^3$.

Perhaps the most important aspect of the analysis is the presence of weak, but detectable ions with $m/z$ values higher than [D2 + Pt(2,2′-bipy)(Mebipy)$_2$]$^{4+}$ – nH$^+$]$^{(n-4)}$. These are assigned to a 1 : 2 adduct, present as both 6– and 5– anions. It is possible that the lower charge of the mononuclear complex relative to the dinuclear complex has an effect on why this adduct is observed in this case, but not when the dinuclear complex is employed. In particular, the charge neutralisation of D2 upon binding the dinuclear octacation is likely to cause the DNA-binding of a second complex moiety to be less favourable. This might not be so pronounced when the complex bears only a 4+ charge, although other effects, such as the size of the complexes, as well as their binding sites, are also likely to influence the binding. With respect to the latter, the size of the binding site of the mononuclear complex is almost certainly smaller than that of the dinuclear species, allowing for more of the mononuclear complexes to be accommodated on a given length of DNA.

As was the case with the palladium(II) complexes, the platinum(II) analogues were also studied with D2a. The results of these ssDNA-binding experiments are presented in the following Section.
4.5.7 Interaction of platinum(II) complexes with D2a

ESI-MS analysis of a equimolar mixture of D2a and [Pt(tmeda)(PEGda)]^{2+} in 0.01 M NH₄OAc afforded the spectrum presented in Figure 4.13.

![Figure 4.13: ESI-MS of D2a with [Pt(tmeda)(PEGda)]^{2+} (1 : 1 in 0.01 M NH₄OAc). Intensities of ions in the range m/z 1450 – 1650 are magnified ×10; ○ [D2a – 3H^+]^{3–}; ● [D2a + Pt(tmeda)(PEGda)^{2+} – 5H^+]^{3–}.](image)

The most intense ions detected were those assigned to free D2a (here [D2a – 3H^+]^{3–}). The only other ion observed was at m/z 1501, a value consistent with the presence of the adduct [D2a + Pt(tmeda)(PEGda)^{2+} – 5H^+]^{3–}. The low intensity of this ion (relative to that of free D2a) suggests that [Pt(tmeda)(PEGda)]^{2+} has negligible affinity for D2a, comparable to, if not lower, than that for D2. While the exact nature of the binding is not clear, it is possible that the reduced charge of D2a (compared to D2) may be a cause of this weaker binding.‡‡‡

It was anticipated that the bipyridinium complex [Pt₂(2,2′-bipy)₂{4,4′-bipy(CH₂)₄4,4′-bipy}₂]^{8+}, owing to its greater charge and aryl surface area compared to [Pt(tmeda)(PEGda)]^{2+}, would allow it to bind D2a more strongly than the latter species. Indeed, it was found that when a solution containing D2a and [Pt₂(2,2′-bipy)₂{4,4′-bipy(CH₂)₄4,4′-bipy}₂]^{8+} was subjected to ESI-MS, the most intense ion in the resulting spectrum was not of free D2a, but rather D2a bound to one dinuclear complex (Figure 4.14(a)).§§§

‡‡‡ In the absence of complexes, the 16mers (as 10 µM solutions in 0.01 M NH₄OAc) were analysed under identical conditions and the most abundant charge state for D2 was 6–, while that for D2a was 4–.

§§§ A 1 : 1 adduct was also observed when [Pt₂(2,2′-bipy)₂{4,4′-bipy(CH₂)₆4,4′-bipy}₂]^{8+} was used (Appendices Section A.4.1, Figure A.24).
The analysis allowed for the detection of other adduct species, including those containing the fragments \([\text{Pt}(2,2'\text{-bipy})]^2^+\) and \([\text{Pt}(2,2'\text{-bipy})(\text{Mebipy})]^3^+\), suggesting that...
[Pt(2,2′-bipy)(Mebipy)$_2$]$^{4+}$ decomposes under the ESI conditions employed. Such fragmentation, not observed in the analysis of D2/[Pt(2,2′-bipy)(Mebipy)$_2$]$^{4+}$ mixtures, may be due to the increased reactivity of the nucleobases in D2a relative to D2 resulting in the displacement of Mebipy$^+$ ligands.

4.5.8 Comparison of D2-binding affinities of platinum(II) complexes

In order to compare the relative DNA-binding strengths of selected platinum(II) complexes, further ESI-MS experiments were conducted under identical conditions, from which important additional information could be obtained. This was made possible because of the moderate stability and affinity for D2 that the complexes showed under the ionisation conditions. Four complexes were chosen for this study: [Pt(2,2′-bipy)(Mebipy)$_2$]$^{4+}$, [Pt$_2$(2,2′-bipy)$_2${4,4′-bipy(CH$_2$)$_6$4,4′-bipy}$_2$]$^{8+}$, [Pt(2,2′-bipy)(NH$_3$)$_2$]$^{2+}$ and [Pt(en)(PEGda)]$^{2+}$. The PEGda complex, owing to its large cavity (which might potentially encircle D2), was considered to be an important species in the analysis. However, as described in the previous Section, its affinity for D2 was found to be low. As a consequence, a 1 : 5 ([D2] = 10 µM, [complex] = 50 µM) molar ratio was used for each of the mixtures, except for the dinuclear complex, for which a 1 : 2.5 ratio was employed. Higher concentrations of this complex caused an unidentified white material to precipitate from solution. It is possible that this solid might take the form of a [Pt$_2$(2,2′-bipy)$_2${4,4′-bipy(CH$_2$)$_6$4,4′-bipy}$_2$]$^{8+}$ salt of D2, given the high charge of both species. If this were the case, it could in itself be evidence of strong binding. In any event, owing to the different ratio used for the dinuclear complex, the results for this species cannot strictly be compared to those of the other complexes, although some definite conclusions can be drawn (vide infra).

Previous work has demonstrated that a convenient way of comparing DNA-binding is to consider the relative abundances of different DNA/drug adducts. Thus, solutions containing DNA and each compound were prepared in 0.1 M NH$_4$OAc and subjected to ESI-MS analysis (using cone voltage = 70 V), from which the data of interest could be obtained. This was achieved for each adduct by summing the intensities of 6– and 5– ions assigned to this species and dividing by the total intensity of all 6– and 5– ions containing D2 (either free or bound). These two charge states were selected as these ions were typically

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**** All experiments were also repeated at cone voltages of 90 V, 110 V, 130 V and 150 V.
found to be the most intense in the spectra acquired. The analysis is illustrated below in the case of \([\text{Pt}(2,2\text{'-bipy})(\text{Mebipy})_2]^{4+}\), with the ESI mass spectrum given in Figure 4.15.

![Figure 4.15: ESI-MS of D2 with \([\text{Pt}(2,2\text{'-bipy})(\text{Mebipy})_2]^{4+}\) (1 : 5 in 0.1 M NH₄OAc); •• [D2 + Pt(2,2\text{'-bipy})(Mebipy)\text{\textsubscript{2}}]^{4+} - n\text{H}^+)^{[m/z]} (n = 9, 10); ••• [D2 + 2Pt(2,2\text{'-bipy})(Mebipy)\text{\textsubscript{2}}]^{4+} - n\text{H}^+)^{[m/z]} (n = 13, 14); •••• [D2 + 3Pt(2,2\text{'-bipy})(Mebipy)\text{\textsubscript{2}}]^{4+} - n\text{H}^+)^{[m/z]} (n = 17, 18); ••••• [D2 + 4Pt(2,2\text{'-bipy})(Mebipy)\text{\textsubscript{2}}]^{4+} - n\text{H}^+)^{[m/z]} (n = 21, 22).

Two progressions of 6– and 5– ions could be detected, with each ion corresponding to a \(\text{D2}\) molecule bound to between one and four complexes (c.f. Figure 4.12, in which the use of a 1 : 1 molar ratio resulted in binding of up to two complexes). The relative abundance of, for example, the 1 : 2 adduct, is determined by adding the intensities of the 6– and 5– ions of this species (31 and 28 at \(m/z\) 1857 and 2228, respectively) and dividing by the total intensity of all 6– and 5– ions (156), to give a relative abundance = \(\frac{31 + 28}{156} \times 100\% = 38\%\). This was repeated for all ions of \(\text{D2}\) (and its adducts) for each mixture, with the relevant spectra and analyses, including those acquired using other cone voltages, being provided in the Appendices (Section A.4.2, Figures A.25-A.36).

The DNA-binding of the four platinum(tII) complexes was compared to two well-studied DNA-binding agents, distamycin A (150, hereafter referred to simply as distamycin) and daunomycin (151), which were subjected to the same ESI-MS analysis described above. The compounds represent two monobasic (monoprotonated at pH 7.4) organic drugs whose interactions with DNA are known in detail, in particular by means of extensive mass spectrometric investigations.\(^{39}\)

The naturally-occurring tripeptide distamycin (150) is a minor groove binder and ESI-MS studies (both positive- and negative-ion) on its interactions with DNA have shown it to possess selectivity for AT base-pairs.\(^{33,39,40}\) In addition to electrostatic forces, the amide
groups of this antibiotic serve as H-bond donors to N$_3$\textsubscript{adenine} and O$_2$\textsubscript{thymine}. In contrast, the chemotherapeutic agent daunomycin (151) contains an intercalating anthraquinone fragment, as well as a minor groove-binding aminosugar (daunosamine) moiety. Daunomycin, because of its delocalised, polarisable electronic structure and electron-deficient nature, can bind DNA strongly, showing preference for GC base-pairs. Mass spectra acquired using distamycin and daunomycin are presented below in Figures 4.16(a) and 4.16(b), respectively.

As expected, distamycin was found to have a high affinity towards D2, a fact evidenced by the high intensities of the ions assigned to 1 : 2 and 1 : 4 adducts and the absence of free D2. The binding of distamycin in pairs suggests cooperative interactions are involved which, for example, would cause the binding of a second molecule to D2 to be more favourable than the first. Indeed, it has been reported that the expansion of the DNA minor groove allows for the accommodation of two distamycin molecules. A similar result to that documented here was reported by Beck and co-workers, in which a 1 : 3 D2/distamycin mixture (in 0.1 M NH$_4$OAc) afforded exclusively 1 : 2 adducts in the negative-ion ESI mass spectrum. No 1 : 4 adducts could be detected in their analysis, a result most likely related to the lower molar ratio of distamycin used in their study.
Daunomycin also exhibited strong binding to D2 with adducts containing up to five such intercalator species being detected. The distribution of the relevant intensities is very similar to previous studies with other 16mer duplexes, and is quite feasible given that the average size of a DNA-binding site of daunomycin is two base-pairs.\textsuperscript{29,45}

With the analysis of the four complexes and two organic drugs complete, a plot of the relative abundances of DNA/drug adducts for each agent could be generated (Figure 4.17).

As was expected given the results presented in Section 4.5.6, [Pt(en)(PEGda)]\textsuperscript{2+} was found to be a weak D2-binder, with 1 : 1 adducts being present at 5% relative abundance. Indeed, most of the intensity assigned to ions containing D2 (95%) could be attributed to free D2. The complex [Pt(2,2′-bipy)(NH\textsubscript{3})\textsubscript{2}]\textsuperscript{2+} afforded adducts containing up to four of these species bound to D2, with a much lower relative abundance of free D2 being observed (21%). As a consequence, it can be concluded that [Pt(en)(PEGda)]\textsuperscript{2+} binds D2 with lesser strength than does [Pt(2,2′-bipy)(NH\textsubscript{3})\textsubscript{2}]\textsuperscript{2+}. Another complex which could bind D2 at a 1 : 4 ratio was [Pt(2,2′-bipy)(Mebipy)\textsubscript{2}]\textsuperscript{4+}. When these tetracations were used, no free D2 could be detected, which suggested the bipyridinium compound to bind more strongly than

\textsuperscript{++} Saturation of a 16 base-pair dsDNA sequence typically occurs once eight daunomycin molecules are bound.\textsuperscript{29}
[Pt(2,2'-bipy)(NH₃)₂]²⁺. This conclusion was also supported by the 1 : 2 and 1 : 3 adducts having greater relative intensities in the case of the bipyridinium complex. It was considered likely that the high charge and aryl surface area of [Pt(2,2'-bipy)(Mebipy)₂]⁴⁺ contribute to this high affinity for D₂.

The binding profile of daunomycin is similar to that for [Pt(2,2'-bipy)(Mebipy)₂]⁴⁺, although it is shifted to a greater number of species being bound to D₂. Considering that ions incorporating up to five daunomycin molecules could be detected, and taking into account the relative intensities of 1 : 4 adducts (36% and 10% for daunomycin and [Pt(2,2'-bipy)(Mebipy)₂]⁴⁺, respectively), it is clear that daunomycin possesses a higher affinity for D₂ than does the tetracation. It is possible that this is due to the interaction of D₂ with [Pt(2,2'-bipy)(Mebipy)₂]⁴⁺ being weaker than that with daunomycin, although other influences, including the charge differences and the size of the binding site must also be considered. For example, the DNA-binding site of the tetracation might be larger than that of daunomycin, and thus it is feasible that the D₂-binding of the complex could be stronger than that of daunomycin. In this case the larger binding site of the tetracation indicates that fewer species can be accommodated on a single D₂ molecule, relative to daunomycin.

As with the other species, the binding curve for [Pt₂(2,2'-bipy)₂{4,4'-bipy(CH₂)₆4,4'-bipy}₂]⁶⁺ is also given, although, as mentioned above, the lower concentration at which it was used prevents a close analysis. Nevertheless, the presence of this complex at 25 µM (with 10 µM D₂) allowed for adducts containing two dinuclear species bound to D₂ to be observed, the relevant spectrum being presented in Figure 4.18.

![Figure 4.18: ESI-MS of D2 with [Pt₂(2,2'-bipy)₂{4,4'-bipy(CH₂)₆4,4'-bipy}₂]⁶⁺ (1 : 2.5 in 0.1 M NH₄OAc); • [D₂ – nH⁺]ⁿ⁺ (n = 5, 6); □ [D₂a – 3H⁺]³⁺; □ [D₂b – 3H⁺]³⁺; * [D₂ + Pt₂(2,2'-bipy)₂{4,4'-bipy(CH₂)₆4,4'-bipy}₂]⁶⁺ – nH⁺]ⁿ⁻⁻; ▪ [D₂ + 2{Pt₂(2,2'-bipy)₂{4,4'-bipy(CH₂)₆4,4'-bipy}₂]⁶⁺ – nH⁺]ⁿ⁻⁻](image)
When a 1 : 1 molar ratio of the related butylene derivative was used, the complex did not undergo fragmentation, with the only adducts that could be observed being of a 1 : 1 stoichiometry (Figure 4.11, vide supra). In contrast, the use 25 µM complex allows for decomposition of this species to be observed, as evidenced by the detection of several weak adduct ions incorporating fragments of the metallocycle. Thus, the lower concentration of $[\text{Pt}_2(2,2'$-bipy)$_2(4,4'$-bipy)$_2]^{8+}$ used, in addition to fragmentation processes competing with the $\text{D}_2$-binding of intact octacations, does not allow much information to be gleaned from the analysis. Despite this, it is clear that $[\text{Pt}_2(2,2'$-bipy)$_2(4,4'$-bipy)$_2]^{8+}$ has a much higher propensity than $[\text{Pt}(\text{en})(\text{PEGda})]^{2+}$ to form $\text{D}_2$ adducts, a result which is probably due to the charge and extended $\pi$-systems of the former.

Despite the complexes of PEGda being weak binders of $\text{D}_2$, further ESI-MS studies of the interactions these species have with DNA were nevertheless undertaken. This work is described in the following Section.

### 4.5.9 Interaction of PEGda complexes with $\text{D}_2'$

In addition to the studies presented above concerning the binding of $\text{D}_2$, preliminary experiments were also conducted using the related sequence $\text{D}_2'$. This 16mer has a sequence of nucleobases identical to $\text{D}_2$, but is biotinylated at each 5' terminus. It could be prepared by annealing equimolar amounts of the biotinylated single strands $\text{D}_2'a$ and $\text{D}_2'b$; the ESI mass spectra of the two individual strands, as well as the duplex $\text{D}_2'$ are provided in the Appendices (Section A.4.3, Figures A.37-A.39).

The biotin groups present in $\text{D}_2'$ allow it to bind two avidin proteins, one at either end of the duplex. It was thought that if PEGda macrocycles of the type $[\text{Pt}(N,N)(\text{PEGda})]^{2+}$ were to thread $\text{D}_2'$, then these pseudorotaxanes might be converted to rotaxanes by using streptavidin ($d_{\text{streptavidin}} \approx 4.6 \text{ nm}$) as a stopper (Scheme 4.1). While the strong non-covalent
biotin-avidin interaction ($K_D \approx 10^{-15}$ M)$^{47}$ has recently been employed in the generation of pseudorotaxanes, as mentioned in the Introduction to this Thesis, the use of DNA as the thread in a synthetic rotaxane would be without precedent.

Scheme 4.1: Proposed synthesis of a [2]rotaxane comprising D2', a macrocycle and two avidin molecules, all of which are held together by non-covalent interactions. In principle, any number of macrocycles might thread D2', with [n]rotaxanes being the general description of the final product(s).

If unambiguous characterisation of the resultant product(s) could be achieved, then conclusions could be drawn regarding whether or not the interactions of D2 with [Pt(N,N)(PEGda)]$^{2+}$ are of a topological nature. For example, if threading does not occur, the macrocyclic ‘wheel’ and stoppered ‘axle’ would not be interlocked, although the two components might nevertheless remain weakly associated with each other. The use of, for example, ESI-MS might allow for this situation to be distinguished from that depicted in Scheme 4.1.

The first and most crucial step in the synthesis involves the characterisation of a mixture possibly containing D2'-based pseudorotaxanes. The complexes of PEGda were considered the most likely candidates for this purpose and the binding of these to D2' was investigated with two such macrocycles, [Pt(tmeda)(PEGda)]$^{2+}$ and [Pt(2,2'-bipy)(PEGda)]$^{2+}$. The ESI mass spectrum of D2' in the presence of the former (at a 1 : 5 ratio in 0.1 M NH$_4$OAc) is given below (Figure 4.19).

Figure 4.19: ESI-MS of D2' with [Pt(tmeda)(PEGda)]$^{2+}$ (1 : 5 in 0.01 M NH$_4$OAc); • [D2' – nH]$^{n-}$ (n = 5, 6); ○ [D2'a – 3H$^+$]$^{3+}$; □ [D2'b – 3H$^+$]$^{3+}$; ◦ [D2' + Pt(tmeda)(PEGda)]$^{2+}$ – nH$^+$(n = 7, 8); ○ [D2' + PEGda – 6H$^+$]$^{6+}$; • [D2' + Pt(tmeda)(PEGda)]$^{2+}$ + Pt(tmeda)$^{2+}$ – 9H$^+$]$.^{3+}$.
While the most intense ions in the spectrum are assigned to free D2’ (m/z 1817 and 2180 are assigned to the 6– and 5– charge states, respectively), the detection of other ions could be attributed to the formation of new species. In particular, the presence of the 1 : 1 adduct [D2’ + Pt(tmeda)(PEGda)]^{2+} – nH^+(m–2)− (n = 7, 8) could be confirmed, although the low intensity of its ions relative to those of D2’ indicate that the binding is of a weak nature. Adducts incorporating the individual fragments PEGda and [Pt(tmeda)]^{2+} were also observed, suggesting that decomposition was occurring under the conditions employed. Apart from differences in m/z values resulting from biotinylation, the spectrum in Figure 4.19 is remarkably similar to that presented in Figure 4.9, obtained using D2. Thus, it is highly likely that the biotin groups do not play a major role in the binding of D2’ by [Pt(tmeda)(PEGda)]^{2+}.

The ESI-MS analysis was repeated for [Pt(2,2’-bipy)(PEGda)]^{2+}, with the spectrum presented in Figure 4.20. This complex was found to bind D2’ with some strength, as indicated by the detection of ions consistent with the interaction of up to four macrocycles with a single duplex oligonucleotide. Two progressions of 6– and 5– ions with increasing m/z values were observed, these representing D2’ binding to successive [Pt(2,2’-bipy)(PEGda)]^{2+} complexes.†††† Apart from the observation of ions corresponding to D2’ and its single strands D2’a and D2’b, evidence could also be obtained for the fragmentation of [Pt(2,2’-bipy)(PEGda)]^{2+}, as well as its interaction with D2’a.

Figure 4.20: ESI-MS of D2’ with [Pt(2,2’-bipy)(PEGda)]^{2+} (1 : 5 in 0.01 M NH4OAc); †††† [D2’ – nH^+]^{m−} (n = 6, 8); ○* [D2’a – nH^+]^{m−} (n = 3, 4); ○b [D2’b – 4H^+]^{m+}; •* [D2’ + Pt(2,2’-bipy)(PEGda)]^{2+} – nH^+(m–2)− (n = 7, 8); •* [D2’ + 2Pt(2,2’-bipy)(PEGda)]^{2+} – nH^+(m–4)− (n = 9, 10); •* [D2’ + 3Pt(2,2’-bipy)(PEGda)]^{2+} – 12H^+]^{m+}; •* [D2’ + 4Pt(2,2’-bipy)(PEGda)]^{2+} – 14H^+]^{m+}; •* [D2’ + Pt(2,2’-bipy)(PEGda)]^{2+} – nH^+(m–6)− (n = 11, 12); •* [D2’ + 2Pt(2,2’-bipy)(PEGda)]^{2+} – 16H^+]^{m+}; ○* [D2’a + Pt(2,2’-bipy)(PEGda)]^{2+} – nH^+(m–2)− (n = 6, 8); ○b [D2’a – nH^+]^{m−} (n = 3, 4); ○* [D2’b – 4H^+]^{m+}; •* [D2’ + Pt(2,2’-bipy)(PEGda)]^{2+} – nH^+(m–2)− (n = 7, 8); •* [D2’ + 2Pt(2,2’-bipy)(PEGda)]^{2+} – nH^+(m–4)− (n = 9, 10); •* [D2’ + 3Pt(2,2’-bipy)(PEGda)]^{2+} – 12H^+]^{m+}; •* [D2’ + 4Pt(2,2’-bipy)(PEGda)]^{2+} – 14H^+]^{m+}; •* [D2’ + Pt(2,2’-bipy)(PEGda)]^{2+} – nH^+(m–6)− (n = 11, 12); •* [D2’ + 2Pt(2,2’-bipy)(PEGda)]^{2+} – 16H^+]^{m+}; ○* [D2’a + Pt(2,2’-bipy)(PEGda)]^{2+} – nH^+(m–2)− (n = 6, 8); ○b [D2’a – nH^+]^{m−} (n = 3, 4). The analysis may suggest that more than four complexes can bind a single D2’ duplex, although the poor resolution in the range m/z > 2900 prevents any further assignment of the spectrum.
The observation that \([\text{Pt}(2,2\text{'-bipy})(\text{PEGda})]^2+\) possesses moderate affinity for \(\text{D}2\text{'-}\) came as a very surprising result, especially considering that no adduct ions could be detected when the non-biotinylated dsDNA \(\text{D}2\) was used instead (see Appendices, Section A.4.1, Figure A.23).

Given the contrast in binding it seems probable that the biotin groups present in \(\text{D}2\text{'-}\) interact in some fashion with \([\text{Pt}(2,2\text{'-bipy})(\text{PEGda})]^2+\), although details of this binding, which may involve the thioether group, are not well understood. Treatment of the \(\text{D}2\text{'-}/[\text{Pt}(2,2\text{'-bipy})(\text{PEGda})]^2+\) mixture with excess streptavidin (5 equiv. in 0.1 mM \(\text{NH}_4\text{OAc}\)) was performed, with the aim of determining whether or not interlocked species might be formed. However, ESI-MS analysis failed to indicate the presence of any \(\text{D}2\text{'-}\) (either free or bound to streptavidin), and the characterisation of this system has so far proven to be intractable. It is possible that further optimisation of the conditions, including \(\text{pH}\), is required to facilitate a clean reaction.

### 4.5.10 Discussion

It must be noted that the mass spectrometric observation of ions assigned to a non-covalently bound aggregate alone gives no information regarding the interactions holding the species together. Indeed, such species might result from the components participating in either non-specific gas-phase interactions or binding of a more particular nature. With respect to dsDNA, both experimental\(^{49}\) and theoretical evidence\(^{50}\) has confirmed the latter to be true – i.e. Watson-Crick base-pairing can be conserved during the ionisation of oligonucleotides. Hence, ions with \(m/z\) values consistent with the presence of dsDNA can be genuinely assigned to the double-helical structures one would expect to be prevalent in solution. However, questions remain as to the nature of the DNA adducts, and the interactions holding these together. For example, the mode of binding present in ions of the type \([\text{D}2 + \text{M}(N,N)(\text{PEGda})]^2+ - \text{nH}^+\text{[(a-2)-]}\) is not known, and it is presently unclear whether these can be assigned to [2]pseudorotaxanes or if they are products featuring \(\text{M}(N,N)(\text{PEGda})^2+\) bound to the surface of \(\text{D}2\).

An aspect of the characterisation not mentioned in this Thesis until now is the effect ESI-MS analysis might have on the molecules present in reaction mixtures. Importantly, the distribution of species detected by mass spectrometry need not be reflective of the equilibrium state in solution.\(^{33}\) Moreover, the ionisation process may lead to the formation of new species not present in solution, and, conversely, species present in solution may undergo fragmentation before they can be detected in the gas phase. This latter aspect
appeared to be particularly significant when the labile palladium(II) complexes were employed. As a consequence, efforts were made to characterise the behaviour of the macrocycles and their DNA adducts in solution, rather than in the gas phase. The solution experiments were performed using the platinum(II) complexes, as since their relative affinities for DNA had already been investigated using ESI-MS, it was anticipated that comparisons might be drawn from the separate studies.

4.6 Thermal denaturation experiments

4.6.1 Background

Thermal denaturation refers to the heat-induced dissociation of DNA into its constituent strands. In the case of dsDNA, this involves the breaking of H-bonds between AT and GC base-pairs at elevated temperatures. This reversible, endothermic process is often referred to as DNA ‘melting’, and the temperature at which one half of the DNA strands exist as dsDNA is known as the melting temperature ($T_m$).\textsuperscript{51} As one would expect, this temperature is dependent on the DNA sequence employed, with longer DNA and DNA incorporating a high proportion of GC base-pairs typically having higher melting temperatures than shorter or AT-rich DNA.

The stacked arrangement of nucleobases in dsDNA results in a decrease in UV absorptivity, relative to free ssDNA.\textsuperscript{52} As a result, thermal denaturation can be conveniently studied in solution by monitoring the absorbance at 260 nm of a given solution while the temperature is varied. If the melting process is monophasic, plots of absorbance versus temperature are of a sigmoidal shape, with the point of inflexion corresponding to $T_m$. As a result, data can be fitted to sigmoidal equations, with differentiation allowing for accurate estimation of $T_m$, which, for CT-DNA, was found to be 60.0°C (Figure 4.21).\textsuperscript{§§§§}

\textsuperscript{§§§§} This is estimated from the x-intercept of the 2nd derivative, which gives the point of inflexion of the melting profile.
The binding of small molecules to a given dsDNA sequence can perturb the melting of the system, resulting in a change in the melting profile. In particular, measuring and comparing the melting temperature both in the presence \( (T_{m}^c) \) and absence of these molecules \( (T_{m}^o) \) represents a sensitive means of gathering information regarding the interactions at play. The difference between these values \( (\Delta T_m = T_{m}^c - T_{m}^o) \), while not directly implicating a particular interaction, can provide evidence that is consistent with certain modes of binding (which might be confirmed by other means). For example, it has been reported that the covalent binding of dsDNA to drugs such as cisplatin destabilises the double helix, resulting in negative \( \Delta T_m \) values.\(^{53} \) Conversely, compounds that intercalate or bind to either of the DNA grooves typically stabilise duplex DNA, leading to positive \( \Delta T_m \) values being recorded.\(^{54} \) As it was thought that some of the compounds prepared in the present study (those with 2,2'-bipy ligands) might intercalate DNA, it was expected that the latter effect, in which DNA is stabilised, would be observed. It was also believed that a compound encircling DNA might potentially cause the melting temperature to increase, as the dissociation of the two strands would be hindered by this topological interaction.

### 4.6.2 Experimental approach and results

The thermal denaturation of DNA in the presence of platinum(II) complexes was studied using a procedure similar to that described by Cusumano and co-workers.\(^{20} \) Mixtures of CT-DNA and complexes ([base-pairs]/[complex] = 10) were prepared in phosphate-buffered
saline solution (pH 7.4) and their thermal denaturation monitored spectrophotometrically over the temperature range 37 – 100°C with the solutions being held at 37°C for 30 min prior to heating. Data were fitted to sigmoidal plots using Origin 7.0®, with the maxima of the first derivatives occurring at $T_m^c$. Examples of such derivative plots are given below both for CT-DNA and a CT-DNA mixture containing $[\text{Pt}_2(2,2′\text{-bipy})_2\{4,4′\text{-bipy(CH}_2)_64,4′\text{-bipy}\}_2](\text{NO}_3)_8$ (110) (Figure 4.22).

![Figure 4.22: Comparison of the 1st derivates of melting data for CT-DNA alone and in the presence of the complex $[\text{Pt}_2(2,2′\text{-bipy})_2\{4,4′\text{-bipy(CH}_2)_64,4′\text{-bipy}\}_2]\text{NO}_3^8$.](image)

The data acquired for CT-DNA in the absence of metal complex ($T_m^o$) was subtracted from these to obtain $\Delta T_m$. Measurements were also taken for all complexes in the absence of DNA and in each case it was found that these solutions had absorbances which were temperature invariant. This is consistent with the complexes having significant stability in the buffer solution used and allowed for reliable measurement of $\Delta T_m$.

The compounds employed in the study, along with the associated $\Delta T_m$ values, are given in Table 4.2 (sample melting curves for each compound are given in the Appendices, Section A.5). The known intercalator ethidium bromide (EtdBr, 152) was also used, in order to validate the study and allow for comparison with previously reported results obtained under different conditions.
Table 4.2: Changes in the melting temperature of CT-DNA in the presence of selected compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\Delta T_m / ^\circ \text{C}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEGda</td>
<td>-0.7</td>
</tr>
<tr>
<td>[Pt(en)(PEGda)]$_2$(NO$_3$)$_2$ (128)</td>
<td>0.6</td>
</tr>
<tr>
<td>[Pt(tmeda)(PEGda)]$_2$(NO$_3$)$_2$ (127)</td>
<td>0.8</td>
</tr>
<tr>
<td>[Pt(2,2'-bipy)(NH$_3$)$_2$(NO$_3$)$_2$ (146)</td>
<td>5.4</td>
</tr>
<tr>
<td>[Pt(2,2'-bipy)(PEGda)]$_2$(NO$_3$)$_2$ (126)</td>
<td>5.8</td>
</tr>
<tr>
<td>[Pt(2,2'-bipy)(Mebipy)]$_2$(NO$_3$)$_4$ (148)</td>
<td>11.3</td>
</tr>
<tr>
<td>EtdBr (152)</td>
<td>12.6</td>
</tr>
<tr>
<td>[Pt$_2$(2,2'-bipy)$_2$(4,4'-bipy(CH$_2$)$_6$4,4'-bipy)$_2$(NO$_3$)$_6$ (110)</td>
<td>25.5</td>
</tr>
</tbody>
</table>

4.6.3 Discussion

The free ligand PEGda and its complexes [Pt(tmeda)(PEGda)]$^{2+}$ and [Pt(en)(PEGda)]$^{2+}$ were each found to have a negligible effect on the melting of CT-DNA. This is consistent with these compounds interacting only very weakly (perhaps by electrostatic means) with DNA, at the concentrations used. The related complex [Pt(2,2'-bipy)(PEGda)]$^{2+}$, was found to stabilise dsDNA, as evidenced by the associated $\Delta T_m$ of 5.8°C. This value is suggestive of intercalation, a binding mode made possible by the 2,2'-bipy co-ligand. When instead the related 2,2'-bipy complex [Pt(2,2'-bipy)(NH$_3$)$_2$]$^{2+}$ was employed, a comparable increase in the DNA melting temperature (5.2°C) was observed. The slight difference can be attributed to the polyether chain of PEGda, with the presence of this group potentially allowing for several weak interactions with DNA that lead to its stabilisation. Apart from participating in non-specific van der Waals forces (which are non-trivial when large species are concerned), the PEGda O atoms can act as H-bond acceptors with nucleobases (N$_{\text{exocyclic}}$–H···O), and this binding might take place in the major (with A and C bases) and/or minor grooves (with G). It was thought that if dsDNA was being encircled by the macrocycle, a high $\Delta T_m$ would be observed, owing to the two strands being mechanically held together. In view of the above melting results it seems unlikely that this is occurring in the present system, a result which is unsurprising given the conformational flexibility of the [Pt(2,2'-bipy)(PEGda)]$^{2+}$ macrocycle and, more importantly, the length of CT-DNA (>20,000 base-pairs). This latter aspect most likely causes threading to be a very slow

***** Using similar conditions to those employed in the present study, Cusumano and co-workers found that the complex [Pt(2,2'-bipy)(en)]$^{2+}$ had a similar $\Delta T_m$ value to that for EtdBr. Surprisingly, the melting studies in this work suggest that the analogue [Pt(2,2'-bipy)(NH$_3$)$_2$]$^{2+}$ has a much lower affinity for DNA.

††††† As specified by Sigma-Aldrich, from which CT-DNA was purchased.
process and, in any case, intercalation may still be a more favourable interaction (these two modes of interaction might be mutually exclusive).

In contrast to the complexes of PEGda, the dinuclear metallocycle \([\text{Pt}_2(2,2'-\text{bipy})_2\{4,4'\text{-bipy(CH}_2)_64,4'\text{-bipy}\}_3]^{8+}\) was found to cause a marked increase in the stability of dsDNA \((\Delta T_m = 25.5^\circ\text{C})\). As mentioned above, while \(\Delta T_m\) values do not confirm or rule out a mode of DNA-binding, when these values are high it is often the case that intercalative interactions are at play.\(^{20}\) It is possible that this compound behaves as a bis(intercalator), in which both \([\text{Pt}(2,2'-\text{bipy})]^{2+}\) fragments bind the same (or separate) DNA molecule. The high charge of the dinuclear complex, as well as its large aromatic surface area undoubtedly allow for this strong binding to occur.

The tetracation \([\text{Pt}(2,2'-\text{bipy})(\text{Mebipy})_2]^{4+}\) was also found to stabilise dsDNA \((\Delta T_m = 11.3^\circ\text{C})\), albeit to a lesser extent than the dinuclear species \([\text{Pt}_2(2,2'-\text{bipy})_2\{4,4'\text{-bipy(CH}_2)_64,4'\text{-bipy}\}_2]^{8+}\), of which it represents roughly half. Its reduced size and charge, relative to the dinuclear complex, most likely cause its interactions with DNA to be weaker.\(^{56}\) Nevertheless, \([\text{Pt}(2,2'-\text{bipy})(\text{Mebipy})_2]^{4+}\) appears to bind DNA with a strength comparable to that of Etd\(^+\) \((\Delta T_m = 12.6^\circ\text{C})\) and it is likely that this binding involves intercalation.

**4.7 Concluding remarks**

The conclusions drawn from both ESI-MS and thermal denaturation studies of DNA/metal complex mixtures were found to be largely in agreement. More specifically, both methods suggest that the platinum(II) complexes of PEGda have only very low affinities for nucleic acids. In contrast, the bipyridinium complexes \([\text{Pt}_2(2,2'-\text{bipy})_2\{4,4'\text{-bipy(CH}_2)_64,4'\text{-bipy}\}_2]^{8+}\) and \([\text{Pt}(2,2'-\text{bipy})(\text{Mebipy})_2]^{4+}\) interact strongly with DNA, a result attributed to their greater number of aryl groups and positive charge. Nevertheless, some differences between ESI-MS and thermal denaturation data were observed. In particular, mass spectrometric analyses indicated \([\text{Pt}(2,2'-\text{bipy})(\text{NH}_3)_2]^{2+}\) binds more strongly to DNA than do either of the metallocycles \([\text{Pt}(2,2'-\text{bipy})(\text{PEGda})]^{2+}\) or \([\text{Pt}_2(2,2'-\text{bipy})_2\{4,4'\text{-bipy(CH}_2)_64,4'\text{-bipy}\}_2]^{8+}\).

\(^{+++}\) Results with complexes of PEGda do not differ greatly when longer incubation times (60 min instead of 30 min) were used. The use of even longer times was not investigated, owing to the possible degradation of DNA.

\(^{####}\) Indeed, the size of \(\Delta T_m\) can be interpreted in terms of the extent of intercalation. In conjunction with other data, thermodynamic parameters, such as enthalpies of binding, can be obtained, although no such analyses were carried out in the present study.
However, melting data suggest the cyclic complexes bind DNA with equal or greater affinity. This was rationalised in terms of the fragmentation of the cyclic species during ESI-MS, which resulted in the lower intensities of ions assigned to intact complexes binding DNA. In general, however, the above results are consistent with previous comparisons between ESI-MS and UV-vis DNA-binding studies. Indeed, it has recently been shown that the relative binding strengths of metal complexes using these gas and solution phase techniques are comparable, although absolute binding affinities, if they can be determined, might not be.

### 4.8 References

Chapter Four: DNA-binding studies


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Chapter Five: Conclusions and future directions

5.1 Synthesis of bridging ligands and their metal complexes

The present study required the preparation of positively-charged macrocycles of considerable size. With this aim in mind, a new family of conformationally flexible, cationic \( N \)-heterocyclic derivatives was prepared and fully characterised, in five examples by an X-ray structure determination. Using a generalised alkylation protocol, the heterocycles 4,4′-bipy, pyz, apyz and apym could be incorporated into halide salts of organocations, from which further compounds were prepared by anion exchange. The straightforward syntheses allowed for the generation of a large number of analogues, which were engineered in terms of their size, solubility and donor ability. Given the ease with which these salts can be tuned, it is hardly surprising that such compounds are extremely versatile, with related species having been shown to possess interesting anion binding, supramolecular and metal ion coordination chemistry. This final aspect was investigated with the potentially bridging diquaternary salts.

The combination of labile palladium(II) and platinum(II) precursors with diquaternary salts resulted in the synthesis of several new multinuclear derivatives. Bis(4,4′-bipyridinium) dications were incorporated into dinuclear [2 + 2] metalloccycles of formula [\( \text{M}_2(2,2′\text{-bipy})_2\{4,4′\text{-bipy(CH}_2)_{4,4′\text{-bipy}}\}_2\text{]}^{8+} \) (\( \text{M} = \text{Pd}, \text{Pt}; \text{n} = 4, 6 \), \( \text{cis}^{-}\text{[Pd}_2\text{Cl}_4\{4,4′\text{-bipy(CH}_2)_34,4′\text{-bipy}\}_2\}^{4+} \), \( \text{[Pt}_2\text{(dppp)}\{4,4′\text{-bipy(1,2-xylene)4,4′-bipy}\}_2\}^{8+} \) and \( \text{cis}^{-}\text{-[Pt}_2\text{Cl}_4\{4,4′\text{-bipy(1,2-xylene)4,4′-bipy}\}_2\}^{4+} \)). While the bis(pyrazinium) analogues were found to be largely unreactive towards the palladium(II) and platinum(II) precursors used in the study, the doubly deprotonated bis(3-aminopyrazinium) and bis(2-aminopyrimidinium) derivatives, in contrast, served as charge-neutral quadruply-bridging ligands when interacted with \( \text{[Pt(2,2′-bipy)(NO}_3)_2\} \). The tetranuclear complexes \( \text{[Pt}_4(2,2′\text{-bipy})_4\{apyz(CH}_2)_6apyz–2H}_2\}^{8+} \) and \( \text{[Pt}_4(2,2′\text{-bipy})_4\{apym(CH}_2)_5apym–2H}_2\}^{8+} \) that resulted both incorporated Pt(II)···Pt(II) interactions in their bonding.

Larger complexes could be prepared when the conformationally flexible diamine PEGda was interacted with \( \text{cis}^{-}\)-labile palladium(II) and platinum(II) precursors. The mononuclear dications \( \text{[M}_2(N,N)(\text{PEGda})}_2\}^{2+} \) that resulted were found to possess high aqueous solubility, and their 62-membered chelate rings, if in an ‘open’ conformation, were thought to be of a size that might accommodate duplex DNA.
5.2 DNA-binding

Having synthesised a series of large cationic metal complexes, DNA-binding studies were conducted using selected candidates in order to investigate the behaviour of these in the presence of nucleic acids.

Solutions containing either the duplex D2 or free D2a and equimolar amounts of selected palladium(II) complexes were prepared and subjected to ESI-MS analysis. Results from studies of D2/Pd(II) mixtures suggested that extensive fragmentation was occurring under these conditions. In particular, the use of \([\text{Pd(tmeda)}(\text{PEGda})]^2^+\) and \([\text{Pd}_2(2,2′\text{-bipy})_2\{4,4′\text{-bipy(CH}_2)_44,4′\text{-bipy}\}]^8^+\) resulted in the detection of DNA adducts containing, for example, \([\text{Pd(tmeda)}]^2^+\) and \([4,4′\text{-bipy(CH}_2)_44,4′\text{-bipy}]^2^+\), respectively. Similar decomposition was observed with the binding of the single strand D2a, although 1 : 1 adducts of intact complexes could be observed when \([\text{Pd(tmeda)}(\text{PEGda})]^2^+, [\text{Pd}(2,2′\text{-bipy})(\text{PEGda})]^2^+\) or \([\text{Pd}_2(2,2′\text{-bipy})_2\{4,4′\text{-bipy(CH}_2)_44,4′\text{-bipy}\}]^8^+\) was used. However, the relative intensities of these ions were invariably very low, indicating that such adducts are unstable under the ESI-MS conditions employed.

Analogous experiments using platinum(II) derivatives were also performed, and in contrast to those with palladium(II), indicated that the complexes stay intact to a large degree. The ESI-MS analysis of D2/Pt(II) mixtures allowed for the detection of 1 : 1 D2 adducts when either \([\text{Pt(tmeda)}(\text{PEGda})]^2^+, [\text{Pt}(\text{en})(\text{PEGda})]^2^+\) was used. The highly-charged bipyridinium derivative \([\text{Pt}_2(2,2′\text{-bipy})_2\{4,4′\text{-bipy(CH}_2)_44,4′\text{-bipy}\}]^8^+\) also gave rise to 1 : 1 species, although these were of greater relative intensity than in the previous two examples. The difference in binding strength could be ascribed to the greater charge and aryl surface area of the dinuclear species relative to complexes of type \([\text{M}(N,N)(\text{PEGda})]^2^+\). Indeed, it is anticipated that these PEGda complexes would require topological interactions to be at play in order to show any sort of affinity towards DNA. Such interactions, as suggested by MS studies, are either non-existent or of a highly transient nature, no doubt resulting from the conformational flexibility of PEGda. This aspect might cause DNA encirclement to be entropically disfavoured with enthalpic contributions (e.g. electrostatic interactions, H-bonding) not compensating for this. When the mononuclear complex \([\text{Pt}(2,2′\text{-bipy})(\text{Mebipy})]^+\) was employed, an ion containing D2 bound to two tetracations could be observed, consistent with the complex representing ‘half’ of the dinuclear complex mentioned above. A 1 : 2 adduct of D2 could also be observed with \([\text{Pt}(2,2′\text{-bipy})(\text{NH}_3)_2]^2^+,\)
although it was found to be a weaker binder than the tetracationic complex, a result perhaps of its lower charge. Additional investigations using 1:5 (D2 : complex) mixtures afforded results consistent with the above observations and, furthermore, suggested that D2 is capable of binding more molecules of daunomycin than any of the platinum(II) species studied. It is thought that this is in some way due to the higher positive charge of the complexes (and also potentially their larger binding sites) hindering the binding of large numbers of the complexes to D2. The results of binding experiments using D2a and the platinum(II) complexes garnered results not dissimilar to those obtained with D2. However, it was noticed that ions containing D2a bound to fragmented complexes were more noticeable, indicating that the nucleobases in D2a could play more significant roles in mediating decomposition than in the case of the duplex D2, in which the bases participate in Watson-Crick pairing.

In addition to the comprehensive study using D2, experiments were also conducted with the biotinylated analogue D2′, allowing for adducts to be observed with [Pt(tmeda)(PEGda)]^{2+} and [Pt(2,2′-bipy)(PEGda)]^{2+}. In the latter case, up to four macrocycles were found to bind a single duplex, and it is believed that the biotin groups could be involved in binding. However, no products could be detected when this mixture was treated with streptavidin, suggesting that further optimisation of the reaction is necessary. Nevertheless, the work described here represents the first mass spectrometric investigation of the interactions between macrocyclic palladium(II) and/or platinum(II) complexes and oligonucleotides.

A spectrophotometric investigation into the effects platinum(II) complexes have on the thermal denaturation of CT-DNA was also conducted in order to study interactions in the solution phase. The complex [Pt_2(2,2′-bipy)_2[4,4′-bipy(CH_2)_64,4′-bipy]_2]^{8+} and its mononuclear analogue [Pt(2,2′-bipy)(Mebipy)_2]^{4+} were found to stabilise CT-DNA considerably, with the associated ΔT_m values being consistent with intercalative interactions being present. In contrast, [Pt(tmeda)(PEGda)]^{2+} and [Pt(en)(PEGda)]^{2+} (as well as the parent compound PEGda) caused negligible changes in T_m suggesting that these compounds interact only weakly with CT-DNA. The results for the related metalloccycle [Pt(2,2′-bipy)(PEGda)]^{2+} suggested that it interacts moderately with DNA, possibly by intercalation of the [Pt(2,2′-bipy)]^{2+} fragment. This was supported by the analysis with [Pt(2,2′-bipy)(NH_3)_2]^{2+}, for which a similar ΔT_m value was obtained, and indicates that the PEGda ligand is unlikely to play a major role in DNA-binding.
While the findings from ESI-MS experiments were largely in agreement with those from thermal denaturation, some discrepancies between results from the two methods could be found. For example, when comparing the binding of \([\text{Pt}(2,2'\text{-bipy})(\text{NH}_3)_2]^{2+}\) and \([\text{Pt}(2,2'\text{-bipy})(\text{PEGda})]^{2+}\), ESI-MS analyses suggested that the former had a greater affinity for DNA, while the thermal denaturation studies suggested their interactions with DNA to be of comparable strengths. In such cases it was considered likely that the fragmentation of complex species under ESI-MS conditions caused the binding strength of some species (in this case \([\text{Pt}(2,2'\text{-bipy})(\text{PEGda})]^{2+}\)) to be underestimated.

As such, it is clear that both the further development of macrocycle synthesis and the strategic use of characterisation methods are necessary in order to gain detailed information regarding binding and perhaps to identify species capable of encircling dsDNA.

5.3 Future work

The design and synthesis of large metallocyclic architectures with the \(\text{H}_2\text{O}\)-solubility, size and charge required for encircling dsDNA is not a straightforward matter. Ideally, it would be desirable to have access to structures possessing the high positive charge of the dinuclear bipyridinium complexes with the size and solubility properties of the PEGda complexes. Lastly, it is also necessary that these species exhibit some degree of conformational rigidity, analogous to the toroidal proteins, whose preorganised structures no doubt contribute to them being effective hosts for linear dsDNA. Two future synthetic targets, both of which are platinum(II) complexes of bidentate bipyridinium-based ligands, are given below.
Molecular hexagon 152 incorporates six trans-diammineplatinum(II) fragments bridged by six 2,6-bis(4,4′-bipyridinium)-9-thiabicyclo[3.3.1]nonane ligands. Such a compound might be prepared from (±)-2,6-bis(4,4′-bipyridinium)-9-thiabicyclo[3.3.1]nonane nitrate and trans-[Pt(NH$_3$)$_2$(NO$_3$)$_2$], provided no trans → cis isomerism of the platinum(II) centres occurs. Indeed, as mentioned in Chapter Two, Stang and co-workers have prepared similar (although lipophilic) self-assembled hexagonal complexes from the very same bridging ligand. In contrast, a different strategy, namely the cyclisation of a large, somewhat flexible bidentate ligand might also afford a potential ‘nanoshuttle’. This approach was employed in the present study to afford complexes of PEGda and might be exploited for the formation of a highly charged metallocycle such as 153. The synthesis of such a macrocycle would first require the (non-trivial) preparation of a suitably large decaquaternary NO$_3^-$ salt similar to 106, which could afford 153 upon treatment with [Pt(en)(NO$_3$)$_2$].

Given candidate macrocycles amenable to ‘nanoshuttle’ behaviour, the unambiguous characterisation of non-covalent DNA/metal complex adducts that might result remains a challenge. The incubation of a suitable complex, such as [Pt(2,2′-bipy)(PEGda)]$^{2+}$ (or even 152/153) with biotinylated dsDNA D2′, followed by treatment with streptavidin remains an interesting prospect, although, as mentioned above, this approach is not without complications. Such a synthesis would result in the formation of interlocked and/or non-interlocked products, depending on the extent to which the threading of D2′ occurs. The resulting species could then be subjected to ESI-MS (run at different cone voltages) or tandem MS/MS analyses, in order to gauge how easily the macrocyclic ‘wheel’ dissociates from the protein/DNA ‘axle’; thus providing information regarding the presence of topological interactions. Contrasting these results to those obtained when the reaction with streptavidin is performed before incubation with the metallocycle (a sequence which should not generate rotaxanes) might also shed light on the processes involved.

A related method of generating permanently interlocked species is the T4 DNA ligase-mediated cyclisation of linear DNA in the presence of macrocyclic species, which might give rise to [n]catenanes, as described in Chapter One. An interesting prospect would be to study the effect of macrocycle concentration on the number of macrocycles potentially threaded onto the longer DNA duplexes that would be used for this approach.
More generally, analysing the interactions of metallocyclic platinum(II) derivatives with a wider variety of duplexes would afford further evidence regarding the nature of DNA-binding and reveal any inherent sequence selectivity. The complexes prepared in this work could be used for such screening and concrete thermodynamic and kinetic data could be obtained from isothermal titration calorimetric studies.

As has been documented in this Thesis, the results of the synthetic and DNA-binding experiments carried out in the present study have allowed for several conclusions to be drawn regarding the chemistry of metallocyclic species. Despite this, many questions remain unanswered and it is anticipated that further synthesis and DNA work (i.e. more iterations of the design and testing processes) will go some way to addressing these matters. With a view to the future, the successful development of DNA ‘nanoshuttles’ might give rise to functional species that may serve as topological probes of DNA structure and function or novel medicinal agents with unprecedented modes of action. Such prospects will undoubtedly serve as powerful motivations for research in the exciting area of supramolecular DNA recognition to flourish for many years to come.
Chapter Six: Experimental

6.1 Instrumentation

Low resolution ESI-MS data were collected using a Finnigan LCQ detector. High resolution ESI-FT-ICR-MS data were collected using either a Bruker 7.0T or a Bruker Apex 4.7T spectrometer. Solutions containing oligonucleotides were analysed using a Waters Micromass Q-ToF2 Ultima spectrometer (see Section 6.12).

NMR spectra were recorded at 300 K using a Bruker Avance DPX200, DPX300 or DPX400 spectrometer. Unless otherwise stated: chemical shifts (δ) are reported relative to Me₄Si and were referenced to residual solvent signals;¹ data for ¹H, ¹³C{¹H} and ¹⁹⁵Pt (relative to external Na₂[PtCl₄] at –1628 ppm) nuclei were recorded at 300, 75 and 85 MHz, respectively; 2-D spectra were acquired with ¹H and ¹³C channels at 400 and 100 MHz, respectively.

UV-vis data were recorded using a Varian Cary 1E spectrophotometer, except for thermal denaturation experiments, for which a Cary 500 spectrophotometer (equipped with a Cary temperature controller) was used.

Elemental analyses were performed at the Campbell Microanalytical Laboratory, University of Otago, New Zealand.

Single-crystal X-ray diffraction data were collected at the University of Sydney using either a Bruker APEXII-FR591 or a Bruker SMART 1000 CCD diffractometer and processed by either Dr Paul Jensen (30) or Dr Jack Clegg (43, 61, 80, 90). The procedures used, as well as selected crystallographic details, are given in the Appendices (Section A.3).

6.2 Materials

Unless otherwise stated, all chemicals used were obtained from commercial sources,¹ are of reagent grade and were used without further purification. Solutions containing Na₂[PtCl₄] or PdCl₂ were filtered prior to use. Many organic compounds used were hygroscopic and all

¹ The author gratefully acknowledges Johnson-Matthey for the generous loan of K₂[PtCl₄].
solid materials were dried (P₂O₅) under vacuum for at least 24 h prior to use. The compound PEGda (Fluka, oligomeric purity) contained trace impurities, as evidenced by ESI-MS.

The 16mers D₂a, D₂b, D₂’a and D₂’b (Geneworks, HPLC grade) were purified using HPLC (aqueous NH₄OAc→MeCN gradient) as reported previously.\(^2\) In each case, equimolar quantities\(^3\) of appropriate strands (both dissolved in 0.1 M NH₄OAc, pH 7.4) were combined to give a mixture in which the concentration of each of these constituents was 1 mM. This solution was heated at (\(T_m + 20\))ºC and cooled slowly to room temperature to afford a stock solution of the annealed dsDNA, which was used without further purification.\(^8\) CT-DNA (sodium salt, highly polymerised) was resuspended in phosphate-buffered saline solution (1 mM phosphate, 2 mM NaCl, pH 7.4) and its concentration determined spectrophotometrically.\(^4\)**

THF was dried over Na/benzophenone ketyl.\(^5\) DMF (peptide grade) was stored over 4 Å molecular sieves. MilliQ\(^\text{TM}\) H₂O was used for all experiments requiring H₂O.

Cellulose microfilters (0.2 µm) were used when small volumes of aqueous solutions required filtration. When larger volumes required filtration and no acidic species (e.g. those containing aminopyrimidinium and aminopyrazinium groups) were present, solids were removed by filtration through Celite.

### 6.3 Synthesis of quaternary halide salts

The syntheses of ligands of the type [Y(spacer)Y]X₂ (Y = 4,4’-bipy, pyz, apyz, apym; X = Cl, Br) were performed using adaptations of the procedure described by Atalla et al.\(^6\) Unless otherwise specified, the N-heterocyclic nucleophile (12 mmol) and bis(alkyl halide) derivative (3 mmol) were stirred overnight in DMF (10 mL) at 70°C, over which time an off-white precipitate formed. After cooling to room temperature, this was isolated by filtration, washed with DMF (2 mL) and Et₂O (5 mL) and crystallised from a small volume

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\(^{†}\) O,O’-Bis(aminoethyl)heptadeca(ethylene glycol) could be detected (m/z 854 [M + H\(^\text{+}\)]\(^+\)).

\(^{‡}\) Values for [ssDNA] were estimated by measurement of UV absorbance at 260 nm using \(ε_{260}\) values for A, G, C and T of 15200, 12010, 7050 and 8400 M\(^{-1}\)cm\(^{-1}\), respectively.\(^3\)

\(^{§}\) The purification and annealing of D₂a and D₂b to give D₂ was performed by Dr Thitima Urathamakul at the University of Wollongong.

\(^{**}\) CT-DNA concentration, quantified as [base-pairs], was estimated using \(ε_{260} = 13100\) M\(^{-1}\)base-pairs\(^{-1}\)cm\(^{-1}\).\(^4\)
of hot H$_2$O to afford the desired product. Derivatives of 2-aminopyrimidinone did not require crystallisation.

(±)-2,6-Bis(4,4′-bipyridinium)-9-thiabicyclo[3.3.1]nonane chloride (30)

The title compound was prepared from 4,4′-bipyridine and (±)-2,6-dichloro-9-thiabicyclo[3.3.1]nonane (60%, off-white powder). A similar method (using MeCN rather than DMF) has been reported by Sharpless. However, in this preparation the title compound is not purified/characterised, but rather converted directly to the PF$_6$ salt using excess NaPF$_6$. Slow evaporation of an aqueous solution of the title compound afforded single crystals suitable for X-ray crystallographic analysis, details of which are given in the Appendices (Section A.3).

$^1$H NMR (D$_2$O) δ 9.24 (d, 4H, $^3$J$_{H,H}$ = 6.58 Hz, H2), 8.84 (d, 4H, $^3$J$_{H,H}$ = 5.05 Hz, H2′), 8.56 (d, 4H, $^3$J$_{H,H}$ = 6.58 Hz, H3), 7.99 (d, 4H, $^3$J$_{H,H}$ = 5.05 Hz, H3′), 5.84 (m, 2H, H$_{\text{aliphatic}}$), 3.46 (m, 2H, H$_{\text{aliphatic}}$), 3.25 (m, 2H, H$_{\text{aliphatic}}$), 2.55 (m, 6H, H$_{\text{aliphatic}}$) ppm. ESI-MS: $m/z$ 487.13 [M – Cl]$^+$, 226.07 [M – 2Cl]$^{2+}$. Anal. calcd for C$_{28}$H$_{28}$N$_4$Cl$_2$S·4H$_2$O: C, 56.47; H, 6.09; N, 9.41. Found: C, 56.25; H, 6.11; N, 9.19.

1,6-Bis(3-aminopyrazinium)hexane bromide (48)

The title compound was prepared from aminopyrazine and 1,6-dibromohexane (56%, yellow powder).

$^1$H NMR (D$_2$O) δ 8.59 (m, 3H, H5), 8.16 (m, 3H, H6), 7.96 (s, 3H, H2), 4.49 (t, 4H, $^3$J$_{H,H}$ = 7.5 Hz, H$_{\text{hexylene}}$), 2.04 (m, 4H, H$_{\text{hexyleneβ}}$), 1.46 (m, 4H, H$_{\text{hexyleneγ}}$) ppm. $^{13}$C($^1$H) NMR (D$_2$O) δ 159.06, 149.38, 125.52, 123.58 (t, $^1$I$_{C,N}$ = 8.0 Hz), 62.64, 30.24, 25.20 ppm. ESI-MS: $m/z$ 353.20 [M – Br]$^+$, 273.20 [M – H – 2Br]$^{2+}$, 137.20 [M – 2Br]$^{2+}$. Anal. calcd for C$_{14}$H$_{22}$N$_6$Br$_2$: C, 38.73; H, 5.11; N, 19.36. Found: C, 38.89; H, 5.39; N, 19.16.
1,3,5-Tris(3-aminopyraziniummethyl)-2,4,6-trimethylbenzene bromide monohydrate (53)

Aminopyrazine (350 mg, 3.68 mmol) and 1,3,5-tris(bromomethyl)-2,4,6-trimethylbenzene (147 mg, 0.368 mmol) were stirred overnight in DMF (2 mL) at 70°C. After cooling to room temperature, the solid was isolated by filtration, washed with DMF (0.5 mL) and Et₂O (1 mL) and crystallised from EtOH/H₂O to afford the title compound as a colourless crystals (139 mg, 54%).

\[ ^1H \text{NMR (D}_2\text{O, relative to residual EtOH)} \delta 8.64 (m, 3H, H5), 8.12 (m, 3H, H6), 7.94 (s, 3H, H2), 6.02 (s, 6H, CH₂), 2.40 (s, 9H, CH₃) \text{ ppm.} \]

\[ ^{13}C\{^1H\} \text{NMR (D}_2\text{O)} \delta 159.63, 150.1, 145.71, 127.91, 124.40, 122.96, 60.02, 17.13 \text{ ppm.} \]

ESI-MS: \( m/z \) 603.67 [M – Br⁻ – H⁺]⁺. Anal. calcd for C₂₄H₃₀N₉Br₃·H₂O: C, 41.05; H, 4.59; N, 17.95. Found: C, 41.18; H, 4.60; N, 17.34.

1,2-Bis(2-aminopyrimidinium)ethane bromide (62)

The title compound was prepared from 2-aminopyrimidine and 1,2-dibromoethane (71%, off-white powder).

\[ ^1H \text{NMR (D}_2\text{O)} \delta 8.76 (m, 2H, H4), 8.54 (m, 2H, H6), 7.04 (m, 2H, H5), 4.10 (t, 4H, \text{J}H,H = 9.14 \text{ Hz, CH}_2) \text{ ppm.} \]


1,4-Bis(2-aminopyrimidinium)butane bromide (63)

The title compound was prepared from 2-aminopyrimidine and 1,4-dibromobutane (76%, white powder).

\[ ^1H \text{NMR (400 MHz, D}_2\text{O)} \delta 8.82 (dd, 2H, \text{J}H,H = 4.4 \text{ Hz, J}_H,H = 2.0 \text{ Hz, H4}), 8.40 (dd, 2H, \text{J}H,H = 6.4 \text{ Hz, J}_H,H = 2.0 \text{ Hz, H6), 7.14 (dd, 2H, J}_H,H = 6.4 \text{ Hz, J}_H,H = 4.4 \text{ Hz, H5), 4.28 (m, 4H, H}_\text{butylene}, 2.04 (m, 4H, H}_{\text{butylene}} \text{ ppm.} \]

1,5-Bis(2-aminopyrimidinium)pentane bromide (66)

The title compound was prepared from 2-aminopyrimidine and 1,5-dibromopentane (22%, colourless crystals). Significant loss of yield was due to crystallisation from H₂O.

¹H NMR (300 MHz, D₂O) \( \delta \) 8.85 (m, 2H, H₄), 8.43 (m, 2H, H₆), 7.17 (m, 2H, H₅), 4.25 (t, 4H, \( ^3J_{H,H} = 6.9 \) Hz, H₆-pentylene), 2.02 (m, 4H, H₆-pentylene), 1.56 (m, 2H, H₆-pentylene) ppm.


1,6-Bis(2-aminopyrimidinium)hexane bromide (69)

The title compound was prepared from 2-aminopyrimidine and 1,6-dibromohexane (66%, off-white powder).

¹H NMR (400 MHz, D₂O) \( \delta \) 8.79 (dd, 2H, \( ^3J_{H,H} = 4.4 \) Hz, \( ^4J_{H,H} = 2.0 \) Hz, H₄), 8.38 (dd, 2H, \( ^3J_{H,H} = 6.8 \) Hz, \( ^4J_{H,H} = 2.0 \) Hz, H₆), 7.11 (dd, 2H, \( ^3J_{H,H} = 6.8 \) Hz, \( ^3J_{H,H} = 4.4 \) Hz, H₅), 4.19 (t, 4H, \( ^3J_{H,H} = 7.2 \) Hz, H₆-hexylene), 1.91 (m, 4H, H₆-hexylene), 1.47 (m, 4H, H₆-hexylene) ppm.

¹³C{¹H} NMR (D₂O) \( \delta \) 166.0, 155.5, 149.9, 111.4, 54.6, 26.0, 25.0 ppm. ESI-MS: \( m/z \) 273.13 [M – 2Br⁻ – H⁺], 137.07 [M – 2Br⁻]²⁺. Anal. calcd for C₁₄H₂₂N₆Br₂: C, 38.73; H, 5.11; N, 19.36. Found: C, 38.50; H, 5.12; N, 19.10.

1,8-Bis(2-aminopyrimidinium)octane bromide (72)

The title compound was prepared from 2-aminopyrimidine and 1,8-dibromooctane (73%, white powder).

¹H NMR (D₂O) \( \delta \) 8.76 (dd, 2H, \( ^3J_{H,H} = 4.4 \) Hz, \( ^4J_{H,H} = 1.9 \) Hz, H₄), 8.33 (dd, 2H, \( ^3J_{H,H} = 6.6 \) Hz, \( ^4J_{H,H} = 1.97 \) Hz, H₆), 7.07 (dd, 2H, \( ^3J_{H,H} = 6.6 \) Hz, \( ^3J_{H,H} = 4.4 \) Hz, H₅), 4.14 (t, 4H, \( ^3J_{H,H} = 7.6 \) Hz, H₆-octylene), 1.86 (m, 4H, H₆-octylene), 1.35 (m, 8H, H₆-octylene) ppm. ¹³C{¹H} NMR (D₂O) \( \delta \) 166.25, 155.86, 150.31, 111.64, 55.12, 28.40, 26.45, 25.56 ppm. ESI-MS: \( m/z \) 380.93 [M – Br⁻], 301.27 [M – 2Br⁻ – H⁺], 151.93 [M – 2Br⁻]²⁺. Anal. calcd for C₁₆H₂₆N₆Br₂·2H₂O: C, 40.02; H, 5.88; N, 17.50. Found: C, 40.05; H, 5.51; N, 17.28.
1,2-Bis(1-methyl-2-aminopyrimidinium)benzene bromide (75)

The title compound was prepared from 2-aminopyrimidine and 1,2-bis(bromomethyl)benzene (67%, white powder).

\(^1\)H NMR (400 MHz, D\(_2\)O, relative to DMF) \(\delta 8.92\) (dd, 2H, \(^3\)J\(_{H,H}\) = 4.1 Hz, \(^4\)J\(_{H,H}\) = 1.9 Hz, H4), 8.26 (dd, 2H, \(^3\)J\(_{H,H}\) = 6.2 Hz, \(^4\)J\(_{H,H}\) = 2.0 Hz, H6), 7.47 (m, 2H, H3\(_{xylyl}\)), 7.23 (br, s, 4H, NH\(_2\)), 7.09 (m, 2H, H4\(_{xylyl}\)), 7.03 (dd, 2H, \(^3\)J\(_{H,H}\) = 6.4 Hz, \(^3\)J\(_{H,H}\) = 4.4 Hz, H5), 5.08 (s, 4H, CH\(_2\)) ppm.

\(^{13}\)C\{\(^1\)H\} NMR (100 MHz, D\(_2\)O, relative to DMF) \(\delta 167.5, 156.7, 149.7, 130.9, 129.5, 128.7, 112.4, 54.9\) ppm. ESI-MS: m/z 293.07 [M – 2Br – H\(^{+}\)]\(^+\), 198.27 [M – 2Br – apym – H\(^{+}\)]\(^+\). Anal. calcd for C\(_{16}\)H\(_{18}\)N\(_6\)Br\(_2\): C, 42.31; H, 3.99; N, 18.50. Found: C, 42.42; H, 4.00; N, 18.60.

1,3-Bis(1-methyl-2-aminopyrimidinium)benzene bromide (78)

The title compound was prepared from 2-aminopyrimidine and 1,3-bis(bromomethyl)benzene (69%, white powder).

\(^1\)H NMR (D\(_2\)O) \(\delta 8.88\) (m, 2H, H4\(_{apym}\)), 8.63 (m, 2H, H6\(_{apym}\)), 7.63 (t, 1H, \(^3\)J\(_{H,H}\) = 7.58 Hz, H5\(_{xylylene}\)), 7.47 (d, 4H, \(^3\)J\(_{H,H}\) = 7.58 Hz, H4\(_{xylylene}\)), 7.23 (s, 1H, H2\(_{xylylene}\)), 7.17 (m, 2H, H5\(_{apym}\)), 5.47 (s, 4H, CH\(_2\)) ppm. Traces of DMF present.

\(^{13}\)C\{\(^1\)H\} NMR (D\(_2\)O) \(\delta 167.2\) (C4\(_{apym}\)), 156.5 (C2\(_{apym}\)), 150.2 (C6\(_{apym}\)), 132.5 (C1\(_{xylylene}\)), 131.3 (C5\(_{xylylene}\)), 129.7 (C4\(_{xylylene}\)), 127.9 (C2\(_{xylylene}\)), 112.3 (C5\(_{apym}\)), 57.3 (CH\(_2\)) ppm. For 2-D COSY and HSQC, see Section A.1. ESI-MS: m/z 827.00 [2M – Br\(^{+}\)]\(^+\), 746.67 [2M – 2Br\(^{–}\) – H\(^{+}\)]\(^+\), 666.67 [2M – 3Br\(^{–}\) – 2H\(^{+}\)]\(^+\), 584.87 [2M – 4Br\(^{–}\) – 3H\(^{+}\)]\(^+\), 293.13 [M – 2Br\(^{–}\) – H\(^{+}\)]\(^+\), 198.20 [M – apym – 2Br\(^{–}\) – H\(^{+}\)]\(^+\), 147.20 [M – 2Br\(^{–}\)]\(^2\)\(^+\). Anal. calcd for C\(_{16}\)H\(_{18}\)Br\(_2\)N\(_6\): C, 42.31; H, 3.99; N, 18.50. Found: C, 42.13; H, 3.97; N, 18.42.
2,6-Bis(1-methyl-2-aminopyrimidinium)pyridine chloride (83)

2,6-Bis(chloromethyl)pyridine (0.447 g, 2.54 mmol) and 2-aminopyrimidine (1.21 g, 12.7 mmol) were dissolved in DMF (2 mL) and the mixture stirred overnight at 70°C. The precipitate was isolated by filtration and washed with DMF (1 mL) and Et₂O (1 mL). The crude solid was dissolved in H₂O (5 mL) and the solution was allowed to evaporate slowly, affording the product as colourless needles (0.411 g, 44%).

\(^1\)H NMR (D₂O) \(\delta\) 8.88 (dd, 2H, \(^3\)J\(_{H,H}\) = 4.40 Hz, \(^4\)J\(_{H,H}\) = 2.03 Hz, H4) 8.30 (dd, 2H, \(^3\)J\(_{H,H}\) = 6.61 Hz, \(^4\)J\(_{H,H}\) = 2.03 Hz, H6) 8.04 (t, 1H, \(^3\)J\(_{H,H}\) = 7.81 Hz, H₄₅⁶) 7.59 (d, 2H, \(^3\)J\(_{H,H}\) = 7.81 Hz, H₃₄₅) 7.12 (dd, 2H, \(^3\)J\(_{H,H}\) = 6.61 Hz, \(^4\)J\(_{H,H}\) = 4.40 Hz, H5) 5.58 (s, 4H, CH₂) ppm. Traces of DMF present.

\(^{13}\)C\({}^1\)H NMR (D₂O) \(\delta\) 167.08, 156.48, 151.25, 150.76, 139.32, 122.65, 111.57, 57.54 ppm. ESI-MS: \(m/z\) 294.13 [M – 2Cl– H⁴⁺]+, 199.27 [M – 2Cl – H⁺ – apym]⁺, 147.60 [M – 2Cl]²⁺. Anal. calcd for C₁₅H₁₇Cl₂N₇·1.25H₂O: C, 46.34; H, 5.06; N, 25.22. Found: C, 46.19; H, 4.99; N, 25.05.

1,3,5-Tris[1-(2-aminopyrimidiniummethyl)]-2,4,6-trimethylbenzene bromide (85)

The title compound was prepared analogously to 1,3,5-tris(3-aminopyraziniummethyl)-2,4,6-trimethylbenzene monohydrate (53), using 2-aminopyrimidine instead of aminopyrazine (73%, white powder).

\(^1\)H NMR (D₂O) \(\delta\) 8.87 (m, 3H, H₄), 7.96 (m, 3H, H₆), 7.10 (m, 3H, H₅), 5.40 (s, 6H, CH₂), 2.31 (s, 9H, CH₃) ppm. Traces of DMF present. \(^{13}\)C\({}^1\)H NMR (100 MHz, D₂O) \(\delta\) 165.8, 156.3, 145.6, 144.6, 127.0, 112.0, 51.7, 16.3 ppm. ESI-MS: \(m/z\) 442.00 [M – 3Br⁺]+, 347.13 [M – 3Br – 2H⁺ – apym]⁺, 252.07 [M – 3Br – 2H⁺ – 2apym]⁺, 221.53 [M – 3Br – H⁺]²⁺, 174.07 [M – 3Br – H⁺ – apym]²⁺. Anal. calcd for C₂₄H₂₉N₉Br₃·4H₂O: C, 38.11; H, 5.06; N, 16.67. Found: C, 38.21; H, 4.96; N, 16.61.
1,2,4,5-Tetrakis[1-(2-aminopyrimidiniummethyl)]benzene bromide (88)

2-Aminopyrimidine (475 mg, 5.00 mmol) and 1,2,4,5-tetrakis(bromomethyl)benzene (225 mg, 0.500 mmol) were stirred in DMF (2 mL) for 48 h at 70°C. After cooling to room temperature, this was isolated by filtration, washed with DMF (0.5 mL) and Et₂O (1 mL) and dried in vacuo to afford the crude product as a white powder (87 mg, 21%).

¹H NMR (D₂O) δ 8.87 (m, 4H, H₄), 8.34 (m, 4H, H₆), 7.19 (m, 4H, H₅), 6.00 (s, 2H, CH), 5.59 (s, 8H, CH₂), 2.31 (s, 9H, CH₃) ppm. Traces of DMF and Et₂O present.


6.4 Synthesis of bis(bromoalkyl) diquaternary Br⁻ salts

The syntheses of ligands of type [Br(CH₂)ₙ4,4′-bipy(CH₂)ₙBr]Br₂ (97: n = 4; 98: n = 5; 99: n = 6) were performed using adaptations of a procedure reported for the dodecyl derivative (n = 12).⁸ A mixture of α,ω-dibromoalkane (50 mmol), 4,4′-bipyridine (10 mmol) and DMF (30 mL) was stirred for 48 h at 70°C, over which time a precipitate formed. After cooling to room temperature, this was isolated by filtration, washed with DMF (20 mL) and Me₂CO (20 mL) and dried in vacuo to give the desired product as a crude solid.††

1,1′-Bis(4-bromobutyl)-4,4′-bipyridinium bromide (97)

A synthesis of the title compound has been reported previously, but it resulted in an inseparable mixture containing this compound. It was prepared from 1,4-dibromobutane and 4,4′-bipyridine (90%, yellow powder).

¹H NMR (D₂O) δ 9.17 (d, 4H, 3J_H,H = 6.4 Hz, H₂’), 8.59 (d, 4H, 3J_H,H = 6.2 Hz, H₃’), 4.84 (obscured by HOD peak, 4H, H₄), 3.58 (t, 4H, 3J_H,H = 6.4 Hz, H₁), 2.29 (m, 4H, H₃), 2.01 (m, 4H, H₂) ppm. ESI-MS: m/z 508.67 [M – Br⁻]⁺.

†† The solid products were found to contain trace amounts of [4,4′-bipy(CH₂)ₙ4,4′-bipy]Br₂ as well DMF and Me₂CO solvate. They were used without further purification.
1,1′-Bis(5-bromopentyl)-4,4′-bipyridinium bromide (98)

The title compound was prepared from 1,5-dibromopentane and 4,4′-bipyridine (87%, yellow powder).

\[ ^1H \text{ NMR } (D_2O) \delta 9.19 \text{ (d, 4H, } J_{H,H} = 6.7 \text{ Hz, H2’}, 8.62 \text{ (d, 4H, } J_{H,H} = 5.9 \text{ Hz, H3’}), 4.83 \text{ (obscured by HOD peak, 4H, H5}), 3.57 \text{ (t, 4H, } J_{H,H} = 6.5 \text{ Hz, H1}), 2.16 \text{ (m, 4H, H4), 1.99 (m, 4H, H2), 1.59 (m, 4H, H3) ppm. ESI-MS: } m/z 536.47 [M – Br]^{+}, 228.00 [M – 2Br]^2^{+}. \]

1,1′-Bis(6-bromohexyl)-4,4′-bipyridinium bromide (99)

The title compound was prepared from 1,6-dibromohexane and 4,4′-bipyridine (81%, yellow powder).

\[ ^1H \text{ NMR } (D_2O) \delta 9.17 \text{ (d, 4H, } J_{H,H} = 6.5 \text{ Hz, H2’}, 8.60 \text{ (d, 4H, } J_{H,H} = 6.2 \text{ Hz, H3’}), 4.78 \text{ (obscured by HOD peak, 4H, H6}), 3.54 \text{ (t, 4H, } J_{H,H} = 6.6 \text{ Hz, H1}), 2.16 \text{ (m, 4H, H5), 1.90 (m, 4H, H2), 1.60-1.40 (m, 8H, H3,4) ppm. ESI-MS: } m/z 564.80 [M – Br]^{+}, 242.13 [M – 2Br]^2^{+}. \]

6.5 Synthesis of tetraquaternary Br’ salts

The compounds 100 and 101 were synthesised using a procedure similar to that previously described for the dodecyl derivative. A mixture of the 1,1′-bis(ω-bromoalkyl)-4,4′-bipyridinium bromide (3.40 mmol) and 4,4′-bipyridine (5.74 g, 34.0 mmol) in DMF/MeOH (2 : 1, 100 mL) was stirred at reflux for 48 h. The mixture was allowed to cool, and MeOH was removed in vacuo. The precipitate was isolated by filtration, washed with DMF (5 mL), Et₂O (10 mL) and crystallised from H₂O to give the tetraquaternary salt as a pure solid.
1,1′-Bis[4-(4,4′-bipyridinium)butyl]-4,4′-bipyridinium bromide (100)

The title compound was prepared from 1,1′-bis(4-bromobutyl)-4,4′-bipyridinium bromide (97) (27%, tan fibres).

\[^1\text{H} \text{NMR} \ (\text{D}_2\text{O}) \ \delta\ 9.20\ (d, 4H, J_{ HH} = 6.7 \text{ Hz}, \text{H}2′′),\ 9.05\ (d, 4H, J_{ HH} = 6.7 \text{ Hz}, \text{H}2′′′),\ 8.80\ (d, 4H, J_{ HH} = 6.0 \text{ Hz}, \text{H}2′),\ 8.62\ (d, 4H, J_{ HH} = 6.6 \text{ Hz}, \text{H}3′′′),\ 8.46\ (d, 4H, J_{ HH} = 6.7 \text{ Hz}, \text{H}3′′′′),\ 7.95\ (d, 4H, J_{ HH} = 6.0 \text{ Hz}, \text{H}3′),\ 4.88\ (m, 4H, \text{H}1),\ 4.81\ (\text{obscured by HOD peak},\ 4H, \text{H}4),\ 2.29\ (m, 8H, \text{H}2,3) \text{ ppm.} \[^{13}\text{C}\{^1\text{H}\} \text{NMR} \ (\text{D}_2\text{O}) \ \delta\ 154.49,\ 150.67,\ 150.42,\ 145.96,\ 145.24,\ 142.90,\ 127.70,\ 126.67,\ 122.92,\ 61.52,\ 60.90,\ 27.80,\ 27.75 \text{ ppm.} \]

ESI-MS: \(m/z\) 820.93 \([\text{M – Br}]^+\), 370.00 \([\text{M – 2Br}]^{2+}\). Anal. calcd for C\(_{38}\)H\(_{40}\)Br\(_4\)N\(_6\)·3.5H\(_2\)O: C, 47.37; H, 4.92; N, 8.72. Found: C, 47.41; H, 4.95; N, 8.68.

1,1′-Bis[6-(4,4′-bipyridinium)hexyl]-4,4′-bipyridinium bromide (101)

The title compound was prepared from 1,1′-bis(6-bromohexyl)-4,4′-bipyridinium bromide (99) (19%, yellow powder).

\[^1\text{H} \text{NMR} \ (\text{D}_2\text{O}) \ \delta\ 9.16\ (d, 4H, J_{ HH} = 6.5 \text{ Hz}, \text{H}2′′),\ 9.00\ (d, 4H, J_{ HH} = 6.5 \text{ Hz}, \text{H}2′′′),\ 8.78\ (d, 4H, J_{ HH} = 5.3 \text{ Hz}, \text{H}2′),\ 8.58\ (d, 4H, J_{ HH} = 6.4 \text{ Hz}, \text{H}3′′′),\ 8.43\ (d, 4H, J_{ HH} = 6.4 \text{ Hz}, \text{H}3′′′′),\ 7.93\ (d, 4H, J_{ HH} = 5.8 \text{ Hz}, \text{H}3′),\ 4.75 – 4.69\ (\text{obscured by HOD peak},\ 8H, \text{H}1,6),\ 2.14\ (m, 8H, \text{H}2,5),\ 1.52\ (m, 8H, \text{H}3,4) \text{ ppm.} \[^{13}\text{C}\{^1\text{H}\} \text{NMR} \ (\text{D}_2\text{O}) \ \delta\ 154.49,\ 150.43,\ 149.90,\ 145.88,\ 145.18,\ 142.98,\ 127.48,\ 126.49,\ 122.91,\ 62.44,\ 61.86,\ 30.82,\ 30.73,\ 25.30 (2 \text{superimposed resonances}) \text{ ppm.} \]

ESI-MS: \(m/z\) 876.80 [M – Br]\(^+\), 397.93 [M – 2Br]\(^{2+}\), 238.87 [M – 3Br]\(^{3+}\). Anal. calcd for C\(_{42}\)H\(_{48}\)Br\(_4\)N\(_6\)·7H\(_2\)O: C, 46.60; H, 5.77; N, 7.76. Found: C, 46.58; H, 5.80; N, 7.68.

6.6 Synthesis of NO\(_3^–\) salts

Typically, the halide salt (1 mmol) was dissolved in the minimum amount of warm H\(_2\)O, and treated with AgNO\(_3\) (1 mmol/mmole\(_{\text{halide ion}}\)) in H\(_2\)O (5 mL). The mixture was filtered, and concentrated to ~3 mL on a hotplate, filtering through cellulose if necessary. The
solvent was removed and, if required, the solid could be crystallised from H₂O to give the pure NO₃⁻ salt.

1-Methyl-4,4′-bipyridinium nitrate (15)

The title compound was prepared from 1-methyl-4,4′-bipyridinium iodide (14)\(^{10\dagger\dagger}\) (31%, colourless crystals).

\(^1\)H NMR (D₂O) \(\delta\) 8.92 (d, 2H, \(3J_{H,H} = 6.76\) Hz, H2), 8.72 (d, 2H, \(3J_{H,H} = 4.43\) Hz, H2'), 8.36 (d, 2H, \(3J_{H,H} = 6.76\) Hz, H3), 7.87 (d, 2H, \(3J_{H,H} = 6.76\) Hz, H3'), 4.47 (s, 3H, CH₃) ppm.

\(^{13}\)C{\(^1\)H} NMR (D₂O) \(\delta\) 153.72, 150.37, 146.02, 142.86, 126.04, 122.79, 48.23 ppm.

ESI-MS: \(m/z\) 171.00 \([M – NO₃⁻]^+\). Anal. calcd for C₁₁H₁₁N₃O₃: C, 56.65; H, 4.75; N, 18.02. Found: C, 56.94; H, 4.99; N, 18.30.

1,2-Bis(4,4′-bipyridinium)ethane nitrate (17)

The title compound was prepared from 1,2-bis(4,4′-bipyridinium)ethane bromide (16)\(^{11}\) (95%, colourless crystals)

\(^1\)H NMR (D₂O) \(\delta\) 9.03 (d, 4H, \(3J_{H,H} = 6.6\) Hz, H2), 8.81 (d, 4H, \(3J_{H,H} = 5.5\) Hz, H2'), 8.52 (d, 4H, \(3J_{H,H} = 6.6\) Hz, H3), 7.95 (d, 4H, \(3J_{H,H} = 5.5\) Hz, H3'), 5.38 (s, 4H, CH₂) ppm.

\(^{13}\)C{\(^1\)H} NMR (D₂O) \(\delta\) 156.00, 150.40, 145.68, 142.60, 127.28, 122.99, 60.11 ppm.

ESI-MS: \(m/z\) 401.87 \([M – NO₃⁻]^+\), 170.27 \([M – 2NO₃⁻]^{2+}\). Anal. calcd for C₂₂H₂₀N₆O₆: C, 56.89; H, 4.34; N, 18.10. Found: C, 56.51; H, 4.41; N, 17.94.

1,3-Bis(4,4′-bipyridinium)propane nitrate (19)

The title compound was prepared from 1,3-bis(4,4′-bipyridinium)propylene bromide (18)\(^6\) (51%, off-white powder). The crude product was purified by dissolution in warm EtOH/H₂O (1 : 1) and precipitation with Me₂CO, resulting in the lower yield.

\(^1\)H NMR (D₂O) \(\delta\) 9.08 (d, 4H, \(3J_{H,H} = 5.96\) Hz, H2), 8.76 (d, 4H, \(3J_{H,H} = 4.33\) Hz, H2'), 8.46 (d, 4H, \(3J_{H,H} = 5.96\) Hz, H3), 7.91 (d, 4H, \(3J_{H,H} = 4.33\) Hz, H3'), 4.96 (t, 4H, \(3J_{H,H} = 7.31\) Hz, H\(_{\text{propylene}}\)), 2.96 (q, 2H, \(3J_{H,H} = 7.31\) Hz, H\(_{\text{propylene}}\)) ppm. \(^{13}\)C{\(^1\)H} NMR (D₂O) \(\delta\) 154.71,

\(^{\dagger\dagger}\) This compound contained traces of 1,1′-dimethyl-4,4′-bipyridinium iodide and could not be purified by repeated crystallisation from EtOH or H₂O.
150.65, 145.41, 142.76, 126.76, 122.92, 58.41, 31.96 ppm. ESI-MS: \textit{m/z} 415.00 [M – NO$_3$]$^+$, 177.27 [M – 2NO$_3$]$^{2+}$. Anal. calcd for C$_{38}$H$_{40}$Br$_4$N$_6$·H$_2$O: C, 55.64; H, 4.87; N, 16.93. Found: C, 55.46; H, 4.90; N, 16.93.

1,4-Bis(4,4′-bipyridinium)butane nitrate (22)

The title compound was prepared from 1,4-bis(4,4′-bipyridinium)butylene bromide (21)$^6$ (51%, colourless crystals).

$^1$H NMR (200 MHz, D$_2$O) δ 9.01 (d, 4H, $^3$J$_{H,H}$ = 6.0 Hz, H2), 8.79 (d, 4H, $^3$J$_{H,H}$ = 4.3 Hz, H2′), 8.46 (d, 4H, $^3$J$_{H,H}$ = 6.0 Hz, H3), 7.85 (d, 4H, $^3$J$_{H,H}$ = 4.3 Hz, H3′), 4.79 (obscured by HOD peak, m, 4H, H$_{\text{butylene} \alpha}$), 2.96 (m, 4H, H$_{\text{butylene} \beta}$) ppm. ESI-MS: \textit{m/z} 430.0 [M – NO$_3$]$^+$, 184.2 [M – 2NO$_3$]$^{2+}$.

1,6-Bis(4,4′-bipyridinium)hexane nitrate (25)

The title compound was prepared from 1,6-bis(4,4′-bipyridinium)hexane bromide (24)$^{11}$ (53%, colourless crystals).

$^1$H NMR (D$_2$O) δ 8.98 (d, 4H, $^3$J$_{H,H}$ = 6.76 Hz, H2), 8.76 (d, 4H, $^3$J$_{H,H}$ = 5.37 Hz, H2′), 8.40 (d, 4H, $^3$J$_{H,H}$ = 6.76 Hz, H3), 7.89 (d, 4H, $^3$J$_{H,H}$ = 5.37 Hz, H3′), 4.70 (t, 4H, $^3$J$_{H,H}$ = 7.20 Hz, H$_{\text{hexylene} \alpha}$), 2.12 (m, 4H, H$_{\text{hexylene} \beta}$), 1.49 (m, 4H, H$_{\text{hexylene} \gamma}$) ppm. $^{13}$C{$^1$H} NMR (D$_2$O) δ 154.24, 150.47, 125.15, 142.92, 126.41, 122.83, 61.922, 30.61, 25.33 ppm. ESI-MS: \textit{m/z} 457.67 [M – NO$_3$]$^+$, 197.67 [M – 2NO$_3$]$^{2+}$. Anal. calcd for C$_{26}$H$_{26}$O$_6$N$_6$: C, 59.99; H, 5.42; N, 16.14. Found: C, 59.79; H, 5.44; N, 16.02.

1,2-Bis(4,4′-bipyridiniummethyl)benzene nitrate (28)

The title compound was prepared from 1,2-bis(4,4′-bipyridiniummethyl)benzene bromide (27)$^{11}$ (90%, yellow powder).

$^1$H NMR (D$_2$O) δ 8.79 (d, 4H, $^3$J$_{H,H}$ = 6.7 Hz, H2), 8.58 (d, 4H, $^3$J$_{H,H}$ = 6.1 Hz, H2′), 8.25 (d, 4H, $^3$J$_{H,H}$ = 6.7 Hz, H3), 7.84 (m, 2H, H$_{\text{xylyl} \alpha}$), 7.77 (m, 2H, H$_{\text{xylyl} \beta}$), 7.66 (d, 4H, $^3$J$_{H,H}$ = 6.1 Hz, H3′), 6.13 (s, 4H, CH$_2$) ppm. $^{13}$C{$^1$H} NMR (D$_2$O) δ 155.48, 151.38, 145.91, 142.45, 135.41, 133.55, 132.09, 127.33, 123.47, 62.81 ppm. ESI-MS: \textit{m/z} 478.13
$\text{[M – NO}_3^+\text{]}^{2+}$, 208.20 $\text{[M – 2NO}_3^-\text{]}^{2+}$. Anal. calcd for C\textsubscript{28}H\textsubscript{24}N\textsubscript{6}O\textsubscript{6}·0.5H\textsubscript{2}O: C, 61.18; H, 4.59; N, 15.30. Found: C, 61.20; H, 4.64; N, 15.22.

\((\pm)-2,6\text{-Bis(4,4′-bipyridinium)-9-thiabicyclo[3.3.1]nonane nitrate (31)}\)

The title compound was prepared from $\pm$-2,6-bis(4,4′-bipyridinium)-9-thiabicyclo[3.3.1]nonane chloride (30) (93%, white powder).

$^1\text{H NMR (D}_2\text{O) } \delta$ 9.23 (d, 4H, $^3J_{H,H} = 6.36$ Hz, H2), 8.85 (d, 4H, $^3J_{H,H} = 4.60$ Hz, H2′), 8.56 (d, 4H, $^3J_{H,H} = 6.36$ Hz, H3), 7.97 (d, 4H, $^3J_{H,H} = 4.60$ Hz, H3′), 5.83 (m, 2H, H\textsubscript{aliphatic}), 3.45 (m, 2H, H\textsubscript{aliphatic}), 3.21 (m, 2H, H\textsubscript{aliphatic}), 2.52 (m, 6H, H\textsubscript{aliphatic}) ppm. Traces of DMF present.

$^{13}\text{C}$$\{}^1\text{H}\text{NMR (D}_2\text{O) } \delta$ 155.34, 150.62, 144.33, 142.99, 127.06, 123.12, 74.04, 36.56, 27.17, 25.33 ppm. ESI-MS: $m/\text{z}$ 514.00 $\text{[M – NO}_3^-\text{]}^{2+}$, 225.90 $\text{[M – 2NO}_3^-\text{]}^{2+}$. Anal. calcd for C\textsubscript{28}H\textsubscript{28}N\textsubscript{6}O\textsubscript{6}S·5H\textsubscript{2}O: C, 50.44; H, 5.74; N, 12.61. Found: C, 50.46; H, 5.28; N, 12.63.

1,6-Bis(pyrazinium)hexane nitrate (41)

The title compound was prepared from 1,6-bis(pyrazinium)hexane bromide (39)\textsuperscript{11} (99%, white powder).

$^1\text{H NMR (200 MHz, D}_2\text{O) } \delta$ 9.49 (m, 4H, H3), 9.09 (m, 4H, H2), 4.74 (observed by HOD peak, m, 4H, H\textsubscript{hexylene}), 2.12 (m, 4H, H\textsubscript{hexylene}), 1.51 (m, 4H, H\textsubscript{hexylene}) ppm. $^{13}\text{C}$$\{}^1\text{H}\text{NMR (D}_2\text{O) } \delta$ 151.38, 137.61 (t, $^1J_{C,N} = 7.8$ Hz), 63.32, 30.56, 27.17, 25.19 ppm. ESI-MS: $m/\text{z}$ 122.13 $\text{[M – 2NO}_3^-\text{]}^{2+}$. Anal. calcd for C\textsubscript{14}H\textsubscript{20}N\textsubscript{6}O\textsubscript{6}·0.33H\textsubscript{2}O: C, 44.92; H, 5.74; N, 22.45. Found: C, 44.91; H, 5.35; N, 22.41.

1-Methyl-3-aminopyrazinium nitrate (43)

The title compound was prepared from 1-methyl-3-aminopyrazinium iodide (42)\textsuperscript{12} (99%, colourless crystals). Slow evaporation of an aqueous solution of the title compound afforded single crystals suitable for X-ray crystallographic analysis, details of which are given in the Appendices (Section A.3).

$^1\text{H NMR (D}_2\text{O) } \delta$ 8.56 (m, 1H, H5), 8.11 (m, 1H, H6), 7.12 (s, 1H, H2), 4.28 (s, 3H, CH\textsubscript{3}) ppm. $^{13}\text{C}$$\{}^1\text{H}\text{NMR (D}_2\text{O) } \delta$ 158.97, 149.10, 126.55 (t, $^1J_{C,N} = 11.18$ Hz),
124.52 (t, $J_{\text{C,N}} = 8.85$ Hz) 48.83 (t, $J_{\text{C,N}} = 5.1$ Hz) ppm. ESI-MS: $m/z$ 110.00 [M – NO$_3$]$^+$. Anal. calcd for C$_5$H$_8$O$_3$N$_4$: C, 34.17; H, 4.82; N, 31.88. Found: C, 34.10; H, 4.64; N, 31.77.

1,6-Bis(3-aminopyrazinium)hexane nitrate (49)

The title compound was prepared from 1,6-bis(3-aminopyrazinium)hexane bromide (48) (96%, tan crystals).

$^1$H NMR (D$_2$O) $\delta$ 8.58 (m, 2H, H$_5$), 8.15 (s, 2H, H$_6$), 7.95 (d, 2H, $J_{\text{H,H}} = 2.8$ Hz, H$_2$), 4.48 (t, 4H, $J_{\text{H,H}} = 7.4$ Hz, H$_{\text{hexylene,\alpha}}$), 2.03 (m, 4H, H$_{\text{hexylene,\beta}}$), 1.45 (m, 4H, H$_{\text{hexylene,\gamma}}$) ppm. $^{13}$C{$^1$H} NMR (D$_2$O) $\delta$ 159.09, 149.38, 125.52, 123.56, 62.65, 30.22, 25.19 ppm. ESI-MS: $m/z$ 336.1 [M – NO$_3$]$^+$, 273.0 [M – H$^+$ – 2NO$_3$]$^+$, 137.2 [M – 2NO$_3$]$^{2+}$. Anal. calcd for C$_{14}$H$_{22}$N$_8$O$_6$: C, 42.21; H, 5.57; N, 28.13. Found: C, 42.28; H, 5.57; N, 27.86.

1,3,5-Tris(3-aminopyraziniummethyl)-2,4,6-trimethylbenzene nitrate (54)

The title compound was prepared from 1,3,5-tris(4-aminopyraziniummethyl)-2,4,6-trimethylbenzene bromide (53) (97%, white powder).

$^1$H NMR (D$_2$O) $\delta$ 8.59 (m, 3H, H$_5$), 7.96 (m, 3H, H$_6$), 7.82 (s, 3H, H$_2$), 5.87 (s, 6H, CH$_3$), 2.35 (s, 9H, CH$_3$) ppm. $^{13}$C{$^1$H} NMR (D$_2$O) $\delta$ 159.43, 149.85, 145.41, 127.74, 124.34, 122.48, 59.70, 16.65 ppm. ESI-MS: $m/z$ 567.47 [M – NO$_3$]$^+$, 504.63 [M – 2NO$_3^-$ – H$^+$]$^+$, 441.60 [M – 3NO$_3^-$ – 2H$^+$]$^+$, 409.53 [M – 2NO$_3^-$ – H$^+$ – apyz]$^+$, 346.60 [M – 3NO$_3^-$ – 2H$^+$ – apyz]$^+$, 252.53 [M – 2NO$_3^-$]$^{2+}$, 173.47 [M – 3NO$_3^-$ – H$^+$ – apyz]$^{2+}$, 147.60 [M – 3NO$_3^-$]$^{3+}$. Anal. calcd for C$_{24}$H$_{30}$O$_9$N$_{12}$·1.5H$_2$O: C, 43.84; H, 5.05; N, 25.56. Found: C, 43.91; H, 4.87; N, 25.52.

1-Methyl-2-aminopyridinium nitrate (57)

The title compound was prepared from 1-methyl-2-aminopyridinium iodide (56)$^{13}$ (97%, colourless crystals).

$^1$H NMR (D$_2$O) $\delta$ 8.82 (dd, 1H, $J_{\text{H,H}} = 4.49$ Hz, $J_{\text{H,H}} = 2.00$ Hz, H$_4$), 8.39 (dd, 1H, $J_{\text{H,H}} = 6.59$ Hz, $J_{\text{H,H}} = 2.00$ Hz, H$_6$), 7.12 (dd, 1H, $J_{\text{H,H}} = 6.59$ Hz, $J_{\text{H,H}} = 4.49$ Hz, H$_5$) 3.86 (s, 3H, CH$_3$) ppm. $^{13}$C{$^1$H} NMR (D$_2$O) $\delta$ 166.37, 156.67, 151.19,
111.50, 42.06 ppm. ESI-MS: \( m/z \) 110.00 [M – NO\(_3\)]\(^+\). Anal. calcd for C\(_5\)H\(_8\)O\(_3\)N\(_4\)·0.2H\(_2\)O: C, 34.17; H, 4.82; N, 31.88. Found: C, 34.33; H, 4.68; N, 31.87.

1,4-Bis(2-aminopyrimidinium)butane nitrate (64)

The title compound was prepared from 1,4-bis(2-aminopyrimidinium)butane bromide (63) (91%, white powder).

\(^1\)H NMR (D\(_2\)O) \( \delta \) 8.82 (dd, 2H, \(^3\)J\(_{H,H}\) = 3.3 Hz, \(^4\)J\(_{H,H}\) = 1.5 Hz, H4), 8.38 (dd, 2H, \(^3\)J\(_{H,H}\) = 5.0 Hz, \(^4\)J\(_{H,H}\) = 1.6 Hz, H6), 7.13 (dd, 2H, \(^3\)J\(_{H,H}\) = 5.0 Hz, \(^3\)J\(_{H,H}\) = 3.4 Hz, H5), 4.26 (m, 4H, H\(_{\text{butylene}}\alpha\)), 2.03 (m, 4H, H\(_{\text{butylene}}\beta\)) ppm.

\(^{13}\)C{\(^1\)H} NMR (D\(_2\)O) \( \delta \) 166.3, 155.5, 149.8, 111.4, 53.8, 23.0 ppm. ESI-MS: \( m/z \) 245.07 [M – 2NO\(_3\) – H\(^+\)]\(^+\), 123.13 [M – 2NO\(_3\)]\(^2+\).

1,5-Bis(2-aminopyrimidinium)pentane nitrate (67)

The title compound was prepared from 1,5-bis(2-aminopyrimidinium)pentane bromide (66) (78%, white powder).

\(^1\)H NMR (300 MHz, D\(_2\)O) \( \delta \) 8.81 (dd, 2H, \(^3\)J\(_{H,H}\) = 4.32 Hz, \(^4\)J\(_{H,H}\) = 1.75 Hz, H4), 8.37 (dd, 2H, \(^3\)J\(_{H,H}\) = 6.58 Hz, \(^4\)J\(_{H,H}\) = 1.66 Hz, H6), 7.13 (dd, 2H, \(^3\)J\(_{H,H}\) = 6.42 Hz, \(^3\)J\(_{H,H}\) = 4.76 Hz, H4), 4.21 (t, 4H, H\(_{\text{pentylene}}\alpha\)), 1.97 (m, 4H, H\(_{\text{pentylene}}\beta\)), 1.51 (qu, 2H, \(^3\)J\(_{H,H}\) = 7.18 Hz, H\(_{\text{pentylene}}\gamma\)) ppm. \(^{13}\)C{\(^1\)H} NMR (D\(_2\)O) \( \delta \) 166.50, 155.94, 150.21, 111.77, 54.67, 26.20, 22.57 ppm. ESI-MS: \( m/z \) 259.07 [M – 2NO\(_3\) – H\(^+\)]\(^+\), 130.20 [M – 2NO\(_3\)]\(^+\). Anal. calcd for C\(_{13}\)H\(_{20}\)O\(_6\)N\(_8\)·1.66H\(_2\)O: C, 37.69; H, 5.67; N, 27.05. Found: C, 37.38; H, 5.40; N, 27.16.

1,6-Bis(2-aminopyrimidinium)hexane nitrate (70)

The title compound was prepared from 1,6-bis(2-aminopyrimidinium)hexane bromide (69) (95%, white powder).

\(^1\)H NMR (200 MHz, D\(_2\)O) \( \delta \) 8.79 (dd, 2H, \(^3\)J\(_{H,H}\) = 4.4 Hz, \(^4\)J\(_{H,H}\) = 1.9 Hz, H4), 8.37 (dd, 2H, \(^3\)J\(_{H,H}\) = 6.6 Hz, \(^4\)J\(_{H,H}\) = 1.9 Hz, H6), 7.11 (dd, 2H, \(^3\)J\(_{H,H}\) = 6.6 Hz, \(^3\)J\(_{H,H}\) = 4.4 Hz, H5), 4.19 (t, 4H, \(^3\)J\(_{H,H}\) = 7.3 Hz, H\(_{\text{hexylene}}\)), 1.91 (m, 4H, H\(_{\text{hexylene}}\)), 1.48 (m, 4H, H\(_{\text{hexylene}}\)) ppm. \(^{13}\)C{\(^1\)H} NMR (D\(_2\)O) \( \delta \) 167.41, 156.95, 151.27, 112.75, 55.97, 27.39, 26.42 ppm. ESI-MS:
\[ m/z\ 273.20 \ [M – 2NO_3^- – H^+]^+,\ 178.13 \ [M – apym – 2NO_3^- – H^+]^+,\ 137.20 \ [M – 2NO_3^-]^2+. \]

Anal. calcd for C_{14}H_{22}N_{8}O_{6}·H_{2}O: C, 40.38; H, 5.81; N, 26.91. Found: C, 40.61; H, 5.72; N, 27.01.

### 1,8-Bis(2-aminopyrimidinium)octane nitrate (73)

The title compound was prepared from 1,8-bis(2-aminopyrimidinium)octane bromide (72) (97%, white powder).

\[^1H\ NMR\ (D_2O)\ \delta\ 8.79\ (m, 2H, H_4),\ 8.36\ (m, 2H, H_6),\ 7.11\ (m, 2H, H_5),\ 4.17\ (t, 4H, J_{H,H} = 7.65\ Hz, H_{octylene}^\alpha),\ 1.89\ (m, 4H, H_{octylene}^\beta),\ 1.38\ (m, 8H, H_{octylene}^\gamma, \delta)\ ppm.\]

\[^{13}C\{^1H\}\ NMR\ (D_2O)\ \delta\ 166.19,\ 155.91,\ 150.29,\ 111.67,\ 55.12,\ 28.42,\ 26.57,\ 25.59\ ppm.\ ESI-MS: m/z\ 363.80 \ [M – NO_3^-]^+,\ 301.27 \ [M – 2NO_3^- – H^+]^+,\ 151.00 \ [M – 2NO_3^-]^2+.\ Anal. calcd for C_{16}H_{26}N_{8}O_{6}: C, 43.24; H, 6.35; N, 25.21. Found: C, 43.14; H, 6.17; N, 24.95.

### 1,2-Bis(1-methyl-2-aminopyrimidinium)benzene nitrate (76)

The title compound was prepared from 1,2-bis(1-methyl-2-aminopyrimidinium)benzene bromide (75) (60%, colourless needles).

\[^1H\ NMR\ (400\ MHz, D_2O)\ \delta\ 9.08\ (dd, 2H, J_{H,H} = 4.4\ Hz, H_4),\ 8.37\ (dd, 2H, J_{H,H} = 6.7\ Hz, J_{H,H} = 2.0\ Hz, H_6),\ 7.76\ (dd, 2H, J_{H,H} = 3.3\ Hz, H_3_s),\ 7.34\ (dd, 2H, J_{H,H} = 3.3\ Hz, J_{H,H} = 5.7\ Hz, J_{H,H} = 5.7\ Hz, H_4_s),\ 7.34\ (dd, 2H, J_{H,H} = 6.7\ Hz, J_{H,H} = 5.7\ Hz, H_5_s),\ 5.63\ (s, 4H, CH_2)\ ppm.\]

\[^{13}C\{^1H\}\ NMR\ (100\ MHz, D_2O)\ \delta\ 167.11,\ 155.91,\ 150.29,\ 111.67,\ 129.06,\ 128.52,\ 112.01,\ 54.31\ ppm.\ For\ 2-D\ COSY,\ see\ Section\ A.1.\ ESI-MS: m/z\ 293.00 \ [M – 2NO_3^- – H^+]^+,\ 198.20 \ [M – 2NO_3^- – apym – H^+]^+.\ Anal. calcd for C_{16}H_{18}N_{8}O_{6}: C, 45.93; H, 4.34; N, 26.78. Found: C, 45.94; H, 4.350; N, 26.61.

### 1,3-Bis(1-methyl-2-aminopyrimidinium)benzene nitrate (79)

The title compound was prepared from 1,3-bis(1-methyl-2-aminopyrimidinium)benzene bromide (78) (96%, white powder).

\[^1H\ NMR\ (D_2O)\ \delta\ 8.88\ (m, 2H, H_4),\ 8.34\ (m, 2H, H_6),\ 7.64\ (t, 1H, J_{H,H} = 7.59\ Hz, H_5_s),\ 7.46\ (d, 4H, J_{H,H} = 7.59\ Hz, H_4_s),\ 7.21\ (s, 1H, H_2_s)\ ppm.\]
(m, 2H, H\textsubscript{5\textsubscript{apym}}), 5.46 (s, 4H, CH\textsubscript{2}) ppm. Traces of DMF present. \textsuperscript{13}C\{\textsuperscript{1}H\} NMR (D\textsubscript{2}O) \(\delta\) 167.15, 156.30, 150.05, 132.50, 127.59, 127.62, 112.12, 57.19 ppm. ESI-MS: \(m/z\) 584.93 [2M – 4NO\textsubscript{3} – H\textsuperscript{+}]\textsuperscript{+}, 293.13 [M – 2NO\textsubscript{3} – H\textsuperscript{+}]\textsuperscript{+}, 198.27 [M – apym – 2NO\textsubscript{3} – H\textsuperscript{+}]\textsuperscript{+}, 147.13 [M – 2NO\textsubscript{3}]\textsuperscript{2+}. Anal. calcd for C\textsubscript{16}H\textsubscript{18}O\textsubscript{6}N\textsubscript{8}: C, 44.97; H, 4.48; N, 26.22. Found: C, 45.09; H, 4.43; N, 26.22.

### 2,6-Bis(1-methyl-2-aminopyrimidinium)pyridine nitrate (84)

The title compound was prepared from 2,6-bis(1-methyl-2-aminopyrimidinium)pyridine chloride (83) (93%, white powder).

\(\textsuperscript{1}H\) NMR (D\textsubscript{2}O) \(\delta\) 8.87 (dd, 2H, \(J_{HH} = 4.46\) Hz, \(J_{HH} = 1.99\) Hz, H4), 8.28 (dd, 2H, \(J_{HH} = 6.59\) Hz, \(J_{HH} = 1.99\) Hz, H6), 8.02 (t, 1H, \(J_{HH} = 7.82\) Hz, H4), 7.57 (d, 2H, \(J_{HH} = 7.82\) Hz, H3), 7.10 (dd, 2H, \(J_{HH} = 6.59\) Hz, \(J_{HH} = 4.46\) Hz, H5), 5.56 (s, 4H, CH\textsubscript{2}) ppm. \textsuperscript{13}C\{\textsuperscript{1}H\} NMR (D\textsubscript{2}O) \(\delta\) 167.24, 156.65, 151.41, 150.94, 139.58, 122.77, 111.704, 57.68 ppm. ESI-MS: \(m/z\) 294.13 [M – 2NO\textsubscript{3} – H\textsuperscript{+}]\textsuperscript{+}, 199.2 [M – 2NO\textsubscript{3} – H\textsuperscript{+} – apym]\textsuperscript{+}, 147.73 [M – 2NO\textsubscript{3}]\textsuperscript{2+}. Anal. calcd for C\textsubscript{15}H\textsubscript{17}N\textsubscript{9}O\textsubscript{6}·0.33H\textsubscript{2}O: C, 42.36; H, 4.19; N, 29.64. Found: C, 42.35; H, 4.07; N, 29.62.

### 1,3,5-Tris[1-(2-pyrimidiniummethyl)]-2,4,6-trimethylbenzene nitrate (86)

The title compound was prepared from 1,3,5-tris[1-(2-pyrimidiniummethyl)]-2,4,6-trimethylbenzene bromide (85) (95%, white powder).

\(\textsuperscript{1}H\) NMR (D\textsubscript{2}O) \(\delta\) 8.85 (m, 3H, H4), 7.83 (m, 3H, H6), 7.06 (m, 3H, H5), 5.38 (s, 6H, CH\textsubscript{2}), 2.29 (s, 9H, CH\textsubscript{3}) ppm. \textsuperscript{13}C\{\textsuperscript{1}H\} NMR (D\textsubscript{2}O) \(\delta\) 166.22, 156.65, 151.41, 150.94, 139.58, 122.77, 111.704, 57.68 ppm. ESI-MS: \(m/z\) 442.00 [M – 3NO\textsubscript{3} – 2H\textsuperscript{+}]\textsuperscript{+}, 347.13 [M – 3NO\textsubscript{3} – 2H\textsuperscript{+} – apym]\textsuperscript{+}, 284.07 [M – 2NO\textsubscript{3}]\textsuperscript{2+}, 252.20 [M – 3NO\textsubscript{3} – 2H\textsuperscript{+} – 2apym]\textsuperscript{+}, 221.47 [M – 3NO\textsubscript{3} – H\textsuperscript{+}]\textsuperscript{2+}, 174.07 [M – 3NO\textsubscript{3} – H\textsuperscript{+} – apym]\textsuperscript{2+}. Anal. calcd for C\textsubscript{24}H\textsubscript{30}O\textsubscript{9}N\textsubscript{12}·3H\textsubscript{2}O: C, 42.10; H, 5.30; N, 24.55. Found: C, 41.73; H, 4.74; N, 24.30.
1-(2-Pyrimidylaminomethyl)-2-(pyrimidiniummethyl)benzene nitrate (90)

The title compound was prepared from 1,2-bis(1-methyl-2-aminopyrimidinium)benzene bromide (75) (60%, colourless prismatic crystals). The mixture was warmed at 70°C for 10 min in order to effect the rearrangement. Slow evaporation of the resulting solution afforded single crystals suitable for X-ray crystallographic analysis, details of which are given in the Appendices (Section A.3).

$^1$H NMR (D$_2$O) $\delta$ 8.64 (dd, 1H, $^3$J$_{H,H}$ = 4.35 Hz, $^4$J$_{H,H}$ = 1.97 Hz, H$_4$aminopyridinium), 8.24 (d, 2H, $^3$J$_{H,H}$ = 4.93 Hz, H$_3$aminopyridine), 7.82 (dd, 1H, $^3$J$_{H,H}$ = 6.80 Hz, $^4$J$_{H,H}$ = 1.97 Hz, H$_6$aminopyridinium), 7.63-7.42 (m, 4H, H phenylene), 6.93 (dd, 1H, $^3$J$_{H,H}$ = 6.80 Hz, $^3$J$_{H,H}$ = 4.35 Hz, H$_5$aminopyridinium), 6.76 (t, 1H, $^3$J$_{H,H}$ = 4.93 Hz, H$_4$aminopyridinium), 5.27 (s, 2H, aminopyridinium CH$_2$), 4.66 (s, 2H, aminopyridine CH$_2$) ppm. For 2-D COSY, see Section A.1. ESI-MS: m/z 293.07 [M – NO$_3$]$^+$, 198.27 [M – NO$_3$ – apym]$^+$. Anal. calcd for C$_{16}$H$_{17}$O$_3$N$_7$·1.8H$_2$O: C, 49.56; H, 5.35; N, 25.28. Found: C, 49.42; H, 5.25; N, 25.49.

1,1′-Bis[4-(4,4′-bipyridinium)butyl]-4,4′-bipyridinium nitrate (102)

The title compound was prepared from 1,1′-bis[4-(4,4′-bipyridinium)butyl]-4,4′-bipyridinium bromide (100) (72%, colourless crystals).

$^1$H NMR (D$_2$O) $\delta$ 9.15 (d, 4H, $^3$J$_{H,H}$ = 6.7 Hz, H2′′), 9.01 (d, 4H, $^3$J$_{H,H}$ = 6.8 Hz, H2′′′), 8.81 (m, 4H, H2′), 8.58 (d, 4H, $^3$J$_{H,H}$ = 6.7 Hz, H3′′′), 8.44 (d, 4H, $^3$J$_{H,H}$ = 6.7 Hz, H3′′′), 7.94 (d, 4H, $^3$J$_{H,H}$ = 5.6 Hz, H3′), 4.85 (m, 4H, H1), 4.83 (obscured by HOD peak, 4H, H4), 2.26 (m, 8H, H2,3) ppm. $^{13}$C($^1$H) NMR (D$_2$O) $\delta$ 154.50, 150.64 (2 resonances), 145.93, 145.21, 143.07, 127.53, 126.62, 123.02, 61.54, 60.94, 27.75, 27.69 ppm. ESI-MS: m/z 766.13 [M – NO$_3$]$^+$, 352.07 [M – 2NO$_3$]$^{2+}$, 214.00 [M – 3NO$_3$]$^{3+}$. Anal. calcd for C$_{38}$H$_{40}$O$_{12}$N$_{10}$·6H$_2$O: C, 48.72; H, 5.59; N, 14.95. Found: C, 48.49; H, 5.23; N, 15.18.
**1,1'-Bis[6-(4,4'-bipyridinium)hexyl]-4,4'-bipyridinium nitrate (104)**

The title compound was prepared from 1,1'-bis[6-(4,4'-bipyridinium)hexyl]-4,4'-bipyridinium bromide (101) (70%, white powder).

$^1$H NMR (D$_2$O) \( \delta \) 9.13 (d, 4H, \( J_{H,H} = 6.4 \) Hz, H2"'), 8.98 (d, 4H, \( J_{H,H} = 6.5 \) Hz, H2"”), 8.81 (m, 4H, H2'), 8.56 (d, 4H, \( J_{H,H} = 6.1 \) Hz, H3"”), 8.43 (d, 4H, \( J_{H,H} = 6.3 \) Hz, H3"”), 7.95 (d, 4H, \( J_{H,H} = 4.8 \) Hz, H3"'), 4.76 – 4.68 (obscured by HOD peak, 8H, H1,6), 2.13 (m, 8H, H2,5), 1.51 (m, 8H, H3,4) ppm. $^{13}$C{\(^1\)H} NMR (D$_2$O) \( \delta \) 154.11, 150.71, 150.44, 145.83, 145.14, 143.17, 127.33, 126.45, 123.01, 62.41, 61.85, 30.79, 30.70, 25.27 ppm. For 2-D COSY, see Section A.1. ESI-MS: \( m/z \) 1707.87 [2M – NO$_3$]$^+$, 1412.67 [5M – 3NO$_3$]$^{3+}$, 1265.00 [3M – 2NO$_3$]$^{3+}$, 1117.67 [4M – 3NO$_3$]$^{3+}$, 822.33 [M – NO$_3$]$^+$, 380.13 [M – 2NO$_3$]$^{2+}$. Anal. calcd for C$_{42}$H$_{48}$N$_{10}$O$_{12}$·9H$_2$O: C, 48.18; H, 6.35; N, 13.38. Found: C, 47.97; H, 5.88; N, 13.27.

6.7 *Synthesis of PF$_6$\textsuperscript{-} salts*

Typically, a halide salt (0.5 mmol) was dissolved in the minimum amount of H$_2$O and was treated with KPF$_6$ (saturated aqueous solution, 50 mL). The mixture was allowed to stand overnight and the precipitate which formed was isolated by filtration, washed with H$_2$O (2 mL) and dried *in vacuo* to give the PF$_6$\textsuperscript{-} salt as a white/off-white powder.

**1,4-Bis(4,4'-bipyridinium)butane hexafluorophosphate (23)**

The title compound was prepared from 1,4-bis(4,4'-bipyridinium)butane bromide (21) (51%, white powder).

$^1$H NMR (CD$_3$CN) \( \delta \) 8.86 (d, 4H, \( J_{H,H} = 6.0 \) Hz, H2), 8.74 (d, 4H, \( J_{H,H} = 7.2 \) Hz, H2'), 8.33 (d, 4H, \( J_{H,H} = 6.0 \) Hz, H3), 7.79 (d, 4H, \( J_{H,H} = 7.2 \) Hz, H3"'), 4.59 (m, 4H, H$_{\text{butylene}}$), 2.07 (m, 4H, H$_{\text{butylene}}$) ppm. $^{13}$C{\(^1\)H} NMR (CD$_3$CN) \( \delta \) 154.73, 151.57, 145.65, 142.14, 126.75, 122.65, 61.09, 27.85 ppm. ESI-MS: \( m/z \) 513.0 [M – PF$_6$]$^+$, 184.2 [M – 2PF$_6$]$^{2+}$. Anal. calcd for C$_{24}$H$_{24}$N$_4$P$_2$F$_{12}$: C, 43.78; H, 3.67; N, 8.51. Found: C, 43.79; H, 3.87; N, 8.44.
1,4-Bis(pyzarinium)butane hexafluorophosphate (35)

The title compound was prepared from 1,4-bis(pyzarinium)butane bromide (34)\(^{11}\) (48%, white powder).

\(^1\)H NMR (CD\(_3\)CN) \(\delta\) 9.42 (m, 4H, H\(_3\)), 8.68 (d, 4H, \(^3\)J\(_{H,H}\) = 4.2 Hz, H\(_2\)), 4.60 (m, 4H, H\(_{\text{butylene}}\)), 2.06 (m, 4H, H\(_{\text{butylene}}\)) ppm. \(^{13}\)C\(^{\{\text{1}\}H}\) NMR (CD\(_3\)CN) \(\delta\) 152.41, 137.61 (t, \(^1\)J\(_{C,N}\) = 8.8 Hz), 62.46, 27.69 ppm. ESI-MS: \(m/z\) 867.00 [2M – PF\(_6\)]\(^+\), 361.00 [M – PF\(_6\)]\(^+\), 108.2 [M – 2PF\(_6\)]\(^2+\). Anal. calcd for C\(_{12}\)H\(_{16}\)N\(_4\)P\(_2\)F\(_{12}\)·0.5H\(_2\)O: C, 27.97; H, 3.33; N, 10.87. Found: C, 27.85; H, 3.28; N, 10.59.

1,6-Bis(pyzarinium)hexane hexafluorophosphate (41)

The title compound was prepared from 1,6-bis(pyzarinium)hexane bromide (39)\(^{11}\) (45%, white powder).

\(^1\)H NMR (CD\(_3\)CN) \(\delta\) 9.40 (m, 4H, H\(_3\)), 8.68 (d, 4H, \(^3\)J\(_{H,H}\) = 4.2 Hz, H\(_2\)), 4.54 (t, 4H, \(^3\)J\(_{H,H}\) = 7.8 Hz, H\(_{\text{hexylene}}\)), 2.10 (obscured by HOD peak, m, 4H, H\(_{\text{hexylene}}\)), 1.36 (m, 4H, H\(_{\text{hexylene}}\)) ppm. \(^{13}\)C\(^{\{\text{1}\}H}\) NMR (CD\(_3\)CN) \(\delta\) 151.09, 136.35 (t, \(^1\)J\(_{C,N}\) = 8.6 Hz), 62.20, 29.85, 24.49 ppm. ESI-MS: \(m/z\) 922.93 [2M – PF\(_6\)]\(^+\), 389.07 [M – PF\(_6\)]\(^+\), 122.13 [M – 2PF\(_6\)]\(^2+\). Anal. calcd for C\(_{14}\)H\(_{20}\)F\(_{12}\)N\(_4\)P\(_2\): C, 31.47; H, 3.77; N, 10.49. Found: C, 31.42; H, 3.64; N, 10.37.

1,5-Bis(3-aminopyrazinium)pentane hexafluorophosphate (47)

The title compound was prepared from 1,5-bis(3-aminopyrazinium)pentane bromide (45) (69%, off-white crystals).

\(^1\)H NMR (CD\(_3\)CN) \(\delta\) 8.52 (m, 2H, H\(_5\)), 7.74 (s, 2H, H\(_2\)), 7.70 (m, 2H, H\(_6\)), 6.34 (br, s, 4H, NH\(_2\)), 4.27 (t, 4H, \(^3\)J\(_{H,H}\) = 7.38 Hz, H\(_{\text{pentylene}}\)), 1.90 (m, 4H, H\(_{\text{pentylene}}\)), 1.33 (q, 2H, \(^3\)J\(_{H,H}\) = 8.09 Hz, H\(_{\text{pentylene}}\)) ppm. \(^{13}\)C\(^{\{\text{1}\}H}\) NMR (CD\(_3\)CN) \(\delta\) 159.95, 150.62, 124.59, 118.38, 62.71, 30.43, 23.00 ppm. ESI-MS: \(m/z\) 405.13 [M – PF\(_6\)]\(^+\), 259.20 [M – 2PF\(_6\) – H\(^+\)]\(^+\), 130.20 [M – 2PF\(_6\)]\(^+\). Anal. calcd for C\(_{13}\)H\(_{20}\)P\(_2\)F\(_{12}\)N\(_4\): C, 28.38; H, 3.66; N, 15.27. Found: C, 28.47; H, 3.59; N, 15.09.
1,4-Bis(1-methyl-3-aminopyrazinium)benzene hexafluorophosphate (52)

The title compound was prepared from 1,4-bis(1-methyl-3-aminopyrazinium)benzene bromide (51) (65%, off-white powder).

$^1$H NMR (CD$_3$CN) $\delta$ 8.76 (m, 2H, H5), 7.86 (m, 2H, H6), 7.77 (m, 2H, H2), 7.55 (s, 4H, HxylyleneCH), 6.46 (br, s, 4H, NH$_2$), 5.55 (s, 4H, CH$_2$) ppm. $^{13}$C{$^1$H} NMR (CD$_3$CN) $\delta$ 159.84, 150.77, 134.44, 131.40, 124.64, 124.15, 64.93 ppm. ESI-MS: $m/z$ 438.93 [M – PF$_6$]$^+$, 292.87 [M – 2PF$_6$ – H$^+$]$^+$, 146.93 [M – 2PF$_6$]$^{2+}$. Anal. calcd for C$_{16}$H$_{18}$N$_6$F$_{12}$P$_2$: C, 32.89; H, 3.11; N, 14.34. Found: C, 32.95; H, 3.28; N, 14.19.

1,3,5-Tris(3-aminopyraziniummethyl)-2,4,6-trimethylbenzene hexafluorophosphate (55)

The title compound was prepared from 1,3,5-tris(4-aminopyraziniummethyl)-2,4,6-trimethylbenzene bromide (53) (75%, white powder).

$^1$H NMR (CD$_3$CN) $\delta$ 8.58 (m, 3H, H5) 7.67 (m, 3H, H6), 7.54 (s, 3H, H2), 6.35 (br, s, 6H, NH$_2$), 5.75 (s, 6H, CH$_2$), 2.25 (s, 9H, CH$_3$) ppm. $^{13}$C{$^1$H} NMR (CD$_3$CN) $\delta$ 160.20, 150.86, 128.24, 123.58, 123.44, 60.16, 17.52 ppm. ESI-MS: $m/z$ 733.60 [M – PF$_6$]$^+$, 587.47 [M – 2PF$_6$ – H$^+$]$^+$, 492.40 [M – 2PF$_6$ – apyz – H$^+$]$^+$, 441.47 [M – 3PF$_6$ – 2H$^+$]$^+$, 346.60 [M – 3PF$_6$ – 2H$^+$ – apyz]$^+$, 294.00 [M – 2PF$_6$]$^{2+}$, 173.47 [M – 3PF$_6$ – H$^+$ – apyz]$^{2+}$, 147.60 [M – 3PF$_6$]$^{3+}$. Anal. calcd for C$_{24}$H$_{30}$F$_{18}$P$_3$N$_9$: C, 32.78; H, 3.44; N, 14.33. Found: C, 32.85; H, 3.28; N, 14.12.

1-Benzyl-2-aminopyrazinium hexafluorophosphate (61)

The title compound was prepared from 1-benzyl-2-aminopyrazinium bromide (59) (38%, colourless crystals). One crystal was selected for X-ray analysis crystallographic analysis, details of which are given in the Appendices (Section A.3).

$^1$H NMR (CD$_3$CN) $\delta$ 8.77 (dd, 1H, $^3$J$_{H,H} = 4.3$ Hz, $^4$J$_{H,H} = 1.8$ Hz, H4), 8.05 (dd, 1H, $^3$J$_{H,H} = 6.6$ Hz, $^4$J$_{H,H} = 1.8$ Hz, H6), 7.43 (m, 3H, Bn), 7.27 (m, 2H, Bn), 7.19 (br, s, 2H, NH$_2$), 7.03 (dd, 1H, $^3$J$_{H,H} = 6.6$ Hz, $^3$J$_{H,H} = 4.3$ Hz, H5) ppm.
$^{13}$C\{\textit{H}\} NMR (CD$_3$CN) $\delta$ 167.57, 156.88, 149.95, 131.14, 130.52, 130.43, 129.38, 113.03, 58.05 ppm. ESI-MS: $m/z$ 516.73 [2M – PF$_6$]$^+$, 185.80 [M – 2PF$_6$]$^{2+}$. Anal. calcd for C$_{11}$H$_{12}$N$_3$PF$_6$: C, 39.89; H, 3.65; N, 12.69. Found: C, 40.10; H, 3.74; N, 12.90.

**1,4-Bis(2-aminopyrimidinium)butane hexafluorophosphate (65)**

The title compound was prepared from 1,4-bis(2-aminopyrimidinium)butane bromide (63) (64%, white powder).

$^1$H NMR (400 MHz, CD$_3$CN) $\delta$ 8.67 (dd, 2H, $^3$J$_{H,H}$ = 4.4 Hz, $^4$J$_{H,H}$ = 2.0 Hz, H4), 8.01 (dd, 2H, $^3$J$_{H,H}$ = 6.8 Hz, $^4$J$_{H,H}$ = 2.0 Hz, H6), 7.17 (br, s, 4H, NH$_2$), 6.97 (dd, 2H, $^3$J$_{H,H}$ = 6.8 Hz, $^4$J$_{H,H}$ = 4.4 Hz, H5), 3.95 (m, 4H, H$_{\text{butylene}}$α), 1.76 (m, 4H, H$_{\text{butylene}}$β) ppm.

$^{13}$C\{\textit{H}\} NMR (100 MHz, CD$_3$CN) $\delta$ 167.6, 156.7, 150.6, 113.0, 54.9, 24.1 ppm. ESI-MS: $m/z$ 235.00 [M – 2PF$_6$ – H]$^+$, 123.07 [M – 2PF$_6$]$^{2+}$. Anal. calcd for C$_{12}$H$_{18}$N$_6$P$_2$F$_{12}$: C, 26.88; H, 3.38; N, 15.67. Found: C, 26.91; H, 3.40; N, 15.62.

**1,5-Bis(2-aminopyrimidinium)pentane hexafluorophosphate (69)**

The title compound was prepared from 1,5-bis(2-aminopyrimidinium)pentane bromide (67) (71%, colourless crystals).

$^1$H NMR (300 MHz, CD$_3$CN) $\delta$ 8.75 (dd, 2H, $^3$J$_{H,H}$ = 4.36 Hz, $^4$J$_{H,H}$ = 2.03 Hz, H4), 8.12 (dd, 2H, $^3$J$_{H,H}$ = 6.59 Hz, $^4$J$_{H,H}$ = 1.83 Hz, H6), 7.21 (br, s, 4H, NH$_2$), 7.05 (dd, 2H, $^3$J$_{H,H}$ = 6.56 Hz, $^4$J$_{H,H}$ = 4.45 Hz, H4), 3.98 (t, 4H, $^3$J$_{H,H}$ = 7.45 Hz, H$_{\text{pentylene}}$α), 1.81 (m, 4H, H$_{\text{pentylene}}$β), 1.41 (m, 2H, H$_{\text{pentylene}}$γ) ppm. $^{13}$C\{\textit{H}\} NMR (100 MHz, CD$_3$CN) $\delta$ 167.6, 156.7, 150.6, 113.0, 54.9, 24.1 ppm. ESI-MS: $m/z$ 235.00 [M – 2PF$_6$ – H]$^+$, 123.07 [M – 2PF$_6$]$^{2+}$. Anal. calcd for C$_{13}$H$_{20}$N$_6$P$_2$F$_{12}$: C, 26.88; H, 3.38; N, 15.67. Found: C, 26.91; H, 3.40; N, 15.62.

**1,6-Bis(3-aminopyrazinium)hexane hexafluorophosphate (71)**

The title compound was prepared from 1,6-bis(3-aminopyrazinium)hexane bromide (69) (43%, off-white fibres).

$^1$H NMR (CD$_3$CN) $\delta$ 8.57 (m, 2H, H5), 7.79 (m, 2H, H6), 7.73 (m, 2H, H2), 6.43 (br, s, 4H, NH$_2$), 4.29 (t, 4H, $^3$J$_{H,H}$ = 7.5 Hz, H$_{\text{pentylene}}$), 2.11 (overlapping HOD peak, m, 4H, H$_{\text{pentylene}}$β), 1.41 (m, 2H, H$_{\text{pentylene}}$γ) ppm. $^{13}$C\{\textit{H}\} NMR (CD$_3$CN) $\delta$ 167.33, 156.62, 150.46, 112.88, 55.26, 26.77, 23.09 ppm. ESI-MS: $m/z$ 259.13 [M – 2PF$_6$ – H]$^+$, 130.20 [M – 2PF$_6$]$^{2+}$. Anal. calcd for C$_{13}$H$_{20}$N$_6$P$_2$F$_{12}$: C, 28.42; H, 3.86; N, 15.32. Found: C, 28.42; H, 3.86; N, 15.32.
1.36 (m, 4H, H$_{hexylene}$) ppm. $^{13}$C\textsuperscript{1}H NMR (CD$_3$CN) $\delta$ 159.87, 150.52, 125.52, 124.53, 62.90, 30.80, 25.76 ppm. ESI-MS: $m/z$ 137.2 [M – 2PF$_6$]$^{2+}$. Anal. calcd for C$_{14}$H$_{22}$F$_{12}$N$_6$P$_2$·0.33H$_2$O: C, 29.49; H, 4.01; N, 14.74. Found: C, 29.99; H, 4.35; N, 14.79.

1,8-Bis(2-aminopyrimidinium)octane hexafluorophosphate (74)

The title compound was prepared from 1,8-bis(2-aminopyrimidinium)octane bromide (70) (79%, white powder).

$^1$H NMR (CD$_3$CN) $\delta$ 8.77 (dd, 2H, $^3$J$_{H,H}$ = 4.38 Hz, $^4$J$_{H,H}$ = 2.06 Hz, H$_4$), 8.16 (dd, 2H, $^3$J$_{H,H}$ = 6.60 Hz, $^4$J$_{H,H}$ = 2.06 Hz, H$_6$), 7.24 (br, s, 4H, NH$_2$), 7.07 (dd, 2H, $^3$J$_{H,H}$ = 6.60 Hz, $^4$J$_{H,H}$ = 4.38 Hz, H$_4$), 4.01 (t, 4H, $^3$J$_{H,H}$ = 7.60 Hz, H$_{octylene}$), 1.80 (m, 4H, H$_{octylene}$), 1.37 (m, 8H, H$_{octylene}$) ppm. $^{13}$C\textsuperscript{1}H NMR (CD$_3$CN) $\delta$ 167.17, 156.58, 150.41, 112.83, 55.63, 29.44, 27.37, 26.47 ppm. ESI-MS: $m/z$ 446.87 [M – PF$_6$]$,^{2+}$, 301.07 [M – 2PF$_6$ – H$^+$]$^+$, 151.93 [M – 2PF$_6$]$^{2+}$. Anal. calcd for C$_{16}$H$_{26}$F$_{12}$N$_6$P$_2$: C, 32.44; H, 4.42; N, 14.19. Found: C, 32.44; H, 4.30; N, 13.94.

1,2-Bis(1-methyl-2-aminopyrimidinium)benzene hexafluorophosphate (77)

The title compound was prepared from 1,2-bis(1-methyl-2-aminopyrimidinium)benzene bromide (75) (70%, white powder).

$^1$H NMR (400 MHz, CD$_3$CN) $\delta$ 8.79 (dd, 2H, $^3$J$_{H,H}$ = 4.4 Hz, $^4$J$_{H,H}$ = 2.0 Hz, H$_4$), 7.81 (dd, 2H, $^3$J$_{H,H}$ = 6.4 Hz, $^4$J$_{H,H}$ = 2.0 Hz, H$_6$), 7.47 (m, 2H, H$_{3xylyl}$), 7.23 (br, s, 4H, NH$_2$), 7.09 (m, 2H, H$_{4xylyl}$), 7.03 (dd, 2H, $^3$J$_{H,H}$ = 6.4 Hz, $^4$J$_{H,H}$ = 4.4 Hz, H$_5$), 5.08 (s, 4H, CH$_2$) ppm. $^{13}$C\textsuperscript{1}H NMR (100 MHz, CD$_3$CN) $\delta$ 168.1, 157.2, 149.5, 131.8, 130.2, 129.9, 113.5, 55.1 ppm. ESI-MS: $m/z$ 293.0 [M – 2PF$_6$ – H$^+$]$^+$, 198.2 [M – 2PF$_6$ – apym – H$^+$]$^+$, 123.1 [M – 2PF$_6$]$^{2+}$. Anal. calcd for C$_{18}$H$_{18}$N$_6$P$_2$: C, 38.89; H, 3.11; N, 14.38. Found: C, 38.05; H, 3.33; N, 14.54.
1,3-Bis(1-methyl-2-aminopyrimidinium)benzene hexafluorophosphate (80)

The title compound was prepared from 1,3-bis(1-methyl-2-aminopyrimidinium)benzene bromide (78) (51%, colourless crystals).

\[
\begin{align*}
\text{NH}_2 & \quad 2\text{PF}_6^- \\
\text{N} & \quad \text{N} & \quad \text{N} & \quad \text{N}
\end{align*}
\]

\[1^H \text{ NMR (CD}_3\text{CN) } \delta 8.85 \text{ (dd, 2H, } ^3J_{\text{HH}} = 4.33 \text{ Hz, } ^4J_{\text{HH}} = 1.95 \text{ Hz, H4)}, 8.10 \text{ (dd, 2H, } ^3J_{\text{HH}} = 6.66 \text{ Hz, } ^4J_{\text{HH}} = 1.95 \text{ Hz, H6)}, 7.58 \text{ (t, 1H, } ^3J_{\text{HH}} = 7.65 \text{ Hz, H5}_{\text{xylylene}}), 7.41 \text{ (d, 4H, } ^3J_{\text{HH}} = 7.65 \text{ Hz, H4}_{\text{xylylene}}), 7.22 \text{ (br, s, 4H, NH}_2), 7.14 \text{ (s, 1H, H2}_{\text{xylylene}}), 7.12 \text{ (dd, 2H, } ^3J_{\text{HH}} = 6.66 \text{ Hz, } ^3J_{\text{HH}} = 4.33 \text{ Hz, H5)}, 5.25 \text{ (s, 4H, CH}_2\text{)} \text{ ppm. } ^{13}\text{C}[^{1}H] \text{ NMR (CD}_3\text{CN) } \delta 167.88, 156.97, 150.23, 132.75, 131.57, 130.5, 129.00, 113.20, 57.36 \text{ ppm. ESI-MS: } m/z 439.00 \text{ [M – PF}_6^-], 293.20 \text{ [M – 2PF}_6^- – H}^+\text{, 198.27 [M – apym – 2PF}_6^- – H}^+\text{, 147.12 [M – 2PF}_6^-]^{2+}. \text{ Anal. calcd for C}_{16}\text{H}_{18}\text{P}_{2}\text{F}_{12}\text{N}_{6}: C, 32.89; H, 3.11; N, 14.38. Found: C, 32.99; H, 2.89; N, 14.28.}

1,4-Bis(1-methyl-2-aminopyrimidinium)benzene hexafluorophosphate (82)

The title compound was prepared from 1,4-bis(1-methyl-2-aminopyrimidinium)benzene bromide (81) (62%, white powder).

\[
\begin{align*}
\text{NH}_2 & \quad 2\text{PF}_6^- \\
\text{N} & \quad \text{N} & \quad \text{N} & \quad \text{N}
\end{align*}
\]

\[1^H \text{ NMR (CD}_3\text{CN) } \delta 8.80 \text{ (dd, 2H, } ^3J_{\text{HH}} = 4.2 \text{ Hz, } ^4J_{\text{HH}} = 1.8 \text{ Hz, H4}), 8.08 \text{ (dd, 2H, } ^3J_{\text{HH}} = 6.6 \text{ Hz, } ^4J_{\text{HH}} = 1.8 \text{ Hz, H6)}, 7.33 \text{ (s, 4H, H}_{\text{xylylene}}), 7.22 \text{ (br, s, 4H, NH}_2), 7.07 \text{ (dd, 2H, } ^3J_{\text{HH}} = 6.6 \text{ Hz, } ^3J_{\text{HH}} = 4.2 \text{ Hz, H5}, 5.23 \text{ (s, 4H, CH}_2\text{)} \text{ ppm. } ^{13}\text{C}[^{1}H] \text{ NMR (CD}_3\text{CN) } \delta 167.81, 156.91, 150.08, 132.71, 130.17, 113.10, 57.51 \text{ ppm. ESI-MS: } m/z 438.60 \text{ [M – PF}_6^-], 292.73 \text{ [M – 2PF}_6^- – H}^+\text{, 197.93 [M – apym – 2PF}_6^- – H}^+\text{, 146.87 [M – 2PF}_6^-]^{2+}. \text{ Anal. calcd for C}_{16}\text{H}_{18}\text{P}_{2}\text{F}_{6}\text{N}_{6}: C, 42.11; H, 4.20; N, 18.42. Found: C, 42.04; H, 4.40; N, 18.32 (this suggests loss of HPF}_6).
1,3,5-Tris[1-(2-aminopyrimidiniummethyl)]-2,4,6-trimethylbenzene hexafluorophosphate (87)

The title compound was prepared from 1,3,5-tris[1-(2-aminopyrimidiniummethyl)]-2,4,6-trimethylbenzene bromide (85) (48%, white powder).

$^1$H NMR (CD$_3$CN) $\delta$ 8.80 (m, 3H, H4), 7.66 (m, 3H, H6), 7.51 (br, s, 6H, NH$_2$), 7.02 (m, 3H, H5), 5.12 (s, 6H, CH$_2$), 2.17 (s, 9H, CH$_3$) ppm. $^{13}$C{$_1$H} NMR (CD$_3$CN) $\delta$ 167.02, 156.40, 146.26, 145.85, 127.85, 113.28, 52.56, 17.30 ppm. ESI-MS: $m/z$ 734.07 [M – PF$_6$]+, 587.93 [M – 2PF$_6$ – H$^+$]+, 442.13 [M – 3PF$_6$ – 2H$^+$]+, 347.00 [M – 3PF$_6$ – 2H$^+$ – apym]$^+$, 294.47 [M – 2PF$_6$]$^+$, 221.47 [M – 3PF$_6$ – H$^+$]$^+$, 174.07 [M – 3PF$_6$ – H$^+$ – apym]$^2$. Anal. calcd for C$_{24}$H$_{30}$F$_{18}$P$_3$N$_9$·1.80H$_2$O: C, 31.61; H, 3.71; N, 13.82. Found: C, 31.42; H, 3.43; N, 13.57.

1,1′-Bis[4-(4,4′-bipyridinium)butyl]-4,4′-bipyridinium hexafluorophosphate (103)

The title compound was prepared from 1,1′-bis[4-(4,4′-bipyridinium)butyl]-4,4′-bipyridinium bromide (100) (89%, brown powder).

$^1$H NMR (CD$_3$CN) $\delta$ 8.93 (d, 4H, $^3$J$_{HH} = 5.9$ Hz, H2′), 8.82 (d, 4H, $^3$J$_{HH} = 5.9$ Hz, H2′), 8.79 (d, 4H, $^3$J$_{HH} = 6.8$ Hz, H2″), 8.41 (d, 4H, $^3$J$_{HH} = 6.0$ Hz, H3′), 8.33 (d, 4H, $^3$J$_{HH} = 6.7$ Hz, H3″), 7.81 (dd, 4H, $^3$J$_{HH} = 4.6$ Hz, $^5$J$_{HH} = 1.4$ Hz, H3′), 4.68 (m, 4H, H1), 4.62 (m, 4H, H4), 2.11 (m, 8H, H2,3) ppm. $^{13}$C{$_1$H} NMR (CD$_3$CN) $\delta$ 154.95, 151.76, 150.88, 146.42, 145.74, 142.16, 128.02, 126.88, 122.69, 61.80, 61.18, 27.99, 27.93 ppm. For 2-D COSY, see Section A.1. ESI-MS: $m/z$ 1015.13 [M – PF$_6$]$^+$, 435.07 [M – 2PF$_6$]$^2$+, 241.73 [M – 3PF$_6$]$^3$. Anal. calcd for C$_{38}$H$_{40}$P$_4$F$_{24}$N$_6$·3H$_2$O: C, 37.57; H, 3.82; N, 6.92. Found: C, 37.38; H, 3.59; N, 6.70.
1,1’-Bis[6-(4,4’-bipyridinium)hexyl]-4,4’-bipyridinium hexafluorophosphate (105)

The title compound was prepared from 1,1’-bis[6-(4,4’-bipyridinium)hexyl]-4,4’-bipyridinium bromide (101) (45%, off-white powder).

\[ \text{H}^1 \text{NMR (acetone-}d_6 \text{)} \delta 9.35 (d, 4H, J_{HH} = 6.6 \text{ Hz, } H2''), 9.20 (d, 4H, J_{HH} = 6.5 \text{ Hz, } H2'), 9.03 (m, 4H, H2''''), 8.75 (d, 4H, J_{HH} = 6.3 \text{ Hz, } H3'''), 8.62 (d, 4H, J_{HH} = 6.4 \text{ Hz, } H3''''), 8.12 (m, 4H, H3'), 4.94 (t, 4H, J_{HH} = 7.9 \text{ Hz, } H1), 4.86 (t, 4H, J_{HH} = 7.5 \text{ Hz, } H6), 2.23 - 2.21 (m, 8H, H2,5), 1.60 (m, 8H, H3,4) \text{ ppm.} \]

\[ \text{13C}\{\text{1H}}\text{NMR (CD}_3\text{CN)} \delta 155.03, 151.95, 150.91, 146.47, 145.80, 142.28, 128.12, 126.97, 122.78, 62.80, 62.19, 31.44, 31.36, 25.79 (2 resonances) \text{ ppm. ESI-MS: } m/z 1071.33 [M – PF}_6\text{]+, 463.40 [M – 2PF}_6\text{]^{2+}.} \]

(±)-2,6-Bis(4,4’-bipyridinium)-9-thiabicyclo[3.3.1]nonane perchlorate (33)

A solution of (±)-2,6-bis(4,4’-bipyridinium)-9-thiabicyclo[3.3.1]nonane chloride (30, 0.50 g, 0.96 mmol) in warm H\textsubscript{2}O (5 mL) was treated with LiClO\textsubscript{4} (saturated aqueous solution, 5 mL) and the mixture allowed to stand overnight, over which time a precipitate formed. This was isolated by filtration, washed with H\textsubscript{2}O (2 mL) and dried \textit{in vacuo} to give the product as a white powder (0.31 g, 50%).

\[ \text{H}^1 \text{NMR (D}_2\text{O)} \delta 9.18 (d, 4H, J_{HH} = 10.34 \text{ Hz, } H2), 8.78 (d, 4H, J_{HH} = 6.63 \text{ Hz, } H2'), 8.51 (d, 4H, J_{HH} = 10.34 \text{ Hz, } H3), 7.94 (d, 4H, J_{HH} = 6.63 \text{ Hz, } H3''), 5.83 (m, 2H, H_{aliphatic}), 3.45 (m, 2H, H_{aliphatic}), 3.21 (m, 2H, H_{aliphatic}), 2.52 (m, 6H, H_{aliphatic}) \text{ ppm.} \]

\[ \text{13C}\{\text{1H}}\text{NMR (DMSO-}d_6 \text{)} \delta 153.5, 151.24, 144.60, 141.05, 126.16, 122.40, 72.51, 35.70, 27.05, 23.63 \text{ ppm. ESI-MS: } m/z 550.87 [M – ClO}_4\text{]+, 225.80 [M – 2ClO}_4\text{]^{2+}. \text{ Anal. calcd for } C_{28}H_{28}Cl_2N_4O_8S·3\text{H}_2\text{O: C, 47.42; H, 4.94; N, 7.90. Found: C, 47.13; H, 4.20; N, 7.81.} \]
6.8 Dimroth rearrangements

1,2-Bis[(2-pyrimidylamino)methyl]benzene (91)

1,2-Bis(1-methyl-2-aminopyrimidinium)benzene bromide (75, 182 mg, 0.400 mmol) was suspended in NaOH (1 M aqueous solution, 10 mL) and the mixture stirred at 60°C for 2 h, before being allowed to cool. The precipitate was isolated by centrifugation, washed with H₂O (2 × 1 mL) and dried in vacuo to give the product as a yellow powder (109 mg, 93%).

¹H NMR (DMSO-d₆) δ 8.20 (d, 4H, ³J_H,H = 4.7 Hz, H₄), 7.28 (dd, 2H, ³J_H,H = 5.3 Hz, ⁴J_H,H = 3.5 Hz, H₃xylylene), 7.13 (dd, 2H, ³J_H,H = 5.5 Hz, ⁴J_H,H = 3.5 Hz, H₄xylylene), 6.41 (t, 2H, ³J_H,H = 4.3 Hz, H₅), 4.53 (s, 4H, CH₂) ppm. ¹³C{¹H} NMR (DMSO-d₆) δ 162.46, 157.88, 137.94, 127.43, 126.31, 109.09, 42.13 ppm. ESI-MS: m/z 804.07 [3M – apym + Na⁺], 781.87 [3M – apym + Na⁺]⁺, 607.13 [2M + Na⁺]⁺, 584.87 [2M + H⁺]⁺, 512.13 [2M – apym + H⁺]⁺, 490.20 [2M – apym + H⁺]⁺, 293.00 [M + H⁺]⁺.

1,4-Bis[(2-pyrimidylamino)methyl]benzene (92)

A method for the synthesis of this compound, from 1,4-bis(aminomethyl)benzene and 2-chloropyrimidine, has been reported previously. However, this compound was instead prepared using a procedure similar to that described for 91, starting from 1,4-bis(1-methyl-2-aminopyrimidinium)benzene bromide (81) and heating the mixture for 24 h (84%, yellow powder).

¹H NMR (DMSO-d₆) δ 8.24 (d, 4H, ³J_H,H = 4.7 Hz, H₄), 7.62 (br, s, 2H, NH exchanges with D₂O), 7.21 (s, 4H, Hₓylylene), 6.54 (t, 2H, ³J_H,H = 4.7 Hz, H₅), 4.44 (s, 4H, CH₂) ppm. ESI-MS: m/z 804.20 [3M – apym + Na⁺], 782.87 [3M – apym + Na⁺]⁺, 607.13 [2M + Na⁺]⁺, 584.67 [2M + H⁺]⁺, 490.27 [2M – apym + H⁺]⁺, 293.07 [M + H⁺]⁺, 198.20 [M – apym + H⁺]⁺.

1,8-Bis(2-pyrimidylamino)octane (93)

The title compound was prepared using a procedure similar to that for 91, instead starting from 1,8-bis(2-aminopyrimidinium)octane bromide (72) and heating the mixture for 24 h (64%, off-white powder).
$^1$H NMR (DMSO-<sub>d</sub>6) δ 8.22 (d, 4H, $^3$J<sub>H,H</sub> = 4.7 Hz, H4), 7.05 (t, 2H, $^3$J<sub>H,H</sub> = 5.3 Hz, NH), 6.50 (t, 2H, $^3$J<sub>H,H</sub> = 4.7 Hz, H5), 3.23 (m, 4H, H<sub>octyleneα</sub>), 1.49 (m, 4H, H<sub>octyleneβ</sub>), 1.27 (m, 8H, H<sub>octyleneγ,δ</sub>) ppm. $^{13}$C{$^1$H} NMR (DMSO-<sub>d</sub>6) δ 162.34, 157.85, 109.62, 40.57, 28.93, 28.85, 26.45 ppm. ESI-MS: $m/z$ 301.40 [M + H$^+$]+.

2,6-Bis[(2-pyrimidylamino)methyl]pyridine (94)

This compound was prepared using a procedure similar to that for 91, instead starting from 2,6-bis(1-methyl-2-aminopyrimidinium)pyridine chloride (83) and heating for 24 h (85%, off-white powder).

$^1$H NMR (DMSO-<sub>d</sub>6) δ 8.27 (d, 4H, $^3$J<sub>H,H</sub> = 4.7 Hz, H<sub>apym</sub>), 7.69 (t, 2H, $^3$J<sub>H,H</sub> = 6.0 Hz, NH), 7.63 (t, 1H, $^3$J<sub>H,H</sub> = 7.8 Hz, H<sub>py</sub>), 7.10 (d, 2H, $^3$J<sub>H,H</sub> = 7.7 Hz, H<sub>py</sub>), 6.59 (t, 2H, $^3$J<sub>H,H</sub> = 4.7 Hz, H<sub>apym</sub>), 4.56 (d, 4H, $^3$J<sub>H,H</sub> = 6.2 Hz, CH<sub>2</sub>) ppm. $^{13}$C{$^1$H} NMR (DMSO-<sub>d</sub>6) δ 162.23, 158.87, 158.01, 136.99, 118.33, 110.44, 45.98 ppm. ESI-MS: $m/z$ 609.00 [2M + Na$^+$]+, 294.27 [M + H$^+$]+.

1,3,5-Tris[(2-pyrimidylamino)methyl]-2,4,6-trimethylbenzene (95)

1,3,5-Tris(1-(2-aminopyridiniummethyl)benzene bromide (85, 73.5 mg, 0.100 mmol) was suspended in NaOH (1 M aqueous solution, 4 mL) and the mixture was stirred at 60°C for 24 h, before being allowed to cool. The precipitate was isolated by centrifugation, washed with H<sub>2</sub>O (2 × 0.5 mL) and dried in vacuo to give the product as a yellow powder (40 mg, 81%).

$^1$H NMR (DMF-<sub>d</sub>7) δ 8.35 (d, 6H, $^3$J<sub>H,H</sub> = 4.5 Hz, H4), 6.75 (br, s, 3H, NH), 6.63 (d, 3H, $^3$J<sub>H,H</sub> = 4.6 Hz, H5), 4.64 (s, 6H, CH<sub>2</sub>), 2.46 (s, 9H, CH<sub>3</sub>) ppm. ESI-MS: $m/z$ 1765.07 [4M + H$^+$]+, 1324.93 [3M + H$^+$]+, 905.33 [2M + Na$^+$]+, 883.07 [2M + H$^+$]+, 464.20 [M + Na$^+$]+, 442.07 [M + H$^+$]+, 347.13 [M – apym + H$^+$]+.
1,2,4,5-Tetrakis[(2-pyrimidylamino)methyl]benzene (96)

1,2,4,5-Tetrakis[1-(2-aminopyrimidiniummethyl)]benzene bromide (88) (24.9 mg, 30.0 µmol) was suspended in NaOH (1 M aqueous solution, 2 mL) and the mixture stirred at 60°C for 48 h, before being allowed to cool. The precipitate was isolated by centrifugation, washed with H₂O (2 × 0.5 mL) and dried in vacuo to give the product as a yellow powder (11.2 mg, 74%).

¹H NMR (DMSO-d₆) δ 8.21 (d, 8H, ³Jₕ,ₕ = 4.7 Hz, H₄), 7.66 (t, 4H, ³Jₕ,ₕ = 5.6 Hz, NH), 7.28 (s, 2H, CH), 4.47 (d, 8H, ³Jₕ,ₕ = 5.8 Hz, CH₂) ppm. Traces of DMF present. ESI-MS: m/z 507.20 [M + H⁺].

6.9 Synthesis of palladium(II) and platinum(II) complexes of N-heterocyclic ligands

[Pd₂(2,2'-bipy)₂{4,4'-bipy(CH₂)₄4,4'-bipy}₂]PF₆₈ (107)

A solution of 1,4-bis(4,4'-bipyridinium)butane nitrate (22, 9.85 mg, 20.0 µmol) in H₂O (1 mL) was treated with [Pd(2,2'-bipy)(NO₃)₂]¹⁵ (7.73 mg, 20.0 µmol) and the suspension stirred for 24 h at 80°C. The mixture was filtered through cellulose, and the yellow solution treated with KPF₆ (saturated aqueous solution, 20 mL). The precipitate was isolated by filtration, washed with cold H₂O (0.5 mL) and dried in vacuo to give the product as an off-white solid (17.7 mg, 73%).

¹H NMR (CD₃CN) δ 9.22 (d, 8H, ³Jₕ,ₕ = 6.6 Hz, H₂), 8.81 (d, 8H, ³Jₕ,ₕ = 6.7 Hz, H₂'), 8.43 (m, 8H, H₄bipy,H₆bipy), 8.29 (d, 8H, ³Jₕ,ₕ = 6.7 Hz, H₃), 8.29 (d, 8H, ³Jₕ,ₕ = 6.7 Hz, H₃'), 7.63 (dd, 4H, ³Jₕ,ₕ = 6.5 Hz, ⁴Jₕ,ₕ = 1.4 Hz, H₅bipy), 7.41 (d, 4H, ³Jₕ,ₕ = 5.4 Hz, H₃bipy), 4.67 (m, 8H, H₈butylene), 2.11 ppm. Anal. calcd for C₆₈H₆₄F₄₈N₁₂P₈Pd₂·3H₂O: C, 32.99; H, 2.85; N, 6.79. Found: C, 32.70; H, 2.81; N, 6.65.
The title compound was prepared analogously to 107, using [Pt(2,2'-bipy)(NO3)2]15 instead of [Pd(2,2'-bipy)(NO3)2] and heating for 48 h (white powder, 19.4 mg, 74%).

1H NMR (CD3CN) δ 9.18 (d, 8H, 3JH,H = 5.1 Hz, H2), 8.79 (d, 8H, 3JH,H = 6.9 Hz, H2'), 8.29 (d, 8H, 3JH,H = 6.3 Hz, H3'), 8.24 (d, 8H, 3JH,H = 6.6 Hz, H3), 8.29 (d, 8H, 3JH,H = 6.9 Hz, H3), 7.58 (m, 8H, Hbipy, H6bipy), 4.54 (t, 8H, 3JH,H = 7.2 Hz, Hhexylene), 1.97 (m, 8H, Hhexylene), 1.40 (m, 8H, Hhexylene) ppm.


The title compound was prepared analogously to 107, using 1,6-bis(4,4'-bipyridinium)hexane nitrate (25) instead of the butane derivative (white powder, 17.2 mg, 69%).

1H NMR (CD3CN) δ 9.17 (d, 8H, 3JH,H = 6.3 Hz, H2), 8.79 (d, 8H, 3JH,H = 6.6 Hz, H2'), 8.35 (m, 8H, H4bipy, H6bipy), 8.24 (d, 8H, 3JH,H = 6.6 Hz, H3'), 8.29 (d, 8H, 3JH,H = 6.3 Hz, H3), 7.58 (m, 4H, H5bipy), 7.39 (d, 4H, 3JH,H = 5.1 Hz, H3bipy), 4.54 (t, 8H, 3JH,H = 7.2 Hz, Hhexylene), 1.97 (m, 8H, Hhexylene), 1.40 (m, 8H, Hhexylene) ppm.

The title compound was prepared analogously to \textsuperscript{108}, using 1,6-bis(4,4′-bipyridinium)hexane nitrate (\textsuperscript{25}) instead of the butane derivative (white powder, 20.9 mg, 79%).

\textsuperscript{1}H NMR (CD\textsubscript{3}CN) \(\delta\) 9.17 (d, 8H, \(^3J_{HH} = 6.4\) Hz, H2), 8.78 (d, 8H, \(^3J_{HH} = 6.4\) Hz, H3), 8.06 (d, 8H, \(^3J_{HH} = 6.4\) Hz, H3′), 7.58 (m, 8H, H\textsubscript{4}bipy, H\textsubscript{6}bipy), 7.39 (d, 4H, \(^3J_{HH} = 5.4\) Hz, H3\textsubscript{bipy}), 4.54 (t, 8H, \(^3J_{HH} = 4.5\) Hz, H\textsubscript{hexylene}\(\alpha\)), 1.97 (m, 8H, H\textsubscript{hexylene}\(\beta\)), 1.40 (m, 8H, H\textsubscript{hexylene}\(\gamma\)) ppm.

ESI-FT-ICR-MS: \(m/z\) calcd for C\textsubscript{72}H\textsubscript{72}F\textsubscript{36}N\textsubscript{12}P\textsubscript{6}Pt\textsubscript{2}\textsuperscript{2+}, [M – 2PF\textsubscript{6} – ]\textsuperscript{2+}: 1182.156840. Found: 1182.153886, calcd for C\textsubscript{72}H\textsubscript{72}F\textsubscript{30}N\textsubscript{12}P\textsubscript{5}Pt\textsubscript{2}\textsuperscript{3+}, [M – 3PF\textsubscript{6} – ]\textsuperscript{3+}: 739.782983. Found: 739.781613. Anal. calcd for C\textsubscript{72}H\textsubscript{72}F\textsubscript{48}N\textsubscript{12}P\textsubscript{8}Pt\textsubscript{2}: C, 32.57; H, 2.73; N, 6.33. Found: C, 32.53; H, 2.99; N, 6.29.

1,2-Bis(4,4′-bipyridiniummethyl)benzene hexafluorophosphate\textsuperscript{16} (\textsuperscript{29}, 7.07 mg, 10.0 \(\mu\)mol) and [Pt(dppp)(OTf)\textsubscript{2}]\textsuperscript{17} (9.06 mg, 10.0 \(\mu\)mol) were dissolved in CH\textsubscript{3}CN (1 mL) and the solution was stirred for 24 h at 60°C. The solvent was allowed to evaporate slowly, and the residue dried \textit{in vacuo} to give the product as a white solid (16.41 mg, 99%).

\textsuperscript{1}H NMR (CD\textsubscript{3}CN) \(\delta\) 8.79 (m, 8H), 8.08 (m, 4H), 7.58 (m, 16H), 7.44 (m, 6H), 7.38 (m, 8H), 7.21 (m, 2H), 5.86 (m, 4H, NCH\textsubscript{2}), 5.66 (m, 4H, NCH\textsubscript{2}′), \textsuperscript{31}P\textsuperscript{1}H NMR (121 MHz, CD\textsubscript{3}CN) \(\delta\) –13.61 (s, PPt), –13.61 (d, \(^1J_{PP-t} = 3028\) Hz, PPt), –143.40 (septet, \(^1J_{P,F} = 707\) Hz, PF\textsubscript{6} – ) ppm. ESI-FT-ICR-
MS: \( m/z \) calcd for C\(_{111}\)H\(_{100}\)Pt\(_2\)F\(_{27}\)N\(_8\)O\(_3\)P\(_8\)S\(_3^+\), [M – 3OTf\(^{3+}\)]: 928.48171. Found: 928.48223. Anal. calcd for C\(_{114}\)H\(_{100}\)F\(_{36}\)N\(_8\)O\(_{12}\)Pt\(_2\)S\(_4^+\)5H\(_2\)O: C, 41.32; H, 3.35; N, 3.38. Found: C, 41.20; H, 3.33; N, 3.39.

\( \text{cis-[Pt}_2\text{Cl}_4\{4,4'-\text{bipy(o-xyylene)} 4,4'-\text{bipy}\}_2](\text{PF}_6)_4 \) (112)

A solution of 1,2-bis(4,4'-bipyridiniummethyl)benzene hexafluorophosphate\(^{16} \) (29, 7.07 mg, 10.0 \( \mu \)mol) in CH\(_3\)CN (1 mL) was treated with \( \text{cis-[Pt(PhCN)}_2\text{Cl}_2] \) (4.72 mg, 10.0 \( \mu \)mol) and the suspension was stirred for 24 h at 60°C. The solvent was allowed to evaporate slowly, and the solid was washed with Et\(_2\)O (0.5 mL) and dried \textit{in vacuo} to give the product as a yellow solid (9.94 mg, 97%).

ESI-FT-ICR-MS: \( m/z \) calcd for C\(_{56}\)H\(_{48}\)N\(_8\)PF\(_6\)Cl\(_4\)Pt\(_2^3^+\), [M – 3PF\(_6^3^+\)]: 503.05527. Found: 503.05560. Anal. calcd for C\(_{56}\)H\(_{48}\)N\(_8\)P\(_4\)F\(_{24}\)Cl\(_4\)Pt\(_2\)·PhCN: C, 35.80; H, 2.55; N, 5.96. Found: C, 35.99; H, 2.86; N, 5.99.

\( \text{cis-[Pd}_2\text{Cl}_4\{4,4'-\text{bipy(CH}_2)_24,4'-\text{bipy}\}_2](\text{PF}_6)_4 \) (113)

A solution of 1,3-bis(4,4'-bipyridinium)propane hexafluorophosphate (20, 6.44 mg, 10.0 \( \mu \)mol) in CH\(_3\)CN (1 mL) was treated with [Pd(PhCN)_2Cl_2] (3.83 mg, 10.0 \( \mu \)mol) and the suspension stirred for 24 h at 60°C. The solution was allowed to evaporate slowly, and the solid was washed with Et\(_2\)O (0.5 mL) and dried \textit{in vacuo} to give the product as a yellow solid (8.29 mg, 94%).

\(^1\)H NMR (CD\(_3\)CN) \( \delta \) 9.04 (d, 8H, \( ^3J_{H,H} = 5.9 \) Hz, H2), 8.82 (d, 8H, \( ^3J_{H,H} = 6.1 \) Hz, H2'), 8.34 (d, 8H, \( ^3J_{H,H} = 6.1 \) Hz, H3'), 7.86 (d, 8H, \( ^3J_{H,H} = 5.9 \) Hz, H3), 7.67 (m, 12H, H2,4benzonitrile), 7.50 (m, 8H, H3benzonitrile), 4.70 (m, 8H, H\(_{\text{propylene}}\)), 2.70 (m, 4H, H\(_{\text{propylene}}\)) ppm.

ESI-FT-ICR-MS: \( m/z \) 677.97932 [M – 2PF\(_6\)]\(^2^+\). Anal. calcd for
C_{48}H_{44}F_{24}N_{8}P_{4}Pd_2·PhCN·H_2O: C, 36.08; H, 2.91; N, 7.14. Found: C, 35.74; H, 3.02; N, 7.03.

$[\text{Pt}_4(2,2′\text{-bipy})_4\{\text{apyz(CH}_2)_6\text{apyz−2H}_2\}_2](\text{PF}_6)_8$ (119)

A solution of 1,6-bis(3-aminopyrazinium)hexane nitrate (49, 7.96 mg, 20.0 µmol) in H_2O (1 mL) was treated with $[\text{Pt}(2,2′\text{-bipy})(\text{NO}_3)_2]_2$ (19.01 mg, 40.0 µmol) and the suspension was stirred for 72 h at 80°C. The red solution was treated with KPF_6 (saturated aqueous solution, 15 mL) and allowed to stand for 30 min. The precipitate was isolated by filtration, washed with cold H_2O (1 mL) and dried in vacuo to give the product as an orange-red solid (19.63 mg, 61%).

$^1$H NMR (D_2O, of NO_3^− salt) $\delta$ 8.80–7.20 (m, 44H, H_{bipy,apyz}), 4.36 (m, 8H, H_{hexylene}), 1.92 (m, 8H, H_{hexylene}), 1.42 (m, 8H, H_{hexylene}) ppm. $^{195}$Pt NMR (D_2O, of NO_3^− salt) $\delta$ –2360.8 ppm. ESI-FT-ICR-MS: m/z calcd for C_{68}H_{72}F_{30}N_{20}P_{8}Pt_{4}^{3+}, [M – 3PF_6]^3+: 891.768257. Found: 891.768992. Anal. calcd for C_{68}H_{72}F_{30}N_{20}P_{8}Pt_{4}·6H_2O: C, 25.38; H, 2.63; N, 8.71. Found: C, 25.57; H, 2.66; N, 8.49. UV-vis (0.014 mM in H_2O): $\lambda$ = 305 nm, $\varepsilon$ = 6.82×10^4 M^{-1}cm^{-1}; $\lambda$ = 428 nm, $\varepsilon$ = 8.36×10^3 M^{-1}cm^{-1}.

$[\text{Pt}_2(2,2′\text{-bipy})_2(\text{Meapyz−H}_2)](\text{PF}_6)_4$ (122)

A solution of 1-methyl-3-aminopyrazinium nitrate (43, 6.88 mg, 40.0 µmol) in H_2O (1 mL) was treated with $[\text{Pt}(2,2′\text{-bipy})(\text{NO}_3)_2]_2$ (19.01 mg, 40.0 µmol) and the suspension was stirred for 72 h at 80°C. The red solution was treated with KPF_6 (saturated aqueous solution, 15 mL) and allowed to stand for 30 min. The precipitate was isolated by filtration, washed with cold H_2O (1 mL) and dried in vacuo to give the product as an orange-red solid (19.44 mg, 64%).

$^1$H NMR (D_2O, of NO_3^− salt) $\delta$ 8.70–7.30 (m, 22H, H_{bipy,apyz}), 4.20 (m, 6H, CH_3) ppm. $^{195}$Pt NMR (D_2O, of NO_3^− salt) $\delta$ –2358 ppm. Anal. calcd for C_{66}H_{72}F_{48}N_{20}P_{8}Pt_{4}·H_2O: C, 25.38; H, 2.63; N, 8.71. Found: C, 25.57; H, 2.66; N, 8.49. UV-vis (0.031 mM in H_2O): $\lambda$ = 305 nm, $\varepsilon$ = 6.82×10^4 M^{-1}cm^{-1}; $\lambda$ = 428 nm, $\varepsilon$ = 2.90×10^3 M^{-1}cm^{-1}. 

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[Pt$_4$(2,2'-bipy)$_4$(apym(CH$_2$)$_5$apym–2H)$_2$](PF$_6$)$_8$ (124)

A solution of 1,5-bis(3-aminopyrimidinium)pentane nitrate (67, 9.00 mg, 20.0 µmol) in H$_2$O (1 mL) was treated with [Pt(2,2'-bipy)(NO$_3$)$_2$] (19.01 mg, 40.0 µmol) and the suspension was stirred for 72 h at 80°C. The orange solution was treated with KPF$_6$ (saturated aqueous solution, 15 mL) and allowed to stand for 30 min. The precipitate was isolated by filtration, washed with cold H$_2$O (1 mL) and dried in vacuo to give the product as an orange solid (21.57 mg, 70%).

$^1$H NMR (D$_2$O, of NO$_3^-$ salt) δ 9.48 (d, 4H, $^3$J$_{HH}$ = 5.3 Hz, H$_6$apym), 8.74 (d, 4H, $^3$J$_{HH}$ = 6.1 Hz, H$_4$apym), 8.70 (d, 4H, $^3$J$_{HH}$ = 5.7 Hz, H$_{bipy}$), 8.4–8.15 (m, 12H, H$_{bipy}$), 8.13 (d, 4H, $^3$J$_{HH}$ = 8.1 Hz, H$_{bipy}$), 7.67 (t, 4H, $^3$J$_{HH}$ = 6.6 Hz, H$_{bipy}$), 7.28 (t, 4H, $^3$J$_{HH}$ = 6.6 Hz, H$_{bipy}$), 7.12 (t, 4H, $^3$J$_{HH}$ = 5.9 Hz, H$_5$apym), 4.75 (m, 4H, H$_{pentylene\alpha}$), 4.33 (m, 4H, H$_{pentylene\alpha'}$), ***2.0–1.2 (m, 12H, H$_{pentylene\beta}$, H$_{pentylene\gamma}$) ppm.

$^{195}$Pt NMR (D$_2$O, of NO$_3^-$ salt) δ –2253.7 ppm. ESI-FT-ICR-MS: m/z calcd for C$_{66}$H$_{68}$F$_{48}$N$_{20}$P$_2$Pt$_4^{2+}$, [M – 2PF$_6$]$^{2+}$: 1395.61755. Found: 1395.61458. calcd for C$_{33}$H$_{34}$F$_{18}$N$_{10}$P$_3$Pt$_2^{+}$, [½M – PF$_6$]$^{+}$: 1395.11721. Found: 1395.11783 (the intensities of both ions are comparable). Anal. calcd for C$_{66}$H$_{68}$F$_{48}$N$_{20}$P$_2$Pt$_4$6H$_2$O: C, 25.73; H, 2.22; N, 9.09. Found: C, 25.97; H, 2.36; N, 9.16.

UV-vis (0.120 mM in H$_2$O): $\lambda$ = 358 nm, $\varepsilon$ = 1.72×10$^4$ M$^{-1}$cm$^{-1}$; $\lambda$ = 433 nm, $\varepsilon$ = 4.91×10$^3$ M$^{-1}$cm$^{-1}$. For 2D COSY and UV-vis spectrum, see Sections A.1 and A.2.

6.10 Synthesis of palladium(II) and platinum(II) complexes of PEGda

[Pd(2,2'-bipy)(PEGda)](NO$_3$)$_2$ (125)

[Pd(2,2'-bipy)(NO$_3$)$_2$] (7.73 mg, 20.0 µmol) was added to PEGda (6.94 mg, 20.0 µmol) in H$_2$O (5 mL) and the suspension was stirred for 6 h at 60°C, over which time [Pd(2,2'-bipy)(NO$_3$)$_2$] had dissolved. The pale-yellow solution was then filtered through cellulose to afford a stock solution of the title complex (assumed to be quantitative).

***These protons become diastereotopic upon complexation due to the restricted rotation of the bound ligands.
$^1$H NMR (D$_2$O) $\delta$ 9.1-8.0 (m, 6H, H$_6$-bipy, H$_3$-bipy, H$_4$-bipy), 7.7 (m, 2H, H$_5$-bipy), 6.46 (br, s, 4H, NH$_2$), 4.00-3.40 (m, 76H, (CH$_2$OCH$_2$)$_{19}$), 3.25 (t, 4H, $^3$J$_{H,H}$ = 5.77 Hz, CH$_2$NH$_2$) ppm. ESI-FT-ICR-MS: $m/z$ calcd for C$_{50}$H$_{92}$N$_4$O$_{19}$Pd$^{2+}$, [M – 2NO$_3^-$]$^{2+}$: 579.27006. Found: 579.27153.

$[\text{Pt}(2,2'$-bipy)(PEGda)](\text{NO}_3)_2$ (126)

[Pt(2,2'-bipy)(NO$_3$)$_2$] (10.6 mg, 22.4 µmol) and PEGda (20.1 mg, 22.4 µmol) were dissolved in DMF (5 mL) and stirred for 48 h at 60°C after which the yellow solution was evaporated to dryness. The residue was dissolved in H$_2$O (1 mL) and the mixture filtered through cellulose to afford a stock solution of the title complex (assumed to be quantitative).

$^1$H NMR (DMF-d$_7$) $\delta$ 9.11 (d, 2H, $^3$J$_{H,H}$ = 5.63 Hz, H$_6$-bipy), 8.90 (d, 2H, $^3$J$_{H,H}$ = 7.55 Hz, H$_3$-bipy), 8.66 (m, 2H, H$_4$-bipy), 8.07 (m, 2H, H$_5$-bipy), 6.46 (br, s, 4H, NH$_2$), 4.00-3.40 (m, 76H, (CH$_2$OCH$_2$)$_{19}$), 3.36 (m, 4H, CH$_2$NH$_2$) ppm. 195Pt NMR (DMF-d$_7$) $\delta$ –2660.6 ppm. ESI-FT-ICR-MS: $m/z$ calcd for C$_{50}$H$_{92}$N$_4$O$_{19}$Pd$^{2+}$, [M – 2NO$_3^-$]$^{2+}$: 624.30133. Found: 624.29932.

$[\text{Pt(tmeda)}(\text{PEGda})](\text{NO}_3)_2$ (127)

[Pt(tmeda)$\text{I}_2$]$^{18}$ (15.9 mg, 28.2 µmol) was suspended in DMF (3 mL) and treated with AgNO$_3$ (9.54 mg, 56.2 µmol) in DMF (3 mL). The mixture was stirred in the absence of light for 24 h at 55°C. AgI was removed by filtration, and the colourless filtrate was added to PEGda (25.3 mg, 28.2 µmol). The solution was stirred for 72 h at 55°C, after which it was evaporated to dryness. This was dissolved in H$_2$O (1 mL) and the mixture filtered through cellulose to afford a stock solution of the title complex (assumed to be quantitative).

$^1$H NMR (DMF-d$_7$) $\delta$ 5.51 (br, s, 4H, NH$_2$), 4.00-3.40 (m, 76H, (CH$_2$OCH$_2$)$_{19}$), 3.30 (m, 4H, CH$_2$NH$_2$), 2.97 (s, 12H, CH$_3$(tmeda)), 2.90 (s, 4H, CH$_2$(tmeda)) ppm. 195Pt NMR (DMF-d$_7$) $\delta$ –2695.5 ppm. ESI-FT-ICR-MS: $m/z$ calcd for C$_{46}$H$_{100}$N$_5$O$_{22}$Pt$^+$, [M – NO$_3^-$]$^+$: 1269.65022. Found: 1269.64911.
[Pt(en)(PEGda)](NO₃)₂ (128)

[Pt(en)I₂]¹⁸ (13.40 mg, 26.33 µmol) was suspended in DMF (3 mL) and treated with AgNO₃ (8.88 mg, 52.26 µmol) in DMF (3 mL). The mixture was stirred in the absence of light for 24 h at 55°C. AgI was removed by filtration, and the colourless filtrate was added to PEGda (23.61 mg, 26.33 µmol) in DMF (1 mL). The solution was stirred for 72 h at 55°C, after which it was evaporated to dryness to give a pale yellow oil which was used without further purification (assumed to be quantitative).

¹H NMR (DMF-d₇) δ 5.72 (br, s, 4H, NH₂), 5.44 (br, s, 4H, NH₂), 3.90–3.40 (m, 76H, (CH₂OCH₂)₁₉), 3.03 (m, 4H, CH₂NH₂), 2.75 (br, s, 4H, CH₂(en)) ppm.¹⁹⁵Pt NMR (DMF-d₇) δ −2870 ppm. ESI-FT-ICR-MS: m/z calcld for C₄₂H₹₂N₅O₂₂Pt⁺, [M – NO₃]⁺: 1213.58762. Found: 1213.58575.

6.11 Synthesis of palladium(II) and platinum(II) acyclic model complexes

[Pt(2,2'-bipy)(NH₃)₂](NO₃)₂ (146)

[Pt(2,2'-bipy)(NO₃)₂] (18.25 mg, 38.4 µmol) was suspended in H₂O (0.3 mL) and treated with NH₃ (28% aqueous solution, 26 mg, ~430 µmol). The suspension was stirred for 4 h at 70°C and allowed to cool. The mixture was filtered through cellulose, and the resulting pale-yellow solution was allowed to evaporate slowly to give the product as a pale yellow powder (16.9 mg, 86%).

¹H NMR (DMSO-d₆) δ 8.71 (m, 4H, H₃, H₆), 8.53 (m, 2H, H₄), 7.96 (m, 2H, H₅), 5.28 (br, s, 6H, NH₃) ppm.¹³C{¹H} NMR (DMSO-d₆) δ 156.4, 149.5, 141.8, 127.8, 124.6 ppm.¹⁹⁵Pt NMR (DMSO-d₆) δ −2604 ppm. ESI-FT-ICR-MS: m/z calcld for C₂₀H₂₈N₁₁O₉Pt₂⁺, [2M – NO₃]⁺: 956.13615. Found: 956.13360. Anal. calcd for C₁₀H₁₄N₆O₆Pt: C, 23.58; H, 2.77; N, 16.50. Found: C, 23.77; H, 2.91; N, 16.51.
[**Pd**(2,2′-bipy)(Mebipy)$_2$](NO$_3$)$_4$ (147)

[**Pd**(2,2′-bipy)(NO$_3$)$_2$] (15.0 mg, 38.8 µmol) was added to a solution of 1-methyl-4,4′-bipyridinium nitrate (15, 18.1 mg, 77.6 µmol) in H$_2$O (0.5 mL) and the suspension was stirred for 2 h at 80°C, over which time [**Pd**(2,2′-bipy)(NO$_3$)$_2$] had dissolved. The pale-yellow solution was filtered through cellulose, and slowly evaporated to give the product as a pale-yellow powder (34.5 mg, 99%).

$^1$H NMR (D$_2$O) $\delta$ 9.44 (d, 4H, $^3$J$_{H,H} = 6.01$ Hz, H$_2$Mebipy), 9.00 (d, 4H, $^3$J$_{H,H} = 6.47$ Hz, H$_2$′Mebipy), 8.51 (d, 2H, $^3$J$_{H,H} = 7.96$ Hz, H$_6$bipy), 8.45 (d, 4H, $^3$J$_{H,H} = 6.47$ Hz H$_3$′Mebipy), 8.42 (obscured, 2H, H$_4$′bipy), 8.28 (d, 4H, $^3$J$_{H,H} = 6.01$ Hz, H$_3$Mebipy), 7.64 (t, 2H, $^3$J$_{H,H} = 6.19$ Hz, H$_5$bipy), 7.54 (dd, 2H, $^3$J$_{H,H} = 5.44$ Hz, H$_3$′bipy), 4.50 (s, 3H, CH$_3$) ppm. $^{13}$C{[$^1$H]} NMR (100 MHz, D$_2$O) $\delta$ 156.4, 152, 150.7, 149.5, 146.4, 145.7, 142.4, 127.7, 126.1, 125.9, 124.0, 47.8 ppm. For 2D COSY, HSQC and HMBC see Section A.1. ESI-MS: $m/z$ 789.52 [M − NO$_3$]$^+$. Anal. calcd for C$_{32}$H$_{30}$N$_{10}$O$_{12}$Pd·2.5H$_2$O: C, 42.80; H, 3.93; N, 15.60. Found: C, 42.60; H, 3.90; N, 15.51.

[**Pt**(2,2′-bipy)(Mebipy)$_2$](NO$_3$)$_4$ (148)

This complex has been reported previously as its ClO$_4$ salt.$^{19}$ The NO$_3^-$ salt was prepared analogously to 147, using [Pt(2,2′-bipy)(NO$_3$)$_2$] instead of [**Pd**(2,2′-bipy)(NO$_3$)$_2$] and stirring overnight (97%, pale yellow powder).

$^1$H NMR (D$_2$O) $\delta$ 9.44 (d, 4H, $^3$J$_{H,H} = 5.51$ Hz, H$_2$Mebipy), 9.02 (d, 4H, $^3$J$_{H,H} = 6.79$ Hz, H$_2$′Mebipy), 8.51 (d, 2H, $^3$J$_{H,H} = 7.44$ Hz, H$_6$bipy), 8.48 (d, 4H, $^3$J$_{H,H} = 6.79$ Hz H$_3$′Mebipy), 8.28 (dd, 4H, $^3$J$_{H,H} = 5.51$ Hz, H$_3$Mebipy), 7.82 (d, 2H, $^3$J$_{H,H} = 5.50$ Hz, H$_5$′bipy), 7.70 (td, 2H, $^3$J$_{H,H} = 7.40$ Hz, H$_5$bipy), 4.52 (s, 3H, CH$_3$) ppm. $^{13}$C{[$^1$H]} NMR (100 MHz, D$_2$O) $\delta$ 153.3, 150.8, 149.5, 146.8, 146.1, 142.8, 128.2, 126.8, 126.2, 124.4, 48.2 ppm. $^{195}$Pt NMR (D$_2$O) $\delta$ −2522.6 ppm. For 2D COSY, HSQC and HMBC see
Section A.1. ESI-MS: \( m/z \) 879.64 [M – NO\(_3\)]\(^+\); 1944 [2M + NO\(_3\)]\(^-\), 1003.02 [M + NO\(_3\)]\(^-\).††† Anal. calcd for C\(_{32}\)H\(_{30}\)N\(_{10}\)O\(_{12}\)Pt·3.5H\(_2\)O: C, 38.25; H, 3.71; N, 13.94. Found: C, 38.09; H, 3.41; N, 13.77.

\[ \text{[Pd(phen)(Mebipy)\(_2\)](NO\(_3\))\(_4\) (149)} \]

The title compound was prepared analogously to 147, using [Pd(phen)(NO\(_3\))\(_2\)]\(^+\) instead of [Pd(2,2′-bipy)(NO\(_3\))\(_2\)] (98%, pale yellow powder). \(^1\)H NMR (D\(_2\)O) \( \delta \) 9.49 (d, 4H, \( ^3J_{HH} = 6.37 \text{ Hz} \), H\(_2\)Mebipy), 8.99 (d, 4H, \( ^3J_{HH} = 6.53 \text{ Hz} \), H\(_2\)′Mebipy), 8.91 (dd, 2H, \( ^3J_{HH} = 6.43 \text{ Hz} \), \( ^3J_{HH} = 2.93 \text{ Hz} \), H\(_3\)Mebipy), 8.45 (d, 4H, \( ^3J_{HH} = 6.53 \text{ Hz} \) H\(_3\)′Mebipy), 8.29 (d, 2H, \( ^3J_{HH} = 6.43 \text{ Hz} \), H\(_3\)phen), 7.92-7.88 (4H, H\(_2\), H\(_4\)phen), 4.50 (s, 3H, CH\(_3\)) ppm. \(^{13}\)C\({}^{1}\)H NMR (100 MHz D\(_2\)O) \( \delta \) 152.59, 151.05, 150.45, 146.92, 146.82, 146.04, 141.70, 131.12, 128.04, 126.41, 126.25, 126.09, 48.13 ppm. For 2-D COSY, HSQC and HMBC see Section A.1. Anal. calcd for C\(_{34}\)H\(_{30}\)N\(_{10}\)O\(_{12}\)Pd·2.5H\(_2\)O: C, 44.29; H, 3.83; N, 15.19. Found: C, 44.54; H, 3.84; N, 15.16.

### 6.12 Biomolecular mass spectrometry

Freshly prepared stock solutions of DNA (1000 \( \mu \)M in 0.1 M NH\(_4\)OAc) and metal complexes (200 \( \mu \)M in either 0.01 or 0.1 M NH\(_4\)OAc) were prepared. These were combined and diluted with either 0.01 or 0.1 M NH\(_4\)OAc to give mixtures in which the final concentration of DNA was 10 \( \mu \)M (pH \( \approx \) 7.0 or 7.4 for 0.01 and 0.1 M NH\(_4\)OAc, respectively). The solutions were mixed by vortex and left at room temperature for at least 15 min before being analysed. Negative-ion ESI mass spectra were acquired using a Waters Micromass Q-ToF2 Ultima spectrometer equipped with a Z-spray probe, calibrated over the appropriate range with CsI (750 \( \mu \)M). Unless otherwise stated, the conditions used were: \( V_{\text{capillary}} = 2.5 \text{ V} \), \( V_{\text{cone}} = 70 \text{ V} \), \( T_{\text{source}} = 25^\circ\text{C} \), \( T_{\text{desolvation}} = 100^\circ\text{C} \), collision energy = 2. Spectra were acquired by summing 20 scans (10 scans for the comparative binding studies of D2).

††† Data for 147 and 148 were collected using a Micromass Q-ToF2 Ultima spectrometer.
6.13 Thermal denaturation

Thermal denaturation experiments of DNA in the presence of metal complexes were performed using a procedure similar to that employed by Cusumano and co-workers.²⁰ Mixtures of CT-DNA and complexes ([DNA base pairs] = 78 µM, [complex] = 7.8 µM) were prepared in phosphate-buffered saline solution (1 mM phosphate, 2 mM NaCl, pH 7.4) and their thermal denaturation monitored by recording absorbance values at 260 nm over the temperature range 37 – 100ºC. The solutions were held at 37ºC for 30 min prior to heating at 0.5ºC/min and absorbance readings were taken every 2 min (1ºC). Data were fitted to sigmoidal plots using origin 7.0®, and $T_m$ values were estimated from the $x$-intercepts of 2nd derivative plots. All values are averaged over at least 2 runs for each mixture. These were compared to values obtained for CT-DNA in the absence of metal complex.

6.14 References

Appendices

A.1 2-D NMR spectra

Figure A.1: $^1$H-$^1$H COSY (400 MHz, D$_2$O) spectrum of 1,2-bis(1-methyl-2-aminopyrimidinium)benzene nitrate (76).

Figure A.2: $^1$H-$^1$H COSY (300 MHz, D$_2$O) spectrum of 1,3-bis(1-methyl-2-aminopyrimidinium)benzene bromide (78).
Figure A.3: $^1$H-$^{13}$C HSQC (300 MHz, D$_2$O) spectrum of 1,3-bis(1-methyl-2-aminopyrimidinium)benzene bromide (78).

Figure A.4: $^1$H-$^1$H COSY (300 MHz, D$_2$O) spectrum of 1,1'-bis[4-(4,4'-bipyridinium)butyl]-4,4'-bipyridinium bromide (100).
Figure A.5: $^1$H-$^1$H COSY (300 MHz, D$_2$O) spectrum of 1,1$'$-bis[4-(4,4$'$-bipyridinium)butyl]-4,4$'$-bipyridinium hexafluorophosphate (103).

Figure A.6: $^1$H-$^1$H COSY (300 MHz, D$_2$O) spectrum of 1,1$'$-bis[6-(4,4$'$-bipyridinium)hexyl]-4,4$'$-bipyridinium nitrate (104).

Figure A.7: $^1$H-$^1$H COSY (300 MHz, D$_2$O) spectrum of [Pt$_4$(2,2$'$-bipy)$_4$(apym(CH$_2$)$_3$apym–2H)$_2$](PF$_6$)$_8$ (124).
Figure A.8: $^1$H-$^1$H COSY (400 MHz, D$_2$O) spectrum of [Pd(2,2'-bipy)(Mebipy)$_2$](NO$_3$)$_4$ (147).

Figure A.9: $^1$H-$^{13}$C HSQC (400 MHz, D$_2$O) spectrum of [Pd(2,2'-bipy)(Mebipy)$_2$](NO$_3$)$_4$ (147).

Figure A.10: $^1$H-$^{13}$C HMBC (400 MHz, D$_2$O) spectrum of [Pd(2,2'-bipy)(Mebipy)$_2$](NO$_3$)$_4$ (147).
Figure A.11: $^1$H-$^1$H COSY (400 MHz, D$_2$O) spectrum of [Pt(2,2'-bipy)(Mebipy)$_2$](NO$_3$)$_4$ (148).

Figure A.12: $^1$H-$^1$C HSQC (400 MHz, D$_2$O) spectrum of [Pt(2,2'-bipy)(Mebipy)$_2$](NO$_3$)$_4$ (148).

Figure A.13: $^1$H-$^1$C HMBC (400 MHz, D$_2$O) spectrum of [Pt(2,2'-bipy)(Mebipy)$_2$](NO$_3$)$_4$ (148).
Appendices

Figure A.14: $^1\text{H}^1\text{H}$ COSY (400 MHz, D$_2$O) spectrum of [Pd(phen)(Mebipy)$_2$](NO$_3$)$_4$ (149).

Figure A.15: $^1\text{H}^1\text{C}$ HSQC (400 MHz, D$_2$O) spectrum of [Pd(phen)(Mebipy)$_2$](NO$_3$)$_4$ (149).

Figure A.16: $^1\text{H}^1\text{C}$ HMBC (400 MHz, D$_2$O) spectrum of [Pd(phen)(Mebipy)$_2$](NO$_3$)$_4$ (149).
A.2 UV-visible spectra

Figure A.18: UV-visible spectrum of \([\text{Pt}_4(2,2'-\text{bipy})_4\{\text{apym}(\text{CH}_2)\text{apym}–2\text{H}\}_2\text{][NO}_3\}_8\) (124 as NO$_3^-$ salt, 0.120 mM in H$_2$O).

A.3 X-ray crystallographic procedures and results

(±)-2,6-Bis(4,4'-bipyridinium)-9-thiabicyclo[3.3.1]nonane chloride (30)

A colourless needle-like crystal of the title compound was attached with Exxon Paratone N, to a short length of fibre supported on a thin piece of copper wire inserted in a copper
mounting pin. The crystal was quenched in a cold N₂ gas stream from an Oxford Cryosystems Cryostream. A Bruker-Nonius FR591 Kappa APEX II diffractometer employing graphite monochromated MoKα radiation generated from a fine-focus rotating anode was used for the data collection. Cell constants were obtained from a least squares refinement against 8715 reflections located between 5.28 and 59.92° 2θ. Data were collected at 150(2) K with φ and ω scans to 60.54° 2θ. The data integration and reduction were undertaken with SAINT and XPREP, and subsequent computations were carried out with the X-Seed³ graphical user interface. An empirical absorption correction determined with SADABS³ was applied to the data.

The structure was solved in the space group P2₁2₁2₁ by direct methods with SHELXS-97,⁴ and extended and refined with SHELXL-97.⁴ The non-H atoms in the asymmetric unit were modelled with anisotropic displacement parameters. A riding atom model with group displacement parameters was used for the H atoms. The absolute structure was established with the Flack parameter⁵ refining to 0.45(10).

Formula C₂₈H₄₅.₅₀Cl₂N₄O₈.₇₅S, M 681.14 Da, orthorhombic, space group P2₁2₁2₁, a 6.9765(2), b 19.7846(5), c 24.6467(6) Å, V 3401.92(15) Å³, Dc 1.330 g cm⁻³, Z 4, crystal size 0.33×0.08×0.08 mm, colour colourless, habit needle, temperature 150(2) K, λ(MoKα) 0.71073 Å, μ(MoKα) 0.306 mm⁻¹, T(SADABS)min,max 0.894, 0.976, 2θmax 60.54, hkl range -9 9, -27 26, -34 34, N 89460, Nind 9994(Rmerge 0.0485), Nobs 9322(I > 2σ(I)), Nvar 440, residuals* R1(F) 0.0849, wR2(F³) 0.2352, GoF(all) 1.128, Δρmin,max -0.647, 1.119 e⁻ Å⁻³.

A colourless block-like crystal of the title compound was mounted on a Bruker SMART 1000 CCD diffractometer employing graphite monochromated MoKα radiation generated from a sealed tube. Cell constants were obtained from a least squares refinement against 4414 reflections located between 7.21 and 56.56° 2θ. Data were collected at 150(2) K with ω scans to 56.58° 2θ. The data integration and reduction were undertaken with SAINT and XPREP, and subsequent computations were carried out with the WinGX⁶ graphical user interface.
interface. The intensities of 88 standard reflections recollected at the end of the experiment changed by 16 during the data collection and a correction was accordingly applied to the data. An empirical absorption correction determined with SADABS was applied to the data.

The structure was solved in the space group \(P3_2\) by direct methods with SIR97\(^7\) and extended and refined with SHELXL-97. The non-H atoms in the asymmetric unit were modelled with anisotropic displacement parameters. Of the 8 H atoms included in the model 2 were located and modelled with isotropic displacement parameters, and a riding atom model was used for the remainder. The absolute structure was established with the Flack parameter refining to 0.074(1).

Formula \(C_5H_8N_4O_3\), \(M 172.15\) Da, trigonal, space group \(P3_2\), \(a 6.1596(7)\), \(b 6.1596(7)\), \(c 16.966(4)\) Å, \(V 557.46(16)\) Å\(^3\), \(D_c 1.538\) gcm\(^{-3}\), \(Z 3\), crystal size 0.600×0.570×0.560 mm, colour colourless, habit block, temperature 150(2) K, \(\lambda(MoK\alpha) 0.71073\) Å, \(\mu(MoK\alpha) 0.128\) mm\(^{-1}\), \(T(SADABS)_{\text{min,max}} 0.834, 0.931\), \(2\theta_{\text{max}} 56.58, hkl\) range -8 8, -8 8, -22 21, \(N\text{ind} 5431, N_{\text{obs}} 1740(I > 2\sigma(I)), N_{\text{var}} 116\), residuals \(R1(F) 0.0266, wR2(F^2) 0.0690, \text{GoF(all)} 0.983, \Delta\rho_{\text{min,max}} -0.270, 0.164\) eÅ\(^{-3}\).

\[\begin{align*}
\text{w} & = \frac{1}{\sigma^2(F_o^2)+0.0509P^2 + 0.0552P}; \ P = (F_o^2 +2F_c^2)/3
\end{align*}\]

**1-Benzyl-2-aminopyrazinium hexafluorophosphate (61)**

A colourless prism-like crystal of the title compound was mounted on a Bruker APEXII-FR591 diffractometer employing graphite monochromated MoK\(\alpha\) radiation generated from a rotating anode. Cell constants were obtained from a least squares refinement against 574 reflections located between 7.98 and 59.32\(^\circ\) \(2\theta\). Data were collected at 150(2) K with \(\omega+\phi\) scans to 60.02\(^\circ\) \(2\theta\). The data integration and reduction were undertaken with SAINT and XPREP, and subsequent computations were carried out with the WinGX graphical user interface. An empirical absorption correction determined with SADABS was applied to the data.

The structure was solved in the space group \(C2/c\) by direct methods with SIR97, and extended and refined with SHELXL-97. Of the 40 non-H atom sites in the asymmetric unit, 16 were modelled with anisotropic displacement parameters and the rest were modelled...
with isotropic displacement parameters. Of the 12 H atoms included in the model 2 were located and modelled with isotropic displacement parameters, and a riding atom model was used for the remainder.

Formula \( \text{C}_{11}\text{H}_{12}\text{F}_{6}\text{N}_{3}\text{P} \), M 331.21 Da, monoclinic, space group \( \text{C}2/c \), \( a = 19.106(4) \), \( \text{b} = 8.6832(17) \), \( c = 17.974(5) \, \AA \), \( \beta = 117.261(6) \), \( V = 2650.8(11) \, \AA^3 \), \( D_c = 1.660 \, \text{g cm}^{-3} \), \( Z = 8 \), crystal size 0.435×0.340×0.235 mm, colour colourless, habit prism, temperature 150(2) K, \( \lambda(\text{MoK} \alpha) = 0.71073 \, \AA \), \( \mu(\text{MoK} \alpha) = 0.275 \, \text{mm}^{-1} \), \( T(\text{SADABS})_{\text{min,max}} = 0.808, 0.937, \theta_{\text{max}} = 60.02 \), \( hkl \) range -26 26, -12 12, -25 24, \( N = 17110 \), \( N_{\text{ind}} = 3842 \, (R_{\text{merge}} = 0.0461) \), \( N_{\text{obs}} = 2961(I > 2\sigma(I)) \), \( N_{\text{var}} = 218 \), residuals \( R1(F) = 0.0633 \), \( wR2(F^2) = 0.1715 \), \( \text{GoF(all)} = 1.036 \), \( \Delta \rho_{\text{min,max}} = -0.838, 0.697 \, \text{e}^{-3} \).

\[
w = \frac{1}{([\sigma^2(F_o^2)] + (0.0751P)^2) + 7.7923P}
\]

1,3-Bis(1-methyl-2-aminopyrimidinium)benzene triflate hexafluorophosphate (80°)

The mixed salt [apym\((m\text{-xylylene})\text{apym}\)](OTf)\(_{1.95}\)(PF\(_6\))\(_{0.05}\) formed as colourless crystals on slow evaporation of a CD\(_3\text{CN}\) solution containing equimolar amounts of [Pt(dppp)(OTf)\(_2\)] and [apym\((m\text{-xylylene})\text{apym}\)](PF\(_6\))\(_2\) (80).

One of the block-like crystals was mounted on a Bruker APEXII-FR591 diffractometer employing graphite monochromated MoK\( \alpha \) radiation generated from a rotating anode. Cell constants were obtained from a least squares refinement against 7706 reflections located between 4.61 and 78.94° \( \theta \). Data were collected at 150(2) K with \( \omega + \phi \) scans to 80.10° \( \theta \). The data integration and reduction were undertaken with SAINT and XPREP, and subsequent computations were carried out with the WinGX graphical user interface. An empirical absorption correction determined with SADABS was applied to the data.

The structure was solved in the space group \( \text{P1} \) by direct methods with SIR97, and extended and refined with SHELXL-97. Of the 45 non-H atom sites in the asymmetric unit, 38 were modelled with anisotropic displacement parameters and the rest were modelled with isotropic displacement parameters. A riding atom model with group displacement parameters was used for the H atoms.
Appendices

Formula $C_{17.95}H_{18.13}N_6O_{5.88}P_{0.06}S_{1.95}$, M 592.32 Da, triclinic, space group $P\bar{1}$, $a$ 9.6224(4), $b$ 11.2521(5), $c$ 12.3853(5) Å, $\alpha$ 98.435(2), $\beta$ 112.739(2), $\gamma$ 100.330(2)°, $V$ 1181.73(9) Å$^3$, $D_c$ 1.665 g cm$^{-3}$, $Z$ 2, crystal size 0.300×0.250×0.200 mm, colour colourless, habit block, temperature 150(2) K, $\lambda$(MoK$\alpha$) 0.71073 Å, $\mu$(MoK$\alpha$) 0.322 mm$^{-1}$, $T$(SADABS)$_{\text{min, max}}$ 0.859, 0.937, $2\theta_{\text{max}}$ 80.10, $hkl$ range -17 17, -20 20, -22 22, $N$ 79449, $N_{\text{ind}}$ 14574 ($R_{\text{merge}}$ 0.0305), $N_{\text{obs}}$ 12323 ($I > 2\sigma(I)$), $N_{\text{var}}$ 365, residuals $R1(F)$ 0.0357, $wR2(F^2)$ 0.0982, GoF(all) 1.025, $\Delta\rho_{\text{min, max}}$ -0.384, 0.577 e Å$^{-3}$.

$$w = 1/[\sigma^2(F_o^2)+(0.0496P)^2 + 0.2401P]$$

Figure A.19: ORTEP of $80'$, with ellipsoids drawn at the 50% probability level. The OTf$^-$ and PF$_6^-$ anions are omitted for clarity.

![Figure A.19](image)

Figure A. 20: The solid state packing of $80'$, featuring 1-D chains held together by N–H···N and N–H···O H-bonds. In turn, these chains are linked by C–Hphenylene···O interactions to form 2-D sheets. The sheets are linked by anion-π interactions ($r_{\text{centroid-O(4)}} = 3.02$ Å, $r_{\text{centroid-F(6)}} = 2.99$ Å).

1-(2-Pyrimidylaminomethyl)-2-(pyrimidiniummethyl)benzene nitrate (90)

A colourless prism-like crystal of the title compound was mounted on a Bruker APEXII-FR591 diffractometer employing graphite monochromated MoK$\alpha$ radiation generated from a rotating anode. Cell constants were obtained from a least squares
refinement against 437 reflections located between 4.87 and 48.98° 2θ. Data were collected at 150(2) K with ω+φ scans to 63.48° 2θ. The data integration and reduction were undertaken with SAINT and XPREP, and subsequent computations were carried out with the WinGX graphical user interface. An empirical absorption correction determined with SADABS was applied to the data.

The structure was solved in the space group $P2_1/n$ by direct methods with SIR97, and extended and refined with SHELXL-97. Of the 32 non-H atom sites in the asymmetric unit, 29 were modelled with anisotropic displacement parameters and the rest were modelled with isotropic displacement parameters. Of the 23 H atoms included in the model 9 were located and modelled with isotropic displacement parameters, and a riding atom model was used for the remainder.

Formula $C_{16}H_{23}N_7O_6$, M 409.41 Da, monoclinic, space group $P2_1/n$, $a$ 10.614(2), $b$ 10.482(3), $c$ 17.966(5) Å, $β$ 100.303(9), $V$ 1966.6(9) Å³, $D_c$ 1.383 g cm⁻³, $Z$ 4, crystal size 0.190×0.119×0.099 mm, colour colourless, habit prism, temperature 150(2) K, $λ$(MoKα) 0.71073 Å, $μ$(MoKα) 0.108 mm⁻¹, $T$(SADABS)$_{\text{min,max}}$ 0.8539, 0.99, $θ_{\text{max}}$ 63.48, $hkl$ range -15 15, -15 15, -26 26, $N$ 31881, $N_{\text{ind}}$ 6621($R_{\text{merge}}$ 0.0815), $N_{\text{obs}}$ 3165(I > 2$σ(I)$), $N_{\text{var}}$ 299, residuals $R1(F)$ 0.0642, $wR2(F^2)$ 0.1804, GoF(all) 1.029, $Δρ_{\text{min,max}}$ -0.560, 0.547 e Å⁻³.

$$w = 1/[σ^2(F_o^2) + (0.0707P)^2 + 0.2567P]$$
A.4 Negative-ion ESI mass spectra of oligonucleotide solutions

A.4.1 Spectra of mixtures containing D2/D2a and palladium(II)/platinum(II) complexes

Figure A.21: ESI-MS of D2 with [Pd(tmeda)(PEGda)]^{2+} (1 : 1) in (a) 0.01 M NH₄OAc or (b) H₂O; • [D2 – nH^n–] (n = 5, 6); ◦ [D2a – 3H^+]^3–; □ [D2b – 3H^+]^3–; ↔ [D2 + Pd(tmeda)(PEGda)]^{2+} – 5H^+; ↔ [D2 + PEGda – 3H^+]^3–; ↔ [D2 + Pd(tmeda)]^{2+} – nH+]^{(n-2)} (n = 7, 8); ↔ [D2 + 2Pd(tmeda)]^{2+} – nH+]^{(n-4)} (n = 9, 10); ↔ [D2a + Pd(tmeda)]^{2+} – 5H^+; ↔ [D2b + Pd(tmeda)]^{2+} – 5H^+.

Figure A.22: ESI-MS of D2 with [Pt(en)(PEGda)]^{2+} (1 : 1 in 0.01 M NH₄OAc); • [D2 – nH^n–] (n = 4, 5, 6); ◦ [D2a – 3H^+]^3–; □ [D2b – 3H^+]^3–; ↔ [D2 + Pt(en)(PEGda)]^{2+} – 7H^+.

Figure A.23: ESI-MS of D2 with [Pt(2,2′-bipy)(PEGda)]^{2+} (1 : 1 in 0.01 M NH₄OAc); • [D2 – nH^n–] (n = 5, 6); × ion present from previous acquisition.
Figure A.24: ESI-MS of D2a with [Pt₂(2,2′-bipy)₂(4,4′-bipy(CH₂)₆4,4′-bipy)]₈⁺ (1 : 1 in 0.01 M NH₄OAc); ◊ [D2a – nH⁺]ⁿ⁻ (n = 3, 4, 5, 6); ◄ [D2a + Pt₂(2,2′-bipy)₂(4,4′-bipy(CH₂)₆4,4′-bipy)]₈⁺ – 11H⁺]⁻; ◄ [D2a + Pt₂(2,2′-bipy)₂(4,4′-bipy(CH₂)₆4,4′-bipy)]₂₈⁺ – 7H⁺]⁻.
A.4.2 Spectra obtained for comparison of binding affinities of platinum(II) complexes towards D2

Figure A.25: ESI-MS of D2 with daunomycin (1 : 5 in 0.1 M NH₄OAc) with cone at (a) 150 V, (b) 130 V, (c) 110 V, (d) 90 V, (e) 70 V.

Figure A.26: D2-binding profile for daunomycin (1 : 5 in 0.1 M NH₄OAc).
Figure A.27: ESI-MS of D2 with distamycin (1 : 5 in 0.1 M NH₄OAc) with cone at (a) 70 V, (b) 90 V, (c) 110 V, (d) 130 V, (e) 150 V.

Figure A.28: D2-binding profile for distamycin (1 : 5 in 0.1 M NH₄OAc).
Figure A.29: ESI-MS of D2 with $[\text{Pt}(2,2\text{'}-\text{bipy})(\text{Mebipy})_2]^{4+}$ (1 : 5 in 0.1 M NH$_4$OAc) with cone at (a) 150 V, (b) 130 V, (c) 110 V, (d) 90 V, (e) 70 V.

Figure A.30: D2-binding profile for $[\text{Pt}(2,2\text{'}-\text{bipy})(\text{Mebipy})_2]^{4+}$ (1 : 5 in 0.1 M NH$_4$OAc).
Figure A.31: ESI-MS of D2 with [Pt(2,2'-bipy)(NH$_3$)$_2$]$^{2+}$ (1 : 5 in 0.1 M NH$_4$OAc) with cone at (a) 150 V, (b) 130 V, (c) 110 V, (d) 90 V, (e) 70 V.

Figure A.32: D2-binding profile for [Pt(2,2'-bipy)(NH$_3$)$_2$]$^{2+}$ (1 : 5 in 0.1 M NH$_4$OAc).
Figure A.33: ESI-MS of D2 with [Pt(en)(PEGda)]^{2+} (1 : 5 in 0.1 M NH\textsubscript{4}OAc) with cone at (a) 150 V, (b) 130 V, (c) 110 V, (d) 90 V, (e) 70 V.

Figure A.34: D2-binding profile for [Pt(en)(PEGda)]^{2+} (1 : 5 in 0.1 M NH\textsubscript{4}OAc).
Figure A.35: ESI-MS of D2 with [Pt(2,2′-bipy)2(4,4′-bipy(CH2)64,4′-bipy)]8+ (1 : 2.5 in 0.1 M NH₄OAc) with cone at (a) 150 V, (b) 130 V, (c) 110 V, (d) 90 V, (e) 70 V.

Figure A.36: D2-binding profile for [Pt(2,2′-bipy)2(4,4′-bipy(CH2)64,4′-bipy)]8+ (1 : 5 in 0.1 M NH₄OAc).
A.4.3 Spectra of biotinylated DNA

Figure A.37: ESI-MS of D2’a (in 0.05 M NH₄OAc); \( \circ D2’a \) [D2’a – nH⁺]ⁿ⁻ (n = 3, 4, 5, 6, 7).

Figure A.38: ESI-MS of D2’b (in 0.05 M NH₄OAc); \( \circ D2’b \) [D2’b – nH⁺]ⁿ⁻ (n = 3, 4, 5, 6, 7).

Figure A.39: ESI-MS of D2’ (in 0.05 M NH₄OAc); \( \circ D2’ \) [D2’ – nH⁺]ⁿ⁻ (n = 5, 6).
A.5 Thermal denaturation data

Sample melting profiles of mixtures containing DNA and given below. Traces of raw data (black), sigmoidal fits (red) and their 1st (blue) and 2nd derivatives (green) are provided in each case.

![Figure A.40: DNA melting profile for [Pt₂(2,2'-bipy)₂{4,4'-bipy(CH₂₄,4',4'  bipy)₂](NO₃)₈ (110 as NO₃⁻ salt).](image)

![Figure A.41: DNA melting profile for [Pt(2,2'-bipy)(PEGda)](NO₃)₂ (126).](image)
Figure A.42: DNA melting profile for $[\text{Pt(tmeda)(PEGda)}](\text{NO}_3)_2$ (127).

Figure A.43: DNA melting profile for $[\text{Pt(en)(PEGda)}](\text{NO}_3)_2$ (128).

Figure A.44: DNA melting profile for PEGda.
Figure A.45: DNA melting profile for [Pt(2,2'-bipy)(NH₃)₂][NO₃]₂ (146).

Figure A.46: DNA melting profile for [Pt(2,2'-bipy)(Mebipy)₂][NO₃]₄ (148).

Figure A.47: DNA melting profile for EtdBr (152).
A.6 References