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Tandem Mass Spectrometry for Characterization of Covalent Adducts of DNA with Anti-cancer Therapeutics

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Abstract

The chemotherapeutic activities of many anticancer and antibacterial drugs arise from their interactions with nucleic acid substrates. Some of these ligands interact with DNA in a way that causes conformational changes or damage to the nucleic acid targets, ultimately altering recognition by key DNA-specific enzymes, interfering with DNA transcription or prohibiting replication, and terminating cell growth and proliferation. The design and synthesis of ligands that bind to nucleic acids remains a dynamic field in medicinal chemistry and pharmaceutical research. The quest for more selective and efficacious DNA-interactive anti-cancer chemotherapeutics has likewise catalyzed the need for sensitive analytical methods that can provide structural information about the nature of the resulting DNA adducts and provide insight into the mechanistic pathways of the DNA/drug interactions and the impact on the cellular processes in biological systems. This review focuses on the array of tandem mass spectrometric strategies developed and applied for characterization of covalent adducts formed between DNA and anti-cancer ligands.

Keywords

tandem mass spectrometry; nucleic acid; oligodeoxynucleotide; adduct; anti-cancer drug

I. Introduction

Many anticancer therapeutics in clinical use function by interacting with DNA, not only those that bind to DNA through non-covalent interactions (Bischoff and Hoffmann, 2002) but also those that form covalent adducts such as via alkylation or inter- and intra-strand crosslinking (Atkinson et al., 2007; Goff and Thorson, 2010; Kumar et al., 2001; Nicolaou and Dai, 1991; Smith and Nicolaou, 1996; Palom et al., 2002; Patterson et al., 2007; Reedijk and Lohman, 1985; Reedijk, 1987; Singleton et al., 2009; Tomasz et al., 1998; Waring, 1986; Winski et al., 1998). Drug-induced damage to DNA can cause replication and transcription machinery to incorrectly process the genetic code, as well as activation of repair processes that lead to double- or single-strand breaks, the latter resulting in chromosome aberrations and ultimately cell death (Chaney and Sancar, 1996). A monoadduct is formed when a drug covalently binds to a single site of a nucleotide. DNA crosslinks arise from the reaction of the drug with a second site of the nucleic acid, although this reaction is usually slower than the formation of a monoadduct or the first covalent bond to a nucleotide (McHugh et al., 2001). An interstrand crosslink occurs when the drug interacts with two nucleotides on opposing DNA strands, and an intrastrand crosslink occurs when a drug moiety binds to two nucleobases on the same DNA strand. Interstrand

crosslinks induce cytotoxicity by resulting in double-strand breaks or prevention of strand separation during replication (McKeown et al., 2007; Nicolaou and Dai, 1991). A conceptual illustration of a monoadduct and both interstrand and intrastrand crosslinks is shown in Figure 1.

Non-covalent complexes, such as those arising from intercalation or hydrogen-bonding interactions in the major or minor grooves of DNA (Bischoff and Hoffmann, 2002), cannot be readily differentiated from covalent adducts based solely on m/z value or specific fragmentation patterns (although non-covalent complexes do tend to be more weakly bound and often disassemble into individual DNA and drug constituents upon CID in the gas phase). Introduction of DNA complexes via liquid chromatography (LC) prior to mass spectrometric detection will frequently degrade all the non-covalent interactions, thus causing detachment of non-covalently bound drugs from the DNA and even separation of the DNA strands of duplexes. In this case, only covalently-bound adducts and crosslinks will survive the LC conditions before MS analysis. The non-covalent complexes are not discussed further in this study.

II. REACTIVE SITES OF NUCLEOTIDES

The four nucleosides comprising DNA are shown in Figure 2. The reactive sites of nucleosides have been extensively studied. Guanine has shown particularly high reactivity at the N7 site (Figure 2) which serves as the critical binding site for several therapeutic agents. For example, nitrogen mustards (Mehta et al., 1980) bind to guanine N7 as monoadducts, and N7 serves as the first binding site in the formation of crosslinks for a number of bioreductive prodrugs like anthraquinones (Winski et al., 1998) and platinum derivatives like cisplatin (Reedijk, 1987). A second reactive site of guanine is the 2-amino group which is the binding site of the antibiotic mitomycin C (Borowy-Borowski et al., 1990; Tomasz et al., 1998), leading to formation of both monoadducts and crosslinks. Eneidyenes bind in the minor groove of DNA and cause strand scission, discussed more later, that hinges on alkylation of guanine at the N7 and the C8 sites (Nicolaou and Dai, 1991). Interestingly, while anticancer agents preferentially bind at the N7 site and 2-amino group of guanine, carcinogenic compounds that enter the cell commonly are metabolized to DNA-alkylating electrophiles and bind to guanine O6 as opposed to a nitrogen site (Ford and Scribner, 1990). The N3 position is the most reactive site of adenine. For example, antibiotic antitumor agent CC-1065 forms a monoadduct with DNA via interaction of the cyclopropyl group at adenine N3 (Scahill et al., 1990), and the cytotoxicity of duocarmycin A arises from formation of a monoadduct involving reaction of the cyclopropane functionality with the N3 position of adenine (Boger et al., 1991; Boger et al., 1992). Cytosine exhibits reactivity at the N3 site with platinum derivative drugs like cisplatin (Sherman and Lippard, 1987). Thymine is also frequently involved in therapeutic DNA binding. For example psoralen derivatives engage in inter- and intrastrand crosslinking at the 5,6 double bond of thymine (Lai et al., 2008).

There are numerous drugs whose pharmacological activities arise from covalent interactions with DNA. Antibiotic drugs include mitomycin (Borowy-Browoski et al., 1990), hedamycin (Hansen, 1995), acridine ligands (Lerman, 1961), enediynes (Nicolaou and Dai, 1991), and nitrogen mustards (Colvin et al., 1976). Antitumor drugs include tamoxifen (Phillips et al., 1994), platinum derivatives (Reedijk, 1987) and prodrugs like aziridinyl benzoquinones (Winski, 1998). Figure 3 depicts the structures of various anticancer drugs.

III. Methods for Sequencing DNA and Identification of DNA Adducts

The primary sequence of nucleic acids can be determined using the well-established Sanger method, now an automated system typically utilizing fluorescent dye-terminator sequencing (Sanger, 1997; Prober, 1987). The Sanger method employs the inhibitory properties of 2',3'-dideoxynucleotide triphosphates used in the elongation of primer strands from the template sequence to be determined. When a 2',3'-dideoxynucleotide triphosphate analog becomes incorporated into the growing template sequence in place of a deoxynucleotide, the absence of the 3'-hydroxyl on the 2',3'-dideoxynucleotide triphosphate leads to the termination of sequence extension by DNA polymerase. In the current automated format, a different fluorescent dye molecule is attached to each dideoxynucleotide triphosphate analog and upon enzymatic digestion a set of nested fragments terminated by dye molecules is separated by high resolution gel electrophoresis and detected based on fluorescence (Metzker, 2005). In this way, the original DNA sequence is determined from the fluorescence emission of the separated bands. Tandem mass spectrometry has also been used to sequence nucleic acids with up to 108 nucleotides based on monitoring the conventional *a* – *B* and *w* ions created upon collision induced dissociation, as described in more detail in the next section (Little and McLafferty, 1995; Little et al., 1996).

Mapping modifications of nucleic acids is significantly more challenging. Modifications of DNA and RNA have been detected and identified using ³²P-post labeling (Gupta et al., 1987; David, 1997; Randerath et al., 1981) with enzymatic digestion followed by gel electrophoresis (Cheng et al., 1996), and HPLC-UV-Vis detection (Palom et al., 2002). The ³²P-post labeling procedure consists of several steps after adduct formation, including DNA digestion to form 3'-phosphonucleotides, kinase enzyme-mediated 5'-post labeling with radioactive [γ -³²P]ATP, and then chromatographic separation of labeled nucleotides. Detection limits as low as 1 modification in 10¹⁰ nucleotides (David, 1997) have been reported. The 3',5'-bisphosphate nucleosides labeled with ³²P are detected and quantified by measuring the ³²P-radioactive decay after electrophoresis. The radioactively-labeled DNA is enzymatically digested into smaller fragments and separated on an agarose gel. Separation of DNA fragments by gel electrophoresis occurs because each fragment has varying numbers of negatively charged phosphate groups, thus influencing their mobilities in the presence of the electric field. Although this robust technique allows direct quantification of DNA adducts, it is time-consuming, can require up to 45 hours of autoradiography (Randerath et al., 1981), and has not been extensively used for human cytotoxic DNA-drug adducts (David, 1997).

DNA adducts have historically been determined by HPLC with UV-Vis detection, and today this technique is commonly used as a front-end separation method prior to analysis by mass spectrometry. The first chromatographic separations of DNA were performed on calcium phosphate columns by phosphate buffer elution in 1957 by Main and Cole who successfully separated photodegraded nucleotide fragments from untreated DNA (Main and Cole, 1957). For this method, cellular DNA is extracted in a series of steps beginning with cell lysis of a centrifuged cell pellet on ice with a protease, for example protease K, addition of sodium acetate to stabilize the helix, and precipitation of DNA by addition of cold isopropanol before lyophilization and resuspension in a buffer (Pfeifer, 1996). Cellular DNA is enzymatically degraded into nucleotides using enzymes such as P₁ nuclease before analysis by HPLC (Wietstock, 1995). Mononucleotides are identified via UV absorbance at 260 nm, and quantified by peak integration and comparison to an internal standard. Currently, most DNA adducts are separated from unreacted DNA by reversed phase HPLC, and fractions are detected by UV absorbance. Liquid chromatography is well established and has a long history of DNA adduct identification both on its own and as an online separation for mass spectrometers (Beranek et al., 1980; Cadet et al., 1988; Cummings et al., 1991; Paz et al.,

2008; Pierce et al., 2010; Singleton et al., 2009; Wickham et al., 1995; Wu et al., 2001). A recent overview of the use of mass spectrometry for characterization of carcinogenic DNA adducts was presented (Lemiere 2010), and such types of adducts are not further addressed in this report.

IV. Mass Spectrometry For Analysis Of Nucleic Acids

With the increased emphasis on understanding the structures and functions of biological molecules on a cellular level, the need for higher throughput methods with greater sensitivity has propelled the development of mass spectrometry for the structural characterization of nucleic acids (Fabris, 2010) and adducts. Tandem mass spectrometry offers perhaps the single best method to pinpoint the sites of attachment of drugs to DNA without the significant sensitivity limitations of NMR methods. The first hurdle encountered with respect to the application of mass spectrometry for the analysis of nucleic acids was the lack of suitable ionization methods for thermally labile molecules. The earliest ionization methods that proved successful for nucleic acids were Cf plasma desorption and fast atom bombardment (FAB). Developed by R. D. Macfarlane and D. F. Torgerson, ^{252}Cf plasma desorption mass spectrometer (PDMS) exploited the high energy deposition from the spontaneous fission of ^{252}Cf to desorb molecules from Cf-doped metal foils (Macfarlane and Torgerson, 1976). McNeal et al. analyzed small protected oligonucleotides McNeal et al., 1980, McNeal and Macfarlane, 1981) and reported ionization of a decanucleotide (McNeal et al., 1980). Barber et al. developed fast atom bombardment (FAB) which entailed the bombardment of samples entrained in a viscous glycerol matrix with neutral molecules, thus leading to volatilization and ionization of the analyte (Barber et al., 1981). FAB was an improvement over ^{252}Cf PDMS with respect to the ease of sample preparation. Grotjahn et al. (Grotjahn et al., 1982) used FAB-MS to sequence a decanucleotide, and later Cerny et al. (Cerny et al., 1986) used collision induced dissociation (CID) to sequence dinucleotides and proposed a mechanism for base loss. The Cf-PD and FAB methods required rather significant sample quantities and were not effective for molecules over 4000 Da. With the advent of MALDI and ESI in the late 1980's, these ionization methods revolutionized the field of biological mass spectrometry because of their robust ability to transfer much larger molecules into the gas phase.

A. Maldi

Matrix assisted laser desorption ionization (MALDI), a method utilizing a pulsed laser to desorb molecule co-crystallized with a large excess of an absorbing matrix, was reported by Hillenkamp et al. in 1991 (Hettich and Buchanan, 1991; Hillenkamp et al., 1991). The successful analysis of nucleic acids by MALDI hinged on the discovery of appropriate matrices that promoted volatilization and ionization of oligonucleotides, typically different matrixes than optimally used for proteins. Some common matrices for nucleic acids include 3-hydroxypicolinic acid, 2,5-dihydroxybenzoic acid, α -cyano-4-hydroxy-cinnamic acid methyl ester (Nordhoff et al., 1994; Zhu et al., 1995). MALDI can be performed in either the positive or negative mode by varying the nature of the matrix (acidic or basic), but is typically performed in the positive mode to achieve greater signal intensity and fewer metal cation adducts (Sauer, 2007). Detecting oligonucleotides in the negative mode by MALDI is challenging. The poor ionization and desorption efficiency for oligonucleotides in the negative mode with both IR and UV laser ionization is hypothesized to be due to a lack of homogeneity of the analyte within the solid matrix and the instability of the N-glycosidic bonds between the sugar moiety and the nucleobase (Kristiansen et al, 1994). Negative mode MALDI analysis can result in unwanted fragmentation of longer sequences, the extent of which is highly dependent on base composition (Zhu et al., 1995).

There have been many studies to find new matrices better suited for nucleotide and nucleotide-adduct analysis. In 2003 Armstrong et al. demonstrated that using organic ionic salt solutions result in increased signal-to-noise for short (10–12 base pairs) synthetic DNA oligomers in the positive mode (Carda-Broch et al., 2003). The most successful matrices were 3-hydroxypicolinic acid mixed with ammonium citrate, now the gold standard matrix for nucleotides. Other organic salts with acid, alcohol, and aldehyde functional groups also increased signal-to-noise of the nucleotides.

Several studies have reported the use of MALDI-TOF-MS to study DNA oligomers and to verify drug-DNA crosslinks. For example, the detection of an intact mechlorethamine-crosslinked duplex reported by Rojsitthisak (Rojsitthisak et al., 2011). Mechlorethamine is a type of nitrogen mustard which has been used clinically for chemotherapeutic applications as it prevents DNA replication, thus affording its significant toxicity. Mechlorethamine crosslinks DNA preferentially at a mismatch C-C base pairs (Romero et al., 1999). Rojsitthisak et al. used negative mode MALDI to detect the molecular ion of a short synthetic duplex with a C-C mismatch base pair, as described in a later section (Rojsitthisak et al., 2011).

B. ESI

Electrospray ionization (ESI), an electrostatic nebulization process, was integrated with mass spectrometry in 1984 by Fenn et al. (Yamashita and Fenn, 1984) and has since been used in a variety of studies involving nucleic acids and anticancer drugs (Chu, 1994; Palom et al., 2002; Smith and Brodbelt, 2010; Wang and Wang, 2003; Wickham et al., 1995). Analysis of oligonucleotides is commonly performed in the negative mode due to the highly anionic/acidic character of the phosphate backbone. ESI was first demonstrated for the analysis of nucleic acids in 1991 (Stults et al., 1991), and it has become the most popular method for ionization of DNA, RNA, and their adducts.

The dominant charge state of the oligonucleotides observed upon ESI is dependent upon the length of the oligomer, the pH of the solution, and additives to the ESI solution. For example, typically 14mer single strand oligonucleotides are observed in the -3 and -4 charge states, whereas double strand 14mers are observed in the -5 through -8 charge states. Ammonium acetate is a common additive that favors lower charge states (Griffeycor et al., 1997), while piperidine favors highly charged oligonucleotides with a larger charge envelope (Greig and Griffey, 1995; Muddiman et al., 1996). Figure 4 shows a representative ESI mass spectrum of an 18mer DNA duplex, 5'-GCGTATAGTACTAGTGCG-3' (ssa) • 5'-CGCACTAGTACTATACGC-3' (ssb), that was acquired for a solution containing 3 μ M DNA in 50 mM ammonium acetate without the addition of any organic co-solvent such as methanol. The dominant charge states of the intact duplex are 6- and 7-, and low abundance single strand ions are observed in the 4- charge state.

Complex biological molecules, like covalently-linked double stranded DNA segments, are readily analyzed by ESI-MS. Drug/DNA binding affinity, sequence selectivity, stoichiometry, and binding site identification can be evaluated by ESI-MS, and the findings are in agreement with traditional methods of identification. An extensive overview of the use of mass spectrometry for characterization of unmodified nucleotides and nucleic acids has been reported (Banoub et al. 2005). Wickham et al. were the first to report the identification of a covalent crosslink between a duplex containing six base pairs and cisplatin via ESI-MS (Wickham et al., 1995). Two years later in 1997, Wickham et al. set the foundation for employing tandem MS with ESI for analysis of covalent DNA/drug adducts in a study that identified the site of alkylation of a nucleobase by hedamycin, an anticancer drug that both intercalates and forms covalent bonds with DNA (Iannitti et al., 1997).

V. Tandem Mass Spectrometry of Nucleic Acids

Tandem MS is a versatile tool for sequencing nucleic acids and for providing specific connectivity information about DNA adducts via diagnostic fragment ions. The most popular MS/MS method is collision induced dissociation (CID) in which activation occurs as the kinetic energy of the analyte ion is translated into an internal energy upon collision with a neutral target gas. McLuckey et al. (McLuckey et al., 1992) created a standard nomenclature for oligonucleotide fragment ions by analyzing multiply charged oligonucleotides by tandem MS. Diagnostic backbone fragment ions of oligonucleotides are produced upon cleavage of the phosphodiester bonds as shown in Figure 5. The most common oligonucleotide fragment ion types produced by CID are base loss ions (which are uninformative with respect to sequencing nucleic acids) as well as complementary $a - B$ and w ions which are generated after the initial base loss. Upon activation, the loss of a neutral nucleobase, preferentially adenine over guanine, is followed by cleavage of the phosphodiester backbone at the 3' C-O bond of the depurinated sugar, as shown in Figure 6.

Electron capture dissociation (ECD) is an alternative activation method that alleviates the uninformative losses of nucleotide bases (Zubarev et al., 1998). Originally developed for FTICR-MS, ECD relies on the capture of an electron by the nucleotide (which must be positively charged). ECD typically produces w^+/d^+ ions and radical a/z ions (Hakansson et al., 2003) but cleavage is biased by base content in terms of which nucleobase is carrying charge for efficient electron capture (Chan et al., 2009). Electron transfer dissociation (ETD) is an activation method that is analogous to ECD with the exception that the source of electrons is supplied by a radical anion reagent, typically fluoranthene (Syka et al., 2004). One predominant result of ETD of oligonucleotides is charge reduction, meaning that the oligonucleotide captures an electron but does not dissociate (McLuckey and Stephenson, 1998). ETD does not lead to significant production of base loss ions, and the main diagnostic sequence ions are w/d ions. However, when combined with subsequent CID or IRMPD to activate the intact charge-reduced precursors, a rich array of w , d , a and z fragment ions are observed and modified sites of DNA can be pinpointed (Smith and Brodbelt, 2009; Smith and Brodbelt, 2010).

Infrared multiphoton dissociation (IRMPD) entails the irradiation of ions with IR photons to cause vibrational excitation and dissociation (Little et al., 1994). The large photoabsorption cross-section of phosphate groups comprising the oligonucleotide backbone at laser wavelength 10.6 μm makes IRMPD a natural fit for characterization of nucleic acids (Wilson and Brodbelt, 2007). Upon IRMPD, the predominant fragment ions produced are $a - B$ and w ions as the efficient IR absorption by the initial base loss ions readily leads to secondary dissociation and conversion of the base loss ions into the more informative $a - B$ and w ions (Wilson and Brodbelt, 2007).

Ultraviolet photodissociation (UVPD) uses higher energy UV photons for excitation. McLafferty et al. first demonstrated UVPD of a 30mer poly-thymine oligonucleotide in an FTICR mass spectrometer by using a pulsed 193 nm excimer laser (Guan et al., 1996). Absorption of UV photons promoted electron photodetachment of multi-deprotonated nucleic acid anions and formation of w and a ions as opposed to the common w and $a - B$ ions observed upon CID. The intact charge-reduced precursors produced by UVPD may be subjected to CID in a process termed electron photodetachment (EPD), and it results in formation of w , d , $a^\bullet$, and $z^\bullet$ ions (Gabelica et al., 2006). UVPD creates a more diverse array of diagnostic fragment ions than any other activation method (Smith and Brodbelt, 2010).

Examples of the MS/MS spectra generated by four activation methods for a 12-mer oligonucleotide are shown in Figure 7. Figure 7 illustrates the fragmentation afforded by

CID, IRMPD, UVPD and EPD for the sequence 5'-GTATGACTCGCA-3' in the 4- charge state (Smith and Brodbelt, 2010).

VI. Characterization of DNA/Drug adducts by Tandem Mass Spectrometry

Prior to the development of ESI and its application for the analysis of DNA adducts, several covalent DNA/drug adducts were characterized by FAB-MS, typically in conjunction with structural characterization of the adducts by CID (Cerny et al., 1986; Cerny et al., 1987; Colvin et al., 1976; Cushnir et al., 1990; Cushnir et al., 1993; Grotjahn et al., 1982; Vu et al., 1981; Wei et al., 1999). These early reports demonstrated the feasibility of analyzing DNA adducts by tandem mass spectrometry and established the groundwork for the subsequent ESI-MS/MS studies over the past 15 years. The first study demonstrating the use of ESI and tandem mass spectrometry for structural elucidation of DNA/drug adducts was reported in 1997 (Ianitti et al., 1997). An antitumor antibiotic, hedamycin, that alkylates double-stranded DNA preferentially at guanine bases in 5'-CGT'3'- and 5'-CGG-3' sequences in the major groove was the focus of this study. The dominant fragmentation pathways upon CID entailed the loss of the hedamycin-alkylated guanine by N-glycosidic bond scission as well as cleavage of the 3' C-O bond of the depurinated nucleotide.

Figure 8 shows the CID spectrum obtained for the adduct formed between the hexamer, CACGTG, and hedamycin in the 2- charge state (Ianitti et al., 1997). The main fragment ions were readily assigned based on the cleavages shown in the inset of the Figure 8. This highly specific set of pathways demonstrated that dissociation was directed by the presence of the hedamycin adduction site. In contrast, comparable unmodified oligonucleotides yielded a far greater array of fragment ions arising from cleavages of other nucleobases and along the phosphate ester backbone. This first study established the utility of ESI-MS/MS strategies for characterization of covalent DNA-drug adducts, as described in the following sections for other DNA-interactive agents. As illustrated in the examples below, tandem mass spectrometry is most often used to elucidate the points of attachment of drugs to specific bases. In the majority of cases, the drug itself does not cleave or decompose, but instead remains attached to a specific base throughout the MS/MS process. The drug adduct does not generally cause significant changes in the types or distributions of product ions, although frequently cleavages close to the modified nucleotide are enhanced. For most of the examples described below, MS/MS was used to assess the sites of attachment linking the drug to the nucleic acid to support mechanistic or structural investigations. The DNA sequences were chosen strategically to include diversity (similar to human DNA), to create specific isolated reactive sites (such as a single thymine within a stretch of guanines), or also to ensure stable duplex formation via a balanced array of base-paired AT and GC nucleotides.

A. Mitomycin

The mitomycin family is a class of broad spectrum antibiotics as well as bioreductive alkylating agents (Tomasz and Palom, 1997). Mitomycin C (MC, see structure in Figure 3)) has anticancer properties by inducing cross-links in DNA (Palom et al., 2002; Paz et al., 2008). MC is a bifunctional alkylating agent that binds preferentially to the 2-amino group (N²) of guanines in CpG sequences via both intra- and inter-strand crosslinking (Tomasz et al., 1998), an outcome which ultimately interferes with DNA synthesis and thus endows MC with its considerable cytotoxicity. The pathway proposed for reaction of MC with DNA is shown in Figure 9. It is hypothesized that MC interacts with guanines in the minor groove of DNA prior to adduction to one guanine or two guanines at the 5'-CpG site in opposing strands. Reaction with CpG segments is preferred over NpG segments, where N can be any nucleotide (Kumar et al., 1997). MC requires reduction for activation, a process promoted

by a variety of cellular reductases that are favored under the often hypoxic environment of cancer cells.

Tandem mass spectrometry has been used to characterize the resulting MC-crosslinked and monoadduct products (Paz et al., 2008; Wang and Wang, 2003). Wang developed a strategy for elucidation of DNA/MC crosslinks based on nuclease P1 digestion of MC-crosslinked DNA and CID of the products (Wang and Wang, 2003). Duplexes consisting of self-complementary oligodeoxynucleotides were incubated with MC and a reducing agent (sodium hydrosulfite), and the resulting DNA/MC products were separated by HPLC prior to nuclease P1 digestion into crosslinked tetranucleotides (i.e. two d(pGpT) units with the guanines covalently linked through MC) (Wang and Wang, 2003). The resulting tetranucleotide/MC crosslinked species were subjected to CID after ESI in the negative mode. CID of the doubly deprotonated DNA/MC crosslink resulted in cleavage of the glycosidic bond of the modified deoxyguanosine, giving a pair of complementary product ions (one containing a single T nucleotide with an extra ribose-phosphate and the other containing three nucleotides corresponding to the G-MC-G-T product. Losses of water and HPO_3 were also predominant processes. MS/MS/MS experiments and high resolution mass assignments by FTICR-MS were used to support the proposed pathways, as well as confirming that the crosslink involved two guanines, not other nucleobases. This strategy was also successfully used to differentiate the two orientation isomers of the DNA/MC crosslinks. Figure 10 shows the fragmentation patterns that lead to the identification two MC-crosslinked tetranucleotide orientational isomers ($1''/10''$ versus $10''/1''$) generated upon interaction of MC with duplex d[TATATCGCTTTT]•d[AAAAGCGATATA] prior to nuclease P1 digestion. Cleavage of the guanine glycosidic bond was more facile at the $1''$ position than at the $10''$ position of MC.

Wang extended this work by utilizing CID to characterize intact MC-crosslinked duplexes by FT-ICR mass spectrometry (Wang et al., 2004). Each duplex was incubated with MC and then isolated by HPLC prior to direct infusion ESI in the negative mode. The MC-crosslinked duplexes yielded w_n and $a_n - B$ product ions for which the sites of MC-binding were revealed by mass shifts of specific product ions in comparison to the product ions of unmodified oligonucleotides. The crosslinked duplexes also underwent characteristic glycosidic bond cleavage of the MC-modified 2'-deoxyguanosine to yield diagnostic $[ss - G]$ and $[ss + MC + G]$ products.

Paz et al. generated a number of DNA/MC products formed upon incubation of several types of cell lines, include mouse tumor cells, human fibroblasts, and human breast cancer cells with MC in vitro under hypoxic conditions (Paz et al., 2008). After isolation and digestion of the nuclear DNA to the nucleoside level using nuclease P1, phosphodiesterase, and phosphatase, the modified nucleosides were isolated from unmodified nucleosides using a C18 cartridge (Paz et al., 2008). Authentic standards were prepared by incubation of 14-mer duplexes with MC, digestion, and separation of the products by HPLC. The various DNA adducts and crosslinks were subsequently characterized by CID upon ESI in the positive mode. Figure 11 shows the CID mass spectrum of one presumed interstrand crosslinked species. The primary fragments arose from the loss of each deoxyribose moiety, and this type of pathway was consistent with the dissociation of covalently bound nucleoside/MC products rather than weakly bound non-covalent complexes.

B. Mustards

Mustards have a long history of use as anticancer agents (Colvin et al., 1976; Kumar et al., 1997; Randerath et al., 1981; Wang et al., 2004). Mustards are known to alkylate guanine bases at the N7 position and less commonly at cytosines. Nitrogen mustards react with DNA

in the form of a positive aziridinium ion upon spontaneous decomposition and loss of chloride (Kohn et al., 1987).

The interactions of a series of sulfur-containing acridine mustards with DNA were evaluated by ESI-MS/MS in a study reported by Smith et al. (Smith et al., 2009). The relative DNA binding affinities and sequence selectivities were assessed for an array of acridine mustards interacting with a series of duplexes. Duplexes composed of 14-mer oligodeoxynucleotides were incubated with each mustard derivative, then analyzed by ESI-MS/MS in the negative mode using both CID and infrared multiphoton dissociation (IRMPD). IRMPD was particularly useful because it promoted strand separation with retention of the mustard on one strand along with inclusion of a guanine base from the other strand, thus indicative of covalent binding, as well as yielding diagnostic sequence ions from each strand at higher photoirradiation times (Figure 12). In the latter case, the longer photoactivation period resulted in secondary dissociation of the individual oligonucleotide strands, producing the conventional $a - B$ and w ions that allow each strand to be sequenced as shown in Figure 12. Moreover, some of the $a - B$ and w ions retained the mustard moiety as evidenced by the detection of product ions that were mass shifted relative to the expected $a - B$ and w ions for the unmodified oligonucleotides. The IRMPD mass spectra were thus used to pinpoint the sites of alkylation at specific guanines. Figure 13 summarizes the sequence coverage obtained for each duplex by IRMPD, and the site of acridine sulfur mustard adduction is highlighted in bold font.

A recent study by Rojsitthisak et al. illustrated the use of MS/MS methods to identify the connectivities of mismatched cytosine bases crosslinked by the nitrogen mustard mechlorethamine (Rojsitthisak et al., 2011). Mismatched base pairs may arise from DNA damage or mutation, and self-regulating enzymes may not recognize the mismatch, thus preventing repair of the mismatches. The ability of mechlorethamine to form an interstrand crosslink with a duplex comprised of d[CTCACACCGTGGTTC] and its complementary strand was elucidated based on MALDI/MS and ESI/MS/MS (Rojsitthisak et al., 2011). Mechlorethamine was incubated with the duplex, and the resulting crosslinked duplexes were purified by HPLC. The intact crosslinked product was detected by MALDI, and then the product was enzymatically digested to the nucleoside level using phosphatase and phosphodiesterase prior to ESI-MS/MS in the positive mode. The hydrolytic crosslinked product, N3dC-mechlorethamine-N3dC, dissociated by elimination of deoxycytidine in conjunction with the loss of ammonia or both ammonia and deoxyribose, as shown in Figure 14, thus affording a diagnostic fragmentation pattern.

C. Cisplatin

Cisplatin, cis-diamminedichloroplatinum (II), is a powerful anticancer therapeutic currently used to treat many forms of cancer including esophageal, bladder, testicular, head and neck, and small cell lung cancer (see structure in Figure 3) (Reedijk and Lohman, 1987; Reedijk, 1987; Rosenberg, 1967). Rosenberg et al. is credited with revisiting the anticancer potential of square planar platinum drugs by investigating the effect of charge state and isomerization upon *E. coli* growth and finding the platinum (II) moiety with a net 2- charge completely inhibited bacterial growth, and the cis-isomer was the most highly cytotoxic (Rosenberg et al., 1967). Cisplatin preferentially binds to N7 of guanine and to a lesser extent N7 of adenine and N3 of cytosine (Wing et al., 1984). The cytotoxic properties of cisplatin are due to the intrastrand crosslink formed between adjacent guanines (5'-GG-3'), resulting in fragmentation of cellular DNA into large segments and the onset of apoptosis (Chu, 1994).

Early mass spectrometry studies of cisplatin/DNA adducts reported that loss of ammonia from cisplatin was a dominant fragmentation pathway as well as fragments containing platinum and one or both guanine residues (Martin et al., 1991). The fragmentation patterns

of cisplatin/1,2-intra-strand DNA crosslinks were investigated by ESI-CID-MS in a more elaborate recent study by Nyakas et al. (Nyakas et al., 2009). 6-mer oligodeoxynucleotides were reacted with cisplatin in a 1.2:1 molar ratio at 37°C for 24 hours prior to their analysis using a time-of-flight mass spectrometer equipped with nanoelectrospray ionization. Platinated fragments were produced upon CID, in agreement with earlier studies, and an increase in the abundance of *w* ions was noted, indicating a preference for cleavage of the 3'-OH phosphodiester bond next to the intrastrand crosslink as shown in Figure 15.

The CID spectrum shown in Figure 15 displays the platinated *a* ions and an increased abundance of complementary *w* ions, features that were also noted for the other 6-mer oligonucleotides in the study. Moreover, the cisplatin adduct exhibited a lower extent of fragmentation and therefore was hypothesized to reflect a more stabilized form of DNA. Platinated guanine fragments were detected in low abundance in the CID spectra, providing evidence of cleavage of the N-glycosidic bond which is typical upon CID activation of oligonucleotides. Figure 16 shows one of the fragmentation pathways elucidated by Nyakas et al. (Nyakas et al., 2009).

Extending this work, Stucki et al. recently investigated the fragmentation pathways of quadruplex DNA-containing cisplatin adducts upon CID (Stucki et al., 2011). Quadruplex DNA is composed of square planar G-tetrads stabilized by interactions with embedded cations. This type of DNA is found at the end of human telomeres and serves as an important regulatory recognition factor for telomerase, an enzyme that is over-expressed in cancer cells and causes increased cell growth (Schaffitzel et al., 2001). It is known that cisplatin binds to the loops of the quadruplex structure as opposed to the square planar G-tetrad (Redon et al., 2003). The quadruplexes were platinated by reacting annealed quadruplexes with cisplatin in 1:1 molar ratio and incubating at 37°C for 24 hours prior to analysis by ESI-CID-MS (Stucki et al., 2011). The dissociation behavior was found to vary based on the type of quadruplex: tetramolecular (four individual DNA strands comprising G-tetrads), bimolecular (two strands folded and Hoogsteen base-pair stabilized to form G-tetrads) or monomolecular (one looped strand stabilized by Hoogsteen base-pairing with itself to form G-tetrads). Dissociation of singly-platinated tetramolecular quadruplexes resulted in guanine base loss and loss of an unplatinated single strand. Bimolecular monoplaminated quadruplexes dissociated primarily via strand separation (Stucki, 2011). Monomolecular quadruplexes reacted to form singly or doubly platinated adducts and dissociation led to platinated *a-B* and *w* ions. Figure 17 shows the CID mass spectrum of the monomolecular quadruplex with one cisplatin adduct, and the inset shows the quadruplex/cisplatin molecular ion region of the ESI-MS spectrum (Stucki et al., 2011).

Smith et al. reported the use of hybrid activation methods to increasing the diversity of fragment ions produced from modified nucleic acids, as demonstrated for ODN TTCCGGTCCT (Smith and Brodbelt, 2010). Hybrid activation methods exploited electron transfer reactions to generate charge-reduced odd electron DNA ions prior to activation using CID, IRMPD, or UVPD. These hybrid MS/MS methods led to broader distributions of diagnostic product ions, an outcome that proved beneficial for pinpointing modification sites of DNA. A comparison of the IRMPD mass spectrum and the ET-IRMPD mass spectrum of the TTCCGGTCCT/cisplatin adduct in the 3+ charge state is shown in Figure 18. The IRMPD spectra displayed a few characteristic *a-B* and *w* ions, with the *w*₆, *a*_{8-B}, *a*_{9-B}, and *w*₉ ions all retaining the platination modification. ET-IRMPD led to new products, including *a*, *d*, *z*, and *w* ions, that more specifically mapped the adduction site and confirmed that radical precursors may follow unique dissociation pathways not observed for the more conventional closed-shelled ions (Smith and Brodbelt, 2010).

In an FT-ICR-MS study, Egger et al. investigated the binding mode of cisplatin and the kinetics of its reaction with two 13-mer duplex sequences ds(GTATTGGCACGTA) and ds(GTACCGGTGTGTA), utilizing IRMPD and CID for structural characterization of the resulting products (Egger et al., 2008). The duplexes were chosen to allow elucidation of the crosslinking preference of cisplatin such that the first duplex contained one favored platination site (GG in single strand S1) and the complementary strand contained a less favored binding site (GTG in single strand S1c), whereas both single strands of the second duplex contained GG sites (S2 and S2c). The binding kinetics were measured over a 50 hour period of cisplatin incubation with DNA in a 3:1 molar ratio, and the platinated products were monitored as single strands in the negative mode. It was found that the monoadduct was formed with S1 after 1 hour of incubation and with S1c after 3 hours of incubation. The deconvolved mass spectra of the adduct formation in the kinetic study is shown in Figure 19. The results of the kinetic study unambiguously demonstrated the formation of the monoadduct prior to crosslinking. Unreacted S1c ions were observed at 60% relative abundance throughout the duration of the reaction monitoring, indicating that the 5'-GTG was a less significant binding site than the known 5'-TGGC binding site in S1. The relative abundance of the latter diminished to 3% over the course of the experiment. The CID and IRMPD spectra acquired for both duplexes resulted in similar fragmentation patterns but with ions of different abundances. Platinated fragment ions included w_{10} , a_6 -G, single strand, and single strand with the loss of adenine (Egger et al., 2008).

D. Psoralens

Psoralens are naturally bifunctional crosslinking furocoumarins that have been used for thousands of years for the treatment of skin diseases and are currently used for treatment of psoriasis, vitiligo and cutaneous T-cell lymphoma (Parrish et al., 1974; Scott et al., 1976; Wolf et al., 2006). With respect to their interactions with nucleic acids, psoralens first intercalate with DNA, then react with the 5,6 bond of a pyrimidine base, preferentially thymine, upon UV photoactivation. The 3,4 bond of the pyrone group of the psoralen undergoes cycloaddition with thymine, and crosslinks are formed upon absorption of a second photon to promote reaction of the 4',5' double bond of the furan of psoralen with a second thymine (Lown and Sim, 1978; Oroskar et al., 1994; Straub et al., 1981). Representative psoralen structures are shown in Figure 20.

Wang et al. studied the fragmentation patterns of 4,5',8-trimethylpsoralen (TMP)-crosslinked DNA duplexes by ESI-CID-MS (Wang et al., 2004). Self-complementary duplexes dCGCGCTAGCGCG with one psoralen binding site, 5'-TA-3', were annealed and reacted in 100 μ L of EDTA, NaCl and Tris buffer with 1 μ L of saturated TMP in ethanol. The reaction mixture was irradiated at 365 nm for 40 minutes on ice and separated by preparative HPLC. Upon CID, some of the backbone fragments were mass-shifted, indicating incorporation of the psoralen moiety. Figure 21 shows the CID mass spectrum obtained for this duplex crosslinked by TMP, where the intact crosslinked product is denoted by XL in the labeled product ions. The predominant backbone fragments were $a - B$ and w ions, typical of CID fragmentation of DNA, for which ions w_7 , w_9 , w_{10} , w_{11} , and $[a_7 - A]$, $[a_8 - G]$, $[a_9 - C]$, and $[a_{10} - G]$ contained TMP plus the opposing thymine base, indicative of an interstrand crosslink.

Smith and Brodbelt further explored the characterization of DNA crosslinked with different psoralen analogs by ESI-MS/MS using both CID and IRMPD (Smith et al., 2010). Duplex DNA was incubated with each psoralen in the dark for one hour followed by UV photoirradiation at 365 nm for 30 min. The binding stoichiometries of the psoralens (see Figure 19), including 5-methoxypsoralen (5-MOP), 8-methoxypsoralen (8-MOP), 4,5',8-trimethylpsoralen (TMP), and psoralen (P), with a variety of duplexes were 1:1, but 4'-aminomethyl-4,5',8-trimethylpsoralen (AMP) yielded products containing up to five

molecules of AMP per duplex, indicative of non-covalent complexation. Four psoralens, P, 8-MOP, 5-MOP and TMP, demonstrated preferred binding to 5'-TA sites over 5'-AT sites. The resulting psoralen adducts were characterized by CID and IRMPD. At shorter activation times, the IRMPD spectra resembled the conventional CID spectra with the losses of neutral nucleobases observed as the dominant fragmentation pathways. However, applying longer IRMPD activation times resulted in secondary dissociation of the base loss products, thus converting them to more diagnostic sequence ions. Figure 22 demonstrates the fragmentation of the 8-MOP/DNA crosslinked species via IRMPD at 9 ms irradiation time. Evidence of crosslink formation is seen by the retention of the 8-MOP moiety on the characteristic $a - B$ and w fragment ions, as well as ions containing an intact single strand, 8-MOP and a segment from the opposing strand. The specific sites of psoralen adduction were identified based on detection of complementary ($ss + psoralen + w$) and ($ss + psoralen + a - B$) ions arising from specific thymine binding sites (Smith et al, 2010).

E. Bioreductive Prodrugs

Many anticancer therapeutics exhibit a high degree of cytotoxicity to non-cancerous proliferating cells as a result of non-selective interactions with cellular DNA (Singh et al., 2008). This promiscuous reaction with DNA accounts for many of the numerous deleterious side-effects of chemotherapy and has accelerated the search for drugs that are more selective for cancer cells. Prodrugs, first introduced by Albert (Albert, 1958) and Harper (Harper, 1962), are drugs that are chemically altered to enter cells in an inert, nontoxic form and only via enzymatic interactions or through extra-cellular conditions become activated into the toxic, therapeutic form of the drug, thereby increasing the specificity for cancer cells. Cancerous cells typically have a hypoxic, reducing environment, a factor that has promoted the development of bioreductive prodrugs, specifically drugs that are reduced in a low-oxygen environment and are unreactive in healthy, well oxygenated cells (McKeown et al., 2007). There are several class of prodrugs that have been developed, including those based on quinones, nitroaromatics, and hetero- and aromatic-N-oxides (McKeown et al., 2007).

One class of quinones currently used in clinical studies is aziridinylbenzoquinones, compounds that achieve cellular toxicity by formation of DNA interstrand crosslinks that prevent strand separation during DNA replication (Winski et al., 1998). The quinone moiety is reduced in the hypoxic environment of cancer cells into the hydroquinone form, which leads to an increased basicity and thereby protonation of the aziridine rings. The opening of the aziridine rings is a two electron process that results in monoadduction to DNA first, followed by interstrand crosslinking. A likely pathway of DNA adduct formation is shown in Figure 23.

A recent study by Pierce et al. investigated DNA adduct formation of 2,5-diaziridinyl-3-[hydroxymethyl]-6-methyl-1,4-benzoquinone (RH1) (Pierce et al., 2010). Upon reduction of the aziridine ring of RH1, the opened ring forms a covalent bond at N7 guanine, preferentially at 5'-GNC or 5'-GNNC sites, where N can be any nucleobase. Eight duplexes were investigated in the study by Pierce et al. to evaluate sequence selectivity and binding affinity, as well to compare the reactivity of RH1 relative to a phenyl-substituted analog, 2,5-diaziridinyl-3-[hydroxymethyl]-6-phenyl-1,4-benzoquinone (PhRH1) (Pierce et al., 2010). The crosslinking reaction with each duplex was initiated by incubation of duplex DNA with each of the two RH1 analogs at a 50:1 concentration ratio in 100 mM TRIS buffer under argon, then reduction by addition of sodium hydrosulfite solution at pH 6 over a 50 minute period. The reaction was quenched by exposure to air, and the products subsequently analyzed by HPLC-MS/MS. The abundances of crosslinks formed by PhRH1 were lower than those observed for RH1, as expected due to the steric hindrance of the phenyl group and possible increased electronic stability afforded by the conjugated ring, thereby decreasing the effective reduction of the drug moiety.

Analysis of the products by CID and IRMPD allowed identification of the crosslinking site, as shown in Figure 24. CID of the RH1/duplex crosslink seen in Figure 24a resulted in the formation of backbone fragment ions a_6^- and w_8^- from each single strand, consistent with preferential cleavage at the crosslink location (Pierce et al., 2010). Strand scission that led to products containing RH1 and the crosslinked guanine from the opposing strand occurred for both single strands of the duplex which indicated that RH1 remained equally associated with either single strand upon strand separation. The CID mass spectrum also showed uninformative nucleobase loss (guanine) for each single strand. The primary fragments seen in Figure 24b by IRMPD also arise from cleavage at the site of crosslink formation. The spectra produced for crosslinked duplexes were dependent on the IR irradiation time used for ion activation. Very short photoirradiation times resulted primarily in the loss of water, slightly longer (3 ms) irradiation times resulted in CID-like spectra for which fragmentation was primarily due to strand separation and retention of the RH1 moiety, and longer irradiation times (5 ms) resulted in secondary fragmentation of the single strands upon separation and formation of backbone ions at the site of crosslinking. The production of ions a_6^- and w_8^{2-} without observation of other backbone fragment ions for either activation method demonstrated the preferential cleavage at the site of crosslink.

F. Eneidyne

Eneidyne exhibit significant cytotoxicity upon interaction with DNA, resulting in scission of the double helix (Zhang et al., 2011). These drugs include the natural product calicheamicins, neocarzinostats, and dynamicins isolated from bacteria (Nicolaou et al., 1993). Cytotoxicity is achieved by Bergman cycloaromatization of the base unit (*Z*)-1,5-diyne-3-ene in the minor groove of DNA, which after a series of reactions leads to a 1,4-benzoid diradical that easily strips hydrogen atoms off the adjacent sugar phosphate backbones of helical DNA, resulting in scission of the double helix (Smith and Nicolaou, 1996). Breakage of cellular DNA affords extremely high cytotoxicity as anticancer therapeutics, but due to the nonselective nature of DNA interaction with eneidyne these drugs have not become a prominent clinical therapy (Kumar et al., 2001; Lawrence, 1996; Nicolaou et al., 1993). The mechanism of enediyne diradical formation is shown in Figure 25.

Sherman et al. reported adduct formation of 4-aza-3-ene-1,6-diyne (Ene-1, **1**, see Figure 26) with single strand oligonucleotides (Sherman et al., 2006), the first study to use ESI-MS to identify adducts formed by eneidyne. Ene-1 preferentially binds to and subsequently cleaves at 2'-deoxycytosine residues (Tuntiwechapikul et al., 2002), and thus duplexes of dC₈ and thymine 6-mers containing a single cytosine nucleobase were selected for reactions with Ene-1 by incubation at 37°C for 24 hours, prior to desalting by C₁₈ cartridges and ESI-MS/MS analysis (Sherman et al., 2006). Two sequences, 5'-CT₅ and 5'-T₂CT₃, were investigated to determine the binding selectivity of Ene-1. The mass spectrum of 5'-CT₅ after incubation at pH 6 with Ene-1 showed evidence of intact 5'-CT₅/Ene-1 products as well as dT₅ alone (with C cleaved) and dT₅ with one or two Ene-1 attached. The C-cleavage occurred 3'-OH of the deoxycytosine in the 5'-CT₅ sequence with high selectivity, supporting the purported cleavage pathway of enediyne strand scission. A representative CID spectrum of the Ene-1 adduct (dCT₅ - 4-H + **1**)³⁻ is shown in Figure 26.

The CID mass spectrum in Figure 26 shows several different $w_n + \mathbf{1}$ ions indicating that although only one molecule of Ene-1 was bound to dCT₅, the precursor ion was likely a mixture of species with Ene-1 attached at different thymine locations. Ene-1 was expected to bind preferentially to cytosine but apparently also interacted with thymine as shown by the MS/MS data. Based on the lack of complementary $a - B$ ions, it was suggested that the positive charge of Ene-1 remained localized on the cytosine in dCT₅. There were some

unidentified peaks in the MS/MS spectra which were attributed to dissociation of the Ene-1 moiety upon activation (Sherman et al., 2006).

To further decipher the interactions between nucleic acids and Ene-1, additional experiments were undertaken using the smaller model dinucleotide dCC (Sherman et al., 2006). After incubation of dCC with Ene-1 at pH 6, products corresponding to $(\text{dCC} + 1)^-$ and $(\text{dCC} + 2 \cdot 1)^-$ were observed. A possible structure for the $(\text{dCC} + 1)^-$ adduct based upon the resulting CID data is given in Figure 27, and the proposed cleavages and m/z values of the product ions are consistent with covalent binding of the ene-diyne to the cytosine nucleobase via nucleophilic attack of the phenylethynyl group of Ene-1 by the cytosine amine.

VII. Conclusions

The ability to characterize DNA/drug adducts is critical for understanding how drugs react with DNA and for aiding the design of more specific chemotherapy agents. Tandem mass spectrometry has been a key analytical tool for mapping DNA adduction sites with high sensitivity. The small sample volume and high throughput capabilities of mass spectrometry are ideal for extension to studies of cellular extracts, especially with the development of increasingly sensitive chromatographic methods and the high resolution/high mass accuracy capabilities of Q-TOF, FTICR, and Orbitrap instruments which facilitate the assignment of molecular compositions of ions. Moreover, alternative activation methods, such as photodissociation and electron-based methods, can provide more detailed fragment ions maps that allow the sites of drug adduction to be pinpointed with confidence. It is anticipated that the growing availability of these high performance mass spectrometric tools should accelerate the applications to DNA adducts across many disciplines and allow even more challenging structural, mechanistic, and functional questions related to the interaction of anti-cancer drugs to nucleic acids to be addressed.

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Abbreviations

CID	collision induced dissociation
ECD	electron capture dissociation
EDD	electron detachment dissociation
ETD	electron transfer dissociation
IRMPD	infrared multiphoton dissociation
ODN	Oligodeoxynucleotide
UVPD	ultraviolet photodissociation

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Figure 1.
Monoadduct, inter- and intra-strand crosslinks in DNA

Figure 2. Structures of guanine, adenine, cytosine, and thymine monophosphate are shown with numeric atom labels.

Mitomycin C, 334.1 Da

Psoralen, 186.2 Da

Acridine mustard, 361.1 Da

4-aza-3-ene-1,6-diyne, 489.1 Da

Hedamycin, 748.4 Da

RH1, 234.1 Da

Cisplatin, 298.0 Da

Tamoxifen, 371.5 Da

Figure 3.
Structures of various anticancer drugs with molecular weight (Da).

Figure 4.
ESI mass spectrum of 18mer duplex (DS), 5'-GCGTATAGTACTAGTGCG-3' (ssa) • 5'-CGCACTAGTACTATACGC-3' (ssb).

Figure 5.
Fragment ion nomenclature for DNA

Figure 6.
Mechanism of formation of $a-B$ and complementary w -ions.

Figure 7. Mass spectra of fragmentation patterns of 5'-GTATGACTCGCA-3' by different dissociation methods. Reprinted (adapted) with permission from Smith and Brodbelt, 2010. Copyright (2010) American Chemical Society.

Figure 8. CID mass spectrum of CACGTG/ hedamycin adduct (2-). Reprinted (adapted) with permission from Iannitti et al. 1997. Copyright (1997) American Chemical Society.

Figure 9. Interaction of mitomycin C with DNA. Reprinted (adapted) with permission from Kumar et al. 1997. Copyright (1997) American Chemical Society.

Figure 10.

CID mass spectrum of MC-crosslinked tetranucleotide fragments from non-self-complementary duplex d[TATATCGCTTTT]•d[AAAAGCGATATA] showing two orientations of MC-adduct isomers. Reprinted (adapted) with permission from Wang and Wang 2003. Copyright (2003) American Chemical Society.

Figure 11.

CID mass spectrum of the interstrand crosslink of mitomycin C with 5'-ATATACGCGTATAT-3' in positive mode, showing loss of one and two deoxyribose units respectively. Reprinted (adapted) with permissions from Paz et al. 2008. Copyright (2008) American Chemical Society.

Figure 12.

IRMPD mass spectrum of acridine sulfur mustard interstrand crosslink with duplex 5'-GGCGTCGGCGTCGC-3'•5'-CGACGCCGACGCC-3'. Reprinted (adapted) from Smith et al, 2009. Copyright (2009) Royal Society of Chemistry.

Duplex 1

Duplex 2

Duplex 3

Duplex 4

Figure 13. IRMPD-MS/MS fragmentation patterns of four unique DNA duplexes with acridine sulfur mustard AS4 (structure shown in Figure 12). Bold font is likely site of adduction per MS/MS data. Reprinted (adapted) from Smith et al, 2009. Copyright (2009) Royal Society of Chemistry.

Figure 14.

A) CID mass spectrum of mechlorethamine crosslink with duplex d[CTCACACCGTGGTTC]•d[GAACCACCGTGTGAG] with a C-C mismatch adduct site and B) proposed product ion structures. Reprinted with permissions from Rojsitthisak et al. 2011. Copyright (2011) *PLoS ONE*.

Figure 15.

CID spectra of single strand DNA 6mer and its cisplatin product. The 6mer yields a dominant w_1^- ion, and the platinated 6mer spectrum shows an enhanced w_2^- ion consistent with cleavage next to the intrastrand crosslink. Reproduced with permission from Nyakas et al, 2009. Copyright (2009) Elsevier.

Mechanism 1

Figure 16. Fragmentation mechanism of cisplatin DNA Reproduced with permission from Nyakas et al., *JASMS* 2009, 20, 792–804. Copyright (2009) Elsevier.

Figure 17. CID mass spectrum of platinated monomolecular quadruplex [d(TGGGGGGT)]₄ showing platinated fragments of *a*-B and *w* ions. The inset shows the ESI-MS spectrum. Reprinted (adapted) from Stucki et al. 2011. Copyright (2011) Elsevier.

Figure 18. MS/MS spectra of TTCCGGTCCT + cis-platin adduct by IRMPD of 3+ (4 ms, 10 W), and ET-IRMPD 3+• (ET 50 ms, IRMPD 4 ms, ~10 W). Precursor ions are noted with a star. Bolded, italicized product ions retain the modification. On the right, a summary of the product ions formed from IRMPD and ET-IRMPD for the cis-platin modified single strand 10-mer is given, in which the slash marks represent cleavages at that location. Reprinted from Smith and Brodbelt, 2011. Copyright (2011) American Chemical Society.

Figure 19.

Mass deconvolution of cisplatin crosslinking to S1: dGTATTGGCAGTA and S_C1: dTACGTGCCAATAC at a 3:1 molar ratio and incubation for 50 hours. Reprinted (adapted) from Egger et al. 2008. Copyright (2008) American Chemical Society

Figure 20.
Psoralen structures and molecular weights (Da)

Figure 21.

CID mass spectrum of $d[CGCGCTAGCGCG]_2$ crosslinked by TMP, the crosslink is denoted by XL. Reproduced with permissions from Wang and Wang, 2004. Copyright (2004) Elsevier.

Figure 22.

IRMPD spectrum of the 8-MOP/DNA crosslink (6-), precursor denoted by star, where subscript ss_C denotes C-rich strand and ss_G denotes G-rich strand of duplex, D1, 5'-GCGGGGTAGGGGCG (ss_G) • 5'-CGCCCCCTACCCCGC (ss_C) and scheme of fragmentation with 8-MOP and D1. Reprinted (adapted) with permissions from Smith and Brodbelt 2010. Copyright (2010) Royal Society of Chemistry.

Figure 23. Mechanism of reduction of RH1/DNA adduct formation. Reprinted (adapted) with permission from Hargreaves and Hartley, 2000. Copyright (2000) Frontier Biosciences.

Figure 24.

MS/MS spectra of RH1/DNA adduct comprised of the duplex 5'-AAATTGAACAAATT-3' (ssa) and complementary strand 3'-TTTAACTTCTTTAA-5' (ssb) 5'-GAAC-3'. (a) CID spectrum of RH1/DNA adduct 6- precursor, and (b) IRMPD at 5 ms irradiation time of RH1/DNA adduct 6- precursor, bold face italics are fragments from ssa. Reprinted from Pierce et al. 2010. Copyright (2010) American Chemical Society.

Figure 25.

Route of (Z)-1,5-diyne-3-ene diradical formation Reprinted from Smith et al. 1996.

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Figure 26.

Representative CID spectrum of Ene-1/ dCT_5 adduct, precursor ion $(dCT_5 - 4 \cdot H + 1)^{3-}$ of 697 m/z. Reprinted (adapted) with permissions from Sherman et al., 2006. Copyright (2006) Springer.

Figure 27.
Proposed structure and fragmentation of $(dCC + 1)^-$ (m/z 747) upon CID activation.
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