Ruthenium versus Platinum: Interactions of Anticancer Metallodrugs with Duplex Oligonucleotides Characterised by ESI-MS

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Supporting Information

MALDI-MS of double stranded oligonucleotides

The use of MALDI-MS was evaluated for the analysis of duplex DNA using different matrixes. As can be seen in Figures S1 and S2, no peaks corresponding to intact duplex DNA were detected in either positive or negative ion mode as dissociation into the corresponding single strands takes place, combined with neutral base loss (depending on the matrix used). Major peaks are labelled in the spectra.

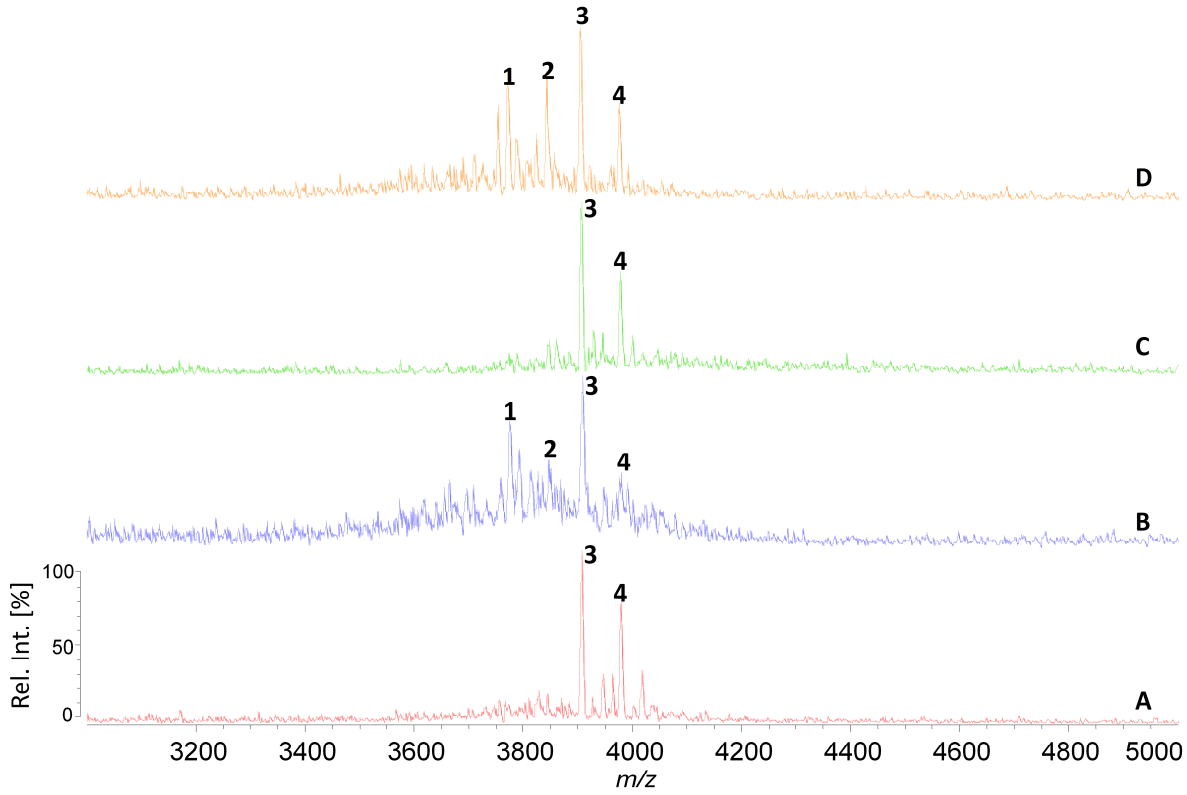

Figure S1. MALDI-MS of DS1 in positive ion mode using different matrices. A: nicotinic acid combined with anthranilic acid. B: 6-aza-thiothymine (ATT). C: combination of 3-hydroxypicolinic acid (HPA) with picolinic acid. D: trihydroxyacetophenone (THAP). Peak identification: **1** $[SS1b\text{-}T]^+$, **2** $[SS1a\text{-}A]^+$, **3** $[SS1b]^+$, **4** $[SS1a]^+$. Additional minor peaks originate from adduct formation with matrix components, and sodium and potassium ions.

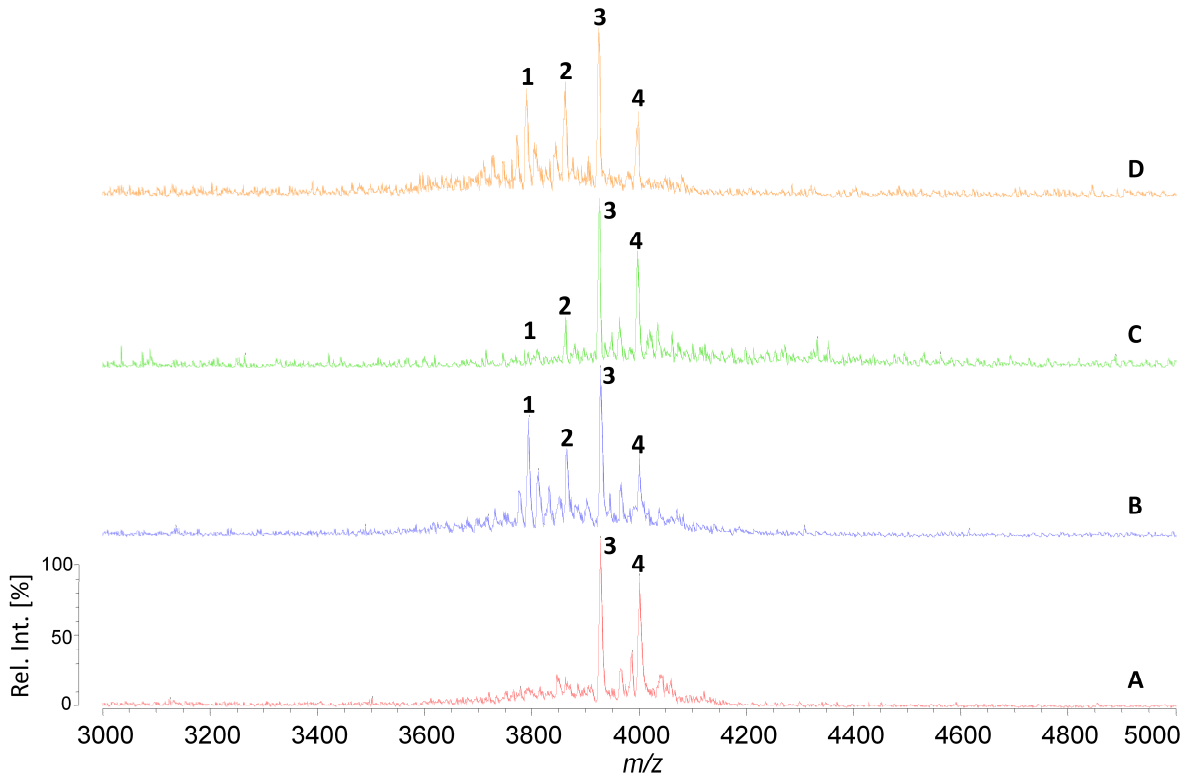

Figure S2. MALDI-MS of DS1 in negative ion mode using different matrices. A: nicotinic acid combined with anthranilic acid. B: 6-aza-thiothymine (ATT). C: combination of 3-hydroxypicolinic acid (HPA) with picolinic acid. D: trihydroxyacetophenone (THAP). **1** [SS1b-T]$^-$, **2** [SS1a-A]$^-$, **3** [SS1b]$^-$, **4** [SS1a]$^-$. Additional minor peaks stem from adduct formation with matrix components, and sodium and potassium ions.

Optimisation of ESI-MS conditions for the analysis of double stranded oligonucleotides

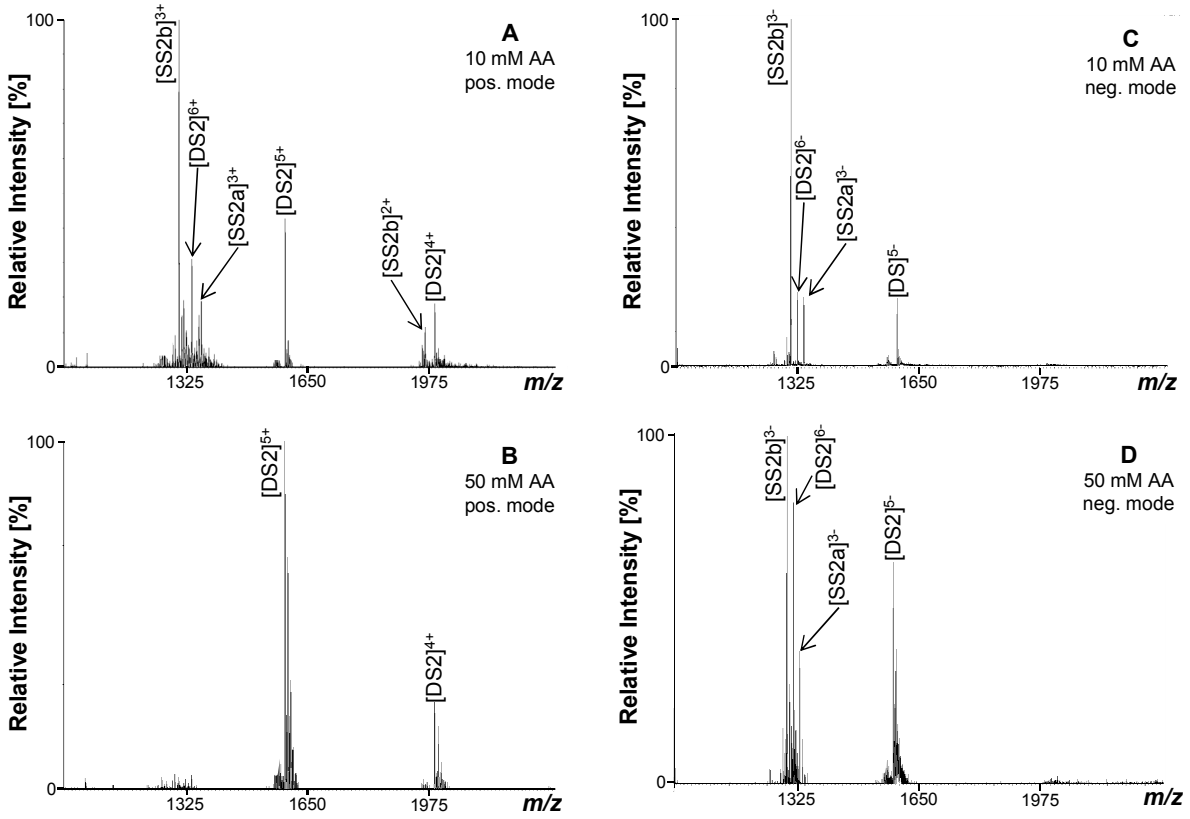

Figure S3. Comparison of different ionisation modes for the analysis of intact duplex DNA (DS2) showing stabilisation of the double strand in positive ion mode at an ammonium acetate (AA) concentration of 50 mM. Concentration of methanol was fixed at 50% for all experiments. Segments show full scan ESI mass spectra in the range from 1000-2300 *m/z*.

Positive Ion Mode ESI-MS for the Characterisation of Adducts of Duplex-DNA with Ruthenium Drugs

Figure S4 shows a positive ion mode spectrum of DS3 incubated with RAPTA-T. Adduct formation with the metallodrug leads to the dissociation of the duplex into the corresponding single strands, probably due to severe distortion of the secondary structure resulting from multidentate binding of ruthenium (note that after a prolonged incubation period, the majority of the adducts are formed with the Ru(PTA) moiety). A similar behavior is observable for the other ruthenium-based compounds. This is in contrast to the platinum-based metallodrugs, where no such dissociation of the duplex occurs.

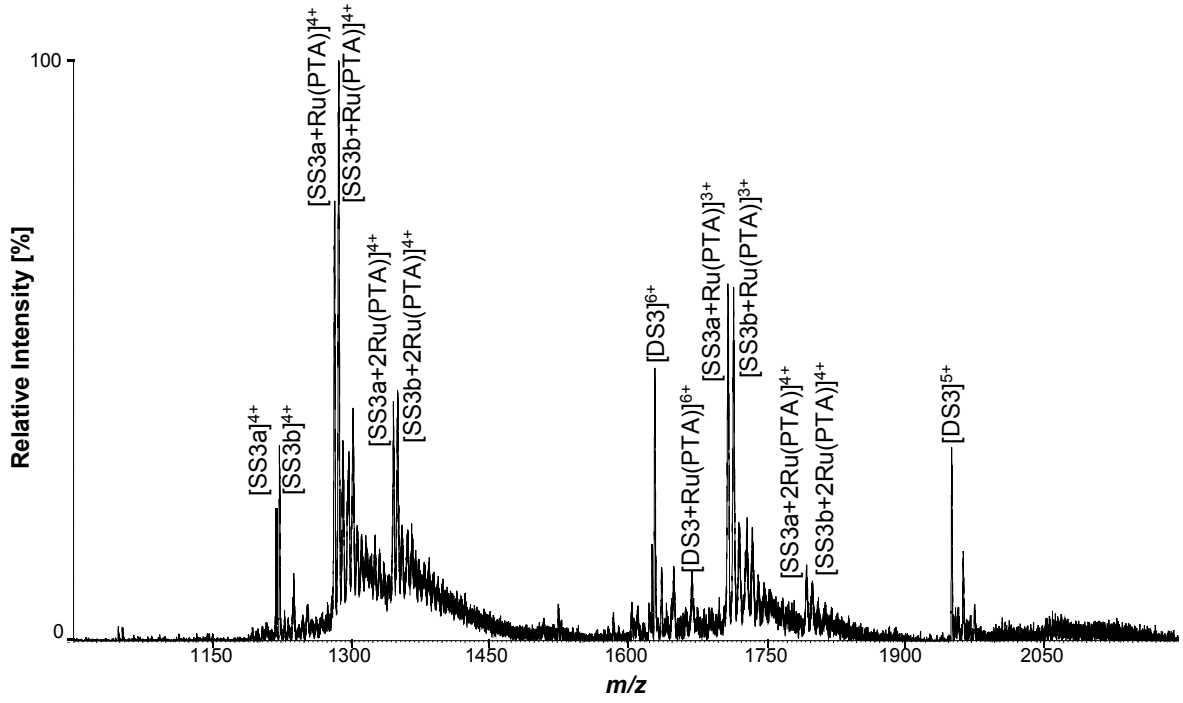

Figure S4. DS3 incubated with RAPTA-T at a drug:duplex ratio of 5:1 for 5 days showing the dissociation of the duplex in its corresponding single strands upon adduct formation with the metallodrug.

Elucidation of the metal compound binding sites on oligonucleotides using CID

For clarity, only fragments with high abundance and those essential for the identification of binding site of the metal are labeled in the following figures S5-S7.

In Figure S5, a segment of the CID spectrum obtained for the precursor ion $[SS1b+Ru(Im)]^{3-}$ (NAMI-A) is displayed (M=SS1b). Upon fragmentation, loss of the imidazole ligand takes places and the modified product ions contain only the ruthenium ion without ligands. As expected, all (a_n-B_n)-fragment from the 5' terminus up to (a_6-G) (peaks at 817.5 und 1636.0 *m/z* for the doubly and singly charged species, respectively) are unmodified, whereas all further (a_n-B_n) fragments, which contain the G6 residue, are ruthenated (e.g. $[a_7\text{-G+Ru}]^{2-}$ at 1113.0 *m/z*]. For w_n fragments starting from the 3' terminus up to w_7 (without G6) no adduct formation is detectable, whereas w_9 and w_{10} (containing G6; peaks at 1429.5 and 1594.0 *m/z*) are modified. No peak could be assigned to either modified or unmodified w_8. Combining this information suggests G6 as the major ruthenium-binding residue.

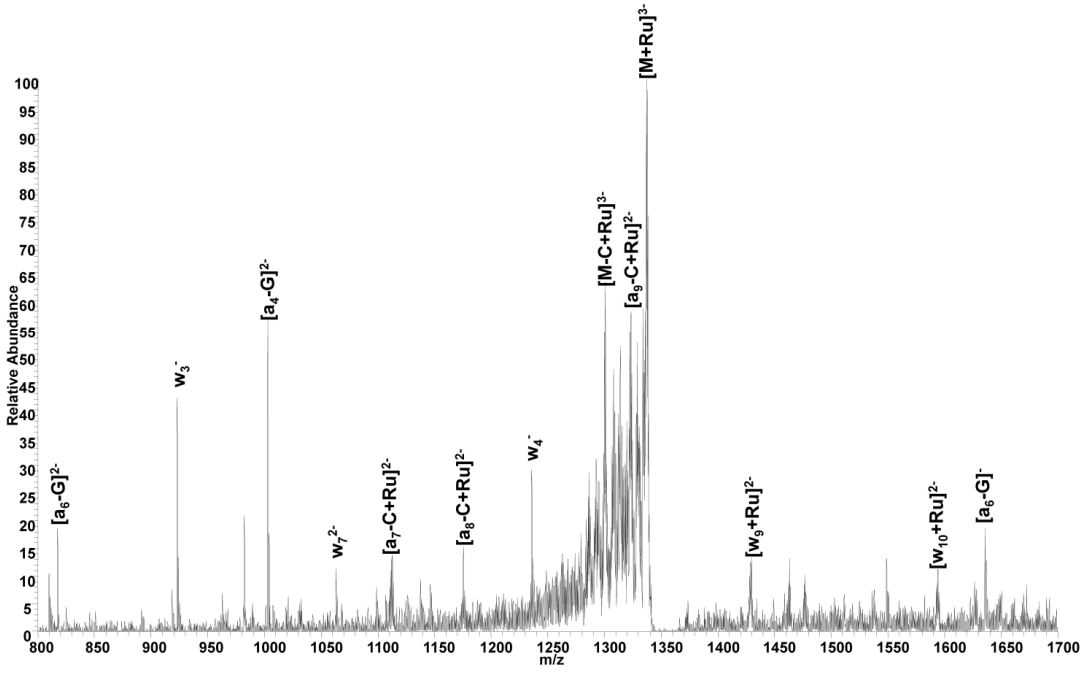

Figure S5. CID spectrum of SS1b modified by NAMI-A in the range from 800 to 1700 *m/z*, identifying G6 as the major binding site.

In Figure S6, a segment of the CID spectrum obtained for the precursor ion $[SS2a+Ru(Ind)]^{3-}$ (KP1019) is displayed (M=SS2a). Upon fragmentation, loss of the indazole ligand takes places and the modified product ions contain only the ruthenium ion without ligands. Whereas the (a_6-G) fragment was found in its original form (1617.3 *m/z*), a ruthenium adduct was detected for (a_7-G) and (a_8-T) (peaks at 1997.5 and 1233.3 *m/z*). For the w_n series, w_6 is unmodified, whereas w_7 is ruthenated. This points to G6 as the major binding site for KP1019.

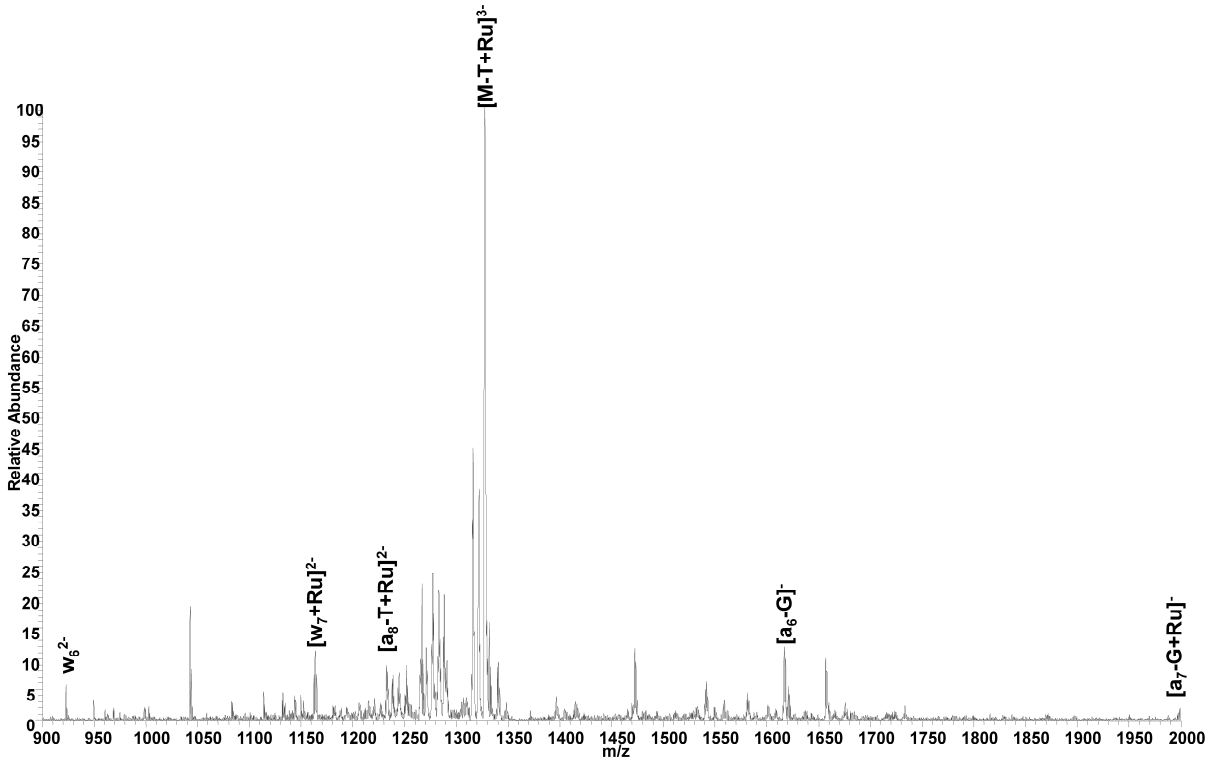

Figure S6. CID spectrum of SS2a modified by KP1019 in the range from 900 to 2000 *m/z*, identifying G7 as the major binding site.

In Figure S7, a segment of the CID spectrum obtained for $[SS3b+Pt(NH_3)_2]^{3-}$ (cisplatin) is displayed (M=SS3b). Again starting from the 5' terminus, fragments up to (a_8-G) (1129.9 *m/z*) were not found to be platinated, whereas (a_n:B_n) fragments with n>8 contained a $Pt(NH_3)_2$ moiety (e.g. $[a_9\text{-C}+Pt(NH_3)_2]^{2-}$ at 1410.9 *m/z*). For fragments from the 3' terminus, product ions up to w_8 (1235.0 *m/z*) are unmodified, whereas w_9 (1513.0 *m/z*) and all further w_n ions are platinated. This suggests G8 as the major binding site for cisplatin.

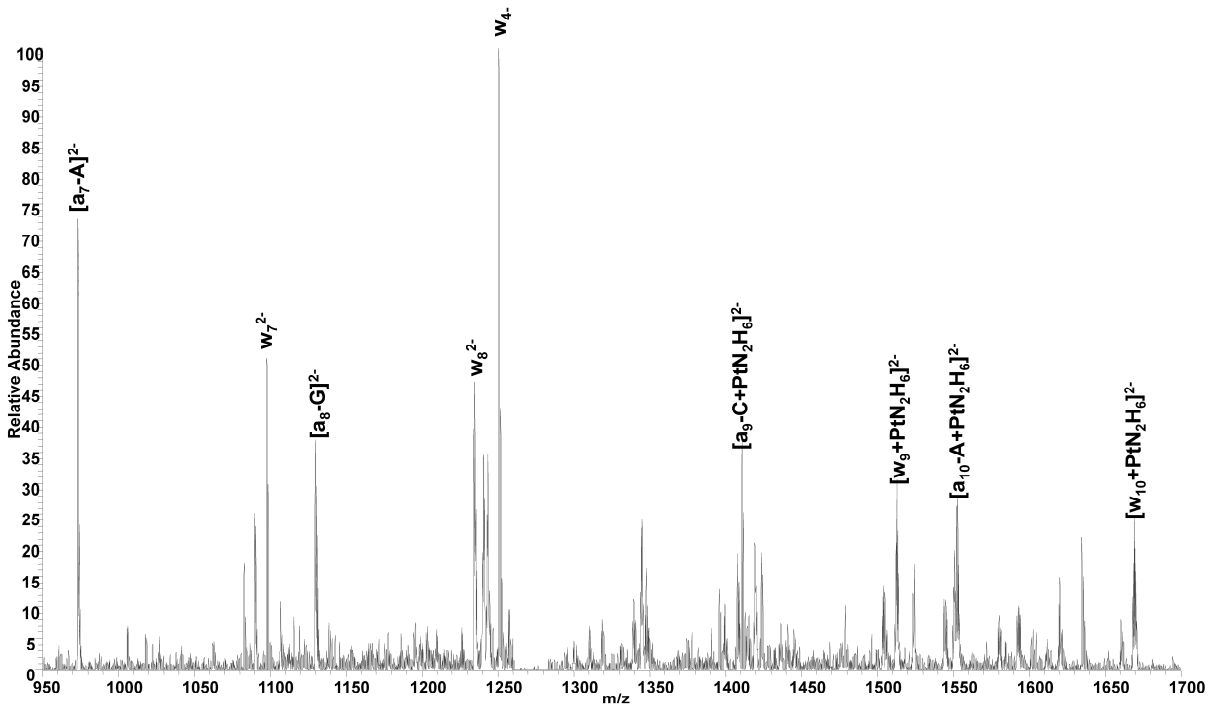

Figure S7. CID spectrum of SS3b modified by cisplatin in the range from 950 to 1700 *m/z*, identifying G8 as the major binding site.