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Identification and Discrimination of Binding Sites of an Organoruthenium Anticancer Complex to Single-Stranded Oligonucleotides by Tandem Mass Spectrometry

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Electronic Supplementary Information

Table S1. Fragment ions detected by negative ESI-MS/MS for ruthenated ODN **I**. **1'**

= $[(\eta^6\text{-bip})\text{Ru}(\text{en})]^{2+}$ (bip = biphenyl; en = ethylenediamine), **I** = 5'-CCCA₄G₅CCC-3'.

The results indicate that G₅ is the binding site of complex **1** on **I**.

Fragment ions	Observed (<i>m/z</i>)	Calculated (<i>m/z</i>)
$[\text{a}_2 - \text{C}_2]^-$	386.07	386.08
$[\text{a}_3 - \text{C}_3]^-$	675.11	675.12
$[\text{a}_4 - \text{A}_4]^{2-}$	481.57	481.58
$[\text{a}_4 - \text{A}_4]^-$	964.15	964.17
$[\text{a}_5 - \text{G}_5]^{2-}$	638.09	638.11
$\{[\text{a}_6 + \mathbf{1}'] - \text{C}_6 - \text{en}\}^{2-}$	929.61	929.62
$\{[\text{a}_7 + \mathbf{1}'] - \text{C}_7 - \text{en}\}^{2-}$	1074.10	1074.14
$\{[\text{a}_7 + \mathbf{1}'] - \text{C}_7 - \text{bip}\}^{2-}$	1027.12	1027.14
$\{[\text{a}_7 + \mathbf{1}'] - \text{C}_7 - \text{bip} - \text{en}\}^{2-}$	997.09	997.10
$\{[\mathbf{I} + \mathbf{1}'] - \text{C}_8 - \text{en}\}^{3-}$	818.09	818.10
w_1^-	306.04	306.05
w_2^-	595.09	595.09
w_3^{2-}	441.56	441.57
w_4^{2-}	606.09	606.09
$\{[w_4 + \mathbf{1}'] - \text{bip} - \text{en}\}^{2-}$	656.04	656.04
$\{[w_5 + \mathbf{1}'] - \text{en}\}^{2-}$	889.58	889.61
$\{[w_6 + \mathbf{1}'] - \text{en}\}^{2-}$	1034.11	1034.13
$\{[w_6 + \mathbf{1}'] - \text{bip} - \text{en}\}^{2-}$	957.05	957.09
w_7^{3-}	700.73	700.77
$\{[w_7 + \mathbf{1}'] - \text{en}\}^{3-}$	785.42	785.43
$\{[w_7 + \mathbf{1}'] - \text{bip} - \text{en}\}^{3-}$	734.05	734.07
$[\mathbf{I} + \mathbf{1}']^{3-}$	875.11	875.15
$\{[\mathbf{I} + \mathbf{1}'] - \text{en}\}^{3-}$	855.11	855.12
$\{[\mathbf{I} + \mathbf{1}'] - \text{bip}\}^{3-}$	823.77	823.79
$\{[\mathbf{I} + \mathbf{1}'] - \text{bip} - \text{en}\}^{3-}$	803.75	803.77

Table S2. Fragment ions detected by negative ESI-MS/MS for ruthenated ODN **II**. **1'**

= $[(\eta^6\text{-bip})\text{Ru}(\text{en})]^{2+}$ (bip = biphenyl; en = ethylenediamine), **II** = 5'-CCCG₄A₅CCC -

3'. The results indicate that G₄ is the binding site of complex **1** on **II**.

Fragment ions	Observed (m/z)	Calculated (m/z)
$[\text{a}_2 - \text{C}_2]^-$	386.07	386.08
$[\text{a}_3 - \text{C}_3]^-$	675.10	675.12
$[\text{a}_4 - \text{G}_4]^{2-}$	481.58	481.58
$\{[\text{a}_5 + \mathbf{1}'] - \text{A}_5 - \text{bip} - \text{en}\}^{2-}$	696.06	696.05
$\{[\text{a}_6 + \mathbf{1}'] - \text{C}_6 - \text{en}\}^{2-}$	929.61	929.62
$\{[\text{a}_7 + \mathbf{1}'] - \text{C}_7 - \text{en}\}^{2-}$	1074.10	1074.14
$\{[\text{a}_7 + \mathbf{1}'] - \text{C}_7 - \text{bip} - \text{en}\}^{2-}$	997.09	997.10
$\{[\mathbf{II} + \mathbf{1}'] - \text{C}_8 - \text{en}\}^{3-}$	818.07	818.10
w_1^-	306.05	306.05
w_2^-	595.10	595.09
w_3^{2-}	441.57	441.57
w_4^{2-}	598.08	598.09
w_5^{2-}	762.60	762.62
$\{[w_5 + \mathbf{1}'] - \text{en}\}^{2-}$	889.58	889.61
$\{[w_6 + \mathbf{1}'] - \text{en}\}^{2-}$	1034.12	1034.13
$\{[w_6 + \mathbf{1}'] - \text{bip} - \text{en}\}^{2-}$	957.06	957.09
$\{[w_7 + \mathbf{1}'] - \text{en}\}^{3-}$	785.41	785.43
$[\mathbf{II} + \mathbf{1}']^{3-}$	875.14	875.15
$\{[\mathbf{II} + \mathbf{1}'] - \text{en}\}^{3-}$	855.11	855.12
$\{[\mathbf{II} + \mathbf{1}'] - \text{bip}\}^{3-}$	823.77	823.79
$\{[\mathbf{II} + \mathbf{1}'] - \text{bip} - \text{en}\}^{3-}$	803.74	803.77

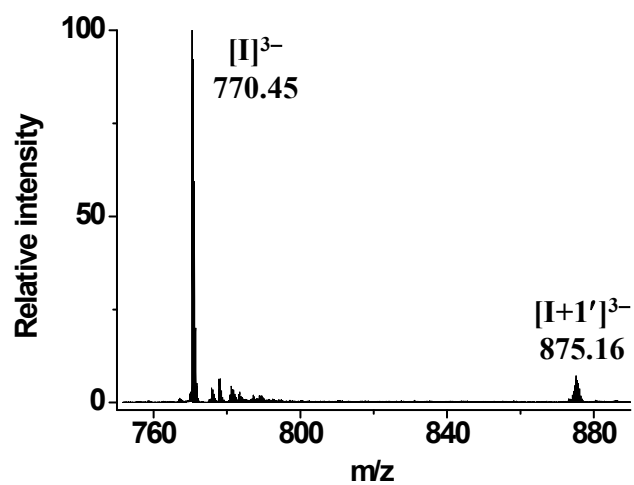


Figure S1. Mass spectrum for reaction mixtures of complex **1** and ODN **I** incubated at 310 K for 24 h with the molar ratio of $[1]/[I] = 0.2$. $1' = (\eta^6\text{-bip})\text{Ru}(\text{en})^{2+}$; The calculated m/z for $[I]^{3-}$ and $[I+1']^{3-}$ were 770.47 and 875.15, respectively.

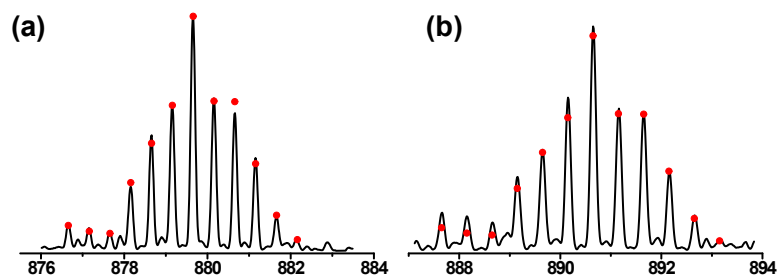


Figure S2. Isotopic models (red dots, for which the values of x and y correspond to the m/z value and intensity of the respective isotopic ion peak, respectively) and mass spectra (lines) for 5'-exonuclease ladders: (a) $[F_4'+\mathbf{1}']^{2-}$ and (b) $\{[F_4'+\mathbf{1}']+\text{Na}^+\}^{2-}$ produced by BSP digestion of the ruthenated ODN $[\mathbf{I}+\mathbf{1}']$. $F_4' = 5'\text{-A}_4\text{G}_5\text{CCC-3}'$; $\mathbf{1}' = [(\eta^6\text{-bip})\text{Ru}(\text{en})]^{2+}$.

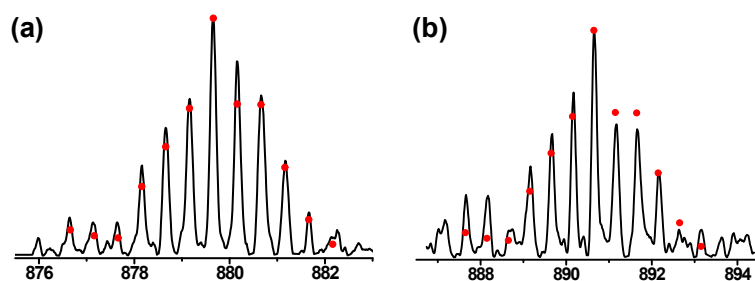


Figure S3. Isotopic models (red dots, for which the values of x and y correspond to the m/z value and intensity of the respective isotopic ion peak, respectively) and mass spectra (lines) for 3'-exonuclease ladders: (a) $[F_5+1']^{2-}$ and (b) $\{[F_5+1']+\text{Na}^+\}^{2-}$ produced by SVP digestion of the ruthenated ODN $[I+1']$. $F_5 = 5'\text{-CCCA}_4\text{G}_5\text{-}3'$; $1' = [(\eta^6\text{-bip})\text{Ru}(\text{en})]^{2+}$.

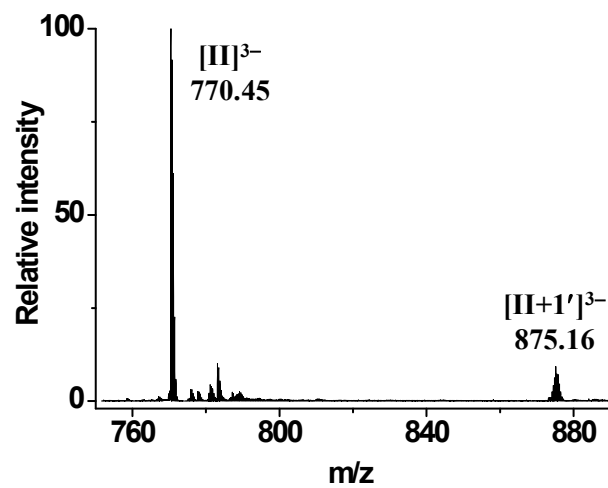


Figure S4. Mass spectrum for reaction mixtures of complex **1** and ODN **II** incubated at 310 K for 24 h with the molar ratio of $[1]/[II] = 0.2$. $1' = (\eta^6\text{-bip})\text{Ru}(\text{en})^{2+}$; The calculated m/z for $[II]^{3-}$ and $[II+1']^{3-}$ were 770.47 and 875.15, respectively.

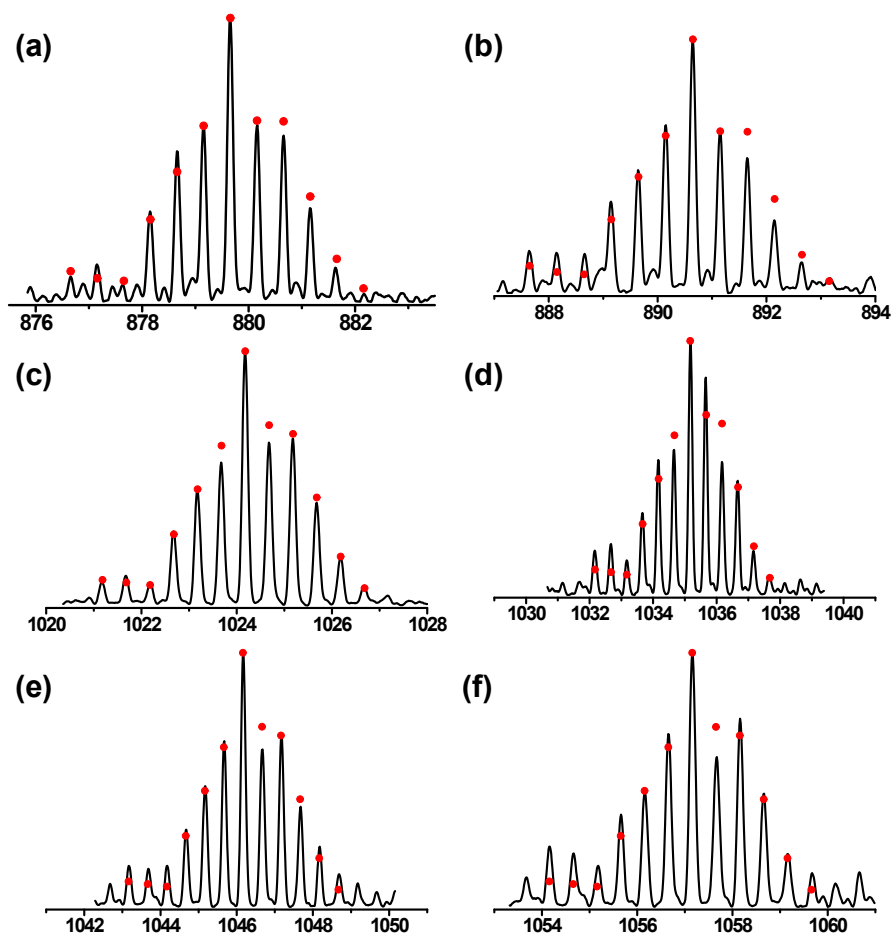


Figure S5. Isotopic models (red dots, for which the values of x and y correspond to the m/z value and intensity of the respective isotopic ion peak, respectively) and mass spectra (lines) for 5'-exonuclease ladders: (a) $[F_4'+1']^{2-}$, (b) $\{[F_4'+1']+\text{Na}^+\}^{2-}$; (c) $[F_3'+1']^{2-}$; (d) $\{[F_3'+1']+\text{Na}^+\}^{2-}$; (e) $\{[F_3'+1']+2\text{Na}^+\}^{2-}$ and (f) $\{[F_3'+1']+3\text{Na}^+\}^{2-}$ produced by BSP digestion of the ruthenated ODN $[\mathbf{II}+1']$. $F_3' = 5'-\text{C}_3\text{G}_4\text{A}_5\text{CCC}-3'$; $F_4' = 5'-\text{G}_4\text{A}_5\text{CCC}-3'$; $1' = [(\eta^6\text{-bip})\text{Ru}(\text{en})]^{2+}$.

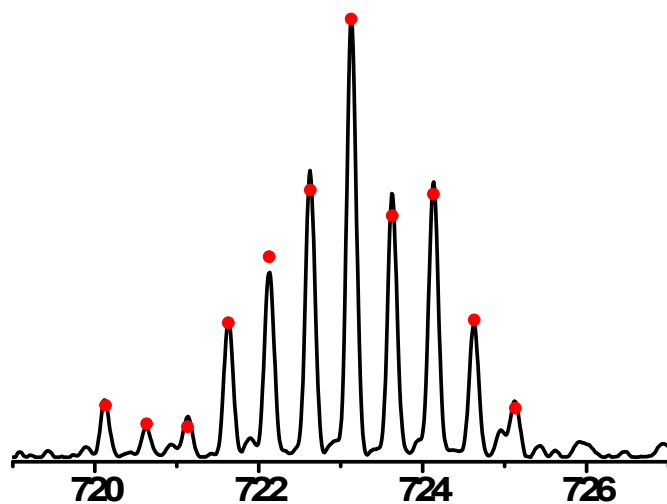


Figure S6. Isotopic models (red dots, for which the values of x and y correspond to the m/z value and intensity of the respective isotopic ion peak, respectively) and mass spectra (lines) for 3'-exonuclease ladders $[F_4+1']^{2-}$ produced by SVP digestion of the ruthenated ODN $[II+1']$. $F_4 = 5'$ -CCCG₄-3'; $1' = [(\eta^6\text{-bip})\text{Ru}(\text{en})]^{2+}$.

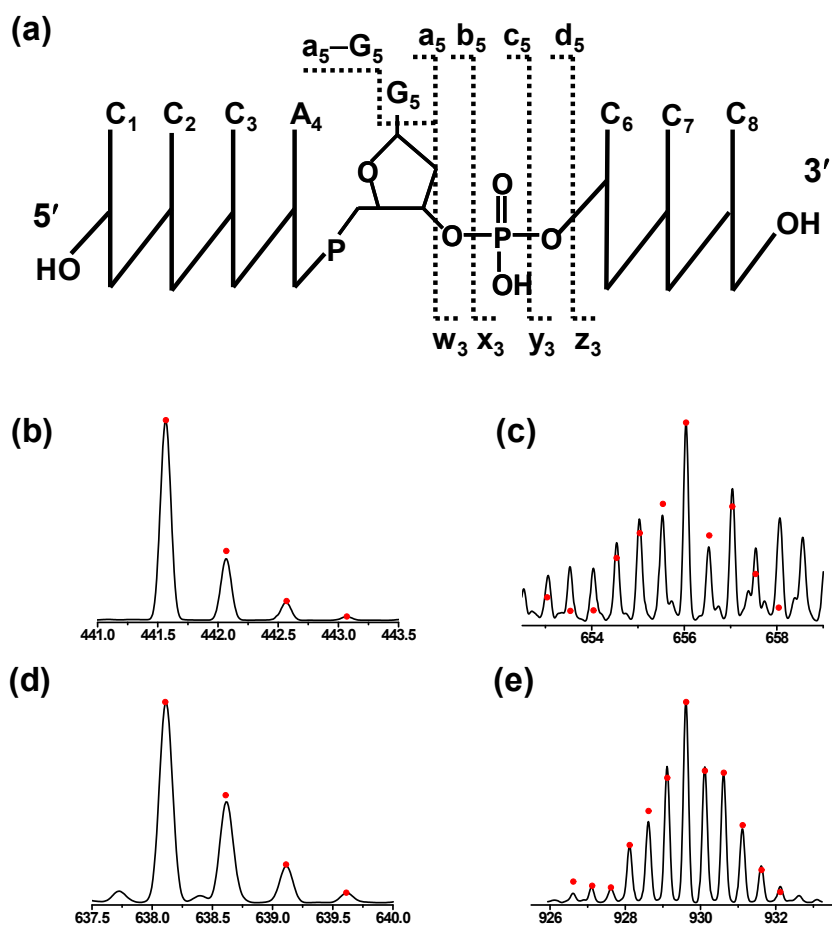


Figure S7. (a) Schematic diagram of MS/MS fragmentation of single-stranded ODN **I**; (b – e) Isotopic models (red dots, for which the values of x and y correspond to the m/z value and intensity of the respective isotopic ion peak, respectively) and mass spectra (lines) for CID fragment ions (b) w_3^{2-} , (c) $\{[w_4+1'] - \text{bip} - \text{en}\}^{2-}$, (d) $[a_5 - G_5]^{2-}$ and (e) $\{[a_6+1'] - C_6 - \text{en}\}^{2-}$ of the ruthenated ODN $[\mathbf{I}+1']$. $1' = [(\eta^6\text{-bip})\text{Ru}(\text{en})]^{2+}$.

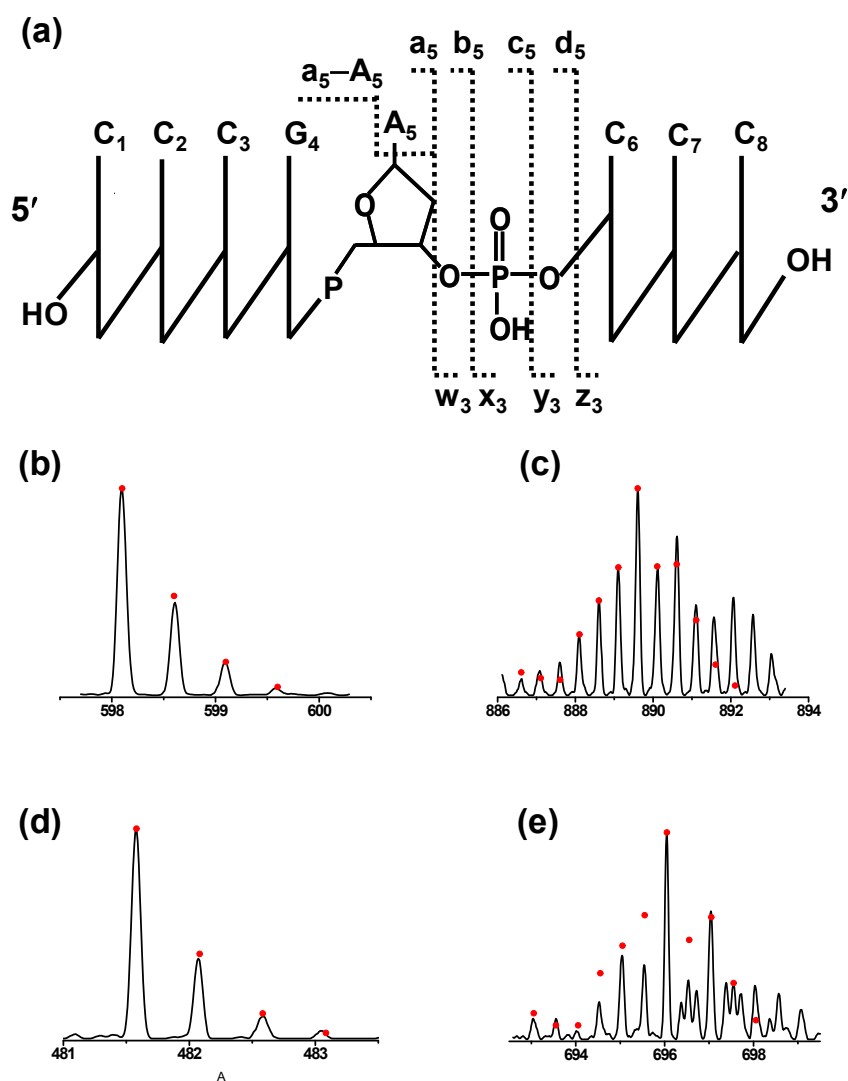


Figure S8. (a) Schematic diagram of MS/MS fragmentation of single-stranded ODN **II**; (b – e) Isotopic models (red dots, for which the values of x and y correspond to the m/z value and intensity of the respective isotopic ion peak, respectively) and mass spectra (lines) for CID fragment ions (b) w_4^{2-} , (c) $\{[w_5+1'] - en\}^{2-}$, (d) $[a_4 - G_4]^{2-}$ and (e) $\{[a_5+1'] - A_5 - en\}^{2-}$ of the ruthenated ODN $[\mathbf{II}+1']$. $1' = [(\eta^6\text{-bip})\text{Ru}(\text{en})]^{2+}$.