Versatile Ruthenium Complexes Based on 2,2'-Bipyridine Modified Peptoids

Maria Baskin, Larisa Panz and Galia Maayan

Schulich Faculty of Chemistry, Technion- Israel Institute of Technology, Haifa, Israel, 32000

Materials:

Rink Amide resin was supplied by Novabiochem; Trifluoroacetic acid (TFA) was supplied by Alfa Aesar; Benzylamine, (S)-(-)-1-Phenylethylamine (Nspe) and (R)-(+)-1-Phenylethylamine (Nrpe) were supplied by Acros; Bromoacetic acid was supplied by MERCK; N,N'diisopropylcarbodiimide (DIC), piperidine, 6-Bromo-2,2'-bipyridine, Ruthenium(III) chloride hydrate, acetonitrile (ACN) and water HPLC grade solvents were supplied by Sigma-Aldrich; dimethylforamide (DMF) and dichloromethane (DCM) solvents were supplied by Bio-Lab Itd; Ethyl alcohol absolute was purchased from Carlo Erba; $Ru(bipy)_3PF_6$ was synthesized according to previously reported protocol.[1] The purchased reagents and solvents were used without additional purification.

Instrumentation:

Peptoid oligomers and their ruthenium complexes were analyzed by reversed-phase HPLC (analytical C18(2) column, Phenomenex, Luna 5μm, 100 Å, 2.0x50 mm) on a Jasco UV-2075 PLUS detector. A linear gradient of 5–95% ACN in water (0.1% TFA) over 10 min was used at a flow rate of 700 μL/min. Preparative HPLC was performed using a AXIA Packed C18(2) column (Phenomenex, Luna 15μm, 100 Å, 21.20x100mm). Peaks were eluted with a linear gradient of 5–95% ACN in water (0.1% TFA) over 50 min at a flow rate of 5 mL/min. Mass spectrometry of peptoid oligomers was performed on a Advion expression CMS mass spectrometer under electrospray ionization (ESI), direct probe ACN:H_2O (95:5), flow rate 0.2 ml/min. Analysis of ruthenium complexes was performed on Autoflex III smartbeam MALDI Bruker (matrix DCTB) and on a Waters LCT Premier mass spectrometer under electrospray ionization (ESI), direct probe ACN:H_2O (70:30), flow rate 0.3 ml/min. UV measurements were performed using an

Agilent Cary 60 UV-Vis spectrophotometer. CD measurements were performed using a circular dichroism spectrometer Applied Photophysics chirascan. Electrochemistry was carried out on Iviumstat XRe potentiostat. ^{1}H-NMR and ^{13}C-NMR measurements were performed on Bruker spectrometer AVIII400 using $CDCl_3$ as a solvent. Data processing was done with the softwares Excel and KaleidaGraph.

Synthesis and Purification of the Peptoid Oligomers

Solid-phase synthesis of Peptoid oligomers was carried out manually in fritted syringes on Rink amide resin at room temperature using the previously reported peptoid sub-monomer protocol.[2] Peptoid synthesis was performed with alternating bromoacylation and amine displacement steps until peptoid oligomers of desired sequence were obtained. Cyclization process was performed according to the previously reported procedure.[3] After the peptoid synthesis, the products were cleaved from the resin by treatment with 95% trifluoroacetic acid (TFA) in water (50 mL g^{-1} resin) for 30 minutes. After filtration, the cleavage mixture was concentrated in vacuum and cleaved samples were then re-suspended in 50% acetonitrile in water and lyophilized to powders. Peptoids and their ruthenium complexes were purified by preparative High Performance Liquid Chromatography (HPLC) using a C18 column. Products were detected by UV absorbance at 230 nm during a linear gradient conducted from 5% to 95% solvent B (0.1% TFA in HPLC grade acetonitrile) over solvent A (0.1% TFA in HPLC grade water) in 50 minutes with a flow rate of 5 mL min^{-1}. Purified products were analyzed by reversed-phase HPLC (C18 column) with a linear gradient of 5–95% ACN in water (0.1% TFA) over 10 min at a flow rate of 700 μL/min and 214 nm UV absorbance.

Circular Dichroism

Approximately 500 μL solutions (5 mM in ACN) of lyophilized peptoids powders and their Ru(II) complexes were prepared immediately before CD measurements. CD scans were performed at room temperature at concentration of 30-200μM in solution of ACN. The spectra were obtained by averaging 4 scans per sample in a fused quartz cell (path length = 0.1 cm), over the 370 to 190 nm region at a step of 1nm (scan rate=1 sec/step).

Cyclic Voltammetry

Cyclic voltammograms were obtained at room temperature in acetonitrile with 0.1 M TBA-PF_6 as the supporting electrolyte. The CV traces were recorded at 0.5 mM for (**L1B**)$_3$Ru, (**C3B**)Ru, (bipy-DM)$_3$Ru and (bipy)$_3$Ru and 0.25 mM for (**L2B**)$_3$Ru$_2$ complex concentrations using a glassy carbon working electrode, platinum counter electrode and $Ag/AgNO_3$ reference electrode at a scan rate of 100 mV s^{-1}.

UV-VIS Spectroscopy

UV-VIS experiments of the peptoid olygomers and Ru(II) complexes were performed in ACN solution using 17-50 μM concentration and measuring the spectra from 200-800 nm using an Agilent Cary 60 UV-Vis spectrophotometer.

Table S1. Peptoid oligomer sequences.

*N*spe =(S)-(-)-1-phenylethylamine, *N*rpe =(R)-(+)-1-phenylethylamine, Nbp-(2,2'-bipyridine-3'-yloxy) ethylamine, Npl- chloropropylamine, Npm = N-benzylamine

Peptoid oligomers	Molecular weight Calc: Found (gr/mol)
Tetramer (Npm- Npm- Nbp- Npm)	713.80 : 714.00
L1B (Nspe-Nspe-Nspe-Nspe-Nbp) linear	917.10: 917.89
L2B (Nspe- Nbp -Nspe-Nspe-Nbp- Nspe) linear	1172.38 : 1172.57
L3B (Npl -Nbp -Nspe- Nbp - Nspe- Nbp- Nspe) linear	1400.02 : 1400.39
C3B (Nbp -Nspe- Nbp - Nspe- Nbp- Nspe) cyclic	1363.56 : 1363.54
R-L1B (Nrpe-Nrpe-Nrpe-Nrpe-Nbp) linear	917.10: 917.34
R-L2B (Nrpe- Nbp -Nrpe-Nrpe-Nbp- Nrpe) linear	1172.38 : 1172.30
R-C3B (Nbp -Nrpe- Nbp - Nrpe- Nbp- Nrpe) cyclic	1363.56 : 1363.14
Di-L1B (Nspe- Nbp)	433.50 : 434.18

Table S2. Ru(II) complexes.

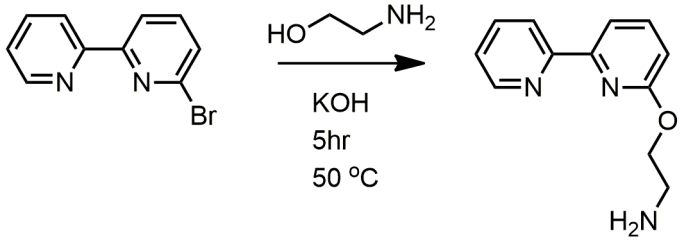
Peptoid and Ru(II) complexes	Molecular weight Calc: Found: Computed (gr/mol)	E_p^{ox} (V, vs. $Ag/AgNO_3$)
(**L1B**)$_3$Ru	2852.38 : 1427.04 (m/2z) : 1427.16 (m/2z)	0.94
(L2B)$_3$Ru$_2$-PF_6 **(L2B)$_3$**Ru$_2$- $(PF_6)_3$	3864.24 : 966.36 (m/4z) : 966.87 (m/4z) 4154.16 : 1039.61 (m/4z) : 1039.35 (m/4z)	0.88
(**C3B**)Ru	1465.64 (m/z) : 1465.71 (m/z)	0.90
(**R-L1B**)$_3$Ru	2852.38 : 1426.64 (m/2z) : 1427.15 (m/2z)	N/A
(R-L2B)$_3$Ru$_2$-PF_6	3864.24 : 966.37 (m/4z) : 966.62 (m/4z)	N/A
(**R-C3B**)Ru	1465.64 (m/z) : 1465.76 (m/z)	N/A
(**Di-L1B**)$_3$Ru	1401.58 : 1440.44 (m/z+K^+) : 1440.50 (m/z+K^+)	0.88
(**bp-DM**)$_3$Ru	834.34 (m/z) : 834.33 (m/z)	1.22

Synthetic procedure for Nbp:

Nbp = 2-(2, 2'-bipyridine-6-yloxy) ethylamine was synthesized by S_N2 reaction between ethanolamine deprotonated by potassium hydroxide in DMSO solution and 6-Bromo-2,2'-bipyridine.

2,2'- Bipyridineamine was synthesized according to similar procedure previously published.[4] To a solution of DMSO (5 ml) with KOH (0.28 gr) and ethanolamine (100 μL) 6-Bromo-2,2'-bipyridine (0.255 gr) was added and the solution was stirred for five hours at 50 °C (Scheme 1). To The reaction mixture was then added 40ml of methylene chloride and washed three times with water, dried over Na_2SO_4 and the solvent was removed (0.2 gr, yield 86%). The product analyzed by ^{1}H NMR (400 MHz, CDCl3): δ 8.67 (1H,dd) δ 8.38 (1H,m) δ 8.00 (1H,d) δ 7.82 (1H,d) δ 7.70 (1H, t) δ 7.29 (1H,m) δ

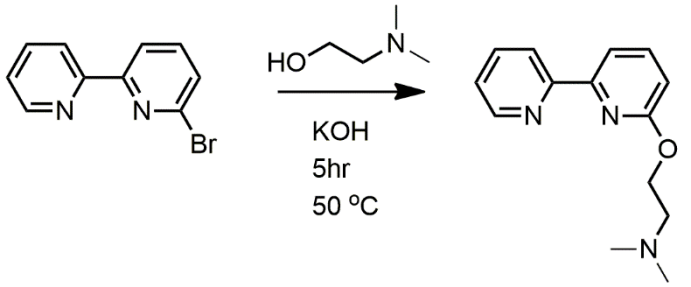
6.80 (1H,d) δ 4.46 (2H, t) δ 3.13 (2H, t) and ^{13}C NMR (100MHz, $CDCl_3$) δ 163.35, 156.16, 153.37, 149.43, 139.22, 137.13, 123.90, 121.12, 113.69, 111.37, 67.98, 41.53. ESI-MS calculated: 215.3; found: 216.1.



Scheme 1: Synthetic routs to the primary amine Nbp.

Synthetic procedure for bpDM:

To a solution of DMSO (5 ml) with KOH (0.17 gr) and 2-Dimethylaminoethanol (85 µL) 6-Bromo-2,2'-bipyridine (0.15 gr) was added and the solution was stirred for five hours at 50 °C (Scheme 2). To The reaction mixture was then added 40ml of methylene chloride and washed three times with water, dried over Na_2SO_4 and the solvent was removed (0.11 gr, yield 71%). The product analyzed by ^{1}H NMR (400 MHz, CDCl3): δ 8.57 (1H, d) δ 8.3 (1H,ג) δ 7.94 (1H,d) δ 7.71 (1H,t) δ 7.64 (1H, t) δ 7.20 (1H,t) δ 6.70 (1H,d) δ 4.48 (2H, t) δ 2.7 (2H, t) δ 2.29 (6H, s) and ^{13}C NMR (100MHz, $CDCl_3$) δ 163.10, 156.09, 153.30, 149.09, 139.42, 136.74, 123.48, 120.93, 113.75, 111.59, 67.39, 58.28, 45.95.



Scheme 2: Synthetic routs to the tertiary amine bpDM.

References:

1. J. V. Caspar and T. J. Meyer, *J. Am. Chem. Soc.* 1983, **105**, 5583-5590.
2. R. N. Zuckermann, J. M. Kerr, S. B. W. Kent and W. H. Moosm *J. Am. Chem. Soc.,* 1992, **114**, 10646-10647.
3. P. J. Kaniraj and G. Maayan, *Org. Lett.,* 2015, **17**, 2110−2113.

4. G. Maayan, B. Yoo and K. Kirshenbaum, *Tetrahedron Letters,* 2008, **49**, 335-338.

Synthesis of Ruthenium complexes

General: To the flask of 25 ml peptoid olygomers or bpDM ligand in dry EtOH were added and the solutions were heated to 70 ^{o}C. Then, desired equivalent of $RuCl_3$ hydrate was added and the mixtures were refluxed for 24 hours in a case of **L2B**, **R-L2B, C3B** and **R-C3B** and 30 hours in a case of **L1B**, **R-L1B**, **Di-L1B** and **bpDM** under nitrogen atmosphere. The complexes (orange-red) were precipitated by addition of aqueous NH_4PF_6, washed twice with water and lyophilized overnight. Peptoid-Ru complexes were then dissolved in 50% ACN in water and purified by preparative HPLC using standard peptoid purification process. The fractions collected from HPLC were lyophilized to yield a dark orange solid. (**bpDM**)$_3$Ru complex was purified by alumina column chromatography using ACN\toluene (50:50 v/v) as a mobile phase.

(**L1B**)$_3$Ru: 20 mg of **L1B** in 2 ml of dry EtOH and 0.3 equiv. of Ruthenium chloride hydrate. (6.8 mg, 30% yield).

(**L2B**)$_3$Ru$_2$: 10 mg of **L2B** in 1.2 ml of dry EtOH and 0.70 equiv. of Ruthenium chloride hydrate. (3.7 mg, 30% yield).

(**C3B**)Ru: 10 mg of **C3B** in 1.2 ml of dry EtOH and 1 equiv. of Ruthenium chloride hydrate. (6.4 mg, 54% yield).

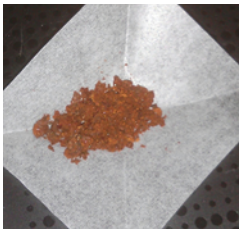
(**R-L1B**)$_3$Ru: 10 mg of **R-L1B** in 2 ml of dry EtOH and 0.3 equiv. of Ruthenium chloride hydrate. (3.2 mg, 28% yield).

(**R-L2B**)$_3$Ru$_2$: 15 mg of **R-L2B** in 1.2 ml of dry EtOH and 0.70 equiv. of Ruthenium chloride hydrate. (5.9 mg, 32% yield).

(**R-C3B**)Ru: 10 mg of **R-C3B** in 1.2 ml of dry EtOH and 1 equiv. of Ruthenium chloride hydrate. (3.4 mg, 29% yield).

(**Di-L1B**)Ru: 30 mg of **Di-L1B** in 2 ml of dry EtOH and 0.3 equiv. of Ruthenium chloride hydrate. (15 mg, 38% yield).

(**bpDM**)$_3$Ru: 40 mg of **bipy-DM** in 2 ml of dry EtOH and 0.3 equiv. of Ruthenium chloride hydrate. (17 mg, 37% yield).



HPLC of peptoid oligomers and their Ru(II) complexes

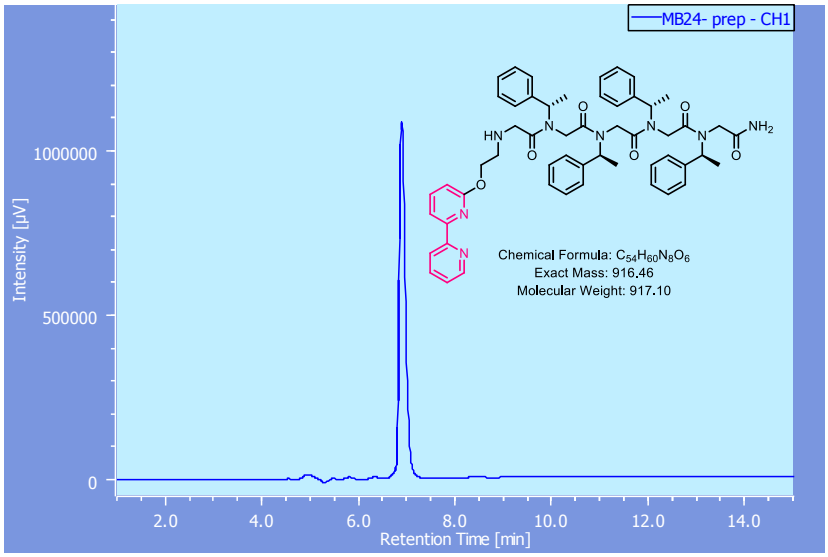


Figure S1. HPLC traces of purified peptoid oligomer **L1B** at 214nm.

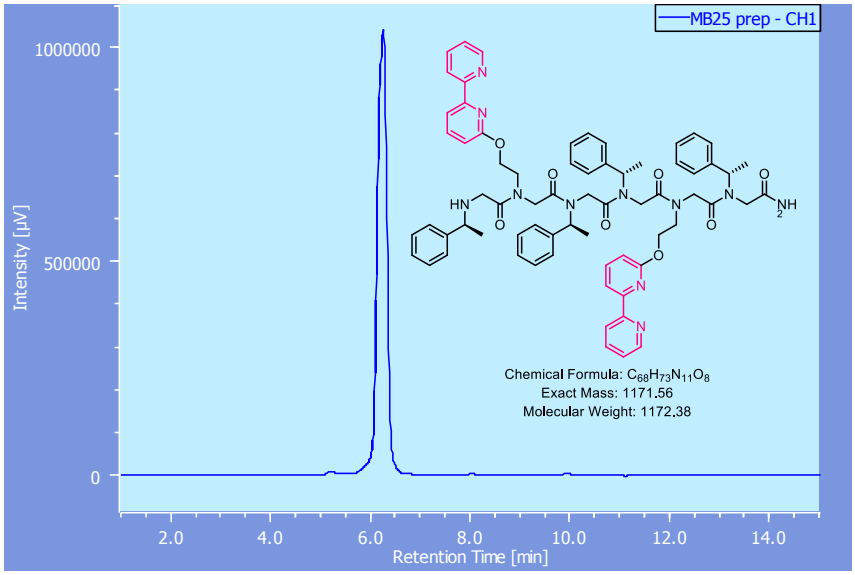


Figure S2. HPLC traces of purified peptoid oligomer **L2B** at 214nm.

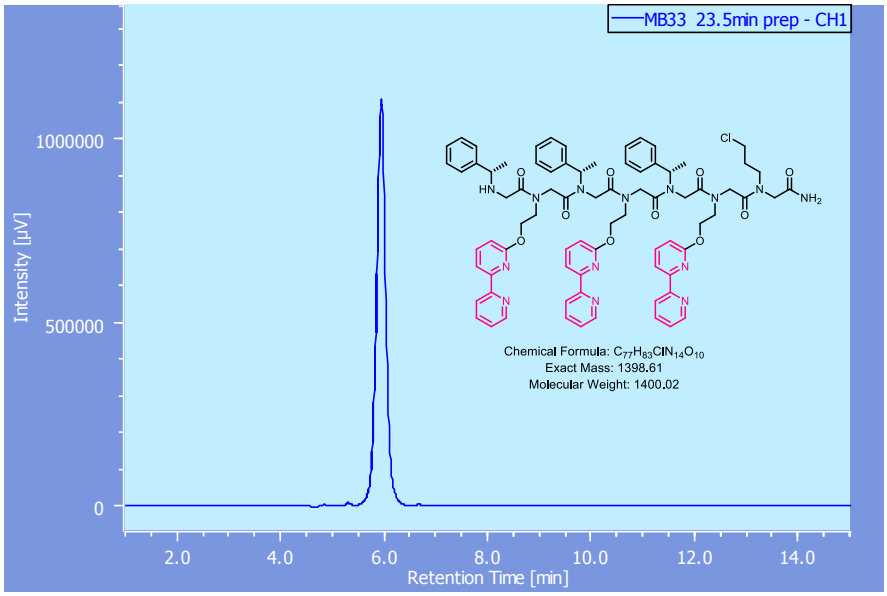


Figure S3. HPLC traces of purified peptoid oligomer **L3B** at 214nm.

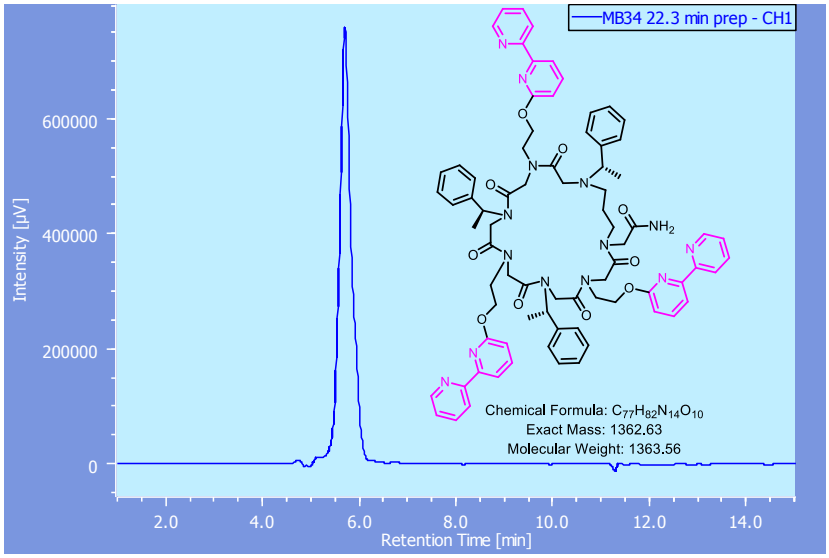


Figure S4. HPLC traces of purified peptoid oligomer **C3B** at 214nm.

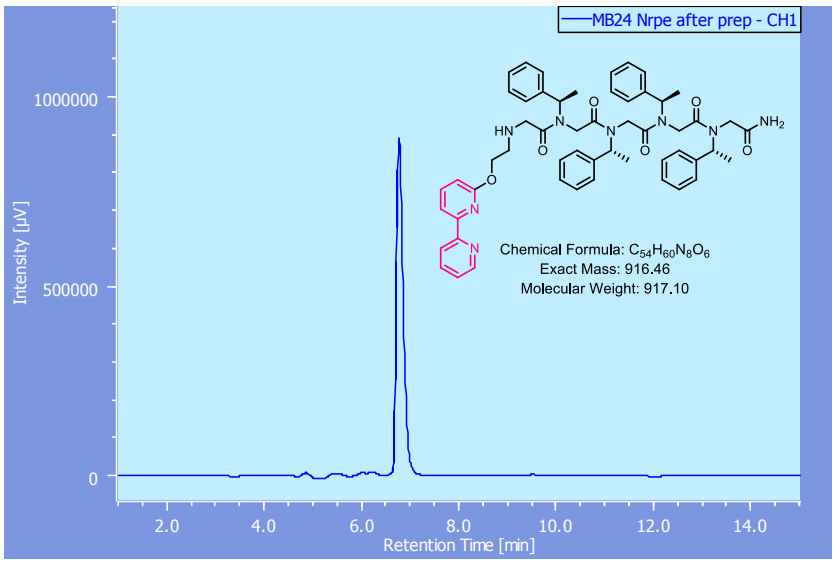


Figure S5. HPLC traces of purified peptoid oligomer **R-L1B** at 214nm.

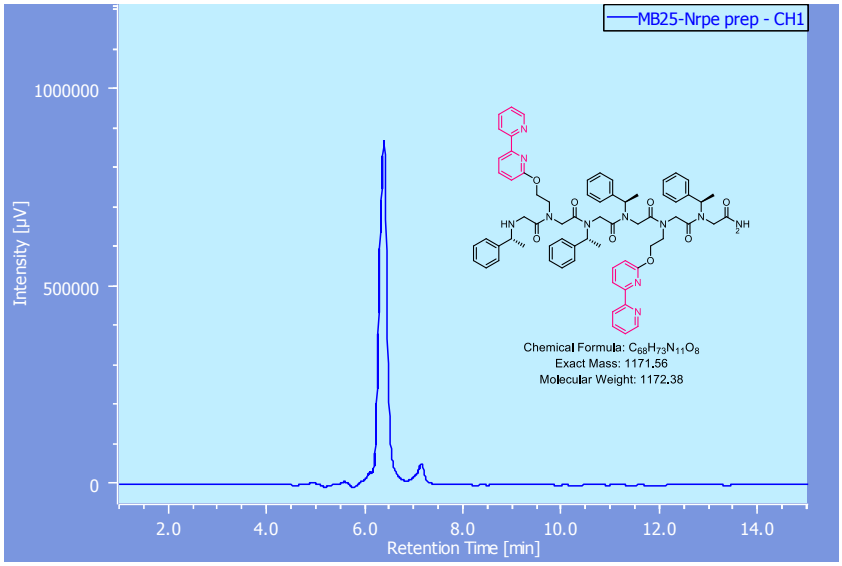


Figure S6. HPLC traces of purified peptoid oligomer **R-L2B** at 214nm.

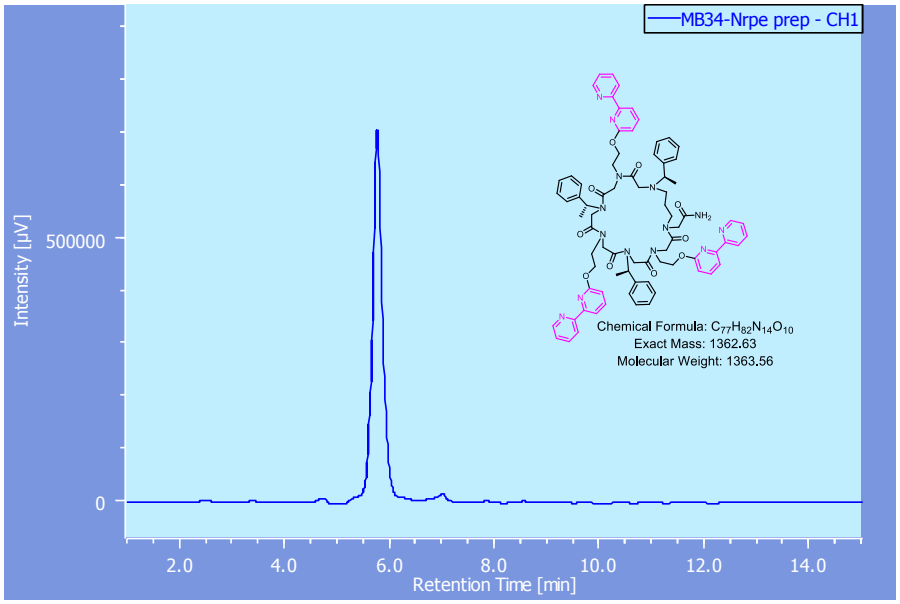


Figure S7. HPLC traces of purified peptoid oligomer **R-C3B** at 214nm.

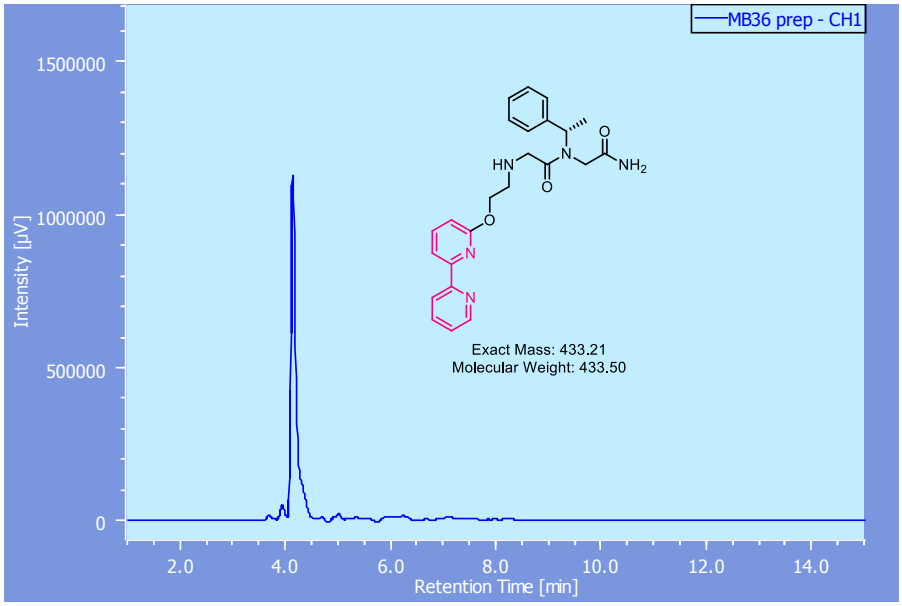


Figure S8. HPLC traces of purified peptoid oligomer **Di-L1B** at 214nm.

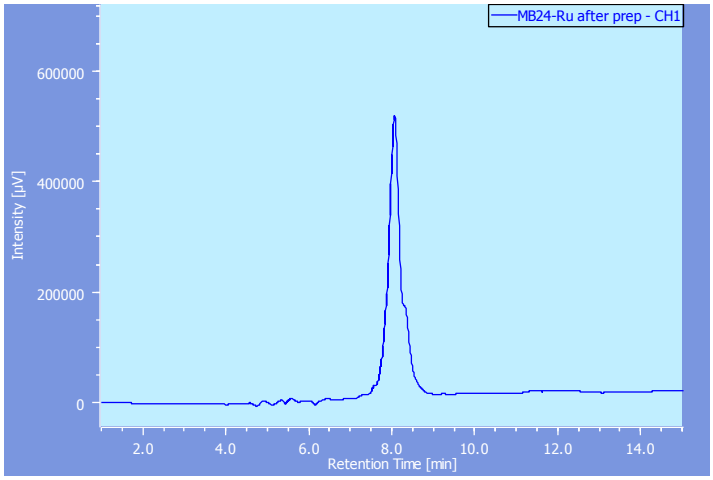


Figure S9. HPLC traces of purified metallopeptoid complex (**L1B**)$_3$Ru at 214nm.

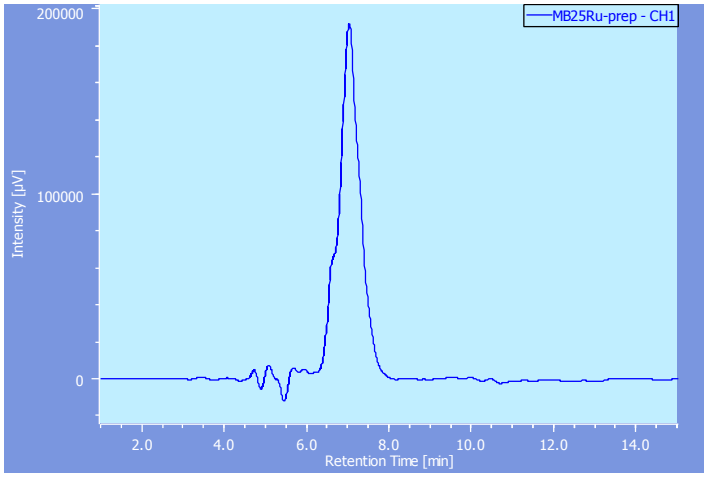


Figure S10. HPLC traces of purified metallopeptoid complex (**L2B**)$_3$Ru$_2$ at 214nm.

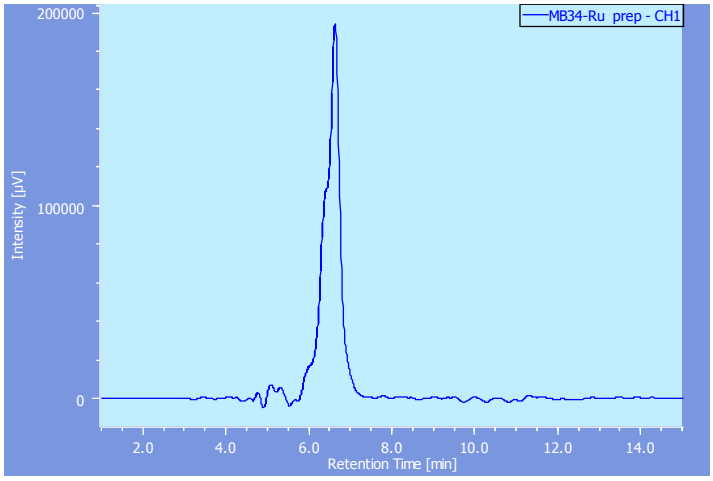


Figure S11. HPLC traces of purified metallopeptoid complex (**C3B**)Ru at 214nm.

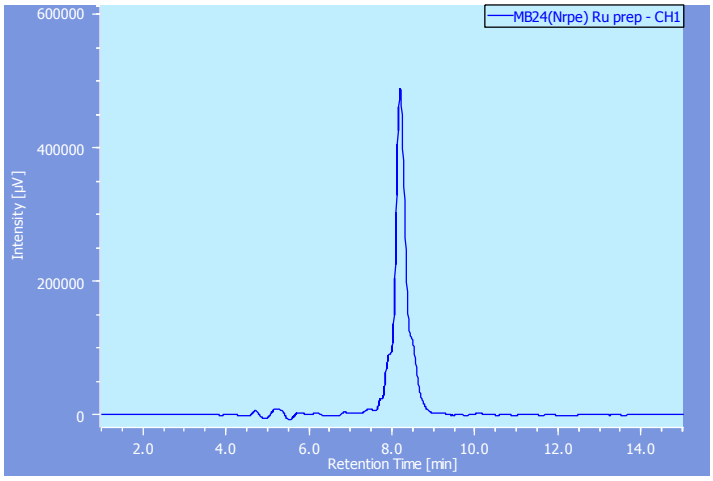


Figure S12. HPLC traces of purified metallopeptoid complex (**R-L1B**)$_3$Ru at 214nm.

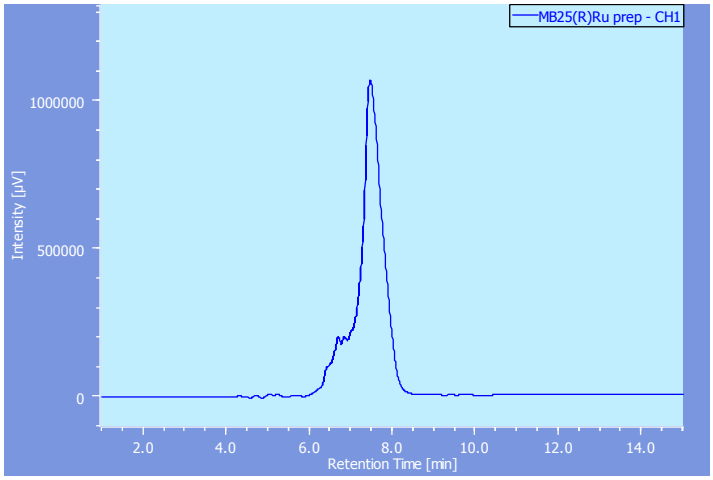


Figure S13. HPLC traces of purified metallopeptoid complex (**R-L2B**)$_3$Ru$_2$ at 214nm.

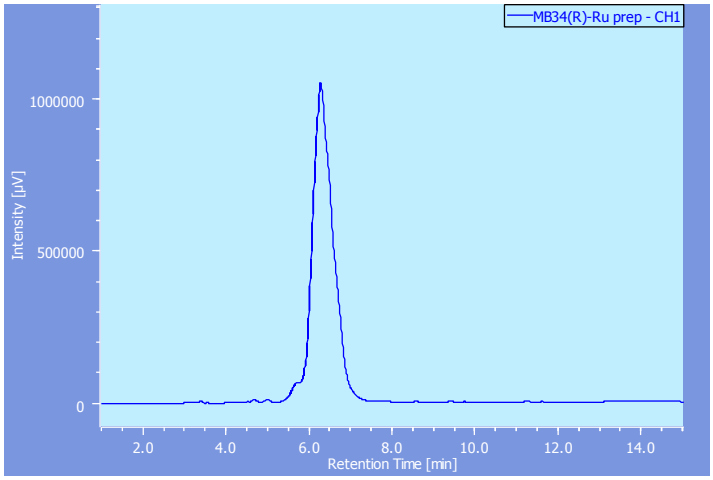


Figure S14. HPLC traces of purified metallopeptoid complex (**R-C3B**)Ru at 214nm.

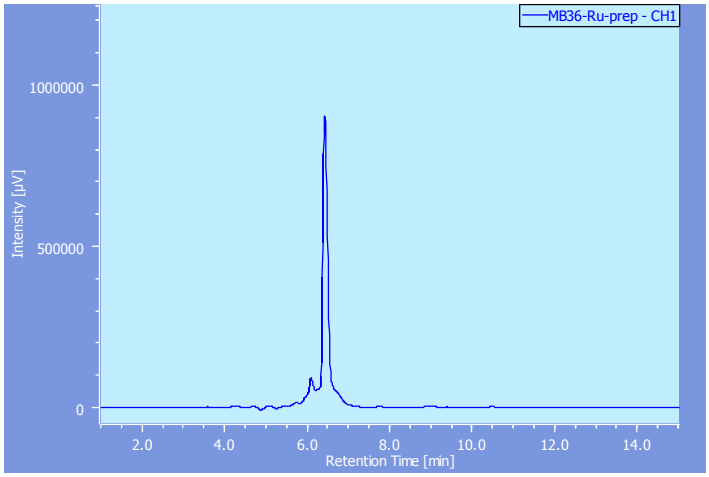


Figure S15. HPLC traces of purified metallopeptoid complex (**Di-L1B**)$_3$Ru at 214nm.

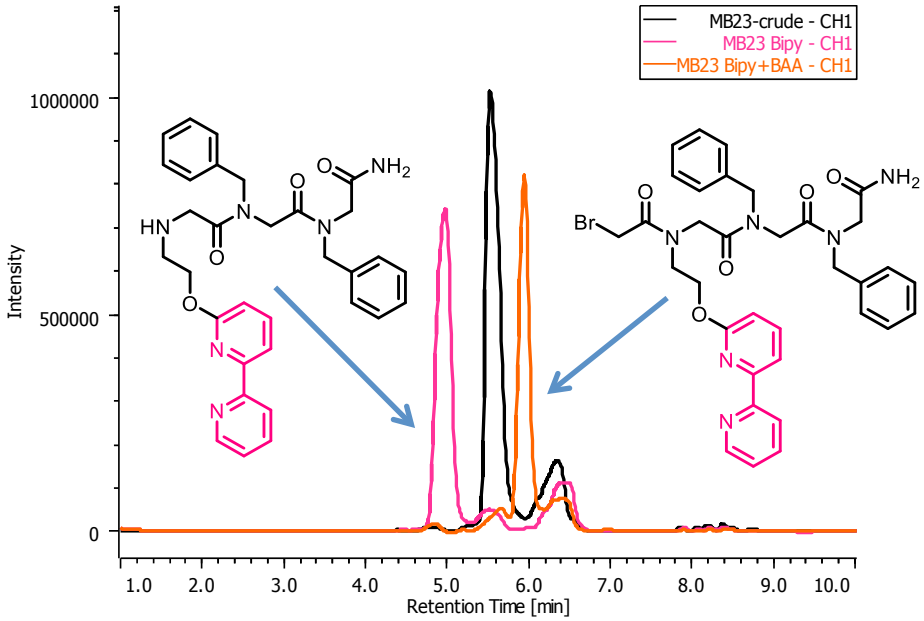


Figure S16. HPLC analyses at 214nm of crude trimer after addition of Bipy-NH_2 (pink) after acetylation (orange) and after substitution with benzylamine to create final crude peptoid tetramer (black).

UV-Vis

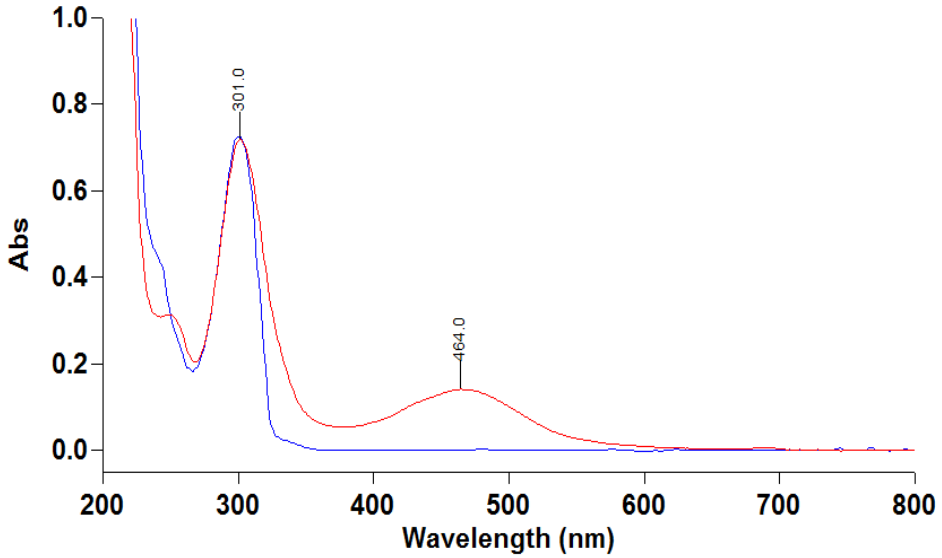


Figure S17. UV-Vis of **R-L1B** free preptoid (blue, 50 μM) and (**R-L1B**)$_3$Ru complex (red, 17 μM).

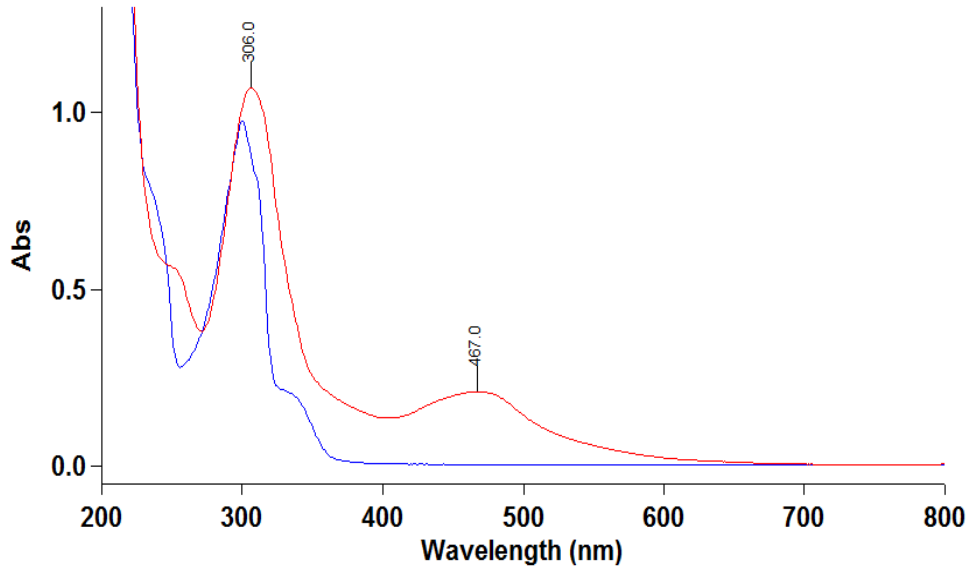


Figure S18. UV-Vis of **R-L2B** free preptoid (blue, 50 μM) and (**R-L2B**)$_3$Ru$_2$ complex (red, 17 μM).

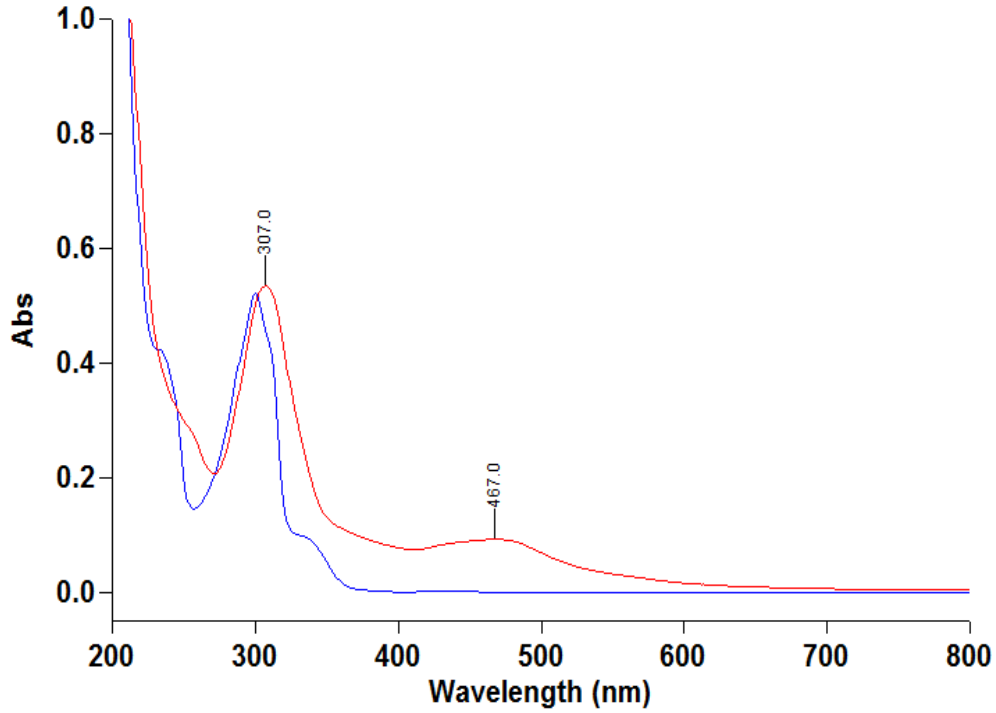


Figure S19. UV-Vis of **R-C3B** free preptoid (blue, 17 μM) and (**R-C3B**)Ru complex (red, 17 μM).

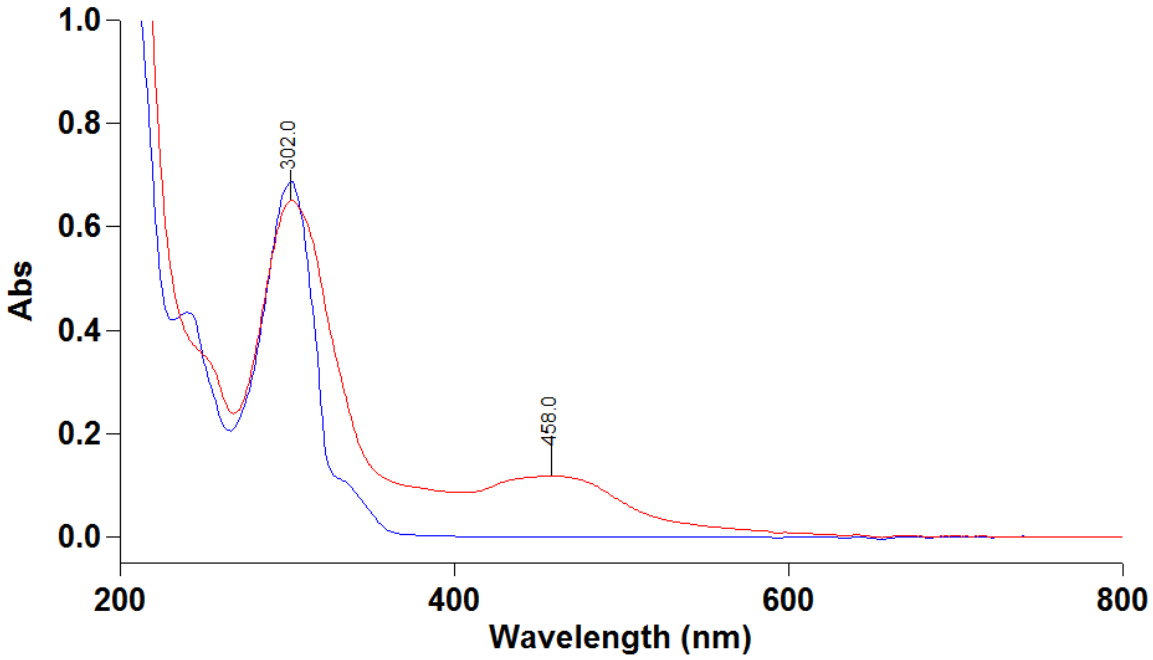


Figure S20. UV-Vis of **Di-L1B** free preptoid (blue, 17 μM) and (**Di-L1B**)$_3$Ru complex (red, 17 μM).

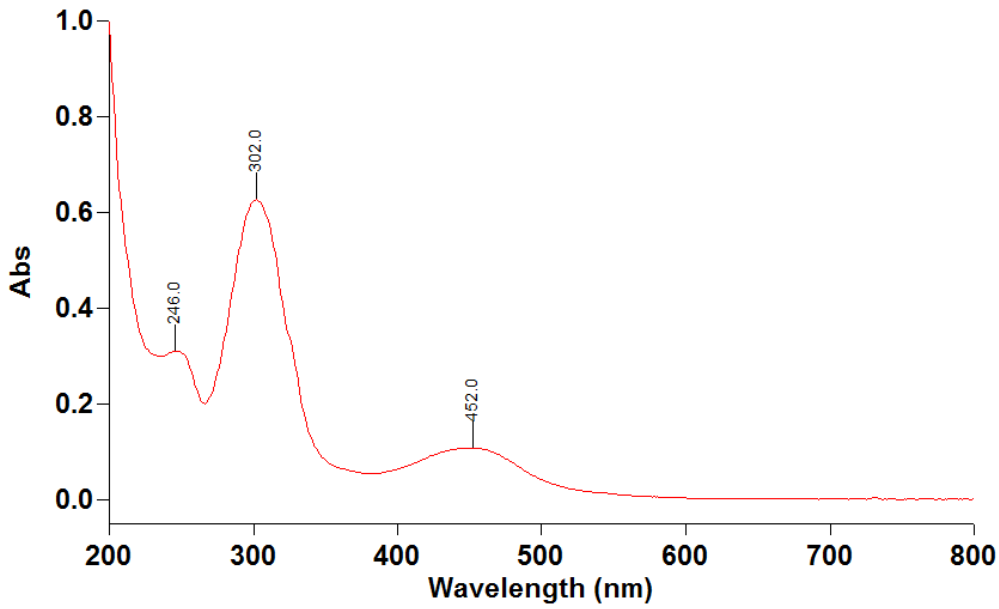


Figure S21. UV-Vis of (**bp-DM**)$_3$Ru complex (red, 17 μM).

ESI-MS of the peptoid oligomers:

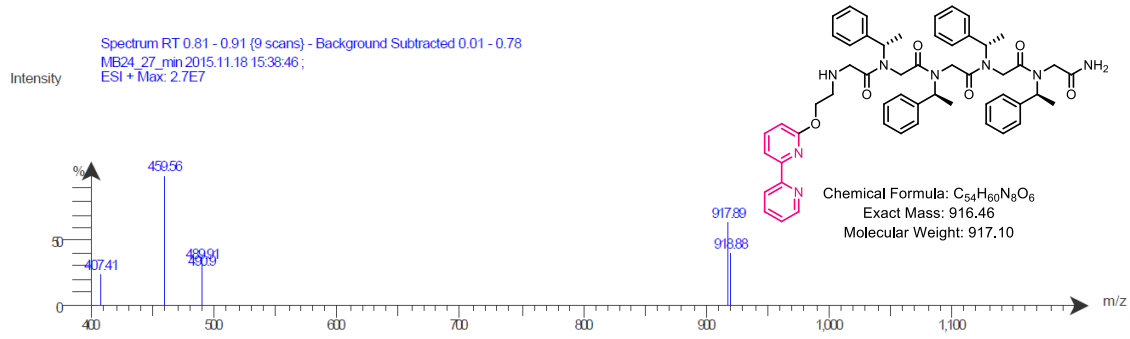


Figure S22. ESI-MS traces of peptoid oligomer **L1B**

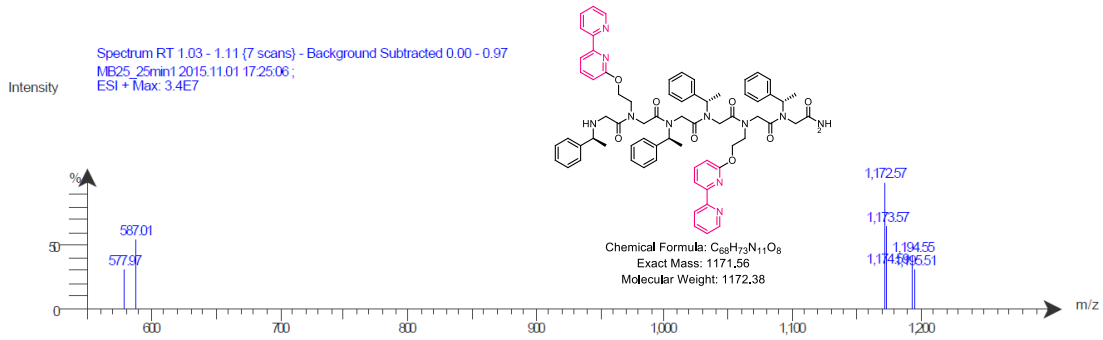


Figure S23. ESI-MS traces of peptoid oligomer **L2B**

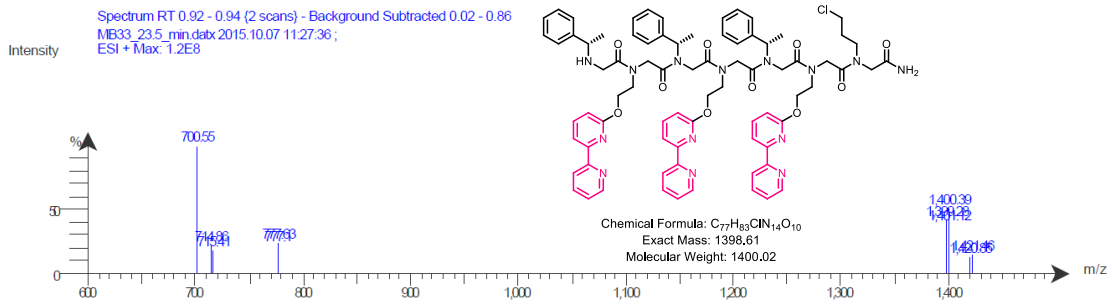


Figure S24. ESI-MS traces of peptoid oligomer **L3B**

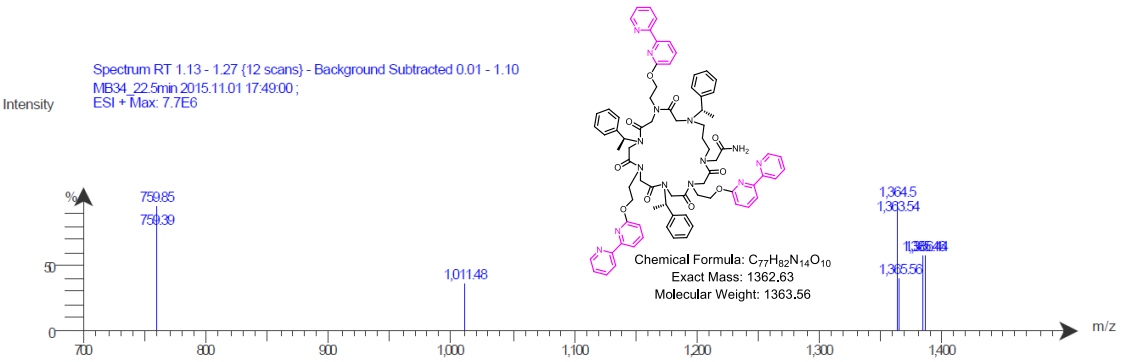


Figure S25. ESI-MS traces of peptoid oligomer **C3B**

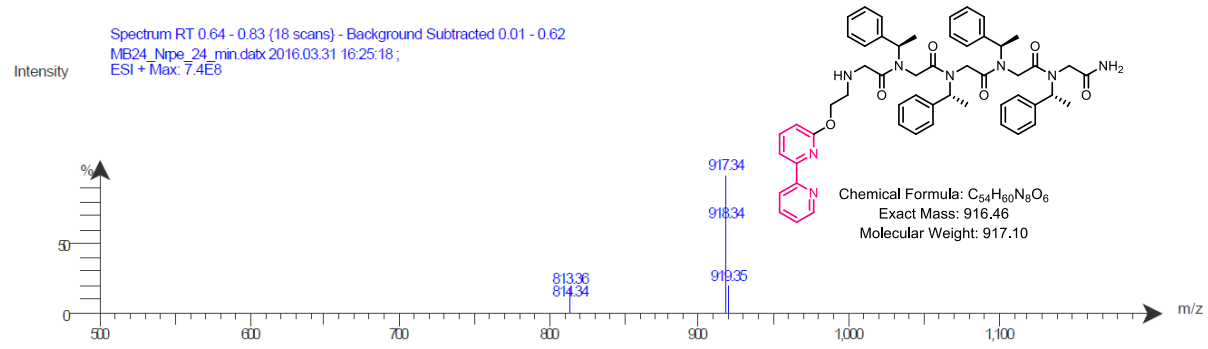


Figure S26. ESI-MS traces of peptoid oligomer **R-L1B**

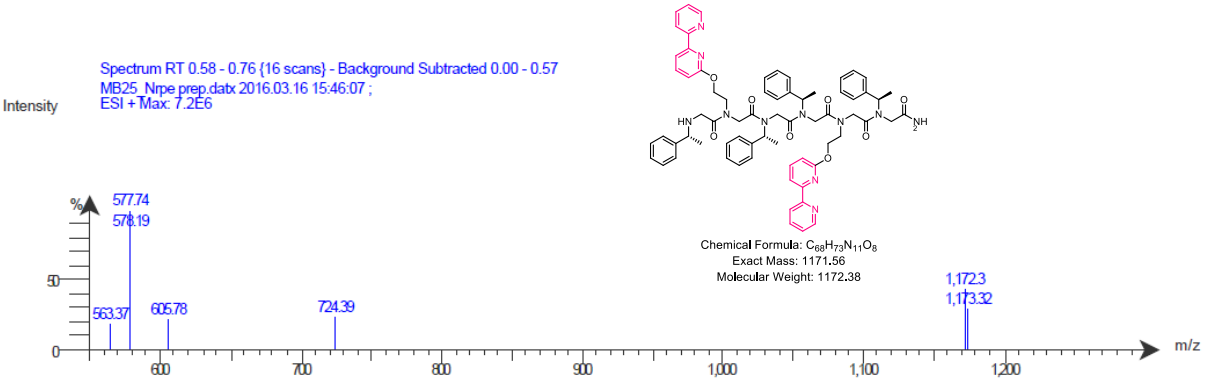


Figure S27. ESI-MS traces of peptoid oligomer **R-L2B**

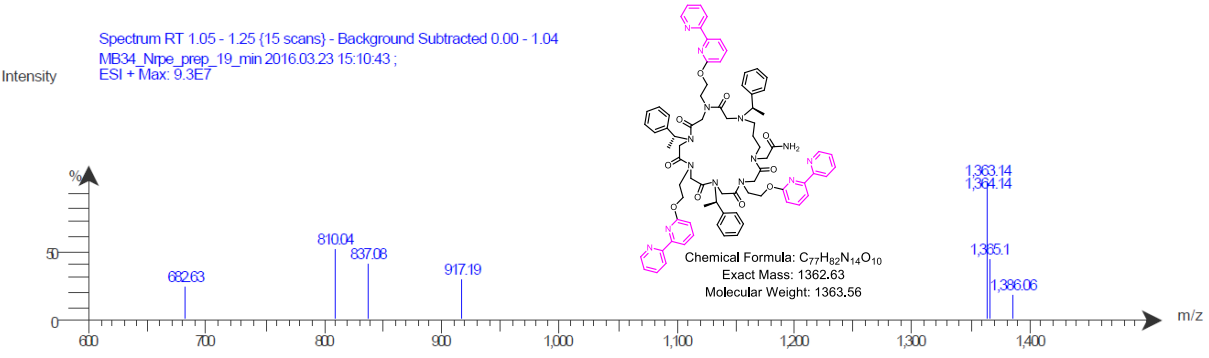


Figure S28. ESI-MS traces of peptoid oligomer **R-C3B**

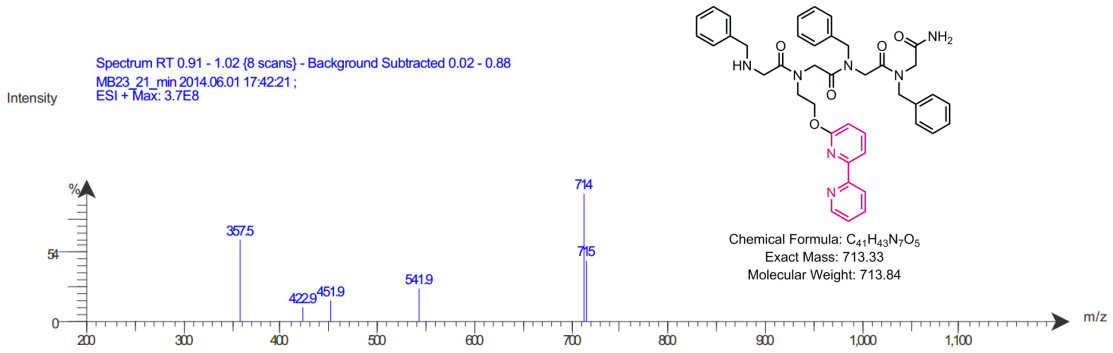


Figure S29. ESI-MS traces of peptoid oligomer tetramer

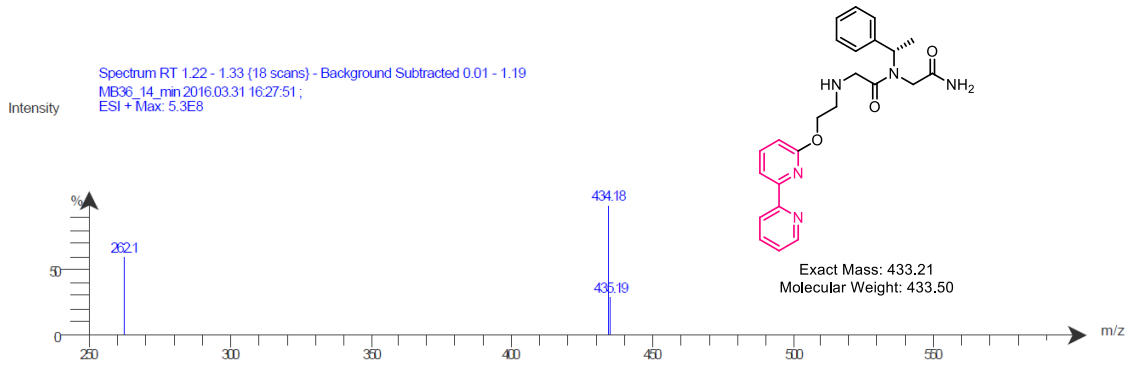


Figure S30. ESI-MS traces of peptoid **Di-L1B**.

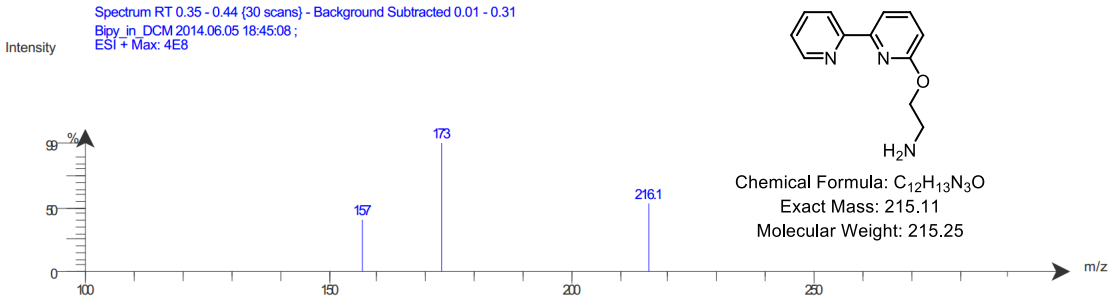


Figure S31. ESI-MS traces of the Nbp ligand

ESI-MS and MALDI of Ruthenium complexes:

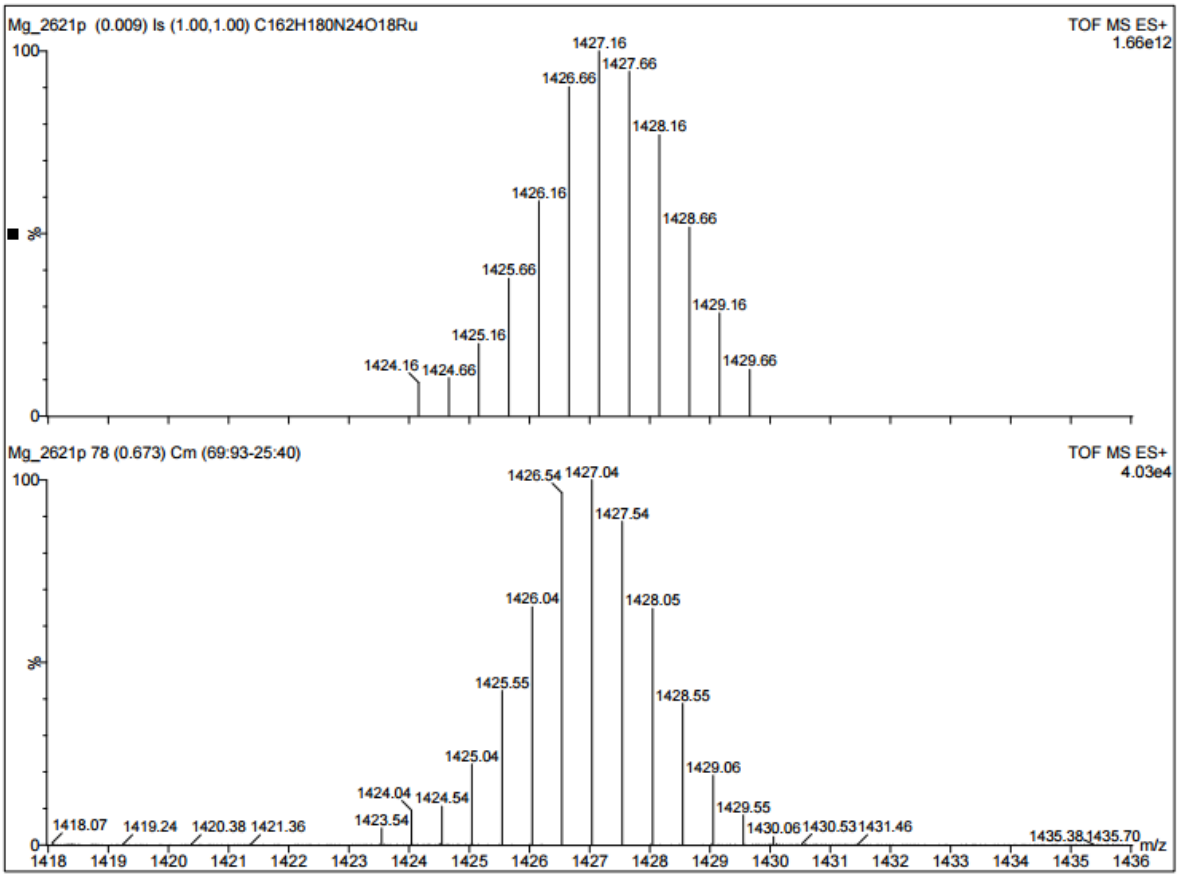


Figure S32. ESI-MS m/2z traces of (**L1B**)$_3$Ru complex (bottom) and computed ESI-MS spectrum (top).

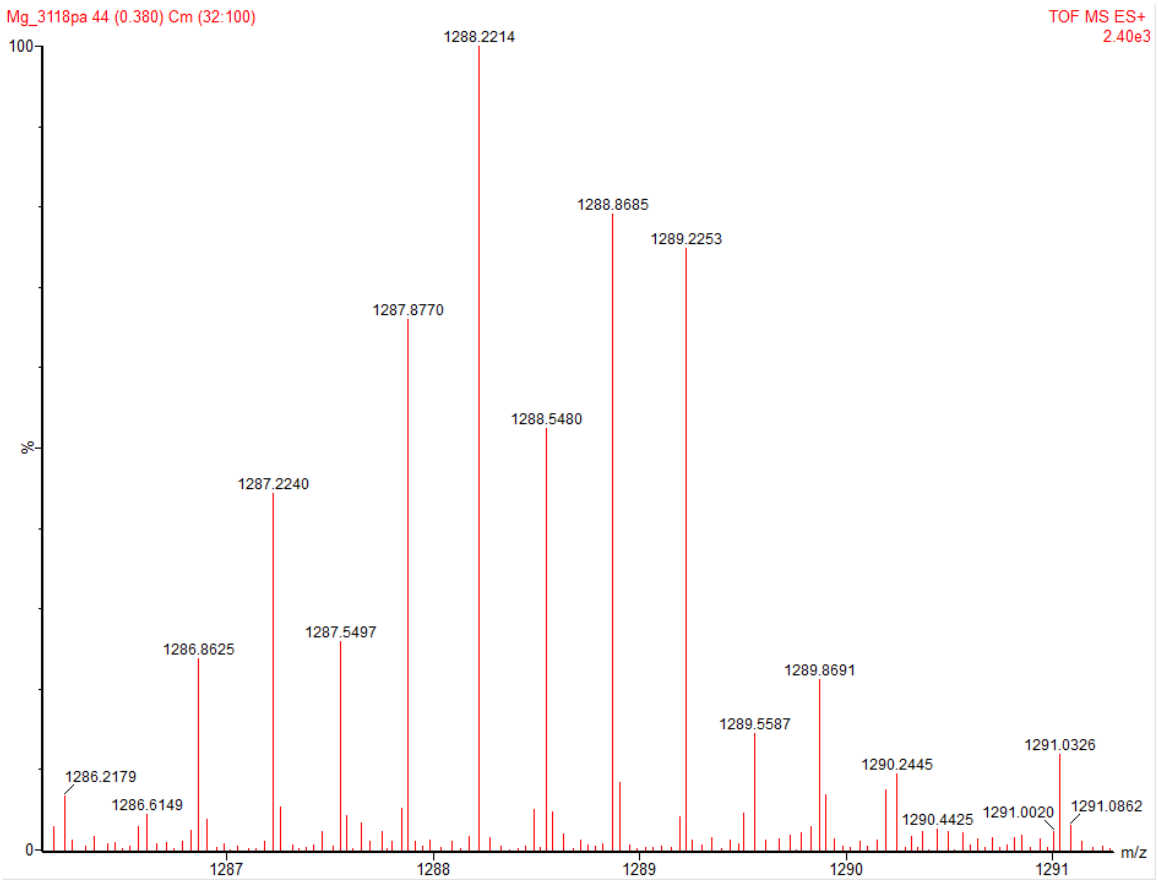


Figure S33. ESI-MS m/3z traces of (**L2B**)$_3$Ru$_2$-PF$_6$ complex.

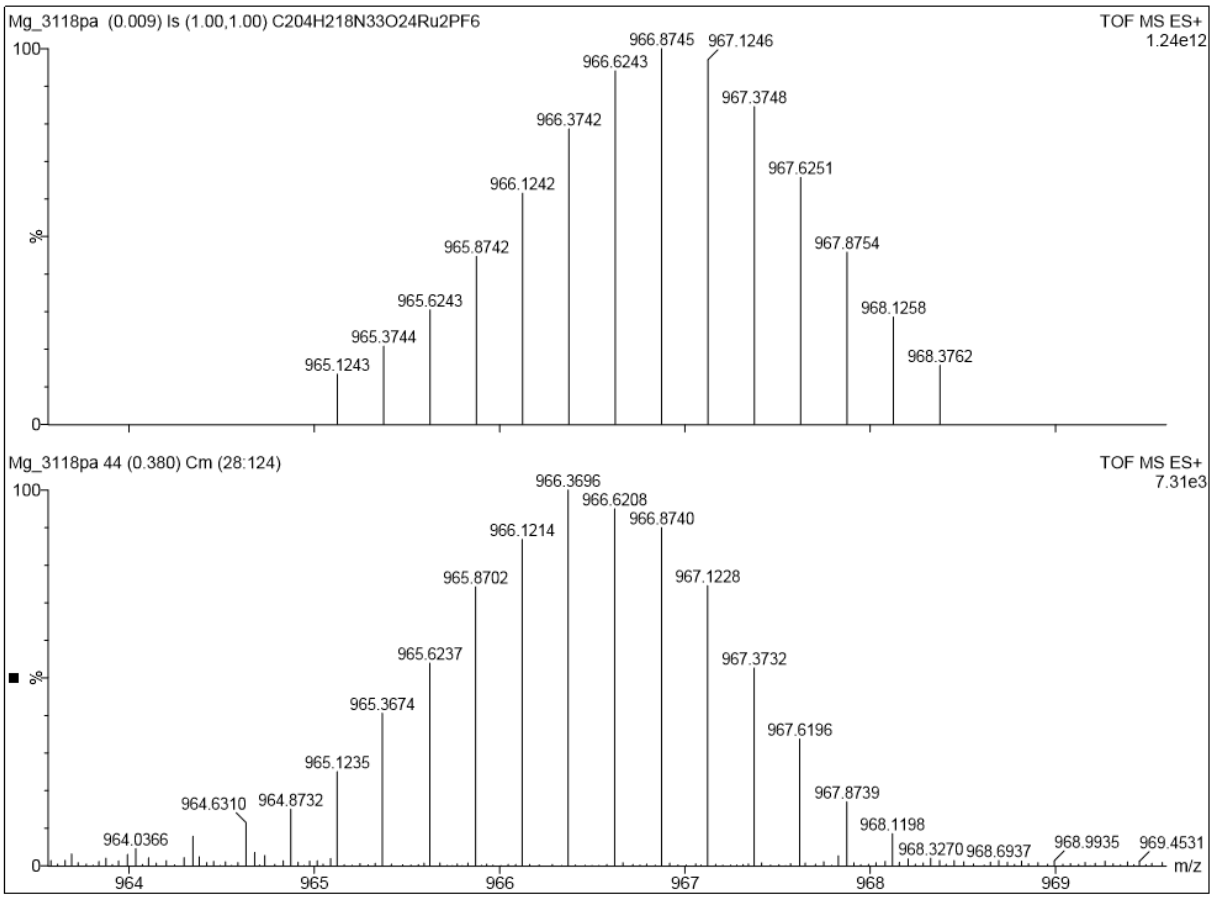


Figure S34. ESI-MS m+H/4z traces of (**L2B**)$_3$Ru$_2$-PF$_6$ complex (bottom) and computed ESI-MS spectrum (top).

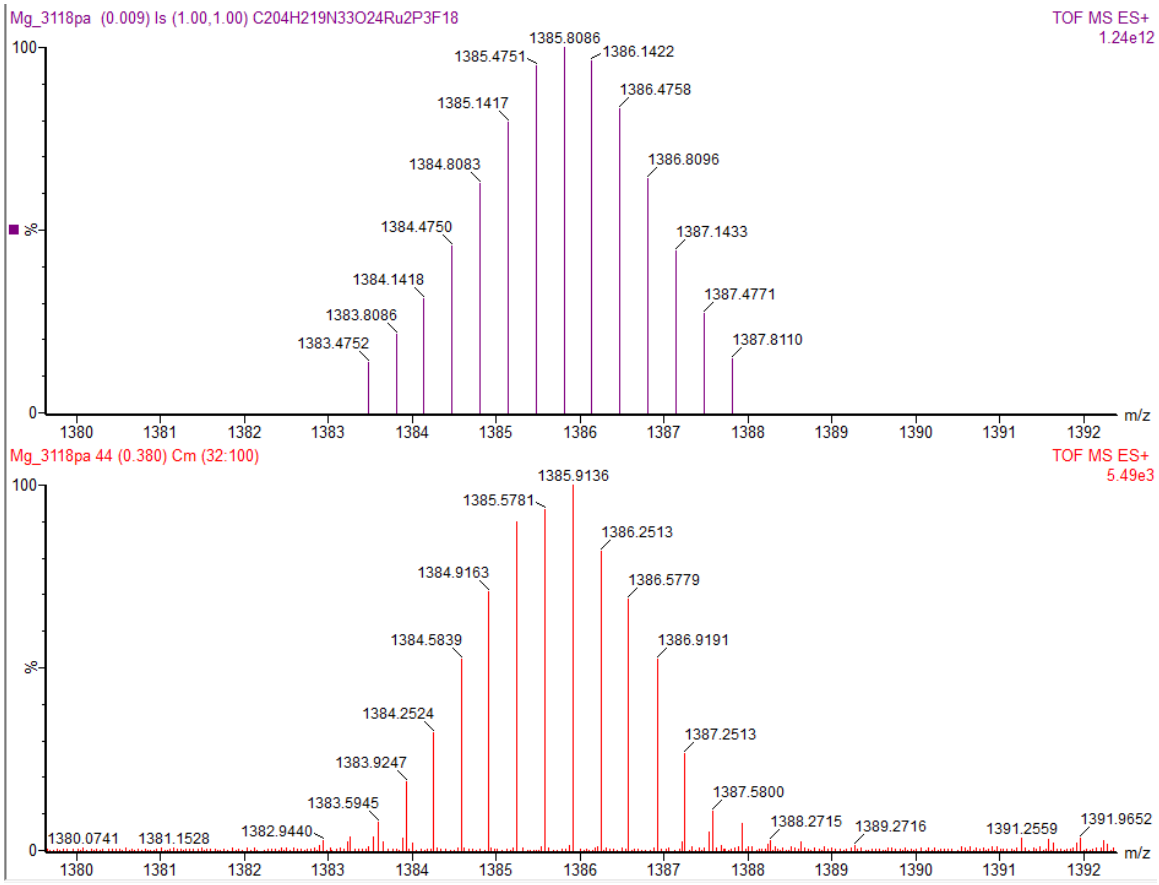


Figure S35. ESI-MS m+2H/3z traces of (**L2B**)$_3$Ru$_2$-(PF$_6$)$_3$ complex (bottom) and computed ESI-MS spectrum (top).

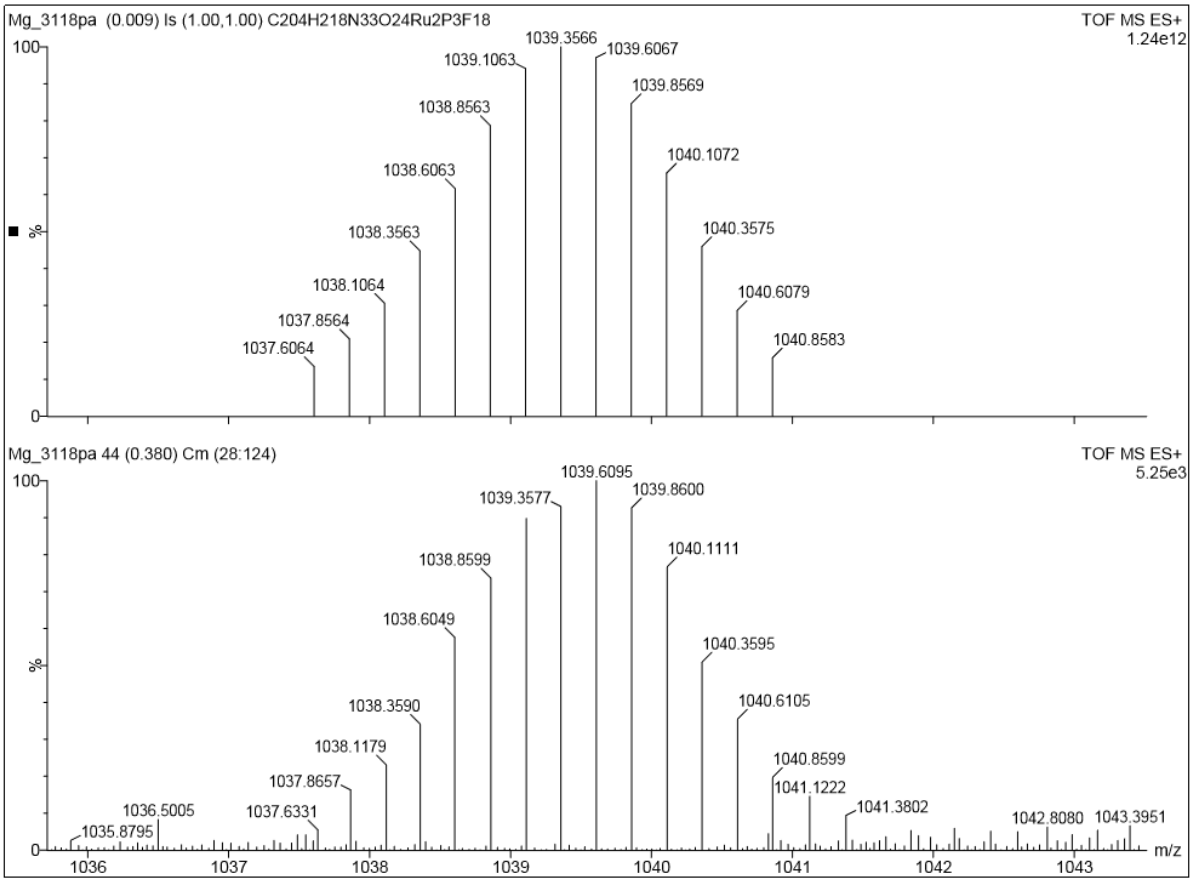


Figure S36. ESI-MS m+3H/4z traces of (**L2B**)$_3$Ru$_2$-(PF$_6$)$_3$ complex (bottom) and computed ESI-MS spectrum (top).

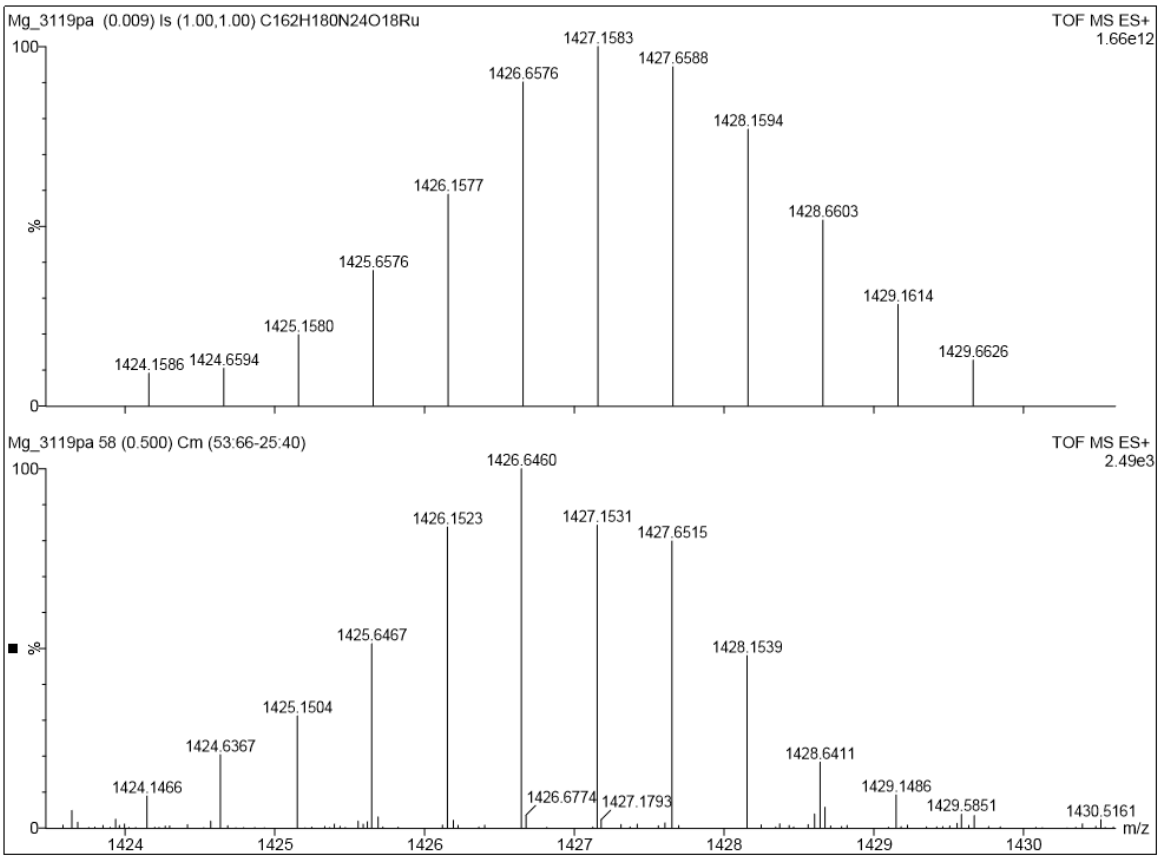


Figure S37. ESI-MS m/2z traces of (**R-L1B**)$_3$Ru complex (bottom) and computed ESI-MS spectrum (top).

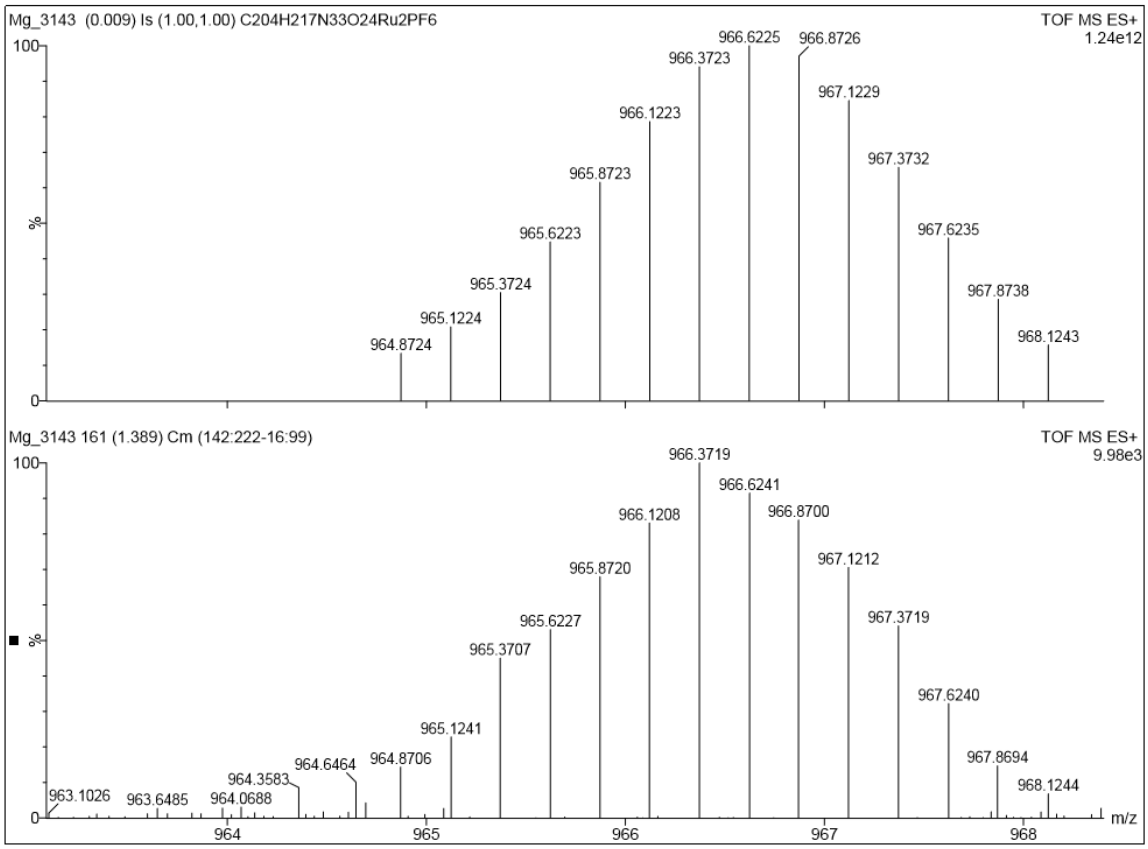


Figure S38. ESI-MS m/4z traces of (**R-L2B**)$_3$Ru$_2$-PF$_6$ complex (bottom) and computed ESI-MS spectrum (top).

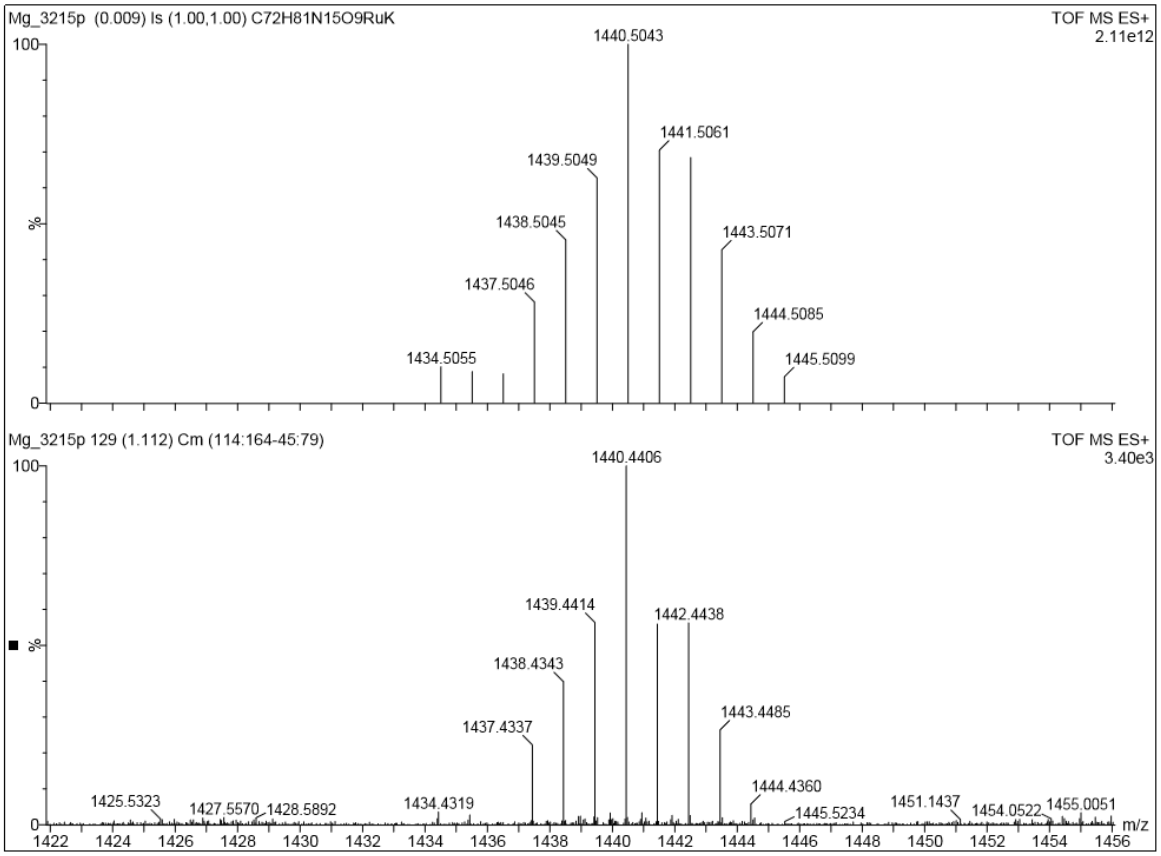


Figure S39. ESI-MS m/z traces of (**Di-L2B**)$_3$Ru$_2$-K$^+$ complex (bottom) and computed ESI-MS spectrum (top).

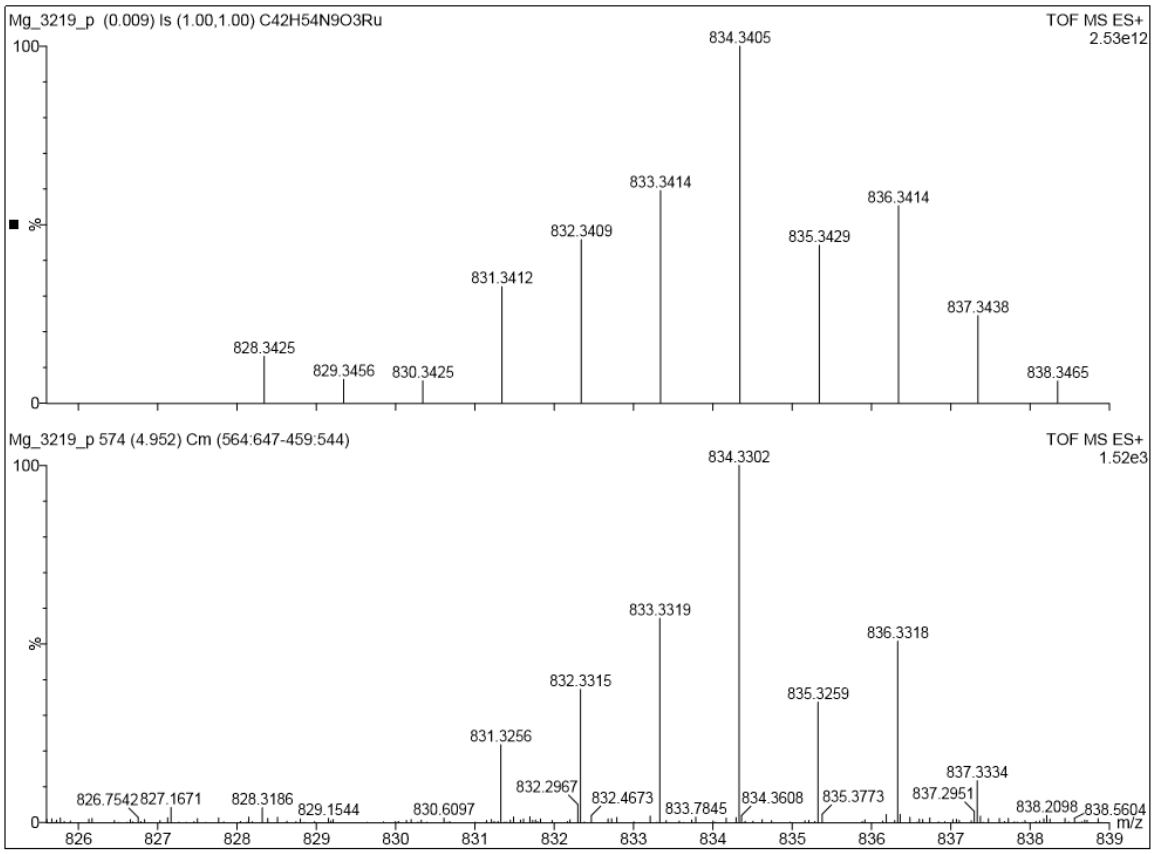


Figure S40. ESI-MS m/z traces of (**bp-DM**)$_3$Ru$_2$ complex (bottom) and computed ESI-MS spectrum (top).

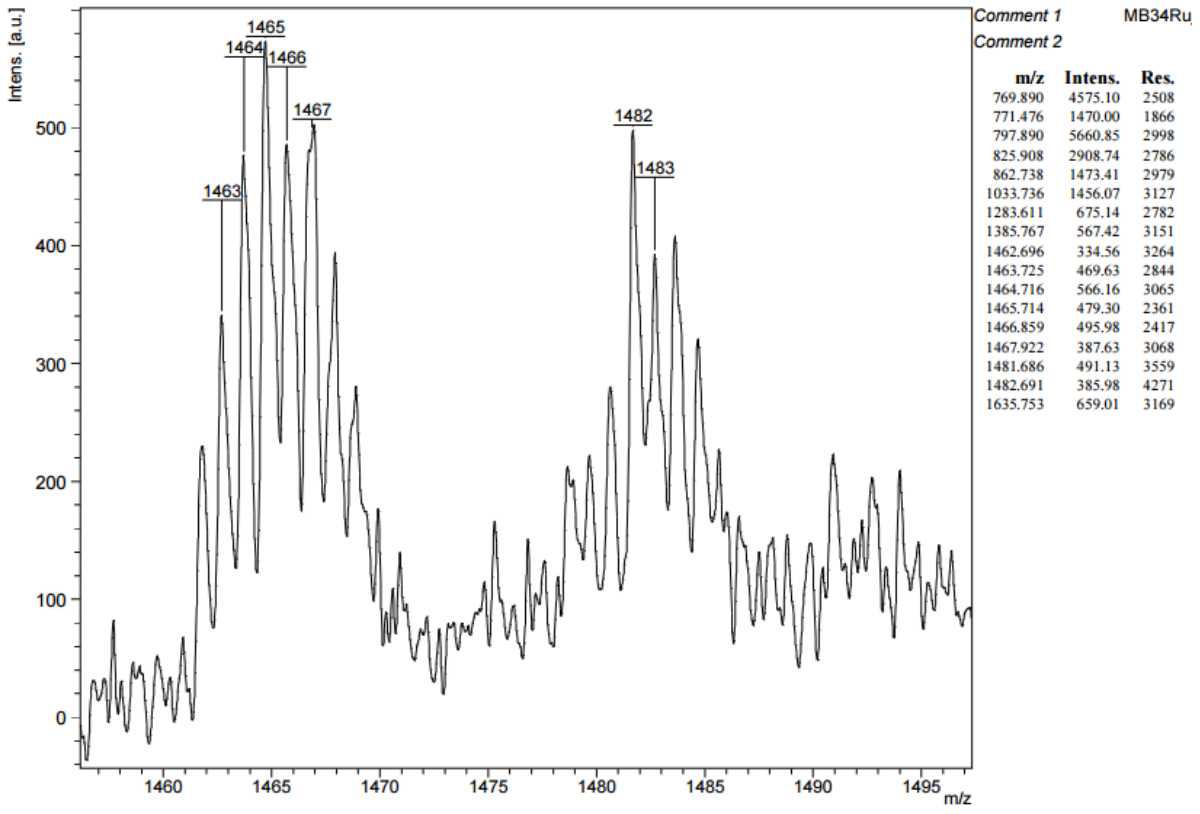


Figure S41. MALDI traces of (**C3B**)Ru complex.

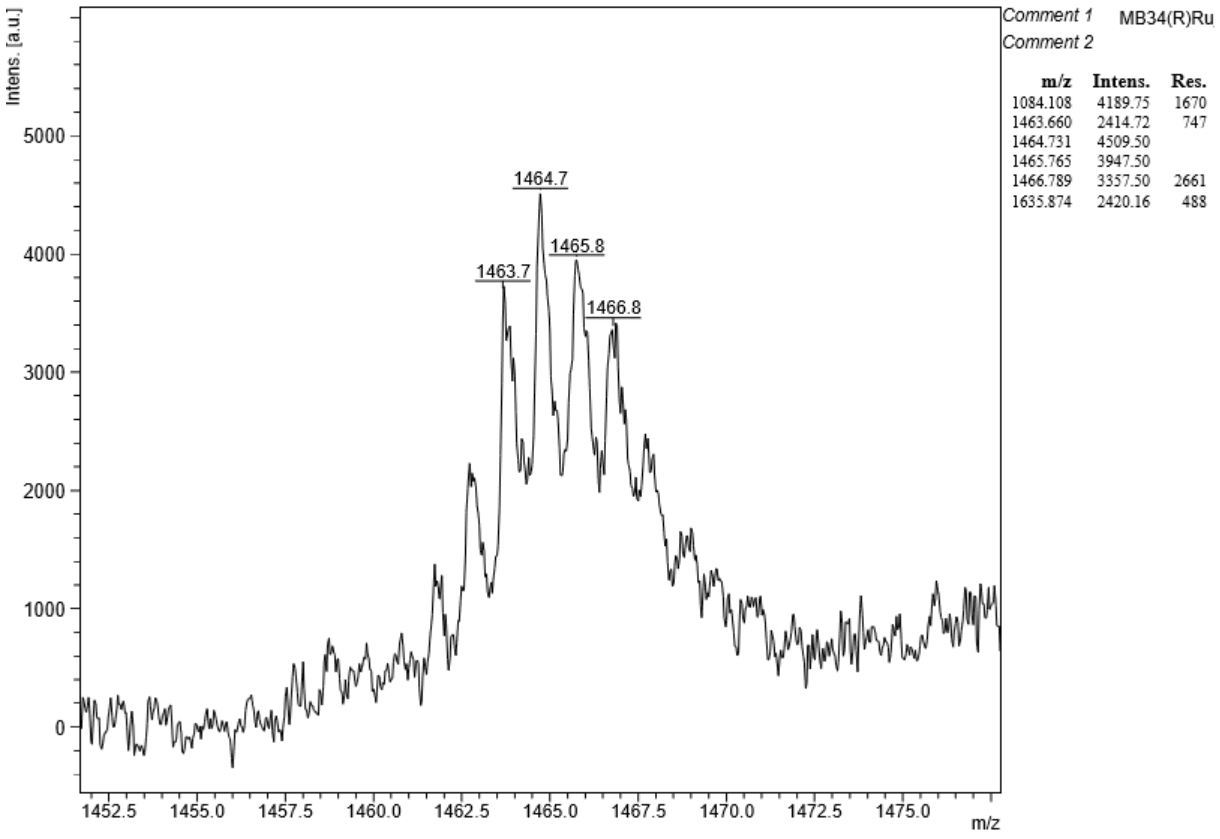


Figure S42. MALDI traces of (**R-C3B**)Ru complex.

NMR

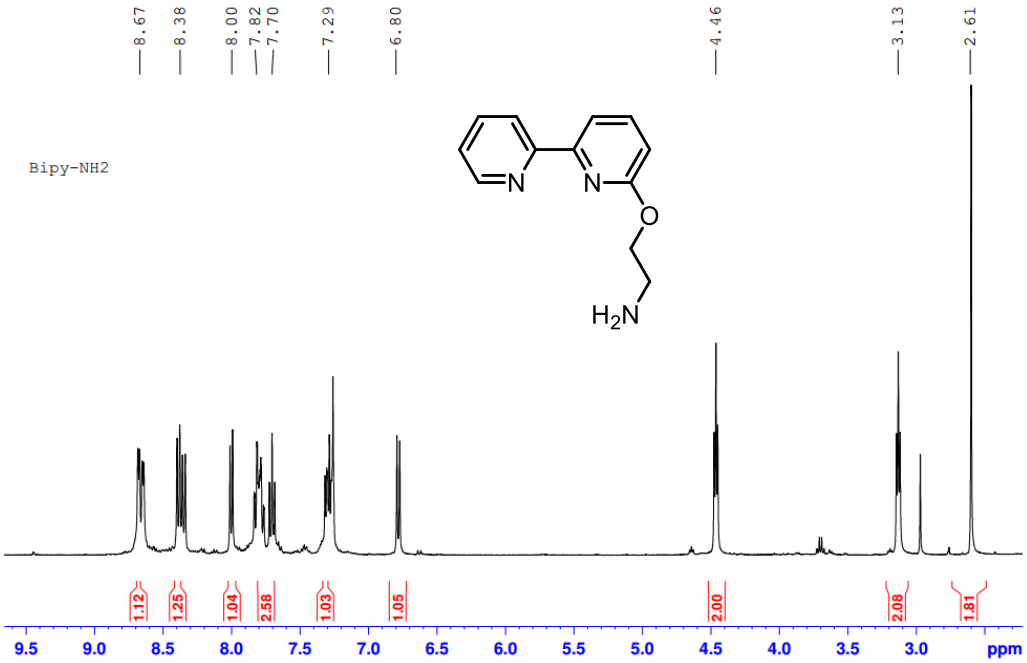


Figure S43. ^{1}H-NMR spectrum of the Nbp ligand.

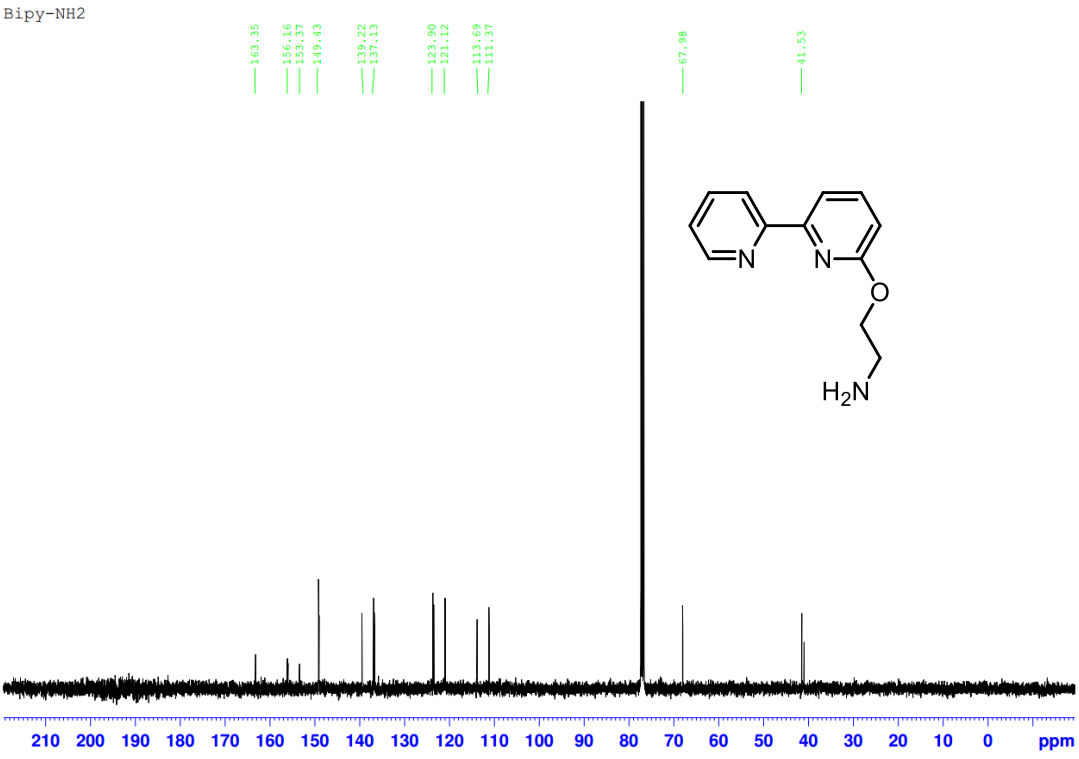


Figure S44. ^{13}C-NMR spectrum of the Nbp ligand.

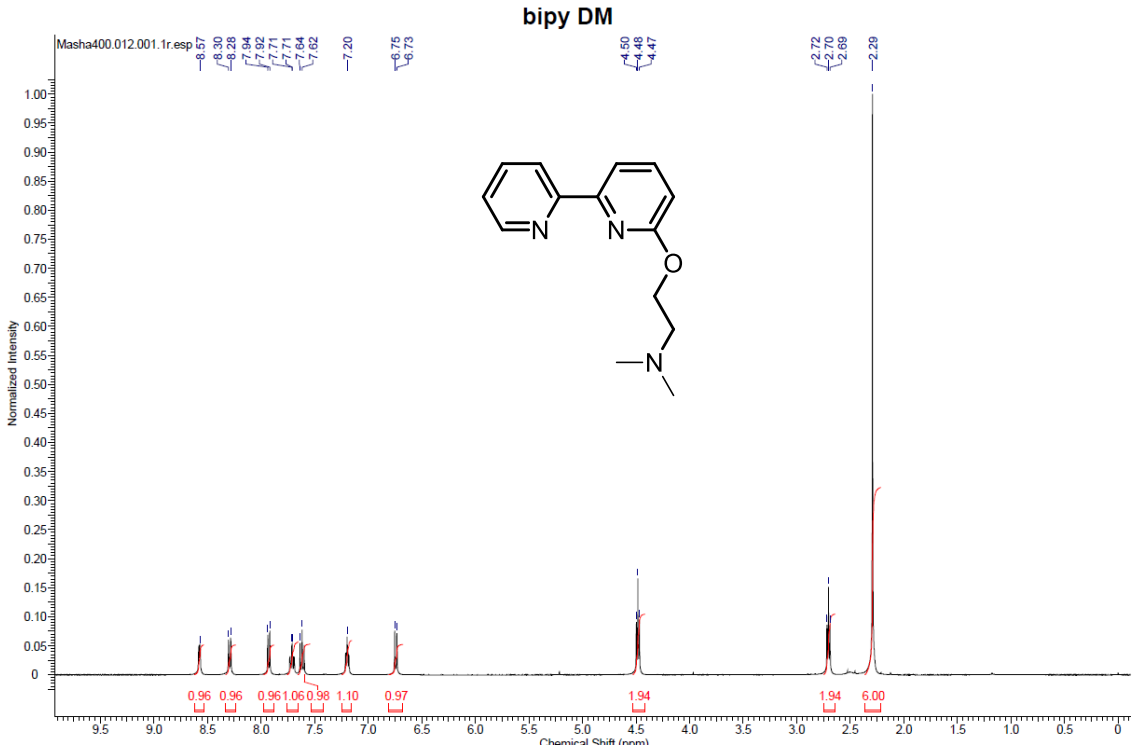


Figure S45. [1]H-NMR spectrum of the bpDM ligand.

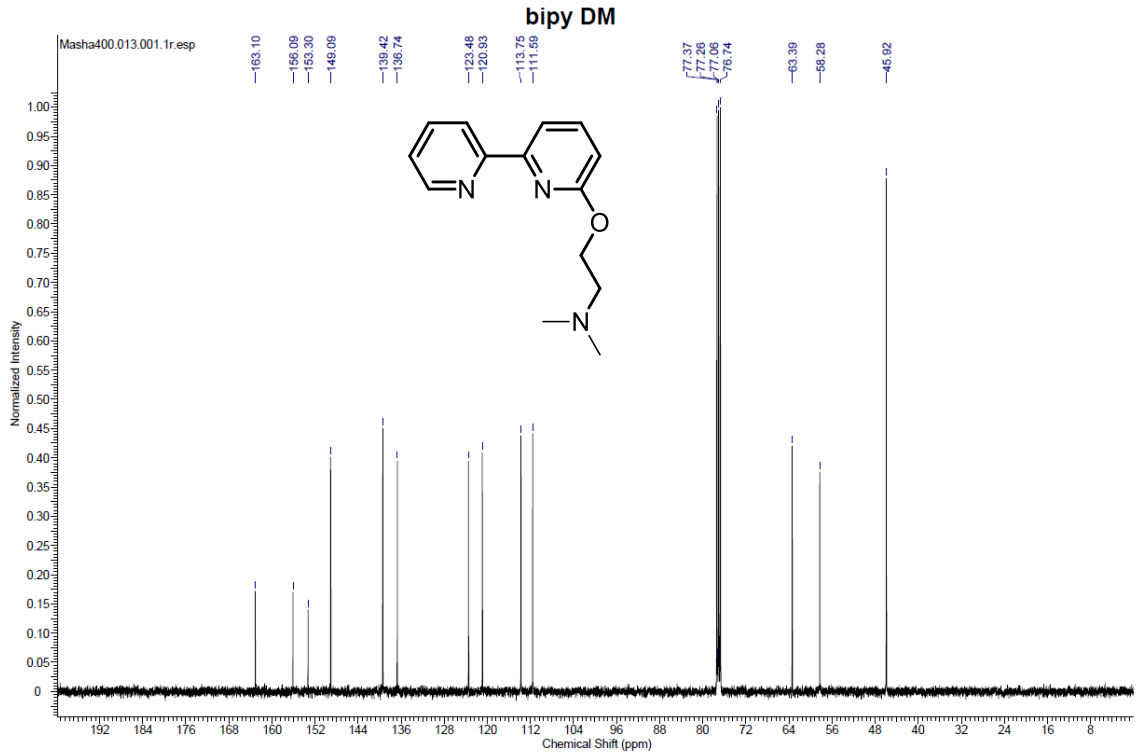


Figure S46. [13]C-NMR spectrum of the bpDM ligand.