Supporting information

Group 13 ion coordination to pyridyl models NAD$^+$ reduction potentials

Leo W. T. Parsons, James C. Fettinger, and Louise A. Berben*

Department of Chemistry, University of California, Davis CA 95616

e-mail: laberben@ucdavis.edu

Table of Contents

1. Experimental Section

Preparation of Compounds. All manipulations were carried out using standard Schlenk or glovebox techniques under a dinitrogen atmosphere. Unless otherwise noted, solvents were deoxygenated and dried by thorough sparging with Ar gas followed by passage through an activated alumina column. Deuterated solvents were purchased from Cambridge Isotopes Laboratories, Inc. and were degassed and stored over activated 3 Å molecular sieves prior to use. 2,6-bis(5-isobutyl-1-isopropyl-1H-pyrazol-3-yl)pyridine (pz_2P),[1] and **A**-$AlCl_4$,[1] were synthesized according to literature methods. All other reagents were purchased from commercial vendors and used without further purification.

[pz$_2$(*N*-MeP)]OTf (1-OTf). To a solution of 250 mg (0.614 mmol) pz_2P in dry toluene in an ice bath was added 74 μL methyl triflate and stirred overnight under a nitrogen atmosphere. The resulting white precipitate was collected, washed with ether, to remove any remaining pz_2P, and dried in vacuo to afford 216 mg (83 % yield) of **2**-OTf. ^1H NMR (600 MHz, $CDCl_3$): δ 8.46 (t, J = 8.0 Hz, 1H), 8.09 (d, J = 8.0 Hz, 2H), 6.68 (s, 2H), 4.54 (hept, J = 6.7 Hz, 2H), 4.50 (s, 3H), 2.57 (d, J = 7.2 Hz, 4H), 1.95 (hept, J = 6.8 Hz, 2H), 1.50 (d, J = 6.5 Hz, 12H), 0.98 (d, J = 6.5 Hz, 12H). ^1H NMR (600 MHz, C_6D_6) δ 7.61 (m, 1H), 7.52 (d, J = 8.1 Hz, 2H), 7.35 (s, 2H), 4.18 (s, 3H), 3.94 (m, 2H), 2.19 (d, J = 7.1 Hz, 4H), 1.91 (m, 2H), 1.21 (d, J = 6.4 Hz, 12H), 0.88 (d, J = 6.4 Hz, 12H). ^1H NMR (400 MHz, THF-d_8): δ 8.51 (t, J = 8.0 Hz, 1H), 8.24 (d, J = 7.9 Hz, 2H), 7.02 (s, 2H), 4.71 (hept, J = 6.4 Hz, 2H), 4.57 (s, 3H), 2.67 (d, J = 7.2 Hz, 4H), 2.04 (hept, J = 6.8 Hz, 2H), 1.52 (d, J = 6.5 Hz, 12H), 1.02 (d, J = 6.6 Hz, 12H). ^{13}C NMR (151 MHz, $CDCl_3$) δ: 150.56, 144.14, 143.86, 141.82, 127.72, 120.03, 108.49, 50.75, 47.04, 34.23, 28.33, 22.81, 22.40. (ESI-HRMS) m/z: $[M]^+$ Calcd for $C_{26}H_{40}N_5$ 422.3287, Found 422.3265.

[(pz$_2$P)GaCl$_2$]GaCl$_4$ (2-GaCl$_4$). To a solution of 200 mg (0.490 mmol) of pz_2P in THF was added 180 mg (1.02 mmol) $GaCl_3$ in THF. The resulting solution was stirred for 1 hour. The solvent was removed in vacuo, and the solid residue was triturated with ether to afford 303 mg of **1**-$GaCl_4$ as a white powder (80 % yield). ^1H NMR (600 MHz, C_6D_6): δ 8.03 (t, J = 7.9 Hz, 1H), 7.84 (d, J = 7.8 Hz, 2H), 7.01 (s, 2H), 5.17 (hept, J = 7.4 Hz, 2H), 2.29 (d, J = 7.2 Hz, 4H), 2.05 (hept, J = 6.8 Hz, 4H), 1.27 (d, J = 7.0 Hz, 12H), 0.91 (d, J = 5.9 Hz, 12H) ^1H NMR (400 MHz, THF-d_8): δ 8.70 (t, J = 7.9 Hz, 1H), 8.34 (d, J = 7.9 Hz, 2H), 7.29 (s, 2H), 5.34 (hept, J = 7.1 Hz, 2H), 2.86 (d, J = 7.3 Hz, 4H), 2.21 (hept, J = 6.7 Hz, 2H), 1.72 (d, J = 7.1 Hz, 12H), 1.10 (d, J = 6.6 Hz, 12H). ^{13}C NMR (151 MHz, C_6D_6) δ 150.23, 147.25, 143.87, 140.54, 122.26, 106.12, 56.48, 35.07, 27.87, 22.13, 20.97. Anal. Calcd. for $C_{25}H_{37}Cl_6N_5Ga_2$: C, 39.52; H, 4.91; N, 9.22. Found: C, 39.23; H, 5.08; N, 9.37.

[(pz$_2$P)GaCl$_2$]BPh$_4$ (2-BPh$_4$). To a solution of 300 mg (0.395 mmol) of complex **1**-$GaCl_4$ in THF was added 202 mg (0.593 mmol) sodium tetraphenylborate in THF. The colorless solution turned clear yellow and was stirred for 1 hour then concentrated in vacuo. The concentrate was triturated with ether

and washed with MeCN to remove excess NaBPh$_4$ (236 mg, 69 % yield). Crystals of **1**-BPh$_4$ suitable for X-ray diffraction were grown as yellow blocks from a saturated THF solution layered with hexane. ^{1}H NMR (600 MHz, CDCl$_3$): δ 7.44 – 7.36 (m, 8H), 7.12 (t, J = 7.9 Hz, 1H), 6.88 (t, J = 7.4 Hz, 8H), 6.73 – 6.70 (t, 4H), 6.64 (d, J = 7.9 Hz, 2H), 5.11 (hept, J = 7.1 Hz, 2H), 2.54 (d, J = 7.2 Hz, 4H), 1.93 (hept, J = 6.8 Hz, 2H), 1.56 (d, J = 7.1 Hz, 12H), 0.94 (d, J = 6.6 Hz, 12H) ^{13}C NMR (151 MHz, CDCl$_3$) δ 164.55, 150.20, 147.86, 143.78, 140.50, 138.28, 125.80, 121.93, 121.66, 105.50, 57.11, 35.52, 28.34, 22.61, 21.76. Anal. Calcd for C$_{49}$H$_{57}$BCl$_2$N$_5$Ga: C, 67.85; H, 6.62; N, 8.07. Found: C, 67.72; H, 6.75; N, 8.07.

X-ray Structure Determinations. X-ray diffraction studies were carried out on a Bruker SMART APEX Duo diffractometer equipped with a CCD detector.[2] Measurements were carried out at −175 °C using Mo Kα (0.71073 Å) and Cu Kα (1.54178 Å) radiation. Crystals were mounted on a Kaptan loop with paratone-N oil. The initial lattice parameters were obtained from least-squares analysis of more than 100 centered reflections; these parameters were later refined against all data. Data were integrated and corrected for Lorentz polarization effects using SAINT[3] and were corrected for absorption effects using SADABS2.3.[4] Space-group assignments were based on systematic absences, E statistics, and the successful refinement of the structures. Structures were solved by direct methods with the aid of successive difference Fourier maps and were refined against all data using the SHELXTL 2014/7 software package.[5] Thermal parameters for all non-H atoms were refined anisotropically. H atoms, where added, were assigned to ideal positions, and refined using a riding model with an isotropic thermal parameter 1.2 times that of the attached C atom (1.5 times for methyl H atoms).

Other Physical Measurements. ^{1}H and ^{13}C NMR spectra were recorded at ambient temperature using a Varian 600 MHz spectrometer and Bruker 400 MHz spectrometer. Chemical shifts were referenced to a residual solvent. Elemental analyses were performed by the Microanalytical Laboratory at The University of California, Berkeley. Samples were injected into a mixture of 50% MeOH and 0.1% Formic Acid/H20 at a flow of 200 ul/min. Source parameters were 5kV spray voltage, capillary temperature of 275 C and sheath gas setting of 20. Spectral data were acquired at a resolution setting of 100,000 FWHM with the lockmass feature which typically results in a mass accuracy < 2 ppm. Cyclic voltammograms (CVs) were recorded in a nitrogen atmosphere using Schlenk air-free techniques. A CH Instruments Electrochemical Analyzer Model 620D with a glassy carbon working electrode (CH Instruments, nominal surface area of 0.071 cm^2), a platinum wire auxiliary electrode, and an Ag/AgNO3 (0.001M) non-aqueous reference electrode with a Vycor tip. All potentials are referenced to the SCE couple, and ferrocene was used as an external standard where the E½ of ferrocene/ferrocenium is +0.56 V vs. SCE in 0.3 M Bu$_4$NPF$_6$ THF or +0.40 V vs. SCE in 0.1 M Bu$_4$NPF$_6$ MeCN.[6] Bu$_4$NPF$_6$ was recrystallized from ethanol and placed under vacuum for 72 hours before electrolyte solutions were made.

Electrolyte solutions were stored over 3 Å molecular sieves for at least 48 hours before use. Sieves were activated by heating under vacuum at 270°C for at least 72 hours.

2. Calculations and Equations

Calculation S1. Randles-Sevick Method for diffusion-controlled process

Plots of the cathodic current density, j_{pc}, (A cm^{-2}) vs the square root of the scan rate, $\upsilon^{1/2}$, (Vs^{-1}) which produce a straight line are consistent with a diffusion controlled one electron event as described by the Randles-Sevick equation (eq. S4)[7,8,9].

$$j_p = 0.4463nFC(\frac{nFD}{RT})^{0.5}\upsilon^{0.5}$$

(equation S4)

Where n is the number of electrons transferred in the redox event (assumed n = 1 as outlined in equation 1) D is the diffusion coefficient (cm^2 s^{-1}), C is the bulk concentration (mol cm^{-3}), F, R and T are Faraday's constant (C mol^{-1}), the ideal gas constatnt (J K^{-1} mol^{-1}), and temperature (K), respectively.

Calculation S2. Method for preparation of Figure S4.

Using the "advanced search query builder" on the protein data bank (PDB) website, the following two structural attributes were searched 1) comp ID = NAD and 2) Data collection resolution < 2 Å. This search returned 620 protein structures. These structures were then downloaded in .pdb format from the PDB in 13 batches. Using the terminal emulator for macOS each downloaded batch was uncompressed, and global regular expression print "grep" command used to transfer the NAD coordinates to a new .pdb file. The grep command was again used to remove the HETATM text before the NAD coordinates and the resulting file saved again in .pdb format. These files were then opened in mercury 2020.3.1 downloaded from the Cambridge Crystallographic Data Center. The bond list feature of mercury was then used to export the bond distances for the NAD molecules as a .csv file. Excel was then used to compile these bond distances and the arithmetic mean for the C_o-N_{py}, C_o-C_m and C_m-C_p bonds calculated and reported in Figure 2. Excel was also used to calculate the standard deviations for these structures and gave values of 0.0896, 0.0981 and 0.0963 for C_o-N_{py}, C_o-C_m and C_m-C_p respectively.

2. Tables

Table S1. Crystallographic data for compounds **1**-OTf and **2**- BPh_4.

	1-OTf [a]	**2**-BPh_4 [b]
Formula	$C_{27}H_{40}F_3N_5O_3S$	$C_{49}H_{57}BCl_2GaN_5$
Crystal size	$0.552 \times 0.162 \times 0.143$	$0.406 \times 0.278 \times 0.192$
Formula weight, g mol^{-1}	571.70	867.42
Space group	P-1	$P2_1$
a, Å	10.5277(3)	9.3560(8)
b, Å	12.2580(3)	15.4776(13)
c, Å	14.2389(4)	16.0042(14)
α, deg	110.4846(8)	90
β, deg	91.5578(8)	106.6450(14)
γ, deg	104.6039(9)	90
V, Å^3	1652.15(8)	2220.4(3)
Z	2	2
T, K	100(2)	90(2)
ρ, calcd, g cm^{-3}	1.149	1.297
Refl. Collected / $2\theta_{max}$	11526 / 137.18	14392 / 54.99
Unique refl. / $I > 2\sigma(I)$	6081 / 5944	10122 / 7833
No. Parameters / restraints	398 / 31	547 / 21
λ,Å/μKα,cm^{-1}	1.54178	0.71073
R_1/GOF	0.0421 / 1.026	0.0464 / 1.007
$wR_2(I > 2\sigma(I))^b$	0.1142	0.0858
Residual density, e Å^{-3}	0.285 / -0.403	0.665 / -0.425

[a] Obtained with graphite-monochromated Cu Kα (λ = 1.54178 Å) radiation. [b] Obtained with graphite-monochromated Mo Kα (λ = 0.71073 Å) radiation. [c] $R1 = \Sigma|| F_o |-F_c||/\Sigma|F_o |$, $wR2 = \{\Sigma[w(F_o^2-F_c^2)^2]/ \Sigma [w(F_o^2)^2]\}^{1/2}$.

Table S2. Selected bond length and angles for **1**$^+$ and **2**$^+$.

	1$^+$	**2**$^+$
N_{pz}-C_{pz}	1.340(2)	1.346(7)
C_{pz}-C_o	1.466(2)	1.465(7)
N_{py}-C_o	1.372(2)	1.346(6)
C_o-C_m	1.390(2)	1.377(7)
C_m-C_{py}	1.370(2)	1.384(6)
C_{py}-$C_{m'}$	1.386(3)	1.374(7)
$C_{m'}$-$C_{o'}$	1.377(2)	1.381(7)
N_{py}-$C_{o'}$	1.374(2)	1.354(5)
$C_{o'}$-$C_{pz'}$	1.471(2)	1.453(7)
$N_{pz'}$-$C_{pz'}$	1.338(2)	1.338(6)
N_{py}-X	1.502(2)	1.986(3)
τ_5 [10]	-	0.57

3. Figures
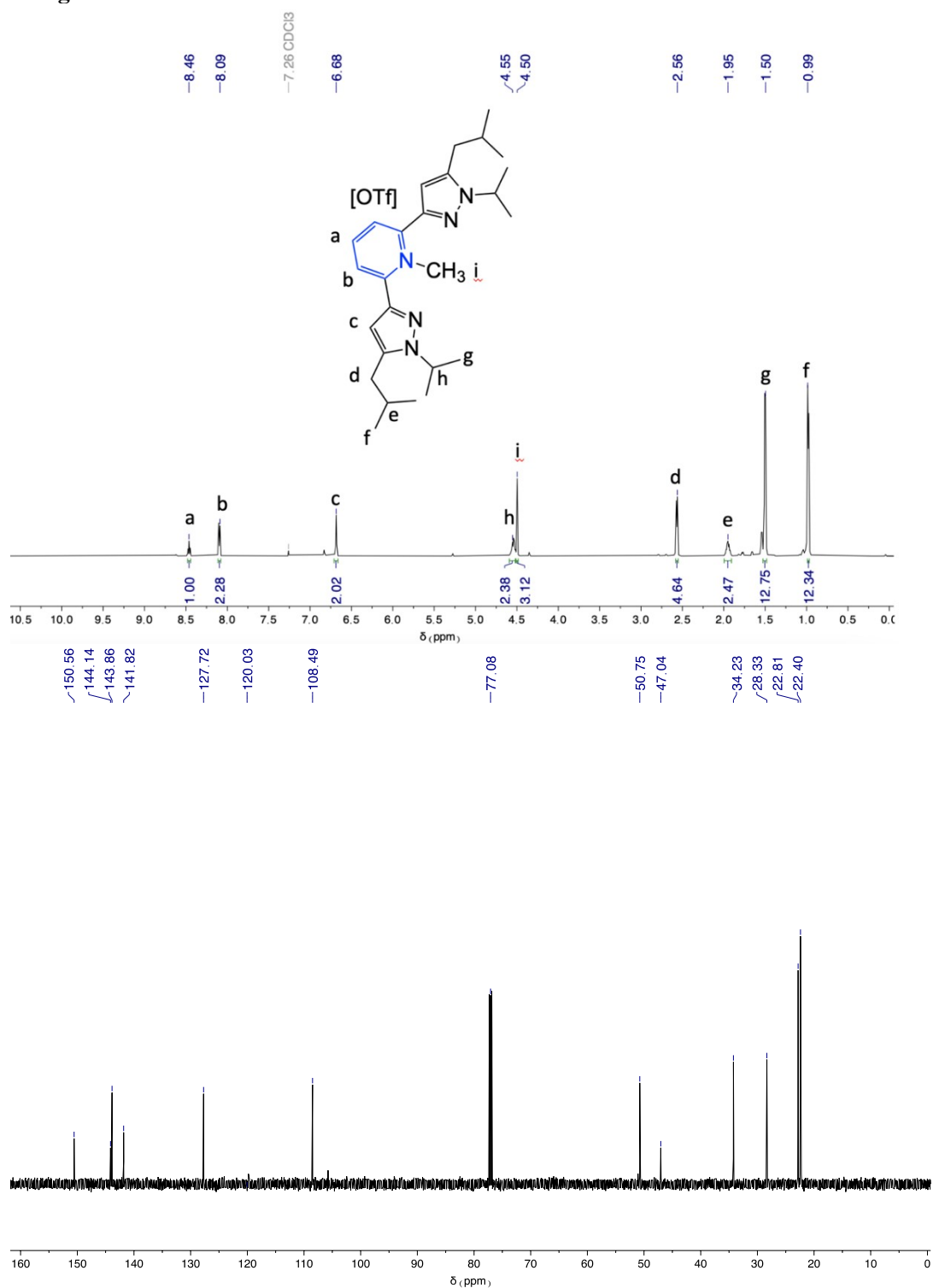
Figure S1. ^{1}H NMR (top) and ^{13}C NMR (bottom) spectra of **1**-OTf collected in CDCl$_3$.
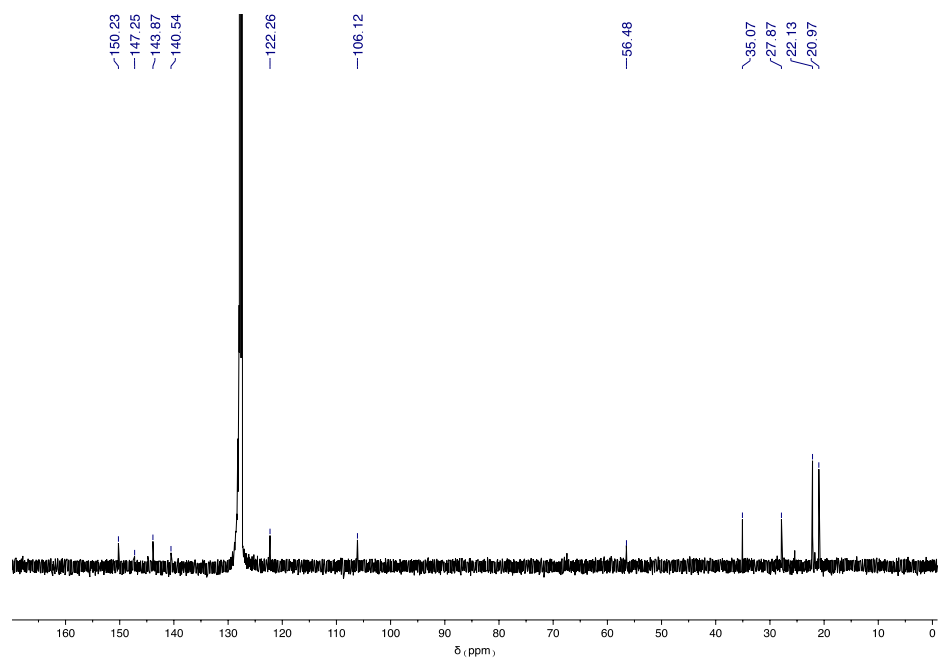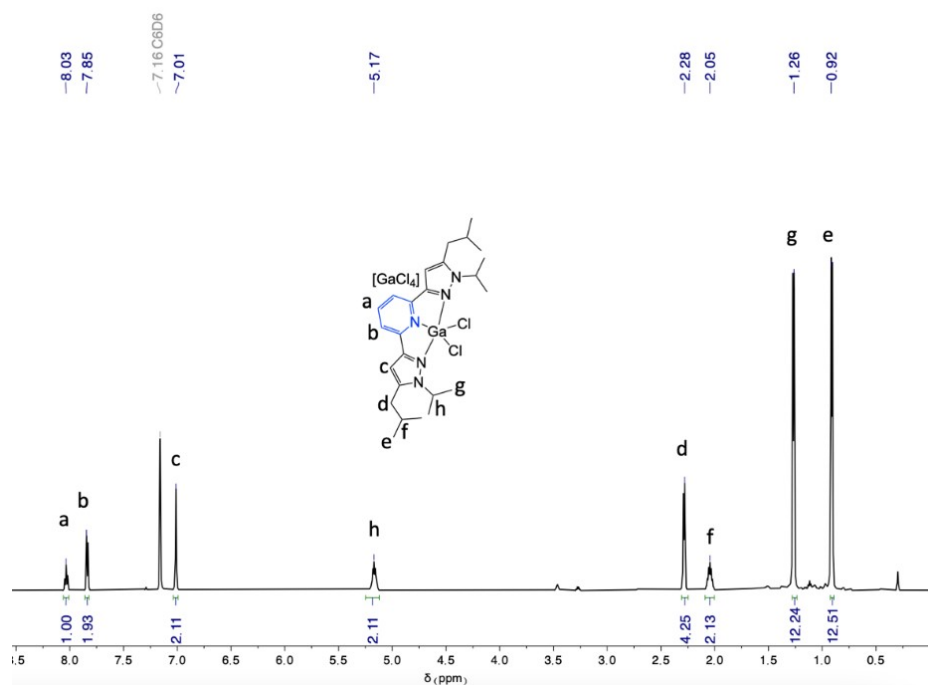
Figure S2. ^1H NMR (top) and ^{13}C NMR (bottom) spectra of **2**-GaCl$_4$ collected in C$_6$D$_6$.
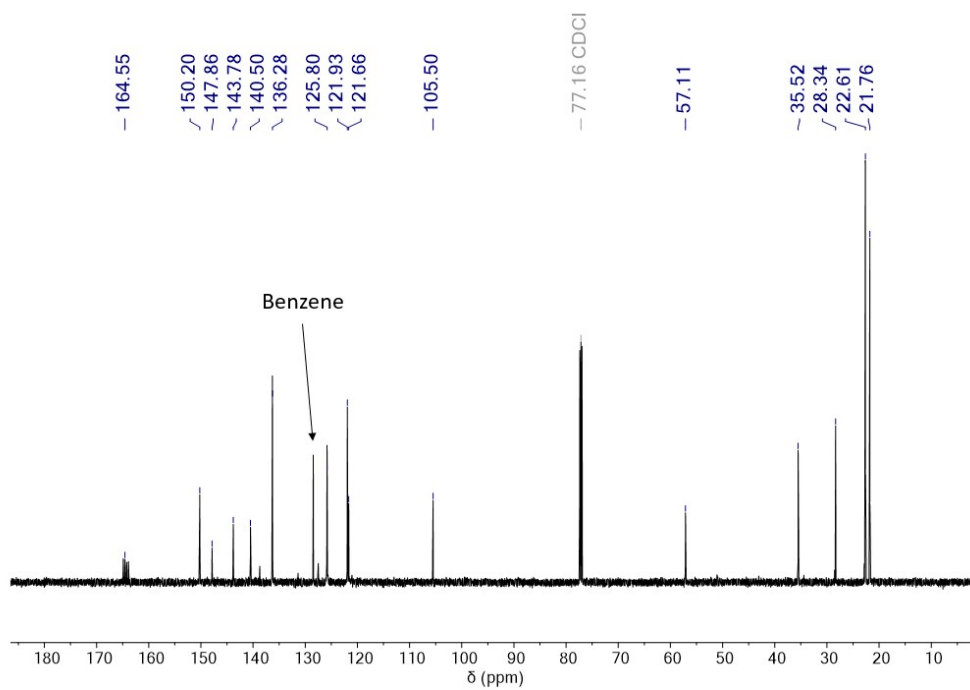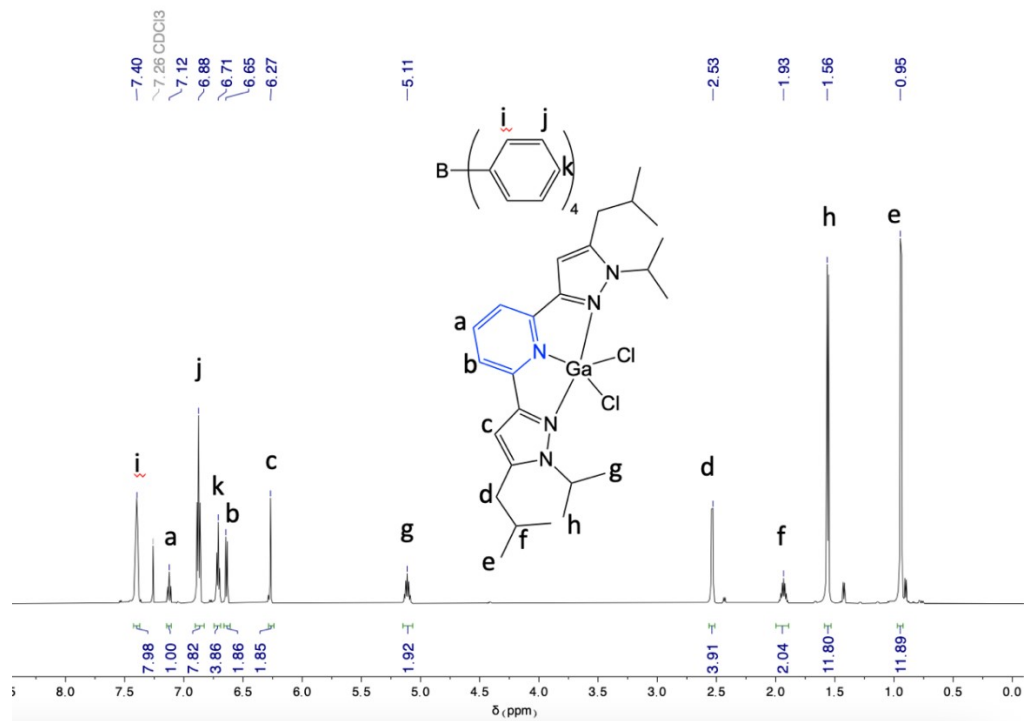
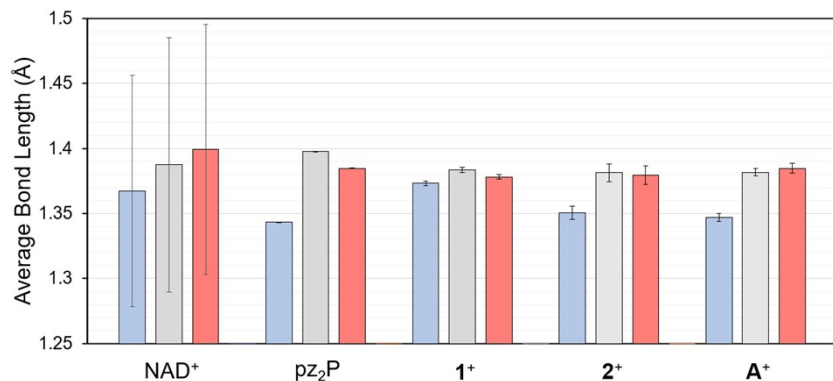**Figure S3.** ^{1}H NMR (top) and ^{13}C NMR (bottom) spectra of **2**-BPh$_4$ collected in CDCl$_3$.

Figure S4: Average bond lengths for the C_o-N_{py} (blue), C_m-C_o (grey) and C_m-C_p (red) bonds in the pyridine ring for NAD^+, pz_2P, $\mathbf{1^+}$, $\mathbf{2^+}$ and $\mathbf{A^+}$ (esd represented by error bars). Bond lengths for and NAD^+ averaged from reported crystal structures of NAD^+ dependent protein structures crystalized with NAD^+ (see Calculation S2 for details). Standard deviations of the bonds for surveyed NAD^+ structures were too large to draw meaningful comparisons. Atom labelling scheme was defined in Chart 1.
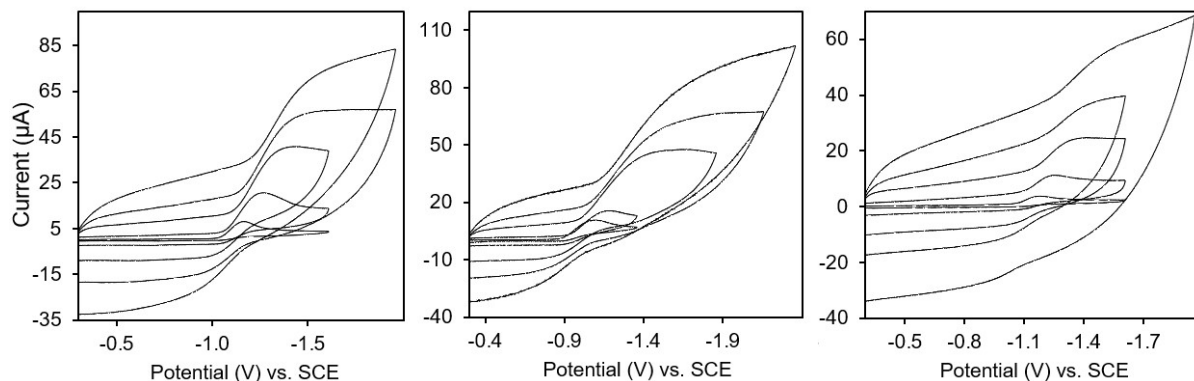

Figure S5: Cyclic voltammograms of 1.0 mM $\mathbf{1}$-OTf (left), $\mathbf{2}$-GaCl$_4$ (middle), $\mathbf{A}$-AlCl$_4$ (right) in 0.3 M Bu$_4$NBF$_4$ THF solution. Scan rate of 0.1, 1, 5, 10 and 20 Vs^{-1}.
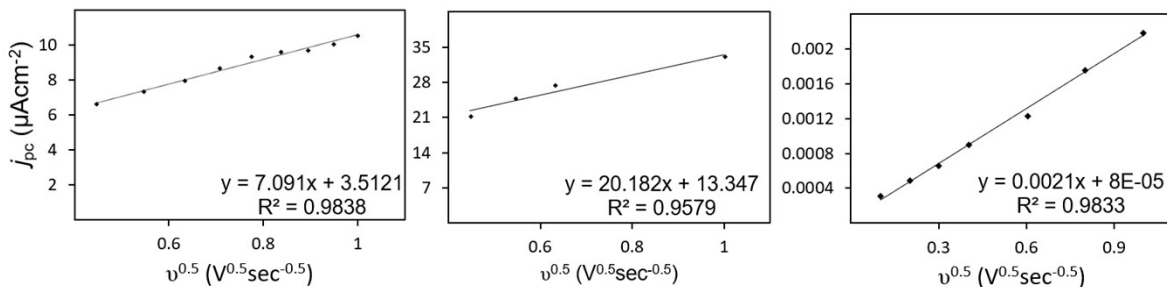
Figure S6: Plots of the j_{pc} vs $\upsilon^{0.5}$ for $\mathbf{1}$-OTf (left), $\mathbf{2}$-GaCl$_4$ (middle), $\mathbf{A}$-AlCl$_4$ (right) in 0.3 M Bu$_4$NBF$_4$ THF solution. Scan rates ranging from 0.1-1 Vs^{-1}. Data points taken from CVs in Figure 4.
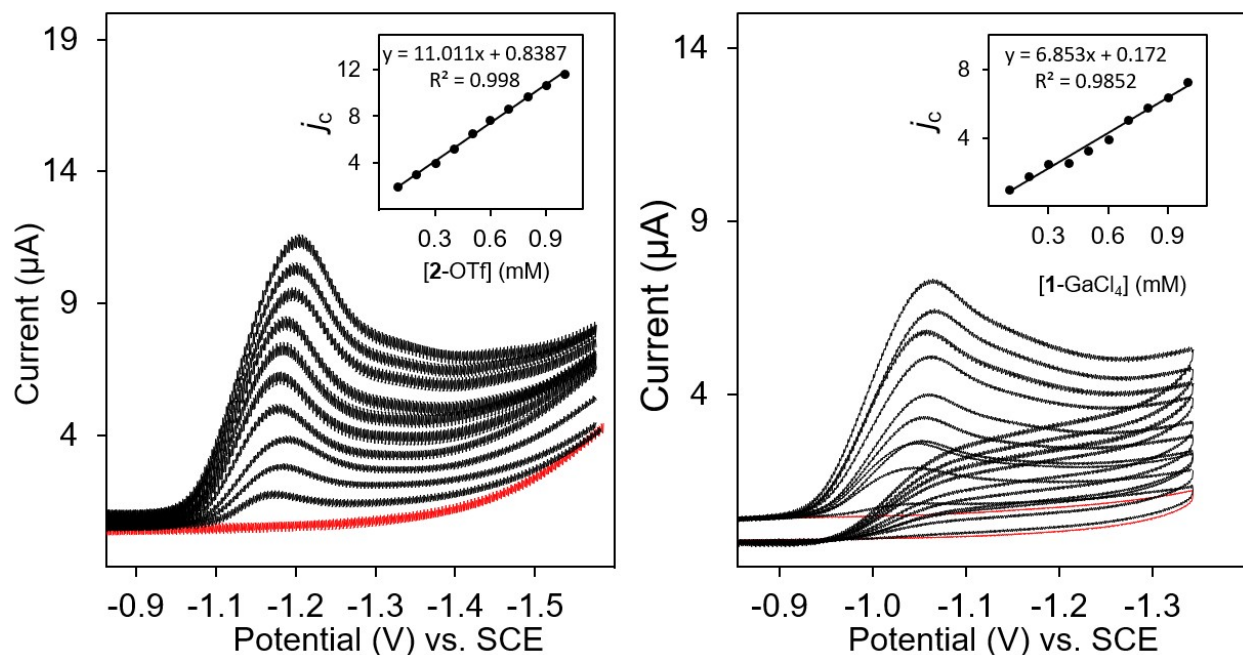
Figure S7: (left) Linear sweep voltammograms of 1.0 mM [Et$_3$NH][BF$_4$] (red trace) with aliquots of **2**-OTf, (black lines) in 0.3 M Bu$_4$NBF$_4$ THF solution. Inset: j_c vs [**1**-OTf]. (right) CVs of 1.0 mM [Et$_3$NH][BF$_4$] (red trace) with aliquots of **1**-GaCl$_4$, (black lines) in 0.3 M Bu$_4$NBF$_4$ THF solution. Inset: j_c vs [**2**-GaCl$_4$]. Data shows the order with respect to catalyst as 1st order in support of equation 2, main text.

4. References

[1] T. J. Sherbow, J. C. Fettinger and L. A. Berben, *Inorg. Chem.,* 2017, **56**, 8651-8660.

[2] SMART Software Users Guide, Version 5.1, Bruker Analytical X-Ray Systems, Inc.; Madison, WI 1999.

[3] SAINT Software Users Guide, Version 7.0, Bruker Analytical X-Ray Systems, Inc.; Madison, WI 1999.

[4] Sheldrick, G. M. SADABS, Version 2.03, Bruker Analytical X-Ray Systems, Inc.; Madison, WI 2000.

[5] Sheldrick, G. M. SHELXTL Version 6.12, Bruker Analytical X-Ray Systems, Inc.; Madison, WI 1999.

[6] N. G. Connelly and W. E. Geiger, *Chem. Rev.,* 1996, **96**, 877-910.

[7] A. Taheri and L. A. Berben, *Inorg. Chem.*, 2016, **55**, 378-385.

[8] , J. E. B. Randles, *Trans. Faraday Soc.*, 1948, **44**, 327−338.

[9] A. J. Bard and L. R. Faulkner, *Electrochemical Methods: Fundamentals and Applications*, 2nd ed.; John Wiley and Sons: New York, 2001.

[10] A. W. Addison, T. N. Rao, J. Reedijk, J. van Rijin and G. C. Verschoor, *J. Chem Soc Dalton Trans*., 1984, **7**, 1349-1356.