**Reductive Alkylation of Proteins Using Iridium Catalyzed Transfer Hydrogenation**

Jesse M. McFarland and Matthew B. Francis*

*Department of Chemistry, University of California, Berkeley, CA 94720-1460, and*

*Material Science Division, Lawrence Berkeley National Labs, Berkeley, CA 94720-1460*

## Supporting Information

### General Procedures and Materials

Unless otherwise noted, all chemicals were obtained from commercial sources and used without further purification. Analytical thin layer chromatography (TLC) was performed on EM Reagent 0.25 mm silica Gel 60-$F_{254}$ plates with visualization by ultraviolet (UV) irradiation at 254 nm and/or staining with phosphomolybdic acid or dinitrophenyl hydrazine. Purifications by flash chromatography were performed using EM silica gel 60 (230-400 mesh). The eluting system for each purification was determined by TLC analysis. Chromatography solvents were used without distillation. All reactions were carried out under a nitrogen atmosphere in oven dried glassware unless otherwise noted. All organic solvents were removed under reduced pressure using a rotary evaporator. Tetrahydrofuran (THF) was distilled under a nitrogen atmosphere from sodium/benzophenone. Dichloromethane ($CH_2Cl_2$) was distilled under a nitrogen atmosphere from calcium hydride. Water (dd$H_2O$) used in biological procedures or as a reaction solvent was deionized using a NANOpure$^{TM}$ purification system (Barnstead, USA). Lysozyme (L-7001) from chicken egg white, cytochrome C (C-7752) from horse heart, myoglobin (M 1882) from horse heart, and ribonuclease A (R-5500) from bovine pancreas were purchased from Sigma and used without further purification. α-Chymotripsynogen A (#100477) was purchased from MP Biomedicals, Inc. and used without further purification. $[Cp^*IrCl_2]_2$ was purchased from Strem and used without further purification.

### Instrumentation and Sample Analysis Preparations

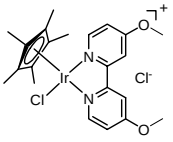
***NMR.*** $^1H$ and $^{13}C$ spectra were measured with a Bruker DRX-500 (500 MHz) spectrometer or a Bruker AVB-400 (400 MHz) spectrometer as noted. High temperature experiments were performed on a Bruker AVB-400 (400 MHz). $^1H$ NMR chemical shifts are reported as δ in units of parts per million (ppm) relative to methanol-$d_4$ (δ 3.31, septet) or chloroform-*d* (δ 7.26, singlet). Multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), dt (doublet of triplets), or m (multiplet). Coupling constants are reported as a *J* value in Hertz (Hz). The number of protons (n) for a given resonance is indicated as nH, and is based on spectral integration values. $^{13}C$ NMR chemical shifts are reported as δ in units of parts per million (ppm) relative to methanol-$d_4$ (δ 49.0, septet) or chloroform-*d* (δ 77.0, triplet).

***Mass Spectrometry.*** Fast Atom Bombardment (FAB) and Electron Impact (EI) mass spectra were obtained at the UC Berkeley Mass Spectrometry Facility. Matrix assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) was performed on a Voyager-DE$^{TM}$ system (PerSeptive Biosystems, USA). All samples were co-crystallized using a sinapinic acid solution (10 mg/mL in 1:1 MeCN:dd$H_2O$ with

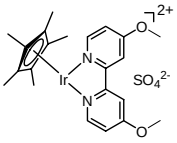
0.1% TFA) or α-CHCA solution (10 mg/mL in 1:1 MeCN:dd$H_2O$ with 0.1% TFA). Electrospray LC/MS analysis was performed using an API 150EX system (Applied Biosystems, USA) equipped with a Turbospray source and an Agilent 1100 series LC pump. Protein chromatography was performed using a Phenomenex Jupiter® 5μ C5 300 Å reversed phase column (2.0 mm x 150 mm) with a MeCN:dd$H_2O$ gradient mobile phase containing 0.1% formic acid (250 μL/min). Protein mass reconstruction was performed on the charge ladder with Analyst software (version 1.3.1, Applied Biosystems). MS/MS analyses were performed on a 4700 Proteomics Analyzer MALDI TOF-TOF system (Applied Biosystems, USA).

***Gel Analyses.*** For protein analysis, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was accomplished on a Mini-Protean apparatus (Bio-Rad, USA), following the general protocol of Laemmli.[1] Commercially available markers (Bio-Rad, USA) were applied to at least one lane of each gel for calculation of apparent molecular weights. Visualization of protein bands was accomplished by staining with Coomassie® Brilliant Blue R-250 (Bio-Rad, USA). Gel imaging was performed on an EpiChem3 Darkroom system (UVP, USA). Protein reaction conversion was estimated from standard optical density measurements of the observed gel bands with LabWorks™ software (version 4.0.0.8, UVP).

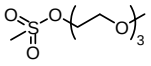
## Experimental



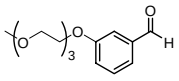

**Cp*Ir(4,4'-dimethoxy-2,2'-bipyridine)$Cl_2$ (7).** An adapted procedure of Dadci, et al was used.[2] 16.0 mg of dichloro(pentamethylcyclopentadienyl)iridium (III) dimer (20.1 μmol), 8.7 mg of 4,4'-dimethoxy-2,2'-bipyridine (40.2 μmol) and 2 mL of methanol were combined in a scintillation vial charged with a magnetic stir bar. The heterogeneous mixture was stirred at room temperature until it became homogeneous (<10 min). The solution was concentrated under reduced pressure and the residue was redissolved in a minimum amount of methylene chloride. The product was then precipitated by the dropwise addition of hexanes until no more precipitate appeared. The precipitate was collected by filtration, washed with three 1 mL portions of hexanes and dried *in vacuo* to yield the product as a light yellow solid (24.0 mg, 97% yield). $^1$H NMR (500 MHz, $CDCl_3$): δ 9.13 (d, 2H, *J* = 2.5), 8.38 (d, 2H, *J* = 6.5), 7.09 (dd, 2H, *J* = 6.5, 2.7), 4.39 (s, 6H), 1.65 (s, 15H). $^{13}$C NMR (125 MHz, $CDCl_3$): δ 169.1, 157.9, 149.9, 117.1, 111.2, 88.0, 59.3, 8.7. HRMS (FAB) calculated for $C_{22}H_{27}ClIrN_2O_2$ ([M – Cl]$^+$) 577.1367, found 577.1374.



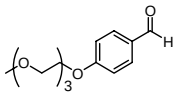

**Cp*Ir(4,4'- dimethoxy-2,2'-bipyridine)$SO_4$ (1b).** An adapted procedure of Dadci, et al was used.[2] 16.4 mg of **7** (26.7 μmol), 8.4 mg of silver (I) sulfate (26.9 μmol), and 2 mL of dd$H_2O$ were combined in a scintillation vial charged with a magnetic stir bar. The heterogeneous mixture was stirred overnight at room temperature. The mixture was filtered to remove the precipitate, and the collected material was washed with three 1 mL portions of dd$H_2O$. The filtrate and washings were combined and the solvent was removed under reduced pressure. The product was obtained as a dull yellow solid (16.3 mg, 95% yield). $^1$H NMR (500 MHz, $D_3COD$): δ 8.80 (d, 2H, *J* = 6.5), 7.94 (s, 2H), 7.21 (d, 2H, *J* = 6.2), 3.96 (s, 6H), 1.56 (s, 15H). $^{13}$C NMR (125 MHz, $D_3COD$): δ 170.1, 159.4, 154.0, 115.4, 111.1, 88.7, 57.6, 8.6. HRMS (FAB) calculated for $C_{22}H_{28}IrN_2O_6S$ ([MH]$^+$) 639.1274, found 639.1265.



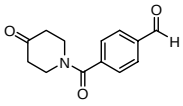
**Methane sulfonyl tri(ethylene glycol) monomethyl ether (8).** To a 250-mL round-bottom flask equipped with a Teflon magnetic stirring bar was added tri(ethylene glycol) monomethyl ether (7.8 mL, 50 mmol), *N*,*N*-diisopropylethylamine (9.6 mL, 55 mmol), and dichloromethane (125 mL). The mixture was cooled to 0 °C and then methane sulfonyl chloride (4.3 mL, 55 mmol) was added *via* syringe. The reaction mixture was stirred for 1.5 h at 0 °C. The reaction solution was transferred to a separatory funnel and washed with 100 mL of brine. The organic phase was concentrated under reduced pressure and the residue was partitioned between hexanes and water in a separatory funnel. After isolating the aqueous phase, NaCl (10 g) was added, and the solution was extracted with three 50 mL portions of dichloromethane. The combined organic phases were dried over anhydrous $Na_2SO_4$, filtered, and concentrated under reduced pressure to afford the product as a yellow oil (12.15 g, quantitative yield) which was used without further purification. The NMR spectra matched literature values.[3] $^1$H NMR (500 MHz, $CDCl_3$): δ 4.35 (t, 2H, $J$ = 4.5), 3.73 (t, 2H, $J$ = 4.5), 3.63 (m, 6H), 3.52 (m, 2H), 3.34 (s, 3H), 3.05 (s, 3H). $^{13}$C NMR (125 MHz, $CDCl_3$): δ 71.8, 70.5, 70.44, 70.42, 69.3, 68.9, 58.9, 37.6.



**3-[tri(ethylene glycol) monomethyl ether] benzaldehyde (4d).** In a 20 mL scintillation vial equipped with a Teflon magnetic stirring bar were combined mesylate **8** (484 mg, 2.00 mmol), 3-hydroxybenzaldehyde (250 mg, 2.05 mmol), cesium carbonate (666 mg, 2.05 mmol), and THF (5 mL). The mixture was heated to 60 °C for 18 h. The reaction was not complete by TLC, so the reaction was heated to 70 °C for an additional 24 h. The mixture was cooled to room temperature, and filtered to remove any solids. The filtrate was concentrated under reduced pressure and the resulting oil was purified by silica gel chromatography eluting with 2:3 to 7:3 ethyl acetate/hexanes. Fractions containing product were combined and concentrated under reduced pressure and dried *in vacuo*. The product was obtained as a clear oil (343 mg, 64% yield). $^1$H NMR (500 MHz, $CDCl_3$): δ 9.96 (s, 1H), 7.45 (m , 2H), 7.39 (app. s, 1H), 7.21 (m, 1H), 4.19 (t, 2H, $J$ = 4.6), 3.88 (t, 2H, $J$ = 4.5), 3.73 (m, 2H), 3.68 (m, 2H), 3.64 (m, 2H), 3.54 (m, 2H), 3.37 (s, 3H). $^{13}$C NMR (500 MHz, $CDCl_3$): δ 192.1, 159.3, 137.7, 130.0, 123.5, 122.0, 112.9, 71.0, 70.8, 70.6, 70.5, 69.5, 67.6, 59.0. HRMS (FAB) calculated for $C_{14}H_{21}O_5$ ($[MH]^+$) 269.1389, found 269.1390.



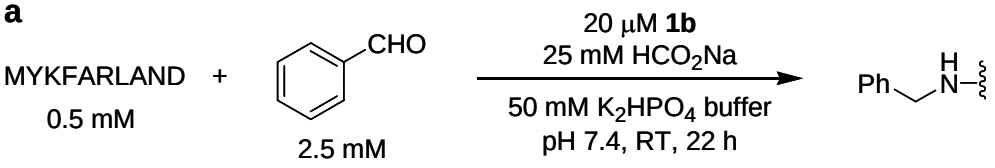
**4-[tri(ethylene glycol) monomethyl ether] benzaldehyde (4e).** In a 20 mL scintillation vial equipped with a Teflon magnetic stirring bar were combined mesylate **8** (484 mg, 2.00 mmol), 4-hydroxybenzaldehyde (250 mg, 2.05 mmol), cesium carbonate (666 mg, 2.05 mmol), and THF (5 mL). The mixture was heated to 60 °C for 18 h. The reaction was not complete by TLC, so the reaction was heated to 70 °C for an additional 24 h. The mixture was cooled to room temperature, and filtered to remove any solids. The filtrate was concentrated under reduced pressure and the resulting oil was purified by silica gel chromatography eluting with 2:3 to 7:3 ethyl acetate/hexanes. Fractions containing product were combined and concentrated under reduced pressure and dried *in vacuo*. The product was obtained as a yellow oil (452 mg, 84% yield). $^1$H NMR (500 MHz, $CDCl_3$): δ 9.89 (s, 1H), 7.81 (d, 2H, $J$ = 8.8), 7.02 (d, 2H, $J$ = 8.7), 4.20 (t, 2H, $J$ = 4.7), 3.88 (t, 2H, $J$ = 4.9), 3.73 (m, 2H), 3.67 (m, 2H), 3.63 (m, 2H), 3.54 (m, 2H), 3.36 (s, 3H). $^{13}$C NMR (125 MHz, $CDCl_3$): δ 190.8, 163.8, 131.9, 130.0, 114.8, 71.9, 70.8, 70.6, 70.5, 69.4, 57.7, 59.0. HRMS (FAB) calculated for $C_{14}H_{21}O_5$ ($[MH]^+$) 269.1389, found 269.1393.



**4-(4-Oxo-piperidine-1-carbonyl)-benzaldehyde (4i).** In a 50 mL round-bottom flask equipped with a Teflon magnetic stirring bar was combined 4-carboxybenzaldehyde (600 mg, 4.00 mmol), 4-piperidone hydrochloride monohydrate (618 mg, 4.02 mmol), diisopropylethylamine (516 mg, 3.99 mmol), 4-(*N*,*N'*-dimethylamino)pyridine (100 mg, 0.82 mmol), and methylene chloride (15 mL). The solution was cooled to 0 °C and dicyclohexylcarbodiimide (880 mg, 4.27 mmol) was added. The reaction was stirred at 0 °C and slowly warmed to room temperature over 3 h. The resulting slurry was filtered to remove the precipitate and the filtrate was concentrated under reduced pressure. The solid was redissolved in methylene chloride and filtered. The filtrate was then purified by column chromatography eluting with 1:1 to 4:1 ethyl acetate/hexanes. Fractions containing product were combined and concentrated under reduced pressure and the resulting solid was dried *in vacuo*. The product was obtained as a white solid (466 mg, 50% yield). $^{1}$H NMR (400 MHz, $CDCl_3$): δ 10.05 (s, 1H), 7.94 (d, 2H, $J$ = 8.2), 7.62 (d, 2H, $J$ = 8.1), 4.05 (br s, 2H), 3.70 (br s, 2H), 2.62 (br, 2H), 2.43 (br, 2H). $^{13}$C NMR (125 MHz, $CDCl_3$, 50 °C): δ 205.4, 190.9, 169.4, 140.9, 137.5, 129.9, 127.5, 40.9 (-$CH_2CH_2$- peaks coalesce). HRMS (FAB) calculated for $C_{13}H_{14}NO_5$ ([MH]$^+$) 232.0974, found 232.0976. Anal. Calcd: C 67.52; H 5.67; N 6.06. Found: C 67.52; H 5.78; N 5.98.

**General Procedure for Peptide Modification.** In a 0.6 mL Eppendorf tube were combined 2.50 μL of peptide solution (2.50 mM in $ddH_2O$), 3.13 μL of aldehyde solution (10.0 mM in *tert*-butyl alcohol/$ddH_2O$ solution), 0.500 μL of catalyst solution (0.50 mM in $ddH_2O$), 1.250 μL of buffer solution (0.50 M phosphate/0.25 M formate, pH 7.4), and 5.13 μL of $ddH_2O$. The solution was incubated at 22 °C for 16-22 h. The resulting mixture was then analyzed by MALDI-TOF without purification (Figure **S1**). In the event that unreduced imine peaks were observed, the reaction mixture was incubated with 10 mM (final concentration) benzyloxyamine at room temperature or 37 °C for 1-48 h.

**a**



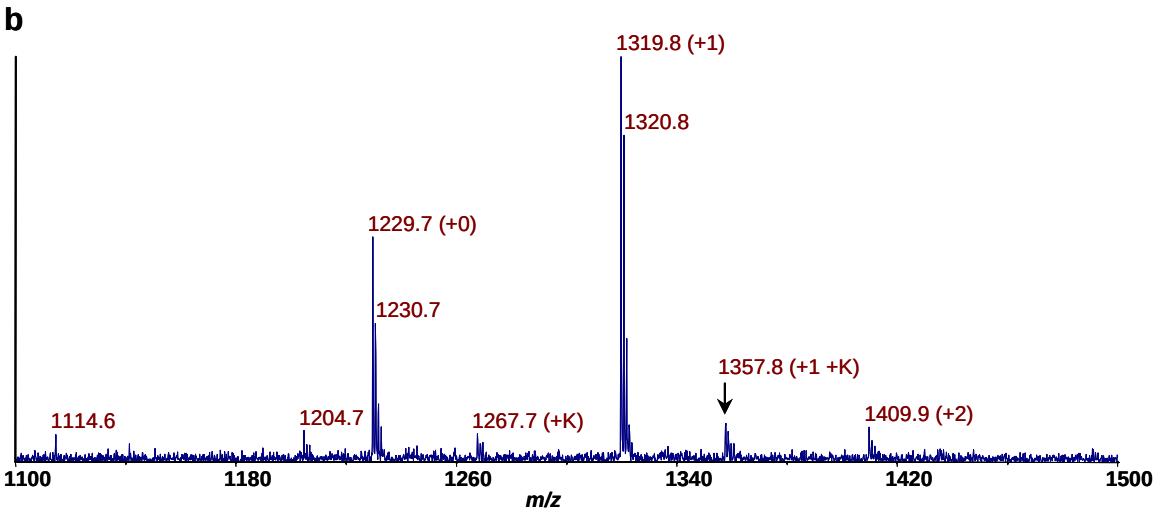



Figure S1. **(a)** Reductive alkylation of peptide MYKFARLAND with benzaldehyde, **(b)** the resulting MALDI mass-spectrum.

**Confirmation of Residue Selectivity.** The peptide reaction mixture was diluted 1:1000 in 10 mg/mL CHCA solution and analyzed on a MALDI TOF-TOF instrument. For the MYKFARLAND peptide reacted with **4i**, the MS/MS spectrum of the *m*/*z* 1658 (+2 mod.) peak is shown. The spectrum shows that both the lysine and *N*-terminal amine reacted (Figure **S2**).

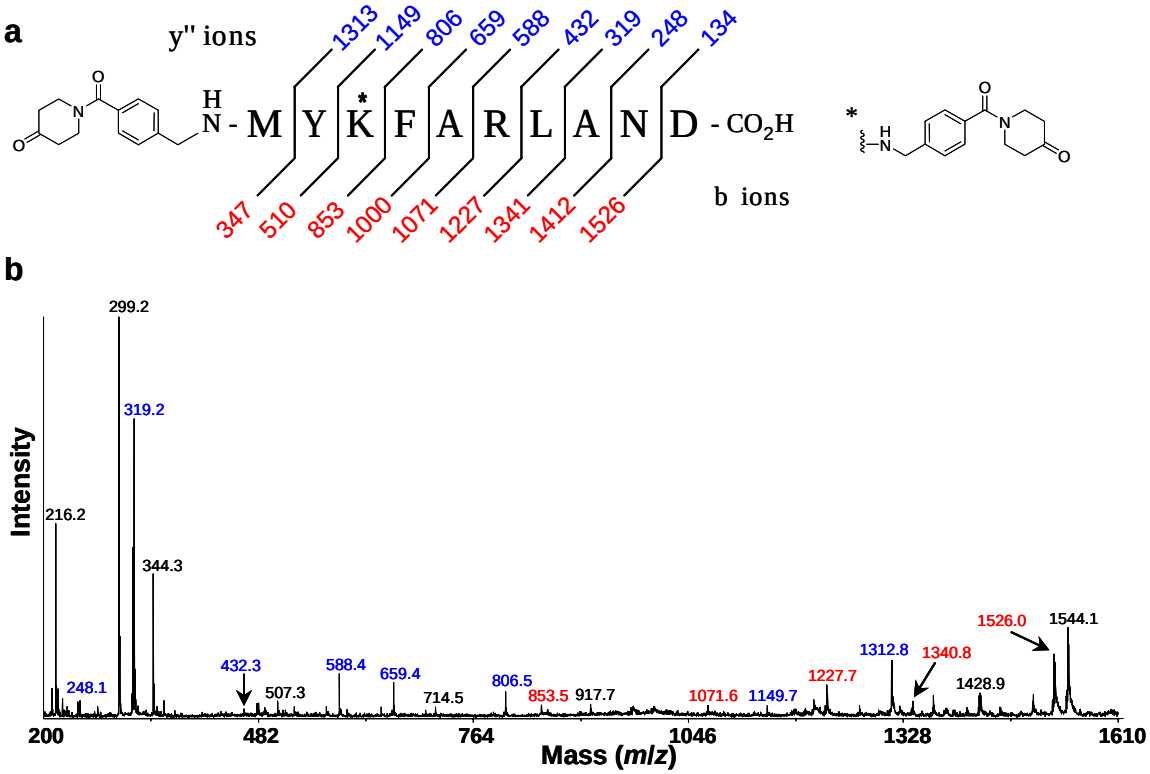



Figure S2. **(a)** Expected masses for MYKFARLAND peptide fragments, +2 modifications with **4i** under standard reaction conditions. **(b)** Maldi TOF-TOF spectrum of peptide MYKFARLAND modified with **4i**, *m*/*z* 1658 (+2 modifications).

**General Procedure for Protein Modification.** In a 1.5 mL Eppendorf tube were combined 75.0 μL of protein solution (400 μM in 200 mM phosphate with 100 mM formate, pH 7.4), 0.300 μL of aldehyde solution (1.0 M in *tert*-butyl alcohol/dd$H_2O$ solution), 0.600 μL of catalyst solution (5.0 mM in dd$H_2O$), and 224.0 μL of dd$H_2O$. The solution was incubated at 22 to 37 °C for 16-22 h. A portion of the resulting mixture was diluted two-fold with dd$H_2O$ and analyzed by LCMS.

**General Procedure for Protein Control Reaction.** To verify the role of complex **1b** in the reaction, a control experiment lacking **1b** was performed under conditions identical to those described above. The control reaction mixture was incubated at 37 °C for 22 h. A portion of the mixture was diluted two-fold with dd$H_2O$ and analyzed by LCMS.

**General Procedure for Oxime Formation on Protein.** To a gel-filtration purified solution of ketone labeled protein (50 μM) in 10 mM potassium phosphate, pH 6.5, was added benzyloxyamine (10 mM final concentration). The solution was incubated at room temperature to 37 °C for up to 20 h. The solution was directly analyzed by LCMS (Figure **S3**). The quantitative conversion to the oxime product indicated by the mass spectrum is further evidence that ketones are not reduced by hydride complex **2b**.

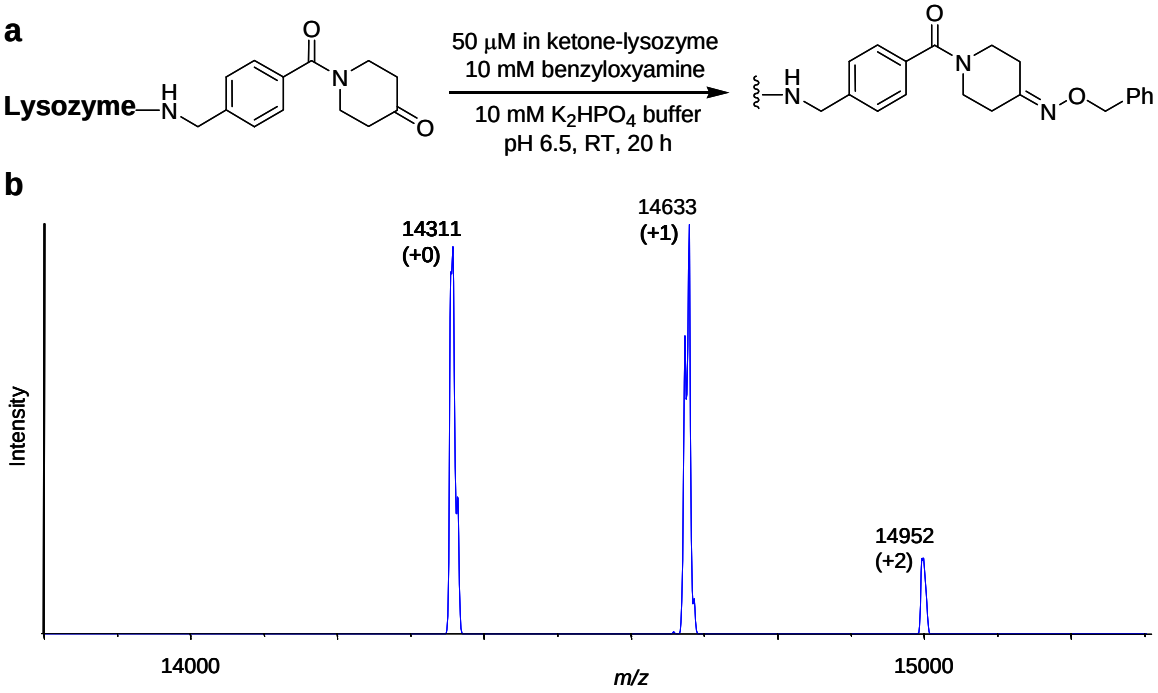



Figure S3. (a) Reaction conditions for the modification of ketone labeled lysozyme with benzyloxyamine. (b) The resulting mass spectrum shows quantitative conversion to the expected oxime product.

**UV-Vis analysis of protein-catalyst mixture.** A solution of lysozyme (40 μM) and catalyst **1b** (50 μM), was prepared in 10 mM potassium phosphate, buffered to pH 7.4. 300 μL of the solution was diluted two-fold with 10 mM phosphate buffer, pH 7.4 and the absorbance spectra was acquired. The stock solution was left at RT for 15 min and then purified by gel-filtration (Nap-5, pre-equilibrated with 10 mM phosphate, pH 7.4) to remove small molecules. The absorbance spectrum of the resulting solution was then

acquired (Figure S5). Also, an absorbance spectrum of complex **1b** (25 μM) in 10 mM phosphate, pH 7.4 was acquired (Figure S4).

**UV-Vis analysis of protein.** A control experiment was performed as above without complex **1b** to determine the amount of protein lost during gel-filtration. The UV-Vis spectra from before and after gel-filtration showed minimal protein loss.

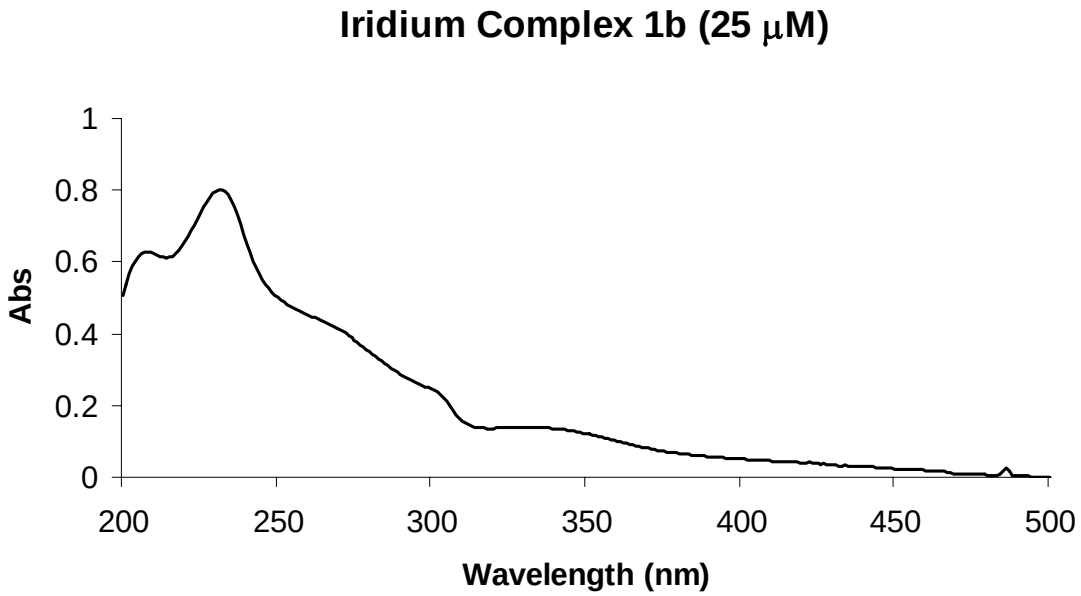



Figure S4. UV-Vis spectra of complex **1b** in 10 mM potassium phosphate, pH 7.4.

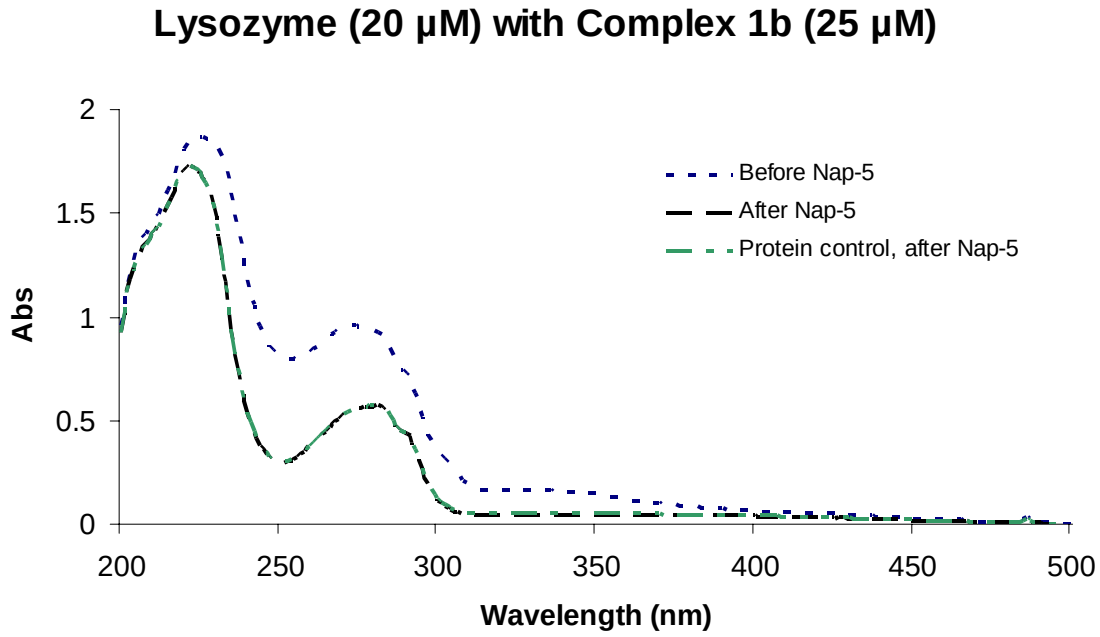



Figure S5. UV-Vis spectra for the mixture of complex **1b** (20 μM) with lysozyme (25 μM) in 10 mM phosphate, pH 7.4, before and after gel-filtration. Also shown is the control experiment without catalyst, after gel-filtration. Abs(325) = 0.16 before purification. Abs(325) = 0.05 after purification. For the control experiment, Abs(325) = 0.05 after purification, indicating that little to no **1b** remains in solution.

**Determination of aldehyde reduction by catalyst 1b.** Complex **1a** was reported previously to be highly selective for the reduction of imines in the presence of α-keto acids. We were concerned that the more electrophilic nature of aldehydes would cause them to be susceptible to reduction under standard reaction conditions. In addition, the higher activity of complex **1b** leads us to believe that aldehyde reduction may be a problem in the reaction. To test this, a solution of benzaldehyde (10 mM), **1b** (500 μM), and sodium formate (25 mM), buffered to pH 7.4 in potassium phosphate (50 mM) in $D_2O$/dd$H_2O$ was incubated at 37 °C for 22 h. The $^1$H NMR spectrum was acquired before and after incubation. Complete disappearance of the aldehyde singlet indicated the complete formation of the reduction product. In addition, the resulting solution was diluted 1:10 into a solution of lysozyme (100 μM) in phosphate/formate buffer (pH 7.4). The resulting mixture was incubated overnight at 37 °C and then analyzed by LCMS. No protein conjugates were observed.

---

[1] Laemmli, U. K. *Nature* **1970**, *227*, 680.
[2] Dadci, L.; Elias, H.; Frey, U.; Hörnig, A.; Koelle, U.; Merbach, A. E.; Paulus, H.; Schneider, J. S. *Inorg. Chem.* **1995**, *34*, 306-315.
[3] Schmidt, M.; Amstutz, R.; Crass, G.; Seebach, D. *Chem. Ber.* **1980**, *113*, 1691-1707.