Targeting Peptides with an Iron-Based Oxidant: Cleavage of the Amino Acid Backbone and Oxidation of Side Chains

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Supporting Information

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Part A. General Considerations

All reagents were purchased from commercial suppliers and used as received. NMR spectra were recorded on a Varian FT-NMR Mercury-400 or 500 MHz Spectrometer. Mass spectra were recorded on a Waters ZQ2000 single quadrupole mass spectrometer using an electrospray ionization source. IR spectra were recorded on a Nicolet FT-IR spectrophotometer. HPLC was performed on an Agilent 1200 Preparative Purification System equipped with a multi-wavelength detector. UV-vis spectra were recorded on a Varian Cary 50 spectrophotometer and a 10 mm path length cell was used for the experiments. The complexes \([\text{Fe}^{\text{II}}(\text{N4Py})(\text{CH}_3\text{CN})](\text{ClO}_4)_2\) (8),\[^1\] [\(\text{Fe}^{\text{II}}(\text{TPA})(\text{OTf})_2\)] (9),\[^2\] and [\(\text{Fe}^{\text{II}}(\text{BPMEN})(\text{OTf})_2\)] (10)\[^3\] (see Figure S1 for ligand structures) used in this study were synthesized according to the literature procedures. N-Acetyl-amino acids were synthesized from commercially available amino acids using standard literature procedures.\[^4\] EDTA-based Chelex 100 resin was purchased from BIO-RAD Laboratories, Inc. All reactions were performed under ambient atmosphere unless otherwise noted. Anaerobic reactions were performed in Schlenk tubes. These reactions were deoxygenated by performing five vacuum-backfill cycles with Ar and were run under a constant purge of Ar.

*Caution: Perchlorate salts are potentially explosive and should be handled with care.*
Compound abbreviations are as follows:

N4Py: \(N,N\)-bis(2-pyridylmethyl)-\(N\)-(bis-2-pyridylmethyl)amine

TPA: Tris(2-pyridylmethyl)amine

BPMEN: \(N,N'\)-dimethyl-\(N,N'\)-bis(2-pyridylmethyl)ethane-1,2-diamine

DCC: Dicyclohexylcarbodiimide

HBTU: 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate

HOBt: Hydroxybenzotriazole

EDAC: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride

Figure S1. Structures of N4Py, TPA and BPMEN.
**Part B.** Experimental procedures and tabulated characterization data for new compounds

![Chemical Structure](image)

**2-Acetamido-**N-*tert*-butylacetamide (1). A mixture of *N*-acetyl-glycine (5.00 g, 42.7 mmol), *p*-nitrophenol (5.93 g, 42.7 mmol), DCC (8.80 g, 42.7 mmol), and CH$_2$Cl$_2$ (75 mL) was heated to 50 °C under an inert atmosphere with vigorous stirring for 2 h. After cooling to rt, the reaction mixture was filtered through a bed of celite, and the filter cake was washed with CH$_2$Cl$_2$ (25 mL). The filtrate was concentrated to give crude ester (13.00 g). The crude ester was purified by recrystallization from hot ethyl acetate (75 mL) to give the *p*-nitrophenol ester (4.50 g, 44%) as a light yellow solid. $^1$H NMR (CDCl$_3$) $\delta$ 8.26 (dd, $J = 7.3, 2.4$ Hz, 2H), 7.29 (dd, $J = 7.3, 2.4$ Hz, 2H), 6.05 (brs, 1H), 4.30 (d, $J = 5.6$ Hz, 2H), 2.08 (s, 3H).

A mixture of the *p*-nitrophenol ester (4.50 g, 18.9 mmol) and CH$_2$Cl$_2$ (100 mL) was stirred for 5 min and the mixture was filtered through a bed of celite to remove any undissolved portion. *tert*-Butylamine (4.4 mL, 42 mmol) was added to the CH$_2$Cl$_2$ solution, resulting in formation of bright yellow precipitate. The reaction was maintained at rt, and more *tert*-butylamine (2.2 mL, 21 mmol) was added, until TLC analysis of the reaction (30 min) indicated that the *p*-nitrophenol ester was consumed. The reaction mixture was filtered through a bed of celite, and the filter cake was washed with CH$_2$Cl$_2$ (15 mL). The filtrate was concentrated to give crude product as a yellow solid (2.50 g). The crude product was purified by recrystallization from hot ethyl acetate (100 mL) to give 1 (2.0 g, 61%) as a white solid. m.p. = 161-162 °C; $^1$H NMR (DMSO-d$_6$) $\delta$ 7.94
(brs, 1H), 7.41 (s, 1H), 3.59 (d, J = 6.5 Hz, 2H), 1.82 (s, 3H), 1.23 (s, 9H); $^{13}$C NMR (DMSO-d$_6$) δ 169.3, 168.1, 50.0, 42.2, 28.5, 22.4; IR (KBr) 3280, 3083, 2972, 2929, 2851, 1638, 1561, 1426, 1371, 1282 cm$^{-1}$; LRMS (ESMS) calcd for C$_8$H$_{17}$N$_2$O$_2$ (M+H)$^+$: 173, found: 173.

![Chemical Formula: C$_8$H$_{14}$D$_2$N$_2$O$_2$
Molecular Weight: 174.24](image)

**2,2-$d_2$-Acetamido-N-tert-butylacetamide (2,2-$d_2$-1).** A solution of glycine-$2,2$-$d_2$ (1.0 g, 13 mmol), acetic anhydride (15 mL, 160 mmol), and D$_2$O (2.5 mL) was stirred at rt. After 3h, the reaction flask was cooled to −20 °C for 0.5 h. The white solid formed was collected by filtration and dried in a vacuum desiccator over P$_2$O$_5$ and KOH to yield N-acetyl-glycine-$d_2$ (0.400 g, 25%).

*tert*-Butylamine (1.0 ml, 10 mmol) was added dropwise to a mixture of N-acetyl-glycine-$2,2$-$d_2$ (0.4 g, 3.4 mmol), HBTU (1.07 g, 3.36 mmol), and THF (50 mL). This reaction mixture was vigorously stirred at rt under an inert atmosphere. After 2h, the THF was removed under reduced pressure and the resulting residue was dissolved in 10 mL of water and extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with NH$_4$Cl solution (1 × 20 mL), 5% NaHCO$_3$ solution (1 × 20 mL), dried (Na$_2$SO$_4$), and concentrated. Crude product was purified by silica gel chromatography (5% MeOH:CHCl$_3$) followed by recrystallization from EtOAc to give 2,2-$d_2$-1 (0.100 g, 17%) as a white solid. $^1$H NMR (CDCl$_3$) δ 7.90 (s, 1H), 7.39 (s, 1H), 3.58 (d, J = 5.8 Hz, 0.1 H,
1.82 (s, 3H), 1.23 (s, 9H); LRMS (ESMS) calcd for C₈H₁₄D₂N₂O₂Na (M+Na)⁺: 197, found: 197.

2-Acetamido-N-tert-butylpropanamide (2). A mixture of N-acetyl-alanine (10.00 g, 76.26 mmol), p-nitrophenol (10.60 g, 76.26 mmol), DCC (15.73 g, 76.26 mmol), and EtOAc (150 mL) was vigorously stirred under an inert atmosphere. After stirring at rt for 6 h, the reaction mixture was kept in freezer for overnight. After warming to rt, the reaction mixture was filtered through a bed of celite. The filtrate was concentrated to give the p-nitrophenol ester (8.0 g, 42%) as a light yellow solid. ¹H NMR (CDCl₃) δ 8.27 (d, J = 9.7 Hz, 2H), 7.29 (d, J = 9.7 Hz, 2H), 6.07 (brs, 1H), 4.79 (m, 1H), 2.07 (s, 3H), 1.59 (d, J = 7.3 Hz, 3H).

A mixture of p-nitrophenol ester (8.00 g, 31.7 mmol), tert-butylamine (13.5 mL, 128 mmol), and CH₂Cl₂ (100 mL) was vigorously stirred under an inert atmosphere. After stirring the reaction mixture at rt for 3 h, the reaction mixture was filtered through a bed of celite, and the filter cake was washed with CH₂Cl₂. The filtrate was concentrated to give crude product as a dark yellow solid. The crude product was purified by recrystallization from hot ethanol to give 2 (4.2 g, 71%) as a white solid. m.p. = 159-160 °C; ¹H NMR (CDCl₃) δ 6.32 (brs, 1H), 6.06 (brs, 1H), 4.37-4.31 (m, 1H), 1.98 (s, 3H), 1.35-1.29 (m, 12H); ¹³C NMR (CDCl₃) δ 171.6, 169.9, 51.3, 49.2, 28.6, 23.2, 18.7; IR
(KBr) 3287, 3084, 2977, 1682, 1633, 1561, 1541, 1456, 1393, 1367, 1256, 1229, 701, 602 cm⁻¹; LRMS (ESMS) calcd for C₉H₁₉N₂O₂ (M+H)⁺: 187, found: 187.

**2-Acetamido-<i>N</i>-<i>tert</i>-butyl-3-methylbutanamide (3).** A mixture of <i>N</i>-acetyl-valine (3.00 g, 18.8 mmol), <i>p</i>-nitrophenol (2.62 g, 18.8 mmol), DCC (3.88 g, 18.8 mmol), and CH₂Cl₂ (50 mL) was heated to 50 °C under an inert atmosphere with vigorous stirring for 3 h. After cooling to rt, the reaction mixture was filtered through a bed of celite, and the filter cake was washed with CH₂Cl₂. The filtrate was concentrated to give the <i>p</i>-nitrophenol ester (4.2 g, 79%) as an oil. ¹H NMR (CDCl₃) δ 8.26 (d, <i>J</i> = 9.1 Hz, 2H), 7.26 (d, <i>J</i> = 9.1 Hz, 2H), 5.97 (brs, 1H), 4.75 (dd, <i>J</i> = 8.2, 5.1 Hz, 1H), 2.36-2.29 (m, 1H), 2.08 (s, 3H), 1.05 (q, <i>J</i> = 9.7, 6.7 Hz, 6H).

A mixture of the <i>p</i>-nitrophenol ester (4.2 g, 14.9 mmol), <i>tert</i>-butylamine (11.3 mL, 107 mmol), and CH₂Cl₂ (50 mL) was vigorously stirred under an inert atmosphere. After stirring the reaction mixture at rt for 16 h, the reaction mixture was filtered through a bed of celite, and the filter cake was washed with CH₂Cl₂. The filtrate was concentrated to give crude product as a dark yellow solid. The crude product was purified by recrystallization from ethyl acetate (100 mL) to give 3 (1.3 g, 40%). m.p. = 193-194 °C; ¹H NMR (CDCl₃) δ 6.28 (brs, 1H), 5.74 (brs, 1H), 4.05 (appt, <i>J</i> = 8.1 Hz, 1H), 2.03-1.94 (m, 4H), 1.32 (s, 9H), 0.91 (dd, <i>J</i> = 6.4, 2.4 Hz, 6H); ¹³C NMR (DMSO-d₆) δ 170.5,
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168.9, 57.7, 50.0, 30.9, 28.4, 22.5, 19.1, 18.2; IR (thin film) 3429, 3277, 3083, 2965, 1637, 1560, 1381, 1367 cm$^{-1}$; LRMS (ESMS) calcd for C$_{11}$H$_{23}$N$_{2}$O$_{2}$ (M+H)$^+$: 215, found: 215.

2-Acetamido-N-tert-butyl-3-phenylpropanamide (4). A mixture of N-acetyl-phenylalanine (14.0 g, 67.6 mmol), p-nitrophenol (9.39 g, 67.6 mmol), DCC (13.93 g, 67.60 mmol), and CH$_2$Cl$_2$ (215 mL) was heated to 50 °C under an inert atmosphere with vigorous stirring for 8 h. After cooling to rt, the reaction mixture was filtered through a bed of celite. The filtrate was concentrated to give crude product (18.50 g). The crude product was purified by recrystallization from hot ethyl acetate to give the p-nitrophenol ester (14.00 g, 63%) as a light yellow solid. $^1$H NMR (CDCl$_3$) δ 8.22 (d, $J$ = 8.9 Hz, 2H), 7.36-7.13 (m, 5H), 7.11 (d, $J$ = 8.9 Hz, 2H), 5.95 (d, $J$ = 7.3 Hz, 1H), 5.04 (dd, $J$ = 13.7, 6.5 Hz, 1H), 3.29-3.18 (m, 2H), 2.02 (s, 3H).

A mixture of p-nitrophenol ester (5.00 g, 15.2 mmol), tert-butylamine (8.0 mL, 75 mmol), and CH$_2$Cl$_2$ (150 mL) was heated to 50 °C under an inert atmosphere with vigorous stirring, resulting in formation of bright yellow precipitate, for 12 h. After cooling to rt, the reaction mixture was filtered through a bed of celite. The filtrate was concentrated to give a dark yellow crude product (5.00 g). The crude product was
purified by recrystallization from hot ethanol (75 mL) to give 4 (2.5 g, 62%) as a white solid. m.p. = 220-221 °C; \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 7.31-7.21 (m, 5H), 6.49 (d, \(J = 7.3\) Hz, 1H), 5.30 (brs, 1H), 4.51-4.45 (m, 1H), 3.10 (dd, \(J = 13.0, 5.6\) Hz, 1H), 2.85 (dd, \(J = 13.3, 9.3\) Hz, 1H), 1.98 (s, 3H), 1.16 (s, 9H); \(^{13}\)C NMR (DMSO-d\(_6\)) \(\delta\) 170.5, 168.9, 137.9, 129.3, 127.9, 126.2, 54.1, 50.1, 38.4, 28.4, 22.5; IR (KBr) 3265, 3080, 2966, 1671, 1643, 1561, 1449, 1392, 1366, 1286, 741, 701 cm\(^{-1}\); LRMS (ESMS) calcd for C\(_{15}\)H\(_{23}\)N\(_2\)O\(_2\) (M+H\(^+\)): 263, found: 263.

**2-Acetamido-\(N\)-\(\text{tert}\)-butyl-3-(4-hydroxyphenyl)propanamide (5).** A mixture of \(N\)-acetyl-tyrosine (6.5 g, 29 mmol), \(\text{tert}\)-butylamine (6.5 mL, 62 mmol), HOBT (4.0 g, 30 mmol), EDAC (5.6 g, 30 mmol), and DMF (150 mL) was vigorously stirred under an inert atmosphere. After stirring for 20 h at rt, DMF was removed under reduced pressure. Water (50 mL) was added to the residue and the mixture was extracted with EtOAc (3 \(\times\) 50 mL). The combined organic layers were washed with 1M HCl solution (1 \(\times\) 50 mL), 5% NaHCO\(_3\) solution (1 \(\times\) 50 mL), brine solution (1 \(\times\) 50 mL), dried (Na\(_2\)SO\(_4\)), and concentrated to give crude product. The crude product was purified by recrystallization from hot ethyl acetate to give 5 (3.0 g, 37%) as a white solid. m.p. = 203-204 °C; \(^1\)H NMR (DMSO-d\(_6\)) \(\delta\) 9.19 (s, 1H), 7.89 (d, \(J = 8.1\) Hz, 1H), 7.43 (s, 1H), 6.99 (d, \(J = 8.1\) Hz, 1H).
Hz, 2H), 6.61 (d, J = 8.9 Hz, 2H), 4.39-4.33 (m, 1H), 2.72 (dd, J = 13.8, 5.7 Hz, 1H), 2.59 (dd, J = 13.8, 8.9 Hz, 1H), 1.74 (s, 3H), 1.18 (s, 9H); \(^{13}\)C NMR (DMSO-d6) \(\delta\) 170.7, 168.7, 155.6, 130.2, 128.0, 114.7, 54.4, 50.0, 37.5, 28.4, 22.5; IR (KBr) 3335, 3266, 3090, 2975, 1650, 1627, 1591, 1550, 1517, 1451, 1392, 1365, 1269, 1225 cm\(^{-1}\); LRMS (ESMS) calcd for C\(_{15}\)H\(_{23}\)N\(_2\)O\(_3\) (M+H): 279, found: 279.

2-Aacetamido-\(\text{N-\text{tert}-\text{butyl-3-(1H-indol-3-yl)propanamide (6).}\)}\ A mixture of \(\text{N-acetyl-tryptophan (10.00 g, 40.60 mmol), p-nitrophenol (5.64 g, 40.6 mmol), DCC (8.37 g, 40.6 mmol), and EtOAc (150 mL) was vigorously stirred under an inert atmosphere. After stirring at rt for 6 h, the reaction mixture was kept in the freezer overnight. The reaction mixture was filtered through a bed of celite and the filtrate was concentrated to give the p-nitrophenol ester (10.00 g, 67%) as a light yellow solid. \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 8.25 (brs, 1H), 8.19 (d, J = 9.7 Hz, 1H), 7.58 (d, J = 8.1 Hz, 1H), 7.41 (d, J = 8.1 Hz, 1H), 7.25-7.09 (m, 5H), 7.03 (d, J = 8.9 Hz, 1H), 6.07 (brd, J = 6.5 Hz, 1H), 5.14-5.12 (m, 1H), 3.49-3.44 (m, 2H), 2.02 (s, 3H).

A mixture of p-nitrophenol ester (10.00 g, 27.22 mmol), tert-butylamine (20 mL, 189 mmol), and CH\(_2\)Cl\(_2\) (100 mL) was heated to 50 °C under an inert atmosphere with vigorous stirring for 3 h. After cooling to rt, the reaction mixture was filtered through a
bed of celite, and the filter cake was washed with CH$_2$Cl$_2$. The filtrate was concentrated to give crude product as a brown solid. The crude product was purified by recrystallization from hot ethanol to give 6 (1.3 g, 16%) as a white solid. m.p. = 209-210 °C; $^1$H NMR (DMSO-d$_6$) δ 10.76 (brs, 1H), 7.92 (d, $J$ = 8.1 Hz, 1H), 7.59 (d, $J$ = 8.1 Hz, 1H), 7.44 (s, 1H), 7.29 (d, $J$ = 8.1 Hz, 1H), 7.09 (d, $J$ = 1.6 Hz, 1H), 7.03 (t, $J$ = 8.1 Hz, 1H), 6.95 (t, $J$ = 8.1 Hz, 1H), 4.51-4.46 (m, 1H), 2.99 (dd, $J$ = 14.6, 5.7 Hz, 1H), 2.86 (dd, $J$ = 14.6, 8.1 Hz, 1H), 1.76 (s, 3H), 1.19 (s, 9H); $^{13}$C NMR (CDCl$_3$ + DMSO-d$_6$) δ 169.6, 168.6, 135.2, 126.4, 122.2, 120.0, 117.5, 117.4, 110.2, 109.2, 52.9, 49.5, 27.4, 26.9, 21.8; IR (thin film) 3415, 3268, 3083, 2975, 1664, 1635, 1546, 1478, 1461, 1391, 1359, 1316, 1291, 1243, 1222, 741 cm$^{-1}$; LRMS (ESMS) calcd for C$_{17}$H$_{24}$N$_3$O$_2$ (M+H)$^+$: 302, found: 302.

2-Acetamido-$N$-tert-butyl-4-(methylthio)butanamide (7). A mixture of $N$-acetyl-methionine (1.0 g, 5.2 mmol), tert-butylamine (0.55 mL, 5.20 mmol), DCC (1.07 g, 5.20 mmol), and CH$_2$Cl$_2$ (25 mL) was vigorously stirred under an inert atmosphere. After stirring for 24 h at rt, the reaction mixture was filtered through a bed of celite and the filtrate was concentrated to give crude product. The crude product was purified by recrystallization from hot EtOAc to give 7 (0.58 g, 45%) as a white solid. m.p. = 154-
155 °C; $^1$H NMR (DMSO-$d_6$) δ 7.92 (d, $J = 8.9$ Hz, 1H), 7.50 (brs, 1H), 4.28-4.22 (m, 1H), 2.42-2.30 (m, 2H), 2.01 (s, 3H), 1.82 (s, 3H), 1.80-1.64 (m, 2H), 1.22 (s, 9H); $^{13}$C NMR (DMSO-$d_6$) δ 170.5, 169.0, 52.1, 50.0, 32.6, 29.6, 28.4, 22.5, 14.6; IR (thin film) 3276, 3080, 2927, 2854, 2360, 2341, 2121, 1636, 1557, 1449, 1391, 1365, 1306, 1292, 1260, 1226, 1124, 812 cm$^{-1}$; LRMS (ESMS) calcd for C$_{11}$H$_{23}$N$_2$O$_2$S (M+H)$^+$: 247, found: 247.

**Oxidation studies of glycine substrate 1 with [Fe$^{II}$(N4Py)CH$_3$CN](ClO$_4$)$_2$ (8) and oxone.** An orange-red color mixture of 1 (20 mg, 116 µmol), [Fe$^{II}$(N4Py)CH$_3$CN](ClO$_4$)$_2$ (0.7 mg, 1.0 µmol), and D$_2$O-CD$_3$CN (4 mL, 3:1) was stirred under an inert atmosphere at rt. After stirring for 5 min, Oxone (330 mg, 536 µmol) in 2.0 mL of D$_2$O was delivered by syringe pump over 1 h. The formation of transient purple color followed by green color was observed during the addition of oxone solution. After stirring for 1 h, the reaction mixture was filtered and $p$-nitrophenol (36 µmol) was added and the solution was analyzed by $^1$HNMR spectroscopy. Yields of the starting material 1 (17%), aldehyde 11 (41%), aldehyde 12 (11%), $\alpha$-hydroxyglycine derivative 13 (5%) and glyoxamide 14 (5%) products were determined by $^1$HNMR analysis with $p$-nitrophenol as an internal standard by integrating peaks at 8.91 (11), 5.32 (12), 5.68 (13), 2.23 (14), and 6.93 ($p$-nitrophenol) ppm. In a separate experiment, this reaction was carried out under argon and the yields are as follows, starting material 1 (17%), aldehyde 11 (31%), aldehyde 12 (9%), $\alpha$-hydroxyglycine derivative 13 (12%) and glyoxamide 14 (5%). The identities of compounds 11 and 12 were confirmed by comparison of their $^1$H NMR and ESMS spectra with literature data.$^{5,6}$ In a separate experiment performed using H$_2$O and MeCN
solvents compounds 13 and 14 were isolated by reverse phase HPLC after extracting the reaction mixture with CHCl₃.

2-Acetamido-N-tert-butyl-2-hydroxyacetamide (13). HPLC column Zorbax XDB-C18, 21.2 X 150 mm, 5 micron equipped with guard column Zorbax XDB-C18, 21.2 mm, 5 micron, flow rate = 20 mL/min, gradient elution 0-3 min 5% MeCN:H₂O, 3-15 min 95% MeCN:H₂O, Tᵣ = 2.5 min; ¹H NMR (DMSO-d₆) δ 8.41 (d, J = 8.9 Hz, 1H), 7.29 (s, 1H), 6.28 (d, J = 5.6 Hz, 1H); 5.31 (dd, J = 8.1, 5.6 Hz, 1H), 1.83 (s, 3H), 1.26 (s, 9H); ¹³C NMR (DMSO-d₆) δ 169.5, 168.7, 71.4, 50.0, 28.3, 22.7; IR (thin film) 3334, 3290, 2974, 1642, 1523, 1365, 1056 cm⁻¹; LRMS (ESMS) calcd for C₈H₁₄N₂O₃ (M+H)⁺: 189, found: 189.

N¹-acetyl-N²-tert-butyloxalamide (14). HPLC column Zorbax XDB-C18, 21.2 X 150 mm, 5 micron equipped with guard column Zorbax XDB-C18, 21.2 mm, 5 micron, flow
rate = 20 mL/min, gradient elution 0-3 min 5% MeCN:H$_2$O, 3-15 min 5-95% MeCN:H$_2$O, $T_R = 9.6$ min; $^1$H NMR (CDCl$_3$) $\delta$ 9.65 (brs, 1H), 7.20 (brs, 1H), 2.43 (s, 3H), 1.37 (s, 9H); $^{13}$C NMR (CDCl$_3$) $\delta$ 170.7, 159.2, 157.1, 52.0, 28.1, 24.9; IR (thin film) 3339, 2973, 1772, 1719, 1689, 1538, 1473, 1395, 1370, 1260, 1226, 1027, 820, 741, 668, 582, 531 cm$^{-1}$; LRMS (ESMS) calcd for C$_8$H$_{14}$N$_2$O$_3$Na(M+Na)$^+$: 209, found: 209.

**Oxidation studies of phenylalanine substrate 4 with [Fe$^{II}$(N4Py)CH$_3$CN](ClO$_4$)$_2$ (8) and oxone.** An orange-red color mixture of 4 (50 mg, 190 µmol), [Fe$^{II}$(N4Py)CH$_3$CN](ClO$_4$)$_2$ (12.6 mg, 19.0 µmol), and H$_2$O:CH$_3$CN (4 mL, 3:1) was stirred at rt. After stirring for 5 min, oxone (352 mg, 572 µmol) in 1 mL of H$_2$O was delivered by syringe pump over 1 h. The formation of transient purple color followed by light green color was observed during the addition of oxone solution. After stirring for another 1 h, aqueous Na$_2$EDTA solution (5 mL) and water (20 mL) were added to reaction mixture and the mixture was extracted with chloroform (3 x 20 mL). The combined organic layers were dried (Na$_2$SO$_4$), filtered and concentrated to give a gummy solid. Compound 15 was determined to be the major product by $^1$HNMR analysis, and the yield of 15 was measured to be (17%) with 1,3,5-tribromobenzene as an internal standard by integrating peaks at 7.60 (15) and 6.78 (1,3,5-tribromobenzene) ppm. In a separate experiment compound 15 was isolated by reverse phase HPLC for characterization purposes.
(S)-2-Acetamido-\textit{N-tert}-butyl-3-(3,4-dioxocyclohexa-1,5-dienyl)propanamide (15).

HPLC column Zorbax XDB-C18, 21.2 X 150 mm, 5 micron equipped with guard column Zorbax XDB-C18, 21.2 mm, 5 micron, flow rate = 20 mL/min, gradient elution 0-3 min 5% MeCN:H₂O, 3-15 min 5-95% MeCN:H₂O, Tᵣ = 9.2 min; \(^1\)H NMR (CDCl₃) 6.79 (d, \(J = 9.7\) Hz, 1H), 6.70 (dd, \(J = 9.7, 2.4\) Hz, 1H), 6.54 (d, \(J = 2.4, 1\)H), 6.30 (d, \(J = 7.2\) Hz, 1H), 5.85 (brs, 1H), 4.46 (m, 1H), 2.91 (dd, \(J = 12.5, 3.6\) Hz, 1H), 2.52 (dd, \(J = 13.3, 9.3\) Hz, 1H), 1.92 (s, 3H), 1.32 (s, 9H); \(^{13}\)C NMR (CDCl₃) 187.3, 187.2, 170.2, 169.2, 144.8, 136.7, 136.5, 134.0, 52.5, 51.8, 34.4, 28.6, 23.0; IR (thin film) 3291, 3079, 2970, 1655, 1540, 1456, 1366, 1291, 1224, 1082, 913, 731 cm\(^{-1}\); LRMS (ESMS) calcd for C\(_{15}\)H\(_{21}\)N\(_2\)O\(_4\)(M+H): 293, found: 293.

\textbf{Oxidation studies of tryptophan substrate 6 with [Fe\(^{II}\)(N\(_4\)Py)CH\(_3\)CN](ClO\(_4\))\(_2\) (8) and oxone.}\ An orange-red color mixture of 6 (200 mg, 663 µmol), [Fe\(^{II}\)(N\(_4\)Py)CH\(_3\)CN](ClO\(_4\))\(_2\) (4.4 mg, 6.6 µmol), and H\(_2\)O-CH\(_3\)CN (40 mL, 3:1) was stirred under open air at rt. After stirring for 5 min, oxone (1.22 g, 1.99 mmol) in 10 mL of H\(_2\)O was delivered by syringe pump over 1 h. The formation of a transient purple color was observed followed by a dark brown color. After stirring for 1 h, aqueous Na\(_2\)EDTA solution (50 mL) and water (200 mL) were added to reaction mixture and the aqueous
layer was extracted with chloroform (3 × 200 mL). The combined organic layers were dried (Na$_2$SO$_4$), filtered and concentrated to give crude (127 mg) as a dark brown gummy liquid. Compound 16 was determined to be the major product by $^1$HNMR analysis of the crude reaction mixture, and the yield of 16 (45 mg, 19%) was measured based on isolated yield from silica gel chromatography (30-40% EtOAc:hexanes). The structure of 16 is supported by the following data:

$^1$H NMR spectrum shows

a) two doublets and two triplets in the aromatic region, indicating the presence of a disubstituted aromatic ring

b) resonances at 7.36 and 6.48 ppm are lost upon addition of CD$_3$OD, indicating that only two exchangeable protons are present

c) the $\alpha$-CH, which has a resonance at 4.75 ppm, is only coupled to 1 CH of the adjacent CH$_2$ unit, which indicates that free rotation is not possible and a ring has formed; also this $\alpha$-CH proton is not coupled to an adjacent NH as observed with other products

$^{13}$C NMR and DEPT data show

a) three carbonyl resonances at 172.6, 172.2 and 171.4 ppm

b) a tetrasubstituted carbon at 77.6 ppm, consistent with the tertiary alcohol

c) one CH$_2$ unit (36.7 ppm) and one CH unit (57.8 ppm)
1-Acetyl-N-tert-butyl-4-hydroxy-4-(2-nitrophenyl)-5-oxopyrididine-2-carboxamide (16). $^1$H NMR (CDCl$_3$) $\delta$ 8.31 (dd, $J = 7.9$, 1.2 Hz, 1H), 8.05 (dd, $J = 8.2$, 1.2 Hz, 1H), 7.72 (dt, $J = 7.9$, 1.2 Hz, 1H), 7.50 (dt, $J = 8.2$, 1.5 Hz, 1H), 7.36 (brs, 1H), 6.48 (brs, 1H), 4.75 (d, $J = 9.1$ Hz, 1H), 2.90 (dd, $J = 14.3$, 9.1 Hz, 1H), 2.53 (s, 3H), 2.31 (d, $J = 14.3$ Hz, 1H), 1.37 (s, 9H); $^{13}$C NMR (CDCl$_3$) 172.6, 172.2, 171.4, 146.8, 134.8, 130.0, 129.2, 125.3, 77.6, 57.8, 52.5, 36.7, 28.3, 24.8; IR (thin film) 3582, 3354, 2970, 2359, 1757, 1713, 1652, 1529, 1344, 1269 cm$^{-1}$; LRMS (ESMS) calcd for C$_{17}$H$_{22}$N$_{3}$O$_{6}$ (M+H)$^+$: 364, found: 364.

Oxidation studies of methionine substrate 7 with [Fe$^{II}$(N4Py)CH$_3$CN](ClO$_4$)$_2$ (8) and oxone. An orange-red color mixture of 7 (50 mg, 203 $\mu$mol), [Fe$^{II}$(N4Py)CH$_3$CN](ClO$_4$)$_2$ (1.34 mg, 2.03 $\mu$mol), and H$_2$O:CH$_3$CN (10 mL, 3:1) was stirred under open air at rt. After stirring for 5 min, oxone (370 mg, 610 $\mu$mol) in 2.5 mL of water was delivered by syringe pump over 1 h. The formation of transient purple color followed by green color solution was observed during the addition of oxone solution. After stirring for another 1 h, 10 mL of aqueous Na$_2$EDTA solution was added to reaction mixture and reaction mixture was extracted with chloroform (3 x 50 mL). The combined organic layers were dried (Na$_2$SO$_4$), and concentrated to give brown color crude (65 mg) as a gummy solid.
Crude product was purified by column chromatography (MeOH-CHCl₃ gradient system) to give sulfone 16 (55 mg, 97%) as a white solid.

\[(S)-2\text{-acetamido-}N\text{-tert-butyl-4-(methylsulfonyl)butanamide (17).}\]

\[^{1}H\text{ NMR (CDCl}_3\text{)} \delta 6.58 (d, J = 7 Hz, 1H), 6.31 (s, 1H), 4.52 (dd, J = 13.8, 6.4 Hz, 1H), 3.25 (m, 1H), 3.07 (m, 1H), 2.97 (s, 3H), 2.19 (m, 2H), 2.00 (s, 3H), 1.33 (s, 9H); ^{13}C\text{ NMR (CDCl}_3\text{)} \delta 170.5, 169.2, 51.6, 51.4, 51.2, 40.6, 28.5, 26.2, 23.1; \text{IR (thin film) 3286, 3079, 2964, 1651, 1555, 1455, 1392, 1365, 1301, 1127, 1019, 800 cm}^{-1}; \text{LRMS (ESMS) calcd for C}_{11}\text{H}_{22}\text{N}_{2}\text{O}_{4}\text{SNa (M+Na)}^{+}: 301, \text{found: 301.}\]
Supporting Information Ekkati, Kodanko*

Part C. Kinetic Studies.

Reactions of the species $[\text{Fe}^{IV}(\text{O})(\text{N4Py})]^{2+}$ with amino acid substrates 1-7 were conducted at 25 °C under pseudo-first-order conditions. Rate constants represent the average of at least three runs. The reactions were monitored using a UV-vis spectrophotometer and were performed using the following standard procedure. A 2mM solution of $[\text{Fe}^{II}(\text{N4Py})(\text{MeCN})](\text{ClO}_4)_2$ in 3:1 H$_2$O:MeCN (1.0 mL, 20 µmol) was treated with a solution of oxone in H$_2$O (100 µL, 20 µmol, 1 equiv, 0.2 M). The solution turned from red to green and was aged for 10 min to allow for generation of $[\text{Fe}^{IV}(\text{O})(\text{N4Py})]^{2+}$ to reach completion, as judged by maximization of absorbance at 680 nm and disappearance of the absorption band at 450 nm, which corresponds with the Fe$^{II}$ species. Next, the green solution of $[\text{Fe}^{IV}(\text{O})(\text{N4Py})]^{2+}$ was treated a solution of amino acid in 1:3 H$_2$O:MeCN (900 µL, 200 µmol, 10 equiv, 0.2 M). After mixing (10 sec), the reaction was monitored by UV-vis spectroscopy (Figure S2). The absorbance traces for the decomposition of $[\text{Fe}^{IV}(\text{O})(\text{N4Py})]^{2+}$ ($\lambda = 680$ nm) showed first-order decay and fit well to the single exponential equation $[A = \Delta A(1-e^{-kt}) + A_0])$ for substrates 1, 6 and 7 (Table 1, Figures S2-S5). With substrate 5, decomposition of the $[\text{Fe}^{IV}(\text{O})(\text{N4Py})]^{2+}$ was complete immediately upon mixing (10 sec), as judged by disappearance of the absorption band at 680 nm. With substrate 4, decay of the signal at 680 nm could not be fit satisfactorily to the first-order rate equation, but qualitatively decay was slower than with 1. Substrates 2 and 3 did not cause absorbance at 680 nm to decay faster than a control experiment in which no substrate was added.
Figure S2. Spectral changes upon mixing 6 (10 equiv) with a solution of [Fe$^{IV}$(O)(N4Py)]$^{2+}$ in 1:1 H$_2$O:MeCN (final concentration = 1 mM) at 25 °C.

Figure S3. Time course of the decay of [Fe$^{IV}$(O)(N4Py)]$^{2+}$ (1 mM) upon treatment with substrate 6 (10 equiv) in 1:1 H$_2$O:MeCN at 25 °C (absorbance at 680 nm = red line, calculated fit = blue line, $k_{obs} = 17.0(5) \times 10^{-3}$ s$^{-1}$).
**Figure S4.** Time course of the decay of [Fe$^{IV}$(O)(N4Py)]$^{2+}$ (1 mM) upon treatment with substrate 7 (10 equiv) in 1:1 H$_2$O:MeCN at 25 °C (absorbance at 680 nm = red line, calculated fit = blue line, $k_{obs} = 3.15(8) \times 10^{-3} \, \text{s}^{-1}$).

**Figure S5.** Time course of the decay of [Fe$^{IV}$(O)(N4Py)]$^{2+}$ (1 mM) upon treatment with substrate 1 (10 equiv) in 1:1 H$_2$O:MeCN at 25 °C (absorbance at 680 nm = red line, calculated fit = blue line, $k_{obs} = 5.8(2) \times 10^{-5} \, \text{s}^{-1}$).
Figure S6. Time course of the decay of [Fe$^{IV}$(O)(N4Py)]$^{2+}$ (1 mM) upon treatment with substrate 2,2-$d_2$-1 (10 equiv) in 1:1 H$_2$O:MeCN at 25 °C (absorbance at 680 nm = red line, calculated fit = blue line, $k_{obs} = 1.2(2) \times 10^{-5}$ s$^{-1}$).

Figure S6. Time course of the decay of [Fe$^{IV}$(O)(N4Py)]$^{2+}$ (1 mM) upon treatment with substrate 4 in 1:1 H$_2$O:MeCN at 25 °C (absorbance at 680 nm = red line).
Part D. References


Part E. $^1$H and $^{13}$C NMR spectra for all new compounds
Supporting Information Ekkati, Kodanko*
Supporting Information Ekkati, Kodanko*
Chemical Formula: C₉H₇NO₂S
Molecular Weight: 246.37
Supporting Information Ekkati, Kodanko*
Supporting Information Ekkati, Kodanko*
Chemical Formula: C_{17}H_{20}NO_{3}S
Molecular Weight: 378.37