Supporting Information

Mode of iron(III) chelation by hexadentate hydroxypyrindinones

Tao Zhou, Robert Hider, Xiaole Kong*

*a Department of Applied Chemistry, School of Food Science and Biotechnology, Zhejiang Gongshang University, Hangzhou, Zhejiang, 310018, China

*b Institute of Pharmaceutical Science, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London, SE1 9NH, UK

E-mail: xiao.kong@kcl.ac.uk

Contents

**Figure S1.** High resolution mass spectrum of peak 779 provides evidence of doubly charged complex \([\text{Ga}^{III}(L-3H)+2\text{K}]^{2+}\). The complex was prepared from a 1:1 molar ratio of gallium chloride and \(\text{NTA(BuHP)}_3\) (4) (100µM) in water with stepwise addition of KOH(0.1M) to pH7.0. Sample was then diluted 10 fold in 1% formic acid and inject into the instrument........................................S2

**Procedure for the synthesis of NTA(BuHP)_3..........................................................S3

**MS experimental.................................................................S3-S4
Figure S1. High resolution mass spectrum of peak 779 provides evidence of doubly charged complex \([\text{Ga}_{2}^{III}(L-3\text{H})_2\text{K}]^{2+}\). The complex was prepared from a 1:1 molar ratio of gallium chloride and NTA(BuHP)\(_3\) (4) (100µM) in water with stepwise addition of KOH(0.1M) to pH7.0. Sample was then diluted 10 fold in 1% formic acid and inject into the instrument.
Synthesis of NTA(BuHP)$_3$ (4): A mixture of nitrilotriacetic acid (0.191g, 1mmol), dihydrochloric salt of 1-(4-aminobutyl)-3-(benzyloxy)-2-methylpyridin-4(1H)-one (1.292g, 3.6mmol), 1-hydroxybenzotriazole (0.551g, 3.6mmol), 1,3-dicyclohexylcarbodiimide (0.742g, 3.6mmol) and N-methyl morpholine (0.79mL) in DMF (15mL) was stirred at room temperature for 2 days. After filtration, the filtrate was concentrated and the residue was purified by silica column chromatography using CH$_2$Cl$_2$/MeOH (6:1 to 2:1) as an eluent to provide benzyl protected NTA(BuHP)$_3$ as a pale yellow solid (0.52g, 52% yield). To a suspension of the protected NTA(BuHP)$_3$ (0.18g) and concentrated hydrochloric acid (0.15 mL) in MeOH (20 mL) was added 5 % Pd/C (0.09 g). Hydrogenation was carried out at 30 psi H$_2$ for 3 h. After filtration to remove the catalyst, the filtrate was concentrated to dryness. The residue was purified by crystallization from methanol/acetone. The hydrochloride of NTA(BuHP)$_3$ was obtained as a white solid (0.138g, 88% yield). $^1$H NMR (DMSO-$d_6$, 400MHz) $\delta$ 1.48 (m, 6H, CH$_2$), 1.74 (m, 6H, CH$_2$), 2.54 (s, 9H, CH$_3$), 3.12 (m, 6H, CH$_2$), 3.55 (br, 6H, CH$_2$), 4.35 (t, $J$=7.6Hz, 6H, CH$_2$), 7.35 (d, $J$=7.2Hz, 3H, C5-H in pyridinone), 8.32 (d, $J$=7.2Hz, 3H, C6-H in pyridinone), 8.81 (t, $J$=6.0Hz, 3H, NH); $^{13}$C NMR (DMSO-$d_6$, 100MHz) $\delta$ 12.44 (CH$_3$), 25.57 (CH$_2$), 26.97 (CH$_2$), 30.68 (CH$_2$), 37.58 (CH$_2$), 55.43 (NCH$_2$CO), 110.71 (C-5H in pyridinone), 137.97 (C-2 in pyridinone), 141.34 (C-3 in pyridinone), 142.93 (C-6 in pyridinone), 158.47 (C-4 in pyridinone), 206.47 (CONH). ESI-MS: $m/z$ 726 [M+H]$^+$.

**MS Instruments:**

**High resolution mass spectrometry** Thermo Exactive Benchtop Orbitrap with Instrument settings:
Sheath Gas flow rate: 10; Aux gas flow rate: 0; Sweep gas flow rate: 0; Sprayvoltage: 4 kV; Capillary temp 250 °C; Capillary voltage: 60 V; Tube lens voltage: 120 V; Skimmer voltage: 25 V; Heater temp: 30 °C; Ion source: ESI positive mode; Resolution: Ultra High; Fragmentation: HCD Gas on.

**Electrospray ionization mass spectrometry** Samples were directly infused into a LCQ Deca XP ion trap mass spectrometer (ThermoFinnigan, San Jose, USA) using a 250 µl syringe at a flow rate of 5.0 µl/min. The instrument was operated in positive ion mode employing the following conditions:
source voltage 4.5 kV; capillary voltage 25 V; capillary temperature 100 to 300 °C and tube lens voltage 10 V.

**ESI-MS of the Gallium complex of NTA(BuHP)_3:**

The MS displays a major peak at 831.2 (Figure S1A) which corresponds to complex consisting of a 1:1 molar ratio of gallium and NTA(BuHP)_3 (4), namely [Ga^{III}(L-3H)+K]^+. With high resolution MS (Figure S1B), the peak was found to display a typical gallium isotope distribution, namely 830.24, 830.75, 831.24, 831.75, 832.24, 832.75, 833.24, where the peaks are separated by half mass units. This confirms that the peak 831.2 corresponds to a doubly charged dimer of mass 1662.4 corresponding to the complex [Ga_2^{III}(L-3H)_2+2K]^2+. 