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SYNTHETIC STUDIES INTO 3-HYDROXY-2(1)-PYRIDINONE BASED HEXADENTATE METAL(III) ION CHELATORS

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Abstract: An improved synthesis and purification of the hexadentate chelators, N,N,N,-tris[2-(3-hydroxy-2-oxo-1,2-dihydropyridin-1-yl)acetamido]ethylamine, **8a** and N,N,N,-tris[2-(3-hydroxy-4-methyl-2-oxo-1,2-dihydropyridin-1-yl)acetamido]ethylamine, **8b** is described.

Desferrioxamine B[1], a naturally occurring siderophore that promotes the excretion of iron[2] from iron-overloaded patients[3-4] has led to a world-wide program of research directed towards the development of synthetic siderophore analogues. Although prescribed clinically, it possesses a number of side-effects.[5-7] Research has endeavoured to address the fundamental properties of potential synthetic substitutes by tailoring parameters such as metal(III) ion specificity and toxicity to give an improved performance in chelation therapy. In response, a range of novel bidentate and hexadentate ligands containing the 3-hydroxy-2(1H)-pyridinone moiety have been designed and synthesised[8] with properties suited for use as effective orally active iron(III) chelation agents. The hexadentate chelator, N,N,N,-tris[2-(3-hydroxy-2-oxo-1,2-dihydropyridin-1-yl)acetamido]ethylamine (**8a**, Fig.1) which provides octahedral co-ordination of iron(III) has been shown to be efficacious in the intracellular mobilisation of iron in both the *in vitro* and *in vivo* system.

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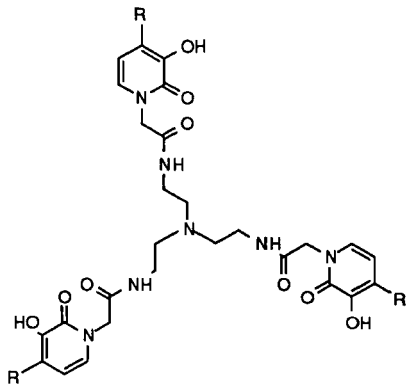


Figure 1: The structures of the hexadentate chelators a) N,N,N,-tris[2-(3-hydroxy-2-oxo-1,2-dihydropyridin-1-yl)acetamido]ethylamine (R = H, **8a**) and b) N,N,N-tris [2-(3-hydroxy-4-methyl-2-oxo-1,2-dihydropyridin-1-yl)acetamido]ethylamine (R= CH_3, **8b**).

This communication reports an improved synthesis and purification of **8a** and discloses for the first time the synthesis of a new analogue of **8a** in which a methyl substituent has been introduced onto each of the bidentate chelating moieties to furnish N,N,N-tris[2-(3-hydroxy-4-methyl-2-oxo-1,2-dihydropyridin-1-yl)acetamido]ethylamine (**8b**, Fig.1). Hexadentate **8a** was synthesised by the amide coupling of three 3-hydroxy-2(1H)-pyridinone bidentate moieties to the tripodal tetraamine, tris(2-aminoethyl)amine. The protection of the hydroxyl functionality as a benzyl ether as described in an earlier publication[8] was found to be superfluous. The active ester of the intermediate carboxylic acid was formed with N-hydroxyphthalimide rather than N-hydroxysuccinimide to furnish an active ester which was soluble in tetrahydrofuran.

Coupling was attained by condensing each of the primary amine functions of the tripodal amine, tris(2-aminoethyl)amine with one molecule of the activated ester. Anion exchange chromatography was used to purify the chelator. The introduction of the methyl substituent on **8b** was effected by aminomethylation of the pyridin-2-one ethyl ester. Hydrogenolysis of the resultant Mannich base afforded the alkyl substituted ester which was saponified to furnish a carboxylic acid. Subsequent amide coupling was undertaken as reported for **8a**. The resultant hexadentate chelator **8b** did not require anion exchange chromatographic purification.

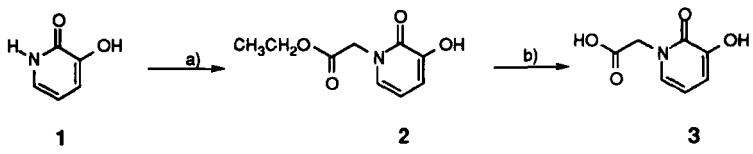
RESULTS AND DISCUSSION

3-Hydroxy-2(1H)-pyridinone **1** was cleanly converted to the N-substituted ethyl ester, 1-ethoxycarbonylmethyl-3-hydroxy-2(1H)-pyridinone **2** as described in the literature. The conversion of the crystalline ester **9**9 to the desired carboxylic acid, 1-carboxylmethyl-3-hydroxy-2(1H)-pyridinone, **3** was effected by base saponification (Scheme 1).

In the earlier synthesis[8], the ester **2** was saponified and the 3-hydroxy moiety simultaneously protected by refluxing with an ethanolic mixture of benzyl chloride and sodium hydroxide to afford the 3-benzyloxy derivative. The need to mask the 3-hydroxy moiety has now been shown to be superfluous thus simplifying the syntheses of the hexadentate chelators **8a** and **8b**.

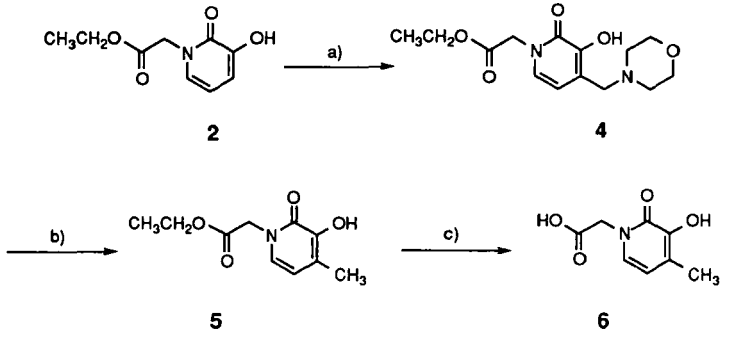
The synthetic route envisioned for the preparation of the hexadentate chelator **8b** necessitiated the regioselective introduction of a methyl substituent. It has been demonstrated that 3-hydroxy-2(1H)-pyridinones readily undergo monoamino-methylation exclusively at the position *ortho* to the 3-hydroxy function[9-10] under facile conditions to furnish 4-aminomethyl-3-hydroxy-2(1H)-pyridinone Mannich bases.[11] The corresponding Mannich reaction of the ethyl ester **2** with three mole equivalents each of formaldehyde and morpholine gave a crystalline product readily identified as 1-ethoxycarbonylmethyl-3-hydroxy-4-morpholinomethyl-2(1H)-pyridinone **4**. Mannich base **4** was readily further transformed to the methyl intermediate 1-ethoxy carbonylmethyl-3-hydroxy-4-methyl-2(1H)-pyridinone **5** under standard palladium catalysed transfer hydrogenolysis conditions. The target carboxylic acid, 1-carboxylmethyl-3-hydroxy-4-methyl-2(1H)-pyridinone **6** was finally realised upon base saponification of the ester in a manner described previously (Scheme 2).

All of the 3-hydroxy-2(1H)-pyridinone chelators were isolated as crystalline solids and characterised according to conventional procedures. An analysis of the proton NMR spectra reveals readily assignable resonances which are characteristic of this class of compound.[9] The signal attributable to the H-4 proton in the derivatives **2** and **3** (δ 6.78 and δ 6.72 respectively) is no longer apparent in the substituted compounds **4**, **5** and **6** thus confirming that 4-substitution has occurred. The bidentates **5** and **6** are further characterised by the appearance of an unambiguous singlet (δ 2.01 and δ 2.16 respectively) corresponding to the methyl substituent. In addition to the preservation of the characteristic four band infrared spectral profile (1600-1400 cm^{-1}) of the pyridinones,[12-16] a new absorption band ($\nu_{C=O}$, 1705-1743 cm^{-1}) is observed for the



Reagents and conditions: a) $BrCH_2CO_2Et$ (5 mol. equiv.), N_2, reflux, 12 hours; b) 4 M NaOH, c. HCl dropwise with cooling.

Scheme 1

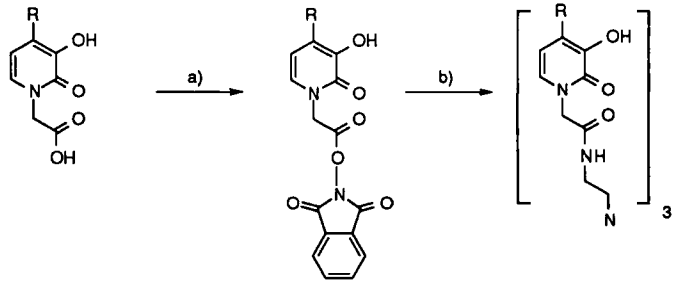


Reagents and conditions: 37% formaldehyde (3 mol. equiv.), morpholine (3 mol. equiv.), 96% ethanol, 2 hrs, r.t; b) 10% palladium-on-carbon catalyst, cyclohexene, absolute ethanol, reflux, 24 hours; c) 4 M NaOH, c. HCl dropwise with cooling.

Scheme 2

carbonyl component of the ester derivatives **2**, **4** and **5** which shifts to shorter wavelengths in the carboxylic acid forms, **3** and **6** (1705-1717 cm^{-1}).

Carboxylic acids **3** and **6** were converted to active esters using dicyclohexylcarbodiimide (DCCI) as the activating reagent to facilitate amide coupling. In the earlier synthesis,[8] the ester of N-hydroxysuccinimide was employed but led to



R = H, **3** R = H, **7a** R = H, **8a**

R = CH_3, **6** R = CH_3, **7b** R = CH_3, **8b**

Reagents and conditions: a) N-hydroxyphthalimide in THF (1.5 mol. equiv.), 0°C, 20 mins., dicyclohexylcarbodiimide in THF (1.5 mol. equiv.), 0°C, 20 mins followed by 1 hour at room temperature; b) Tris(2-aminoethyl)amine (0.2 mol. equiv.), triethylamine (3.0 mol. equiv.), THF, room temperature, 1 hour.

Scheme 3

active esters which were insoluble in tetrahydrofuran (THF) and precipitated with the side-product, dicyclohexylurea (DCU). This step has been modified by use of an ester of N-hydroxyphthalimide furnishing THF-soluble phthalimide active esters, 3-hydroxy-1-phthalimyl-oxycarbonylmethyl-2(1H)-pyridinone **7a** and 3-hydroxy-4-methyl-1-phthalimyl-oxycarbonylmethyl-2(1H)-pyridinone **7b** from which DCU could be removed by gravity filtration.

The condensation of the active esters **7a** and **7b** with the tripodal amine was facilitated by maintaining the molar ratio of the activated ester in excess of 5:1 with respect to the tetraamine (Scheme 3).

Triethylamine was introduced to function as a sacrificial amine and promote the addition of the tetraamine to the active esters.

The reaction of active ester **7a** initially resulted in the precipitation of a bright, orange coloured solid. This solid was re-dissolved in ethanol and following removal of the solvent, sufficient base was added to dissolve the solids and hydrolyse any remaining active ester. The THF-insoluble by-product, N-hydroxyphthalimide was

removed by filtration and the basified filtrate containing crude **8a** purified by anion exchange chromatography. Phthalimide active ester **7b** was reacted similarly. However, the desired hexadentate chelator **8b** precipitated as an off-white powder.

The application of synthetic resins as ion exchangers was utilised for the purification of the hexadentate chelator, N,N,N,-tris[2-(3-hydroxy-2-oxo-1,2-dihydropyridin-1-yl)acetamido]-ethylamine, **8a**. For the purpose of this work, a quaternary ammonium cellulose (trimethylhydroxy-propylcellulose, hydrochloride salt) anion exchanger (Whatman QA 92) was employed in a batch method.

The net charge of the hexadentate chelator **8a** between the range pH 0-14 was initially calculated from the four pK_a values previously reported.[17] A plot of net charge versus pH generated from the data (not shown) shows that in the range pH > 11, the hexadentate chelator has a net charge of -3.0 and would therefore be expected to bind strongly to the exchange medium whilst permitting any impurities to be washed away. Contrastingly, in the range pH < 4, the net charge is +1.0 and the chelator should be readily recovered by elution. By the application of this technique, an excellent separation of the hexadentate **8a** from known contaminants including traces of the iron(III) complex was achieved. It is anticipated that the technique of anion exchange chromatographic purification may be extended to a large range of oligodentate chelators based on 3-hydroxy-2(1H)-pyridinones.

SUMMARY

The omission of a protecting group on the 3-hydroxy function causes no apparent decrease in the overall yield and makes a considerable simplification to the synthesis of **8a**. The hydroxy group of N-hydroxyphthalimide appears to react much faster with the adduct of dicyclohexylcarbodiimide and the free carboxylic acid than the 3-hydroxy moiety of the pyridinone. It is possible that even if an ester with 1-carboxymethyl-3-hydroxy-2(1H)-pyridinone is formed, it is also an active ester susceptible to amine nucleophiles and therefore giving no final side products. Anion exchange chromatography gives an excellent separation of the free chelator from known impurities including a trace of the iron(III) complex.

A new hexadentate chelator, N,N,N-tris [2-(3-hydroxy-4-methyl-2-oxo-1,2-dihydropyridin-1-yl) acetamido]ethylamine containing three methyl substituents was synthesised utilising the Mannich reaction. Further investigations into the synthesis of the hexadentate chelators based on substituted 3-hydroxy-2(1H)-pyridinones are planned.

EXPERIMENTAL

Melting points were determined on a Gallenkamp capillary melting point apparatus and are uncorrected. The infrared spectra were determined as potassium bromide (KBr) discs in the range 4000-500 cm^{-1} with a Perkin Elmer 1605 FT-IR spectrometer and were referenced to polystyrene film. Microanalyses (C, H, N,) were carried out by the Microanalytical Section of the Department of Chemistry, University College London. The proton nuclear magnetic resonance (^1H NMR) spectra were recorded in DMSO-d_6 on a Varian VXR-400 (400 MHz) spectrometer with the chemical shifts reported in parts per million (δ). The residual protic solvent signal i.e. $C_2D_5\underline{H}S{=}O$ (δ_H = 2.52 ppm) was used as the internal reference. The carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded at 100 MHz using the residual resonances of $(\underline{C}D_3)_2S{=}O$ (δ_C = 39.7 ppm) as an internal reference. Compounds run in D_2O were referenced to 3-(trimethylsilyl)-1-propanesulphonic acid, sodium salt) All reagents were used as received unless otherwise stated.

1-Ethoxycarbonylmethyl-3-hydroxy-2(1H)-pyridinone, 2

Compound **2** was prepared from 3-hydroxy-2(1H)-pyridinone **1** and ethyl bromoacetate according to the literature procedure.[8] Yield = 75%. Melting point: 151-153 °C (lit[8] mp. 150-152 °C). ^1H NMR (400 MHz, DMSO-d_6) δ 1.19 (t, 3H, $^3J_{H,H}$ = 7.6 Hz, $C\underline{H}_3CH_2O$-), 4.13 (q, 2H, $^3J_{H,H}$ = 7.6 Hz, $CH_3C\underline{H}_2O$-), 4.70 (s, 2H, $NC\underline{H}_2CO$),6.10 (t, 1H, $^3J_{H,H}$ = 6.4 Hz, H-5), 6.78 (d, 1H, $^3J_{4,5}$ = 7.2 Hz, H-4), 7.13 (d, 1H, $^3J_{5,6}$ = 7.0 Hz, H-6), OH group exchanged. ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.08 (CH_3 of ester), 50.34 (-$N\underline{C}H_2CO$-), 60.98 (CH_2 of ester), 105.24 (C-5), 115.38 (C-6), 128.89 (C-4), 146.69 (C-3), 157.91 (C-2), 168.05 ($\underline{C}OCH_2$). IR (KBr, cm^{-1}): Selected data: 1743 ($\nu_{C=O}$, ester), 1662 ($\nu_{C=O}$), 1609 ($\nu_{C=O}$),1551 ($\nu_{C=C}$), 1458 ($\nu_{C=C}$). Elemental analysis: $C_9H_{11}O_4N$ requires C, 54.82%; H, 5.62%; N, 7.10%. Found C, 54.50%; H, 5.46%; N, 6.91%.

1-Carboxylmethyl-3-hydroxy-2(1H)-pyridinone, 3

Compound **2** (9.86 g, 50 mmol) was dissolved in aqueous sodium hydroxide (4M, 50 mL). After 20 minutes, concentrated hydrochloric acid was added dropwise with cooling until a faint precipitate just appeared. Upon standing, crystals of **3** precipitated (7.02 g, 83%). Melting point: 201-203 °C.^1H NMR (400 MHz, DMSO-d_6) δ4.63 (s, 2H, $NC\underline{H}_2CO$), 6.08 (t, 1H, $^3J_{H,H}$ = 6.4 Hz, H-5), 6.72 (d, 1H, $^3J_{4,5}$ = 7.2 Hz, H-4),

7.12 (d, 1H, $^3J_{5,6}$ = 7.0 Hz, H-6), 9.16 (s, 1H, ring OH), COOH group not observed. ^{13}C NMR (100 MHz, DMSO-d_6) δ 50.32 (-N$\underline{C}H_2CO$-), 105.13 (C-5), 115.35 (C-6) 129.07 (C-4), 146.72 (C-3), 157.99 (C-2), 169.40 ($\underline{C}OCH_2$). IR (KBr, cm^{-1}): Selected data: 1705 (ν C=O, acid), 1652 (ν C=O, pyridinone), 1599 (ν C=O, pyridinone),1560 (ν C=C), 1462 (ν C=C). Elemental analysis: $C_7H_7O_4N$ requires C, 49.71%; H, 4.17%; N, 8.28%. Found C, 50.08%; H, 3.97%; N, 7.92%.

1-Ethoxycarbonylmethyl-3-hydroxy-4-morpholinomethyl-2(1H)-pyridinone, 4

Compound **2** (3.00 g, 15.2 mmol) was stirred into aqueous ethanol (96%, 30 mL) at room temperature. Aqueous formaldehyde (37%, 3.70 g, 45.6 mmol) and morpholine (3.97 g, 45.6 mmol) were mixed with cooling for 30 minutes and then added dropwise to the ethanolic mixture prepared above. Stirring was maintained for 2 hours and then left to stand overnight during which time a solid precipitated. The precipitate was filtered off, washed with acetone and recrystallised from 96% ethanol to furnish **2** as a white crystalline solid (3.56 g, 79%). Melting point: 147-149 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.19 (t, 3H, $^3J_{H,H}$ = 7.0 Hz, $CH_3\underline{C}H_2O$-), 2.36 (sbr, 4H, 3/5-M), 3.36 (s, 2H, $C\underline{H}_2$), 3.57 (sbr, 4H, 2/6-M), 4.14 (q, 2H, $^3J_{H,H}$ = 7.1 Hz, $CH_3C\underline{H}_2O$-), 4.69 (s, 2H, $NC\underline{H}_2CO$), 6.21 (d, 1H, $^3J_{5,6}$ = 7.2 Hz, H-5), 7.09 (d, 1H, H-6), OH group exchanged. ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.07 (CH_3 of ester), 50.21 (-N$\underline{C}H_2CO$-), 53.28 (3-M), 55.24 (CH_2N), 60.96 (CH_2 of ester), 66.19 (2-M), 106.60 (C-5), 125.00 (C-6), 127.70 (C-4),144.17 (C-3), 157.37 (C-2), 168.05 ($\underline{C}OCH_2$). IR (KBr, cm^{-1}): Selected data: 1737 (ν C=O, ester), 1665 (ν C=O), 1604 (ν C=O),1558 (ν C=C), 1454 (ν C=C). Elemental analysis: $C_{14}H_{20}O_5N_2$ requires C, 57.75%; H, 6.80%; N, 9.45%. Found C, 57.87%; H, 6.81%; N, 9.33%.

1-Ethoxycarbonylmethyl-3-hydroxy-4-methyl-2(1H)-pyridinone, 5

Compound **4** (5.00 g, 16.87 mmol) was stirred into a mixture of cyclohexene (80 mL) and absolute ethanol (40 mL). After 30 minutes, the reaction mixture was treated with 10% palladium-on-carbon catalyst (1.0 g) and the mixture refluxed for 24 hours after which time TLC analysis indicated the consumption of the Mannich base. After removing the catalyst by gravity filtration, the filtrate was concentrated under diminished pressure to afford an off-white crystalline solid. Subsequent recrystallisation from absolute ethanol furnished **5** as fluffy, needle crystals (2.60 g, 73%). Melting point: 157-159 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ1.19 (t, 3H, $^3J_{H,H}$ = 7.0 Hz, $-OCH_2C\underline{H}_3$), 2.01 (s, 3H, ring $C\underline{H}_3$), 4.13 (q, 2H, $^3J_{H,H}$ = 7.2 Hz,

-OC$\underline{H}_2$CH$_3$), 4.68 (s, 2H, -NC$\underline{H}_2$-), 6.06 (d, 1H, $^3J_{5,6}$ = 6.8 Hz, H-5), 7.06 (d, 1H, H-6), 8.76 (s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6) δ14.09 (CH$_3$ of ester), 14.87 (ring CH$_3$), 50.16 (-N$\underline{C}$H$_2$-), 60.95 ($\underline{C}$H$_2$ of ester), 108.72 (C-5), 125.50 (C-6), 127.50 (C-4), 143.27 (C-3), 157.21 (C-2), 168.12 ($\underline{C}$OCH$_2$). IR (KBr, cm^{-1}): Selected data: 1705 (ν C=O, ester), 1652 (ν C=O, pyridinone), 1599 (ν C=O, pyridinone), 1560 (ν C=C, 1462 (ν C=C). Elemental analysis: $C_{10}H_{13}O_4N$ requires C, 56.86%; H, 6.20%; N, 6.63%. Found C, 56.63%; H, 5.98%; N, 6.46%.

1-Carboxylmethyl-3-hydroxy-4-methyl-2(1H)-pyridinone, 6

Compound **5** was treated in an similar manner to that described for **3** furnishing **6** as small crystals (Yield = 80%). Melting point: 246 °C (decomp.). ^{1}H NMR (400 MHz, D$_2$O) δ 2.16 (s, 3H, CH$_3$), 4.53 (s, 2H, -NC$\underline{H}_2$-), 6.33 (d, 1H, $^3J_{5,6}$ = 7.2 Hz, H-5), 7.02 (d, 1H, H-6), OH groups exchanged. ^{13}C NMR (100 MHz, D$_2$O) δ17.32 ($\underline{C}$H$_3$), 55.84 (-N$\underline{C}$H$_2$-), 114.11 (C-5), 130.92 (C-6), 132.99 (C-4), 145.26 (C-3), 160.50 (C-2), 177.27 ($\underline{C}$OCH$_2$). IR (KBr, cm^{-1}): Selected data: 1717 (ν C=O, acid), 1653 (ν C=O, pyridinone), 1586 (ν C=O, pyridinone), 1540 (ν C=C), 1472 (ν C=C). Elemental analysis: $C_8H_9O_4N$ requires C, 52.46%; H, 4.95%; N, 7.65%. Found C, 52.20%; H, 5.10%; N, 7.41%.

General procedure for the synthesis of phthalimide active esters, 7a and 7b.

To a solution of the free acid, **3** or **6** (0.02 mol) and N-hydroxyphthalimide (4.89 g, 0.03 mol) in cooled (0°C) tetrahydrofuran (100 mL) was added a THF solution (15 mL) of dicyclohexylcarbodiimide (DCCI, 6.19 g, 0.03 mol). Stirring was maintained at 0°C for 20 minutes during which time a white precipitate of dicyclohexylurea (DCU) appeared. The solution was permitted to warm to room temperature and stirred for a further 1 hour prior to removing the THF-insoluble DCU via filtration. The resultant filtrate containing **7a** or **7b** was used immediately in the next step.

N, N, N-tris[2-(3-hydroxy-2-oxo-1,2-dihydropyridin-1-yl)acetamido]ethylamine, 8a

To the filtrate containing **7a** was added a THF solution (15 mL) of tris(2-aminoethyl)amine (0.57 g, 4 mmol) and triethylamine (6.07 g, 60 mmol) dropwise over several minutes at room temperature. Stirring was maintained for 1 hour during which time a bright orange coloured solid precipitated. The product was re-dissolved in absolute ethanol and stirring continued overnight. The reaction mixture was evaporated to dryness affording crude **8a** as an orange solid. Sufficient aqueous

sodium hydroxide (1 M) was added to dissolve the crude chelator and hydrolyse any remaining phthalimide active ester. The THF-insoluble side product, N-hydroxy phthalimide was removed by filtration and the filtrate containing the desired product purified via anion exchange chromatography.

Anion Exchange Chromatography

The filtrate was added to a slurry of Whatman QA92 quaternary ammonium cellulose anion exchange resin (10 g) in purified water (40 mL) and carefully titrated to pH 11 with aqueous sodium hydroxide and allowed to stand with intermittent stirring. After 1 hour the resin was filtered and washed twice with distilled water (40 mL). The anion exchange resin was re-suspended in water and titrated to pH 4 with aqueous hydrochloric acid. After standing for 1 hour with intermittent stirring, the resin was re-filtered and washed once with water (20 mL). The filtrate and acidic washings were subsequently combined and lyophilised affording **8a** as a colourles solid (0.79 g, 33% based on tris(2-aminoethyl)amine). Melting point: 180-182 ^{o}C (lit [8] mp. 178-180^{o}C, decomp.). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.17 (sbr, 6H, 3 x NHC$\underline{H}_2$), 3.29 (sbr, 6H, 3 x C$\underline{H}_2$N), 4.47 (s, 6H, 3 x NC$\underline{H}_2$CO),5.92 (t, 3H, $^{3}J_{H,H}$ = 6.8 Hz, 3 x H-5), 6.56 (d, 3H, $^{3}J_{4,5}$ = 5.6 Hz, 3 x H-4), 6.93 (d, 3H, $^{3}J_{5,6}$ = 6.8 Hz, 3 x H-6), 8.22 (sbr, 3H, 3 x N$\underline{H}$), 8.92 (sbr, 3H, 3 x OH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 36.81 (C-10), 51.38 (C-9), 53.18 (C-7), 104.85 (C-5), 115.18 (C-6), 129.55 (C-4), 146.60 (C-3), 157.92 (C-2), 167.07 (C-8). IR (KBr, cm^{-1}):Selected data: 1713 (ν C=O),1651(ν C=O, pyridinone), 1590 (ν C=O, pyridinone), 1552 (ν C=C), 1462 (ν C=C). Elemental analysis: $C_{27}H_{33}O_9N_7.1/2\ H_2O$ requires C, 53.28%; H, 5.63%; N, 16.11%. Found C, 53.61%; H, 5.90%; N, 16.11%.

N, N, N-tris[2-(3-hydroxy-4-methyl-2-oxo-1,2-dihydropyridin-1-yl)acetamido] ethylamine, 8b

To the filtrate containing **7b** was added a THF solution (10 mL) of tris(2-aminoethyl)amine (0.57 g, 4 mmol) and triethylamine (6.07 g, 60 mmol) dropwise over several minutes. The reaction mixture was stirred at room temperature for 1 hour prior to treatment with absolute ethanol (50 mL) and stirring continued overnight after which time **8b** precipitated as an off-white solid. The solid was filtered off, washed thoroughly with absolute ethanol and dried *in vacuo* over P_2O_5 and KOH pellets affording **8b** as a fine white powder (1.03 g, 40% based on tris(2-aminoethyl)amine). Melting point: 231-233^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.99 (sbr, 9H, 3 x C$\underline{H}_3$), 3.14 (sbr, 6H, 3 x

NHCH_2), 3.36 (sbr, 6H, 3 x CH_2N), 4.51 (sbr, 6H, 3 x NCH_2CO),6.00 (d, 3H, $^3J_{5,6}$ = 6.8 Hz, 3 x H-5), 6.97 (d, 3H, 3 x H-6), 8.03 (sbr, 3H, 3 x NH), 8.57 (sbr, 3H, 3 x OH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.85 (CH_3), 37.24 (C-10), 51.09 (C-9), 53.29 (C-7), 108.30 (C-5), 125.16 (C-6), 128.10 (C-4), 143.17 (C-3), 157.24 (C-2), 166.96 (C-8). IR (KBr, cm^{-1}): Selected data: 1700 (ν C=O), 1654 (ν C=O, pyridinone), 1593 (ν C=O, pyridinone), 1558 (ν C=C), 1464 (ν C=C). Elemental analysis: $C_{33}H_{39}O_9N_7.1/2$ H_2O requires C, 57.72%; H, 5.87%; N, 14.28%. Found C, 57.87%; H, 5.53%; N, 14.13%.

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