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BIS(5-HYDROXY-2-HYDROXYMETHYL-PYRAN-4-ONE-6-YL)METHANE: A NOVEL LIGAND FOR THE INTRACELLULAR MOBILISATION OF FERRITIN-BOUND IRON

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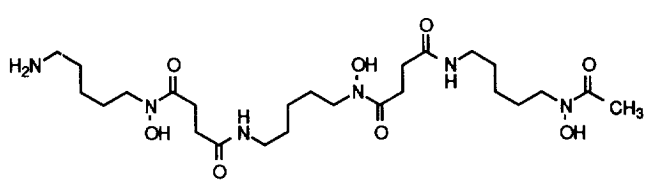
Abstract: The efficacy of a novel tetradentate iron(III) ligand for the in vitro mobilisation of ferritin-bound iron is measured in direct comparison to the clinically approved agents, 1,2-dimethyl-3-hydroxypyridin-4-one and desferrioxamine.

Desferrioxamine B[1] (DFO, **1**), a linear trishydroxamic acid siderophore is the most widely studied iron(III) chelating ligand. Although it binds a number of different metal(III) ions, it exhibits a high specificity for iron(III) forming a very stable hexacoordinate complex (formation constant, $K = 3 \times 10^{30}$).[2] Since DFO was first demonstrated to increase iron excretion,[3] it has become the current drug of choice for transfusional iron overload therapy[4] in the form of the methane sulphonate salt (Desferal®, Ciba-Geigy). Unfortunately, DFO is beset with disadvantages namely oral inactivity, rapid degradation in the hepatobiliary system, and poor patient compliance.[5]



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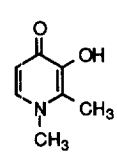

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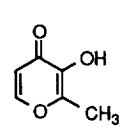

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In response, a number of *N*-alkyl-3-hydroxypyridin-4-one bidentate chelators have been developed as potential substitutes.[6] The specific chelator, 1,2-dimethyl-3-hydroxypyridin-4-one (deferiprone, **2**) also coordinates iron(III) with a formation constant which is five times the order of magnitude of DFO.[7] Ligand **2** has been extensively tested for in vitro iron(III) mobilisation in mammalian cell systems and in vivo in animals although some toxic effects are known.[8]

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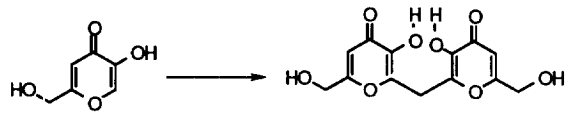
Bis(5-hydroxy-2-hydroxymethyl-pyran-4-one-6-yl)methane[9] **5** was prepared from kojic acid **4** in an improved single facile step by a modification of the Mannich reaction in which the aminomethylation reaction was suppressed in favour of a dimeric, Bakelite-type structure (Scheme 1).



4 **5**

Scheme 1: Reagents and conditions: Kojic acid (1 equiv), formaldehyde (37 wt. % in water, 0.5 equiv), dimethylamine (40 wt. % in water, 0.5 equiv), 96% EtOH, room temperature, 24 h.[10]

The potential of ligand **5** as a suitable ligand for the in vitro mobilisation of ferritin-bound iron in the mammalian cell system was assessed by the application of a hepatocyte cell system assay used to permit the simultaneous comparison of cellular iron uptake and toxicity.[11] Whereas the assay has been used acutely,[12] it is now considered that a more meaningful result may be attained if the cultured cells are exposed to the chelator over a number of days.

Hepatocytes were isolated by collagenase perfusion of the livers of adult male Wister rats.[13] The cells were subsequently established in culture on collagen coated Petri dishes at around 2 x 10^6 cells per dish in a serum-free culture medium.[14] Viable hepatocytes were labelled and iron loaded by incubation with radio-labelled ^{59}Fe-ferritin for 24 h.[15] The resultant preparations were incubated subsequently with the following chelators: (a) DFO, **1**; (b) deferiprone, **2**; (c) 3-hydroxy-2-methyl-pyran-4-one (maltol, **3**) and the test compound **5** at a concentration of 50 mM in fresh medium for 2, 4, 6, 8, or 10 days and the intracellular iron determined and compared with control incubations containing no chelator. The concentration of 50 mM was chosen since **1** is known to be fully effective at this level. At this concentration, ligand **3** is only partially effective and is used to test the sensitivity of the system. In order to measure the time course of ^{59}Fe efflux in the presence of the chelators, the media was removed for ^{59}Fe counting and replaced with fresh medium.

At 50 mM, the hexadentate chelator **1** mobilised 90% of the intracellular iron in 10 days whereas the control bidentate chelators **2** and **3** mobilised 100% and 20% respectively (Fig.1). These results were expected and acted as good controls for tests with the experimental chelator. Tetradentate **5** also achieved 100% cell depletion of iron following the same time course as **1** and **2** suggesting that the tetradentate chelator is efficacious in the in vitro system. It is likely that the free ligand and its iron(III) complex(es) cross the cell membrane. The data also suggests that **5** may have a flatter, and from a clinical viewpoint, more manageable dose-response relationship. Although not reported here, the more lipophilic chelators have been shown to enhance the rate of iron mobilisation and this explains their apparent potency in short term tests. However, over a longer test period, less lipophilic chelators would be expected to be at least as effective.

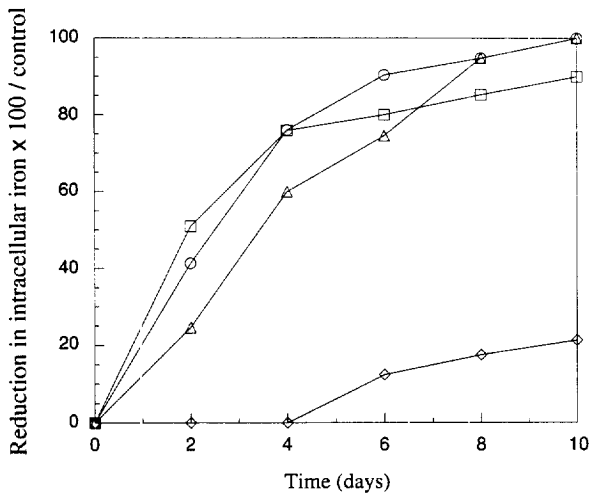


Figure 1: Cellular iron mobilisation. Iron-59 radio-labelled hepatocytes incubated with 50 mM concentrations of the following chelators: (a) desferrioxamine, **1** (□); (b) 1,2-dimethyl-3-hydroxypyridin-4-one, **2** (o); (c) 3-hydroxy-2-methyl-pyran-4-one, **3** (◊) and (d) bis(5-hydroxy-2-hydroxymethyl-pyran-4-one-6-yl) methane, **5** (Δ). Each value is the mean of three observations. Standard deviations less than 10% of the mean.

The results of this preliminary in vitro study support the case that more hydrophilic chelators may be interesting as iron(III) mobilisation agents. The test employed here does not address whether ligand **5** has acceptable bioavailability or toxicity in vivo. This would need to be investigated in a more appropriate model system.

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10. Typical experimental procedure for compound **5**: 5-Hydroxy-2-hydroxymethyl-pyran-4-one **4** (2.00 g, 14 mmol) was stirred into aqueous ethanol (96%, 20 mL) at room temperature. Aqueous dimethylamine (40%, 0.80 g, 7.1 mmol) and aqueous formaldehyde (37%, 0.58 g, 7.1 mmol) were mixed with ethanol (2 mL) for 30 minutes and then added dropwise to the ethanolic mixture prepared above over several minutes. Stirring was maintained at room temperature for 24 h during which time a fine white precipitate appeared. Upon refrigeration for 6 h, the reaction mixture was filtered and the resultant solid recrystallised from hot water to furnish **5** as a fine white powdery solid. Analytical data for **5**: mp: 245–246 °C (lit[9]. mp: 248.3–249 °C, corrected); IR (KBr): 3177, 3059, 1662, 1631, 1583, 1473, 1227, 1142, 1074, 864 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 4.03 (s, 2H, -CH_2-), 4.24 (sbr, 4H, 2 x $C\underline{H_2}OH$), 5.65 (sbr, 2H, 2 x $CH_2\underline{OH}$), 6.30 (s, 2H, 2 x H-3), 9.08 (sbr, 2H, 2 x OH); ^{13}C NMR (100 MHz) δ 26.85 (-CH_2-), 59.44 (CH_2OH), 109.06 (C-3), 142.64 (C-6), 145.46 (C-5), 167.46 (C-2), 173.43 (C-4); MS (FAB): MH^+ (297, 94%); Anal. calcd for $C_{13}H_{12}O_8$: C, 52.71; H, 4.08%. Found C, 52.53; H, 4.35%
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