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THE DESIGN OF MAGNETIC RESONANCE CONTRAST AGENTS : NEW IRON (III) DIHYDROXAMATE COMPLEXES

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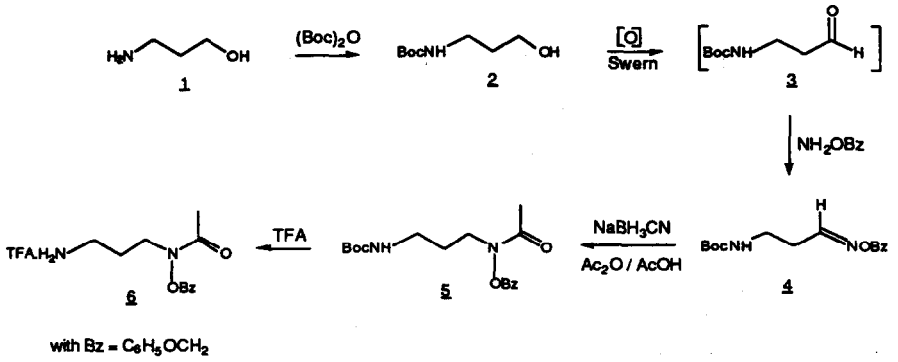
We have prepared a series of dihydroxamates and their iron (III) complexes in view of obtaining new NMR imaging agents. Four new dihydroxamic acid ligands have been synthesized by condensation of 4-substituted-2,6-pyridine dicarboxylic acids with N-aminopropyl-N-acetylhydroxylamine, and the iron (III) complexes exhibit high stability over the pH range 3 to 7 and a binuclear structure Fe_2L_3 at pH7. The presence of two non-interacting paramagnetic centers per mole should allow a good compromise between an efficient relaxivity and limited toxicity.

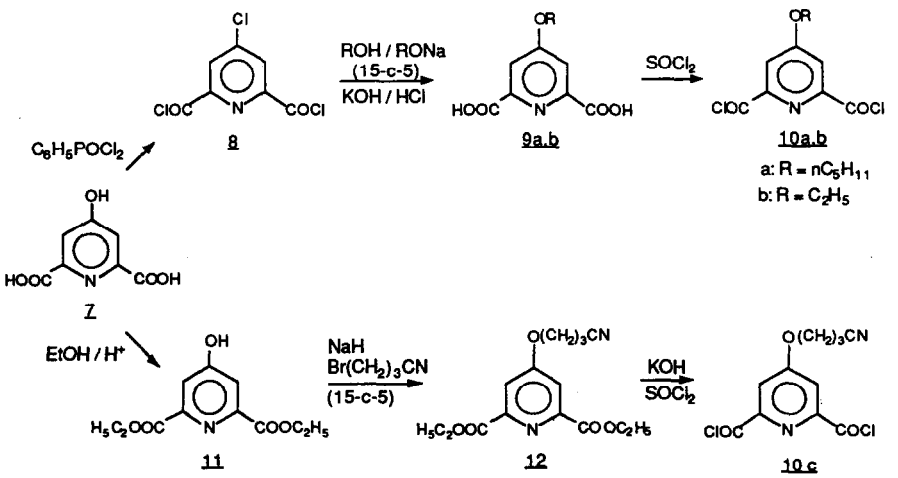
Contrast agents for NMR imaging in medical diagnosis have become increasingly important in recent years. These compounds, which must be paramagnetic in order to decrease the relaxation times of tissue water protons, may be placed in two classes: stable organic free radicals (e.g. nitroxides) and metal complexes [1]. Among the paramagnetic metal ions which are of interest in this respect because of their magnetic properties (Mn^{2+}, Cr^{3+}, V^{4+}, Fe^{3+},Gd^{3+},...), iron (III) is the less toxic and its physiological behaviour is well known [2]. We report here the synthesis of new iron (III) dihydroxamic acid complexes. The ligands were designed as structural analogues of rhodotorulic acid, a natural siderophore which forms a highly stable Fe(III) complex of stoechiometry Fe_2L_3 between pH3 and pH12 [3]. Rhodotorulic acid is not amenable to structural variations which can promote a variation in biodistribution of the Fe(III) complex. Our choice of a substituted pyridine moiety was dictated by the possibility of binding two hydroxamic acid groups in positions 2 and 6, while at the same time introducing substituents of varying polarity in position 4 which might induce specific biodistributions for the corresponding iron(III) complexes. The synthesis of the ligands was realised in three subsequent steps (schemes I,II and III).

Scheme I: Synthesis of the protected hydroxamic "arm":



The synthesis of the trifluoroacetate of amino-1-(acetyl-amino-benzyloxy)-3 propane was performed and optimized by taking advantage of previous work by Miller et al.[4]. 1-Amino-3-propanol 1 was converted to its Boc derivative 2 [5] and then oxidized to the aldehyde 3 using the Swern reagent [6]. Compound 3 was not purified, due to its instability, and was reacted with O-benzylhydroxylamine in an H_2O-methanol mixture at pH 5. After column chromatography on silica gel, a mixture of the syn and anti isomers of the benzyloxime 4 was obtained and then reduced by $NaBH_3CN$ in acetic acid in the presence of excess acetic anhydride to yield compound 5, which was also purified by column chromatography [7]. Treatment of 5 with trifluoroacetic acid led to the deprotected amine 6 as its trifluoroacetate (overall yield from 1: 38 %).

Scheme II: Preparation of the trisubstituted pyridinic moieties 10a, b and c:

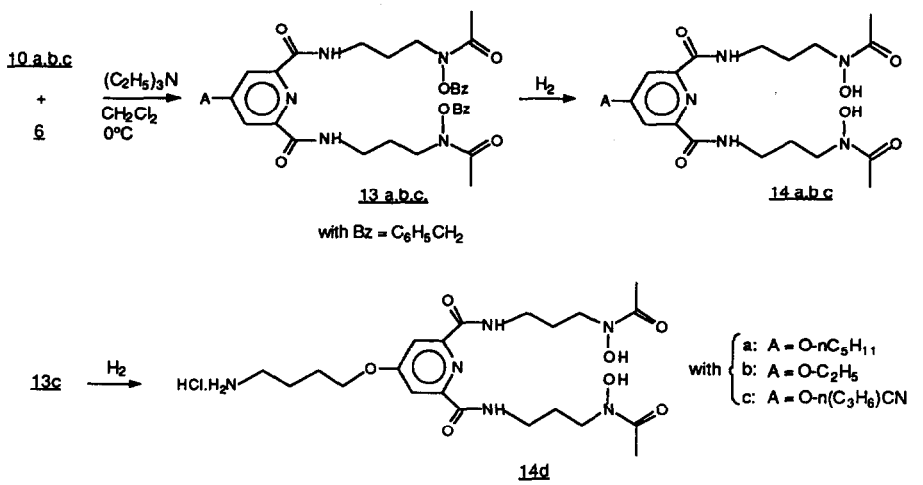


Two different sequences have been used. In the first, chelidamic acid 7 was reacted with $C_6H_5PCl_2$ at 120°C for two hours [8] to give after purification by sublimation compound 8 (yield

57%). Treatment of 8 by sodium n-pentoxide or ethoxide in the corresponding alcohol in the presence of catalytic amounts of the crown ether (15-c-5) followed by saponification led to the para-substituted diacids 9a and b (yields 72-75%). The latter were reacted with $SOCl_2$ at reflux in the presence of catalytic amounts of DMF to yield the acid chlorides 10a and b (yields 80-85%) [9].

The second sequence starts with the diethyl ester of chelidamic ester 11 which was converted to its sodium phenoxyde. Reaction of the latter with 4-bromobutyronitrile (1,1 equivalent) in the presence of catalytic amount of the crown ether (15-c-5) gave compound 12 (yield 60%) which was converted to the corresponding acid chloride 10c [10].

Scheme III: Synthesis of ligands 14a-d:



Condensation of the hydroxamic "arm" 6 with the acid dichlorides 10a,b and c in CH_2Cl_2 at 0°C in the presence of $(C_2H_5)_3N$ (4 equivalents) [3] led to the amides 13a, b and c (yields 30-50%). Catalytic hydrogenolysis (CH_3OH, Pd/C 10%, H_2 1atm., 4 to 8 hours) of 13a, b and c led to the free hydroxamic acid (yields: 70-85%). Hydrogenolysis of 13c using the conditions described by Bergeron et al. [11] allows the simultaneous reduction of the nitrile function to give amine 14d isolated as its hydrochloride. All products synthesized gave elemental analysis and spectroscopic data (IR, NMR, mass) in agreement with their proposed structure [12].

Iron(III) complexes of ligands 14a-d have been formed in situ and characterized at various pH using ESR, UV-visible spectrophotometry and cyclic voltammetry. Between pH3 and pH7, the predominant species has the stoechiometry Fe_2L_3 (λmax = 420 nm, ϵ_M = 2460-2720 $M^{-1}.cm^{-1}$) with two high spin Fe(III) centers. The Fe(III)/Fe(II) reduction potential ($E_{1/2}$ = - 0.65V vs SCE) was nearly identical to that of the Fe(III) complex of the rhodotorulic acid complex [2]. This suggests a similarity of structure and stability of the natural and synthetic ligands. The relaxivity of the Fe(III) complex of ligand 14d was measured and compared to the relaxivity of the contrast agent Gd(DTPA) in the same

conditions. The results indicate that the former complex, though five times less efficient than Gd(DTPA), can be considered as a potential NMR imaging agent.

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12.- **14a** Mp : 133-135°C. ^1H NMR (CD_3OD): 0.84 (t, J = 6.9, 3H); 1.33 (m, 4H); 1.82 (m, 6H); 2.01 (s, 6H), 3.33 (m, J = 6.6, 4H); 3.63 (t, J = 6.6, 4H); 4.08 (t, J = 6.9, 2H); 7.62 (s, 2H).
Mass Spectrometry (C.I./ NH_3): m/z = 482 ($M + H^+$).

14b Mp : 138-140°C.^1H NMR (CD_3OD): 1.35 (t, J = 6.9, 3H); 1.82 (m, 4H); 2.01 (s, 6H); 3.35 (t, J = 6.4, 4H); 3.63 (t, J = 6.3, 4H); 4.15 (quartet, J = 7, 2H) ; 7.65(s, 2H).
Mass Spectrometry (C.I./ NH_3): m/z = 396 ($M + H^+ - 44$)(100).

14c Mp: 137-139°C.^1H NMR (CD_3OD): 1.83 (quintet, J = 6.6, 4H); 2.02 (s, 6H); 2.10 (quintet, J = 7, 2H) ; 2.58 (t, J = 7, 2H); 3.36 (t, J = 6.6, 4H); 3.63 (t, J = 6.6, 4H); 4.20 (t, J = 5.8, 2H); 7.67 (s, 2H).
Mass Spectrometry (C.I./ NH_3): m/z = 479 ($M + H^+$).

14d Mp 110 - 112°C.^1H NMR (CD_3OD) :1.96 (m, 8H) ; 2.09 (s, 6H) ; 3.00 (t, J = 6.5, 2H) ; 3.43 (t, J = 6.5, 4H) ; 3.70 (t, J = 6.5, 4H) ; 4.22 (t, J = 5, 2H) ; 7.7 (s, 2H).
Mass Spectrometry (C.I./ NH_3) with M' = M - HCl: m/z = 483 ($M' + H^+$)(74), 439 (($M' - COCH_3$) + H^+)(18.5), 425 (($M' - C_3H_6NH_2$) + H^+)(100).

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