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Synthesis of 1,4-dihydro-2-methyl-4-oxo-nicotinic acid: Ochiai's route failed

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Abstract—The synthesis of 1,4-dihydro-2-methyl- and 1,4-dihydro-1,2-dimethyl-4-oxo-nicotinic acids was accomplished following a route other than Ochiai's procedure, which yielded the isomer 1,6-dihydro-2-methyl-6-oxo-nicotinic acid ethyl ester, and not the 4-oxo-derivative, as reported. Analytical data confirmed the identity of the two isomer oxo-nicotinic acids. UV–vis and potentiometric preliminary data showed that Al(III) does not form complexes with 1,6-dihydro-2-methyl-6-oxo-nicotinic acid ethyl ester in solution, as expected, but with 1,4-dihydro-2-methyl-4-oxo-nicotinic acid.

1. Introduction

Therapy for metal overload pathologies usually involves administration of suitable chelating agents to remove the metal from the body selectively. Regarding aluminum(III) and iron(III), medical research constantly emphasizes the need for new safe, efficient, and orally effective chelators.[1–3]

Hydroxypyridinecarboxyl acids are a class of compounds, which have been considered for metal chelation therapy. Their structure suggests good binding capacity toward aluminum(III) and iron(III), and interactions between 2,3 and 3,2- and 3,4 and 4,3-hydroxypyridinecarboxyl acids with aluminum(III) and iron(III) have been studied.[4,5] The thermodynamic data and chelation efficiencies of these compounds are such that they cannot be considered in aluminum(III) chelation therapy. Synthesis of more lipophilic compounds was indicated and thus, in analogy with deferiprone or 1,2-dimethyl-3-hydroxy-pyridin-4-one, an effective oral chelation therapy, monomethyl- and dimethyl-derivatives of hydroxypyridinecarboxyl acids, was designed. Among these acids, new 1,4-dihydro-2-methyl-4-oxo-nicotinic acid **7** and the corresponding *N*-methyl derivative **8** were proposed for synthesis. At first, we adopted Ochiai's procedure, the only one reported in the literature,[6] which would have allowed us to prepare the methyl ester of the desired hydroxypyridinecarboxyl acid by only one reaction between aminomethylenemalonate and ethyl acetoacetate. However, in all conditions applied, we obtained the isomer 1,6-dihydro-2-methyl-6-oxo-nicotinic acid ethyl ester **1**. In the present work, we intend to demonstrate that the compound obtained using Ochiai's method is not the ethyl ester 1,4-dihydro-2-methyl-4-oxo-nicotinic acid but its isomer **1**. In this connection, we report a successful multi-step synthesis providing 1,4-dihydro-2-methyl-4-oxo-nicotinic acid (**7**) and its *N*-methyl derivative. Exhaustive analytical data (NMR, IR, UV, MS, mp) and preliminary potentiometric and UV–vis results on the complexation properties of the two ligands toward Al(III) are reported.

2. Results and discussion

In our first attempt at synthesizing 1,4-dihydro-2-methyl-4-oxo-nicotinic acid **7**, we adopted the one-step method described by Ochiai[6] for synthesis of its ethyl ester, by reacting diethyl aminomethylenemalonate and ethyl acetoacetate in the presence of bubbled HCl dry gas at room temperature (Scheme 1), and easily obtained the corresponding acid by alkaline hydrolysis. Ochiai conditions allowed us to isolate only the isomer 1,6-dihydro-2-methyl-6-oxo-pyridine-3-carboxyl acid ethyl ester (**1**) in 44% yield. Varying the molar ratio of the two reagents or increasing/decreasing the reaction temperature never isolated the desired ethyl ester of **7**.

Very recently, researchers have obtained identical results with Ochiai's reaction for 1,4-dihydro-2-methyl-4-oxo-pyridine-3-carboxyl acid ethyl ester and proposed a four-step pathway leading to the target.[7]

Analytical data for isomer ester **1** were consistent with those reported in the literature for the same compound (IR,

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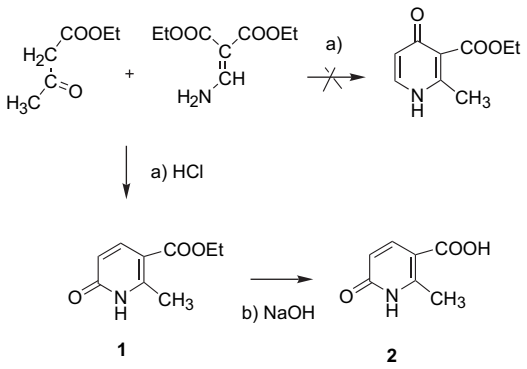


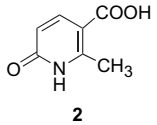
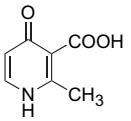
Scheme 1.

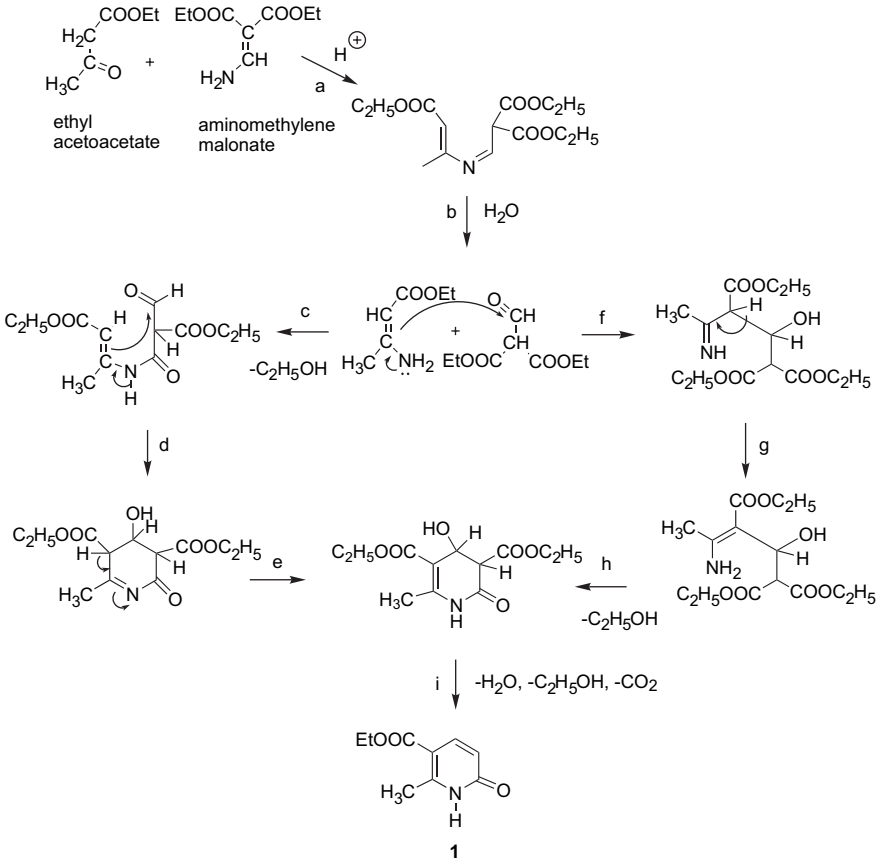
^{1}H NMR, mp) which, however, had been synthesized by means of other methods.[8–11] In particular, it has been obtained by cyclization of a dienamino ester, which resulted from the reaction between a β-amino-croton ester and a propiol ester.[8]

It is not the aim of this paper to speculate on the mechanism involved in the formation of 6-oxo-nicotinic derivative **1** by means of a reaction between aminomethylenmalonate and ethyl acetoacetate, but we suggest two similar possible mechanisms, described in Scheme 2. Briefly, after condensation between aminomethylenmalonate and ethyl acetoacetate (a), transamination may occur (a plus b), forming ethyl 3-amino-butenoate and formyl malonate, followed by amide formation (c) with one of the ester functions in the malonate derivative and ring closure (d, e). An alternative (which leads to the same thing) is first alkylation of ethyl 3-amino-butenoate by the formyl group in malonate (f) and then ring closure by amide formation (g, h). Lastly, water loss, ester hydrolysis, and carbon dioxide loss (i) affords compound **1**.

Subsequently, 5-carboxyl ethyl ester **1** is transformed by means of alkaline hydrolysis (NaOH 2 N) into the corresponding acid **2**, to yield a compound to compare with target acid **7** (Table 1).

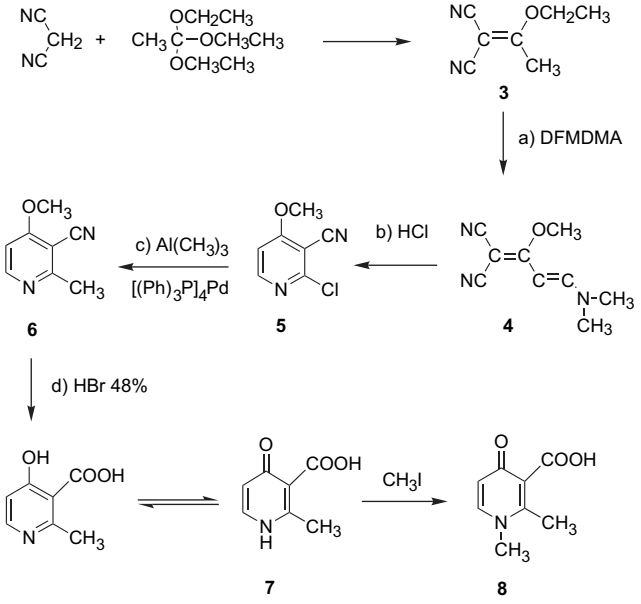
Table 1. Comparison of significant analytical data of the two isomer acids **2** and **7**

Analysis	 **2**	 **7**
UV (H_2O) (nm) pH 1	262 (14,209)	245 (6162)
IR (KBr) (cm^{-1})	1668 (carboxyl C=O), 1650 (amide C=O)	1721.27 (carboxyl C=O), 1646.41 (carbonyl C=O)
^{1}H NMR (δ)	6.16 (d, 1H, J=9.72 Hz, HC-5), 7.79 (d, 1H, J=9.72 Hz, HC-4)	6.66 (d, 1H, J=7.5 Hz, HC-5), 7.96 (d, 1H, J=7.5 Hz, HC-6)
^{13}C NMR (δ)	165.6 (carboxyl C=O), 155.9 (amide C=O)	167 (carboxyl C=O), 180 (carbonyl C=O)
Mp	153 °C dec	180 °C dec
HRMS	MH^+ 154	MH^+ 154



Scheme 2.

The synthesis of definite 1,4-dihydro-2-methyl-4-oxo-nicotinic acid **7** was accomplished partly by taking advantage of a known method already used by us to prepare 4-oxo-nicotinic acid[5,12] (Scheme 3). We obtained a useful intermediate, 2-chloro-4-methoxy-nicotinonitrile **5**, starting from condensation of malodinitrile and triethyl orthoacetate to give 1,1-dicyano-2-ethoxypropene (**3**), which was reacted with DMF–DMA in methanol, yielding 1,1-dicyano-4-(*N*,*N*-dimethylamino)-2-methoxy-1,3-butadiene (**4**). The latter was cyclized to chloro-derivative **5** (70%), the key precursor for target acid **7**, which was obtained from it in two steps: (a) introduction of a methyl group by the cross-coupling reaction of **5** with $Al(CH_3)_3$ and $(Ph)_3P_4Pd$ as catalyst in dry dioxane to give 2-methyl-4-methoxy-nicotinonitrile (**6**) in optimum yield; (b) hydrolysis by HBr 48% to the final compound **7** (40% yield). Acid **7** was also N-methylated by the conventional method,[13] using CH_3I in DMF to furnish 1,2-dimethyl-4-oxo-nicotinic acid **8**.



Scheme 3.

Data on the characterization of the two isomer acids **2** and **7** by UV, IR, ^1H, and ^{13}C NMR, plus melting points, are listed in Table 1, so that, by comparing their analytical data, we can state that the Ochiai compound is the isomer 1,6-dihydro-6-oxo-pyridine derivative **1**. As shown, the IR and ^{13}C NMR values for the carbonyl C=O of **2**, 1665 cm^{-1} and δ 167, respectively, are consistent with an amide C=O, whereas for **7** the same signals occur at 1646.5 cm^{-1} and δ 180, according to a cyclic ketone structure. Moreover, ^1H NMR coupling constants for H-4 and H-5 (9.5 Hz) of **2** are higher than those for H-5 and H-6 (7.5 Hz) of **7**, in agreement with literature data on α-pyridinone.[8]

NOESY, HMQC, and HMBC 2D NMR experiments were also performed with **1** in order to confirm further the structure of the obtained compounds. In the NOESY spectrum, a weak correlation was observed between the doublet at δ 7.79 (H-4) and the triplet at δ 1.26 (methyl signal of the ethyl residue). Further evidence was represented by the diagnostic HMBC correlations between the proton signal

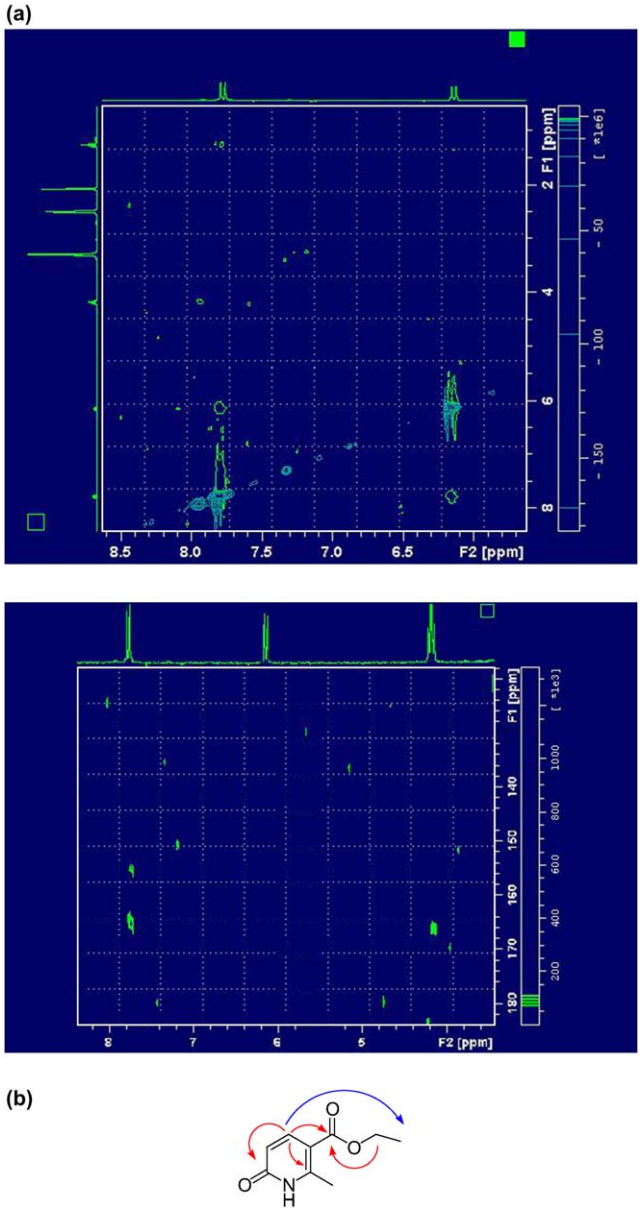


Figure 1. (a) NOESY and HMBC spectra of compound **1**; (b) Blue arrow: NOESY correlation; red arrows: diagnostic HMBC correlations described in text.

at δ 7.79 (H-4) and the carbon resonances at δ 155.9 (C-6) and 165.6 (carboxyl C=O) and between the CH_2 of the ethoxy group at δ 4.18 and the carbon resonance at δ 165.6 (C=O) (Fig. 1, a and b).

Lastly, as regards melting points, the breakdown of **2** at 153 °C is consistent with its decarboxylation, which occurs more readily than in **7** (180 °C), in which the carbonyl and carboxyl groups are next to each other.

Further evidence of the identity of the two isomers came from comparing their capacity to form complexes with a hard metal ion like aluminum(III). Hard metal ions can form reasonably stable complexes with hard functional groups, such as phenol and carboxyl oxygens, provided that a chelated complex forms, i.e., that two or more groups can bind simultaneously with the metal ion[14] to form a

five- or six-membered ring. If this is not the case then very weak monodentate complexes form (the only exceptions being phosphonate oxygens at acidic pH values, which form reasonably stable monodentate complexes). This property can be used to discriminate between isomers **2** and **7**, as only **7** has the carboxyl and phenol groups in *ortho*, and is thus able to chelate the metal ion.

The formation of complexes is easily detected by UV–vis analysis. Figure 2 shows UV–vis spectra obtained at the same pH value (4.1) for one solution containing **2** and Al(III) and another containing only **2**. A pH value of 4.1 was chosen to ensure reasonable complex formation (complexes may not form at more acidic values) and to avoid the precipitation of aluminum hydroxide (expected at pH>4.5–5 if complex stability constants are not sufficiently high). Nevertheless, the two spectra are practically identical, indicating that the ligand is not complexed.

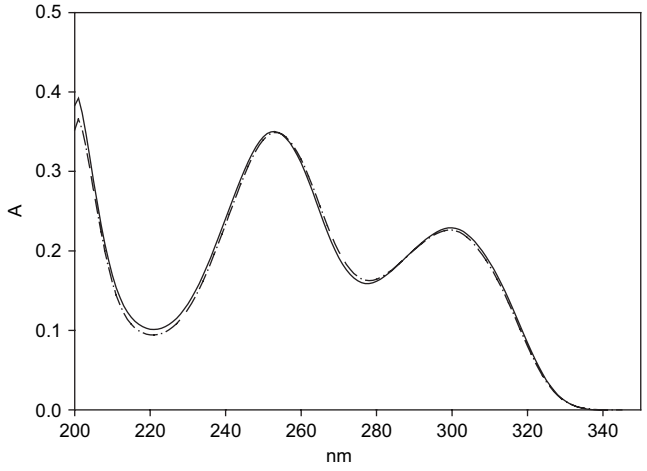


Figure 2. UV–vis spectra for one solution containing ligand alone, pH=4.08 (solid line) ($[L]_0=3.39\times10^{-4}$ m), and another containing ligand with metal ion, pH=4.13 (dash-dotted line) ($[L]_0=3.39\times10^{-4}$ m, $[Al^{3+}]_0=9.86\times10^{-5}$ m).

As regards potentiometric titrations, formation of the complexes is competitive with acid–base equilibria, so that the acidity constants of the ligand were determined first (the acid–base properties of Al^{3+} were known from previous data[15]). The results were $pK_1=0.308\pm0.008$ (OH), $pK_2=3.758\pm0.008$ (COOH), $pK_3=11.63\pm0.02$ (NH) (note that pK_a values alone cannot discriminate between **2** and **7**, as very similar values are expected from both isomers).

Figure 3 shows an experimental potentiometric titration for a solution containing **2** and Al^{3+}. The overlapping theoretical curve was calculated solely on the basis of the acid–base properties of the ligand and the metal ion, thus assuming no metal–ligand species. The theoretical curve almost coincides with the experimental data, demonstrating that, in this system, there is no (or very little) complexation at any pH (values higher than 4.5–5 could not be reached, due to precipitation of $Al(OH)_3$).

Different results were obtained for isomer **7**. The complete results and discussion will be provided elsewhere.[16] Figure 4 shows an experimental potentiometric titration for

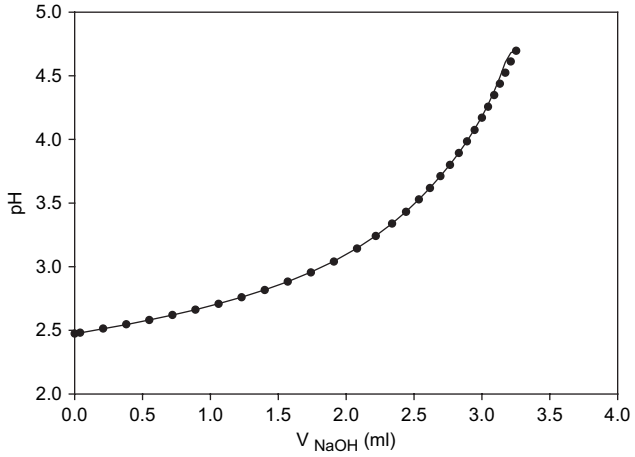


Figure 3. Theoretical (line) and experimental (circles) potentiometric titration curves for a solution containing Al^{3+} (3.81×10^{-4} m) and **2** (1.14×10^{-3} m).

a solution containing **7** and Al^{3+}. The overlapping theoretical curve was calculated presuming no complex formation. The differences between the two curves are large, especially at higher pH values. Assuming the formation of strong complexes of the type AlL, AlL_2, and AlL_3 leads to a far better match between the two curves.[16] Moreover, in solutions containing **7** and Al^{3+}, precipitation of $Al(OH)_3$ was only observed at [L]/[Al] ratios of less than 3.

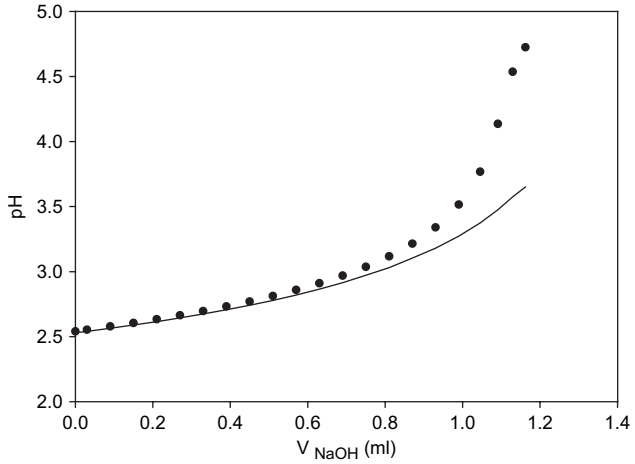


Figure 4. Theoretical (line) and experimental (circles) potentiometric titration curve for a solution containing Al^{3+} (3.11×10^{-3} m) and **7** (9.70×10^{-3} m).

These preliminary complexometric results undoubtedly support our structural assignments for **2** and **7**. The significant complexation ability of **7** toward Al^{3+} confirms that, in this compound, the two coordinating groups, COOH and OH, are in *ortho*, whereas this is not the case for **2**.

3. Experimental

3.1. General

Melting points were determined on a Gallenkamp MFB 595 010M/B capillary melting point apparatus, and are not corrected. Infrared spectra were recorded on a Perkin–Elmer

1760 FTIR spectrometer using potassium bromide pressed disks; all values are expressed in cm^{-1}. UV–vis spectra were recorded on a Perkin–Elmer Lambda UV/VIS spectrometer. ^1H NMR spectra were recorded on Varian Gemini (200 MHz) and Bruker (300 MHz) spectrometers, using the indicated solvents; chemical shifts are reported in δ (parts per million) downfield from tetramethylsilane as internal reference. Coupling constants are given in hertz. In the case of multiplets, chemical shift was measured starting from the approximate center. Integrals were satisfactorily in line with those expected on the basis of compound structure. Elemental analyses were performed in the Microanalytical Laboratory, Department of Pharmaceutical Sciences, University of Padova, using a Perkin–Elmer elemental analyzer model 240B; results fell in the range of calculated values ±0.4%. Mass spectra were obtained on a Mat 112 Varian Mat Bremen (70 eV) mass spectrometer and Applied Biosystems Mariner System 5220 LC/Ms (nozzle potential 250.00). Column flash chromatography was performed on Merck silica gels (250–400 mesh ASTM). Chemical reactions were monitored by analytical thin-layer chromatography (TLC) using Merck silica gel 60 F-254 glass plates with a 9:1 dichloromethane/methanol mixture as eluant, unless otherwise specified.

Solutions were concentrated in a rotary evaporator under reduced pressure. Starting materials were purchased from Aldrich Chimica and Acros, and solvents from Carlo Erba, Fluka and Lab-Scan. DMSO was made anhydrous by refluxing with CaO for 8 h and then by distillation under vacuum and storage on molecular sieves. Dioxane was dried by leaving it on KOH pellets overnight and then storing it on metallic Na.

3.1.1. Synthesis of 1,6-dihydro-2-methyl-6-oxo-nicotinic acid ethyl ester (1): Ochiai's procedure. A mixture of aminomethylenemalonic acid diethyl ester (1 g, 5.34 mmol) and ethyl acetoacetate (0.68 ml, 5.34 mmol) was stirred at room temperature to give a homogeneous suspension and then, after cooling at 0 °C, HCl gas was bubbled until saturation and complete solubilization. The yellow solution was then allowed to reach room temperature and left for 4–5 days. By this time, a yellow crystalline product had formed, which was collected and recrystallized from methanol/acetone 2:8, yielding 44% of pure compound. Mp 213 °C (lit.[6,7,10] 207 °C, lit.[10] 222 °C); R_f 0.34 (chloroform/methanol 95:5); IR (KBr) 3350 (NH), 1705 (ester CO), 1645 (amide CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.26 (t, 3H, CH_3), 2.52 (s, 3H, CH_3), 4.18 (q, 2H, J=7.0 Hz, CH_2O), 6.19 (d, 1H, $J_{4,5}$=9.66 Hz, H-5), 7.79 (d, 1H, $J_{5,4}$=9.59 Hz, H-4), 12.02 (s, 1H, H-1); ^{13}C NMR (DMSO-d_6) δ 165.6 (carboxyl C═O), 155.9 (C-6), 142.8 (C-4), 140.66 (C-2), 117.96 (C-5), 115.82 (C-3), 60.87 (CH_2), 15.96 (CH_3), 14.28 (CH_3); HRMS [MH^+] 182.12; Anal. Calcd for $C_9H_{11}NO_3$: C, 59.66; H, 6.12; N, 7.73; found: C, 59.45; H, 6.13; N, 7.64.

3.1.2. Synthesis of 1,6-dihydro-2-methyl-6-oxo-nicotinic acid (2). A solution of 1.2 g (6.62 mmol) of ethyl ester **1** in NaOH 0.5 M (3–4 ml) was heated at 70–80 °C for 3 h. After cooling, the solution was slowly acidified with HCl 2 N until precipitation was complete, and placed at 0 °C overnight. The white precipitate was collected, washed with water, and recrystallized from water. Yield 74%; mp 153 °C (dec); R_f 0.2 (methanol); UV–vis 262 (14,209) nm; IR (KBr) 3320 (NH), 1668 (carboxyl C═O), 1650 (amide C═O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.52 (s, 3H, CH_3), 6.1 (d, 1H, $J_{5,4}$=9.66 Hz, H-5), 7.81 (d, 1H, $J_{4,5}$=9.66 Hz, H-4), 11.96 (s, 1H, HN-1); ^1H NMR (MeOD) δ 2.63 (s, 3H, CH_3), 6.33 (d, 1H, $J_{4,5}$=9.70 Hz, HC-4), 8.04 (d, 1H, $J_{5,4}$=9.72 Hz, HC-5); ^{13}C NMR (DMSO-d_6) δ 170.37 (carboxyl C═O), 163.45 (C-6), 143.5 (C-2), 142.8 (C-4), 117.96 (C-5), 115.82 (C-3), 15.96 (CH_3); HRMS [MH^+] 154.129; Anal. Calcd for $C_7H_7NO_3$: C, 54.90; H, 4.61; N, 9.15; found: C, 54.78; H, 4.33; N, 9.00.

3.1.3. Synthesis of 2-chloro-4-methoxy-nicotinonitrile (5). Following the reported route,[12] 10 g (151.37 mmol) of malodinitrile were reacted with 27.1 g (167.05 mmol) of triethyl orthoacetate at 90–95 °C for 45 min. The mixture was then evaporated at 60 °C, giving a pink product of 1,1-dicyano-2-ethoxypropene (**3**). Yield 99%; mp 91–92 °C [lit.[17] 88.5–89.5 °C].

A solution obtained by heating a mixture of **3** (7.4 g, 54.35 mmol) in 17 ml of MeOH at 50 °C, was added with DMF–DMA (10.92 g, 82.05 mmol) and the resulting mixture was refluxed for 1.5 h. After cooling at room temperature, a dark red precipitate formed, which was collected, washed with cold MeOH, and dried, yielding 52% of **4**, mp 133 °C (lit.[12] 130 °C).

1,1-Dicyano-4-(*N*,*N*-dimethylamino)-2-methoxy-1,3-butadiene (**4**) (5 g, 28.21 mmol) in 100 ml of MeOH was submitted to cyclization in acid conditions by HCl gas, with vigorous stirring, at 15 °C. At the end of the reaction, water/ice (800 g) was added and a white precipitate separated from the solution. This was collected, washed with cold water, and dried. The filtrate was concentrated under vacuum and basified with NaOH 2 N, and a further product formed. Overall yield was 70% of almost pure **5**; mp 175–176 °C (methanol) (lit.[12] 168 °C).

3.1.4. Synthesis of 2-methyl-4-methoxy-nicotinonitrile (6). 2-Chloro-4-methoxy-nicotinonitrile (2 g, 11.86 mmol) and 85 ml of anhydrous dioxane (KOH, Na) were placed in a 250-ml two-necked round-bottomed flask. After dissolving by slight heating, 6 ml (56.84 mmol) of $(CH_3)_3Al$, 2 M *n*-hexane solution and then 0.224 g (0.1916 mmol) of $[(Ph_3)P]_4Pd$ as catalyst were added. The mixture was refluxed for 4 h under an inert atmosphere of N_2, checking the ongoing reaction by TLC analysis (chloroform/methanol 95:5). At the end, the cooled reaction mixture was acidified with HCl 2 N and the solvent evaporated off. The residue was treated with water, basified with NaOH 20%, and the mixture extracted with diethyl ether. The combined extracts, washed with water and dried over anhydrous Na_2SO_4, were evaporated until dry. Yield 99%; mp 117 °C; R_f ($CHCl_3$/MeOH 95:5); ^1H NMR (DMSO-d_6) δ 2.53 (s, 3H, CH_3), 4.05 (s, 3H, OCH_3), 7.39 (d, 1H, $J_{5,6}$=6.1 Hz, H-5), 8.56 (d, 1H, $J_{6,5}$=6.1 Hz, H-6); HRMS [MH^+] 147.15; Anal. Calcd for $C_8H_8N_2O$: C, 64.85; H, 5.44; N, 18.91; found: C, 64.69; H, 5.38; N, 18.86.

3.1.5. Synthesis of 1,4-dihydro-2-methyl-4-oxo-nicotinic acid (7). In a 50-ml flask, 1 g (6.84 mmol) of 2-methyl-4-methoxy-nicotinonitrile and 17 ml of HBr 48% water solution were heated at 70–80 °C for 1 h and then refluxed,

checking the course of reaction by TLC analysis (chloroform/methanol 9:1). Although refluxing lasted some days, the reaction had not finished, so refluxing was stopped, the mixture cooled, and worked up as follows. It was basified with NaOH 20% (pH 8–9), extracted with ethyl acetate in order to remove the starting material, and the aqueous phase acidified with HCl 2 N (pH 3). In this way, a fine white precipitate formed, which was left at 0–4 °C overnight. The next day, it was collected, washed with water, and dried. Yield 45%; mp 180 °C (dec); R_f 0.2 (methanol); UV–vis 245 nm (6162); IR (KBr) 3395 (NH), 1721 (carboxyl C═O), 1646.41 (carbonyl C═O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.76 (s, 3H, CH_3), 6.66 (d, 1H, $J_{5,6}$=7.06 Hz, H-5), 7.96 (d, 1H, $J_{6,5}$=7.25 Hz, H-6), 12.82 (s, 1H, HN-1), 16.15 (s, 1H, OH); ^{13}C NMR (DMSO-d_6) δ 180 (C-4), 167.1 (carboxyl C═O), 156.59 (C-2), 139.45 (C-6), 116.36 (C-5), 113.50 (C-3), 20.16 (CH_3); HRMS [MH^+] 154.12, [M−1] 152.0; Anal. Calcd for $C_7H_7NO_3$: C, 54.90; H, 4.61; N, 9.15; found: C, 54.95; H, 4.53; N, 9.02.

3.1.6. Synthesis of 1,4-dihydro-1,2-dimethyl-4-oxo-nicotinic acid (8). 2-Methyl-4-oxo-1,4-dihydro-pyridine-3-carboxyl acid (**7**) (0.900 g, 5.88 mmol) in 45 ml of DMF was added with 1.8 ml (29.1 mmol) of CH_3I and the mixture heated at 100 °C for 2 h. After cooling, the solvent was evaporated off and the residue was recrystallized with methanol. Yield 60%; mp 271 °C; R_f 0.2 (methanol); IR (KBr) 1718.6 (CO), 1655.6 (carbonyl); ^1H NMR (DMSO-d_6) δ 2.76 (s, 3H, CH_3), 3.45 (s, 3H, N–CH_3), 6.66 (d, 1H, $J_{5,6}$=7.06 Hz, H-5), 7.96 (d, 1H, $J_{6,5}$=7.25 Hz, H-6), 16.15 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ 180 (C-4), 170.1 (carboxyl C═O), 163.4 (C-2), 142.2 (C-6), 116.2 (C-5), 114.6 (C-3), 33.2 (CH_3), 19.3 (CH_3); HRMS [MH^+] 168.05; Anal. Calcd for $C_6H_9NO_3$: C, 57.48; H, 5.43; N, 8.38; found: C, 57.32; H, 5.41; N, 8.25.

4. Complexometric measurements

The experimental apparatus, reagents, and measurement methods were almost the same as those previously reported.[4] A summary follows, with details given only when they differ from those already described.

All analyte concentrations are expressed in the molality scale (mol/kg of water).

Potentiometric measurements were performed on a Radiometer ABU93 Triburette apparatus; UV–vis spectra were recorded on a Perkin–Elmer Lambda 25 instrument.

Working solutions of HCl (0.2 m), NaOH (0.2 m), and $AlCl_3$ (0.1 m, containing HCl 0.3 m) were prepared and standardized as previously described.[4] The water solubility of **2** and **7** is low ($<2\times10^{-3}$ m): working solutions at higher concentrations (4.4×10^{-3} m and 6.5×10^{-3} m) could be prepared upon slight basification. The ionic strength of all solutions was adjusted to 0.6 m (≈0.594 M) (Na)Cl.

Potentiometric titrations were carried out at 25±0.1 °C. Duplicate potentiometric measurements were carried out using two glass electrodes (Radiometer pHG201 and BDH 309/1015/02) and a Ag/AgCl/0.6 m NaCl reference electrode.

Solutions for UV–vis analysis were prepared in the same cell used for potentiometric titrations and, after fixing the pH, the solutions were transferred into a cuvette (1 cm path length). A 0.6 m NaCl solution was used as blank.

All stability constants were calculated using the PITMAP program.[18]

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