CONVENIENT ACCESS TO 3,4,5-TRISUBSTITUTED PYRIDINES

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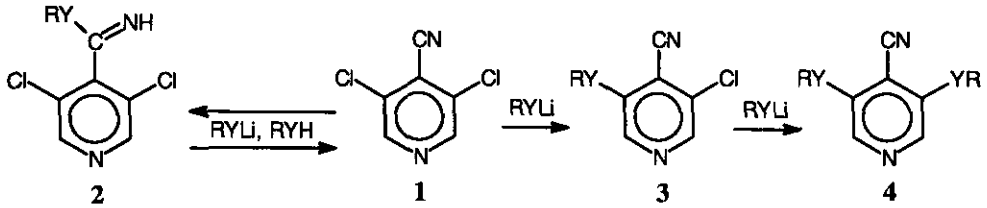
Abstract - The reaction of 3,5-dichloro-4-pyridinecarbonitrile towards nucleophilic substitution of chlorine atoms and nucleophilic addition to the cyano group has been studied in presence of different nucleophiles and solvents in order to evidence the best conditions for the synthesis of 3,4,5-trifunctionalized pyridines. Besides the products of substitution of only one chlorine atom, 3,5-bis(ethylthio)-4-pyridinecarbonitrile and 3,5-bis(*tert*-butylthio)-4-pyridinecarbonitrile have been prepared in high yields and the reduction of their cyano to amino group has been studied in order to obtain model molecules and new selective inhibitors of extramitochondrial amine oxidase enzymes.

Our previous researches allowed us to prepare the first benzylamine oxidase inhibitors,[1] highly selective with respect to other members of the same copper containing amine oxidase enzyme class, having benzylamine or 4-aminomethylpyridine structure, with IC_{50}(M) values in the range 10^{-7}-10^{-8}, very useful for many pharmacological studies. The best ones for their low toxicity are pyridine derivatives[2] and the most used in pharmacological assays is the 3,5-diethoxy-4-aminomethylpyridine[3] known as B24. An advantageous procedure for the preparation of the requested 3,4,5-trifunctionalized pyridines was attained

through the synthesis and use of a key reagent such as 3,5-dichloro-4-pyridinecarbonitrile (**1**) resulted particularly prone to nucleophilic substitution reactions.[2,4] Stimulated by the increasing interest for such inhibitors and by the wish of studying the sulphur complexing property effect of molecules able to interact with copper containing amine oxidases, we planned to prepare proper sulphur containing 3,4,5-trisubstituted pyridine derivatives by exploring the usefulness of the same key reagent. The synthesis of the new compounds and some original findings on the reactivity of **1** are the object of the present note.

The substitution of the chlorine atoms by nucleophiles such as alkoxides in 3,5-dichloropyridine is largely unsatisfactory for preparative purposes according to the modest activation towards nucleophilic attack of the positions 3 and 5 in the pyridine ring,[2] while the introduction in the position 4 of an electron-withdrawing substituent like a cyano, ester, or acetyl group[5,6] remarkably increases their reactivity, opening the way to useful preparative routes. Nevertheless, it must be considered that such substrates may undergo nucleophilic attack also at the electron-withdrawing group as observed for instance in the reaction of 3-chloro-4-pyridinecarbonitrile with sodium alkoxide which under solvent control forms imino ether in alcohol and 3-alkoxy-4-pyridinecarbonitrile in DMF.[5]

Wishing now to resort to the reaction of **1** with thiolates for a satisfactory substitution of one or both chlorine atoms, aimed to the preparation of unsymmetrical or symmetrical derivatives, it appeared opportune to explore preliminarly solvent effects and general aspects of the reactivity of **1** towards nucleophiles. Therefore, we examined through gc/ms analysis the reaction of **1** with lithium ethoxide and lithium ethanethiolate in some protic and aprotic solvents. The obtained data accounted for the following reaction pattern (Scheme 1), where compound (**1**) forms the imino ether (**2**) through an equilibrium reaction and produces the derivatives (**3**) and (**4**) by chlorine substitution.

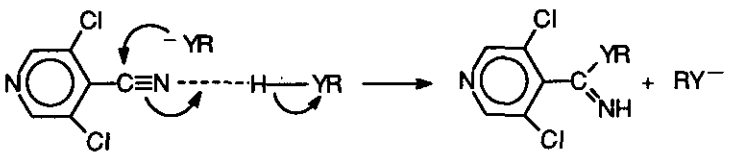


2 **1** **3** **4**

Y = O, S R= ethyl

Scheme 1

The gradual transformation of **1** into **3** and **3** into **4** was observed in both protic and aprotic solvents such as ethanol, ethanethiol, DMF, THF, Et_3N with reaction rates increasing with dielectric constant of the solvent, so for the preparation of **4** DMF was confirmed the most effective medium[2,5] among those examined. The equilibrium between **1** and **2** was observed only when the solvent was the conjugated acid of the used nucleophile like ethanol with lithium ethoxide or ethanethiol with lithium ethanethiolate, while for instance, in the temperature range examined, lithium ethanethiolate in the protic solvent ethanol easily substituted the chlorine atoms, but did not show any attack at the cyano group. With excess of nucleophile in presence of the conjugated acid as solvent, at temperatures lower than +10°C only the equilibrium reaction is practically active, so **1** disappears in favour of the formation of **2** which may be separated. At higher temperatures a gradual variation in time of the reaction mixture composition is observed that accounts for the rapid formation of **2** with contemporaneous lowering of **1**, then the appearance of **3** with decrease of **2** and **1** and at last, when **1** and **2** are vanished, the slow appearance of **4** contemporaneous to the decrease of **3**, which if desired may be separated in good yields before the formation of **4**. With the nucleophile in catalitic amount the equilibrium reaction is still very active and it is in the best practical condition for the preparation of **2**. The need of the couple protic solvent/conjugated base for the reaction at the cyano group, already observed in different processes,[7] could involve an assisted mechanism based on solvent hydrogen bonding at the same cyano group[8] and free base nucleophilic attack, implying regeneration of the free base (Scheme 2).



Scheme 2

Pure ethyl 3,5-dichloro-4-pyridinecarboximidate (**2**, R=ethyl, Y=O) treated with lithium ethoxide in ethanol at -10°C clearly showed the formation of **1** definitively proving the equilibrium reaction between **1** and **2**. A stable or transient iminoether or iminothioether compound with only one chlorine atom substituted by the nucleophile was never observed in accordance with the fact that under the reaction conditions the nucleophilic aromatic substitution cannot involve compound **2**, but only **1** and **3**, while **2** disappears in force of the equilibrium reaction.

In presence of aprotic solvents the reaction of **1** with lithium ethoxide or lithium ethanethiolate, the formation of **2** lacking, allows to observe the lowering of **1** and the appearance of **3** then of **4**, with greater delay in the formation of **4** at lower dielectric constants, so, from a preparative stand point, **3** and **4** are better obtained respectively in THF and DMF.

After such new acquirements, useful preparations of **3** and **4** from **1** were achieved with lithium ethanethiolate and lithium *tert*-butylthiolate in THF and DMF. The reaction of **1** with lithium ethanethiolate in THF afforded 3-chloro-5-ethylthio-4-pyridinecarbonitrile (**3**, R=ethyl, Y=S) in 88% yield and that in DMF 3,5-bis(ethylthio)-4-pyridinecarbonitrile (**4**, R=ethyl, Y=S) in 87% yield. The reaction of **1** with lithium *tert*-butylthiolate in THF produced 3-chloro-5-(*tert*-butylthio)-4-pyridinecarbonitrile (**3**, R=*tert*-butyl, Y=S) in 87% yield and that in DMF 3,5-bis(*tert*-butylthio)-4-pyridinecarbonitrile (**4**, R=*tert*-butyl, Y=S) in 90% yield.

The desired aminomethylpyridine products for pharmacological assays, which are affording very positive preliminary tests for selective actions on benzylamine oxidase and diamine oxidase, were prepared after several attempts by reducing the cyano group to amine in the compound (**4**) (R= ethyl, Y=S) with gaseous diborane and in the compound (**4**) (R=*tert*-butyl, Y=S) with aluminum hydride.[9] Reversed procedures failed probably because diborane in presence of bulky *tert*-butyl groups is hindered from forming the intermediate borazole ring[10] and aluminum hydride in presence of ethyl groups promotes the substitution of an ethylthio function rather than the attack at the cyano group. The prepared 3,5-bis(ethylthio)-4-aminomethylpyridine and 3,5-bis(*tert*-butylthio)-4-aminomethylpyridine with stoichiometric gaseous HCl were transformed into the corresponding crystalline hydrochlorides.

EXPERIMENTAL

Melting points were determined on a Reichert-Thermovar hot stage apparatus and are uncorrected. ^{1}H-Nmr spectra were obtained on Bruker WM-300 or AcP-300 spectrometer. Chemical shifts are reported on the δ scale and are referred to TMS. Mass spectra were recorded on a Hewlett-Packard GC-MSD 5972 instrument. Ir spectra were recorded on a 1330 Perkin Elmer spectrophotometer and Perkin Elmer FT-IR spectrophotometer Paragon 1000 PC as KBr pellets or films.

Lithium ethoxide, lithium ethanethiolate and lithium *tert*-butylthiolate were prepared in homogeneous phase from a commercial hexane solution of butyllithium by addition at -60°C of the proper alcohol or

thiol in slight excess and evaporation at room temperature to obtain noticeably pure solid salts.

Reaction of 3,5-dichloro-4-pyridinecarbonitrile (1) with lithium ethoxide or lithium ethanethiolate. The reaction in various protic and aprotic solvents was allowed to proceed in thermostatic bath at different temperatures in the range from -30 to +50°C, with nucleophile concentrations in the range 0.05-0.8 N. At different reaction times samples were drawn from the solution and submitted to gc/ms analysis after a rapid process of quenching with water, extraction with ether and drying with Na_2SO_4 to avoid hydrolysis of the cyano group. Experiments carried out delaying the extraction of the quenched samples allowed us to individuate by gc/ms 3,5-dichloro-4-pyridinecarboxamide[11] which was also isolated by column chromatography on Merck aluminum oxide, activity I, neutral (70-230 mesh) with chloroform as eluent. The chromatographed 3,5-dichloro-4-pyridinecarboxamide showed mp 208-210°C; ms (m/z) 191 (8%, M^+), 190 (96%), 174 (100%), 146 (18%); ir (KBr, ν cm^{-1}) 3355, 3167, 1678, 1628, 827, 601; ^{1}H-nmr ($CDCl_3$, δ ppm) 4.89 (s, 2H), 8.59 (s, 2H). For the reaction carried out in ethanethiol the drawn samples were rapidly evaporated at reduced pressure up to the removal of all the volatiles before the addition of water.

Ethyl 3,5-dichloro-4-pyridinecarboximidate (**2**, R=ethyl, Y=O). In a thermostatic bath at 20°C a solution of **1** (240 mg, 1.39 mmol) in anhydrous ethanol (10 ml) was treated with a 1.6 N hexane solution of butyllithium (0.02 ml, 0.032 mmol) (molar ratio 43.4 : 1) and allowed to react for 4 h. According to gc/ms analysis, already after 45 min a constant reaction equilibrium was attained corresponding at 27% of **1** and 73% of **2** (R=ethyl, Y=O). The reaction mixture was in turn submitted to addition of water, extraction with ether, anhydrification with Na_2SO_4, evaporation of the solvent and column chromatography on Merck 60 silica gel (70-230 mesh) with eluent hexane-chloroform 50:50. The chromatography afforded unreacted **1** (59 mg, 24%) and crystalline **2** (R=ethyl, Y=O) (184 mg, 60%) having mp 63-65°C; ms (m/z) 218 (<1%, M^+), 190 (46%), 174 (100%), 139 (52%), 137 (33%); ir (KBr, ν cm^{-1}) 3269, 1656, 1067, 823, 594; ^{1}H-nmr ($CDCl_3$, δ ppm) 1.37 (t, J=7.0 Hz, 3H), 4.26 (q, J=7.0 Hz, 2H), 7.49 (s, 1H), 8.47 (s, 2H). *Anal.* Calcd for $C_8H_8N_2OCl_2$: C,43.86; H, 3.68; N, 12.79. Found: C, 44.26; H, 3.82; N, 12.69.

3-Chloro-5-ethylthio-4-pyridinecarbonitrile (**3**, R=ethyl, Y=S) and **3-chloro-5-(*tert*-butylthio)4-pyridinecarbonitrile** (**3**, R=*tert*-butyl, Y=S). Compound (**1**) (500 mg, 2.89 mmol) was allowed to react at

20°C for 30 min under nitrogen with 57 ml of 0.2 N solution in THF of lithium ethanethiolate or lithium *tert*-butylthiolate, then the reaction mixture was submitted to the following treatments: addition of water, rapid extraction with ether, anhydrification with Na_2SO_4, evaporation to dryness and purification by column chromatography through Merck 60 silica gel (70-230 mesh) with hexane-chloroform (50:50) as eluent. The obtained **3** (R=ethyl, Y=S) weighed 505 mg (88%). mp 73-75°C; ms (m/z) 198 (50%, M^+), 172 (34%), 170 (100%); ir (KBr, ν cm^{-1}) 2225, 1410, 1232, 821, 580; ^{1}H-nmr ($CDCl_3$, δ ppm) 1.42 (t, J=7.5 Hz, 3H), 3.16 (q, J=7.5 Hz, 2H), 8.53 (s, 1H), 8.54 (s, 1H). *Anal.* Calcd for $C_8H_7N_2ClS$: C, 48.37; H, 3.55; N, 14.10. Found: C, 48.03; H, 3.65; N, 13.98. With Merck 60 silica gel (230-400 mesh) and hexane-chloroform (40:60) as eluent the obtained **3** (R=*tert*-butyl, Y=S) weighed 571 mg (87%). mp 46-48°C; ms (m/z) 226 (4%, M^+), 170 (6%), 57 (100%); ir (KBr, ν cm^{-1}) 2239, 1434, 1221, 821, 586; ^{1}H-nmr ($CDCl_3$, δ ppm) 1.40 (s, 9H), 8.76 (s, 1H), 8.78 (s, 1H). *Anal.* Calcd for $C_{10}H_{11}N_2ClS$: C, 52.98; H, 4.89; N, 12.36. Found: C, 53.07; H, 4.95; N, 12.41.

3,5-Bis(ethylthio)-4-pyridinecarbonitrile (**4**, R=ethyl, Y=S) and **3,5-bis(*tert*-butylthio)-4-pyridinecarbonitrile** (**4**, R=*tert*-butyl, Y=S). Compound (**1**) (1.030 g, 5.95 mmol) was allowed to react at 0°C for 10 min under nitrogen with 30 ml of 0.8 N solution in DMF of lithium ethanethiolate or lithium *tert*-butylthiolate, then the reaction mixture was submitted to the following treatments: evaporation at reduced pressure, addition of water, rapid extraction with chloroform, anhydrification with Na_2SO_4, evaporation up to the removal of the solvent and separation by column chromatography with Merck 60 silica gel (70-230 mesh). The compound (**4**) (R=ethyl, Y=S) obtained with hexane-chloroform (50:50) as eluent weighed 1.160 g (87%). mp 92-93°C; ms (m/z) 224 (100%, M^+), 195 (20%), 163 (32%); ir (KBr, ν cm^{-1}) 2220, 1414, 1228, 818, 578; ^{1}H-nmr ($CDCl_3$, δ ppm) 1.40 (t, J=7.4 Hz, 6H), 3.15 (q, J=7.4 Hz, 4H), 8.44 (s, 2H). *Anal.* Calcd for $C_{10}H_{12}N_2S_2$: C, 53.54; H, 5.39; N, 12.49. Found: C, 53.76; H, 5.52; N, 12.60. The compound (**4**) (R=*tert*-butyl, Y=S) obtained with hexane-chloroform (20:80) as eluent weighed 1.500 g (90%). mp 90-91°C; ms (m/z) 280 (5%, M^+), 224 (8%), 168 (27%), 57 (100%); ir (KBr, ν cm^{-1}) 2233, 1415, 1222, 822, 589; ^{1}H-nmr ($CDCl_3$, δ ppm) 1.39 (s, 18H), 8.54 (s, 2H). *Anal.* Calcd for $C_{14}H_{20}N_2S_2$: C, 59.96; H, 7.19; N, 9.99. Found: C, 59.72; H, 7.15; N, 10.09.

3,5-Bis(ethylthio)-4-aminomethylpyridine dihydrochloride. A solution of **4** (R=ethyl, Y=S) (412 mg, 1.84 mmol) in 8 ml of THF cooled to -70°C in autoclave was added with gaseous diborane (5.58 mmol)

and allowed to react at room temperature for 2 days. The reaction mixture was submitted to the following treatments: addition of water (6 ml), extraction with chloroform, anhydrification with Na_2SO_4, evaporation of the solvent and column chromatography on Merck aluminum oxide, activity I, neutral (70-230 mesh) with hexane-chloroform (40:60) as eluent. The oily 3,5-*bis*(ethylthio)-4-*aminomethylpyridine* weighed 246.6 mg (59%). Ms (m/z) 228 (38%, M^+), 199 (100%). A sample of the obtained amine (200 mg, 0.88 mmol) in 10 ml of anhydrous ether was treated under stirring with 1.2 ml of anhydrous 0.65 N ethereal solution of gaseous HCl. The solid dihydrochloride was filtered, washed with ether and crystallized from 2-propanol (208 mg, 79%); mp 130-140°C (decomp.); ir (KBr, ν cm^{-1}) 3425, 1419, 1224, 818, 567; ^{1}H-nmr (DMSO-d_6, δ ppm) 1.24 (t, J=7.3 Hz, 6H), 3.09 (q, J=7.3 Hz, 4H), 4.24 (br s, 2H); 8.54 (s, 3H); 8.58 (s, 2H). *Anal.* Calcd for $C_{10}H_{18}N_2Cl_2S_2$: C, 39.86; H, 6.02; N, 9.30. Found: C, 39.49; H, 6.18; N, 9.52.

3,5-Bis(*tert*-butylthio)-4-aminomethylpyridine dihydrochloride. A solution of **4** (R=*tert*-butyl, Y=S) (410 mg, 1.46 mmol) in 5 ml of THF was treated under nitrogen with 4 ml of a 1.26 N solution of aluminum hydride in THF and allowed to react at room temperature for 40 h. The reaction mixture was submitted to the following treatments: addition of water (5 ml), extraction with chloroform, anhydrification with Na_2SO_4, evaporation of the solvent and purification by preparative layer chromatography on Alltech alumina N (15 μm) with hexane-chloroform (60:40). The oily 3,5-bis(*tert*-butylthio)-4-aminomethylpyridine weighed 227.6 mg (55%); ms (m/z) 284(2%, M^+), 171 (100%). A sample of the obtained amine (200 mg, 0.70 mmol) in 10 ml of anhydrous ether was treated under stirring with 1 ml of anhydrous 0.65 N ethereal solution of gaseous HCl. The solid dihydrochloride was filtered, washed with ether and crystallized from ethanol (112.6 mg, 45%). mp 170-175°C (decomp.); ir (KBr, ν cm^{-1}) 3419, 1416, 1215, 822, 574; ^{1}H-nmr (DMSO-d_6, δ ppm) 1.26 (s, 18H); 4.59 (m, 2H); 8.48 (s, 2H); 8.81 (br s, 3H). *Anal.* Calcd for $C_{14}H_{26}N_2Cl_2S_2$: C, 47.05; H, 7.33; N, 7.84. Found: C, 46.68; H, 7.15; N, 8.04.

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