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Synthesis of Imino-Amide as Models of associated organic ligands to glucose tolerant factor activity

Síntesis de ligantes Imino-Amida como modelos de ligantes orgánicos asociados a actividad factor tolerante a la glucosa

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Abstract

The molecular structure of the Tolerant Factor Glucose (FTG) has been associated with a metal center of chromium bound in a coordinated manner to ligands Electrodonators such as amino acids and nicotinic acid. In this sense, in this article, the synthesis is carried out of ligands with potential applications such as FTG at Coordinated to the center of chromium (III). A compound Imino-enol (3) from benzylamine (2) and acetoacetate Ethyl (1) in anhydrous methanol. Likewise, the synthesis of an Amine quaternary amine (5), derived from dicyandiamide (4), Where the metal center served as a catalyst for A condensation reaction and not as Lewis acid forming coordination compounds.

Keywords: GTF; chromium; imine-enol; dicyandiamide.

Introducción

The study of coordination compounds of the chromium (III) metal ion became important after it was discovered that these species are part of a metabolic agent called FTG glucose-tolerant factor, reported by Mertz and Schwarz in 1955 (Vincent, 2001).

In this sense, the relevance of the discovery lies in the fact that the biochemistry of Chromium (III) exerts an important role in the control of the metabolism of carbohydrates and lipids in the organism (Vincent, 2000, Sharma *et al*., 2011). This process is mediated by one or more coordination compounds of the metal ion with amino acids such as aspartate, glutamate, glycine, and cysteine for the case of uromodulin (Chen *et al*., 2011, Gómez García & Magaña Garns, 2004). The structure of X-ray diffraction has not been reported for FTG, however it is proposed that the coordination sphere is formed by chromium (III)

coordinated to nicotinic acid and amino acids as cysteine or glutathione peptide residues (Chen *et al*., 2011 ; E-Learning Chemistry: Homepage, 2013).

The study of the biochemistry of vanadium (III, IV, and V) and zinc (II) has shown its participation in the metabolism of carbohydrates and lipids, as well as chromium (Goméz García, & Magaña Garns, 2004; Alvino De la Sota & Pacheco Calderón, 2007). Vanadium began to be used in 1899 as an oral drug in the form of sodium metavanadate for diabetic patients, reporting activity (Alvino De la Sota & Pacheco Calderón, 2007). Currently, vanadium research advances the design of vanadyl (III) ion coordination compounds with organic molecules that improve bioabsorption with respect to the use of inorganic salts. The efficacy of these compounds has been evaluated in vitro in adipose cells isolated from rats, associating the biological activity with a binding to the β receptor that activates the adenylate cyclase that transforms ATP into adenosine 3′, 5′-cyclic monophosphate, which In turn, it activates some protein kinases and lipases (Alvino De la Sota & Pacheco Calderón 2007).

The investigation of new molecules that function as drugs in the treatment of diabetes mellitus (I and II) and its associated complications have also been oriented on organic compounds. Aminoguanidine has been studied as a compound capable of preventing vascular dysfunctions caused by diabetes (Corbett *et al*., 1992). Metformin is currently used as a medication in the treatment of side effects of diabetes such as obesity, hypertension, among others. It is also used as a drug in the treatment of diabetes mellitus type 2 because metformin does not directly affect insulin levels but regulates carbohydrate levels in the bloodstream (Palumbo, 1998, Chan *et al*., 2007). Arylsulfone derivative compounds have been used in the treatment of obesity generated by DM (Greenwood, 1971, Imperial College of London, 1956, Fang *et al*., 2006).

Next, the formation reaction of the imino ester with structural similarity to the binders associated with FTG activity is presented, a detailed product in the context of the article, Figure 1.

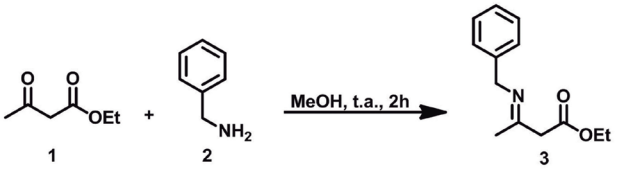


Figure 1. Scheme of iminoester synthesis.

On the other hand, the homogeneous catalytic processes associated with transition metals, constitute a tool for industrial and technological development. Processes such as carbonylation, addition, oxidation, and polymerization of olefins; condensation and hydrolysis are part of the catalysis where a metallic center intervenes. The reaction conditions of the aminoguanidine nitrate binder and the chromium (III) metal salt allowed to crystallize a hydrolyzed derivative as quaternary ammonium salt, whose charge is stabilized with the chloride counterion, which comes from the metal salt as a starting reagent in the synthesis of coordination compounds. The quaternary ammonium cation has the structure NR +, where R can be an alkyl group or an aryl group. These ions are permanently charged, independent of the pH of the solution.

Experimental

Instrumental conditions

The substances were weighed on an analytical balance OHAUS Pioneer PA214. IR spectra of 4000 to 400 cm-1 were taken on the Agilent Technologies Cary 630 FTIR IR spectroscopy. The electronic scans from 800 to 200 nm were collected in a UV-vis Thermo Scientific Evolution 201 spectrophotometer. The 1H and 13C NMR spectra were obtained from Agilent Technologies NMR-vnmrs 400 nuclear magnetic resonance spectroscopy, taken at 400 MHz and using CDCl3 as a solvent, the measurement was made at the National Autonomous University de México UNAM, postgraduate studies unit, Faculty of Chemistry. A Thermo Scientific IA9300X1 digital flushometer with a temperature range of 10 to 400 °C, 12 V, and 45 W was used. For the characterization of the monocrystalline compound, Bruker P4 X-ray diffraction equipment was used, at room temperature, with the Mokα radiation (λ = 0.71073 A°), with standard measurement conditions and applying an absorption correction. The structures were solved by direct methods. Finally, the structural models, including anisotropic thermal agitation parameters, were refined by least squares, with the hydrogen atoms placed in calculated positions. In the last refinement cycles, a weighting scheme was applied to the diffraction data and the effect of the secondary extinction was corrected by a semi-empirical formula. The calculations and refinement of the structure were carried out in the WINGX program and the equipment was used in collaboration with the Postgraduate Studies Unit of the Faculty of Chemistry (UNAM).

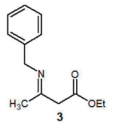
Materials

99 % Benzylamine (MERCK), 99 % ethyl acetoacetate (MERCK), CrCl3 • 6H2O 99 % (MERCK), 99 % NaCl (MERCK), hexane HPLC (MERCK), ethyl acetate HPLC (MERCK), 99 % methanol (MERCK), metallic magnesium (JT Baker) chips and iodine crystals (JT Baker), aminoguanidine nitrate (MERCK).

The anhydrous methanol was obtained by distilling a mixture of methanol, magnesium chips and iodine crystals. The procedure adapted from Vogel's practical organic chemistry book (Vogel, Furniss, Hannaford, Smith & Tatchell, 1989).

Synthesis

Synthesis of Iminoester 3



A a solution of ethyl acetoacetate (127 µL, 1 mmol) in 3.6 mL of anhydrous methanol at room temperature was added benzylamine (109 µL, 1 mmol) in a 50 mL distillation balloon, the reaction was left in constant stirring during 2 hours in reflux assembly. Subsequently, the reaction product was left under constant stirring for 6 hours to evaporate the methanol. Then the mixture was left to stand and after 24 hours crystals of the compound were obtained (3) ocher yellow. The monitoring of the reaction is performed by TLC for 2 hours (6:4 hexane/acetate de etilo). IR (estado sólido, cm-1): 3357 (ν O-H) 3062 (ν C-H), 1660 (ν C=N). RMN1H (300 MHz, Chloroform-d) δ 8.96 (s, 1H), 7.37 – 7.24 (m, 5H), 4.55 (s, 1H), 4.43 (d, J = 6.4 Hz, 2H), 4.11 (c, J = 7.1 Hz, 2H), 1.92 (s, 3H), 1.26 (t, J = 7.1 Hz, 3H). RMN 13C (75 MHz, Chloroform-d) δ 170.57, 161.78, 138.74, 128.76, 127.46 – 127.04 (m), 126.69, 83.18, 58.37, 46.77, 19.36, 14.63. EM (IE, M+/z): 219.

Synthesis of the quaternary amine

In the particular case of the dicyandiamide binder, the reaction conditions differ from the conventional quaternization. The reaction conditions that were carried out are shown in Figure 2.

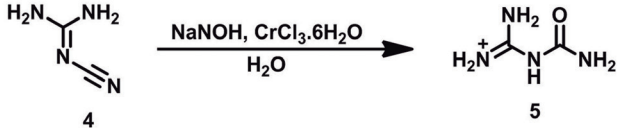


Figure 2. A synthesis scheme of the quaternary amine

Results and Discussion

• *Imino ester 3*

Carbonyl compounds, and especially aldehydes and ketones, have a tautomeric keto-enol balance due to the presence of alpha hydrogens to the slightly acidic carbonyl group. In most cases, this equilibrium is displaced towards the keto form because this type of compound is much more stable than its enolic counterpart, but in some very specific cases it is observed that the enolic tautomer is favored. Spectroscopic evidence shows that iminoester 3 is actually in its enol form because the hydrogen of the OH group forms a hydrogen bond with the N of the imino group, forming a six-member intermediate thermodynamically more stable to its ketonic tautomer (Figure 3).

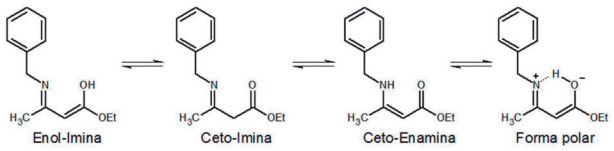


Figure 3. Tautomerism of compound 3

IR spectroscopy

The expected product (3) is a keto-imine where a tautomerism may occur due to the acid hydrogens of the methylene which is found in the α carbon with respect to the ester carbonyl, thus generating enolimine or keto-enamine, but the enol-imine tautomer prevails over keto-enamine, because oxygen is more electronegative and thus easier to extract said methylene proton (Alcântara, Barroso, & Piló-Veloso, 2002, Ferraz & Gonçalo, 2007). The IR spectrum confirms this approach where a voltage vibration is observed at 3357 cm-1 for the free OH bond, while at 1660 cm-1 the voltage vibration C = N is found, thus establishing the presence of the imino group and not of the carbonyl group, because if this group emerges the characteristic vibration would appear above 1700 cm-1. Figure 4.

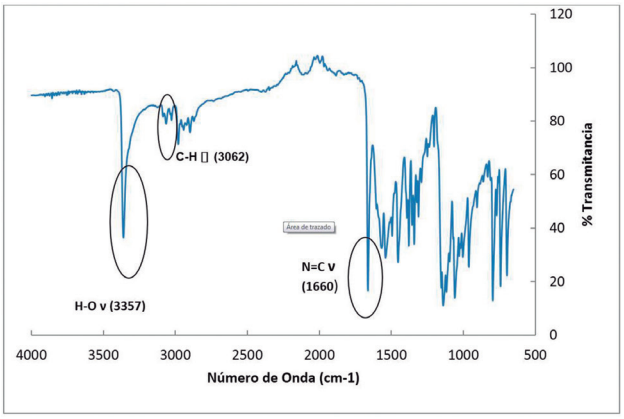


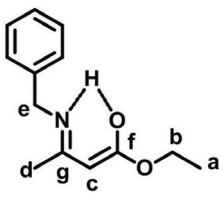
Figure 4. IR spectrum of imino-enol

1H and 13C NMR spectroscopy

In the 1 H-NMR spectrum, the triple hydrogen-a and quadruple hydrogen-b signals have a coupling constant of equal value (J = 7.1 Hz), indicating that the hydrogens are coupled, being the protons belonging to the ethyl ester group. Due to the presence of an electronegative atom, the quadruple signal moves to a smaller field, as observed in the 1H spectrum. The evidence that allows us to ensure that the compound is in its tautomeric form are the signals of the hydrogen-c that is presented as a simple signal at 4.55 ppm, the benzylic-e hydrogens that appear as a double due to the coupling that they reveal with the H that makes a bridge with the N and of course the NH that is located at 8.96 ppm. Additionally, the five aromatic hydrogens are observed in the region of 7.37-7.24 ppm (Table 1, Figure 5).

Table 1. 1H NMR spectrum signals of aminophenol.

Type of signal	Displacement (ppm)	Hydrogens
Triplete (a)	1.26	3
Cuartete (b)	4.11	2
singulete (c)	4.55	1
singulete (d)	1.92	3
Doblete (e)	4.43	2
Multiplete (Ar-H)	7.37-7.24	5
Singlete (N-H)	8.96	1



Compuesto Enol-Imina

Figure 5. Assignment of imino-enol signals (3)

In the 13 C-NMR spectra, 13 characteristic imino-enol signals are observed. In 170.6 and 161.8 ppm, the carbons of the imino group and the enolic carbon are shown respectively, in the region of 138.7-126.7 ppm the six aromatic carbons are found. Additionally, the benzylic carbon-83.1 ppm and the aliphatic carbons b, c, d and a are observed at 58.4, 46.8, 19.4 and 14.6 ppm respectively (Table 2).

Table 2. 13C NMR spectrum signals of imino-enol (3)

Type of Carbon	Displacement (ppm)
Carbon-g	170.6
Carbon-f	161.8
Carbon-Ar	138.7. 128.8. 128.9. 127.3126.7
Carbon-e	83.1
Carbon-b	58.4
Carbon-c	46.8
Carbon-d	19.4
Carbon-a	14.6

The enol-imine tautomer may have intramolecular hydrogen bonds with the N of the amino group, thus generating that the molecule closes in a cycle of 6 members that are particularly stable (Spedaletti, Vega Hissi, Andrada, Estrada & Garro Martínez, 2014), favoring the enol form in front of the keto and avoiding, that the reduction processes complete up to the amino ester, confirming. Therefore, a method must be used alternate reduction of carbonyl group as described by Giuseppe Bartoli being a reduction of Bouveault-- Blanc (Bartoli, Cimarelli, Marcantoni, Palmieri, & Petrini, 1994, Sutton, 1975), Figure 6.

Gas chromatography - mass spectrometry

In the chromatogram obtained, three peaks are observed, one corresponding to compound 3, another to the product of the transesterification of ethyl ester with methanol used as solvent, obtaining a methyl ester of compound 3. The third chromatographic peak is attributable to derived contamination of the use of organic solvents in plastic material used during the laboratory procedure in obtaining compound 3. (Table 3).

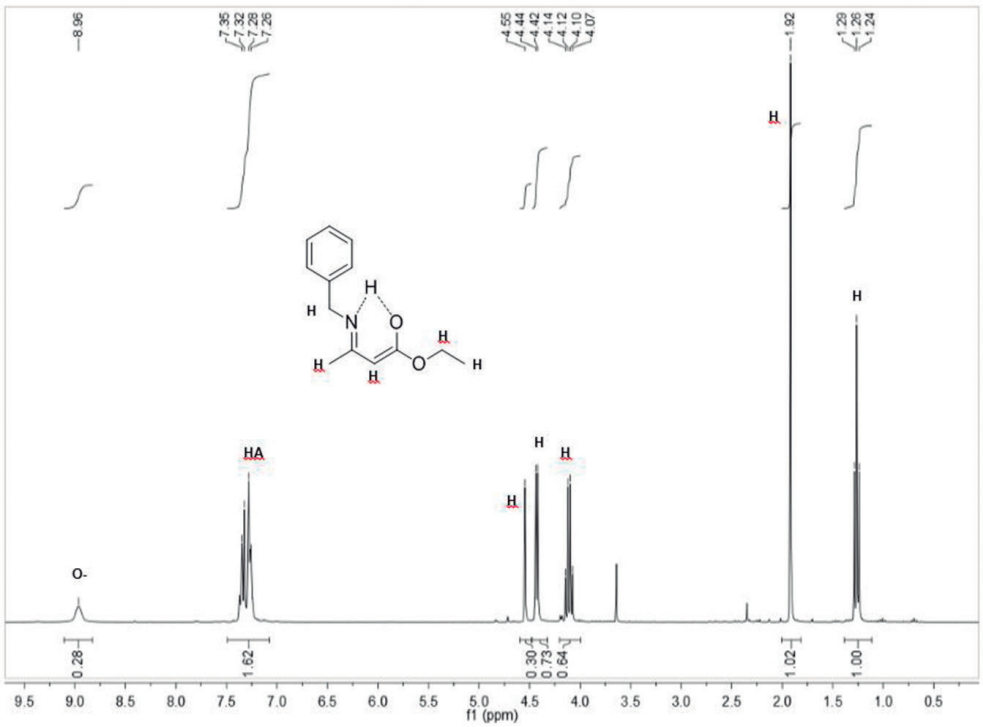


Figure 6. 13C NMR spectrum of imino-enol (3)

Quaternary amine

X-ray diffraction of monocrystal

The structural elucidation of the quaternary salt was possible since crystals suitable for monocrystal X-ray diffraction were obtained. The reflections were collected in the Postgraduate Studies Unit of the Faculty of Chemistry (UNAM) in collaboration with Dr. Marcos Flores. Next, all the structural details are described, as well as the tables with the respective distances and angles between the atoms that make up the quaternary amine (Figure 7).

The ORTEP diagram was obtained from the refinement of the structure by X-ray diffraction with the WIN GX program and the SHELL method for heavy atoms. The network parameters that describe the cell are observed in Table 4.

In the crystalline cell, there are four molecules per cell in a monoclinic system, where the chloride compensates the charge of the amine, (Figure 8).

The intermolecular interactions are numerous, this allows us to show that the binder is strongly stabilized. The interactions are classified with moderate directionality, with an angle greater than 170° from heavy atom to heavy atom and a distance around 3.2 A, for N-H ..... Cl and NH ..... .O. The three-dimensional vision allows observing a zigzag arrangement in which an amine molecule is located on the a axis and the posterior one on the b axis periodically, (Figure 9 a and b).

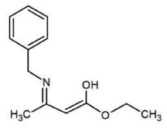
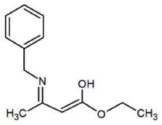
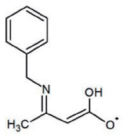
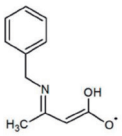
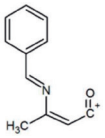
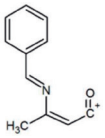
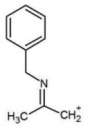
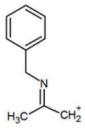
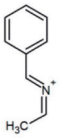
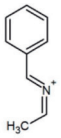
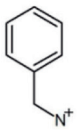
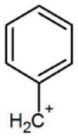
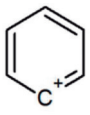
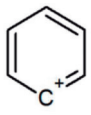
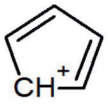
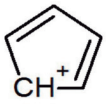
It should be mentioned that the structure obtained from X-ray diffraction, allows demonstrating the stabilization of a quaternary amine, where conventional quartering was not used as a synthesis process, but a mechanism of hydrolysis, decarboxylation is proposed and nucleophilic attack with the release of the urea group. The reaction was carried out in the absence of the metal salt and the product was not obtained. This concludes that the metal center has significant activity in the reaction, a role that will be addressed in new studies around the metal center.

The proposed mechanism by which the quaternary salt was obtained consists of the following steps:

The cyano group of compound 4 is hydrolyzed in basic medium to obtain the corresponding carboxylic acid

Subsequently, the decarboxylation of intermediate A is carried out to obtain the zwitterion B, which by a proton transfer leads to the formation of intermediate B'. Intermediary B 'effects a nucleophilic attack on carboxylic acid A to obtain the condensation product D, by loss of a molecule of water.

Table 3. Patterns of fragmentation of aminophenol (3)

Iminoester de etilo		Iminoester de metilo	
m/z	Fragment	m/z	Fragment
219		205	
190		190	
172		172	
146		146	
131		131	
105		105	
91		91	
77		77	
65		65	

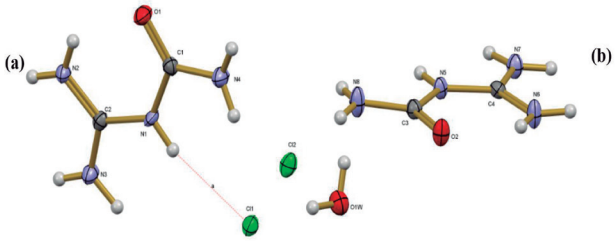


Figure 7. a) *ORTEP diagram of the quaternary amine and* ***b)*** *Two-dimensional scheme*

Table 4. Parameters of the quaternary amine

Crystal systemcristalino	Monoclínic
Space group	P-21
Network parameters	a= 6.4849 Å
	b=29.868 Å
	c=6.6037 Å
	α= 90°
	β= 96.719°
	λ = 90°
Z	4
Volume	1270.29 Å^3

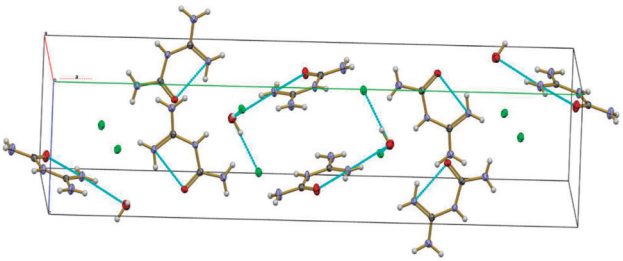


Figure 8. Crystal cell of the quaternary amine

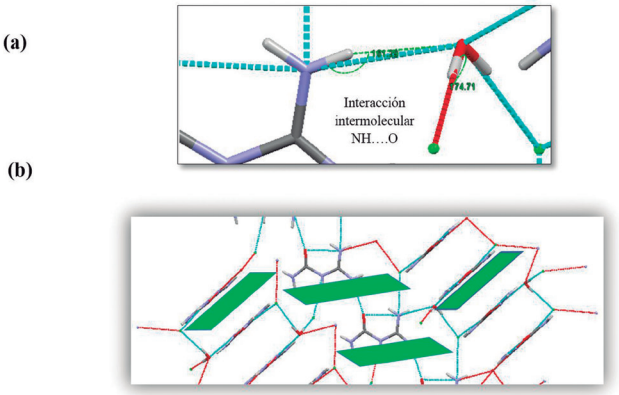


Figure 9. a) Intermolecular interactions and **b)** Crystal arrangement for quaternary amine

Finally, the base present in the medium attacks the imine carbon of intermediate D to obtain the quaternary salt 5 due to the loss of a urea molecule. (Figure 10).

The steps proposed in this reaction mechanism have transformations well known in organic chemistry that can be observed in many of the mechanisms addressed in the text "Strategic Applications of Named Reactions in Organic Synthesis: Background and Detailed Mechanisms", (Kürti , & Czakó, 2005).

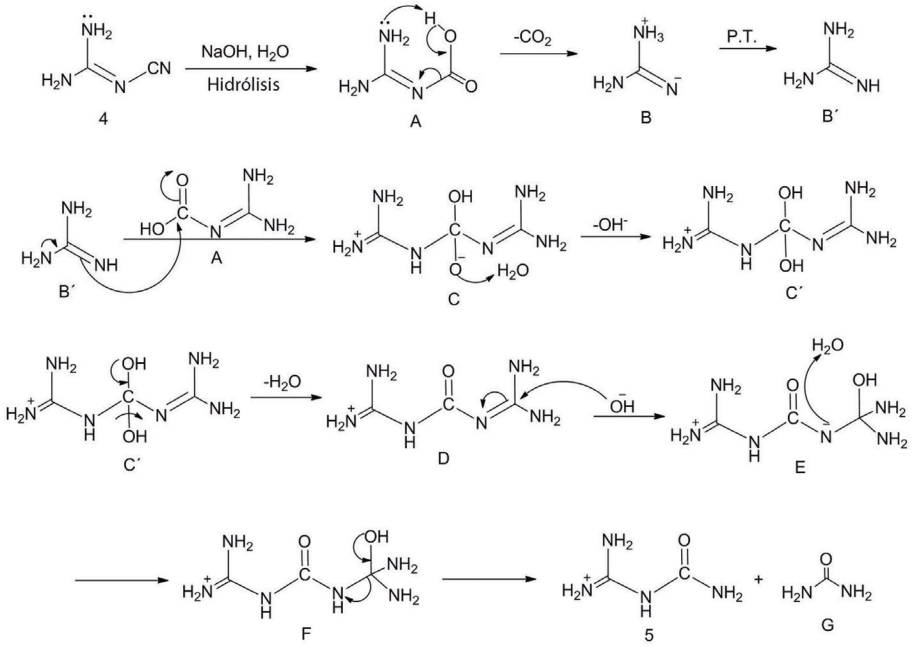


Figure 10. Proposed mechanism for obtaining quaternary amine from dicyanodiamide

Conclusions

The compound obtained was iminoester (3) as a solid, which represents tautomerism that improves imina-enol presence. Additionally a mechanism is presented for the obtaining of anima quaternions from the dicyandiamide, where the presence of the nonmetallic chrome (III) shows that there is an effect over the synthesis, which will be addressed in future issues. This paper provides mainly synthesis of new links which are potential Lewis bases in coordination to chrome (III), stabilizing biometric systems a glucose tolerant factor.

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