Understanding OxymaPure As A Peptide Coupling Additive. A Guide To New Oxyma Derivatives

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Abstract

An *in-silico* study, using the GALAS algorithm available in ACD/Percepta, was performed to calculate the pK_a(s) of the various oximes with potential application as peptide coupling additives. Among the known oximes and predicted structures, OxymaPure is superior based on the pK_a values calculated, confirming the results described in the literature and validating this algorithm for further uses in that field. Among the non-described oximes, based on pK_a calculation, ethyl 2-(hydroxyimino)-2-nitroacetate seems to be a potential candidate to be used as an additive during peptide coupling.

Introduction

The amide/peptide bond is almost exclusive in peptide structures, but its presence is also the most common in organic compounds with pharmaceutical interest as reflected in independent reports of the Centres of Excellence for Drug Discovery (CEDD) at GlaxoSmithKline (GSK) and of the University of Manchester.[1, 2] Although it looks simple, the reaction of a carboxylic acid and amine to render the amide/peptide bond is not so straightforward and requires activation of one of the two components. While activation of the amino function has been increasingly studied in recent years, historically the majority of amide/peptide bonds considered within the pharmaceutical industry are obtained *via* the activation of the carboxylic acid group.[3]

The leitmotif of this long journey of the carboxylic group activation is “reactivity/stability”. Thus, the activation should be strong enough to allow amide/peptide formation, but with sufficient stability to allow the reaction before decomposition and to avoid or minimize undesired side reactions.[3] The pioneer works of Fisher and Curtius exemplified this dichotomy. While Fisher proposed the acyl chloride as the activating method,[4] Curtius developed a less strong activation, the acyl azide,[5] which was the method of choice for peptide/amide formation until the early 1960s. Unfortunately, neither were exempt from side reactions.[6]

A real breakthrough was the development of the carbodiimide reagents by Sheehan;[7] still the most popular coupling method. Initially, the carboxylic acid reacts with carbodiimides and forms a reactive O-acylisourea intermediate. Then, this intermediate reacts with the nucleophilic amine and forms corresponding amides/peptides (Scheme 1). In parallel, Bodanszky introduced the concept of active esters,[8] taking as a model the *p*-nitrophenyl esters, which react smoothly with amines giving the amide/peptide bond. With time, the use of carbodiimides have facilitated the preparation of active esters, which could be purified, stored for a long period of time, and even commercialized.

In 1970, König and Geiger proposed the use of 1-hydroxybenzotriazole (HOBt) as an additive during carbodiimide activation.[9] The HOBt reacts instantly with the O-acylisourea intermediate rendering *in situ* the corresponding OBt active species. The OBt active species, which can be found on different isoforms, are described to be very reactive and difficult to isolate (see below). The presence of HOBt during the mediated carbodiimide couplings translates to better yields and less racemization of the carboxylic

moiety. Although it is commonly thought that this better performance of the carbodiimide in the presence of HOBt is due to the higher reactivity of the OBt active species compared to the O-acylisourea, in fact the opposite is true. The intermediate O-acylisourea is more reactive than the OBt active species. O-acylisourea avoids the formation of a rearrangement side-reaction that renders the inactive *N*-acylurea and the formation of the oxazolone, which is less reactive than the OBt active species and, in addition, provokes racemization (Scheme 1).

For many years, the active species involved in all coupling reactions were OBt or OBt derivatives, mainly 6-chloro-1-hydroxybenzotriazole (6-Cl-HOBt) and 7-aza-1-hydroxybenzotriazole (HOAt), and the related 1-oxo-2-hydroxydihydrobenzotriazine (HODhbt, HOOBt). These additives are being used either as additives in carbodiimide mediated couplings or as stand-alone reagents such as *N*-[(1H-benzotriazol-1-yl)(dimethylamino)-methylene]-N-methylmethanaminium hexafluorophosphate *N*-oxide (HBTU), *N*-[6-chloro(1H-benzotriazol-1-yl)-(dimethylamino)methylene]-N-methylmethanaminium hexafluorophosphate *N*-oxide (6-Cl-HBTU, HCTU), and N-[(dimethylamino)-1H-1,2,3-triazolo[4,5-b]-pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) as aminium salts;[10, 11],[12, 13],[14, 15] and benzotriazol-1-yloxytri(pyrrolidino) phosphonium hexafluorophosphate (PyBOP), (6-chloro-benzotriazol-1-yloxy)tris(pyrrolidino) phosphonium hexafluorophosphate (PyClock), and (7-azabenzotriazol-1-yl)oxy]tris(pyrrolidino) phosphonium hexafluorophosphate (PyAOP) as phosphonium salts (Figure 1).[16], [17]

However, after September 11, 2001, the potentially explosive character of HOBt and its triazole/triazine-related additives was reported.[18] These compounds were recategorized under a Class 1 explosive category, making their transportation difficult.[18]

In this context, our groups started a broad research project with the goal of developing another family of safe and efficient additives, based on a different template. Our premise for developing it were, first, retaining the N-OH as the leaving group, because phenols were reported in the literature to have the worst performance, and, second, avoiding the presence of several N atoms in a row to minimize the risk of explosion.

Our first results using *N*-OH heterocycles were not very positive, because although the additives developed were useful, their performance was far inferior to that of 1-hydroxybenzotriazoles. Then, we investigated the oxime series proposed by Itoh, in particular, the ethyl 2-hydroximino-2-cyanoacetate (OxymaPure (**1**)), [19] which looked promising and whose performance was also evaluated by Izdebski.[20] Since then, OxymaPure and its stand-alone derivatives, 1-[(1-(cyano-2-ethoxy-2-oxoethylideneaminooxy)-dimethylamino-morpholinomethylene)] methanaminium hexafluorophosphate (COMU)[21] and O-[(cyano(ethoxycarbonyl)imidazolidi)-amino]-yloxytri(pyrrolidino) phosphonium hexafluorophosphate (PyOxim),[22] are the reagents of choice for making any peptide bond. These derivatives have been shown to be superior to HOBt derivatives and in some cases very close to the HOAt derivatives in terms of yield and minimization of racemization.

Results And Discussions

In our continuous efforts to develop different additives fulfilling our lemma “Choosing the Right Peptide Coupling Reagent for Each Reaction”, we have prepared and assayed different oxime analogs. Although OxymaPure has been shown to be unbeatable, some of the new oxime-based derivatives have been found to possess interesting properties. Thus, Oxyma-B (**9**)[23] has shown to be even better than OxymaPure in minimizing racemization and Amox (**4**)[24] to be very convenient for the protection of amines with the 9-fluorenylmethyloxycarbonyl (Fmoc) group avoiding the formation of dimers associated to the high reactivity of the active species, mainly the chloride derivative.

It is well known that the quality of an active ester is intrinsically associated with the strength of the conjugate acid. In that regard and to rationalize our previous results, and more importantly for the development of new ones, we have performed an *in silico* study using ACD/Percepta software[25] and the pK_a GALAS algorithm available in it to calculate the acid ionization constant values of the various oximes and other additives (Table 1).[26] Like the pK_a Classic method, which is a variation of a classical Hammett-Taft approach and is available as an alternative within said software, the GALAS algorithm is based on analogous fundamental considerations.[26-28] However, instead of largely relying on equations and parameters quantified by other authors, it is developed entirely in-house by ACD/Labs, parameterized “from scratch” using an internal training set of >18,000 compounds with available experimental pK_a measurement data. The custom nature of the pK_a GALAS model allows for greater flexibility in using various ad-hoc adjustments and modifications, going beyond the scope of the concepts considered in the classic Hammett-Taft approach where needed. One of them is the concept of so-called “fundamental micro constant”—a micro-pK_a value for an ionizable group in a hypothetical state of an uncharged molecule, which is then used to calculate a corresponding micro constant for that group in any protonation state by introducing the corrections for charges. In total, the algorithm utilizes a database of 4600 ionization centers, a set of ca. 500 various interaction constants and four interaction calculation methods for different types of interactions, producing a full range of micro constants from which pK_a macro constants are obtained. The latter are experimentally measurable values associated with a particular ionization stage of any given ionizable group. Very often, when ionizable groups in a particular protonation state possess pK_a micro constants of comparable magnitude, several of them undergo (de)protonation simultaneously in an isolated ionization stage and make a collective influence toward the corresponding macro-pK_a value. pK_a GALAS provides full and detailed insights into this relationship between the macroscopic pK_a values of the molecule and the microscopic pK_a constants of individual groups and extent of their dissociation in each ionization stage. This was the main reason for selecting pK_a GALAS versus pK_a Classic for this investigation.

First, the pK_a of some non-oxime additives were calculated (Table 1). However, using this method 1-hydroxybenzotriazole-based additives did not show any pK_a values. The 1-hydroxybenzotriazoles can form zwitterionic species (HB^+A^-) via two tautomeric equilibria (Figure 4). Those zwitterionic species

possesses zero net charge and show low or negative pK_a values.[29] pK_a values found in the literature for HOBt and HOAt are 4.60 and 3.28, respectively.[30]

Then, pK_a of some oxime coupling reagent additives reported by our group and others were calculated, then of some oximes described in the literature or commercially available, and finally, some unknown oximes. The pK_a values of oximes are divided into four categories and indicated with color code (if pK_a values < 4—yellow, 4 to 5—dark green, 5 to 7—light green, 7 to 9—light orange, > 9—brown).

The first conclusion that we can get from Table 1 is that overall, the results obtained agree with what was expected. Thus, OxymaPure (**1**) and their close ester derivatives (**2, 3**) are experimentally considered to be the best and this correlates with their acidity, which is also superior for the most part compared to the other derivatives. In this regard, our group has demonstrated that OxymaPure is more efficient than Amox (**4**), *N*-Oxyma (**5**), Dmox (**6**), PipOX (**7**), MorOx (**8**), Oxyma-B (**9**), and Oxyma-T (**10**),[23, 24, 35] and the calculation outlined in Table 1 confirm that all of them have a higher pK_a. Of course, the acidity of the oxime depends on the electron-withdrawing groups adjacent to oxime. Among the oximes described, the presence of cyano is key for their acidity, and the pair cyano-ester (**1-3**) is superior to cyano-amide (**4-8**), and these to the cyano-aromatic group (**11-14**). The superiority of OxymaPure (**1**) over HOPO (**22**) can also been explained by the higher acidity of the former.

The surprising results are the acidity of Oxyma-B, because it is considered to be a substitute for OxymaPure but its acidity is not very high. However, its good performance could be explained by the presence of the carbonyl groups oriented in the same direction as the *N*-OH group in Oxyma-B playing an assisted basic catalytic role, thereby enhancing the nucleophilicity of the amine function during the coupling (Figure 5). A similar effect has been described for HONM (**19**), HOAt, and *N*-ethoxycarbonyl-2-ethoxy-1,3-dihydroquinoline (EEDQ).

Amox, which has an acidity lower than OxymaPure, has demonstrated that when used in combination with 9-fluorenylmethanol as a mixed carbonate (Fmoc-Amox) it is able to introduce the Fmoc group in amino acids without the formation of dipeptides as occurs to a greater extent with Fmoc-Cl and a lesser extent with Fmoc-OxymaPure (Figure 6). In this same regard, hydroxyimino-2-phenylacetonitrile (**11**), which forms part of Boc-ON [2-(Boc-oxyimino)-2-phenylacetonitrile] and was proposed by Ito for the safe protection of amines with tert-butoxycarbonyl (Boc) group,[36-38] shows a pK_a that confirms its moderate reactivity and therefore the absence of formation of Boc-dipeptides during the introduction of the Boc group (Figure 6). Finally, the pK_a of HOSu also confirms that Fmoc-OSu is a good reagent to avoid this side-reaction.

Our group has demonstrated that the oxime derivative of Meldrum's acid (HONM) reacts with DIC rendering the corresponding adduct (Figure 7). Because this reaction is preferred, HONM is not a good additive in combination with DIC for peptide coupling, since it mostly reacts with DIC leading to peptide formation in low yield.[40]

Recently, Kolis and co-workers have observed that OxymaPure also reacts with DIC.[54] Although, in this case, the formation of the adduct is taking place to a much lesser extent than with HONM, it can cyclize with the generation of HCN (Figure 8). These results have been corroborated by Pawlas[55] and co-workers, and our own group.[56, 57]

In this context, and although this side-reaction takes place to a very low extent and in only certain cases, there is interest in finding oxime derivatives with no cyano groups. Taking into account both the availability of their synthesis and the pK_a, out of four nitro derivatives (**46-49**) only one ethyl—2-(hydroxyimino)-2-nitroacetate (**46**)—fulfils those requirements. Admittedly, the high value of uncertainty, indicating a relatively lower quality of pK_a predictions for these nitro derivatives, could be the source of some concern. However, absolute values aside, the error margin being essentially equal for these four compounds (**46-49**), and cyano and nitro groups being very similar in their electronic activity profile, allows for an interpretation of the general trends. The latter for the group of four nitro compounds (**46-49**) is fully in line with common chemical intuition, and the corresponding trends in the series of cyano analogs, which are predicted with a much higher certainty, i.e., that a dinitro compound, just as a dicyano one, will be more acidic compared to a mononitro/monocyano derivative, and the latter, in its own turn, will be a stronger acid than a mononitro/monocyano-phenyl analog. Specifically, pK_a (**47**) << pK_a (**46**) << pK_a (**48**) ~ pK_a (**49**) is analogous to pK_a (**15**) << pK_a (**1**) << pK_a (**11**). In this context, concerns regarding prediction accuracy do not interfere with the conclusion, that ethyl 2-(hydroxyimino)-2-nitroacetate (**46**) should be the most promising cyano-free alternative candidate of all nitro compounds considered here.

Conclusions

In silico study using the pK_a GALAS algorithm available in ACD/Percepta has allowed us to calculate the pK_a values of the various oximes and other peptide coupling additives. This study has allowed us to confirm the superiority over other oximes as described by our group and others in the literature, and helps to rationalize the absence of formation of protected dipeptides when the protecting group is introduced by mixed carbonates of the skeleton of the protecting group and HOSu, Amox, and hydroxyimino-2-phenylacetonitrile. Furthermore, this method has allowed us to identify compound **46** as a potential substitute for OxymaPure (Figure 9).

Declarations

Availability Data

The datasets during and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

Andrius Sazonovas works for Advanced Chemistry Development, Inc (ACD/Labs), the rest of authors declare no competing financial interest.

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Authors' contributions

S.R.M and A.S. carried out the experimental work and prepared the first draft of the manuscript. A-E.F., B.G.T and F.A. conceived and designed the study, and wrote the last version of the manuscript. A.S. contributed to data corrections and provided revisions to the paper

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Table

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Schemes 1

Schemes 1 is available in the Supplemental Files section

Figures

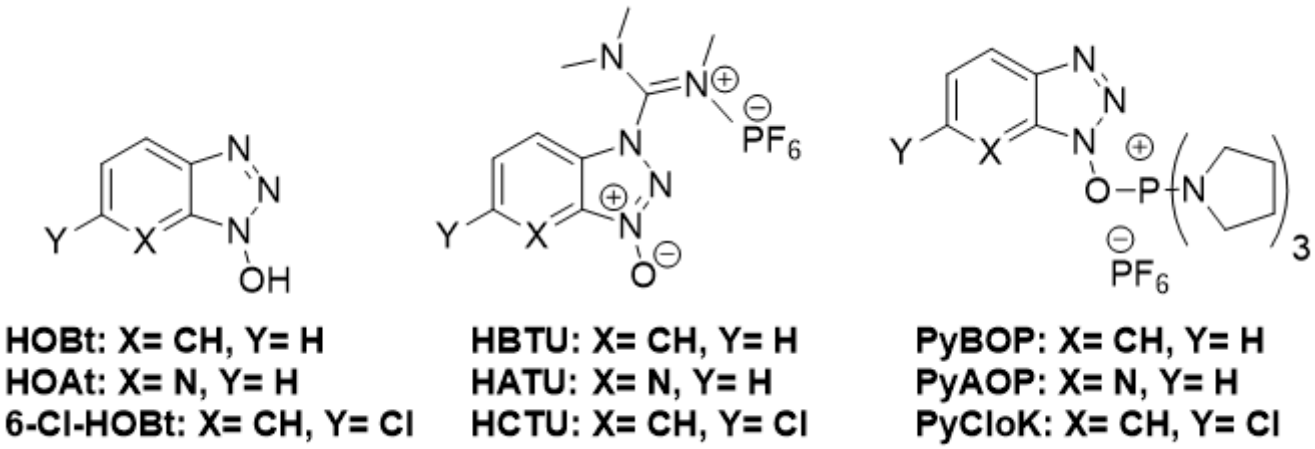

Figure 1

Some important benzotriazole additives and benzotriazole-based coupling reagents

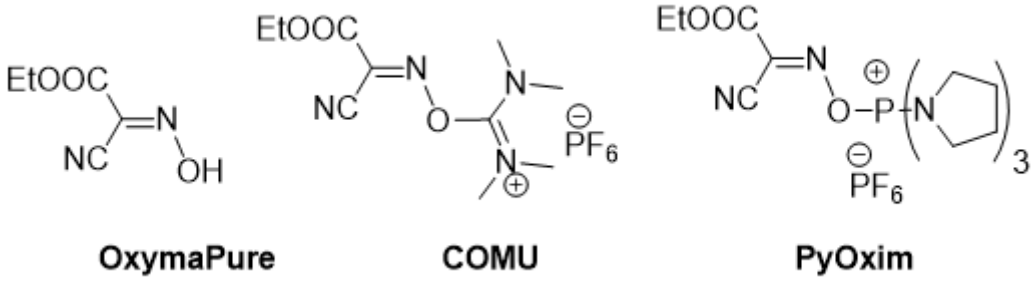

Figure 2

OxymaPure and OxymaPure based stand-alone coupling reagents

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Figure 3

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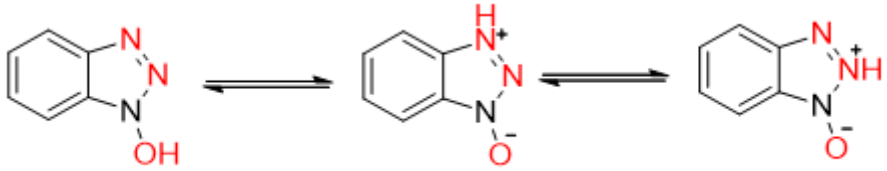

Figure 4

Tautomerism of 1-hydroxybenzotriazoles.

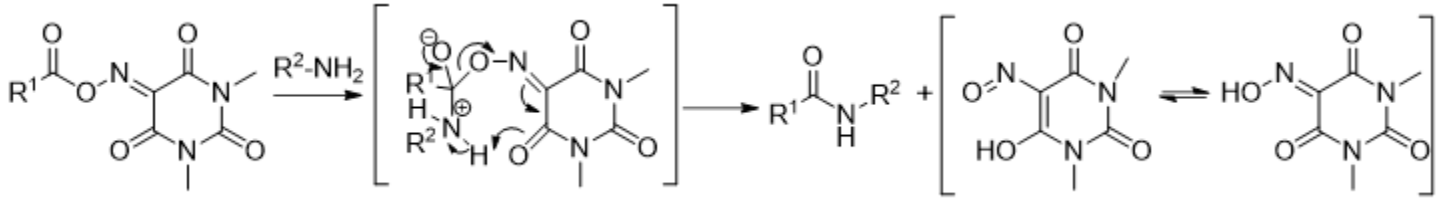

Figure 5

Assisted basic catalysis involved in the coupling through Oxyma-B active ester

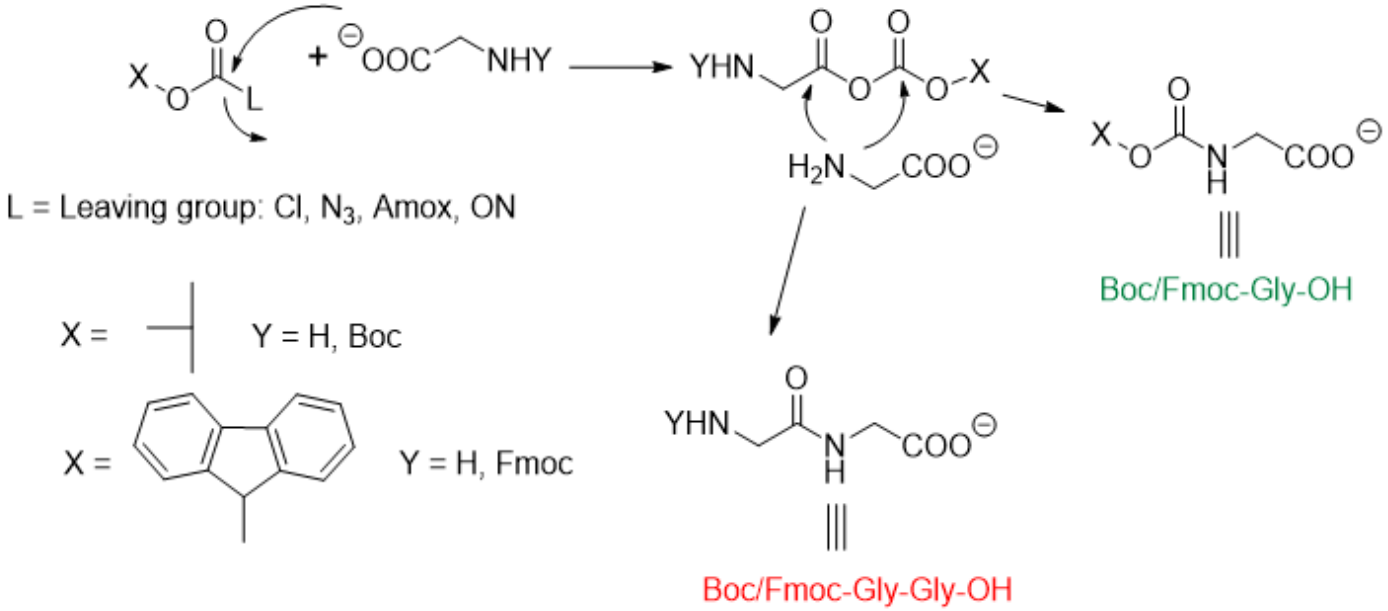

Figure 6

Undesired formation of N-protected dipeptides during the protection reaction

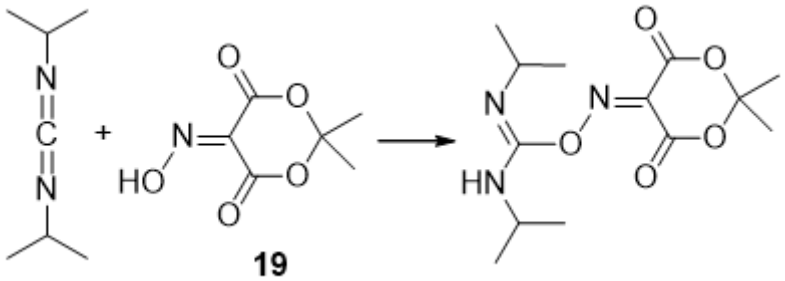

Figure 7

The reaction of HONM (19) with DIC

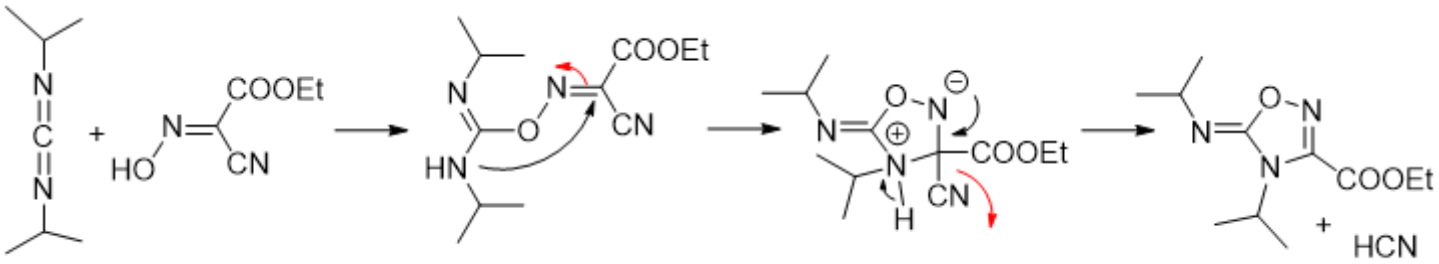

Figure 8

Formation of the adduct and posterior cyclization with the generation of HCN

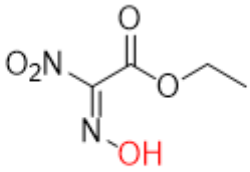

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Figure 9

Structure of the ethyl (E)-2-(hydroxyimino)-2-nitroacetate (46)

Supplementary Files

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- Scheme1.png
- Table1.docx