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/PhysChem Suite, was performed to calculate the pK_a(s) of 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 use in that field. Among the undescribed 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. 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.

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 to avoid or minimize undesired side reactions. The pioneer studies of Fisher and Curtius exemplified this dichotomy. While Fisher proposed the acyl chloride as the activating method, Curtius developed less strong activation, the acyl azide, which was the method of choice for peptide/amide formation until the early 1960s. Unfortunately, neither were exempt from side reactions.

A real breakthrough was the development of the carbodiimide reagents by Sheehan, which is still the most popular coupling method. Initially, the carboxylic acid reacts with carbodiimides and forms a reactive O-aclylisourea (1) intermediate. Then, this intermediate reacts with the nucleophilic amine and forms the corresponding amides/peptides (Scheme 1). In parallel, Bodanszky introduced the concept of active esters, taking as a model the p-nitrophenyl esters, which react smoothly with amines giving the amide/peptide bond. With time, the use of carbodiimides has 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. 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 coupling 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 O-aclylisourea (1), in fact, the opposite is true. The intermediate O-aclylisourea (1) is more reactive than the OBt active species (4). O-aclylisourea (1) avoids the formation of a rearrangement side reaction that renders the inactive N-acylurea (2) and the formation of the oxazolone (3), which is less reactive than the

Received: November 10, 2021
Accepted: January 27, 2022
Published: February 9, 2022
OBt active species (4) 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 (HODhbt, HOObt). These additives are being used either as additives in carbodiimide-mediated coupling 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; 10−15 and benzotriazol-1-yl)oxy]tris(pyrrolidino) phosphonium hexafluorophosphate (PyBOP), (6-chloro-benzotriazol-1-yloxy)tris(pyrrolidino) phosphonium hexafluorophosphate (PyClock), and (7-azabenztixazol-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 was, 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-cyano-2-ethoxy-2-oxoethylideneaminooxy)-dimethylamino-morpholino-carbenium hexafluorophosphate (COMU)21 and (1-cyano-2-ethoxy-2-oxethylideneaminooxy)-tri-1-pyrrolidinophosphonio hexafluorophosphate (PyOxim) (Figure 2),22 are the reagents of choice for making any peptide bond. These derivatives have been shown to be superior to HOAt derivatives and in some cases very close to the HOAt derivatives in terms of yield and minimization of racemization.

**RESULTS AND DISCUSSION**

In our continuous efforts to develop different additives fulfilling our lemma “Choosing the Right Peptide Coupling
Table 1. Calculated pKₐ of Some Coupling Additives Using the pKₐ GALAS Prediction Algorithm from ACD/PhysChem Suite[^25]

| Sl. No | IUPAC name | Structure | Abbreviation | GALAS         | Ref  |
|-------|------------|-----------|--------------|---------------|-----|
| 1     | Ethyl 2-cyano-2-(hydroxyimino)acetate | ![Structure](image1) | OxymaPure | ![GALAS](image2) (4.5±0.4) | 19, 31 |
| 2     | tert-Butyl 2-cyano-2-(hydroxyimino)acetate | ![Structure](image3) | - | ![GALAS](image4) (4.5±0.4) | 32 |
| 3     | (2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 2-cyano-2-(hydroxyimino)acetate | ![Structure](image5) | - | ![GALAS](image6) (4.5±0.4) | 33 |
| 4     | 2-Amino-N-hydroxy-2-oxoacetimidoyl cyanide | ![Structure](image7) | Amox | ![GALAS](image8) (6.3±0.5) | 24 |
| 5     | 2-(Ethylamino)-N-hydroxy-2-oxoacetimidoyl cyanide | ![Structure](image9) | N-Oxyma | ![GALAS](image10) (6.3±0.5) | 24 |
| 6     | 2-(Dimethylamino)-N-hydroxy-2-oxoacetimidoyl cyanide | ![Structure](image11) | DmOX | ![GALAS](image12) (6.3±0.5) | 24 |
| 7     | N-Dydroxy-2-oxo-2-(piperidin-1-yl)acetimidoyl cyanide | ![Structure](image13) | PIPOX | ![GALAS](image14) (6.3±0.5) | 24 |
| 8     | N-Hydroxy-2-morpholino-2-oxoacetimidoyl cyanide | ![Structure](image15) | MorOX | ![GALAS](image16) (6.1±0.5) | 24 |
| Sl. No | IUPAC name                                                                 | Structure | Abbreviation | GALAS | Ref  |
|-------|----------------------------------------------------------------------------|-----------|--------------|-------|------|
| 9     | 5-(Hydroxyimino)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione            | ![Structure](image1) | Oxyma-B      | ![GALAS](image2) | 33   |
| 10    | 1,3-Diethyl-5-(hydroxyimino)-2-thioxothiophenylhydrazine-4,6(1H,3H)-dione | ![Structure](image3) | Oxyma-T      | ![GALAS](image4) | 34   |
| 11    | Hydroxyimino-2-phenylacetonitrile                                          | ![Structure](image5) | -            | ![GALAS](image6) | 35,37 |
| 12    | Hydroxyimino-2-(4-chlorophenyl)acetonitrile                                | ![Structure](image7) | -            | ![GALAS](image8) | 36   |
| 13    | Hydroxypicolinimidoyl cyanide                                              | ![Structure](image9) | -            | ![GALAS](image10) | 31,35 |
| 14    | Hydroxyimino-2-(1-naphthyl)phenylacetonitrile                             | ![Structure](image11) | -            | ![GALAS](image12) | 36   |
| 15    | Hydroxycarbonimidoyl dicyanide                                             | ![Structure](image13) | -            | ![GALAS](image14) | 31   |
| Sl. No | IUPAC name                          | Structure | Abbreviation | GALAS               | Ref |
|-------|------------------------------------|-----------|--------------|---------------------|-----|
| 16    | 1-Ethyl 3-methyl-2-(hydroxyimino)malonate | ![Structure](image1) | -            | ![GALAS](image2) (7.2±0.4) | 36  |
| 17    | Diethyl (hydroxyimino)malonate      | ![Structure](image3) | -            | ![GALAS](image4) (7.2±0.4) | 36  |
| 18    | Diisopropyl (hydroxyimino)malonate  | ![Structure](image5) | -            | ![GALAS](image6) (7.2±0.4) | 38  |
| 19    | 5-(Hydroxyimino)-2,2-dimethyl-1,3-dioxane-4,6-dione | ![Structure](image7) | HONM         | ![GALAS](image8) (6.1±1.2) | 39  |
| 20    | 1-Hydroxyprrolidine-2,5-dione       | ![Structure](image9) | HOSu         | ![GALAS](image10) (7.7±0.4) | 40  |
| 21    | (4S,7S)-2-Hydroxy-3a,4,7,7a-tetrahydro-1H-4,7-methanoisoindole-1,3(2H)-dione | ![Structure](image11) | HONB         | ![GALAS](image12) (7.7±0.4) | 41  |
| 22    | 1-Hydroxyprydin-2(1H)-one          | ![Structure](image13) | HOPO         | ![GALAS](image14) (6.0±0.4) | 42  |
| 23    | 2-Phenyl-1H-benzot[d]imidazol-1-ol  | ![Structure](image15) | HOBI         | ![GALAS](image16) (7.7±0.9) | 43  |
| Sl. No | IUPAC name                                      | Structure | Abbreviation | GALAS          | Ref |
|-------|------------------------------------------------|-----------|--------------|----------------|-----|
| 24    | 6-Chloro-2-phenyl-1H-benzof[d]imidazol-1-ol    | ![Structure](image1) | 6-Cl-HOBI    | ![GALAS](image2) (7.1±0.9) | 42  |
| 25    | 3-Hydroxypyrido[3,2-d]pyrimidin-4(3H)-one      | ![Structure](image3) | HODhad       | ![GALAS](image4) (5.8±0.5) | 43  |
| 26    | 2H-Tetrazol-2-ol                               | ![Structure](image5) | -            | ![GALAS](image6) (8.2±0.8) | 44  |
| 27    | 1-Hydroxyindolin-2-one                         | ![Structure](image7) | HOI          | ![GALAS](image8) (8.3±0.4) | 42  |
| 28    | 2-(Hydroxyimino)-2-(pyrazin-2-yl)acetamide     | ![Structure](image9) | -            | ![GALAS](image10) (9.4±0.4) | -   |
| 29    | Ethyl-2-(hydroxyimino)-2-(pyrazin-2-yl)acetate | ![Structure](image11) | -            | ![GALAS](image12) (9.6±1.0) | -   |
| 30    | Ethyl-2-(furan-2-yl)-2-(hydroxyimino)acetate   | ![Structure](image13) | -            | ![GALAS](image14) (9.6±1.0) | -   |
| 31    | 2-(Furan-2-yl)-2-(hydroxyimino)acetamide       | ![Structure](image15) | -            | ![GALAS](image16) (9.4±0.4) | -   |
| Sl. No | IUPAC name                       | Structure | Abbreviation | GALAS       | Ref |
|-------|----------------------------------|-----------|--------------|-------------|-----|
| 32    | Ethyl 2-(hydroxyimino)-2-(thiazol-2-yl)acetate | ![Structure](image1) | -            | ![GALAS](image2) (8.7±0.4) | -   |
| 33    | 2-(Hydroxyimino)-2-(thiazol-2-yl)acetamide | ![Structure](image3) | -            | ![GALAS](image4) (8.7±0.4) | -   |
| 34    | Ethyl 2-(hydroxyimino)-2-(pyrimdin-2-yl)acetate | ![Structure](image5) | -            | ![GALAS](image6) (9.6±1.0) | -   |
| 35    | 2-(Hydroxyimino)-2-(pyrimdin-2-yl)acetamide | ![Structure](image7) | -            | ![GALAS](image8) (9.4±0.4) | -   |
| 36    | 2-(Hydroxyimino)-2-(pyrimdin-2-yl)ethanethioamide | ![Structure](image9) | -            | ![GALAS](image10) (10.5±0.9) | -   |
| 37    | Ethyl 2-(furan-2-yl)-2-(hydroxyimino)acetate | ![Structure](image11) | -            | ![GALAS](image12) (9.6±1.0) | -   |
| 38    | 2-(Hydroxyimino)-2-(1H-pyrrol-2-yl)acetamide | ![Structure](image13) | -            | ![GALAS](image14) (9.4±0.4) | -   |
| 39    | 2-(Hydroxyimino)-2-(thiophen-2-yl)acetamide | ![Structure](image15) | -            | ![GALAS](image16) (9.4±0.4) | -   |
| Sl. No. | IUPAC name                                      | Structure       | Abbreviation | GALAS        | Ref  |
|--------|-----------------------------------------------|-----------------|--------------|--------------|------|
| 40     | Ethyl 2-(hydroxyimino)-2-(1H-pyrrol-2-yl)acetate | ![Structure](image1.png) | -            | ![GALAS](image2.png) (9.6±1.0) | -    |
| 41     | Ethyl 2-(hydroxyimino)-2-(thiophen-2-yl)acetate | ![Structure](image3.png) | -            | ![GALAS](image4.png) (9.6±1.0) | -    |
| 42     | Ethyl 2-(hydroxyimino)-2-(pyridin-2-yl)acetate | ![Structure](image5.png) | -            | ![GALAS](image6.png) (9.6±1.0) | 35   |
| 43     | Ethyl 2-(hydroxyimino)-2-(pyridin-4-yl)acetate | ![Structure](image7.png) | -            | ![GALAS](image8.png) (9.6±1.0) | -    |
| 44     | 2-(Hydroxyimino)but-3-ynamide                 | ![Structure](image9.png) | -            | ![GALAS](image10.png) (9.4±0.4) | -    |
| 45     | Ethyl 2-(hydroxyimino)but-3-ynoate            | ![Structure](image11.png) | -            | ![GALAS](image12.png) (9.6±1.0) | -    |
| 46     | Ethyl 2-(hydroxyimino)-2-nitroacetate         | ![Structure](image13.png) | -            | ![GALAS](image14.png) (4.7±1.3) | 43, 46|
| 47     | Dinitromethane oxide                          | ![Structure](image15.png) | -            | ![GALAS](image16.png) (2.7±1.5) | 47   |
| Sl. No | IUPAC name                                      | Structure | Abbreviation | GALAS       | Ref  |
|-------|------------------------------------------------|-----------|--------------|-------------|------|
| 48    | Nitro(phenyl)methanone oxime                    | \begin{align*}
\text{O}_2\text{N} & \text{N} \\
\text{N} & \text{OH}
\end{align*} | -            | \begin{align*}
\text{O}_2\text{N} & \text{N} \\
\text{N} & \text{O} \\
\text{OH}
\end{align*} | (7.6±1.1) | 45   |
| 49    | 1-Nitro-3-phenylpropan-1-one oxime              | \begin{align*}
\text{O}_2\text{N} & \text{N} \\
\text{N} & \text{O} \\
\text{OH}
\end{align*} | -            | \begin{align*}
\text{O}_2\text{N} & \text{N} \\
\text{N} & \text{O} \\
\text{OH}
\end{align*} | (7.4±1.2) | 45   |
| 50    | 1-(Hydroxyimino)-N,N-dimethyl-1-phenylmethanesulfonamide | \begin{align*}
\text{S} & \text{N} \\
\text{N} & \text{O} \\
\text{OH}
\end{align*} | -            | \begin{align*}
\text{S} & \text{N} \\
\text{N} & \text{O} \\
\text{OH}
\end{align*} | (8.9±1.1) | -    |
| 51    | Ethyl 2-(hydroxyimino)-2-sulfamoylacetate       | \begin{align*}
\text{H}_2\text{N} & \text{S} \\
\text{O} & \text{N} \\
\text{O} \\
\text{OH}
\end{align*} | -            | \begin{align*}
\text{H}_2\text{N} & \text{S} \\
\text{O} & \text{N} \\
\text{O} \\
\text{OH}
\end{align*} | (5.8±1.3) | -    |
| 52    | (Hydroxyimino)(methylsulfonyl)methanesulfonamide | \begin{align*}
\text{S} & \text{S} \\
\text{NH}_2 & \text{N} \\
\text{O} & \text{N} \\
\text{OH}
\end{align*} | -            | \begin{align*}
\text{S} & \text{S} \\
\text{NH}_2 & \text{N} \\
\text{O} & \text{N} \\
\text{OH}
\end{align*} | (4.8±1.3) | -    |
| 53    | 2-(Hydroxyimino)-2-(methylsulfonyl)acetamide    | \begin{align*}
\text{S} & \text{S} \\
\text{NH}_2 & \text{N} \\
\text{O} & \text{N} \\
\text{OH}
\end{align*} | -            | \begin{align*}
\text{S} & \text{S} \\
\text{NH}_2 & \text{N} \\
\text{O} & \text{N} \\
\text{OH}
\end{align*} | (9.4±0.5) | -    |
| 54    | Ethyl 2-(hydroxyimino)-2-((trifluoromethyl)sulfonyl)acetate | \begin{align*}
\text{F} & \text{F} \\
\text{S} & \text{O} \\
\text{N} & \text{O} \\
\text{OH}
\end{align*} | -            | \begin{align*}
\text{F} & \text{F} \\
\text{S} & \text{O} \\
\text{N} & \text{O} \\
\text{OH}
\end{align*} | (4.2±1.4) | -    |
| Sl. No | IUPAC name                                                                 | Structure | Abbreviation | GALAS                        | Ref |
|-------|---------------------------------------------------------------------------|-----------|--------------|-------------------------------|-----|
| 55    | 2-Ethoxy-N-hydroxy-2-oxoacetimidic trifluoromethanesulfonic anhydride      | ![Structure](image1) | -            | ![Structure](image2) (7.4±1.2) | -   |
| 56    | Diphenylmethanone oxime                                                   | ![Structure](image3) | -            | ![Structure](image4) (11.1±0.4) | 36  |
| 57    | 2-((Hydroxyimino)(pyridin-2-yl)methyl)-1-methylpyridin-1-ium iodide         | ![Structure](image5) | -            | ![Structure](image6) (5.6±1.3)  | 48  |
| 58    | 9H-Fluoren-9-one oxime                                                    | ![Structure](image7) | -            | ![Structure](image8) (11.1±0.4) | 47  |
| 59    | Anthracene-9,10-dione dioxime                                             | ![Structure](image9) | -            | ![Structure](image10) (7.1±0.9)  | 49  |
| 60    | Pyrido[3,4-g]isoquinoline-5,10-dione dioxime                              | ![Structure](image11) | -            | ![Structure](image12) (5.5±0.8)  | 49  |
| 61    | Imidazolidin-2-one oxime                                                  | ![Structure](image13) | -            | ![Structure](image14) (12.5±0.8) | 50  |
| Sl. No | IUPAC name                          | Structure                                                                 | Abbreviation | GALAS       | Ref  |
|-------|-------------------------------------|---------------------------------------------------------------------------|--------------|-------------|------|
| 62    | 1,3-Dihydro-2H-imidazol-2-one oxime | ![Structure](image)                                                       | -            | ![GALAS](image) (8.2±0.8) | 50   |
| 63    | Oxazolidin-2-one oxime              | ![Structure](image)                                                       | -            | ![GALAS](image) (12.5±0.8)  | -    |
| 64    | Oxazol-2(3H)-one oxime              | ![Structure](image)                                                       | -            | ![GALAS](image) (6.6±0.9)   | -    |
| 65    | 2-(Hydroxyimino) malonamide         | ![Structure](image)                                                       | -            | ![GALAS](image) (9.4±0.4)   | -    |
| 66    | N,N-Dihydroxy-2-(hydroxyimino)malonamide | ![Structure](image)                                                     | -            | ![GALAS](image) (7.7±0.6)   | -    |
| 67    | Butane-2,3-dione dioxime            | ![Structure](image)                                                       | -            | ![GALAS](image) (12.1±0.5)  | 47   |
| 68    | N,N,Dihydroxyoxalimidoyl dichloride | ![Structure](image)                                                       | -            | ![GALAS](image) (5.8±1.2)   | 47   |
| 69    | N'-Hydroxypyrazine-2-carboximidamide| ![Structure](image)                                                       | -            | ![GALAS](image) (12.5±0.8)  | 49   |
| Sl. No | IUPAC name | Structure | Abbreviation | GALAS          | Ref   |
|-------|------------|-----------|--------------|----------------|-------|
| 70    | 3-Methyl-1,2,4-oxadiazole-5-carbaldehyde oxime | ![Structure](image1.png) | -            | ![GALAS](image2.png) (8.0±0.4) | 31    |
| 71    | 3-(Hydroxyimino)-1-phenylindolin-2-one | ![Structure](image3.png) | -            | ![GALAS](image4.png) (9.0±0.4) | 52    |
| 72    | 3-(Hydroxyimino)-1-methylindolin-2-one | ![Structure](image5.png) | -            | ![GALAS](image6.png) (9.4±0.4) | 52    |
| 73    | 2-((Hydroxyimino)methyl)-1-methylpyridin-1-ium | ![Structure](image7.png) | -            | ![GALAS](image8.png) (8.0±0.4) | 68    |
| 74    | 2,3,4,5,6-Pentafluorophenol | ![Structure](image9.png) | -            | ![GALAS](image10.png) (4.9±0.4) |       |
| 75    | 2,3,5-Trichlorophenol | ![Structure](image11.png) | -            | ![GALAS](image12.png) (6.4±0.4) |       |
| 76    | 4-Nitrophenol | ![Structure](image13.png) | -            | ![GALAS](image14.png) (7.2±0.4) |       |
Reagent for Each Reaction’, we have prepared and assayed different oxime analogues. 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) has shown to be even better than OxymaPure in minimizing racemization and Amox (4) to be very convenient for the protection of amines with the 9-fluorenylmethyloxycarbonyl (Fmoc) group avoiding the formation of dimers associated with 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 this 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/PhysChem Suite software and the pK\(_a\) GALAS algorithm available in it to calculate the acid ionization constant values of various oximes and other additives (Table 1). Like the pK\(_a\) Classic method, which is a variation of a classical Hammett-Taft approach and is available as an alternative within the said software, the GALAS algorithm is based on analogous fundamental considerations. 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 the so-called “fundamental microconstant”—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 microconstant 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 microconstants from which pK\(_a\) macroconstants 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\(_a\) microconstants 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 the 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\(_a\) values of some nonoxime 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 the zwitterionic species (HB\(^{\ddagger}\)) via two tautomeric equilibria (Figure 3). This zwitterionic species possesses a zero net charge and shows low or negative pK\(_a\) values. pK\(_a\) values found in the literature for HOBT and HOAt are 4.60 and 3.28, respectively.

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 a color code (if pK\(_a\) of some nonoxime additives 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 a color code (if pK\(_a\) values found in the literature for HOBT and HOAt are 4.60 and 3.28, respectively.

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). The calculation outlined in Table 1 confirms 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 be 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

Table 1. continued

| Sl. No | IUPAC name | Structure | Abbreviation | GALAS | Ref |
|-------|------------|-----------|--------------|-------|-----|
| 77    | Phenol     | ![Phenol](image) |             |       |     |

“Table 1. continued. The uncertainty of the prediction reported after the ± sign can be used as a reference point for the prediction quality with the value of 0.4 indicating the highest accuracy offered by the algorithm.”

![Figure 3. Tautomism of 1-hydroxybenzotriazoles.](image)
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 4). 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 5). 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 the tert-butoxycarbonyl (Boc) group, shows a pKₐ that confirms its moderate reactivity and therefore the absence of formation of Boc-dipeptides during the introduction of the Boc group (Figure 5). Finally, the pKₐ 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 6). 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.

Recently, Kolis and co-workers have observed that OxymaPure also reacts with DIC. Although, in this case, the formation of the adduct takes place to a much lesser extent than with HONM, it can cyclize with the generation of HCN (Figure 7). These results have been corroborated by Pawlas and co-workers, and our own group.

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 pKₐ of four nitro derivatives (46−49) only one ethyl-2-(hydroxyimino)-2-nitroacetate (46)—fulfils those requirements. Admittedly, the high value of uncertainty, indicating relatively lower quality of pKₐ 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 analogues, 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 analogue. Specifically, pKₐ (47) ≪ pKₐ (46) ≪ pKₐ (48) ∼ pKₐ (49) is analogous to pKₐ (15) ≪ pKₐ (1) ≪ pKₐ (11). In this context, concerns regarding the 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

The in silico study using the pKₐ GALAS algorithm available in ACD/PhysChem Suite has allowed us to calculate the pKₐ values of 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-phenylacetetonitrile. Furthermore, this method has allowed us to identify compound 46 as a potential substitute for OxymaPure (Figure 8).38-45 46

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.1c06342

Author Contributions
S.R.M. and A.S. contributed equally to this work. 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.

Funding
The work was funded by the National Research Foundation (NRF) (Blue Sky’s Research Programme # 120386).

Notes
The authors declare no competing financial interest. The data sets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

ACKNOWLEDGMENTS
The authors wish to thank Sanji K. Bhal (Advanced Chemistry Development, Inc, ACD/Labs) for the help with the review of the article manuscript.

REFERENCES

1) Cooper, T. W. J.; Campbell, I. B.; Macdonald, S. J. F. Factors Determining the Selection of Organic Reactions by Medicinal Chemists and the Use of These Reactions in Arrays (Small Focused Libraries). Angew. Chem., Int. Ed. 2010, 49, 8082–8091.
2) Roughley, S. D.; Jordan, A. M. The Medicinal Chemist's Toolbox: An Analysis of Reactions Used in the Pursuit of Drug Candidates. J. Med. Chem. 2011, 54, 3451–3479.
3) El-Faham, A.; Albericio, F. Peptide Coupling Reagents, More than a Letter Soup. Chem. Rev. 2011, 111, 6557–6602.
4) Fischer, E. Synthese von Polypeptiden. XVII. Ber. Dtsch. Chem. Ges. 1907, 40, 1754–1767.
5) Curtius, T. Synthetische Versuche mit Hippurazid. Ber. Dtsch. Chem. Ges. 1902, 35, 3226–3228.
6) Kumar, A.; Sharma, A.; Haimov, E.; El-Faham, A.; de la Torre, B. G.; Albericio, F. Fmoc-Amox, A Suitable Reagent for the Introduction of Fmoc. Org. Process Res. Dev. 2017, 21, 1533–1541.
7) Sheehan, J. C.; Hess, G. P. A New Method of Forming Peptide Bonds. J. Am. Chem. Soc. 1955, 77, 1067–1068.
8) Bodanszky, M. Synthesis of Peptides by Aminolysis of Nitrophenyl Esters. Nature 1955, 175, 685.
9) König, W.; Geiger, R. Eine neue Methode zur Synthese von Peptiden: Aktivierung der Carboxylgruppe mit Dicyclohexycarbodiimid unter Zusatz von 1-Hydroxy-benzotriazolen. Chem. Ber. 1970, 103, 788–798.
10) Sureshbabu, V. V.; Lalithamba, H. S.; Narendra, N.; Hemantha, H. P. New and simple synthesis of acid azides, ureas and carbamates from carboxylic acids: application of peptide coupling agents EDC and HBTU. Org. Biomol. Chem. 2010, 8, 835–840.
11) Speicher, A.; Klaus, T.; Eicher, T. O-(1-Benzotriazolyl)-N,N′,N,N′-tetramethyllumoniumhexafluorophosphat (HBTU) und O-(7-Aza-1-benzotriazolyl)-N,N′,N′-tetramethyllumoniumhexafluoro-
phosphat (HATU) – Zwei moderne Kupplungsreagenzien zur Peptidsynthese. J. Prakt. Chem. 1998, 340, 581–583.

(12) Abdelmoty, I.; Albericio, F.; Carpino, L. A.; Foxman, B. M.; Kates, S. A. Structural studies of reagents for peptide bond formation: Crystal and molecular structures of HBTU and HATU. Lett. Pept. Sci. 1994, 1, 57–67.

(13) Reszka, P.; Methling, K.; Lalk, M.; Xiao, Z.; Weiss, K.; BednarSKI, P. J. Control of aspartate epimerization during the coupling of caspase specific tetrapeptides with aromatic amines by using N-[(dimethylamino)-1H-1,2,3-triazolo[4,5-b]-pyridin-1-yl]-methylene-N-methylaminium hexafluorophosphate N-oxide (HATU) as a coupling reagent. Tetrahedron: Asymmetry 2008, 19, 49–59.

(14) Marder, O.; Shvo, Y.; Albericio, F. HCTU and TCTU: New Coupling Reagents—Development and Industrial Aspects. Chem. Oggi 2002, 20, 37–41.

(15) Hood, C. A.; Fuentes, G.; Patell, H.; Page, K.; Menakuru, M.; Park, J. H. Fast conventional Fmoc solid-phase peptide synthesis with HCTU. J. Pept. Sci. 2008, 14, 97–101.

(16) Albericio, F.; Bofill, J. M.; El-Faham, A.; Kates, S. A. Use of onium salt-based coupling reagents in peptide synthesis. J. Org. Chem. 1998, 63, 9678–9683.

(17) Albericio, F.; Cases, M.; Alina, J.; Triolo, S. A.; Carpino, L. A.; Kates, S. A. On the use of PyAOP, a phosphonium salt derived from HOAt, in solid-phase peptide synthesis. Tetrahedron Lett. 1997, 38, 4853–4856.

(18) Wehrstedt, K. D.; Wendrey, P. A.; Heitkamp, D. Explosive properties of 1-hydroxybenzotriazoles. J. Hazard. Mater. 2005, 126, 1–7.

(19) Subirós-Funosas, R.; Prohens, R.; Barbas, R.; El-Faham, A.; Albericio, F. Oxyma: An Efficient Additive for Peptide Synthesis to Replace the Benzotriazol-based HOBT and HOAt with a Lower Risk of Explosion. Chem. - Eur. J. 2009, 15, 9394–9403.

(20) Izedinski, J. New Reagents Suppressing Racemization in Peptide Synthesis by the DCC Method. Pol. J. Chem. 1979, 1049–1057.

(21) El-Faham, A.; Funosas, R. S.; Prohens, R.; Albericio, F. COMU: a safer and more effective replacement for benzotriazol-based urea-coupling reagents. Chem. - Eur. J. 2009, 15, 9404–9416.

(22) Subirós-Funosas, R.; El-Faham, A.; Albericio, F. PyOxB and PyOx:B: the oxyma-based novel family of phosphonium salts. Org. Biomol. Chem. 2010, 8, 3665–3673.

(23) Jad, Y. E.; Khattab, S. N.; de la Torre, B. G.; Govender, T.; Kruger, H. G.; El-Faham, A.; Albericio, F. Oxyma-B, an excellent racemization suppressor for peptide synthesis. Org. Biomol. Chem. 2014, 12, 8379–8385.

(24) Khattab, S. N.; Subirós-Funosas, R.; El-Faham, A.; Albericio, F. Screening of N-alkyl-cyanoacetamido oximes as substrates for N-hydroxysuccinimide. ChemistryOpen 2012, 1, 147–152.

(25) ACD/PhysChem Release, ver. 2020.1.2; Advanced Chemistry Development, Inc.: Toronto, ON, Canada, 2020; www.acdlabs.com.

(26) Ribeiro, A. R.; Schmidt, T. C. Determination of acid dissociation constants (pKₐ) of cephalosporin antibiotics: Computational and experimental approaches. Chemosphere 2017, 169, 524–533.

(27) Ráfols, C.; Subirats, X.; Rubio, J.; Rosés, M.; Bosch, E. Lipophilicity of amphoteric and zwiterionic compounds: A comparative study of determination methods. Talanta 2017, 162, 293–299.

(28) Kalliokoski, T.; Sinervo, K. Predicting pKₐ for Small Molecules on Public and In-house Datasets Using Fast Prediction Methods Combined with Data Fusion. Mol. Inf. 2019, 38, No. 1800163.

(29) Lin, C.-E.; Deng, Y.; Jiao, W.-S.; Sun, S.-W.; Lin, W.-Y.; Chen, C.-C. Electrophoretic behavior and pKₐ determination of quinolones with a piperazinyl substituent by capillary zone electrophoresis. J. Chromatogr. A 2004, 1051, 283–290.

(30) Fathallah, M. F.; Khattab, S. N. Spectrophotometric determination of pKₐ’s of 1-hydroxybenzotriazole and oxime derivatives in 95% acetonitrile-water. J. Chem. Soc. Pak. 2011, 33, 324–332.
(50) Mehio, N.; Lashely, M. A.; Nugent, J. W.; Tucker, L.; Correia, B.; Do-Thanh, C.-L.; Dai, S.; Hancock, R. D.; Bryantsev, V. S. Acidity of the Amidoxime Functional Group in Aqueous Solution: A Combined Experimental and Computational Study. J. Phys. Chem. B 2015, 119, 3567–3576.

(51) Bedford, C. D.; Howd, R. A.; Dailey, O. D.; Miller, A.; Nolen, H. W., III; Kenley, R. A.; Kern, J. R.; Winterle, J. S. Nonquaternary cholinesterase reactivators. 3. 3(5)-Substituted 1,2,4-oxadiazol-5(3)-aldoximes and 1,2,4-oxadiazole-5(3)-thiocarbohydroximates as reactivators of organophosphonate-inhibited eel and human acetylcholinesterase in vitro. J. Med. Chem. 1986, 29, 2174–2183.

(52) Sin, N.; Venables, B. L.; Liu, X.; Huang, S.; Gao, Q.; Ng, A.; Dalterio, R.; Rajamani, R.; Meanwell, N. A. The alkylation of isatin-derived oximes: Spectroscopic and X-ray crystallographic structural characterization of oxime and nitrone products. J. Heterocycl. Chem. 2009, 46, 432–442.

(53) McFarland, A. D.; Buser, J. Y.; Embry, M. C.; Held, C. B.; Kolis, S. P. Generation of Hydrogen Cyanide from the Reaction of Oxyma (Ethyl Cyano(hydroxyimino)acetate) and DIC (Diisopropylcarbodiimide). Org. Process Res. Dev. 2019, 23, 2099–2105.

(54) Erny, M.; Lundqvist, M.; Rasmussen, J. H.; Ludemann-Hombourger, O.; Bihel, F.; Pawlas, J. Minimizing HCN in DIC/Oxyma-Mediated Amide Bond-Forming Reactions. Org. Process Res. Dev. 2020, 24, 1341–1349.

(55) Manne, S. R.; Luna, O.; Acosta, G. A.; Royo, M.; El-Faham, A.; Orosz, G.; de la Torre, B. G.; Albercio, F. Amide Formation: Choosing the Safer Carbodiimide in Combination with OxymaPure to Avoid HCN Release. Org. Lett. 2021, 23, 6900–6904.

(56) Manne, S. R.; El-Faham, A.; de la Torre, B. G.; Albercio, F. Minimizing side reactions during amide formation using DIC and oxymapure in solid-phase peptide synthesis. Tetrahedron Lett. 2021, 85, No. 153462.