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Article

Understanding OxymaPure as a Peptide Coupling Additive: A Guide to New Oxyma Derivatives

Srinivasa Rao Manne,[○] Anamika Sharma,[○] Andrius Sazonovas, Ayman El-Faham, Beatriz G. de la Torre, and Fernando Albericio*

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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 nondescribed 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 studies of Fisher and Curtius exemplified this dichotomy. While Fisher proposed the acyl chloride as the activating method,[4] Curtius developed 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] which is still the most popular coupling method. Initially, the carboxylic acid reacts with carbodiimides and forms a reactive *O*-acylisourea (**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,[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 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.[9] 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*-acylisourea (**1**), in fact, the opposite is true. The intermediate *O*-acylisourea (**1**) is more reactive than the OBt active species (**4**). *O*-acylisourea (**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

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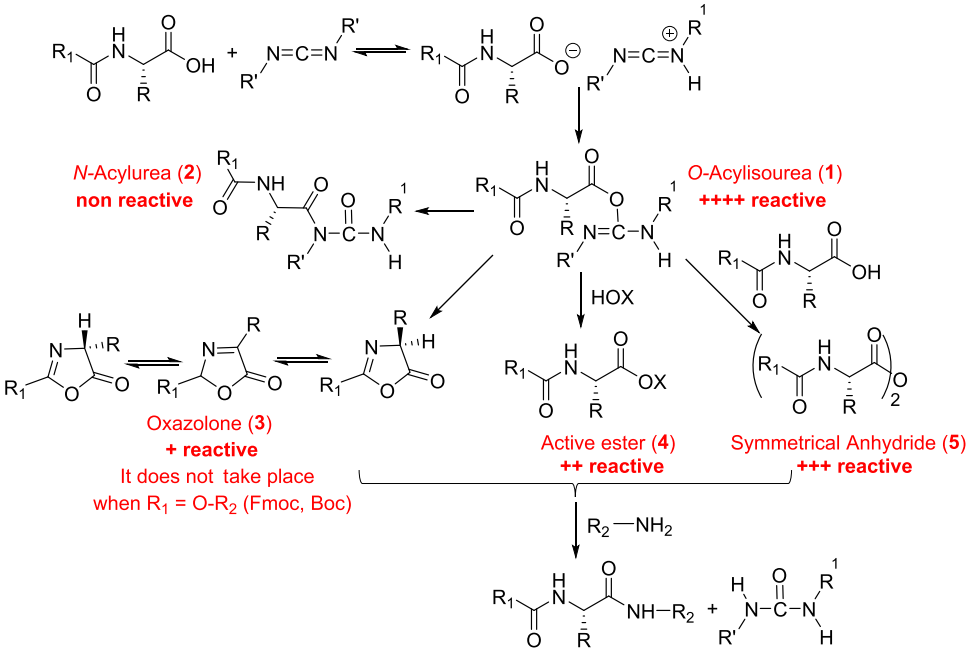
Scheme 1. Mechanism of Activation of Carbodiimides and the Role of Adding a Coupling Additive (HOX)



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 (HOAt), and the related 1-oxo-2-hydroxydihydrobenzotriazine (HODhbt, HOOBt). These additives are being used either as additives in carbodiimide-mediated coupling or as stand-alone reagents such as *N*-[(1*H*-benzotriazol-1-yl)-(dimethylamino)-methylene]-N-methylmethanaminium hexafluorophosphate *N*-oxide (HBTU), *N*-[6-chloro(1*H*-benzotriazol-1-yl)-(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (6-Cl-HBTU, HCTU), and *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]-pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) as aminium salts;[10−15] 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]

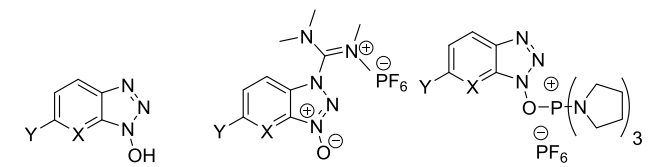


Figure 1. Some important benzotriazole additives and benzotriazole-based coupling reagents.

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-oxoethylideneaminooxy)-tri-1-pyrrolidinophosphonium hexafluorophosphate (PyOxim) (Figure 2),[22] are the

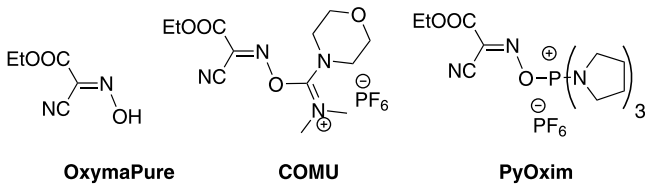


Figure 2. OxymaPure and OxymaPure-based stand-alone coupling reagents.

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 DISCUSSION

In our continuous efforts to develop different additives fulfilling our lemma "Choosing the Right Peptide Coupling

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Table 1. Calculated pK_a of Some Coupling Additives Using the pK_a GALAS Prediction Algorithm from ACD/PhysChem Suite[a25]

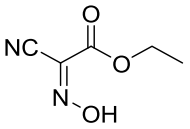
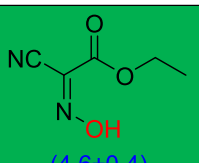
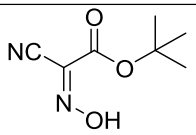
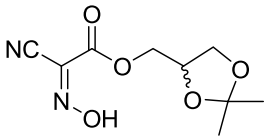
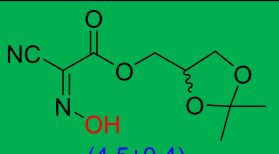
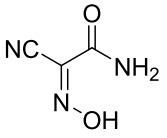
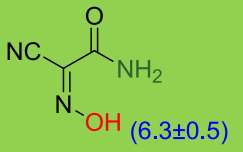
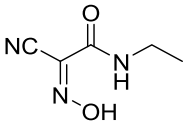
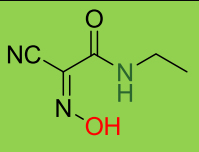
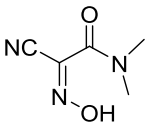
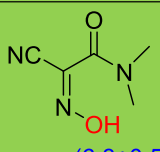
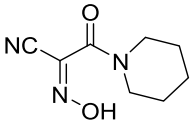
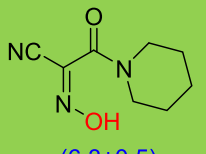
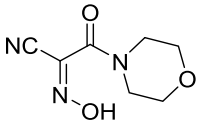
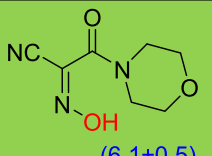
Sl. No	IUPAC name	Structure	Abbreviation	GALAS	Ref
1	Ethyl 2-cyano-2-(hydroxyimino)acetate		OxymaPure	 (4.6±0.4)	19, 31
2	*t*ert-Butyl 2-cyano-2-(hydroxyimino)acetate		-	 (4.6±0.4)	32
3	(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 2-cyano-2-(hydroxyimino)acetate		-	 (4.5±0.4)	33
4	2-Amino-N-hydroxy-2-oxoacetimidoyl cyanide		Amox	 (6.3±0.5)	24
5	2-(Ethylamino)-N-hydroxy-2-oxoacetimidoyl cyanide		*N*-Oxyma	 (6.3±0.5)	24
6	2-(Dimethylamino)-N-hydroxy-2-oxoacetimidoyl cyanide		DmOX	 (6.3±0.5)	24
7	N-Dydroxy-2-oxo-2-(piperidin-1-yl)acetimidoyl cyanide		PipOX	 (6.3±0.5)	24
8	N-Hydroxy-2-morpholino-2-oxoacetimidoyl cyanide		MorOX	 (6.1±0.5)	24

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Table 1. continued

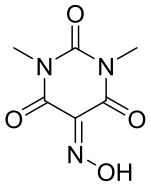
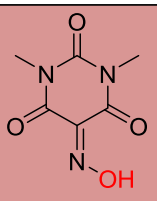
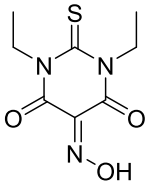
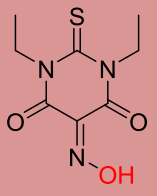
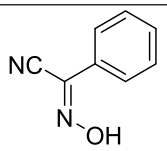
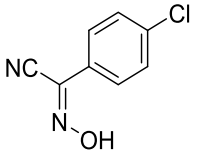
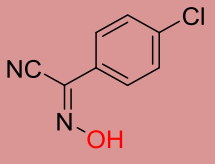
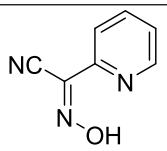
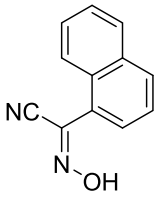
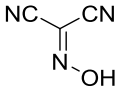
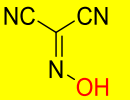
Sl. No	IUPAC name	Structure	Abbreviation	GALAS	Ref
9	5-(Hydroxyimino)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione		Oxyma-B	 (8.2±0.5)	23
10	1,3-Diethyl-5-(hydroxyimino)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione		Oxyma-T	 (8.2±0.5)	34
11	Hydroxyimino-2-phenylacetonitrile		-	 (8.2±0.4)	35-37
12	Hydroxyimino-2-(4-chloro)phenylacetonitrile		-	 (8.0±0.4)	36
13	Hydroxypicolinimidoyl cyanide		-	 (8.2±0.4)	31, 35
14	Hydroxyimino-2-(1-naphtyl)phenylacetonitrile		-	 (8.2±0.4)	36
15	Hydroxycarbonimidoyl dicyanide		-	 (3.8±0.7)	31

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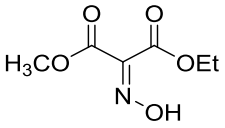
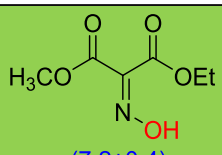
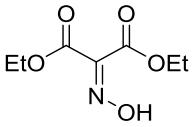
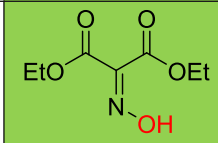
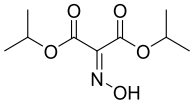
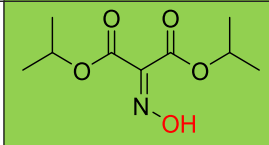
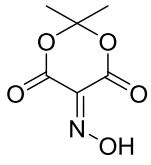
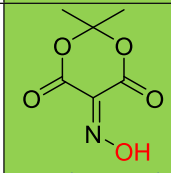
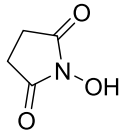
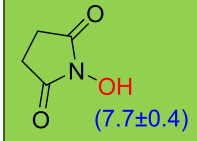
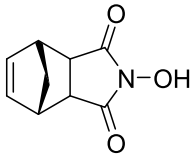
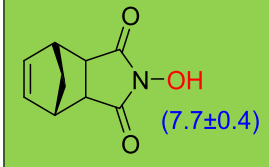
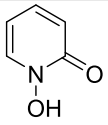
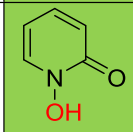
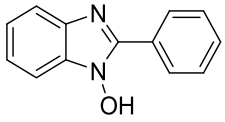
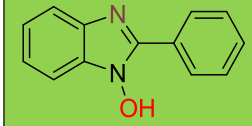
Table 1. continued

Sl. No	IUPAC name	Structure	Abbreviation	GALAS	Ref
16	1-Ethyl 3-methyl-2-(hydroxyimino)malonate		-	 (7.2±0.4)	36
17	Diethyl 2-(hydroxyimino)malonate		-	 (7.2±0.4)	36
18	Diisopropyl 2-(hydroxyimino)malonate		-	 (7.2±0.4)	38
19	5-(Hydroxyimino)-2,2-dimethyl-1,3-dioxane-4,6-dione		HONM	 (6.1±1.2)	39
20	1-Hydroxypyrrolidine-2,5-dione		HOSu	 (7.7±0.4)	40
21	(4S,7S)-2-Hydroxy-3a,4,7,7a-tetrahydro-1H-4,7-methanoisoindole-1,3(2H)-dione		HONB	 (7.7±0.4)	41
22	1-Hydroxypyridin-2(1H)-one		HOPO	 (6.0±0.4)	42
23	2-Phenyl-1H-benzo[d]imidazol-1-ol		HOBI	 (7.7±0.9)	42

https://doi.org/10.1021/acsomega.1c06342
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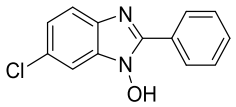
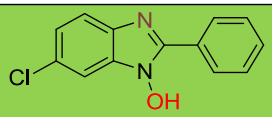
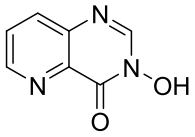
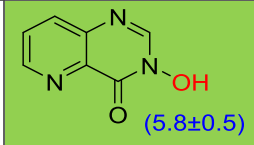
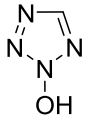
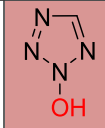
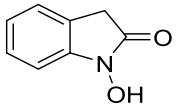
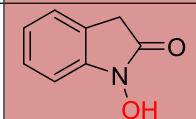
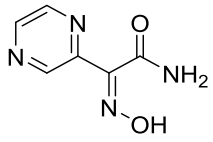
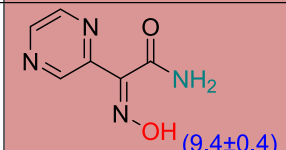
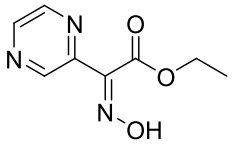
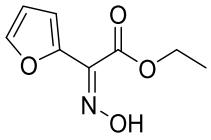
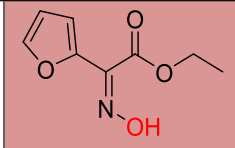
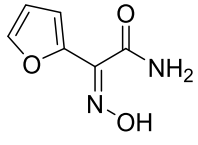
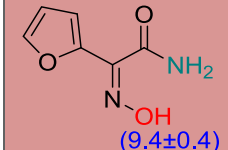
Table 1. continued

Sl. No	IUPAC name	Structure	Abbreviation	GALAS	Ref
24	6-Chloro-2-phenyl-1H-benzo[d]imidazol-1-ol		6-Cl-HOBI	 (7.1±0.9)	42
25	3-Hydroxypyrido[3,2-d]pyrimidin-4(3H)-one		HODhad	 (5.8±0.5)	43
26	2H-Tetrazol-2-ol		-	 (8.2±0.8)	44
27	1-Hydroxyindolin-2-one		HOI	 (8.3±0.4)	42
28	2-(Hydroxyimino)-2-(pyrazin-2-yl)acetamide		-	 (9.4±0.4)	-
29	Ethyl-2-(hydroxyimino)-2-(pyrazin-2-yl)acetate		-	 (9.6±1.0)	-
30	Ethyl-2-(furan-2-yl)-2-(hydroxyimino)acetate		-	 (9.6±1.0)	-
31	2-(Furan-2-yl)-2-(hydroxyimino)acetamide		-	 (9.4±0.4)	-

https://doi.org/10.1021/acsomega.1c06342
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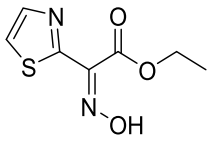
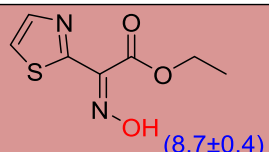
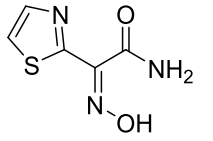
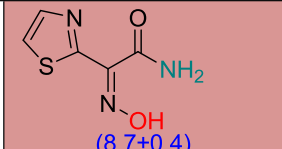
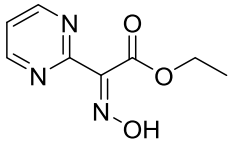
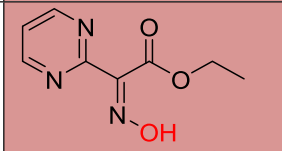
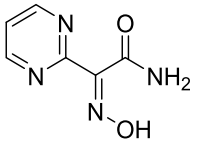
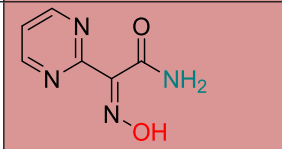
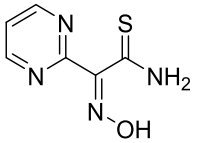
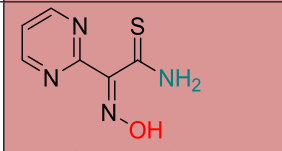
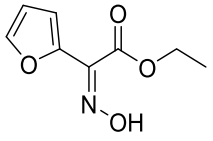
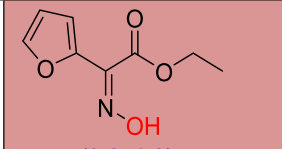
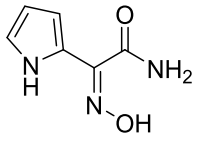
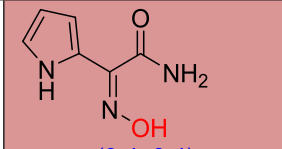
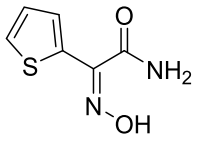
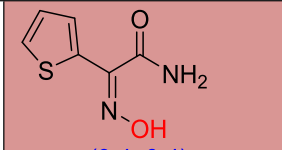
Table 1. continued

Sl. No	IUPAC name	Structure	Abbreviation	GALAS	Ref
32	Ethyl 2-(hydroxyimino)-2-(thiazol-2-yl)acetate		-	 (8.7±0.4)	-
33	2-(Hydroxyimino)-2-(thiazol-2-yl)acetamide		-	 (8.7±0.4)	-
34	Ethyl 2-(hydroxyimino)-2-(pyrimidin-2-yl)acetate		-	 (9.6±1.0)	-
35	2-(Hydroxyimino)-2-(pyrimidin-2-yl)acetamide		-	 (9.4±0.4)	-
36	2-(Hydroxyimino)-2-(pyrimidin-2-yl)ethanethioamide		-	 (10.5±0.9)	-
37	Ethyl 2-(furan-2-yl)-2-(hydroxyimino)acetate		-	 (9.6±1.0)	-
38	2-(Hydroxyimino)-2-(1H-pyrrol-2-yl)acetamide		-	 (9.4±0.4)	-
39	2-(Hydroxyimino)-2-(thiophen-2-yl)acetamide		-	 (9.4±0.4)	-

https://doi.org/10.1021/acsomega.1c06342
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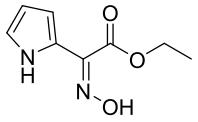
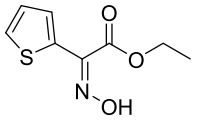
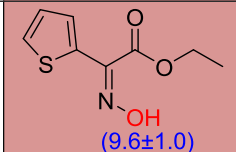
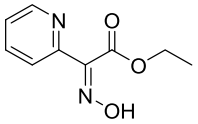
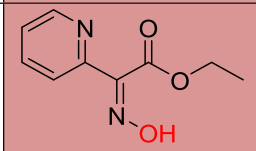
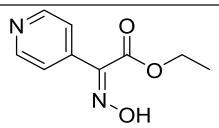
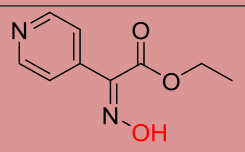
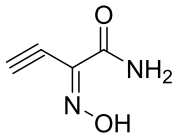
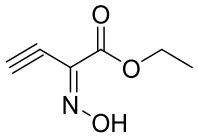
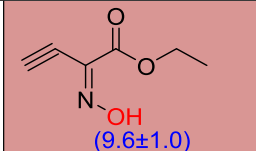
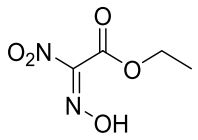
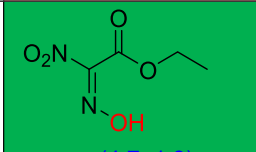
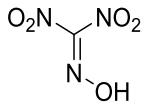
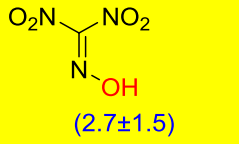
Table 1. continued

Sl. No	IUPAC name	Structure	Abbreviation	GALAS	Ref
40	Ethyl 2-(hydroxyimino)-2-(1H-pyrrol-2-yl)acetate		-	 (9.6±1.0)	-
41	Ethyl 2-(hydroxyimino)-2-(thiophen-2-yl)acetate		-	 (9.6±1.0)	-
42	Ethyl 2-(hydroxyimino)-2-(pyridin-2-yl)acetate		-	 (9.6±1.0)	35
43	Ethyl 2-(hydroxyimino)-2-(pyridin-4-yl)acetate		-	 (9.6±1.0)	-
44	2-(Hydroxyimino)but-3-ynamide		-	 (9.4±0.4)	-
45	Ethyl 2-(hydroxyimino)but-3-ynoate		-	 (9.6±1.0)	-
46	Ethyl 2-(hydroxyimino)-2-nitroacetate		-	 (4.7±1.3)	45, 46
47	Dinitromethanone oxime		-	 (2.7±1.5)	47

https://doi.org/10.1021/acsomega.1c06342
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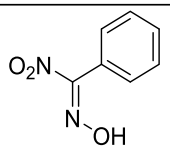
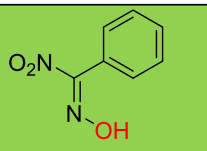
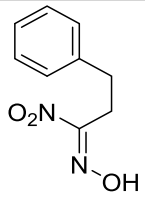
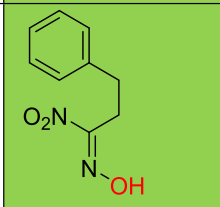
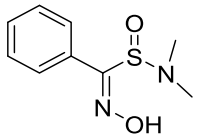
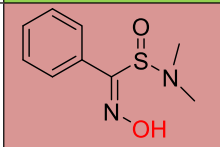
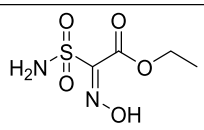
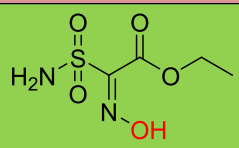
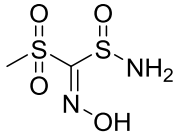
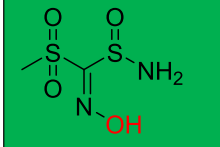
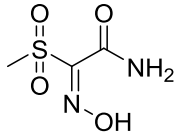
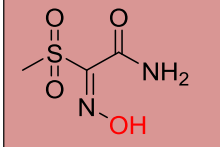
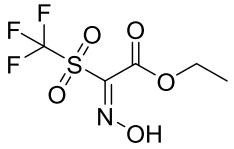
Table 1. continued

Sl. No	IUPAC name	Structure	Abbreviation	GALAS	Ref
48	Nitro(phenyl)methanone oxime		-	 (7.6±1.1)	45
49	1-Nitro-3-phenylpropan-1-one oxime		-	 (7.4±1.2)	45
50	1-(Hydroxyimino)-N,N-dimethyl-1-phenylmethanesulfinamide		-	 (8.9±1.1)	-
51	Ethyl 2-(hydroxyimino)-2-sulfamoylacetate		-	 (5.8±1.3)	-
52	(Hydroxyimino)(methylsulfonyl)methanesulfinamide		-	 (4.8±1.3)	-
53	2-(Hydroxyimino)-2-(methylsulfonyl)acetamide		-	 (9.4±0.5)	-
54	Ethyl 2-(hydroxyimino)-2-((trifluoromethyl)sulfonyl)acetate		-	 (4.2±1.4)	-

https://doi.org/10.1021/acsomega.1c06342
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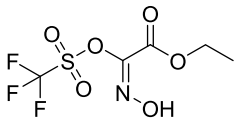
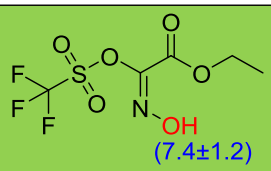
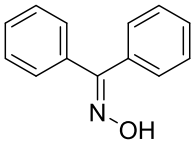
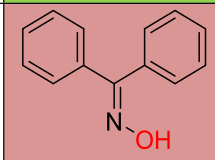
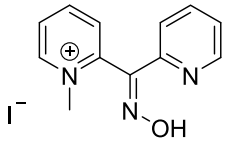
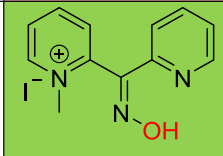
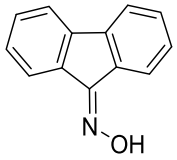
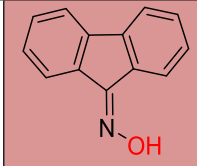
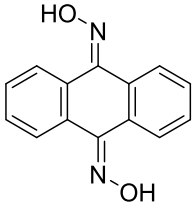
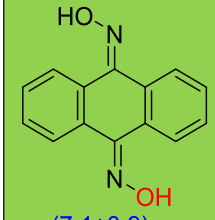
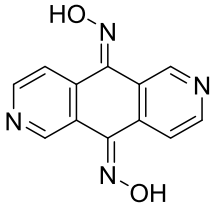
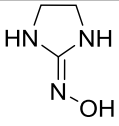
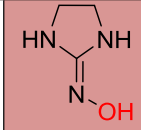
Table 1. continued

Sl. No	IUPAC name	Structure	Abbreviation	GALAS	Ref
55	2-Ethoxy-N-hydroxy-2-oxoacetimidic trifluoromethanesulfonic anhydride		-	 (7.4±1.2)	-
56	Diphenylmethanone oxime		-	 (11.1±0.4)	36
57	2-((Hydroxyimino)(pyridin-2-yl)methyl)-1-methylpyridin-1-ium iodide		-	 (5.6±1.3)	48
58	9H-Fluoren-9-one oxime		-	 (11.1±0.4)	47
59	Anthracene-9,10-dione dioxime		-	 (7.1±0.9)	49
60	Pyrido[3,4-g]isoquinoline-5,10-dione dioxime		-	 (5.5±0.8)	49
61	Imidazolidin-2-one oxime		-	 (12.5±0.8)	50

https://doi.org/10.1021/acsomega.1c06342
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Table 1. continued

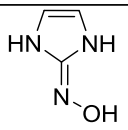
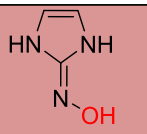
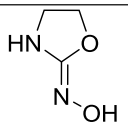
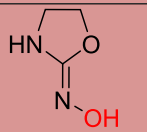
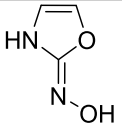
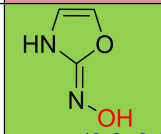
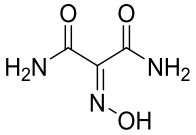
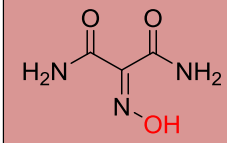
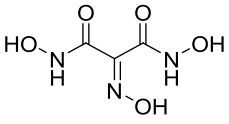
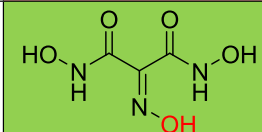
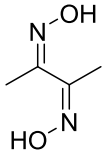
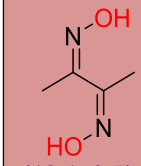
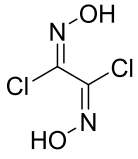
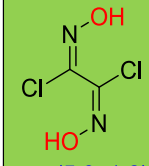
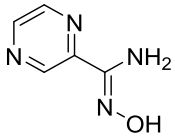
Sl. No	IUPAC name	Structure	Abbreviation	GALAS	Ref
62	1,3-Dihydro-2H-imidazol-2-one oxime		-	 (8.2±0.8)	[50]
63	Oxazolidin-2-one oxime		-	 (12.5±0.8)	-
64	Oxazol-2(3H)-one oxime		-	 (6.6±0.9)	-
65	2-(Hydroxyimino) malonamide		-	 (9.4±0.4)	-
66	N^1,N^3-Dihydroxy-2-(hydroxyimino)malonamide		-	 (7.7±0.6)	-
67	Butane-2,3-dione dioxime		-	 (12.1±0.5)	[47]
68	N'1,N'2-Dihydroxyoxalimidoyl dichloride		-	 (5.8±1.2)	[47]
69	N'-Hydroxypyrazine-2-carboximidamide		-	 (12.5±0.8)	[49]

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Table 1. continued

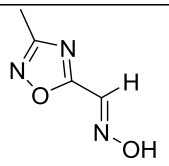
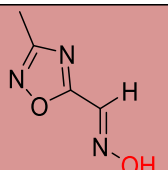
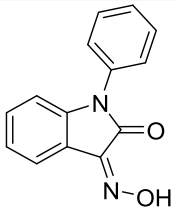
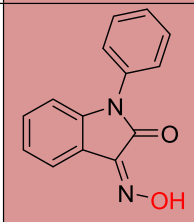
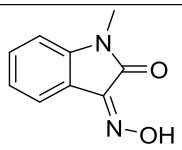
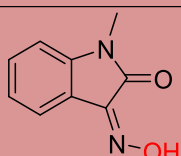
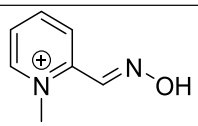
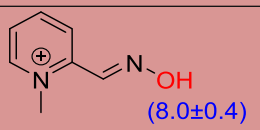
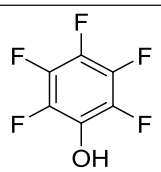
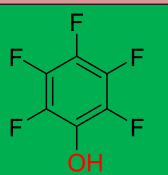
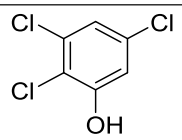
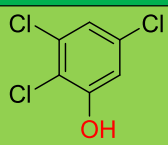
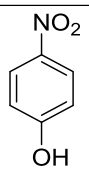
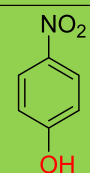
Sl. No	IUPAC name	Structure	Abbreviation	GALAS	Ref
70	3-Methyl-1,2,4-oxadiazole-5-carbaldehyde oxime		-	 (8.0±0.4)	51
71	3-(Hydroxyimino)-1-phenylindolin-2-one		-	 (9.0±0.4)	52
72	3-(Hydroxyimino)-1-methylindolin-2-one		-	 (9.4±0.4)	52
73	2-((Hydroxyimino)methyl)-1-methylpyridin-1-ium		-	 (8.0±0.4)	48
74	2,3,4,5,6-Pentafluorophenol		-	 (4.9±0.4)	
75	2,3,5-Trichlorophenol		-	 (6.4±0.4)	
76	4-Nitrophenol		-	 (7.2±0.4)	

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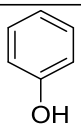
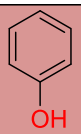
Table 1. continued

Sl. No	IUPAC name	Structure	Abbreviation	GALAS	Ref
77	Phenol			 (10.0±0.4)	

[a]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.

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**)[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 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[25] and the pK_a GALAS algorithm available in it to calculate the acid ionization constant values of 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 the 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 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^+A^-) via two tautomeric equilibria (Figure 3). This

Figure 3. Tautomerism of 1-hydroxybenzotriazoles.

zwitterionic species possesses a zero net charge and shows 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 a 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**),[23,24,31−34] and 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

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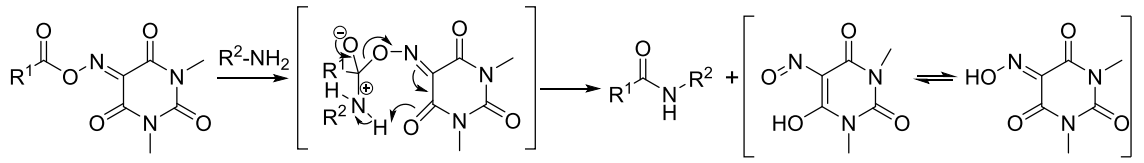

Figure 4. Assisted basic catalysis involved in the coupling through the Oxyma-B active ester.

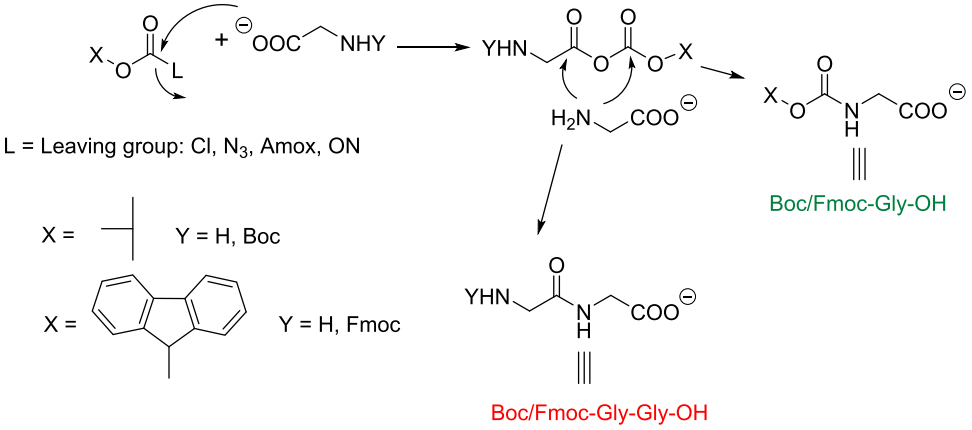

Figure 5. Undesired formation of *N*-protected dipeptides during the protection reaction.

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,[35−37] 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 5). 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 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.[53] 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[54] and co-workers, and our own group.[55,56]

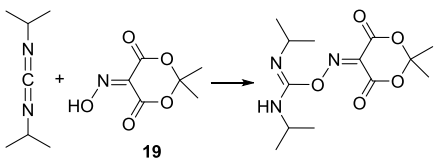

Figure 6. Reaction of HONM (**19**) with DIC.

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 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 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, 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 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 pK_a GALAS algorithm available in ACD/PhysChem Suite has allowed us to calculate the pK_a 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

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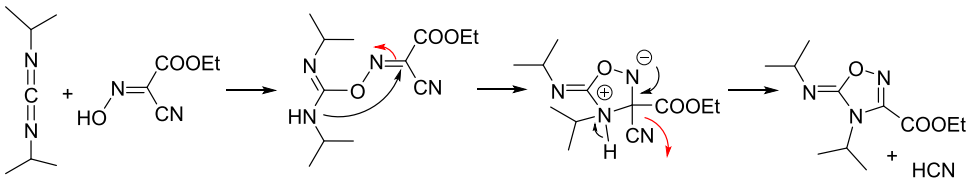
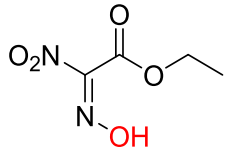


Figure 7. Formation of the adduct and posterior cyclization with the generation of HCN.

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 8).[38−4546−]



46

Figure 8. Structure of ethyl (*E*)-2-(hydroxyimino)-2-nitroacetate (**46**).

AUTHOR INFORMATION

Corresponding Author

Fernando Albericio − *Peptide Science Laboratory, School of Chemistry and Physics, University of KwaZulu-Natal, Durban 4000, South Africa; Institute for Advanced Chemistry of Catalonia (IQAC-CSIC), 08034 Barcelona, Spain; CIBER-BBN, Networking Centre on Bioengineering, Biomaterials and Nanomedicine, and Department of Organic Chemistry, University of Barcelona, 08028 Barcelona, Spain;* orcid.org/0000-0002-8946-0462; Email: albericio@ukzn.ac.za

Authors

Srinivasa Rao Manne − *Peptide Science Laboratory, School of Chemistry and Physics, University of KwaZulu-Natal, Durban 4000, South Africa*

Anamika Sharma − *Peptide Science Laboratory, School of Chemistry and Physics, University of KwaZulu-Natal, Durban 4000, South Africa; KwaZulu-Natal Research Innovation and Sequencing Platform (KRISP), School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban 4041, South Africa; Department of Chemistry, Prayoga Institute of Education Research (PIER), Bangalore 560082, India*

Andrius Sazonovas − *Advanced Chemistry Development, Inc. (ACD/Labs), Toronto, Ontario M5C 1B5, Canada*

Ayman El-Faham − *Department of Chemistry, Faculty of Science, Alexandria University, Alexandria 21321, Egypt;* orcid.org/0000-0002-3951-2754

Beatriz G. de la Torre − *Peptide Science Laboratory, School of Chemistry and Physics, University of KwaZulu-Natal, Durban 4000, South Africa; KwaZulu-Natal Research Innovation and Sequencing Platform (KRISP), School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban 4041, South Africa;* orcid.org/0000-0001-8521-9172

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.

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Notes

The authors declare no competing financial interest.

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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) Bodánszky, 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 Dicyclohexylcarbodiimid 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*′-tetramethyluroniumhexafluorophosphat (HBTU) und *O*-(7-Aza-1-benzotriazolyl)-*N*,*N*,*N*′,*N*′-tetramethyluroniumhexafluoro-

https://doi.org/10.1021/acsomega.1c06342
ACS Omega 2022, 7, 6007−6023

phosphat (HATU) − Zwei moderne Kupplungsreagenzien zur Peptidsynthese. *J. Prakt. Chem./Chem.-Ztg.* **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.; Weisz, K.; Bednarski, P. J. Control of aspartate epimerization during the coupling of caspase specific tetrapeptides with aromatic amines by using *N*-[[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]-pyridin-1-yl]methylene]-*N*-methylmethanaminium 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. *Chim. Oggi* **2002**, *20*, 37−41.
(15) Hood, C. A.; Fuentes, G.; Patel, 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.; Alsina, 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.; Wandrey, 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 Benzotriazole-Based HOBt and HOAt with a Lower Risk of Explosion. *Chem. - Eur. J.* **2009**, *15*, 9394−9403.
(20) Izdebski, 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 benzotriazole-based uronium coupling reagents. *Chem. - Eur. J.* **2009**, *15*, 9404−9416.
(22) Subirós-Funosas, R.; El-Faham, A.; Albericio, F. PyOxP and PyOxB: 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 substitutes 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 (pK_a) of cephalosporin antibiotics: Computational and experimental approaches. *Chemosphere* **2017**, *169*, 524−533.
(27) Ràfols, C.; Subirats, X.; Rubio, J.; Rosés, M.; Bosch, E. Lipophilicity of amphoteric and zwitterionic compounds: A comparative study of determination methods. *Talanta* **2017**, *162*, 293−299.
(28) Kalliokoski, T.; Sinervo, K. Predicting pK_a 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., Jr; Liao, W.-S.; Sun, S.-W.; Lin, W.-Y.; Chen, C.-C. Electrophoretic behavior and pK_a 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 pK_a's of 1-hydroxybenzotriazole and oxime derivatives in 95% acetonitrile-water. *J. Chem. Soc. Pak.* **2011**, *33*, 324−332.
(31) El-Faham, A.; Funosas, R. S.; Prohens, R.; Albericio, F. COMU: A Safer and More Effective Replacement for Benzotriazole-Based Uronium Coupling Reagents. *Chem. - Eur. J.* **2009**, *15*, 9404−9416.
(32) Duguay, G.; Guémas, J.-P.; Meslin, J.-C.; Pradère, J.-P.; Reliquet, F.; Reliquet, A.; Tea-Gokou, C.; Quiniou, H.; Rabiller, C. Heteroatomic chains and their products of cyclisation. IV. t-butyl-2-phthalimido-2-(3,6-dihydro-1,3-2*H*-thiazine-2-yliden)-acetates substituted in position 5 by a functional group. *J. Heterocycl. Chem.* **1980**, *17*, 767−770.
(33) Wang, Q.; Wang, Y.; Kurosu, M. A New Oxyma Derivative for Nonracemizable Amide-Forming Reactions in Water. *Org. Lett.* **2012**, *14*, 3372−3375.
(34) Jad, Y. E.; de la Torre, B. G.; Govender, T.; Kruger, H. G.; El-Faham, A.; Albericio, F. Oxyma-T, expanding the arsenal of coupling reagents. *Tetrahedron Lett.* **2016**, *57*, 3523−3525.
(35) El-Faham, A.; Elnakdy, Y. A.; El Gazzar, S. A. M.; Abd El-Rahman, M. M.; Khattab, S. N. Synthesis, Characterization and Anti-proliferation Activities of Novel Cyano Oximino Sulfonate Esters. *Chem. Pharm. Bull.* **2014**, *62*, 373−378.
(36) Masumi, I.; Daijiro, H.; Takashi, K. Peptides. VI. Some Oxime Carbonates as Novel t-Butoxycarbonylating Reagents. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 718−721.
(37) Itoh, M.; Hagiwara, D.; Kamiya, T. A new tert-butyloxycarbonylating reagent, 2-*tert*-butyloxycarbonyloxyimino-2-phenylacetonitrile. *Tetrahedron Lett.* **1975**, *16*, 4393−4394.
(38) Kattamuri, P. V.; Yin, J.; Siriwongsup, S.; Kwon, D.-H.; Ess, D. H.; Li, Q.; Li, G.; Yousufuddin, M.; Richardson, P. F.; Sutton, S. C.; Kürti, L. Practical Singly and Doubly Electrophilic Aminating Agents: A New, More Sustainable Platform for Carbon−Nitrogen Bond Formation. *J. Am. Chem. Soc.* **2017**, *139*, 11184−11196.
(39) El-Faham, A.; Subirós-Funosas, R.; Albericio, F. A Novel Family of Onium Salts Based Upon Isonitroso Meldrum's Acid Proves Useful as Peptide Coupling Reagents. *Eur. J. Org. Chem.* **2010**, *2010*, 3641−3649.
(40) Sheehan, J. C.; Johnson, D. A. The synthesis of substituted penicillins and simpler structural analogs. VIII. Phthalimidomalonaldehydic esters: Synthesis and condensation with penicillamine. *J. Am. Chem. Soc.* **1954**, *76*, 158−160.
(41) Galanis, A. S.; Albericio, F.; Grøtli, M. Solid-phase peptide synthesis in water using microwave-assisted heating. *Org. Lett.* **2009**, *11*, 4488−4491.
(42) El-Faham, A.; Albericio, F. Synthesis and Application of *N*-Hydroxylamine Derivatives as Potential Replacements for HOBt. *Eur. J. Org. Chem.* **2009**, *2009*, 1499−1501.
(43) Carpino, L. A.; Xia, J.; El-Faham, A. 3-Hydroxy-4-oxo-3,4-dihydro-5-azabenzo-1,2,3-triazene. *J. Org. Chem.* **2004**, *69*, 54−61.
(44) Spetzler, J. C.; Meldal, M.; Felding, J.; Vedsø, P.; Begtrup, M. Novel acylation catalysts in peptide synthesis: derivatives of *N*-hydroxytriazoles and *N*-hydroxytetrazoles. *J. Chem. Soc., Perkin Trans. 1* **1998**, 1727−1732.
(45) Matt, C.; Gissot, A.; Wagner, A.; Mioskowski, C. Nitrolic acids: efficient precursors of nitrile oxides under neutral conditions. *Tetrahedron Lett.* **2000**, *41*, 1191−1194.
(46) Zhou, L.; Haorah, J.; Chen, S. C.; Wang, X.; Kolar, C.; Lawson, T. A.; Mirvish, S. S. Nitrosation of Glycine Ethyl Ester and Ethyl Diazoacetate To Give the Alkylating Agent and Mutagen Ethyl Chloro(hydroximino)acetate. *Chem. Res. Toxicol.* **2004**, *17*, 416−423.
(47) Chong, S.-S.; Fu, Y.; Liu, L.; Guo, Q.-X. O−H Bond Dissociation Enthalpies of Oximes: A Theoretical Assessment and Experimental Implications. *J. Phys. Chem. A* **2007**, *111*, 13112−13125.
(48) Kuča, K.; Pícha, J.; Cabal, J.; Liška, F. Synthesis of the three monopyridinium oximes and evaluation of their potency to reactivate acetylcholinesterase inhibited by nerve agents. *J. Appl. Biomed.* **2004**, *2*, 51−56.
(49) Reddy, D. S.; Kongot, M.; Netalkar, S. P.; Kurjogi, M. M.; Kumar, R.; Avecilla, F.; Kumar, A. Synthesis and evaluation of novel coumarin-oxime ethers as potential anti-tubercular agents: Their DNA cleavage ability and BSA interaction study. *Eur. J. Med. Chem.* **2018**, *150*, 864−875.

https://doi.org/10.1021/acsomega.1c06342
ACS Omega 2022, 7, 6007−6023

(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.; Albericio, 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.; Albericio, F. Minimizing side reactions during amide formation using DIC and oxymapure in solid-phase peptide synthesis. *Tetrahedron Lett.* **2021**, *85*, No. 153462.

https://doi.org/10.1021/acsomega.1c06342
ACS Omega 2022, 7, 6007−6023