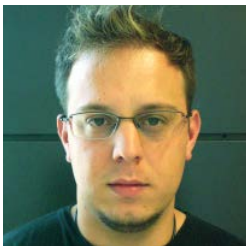
Advances in Acylation Methodologies Enabled by Oxyma-Based Reagents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Abstract. The aim of this review is to cover recent advances in acylation chemistry (peptide, amide, and ester bond formation) in which ethyl 2-cyano-2-(hydroxyimino)acetate (Oxyma)-derived reagents are involved. The distinct applications of each class of Oxyma derivatives will be discussed and compared to the behavior of reputed standards in the field.

Outline

1. Introduction

Oximes are some of the most versatile building blocks in organic and organometallic chemistry. The substituents at the α position account for the specific properties of each oxime, such as dissociation constant, solubility, and chelating ability. Oximes are highly polar compounds of moderate acidity, and have a broad range of applications in chemistry and biology. For example, oxime-containing molecules are responsible for the prevention of biofouling in marine submerged materials, and display growth-regulating and fungicide activities in plants.[1,2] Due to their high physiological stability, oximes are also present in prodrugs and natural antibiotics.[3] In synthesis, oximes are involved in alkyl-transfer reactions, in the construction of palladium precatalysts for

carbon–carbon cross-coupling, and in electrocatalysis.[4] In addition, chemoselective protein ligation has recently been envisaged by connecting peptide fragments through an oxime bridge.[5]

Since the 1970s, the beneficial uses of cyanooximes—lacking the typical instability of oximes possessing an α hydrogen—have been extensively investigated in diverse fields of research.[6–12] Their high acidity translates into remarkable aqueous solubility and into the bright yellow color of the resulting anion—a consequence of a UV transition similar to that of the nitro group.[6] Some of the most prominent cyanooximes, bearing distinct electron-withdrawing moieties are depicted in **Figure 1**. The acidic nature of the cyanooximes with electron-withdrawing substituents (e.g.; AmOx (**8**), pK_a = 5.2; Oxyma (**6**), pK_a = 4.60) has led researchers to develop activated derivatives for acylation reactions on the basis of their potential as leaving groups.[13–18] Of all the oxime derivatives examined as acylation promoters, Oxyma (**6**) offers the best balance between reactivity and stability, in addition to its high solubility in a broad spectrum of solvents.[14] These desirable properties and its commercial availability on a large scale have made

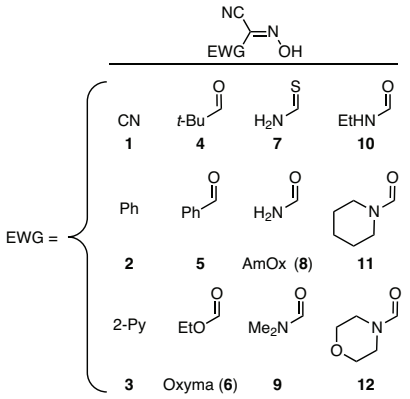


Figure 1. Structures of the Better Known Cyanooximes.

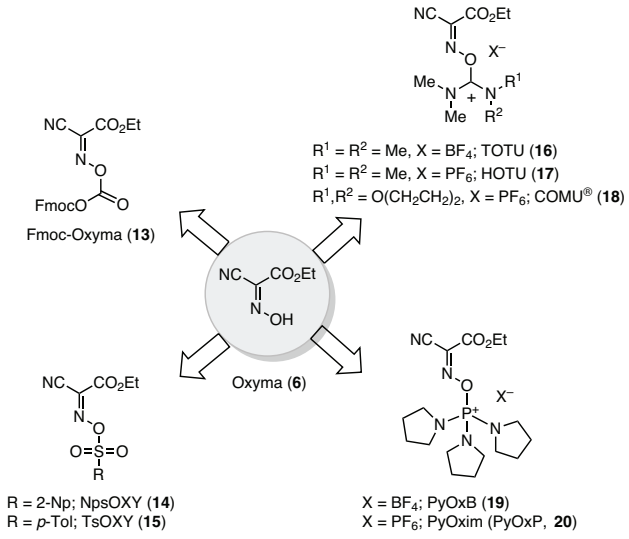


Figure 2. Oxyma (**6**)-Based Family of Peptide Coupling Reagents. *(Ref. 16,17,19–22)*

it a good candidate for investigating its acylation behavior.[19] In spite of its early use as an epimerization-suppressing additive,[14] Oxyma (**6**) remained unnoticed as a coupling reagent in the ensuing decades. Following the reevaluation of Oxyma (**6**) and other acidic oximes by DeGrado's group[20a] and ours,[17,20b,c,21,22] Oxyma (**6**) and its derived coupling reagents (**Figure 2**) have emerged as worthy alternatives to benzotriazoles,[13,14] which had hitherto dominated the field of acyl-transfer reactions.[20–22] In a short period of time, this oxime scaffold has rapidly been adopted in research laboratories to effect a broad range of acylations.[23,24] In the past few years, although some authors have surveyed the available acylation strategies for forming peptide and amide bonds, cyanooximes such as Oxyma (**6**) were either not included, or were described in a very limited number of applications.[25,26] This review offers a unique perspective on Oxyma-based coupling reagents, by focusing on their recently discovered acylation possibilities and by including some of their applications outside of peptide chemistry.

2. Oxyma-Based Coupling Reagents

The set of Oxyma-derived coupling reagents includes motifs with varying degrees of electrophilic character, resulting in distinct acylation abilities and diverse applications.[25,26] Thus, uronium salts (e.g., **16–18**) contain a markedly reactive carbocation core, and stand out as the preferred choice when powerful activation is required.[25,26] Although the tetramethylamino ones, TOTU (**16**) and HOTU (**17**), were described as peptide coupling reagents in the early 1990s[16]—mimicking the structure of benzotriazolium oxide based uronium salts—they did not gain acceptance in assisting peptide bond formation, and suffered a similar fate to that of the parent Oxyma (**6**) at that time. More recently, the inclusion of morpholine as the proton acceptor moiety in the electron-deficient uronium fragment resulted in COMU® (**18**), with enhanced solubility and acylation potency.[21,27] Oxyma-based phosphonium salts have also been investigated, and found to be especially suited for slow couplings and cyclizations. The tetrafluoroborate (PyOxB, **19**) and hexafluorophosphate (PyOxP, **20**, commercially sold as PyOxim) versions have been designed to stabilize the positively charged phosphorus center.[22] Whereas PyOxB (**19**) has only recently been introduced, the synthesis and characterization of PyOxim (**20**) had already been reported by Hoffmann et al. in 2003, although it was inexplicably not tested at that time.[28] The influence of the counteranion is far from being trivial, since this feature has direct impact on the physical properties (solubility and hydrolytic stability) and, therefore, on the appropriateness of the salt as a cyclization-promoting reagent.[22] Further derivatization of the Oxyma building block with the arylsulfonyl group was accomplished in 2010 by Khattab, resulting in milder activating reagents.[29] The sulfonate esters containing the 2-naphthalene- or *para*-toluenesulfonyl moiety, NpsOXY (**14**) and TsOXY (**15**), exhibit their strongest performance at short preactivation times, thereby ensuring retention of configuration of nearby chiral centers.[29] A completely different aim is achieved with the development of Oxyma carbonates, which contain in their structures the acyl group that is to be transferred to the potential nucleophile.[17] Together with analogues featuring other relevant acidic oximes, Fmoc-Oxyma (**13**) stands out as a reliable reagent for introducing the Fmoc protecting group with minimal impact on oligomers.[17]

Oxyma (**6**) is simply, rapidly, and almost quantitatively accessed in one step from the active-methylene compound ethyl cyanoacetate (**21**) (**Scheme 1**).[16,17,21a,22,29] In this modified Meyer nitrosation, nitrous acid is generated in situ from the reaction of sodium nitrite with an organic

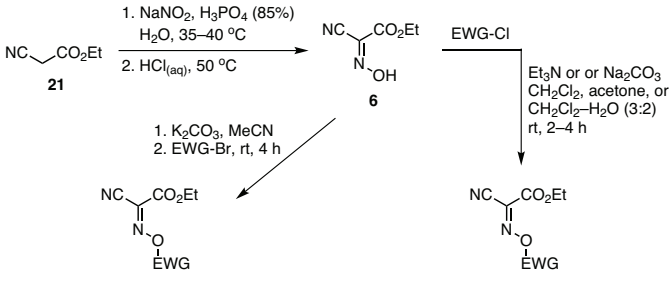
acid (acetic or phosphoric acid) at low temperature.[10e,19,30] Oxyma (**6**), isolated as a crystalline white solid, exists in the oxime form as shown by IR spectroscopic analysis.[10e,19,31] However, in the anionic state (salt), or in solution if the pH is strongly basic, the tautomeric nitroso form is prevalent.[10e,19] Standard solutions of Oxyma (**6**) in DMF or acetonitrile do not shift the equilibrium to the nitroso species, even after prolonged storage or under moderate heating.[32] Subsequent O-functionalization of Oxyma (**6**) with electron-withdrawing groups takes place in the presence of a mild base, and yields the corresponding derivatives in excellent yields and purities (see Scheme 1).[16,17,21a,29] It is worth noting that TOTU (**16**), HOTU (**17**), and COMU® (**18**) are obtained as the O-form isomer (uronium salts), which is more reactive than the typical benzotriazolic N-form (aminium salts).[21a,33] In the case of phosphonium salts PyOxB (**19**) and PyOxP (**20**), an innovative one-pot procedure was implemented, in which the potassium salt of Oxyma (**6**) is formed in situ and then the bromophosphonium salt is added, resulting in enhanced yields in comparison to the original Hoffmann approach.[22,28]

A remarkable feature of Oxyma (**6**)-based reagents is their approximately three-fold enhanced solubility in DMF when compared to their benzotriazole counterparts. This allows the preparation of more concentrated solutions to achieve greater acylation rates.[21,22,34,35] In this regard, not only does the oxime leaving group play an important role, but so does the nature of the electrophilic core.[27] Thus, the morpholine moiety within COMU® (**18**) is responsible for the 50% increased solubility in DMF in comparison to HOTU (**17**).[21,27] Phosphonium salts derived from Oxyma (**6**) also show a high solubility in DCM.[22] Moreover, the highly polar nature of Oxyma (**6**) influences the water solubility of its derivatives, which is essential to removing the coupling byproducts in solution-based acylations.[20b,21c] A risk assessment on Oxyma (**6**) and COMU® (**18**) by means of DSC and ARC calorimetric experiments proved that the likeliness of thermal runaways with these compounds was considerably lower than those with benzotriazole-based reagents, because they decompose in a controlled manner with a relatively low pressure release in contrast to the behavior of benzotriazoles.[20a,21a,36]

3. Assisting Peptide Bond Formation

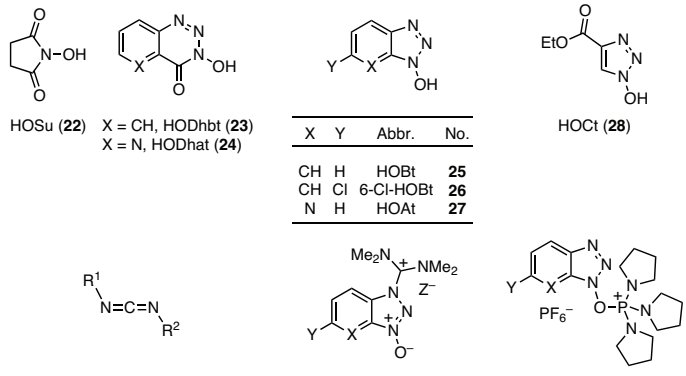
The field of peptide synthesis is continually integrating the latest advances in process technology.[37] One of the areas that have garnered considerable attention is the optimization of the chemical tools promoting amino acid assembly through the formation of an amide bond (referred to as peptide bond in this context).[25] Given the propensity of amino acids to undergo epimerization at the C_α position under strong acid activation, and the subsequent impact of the epimerization on the final purity of the target peptide, acid halides are commonly dismissed for this purpose—in contrast to their role in conventional amide-bond-formation methods.[25,38] Another drawback besets acid azides, the other class of possible acylation reagents, which have been associated with explosive incidents when dried.[25]

In the search for more balanced acylation strategies, several amino acid active esters have been introduced; these are mainly generated in situ by using a combination of carbodiimide and additive, or by utilizing standalone coupling reagents (**Figure 3**).[19,25] With the exception of electron-withdrawing phenols and triazines, the majority of active esters are based on *N*-hydroxylamines as leaving groups, such as triazoles, benzotriazines, succinimides, and especially benzotriazoles.[19,39] Although carbodiimides [DCC (**29**) and DIC (**30**) in the solid phase; and typically EDC (**31**) in solution] were originally proposed as sole acylation reagents in the coupling medium, the high levels of *N*-acylisourea and epimerized stereoisomers prompted the use of the abovementioned *N*-hydroxylamines as additives, with the aim of favoring the presence of *N*-hydroxylamine active esters in situ.[40] In this scenario, benzotriazoles have prevailed due to the versatility of their scaffold and their generally accessible prices. The pioneering work of König and Geiger in the early 1970s with the introduction of HOBt (**25**) as racemization-suppressing additive continued for a few years later with the implementation of HOAt (**27**, the most potent and expensive analogue) and 6-Cl-HOBt (**26**, analogue of medium reactivity and more accessible potency-to-cost ratio).[41–43] Although its reactivity is somewhat lower than that of HOBt (**25**), succinimide [HOSu (**22**)] has also attracted much interest because its water solubility allows the acylation to be performed in aqueous media.[44] Benzotriazines HODhbt (**23**) and HODhat (**24**) were initially well received because their performance approached that of HOAt (**27**); however, ring-opening side reactions compromised their continued use.[41a,45] Other



Abbr.	No.	Yield	Ref.
Fmoc-Oxyma	**13**	91%	*17*
NpsOXY	**14**	92%	*29*
TsOXY	**15**	86%	*29*
TOTU	**16**	44%	*16*
HOTU	**17**	82%	*21a*
COMU®	**18**	89%	*21a*

Scheme 1. Synthesis of Oxyma (**6**) and Derived Reagents.



X	Y	Abbr.	No.
CH	H	HOBt	**25**
CH	Cl	6-Cl-HOBt	**26**
N	H	HOAt	**27**

R^1	R^2	Abbr.	No.
Cy	Cy	DCC	**29**
i-Pr	*i*-Pr	DIC	**30**
Et	$(H_2C)_3NHMe_2^+ Cl^-$	EDC	**31**

X	Y	Z	Abbr.	No.
CH	H	PF_6	HBTU	**32**
CH	H	BF_4	TBTU	**33**
CH	Cl	PF_6	HCTU	**34**
CH	Cl	BF_4	TCTU	**35**
N	H	PF_6	HATU	**36**

X	Y	Abbr.	No.
CH	H	PyBOP®	**37**
N	H	PyAOP	**38**
CH	Cl	PyClock®	**39**

Figure 3. Most Relevant *N*-Hydroxylamines, Carbodiimides, and Onium Salts Employed in Peptide-Bond Formation.

templates, such as triazole HOCt (**28**), which was introduced by Jiang et al., have also shown promising performance.[46] Although they were part of a mild and reliable coupling methodology, carbodiimides have slowly been replaced by the more powerful onium (aminium/uronium and phosphonium) salts.[25] Those standalone coupling reagents contain an *N*-hydroxylamine moiety in their structure and, like the corresponding additives, the predominant ones have a benzotriazole core. Aminium salts HBTU/TBTU (**32**/**33**), HCTU/TCTU (**34**/**35**), and HATU (**36**) have been associated with demanding couplings; whereas phosphonium salts PyBOP® (**37**), PyAOP (**38**), and PyClock® (**39**) are preferred in slow-rate acylations.[42,47,48]

In spite of their reliability in routine peptide couplings, some of these highly reactive species still give rise to incomplete acylations in difficult sequences, detrimental side reactions, and/or safety concerns.[25,36] Particularly troublesome has been the consideration of benzotriazole-based reagents as Class 1 explosives, which has severely restricted their overseas transport.[36] Alternative, groundbreaking strategies for the assembly of demanding amino acids have been recently devised, such as isonitrile-mediated peptide bond formation (Danishefsky's group)[49] or umpolung stereoselective peptide synthesis (Johnston's lab).[50] However, the limited availability of the corresponding building blocks may hinder their wide adoption in the field.[49,50] The need for chemical tools that rapidly accomplish the elongation of the peptide chain in a safe and efficient manner has led our group to reevaluate Oxyma (**6**) and its derivatives for this purpose, and has led to the development of excellent alternatives to classical methods.

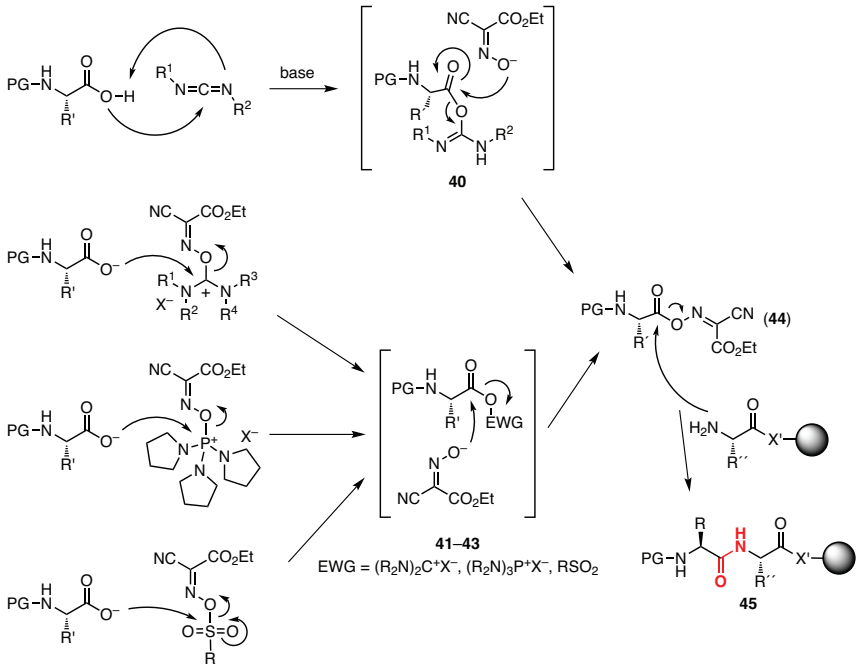
3.1. Manual Synthesis

3.1.1. Linear Couplings

Most of the coupling steps performed in a peptide synthesis laboratory are carried out in a linear fashion, anchoring the growing peptide chain onto a polymeric solid support.[51a] Therefore, the majority of experiments employing Oxyma (**6**), either as additive to carbodiimides or contained in standalone coupling reagents, have been performed as linear syntheses. The proposed mechanism of amino acid activation with Oxyma-based reagents and subsequent aminolysis is detailed in **Scheme 2**.[51b] It must be stressed that, regardless of the chosen coupling strategy, the active species that is ultimately generated in situ corresponds to the Oxyma amino acid ester **44** (if aminolysis does not occur in the precursor to **44**). In the case of carbodiimides, a strongly activated *O*-acylisourea intermediate (**40**) is primarily formed after deprotonation of the carboxylic acid by the carbodiimide and its attachment to the electron-deficient carbon center of the carbodiimide.[19,25] Reaction of Oxyma (**6**) with **40** converts it into the oxime active ester **44**.

A slightly different mechanism takes place with standalone coupling reagents, which, unlike carbodiimides, require the presence of base to initiate the acylation process.[25] Nucleophilic attack of the carboxylate anion onto the electrophilic center (carbon, phosphorus, or sulfur) generates a highly reactive species (**41**–**43**) which is not isolated. This reactive species undergoes acyl transfer with Oxyma (**6**), which is previously released as the leaving group in the first step of the process. In the last step, aminolysis of the Oxyma active ester (**44**) leads to the desired peptide **45**. In all cases, the driving force for the acylation is the highly stable byproduct (urea, phosphoramide, or sulfonate) released after reaction of the Oxyma anion with the first intermediate.[25] Although all strategies form the oxime active ester **44**, different types of reagent are associated with varying acylation rates and potencies, which consequently leads to the assumption that the formation of the Oxyma active ester **44** (i.e., the attachment of carboxylate to the reactive core of the Oxyma reagent) is rate-determining.

Epimerization is the most troublesome side reaction faced by



Scheme 2. Proposed Mechanism of Peptide-Bond Formation Using Various Oxyma (**6**)-Based Coupling Reagents. (Ref. 51b)

peptide chemists, which can take place through oxazolone formation or via enolization of the linear peptide.[14b,25d,52] Given that separation of epimerized byproducts from the target peptide by chromatographic methods is extremely challenging, the loss of chiral integrity has a direct impact on the final purity of the compound, which raises major concerns in the manufacture of bioactive peptides.[53] Indeed, the capacity of a coupling reagent to reduce the proportion of stereomutated analogues is a decisive factor in the activator selection process.[52,54] Usually, strong activation methods (such as those utilizing acyl halides) are avoided, and carbodiimide-based approaches are employed in combination with additives to balance the reactivity of the active species.[19,38,41a,b] The outstanding potential of Oxyma (**6**) to retain the optical purity of the activated amino acid had already been envisaged a few decades ago separately by Itoh and Izdebski.[13,14] First, Itoh reported full suppression of epimerization in the coupling of **Z-Gly-Phe-OH** to H-Gly-OEt by utilizing DCC–Oxyma, and a greater reduction of stereomutation in the assembly of **Ac-Ile-Gly-OEt**, when this coupling cocktail is employed than when HOBt (**25**) or HOSu (**22**) is used.[13] Shortly thereafter, Izdebski obtained moderate epimerization control with Oxyma when the Young peptide model was utilized.[14] However, the extraordinary ability of this oxime to minimize loss of chirality was not unambiguously proved until 2009, when our group invested efforts in examining in depth this remarkable feature of Oxyma and its derived coupling reagents, either in stepwise or segment couplings.[20b,21a,22,29]

3.1.1.1. [1 + 1] Couplings

Selection of an adequate peptide model is central to highlighting the relative ability of various reagents to promote retention of chirality. To that end, the activation of epimerization-prone α-phenylglycine (Phg) provides a suitable scenario for evaluating the performance of a coupling activator in stepwise peptide synthesis.[55] Thus, Oxyma-based activators have been evaluated in the coupling of Z-Phg-OH with H-Pro-NH_2 in solution to form **Z-Phg-Pro-NH_2**.[56] Remarkably, the extent of **Z-D-Phg-Pro-NH_2** formed using DIC (**30**)–Oxyma (**6**), COMU® (**18**), HOTU (**17**), PyOxB (**19**), or PyOxim (**20**) was maintained in all cases below 1%.[20a,21a,22]

Hence, the performance of each subfamily of Oxyma-derived reagents exceeds that of the corresponding benzotriazoles, even HOAt-based ones, which represents a hallmark in the field. Particularly impressive is the almost negligible stereomutation level obtained with Oxyma-containing uronium salts HOTU (**17**) and COMU® (**18**) (0.17% and 0.12%, respectively), which thus allow a substantially higher conservation of chirality than HBTU (**32**) and HATU (**36**) (8.2% and 3.1%, respectively).[21a] Moreover, uronium salt COMU® (**18**) affords **Z-Phg-Pro-NH_2** in 93% yield also when only 1 equivalent of base is employed, owing to its morpholine-based moiety.[21a,27] Although Oxyma-based phosphonium salts gave rise to slightly less optically pure crudes than HOTU (**17**) and COMU® (**18**), PyOxim (**20**) induced a two-fold higher retention of configuration than the tetrafluoroborate analogue PyOxB (**19**) (0.3% and 0.6% stereomutation, respectively)—still, both surpassed the performance of HOBt (**25**), 6-Cl-HOBt (**26**), and HOAt (**27**) (5.8%, 1.6%, and 2.2% stereomutation, respectively).[22] In a solid-phase approach to the same dipeptide, the notorious difference in epimerization reduction between PyOxim (**20**) or PyOxB (**19**) and benzotriazole-based phosphonium salts was even more pronounced.[22] Interestingly, both base-mediated coupling strategies (utilizing uronium and phosphonium salts) afforded less epimerized dipeptide than DIC (**30**)–Oxyma (**6**), which required a short preactivation to ensure full acid activation.[20a] Nevertheless, the content of DL stereoisomer when using Oxyma (**6**) as additive was much lower than in analogous syntheses using HOAt (**27**) and HOBt (**25**) (1.0% vs 3.3% and 9.3% stereomutation, respectively).[20a] In addition, the presence of Oxyma (**6**) in the coupling medium not only favors preservation of chirality in carbodiimide-based peptide bond formation, but also when combined to coupling reagents, such as in fluoroformamidinium salt TFFH, because the additive speeds up the transition to the less reactive active ester.[57] Thus, addition of Oxyma (**6**) to TFFH coupling mixtures promotes a huge reduction of epimerization (from 7.4% to 0.5%).[57]

Oxyma-based reagents continued to prove their great capacity to preserve optical configuration during the stepwise assembly of dipeptide **Z-Phe-Val-OMe**. Although less epimerization-prone than Z-Phg-OH, this model system allowed a clear visualization of the effectiveness of Oxyma-sulfonates NpsOXY (**14**) and TsOXY (**15**), and the tetramethyluronium salt HOTU (**17**).[29,58] NpsOXY (**14**) not only led to more effective control of epimerization than benzotriazole counterparts NpsOBt and NpsOAt (4.8% vs 12.9% and 6.7% stereomutation, respectively), it also afforded the dipeptide in considerably higher yields (92% vs 69% and 54%, respectively).[29] Moreover, the overall process was much faster with the Oxyma-derived reagent, because long preactivation times were avoided.[29] A similar scenario was followed with the tosyl analogue TsOXY (**15**), which slightly underperformed the HOBt analogue in the percentage of DL isomer present (2.0% vs 1.4%), but gave a better yield of the desired dipeptide (91% vs 52%).[29] An even more impressive performance was provided by HOTU (**17**) in the same reaction (using Z-Phe-OH), affording less than 1% of the dipeptide epimer.[58] The extraordinary retention of configuration observed with Oxyma-based reagents is evident when compared to that afforded by HOBt-based coupling systems [DCC (**29**)–HOBt (**25**), BOP, and TBTU (**33**)], which produced 39–44% of the epimer.[47c] NpsOXY (**14**) and TsOXY (**15**) also showed a great capacity to prevent loss of chirality and to give higher isolated yields than benzotriazole reagents in the stepwise assembly of **Z-Phe-Ala-OMe**, **Z-Val-Val-OMe**, and **Z-Val-Ala-OMe**.[29] Oxyma (**6**) has also been employed to reduce the extent of Cys isomerization in the on-resin elongation of **H-Gly-Cys-Phe-NH_2**, achieving epimerization suppression comparable to that of HOAt (**27**) (0.1% epimer with 5 min preactivation) and higher yields.[20a]

3.1.1.2. [2 + 1] Couplings

Loss of chiral integrity is particularly severe during activation of peptide fragments (even at the dipeptide stage), since the concentration of the epimerization-prone oxazolone intermediate is higher here than in the coupling of urethane-protected amino acids.[25] Therefore, the ability of Oxyma-based reagents to enhance the optical purity in segment couplings has also been examined. The activation of dipeptide **Z-Phe-Val-OH**, and its subsequent coupling with H-Pro-NH_2 to give **Z-Phe-Val-Pro-NH_2**, was employed as a model system, which gave rise to higher epimerization than the stepwise coupling.[59] In this type of coupling, the nature of the base plays an important role in the preservation of chirality of the activated dipeptide starting material (**Z-Phe-Val-OH**). For example, peptide crudes of higher optical purity were obtained with TMP (2,4,6-trimethylpyridine) than with DIEA:[21a,57] Employing 2 equiv of DIEA, COMU® (**18**) and HOTU (**17**) each led to a higher retention of configuration than HBTU (**32**), but both were less efficient than HATU (**36**) (19.3% and 23.6% vs 27.7% and 13.9% stereomutation, respectively).[21a] In contrast, when only 1 equiv of TMP was used, the level of stereomutation was uniformly decreased, with COMU® (**18**) producing the smallest percentage of

LDL epimer (3.5%) while maintaining yields over 90%.[21a] A similar level of control of optical purity was observed using PyOxim (**20**) and PyOxB (**19**) (5.7% and 5.3% stereomutation, respectively), which improved upon the epimerization degree obtained with PyBOP® (**37**) and PyClock® (**39**) (12.5% and 8.6% stereomutation, respectively).[22] Furthermore, the addition of Oxyma (**6**) to PyOxim (**20**) resulted in further reduction of the impact of epimerization to a level (3.4%) similar to that obtained with COMU® (**18**) and 1 equiv of TMP.[21a,22] A comparable performance was observed vis-à-vis the corresponding *N*-hydroxylamines, with Oxyma (**6**) performing at a level (3.8% stereomutation) close to that (2.1% stereomutation) of HOAt (**27**).[20a] In the assembly of **Z-Phg-Pro-NH$_2$**, the use of Oxyma (**6**) as additive to fluoroformamidinium salts reduces further the impact of the LDL epimer from 23% to 2.8%, thereby standing out as a promising low-epimerization approach.[57] Other fragment systems were investigated, such as the [2 + 1] assembly of tripeptides **Z-Gly-Phe-Ala-OMe**, **Z-Gly-Phe-Val-OMe**, and **Z-Gly-Val-Val-OMe**.[58] Using these peptide platforms, HOTU (**17**) achieved an extraordinarily low degree of DL epimer (<1%), in contrast to the poor retention of configuration induced by cyano-2-pyridyloxime- and triazine-based reagents (6–50% stereomutation).[58] In addition, PyOxim (**20**) showed a considerably greater conservation of chirality than PyBOP® (**37**), PyAOP (**38**), and PyClock® (**39**) in the [3 + 3] synthesis of **Z-Gly-Gly-Val-Pro-Gly-Gly-NH$_2$**.[22]

Coupling reagents based on Oxyma (**6**) stand out as the preferred acylating species for assembling sterically demanding sequences.[20,21a,22] Thus, Oxyma (**6**), COMU® (**18**), HOTU (**17**), and PyOxim (**20**) displayed an impressive performance in the coupling of *N*Me-amino acids and Aib residues.[20,21a,22] Remarkably, the acylation capacity of Oxyma-derived reagents often exceeds that of HOAt-based analogues, and the gap in the performance of both classes of activator increases as the steric hindrance of the amino acid increases.[20,21a,22] Steric interactions can account for this behavior given the rigidity of the benzotriazole core. An excellent platform to test the coupling efficiency of a given reagent is the Leu-enkephalin pentapeptide, an endogeneous hormone, modified at the two central Gly residues (**H-Tyr-AA-AA-Phe-Leu-NH$_2$**).[47c] In the on-resin elongation of the *N*Me-Gly analogue, Oxyma (**6**) afforded 91% of the pentapeptide with short, 5-min coupling times—a performance superior to that of HOBt (**25**) in the same reaction model.[20a] Oxyma (**6**) even surpassed the acylation abiliy of HOAt (**27**) in the synthesis of the *N*Me-Ala-enkephalin pentapeptide (79% vs 74% using 30-min coupling times),[20a] whereas in the assembly of the *N*Me-Leu derivative, Oxyma-based COMU® (**18**) performed at an intermediate level between HOAt- and HOBt-containing aminium salts.[21a] Taking advantage of their extraordinary capacity to assemble N-methylated residues, COMU® (**18**) and Oxyma (**6**) were recently combined and proved more efficient than the HATU (**36**)–HOAt (**27**) system in a recent linear sequence leading to an *N*Me-rich cyclic antitumor depsipeptide. Furthermore, the COMU® (**18**)–Oxyma (**6**) system was compatible with the activation of Alloc-based residues.[35]

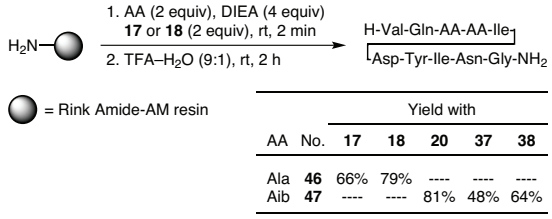
In light of the steric and conformational restrictions that an α,α-disubstituted amino acid residue such as Aib would introduce in peptide sequences, several Aib-containing peptide models have been employed to further investigate the capacity of Oxyma-based reagents to assist in the coupling of bulky junctions.[60,61] In particular, the elongation of the Aib-enkephalin pentapeptide has been broadly used to amplify differences in the performance of coupling reagents.[47a,62] Oxyma (**6**), COMU® (**18**), HOTU (**17**), and PyOxim (**20**) displayed an outstanding efficiency in the manual SPPS elongation of Aib-Aib-containing peptide **H-Tyr-Aib-Aib-Phe-Leu-NH$_2$**, often reaching percentages of the target product close to completion.[20,21a,22,57,63] In all cases, a clear superiority of Oxyma-based reagents over HOAt-, and HOBt-derived ones was observed. Using a carbodiimide approach, Oxyma (**6**) as additive rendered a much higher content of the target pentapeptide than HOBt (**25**) and HOAt (**27**), regardless of the coupling time applied (69% vs 19% and 55% of pentapeptide after 1-h double couplings).[20] The same trend was observed with the corresponding uronium salts, with HOTU (**17**) and COMU® (**18**) yielding an impressive 99.0% and 99.7% of the Aib-enkephalin pentapeptide [vs 83.0% and 47.0% for HATU (**36**) and HBTU (**32**), respectively].[21a] In addition, both Oxyma-based uronium salts accomplished conversions higher than 87% with substantially reduced protocols.[21a] Similarly, PyOxim (**20**) showed greater acylation capacity than PyBOP® (**37**), PyAOP (**38**), and PyClock® (**39**) in this Aib-Aib linear system (98% vs 49%, 85%, and 77% of **H-Tyr-Aib-Aib-Phe-Leu-NH$_2$** after 30-min double couplings).[22] Moreover, the addition of Oxyma (**6**) to other standalone coupling reagents enhanced their efficiency to a greater extent than HOBt (**25**) and HOAt (**27**) did.[57,63] Thus, the combination of TFFH and Oxyma (**6**) raised the percentage of the target Aib-peptide in comparison to TFFH alone (98% vs 95%).[57] A more dramatic increase in the yield of **H-Tyr-Aib-Aib-Phe-Leu-NH$_2$** was observed with triazine-based DFET by the inclusion of Oxyma (**6**) in the reaction mixture (94% vs 55%).[63] Recently, several cyanoacetamidooximes (**8–12**) showed promising performance in this peptide system, as replacements for HOSu (**22**).[64] Dimeric versions of this scaffold, 3,4- and 8,9-enkephalin decapeptides, have been employed to compare the performance of various phosphonium salt coupling reagents: PyOxim (**20**) produced the highest percentage (95–96%) of the desired peptide.[22]

Oxyma-based reagents have also been tested in the solid-phase elongation of ACP (65–74) decapeptide, a commonly established model platform, either in its naturally ocurring sequence (**46**) or as the Aib^{67}-Aib^{68} analogue (**47**) (**eq 1**).[21a,22,59,65] HOTU (**17**) and COMU® (**18**) were compared in the synthesis of the unmodified decapeptide, **46**, under a fast acylation protocol consisting of 2-min couplings.[21a] Under these conditions, COMU® (**18**) produced ACP (**46**) to a higher extent than HOTU (**17**) (79% vs 66%), demonstrating the enhanced reactivity of the dimethylmorpholino skeleton.[21a] In comparison to a previous synthesis carried out under identical coupling conditions, COMU® (**18**) led to a percentage of ACP (**46**) similar to that by HATU (**36**), but much higher than that by HBTU (**32**) (80% and 46% of **46**, respectively).[66] The low acylation extent obtained with phosphate DEPBT (6%) highlights the difficulty of this synthesis.[45b] The preparation of the Aib^{67}-Aib^{68} derivative (**47**) proved even more demanding, requiring longer coupling times to obtain a similar percentage of the desired peptide.[22] However, the Oxyma-based phosphonium salt, PyOxim (**20**), outperformed the benzotriazole analogues PyBOP® (**37**) and PyAOP (**38**) (81% vs 48 and 64%), giving rise to minimal amounts of des-Aib.[22]

The benefits of using Oxyma-based reagents also extend to solution-phase approaches, as has been proven in the previous discussion of epimerization systems.[20,21a,22,29] In 1991, Breipohl and König showed the suitability of Oxyma-based TOTU (**16**) and HOTU (**17**) for the preparation of various peptides in solution, such as **Fmoc-D-Hyp-Gly-O*t*Bu** and **Fmoc-Leu-Arg-Pro-azaGly-NH$_2$**, the latter in a [2 + 2] fashion.[16] Additionally, TOTU (**16**) was employed in the activation of aromatic acids, like benzophenone-4-carboxylic acid, and subsequent coupling to the ε-amino group of Lys.[16] One of the most remarkable advantages over benzotriazole-based reagents is the

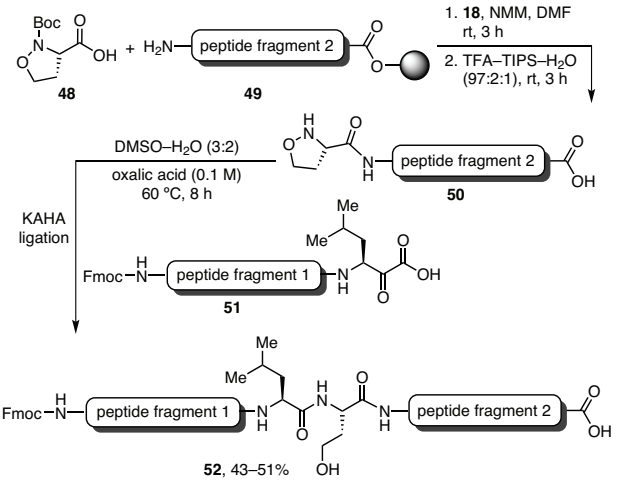
enhanced solubility of Oxyma-derived byproducts, which results in the preparation of more concentrated coupling mixtures and easier byproduct removal during workup.[21,22,34,35,67] The outstanding acylation potential of COMU® (**18**) and HOTU (**17**) vis-à-vis HATU (**36**) was highlighted in dipeptide models containing Val and Aib residues.[21a] Generally, Oxyma-based uronium salts showed faster acylation rates, better yields, and required less of the base during assembly of **Fmoc-Val-Val-NH$_2$** and **Z-Aib-Val-OMe**.[21]

The scope of applications of Oxyma-based reagents extends to Boc-based peptide synthesis.[57,68] An initial report by Khattab in 2010 confirmed the compatibility of Oxyma (**6**) with this protecting group, in a solution-phase approach to Leu-enkephalin pentapeptide using Boc-amino acids (except for the *N*-terminal Tyr residue).[57] Thus, TFFH–Oxyma (**6**) mediated couplings and TFA–DCM (1:1) deprotection cycles afforded the target peptide in high purity (98.3% by HPLC) and 69% yield.[57] Additionally, in a recently published work, Alloc- and Boc-protected γ-aminoproline residues were stepwise and alternately assembled in the solid phase by means of DIC (**30**)–Oxyma (**6**) couplings.[68c] However, in comparison with HOBt (**25**) and HOAt (**27**) based coupling reagents, the superiority of the Oxyma (**6**) template is not as evident here as it is in Fmoc-based peptide synthesis. Hence, Alewood and collaborators compared the performance of HBTU (**32**), HCTU (**34**), and COMU® (**18**) in Boc SPPS using different solid supports and fast coupling protocols.[68a] Although COMU® (**18**) performed better in PEG-based resins than in polystyrene ones, its coupling efficiency did not exceed that of HBTU (**32**) or HCTU (**34**) in any of the cases investigated.[68a] Bearing in mind that Boc-amino acids are much less bulky than the Fmoc analogues, the steric bulk of the coupling reagent (hypothetically favoring Oxyma) should not play a crucial role in the activation step. Finally, a groundbreaking ligation methodology for chemical protein synthesis, based on 5-oxaproline, has been described very recently.[68b] In the key coupling of the precious (*S*)-*N*-Boc-5-oxaproline (**48**) onto the solid-phase-attached peptide chain (**49**), COMU® (**18**) was preferred over HCTU (**34**) (**Scheme 3**).[68b] After cleavage from the resin, the oxaproline-peptide (**50**) was chemoselectively ligated with an α-keto acid fragment (**51**) to yield the target sequence (**52**). COMU® (**18**) was also employed in the Fmoc-SPSS of both peptide fragments, when difficult junctions required stronger activation than possible with HCTU (**34**).[68b]



AA	No.	Yield with 17	18	20	37	38
Ala	**46**	66%	79%	----	----	----
Aib	**47**	----	----	81%	48%	64%

eq 1 *(Ref. 21a,22)*



Scheme 3. COMU® (**18**) in the Implementation of Boc-protected KAHA Ligation. *(Ref. 68b)*

Additional examples of the benefits of using Oxyma-based reagents include: (i) Sawada and Gellman's solid-phase elongation of a γ-amino acid containing 14-mer peptide designed to resemble α-helix motifs.[69] (ii) The use of Oxyma (**6**) by Royo's and Feliu's groups to acylate constrained γ-aminoPro foldamers en route to a battery of dual antimicrobial peptide–cell-penetrating peptide antitumor compounds.[68c] (iii) The use of COMU® (**18**) to synthesize two bioactive cyclopeptides on Barlos's 2-chlorotrityl solid support,[70] which illustrates the full compatibility of Oxyma-based reagents with acid-sensitive resins.[35,71] (iv) COMU® (**18**) has been employed in an optimized protocol for the synthesis of a complement 5a antagonist cyclopeptide, active against Alzheimer's disease and sclerosis.[71] (v) The great solubility of Oxyma (**6**) in organic solvents has prompted its use in the evaluation of green alternatives to DMF.[32] Thus, employing DIC (**30**)–Oxyma (**6**) assisted couplings, the suitability of acetonitrile as solvent, in combination with PEG-based resins, was surveyed in the assembly of several peptides, including ACP (65–74) (**46**) and Leu-enkephalin.[72] The extent of epimerization during assembly of Fmoc-Phe-OH onto H-Leu-Rink-resin was kept at minimal levels using Oxyma (**6**) in this solvent.[72] A few decades ago, DIC (**30**)–Oxyma (**6**) assisted peptide couplings in THF were also reported.[13b]

3.1.2. Cyclizations

The markedly slow acylation rate in couplings leading to cyclic peptides determines their particular methodological requirements. Thus, the use of uronium salts to activate the carboxylic acid is dismissed in these cases, since guanidylation of the amino group occurs to a great extent as result of the notorious electrophilicity of the reagent's skeleton.[73] In contrast to linear couplings, peptide cyclizations are generally carried out in high dilution in order to avoid the formation of unwanted linear or cyclic dimers. To this end, phosphates (e.g., DEBPT), phosphoryl azides (e.g., DPPA), phosphonic acid anhydrides (e.g., T3P), carbodiimides, and phosphonium salts are the most convenient reagents.[47,74] Acylating agents based on the Oxyma scaffold (**6**)—among which DIC (**30**)–Oxyma (**6**) and the corresponding phosphonium salts **19** and **20** are worth noting—have also been sporadically used in peptide cyclization steps. As would be expected, uronium salt COMU® (**18**) is not the best suited for this type of coupling, since the *N*-terminal guanidylated peptide is obtained as the major if not only product.[22,75]

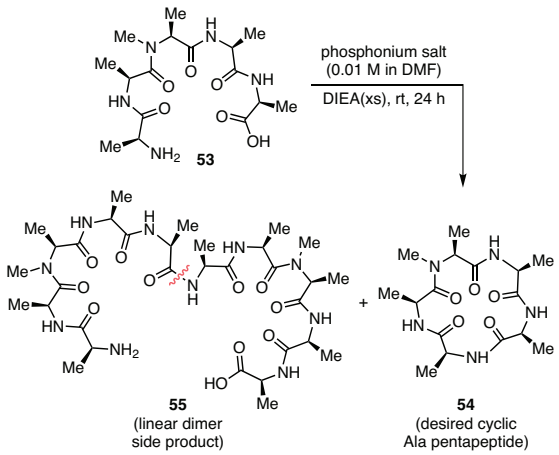
The Oxyma (**6**)-derived phosphonium salts PyOxB (**19**) and PyOxim (**20**) have been developed as safer alternatives to benzotriazole-based PyBOP® (**37**), PyAOP (**38**), and PyClock® (**39**).[22] PyOxB (**19**) and PyOxim (**20**) exhibit improved solubility, enhanced capacity to retain optical purity, and increased acylation potency in linear couplings over benzotriazole-containing reagents.[22] In cyclizations, however, hydrolytic stability of the coupling reagent is of utmost importance

given that the acylation rate is very slow and couplings usually take several hours to complete. Therefore, in view of the poor stability of the tetrafluoroborate salt PyOxB (**19**) (25% reagent left in DMF after 5 hours), the performance of this analogue in slow couplings is compromised.[22] On the other hand, PyOxim (**20**) shows higher stability in acetone and DMF than all benzotriazole counterparts, consequently standing out as a promising choice for cyclizations. To practically test the performance of PyOxB (**19**) and PyOxim (**20**) in cyclic couplings, the linear peptide (**53**) was mixed with the reagent and excess DIEA in DMF and, after 24 hours, the proportion of cyclic material (**54**) and linear dimer (**55**) was determined by HPLC (**eq 2**).[22,76] As envisaged, the level of cyclic peptide (**54**) in the PyOxB (**19**)-promoted reaction was one of the lowest, comparable to its level from the PyBOP® (**37**)-assisted synthesis (47% and 43% of **54**, respectively). In contrast, PyOxim (**20**) afforded the highest purity of **54** (70% vs 54% with PyAOP) among the phosphonium salts tested.[22]

In a recent publication, Hinou et al. considered the use of DIC (**30**)–Oxyma (**6**) in cyclizations performed in fluorinated solvents such as 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and 2,2,2-trifluoroethanol (TFE).[75] As a result of the hydrogen-bond acceptor nature of these solvents, the twin effects of enhanced reagent solubility and boosted cyclization rate were observed.[75,77] Thus, HFIP–DCM and TFE–DCM solvent systems were chiefly investigated during cyclization to the hexapeptide core (**57**) of an antifreeze glycopeptide at 10 mM concentration (**eq 3**).[78] For example, in TFE–DCM (1:1) as opposed to HFIP–DCM, Oxyma (**6**) was the most effective additive (lowest yield of intermolecular coupling product **59**), providing 84% of cyclic material **57** in 1 h, thereby surpassing the cyclization abilities of HOBt (**25**) and HOAt (**27**). The authors attributed these results to the favorable steric factors in the case of DIC (**30**)–Oxyma (**6**).[20a,21a,75]

3.2. Automated Conventional Synthesis

One of the advantages of the introduction of solid-phase synthesis in peptide research is the possibility of applying automated technologies, as result of the iterative coupling–deprotection cycles for peptide elongation. Nowadays, peptide synthesizers offer advanced protocols for allowing fast assembly of long sequences, which would be tedious in a manual approach.[54] Most of them are compatible with Boc- and Fmoc-SPPS, and some are designed to enable parallel synthesis or assistance by microwave or infrared heating. Taking into account that solutions of coupling reagents in DMF or NMP need to be functional for several hours during the automated assembly, hydrolytic stability of the reagents is pivotal to the success of the process. Therefore, the hydrolytic stability of Oxyma-based reagents will be discussed.



No.	Phos. Salt	54	55
20	PyOxim	70%	20%
39	PyClock®	61%	24%
38	PyAOP	54%	36%
19	PyOxB	47%	38%
37	PyBOP®	43%	29%

eq 2 *(Ref. 22)*

In 2006, an exhaustive study by Hachmann and Lebl of the performance of various coupling reagents in the automated preparation of difficult sequences concluded that carbodiimides were the most suitable reagents for use in peptide synthesizers, given their prolonged stability and the high purity of the final peptide.[54] With regard to Oxyma (**6**), our group has shown that its solutions in DMF, MeCN, and NMP are stable even at 40 ºC for at least 30 days.[32] Our group investigated the compatibility of the DIC (**30**)–Oxyma (**6**) coupling system with the ABI 433A peptide synthesizer, using a Fmoc/*t*-Bu strategy on a 0.1 mmol scale.[20a] ACP (65–74) decapeptide **46** was selected as peptide model, in view of its demanding sequence.[47a,60] For comparison purposes, 0.2 M DMF solutions of Oxyma (**6**), HOBt (**25**), and HOAt (**27**) were prepared, which allowed a clear visualization of their distinct acylation abilities.[20a] Oxyma (**6**) gave rise to almost 70% of the target decapeptide **46** and one of the lowest contents of des-Val deletion peptide (2.1%), thereby performing at an intermediate level between HOBt (**25**) and HOAt (**27**) (62% and 72% of **46**, respectively). It is worth noting that the efficiency of DIC (**30**)–Oxyma (**6**) is thus similar to that obtained a few years ago with more powerful onium salts in the same peptidic target, also conducted in an automated approach with tilted plate centrifugator.[20,54]

With respect to the use of onium salts with peptide robots, the stability of the reagent to hydrolysis in organic solution is an essential factor to consider. Unfortunately, questions have been raised[34,79] as to whether commercial samples of Oxyma-based uronium salts are as stable in solution in an open vial as the stability studies of the lab-synthesized versions had indicated.[21a] Recently, Behrentd and co-workers investigated the stability of various reagents in DMF-d_7 by means of ^{1}H NMR in open and closed vials.[34] These authors reported that, in an open vial, approximately 50% of COMU® (**18**) remained active after 24 hours and that there was only 14% of reagent left after 2 days.[34] With regard to TOTU (**16**), stability was improved in comparison to COMU® (**18**, 72% remaining after 1 day), but hydrolysis was still much faster than those of benzotriazole salts, which remained almost unaltered after 2 days. A similar trend could be observed in closed-vial experiments, with COMU® (**18**) being less stable than the rest of the uronium salts.[34] Under these conditions that mimick those of automated synthesizers, there was still 67% of COMU® (**18**) left after 2 days, which would result in efficient peptide assembly of most sequences.[34] Even more dramatic results were obtained in a similar HPLC-based study conducted by Jensen's group,[79a] who found that, whereas 95% of HBTU (**32**) remains in DMF solution after 2 days in an open vial, COMU® (**18**) is completely hydrolyzed after 5 hours. In other words, the half-life of COMU® (**18**) in DMF is 3 hours, whereas those of HBTU (**32**) and HATU (**36**) are a few days each.[79b] However, in closed containers, the percentage of COMU® (**18**) after 23 hours rises to 85%.[79a] These authors also showed that the stability of Boc-Ala-OH active esters generated with COMU® (**18**) is much lower than the one formed with HBTU (**32**).[79] In order to evaluate the effect of solvent purity on the large discrepancy between the results obtained

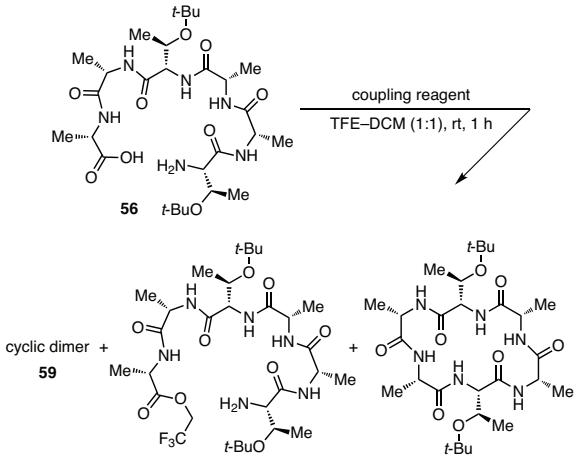
in our group and those obtained by others, a commercial sample of COMU® (**18**) was dissolved in DMF batches of varied purity and its content was checked by HPLC in a closed vial.[32] Although residual water in the solvent enhances the breakdown rate of the reagent, the most relevant factor is the presence of free amines in DMF, which can be removed by aspiration.[21c,32,79b] Nonetheless, the stability values are still far from those of our freshly synthesized material.[21] Traces of chloroformamidinium salt, morpholine, or impurities from the potassium salt of Oxyma (**6**) in the multikilogram-scale synthesis of COMU® are likely to be responsible for such distinct behavior from our samples. In contrast to COMU® (**18**), the stability of the Oxyma-based *phosphonium* salts (**19**, **20**) produced in our group was confirmed in the Behrentd evaluation.[22,34] Hence, PyOxim (**20**) was hydrolyzed in DMF to a similar extent as PyBOP® (**37**) in open vial after 24 hours.[34] Comparison in a closed vial showed that PyOxim (**20**) is slightly more stable than PyBOP® (**37**) (86% vs 81% remaining after 24 h).[34]

The reduced stability of some Oxyma-based onium salts has not decreased their efficiency in the assembly of demanding sequences in conventional peptide synthesizers (without heating or microwave irradiation).[34,80] TOTU (**16**), COMU® (**18**), and PyOxim (**20**) were tested in the manual assembly of the MeLeu-analogue of Leu-enkephalin pentapeptide (**H-Tyr-MeLeu-MeLeu-Phe-Leu-NH$_2$**) in an ABI 433A automated robot.[21a,34] Surprisingly, in spite of the high hydrolysis rates of COMU® (**18**) and TOTU (**16**) reported in the same communication, both were considerably more efficient than HOBt (**25**) and 6-Cl-HOBt (**26**), and were only surpassed by HATU (**36**).[34] PyOxim (**20**) afforded the target peptide in a yield (40%) comparable to those obtained with COMU® (**18**) and TOTU (**16**), and much higher than that achieved with PyBOP® (4%).[34] A recent publication by Chantell and colleagues supports the compatibility of Oxyma-based reagents with peptide synthesizers applying fast protocols.[80,81] COMU® (**18**) and PyOxim (**20**) were evaluated in the automated assembly of several demanding sequences; including ACP decapeptide (**46**), G-LHRH, GHRP-6, 9Pbw0, and linear oxytocin; in a SYMPHONY® robot with short, 2- and 20-min couplings.[80] COMU® (**18**) was particularly suited to perform fast peptide synthesis and afforded the highest purities, regardless of the peptide model tested.[80] On the other hand, PyOxim (**20**) had an acylation potency comparable to that of PyClock® (**39**) and PyBOP® (**37**) in 20-min couplings, although its activation rate was slower than those of other reagents.[80] For example, while PyOxim (**20**) gave rise to purities of 54% and 90% of hexapeptide GHRP-6 using 2- and 20-min couplings, respectively, COMU® (**18**) rendered 92% and 94% of GHRP-6, surpassing the efficiency of HATU (**36**) and HCTU (**34**).

3.3. Microwave-Assisted Synthesis

The implementation of microwave irradiation in peptide synthesis is a powerful tool to accelerate coupling and deprotection steps.[82] Since its introduction in the field by Wang and colleagues in 1991, microwave-assisted SPPS has evolved steadily, and, nowadays, the technique is commonly combined with automated synthesizers to rapidly achieve difficult syntheses in high yields.[82a,83] One of the most remarkable advantages offered by microwave irradiation is the precise control of the temperature during coupling and temporary-group removal. Furthermore, during the elongation of long or hydrophobic sequences, chain aggregation is avoided as a result of the nonthermal dipolar polarization effect,[84] even though thermal and nonthermal effects are still not easily distinguished.[85] Our group was interested in combining Oxyma-containing reagents, mainly Oxyma (**6**) and COMU® (**18**), and microwave-assisted peptide synthesizers in order to develop an ultimate synthetic methodology.[21b] In the original communication, we reported the low thermal stability of Oxyma (**6**) and COMU® (**18**), which is connected to their low decomposition onset,[20,21] and calorimetric measurements recommend keeping the coupling temperatures at values close to room temperature (≤74 ºC for Oxyma and ≤41 ºC for COMU®).[20,21] However, no safety incidents have been reported by our group or others when Oxyma (**6**) and COMU® (**18**) have been heated at 50–80 ºC in the process of assisting peptide coupling steps, consistent with the nonhazardous decomposition profile of both Oxyma-based reagents.[20,21,79,82a,86] In addition, the stability of Oxyma (**6**) to amine nucleophiles was tested under microwave irradiation at 80 ºC, and it was found that addition byproducts only arose when extreme conditions were employed.[20a] Based on these findings, we assessed the efficiency of COMU® (**18**) in a CEM® Liberty peptide synthesizer.[21b] In 6-min couplings at 80 ºC, COMU® (**18**) afforded an impressive amount (92%) of the demanding Aib-pentapeptide (**H-Tyr-Aib-Aib-Phe-Leu-NH$_2$**), showing greater efficiency in this system than HATU (**36**) and HBTU (**32**), which produced only 79% and 23% of the target peptide, respectively.[21b] After our initial report, other groups have reported exciting results from combining COMU® (**18**) with microwave irradiation.[79a,86a,b,e]

Jensen's group has extensively revised the compatibility of COMU® (**18**) with microwave-assisted automated synthesizers, in particular using a Syro *Wave*™ robot, in the assembly of sterically encumbered sequences,[79a,86a–d] such as the elongation of the Jung–Redemann sequence (**H-Trp-Phe-Thr-Thr-Leu-Ile-Ser-Thr-Ile-Met-NH$_2$**), a decapeptide based on the MuLV CTL epitope.[79a,86c,d,87] In a recent communication, the capacity of stock solutions of COMU® (**18**), HBTU (**32**), and HATU (**36**) to assist the assembly of the abovementioned decapeptide was analyzed.[79] Unfortunately, a solution of COMU® (**18**) in standard DMF stored 4 hours was unable



No.	Reagent	57	58	59
30	DIC	5%	89%	1%
30,27	DIC–HOAt	82%	3%	11%
30,25	DIC–HOBt	78%	10%	11%
30,6	DIC–Oxyma	85%	3%	6%

Yields of **57**–**59** were estimated from HPLC peak areas at 220 nm.

eq 3 *(Ref. 78)*

to produce the target peptide, although the use of anhydrous solvents raised the percentage to 39%.[79b] Benzotriazole-based reagents showed a consistent performance over time, although HATU (**36**), similarly to COMU® (**18**), rendered none of the decapeptide after 48 h of storage.[79b] However, employing fresh solutions, COMU® (**18**) was clearly superior to HBTU (**32**) and comparable to HATU (**36**) (56% vs 46% and 60% decapeptide, respectively), while DIC (**30**)–Oxyma (**6**) was the preferred coupling system (54–58% of decapeptide, regardless of reagent storage time).[79] According to the authors, the low hydrolytic stability of COMU® (**18**) can be solved in automated synthesizers by placing anhydrous solutions in closed vials.[79b] For example, after 24 hours of storage in closed vials, stock solutions of COMU® (**18**) afforded Leu-enkephalin pentapeptide **H-Tyr-Gly-Gly-Phe-Leu-NH$_2$** in good yields.[79b] The Jung–Redemann model decapeptide was further utilized to study the performance of several activators in microwave synthesizers, using HOBt (**25**)–HOAt (**27**) (4:1) to solubilize various onium salts.[86c,d] Once again, the acylation ability of COMU® (**18**) surpassed that of HOBt (**25**)-, and HOAt (**27**)-derived aminium and phosphonium salts, affording the Jung–Redemann decapeptide in 70% purity.[86c,d] The same group next checked the capacity of COMU® (**18**) to assist the assembly of *N*Me residues in a Syro *Wave*™ microwave synthesizer.[86a,b] Here, COMU® (**18**) was slightly less efficient than HATU (**36**)–HOAt (**27**) and DIC (**30**)–HOAt (**27**) systems (59% vs 75% and 76% respectively) in the solid-phase elongation of **H-MeAla-MeIle-MeGly-NH$_2$** tripeptide by means of 20-min couplings at 75 ºC.[86b] Nonetheless, in a separate communication, the authors reported that the coupling of Fmoc-Ala-OH onto the highly demanding **H-MeIle-Gly-Tyr-Gly-Gly-Phe-Leu-peptidyl** resin is preferrably conducted using COMU® (**18**) than any of the HOAt (**27**)-based systems (86% vs 62% and 76%, respectively).[86a] Following the same trend, COMU® (**18**) and DIC (**30**)–Oxyma (**6**) exhibited a higher acylation potency than HATU (**36**) in the challenging coupling of Fmoc-Aib-OH with resin-bound **H-Aib-Ile-Asp(O*t*-Bu)-Tyr(O*t*-Bu)-Ile-Asn(Trt)-Gly** (87% and 90% conversion vs 72% for HATU (**36**) after 20-min couplings at 75 ºC in the Syro *Wave*™).[79b]

Recently, COMU® (**18**) performed better than HBTU (**32**) in a microwave-mediated manual approach using a 2-chlorotrityl resin to prepare a linear RGD-based pentapeptide as a precursor to a cyclic $\alpha_v\beta_3$ integrin-specific targeting ligand that has potential applications in tumor imaging.[86e,88] Hence, Yamada, Shimizu, and co-workers obtained the target linear peptide in high purity and 84% isolated yield by means of 10-min couplings under controlled microwave heating at 50 ºC (**Scheme 4**), thus improving on the HBTU (**32**)-mediated original process (39% yield), which rendered incomplete couplings.[86e] Moreover, the authors applied the same COMU® (**18**)-based microwave methodology to assemble the N-methylated RGD-based cilengitide peptide.[89] In contrast to HATU (**36**), which required triple coupling reactions, COMU® (**18**) successfully produced the desired peptide with only double couplings.[86a]

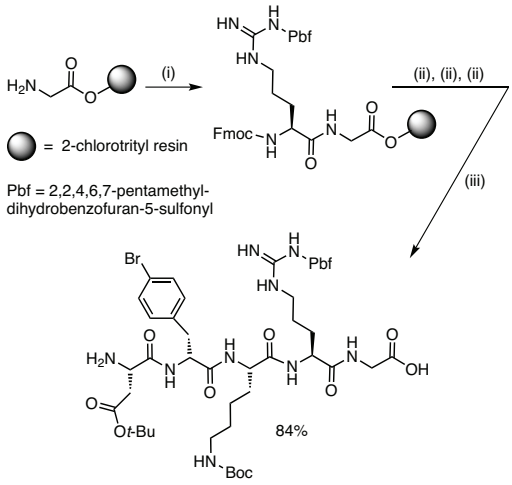
4. Assisting Amide Bond Formation

Besides its important role in biologically relevant molecules, such as proteins or heterocyclic natural products,[90] the amide bond is also of utmost importance in industry and, particularly, in the development of active pharmaceutical ingredients (APIs).[91] The most utilized nonenzymatic synthesis of amide bonds is the reaction between an acyl chloride and an amine; however, the strong reaction conditions employed are often not compatible with sensitive protecting groups.[92] Thus, alternative methods to form an amide bond under mild conditions have been pursued.[25b,d,93] Recently, new approaches have been reported that employ $AlMe_3$ to catalyze the reaction between an unactivated acid and an amine or ones that utilize fluorous Mukaiyama reagents at room temperature.[94]

In addition, acyl and aminyl radicals have recently been reported to generate amide bonds under oxidizing conditions, although aromatic or conjugated aldehyde precursors are required.[95] Alternative acylating strategies, recently described in the literature, consist of using carbonyldiimidazole (CDI) and pyridinium salts as starting materials, or utilizing acyltrifluoroborates and hydroxylamines in water to effect chemoselective amidation at room temperature.[96] Oxygen-containing precursors other than carboxylic acids, such as aldehydes or alcohols, have also been surveyed.[95,97] Transition-metal-mediated catalysis, achieving accurate chemoselectivity and regioselectivity, has also been described.[97b,c,98]

In a "green chemistry" approach, Nageswar and co-workers employed a bioglycerol-based recyclable carbon catalyst to obtain amides from aldehydes and hydroxylamine hydrochloride in good yields.[97a] To avoid the use of hazardous organic solvents, additional "green chemistry" methods have been developed for the acylation of the amino group: One example is a solvent-free CDI amidation that shortens reaction times and is suitable for Boc protection.[99] Another example is an elegant conversion of aldehydes into amides by using a copper catalyst in aqueous media.[97b]

While the preceding amidation strategies are valid and useful, coupling reagents that are traditionally employed in peptide synthesis have nevertheless been adopted in organic synthesis to effect the acylation of amino groups, given the mild reaction conditions and strong activation offered by these reagents. In recent years, Oxyma-based reagents, mainly Oxyma (**6**) and COMU® (**18**), have enjoyed great popularity in the organic chemistry community, which has applied their outstanding acylation capacity to establish new amide bonds in diverse chemical environments.

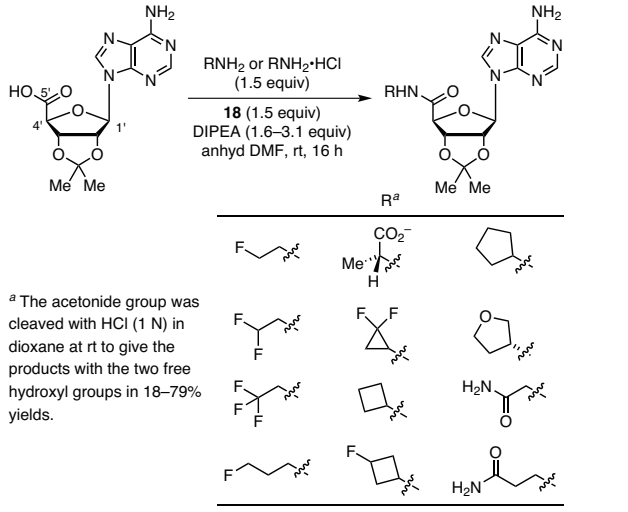


(i) Fmoc-Arg(Pbf)-OH (5 equiv), **18** (5 equiv), DIEA (10 equiv), DMF, μw, 50 ºC, 10 min. (ii) (a) piperidine (20% in DMF), μw, 50 ºC, 3 min; (b) Fmoc-Lys(Boc)-OH (5 equiv), **18** (5 equiv), DIEA (10 equiv), DMF, μw, 50 ºC, 10 min. (iii) (a) piperidine (20% in DMF), μw, 50 ºC, 3 min; (b) HOAc–TFE–DCM (1:1:4), rt, 1.5 h.

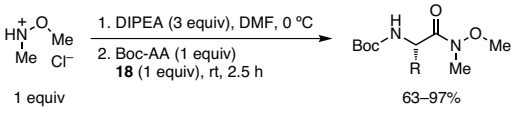
Scheme 4. COMU® (**18**)-Mediated Solid-Phase Synthesis of a Linear Integrin Ligand. *(Ref. 86e)*

4.1. Nucleoside-5′-carboxamide Synthesis

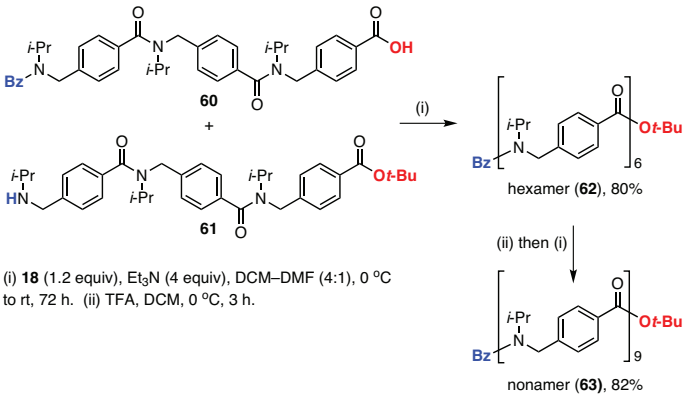
In a joint publication, Jacobson's and Katritch's groups disclosed that COMU® (**18**) is useful in the synthesis of several bioactive adenosine-5'-carboxamido analogues (**eq 4**),[100] which act as agonists to various subfamilies of Adenosine Receptors (ARs) in the submicromolar range. AR targeting is of utmost biological relevance since this receptor is regarded as a therapeutic target for cancer, cardiovascular, and neurodegenerative diseases among others, and some of its agonists are used in myocardial imaging.[101] Based on a rationally designed approach, the affinities of various 5'-carboxamido ligands (differing in the nature of the N-alkyl substitution) for adenosine receptors A_1, A_{2A}, and A_3 were calculated and the most promising ligands were chemically prepared.[100] Remarkably, the sensitive adenosine 5'-carboxylic acid starting material (containing a chiral C_α center) maintained its chiral integrity in this COMU® (**18**)-assisted acylation. Furthermore, COMU® (**18**) was compatible with halogenated, constrained, cyclic, and chiral amines; and promoted selective acylation in the presence of primary amides and free carboxylic acids.[100]



eq 4 *(Ref. 100)*



eq 5 *(Ref. 67)*



Scheme 5. COMU® (**18**)-Mediated Submonomer Approach to Oligoaryloamides in Solution. *(Ref. 104a)*

4.2. Weinreb Amide Synthesis

N-Methyl-*N*-methoxycarboxamides (commonly known as Weinreb amides) are a unique class of amides, which are synthetically appreciated for enabling the reduction of carboxyl groups to aldehydes or ketones.[102] Tyrrell's group has reported the application of COMU® (**18**) in the safe and efficient transformation of Boc-amino acids into the corresponding *C*-terminal Weinreb amides.[67] The amides were obtained in 63–97% yields by adding COMU® (**18**) and the Boc-amino acid to a DMF solution of the free *N*-methoxy-*N*-methylamine, generated in situ from the hydrochloride salt using excess DIPEA at low temperature (**eq 5**).[67]

COMU® (**18**)-mediated Weinreb amide synthesis offers several benefits. Firstly, the progress of the coupling reaction can be visually monitored by a color change from yellow to orange (pink to colorless using TMP).[21,67] Secondly, COMU® (**18**) effects fast acylation rates (complete conversion in less than 3 hours), exceeding the performance of triazine-based CDMT, which required longer coupling times in the synthesis of the Boc-alanine analogue.[67] Thirdly, the enhanced water solubility of COMU® byproducts allows the isolation of crudes that do not require further purification by column chromatography, as verified by NMR. Fourthly, COMU® (**18**) allowed the optical purity of the amino acid substrate to be preserved: only <1% of the epimeric product was detected by chiral HPLC, reaffirming the results obtained previously in our group.[21a,27,67]

4.3. Oligobenzamide Synthesis

Oligomeric N-alkylated aminomethyl benzamides (extended peptoids or arylopeptoids) form one of the least studied peptidomimetic templates, which, nevertheless, have great potential as foldamers.[103] Hjelmgaard et al. optimized the synthetic route towards *para*- and *meta*-arylopeptoids in solution and in the solid phase using Oxyma-based COMU® (**18**).[104] In the preliminary solution-phase studies, the authors introduced a novel submonomer approach, consisting in iterative acylation–substitution cycles employing isopropyl as model nitrogen side chain to assemble trimeric *para*- and *meta*-arylopeptoids starting from 4-(bromomethyl)benzoyl bromide.[104a] However, in order to form longer oligomers, a trimeric-fragment approach was envisaged, in analogy to peptide segment coupling (**Scheme 5**).[104a] COMU® (**18**) and HOTU (**17**) effected a faster acylation rate than HATU (**36**) and especially faster than PyBOP® (**37**), PyBroP®, DPPA, and DIC (**30**), with COMU® (**18**) forming the active species in less than 2 minutes.[104a] The nature of the solvent was highly relevant, with DCM–DMF and DCM–NMP mixtures being preferred over DCM alone.[104a] Using the optimized combination of solvents, COMU® (**18**) achieved 88–90% conversion in 24 hours, whereas HATU (**36**) rendered only 65–75% conversion.[104a] Thus, hexa- (**62**) and nonameric (**63**) *para*-arylopeptoids were assembled in 55–82% yields by employing COMU® (**18**)-mediated acylations in DCM–DMF (4:1).[104a] NMR experiments showed that the hexa- and nonameric arylopeptoids were predominantly in the cis form, especially as the steric hindrance of the side chain increased. Unfortunately, the difficult separation of dimethylmorpholinourea from the meta analogues led the authors to employ HATU (**36**) for the elongation of these meta analogues.[104a]

The preceding solution-phase approach was extended to the solid phase by utilizing 2-chlorotrityl or Rink amide-polystyrene resins.[104b] Following optimization of the previous solution-phase studies by building chloromethyl submonomer analogues, heterooligomeric *meta*- and *para*-arylopeptoids were elongated stepwise on-resin by means of COMU® (**18**) activation (**Scheme 6**).[104b] Once again, acylation was fastest with COMU® (**18**), which led to a two-fold, four-fold, and six-fold higher rate than with HOTU (**17**), HATU (**36**), and PyBroP®, respectively—the most prominent non-Oxyma reagents tested. Remarkably, meta isomers were successfully produced with COMU® (**18**) in the solid-phase technique, in contrast to the results observed in solution.[104] In the subsequent steps, monomeric units bearing isopropyl and 2-morpholinoethyl side chains were introduced to afford the corresponding dimer (**67**) and trimer (**68**). Iterative COMU® (**18**)-assisted acylation–substitution steps allowed the isolation of a dodecameric heteroarylopeptoid (**69**).[104b]

4.4. Amino Group Conjugation

One of the most recently surveyed applications of Oxyma (**6**) as acylating reagent is the functionalization of amino-bearing species such as particles with linkers, fluorophores, and biologically active entities, with implications in imaging, sensing, chemical biology, and drug delivery, to name a few.[68c,105–107] In particular, Bradley's group has taken advantage of the acylation potential of Oxyma (**6**) to modify amino-grafted micro- and nanoparticles, together with intrinsically fluorescent particles.[105] In their first communication, the authors disclosed a novel technique (zeta potential analysis) to monitor the extent of chemical modifications directly on-particle.[105a] With the aim of demonstrating the effectiveness of this technique that is based on the electric field generated by charged particles, aminomethyl submicron polystyrene beads were modified with compounds bearing anionic, cationic, or neutral moieties.[105a] Thus, aryl, alkyl, aminoacyl, and aminopoly(ethylene glycol) chains were attached to the amino group of microparticles using DIC (**30**)–Oxyma (**6**), thereby showing the great versatility of substrate acid activation (**Scheme 7**).[105a] Noteworthy in the assembly of the 6-hydroxycaprioic acid derivative is the selectivity between hydroxy and amino acylation that was achieved. As further proof of the compatibility of Oxyma (**6**) with microwave heating, a 10-min preactivated solution of the carboxylic acid was added to a suspension of the particles in DMF, followed by microwave irradiation at 60 ºC.[105a] The potency of this acylation methodology was demonstrated in the application of short, 20-min coupling times to efficiently obtain the altered particles. In addition, PEG-containing particles were subsequently modified with NTA-adipic acid linkers using Oxyma (**6**)-mediated couplings, in order to improve zeta potential measurements.[105a] Following this study, Bradley and colleagues developed fluorescent particles by copolymerization of polystyrene with diverse fluorescein-based units, a typical fluorescence imaging label.[105b,108] Once particles of different sizes were obtained, the aforementioned zeta potential technique was applied to characterize the extent of introduction of PEG units by means of DIC (**30**)–Oxyma (**6**)-mediated couplings at 60 ºC with microwave irradiation.[105b] In the same report, Oxyma (**6**) was responsible for the successful conjugation of aminopolystyrene beads with 5- or 6-fluorescein as a control experiment.[105b]

Recently, an additional example of the use of Oxyma (**6**) to assist in the labelling of amino groups with PEG linkers has been provided by Rosés et al.[68c] The aim of their research was to develop a repurposing approach to the CECMEL-11 antimicrobial undecapeptide, by testing the anticancer activity of a series of conjugates with a cell-penetrating peptide to enhance targeted cell delivery.[68c,109] The synthetic strategy consisted, first, of the solid-phase assembly of a hexapeptide γ-aminoproline foldamer featuring alternate N_α-alkyl chains and functioning as a cell-penetrating peptide (**70**). After removal of the N_γ-terminal Fmoc protecting group, Oxyma (**6**) was employed to introduce 8-Fmoc-amino-3,6-dioxaoctanoic acid (Fmoc-NH-PEG-CO_2H, **71**), proving its capacity to activate flexible carboxylic acids (**Scheme 8**).[68c] Finally, several antimicrobial peptides, chiefly differing in the nature of two residues, were stepwise assembled on the PEG-CPP-resin (**72**).[68c] The various antimicrobial-PEG-CPP conjugates (**73**) showed significant activity against MDA-MB-231 human breast tumor cells and specific delivery, in addition to low cytotoxicity, in normal human cells.[68c] In a similar labelling context, Della Ciana suggested the use of Oxyma-based COMU® (**18**) in the application of a novel approach to conjugate amino-containing bioactive compounds with biomarkers.[107]

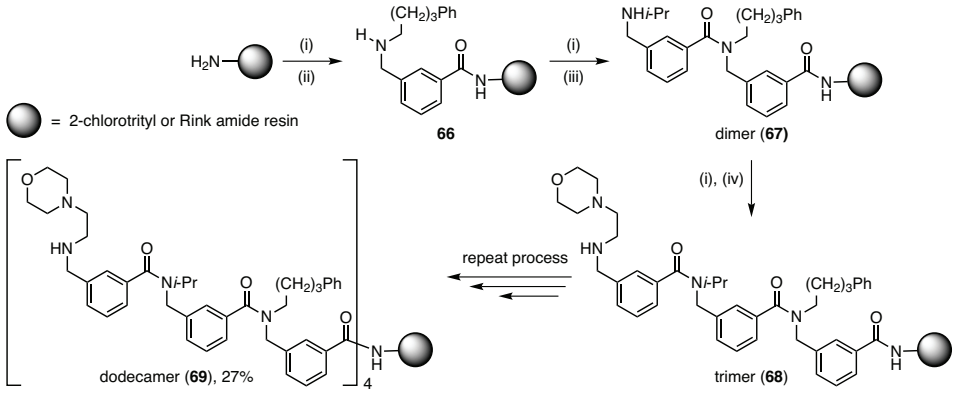
4.5. Other Amide Scaffolds

In contrast to aliphatic amines, anilines are strongly deactivated towards nucleophilic attack and, therefore, are challenging substrates for testing the acylation potency of a coupling reagent. In 2010, we examined COMU® (**18**), together with an analogue featuring isonitroso Meldrum's acid, in the acylation of *para*-chloroaniline with Z-Aib-OH.[110a] In spite of this highly demanding junction, combining a poorly reactive amine and a sterically encumbered acid, COMU® (**18**) rendered 89% of the anilide in only 3 hours and an impressive 98% after 1 day.[110a] The acylation rate and yield were much higher than those with the HOBt analogue and slightly superior to those with the HOAt derivative, although the isonitroso Meldrum's acid counterpart was the most reactive of all.[110a] Another example of aniline acylation was reported by Brandt and Blagg, who designed monoenomycins as simpler versions of the antitumor macrocycle trienomycin A that still retain its potent anticancer activity.[110b,111] The authors employed COMU® (**18**) to efficiently form the anilide intermediate selectively over the ester in 93% yield, showcasing another useful application COMU® (**18**) (**Scheme 9**).[110b]

Similarly to RGD (Arg-Gly-Asp) peptides, LDV (Leu-Asp-Val)-containing sequences specifically bind integrin receptors, in this case $\alpha_4\beta_1$, which is overexpressed in leukemia cells.[112] Recently, Oxyma-based activators were involved in the functionalization of LDV peptidomimetics with oligo(ethylene glycol) (OEG) linkers,[113] whereby carboxylic moieties were labelled with OEG by means of COMU® (**18**)-mediated acylations in moderate-to-high yields (50–80%).[113] Finally, COMU® (**18**) has been applied in the acylation of aromatic amines and anilines for *C*-terminal peptide modification.[114] *C*-Terminal amides are of biological interest, prompting several strategies to be devised for their attachment to the peptide chain.[115] The approach of Kwiatkowska et al. consists of the incorporation of Fmoc-Lys-OAllyl into 2-chlorotrityl through the side chain of lysine, followed by Boc protection.[114] After allyl removal, COMU® (**18**) is employed to attach the diverse amines to the *C*-terminal peptide chain.[114]

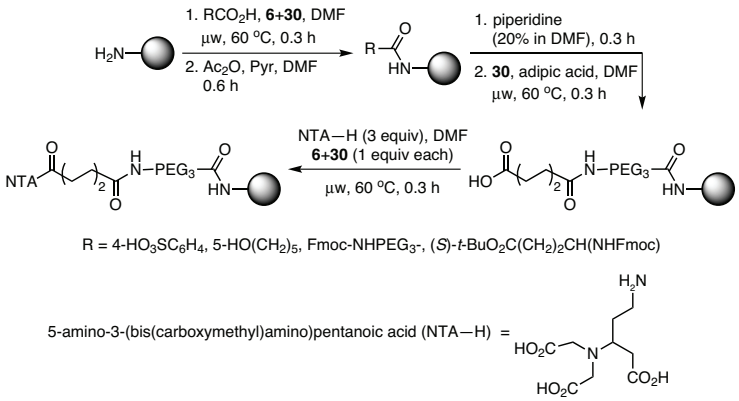
5. Assisting Ester Bond Formation

In contrast to the more nucleophilic amines, alcohols require an activating species of enhanced reactivity in order to undergo acylation, as demonstrated earlier by the fact that COMU® (**18**) preferably reacts with poorly reactive anilines rather than with primary alcohols, when both are present in the same structure.[110b] Additionally, alcohol acylation must compete with water or other protic solvents and,

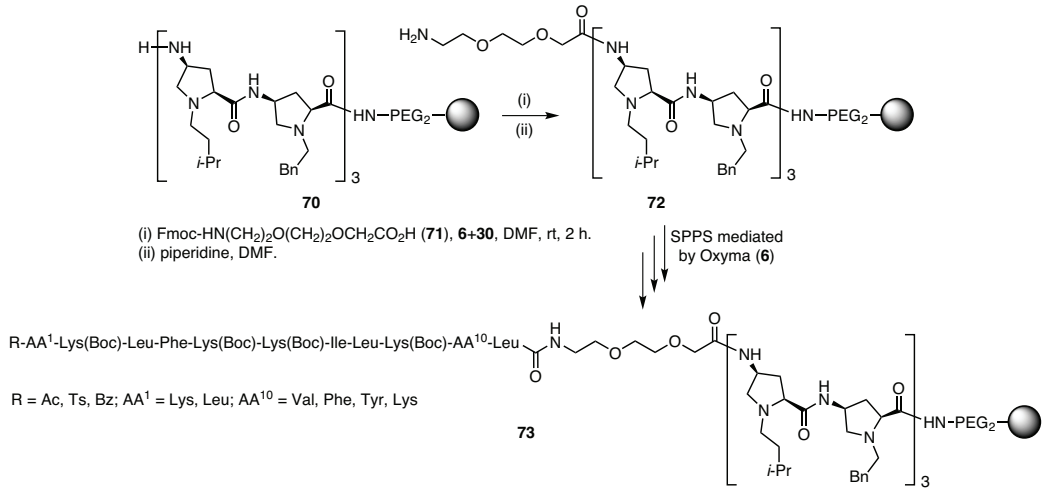


(i) 3-ClCH$_2$C$_6$H$_4$CO$_2$H (**64**) (3 equiv), **18** (3.5 equiv), DIPEA (7 equiv), NMP, rt, 0.3 h. (ii) Ph(CH$_2$)$_4$NH$_2$ (65, 20 equiv), DMSO, 50°C, 1 h. (iii) *i*-PrNH$_2$ (20 equiv), DMSO, 50 °C, 1 h. (iv) O(CH$_2$CH$_2$)$_2$N(CH$_2$)$_2$NH$_2$ (20 equiv), DMSO, 50 °C, 1 h.

Scheme 6. COMU® (**18**)-Mediated Approach to Heteroaryloamides on Solid Phase. *(Ref. 104b)*

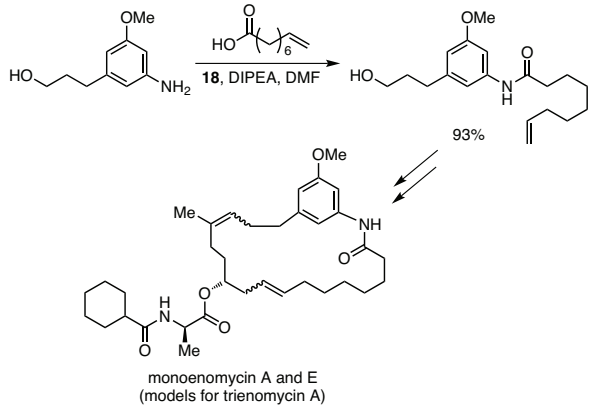


Scheme 7. Oxyma-Mediated Functionalization of Amino-Grafted Microparticles. *(Ref. 105a)*

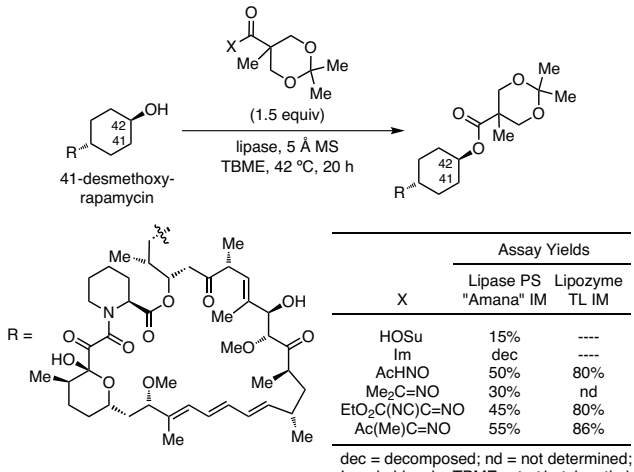


Scheme 8. Oxyma-Based Solid-Phase Synthesis of Various Antimicrobial-CPP Conjugates. *(Ref. 68c)*

consequently, a completely inert atmosphere needs to be set prior to esterification. In spite of the growing interest in aldehydes as substrates for esterification under oxidative conditions—either catalyzed by transition metals or by proline-derived organocatalysts—the best strategies for ester bond formation have focused on acyl transfer from an activated carboxylic acid.[116] A large number of traditional methods are available and generally employ harsh reaction conditions and/or lead to low regioselectivities, such as what happens in the Fischer esterification with acyl chlorides and anhydrides, or in the Bayer–Villiger oxidation.[117] However, when the alcohol to be acylated also contains an acid- or base-sensitive functionality, or is highly sterically hindered, the arsenal of suitable methodologies is reduced. Such substrates are more conveniently acylated by using Mitsunobu's conditions, Mukaiyama's 2-halopyridinium salts, CDI esters with Brønsted acid and/or pyridine catalysis, phosphate–carboxylate mixed anhydrides, or, more recently, hypervalent iodine(III) iodosodilactones in conjuction with DMAP and PPh_3.[118]



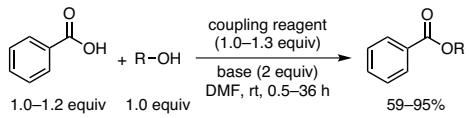
Scheme 9. Application of COMU® (**18**) in Anilide Formation en Route to Monoenomycin A and E. *(Ref. 110b)*



X	Assay Yields: Lipase PS "Amana" IM	Assay Yields: Lipozyme TL IM
HOSu	15%	----
Im	dec	----
AcHNO	50%	80%
Me_2C=NO	30%	nd
$EtO_2C(NC)C$=NO	45%	80%
Ac(Me)C=NO	55%	86%

dec = decomposed; nd = not determined; Im = imidazole; TBME = *tert*-butyl methyl ether.

eq 6 *(Ref. 120a)*



R = *i*-Pr, *t*-Bu, *c*-Pent, 4-$IC_6H_4O(CH_2)_2$
Base = DBU, DIPEA, MTBD
Coupling reagent = TATU, TBTU (**33**), COMU® (**18**)

eq 7 *(Ref. 120b)*

In recent years, coupling reagents commonly employed in peptide synthesis have been proposed as milder alternatives for ester bond formation, since most of them can be stored for several weeks, and acylations can usually be carried out at room temperature. In the past decade, aminium salts TBTU (**33**) and HBTU (**32**), together with HOBt (**25**), in combination with DCC (**29**)–DMAP, were investigated and found to perform satisfactorily.[119] In light of the previously described acylation results with Oxyma-based reagents in peptide bond formation, the recycling of these efficient activators in alcohol acylations stands out as a promising approach.[20–22] Indeed, in the short period of time since we first disclosed the implementation of this approach, other groups have also reported its use for efficient ester bond formation.[120] The esterification pathway with Oxyma-based reagents is very similar to the one depicted in Scheme 2 for the acylation of amides. As in the corresponding peptide bond formation, the carboxylate anion reacts with the electrophilic portion of the uronium salt, as in HOTU (**17**) or COMU® (**18**), resulting in an *O*-acylisourea intermediate which immediately undergoes nucleophilic attack by Oxyma (**6**) that is present in the medium. The Oxyma-actived ester is then subjected to alcoholysis by the substrate alcohol, giving rise to the desired ester bond and Oxyma (and urea) as byproducts.

5.1. Lypase-Catalyzed Acylation of Secondary Alcohols

A remarkable acylation strategy combining biocatalysts and *N*-hydroxylamine esters was reported by Storz and colleagues[120a] in 2010 as an alternative to the hazardous vinyl ester approach,[121] for the selective acylation of alcohols in stereochemically complex macrocycles. The authors proved that certain lipases are able to promote the esterification of secondary alcohols regioselectively in the presence of other secondary or tertiary alcohols,[120a] as in the extraordinarily difficult C42-esterificaction of 41-desmethoxy-rapamycin (a structural feature present in the members of the rapamycin family of polyketide macrocycles with the highest antitumor activity[122]) with a quaternary carboxylic acid (**eq 6**).[120a] Thus, in conjuction with *Burkholderia cepacia*, the Oxyma ester achieved a more efficient acylation of the C42 alcohol than the corresponding HOSu and imidazole esters. In comparison to the other oxime ester (55%) and hydroxamate (50%), the Oxyma ester slightly underperformed (45%) in combination with *Burkholderia cepacia*, although an impressive increase in yield (80%) was achieved by switching the biocatalyst to *Thermomyces lanuginosus*.[120a]

5.2. Selective Acylation of Tertiary Alcohols

Recently, a thorough study of the esterification ability of onium salts typically employed in peptide bond formation has been conducted by Twibanire and Grindley.[120b] The acylation of primary, secondary, and tertiary alcohols with various carboxylic acids at room temperature was screened with COMU® (**18**), TATU, and TBTU (**33**) (**eq 7**).[21a,120b] Using primary alcohols, benzotriazole derivatives displayed higher acylation efficiencies than COMU® (**18**), which required much longer esterification times.[120b] However, COMU® (**18**) achieved higher conversions than HOBt-based TBTU (**33**) when secondary alcohols,

such as isopropanol and cyclopentanol, were employed. The nature of the organic base exerted a great impact on the performance of the reagents, with the majority of the acylations being carried out in the presence of DBU, since esterification with DIEA did not occur using TATU and TBTU (**33**), in contrast to COMU® (**18**). However, only MTBD (7-methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene)[123] was capable of promoting the acylation of tertiary alcohols with COMU® (**18**), which did not react in the presence of DBU,[120b] while the highly reactive TATU showed no conversion even when employing MTBD. Furthermore, COMU® (**18**) could be used without preactivation of the carboxylic acid, in contrast to the 30-min preincubation time required by the benzotriazole derivatives. According to the authors, the outstanding potency of COMU® (**18**) in the acylation of tertiary alcohols is proof that its rate-determining step depends on the substrate, switching to the final alcoholysis of the Oxyma-active ester with the stronger base MTBD.[120b]

6. Introduction of Urethane-type Protecting Groups

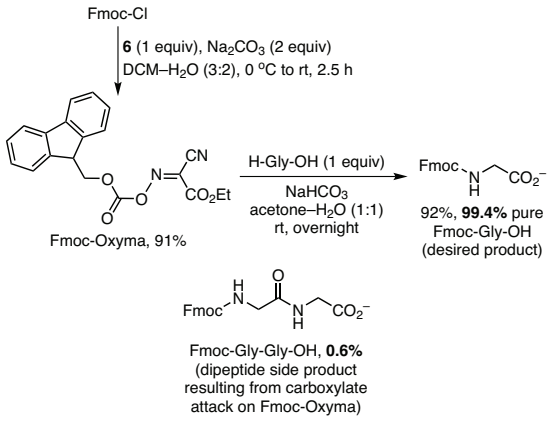
Oxime moieties have recently addressed fundamental issues in the introduction of amino acid blocking groups.[17,18] Orthogonal masking is essential in peptide chemistry and, therefore, several N-protecting groups have been developed such as Boc, Fmoc, Alloc, Z, or pNZ, which present a broad range of deprotection conditions.[124] Among these, Fmoc-based SPPS is slowly but firmly replacing Boc chemistry in virtue of its milder deprotection and peptide-cleavage conditions.[125] However, its introduction into amino acid building blocks is not without troublesome side reactions.[124] Although the use of the chloroformate (Fmoc-Cl) represents the most potent strategy, it often contaminates the Fmoc-amino acid with substantial amounts of oligomers as a result of its strong activation.[126] Moreover, many milder approaches, such as the use of the azidoformate (Fmoc-N_3) and *N*-hydroxysuccinimido carbonate (Fmoc-OSu) are scattered in the literature.[125,127] Although these Fmoc-introducing reagents succeed in controlling oligomer formation, they are explosion-prone or give rise to other side reactions (Lossen rearrangement).[128] Other approximations include Fmoc introduction with 2-MBT, 5-norbornene-2,3-dicarboximido, pentafluorophenyl, and HOBt carbonates; with symmetrical pyrocarbonates, or via in situ bis(trimethylsilylation) steps.[129]

In 1977, Itoh opened the way for the implementation of oximes as leaving groups in active carbonates for urethane-type protection.[15] In that early work, α-phenylcyanooxime (**2**) was proposed for Boc protection (Boc-ON), which still stands today as an alternative to Boc-anhydride and azidoformate.[15,130] Among several oxime templates tested, Boc-ON showed enough stability to be isolated in a crystalline form.[15] The oxime-based reagent was used to promote N_α-Boc protection of various amino acids including Arg, Cys, Ile, Met, Phg, Pro, and Trp in just 5 hours and 80–99% yields. Moreover, the corresponding oxime could be easily removed from the medium. In contrast to the azidoformate, Boc-ON allowed complete protection of glycine in 2 hours at room temperature, whereas the former required 20 hours at 40 ºC.[15]

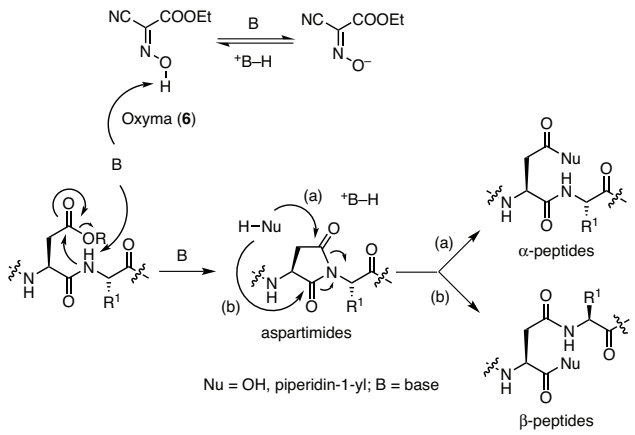
More than 30 years later, oximes were again included in carbonates for N_α-protection.[17] Various oxime templates (**1**, **2**, **6**) were considered for the construction of Fmoc-carbonates. In view of the extraordinary performance of Oxyma (**6**) in diverse acylation steps, its carbonate analogue was included in the designed set of reagents (**Scheme 10**).[17] Thus, Fmoc-Oxyma was successfully obtained in 91% yield after recrystallization, by reaction with Fmoc-chloroformate under Schotten–Bauman basic conditions.[17] Its performance was subsequently tested in the protection of H-Gly-OH, which stands out as the most challenging residue for Fmoc introduction, since its low steric hindrance promotes the presence of Fmoc-dipeptides.[127b,129a] Thus, Fmoc-Oxyma rendered Fmoc-Gly-OH in high yield (92.1%) and purity (99.4%) in the presence of $NaHCO_3$ at controlled pH,[17] and the level of Fmoc-dipeptide (**Fmoc-Gly-Gly-OH**) side product was minimal. An even lower impact of oligomerization was found in the crude obtained with cyano-2-pyridyloxime (**3**) (0.01%), as a result of its moderate acidity, which was also utilized to build an efficient Alloc-oxime carbonate.[17] An additional advantage of Fmoc-Oxyma was its facile removal during workup. Very recently, cyanoacetamido scaffolds (featuring unsubstituted or *N*-piperidinyl-, *N*-morpholinyl-, or *N*-ethyl-substituted amides) were surveyed for the introduction of Fmoc, given their simpler synthetic accessibility in comparison to the 2-pyridyl analogue.[18] The moderate activation of these oximes translated into minimal dipeptide formation (<0.17%).

7. Other Applications in Peptide Chemistry

The acidity of *N*-hydroxylamines, including oximes, is rarely high in the context of organic molecules.[19] In particular, the dissociation constant of Oxyma (**6**) ($pK_a = 4.60$) is comparable to that of acetic acid ($pK_a = 4.75$).[13a] Thus, apart from serving as an excellent leaving group in acylations, Oxyma (**6**) can be advantageous in other branches of peptide chemistry.[20b,131] Its acidic nature helps prevent the occurrence of base-catalyzed side reactions such as aspartimide formation and proline-derived overcoupling.[131] The undesired intramolecular cyclization of Asp residues leading to an aminosuccinyl-modified backbone (commonly referred to as aspartimides) is one of the most troublesome side reactions in peptide synthesis.[132] Although acid-catalyzed mechanisms are reported in the Boc-approach, aspartimide formation can be dramatic in Fmoc SPPS, since this side reaction takes place in every deprotection cycle once Asp is incorporated into the sequence.[133] Hence, the presence of base accelerates nucleophilic attack of the backbone amide of the Asp residue onto the β-carboxy ester side chain, resulting in an intermediate containing an aminosuccinyl moiety.[131,134] Attack of nucleophiles such as piperidine or water onto this intermediate aspartimide peptide results in the corresponding modified α- and β-peptides.[133c,135] In order to reduce the extent of this unwanted side reaction, efforts have been dedicated to enhancing the steric hindrance of the base or β-side-chain protecting group by introducing pseudoprolines or attaching



Scheme 10. Synthesis and Performance of Fmoc-Oxyma in the Protection of H-Gly-OH. *(Ref. 17)*



Scheme 11. Proposed Oxyma (**6**)-Mediated Mechanism of Inhibition of Undesirable Aspartimides and Derived Products. *(Ref. 131,134)*

amide backbone protectants.[132] Although some of these approaches completely suppress aspartimide and piperidide formation, they are difficult to implement in routine SPPS. A simpler and effective alternative consists in the addition of acidic *N*-hydroxylamines, such as HOBt (**25**), and polyhalogenated phenols to the Fmoc deprotection cocktail.[133b,135,136] Considering the successful implementation of Oxyma (**6**) in the plethora of coupling activators, we envisaged that this oxime could be valuable in reducing the impact of aspartimide formation. Indeed, we compared the behavior of Oxyma (**6**), HOBt (**25**), and HOAt (**27**) in a piperidine cocktail employed to remove Fmoc from resin-bound **Fmoc-Ala-Orn-Asp-Gly-Tyr-Ile-NH$_2$**.[131] This sequence is well suited to checking the performance of *N*-hydroxylamines, since it combines a strongly aspartimide-prone Asp-Gly junction and Asp-Gly-Tyr-Ile domain.[133a] After a double 6 + 6 hour treatment and cleavage from the resin, Oxyma (**6**) yielded the highest percentage of desired the α-peptide, independently of the concentration of additive in DMF.[131] The mechanim of action remains unknown, although the most accepted hypothesis is competition with the backbone amide for the base in the medium when Oxyma (**6**) is added.[133b]

In addition, Oxyma (**6**) has also been investigated in the prevention of amino acid overattachment caused by the basicity of proline (pK_a = 10.6), an unprecedented side reaction in peptide chemistry.[20b,131] As a result, we undertook the evaluation of proline-based overcoupling during solid-phase elongation of Pro-enkephalin (**H-Tyr-Pro-Pro-Phe-Leu-NH$_2$**), by comparing the effect induced by Oxyma (**6**) with those of HOBt (**25**) and HOAt (**27**).[131] The experimental approach consisted in mixing peptides presenting Pro at the *N*-terminus with the corresponding Fmoc-amino acid (Pro and Tyr) for 2 hours, followed by addition of carbodiimide and a 0.1 M solution of the *N*-hydroxylamine in DMF. All additives slightly increased the purity of the target Pro-enkephalin, with Oxyma (**6**) reducing almost completely the content of +Pro hexapeptide (0.39%).[131]

8. Conclusions

This review highlighted the increasing use of Oxyma (**6**) in peptide, amide, and ester chemistry, owing to its outstanding acylation potential vis-à-vis other activators in the coupling reaction. Although there is still room for improvement in certain of their aspects (i.e., uronium salt stability), Oxyma-based reagents are versatile acylating agents and stand out as cost-saving and efficient alternatives to benzotriazole-based reagents. In view of the great acceptance that Oxyma (**6**) has received in such a short period of time, further exciting applications will no doubt follow in the near future.

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