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K-Oxyma: a Strong Acylation-Promoting, 2-CTC Resin-Friendly Coupling Additive

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Here we present a new formulation of the recently introduced OxymaPure additive for peptide bond formation, in which the *N*-hydroxylamine group is replaced by a potassium salt. The complete suppression of its acidity converts K-Oxyma into the most suitable coupling choice when peptides are assembled on highly acid-labile solid-supports. The coupling efficiency and diminished prospects for epimerization are conserved relative to OxymaPure. In addition, K-Oxyma displays excellent solubility in a variety of organic solvents and undergoes safer decomposition than classical 1-hydroxybenzotriazole additives.

Introduction

Among existing peptide coupling processes, the carbodiimide-additive approach is regarded as a mild strategy for peptide synthesis.[1,2] This methodology allows the presence of base to stand as the peptide chemist's choice, depending on whether low epimerization or enhanced yields are the primary objective.[3] Even though acid activation is not as powerful as stand-alone coupling reagents, the carbodiimide approach is more reliable in automated synthesis.[4] Typically, *N*-hydroxylamine-based additives are added to the carbodiimide acylating cocktail.[1,5] The positive effect of additives is explained by the formation of a transient active ester that is slightly less reactive than previously formed intermediates yet reactive enough to successfully lead to the desired peptide bond with simultaneous reduction or even suppression of undesired *N*-acylation and epimerization.[1,2,6]

Historically, the benzotriazole family of additives prevailed over other templates.[1,2] The main scaffold, HOBt (**1**), was the first to be unveiled by König and Geiger in the 1970s, and some years later, the more potent analogues HOAt (**2**) and 6-Cl-HOBt (**3**) were added to the myriad of benzotriazolic additives to carbodiimides (Figure 1).[7–9] In 2009, a more thermally stable and, at least, equally efficient alternative was introduced after a number of previous unfortunate attempts a few decades ago, ethyl 2-cyano-2-hydroxyimino acetate (OxymaPure, **4**) (Figure 1).[10] This oxime-based scaffold has been employed to generate several

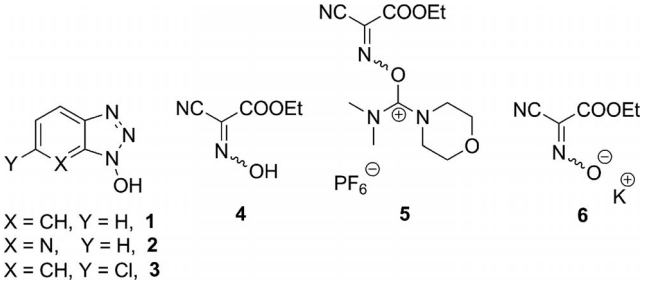

Figure 1. Structure of benzotriazole additives **1**–**3** and OxymaPure-based additives and coupling reagents **4**–**6**.

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derived stand-alone coupling reagents for peptide bond formation; examples include uronium salt COMU (**5**, Figure 1), which shows similar activity to HOAt-based counterparts.[11] Most of these reagents have reached the market at competitive prices, especially OxymaPure (**4**), which is easily and rapidly produced from ethyl cyanoacetate.[5,12] The impact of Oxyma-based coupling reagents has been so striking that in the following years analogues displaying greater suppression of epimerization have been designed by other research groups.[13,14]

Key to the performance of this cyanooxime (**4**) is its mild acidic character, comparable to that of HOBt (**1**, pK_a = 4.6). This property results in optimal leaving group ability during reactions such as substitutions; examples include amino acid acylations.[15] However, such acidity may lead to unwanted side reactions in certain scenarios. Thus, the compatibility of extremely acid-labile resins with *N*-hydroxylamine-type additives was not tested until our group determined that peptide cleavage does not occur in prolonged treatments.[16] Recently, a more in-depth study showed that peptide-resins were significantly more sensitive to HOAt (**2**) than HOBt (**1**) and OxymaPure (**4**), although all additives were found to effect premature peptide release.[17] Moreover, the relative stability of the peptide-resin depends upon the nature of the residue at the C-terminus and is much higher than resin-bound organic molecules.[17] Contrary to the effects produced by additives, when benzotriazole-based stand-alone coupling reagents were employed in conjunction with an excess of base no scission was observed.[17] This hypothesis (acidic cleavage due to *N*–OH moiety) was confirmed upon observation that the combination of COMU (**5**)/OxymaPure (**4**), which requires basic medium, does not cause premature cleavage from the resin.[18,19]

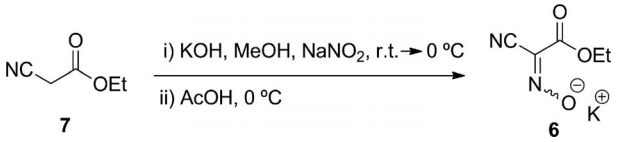
Thus, given the problematic acidity of *N*-hydroxylamines during solid-supported peptide elongations, our group sought to identify effective alternatives to the use of OxymaPure (**4**). We sought, however, to retain the highly reactive cyanooxime scaffold characteristic of **4**.

At a glance, the simplest proposal would be to use the oxime structure as an organic salt, therefore eliminating the source of acidity. In fact, the isolation and use of other oxime potassium salts had been previously reported by our group. The solid nature of such compounds simplifies the handling of such materials and helps to preserve their reactivity.[20] In the case of OxymaPure (**4**), there is no record of the use of the OxymaPure potassium salt as an additive to carbodiimide for peptide synthesis. However, its synthesis and isolation has been described in the past; the salt has been used to generate unique oxime metal complexes and also as building block for heterocycles.[21–23] In some recent patents in the field of peptide synthesis, the OxymaPure potassium salt (referred to as K-Oxyma, **6**, Figure 1) was merely employed as a precursor to OxymaPure-based onium salts but not as an additive to be used in conjuction with carbodiimides.[24,25] Herein we detail the synthesis, characterization, as well as the efficiency and thermal properties of K-Oxyma (**6**) as an acylation-assisting reagent in peptide bond formation.

Results and Discussion

Preparation and Properties

In a fashion similar to the production of OxymaPure (**4**), potassium salt derivative **6** is obtained in a direct reaction from commercially available ethyl cyanoacetate (**7**) (Scheme 1).[5,12] By means of a modified Meyer nitrosation in the presence of potassium hydroxide to generate the corresponding salt, followed by crystallization in acidic aqueous medium, K-Oxyma (**6**) is readily afforded in one-pot as a yellow-colored solid. The correct structure was confirmed by means of ^{1}H- and ^{13}C-NMR data which slightly differed from those of **4** in the chemical shift of the ethyl group (4.2 vs. 4.3 ppm respectively). The quantitative presence of potassium ion in **6** was identified by means of ICP, and also by IR spectroscopy, clearly depicting the absence of the broad *N*–OH band and the appearance of several other characteristic bands.[23] In addition, the melting point increase clearly indicated a change in the original *N*-OH oximic structure (127–129 °C vs. 150–153 °C).



Scheme 1. Synthetic access to K-Oxyma (**6**) from ethyl cyanoacetate (**7**).

One of the most celebrated features of **4** is its high solubility not only in organic solvents commonly employed in peptide synthesis, but also in aqueous environments, facilitating removal of excess reagents employed during solution-phase approaches.[10,26] Given the relevance of this issue to the overall performance of the reagent, a direct comparison between OxymaPure (**4**) and K-Oxyma (**6**) was conducted in a variety of solvents at different temperatures (Table 1). K-Oxyma (**6**) showed approximately a two-fold enhancement of solubility relative to OxymaPure (**4**) in polar aprotic solvents such as NMP, DMA and DMF at room temperature. In addition, **6** also displayed water solubility comparable to that of **4**, which was found to increase at 35 °C.

Table 1. Solubility of OxymaPure (**4**) and K-Oxyma (**6**) in various solvents at different temperatures.

Solvent	Additive	Temperature [°C]	Solubility [g/mL]
NMP	OxymaPure (**4**)	r.t	0.249
	K-Oxyma (**6**)	r.t	0.323
DMA	OxymaPure (**4**)	r.t	0.170
	K-Oxyma (**6**)	r.t	0.312
DMF	OxymaPure (**4**)	r.t	0.197
	K-Oxyma (**6**)	r.t	0.449
H_2O	OxymaPure (**4**)	r.t	0.046
		35	0.149
	K-Oxyma (**6**)	35	0.140
EtOH	OxymaPure (**4**)	r.t	<0.01
		35	0.032
	K-Oxyma (**6**)	50	0.022

On the other hand, only partial solubility of both oxime additives in EtOH was found at temperatures above room temp. (45–50 °C in the case of the potassium salt). Furthermore, K-Oxyma was also highly soluble in CH_3CN, and partially soluble in less polar solvents like DCM.

Epimerization Control

The retention of optical configuration during peptide assembly is a critical issue, especially in the preparation of bioactive peptides.[27,28] In that sense, one of the most remarkable features of OxymaPure (**4**) is the increase in product optical purity relative to products of chemistry involving HOBt (**1**) and even HOAt (**2**).[10,12] Usually peptide elongation takes place by sequential incorporation of *N*-terminal protected amino acids rather than convergence of peptide fragments. Therefore, in order to monitor epimerization induced by K-Oxyma (**6**), the sensitive stepwise model Z-Phg-Pro-NH_2 was selected (Table 2).[29,30]Various preactivation times and solvents were scattered with the aim of finding the optimal epimerization-free coupling conditions, when used in conjunction with EDC·HCl at 0 °C.

Table 2. Impact of epimerization during solution-phase coupling of Z-Phg-OH to H-Pro-NH_2 using K-Oxyma in various solvents and preactivation times.

Additive	Solvent	Preactivation time [min]	Yield [%]	DL [%]	Z-Phg-OH [%]
	DMF	–	88	1.2	–
	DCM	–	85	0.8	5.3
K-Oxyma (**6**)	DMF/DCM 1:1	–	88	1.3	0.5
	DMF	3	89	20.5	–
	DCM	3	87	45.4	–
HOBt (**1**)[a,b]	DMF	–	82	9.3	–
HOAt (**2**)[a,b]	DMF	–	81	3.3	–
Oxyma (**4**)[a,b]	DMF	–	90	1.0	0.5
	DMF	2	88	1.1	–

[a] Data extracted from ref. 10. [b] DIC was used as carbodiimide in a solid-phase approach.

Noteworthy, K-Oxyma (**6**) was able to maintain the degree of epimerization in this model at values close to 1%, regardless of solvent used. In comparison, HOBt (**1**) and HOAt (**2**) produced 3 and 9% of the undesired DL epimer during generation of the same dipeptide. However, whereas the use of DMF as solvent rendered full conversion, the use of DCM led to small amounts of initial unreacted acid as a result of the low solubility of K-Oxyma (**6**) in this solvent. The inclusion of DMF in the solvent mixture partially solved this issue of remaining starting materials (Table 2).

Nonetheless, the most dramatic effect was observed when employing short 3-min preactivation times. Under these conditions, even though no starting acid was detected with any of the solvents employed, the content of DL epimer greatly increased to unacceptable levels. This could be due to the mild basic nature of the oximate anion.[15] Particularly severe was the scenario of attempted coupling in DCM (45.4% epimerization). The low solubility of **6** in DCM appears to promote slow conversion to the active ester, which seriously compromises optical integrity. Therefore, despite its powerful epimerization-reduction capacity, K-Oxyma (**6**) must still be used with caution to avoid potentially detrimental preactivation events.

Coupling Efficiency and Peptide-CTC Resin Stability

Once the ability of **6** to reduce epimerization had been analyzed, the next step was to evaluate acylation potency in the assembly of difficult sequences. Since one of the most relevant anticipated features of **6** is its compatibility with acid-sensitive solid supports after prolonged coupling steps, 2-chlorotritylchloride (CTC) resin, known to allow generation of protected peptide acids, was selected to evaluate the efficiency of **6** in solid-phase peptide synthesis.[31]

On a first attempt, the relative performance of **4** and **6** was determined in the solid-phase assembly of myelin basic protein (MBP) 104–118 (H-Gly-Lys-Gly-Arg-Gly-Leu-Ser-Leu-Ser-Arg-Phe-Ser-Trp-Gly-Ala-OH).[32] Once the first residue (Fmoc-Ala-OH) was incorporated into the solid-support, the Ala-CTC resin was partitioned into two separate syringe reactors, differing only in coupling additive (**4** vs. **6**). After peptide cleavage from the resin, a considerable difference in weight was observed (Table 3). The use of K-Oxyma (**6**) afforded the target peptide in 90% yield. Conversely, the coupling reaction containing *N*-OH additive **4** rendered only 63% of the desired compound. Given that both compounds share the same scaffold, it is clear that the absence of the acidic *N*-OH moiety beneficial; its removal helps to avoid premature release from the resin. Furthermore, the purity of the isolated material was also slightly higher when using potassium salt **6** versus its acid **4** (91 vs. 88%).

Table 3. Coupling efficiency of K-Oxyma in the preparation of difficult sequences and stability of peptide-resins.

Sequence	Additive	Yield [%]	Purity [%]
MBP (104–118)	OxymaPure (**4**)	63	88
	K-Oxyma (**6**)	90	91
Cyclo (Arg-Gly-Asp-DPhe-Cys)	K-Oxyma (**6**)	68	95
Aib-ACP	K-Oxyma (**6**)	75	84

The peptide bond-assisting capacity of K-Oxyma (**6**) was further tested in the elongation of two difficult sequences: *cyclo*-(Arg-Gly-Asp-DPhe-Cys) and Aib-ACP. The first of these models contains the integrin-specific RGD motif, broadly used in anticancer therapy.[33] Using 90-min couplings with DIC/**6**, the cyclic RGD peptide was rendered in high purity and in nearly 70% yield (Table 3). Similarly, the Aib^{67}, Aib^{68}-modified ACP decapeptide is a demanding sequence, given the steric hindrance induced by this α,α-disubstituted residue.[34] In spite of this difficulty, K-Oxyma (**6**) afforded the desired decapeptide in 75% yield and with

84% purity. In solution-phase, K-Oxyma (**6**) also exhibited its great acylation potency in the assembly of sterically demanding dipeptide Z-Aib-Val-OMe, affording a single peak with the desired mass in only 5 h.

Thermal Stability

Since benzotriazole-based additives have been reported as potentially explosive, we decided to address the thermal safety of K-Oxyma (**6**) by comparing its thermal properties with those of HOBt (**1**) HOAt (**2**), and OxymaPure (**4**), as had been recently reported by some of us.[10,35,36] Thus, the thermal stability of K-Oxyma (**6**) was studied both by DSC and Adiabatic calorimetry (ARC).[37,38]

Differential Scanning Calorimetry (DSC) experiments were carried out for K-Oxyma (**6**) and OxymaPure (**4**) in closed crucibles under an N_2 atmosphere, from 30 to 300 °C and at a heating rate of 10 °C/min. Both compounds showed similar thermal profiles consisting of a melting process accompanied by slow decomposition. However, K-Oxyma (**6**) showed a slightly higher thermal stability than OxymaPure (**4**) since its melting point was found to be 15 °C higher (145 °C and 130 °C respectively) while possessing a similar enthalpy of decomposition (543 J/g and 531 J/g respectively) (Figure 2). For both **4** and **6** the thermal profiles were found to be safer than those of HOBt (**1**) and HOAt (**2**); both **1** and **2** show much faster and intense decomposition processes.

Adiabatic calorimetry was also carried out to compare the stability of the compounds under study in adiabatic conditions.[38,39] In particular, pressure rise associated with decomposition can be measured to assess the safety of different samples when a thermal runaway is produced. We decided to apply the ARC technique with the "heat–wait–seek" method to solutions of K-Oxyma (**6**), HOBt (**1**), HOAt (**2**) and OxymaPure (**4**) at the same concentration in dimethylformamide, a typical solvent used in peptide coupling reactions. No significant exotherms were detected at the range of temperatures studied (30 to 300 °C). However, the pressure rises observed for K-Oxyma (**6**) and OxymaPure (**4**) are markedly lower (51 and 48 bar, respectively) than those of HOBt (**1**) and HOAt (**2**) (84 and 122 bar, respectively) under the same experimental conditions (Figure 3). This combination of DSC and ARC assays confirm that **6** is a much safer additive from a thermal point of view than benzotriazoles and that it shows thermal behaviour similar to its precursor **4**.

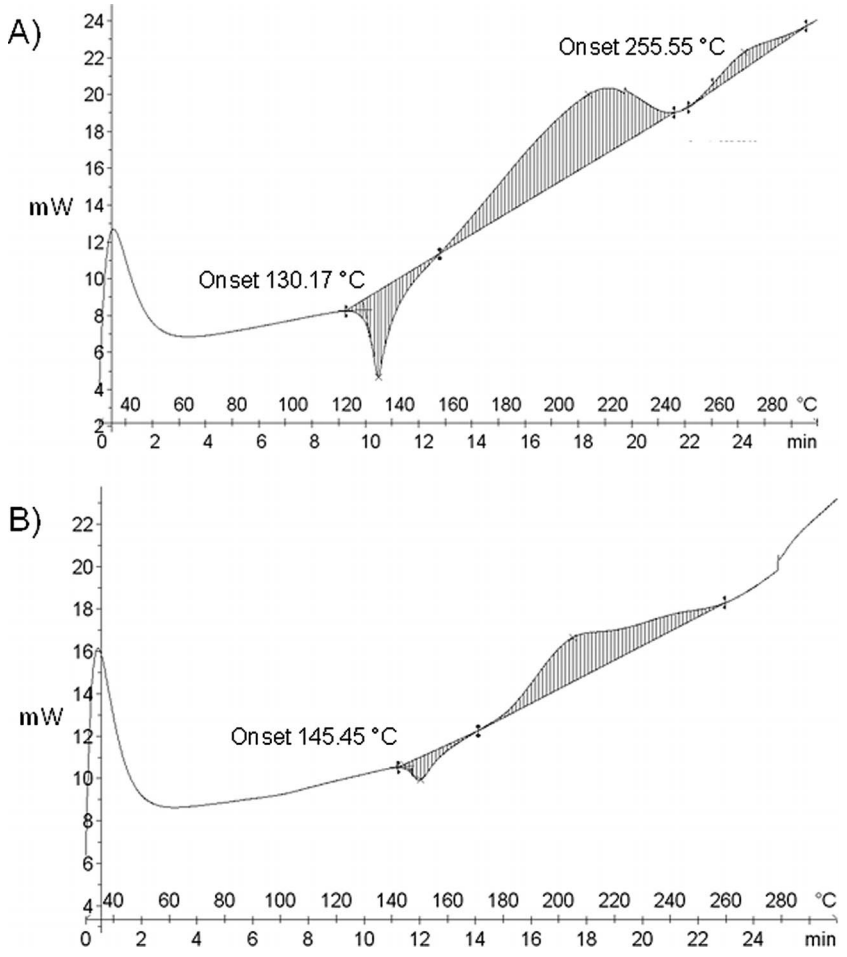


Figure 2. Thermograms showing heat flow vs. temperature and time for DSC experiments with A) OxymaPure, and B) K-Oxyma.

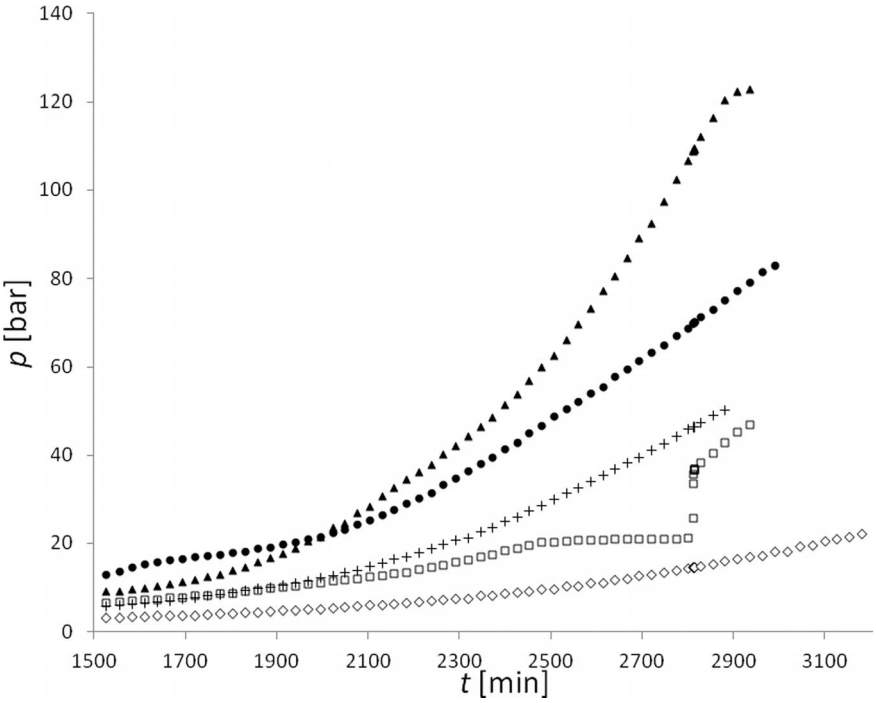


Figure 3. Decomposition profiles of 0.2 M solutions of HOBt (●), HOAt (▲), OxymaPure (□) and K-Oxyma (+) in DMF, together with the solvent (◇) and showing released pressure (bar) as a function of time (min) during ARC experiments.

Conclusions

In a netshell, a potent additive for use in peptide bond formation based in the successful OxymaPure scaffold has been prepared and tested. Although it conserves the acylation ability of OxymaPure, potassium salt **6** prevents premature cleavage of peptide-resins from extremely acid-labile solid supports, such as the widely known CTC resin. Furthermore, the solubility of the additive in organic solvents is enhanced relative to the parent *N*-OH compound (**4**), which is of great importance in the preparation of coupling mixtures. Similar to oxime **4**, K-Oxyma **6** shows higher thermal stability and a lower pressure release than parent benzotriazoles HOBt and HOAt. Consequently, the likeliness of an explosive event is substantially lower with **6** (and **4**) relative to the benzotriazoles in question. Consequently, K-Oxyma **6** represents a preferred coupling alternative to **4** and appears to be the best coupling additive choice when using acid-sensitive solid supports.

Experimental Section

Materials and General Methods: The solvents used were of HPLC reagent grade. Melting points were determined with a Buchi B540 apparatus and are uncorrected. Infrared (IR) spectra were recorded using a Perkin–Elmer 1600 series Fourier transform instrument as KBr pellets. Nuclear Magnetic resonance spectra (^{1}H- and ^{13}C-NMR spectra) were recorded with a Mercury 400 MHz spectrometer with chemical shift values reported in δ units (ppm) relative to an internal standard. Follow-up of the reactions and checks of the purity of the compounds were done by TLC on silica gel-protected aluminum sheets (Type 60 GF254, Merck) and the spots were detected by UV light at (λ = 254) nm for a few seconds. HPLC separation was performed with an instrument consisting of a Waters 600 controller, a Waters 600 pump, and a Waters 2487 dual λ absorbance detector. Samples for HRMS determination, either K-Oxyma or model peptides, were prepared by dissolving the compound in 100 μL of CH_3CN followed by 1:100 dilution in H_2O/CH_3CN (1:1). Analyses were determined with a LTQ-FT Ultra mass spectrometer from Thermo Scientific by introducing the sample by direct infusion into a NanoESI chip (nanoESI positive ionization, 1.7 kV capillary voltage, 44 V capillary voltage, 200 °C capillary temperature, 100–1500 *m*/*z*). Data were acquired with Xcalibur software, vs.2.0SR2 (ThermoScientific). Elemental compositions from experimental exact mass monoisotopic values were obtained with a dedicated algorithm integrated in Xcalibur software (vs. 2.0SR2).

Method for Preparation of K-Oxyma: A solution of potassium hydroxide (5 g, 0.089 mol) in ethanol (30 mL) was poured into ethyl cyanoacetate (50 mL, 0.47 mol) under nitrogen. Then, sodium nitrite (40 g, 0.58 mol) was added to the reaction mixture, and this was stirred about 10–15 min at room temp. After cooling the reaction mass to 0–5 °C, acetic acid was slowly added and the mixture was stirred at 0–5 °C for 1–2 h. The crystallized solid was filtered to afford ethyl cyanoglyoxylate-2-oxime potassium salt (K-Oxyma) as a bright yellow powder (30 g, 0.17 mol, 35% yield) (m.p. 150–153 °C). Moisture content according to KF: 0.4%. Purity of 99.19% according to HPLC, with relative absorption max. at 235 nm, using a XBridge BEH C18 column, 3.5 μm, 4.6 × 100 mm, with 5–100% gradient of 0.036% TFA in CH_3CN/0.045% TFA in H_2O over 8 min, with flow = 1.0 mL/min, detection at 235 nm (t_R = 4.9 min). LCMS analysis was conducted using a Waters 2998 PDA apparatus equipped with Micromass ZQ detector, Waters SunFire C18 (3.5 μm, 2.1 × 100 mm) column and a 5–100% gradient of 0.1% formic acid in CH_3CN/0.1% TFA in H_2O over 8 min, with flow = 0.3 mL/min, *m*/*z* = $[M + H]^+$ = 142. HRMS analysis: *m*/*z* = 141.0303 $[M - H]^-$ for $C_5H_5N_2O_3$; *m*/*z* = 321.0250 $[2M + K - H]^-$ for $C_{10}H_{10}KN_4O_6$. IR (KBr): $\tilde{\nu}$ = 3187, 3127, 2989, 2233,

1728, 1631, 1580, 1472, 1433, 1373, 1314, 1167, 1067, 1004, 853, 768, 752, 519, 449 cm^{-1}. ^1H NMR ([D_6]DMSO): δ = 1.28 (*t*, 3 H, CH_3), 4.30 (*q*, 2 H, CH_2) ppm. ^{13}C NMR ([D_6]DMSO): δ = 14.5, 59.4, 114.2, 126.4, 164.4 ppm.

Determination of Potassium Content of K-Oxyma: The precise potassium content within K-Oxyma was determined by means of the ICP technique. 4.025 mg of K-Oxyma were exactly weighted in a MX5 balance and dissolved in 25 mL of HNO_3 (1% in H_2O). A 1:10 dilution was required for the ICP determination. Expected result: 34.99 ppm; Experimental result: 31.11 ppm.

Solubility Assays: Solubility tests of OxymaPure and K-Oxyma were performed as follows: 1 mL of every solvent was taken, and known amounts of Oxyma salt were added with stirring until no more solid was soluble. In some cases heating was needed (see Table 1).

Epimerization Assays in Solution-Phase Using 3-min Preactivation: Z-Phg-OH (0.5 mmol), K-Oxyma (0.5 mmol), EDCl·HCl (0.5 mmol) were mixed together in solvent (3 mL, DMF or DCM) at 0 °C and stirred at this temperature for 3 min, then H-Pro-NH_2 (0.5 mmol) was added and the reaction mixture was stirred at 0 °C for 1 h and 5 h at room temp. Ethyl acetate was added (50 mL), washed with 10% HCl (2 × 10 mL), Na_2CO_3 (2 × 10 mL), satd. NaCl (2 × 10 mL), dried with a $MgSO_4$ filter and then the solvent was removed under vacuum, to give an oily product which solidified on standing at room temp. The degree of epimerization was analyzed by means of HPLC, using Agilent Technologies with Column Zobrax Eclipse Plus 3.5μ (4.6 × 100 mm) and 20–50% CH_3CN/H_2O, 0.1% TFA gradient in 20 min, with a flow rate of 1 mL/min, λ = 220 nm. t_R(LL) = 10.70; t_R(DL) = 11.51.

Epimerization Assays in Solution-Phase without Preactivation: Z-Phg-OH (0.5 mmol), K-Oxyma (0.5 mmol), H-Pro-NH_2 (0.5 mmol) were mixed together in solvent [3 mL, DMF or DCM or DMF-DCM (1:1)] at 0 °C and then EDCl·HCl (0.5 mmol) was added and the reaction mixture was stirred at 0 °C for 1 h and 5 h at room temp. Ethyl acetate was added (50 mL) and then washed with 10% HCl (2 × 10 mL), Na_2CO_3 (2 × 10 mL), and then satd. NaCl (2 × 10 mL), dried with a $MgSO_4$ filter and then the solvent was removed under vacuum, to give an oily product which solidified on standing at room temp. The degree of epimerization was analyzed by means of HPLC, using Agilent Technologies with Column Zobrax Eclipse Plus 3.5μ (4.6 × 100 mm) and 20–50% CH_3CN-H_2O, 0.1% TFA gradient in 20 min, with Flow rate = 1 mL/min, λ = 220 nm. t_R(LL) = 10.70; t_R(DL) = 11.51.

Solid-Phase Assembly of MBP (104–118): 2-CTC resin (2.002 g, 1.54 mmol/g) was placed in a 50 mL polypropylene syringe fitted with a polyethylene filter disc. The resin was washed with DMF (3 × 10 mL, 0.5 min) followed by CH_2Cl_2 (3 × 10 mL, 0.5 min) and then a solution of Fmoc-L-Ala-OH (400 mg, 1.2 mmol) and DIEA (0.51 mL, 3 mmol) in CH_2Cl_2 (1.5 mL) was added. The mixture was then stirred for 2–3 min, DIEA (1.53 mL, 9 mmol), was added to resin and the mixture then stirred at room temp. on a shaker for 1 h. The reaction was completed by addition of MeOH (1.8 mL) after stirring for 30 min. The Fmoc-L-Ala-O-TrtCl resin was subjected to washings/treatments with DMF (3 × 10 mL, 0.5 min) and CH_2Cl_2 (3 × 10 mL, 0.5 min) and was then incubated three times with 20% piperidine/DMF (3 × 10 mL, v/v) for 5 min and followed by extensive washing with DMF (3 × 10 mL) and CH_2Cl_2 (3 × 10 mL). At this point the resin was divided into two equal portions (1.76 g and 1.80 g). The following Fmoc-amino acids (3 equiv.) were added sequentially to the H_2N-L-Ala-O-TrtCl resin described above using either K-Oxyma (324 mg, 1.8 mmol, 3 equiv.) or OxymaPure (256 mg, 1.8 mmol, 3 equiv.) and DIC (0.28 mL, 1.8 mmol, 3 equiv.) in DMF (2.0 mL). In all cases, couplings were complete after 90 min as measured by Kaiser test. Removal of the Fmoc group and washings were carried out as described above. Final resin weight in the case of K-Oxyma was 3.04 g (1.28 g increase) and with OxymaPure was 2.31 g (513 mg increase). The protected peptide was cleaved from the resin by TFA/TIS/H_2O (95:2.5:2.5) (3 × 30 min), the filtrate was collected in 250 mL round-bottomed flask containing 50 mL of TFA/TIS/H_2O. This mixture was stirred for 2 h at room temp. and TFA was removed by evaporation under reduced pressure. The residue was then added dropwise to cold diethyl ether. A white precipitate appeared that was centrifuged and washed with cold diethyl ether three times. Acetonitrile was then added to dissolve this solid until the solution turned clear and the mixture was then lyophilized to give 851 mg (90%, in the case of K-Oxyma) and 593 mg (63%, in the case of OxymaPure) crude peptide. HPLC analysis of MBP (104–118) was performed using an XBridge BEH130 C18 column, 3.5 μm, 4.6 × 100 mm, with 10–60% gradient of 0.036% TFA in CH_3CN/0.045% TFA in H_2O over 8 min, with flow = 1.0 mL/min, detection at 220 nm (t_R = 4.9 min). The crude obtained with OxymaPure was of 88% purity, whereas the one afforded by K-Oxyma was 91% pure. LCMS analysis was conducted using a Waters 2998 PDA apparatus equipped with a Micromass ZQ detector, Waters SunFire C18 (3.5 μm, 2.1 × 100 mm) column and a 5–100% gradient of 0.1% formic acid in CH_3CN/0.1% TFA in H_2O over 8 min, with flow = 0.3 mL/min, *m*/*z* = $[M + 2H]^{2+}$ = 790.32. HRMS analysis: *m*/*z* = 1577.84238 $[M + H]^+$for $C_{70}H_{111}N_{23}O_{19}$.

Solid-Phase Assembly of Cyclo (Arg-Gly-Asp-DPhe-Cys): Synthesis was performed in 2-CTC (0.5 g, loading: 0.5×10^{-3} mmol/g) resin, using coupling and deprotection conditions as described in the generation of MBP (104–118). All couplings were conducted using the DIC/K-Oxyma system in DMF in 90 min. After cleavage, 195 mg of crude peptide were isolated (68% yield). Purity was checked by HPLC using an XBridge BEH130 C18 column, 3.5 μm, 4.6 × 100 mm, with 50–100% gradient of 0.036% TFA in CH_3CN/0.045% TFA in H_2O over 8 min, with flow = 1.0 mL/min, detection at 220 nm (t_R = 4.0 min), resulting in 95% purity. LCMS analysis was conducted using a Waters 2998 PDA apparatus equipped with a Micromass ZQ detector, Waters SunFire C18 (3.5 μm, 2.1 × 100 mm) column and a 5–100% gradient of 0.1% formic acid in CH_3CN/0.1% TFA in H_2O over 8 min, with flow = 0.3 mL/min, *m*/*z* = $[M + H]^+$ = 579.16. HRMS analysis: *m*/*z* = 579.23485 $[M + H]^+$ for $C_{24}H_{35}N_8O_7S_1$.

Solid-Phase Assembly of Aib-ACP: Synthesis was performed in an H-Rinkamide-ChemMatrix (0.2 g, loading: 0.52×10^{-3} mmol/g) resin, using coupling and deprotection conditions as described in the generation of MBP (104–118). All couplings were conducted using the DIC/K-Oxyma system in DMF in 90 min. After the cleavage step, 85 mg were isolated (75% yield). Purity was checked by HPLC using XBridge BEH130 C18 column, 3.5 μm, 4.6 × 100 mm, with 10–60% gradient of 0.036% TFA in CH_3CN/0.045% TFA in H_2O over 8 min, with flow = 1.0 mL/min, detection at 220 nm (t_R = 4.6 min), resulting in 84% purity. LCMS analysis was conducted using a Waters 2998 PDA apparatus equipped with a Micromass ZQ detector, Waters SunFire C18 (3.5 μm, 2.1 × 100 mm) column and a 5–100% gradient of 0.1% formic acid in CH_3CN/0.1% TFA in H_2O over 8 min, with flow = 0.3 mL/min, *m*/*z* = $[M + H]^+$ = 1090.6. HRMS analysis: *m*/*z* = 1090.58850 $[M + H]^+$for $C_{98}H_{160}N_{26}O_{30}$.

Solution-Phase Assembly of Z-Aib-Val-OMe: Z-Aib-OH (0.5 mmol), K-Oxyma (0.5 mmol), EDCl·HCl (0.5 mmol) were mixed together in 3 mL solvent (DMF or DCM) at 0 °C and stirred

at this temperature for 3 min, then H-Val-OMe·HCl (0.5 mmol) was added and the reaction mixture was stirred at 0 °C for 1 h and 5 h at room temp. Ethyl acetate was added (50 mL) and then washed with 10% HCl (2 × 10 mL), Na_2CO_3 (2 × 10 mL), and then satd. NaCl (2 × 10 mL), dried with a $MgSO_4$ filter and then the solvent was removed under vacuum to give an oily product which solidified on standing at room temp. The degree of epimerization was analyzed by means of HPLC, using Agilent Technologies with Column Zobrax Eclipse Plus 3.5μ (4.6 × 100 mm) and 20–50% CH_3CN-H_2O, 0.1% TFA gradient in 20 min, with Flow rate = 1 mL/min, λ = 220 nm. t_R = 17.82.

General Procedure for Dynamic Differential Scanning Calorimetry Assays: Samples of benzotriazole and oxime-based additive (1 mg) were heated from 30–300 °C at a heating rate of 10 °C/min in a closed high-pressure crucible with N_2 flow in a Mettler Toledo DSC-30 differential scanning calorimeter. Diagrams showing heat flow as a function of temperature and time were obtained (Figure 2).

General Procedure for ARC Experiments: Adiabatic experiments were carried out in an Accelerating Rate Calorimeter (ARC) from Thermal Hazard Technology, in ARC TC-HC-MCQ (Hastelloy) test cells. 0.2 M solutions of additives (4 mL) in DMF were introduced into the calorimetric test cell at room temperature, without stirring. A test blank of the pressure released by the organic solvent was also measured. The cell was heated at the initial temperature (30 °C) and the "heat–wait–seek" method was applied; this consisted of heating the sample by 5 °C and, after 15 min of equilibrium, measuring whether self-heating was occurring at a rate higher than 0.02 °C/min (default sensitivity threshold). When self-heating was detected, the system was changed to adiabatic mode. After decomposition, the assay was stopped when the temperature rose above 300 °C.

Supporting Information (see footnote on the first page of this article): ^{1}H- and ^{13}C-NMR spectra of K-Oxyma and HPLCs of the synthesized peptides.

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