Luxembourg Bio Technologies Ltd.

Oxyma Pure: a safe and efficient substitute for HOBt and HOAt

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1.Introduction

Benzotriazole-based additives, especially HOBt[1] and HOAt[2], have been widely used along with carbodiimides or stand-alone coupling reagents, in order to suppress racemization or other side reactions. Nonetheless, recent studies[3] revealed the explosive properties of such compounds that led to their classification as Class 1 (transport of explosive substances), thereby increasing transport restrictions. The need for a new family of safe and potent additives has therefore become of marked importance in peptide synthesis. In that scenario, *ethyl 2-hydroxyimino-2-cyanoacetate* (Oxyma pure, **Fig.1**), which had been previously reported[4], has been evaluated in terms of racemization control, efficiency in difficult couplings and safety profile.

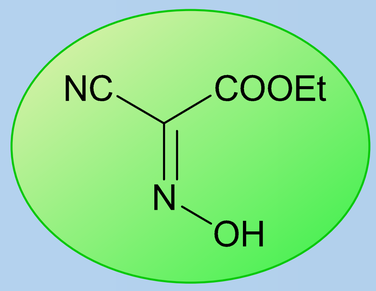


Figure 1. Structure of oxyma pure

2.Racemization control

The ability to suppress racemization by Oxyma Pure was studied and compared to HOBt, HOAt and HOP (N-hydroxy-2-pyridone) using DIC in solution phase. Two peptide model systems were chosen, involving both stepwise coupling (Z-Phg-Pro-NH_2) and [2+1] segment coupling (Z-Phe-Val-Pro-NH_2)[5]. In some cases, preactivation took place for 2 minutes prior to the addtion as displayed in **Table 1** and **Table 2**. Results showed that Oxyma Pure induces low racemization levels in both systems, especially in the stepwise coupling (1.0% and 1.1% of DL epimer), in which is even superior to HOAt

Table 1. Z-Phg-Pro-NH_2

Additive	Yield(%)	DL(%)
DIC/HOP	88.2	17.4
DIC/HOP 2min PT	83.4	26.1
DIC/HOAt	81.4	3.3
DIC/HOBt	81.9	9.3
DIC/Oxyma Pure	**89.9**	**1.0**
DIC/Oxyma Pure 2min PT	**88.2**	**1.1**

Table 2. Z-Phe-Val-Pro-NH_2

Additive	Yield(%)	DL(%)
DIC/HOP	88.5	45.1
DIC/HOAt	86.1	2.1
DIC/HOBt	78.8	8.9
DIC/Oxyma Pure 2min PT	**89.8**	**3.8**

3. Efficiency in difficult couplings

The performance of Oxyma Pure in obtaining a modified Leu-enkephalin pentapeptide (H-Tyr-Aib-Aib-Phe-Leu-NH_2)[6] was tested. This model peptide, containing Aib (α-aminoisobutyric acid) residues, proved to be the most demanding one, and so bigger differences among HOAt, HOBt and Oxyma Pure arose in terms of ratio pentapeptide/deletion peptides observed. Incorporation of Tyr and Aib to a previously elongated H-Aib-Phe-Leu-resin tripeptide was followed, using DIC/additive/Fmoc-aminoacid (3eq) with a 3 min PT to ensure formation of the corresponding active esters. Different coupling conditions were studied (**Table 3**). In all cases HOBt gave rise to the lowest percentages of pentapeptide obtained (des-Tyr and tripeptide were detected even after 1 hour coupling). Oxyma Pure showed greater efficiency than HOAt in every experiment.

Table 3. H-Tyr-Aib-Aib-Phe-Leu-NH_2

Coupling time	additive	Pentapeptide (%)	des-Aib (%)	des-Tyr(%)	Tripeptide (%)
30 minutes	HOAt	11.3	86.1	1.8	0.8
	HOBt	3.0	91.0	0.9	5.1
	Oxyma Pure	**28.0**	**70.5**	**1.1**	**0.4**
1 hour	HOAt	28.7	71.3	--	--
	HOBt	9.8	86.9	1.6	1.7
	Oxyma Pure	**55.7**	**44.3**	**--**	**--**
1 hour double coupling	HOAt	55.0	45.0	--	--
	HOBt	18.9	80.6	0.5	--
	Oxyma Pure	**69.0**	**31.0**	**--**	**--**

4. Differential Scanning Calorimetry Assays

For a compound to be explosive, a fast release of pressure is necessary when decomposing. To address this issue, Differential Scanning Calorimetry (DSC) tests comparing HOAt, HOBt and Oxyma Pure were carried out. In this experiment, information is given concerning the kinetics of decomposition. The sample is heated from 30ºC to 300ºC at a constant heating rate of 10ºC/min in a closed crucible with N_2 flow. A diagram with released dH vs temperature/ time is obtained. Benzotriazole-based additives displayed similar profiles, (**Fig.2** and **Fig.3**) revealing that decomposition takes place in a short period of time, whereas Oxyma Pure (**Fig.4**) decomposed much slower.

Fig 2. HOAt

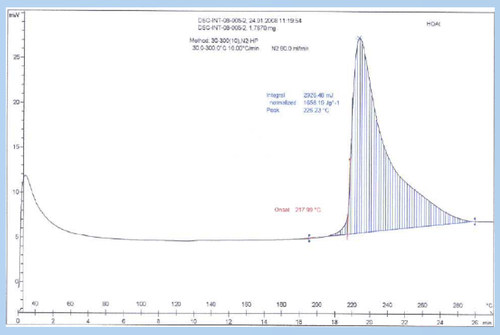


Fig 3. HOBt

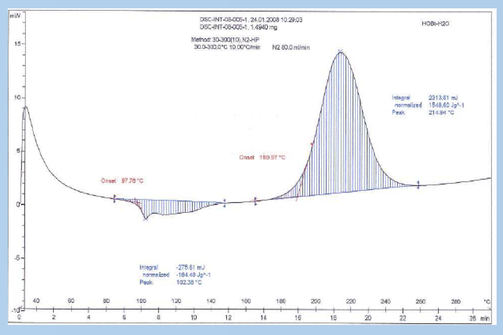
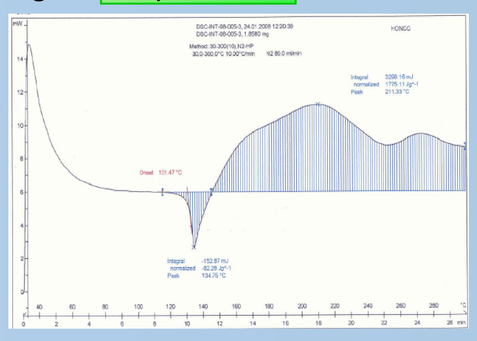


Fig 4. Oxyma Pure



5. Adiabatic Calorimetry Assays

Complementary to the above mentioned DSC experiments are the adiabatic calorimetry assays run on an Accelerating Rate Calorimeter (ARC) applying the "heat-wait-seek" method. The sensitivity threshold was set to 0.02ºC/min. Data is given regarding the pressure released in the decomposition process (temperature in red or pressure in blue vs time). Whereas in the experiment with the benzotriazole-based additives HOAt and HOBt (**Fig.5** and **Fig.6**) 170-180 bar pressure is detected, due to N_2 release, in the case of Oxyma Pure only 60 bar pressure (**Fig.7**) was observed.

Fig 5. HOAt

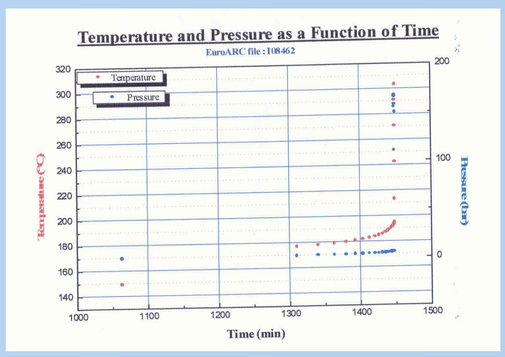


Fig 6 HOBt

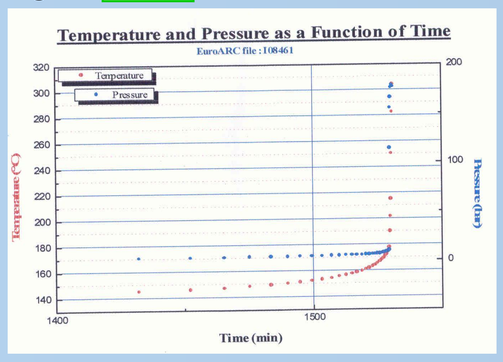
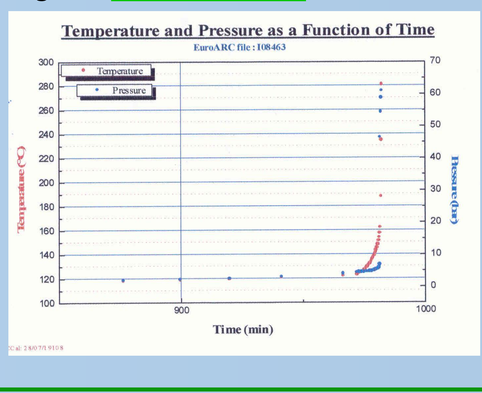


Fig 7. Oxyma Pure



Conclusions

Results on Oxyma Pure have shown that:

- The suppresion of racemization is at least comparable to HOAt
- The obtention of sterically hindered peptides is more efficient than HOAt and HOBt.
- The likeliness of an explosion can not be compared to HOAt or HOBt, because it decomposes slowly releasing low pressure

References

[1] König, W.; Geiger, R.; *Chem.Ber.* **1970**, 103, 788-798 and 2034-2040

[2] Carpino, L.A.; *J.Am.Chem.Soc*. **1993,** 115, 4397-4398

[3] Wehrstedt et al., *J. Hazard. Mat*. **2005,** *A126*, 1-7

[4] a) Izdebski, J., *Pol.J.Chem.* **1979**, 53, 1049-1057; b) Itoh, M. , *Bull.Chem. Soc. Japan*, **1973**, 46, 2219-2221

[5] a) El-Faham, A.; Albericio, F. *Org. Lett.* **2007**, *9,* 4475-4477., b) El-Faham, A.; Albericio, *J. Org. Chem.,* **2008**, *73*, 2731-2737.

[6] Carpino, L A.; El-Faham, A.; Minor, C. A.; Albericio, F. *J. Chem. Soc., Chem. Comm.* **1994**, pp. 201-203

Acknowledgements

This work was partially supported by CICYT (CTQ2006-03794/BQU), the Generalitat de Catalunya (2005SGR 00662), the Institute for Research in Biomedicine, and the Barcelona Science Park. RS-F thanks Ministerio de Educación y Ciencia for a FPU PhD fellowship. We also thank the Calorimetry Platform at Barcelona Science Park for their support in the DSC and ARC experiments.