DEVELOPMENT OF NOVELS PEPTIDE COUPLING REAGENTS BASED ON 1,3-DIMETHYLBARBUTRIC ACID, THE OXYMA-B FAMILY

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Introdution

After the identification of the potential explosively of benzotriazole derivatives, mainly HOBt and HOAt [1], which are used as additive in carbodiimide based peptide formation, there has been a large development of new additives. Some time ago, some of us proposed the OxymaPure as a superior peptide coupling additive. OxymaPure has been showed always superior to HOBt and even in some cases superior to HOAt [2].

More recently, a new family of uronium salts based on isonitroso Meldrum's acid (HONM) was reported as stand-alone coupling reagents [3]. HONM shows a special orientation of the carbonyl moiety, which can play an assisted basic catalytic role by enhancing the nucleophilicity of the amino function during coupling. This modification should enhance the reactivity of the oxime-based additive as a result of its more powerful electron-withdrawing effect compared with OxymaPure. Although, HONM is very reactive and even reacts with carbodiimides, which translates into a consumption of both coupling reagents with decreased of yields, its uronium salts showed a rather increased reactivity when compared with classical coupling reagents [3].

With the objective of fine-tuning the performance of OxymPure and with the idea of removing the ethyl ester of which some colleagues has suggested us the interest of having in our tools of synthetic reagents a similar derivative of OxymaPure with similar synthetic performance chemistry, but much better performance and the absence of potential side reactions.

Herein, we are presenting a new additive, its potassium salt and its uronium salts. This new additive, Oxyma-B, is an oxime derived from 1,3-dimethylbarbituric acid, showing a similar cyclic structure as HONM with a special orientation of the carbonyl moiety, which can play an assisted basic catalytic role by enhancing the nucleophilicity of the amino function during coupling. In addition, Oxyma-B does not show any ester moiety in its structure and, therefore, there is no risk of any side-reaction.

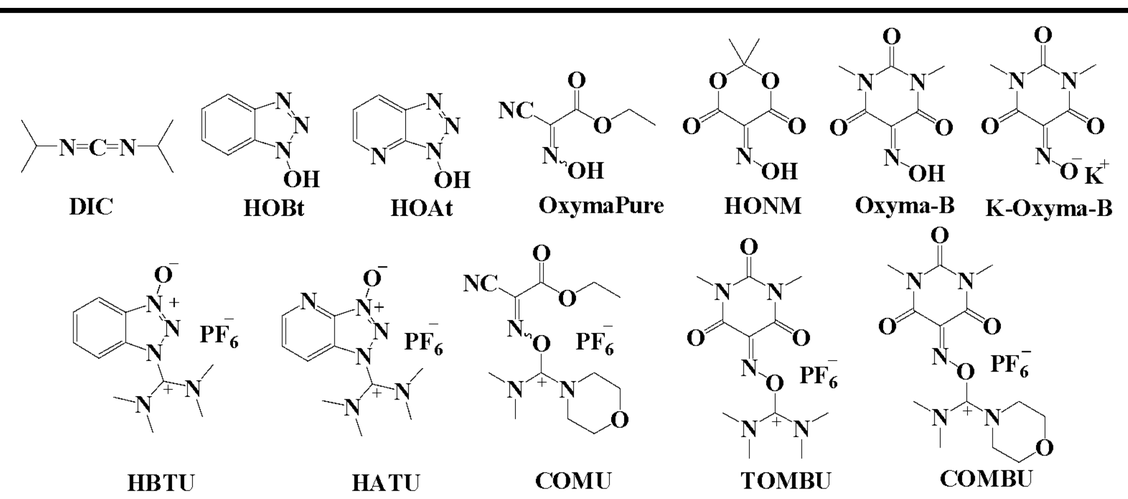

Table 1.Yield and racemization during the formation of the formation of Z-Phg-Pro-NH_2 and Z-Phe-Val-Pro-NH_2 using DIC-mediated mediated coupling method in combination with different additives through [1+1] stepwise and [2+1] segment solution-phase coupling, respectively.[a]

Entry	Peptide model	Coupling reagent	Yield (%) [b]	DL/LL or LDL/LL L (%)[c]
1	Z-Phg-Pro-NH_2	DIC/HOBt	94.3	11.0
2		DIC/HOAt	91.5	3.9
3		DIC/OxymaPure	94.4	0.9
4		DIC/Oxyma-B	90.0	1.0
5		DIC/K-Oxyma-B	89.2	1.2
6	Z-Phe-Val-Pro-NH_2	DIC/HOBt	96.3	14.8
7		DIC/HOAt	97.6	5.9
8		DIC/OxymaPure	91.9	7.7
9		DIC/Oxyma-B	90.7	5.1
10		DIC/K-Oxyma-B	93.3	9.7

[a] Couplings were performed without preactivation in DMF at room temperature.[b] Conversion yield calculated by HPLC. Retention times of starting materials and products were identified by injection of pure sample. [c] Retention times for each epimer were identified after co-injection with a pure LL and DL or LLL and LDL samples.

Since Oxyma-B showed the best performance in reducing racemization , the next models is for testing the racemization during solid-phase assembling of serine, cysteine and histidine residues because of their unusual racemization sensitivity during solid-phase synthesis [4].

Table 2. Racemization studies on the solid-phase assembling of H-Gly-AA-Phe-NH_2 (where AA=Ser, Cys, Cys(Acm) or His).[a]

Entry	Peptide model	Coupling reagent	DL/LL (%)
1	H-Gly-Ser-Phe-NH_2	DIC/HOBt	3.3
2		DIC/HOAt	0.4
3		DIC/OxymaPure	0.4
4		DIC/Oxyma-B	0.3
5	H-Gly-Cys-Phe-NH_2	DIC/HOBt	0.5
6		DIC/HOAt	0.4
7		DIC/OxymaPure	0.3
8		DIC/Oxyma-B	0.3
9	H-Gly-Cys(Acm)-Phe-NH_2	DIC/HOBt	0.4
10		DIC/HOAt	0.3
11		DIC/OxymaPure	0.3
12		DIC/Oxyma-B	0.3
13	H-Gly-His-Phe-NH_2	DIC/HOBt	1.1
14		DIC/HOAt	1.9
15		DIC/OxymaPure	3.0
16		DIC/Oxyma-B	1.0

[a] Couplings were performed with 5 min activation and one hour coupling time in DMF at room temperature.

Table 3.Yield and racemization during the formation of the formation of Z-Phg-Pro-NH_2 and Z-Phe-Val-Pro-NH_2 using uronium/aminium-type coupling reagents through [1+1] stepwise and [2+1] segment solution-phase coupling, respectively.[a]

Entry	Peptide model	Coupling reagent	Yield (%) [b]	DL/LL or LDL/LLL (%)[c]
1	Z-Phg-Pro-NH_2	HBTU	93.1	7.5
2		HATU	95.5	3.9
3		COMU	98.2	0.9
4		TOMBU	98.5	0.7
5		COMBU	93.2	0.9
6	Z-Phe-Val-Pro-NH_2	HBTU	96.4	43.2
7		HATU	98.4	7.5
8		COMU	98.0	16.7
9		TOMBU	91.4	15.3
10		COMBU	89.2	20.3

[a] Couplings were performed without preactivation in DMF at room temperature.[b] Conversion yield calculated by HPLC. Retention times of starting materials and products were identified by injection of pure sample. [c] Retention times for each epimer were identified after co-injection with a pure LL and DL or LLL and LDL samples.

Solid-phase peptide synthesis

Table 4. Percentage of tetrapeptide des-Aib (H-Tyr-Aib-Phe-Leu-NH_2) during solid-phase assembling of pentapeptide (H-Tyr-Aib-Aib-Phe-Leu-NH_2) using DIC-mediated mediated coupling method in combination with different additives.[a]

Entry	Coupling reagent	Preactivation time	Penta (%)	des-Aib (%) [b]
1	DIC/HOBt	3 min	8.4	83.1
2	DIC/HOAt	3 min	37.5	60.2
3	DIC/OxymaPure	3 min	42.8	50.4
4	DIC/Oxyma-B	No preactivation	19.4	72.6
5	DIC/Oxyma-B	3 min	26.4	61.1
6	DIC/Oxyma-B	3 min[c]	10.5	79.7
7	DIC/Oxyma-B	7 min	9.4	82.8
8	DIC/K-Oxyma-B	3 min	20.7	74.0

[a] One-hour coupling times were generally applied, except for Aib-Aib (one- hour double coupling). [b] Deletion tetrapeptide (des-Aib) was identified by peak overlap in HPLC with an authentic sample obtained in solid phase. [c] 0.1% DIEA was used.

Table 5. Percentage of tetrapeptide des-Aib (H-Tyr-Aib-Phe-Leu-NH_2) during solid-phase assembling of pentapeptide (H-Tyr-Aib-Aib-Phe-Leu-NH_2) using uronium/aminium-type coupling reagents.[a]

Entry	Coupling reagent	Base (equiv.)	Penta (%)	des-Aib (%) [b]
1	HBTU	DIEA (2)	53.15	46.84
2	HATU	DIEA (2)	97.90	2.09
3	COMU	DIEA (2)	99.2	0.8
4	TOMBU	DIEA (2)	90.41	9.56
5	COMBU	DIEA (2)	82.5	17.5
6	COMBU	DIEA (2)[c]	83.5	16.5

[a] 30 min coupling times were generally applied, except for Aib-Aib (30 min double coupling). [b] Deletion tetrapeptide (des-Aib) was identified by peak overlap in HPLC with an authentic sample obtained in solid phase. [c] Fmoc-amino acids were preactivated with only 1 equiv DIEA for 15-30 s, with addition of another 1 equiv onto the resin after the first addition.

Table 6. Closed vials hydrolytic stability of uronium/aminium-type coupling reagents in DMF.

Entry	Coupling reagent	2 min	1 h	4 h	6	24 h	48 h
1	HBTU	100	100	100	100	100	100
2	HATU	100	100	100	100	100	99.2
3	COMU	93.4	79.1	45.1	31.6	2.5	0
4	TOMBU	97.1	95.5	89.2	83.9	34.9	13.3
5	COMBU	88.4	85.1	70.8	60.9	9.5	0

Acknowledgments

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Conclusions

- Oxyma-B has showed a performance similar to OxymaPure in terms of conversion, this means superior in all cases to HOBt and in some even to HOAt. Oxyma-B is a superior racemization suppressor than OxymaPure and even more superior than HOAt.
- In solid-phase peptide synthesis, Oxyma-B and K-Oxyma-B rendered the product in higher purity than HOBt but in lower purity than HOAt and OxymaPure.
- TOMBU and COMBU rendered the product rendered the product in higher purity than HBTU and HATU and similar to COMU in stepwise coupling. In segment coupling, TOMBU and COMBU rendered the product rendered the product in higher purity than HBTU, similar to COMU and lower than HATU.
- In solid-phase peptide synthesis, TOMBU and COMBU rendered the product in higher purity than HBTU but in lower purity than HATU and COMU.
- TOMBU and COMBU are less stable than HBTU and HATU in DMF but they are more stable than COMU.

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