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A Non-Explosive Replacement for Benzotriazole Based Coupling Reagents

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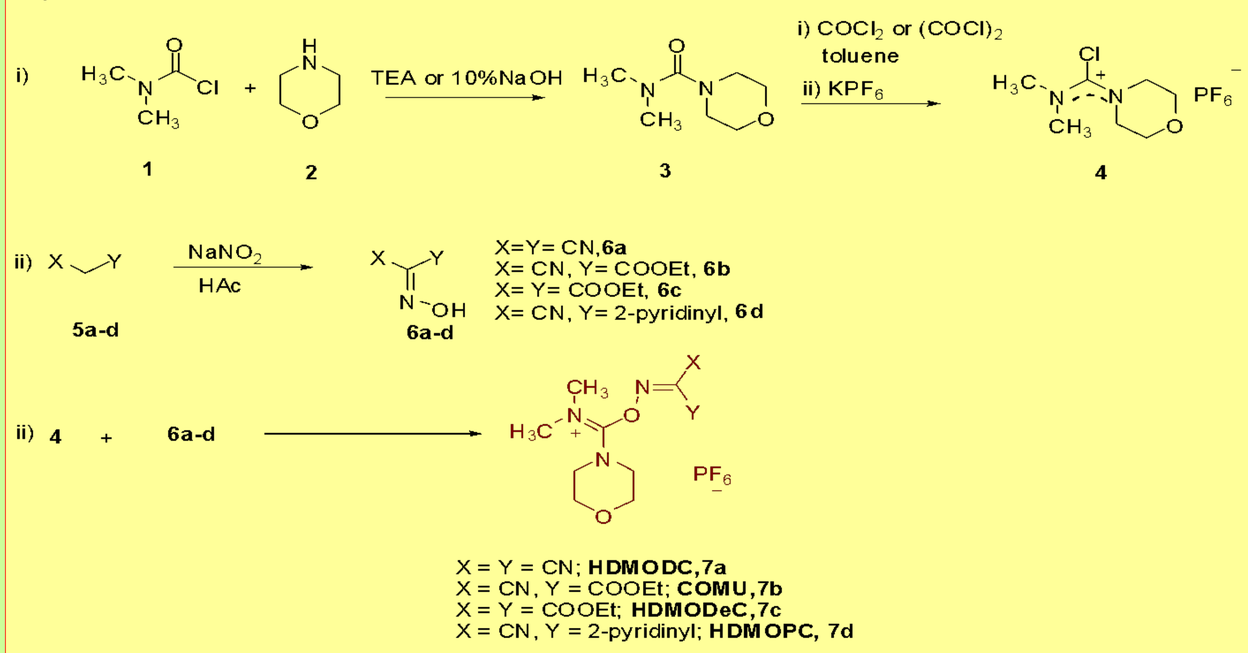
Introduction

Peptide synthesis is based on the proper combination of protecting groups and in the right choice of the right coupling method.1 Nowadays, almost all peptide bond formed are carried out in the presence of 1-hydroxybenzotriazole (HOBt)2 or its derivatives (HOAt,3 Cl-HOBt4). Recent reports have confirmed the explosive properties of HOBt derivatives.7 Thus, a replacement of HOBt should be found for preparation of peptides for research purposes and more important, for the production of peptide based APIs. Herein, several alternatives to HOBt will be discussed taking into account the explosively properties. Examples on the use of the non-explosive replacement for HOBt together with uronium salts incorporated with the proton acceptor will be discussed.

Synthesis of Uronium Salts

The corresponding non-symmetrical uronium salts were prepared as shown in Scheme 1



Scheme 1: Synthesis of Non-symmetric Uronium Salts

To determine the compatibility of the new coupling reagents with peptide synthesis in both manual and automatic mode, their solubility and stability in solution and in solid state was tested (Table 1 and 2)

Table1.Hydrolytic stability of immonium-type coupling reagents in DMF

Coupling Reagent	5 h	24 h	48 h
HATU	99 %	95 %	76 %
HBTU	100 %	98 %	86 %
HOTU	100 %	95 %	84 %
COMU(**7b**)	100 %	100 %	93 %

Table 2. Effect of Oxygen on the solubility of the uronium type coupling reagents

Coupling Reagent	Wt/1mL	Molarity
HATU	0.165	0.43
HBTU	0.175	0.46
HOTU	0.420	1.09
COMU (7b)	0.620	1.44
HTODC	0.410	1.20
HDMODC (**7a**)	0.520	1.36
HDMOPC (**7d**)	0.430	0.98

Best Results was obtained with COMU (Figure 1)

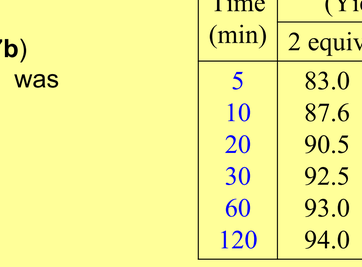
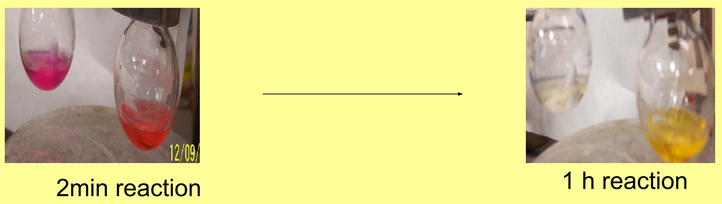


Figure 1: Structure of COMU

the oxime derivatives **7b** (**COMU**) gave a color which makes the reaction could be followed for completion (figure 2).



2min reaction — 1 h reaction

Figure 2

The efficiency of the new coupling reagent (**7b**) for coupling of hindered amino acid was examined using the model system **(Fmoc-Val-OH + H-Val-NH_2)** (Table 3).

Table 3: Extent of Coupling of Fmoc-Val-Val-NH_2

Time (min)	HATU (Yield %)		COMU (Yield %)	
	2 equiv	1 equiv	2 equiv	1 equiv
5	83.0	70.0	95.1	82.0
10	87.6	76.0	96.0	86.0
20	90.5	80.0	98.0	90.1
30	92.5	82.0	98.5	94.5
60	93.0	82.0	100.0	96.0
120	94.0	83.0	100.0	98.0

To investigate the configuration retention induced for the new coupling reagents, several previously studied model peptide systems were examined (Table 4)

Table 4

AA	Coupling Reagent	Base (equiv.)	Yiel(%)	LDL (%)
Z-Phg-Pro-NH_2	HATU	DIEA (2)	78.4	3.1
	HBTU	DIEA (2)	80.2	8.2
	HOTU	DIEA (2)	78.9	0.17
	COMU (7b)	DIEA (2)	88.2	0.12
	HDMODC (**7a**)	DIEA (2)	90.1	0.40
	HDMOPC (**7d**)	DIEA (2)	86.0	13.6
			85.8	13.9
Z-Phe-Val-Pro-NH_2	HATU	DIEA (2)	83.2	11.0
		DIEA (1)	78.0	5.3
		TMP (2)	76.1	4.9
	HBTU	DIEA (2)	78.6	27.7
		DIEA (1)	81.2	16.3
		TMP (2)	91.2	14.2
	HOTU (**7a**)	DIEA (2)	88.7	23.6
		TMP (2)	80.3	7.4
		TMP (1)	91.3	7.5
	COMU (7b)	DIEA (2)	89.8	19.3
		TMP (2)	90.3	7.0
		TMP (1)	88.0	3.5
	HDMODC (**7a**)	TMP (2)	90.0	17.9
	HDMOPC (**7d**)	TMP (2)	87.2	43.6

In a more demanding example, H-Tyr-Aib-Aib-Phe-Leu-NH2 was manually assembled on Fmoc-Rink Amide.AM-resin using amino acid/activator (3 eq.), DIEA (6 eq.) or (3 eq.), using 30 min coupling time except for the case of Aib-Aib, for which 1 h was used. The best results were obtained with 7b (HDMOC) (Table 5, Figure 3)

Table 5

Coupling Reagent	Base (equiv.)	Penta (%)	Des-Aib (%) tetra
HATU	DIEA (2)	83.0	17
HBTU	DIEA (2)	47.0	53
HTOC	DIEA (2)	99.0	1.0
COMU (7b)	**DIEA (2)**	**99.7**	**0.26**
HDMODC	DIEA (2)	95.3	4.7
HDMOPC	DIEA (2)	41.3	58.3

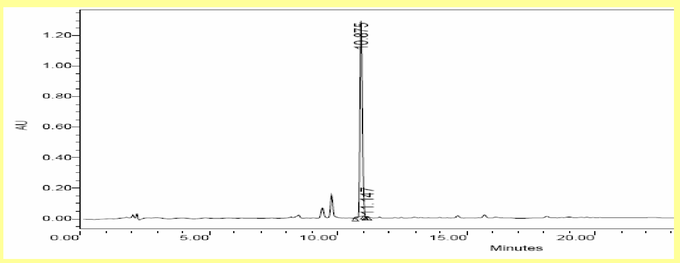


Figure 3

HPLC traces of H-Tyr-Aib-Aib-Phe-Leu-NH_2

Conclusions

In conclusion, herein new families of uronium-type coupling reagents that differ in their carbocation skeleton structure as well as the leaving group have been described. The presence of the morpholino group has a marked influence on the polarity of the carbon skeleton, which affects the solubility and stability as well as the reactivity of the reagent These results should be taken into account when coupling reagents are placed in open vessels, such as in some automatic synthesizers. Remarkably, HONC (6b) derivative gave equally good results as the aza derivatives and performed extremely well in the presence of only 1 eq. of base.