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Novel Proton Acceptor Immonium-Type Coupling Reagents: Application in Solution and Solid-Phase Peptide Synthesis

Ayman El-Faham*,†,‡ and Fernando Albericio*,†,§

Institute for Research in Biomedicine, Barcelona Science Park, Josep Samitier 1, 08028-Barcelona, Spain, Department of Chemistry, Faculty of Science, Alexandria University, Ibrahimia 21321, Alexandria, Egypt, and Department of Organic Chemistry, University of Barcelona, Martí i Franqués 1-11, 08028-Barcelona, Spain

albericio@pcb.ub.es; aymanel_faham@hotmail.com

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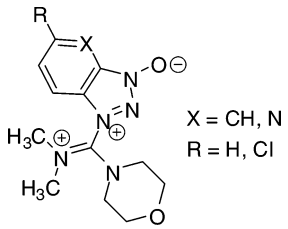
ABSTRACT



A novel proton acceptor coupling reagent shows superiority to those described previously. The oxygen in the carbocation moiety confers more solubility to the reagent. Furthermore, it enhances coupling yields and decreases racemization, allowing the use of 1 equiv of base.

The most widely used coupling reagents are carbodiimides, immonium and phosphonium salts, and *N*-triazinylammonium salts.[1] Immonium salts, such as HATU, HAPyU, HBTU, HCTU, or TFFH,[2] which possibly are the most powerful,[3] are formed by a leaving group and a carbocation skeleton. Even though a substantial amount of work has been done in the past few years to identify the best leaving group,[1,4] less attention has been paid to the nature of the carbocation part.[3,5] The first study on the design, preparation, and application of novel immonium salts, which incorporate a proton acceptor in their carbocation skeleton, is described herein.

Couplings involving immonium salts (Figure 1) are carried out with an excess of base, at least 2 equiv, and in the presence of 1 equiv of the hydroxylamine derivative.[1c] In this regard, we thought that the incorporation of a proton

† Barcelona Science Park.
‡ Alexandria University.
§ University of Barcelona.

(1) (a) Albericio, F.; Carpino, L. A. In *Methods in Enzymology, Solid-Phase Peptide Synthesis*; Fields, G. B., Ed.; Academic Press: Orlando, 1997; Vol. 289, pp 104−126. (b) Humphrey, J. M.; Chamberlin, A. R. *Chem. Rev.* **1997**, *97*, 2243−2266. (c) Kaminski, Z. J. *Biopolymers* **2000**, *55*, 140−165. (d) Albericio, F.; Chinchilla, R.; Dodsworth, D. J.; Najera, C. *Org. Prep., Proc. Int.* **2001**, *33*, 203−303. (e) Han, S.-Y.; Kim, Y.-A. *Tetrahedron* **2004**, *60*, 2447−2467.

(2) Abbreviations not defined in the text: Boc, *tert*-butyloxycarbonyl; Cl-HOBt, 6-chloro-1-hydroxybenzotriazole; DCM, dichloromethane; DIEA, diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; Et_3N, *N*,*N*,*N*-triethylamine; Fmoc, fluorenylmethoxycarbonyl; HATU, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HBTU, *N*-[(1*H*-benzotriazol-1-yl)-(dimethylamino)methylene] *N*-methylmethanaminium hexafluorophosphate *N*-oxide; HCTU, *N*-[(1*H*-6-chloro-benzotriazol-1-yl)-(dimethylamino)methylene] *N*-methylmethanaminium hexafluorophosphate *N*-oxide; 6-HDMCB, 1-((dimethylimino)(morpholino)methyl)3-*H*-6-chlorobenzo[1,2,3]triazolo-1-ium-3-olate hexafluorophosphate; HMDA, 1-((dimethylimino)(morpholino)methyl)3-*H*-[1,2,3]triazolo[4,5-*b*]pyridine-1-3-olate hexafluorophosphate; HMDB, 1-((dimethylimino)(morpholino)methyl)3-*H*-benzo[1,2,3]triazolo-1-ium-3-olate hexafluorophosphate; HOAt, 7-aza-1-hydroxybenzotriazole; HOBt, 1-hydroxybenzotriazole; TFFH, tetramethylfluoroformamidinium hexafluorophosphate; TFA, trifluoroacetic acid; TMP, 2,4,6-trimethylpyridine (collidine); Z, benzyloxycarbonyl. Amino acids and peptides are abbreviated and designated following the rules of the IUPAC-IUB Commission of Biochemical Nomenclature [*J. Biol. Chem.* **1972**, *247*, 977].

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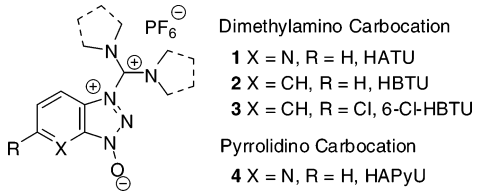


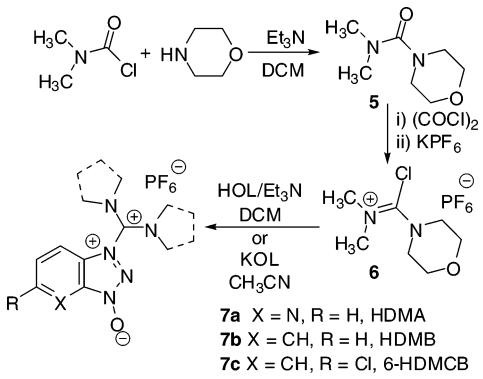
Figure 1. Representative immonium salt coupling reagents.

acceptor could have influenced the coupling reagent performance in terms of reactivity and racemization control, as well as its solubility and stability.

The unsymmetrical morpholine-based immonium salts can be readily prepared by treating *N*,*N*-dimethyl carbamoyl chloride with morpholine to give the corresponding urea derivatives **5**.[6] Then, the urea derivative reacts with oxalyl chloride to yield the corresponding chloro salts (Scheme 1), which are stabilized by the formation of a PF_6 salt **6**. Subsequent reaction with KOXt or HOXt in the presence of a tertiary amine such as Et_3N affords the desired compound **7** as crystalline and shelf-stable solids.[7] X-ray crystallography showed that both HDMA and HDMB were in the *N* form, and this agreed with the ^{13}C NMR spectral published data.[8]

The first property to be taken into account is the solubility of the coupling reagent, which has a great influence on the reaction yield and its performance in the automatic synthesizers. Thus, the presence of the oxygen atom in the carbon

Scheme 1. Pathway Used for the Preparation of Novel Proton Acceptor Immonium-Type Coupling Reagents



skeleton is of marked importance to the solubility of the compound, increasing its solubility. Accordingly, morpholino derivatives **7** are more soluble than dimethylamino derivatives **1** and **2**, where the salt 6-HDMCB (**7c**) is more soluble and can be used to prepare up to a 1 M solution, while morpholino salts derived from HOAt **7a** or HOBt **7b** can be used to prepare up to a 0.75 M solution, as compared to other dimethylamino derivatives **1**−**3**, which can be used to prepare up to a 0.5 M solution in DMF. This enhanced solubility should help the removal during the workup after the coupling of the urea formed in a solution mode approach.

Table 1 shows examples illustrating the effectiveness of morpholino derivatives **7** in reducing racemization. All

Table 1. Extent of Conversion and Racemization during Coupling of Z-Phg-OH or Z-Phe-Val-OH and H-Pro-NH_2 Using Immonium Salt-Based Reagents[a]

no.	AA_1	AA_2	coupling reagent	base (equiv)	yield	D−L isomer (%)
1	Z-Phg-OH	H-Pro-NH_2	HATU	DIEA (2)	78.4	3.1
				DIEA (1)	74.8	2.4
				TMP (2)	77.9	2.1
2	Z-Phg-OH	H-Pro-NH_2	HMDA	DIEA (2)	81.2	1.6
				DIEA (1)	82.3	1.6
				TMP (2)	80.3	3.9
3	Z-Phg-OH	H-Pro-NH_2	HBTU	DIEA (2)	80.2	8.2
				DIEA (1)	75.0	5.3
				TMP (2)	81.2	6.4
4	Z-Phg-OH	H-Pro-NH_2	HMDB	DIEA (2)	80.8	3.8
				DIEA (1)	82.3	3.1
				TMP (2)	79.9	7.8
5	Z-Phg-OH	H-Pro-NH_2	6-HDMCB	DIEA (2)	84.5	1.5
6	Z-Phe-Val-OH	H-Pro-NH_2	HATU	DIEA (2)	85.8	13.9
				DIEA (1)	83.2	11.0
				TMP (2)	78.0	5.3
7	Z-Phe-Val-OH	H-Pro-NH_2	HMDA	DIEA (2)	89.3	10.5
				DIEA (1)	87.4	5.1
				TMP (2)	86.2	3.7
8	Z-Phe-Val-OH	H-Pro-NH_2	HBTU	DIEA (2)	89.7	27.7
				DIEA (1)	78.6	16.3
				TMP (2)	81.2	14.2
9	Z-Phe-Val-OH	H-Pro-NH_2	HMDB	DIEA (2)	88.7	20.3
				DIEA (1)	86.3	11.5
				TMP (2)	87.1	13.3

[a] conc = 0.1 mM, calculated by HPLC.

morpholino derivatives (nos. 2, 4, 5, 7, and 9) result in improved control of racemization when compared to their tetramethylamino counterparts (nos. 1, 3, 6, and 8). Interestingly, morpholino derivatives perform especially well with just 1 equiv of base. Thus, yields are similar or even slightly better than those obtained when 2 equiv of base and/or tetramethylamino derivatives are used.

To compare the relative coupling rates for various reagents and the effect of the oxygen as a proton acceptor, reaction of Fmoc-Val-OH with H-Val-NH_2 in the presence of 1 or 2 equiv of DIEA was carried out. Table 2 indicates that the morpholino derivative (HDMA) showed a better coupling rate in both cases (1 and 2 equiv of DIEA). This indicates that the oxygen atom increases reactivity and acts as a proton acceptor during the coupling step.

Table 2. Extent of Conversion in the Preparation of Fmoc-Val-Val-NH_2 Using HATU/HDMA and DIEA (1 Equiv/2 Equiv) as a Base in DMF (Calculated by HPLC)

	HATU yield (%)		HDMA yield (%)	
time (min)	2 equiv	1 equiv	2 equiv	1 equiv
5	83.0	70.0	94.8	80.0
10	87.6	76.0	95.2	85.0
20	90.5	80.0	96.4	90.0
30	92.5	82.0	98.0	93.5
60	93.0	82.0	99.0	95.5
120	94.0	83.0	99.0	96.0

To demonstrate the effectiveness of the oxygen on the carbon skeleton of the new reagents in the solution phase, the pentapeptide H-Tyr-Gly-Gly-Phe-Leu-NH_2 was prepared in solution without isolation and purification of the intermediates using Boc chemistry and HDMB (**7b**) as a coupling reagent. The purity of the pentapeptide tested by HPLC analysis and HPLC-MS was excellent (Figure 2). The HPLC-MS showed the correct mass for the pentapeptide. No traces of the urea were detected confirming the high solubility of these derivatives.

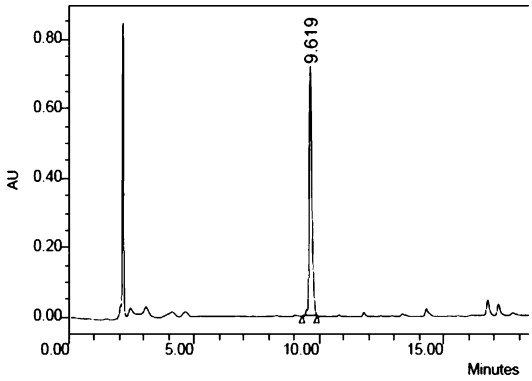


Figure 2. HPLC for the crude pentapeptide prepared in solution.

In a more demanding example, H-Tyr-Aib-Aib-Phe-Leu-NH_2 was manually solid-phase assembled on Fmoc-Rink Amide-AM-resin using amino acid/activator (3 equiv) and DIEA (6 equiv) for a 30 min coupling, except for Aib-Aib, which required 1 h. Yields for coupling of Fmoc-Aib-OH onto H-Aib-Phe-Leu-NH-Rink Amide-AM-resin were determined by reverse-phase HPLC analysis (integration of pentapeptide vs tetrapeptide) after cleavage of the peptide from the resin. The best results were obtained with 6-HDMCB (**7c**) (99% coupling) and HDMA (**7a**) (98% coupling) and HDMB (**7b**) (89% coupling) over HATU (**1**) (83% coupling) and HBTU (**2**) (47% coupling).

In conclusion, the novel proton acceptor coupling reagents showed superiority to those described previously.[9] The oxygen in the carbocation moiety confers more solubility, to the reagent, allowing us to carry out the reaction at higher concentration and facilitating the removal of side products when chemistry is carried out in solution. Furthermore, it enhances coupling yields and decreases racemization, allowing the use of just 1 equiv of base. Results obtained with HMDB and 6-HDMCB, which contain the less reactive HOBt and Cl-HOBt, match those obtained with HATU, which contains the most effective and expensive HOAt.

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Supporting Information Available: Experimental procedures and characterization material. This material is available free of charge via the Internet at http://pubs.acs.org.

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(4) Immonium salt derivatives of HOAt, such as HATU and HAPyU, have been shown to be the most efficient in terms of both reactivity and controlling racemization. This has been interpreted by the electron-withdrawing influence of the nitrogen atom that effects stabilization of the leaving group leading to greater reactivity. Second, the nitrogen makes a classic neighboring group effect feasible which can both speed up the reactivity and reduce loss of configuration. (a) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397−4398. (b) Carpino, L. A.; El-Faham, A.; Minor, C. A.; Albericio, F. *J. Chem. Soc., Chem. Commun.* **1993**, 201−203.

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(6) Reactions of urea **6** with other secondary amines did not led to positive results [thiomorpholine, poor coupling reagent; *N*-methylpiperazine, the target compound was not obtained].

(7) Patent pending.

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(9) Interestingly, Kaminski, Papini, and co-workers have described the *N*-methylmorpholinium derivative as the reagent of choice of its family of reagents for their independent triazinylammonium system. Kaminski, Z. J.; Kolesinska, B.; Kolesinska, J.; Sabatino, G.; Chelli, M.; Rovero, P.; Blaszczyk, M.; Glowka, M. L.; Papini, A. M. *J. Am. Chem. Soc.* **2005**, *127*, 16912−16920.