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Morpholine-Based Immonium and Halogenoamidinium Salts as Coupling Reagents in Peptide Synthesis[1]

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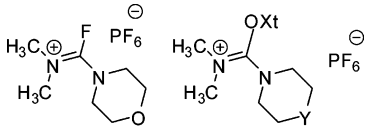

Here we describe a new family of *N*-form immonium-type coupling reagents that differ in their carbocation skeleton structure. The *N*-methylpiperazine derivative failed to form immonium salts, while the thiomorpholine derivative did not give better results than the coupling reagents currently used. The presence of the morpholine had a marked influence on the solubility and stability as well as the reactivity of the reagent. Finally, the fluoroamidinium salt performed extremely well in the presence of only 1 equiv of base, thereby confirming the effect of the proton acceptor in the reaction.

Introduction

Peptide synthesis depends on the proper combination of protecting groups and the right choice of the coupling method.[2] The two main classes of coupling techniques are (i) those that require in situ activation of the carboxylic acid and (ii) those that require an activated species that has previously been prepared (usually from an in situ activation step), isolated, purified, and characterized.[3] Among the first, the predominance of carbodiimides is being replaced with the stand-alone reagents, such as immonium and phosphonium salts, phosphates, and phosphinates.[3] Immonium salts,[4] probably the most widely used and most powerful salts,[5] are formed by a leaving group and an immonium skeleton (Figure 1). The mechanism involves an attack of the carboxylate on the electron-deficient carbon of the immonium salt to give the corresponding acyloxyamidinium salt, which is unstable and reacts with the leaving group originally presented in the immonium coupling reagent to give the active species, which undergoes aminolysis to give the target amide bond (Scheme 1). The driving force of this process is the generation of urea. Couplings involving immonium salts are carried out with an excess of base, which can promote racemization.

Although a considerable amount of work has been done to identify the best leaving groups,[3,6] less attention has been paid

(1) Abbreviations not defined in text: Aib, α-aminoisobutyric acid; DCM, dicloromethane; DIEA, *N*,*N*-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; HOBt, 1-hydroxybenzotriazole; HOAt, 7-aza-1-hydroxybenzotriazole; HOPfp, pentafluorophenol; 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; HAM_2PipU, *O*-(1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-yl)-1,1-dimethyl-3,3-pentamethyleneuronium hexafluorophosphate; HBM_2PipU, *O*-(1*H*-benzotriazol-1-yl)-1,1-dimethyl-3,3-pentamethyleneuronium hexafluorophosphate; TFFH, tetramethylfluoroformamidinium hexafluorophosphate, TFA = trifluoroacetic acid; TMP, 2,4,6-trimethylpyridine (collidine); Z, benzyloxycarbonyl; Boc, *tert*-butyloxycarbonyl. DMCH, *N*-(Chloro(morpholino)methylene)-*N*-methylmethanaminium hexafluorophosphate; HDMA, 1-((dimethylamino)-(morpholino)methylene)-1*H*-[1,2,3]triazolo[4,5-*b*]pyridinium hexafluorophosphate 3-oxide; HDMB, 1-((dimethylamino)(morpholino)methylene)-1*H*-benzotriazolium hexafluorophosphate 3-oxide; 4-HDMA, 3-((dimethylamino)-(morpholino)methylene)-1*H*-[1,2,3]triazolo[4,5-*b*]pyridinium hexafluorophosphate 1-oxide; 6-HDMCB, 6-chloro-1-((dimethylamino)(morpholino)-methylene)-1*H*-benzotriazolium hexafluorophosphate 3-oxide; 6-HDMFB, 6-trifluoromethyl-1-((dimethylamino)(morpholino)methylene)-1*H*-benzotriazolium hexafluorophosphate 3-oxide; HDMPfp, 1-((dimethyamino)-(morpholino))oxypentafluorophenyl metheniminium hexafluorophosphate; HDMS, 1-((dimethyamino)(morpholino))oxypyrrolidine-2,5-dione methanaminium hexafluorophosphate; HDTMA, 1-((dimethylamino)(thiomorpholino)methylene)-1*H*-[1,2,3]triazolo[4,5-*b*]pyridinium hexafluorophosphate 3-oxide; HDTMB, 1-((dimethylamino)(thiomorpholino)methylene)-1*H*-benzotriazolium hexafluorophosphate 3-oxide; DMFH, *N*-(fluoro(morpholino)methylene)-*N*-methylmethanaminium hexafluorophosphate. 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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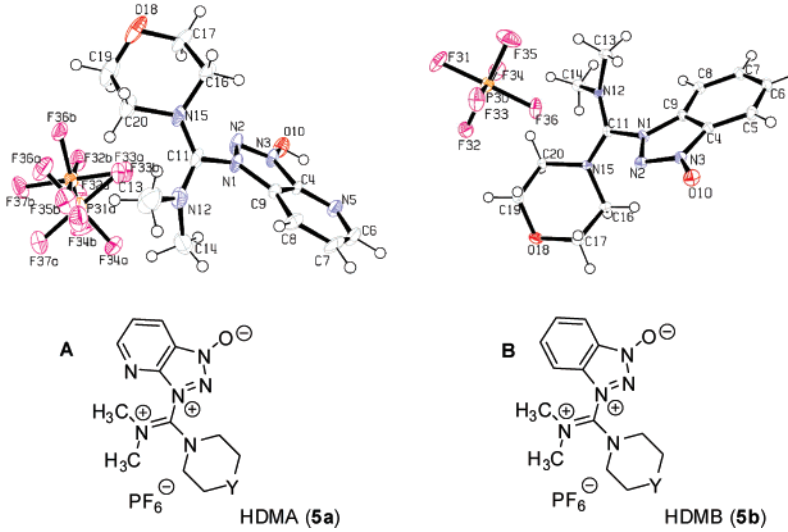
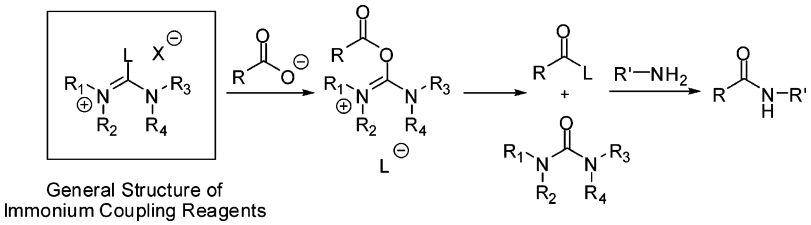


FIGURE 1. X-ray crystallography of HDMA (A) and HDMB (B).

SCHEME 1. General Structure of Immonium Coupling Reagents and Mechanism of the Amide Formation Using These Reagents



to the immonium part. Furthermore, in these cases, no additional heteroatoms have been included.[3,6a,i,l,o,7] Recently, we have shown that the incorporation of a proton acceptor in the immonium part showed superiority to those described previously.[8] Herein, this concept has been extended to other leaving groups, paying special attention to the use of halogens such as F and Cl.

Results and Discussion

Taking a *N*-containing 6-membered ring structure as a base, the corresponding nonsymmetrical immonium salts containing O, S, and NCH_3 were prepared by treating *N*,*N*-dimethylcarbamoyl chloride with the morpholine (**2i**), thiomorpholine (**2ii**), and *N*-methylpiperazine (**2iii**) to give the urea derivatives **3** (Scheme 2). Urea derivatives (**3i**,**ii**) from morpholine and thiomorpholine were reacted with oxalyl chloride to yield the corresponding chloro salts, which were stabilized by the formation of PF_6 salts (**4i**,**ii**). Subsequent reaction with the *N*-hydroxylamines (**8a**−**f**) and the pentafluorophenol (**8g**) in the form of the potassium salt or in the presence of Et_3N rendered the target compounds (**5**, **6**).

Furthermore, reaction of the urea derived from the *N*-methylpiperazine (**3iii**) with oxalyl chloride did not give the

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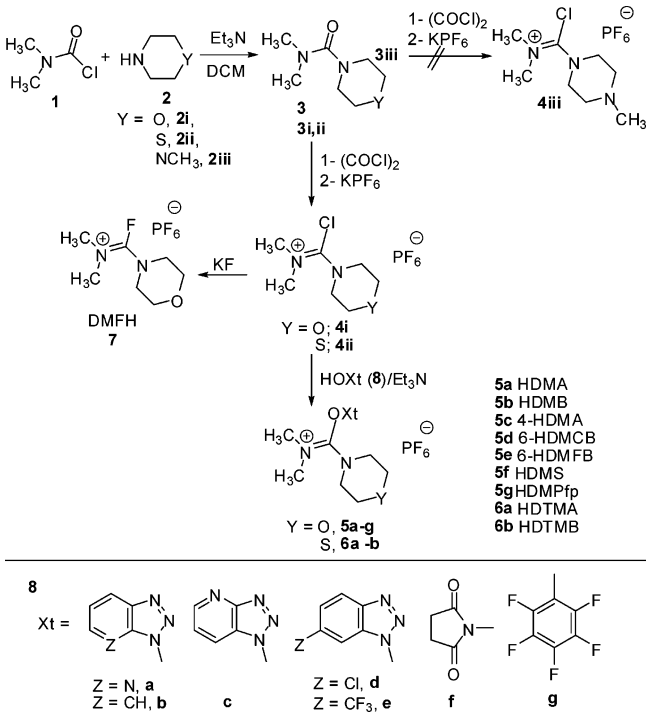
SCHEME 2. Procedure Followed for the Preparation of the Novel Proton Acceptor Immonium-Type Coupling Reagents



expected product, but only an oily residue that could not be purified or identified by NMR spectra.

Finally, the chloro salts (**4i**) were converted into their corresponding fluoride (**7**) by reaction with KF (Scheme 2).

X-ray crystallography showed that both HDMA (**5a**) and HDMB (**5b**) were in the *N*-form, as expected when compared with similar derivatives.[9] Furthermore, the ^{13}C NMR for these two compounds indicated displacements of the *imino carbon* [C═N^+(Me_2)] 149.65 ppm for HDMA (**5a**) and 150.65 ppm for HDMB (**5b**), which are consistent with those reported [151.63 and 151.42 ppm respectively for the counterparts HATU (**9**) and HBTU (**10**)[10]] for this kind of compound.[11]

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(10) Representative immonium salt coupling reagents:

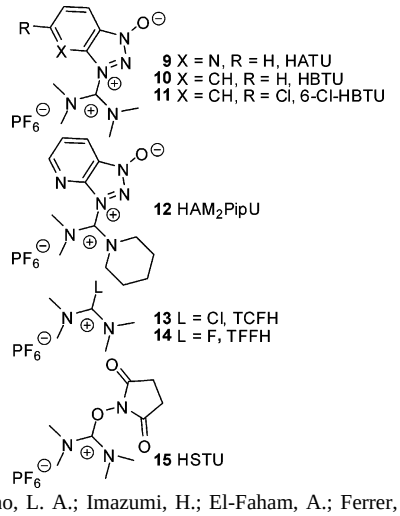

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TABLE 1. Hydrolytic Stability of Immonium-Type Coupling Reagents in DMF (Open Vials)

entry	coupling reagent	5 h (%)	24 h (%)	48 h (%)
1	HATU (**9**)	99	95	76
2	HBTU (**10**)	100	98	86
3	HDMA (**5a**)	100	96	83
4	HDMB (**5b**)	100	100	90

TABLE 2. Solubility in DMF of the Immonium-Type Coupling Reagents

entry	coupling reagent	wt/1 mL	molarity
1	HATU (**9**)	0.165	0.43
2	HBTU (**10**)	0.175	0.46
3	HCTU (**11**)	0.207	0.50
4	HDMA (**5a**)	0.285	0.68
5	HDMB (**5b**)	0.350	0.83
6	6-HDMCB (**5d**)	0.456	1.00
7	6-HDMFB (**5e**)	0.475	1.02

To determine the compatibility of the new coupling reagents (**5**) with peptide synthesis in both manual and automatic mode, their solubility and stability in solution and in the solid state was examined via ^1H NMR analysis. Table 1 indicates that the oxygen in the structure increases the stability of the coupling reagent when compared with the tetramethyl derivatives (entries 1 vs 3 and 2 vs 4). Reagents **5a**,**b** showed stability greater than 95% in a closed vial. The presence of the oxygen in the skeleton showed the most remarkable effects on the stability of the most hygroscopic and less stable chloro derivatives (**4i**). The NMR spectra showed only 18% hydrolysis (2% after 1 week and 10% after 1 month) after 2 months of storage at room temperature in a closed plastic vial.[12] These observations have a practical implication for both solid-phase and solution strategies. Thus, if the activation of a carboxylic acid is slow, the coupling reagents will be degraded and no longer be able to activate the carboxyl function. This is crucial for cyclization steps or in convergent strategies during the fragment coupling steps because yields tend to be lower than for other couplings.

Table 2 indicates that the presence of the oxygen atom in the carbon skeleton is of marked importance for the solubility of the compound. Thus, all morpholine derivatives (**5**) were more soluble than the dimethylamine ones (**9**−**11**) (entries 1 vs 4, 2 vs 5, 3 vs 6). Furthermore, 6-HDMCB (**5d**) and 6-HDMFB (**5e**) were the most soluble and could be used to prepare up to 1 M solution. This enhanced solubility should be used to prepare more concentrated solution to enhance coupling rates and to remove the excess and the urea side products during the workup after the coupling in a solution mode approach.

The efficacy of the new morpholine derivatives to reduce racemization was examined using two models systems (Z-Phg-OH + H-Pro-NH_2 and Z-Phe-Val-OH + H-Pro-NH_2) in solution.[13] Table 3 [(1 + 1) model] indicates that in all cases the morpholine derivatives resulted in improved control of racemization when compared with their tetramethylamino counterparts (entries 2, 5, 14, 16 vs 1, 4, 13, 15, respectively). A good example is the case of the chloro salts (entries 16 vs 15) where racemization decreased from 29.9% to 6.9%. For the morpholine derivatives (entries 2, 5, 14), the use of only 1 equiv

(12) In contrast, the tetramethyl (TCFH) derivatives have been described as very hygroscopic compounds (El-Faham, A. *Chem. Lett.* **1998**, *7*, 671−673.

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TABLE 3. Yield and Racemization during the Formation of Z-Phg-Pro-NH_2 in DMF[a]

entry	coupling reagent	base (equiv)	yield (%)	DL (%)	ref
1	HATU (**9**)	DIEA (2)	78.4	3.1	8
		DIEA (1)	74.8	2.4	
2	HDMA (**5a**)	DIEA (2)	81.2	1.6	8
		DIEA (1)	82.3	1.6	
3	HDTMA (**6a**)[b]	DIEA (2)	83.0	2.1	
4	HBTU (**10**)	DIEA (2)	80.2	8.2	8
		DIEA (1)	75.0	5.3	
5	HDMB (**5b**)	DIEA (2)	80.8	3.8	8
		DIEA (1)	82.3	3.1	
6	HDTMB (**6b**)[b]	DIEA (2)	81.3	3.5	
7	6-HDMCB (**5d**)	DIEA (2)	84.5	1.5	8
8	6-HDMFB (**5e**)	DIEA (2)	84.1	0.6	
9	HDMPfp (**5g**)	DIEA (2)	80.1	15.7	
10	HDMS (**5f**)	DIEA (2)	79.6	14.9	
11	HSTU (**15**)[b]	DIEA (2)	80.0	18.9	
12	4-HDMA (**5c**)	DIEA (2)	83.7	3.7	
13	TFFH (**14**)	DIEA (2)	80.1	7.1	
		DIEA (1)	77.4	6.5	
14	DMFH (**7**)	DIEA (2)	82.6	4.9	
		DIEA (1)	83.7	1.2	
15	TCFH (**13**)	DIEA (2)	80.0	29.9	
16	DMCH (**4i**)	DIEA (2)	81.9	6.9	
		DIEA (1)	80.3	5.2	
17	HAM_2PipU (**12**)	DIEA (2)	80.3	3.3	
		DIEA (1)	78.4	2.4	

[a] Couplings were carried out without preactivation, as described in the Experimental Section. LL and DL forms of the test dipeptide have been described elsewhere.[13] The t_R of LL and DL were identified by coinjection with pure samples of LL. [b] The HPLC showed a peak at 5.6 min for the urea, which indicates that the thiomorpholine urea derivatives do not wash out completely during the normal work up.

TABLE 4. Yield and Racemization during the Formation of Z-Phe-Val-Pro-NH_2 (2 + 1) in DMF[a]

entry	coupling reagent	base (equiv)	yield (%)	LDL (%)	ref
1	HATU (**9**)	DIEA (2)	85.8	13.9	8
		DIEA (1)	83.2	11.0	
		TMP (2)	78.0	5.3	
		TMP (1)	76.1	4.9	
2	HDMA (**5a**)	DIEA (2)	89.3	10.5	8
		DIEA (1)	87.4	5.1	
		TMP (2)	86.2	3.7	
		TMP (1)	84.1	3.8	
3	HDTMA (**6a**)	DIEA (2)	81.3	22.6	
4	HBTU (**10**)	DIEA (2)	89.7	27.7	8
		DIEA (1)	78.6	16.3	
		TMP (2)	81.2	14.2	
5	HDMB (**5b**)	DIEA (2)	88.7	20.3	8
		DIEA (1)	86.3	11.5	
		TMP (2)	87.1	13.3	
		TMP (1)	80.1	10.5	
6	HDTMB (**6b**)	DIEA (2)	82.2	29.8	
7	6-HDMCB (**5d**)	DIEA (1)	79.9	13.9	
8	6-HDMFB (**5e**)	DIEA (2)	81.3	31.4	
9	HDMPfp (**5g**)	DIEA (2)	75.8	37.1	
10	HDMS (**5f**)	DIEA (2)	78.3	35.2	
11	HSTU(**15**)[b]	DIEA (2)	89.1	37.8	
12	4-HDMA (**5c**)	DIEA (2)	80.0	28.9	
13	HAM_2PipU (**12**)	DIEA (2)	88.9	11.3	
		DIEA (1)	76.5	10.6	
		TMP (2)	85.5	4.1	

[a] LLL and DL forms of the test tripeptide have been described elsewhere[13] and coinjected with authentic and pure samples. [b] Extra peaks were observed at 17.3 min (25.0%) and at 17.6 min (6.1%).

TABLE 5. Yield and Racemization during the Formation of Z-Gly-Phe-Pro-NH_2, Z-Gly-Phe-Ala-OMe, and Z-Gly-Phe-Val-OMe (2 + 1) in DMF (Solution-Phase Synthesis)[a]

entry	peptide	coupling reagent	base (equiv)	yield (%)	l (%)
1	Z-Gly-Phe-Pro-NH_2	HATU (**9**)	DIEA (2)	90.1	1.6
			DIEA (1)	80.1	0.9
			TMP (2)	86.8	0.9
			TMP (1)	76.9	0.8
2		HDMA (**5a**)	DIEA (2)	89.9	1.0
			DIEA (1)	88.9	0.9
			TMP (2)	86.9	0.9
3		HDTMA (**6a**)	DIEA (2)	78.8	1.6
4		HBTU (**10**)	DIEA (2)	88.9	5.9
			DIEA (1)	78.9	4.1
			TMP (2)	84.8	3.6
5		HDMB (**5b**)	DIEA (2)	89.6	2.8
			DIEA (1)	88.4	2.1
			TMP (2)	87.3	2.9
6		HDTMB (**6b**)	DIEA (2)	79.1	3.9
7		4-HDMA (**5c**)	DIEA (2)	90.3	3.6
8		6-HDMCB (**5d**)	DIEA (2)	78.9	1.5
9		6-HDMFB (**5e**)	DIEA (2)	84.5	2.8
10		HSTU (**15**)	DIEA (2)	91.2	12.5
11		HDMS (**5f**)	DIEA (2)	83.4	11.3
12		HDMPfp (**5g**)	DIEA (2)	84.1	32.0
13		HPyOPfp	DIEA (2)	85.6	33.7
14	Z-Gly-Phe-Ala-OMe	HDMA (**5a**)	DIEA (2)	90.1	0.06
			TMP (2)	89.3	0.12
15		HDMB (**5b**)	DIEA (2)	89.8	0.03
			TMP (2)	88.9	0.11
16	Z-Gly-Phe-Val-OMe	HATU (**9**)	DIEA (2)	90.1	1.56
			TMP (2)	86.8	0.82
18		HDMA (**5a**)	DIEA (2)	89.9	0.65
			TMP (2)	88.6	0.65
17		HBTU (**10**)	DIEA (2)	88.9	5.90
			TMP (2)	84.8	3.57
19		HDMB (**5b**)	DIEA (2)	89.7	2.90
			TMP (2)	88.3	3.39

[a] LL and DL forms of the test dipeptide have been described elsewhere. The t_R of LL and DL were identified by coinjection with authentic and pure samples of LL.

of base caused a slight increase in yield and a decrease in racemization, while for the tetramethylamine derivatives (entries 1, 4, 13) the decrease in racemization was also accompanied by a decrease in yield.[14] In this model, the thiomorpholine derivatives showed a similar performance to the morpholine ones (entries 3, 6 vs 2, 4). Interestingly, the chloro (**5d**, entry 7) and the trifluoromethyl (**5e**, entry 8), which contains the less reactive and expensive benzotriazoles when compared with the aza derivatives, performed well and are good alternatives to the aza derivatives. The compounds that contained less reactive leaving groups rendered more racemization (entries 9−12). Finally, HDMA (**5a**) compared favorably with HAM_2PipU (**12**), which contains a piperidine ring instead of the morpholine to discharge the possible six-membered ring effect.

A similar tendency was observed when the most demanding [2 + 1], Z-Phe-Val-OH + H-Pro-NH_2, model was used. The largest discrepancy with the previous experiments is the poorer behavior of the thiomorphonilo derivatives (entries 3, 6) as indicated in Table 4. As expected, the use of the less basic TMP considerably reduced the level of racemization when compared with DIEA.

For the rather nonsensitive case of segment coupling, such as Z-Gly-Phe-OH to H-Val-OMe, Z-Phe-Val-OH to H-Ala-OMe, and Z-Gly-Phe-OH to H-Pro-NH_2 leading to corresponding tripeptides,[13] the immonium salts derived from morpholine again performed better than diemthylamino derivatives (Table 5).

In order to demonstrate the effectiveness of the new morpholine coupling reagents in solid-phase mode, the nonapeptide H-Met-Pro-Pro-Glu-Val-Lys-Phe-Leu-NH_2 was assembled on the solid-phase. The peptide was manually elongated on an Fmoc-Rink-Amide-resin. Coupling times were reduced (15 min) as well as the excess of reagents [3 equiv for the coupling reagent and 6 equiv for DIEA (3 equiv for the fluoro derivative DMFH)] with preactivation time (5 min). Peptide purity as determined by reversed-phase HPLC analysis: HDMA (**5a**), 88%; HATU (**9**), 84%; HDMB (**5b**), 91%; HBTU (**10**), 81%;

(14) The most unstable chloro derivative (**4i**, entry 16) does not follow this trend.

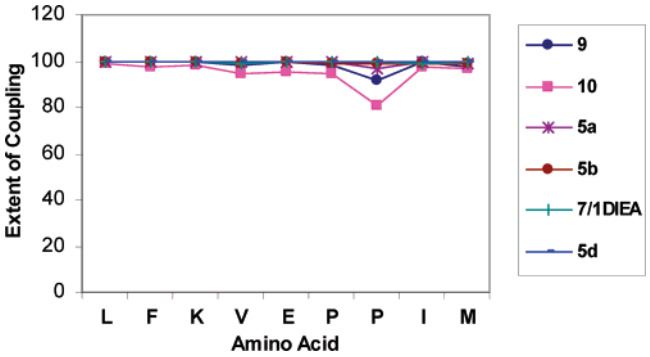

FIGURE 2. Extent of coupling with 1−2 min of preactivation in the presence of 6 equiv of DIEA, Fmoc-amino acids, and coupling reagents (3 equiv), except for DMFH (**7**) (5 equiv), 15 min coupling.

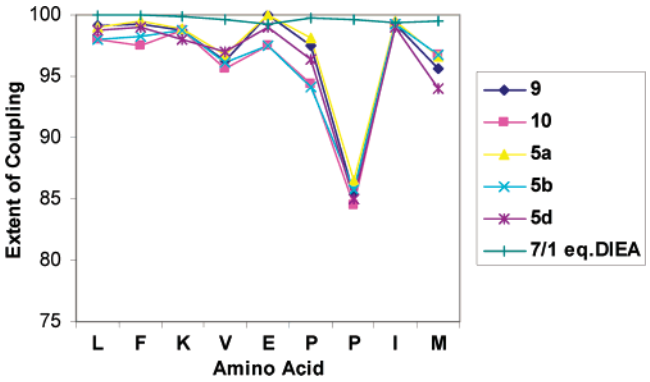

FIGURE 3. Extent of coupling with 7 min preactivation in the presence of 6 equiv of DIEA, Fmoc-amino acidsv and coupling reagents (3 equiv) except for DMFH (**7**) (5 equiv), 15 min coupling time.

6-HDMCB (**5d**), 91%; DMFH (**7**), 89%. Furthermore, the extent of each coupling was calculated using the UV spectroscopy for the dibenzofulvene (DBF) adduct at λmax of 300.5 (Figures 2 and 3). Again, the morpholine derivatives gave purity in all cases around 90%, while the purity of the tetramethylamine derivatives (**9**, **10**) was around 81−84%. Again, HDMB (**5b**) and 6-HDMCB (**5d**) performed similarly to the aza derivatives HDMA (**5a**) and HATU (**9**), which contain the most expensive HOAt. Remarkably interesting is the result with DMFH (**7**), which performed extremely well with only 1 equiv of base.

In a more demanding example, H-Tyr-Aib-Aib-Phe-Leu-NH_2[6d] was manually assembled on Fmoc-Rink Amide.AM-resin using amino acid/activator (3 equiv), DIEA (6 equiv) or (3 equiv), using 30 min coupling time except for the case of Aib-Aib, for which 1 h was used. Yields for the coupling of Fmoc-Aib-OH onto the Aib-containing resin were determined by reversed-phase HPLC analysis, after cleavage of the peptide from the resin (Table 6). The best results were obtained with HDMA (**5a**) and 6-HDMCB (**5d**) with 2 equiv of base, while the fluoro salt DMFH (**7**) gave better results with 1 equiv of base. In addition, DMCH (**4**) in the presence of 6-ClHOBt gave equally good results as **5a** and **7**. Again, the thiomorpholine derivatives (**6a**,**b**) gave slightly worse results when compared with the morpholine ones.

Conclusions

In conclusion, herein a new family of *N*-form immonium-type coupling reagents that differ in their carbocation skeleton structure 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 (yield and control of the racemization). The salt derived from morpholine derivatives are the most stable to air. These results should be taken into account when coupling reagents are placed in open vessels, such as in some automatic synthesizers. HOAt derivatives were confirmed to be superior to HOBt ones in terms of both coupling yield and retention of configuration for all cases. Remarkably, the 6-ClHOBt derivative gave equally good results as the aza derivatives. Finally, the fluoride salt performed extremely well in the presence of only 1 equiv of base, thereby confirming the effect of the O proton acceptor in the reaction.

TABLE 6. Yield for the Coupling of Fmoc-Aib-OH onto the Aib-Phe-Leu-NH-resin Calculated by Tyr-Aib-Aib-Phe-Leu-NH_2 vs Tyr-Aib-Phe-Leu-NH_2[a]

entry	coupling reagent	base (equiv)	penta (%)	Des-Aib (%)	ref
1	HATU (**9**)	DIEA (2)	83	17	8
		DIEA (1)	68	32	
2	HDMA (**5a**)	DIEA (2)	98	2	8
		DIEA (1)	90	10	
3	HDTMA (**6a**)	DIEA (2)	80	20	
4	HBTU (**10**)	DIEA (2)	47	53	8
		DIEA (1)	33	67	
5	HDMB (**5b**)	DIEA (2)	89	10	8
		DIEA (1)	64	36	
6	HDTMB (**6b**)	DIEA (2)	75	25	
7	6-HDMCB (**5d**)	DIEA (2)	99	<1	8
		DIEA (1)	39	62	
8	6-HDMFB (**5e**)	DIEA (2)	78	22	
		DIEA (1)	30	70	
9	4-HDMA (**5c**)	DIEA (2)	98	2	
10	TFFH (**13**)	DIEA (2)	95	5	
		DIEA (1)	94	6	
11	DMFH (**7**)	DIEA (2)	96	4	
		DIEA (1)	99	<1	
12	TCFH (**13**)	DIEA (2)	9	91	
		DIEA (1)	10	90	
13	DMCH (**4i**)	DIEA (2)	43	56	
		DIEA (1)	49	51	
14	DMCH (**9**)/6-Cl-HOBt	DIEA (2)	99	<1	

[a] Tetrapeptide (des-Aib) was confirmed by peak overlap in the presence of an authentic sample. The crude H-Tyr-Aib-Aib-Phe-Leu-NH_2 was analyzed by HPLC. HPLC−MS showed the right mass for the penta at 612.0.

Experimental Section

General Procedure for the Preparation of Urea Derivatives.[6g] *N*,*N*-Dialkylcarbamoyl chloride (0.6 mol) was added dropwise to a stirring mixture of secondary amine (0.5 mol) and triethylamine (TEA) (0.5 mol)[15] in DCM (400 mL) at 0 °C. When the addition was completed, the mixture was stirred for 3−4 h at room temperature. The reaction mixture was basified with 10% NaOH, and then the organic layer was collected and the aqueous layer washed with 100 mL of DCM. The combined DCM solution was washed with H_2O (2 × 100 mL) and saturated solution of NaCl (2 × 100 mL). Finally, the organic solvent was dried over anhydrous $MgSO_4$ and filtered, and the solvent was removed under reduced pressure. The oily residue obtained was purified by vacuum distillation.

***N*,*N*-Dimethyl-4-morpholinecarboxamide, 3i.**[16] The urea derivative was distilled and collected at 127−129 °C as a colorless

(15) Alternatively, NaOH can be used instead of TEA (ref 8).

(16) Solomo, T. (Ciba-Geigy A.-G.). Ger. Offen. 2206366 19720831, 1972, Patent written in German. Application: DE 72-2206366 19720210.

oil with a 92.4% yield. ^{1}H NMR ($CDCl_3$): δ 2.84 (s, 6H, 2 CH_3), 3.22−3.20 (m, 4H, 2CH_2), 3.68−3.70 (m, 4H, 2CH_2) ppm. ^{13}C NMR ($CDCl_3$): δ 38.6, 47.5, 66.9, 165.0 ppm.

***N,N*-Dimethyl-4-thiomorpholinecarboxamide, 3ii.**[16] The pure urea was obtained as light brown crystals with an 89.5% yield. Mp: 61−62 °C. ^{1}H NMR ($CDCl_3$): δ 2.62−2.65 (m, 4H, 2CH_2), 2.81 (s, 6H, 2CH_3), 3.48−3.50 (m, 4H, 2CH_2) ppm. ^{13}C NMR ($CDCl_3$): δ 27.4, 38.77, 5.38, 165.2 ppm. Anal. Calcd for $C_7H_{14}N_2$-OS: C, 48.25; H, 8.10; N, 16.08. Found: C, 48.35; H, 8.15, N, 16.18.

***N,N*-4-Trimethyl-1-piperazinecarboxamide, 3iii.**[17,18] The pure urea derivative was collected at 120−122 °C as a pale yellow oil (1−2 mmHg) following method A, in 87.4% yield (74.7 g). IR (BaF_2): 2936−2791 (Sp,[3] H), 1650 (CO) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 2.20 (s, 3H, CH_3), 2.31−2.34 (m, 4H, 2CH_2), 2.75 (s, 6H, 2CH_3), 3.18−3.20 (m, 4H, 2CH_2) ppm. ^{13}C NMR ($CDCl_3$): δ 38.7, 46.4, 46.9, 55.1, 165.0 ppm.

***N*-(Chloro(morpholino)methylene)-*N*-methylmethanaminium Hexafluorophosphate (DMCH, 4i).**[8] Oxalyl chloride (100 mmol) in dichloromethane (DCM) (100 mL) was added dropwise to a solution of urea derivative **3i** (100 mmol) in dry DCM (200 mL) at room temperature over 5 min. The reaction mixture was stirred under reflux for 3 h, the solvent was removed under vacuum, and the residue was washed with anhydrous ether (2 × 100 mL) and then bubbled with N_2 to remove excess of the ether. The white solid obtained was dissolved in DCM (500 mL), and a saturated aqueous KPF_6 solution (18.4 g in 50 mL H_2O) was added at room temperature with vigorous stirring for 10−15 min. The organic layer was collected, washed once with water (50 mL), dried over anhydrous $MgSO_4$, and filtered, and the solvent was then removed under reduced pressure to give a white solid which recrystallized from DCM ether to give white crystals (28.9 g, 89.6% yield). Mp: 94−95 °C. ^{1}H NMR (CD_3COCD_3): δ 3.39 (s, 6H, 2CH_3), 3.75(t, 4H, 2CH_2), 3.86 (t, 4H, 2CH_2) ppm. ^{13}C NMR (CD_3COCD_3): δ 44.4, 52.8, 66.0 ppm. Anal. Calcd for $C_7H_{14}ClF_6N_2OP$ (322): C, 26.06; H, 4.37; N, 8.68. Found: C, 25.94; H, 4.43; N, 8.79.

General Method for the Synthesis of Immonium-Type Coupling Reagents, 5a−g, 6a,b. The chloro salt **4** (6.45 g) was added to a solution of HOXt (**8**) (20 mmol) and TEA (20 mmol, 2.8 g) in DCM (50 mL) or KOXt (20 mmol) in CH_3CN (40 mL) at 0 °C. The reaction mixture was stirred at this temperature and then left at room temprature overnight. The solid was filtered and washed with cooled DCM. The white solid was recrystallized from CH_3CN−ether.

1-((Dimethylamino)(morpholino)methylene)-1*H*-[1,3]triazolo-[4,5-*b*]pyridinium Hexafluorophosphate 3-Oxide (HDMA, 5a).[8] The product was obtained as a white solid (91.2% yield). Mp: 194−195 °C dec. ^{1}H NMR (CD_3COCD_3): δ 3.27 (s, 3H, CH_3), 3.64 (s, 3H, CH_3), 3.85−3.89 (m, 4H, 2CH_2), 4.00−4.07 (m, 4H, 2CH_2), 7.93−7.96 (dd, 1H, ar), 8.44−8.47 (dd,1H, ar), 8.76−8.77 (dd, 1H, ar) ppm.[13] C NMR (CD_3COCD_3): δ 41.7, 42.3, 50.8, 51.6, 66.2, 66.4, 124.4, 127.84, 149.65 ppm. MS *m*/*z* using MALDI with ACH matrix 422.2.Anal. Calcd for $C_{12}H_{17}F_6N_6O_2P$ (422): C, 34.13; H, 4.06; N, 19.90. Found: C, 34.31; H, 4.17; N, 20.13.

1-((Dimethylamino)(morpholino)methylene)-1*H*-benzotriazolium hexafluorophosphate 3-Oxide (HDMB, 5b).[8] The product was obtained as a white solid (7.54 g, 88.27% yield). Mp: 196−197 °C dec. ^{1}H NMR (CD_3COCD_3): δ 3.22 (s, 3H, CH_3), 3.51 (s, 3H, CH_3), 3.66−3.88 (m, 4H, 2CH_2), 4.03−4.06 (m, 4H, 2CH_2), 7.65−7.96 (dt, 1H, ar), 7.86−7.92 (dm,2H, ar), 7.98.7.99 (dd, 1H, ar) ppm. ^{13}C NMR (CD_3COCD_3): δ 41.8, 42.2, 50.8, 51.5, 66.2, 66.4, 110.0, 114.6, 127.5, 133.5 ppm. Anal. Calcd for $C_{13}H_{18}F_6N_5O_2P$ (421): C, 37.06; H; 4.31; N, 16.62. Found: C, 36.98; H, 4.26; N, 16.73. MS *m*/*z* using MALDI with ACH matrix 421.23.

3-((Dimethylamino)(morpholino)methylene)-1*H*-[1,3]triazolo-[4,5-*b*]pyridinium Hexafluorophosphate 1-Oxide (4-HDMA, 5c). The product was obtained as a pale yellow solid (88.9% yield). Mp: 208−210 °C dec. ^{1}H NMR (CD_3COCD_3): δ 3.30 (s, 3H, CH_3), 3.71 (s, 3H, CH_3), 3.75−4.02 (m, 4H, 2CH_2), 4.11−4.16 (m, 4H, 2CH_2),7.86−7.89 (dd, 1H,ar), 8.58−8.61 (dd,1H,ar), 9.09−9.11 (dd,1H,ar) ppm. ^{13}C NMR (CD_3COCD_3): δ 42.1, 42.5, 50.7, 51.8, 66.1, 123.3, 126.3, 155.4 ppm. Anal. Calcd for $C_{12}H_{17}F_6N_6O_2P$ (422): C, 34.13; H, 4.06; N, 19.90. Found: C, 34.22; H, 4.11; N, 19.78.

6-Chloro-1-((dimethylamino)(morpholino)methylene)-1*H*-benzotriazolium hexafluorophosphate 3-Oxide (6-HDMCB, 5d).[8] The product was obtained as a white solid (93.5% yield). Mp: 193−194 °C dec. ^{1}H NMR (CD_3COCD_3): δ 3.31(s, 3H, CH_3), 3.69(s, 3H, CH_3), 3.94−3.951(m, 4H, 2CH_2), 4.12−4.14 (m, 4H, 2CH_2), 7.96−8.03 (qd, 2H, ar), 8.12−8.13 (dd,1H, ar) ppm. ^{13}C NMR (CD_3COCD_3): δ 41.8, 42.3, 50.8, 51.8, 66.2, 66.4, 115.8, 116.2, 132.8, 133.9, 150.5 ppm. Anal. Calcd for $C_{13}H_{17}ClF_6N_5O_2P$ (455): C, 34.26; H, 3.76; N, 15.37. Found: C, 34.39; H, 3.83; N, 15.54.

6-Trifluoromethyl-1-((dimethylamino)(morpholino)methylene)-1*H*-benzotriazolium Hexafluorophosphate 3-Oxide (6-HDMFB, 5e). The product was obtained as a white solid (81.5% yield). Mp: 194−195 °C. ^{1}H NMR (CD_3COCD_3): δ 3.34 (s, 3H, CH_3), 3.72 (s, 3H, CH_3), 3.95−3.961 (m, 4H, 2CH_2), 4.08−4.15 (m, 4H, 2CH_2), 8.24−8.27 (qd, 2H, ar), 8.43 (t,1H, ar) ppm. ^{13}C NMR (CD_3-$COCD_3$): δ 41.8, 42.4, 50.9, 51.7, 66.2, 66.4, 114.5, 116.4, 129.9, 150.5 ppm. Anal. Calcd for $C_{14}H_{17}F_9N_5O_2P$ (489): C, 34.37; H, 3.50; N, 14.31. Found: C, 34.49; H, 3.63; N, 14.50.

1-((Dimethyamino)(morpholino))oxypentafluorophenyl Methanaminium Hexafluorophosphate (HDMPfp, 5f). The product was obtained as a white solid (91% yield). Mp: 202−203 °C. ^{1}H NMR (CD_3COCD_3): δ 3.38 (s, 6H, CH_3), 3.80−3.83 (m, 4H, CH_2), 3.86−3.89 (m, 4H, 2CH_2) ppm. ^{13}C NMR (CD_3COCD_3): δ 40.6, 49.3, 65.7, 159.7 ppm. Anal. Calcd for $C_{13}H_{15}F_{11}N_2O_2P$ (471): C, 33.13; H, 3.21; N, 5.94. Found: C, 33.33; H, 3.13; N, 6.12.

1-((Dimethyamino)(morpholino))oxypyrrolidine-2,5-dione Methanaminium Hexafluorophosphate (HDMS, 5g). The product was obtained as a white solid (78.3% yield). Mp: 192−194 °C. ^{1}H NMR (CD_3COCD_3): δ 3.03 (s, 4H, 2CH_2), 3.35 (s, 6H, 2CH_3), 3.82−3.85 (m, 8H, 4CH_2) ppm. ^{13}C NMR (CD_3COCD_3): δ 25.9, 49.4, 65.8, 161.5, 170.2 ppm. Anal. Calcd for $C_{11}H_{19}F_6N_3O_4P$ (402): C, 32.84; H, 4.76; N, 10.45. Found: C, 32.69; H, 4.70; N, 10.58.

1-((Dimethylamino)(thiomorpholino)methylene)-1*H*-[1,3]triazolo[4,5-*b*]pyridinium Hexafluorophosphate 3-Oxide (HDTMA, 6a). The crude chloro salt (**4ii**)[19] (20 mmol) at 0 °C was added to a solution of HOAt (2.72 g, 20 mmol) and TEA (2.8 g, 20 mmol) in DCM (50 mL). The reaction mixture was stirred at this temperature under N_2 and left at room temperature overnight. The solid was filtered and washed with cooled DCM. The pale yellow solid was recrystallized from CH_3CN ether to give white crystals (75.6%). Mp: 197−199 °C dec. ^{1}H NMR (CD_3COCD_3): δ 2.76−2.77 (m, 1H, CH), 2.97−3.01 (dt, 1H, CH), 3.14−3.23 (m, 2H, CH_2), 3.56 (s, 3H, CH_3), 3.71 (s, 3H, CH_3), 3.72−3.73 (dt, 1H, CH), 3.96−4.00 (dt, 1H, CH), 4.11−4.18 (m, 1H, CH), 4.39−4.45 (dt, 1H, CH), 8.00−8.03 (dd, 1H, ar), 8.47−8.50 (dd,1H, ar), 8.84−8.85 (dd, 1H, ar) ppm. ^{13}C NMR (CD_3COCD_3): δ 14.7, 27.0, 38.8, 45.5, 59.7, 120.5, 128.7, 135.2, 140.2, 150.2 ppm. Anal. Calcd for $C_{12}H_{17}F_6N_6OPS$ (438): C, 32.88; H, 3.91; N, 19.17. Found: C, 32.67; H, 4.03; N, 19.31.

1-((Dimethylamino)(thiomorpholino)methylene)-1*H*-benzotriazolium Hexafluorophosphate 3-Oxide (HDTMB, 6b). The white solid obtained was recrystallized from CH_3CN ether to give white crystals (80.1% yield). Mp: 190−191 °C dec. ^{1}H NMR (CD_3-$COCD_3$): δ 2.67−2.70 (m, 1H, CH), 2.91−2.96 (m, 1H, CH), 2.97 (s, 3H, CH_3), 3.33−3.48 (m, 5H, CH_2, CH_3), 3.65−3.69 (m, 1H,

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(19) **4ii** was used directly without storage because it is very sensitive to the moisture. The reaction should be under nitrogen atmosphere.

CH), 3.88−3.95 m, 1H, CH), 4.09−4.12 (m, 1H, CH), 8.00−8.03 (dd, 1H, ar), 7.68−7.72 (td,1H, ar), 7.86−7.88 (d, 1H, ar), 7.93−7.97 (d, 1H, ar), 8.08 (d, 1H, ar) ppm. ^{13}C NMR (CD_3COCD_3): δ 14.7, 27.3, 40.6, 40.8, 42.8, 43.1, 52.9, 53.2, 115.4, 116.5, 127.9, 133.6, 150.8 ppm. Anal. Calcd for $C_{13}H_{18}F_6N_5OPS$ (437): C, 35.70; H, 4.15; N, 16.01. Found: C, 35.87; H, 4.06; N, 16.22.

***N*-(Fluoro(morpholino)methylene)-*N*-methylmethanaminium Hexafluorophosphate (DMFH, 7).** Predried KF (0.3 mol) was added to a stirring solution of chloroformamidinium salt **4i** (0.1 mol) in dry CH_3CN (200 mL). The reaction mixture was stirred at room temperature for 6 h, filtered, and washed with CH_3CN (2 × 50 mL). The CH_3CN solutions were combined and concentrated under vacuum, and the residue was dissolved in hot DCM and then filtered. The filtrate was concentrated to approximately 1/3 of the original volume, and ether was added with vigorous stirring to promote the formation of a white solid (89.5 yield). Mp: 92−93 °C. ^{1}H NMR (CH_3CN-d_6): δ 3.31 (d, 6H, $2CH_3$), 3.70−3.81 (m, 4H, $2CH_2$) 3.84−3.86 (m, 4H, $2CH_2$) ppm.^{13} C NMR (acetone-d_6): 39.3, 43.6, 159.6 ppm. Anal. Calcd for $C_7H_{14}F_7N_2OP$ (306): C, 27.46; H, 4.61; N, 9.15. Found: C, 27.55; H, 4.64; N, 9.33.

Model Segment Coupling Reaction. Test couplings were carried out as previously described for Z-Phg-Pro-NH_2,[20] Z-Phe-Val-Pro-NH_2,[6f,20] Z-Gly-Phe-Val-OMe,[20] Z-Phe-Val-Ala-OMe,[20] and Z-Gly-Phe-Pro-NH_2.[6f,20]

Solid-Phase Synthesis of H-Met-Pro-Pro-Glu-Val-Lys-Phe-Leu-NH_2. The peptide was manually elongated on an Fmoc-Rink Amide-resin (0.7 mmol/g). Coupling times were 15 min; excesses and reagents were 3 equiv for the Fmoc-amino acids and the coupling reagent and 6 equiv for DIEA; pre-activation time 1−2 min. Peptide purity was determined after cleavage of the peptide from the resin by treatment with TFA−H_2O (9:1) for 2 h at room temperature by reverse-phase HPLC analysis (using Symmetry Waters C_{18} 4 μ, 4.6 × 150 mm.), linear gradient over 30 min of 10 to 90% CH_3CN in H_2O/0.1% TFA, flow rate 1.0 mL/min, t_R nonapeptide = 10.95 min).

Analysis of the Extent of Coupling Using UV during the Solid-Phase Synthesis of H-Met-Pro-Pro-Glu-Val-Lys-Phe-Leu-NH_2. After each coupling, 4−6 mg of the resin was taken, washed with DMF, DCM, and ether, and then dried under vacuum to obtain the exact weight. The dried resin was treated with 20% piperidine in DMF (2 mL) for 10 min and then diluted with ethanol up to 25 mL. The extent of coupling was calculated according to the λ_{max} at 300.5 for the dibenzofulvene adduct.

Solid-Phase Synthesis of H-Tyr-Aib-Aib-Phe-Leu-NH_2.[8] The pentapeptide was manually assembled on a Fmoc-PAL-PEG-PS-resin (0.18 mmol/g) with the preactivation time as indicated in Table 6, using Fmoc-amino acid (4 equiv), coupling reagent (4 equiv), and DIEA (8 equiv) in DMF at a total concentration of 0.3 M. A coupling time of 30 min was used for all amino acids except for Aib-Aib, for which the time was 60 min. The peptide was cleaved from the resin using TFA−H_2O (9:1) at room temperature for 2 h. The solution was filtered, and the TFA was removed under vacuum. The crude peptide was precipitated by addition of cold ether and analyzed on HPLC [Waters Symmetry Column C18, 5 μm, 4.6 × 150 mm, linear gradient of CH_3CN and H_2O containing 0.1% TFA each one, from 10% to 90% in 25 min, detection at 220 nm.] The t_R for the pentapeptide and for the des-Aib tetrapeptide was 8.80 and 9.10 min, respectively.

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

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