DOI: 10.1002/ejoc.200500630

Design and Synthesis of New Immonium-Type Coupling Reagents[‡]

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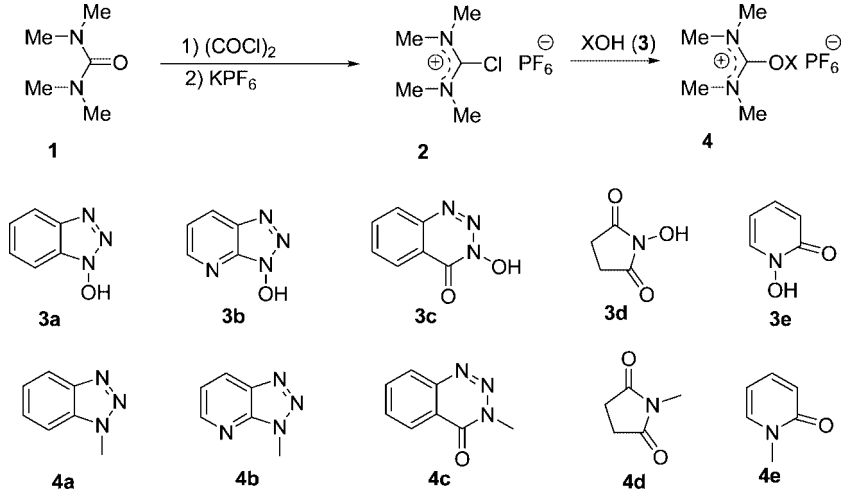
Keywords: Amides / Combinatorial chemistry / Peptides / Racemization / Solid-phase synthesis

A new family of immonium-type coupling reagents is described here. The differences in the carbocation skeletons of these reagents can be correlated with differences in stability and thus reactivity. The dihydroimidazole derivatives are highly unstable to air, whereas the salts derived from dimethylamine are the most stable and the pyrrolidino derivatives are of intermediate stability. These results should be taken into account mainly when coupling reagents are deposited in open vessels, such in some automatic synthesizers. As regards both coupling yield and retention of configuration, HOAt derivatives have been confirmed to be superior to those of HOBt in all cases. For peptides containing hindered residues, fluoroformamidinium salts are more convenient than the HOAt-based reagents.

The activation of a carboxylic acid for the formation of an amide bond is usually carried out using the so-called peptide coupling reagents.[2] During the last decade there has been an evolution in the development of new activation methods and their application to both solution and solid-phase methodologies.[2a,3] The formerly predominant carbodiimide and active-ester techniques have been replaced with onium salts based upon 1-hydroxybenzotriazole (HOBt, **3a**)[4] and 7-aza-1-hydroxybenzotriazole (HOAt, **3b**).[5] Among current methods of choice for peptide coupling reagents are the aminium/uronium derivatives (also known as Knorr reagents[6]), which have become popular because of their high efficiency and low tendency towards racemization of the amino acid or peptide residue. These salts are prepared by reaction of a compound of the type HOX (HOBt **3a**, HOAt **3b**, HODbht **3c**, and HOSu **3d**) with the chloroformamidinium salt **2** derived from an urea such as tetramethylurea (TMU, **1**; Scheme 1). Examples of these common aminium/uronium salts are the 1-hydroxybenzotriazole (HBTU) derivatives **4a**,[6,7] the 7-aza-1-hy-



Scheme 1.

[‡] For abbreviations see ref.[1]

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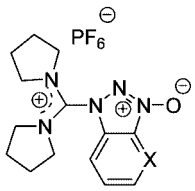
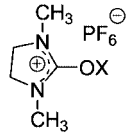
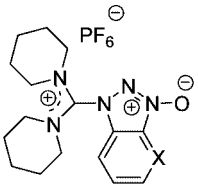


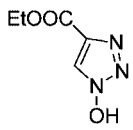
X = CH; **5**
X = N; **6**



X = OBt; **7**
X = OAt; **8**



X = CH; **9a**
X = N; **9b**



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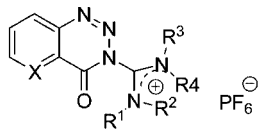
droxybenzotriazole (HATU) derivatives **4b**,[5] the 3-hydroxy-3,4-dihydro-1,2,3-benzotriazin-4-one (HDTU) derivatives **4c**,[6,8] the 1-hydroxy-1,2-dihydro-2-pyridone (HPTU) derivatives **4e**,[9] and the hydroxysuccinimide (HSTU) derivatives **4d**.[10] The majority of these compounds are commercially available. Further investigation has led to other aminium-type reagents such as HBPyU (**5**),[11] HAPyU (**6**),[12] HBMDU (**7**),[13] HAMDU (**8**),[12] HBPipU (**9a**),[12] and HAPipU (**9b**).[12]

In 1998, Ramage et al. reported the synthesis of HOCt (**10**),[14] which was designed for Fmoc solid-phase peptide synthesis. HOCt exhibits high coupling efficiency, but it should be used only as an additive in combination with carbodiimide reagents because its corresponding Knorr reagent decomposes during synthesis. This has also been observed during the preparation of imidazolidonium-type reagents.[9]

Recently, Xu and Li[15] have reported an alternative pathway to enhance the coupling efficiency by modifying the carbon skeleton of uronium salts by replacing one of the substituted amino groups with a hydrogen, alkyl, or aryl group (Scheme 2). The authors described that using these types of immonium reagents to synthesize peptides not only enhanced the coupling efficiency but also substantially suppressed the extent of racemization under relatively mild conditions.[15]

More recently, Carpino et al.[16] reported the new coupling reagents HDATU (**11**), and HDAPyU (**12**), which they prepared by a method analogous to that used for the preparation of HDTU (**4c**),[8] which is the HODhbt uronium salt reagent. As expected, **11** and **12** are more reactive than **4c**.

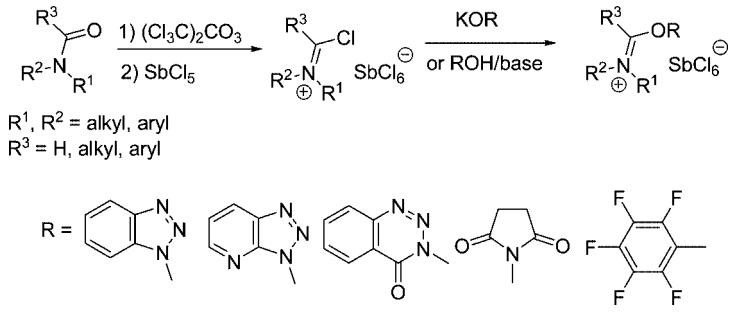


X = N; $R^1 = R^2 = R^3 = R^4 = CH_3$, HDATU; **11**
X = N; $R^1 = R^2 = R^3 = R^4 = (CH_2)_4$, HDAPyU; **12**
X = CH; $R^1 = R^2 = R^3 = R^4 = CH_3$, HDTU; **4c**

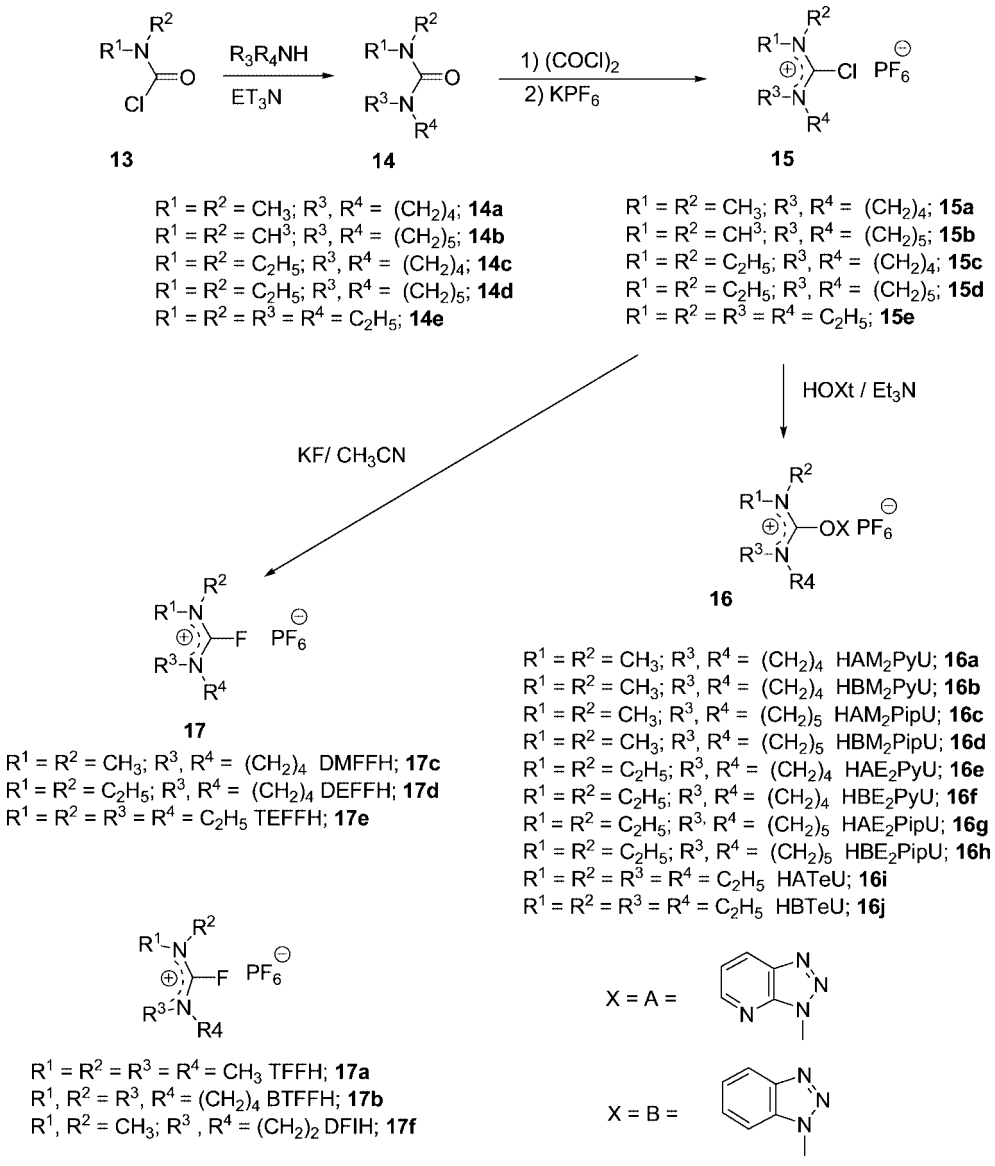
Onium salt mediated amide formation generally involves two steps: activation, in which the coupling reagent reacts with an N-protected amino acid to form an active carboxyl, and coupling, whereby the active carboxyl reacts with the amino component to form the peptide bond.[2,17] Thus, the coupling efficiency depends on the nature of the leaving group of the onium salt, which is also the leaving group of the active carboxyl. Therefore, the coupling efficiency can be enhanced by modifying the leaving group of the onium salts. This approach has proven successful for cases in which HOAt-based onium salts were substituted for HOBt-, HOPfp-, HOSu-, or HODhbt-derived onium salts.[2,6,7,8,13,18]

We present here a new approach to enhance the coupling efficiency based on the modification of the structures of the carbocation skeleton moiety of aminium/uronium-type reagents, which feature relatively high reactivity and low racemization during peptide bond formation.

These uronium-type reagents can be readily prepared by treating *N*,*N*-dialkylcarbamoyl chloride (**13**) with secondary



Scheme 2.



Scheme 3.

amines such as diethylamine, pyrrolidine, or piperidine to give the corresponding urea derivatives **14** (Scheme 3, Table 16). Then, the urea derivatives react with oxalyl chloride to yield the corresponding chloro-salts **15** (Scheme 3, Table 17), which are stabilized by the formation of a PF_6 salt. Subsequent reaction with HOXt (**3a** or **3b**) in the presence of a tertiary amine such as NEt_3 affords the desired compounds **16** as crystalline, stable solids (Scheme 3, Tables 18 and 19).

Furthermore, the chloride salts **15** can be converted into their corresponding fluorides **17** by treatment with KF (3 equiv.) in acetonitrile at 60 °C for 3–4 h (Scheme 3, Table 20).

Stability of HOXt Uronium-Type Reagents

In order to determine the compatibility of HOAt-based coupling reagents with automated peptide synthesizers, their stability in solution and in the solid state was examined by HPLC and ^{1}H NMR analysis. Furthermore, evaluation of a given compound's stability in solution can provide information about its reactivity.[17] HATU (**4b**), HAPyU (**6**), HAM_2PyU (**16a**), HAM_2PipU (**16c**), HAE_2-PyU (**16e**), HAE_2PipU (**16g**), HATeU (**16i**), TFFH (**17a**), BTFFH (**17b**), DMFFH (**17c**), DEFFH (**17d**), and TEFFH (**17e**) are stable solids at 25 °C. DMF solutions (0.6 M) of HATU (**4b**), HAPyU (**6**), HAM_2PyU (**16a**), HAM_2PipU (**16c**), HAE_2PyU (**16e**), HAE_2PipU (**16g**), and HATeU (**16i**) are stable under nitrogen at 25 °C for 3–4 weeks. DMF solutions (0.6 M) of the reagents shown in Table 6 exposed to the atmosphere are stable for 1–2 weeks. DMF solutions (0.6 M) of the fluoride salts TFFH (**17a**), BTFFH (**17b**), DMFFH (**17c**), DEFFH (**17d**), and TEFFH (**17e**), are stable for 1–2 days, but in the presence of DIEA (0.6 M) they are only stable for 1 h, as determined by ^{1}H NMR

spectroscopy. The data in Table 1 show that, as expected, aza derivatives are less stable, and therefore more reactive, than the benzotriazole analogs.[17]

Table 1. Hydrolytic stability of DMF solutions of uronium salt derivatives in open vials.[a]

Coupling reagent	5 h	24 h	48 h
HATU (**4b**)	99	95	76
HBTU (**4a**)	100	98	86
HAPyU (**6**)	96	79	20
HBPyU (**5**)	97	84	40
HAPipU (**9b**)	98	95	81
HAMDU (**8**)	0	0	0
HBMDU (**7**)	0	0	0
HATeU (**16i**)	100	92	85
HBTeU (**16j**)	100	95	88
HAM_2PyU (**16a**)	98	85	50
HBM_2PyU (**16b**)	100	90	83
HAE_2PyU (**16e**)	100	88	60

[a] All reagents have a stability greater than 95% in a closed vial, but HAMDU (**8**) and HBMU (**7**) are totally unstable.

Furthermore, the nature of the carbon skeleton of a compound is of marked importance to the stability of the compound. Both dihydroimidazole [HAMDU (**8**), HBMDU (**7**)] derivatives are very unstable, whereas their corresponding dimethylamine salts are the most stable and the pyrrolidino derivatives are of intermediate stability. These results should be taken into account mainly for those syntheses carried out in automatic synthesizers, where the coupling reagents are dispensed in open vessels. As expected, all the coupling reagents are more stable when their DMF solutions are stored under N_2,[19] conditions which are employed in some automatic synthesizers (Table 2).

Table 2. Hydrolytic stabilty of uronium salt derivatives in DMF under nitrogen.

Coupling reagent	1 day	3 days	7 days	24 days
HATU (**4b**)	100	98	92	87
HAPyU (**6**)	100	96	84	68
HAPipU (**9b**)	100	98	88	72
HATeU (**16i**)	100	100	94	90
HAM_2PyU (**16a**)	100	98	88	75
HAE_2PyU (**16e**)	100	100	94	89
HAM_2PipU (**16c**)	100	100	95	90

The stability of these compounds was also examined in the presence of DIEA (Table 3) because peptide bond formation is usually carried out in the presence of at least one extra equivalent of base. Analysis of these results confirms that the various coupling reagents rapidly degrade in the absence of a carboxylic acid function.

This observation has practical consequences for both solid-phase and solution strategies. Thus, if activation of a carboxylic acid is slow, then the coupling reagents will be degraded and will no longer be able to activate the carboxyl function. Under these conditions, aza derivatives are more labile than the benzotriazole derivatives and pyrrolidino derivatives are more labile than dimethylamino and diethylamino derivatives. This is important for cyclization steps or in convergent strategies during the fragment coupling steps because the yields tend to be lower than for other couplings.

Table 3. Hydrolytic stability of DMF solutions of uronium salt derivatives in the presence of DIEA.

Coupling reagent	2 min	30 min	60 min
HATU (**4b**)	93	62	36
HBTU (**4a**)	95	76	62
HAPyU (**6**)	59	37	9
HAPipU (**9b**)	65	65	43
HATeU (**16i**)	95	67	43
HBTeU (**16j**)	97	79	66
HAM_2PyU (**16a**)	80	53	28
HBM_2PyU (**16b**)	90	77	62
HAE_2PyU (**16e**)	84	59	38
HAM_2PipU (**16c**)	83	66	36

Activation of Z-Aib-OH

As mentioned above, the formation of a peptide bond between two amino acids involves two steps. The first step is the activation of the carboxyl group of one residue, and the second step is the nucleophilic attack of the amino group of the other amino acid derivative at the active carboxylic group. To study the activation step of the carboxylic acid, Z-Aib-OH was chosen because of its sterically hindered carboxyl group.

Reaction of Z-Aib-OH with *p*-chloroaniline (PCA), a rather poor nucleophilic amine, in the presence of a coupling reagent was used to compare the relative coupling rates for various reagents. As the formation of intermediate Z-Aib-OXt (active ester) is usually very fast (Table 4), half-lives were determined by ^1H NMR spectroscopy by monitoring the disappearance of the benzylic CH_2 residue (δ = 5.2 ppm) of the active ester and the appearance of the benzylic unit (δ = 5.02 ppm) of the Z-Aib-PCA formed (Table 5).

Table 4. Rate of formation of active ester (Z-Aib-OAt) in DMF as solvent with TMP as base.[a]

Coupling reagent	**4b**	**6**	**16a**	**16e**	**16i**	**16c**	**16g**
$t_{1/2}$ [min]	3	≈2	≈2	≈2	7	4	4

[a] $t_{1/2}$ of formation of active ester by all of the coupling reagents is less than 2 min (almost complete conversion within 2 min) when DIEA is used, as opposed to 5–8 min when TMP is used, or around 16 min for the case of tetraethyl derivatives.

Table 5. Approximate half-lives for the disappearance of Z-Aib-OXt in DMF in the presence of *p*-chloroaniline.

$t_{1/2}$	DMF
HATU (**4b**)	75–85 min
HBTU (**4a**)	10–11 h
HAPyU (**6**)	70–80 min
HAM_2PyU (**16a**)	70–80 min
HBM_2PyU (**16b**)	9–10 h
HAE_2PyU (**16e**)	90 min
HAM_2PipU (**16c**)	100 min
HAE_2PipU (**16g**)	100 min
HATeU (**16i**)	100 min

The results shown in Table 5 are in agreement with the previously discussed observation (Table 1). Thus, aminium salts derived from HOAt (**4b**) are more effective than those derived from HOBt (**4a**). Examination of the activation at 2 min indicates that the aza derivatives are more reactive than their corresponding benzotriazole analogs (the NMR spectra show a 10–15% conversion for the aza derivatives but almost no reaction for the those of HOBt). Furthermore, formation of the active-ester species competes with hydrolysis of the coupling reagents. Thus, the most reactive onium salt, HAMDU, gives the poorest activation as compared to the less reactive HATU (**4b**), HATeU (**16i**), HAM_2-PyU (**16a**), and HAPyU (**6**), due to the fact that after a few seconds no activation reagent is present in the medium.

Z-Phy-Pro-NH_2 **18**

Z-Phe-Val-Pro-NH_2 **19**

Z-Gly-Phe-Val-OMe **20**

Z-Phe-Val-Ala-OMe **21**

Z-Gly-Phe-Pro-NH_2 **22**

Z-Gly-Gly-Val-Ala-Gly-Gly-NH_2 **23**

Racemization

To investigate the configuration retention induced for the new coupling reagents, several previously studied model peptide systems **18**–**23** were examined.[12]

These models involve stepwise coupling and [2+1] segment coupling as well. For the sensitive coupling of the Z-Phg-OH to H-Pro-NH_2 to give **18**, HATU (**4b**), HAPyU (**6**), HAM_2PyU (**16a**), HAM_2PipU (**16c**), HAE_2PyU (**16e**), HAE_2PipU (**16g**), and HATeU (**16i**) give a greater conser-

Table 6. Yield and racemization during the formation of Z-Phg-Pro-NH_2 in DMF (solution-phase synthesis).[a]

Base	4b	4a	6	16[a]	16b	16e	16f	16i	16j
TMP									
Yield [%]	77.9	81.2	78.9	80.1	80.4	78.1	78.1	85.2	86.1
DL [%]	2.1	6.4	2.3	2.9	3.7	4.9	6.2	3.5, 3.0	6.8, 6.7
DIEA									
Yield [%]	78.4	80.1	77.9	82.3	80.4	82.3	78.7	88.1	84.6
DL [%]	3.1	8.2	3.2	2.7	3.4	4.1	4.9	1.5, 1.7	6.2, 6.3

[a] The LL and DL forms of the test tripeptide have been described elsewhere.[12] The t_R's of LL and DL were determined by co-injection with authentic and pure samples of LL.

Table 7. Yield and racemization during the formation of Z-Phe-Val-Pro-NH_2 (2 + 1) in DMF (solution-phase synthesis).[a]

Base	4b	4a	6	16a	16b	16e	16f	16i	16j
TMP									
Yield [%]	83.2	81.2	85.5	82.1	83.4	83.2	84.5	86.3	84.5
DL [%]	5.3	14.2	3.5	4.1	14.2	4.9	15.7	9.0	18.3
DIEA									
Yield [%]	85.8	89.7	88.9	83.6	83.9	85.1	86.1	89.0	89.3
DL [%]	13.9	27.4	10.8	13.6	30.1	14.9	30.4	16.0	37.3

[a] The LLL and LDL forms of the test tripeptide have been described elsewhere.[8] The t_R's of LLL and LDL were determined by co-injection with authentic and pure samples.

Table 8. Yield and racemization during formation of Z-Phe-Val-Pro-NH_2 **19** (2 + 1) in DMF (solid-phase synthesis).[a]

Base	4b	4a	6	16a	16b	16e	16f	16i	16j
TMP									
Yield [%]	93.9	95.2	93.4	93.2	93.4	92.1	92.3	92.1	91.2
DL [%]	9.4	13.6	8.8	16.9	22.9	17.2	29.8	22.2	39.5
DIEA									
Yield [%]	98.1	97.6	95.7	94.6	95.3	96.5	–	96.3	–
DL [%]	22.2	24.8	15.6	18.9	26.7	23.4		25.3	

[a] The LLL and LDL forms of the test tripeptide have been described elsewhere.[8] The t_R's of LLL and LDL were determined by co-injection with authentic and pure samples.

vation of chirality than their benzotriazole analogs. Likewise, HAM_2PyU (**16a**) performs slightly better than HAPyU (**6**; Table 6).

For the well-studied segment coupling of Z-Phe-Val-OH to H-Pro-NH_2, which leads to tripeptide **19**,[8,12,16] the best results were obtained with HATU (**4b**), HAPyU (**6**), HAM_2-PyU (**16a**), and other HOAt derivatives in both solution (Table 7) and on a solid phase (Table 8).

For the rather non-sensitive case of segment coupling of Z-Gly-Phe-OH to H-Pro-NH_2, which leads to tripeptide **22**, HOAt-derived reagents again perform better than their HOBt analogues (Table 9).

Studies with tripeptide models **20** and **21** also confirmed the previous results (Tables 10 and 11).

Finally, the test hexapeptide Z-Gly-Gly-Val-Ala-Gly-Gly-NH_2 (**23**) was assembled on a solid phase by coupling Z-Gly-Gly-Val-OH to H-Ala-Gly-Gly-PAL-resin. This coupling has previously been shown to be a sensitive test for checking the performance of coupling reagents and bases (Table 12).[12]

Rate of Formation of the Protected Amino Acid Fluorides

N-Protected amino acid (0.1 mmol), DIEA (0.1 mmol), and fluoroformamidinium salt (0.1 mmol) were dissolved in the corresponding solvent (DMF or DCM, 0.5 mL). The reaction mixture was monitored by IR spectroscopy, which

Table 9. Yield and racemization during the formation of Z-Gly-Phe-Pro-NH_2 **22** (2 + 1) in DMF (solution-phase synthesis).[a]

Base	4b	4a	6	16a	16b	16e	16f	16i	16j
TMP									
Yield [%]	86.8	84.8	86.8	88.6	86.1	79.8	78.1	78.0	76.9
DL [%]	0.8	3.6	0.6	0.6	3.8	0.9	2.6	1.3	3.1
DIEA									
Yield [%]	90.1	88.9	89.8	88.9	86.1	83.3	79.3	80.1	78.9
DL [%]	1.6	5.9	1.3	1.0	4.7	1.2	3.9	1.8	3.9

[a] The LLL and LDL forms of the test tripeptide have been described elsewhere.[8] The t_R's of LLL and LDL were determined by co-injection with authentic and pure samples.

Table 10. Yield and racemization during the formation of Z-Gly-Phe-Val-OMe **20** (2 + 1) in DMF (solution-phase synthesis).[a]

Base	4b	4a	6	16a	16b	16e	16f	16i	16j
TMP									
Yield [%]	90.1	84.8	86.6	85.1	84.4	86.1	84.2	86.3	87.1
DL [%]	0.9	5.9	0.6	0.9	4.5	0.8	4.1	0.8	4.8
DIEA									
Yield [%]	86.2	88.9	84.1	85.0	86.4	82.3	82.7	87.1	86.3
DL [%]	1.6	3.6	1.3	1.6	3.4	1.8	10.1	1.5	11.8

[a] The LLL and LDL forms of the test tripeptide have been described elsewhere.[8] The t_R's of LLL and LDL were determined by co-injection with authentic and pure samples.

Table 11. Yield and racemization during the formation of Z-Phe-Val-Ala-OMe **21** (2 + 1) in DMF (solution-phase synthesis).[a]

Base	4b	4a	6	16a	16b	16e	16f	16i	16j
TMP									
Yield [%]	82.1	83.3	83.2	80.1	83.4	82.2	80.2	89.3	85.6
DL [%]	<0.1	2.9	<0.1	0.6	3.5	0.4	10.4	0.39	12.4
DIEA									
Yield [%]	83.2	88.9	84.1	85.0	86.4	82.3	84.7	86.2	90.1
DL [%]	2.1	5.6	2.3	2.6	6.4	2.8	13.1	2.8	16.1

[a] The LLL and LDL forms of the test tripeptide have been described elsewhere.[8] The t_R's of LLL and LDL were determined by co-injection with authentic and pure samples.

Table 12. Yield and racemization during the formation of Z-Gly-Gly-Val-Ala-Gly-Gly-NH_2-resin (3 + 3) in DMF (solid-phase synthesis).[a]

Base	4b	4a	6	16a	16b	16e	16f	16i	16j
TMP									
Yield [%]	97.8	94.8	97.9	96.3	93.4	95.6	95.2	96.2	95.6
DL [%]	2.1	4.6	2.3	2.3	11.3	9.4	12.5	9.9	12.8
DIEA									
Yield [%]	92.1	95.9	96.7	–	–	–	–	–	–
DL [%]	29.9	36.8	41.4						

[a] The test tripeptide has been described elsewhere.[8,12] The t_R's of LLL and LDL were determined by co-injection with authentic and pure samples.

allows facile tracking of the formation of the corresponding acid fluoride (1844 cm^{-1}). For unhindered amino acids such as Ala, Leu, Gly, His, Phe, Ser, and Met, three to seven minutes is sufficient for complete conversion to the acid fluoride in DMF or DCM. In the case of hindered amino acids such as Val or Ile seven to ten minutes is necessary, and in the case of the most hindered residue, Aib, the complete conversion takes about 25 min. However, even in these latter cases (Val, Ile, Aib), more than 50–75% of acid fluoride was formed after seven minutes of preactivation. Furthermore, the rate of formation can be increased by using two equivalents of DIEA. Thus, for Val and Ile the acid fluoride formation is almost complete within seven minutes. Based on these experiments, the recommended maximum preactivation time for the salts is 7–10 min.

Based on IR studies, the order of reactivity is as follows: TFFH (**17a**), BTFFH (**17b**) ≈ DMFFH (**17c**), DEFFH (**17d**), TEFFH (**17e**) > DFIH (**17f**). The poor results obtained for DFIH are due to its decomposition during preactivation.

Comparison of Coupling Techniques

In order to demonstrate the effectiveness of the new HOAt-based reagents and compare their performance to that of HOBt analogues, the common decapeptide model ACP(65–74) was assembled on a solid phase.[19] The peptide was manually elongated on an Fmoc-Gly-Wang-PEG-PS-resin. Coupling times were shortened and excesses of reagents were reduced in order to highlight the differences between the various coupling reagents studied, as described previously.[19] Under these conditions, incomplete incorporation was detected for Ile^{72} onto Asn, Ile^{69} onto Asp, and Val onto Gln. Peptide purity was determined by reverse-phase HPLC analysis (Table 13) after cleavage of the peptide from the resin by treatment with TFA/H_2O (9:1) for 2 h at room temperature.

The results outlined in Table 13 reflect the superiority of aza derivatives to HOBt analogs. Furthermore, long preactivation times are shown to be detrimental. Finally, although all aza derivatives gave good results, HATU (**4b**), HAPyU (**6**), HAM_2PyU (**16a**), and HAE_2PyU (**16e**) gave the best results (>80% purity).

Hindered Amino Acids

In a more demanding example, H-Tyr-Aib-Aib-Phe-Leu-NH_2 was manually assembled on PAL-PEG-PS-resin using amino acid/activator (4 equiv.), base (8 equiv.), with a 30-min coupling time, except for the case of Aib-Aib, which required 1 h. Peptide purity was determined by reverse-phase HPLC analysis after cleavage of the peptide from the resin by treatment with TFA/H_2O (9:1) for 2 h at room temperature (Tables 14 and 15).

The results shown in Table 14 confirm that aza derivatives are more convenient for the synthesis of these demanding peptides, and that preactivation can be detrimental to the quality of the final product.

The same synthesis was carried out with fluoroformamidinium salts. The results, which are shown in Table 15, indicate that for this peptide these salts are more convenient than HOAt-based uronium salts.

In conclusion, a new family of immonium-type coupling reagents that differ in the structure of their carbocation

Table 13. Purity of ACP(65–74) using a 1.5-fold excess of amino acids and reagents and 1.5-min coupling time.[a]

Coupling Reagent	P.T.[b]	Yield [%]	ACP	Des-Asn	Des-Val	Des-Ala	Des-Ile^{69}	Des-Ile^{72}	Des-Ile, Ile
HATU (**4b**)	20–30 s	71.9	80.6	0.7	1.8	1.1	2.45	–	–
HATU (**4b**)	7 min	70.3	80.7	10.5	0.5	3.2	1.1	1.4	–
HBTU (**4a**)	20–30 s	70.5	45.8	0.8	2.6	1.2	6.4	0.9	5.8
HBTU (**4a**)	7 min	70.0	48.9	10.5	0.9	3.8	4.5	5.6	7.3
HAPyU (**6**)	20–30 s	73.1	88.1	0.64	1.5	0.66	0.9	–	–
HAPyU (**6**)	7 min	73.4	70.1	10.4	0.8	1.5	4.9	3.2	0.5
HBPyU (**7**)	20–30 s	70.0	55.7	0.8	2.6	1.1	5.3	0.7	4.3
HBPyU (**7**)	7 min	70.0	54.0	10.3	1.0	3.1	5.8	5.6	6.7
HAM_2PyU (**16a**)	20–30 s	71.2	81.3	0.79	1.53	0.43	1.23	–	–
HAM_2PipU (**16c**)	20–30 s	70.2	78.3	2.5	1.83	0.98	1.53	–	–
HB M_2PyU (**16b**)	20–30 s	70.9	58.9	0.76	2.4	1.0	4.9	0.8	4.2
HBM_2PyU (**16b**)	7 min	76.0	60.2	10.1	2.5	5.8	6.1	4.5	6.8
HAM_2PyU (**16a**)	7 min	78.0	71.9	12.0	2.0	5.3	1.0	–	–
HAM_2PipU (**16c**)	7 min	78.0	68.9	13.0	2.5	5.8	1.6	–	–
HAE_2PyU (**16e**)	20–30 s	70.1	79.7	7.77	2.44	1.6	2.65	2.82	2.16
HAE_2PyU (**16e**)	7 min	67.3	60.3	6.8	1.4	14.9	2.2	2.1	1.7
HATeU (**16i**)	20–30 s	68.6	72.5	1.6	10.6	0.8	11.9	2.7	–
HATeU (**16i**)	7 min	69.1	66.8	7.5	5.7	3.6	5.2	2.7	2.6
HBTeU (**16j**)	20–30 s	66.0	42.3	2.3	11.0	1.3	13.8	3.4	6.3
HBTeU (**16j**)	7 min	68.0	40.1	10.5	5.8	5.2	6.1	5.9	7.9
HAE_2PipU (**16g**)	20–30 s	70.1	75.6	1.6	9.4	0.6	10.1	2.6	–
HAE_2PipU (**16g**)	7 min	69.3	63.3	7.6	2.4	15.8	2.7	2.6	1.9

[a] All peptides (ACP and des-amino acid structures) were confirmed by peak overlap in the presence of authentic samples prepared by standard protocols and identified by mass spectral analysis on a Perseptive Biosystems Voyager DE type MALDI-TOF instrument using sinapinic acid as matrix. Crude ACP(65–74) was analyzed by HPLC [Nova Pak C_{18} 60-Å column (3.9 × 150 mm, 4 μm), linear gradient over 25 min of 5 to 35% CH_3CN in H_2O/0.1% TFA; flow rate: 1.0 mL min^{-1}]. [b] P.T: preactivation time.

Table 14. Percentage of des-Aib (4-mer) obtained during solid-phase assembly of the pentapeptide.[a]

Coupling reagent	HATU (**4b**) (HBTU, **4a**)	HAPyU (**6**) (HBPyU, **5**)	HAM_2PyU (**16a**) (HBM_2PyU, **16b**)	HAE_2PyU (**16e**) (HBE_2PyU, **16f**)	HATeU (**16i**) (HBTeU, **16j**)
20–30 s P.T.[b]	7.9	23	18.9	21.2	22.3
	(43)	(42)	(45)	(44)	(45)
7 min P.T.[b]	22	24	23.6	25.1	24.2
	(58)	(57)	(59)	(57)	(58)

[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 [Nova Pak C_{18} 60-Å column (3.9 × 150 mm, 4 μm), linear gradient over 25 min of 10 to 90% CH_3CN in H_2O/0.1% TFA; flow rate: 1.0 mL min^{-1}]. [b] P.T.: preactivation time.

Table 15. Pentapeptide purity and relative amount of des-Aib for solid-phase syntheses.

Coupling reagent	TFFH (**17a**)	BTFFH (**17b**)	DFIH (**17f**)	DMFFH (**17c**)	DEFFH (**17d**)	TEFFH (**17e**)
Pentapep [%]	95.2	95.8	92.2	94.6	94.7	94.9
Des-Aib [%]	4.8	4.2	7.8	5.4	5.3	5.1

skeletons has been described. These differences have a marked influence on the stability of the reagent. The salts derived from dimethylamine are the most stable to air, whereas the pyrrolidino derivatives are the least stable and the remaining derivatives are of intermediate stability. These results should be taken into account mainly when coupling reagents are deposited in open vessels, such as in some automatic synthesizers. HOAt derivatives are confirmed to be superior to HOBt derivatives in terms of both coupling yield and retention of configuration for all cases. Although all aza derivatives gave good results, HATU (**4b**), HAPyU (**6**), HAM_2PyU (**16a**), and HAE_2PyU (**16e**) gave the best results. For peptides containing the rather hindered Aib residue, the fluoroformamidinium salts proved to be more convenient than the HOAt-based reagents.

Experimental Section

General: TLC was performed on silica plates (8 × 4 cm) from Albet using suitable solvent systems and visualization with a Spectroline UV Lamp Model CM-10 (254 nm). Melting points were obtained in open capillary tubes using a Gallenkamp Sanyo melting point apparatus and are uncorrected. IR spectra were recorded with a Shimadzu 8300 series Fourier Transformer instrument as KBr pellets. The absorption bands ($\tilde{\nu}_{max}$) are given in wavenumbers (cm^{-1}). NMR spectra were recorded with a Bruker Avance 300 MHz spectrometer at room temperature. Tetramethylsilane (TMS) was used as reference for all NMR spectra, with chemical shifts reported in ppm relative to TMS. Elemental analyses were carried out at the Microanalytical Laboratories of the Beirut Arab University, Lebanon. All solvents used for recrystallization, extraction, column chromatography, and TLC were of commercial grade, distilled before use, and stored under dry conditions.

The model peptides Z-Phg-Pro-NH_2 (**18**),[12] Z-Phe-Val-Pro-NH_2 (**19**),[8,12] Z-Gly-Phe-Val-OMe (**20**),[12] Z-Phe-Val-Ala-OMe (**21**),[12] Z-Gly-Phe-Pro-NH_2 (**22**),[8,12] and Z-Gly-Gly-Val-Ala-Gly-NH_2 (**23**)[8,12] were analyzed according to previously described methods.

General Procedure for the Preparation of Urea Derivatives: *N*,*N*-Dialkylcarbamoyl chloride (0.6 mol) was added dropwise to a stirring mixture of the secondary amine (0.5 mol) and triethylamine

Table 16. Yield, b.p., ^{1}H NMR spectroscopic, and elemental analytical data of urea derivatives.

Compd.	Yield [%]	B.p. [°C]	^{1}H NMR ($CDCl_3$) δ [ppm]	Elemental analysis: calcd. (found) C	H	N
14a	79	85	1.7–2.1 (m, 4 H), 2.83 (s, 6 H), 3.35 (m, 4 H)	59.12 (59.21)	9.92 (9.76)	19.7 (19.51)
14b	78	93	1.6–2.1 (m, 6 H), 2.83 (s, 6 H), 3.35 (m, 4 H)	61.50 (61.32)	10.32 (10.33)	17.93 (18.11)
14c	81	90	1.2 (t, 6 H), 1.8–2.1 (m, 4 H), 3.1–3.7 (m, 8 H)	63.49 (63.42)	10.66 (10.43)	16.45 (16.51)
14d	81	97	1.2 (t, 6 H), 1.8–2.1 (m, 6 H), 3.1–3.7 (m, 8 H)	65.18 (65.43)	10.49 (11.05)	15.20 (15.41)
14e	85	75	1.1 (t, 12 H), 3.18 (q, 8 H)	62.75 (62.85)	11.70 (11.69)	16.26 (16.35)

Table 17. Yield, m.p., ^{1}H NMR spectroscopic, and elemental analytical data of chloroformamidinium salts.

Compd.	Yield [%]	M.p. [°C]	^{1}H NMR ($CDCl_3$) δ [ppm]	Elemental analysis: calcd. (found) C	H	N
15a	89	93–95	2.0–2.13 (m, 4 H), 3.47 (s, 6 H), 3.9–4.0 (m, 4 H)	27.41(27.37)	4.60 (4.52)	9.14 (9.01)
15b	88	99–101	1.8–2.1 (m, 6 H), 3.27 (s, 6 H), 3.9–4.0 (m, 4 H)	29.95 (29.73)	5.03(4.92)	8.74(8.81)
15c	90	141–1431	1.39 (t, 6 H), 2.0–2.5 (m, 4 H), 3.9 (q, 4 H), 4.14–4.29 (m, 4 H)	32.29 (32.14)	5.42(5.29)	8.37 (8.31)
15d	90	163–165	1.39 (t, 6 H), 1.8–2.3 (m, 6 H), 3.9 (q, 4 H), 4.14–4.29 (m, 4 H)	34.43(34.24)	5.74(5.79)	8.03 (8.11)
15e	88	104–106	1.38 (t, 12 H), 3.72 (q, 8 H)	32.11 (32.21)	5.99(5.90)	8.32 (8.41)

Table 18. Yield, m.p., ^{1}H NMR spectroscopic, and elemental analytical data of uronium salts derived from HOAt.

Compd.	Yield [%]	M.p. [°C]	^{1}H NMR ($CDCl_3$) δ [ppm]	Elemental analysis: calcd. (found) C	H	N
16a	86	181 (dec)	1.9–2.18 (m, 4 H), 3.19 (s, 6 H), 3.8–3.95 (m, 4 H), 7.91 (dd, 1 H), 8.14 (dd, 1 H), 8.8 (dd, 1 H)	35.48 (35.38)	4.22 (4.13)	20.69 (20.49)
16c	83	188 (dec)	1.9–2.18 (m, 6 H), 3.19 (s, 6 H), 3.8–3.95 (m, 4 H), 7.91 (dd, 1 H), 8.14 (dd, 1 H), 8.8 (dd, 1 H)	37.15 (37.38)	4.56 (4.63)	20.00 (20.29)
16e	86	176 (dec)	1.3 (t, 6 H), 1.9–2.2 (m, 4 H), 3.4–3.89 (m, 8 H), 7.95 (dd, 1 H), 8.1 (dd, 1 H), 8.8 (dd, 1 H)	38.72 (38.60)	4.87 (4.80)	19.35 (19.19)
16g	88	182–183	1.3 (t, 6 H), 1.9–2.2 (m, 6 H), 3.4–3.89 (m, 8 H), 7.95 (dd, 1 H), 8.1 (dd, 1 H), 8.8 (dd, 1 H)	40.18 (40.31)	5.17 (5.21)	18.75 (18.89)
16i	89	168–170	1.12 (t, 6 H), 1.46 (t, 6 H), 3.39 (q, 4 H), 3.7 (q, 4 H), 7.92 (dd, 1 H), 8.0 (dd, 1 H), 8.85 (dd, 1 H)	38.54 (38.46)	5.31 (5.23)	19.26 (19.12)

Table 19. Yield, m.p., ^{1}H NMR spectroscopic, and elemental analytical data of uronium salts derived from HOBt.

Compd.	Yield [%]	M.p. [°C]	^{1}H NMR ($CDCl_3$) δ [ppm]	Elemental analysis: calcd. (found) C	H	N
16b	83	153–155	1.9–2.2 (m, 4 H), 3.2 (s, 6 H), 3.8–4.0 (m, 4 H), 7.6 (d, 1 H), 7.92 (d, 1 H), 7.96 (d, 1 H), 8.03 (d, 1 H)	38.53 (38.4)	4.48 (4.39)	17.28 (17.18)
16d	84	167–168	1.7–2.2 (m, 6 H), 3.2 (s, 6 H), 3.8–4.0 (m, 4 H), 7.6 (d, 1 H), 7.92 (d, 1 H), 7.96 (d, 1 H), 8.03 (d, 1 H)	40.10 (39.88)	4.81 (4.58)	16.70 (16.82)
16f	85	118–120	1.3 (t, 6 H), 1.9–2.2 (m, 4 H), 3.6–4.1 (m, 8 H), 7.6 (d, 1 H), 7.92 (d, 1 H), 7.96 (d, 1 H), 8.03 (d, 1 H)	41.58 (41.42)	5.18 (4.93)	16.16 (16.01)
16h	82	132–135	1.3 (t, 6 H), 1.6–2.1 (m, 6 H), 3.4–3.9 (m, 8 H), 7.6 (d, 1 H), 7.92 (d, 1 H), 7.96 (d, 1 H), 8.03 (d, 1 H)	42.95 (42.86)	4.92 (5.01)	15.66 (15.73)
16j	87	154–156	1.18 (t, 12 H), 3.42 (q, 8 H), 7.6 (d, 1 H), 7.92 (d, 1 H), 7.96 (d, 1 H), 8.03 (d, 1 H)	41.38 (41.45)	5.56 (5.50)	16.09 (15.98)

Table 20. Yield, m.p., ^{1}H NMR spectroscopic, and elemental analytical data of fluoroformamidinium salts.

Compd.	Yield [%]	M.p. [°C]	^{1}H NMR ($CDCl_3$) δ [ppm]	Elemental analysis: calcd. (found) C	H	N
17a	92	110	3.35 (d)			
17b	91	153	1.98–2.03 (m, 8 H), 3.75–3.84 (m, 8 H)			
17c	93	115	1.97–2.04 (m, 4 H), 3.36(d, 6 H), 3.77–3.82 (m, 4 H)	28.98 (28.83)	4.86 (4.79)	9.65 (9.70)
17d	92	108	1.41 (t, 6 H), 1.98–2.0 (m, 4 H), 3.78 (dq, 4 H), 3.94–4.05 (m, CH_2)	33.97(33.85)	5.70 (5.61)	8.80 (8.78)
17e	83	118	1.29 (t, 12 H), 3.58 (dq, 8 H)	33.76 (33.81)	6.30 (6.24)	8.75 (8.82)
17f	89	168	2.9 (s, 6 H), 3.88 (d, 4 H)			

(0.5 mol) in dry DCM (400 mL) at 0 °C. When the addition was complete, the mixture was stirred overnight at room temperature. The reaction mixture was then washed with H_2O (100 mL) in order to dissolve the inorganic salt formed ($Et_3N{\cdot}HCl$), and the mixture was subsequently washed with 10% aqueous HCl, saturated aqueous Na_2CO_3, H_2O, and saturated aqueous NaCl solution (100 mL each). Finally, the organic solvent was dried with anhydrous Na_2SO_4, filtered, and the solvent removed under reduced pressure. The oily residue obtained was purified by vacuum distillation. Analytical data for the urea derivatives are given in Table 16, those of the chloroformamidinium salts in Table 17, those of uronium salts derived from HOAt in Table 18, those of uronium salts derived from HOBt in Table 19, and those of fluoroformamidinium salts in Table 20.

General Procedure for the Preparation of Chloroformamidinium Salts:[20] Oxalyl chloride (100 mmol) was added dropwise to a solution of the urea derivative (100 mmol) in dry DCM (300 mL) at room temperature over a period of 5 min. The reaction mixture was stirred under reflux for 3 h, and the solvent was removed under reduced pressure. The residue was washed with anhydrous diethyl ether (2 × 100 mL), then bubbled with N_2 to remove the diethyl ether. The residue obtained was dissolved in DCM, and a saturated aqueous potassium hexafluorophosphate (KPF_6) solution was added at room temperature with vigorous stirring for 10–15 min. The organic layer was collected, washed once with water (100 mL), dried with anhydrous $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude product was recrystallized from DCM/diethyl ether.

Synthesis of Fluoroformamidinium Salts:[20] Pre-dried KF (0.3 mol) was added to a stirring solution of chloroformamidinium salt (0.1 mol) in dry CH_3CN (200 mL). The reaction mixture was heated at 60 °C for 3 h, then cooled down to room temp., 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 then filtered while hot. The filtrate was concentrated to approximately one-third of the original volume and diethyl ether was added with vigorous stirring to promote the formation of a white solid. No further crystallization was needed. All of the fluoroformamidinium salts were stored in capped plastic vials at room temperature for about six months then tested. Neither hydrolysis nor urea formation was observed by ^{1}H NMR spectroscopy.

Synthesis of Uronium/Aminium Salts: The chloroformamidinium salt (5 mmol) was added to a stirring solution of HOXt (5 mmol) and triethylamine (5 mmol) in dry DCM (50 mL). The reaction mixture was stirred at room temperature overnight, filtered, washed with DCM (10 mL), and the residue was recrystallized from CH_3CN/diethyl ether.

Model Segment Coupling Reaction: Test couplings were carried out as described previously for Z-Phg-Pro-NH_2 (**18**),[12] Z-Phe-Val-Pro-NH_2 (**19**),[8,12] Z-Gly-Phe-Val-OMe (**20**),[12] Z-Phe-Val-Ala-OMe (**21**),[12] Z-Gly-Phe-Pro-NH_2 (**22**),[8,12] and Z-Gly-Gly-Val-Ala-Gly-Gly-NH_2 (**23**).[8,12]

ACP(65–74) (H-Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly-OH) Synthesis:[19] The peptide was manually assembled on an Fmoc-Gly-Wang-PEG-PS-resin (0.17 mmol g^{-1}) with Fmoc-amino acids (1.5-fold excess), coupling reagent (1.5-fold excess), and DIEA (threefold excess). The preactivation times used are given in Table 18, the coupling time was 1.5 min for each amino acid, and the deprotection (piperidine/DMF, 2:8) time was 7 min. The peptide was cleaved from the resin with TFA/H_2O (9:1) for 2 h at room temperature. The crude peptide was run on HPLC using the following conditions: linear gradient of CH_3CN and H_2O containing 0.1% TFA each, from 5% to 35% in 25 min, C-18 Nova Pak column (4 μm, 60 Å, 3.9 × 150 mm), detection at 220 nm.

Solid-Phase Synthesis of H-Tyr-Aib-Aib-Phe-Leu-NH_2 Using Uronium Salts:[19] The pentapeptide was manually assembled on an Fmoc-PAL-PEG-PS-resin (0.18 mmol g^{-1}) with the preactivation times in Table 19, 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 Aib-Aib, for which the time was 60 min. The peptide was cleaved from the resin with TFA/H_2O (9:1) at room temperature for 2 h. The solution was then filtered and the TFA removed under vacuum. The crude peptide was precipitated by addition of cold diethyl ether, and run on HPLC [C-18 Nova Pak column (4 μm, 60 Å, 3.9 × 15 mm), linear gradient of CH_3CN and H_2O containing 0.1% TFA each, from 10% to 90% in 25 min, detection at 220 nm.] The retention times for the pentapeptide and for the des-Aib tetrapeptide were 12.03 and 12.30 min, respectively.

Solid-Phase Synthesis of H-Tyr-Aib-Aib-Phe-Leu-NH_2 Using Fluoroformamidinium Salts: The pentapeptide was manually assembled on an Fmoc-PAL-PEG-PS-resin (0.18 mmol g^{-1}) with 7 min preactivation time using Fmoc-amino acid (5 equiv.), coupling reagent (5 equiv.), and DIEA (10 equiv.) in DMF at a total concentration of 0.3 M. The coupling time used was 30 min for all amino acids, except for Aib-Aib, which required 60 min. The pentapeptide was cleaved from the resin with TFA/H_2O (9:1) at room temperature for 2 h. The solution was then filtered and the TFA removed under vacuum. The addition of cold diethyl ether precipitated the pentapeptide. The crude peptide was run on HPLC using a C-18 Nova Pak column (4 μm, 60 Å, 3.9 × 15 mm), linear gradient of CH_3CN and H_2O containing 0.1% TFA each, from 10% to 90% in 25 min, detection at 220 nm. The retention times for the pentapeptide and for the des-Aib tetrapeptide were 12.03 and 12.30 min, respectively.

Acknowledgments

Prof. L. A. Carpino, University of Massachusetts, Amherst, USA, is thanked for his support and advice. The authors are indebted to the National Council for Scientific Research (CNRS-48-08-03) for partial support of the work in Lebanon.

[1] Abbreviations not defined in text: Aib = α-aminoisobutyric acid; ACP = acyl carrier protein decapeptide (65–74); DCC = dicyclohexylcarbodiimide; DCM = dichloromethane; DIC = diisopropylcarbodiimide; DIEA = diisopropylethylamine; DMF = dimethyl formamide; HOBt = 1-hydroxybenzotriazole; HOAt = 7-aza-1-hydroxybenzotriazole; HOPfp = pentafluorophenol; HAPyU = 1-(1-pyrrolidinyl-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene)-*N*-methylmethanaminium) hexafluorophosphate *N*-oxide; 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; HBPyU = *N*-[(1*H*-benzotriazol-1-yl)-bis(pyrrolidinyl)-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HDTU = *O*-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-1,2,3,3-tetramethyluronium hexafluorophosphate; HAM_2PyU = *O*-(1*H*-1,2,3-triazolo[4,5-b]pyridin-1-yl)-1,1-dimethyl-3,3-tetramethyleneuronium hexafluorophosphate; HBM_2PyU = *O*-(1*H*-benzotriazol-1-yl)-1,1-dimethyl-3,3-tetramethyleneuronium hexafluorophosphate; HAE_2PyU = *O*-(1*H*-1,2,3-triazolo[4,5-b]pyridin-1-yl)-1,1-diethyl-3,3-tetramethyleneuronium hexafluorophosphate; HBE_2PyU = *O*-(1*H*-benzotriazol-1-yl)-1,1-diethyl-3,3-tetramethyleneuronium hexafluorophosphate; HATeU = *O*-(1*H*-1,2,3-triazolo[4,5-b]pyridin-1-yl)-1,1,3,3-tetraethyluronium hexafluorophosphate; HBTeU = *O*-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetraethyluronium hexafluorophosphate; TFFH = tetramethylfluoroformamidinium hexafluorophosphate; DmFFH = 1,2-dimethyl-3,3-tetramethylenefluoroformamidinium hexafluorophosphate; DeFFH = 1,2-diethyl-3,3-tetramethylenefluoroformamidinium hexafluorophosphate; TeFFH = tetraethylfluoroformamidinium 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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Received: August 18, 2005
Published Online: January 16, 2006

