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BOP-OXy, BOP-OBt, and BOP-OAt: novel organophosphinic coupling reagents useful for solution and solid-phase peptide synthesis

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Stand-alone coupling reagents derived from bis(2-oxo-3-oxazolidinyl)phosphorodiamidic chloride show efficient performance in solution and SPPS. In particular, the Oxyma Pure (Luxembourg Biotech., Tel Aviv, Israel) derivative shows the additional advantage of being highly soluble in DMF and even fairly soluble in CH_3CN, which can extend its use for the synthesis of complex peptides. These new stand-alone coupling reagents have the advantage of not bearing any counteranion such as PF_6 or BH_4, whose presence can jeopardize the purification of final peptides prepared in solution. Copyright © 2013 European Peptide Society and John Wiley & Sons, Ltd.

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Keywords: Oxyma Pure; phosphorous reagent; amino acids; solid-phase peptide synthesis; BOP-Cl

Introduction

Takeuchi and Yamada [1] reported in 1974 DPPA (**1**) as the first mixed carboxylic phosphoric anhydride method for peptide bond formation. DPPA was followed by the development of various organophosphorous reagents [2–5].

The next generation of organophosphinic reagents was represented by thiophosphinic-type coupling reagents such as dimethylphosphinothioyl azide (MPTA, **2**) and 3-dimethylphosphinothioyl-2(3*H*)-oxazolone (MPTO, **3**) (Figure 1) [6]. Because MPTA (**2**) generates a carbamoyl azide or urea derivative as by-product, Ueki introduced MPTO (**3**), in which the azide group of MTPA is replaced by a 2-oxazolone group. On the basis of the earlier development of organophosphorous reagents, considerable effort has been focused on developing various coupling reagents of a similar kind [7–17]. In the last decade [18,19], phosphoric acid diethyl 2-phenylbenzimidazol-1-yl ester (DOEPBI, **4**), diphenylphosphinic acid 2-phenylbenzimidazol-1-yl ester (DPPBI, **5**), diphenyl 2-phenyl-1*H*-benzo[*d*]imidazol-1-yl phosphate (DOPPBI, **6**) [19], and TFMS-DEP (**7**) [20] (Figure 2) have been reported as highly efficient coupling reagents. Their efficiency was evaluated through the synthesis of a range of amide-containing compounds and peptides, and the extent of racemization was found to be negligible.

Bis(2-oxo-3-oxazolidinyl)phosphorodiamidic chloride (BOP-Cl, **8**) is the most widely used peptide coupling reagent of the organophosphinic family (Figure 3) [21]. BOP-Cl (**8**) [22] is a well-known excellent dehydrating reagent in peptide synthesis, featuring enhanced stability, lesser corrosive character, and more easiness to handle than other activating agents.

However, despite such excellent performance, the use of BOP-Cl in peptide synthesis has been limited to a few cases involving *N*-methyl amino acids [22]. Furthermore, it is commonly regarded that BOP-Cl does not work properly in solid phase. This can be because activation of the urethane-protected amino acid with BOP-Cl is converted first to the acid chloride and then to the oxazolone, which is a rather poor acylating derivative, and in addition is accompanied by racemization [23].

Herein, the use of the BOP moiety in peptide synthesis in both solution and solid phase is expanded through the introduction of the most common additives to carbodiimides: HOBt (**9**) [24], HOAt (**10**) [25–27], and Oxyma Pure (**11**) [28–30]. HOBt and its

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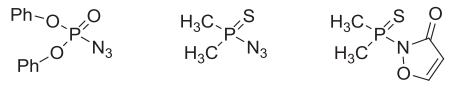


Figure 1. Organophosphorous reagents: DPPA (**1**), MPTA (**2**), and MPTO (**3**).

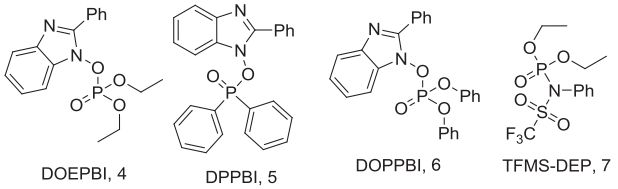


Figure 2. Organophosphorous reagents: DOEPBI (**4**), DPPBI (**5**), DOPPBI (**6**), and TFMS-DEP (**7**).

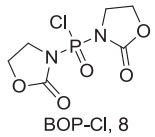


Figure 3. Bis(2-oxo-3-oxazolidinyl)phosphorodiamidic chloride (BOP-Cl, **8**).

more potent aza derivative HOAt are being discontinued for safety reasons [31]. Oxyma Pure does not show that problem, and it has been shown to be superior in all cases to HOBt and even in many cases with the same performance than HOAt [28,32–40]. In addition, the derivative of the amide counterpart, *N*-Oxyma (**12**) [41], was also prepared.

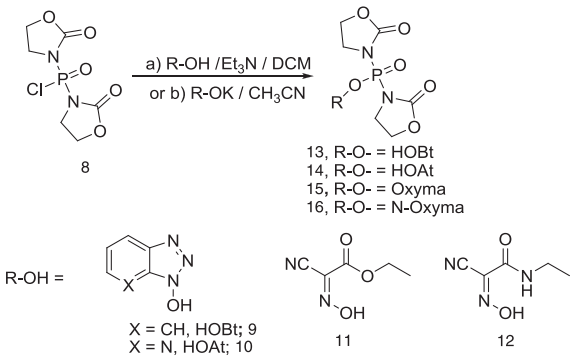
Results and Discussion

Synthesis of Phosphinic Coupling Reagents

The synthetic strategy consists in the reaction of BOP-Cl (**8**) with HOBt (**9**), HOAt (**10**), Oxyma Pure (**11**), and *N*-Oxyma (**12**) in DCM in presence of TEA as base to afford the corresponding desired phosphinic coupling reagents (**13–16**) (Scheme 1).

Solubility of the Novel Phosphinic Reagents

In similarity to most organic processes, the solubility of the reactants plays a crucial role in the conversion. Although DMF is the common solvent of choice for both the solid phase and solution strategies of peptide synthesis, the use of greener solvent alternatives has been



Scheme 1. Synthesis of phosphinic derivatives (**13–16**).

Table 1. Solubility of the organophosphinic coupling reagents in different solvents

Entry	Coupling reagent	Solubility (molarity)			
		DMF	DCM	CH_3CN	THF
1	BOP-Cl (**8**)	0.19	0.06	0.12	Insoluble[a]
2	BOP-OBt (**13**)	0.01	Insoluble[a]	0.006	Insoluble[a]
3	BOP-OAt (**14**)	0.12	Insoluble[a]	0.02	Insoluble[a]
4	BOP-OXy (**15**)	1.18	0.06	0.72	0.06
5	BOP-*N*-OXy (**16**)	0.24	Insoluble[a]	0.35[b]	0.03[b]
6	COMU	1.44	—	—	—
7	HATU	0.43	—	—	—
8	HBTU	0.46	—	—	—

[a]Compounds labeled as 'insoluble' refer to 30 mg that could not be dissolved in 15 ml of solvent (<0.002 M).
[b]Solutions of BOP-*N*-Oxyma show a characteristic brown color.

recently of interest, especially CH_3CN and THF [42]. In this sense, a solubility study in different solvents has been carried out.

As it is shown in Table 1, BOP-OXy is almost as soluble as (1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU) [33] and three times more soluble than HATU and HBTU. Furthermore and regardless of solvent used, the Oxyma Pure-based reagent is more soluble than the OAt/OBt ones, showing a relatively good solubility in CH_3CN (better than HATU/HBTU in DMF). This result could enlarge the use of BOP-OXy for synthesis of complex peptides.

Epimerization Control

In order to check the impact of epimerization in solution when using the novel phosphinic coupling reagents, BOP-Cl (**8**), BOP-OBt (**13**), BOP-OAt (**14**), BOP-OXy (**15**), and BOP-NOXy (**16**), the model peptides (**17–19**) were selected (Figure 4). These peptides are described to give rise to considerable racemization during stepwise and [2 + 1] fragment couplings [43,44]. After coupling of equimolar quantities of the amine- and acid-containing counterparts and aqueous work-up, the impact of epimerization for the three peptides (**17–19**) was determined by HPLC, using optimized conditions that give clear separation of the two epimers.

The performance of the activating reagents (**13–16**) in a stepwise coupling was examined during the assembly of Z-Phg-OH onto H-Pro-NH_2 (**17**) (Table 2) [43]. The Phg residue shows a high sensitivity toward epimerization, because of the high stability of the counteranion. In this model, remarkably, most of the phosphonic derivatives (**13–16**) were able to maintain the desired chirality of the test dipeptide (entries 6–8). In particular, BOP-OXy (**15**) was the most efficient in reducing epimerization (entry 7).

The retention of configuration during the [2 + 1] segment coupling of dipeptide Z-Gly-Phe-OH onto H-Pro-NH_2 to yield Z-Gly-Phe-Pro-NH_2 (**18**) was higher than in the previous stepwise model as has been also observed in other works for segment couplings (Table 3) [28,33,45]. The use of 2,4,6-trimethylpyridine (TMP) in these experiments is recommended because it reduces racemization in segment couplings more efficiently than other bases such as DIEA [46].

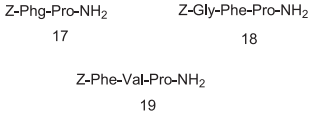


Figure 4. Peptide models used to test the reduction of epimerization induced by the phosphinic coupling reagents.

Journal of PeptideScience

Table 2. Yield and epimerization during the formation of Z-Phg-Pro-NH_2 (**17**) in DMF at room temperature by using the phosphinic coupling reagents (solution phase synthesis)[a]

Entry	Coupling reagent	Pre-activation	Yield [%]	DL [%][b]
1	BOP-Cl (**8**)	2 min	86.0	3.47
2	BOP-Cl/HOBt	2 min	83.0	5.67
3	BOP-Cl/HOAt	2 min	86.0	2.78
4	BOP-Cl/Oxyma Pure	1 min	81.0	1.17
5	BOP-OBt (**13**)		78.0	6.1
6	BOP-OAt (**14**)		86.0	1.4
7	BOP-OXy (**15**)		90.0	0.16
8	BOP-*N*-OXy (**16**)		81.0	1.2

[a]2 equiv DIEA was used.
[b]The LL and DL epimers of this peptide model have been described in the literature [43]. Retention times for each epimer were identified after co-injection with a pure LL sample.

Table 3. Yield and epimerization during the [2 + 1] formation of Z-Gly-Phe-Pro-NH_2 (**18**) in DMF at room temperature by using the phosphinic coupling reagents (solution phase synthesis)[a]

Entry	Coupling reagent	Base	Yield [%]	DL [%][b]
1	BOP-Cl (**8**)	TMP (2 equiv)	83.0	15.1
2	BOP-OAt (**14**)	TMP (2 equiv)	83.0	1.1
3	BOP-OXy (**15**)	TMP (2 equiv)	95.0	1.8
4	BOP-NOXy (**16**)	TMP (2 equiv)	80.0	6.6

[a]Coupling was conducted without pre-activation time.
[b]The LLL and LDL epimers of this tripeptide model have been described in the literature [44]. Retention times for each epimer were identified after co-injection with a pure sample.

Best results were obtained with BOP-OAt (**14**) (entry 2), whereas BOP-OXy (**15**) performed closely (entry 3). All BOP-OR phosphonic reagents superseded in configuration control that of the parent BOP-Cl (entry 1).

Similar tendencies were observed in the [2 + 1] segment coupling (Z-Phe-Val-OH onto H-Pro-NH_2, **19**) (Table 4). In this model system, BOP-Cl (**8**) was the poorest racemization-suppressing reagent of the compounds tested, with a percentage of LDL epimer of nearly 35% (Table 4, entry 1). Best results were obtained with BOP-OAt (**14**) (entry 6), whereas BOP-Oxy (**15**) provided remarkably low epimerization (entry 8). In all cases (Tables 2–4), the use of a mixture of BOP-Cl and the *N*-hydroxy derivative provides poorer results than when the coupling reagent is used alone.

Coupling Potency

To conclude with the evaluation of the novel organophosphinic reagents, coupling efficiency assays were designed. Thus, the pentapeptide H-Tyr-Aib-Aib-Phe-Leu-NH_2 (**20**, Table 5) was assembled in solid phase by means of 30-min couplings, except in the formation of Aib-Aib peptide bond (30-min double coupling) [47]. Misincorporation of one Aib residue (des-Aib) is the most important side reaction and therefore gives a clear reference of the quality of coupling reagent (Table 5). BOP-OXy exhibited closer coupling potency to BOP-OAt with enhanced purity (entries 4 and 3), achieving an impressive 93.1% and 90.5% yield of **20**, respectively. In contrast, BOP-Cl and BOP-OBt gave the worst results (entries 1 and 2), providing only 10.9% and 33.3% yield of the pentapeptide **20**,

Table 4. Yield and epimerization during the [2 + 1] formation of Z-Phe-Val-Pro-NH_2 (**19**) in DMF at room temperature by using the phosphinic coupling reagents (solution phase synthesis)[a]

Entry	Coupling reagent	Base	Yield [%]	DL [%][b]
1	BOP-Cl (**8**)	DIEA (2 equiv)	87.0	35.2
2	BOP-Cl/HOBt	DIEA (2 equiv)	86.0	18.3
3	BOP-Cl/HOAt	DIEA (2 equiv)	85.0	6.9
4	BOP-Cl/Oxyma Pure	TMP (2 equiv)	83.0	12.9
5	BOP-OBt (**13**)	TMP (2 equiv)	80.3	16.1
6	BOP-OAt (**14**)	TMP (2 equiv)	87.0	2.7
7	BOP-OXy (**15**)	DIEA (2 equiv)	92.0	20.4
8	BOP-OXy (**15**)	TMP (2 equiv)	87.0	9.2

[a]No pre-activation was performed.
[b]The LLL and LDL epimers of this tripeptide model have been described in the literature [44]. The retention times for each epimer were identified after co-injection with a pure sample.

Table 5. Impact of des-Aib (H-Tyr-Aib-Phe-Leu-NH_2) tetrapeptide during solid-phase assembly of the pentapeptide H-Tyr-Aib-Aib-Phe-Leu-NH_2 (20) by using the phosphinic coupling reagents[a]

Entry	Coupling reagent	Yield [%]	Pentapeptide **20** [%]	Des-Aib [%][b]
1	BOP-Cl[c] (**8**)	75	10.9	78.0
2	BOP-OBt (**13**)	85	33.3	66.8
3	BOP-OAt (**14**)[d]	89	90.5	5.6
4	BOP-OXy (**15**)	89	93.1	6.9
5	BOP-NOXy (**16**)	86	77.5	22.5

[a]1–2 min pre-activation and 30-min coupling times were generally applied, except for Aib-Aib (30-min double coupling)
[b]Deletion tetrapeptide des-Aib was identified by peak overlap in HPLC with an authentic sample obtained in solid phase.
[c]Extra peaks were observed at 5.38 (4.05%), 5.71 (2.28%), 6.16 (3.44%), and 6.54 min (1.26%).
[d]Extra peak was observed at 6.25 min (3.69%).

respectively (entries 1,2 vs 4,3). These results confirm that BOP-Cl is not suitable at all for SPPS.

H-Tyr-Aib-Aib-Phe-Leu-NH2

20

Conclusions

In conclusion, BOP-based stand-alone coupling reagents can be used in both solution and solid-phase approaches. BOP-OXy showed superiority to BOP-OAt in reducing epimerization in stepwise couplings, whereas in 2 + 1 segment couplings performed closely to BOP-OAt. Concerning coupling potency, BOP-OXy gave close results to BOP-OAt in the synthesis of the H-Tyr-Aib-Aib-Phe-Leu-NH_2 pentapeptide, whereas the worst results were obtained with BOP-Cl. BOP-OXy showed advantageous performance over BOP-NOXy in all cases, highlighting the ester to amide difference in reactivity with regard to BOP-OXy. An additional advantage of BOP-OXy compared with OAt derivative is its superior solubility in DMF and even in CH_3CN, a feature that can expand its use for the preparation of complex peptides. Finally, these new stand-alone coupling reagents have the advantage of not bearing any counteranion such as PF_6 or BH_4, whose presence can jeopardize the purification of final peptides prepared in solution.

Experimental Section

Materials and General Methods

The solvents used were of HPLC reagent grade. Melting points were determined with a Buchi B-540 apparatus (BUCHI Labortechnick GmbH, Essen, Germany) and are uncorrected. NMR spectra (^{1}H NMR and ^{13}C NMR spectra) were recorded on Varian Mercury 400 MHz spectrometer (Vernon Hills, IL, USA) with chemical shift values reported in δ units (ppm) relative to an internal standard. Follow-up of the reactions and checks of the purity of the compounds was performed by TLC on silica gel-protected aluminum sheets (Type 60 GF254, Merck Millipore, Bedford, MA, USA), and the spots were detected by exposure to UV-lamp at λ 254 nm for a few seconds. For analytical separation and characterization, a reverse-phase Waters 2695 HPLC separation module, coupled to a Waters 996 PDA UV detector (Milford, MA, USA), was used. Chromatograms were processed with Empower software. Peptide mass was detected by means of an HPLC-PDA system as described earlier, coupled to a Waters Micromass ZQ mass detector, using the MassLynx 4.1 software (Milford, MA, USA).

General Procedure for Synthesis of Phosphonic Derivatives (13–16)

Method A

To a solution of *N*-hydroxy derivatives (10 mmol) and TEA (10 mmol) in dry DCM (20 ml) was added the BOP-Cl (10 mmol) at 0 °C. The reaction mixture was stirred at this temperature for 1 h and cooled to room temperature. Then, the reaction mixture was stirred for 6 h under N_2 at room temperature, filtered from the precipitate, and washed with 10 ml of DCM to afford a white solid in pure state as indicated from the NMR. To the filtrate, 50 ml of DCM was added and washed with 10% $NaHCO_3$ (2 × 10 ml), 1 N HCl (2 × 10 ml), and saturated NaCl (2 × 10 ml) and dried. The solvent was removed under vacuum, and the residue was crystallized from DCM/hexane to afford extra 10% yield.

Method B

BOP-Cl (10 mmol) was added to a suspension of the potassium salt of *N*-hydroxy derivatives (10 mmol) in CH_3CN at 0 °C. The reaction mixture was stirred at this temperature for 1 h and cooled to room temperature with stirring for 6 h, under N_2. It was filtered from the precipitate and washed with 20 ml CH_3CN, and then the solvent was removed under vacuum. The residue was recrystallized from DCM/hexane or CH_3CN/ether to give a white solid in pure state as indicated from the NMR.

1H-Benzo[d][1,2,3]triazol-1-yl bis(2-oxooxazolidin-3-yl)phosphinate (BOP-OBt, 13)

Method A: The product was obtained as a white solid, mp 178–181 °C, in 2.3 g (65.0%) yield. ^{1}H NMR (acetone-d_6): δ 3.83 (t, 4H, $2CH_2$), 4.29 (t, 4H, $2CH_2$), 7.41 (t, 1H, CH), 7.54 (t, 1H, CH), 7.73 (d, 1H, CH), 7.95 (d, 1H, CH). ^{13}C NMR (acetone-d_6): δ 46.05, 64.33, 121.40, 129.53, 149.22, 157.11. Anal. Calcd for $C_{12}H_{12}N_5O_6P$ (353.23): C, 40.80; H, 3.42; N, 19.83. Found: C, 40.57; H, 3.20; N, 20.05.

3H-[1,2,3]Triazolo[4,5-b]pyridin-3-yl bis(2-oxooxazolidin-3-yl)phosphinate (BOP-OAt, 14)

Method A: The product was obtained as a white solid, mp 184–185 °C, in 2.7 g (76.3%) yield. ^{1}H NMR (acetone-d_6): δ 3.81 (t, 4H, $2CH_2$), 4.26 (t, 4H, $2CH_2$), 7.50 (dd, 1H, CH), 8.50 (d, 1H, CH), 8.75 (dd, 1H, CH). ^{13}C NMR (acetone-d_6): δ 46.01, 46.05, 64.24, 64.33, 121.40, 129.53, 135.30, 149.22, 151.80, 157.12. Anal. Cacld for $C_{11}H_{11}N_6O_6P$ (354.22): C, 37.30; H, 3.13; N, 23.73. Found: C, 37.55; H, 3.00; N, 23.91.

Ethyl 2-(bis(2-oxooxazolidin-3-yl)phosphoryloxyimino)-2-cyanoacetate (BOP-OXy, 15)

Methods A and B: The product was obtained as a white powder, mp 152–153 °C, in 2.1 g (73.3%) yield. ^{1}H NMR ($CDCl_3$): δ 1.45 (t, 3H, CH_3), 4.22 (t, 4H, $2CH_2$), 4.49 (q, 2H, CH_2), 4.58 (t, 4H, $2CH_2$). ^{13}C NMR ($CDCl_3$): δ 14.14, 45.48, 46.05, 64.77, 65.38, 106.47, 136.43, 155.48, 156.68. Anal. Calcd for $C_{11}H_{13}N_4O_8P$ (360.22): C, 36.68; H, 3.64; N, 15.55. Found: C, 36.82; H, 3.61; N, 15.86.

N-(Bis(2-oxooxazolidin-3-yl)phosphoryloxy)-2-(ethyl amino)-2-oxoacetimidoyl cyanide (BOP-NOXy, 16)

Method A: The product was obtained as a white powder, mp 135–137 °C, in 2.98 g (83.0%). ^{1}H NMR (acetone-d_6): δ 1.18 (t, 3H, CH_3), 3.39 (t, 4H, $2CH_2$), 4.17 (q, 2H, CH_2), 4.60 (t, 4H, $2CH_2$). ^{13}C NMR (acetone-d_6): δ 14.03, 35.09, 45.37, 45.41, 65.23, 65.33, 106.89, 155.48. Anal. Calcd for $C_{11}H_{14}N_5O_7P$ (359.23): C, 36.78; H, 3.93; N, 19.50. Found: C, 36.59; H, 3.88; N, 19.81.

General Method for the Racemization Experiments Using the New Phosphonic Derivatives (BOP-Cl, BOP-OBt, BOP-OAt, BOP-OXy, and BOP-NOXy derivatives) [28,33,35,36,45]

Some 0.125 mmol of an acid (Z-Phg-OH, Z-Gly-Phe-OH, or Z-Phe-Val-OH) and (20 µl) 0.125 mmol BOP-Cl, BOP-OBt, BOP-OAt, BOP-OXy, and BOP-NOXy were dissolved in 2 ml DMF at 0 °C. Then, 0.125 mmol of H-Pro-NH_2 was added. The reaction mixture was stirred at 0 °C for 1 h and at room temperature overnight. The reaction mixture was diluted with 25 ml of ethyl acetate and extracted with 1 N HCl (2 × 5 ml), 1 N $NaHCO_3$ (2 × 5 ml), and saturated NaCl (2 × 5 ml). It was then dried over anhydrous $MgSO_4$. The solvent was then removed, and the crude peptide was directly analyzed by HPLC.

Z-Phg-Pro-NH_2 17

A linear gradient of 25–50% CH_3CN/H_2O and 0.1% TFA over 20 min was applied, with a flow rate of 1.0 ml/min and detection at 220 nm using a Waters 996 PDA detector and a Sunfire C_{18} column 3.5 µm, 4.6 × 100 mm (Milford, MA, USA), $t_{R\ LL}$ = 9.89 min, $t_{R\ DL}$ = 10.50 min.

Z-Gly-Phe-Pro-NH_2 18

A linear gradient of 20–70% CH_3CN/H_2O and 0.1% TFA over 8 min was applied, with detection at 220 nm using a Waters 996 PDA detector and a Sunfire C_{18} column 3.5 µm, 4.6 × 100 mm, $t_{R\ LLL}$ = 5.61 min, $t_{R\ LDL}$ = 5.94 min.

Z-Phe-Val-Pro-NH_2 19

A linear gradient of 20–70% CH_3CN/H_2O and 0.1% TFA over 8 min was applied, with a flow rate of 1.0 ml/min and detection at 220 nm using a Waters 996 PDA detector and a Sunfire C_{18} column 3.5 µm, 4.6 × 100 mm, $t_{R\ LLL}$ = 6.81 min, $t_{R\ LDL}$ = 7.24 min.

Synthesis of H-Tyr-Aib-Aib-Phe-Leu-NH_2 (20) in solid phase [28,33,35,36,45]

The pentapeptide H-Tyr-Aib-Aib-Phe-Leu-NH_2 (**20**) was prepared following a published procedure. The synthesis was carried out in a plastic syringe that was attached to a vacuum manifold so as to effect rapid removal of reagents and solvent. The resin

Journal of PeptideScience

Fmoc-Rink-amide-AM-PS with loading 0.75 mmol/g (100 mg) was washed with DCM and DMF (2 × 10 ml each), deblocked with 20% piperidine in DMF (10 ml) for 10 min, and washed with DMF (2 × 10 ml), DCM (2 × 10 ml), and then with DMF (2 × 10 ml). Fmoc-Leu-OH (3 equiv), coupling reagent (3 equiv), and DIEA (6 equiv) were mixed in DMF (0.4 ml), pre-activated for 1–2 min, and then added to the resin. The resulting slurry was stirred slowly for 30–60 min and allowed to couple for 30 min (30-min double coupling for Aib-Aib). The ninhydrin test was performed during the introduction of the first residue (negative). The resin was subsequently washed with DMF and then deblocked by 20% piperidine in DMF for 7 min. Next, the peptide resin was washed with DMF, DCM, and DMF, and coupled with the next amino acid. Coupling and deblocking steps were repeated to provide the pentapeptide. The peptide was cleaved from the resin by TFA/H_2O (9 : 1) at room temperature for 2 h. TFA was removed in vacuum, and the crude peptide was precipitated with ether. The crude weight was recorded, and the percentage of des-Aib (Tyr-Aib-Phe-Leu-NH_2) during solid-phase assembly of the pentapeptide (Tyr-Aib-Aib-Phe-Leu-NH_2) was confirmed by peak overlap in the presence of an authentic sample. The crude H-Tyr-Aib-Aib-Phe-Leu-NH_2 (**20**) was analyzed by HPLC analysis using a Sunfire C_{18} column (3.5 μm, 4.6 × 100 mm) with a 996 PDA detector at 220 nm, flow rate 1 ml/min, using a linear gradient of 10/900 in 20 min. CH_3CN/H_2O/0.1TFA. t_R (pentapeptide) = 7.73 min, $[M + H]^+ = 611.35$; t_R (tetrapeptide, des-Aib) = 7.98 min, $[M + H]^+ = 526.30$, none of the des-Tyr (448) or the tripeptide (363) was observed.

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References

1 Takeuchi Y, Yamada S-I. Amino acids and peptides. VII. Phosphorus in organic synthesis. II. A new method for the synthesis of peptides using the adducts of phosphorus compounds and tetrahalomethanes. *Chem. Pharm. Bull.* 1974; **22**: 832–840.
2 Jackson AG, Kenner GW, Moore GA, Ramage R, Thorpe WD. Activation of carboxylic acids as diphenylphosphinic mixed anhydrides: application to peptide chemistry. *Tetrahedron Lett.* 1976; **17**: 3627–3630.
3 Katoh T, Ueki M. 3-Dimethylphosphinothioyl-2(3*H*)-oxazolone (MPTO) a promising new reagent for racemization-free couplings. *Int. J. Pept. Protein Res.* 1993; **42**: 264–269.
4 Ueki M, Inazu T, Ikeda S. Phosphinyl- and phosphinothioylamino acids and peptides. V. Preparation of dimethylphosphinothioylamino acids and solid phase peptide synthesis. *Bull. Chem. Soc. Jpn.* 1979; **52**: 2424–2427.
5 Ueki M, Inazu T. Synthesis of peptides containing hydroxyamino acids by the mixed anhydride method without protecting the hydroxyl functions. *Chem. Lett.* 1982; **11**: 45–48.
6 Kiso Y, Miyazaki T, Satomi M, Hiraiwa H, Akita T. An 'active ester'-type mixed anhydride method for peptide synthesis. Use of the new reagent, norborn-5-ene-2,3-dicarboximido diphenyl phosphate (NDPP). *J. Chem. Soc., Chem. Commun.* 1980; **21**: 1029–1030.
7 Ramage R, Ashton CP, Hopton D, Parrott MJ. A new reagent for the mediation of amide bond formation in peptide synthesis. *Tetrahedron Lett.* 1984; **25**: 4825–4828.
8 Poulos C, Ashton CP, Green J, Ogunjobi OM, Ramage R, Tsegenidis T. Application of carboxylic-phospholanic mixed anhydrides to fragment coupling in peptide synthesis. *Int. J. Pept. Protein Res.* 1992; **40**: 315–321.
9 Miyake M, Kirisawa M, Tokutake N The synthesis of amides, esters, and thioesters. *Chem. Lett.* 1985; **1**: 123–126.
10 Kim S, Chang H, Ko Y-K. Benzotriazol-1-yl diethyl phosphate. A new convenient coupling reagent for the synthesis of amides and peptides. *Tetrahedron* 1985; **26**: 1341–1342.
11 Watanabe Y, Mukaiyama TA. Convenient method for the synthesis of carboxamides and peptides by the use of tetrabutylammonium salts. *Chem. Lett.*, 1981; **10**: 285–288.
12 Mukaiyama T, Kamekawa K, Watanabe Y. One-pot cyclization of a peptide by the use of (5-nitropyridyl)diphenyl phosphinate: the synthesis of cyclic decapeptide gramicidin S. *Chem. Lett.* 1981; **10**: 1367–1370.
13 Kunieda T, Abe Y, Higuchi T, Hirobe M A new reagent for activating carboxyl groups: diphenyl 2-oxo-3-oxazolinylphosphonate. *Tetrahedron Lett.* 1981; **22**: 1257–1258.
14 Ueda M, Oikawa H. 1,2-Benzisoxazol-3-yl diphenyl phosphate a new, reactive activating agent for the synthesis of amides, esters, and peptides via condensation. *J. Org. Chem.* 1985; **50**: 760–763.
15 Fan C-X, Hao X-L, Ye, Y-H. A novel organophosphorus compound as a coupling reagent for peptide synthesis. *Synth. Commun.* 1996; **26**: 1455–1457.
16 Li H, Jian X, Ye Y-H, Fan C, Romoff T, Goodman M. 3-(Diethoxyphosphoryl oxy)-1,2,3-benzotriazin-4(3*H*)-one (DEPBT): a new coupling reagent with remarkable resistance to racemization. *Org. Lett.* 1999; **1**: 91–94.
17 Xie H-B, Tian G-L, Ye Y-H. Application of an organophosphorus reagent depbt for synthesis of cycloheptapeptide. *Synth. Commun.* 2000; **30**: 4233–4240.
18 Gardiner JM, Procter J. Synthesis of *N*-alkoxybenzimidazoles with differentiated C2 and *O*-substituents. *Tetrahedron Lett.* 2001; **42**: 5109–5111.
19 Kokare ND, Nagawade RR, Rane VP, Shinde DB. Organophosphorus esters of 1-hydroxy-2-phenylbenzimidazole: synthesis and utilization as novel peptide coupling reagents. *Synthesis* 2007; **5**: 766–772.
20 Yasuhara T, Nagaoka YT, Tomioka K. An activated phosphate for an efficient amide and peptide coupling reagent. *J. Chem. Soc., Perkin Trans. 1* 2000, **17**: 2901–2902.
21 Diago-Messeguer J, Palomo-Coll AL, Fernandez-Lizarbe JR, Zugaza-Bilbao A. A new reagent for activating carboxyl groups; preparation and reactions of *N,N*-bis[2-oxo-3-ox-azolidinyl]phosphorodiamidic chloride. *Synthesis* 1980; **7**: 547–551.
22 Van der Auwera C, Anteunis MJO. *N,N'*-bis(2-oxo-3-oxazolidinyl) phosphinic chloride (BOP-Cl) a superb reagent for coupling at and with iminoacid residues. *Int. J. Pept. Protein.* 1987; **29**: 574–588.
23 Carpino LA, Ionesco D, El-Faham A, Henklein P, Wenschuh H, Bienert M, Beyermann M Protected amino acid chlorides vs protected amino acid fluorides: reactivity comparisons. *Tetrahedron Lett.* 1998; **39**: 241–244.
24 Sheehan JC, Hess GP. A new method of forming peptide bonds. *J. Am. Chem. Soc.* 1955; **77**: 1067–1068.
25 Carpino, LA. 1-Hydroxy-7-azabenzotriazole. An efficient peptide coupling additive. *J. Am. Chem. Soc.* 1993; **115**: 4397–4398.
26 Carpino LA, El-Faham A, Minor CA, Albericio F. Advantageous applications of azabenzotriazole (triazopyridine)-based coupling reagents to solid phase peptide synthesis. *J. Chem. Soc., Chem. Commun.* 1994; **2**: 201–203.
27 Carpino LA, El-Faham A, Albericio F. Racemization studies during solid phase peptide synthesis using azabenzotriazole-based coupling reagents. *Tetrahedron Lett.* 1994; **35**: 2279–2282.
28 Subirós-Funosas R, Prohens R, Barbas R, El-Faham A, Albericio F. Oxyma: an efficient additive for peptide synthesis to replace benzotriazole-based HOBt and HOAt with a lower risk of explosion. *Chem. Eur. J.* 2009; **15**: 9394–9403.
29 Subiros-Funosas R, El-Faham A, Albericio F. Aspartimide formation in peptide chemistry: occurrence, prevention strategies and the role of *N*-hydroxylamines. *Tetrahedron* 2011; **67**: 8595–8606.
30 Subirós-Funosas R, Khattab SN, Nieto-Rodriguez L, El-Faham A, Albericio F. Oxyma as cyanooxime building block for the construction of versatile reagents assisting acylation reactions in peptide and organic chemistry. *Aldrichimica* 2013; **46**: 20–40.
31 Wehrstedt KD, Wandrey PA, Heitkamp D. Explosive properties of 1-hydroxybenzotriazoles. *J. Hazard.Mater.* 2005; **126**: 1–7.
32 Subiros-Funosas R, Albericio F. In *Chemistry of Hydroxylamines, Oximes and Hydroxamic Acids*. Rappoport Z, Liebman JF (eds.). 2011; **2**: (Pt. 2), 623–730.

33 El-Faham A, Subirós-Funosas R, Prohens R, Albericio F. COMU a safer and more effective replacement for benzotriazole-based uronium coupling reagents. *Chem.Eur. J.* 2009; **15**: 9404–9416.
34 Subirós-Funosas R, Acosta GA, El-Faham A, Albericio F. Microwave irradiation and COMU a superior tool for solid phase peptide synthesis. *Tetrahedron Lett.* 2009; **50**: 6200–6202.
35 El-Faham A, Albericio F. Synthesis and application of *N*-hydroxyamine derivatives as potential replacement for HOBt. *Eu. J. Org. Chem.* 2009; 1499–1501.
36 El-Faham A, Albericio F. COMU: a third generation of uronium-type based coupling reagent. *J. Peptide Sci.* 2010; **16**: 6–9.
37 Khattab SN. Sulfonate esters of 1-hydroxypyridin-2(1*H*)-one and ethyl 2-cyano-2-(hydroxyimino)acetate (Oxyma) as effective peptide coupling reagents for replacing HOBt and HOAt. *Chem. Pharm. Bull.* 2010; **58**: 501–506.
38 Khattab SN. 2-Cyano-2-(hydroxyimino)acetate (Oxyma): an efficient and convenient additive used with tetramethylfluoroformamidinium hexafluorophosphate (TFFH) to replace 1-hydroxybenzotriazole (HOBt) and 1-hydroxy-7-azabenzotriazole (HOAt) during peptide synthesis. *Bull. Chem. Soc. Japan* 2010; **83**: 1374–1379.
39 Subiros-Funosas R, El-Faham A, Albericio F PyOxP and PyOxB: the Oxyma-based novel family of phosphonium salts. *Org. Biomol. Chem.* 2010; **8**: 3665–3673.
40 El-Faham A, Albericio F. Peptide coupling reagents, more than a letter soup. *Chem. Rev.* 2011; **111**: 6557–6602.
41 Khattab SN, Subirós-Funosas R, El-Faham A, Albericio F. Screening of *N*-alkyl-cyanoacetamido oximes as substitutes for *N*-hydroxysuccinimide. *ChemistryOpen* 2012; **1**: 147–152.
42 Acosta GA, del Fresno M, Paradis-Bas M, Rigau-De LM, Cote S, Royo M, Albericio F. Solid-phase peptide synthesis using acetonitrile as a solvent in combination with PEG-based resins. *J. Pept. Sci.* 2009, **15**: 629–633.
43 Wenschuh H, Beyermann M, Haber H, Seydel JK, Krause E, Bienert M, Carpino LA, El-Faham A, Albericio F Stepwise automated solid phase synthesis of naturally occurring peptaibols using FMOC amino acid fluorides. *J. Org. Chem.* 1995; **60**: 405–410.
44 Carpino LA, El-Faham A, Albericio F. Efficiency in peptide coupling: 1-hydroxy-7-azabenzotriazole *vs* 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine. *J. Org. Chem.* 1995; **60**: 3561–3564.
45 El-Faham A, Albericio F. Morpholine-based immonium and halogenoamidinium salts as coupling reagents in peptide synthesis. *J. Org. Chem.* 2008; **73**: 2731–2737.
46 Carpino LA, El-Faham A. Effect of tertiary bases on O-benzotriazolyluronium salt-induced peptide segment coupling. *J. Org. Chem.* 1994; **59**: 695–698.
47 Carpino LA, Ionescu D, El-Faham A, Beyermann M, Henklein P, Hanay C, Wenschuh H, Bienert M. Complex polyfluoride additive in Fmoc-amino acid fluoride coupling processes. Enhanced reactivity and avoidance of stereomutation. *Org. Lett.* 2003; **5**: 975–977.

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