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ARTICLE TYPE

Peptide Synthesis Beyond DMF:THF and ACN as Excellent and Friendlier Alternatives&

Yahya E. Jad,[a] Gerardo A. Acosta,[b,c] Sherine N. Khattab,[d] Beatriz G. de la Torre,[a,e] Thavendran Govender,[a] Hendrik G. Kruger,[a] Ayman El-Faham*[d,f] and Fernando Albericio*[a,b,c,e,f,g,h]

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To date, DMF has been considered the only solvent suitable for peptide synthesis. Herewe demonstrate the capacity of THF and ACN, which are friendlier solvents than DMF, to yield the product in higher purity than DMF. Using various peptide models, both THF and ACN reducedracemization in solution-phase and solid-phase synthesis when compared with DMF. Moreover, the use of ACN and THF in the solid-phase peptide synthesis of hindered peptides, such as Aib-enkephalinepentapeptide and Aib-ACP decapeptide, in combination with a complete polyethylene glycol resin (ChemMatrix),gave a better coupling efficiency than DMF.

Introduction

Peptide bond (also known as amide bond) formation is the key step in the formation of biologically active compounds such as peptides and proteins. Therefore, advances in the methods used for this purpose are called for.[1, 2] In 1999, an analysis of a comprehensive medical chemistry database revealed the presence of amide groups in more than 25% of known drugs.[3] According to a more recent survey performed by the Pfizer-Groton pharmaceutical group in 2005, for the period 1997-2002, about 38% of interconversion reactions of carboxylic acid derivatives isbased on amide bond formation.[4] Moreover, another survey of chemical syntheses carried out within the R&D departments of the world's largest pharmaceutical companies (GlaxoSmithKline, AstraZeneca and Pfizer) concluded that 66% of the acylation reaction involves amide bond formation.[5] Similarly, in 2011, Roughley's group analyzed the most common reactions used by the same pharmaceutical companies. According to this analysis, the amide bond ranked first with respect to frequency of use, accounting for 16% of all reactions performed, and with the amide linkage present in 54% of the compounds set analyzed.[6, 7]

Various papers have addressed the development of peptide coupling reagents.[8-12] Nowadays, there is an arsenal of such compounds available, thus providing chemists with a wide variety to choose from for the coupling reaction of interest. Generally, DCM (dichloromethane), DMF (*N*,*N*-dimethylformamide), and NMP (*N*-methyl-2-pyrrolidone) are usually used as solvents during the peptide-bond formation reaction. In fact, and according to a another survey, about 83% of peptide bond formation is achieved using DCM or DMF as a solvent.[7] Historically, during the 60s,DCM was the reagent of choice for performing the entire solid-phase synthesis. Later, DMF and, to a lesser extent, NMP were the only solvents used. However, these reagents have several limitations. First, amino acid derivatives and coupling reagents, except carbodiimides, have poor solubility in DCM. Moreover, DCM reacts with primary, secondary, and tertiary amines such as piperidine,[13, 14] which is widely used to remove the Fmoc group in SPPS. Although amino acid derivatives and coupling reagents are soluble in DMF and DMF does not react with piperidine, this reagent can decompose into formaldehyde and dimethylamine, which can jeopardize peptide synthesis. Finally, the search for alternatives to DMF and NMP is mandatory as the implementation of REACH will restrict the use of these compounds.

Given that solvent use consistently accounts for between 80 and 90% of mass utilization in typical batch operations involving pharmaceutical chemicals and within these same operations solvents play a dominant role in the overall toxicity profile of any given process,[15]several studies drew up a classification system of solvents on the basis of the environmental risk posed. Accordingly, DMF and NMP were defined as undesirable solvents; in contrast, THF (tetrahydrofuran) and ACN (acetonitrile) were deemed usable solvents. In fact, these studies reported that ACN is a suitablereplacement for dipolar aprotic solvents such as DMF, NMP, and also DMA (*N*,*N*-dimethylacetamide).[16]

Several years ago, some of the authors of the present study demonstrated that ACN,in combination with a totally polyethyleneglycol-based resin, is agood alternative to DMF and NMP.[17] The capacity of solvents to swell the resin is considered one of the main requirements of solvents used in solid-phasepeptide synthesis. In general, polyethlyeneglycol-based resin swells better than polystyrene in response to all solvents.[18, 19] Here we discuss the use of THF and ACN in solid-phase and solution-phase peptide synthesis.

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Results and Discussion

In this study, we chosea DIC (*N*,*N*′-diisopropylcarbodiimide)-mediated coupling method in combination with HOBt**1** (1-hydroxybenzotriazole, the most classical benzotriazole additive),[20]HOAt**2** (1-hydroxy-7-azabenzotriazole, the most reactive and expensive additive),[21]OxymaPure**3** (ethyl 2-cyano-2-(hydroxyimino)acetate, which showedsuperiority in all cases to HOBt and in many cases the same performance asHOAt)[22, 23] or Oxyma-B **4** (5-(hydroxyimino)-1,3-dimethylpyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione, which showed superiority in racemization suppression in comparison to HOBt**1** and even HOAt**2** and OxymaPure**3**).[24]

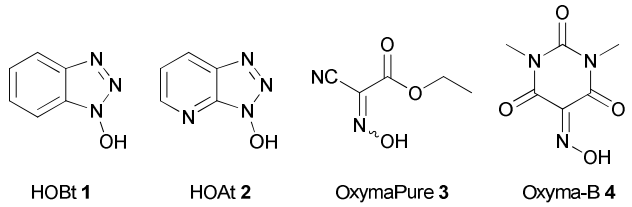

Fig. 1 Structure of the additives used with carbodiimide in this study.

The key parameters used to evaluate the effect of solvent on the coupling reaction are racemization and coupling performance. To address racemization, we selected the previously studied peptide coupling models, namely (1+1) stepwise coupling (Z-Phg-Pro-NH_2, **5**) and (2+1) segment coupling (Z-Phe-Val-Pro-NH_2, **6**) in solution. In the (1+1) model, the α-phenyl moiety in phenylglycine ensured high sensitivity towards racemization. In contrast, the (2+1) model was more prone to racemization since the formation of oxazolone, which occurs during the activation of dipeptide acid,was promoted as a result of the electron-donating effect of the *N*-aminoacyl substitution.[22, 25, 26]

Table 1 Yield and racemization during the formation of Z-Phg-Pro-NH_2**5** (solution-phase synthesis).[a]

Entry	Coupling reagent	Solvent	Yield (%)[b]	DL (%)[c]
1	DIC/HOBt (**1**)	DMF	94.4	9.9
2		THF	93.8	8.0
3		ACN	94.6	4.2
4	DIC/HOAt (**2**)	DMF	91.8	3.7
5		THF	94.1	2.3
6		ACN	94.1	2.6
7	DIC/OxymaPure (**3**)	DMF	94.4	0.9
8		THF	93.5	0.6
9		ACN	95.8	0.6
10	DIC/Oxyma-B (**4**)	DMF	91.0	1.0
11		THF	94.6	1.1
12		ACN	95.0	0.3

[a]Couplings were performed without preactivation at room temperature.[b]Conversion yield calculated by HPLC. Retention times of Z-Phg-OH and Z-Phg-Pro-NH_2 were identified by injection of pure sample.[c]Retention times for each epimer were identified after co-injection with a pure LL and DL sample onto reverse-phase HPLC using linear gradient of 25 to 50% 0.1% TFA in CH_3CN/ 0.1% TFA in H_2O over 15 min, detection at 220 nm and a Phenomex C_{18}(3 μm,4.6 × 50 mm) column, t_R(LL) = 6.4 min, t_R(DL) = 6.8 min.

Regarding the first model **5**, THF and ACN suppressed racemization more than DMF. In addition, ACN afforded a higher coupling efficiency than DMF and THF in all cases. Moreover, OxymaPure and Oxyma-B showed excellent results compared to HOBt and even HOAt in terms of reducing racemization in all solvents.

In the case of (2+1) segment coupling model**6**, THF suppressed racemization more than DMF and ACN when benzotriazole derivatives were used. On the other hand, ACN suppressed the racemization more than DMF and THF when OxymaPure or Oxyma-B was used. However, and in all cases, both THF and ACN showed greater suppression of racemizationthan DMF.

Table 2 Yield and racemization during the formation of Z-Phe-Val-Pro-NH_2**6** (solution-phase synthesis).[a]

Entry	Coupling reagent	Solvent	Yield (%)[b]	LDL (%)[c]
1	DIC/HOBt (**1**)	DMF	96.7	12.9
2		THF	92.6	4.6
3		ACN	96.8	9.5
4	DIC/HOAt (**2**)	DMF	97.7	5.6
5		THF	94.4	0.9
6		ACN	96.9	1.7
7	DIC/OxymaPure (**3**)	DMF	92.4	7.2
8		THF	91.9	1.8
9		ACN	96.2	0.7
10	DIC/Oxyma-B (**4**)	DMF	91.1	4.9
11		THF	88.0	2.2
12		ACN	94.4	0.5

[a]Couplings were performed without preactivation at room temperature.[b]Conversion yield calculated by HPLC. Retention times of Z-Phe-Val-OH and Z-Phe-Val-Pro-NH_2 were identified by injection of pure sample.[c]Retention times for each epimer were identified after co-injection with apure LLL and LDL sample onto reverse-phase HPLC using linear gradient of 30 to 60% 0.1% TFA in CH_3CN/ 0.1% TFA in H_2O over 15 min, detection at 220 nm and a Phenomex C_{18}(3 μm,4.6 × 50 mm) column, t_R(LLL) = 5.8 min, t_R(LDL) = 6.9 min.

After evaluation of the racemization test in solution-phase peptide synthesis, the next step was to test racemization during solid-phase assembly of serine and cysteine residues, because of their unusual racemization sensitivity during solid-phase synthesis.[27-29] In addition, the solid-phase strategy is most commonly used for peptide synthesis for research purposes and even for large-scale production processes.[30]

Table 3 Racemization studies on the solid-phase assembling of H-Gly-Ser-Phe-NH_2**7** (solid-phase synthesis).[a]

Entry	Coupling reagent	Solvent	DL (%)[b]
1	DIC/HOBt (**1**)	DMF	0.6
2		THF	0.3
3		ACN	0.3
4	DIC/HOAt (**2**)	DMF	0.3
5		THF	0.2
6		ACN	0.3
7	DIC/OxymaPure (**3**)	DMF	0.4
8		THF	0.2
9		ACN	0.2
10	DIC/Oxyma-B (**4**)	DMF	0.3
11		THF	0.2
12		ACN	0.3

[a] Couplings were performed 5 min preactivation at room temperature with 1 h coupling times and H-RinkAmide-AM-ChemMatrix resin.[b]Retention times for each epimer were identified after co-injection with a pure LL and DL sample onto reverse-phase HPLC using linear gradient of 0 to 30% 0.1% TFA in CH_3CN/ 0.1% TFA in H_2O over 15 min, detection at 220 nm anda Phenomex C_{18}(3 μm,4.6 × 50 mm) column, t_R(LL) = 5.5 min, t_R(DL) = 6.1 min.

The tripeptide models H-Gly-Ser-Phe-NH_2**7** and H-Gly-Cys-Phe-

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NH_2**8** were manually assembled by stepwise solid-phase synthesis using 5 min preactivation and Ser(tBu) and Cys(Trt) as protecting group for **7** and **8**, respectively.[22, 31, 32] Again, THF and ACN achieved greater suppression of racemization than DMF in both cases.

Table 4. Racemization studies on the solid-phase assembly of H-Gly-Cys-Phe-NH_2 **8** (solid-phase synthesis).[a]

Entry	Coupling reagent	Solvent	DL (%)[b]
1	DIC/HOBt (**1**)	DMF	0.4
2		THF	0.3
3		ACN	0.4
4	DIC/HOAt (**2**)	DMF	0.3
5		THF	0.2
6		ACN	0.3
7	DIC/OxymaPure (**3**)	DMF	0.3
8		THF	0.2
9		ACN	0.3
10	DIC/Oxyma-B (**4**)	DMF	0.3
11		THF	0.3
12		ACN	0.3

[a]Couplings were performed 5 min preactivation at room temperature with 1 h coupling times and H-RinkAmide-AM-ChemMatrix resin.[b]Retention times for each epimer were identified after co-injection with a pure LL and DL sample onto reverse-phase HPLC using linear gradient of 0 to 40% 0.1% TFA in CH_3CN/ 0.1% TFA in H_2O over 15 min, detection at 220 nm and a Phenomex C_{18}(3 µm,4.6 × 50 mm) column, t_R(LL) = 6.7 min, t_R(DL) = 7.95 min.

In order to demonstrate the efficiency of THF and ACN in peptide synthesis, Aib-enkephaline pentapeptide **9** was used as a model for solid-phase peptide synthesis.[22, 25, 26, 33] Misincorporation of one Aib residue to give (des-Aib) is the most important side reaction, which is caused by the sterically hindered nature of the Aib residue. Therefore, a clear difference between the performances of coupling potency in different solventswill be achieved.

For this purpose, **9** was manually assembled stepwise on Fmoc-RinkAmide-AM-ChemMatrix-resin by means of a 1-h coupling (except Aib-Aib where a 1-h double coupling was applied) with an excess of 3 equivalents of Fmoc-amino acid/additive/carbodiimide. THF and ACN gave better results than DMF in all cases (Table 5). Moreover, OxymaPure **3** in combination with THF or ACN showed a spectacular coupling efficiency (over 90% in both cases *vs.* 53% when DMF was used).

Table 5. Percentage of tetrapeptide des-Aib (H-Tyr-Aib-Phe-Leu-NH_2) during solid-phase assembly of pentapeptide **9** (H-Tyr-Aib-Aib-Phe-Leu-NH_2).[a]

Entry	Coupling reagent	Solvent	Pentapeptide (%)	des-Aib(%)[b]
1	DIC/HOBt (**1**)	DMF	4.7	89.9
2		THF	9.6	90.4
3		ACN	12.4	84.4
4	DIC/HOAt (**2**)	DMF	18.2	78.5
5		THF	45.9	54.1
6		ACN	53.6	41.5
7	DIC/OxymaPure (**3**)	DMF	53.0	47.0
8		THF	93.6	6.4
9		ACN	91.8	8.2
10	DIC/Oxyma-B (**4**)	DMF	19.6	80.4
11		THF	62.6	37.4
12		ACN	70.3	29.7

[a]1-h coupling times were generally applied, except for Aib-Aib (1-h double coupling).[b]Deletion tetrapeptide (des-Aib) was identified by peak overlap in HPLC with an authentic sample obtained in solid phase. The crude H-Tyr-Aib-Aib-Phe-Leu-NH_2 was analyzed by reverse-phase HPLC using linear gradient of 20 to 40% 0.1% TFA in CH_3CN/ 0.1% TFA in H_2O over 15 min, detection at 220 nm and a Phenomex C_{18}(3 µm,4.6 × 50mm) column, t_R = 6.68 (pentapeptide), 6.78 (des-Aib) min.

The efficency of using THF or ACN in solid-phase peptide synthesis was further tested in a longer synthesis of Aib^{67}, Aib^{68}-modified ACP decapeptide **10** model (H-Val-Gln-Aib^{67}-Aib^{68}-Ile-Asp-Tyr-Ile-Asn-Gly-NH_2).[34-36]To the best of our knowledge, unmodified ACP decapeptide has frequently beenused fortestingnew protocols.[17, 37-39]In this study, two Aib residuesreplaced the two consecutive Ala residues in the normal ACP decapeptide in order to make it a more difficult sequence and as a result allow a clear observation of the effect of solvent on the synthesis of a long peptide.Decapeptide **10** was manually assembledstepwise on Fmoc-RinkAmide-AM-ChemMatrix-resin by means of a 1-h coupling (except Aib-Aib where a 2-h double coupling was applied) with an excess of 3 equivalents of Fmoc-amino acid/additive/carbodiimide.

Table 6 Solid-phase synthesis of Aib^{67}, Aib^{68}-modified ACP **10**(H-Val-Gln-Aib-Aib-Ile^{69}-Asp-Tyr-Ile^{72}-Asn-Gly-NH_2).[a, b]

Entry	Coupling reagent	Solvent	Decapeptide (%)	des-Aib (%)	des-Aib-Ile^{72} (%)	des-Gln (%)	Byproduct (%)[c]
1	DIC/HOBt (**1**)	DMF	8.2	38.6	-	2.9	42.1
2		THF	4.9	32.8	1.9	1.2	49.3
3		ACN	7.1	46.3	8.2	1.8	31.3
4	DIC/HOAt (**2**)	DMF	23.8	53.0	-	2.7	14.2
5		THF	26.5	56.7	-	2.7	12.2
6		ACN	33.7	51.3	-	1.4	12.9
7	DIC/OxymaPure (**3**)	DMF	37.8	34.0	-	2.7	21.9
8		THF	69.8	26.8	-	-	3.4
9		ACN	49.6	47.4	-	-	3.0
10	DIC/Oxyma-B (**4**)	DMF	10.6	33.5	-	0.8	34.5
11		THF	59.7	10.7	-	4.4	18.3
12		ACN	47.3	43.3	-	2.0	2.4

[a]1-h coupling times with 3-min preactivation were generally applied, except for Aib-Aib (2-h double coupling).[b]The crude decapeptide was analyzed by reverse-phase HPLC using linear gradient of 10 to 50% 0.1% TFA in CH_3CN/ 0.1% TFA in H_2O over 15 min, detection at 220 nm and a Phenomex C_{18}(3 µm,4.6 × 50mm) column, t_R = 6.8 (decapeptide), 6.9 (des-Aib),4.5 (des-Aib-Ile^{72}) and 7.8 min (des-Gln). [c] This byproduct showed 778 m/z $[M+H]^+$, which corresponds to des-Aib-Ile-Asn. However, its retention time did not match that of either des-Aib-Ile^{69}-Asn (t_R = 4.5 min) or des-Aib-Ile^{72}-Asn (t_R = 4.8 min). Also, a pure sample of des-Aib-Ile^{72}-Asn was mixed with crude product, which was obtained from HOBt and THF, and it showed two peaks on the HPLC (for more details, see supporting information).

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In all cases, both THF and ACN yielded better purity of the decapeptide **10** than DMF. Moreover, OymaPure with THF as a solvent rendered the best result in this study (69.8%, entry 8 in Table 6). Moreover, THF and ACN suppressed the formation of byproducts in all cases.

Conclusions

DMF and, to a lesser extent, NMP are considered the solvents of choice for peptide/amide formation. However, we have demonstrated that THF and ACN, which are friendlier solvents than DMF/NMP, are excellent alternatives to DMF/NMP in terms of minimization of racemization and coupling yield in both solution and solid-phase peptide synthesis. Finally, the combination of THF/ACN as solvents, DIC/OxymaPure, and the ChemMatrix resin gave the best results in term of yield purity and less racemization.

Experimental

Materials

The solvents used were of HPLC reagent grade. Chemicals and amino acid derivatives were purchased from Sigma-Aldrich, Fluka, Gl Biochem (Shanghai) Ltd., Iris Biotech GmbH, or Merck Millipore. The following coupling reagents were used: DIC (Fluka, lot number BCBK8348V);HOBt (GlBiochem (Shanghai) Ltd., Lot number GLS110604-00602); HOAt (Gl Biochem (Shanghai) Ltd., Lot number GLS121115-00601); and OxymaPure (Luxembourg Biotech., Batch number 1301117008). Analytical HPLC was performed on an Agilent 1100 system, and Chemstation software was used for data processing. LC-MS was performed on Shimadiz 2020 UFLC-MS using an YMC-Triart C_{18} (5 µm, 4.6 × 150 mm) column and data processing was carried out by LabSolution software. Buffer A: 0.1% formic acid in H_2O; buffer B: 0.1% formic acid in CH_3CN.

General Method for the Racemization Experiments[33, 36]

0.125 mmol of an acid (Z-Phg-OH or Z-Phe-Val-OH), 0.125 mmol of H-Pro-NH_2, and 0.125 mmol of the corresponding additive were dissolved in a solvent, and the solution was cooled in an ice bath and treated with 0.125 mmol of DIC. The mixture was stirred at 0°C for 1 h and at room temperature overnight. An aliquot (10 µL) of the solution was then picked up and diluted to 1 mL with a mixture of CH_3CN/H_2O (1:2), and 5µL was injected into a reverse-phase HPLC apparatus.

Z-Phg-Pro-NH_2

A linear gradient of 25–50% 0.1% TFA in CH_3CN/ 0.1% TFA in H_2O over 15 min was applied, with a flow rate of 1.0 mL/min and detection at 220nm using a Phenomex C_{18} (3 µm,4.6 × 50 mm) column, t_R(LL) = 6.4 min, t_R(DL) = 6.8 min, t_R(Z-Phg-OH) = 9.1 min.

Z-Phe-Val-Pro-NH_2

A linear gradient of 30–60% 0.1% TFA in CH_3CN/ 0.1% TFA in H_2O over 15 min was applied, with a flow rate of 1.0 mL/min and detection at 220 nm using a Phenomex C_{18} (3 µm, 4.6 × 50 mm) column, t_R(LLL) = 5.8 min, t_R(LDL) = 6.9 min, t_R(Z-Phe-Val OH) = 8.1 min.

Study of serine racemization during assembly of H-Gly-Ser-Phe-NH_2 on solid phase[29, 31]

Experiments consisted of the study of the stepwise coupling of Ser and Gly residues onto previously formed H-Phe-RinkAmide-AM-ChemMatrix-resin (0.52 $mmolg^{-1}$, 100 mg), with the use of the Fmoc/*t*Bu and Ser(^{t}Bu) protection strategy. Glycine was introduced in order to achieve better separation of LL and DL isomers than des-Gly dipeptides. Coupling times of 1 h were used after 5 min preactivation of a solution of Fmoc-amino acids (3 equiv.), the corresponding additive (3 equiv.), and DIC (3equiv.) in a minimum amount of solvent (DMF, THF or ACN) at room temperature. Fmoc removal was carried out with 20% piperidine in DMF for 7 min. The peptide chain was released from the resin by treatment with TFA/H_2O/TIS (95:2.5:2.5) for 1h at room temperature. The colorless solution was filtered, and the resin was washed with CH_2Cl_2 (3×0.5 mL). The solvent and residues from the cleavage cocktail were concentrated under nitrogen. The crude peptide was precipitated with cold Et_2O (3×5 mL) and, after being lyophilized, was analyzed by reversed-phase HPLC, with the use of a Phenomex C_{18} (3 µm, 4.6 × 50 mm) column, linear gradient 0 to 30% of 0.1% TFA in CH_3CN/0.1% TFA in H_2O over 15 min, with detection at 220 nm. The t_R values of the LL and DL epimers were5.55 min and 6.06 min, respectively. LC–MS showed the expected mass for the tripeptide at m/z = 309.0.

Study of cysteine racemization during assembly of H-Gly-Cys-Phe-NH_2 on solid phase[22, 27, 29]

Experiments consisted of the study of the stepwise coupling of Cys and Gly residues onto previously formed H-Phe-RinkAmide-AM-ChemMatrix-resin (0.52 $mmolg^{-1}$, 100 mg), with the use of the Fmoc/*t*Bu and the Cys(Trt) protection strategy. Glycine was introduced in order to achieve better separation of LL and DL isomers than des-Gly dipeptides. Coupling times of 1 h were used after 5 min preactivation of a solution of Fmoc-amino acids (3 equiv.), the corresponding additive (3 equiv.), and DIC (3 equiv.) in minimum amount of solvent (DMF, THF or ACN) at room temperature. Fmoc removal was carried out with 20% piperidine in DMF for 7 min. The peptide chain was released from the resin by treatment with TFA/H_2O/TIS (95:2.5:2.5) for 1 h at room temperature. The colorless solution was filtered and the resin was washed with CH_2Cl_2 (3×0.5 mL). The solvent and residues from the cleavage cocktail were concentrated under nitrogen. The crude peptide was precipitated with cold Et_2O (3×5 mL) and, after being lyophilized, was analyzed by reversed-phase HPLC, with the use of a Phenomex C_{18} (3 µm,4.6 × 50 mm) column, linear gradient 0 to 40% of 0.1% TFA in CH_3CN/0.1% TFA in H_2O over 15 min, with detection at 220 nm. The t_R values of the

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LL and DL epimers were 6.79 and7.95 min, respectively. LC–MS showed the expected mass for the tripeptide at m/z = 325.0.

Solid-Phase Synthesis of H-Tyr-Aib-Aib-Phe-Leu-NH_2[33, 35, 37]

The synthesis was carried out in a plastic syringe, attached to a vacuum manifold so as to effect rapid removal of reagents and solvent. The Fmoc-RinkAmide-AM-PEG resin (0.58 $mmolg^{-1}$, 50 mg)was washed with DMF, DCM, and DMF (2×10 mL each) and then treated with 20% piperidine in DMF (10 mL) for 10 min. The resin was then washed with DMF and DCM, and then with solvent used during the coupling step (2×10 mL each). The resin was thenacylated with a solution of Fmoc-Leu-OH (3 equiv.), the corresponding additive (3 equiv.) and DIC (3 equiv.) in minimum amount of solvent (DMF, THF or ACN) at room temperature and preactivated for 3 min. After peptide coupling, the resin was washed with DMF, DCM, and DMF and then deblocked by treatment with 20% piperidine in DMF for 7 min. Next, washing and coupling with the next amino acid, as explained before, and deblocking, was repeated to obtain the pentapeptide. The peptide was cleaved from the resin with TFA/H_2O (9:1) at room temperature for 2 h. TFA was removed under nitrogen, and the crude peptide was purified with cold Et_2O (3×10 mL) and lyophilized. The ratio of the penta- and tetra-peptide was determined by HPLC analysis by using a Phenomex C_{18} (3 µm, 4.6 × 50 mm) column, with a linear gradient of 20 to 40% of 0.1% TFA in CH_3CN/0.1 %TFA in H_2O over 15 min, flow rate = 1.0 $mLmin^{-1}$, detection at 220 nm. The t_R values for pentapepide and des-Aib were 6.68 min and 6.78 min, respectively. LC–MS showed the expected mass for the pentapeptide at m/z = 611.0, and also for des-Aib at m/z = 526.

Solid-Phase Synthesis of Aib[67], Aib[68]-modified ACP (65-74) decapeptide [H-Val-Gln-Aib[67]-Aib[68]-Ile-Asp-Tyr-Ile-Asn-Gly-NH_2][35, 36]

The synthesis was carried out in a plastic syringe, attached to a vacuum manifold so as to effect rapid removal of reagents and solvent. The Fmoc-RinkAmide-AM-PEG resin (0.52 $mmolg^{-1}$, 50 mg), was washed with DMF, DCM, and DMF (2×10 mL each) and then treated with 20% piperidine in DMF (10 mL) for 10 min. The resin was then washed with DMF, and DCM, and then with the solvent used during the coupling step(2×10 mL each). The resin was thenacylated with a solution of Fmoc-Gly-OH (3 equiv.), the corresponding additive (3 equiv.), and DIC (3 equiv.) in minimum amount of solvent (DMF, THF or ACN) at room temperature and preactivated for 3 min. After peptide coupling, the resin was washed with DMF and then deblocked by treatment with 20% piperidine in DMF for 7 min. Next, washing and coupling with the next amino acid, as explained before, and deblocking, was repeated to obtain the decapeptide. The peptide was cleaved from the resin with TFA/H_2O (9:1) at room temperature for 2 h. TFA was removed under nitrogen, and the crude peptide was purified with cold Et_2O (3×10 mL) and lyophilized. The purity of product was determined by HPLC analysis by using a Phenomex C_{18} (3 µm, 4.6 × 50 mm) column, with a linear gradient of 10 to 50% of 0.1% TFA in CH_3CN/0.1 %TFA in H_2O over 15 min, flow rate = 1.0 $mLmin^{-1}$, detection at 220 nm. The t_R values for decapepide, des-Aib, des-Aib-Ile[72] and des-Gln were 6.8 (decapeptide), 6.9(des-Aib), 4.5 (des-Aib-Ile[72]) and 7.8 min (des-Gln), respectively. LC–MS showed the expected mass for the decapeptide at m/z = 1090, des-Aib at m/z = 1005, des-Aib-Ile[72] at m/z = 892 and des-Glnat m/z = 963.

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Notes and references

[a]*Catalysis and Peptide Research Unit, School of Health Sciences, University ofKwazulu-Natal, Durban 4001, South Africa.*
[b]*Institute for Research in Biomedicine-Barcelona,08028-Barcelona, Spain. E-mail: albericio@irbbarcelona.org*
[c]*CIBER-BBN, Networking Centre on Bioengineering, Biomaterials and Nanomedicine, Barcelona Science Park, 08028-Barcelona, Spain*
[d]*Department of Chemistry, Faculty of Science, Alexandria UniversityP.O. Box 426, Ibrahimia, Alexandria 21321, Egypt.E-mail: aelfaham@ksu.edu.sa*
[e]*School of Chemistry, Yachay Tech, Yachay City of Knowledge, 100119-Urcuqui, Ecuador. E-mail: falbericio@yachaytech.edu.ec*
[f]*Department of Chemistry, College of Science, King Saud UniversityP.O. Box 2455,Riyadh11451, Saudi Arabia*
[g]*School of Chemistry and Physics, University ofKwazulu-Natal, Durban 4001, South Africa*
[h]*Department of Organic Chemistry, University of Barcelona, 08028-Barcelona, Spain*
[&]Although the term "green" could not be strictly applied to THF and ACN, both could be more considered "greener" or the friendlier use than DMF
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1. T. Cupido, J. Tulla-Puche, J. Spengler and F. Albericio, *Curr. Opin. Drug Discovery Dev.*, 2007, **10**, 768-783.
2. A. A. Zompra, A. S. Galanis, O. Werbitzky and F. Albericio, *Future Med. Chem.*, 2009, **1**, 361-377.
3. A. K. Ghose, V. N. Viswanadhan and J. J. Wendoloski, *J. Comb. Chem.*, 1999, **1**, 55-68.
4. R. W. Dugger, J. A. Ragan and D. H. B. Ripin, *Org. Process Res. Dev.*, 2005, **9**, 253-258.
5. J. S. Carey, D. Laffan, C. Thomson and M. T. Williams, *Org. Biomol. Chem.*, 2006, **4**, 2337-2347.
6. S. D. Roughley and A. M. Jordan, *J. Med. Chem.*, 2011, **54**, 3451-3479.
7. D. S. MacMillan, J. Murray, H. F. Sneddon, C. Jamieson and A. J. B. Watson, *Green Chem.*, 2013, **15**, 596-600.
8. F. Albericio and L. A. Carpino, in *Methods Enzymol.*, ed. B. F. Gregg, Academic Press, 1997, vol. Volume 289, pp. 104-126.
9. S.-Y. Han and Y.-A. Kim, *Tetrahedron*, 2004, **60**, 2447-2467.
10. C. A. G. N. Montalbetti and V. Falque, *Tetrahedron*, 2005, **61**, 10827-10852.
11. E. Valeur and M. Bradley, *Chem. Soc. Rev.*, 2009, **38**, 606-631.
12. A. El-Faham and F. Albericio, *Chem. Rev.*, 2011, **111**, 6557-6602.

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DOI: 10.1039/C4OB02046D

13. J. E. Mills, C. A. Maryanoff, D. F. McComsey, R. C. Stanzione and L. Scott, *J. Org. Chem.*, 1987, **52**, 1857-1859.
14. A. B. Rudine, M. G. Walter and C. C. Wamser, *J. Org. Chem.*, 2010, **75**, 4292-4295.
15. D. J. C. Constable, C. Jimenez-Gonzalez and R. K. Henderson, *Org. Process Res. Dev.*, 2007, **11**, 133-137.
16. K. Alfonsi, J. Colberg, P. J. Dunn, T. Fevig, S. Jennings, T. A. Johnson, H. P. Kleine, C. Knight, M. A. Nagy, D. A. Perry and M. Stefaniak, *Green Chem.*, 2008, **10**, 31-36.
17. G. A. Acosta, M. del Fresno, M. Paradis-Bas, M. Rigau-DeLlobet, S. Cote, M. Royo and F. Albericio, *J. Pept. Sci.*, 2009, **15**, 629-633.
18. F. Garcia-Martin, M. Quintanar-Audelo, Y. Garcia-Ramos, L. J. Cruz, C. Gravel, R. Furic, S. Cote, J. Tulla-Puche and F. Albericio, *J. Comb. Chem.*, 2006, **8**, 213-220.
19. Y. Garcia-Ramos, M. Paradis-Bas, J. Tulla-Puche and F. Albericio, *J. Pept. Sci.*, 2010, **16**, 675-678.
20. W. König and R. Geiger, *Chem. Ber.*, 1970, **103**, 788-798.
21. L. A. Carpino, *J. Am. Chem. Soc.*, 1993, **115**, 4397-4398.
22. R. Subirós-Funosas, R. Prohens, R. Barbas, A. El-Faham and F. Albericio, *Chem. Eur. J.*, 2009, **15**, 9394-9403.
23. R. Subiros-Funosas, S. N. Khattab, L. Nieto-Rodriguez, A. El-Faham and F. Albericio, *Aldrichim. Acta*, 2013, **46**, 21-40.
24. Y. E. Jad, S. N. Khattab, B. G. de la Torre, T. Govender, H. G. Kruger, A. El-Faham and F. Albericio. *Org. Biomol. Chem.*,**12**, 8379-8385 (2014).
25. A. El-Faham and F. Albericio, *Org. Lett.*, 2007, **9**, 4475-4477.
26. A. El-Faham and F. Albericio, *J. Org. Chem.*, 2008, **73**, 2731-2737.
27. T. Kaiser, G. J. Nicholson, H. J. Kohlbau and W. Voelter, *Tetrahedron Lett.*, 1996, **37**, 1187-1190.
28. N. Robertson, L. Jiang and R. Ramage, *Tetrahedron*, 1999, **55**, 2713-2720.
29. W. Van Den Nest, S. Yuval and F. Albericio, *J. Pept. Sci.*, 2001, **7**, 115-120.
30. T. Bruckdorfer, O. Marder and F. Albericio, *Curr. Pharm. Biotechnol.*, 2004, **5**, 29-43.
31. A. Di Fenza, M. Tancredi, C. Galoppini and P. Rovero, *Tetrahedron Lett.*, 1998, **39**, 8529-8532.
32. Y. Han, F. Albericio and G. Barany, *J. Org. Chem.*, 1997, **62**, 4307.
33. S. N. Khattab, R. Subiros-Funosas, A. El-Faham and F. Albericio, *ChemistryOpen*, 2012, **1**, 147-152.
34. L. A. Carpino, D. Ionescu, A. El-Faham, M. Beyermann, P. Henklein, C. Hanay, H. Wenschuh and M. Bienert, *Org. Lett.*, 2003, **5**, 975-977.
35. A. El-Faham, R. Subirós-Funosas and F. Albericio, *Eur. J. Org. Chem.*, 2010, **2010**, 3641-3649.
36. P. Cherkupally, G. A. Acosta, L. Nieto-Rodriguez, J. Spengler, H. Rodriguez, S. N. Khattab, A. El-Faham, M. Shamis, Y. Luxembourg, R. Prohens, R. Subiros-Funosas and F. Albericio, *Eur. J. Org. Chem.*, 2013, **2013**, 6372-6378.
37. A. El-Faham, R. S. Funosas, R. Prohens and F. Albericio, *Chem. Eur. J.*, 2009, **15**, 9404-9416.
38. C. A. Chantell, M. A. Onaiyekan and M. Menakuru, *J. Pept. Sci.*, 2012, **18**, 88-91.
39. J. M. Collins, K. A. Porter, S. K. Singh and G. S. Vanier, *Org. Lett.*, 2014, **16**, 940-943.