PAPER

Check for updates

A practical method for the synthesis of small peptides using DCC and HOBt as activators in H₂O–THF while avoiding the use of protecting groups†

Karina Herrera-Guzmán,[a] Miguel Ángel Jaime-Vasconcelos,[a] Eréndira Torales,[a] Itzel Chacón,[a] Rubén Gaviño,[a] Eréndira García-Ríos,[a] Jorge Cárdenas [ID] *[a] and José A. Morales-Serna [ID] *[b]

The synthesis of peptides in solution proceeds through successive steps involving the removal of a protecting group and the formation of the peptide bond. While most methodological efforts have focused on the development of new protecting groups and coupling agents, methodologies based on minimal protecting groups have been less explored. In this research, a peptide synthesis methodology was developed using DCC and HOBt in THF–H₂O, avoiding the use of protecting groups, reducing reaction times, and reusing HOBt during successive couplings. The reaction conditions allow the production of peptides that can directly serve as the starting material for the next coupling, leading to the creation of small peptide sequences, which in turn are precursors to biologically important molecules. Here we explore the example of Sansalvamide as a template for other active peptides. Unlike SPPS, our methodology constructs the sequence from the N-terminus to C-terminus. This unique approach could streamline peptide synthesis and facilitate the development of complex peptides efficiently.
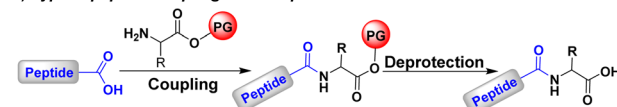
Introduction

Peptide synthesis is at the cutting edge of contemporary research, as peptides play an important role in many fields. Peptide-based materials with diverse functionalities address biomaterials with significant interest because they complement small molecule therapeutics.[1] However, peptide synthesis remains a challenging task due to the inherent thermodynamic limitations of amide bond formation. From an energetic standpoint, the formation of an amide from a carboxylic acid and an amine is thermodynamically unfavourable.[2] To facilitate this process, it is essential to exploit carboxylic acid derivatives, such as acyl halides, anhydrides, and esters.[3–5] For peptide bond formation, the most common approach involves activating the carboxylic acid using coupling agents, which enables bond formation under mild reaction conditions.[6–9]
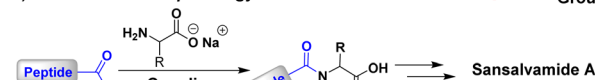
The synthesis of peptides with a defined sequence of amino acids is a demanding task, despite it only involves forming a series of amide bonds to build the desired peptide sequence.[10,11]

This complexity arises from the need to protect functional groups not involved in the amide bond formation[12,13] and in order to prevent undesired side reactions, such as the formation of *N*-acyl ureas, oxazolones,[14] and peptide epimers.[15–17] Peptide bond formation typically proceeds through successive coupling and deprotection steps,[18–21] requiring two reactions for each new C–N bond (Scheme 1a). While the correct choice of protecting groups ensures the desired sequence, the appropriate selection of coupling agents and reaction conditions is crucial to avoid racemisation or epimerisation of the final product.[12–17]

Scheme 1 Synthetic strategy.

[a]Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Ciudad de Mexico, 04510, Mexico. E-mail: rjcp@unam.mx

[b]Centro de Investigaciones Científicas, Instituto de Química Aplicada, Universidad del Papaloapan, Tuxtepec, Oaxaca, 68301, Mexico. E-mail: joseantonio.moralesserna@gmail.com

† Electronic supplementary information (ESI) available: NMR data and spectra of the synthesised compounds. See DOI: https://doi.org/10.1039/d4ra07847k

Although established methodologies exist,[11,21] recent efforts have focused on developing new coupling strategies,[22–30] coupling agents,[31–41] and protecting groups,[42–44] as well as on establishing synthetic protocols where amino acids are mostly free of protecting groups[45–49] and employing environmentally friendly solvents.[50–56] Here, we propose a strategy for synthesising small peptides to contribute to this kind of methodologies. Our approach is based on the use of *N,N'*-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) in a THF–H$_2$O mixture. This approach eliminates the need for protecting groups and, consequently, extra deprotection steps (Scheme 1b). Furthermore, the peptide obtained from the reaction can be transferred directly to the next coupling without purification. This straightforward approach resembles solid-phase peptide synthesis (SPPS) since intermediates are not isolated.[57–60] Nonetheless, it is important to highlight the absence of intermediate deprotection steps.[61–65]

Another significant contribution from our strategy is the use of DCC as a coupling agent. Despite being cost-effective compared to other water-soluble carbodiimides, DCC is often avoided due to the challenges associated with removing its byproduct, 1,3-dicyclohexyl urea (DCU).[66] To facilitate the removal, we took advantage of the environmentally friendly solvent mixture THF : H$_2$O as precipitation and elimination of DCU is very easy by filtration, avoiding the need for chromatographic techniques. Additionally, our approach allows for the reuse of HOBt during peptide chain growth, as previously reported.[67]

Given the current interest in developing efficient protocols for synthesising cyclic peptides[68,69] and the need for synthetic samples in biological assays,[70–74] we aim to apply our methodology to the synthesis of different depsipeptides, focusing first on Sansalvamide A as target.[75] This application will help to demonstrate the potential scope and impact of our work.
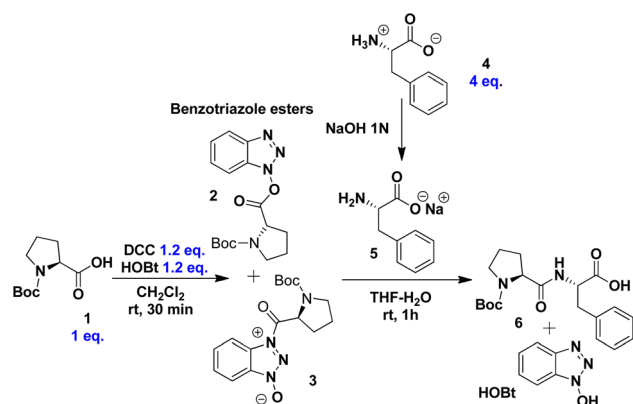
Results and discussion

To establish optimal reaction conditions, we first evaluated various conditions for synthesising the dipeptide *N*-Boc-Pro-Phe-OH **6** from *N*-Boc-Pro **1** and the zwitterion of L-phenylalanine **4**. The process begins with the formation of benzotriazole

esters **2** and **3** through the reaction of DCC and HOBt in CH$_2$Cl$_2$ (Scheme 2). After 30 minutes, thin-layer chromatography (TLC) confirmed that the conversion to activated esters was complete. Dichloromethane was chosen for this step due to its effectiveness in quantitatively forming these intermediates.[76–79] Following solvent removal, the mixture of esters **2** and **3** was redissolved in THF exploring its water miscibility, a key feature in peptide bond formation under our reaction conditions.

Meanwhile, the zwitterion of L-phenylalanine **4** was dissolved in THF. To obtain the predominant carboxylate form **5**, an equimolar amount of a 1 N aqueous solution of NaOH was added. Consequently, the amino group acts as the most nucleophilic species for peptide bond formation. To form dipeptide **6**, the solution containing **5** was slowly added to the mixture of benzotriazol esters **2** and **3**, with constant stirring for 5 minutes. After stirring for an additional 55 minutes, the reaction mixture was filtered to remove DCU, yielding a product consisting of dipeptide **6** and HOBt (Scheme 2).

Next, we established a kinetic profile varying the equivalents of **4**, which helped determine the highest yield of reaction when an excess of 4 equivalents of zwitterion were added (Fig. 1a). Additionally, the reaction yield for **6** reached a maximum of 80% with no further increase despite extended reaction time (Fig. 1b). Yields were quantified by ^{1}H NMR of the crude reaction mixture.
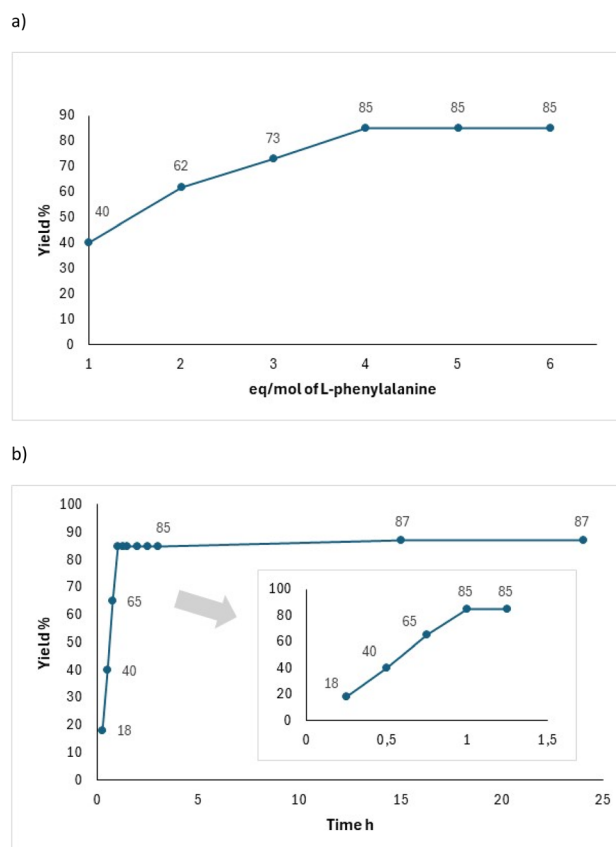

Fig. 1 Reaction kinetic to obtain **6**: (a) varying equivalents of L-phenylalanine **4** and (b) varying the reaction time.

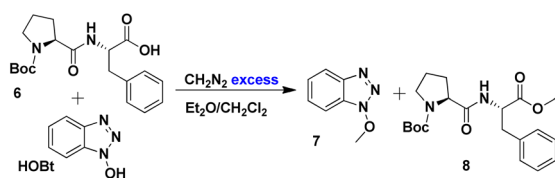Scheme 2 Synthesis of dipeptide **6**.

The mixture of HOBt and dipeptide **6** underwent a methylation reaction with diazomethane, resulting in the quantitative formation of the methylated derivatives of benzotriazole **7** and dipeptide **8** (Scheme 3). The reaction products were separated by column chromatography, and **8** was characterised by NMR, with no epimer formation observed. The optical rotation reported for compound **8** in methanol was −53.4° ($\lambda = 589$ nm),[80] while our value at 25 °C was −53.8° ($\lambda = 589$ nm), establishing that no epimerisation occurred during peptide bond formation.

To further explore our methodology, we synthesised peptides **9–15** from Boc-L-Pro **1**, with yields determined by ^{1}H NMR (Table 1). The lowest yield (50%) was observed with non-chiral glycine, while the highest (80%) was seen with leucine.

Next, we focused on synthesising Sansalvamide A **23** to demonstrate the applicability of our peptide chain elongation strategy to biologically relevant molecules. Sansalvamide A is a cyclopenta depsipeptide isolated from the fungus *Fusarium* sp.,[75] noted for its inhibitory activity against the topoisomerase of the *Molluscum contagiosum*[81] virus and its cytotoxicity against various colon cancer cell lines (COLO 205, SKMEL-2, and HCT 116, HT-29).[82–84]
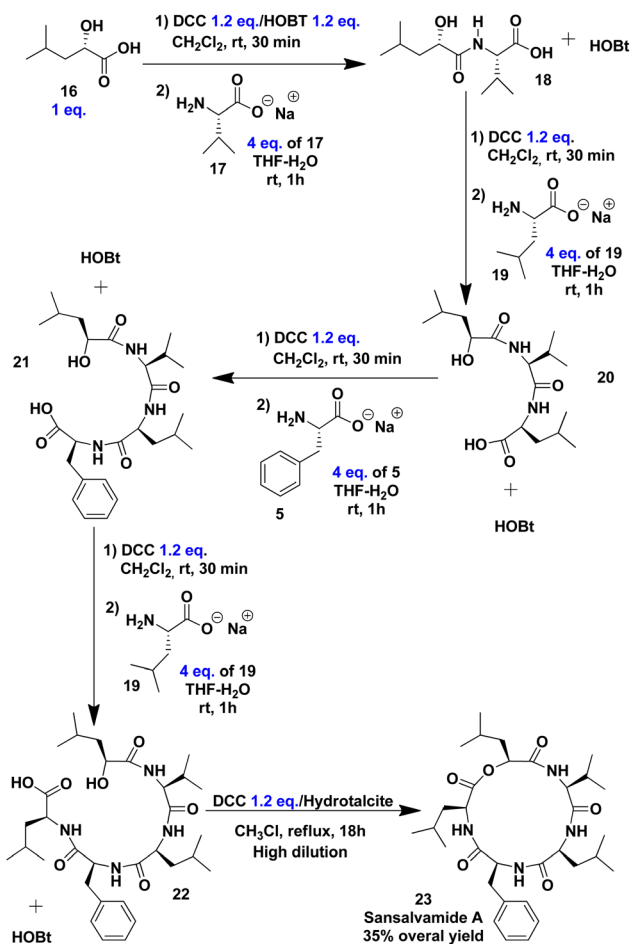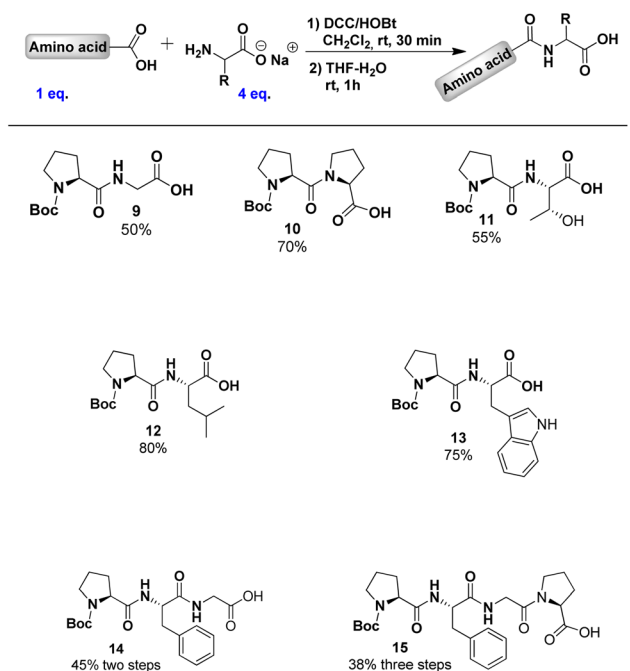
The initial step in synthesising Sansalvamide A involved coupling α-hydroxy acid **16** with the zwitterion of L-valine **17** (Scheme 4). The corresponding benzotriazole esters of **16** were first formed in the presence of DCC and HOBt in CH_2Cl_2. The formation of activated esters was verified by TLC, and after 30 minutes of reaction, the conversion was found to be quantitative. After solvent removal, the mixture of benzotriazole esters was dissolved in THF. As established with **4**, L-valine **17** was meanwhile being dissolved in a THF/aqueous NaOH solution. Following the addition of **16** to **17**, dipeptide **18** in a mixture with HOBt was used without further purification for the next step. Subsequent coupling steps lead to completion of the primary structure of Sansalvamide A until peptide **22** without intermediate purification. Peptide **22** is the direct precursor of the cyclodepsipeptide Sansalvamide A (Scheme 4). The formation of each peptide bond was monitored *via* ^{1}H NMR analysis of the crude reaction mixture.

The macrolactonization as the final step took place under high-dilution conditions in the presence of DCC and hydrotalcite, a solid catalytic material serving as a solid base.[85] The resulting mixture was purified by column chromatography, yielding cyclodepsipeptide **23** as a white solid, which was characterised by NMR and electrospray ionisation ion trap (ESI-IT) mass spectrometry (see ESI†). The NMR data matched those reported for the natural product (Table 2), and the purity of **23** was confirmed by HPLC, with no epimers detected.

Table 1 Scope of the method for synthesising different peptides

Scheme 3 Methylation reaction with diazomethane.

Scheme 4 Synthesis of Sansalvamide A.

Table 2 Comparison of ^{1}H and ^{13}C NMR data from this study with literature values for Sansalvamide A

	^{1}H NMR (CD$_3$OD)		^{13}C NMR (CD$_3$OD)	
	Ref. 74	This work	Ref. 74	This work
OLeu				
C=O			172.9	172.8
α-CH	5.06 (dd, J = 8.8, 4.9 Hz)	5.06 (dd, J = 9, 4.8 Hz)	76.2	76.3
β-CH$_2$	1.76–1.88 (m)	1.62–1.88 (m)	42.1	41.8
	1.58–1.64 (m)			
γ-CH	1.66–1.78 (m)	1.62–1.67 (m)	26.1	26.1
δ-CH$_3$	0.99 (d, J = 6.4 Hz)	0.99 (d, J = 6.4 Hz)	23.4	23.3
	0.96 (d, J = 6.4 Hz)	0.96 (d, J = 6.4 Hz)	22.2	22.1
Val				
C=O			173.8	174.0
α-CH	4.09 (d, J = 8.8 Hz)	4.09 (d, J = 8.5 Hz)	60.9	60.7
β-CH	2.10 (m)	2.07 (m)	32.2	32.1
γ-CH$_3$	0.96 (d, J = 6.4 Hz)	0.92 (d, J = 6.6 Hz)	20.0	19.9
	0.92 (d, J = 6.8 Hz)	0.86 (d, J = 6.6 Hz)	19.0	18.8
Leu-1				
C=O			174.1	174.09
α-CH	3.83 (br dd, J = 8.8, 5.4 Hz)	3.74 (dd, J = 9, 5.2 Hz)	56.2	56.4
β-CH$_2$	1.72 (m)	1.72 (m)	40.1	39.7
	1.38 (m)	1.38 (m)		
γ-CH	1.41 (m)	1.41 (m)	26.2	26.2
δ-CH$_3$	0.85 (d, J = 6.4 Hz)	0.85 (d, J = 6.5 Hz)	23.2	23.1
	0.81 (d, J = 5.9 Hz)	0.81 (d, J = 6.2 Hz)	22.2	22.1
Phe				
C=O			173.7	173.6
α-CH	4.60 (dd, J = 10.8, 4.4 Hz)	4.57 (dd, J = 10.8, 4.8 Hz)	58.1	58.1
β-CH$_2$	3.23 (dd, J = 13.9, 4.6 Hz)	3.23 (dd, J = 13.9, 4.8 Hz)	38.4	38.1
	3.06 (dd, J = 13.7, 10.7 Hz)	3.07 (dd, J = 13.9, 10.8 Hz)		
1 Ar			138.8	138.7
2 and 6 Ar	7.25–7.32 (m)	7.22–7.29 (m)	130.4	130.2
3 and 5 Ar	7.25–7.32 (m)	7.22–7.29 (m)	129.7	129.7
4 Ar	7.22 (m)	7.22–7.29 (m)	128.0	128.0
Leu-2				
C=O			171.3	171.4
α-CH	4.73 (dd, J = 9.8, 5.9 Hz)	4.72 (dd, J = 9.7, 5.8 Hz)	52.6	52.5
β-CH$_2$	1.64–1.86 (m)	1.64–1.86 (m)	41.6	41.4
γ-CH	1.62 (m)	1.62 (m)	26.0	25.9
δ-CH$_3$	0.99 (d, J = 6.4 Hz)	0.99 (d, J = 6.4 Hz)	23.5	23.5
	0.96 (d, J = 6.4 Hz)	0.96 (d, J = 6.4 Hz)	22.4	22.4

After purifying the final product, the overall yield of the process was 35%. To enhance this yield, we tested THF instead of CH$_2$Cl$_2$ for benzotriazole ester formation, but the overall yield dropped to 20%. A comparison of our methodology with those previously reported for Sansalvamide A showed our yield is not different from those works using LPPS[86] or SPPS.[87,88] Moreover, the time required for peptide bond formation is strikingly reduced, as detailed in Table 3.

Importantly, while DCC is typically avoided due to challenges in removing its byproduct, DCU, our use of a THF–H$_2$O solvent system facilitates its precipitation and removal by filtration. Furthermore, our methodology minimises waste generation, as HOBt is reused throughout the process. These achievements of our approach undoubtedly represent significant environmental and economic benefits.[89–93]

Table 3 Comparison of the efficiency of various routes for synthesising Sansalvamide A

	Strategy			
	LPPS[a]	LPPS[a]	SPPS[b]	SPPS[b]
Coupling reagent	DCC/HOBt	EDC/HOBt	HBTU	PyBOP
Reuse of coupling reagent	No/Yes	No/No	No	No
Solvent	THF/H$_2$O	CH$_2$Cl$_2$	NMP	CH$_3$CN
Time for coupling (h)	1.5	16	6	18
Steps	5	9	13	12
Overall yield (%)	35	45	40	13
Reference	This work	86	87	88

[a] LPPS: Liquid Phase Peptide Synthesis. [b] SPPS: Solid Phase Peptide Synthesis.

Conclusions

This work focused on the development of an efficient methodology for the coupling of peptides without protective groups in a THF–H_2O system. Our approach not only allows the coupling of amino acids in a shorter time than other methodologies, but it is also cost-effective due to the use of DCC and recycling of HOBt. This results in a significant reduction in the waste produced during the reaction.

Finally, we tested this methodology with the total synthesis of Sansalvamide A, a cyclodepsipeptide with anticancer activity. We strongly believe this approach will facilitate the development of libraries for the study of cyclopeptides, thus contributing to the advancement of research on biologically active compounds.

Experimental

General information

All the chemicals were purchased from Sigma-Aldrich and used without further purification. Yields refer to materials that are chromatographically and spectroscopically homogeneous (^{1}H and ^{13}C) unless otherwise stated. Reactions were monitored by TLC on 0.25 mm Macherey Nagel silica gel plates. Developed TLC plates were visualised under a short-wave UV lamp and by heating plates dipped in $Ce(SO_4)_2$ or ninhydrin reagent 2% ethanol solution. Flash column chromatography (FCC) was performed using flash silica gel (230–400) with solvent polarity correlated to TLC mobility. Optical rotations were measured at 598 nm on a Jasco DIP-370 digital polarimeter using a 100 mm cell. NMR experiments were conducted on aVarian-Gemini 200 MHz, Varian Unity 300 MHz and, Bruker-Avance 300 MHz instruments, using $CDCl_3$ (99.9% D) as the solvent. Chemical shifts (δ) were referenced to internal standards $CDCl_3$ (7.26 ppm ^{1}H, 77.0 ppm ^{13}C) or Me_4Si as an internal reference (0.00 ppm). Chemical shifts are reported in parts per million (ppm). HPLC/MS were collected using a Waters 1525 chromatograph coupled with a Bruker Esquire 600 ESI-IT using a ZORBAX Eclipse Plus C18 3.5 μm 2.1 × 100 mm (Agilent), the mobile phase flow-rate was 0.2 mL min^{-1} and the detection range was 200–600 nm. Elution solvents were A (water/formic acid 99.9/0.1, v/v) and B (methanol) and the elution program was from 40 to 100% of B during 15 min followed by isocratic elution with 100% of B during 7 min.

General procedure for peptide bond formation

Synthesis of peptide Boc-Pro-Phe-OH 6. Flask A: a solution of N-Boc-L-Pro **1** (1 g, 4.651 mmol), DCC (1.149 g, 5.581 mmol) and HOBt (0.785 g, 5.581 mmol) in CH_2Cl_2 (30 mL) was stirred at room temperature for 30 minutes. CH_2Cl_2 was then evaporated, and the residue was dissolved in THF (50 mL). Flask B: to a solution of zwitterion of L-phenylalanine **4** (3.069 g, 18.604 mmol) in THF (30 mL), 18.6 mL of 1 N NaOH(aq.) was added, and the mixture was stirred for 5 minutes at room temperature. The solution from flask B was added to flask A over 5 minutes while stirring at room temperature. After 55 minutes, the reaction mixture was filtered to eliminate DCU. The filtrate was

placed in a separatory funnel and extracted with EtOAc (2 × 50 mL). The aqueous phase was adjusted to pH 3 and extracted with EtOAc (3 × 50 mL). The organic phases were combined and washed with brine (3 × 50 mL), dried over anhydrous Na_2SO_4, filtered and, concentrated under reduced pressure. The crude product contains peptide **6** (85% yield) and HOBt. This procedure was also followed to obtain peptides **9–13**.

Synthesis of peptide Boc-Pro-Phe-Gly-OH 14. Flask A: a solution containing peptide Boc-Pro-Phe-OH **6** (1.431 g, 3.953 mmol) and HOBt in CH_2Cl_2 (30 mL) was stirred, and DCC (0.977 g, 4.743 mmol) was added under constant stirring at room temperature for 30 minutes. CH_2Cl_2 was then evaporated, and the residue was dissolved in THF (50 mL). Flask B: to a solution of zwitterion of glycine (1.185 g, 15.812 mmol) in THF (30 mL), 15.8 mL of 1 N NaOH(aq.) was added, and the mixture was stirred for 5 minutes at room temperature. The solution from flask B was then added to flask A over a period of 5 minutes at room temperature. After 55 minutes, the mixture was filtered to eliminate DCU. The filtrate was placed in a separatory funnel and extracted with EtOAc (2 × 50 mL). The aqueous phase was adjusted to pH 3 and subsequently extracted with EtOAc (3 × 50 mL). The organic phases were combined and washed with brine (3 × 50 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product contains the Boc-Pro-Phe-Gly-OH **14** and HOBt. The yield was determined by ^{1}H NMR (45% two steps).

Synthesis of peptide Boc-Pro-Phe-Gly-Pro-OH 15. Flask A: a solution contained the mixture of peptide Boc-Pro-Phe-Gly-OH **14** (0.744 g, 1.775 mmol) and HOBt in CH_2Cl_2 (30 mL) was stirred, and DCC (0.437 g, 2.124 mmol) was added under constant stirring at room temperature. After 30 minutes, CH_2Cl_2 was evaporated under vacuum, and the residue was dissolved in THF (50 mL). Flask B: to a solution of zwitterion of proline (0.816 g, 7.100 mmol) in THF (15 mL), 7.1 mL of 1 N NaOH(aq.) was added, and the mixture was stirred for 5 minutes at room temperature. The solution from flask B was added to flask A. The reaction was left for 55 minutes under constant stirring. DCU was then eliminated by filtration of the reaction mixture. The filtrate was placed in a separatory funnel and extracted with EtOAc (2 × 50 mL). The aqueous phase was adjusted to pH 3 and subsequently extracted with EtOAc (3 × 50 mL). The organic phases were combined and washed with brine (3 × 50 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product contains the Boc-Pro-Phe-Gly-Pro-OH **15** and HOBt. The yield was determined by ^{1}H NMR (38% three steps).

Preparation of diazomethane

N-Nitroso-N-methylurea (250 mg) was added to ethyl ether (20 mL), and suspended over a 50% aqueous solution of KOH (6 mL) at 0 °C. The reaction mixture was stirred for 20 minutes, then transferred to a separatory funnel where the phases were separated. The ether phase was dried over KOH pellets.

Synthesis of methylated derivatives of 7 and 8. The mixture of BOC-Pro-Phe-OH **6** (1.431 g, 3.953 mmol) and HOBt was dissolved in 50 mL of Et_2O–CH_2Cl_2 (1 : 1), and the ethereal solution of

diazomethane was added at 0 °C. The reaction mixture was stirred for 15 minutes and then left at room temperature for 20 hours. The solvents were eliminated under reduced pressure, and the crude reaction mixture crude was separated by column chromatography. The dipeptide **8** was obtained from the elution mixture of EtOAc–hexane (20 : 80). The product was recrystallised from an Et$_2$O–hexane mixture, yielding a white product (99% yield) with a melting point of 67–69 °C.

Synthesis of Sansalvamide A 24

General procedure for peptide coupling

Synthesis of 18. Flask A: a solution of α-hydroxy acid **16** (1 g, 7.575 mmol), DCC (1.872 g, 9.090 mmol) and, HOBt (1.227 g, 9.090 mmol) in CH$_2$Cl$_2$ (30 mL) was stirred at room temperature for 30 minutes. CH$_2$Cl$_2$ was evaporated under reduced pressure, and the residue was dissolved in THF (50 mL). Flask B: to a solution of zwitterion of L-valine **17** (3.545 g, 30.300 mmol) in THF (60 mL), 30 mL of 1 N NaOH was added, and the mixture was stirred for 5 minutes at room temperature. The solution from flask B was added to flask A over 5 minutes at room temperature. After 55 minutes, the reaction mixture was filtered, and the filtrate was placed in a separatory funnel to be extracted with EtOAc (2 × 50 mL). The aqueous phase was adjusted to pH 3 and subsequently extracted with EtOAc (3 × 50 mL). The organic phases were combined and washed with brine (3 × 50 mL), dried over anhydrous Na$_2$SO$_4$, and concentrated under reduced pressure. The crude product contains peptide **18** and HOBt. The yield (90%) was quantified by ^{1}H NMR of the crude reaction mixture, and the mixture was used without purification in the next step.

Synthesis of 20. Following the general procedure, the reaction was carried out with zwitterion of L-leucine **19** (3.572 g, 27.268 mmol) and DCC (1.685 g, 8.180 mmol). Yield (85%) was determined by ^{1}H NMR of the crude reaction mixture, and the mixture was used without purification in the next step.

Synthesis of 21. Following the general procedure, the reaction was carried out with zwitterion of L-phenylalanine **5** (3.824 g, 23.176 mmol) and DCC (1.432 g, 6.952 mmol). Yield (80%) was quantified by ^{1}H NMR of the crude reaction mixture, and peptide **21** was used in the next step without purification.

Synthesis of 22. Following the general procedure, the reaction was carried out with zwitterion of L-leucine **19** (2.428 g, 18.540 mmol) and DCC (1.145 g, 5.562 mmol). Peptide **22** (77%) and HOBt as a white solid were obtained. Peptide **22** was quantified by ^{1}H NMR from the crude reaction mixture and used in the next step without purification.

Synthesis of depsipeptide 24 via a macrolactonization reaction. A solution of DCC (881 mg, 4.281 mmol) and hydrotalcite (200 mg) in ethanol-free chloroform (100 mL) was brought to reflux. A solution of peptide **22** and HOBt in 20 mL of THF was infused *via* a syringe pump over 18 h, the reaction mixture was then filtered and evaporated. It was then diluted with EtOAc (75 mL), washed with 10% citric acid solution (2 × 50 mL), 10% NaHCO$_3$ solution (2 × 50 mL), brine (2 × 50 mL), dried over Na$_2$SO$_4$ and concentrated under vacuum. The crude product was purified through a silica gel column chromatography, first eluting with hexane and

then with 70% EtOAc–hexane. Finally, Sansalvamide A **23** was obtained as white solid (1.553 g, 75%, m.p. 144–146 °C).

Data availability

The data supporting this article have been included as part of the ESI.†

Author contributions

J. C. and J. A. M. S. conceived the project and acquired the funds. M. A. J. V., R. G., J. C., and J. A. M. S. designed the experiments. M. A. J. V., K. H. G., E. T., I. C., R. G., E. G. R., J. C., and J. A. M. S. conducted the experimental work. J. C. and J. A. M. S. coordinated the whole project. M. A. J. V., K. H. G., R. G., E. G. R., J. C., and J. A. M. S. wrote the manuscript. All the authors contributed to the discussions.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

Notes and references

1 M. Muttenthaler, G. F. King, D. J. Adams and P. F. Alewood, Trends in peptide drug discovery, *Nat. Rev. Drug Discovery*, 2021, **20**, 309–325.

2 P. Gao, M. M. Rahman, A. Zamalloa, J. Feliciano and M. Szostak, Classes of amides that undergo selective N–C amide bond activation: the emergence of ground-state destabilization, *J. Org. Chem.*, 2022, **88**, 13371–13391.

3 P. Acosta-Guzmán, A. Ojeda-Porras and D. Gamba-Sánchez, Contemporary approaches for amide bond formation, *Adv. Synth. Catal.*, 2023, **365**, 4359–4391.

4 E. Massolo, M. Pirola and M. Benaglia, Amide bond formation strategies: latest advances on a dateless transformation, *Eur. J. Org Chem.*, 2020, 4641–4651.

5 J. Singh and A. Sharma, Green and sustainable visible light-mediated formation of amide bonds: an emerging niche in organic chemistry, *New J. Chem.*, 2022, **46**, 16220–16242.

6 J. Yang, H. Huang and J. Zhao, Active ester-based peptide bond formation and its application in peptide synthesis, *Org. Chem. Front.*, 2023, **10**, 1817–1846.

7 T. I. Al-Warhi, H. M. Al-Hazimi and A. El-Faham, Recent development in peptide coupling reagents, *J. Saudi Chem. Soc.*, 2012, **16**, 97–116.

8 S. Y. Han and Y. A. Kim, Recent development of peptide coupling reagents in organic synthesis, *Tetrahedron*, 2004, **60**, 2447–2467.

9 E. Valeur and M. Bradley, Amide bond formation: beyond the myth of coupling reagents, *Chem. Soc. Rev.*, 2009, **38**, 606–631.

10 A. Sharma, A. Kumar, B. G. De La Torre and F. Albericio, Liquid-phase peptide synthesis (LPPS): a third wave for the preparation of peptides, *Chem. Rev.*, 2022, **122**, 13516–13546.

11 P. Lloyd-Williams, F. Albericio and E. Giralt, in *Chemical Approaches to the Synthesis of Peptides and Proteins*, CRC Press, Boca Raton, 1997.

12 I. Ramakrishna, T. Hattori, T. Ishiguro and H. Yamamoto, Triisobutylaluminium-mediated regioselective protection of sterically hindered amide NH of cyclo-(AA-Gly): key building block for next-generation peptide synthesis, *Synlett*, 2024, **35**, 1113–1120.

13 H. Itoh and M. Inoue, Full solid-phase total synthesis of macrocyclic natural peptides using four-dimensionally orthogonal protective groups, *Org. Biomol. Chem.*, 2019, **17**, 6519–6527.

14 S. R. Manne, D. C. Akintayo, O. Luna, A. El-Faham, B. G. De La Torre and F. Albericio, Tert-butylethylcarbodiimide as an efficient substitute for diisopropylcarbodiimide in solid-phase peptide synthesis: understanding the side reaction of carbodiimides with OxymaPure, *Org. Process Res. Dev.*, 2022, **26**, 2894–2899.

15 V. Di Matteo, G. Esposito, V. Costantino, G. Della Sala, R. Teta and A. Mangoni, When synthesis gets it wrong: unexpected epimerization using PyBOP in the synthesis of the cyclic peptide Thermoactinoamide A, *J. Nat. Prod.*, 2024, **87**, 948–953.

16 M. Kobayashi, K. Fujita, K. Matsuda and T. Wakimoto, Streamlined chemoenzymatic synthesis of cyclic peptides by non-ribosomal peptide cyclases, *J. Am. Chem. Soc.*, 2023, **145**, 3270–3275.

17 G. Liu, Y. Guo, M. Wang and Y. Gao, Recent advances in asymmetric synthesis of chiral amides and peptides: racemization-free coupling reagents, *Org. Biomol. Chem.*, 2024, **24**, 4420–4435.

18 M. Anand, M. Alagar, J. Ranjitha and V. Selvaraj, Total synthesis and anticancer activity of a cyclic heptapeptide from marine sponge using water soluble peptide coupling agent EDC, *Arabian J. Chem.*, 2019, **12**, 2782–2787.

19 C. A. Arbour, L. G. Mendoza and J. L. Stockdill, Recent advances in the synthesis of C-terminally modified peptides, *Org. Biomol. Chem.*, 2020, **18**, 7253–7272.

20 I. F. Eggen, F. T. Bakelaar, A. Petersen, P. B. W. Ten Kortenaar, N. H. S. Ankone, H. E. J. M. Bijsterveld, G. H. L. Bours, F. E. Bellaj, M. J. Hartsuiker, G. J. Kuiper and E. J. M. Ter Voert, A novel method for repetitive peptide synthesis in solution without isolation of intermediates, *J. Pept. Sci.*, 2005, **11**, 633–641.

21 G. K. Rathod, R. Misra and R. Jain, Advances in Peptide Synthesis, in *Recent Advances in Pharmaceutical Innovation and Research*, ed. P. P. Singh, Springer, Singapore, 2023, pp. 193–213.

22 A. Yamada, T. Takei, T. Kawakami, Y. Taniguchi, K. Sekiguchi and H. Hojo, Application of cysteinyl prolyl ester for the synthesis of cyclic peptides containing an RGD sequence and their biological activity measurement, *Front. Chem.*, 2024, **12**, 1391678.

23 X. Y. Liu, W. Cai, N. Ronceray, A. Radenovic, B. Fierz and J. Waser, Synthesis of fluorescent cyclic peptides *via* gold (I)-catalyzed macrocyclization, *J. Am. Chem. Soc.*, 2023, **145**, 26525–26531.

24 A. J. Mijalis, D. A. Thomas III, M. D. Simon, A. Adamo, R. Beaumont, K. F. Jensen and B. L. Pentelute, A fully automated flow-based approach for accelerated peptide synthesis, *Nat. Chem. Biol.*, 2017, **13**, 464–466.

25 Y. Mifune, H. Nakamura and S. Fuse, A rapid and clean synthetic approach to cyclic peptides *via* micro-flow peptide chain elongation and photochemical cyclization: synthesis of a cyclic RGD peptide, *Org. Biomol. Chem.*, 2016, **14**, 11244–11249.

26 H. Li, J. Li, J. Chao, Z. Zhang and C. Qin, Head-to-tail cyclization for the synthesis of naturally occurring cyclic peptides on organophosphorus small-molecular supports, *Org. Chem. Front.*, 2022, **9**, 946–952.

27 H. Y. Chow, Y. Zhang, E. Matheson and X. Li, Ligation technologies for the synthesis of cyclic peptides, *Chem. Rev.*, 2019, **119**, 9971–10001.

28 A. Wu, I. Ramakrishna, T. Hattori and H. Yamamoto, Silicon-based hydrophobic tags applied in liquid-phase peptide synthesis: protected DRGN-1 and poly alanine chain synthesis, *Org. Biomol. Chem.*, 2022, **20**, 8685–8692.

29 A. Wróblewska, I. I. Bak-Sypien, P. Paluch, E. Wielgus, J. Zając, A. Jeziorna, S. Kaźmierski and M. J. Potrzebowski, Solvent-free mechanosynthesis of oligopeptides by coupling peptide segments of different lengths–elucidating the role of cesium carbonate in ball mill processes, *Chem.–Eur. J.*, 2024, **30**, e202400177.

30 A. K. Mishra, G. Parvari, S. K. Santra, A. Bazylevich, O. Dorfman, J. Rahamim and A. M. Szpilman, Solar and visible light assisted peptide coupling, *Angew. Chem., Int. Ed.*, 2021, **60**, 12406–12412.

31 A. Sakurada, M. Sato, K. Higashida and M. Sawamura, Gold–zinc co–catalyzed alkynoate hydrocarboxylation with *N*-protected amino acids for preparation of storable acylating reagents and racemization–free peptide synthesis, *Adv. Synth. Catal.*, 2024, **366**, 2507–2513.

32 J. Yang, D. Zhang, Y. Chang, B. Zhang, P. Shen, C. Han and J. Zhao, TFPN–mediated racemization/epimerization–free amide and peptide bond formation, *Org. Chem. Front.*, 2024, **11**, 5422–5428.

33 J. Xu, S. Yuan, M. Miao and Z. Chen, 1-Hydroxybenzotriazole-assisted, N-heterocyclic carbene catalyzed β-functionalization of saturated carboxylic esters: access to spirooxindole lactones, *J. Org. Chem.*, 2016, **81**, 11454–11460.

34 T. Nobuta, N. Tsuchiya, Y. Suto and N. Yamagiwa, Hexylsilane-mediated direct amidation of amino acids with a catalytic amount of 1,2,4-triazole, *Org. Biomol. Chem.*, 2024, **22**, 703–707.

35 S. Xu, D. Jiang, Z. Peng, L. Hu, T. Liu, L. Zhao and J. Zhao, Ynamide-mediated peptide bond formation: mechanistic study and synthetic applications, *Angew. Chem., Int. Ed.*, 2022, **61**, e202212247.

36 J. R. Dunetz, J. Magano and G. A. Weisenburger, Large–scale applications of amide coupling reagents for the synthesis of pharmaceuticals, *Org. Process Res. Dev.*, 2016, **20**, 140–177.

37 J. García-Gros, Y. Cajal, A. M. Marqués and F. Rabanal, Synthesis of the antimicrobial peptide Murepavadin using novel coupling agents, *Biomolecules*, 2024, **14**, 526.

38 D. Uehara, S. Adachi, A. Tsubouchi, Y. Okada, V. V. Zhdankin, A. Yoshimura and A. Saito, Peptide coupling using recyclable bicyclic benziodazolone, *Chem. Commun.*, 2024, **60**, 956–959.

39 K. M. Freiberg, R. D. Kavthe, R. M. Thomas, D. M. Fialho, P. Dee, M. Scurria and B. H. Lipshutz, Direct formation of amide/peptide bonds from carboxylic acids: no traditional coupling reagents, 1–Pot, and green, *Chem. Sci.*, 2023, **14**, 3462–3469.

40 O. Al Musaimi, R. Wisdom, P. Talbiersky, B. G. De La Torre and F. Albericio, Propylphosphonic anhydride (T3P®) as coupling reagent for solid–phase peptide synthesis, *ChemistrySelect*, 2021, **6**, 2649–2657.

41 D. S. MacMillan, J. Murray, H. F. Sneddon, C. Jamieson and A. J. Watson, Evaluation of alternative solvents in common amide coupling reactions: replacement of dichloromethane and *N,N*-dimethylformamide, *Green Chem.*, 2013, **15**, 596–600.

42 W. Li, N. M. O'Brien-Simpson, M. A. Hossain and J. D. Wade, The 9–fluorenylmethoxycarbonyl (Fmoc) group in chemical peptide synthesis–its past, present, and future, *Aust. J. Chem.*, 2019, **73**, 271–276.

43 D. Takahashi, T. Inomata and T. Fukui, AJIPHASE®: a highly efficient synthetic method for one–pot peptide elongation in the solution phase by an Fmoc strategy, *Angew. Chem.*, 2017, **129**, 7911–7915.

44 T. Yamamoto, T. C. Chang and K. Tanaka, Epoc group: transformable protecting group with Gold (III)–catalyzed fluorene formation, *Chem. Sci.*, 2021, **12**, 10703–10709.

45 T. Hattori and H. Yamamoto, Peptide bond formation between unprotected amino acids: convergent synthesis of oligopeptides, *J. Am. Chem. Soc.*, 2024, **146**, 25738–25744.

46 C. Palomo, A. L. Palomo, F. Palomo and A. Mielgo, Soluble α–amino acid salts in acetonitrile: practical technology for the production of some dipeptides, *Org. Lett.*, 2002, **4**, 4005–4008.

47 A. Nagaya, S. Murase, Y. Mimori, K. Wakui, M. Yoshino, A. Matsuda and N. Nishizawa, Extended solution–phase peptide synthesis strategy using isostearyl–mixed anhydride coupling and a new C–terminal silyl ester–protecting group for *N*-methylated cyclic peptide production, *Org. Process Res. Dev.*, 2021, **25**, 2029–2038.

48 M. Diamandas, R. Moreira and S. D. Taylor, Solid–phase total synthesis of dehydrotryptophan–bearing cyclic peptides Tunicyclin B, Sclerotide A, CDA3a, and CDA4a using a protected β–hydroxytryptophan building block, *Org. Lett.*, 2021, **23**, 3048–3052.

49 K. Hojo, Y. Manabe, T. Uda and Y. Tsuda, Water–based solid–phase peptide synthesis without hydroxy side chain protection, *J. Org. Chem.*, 2022, **87**, 11362–11368.

50 A. S. Galanis, F. Albericio and M. Grøtli, Solid–phase peptide synthesis in water using microwave–assisted heating, *Org. Lett.*, 2009, **11**, 4488–4491.

51 A. S. Galanis, F. Albericio and M. Grøtli, Solid–phase peptide synthesis in water using microwave–assisted heating, *Org. Lett.*, 2009, **11**, 4488–4491.

52 T. M. Postma and F. Albericio, *N*-Chlorosuccinimide, an efficient peptide disulfide bond–forming reagent in aqueous solution, *RSC Adv.*, 2013, **3**, 14277–14280.

53 R. S. Dhiman, L. G. Opinska and R. Kluger, Biomimetic peptide bond formation in water with aminoacyl phosphate esters, *Org. Biomol. Chem.*, 2011, **9**, 5645–5647.

54 M. Cortes-Clerget, J. Y. Berthon, I. Krolikiewicz-Renimel, L. Chaisemartin and B. H. Lipshutz, Tandem deprotection/coupling for peptide synthesis in water at room temperature, *Green Chem.*, 2017, **19**, 4263–4267.

55 S. B. Lawrenson, R. Arav and M. North, The greening of peptide synthesis, *Green Chem.*, 2017, **19**, 1685–1691.

56 S. Hazra, F. Gallou and S. Handa, Water: An underestimated solvent for amide bond–forming reactions, *ACS Sustainable Chem. Eng.*, 2022, **10**, 5299–5306.

57 W. Hou, X. Zhang and C. F. Liu, Progress in chemical synthesis of peptides and proteins, *Trans. Tianjin Univ.*, 2017, **23**, 401–419.

58 L. Posada, L. Rey, J. Villalba, S. Colombo, L. Aubriot, N. Badagian and G. Serra, Cyclopeptides natural products as herbicides and inhibitors of cyanobacteria: Synthesis of Versicotides E and F, *ChemistrySelect*, 2022, **7**, e202201956.

59 M. I. Muhajir, A. Hardianto, J. Al-Anshori, D. Sumiarsa, T. Mayanti, N. Nurlelasari and R. Maharani, Total synthesis of Nocardiotide A by using a combination of solid– and solution–phase methods, *ChemistrySelect*, 2021, **6**, 12941–12946.

60 H. Luo, H. Yin, C. Tang, P. Wang and F. Liang, Synthesis of cyclic peptide Reniochalistatin E and conformational isomers, *Chin. Chem. Lett.*, 2018, **29**, 1143–1146.

61 S. R. Tivari, S. V. Kokate, E. M. Sobhia, S. G. Kumar, U. B. Shelar and Y. S. Jadeja, A series of novel bioactive cyclic peptides: synthesis by head-to-tail cyclization approach, antimicrobial activity and molecular docking studies, *ChemistrySelect*, 2022, **7**, e202201481.

62 N. Jain and S. H. Friedman, A tetra-orthogonal strategy for the efficient synthesis of scaffolds based on cyclic peptides, *Int. J. Pept. Res. Ther.*, 2018, **24**, 535–542.

63 C. Fagundez, D. Sellanes and G. Serra, Synthesis of cyclic peptides as potential anti-malarials, *ACS Comb. Sci.*, 2018, **20**, 212–219.

64 H. Masui and S. Fuse, Recent advances in the solid-and solution-phase synthesis of peptides and proteins using microflow technology, *Org. Process Res. Dev.*, 2022, **26**, 1751–1765.

65 S. Noki, B. G. de la Torre and F. Albericio, Safety-catch linkers for solid-phase peptide synthesis, *Molecules*, 2024, **29**, 1429.

66 H. Nzama, S. R. Manne, O. Marder, G. Orosz, B. G. de la Torre and F. Albericio, Unveiling the quaternary carbodiimide

symphony: harmonizing green chemistry in peptide synthesis, *Green Chem. Lett. Rev.*, 2024, **17**, 2392826.

67 Y. J. Pu, R. K. Vaid, S. K. Boini, R. W. Towsley, C. W. Doecke and D. Mitchell, A Practical method for functionalized peptide or amide bond formation in aqueous–ethanol media with EDC as activator, *Org. Process Res. Dev.*, 2009, **13**, 310–314.

68 A. Méndez-Ardoy, I. Insua, J. R. Granja and J. Montenegro, Cyclization and self-assembly of cyclic peptides, in *Peptide Macrocycles. Methods and Protocols*, ed. M. B. Coppock and A. J. Winton, Humana, New York, 2022, pp. 449–466.

69 S. C. Stolze and M. Kaiser, Case studies of the synthesis of bioactive cyclodepsipeptide natural products, *Molecules*, 2013, **18**, 1337–1367.

70 N. Lalani, S. Tivari, V. Jain and Y. Jadeja, Review on therapeutic potential of peptides: advancements in synthesis methods, linear and cyclic peptides, and strategies for overcoming challenges, *Pept. Sci.*, 2024, e24343.

71 D. S. Nielsen, N. E. Shepherd, W. Xu, A. J. Lucke, M. J. Stoermer and D. P. Fairlie, Orally absorbed cyclic peptides, *Chem. Rev.*, 2017, **117**, 8094–8128.

72 A. Zorzi, K. Deyle and C. Heinis, Cyclic peptide therapeutics: past, present and future, *Curr. Opin. Chem. Biol.*, 2017, **38**, 24–29.

73 M. R. Naylor, A. T. Bockus, M. J. Blanco and R. S. Lokey, Cyclic peptide natural products chart the frontier of oral bioavailability in the pursuit of undruggable targets, *Curr. Opin. Chem. Biol.*, 2017, **38**, 141–147.

74 Y. Lee, C. Phat and S. C. Hong, Structural diversity of marine cyclic peptides and their molecular mechanisms for anticancer, antibacterial, antifungal, and other clinical applications, *Peptides*, 2017, **95**, 94–105.

75 G. N. Belofsky, P. R. Jensen and W. Fenical, Sansalvamide: a new cytotoxic cyclic depsipeptide produced by a marine fungus of the genus Fusarium, *Tetrahedron Lett.*, 1999, **40**, 2913–2916.

76 M. C. Sheikh, S. Takagi, T. Yoshimura and H. Morita, Mechanistic studies of DCC/HOBt-mediated reaction of 3-phenylpropionic acid with benzyl alcohol and studies on the reactivities of 'active ester' and the related derivatives with nucleophiles, *Tetrahedron*, 2010, **66**, 7272–7278.

77 J. A. Morales-Serna, A. Sauza, G. P. de Jesús, R. Gaviño, G. G. de la Mora and J. Cardenas, Facile and efficient addition of terminal alkynes to benzotriazole esters: synthesis of d-erythro-sphingosine using ynones as the key intermediate, *Tetrahedron Lett.*, 2013, **54**, 7111–7114.

78 J. A. Morales-Serna, E. García-Ríos, J. Bernal, E. Paleo, R. Gaviño and J. Cardenas, Reduction of carboxylic acids using esters of benzotriazole as high-reactivity intermediates, *Synthesis*, 2011, 1375–1382.

79 J. A. Morales-Serna, A. Vera, E. Paleo, E. García-Ríos, R. Gaviño, G. G. de la Mora and J. Cardenas, Using benzotriazole esters as a strategy in the esterification of tertiary alcohols, *Synthesis*, 2010, 4261–4267.

80 L. A. Carpino, E. S. M. Mansour and D. Sadat-Aalaee, tert-Butyloxycarbonyl and benzyloxycarbonyl amino acid fluorides. New, stable rapid-acting acylating agents for peptide synthesis, *J. Org. Chem.*, 1991, **56**, 2611–2614.

81 Y. Hwang, D. Rowley, D. Rhodes, J. Gertsch, W. Fenical and F. Bushman, Mechanism of inhibition of a poxvirus topoisomerase by the marine natural product Sansalvamide A, *Mol. Pharmacol.*, 1999, **55**, 1049–1053.

82 B. K. Chagaleti, K. Baby, S. I. Pena-Corona, G. Leyva-Gomez, S. M. Sindhoor, N. R. Naveen, J. Jose, A. A. Aldahish, J. Sharifi-Rad and D. Calina, Anti-cancer properties of Sansalvamide A, its derivatives, and analogs: an updated review, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 2024, **397**, 7337–7351.

83 C. L. Carroll, J. V. Johnston, A. Kekec, J. D. Brown, E. Parry, J. Cajica and S. R. McAlpine, Synthesis and cytotoxicity of novel Sansalvamide A derivatives, *Org. Lett.*, 2005, **7**, 3481–3484.

84 K. Otrubova, T. J. Styers, P. S. Pan, R. Rodriguez, K. L. McGuire and S. R. McAlpine, Synthesis and novel structure–activity relationships of potent Sansalvamide A derivatives, *Chem. Commun.*, 2006, 1033–1034.

85 J. A. Morales-Serna, M. Á. Jaime-Vasconcelos, E. García-Ríos, A. Cruz, D. Angeles-Beltrán, L. Lomas-Romero, G. E. Negrón-Silva and J. Cárdenas, Efficient activity of magnesium–aluminium hydrotalcite in the synthesis of amides, *RSC Adv.*, 2013, **3**, 23046–23050.

86 J. A. Morales-Serna, E. Sánchez, R. Velázquez, J. Bernal, E. García-Ríos, R. Gaviño and J. Cárdenas, Highly efficient macrolactonization of ω-hydroxy acids using benzotriazole esters: synthesis of Sansalvamide A, *Org. Biomol. Chem.*, 2010, **8**, 4940–4948.

87 Y. Lee and R. B. Silverman, Rapid, high-yield, solid-phase synthesis of the antitumor antibiotic Sansalvamide A using a side-chain-tethered phenylalanine building block, *Org. Lett.*, 2000, **2**, 3743–3746.

88 L. Chen, M. Zheng, Y. Zhou, H. Liu and H. Jiang, Ionic-liquid-supported total synthesis of Sansalvamide A peptide, *Synth. Commun.*, 2008, **38**, 239–248.

89 O. Al Musaimi, B. G. de la Torre and F. Albericio, Greening Fmoc/tBu solid-phase peptide synthesis, *Green Chem.*, 2020, **22**, 996–1018.

90 L. Ferrazzano, M. Catani, A. Cavazzini, G. Martelli, D. Corbisiero, P. Cantelmi and A. Tolomelli, Sustainability in peptide chemistry: current synthesis and purification technologies and future challenges, *Green Chem.*, 2022, **24**, 975–1020.

91 M. H. A. Somehsaraie, V. F. Vavsari, M. Kamangar and S. Balalaie, Chemical wastes in the peptide synthesis process and ways to reduce them, *Iran. J. Pharm. Res.*, 2022, **21**, e123879.

92 K. Venkateswarlu, Ashes from organic waste as reagents in synthetic chemistry: a review, *Environ. Chem. Lett.*, 2021, **19**, 3887–3950.

93 J. Lopez, F. Gallou, I. Kekessie, K. Wegner, I. Martinez, M. E. Kopach and L. Vandenbos, Process Mass Intensity (PMI): A holistic analysis in current peptide manufacturing processes, informing sustainability in peptide synthesis, *J. Org. Chem.*, 2024, **89**, 4261–4282.