Review

Marine Natural Peptides: Determination of Absolute Configuration Using Liquid Chromatography Methods and Evaluation of Bioactivities

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Abstract: Over the last decades, many naturally occurring peptides have attracted the attention of medicinal chemists due to their promising applicability as pharmaceuticals or as models for drugs used in therapeutics. Marine peptides are chiral molecules comprising different amino acid residues. Therefore, it is essential to establish the configuration of the stereogenic carbon of their amino acid constituents for a total characterization and further synthesis to obtain higher amount of the bioactive marine peptides or as a basis for structural modifications for more potent derivatives. Moreover, it is also a crucial issue taking into account the mechanisms of molecular recognition and the influence of molecular three-dimensionality in this process. In this review, a literature survey covering the report on the determination of absolute configuration of the amino acid residues of diverse marine peptides by chromatographic methodologies is presented. A brief summary of their biological activities was also included emphasizing to the most promising marine peptides. A case study describing an experience of our group was also included.

Keywords: absolute configuration; bioactivity; chiral HPLC; Marfey’s method; marine peptides; stereochemistry

1. Introduction

In recent years, it has become well known that the oceans represent a rich source of structurally unique bioactive compounds from the perspective of potential therapeutic agents [1,2]. Bioactive compounds can be isolated from a myriad of marine invertebrates such as mollusks, sponges, tunicates and bryozoans, in addition to algae and marine microorganisms, especially cyanobacteria, bacteria and fungi [3–5].

Over the last decades, novel bioactive compounds from marine organisms with important bioactivities, such as antifungal, antibacterial, cytotoxic and anti-inflammatory properties, have been widely explored, and many of them are considered as lead compounds for drug discovery as well as biologically useful agents in pharmaceutical research [6–10]. In fact, owing to their pharmacological
potential, either directly as drugs or as models for molecular modifications and/or total synthesis, marine natural products are certainly an interesting source, exploited by many researchers [11].

Ziconotide (Prialt®), a peptide first isolated from the venom of the cone snail (Conus magus), and trabectedin (Yondelis®), an alkaloid originally isolated from a marine tunicate Ectenascidia turbinata and now obtained by semisynthesis, are examples of marine natural products that have already been approved as human therapeutics [3,12–14]. Ziconotide is an analgesic used for treatment of patients suffering from chronic pain, and trabectedin for the treatment of soft tissue sarcomas and ovarian cancer.

In terms of the overall number of marine natural products, peptides are one of the most described due to their novel chemistry and diverse biological properties [15]. Actually, marine peptides are known to exhibit various biological activities such as antiviral, antiproliferative, antioxidant, anticancer, antidiabetic, anti-obesity, anticoagulant, antihypertensive, and calcium-binding activities [6,15–17].

Marine peptides are chiral molecules comprising different amino acid residue subunits. For their total characterization, and taking into account the mechanisms of molecular recognition and the influence of molecular three-dimensionality in this process, it is essential to define the configuration of the amino acids components of the peptide fractions, isolated from marine sources. Besides, it is also crucial to obtain the bioactive marine peptides by synthesis in order to achieve higher amount of compound for future assays or as a basis for structural modifications to obtain more potent derivatives.

Nowadays, there are different methodologies for the determination of the absolute configuration of amino acids, such as X-ray crystallography, NMR techniques, vibrational circular dichroism (VCD), enantioselective chromatography, optical rotatory dispersion (ORD), among others [18–26].

For the determination of the absolute configuration of amino acid residues of marine peptides, separation methodologies by using Marfey’s method, chiral high performance liquid chromatography (HPLC) analysis or both have proved to be suitable and the most described, as will be shown in this review. Regardless of the method used, the evaluation of peptides stereochemistry is based on the determination of the amino acid composition in peptide hydrolysates. Two main steps are involved, specifically the total or partial hydrolysis of peptides to obtain amino acid residues followed by their analysis by comparison with appropriate standards [27] (Figure 1).

Marfey’s method was first reported by Marfey in 1984 [28]. After the acid hydrolysis of peptides, the amino acid residues are derivatized with chiral Marfey’s reagents such as 1-fluoro-2-4-dinitrophenyl-5-\(L\)-alanine amide (FDAA) or 1-fluoro-2-4-dinitrophenyl-5-\(D\),\(L\)-leucine amide (FDLA). Subsequent analysis via reverse phase liquid chromatography (LC), using generally C\(_{18}\) columns, and by comparison the retention times of the derivatized amino acids with suitable standards, afforded the stereochemistry of the peptides [29–31]. This method is often used for determination of the absolute configuration of amino acids, mainly because it is a simple method, offering a better resolution when compared to chiral HPLC methodologies; furthermore, several derivatization agents, such as FDAA and FDLA, are commercially available. However, this methodology has some disadvantages, including low availability of some standards, and the possibility of occurring racemization of the analyte during the derivatization reaction, prior to the chromatographic analysis [30,31].

The chiral analysis by HPLC is based on a formation of transient diastereomeric complexes between the amino acids present in the hydrolysates and the chiral stationary phase (CSP) employed, being the less stable complex the first to elute [32]. There are several types of CSPs, such as polysaccharide-based, Pirkle-type, protein-based, macrocyclic antibiotic-based, crown ether-based, ligand exchange type, among others [33–35]; however, the last three types are the most used for the separation of primary amine-containing compounds and amino acids [36,37]. Chiral HPLC offers several advantages, when comparing to Marfey’s method, including the direct analysis of the amino acid hydrolysates without further derivatization; moreover, the analysis often provides quicker results. However, poor chemical sensitivity, low sample capacity, and low availability and expensiveness of commercial chiral columns are some of the disadvantages of chiral HPLC method [38].
Figure 1. Schematic presentation of the methodologies generally used for determination of the configuration of amino acid residues of marine peptides. HPLC—High Performance Liquid Chromatography; CSP—Chiral Stationary Phase; FDAA—1-Fluoro-2-4-dinitrophenyl-5-D, L-alanine amide; FDLA—1-Fluoro-2-4-dinitrophenyl-5-D, L-leucine amide.

A number of reviews on marine peptides have appeared in recent years, focusing mainly on their biological activities, applications and biosynthesis as well as isolation procedures [16,39–57]. In this review, several works related to the methods used for determination of the absolute configuration of marine peptides by chromatographic methods are presented in different sections according to the source of the marine peptides. Diverse types of peptides such as cyclic peptides, cyclic depsipeptides and lipopeptides are reported. A literature survey covering all the reports on liquid chromatographic methods (Marfey’s method and chiral HPLC) is presented (from 1996 to 2017). Furthermore, a case study describing an experience of our group is included.

2. Peptides from Marine Cyanobacteria and Other Bacteria

Cyanobacteria (blue-green algae), the most ancient known microorganisms on Earth, are a rich source of novel secondary metabolites possessing a broad spectrum of biological activities including antitumor, antibacterial, anticoagulant, antifungal, antiviral, antimalarial, antiprotozoal, and anti-inflammatory activities [58]. Currently, cyanobacteria are one of the most interesting sources of novel marine compounds [59]. Actually, the number of biologically active cyclic peptides, depsipeptides, lipopeptides, and other acyclic or small peptides, many of which containing unusual amino acid residues or modified amino acid units, is impressive. In addition to cyanobacteria, this type of compounds has also been isolated from other marine-derived bacteria.
2.1. Cyclic Peptides

Scattered publications concerning the stereochemistry determination of the amino acid residues of several cyclic peptides, isolated from marine cyanobacteria and other bacteria, were reported (Table 1). Marfey’s method, using FDAA as derivatization reagent, allowed the successful determination of the absolute configuration of the amino acid residues of cyclic peptides 1–4 (Figure 2).

For the new cyclic tetrapeptide 1 isolated from the bacterium *Nocardiopsis* sp. [60], the absolute configuration of all the amino acid residues was found to be L. Similarly, the absolute configuration of the amino acid residues of three novel anabaenopeptins labeled NZ825 (2), NZ841 (3), and NZ857 (4) [61], were successfully determined by Marfey’s method combined with HPLC.

However, as Marfey’s method was not accurate enough to determine the absolute configuration of all the amino acid residues of some cyclic peptides 5–16 (Figure 2), it was necessary to associate this method with chiral HPLC.

Figure 2. Structure of cyclic peptides 1–16, isolated from marine cyanobacteria and other bacteria, whose stereochemistry determination of their amino acids was performed by Marfey’s method (compounds 1–4) and by a combination of both Marfey’s method and chiral HPLC (compounds 5–16).
This strategy, i.e., using a ligand exchange-type CSP in chiral HPLC associated with Marfey’s method, was used for the determination of amino acids stereochemistry of several cyclic peptides, including aurilide B (5) and C (6), isolated from the cyanobacterium Lyngbya majuscula [62], urukthapelstatin A (7), isolated from a culture broth of thermoactinomycetaceae bacterium Marinecharinymyces asporophorigenes YM11-542 [63], pompanopeptins A (8) and B (9), isolated from the cyanobacterium Lyngbya confervoides [64], marthiapeptide A (10) isolated from the deep South China Sea-derived Marinactinospora thermotolerance SCSIO 00652 [65], norcardiamides A (11) and B (12), isolated from the marine-derived actinomycete Nocardiopsis sp. CNX037 [66], destomides B–D (13–15), isolated from the deep South China Sea-derived Streptomyces scopoliridis SCSIO ZJ46 [67], and jandolide (16) isolated from the marine cyanobacterium Okeania sp. [68].

The cyclic peptides aurilides B (5) and C (6) were reported to have the in vitro cytotoxicity toward NCI-H460, human lung tumor, and neuro-2a mouse neuroblastoma cell lines, with lethal concentration 50 (LC50) values between 0.01 and 0.13 µM [62]. Aurilide B (5) was evaluated in the NCI 60 cell line panel and was found to exhibit a high level of cytotoxicity, particularly against leukemia, renal, and prostate cancer cell lines [62]. The cyclic peptide pompanopeptin A (8) was shown to exhibit trypsin inhibitory activity with an IC50 value of 2.4 ± 0.4 µg/mL [64]. A polythiazole cyclopeptide, marthiapeptide A (10) showed antibacterial activity against a panel of Gram-positive bacteria with minimum inhibitory concentration (MIC) values ranging from 2.0 to 8.0 µg/mL, and strong cytotoxicity against a panel of human cancer cell lines with IC50 values ranging from 0.38 to 0.52 µM [65]. The cyclohexapeptide destomide B (13) also showed antimicrobial activity against Staphylococcus aureus ATCC 29213, Streptococcus pneumoniae NCTC 7466 and MRSE shhs-E1 with MIC values of 16.0, 12.5, 32.0 µg/mL, respectively [67]. A cyclic polyketide-peptide hybrid, jandolide (16) exhibited potent antitrypanosomal activity with an IC50 value of 47 nM [68].

Recently, the configuration of the amino acids of a cytotoxic cyanobactin, wewakazole B (17), isolated from the cyanobacterium Moorea producens (Figure 3), was determined using only chiral HPLC [69]. Two different types of CSPs, under reverse phase mode, were used to perform the analysis. A macrocyclic antibiotic-based CSP afforded the assignment of the l-configuration for its Ala, Phe, and Pro residues, while a ligand exchange type CSP clearly identified the presence of L-Ile, which could not be distinguished by the first CSP [69].

![Figure 3. Structure of wewakazole B (17) isolated from a marine cyanobacteria.](image-url)
| Peptide          | Source                              | aa Composition | Chromatographic Conditions                                                                 | Biological Activities                                      | Refs. |
|------------------|-------------------------------------|----------------|--------------------------------------------------------------------------------------------|------------------------------------------------------------|-------|
| Tetrapeptide (1) | Bacterium Nocardiopsis sp.          | 1-Ile, L-Leu, L-Pro | Marfey’s method (FDAA) combined with HPLC C<sub>18</sub> (YMC-ODS-A) (4.5 × 250 mm) Flow rate: 0.8 mL/min; UV detection at 340 nm MP: ACN (aq) (0–50% (v/v)) with 0.1% TFA | Cytotoxicity toward the leukemia cell-line K-562             | [60]  |
| Anabaenopeptins  | Cyanobacterium Anabaena sp.         | 1-Ile, D-Lys, L-Phe; 2: 1-Hph; 3: 1-Hph, L-Hty; 4: L-Hty | Marfey’s method (FDAA) combined with HPLC Merck Chromolith performance RP-18e, (4.6 × 100 mm) MP: 50 mM TEAP buffer (pH 3)/ACN (9:1 to 1:1 v/v) | No inhibition of serine proteases                           | [61]  |
| Aurilides B (5)  | Cyanobacterium Lyngbya majuscula    | L-Val, N-Me-L-Ile, L-Ile | Ligand Exchange Type CSP; Phenomenex Chirex 3126 (D) (4.6 × 250 mm); Flow rate: 1.0 mL/min; UV detection at 254 nm MP: 2 mM CuSO<sub>4</sub> in ACN/H<sub>2</sub>O (5/95 v/v) or 2 mM CuSO<sub>4</sub> in ACN/H<sub>2</sub>O (15/85 v/v) | Cytotoxicity against NCI-H460 and neuro-2a mouse neuroblastoma cell lines | [62]  |
| Pompanopeptins A (8) and B (9) | Cyanobacterium Lyngbya conforoides | 8: L-Val, L-Thr, L-Met (O), S-Alph, L-Ile, L-Arg 9: L-Ile | Ligand Exchange Type CSP; Phenomenex Chirex 3126 N,S-dioctyl(-)-perillicamine, 5 µm (4.6 × 250 mm) Flow rate: 1.0 mL/min; UV detection at 254 nm MP: 2 mM CuSO<sub>4</sub> or 2 mM CuSO<sub>4</sub>/ACN (95:5 v/v) | 8: Trypsin inhibitory activity                             | [64]  |

Table 1. Cyclic peptides from marine cyanobacteria and other bacteria.
Table 1. Cont.

| Peptide Source | aa Composition | Chromatographic Conditions | Biological Activities | Refs. |
|----------------|----------------|----------------------------|-----------------------|-------|
| Marthiapeptide A (10) | Deep sea-derived *Marinactinospora thermotolerans* SCSIO 00652 | 1-Ile | Ligand Exchange Type CSP; MCIGELCR10W (4.6 × 150 mm); Flow rate: 0.5 mL/min; UV detection at 254 nm; MP: 2 mM CuSO₄ solution | Antibacterial and cytotoxic activities | [65] |
| | | D-Phe, L-Ile | Marfey’s method (FDAA) combined with HPLC; Zorbax SB-C₈ column, 5 µm (2.1 × 30 mm) | | |
| Nocardiamides A (11) and B (12) | Marine-derived Actinomycete *Nocardiosis sp.* CNX037 | L-Tyr, D-Leu, D- and L-Val | Marfey’s method (FDAA or FDLA) combined with HPLC; Conditions not described | Antimicrobial activity and no cytotoxicity against HCT-116 cell line | [66] |
| | | 11: L-Ile | Ligand Exchange Type CSP; MCIGELCRS10W (4.6 × 250 mm); Flow rate: 0.5 mL/min; UV detection at 254 nm; MP: 2 mM CuSO₄/H₂O | | |
| Destomides B–D (13–15) | Deep sea-derived *Streptomyces scopuliridis* SCSIO ZJ146 | 1-Asn, D-Leu | Marfey’s method (FDAA) combined with HPLC | 13: Antimicrobial activity against *Staphylococcus aureus* ATCC 29213, *Streptococcus pneumoniae* NCTC 7466 and MRSE shhs-E1 | [67] |
| | | 13: L-Trp, L-Val, L-Leu; 14: L-Gly, L-Ile, 15: L-Gly, L-Ile, L-Leu | Phenomenex ODS column, 5 µm (4.6 × 150 mm) | Flow rate: 1.0 mL/min; UV detection at 340 nm MP: ACN:H₂O:TFA (15:85:0.1 to 90:10:0.1) | 13–15: no cytotoxicity |
| Janadolide (16) | Cyanobacterium *Okeania* sp. | N-Me-L-Leu, L-Pro, L-Val | Marfey’s method (FDAA) combined with HPLC | Antitrypanosomal activity | [68] |
| | | | Cosmolos Cholesterol (4.6 × 50 mm); Flow rate: 1.0 mL/min; UV detection at 254 nm; MP: 2.0 mM CuSO₄ | | |
| Wewakazole B (17) | Cyanobacterium *Moorea producens* | N-Me-L-Ala | Marfey’s method (FDAA) combined with HPLC | Cytotoxicity against MCF7 and human 460 lung cancer cell lines | [69] |

aa—Amino acid; FDAA—1-Fluoro-2-4-dinitrophenyl-5-L-alanine amide; ESI—Electrospray Ionization; LC—Liquid Chromatography; MS—Mass spectrometry; HPLC—High Performance Liquid Chromatography; MP—Mobile Phase; TEAP—Triethylammonium phosphate; ACN—Acetonitrile; CPA—Carboxypeptidase A; TFA—Trifluoroacetic acid; MeOH—Methanol; TEA—Triethylamine; IPA—Isopropyl alcohol; FDLA—1-fluoro-2-4-dinitrophenyl-5-D,L-leucine amide; NaOAc—Sodium acetate; NH₄OAc—Ammonium acetate.
2.2. Cyclic Depsipeptides

As mentioned above, there are many publications describing the isolation and characterization, including the determination of the stereochemistry of their amino acids, of new cyclic depsipeptides from marine cyanobacteria and other bacteria (Table 2). However, contrary to cyclic peptides, several works reported the use of chiral HPLC as the only method for determination of the configuration of amino acids. Figure 4 shows the structure of cyclic depsipeptides 18–46, isolated from marine cyanobacteria and other bacteria, whose stereochemistry of the amino acids was determined only by this method.

The ligand exchange-type CSPs were the most widely used by different research groups. Cai et al. employed a penicillamine ligand exchange-type CSP to determine the absolute configuration of the amino acids constituent of malevamide B (18) and C (19) isolated from the cyanobacterium *Symproca lae-t-viridis* [71]. Three different mobile phases in reverse phase elution mode were used. Nevertheless, the stereochemistry of Amha and Amoa residues present in both compounds were not determined [71]. The same CSP was employed to establish that all the amino acids of the cytotoxic depsipeptide lyngbyapeptin B (20) [72], tasipeptins A (21) and B (22) [73], wewakamide A (23) [74], cosamamide A (24) and B (25) [75], and the antiparasitic depsipeptides dudawelamides A–D (26–29) [76], isolated from cyanobacteria *Lyngbya majuscula*, *Symproca* sp., *Lyngbya semiplena*, *Lyngbya majuscula*, and *Moorea producens*, respectively, has L-configuration. The only exception was for allo-Hiva amino acid of dudawelamide C (29), which has D-configuration [76]. The configuration of the amino acids of the cyclic depsipeptides pitipeptolides A (30) and B (31), isolated from cyanobacterium *Lyngbya majuscula*, was assigned to be L by a ligand exchange-type CSP comprising *N*,*N*-dioctyl-L-alanine as chiral selector (Chiralpack MA (+) from Daicel) and different proportion of CuSO₄:ACN as mobile phase [77]. By using the same CSP, the absolute configuration of three new cyclic depsipeptides, kohamamides A–C (32–34) were also successfully established [78].

Zhou et al. [79] described the determination of the absolute configuration of new anti-infective cycloheptadepsipeptides marformycins A–F (35–40), produced by the deep sea-derived *Streptomyces drozdowiczii* SCSIO 1014, using a ligand exchange type CSP containing the same chiral selector as the previous ones (*N*,*N*-dioctyl-L(or D)-alanine) but purchased from Mitsubishi Chemical Corporation (MCI GEL CRS10W). Another type of CSP, specifically the macrocyclic antibiotic-based Chirobiotic TAG, confirmed the presence of L-Pro and L-Val in an unusual cyclic depsipeptide, pitiprolamide (41), isolated from *Lyngbya majuscula* [80]. Interestingly, in some works, more than one CSP were employed to elucidate the configuration of all the amino acids contained in the hydrolysates of cyclic depsipeptides. For example, two different types of ligand exchange type CSPs were used to elucidate the stereochemistry of the amino acid residues of palau’amide (42), depsipeptide with strong cytotoxicity against KB cell line (IC₅₀ value of 13 nM) [81].

In the case of pitipeptolides C–F (43–46), which were isolated from the cyanobacterium *Lyngbya majuscula*, the configuration of most of the amino acid residues was determined using the macrocyclic antibiotic-based Chirobiotic TAG under reverse phase elution conditions [82]. Then, the *N*,*N*-dioctyl-L-alanine ligand exchange CSP Chiralpack MA (+), under the same elution mode, was used for the assignment of S configuration for Hiva residue [82].
Figure 4. Structure of cyclic depsipeptides 18–46, isolated from marine cyanobacteria and other bacteria, whose stereochemistry of their amino acids was determined only by chiral HPLC.
| Peptide          | Source                        | aa Composition                           | Chromatographic Conditions                                                                 | Biological Activities                                                                 | Refs.     |
|------------------|-------------------------------|------------------------------------------|------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|-----------|
| Malevamides      | *Cyanobacterium Symploca laevis-radicis* | 1-Pro, N-Me-t-Val, N-Me-t-Phe            | Ligand Exchange Type CSP; Chirex (D) Phenomenex 00G-3126D (4.6 × 250 mm) Flow rate: 1.0 and 0.8 mL/min; UV detection at 254 nm | Inactive against P-388, A-549 and HT-29 cancer cells                                  | [71]      |
|                  |                               | 18: I-Le, N-Me-t-Ala, N-Me-d-Val, 1-Val, (R)-Hiva; 19: t-Ala, N-3Me-t-Ser, t-Leu, N-Me-d-Ala, N-Me-t-Ile, (5)-Hiva |                                                                                             |                                                                                       |           |
| Lyngbyaseptin A  | *Cyanobacterium Lyngbya majuscula* | N-Me-t-Ile, N-Me-t-Leu, N,3-diMe-t-Tyr   | Ligand Exchange Type CSP; Chirex (D) Phenomenex 00G-3126D (4.6 × 250 mm) MP: 2 mM CuSO4 Flow: 0.8 mL/min; UV detection at 254 nm | Cytotoxicity against KB and LoVo cells                                                | [72]      |
| and B (20)       |                               |                                         |                                                                                             |                                                                                        |           |
| Tasipeptins A (21) and B (22) | *Cyanobacterium Symploca sp.* | 1-Thr, 1-Val, 1-Leu, 1-Glu, N-Me-t-Phe | Ligand Exchange Type CSP; Phenomenex Chirex Phase 3126 (D) (4.6 × 250 mm) MP: 2 mM CuSO4/2 mM CuSO4/ACN (95.5:85.15:0.970) UV detection at 254 nm | Cytotoxicity toward KB cells                                                           | [73]      |
| Wewakamide A (23) | *Cyanobacteria Lyngbya semiplena and Lyngbya majuscula* | 1-M-Ala, 1-Pro, 1-Val, 1-Me-Leu, 1-Phe, 1-Me-Il, 1-Hiv | Ligand Exchange Type CSP; Phenomenex Chirex 3126 (D) (4.6 × 250 mm); MP: 2 mM CuSO4 in H2O or 2 mM CuSO4 in ACN/H2O (15:85 or 5:95 v/v) Flow rate: 0.7:0,8:1,0 mL/min; UV detection at 254 nm | Brine shrimp toxicity                                                                | [74]      |
| Cocosamide A (24) and B (25) | *Cyanobacterium Lyngbya majuscula* | 1-Pro, 1-Val, N-Me-t-Phe | Ligand Exchange Type CSP; Phenomenex Chirex (D), Penicillamine, 5 μm (4.6 × 250 mm) MP: 2.0 mM CuSO4/ACN (85:15:90:10 v/v) Flow rate: 1.0 mL/min; UV detection at 254 nm | Cytotoxicity against MCF-7 (breast cancer) and HT-29 (colon cancer) cells               | [75]      |
| Dudawalamides A–D (26–29) | *Cyanobacterium Moorea producens* | 1-Dhoya, 1-Hiva, 1-Val 29: 3-allo-Hiva | Ligand Exchange Type CSP; Chirex Phase 3126 (D) 5 μm (4.6 × 250 mm); MP: 2 mM CuSO4/ACN (95:5:85;15:95:12.5:5 v/v/v), ACN/H2O/HCOOH (30:70.1: or 70:30.1:0.1 v/v/v) Flow rate: 0.8 mL/min; UV detection at 340 nm | Antiparasitic activity                                                              | [76]      |
| Pitipeptides A (30) and B (31) | *Cyanobacterium Lyngbya majuscula* | 1-Gly, 1-Pro, 1-Val, 1-Ile, N-Me-t-Phe, (25,35),3Omp | Ligand Exchange Type CSP; Chiralpak MA (+) (4.6 × 50 mm); MP: 2 mM CuSO4/ACN (90:10 or 85:15 v/v); Flow rate: 1.0 mL/min; UV detection at 254 nm | Cytotoxicity, antimonycobacterial and elastase inhibitory activities                  | [77]      |
| Kohamamides A–C (32–34) | *Cyanobacterium Oleania sp.* | 1-Pro, 1-Ala, N-Me-t-Val, 1-Leu 32: 1-Ile | Ligand Exchange Type CSP; Chiralpak MA (+) (4.6 × 250 mm); MP: 2 mM CuSO4/ACN: 2 mM CuSO4 (15:85:5 v/v); Flow rate: 1.0 mL/min; UV detection at 254 nm | No growth inhibition against HeLa and HL60 cells                                        | [78]      |
| Marformycins A–F (35–40) | *Deep sea-derived Streptomyces drozdowiczii* | 35: 3-allo-Ile, 1-Val; 36: 3-allo-Ile, 1-allo-Ile, 1-allo-Ile, 1-allo-Ile, 1-Leu; 37: 1-Val; 38: 3-allo-Ile, 1-allo-Ile, 1-Leu; 39 and 40: 1-Thr, 1-Val, 1-DVal, 1-Leu | Ligand Exchange Type CSP; MCIGELCRS10W (4.6 × 50 mm); MP: 2 mM CuSO4 in H2O Flow rate: 0.5 mL/min; UV detection at 254 nm | Anti-infective activity against Micrococcus luteus                                      | [79]      |
| Peptide          | Source                  | aa Composition          | Chromatographic Conditions                                                                 | Biological Activities                                                                 | Refs.       |
|------------------|-------------------------|-------------------------|--------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|-------------|
| Pitiprolamide    | Cyanobacterium Lyngbya majuscula | 1-Pro, 1-Val            | Macrocyclic Antibiotic Type CSP; Chirobiotic TAG (4.6 × 250 mm); MP: MeOH/10 mM NH₄OAc (40:60 v/v) (pH 5.6); Flow rate: 0.5 mL/min | Cytotoxicity against CT116 and MCF7 cancer cell lines and antibacterial activity        | [80]        |
| Palau’amide      | Cyanobacterium Lyngbya sp. | 1-Ala, 1-Ile, N-Me-t-Ala, N-Me-D-Phe and D-hydroxyisocaproic acid | Ligand Exchange Type CSP; Phenomenex Chirex Phase 3126 (D) (4.6 × 250 mm); MP: 1 mM CuSO₄, 2 mM CuSO₄/ACN (95:5 or 85:15 v/v); Flow rate: 0.8 mL/min; UV detection at 254 nm | Cytotoxicity against KB cell line                                                      | [81]        |
| Pitipeptolides C–F | Cyanobacterium Lyngbya majuscula | 1-Pro, 1-Val, 1-Ile, 1-Phe, N-Me-t-Phe | Macrocyclic Antibiotic Type CSP; Chirobiotic TAG (4.6 × 250 mm); MP: MeOH/10 mM NH₄OAc (40:60 v/v) (pH 5.6); Flow rate: 0.5 mL/min; UV detection at 254 nm | 46: Active against Mycobacterium tuberculosis                                          | [82]        |
| Ulongapeptin     | Cyanobacterium Lyngbya sp. | 1-Lactic acid, 1-Val, N-Me-t-Val, N-Me-D-Val, N-Me-D-Phe | Ligand Exchange Type CSP; Phenomenex Chirex Phase 3126 (D), 4.6 × 250 mm; MP: 2 mM CuSO₄, 2 mM CuSO₄/ACN (95:5 or 85:15 v/v); Flow rate: 1.00 mL/min; UV detection at 254 nm | Cytotoxicity against KB cells                                                          | [83]        |
| Largamides A–H   | Cyanobacterium Oscillatoria sp. | 1-Val, N-Me-t-Val, N-Me-D-Val, 2-hydroxy-3-methylvaleric acid N-Me-t-Ala | Marfey’s method (FDLA) combined with HPLC YMC-Pack AQ-ODS (10 × 250 mm); MP: 50% ACN in 0.01 N TFA; Flow rate: 2.5 mL/min; UV detection at 254 nm | Chymotrypsin inhibition                                                               | [84]        |

| D-Glyceric acid  | Cyanobacterium Limosa sp. | D-Glyceric acid          | Marfey’s method (FDLA) combined with HPLC Phenomenex Jupiter Proteo C₁₂ column, 4 µm (4.6 × 150 mm); MP: ACN containing 0.01 M TFA; Flow 0.5 mL/min; UV detection at 254 nm | Chymotrypsin inhibition                                                               | [84]        |
| Peptide | Source | aa Composition | Chromatographic Conditions | Biological Activities | Refs. |
|---------|--------|----------------|-----------------------------|-----------------------|-------|
| Trungapeptins A–C ([56–58]) | Cyanobacterium *Lyngbya majuscula* | 1-Val, 1-N-MeVal, 1-alloLeu, 1-Pro | Marfey’s method (FDLA) combined with HPLC. Alltech Econosil C18; MP A: 40% ACN with 0.04% TFA. MP B: 37.5% ACN with 0.05% TFA. Flow rate: 1.0 mL/min; UV detection at 254 nm | Brine shrimp toxicity and ichthyotoxicity | [85] |
| Phenyllactic acid (S) | | | Ligand Exchange Type CSP; CHIRALPAK MA (+) (4.6 × 50 mm); MP: 2 mM CuSO₄/ACN (85:15) Flow rate: 0.5 mL/min; UV detection at 254 nm | | |
| Carriebowmide ([59]) | Cyanobacterium *Lyngbya polychroa* | 1-Ala, N-Me-1-Leu, N-Me-D-Phe, 1-Phe, 1-Met | Ligand Exchange Type CSP; Phenomenex, Chirex (D) Penicillamine, 5 µM (4.6 × 250 mm); MP: 2.0 mM CuSO₄-ACN (95:5, 90:10, or 85:15 v/v) Flow rate: 0.8 or 1.0 mL/min; UV detection at 254 nm | Lipophilic extract reduced feeding on agar food pellets | [86] |
| R-Hmba | | | Ligand Exchange Type CSP; Chiralpak MA (+) (4.6 × 250 mm); MP: 2.0 mM CuSO₄-ACN (90:10 v/v) Flow rate: 1.0 mL/min; UV detection at 254 nm | | |
| 1-Aba | | | Ligand Exchange Type CSP; Phenomenex, Chirex (D) Penicillamine, 5 µM (4.6 × 250 mm); MP: 2.0 mM CuSO₄ Flow rate: 1.0 mL/min; UV detection at 254 nm | | |
| (2R,3R)-Amha | | | Marfey’s method (FDAA) combined with HPLC. Atlantis, C18, (3.0 × 250 mm); MP: 50 mM NH₄COOCH₃(aq)-ACN (70:30 v/v) Flow rate: 1.0 mL/min; UV detection at 254 nm | | |
| Symplecamide A ([60]) | Cyanobacterium *Symplaca sp.* | 1-Val, 1-Thr, 1-Ile, 1-Cit, 1-Gln, 1-Btyr, 1-But | Marfey’s method (FDAA) combined with HPLC. Phenomenex Jupiter C18 column (4.6 × 250 mm) MP: ACN:H₂O:HOAc (15:85:0.2) to 1:1:0.2 v/v/v Flow rate: 0.5 mL/min; UV detection at 340 nm | Cytoxicity and antimicrobial activities | [87] |
| Kempopeptins A ([61]) and B ([62]) | Cyanobacterium *Lyngbya sp.* | 61: N-O-dMe-Br-L-Tyr | Marfey’s method (FDLA) combined with HPLC Conditions not described | Elastase and chymotrypsin inhibitor | [88] |
| | | 61: N-Me-t-Tyr, t-Val, t-Thr-2, t-Pro, t-Phe, t-Ahp, t-Leu | | | |
| | | 62: t-Lys, t-Thr, t-Val, t-Ile | Ligand Exchange Type CSP; Phenomenex Chirex Phase 3126 N,3-dioxyl-(D)-penicillamine column, 5 µM (4.6 × 250 mm); MP: 2 mM CuSO₄ in H₂O-ACN (95:5 v/v) or 2 mM CuSO₄ Flow rate: 1.0 mL/min; UV detection at 254 nm | | |
| Peptide          | Source                                      | aa Composition          | Chromatographic Conditions                                                                 | Biological Activities                                                                 | Refs. |
|------------------|---------------------------------------------|-------------------------|--------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|-------|
| Tiglicamides A–C | Cyanobacterium *Lyngbya confervoides*       | 1-Ala, L-Thr, L-Val, D-Glu, D-Tyr; 63: 1-Htyr; 65: 1-Met (O) | Ligand Exchange Type CSP; Phenomenex, Chirex 3126, 5 μm (4.6 × 250 mm); Mobile Phase: 2 mM CuSO₄; Flow rate: 1.0 mL/min; UV detection at 254 nm | Porcine pancreatic elastase inhibition                                                 | [89]  |
|                  |                                             | 65: L-Phe                | Marfey’s method (FDLA) combined with HPLC; Stefanini Alltech Alltima HP C₁₈, 5 μm (4.6 × 250 mm); MP: 50–100% MeOH in 0.1% (v/v) aqueous TFA; Flow rate: 0.8 mL/min; PDA detection at 200–500 nm |                                                                                       |       |
| Hantupeptin B    | Cyanobacterium *Lyngbya majuscula*          | 1-Pro, L-Val, N-Me-L-Val, N-Me-L-Val, L-Pro | Marfey’s method (FDAA) combined with HPLC; Phenomenex, Luna, 5 μm, 200 × 150 mm; MP: ACN in 0.1% (v/v) aqueous HCOOH; Flow rate: 0.2 mL/min | Cytotoxicity against MOLT-4 (leukemic) and MCF-7 (breast cancer) cell lines            | [90]  |
|                  |                                             | 1,3-phenyllactic acid (S)| Ligand Exchange Type CSP; Chiralpak MA (+) (4.6 × 500 mm); MP: 2 mM CuSO₄/ACN (85:15 v/v); Flow rate: 0.7 mL/min; UV detection at 218 nm |                                                                                       |       |
| Palmyramide A    | Cyanobacterium *(Lyngbya majuscula)* and a red alga *Centroceras* sp., complex | 1-Val, N-Me-L-Val, L-Pro | Marfey’s method (FDAA) combined with HPLC; Merck LiChroprep 100 RP-18 4.0 × 125 mm; MP: ACN/H₂O/HCOOH (30:70:1.0 to 70:30:0.1 v/v/v) or 2.0 mM CuSO₄ in H₂O; Flow rate: 0.7 mL/min; UV detection at 254 nm | Sodium channel blocking activity in neuro-2a cells and cytotoxic activity in H-460 (human lung carcinoma) cells | [91]  |
|                  |                                             | 1-Lac, L-Pla             | Ligand Exchange Type CSP; Phenomenex Chirex 3126 (4.6 × 250 mm); Conditions not described |                                                                                       |       |
| Veraguamides A–G | Cyanobacterium *Symploca cf. hydnoides*     | 68–71, 73 and 74: 1-Val, N-Me-L-Val, L-Pro; 70: (2S,3R) Br-Hmoya; 71: N-Me-L-ile, 72: 1-Ile, N-Me-L-Val, N-Me-L-ile, 1-Pro | Macrocyclic Antibiotic Type CSP; Chirobiotic TAG (4.6 × 250 mm); MP: MeOH/10 mM NH₄OAc (40:60 v/v) (pH 5.6); Flow rate: 0.5 mL/min | Cytotoxic activity against HT29 (colorectal adenocarcinoma) and HeLa (cervical carcinoma) cell lines | [92]  |
|                  |                                             | 74: 2S:3R dpv             | Marfey’s method (FDAA) combined with HPLC; Phenomenex Synergi Hydro-RP (4.6 × 150 mm); MP: MeOH/H₂O/HCOOH (40–100% MeOH: 0.1% HCOOH); Flow rate: 0.5 mL/min |                                                                                       |       |
| Peptide | Source | aa Composition | Chromatographic Conditions | Biological Activities | Refs. |
|---------|--------|----------------|-----------------------------|-----------------------|-------|
| Porpoisamides A (75) and B (76) | Cyanobacterium *Lyngbya* sp. | 75: (2S,3R)-Amoa, 76: (2R,3R)-Amoa | Ligand Exchange Type CSP; Chiralpak MA (+) (4.6 × 50 mm); MP: ACN/2 mM CuSO\(_4\) (10:90 v/v); Flow: 1.0 mL/min; UV detection at 254 nm | Cytotoxicity against HCT 116 (colorectal carcinoma) and U2OS (osteosarcoma) cells | [93] |
| Companeramides A (77) and B (78) | Cyanobacterial assemblage collected from Coiba National Park, Panama | 77: t-Ala, N-Me-t-Ala, t-Pro, t-Ile, N-Me-t-Leu, and N-Me-t-Val, 78: t-Pro, N-Me-t-Val, t-Ile, d- and N-Me-t-Ala; S-Hiva | Marfey’s method (FDAA) combined with HPLC C\(_{18}\) column (3.9 × 150 mm); MP: 40 mM NH\(_4\)Ac (pH 5.2):ACN (9:1 to 1:1 v/v); Flow: 1.0 mL/min; UV detection at 340 nm | Antiplasmodial activity against *Plasmodium falciparum* | [94] |
| Piperazimycins A–C (79–81) | Fermentation broth of a *Streptomyces* sp. | (5)-AMNA, (5,5)-OHPip1, (R,R)-OHPip2, 79: (S)-aMeSer | Marfey’s method (FDAA) combined with HPLC C\(_{18}\) MP: ACN in H\(_2\)O (10–100%); Flow: 1.0 mL/min; UV detection: 210, 254, 340 nm | 79: Active against diverse cancer cell lines | [95] |
| Grassypeptolides D (82) and E (83) | Red sea cyanobacterium *Leptolyngbya* sp. | d-allo-Thr, N-Me-t-Leu, t-Thr, N-Me-t-Leu | Marfey’s method (FDAA) combined with HPLC Gemini C\(_{18}\) 110 A, 5 \(\mu\)m (4.6 × 250 mm) | Cytotoxicity against HeLa and mouse neuro-2a blastoma cells | [96] |
Table 2. Cont.

| Peptide Source | aa Composition Chromatographic Conditions | Biological Activities Refs. |
|----------------|------------------------------------------|-----------------------------|
| **Fijimycins A–C** (84–86) | Fermentation broth of *Streptomyces* sp. strain CNS-575 | | |
| 84: D-PhSar, l-Ala, l-DiMe-Leu, Sar, d-Hyp, d-Leu, l-Thr; 85: l-N-Me-Leu, l-Ala, l-DiMe-Leu, Sar, d-Hyp, d-Leu, l-Thr; 86: l-PhSar, l-Ser, l-DiMeLeu, Sar, d-Hyp, d-Leu, l-Thr | Marfey’s method (FDAA) combined with HPLC C18 column, Luna (4.6 × 100 mm) MP: ACN:H2O:TFA (10:90:1 to 50:50:1 v/v/v) Flow rate: 0.7 mL/min; UV detection at 340 nm | Antibacterial activity against three MRSA strains of *Staphylococcus aureus* [97] |
| **Itralamides A (87)** and B (88), and Carriebowmide sulfone (89) | Cyanobacterium *Lyngbya majuscula* | | |
| 87: l-Ala, d-Ala, N-Me-l-Ala, N-Me-d-Phe, N-Me-l-Thr, N-Me-l-Val | Marfey’s method (FDLA) combined with HPLC Eclipse XDB-18, Agilent (4.6 × 150 mm) MP: ACN:H2O:HOAc (20:80:0.1 to 80:20:0.1 v/v/v) Flow rate: 0.8 mL/min; Detection by ESI-MS | 88: Cytotoxicity against HEK293 (human embryonic kidney) cell line [98] |
| 88: N-Me-l-Ala, N-Me-d-Phe, N-Me-l-Thr, d-Val | Marfey’s method (FDLA) combined with HPLC Luna C18, Phenomenex, 5 µm (4.6 × 250 mm) MP: ACN:H2O:HOAc (20:80:0.1 to 90:10:0.1 v/v/v) Flow rate: 0.8 mL/min | | |
| 89: (2S,3R)-AMHA | Marfey’s method (FDLA) combined with HPLC-PDA dC18, 5 µm (3.0 × 250 mm); MP: ACN:H2O:HOAc (0:100:0.1 to 50:50:0.1 v/v/v); Flow rate: 0.3 mL/min | Highly toxic to H460 (human lung cancer) cells [99] |
| **Viequeamide A** (90) | Marine button cyanobacterium *Rivularia* sp. | Marfey’s method (FDLA) combined with HPLC Conditions not described | | |
| 1-Val, l-Thr, N-Me-l-Val, 1-Pro | | Highly toxic to H460 (human lung cancer) cells [99] |
| **Ngercheumicin** F–I (91–94) | Photobacterium related to *Photobacterium halotolerans* | Marfey’s method (FDLA) combined with HPLC Dionex RSLC Ultimate 300 with a diode array detector Kinetex C18 column, 2.6 µm at 60 °C (2.1 × 150 mm) ACN:H2O:TFA (0:100:0.1 to 50:50:0.1 v/v/v) Flow rate: 0.8 mL/min | 91–93: *null* inhibiting activities [100] |

**aa**—Amino acid; FDAA—1-Fluoro-2-4-dinitrophenyl-5-L-alanine amide; LC—Liquid Chromatography; MS—Mass Spectrometry; HPLC—High Performance Liquid Chromatography; MP—Mobile Phase; TEAP—Triethylammonium phosphate; ACN—Acetonitrile; TFA—Trifluoroacetic acid; MeOH—Methanol; TEA—Triethylamine; IPA—Isopropyl alcohol; FDLA—1-Fluoro-2-4-dinitrophenyl-5-D,L-leucine amide; NaOAc—Sodium acetate; NH4OAc—Ammonium acetate.
The concurrent applicability of chiral HPLC and Marfey’s methods for determination of the absolute configuration of all the amino acid residues of cyclic depsipeptides 47–78 (Figure 5) was also described in several reports, among which ten described the use of ligand exchange-type CSPs to perform the analysis in association with Marfey’s method [71,72,74–76,78,79,81]. Furthermore, the use of macrocyclic antibiotic-based CSPs was reported by Montaser et al. [82].

Considering the biological activities of cyclic depsipeptides, whose stereochemistry of the amino acids was determined by a combination of Marfey’s method and chiral HPLC, it is worth mentioning the following compounds. Ulongapeptin (47), isolated from a Palauan marine cyanobacterium *Lyngbya* sp. displayed significant cytotoxic activity against KB cells with IC$_{50}$ value of 0.63 µM [83]. Largamides A–H (48–55), isolated from the marine cyanobacterium *Oscillatoria* sp., inhibited chymotrypsin with IC$_{50}$ values ranging from 4 to 25 µM [84]. Symlocamide A (60), isolated from the marine cyanobacterium *Symploca* sp., showed cytotoxicity against NCI-460, non-small cell lung cancer cells (IC$_{50}$ = 40 nM), and neuro-2a mouse neuroblastoma cells (IC$_{50}$ = 29 nM). It was also reported that 60 was active against three tropical parasites: malaria (*Plasmodium falciparum*, IC$_{50}$ = 0.95 µM), chagas disease, (*Trypanosoma cruzi*, IC$_{50}$ > 9.5 µM), and leishmaniasis (*Leishmania donovani*, IC$_{50}$ > 9.5 µM) [87]. It was found that, kempopeptins A (61) and B (62), isolated from the marine cyanobacterium *Lyngbya* sp., exhibited inhibitory activity against elastase and chymotrypsin with IC$_{50}$ values of 0.32 µM and 2.6 µM, respectively [88]. Palmyramide A (67), isolated from the marine cyanobacterium *Lyngbya majuscula*, showed sodium channel blocking activity in the neuro-2a cells as well as cytotoxic activity in H-460 human lung carcinoma cell line [91]. Companeramides A (77) and B (78), isolated from a marine cyanobacterial assemblage comprising a small filament *Leptolyngbya* species, showed high nanomolar in vitro antiplasmodial activity against *Plasmodium falciparum* strains D6, Dd2, and 7G8 [94].

Moreover, HPLC analysis after derivatization with a Marfey’s reagent has been reported as the only method to determine the stereochemistry of the amino acid residues of cyclic depsipeptides 79–94 (Figure 6). FDAA was used as derivatization reagent for piperazimycins A–C (79–81), cyclic hexadepsipeptides isolated from the fermentation broth of a marine-derived bacterium *Streptomyces* sp. Strain, collected from a sediment [95], grassypeptolides D (82) and E (83), cyclic depsipeptides isolated from the marine cyanobacterium *Leptolyngbya* sp. [96], fijimycins A–C (84–86), cyclic depsipeptides isolated from a marine bacteria *Streptomyces* sp. [97]. The Marfey’s reagent FDLA was employed for the assignment of the absolute configuration of the amino acid residues of several cyclic depsipeptides such as itralamide A (87) and B (88) and carriebowmide sulfone (89), isolated from the marine cyanobacterium *Lyngbya majuscula* [98], viequeamide A (90), isolated from the marine button cyanobacterium (*Rivularia* sp.) [99], ngercheumicins F–I (91–94) [100].

Many cyclic depsipeptides whose stereochemistry of their amino acids was determined only by Marfey’s method, exhibited various interesting biological activities. Thus, piperazimycin A (79) was found to exhibit potent cytotoxicity against a panel of sixty cancer cell lines (mean values of growth inhibition (GI$_{50}$) = 100 nM, and LC$_{50}$ = 2 µM) [95]. While, grassypeptolides D (82) and E (83) exhibited significant cytotoxicity to HeLa (IC$_{50}$ = 335 and 192 nM, respectively) and mouse neuro-2a blastoma (IC$_{50}$ = 559 and 407 nM, respectively) cell lines [96], itralamide B (88) was active against HEK293 cells (IC$_{50}$ value of 6 ± 1 µM) [98]. Fijimycins A–C (84–86) exhibited strong growth inhibitory activity against three MRSA strains in a concentration range of 4–32 µg/mL$^{-1}$ [97].

### 2.3. Lipopeptides

To the best of our knowledge, there are only two reports describing simultaneously the isolation and characterization of lipopeptides from marine cyanobacteria (Figure 7) as well as the stereochemistry determination of the amino acids present in their hydrolysates (Table 3).
Figure 5. Structure of cyclic depsipeptides 47–78, isolated from marine cyanobacteria and other bacteria, whose stereochemistry of their amino acids was determined by a combination of Marfey’s method and chiral HPLC.
Figure 6. Structure of cyclic depsipeptides 79–94, isolated from marine cyanobacteria and other bacteria, whose stereochemistry of their amino acids was determined by Marfey’s method.

The configuration of N-Me-Hph of the lipopeptide antillatoxin B (95), isolated from the cyanobacterium *Lyngbya majuscula*, was assigned as L using FDAA as Marfey’s derivatization reagent [101]. Compound 95 exhibited significant sodium channel activation (EC\(_{50} = 1.77 \mu M\)) and ichthyotoxicity (LC\(_{50} = 1 \mu M\)) [101]. The hydrolysates of lipopeptides lobocyclamides A–C (96–98), isolated from the cyanobacterium *Lyngbya confervoides*, were analyzed by either direct chiral HPLC, using the D-penicillamine ligand exchange type CSP or by prior derivatization by Marfey’s method and reverse phase HPLC [102]. Both compounds displayed modest in vitro antifungal activity against a panel of *Candida* sp., including two fluconazole-resistant strains. Interestingly, synergistic antifungal activity was also observed [102].
Peptides from Marine-Derived Fungi

Marine fungi have been isolated from various marine sources like algae, marine invertebrates, sediment or water, mangroves and sponges. Most of the fungal species isolated from marine sponges are related to the genera *Aspergillus* and *Penicillium* [103]. Marine fungi are a rich source of structurally unique and biologically active compounds with a wide range of biological activities, such as antimalarial, anticancer, antifungal, antibacterial, cytotoxicity and among others [104]. More than

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**Figure 7.** Structure of lipopeptides 95–98, isolated from marine cyanobacteria.

**Table 3.** Lipopeptides from marine cyanobacteria.

| Peptide           | Source                      | aa Composition | Chromatographic Conditions | Biological Activities                          | Ref. |
|-------------------|-----------------------------|----------------|------------------------------|-----------------------------------------------|------|
| Antillatoxin B (95) | Cyanobacterium *Lyngbya majuscula* | N-Me-t-Hph     | Marfey’s method (FDAA) combined with HPLC Waters Nova-Pak C18 (3.9 x 150 mm), MP: 10 to 50% ACN in H2O with 0.05% TFA, UV detection at 340 nm | Sodium channel-activating and ichthyotoxic activities | [101] |
| Lobocyclamides A–C (96–98) | Cyanobacterium *Lyngbya confervoides* | 96: S-Ile, S-allo-Ile, S-Leu, R-β-Aoa, S-Ser, R-Tyr, S-Hse, R-Hpr | Ligand Exchange Type CSP Chirex 3126 (t)-penicillamine column; MP: 2 mM aq CuSO4/ACN (1:99, 95:5 or 86:14 v/v), Flow rate: 1.15–1.20 mL/min, UV detection at 254 nm | Antifungal activity against a panel of *Candida* sp. | [102] |

aa—Amino acid; FDAA—1-Fluoro-2-4-dinitrophenyl-L-alanine amide; HPLC—High Performance Liquid Chromatography; MP—Mobile Phase; ACN—Acetonitrile; TFA—Trifluoracetic acid.
1000 compounds have been already isolated from marine derived fungi and among them around 150–200 new compounds were bioactive [103,104].

3.1. Cyclic Peptides

A large number of cyclic peptides have been isolated from marine-derived fungi (Figure 8) and Table 4 shows the marine fungal cyclic peptides whose stereochemistry of their amino acid residues were determined. To the best of our knowledge, only three reports described the use of FDAA and FDLA as Marfey's derivatization reagents, specifically for analysis of the peptides 99–112.

The cyclic peptide cyclo-(L-leucyl)-trans-4-hydroxyl-L-prolyl-D-leucyl-trans-4-hydroxy-L-proline) (99), isolated from the marine mangrove-derived fungi Phomopsis sp. K38, and Alternaria sp. E33, was found to exhibit antifungal activity, particularly the fungus Helminthosporium sativum. By using a combination of Marfey’s method and a reverse phase HPLC, the presence of 4-OH-L-Pro and both L- and D-Leu residues in its structure was confirmed [105]. Scytalidamides A (100) and B (101), and clonostachysins A (102) and B (103), isolated from marine sponge derived fungus Clonostachys rogersoniana strain HJK9, were found to comprise L-configuration for all their amino acids [106,107]. Scytalidamides A (100) and B (101) showed cytotoxicity against human colon carcinoma tumor cell line (HCT-116) with IC50 values of 2.7 and 11.0 µM, respectively, and the NCI 60 cell-line, with 7.9 and 4.1 µM GI-50, respectively [106], while clonostachysins A (102) and B (103) exhibited inhibitory effect on the Prorocentrum micans alga at concentration higher than 30 µM [107].

Figure 8. Structure of cyclic peptides 99–112, isolated from marine-derived fungi.
Table 4. Cyclic peptides from marine-derived fungi.

| Peptide                           | Source                                      | aa Composition | Chromatographic Conditions                                      | Biological Activities                                      | Ref. |
|-----------------------------------|---------------------------------------------|----------------|----------------------------------------------------------------|-----------------------------------------------------------|------|
| Cyclo-L-leucyl-trans-4-hydroxy-L-prolyl-L-leucyl-trans-4-hydroxy-L-proline (99) | Marine mangrove-derived fungi *Phomopsis* sp. K38 and *Alternaria* sp. E33 | 4-OH-L-Pro, D-Leu, L-Leu, L-Thr | Marfey’s method (FDAA) combined with LC/MS                      | Inhibition against four crop-threatening fungi [105]       |      |
| Scytalidamides A (100) and B (101) | Marine Fungus of the genus *Scytalidium*    | L-Phe, N-Me-L-Phe, L-Leu, N-Me-L-Leu, L-Pro, 3-Me-L-Pro | Marfey’s method (FDLA) combined with HPLC                      | Cytotoxicity against HCT-116 and NCI 60 cell lines [106] |      |
| Clonostachysins A (102) and B (103) | Marine sponge-derived fungus *Clonostachys rogersianus* strain HJK9 | N-Me-L-Ile, N-Me-L-Leu, L-Pro, L-Gly, N-Me-L-Tyr, N-Me-L-Ala 102: N-Me-L-Val; 103: N-Me-L-Ile | Marfey’s method (FDLA) combined with LC-ESI MS/MS; Conditions not described | Inhibitory effect on dinoflagellate *Prorocentrum micans* [107] |      |
| Asperterrestide A (104)            | Marine-derived fungus *Aspergillus terreus* SCSGAF0162 | D-Ala | Marfey’s method (FDAA) combined with HPLC                      | Cytotoxicity against U937 and MOLT4 human carcinoma cell lines and inhibitory effects on influenza virus [108] |      |
| Sclerotides A (105) and B (106)    | Marine-derived fungus *Aspergillus sclerotiorum* PT06-1 | L-Thr, L-Ala, D-Phe, D-Ser | Crown Ether CSP; Crownpak CR (+); MP: 2 mM CuSO₄·H₂O solution Flow rate: 1.0 mL/min; UV detection at 254 nm | 105 and 106: Antifungal activity; 106: Cytotoxicity and antibacterial activity [109] |      |
| Cordyheptapeptides C-E (107–109)  | Marine-derived fungus *Acremonium perisicum* SCSIO 115 | N-Me-L-Tyr, L-Phe, L-Pro, L-Leu 107–109: N-Me-D-Phe, L-Val 109: N-Me-L-Gly, N-Me-D-Tyr, L-allo-Ile | Crown Ether Chiral CSP; Crownpak CR (+); MP: 2 mM CuSO₄·H₂O solution Flow rate: 1.0 mL/min; UV detection at 254 nm | 107 and 109: Cytotoxicity against SF-268, MCF-7, and NCI-460 tumor cell lines [110] |      |
| Similanamide (110)                 | Marine sponge-associated fungus *Aspergillus similanensis* KUFA 0013 | L-Ala, D-Leu, L-Val, N-Me-L-Leu, D-pipecolic acid | Macrocyclic Antibiotic Type CSP; Chirobiotic T, 5 µm (46.6 × 150 mm); MP: MeOH-H₂O-CH₃COOH (70:30:0.02 v/v/v); Flow rate: 1.0 mL/min; UV detection at 210 nm | Cytotoxicity against MCF-7, NCI-H460 and A373 tumor cell lines [111] |      |
| Sartoryglabramide A (111) and B (112) | Marine sponge-associated fungus *Neosartorya glabra* KUFA 0702 | L-Phe, L-Pro 111: L-Trp | Macrocyclic Antibiotic Type CSP; Chirobiotic T, 5 µm (46.6 × 150 mm); MP: MeOH-H₂O (80:20 v/v) Flow rate: 1.0 mL/min; UV detection at 210 nm | Neither antibacterial nor antifungal activity [112] |      |

aa—Amino acid; FDAA—1-Fluoro-2-4-dinitrophenyl-5-l-alanine amide; HPLC—High Performance Liquid Chromatography; MP—Mobile Phase; ACN—Acetonitrile; TFA—Trifluoroacetic acid; MeOH—Methanol; FDLA—1-fluoro-2-4-dinitrophenyl-5-D-l-leucine amide.
Both Marfey’s method and chiral HPLC analysis were also used for the analysis of the absolute configuration of the amino acids of asperterrestride A (104), a cyclic peptide isolated from the marine-derived fungus Aspergillus terreus SCGAF0162 which revealed the presence of D-Ala in its structure [108]. Nevertheless, it was not possible to distinguish between D-Ile and D-allo-Ile. Compound 104 showed promising inhibitory effects to the influenza virus strains A/WSN/33, and A/Hong Kong/8/68 (IC₅₀ values of 15 and 8.1 µM, respectively) as well as cytotoxicity against U937 and MOLT4 cell lines (IC₅₀ values of 6.5 and 6.2 µM, respectively) [108].

There are some reports describing the application of different types of CSPs, including crown ethers and macrocyclic antibiotics, for a chiral HPLC as the only method for analysis of the absolute configuration of the amino acids of peptides. Thus, the determination of the stereochemistry of the amino acids in the cyclic peptides sclerotides A (105) and B (106), isolated from the marine-derived fungus Aspergillus sclerotiorum PT06-1 [109], and cordyheptapeptides C–E (107–109), isolated from the marine-derived fungus Acremonium persicinum SCSIO 115 [110], was achieved via chiral HPLC analysis of the hydrolysates using the crown ether-based CSP Crownpak CR (+). Sclerotides A (105) and B (106) were found to comprise L-Thr, L-Ala, D-Phe, and D-Ser [109]. Moreover, the presence of N-Me-D-Gly, and L-Val in cordyheptapeptides C (107) and D (108) and N-Me-L-Gly, N-Me-D-Tyr, and L-allo-Ile in cordyheptapeptide E (109) was confirmed, in addition to the present of other amino acids common to the three cyclic peptides [110]. Sclerotides A (105) and B (106) displayed antifungal activity against Candida albicans, with MIC values of 7.0 and 3.5 µM, respectively. Furthermore, sclerotide B (106) also exhibited cytotoxicity against HL-60 cell line as well as antibacterial activity against Pseudomonas aeruginosa [109] whereas cordyheptapeptides C (107) and E (109) exhibited cytotoxic activity against SF-268 (IC₅₀ values of 3.7 and 3.2 µM, respectively), MCF-7 (IC₅₀ values of 3.0 and 2.7 µM, respectively), and NCI-H460 (IC₅₀ values of 11.6 and 4.5 µM, respectively) tumor cell lines [110]. Recently, the macrocyclic antibiotic-based CSP Chirobiotic T was employed in our group to determine the stereochemistry of amino acid residues of a new cyclic hexapeptide, similanamide (110), isolated from a marine sponge-associated fungus Aspergillus similaneinensis KUFA 0013 [111] which confirmed the presence of L-Ala, L-Leu, L-Val and D-pipeolic acid as its amino acids constituent. By using a similar approach, the absolute configuration of all the amino acids of two new cyclotetrapeptides, sartoryglabramides A (111) and B (112), isolated from the marine sponge-associated fungus Neosartorya glabra KUFA 0702, were assigned to be L-configuration in both cyclic peptides [112]. Further details are described in the case-study presented below.

3.2. Cyclic Depsipeptides

Most of the works describing the stereochemistry determination of amino acid residues of cyclic depsipeptides, isolated from marine fungus (Figure 9), employed Marfey’s method coupled with HPLC, using FDAA or FDLA as derivatization reagents (Table 5).

The structures of exumolides A (113) and B (114), cyclic depsipeptides isolated from the marine fungus of the genus Scytalidium, were confirmed to have L-Pro, L-Phe and N-Me-L-Leu in their composition [113]. Moreover, guangomide A (115), isolated from an unidentified sponge-derived fungus, was found to comprise N-Me-D-Phe [114]. The absolute configuration of common amino acid residues in destruxin E chlorohydrin (116) and pseudodestruxin C (117), isolated from the marine-derived fungus Beauveria felina, indicated the presence of N-Me-L-Ala and L-Ile in 116, L-Phe in 117, and N-Me-L-Val in both cyclic depsipeptides [115]. Furthermore, the absolute configuration of amino acid residues in zygosporamide (118), isolated from the marine-derived fungus Zygosporium masonii [116], petriellin A (119), isolated from the coprophilous fungus Petriella sordida [117], alternaramdie (120), isolated from the marine derived fungus Alternaria sp. SF-5016 [118], petrosifungins A (121) and B (122), isolated from a Penicillium brevicompac-tum strain of the Mediterranean sponge Petrosia ficiformis Poirot [119], were also successfully determined by Marfey’s method coupled with HPLC. Zygosporamide (118) displayed cytotoxic activity against RXF 393 and SF-268 cancer cell lines, with mean values of GI-50 of 6.0 and <5.6 nM, respectively [116].
whereas guangomide A (115) [114] and alternaramdie (120) [118] showed antibacterial activity against *Staphylococcus epidermidis* and *Staphylococcus aureus*, respectively.

In the last few years, ultra-high-pressure liquid chromatography (UHPLC) is becoming an essential technique for ultra-fast separations, since it offers many benefits, including high efficiency in short analysis time and low solvent consumption [120,121]. Thus, the absolute configuration of the amino acid residues of oryzamides A–E (123–127), isolated from the sponge-derived fungus *Nigrospora oryzae PF18*, was achieved by Marfey’s analysis with FDLA, combined with UHPLC [122].

Figure 9. Structure of cyclic depsipeptides 113–131, isolated from marine-derived fungi.
Table 5. Cyclic depsipeptides from marine-derived fungi.

| Peptide                  | Source                                          | aa Composition | Chromatographic Conditions                                                                 | Biological Activities                                                                 | Ref. |
|--------------------------|-------------------------------------------------|----------------|---------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|------|
| Exomulides A (113) and B (114) | Fungus of the genus Septalidium sp.              | t-Pro, l-Phe, N-Me-l-Leu | Marfey’s method (FDAA) combined with HPLC HP Hewlett Packard 1090 Dode Array, 5 µm (10 × 250 mm); MP: 10–50%aq ACN (0.1% TFA); Flow rate: 1.0 mL/min; UV detection at 340 nm | Antimicrobial activity against unicellular chlorophyte Dunaliella sp                   | [113]|
| Guangomide A (115)       | Sponge-derived fungus                            | N-Me-d-Phe       | Marfey’s method (FDAA) combined with HPLC Alltech Altima C18 column, 5 µm (10 × 250 mm); MP: ACN:H2O (4:1 to 1.1 v/v); Flow rate: 1.0 mL/min; UV detection at 340 nm | Antibacterial activity against Staphylococcus epidermidis and Enterococcus durans        | [114]|
| Destruxin E chlorohydrin (116) and pseudodestruxin C (117) | Marine-derived fungus Beauveria felina          | N-Me-l-Val 116: N-Me-l-Ala, l-Ile 117: l-Phe | Marfey’s method (FDAA) combined with HPLC C18 column, 5 µm (4.6 × 250 mm); MP: 10–20% ACN in 0.1 M NH4OAc (pH = 5); Flow rate: 1.0 mL/min; UV detection at 340 nm | Cytotoxicity in NCI’s 60 cell line panel                                                | [115]|
| Zygosporamide (118)      | Marine-derived fungus Zygosporum massonii        | l-Phe, l-Leu, d-Leu | Marfey’s method (FDA) combined with HPLC C18, Agilent column, 5 µm (4.6 × 250 mm); MP: 10–50% ACN (0.1% TFA); Flow rate: 3.0 mL/min; UV detection at 340 nm | Cytotoxicity in XRF 393 and SF-268 cancer cell lines                                      | [116]|
| Petriellin A (119)       | Coprophilous fungus Petriella sorida             | N-Me-l-Ile, N-Me-l-Thr d-Phenyl lactate | Marfey’s method (FDA) combined with HPLC C18 column (4.6 × 250 mm); Conditions not described; UV detection at 260 nm | Antifungal activity                                                                   | [117]|
| Alternaramide (120)      | Marine derived fungus Alternaria sp. SF-5016     | l-Pro, d-Phe     | Marfey’s method (FDA) combined with HPLC Capcell Pak C18 column; MP: 30–60% ACN in H2O (0.1% HCOOH); Flow rate: 1.0 mL/min | Antibacterial activity against Bacillus subtilis and Staphylococcus aureus              | [118]|
| Petrosifungins A (121) and B (122) | Penicillium brevicompactum                       | l-Val, l-Pro, l-Thr, l-pippecolic acids | Marfey’s method (FDA) combined with HPLC C18 column, Waters, 5 µm (2.1 × 150 mm); MP: H2O or ACN (0.05% TFA); Flow rate: 1.0 mL/min | Not described                                                                         | [119]|
| Orzyamides A–E (123–127) | Sponge-Derived fungus Nigrospora oryzae PF18    | l-Ala, d-Leu, l-Val 123: l-Leu, 124: l-Tyr 125 and 126: l-Met, 127: l-Phe | Marfey’s method (FDCA) combined with UHPLC Acquity UHPLC BEH column, 1.7 µm (2.1 × 250 mm); MP: 10–100% ACN in H2O with 0.1% HCOOH; Flow rate: 1.0 mL/min | No cytotoxicity, antibacterial, antiparasitic, and NF-48 activities                   | [122]|
| Spiecillamide A (128) and B (129) | Marine-derived fungus Spiecillum roseum          | N-Me-d-Phe, N-Me-l-Ala, l-Ala | Marfey’s method (FDA) combined with HPLC C18 column; Macherey-Nagel Nucleodur 100, 5 µm (2.0 × 125 mm); MP: MeOH:H2O (10:90 v/v or to 100% MeOH) or 100% MeOH with NH4Ac, 2 minol | Not described                                                                         | [123]|
| Depsipeptides 1962A (130) and 1962B (131) | Endophytic fungus Kandelia candel               | l-Tyr, l-Val, d-Leu, (S)-O-Leu | Crown Ether CSP, Crownpak CR (+) column (0.4 × 150 mm); MP: 2 mM CuSO4 aq. solutions | Activity against MCF-7 tumor cell line                                                   | [131]|

aa—Amino acid; FDAA—1-Fluoro-2-4-dinitrophenyl-5-l-alanine amide; HPLC—High Performance Liquid Chromatography; MP—Mobile Phase; ACN—Acetonitrile; TFA—Trifluoroacetic acid; MeOH—Methanol; TEA—Triethylamine; FDLA—1-Fluoro-2-4-dinitrophenyl-5-D,L-l-leucine amide; NaOAc—Sodium acetate; NH4OAc—Ammonium acetate.
Spicellamides A (128) and B (129), which were isolated from the marine-derived fungus *Spicellum roseum*, exhibited cytotoxicity against rat neuroblastoma B104 cell line, with an IC$_{50}$ value of 6.2 µg/mL for spicellamide B (129) [123]. It is interesting to note that Marfey’s method was not suitable for the determination of the configuration of all amino acid residues of these two peptides. Therefore, a chiral HPLC approach was also employed, using a ligand exchange type CSP [123]. Furthermore, the chiral HPLC, using the crown ether-based CSP Crownpak CR (+), was used as the only method for determination of the configuration of the amino acids residues to confirm the presence of L-Tyr, L-Val, D-Leu, and (S)-O-Leu in the cyclic depsipeptides 1962 A (130) and B (131), isolated from the endophytic fungus *Kandelia candel* [124]. The cyclic depsipeptide 1962 A (130) exhibited growth inhibitory activity against the human breast cancer cell line, MCF-7, with IC$_{50}$ of 100 µg/mL [124].

4. Peptides from Marine Sponges

Marine sponges are an important source of new metabolites from the marine environment [125]. They are considered one of the most prolific sources of novel bioactive compounds, such as terpenoids, alkaloids, macrolides, nucleoside derivatives, polyethers, fatty acids, sterols, peroxides and other numerous organic compounds [17,126]. In addition, cyclic peptides and depsipeptides have also been isolated from marine sponges. Most bioactive compounds from sponges displayed myriad of biological activities including anti-inflammatory, antibiotic, antitumor, antimalarial, antiviral, antifouling, and immuno- or neurosuppressive [127]. However, a significant number of marine natural products isolated from sponges were tested for the anticancer activity, and many of them were successfully undergoing to preclinical and clinical trials [126,128]. More recently, among bioactive compounds discovered from marine sponges, bioactive peptides have aroused attention of many researchers [8,17].

4.1. Cyclic Peptides

Several works reported the determination of the stereochemistry of the amino acid residues of diverse peptides isolated from marine sponges (Figures 10 and 11), most of which described the application of Marfey’s method, using FDAA as the derivatization reagent (Table 6). By using Marfey’s method, Randazzo et al. [129] have showed that a 16-membered cyclic peptide, haliclamide (132), isolated from the Vanuatu marine sponge *Haliclona sp.*, comprised the amino acid N-Me-L-Phe. The absolute configuration analysis of the amino acid residues of microsclerodermins J (133) and K (134), isolated from the sponge *Microsclerodermia herdmani*, indicated, besides the amino acids common to both microsclerodermins, the presence of L-Phe, and L-Gly in 133, and L-Val, and L-Ala in 134 [130]. Moreover, in the case of euryjanicins E–G (135–137), isolated from the Caribbean sponge *Prosuberites laughlini* [131], chujamide A (138), isolated from *Suberites waedoensis* [132], and kapakahines A–D (139–142), isolated from *Cribrochalina olemda* [133], all the amino acid residues were proved to have the L configuration. However, except for D-Phe, all the amino acid residues of koshikamide B (143), isolated from the marine sponge *Theonella sp.*, were shown to possess L-configuration [134]. Furthermore, perthamides C–F (144–147), isolated from the sponge marine *Theonella swinhoei*, were found to comprise L-ThrOMe, and L-Phe; while perthamides C (144) and D (145) also comprise in their structures L-Asp, and (2R,3S)-βOHAsp [135,136]. Marfey’s method was also successfully used for evaluation of the stereochemistry of the amino acids of the cyclic peptides stylisins 1 (148) and 2 (149), stylissatins B–D (152–154), and carteritins A (150) and B (151), isolated from marine sponge *Stylissa sp.* [137–139], as well as of callyaerin G (155), isolated from the marine sponge *Callyspongia aerizusa* [140].

The marine sponge cyclic peptides whose configuration of their amino acids constituent was determined by Marfey’s method, were found to display interesting biological activities. For examples, haliclamide (132) exhibited cytotoxicity against NSCLC-N6 cell line, with an IC$_{50}$ value of 4.0 µg/mL [129], while koshikamide B (143) showed growth inhibitory activity against P388 and HCT-116 cell lines, with IC$_{50}$ values of 0.45 and 7.5 µg/mL, respectively [134]. Callyaerin G (155) also exhibited cytotoxicity against mouse lymphoma cell line (L5178Y), and HeLa cell line, with ED$_{50}$ values of 0.53 and 5.4 µg/mL,
respectively [140]. Moreover, perthamides C (144), D (145) and F (147) showed anti-inflammatory activity, with perthamide F (147) having a promising antipsoriatic effect [135,136].

Figure 10. Structure of cyclic peptides 132–151, isolated from marine sponges.
The simultaneous application of Marfey’s method, using FDAA as derivatization reagent, and chiral HPLC, using a ligand exchange type CSP, afforded the total assignment of the configuration of all the amino acid residues of reniochalistatins A–E (156–160) [141]. Reniochalistatins A–E (156–160), the cyclic peptides isolated from the marine sponge *Reniochalina stalagmitis*, were found to have all the amino acid residues with L configuration, including L-Asn and L-Trp in reniochalistatins A (156) and E (160) respectively [141]. The octapeptide reniochalistatin E (160) exhibited cytotoxicity towards myeloma RPMI-8226, and gastric MGC-803 cell lines (IC50 values of 4.9 and 9.7 µM, respectively) [141].

Phakellistatins 15–18 (161–164) were analysed only by chiral HPLC, using the ligand exchange type Chirex 3126 D-penicillamine CSP, being able to identify that all the amino acids presented L-configuration. Furthermore, phakellistatins 15 (161) and 16 (162) exhibited cytotoxicity against P388 cancer cell line, with IC50 values of 8.5 and 5.4 µM, respectively, while phakellistatin 16 (162) was also active against BEL-7402 cancer cell line, with an IC50 value of 14.3 µM [142].

![Figure 11. Structure of cyclic peptides 152–164, isolated from marine sponges.](image-url)
| Peptide Source | Peptide | aa Composition | Chromatographic Conditions | Biological Activities | Ref. |
|----------------|---------|----------------|----------------------------|-----------------------|------|
| Vanuatu marine sponge *Haliclona* sp. | Haliclamide (132) | N-Me-t-Ile-Phe | Marfey’s method (FDAA) combined with HPLC Vydac C18; MP: ACN in H₂O with 0.1% TFA (9:1 to 1:1 v/v); UV detection at 340 nm | Cytotoxicity against NSCLC-N6 carcinoma cell line | [129] |
| Deep water sponge *Microscleroderma herdsmani* | Microsclerodermins J (133) and K (134) | 1-Ile, 1-Thr | Marfey’s method (FDAA) combined with HPLC Cosmosol C₁₈ column, 5 µm (4.6 × 150 mm); Flow rate: 1.0 mL/min; UV detection at 340 nm | Activity against opportunistic pathogenic fungi | [130] |
| The Caribbean Sponge *Prosuberites lauglini* | Euryjanicins E–G (135–137) | 1-Pro, 1-Ile, 1-Phe | Marfey’s method (FDAA) combined with HPLC Cosmosol C₁₈ column, 5 µm (4.6 × 150 mm); Flow rate: 1.0 mL/min; UV detection at 340 nm | No significant activity cytotoxicity against the National Cancer Institute 60 tumor cell line panel | [131] |
| Marine sponge *S. carteri* | Chujamide A (138) | 1-Pro, 1-Tyr, 1-Cys, 1-Leu, 1-Phe, 1-Ile (S) | Marfey’s method (FDAA) combined with HPLC ESI-LC/MS YMC ODS-A column, 5 µm (4.6 × 250 mm); MP: H₂O:ACN (80:20 to 30:70 v/v); Flow rate: 0.7 mL/min; UV detection at 360 nm | Weak cytotoxicity against A549 and K562 cell lines | [132] |
| Marine Sponge *Cribrochalina olemda* | Kapakahines A–D (139–142) | 1-Val, 1-Ile, 1-Leu, 1-Trp, 1-Phe, 1-Ala, 1-Pro, 1-Try | Marfey’s method (FDAA) combined with HPLC Cosmosol C₁₈-MS column, 5 µm (4.6 × 250 mm); MP: 37.5% ACN in 0.05% TFA or 20% or 38% ACN in 50 mM NH₄OAc | 139–141: Cytotoxicity against P388 cell line | [133] |
| Marine sponge *Theonella sp.* | Koshikamide B (143) | δ-Phe, 1-Thr, N-Me-1-Val, N-Me-1-Asn, N-Me-1-Leu | Marfey’s method (FDAA) combined with HPLC ODS HPLC (10 × 250 mm); MP: ACN: H₂O: TFA (25:75:0.05 to 55:45:0.05 v/v/v); Flow rate: 1.0 mL/min; UV detection at 340 nm | Cytotoxicity against P388 and HCT-116 tumor cell lines | [134] |
| Solomon Lithistid sponge *Theonella swinhoei* | Perthamides C (144) and D (145) | 1-Asp, 1-ThrOMe, (2R,3S)-βOHAsp, 1-Phe | Marfey’s method (FDAA) combined with HPLC Cosmosol C₁₈-Ms column (1.8 × 25 mm); MP: 10–50% ac ACN with 5% HCOOH and 0.05% TFA; Flow rate: 0.15 mL/min | Anti-inflammatory activity | [135] |
| Polar extracts of the sponge *Theonella swinhoei* | Perthamides E (146) and F (147) | 1-46: 1-ThrOMe, 1-47: 1-Phe | Marfey’s method (FDAA) combined with HPLC Cosmosol C₁₈ column, (1.8 × 25 mm); MP: 10–50% ac ACN with 5% HCOOH and 0.05% TFA; Flow rate: 0.15 mL/min | 147: IL-8 release inhibition | [136] |
| Jamaican sponge *Stylissa caribica* | Stylisins 1 (148) and 2 (149) | 1-Pro, 1-Tyr, 1-Ile | Marfey’s method (FDAA) combined with HPLC Cosmosol C₁₈, MS (4.6 × 250 mm); MP: H₂O:TFA (100:0.1) to ACN:H₂O: TFA (50:50:0.1 v/v/v); Flow rate: 1.0 mL/min; UV detection at 340 nm | No antimicrobial, antimalarial, anticancer, anti-HIV-1, anti-Mtb and anti-inflammatory activities | [137] |
| Marine sponge *Stylissa carteri* | Carteritins A (150) and B (151) | 150: 1-Pro, 1-Phe, 1-Ile, 1-Pro (trans), 1-Pro (cis); 1-Glu, 1-Tyr; 151: 1-Pro (trans), 1-Leu, 1-Tyr, 1-Pro(cis) | Marfey’s method (FDAA) combined with HPLC Cosmosol C₁₈, MS (4.6 × 250 mm); MP: H₂O:TFA (100:0.1) to ACN:H₂O: TFA (50:50:0.1 v/v/v); Flow rate: 1.0 mL/min; UV detection at 340 nm | 150: Cytotoxicity against HeLa, HCT116, and RAW264 cells | [139] |
| Peptide          | Source                     | aa Composition       | Chromatographic Conditions                                                                 | Biological Activities                                                                 | Ref. |
|------------------|----------------------------|----------------------|-----------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|------|
| Stylissatins B–D (152–154) | Marine sponge *Stylissa massa* | 1-Pro, 1-Phe, 1-Leu   | Marfey’s method (FDAA) combined with HPLC                                                   | 152: Inhibitory effects against a panel of human tumor cell lines including HCT-116, HepG2, BGC-823, NCI-H1650, A2780, and MCF7 | [138]|
|                  |                            | 152: t-His            | Thermo BDS Hypersil C18 column, 5 µm (4.6 × 150 mm); MP: 30–70% MeOH:H2O (H3PO4)             |                                                                                       |      |
|                  |                            | 153–154: 1-Asp, 1-Val | Flow rate: 1.0 mL/min; UV detection at 340 nm                                               |                                                                                       |      |
| Callyaerin G (155) | Indonesian sponge *Callyspongia aerizusa* | 1-Pro, 1-Leu, 1-Phe, 1-FGly | Marfey’s method (FDAA) combined with HPLC/MS; Conditions not described                      | Cytotoxicity against L5178Y, Hela, and PC12                                             | [140]|
| Reniochalistatins A–E (156–160) | Marine sponge *Reniochalina stalagmitis* | 1-Pro, 1-Phe, 1-Val, 1-Leu, 1-Ile, 1-Tyr | Ligand Exchange Type CSP; MCI GELCRS 10 W (4.6 × 50 mm); MP: 2 mM CuSO₄·H₂O solution Flow rate: 1.0 mL/min; UV detection at 254 nm | 160: Cytotoxicity against RPMI-8226, MGC-803, HL-60, HepG2, and HeLa | [141]|
|                  |                            | 156: 1-Asn             | Marfey’s method (FDAA) combined with HPLC YMC-Park Pro C18, 5 µm (4.6 × 250 mm) MP: 2 mM CuSO₄·H₂O solution Flow rate: 1.0 mL/min; UV detection at 254 nm |                                                                                       |      |
|                  |                            | 160: 1-Trp             |                                                                                               |                                                                                       |      |
| Phakellistatins 15–18 (161–164) | South china sea sponge *Phakellia fusca* | 1-Pro 161: 1-Trp, 1-Ile, 1-Leu, 1-Thr; 162: 1-Phe, 1-Asp, 1-Ser, 1-Arg, 1-Ala, 1-Val, 1-Thr, 1-Tyr; 163: 1-Trp, 1-Val, 1-Ile, 1-Val, 1-Ile, 1-Tyr, 1-Tyr, 1-Ile, 1-Phe | Ligand-exchange type CSP; Chirex 3126 (d)-penicillamine column MP:aq 2 mM CuSO₄·MeOH (85:15 to 70:30 v/v) or aq 1 mM/0.5 mM CuSO₄·H₂O; Flow rate: 0.5 or 1.0 mL/min | 161: Cytotoxicity against P388 cancer cell line 162: Cytotoxicity against P388 and BEL-7402 cancer cell lines | [142]|

aa—Amino acid; FDAA—1-Fluoro-2,4-dinitrophenyl-5-L-alanine amide; LC—Liquid Chromatography; MS—Mass spectrometry; HPLC—High Performance Liquid Chromatography; MP—Mobile Phase; TEAP—Triethylammonium phosphate; ACN—Acetonitrile; TFA—Trifluoracetic acid; MeOH—Methanol; TEA—Triethylamine; IPA—Isopropyl alcohol; NaOAc—Sodium acetate; NH₄OAc—Ammonium acetate.
4.2. Cyclic Depsipeptides

A number of cyclic depsipeptides (Figures 12 and 13), have been reported from marine sponges and Marfey’s method using FDAA as the derivatization reagent was the most used for the determination of absolute configuration of the amino acid residues. Table 7 gives some examples of the cyclic depsipeptides, isolated from marine sponges, whose stereochemistry of their amino acid residues was determined by Marfey’s method. By application of this method, callipeltins B (165) and C (166), isolated from the marine lithistida sponge Callipelta sp., were found to have in their structure L-Ala, N-Me-L-Ala, L-Leu, L-Thr and D-Arg [143]. For halipeptins A (167) and B (168), isolated from the marine sponge Haliclona sp., the referred method was only able to determine the configuration for L-Ala [144]. Marfey’s method was successfully used to determine the absolute configuration of the amino acid constituents of several marine sponge cyclic peptides including phoriospongin A (169) and B (170), isolated from the marine sponges Phoriospongia sp. and Callyspongia bilamellata [145], mirabamides A–D (171–174), isolated from the marine sponge Siliquariaspongia mirabilis [146], and neamphamides B–D (175–177), isolated from the marine sponge Naemphius huxleyi [147]. Furthermore, the stereochemistry determination of amino acid residues in pipercolidespins A (178) and B (179), isolated from the marine sponge Homophymia lamellose, confirmed the presence of several L and D amino acid residues, besides the (3S,4R) diMe-L-Glu and (2S,3S)-EtO-Asp present in both peptides [148]. Stellatolide A (180), a cyclic depsipeptide isolated from Ecionemia acervus, was found to have N-Me-D-Ser and D- allo-Thr, among other L-configured amino acids [149]. The amino acid constituents of the cyclic depsipeptides cyclolithistide A (181) and nagahamide A (182), both isolated from the sponge Theonella swinhoei, were all found to have the S or L-configuration, and the 3-amino-5-hydroxybenzoic acid (AHBA) residue in nagahamide A (182) was established to have 3S configuration [150,151].

Almost all the cyclic peptides isolated from marine sponges displayed a variety of biological activities. Thus, callipeltin C (166) [143], cyclolithistide A (181) [150], and mirabamides A–D (171–174) [146] exhibited growth inhibitory activity against Candida albicans. Moreover, mirabamides A–D (171–174) also exhibited potent anti-HIV activities towards several HIV strains [146] whereas neamphamides B–D (175–177) displayed cytotoxic activity against several human cancer cell lines, including A549, HeLa, LNCalP, PC3, HEK, and NFF, with IC50 values ranging from 88 to 370 nM [147].

A simultaneous use of Marfey’s method and chiral HPLC analysis for stereochemical analysis of the amino acids of this type of peptides have been reported (Table 7). For examples, the absolute configuration of the amino acids of theopapuamides B (183) and C (184) and celebesides A–C (185–187), isolated from an Indonesian sponge Siliqurias-pongia mirabilis, was successful assigned by HPLC-MS analysis of FDAA derivatives as well as via chiral HPLC analysis using a ligand exchange type CSP [152]. In the case of theopapuamide (188), isolated from a Papua New Guinea Lithistid Sponge Theonella swinhoei, Marfey’s method was used to confirm the presence of D- allo-Thr, whereas chiral HPLC using a ligand exchange type CSP, revealed the presence of N-Me-L-Leu, D-Asp, L-Leu and N-Me-L-Glu in its structure [153]. The absolute configuration of the amino acid residues of a new sulfated cyclic depsipeptide, mutremdamide A (189) and six new highly N-methylated peptides, koshikamides C–H (190–195), isolated from different deep-water specimens of Theonella swinhoei and Theonella cupola, was also established by using both approaches. However, two different columns (C12 and C18) were used in Marfey’s method. By using chiral HPLC, it was possible to identify the amino acid residue N-Me- allo-L-Ile in koshikamide H (195) [154]. These cyclic peptides showed interesting biological activities. While theopapuamide (188) was cytotoxic against CEM-TART and HCT cell lines (IC50 values of 0.5 and 0.9 µM, respectively) [153], koshikamides F (193) and H (195) were active against a CCR5-using viral envelope, with IC50 values of 2.3 and 5.5 µM [154].
Figure 12. Structure of cyclic depsipeptides 165–179, isolated from marine sponges.
Figure 13. Structure of cyclic depsipeptides 180–195, isolated from marine sponges.
| Peptide Type       | Peptide Source                  | aa Composition                              | Chromatographic conditions                                                                 | Biological activities                                                                 | Ref.       |
|-------------------|---------------------------------|---------------------------------------------|------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|------------|
| Cyclic depsipeptides from marine sponges.                                                                                                                                           |                                                                                         |                                                                                         |            |
| Callipeltins B (165) and C (166) | Callipella sp. | L-Ala, D-Arg, L-Thr, N-Me-L-Ala, L-Leu | Marfey's method (FDAA) combined with HPLC; Column not described; MP: TEAP (50 mL, pH 3.0):ACN 90–50% TEAP; Flow rate: 2.0 mL/min; UV detection at 340 nm | Cytotoxicity; Growth inhibitory activity against Candida albicans | [143] |
| Halipeptins A (167) and B (168) | Haliclona species | L-Ala | Marfey's method (FDAA) combined with HPLC; Vydac C18 column; MP: H2O (1.0% TFA):ACN (0:1 to 1:1 v/v); UV detection at 340 nm | Anti-inflammatory activity | [144] |
| Phoriospongin A (169) and B (170) | Phoriospongia sp. and Callyspongia bilamellata | D-Asp, D-allo-Thr, D-Ala, L-Phe, D-Leu, D-nor-Val, N-Me-D-nor-Val, 170: N-Me-L-Leu | Marfey's method (FDAA) combined with HPLC; Phenomenex Jupiter Proteo C18 column, 4 μm (4.6 × 150 mm); MP: 25–70% ACN; Flow rate: 0.5 mL/min | Nematocidal activity against the parasite Haemonchus contortus | [145] |
| Mirabamides A–D (171–174) | Siliquarias-pongia mirabilis | N-Me-L-Thr, L-Thr, L-Ala, D-3-O-MeAla, (2R,3R)-3-Oh-Leu, (35,4R)-diMe-I-Glu, (25,3R)-diaminobutanoic acid; 174: L-HP | Marfey's method (FDAA) combined with HPLC; Phenomenex Luna Column C8 column, 5 μm (4.6 × 250 mm); Flow rate: 1.0 mL/min; UV detection at 340 nm | Growth inhibition of human cell lines: A549, HeLa, LNCAp; PC3, and NFF | [147] |
| Neamphamides B (175), C (176) and D (177) | Neanphius huxleyi | D-Arg, L-Asn, L-Hpr, L-Leu, D-allo-Thr, 175 and 177: N-Me-L-Gln, 176: N-Me-L-Glu | Marfey's method (FDAA) combined with HPLC; Phenomenex Luna Column C8, 3 μm (2.0 × 150 mm); MP: H2O:ACN:HCOOH (100:0:0.1 to 0:100:0.1 v/v/v); UV detection at 340 nm | Cytotoxicity against human tumor cell lines (A-549, HT-29, and MDA-MB-231) | [148] |
| Piceolidepsins A (178) and B (179) | Homoplymnia lamellosa | D-Asp, L-Leu, L-Lys, D-allo-Thr, (35,4R) diMe-I-Glu, (25,3S) EtO-Asp, N-Me-L-Glu, L-Pip | Marfey's method (FDAA) combined with HPLC; Phenomenex Jupiter Proteo C18 column, 4 μm (4.6 × 150 mm); MP: 20–50% ACN (0.04% TFA); Flow rate: 0.8 mL/min | In vitro antiproliferative activity | [149] |
| Stellolide A (180) | Ecionemia acervus | L-Ala, L-Asp, L-Leu, N-Me-1-Glu, N-Me-D-Ser, D-allo-Thr | Marfey's method (FDAA) combined with HPLC; Hewlett-Packard Hypersil BDS-C8, 4 μm (4.0 × 100 mm); MP: H2O (0.1% TFA):ACN (90:10 to 50:50 v/v); Flow rate: 1.0 mL/min | In vitro antiproliferative activity | [149] |
| Cyclolithistide A (181) | Theonella swinhoei | nor-S-Val, S-Phe, S-Gln, N-Me-S-Leu, S-Ala, S-Allo-S-Thr | Marfey's method (FDAA) combined with HPLC; ODS (4.6 × 250 mm); MP: 100% H2O; Flow rate: 2.0 mL/min; UV detection at 210 nm | Antibacterial activity against Candida albicans (ATCC 24433) | [150] |
| Nagahamide A (182) | Theonella swinhoei | L-Val, 35-3AHBA | Marfey's method (FDAA) combined with HPLC; ODS column (4.6 × 250 mm); Conditions not described | Antibacterial activity | [151] |
| Theopapuamides B (183) and C (184), Celebesides A–C (185–187) | Siliquarias-pongia mirabilis | 185: L-βMeAsn | Marfey's method (FDAA) combined with HPLC; Conditions not described; MP: 25–70% ACN with 0.01 M TFA; Flow rate: 0.5 mL/min | 185: Inhibits HIV-1 Entry; 183–185: Cytotoxic to human colon tumor cell line (HCT-116); 183 and 185: Antibacterial activity against Candida albicans | [152] |
Table 7. Cont.

| Peptide                   | Source                           | aa Composition                                 | Chromatographic conditions                                                                 | Biological activities                  | Ref. |
|---------------------------|----------------------------------|------------------------------------------------|-------------------------------------------------------------------------------------------|----------------------------------------|------|
| Theopapuamide (188)       | Lithistid sponge Theonella swinhoei | N-Me-t-Leu, d-Asp, t-Leu, N-Me-t-Glu          | Ligand Exchange Type CSP Chirex Phase 3126 (D), 5 µm (4.6 × 250 mm); MP: IPA: 2 mM CuSO₄ (5:95 v/v) | Cytotoxicity against CEM-TART and HCT-cell lines | [153]|
|                          |                                  | d-allo-Thr                                    | Marfey’s method (FDAA) combined with HPLCPhenomenex C₁₈, 5 µm (4.6 × 250 mm); MP: 10-50% ACN in H₂O (0.05% TFA); Flow rate: 1.0 mL/min; UV detection at 340 nm |                                         |      |
| Mutremdamide A (189) and  | Koshikamides C–H (190–195)        | 189: N-Me-t-Val; 190: N-Me-t-Val, N-Me-t-Asn,  | Marfey’s method (FDAA) combined with HPLC LC-MS analysis using a C₁₂ column, 4 µm (4.6 × 250 mm); MP: ACN with 0.01% TFA; Flow rate: 0.5 mL/min |                                         | [154]|
| Koshikamides C–H (190–195) |                                  | N-Me-t-Leu, t-Pro, N-Me-allo-t-Ile, d-Phe      |                                                                                           |                                         |      |
|                          |                                  | 191 and 192: N-Me-allo-t-Ile, N-Me-t-Val; 192–194: N-Me-allo-t-Ile, t-Ala 1, d-Ala2, t-Asn | Marfey’s method (FDAA) combined with HPLC LC-MS, C₁₄ column, 4 µm (4.6 × 250 mm); MP: 20 mM buffer (AF):ACN (5:1 to 3:7 v/v); Flow rate: 0.5 mL/min |                                         |      |
|                          |                                  | 195: N-Me-allo-t-Ile                         | Chiral HPLC (column not described); MP: 1 mM CuSO₄:ACN (95:5 v/v); Flow rate: 0.5 mL/min; UV detection at 254 nm |                                         |      |

aa—Amino acid; FDAA—1-Fluoro-2,4-dinitrophenyl-5-L-alanine amide; LC—Liquid Chromatography; MS—Mass spectrometry; HPLC—High Performance Liquid Chromatography; MP—Mobile Phase; TEAP—Triethylammonium phosphate; ACN—Acetonitrile; TFA—Trifluoracetic acid; MeOH—Methanol; TEA—Triethylamine; NaOAc—Sodium acetate; NH₄OAc—Ammonium acetate.
4.3. Lipopeptides

The absolute configuration of the amino acids of new N-sulfoureylated lipopeptides sulfolipodiscamides A–C (196–198), isolated from the n-butanol fraction of the marine sponge Discoderma kiiensis (Figure 14), was determined by Marfey’s method to be L-Uda and L-Gly (Table 8). Compound 196 was found to be cytotoxic against the murine leukemia cell line P388 with an IC₅₀ value of 15 µM [155].

![Figure 14. Structure of cyclic lipopeptides 196–198, isolated from marine sponges.](image)

| Peptide | Source | aa Composition | Chromatographic Conditions | Biological Activity | Ref. |
|---------|--------|----------------|----------------------------|---------------------|------|
| Sulfolipodiscamides A–C (196–198) | Sponge Discoderma kiiensis | L-Uda, L-Gly | Marfey’s method (FDAA) combined with HPLC Cosmosil C₁₈-MSII column (4.6 × 250 mm); MP: 100 mM NaClO₄ in 60% ACN; Flow rate: 0.8 mL/min | 196: Cytotoxicity against P388 cell line | [155] |

| Peptide | Source | aa Composition | Chromatographic Conditions | Biological Activity | Ref. |
|---------|--------|----------------|----------------------------|---------------------|------|
| Sulfolipodiscamide A (196) | | | | 196: Cytotoxicity against P388 cell line | [155] |
| Sulfolipodiscamide B (197) | | | | 197: Cytotoxicity against P388 cell line | [156] |
| Sulfolipodiscamide C (198) | | | | 198: Cytotoxicity against P388 cell line | [157] |

aa—Amino acid; FDAA—1-Fluoro-2,4-dinitrophenyl-5-L-alanine amide; HPLC—High Performance Liquid Chromatography; MP—Mobile Phase; ACN—Acetonitrile.

5. Peptides from Other Marine Invertebrates and Algae

A number of diverse bioactive peptides such as cyclic peptides, cyclic depsipeptides and linear peptides have been isolated from other marine invertebrates including ascidians, commonly called tunicates, mollusks, among others [17]. Moreover, the potential applications of many bioactive compounds from marine algae, mainly red and brown as well as some green algae, were reported [156].

5.1. Cyclic Peptides

To the best of our knowledge, only five works described the analysis of the stereochemistry of the cyclic peptides from marine invertebrates and algae (Figure 15). In all reported works, Marfey’s method was employed (Table 9). Among these, the determination of the absolute configuration of the cyclic hexapeptides didmolamides A (199) and B (200) and mollamides B (201) and C (202), isolated from the marine ascidian Didemnum molle from Madagascar and Indonesia, respectively, was performed by Marfey’s method using FDAA as derivatization reagent [157,158]. These compounds showed interesting biological activities, particularly, cytotoxicity against A549, HT29 MEL28 tumor cell lines, with IC₅₀ values ranging from 10 to 20 µg/mL for didmolamides A (199) and B (200) [157] while 201 showed antimalarial activity against Plasmodium falciprum, clones D6 and W2, with IC₅₀ values of 2.0 and 21 µg/mL, respectively [158].

Furthermore, the stereochemical determination of antatollamides A (203) and B (204), isolated from the marine ascidian Didemnum molle, sanguinamide A (205), isolated from the sea slug

Table 8. Lipopeptides from marine sponge.
Hexabranchus sanguineus, and gamakamide E (206), isolated from the oysters Crassostrea gigas, was carried out by Marfey’s method using FDLA as a derivatization reagent. The analysis demonstrated that most of their amino acids have the L-configuration, with the exception of D-Ala and D-Lys in antatollamides A (203) and B (204), and gamakamide E (206), respectively [159–161].

![Peptide structures](image)

**Figure 15.** Structure of cyclic peptides 199–206, isolated from marine invertebrates and marine algae.

| Peptide          | Source             | aa Composition          | Chromatographic Conditions                          | Biological Activities                       | Ref. |
|------------------|--------------------|-------------------------|-----------------------------------------------------|---------------------------------------------|------|
| Didomolamides     | Ascidian Didemnum molle | l-Thr, l-Ala, l-Phe     | Marfey’s method (FDAA) combined with HPLC; MP: 50 mM (TEAP) buffer pH 3; ACN (9:1 to 1:1 v/v); Flow rate: 1.0 mL/min; UV detection at 340 nm | Cytotoxicity against AS49, HT29 MEL28 tumor cell lines | [157] |
| A (199) and B (200) |                    | 200: l-Tzi             |                                                     |                                             |      |
| Mollamides B (201) and C (202) | Tunicate Didemnum molle | l-Thr, l-Ile, l-Pro | Marfey’s method (FDAA) combined with HPLC; MP: 50 mM (TEAP) buffer pH 3; ACN (9:1 to 1:1 v/v) or 40 mM NH4OAc, 70% ACN, and 30% MeOH (98:2 to 66:34 v/v); Flow rate: 1.0 or 0.8 mL/min; UV detection at 340 nm | 201: Activity against HIV, Plasmodium falciparum, Leishmania donovani, and cytotoxicity against H460, MCF7, SF-268 cell lines | [158] |
| (201) and C (202) |                    | 201: l-Val, l-Phe      |                                                     |                                             |      |
| Antatollamides     | Ascidian Didemnum molle | l-Ile, l-Phe, l-Val, l-Pro, D-Ala | Marfey’s method (FDLA) combined with HPLC/MS; Hypersil Gold C18 column, 1.9 µm (2.1 × 50 mm); MP: H2O 0.1%; HCOOH:ACN (85:15 to 55:45 v/v); Flow rate: 0.5 mL/min | 203: Weak cytotoxicity against a chronic lymphocytic leukemia cell line | [159] |
| A (203) and B (204) |                    | 204: l-Pro             |                                                     |                                             |      |

**Table 9.** Cyclic peptides from marine invertebrates and algae.
5.2. Cyclic Depsipeptides

To the best of our knowledge, only four works reported the determination of the stereochemistry of amino acid constituents of the cyclic depsipeptides from marine invertebrates and algae (Figure 16). Among these, three employed only Marfey’s method, specifically for peptides 207–216. However, for peptide 217, Marfey’s method was not efficient and, as a consequence, a ligand exchange type CSP was also used for complete determination of the configuration of its amino acids (Table 10).

The determination of the absolute configuration of the amino acids in kahalalides A–F (207–212), isolated from the marine mollusk *Elysia rufescens*, was performed by using FDLA as the derivatization reagent and the presence of diverse residues of L- and D-Val in these peptides was confirmed [162]. Using FDAA as the Marfey derivatization reagent, the absolute configuration of tamandarins A (213) and B (214), isolated from an unidentified Brazilian marine ascidian of the family Didemnidae [163], and kahalalides P (215) and Q (216), isolated from green algae *Bryopsis* species [164] were elucidated. In the case of kahalalide O (217), the absolute configuration of its amino acid constituents was determined by Marfey’s method and chiral HPLC analysis, using a ligand exchange type CSP [165]. Tamandarin A (213) was found to display cytotoxicity against BX-PC3, DU-145, and UMSCC10b human cancer cell lines, with IC50 values of 1.79, 1.36, and 0.99 µg/mL, respectively [163].

![Figure 16. Cont.](image_url)
Figure 16. Structure of cyclic depsipeptides 207–217, isolated from marine invertebrates and marine algae.

Table 10. Cyclic depsipeptides from marine invertebrates and algae.

| Peptide          | Source                          | aa Composition | Chromatographic Conditions | Biological Activities                                                                 | Ref. |
|------------------|---------------------------------|----------------|----------------------------|----------------------------------------------------------------------------------------|------|
| Kahalalides A–F | Mollusk *Elysia rufescens*      | D-Val-5, 208:  | Marfey’s method (FDLA) combined with HPLC | 207: Antimalarial activity                                                             | [162]|
| (207–212)        |                                 | D-Val-1, 2-Val-2, d-allo-Thr-1, 209: | COSMOSIL 5C$_{18}$-AR; MP: ACN:H$_2$O:TFA (42:6:0.05 v/v/v) or ACN:H$_2$O:50 mM NH$_4$OAc (20:80:0.01 v/v/v) | 211: Activity against RSV II virus                                                  |      |
|                  |                                 | L-Val-3, D-Val-4, l-Thr-2, 210: | |                                                                                       |      |
|                  |                                 | D-Val-2, d-allo-Thr-1 | |                                                                                       |      |
| Tamandarin A     | Ascidian of the Didemniidae     | 213: S-Lac, l-Pro, N-Me-O-Leu, l-Thr, (3.5R,5S)-1st | Marfey’s method (FDAA) combined with HPLC | 213: Cytotoxicity against various human cancer cell lines                             | [163]|
| (213) and B (214)|                                | 214: S-Lac, l-Pro, N-Me-O-Leu, l-Thr (3.5R)-Nst | Hewlett-Packard ODS Hypersil 5 μm (4.6 × 200 mm); MP: 0.1% TFA in H$_2$O or MeOH; Flow rate: 1.0 mL/min; UV detection at 340 nm |                                                                                       |      |
| Kahalalides P    | Green alga *Bryopsis sp.*       | l-Asp, l-Val, | Marfey’s method (FDAA) combined with HPLC | No antimicrobial and no hemolytic activities                                             | [164]|
| (215) and Q (216)|                                | d-Leu, l-Ser, l-Hyp, l-Pro, l-Lys | COSMOSIL 5C$_{18}$-AR-II (4.6 × 250 mm); MP: 0.1 M NH$_4$OAc pH 3 or 90% aq ACN |                                                                                       |      |
| Kahalalide O     | Mollusk *Elysia ornata* and green | l-Ile, l-Thr, | Ligand Exchange Type CSP Chirex | No growth inhibition of P388, A549, HT29 and MEL28 cancer cell lines                  | [165]|
| (217)            | alga *Bryopsis sp.*             | d-allo-Thr, d-Tyr, l-Val | (D) Penicillamine Column (4.6 × 250 mm); MP: 1.9 mM CuSO$_4$ in ACN:H$_2$O:50 mM or 2.0 mM CuSO$_4$ in H$_2$O; UV detection at 254 nm |                                                                                       |      |
|                  |                                 | d-Trp | Marfey’s method (FDAA) combined with HPLC |                                                                                       |      |
|                  |                                 | | COSMOSIL 5C$_{18}$-AR; MP: ACN:H$_2$O:TFA (37.5:62.5:0.05 v/v/v); Flow rate: 1.0 mL/min |                                                                                       |      |

aa—Amino acid; FDAA—1-Fluoro-2,4-dinitrophenyl-5-L-alanine amide; HPLC—High Performance Liquid Chromatography; MP—Mobile Phase; ACN—Acetonitrile; FDLA—1-fluoro-2,4-dinitrophenyl-5-D, L-leucine amide; TFA—Trifluoroacetic acid; MeOH—Methanol; TEA—Triethylamine; NH$_4$OAc—Ammonium acetate.
5.3. Lipopeptides

For lipopeptides isolated from other marine invertebrates and algae, there are only two works which reported the use of a chiral HPLC for the stereochemistry determination of the amino acid residues (Table 11) of the peptides 218–221 (Figure 17).

![Figure 17. Structure of lipopeptides 218–221, isolated from marine invertebrates and marine algae.](image)

**Table 11.** Lipopeptides from marine invertebrates and algae.

| Peptide          | Source       | aa Composition | Chromatographic Conditions                                      | Biological Activities                  | Ref. |
|------------------|--------------|----------------|---------------------------------------------------------------|----------------------------------------|------|
| Eudistomides A   | Ascidian     | l-Pro, l-Ala,  | Ligand Exchange Type CSP                                        | No activity reported                   | [166]|
| (218) and B (219)| *Eudistoma*  | l-Leu          | Phenomenex Chirex 3126 (D)                                      |                                        |      |
|                  | sp.          |                | (4.6 × 250 mm); MP: 2 mM CuSO₄, 2 mM CuSO₄:ACN (95:5 or 85:15 v/v); Flow rate: 1.0 mL/min; UV detection at 254 nm |                                        |      |
| Eudistomide B    | *Eudistoma*  | l-Cyp          |                                                                 |                                        |      |
| (219)            | sp.          |                |                                                              |                                        |      |
| Memamamides A    | Green algae  | l-Leu, l-Pro,  | Ligand Exchange Type CSP                                        | No growth inhibitory activity against HeLa and HL60 cell lines | [167]|
| (220) and B (221)| *Derbesia*   | d-Ala, l-Thr,  | Phenomenex Chirex 3126 (D)                                      |                                        |      |
|                  | marina       | l-Val, d-Phe,  | (4.6 × 50 mm); MP: 2.0 mM CuSO₄; Flow rate: 1.0 mL/min; UV detection at 254 nm |                                        |      |
|                  |              | d-Ser          |                                                              |                                        |      |

aa—Amino acid; MP—Mobile Phase; ACN—Acetonitrile.

Chiral HPLC analysis by using a ligand exchange type CSP (Phenomenex Chirex Phase 3126) was used to determine the configuration of the amino acid residues in eudistomides A (218) and B (219), isolated from an ascidian *Eudistoma* sp. It was possible to verify the presence of l-Pro, l-Ala and l-Leu in both compounds as well as the presence of l-Cyp in eudistomide A (218) [166]. Similarly, a chiral HPLC analysis using a ligand exchange type CSP (CHIRALPAK (MA+)) was able to confirm the presence of four L-amino acid residues and D-Ala, D-Phe, and D-Ser in mebamamides A (220) and B (221), isolated from the green alga *Derbesia marina* [167].
6. Case-Study: Chiral HPLC in the Analysis of the Stereochemistry of Cyclopeptides Isolated from Marine Sponge-Associated Fungi

Recently, the determination of the stereochemistry of the amino acid residues of three bioactive marine natural products, by chiral HPLC analysis of their acidic hydrolysates, using appropriate D- and l-amino acid standards was achieved in our group [111,112]. The marine sponge-associated fungus *Aspergillus similanensis* KUFA 0013 was the source of the cyclohexapeptide similanamide (110) (Figure 8), while cyclotetrapeptides sartoryglabramides A (111) and B (112) (Figure 8) were isolated from the marine sponge-associated fungus *Neosartorya glabra* KUFA 0702. The enantioseparations of the amino acids were successfully performed on Chirobiotic T column under reverse phase elution conditions. Actually, the teicoplanin selector of this column has several characteristic features that make it suitable for amino acid analysis [168,169]. Figure 18 shows selected chromatograms of the enantioseparation of standard amino acids.

![Figure 18. Chromatograms of enantiomeric mixture of DL-Ala (A), DL-pipecolic acid (B), and DL-Val (C). Column, Chirobiotic T; Mobile phase, MeOH:H₂O:acetic acid (70:30:0.02 v/v/v); Flow rate, 1.0 mL/min; UV detection, 210 nm.](image)

The elution order of all the standard enantiomers of amino acids was confirmed by injecting solutions of the racemic or enantiomeric mixtures of amino acids and then each enantiomer separately. As an example, Figure 19 shows the chromatograms obtained during the method development for the determination of the elution order of Ala. As expected, the D-enantiomer was always more strongly retained than the corresponding L-enantiomer on Chirobiotic T column [168]. Mixed HPLC analyses of the acidic hydrolysates with appropriate standard amino acids (co-injection) (Table 12), confirmed the stereochemistry of the amino acids of the three cyclopeptides [111,112]. Chiral HPLC technique demonstrated to be decisive leading to the unambiguous elucidation of the amino acid constituents of the three marine natural products.

Additionally, the in vitro growth inhibitory activity against MCF-7, breast adenocarcinoma, NCI-H460, non-small cell lung cancer and A373, melanoma, cell lines, as well as antibacterial activity against reference strains and the environmental multidrug-resistant isolates (MRS and VRE) were evaluated for cyclopeptide 110. Only weak activity against the three cancer cell lines was observed [111]. Moreover, cyclopeptides 111 and 112 were tested for their antifungal activity against filamentous (*Aspergillus fumigatus* ATCC 46645), dermatophyte (*Trichophyton rubrum* ATCC FFS) and yeast (*Candida albicans* ATCC 10231), as well as for their antibacterial activity against Gram-positive (*Escherichia coli* ATCC 25922) and Gram-negative (*Staphylococcus aureus* ATCC 25923) bacteria. None of them exhibited antibacterial or antifungal activities [112].
In summary, concerning all the reported studies surveyed in this review, which are related to the determination of the absolute configuration of the marine peptides, their distribution according to the methods used, is shown in Figure 20. It is possible to conclude that Marfey’s method is the most employed accounting for 52% of the reported studies, while only 21% of the studies described the use of chiral HPLC analysis. Moreover, 27% of the studies included the application of both methods. In fact, in some cases, the complementarity of both methods demonstrated to be crucial for the stereochemical analysis of all the amino acid residues.

7. Conclusions

Figure 19. Chromatograms of enantiomeric mixture of D/L-Ala (a), L-Ala (b), and D-Ala (c). Column, Chirobiotic T; Mobile phase, MeOH:H₂O:acetic acid (70:30:0.02 v/v/v); Flow rate, 1.0 mL/min; UV detection, 210 nm.

Table 12. Chiral HPLC analysis of the acidic hydrolysates of 110, 111 and 112 by co-injection with amino acids standards.

| Retention Time (min) | Retention Time (min) |
|----------------------|----------------------|
| D-Trp (A) 4.51       | Acidic hydrolysate of 110 (B) 6.59, 7.20, 8.09, 8.83, 9.67, 10.57, 14.69 |
| L-Trp (A) 6.60       | Acidic hydrolysate of 110 + DL-Val (co-injection) (B) 6.61, 7.31, 8.30, 8.10, 8.84, 9.70, 10.50, 14.95 |
| L-Val (B) 8.32       | Acidic hydrolysate of 110 + DL-Ala (co-injection) (B) 6.59, 7.19, 8.04, 8.81, 9.37, 9.70, 10.50, 14.90 |
| L-Ala (B) 7.66       | Acidic hydrolysate of 110 + DL-Leu (co-injection) (B) 6.60, 6.76, 7.26, 8.04, 8.83, 9.70, 10.54, 15.02 |

Table continued...

Column, Chirobiotic T; Mobile phase, MeOH:H₂O:acetic acid (80:20 v/v/v) (A) or MeOH:H₂O:acetic acid (70:30:0.02 v/v/v) (B); Flow rate, 1.0 mL/min (A) or 0.5 mL/min (B); UV detection, 210 nm.
Figure 20. Distribution of the reported studies concerning the determination of the stereochemistry of marine peptides according to the methods used.

Figure 21 compares the reported studies before and after 2007. Interestingly, it is possible to observe that in the last ten years, Marfey’s method is still the most used for determination of the absolute configuration of amino acid residues in marine peptides. However, it is important to point out a notable increase of the number of studies related to a chiral HPLC analysis, either as the only method or in a combination with Marfey’s method.

Figure 21. Distribution of the studies concerning the determination of the stereochemistry of marine peptides according to the method used before (A) and after 2007 (B).

In our opinion, the current trend is to use chiral HPLC for stereochemical analysis due to many advantages of this method. For examples, there is no need for prior derivatization, it requires much less sample manipulation and the results are more rapid to obtain. In contrast, Marfey’s method involves time-consuming and labor-intensive procedure.

We believe that the reasons that can justify the actual low number of studies using chiral HPLC is due to the price of the commercially available CSPs and the fact that there is no universal CSP, i.e., one CSP can only separate a limited number of chiral compounds and, in many cases, the choice of CSP may become a very difficult task.

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