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Synthesis of Cyclopeptides Analogues of Natural Products and Evaluation as Herbicides and Inhibitors of Cyanobacteria

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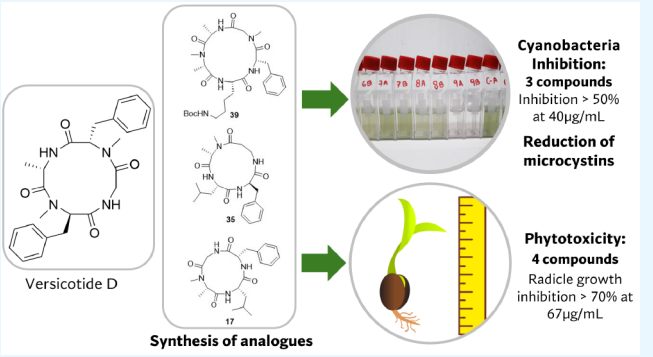
ABSTRACT: Natural products derived from plants or microorganisms have been considered as eco-friendly herbicides with application in crop protection. Several natural cyclopeptides have been reported as herbicides, while others have been identified as inhibitors of cyanobacteria. In this work, the syntheses of cyclotetrapeptides and cyclopentapeptides analogues of natural products were successfully performed by solid-phase peptide synthesis of their linear precursor and solution-phase macrolactamization. Four of the obtained peptides and cyclopeptides present phytotoxicity with more than 70% of radicle growth inhibition at 67 μg/mL. In addition, evaluation of 20 peptides and cyclopeptides, as inhibitors of cyanobacteria, rendered five active compounds that reduced the concentration of microcystins in the culture medium.



1. INTRODUCTION

Weeds produce the highest potential loss (34%) to crop production, compared with insect pests (losses of 18%) and pathogens (losses of 16%).[1] Chemical weed control using herbicides has been widely adopted to prevent their growth in a variety of crops.[2] Unfortunately, the high dependence on herbicides produces residues in crops, and has led to adverse effects on the environment along with ecological impacts such as contamination in the underground water and aboveground lakes and rivers. The above added to hydrological alterations and to the global warming promote cyanobacteria blooms which have increased globally over the past decade.[3] Cyanobacteria produce cyanotoxins, such as microcystins, that can cause adverse effects on the liver and digestive and nervous systems in animals and humans.[4] In addition, the taste and odorous compounds produced by cyanobacteria impair reservoirs for drinking water and recreational lakes.[5] Many reports showed that some of the world's largest and important water bodies, such as Lake Erie in USA, Lake Biwa in Japan, Lake Taihu in China, the Baltic Sea in Europe, Río de la Plata in South America, and Lake Victoria in Africa are currently threatened by cyanobacterial blooms.[6] In particular, during 2019 summer, one of the most important blooms in the Uruguayan coast comprised 500 km of the Río de la Plata coast and reached the Atlantic Ocean.[7]

Therefore, the need for new herbicides with higher eco-friendly profiles is urgent. Natural products derived from plants or microorganisms have emerged as an alternative for sustainable agriculture. They show insecticidal, fungicidal, herbicidal, nematicidal, or antiphytoviral activities.[8] In particular, naturally occurring and synthetic peptides and cyclopeptides with phytotoxic, herbicidal, or plantgrowth-regulating activities have been considered as eco-friendly herbicides with application in crop protection.[9] During the last years, the research and development of cyclopeptides as new drug candidates has increased as they present valuable properties compared with their linear precursors. Among other advantages, cyclopeptides present improved metabolic stability, selectivity to the receptors, and can adopt bioactive conformations.[10] Moreover, it was demonstrated that multiple backbone *N*-methylation of cyclic peptides remarkably improves their cell permeability.[11]

Examples of bioactive cyclopeptides with phytotoxic activity are destruxin B (**1**),[12] tentoxin (**2**),[13] and versicotide D (**3**)[14] (Figure 1). Tentoxin, a cyclic tetrapeptide produced by the fungus *Alternaria alternata* (previously called *A. tenuis*), causes seedling chlorosis. The natural cyclopeptide versicotide D isolated from *Aspergillus versicolor* LZD-14-1 shows low cytotoxicity on HepG2 cells,[15] high phytotoxicity, and the

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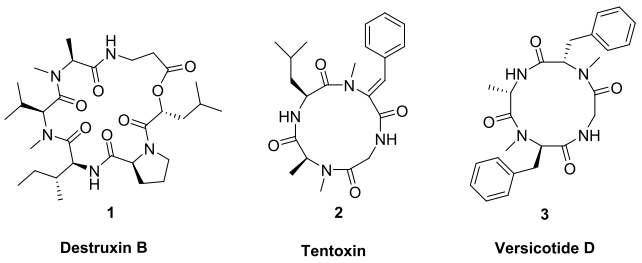

Figure 1. Cyclopeptide natural products with herbicidal activity: destruxin, tentoxin, and versicotide D.

ability to inhibit cyanobacteria population with a substantial depletion of the concentration of microcystins in the media.[16]

Previously, our group reported the phytotoxic evaluation of peptide precursors and cyclotetrapeptides analogues of tentoxin and versicotide D (Table 1).[17]

Table 1. Tetra and Cyclotetrapeptides Analogues of Natural Products Reported as Herbicides by Our Group

sequence	peptide precursor	cyclopeptide
Ala-Leu-D-Phe-Gly	**4**	**16**
N-MeAla-Leu-Phe-Gly	**5**	**17**
Ala-Leu-Phe-*N*-MeGly	**6**	**18**
Ala-Leu-*N*-Me-D-Phe-Gly	**7**	**19**
N-MeAla-Leu-D-Phe-Gly	**8**	**20**
Ala-Leu-D-Phe-*N*-MeGly	**9**	**21**
Ala-Leu-*N*-MePhe-*N*-MeGly	**10**	**22**
N-MeAla-Leu-Phe-*N*-MeGly	**11**	**23**
N-MeAla-Leu-*N*-MePhe-Gly	**12**	**24**
N-MeAla-Phe-*N*-MePhe-Gly	**13**	**25**
N-Me-D-Phe-Ala-Phe-*N*-MeGly	**14**	**26**
Phe-*N*-MeGly-Cys(Bn)-*N*-MeGly	**15**	**27**

In this work, we present the synthesis of new analogues, with a focus on the influence in the phytotoxic activity given by the change of Gly by *β*-Ala in the peptide sequence, the increase in ring size by the addition of Ala to obtain cyclopentapeptides, and the change of Leu by Lys. Also, the inhibition of cyanobacterial population growth and the concentration of microcystins in the culture medium of these synthesized compounds and of the previously reported peptides[17] are presented. The concentration of microcystins in the culture medium is relevant to estimate the possible cell breakage and toxin release.[18]

2. MATERIALS AND METHODS

For general experimental procedures, see the Supporting Information.

2.1. NH_2-Ala-Leu-D-Phe-*β*-Ala-OH (28). The trifluoroacetate salt of NH_2-Ala-Leu-D-Phe-*β*-Ala-OH was obtained following the general SPPS procedure. 369 mg (0.69 mmol, 95%) was obtained as a white solid. ^{1}H NMR (400 MHz, D_2O), *δ* (ppm): 7.41−7.35 (m, 2H), 7.34−7.25 (m, 3H), 4.65 (dd, *J* = 10.1, 5.8 Hz, 1H), 4.22 (dd, *J* = 8.5, 6.4 Hz, 1H), 4.05 (q, *J* = 7.05 Hz, 1H), 3.35−3.33 (m, 2H), 3.27 (dd, *J* = 14.0, 5.8 Hz, 1H), 2.90 (dd, *J* = 14.0, 10.2 Hz, 1H), 2.61−2.46 (m, 2H), 1.48 (d, *J* = 7.1 Hz, 3H), 1.39−1.29 (m, 1H), 1.27−1.08 (m, 2H), 0.78 (d, *J* = 6.5 Hz, 3H), 0.76 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (100 MHz, D_2O), *δ* (ppm): 176.0, 174.3, 172.8, 170.7, 136.4, 129.0, 128.8, 127.2, 54.8, 52.7, 48.7, 39.4, 36.9, 35.1, 33.3, 25.1, 23.9, 21.7, 21.1, 16.5. ESI-MS calc. for $C_{21}H_{33}N_4O_5$: 421.24 ([M + H]$^+$); found 421.35. The purity (92%) was determined by HPLC.

2.2. NH-MeAla-Leu-D-Phe-*β*-Ala-OH (29). The trifluoroacetate salt of NH-MeAla-Leu-D-Phe-*β*-Ala-OH was obtained following the general SPPS procedure. 370 mg (0.67 mmol, 96%) was obtained as a white solid. ^{1}H NMR (400 MHz, D_2O), *δ* (ppm): 7.40−7.23 (m, 5H), 4.64 (dd, *J* = 10.0, 5.7 Hz, 1H), 4.24 (dd, *J* = 8.8, 6.0 Hz, 1H), 3.91 (q, *J* = 7.0 Hz, 1H), 3.54−3.37 (m, 2H), 3.25 (dd, *J* = 14.0, 5.8 Hz, 1H), 2.90 (dd, *J* = 14.0, 10.1 Hz, 1H), 2.67 (s, 3H), 2.63−2.48 (m, 2H), 1.47 (d, *J* = 7.0 Hz, 3H), 1.40−1.27 (m, 1H), 1.25−1.12 (m, 2H), 0.78 (d, *J* = 6.3 Hz, 3H), 0.76 (d, *J* = 6.1 Hz, 3H). ^{13}C NMR (100 MHz, D_2O), *δ* (ppm): 175.9, 174.1, 172.8, 169.7, 136.4, 129.0, 128.8, 127.2, 56.9, 54.8, 52.6, 39.4, 37.0, 35.1, 33.2, 31.0, 24.0, 21.7, 21.0, 15.3. ESI-MS *m*/*z* calc. for $C_{22}H_{35}N_4O_5$: 435.26 ([M + H]$^+$); found 435.30. The purity (92%) was determined by HPLC.

2.3. NH_2-Ala-*N*-MeAla-Leu-Phe-Gly-OH (30). The trifluoroacetate salt of NH_2-Ala-*N*-MeAla-Leu-Phe-Gly-OH was obtained following the general SPPS procedure. 237 mg (0.39 mmol, 92%) was obtained as a white solid. ^{1}H NMR (400 MHz, D_2O), *δ* (ppm): 7.43−7.25 (m, 5H), 4.9−4.85 (m, 1H), 4.71 (dd, *J* = 9.0, 6.0 Hz, 1H), 4.49 (q, *J* = 7.0 Hz, 1H), 4.32−4.23 (m, 1H), 3.99−3.94 (m, 2H), 3.21 (dd, *J* = 13.9, 5.9 Hz, 1H), 3.03 (dd, *J* = 13.8, 8.8 Hz, 1H), 3.01 (s, 3H), 1.54−1.40 (m, 3H), 1.50 (d, *J* = 7.0 Hz, 3H), 1.36 (d, *J* = 7.2 Hz, 3H), 0.89 (d, *J* = 6.0 Hz, 3H), 0.83 (d, *J* = 6.0 Hz, 3H). ^{13}C NMR (100 MHz, D_2O), *δ* (ppm): 174.2, 174.1, 173.0, 173.0, 170.7, 136.4, 129.3, 128.7, 127.1, 54.4, 52.4, 51.1, 47.4, 41.1, 39.5, 37.0, 31.1, 24.2, 21.9, 20.6, 15.1, 13.1. ESI-MS calc. for $C_{24}H_{38}N_5O_5$: 492.28 ([M + H]$^+$); found 492.35. The purity (96%) was determined by HPLC.

2.4. NH_2-Ala-*N*-MeAla-Leu-*N*-MePhe-Gly-OH (31). The trifluoroacetate salt of NH_2-Ala-*N*-MeAla-Leu-*N*-MePhe-Gly-OH was obtained following the general SPPS procedure. 199 mg (0.32 mmol, 97%) was obtained as a white solid. ^{1}H NMR (400 MHz, D_2O), two conformers a:b were observed in a ratio of 1:0.2, *δ* (ppm): 7.50−7.23 (m, 5H), 5.23 (dd, *J* = 10.9, 5.3 Hz, 1Ha), 5.17 (dd, *J* = 10.8, 5.9 Hz, 1Hb), 5.00−4.83 (m, 1H), 4.76−4.67 (m, 1H), 4.53−4.45 (m, 1Ha), 4.45−4.39 (m, 1Hb), 4.12 (d, *J* = 17.0 Hz, 1Hb), 4.02 (d, *J* = 17.8 Hz, 1Hb), 3.99 (s, 2Ha), 3.40−3.27 (m, 1H), 3.18−3.07 (m, 1H), 3.03 (s, 3H), 3.00−2.95 (m, 3H), 2.79 (m, 1H), 1.57−1.44 (m, 3Ha, 2Hb), 1.50 (d, *J* = 7.9 Hz, 3Ha), 1.46 (d, *J* = 7.2 Hz, 3Hb), 1.36−1.31 (m, 3Hb), 1.31 (d, *J* = 7.4 Hz, 3Ha), 1.26−1.15 (m, 1Hb), 0.89 (d, *J* = 6.4 Hz, 3Ha), 0.85 (d, *J* = 6.4 Hz, 3Ha), 0.68 (d, *J* = 6.6 Hz, 3Hb), 0.64 (d, *J* = 6.5 Hz, 3Hb). ^{13}C NMR (100 MHz, D_2O), two conformers a:b were observed in a ratio of 1:0.2, *δ* (ppm): 175.0, 174.3, 173.3, 173.2, 172.6, 172.3, 172.1, 171.5, 170.7, 137.0, 136.7, 129.4, 129.3, 129.1, 129.1, 128.6, 127.5, 126.9, 62.9, 59.9, 55.4, 53.3, 48.4, 47.5, 47.3, 41.5, 41.2, 39.3, 37.5, 33.3, 33.2, 33.0, 30.6, 30.5, 29.6, 24.3, 23.7, 22.3, 22.3, 20.3, 20.1, 19.7, 15.1, 15.1, 14.3, 13.3, 13.1. ESI-MS calc. for $C_{25}H_{40}N_5O_6$: 506.29 ([M + H]$^+$); found 506.30. The purity (93%) was determined by HPLC.

2.5. NH-Ala-*N*-MeAla-Leu-Phe-*N*-MeGly-OH (32). The trifluoroacetate salt of NH_2-Ala-*N*-MeAla-Leu-Phe-*N*-MeGly-OH was obtained following the general SPPS procedure. 248 mg (0.49 mmol, 81%) was obtained as a white solid. ^{1}H NMR (400 MHz, D_2O), *δ* (ppm): 7.38−7.08 (m, 5H), 5.06 (dd, *J* = 8.4, 6.1 Hz, 1H), 4.83−4.73 (m, 1H), 4.40 (q, *J* = 7.1 Hz, 1H), 4.24−4.16 (m, 1H), 4.15−4.11 (m, 2H), 3.10−3.02 (m, 1H),

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3.01 (s, 3H), 2.95 (s, 3H), 2.91−2.86 (m, 1H), 1.53−1.30 (m, 6H), 1.28 (d, J = 7.2 Hz, 3H), 0.81 (d, J = 5.9 Hz, 3H), 0.75 (d, J = 6.0 Hz, 3H). ^{13}C NMR (100 MHz, D_2O), δ (ppm): 173.6, 172.9, 172.8, 172.5, 170.7, 136.1, 129.5, 128.6, 127.1, 53.9, 52.4, 50.6, 50.2, 47.4, 39.6, 36.4, 31.0, 30.2, 24.3, 22.0, 20.6, 15.1, 13.2. ESI-MS calc. for $C_{25}H_{40}N_5O_6$: 506.29 ([M + H]$^+$); found 506.40. The purity (83%) was determined by HPLC.

2.6. NH_2-Ala-*N*-MeAla-Lys(Boc)-*N*-MeGly-OH (33). The trifluoroacetate salt of NH_2-Ala-*N*-MeAla-Lys(Boc)-Phe-*N*-MeGly-OH was obtained following the general SPPS procedure. 311 mg (0.50 mmol, 92%) was obtained as a white solid. ^{1}H NMR (400 MHz, D_2O), δ (ppm): 7.44−7.19 (m, 5H), 5.15 (dd, J = 8.3, 6.1 Hz, 0.7H), 4.96 (dd, J = 8.2, 6.3 Hz, 0.3H), 4.49 (q, J = 7.0 Hz, 1H), 4.33−4.19 (m, 1H), 4.13 (d, J = 17.6 Hz, 1H), 4.05 (d, J = 17.6 Hz, 1H), 3.12 (dd, J = 13.6, 6.2 Hz, 1H), 3.08 (s, 2H), 3.02 (s, 2H), 3.00 (s, 1H), 3.00−2.91 (m, 4H), 2.84 (s, 1H), 2.83−2.78 (m, 1H), 1.79−1.57 (m, 4H), 1.50 (d, J = 6.4 Hz, 3H), 1.39 (d, J = 7.0 Hz, 3H), 1.37−1.27 (m, 1H), 1.23 (s, 8H). ^{13}C NMR (100 MHz, D_2O), δ (ppm): 173.1, 172.9, 172.8, 172.5, 170.8, 164.9, 136.0, 129.4, 128.6, 127.1, 69.7, 54.2, 53.5, 50.6, 50.2, 47.4, 39.1, 36.9, 36.6, 31.3, 30.3, 29.6, 26.2, 22.0, 15.1, 13.5. ESI-MS calc. for $C_{30}H_{48}N_6O_8$: 621.40 ([M + H]$^+$); found 621.50. The purity (97%) was determined by HPLC.

2.7. *Cyclo*-[Ala-Leu-D-Phe-β-Ala] (34). Solution-phase macrocyclization was carried out following Method I of the general procedure (dilution 3 mM, 4 days), starting from the trifluoroacetate salt NH_2-Ala-Leu-D-Phe-β-Ala-OH (250 mg, 0.47 mmol); HBTU was used as coupling reagent. When the reaction mixture was washed with a solution of 5% HCl, the formation of a precipitate was observed, which was the desired product. White solid (Y = 30%). ^{1}H NMR (400 MHz, $DMSO_{d\text{-}6}$) δ (ppm): 8.28−8.17 (m, 1H), 8.15−8.06 (m, 1H), 7.98 (bs, 1H), 7.95−7.86 (m, 1H), 7.30−7.10 (m, 5H), 4.49−4.35 (m, 1H), 4.33−4.23 (m, 1H), 4.21−4.09 (m, 1H), 3.29−3.15 (m, 2H), 3.02 (d, J = 11.1 Hz, 1H), 2.74 (d, J = 11.2 Hz, 1H), 2.36−2.25 (m, 2H), 1.26−1.17 (m, 3H), 1.15 (d, J = 6.9 Hz, 3H), 0.73 (d, J = 4.9 Hz, 3H), 0.70 (d, J = 5.0 Hz, 3H). ^{13}C NMR (100 MHz, $DMSO_{d\text{-}6}$) δ (ppm): 172.8, 172.2, 171.4, 170.7, 138.4, 129.6, 128.4, 126.6, 54.6, 51.9, 51.7, 48.6, 41.1, 35.9, 35.5, 25.6, 24.4, 23.2, 23.3, 18.4. The compound presented solubility problems and was not soluble in commonly used solvents like MeCN and MeOH, and due to this, the purity using HPLC and ESI-MS analyses could not be performed.

2.8. *Cyclo*-[*N*-MeAla-Leu-D-Phe-β-Ala] (35). Solution-phase macrocyclization was carried out following Method I of the general procedure (dilution 3 mM, 5 days), starting from the trifluoroacetate salt NH-MeAla-Leu-D-Phe-β-Ala-OH (250 mg, 0.46 mmol); HATU was used as coupling reagent. Purification by flash chromatography, and EtOAc as mobile phase rendered the desired macrocycle. Yellow oil (Y = 90%). Rf = 0.2 (EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$), multiple conformers were observed, δ (ppm): 7.48 (d, J = 7.4 Hz, 0.2H), 7.35−7.13 (m, 5H), 7.05−6.99 (m, 0.3H), 6.99−6.91 (m, 0.5H), 6.74−6.69 (m, 0.1H), 6.45−6.40 (m, 0.1H), 6.27 (d, J = 6.9 Hz, 0.2H), 4.77−4.50 (m, 1H), 4.45−4.31 (m, 0.7H), 4.31−4.22 (m, 0.3H), 4.21−4.10 (m, 0.2H), 4.04 (q, J = 7.0 Hz, 0.2H), 3.86−3.77 (m, 0.3H), 3.70−3.62 (m, 0.9H), 3.52−3.40 (m, 1.3H), 3.28 (dd, J = 14.5, 5.0 Hz, 0.8H), 3.12−3.02 (m, 1.5H), 2.97−2.82 (m, 1.6H), 2.75 (s, 1H), 2.7−2.4 (m, 1.5H), 1.70−1.10 (m, 6H), 0.99−0.62 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 173.7, 173.4, 173.2, 172.9, 172.6, 171.7, 171.1, 171.0, 170.6, 137.2, 136.7, 129.2, 129.2, 128.9, 128.7, 128.7, 128.6, 128.5, 127.2, 126.8, 57.2, 55.7, 53.9, 53.0, 52.2, 51.9, 51.8, 40.2, 39.9, 38.5, 37.0, 35.9, 35.2, 34.6, 33.8, 32.4, 30.3, 29.7, 29.1, 25.0, 24.6, 22.6, 22.4, 22.0, 15.0, 13.2. ESI-MS calc. for $C_{22}H_{33}N_4O_4$: 417.24 ([M + H]$^+$); found 417.30. The purity (92%) was determined by HPLC.

2.9. *Cyclo*-[Ala-*N*-MeAla-Leu-Phe-Gly] (36). Solution-phase macrocyclization was carried out following Method I of the general procedure (dilution 5 mM, 5 days), starting from the trifluoroacetate salt NH_2-Ala-*N*-MeAla-Leu-Phe-Gly-OH (237 mg, 0.39 mmol); HBTU was used as coupling reagent. Purification by flash chromatography, and $CHCl_3$:MeOH (3:0.1) as mobile phase rendered the desired macrocycle. White solid (Y = 73%). Rf = 0.4 ($CHCl_3$:MeOH, 3:0.1). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.62 (d, J = 7.7 Hz, 1H), 7.29−7.16 (m, 5H), 7.10−7.00 (m, 2H), 6.13 (d, J = 5.3 Hz, 1H), 4.87−4.77 (m, 1H), 4.65 (q, J = 7.6 Hz, 1H), 4.48 (dd, J = 14.4, 8.9 Hz, 1H), 4.23−4.16 (m, 1H), 3.48 (q, J = 7.04 Hz, 1H), 3.32 (dd, J = 13.6, 7.7, 1H), 3.19 (s, 3H), 3.14 (dd, J = 14.3, 3.9 Hz, 1H), 3.01 (dd, J = 13.5, 7.0 Hz, 1H), 1.68−1.59 (m, 1H), 1.56 (d, J = 7.0 Hz, 3H), 1.51−1.34 (m, 2H), 1.28 (d, J = 6.9 Hz, 3H), 0.92 (d, J = 6.4 Hz, 3H), 0.87 (d, J = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 173.8, 172.0, 171.7, 171.0, 170.5, 137.5, 129.5, 128.3, 126.5, 62.2, 54.8, 53.5, 45.3, 44.4, 40.7, 38.6, 38.4, 37.0, 25.1, 23.1, 21.5, 21.5, 17.3, 13.6. ESI-MS calc. for $C_{24}H_{35}N_5O_5$: 474.27 ([M + H]$^+$); found 474.35. The purity (93%) was determined by HPLC.

2.10. *Cyclo*-[Ala-*N*-MeAla-Leu-*N*-MePhe-Gly] (37). Solution-phase macrocyclization was carried out following Method I of the general procedure (dilution 5 mM, 2 days), starting from the trifluoroacetate salt H_2N-Ala-*N*-MeAla-Leu-*N*-MePhe-Gly-OH (100 mg, 0.16 mmol); HBTU was used as coupling reagent. Purification by flash chromatography, EtOAc, rendered the desired macrocycle. White solid (Y = 63%). Rf = 0.3 (EtOAc). ^{1}H NMR (400 MHz, $DMSO_{d\text{-}6}$), two conformers a:b were observed in a ratio of 1:1, δ (ppm): 8.20−8.15 (m, 1Hb), 8.10 (d, J = 7.55 Hz, 1Ha), 7.99−7.90 (m, 1Hb), 7.89−7.79 (m, 1Hb), 7,60 (m, 1Ha), 7.42−7.35 (m, 1Hb), 7.27 (dd, J = 8.9, 4.4 Hz, 1Ha), 7.15−7.08 (m, 2Ha,b), 7.07−6.96 (m, 3Ha,b), 4.77−4.67 (m, 1Hb), 4.63−4.55 (m, 1Hb), 4.54−4.45 (m, 1Hb), 4.22 (p, J = 6.5 Hz, 1Ha), 4.12 (dd, J = 11.8, 3.0 Hz, 1Ha), 4.02−3.91 (m, 2Ha), 3.80 (dd, J = 14.7, 8.9 Hz, 1Ha), 3.53 (dd, J = 14.7, 4.5 Hz, 1Ha,b), 3.10−2.98 (m, 2Hb), 2.84−2.68 (m, 2Ha,b), 2.63 (s, 3Ha,b), 2.52 (s, 3Ha), 2.37 (s, 3Hb), 1.29 (dt, 13.1, 6.5 Hz, 1Ha,b), 1.10 (d, J = 7.0 Hz, 3Ha,b), 0.99 (d, J = 6.3 Hz, 3Ha, b), 0.97−0.91 (m, 1Ha,b), 0.82 (ddq, J = 19.4, 12.9, 6.5 Hz, 1Ha,b), 0.73−0.59 (m, 2Ha,b), 0.44 (d, J = 6.3 Hz, 2Ha,b), 0.36 (d, J = 6.4 Hz, 2Ha,b). ^{13}C NMR (100 MHz, $DMSO_{d\text{-}6}$) δ (ppm): 170.9, 170.5, 168.8, 168.8, 168.8, 138.2, 129.2, 129.1, 127.1, 62.6, 55.7, 47.3, 46.1, 44.3, 42.3, 38.7, 30.9, 30.3, 24.5, 23.0, 22.9, 17.8, 15.2. ESI-MS calc. for $C_{25}H_{38}N_5O_5$: 488.29 ([M + H]$^+$); found 488.45. The purity (91%) was determined by HPLC.

2.11. *Cyclo*-[Ala-*N*-MeAla-Leu-Phe-*N*-MeGly] (38). Solution-phase macrocyclization was carried out following Method II of the general procedure (dilution 5 mM, 1 days), starting from the trifluoroacetate salt H_2N-Ala-*N*-MeAla-Leu-Phe-*N*-MeGly-OH (140 mg, 0.22 mmol). Purification by flash chromatography, and EtOAc as mobile phase rendered the desired macrocycle. White solid (Y = 50%). Rf = 0.35 (EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.78 (d, J = 8.4 Hz, 1H), 7.39−7.21 (m, 5H), 7.21−7.14 (m, 1H), 5.80

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(d, J = 7.4 Hz, 1H), 5.10 (td, J = 8.7, 5.5 Hz, 1H), 4.94−4.83 (m, 2H), 4.33−4.22 (m, 1H), 3.50 (q, J = 7.0 Hz, 1H), 3.30 (dd, J = 13.2, 9.0 Hz, 1H), 3.25 (s, 3H), 2.94−2.82 (m, 5H), 1.80−1.68 (m, 1H), 1.60 (dd, J = 11.5, 6.4 Hz, 1H), 1.53 (d, J = 7.0 Hz, 3H), 1.46−1.35 (m, 1H), 1.29 (d, J = 7.1 Hz, 3H), 0.94 (d, J = 6.6HZ, 3H), 0.88 (d, J = 6.5HZ, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 173.8, 171.0, 170.8, 170.0, 169.2, 137.7, 129.8, 128.2, 126.4, 61.6, 53.3, 52.9, 51.1, 44.9, 40.7, 38.2, 36.0, 25.2, 23.2, 21.3, 17.4, 13.2. ESI-MS calc. for $C_{25}H_{38}N_5O_5$: 488.60 ($[M + H]^+$); found 488.30. The purity (98%) was determined by HPLC.

2.12. *Cyclo*-[Ala-*N*-MeAla-Lys(boc)-Phe-*N*-MeGly] (39). Solution-phase macrocyclization was carried out following Method II of the general procedure (dilution 5 mM, 3 days), starting from the trifluoroacetate salt H_2N-Ala-*N*-MeAla-Lys(Boc)-Phe-*N*-MeGly-OH (140 mg, 0.21 mmol). Purification by flash chromatography, and EtOAc as mobile phase rendered the desired macrocycle. White solid (Y = 53%). Rf = 0.30 (EtOAc:MeOH, 9:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.81 (d, J = 8.5 Hz, 1H), 7.35−7.27 (m, 3H), 7.26−7.23 (m, 2H), 7.21−7.15 (m, 1H), 5.98 (d, J = 6.2 Hz, 1H), 5.15−5.06 (m, 1H), 4.92−4.81 (m, 2H), 4.63−4.53 (m, 1H), 4.26−4.17 (m, 1H), 3.49 (q, J = 7.0 Hz, 1H), 3.31 (dd, J = 13.2, 8.9 Hz, 1H), 3.25 (s, 3H), 3.13−3.05 (m, 2H), 2.93−2.84 (m, 5H), 1.92−1.77 (m, 1H), 1.68−1.58 (m, 1H), 1.55 (d, J = 7.0 Hz, 3H), 1.49−1.46 (m, 2H), 1.43 (s, 9H), 1.32−1.27 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 173.8, 170.8, 170.5, 170.3, 169.3, 156.2, 137.6, 129.8, 128.2, 126.4, 79.2, 61.6, 54.4, 53.3, 51.1, 45.0, 40.1, 38.3, 38.2, 36.0, 31.3, 29.8, 28.4, 23.2, 17.3, 13.2. ESI-MS calc. for $C_{30}H_{47}N_6O_7$: 603.30 ($[M + H]^+$); found 603.40. The purity (93%) was determined by HPLC.

2.12.1. Herbicidal Activity. The experiments to determine the herbicidal activity of the cyclopeptide compounds were carried out on *Lolium multiflorum* (Ray grass) plants. Germination, root length, and leaf development were evaluated compared to a control without herbicide, a negative control (100 μL DMSO in 15 mL agar, used as solvent), and an herbicide control (1/8 of the commercial dose of the herbicide *S*-metolachlor).

Serial experiments were conducted using the agar germination methodology, where the tested compounds and the respective controls were placed in glass Petri dishes (6 cm diameter), in three replicates per treatment. Ten Ryegrass seeds were germinated in a growth chamber (20 °C, day/night temperature). The seeds were previously sterilized by immersing them in 70% alcohol for 10 s. When distributed in the Petri dish on the agar, the seeds were placed in such a way as to ensure that they remained submerged in the solution.

The agar−water solution was prepared at 0.3%; 3 g of agar was placed in 1 L of deionized water and the solution was autoclaved at 100 °C for 45 min. Once the agar medium had cooled to approximately 60 °C, the solutions were prepared.

The negative control was prepared by adding 100 μL of DMSO per plate in 15 mL of agar and then the seeds were distributed as mentioned above. A control without DMSO was also carried out to check that the product was not altering the correct development of the Ryegrass seeds. For this test, 15 mL of agar was placed in each Petri dish and, before it solidified, the seeds of the species evaluated were placed on top. The herbicide treatment, positive control—Control *S*-metolachlor (960 g/L)—was carried out for a conversion of 1/8 of the dose of 1 L/ha of commercial product. For this purpose, a stock solution of *S*-metolachlor was prepared by placing 0.28 mL of the herbicide in a volumetric flask and topping up to 1000 mL. 25 mL of this stock solution was taken, placed in a volumetric flask, and brought to 200 mL, thus generating the 1/8× solution of *S*-metolachlor. A volume of 3 mL of 1/8× herbicide solution was mixed with 45 mL of the agar solution to bring 16 mL into each Petri dish. This ensured that there was 1 mL of 1/8× *S*- metolachlor solution per plate: 0.28 μL of herbicide solution. Seeds were arranged in the same way. Using the same method, the agar media corresponding to each plate (15 mL) was mixed with the cyclopeptides dissolved in 100 μL of DMSO.

Germination, root length, and leaf development were evaluated 12 days after preparation. The variables germinated plants and plants with developed leaves of the total number of plants placed to germinate were analyzed by fitting a generalized linear model since they presented a binomial distribution. Glinmix procedure of the SAS statistical package was used. Based on the model and for the comparison of the treatments with the different controls, the contrasts of interest were carried out. The effect of the treatments on the root length variable was studied by comparing means using a Tukey test (p-value <0.05) in INFOSTAT.

2.12.2. Cyanobacteria Inhibition. Cyanobacterial culture. *Microcystis aeruginosa* strain, not axenic and previously isolated from Río de la Plata, Uruguay, was grown in BG11 medium at a temperature of 25 °C and light intensity of 60 μmol photon m^{-2} s^{-1} with 16:8 light:dark photoperiod. The strain was inspected periodically for bacterial contamination. For the experiments of growth inhibition screening and IG50 of the cyclopeptide compounds, a volume of the original culture grown in exponential phase was diluted in BG11 medium to achieve an initial chlorophyll a of 65 μg L^{-1}. The diluted culture was divided into aliquots of 25 mL and then placed in sterile Greiner culture flasks of 50 mL with filter to allow gas exchange. Each treatment was run in duplicates during 10 days. The negative control (DMSO control) was prepared by adding 100 μL of DMSO to 25 mL of cyanobacterial culture. For positive control, a final concentration of 30 μg/mL of colistine diluted in 100 μL DMSO was used. The same dilution in DMSO was used for screening the inhibitory effect of the compounds. The IG_{50} curve was performed by serial dilutions to raise six concentrations. For IG_{50} curve, 9.0 mg of compound **5** was diluted in 450 μL DMSO. Aliquots of 100 μL, 50 μL, 25 μL, 15 μL, and 7 μL of **5**-DMSO solutions were diluted in 25 mL cyanobacterial culture in duplicates. Optical density was followed periodically and measured in a dark chamber with a LED light source and a 2π visible light sensor (LI-192 quantum sensor Li-Cor, USA). In vivo chlorophyll a was evaluated fluorometrically at initial and final time (PhycoLA, BBE, Moldaenke, Germany)

2.12.3. Microcystins Determination. Samples of the Microcystis cultures incubated with the compounds dissolved in DMSO, as well as positive (colistine) and negative controls (DMSO) were freezed and thawed three times to lyse the cells and release the intracellular toxins. After filtration (0.22 μm), samples were analyzed by ELISA, using a llama nanobody (clon A2), as described in Pírez-Schirmer et al. Briefly, samples and standards (150 μL) were previously mixed in a dilution plate with 34 μL of 1 M Tris buffer pH 7.5 containing 0.27 M NaCl, 0.27 M EDTA, and 1% BSA. Then, 50 μL of each mixture was dispensed by triplicate in an ELISA plate previously coated with 100 μL/well of a 60 μg/L MC-LR-

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ACS Omega 2024, 9, 20167−20175

Bovin Seroalbumin conjugate solution (MC-LR-BSA) in saline phosphate buffer (PBS) and blocked with 0.5% gelatin in PBS. The samples were incubated with 50 μL of the nanobody solution (7 ng/mL). After incubation and washing steps, the bound nanobody was detected with a streptavidin-peroxidase conjugate (Pierce, 1/10.000) and a peroxidase enzyme substrate solution (H_2O_2 and 3,3′,5,5′-tetramethylbenzidine, TMB) in acetate buffer with pH 5.5. After 15 min, the enzyme reaction was stopped by the addition of 50 μL of 2N H_2SO_4, and the absorbance was read at 450 nm using a Fluostar Optima Reader (BMG, Ortenberg, GE). Calibration curves were prepared with MC-LR (Abraxis) in the concentration range: 0.2−2.5 μg/L. The percentage of absorbance of each of the 20 standard with respect to the absorbance of the zero (% A/A_o) was plotted versus the MC-LR concentration and log−linear fitted by GraphPad prism 7 software. Quality controls included Certified Microcystin-LR Reference Standard (National Research Council of Canada) and blanks. To evaluate recovery, samples were fortified with 1 μg/L MC-LR and analyzed, and the results were in the range of 88−130%.

3. RESULTS AND DISCUSSION

3.1. Synthesis. The syntheses of the linear peptides, **28**−**33** (Table 2) were carried out through SPPS methodology, employing the 2-chlorotrityl chloride resin (2-CTC resin) and were based on the Fmoc-strategy; the general procedure is shown in Scheme 1. In all the cases, the desired peptides were obtained in very good yields and purities.

Table 2. Peptides Obtained by SPPS

peptide	yield (%)	purity (%)[a]
Ala-Leu-D-Phe-β-Ala (**28**)	95	92
N-MeAla-Leu-D-Phe-β-Ala (**29**)	96	92
Ala-*N*-MeAla-Leu-Phe-Gly (**30**)	92	96
Ala-*N*-MeAla-Leu-*N*-MePhe-Gly (**31**)	97	93
Ala-*N*-MeAla-Leu-Phe-*N*-MeGly (**32**)	81	83
Ala-*N*-MeAla-Lys(Boc)-Phe-*N*-MeGly (**33**)	92	97

[a]Purity was determined by HPLC analysis.

Glycine, *N*-MeGly, or β-Alanine was chosen to start the peptide sequences. In this way, steric hindrance is diminished and the possibility of racemization in the subsequent macrocyclization reaction is avoided. Even though, these amino acids should favor the diketopiperazine (DPK) formation, an important side reaction that produces the peptide cleavage from resin. In all cases, the resin loading was achieved with a solution of the Fmoc-AA−OH in CH_2Cl_2 and an excess of DIPEA.

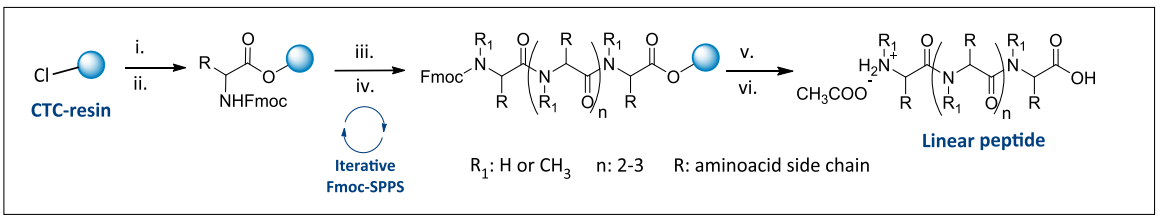
For the peptide chain elongation, successive deprotection and coupling steps were carried out. Fmoc group deprotection was achieved with a solution of 20% piperidine in DMF. For the amide reaction, HBTU or HATU was chosen as coupling reagent. This choice was made based on the nature of the amine involved in the reaction, HBTU being for primary amines and HATU for secondary amines. All reactions were monitored by HPLC. In case of incomplete deprotection or coupling, additional steps were required. For peptide **31**, special precautions were taken during Fmoc-*N*-MePhe coupling and deprotection steps due to the presence of an *N*-MeAA as the second amino acid that can promote the diketopiperazine formation. Moreover, despite the use of 2-CTC resin, which contains a bulky trityl group that can decrease the diketopiperazine formation, short times during Fmoc-*N*-MePhe deprotection were employed.

After resin treatment with a solution of TFA 1% in CH_2Cl_2, the desired linear peptides were obtained as trifluoracetate salts. The structural elucidation was performed using NMR and HPLC-MS, and the purity was analyzed by HPLC. In the majority of cases, yields exceeding 90% were attained, indicating that the formation of DKP did not occur, at least to a significant extent. Characterization data of compounds can be found in the Supporting Information.

The macrocyclization reaction of the linear peptides was performed during 2−5 days, using coupling reagents in high diluted conditions, in order to favor the intramolecular head-to-tail reaction (Figure 2). The chosen coupling reagents for the cyclization reaction were HBTU when the *N*-terminal residue was a primary amine and HATU when the N-terminal residue was a secondary amine. In the case of peptides **32** and **33**, the combination of Oxyma/DIC was also employed. After purification, the cyclopeptides were obtained in good yields and adequate purities (Table 3). For **39**, Oxyma/DIC coupling reagents gave rise to the cyclopeptide in only 13% yield. This yield was improved by performing the macrocyclization using HBTU (53%).

The cyclization yields for cyclopentapeptides **36**, **38**, and **37** were higher than those obtained for the corresponding cyclotetrapeptides (**17**, **23**, and **24**, respectively).[17] This fact can be directly related to the ring size. The addition of Ala in the case of pentapeptides would allow a better orientation of the participating groups for the cyclization reaction. In line with this, when comparing the yields for **16** versus **34** and **20** versus **35**,[17] the extra CH_2 given by the β-Ala significantly improves cyclization yields that could be due to an increase in the ring size of the cyclopeptide.

Scheme 1. General Procedure of the Solid-Phase Peptide Synthesis (SPPS) Fmoc-Strategy[a]



[a]CTC-resin: 2-chlorotrityl chloride resin. i. Resin loading: Fmoc-AA−OH, CH_2Cl_2, DIPEA; ii. Capping: MeOH; iii. Deprotection: piperidine 20% in DMF; iv. Coupling: Fmoc-AA−OH, HBTU/DIPEA, or HATU/DIPEA; v. Deprotection: piperidine 20% in DMF; vi. Cleavage: 1% TFA in CH_2Cl_2.

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ACS Omega 2024, 9, 20167−20175

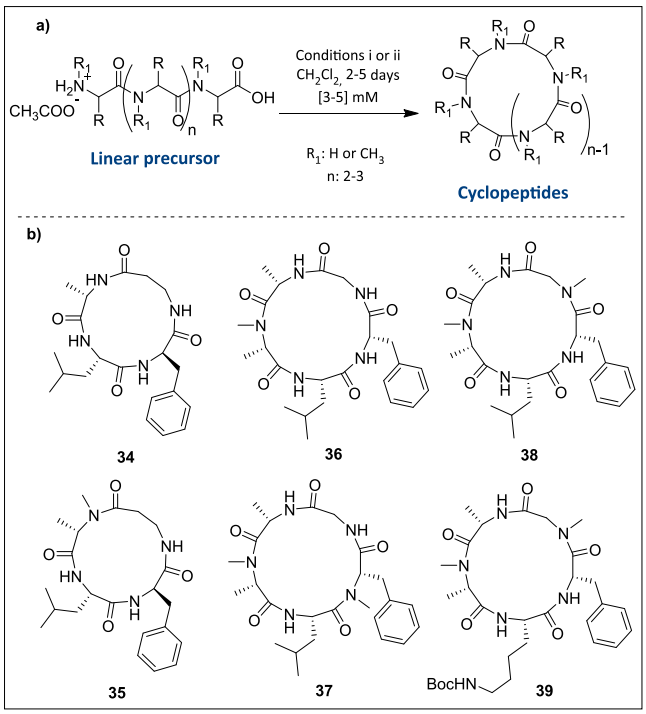

Figure 2. (a) Scheme of macrocyclization in solution phase. Condition i: HBTU or HATU/DIPEA. Condition ii: Oxyma/DIC/DIPEA. (b) Structure of the cyclopeptides synthesized in this work.

Cyclotetrapeptide **35** was obtained in a higher yield (90%). This may be due to the combination of three factors: the presence of *β*-Ala which raises the ring size, and of an *N*-MeAA and a D-amino acid, which are known as turn inducers,[20] capable of leading to improvements in cyclization yields especially of small peptides. For the case of cyclotetrapeptide **34**, despite the presence of *β*-Ala, the low yield can be related to the absence of an *N*-MeAA. This, may also be the reason for its poor solubility.

By comparison of **37** versus **38,** the change in one *N*-MeAA position does not significantly affect the yield of the cyclization reaction.

3.2. Herbicidal Activity. The evaluation of the herbicidal activity was realized through the germination in agar methodology, as was reported before.[17] Ryegrass seeds were germinated with the linear peptides **28**−**30** and **33** and cyclopeptides **34**−**39** at 67 *μ*g/mL. The assay was developed by triplicate with DMSO as negative control and the herbicide commercial solution of *S*-metolachlor as positive control. Three variables were evaluated: germination, leaf development, and radicle length growth. No significant differences were observed between the negative control and the control witness without additive in any of the variables. In the case of germination, no significant differences were found for the compounds with respect to the negative control. The results for leaf development and germination can be found Figures S48 and S49.

Only compounds **34** and **35** affect significantly leaf development (23%; Figure S48). As these cyclopeptides differ in an *N*-MeAA, this change does not seem to have great influence on the activity. As their linear precursors **28** and **29**, respectively, were not active, it could be concluded that cyclization is important for leaf development inhibition. In addition, the presence of *β*-Ala could be playing an important role in leaf development inhibition, because analogues containing Gly instead of *β*-Ala were not active.

The results of the radicle growth inhibition (%) are shown in Figure 3. By setting the criteria that active compounds are

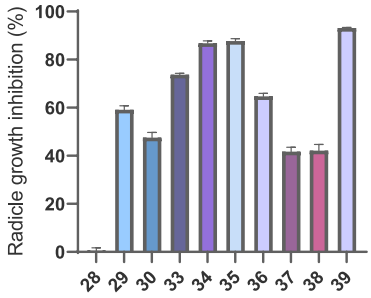

Figure 3. Radicle growth inhibition (%) observed for the compounds at a concentration of 67 *μ*g/mL. Positive control showed 100% inhibition at 2.1 *μ*g/mL.

those that present a radicle growth inhibition greater than 70%, four of the evaluated compounds are considered active. These are the cyclotetrapeptides **34** and **35** with 87% and 88% radicle growth inhibition, respectively, and the linear pentapeptide **33** and its corresponding cyclopentapeptide **39**, with 74% and 93%, respectively.

In general, the cyclotetrapeptides present higher activity than their respective linear peptides. For example, the linear peptide **28** showed 1% of inhibition while its corresponding cyclopeptide (**34**) presents 87%. Considering the reported activity for compound **16** (41% radicle growth inhibition),[17] it is interesting to note that the change of Gly in **16** by *β*-Ala in **34** leads to an increase of inhibition (87%). However, the effect of substitution of Gly in **20** by *β*-Ala in **35** is not so noticeable. The cyclopentapeptides **36**−**38** are not active, neither are their respective linear peptides. By comparison of the results for **36** and **37**, with those obtained for the respective cyclotetrapeptides **17** and **24** (radicle growth inhibition 71% and 81%, respectively), it seems that the increase in the ring size, given by the addition of Ala in the sequence, has a negative effect on the herbicidal activity.

Table 3. Cyclopeptides Obtained by Macrocyclization of Peptide Precursors in Solution Phase

peptide precursor	cyclopeptide	macrocyclization yield (%)	purity (%)[a]
Ala-Leu-D-Phe-*β*-Ala (**28**)	**34**	30	ND
N-MeAla-Leu-D-Phe-*β*-Ala (**29**)	**35**	90	92
Ala-*N*-MeAla-Leu-Phe-Gly (**30**)	**36**	73	93
Ala-*N*-MeAla-Leu-*N*-MePhe-Gly (**31**)	**37**	63	91
Ala-*N*-MeAla-Leu-Phe-*N*-MeGly (**32**)	**38**	50	98
Ala-*N*-MeAla-Lys(Boc)-Phe-*N*-MeGly (**33**)	**39**	53	93

[a]Purity was determined by HPLC analysis at 220 nm.

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ACS Omega 2024, 9, 20167−20175

Among the tested compounds, cyclopeptide **39** presents the highest radicle growth inhibition (93%). Interestingly, the presence of a Boc-Lys (**39**) instead of Leu (**38**) seems relevant to confer increased herbicidal activity.

While previous structure−activity relationship studies on the natural product tentoxin (cyclo(NMe-Ala-Leu-NMe-Δ^z-Phe-Gly) show a strong relevance on the presence of *N*-methyl groups at certain positions,[19] this tendency was observed in the cyclic tetrapeptides previously reported by us (which contain 12 atoms), but is not observed when the size of the cycle increases. For example, for 13- membered ring cyclopeptides **35** and **34**, which differ only in the absence of an *N*-methyl group, both exhibit similar phytotoxic activity (87% and 88% radicle growth inhibition). Furthermore, in cyclic pentapeptides **36** and **37**, the removal of one *N*-Me increases activity.

3.3. Cyanobacteria Inhibition. The obtained and the previously obtained cyclopeptides,[17] and some of the linear peptides were evaluated as cyanobacterial growth inhibitors. The primary screening was performed with a not axenic *Microcystis aeruginosa* strain (MVCC42), isolated from Río de la Plata, Uruguay. The strain was grown in BG11 medium at a temperature of 25 °C and light intensity of 60 μmol photon $m^{-2}s^{-1}$ with a 16:8 light:dark photoperiod. The original culture in exponential growth phase was diluted in the grown medium to achieve an initial chlorophyll a of 65 μg L^{-1}. The diluted culture was divided in aliquots of 25 mL and placed in sterile Greiner culture flask of 50 mL with filter to allow gas exchange. Each treatment was run in duplicates for 7−10 days. In vivo chlorophyll a was evaluated fluorometrically at initial and final time (PhycoLA, bbe, Moldaenke, Germany). Colistin (30 μg/mL) was employed as positive control and DMSO as negative control. The obtained results are shown in Figure 4.

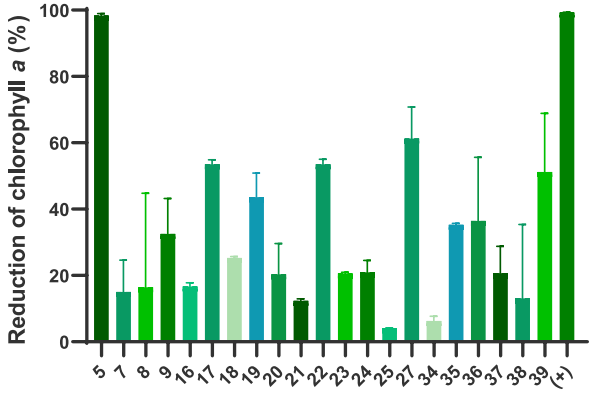

Figure 4. Reduction of chlorophyll a (%) observed for the compounds at a concentration of 40 μg/mL. Colistin (30 μg/mL) was used as positive control (+) and DMSO as negative control.

20 compounds were evaluated at a 40 μg/mL concentration, where 4 are linear peptides (**5**, **7**−**9**) and 16 are cyclopeptides, including cyclotetrapeptides (**16**−**25**, **27**, **34**, and **35**) and cyclopentapeptides (**36**−**39**). Five compounds resulted in a reduction of chlorophyll a (%) greater than 50%. The most active compound was the linear tetrapeptide **5**, with 98% reduction of chlorophyll a, similar to the observed inhibition of the positive control (99%). The cyclization of **5** to obtain **17** decreased the activity against cyanobacteria (53%). However, cyclization of peptide **8** to obtain **20**, which contains D-Phe instead of Phe (**5** and **17**), did not affect the activity (about 20% reduction of chlorophyll a).

Taking as reference the cyclotetrapeptide **17**, the addition of an extra N-Me in compound **24** decreased the activity. In addition, the increase in the ring size of **17** by the addition of Ala in the sequence (**36**) has a negative effect on the bioactivity.

Only one cyclopentapeptide (**39**) was active, indicating that change of Leu in **38** by Boc-Lys (**39**) led to a marked increase in the inhibitory activity.

As compound **5** showed the highest activity, determination of its GI_{50} (growth inhibition 50) was performed. A diluted Microcystis culture (chlorophyll a 77 μg/L) in exponential growth phase was incubated with serial concentrations of the compound in a range of 5.6 to 80 μg/mL. The concentration−response curve was generated on GraphPad prism 8 software using a four-parameter Hil's equation, giving a relative GI_{50} value of 14.01 μg/mL, corresponding to 26 μM (Figure 5).

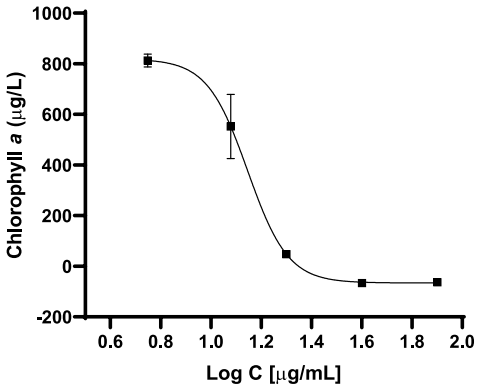

Figure 5. Concentration−response curve of compound **5**. Generated on GraphPad prism 8 software; Hil's equation parameters: top = 896.1, bottom = 10.95, Hil's slope = −5.400, and IC_{50} = 14.01 μg/mL.

3.4. Microcystins Determination. To assess the overall impact of the compounds on toxin production, we determined the concentrations of total microcystins (both cell-bound and soluble) in the culture media. In this way, the potential effect of the compounds to induce cell lysis of the cyanobacteria can be evidenced. To this end, samples of the *Microcystis* (MVCC42) cultures after incubation with the compounds during 7 days were frozen and thawed three times in order to lyse the cells and release the intracellular toxins. The samples were filtered (0.22 μm) and analyzed by ELISA, using a llama nanobody (clon A2).[21] Calibration curves were prepared with MC-LR (Abraxis) in concentration range 0.2−2.5 μg/L. The percentage of absorbance of each standard with respect to the absorbance of the zero (%A/A_o) was plotted versus the MC-LR concentration and log−linear fitted by GrapPad prism 7 software. The obtained results are shown in Figure 6.

The *Microcystis* cultures incubated with compounds that resulted in ≥50% reduction of chlorophyll *a* (**5**, **17**, **22**, **27**, and **39**) were analyzed for microcystins. Consistent with the observed inhibition of cyanobacterial growth, compound **5** showed the highest decrease in microcystins (95%). Notably, this value is very similar to the positive control, colistine (90%). It is also important to note that in all cases, the reduction of cyanobacterial growth was accompanied by an inhibition of the microcystins concentration.

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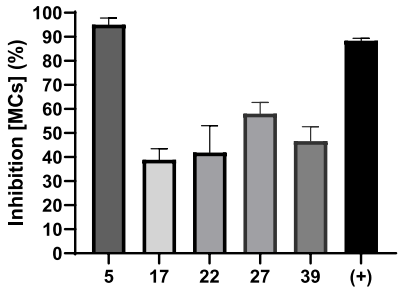

Figure 6. Inhibition of microcystins concentration (%). Percentage calculated with respect to the negative control (DMSO). Colistine treatment (30 μg/mL) as positive control (+).

In conclusion, cyclopeptides analogues of natural products were successfully synthesized by SPPS of their linear precursor and solution-phase macrolactamization.

Four of the newly obtained analogues (**33**, **34**, **35**, and **39**) present phytotoxicity with more than 70% of radicle growth inhibition at 67 μg/mL. In the case of cyclopeptides **34** and **35**, they also affect leaf development. These results could suggest that these compounds would act as herbicides through at least two different mechanisms, which are intended to be studied in the future. In addition, the evaluation of peptides and cyclopeptides as inhibitors of cyanobacteria rendered five active compounds (**5**, **17**, **22**, **27**, and **39**) that reduced the concentration of microcystins in the culture medium.

Taking into account the previous and these results,[17] we can conclude that tetrapeptide **5** and cyclotetrapeptides **17** and **39** are the most promising compounds to investigate these peptides as potential herbicides that also inhibit cyanobacteria growth.

The compounds investigated in this study have a simplified chemical structure compared to tentoxin, a phytotoxic natural product containing dehydrophenylalanine. Unlike tentoxin, which necessitates numerous synthetic steps with low yields,[22] these compounds can be prepared more easily and efficiently. As a result, they hold greater economic appeal for the agriculture industry.

Considering the explored modifications, the obtained results allow the proposal of new potentially more active analogues. Besides that, additional studies with the compounds to evaluate eco-toxicity are in progress and will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c00311.

General experimental information; solid-phase peptide synthesis; solution-phase macrocyclization; characterization data; NMR spectra; ESI-MS; and HPLC data of compounds (PDF)

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Notes

The authors declare no competing financial interest.

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