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Haute Ecole Spécialisée de Suisse occidentale
Fachhochschule Westschweiz
University of Applied Sciences and Arts Western Switzerland

Master of Science HES-SO in Life Sciences

Antimicrobial Cyclodepsipeptides Library : preparation fo 16 lead compounds on Synphase Lanterns

Lopez Alexandro
Applied Bioscience
HES-SO Valais
Advisor: Dr. Mathieu Marc // In collaboration with Dr. Enrico Condemi

Objectives and description

The objectives of this thesis was to develop a synthetic method to produce a library of 4 cyclodepsipetides using a new support for solid phase peptide synthesis (SPPS). Then once the method is established, to transfer the classical SPPS methodology to Micro-Wave system. At the end, to produce a library of 16 cyclic peptides and confirm all target compounds by LC-MS as well as major impurities.
To do this, a proof of concept of the support (Synphase Lantern PHD and Trityl-Ala-Fmoc Synphase Lantern) was first made and then different tests on the efficiency of the reagents used for the synthesis were performed. Only the Trityl-Ala-Fmoc Synphase lantern were retained for the SPPS. Once the reagents were defined for amide (Oxyma/DIC) and ester (DIC/DMAP) bond formation, linear synthesis method were performed and tested. Followed by a purification step and cyclization step to obtain the desired CDPs compounds. Until the 1st CDPs is obtained, the method were transfer to the Micro-Wave device.

Material and methods

Analysis:
Anaylsis was done on UHPLC Agilent 1290 infinity serie equiped with DAD and simple Quad 6130B. The separation was done on a Phenomenex C18 Kinetex (1,7µm 50x2,1mm, Ref. 00B-4475-AN, No°5605-0159) and on a Phenomenex, Column C18 Kinetex® (5µm 100x21,2mm, Ref. 00D-4601-P0-AX, No°754165-1) for the preparative step. The sample analysis was made with 5µL injection, a flow rate of 0.6 mL/min, at 30°C temperature. The eluent is 0.1%TFA in water (A) and 0.1%TFA in ACN (B). The gradient was as follow : 0 to 0,1 min. 5% B, 0,10 to 4,10 min. 95% B, 4,10 to 6,90 min. 95% B, 6,90 to 7 min. 95% B. Then 2.5 min of post run at 5% B. The gradient used for the preparative part, changed according to the peptide to purify.
Synthesis :
For the amide bond coupling reagents we used 20eq AA; 20eq DIC; 20eq Oxyma in DMF and for the ester bond coupling regent, we used 20eq AA; 20eq DIC; 0,2eq DMAP in DMF/DCM (20/80%). We used the Synphase PA D-series Lantern Fmoc-L-Ala-OH on trityl linker 5 µmol as a solid support. And the P12 synthesizer from Activotec. We applied 30 min. for amide bond coupling time and 60 min. for ester bond coupling time. And 3x5 min. deprotection (pip/DMF (2:8)) time reaction and 3x2 min. washing (DMF) time step. The SPPS was as follow : 1) Deprotection step; 2) Washing step; 3) Coupling step; 4) Second washing step (common cyclic synthesis for adding the amino acid). End of the synthesis we made a last washing with DCM and made the cleavage step to recovery the peptide. For the cyclization step we used commonly 20 µmol of purified linear peptide and we poured into a 50mL rounded flask fitted with magnetic stirrer. Then we adding 5eq of BOP-Cl and DMAP and filled with DCM/MeOH (4:1) solution until the 0,5µmol/mL concentration of the peptide is reached. The reaction medium is maintained under agitation for 24 hours. At the end of the synthesis (confirmed by sampling). The peptide is recovered. The method was transferred on the Liberty Blue device following the supplier guideline.

RESULTS

The following table 1 and 2 gives the mass and yields obtained and the figure 1 give the E CDPs LC-NS analysis as example of the result obtained :

Table 1 : Mass obtained and amino acid sequence

Cyclic peptide	Amino acid sequence	MW [g/mol]	Pure cyclic mass [mg]
E	Ala-N^{Me}-Ala-Pro-Ser(Pro)-Gly-Oct.Acid	636,74	7,61
I	Ala-N^{Me}-Ala-Pro-Ser(Pro)-Val-Oct.Acid	678,82	7,51
J	Ala-N^{Me}-Ala-Pro-Ser(Pro)-Leu-Oct.Acid	692,85	8,05
K	Ala-N^{Me}-Ala-Pro-Ser(Pro)-Ile-Oct.Acid	692,85	9,30

RESULTS

Table 2 : Global yields obtained

Step	Global yield	Condition
Linear synthesis	116,57%	Without CDPs and P11 sample
	129,56%	Only sample E-K + reference 2 (Optimized linear synthesis method sample)
1st purification	50,51%	All
	55,29%	Only sample E-K + reference 2 (Optimized linear synthesis method sample)
Cyclization	214,78%	-
2nd purification	27,52%	-
Global yield synthesis	48,06%	-

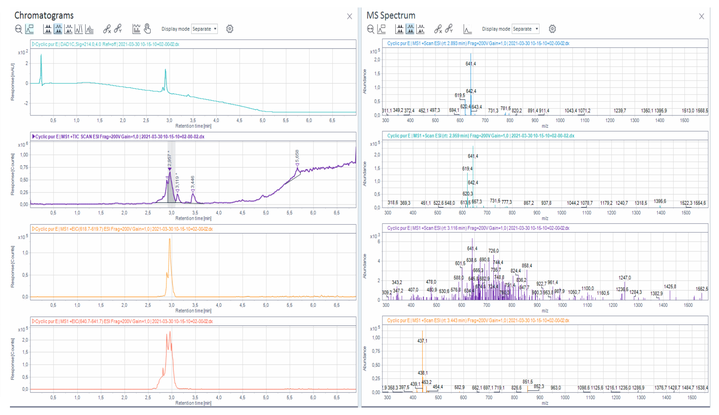

Figure 1 : Purified CDPs E Separated signal, UV 214 nm (top), TIC SCAN (middle top), EIC 618 m/z (middle bottom), EIC 641m/z (bottom) and MS signal

The UV detector confirms the presence of the product peak (RT of 2.95 min.). The overlay with the MS signal confirms the presence of the M^{+H} and M^{+Na} peak of the peptide as well as a good abundance. The peak at 3.45 min is from the eluent. The peak at 2.89 min. would also be a peak of the compound according to the MS spectrum however in this region the majority peak overwrites the less intense signals. It may therefore also be an impurity.

The figure 2 below give the result obtained from the transfered method :

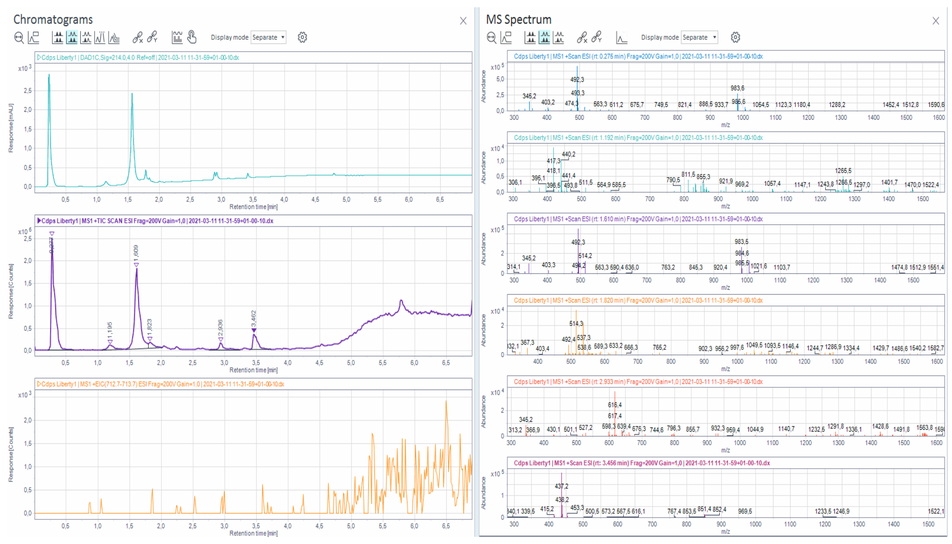

Figure 2 : Sample CDPs liberty 1 Separated signal, UV 214 nm (top), TIC SCAN (middle top), EIC 712 m/z (bottom) and MS signal

The results obtained from the transferred method on the Micro-wave device were not succesful.The target ion (Ala-Ala-Pro-Ser(Pro)-Phe-Oct.Acid) 712 m/z was not observed. No peptide was obtained with the parameter tested.The test showed that the agitation did not allow the synthesis. However, the results obtained from the samples having performed the microwaves assisted synthesis, showed significant UV and MS signals. This means that there was a reaction.

CONCLUSION

The 1st objectives was complete, the production of 4 CDPs were made and the method well established. The second objectives was not reached because the parameter tested the parameters tested did not allow the production of the peptide. The last objectives was partially reached. Only four peptide were made. However, the method works (global yield of 48%). So, it is only a matter of time to produce the library and the final mass obtained are enough to perform the antimicrobial test to confirm the bioactivity of the CDPs obtained. The method adapted on the CEM liberty blue system showed that the synthesis was not possible with the parameter tested but it would be interesting to test other parameters such as: increasing the number of cycles per coupling (e.g., 3x15 min for 1 coupling); varying the microwave power used (e.g., from 70W to 120W then 150W) or increasing the temperature up to 60°C (>70°C to be avoided). The method developed for the synthesis of the peptide uses DMF as main solvent. This solvent is environmentally dangerous, toxic and expensive. Scientists have succeeded in performing the amide bond using phosphonium salt coupled with base and ACN as eluent. The reaction is as follows: AA/PyAOP/DIEA in ACN solvent. In case the production of the peptide goes well, the trial of this method could have brought a benefit for the environment and potentially a money saving because the ACN is 20% cheaper for the same quality and quantity.

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