



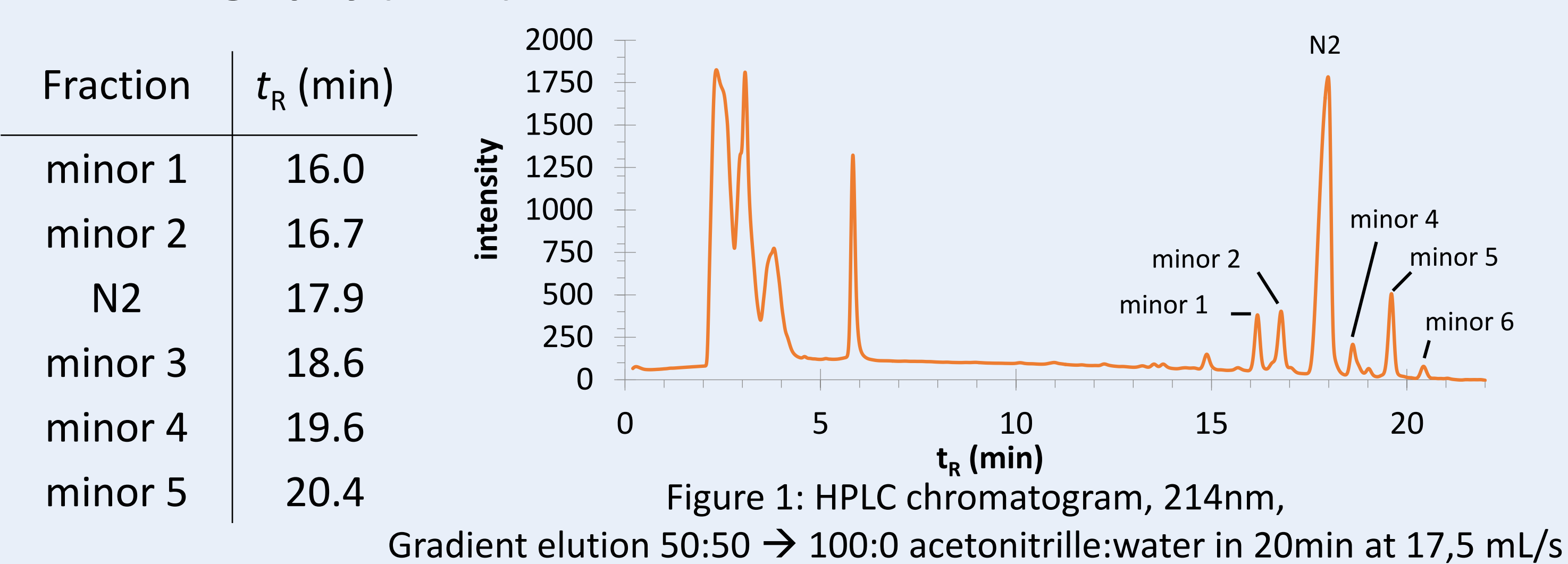
## Introduction

N2 is a new, uncharacterized **cyclic lipodepsipeptide (CLP)**. CLP's are produced by bacteria and have interesting biological activities including antibacterial, antifungal and antiviral properties. During the bachelor project we determined the structure of N2 and one of its minors and established that they belong to **the amphisin group** using high-resolution nuclear magnetic resonance (NMR) spectroscopy. The amphisin group of

CLP's consists of **11 amino acids** and a fatty acid tail at the N-terminus. Nine amino acids form a peptide cycle through the formation of an ester bond at the C-terminus. Since CLP's are able to **self-assemble** in non-polar organic solvents such as in chloroform or dioxane, we also studied the self-assembly of N2 using diffusion NMR<sup>(1,2)</sup>.

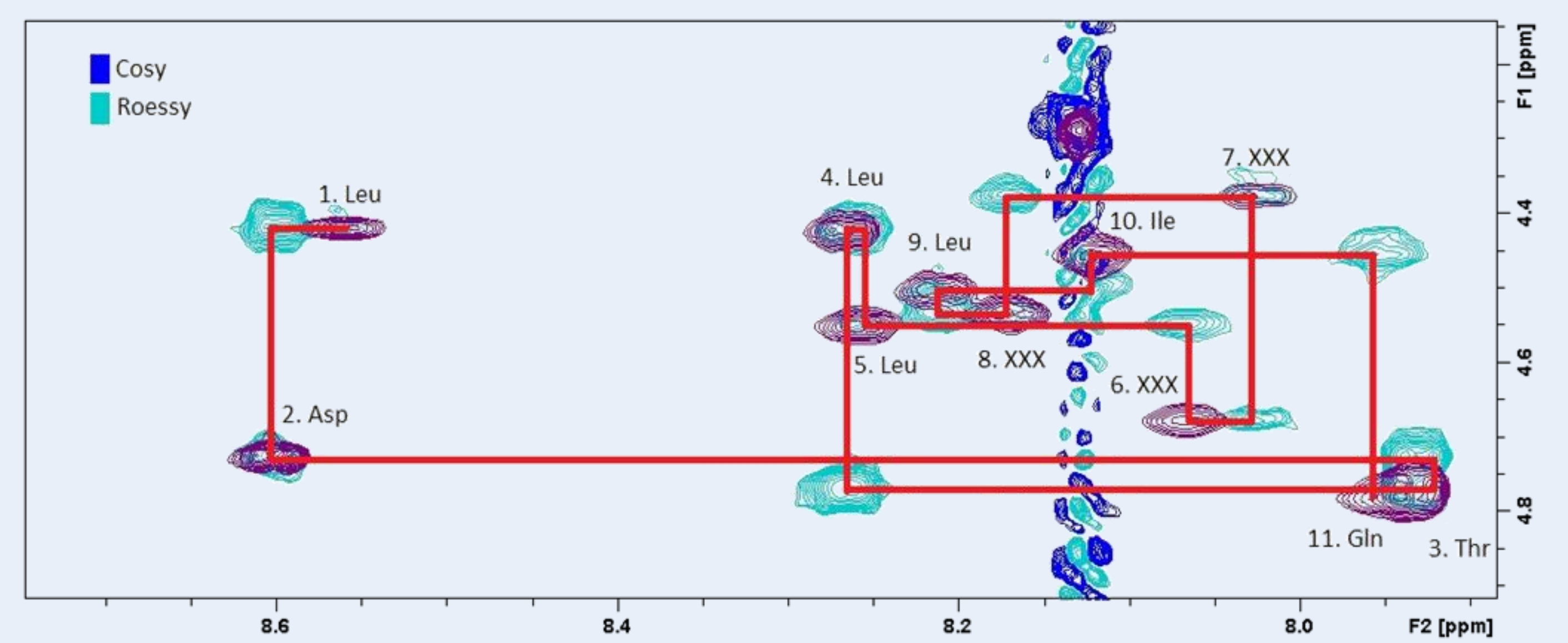
### 1. Separation of N2 and its minors by HPLC

Impure N2 was extracted from *Pseudomonas koreensis* COW8 bacteria and subsequently separated by reversed phase **high pressure liquid chromatography (HPLC)** in N2 and minors. **Six fractions** were collected:



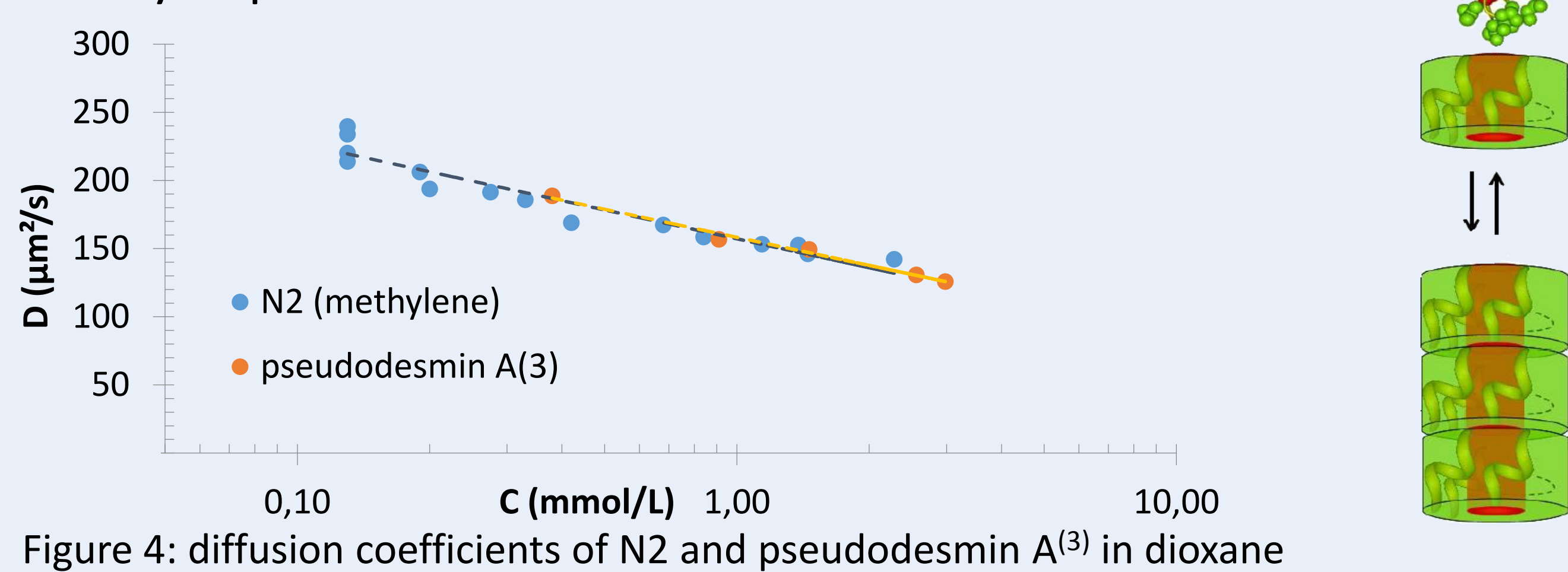
### 2. Determination of the amino acid sequence of N2 and minor 2

To elucidate the structure of N2, we first identified the amino acids present on the basis of the **COSY spectra**. Subsequently, we used the **ROESY spectrum** for sequential assignment (Figure 2). The length and composition of the fatty acid tail was determined using **Mass Spectrometry (MS)**.

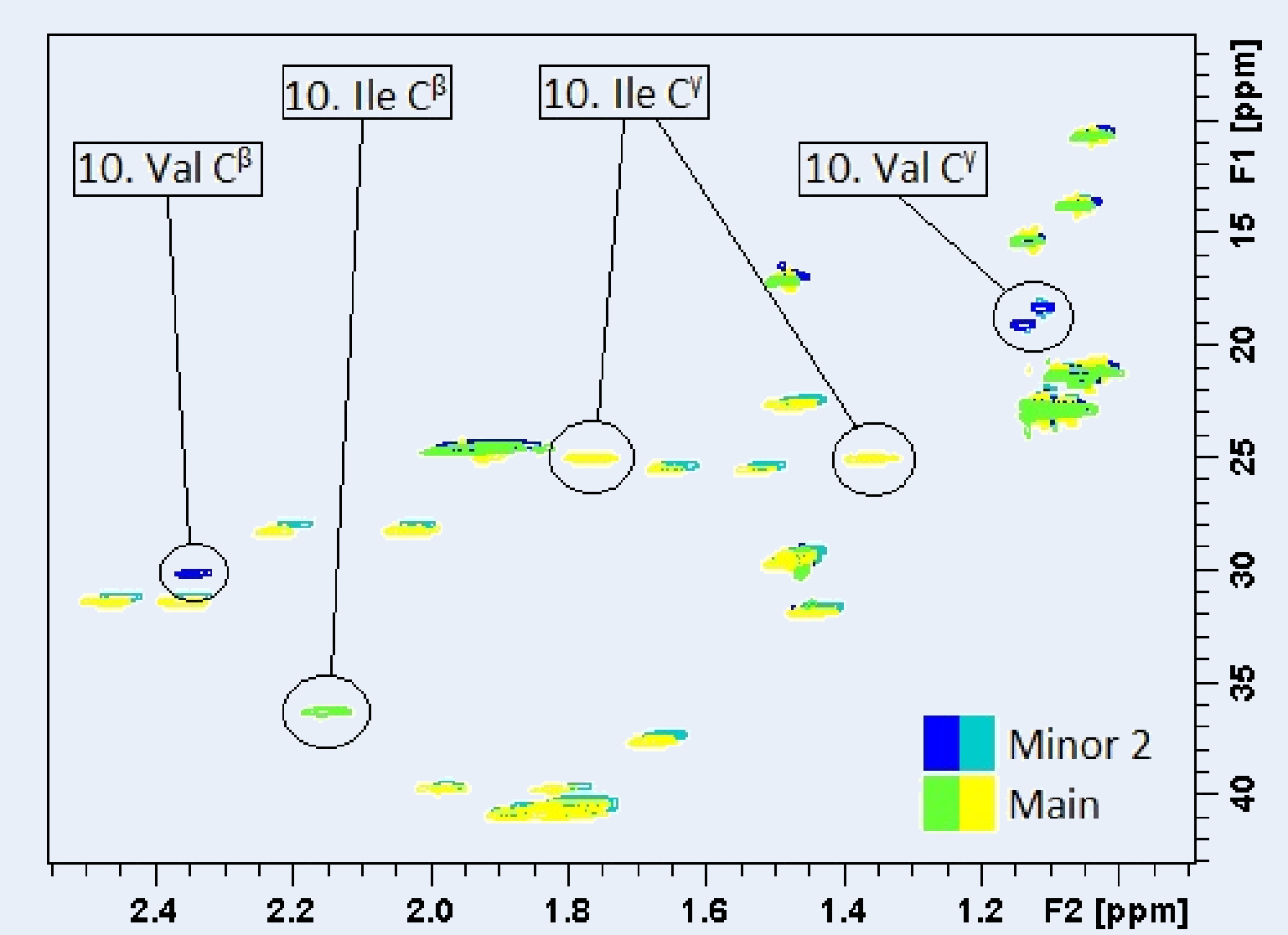


### 3. Self-assembly of cyclic lipodepsipeptide N2

Measuring the **translational diffusion coefficient** of N2 by using pulsed-field gradient (PFG) NMR spectroscopic techniques confirms N2 self-associates into a **supramolecular structure** when exposed to apolar solvents such as dioxane. The self-assembly is **concentration-dependent** and similar to the previously studied self-assembly of pseudodesmin A<sup>(3)</sup>.



In order to determine the difference between N2 and minor 2, we compared their <sup>1</sup>H-<sup>13</sup>C HSQC spectra (Figure 3), which revealed 6 differences. By performing sequential assignment on minor 2, we concluded that this differences were due to the replacement of **Ile** at position 10 by **Val**. This is in agreement with the mass spectrum of minor 2, which showed a **loss of mass of 14 Da** compared to N2.



Name	Fatty acid	1	2	3	4	5	6	7	8	9	10	11
N2	3-hydroxy C10	Leu	Asp	Thr	Leu	Leu	XXX*	XXX*	XXX*	Leu	Ile	Gln
Minor 2	3-hydroxy C10	Leu	Asp	Thr	Leu	Leu	XXX*	XXX*	XXX*	Leu	Val	Gln

\*Due to patent pending, the exact sequence is confidential

### Safety aspects of nuclear magnetic resonance (NMR)

NMR laboratories have two major safety issues: the presence of a **strong magnetic field** and **cryogenic liquids** <sup>(4,5)</sup>.

- The strong magnet attracts ferromagnetic objects. For this reason, **ferromagnetic objects** are not allowed in the area of the magnet and people with metal **prosthesis** or **pacemakers** are not allowed to enter. In addition, the magnetic field can disable magnetic strips. It is an invisible danger, therefore anyone who comes close to the magnet must be aware of the safety issues.
- The two used cryogenic liquids are **nitrogen** and **helium** at respectively 77 K and 4,2 K. Contact with cryogenic liquids can cause **frostbites**. Another danger is **asphyxia**. The oxygen can be expelled through the expansion caused by evaporation and the room will be filled with nitrogen and/or helium. For this reason NMR laboratories must be extra ventilated. Transport of cryogenic liquids in elevators is possible but, for safety issues, not in the presence of people. People working with cryogenic liquids must wear a lab coat, gloves and lab glasses.



### Conclusion

**HPLC:** We isolated N2 and 5 minors. **NMR and MS:** We determined the structure of N2 and minor 2. Amphisin N2 contains: 4 Leu, Asp, Thr, Ile, Gln, 3-hydroxy C10 and 3 confidential amino acids due to patent pending. Minor 2 contains a Val instead of an Ile at position 10. Structure elucidation of all other minors requires further examination. **Self-assembly:** The self-assembly properties of N2 in organic solvents were investigated and found to be similar to other CLP's, such as pseudodesmin A.

### References

- 1) N. Geudens, M. De Vleeschouwer, K. Fehér et al. "Impact of a Stereocentre Inversion in Cyclic Lipodepsipeptides from the Viscosin Group: A Comparative Study of the Viscosinamide and Pseudodesmin Conformation and Self-Assembly." *ChemBioChem* 15.18 (2014): 2736-2746. 2) D. Sinnaeve, P. M. S. Hendrickx, J. Van Hemel et al. "The Solution Structure and Self-Association Properties of the Cyclic Lipodepsipeptide Pseudodesmin A Support Its Pore-Forming Potential." *Chem. Eur. J* 15 (2009): 12653-12662. 3) M. De Vleeschouwer, D. Sinnaeve, J. Van den Begin et al. "Rapid Total Synthesis of Cyclic Lipodepsipeptides as a Premise to Investigate their Self-Assembly and Biological Activity" *Chem. Eur. J.* 20 (2014): 7766 – 7775. 4) E. Butler. *AVANCE Beginners, User Guide* 7 Feb. 2008: 11-14. 5) General Safety Considerations for the Installation and Operation of Superconducting Magnet Systems. Vol. 1.1. Bruker Biospin, March 29 2002.

### Acknowledgements

The 700 MHz is part of the interuniversity NMR facility jointly operated by UGent, UA and VUB. Thanks to the entire NMR and structural analysis unit.