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EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Název: Synthesis of peptides

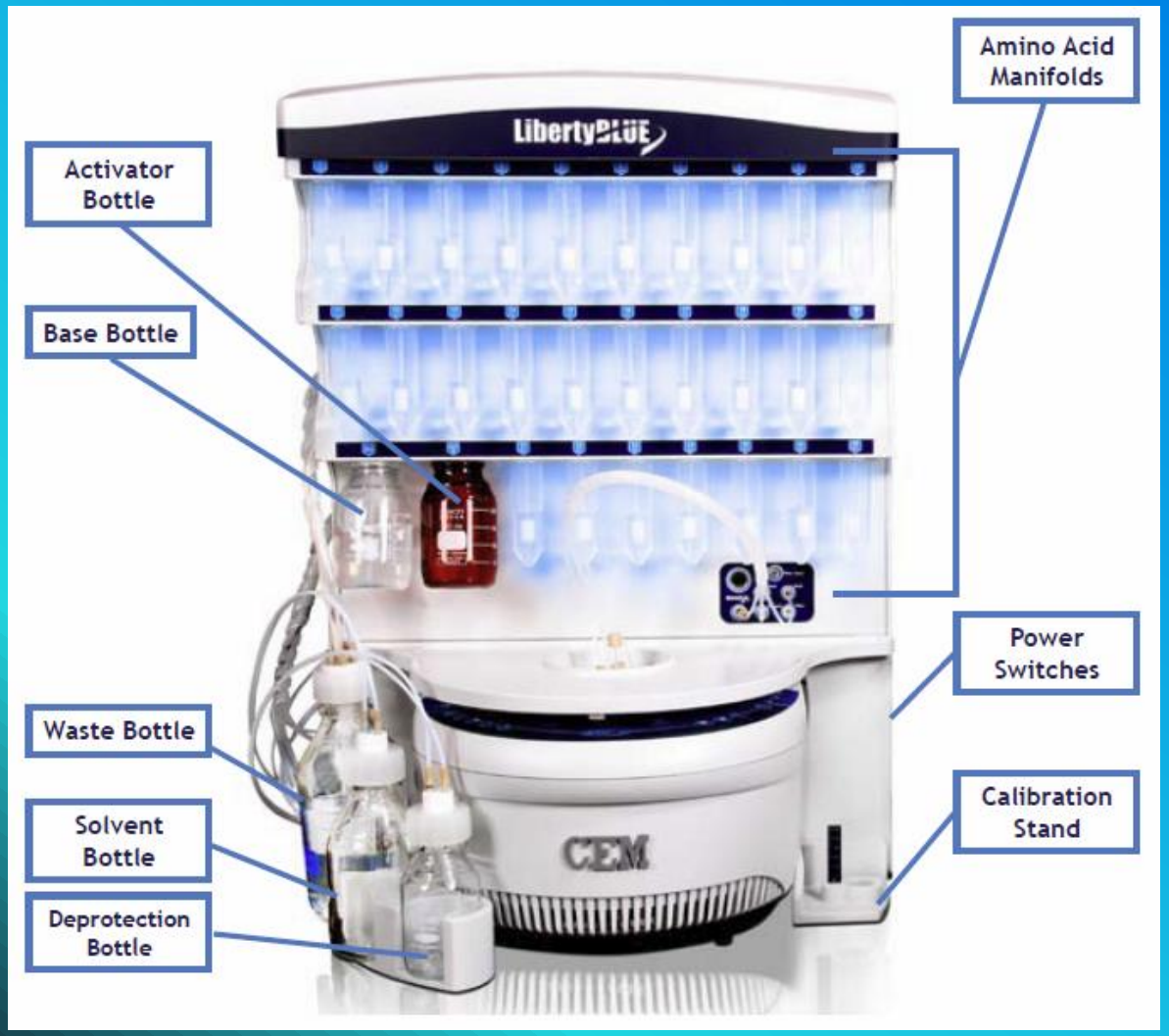
Školitel: Pavel Kopel

Datum: 24.1. 2014

Reg.č.projektu: CZ.1.07/2.4.00/31.0023

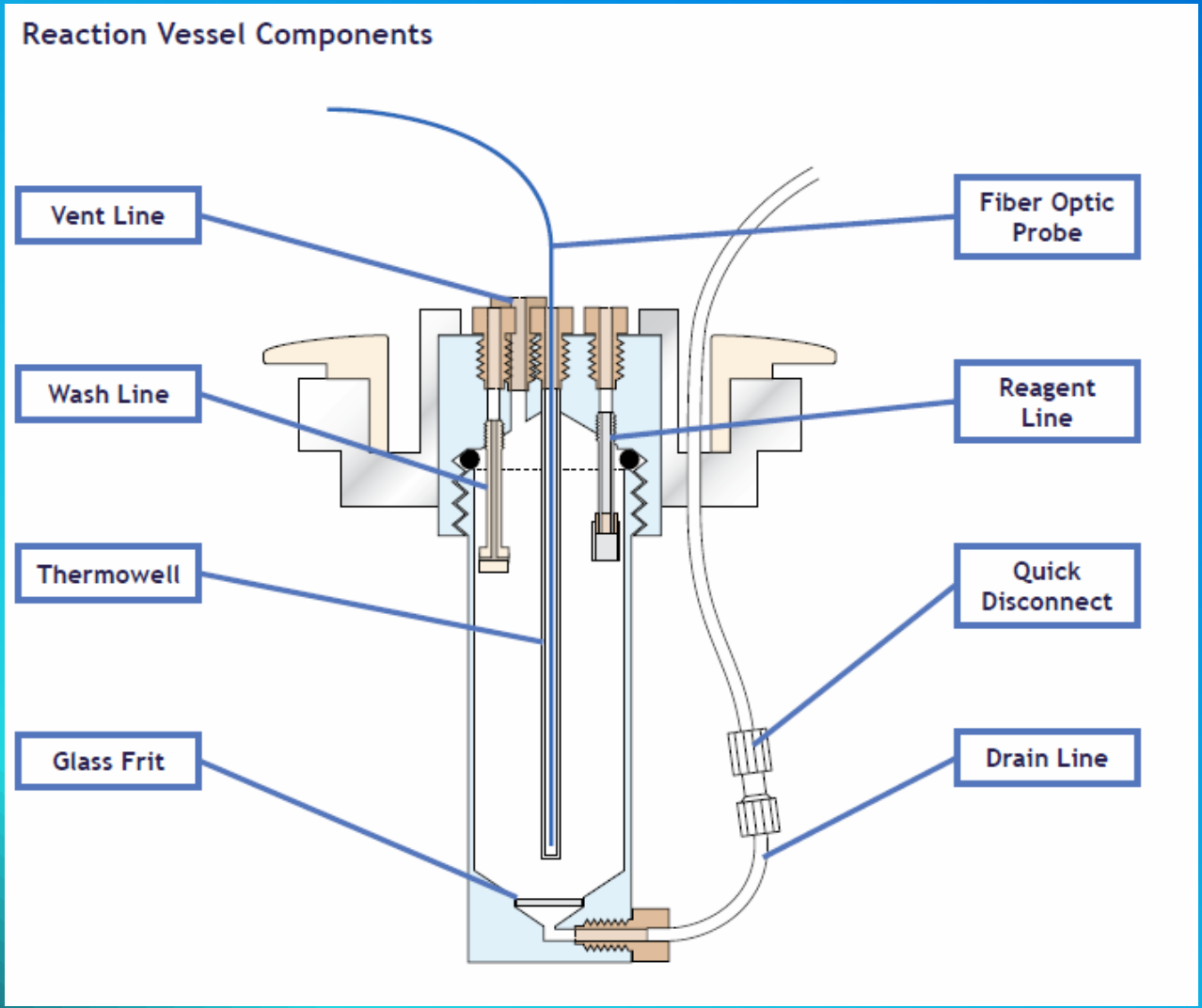
Název projektu: Partnerská síť centra excelentního bionanotechnologického výzkumu

Liberty Blue Automated Microwave Peptide Synthesizer



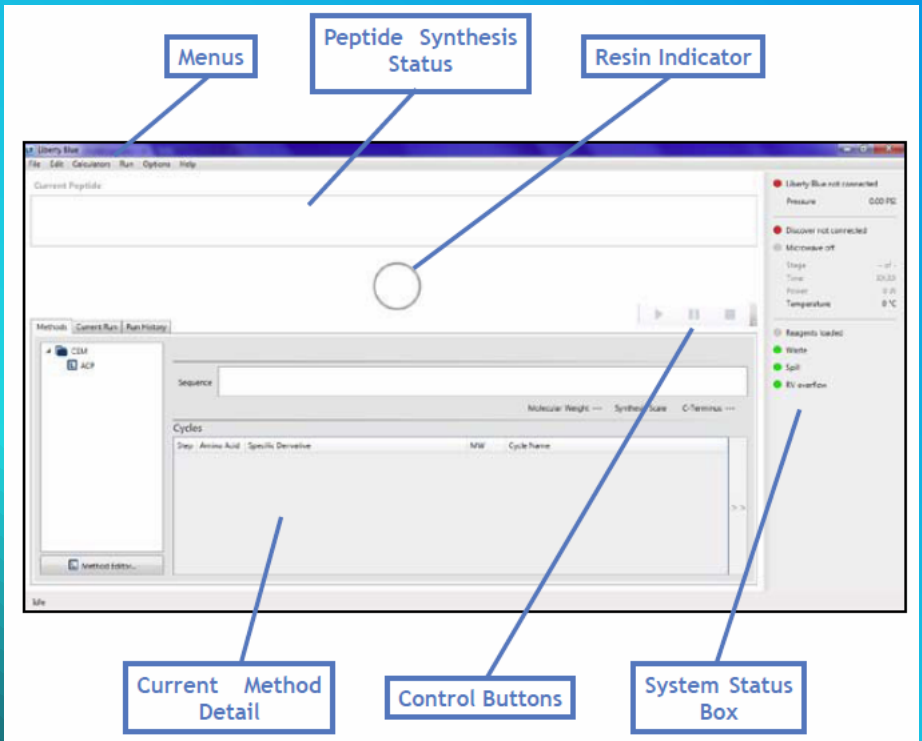
- Liberty Blue Module
- Discover Microwave Reactor
- External Bottles
- Amino Acid Manifolds
- Reaction Vessel
- Fiber Optic Temperature Probe
- Waste Container

Liberty Blue Automated Microwave Peptide Synthesizer



Liberty Blue Software

The operation of the Liberty Blue is controlled through the Liberty Blue application software - external computer connected to the Liberty through an ethernet connection.



Control Buttons

- **Start/Resume**
- **Pause**
- **Stop**

Menus

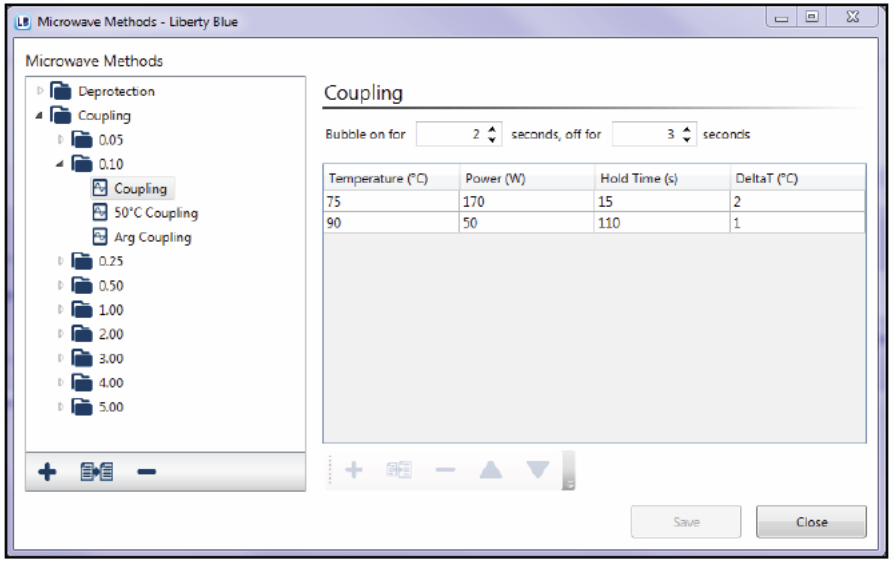
- **File**
- **Edit**
- **Calculators**
- **Run**
- **Options**
- **About**

Indicators

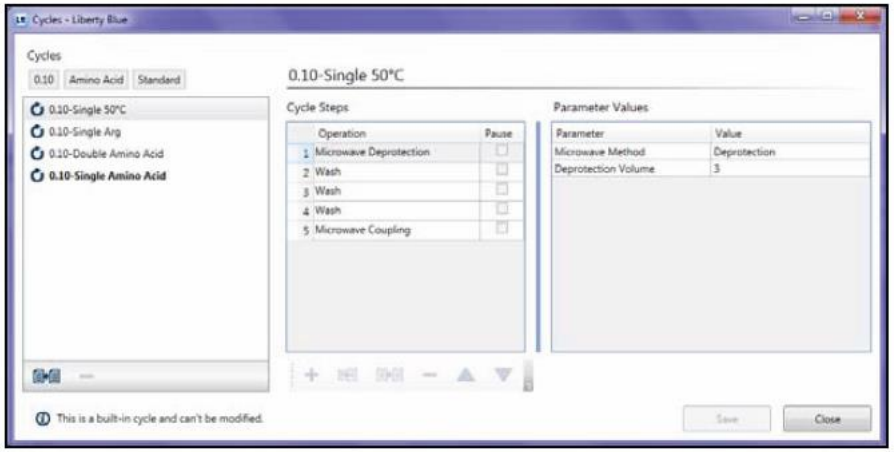
- **Resin Indicator**
- **Peptide Synthesis Status**
- **Current Method**

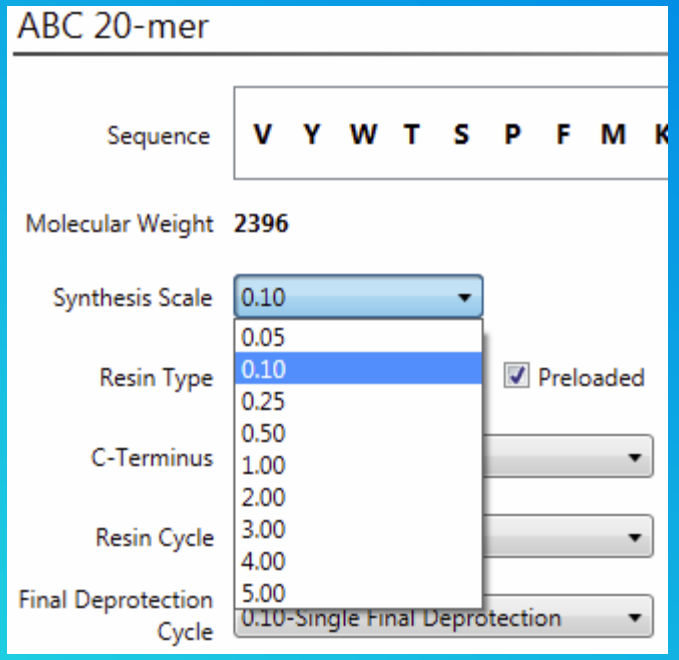
Liberty Blue Editors

Microwave Editor



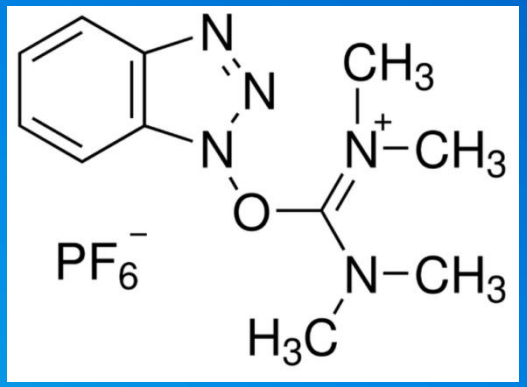
Cycle Editor



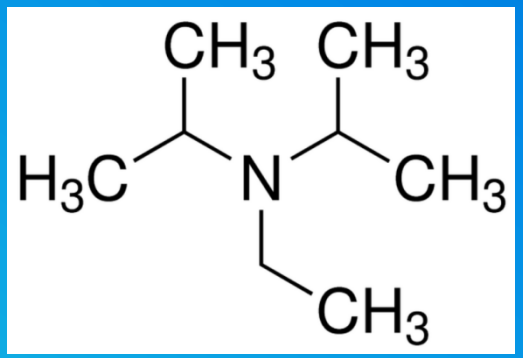


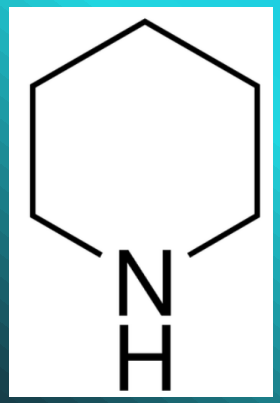
Reagents and solutions

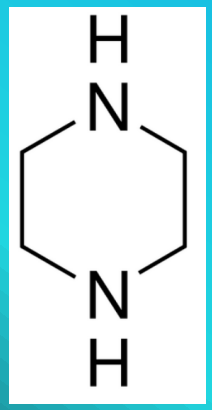
CEM Preference	Reagents	Cycle	Exceptions
1	AA/DIC/Oxyma in DMF	Single Amino Acid	His: Use 50 °C Cycle Arg: Use Double Arg Cycle
2	AA/HBTU/DIEA in DMF	Single Amino Acid	Cys: Use 50 °C Cycle His: Use 50 °C Cycle Arg: Use Double Arg Cycle
3	AA/DIC/Oxyma in NMP	Modified Single Amino Acid	Cys: Use 50 °C Cycle His: Use 50 °C Cycle Arg: Use Double Arg Cycle
4	AA/HBTU/DIEA in NMP	Modified Single Amino Acid	His: Use 50 °C Cycle Arg: Use Double Arg Cycle

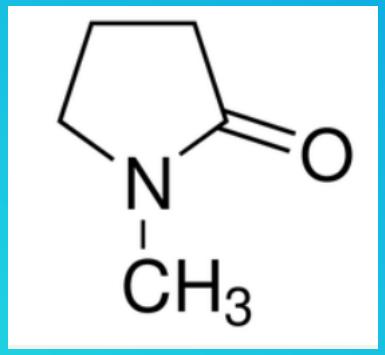


CEM Preference	Deprotection Cocktail
1	10% (w/v) Piperazine in 10:90 (EtOH:NMP)
2	20% Piperidine (v/v) in DMF or NMP



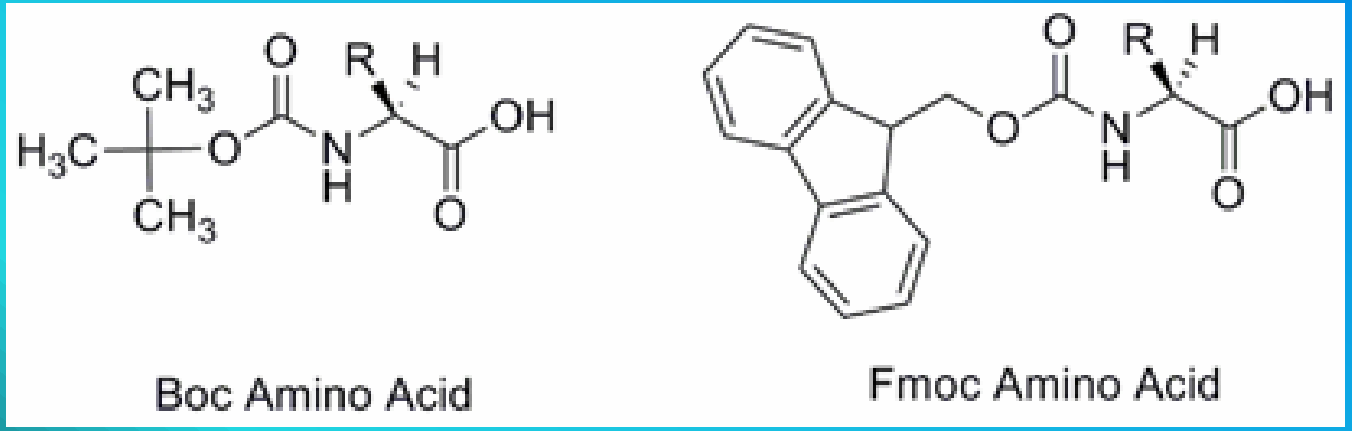






Peptide Synthesis Principles

Solid phase peptide synthesis (Bruce Merrifield, 1963)
The growing peptide chain is assembled on a solid support -resin.
The chain is built from C-terminus to N-terminus
One-by-one by reacting the free amine of the growing chain with the free carboxylic acid of the incoming amino acid.
To prevent unwanted reactions, the amine of the incoming amino acid is masked with a protecting group.
Boc synthesis utilizes the acid-labile *t*-butoxycarbonyl protecting group.
Fmoc synthesis utilizes the base-labile 9-fluoromethyloxycabonyl protecting group.

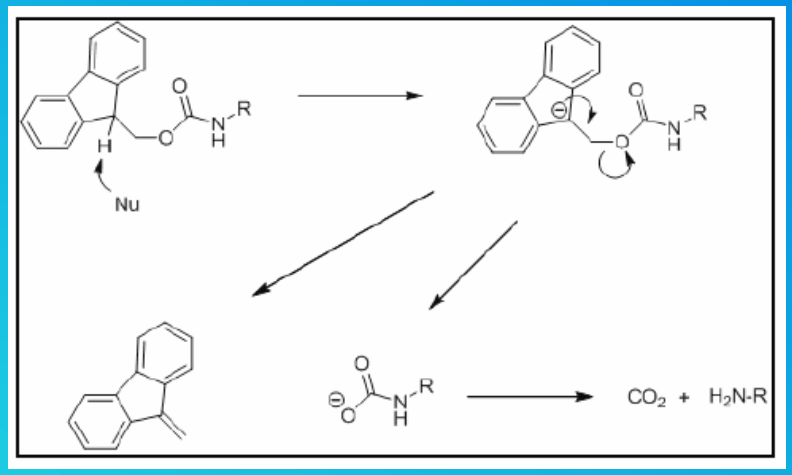


Peptide Synthesis Principles

Solid phase peptide synthesis is accomplished through the repetition of two main reactions **deprotection** of the N-terminus followed by **coupling** of the incoming amino acid

Deprotection

is accomplished with piperidine (piperazine) yielding the free amine



Coupling

Carbodiimide Coupling - the reaction of the incoming amino acid with the growing peptide chain to form an amide bond is accomplished by converting the acid into an activated form.

Final cleavage step - TFA

Peptide Synthesis Results

Maximin H5-N ILGPVLGLVSNTLDDVLGIL Mw 2020

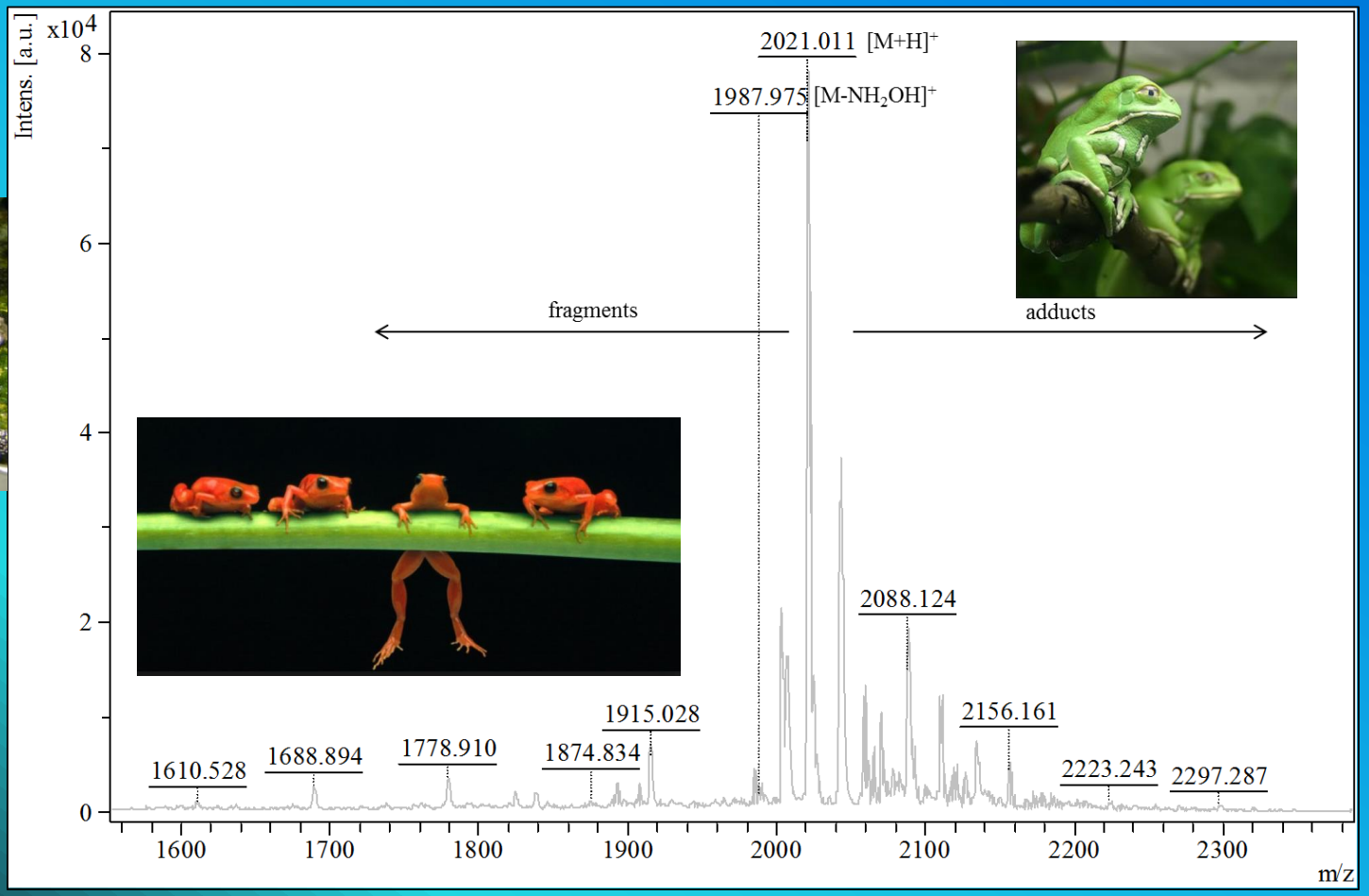
Asparagine, Aspartic acid, Glycine, Isoleucine, Leucine, Proline, Serine, Threonine, Valine.

Activator - HBTU = 2-(1 H-benzotriazol-1-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate

Activator base - DIEA (N,N-Diisopropylethylamine)

Deprotection - piperidine

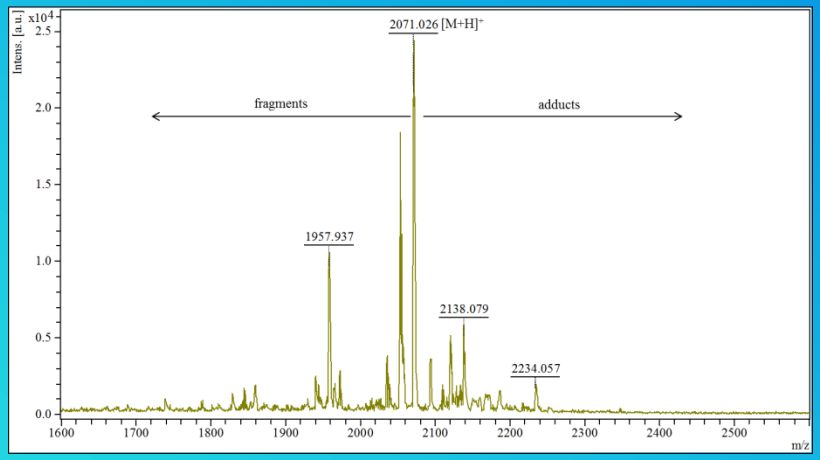
Maximin H5-N (100 µg.ml^{-1}), DHB matrix, reflector positive mode, laser 65%, 2500 averaged subspectra



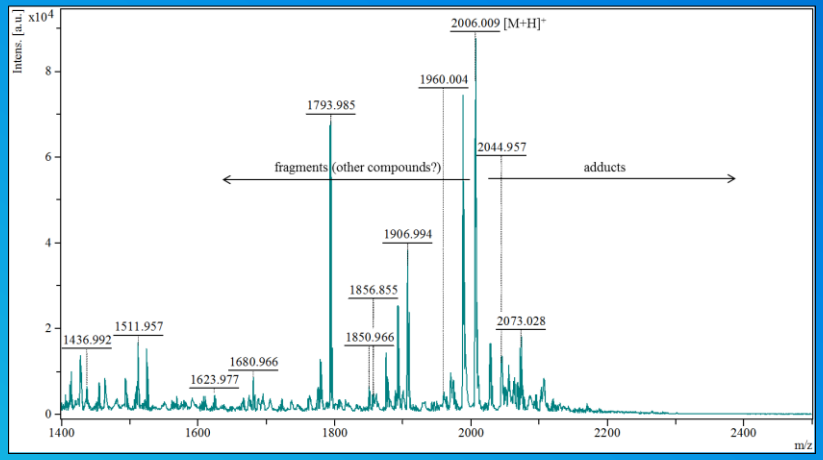
Peptide Synthesis Results

Maximin H5-H	ILGPVLGLVSHTLDDVLGIL	Mw 2071
Maximin H5-V	ILGPVLGLVSVTLDDVLGIL	Mw 2096
Maximin H5-A	ILGPVLGLVSATLDDVLGIL	Mw 1977
CD4	SSGGDPIVTH	Mw 1098

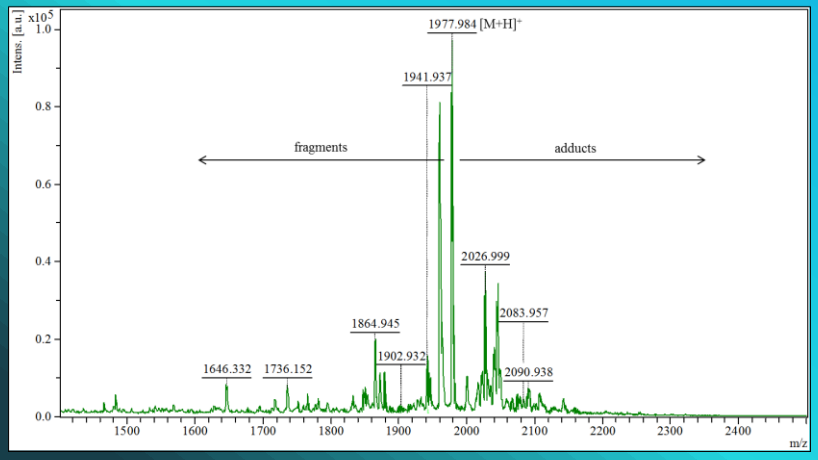
Maximin H5-H (100 µg.ml^{-1}), DHB matrix, reflector positive mode, laser 65%, 2500 averaged subspectra; M is molecule of analyte; H is atom of hydrogen



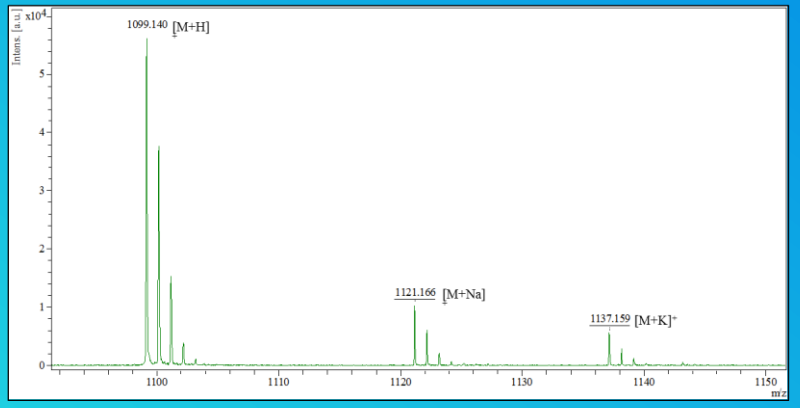
Maximin H5-V (100 µg.ml^{-1}), DHB matrix, reflector positive mode, laser 65%, 2500 averaged subspectra; M is molecule of analyte; H is atom of hydrogen



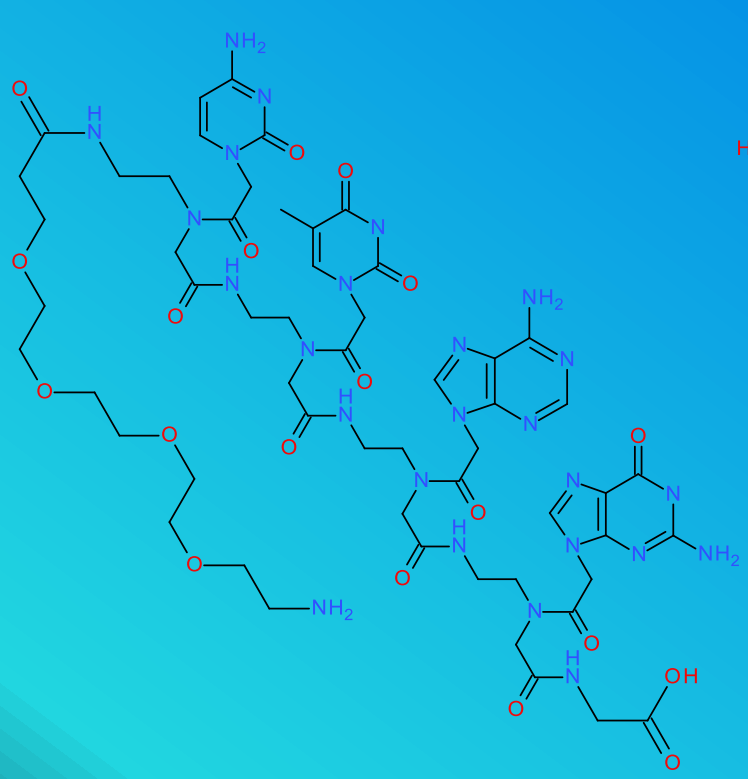
Maximin H5-A (100 µg.ml^{-1}), DHB matrix, reflector positive mode, laser 65%, 2500 averaged subspectra; M is molecule of analyte; H is atom of hydrogen

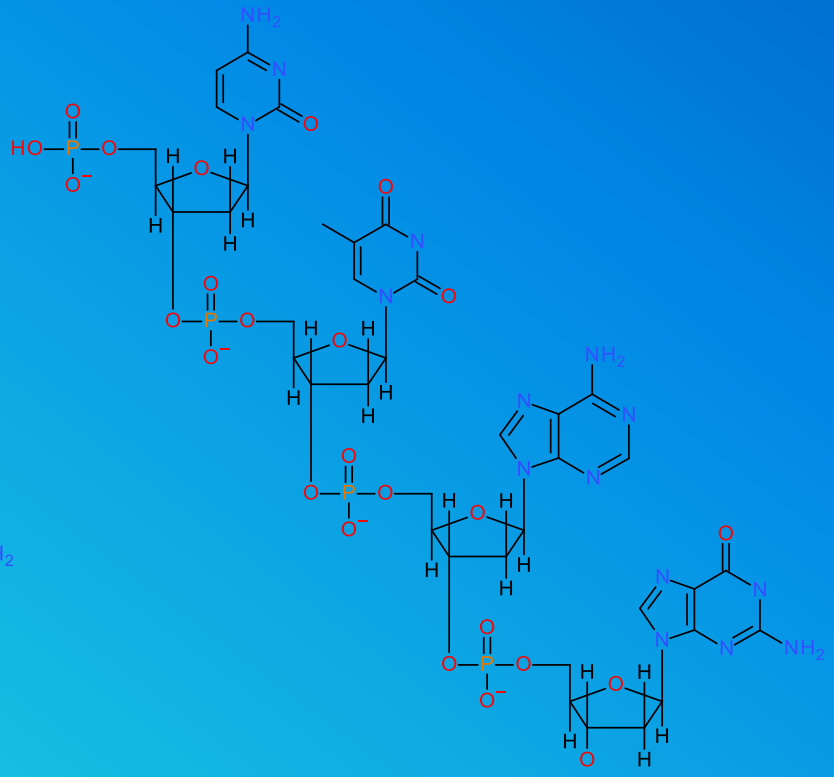


CD4 (SSGGDPEIVTH; M = 1098.13 Da), DHB matrix, reflector positive mode, laser 70 %, 2500 averaged subspectra; M is molecule of analyte; H is atom of hydrogen



Future Challenge of Peptide Synthesis and Others





Acknowledgements

All the members of Laboratory of Metalomics and Nanotechnology

Congratulations to

Amitava
Vedran

CEITEC CZ.1.05/1.1.00/02.0068

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Děkuji Vám za pozornost

Reg.č.projektu: CZ.1.07/2.4.00/31.0023

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