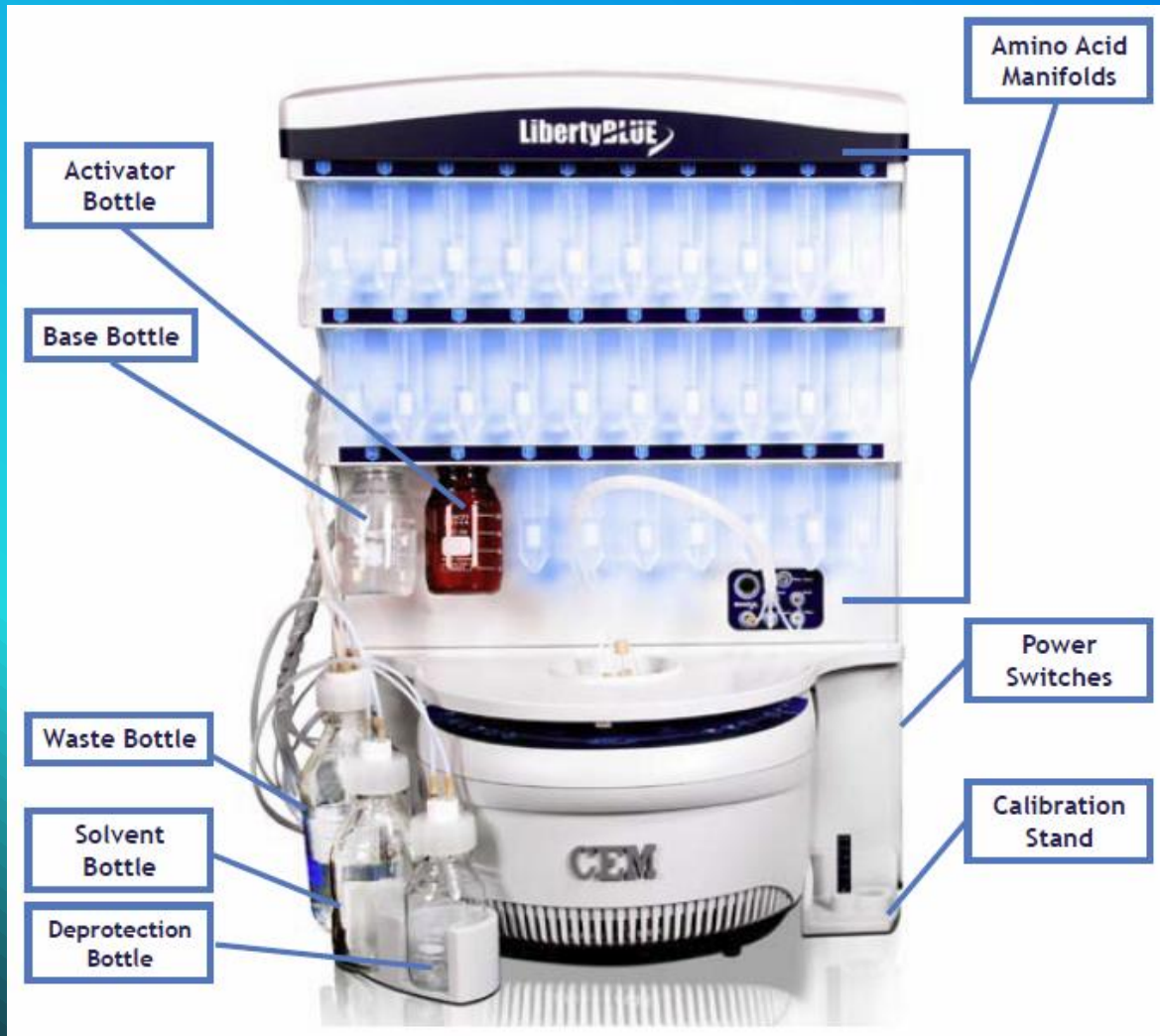


Název: **Synthesis of peptides**

Školitel: **Pavel Kopel**

Datum: **24.1. 2014**

Liberty Blue Automated Microwave Peptide Synthesizer



Liberty Blue Module

Discover Microwave
Reactor

External Bottles

Amino Acid Manifolds

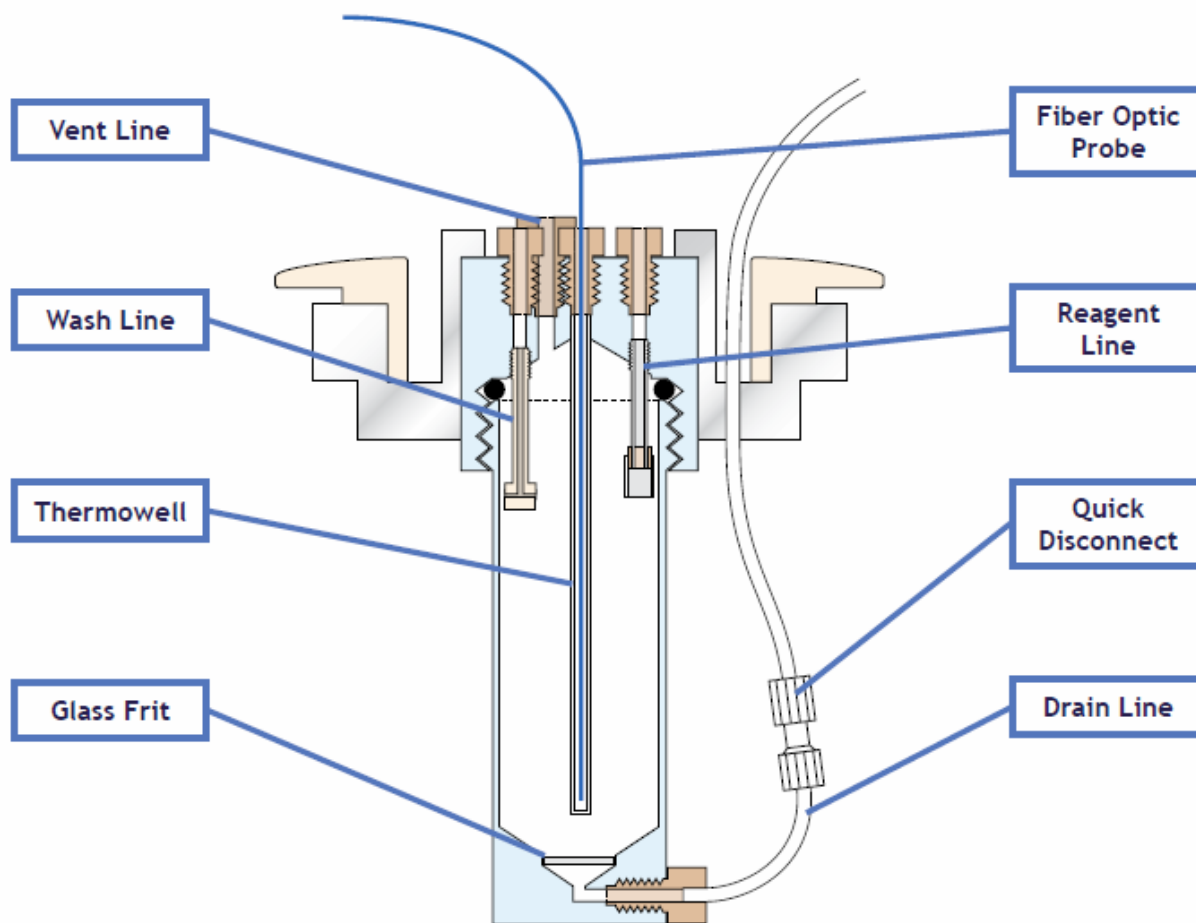
Reaction Vessel

Fiber Optic Temperature
Probe

Waste Container

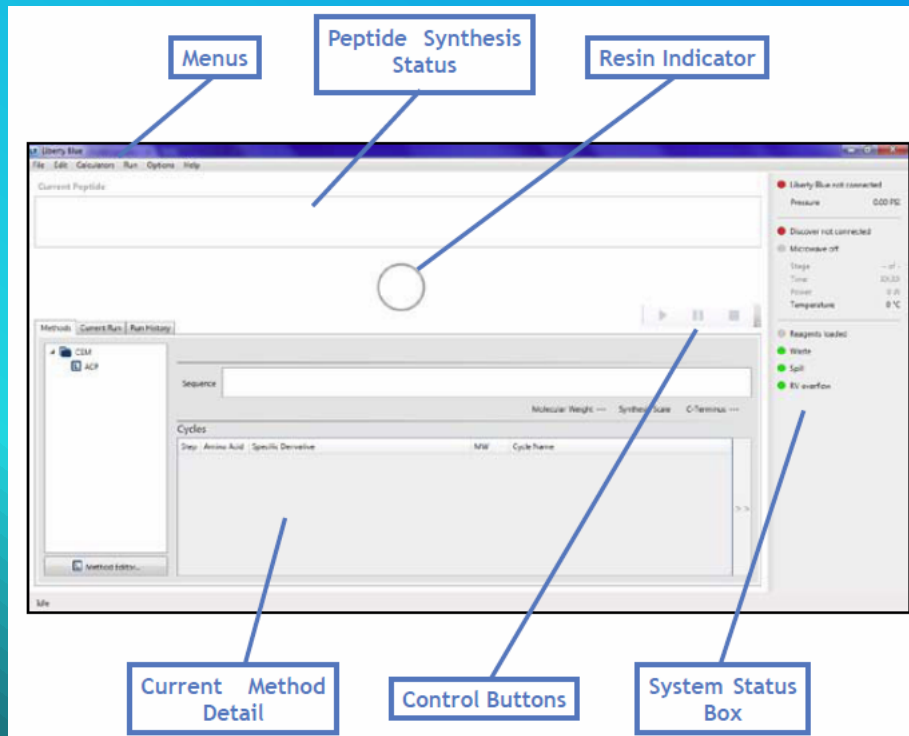
Liberty Blue Automated Microwave Peptide Synthesizer

Reaction Vessel Components



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

Microwave Methods - Liberty Blue

Deprotection

- Coupling
 - 0.05
 - 0.10
 - Coupling
 - 50°C Coupling
 - Arg Coupling
 - 0.25
 - 0.50
 - 1.00
 - 2.00
 - 3.00
 - 4.00
 - 5.00

Coupling

Bubble on for seconds, off for seconds

Temperature (°C)	Power (W)	Hold Time (s)	DeltaT (°C)
75	170	15	2
90	50	110	1

Save Close

Cycle Editor

Cycles - Liberty Blue

0.10 | Amino Acid | Standard

0.10-Single 50°C

0.10-Single 50°C
0.10-Single Arg
0.10-Double Amino Acid
0.10-Single Amino Acid

Cycle Steps

Operation	Pause
1 Microwave Deprotection	<input type="checkbox"/>
2 Wash	<input type="checkbox"/>
3 Wash	<input type="checkbox"/>
4 Wash	<input type="checkbox"/>
5 Microwave Coupling	<input type="checkbox"/>

Parameter Values

Parameter	Value
Microwave Method	Deprotection
Deprotection Volume	3

Save Close

This is a built-in cycle and can't be modified.

Sequence: V Y W T S P F M K L I E H Q C N R A D G

Weight

Scale

minus

Cycle

action

cycle

Overwrite

Delete

A Ala 1	R Arg 2	N Asn 3	D Asp 4	C Cys 5	Q Gln 6	E Glu 7	G Gly 8	H His 9	I Ile 10
L Leu 11	K Lys 12	M Met 13	F Phe 14	P Pro 15	S Ser 16	T Thr 17	W Trp 18	Y Tyr 19	V Val 20
1 EX1 21	2 EX2 22	3 EX3 23	4 EX4 24	5 EX5 25	6 EX6 26	7 EX7 27			

ABC 20-mer

Sequence: V Y W T S P F M K

Molecular Weight: 2396

Synthesis Scale: 0.10

Resin Type: 0.10 Preloaded

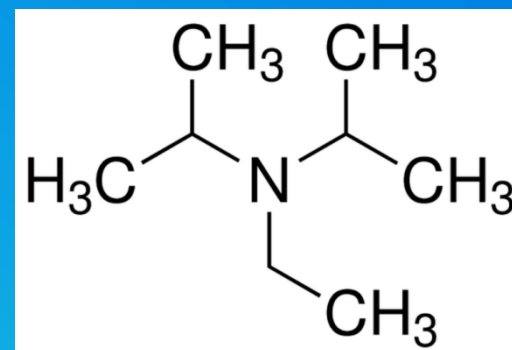
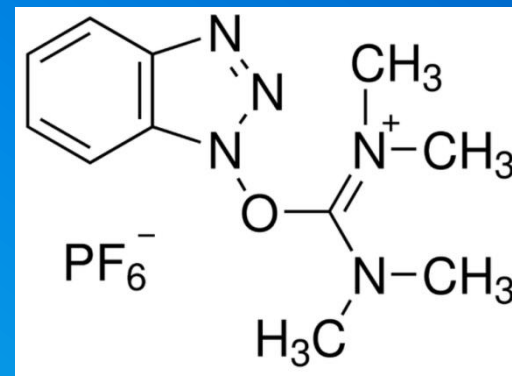
C-Terminus: 1.00

Resin Cycle: 3.00

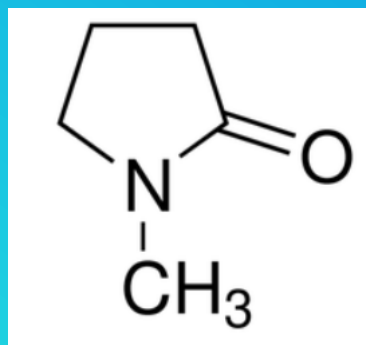
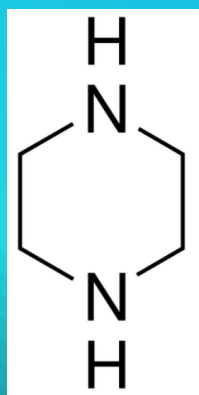
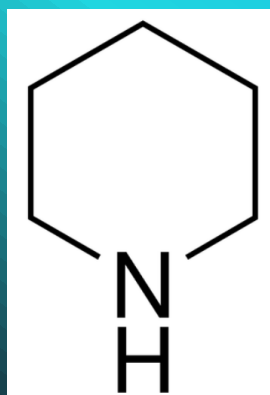
Final Deprotection Cycle: 0.10-Single Final Deprotection

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.

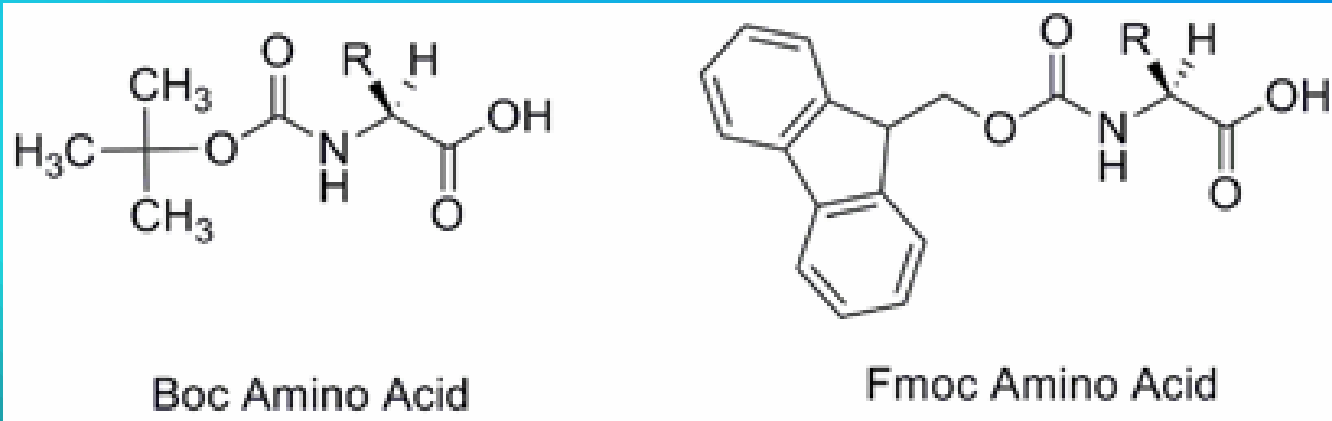
The chain is built from C-terminus to N-terminus

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-fluoromethyloxycarbonyl protecting group.

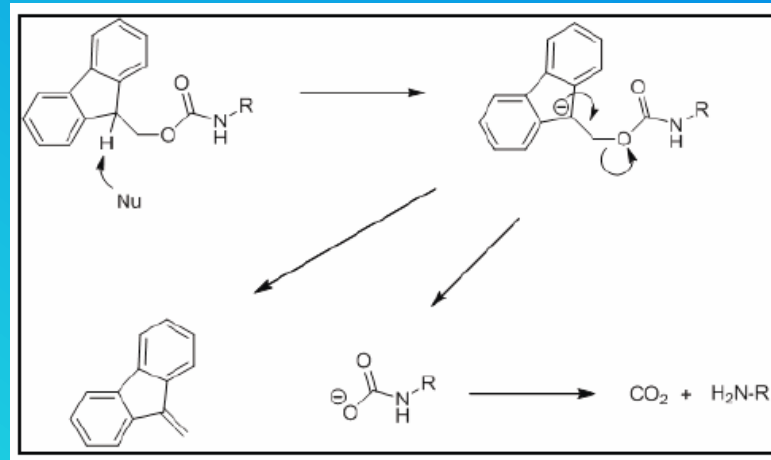


Peptide Synthesis Principles

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

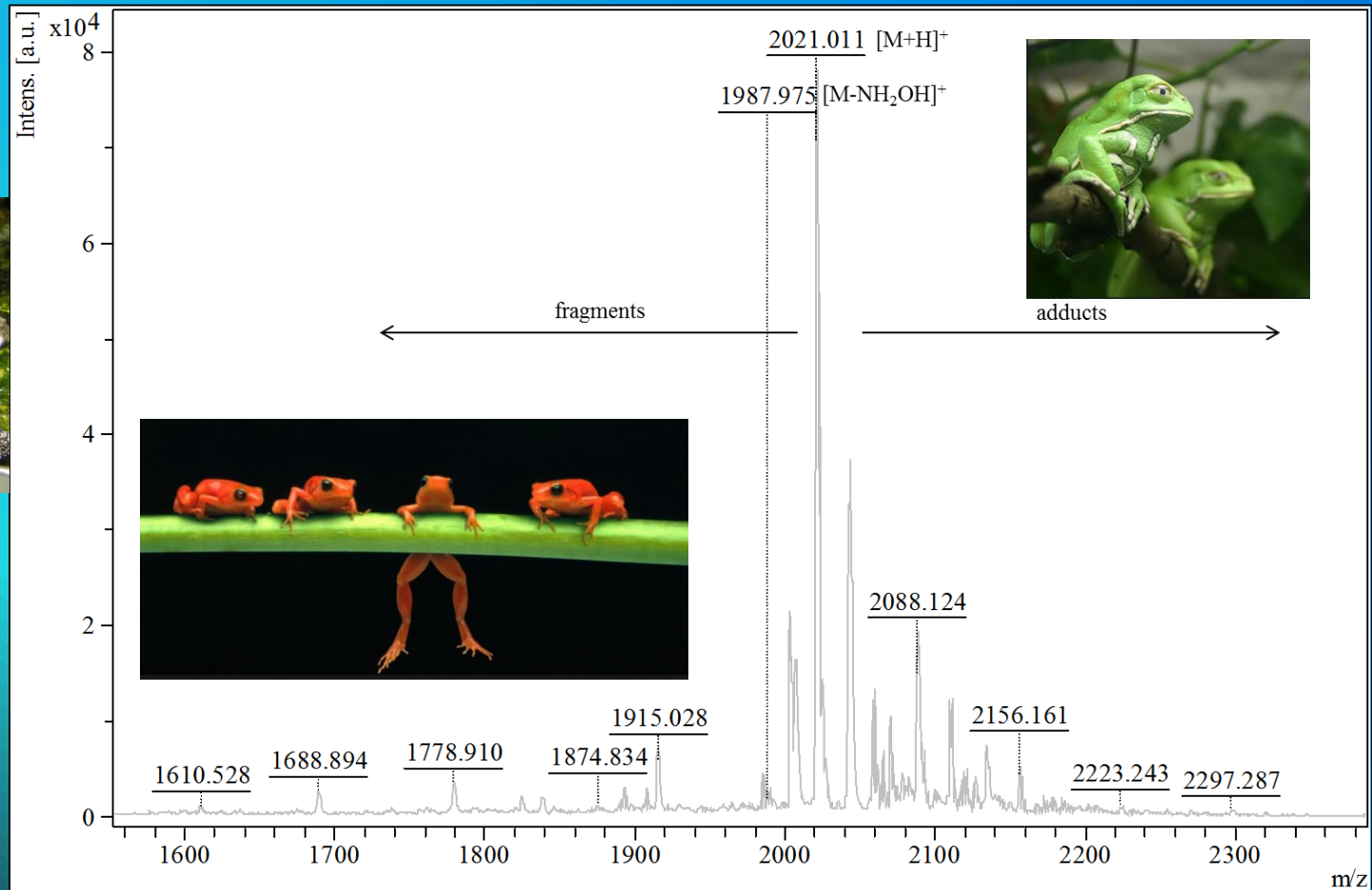
Asparagine, Aspartic acid, Glycine, Isoleucine, Leucine, Proline, Serine, Threonine, Valine.

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

Activator base - DIEA (N,N-Diisopropylethylamine)

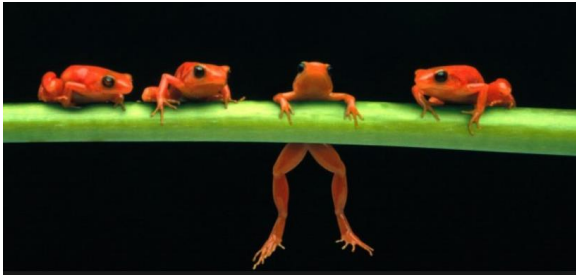
Deprotection - piperidine

Maximin H5-N (100 $\mu\text{g}\cdot\text{ml}^{-1}$), DHB matrix, reflector positive mode, laser 65%, 2500 averaged subspectra



adducts

fragments



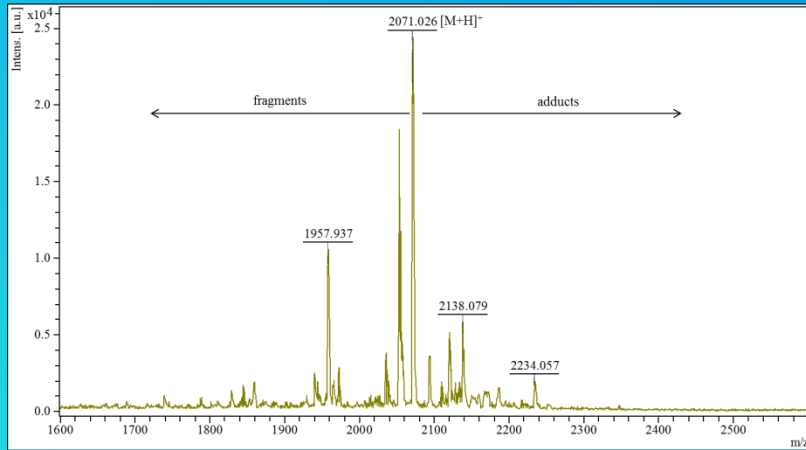
Peptide Synthesis Results

Maximin H5-H
 Maximin H5-V
 Maximin H5-A
 CD4

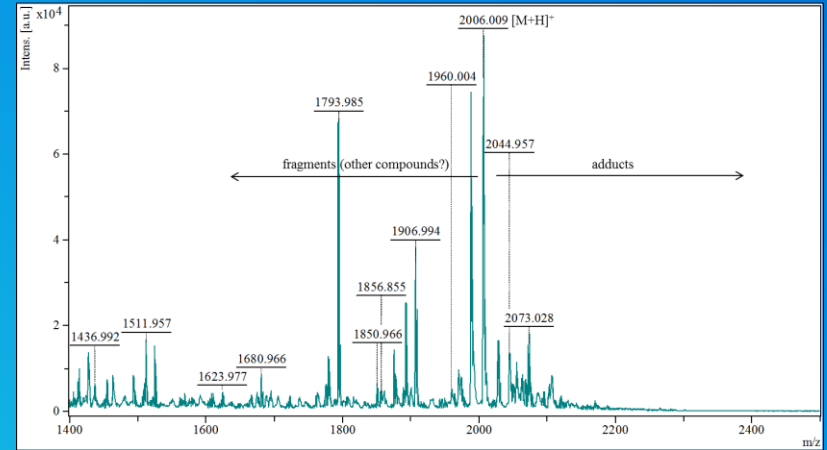
ILGPVLGLV**S**HTLDDVLGIL
 ILGPVLGLV**S**VTLDDVLGIL
 ILGPVLGLV**S**ATLDDVLGIL
 SSGDPV**I**TH

Mw 2071
 Mw 2096
 Mw 1977
 Mw 1098

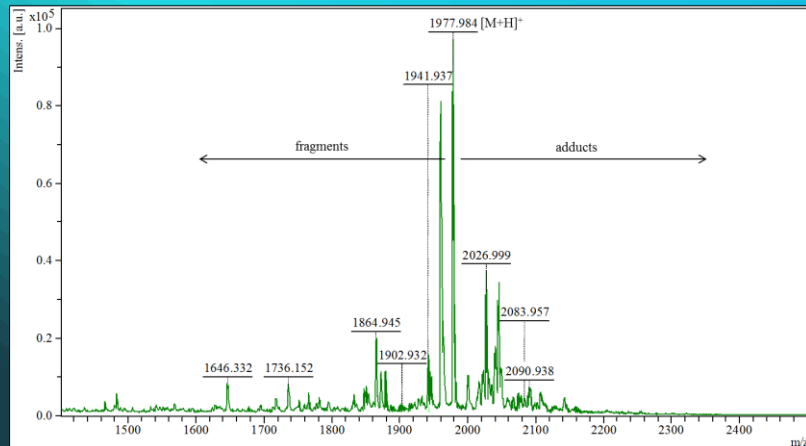
Maximin H5-H (100 µg.ml⁻¹), DHB matrix, reflector positive mode, laser 65%, 2500 averaged spectra;
 M is molecule of analyte; H is atom of hydrogen



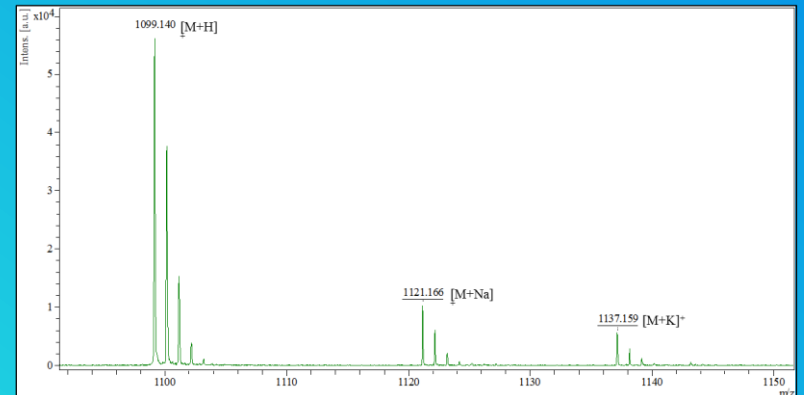
Maximin H5-V (100 µg.ml⁻¹), DHB matrix, reflector positive mode, laser 65%, 2500 averaged spectra;
 M is molecule of analyte; H is atom of hydrogen



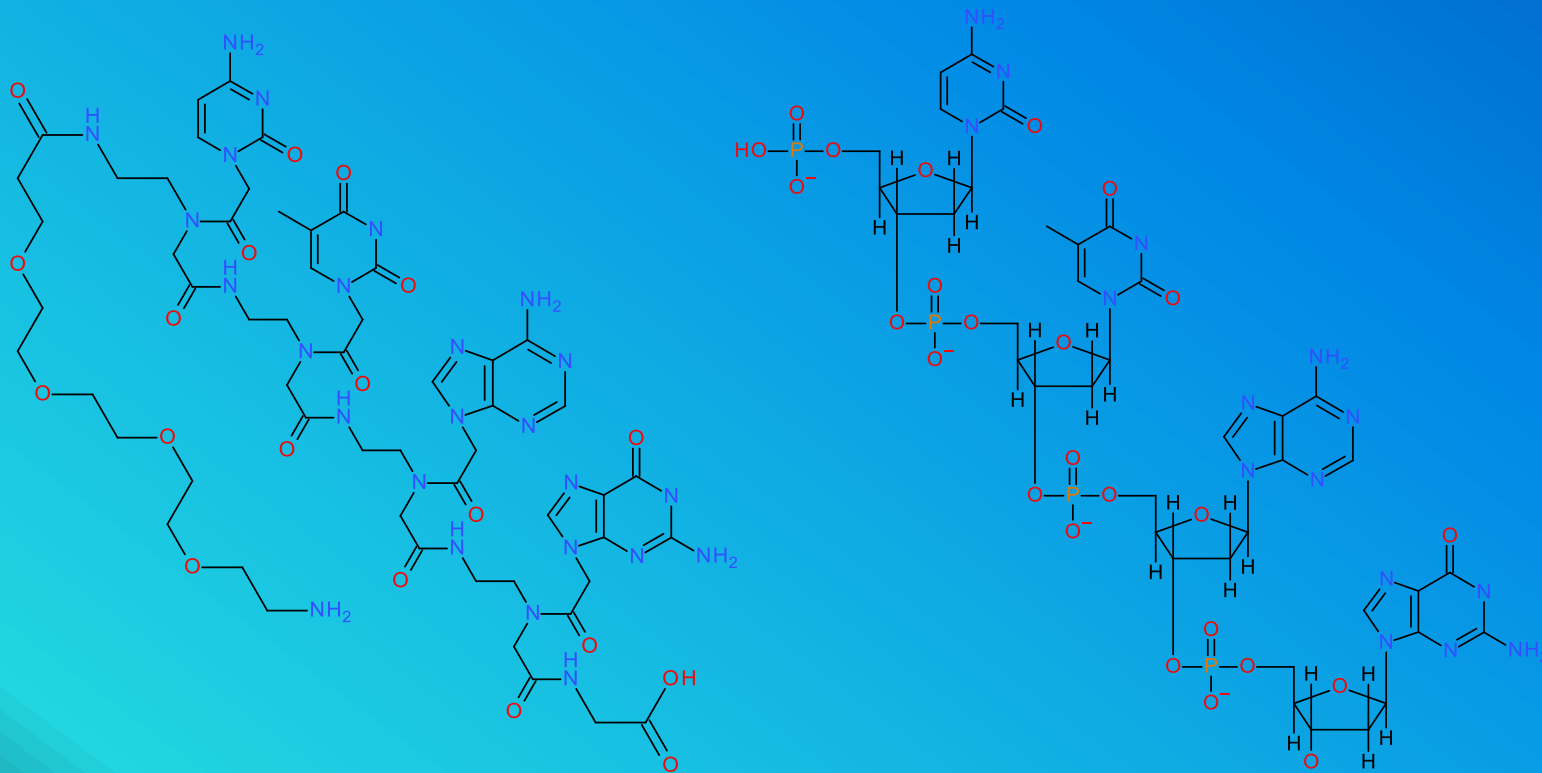
Maximin H5-A (100 µg.ml⁻¹), DHB matrix, reflector positive mode, laser 65%, 2500 averaged spectra;
 M is molecule of analyte; H is atom of hydrogen



CD4 (SSGGDPV**I**TH; M = 1098.13 Da), DHB matrix, reflector positive mode, laser 70 %, 2500 averaged spectra;
 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



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Děkuji Vám za pozornost

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

Název projektu: Partnerská síť centra excelentního bionanotechnologického výzkumu

