The New
Syro *Wave*™
Microwave &
Parallel Peptide Synthesizer
- "The Best of Both Worlds"

Biotage Lunch Seminar
5th IPS/47th JPS, Kyoto
Dec 5th 2010

Amit Mehrotra PhD
Marketing Manager – Peptide Chemistry
Product Manager – Synthesis

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Outline

- Overview of Biotage & MST
- Biotage Peptide Products
  - Peptide Synthesizers
  - ChemMatrix Resins
  - Resolux HPLC Columns
- Microwave Heating
- Syro *Wave*™
- Syro *Wave*™ Application work
- Conclusion

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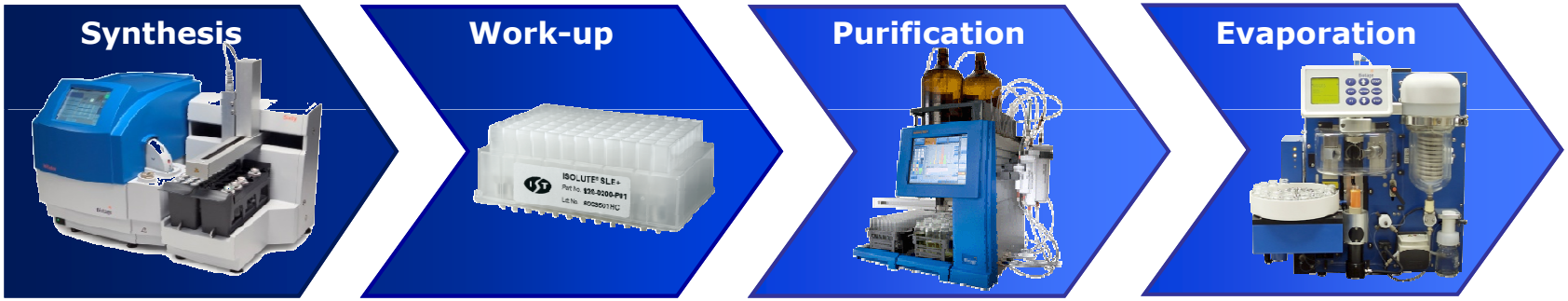
With more than 5,000 discovery chemistry systems installed in over 600 facilities worldwide, Biotage automated systems and consumables work together to increase productivity and improve success rates



Microwave-Assisted Organic Synthesis (MAOS)

Solid-Phase Extraction (SPE)

Automated Flash Purification

Rapid Solvent Evaporation

& PEPTIDES

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MultiSynTech

Biotage entered the peptide synthesis business by partnering with MultiSynTech of Germany and provides technical support with fully trained Field Service Engineers and Application Chemists worldwide

- Dr. Udo Treffer, CEO of MultiSynTech, is a peptide chemist, who developed the Syro I and Syro II robotic peptide synthesizers
- Over 150 systems installed
- Over 300 publications in technical journals using Syro systems for peptide synthesis
- Proven reliable in the most demanding applications

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Microwave Assisted Peptide Synthesis Using Biotage Instruments

Manual SPPS

M. Erdélyi, A. Gogoll, *Rapid microwave-assisted solid phase peptide synthesis*, Synthesis, **2002**, 11, 1592-1596*

M. Brandt, S. Gammeltoft, **K. J. Jensen**, *Microwave heating for solid-phase peptide synthesis: General evaluation and applications to 15-mer phosphopeptides*, International Journal of Peptide Research and Therapeutics, **2006**, 12(4), 349-357

Semi-automated SPPS

S. L. Pedersen, K. K. Sørensen, **K. J. Jensen**, Semi-automated microwave-assisted SPPS: *Optimization of protocols and synthesis of difficult sequences*, Biopolymers (Peptide Science), **2010**, 94, 206-212

Fully automated SPPS

L. Malik, A. P. Tofteng, S. L. Pedersen, K. K. Sørensen and **K. J. Jensen**, *Automated 'X-Y' robot for peptide synthesis with microwave heating: Application to difficult peptide sequences and protein domains*, Journal of Peptide Science, **2010**, 16, 506-512

* Personal Chemistry

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Peptide Market Estimates

- The market for synthetic therapeutic peptides rose from €5.3 billion in 2003 to €8 billion in 2005. It has been estimated that it will reach €11.5 billion in 2013

  Pichereau, C. and Allary, C. (2005) Therapeutic peptides under the spotlight. Eur. Biopharm. Rev. 88–91

- CBI (Commonwealth Biotechnologies Inc) believes the market for peptide drugs will achieve a compound annual growth rate of 7.5 per cent and be worth $13.4bn by 2010 (June 2009)

- The worldwide market for custom peptides is projected to grow at an annual average rate of 11.9%, valuing the market up to $1bn in 2010

  CBI market update, Euroinvestor.co.uk, March 2010

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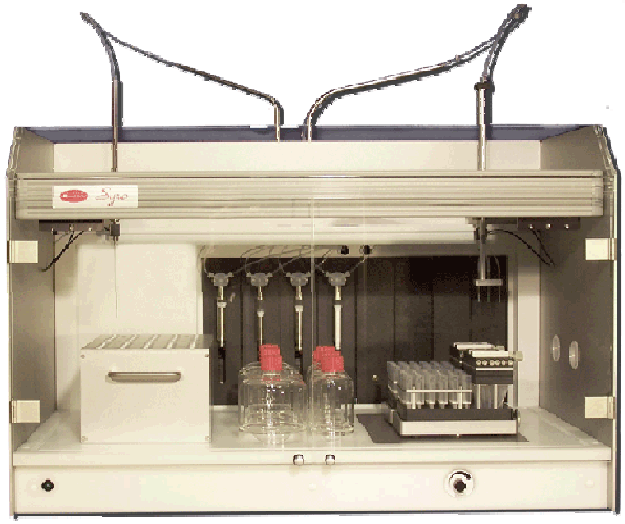
Biotage Peptide Synthesis Systems



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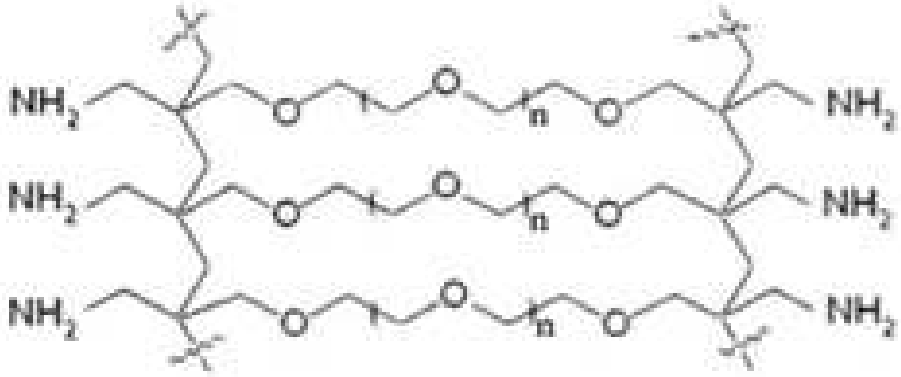
ChemMatrix Resins



If your peptide is : long, complex, or hydrophobic: → **ChemMatrix resin**

- Biotage is now distributing ChemMatrix resins
- Biotage have selected 5 of the most popular linker chemistries for SPPS (Rink, Wang, HMPB, Trityl, PAL)
- ChemMatrix is a patented 100% PEG resin from Matrix Innovation that offers substantial advantages over traditional PS & PEG based resins for SPPS
- Peptides produced with ChemMatrix - higher purity and yields

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Benefits of ChemMatrix Resin

- **Exceptional stability** – more stability for the chemistry needed in peptide synthesis
- **No Leaching** – does not add impurity to the end-users work flow
- **Excellent solvent compatibility** – organic or aqueous, water or otherwise
- **Many choices of linker and also pre-loaded options available** – we have access to a wide choice
- **Proven superior performance** – comparison of synthesis of Protease HIV-1 (99 aa) using Wang-ChemMatrix and PS resin shows significant advantages
- **Microwave compatible** – in peptide synthesizers or manual synthesis

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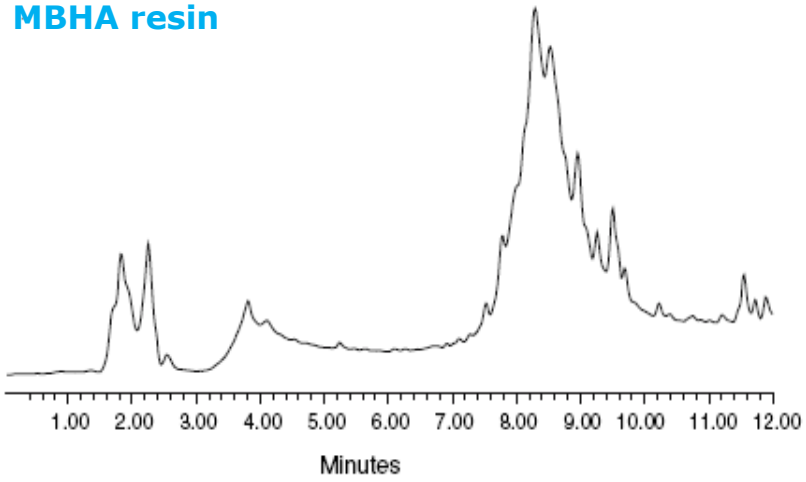
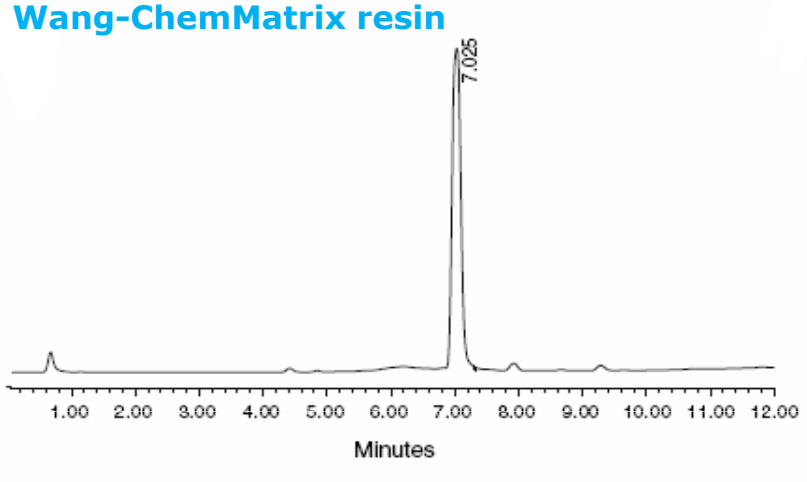
Case Study: Protease HIV-1 (99 aa)

MBHA resin



Wang-ChemMatrix resin



After 78 cycles

- **Scale: 0.1 mmole**
- **Conditions: HATU/HOAt/DIEA**
- **Cleavage: Reagent K, 2 h**

Int. J. Pept. Res. Ther. **2007**, *13*, 221-227

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The Resolux family

- R-P HPLC columns for separation of peptides and proteins
- Analytical, semi-prep & prep columns
- Three product lines, defined on the different pore sizes and each product line is available with two surface chemistries:
  - Resolux 120: A 120 Å media – available with C18 and C8 functional groups
  - Resolux 200: A 200 Å media - available with C18 and C4 functional groups
  - Resolux 300: A 300 Å media - available with C18 and C4 functional groups
- An economical choice

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Microwave Heating Gives…

- Faster and more precise heating
- Faster chemical reactions
- Greater yields and better purities
- Novel reactions

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Why Does Microwave Heating Speed Up Reactions?

Arrhenius Equation: $k = A\, e^{-Ea/RT}$

Conventional		Microwave
23°C / 12 h	=	100°C / 5 min
70° C / 16 h	=	150° C / 5 min

Reaction rate roughly doubles for every 10 °C temperature increase

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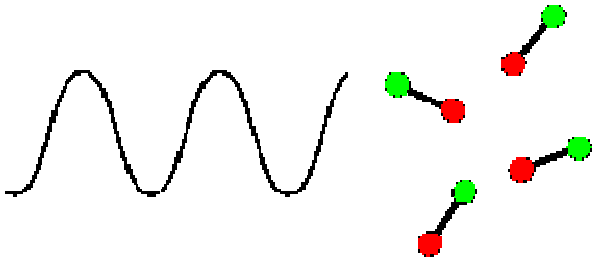
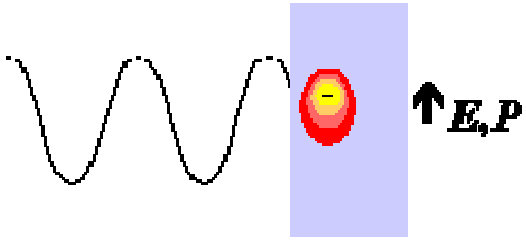
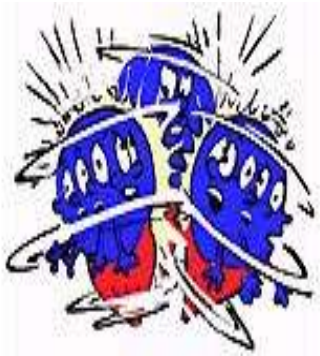
Mechanism of Microwave Heating

Dipolar oscillation



Ionic conduction





Molecular Orientation = Molecular Friction = **Heat**

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Advantages of Microwave Heating

- The rate of heating is generally higher than by conventional means
- No direct contact between the energy source and the reaction mixture
    - No temperature gradient through the sample
- The energy transfer is direct to the absorbing reactants
- Allows reactions to occur in a more controlled manner in a decreased time scale

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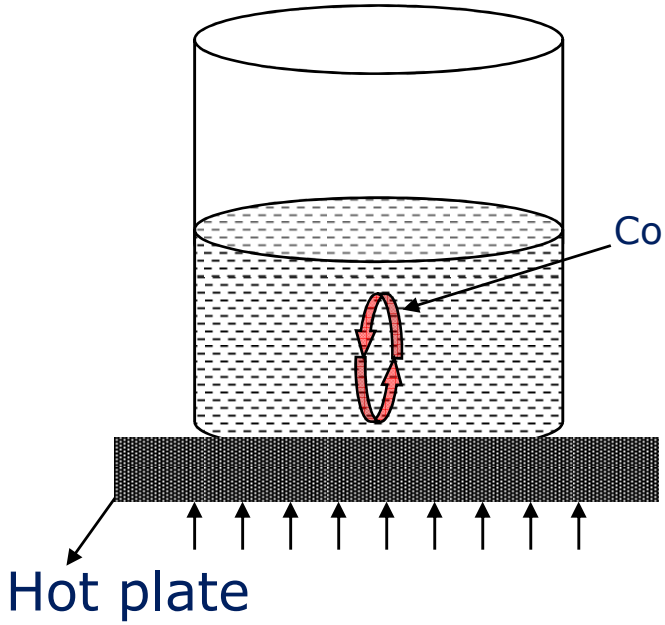
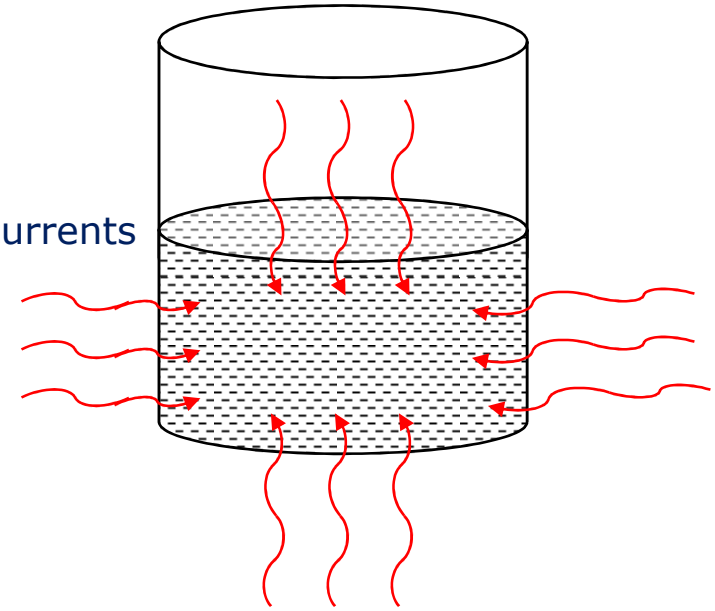
Heating in Synthesis

Indirect heating through vessel



Conventional Heating

Direct heating – transparent vessel



Microwave heating

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Syro *Wave*™ - "The Best of Both Worlds"

This unique Parallel Peptide Synthesizer with Automated Microwave Technology offers "The Best of Both Worlds" for Peptide Chemists

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Syro *Wave*™ - "The Best of Both Worlds"

The Syro *Wave* is the result of a joint development project announced last year between Biotage and MultiSynTech

It combines the proven performance of the established MultiSynTech robotic synthesizer with Biotage's expertise in microwave technology

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Syro *Wave*™ - "The Best of Both Worlds"

- Standalone parallel peptide synthesizers:
  - productivity and cost efficiency
- Standalone microwave peptide synthesizers:
  - difficult or longer peptides
- Inevitably results in:
  - Increased demand on bench space
  - Duplication of computer control systems
  - Multiple operating software platforms to learn and maintain
- Biotage has addressed these shortcomings, with the Syro *Wave*™, containing both parallel and microwave technologies on the same platform

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Biotage Syro *Wave*™ - Specification

Includes PC, Monitor, Printer
& Syro XP Software

- Microwave cavity
- Single robotic arm
- 1 x digital syringe pump
- 2 Vortex mixers: μWave & parallel
- 24 or 48 position reactor block
- Vacuum Pump
- Amino Acid Rack
- Reagent Bottle Rack
- Waste Bottle

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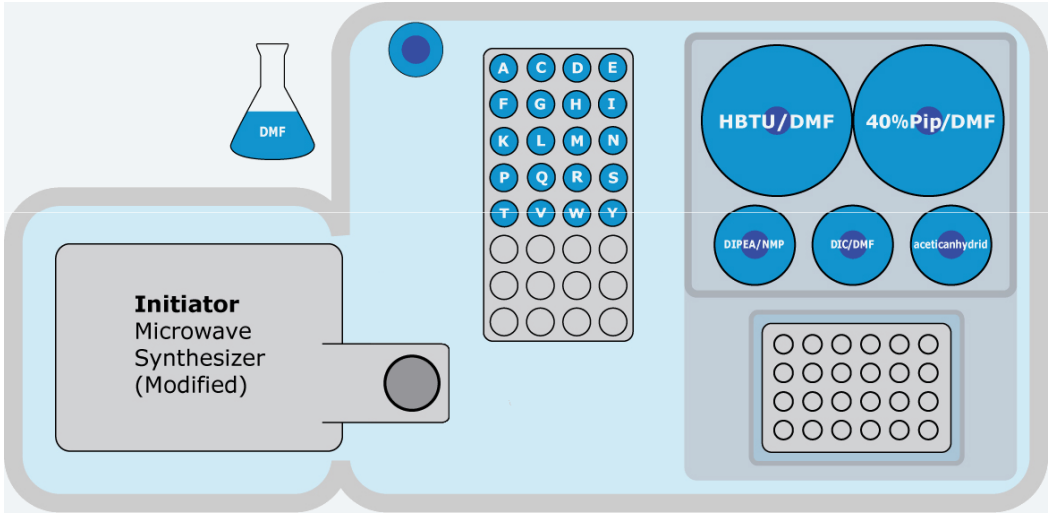
Biotage Syro *Wave*™



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Syro *Wave*™

- The Biotage Syro *Wave*™ is the **first** valve-free peptide synthesizer with integrated microwave heating
- The system offers unequalled productivity for peptide synthesis with the ability to run 24, 48 or 96 reactions in parallel at RT
- The additional microwave cavity can reduce cycle times and synthesize a long peptide in the shortest possible reaction time
- Unlike other systems that use complex valve modules and compressed air transfer of reagents, the Syro *Wave*™ system delivers precise volumes of each reagent with a robotic liquid handler. This design minimizes amino acid consumption and therefore reduces synthesis cost
- Proven history of performance and reliability

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Syro *Wave*™ - Key Features & Benefits

- All amino acids and reagents are delivered by a digital syringe pump for the highest accuracy and minimum reagent use
- Dedicated vortex mixer for MW allows for homogeneous heat distribution in the reactor vial
- Disposable reactor vials - avoid wasting time cleaning reactors or replacing blocked frits
- Separate ("off-line") cleavage & transfer workstation removes any bottlenecks in synthesis

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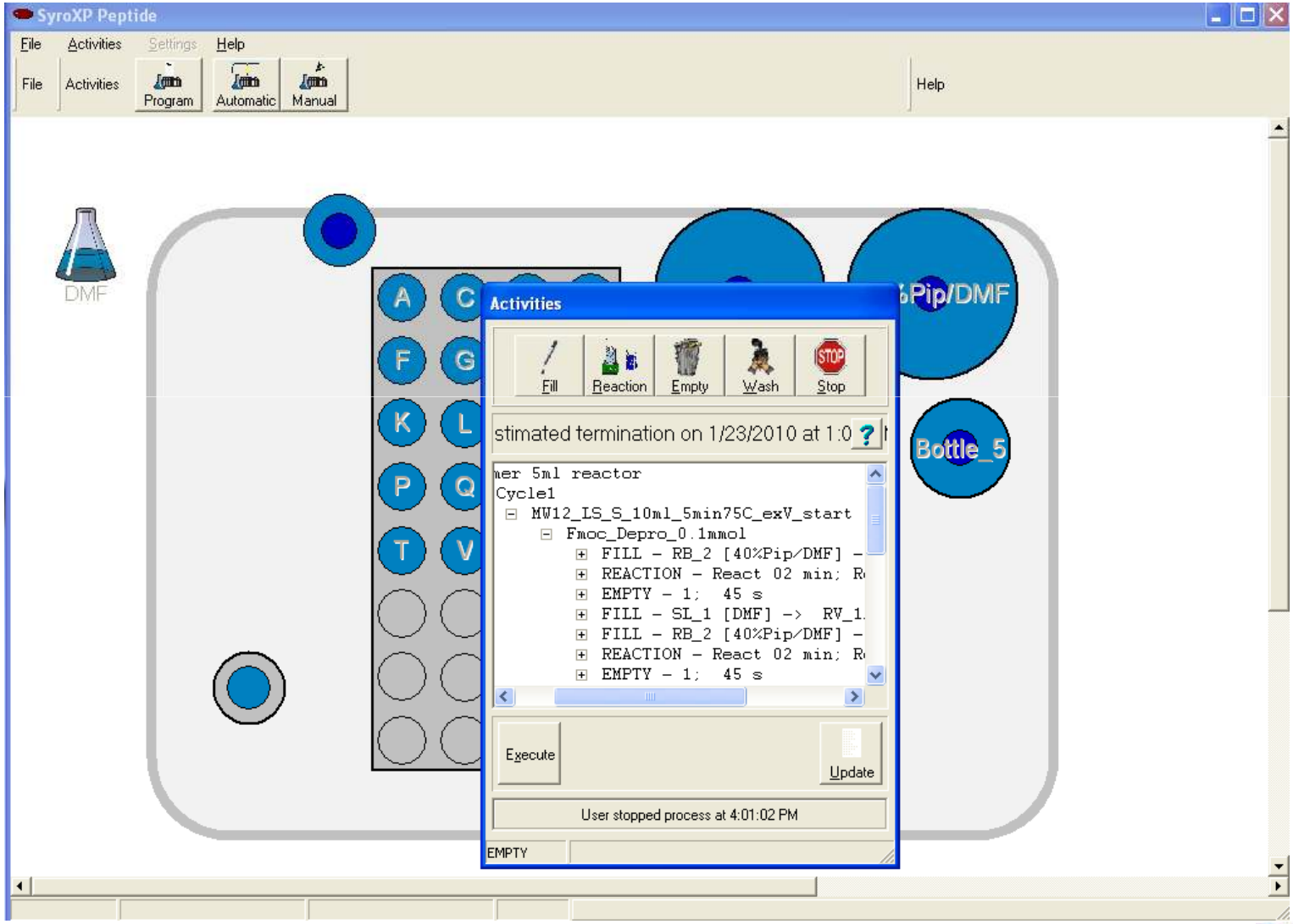
Syro XP Software



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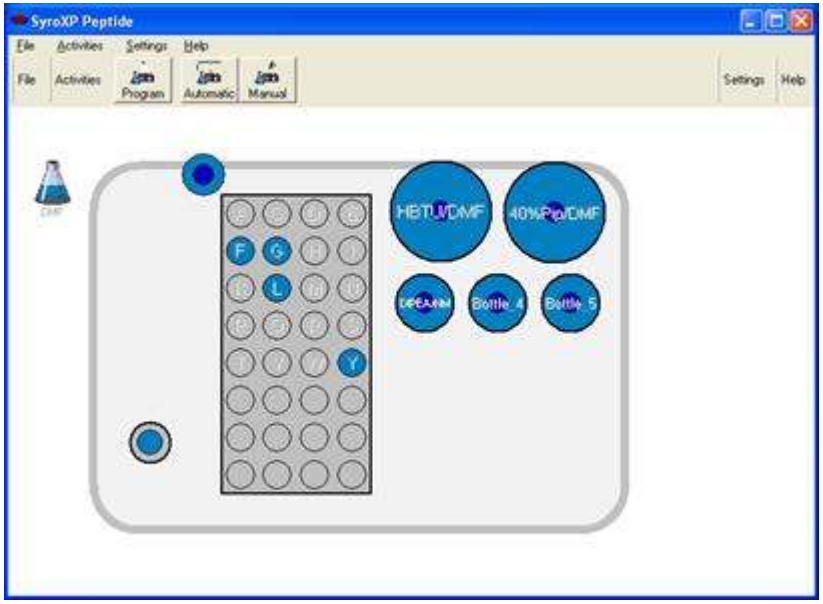
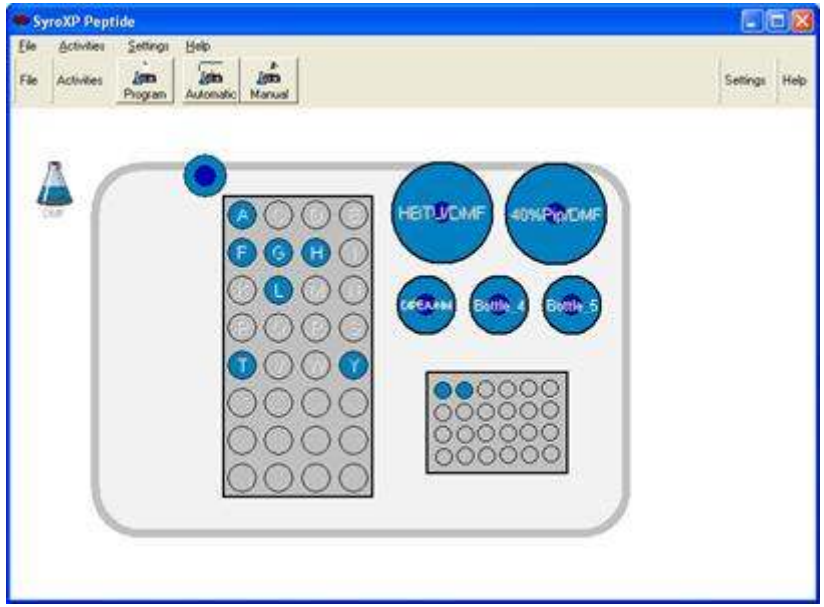
Syro XP Software

The Workplace is an active component of the programming desktop





Chemfile - covers all the commands for a reaction step

Workfile - covers all the commands for an entire synthesis cycle

Synthesis file - containing all the cycles for a complete synthesis

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Consumables & Accessories

Vial Loading Tool

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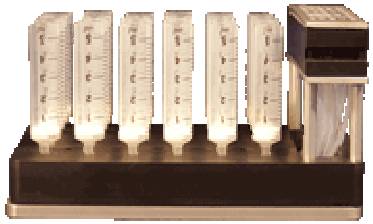
Consumables & Accessories

5 ml PP reactor vial
with PTFE frit
Disposable



50 ml AA vessel
Re-usable/disposable

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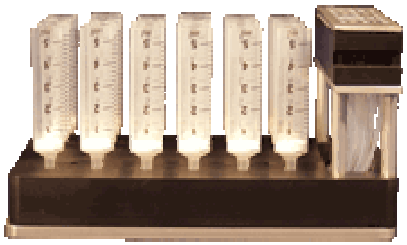
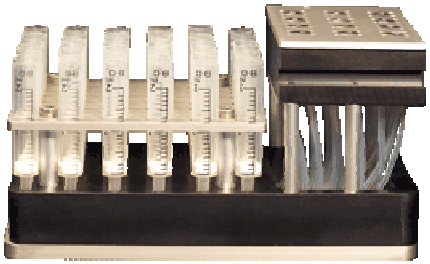
Reactor Block Configurations





24 x reactor block

2-ml reactor vials for 5-50 µmole

or

5 ml reactor vials for 25-150 µmole

or

10 ml reactor vials for 50-300 µmole

1 x Syro *Wave*™ & Syro I, 2 x Syro II

48 x reactor block

2 ml reactor vials for 5-50 µmole

1 x Syro *Wave*™ & Syro I, 2 x Syro II

96 x tip reactor (optional)

0.4 ml pipette tips for 1-5 µmole

1 x Syro *Wave*™ & Syro I, 3 x Syro II

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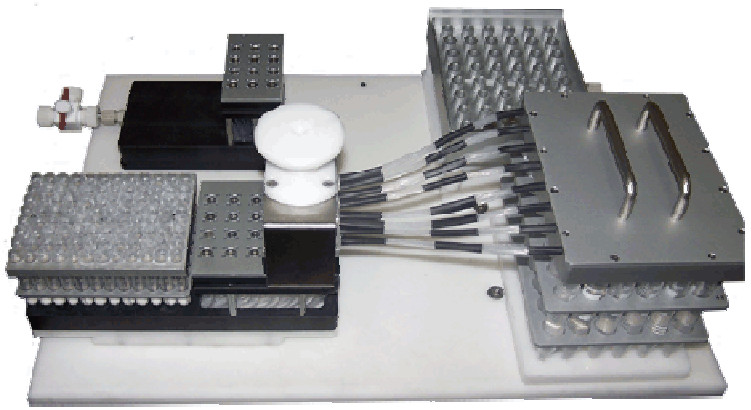
Cleavage & Transfer Workstation



Parallel cleavage & transfer

3-configurations:

- 24 position reactor block
- 48 position reactor block
- 96 position Tip reactor block

- Pressure mediated transfer of the cleavage solution into the transfer rack
- Simple and reliable manual operation
- This off-line cleavage and transfer process improves throughput

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Syro *Wave*™ - Summary of Advantages

- Microwave and parallel peptide synthesis capability
  - 3 systems in 1
    - Single channel microwave peptide synthesizer
    - Parallel peptide synthesizer RT
    - Single channel peptide synthesizer RT
  - versatile solution to synthesize difficult sequences
  - higher yield for long peptides (and short)
  - faster reactions for reduced synthesis time
  - reduced reagent consumption and lower cost
  - improved reliability

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What is a difficult sequence?

So called **"difficult sequences"** are problematic if not impossible to synthesize using standard coupling and deprotection protocols

Difficulties are mainly related to:

- Intra- and/or intermolecular aggregation
- Secondary structure formation
- Steric hindrance of protecting groups which can generate premature termination of the sequence

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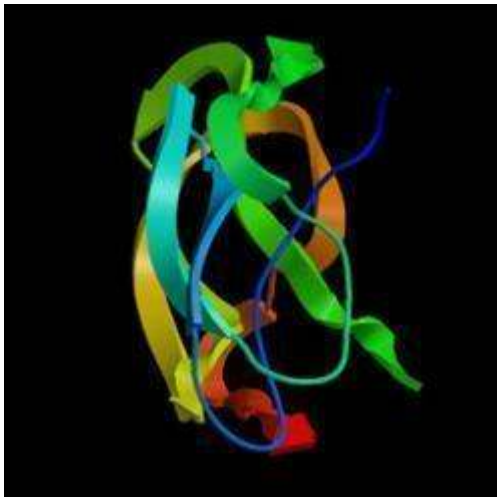
β-amyloid (1-42) peptide

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA



β-amyloid (1-42) peptide is the major component of the neurological plaques in Alzheimer's patients

Synthesis is difficult due to reported on-resin aggregation and folding due to hydrophobic C-terminus

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β-amyloid (1-42) peptide reagents

- **Resin**: Fmoc-RAM-TG resin 0.24 mmol/g loading
- **Amino Acids:** 770-μL of 0.5M Fmoc-AA in DMF with HOBt
- **Coupling:** + 880 μL of HBTU/DMF (0.45 M)
  + 380 μL of DIPEA/NMP (2.0 M)
- **De-protect:** 2000 μL of 40% piperidine in DMF
- **Wash:** DMF
- **Notes:** Fmoc-Phe-OH in 0.5M NMP with HOBt (better solubility than in DMF)

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β-amyloid (1-42) peptide method

- **Synthesis Scale:** 100 µmol
- **Deprotection:** 3 min with 40% piperidine in DMF
  +10 min with 20% piperidine in DMF
- **Wash:** 4 x 30 sec with DMF
- **Coupling:** 1 x 45 min for RT
  or 1 x 5 min @ 75 ^{0}C (microwave)
- **Wash:** 3 x 30 sec with DMF

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Synthesis of β-amyloid (1-42)

H-DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA-NH_2 (**1**)

Different reaction conditions for the synthesis of 1 using RAM-*S*-TG resin (0.24 mmol/g):

Entry	Deprotection*	Coupling** Temperature	Coupling time	HPLC purity*** (%)	Overall synthesis time
1	3 + 10 min, RT	RT	1 x 45 min	54	54 h
2	3 + 10 min, RT	75ºC	1 x 5 min	73	26 h

*Deprotection: 3 min with 40% pip in DMF followed by 10 min with 20% pip in DMF at RT

**Couplings reagent HBTU / HOBt

***Cleavage: TFA-TES (97.5:2.5) 1ml for 2h. Then add EDT (16µl) and TMSBr (13µl) for 15 min.

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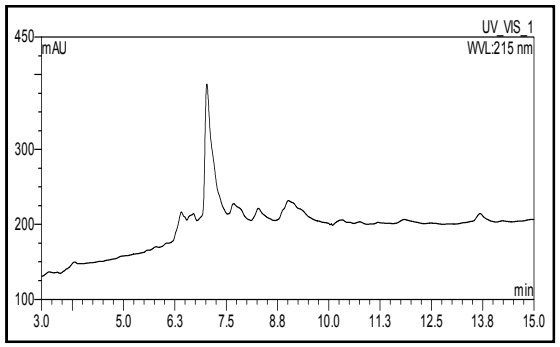
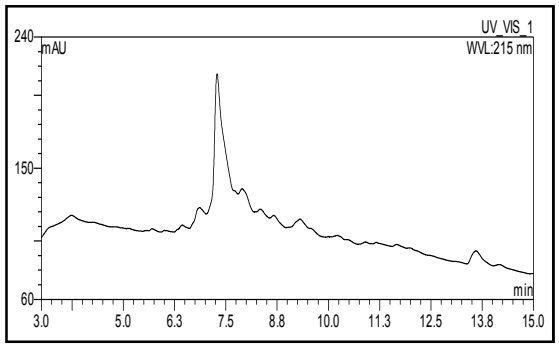
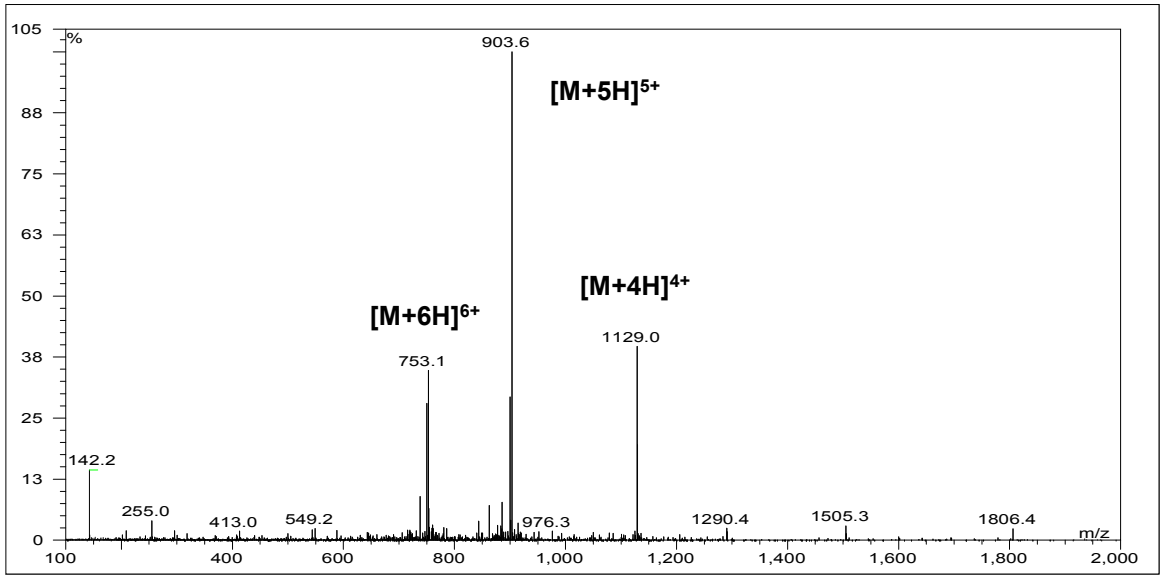
Synthesis of β-amyloid (1-42)

45 min RT, 54% purity



5 min 75 ºC, 73% purity





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LysM Domain - H-LPERVKVVFPL-NH_2

- The LysM (lysine motif) domain is believed to be involved in the regulation of the interaction between plants and rhizobial bacteria to promote plant growth

- The LysM domain is predicted to consists of two α-helices and a two-stranded anti-parallel β-sheet in a β-α-α-β structure

- The peptide H-LPERVKVVFPL-NH_2 is derived from the *C*-terminus of the LysM2 domain and is difficult to synthesize as it contains several β-branched and bulky amino acid residues

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LysM Domain - H-LPERVKVVFPL-NH_2

H-LPERVKVVFPL-NH_2 (**2**)

Different reaction conditions for the synthesis of 2 using RAM-*S*-TG resin (0.24 mmol/g):

Entry	Deprotection*	Coupling** Temperature	Coupling time	HPLC purity*** (%)	Overall synthesis time
1	3 + 10 min,RT	RT	1 x 45 min	52	~14 h
2	3 + 10 min,RT	RT	2 x 45 min	62	~ 24 h
3	3 + 10 min,RT	RT	2 x 120 min	57	~ 52 h
4	3 + 10 min,RT	75ºC	1 x 5 min	70	~ 6½ h
5	3 + 10 min,RT	75ºC	2 x 5 min	71	~ 9½ h
6	3 + 10 min,RT	75ºC	2 x 10 min	77	~ 11½ h

*Deprotection: 3 min with 40%pip in DMF followed by 10 min with 20%pip in DMF
**Coupling reagents HBTU/HOBt/DIEA/AA, (3.8:4.0:7.2:4.0)
***Cleavage: TFA-TES-H_2O (95:2:3) 2h.

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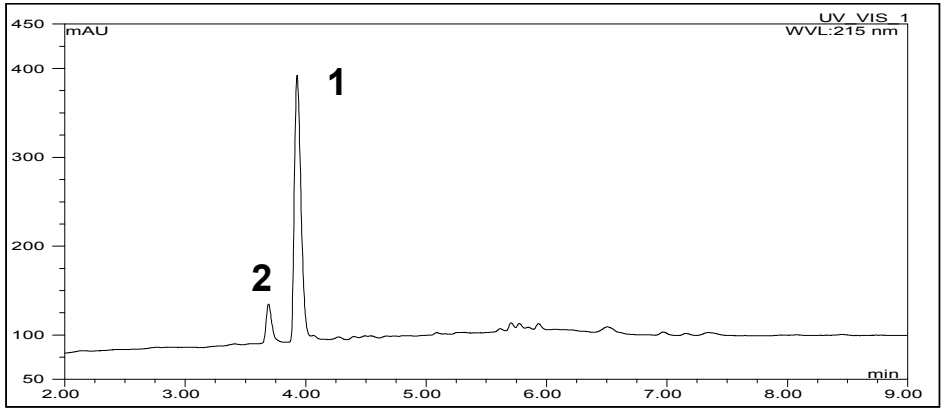
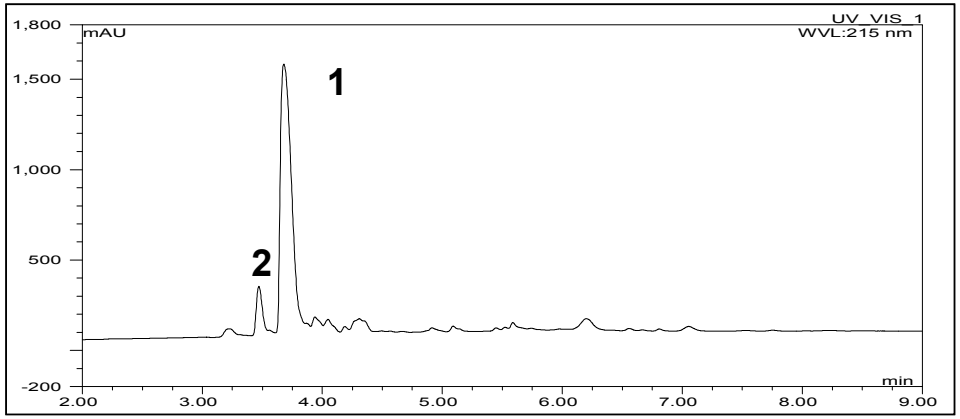
LysM Domain - H-LPERVKVVFPL-NH_2

2 x 45 min at RT **Purity: 62%**



Overall synthesis time: ~24 h

2 x 10 min at 75 °C **Purity: 77%**



Overall synthesis time: ~11½ h

1 = product, 2 = des-Leu(*N*-terminal)

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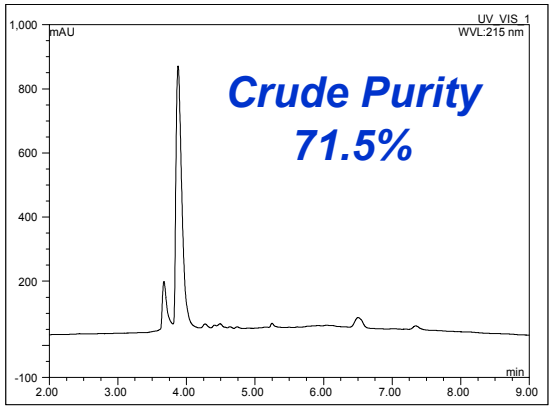
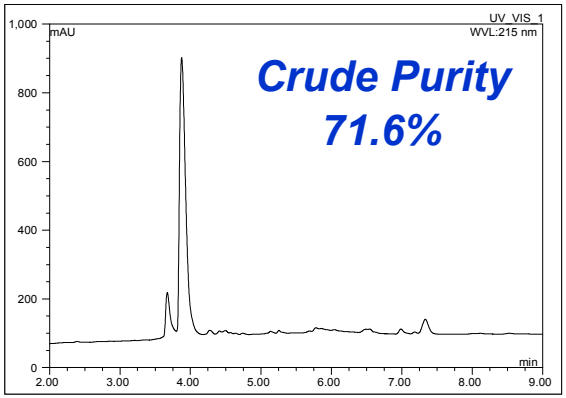
Comparison of Different Reactor Vial Sizes

Synthesis of LysM-Domain C-terminal 11mer: - Coupling: 5 min at 75 ºC
- Deprotection: 3 + 10 min at RT

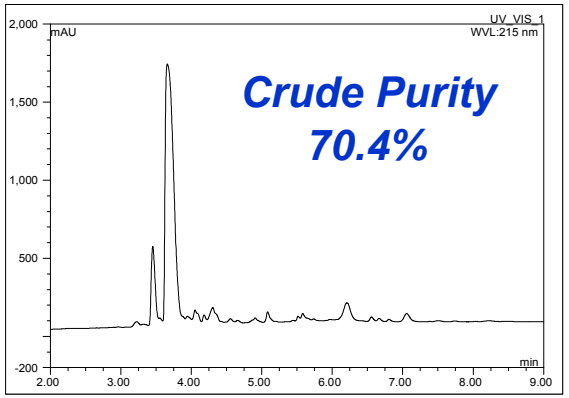
2mL reactor
25 µmol



5mL reactor
50 µmol



10mL reactor
100 µmol



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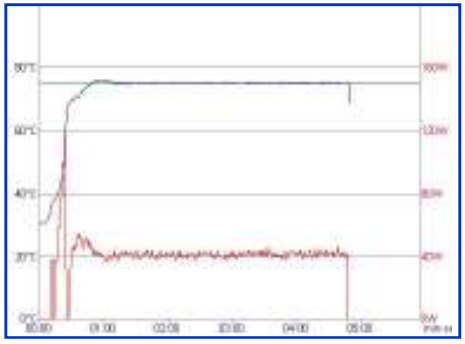
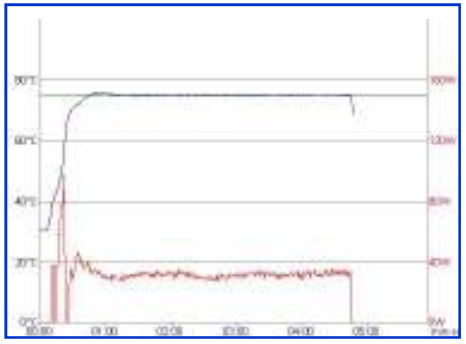
Comparison of Different Reactor Vial Sizes

Synthesis of LysM-Domain C-terminal 11mer: - Coupling: 5 min at 75 ºC
- Deprotection: 3 + 10 min at RT

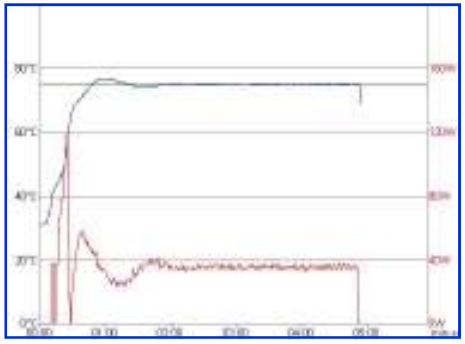
2mL reactor
25 µmol



5mL reactor
50 µmol



10mL reactor
100 µmol



Heating profiles: **Blue = temperature**
Red = power

Biotage

Why are we Interested in *N*-Methylated Amino Acids?

- Exist in many biologically-active natural products
- Help obtain information about backbone conformation
- Offer improved lipophilicity, proteolytic stability and bioavailability
- Potentially useful therapeutics

Biotage

Highly *N*-Methylated Trimer Synthesis

H-MeAla-MeIle-MeGly-NH_2 (**3**)

Different reaction conditions at RT for the synthesis of 3 using RAM-*S*-TG resin (0.24 mmol/g):

Entry	Coupling Temperature	Coupling time	Coupling reagent*	HPLC purity* (%)	Overall synthesis time
1	RT	20 min	DIC/HOAt	<5	2 h 48 min
2	RT	20 min	HBTU/HOBt	<5	2 h 48 min
3	RT	60 min	DIC/HOAt	<5	4 h 48 min
4	RT	60 min	COMU	<5	4 h 48 min
5	RT	60 min	HATU/HOAt	<5	4 h 48 min
6	RT	2 x 60 min	DIC/HOAt	<5	7 h 51 min
7	RT	24 h	DIC/HOAt	39	~74 h

- Deprotection: 3 min with 40%pip in DMF followed by 10 min with 20%pip in DMF.

* Cleavage: TFA-TES-H_2O (95:2:3) 2h.

***J. Pept Sci* 2010, 16, 136-140**

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Highly *N*-Methylated Trimer Synthesis

H-MeAla-MeIle-MeGly-NH_2 (**3**)

Different reaction conditions with microwave heating for the synthesis of 3:

Entry	Coupling Temperature	Coupling time	Coupling reagent*	HPLC purity* (%)	Overall synthesis time
8	75ºC	20 min	HBTU/HOBt	8	2 h 48 min
9	75ºC	20 min	COMU	59	2 h 48 min
10	75ºC	20 min	HATU/HOAt	75	2 h 48 min
11	75ºC	20 min	DIC/HOAt	76	2 h 48 min
12	75ºC	2 x 10 min	DIC/HOAt	80	3 h 10 min

- Deprotection: 3 min with 40%pip in DMF followed by 10 min with 20%pip in DMF.

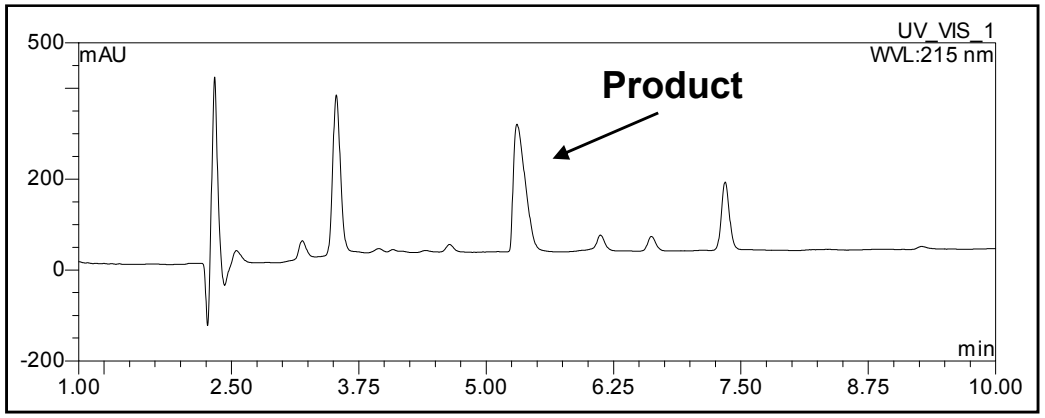
* Cleavage: TFA-TES-H_2O (95:2:3) 2h.

Biotage

Highly *N*-Methylated Trimer Synthesis

H-MeAla-MeIle-MeGly-NH_2 (**3**)

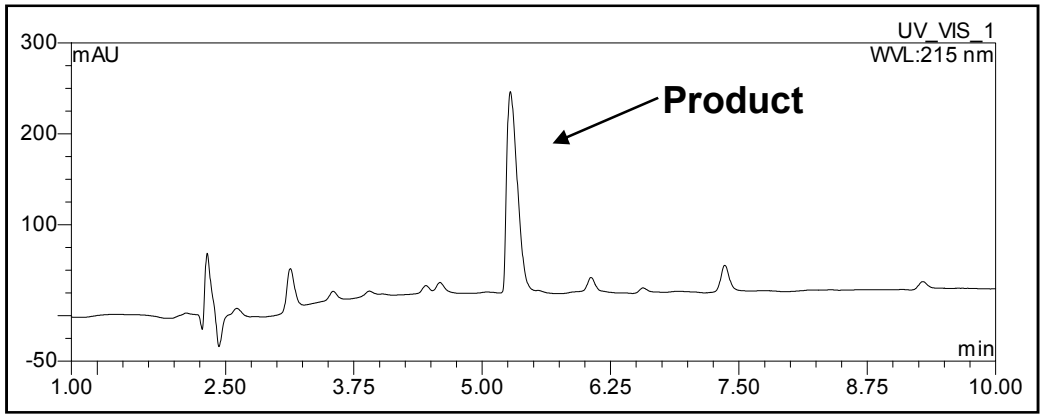
24h at RT



Overall synthesis time: ~74 h

Crude Purity 39%

2 x 10 min at 75°C



Overall synthesis time: ~3 h

Crude Purity 80%

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ACP-(65-74)

H-^{65}Val-^{66}Gln-^{67}Ala-^{68}Ala-^{69}Ile-^{70}Asp-^{71}Tyr-^{72}Ile-^{73}Asn-^{74}Gly-NH_2 (**4**)

Different reaction conditions using HBTU/HOBt for the synthesis of 4:

Coupling conditions:*

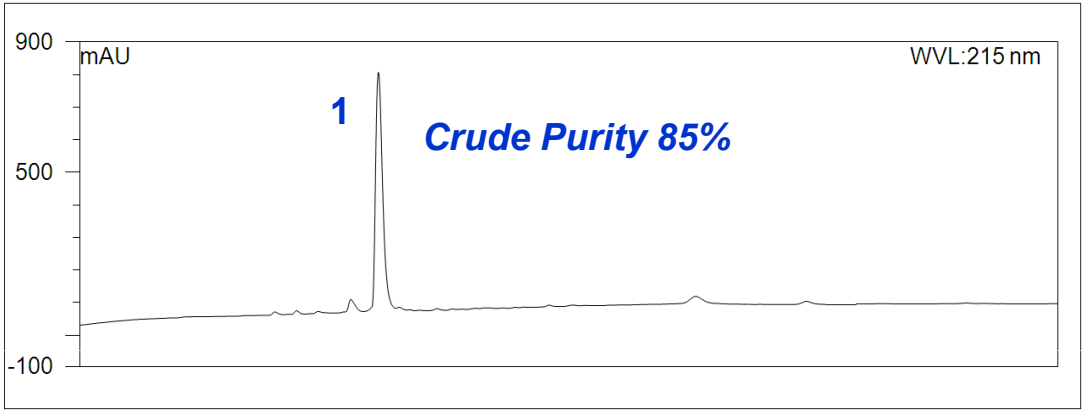
Entry	Residues 65-74	HPLC purity** (%)	Overall synthesis time
1	2 min, RT	85	4 h 53 min
2	**2 min, 75ºC**	**90**	**4 h 53 min**

• Deprotection: 3 min with 40%pip in DMF followed by 10 min with 20%pip in DMF.
* Coupling reagents HBTU/HOBt/DIEA/AA, (5.0:5.2:10:45.2)
** Cleavage: TFA-TES-H_2O (95:2:3) 2h.

Biotage

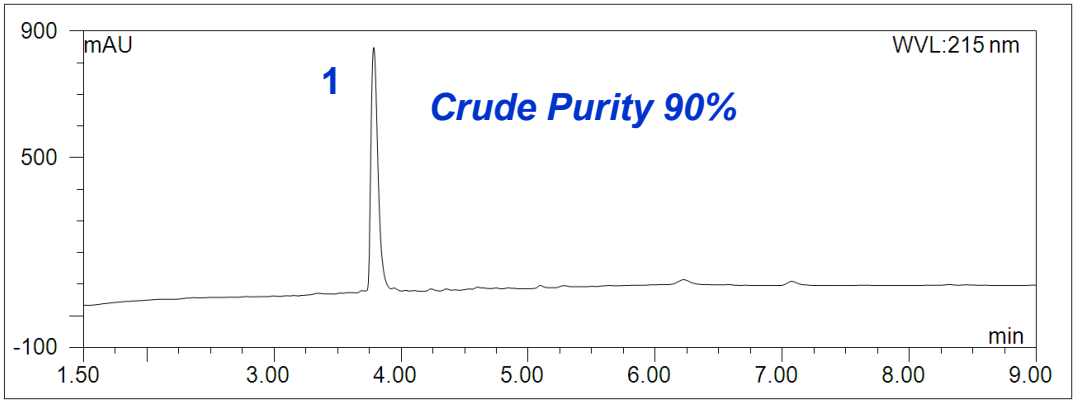
ACP-(65-74)

2 min RT



Overall synthesis time: ~5 h

2 min 75 ºC



Overall synthesis time: ~5 h

Biotage

[^{67}Aib-^{68}Aib]-ACP (65-74)

H-^{65}Val-^{66}Gln-^{67}Aib-^{68}Aib-^{69}Ile-^{70}Asp-^{71}Tyr-^{72}Ile-^{73}Asn-^{74}Gly-NH_2 (**5**)

Initial screening of coupling reagents and conditions for the synthesis of 5 using RAM-*S*-TG resin (0.24 mmol/g):

Coupling conditions:

Entry	Residues 66-68	Residues 65, 69-74	Coupling reagent*	HPLC purity** (%)	Overall synthesis time
1	5 min 75ºC	2 min 75ºC	HATU	0	5 h 43 min
2	5 min 75ºC	2 min 75ºC	COMU	0	5 h 43 min
3	5 min 75ºC	2 min 75ºC	DIC	13	5 h 43 min
4	2 x 5 min 75ºC	2 min 75ºC	DIC	22	5 h 58 min
5	10 min 75ºC	2 min 75ºC	DIC	20	6 h 19 min
6	2 x 10 min 75ºC	2 min 75ºC	DIC	30	6 h 49 min

- Deprotection: 3 min with 40%pip in DMF followed by 10 min with 20%pip in DMF.

* HOAt was used as additive (except for COMU) and DIEA as base. **3 equiv amino acids**.

** Cleavage: TFA-TES-H_2O (95:2:3) 2h.

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[^{67}Aib-^{68}Aib]-ACP (65-74)

H-^{65}Val-^{66}Gln-^{67}Aib-^{68}Aib-^{69}Ile-^{70}Asp-^{71}Tyr-^{72}Ile-^{73}Asn-^{74}Gly-NH_2 (**5**)

Different reaction conditions using DIC/HOAt for the synthesis of 5:

Coupling conditions:*

Entry	Residues 66-68	Residue 65, 69-74	HPLC purity** (%)	Overall synthesis time
7	2 x10 min, 75ºC	2 min 75ºC	59	6 h 49 min
8	**3 x 10 min, 75ºC**	**2 min 75ºC**	**63**	**7 h 39 min**
9	3 x 90 min, RT	10 min RT	42	20 h 35 min
10	3 x 90 min, RT	45 min RT	37	25 h 50 min
11	3 x 10 min, 75ºC	45 min RT	43	13 h 50 min

• Deprotection: 3 min with 40%pip in DMF followed by 10 min with 20%pip in DMF.

* DIC/ HOAt / DIEA. **6 equiv amino acids.**

** Cleavage: TFA-TES-H_2O (95:2:3) 2h.

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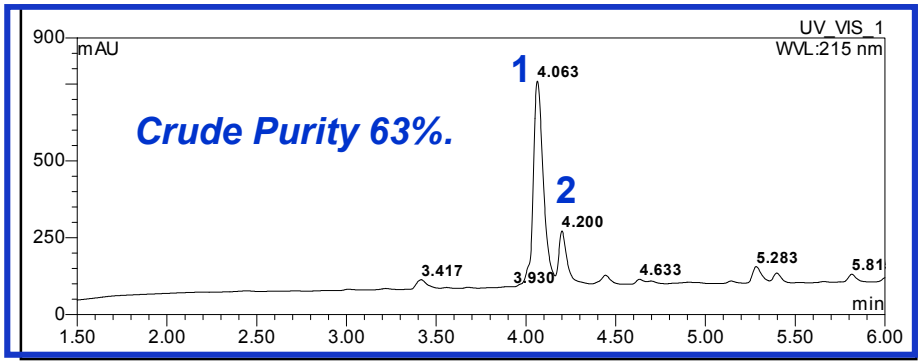
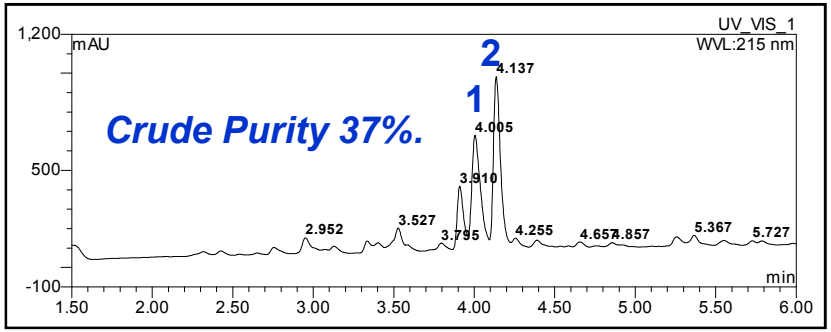
[^{67}Aib-^{68}Aib]-ACP (65-74)

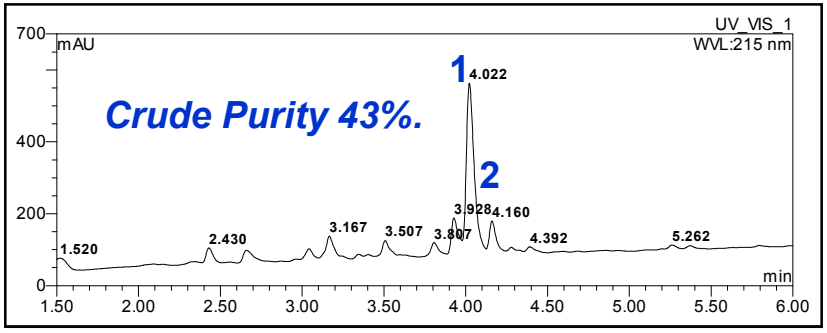
3 x 10min 75 ºC/ 2min 75 ºC



3 x 90 min RT/ 45 min RT



3 x 10min 75 ºC/ 45 min RT



1 = Product, 2 = des-Aib

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Conclusion:
Benefits of Microwave Irradiation in SPPS

- Powerful technique for accelerating the synthesis of peptides and peptidomimetics
- Allows access to the synthesis of difficult peptide sequences
- Improve coupling rates and prevent side reactions
- Use of standard coupling reagents
- Reduction in cost of synthesis
- “Green Chemistry” potential, less harmful solvents

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Syro *Wave*™

Thank you!

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We look forward to meeting you on our Stand

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