

Revolutionary reduction of amino acid excess

> Full automatic synthesis

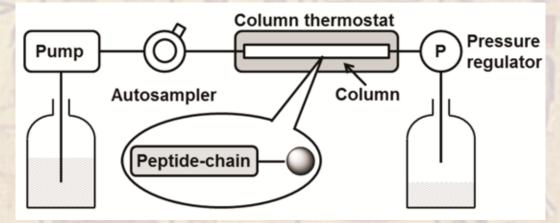
High performance peptide synthesizer HPPS-4000

Continuous flow solid phase peptide synthesis

The high pressure and high temperature in the continuous flow reactor ensure excellent conversion in a short reaction time and using a relatively low amino acid excess. The synthesis is carried out in a continuous flow cell on a column filled by a special solid support. The system is being built up using JASCO LC-4000 HPLC modules providing the appropriate pressure, temperature and solvent resistance. The pump delivers the steady and high pressure liquid flow. N,N-dimethylformamide (DMF) can be typically used both as reaction medium as well as resin washing fluid. An HPLC autosampler is utilized for the injection of coupling and deprotection mixtures into the flow-line, these pass through the reactor as plugs.



The high volume precision of the injection ensures excellent reproducibility. Premixed coupling mixtures are also suitable, but the programmable autosampler is capable to do in situ mixing and delivering of reagents. Performing the mixing steps immediately before the coupling minimizes the possibility of racemisation and decomposition of the activated amino acids. By changing the flow rate the residence time can be influenced, while the fine-tuning of the temperature allows high conversion values and reduced side-product formation. A back-pressure regulator provides the constant pressure in the reactor cell.



START STOP	PALME	CONT RESET MONIT	PLANF 330F - FLUSH	THE
201				Contract of the last
# Type	Sample #	Volume Chromatogram Nan	ne Aco. Time Control Me	tod
I LINK		1000.0 deprotekt_1	14.0	
2 UNK .	1	800.0 tyr	14.0 Peptid14	
3 LNK	35	1000.0 deprotekt_2	14.0 Peptid14	
4 006	2	800.0 pro	14.0 Peptd14	
S UNK	36	1000.0 deprotekt_3	14.0 Peptid14	Control Center - Administrator
6 UNK	3	800.0 ala	14.0 Peptid14	and the second
7 UNK	37	1000.0 deprotekt_4	14.0 Peptd14	Operation View Help
B UNK	14	800.0 gly	14.0 Pepbd14	
9 UNK	38	3000.0 deprotekt_5	14.0 Pepid14	And the second sec
10 LINK	5	800.0 pro_2	14.0 Peptd14	Management Tools
11 UNK	29	3000.0 deprotekt_6	14.0 Peptid14	
12 UNK	6	800.0 tp	14.0 Peptid14	100
13 UNK	10	1000.0 deprotekt_7 800.0 gly_2	14.0 Peptd14 14.0 Peptd14	Project
15 LEK	41	1000.0 deprotekt_8	14.0 Peptd14	State User
16 LINK		800.0 p/s 3	14.0 Peptid14	909 VIN
17 UNK	42	1000.0 deprotekt 9	14.0 Pep6d14	 LC-NetUADC
18 LINK		800.0 asn	14.0 Peptd14	
15 UNK	43		14.0 Pep5d14	Year HPSC lyclem
20 LINK	10	800.0 gly_4	14.0 Pepbd14	100
21 UNK		1000.0 deprotekt_11	14.0 Peptd14	🍏 Tipectral Library
22 LINK	11	800.0 tvs	14.0 Peptid14	Application Template
23 UNK	45	3000.0 deprotekt_12	14.0 Pepid14	white more and one
241096	12	800.0 aep	14.0 Peptid14	System Log
25 UNK	46	1000.0 deprotekt 13	14.0 Peptid14	X
26 LINK	13	800.0 dv.5	14.0 Peptd14	Application Log
27 UNC	47	1000.0 deprotekt_14	14.0 Peptid14	(17)
25 UNK	14	800.0 arg	14.0 Pep6d14	(B) System Information
29 LINK	40	1000.0 deprotekt_15	14.0 Peptid14	System Policy
30 UNK	15	800.0 val	14.0 Peptd14	B Nexus week
31 UNK	40	1000.0 deprotekt_16	14.0 Peptd14	The Module Management
32 UNK	16	000.0 dv.5	14.0 Peptid14	
33 UNK	55	1000,0 deprotekt_17	14.0 Peptid14	
				Carlo Car
				O ChromiliaW
		<u>n 444</u>	- 13 I.	🐺 Management Tools
 HPLC System 			SACAWAR LAW	
System Name		Peptide Synth	Frede	
IP Address		192.168.0.11	192,168.0.11	
MAC Addre	ess		00/C0/28/00/24/8A	
Modules				
Start Mode			LC-Netl/ADC (Start On Injection)	
			AS-4150-L	
Autosampler				
Pump#1			PU-4180	
Oven#1			CO-4060	
Valve/Event			LC-Netl/ADC	

The entire synthesis is automated and computer controlled. The flow rate, temperature, duration of the individual reaction steps, the amino acid sequence and injected volumes are settable in the ChromNav2 software. Selected users receive e-mail notification about eventual malfunctions or at the end of the synthesis process.

The system, by addition of an optional detector, can be utilised as an HPLC system for the purification or analysis of the synthesized products. An other option can be a solvent recycling valve system, by the use of which the coupling mixture can be recirculated through the reaction bed as many times as needed, so minimising the amino acid consumption. In case of trityl linker, the cleavage can be performed by the utilization of a cleavage cocktail containing TFA (max. 1%) or by 20% HFIP (hexafluoroisopropanol) directly in the instrument. In case of other linkers, the resin is removed from the reactor cell and treated off-line with the usual cleavage cocktails.



Properties of the synthesis:

- ♦ Scale: 0.01-0.2 mmol
- Low coupling time
- ◆ Excellent coupling with 1.5x excess of the amino acid
- Excellent conversion with all 20 natural amino acids
- The system can be used efficiently in small scales too
- ♦ 1.1 ml of the coupling mixture is sufficient
- The total used amounts of the Fmoc-protected amino acids are in the range of 14-30 mg (in case of 0.04 mmol scale)
- The crystallization of the Fmoc-protected amino acid in the tubing and in the instrument itself was never observed
- ◆ All conventional linkers are compatible with the system
- Fast synthesis of protected peptides
- The syntheses of difficult sequences and peptides containing artificial amino acids can also be realised.

Properties of the instrument:

- ♦ HPLC based reactor
- No solenoid valve, no complicated valve system, no pneumatic solvent delivery
- No need for gas supply either for pneumatics or inert atmosphere or mixing
- ♦ Wetted surfaces: stainless steel, glass, PEEK
- ◆ Economic maintenance and easy serviceability
- Controlled programmable reactor temperature
- ◆ Full automation, computer controlled system
- ♦ Simple operation

Specification:

- Flow rate: 0.001-10 mL/min, typically: 0.150 mL/min
- Maximal pressure: 700 bar
- Pressure regulation: constant ~70 bar
- Temperature: -15 80°C, typically: 70 °C
- Reaction time: 1-9999 min, typically: 7-30 min
- Amino acid excess: 1-10 equiv., typically 1.5 equiv.
- Coupling agent: heat resistant coupling agents, typically HATU
- Amino acids: all natural and artificial amino acid can be coupled, in a given synthesis the maximum of 80 different amino acid can be coupled
- Protecting group: Fmoc
- Linker: Heat resistant linkers, typically Rink amide or Wang linker
- Solvent: typically N,N-dimethylformamide (DMF)
- Reaction mixture volume: 10-1000 μL
- Required minimum solution volume: 10 µL (microvial)
- Computer controlled system with ChromNav2 software (WIN7 32/64 bit compatible)

Optional modules

- Chromatography detector (UV/VIS, PDA, fluorescent, circular dichroism)
- Solvent recirculation valve system
- Second pump head for programmable solvent switching and gradient chromatography
- Automated, software controlled pressure regulator

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