

# Fast and Effective Synthesis of Peptides

## A Statistical Approach Using EasyMax™

Urs Groth, Fabio Visentin  
Mettler-Toledo AG, Schwerzenbach, Switzerland

No. 2010-002

**Peptides are organic compounds which are formed through linking identical or different amino acids. Due to the chemical nature of the molecules and the associated diverse range of links, peptide synthesis is a complex process, which in the majority of cases results in a time-consuming and costly procedure. It is often the case that peptides can only be crystallized with difficulty, and this is exactly the reason why a high level of purity must be maintained throughout the manufacturing phase.**

Peptide synthesis is naturally subject to a wide range of influences, such as the reaction temperature, solvent, catalyst, as well as concentrations of the substrate and reagent. Consequently the target variables, such as the product composition, purity, yield or selectivity, vary. As a result of the diverse range of influencing factors and the costs and efforts involved in the synthesis, sophisticated concepts and state-of-the-art tools are to be applied as an essential element of peptide synthesis.

Rather than adopting a trial and error approach, whereby each parameter is examined on an individual basis and interactions between these parameters cannot be easily detected, in today's industry the statisti-

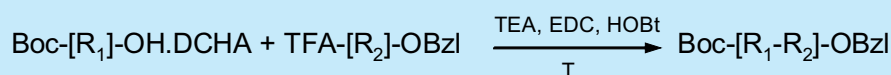
cal Design of Experiments (DoE) is generally applied. In order for the development based on the Design of Experiments concept to be regarded a success, it is essential that any experiments are performed within an accurately controlled framework under accurately maintained and reproducible conditions, thus enabling the target variable (e.g. selectivity or yield) to reliably achieve its optimum value.

The requirements placed on the experiment setup and execution are therefore high and demand a high degree of flexibility, precision and reproducibility. A partially automated synthesis tool, such as the EasyMax provides the necessary support required.

The EasyMax is extremely easy to use and supports the execution of organic syntheses within a precisely controlled framework. The reactor system, which is controlled by means of an easy to operate touchpad, features two fully independent reactors of freely selectable sizes and can be equipped with dosing units, various stirrer types, as well as with analytical measuring probes such as pH measurement, Mid IR or particle characterization. The process performed along with the measured data is recorded in detail. These can be called up and further processed, if required.

### Optimizing peptide synthesis using DoE and EasyMax

Reaction diagram



# Fast and Effective Synthesis of Peptides

## A Statistical Approach Using EasyMax™

Using peptide synthesis from LONZA Peptides based in Braine-l'Alleud, Belgium, the performance of EasyMax is demonstrated in combination with the sophisticated concepts of the statistical Design of Experiments.

### Objectives

- To identify the relevant reaction parameters which are necessary for creating a kinetic model
- To maximise yield and selectivity
- To minimize by-products

### Experiments

Four probable influencing factors were tested (reagents EDC, TEA, HOBt and the temperature at which the experiment was performed).

Factor	Name	Unit of measurement	Type	Lower limit	Upper limit
A	Temp	°C	Numerical	10	45
B	TEA	Eq	Numerical	0	1
C	EDC	Eq	Numerical	1	1.3
D	HOBt	Eq	Numerical	0.1	1.5

A subfactorial Design of Experiments, which comprises a total of 11 experiments, has been developed (design: 2 level factorial, centre points: 3).

Run	T [°C]	Eq TEA	Eq EDC	Eq HOBt
1	45.0	0.0	1.30	0.1
2	27.5	0.5	1.15	0.8
3	27.5	0.5	1.15	0.8
4	10.0	1.0	1.00	1.5
5	10.0	0.0	1.30	1.5
6	10.0	0.0	1.00	0.1
7	45.0	0.0	1.00	1.5
8	27.5	0.5	1.15	0.8
9	45.0	1.0	1.30	1.5
10	45.0	1.0	1.00	0.1
11	10.0	1.0	1.30	0.1

The 11 experiments were performed in pairs in parallel on an EasyMax.

For this purpose, the EasyMax was equipped with 100 mL reactors, a mechanical stirring device with a turbine agitator, a dosing unit and a ReactIR™ 45m system. The composition was determined using HPLC following reaction times of 30, 60 and 90 minutes.



**Figure 1**  
EasyMax 102 reactor system with ReactIR 45m

The following sequence was followed throughout all 11 experiments:

1. Addition of the DMF solvent
2. Setting of the temperature at which the experiment is performed in accordance with the Design of Experiments
3. Assignment of premixed reactants (peptide, EDC and HOBt)
4. Assignment of the TEA
5. Sampling following periods of 30, 60 and 90 minutes for the HPLC analysis

Due to ease of operation and precisely executed and reproducible reaction steps, all experiments could be performed exactly as shown in the Design of Experiments. Additional inspection-based experiments were no longer necessary. Thanks to the sophisticated heating and cooling system, temperatures below room temperature could be controlled easily without the use of cryostats.

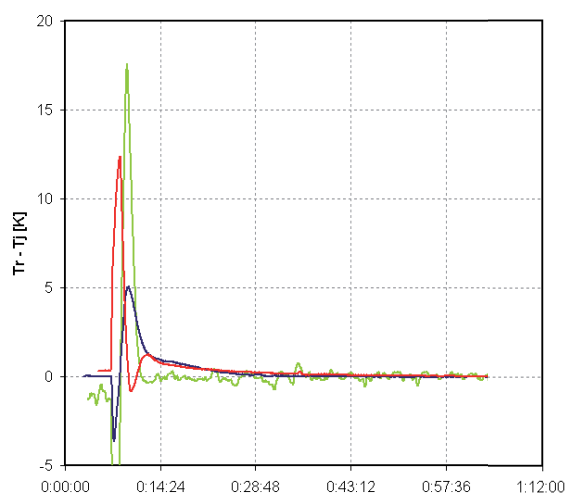
# Fast and Effective Synthesis of Peptides

## A Statistical Approach Using EasyMax™

The dosing of liquid substances was precisely executed in the required manner by means of a dosing unit.

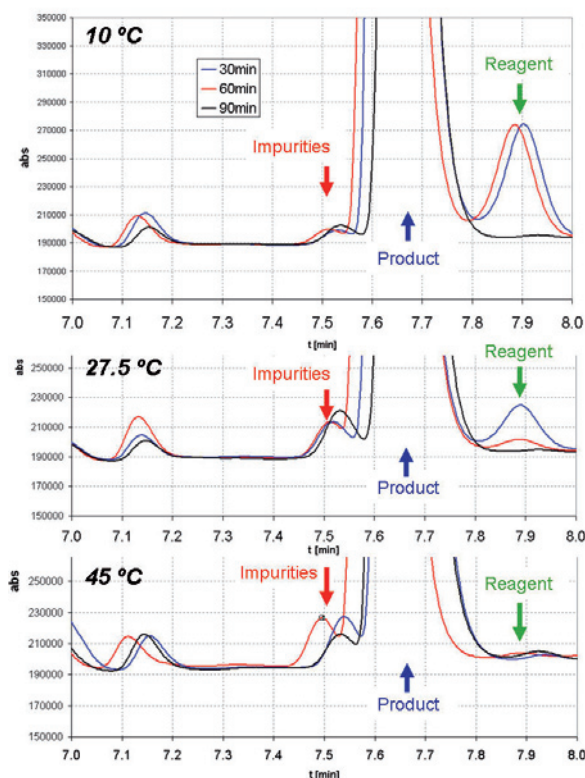
Data was obtained during the experiment on a continual basis, the trends (temperature, dosing, heat balance trend) detected here are presented in the form of graphics and are simultaneously saved for any subsequent re-evaluations.

The following graphic displays the trends from three experiments at various temperatures, thus indicating the reaction sequence in a fast, yet effortless manner.



**Figure 2**  
Heat flow trend = f (temperature)  
Run 1 - fastest reaction at T = 45.0 °C  
Run 2 at T = 27.5 °C  
Run 3 - slowest reaction at T = 10.0 °C

The HPLC analysis following reaction times of 30, 60 and 90 minutes for the runs 1, 2 and 5 highlighted a considerable influence of the reaction temperature on the product composition and the proportion of impurities.



**Figure 3**  
HPLC analysis at various temperatures

While at an experiment temperature of 10.0 °C, the conversion, and thereby the yield was, as expected, at a minimum level after 90 minutes. The lowest proportion of impurities was also measured. A temperature rise for increasing the reaction rate and thereby the conversion is not possible without experiencing losses in terms of the selectivity. This can be clearly seen in the experiments performed at 27.5 °C and 40 °C through the higher proportions of impurities.

As shown in Figure 3, the product concentrations, the unreacted reagents and the by-products created were determined for all the experiments defined in the Design of Experiments.

## Fast and Effective Synthesis of Peptides

### A Statistical Approach Using EasyMax™

In this case, a lower temperature and EDC concentration seem to result in rather low concentrations of impurities. In contrast, low TEA concentrations tend to result in higher concentrations of impurities.

The information gathered from the experiments was used to subsequently perform an analysis of variance (ANOVA test) in order to determine the significance of the parameters.

#### Conclusions

- The applied method of statistical Design of Experiments has proven to be an appropriate way of identifying relevant parameters and has clearly highlighted the influence which the temperature and the reagent concentrations have on the yield and selectivity of the reaction.
- The number of experiments to be performed could be kept to a minimum as a result of the systematic approach.
- The data obtained enabled a kinetic model to be created, which was able to predict the concentration of impurities and thus minimize their occurrence.
- The simple operation of the EasyMax enabled the Design of Experiments to be executed in an efficient manner in no time at all.
- Thanks to the high degree of precision, experiments could be performed in a precise and fast manner to the highest of quality.
- The excellent reproducibility level of the system meant that time-consuming, repetitive experiments were no longer required and manual input could be eliminated to the greatest possible extent.

This article was written with the kind support of Didier Monnaie and Geoffroy Sommen from LONZA Peptides, Braine-l'Alleud, Belgium.

#### Mettler-Toledo AG, AutoChem

Sonnenbergstrasse 74  
CH-8603 Schwerzenbach, Switzerland  
Phone +41-44 806 77 11  
Fax +41-44 806 72 90

Internet [www.mt.com/autochem](http://www.mt.com/autochem)  
E-Mail [autochem@mt.com](mailto:autochem@mt.com)

Subject to technical changes  
©03/2010 Mettler-Toledo AG  
Printed in Switzerland  
MarCom RXE

[www.mt.com/EasyMax](http://www.mt.com/EasyMax)

For more information