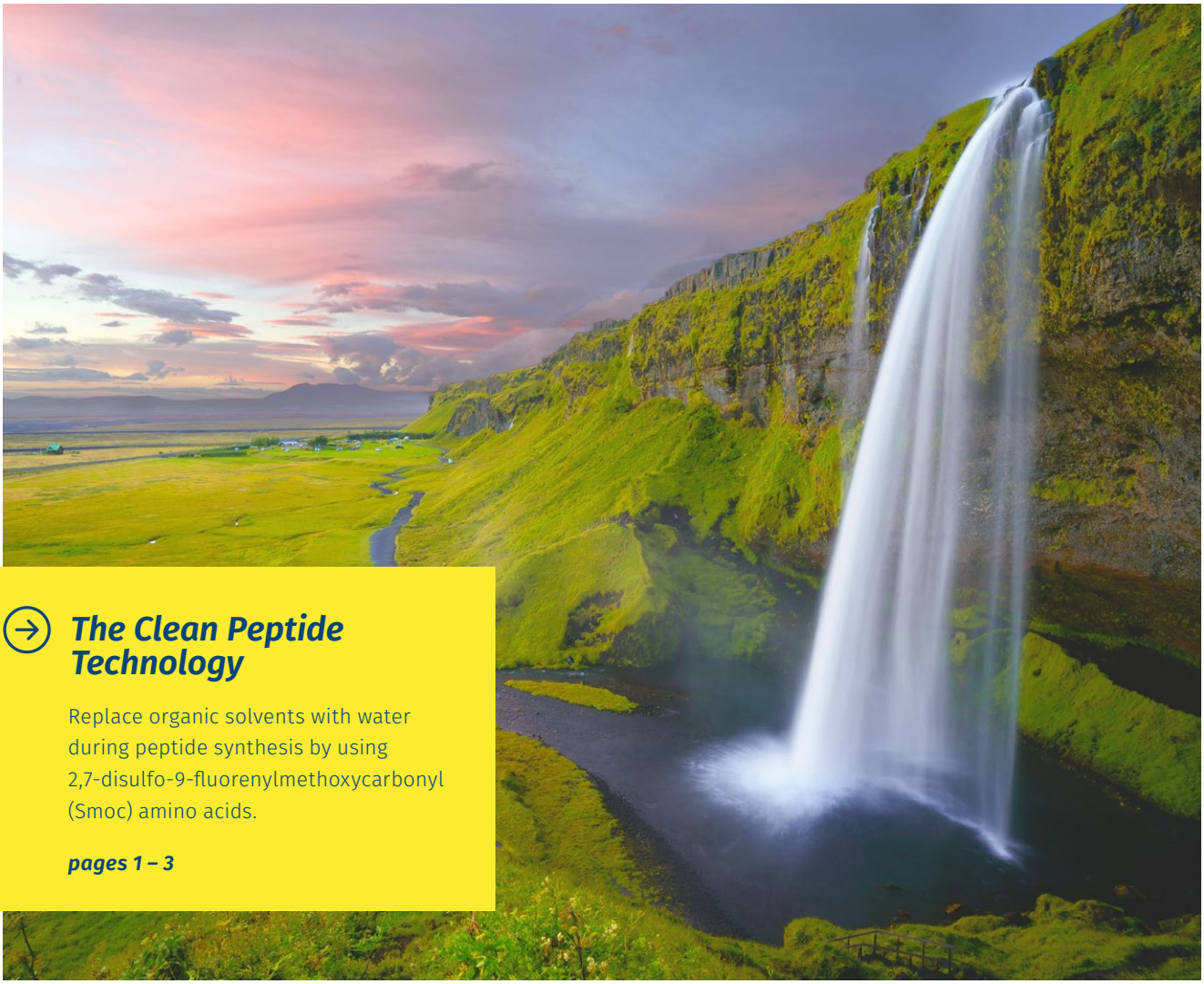
Sulfotools

Iris Biotech

SMOC-AMINO ACIDS

Innovative Peptide Synthesis using Water Instead of Organic Solvents



The Clean Peptide Technology

Replace organic solvents with water during peptide synthesis by using 2,7-disulfo-9-fluorenylmethoxycarbonyl (Smoc) amino acids.

pages 1 – 3

Compatible with water-swellable resins.

pages 1 – 3

Pronounced fluorescence allows real-time monitoring.

page 2

Purification by ion exchange chromatography.

pages 1 – 3

Version: IF13_2

Smoc-Amino Acids

Innovative Peptide Synthesis using Water Instead of Organic Solvents

For the production of synthetic peptides, every year tens of thousands of tons of organic solvents are required in the chemical, cosmetic and pharmaceutical industries. According to the European Chemicals Directive REACH, these solvents are classified as substances of very high concern and their use is associated with significant risks for health and the environment. Following modern regulatory rules, “undesirable” solvents such as DCM, DMF, NMP and THF, which are frequently recommended and employed during SPPS, should be replaced. At the same time, the enormous consumption of solvents and reagents leads to high production costs.

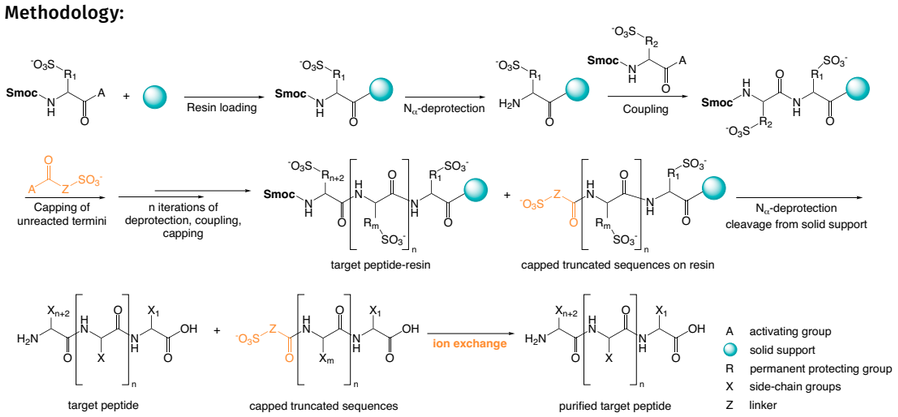
Thus, despite being considered as advanced and efficient technique for peptide production, solid-phase peptide synthesis (SPPS) is associated with severe drawbacks. Therefore, ongoing attempts aim at developing alternative approaches using solvents, which are permitting to reduce the risks for environment and human health.

The Clean Peptide Technology Using Smoc-Amino Acids – eco-friendly peptide manufacturing

Benefits:

- 2,7-disulfo-9-fluorenylmethoxycarbonyl (Smoc) amino acids are water compatible
  → replacement of organic solvents during peptide synthesis
- Certain side chains can remain unprotected
  → more atom-efficient
- Smoc-amino acids show a pronounced fluorescence
  → real-time monitoring during peptide synthesis (λ_{Ex} = 280 nm; λ_{Em} = 340 nm)
- As a solid support, a water-swellable resin can be used

Methodology:



Reaction scheme for solid-phase peptide synthesis using Smoc-amino acids.

Cleavage of the Smoc-group can be achieved using aqueous piperidine, piperazine, sodium hydroxide, ethanolamine, and ammonia to liberate the N-terminus within 5-15 minutes at ambient temperature together with the formation of the respective disulfonated dibenzofulvene and the products of water or base addition.

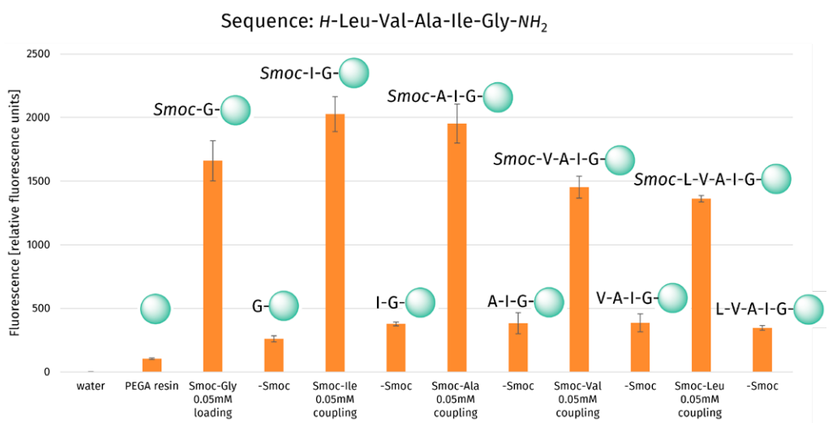
Recommended Coupling Protocols:

	Method 1) *in situ* Oxyma coupling	Method 2) NHS with pre-activation
Preparation & Coupling	3.0 eq. Smoc amino acid, 5.5 eq. EDC, 3.5 eq. Oxyma, 3.0 eq. $NaHCO_3$ in 30% isopropanol or MeCN in water 45 min or 2x25 min for double coupling	NHS ester formation: 3.0 eq. Smoc amino acid, 5.5 eq. EDC, 3.5 eq. NHS in water (pH range 5-6) 25 min For the coupling, add the prepared NHS mixture to the amine, adjust the pH to 8.0 and readjust over time for 15 min.
Wash	2x with water	2x with water
Deprotection	1M NaOH (5 min + 10 min) If ester side chains are present in the sequence use another base, e.g., piperazine or 4-methylpiperidine.	1M NaOH (5 min + 10 min) If ester side chains are present in the sequence use another base, e.g., piperazine or 4-methylpiperidine.

Note:

- Rink amide resin needs to be dry before cleavage from the solid support, otherwise side reactions occur.
- Recommended resins: TentaGel® or other resins suitable for polar solvents

Exemplary Fluorescence Monitoring:



Fluorescence monitoring of the coupling and deprotection steps during Smoc-based SPPS.

Comparison Boc – Fmoc – Smoc

Boc SPPS	*Fmoc SPPS*	*Smoc SPPS*
Boc-Arg(Tos)-OH	*Fmoc-Arg(Pbf)-OH*	*Smoc-Arg-OH*
Boc-Asn(Xan)-OH	*Fmoc-Asn(Trt)-OH*	*Smoc-Asn-OH*
Boc-Asp(OBzl)-OH	*Fmoc-Asp(OtBu)-OH*	*Smoc-Asp(OtBu)-OH*
Boc-Cys(Acm)-OH	*Fmoc-Cys(Trt)-OH*	*Smoc-Cys(Trt)-OH*
Boc-Gln(Xan)-OH	*Fmoc-Gln(Trt)-OH*	*Smoc-Gln-OH*
Boc-Glu(OBzl)-OH	*Fmoc-Glu(OtBu)-OH*	*Smoc-Glu(OtBu)-OH*
Boc-His(Dnp)-OH	*Fmoc-His(Trt)-OH*	*Smoc-His-OH**
Boc-Lys(Cbz)-OH	*Fmoc-Lys(Boc)-OH*	*Smoc-Lys(Boc)-OH*
Boc-Ser(Bzl)-OH	*Fmoc-Ser(tBu)-OH*	*Smoc-Ser(tBu)-OH*
Boc-Thr(Bzl)-OH	*Fmoc-Thr(tBu)-OH*	*Smoc-Thr(tBu)-OH*
Boc-Trp(For)-OH	*Fmoc-Trp(Boc)-OH*	*Smoc-Trp-OH**
Boc-Tyr(Bzl)-OH	*Fmoc-Tyr(tBu)-OH*	*Smoc-Tyr-OH**

** also available with side chain protection*

In comparison to Fmoc SPPS, no increased racemization levels were observed during Smoc SPPS. Regarding the prevention of aspartimide formation during Smoc SPPS in water, reduced temperature is recommended for the deprotection of sequences prone to aspartimide formation.

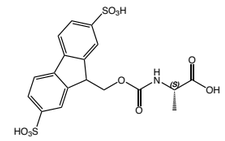
Looking for the right resin?
Download our Booklet!

Product details

SAA1010 Smoc-L-Ala-OH

(((2,7-disulfo-9H-fluoren-9-yl)methoxy)carbonyl)-L-alanine potassium salt

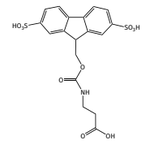
CAS-No. 2442552-59-0 (net)
Formula $C_{18}H_{15}K_2NO_{10}S_2$
Mol. weight 547,63 g/mol



SAA1230 Smoc-beta-Ala-OH

(((2,7-disulfo-9H-fluoren-9-yl)methoxy)carbonyl)-beta-alanine potassium salt

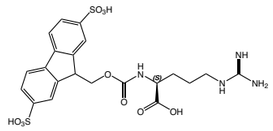
Formula $C_{18}H_{15}K_2NO_{10}S_2$
Mol. weight 547,63 g/mol



SAA1050 Smoc-L-Arg-OH

(((2,7-disulfo-9H-fluoren-9-yl)methoxy)carbonyl)-L-arginine potassium salt

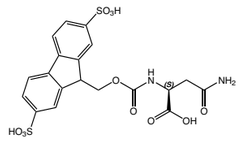
CAS-No. 2337407-38-0 (net)
Formula $C_{21}H_{22}K_2N_4O_{10}S_2$
Mol. weight 632,74 g/mol



SAA1080 Smoc-L-Asn-OH

(((2,7-disulfo-9H-fluoren-9-yl)methoxy)carbonyl)-L-asparagine potassium salt

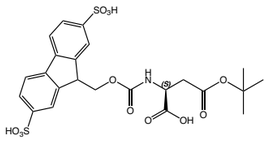
CAS-No. 2337407-22-2 (net)
Formula $C_{19}H_{16}K_2N_2O_{11}S_2$
Mol. weight 590,66 g/mol



SAA1130 Smoc-L-Asp(O*t*Bu)-OH

(((2,7-disulfo-9H-fluoren-9-yl)methoxy)carbonyl)-L-aspartic-acid-beta-*t*-butyl-ester potassium salt

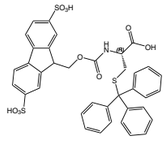
CAS-No. 2337407-41-5 (net)
Formula $C_{23}H_{23}K_2NO_{12}S_2$
Mol. weight 647,75 g/mol



SAA1110 Smoc-L-Cys(Trt)-OH

N-(((2,7-disulfo-9H-fluoren-9-yl)methoxy)carbonyl)-S-trityl-L-cysteine potassium salt

CAS-No. 2442552-68-1 (net)
Formula $C_{37}H_{29}K_2NO_{10}S_3$
Mol. weight 822,01 g/mol

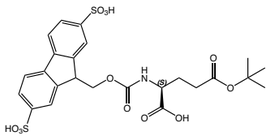


Product details

SAA1120 Smoc-L-Glu(O*t*Bu)-OH

(((2,7-disulfo-9H-fluoren-9-yl)methoxy)carbonyl)-L-glutamic-acid-gamma-*t*-butyl-ester potassium salt

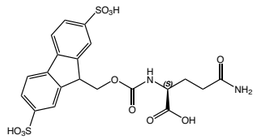
CAS-No.	2442552-71-6 (net)
Formula	$C_{24}H_{25}K_2NO_{12}S_2$
Mol. weight	661,78 g/mol



SAA1070 Smoc-L-Gln-OH

(((2,7-disulfo-9H-fluoren-9-yl)methoxy)carbonyl)-L-glutamine potassium salt

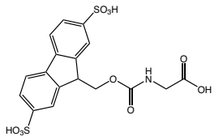
CAS-No.	2337407-39-1 (net)
Formula	$C_{20}H_{18}K_2N_2O_{11}S_2$
Mol. weight	604,68 g/mol



SAA1000 Smoc-Gly-OH

(((2,7-disulfo-9H-fluoren-9-yl)methoxy)carbonyl)glycine potassium salt

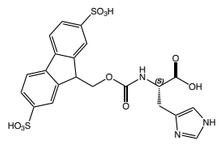
CAS-No.	2337407-26-6 (net)
Formula	$C_{17}H_{13}K_2NO_{10}S_2$
Mol. weight	533,60 g/mol



SAA1140 Smoc-L-His-OH

(((2,7-disulfo-9H-fluoren-9-yl)methoxy)carbonyl)-L-histidine potassium salt

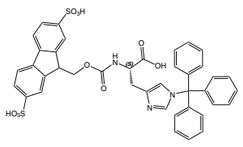
CAS-No.	2442552-74-9 (net)
Formula	$C_{21}H_{17}K_2N_3O_{10}S_2$
Mol. weight	613,69 g/mol



SAA1220 Smoc-L-His(Trt)-OH

N-alpha-(((2,7-disulfo-9H-fluoren-9-yl)methoxy)carbonyl)-N-tau-trityl-L-histidine potassium salt

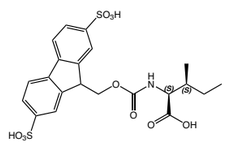
CAS-No.	2442552-76-1 (net)
Formula	$C_{40}H_{31}K_2N_3O_{10}S_2$
Mol. weight	856,02 g/mol



SAA1030 Smoc-L-Ile-OH

(((2,7-disulfo-9H-fluoren-9-yl)methoxy)carbonyl)-L-isoleucine potassium salt

CAS-No.	2337407-24-4 (net)
Formula	$C_{21}H_{21}K_2NO_{10}S_2$
Mol. weight	589,71 g/mol

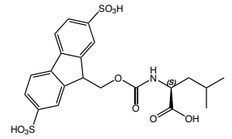


Product details

SAA1040 Smoc-L-Leu-OH

(((2,7-disulfo-9H-fluoren-9-yl)methoxy)carbonyl)-L-leucine potassium salt

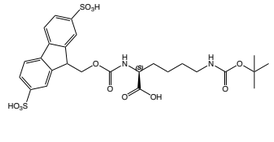
CAS-No.	2337407-36-8 (net)
Formula	$C_{21}H_{21}K_2NO_{10}S_2$
Mol. weight	589,71 g/mol



SAA1190 Smoc-L-Lys(Boc)-OH

N6-(*tert*-butoxycarbonyl)-N2-(((2,7-disulfo-9H-fluoren-9-yl)methoxy)carbonyl)-L-lysine potassium salt

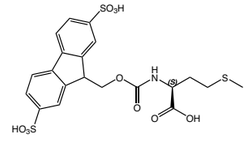
CAS-No.	2442552-82-9 (net)
Formula	$C_{26}H_{30}K_2N_2O_{12}S_2$
Mol. weight	704,84 g/mol



SAA1100 Smoc-L-Met-OH

(((2,7-disulfo-9H-fluoren-9-yl)methoxy)carbonyl)-L-methionine potassium salt

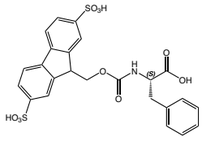
CAS-No.	2442552-84-1 (net)
Formula	$C_{20}H_{19}K_2NO_{10}S_3$
Mol. weight	607,75 g/mol



SAA1060 Smoc-L-Phe-OH

(((2,7-disulfo-9H-fluoren-9-yl)methoxy)carbonyl)-L-phenylalanine potassium salt

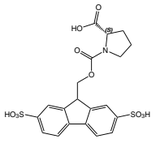
CAS-No.	2442552-86-3 (net)
Formula	$C_{24}H_{19}K_2NO_{10}S_2$
Mol. weight	623,73 g/mol



SAA1150 Smoc-L-Pro-OH

(((2,7-disulfo-9H-fluoren-9-yl)methoxy)carbonyl)-L-proline potassium salt

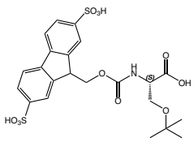
CAS-No.	2337407-20-0 (net)
Formula	$C_{20}H_{17}K_2NO_{10}S_2$
Mol. weight	573,67 g/mol



SAA1170 Smoc-L-Ser(*t*Bu)-OH

O-(*tert*-butyl)-N-(((2,7-disulfo-9H-fluoren-9-yl)methoxy)carbonyl)-L-serine potassium salt

CAS-No.	2337407-37-9 (net)
Formula	$C_{22}H_{23}K_2NO_{11}S_2$
Mol. weight	619,74 g/mol

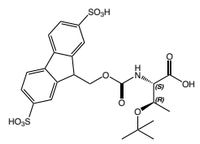


Product details

SAA1160 Smoc-L-Thr(*t*Bu)-OH

O-(*tert*-butyl)-N-(((2,7-disulfo-9H-fluoren-9-yl)methoxy)carbonyl)-L-threonine potassium salt

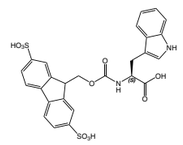
CAS-No. 2442552-94-3 (net)
Formula $C_{23}H_{25}K_2NO_{11}S_2$
Mol. weight 633,77 g/mol



SAA1180 Smoc-L-Trp-OH

(((2,7-disulfo-9H-fluoren-9-yl)methoxy)carbonyl)-L-tryptophan potassium salt

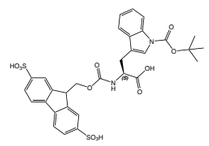
CAS-No. 2442552-96-5 (net)
Formula $C_{26}H_{20}K_2N_2O_{10}S_2$
Mol. weight 662,77 g/mol



SAA1210 Smoc-L-Trp(Boc)-OH

1-(*tert*-butoxycarbonyl)-N-alpha-(((2,7-disulfo-9H-fluoren-9-yl)methoxy)carbonyl)-L-tryptophan potassium salt

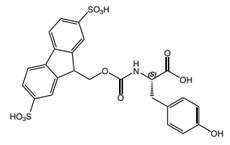
CAS-No. 2442552-98-7 (net)
Formula $C_{31}H_{28}K_2N_2O_{12}S_2$
Mol. weight 762,88 g/mol



SAA1090 Smoc-L-Tyr-OH

(((2,7-disulfo-9H-fluoren-9-yl)methoxy)carbonyl)-L-tyrosine potassium salt

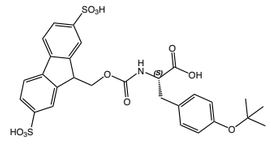
CAS-No. 2337407-33-5 (net)
Formula $C_{24}H_{19}K_2NO_{11}S_2$
Mol. weight 639,73 g/mol



SAA1200 Smoc-L-Tyr(O*t*Bu)-OH

O-(*tert*-butyl)-N-(((2,7-disulfo-9H-fluoren-9-yl)methoxy)carbonyl)-L-tyrosine potassium salt

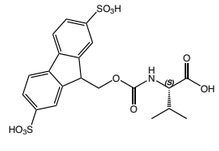
CAS-No. 2442553-00-4 (net)
Formula $C_{28}H_{27}K_2NO_{11}S_2$
Mol. weight 695,84 g/mol



SAA1020 Smoc-L-Val-OH

(((2,7-disulfo-9H-fluoren-9-yl)methoxy)carbonyl)-L-valine potassium salt

CAS-No. 2442553-02-6 (net)
Formula $C_{20}H_{19}K_2NO_{10}S_2$
Mol. weight 575,69 g/mol

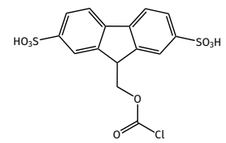


Product details

SAA1240 Smoc-Cl

9-(((chlorocarbonyl)oxy)methyl)-9H-fluorene-2,7-disulfonic acid

CAS-No. 1899144-09-2
Formula $C_{15}H_{11}ClO_8S_2$
Mol. weight 418,83 g/mol



References:

- *Method for peptide synthesis and apparatus for carrying out a method for solid phase synthesis of peptides; S. Knauer, T. M. L. Roese, O. Avrutina, H. Kolmar, C. Uth; 2016, WO 2016 050764.*
- *Sustainable Peptide Synthesis Enabled by a Transient Protecting Group; S. Knauer, N. Koch, C. Uth, R. Meusinger, O. Avrutina, H. Kolmar;* ***Angew. Chem. Int. Ed.*** *2020;* ***59(31)****: 12984-12990. https://doi.org/10.1002/anie.202003676.*
- *Improved method for preparing peptides; S. Knauer; WO 2019 101939.*
- *Method for preparing peptides; S. Knauer; WO 2019 101940.*
- *Novel amino-Li resin for water-based solid-phase peptide synthesis; C. Uth, S. Englert, O. Avrutina, H. Kolmar, S. Knauer;* ***J. Pept. Sci.*** *2023;* ***29(12)****: e3527. https://doi.org/10.1002/psc.3527*

You need more details? Watch the recording of our online workshop about the Smoc technology and its use for peptide synthesis in water.

Notes

Empowering Peptide Innovation