

Novabiochem®

## Enhanced specification Fmoc-amino acids

The new quality standard for peptide building blocks



# Purer Fmoc means purer peptides

## New stunning specifications for the 20 standard Fmoc

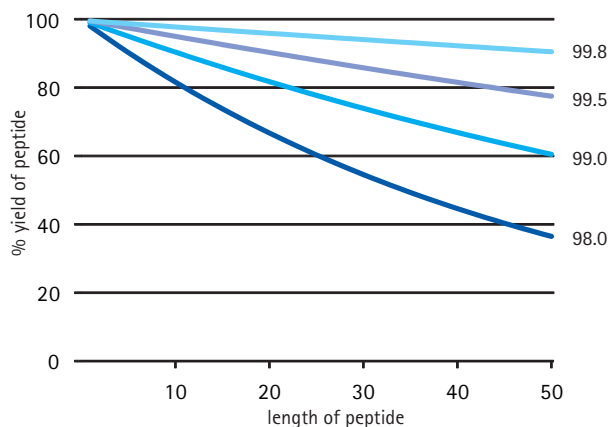
All peptide chemists recognize the key to obtaining high purity peptides is to maximize efficiency of every synthetic step. However, not all realize that obtaining high purity peptides also depends on using starting materials of the highest quality - small improvements in starting material quality can result in dramatic improvements in peptide yield (Figure 1 shows effect of amino acid purity on step-wise yield). That is why Novabiochem is pleased to introduce enhanced specifications for the standard 20 Fmoc-amino acid building blocks:

- HPLC Purity NOW  $\geq 99\%$ \* [\*Except Fmoc-Trp(Boc)-OH], with all significant amino acid related impurities quantified
  - No impurities hidden under product peak gives confidence in product purity
- Enantiomeric purity NOW  $\geq 99.8\%$
- Free amine content  $\leq 0.2\%$ 
  - Improved Fmoc stability during storage
- Acetate content  $\leq 0.02\%$ 
  - Negligible capping by-products

### Purer Fmoc means:

- Higher yields and more easily purified peptides
- More reproducible impurity profile of crude peptides so more easily optimized scale-up and less regulatory issues during GMP peptide manufacture
- Less synthesis failures and more easy trouble-shooting as you can have confidence in the integrity of the amino acid building blocks

Fig. 1: Effect of building block purity on step-wise peptide yield.



- Available from stock in large quantities
- Change control agreements available on request

# Is pure really pure?

## Can you be sure 99% HPLC purity really means your Fmoc is 99% pure?

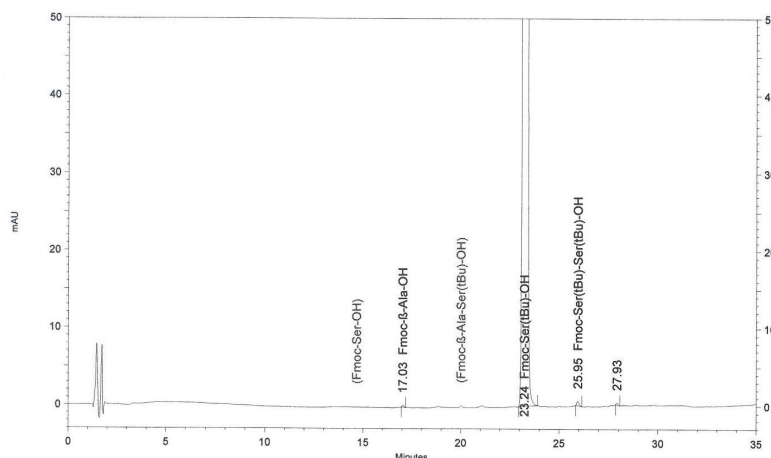
### What about the hidden impurities?

Synthesis of an Fmoc-amino acid produces predictable amino-acid related impurities, the presence of which can negatively effect the outcome of peptide synthesis.

### How can you be sure these impurities are not hiding under your "99%" pure HPLC peak?

By performing HPLC analysis the Novabiochem® way: by employing characterized standards of all amino-acid related impurities and using highly optimized HPLC methods to ensure these are clearly separated from the Fmoc-amino acid, enabling their accurate quantitation.

Fig. 2: Actual HPLC profile of Fmoc-Ser(tBu)-OH, showing amino acid impurities clearly identified.



### But purity does not end with HPLC purity!

Standard HPLC columns cannot separate between D- and L-amino acids, so HPLC analysis of your peptide will always miss enantiomeric impurities. However, small amounts of enantiomeric impurities in your Fmoc-amino acids will have a significant impact on the quality of the target peptide.

	Effect of amino acid enantiomeric purity on peptide purity	
	L-content 99.5%	L-content 99.8%
20mer	90%	96%
40mer	82%	92%
60mer	74%	89%

### Other hidden impurities

**Acetic acid:** Fmoc-amino acids can contain traces of acetic acid, which can cause chain termination during synthesis. For this reason, Novabiochem specifies acetate content of less than 0.02% for all our 20 proteinogenic Fmoc-amino acids.

**Free-amino acid content:** Unlike other suppliers who use an inaccurate TLC-based assay, Novabiochem employs a quantitative GC-based method, to ensure a free amino acid content of less than 0.2%.

Novabiochem® provides standard Fmoc-s with the highest available enantiomeric purity of 99.8%.

# Introducing our new specifications

Certificate of analysis (previous version)



## Certificate of Analysis

8.52019.0000 Fmoc-Ser(tBu)-OH Novabiochem®  
04-12-1033

Batch S6523519

	Spec. Values	Batch Values
Colour (visual)	white to off white	white
Appearance of substance (visual)	powder	powder
Identity (IR)	conforms	passes test
Optical rotation $\alpha$ 25/D (c=1 in EtOAc)	+22.5 - +27.5 °	+26.3 °
Enantiomeric purity	≥ 99.5 %	99.9 %
Purity		
TLC(011A)	≥ 98 %	> 99.0 %
TLC(0811)	≥ 98 %	> 99.0 %
Assay (HPLC, area%)	≥ 98.0 %	99.8 %
Fmoc-B-Ala-OH (HPLC, area%)	lot specific result	< 0.10 %
Fmoc-B-Ala-Ser (tBu)-OH (HPLC, area%)	lot specific result	< 0.10 %
Solubility (25 mmole in 50 ml DMF)	clearly soluble	passes test
UV/VIS (E(450 bis 600 nm))	≤ 40 mAU	6 mAU
Assay (acidimetric)	≥ 97.0 %	98.4 %
Water (K. F.)	≤ 1.0 %	0.2 %
Ethyl acetate (HS-GC)	≤ 0.5 %	< 0.5 %

For TLC-Systems see our General Catalog

### IMPROVED

Specified HPLC purity and enantiomeric purity has been increased to **≥ 99%** and **≥ 99.8%**, respectively, facilitating the production of purer and more consistent quality peptides.

Certificate of analysis (new version)



## Certificate of Analysis

8.52019.0000 Fmoc-Ser(tBu)-OH Novabiochem®

Batch S6671519

	Spec. Values	Batch Values
Colour (visual)	white to off white	white
Appearance of substance (visual)	powder	powder
Colour index (0.5 M in DMF)	≤ 60 Hazen	17 Hazen
Identity (IR)	passes test	passes test
Enantiomeric purity	≥ 99.8 % (a/a)	99.9 % (a/a)
Purity (HPLC)	≥ 99.0 % (a/a)	99.9 % (a/a)
Fmoc-B-Ala-OH (HPLC)	≤ 0.1 % (a/a)	≤ 0.1 % (a/a)
Fmoc-B-Ala-Ser (tBu)-OH (HPLC)	≤ 0.1 % (a/a)	≤ 0.1 % (a/a)
Fmoc-Ser(tBu)-Ser(tBu)-OH (HPLC)	≤ 0.1 % (a/a)	≤ 0.1 % (a/a)
Fmoc-Ser-OH (HPLC)	≤ 0.1 % (a/a)	≤ 0.1 % (a/a)
Assay free amino acid (GC)	≤ 0.2 % (a/a)	≤ 0.2 % (a/a)
Purity (TLC(011A))	≥ 98 %	> 99 %
Purity (TLC(0811))	≥ 98 %	> 99 %
Solubility (25 mmole in 50 ml DMF)	clearly soluble	passes test
Assay (acidimetric)	≥ 98.0 %	99.3 %
Water (K. F.)	≤ 2.0 %	< 0.1 %
Ethyl acetate (HS-GC)	≤ 0.5 %	0.1 %
Acetate (IC)	≤ 0.02 %	≤ 0.02 %

For TLC-Systems see our General Catalog

Responsible laboratory manager quality control

This document has been produced electronically and is valid without a signature.

### IMPROVED

All key amino acid derived impurities (β-alanyl, dipeptides and side-chain unprotected Fmoc-amino acid) are now specified to **≤ 0.1%**. More consistent and high quality Fmoc leads to purer and more easy to purify peptides.

NEW

Free amino acid content is now specified to **≤ 0.2%** using a quantitative GC-based assay instead of qualitative TLC-based assay used by other suppliers. Low free amine content reduces double insertion and improves stability on long term storage.

Ethyl acetate specified to **≤ 0.5%**, minimizing slow production of harmful acetic acid on long term storage.

NEW

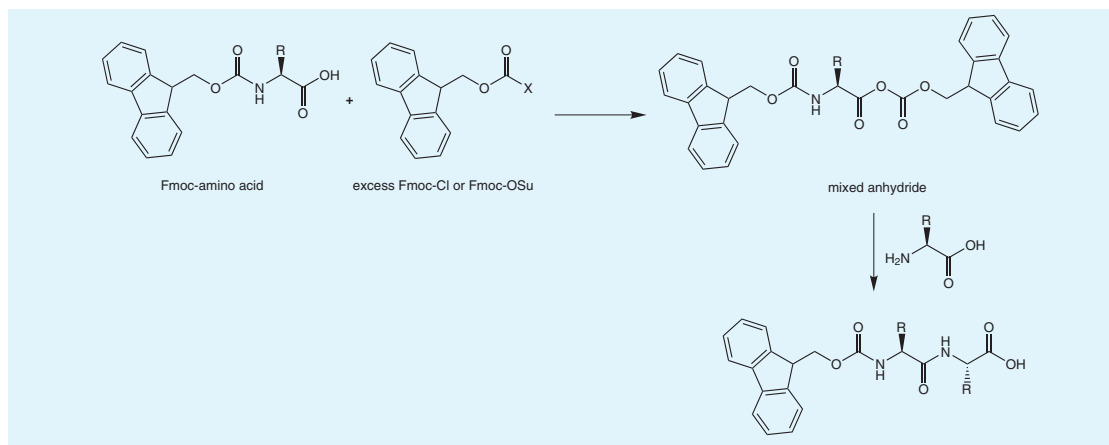
Acetate content specified to **≤ 0.02%** to minimize build up of truncated sequences during synthesis.

# So where do these impurities come from?

The impurities present in Fmoc-amino acids are predictable and mainly arise from side reactions during the attachment of the Fmoc group to the amino acid.

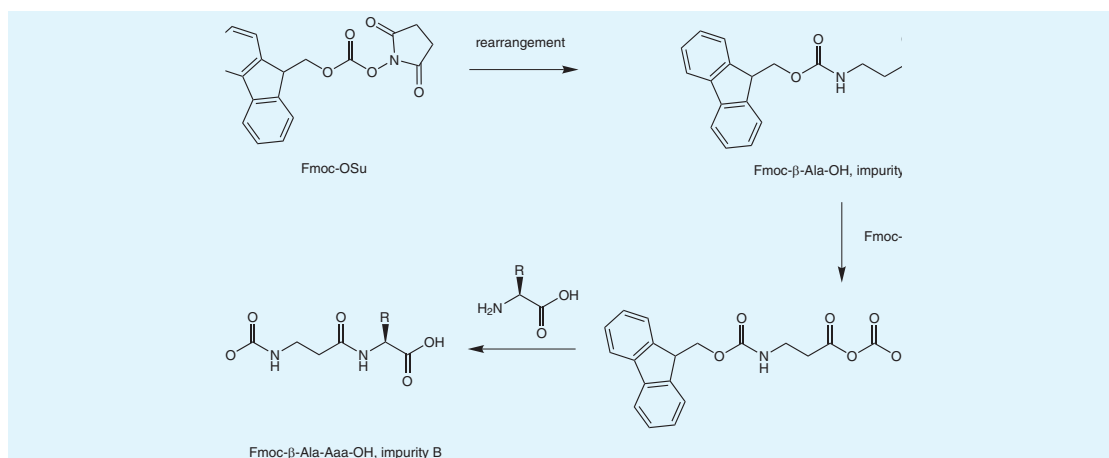
These impurities arise from reaction of reagent used to introduce the Fmoc group with the already formed Fmoc-amino acid. The presence of these impurities leads double insertion of the target amino acid.

## Dipeptides



## β-Alanyl impurities

Ring opening and rearrangement of Fmoc-OSu, which is used for Fmoc introduction, results in the formation of β-alanyl impurities. The presence of these impurities leads to insertion of additional β-alanine residue (B) or β-alanine (A) instead of the target amino acid into the peptide.



## Free amino acid

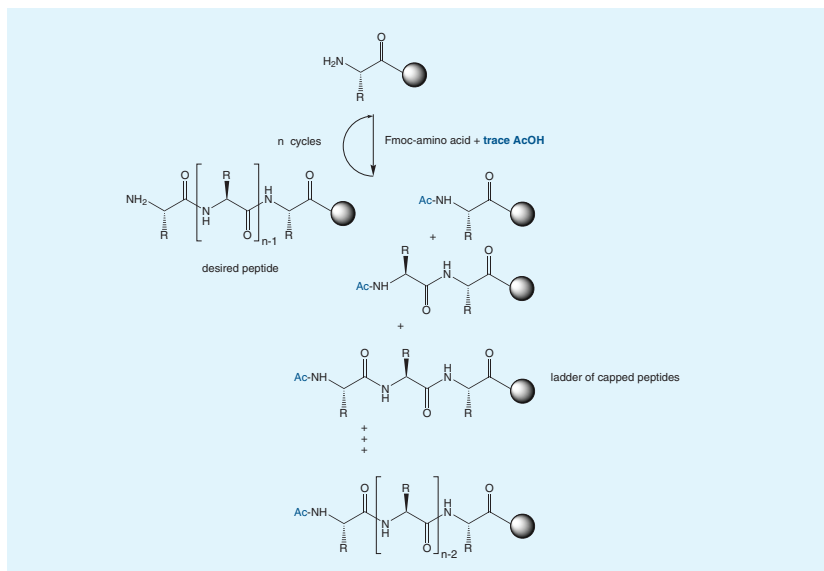
Free amino acids arise from incomplete reaction of the amino acid with the Fmoc reagent. The presence of free amino acid will lead to insertion of multiple copies of the target amino acid into the peptide chain and promote autocatalytic cleavage of the Fmoc group during storage of the Fmoc amino acid derivative. Unlike other suppliers which use an inaccurate TLC-based assay, Novabiochem® employs a quantitative GC-based method, to determine the levels of these impurities.

# Acetic Acid

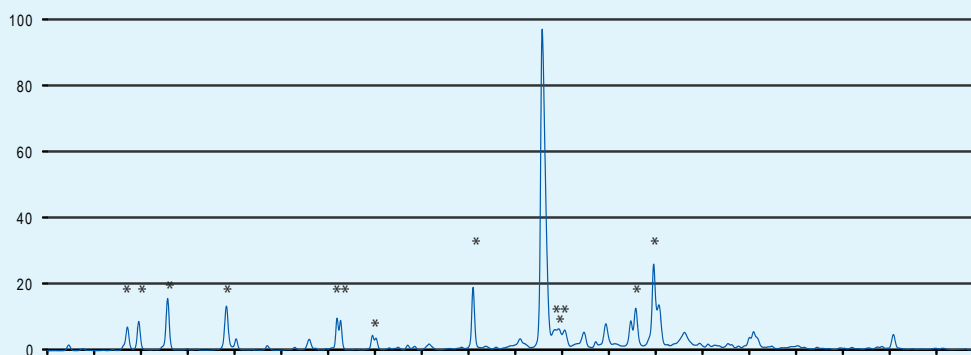
## The hidden killer

Acetic contamination of Fmoc-amino acids results in truncation of the growing peptide during solid phase synthesis. Even traces can have a dramatic effect owing to the small molecule mass of acetic acid and its high reactivity.

Consider Fmoc-Arg(Pbf)-OH contaminated with as little as 0.1% acetic acid. This translates to 1 mol% acetic acid contamination which does not sound too bad. However, when one considers that generally a 5-fold excess of reagents is used and the highly reactive acetic acid will always couple before the amino acid, then this can result in up to 5% chain termination each cycle. If you peptide contains 3 arginine residues then is 15% of chains will be terminated.



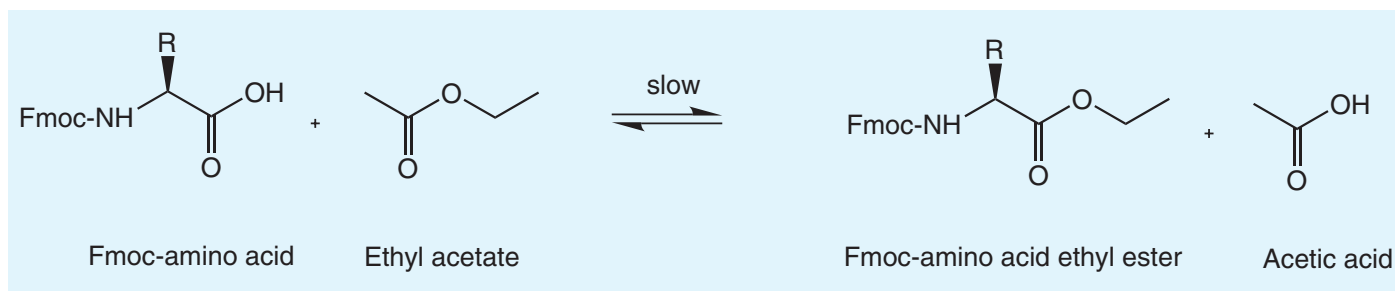
The effect of just 0.1% acetic contamination on the synthesis of a 21mer is illustrated in the HPLC chart below; the truncated by-products are marked by an asterisk.



The presence of acetic acid in Fmoc-amino acids is very hard to detect. It is invisible to HPLC and can not be detected in trace quantities by  $^1\text{H}$  NMR. This is why Merck Millipore has developed its methods for quantitation of acetic acid.

### So where does the acetic acid come from?

Ethyl acetate is a common solvent used in the preparation and crystallization of Fmoc-amino acids. Hydrolysis of this solvent results in formation of acetic acid. Moreover, residual ethyl acetate left after crystallization can slowly transesterify with the solid Fmoc-amino acid, resulting in formation of acetic acid on storage. This is why we specify both acetate and ethyl acetate content of its 20 standard Fmoc-amino acids.



# Ordering information

Product	Size	Cat. No.
Fmoc-Ala-OH	25 g	8520030025
	100 g	8520030100
	250 g	8520030250
	1 kg	8520031000
	10 kg	8520039010
Fmoc-Arg(Pbf)-OH	25 g	8520670025
	100 g	8520670100
	250 g	8520670250
	1 kg	8520671000
	10 kg	8520679010
Fmoc-Asn(Trt)-OH	25 g	8520440025
	100 g	8520440100
	250 g	8520440250
	1 kg	8520441000
	10 kg	8520449010
Fmoc-Asp(OtBu)-OH	25 g	8520050025
	100 g	8520050100
	250 g	8520050250
	1 kg	8520051000
	10 kg	8520059010
Fmoc-Cys(Trt)-OH	25 g	8520080025
	100 g	8520080100
	250 g	8520080250
	1 kg	8520081000
	10 kg	8520089010
Fmoc-Glu(OtBu)-OH	25 g	8520090025
	100 g	8520090100
	250 g	8520090250
	1 kg	8520091000
	10 kg	8520099010
Fmoc-Gln(Trt)-OH	25 g	8520450025
	100 g	8520450100
	250 g	8520450250
	1 kg	8520451000
	10 kg	8520459010
Fmoc-Gly-OH	25 g	8520010025
	100 g	8520010100
	250 g	8520010250
	1 kg	8520011000
	10 kg	8520019010
Fmoc-His(Trt)-OH	25 g	8520320025
	100 g	8520320100
	250 g	8520320250
	1 kg	8520321000
	10 kg	8520329010
Fmoc-Ile-OH	25 g	8520100025
	100 g	8520100100
	250 g	8520100250
	1 kg	8520101000
	10 kg	8520109010

Product	Size	Cat. No.
Fmoc-Leu-OH	25 g	8520110025
	100 g	8520110100
	250 g	8520110250
	1 kg	8520111000
	10 g	8520119010
Fmoc-Lys(Boc)-OH	25 g	8520230025
	100 g	8520230100
	250 g	8520230250
	1 kg	8520231000
	10 kg	8520239010
Fmoc-Met-OH	25 g	8520020025
	100g	8520020100
	250 g	8520020250
	1 kg	8520021000
	10 kg	8520029010
Fmoc-Phe-OH	25 g	8520160025
	100 g	8520160100
	250 g	8520160250
	1 kg	8520161000
	10 kg	8520169010
Fmoc-Pro-OH	25 g	8520170025
	100 g	8520170100
	250 g	8520170250
	1 kg	8520171000
	10 g	8520179010
Fmoc-Ser(tBu)-OH	25 g	8520190025
	100 g	8520190100
	250 g	8520190250
	1 kg	8520191000
	10 kg	8520199010
Fmoc-Thr(tBu)-OH	25 g	8520000025
	100 g	8520190100
	250 g	8520000250
	1 kg	8520001000
	10 kg	8520009010
Fmoc-Trp(Boc)-OH	25 g	8520500025
	100 g	8520500100
	250 g	8520500250
	1 kg	8520501000
	10 kg	8520509010
Fmoc-Tyr(tBu)-OH	25 g	8520200025
	100 g	8520200100
	250 g	8520200250
	1 kg	8520201000
	10 kg	8520209010
Fmoc-Val-OH	25 g	8520210025
	100 g	8520210100
	250 g	8520210250
	1 kg	8520211000
	10 kg	8520219010

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