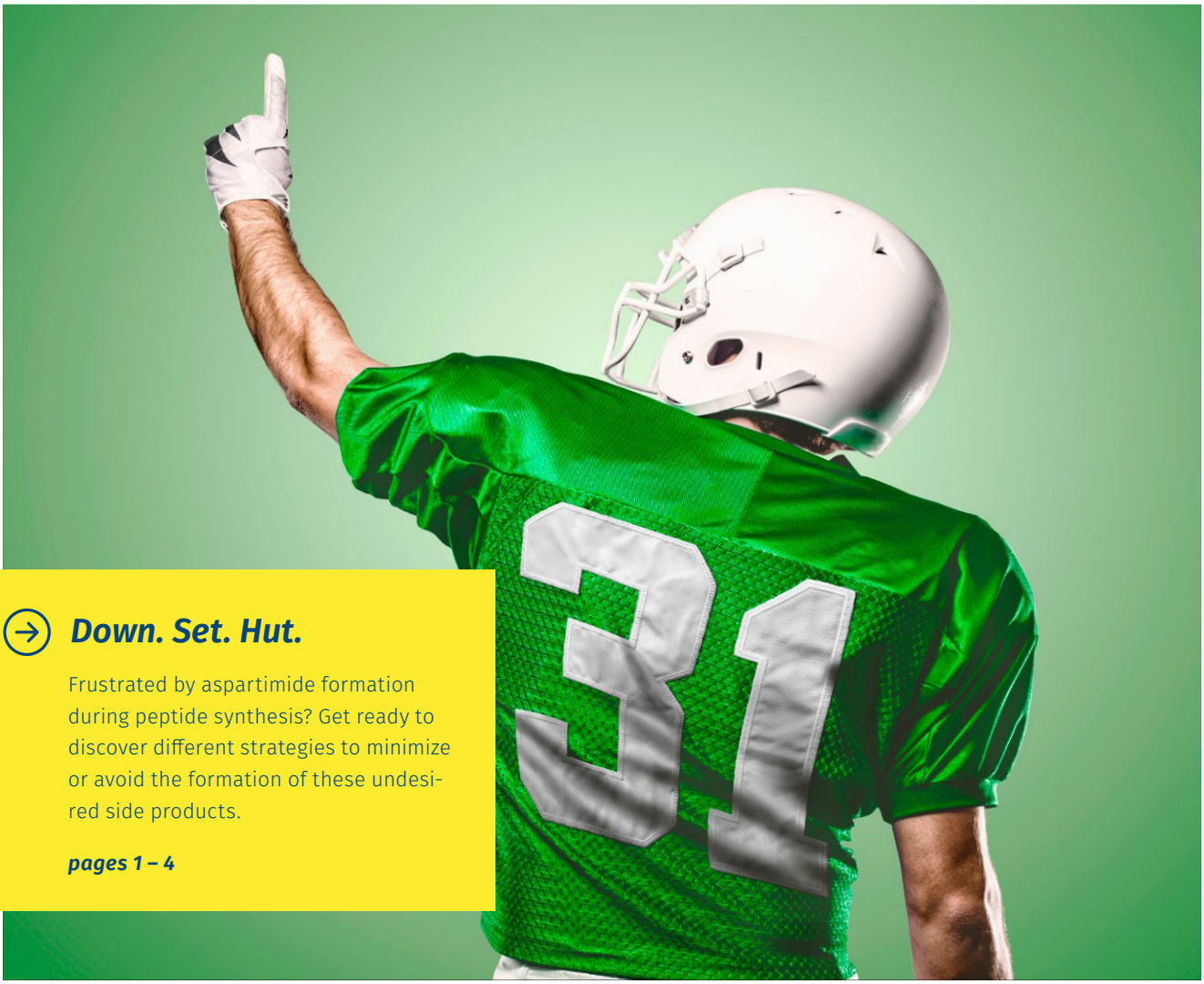
ASPARTIMIDE FORMATION

Measures to Tackle an Undesired Side Reaction



Down. Set. Hut.

Frustrated by aspartimide formation during peptide synthesis? Get ready to discover different strategies to minimize or avoid the formation of these undesired side products.

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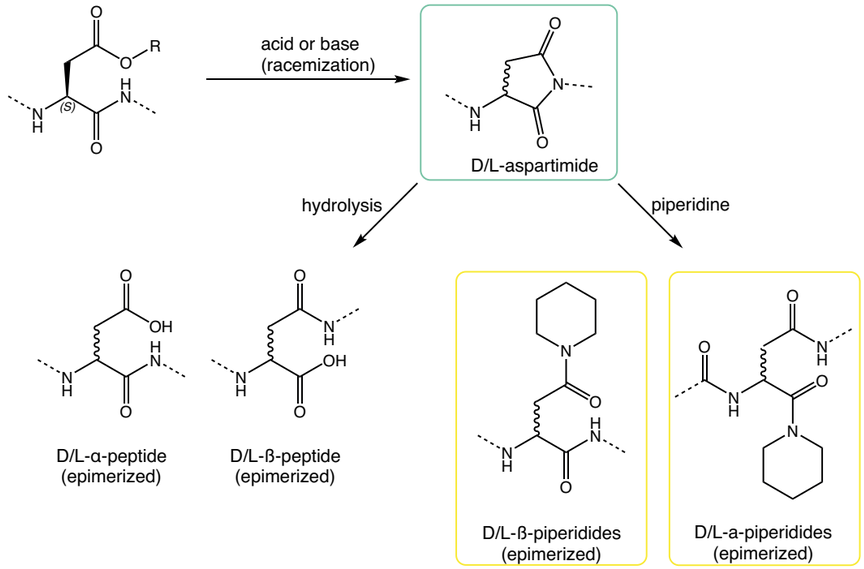
Version: IF18_3

Aspartimide Formation

Measures to Tackle an Undesired Side Reaction

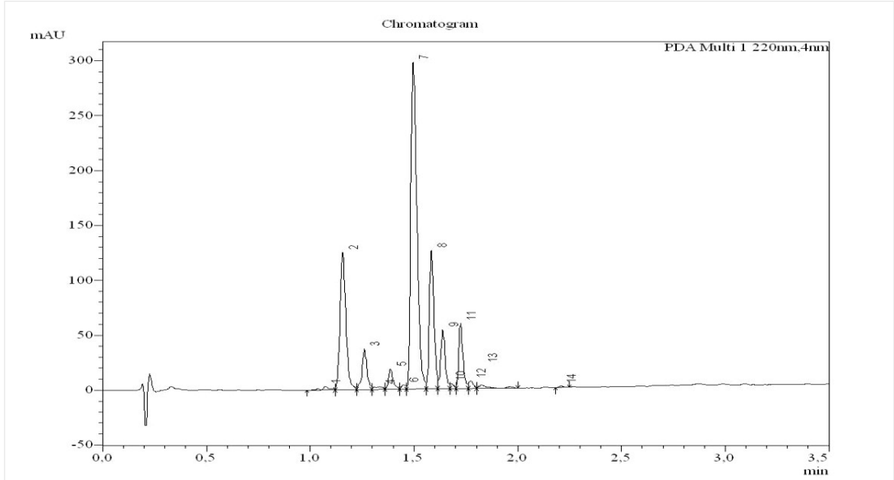
Chemical Background

Although peptide chemistry is constantly developing, the event of aspartimide formation represents a serious challenge during the synthesis of aspartate-containing peptides as it leads to lowered yields, difficult purifications, or even inaccessible sequences. This side reaction is strongly sequence dependent and preferably occurs at Asp-Aaa motifs (Aaa = glycine, aspartic acid, asparagine, glutamine or arginine). In a first step, the cyclic aspartimide is formed upon ring-closure between the nitrogen of the α-carboxyl amide bond and the β-carboxyl side-chain and release of the carboxyl protecting group. The formed aspartimides undergo rapid epimerization followed by ring-opening either by hydrolysis or by virtue of base, leading to (epimerized) α- and β-Asp peptides and corresponding piperidides. As this side reaction is promoted by strong bases such as piperidine, which is commonly used for Fmoc removal, this problem is especially pronounced during Fmoc SPPS.



Aspartimide formation and possible side products.

Thus, aspartimide formation leads to lowered product yields in addition to time- and cost-intensive purification. As some of those by-products are even co-eluting on HPLC due to identical retention times compared to the desired product, they may be virtually impossible to remove rendering certain peptide sequences totally inaccessible. Over the last decades, several approaches – based on the employment of specific building blocks and/or tuning the reaction conditions – have been developed to avoid this side reaction.

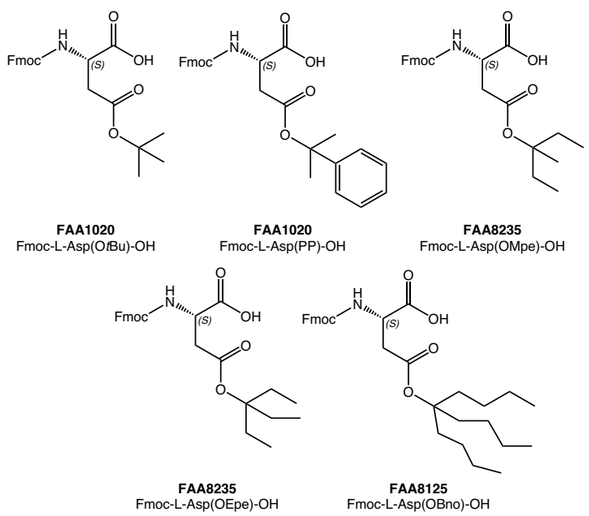


*Chromatogram of the model sequence VK**DG**YI-OH after treatment with 30% piperidine. Peak number 2 – target peptide, 3 – aspartimide, 7/8/9/11 – piperidides.*

Strategies

Use of Sterically Demanding Aspartic Acid Derivatives

Aspartimide formation can be decreased by increasing the steric bulk of the side-chain aspartic acid ester moiety. Besides the classical Fmoc-L-Asp(O*t*Bu)-OH (*FAA1020 on page 5*), Iris Biotech offers bulky ester derivatives with increased steric demand.

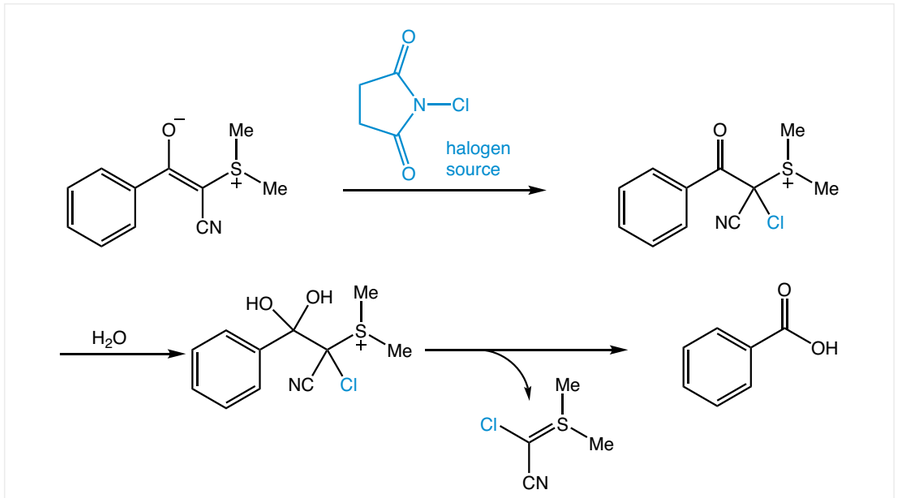


Bulky aspartic acid derivatives offered by Iris Biotech.

Analogs of the *tert*-butyl group in which at least one of the methyl groups is replaced by a bulkier alkyl or aryl substituent help to shield the aspartyl β-carboxyl group and thereby reduce the formation of aspartimide-derived by-products. The more sterically demanding the bulky aspartic acid side-chain protecting group, the lower the degree of aspartimide formation. Thus, our bulky side-chain protecting groups provide considerably more protection against the formation of aspartimide-related by-products than the commonly used O*t*Bu group.

A Cyanosulfurylide-Protected Aspartic Acid

Using cyanosulfurylide (CSY) as aspartic acid side-chain protecting group allows to completely suppress the event of aspartimide formation. In contrast to hydrophobic bulky aspartic acid derivatives, which often suffer from poor solubility and low coupling efficiency, CSY benefits of enhanced solubility. The CSY protecting group can be selectively and quantitatively cleaved from protected or unprotected peptides under aqueous conditions with electrophilic halogen species, e.g., N-chlorosuccinimide, to regenerate the carboxylic acid from the ylide, while being stable towards strong reducing agents, transition metals, strong acids and strong bases. Even though removal of this protecting group can be performed on-resin as well as in solution, the latter is recommended for best results. As cyanosulfurylides are absorbing strongly at 254 nm their cleavage can easily be monitored by HPLC analysis as varying amounts of NCS might be required depending on peptide sequence, purity, and concentration.



Postulated mechanism for the recovery of the free acid from the ylide by virtue of a halogen source.

Even though this building block allows to suppress aspartimide formation, other side products are observed, even when using morpholine as very weak cleaving reagent. Possible side reactions include the oxidation of cysteine, methionine, and tryptophane. Besides, chlorination of tyrosine might occur upon cleavage of the CSY group when using a large excess of N-chloro-succinimide.

Di- and Trimethoxybenzyl Glycine for Amide Backbone Protection

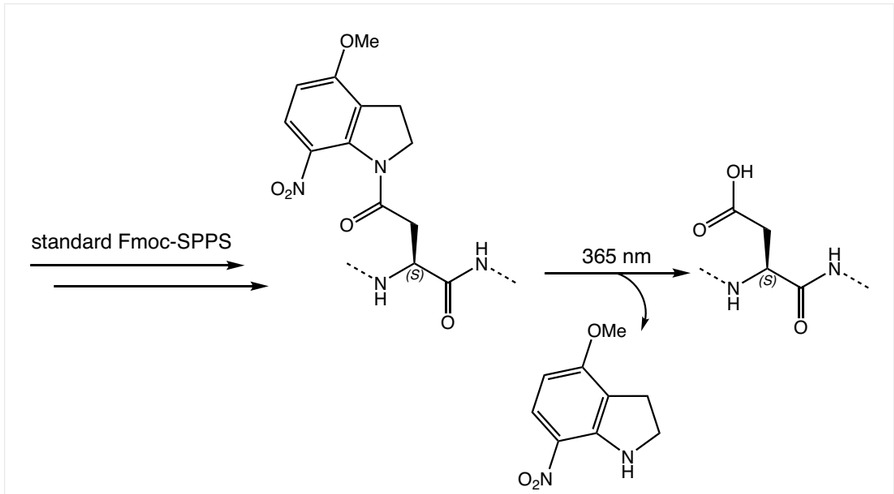
When the aspartic acid within the desired peptide sequence is next to a glycine, the use of di- or trimethoxybenzyl (DMB/TMB) is highly beneficial in order to prevent aspartimide formation. Dmb acts as an auxiliary protecting group temporarily masking the amide nitrogen of a peptide bond. Its efficacy and ease of introduction under standard coupling methods, e.g., PyBOP®/DIPEA or DIPCDI/HOBt, make it a valuable building block. After successful peptide synthesis, the N-Dmb group can be removed by addition of TFA, typically during TFA cleavage of the peptide from the resin. Iris Biotech offers Fmoc-DmbGly-OH (*FAA3390 on page 6*), Fmoc-TmbGly-OH (*FAA3400 on page 6*) as well as the precoupled dipeptide building block Fmoc-L-Asp(*t*Bu)-DmbGly-OH (*FDP1380 on page 6*) for the ease of synthesis. Despite its advantages, as possible side reaction, the Dmb group might react with „in-sequence“ tryptophanes.

Tuning Reaction Conditions

Besides using specific building blocks, also the choice of the reaction conditions has an impact on aspartimide formation. When a weak Fmoc cleaving reagent, e.g.,, morpholine (pK_a morpholine = 8.4) is used, almost no aspartimide formation is observed. However, sometimes this cleavage reagent is not sufficient for complete Fmoc removal, thus, stronger ones, e.g., 30% piperidine (pK_a piperidine = 11.2), have to be used. Depending on the sequence, the addition of acid (e.g., 30% piperidine/0.1 M formic acid) can help to reduce aspartimide formation.

Use of a Photocaged Aspartic Acid

The introduction of a 4-methoxy-7-nitroindoline (MNI) group on the side-chain of aspartic acid helps to efficiently prevent the formation of aminosuccinyl side products and pyrrolidones. The MNI caging group has excellent photochemical properties (two-photon cross section = 0.06 GM) and provides rapid photolysis kinetics at 365 nm (half time of photo release 60.26 ms). The building block containing the MNI protecting group (*FAA9555 on page 6*) can be efficiently incorporated into peptides using standard SPPS protocols, making it a useful tool for the study of biological systems in a triggerable manner.



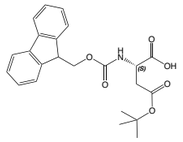
MNI-photocaged aspartic acid and deprotection by irradiation with UV light.

Product details

FAA1020 Fmoc-L-Asp(O*t*Bu)-OH

N-alpha-(9-Fluorenylmethyloxycarbonyl)-L-aspartic acid beta-*t*-butyl ester

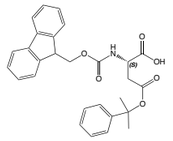
CAS-No. 71989-14-5
Formula $C_{23}H_{25}NO_6$
Mol. weight 411,45 g/mol



FAA1970 Fmoc-L-Asp(OPP)-OH

N-alpha-(9-Fluorenylmethyloxycarbonyl)-L-aspartic acid beta-(2-phenylisopropyl ester)

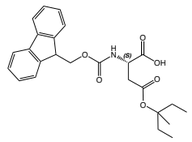
CAS-No. 200336-86-3
Formula $C_{28}H_{27}NO_6$
Mol. weight 473,52 g/mol



FAA8120 Fmoc-L-Asp(OMpe)-OH

N-alpha-(9-Fluorenylmethyloxycarbonyl)-L-aspartic acid beta-3-methylpentyl ester

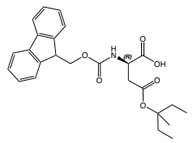
CAS-No. 180675-08-5
Formula $C_{25}H_{29}NO_6$
Mol. weight 439,51 g/mol



FAA9275 Fmoc-D-Asp(OMpe)-OH

N-alpha-(9-Fluorenylmethyloxycarbonyl)-D-aspartic acid beta-3-methylpentyl ester

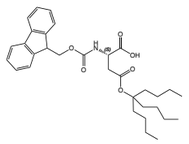
CAS-No. 1926162-97-1
Formula $C_{25}H_{29}NO_6$
Mol. weight 439,51 g/mol



FAA8125 Fmoc-L-Asp(OBno)-OH

N-alpha-(9-Fluorenylmethyloxycarbonyl)-L-aspartic acid beta-(5-butylnon-5-yl) ester

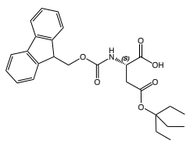
CAS-No. 1799418-06-6
Formula $C_{32}H_{43}NO_6$
Mol. weight 537,70 g/mol



FAA8235 Fmoc-L-Asp(OEpe)-OH

N-alpha-(9-Fluorenylmethyloxycarbonyl)-L-aspartic acid beta-3-ethylpentyl ester

CAS-No. 1799418-01-1
Formula $C_{26}H_{31}NO_6$
Mol. weight 453,54 g/mol

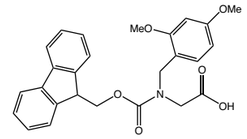


Product details

FAA3390 Fmoc-DmbGly-OH

N-alpha-(9-Fluorenylmethyloxycarbonyl)-N-alpha-(2,4-dimethoxybenzyl)-glycine

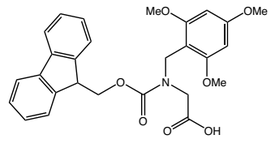
CAS-No. 166881-42-1
Formula $C_{26}H_{25}NO_6$
Mol. weight 447,48 g/mol



FAA3400 Fmoc-TmbGly-OH

N-alpha-(9-Fluorenylmethyloxycarbonyl)-N-alpha-(2,4,6-trimethoxybenzyl)-glycine

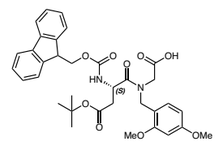
CAS-No. 166881-43-2
Formula $C_{27}H_{27}NO_7$
Mol. weight 477,51 g/mol



FDP1380 Fmoc-L-Asp(*t*Bu)-DmbGly-OH

N-alpha-(9-Fluorenylmethyloxycarbonyl)-L-aspartic acid beta-*t*-butylester-*N'*-(2,4-dimethoxybenzyl)-glycine

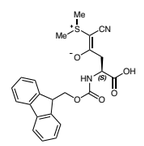
CAS-No. 900152-72-9
Formula $C_{34}H_{38}N_2O_9$
Mol. weight 618,68 g/mol



FAA8480 Fmoc-L-Asp(CSY)-OH

N-alpha-(9-Fluorenylmethyloxycarbonyl)-L-aspartic acid cyanosulfurylide

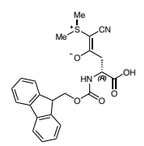
CAS-No. 2379679-90-8
Formula $C_{23}H_{22}N_2O_5S$
Mol. weight 438,50 g/mol



FAA8483 Fmoc-D-Asp(CSY)-OH

N-alpha-(9-Fluorenylmethyloxycarbonyl)-D-aspartic acid cyanosulfurylide

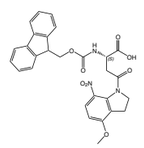
Formula $C_{23}H_{22}N_2O_5S$
Mol. weight 438,50 g/mol



FAA9555 Fmoc-L-Asp(MNI)-OH

(S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-(4-methoxy-7-nitroindolin-1-yl)-4-oxobutanoic acid

CAS-No. 1799532-96-9
Formula $C_{28}H_{25}N_3O_8$
Mol. weight 531,51 g/mol



Aspartimide Formation

References:

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- *New t-butyl based aspartate protecting groups preventing aspartimide formation in Fmoc SPPS; R. Behrendt, S. Huber, R. Marti, P. White;* ***J. Pept. Sci.*** *2015;* ***21(8)****: 680-687. https://doi.org/10.1002/psc.2790*
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- *The aspartimide problem in Fmoc-based SPPS. Part I; M. Mergler, F. Dick, B. Sax, P. Weiler, T. Vorherr;* ***J. Pept. Sci.*** *2003;* ***9(1)****: 36-46. https://doi.org/10.1002/psc.430*
- *A new protecting group for aspartic acid that minimizes piperidine-catalyzed aspartimide formation in Fmoc solid phase peptide synthesis; A. Karlström, A. Undén;* ***Tetrahedron Lett.*** *1996;* ***37(24)****: 4243-4246. https://doi.org/10.1016/0040-4039(96)00807-6*
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- *Prevention of aspartimide formation during peptide synthesis using cyanosulfurylides as carboxylic acid-protecting groups; K. Neumann, J. Farnung, S. Baldauf, J. W. Bode;* ***Nat. Commun.*** *2020;* ***11****: 982. https://doi.org/10.1038/s41467-020-14755-6*
- *Patent EP 2 886 531 B1*
- *Acid-Mediated Prevention of Aspartimide Formation in Solid Phase Peptide Synthesis; T. Michels, R. Dölling, U. Haberkorn, W. Mier;* ***Org. Lett.*** *2012;* ***14(20)****: 5218-5221. https://doi.org/10.1021/ol3007925*

For more details, watch the recording of our workshop about measures to prevent aspartimide formation!