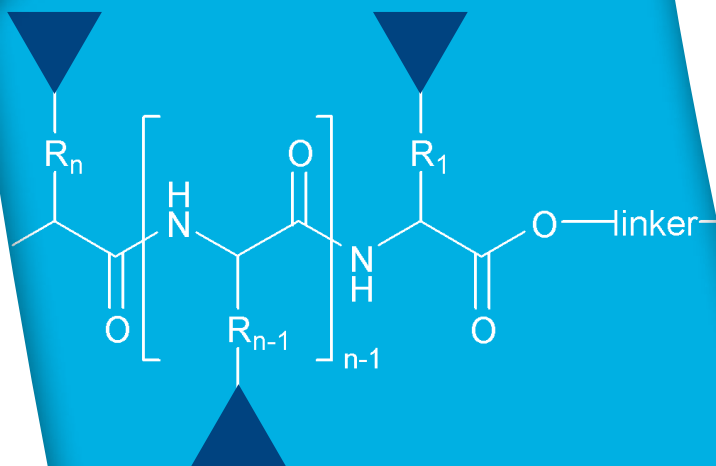


# ORTHOGONALITY OF PROTECTING GROUPS BACHEM

PIONEERING PARTNER FOR PEPTIDES



# ORTHOAGONALITY OF PROTECTING GROUPS

The term “orthogonal” was coined by Barany and Merrifield in 1977 to designate “classes of protecting groups which are removed by differing chemical mechanisms. Therefore they can be removed in any order and in the presence of the other classes. Orthogonal protection schemes allow milder overall reaction conditions as well as the synthesis of partially protected peptides” (G. Barany and R.B. Merrifield). The combination Fmoc/tBu is truly orthogonal whereas, under the conditions of Boc-SPPS, Boc/Bzl is not, as both protecting groups are cleaved by acids. As Boc can be selectively removed in the presence of Z/Bzl, the combination has been termed “quasi-orthogonal”.

## UNUSUAL AMINO ACIDS

In addition to our comprehensive choice of derivatives of all stereoisomers of the 20 canonical amino acids we offer an extensive range of unusual amino acids, which can serve as building blocks for the synthesis of peptide analogs and for use in structure-activity relationship (SAR) studies. Furthermore, these compounds are valuable chiral educts for organic synthesis.

### Orthogonal Protection

Fmoc/tBu probably is the most popular “orthogonal” combination of protecting groups. According to the definition of this term by Barany and Merrifield [1], this combination is truly orthogonal, Fmoc can be removed selectively in the presence of tBu and the latter is split off under conditions leaving the amino-protecting group intact. On the other hand, the pairs Boc/OPp or Fmoc/ivDde are merely quasi-orthogonal because both groups are acid-labile or cleaved by bases, respectively. OPp can be removed selectively in the presence of Boc/tBu by “weak acid”, and Fmoc by piperidine/DMF with preservation of ivDde protection, whereas the order of deprotection cannot be reverted.

Modified peptides may be sensitive towards both bases and acids, so it should be kept in mind that Alloc/OAll and Z/Bzl may be removed under neutral conditions.

Thus, when conceiving the synthesis of more complex or modified peptides (e.g. side-chain cyclized peptides), the tactics of synthesis, i.e. the choice of side-chain

protecting groups and type of resin, has to be considered thoroughly.

For on-resin modification Alloc/OAll, Dde and ivDde/Dmab, Mtt and Mmt/OPp may be selectively cleaved from peptides bound to Wang resin. Alloc can be removed on-resin in the presence of Mmt and vice versa during Fmoc-SPPS. The combination of orthogonal protecting groups allows the synthesis of very complex peptides, impressive examples have been published e.g. by Hirschmann et al. [2] or Royo et al. [3].

Orthogonal protection schemes can also be devised for Boc-SPPS, e.g. Boc/Fm, Boc/allyl. An additional “dimension” of orthogonality, the hydrogenolysis of benzyl-derived protecting groups, is available in solution-phase peptide synthesis. This deblocking method is only rarely used in SPPS. Enzymatically cleavable protecting groups as phenylacetyl have also been employed for peptide modifications, mostly in aqueous solution.

The risk of aspartimide formation has to be kept in mind when considering a postsynthetic modification of the  $\beta$ -carboxy moiety of Asp. This notorious side reaction is facilitated by unhindered  $\beta$ -carboxy protecting groups such as OBzl, OAll or ODmab. The selectively cleavable OPp is nearly as effective as OtBu in suppressing base-catalyzed aspartimide formation [4].

The sterically demanding OMpe group has been developed as an even more efficient tool for preventing this side reaction, but it can't be removed selectively under the conditions of standard Fmoc-SPPS [5].

Even though Glu tends far less than Asp to base-catalyzed cyclization, unhindered  $\gamma$ -carboxyl protective groups as allyl promote the side reaction.

Cysteine requires special consideration when conceiving a synthetic strategy based on multidimensional orthogonal protection, please see our brochure “Cysteine Derivatives” for more details.

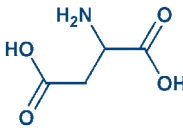
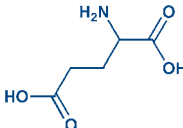
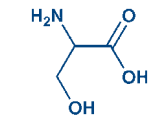
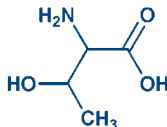
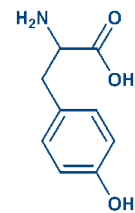
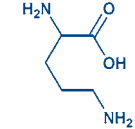
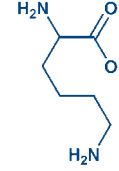
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Orthogonality “Dimension”	Examples for feasible combinations of protecting groups
2nd	Fmoc/tBu
3rd	Fmoc/tBu/allyl
3rd	Fmoc/tBu/Mmt <sup>1</sup>
3rd	Fmoc/tBu/Dde <sup>1</sup>
4th	Fmoc/tBu/Mmt/allyl
4th	Fmoc/tBu/allyl/Dde
5th	Fmoc/tBu/allyl/Dde/Mmt
5th	Fmoc/tBu/allyl/Dde/Bzl <sup>1,2</sup>
5th	Fmoc/tBu/Mmt/allyl/Dde
6th	Fmoc/tBu/Mmt/allyl/Dde/Bzl

<sup>1</sup>partly quasi-orthogonal

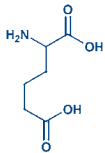
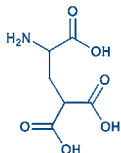
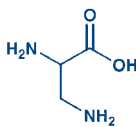
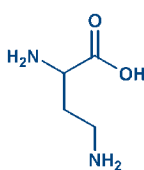
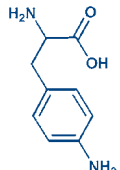
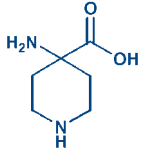
<sup>2</sup>Bzl: hydrogenolytic cleavage

Protection Type		tBu and Others	Different				
Functionality	Typical Cleavage Conditions	95% aq TFA/ Scavengers		1% TFA/DCM			
COOH		Fmoc-Asp(OtBu)-OH <sup>†‡*x</sup>	B-1065	Fmoc-Asp(2-phenylisopropyl ester)-OH	B-2475		
		Fmoc-Asp(OMpe)-OH*	B-3560				
		Fmoc-Asp(OtBu)-OPfp*	B-1110			Fmoc-Asp-ODmb*	B-2175
		Fmoc-Asp(OtBu)-OSu*	B-1075				
		Fmoc-Asp-OtBu*	B-1735				
		Fmoc-N-Me-Asp(OtBu)-OH <sup>†</sup>	B-2195				
COOH		Fmoc-Glu(OtBu)-OH · H <sub>2</sub> O <sup>†‡*x</sup>	B-1315	Fmoc-Glu(2-phenylisopropyl ester)-OH	B-2500		
		Fmoc-Glu(OtBu)-OPfp*	B-1130				
		Fmoc-Glu(OtBu)-OSu	B-1325				
		Fmoc-Glu(OSu)-OtBu	B-2310				
		Fmoc-N-Me-Glu(OtBu)-OH	B-2395				
OH		Fmoc-Ser(tBu)-OH <sup>†‡*x</sup>	B-1235	Fmoc-Ser(Trt)-OH	B-2550		
		Fmoc-Ser(tBu)-OPfp	B-1180				
		Fmoc-Ser(tBu)-ODhbt*	B-1640				
		Fmoc-N-Me-Ser(tBu)-OH	B-3400				
		Fmoc-Ser(BSi)-OH*	B-1225				
		Fmoc-Ser(PO(OBzl)OH)-OH	B-3455				
OH		Fmoc-Thr(tBu)-OH <sup>†‡*x</sup>	B-1245	Fmoc-Thr(Trt)-OH	B-2545		
		Fmoc-Thr(tBu)-OPfp	B-1185				
		Fmoc-Thr(tBu)-ODhbt	B-1635				
		Fmoc-allo-Thr(tBu)-OH*	B-1815				
		Fmoc-allo-Thr(tBu)-ODhbt	B-1810				
		Fmoc-N-Me-Thr(tBu)-OH	B-3420				
		Fmoc-Thr(PO(OBzl)OH)-OH	B-3460				
OH		Fmoc-Tyr(tBu)-OH <sup>†‡*x</sup>	B-1255				
		Fmoc-Tyr(tBu)-OPfp	B-1195				
		Fmoc-Tyr(PO <sub>3</sub> (MDPSE) <sub>2</sub> )-OH	B-2910				
		Fmoc-Tyr(PO <sub>3</sub> Me <sub>2</sub> )-OH <sup>†</sup>	B-1990				
		Fmoc-Tyr(PO(OBzl)OH)-OH	B-3565				
		Fmoc-Tyr(malonyl-di-OtBu)-OH	B-2825				
		Fmoc-Tyr(SO <sub>3</sub> )-OH sodium salt	B-2765				
NH <sub>2</sub>		Fmoc-Orn(Boc)-OH <sup>†*</sup>	B-1090				
		Fmoc-Orn(Boc)-OPfp	B-2155				
		Boc-Orn(Fmoc)-OH*	A-3325				
NH <sub>2</sub>		Fmoc-Lys(Boc)-OH <sup>†‡*x</sup>	B-1080	Fmoc-Lys(Adpoc)-OH	B-2520		
		Fmoc-Lys(Boc)-OPfp*	B-1155			Fmoc-Lys(Mtt)-OH <sup>†‡*x</sup>	B-2535
		Boc-Lys(Fmoc)-OH*	A-1610			Fmoc-Lys(4-methoxytrityl)-OH	B-3215
		Fmoc-Lys(Boc)(Me)-OH	B-3575				
		Fmoc-Lys(Boc)(isopropyl)-OH	B-2455				
		Fmoc-Lys(retro-Abz-Boc)-OH	B-3180				
		Fmoc-Lys(N-Me-Abz-Boc)-OH	B-2515				
		Fmoc-N-Me-Lys(Boc)-OH <sup>†</sup>	B-3685				

<sup>†</sup> also available linked to SASRIN resin <sup>‡</sup> also available linked to Wang resin <sup>\*</sup> H-derivative linked to 2-Chlorotrityl-resin also available  
<sup>\*</sup> D-enantiomer also available

Allyl		Dde and Analogs		Bzl, Analogs and Others	
Pd(PPh <sub>3</sub> ) <sub>4</sub> /PhSiH <sub>3</sub>		2% N <sub>2</sub> H <sub>4</sub> · H <sub>2</sub> O/DMF <sup>§</sup>		Catalytic Hydrogenation <sup>§§</sup>	
Fmoc-Asp(OAll)-OH	B-2325	Fmoc-Asp(ODmab)-OH	B-3240	Fmoc-Asp(OBzl)-OH*	B-1060
Fmoc-Asp-OAll	B-2715			Fmoc-Asp(OBzl)-OPfp	B-1760
				Fmoc-Asp-OBzl	B-2780
Fmoc-Glu(OAll)-OH*	B-3255	Fmoc-Glu(ODmab)-OH	B-3010	Fmoc-Glu(OBzl)-OH*	B-1310
Fmoc-Glu-OAll	B-2720	Fmoc-Glu-ODmab	B-3005		
				Fmoc-Ser(Bzl)-OH*	B-1200
				Fmoc-Thr(Bzl)-OH*	B-1210
				Fmoc-Tyr(Bzl)-OH†	B-1215
				Fmoc-Tyr(2-bromo-Z)-OH	B-2795
				Fmoc-Tyr(2,6-dichloro-Bzl)-OH	B-1280
Fmoc-Orn(Aloc)-OH*	B-2890	Fmoc-Orn(Dde)-OH	B-3185	Z-Orn(Fmoc)-OH*	C-3325
Fmoc-Lys(Aloc)-OH*	B-2240	Fmoc-Lys(Dde)-OH	B-3015	Fmoc-Lys(Z)-OH*	B-1270
		Fmoc-Lys(ivDde)-OH	B-3515	Fmoc-Lys(2-chloro-Z)-OH	B-2790
		Fmoc-Lys(Nde)-OH	B-3380	Z-Lys(Fmoc)-OH*	C-4125
		Dde-Lys(Fmoc)-OH	E-3385		

§ Fmoc is not stable under these cleavage conditions. Dde and analogs may be cleaved selectively in the presence of the other protecting groups listed here §§ Fmoc may not be fully stable in some cases towards hydrogenation

Additional Orthogonally Protected Fmoc Amino Acid Derivatives offered by Bachem			
<b>Aad</b>		$\alpha$ -Aminoadipic Acid	Fmoc-Aad(OtBu)-OH B-2440
<b>Gla</b>		$\gamma$ -Carboxy-glutamic Acid	Fmoc- $\gamma$ -carboxy-Glu(OtBu) <sub>2</sub> -OH* B-1265
<b>Dap</b>		$\alpha,\beta$ -Diaminopropionic Acid	Fmoc-Dap(Adpoc)-OH B-2865 Fmoc-Dap(Aloc)-OH B-2845 Fmoc-Dap(Boc)-OH* B-2380 Fmoc-Dap(ivDde)-OH B-3885 Boc-Dap(Fmoc)-OH* A-3580 Z-Dap(Fmoc)-OH* C-4200
<b>Dab</b>		$\alpha,\gamma$ -Diaminobutyric Acid	Fmoc-Dab(Adpoc)-OH B-2860 Fmoc-Dab(Aloc)-OH B-2850 Fmoc-Dab(Boc)-OH* B-1800 Fmoc-Dab(ivDde)-OH B-4065 Fmoc-Dab(Z)-OH B-3250 Boc-Dab(Fmoc)-OH A-3520
<b>Aph</b>		4-Aminophenylalanine	Fmoc-p-amino-Phe(Boc)-OH* B-1995 Boc-p-amino-Phe(Fmoc)-OH* A-3975
<b>4-Pip</b>		4-Aminopiperidine-4-carboxylic acid	1-Boc-4-(Fmoc-amino)-piperidine-4-carboxylic acid B-3115
<b>Various</b>		p-Carboxyphenylalanine $\alpha$ -Amino-4-thiaadipic Acid 4-Thiahomolysine N-2-Aminoethylglycine $\beta$ -Piperazinyllalanine	Fmoc-p-carboxy-Phe(OtBu)-OH B-3070 Fmoc-Cys(t-butylcarboxymethyl)-OH B-3395 Fmoc-Cys(3-(Boc-amino)-propyl)-OH B-3120 Fmoc-(N- $\beta$ -Boc-aminoethyl)-Gly-OH B-3285 Fmoc- $\beta$ -(1-piperazinyll)-Ala(Boc)-OH B-3725

\* D-enantiomer also available

## Abbreviations

Abz	2-Aminobenzoyl (Anthraniloyl)
Adpoc	1-(1'-Adamantyl)-1-methyl-ethoxycarbonyl
Aloc	Allyloxycarbonyl
Boc	t-Butyloxycarbonyl
Bsi	(or TBDMS) t-Butyldimethylsilyl
Bzl	Benzyl
Dde	1-(4,4-Dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl
Fm	9-Fluorenylmethyl
Fmoc	9-Fluorenylmethyloxycarbonyl
ivDde	1-(4,4-Dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl
MDPSE	2-(Methyl-diphenyl-silyl)ethyl
Me	Methyl
Mmt	4-Methoxytrityl
Mtt	4-Methyltrityl
Nde	1-(4-Nitro-1,3-dioxoindan-2-ylidene)ethyl
OAll	Allyl ester
OBzl	Benzyl ester
ODhbt	3,4-Dihydro-4-oxo-1,2,3-benzotriazine-3yl ester
ODmab	4-(N-[1(4,4-Dimethyl-2,6-dioxocyclohexylidene)-3-methyl-butyl]amino)benzyl ester
ODmb	2,4-Dimethoxybenzyl ester
OMpe	3-Methyl-pent-3-yl ester
OPfp	Pentafluorophenyl ester
OPp	2-Phenylisopropyl ester
OSu	N-Hydroxysuccinimide ester
OtBu	t-Butyl ester
Ph	Phenyl
tBu	t-Butyl
Trt	Trityl
Z	Benzyloxycarbonyl

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