Novabiochem®

Letters: 03/05

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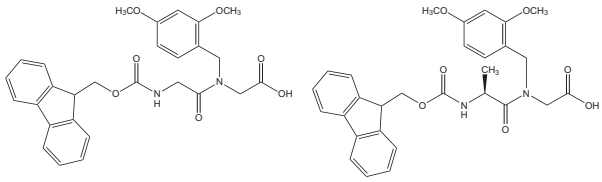
Product Focus: New products for peptide synthesis

NEW Derivatives for enhancing peptide synthesis

Fmoc-Gly-(Dmb)Gly-OH **Fmoc-Ala-(Dmb)Gly-OH**



Features & Benefits

- "Pseudoproline effect" for Ala-Gly- and Gly-Gly-containing sequences
- Compatible with standard Fmoc protocols
- Dmb group removed during TFA cleavage
- Cannot form cyclic lactones

Pseudoproline dipeptides [1] and Hmb derivatives [2] are extremely powerful tools for enhancing synthetic efficiency in Fmoc SPPS, particularly of intractable peptides [3 - 6], long peptides/small proteins [7, 8], and cyclic peptides [9, 10]. They work by exploiting the natural propensity of N-alkyl amino acids [11, 12] (Figure 1) to disrupt the formation of the secondary structures during peptide assembly. The results are better and more predictable acylation and deprotection kinetics, enhanced reaction rates, and improved yields, purities and solubilities of crude products. However, these approaches have certain limitations. Pseudoproline dipeptides can only be used for sequences containing serine or threonine, and the coupling of the amino acid following the Hmb residue can be extremely

difficult. To alleviate some of these shortcomings and expand the scope of the structure breaking N-alkyl amino acids available for Fmoc SPPS, Novabiochem is pleased to introduce Fmoc-Ala-(Dmb)Gly-OH and Fmoc-Gly-(Dmb)Gly-OH, the first in its new range of Dmb dipeptides. These novel derivatives offer the same benefits as pseudoproline dipeptides but for peptide sequences containing Gly.

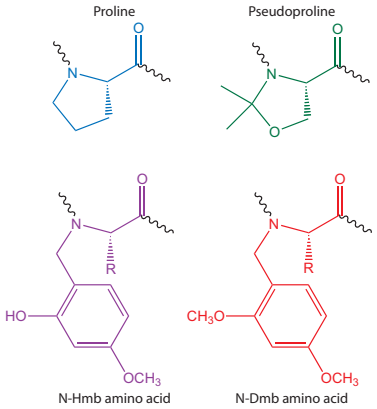


Fig. 1: Secondary structure disrupting N-alkyl amino acids.

Dmb dipeptides are extremely easy to use: simply substitute a Gly residue together with the preceding Ala or Gly residue in the peptide sequence with the appropriate Dmb dipeptide (Figure 2). They are fully compatible with standard coupling methods such as PyBOP®/DIPEA or DIPCDI/HOBt, since unlike analogous Hmb-based derivatives they cannot form cyclic lactones [13]. Removal of the Dmb group and regeneration of the glycine residue occurs during the course of the standard TFA-mediated cleavage reaction. It is important to note, however, that the Dmb cation produced during this process is a very powerful alkylating agent and can cause side-chain modification of unprotected tryptophan residues. Therefore, the use of Fmoc-Trp(Boc) is strongly recommended in such cases.

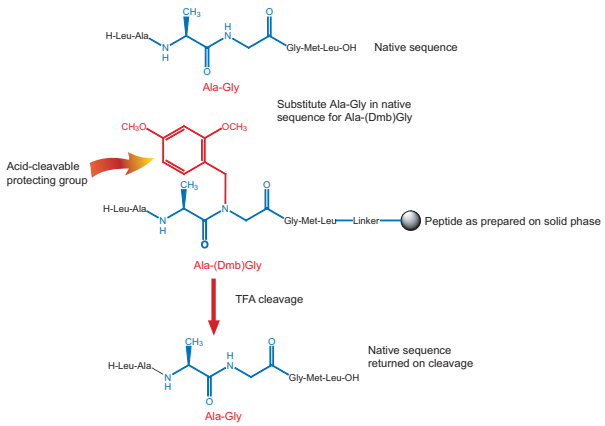


Fig. 2: Principles of using Dmb dipeptides.

To achieve the maximum benefits from the use of Dmb dipeptides, one should observe the following guidelines based on those developed for pseudoproline dipeptides.

- Optimal results are obtained, if the Dmb dipeptides are spaced 5-6 residues apart throughout the sequence.
- The optimum separation between a Dmb dipeptide and a Pro or pseudoproline dipeptide is 5-6 amino acid residues.
- The minimum separation between a Dmb dipeptide and another Dmb/pseudoproline dipeptide or Pro residue is 2 residues.
- Aim to insert a Dmb dipeptide before regions of hydrophobic residues.

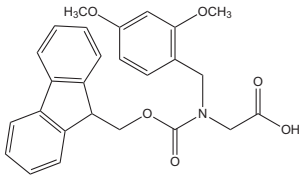
Recently, the use of Fmoc-Gly-(Dmb)Gly-OH was found to be essential for the synthesis of peptides related to nucleolin [14].

04-12-1265	Fmoc-Ala-(Dmb)Gly-OH *NEW*	1 g
04-12-1266	Fmoc-Gly-(Dmb)Gly-OH *NEW*	1 g

Novabiochem's Hmb dipeptides

04-12-1235	Fmoc-Asp(OtBu)-(Hmb)Gly-OH	1 g 5 g
04-12-1253	Fmoc-Gly-(Hmb)Gly-OH	1 g 5 g

Fmoc-(Dmb)Gly-OH



Features & Benefits

- "Pseudoproline effect" for Gly-containing sequences
- Compatible with standard Fmoc protocols
- Dmb group removed during TFA cleavage
- Cannot form cyclic lactones

Fmoc-(Dmb)Gly-OH [2] offers many of the same benefits as the previously described Dmb dipeptides, making it ideal for those situations where the appropriate pre-formed Dmb dipeptide is not available. Like Fmoc-(FmocHmb)Gly-OH, it is useful for preparing peptides containing the aspartimide-prone Asp-Gly sequence, as introduction of the Gly residue using Fmoc-(Dmb)Gly will completely prevent this side reaction [15]. Furthermore, the turn-inducing conformational properties of (Dmb)Gly make it an excellent tool for the preparation of cyclic peptides [16].

The most important application of this derivative, however, is likely to be in the synthesis of hydrophobic amyloid and transmembrane peptides. These sequences are extremely difficult to prepare using conventional methods, and are generally not amenable to pseudoproline substitution

as they rarely contain multiple serine or threonine residues. Glycine, however, does occur frequently in such peptides, often adjacent to hydrophobic residues such as Ile, Val, and Ala. Therefore, substitution of Gly by (Dmb)Gly should prove to be a highly effective method of overcoming these problems. Indeed, using six substitutions of the analogous (Tmob)Gly derivative, Bayer and colleagues were able to prepare a 64-residue transmembrane peptide in remarkable purity by this approach [17].

The coupling of Fmoc-(Dmb)Gly-OH can be achieved using standard methods such as PyBOP®/DIPEA. Following Fmoc removal with piperidine/DMF, the glycine secondary amine can be acylated with Fmoc-amino acids by a single coupling with PyBrOP® or HATU, or by using pre-formed amino acid fluorides [17].

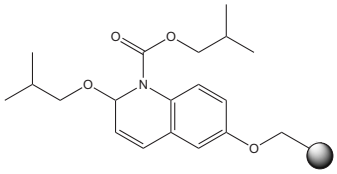
04-12-1268	Fmoc-(Dmb)Gly-OH	1 g
NEW		5 g

Novabiochem's Hmb derivatives

04-12-1127	Fmoc-(FmocHmb)Ala-OH	1 g
		5 g
04-12-1135	Fmoc-(FmocHmb)Gly-OH	1 g
		5 g
04-12-1129	Fmoc-(FmocHmb)Leu-OH	1 g
		5 g
04-12-1148	Fmoc-(FmocHmb)Lys(Boc)-OH	1 g
		5 g
04-12-1187	Fmoc-(FmocHmb)Phe-OH	1 g
		5 g
04-12-1134	Fmoc-(FmocHmb)Val-OH	1 g
		5 g

NEW Polymer-supported coupling reagent

IIDQ-polystyrene



Features & Benefits

- Excellent coupling reagent for hindered carboxylic acids and anilines
- No preactivation step required
- Recyclable

IIDQ-polystyrene (IIDQ-PS) [18] is a polymer-supported version of the IIDQ coupling reagent [19]. IIDQ has many advantages over conventional carbodiimide- or uronium-based reagents: no preactivation step is required, and acid, amine and coupling reagent can be added in any order; in contrast to uronium-based reagents like HBTU, it cannot form guanidinium by-products; and it is totally stable to base.

The treatment of a carboxylic acid with IIDQ-PS in DCM or MeCN rapidly generates *in situ* the corresponding isobutoxycarbonyl mixed anhydride [20]. Attack by nucleophiles preferentially takes place at the less hindered and more electrophilic carbonyl of the carboxylic acid moiety, releasing only volatile carbon dioxide and isobutanol as by-products (Figure 3). If reaction is carried out in the presence of an amine, amide bond formation occurs concurrently with generation of the anhydride. Alternatively, addition of $NaBH_4$ or polymer-supported borohydride to the anhydride will lead directly to the corresponding alcohol.

IIDQ-PS appears to be particularly effective for mediating the acylation of anilines, and has also been found to couple peptide fragments without epimerization. In a comparative study, IIDQ-PS was found to give higher yields and greater purities than HATU, EDC-PS or DCC-PS (Table 1) [21].

IIDQ-PS can be regenerated from spent resin by treatment with isobutyl chloroformate/DIPEA in DCM.

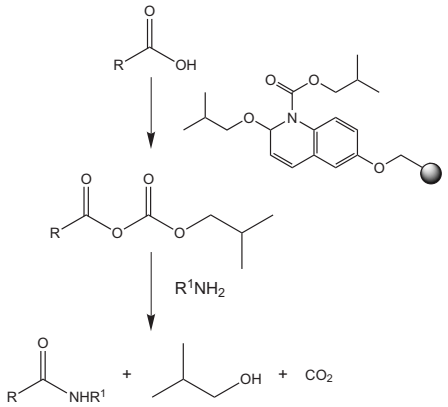
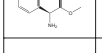


Fig. 3: Generation of mixed anhydrides with IIDQ resin.

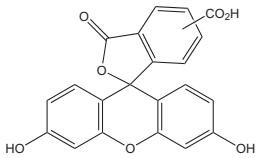
01-64-0469	IIDQ-polystyrene	5 g
NEW		25 g
01-64-0211	N-Cyclohexylcarbodiimide, N'-methyl polystyrene (DCC-PS)	5 g
		25 g

Table 1: Comparison of IIDQ-PS with other commonly used coupling reagents [18].

		IIDQ-PS		HATU		EDC-PS		DCC-PS	
Amine	Acid	Yield	Purity	Yield	Purity	Yield	Purity	Yield	Purity
		69	100	42	100	15	100	16	100
		66	100	45	93	39	87	17	94
		64	100	48	100	24	95	24	86
		60	100	43	100	68	100	41	100
		80	100	69	100	48	100	25	100
		61	100	77	92	49	100	35	100
		89	100	45	100	44	100	28	100
		85	100	68	100	48	81	24	95
		75	100	54	100	33	100	24	100

New Fluorescent dye for peptide-labeling

5(6)-Carboxyfluorescein



This fluorescent dye is a useful tool for preparing fluorescently-labeled peptides and fluorescence-quenched peptide substrates. Novabiochem's 5(6)-carboxyfluorescein (FAM; λ_{ex} 555 nm; λ_{em} 580 nm) is supplied as a mixture of 5- and 6-isomers, making it cost effective for those applications which do not require a single isomer dye.

5(6)-FAM is conveniently introduced during solid phase synthesis by coupling to N-terminal or side-chain amino groups using HOBt or HOAt/DIPCDI in DMF. When it is to be located on a side-chain amino group, the simplest approach is to incorporate an orthogonally-protected derivative, such as Lys(Mtt) or Lys(ivDde), which can be later selectively deprotected on the resin immediately prior to coupling of the dye.

01-63-0149	**5(6)-Carboxyfluorescein**	1 g	€	25.00
NEW		5 g		91.00

Novabiochem's single isomer dyes

01-63-0112	**5-Carboxyfluorescein**	25 mg
		100 mg
01-63-0113	**6-Carboxyfluorescein**	25 mg
		100 mg

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