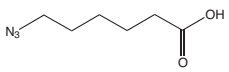
Novabiochem®
Letters: 1/10

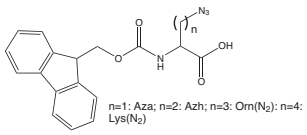
Contents

NEW Reagents for click ligation reactions

6-Azido-hexanoic acid

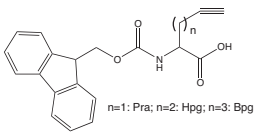


Fmoc-Aza/Azh/Orn(N_2)/Lys(N_2)-OH



n=1: Aza; n=2: Azh; n=3: Orn(N_2); n=4: Lys(N_2)

Fmoc-Bpg/Hpg/Pra-OH



n=1: Pra; n=2: Hpg; n=3: Bpg

Features & Benefits

- High yielding ligation in aqueous media
- Orthogonal to standard methods of ligation
- Building blocks compatible with standard Fmoc SPPS methods

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The Huisgen 1,3-dipolar cycloaddition of azides to alkynes is a well documented reaction for the synthesis of triazoles [1]. However, it was the discovery by Tornøe and Meldal [2] in 2001 of Cu(I) catalysis, and subsequent developments by Meldal [3] and Sharpless laboratories [4], that has transformed this reaction into a powerful and important tool for biomolecule ligation. The reaction is virtually quantitative, relatively insensitive to reaction conditions, orthogonal to other ligation methods, and is compatible with functionalities found in biomolecules. It fulfills Sharpless's [5] definition of "click chemistry", and for this reason has become known as the azide-alkyne click reaction or CuAAC reaction. It has been used in applications as diverse as peptide cyclization, DNA-peptide conjugation, fluorescent dye labeling, and immobilization of molecules to surfaces (see reviews [6, 7]). The reaction is normally conducted using CuBr, or with $CuSO_4$ in the presence of a reducing agent such as ascorbic acid or TCEP to generate the active Cu(I) *in situ*. The reaction can be accelerated by the addition of the tetradendate ligand tris-[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine, TBTA [8].

For azide-alkyne conjugation, the Novabiochem® brand offers 6-azido-hexanoic acid and Fmoc-protected amino acids bearing side-chain azide (Fmoc-Aza-OH, Fmoc-Azh-OH, Fmoc-Orn(N_2)-OH and Fmoc-Lys(N_2)-OH) and alkyne (Fmoc-Pra-OH, Fmoc-Hpg-OH and Fmoc-Bpg-OH) functionalities. They can be introduced using standard coupling methods and are completely stable to piperidine and TFA. The use of thiols in the TFA cleavage mixture should be avoided as this has been shown to lead to azide reduction [9].

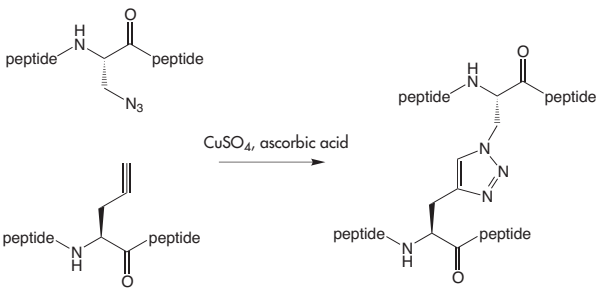
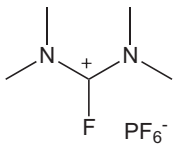


Fig. 1: Azide-alkyne conjugation.

851089	6-Azido-hexanoic acid	1 g
NEW		5 g
852300	Fmoc-Aza-OH	100 mg
NEW		500 mg
852301	Fmoc-Azh-OH	100 mg
NEW		500 mg
852304	Fmoc-Bpg-OH	100 mg
NEW		500 mg
852303	Fmoc-Hpg-OH	100 mg
NEW		500 mg
852306	Fmoc-Lys(N_2)-OH	250 mg
NEW		1 g
852302	Fmoc-Orn(N_2)-OH	100 mg
NEW		500 mg
852260	Fmoc-Pra-OH	1 g
NEW		5 g

NEW Coupling reagents

TFFH



Features & Benefits

- ***In situ* activation reagent for formation of acid fluorides**
- **Excellent coupling reagent for peptides containing hindered amino acids**

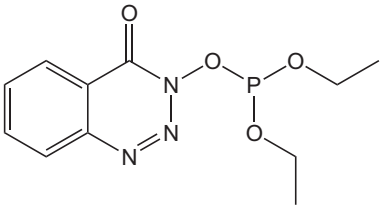
TFFH smoothly converts Fmoc-amino acids in the presence of DIPEA to the corresponding acid fluorides, which can be either isolated or used *in situ* to effect peptide bond formation [10]. TFFH is an extremely effective coupling reagent for the synthesis of peptides containing hindered amino acids, such as the Aib-containing Alamethicins, and of difficult sequences not accessible with TBTU/HOBt coupling [11].

TFFH can be used with automated peptide synthesizers exactly like other *in situ* activating reagents. Typically, Fmoc-amino acid and TFFH are dissolved together in DMF in the presence of 2 eq. of DIPEA; the mixture is then gently agitated for 7 minutes, to allow formation of the acid fluoride, before being transferred to the amino resin.

TFFH has also been used in solution phase to prepare hydrazide, azides, anilides, alcohols and hydroxamates, see [12] for a review.

851090	TFFH	1 g
NEW		5 g
		25 g

DEPBT



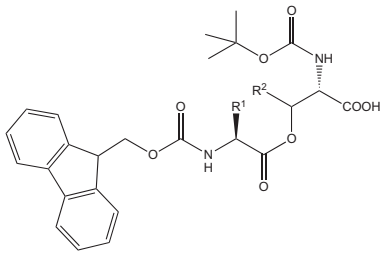
Features & Benefits

- **Reagent for *in situ* generation of racemization-resistant OOBt esters**
- **Coupling reagent of choice of fragment condensation**

DEPBT is an *in situ* coupling reagent for the formation of OOBt esters [13]. These esters are remarkedly resistant to racemization, making DEPBT an ideal choice for mediating peptide fragment and cyclization reactions.

851091	DEPBT	5 g
NEW		25 g
		100 g

NEW Structure breaking isoacyl dipeptides



Boc-Ser(Fmoc-Asp(OtBu))-OH: $R^1 = CH_2COOtBu$; $R^2 = H$
Boc-Thr(Fmoc-Asp(OtBu))-OH: $R^1 = CH_2COOtBu$; $R^2 = CH_3$
Boc-Thr(Fmoc-Arg(Pbf))-OH: $R^1 = (CH_2)_3NHC(=NH)NHPbf$; $R^2 = CH_3$
Boc-Ser(Fmoc-Glu(OtBu))-OH: $R^1 = (CH_2)_2COOtBu$; $R^2 = H$
Boc-Thr(Fmoc-Glu(OtBu))-OH: $R^1 = (CH_2)_2COOtBu$; $R^2 = CH_3$
Boc-Ser(Fmoc-Met)-OH: $R1 = (CH_2)_2SCH_3$; $R^2 = H$
Boc-Thr(Fmoc-Met)-OH: $R1 = (CH_2)_2SCH_3$; $R^2 = CH_3$
Boc-Ser(Fmoc-Val)-OH: $R1 = CH(CH_3)_2$; $R^2 = H$
Boc-Thr(Fmoc-Val)-OH: $R1 = CH(CH_3)_2$; $R^2 = CH_3$

Features & Benefits

- **Improved yields and purities of insoluble aggregated peptides**
- **Purification can be carried out on soluble depsipeptide prior to conversion to native sequence**
- **Use of an isoacyl dipeptide at C-terminus of peptide prevents epimerization during fragment coupling and cyclization reactions**

The Novabiochem® brand is pleased to offer Boc-Ser/Thr(Fmoc-Asp(OtBu))-OH, Boc-Thr(Fmoc-Arg(Pbf))-OH, Boc-Ser/Thr(Fmoc-Glu(OtBu))-OH, Boc-Ser/Thr(Fmoc-Met)-OH and Boc-Ser/Thr(Fmoc-Val)-OH as the latest additions to our range of isoacyl dipeptides.

Isoacyl dipeptides are powerful tools for enhancing synthetic efficiency in Fmoc SPPS which consist of a Boc-protected serine or threonine derivative in which the β-hydroxyl group is acylated by an Fmoc-amino acid [14, 15]. They perform the same role as pseudoproline dipeptides. Substitution of Aaa-Ser or Aaa-Thr in a peptide sequence with an isoacyl dipeptide results in the formation of a depsipeptide analog of the native sequence in which the amide bond between Aaa and Ser or Thr is replaced by an ester linkage. This modification results in a marked change in the conformation of the peptide chain which leads to disruption of aggregation in much the same way as would insertion of a pseudoproline or N-Dmb/Hmb-residue [16 - 18]. However, the real benefits of using isoacyl dipeptides become apparent once the peptide is released from the solid phase. In contrast to pseudoproline dipeptides, the product cleaved when using isoacyl dipeptides is the depsipeptide and not the native peptide sequence (Figure 2). Depsipeptide analogs of aggregation prone peptides have been found to be more soluble and consequently more easily purified than the highly structured native peptide [16 - 18]. For example, isoacyl β-amyloid has a solubility of 15 mg/ml in water, whereas for the natural peptide it is only 0.14 mg/ml [19]. Once the depsipeptide form is purified, it can be easily converted to the native form by adjusting the pH to 7.4. Spontaneous O- to N-acyl migration occurs, with formation of an amide bond between the Ser or Thr residue and the next amino acid [20, 21].

Coupling of isoacyl dipeptides should be carried out using HOBt/DIPCDI in DCM to avoid by-products due to β-elimination [22, 23]. Occasionally, sequence dependent cleavage of the ester bond has been observed. This presumably arises from diketopipiperazine formation during the removal of Fmoc from the residue following introduction of the isoacyl dipeptide. Recently, T. Yoshiya, *et al.* have shown that this side reaction can be eliminated by using 1-methylpyrrolidine/hexamethyleneimine/HOBt in NMP/DMSO [24].

An important further application of isoacyl dipeptides is in fragment condensation as their use at the C-terminus of protected peptides enables coupling [23 - 25] and cyclization [26] to be carried out without risk of epimerization.

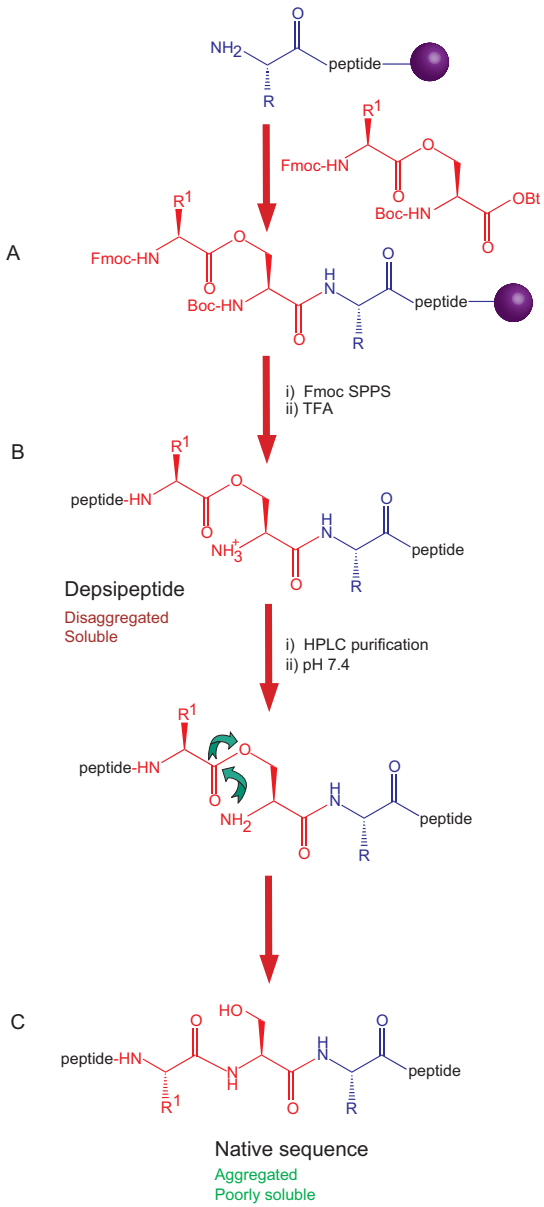


Fig. 2: Use of isoacyl dipeptides.

852298	Boc-Ser(Fmoc-Asp(OtBu))-OH	1 g
NEW		5 g
852295	Boc-Ser(Fmoc-Glu(OtBu))-OH	1 g
NEW		5 g
852293	Boc-Ser(Fmoc-Met)-OH	1 g
NEW		5 g
852290	Boc-Ser(Fmoc-Val)-OH	1 g
NEW		5 g
852294	Boc-Thr(Fmoc-Arg(Pbf))-OH	1 g
NEW		5 g
852297	Boc-Thr(Fmoc-Asp(OtBu))-OH	1 g
NEW		5 g
852296	Boc-Thr(Fmoc-Glu(OtBu))-OH	1 g
NEW		5 g
852292	Boc-Thr(Fmoc-Met)-OH	1 g
NEW		5 g
852253	Boc-Thr(Fmoc-Val)-OH	1 g
NEW		5 g
852174	Boc-Ser(Fmoc-Ala)-OH	1 g
		5 g
852249	Boc-Ser(Fmoc-Arg(Pbf)-OH	1 g
		5 g
852257	Boc-Ser(Fmoc-Asn(Trt)-OH	1 g
		5 g
852256	Boc-Ser(Fmoc-Gln(Trt))-OH	1 g
		5 g
852168	Boc-Ser(Fmoc-Gly)-OH	1 g
		5 g
852250	Boc-Ser(Fmoc-Ile)-OH	1 g
		5 g
852169	Boc-Ser(Fmoc-Phe)-OH	1 g
		5 g
852172	Boc-Ser(Fmoc-Ser(tBu))-OH	1 g
		5 g
852173	Boc-Ser(Fmoc-Thr(tBu))-OH	1 g
		5 g
852170	Boc-Thr(Fmoc-Ala)-OH	1 g
		5 g
852171	Boc-Thr(Fmoc-Gly)-OH	1 g
		5 g
852252	Boc-Thr(Fmoc-Ile)-OH	1 g
		5 g

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