Merck Biosciences

Calbiochem | Novabiochem | Novagen

Novabiochem®

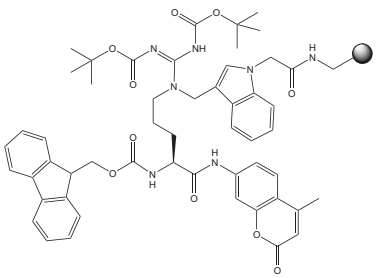
Letters: 02/05

Contents

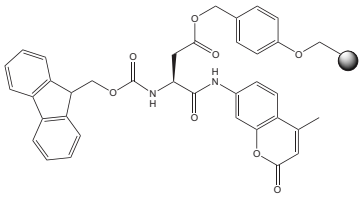
Product Focus: New products for biomolecule labeling

NEW Resins for the synthesis of peptide substrates

Fmoc-Arg(bis-Boc-resin)-AMC



Fmoc-Asp(Wang resin)-AMC



Features & Benefits

- Direct synthesis of peptide fluorescent substrate containing C-terminal Arg-AMC and Asp-AMC
- Compatible with Fmoc SPPS
- TFA cleavage provides peptide-AMC directly
- Ideal for peptide substrate optimization and enzyme profiling

MERCK

Enzyme substrates based on the 7-amino-4-methylcoumarin (AMC) fluorophore are very popular tools for studying protease activity and specificity [1]. In such substrates, the AMC is typically linked to the peptide through formation of an amide bond between the coumarin amine and the carboxyl group of the C-terminal amino-acid residue (Figure 1). Proteolysis of this amide bond liberates free AMC, resulting in a large increase in fluorescence that can be detected at 441 nm upon excitation at 342 nm.

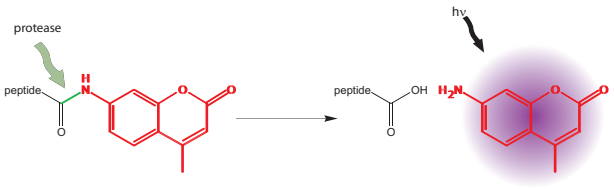


Fig. 1: Principle of AMC-labeled fluorogenic substrates.

The synthesis of peptide-AMC derivatives is particularly problematic owing to the poor nucleophilicity of the AMC amine group. The usual strategy involves first formation of the AMC derivative of the C-terminal amino-acid residue followed by fragment condensation or stepwise elongation. This approach is obviously not amenable to solid phase methods and cannot be applied to the production of enzyme substrate libraries for protease profiling. To overcome these limitations, Novabiochem is introducing a range of resins pre-loaded with amino acid-AMC derivatives, of which the first two products are Fmoc-Asp(Wang resin)-AMC and Fmoc-Arg(bis-Boc-resin)-AMC. Arginine or aspartic acid were selected as these amino acids occur at the P_1 position of endogenous substrates for a number of important proteases, including cathepsins, thrombin, plasmin (Arg), and caspases (Asp).

Fmoc-Asp(Wang resin)-AMC and Fmoc-Arg(bis-Boc-resin)-AMC are extremely simple to use and are fully compatible with standard Fmoc SPPS protocols. The Fmoc group is removed with 20% piperidine in DMF under standard conditions, and the free amine group can be acylated with Fmoc amino acids activated with PyBOP® or TBTU. Following peptide assembly, cleavage with 95% TFA releases the peptide-AMC directly from the solid support without any additional steps.

The use of these new resins is illustrated in the examples given in Figures 2 and 3.

04-12-3912	Fmoc-Arg(bis-Boc-resin)-AMC	500 mg
NEW		
04-12-2177	Fmoc-Asp(Wang resin)-AMC	500 mg
NEW		1 g

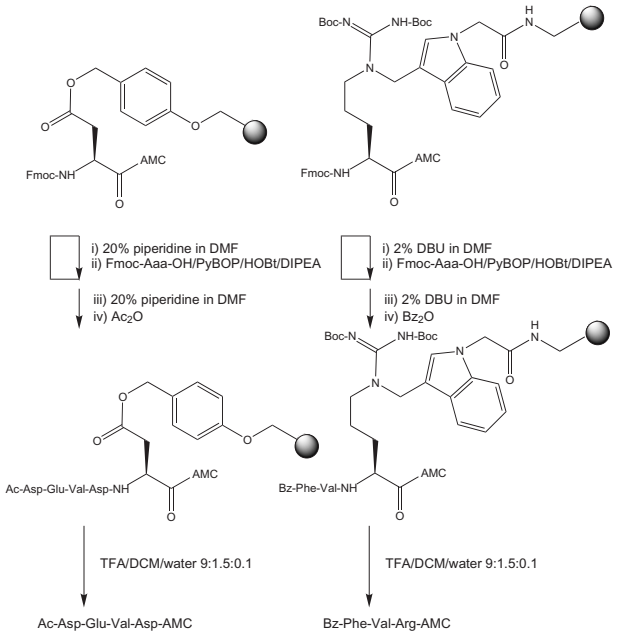


Fig. 2: Synthesis of Ac-Asp-Glu-Val-Asp-AMC using Fmoc-Asp(Wang resin)-AMC and Bz-Phe-Val-Arg-AMC using Fmoc-Arg(bis-Boc-resin)-AMC.

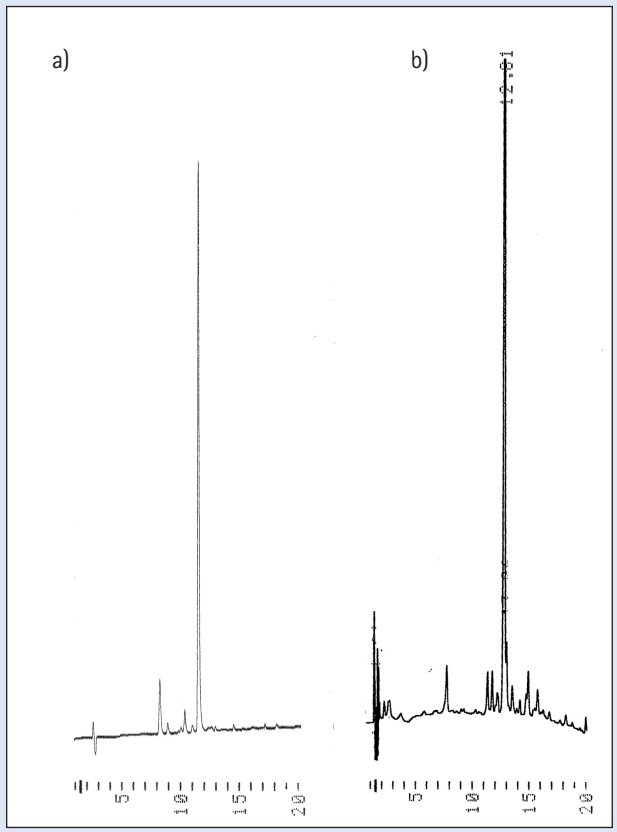
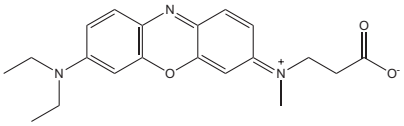


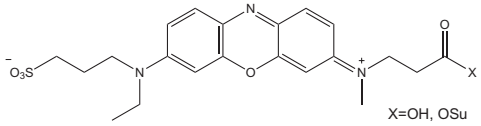
Fig. 3: HPLC elution profiles of a) crude Ac-Asp-Glu-Val-Asp-AMC prepared with Fmoc-Asp(Wang resin)-AMC; b) crude Bz-Phe-Val-Arg-AMC prepared with Fmoc-Arg(bis-Boc-resin)-AMC.

NEW Fluorescent amino-reactive red dyes for biomolecule labeling

Evoblue™ 10



Evoblue™ 30



These dyes are excited by light above 600 nm and fluoresce around 670 nm (Figure 4), making them compatible with low-cost laser diodes and readily-available filter sets.

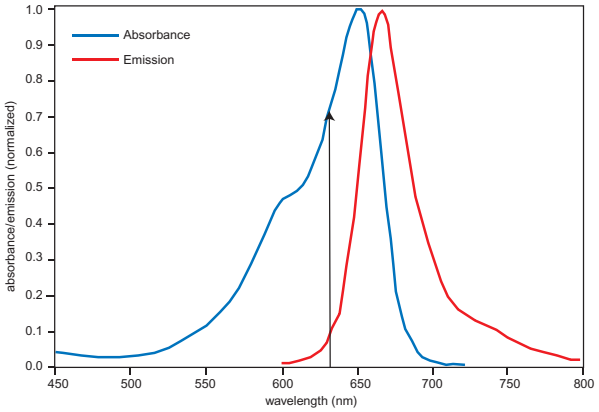


Fig. 4: Absorbance and emission spectra of Evoblue™ 30 in PBS buffer.

EVOblue™ 10 and EVOblue™ 30 belong to a novel class of oxazine-based dyes developed by Evotec OAI for labeling of biomolecules [2]. Both dyes contain carboxylic acid functionalities enabling them to be easily coupled to amine-containing proteins, peptides, oligonucleotides or phospholipids. Their high quantum yield and long wavelength emission and excitation make them ideal for FRET assays, fluorescence-in-situ-hybridization, and confocal fluorescence. EVOblue™ 30 is also available as a water soluble pre-activated OSu-ester, which can be used directly for biomolecule labeling in aqueous solution.

EVOblue™ 30 in particular has high chemical stability compared to other red dyes, making it also suited to solid phase synthesis of peptides and small organic molecules provided excessive exposure to TFA and piperidine is avoided. Introduction of the dye is best carried out as the final step in the synthesis prior to cleavage from the resin.

01-63-0138	EVOblue™ 10	0.5 mg
NEW		1 mg
01-63-0139	EVOblue™ 30	0.5 mg
NEW		1 mg
01-63-0140	EVOblue™ 30-OSu	0.5 mg
NEW		1 mg

These products are for research use only. Any use in diagnostics or therapeutic products, in the manufacture of products for further sale, in the form of kits or any other commercial use requires a license. Please contact Evotec OAI AG for licensing information. These products are covered by the following patent: EP1213328.

Table 1: Features and benefits of Evoblue™ 10 and Evoblue™ 30.

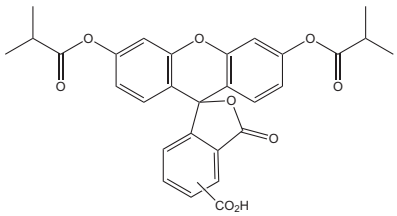
EVOblue™ 10	EVOblue™ 30	Features	Benefits
✔	✔	Amino reactive	Easy labeling of a wide range of biomolecules
✔	✔	Water soluble	Biomolecule compatible
✔		Cell permeable	Compatible with cell staining applications
✔	✔	Excitable 630 nm - 650 nm	Excitable with low-cost laser diodes or HeNe lasers
✔	✔	Emission 670 nm	Compatible with common filters
✔	✔	High fluorescence quantum yield	Bright signals
✔	✔	Solvent and pH stable (pH 1 - 10)	Suitable for a wide range of applications
	✔	No net charge	No electrostatic influence on labeled molecule
✔	✔	Less prone to aggregation	Products have better solubility
	✔	Chemically stable	Compatible with TFA cleavage in Fmoc SPPS
✔	✔	NIR-excitable	Low background fluorescence

19th American Peptide Symposium

Visit our booth at the 19th American Peptide Symposium, in San Diego, to find out more about Novabiochem's new products and to pick-up copies of our latest literature and posters. Whilst there why not take the opportunity to talk to our technical support staff who will be on-hand each day of the conference to answer your questions.

NEW Carboxyfluorescein derivative

5(6)-Carboxyfluorescein diisobutyrate (CFDI)



Features & Benefits

- Fully-protected version of FAM
- Prevents formation of FAM oligomers during coupling
- Better coupling efficiency than FAM
- Isobutyryl groups removed with piperidine

5(6)-Carboxyfluorescein diisobutyrate(CFDI) is a derivative of carboxyfluorescein (FAM) in which the phenolic hydroxyl groups are acylated with isobutyric acid. The protection of these functionalities prevents self-condensation during carboxyl activation and avoids the formation of fluorophore-oligomer containing by-products associated with the use of unprotected FAM. Such oligomer formation not only wastes expensive fluorophore but also reduces the excess of acylating species during label introduction. Furthermore, the presence of these by-products in the final product can later compromise assay sensitivity as they can hydrolyze releasing FAM.

Peptides labeled with CFDI are not fluorescent until the isobutyryl groups are removed. Deprotection of the FAM can be carried out on the solid phase by treatment with piperidine immediately before cleavage. Alternatively, the peptide can be cleaved with the butyryl groups still in place. Carboxyfluorescein diacetate, an analog of CFDI, has been used as an indicator of cell viability [3].

01-63-0147 5(6)-Carboxyfluorescein diisobutyrate 5 mg
NEW

References

1. D. J. Maly, et al. (2002) *Chembiochem*, **3**, 16.
2. J. R. Fries, et al. (2001) *Chimica Oggi*, **19**, 18.
3. J. J. Van der Poel, et al. (1981) *Immunol. Lett.*, **2**, 187.

Merck Biosciences AG · Switzerland
Weidenmattweg 4
4448 Läufelfingen
Phone +41 (62) 285 2525
Fax +41 (62) 285 2520

www.novabiochem.com

NVL205-NP