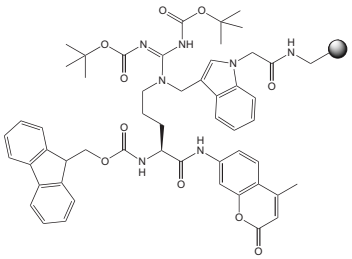
Novabiochem®

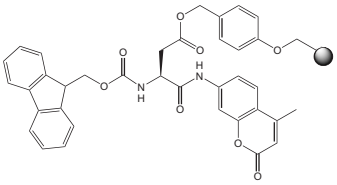
innovations 6/05

Solid phase synthesis of AMC-based enzyme substrates

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



Fmoc-Asp(Wang resin)-AMC



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. 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 (Figure 1).

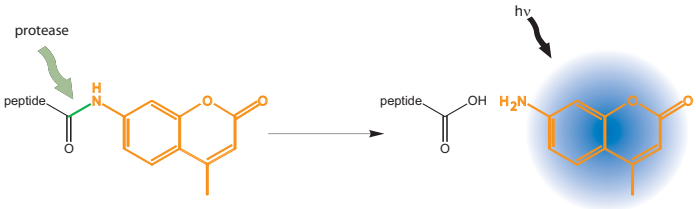


Fig. 1: Principle of AMC-labeled fluorogenic substrates.

Synthesis of C-terminally AMC-labeled peptides

The synthesis of peptide-AMC derivatives is particularly problematic owing to the poor nucleophilicity of the AMC amine group. The usual strategy involves coupling an N-protected derivative of the C-terminal amino-acid to AMC using $POCl_3$/pyridine [2], then removal of the amine protection followed by fragment condensation or stepwise elongation to assemble the full length peptide. 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 resins pre-loaded with amino acid-AMC derivatives that are designed to enable the direct synthesis of peptide-AMCs by Fmoc solid phase peptide synthesis. The first two products in this range 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 P1 position of endogenous substrates for a number of important proteases, including cathepsins, thrombin, plasmin (Arg), and caspases (Asp). Both resins are fully compatible with standard Fmoc SPPS protocols and provide peptides C-terminally labeled with AMC directly from the TFA-mediated cleavage reaction (Figure 2).

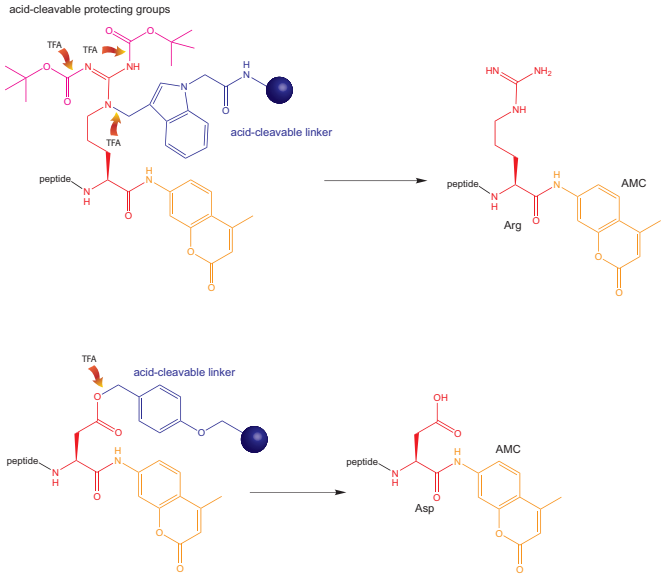


Fig. 2: Design of resins for direct release of peptidyl-Aaa-AMC derivatives by TFA cleavage. 1) Aaa = Arg; 2) Aaa = Asp.

Spectral properties of AMC

The absorbance and fluorescence spectra of AMC is shown in Figure 3. The absorbance and emission maxima are 342 nm and 441 nm, respectively.

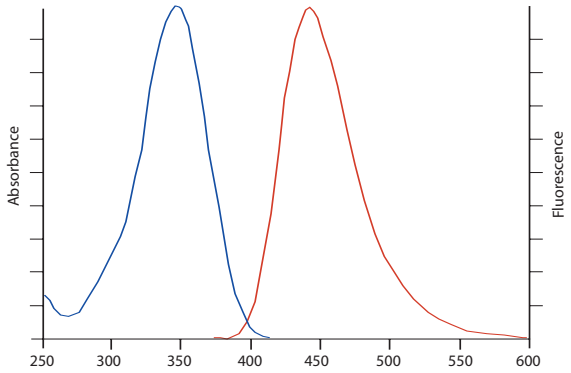


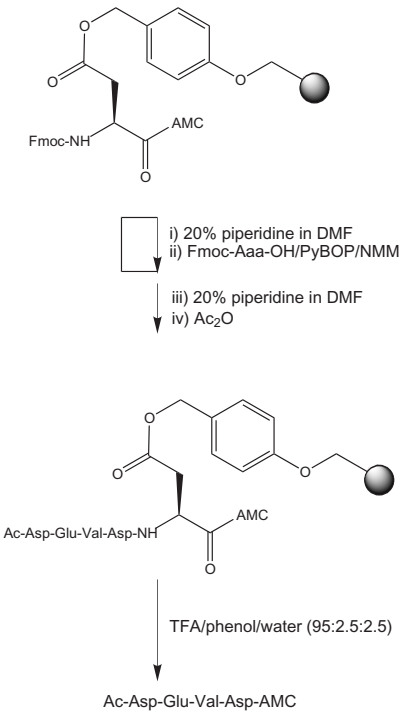
Fig. 3: Absorbance and emission fluorescence spectra of AMC.

Using AMC resins

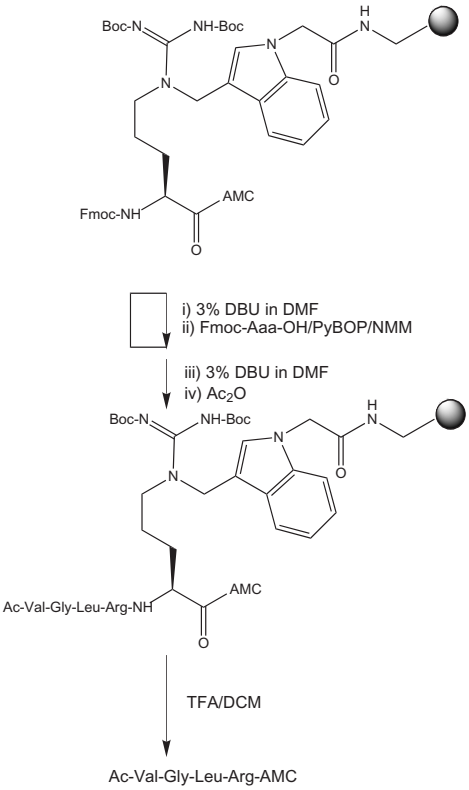
Fmoc-Asp(Wang resin)-AMC and Fmoc-Arg(bis-Boc-resin)-AMC are extremely easy to use. The resins are simply weighed into the peptide synthesis reaction vessel and then swollen for 30 minutes with DMF. For Fmoc group removal, 20% piperidine in DMF is recommended for use with Fmoc-Asp(Wang resin)-AMC, whereas with Fmoc-Arg(bis-Boc-resin)-AMC, best results are obtained if 3% DBU in DMF is used. Due to the hindered nature of the resin-bound AMC-modified amino acid, the next amino acid should be coupled twice using either PyBOP® or TBTU activation to ensure complete acylation. Subsequent amino acids can be introduced using a single coupling reaction. 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 4 and 5.

Synthesis of Ac-Asp-Glu-Val-Asp-AMC



Synthesis of Ac-Val-Gly-Leu-Arg-AMC



Application 1: Synthesis of Ac-Asp-Glu-Val-Asp-AMC using Fmoc-Asp(Wang resin)-AMC

Fmoc-Asp(Wang resin)-AMC (0.4 mmole/g) was swollen in DMF and treated with 20% piperidine in DMF (2 x 3 min) to remove the Fmoc group. Fmoc-Val-OH (5 eq.) was coupled for 1 h using PyBOP® (5 eq.) in the presence of NMM (10 eq.). Subsequent acylation and deprotection reactions were carried out in the same manner except 30 min couplings were used. The labeled peptide was cleaved from the resin by treatment with TFA/phenol/water (95:2.5:2.5) for 2 h. The product was characterized by HPLC (Figure 4) and ES-MS [expected $M+H^+$ 676.4, found 676.4].

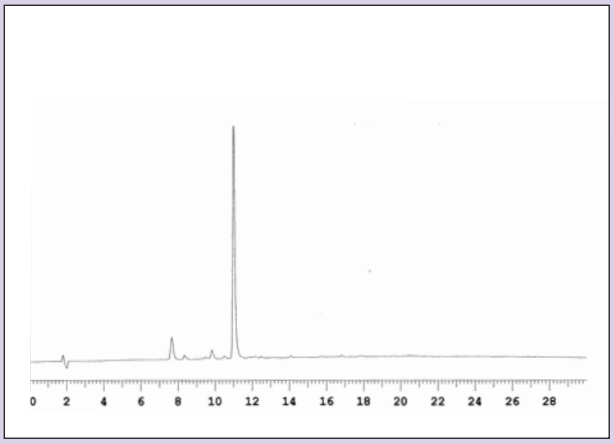


Fig. 4: HPLC elution profile of crude Ac-Asp-Glu-Val-Asp-AMC.

Application 2: Synthesis of Ac-Val-Gly-Leu-Arg-AMC using Fmoc-Arg(bis-Boc-resin)-AMC

Fmoc-Arg(bis-Boc-resin)-AMC (308 mg, 0.08 mmole) was swollen in DMF and treated twice with 3% DBU in DMF (3 min, 10 min) to remove the Fmoc group. Fmoc-Leu-OH (3.1 eq., 0.25 mmole) was coupled twice (50 min, 30 min) using PyBOP® (3.1 eq.) in the presence of NMM (6.2 eq.). Subsequent amino acid additions were carried out in the same manner as above, except that 40 min couplings were used. The N-terminal acetyl group was introduced by treatment of the resin with acetic anhydride (4 eq.) and DIPEA (8 eq.) in THF for 15 min. The labeled peptide was cleaved from the resin by treatment with DCM/TFA (50:50) for 2.5 h. The crude peptide was characterized by HPLC (Figure 5) and ES-MS [expected $M+H^+$ 643.6, found 643.5].

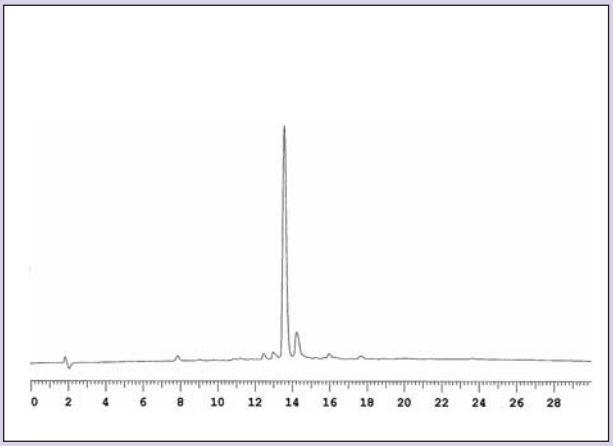


Fig. 5: HPLC elution profile of crude Ac-Val-Gly-Leu-Arg-AMC.

Ordering information

04-12-3912	Fmoc-Arg(bis-Boc-resin)-AMC	500 mg
NEW		
04-12-3915	Fmoc-Asp(Wang resin)-AMC	500 mg
NEW		1 g

Novabiochem's other resins for making chromogenically labeled peptides

04-12-3900	Dansyl NovaTag™ resin	100 mg
		500 mg
04-12-3903	Dnp NovaTag™ resin	100 mg
		500 mg
04-12-3904	EDANS NovaTag™ resin	100 mg
		500 mg
04-12-3902	Mca NovaTag™ resin	100 mg
		500 mg
04-12-3910	Universal NovaTag™ resin	500 mg
		1 g
04-12-3911	Universal PEG NovaTag™ resin	500 mg
		1 g

Novabiochem's other chromogenic derivatives

01-63-0112	5-Carboxyfluorescein	25 mg
		100 mg
01-63-0113	6-Carboxyfluorescein	25 mg
		100 mg
01-63-0149	5(6)-Carboxyfluorescein	1 g
		5 g
01-63-0147	5(6)-Carboxyfluorescein diisobutyrate	100 mg
		500 mg
01-63-0114	5-Carboxy-tetramethylrhodamine	10 mg
		50 mg
01-63-0115	6-Carboxy-tetramethylrhodamine	10 mg
		50 mg
01-63-0134	5(6)-Carboxy-tetramethylrhodamine	100mg
		500 mg
01-63-0105	Dabcyl-OSu	1 g
01-63-0138	EVOblue™ 10	0.5 mg
		1 mg
01-63-0139	EVOblue™ 30	0.5 mg
		1 mg
01-63-0140	EVOblue™ 30-OSu	0.5 mg
		1 mg
04-12-1238	Fmoc-Glu(EDANS)-OH	500 mg
		1 g
04-12-1236	Fmoc-Lys(Dabcyl)-OH	500 mg
		1 g
04-12-1239	Fmoc-Lys(Dnp)-OH	500 mg
		1 g
04-12-1233	Fmoc-Lys(Mca)-OH	500 mg
		1 g
01-63-0111	Mca-OH	1 g
		5 g
01-63-0110	Mca-OSu	1 g

References

1. D. J. Maly, et al. (2002) *Chembiochem*, **3**, 16.
2. D. T. S. Rijkers (1995) *Tetrahedron*, **51**, 11235.

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