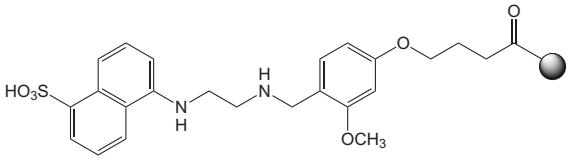
Novabiochem®

innovations 1/05

New tools for SPPS of FRET peptides

EDANS NovaTag™ resin



FRET-based enzyme substrates are highly sensitive tools for probing protease specificity and activity [1], particularly for enzymes such as HIV protease which have a requirement for amino acids on both sides of the cleavage site for substrate recognition (Figure 1).

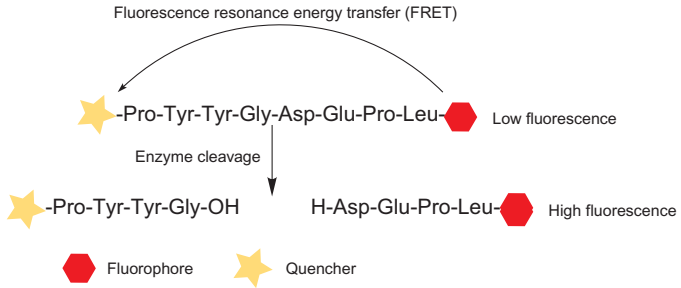


Fig. 1: FRET-based substrates.

One of the most commonly used pairs of fluorophore and quencher is EDANS and Dabcyl, owing to excellent spectral overlap between the emission spectrum of EDANS and absorbance spectrum of Dabcyl [2, 3]. Traditionally, the introduction of the EDANS moiety is achieved either by coupling of a peptide fragment to EDANS in solution [3, 4] or by employing complex and difficult-to-prepare Fmoc-protected amino acids pre-derivatized with EDANS [5, 6]. The first approach is cumbersome and not readily amenable to SPPS, and the second often requires manual intervention to ensure complete coupling of the poorly soluble expensive derivatives. To overcome these limitations, Novabiochem has developed EDANS NovaTag™ resin, which allows for the first time the direct synthesis of C-terminally EDANS-labeled peptides by solid phase synthesis [7]. The EDANS fluorophore is built into the linker, so no additional steps are required for its introduction and the presence of EDANS in every peptide chain is assured from the

outset. The peptide chain is constructed on the benzylic amine, whereas the naphthyl aromatic amine is unreactive under normal conditions employed for chain extension. Treatment with TFA cleaves the benzylic amine releasing the target peptide C-terminally labeled with EDANS (Figure 2).

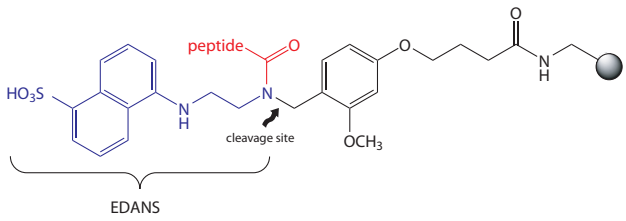
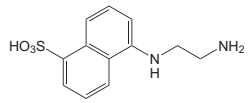


Fig. 2: Loaded EDANS NovaTag™ resin showing point of attachment of peptide and site of cleavage.

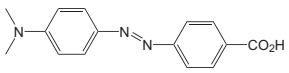
Spectral Properties of EDANS and Dabcyl

EDANS



Absorbance λ_{max}=336 nm, emission=490 nm

Dabcyl



Absorbance λ_{max}=472 nm

The excellent overlap between the absorbance spectrum of Dabcyl and the emission spectrum of EDANS is illustrated in Figure 3. Quenching of the fluorescence of EDANS by Dabcyl is consequently highly efficient, with up to 40-fold enhancements in fluorescence having been observed upon proteolysis of Dabcyl/EDANS-labeled peptides [3].

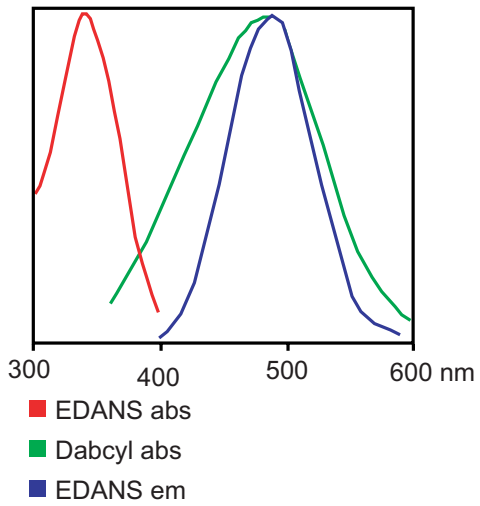


Fig. 3: Absorbance and emission spectra of Dabcyl and EDANS [3].

Using EDANS NovaTag™ resin

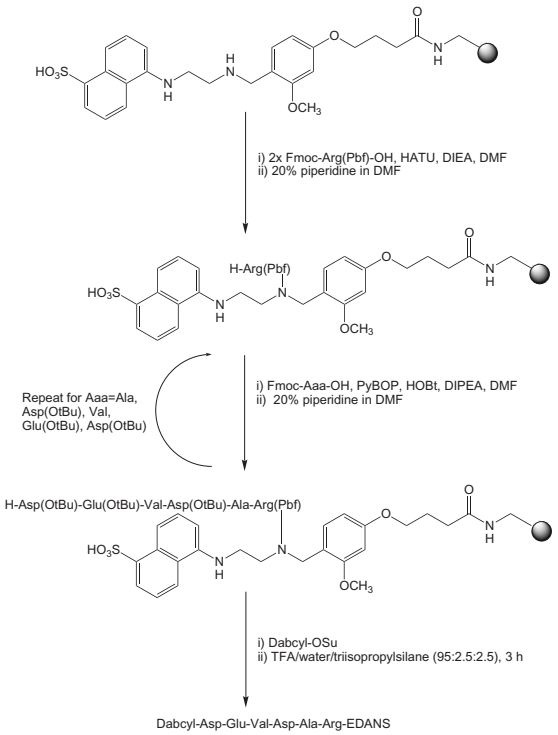
The use of EDANS NovaTag™ resin in the solid phase synthesis of FRET peptides is illustrated by the examples given in Applications 1 and 2. This work has been published in full by Beythien and White [7]. Since the EDANS fluorophore is built into the linker, it becomes linked to the C-terminus of the peptide when the first amino acid is attached to the resin. The Dabcyl quencher group can be introduced to the N-terminus using Dabcyl-OSu in DMSO or DMF in the presence of HOBt. If the Dabcyl group is to be located in the peptide chain, the simplest approach is to introduce Lys(Dabcyl) at the desired position using Fmoc-Lys(Dabcyl)-OH activated with PyBOP®/DIPEA.

The first and most important step in using EDANS NovaTag™ resin is attachment of the first amino acid residue. As this process involves acylation of a resin-bound secondary amine, it is best carried out using HATU activation (Method 1). Pfp esters in the presence of collidine can also be employed, although longer acylation times may be required. It is important that this reaction is carried out to completion, as any unreacted amino groups left on the resin may react in subsequent couplings and lead to the formation of truncated sequences. Following loading, the substitution of the resin should be checked with the Fmoc UV assay, and if necessary, the loading reaction repeated using fresh reagents. Once loaded with the first amino acid, peptide synthesis can be carried out under standard conditions. The use of PyBroP® should be avoided as this can lead to double acylation. Cleavage from the resin can be effected using standard TFA cocktails; however, due to the proximity of the naphthylamine nitrogen to the cleavage site of the linker, product release can sometimes be sluggish. The reaction can be accelerated, if necessary, by the addition of a few drops of TMSBr to the standard TFA cocktail provided water is omitted.

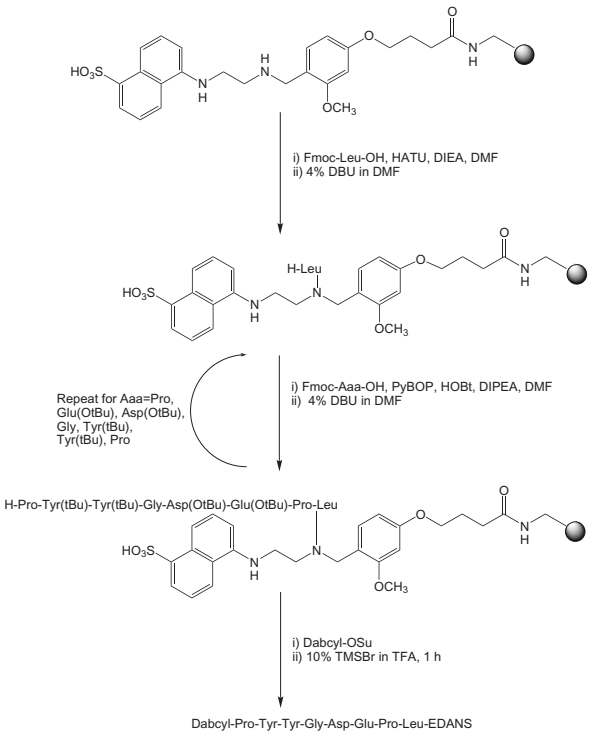
Method 1: Loading EDANS NovaTag™ resin

1. Suspend resin in DMF and leave to swell for 30 min.
2. Dissolve Fmoc-amino acid (2.5 eq.) and HATU (2.5 eq.) in minimum volume of DMF and add to resin. Add DIPEA (5 eq.) and mix.
3. The mixture is left to stand for 2 h with gentle agitation. A sample of resin can be removed and the loading determined using the Fmoc UV assay [8]. Repeat the coupling with fresh reagents if necessary.
4. The resin is removed by filtration, washed with DMF and used immediately in synthesis, or washed further with DCM and then MeOH, dried and stored for later use.

Synthesis of Dabcyl-Asp-Glu-Val-Asp-Ala-Arg-EDANS [7]



Synthesis of Dabcyl-Pro-Tyr-Tyr-Gly-Asp-Glu-Pro-Leu-EDANS [7]



Application 1: Synthesis of Dabcyl-Asp-Glu-Val-Asp-Ala-Arg-EDANS using EDANS NovaTag™ resin

EDANS NovaTag™ resin (188 mg, 0.1 mmole) was loaded with Fmoc-Arg(Pbf)-OH as described in Method 1. Using this resin, H-Asp(OtBu)-Glu(OtBu)-Val-Asp(OtBu)-Ala-Arg(Pbf)-EDANS NovaTag™ resin was prepared automatically on a NovaSyn Crystal peptide synthesizer. All acylation reactions were carried out for 1 h using Fmoc-amino acids (5 eq.) activated with PyBOP® (5 eq.) in the presence of DIPEA (10 eq.) and HOBt (1 eq.). Dabcyl was introduced to the N-terminus using Dabcyl-OSu (2.5 mmole) dissolved in DMSO. The labeled peptide was cleaved from the resin using TFA /TIS/water (95:2.5:2.5) for 3 h and was obtained after ether precipitation in a yield of 68 mg (76%). The crude peptide was analyzed by HPLC (Figure 4) and ES-MS [expected $M+H^+$ 1230, found 1230].

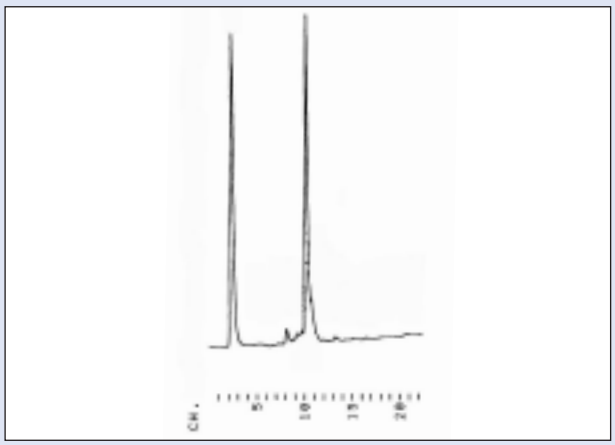


Fig. 4: HPLC elution profile of crude Dabcyl-Asp-Glu-Val-Asp-Ala-Arg-EDANS prepared with EDANS NovaTag™ resin .

Application 2: Synthesis of Dabcyl-Pro-Tyr-Tyr-Gly-Asp-Glu-Pro-Leu-EDANS using EDANS NovaTag™ resin

EDANS NovaTag™ resin (300 mg, 0.16 mmole) was loaded with Fmoc-Leu-OH as described in Method 1. Using this resin, H-Pro-Tyr(tBu)-Tyr(tBu)-Gly-Asp(OtBu)-Glu(OtBu)-Pro-Leu-EDANS NovaTag™ resin was prepared manually. All acylation reactions were carried out for 1 h using Fmoc-amino acids (2 eq.) activated with PyBOP® (2 eq.) in the presence of DIPEA (5.5 eq.) and HOBt (1.3 eq.). Removal of Fmoc was effected by treatment with 4% DBU in DMF. Dabcyl was introduced to the N-terminus using Dabcyl-OSu (120 mg, 0.32 mmole) with HOBt (30 mg, 0.2 mmole) and collidine (500 µl) dissolved in DMF. The labeled peptide was cleaved from the resin using 10% TMSBr in TFA for 1 h. and was obtained after ether precipitation in a yield of 68 mg (63%). The crude peptide was analyzed by HPLC (Figure 5) and ES-MS [expected $M+H^+$ 1453, found 1453].

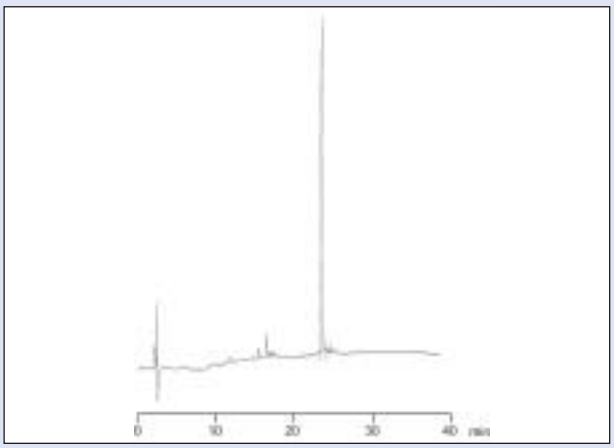


Fig. 5: HPLC elution profile of crude Dabcyl-Pro-Tyr-Tyr-Gly-Asp-Glu-Pro-Leu-EDANS prepared with EDANS NovaTag™ resin .

Ordering information

04-12-3904	**EDANS NovaTag™ resin**	100 mg 500 mg
01-63-0105	**Dabcyl-OSu**	1 g

Other NovaTag™ resins

04-12-3901	**Biotin NovaTag™ resin**	500 mg 1 g
04-12-3908	**Biotin-PEG NovaTag™ resin**	500 mg 1 g
04-12-3900	**Dansyl NovaTag™ resin**	100 mg 500 mg
04-12-3903	**Dnp 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
01-63-0114	**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
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

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7. J. Beythien & P. D. White (2005) *Tetrahedron Lett.*, **46**, 101.
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