Novabiochem®
Reagents for labeling & ligation

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Reagents for peptide ligation & labeling

1 Chemoselective ligation

In spite of the numerous methodological advances made over the last two decades, the preparation of large peptides and proteins by step-wise solid phase synthesis still remains problematic. This is principally due to difficulties in separating the target molecule from the melange of closely related truncated and deletion products which arise during the synthetic process. In addition, the purified products, despite giving the appearance of being homogeneous by HPLC analysis, are often heterogeneous, being contaminated with numerous co-eluting sequences which, because they are individually only present in small amounts, escape detection by mass spectrometry.

These limitations have led a number of different research groups to develop methods based on the conjugation of unprotected peptide segments in aqueous media *via* formation of oxime [1, 2], hydrazone [3], and thiazolidine [4] linkages, utilizing the selectivity of the reaction of aldehydes with hydroxylamines, hydrazines and aminothiols in the presence of protonated amino functions (see Figure 1), triazole formation by azide-alkyne addition [5] or by formation of an amide bond, through the intermediacy of a thioester [6-8] or thiazolidine linkage [9]. The subject has been reviewed by Tam, et al. [10, 11] (the latter paper contains detailed laboratory protocols), Dawson & Kent [12, 13] and Casi & Hilvert [14], and more recently Dirksen & Dawson [15].

Ligation strategies have obvious benefits: by coupling together small to medium sized peptides, which can be produced routinely to a high level of homogeneity, the task of product purification can be greatly simplified; and by using unprotected peptides, the problems of poor fragment solubility normally associated with fragment condensation methods are eliminated.

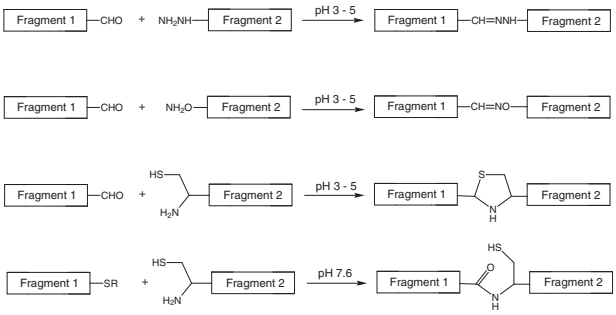


Fig. 1: Approaches to chemoselective ligation.

1.1 Native thiol ligation

Native thiol ligation is perhaps the most powerful ligation procedure since it provides the product containing an amide bond at the site of connection (Figure 2) [6, 7]. This approach generally requires the amino fragment to have a Cys residue at its *N*-terminus or acyl-transfer auxilaries [16] and the C-terminal carboxy group of the carboxy fragment to be present as a thioester. The required thioesters can be prepared either by chemical synthesis (see below), or from larger fragments by splicing from an expressed extein-intein construct [17].

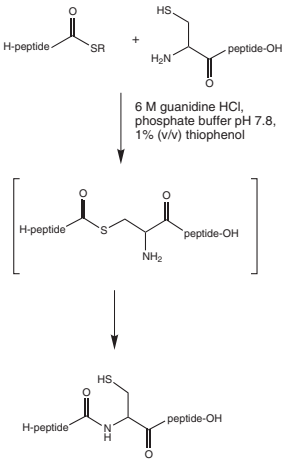


Fig. 2: Native chemical ligation.

1.2 Synthesis of thioesters by Fmoc SPPS

The synthesis of thioesters by Fmoc methods can not be reliably effected directly by solid phase synthesis, owing to the facile cleavage of thioesters by piperidine. To overcome this limitation, a number of innovative strategies have been devised, of which the three most frequently employed are solution synthesis of thioester, use of safety-catch linker and N-to-S acyl migration.

Solution synthesis of thioester

Peptide thioesters can be prepared by coupling of protected peptides to thiols [18] or amino-acid thioesters. Generally, the latter approach is preferred as the reaction is more facile and leads to less epimerization of the *C*-terminal amino acid of the peptide fragment. Recently, Danishefsky and coworkers [19] used peptide thioesters prepared in this way as building blocks for the synthesis of glycosylated EPO by native chemical ligation. Here they prepared fully protected peptide fragments on 2-chlorotrityl resin, incorporating pseudoproline and Dmb-dipeptides to aid solubility and synthesis of the fragments. These fragments were coupled in solution to amino-acid thioesters by Sakabara's method [20] with water-soluble carbodiimide and HOOBt. Treatment of the resultant peptide with TFA yielded the desired peptide-thioester.

Safety-catch approach

Synthesis of peptide thioesters by the safety-catch approach involves the use of a specially designed linker that, after peptide chain extension, can be chemically activated towards thiolysis. The two most frequently used are the sulfamylbutryryl (Ellman) and diaminobenzoyl (Dbz, Dawson) linkers, and these are available from Novabiochem attached to variety of different resins.

Sulfonamide method

This approach, first described by Pessi and coworkers [21] and then later by others [22 - 24], involves displacement of the peptide fragment with a thiol from an alkylated sulfamylbutyryl resin (Figure 3). Following chain assembly the resin is activated by alkylation of the sulfonamide nitrogen, usually treatment with iodoacetonitrile or TMS-CHN_2. Activation with iodoacetonitrile produces a more reactive intermediate, whereas with TMS-CHN_2 the actual process of activation is more efficient. Activation methods have been reviewed in ref. [25]. The resulting *N*-alkyl-*N*-acylsulfonamide is then cleaved by treatment with either benzylmercaptan [21] or ethyl mercaptopropionate /thiophenol [22]. However, the combination of activation with TMS-CHN_2 and displacement with ethyl mercaptopropionate/thiophenol appears to be optimal (Method 1). The use of 2M LiBr in THF as the cleavage solvent has been shown to lead to greatly improved yields of peptide thioester [26].

The resulting protected peptide thioester is then treated with TFA and the appropriate scavengers to give the deprotected peptide ready for ligation. The process of ligation is described in detail in ref. [10].

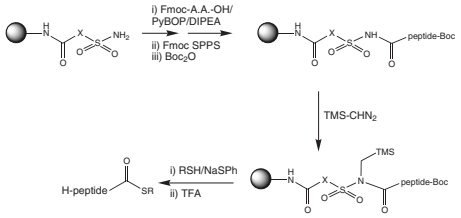


Fig. 3: Synthesis of peptide thioesters using sulfamylbutyryl resin.

Method 1: Thioester ligation with sulfamyl resins

Activation of acylsulfamyl resins

1. Pre-swell the resin (0.1 mmole) in dry THF in a 10 ml polypropylene syringe fitted with a 20 µm polyethylene filter.
2. Add 5 ml of 1 M TMS-CHN_2 in dry hexane/THF (1:1). Cap the syringe.
3. Agitate gently for 2 h. Wash resin with THF and use immediately, or wash with THF, then DCM and dry in vacuo.

Cleavage of thioester

1. Pre-swell methylated resin in DMF for 1 h before use.
2. Add ethyl-3-mercaptopropionate (50 eq.) and sodium thiophenoxide (0.5 eq.), cap the syringe and agitate the mixture gently for 24 h.
3. Remove the resin by filtration and wash it three times with DMF.
4. Combine the filtrates and evaporate to dryness on a rotary evaporator. Triturate the product with ether.
5. Treat the residue with TFA/water/TIS /phenol (88:5:2:5) for 2 h at rt.
6. Add the cleavage solution drop-wise to 10 volumes of cold methyl t-butyl ether (MTBE), and isolate the product by filtration or centrifugation using standard methods. Purify product by RP-HPLC.

Ligation of unprotected peptide fragments

1. Dissolve peptide thioester (1 eq.) and N-terminal-Cys peptide (1 eq.) in a screw-cap tube containing degassed 0.1 M sodium phosphate buffer, pH 7.8. If necessary, the solution can also contain guanidine hydrochloride (up to 6 M) to add dissolution of the peptide components. The final concentration of the peptides should be 2-5 mM.
2. Add thiophenol (1% by volume of total solution), flush with nitrogen, recap tube, and agitate mixture vigorously. The progress of the reaction can be monitored by HPLC. The reaction is typically over in 5-16 h.
3. Acidify the reaction with TFA (0.1% by volume of solution), lyophilize and purify by standard procedures.

Novabiochem offers the sulfamylbutyryl linker attached to AM resin and NovaSyn® TG resin. Loading of these resins with Fmoc-amino acids is best achieved PyBOP® and DIPEA in $CHCl_3$ at -20°C [27] or with DIPCDI/*N*-methylimidazole. In the case of PyBOP® activation, the loading efficiencies are reported to vary from >95% for Cys, Met and His to 44% for Pro, the worst case. Extent of racemizationization for the loading of Fmoc-Phe and Fmoc-Leu by these methods are 0.5% and 0.3%, respectively. Novabiochem® also offers a range of pre-loaded sulfamylbutyryl NovaSyn® TG resins. Here, coupling of the first amino acid to the sulfamyl linker is carried out in solution prior to attachment of the purified, fully characterized Fmoc-amino acid linker to amino NovaSyn® TG. This produces high-quality supports of defined substitution, free from by-products arising from overacylation. The supports can be used directly in automated peptide synthesis without modification of existing protocols.

The sulfonamide method, whilst popular, has been plagued by notoriously low yields. These originate from three sources:

1) incomplete acylation of the resin-bound sulfonamide with the *C*-terminal residue;

2) incomplete alkylation of the sulfonamide, which is usually performed blind and not optimized;

3) incomplete thiolysis, due to the inherent low reactivity of the activated sulfonamide towards thiols and poor solvation of the resin-bound protected peptide.

Recently, a novel dual linker strategy has been developed [28] that involves anchoring of the sulfamylbutyryl linker to a standard acid-labile resin. This approach appears to overcome all the limitations of the sulfonamide method and provides a simple and robust strategy for Fmoc SPPS-based NCL (Figure 4):

The sulfamylbutyryl linker is pre-loaded with the *C*-terminal residue prior to its attachment to the solid phase, thereby overcoming issues with incomplete loading, racemizationization and double addition that can occur during on-resin linker functionalization;

By using Rink amide or Sieber amide resin as the solid support for the pre-loaded linker, the *N*-peptidyl-*N*-methylsulfonamide can be released from the resin by TFA treatment, enabling the progress of the synthesis and extent of methylation to be easily checked by LC-MS;

Moreover and most importantly, the intermediate *N*-peptidyl-*N*-methylsulfonamide can be cleaved from the support and be used as a surrogate thioester directly in NCL reactions, without the need for prior conversion to the thioester.

For this method, Novabiochem offers sulfamylbutyryl linkers pre-loaded with Fmoc-amino acids, which can be attached to any TFA-labile resins, sulfamylbutyryl Rink Amide resins, and Fmoc-amino acid sulfamylbutyryl Rink Amide resins.

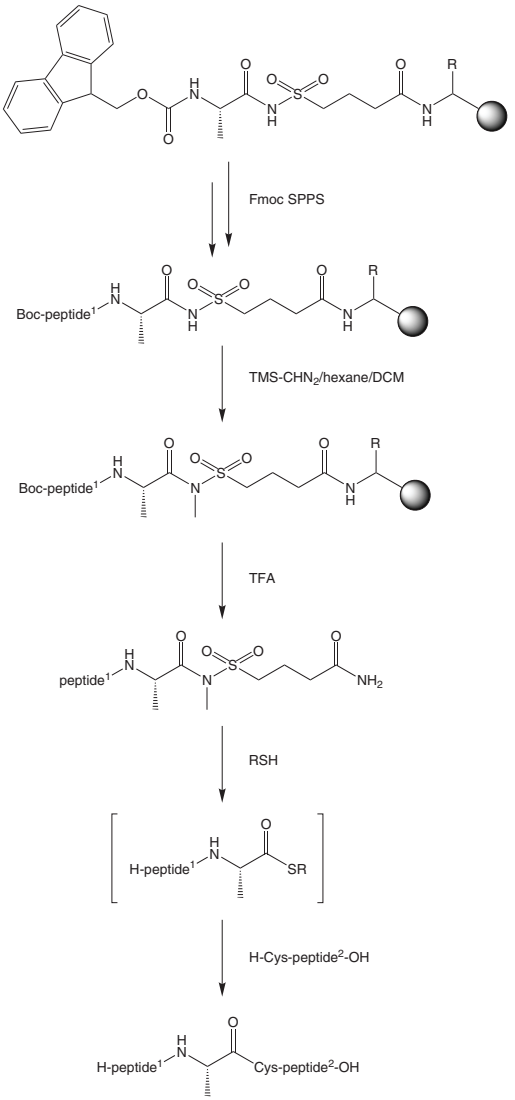


Fig. 4: NCL using sulfamylbutyryl Rink amide resins.

Synthesis of BPTI using sulfamyl method

The double linker strategy is exemplified in the synthesis of BPTI, a 58 residue, small protein containing a full range of side chain functional groups. The strategy of Lu and co-workers [29] was selected for the preparation of BPTI, involving the ligation of two fragments: BPTI(1-37) and BPTI (38-58) (Figure 5).

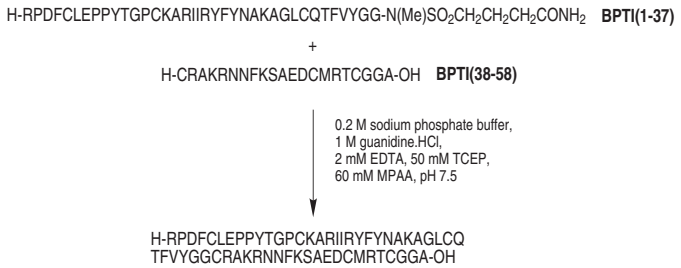


Fig. 5: Synthesis of BPTI.

BPTI(38-58) was assembled on Fmoc-Ala-Wang resin using standard HOBt/DIPCI activation (Application 1).

Application 1: Synthesis of BPTI (38-58)

BPTI(38-58) was assembled using a CS Bio 336 automated synthesizer on Fmoc-Ala-Wang resin using 5-fold excesses of Fmoc-amino acids activated with DIPCDI/HOBt in DMF. A coupling time of 45 min was used throughout. Fmoc removal was effected by three 5 min treatments of 20% piperidine in DMF. Cleavage of the peptide from the resin with concommitant side-chain deprotection was achieved by treatment with TFA/TES/water/EDT (94.5:2.5:2.5:0.5) for 1.5 h. The crude peptide was analyzed (Figure 6) and purified by HPLC.

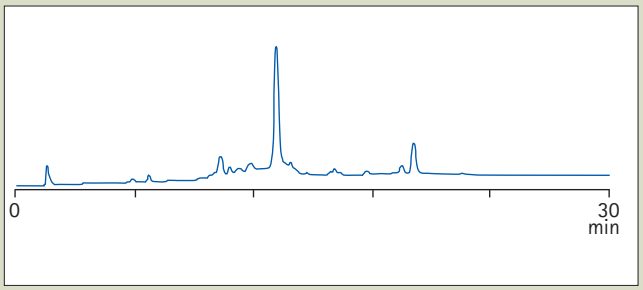


Fig. 6: HPLC profile of BPTI (38-58).

BPTI(1-37) was assembled in a similar manner on Fmoc-Gly-sulfamylbutyryl Sieber Amide resin. Methylation of the linker was effected by treatment with 2M TMS-CHN_2 in hexane/DCM (1:1) for 18 hours. TFA cleavage afforded the deprotected *N*-BPTI(1-37)-*N*-methylsulfonamide. Following purification by RP-HPLC, the two fragments were ligated (Fig. 2) using low millimolar fragment concentration. The reaction was almost complete in 8 h, a time comparable to that observed by Lu and co-workers [29] with classical NCL using a preformed thioester.

Application 2: Synthesis of BPTI (1-37)

BPTI(1-37) was assembled using a CS Bio 336 automated synthesizer on Fmoc-Gly-sulfamylbutyryl Sieber Amide resin using 5-fold excesses of Fmoc-amino acids activated with DIPCDI/HOBt in DMF. A coupling time of 45 min was used throughout. Fmoc removal was effected by three 5 min treatments of 20% piperidine in DMF. A small sample of resin was treated with TFA/TES/water/EDT (94.5:2.5:2.5:0.5) for 1.5 h and the purity of the isolated product checked by RP-HPLC (Figure 7a). The bulk of the resin was then treated overnight with TMS-CHN_2 in hexane/DCM (1:1). *N*-BPTI(1-37)-*N*-methylsulfamylbutyramide was cleaved from the resin by treatment with TFA as described above and the isolated product analyzed by HPLC (Figure 7b). The crude peptide was purified by HPLC.

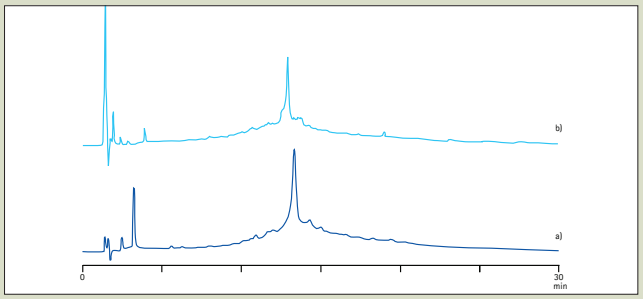


Fig. 7: HPLC profiles of a) *N*-BPTI(1-37)-sulfamyl-butyramide & b) *N*-BPTI(1-37)-*N*-methylsulfamyl-butyramide.

Application 3: Preparation of BPTI (1-58)

N-BPTI(1-37)-*N*-methylsulfamylbutyramide (10 mg, 2 µmol) and BPTI(38-58) (5.8 mg, 2 µmol) were dissolved in 1 mL of degassed 0.2 M sodium phosphate buffer pH 7.5, containing 6 M guanidine·HCl, 2 mM EDTA, 50 mM TCEP, 60 mM MPAA. The solution was heated at 40 °C. The reaction was allowed to stir under Ar for 8 h. HPLC purification yielded linear BPTI (2.6 mg, 16% yield). The product was characterized by MALDI-TOF MS in positive linear mode using CHCA matrix: m/z= 6517.9 [M+H]+ (average isotope composition), calc: 6517.6. Figure 8 shows the monitoring by HPLC of a preliminary sample scale ligation reaction.

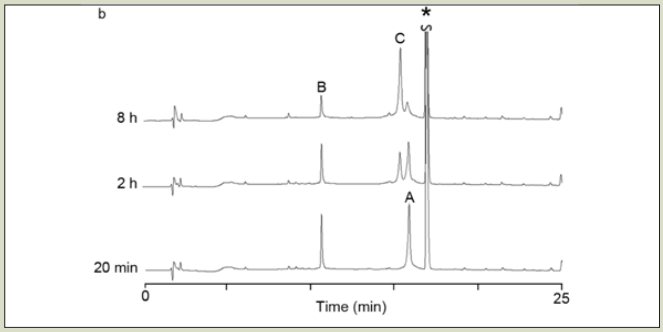
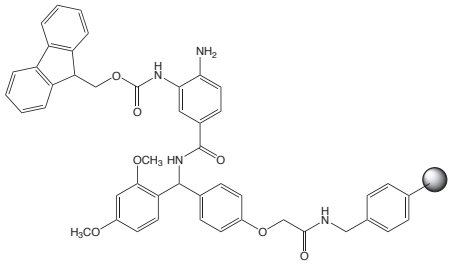


Fig. 8: HPLC profiles of monitoring of a preliminary ligation reaction. *N*-BPTI(1-37)-N-methylsulfamyl-butyramide (A), BPTI(38-58) (B); BPTI(1-58) (C); *MPAA.

Dbz method



This strategy utilizes a 3,4-diaminobenzoic acid (Dbz) attached *via* its carboxyl group to a TFA-cleavable amino functionalized resin [30, 31]. Peptide chain extension is performed on one of the anilino groups, followed by formation of an imidazolidinone (Nbz) with p-nitrophenyl chloroformate, and cleavage form the resin with TFA. The peptide-Nbz is used directly in chemical ligation reactions to generate in situ the desired peptide thioester (Figure 9).

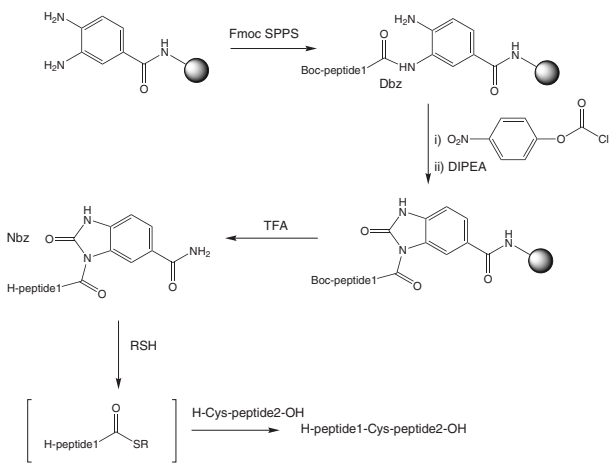


Fig. 9: Synthesis of peptide thioesters using Dawson Dbz AM resin.

Success in using the Dbz strategy depends on being able to fully acylate only one of the two linker amines with the *C*-terminal amino acid residue and to avoid acylation of the unprotected amine during chain extension. Incomplete acylation leads to formation of *C*-terminally truncated peptides as new chains are propagated by acylation of any unreacted amines during subsequent coupling cycles. Whereas, overacylation results to formation of branched peptides with chains growing off both linker amines. Therefore, the selection of acylation method of attachment of the *C*-terminal residue and subsequent couplings is critical if good results are to be obtained (Method 2).

Particularly problematic is the coupling of glycine residues, especially if they occur close to the *C*-terminus of the peptide. This reactive and unhindered amino acid can couple to the free Dbz-amino group if uronium or phosphonium activation is used. In our hands best results are obtained if glycine residues are introduced using Fmoc-Gly-OPfp/HOBt. This precaution may be unnecessary once the peptide is extended beyond 10 residues, as hindrance should reduce the reactivity of unprotected Dbz amine.

Blocking of the *N*-terminal amino group, prior to activation of the linker with *p*-nitrophenyl chloroformate, is essential. The easiest and simplest way is to use a Boc-amino acid to introduce the final residue. If *N*-terminal capping is necessary, it must be done using very mild reagents if blocking of the second amine is to be avoided. Recently, Dawson and coworkers [31c] found *N*-terminal acylation could be performed successfully using *N,N*-diacetylaminoquinazolinone. Boc-N_3 should be suitable for introduction of an *N*-terminal Boc group.

Recently, the use of Alloc protection for blocking the second amino group has been advocated as a way to avoid all issues with branching and truncation [32] (Figure 10). Dbz resins as supplied contain mostly 3-Fmoc-Dbz, with small amounts of 4-Fmoc-Dbz and bis-Fmoc-Dbz. Capping the resin with Alloc-Cl prior to removal of the Fmoc group will thus reduce the maximum potential for branching or truncation to 6%. For hindered amino acids, it has been found necessary to load the resin prior to capping with Alloc. The Alloc group must be cleaved off with Pd(0) before conversion to the Nbz form.

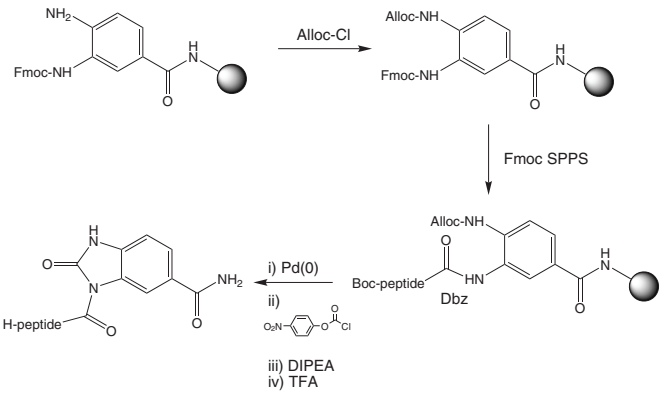


Fig. 10: Alloc protection strategy [32].

Loading of the C-terminal residue

Loading of the *C*-terminal residue is best achieved using Method 2a. With synthesizers that use dry reagents, such as the ABI 443A, loading of the first residue can be automated by using a mixture of Fmoc-amino acid and the appropriate coupling reagent packed in a cartridge or vial, with activation effected by addition of DIPEA in DMF. For subsequent amino acids, the standard pre-dissolved activator can be used. Some optimization of the loading reaction may be required to maximize loading and minimize branching. Treatment of a small sample of loaded resin with TFA and analysis by HPLC of the cleaved product can be helpful in monitoring this process.

Chain extension

In general, strong activators like HATU or HCTU should be avoided as their use can lead to branching. In our hands, HBTU/HOBt appears to work well for coupling of all residues except Gly, where the use of the pre-formed OPfp in conjunction with HOBt gives minimal branching.

Conversion to Nbz

Activation of the linker by conversion to the Nbz is achieved according to Method 2b. The reaction is usually quantitative. With high loaded resins like Dawson Dbz AM resins, some cross-linking of Dbz moieties can occur. In our experience, much cleaner results are obtained with low-loaded resins like Dawson Dbz NovaSyn® TGR resin. Treatment of the Nbz resin with TFA releases the fully deprotected peptide-Nbz, which can be used directly in the NCL reaction. The Nbz peptide is obtained as a mixture of regioisomers.

Method 2: Thioester ligation with Dawson Dbz AM resin

a) Loading

1. Pre-swell the resin (0.1 mmole) in DCM for 60 mins and wash with DMF. Remove Fmoc group with 20% piperidine in DMF and wash with DMF.
2. **Ile, Val, Thr, Pro, Arg:** Add Fmoc-Aaa-OH (0.6 mmole), HATU (0.6 mmole) and DIPEA (0.9 mmole). Agitate gently for 1 h. Wash resin with DMF and repeat coupling.
   Gly: Add Fmoc-Gly-OPfp (0.6 mmole) and HOBt (0.6 mmole). Agitate gently for 1 h.
   Other amino acids: Add Fmoc-Aaa-OH (0.6 mmole), HCTU (0.6 mmole) and DIPEA (0.9 mmole). Agitate gently for 1 h. .
3. Check loading using Method 3-11, p. 3.7. Alternatively, wash a sample of resin with DCM and treat with 95% TFA aq. for 30 min. Anaylze cleaved product by HPLC.

b)Synthesis & Activation

1. Extend peptide chain using HBTU/HOBt/DIPEA activation, except for Gly which should be introduced using Fmoc-Gly-OPfp/HOBt. The N-terminal residue must be introduced using a Boc-amino acid. Wash the resin with DMF and DCM.
2. Add p-nitrophenyl chloroformate (0.5 mmole) in DCM and leave to gently agitate under N2 for 1 h. Wash resin with DCM and add 0.5 M DIPEA in DMF (10 ml) and leave for 30 min. Wash resin with DMF and DCM.
3. Cleave peptide with TFA/water/TIS 95:2.5:2.5 for 3 h.

Ligation of unprotected peptide fragments

1. Dissolve purified peptide-Nbz (1 eq.) and N-terminal-Cys peptide (1.5 eq.) in a screw-cap tube containing degassed ligation buffer (0.2 M phosphate buffer, 6 M guanidine hydrochloride, 0.2 M 4-mercaptophenylacetic acid, 0.02M TCEP, pH 7.0). The final concentration of the peptides should be approximately 2 mM.
2. Monitor the progress of the reaction by HPLC.
3. Acidify the reaction with TFA (0.1% by volume of solution), lyophilize and purify by standard procedures.

N to S Acyl migration/thiol capture

Peptides containing cysteine residues are in equilibrium between the amide and thiodepsipeptide forms. The position of the equilibrium between peptide and thiodepsipeptide is dependent on pH. At neutral pH, the amide form of peptide is strongly favored, but under acidic conditions, the equilibrium is shifted towards the thioester. If an excess of external thiol is present, the thiodepsipeptide can be cleaved to give a peptide thioester (Figure 11). This principle forms the basis of a number of methods for peptide thioester synthesis. In these, *N*-alkylation of the key amide bond, covalent capture of the thiodepsipeptide amine, and enhancement of the local thiol concentration have all been employed to move the position of the equilibrium to favor the thiodepsipeptide, and hence formation of the peptide thioester.

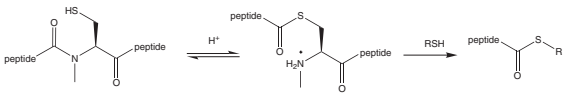


Fig. 11: N to S acyl migration and thiol capture.

One simple and effective approach to NCL based on N to S acyl migration/thiol capture is known as SEA ligation [33 - 35]. Here, peptides are prepared bearing a bis(2-sulfanylethyl)amide (SEA) on their *C*-terminus. These peptide at neutral pH exist as a mixture of amide and thioester forms. In presence of added thiol, the SEA group is cleaved to give the peptide thioester, which can be isolated or used *in situ* for NCL (Figure 12).

For the synthesis of peptide-SEAs, Novabiochem provides SEA-polystyrene resin. Loading of the resin with the *C*-terminal residue is best done using Fmoc-amino acid coupled activated with HATU. After peptide assembly, the peptide-SEA is cleaved from the resin using standard TFA cocktails. To stabilize the peptide and to simplify HPLC analysis and purification in acidic buffers, the SEA peptide should be converted to the disulfide form by air or iodine oxidation (Figure 12). In the presence of a reducing agent such as TCEP, peptide-SEA disulfides undergo rapid NCL or can be converted to thioesters. In the absence of a reducing reagent, peptide-SEA disulfides do not undergo ligation, which has been exploited to perform one-pot three segment ligations [36].

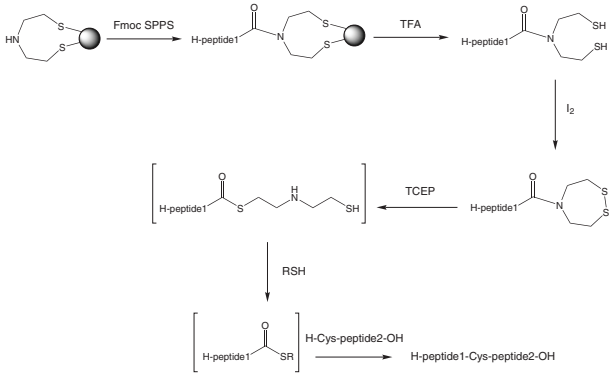


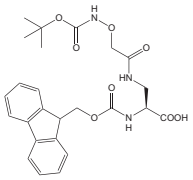
Fig. 12: SEA strategy [33-35].

1.3 Oxime ligation

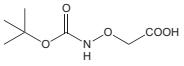
Oxime ligation is a powerful and versatile method for the production of MAPs [1, 2], cyclic peptides [37-40], and peptide-glycopeptide conjugates [41]. At pH ~5 in aqueous media, hydroxylamine-labeled peptides ligate with peptides, lipids, sugars, or other entities possessing an aldehyde functionality. The reaction is extremely selective and is compatible with all the functional groups present in natural amino acids, with the exception of N-terminal cysteine which can undergo thiazolidine formation.

The types of peptide conjugates that can be prepared by oxime ligation are summarized in Figure 13. By incorporating the hydroxylamine and aldehyde component into the same sequence, side-chain to side-chain cyclized peptides are produced [38-42]; the oxime serves in this instance as a kinetically and metabolically stable analog of a cysteine bridge. Peptides conjugated in a side-chain to head manner can be prepared by incorporation of aminoserine into the sequence and utilizing an N-terminal serine residue as the precursor to the glyoxylic acid moiety. MAPs can also be produced in an analogous fashion.

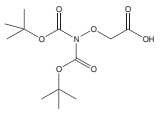
Hydroxylamine-functionalized building blocks



Fmoc-Dpr(Boc-Aoa)-OH



Boc-Aoa-OH



Boc_2-Aoa-OH

For introduction of the hydroxylamine label, Novabiochem® provides Fmoc-Dpr(Boc-Aoa)-OH [40], as well as protected amino-oxy carboxylic acids Boc-Aoa-OH and Boc_2-Aoa-OH. Following cleavage and side-chain deprotection, peptides are produced bearing either a pendant or an *N*-terminal hydroxylamine moiety.

Incorporation of mono-protected hydroxylamine compounds like Boc-Aoa-OH is best achieved using HOBt/DIPCDI or HATU/collidine in order to minimize problems with double acylation of the hydroxylamine nitrogen [42]. Such problems can be avoided using Boc_2-Aoa-OH where the nucleophilicity of the hydroxylamine functionality is completely masked.

Due to the high reactivity of Aoa-labeled peptides, care must be taken to avoid formation of formaldehyde, acetaldehyde and acetone adducts from the corresponding aldehydes and ketones present in the ambient environment. Addition of excess Boc-Aoa-OH to the cleavage mixture as a carbonyl scavenger has been found to be highly effective at protecting the Aoa moiety prior to HPLC [43].

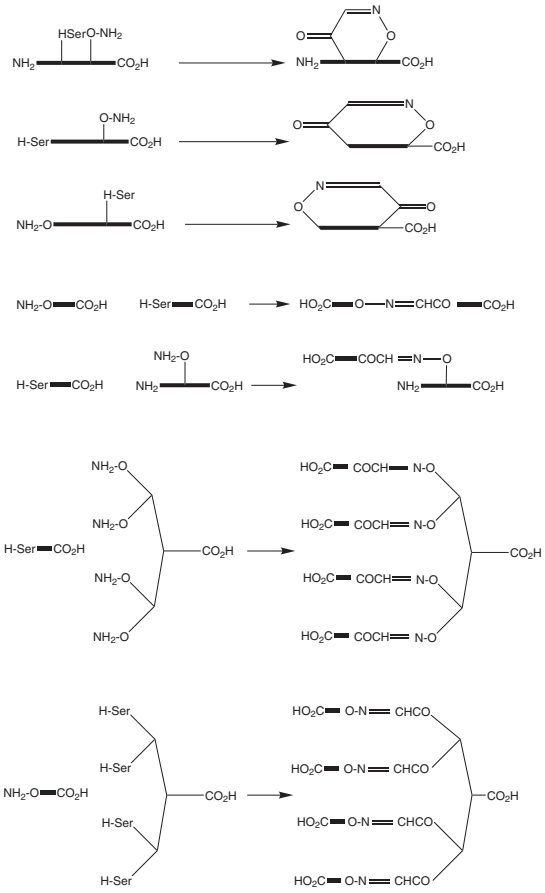
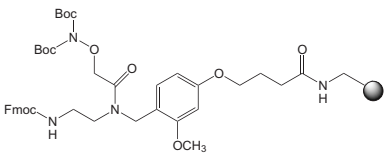


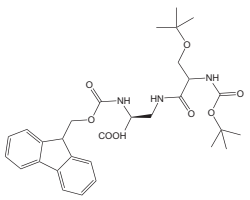
Fig. 13: Oxime ligation.

Hydroxylamine NovaTag™ resin/Hydroxylamine PEG NovaTag™ resin



For the production of peptides which can undergo oxime ligation at their C-terminus, Novabiochem® has developed Hydroxylamine NovaTag™ and Hydroxylamine PEG NovaTag™ resins. The Fmoc group is preferably removed using 2% DBU in DMF. Peptide chain elongation can be achieved using standard acylation methods. Cleavage with 95% TFA furnishes the fully deprotected hydroxylamine-labeled peptide.

Reagent for synthesis of aldehyde components



Fmoc-Dpr(Boc-Ser(tBu))-OH

Peptides bearing aldehyde groups are usually generated *in situ* by periodate oxidation of a precursor peptide containing a 1,2-aminoalcohol group (Figure 14). If the oxidation reaction is carried out in the presence

of the hydroxylamine component, cis-oxime formation with coupling of the peptide fragments occurs concurrently. For peptides containing an *N*-terminal aldehyde group, the most convenient precursor is the corresponding N-terminal serinyl peptide [44, 45]. Side-chain aldehyde groups are generated by oxidation of the peptide containing a Dpr(Ser) residue, which is introduced using Fmoc-Dpr(Boc-Ser(tBu))-OH [37].

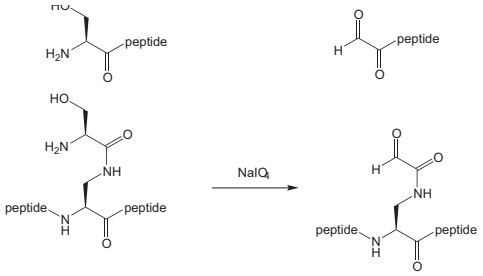
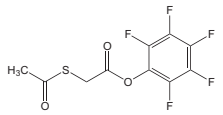


Fig. 14: Periodate oxidation route to N-terminal and side-chain aldehyde groups.

1.4 SAMA-OPfp



This reagent [46] provides an effective means of linking synthetic peptide antigens to MAP core peptides [47] or carrier proteins for the purpose of raising antibodies [48] (Figure 8). Using this technique many of the problems associated with the analysis and purification of MAPs are avoided, since the linear peptide antigen can be fully characterized before conjugation to the preformed lysine tree. For protocols describing the use of SAMA derivatives in the preparation of peptide-protein conjugates and MAPs, see [49]. The derivative is supplied in the form of a Pfp ester, so labeling of an N-terminal amino group, or a lysine side chain, with SAMA is just a matter of treating the peptidyl resin with the reagent in DMF in the presence of HOBt. The S-acetyl group is stable to TFA and remains intact during cleavage of the peptide from the resin. Conjugation is carried out by incubating the SAMA labeled peptide with the bromoacetylated MAP, or protein, in the presence of hydroxylamine. For further details see Method 3.

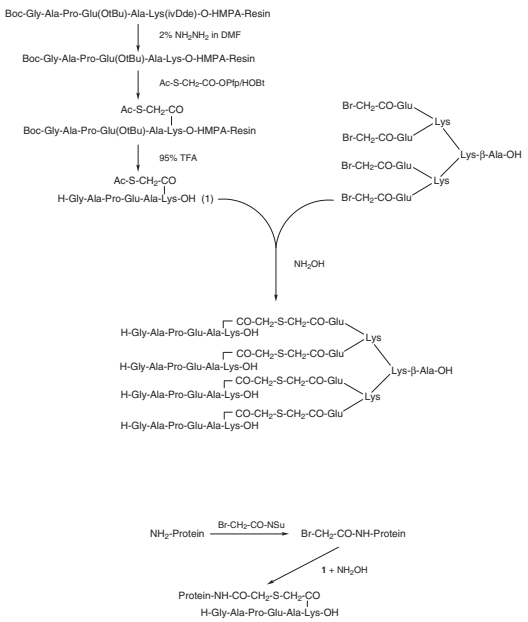


Fig. 15: Applications of SAMA.

Method 3: Use of SAMA

Derivatization with SAMA

1. Assemble the peptide sequence; if the SAMA moiety is to be attached via a lysine side chain then incorporate that residue using Fmoc-Lys(ivDde)-OH and introduce a Boc-amino acid at the N-terminus.
2. Deprotect either the N-terminal amino function or lysine side chain, depending on where the SAMA moiety is to be linked, with 20% piperidine in DMF or 2% hydrazine in DMF, respectively. Wash the resin with DMF.
3. Dissolve SAMA-OPfp (5 eq.) and HOBt (5 eq.) in DMF and add to the resin. Leave to stand with gentle agitation for 1 h. Check the completeness of the reaction with ninhydrin. Repeat the SAMA addition if necessary.

Bromoacetylation

MAP resin

1. Prepare the MAP resin as normal. After completion of the lysine scaffold, introduce a Glu residue; this will greatly improve the solubility of the MAP core.
2. Deprotect this residue with 20% piperidine, and wash the resin with DMF. Dissolve N-succinimidyl bromoacetate (5 eq.) and HOBt (5 eq.) in DMF, and add the solution to the resin. Leave to stand with gentle agitation for 1 h. Check the completeness of the reaction with ninhydrin. Repeat the N-succinimidyl bromoacetate addition if necessary.

Carrier Protein

1. Dissolve the carrier protein (BSA, KLH or ovalbumin; see CALBIOCHEM catalog) in 100 mM phosphate buffer/100 mM NaCl, pH 7.4.
2. Add N-succinimidyl bromoacetate (10 mg/ml) in DMF (final molar ratio of protein to N-succinimidyl bromoacetate of 1:40). Stir for 2 h at rt.
3. Isolate the protein by gel-filtration on a Sephadex G-25 column.

Conjugation

1. Dissolve the SAMA labeled peptide in 2.5% sodium dodecyl sulfate (Calbiochem cat. no. 428015).
2. Add a solution of bromoacetylated carrier protein (1.1 eq. relative to bromoacetyl functions) in 0.1 M sodium phosphate/5 mM EDTA (Calbiochem cat. no. 34103) at pH 6, followed by 2 M NH_2OH (50 eq.) dissolved in the same buffer.
3. Stir at rt for 2 d, and then add 2-aminoethanethiol (6 eq.) in 0.1 M sodium phosphate, pH 6. After a further 16 h, purify the conjugate by gel-filtration on a Sephadex G-25 column.

Related products

851028	Bis-Boc-amino-oxyacetic acid	p. 39
851017	Boc-amino-oxyacetic acid	p. 39
855131	Dawson Dbz AM resin	p. 43
855142	Dawson Dbz NovaSyn® TGR resin	p. 43
852216	Fmoc-Dpr(Boc-Aoa)-OH	p. 40
855021	4-Sulfamylbutyryl AM resin	p. 45
855044	4-Sulfamylbutyryl NovaSyn® TG resin	p. 46
855147	4-Sulfamylbutyryl Rink Amide AM resin	p. 46
855056	Hydroxylamine NovaTag™ resin	p. 41
855144	Hydroxylamine PEG NovaTag™ resin	p. 42
856069	H-Ala-Sulfamylbutyryl NovaSyn® TG resin	p. 46
856191	Fmoc-Ala-4-Sulfamylbutyryl Rink Amide AM resin	p. 47
856078	H-Asn(Trt)-Sulfamylbutyryl NovaSyn® TG resin	p. 47
856070	H-Gln(Trt)-Sulfamylbutyryl NovaSyn® TG resin	p. 47
856068	H-Gly-Sulfamylbutyryl NovaSyn® TG resin	p. 47
856192	Fmoc-Gly-4-Sulfamylbutyryl Rink Amide AM resin	p. 47
856076	H-Ile-Sulfamylbutyryl NovaSyn® TG resin	p. 47
856077	H-Leu-Sulfamylbutyryl NovaSyn® TG resin	p. 48
856074	H-Lys(Boc)-Sulfamylbutyryl NovaSyn® TG resin	p. 48
856079	H-Phe-Sulfamylbutyryl NovaSyn® TG resin	p. 48
856080	H-Thr(tBu)-Sulfamylbutyryl NovaSyn® TG resin	p. 48
856075	H-Val-Sulfamylbutyryl NovaSyn® TG resin	p. 48
851213	Fmoc-Ala-sulfamylbutyryl linker	p. 46
851214	Fmoc-Gly-sulfamylbutyryl linker	p. 46
851215	Fmoc-Ser(tBu)-sulfamylbutyryl linker	p. 46
851016	SAMA-OPfp	p.40
855152	SEA-PS resin	p. 44

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2 Peptide-labeling

Fluorescent- and biotin-labeled peptides are invaluable tools for biochemistry, having numerous applications in enzymology, protein chemistry, immunology and histochemistry. Novabiochem® offers one of the most extensive ranges of labeling reagents for the synthesis of such peptides, including the unique NovaTag™ resins for the production of C-terminally-labeled peptides.

2.1 Chromogen-labeling

The spectral properties of Novabiochem's chromogenic and fluorogenic derivatives are summarized in Table 1.

One of the most important applications of such reagents is in the synthesis of fluorescence-quenched enzyme substrates. These are highly sensitive tools for probing protease specificity and activity, particularly for endoproteases where conventional carboxy-terminally labeled substrates are not always appropriate. Such substrates typically contain a fluorophore and a quencher group attached to either side of the cleavage site. In the intact molecule, the natural fluorescence of the fluorophore is suppressed by the proximity of the quencher through a process called fluorescence resonance energy transfer (FRET). Upon cleavage of the substrate by a protease, the quencher and fluorophore become separated, leading to an increase in fluorescence, which can then be detected spectrophotometrically (Figure 16). Sensitivity is determined primarily by the distance between fluorophore and quencher, which should be in the range 10-100 Å, and the extent of overlap between the absorbance spectrum of the quencher and the emission spectrum of the fluorophore. The recommended fluorophore-quencher pairs are listed in Table 1.

Table 1: Spectral properties of Novabiochem dyes.

Chromophore/ Fluorophore	λ_{max} (nm)	λ_{em} (nm)	Quencher
AMC	342	441	-
Dabcyl	453	-	-
Dansyl	335	526	Dabsyl
Dnp	348	-	-
EDANS	341	471	Dabcyl
Mca	328	393	Dnp
FAM	494	518	Dabcyl
TAMRA	555	580	-

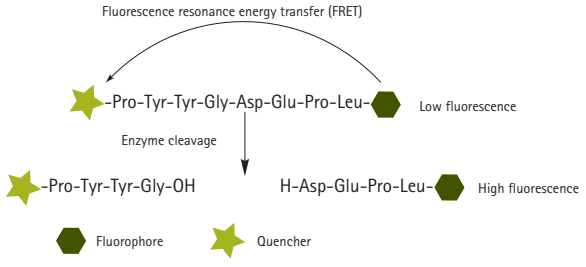
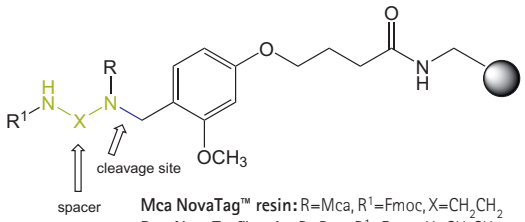


Fig. 16: Fluorescence-quenched peptide substrate.

2.2 NovaTag™ resins



Mca NovaTag™ resin: R=Mca, R^1=Fmoc, X=CH_2CH_2
Dnp NovaTag™ resin: R=Dnp, R^1=Fmoc, X=CH_2CH_2
Dansyl NovaTag™ resin: R=Dansyl, R^1=Fmoc, X=CH_2CH_2
EDANS NovaTag™ resin: R=H, R^1=5-sulfonaphthyl, X=CH_2CH_2
Universal NovaTag™ resin: R=Fmoc, R^1=Mmt, X=CH_2CH_2
Universal PEG NovaTag™ resin: R=Fmoc, R^1=Mmt, X=PEG

Fig. 17: NovaTag™ resins.

Labels are most frequently incorporated at the N-terminus of the peptide during solid phase synthesis as this is synthetically very straightforward using carboxylic acid derivatives of labels such as biotin-OSu, TAMRA, etc. For many applications, however, it is advantageous to place the label at the *C*-terminus, particularly if the *N*-terminus is required for biological activity, or if the peptide contains more than one label.

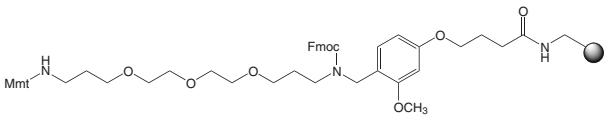
Furthermore, for FRET-based enzyme substrates, it is often preferable to place the fluorophore/quencher pair at the *N*- and *C*-termini, rather than on amino-acid side chains as this avoids modification of the native peptide sequence.

Novabiochem®'s NovaTag™ resins are unique tools designed to streamline the Fmoc SPPS of chromogen and biotin labeled peptides [1]. Each resin contains a standard TFA-cleavable linker which has been modified to incorporate a diamine spacer that is either orthogonally protected or pre-derivatized with a chromogenic label (Fig. 9). This arrangement allows *C*-terminally labeled peptides to be prepared quickly and efficiently with the minimum number of synthetic steps. Pre-loaded resins are available which on cleavage directly provide peptides containing fluorophores (Dansyl, Mca, EDANS) and quencher groups (Dnp) for FRET applications, or affinity labels (biotin, biotin-PEG, hydroxylamine) for bioconjugation and surface immobilization.

The pre-loaded resins are easy to use, and can be generally employed in automated instrumentation without modification of existing Fmoc synthesis protocols. The only exceptions are the EDANS NovaTag™ and Universal NovaTag™ resins where attachment of the first residue must be carried out under modified conditions as this involves acylation of a secondary amine (Method 3-10, page 3.7). Following chain extension and cleavage with TFA in the usual manner, products are obtained containing the appropriate fluorophore or biotin at the C-terminus.

The use of pre-loaded resins avoids the inherent problems of poor solubility and inefficient coupling, as well as the additional steps and costs, that are associated with the introduction of biotin and certain fluorophores in solid phase synthesis. The approach is particularly suited to the production of peptides for high-throughput screening, as it removes the difficulty of ensuring complete label incorporation for all molecules in the array. NovaTag™ resins incorporating different labels and spacer groups can also be custom manufactured.

Universal PEG NovaTag™ resin



For situations where it is not always apparent at the outset which is the optimum label or combination of labels for a given application, Novabiochem® offers the Universal NovaTag™ resins. These supports facilitate the synthesis of peptides bearing any number of different acyl moieties at *N*- and *C*-termini from a single solid phase synthesis (Figure 18). Universal resins are also useful for preparing labeled peptides containing fluorophores that are not compatible with Fmoc SPPS protocols, such as TAMRA and FAM [2], since they allow the labels to be easily introduced as the final step before cleavage. Universal PEG NovaTag™ resin is particularly efficacious for applications where a hydrophilic spacer is required between the peptide and the label and where the peptide solubility is an issue.

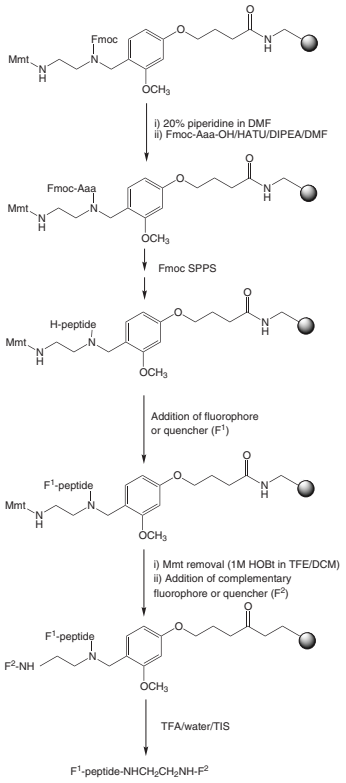


Fig. 18: Synthesis using Universal NovaTag™ resins.

After loading of the first amino acid to the resin-bound secondary amine, chain extension is carried out under standard Fmoc methods. Following synthesis, the resin can be partitioned and each aliquot end-capped with the appropriate carboxyl-functionalized label. The pendant Mmt group is then removed using HOBt/TFE/DCM (Method 4) and the C-terminal label introduced to each resin aliquot. Thus, from a single synthesis any number of label variations for a given sequence can be prepared.

Universal PEG NovaTag™ resin has been used to prepare fluorescently-labled Shc Src homology 2 domain-binding peptides linked to cell

penetrating sequences via a PEG spacer [3] and dimeric SMAC PEG-linked peptides [4]. Universal NovaTag™ resin has been utilized to prepare peptides *C*-terminally modified to ketone for ligation [5].

Method 4: Removal of Mmt group

1. Add 0.6 M HOBt in DCM/TFE (1:1) to resin swollen in DCM.
2. Gently agitate for 1h; solution goes dark red.
3. The solvent is removed by filtration, and steps 1 & 2 are repeated.
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.

Application 4: Synthesis of Dansyl-Pro-Leu-Gly-Leu-$NHCH_2CH_2NH$-Dabcyl using Universal NovaTag™ resin

Fmoc-Leu-OH (3 eq.) was coupled to Universal NovaTag™ resin (0.4 mmole) using PyBrOP® (3 eq.) and DIPEA (10 eq.) in DMF for 18 h. The chain was extended by addition of Gly, Leu and Pro using Fmoc-amino acids (3 eq.) activated with PyBOP®(3 eq.), DIPEA (10 eq.) and HOBt (1 eq.) in DMF for 35 min. Following Fmoc deprotection of Pro, the resin was treated with Dansyl chloride (1.5 eq.) and DIPEA (10 eq.) in THF for 1 h. The Mmt group was then removed as described in Method 4, and the resin was treated with Dabcyl-OSu (2 eq.) and HOBt (0.06 eq.) in DMF for 18 h. Labeled peptide was cleaved from the resin using TFA/DCM/water (50:50:1) for 2 h. The crude peptide was analyzed by HPLC (Figure 19).

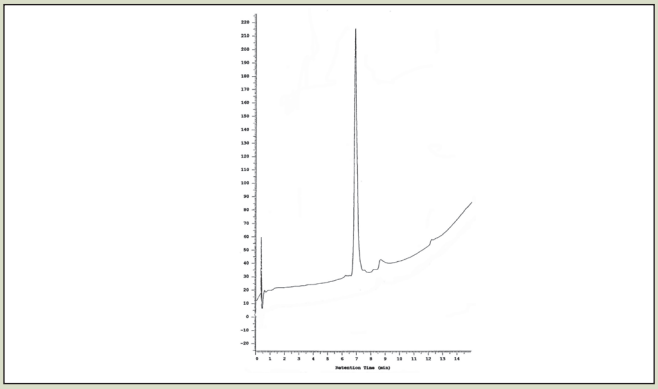
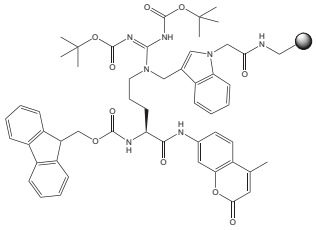


Fig. 19: HPLC elution profile of crude Dansyl-Pro-Leu-Gly-Leu-$NHCH_2CH_2NH$-Dabcyl with Universal NovaTag™ resin.

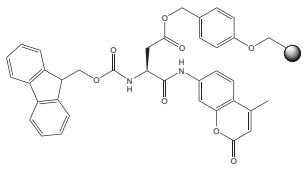
2.3 Reagents for introduction of optical labels

AMC

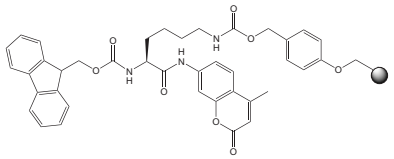
Fmoc-Arg(bis-Boc-resin)-AMC



Fmoc-Asp(Wang resin)-AMC



Fmoc-Lys(carbamate Wang resin)-AMC



Enzyme substrates based on the 7-amino-4-methylcoumarin (AMC) fluorophore are very popular tools for studying protease activity and specificity [6]. 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 20). 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.

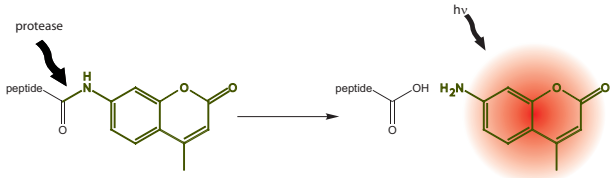


Fig. 20 Principle of AMC-labeled fluorogenic substrates.

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® has introduced a range of resins pre-loaded with amino acid-AMC derivatives: Fmoc-Asp(Wang resin)-AMC; Fmoc-Arg(bis-Boc-resin)-AMC[7]; Fmoc-Lys(carbamate Wang resin)-AMC. Arginine, aspartic acid and lysine were selected as these amino acids occur at the P1 position of endogenous substrates for a number of important proteases.

These resins are extremely simple to use and are fully compatible with standard Fmoc SPPS protocols. In the case of Fmoc-Arg(bis-Boc-resin)-AMC, the use of 2% DBU in DMF for Fmoc removal gives remarkably better results. 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 21 and 22.

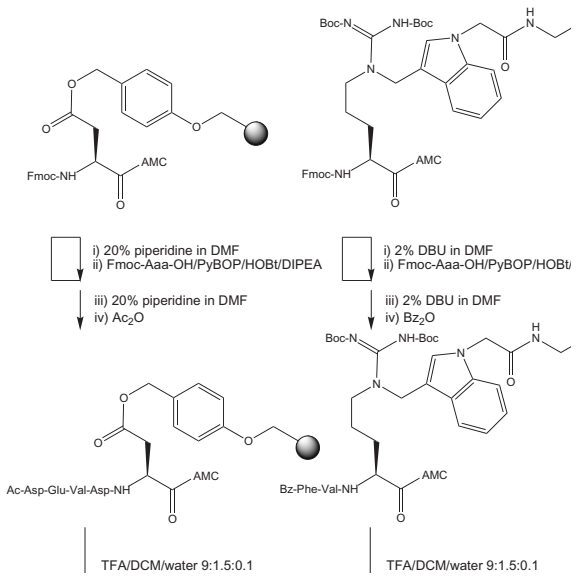


Fig. 21: Synthesis of Ac-Asp-Glu-Val-Asp-AMC using Fmoc-Asp(Wang resin)-AMC and Bz-Phe-Val-Arg-AMC using Fmoc-Arg(bis-Boc-resin)-AMC.

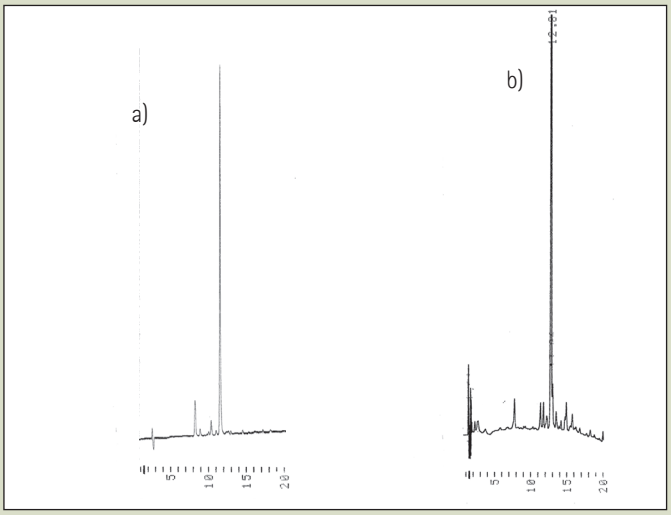
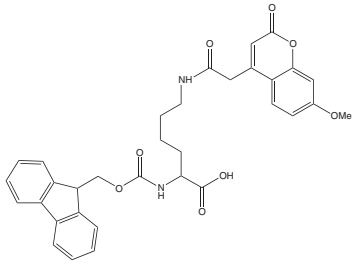


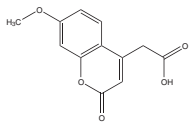
Fig. 22: HPLC elution profiles of a) crude Ac-Asp-Glu-Val-Asp-AMC prepared with Fmoc-Asp(Wang resin)-AMC b) crude Bz-Phe-Val-Arg-AMC prepared with Fmoc-Arg(bis-Boc-resin)-AMC.

7-Methoxycoumarin (Mca)

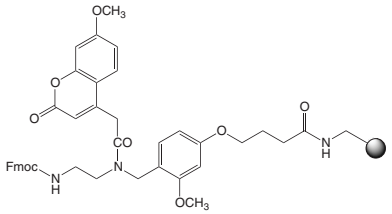
Fmoc-Lys(Mca)-OH



Mca-OH



Mca NovaTag™ resin

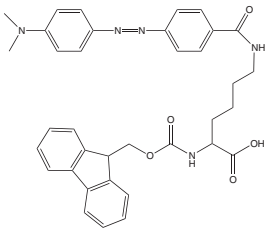


The 7-methoxycoumarin group (Mca) fluoresces at 393 nm when stimulated at 328 nm, and is most commonly used in conjunction with 2,4-dinitrophenyl (Dnp) quenching groups [8]. It can be coupled on solid phase to free amines using Mca-OSu, Mca-OH, or incorporated during chain extension using a pre-formed building block such as Fmoc-Lys(Mca)-OH [9]. Fmoc-Lys(Mca)-OH and Mca-OH can be coupled using any standard coupling method, such as PyBOP®/DIPEA and DIPCDI/HOBt, whereas the preactivated derivative, Mca-OSu, couples best in DMSO or NMP in the presence of HOBt. The coumarin moiety is stable to the standard conditions employed in Fmoc SPPS.

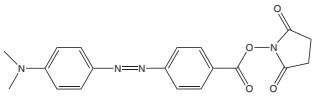
Mca NovaTag™ resin provides peptides C-terminally modified with the Mca fluorophore attached via an ethylene diamine spacer [1]. Following removal of the Fmoc group, the resin-bound primary amine can be loaded with the first amino acid residue using standard activation methods, such as PyBOP, HOBt/DIPCDI. After peptide assembly, treatment with 95% TFA cleaves the Mca peptide directly from the resin. The use of this resin is illustrated in Application 5.

Dabcyl

Fmoc-Lys(Dabcyl)-OH



Dabcyl-OSu



Dabcyl is one of the most frequently utilized quenching groups because of its lack of innate fluorescence and spectral overlap (λ_{max} 453 nm) with a number of commonly-used fluorophores, such as EDANS, Mca, TET, JOE, FAM. In the preparation of fluorescence-quenched peptide substrates, it is most frequently used in conjunction with EDANS as this pairing is particularly efficacious owing to their excellent spectral overlap [10, 11].

Application 5: Synthesis of endopeptidase Glu-C substrate

Mca NovaTag™ resin (155 mg, 0.045 mmole) was swollen in DMF and the Fmoc group removed with 20% piperidine. Peptide assembly was carried out using 30 min couplings of Fmoc-amino acids (6 eq.) activated with PyBOP® (6 eq.) in the presence of DIPEA (12 eq.). The peptide was cleaved from the resin using 95:2.5:2.5 TFA/water/TIS for 3 h and purified directly by HPLC. The purified peptide gave the HPLC profile shown in Figure 23 and was characterized by ES-MS [expected M+H$^+$ 1142.4, found 1142.4].

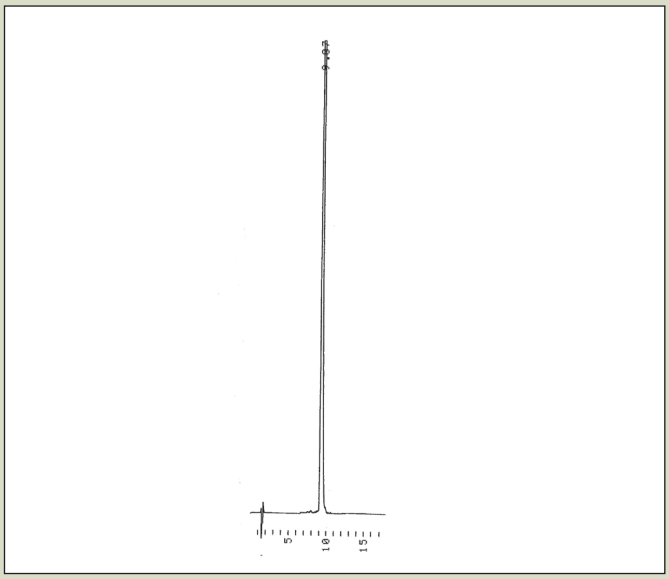
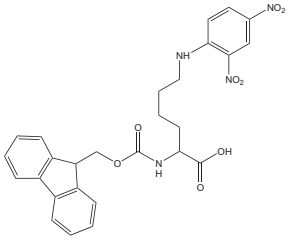


Fig. 23: HPLC elution profile of purified H-Lys(Dnp)-Leu-Glu-Val-Asp-Gly-Trp-NHCH$_2$CH$_2$NH-Mca prepared with Mca NovaTag™ resin.

The Dabcyl group is most conveniently introduced during solid phase synthesis of the substrate. Addition to the N-terminal amino group is best achieved using Dabcyl-OSu in DMSO or NMP in the presence of HOBt. When 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.

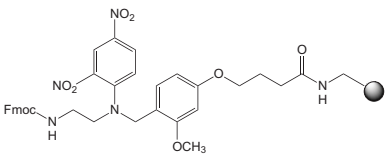
2,4-Dinitrophenyl (Dnp)

Fmoc-Lys(Dnp)-OH



The Dnp group (λ_{max} 348 nm) is the preferred quenching group for the Mca fluorophore [8]. It is most easily incorporated into a peptide as Fmoc-Lys(Dnp)-OH, which can be coupled using any standard activation method.

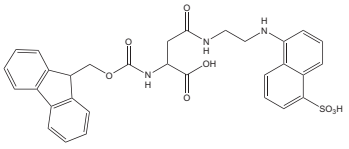
Dnp NovaTag™ resin



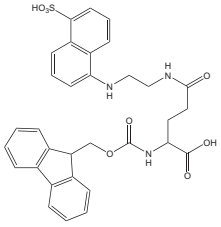
This resin is ideal for the assembly of labeled peptides incorporating a C-terminal Dnp group [1]. Following removal of the Fmoc group, the resin-bound primary amine can be loaded with the first amino acid residue using standard activation methods, such as PyBOP®, HOBt/DIPCDI. After peptide assembly, treatment with 95% TFA cleaves the Dnp peptide directly from the resin. This resin is particularly advantageous for the synthesis of FRET peptides, since the presence of the quencher in every peptide chain minimizes the levels of fluorescent unquenched by-products and so reduces background fluorescence of the product FRET substrates.

EDANS

Fmoc-Asp(EDANS)-OH



Fmoc-Glu(EDANS)-OH



The EDANS/Dabcyl fluorophore-quencher pair is one of the most commonly-used for FRET applications, owing to excellent spectral overlap between the emission spectrum of EDANS (λ_{ex} 341 nm, λ_{em} 471 nm) and absorbance spectrum of Dabcyl (λ_{max} 453 nm) [10, 11] (Figure 24). 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 [10].

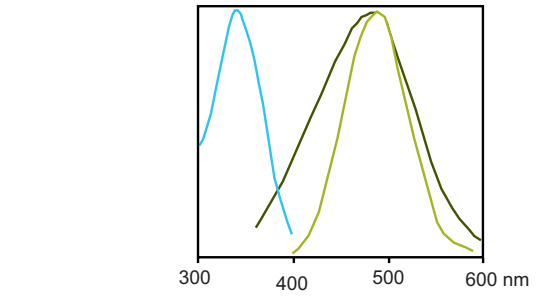


Fig. 24: Absorbance and emission spectra of Dabcyl and EDANS [8].

To incorporate EDANS within the peptide chain, the simplest approach is to use either Fmoc-Asp(EDANS)-OH or Fmoc-Glu(EDANS)-OH during peptide assembly [12, 13]. Introduction of these derivatives during SPPS can be achieved using PyBOP®/DIPEA activation in conjunction with an extended coupling time [13]. Powerful acylating reagents such as PyBrOP should be avoided as their use may lead to acylation of the naphthylamine nitrogen.

EDANS NovaTag™ resin

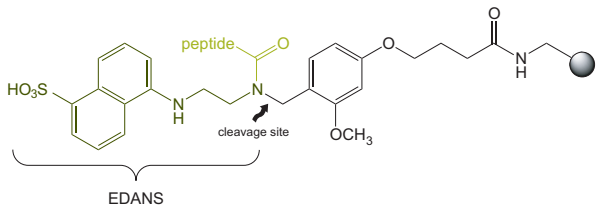


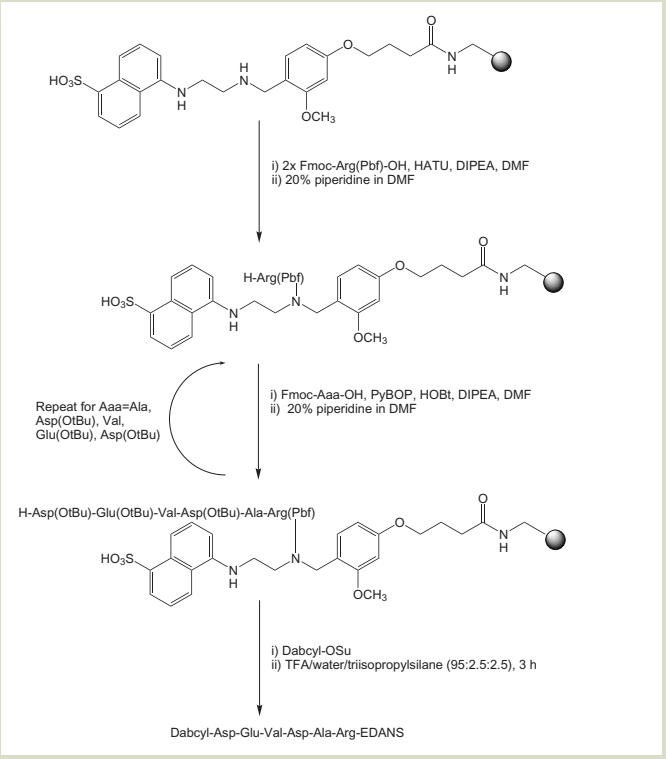
Fig. 25: Loaded EDANS NovaTag™ resin showing point of attachment of peptide and site of cleavage.

Traditionally, the introduction of the EDANS moiety at the C-terminus of a peptide is achieved by coupling of a peptide fragment to EDANS in solution [14]. Novabiochem®'s EDANS NovaTag ™ resin enables for the first time the direct synthesis of C-terminally EDANS-labeled peptides by solid phase synthesis [1b] (Figure 25). The use of EDANS NovaTag™ resin in the solid phase synthesis of FRET peptides is illustrated by the examples given in Applications 3 and 4. 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 5). 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.

EDANS NovaTag™ resin has been recently employed to prepare fluorescently labeled aminoalkane diphenyl phosphonate affinity probes for chymotrypsin- and elastase-like serine proteases [15].

Application 6: 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 5. 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 eq) 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 26) and ES-MS [expected M+H⁺ 1230, found 1230].

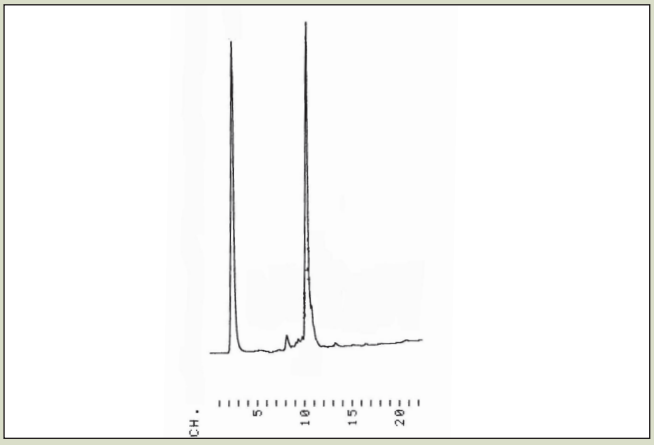
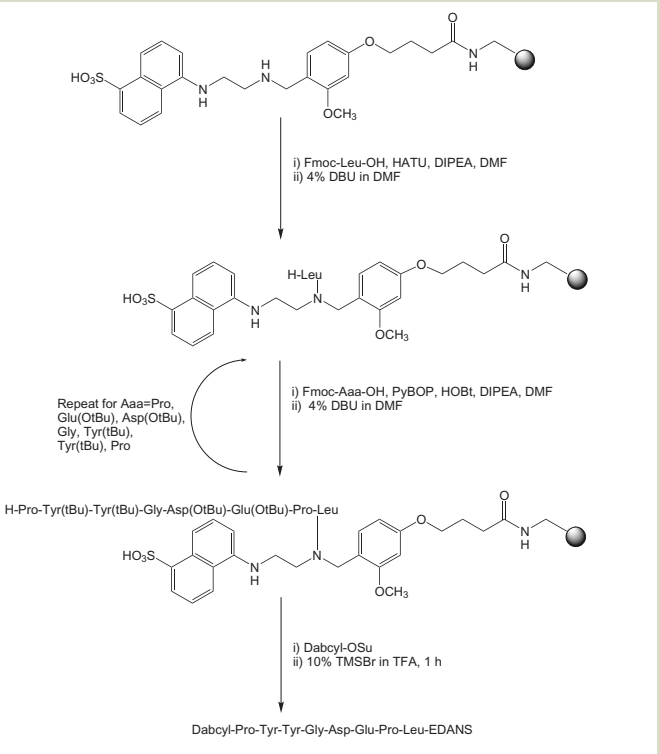


Fig. 26: HPLC elution profile of crude Dabcyl-Asp-Glu-Val-Asp-Ala-Arg-EDANS prepared with EDANS NovaTag™ resin .

Application 7: 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 5. 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 27) and ES-MS [expected M+H$^+$ 1453, found 1453].

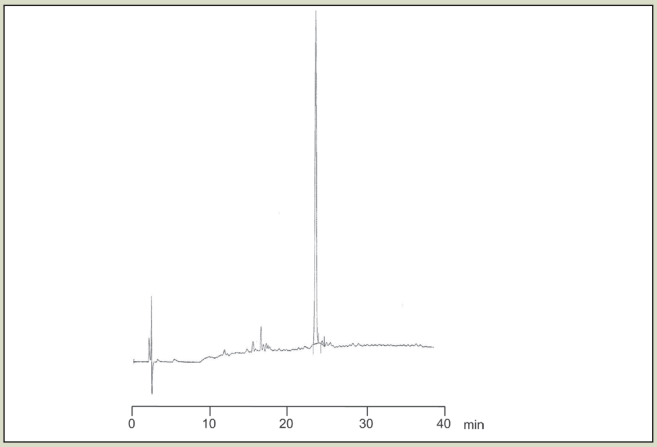
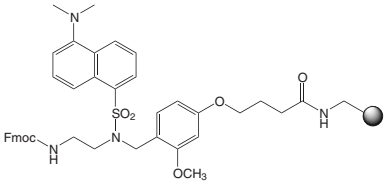


Fig. 27: HPLC elution profile of crude Dabcyl-Pro-Tyr-Tyr-Gly-Asp-Glu-Pro-Leu-EDANS prepared with EDANS NovaTag™ resin .

Method 5: Loading EDANS and Biotin-PEG NovaTag™ resins

1. Suspend resin in DMF and leave to swell for 30 min. (In the case of Biotin-PEG NovaTag™ resin the Fmoc group should be removed at this stage with 20% piperidine in DMF.)
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 [see Method 3-11. page 3.7]. 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.

Dansyl NovaTag™ resin



This resin facilitates the direct synthesis of peptides C-terminally labeled with the Dansyl group (λ_{ex} 335 nm, λ_{em} 526 nm). Following removal of the Fmoc group, the resin-bound primary amine can be loaded with the first amino acid residue using standard activation methods, such as PyBOP®, HOBt/DIPCDI. After peptide assembly, treatment with 95% TFA cleaves the Dansyl peptide directly from the resin (Application 5).

Dansyl NovaTag™ resin has been recently employed to prepare FRET probes for mercury binding protein MerP [16] and functionalized amyloid fibrils [17].

Application 8: Synthesis of H-Asp-Glu-Val-Asp-Ala-Arg-$NHCH_2CH_2NH$-Dansyl using Dansyl NovaTag™ resin

H-Asp(OtBu)-Glu(OtBu)-Val-Asp(OtBu)-Ala-Arg(Pbf)-Dansyl NovaTag™ resin was prepared automatically on a NovaSyn Crystal peptide synthesizer using Dansyl NovaTag™ resin (263 mg, 0.1 mmole). 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 (5 eq.). The labeled peptide was cleaved from the resin using TFA /TIS/water (95:2.5:2.5) for 2.5 h. The crude peptide was analyzed by HPLC (Figure 28) [expected M+H$^+$ 979, found 979].

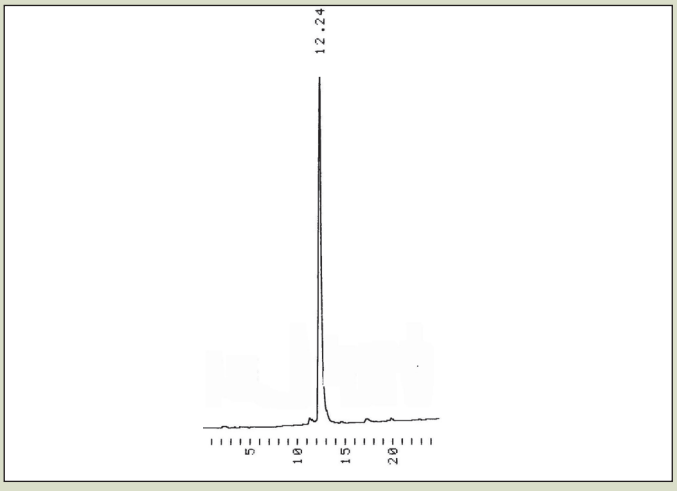
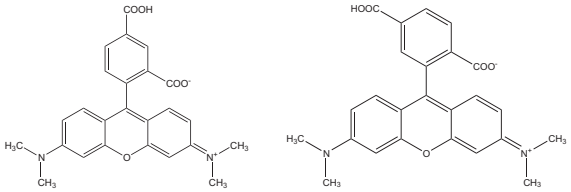


Fig. 28: HPLC elution profile of crude H-Asp-Glu-Val-Asp-Ala-Arg-Dansyl prepared with Dansyl NovaTag™ resin [1].

5-Carboxyfluorescein/6-Carboxyfluorescein

5-Carboxytetramethylrhodamine/6-Carboxytetramethylrhodamine



Novabiochem® supplies carboxyfluorescein (FAM; λ_{ex} 494 nm, λ_{em} 518 nm) and carboxytetramethylrhodamine (TAMRA, λ_{ex} 555 nm; λ_{em} 580 nm) as single isomers, ensuring labeled products of defined chemical structure, as well as greatly assisting product purification and characterization. However, for those applications which do not require a single isomer dye, 5,6-carboxyfluorescein is also available as the cost effective option.

The dyes are most conveniently introduced during solid phase synthesis by coupling to *N*-terminal or side-chain amino groups using HOBt or HOAt/DIPCDI in DMF (Method 6). When one of the dyes 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), that can be later selectively deprotected on the resin immediately prior to coupling of the dye. After addition of FAM and subsequent amino acids, the resin should be washed with 20% piperidine in DMF to remove phenyl esters formed by acylation of the FAM phenolic hydroxyls. Treatment of FAM peptides with hydrazine can result in hydrazone formation. Esters and hydrazone formation can both be avoided if, following the introduction of FAM and the subsequent piperidine treatment, the phenolic hydroxyls are blocked by tritylation with Trt-Cl and DIPEA in DCM [2].

When used together in the same peptide, fluorescence resonance energy transfer (FRET) between FAM and TAMRA results in quenching of the fluorescence of both dyes, making them excellent reagents for FRET applications [18].

Method 6: Coupling of carboxyl-functionalized dyes

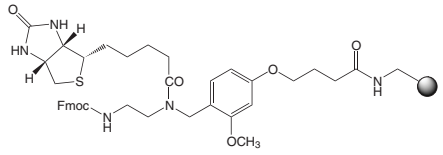
1. Dissolve dye (1.5 eq.) in minimum DMF with HOBt or HOAt (1.5 eq.). If necessary, add DMSO to aid dissolution.
2. Add DIPCDI (1.7 eq.) and stir mixture and leave to stand for 10 min.
3. Add mixture of drained peptidyl resin and agitate gently for 2 h. Check for free amino groups using the Kaiser test. Note: the TNBS test does not work for beads loaded with colored dyes. If the reaction is not complete, leave o/n and then recheck. If after this time it is still not complete, wash resin and repeat with fresh reagents.

2.4 Biotinylating reagents

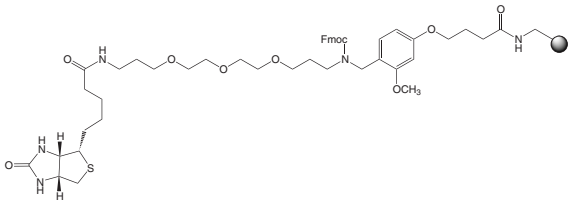
Biotin-labeled peptides have many important applications in immunology and histochemistry, such as affinity purification [19] and FRET-based flow cytometry [20], solid-phase immunoassays [21], and receptor localization [22], that exploit the high affinity of streptavidin and avidin for biotin.

Incorporation of the biotin label is best carried out during solid phase synthesis of the peptide ligand, with the optimum location for the biotin label dependent on the nature of the application.

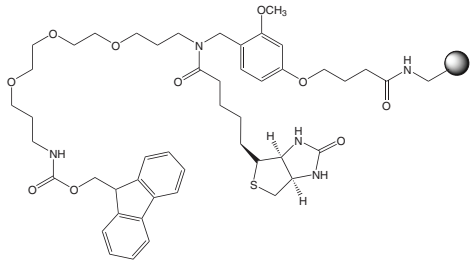
Biotin NovaTag™ resin



Biotin–PEG NovaTag™ resin



Fmoc–PEG Biotin NovaTag™ resin



The biotin label is most frequently located directly on the N-terminal group of the peptide, often without any regard to how this may affect peptide-target interactions, biotin-avidin binding, and the solubility properties of the resultant peptide. In many instances the products are poorly soluble, and have little biological activity and poor affinity for biotin. Problems can also arise during the synthesis of such N-terminally biotinylated peptides due to the poor solubility and reactivity of many of the reagents used for biotin introduction.

Novabiochem®'s biotin-loaded NovaTag™ resins provide a simple and elegant solution to these problems [23-27]. Using these resins, biotinylated peptides are obtained directly following TFA cleavage, without the need for any additional biotinylation steps. Resins incorporate either an ethylenediamine or a 15 atom PEG spacer between the peptide and biotin to reduce steric hindrance.

The use of Biotin-PEG NovaTag™ resin or Fmoc-PEG Biotin NovaTag™ resins are particularly advantageous because not only does the

hydrophilic PEG chain confer better solubility to the peptide biotin conjugate, but its extended conformation leads to better avidin binding which can dramatically improve assay sensitivity as demonstrated in Figure 23. As the biotin is an integral part of the linker, its presence in every peptide chain is assured from the outset.

Using Novabiochem®'s NovaTag™ resins for biotinylated peptide synthesis is extremely easy. Biotin NovaTag™ resin and Fmoc-PEG Biotin NovaTag™ resin can be used directly in an automated synthesizer in the same manner as Rink amide resin. The Fmoc group is removed with 20% piperidine and the peptide assembled on the support using standard protocols. With Biotin-PEG NovaTag™ resin, the procedure is the same except that the first residue should be coupled using HATU as described in Method 5, since this reaction involves acylation of a less reactive secondary amine. Cleavage from the resin can be effected using standard TFA cocktails, providing the C-terminally labeled biotinylated peptide.

The use of Biotin NovaTag™ and Biotin-PEG NovaTag™ resins is illustrated in Applications 9 & 10.

Application 9: Synthesis of H-KKKKXXLLDXXXXXXXXXMKDEE-NH-PEG-NH-biotin (23mer) [28]

Biotin-PEG NovaTag™ resin (345 mg, 0.145 mmole) was swollen in DMF and the Fmoc group removed with 20% piperidine. Peptide assembly was carried out on a Protein Technologies, Inc. Symphony peptide synthesizer using 30 min couplings of Fmoc-amino acids (3 eq.) activated with HCTU (3 eq.) in the presence of NMM (5 eq.). The biotinylated peptide was cleaved from the resin using Reagent K for 2.5 h. The crude peptide gave the HPLC profile shown in Figure 29. The minor component eluting ahead of the main product, is the corresponding methionine sulfoxide peptide.

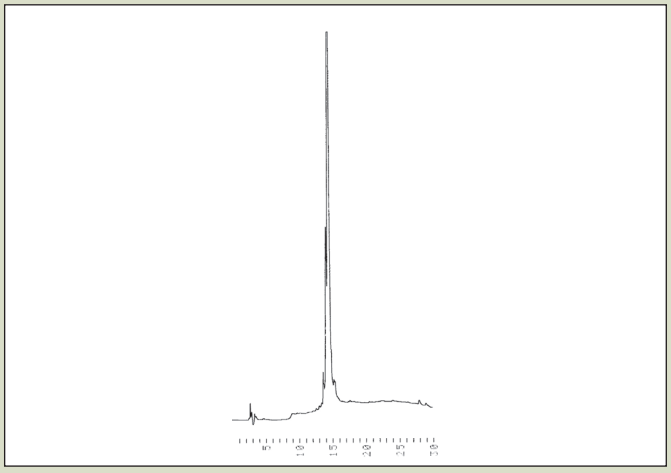


Fig. 29: HPLC elution profile of crude H-KKKKXXLLDXXXXXXXXX-MKDEE-NH-PEG-NH-biotin prepared with Biotin-PEG NovaTag™ resin.

Application 10: Synthesis of H-KKKKXXLLDXXXXXXXXXMKDEE-$NHCH_2CH_2NH$-biotin (23mer) [28]

Biotin NovaTag™ resin (354 mg, 0.145 mmole) was swollen in DMF and the Fmoc group removed with 20% piperidine. Peptide assembly was carried out on a Protein Technologies, Inc. Symphony peptide synthesizer using 30 min couplings of Fmoc-amino acids (3 eq.) activated with HCTU (3 eq.) in the presence of NMM (5 eq.). The biotinylated peptide was cleaved from the resin using Reagent K for 2.5 h. The crude peptide gave the HPLC profile shown in Figure 30. The minor component eluting ahead of the main product, is the corresponding methionine sulfoxide peptide.

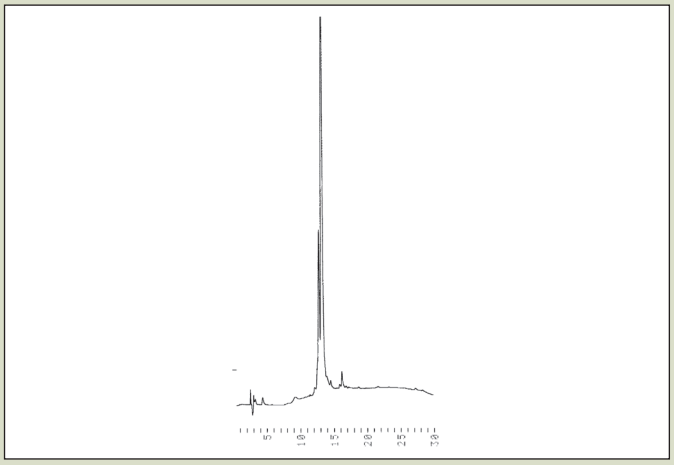


Fig. 30: HPLC elution profile of crude H-KKKKXXLLDXXXXXXXXXM-KDEE-$NHCH_2CH_2NH$-biotin prepared with Biotin NovaTag™ resin.

Biotinylated peptide design

When designing biotinylated peptides for use in assays, two of the most important considerations are the position of the biotin moiety and the nature of the spacer group between the peptide and biotin. This is because these can profoundly effect the strength of peptide-protein and biotin-avidin interactions and consequently the sensitivity of the assay. The importance of correct peptide presentation is illustrated in the following examples taken from developmental work on protein-binding and kinase assays carried out at Merck Pharma KGaA [29].

AlphaScreen™ protein-binding assay

The peptide-protein binding assay was conducted using the AlphaScreen™ technology as shown in Figure 31. *N*- and *C*-terminally biotin-labeled versions of the native peptide ligand immobilized on streptavidin-coated donor beads were screened against acceptor beads loaded with target protein. Only the peptide which was *C*-terminally labeled with PEG-biotin had acceptable solubility in the test buffer and showed significant levels of protein binding (Figure 32).

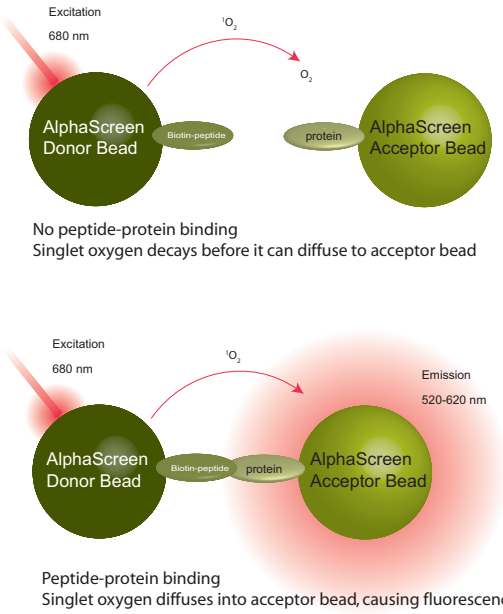


Fig. 31: Principles of the protein-peptide binding AlphaScreen™ assay.

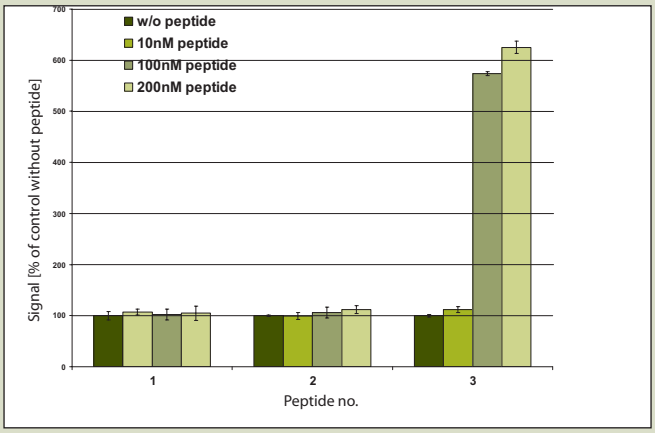


Fig. 32: AlphaScreen protein-binding assay. Peptide 1: N-biotin-XXXXX-NH_2; peptide 2: H-XXXXX-$NHCH_2CH_2NH$-biotin; peptide 3: H-XXXXX-NH-PEG-NH-biotin [28].

Kinase bind assay

N- and *C*-terminally biotin-labeled versions of a kinase substrate were evaluated in the assay shown in Figure 33. Peptides that were C-terminally labeled with biotin were found to give better reponses than those that were labeled on the *N*-terminus, whilst inclusion of a PEG spacer between the peptide and biotin appeared to have little effect (Figure 34).

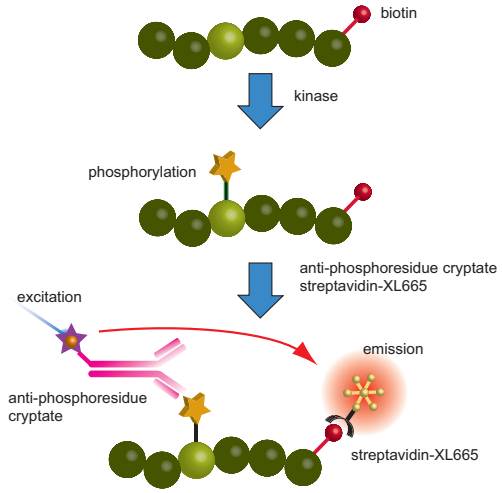


Fig. 33: Principles of the kinase assay.

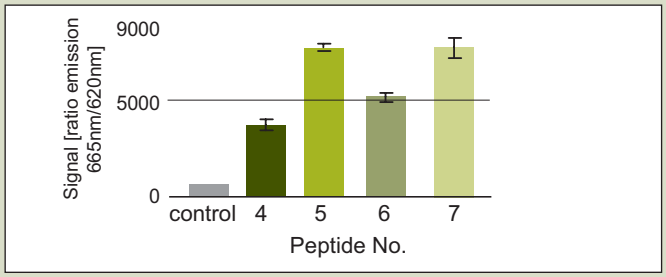
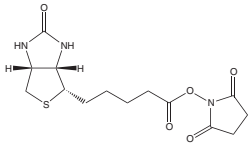
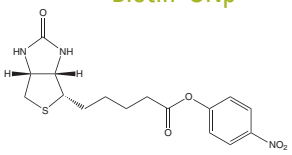


Fig. 34: Kinase assay. Peptide 4: biotin-KKKKXXLLDXXXXXXXXXMKDEE-NH_2; peptide 5: H-KKKKXXLLDXXXXXXXXXMKDEE-$NHCH_2CH_2NH$-biotin; peptide 6; biotin-NH-PEG-KKKKXXLLDXXXXXXXXXMKDEE-NH_2 ; peptide 7: H-KKKKXXLLDXXXXXXXXXMKDEE-NH-PEG-NH-biotin [28].

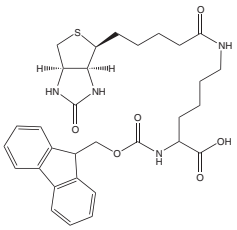
Biotin-OSu



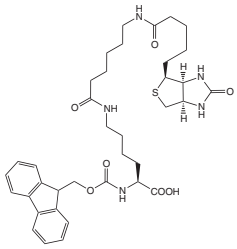
Biotin-ONp



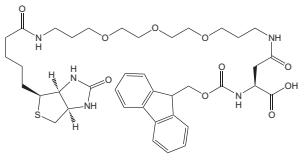
Fmoc-Lys(biotin)-OH



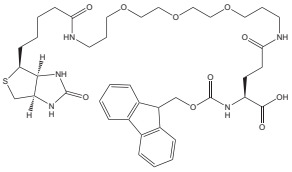
Fmoc-Lys(biotinyl-ε-aminocaproyl)-OH



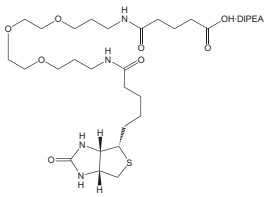
Fmoc-Asp(biotinyl-PEG)-OH



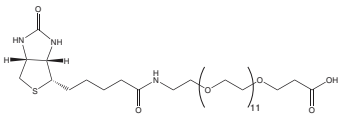
Fmoc-Glu(biotinyl-PEG)-OH



N-Biotinyl-NH-PEG$_2$-COOH



N-Biotinyl-NH-PEG$_{11}$-COOH



For coupling of biotin to amines on the solid phase the use of Biotin-ONp is strongly recommended [30]. As can be seen from Table 2 and Figure 35, the solubility of this reagent in DMF or NMP is much greater than that of Biotin-OSu, and it couples with amines much more rapidly: typically in 40 min as opposed to 12 h for Biotin-OSu.

Alternatively, preformed derivatives such as Fmoc-Lys(biotin)-OH, Fmoc-Lys(biotinyl-ε-aminocaproyl)-OH, Fmoc-Asp(biotinyl-PEG)-OH and Fmoc-Glu(biotinyl-PEG)-OH can be used to introduce biotin at a precise location within a peptide chain. The use of Fmoc-Glu(biotinyl-PEG)-OH is particularly recommended. In contrast to the other derivatives, it possesses good solubility in DMF (Fmoc-Glu(biotinyl-PEG)-OH, 0.5 mmole/ml; Fmoc-Lys(biotin)-OH, <0.05 mole/ml; Fmoc-Lys(biotinyl-ε-aminocaproyl)-OH, <0.05 mmole/ml) and the presence of the PEG chain improves the solubility of the product biotinylated peptide [1b] and helps reduce steric hindrance between peptide and biotin, leading to better avidin binding. It is recommended that NMP or NMP/DMSO be used as the solvents for Fmoc-Lys(biotin)-OH and Fmoc-Lys(biotinyl-ε-aminocaproyl)-OH. For convenience, Fmoc-Lys(biotinyl-ε-aminocaproyl)-OH pre-loaded onto NovaSyn ® TG^R A resin is available.

N-Biotinyl-NH-PEG$_2$-COOH and N-Biotinyl-NH-PEG$_{11}$-COOH offer the same benefits as Fmoc-Glu(biotinyl-PEG)-OH and are ideal for incorporation of biotin-PEG at the *N*-terminus of a peptide. The long 40 atom spacer in the latter has an marked affect on peptide solubility compared to that of Glu(biotinyl-PEG) or *N*-biotinyl-NH-PEG$_2$.

Table 2: Solubilities of Novabiochem®'s biotin derivatives.

	Solubility (mmole/ml)		
Compound	DCM	DMF	NMP
Biotin-OSu	>0.02	0.1	0.15
Biotin-ONp	0.09	0.5	0.5
Fmoc-Lys(biotin)-OH	<0.025	<0.05	0.25
Fmoc-Lys(biotinyl-ε-aminocaproyl)-OH	<0.025	0.05	0.5
Fmoc-Glu(biotinyl-PEG)-OH	<0.025	0.5	0.5

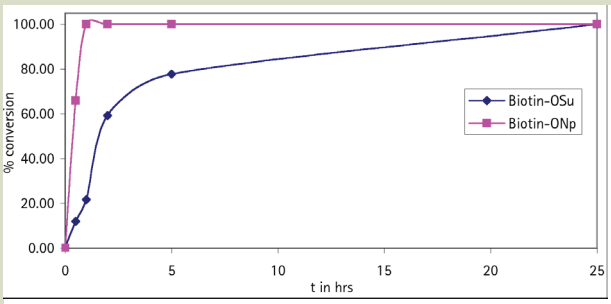


Fig. 35: Coupling rate of Biotin-OSu and Biotin-ONp to H-Asp(OtBu)-Glu(OtBu)-Val-Glu(OtBu)-Wang resin.

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Related products

851209	D(+)-Biotin	p. 33
851027	Biotin-ONp	p. 33
851023	Biotin-OSu	p. 33
855051	Biotin NovaTag™ resin	p. 33
855055	Biotin-PEG NovaTag™ resin	p. 34
855145	Fmoc-PEG Biotin NovaTag™ resin	p. 34
851029	N-Biotinyl-NH-PEG_2-COOH	p. 32
852340	N-Biotinyl-NH-PEG_{11}-COOH	p. 32
851025	5-Carboxyfluorescein	p. 23
851072	6-Carboxyfluorescein	p. 23
851082	5(6)-Carboxyfluorescein	p. 23
851026	5-Carboxy-tetramethylrhodamine	p. 24
851073	6-Carboxy-tetramethylrhodamine	p. 24
851030	5(6)-Carboxy-tetramethylrhodamine	p. 24
851022	Dabcyl-OSu	p. 24
855050	Dansyl NovaTag™ resin	p. 28
855053	Dnp NovaTag™ resin	p. 28
855054	EDANS NovaTag™ resin	p. 29
856081	Fmoc-Arg(bis-Boc-resin)-AMC	p. 25
856146	Fmoc-Asp(Wang-resin)-AMC	p. 25
856147	Fmoc-Lys(carbamate Wang-resin)-AMC	p. 26
852113	Fmoc-Asp(biotinyl-PEG)-OH	p. 35
852102	Fmoc-Glu(biotinyl-PEG)-OH	p. 35
852118	Fmoc-Asp(EDANS)-OH	p. 25
852098	Fmoc-Glu(EDANS)-OH	p. 25
852097	Fmoc-Lys(biotin)-OH	p. 36
852100	Fmoc-Lys(biotinyl-ε-aminocaproyl)-OH	p. 36
856193	Fmoc-Lys(biotinyl-ε-aminocaproyl)-NovaSyn ® TG^R A resin	p. 36
852096	Fmoc-Lys(Dabcyl)-OH	p. 26
852099	Fmoc-Lys(Dnp)-OH	p. 26
852095	Fmoc-Lys(Mca)-OH	p. 27
855052	Mca NovaTag™ resin	p. 29
851071	Mca-OH	p. 27
855057	Universal NovaTag™ resin	p. 30
855058	Universal PEG NovaTag™ resin	p. 31

3. Chemoselective purification tags

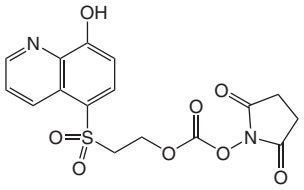
3.1 Introduction

Whilst RP-HPLC is an extremely powerful tool for the purification of small to medium sized peptides, for long peptides the technique lacks the resolution necessary to separate the target molecule from the melange of closely related deletion and truncation products that arise during synthesis. In addition, the purified products, despite giving the appearance of being homogeneous by HPLC analysis, are often microheterogeneous, being contaminated with numerous co-eluting sequences which, because they are individually only present in small amounts, escape detection by mass spectrometry.

One solution is to utilize a combination of chemoselective purification tags [1] and standard RP-HPLC. In the former, unreacted amino groups are capped after each coupling step, converting deletion sequences to shorter truncation sequences. Prior to cleavage of the peptide from the resin, the *N*-terminal amino functionality of the full length peptide is labeled with an affinity tag which permits selective separation of the tagged target peptide from these truncation sequences. Following affinity purification, the tag is cleaved and the desired peptide isolated. The method is especially effective at removing impurities that are closely eluting or hidden under the isolated product peak. Furthermore, removal of these ion-suppressing smaller impurities can greatly enhance the signal of the target peptide in ES-MS. Final polishing by HPLC, removes modified and partially-protected by-products.

Novabiochem® offers three chemoselective purification tags: IMAC Tag, C18 Tag (p-nitrophenyl-2-(octadecylsulfonyl)ethyl carbonate), and 2-biotinyldimedone.

3.2 IMAC Tag



The mechanism of purification using the IMAC Tag [2] is analogous to HisTag affinity purification, the traditional method in use for isolation and purification of recombinant proteins. The IMAC purification method is extremely easy-to-use, gives higher recoveries than RP-HPLC, and is more effective at removing closely eluting impurites. Furthermore, as the purification is an on-off process, it can be readily automated using standard HPLC or FPLC instrumentation.

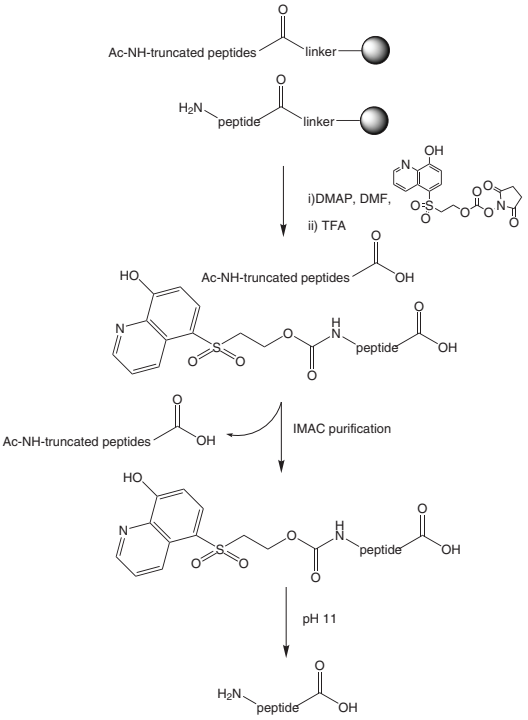
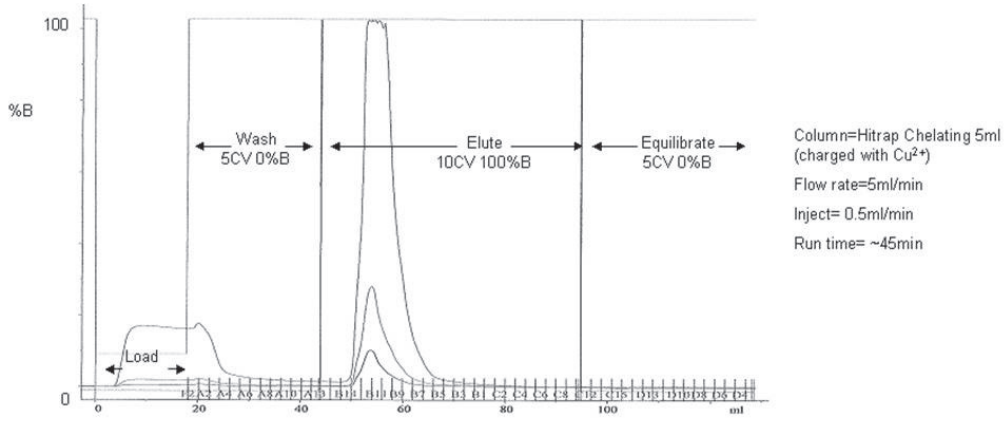


Fig. 36: IMAC purification of peptides.

Fig. 37: A typical IMAC purification trace obtained using a GE Akta Explorer purification system. Untagged truncates elute during the load and wash step and tagged full length product remains bound to the column until the elute step.



The peptide is synthesized using standard methods. After each coupling unreacted amino groups are capped using Ac_2O or Z(Cl)-OSu. Once the final Fmoc group is removed, the IMAC tag is attached to the peptide *via* an ONp carbonate (Figure 36). Peptides are then cleaved from the resin under standard conditions and purified using a column functionalised with iminodiacetic acid (IDA) loaded with Cu^{2+} ions. Tagged peptide is bound to the column at pH 6.5 – 8.0 and truncated sequences are washed away before the tagged peptide is eluted from the column by adjusting the pH to 3.5 (Figure 37). Buffers incorporating sodium phosphate, sodium chloride and urea are used to ensure maximum solubility of substrates.

Once the purified tagged peptide is eluted from the column, the TAG can be removed by simply raising the pH to 11.0 using 2 M NaOH for a short period. The liberated TAG and purified peptide can then be separated using the same IMAC column or an HPLC column for combined isolation and de-salting. As reducing agents are not compatible with IDA, any cysteines present in peptides must be protected during the IMAC step. This can be done simply and reversibly using for example StBu protection.

Protocols

The procedure of attachment of the IMAC and cleavage of tagged peptide from the resin are shown in Method 7.

Method 7: Formation of IMAC-tagged peptide

Attachment of IMAC tag

1. Dissolve Tag-OSu in the minimum volume of DMF and add to pre-swollen resin. Coupling has been shown to be effective using as little as 1 eq Tag-OSu with respect to the initial resin loading.
2. Agitate the mixture for one hour, add a catalytic amount of DMAP and agitate for a further hour.
3. Wash the resin with DMF, DCM and diethyl ether before drying under vacuum.

.

Cleavage of tagged peptide from the resin

1. Cleave non-cysteine containing peptides from the solid phase with concomitant side-chain deprotection by treatment with 90%TFA v/v, 5% H_2O v/v, 2.5% TIS v/v, 2.5% EDT v/v for 4.5 hours.
2. Cleave peptide-resins containing Cys(StBu) by treatment with 90% TFA v/v, 5% H_2O v/v, 5% TIS v/v for 4.5 hours.
3) Work up cleavage reactions in the standard manner by precipitation.

The IMAC purification using a copper (II) loaded IDA column is given in method 8. Columns such as HiTrap Chelating HP worked well for this application.

Method 8: Purification of tagged peptide

Buffer preparation

1. Binding buffer for the IMAC chromatography may consist of 20 mM sodium phosphate, 2-8 M urea (depending on peptide solubility) and 0.5 M NaCl. A pH range of 6.5-8.5 can be used.
2) Elution Buffer should have a pH of 3.5. A suggested buffer within this range is citric acid-sodium phosphate system. Elution buffer should also contain Urea (2-8 M) and NaCl (0.5 M).

IMAC purification

1. Charge column with 0.5 column volumes (CV) of 0.1 M CuSO4 in H20.
2. Wash column with 2 CV H_2O, 7.5 CV elution buffer, 10 CV binding buffer.
3. Solubilise crude tagged peptide in binding buffer.
4. Load sample onto IMAC column.
5. Wash any unbound material from the column with 10 CV binding buffer.
6. Elute tagged peptide from column using 20 CV elution buffer.

Method 9: Removal of tag

1. Combine fractions containing purified tagged product. Adjust to pH 11 with 2 M NaOH. If product contains Cys(StBu) add TCEP up to a concentration of 5 mM to remove S-tBu protecting groups simultaneously.
2. Agitate reaction for 1-2 hours at room temperature and then adjust pH to ~ 4 with 2 M HCl.
3. Separate liberated tag and purified peptide using a second IMAC step or carry out RP-HPLC to combine isolation with desalting.

Example purifications

Application 11: Purification of Gly-GLP (2-36)

Gly-GLP (2-36) was also tagged and purified using IMAC methodology. The crude peptide was shown to contain several impurities identified as capped truncates formed during SPPS (Figure 4). A recovery of 22% pure peptide (89% purity) was obtained after IMAC purification and TAG cleavage. For comparison, purification using HPLC gave pure peptide (81%) with a lower recovery of 5%.

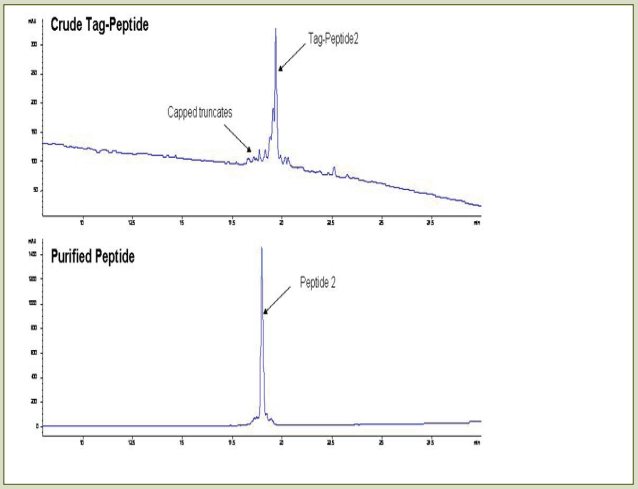


Fig. 38: HPLC profiles of Gly-GLP 2-36 before and after IMAC purification.

Application 12: Purification of Ubiquitin

Ubiquitin was also tagged and purified using IMAC methodology. The crude peptide was shown to contain several impurities identified as capped truncates formed during SPPS (Scheme 4). A recovery of 62% pure peptide (89% purity) was obtained after IMAC purification and TAG cleavage. For comparison, purification using HPLC gave pure peptide (82%) with a lower recovery of 21%..

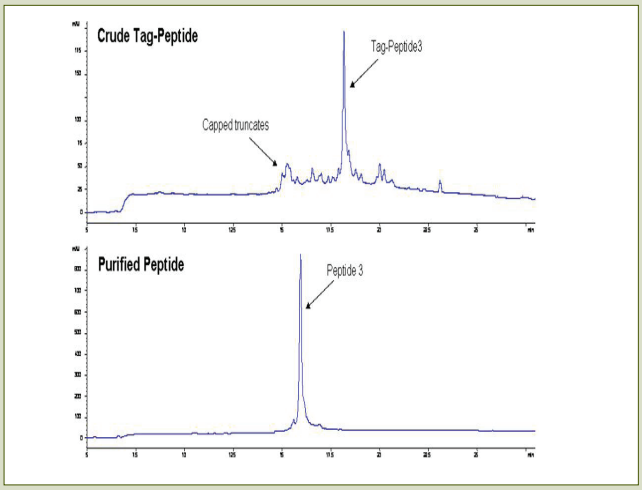
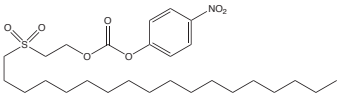


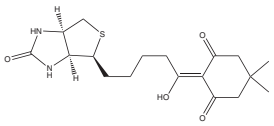
Fig. 39: HPLC profiles of Ubiquitin before and after IMAC purification.

3.3 C18-Tag



This lipophilic tag allows separation of the tagged peptide by HPLC. Typically, tagged peptides elute 5 - 10 minutes later than the capped by-products. Removal of the tag is effected by treatment with 5% aq. ammonia [3] or by Method 9. This tag is useful for the purification of hydrophilic peptides.

3.4 2-Biotinyldimedone



2-Biotinyldimedone tag allows product isolation by biotin-avidin affinity chromatography [4]. Without any pre-activation, it reacts with free primary amino groups to give derivatives that are stable to both acid and base conditions employed in Fmoc SPPS. Labeling of the *N*-terminal amine of resin-bound peptides is simply carried out by incubating overnight the peptidyl resin with a fourfold excess of the reagent in DMF. The biotinylated peptide is obtained following the standard TFA cleavage-deprotection procedure. Following cleavage, this biotin-labeled peptide can be bound to an avidin-coated support, allowing capped fragments to be simply washed away. The purified product can then be eluted with aqueous hydrazine, leaving the used tag attached to the support.

References

1. P.Mascagni in "Fmoc solid phase peptide synthesis - a practical approach",W. C. Chan & P. D.White (Eds), Oxford University Press, 2000, pp. 243.
2. PCT Application No.: PCT/GB2011/000363
3. C. Garcia-Echeverria (1995) *J. Chem. Soc., Chem. Commun.*, 779.
4. B. Kellam, et al. (1997) *Tetrahedron Lett.*, **38**, 5391.

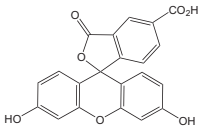
Related products

851069	2-Biotinyldimedone	p. 38
851208	IMAC Tag	p. 38
851092	4-Nitrophenyl-2-(octadecylsulfonyl)ethylcarbonate	p. 38

Product No.	Product	Quantity	Price

Labeling reagents & resins

Chromogenic reagents & resins



851025

5-Carboxyfluorescein

25 mg
100 mg
1 g

5-FAM

NBC No.: 01-63-0112; CAS No.: 76823-03-5; $C_{21}H_{12}O_7$; M.W.: 376.3

TLC: EtOAc:MeOH:H_2O:DIPEA (60:30:10:3), purity: ≥ 98.00%.

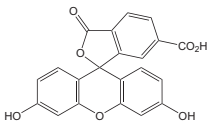
HPLC: purity: ≥ 99.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; protect from light.

This high purity fluorescent dye (λ_{ex} 494 nm, λ_{em} 518 nm) is supplied as a single isomer, which ensures production of labeled products of defined chemical structure, as well as greatly simplifying purification and characterization. FAM should be introduced as the last step in the synthesis to avoid formation of side-products, or be protected by tritylation prior to acylation or hydrazine treatment [1].

ⓘ G 15, M 15

[1] R. Fischer, et al. (2003) *Bioconjugate Chem.*, **14**, 653.



851072

6-Carboxyfluorescein

25 mg
100 mg

6-FAM

NBC No.: 01-63-0113; CAS No.: 3301-79-9; $C_{21}H_{12}O_7$; M.W.: 376.3

TLC: EtOAc:MeOH:H_2O:DIPEA (60:30:10:3), purity: ≥ 95.00%.

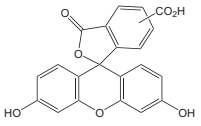
HPLC: purity: ≥ 98.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; protect from light.

This high purity fluorescent dye (λ_{ex} 494 nm, λ_{em} 518 nm) is supplied as a single isomer, which ensures production of labeled products of defined chemical structure, as well as greatly simplifying purification and characterization. FAM should be introduced as the last step in the synthesis to avoid formation of side-products, or protected by tritylation prior to acylation or hydrazine treatment [1].

ⓘ G 15, M 15

[1] R. Fischer, et al. (2003) *Bioconjugate Chem.*, **14**, 653.



851082

5(6)-Carboxyfluorescein

1 g
5 g

NBC No.: 01-63-0149; CAS No.: 72088-94-9; $C_{21}H_{12}O_7$; M.W.: 376.3

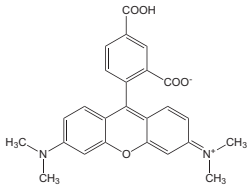
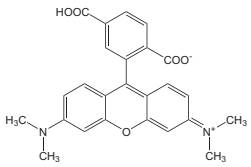
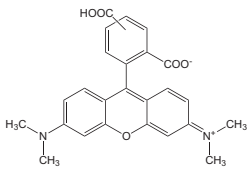
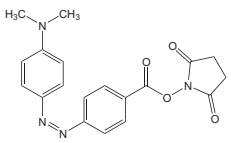
TLC: EtOAc:MeOH:H_2O:DIPEA (60:30:10:3), purity: ≥ 97.00%.

HPLC: purity: ≥ 96.00%.

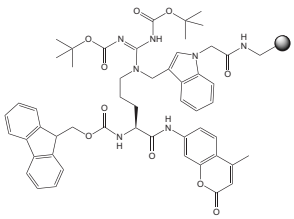
⚠ Prolonged storage: +2 to +8°C; keep cool and dry; protect from light.

Novabiochem®'s 5(6)-carboxyfluorescein (FAM; λ_{ex} 494 nm, λ_{em} 518 nm) is a useful tool for preparing fluorescently-labeled peptides. It 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.

ⓘ G 15, M 15

	Product No.	Product	Quantity	Price
	851026	**5-Carboxytetramethylrhodamine** 5-TAMRA NBC No.: 01-63-0114; CAS No.: 91809-66-4; $C_{25}H_{22}N_2O_5$; M.W.: 430.5 TLC: EtOAc:MeOH:H_2O:DIPEA (60:30:10:3), purity: ≥ 97.00%. HPLC: purity: ≥ 96.00%. ⚠ Prolonged storage: +2 to +8°C; keep cool and dry; protect from light. This fluorescent dye (λ_{ex}555 nm, λ_{em}580 nm) is supplied as a single isomer, which ensures production of labeled products of defined chemical structure, as well as greatly simplifying purification and characterization. ⓘ G 15, M 15	10 mg 50 mg	
	851073	**6-Carboxytetramethylrhodamine** 6-TAMRA NBC No.: 01-63-0115; CAS No.: 91809-67-5; $C_{25}H_{22}N_2O_5$; M.W.: 430.5 TLC: EtOAc:MeOH:H_2O:DIPEA (60:30:10:3), purity: ≥ 97.00%. HPLC: purity: ≥ 96.00%. ⚠ Prolonged storage: +2 to +8°C; keep cool and dry; protect from light. This high purity fluorescent dye (λ_{ex}555 nm, λ_{em}580 nm) is supplied as a single isomer, which ensures production of labeled products of defined chemical structure, as well as greatly simplifying purification and characterization. ⓘ G 15, M 15	10 mg 50 mg	
	851030	**5(6)-Carboxytetramethylrhodamine** 5(6)-TAMRA NBC No.: 01-63-0134; CAS No.: 98181-63-6; $C_{25}H_{22}N_2O_5$; M.W.: 430.5 TLC: $CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 97.00%. HPLC: purity: ≥ 95.00%. ⚠ Prolonged storage: +2 to +8°C; keep cool and dry; protect from light. Novabiochem®'s 5(6)-carboxytetramethylrhodamine (λ_{ex}555 nm, λ_{em} 580 nm) is a useful tool for preparing fluorescently-labeled peptides. It 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. ⓘ G 15, M 15	100 mg 500 mg	
	851022	**Dabcyl-OSu** N-(4-[4'-(Dimethylamino)phenylazo]benzoyloxy)succinimide NBC No.: 01-63-0105; CAS No.: 146998-31-4; $C_{19}H_{18}N_4O_4$; M.W.: 366.4 TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%. HPLC: purity: ≥ 98.00%. ⚠ Prolonged storage: ≤ -20°C; keep cool and dry. Pre-activated derivative for the introduction of the Dabcyl group during SPPS. The Dabcyl group quenches the fluorescence of EDANS, Mca, TET, JOE, FAM fluorophores, making it an extremely useful tool for the synthesis of fluorescence-quenched peptide substrates. ⓘ G 11	1 g	

Product No.	Product	Quantity	Price



856081 **Fmoc-Arg(bis-Boc-resin)-AMC** 500 mg

NBC No.: 04-12-3912

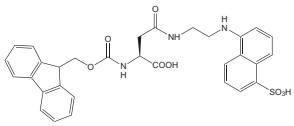
Loading: 0.20 - 0.30 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry; protect from light.

A novel resin for the preparation of peptide substrates based on 3-amino-7-methylcoumarin (AMC) by Fmoc SPPS [1]. Following Fmoc removal with 20% piperidine or 2-3% DBU in DMF, peptide assembly can be effected using standard coupling methods. Treatment with 50%TFA in DCM for 2-3h releases the peptide-AMC directly from the solid phase.

ⓘ G 10, A 11

[1] J. Beythien et al. (2006) *Tetrahedron Lett.*, **47**, 3009.



852118 **Fmoc-Asp(EDANS)-OH** 500 mg 1 g

NBC No.: 04-12-1288; CAS No.: 182253-73-2; $C_{31}H_{29}N_3O_8S$; M.W.: 603.64

TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 95.00%.

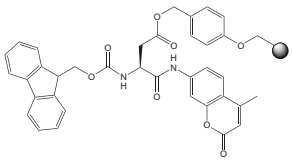
Optical purity: ≥ 99.50% L-enantiomer.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry; keep open bottle under nitrogen.

Fluorescence-labeled amino acid for preparing fluorescence-quenched peptide substrates [1]. Most frequently used in conjunction with Dabcyl quenching group.

ⓘ G 12

[1] L. L. Maggiora, et al. (1992) *J. Med. Chem.*, **35**, 3727.



856146 **Fmoc-Asp(Wang-resin)-AMC** 500 mg 1 g

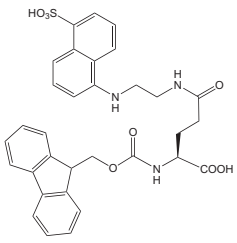
NBC No.: 04-12-3915

Loading: 0.40 - 1.00 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; protect from light.

A novel resin for the preparation of peptide substrates based on 3-amino-7-methylcoumarin (AMC) by Fmoc SPPS. Following Fmoc removal with 20% piperidine, peptide assembly can be effected using standard coupling methods. Treatment with 95% TFA releases the peptide-AMC directly from the solid phase.

ⓘ G 10, A 11



852098 **Fmoc-Glu(EDANS)-OH** 500 mg 1 g

NBC No.: 04-12-1238; CAS No.: 193475-66-0; $C_{32}H_{31}N_3O_8S$; M.W.: 617.7

TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 95.00%.

HPLC: purity: ≥ 95.00%.

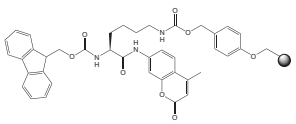
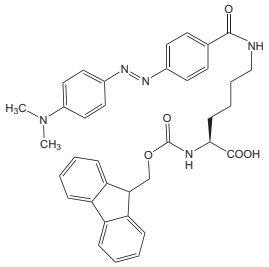
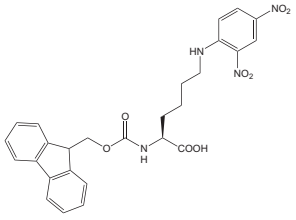
Optical purity: ≥ 99.00% L-enantiomer.

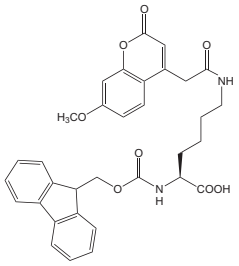
⚠ Prolonged storage: ≤ -20°C; keep cool and dry; keep open bottle under nitrogen.

Fluorescence-labeled amino acid for preparing fluorescence-quenched peptide substrates [1]. Most frequently used in conjunction with Dabcyl quenching group.

ⓘ G 12

[1] J. W. Drijfhout, et al. in "Peptides, Chemistry, Structure & Biology, Proc. 14th American Peptide Symposium", P. T. P. Kaumaya & R. S. Hodges (Eds), Kingswinford, Mayflower Scientific Ltd., 1996, pp. 129.

Product No.	Product	Quantity	Price
856147	 **Fmoc-Lys(carbamate Wang resin)-AMC** NBC No.: 04-12-3917 Loading: 0.40 - 0.60 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF. ⚠ Prolonged storage: +2 to +8°C; keep cool and dry; protect from light. A novel resin for the preparation of peptide substrates based on 3-amino-7-methylcoumarin (AMC) by Fmoc SPPS. Following Fmoc removal with 20% piperidine, peptide assembly can be effected using standard coupling methods. Treatment with 95% TFA releases the peptide-AMC directly from the solid phase. ⓘ G 10, A 11	500 mg 1 g	
852096	 **Fmoc-Lys(Dabcyl)-OH** NBC No.: 04-12-1236; CAS No.: 146998-27-8; $C_{36}H_{37}N_5O_5$; M.W.: 619.7 TLC: $CHCl_3$:MeOH:AcOH:H_2O (85:13:0.5:1.5), purity: ≥ 97.00%. HPLC: purity: ≥ 96.00%. Optical purity: ≥ 99.00% L-enantiomer. ⚠ Prolonged storage: +2 to +8°C; keep cool and dry; protect from light. A modified lysine derivative for the preparation of chromogenically-labeled peptides by Fmoc. The Dabcyl group quenches the fluorescence of EDANS, Mca, TET, JOE, FAM fluorophores, making it an extremely useful tool for the synthesis of fluorescence-quenched peptide substrates. ⓘ G 11	500 mg 1 g	
852099	 **Fmoc-Lys(Dnp)-OH** N-α-Fmoc-N-ε-2,4-dinitrophenyl-L-lysine NBC No.: 04-12-1239; CAS No.: 148083-64-1; $C_{27}H_{26}N_4O_8$; M.W.: 534.5 TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%. CH_3CN:$CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%. HPLC: purity: ≥ 97.00%. ⚠ Prolonged storage: ≤ -20°C; keep cool and dry. A modified lysine derivative for the preparation of chromogenically-labeled peptides by Fmoc. The Dnp group is the preferred quencher for use in conjunction with the Mca fluorophore, making it an extremely useful tool for the synthesis of fluorescence-quenched peptide substrates. ⓘ G 12	500 mg 1 g	

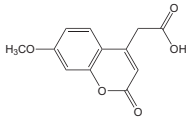
Product No.	Product	Quantity	Price
852095			

Fmoc-Lys(Mca)-OH

500 mg
1 g

N-α-Fmoc-N-ε-7-methoxycoumarin-4-acetyl-L-lysine
NBC No.: 04-12-1233; CAS No.: 386213-32-7; $C_{33}H_{32}N_2O_8$; M.W.: 584.6
TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 97.00%.
HPLC: purity: ≥ 97.00%.
Optical purity: ≥ 99.00% L-enantiomer.
⚠ Prolonged storage: ≤ -20°C; keep cool and dry; protect from light.
A modified lysine derivative for the preparation of fluorogenically-labeled peptides by Fmoc chemistry [1].
The Mca group fluoresces at 405 nm when stimulated at 340 nm, and is most commonly used in conjunction with Dabcyl and 2,4-dinitrophenyl quenching groups.
ⓘ G 11

[1] J. L. Lauer-Fields, et al. (2001) *Biochemistry*, **40**, 5795.



Mca-OH

1 g
5 g

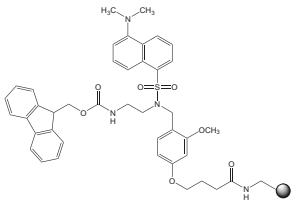
7-Methoxycoumarin-4-acetic acid
NBC No.: 01-63-0111; CAS No.: 629395-72-2; $C_{12}H_{10}O_5$; M.W.: 234.2
TLC: $CHCl_3$:MeOH:AcOH 32% (5:3:1), purity: ≥ 95.00%.
HPLC: purity: ≥ 95.00%.
⚠ Prolonged storage: +2 to +8°C; keep cool and dry; protect from light.
7-Methoxycoumarin (Mca) fluoresces at 405 nm when stimulated at 340 nm, and is most commonly used in conjunction with 2,4-dinitrophenyl quenching group.
ⓘ G 11

Product No.	Product	Quantity	Price

NovaTag™ resins

Novabiochem's NovaTag™ resins are novel supports for the preparation of C-terminally-modified peptides. Pre-loaded resins are available which on cleavage directly provide peptides containing a range of fluorophores (Mca, EDANS) and quencher groups (Dnp, Dabcyl) for FRET applications, or affinity labels (biotin, biotin-PEG, hydroxylamine) for bioconjugation and surface immobilization.

G 9



855050 **Dansyl NovaTag™ resin** 100 mg 500 mg

N-Dansyl-N'-Fmoc-ethylenediamine MPB-AM resin

NBC No.: 04-12-3900

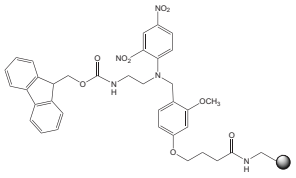
Loading: 0.30 - 0.60 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF.

⚠ Prolonged storage: ≤-20°C; keep cool and dry; protect from light.

Dansyl NovaTag™ resin is an ideal tool for the synthesis of Dansyl-labeled peptides. The resin pre-loaded with the Dansyl chromophore can be used direcly in Fmoc SPPS. Following peptide assembly under standard Fmoc SPPS conditions, cleavage with TFA affords the Dansyl-labeled peptide. This resin has been recently used to dansyl-labeled amyloid-RGD peptides [1] and FRET probes for mercury binding protein MerP [2].

G 14, A 14

[1] S. L. Gras, et al. (2008) *Biomaterials*, **29**, 1553.
[2] B. R. White, et al. (2008) *Analyst*,, **133**, 65.



855053 **Dnp NovaTag™ resin** 100 mg 500 mg

N-Dnp-N'-Fmoc-ethylenediamine MPB-AM resin

NBC No.: 04-12-3903

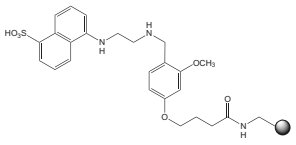
Loading: 0.30 - 0.60 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry; protect from light.

Dnp NovaTag™ resin is an ideal tool for the synthesis of FRET peptide substrates based on the 7-methoxycoumarin (Mca) and 2,4-dintrophenyl (Dnp) fluorophore/quench pair. The resin pre-loaded with the Dnp quencher group can be used direcly in Fmoc SPPS. Following peptide assembly, the Mca group is coupled to the N-terminal N-amino group using Mca-OSu, or introduced to a side-chain of Lys using Fmoc-Lys(Mca)-OH, to give after TFA cleavage the desired FRET peptide substrate.

G 12

Product No.	Product	Quantity	Price



855054 **EDANS NovaTag™ resin** 100 mg, 500 mg

EDANS-MPB-AM resin

NBC No.: 04-12-3904

Loading: 0.30 - 0.60 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin.

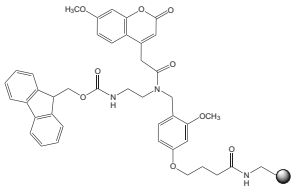
⚠ Prolonged storage: ≤ -20°C; keep cool and dry; protect from light.

The EDANS/dabcyl fluorophore-quencher pair is one of the most commonly-used for FRET peptides, owing to excellent spectral overlap between the emission spectrum of EDANS and absorbance spectrum of dabcyl [1]. The introduction of the EDANS moiety is traditionally achieved either by coupling of a peptide fragment to EDANS in solution [1, 2] or through the use of a pre-formed derivative, such as Fmoc-Glu(EDANS)-OH [3]. Novabiochem's EDANS resin enables for the first time the direct synthesis of C-terminally EDANS-labeled peptides by solid phase synthesis [4]. Loading of the first residue requires acylation of the resin-bound secondary amine and is, therefore, best carried out using HATU activation. Chain extension and cleavage can then be effected using standard methods.

EDANS NovaTag™ resin has been recently employed to prepare fluorescently labeled aminoalkane diphenyl phosphonate affinity probes for chymotrypsin- and elastase-like serine proteases [5].

ⓘ G 13, A 13, 14

[1] G. T. Wang, et al. (1990) *Tetrahedron Lett.*, **31**, 6493.
[2] C. Garcia-Echeverria & D. H. Rich (1992) *Febs Lett.*, **297**, 100.
[3] J. W. Drijfhout, et al. in "Peptides, Chemistry, Structure & Biology, Proc. 14th American Peptide Symposium", P. T. P. Kaumaya & R. S. Hodges (Eds), Kingswinford, Mayflower Scientific Ltd., 1996, pp. 129.
[4] J. Beythien & P. White (2005) *Tetrahedron Lett.*, **46**, 101.
[5] B. F. Gilmore, et al. (2009) *Bioconjugate Chem.*, **20**, 2098.



855052 **Mca NovaTag™ resin** 100 mg, 500 mg

N-Mca-N'-Fmoc-ethylenediamine MPB-AM resin

NBC No.: 04-12-3902

Loading: 0.20 - 0.50 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF.

⚠ Prolonged storage: ≤-20°C; keep cool and dry; protect from light.

Mca NovaTag™ resin is an ideal tool for the synthesis of FRET peptide substrates based on the 7-methoxycoumarin (Mca) and 2,4-dinitrophenyl (Dnp) fluorophore/quench pair. The resin pre-loaded with the Mca fluorophore can be used direcly in Fmoc SPPS. Following peptide assembly, the Dnp group can be introduced using Fmoc-Lys(Dnp)-OH, to give after TFA cleavage the desired FRET peptide substrate.

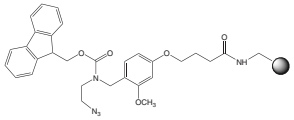
ⓘ G 11, A 12

Product No.	Product	Quantity	Price

Universal resins

When preparing labeled peptides it is not always apparent at the outset which is the optimum combination of fluorophore and quencher or biotin-derivative of a given application. In addition, certain chromophores, such as FAM and TAMRA, are not totally stable to the conditions employed in Fmoc SPPS. For these reasons, Novabiochem® has developed linkers which facilitate the synthesis of peptides bearing any number of different acyl moieties at N- and C-termini from a single solid phase synthesis. After loading of the first amino acid to the resin-bound secondary amine, chain extension is carried out under standard Fmoc methods. Following synthesis, the resin can be partitioned and each aliquot end-capped with the appropriate carboxyl-functionalized label. The pendant protected amine is then deprotected (Mmt: using 1M HOBt in TFE/DCM; azide: by reduction with DDT) and the C-terminal label introduced to each resin aliquot. Thus, from a single synthesis any number of label variations for a given sequence can be prepared.

ⓘ G 9

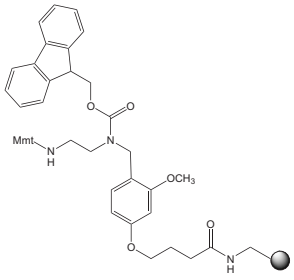


855143 **Azido-NovaTag™ resin** 500 mg, 1 g

⚠ Prolonged storage: ≤ -20°C; keep cool and dry; prevent exposure to light.

Loading: 0.30 - 0.60 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF.

The azido group acts as an orthogonally masked amine; it is stable to both TFA and piperidine but is easily converted to an amine by reduction with phosphines or thiols.



855057 **Universal NovaTag™ resin** 500 mg, 1 g

N-Fmoc-N'-Mmt-ethylenediamine-MPB-AM resin

NBC No.: 04-12-3910

Loading: 0.30 - 0.60 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF.

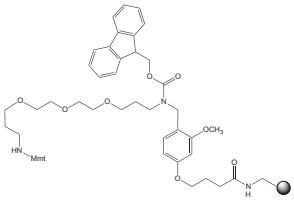
⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

This resin has been recently used to prepare C-terminally modified peptide aldehydes and ketones for ligation [1, 2].

ⓘ G 9, A 10

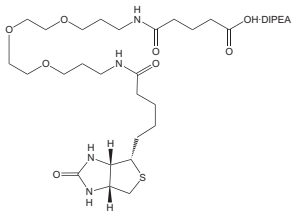
[1] P. Marceau, et al. (2005) *Bioorg. Med. Chem. Lett.*, **15**, 5442.
[2] C. Bure, et al. (2012) *J. Pept. Sci.* , **18**, 147.

Product No.	Product	Quantity	Price
855058	**Universal PEG NovaTag™ resin** N-Fmoc-N'-Mmt-PEG-diamine-MPB-AM resin NBC No.: 04-12-3911 Loading: 0.20 - 0.50 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF. ⚠ Prolonged storage: ≤ -20°C; keep cool and dry. This resin has recently been used to prepare Shc and Src homology domain binding peptoid-peptide hybrids [1] and dimeric SMAC PEG-linked peptides [2]. ⓘ G 9 [1] W. J. Choi (2009) *J. Med. Chem.*, **52**, 1612. [2] Z. Gao, et al. (2007) *J. Biol. Chem.*, **282**, 30718.	500 mg 1 g	



Product No.	Product	Quantity	Price

Biotinylation reagents



851029 **N-Biotinyl-NH-PEG$_2$-COOH · DIPEA (20 atoms)** 500 mg, 1 g

O-(N-Biotinyl-3-aminopropyl)-O'-(N-glutaryl-3-aminopropyl)-diethyleneglycol · DIPEA

NBC No.: 01-63-0133; $C_{25}H_{44}N_4O_8S \cdot C_8H_{19}N$; M.W.: 560.7 · 129.2

Solubility: 50 mg in 1 ml NMP.

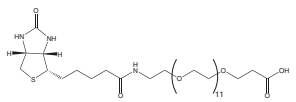
TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 95.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; keep open bottle under nitrogen.

The use of this novel biotin derivative incorporates a hydrophilic 20 atom PEG spacer between the peptide and biotin. This not only greatly improves the solubility of the resultant peptide, but reduces steric hindrance between the peptide and biotin, leading to better avidin binding and higher biological activity. The biotin-PEG derivative can be easily incorporated using any standard coupling method as the final step in SPPS, immediately prior to cleavage of the peptide from the resin.

ⓘ G 18



852340 **N-Biotinyl-NH-PEG$_{11}$-COOH (40 atoms)** 250 mg, 1 g

O-[2-(Biotinylamino)ethyl]-O'-(2-carboxyethyl)undecaethylene glycol

$C_{37}H_{69}N_3O_{16}S$; M.W.: 844.0

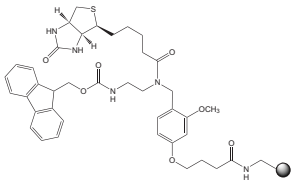
TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry.

The use of this novel biotin derivative incorporates a hydrophilic 40 atom PEG spacer between the peptide and biotin. This not only greatly improves the solubility of the resultant peptide, but reduces steric hindrance between the peptide and biotin, leading to better avidin binding and higher biological activity. The biotin-PEG derivative can be easily incorporated using any standard coupling method as the final step in SPPS, immediately prior to cleavage of the peptide from the resin.

ⓘ G 18

Product No.	Product	Quantity	Price



855051

Biotin NovaTag™ resin

500 mg
1 g

N-Biotin-N'-Fmoc-ethylenediamine MPB-AM resin

NBC No.: 04-12-3901

Loading: 0.35 - 0.60 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF.

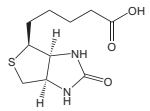
⚠ Prolonged storage: ≤ -20°C; keep cool and dry; keep open bottle under nitrogen.

Biotin NovaTag™ resin is an ideal tool for the synthesis of peptides labeled iwth biotin. As the biotin moiety is incorporated into the linker, its use eliminates the problems associated with biotin-labeling of peptides, namely poor solubility and sluggish coupling kinetics of biotin and its derivatives. Following peptide assembly under standard Fmoc SPPS conditions, cleavage with TFA affords the peptide labeled with biotin. For a recent applications see [1, 2].

[1] M. Carraz, et al. (2009) *Chemisty & Biology*, **16**, 702.

[2] S. Millward, et al. (2007) *Chem. Biol.*, **2**, 625.

ⓘ G 15, A 16



851209

NEW

D(+)-Biotin

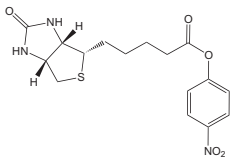
1 g
5 g
25 g

D-Biotin

Vitamin H

CAS No.: 58-85-5; $C_{10}H_{16}N_2O_3S$; M.W.: 244.31

⚠ Prolonged storage: +2 to +8°C



851027

Biotin-ONp

1 g

Biotin p-nitrophenyl ester

5-(2-Oxo-hexahydro-thieno[3,4-d]imidazol-6-yl)-pentanoic acid p-nitrophenyl ester

NBC No.: 01-63-0116; CAS No.: 33755-53-2; $C_{16}H_{19}N_3O_5S$; M.W.: 365.4

TLC: $CH_3CN:CHCl_3$:AcOH (8:1:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 98.00%.

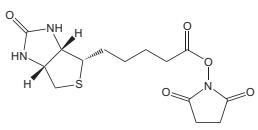
⚠ Prolonged storage: ≤ -20°C; keep cool and dry; keep open bottle under nitrogen.

Pre-activated reagent for the introduction of biotin by solid phase synthesis. More soluble and more reactive than 851023 [1]. This reagents has recently been found to be effective in SPOT synthesis of biotinylated peptides [2].

[1] B. Baumeister, et al. *Int. J. Pept. Res. Ther.*, **11**, 139.

[2] D. Winkler & P. McGeer (2008) *Proteomics*, **8**, 961.

ⓘ G 18,



851023

Biotin-OSu

1 g

N-(Biotinyloxy)succinimide

NBC No.: 01-63-0106; CAS No.: 35013-72-0; $C_{14}H_{19}N_3O_5S$; M.W.: 341.4

solubility: 50 mg in 1 ml NMP.

TLC: $CHCl_3$:MeOH:AcOH (85:10:5), purity: ≥ 97.00%.

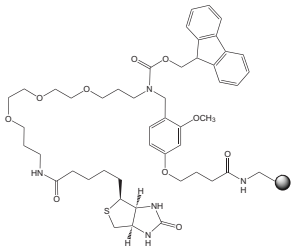
HPLC: purity: ≥ 97.00%.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry; keep open bottle under nitrogen.

Pre-activated reagent for the introduction of biotin by solid phase synthesis.

ⓘ G 18

Product No.	Product	Quantity	Price



855055 **Biotin-PEG NovaTag™ resin** 500 mg, 1 g

N-Biotin-N'-Fmoc-PEG-diamine-MPB-AM resin

NBC No.: 04-12-3908

Loading: 0.20 - 0.50 mmole/g resin; as photometric determination of the Fmoc-chromophore liberated upon treatment with piperidine/DMF.

⚠ Prolonged storage: ≤ -20°C; keep cool and dry; keep open bottle under nitrogen.

Biotin NovaTag™ resin is an ideal tool for the synthesis of peptides labeled iwth biotin. As the biotin moiety is incorporated into the linker, its use eliminates the problems associated with biotin-labeling of peptides, namely poor solubility and sluggish coupling kinetics of biotin and its derivatives.

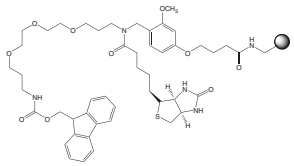
Attachment of the first amino acid should be effected using HATU/DIPEA.

Following peptide assembly under standard Fmoc SPPS conditions, cleavage with TFA affords the peptide labeled with biotin.

Biotin-PEG NovaTag™ resin incorporates a PEG spacer into the peptide, which leads to products having better solubilities compared to those prepared using standard biotin derivatives [1]. Furthermore, the PEG spacer reduces steric hindrance between the peptide and avidin, leading to better binding of biotin. For recent applications, see [2, 3].

ⓘ G 15, A 16

[1] B. Baumeister, et al. (2003) *Biopolymers*, **71**, 339.
[2] J. Parisot, et al. (2009) *J. Sep. Sci.*, **32**, 1613.
[3] S. Shiryaev, et al. (2007) *Biochem. J.*, **401**, 743.



855145 **Fmoc-PEG Biotin NovaTag™ resin** 500 mg, 1 g

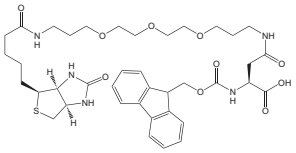
⚠ Prolonged storage: ≤ -20°C; keep cool and dry; keep open bottle under nitrogen.

Loading: 0.20 - 0.50 mmole/g resin; as photometric determination of the Fmoc-chromophore liberated upon treatment with piperidine/DMF.

Fmoc-PEG Biotin NovaTag™ resin offers all the benefits as biotin-PEG NovaTag™ resin (855055) in the synthesis of biotinylated peptides, with the additional advantage that the C-terminal amino acid can be coupled to this resin using any coupling methods.

ⓘ G 15

Product No.	Product	Quantity	Price



852113 **Fmoc-Asp(biotinyl-PEG)-OH** 500 mg, 1 g

N-α-Fmoc-N-γ-(N-biotinyl-3-(2-(2-(3-aminopropyloxy)-ethoxy)-ethoxy)-propyl)-L-asparagine

NBC No.: 04-12-1279; $C_{39}H_{53}N_5O_{10}S$; M.W.: 783.9

Solubility: 1 mmole in 2 ml DMF.

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 95.00%.

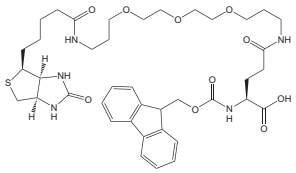
Optical purity: ≥ 99.50% L-enantiomer.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; keep open bottle under nitrogen.

In contrast to Fmoc-Lys(biotin)-OH, this novel biotin-labeled amino acid has excellent solubility in DMF and other solvents used in SPPS [1]. The PEG-spacer restricts hindrance between the peptide and avidin, leading to better biotin binding. Furthermore, the hydrophilic nature of the PEG minimizes non-specific interactions that can arise from the spacer group becoming buried in the hydrophobic pocket of proteins.

ⓘ G 18

[1] B. Baumeister, et al. (2003) *Biopolymers*, **71**, 339.



852102 **Fmoc-Glu(biotinyl-PEG)-OH** 500 mg, 1 g

N-α-Fmoc-N-γ-(N-biotinyl-3-(2-(2-(3-aminopropyloxy)-ethoxy)-ethoxy)-propyl)-L-glutamine

NBC No.: 04-12-1250; CAS No.: 817169-73-6; $C_{40}H_{55}N_5O_{10}S$; M.W.: 798.0

Solubility: 0.2 mmol in 1 ml DMF.

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 97.00%.

HPLC: purity: ≥ 95.00%.

Optical purity: ≥ 99.50% L-enantiomer.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry; keep open bottle under nitrogen.

In contrast to Fmoc-Lys(biotin)-OH, this novel biotin-labeled amino acid has excellent solubility in DMF and other solvents used in SPPS [1]. The PEG-spacer restricts hindrance between the peptide and avidin, leading to better biotin binding. Furthermore, the hydrophilic nature of the PEG minimizes non-specific interactions that can arise from the spacer group becoming buried in the hydrophobic pocket of proteins.

For recent applications, please see [2 - 4].

ⓘ G 18

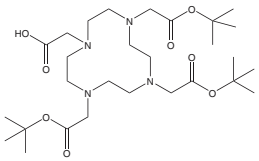
[1] B. Baumeister, et al. (2003) *Biopolymers*, **71**, 339.
[2] X. Zhou et al., (2004) *J. Am. Chem. Soc.*, **126**, 15656.
[3] B. F. Gilmore, et al. (2006) *Biochem. Biophys. Res. Commun.*, **347**, 373.
[4] C. T. Archer, et al. (2005) *Mol. BioSyst.*, **1**, 366.

Product No.	Product	Quantity	Price
852097	**Fmoc-Lys(biotin)-OH** N-α-Fmoc-N-ε-biotinyl-L-lysine NBC No.: 04-12-1237; CAS No.: 146987-10-2; $C_{31}H_{38}N_4O_6S$; M.W.: 594.7 Solubility: 0.1 mmole in 1 ml NMP. TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 95.00%. HPLC: purity: ≥ 95.00%. Optical purity: ≥ 99.50% L-enantiomer. A modified lysine derivative for the preparation of biotin-labeled peptides by Fmoc SPPS. G 18	500 mg 1 g	
852100	**Fmoc-Lys(biotinyl-ε-aminocaproyl)-OH** N-α-Fmoc-N-ε-(biotinylcaproyl)-L-lysine NBC No.: 04-12-1243; CAS No.: 160158-05-4; $C_{37}H_{49}N_5O_7S$; M.W.: 707.9 Solubility: 100mg in 10 ml DMF. TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%. HPLC: purity: ≥ 96.00%. Prolonged storage: +15 to +25°C; keep cool and dry; keep open bottle under nitrogen. A modified lysine derivative for the preparation of biotin-labeled peptides by Fmoc SPPS, in which the biotin is separated from the lysine side-chain by a 6-atom spacer. G 18	500 mg 1 g	
856193	**Fmoc-Lys(biotinyl-ε-aminocaproyl)-NovaSyn® TG^R A resin** NEW	250 mg 1 g	

Product No.	Product	Quantity	Price

Other labeling reagents

Chelators



851200 **DOTA tris-t-Bu ester** 100 mg, 250 mg

Tri-tert-butyl 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate

CAS No.: 137076-54; $C_{28}H_{52}N_4O_8$; M.W.: 572.7

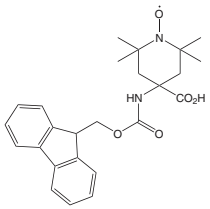
Assay: purity: ≥ 95.00%.

⚠ Prolonged storage: +2 to +8°C

The most convenient reagent for the labeling of a peptide with DOTA [1]. This reagent has good solubility in DMF and DCM, and its free carboxyl group can be easily activated using uronium or phosphonium coupling reagents, enabling it to be attached to peptide amino groups. Removal of the t-butyl ester groups from the DOTA occurs during the course of the TFA-mediated cleavage reaction. The progress of the reaction should be monitored as the cleavage of these esters is slow, owing to the proximity of the basic ring nitrogens.

[1] L. M. De León-Rodríguez & Z. Kovacs (2008) *Bioconjugate Chem*, **19**, 391.

Spin-labeling reagents



852342 **Fmoc-TOAC-OH** 100 mg, 500 mg

2,2,6,6-Tetramethylpiperidine-N-oxyl-4-(9-fluorenylmethyloxycarbonyl-amino)-4-carboxylic acid

CAS No.: 93372-25-9; $C_{25}H_{29}N_2O_5$; M.W.: 437.5

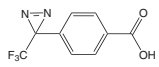
⚠ Prolonged storage: +2 to +8°C; keep cool and dry; prevent exposure to light.

HPLC: purity: ≥ 98.00%.

Fmoc-TOAC-OH is a useful tool for the incorporation of the ESR spin-label TOAC into peptide sequences [1]. Incorporation of this derivative and the following residue is best achieved using HATU activation. TFA/water/TIS should be used for cleavage of TOAC-containing peptides. The use of EDT should be avoided as it can cause permanent reduction of the nitroxide radical [2]. Following cleavage, the TOAC peptide should be treated with aqueous ammonia in air to regenerate the nitroxide from the hydroxylamine that is generated by the TFA treatment.

[1] R. Marchetto, et at. (1993) *J. Am. Chem. Soc.*, **115**, 11042.
[2] L. Martin, et al. (2001) *J. Pept. Res.*, **58**, 424.

Photo-cross linking reagents



851093 **TDBA** 25 mg, 100 mg, 250 mg

4-(3-(Trifluoromethyl)-3H-diazirin-3-yl)benzoic acid

CAS No.: 85559-46-2; $C_9H_5F_3N_2O_2$; M.W.: 230.15

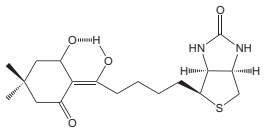
⚠ Prolonged storage: +2 to +8°C; keep cool and dry; prevent exposure to light.

A highly sensitive photocross-linker activated at 300 nm for the preparation of photoactivatable biological probes [1].

[1] M.Nassal, et al. (1983) *Liebigs Ann. Chem.*,, 1510.

Product No.	Product	Quantity	Price

Chemoselective purification reagents



851069 **2-Biotinyldimedone** 1 g, 5 g

NBC No.: 01-63-0108; CAS No.: 194038-08-9; $C_{18}H_{26}N_2O_4S$; M.W.: 366.5

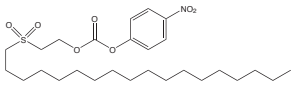
TLC: $CHCl_3$:MeOH:AcOH (90:8:2), purity: ≥ 98.00%.

HPLC: purity: ≥ 99.00%.

A reagent for the reversible labeling of peptides with biotin [1]. Labeling of the N-terminal amine of resin-bound peptides is carried out by incubating overnight with excess reagent in DMF. The biotinylated peptide is obtained following TFA cleavage. Removal of the biotin label is effected by treatment with 5% aqueous hydrazine.

G 19

[1] B. Kellam, et al. (1997) *Tetrahedron Lett.*, **38**, 5391.



851092 **4-Nitrophenyl-2-(octadecylsulfonyl)ethylcarbonate** 250 mg, 1 g

C_{18} Tag

$C_{25}H_{45}O_7NS$; M.W.: 503.7

Solubility: 0.5 mmol in 2 ml DCM.

TLC: EtOAc:Hexane (4:5), purity: ≥ 97.00%.

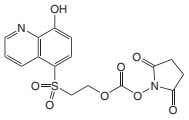
Prolonged storage: +2 to +8°C; keep cool and dry.

4-Nitrophenyl-2-(octadecylsulfonyl)ethylcarbonate is lipophilic peptide purification tag [1]. By capping unreacted sites during synthesis, the N-terminal amino group of the desired peptide can be labeled with this tag. The lipophilic nature of the tag dramatically changes the HPLC characteristics of the product enabling it to be easily separated form capped by-products. Cleavage of the tag can be effected by treatment with 5-10% NH_4OH in TFE. For a detailed discussion on the use of lipophilic purification tags see [2].

G 19, 21

[1] C. Garcia-Echeverria (1995) *J. Chem. Soc., Chem. Commun.*, 779.

[2] P. Mascagni in "Fmoc solid phase peptide synthesis - a practical approach", W. C. Chan & P. D. White (Eds), Oxford, Oxford University Press, 2000, pp. 243.



851208 **IMAC Tag** 250 mg, 1 g

NEW

N-(2-(8-hydroxyquinolin-5-ylsulfonyl)ethoxycarbonyloxy)succinimide

$C_{16}H_{14}N_2O_8S$; M.W.: 394.4

Prolonged storage: ≤-20°C; keep cool and dry.

HPLC: purity: ≥ 98.00%.

Novabiochem®'s new IMAC-based purification Tag provides an simple alternative to the use of RP-HPLC for the purification of long peptides. The method is extremely easy-to-use, gives higher recoveries than RP-HPLC, and because it has a selectivity orthogonal to RP-HPLC, it is more effective at removing closely eluting impurites.

G 19, 20, A 20

Product No.	Product	Quantity	Price

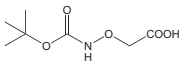
Conjugation & ligation reagents

Novabiochem® provides reagents and amino-acid building blocks that enable incorporation of thiol, hydroxylamine and aldehyde functionalities for oxime [1 - 3], thiazolidine [4], and thioether [5.] conjugation of peptides to biomolecules. Resins for native thiol ligation and synthesis of peptide thioesters are found in the Resins for solid phase peptide synthesis section.

ⓘ G 1

[1] K. Rose, et al. (1994) *J. Am. Chem. Soc.*, **116**, 30.
[2] J. Shao & J. P. Tam (1995) *J. Am. Chem. Soc.*, **117**, 3893.
[3] F. Wahl & M. Mutter (1996) *Tetrahedron Lett.*, **37**, 6861.
[4] C. F. Liu & J. P. Tam (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 6584.
[5] J. W. Drijfhout & P. Hoogerhout in "Fmoc solid phase peptide synthesis - a practical approach", W. C. Chan & P. D. White (Eds), Oxford, Oxford University Press, 2000, pp. 229.

Building blocks



851017 **Boc-amino-oxyacetic acid** 1 g, 5 g

Boc-NH-O-CH_2COOH

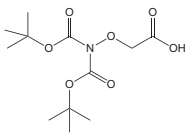
Boc-Aoa-OH

NBC No.: 01-63-0060; CAS No.: 429890-85-5; $C_7H_{13}NO_5$; M.W.: 191.2

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

This reagent can be used to introduce a hydroxylamine functionality to N-terminal or side-chain amino groups. The use of this reagent may lead to double acylation of the hydroxylamine nitrogen. Hydroxylamine-labeled peptides prepared in this manner can be ligated in aqueous solution at pH 3.5 to aldehyde-containing peptides via oxime formation.

ⓘ G 3



851028 **Bis-Boc-amino-oxyacetic acid** 1 g, 5 g

Boc_2-Aoa-OH

NBC No.: 01-63-0129; CAS No.: 293302-31-5; $C_{12}H_{21}NO_7 \cdot H_2O$; M.W.: 291.3 · 18.0

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 97.00%.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

This reagent can be used to introduce a hydroxylamine functionality to N-terminal or side-chain amino groups. Hydroxylamine-labeled peptides prepared in this manner can be ligated in aqueous solution at pH 3.5 to aldehyde-containing peptides via oxime formation.

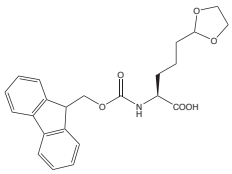
This bis-protected derivative eliminates oligomer formation which can occur with mono-protected hydroxylamine reagents as a result of double acylation.

Coupling of this derivative should be done using an OSu ester.

ⓘ G 3

[1] J. C. Spetzler & T. Hoeg-Jensen (2001) *J. Peptide Sci.*, **7**, 537.

Product No.	Product	Quantity	Price



852305 **Fmoc-Aea-OH** — 100 mg, 500 mg

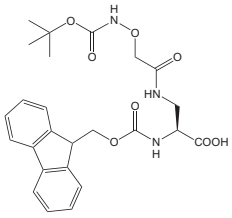
Fmoc-allysine ethylene acetal

CAS No.: 1234692-73-9; $C_{23}H_{25}NO_6$; M.W.: 411.45

Solubility: 1 mmole in 2 ml DMF.

HPLC: purity: ≥ 98.00%.

⚠ Prolonged storage: ≤-20°C; keep cool and dry.



852216 **Fmoc-Dpr(Boc-Aoa)-OH** — 1 g, 5 g, 25 g

N-α-Fmoc-N-β-(N-t.-Boc-amino-oxyacetyl)-L-diaminopropionic acid

NBC No.: 04-12-1185; CAS No.: 600153-12-6; $C_{25}H_{29}N_3O_8$; M.W.: 499.5

Solubility: 1 mmole in 2 ml DMF.

TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%.

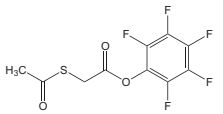
HPLC: purity: ≥ 96.00%.

Optical purity: ≥ 99.50% L-enantiomer.

An excellent derivative for the chemoselective ligation of unprotected peptides in aqueous media [1] via oxime formation. This derivative introduces a hydroxylamine functionality which can couple with aldehyde groups present in another peptide unit.

ⓘ G 3

[1] F. Wahl & M. Mutter (1996) *Tetrahedron Lett.*, **37**, 6861.



851016 **SAMA-OPfp** — 1 g, 5 g

S-Acetylthioglycolic acid pentafluorophenyl ester

NBC No.: 01-63-0041; CAS No.: 129815-48-1; $C_{10}H_5F_5O_3S$; M.W.: 300.1

TLC: Toluene:Dioxane:AcOH (95:25:4), purity: ≥ 98.00%.

⚠ Prolonged storage: ≤-20°C; keep cool and dry.

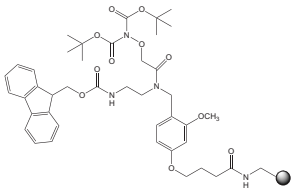
This reagent provides an effective means of linking synthetic peptide antigens to MAP core peptides or carrier proteins for the purpose of raising antibodies. Using this technique many of the problems associated with the analysis and purification of MAPs are avoided, since the linear peptide antigen can be fully characterized before conjugation to the preformed lysine tree.

ⓘ G 7, M 7

[1] J. W. Drijfhout, et al. (1990) *Anal. Biochem.*, **187**, 349.
[2] J. W. Drijfhout (1991) *Int. J. Peptide Protein Res.*, **37**, 27.
[3] H. F. Brugghe, et al. (1994) *Int. J. Peptide Protein Res.*, **43**, 166.

Product No.	Product	Quantity	Price

NovaTag™ resins



855056

Hydroxylamine NovaTag™ resin

500 mg
1 g

N-Boc$_2$-Aoa-N'-Fmoc-ethylenediamine MPB-AM resin

NBC No.: 04-12-3909

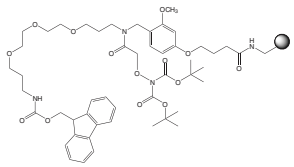
Loading: 0.20 - 0.60 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF. The polymer matrix is copoly (styrene-1 % DVB), 100 -200 mesh.

⚠ Prolonged storage: ≤-20°C; keep cool and dry.

Hydroxylamine NovaTag™ resin is an ideal tool for the synthesis of peptides labeled at the C-terminus with an hydroxylamine group. Such peptides readily undergo oxime formation in aqueous media with aldehyde-modified proteins or peptides, MAP core peptides, or surfaces. For the synthesis of MAPs, this approach avoids many of the problems associated with their purification and analysis, since the linear peptide antigen can be fully characterized prior to conjugation with the pre-formed lysine tree.

ⓘ G 6

Product No.	Product	Quantity	Price
855144	Hydroxylamine PEG NovaTag™ resin ⚠ Prolonged storage: ≤-20°C; keep cool and dry. Loading: 0.20 - 0.60 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF. Hydroxylamine PEG NovaTag™ resin is an ideal tool for the synthesis of peptides labeled at the C-terminus with an hydroxylamine group. Such peptides readily undergo oxime formation in aqueous media with aldehyde-modified proteins or peptides, MAP core peptides, or surfaces. For the synthesis of MAPs, this approach avoids many of the problems associated with their purification and analysis, since the linear peptide antigen can be fully characterized prior to conjugation with the pre-formed lysine tree. ⓘ G 6	500 mg 1 g	



Product No.	Product	Quantity	Price

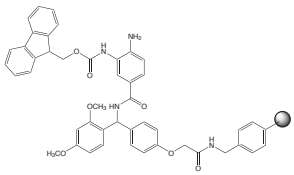
Resins for native chemical ligation by Fmoc SPPS

Dawson resins

Dbz resins are novel suports for the synthesis of peptide thioesters by Fmoc SPPS [1]. After removal of the Fmoc group, the resin is acylated with the first amino acid (see Method 3 page 2 for details) then synthesis is carried out using HBTU/HOBt activation. To avoid branching, especially in case of Fmoc-Gly-OH couplings, protection of the second amino group with allyloxycarbonyl (Alloc) is recommended [2]. Following chain assembly the resin is activated by treatment with p-nitrophenyl chloroformate. Treatment with TFA liberates the fully deprotected peptide benzimidazolinone which can be converted to a thioester with aryl thiol or used directly in native chemical ligation and cyclization reactions. For recent applications see [3-6].

ⓘ G 4, M 5

[1] J. B. Blanco-Canosa (2008) *Angew. Chem. Int. Ed.*, **47**, 6851.
[2] S. K. Mahto, et al. (2011) *ChemBioChem* , **12**, 2488.
[3] Z. Harpaz, et al. (2010) *ChemBioChem*, **11**, 1232.
[4] B. L. Pentelute, et al. (2010) *Chem. Biol.*, **5**, 359.
[5] T. K. Tiefenbrunn, et al. (2010) *Pept. Sci.*, **94**, 405.
[6] S. Gunasekera, et al. (2013) *Int. J. Pept. Res. Ther.*, **19**, 43.

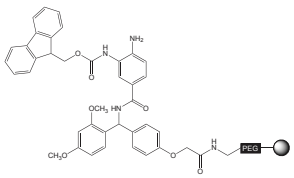


855131 **Dawson Dbz Rink AM resin (100 - 200 mesh)** 1 g / 5 g / 25 g

3-(Fmoc-amino)-4-aminobenzoyl Rink Amide AM resin (100 - 200 mesh)

Loading: 0.40 - 0.80 mmole/g resin; as photometric determination of the Fmoc-chromophore liberated upon treatment with piperidine/DMF.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.



855142 **Dawson Dbz NovaSyn® TGR resin** 1 g / 5 g

Loading: 0.10 - 0.30 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

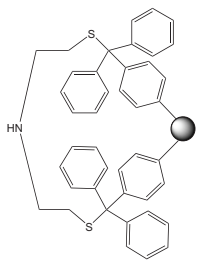
Product No.	Product	Quantity	Price

SEA resins

SEA are useful tools for the synthesis for peptides for used in native chemical ligation reactions (NCL) [1-3]. Loading of the resin with the *C*-terminal amino acid is best done using HATU activation. On cleavage, a peptide is produced bearing a bis(2-sulfanylethyl)amide (SEA) on the *C*-terminus of the peptide. To stabilize the peptide and to simplify HPLC in acidic buffers, the SEA peptide should be converted to the disulfide form by air or iodine oxidation. In the presence of TCEP, cyclic SEA peptides undergo rapid NCL or can be converted to thioesters. SEA disulfide peptides do not undergo ligation in the absence of reducing reagents. This has been exploited to perform one-pot three segment ligations [4].

G 5.5

[1] N. Ollivier, et al. (2010) *Org. Lett.*, **12**, 5238.
[2] J. Dheur, et al. (2011) *J. Org. Chem.*, **76**, 3194.
[3] J. Dheur, et al. (2011) *Org. Lett.*, **13**, 1560.
[4] N. Ollivier, et al. (2012) *Angew. Chem. Int. Ed.*, **51**, 209.



855152 NEW

SEA-PS resin

bis(2-Sulfanylethyl)aminotrityl polystyrene

Loading: 0.10 - 0.16 mmole/g resin; as photometric determination of the Fmoc-chromophore liberated upon treatment with piperidine/DMF. The polymer matrix is: copoly (styrene-1 % DVB), 200-400 mesh.

Quantity	Price
1 g	
5 g	

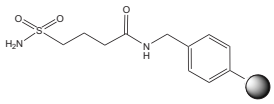
Product No.	Product	Quantity	Price

Sulfamylbutyryl resins & linkers

Sulfamylbutyryl resins facilitate the preparation by Fmoc SPPS of the *C*-terminal peptide thioesters required for native thiol ligation.
The resin-bound sulfamyl group is acylated by carboxylic acids activated with PyBOP® and DIPEA in $CHCl_3$ at -20 °C [1] or DIPCDI and N-methylimidazole in DCM [2]. It can be sometimes problematic to obtain acceptable loadings by these methods, and so it is for this reason that Novabiochem® now offers a number of pre-loaded sulfamylbutyryl resins.
The resin-bound acylsulfonamide is completely stable to basic or strongly nucleophilic reagents, making it compatible with Fmoc SPPS.
Following completion of the synthesis, the sulfonamide can be activated by methylation with TMS-CHN_2, and then cleaved with ethyl mercaptopropionate in the presence of sodium thiophenolate, to yield the appropriate thioester [3]. Activation can be performed with iodoacetonitrile/DIPEA and cleavage by benzylmercaptan [4]. Following removal of side-chain protecting groups with TFA, this material can be used for native thiol ligation. Alternatively, if a sulfamylbutyryl Rink amide resin is used, treatment with TFA generated a peptide N-methylsulfonamide which can be used directly in the NCL reaction without need for prior formation of the thioester. Furthermore, this method enables the progress of the synthesis and the extent of methylation to be checked by performing test cleavages during the synthesis [5].
For further examples of the use of sulfamyl resins in the preparation of thioesters, see [6 - 10]. The use of this type of linker has been reviewed [11, 12].

ⓘ G 1, M 2 A 3, 4

[1] B. J. Backes & J. A.Ellman (1999) *J. Org. Chem.*, **64**, 2322.
[2] Novabiochem Innovations 4/99.
[3] R. Ingenito, et al. (1999) *J. Am. Chem. Soc.*, **121**, 11369.
[4] Y. Shin, et al. (1999) *J. Am. Chem. Soc.*, **121**, 11684.
[5] F. Burlina, et al. (2012) *Chem. Commun.*, 2579.
[6] S. Biancalana, et al. (2001) *Lett. Pept. Sci.*, **7**, 291.
[7] D. Fattori, et al. (2002) Bioorg. Med. Chem. Lett., 12, 1143.
[8] P. Buczek, et al. (2004) Biopolymers, 80, 50.
[9] K. Teruya, et al. (2004) J. Pept. Sci., 10, 479.
[10] D. Macmillan, et al. (2002) Org. Lett., 4, 1467.
[11] P. Heidler & A. Link (2005) Biorg. Med. Chem., 13, 585.
[12] J. C. M. Monbaliu & A. R. Katritzky (2012) *Chem. Commun.*, 11601.

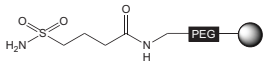
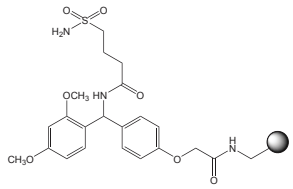
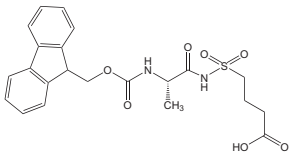
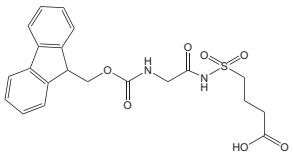
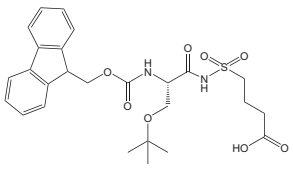
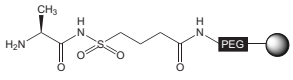


855021 **4-Sulfamylbutyryl AM resin** — 1 g, 5 g, 25 g

NBC No.: 01-64-0152

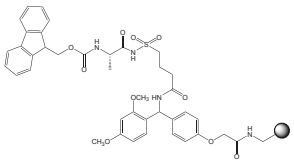
Loading: 0.60 - 1.20 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The polymer matrix is copoly (styrene-1 % DVB), 200 -400 mesh.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

	Product No.	Product	Quantity	Price
	855044	**4-Sulfamylbutyryl NovaSyn® TG resin** NBC No.: 01-64-0458 Loading: 0.15 - 0.30 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The base resin is PEG-PS-copolymer (90 µm), functionalized with an amino group. ⚠ Prolonged storage: +2 to +8°C; keep cool and dry.	1 g 5 g	
	855147	**4-Sulfamylbutyryl Rink Amide AM resin** CAS No.: 878408-63-0; $C_{29}H_{31}NO_8S$; M.W.: 553.6 Loading: 0.60 - 1.20 mmole/g resin; as determined by elemental analysis of sulfur. ⚠ Prolonged storage: +2 to +8°C; keep cool and dry. TFA cleavage, following activation of the linker by methylation, liberates a peptidyl N-methyl-sulfonamide which can be used directly in native chemical ligation reagents without the need for prior conversion to the thioester [1]. ⓘ [1] F. Burlina, et al. (2012) *Chem. Commun.*, 2579.	1 g 5 g	
	851213 NEW	**Fmoc-Ala-sulfamylbutyryl linker** TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%. HPLC: purity: ≥ 97.00%. Optical purity: ≥ 99.50% L-enantiomer. ⚠ Prolonged storage: +2 to +8°C; keep cool and dry. ⓘ A 3, 4	1 g	
	851214 NEW	**Fmoc-Gly-sulfamylbutyryl linker** TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%. HPLC: purity: ≥ 97.00%. Optical purity: ≥ 99.50% L-enantiomer. ⚠ Prolonged storage: +2 to +8°C; keep cool and dry. ⓘ A 3, 4	1 g	
	851215 NEW	**Fmoc-Ser(tBu)-sulfamylbutyryl linker** TLC: $CHCl_3$:MeOH:AcOH 32% (15:4:1), purity: ≥ 98.00%. HPLC: purity: ≥ 97.00%. Optical purity: ≥ 99.50% L-enantiomer. ⓘ A 3, 4	1 g	
	856069	**H-Ala-Sulfamylbutyryl NovaSyn® TG resin** NBC No.: 04-12-3715 Loading: 0.18 - 0.25 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The base resin is amino PEG-PS-polymer (90 µm). ⚠ Prolonged storage: +2 to +8°C; keep cool and dry.	1 g 5 g	

Bulk quantities available, please enquire

Product No.	Product	Quantity	Price



856191

Fmoc-Ala-4-Sulfamylbutyryl Rink Amide AM resin

1 g
5 g

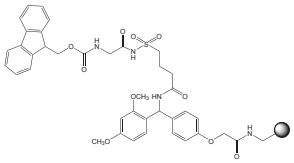
Loading: 0.20 - 0.60 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF.

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

TFA cleavage, following activation of the linker by methylation, liberates a peptidyl N-methyl-sulfonamide which can be used directly in native chemical ligation reagents without the need for prior conversion to the thioester [1].

ⓘ G 2.22

[1] F. Burlina, et al. (2012) *Chem. Commun.*, 2579.



856192

Fmoc-Gly-4-Sulfamylbutyryl Rink Amide AM resin

1 g
5 g

Loading: 0.20 - 0.60 mmole/g resin; by photometric determination of the Fmoc-chromophore liberated upon treatment with DBU/DMF.

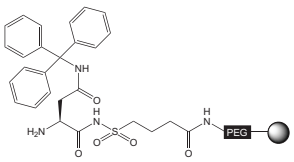
⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

TFA cleavage, following activation of the linker by methylation, liberates a peptidyl N-methyl-sulfonamide which can be used directly in native chemical ligation reagents without the need for prior conversion to the thioester [1].

⚠ Prolonged storage: keep cool and dry.

ⓘ A 3, 4

[1] F. Burlina, et al. (2012) *Chem. Commun.*, 2579.



856078

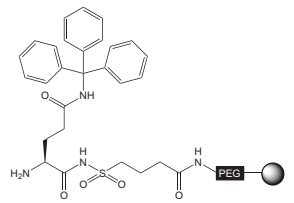
H-Asn(Trt)-Sulfamylbutyryl NovaSyn® TG resin

1 g
5 g

NBC No.: 04-12-3730

Loading: 0.15 - 0.25 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The base resin is amino PEG-PS-polymer (90 µm).

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.



856070

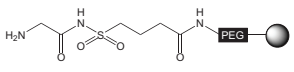
H-Gln(Trt)-Sulfamylbutyryl NovaSyn® TG resin

1 g
5 g

NBC No.: 04-12-3717

Loading: 0.15 - 0.30 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The base resin is amino PEG-PS-polymer (90 µm).

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.



856068

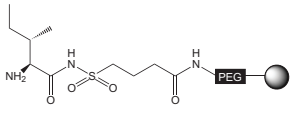
H-Gly-Sulfamylbutyryl NovaSyn® TG resin

1 g
5 g

NBC No.: 04-12-3714

Loading: 0.18 - 0.25 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The base resin is amino PEG-PS-polymer (90 µm).

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.



856076

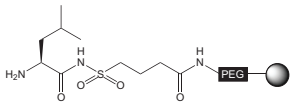
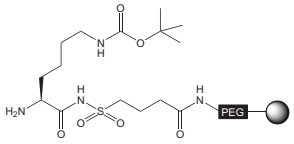
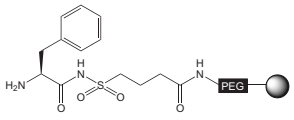
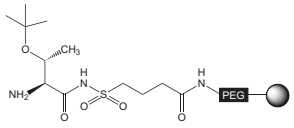
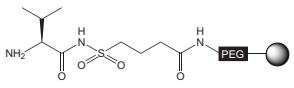
H-Ile-Sulfamylbutyryl NovaSyn® TG resin

1 g
5 g

NBC No.: 04-12-3727

Loading: 0.20 - 0.30 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The base resin is amino PEG-PS-polymer (90 µm).

⚠ Prolonged storage: +2 to +8°C; keep cool and dry.

	Product No.	Product	Quantity	Price
	856077	**H-Leu-Sulfamylbutyryl NovaSyn® TG resin** NBC No.: 04-12-3728 Loading: 0.15 - 0.25 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The base resin is amino PEG-PS-polymer (90 µm). ⚠ Prolonged storage: +2 to +8°C; keep cool and dry.	1 g 5 g	
	856074	**H-Lys(Boc)-Sulfamylbutyryl NovaSyn® TG resin** NBC No.: 04-12-3724 Loading: 0.15 - 0.30 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The base resin is amino PEG-PS-polymer (90 µm). ⚠ Prolonged storage: +2 to +8°C; keep cool and dry.	1 g 5 g	
	856079	**H-Phe-Sulfamylbutyryl NovaSyn® TG resin** NBC No.: 04-12-3731 Loading: 0.18 - 0.25 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. Base resin is amino PEG-PS-polymer (90µm). ⚠ Prolonged storage: +2 to +8°C; keep cool and dry.	1 g 5 g	
	856080	**H-Thr(tBu)-Sulfamylbutyryl NovaSyn® TG resin** NBC No.: 04-12-3732 Loading: 0.20 - 0.30 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. Base resin is amino PEG-PS-polymer (90µm). ⚠ Prolonged storage: +2 to +8°C; keep cool and dry.	1 g 5 g	
	856075	**H-Val-Sulfamylbutyryl NovaSyn® TG resin** NBC No.: 04-12-3726 Loading: 0.15 - 0.25 mmole/g resin; as determined from the substitution of the Fmoc-Leu loaded resin. The base resin is amino PEG-PS-polymer (90 µm). ⚠ Prolonged storage: +2 to +8°C; keep cool and dry.	1 g 5 g	

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