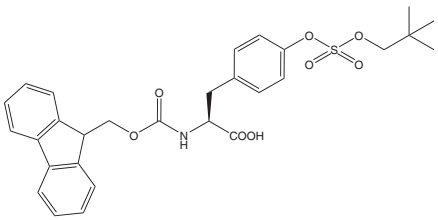
innovations 2 Vol. 12

Novabiochem®
NEW • Approach to sulfoTyr peptides

- Compatible with synthesis standard protocols
- Sulfate protected from hydrolysis during TFA cleavage reaction
- Sulfate unmasked post-cleavage using mild conditions

NEW • Derivative for Fmoc SPPS of sulfotyrosine peptides

Fmoc-Tyr(SO_3nP)-OH



It is believed that up to 1% of all protein tyrosine residues in eukaryotes may be sulfated [1]. However, the biological role of tyrosine sulfation is poorly understood compared to that of other post-translational modifications like phosphorylation. Sulfation is thought to be involved in the modulation of the extracellular protein-protein interactions of secreted and transmembrane proteins. It is also an essential requirement for maintaining the biological activity of a number of peptide hormones such as gastrin II, cholecystokinin, and caerulein.

One of the principal hurdles to studying tyrosine sulfation is the difficulty in obtaining site-specifically sulfated peptides for use as bioloigical probes or antigens for raising antibodies, as until recently the chemical synthesis of sulfotyrosine-containing peptides has been far from routine. This is because tyrosine sulfate esters are rapidly degraded in acid and fragment during mass spectrometry, making their synthesis and characterisation highly problematic. The issue of desulfation has been addressed by employing low temperature TFA cleavage [2] or introducing the sulfate group post cleavage [reviewed in 1]. However, the latter is technically difficult and not compatible with all amino acid side chains and the former is only partially effective. Furthermore, both approaches need careful attention to experimental conditions and are not amenable to high-throughput synthesis.

Recently, a number of research groups have found that protecting the sulfate stabilizes it during the TFA cleavage, enabling standard reaction conditions to be used without significant loss of the sulfate. The use of three protecting groups have been examined in detail: trichloroethyl (TCE) [3, 4], dichlorovinyl (DCV) [4] and neopentyl (nP) [5, 6]. Of these nP protection appears to offer particular promise as the group is stable to piperidine and TFA, but can be removed post-cleavage with either sodium azide or ammonium acetate [6]. In this Innovation, we investigate the utility of nP protection through the application of Fmoc-Tyr(SO_3nP)-OH in the synthesis of a sulfotyrosine-containing peptides.

Fmoc-Tyr(SO_3nP)-OH

Features

- Fmoc-Tyr(SO_3nP)-OH can be introduced using standard Fmoc SPPS coupling methods such as PyBOP, HBTU, or DIPCDI/HOBt
- nP group is stable to TFA, so standard TFA-mediated cleavage reaction affords a partially protected peptide in which the tyrosine sulfo functionality is blocked as a nP ester
- Removal of nP group can be achieved by treatment with either sodium azide in DMSO or 2 M ammonium acetate
- Purification of deprotected peptide is best carried out by RP-HPLC using 0.1 M ammonium acetate-based buffers.

Synthesis using Fmoc-Tyr(SO_3nP)-OH

Assembly

In contrast to the traditional derivatives for introduction of sulfoTyr, such as Fmoc-Tyr(SO_3·$NnBu_4$)-OH and Fmoc-Tyr(SO_3Na)-OH, which require the use of HBTU/DIPEA activation for their introduction, the fully side-chain protected derivative, Fmoc-Tyr(SO_3nP)-OH, can be coupled using any standard coupling method. Furthermore, Fmoc-Tyr(SO_3nP)-OH also has excellent solubility in DMF or NMP, faciliting its use in automated synthesizers without modification of existing protocols.

Cleavage

Whilst peptides containing Tyr(SO_3nP) can be cleaved from the resin with TFA-based cleavage cocktails, extended cleavage times should be avoided as some loss of the nP group can occur during prolonged contact with TFA. Ideally, the progress of the cleavage reaction should be followed by sampling of the reaction and analysis by HPLC. Cleavage times of 1.5 hours generally result in minimal loss of the nP group. Peptides containing Tyr(SO_3nP) are more strongly retained on RP-HPLC columns than the corresponding Tyr and Tyr(SO_3^-) peptides. They can also be detected by positive mode ES-MS and MALDI-MS.

Deprotection of SO_3nP

nP sulfate esters are stable to piperidine but are readily cleaved using small powerful nucleophiles such as azide or cyanide. For peptides the reaction is most conveniently conducted in DMSO or DMF at 50 °C. Overnight treatment is usually sufficient to effect complete reaction. Following reaction, removal of excess azide can be effected by SPE or RP-HPLC, using non-acidic buffers. For some peptides, the nP group can be removed by simply dissolving the peptide in 2 M ammonium acetate and warming the mixture at 37 °C overnight.

Purification and mass spectrometry

For RP-HPLC purification of sulfotyrosine, buffers containing ammonium acetate at pH 7 should be used, as some loss of the sulfate group may occur if standard TFA-containing buffers are used. A gradient formed from 0.1 M ammonium acetate and acetonitrile/0.1 M ammonium acetate (7:3) works well. Repeated lyophilization of the product fractions will be required to fully remove ammonium acetate.

Characterization of sulfopeptides by mass spectrometry is best performed using negative ion mode. With MALDI in positive ion mode, only the desulfated peptide is usually detected. In the case of ES ionization, the target peptide together with the desulfated peptide is generally detected.

Method 1: Cleavage of nP group

Azide method

1. Dissolve the nP protected sulfoTyr peptide in minimum volume of DMSO.
2. Add NaN_3 (10 eq.) relative to peptide. Incubate peptide at 50 °C overnight.
3. Dilute reaction mixture with three volumes of water and apply to a conditioned SPE cartridge.
4. Elute cartridge with 5 column volumes of water, and then elute peptide from the cartridge with MeCN/water 6:4 and lyophilize peptide containing eluates.

Ammonium acetate method

1. Dissolve the nP protected sulfoTyr peptide in minimum volume of 2 M ammonium acetate. MeCN may be added to aid dissolution of the peptide.
2. Incubate peptide at 37 °C overnight.
3. Apply mixture to an RP-HPLC column and elute peptide with a gradient formed between 0.1 M ammonium acetate and MeCN/0.1 M ammonium acetate (7:3).

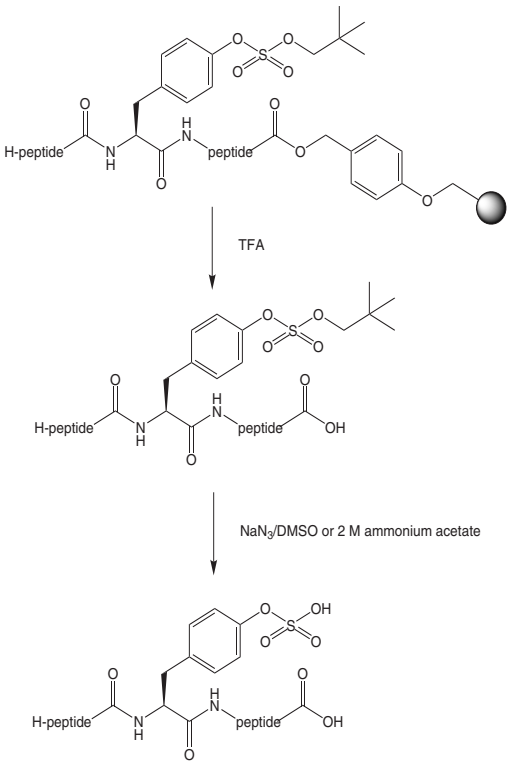


Fig. 1: Synthesis of Tyr(SO_3^-) peptides using Fmoc-Tyr(SO_3nP)-OH

Example syntheses

The utility of Fmoc-Tyr(SO_3nP)-OH was exemplified through the synthesis of two model peptides. In the case of the first example, the nP group was not removed by treatment with ammonium acetate but was easily removed with azide.

Application 1: Synthesis of H-Tyr(SO_3^-)-Glu-Phe-Lys-Lys-Ala-NH_2

H-Tyr(SO_3nP)-Glu(OtBu)-Phe-Lys(Boc)-Lys(Boc)-Ala was assembled using an ABI 433 automated synthesizer on Rink Amide MBHA resin using 10-fold excesses of Fmoc-amino acids activated with HCTU/DIPEA. A coupling time of 60 min was used throughout. Fmoc removal was effected by three 5 min treatments of 20% piperidine in DMF. The resin was treated with TFA/TIS/water(95:2.5:2.5) for 1.5 h and the purity of the isolated product checked by RP-HPLC (Figure 2).

A small sample of H-Tyr(SO_3nP)-Glu-Phe-Lys-Lys-Ala-NH_2 was dissolved in DMSO containing 10 eq. NaN_3. The mixture was heated overnight at 50 °C. HPLC analysis (Figure 3) revealed the removal of the nP group to be almost complete. The reaction mixture was applied to a RP-HPLC column and eluted with a gradient of 0.1 M ammonium acetate/acetonitrile, to afford the desired peptide, ES-MS ($M+H^+$)found 978.6; expected 978.4.

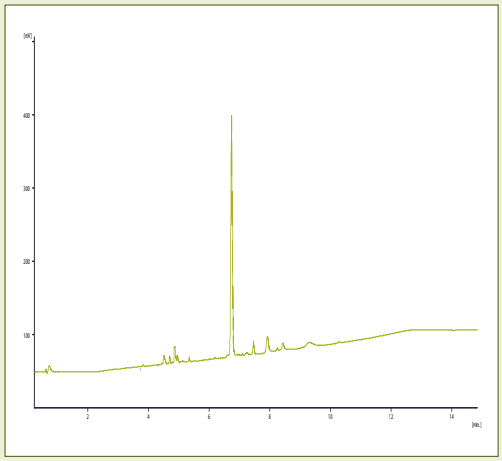


Fig. 2: HPLC profile of crude H-Tyr(SO_3nP)-Glu-Phe-Lys-Lys-Ala-NH_2.

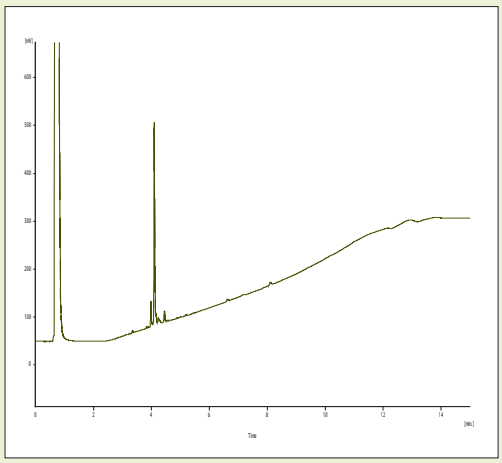


Fig. 3: HPLC profile of crude H-Tyr(SO_3^-)-Glu-Phe-Lys-Lys-Ala-NH_2.

Application 2: Synthesis of H-Tyr-Glu-Tyr(SO_3^-) Leu-Asp-Tyr-Asp-Phe-NH_2

H-Tyr(tBu)-Glu(OtBu)-Tyr(SO_3nP)-Leu-Asp(OtBu)-Tyr(tBu)-Asp(OtBu)-Phe was assembled using an ABI 433 automated synthesizer on Rink Amide SpheriTide resin using 10-fold excesses of Fmoc-amino acids activated with HCTU/DIPEA. A coupling time of 60 min was used throughout. Fmoc removal was effected by three 5 min treatments of 20% piperidine in DMF. The resin was treated with TFA/TIS/water(95:2.5:2.5) for 1.5 h and the purity of the isolated product checked by RP-HPLC (Figure 4) and characterized by ES-MS ($M+H^+$) found 1276.2; expected 1276.4.

A small sample of H-Tyr-Glu-Tyr(SO_3nP)-Leu-Asp-Tyr-Asp-Phe-NH_2 was dissolved in 2 M ammonium acetate/MeCN (2:1) The mixture was incubated overnight at 37 °C. HPLC analysis (Figure 5) revealed the removal of the nP group to be almost complete. The reaction mixture was applied to a RP-HPLC column and eluted with a gradient of 0.1 M ammonium acetate/acetonitrile, to afford the desired peptide, ES-MS ($M+Na^+$)found 1227.6; expected 1227.4.

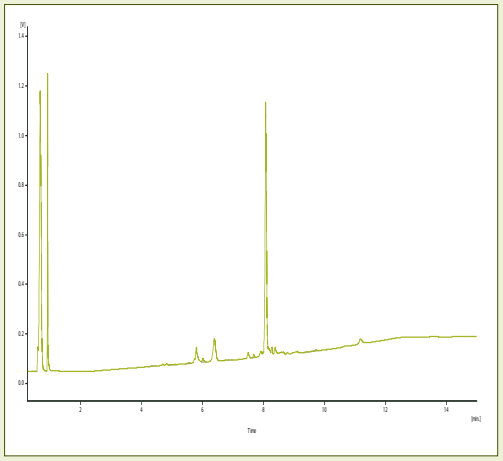


Fig. 4: HPLC profile of crude H-Tyr-Glu-Tyr(SO_3nP)-Leu-Asp-Tyr-Asp-Phe-NH_2.

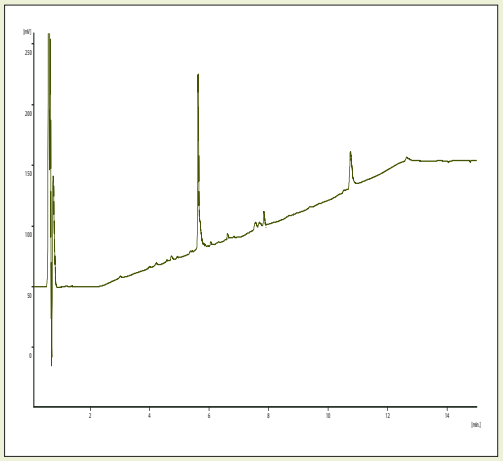


Fig. 5: HPLC profile of crude Fig. 4: HPLC profile of crude H-Tyr-Glu-Tyr(SO_3^-)-Leu-Asp-Tyr-Asp-Phe-NH_2.

Ordering Information

Cat.No.	Product	Contents	Price EUR
852347	Fmoc-Tyr(SO_3nP)-OH	1 g	185.00
NEW		5 g	750.00
	Other sulfotyrosine derivatives		
852103	Fmoc-Tyr(SO_3·NnBu_4)-OH	1 g	170.00
		5 g	680.00

References

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