

**6-Nitro-1- β -Naphthalenesulfonyloxybenzotriazole : A Novel
Coupling Reagent For Peptide Synthesis⁺**

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Abstract : Synthesis of 6-nitro-1- β -naphthalenesulfonyloxybenzotriazole (N-NSBt) and its application as a peptide coupling reagent is being reported. It has been found to be suitable for rapid and quantitative synthesis of optically pure peptides in a stepwise manner.

The use of 1- β -naphthalenesulfonyloxybenzotriazole (NSBt) as an efficient coupling reagent for synthesis of biologically active peptides has been demonstrated in a number of cases¹⁻⁷. Since the reaction time for a peptide coupling with this reagent ranges between 10-12 hrs, an attempt was made to get a more powerful coupling reagent by introducing a strong electron withdrawing group such as nitro, at position 6 of HOBt. The β -naphthalenesulfonate of 6-nitro-1-hydroxybenzotriazole, hereafter called N-NSBt, (Fig. 1) was expected to yield activated esters of N^o-protected amino acids and peptides with greatly enhanced reactivity due to the presence of nitro substituent. We have found that N-NSBt is a highly potent coupling reagent for rapid synthesis of peptides in near quantitative yields whereby reactions are complete within just 2-3 hrs. In this communication, synthesis of this useful reagent and the methodology for getting optically pure peptides with its help are reported along with racemisation studies using ¹H NMR and HPLC techniques.

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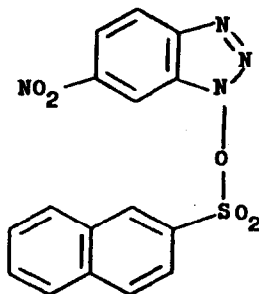


Fig.1. Structure of N-NSBt

For racemisation studies, Ac-Phe-Ala-OMe and Z-Phe-Ala-OMe were used as model peptides. Ac-Phe and Z-Phe were coupled with Ala-OMe using N-NSBt and the crude products obtained after workup were used as such for analysis. Optically pure Ac-Phe-Ala-OMe and Ac-D-Phe-Ala-OMe were synthesized using DCC/HOBt⁸ procedure. On HPLC, Ac-Phe-Ala-OMe obtained by N-NSBt method showed two peaks of nearly equal intensities. Their assignment was done by coinjecting crude Ac-Phe-Ala-OMe obtained with the help of N-NSBt alongwith optically pure Ac-Phe-Ala-OMe and Ac-D-Phe-Ala-OMe. These studies clearly indicated that racemisation did occur during the coupling of Ac-Phe with Ala-OMe in presence of N-NSBt. Moreover, the fraction of D-L racemate was found to be 50% as calculated by ¹H NMR using the method of Weinstein *et al*⁹. This led us to the obvious conclusion that coupling reagent N-NSBt was not suitable for fragment condensation as it caused nearly total racemisation when an acyl type protecting group was used.

Further, the application of N-NSBt to the synthesis of Z-Phe-Ala-OMe having a urethane type protecting group was examined. HPLC and 400 MHz ¹H NMR spectrum of crude Z-Phe-Ala-OMe, both did not exhibit any peak due to the D-L isomer. Since the time required for peptide bond formation with the aid of N-NSBt is merely 2-3 hrs, it is evident that this reagent can be advantageously used for the rapid assembly of larger peptides in a stepwise manner without any detectable racemisation. Several optically pure di- and tripeptides with urethane type protecting groups have been synthesized using N-NSBt in near quantitative yields within 2-3 hrs¹⁰ (Table 1). Like NSBt, it is particularly useful for the synthesis of Gln and Asn peptides.

Thus, introduction of nitro group in NSBt resulted in a powerful peptide coupling reagent which drastically cuts down the reaction time for peptide bond formation and affords optically pure products in excellent yields when stepwise procedure for peptide chain elongation is employed using urethane type protecting groups.

Table 1 - Synthesis of Peptides with N-NSBt

Acid Component	Amino Component	Peptide	Yield (%)
Z-Phe	32% NH ₄ OH ^a	Z-Phe-NH ₂	80
Z-Leu	Gly-NH ₂ ^b	Z-Leu-Gly-NH ₂	87
Z-Gln	Asn-ONBzl ^b	Z-Gln-Asn-ONBzl	76
Boc-Ile.DCHA	Gln-Asn-ONBzl ^c	Boc-Ile-Gln-Asn-ONBzl	80
Boc-Lys(Z).DCHA	Lys(Z)-OBzl	Boc-Lys(Z)-Lys(Z)-OBzl	82
Z-Phe	Phe-OMe ^c	Z-Phe-Phe-OMe	85
Z-Phe	Tyr-OMe ^c	Z-Phe-Tyr-OMe	87
Z-Cys(Trit)DEA	Tyr(Bu ^t)-OBu ^t ^d	Z-Cys(Trit)-Tyr(Bu ^t)-OBu ^t	93

Reactions were carried out in a) THF; b) DMF; c) DMF/EtOAc and d) DMF/CH₂Cl₂

6-Nitro-1-β-naphthalenesulfonyloxybenzotriazole :

A solution of 6-nitro-1-hydroxybenzotriazole (10 g, 55 mmol) in 0.55 N NaOH (100 ml, 55 mmol) was added dropwise to a solution of β-naphthalenesulfonyl chloride (11 g, 48.5 mmol) in acetone (100 ml) at 0°. After 1 hr, the reaction mixture was diluted with water and the product filtered. It was washed thoroughly with water, dried and crystallised from acetone-hexane. Yield 14 g (76%), mp 153-54°, ¹H NMR (CDCl₃) : 8.46 (m, 2H, ArH), 8.15 (m, 2H, ArH) and 8.15-7.66 (m, 6H, ArH).

Ac-Phe-Ala-OMe using N-NSBt :

A solution of Ac-Phe (0.2 g, 0.96 mmol) in dry DMF (2 ml) is treated with NMM (0.206 ml, 1.92 mmol) and mixed with Ala-OMe.HCl (0.14 g,

0.96 mmol) under stirring. N-NSBt (0.33 g, 0.96 mmol) is added to the stirred mixture after 10 min and the reaction continued for 2 hrs at room temperature. After the removal of solvent under reduced pressure, the residue is taken up in EtOAc and organic layer washed successively with 5% NaHCO₃, 1N HCl and finally with water. It was dried over Na₂SO₄ and evaporated to dryness in vacuo.

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Reference and Notes

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