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Use of 1-β-Naphthalenesulfonyloxybenzotriazole As Coupling Reagent in Solid Phase Peptide Synthesis[+]

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Abstract : Application of 1-β-naphthalenesulfonyloxybenzotriazole (NSBt) as an efficient coupling reagent in solid phase is reported. It has been found to be suitable for the rapid and quantitative coupling of various amino acid derivatives.

Among the several coupling reagents used in solid phase peptide synthesis, DCC[1] and BOP[2] have been widely used for peptide bond formation. However, these reagents are not without shortcomings, side reactions such as intramolecular arrangement of acylisourea during DCC activation[3] and formation of carcinogenic byproduct during BOP activation[4] are well known. In this paper details on the use of 1-β-naphthalenesulfonyloxybenzotriazole[5] (NSBt) as coupling reagent in solid phase procedure is being reported. The reagent is a stable, non-hygroscopic solid and has been successfully employed for the synthesis of a variety of biologically active peptides by solution phase procedure[6-10].

In the first instance, coupling properties of NSBt has been evaluated by its capacity to couple Boc-Leu with Gly-Merrifield resin. The rate of reaction was compared with coupling reagents BOP and DCC/HOBt and described in terms of uncoupled amine (Table 1). The coupling efficiency was monitored by quantitative ninhydrin test[11]. As is evident, coupling efficiency of NSBt was found to be superior to DCC/HOBt and comparable with BOP. This experiment was followed by the synthesis of a test peptide Leu-Ala-Gly-Val on Boc-Val-PAM resin (0.35 mmol)[12]. Amino acid analysis indicates 0.438 mmol of peptide/g of substituted resin. Amino acid composition was found to be Leu 1.05, Ala 1.09, Gly 0.96 and Val 1.05. Cleavage of the resin with TFMSA released tetrapeptide in 78% yield. Thus, the tetrapeptide can be prepared in high purity using NSBt as coupling reagent.

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Table 1 - Uncoupled amine

Min	NSBt[a]	BOP	DCC/HOBt
10	8.1%	8.0%	13.7%
20	4.4%	3.9%	8.0%
30	3.1%	3.0%	4.9%
40	2.6%	2.5%	4.0%
50	1.2%	1.4%	2.9%
60	0.7%	0.63%	1.6%

[a]Reaction conditions: 0.8 meq Gly-® 3 equiv Boc-Leu in DMF, 3 equiv NSBt and 3 equiv DIEA.

In another set of experiments Boc-Asn-OH and Boc-Gln-OH which are known to give poor yield during DCC activation, was activated with NSBt and coupled with Ala-Gly-Val-PAM resin. As is evident, coupling yield of higher order was obtained with NSBT than DCC/HOBt.

Boc-Asn-OH + Ala-Gly-Val-PAM-® $\xrightarrow[2\,hr]{}$ Boc-Asn-Ala-Gly-Val-PAM-®

Reagent	Coupling yield
NSBt	74%
BOP	71%
DCC/HOBt	52%

Boc-Gln-OH + Ala-Gly-Val-PAM-® $\xrightarrow[2\,hr]{}$ Boc-Gln-Ala-Gly-Val-PAM-®

Reagent	Coupling yield
NSBt	96%
BOP	95%
DCC/HOBt	70%

In order to further evaluate the applicability of the NSBt reagent for other types of difficult peptide bond formation, we evaluated the yield of coupling observed after the incorporation of the sterically hindered residue Boc-Ile which is often associated with a poor coupling rate. Boc-Ile was incorporated in a model peptide more readily with NSBt than by the DCC procedure. Thus, it is clearly evident that rate of coupling with NSBt although comparable with BOP is superior to DCC/HOBt and can be successfully employed in solid phase procedure for the stepwise synthesis of peptides.

Boc-Ile-OH + Leu-Ala-Gly-Val-PAM-® $\xrightarrow[2\ hr]{}$ Boc-Ile-Ala-Gly-Val-PAM-®

Reagent	Coupling yield
NSBt	95%
BOP	96%
DCC/HOBt	91%

Racemisation studies were carried out by synthesizing Z-Gly-Phe-Val-OMe[13] in a stepwise manner on Merrifield's resin through NSBt activation. It was then analyzed by RP HPLC[14] for any enantiomeric impurity. It was found that <0.2% formation of Z-Gly-D-Phe-Val-OMe had occurred.

Our studies thus indicate that NSBt is an efficient coupling reagent for solid phase peptide synthesis. It can be successfully used with hindered amino acids as well as with Asn and Gln residues. The ease of preparation of NSBt, formation of harmless by product and shelf stability makes it a useful coupling reagent that can be used for the synthesis of peptides both by solid phase and solution phase.

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12. Boc-Val-PAM resin was placed in a reaction vessel on a shaker and treated as follows for the incorporation of each residue: (1) DCM (3x8 ml), (2) TFA:DCM (1:1) (10 ml) for 30 min, (3) DCM (3x8 ml.), (4) 5% DIEA/DCM (3x8 ml) for 10 min, (5) DCM (5x8 ml), (6) DMF (1x8 ml), (7) 3 equi Boc-Gly in DMF (8 ml), 3 equi NSBt, 3 equi DIEA for 2 hr, (8) DMF (2x8 ml), (9) DCM (3x8 ml). At each step the extent of coupling was monitored by ninhydrin test and then second coupling was performed. The coupling yields were: Boc-Gly, 1st coupling 99.2%, 2nd coupling 100%; Boc-Ala 1st coupling 98.8%, 2nd coupling 99.6%; Boc-Leu, 1st coupling 99.1%, 2nd coupling 99.7%.

13. Boc-Val-Merrifield's resin was placed in a reaction vessel and treated successively with Boc-Phe and Z-Gly using NSBt activation in a manner as described in ref. 12. Z-Gly-Phe-Val-® so obtained, was then treated with methanol in the presence of triethylamine to get the protected peptide Z-Gly-Phe-Val-OMe.

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