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3-NITRO-2-PYRIDINESULFENYL GROUP FOR PROTECTION AND ACTIVATION OF THE THIOL FUNCTION OF CYSTEINE

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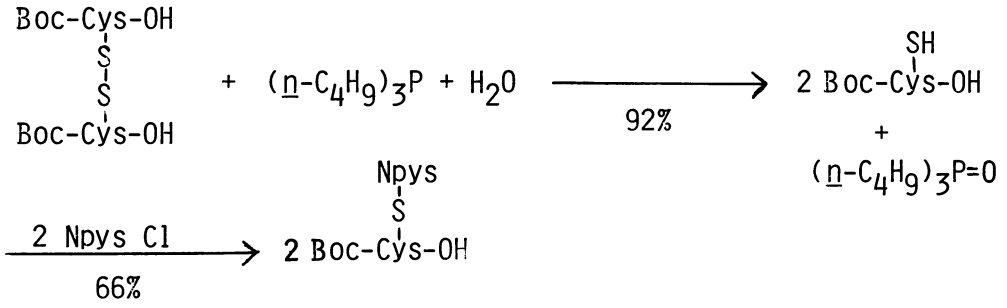
The use of the 3-nitro-2-pyridinesulfenyl (Npys) halide, a versatile reagent in peptide chemistry, for the protection and activation of the thiol function is reported. Boc-Cys(Npys)-OH is prepared starting from bis(N-t-butyloxycarbonyl)-L-cystine. The Npys group is easily removed by treatment with a stoichiometric amount of tri-n-butylphosphine in the presence of water, but it is resistant to acids such as trifluoroacetic acid, HCl, and HF. Importantly, the cysteine residue modified with the Npys group can react selectively with free thiol of cysteine to afford a cystine disulfide bond.

We wish to report a convenient method for the preparation of N-t-butyloxycarbonyl-S-(3-nitro-2-pyridinesulfenyl)-L-cysteine. The 3-nitro-2-pyridinesulfenyl (Npys) group not only acts to protect the thiol function of cysteine, but also the Npys-modified cysteine can react selectively with the free thiol group of another cysteine molecule to afford a new disulfide bond. The Npys group is easily removed under neutral conditions using tertiary phosphine and water, but it is sufficiently resistant to acids such as trifluoroacetic acid, HCl, and HF.

Recently the synthesis of 3-nitro-2-pyridinesulfenyl halides has been reported and these compounds were found to be unusually stable nitrogen-containing heterocyclic sulfenyl halides.[1] One of the authors showed previously that Npys halides react easily with amino, hydroxyl and thiol functions of amino acids to form sulfenamides, sulfenates, and mixed disulfide, respectively and the Npys group can serve for both protection and activation in peptide synthesis.[2]

N-(t-butyloxycarbonyl)-S-(3-nitro-2-pyridinesulfenyl)-L-cysteine (Boc-Cys(Npys)-OH) is easily prepared starting from bis(N-t-butyloxycarbonyl)-L-cystine ((Boc-Cys(S-)-OH)$_2$) according to the following scheme.



In a typical experiment, to a solution of $(\text{Boc-Cys(S-)-OH})_2$ (4.4 g, 10 mmol) in 80 ml of acetone and 20 ml of water, tri-n-butylphosphine[3] (2.02 g, 10 mmol) was added at room temperature with stirring, and the mixture was stirred for 4 hr. The acetone was removed in vacuo and 300 ml of ethyl acetate was added to the residue, followed by successive washing with 5% citric acid and water. After the solution was concentrated in vacuo, the dicyclohexylamine (DCHA) salt of N-t-butyloxycarbonyl-L-cysteine (Boc-Cys(SH)-OH)[6] was precipitated upon addition of DCHA (5.43 g, 30 mmol) and petroleum ether at 0°C: yield, 7.35 g (91.5%), mp 154-6°C, $[\alpha]_D^{22}$+19.3° (c1, methanol). Anal. Found: C, 59.66; H, 9.52; N, 6.96; S, 7.96%. Calcd for $C_{20}H_{38}N_2O_4S$: C, 59.78; H, 9.41; N, 6.78; S, 7.91%. This salt (2.01 g, 5 mmol) was suspended in 200 ml of CH_2Cl_2 and converted into the free form by washing with 5% citric acid, and the solution was dried over sodium sulfate. To the resulting solution, Npys chloride (1.04 g, 5.5 mmol) was added in 4 equal portions while triethylamine (1.11 g, 11 mmol) in 30 ml of CH_2Cl_2 was added dropwise with stirring at 0°C. The reaction mixture was stirred for additional 2 hr at room temperature. The solvent was removed in vacuo, and the residue was dissolved in 150 ml of acetone. The DCHA salt of Boc-Cys(Npys)-OH was precipitated upon addition of 1 ml of DCHA at 0°C. The salt was dissolved in methanol and a small amount of insoluble material was removed by filtration and the solvent was evaporated. The residue was crystallized from ethyl acetate-petroleum ether: yield, 1.84 g (66.3%), mp 150 - 2°C, $[\alpha]_D^{22}$-86.5° (c1, methanol).[2] This salt was suspended in ethyl acetate and converted into the free form by washing with 5% citric acid. Boc-Cys(Npys)-OH was obtained in quantitative yield by addition of petroleum ether after the solution was concentrated in vacuo: mp 153 - 5°C (dec), $[\alpha]_D^{22}$-88.7° (c1, methanol). Anal. Found: C, 41.44; H, 4.62; N, 11.05; S, 17.11%. Calcd for $C_{13}H_{17}N_3O_6S_2$: C, 41.59; H, 4.57; N, 11.20; S, 17.08%.

The S-Npys group is stable toward acids such as trifluoroacetic acid, HCl and HF: no decomposition was observed, as checked by thin layer chromatography on silica gel (n-butanol : acetic acid : H_2O (4 : 1 : 1)), after H-Cys(Npys)-OH was kept at room temperature in trifluoroacetic acid (24 hr), 4M HCl/dioxane (24 hr), or HF (1 hr). However, it is cleaved under neutral conditions using a stoichiometric amount of tri-n-butylphosphine in the presence of water at room temperature or with an excess of 2-mercaptoethanol.

Most important is the fact that cysteine modified with the Npys group can

react selectively with the thiol of cysteine to afford a cystine disulfide bond. Quantitative reaction took place as determined by thin layer chromatography on silica gel ($CHCl_3$: methanol : acetic acid (30 : 1 : 1)) when 1 mmol each of Boc-Cys(Npys)-OH and Boc-Cys(SH)-OH were mixed in 50 ml each of acetone and 0.1 M phosphate buffer (pH 8) for 2 hr and (Boc-Cys(S-)-OH)$_2$ was obtained in 92% yield by acidification to pH 3 and gel filtration on Sephadex LH-20 in methanol. This experimental approach was successfully applied to the modification of papain as shown in Figure 1.

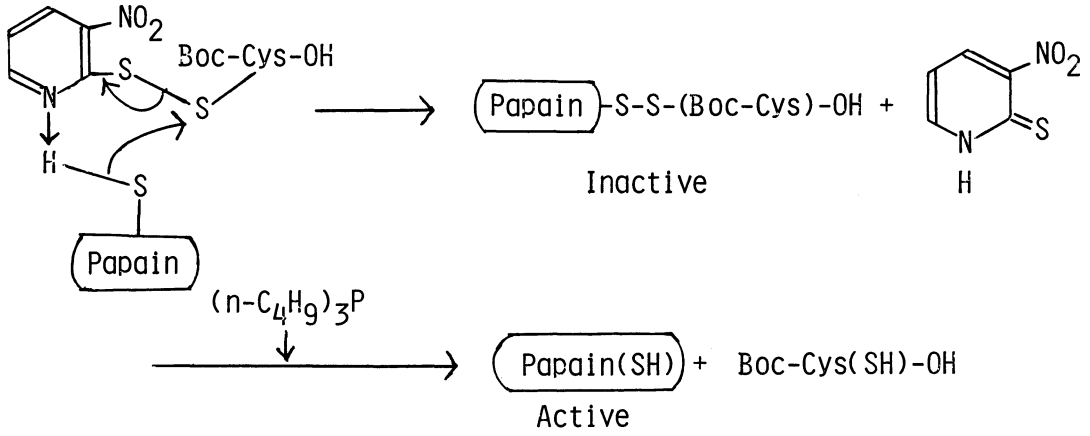


Figure 1. Modification of Papain with Boc-Cys(Npys)-OH

The papain (Worthington, 2 x crystallized) which had been purified by affinity chromatography on a column of agarose-Gly-Gly-Tyr(Bzl)-Arg[7] was incubated with Boc-Cys(Npys)-OH (6.18×10^{-6}M) in 0.5 M Tris buffer (pH 7.5) containing 0.005 M ethylenediaminetetraacetic acid at 25°C. The thiol group in the active site of the enzyme was modified within 10 seconds during which it completely lost enzymic activity.[8] The content of free thiol in the enzyme was easily determined from the amount of 3-nitro-2-pyridinethiol produced by measuring the absorption at 310.4 nm (ε 4,900).[9] The modified enzyme[10] was separated from the reagents by gel filtration on Sephadex G-25. Full enzymic activity was recovered by addition of a stoichiometric amount of tri-n-butylphosphine based on the amount of liberated 3-nitro-2-pyridinethiol.

In conclusion, the 3-nitro-2-pyridinesulfenyl group can offer new options and flexibility in the choice of a protecting group for the thiol function and provide a unique concept of protection and activation in peptide chemistry.

Acknowledgements

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References and Notes

1) R. Matsueda and K. Aiba, Chem. Lett., 1978, 951.

2) R. Matsueda, D. Theodoropoulos, and R. Walter, Peptides, Proceedings of the Sixth American Peptide Symposium (June, 17 - 22, 1979, E. Gross and J. Meienhofer, eds.), pp. 305, Pierce Chemical Co., Rockford (1979). R. Matsueda and R. Walter, Int. J. Peptide Protein Res. 16, 392 (1980).

3) As to the reduction of (Boc-Cys(S-)-OH)$_2$ to Boc-Cys(SH)-OH, Smith et al.[4] have used excess amount of 1,4-butanedithiol. However the process can also be achieved by hydrolysis of the phosphonium salt which is produced by the reaction of disulfide with a stoichiometric amount of tertiary phosphine.[5]

4) C.W. Smith, M.F. Ferger, and W.Y. Chan, J. Med. Chem., 18, 822 (1975).

5) R. Matsueda, H. Maruyama, M. Ueki, and T. Mukaiyama, Bull. Chem. Soc. Japan, 44, 1373 (1971). J.H. Seely, U. Ruegg, and J. Rudinger, Peptides 1972 (H. Hanson and H.D. Jakubke, eds.), pp. 86, North-Holland Publishing Co., Amsterdam (1972).

6) Without isolation of Boc-Cys(SH)-OH, the DCHA salt of Boc-Cys(Npys)-OH was also prepared in 62% yield from (Boc-Cys(S-)-OH)$_2$ by a "one pot" reaction: Npys chloride (1.3 eq) in dioxane was added to the solution containing Boc-Cys(SH)-OH, while 5% $NaHCO_3$ was added to keep the pH of 6 - 7. Boc-Cys(Npys)-OH was extracted with ethyl acetate at pH 4 and precipitated as the DCHA salt.

7) M.O. Funk, Y. Nakagawa, J. Skochdopole, and E.T. Kaiser, Int. J. Pep. Protein Res., 13, 296 (1979).

8) Papain activity was determined spectrophotometrically using benzoyl-DL-arginine-p-nitroanilide according to the literature procedure (R. Arnon, Methods in Enzymology, vol. 19 (G.E. Perlmann and L. Lorand, eds), pp. 226, Academic Press, New York (1970)) with the exception that cysteine was eliminated from the Tris buffer.

9) The values obtained by this method are in good agreement with those obtained by the method using 2,2'-dipyridyl disulfide by K. Brocklehurst and G. Little (Biochem. J., 133, 67 (1973)): for example, this method and the literature method gave values of 4.88×10^{-6}M and 4.82×10^{-6}M, respectively.

10) The modified enzyme is stable since it is blocked in the form of cystine disulfide bond. The native papain is also known to be isolated with Cys-25 involved in a cystine disulfide bond.[8]

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