

ACTIVATION OF CONVENTIONAL *S*-PROTECTING GROUPS OF CYSTEINE BY
CONVERSION INTO THE 3-NITRO-2-PYRIDINESULFENYL (NPYS) GROUP

Rei MATSUEDA,* Susumu HIGASHIDA, Richard J. RIDGE,[†] and Gary R. MATSUEDA[†]

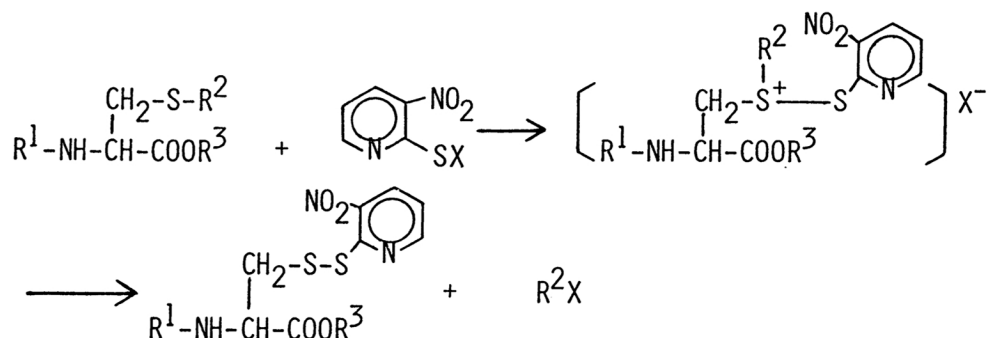
Chemical Research Laboratories, Sankyo Co., Ltd., Shinagawa-ku, Tokyo 140

[†]The Cellular and Molecular Research Laboratory, Massachusetts General Hospital, Boston, Massachusetts 02114, U.S.A.

All of the conventional *S*-protecting groups of cysteine which were tested could be selectively converted to the 3-nitro-2-pyridine-sulfenyl (Npys) group after treatment with an appropriate Npys halide. Unidirectional formation of an unsymmetrical disulfide bond is possible when Cys(Npys) is mixed with a free thiol of another Cys residue. Some of these features were exploited during the solid phase synthesis of lysine⁸-vasopressin.

In our earlier report¹⁾ *N*-*t*-butoxycarbonyl-*S*-(3-nitro-2-pyridinesulfenyl)-L-cysteine (Boc-Cys(Npys)-OH) was prepared from Boc-Cys(SH)-OH with Npys chloride and used subsequently for the reversible inhibition of papain. In this communication, we wish to report on the activation of conventional *S*-protecting groups after selective reaction with Npys halides; and also on the utilization of Cys(Npys) for the solid phase synthesis of lysine⁸-vasopressin.

Conventional *S*-protecting groups are easily converted into the Npys group by the reaction using Npys halides as shown in the following scheme.



In a typical experiment, Npys chloride (228 mg, 1.20 mmol) was added to a solution of Boc-*S*-(4-methoxybenzyl)-L-cysteine (Boc-Cys(Bzl(OMe))-OH, 341 mg, 1.00 mmol) in 200 ml of CH₂Cl₂ at 0°C and the mixture was stirred for 30 min. The CH₂Cl₂ was removed *in vacuo* and Boc-Cys(Npys)-OH (344 mg, 92% yield) was obtained by evaporation of the solvent after preparative layer chromatography²⁾ on silica gel with

$\text{CHCl}_3/\text{MeOH}$ (17:3): mp. $155\sim 158^\circ\text{C}$ (dec.), $[\alpha]_{\text{D}}^{22} -86.2^\circ$ (c 1.00, MeOH).¹⁾ Recrystallization from ethyl acetate-ether gave a product with mp $162\sim 164^\circ\text{C}$ (dec.) and $[\alpha]_{\text{D}}^{22} -86.5^\circ$ (c 1.00, MeOH). When this compound was treated with a stoichiometric amount of tri-*n*-butylphosphine in acetone-water (4:1) for 10 min, complete cleavage of *S*-Npys occurred. The dicyclohexylamine salt of the resulting Boc-Cys(SH)-OH (obtained in 85% yield) showed the expected physical properties.¹⁾ These results show that a facile conversion of Cys(Bzl(OMe)) to Cys(Npys) and the following *S*-Npys cleavage under very mild conditions proceed without detectable racemization. Various *S*-protected cysteine derivatives were also treated with Npys halides to provide the results shown in Table 1.

Table 1. Conversion of Conventional *S*-Protecting Groups into the Npys Group^{a)}

Starting Materials	Npys-X, Eq.	Conditions	Yield, %
Boc-Cys(Bzl)-OH	Cl, 1.2	R.T., 24 hr in CH_2Cl_2	No reaction
Boc-Cys(Bzl(OMe))-OH	"	0°C , 30 min in CH_2Cl_2	92
Boc-Cys(Bzl(Me) ₂)-OH	"	0°C , 30 min in CH_2Cl_2	90
Z-Cys(Bzl(OMe))-Phe-Phe-Gln-Asn-O- <i>t</i> -Bu	"	R.T., 30 min in $\text{CH}_2\text{Cl}_2/\text{CF}_3\text{CH}_2\text{OH}$ (1:1)	85 ^{b)}
Fmoc-Cys(<i>t</i> -Bu)-OH	"	0°C , 30 min in CH_2Cl_2	80 ^{c)}
Boc-Cys(Trt)-OH	"	-30°C , 3 hr in CH_2Cl_2	91
Boc-Cys(Acm)-OH	"	0°C , 30 min in AcOH	63
Z-Cys(Bzl)-OH	Br, 2.0	R.T., 10 hr in CH_2Cl_2	21 ^{d)}
Z-Cys(Bzl)-OH	Cl, 2.0	R.T., 5 hr in $\text{CF}_3\text{CH}_2\text{OH}$	61 ^{d)}
Z-Cys(Bzl)-OH	Br, 2.4	R.T., 3 hr in $\text{CF}_3\text{CH}_2\text{OH}/\text{AcOH}$ (10:1)	73 ^{d)}
Z-Cys(Bzl)-Pro-Leu-Gly-NH ₂	Br, 2.5	"	70 ^{e)}

a) All the products gave satisfactory elemental analyses and amino acid analyses in the case of peptides. Most of the results were presented at the 19th Japanese Peptide Symposium.⁵⁾

b) mp $178\sim 180^\circ\text{C}$, $[\alpha]_{\text{D}}^{22} -83.3^\circ$ (c 1.00, DMF). c) mp $120\sim 122^\circ\text{C}$, $[\alpha]_{\text{D}}^{22} -74.1^\circ$ (c 1.00, MeOH).

d) Isolated as dicyclohexylamine salt; mp $122\sim 123^\circ\text{C}$, $[\alpha]_{\text{D}}^{22} -60.6^\circ$ (c 1.00, MeOH).

e) mp $179\sim 180^\circ\text{C}$, $[\alpha]_{\text{D}}^{22} -79.2^\circ$ (c 1.00, MeOH).

Conventional protecting groups such as *t*-butyl (*t*-Bu), trityl (Trt), acetamidomethyl (Acm), 3,4-dimethylbenzyl (Bzl(Me)₂), and 4-methoxybenzyl (Bzl(OMe)) were converted into the Npys group in good yield by using Npys chloride. However, no conversion took place in the case of the *S*-benzyl (Bzl) group. The starting material was recovered intact quantitatively. This resistance of *S*-Bzl group toward the sulfonyl chloride has also been reported by Moroder *et al.*⁶⁾ in the case of *o*-nitrophenylsulfonyl chloride and by Hiskey *et al.*⁷⁾ in the case of methoxycarbonylsulfonyl chloride. However, we were able to overcome the unreactivity of Cys(Bzl) by using Npys bromide in place of Npys chloride in polar solvent such as

$\text{CF}_3\text{CH}_2\text{OH}$ or $\text{CF}_3\text{CH}_2\text{OH}/\text{AcOH}$ (10:1) in place of CH_2Cl_2 as shown in the bottom of the table. Z-Cys(Bzl)-Pro-Leu-Gly- NH_2 , which was prepared by solid phase synthesis as reported previously,⁴⁾ was converted into Z-Cys(Npys)-Pro-Leu-Gly- NH_2 in 70% yield without affecting the *N*-benzyloxycarbonyl (Z) group. Thus a selective conversion of \underline{S} -Bzl and other *S*-protecting groups into the Npys group was possible by the choice of suitable reaction conditions.

The *S*-Npys group was successfully used for side chain protection of cysteine during the solid phase synthesis of lysine⁸-vasopressin (LVP) on benzhydrylamine resin as shown in Figure 1.

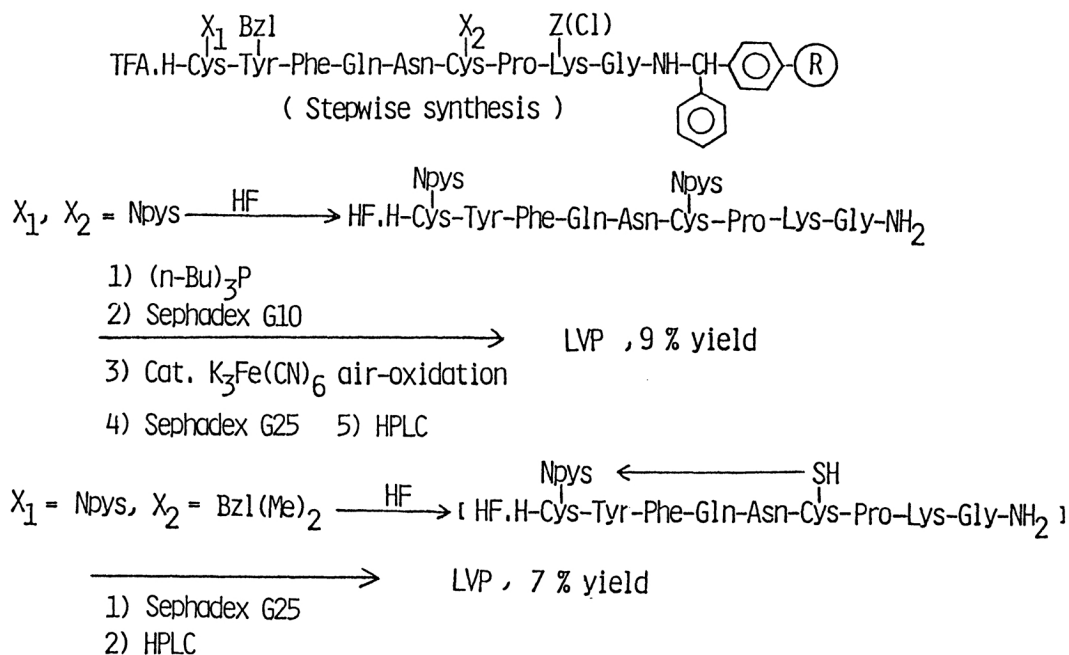


Figure 1. Solid Phase Syntheses of Lysine⁸-vasopressin using *S*-Npys Protecting Group

Starting from glycybenzhydrylamine resin containing 0.50 mmol of Gly, the peptide chain was assembled stepwise using the appropriate Boc-amino acids and dicyclohexylcarbodiimide or Boc-amino acid *p*-nitrophenyl esters (Asn, Gln). Tri-fluoroacetic acid/ CH_2Cl_2 (1:1) was employed for removal of the Boc-group throughout. The *S*-Npys group was found to be stable⁸⁾ in trifluoroacetic acid which was used for Boc-deprotection and also in HF/anisole (9:1) for 45 min at 0°C which was used for cleavage of the nonapeptide from the resin. As shown in Figure 1, removal of the *S*-protecting groups from the di-Npys peptide amide resulting from HF cleavage of peptidyl resin ($\text{X}_1, \text{X}_2 = \text{Npys}$) was accomplished by tributylphosphine in 1-propanol and water. $\text{K}_3\text{Fe(CN)}_6$ mediated⁹⁾ oxidation of the free dithiol-peptide amide; and subsequent purification by gel filtration on Sephadex G-25 in 20% AcOH; and finally reverse-phase HPLC¹⁰⁾ gave pure LVP in 9% yield,¹¹⁾ based on the starting resin (0.61 mmol NH_2 groups/g). The product had the expected amino acid analysis data and was biologically full active on adenylate cyclase activation when compared with Sigma LVP. In the case of the peptidyl resin ($\text{X}_1 = \text{Npys}$, $\text{X}_2 = \text{Bzl(Me)}_2$), Bzl(Me)₂ of Cys⁶ was deprotected by HF treatment, which spontane-

ously displaced the Npys group on Cys¹ and preferentially formed an intramolecular disulfide bond. The pure LVP was obtained in 7% yield.¹²⁾

In conclusion, the versatility of the *S*-Npys groups should offer new possibilities for synthetic strategies for cysteine protection, deprotection and selective disulfide bond formation under mild conditions. These options have been diagrammed in Figure 2.

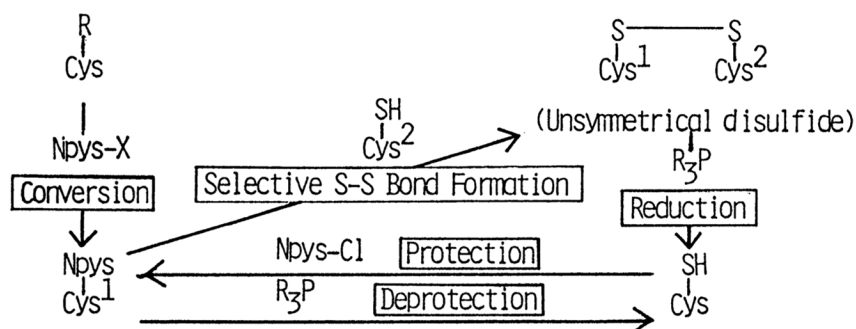


Figure 2. Features of the Npys Protecting Group

References

- 1) R. Matsueda, T. Kimura, E.T. Kaiser, and G.R. Matsueda, Chem. Lett., **1981**, 737.
- 2) The product was isolated by chromatography to check the racemization. The product was also easily isolated by the following procedure: the dicyclohexylamine salt was precipitated from acetone solution as reported previously.¹⁾ The salt was precipitated again after it was once converted into the free form, and obtained in 81% yield: mp 150~152°C, $[\alpha]_D^{22} -62.2^\circ$ (c1.00, MeOH).³⁾
- 3) The value of -86.5° which was determined by a commercial laboratory (Baron Consulting Co.) in the previous reports^{1),4)} is revised to -62.2° , since values of this product and the product of the previous reports are identical by our measurements.
- 4) R. Matsueda and R. Walter, Int. J. Peptide Protein Res., **16**, 392 (1980).
- 5) Peptide Chemistry 1981, Proceedings of the 19th Symposium on Peptide Chemistry, T. Shioiri ed., Protein Research Foundation, Osaka (1982) p.31.
- 6) L. Moroder, F. Marchiori, G. Borin, and E. Scoffone, Biopolymers, **12**, 493 (1973).
- 7) R.G. Hiskey, N. Muthukumaraswamy, and R.R. Vunnam, J. Org. Chem., **40**, 950 (1975).
- 8) Boc-Cys(Npys)-OH is also stable to 5% NaHCO₃/MeOH (1:1) at room temperature but it is hydrolyzed in 1 M NaOH/acetone (1:1) to afford 3-nitro-2-pyridine-thiol and di-Boc-cystine.
- 9) D. Yamashiro, R.L. Noble, and C.H. Li, J. Org. Chem., **38**, 3561 (1973).
- 10) H.P.J. Bennett, C.A. Browne, D. Goltzman, and S. Solomon, Peptides, Proceedings of the Sixth American Peptide Symposium (E. Gross and J. Meienhofer, eds), Pierce Chemical Co., Rockford, Illinois (1979) p. 121.
- 11) In a control experiment ($X_1, X_2 = \text{Bzl}(\text{Me})_2$), LVP was obtained in a similar yield by the HF treatment, the subsequent oxidation and purification.
- 12) R.J. Ridge, G.R. Matsueda, E. Haber, and R. Matsueda, Int. J. Peptide Protein Res., submitted for publication.

(Received March 26, 1982)