# A New Method for the Preparation of 3-Nitro-2-pyridinesulfenyl Chloride and One-Pot Syntheses of *N*(α)-*tert*-butoxycarbonyl-*S*-3-nitro-2-pyridinesulfenyl Derivatives of Cysteine and D-Penicillamine

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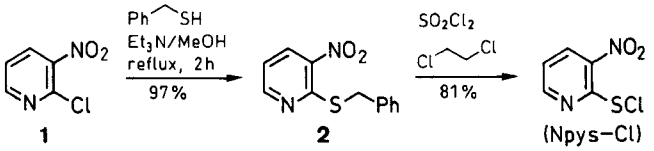
3-Nitro-2-pyridinesulfenyl chloride was prepared by the reaction of benzyl 3-nitro-2-pyridyl sulfides with sulfuryl chloride. One-pot syntheses of *N*(α)-*tert*-butoxycarbonyl-*S*-3-nitro-2-pyridinesulfenyl derivatives of cysteine and D-penicillamine were accomplished using 4-methoxybenzyl 3-nitro-2-pyridyl sulfide.

Thiol protecting groups of cysteine which allow selective cleavage and facilitate the subsequent disulfide bond formation are keys for the successful synthesis of cysteine-containing peptides. 3-Nitro-2-pyridinesulfenyl (Npys) group developed by Matsueda[1] has given a new feature in S–S bond formation that the usual air and iodine oxidations could not achieve. The *S*-Npys group not only has sufficient stability toward the acidic conditions in peptide synthesis but also acts as a kind of activatable protecting group to afford an asymmetric disulfide bond by reaction with a free thiol.[2] In spite of these favorable advantages, supply of *S*-Npys derivatives of cysteine is not so far sufficient. This is probably because a reliable preparative method for 3-nitro-2-pyridinesulfenyl chloride (Npys-Cl) has not been established. Instability of Npys-Cl could be another reason.[3] In this paper we wish to report a new synthetic method for Npys-Cl and its application to one-pot syntheses of *N*(α)-protected *S*-Npys derivatives of cysteine and D-penicillamine (D-Pen).

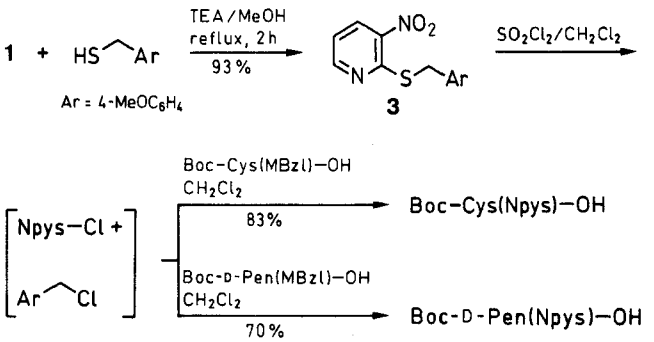
Npys-Cl has been prepared by the reaction of bis(3-nitro-2-pyridyl) disulfide with chlorine.[4] The reaction is often incomplete because of the poor solubility of the disulfide. In addition, lack of a proper purification method made it difficult to obtain the pure material as shown by the two different melting points reported; 217–222 °C by Matsueda et al,[4] and 95 °C by Ploux et al.[5] These facts suggested the use of a more soluble compound as the starting material[3] for the synthesis of Npys-Cl.

Benzyl sulfides as well as disulfides can be used as a source of sulfenyl chlorides.[6] Since sulfides have usually higher solubility than the corresponding disulfides, the benzyl sulfide route has been successfully utilized in preparation of 2,4-dinitrobenzenesulfenyl chloride.[7] Hence, we tried to use benzyl 3-nitro-2-pyridyl sulfide as the starting material.

Benzyl 3-nitro-2-pyridyl sulfide **(2)** was prepared from 2-chloro-3-nitropyridine **(1)** and phenylmethanethiol. Use of triethylamine as a base and an excess of the thiol was essential to obtain the pure material in nearly quantitative yield. Npys-Cl with mp 87.5–90 °C could be obtained in 81 % yield by treating **2** with sulfuryl chloride in dichloroethane. Reaction of this compound with N(α)-*tert*-butoxycarbonyl-*S*-4-methoxybenzylcysteine, Boc-Cys(MBzl)-OH,[8] in dichloromethane gave Boc-Cys (Npys)-OH, quite identical with the authentic sample, in 91 % yield.




The major purpose of this study is to establish a one-pot method for the synthesis of *S*-Npys derivatives of cysteine and penicillamine. Dichloromethane is the best solvent for displacement of MBzl group to Npys group,[9] whereas the cleavage reaction of the benzyl thioether bond with sulfuryl chloride is rather slow in this solvent. To overcome this problem, we prepared 4-methoxybenzyl 3-nitro-2-pyridiyl sulfide **(3)**. Compound **3** has higher reactivity than **2** and generated Npys-Cl in dichloromethane easily by treatment with sulfuryl chloride. After purging hydrogen chloride gas by bubbling argon gas into the solution, Npys-Cl was treated in the same vessel with Boc-Cys(MBzl)-OH to afford Boc-Cys(Npys)-OH in a yield of 83 %. Under the same conditions Boc-D-Pen(Npys)-OH could also be obtained in 70 % yield.




Under similar conditions *N*(α)-9-fluorenylmethoxycarbonyl (Fmoc)[10] *S*-Npys derivatives, Fmoc-Cys(Npys)-OH and Fmoc-D-Pen(Npys)-OH, were obtained in 86 and 78 % yields, respectively, from the corresponding *S*-MBzl derivatives.[11] In these cases, the process of purging HCl gas could be omitted. However, the *S*-Npys group was found to be unstable toward the Fmoc cleaving reagents, 20 % piperidine in dimethylformamide,[12] 2% 1,8-diazabicyclo[5,4,0]undec-7-ene in dimethylformamide,[13] and 0.34 M tetrabutylammonium fluoride in dimethylformamide.[14,15] For possible use of these Fmoc derivatives a new selective cleavage method for Fmoc groups should be developed.

All solvents were distilled before use. 2-Chloro-3-nitropyridine was purchased from Aldrich Chemical. Wakogel C-300 (Wako Pure Chemical Industries, ltd., 200–300 mesh) was used for column chromatography. Silica gel for TLC was purchased from Merck. $^1$H NMR spectra were recorded on JEOL JNM-Ex-270 FT-NMR system (270 MHz).

**Benzyl 3-Nitro-2-pyridyl Sulfide (2):**
A mixture of **1** (450 mg, 2.84 mmol), phenylmethanethiol (0.5 mL, 4.26 mmol) and TEA (594 μL, 4.26 mmol) in MeOH (2 mL) was refluxed for 5 h. After removal of the solvent the product was isolated by column chromatography on silica gel using $CHCl_3$ for elution to give **2** as yellow needles; yield: 693 mg (99%); mp 66.0–67.5 °C.

$^1$H NMR ($CDCl_3$/TMS): $\delta$ = 4.4 (s, 2H, Ar$\underline{C}H_2$), 7.1 (dd, 1H, $J$ = 4.8, 8.1 Hz, 5-$H_{pyridyl}$), 7.2–7.4 (m, 5$H_{arom}$), 8.4 (dd, 1H, $J$ = 1.6, 8.1 Hz, 4-$H_{pyridyl}$), 8.7 (dd, 1H, $J$ = 1.6, 4.8 Hz, 6-$H_{pyridyl}$).

**Npys-Cl:**
To an ice-cooled solution of **2** (935 mg, 3.80 mmol) in $ClCH_2CH_2Cl$ (2 mL) under an atomsphere of Ar, was added $SO_2Cl_2$ (0.4 mL, 4.94 mmol) and pyridine (one drop). The mixture was stirred for 1 h and evaporated. The residue was repeatedly washed with hexane to give a yellow powder; yield: 589 mg (81%); mp 87.5–90.0 °C.

$^1$H NMR ($CDCl_3$/TMS): $\delta$ = 7.4 (1H, dd, $J$ = 4.6, 8.2 Hz, 5-$H_{pyridyl}$), 8.6 (dd, 1H, $J$ = 1.5, 8.2 Hz, 4-$H_{pyridyl}$), 8.9 (dd, 1H, $J$ = 1.5, 4.6 Hz, 6-$H_{pyridyl}$).

**Boc-Cys(Npys)-OH:**
To an ice-cooled solution of Boc-Cys(MBzl)-OH (341 mg, 1.00 mmol) in $CH_2Cl_2$ (200 mL) was added Npys-Cl (228 mg, 1.20 mmol), and the mixture was stirred for 1 h. After evaporation of the solvent the residue was purified by preparative TLC on silica gel using $CHCl_3$/MeOH (17:3) for development; yield: 324 mg (91%); mp 153.5–158.0 °C (dec); $[\alpha]_D^{26}$ −85.6° ($c$ = 1, MeOH); $R_f$ ($CHCl_3$/MeOH 9:1) 0.44. [Lit.[9] mp 155–158 °C (dec), $[\alpha]_D^{22}$ −86.2° ($c$ = 1, MeOH)].

$^1$H NMR ($CDCl_3$/TMS): $\delta$ = 1.4 (s, 9H, $t$-$C_4H_9$), 3.2–3.4 (d, 2H, $\beta$-$CH_2$), 4.2–4.3 (br, 1H, α-H), 6.2–6.3 (br, 1H, NH), 7.5 (dd, 1H, $J$ = 4.5, 8.2 Hz, 5-$H_{pyridyl}$) 8.5 (dd, 1H, $J$ = 1.8, 8.2 Hz, 4$H_{pyridyl}$), 8.9 (dd, 1H, $J$ = 1.8, 4.5 Hz, 6-$H_{pyridyl}$), 9.0–9.2 (br, 1H, $CO_2H$).

**4-Methoxybenzyl 3-Nitro-2-pyridyl Sulfide (3):**
A mixture of **1** (2.18 g, 13.8 mmol), 4-methoxyphenylmethanethiol (2.50 mL, 21.3 mmol) and TEA (2.50 mL, 21.3 mmol) in MeOH (10 mL) was refluxed for 4 h. After removal of the solvent the residue was purified by column chromatography on silica gel using $CHCl_3$ for elution to give yellow needles; yield: 3.54 g (93%); mp 89.2–90.0 °C.

$^1$H NMR ($CDCl_3$/TMS): $\delta$ = 3.8 (s, 3H, $OCH_3$), 4.4 (s, 2H, Ar$\underline{C}H_2$), 6.8–7.3 (m, 5H, $H_{arom}$ + 5-$H_{pyridyl}$), 8.4 (dd, 1H, $J$ = 1.6, 9.3 Hz, 4-$H_{pyridyl}$), 8.7 (dd, 1H, $J$ = 1.6, 5.1 Hz, 6-$H_{pyridyl}$).

**Boc-D-Pen(MBzl)-OH:**
D-Pen(MBzl) was prepared from D-Pen according to the procedure reported for the cysteine derivative[16] and used without purification. Introduction of Boc was performed using the procedure described for the synthesis of Boc-Cys(MBzl)-OH.[8]

mp 93.0–93.3 °C, $[\alpha]_D^{26}$ −0.96° ($c$ = 1, EtOH).

$^1$H NMR ($CDCl_3$/TMS): $\delta$ = 1.3–1.6 (m, 15H, $t$-$C_4H_9$ + $CH_3$), 3.6–3.7 (m, 5H, $OCH_3$ + Ar$\underline{C}H_2$), 4.2–4.3 (br, 1H, α-H), 5.4–5.5 (br, 1H, NH), 6.8 (d, 2$H_{arom}$, $J$ = 8.4 Hz), 7.2 (d, 2$H_{arom}$, $J$ = 8.4 Hz), 7.9–8.2 (br, 1H, $CO_2H$).

**One-Pot Synthesis of Boc-Cys(Npys)-OH:**
To an ice-cooled solution of **3** (2.54 g, 9.22 mmol) in $CH_2Cl_2$ (10 mL) under an atmosphere of Ar was added $SO_2Cl_2$ (0.80 mL, 9.88 mmol), and the mixture was stirred for 20 min. After removal of HCl by bubbling Ar gas, the solution was diluted with $CH_2Cl_2$ (250 mL). To this was added Boc-Cys(MBzl)-OH (2.78 g, 8.14 mmol), and the mixture was stirred for 45 min. After evaporation of the solvent the residue was dissolved in EtOAc (100 mL), and filtered. The filtrate was washed successively with 5% aq citric acid, $H_2O$ and dried. The solution was evaporated and the product was isolated by column chromatography on silica gel using $CHCl_3$/MeOH (80:1 v/v) for elution. (2.52 g) Some fractions containing impurities were purified by preparative TLC on silica gel using $CHCl_3$/MeOH (17:3) for development; total yield: 2.65 g (88%); mp 154–156 °C; $[\alpha]_D^{26}$ −85.5° ($c$ = 1, MeOH).

**One-Pot Synthesis of Boc-D-Pen(Npys)-OH:**
Boc-D-Pen(MBzl)-OH (807 mg, 2.19 mmol) was treated with Npys-Cl generated from **3** (899 mg, 3.28 mmol) under the same conditions used for the cysteine derivative. After washing in the same manner the product was isolated by preparative TLC on silica gel using $CHCl_3$/MeOH (22:5) for development; yield: 621 mg (70%); mp 155.5–158.5 °C; $[\alpha]_D^{25}$ −29.0° ($c$ = 1, EtOH).

$^1$H NMR ($CDCl_3$/TMS): $\delta$ = 1.2–1.6 (m, 15H, $t$-$C_4H_9$, $CH_3$), 4.1–4.2 (br, 1H, α-H), 6.2–6.3 (br, 1H, NH), 7.45 (dd, 1H, $J$ = 4.7, 8.4 Hz, 5-$H_{pyridyl}$), 8.6 (dd, 1H, $J$ = 1.9, 8.4 Hz, 4-$H_{pyridyl}$), 8.9 (dd, 1H, $J$ = 1.9, 4.7 Hz, 6-$H_{pyridyl}$), 9.9–10.2 (br, 1H, $CO_2H$).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