51. Deblocking of *o*-Nitrophenylsulfenyl-Protected Peptides by Ammonium Thiocyanate and (2-Methyl-1-indolyl)acetic acid

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Summary

The thiocyanate cleavage of the N^{α}-*o*-nitrophenylsulfenyl group from peptides in solution or on a solid support proceeds effectively in the presence of (2-methyl-1-indolyl)acetic acid. This scavenger was prepared from 2-methylindole and sodium bromoacetate; it can readily be removed by extraction with base after the cleavage reaction, together with (2-methyl-3-(2-nitrophenylthio)-1-indolyl)acetic acid.

The *o*-nitrophenylsulfenyl (Nps) group introduced into peptide synthesis by *Zervas et al.* [1] has considerable potentialities as a temporary protecting group for the α-amino terminus because its selective removal can be accomplished by nucleophilic reagents. These reagents avoid problems encountered with protecting groups requiring acids for their cleavage, *i.e.* alkylation of the side chains of methionine, tyrosine and also lysine and histidine by carbocations or their reaction products with the deprotecting reagent (*e.g. t*-butyl trifluoroacetate) [2]. Thiolytic cleavage of the Nps group with a number of reagents has been described, and recently 2-mercaptopyridine was shown to enable rapid deprotection [3] [4].

In our hands the method of *Wünsch & Spangenberg* [5] using thiocyanate (rhodanide) in the presence of 2-methylindole proved very valuable for the cleavage of Nps-protected peptides. Thiocyanate ion attacks *N*-terminally bound Nps and reversibly forms *o*-nitrophenylsulfenyl thiocyanate (**1**). The equilibrium is fully displaced in the presence of excess 2-methylindole since **1** is converted into stable 3-(2-nitrophenylthio)-2-methylindole (**2**) and thiocyanate ion. The indole derivative is removed by washing with ether. However, with relatively lipophilic peptides, ether extraction becomes unsatisfactory. Since the two-phase-purification method of peptide synthesis [6] [7] frequently used in our laboratory depends on lipophilic peptide intermediates, it seemed worthwhile to adapt the indole reagent used in [5] and to investigate carboxy derivatives of indole which can be removed by aqueous base after reaction with an Nps residue.

Using Nps-Lys(Boc)-OH as a model, the cleavage rate of a variety of reagents was assessed *(Table 1)*. It is obvious that the 3-position of the indole system should be reserved for the Nps capture and cannot be blocked as in the indoles **3**–**5**. The

substitution at the N-atom of 2-methylindole with a carboxymethyl or carboxylatomethyl group is, on the other hand, advantageous, and compounds **7** and **7a** are as reactive as 2-methylindole.

Table 1. *Removal of the Nps group from Nps-Lys(Boc)-OH dicyclohexylammonium salt with NH_4SCN in the presence of different indole derivatives*

Nr.	Indole derivative	Time for complete reaction[a])
3	(3-Indolyl)acetic acid	> 48 hours
4	3-(3-Indolyl)propionic acid	40 hours
5	L-Tryptophane	> 48 hours
6	2-Methylindole	3 min
7	(2-Methyl-1-indolyl)acetic acid	3 min
7a	Dicyclohexylammonium (2-methyl-1-indolyl)acetate	3 min

[a]) Nps-Lys(Boc)-OH dicyclohexylammonium salt (0.1 mmol) in 3.8 ml of $CH_2Cl_2/CH_3OH/CH_3COOH$ 15:2:2 was mixed with 0.2 mmol of NH_4SCN and 0.2 mmol of indole derivative and stirred at r.t. (approx. 23°). The final volume was 3.9 ml and the molarities therefore 0.0255 and 0.051 for the lysine derivative and the reagents, respectively. Frequently 2 µl aliquots of the reaction solution were withdrawn and immediately chromatographed on silica gel plates (*F254, Merck*, Darmstadt) with $CHCl_3/CH_3OH$ 9:1. The time of disappearance of Nps-Lys(Boc)-OH (Rf 0.4) detected by fluorescence quenching at 254 nm is taken as the time for complete reaction.

The synthesis of **7** and **7a** from the sodium salt of 2-methylindole proceeded essentially according to *Cardillo et al.* [8], who reported alkylations of the indole sodium salt. The reaction with sodium bromoacetate in heterogeneous phase is not adapted for maximum yield. Mostly the salt **7a** was employed for deprotection. The Nps-substituted product (2-methyl-3-(2-nitrophenylthio)-1-indolyl)acetic acid (**8**) was also prepared and shown to be a stable compound of good solubility in many organic solvents and easily extractable from such solutions with aqueous base.

Table 2. *Removal of the N-terminal Nps group from various peptides with NH_4SCN in the presence of dicyclohexylammonium 2-methylindol-1-yl acetate* (**7a**)

Peptide[a])	Time for complete cleavage[b]) [min]
Nps-Lys(Boc)-OSuco	4
Nps-Lys(Boc)-Lys(Boc)-OSuco	7
Nps-Lys(Boc)-[Lys(Boc)]$_3$-Lys(Boc)-O*t*Bu	50
Nps-Lys(Boc)-[Lys(Boc)]$_4$-Lys(Boc)-OSuco	40
Nps-Lys(Boc)-[Lys(Boc)]$_6$-Lys(Boc)-OSuco	60
Nps-Lys(Boc)-ε-Ahx-[Lys(Boc)-ε-Ahx]$_3$-Lys(Fmoc)-Gly-OSuco	60
Nps-Leu-Lys(Z)-Ala-Leu-Lys(Z)-Gly-OEt	30

[a]) OSuco: 3-[4-(5α-cholestan-3β-yl)]$OCH_2C_6H_4CH_2$–O–CO–CH_2CH_2COO; εAhx: 6-aminohexanoic acid; Fmoc = 9-(Methoxycarbonyl)fluorenyl.

[b]) The method of *Table 1* was used. Where appropriate, TLC. was performed with toluene/EtOH 7:3 instead of $CHCl_3/CH_3OH$.

As shown in *Table 2*, the thiocyanate cleavage in the presence of **7a** becomes slower when peptides of increasing size are treated. However, in all cases virtually

homogeneous N^{α}-deprotected peptides were obtained after extraction of the reaction solution with H_2O, 0.2 M K_2CO_3 and 0.1 M HCl. Interestingly, Nps-Lys(Boc) bound to a standard solid support could be N^{α}-deprotected within a relatively short time by treating the resin first with NH_4SCN alone, adding **7a** after several minutes.

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Experimental Part

General. Amino acid derivatives, reagents, solvents, (3-indolyl)acetic acid, 3-(3-indolyl)propionic acid and 2-methylindole were obtained from *Fluka*, Buchs. NaH was washed with hexane before suspension in dry THF. THF was passed through an aluminium oxide *(504C, Fluka)* column before use. Peptides were prepared according to the two-phase-purification method [6] [7] and were taken from ongoing projects. Nps-Lys(Boc)-O-resin was prepared from polystyrene resin, *Merrifield* type, 1% DVB, containing 1.2 mol-equiv. of chloromethyl groups per g, and Nps-Lys(Boc)-O-Cs^+ using the method of *Juillerat & Bargetzi* [9] for preparing Nps-Gly-O-resin. Nps-Cl liberation with HCl indicated the binding of 0.38 mol-equiv. per g. Thin layer chromatography (TLC.) was performed on fluorescent 5×10 cm silica gel plates *60F254, Merck*, Darmstadt, with $CHCl_3/CH_3OH$ 9:1 (A) or 92.5:7.5 (B) or toluene/EtOH, 7:3 (C). Spots were detected as described previously [10]. Melting points are uncorrected. UV. spectra were recorded on a *Pye Unicam SP 8-100* spectrophotometer. ^{1}H-NMR. spectra were obtained in CD_3COCD_3 from a *Varian XL 100* spectrometer using tetramethylsilane as internal standard. Elementary analysis were performed by *H. Frohofer*, University of Zürich.

Preparation of dicyclohexylammonium (2-methyl-1-indolyl)acetate (**7a**). A solution of 2-methylindole (22.5 g, 0.172 mol), recrystallized from $EtOH/H_2O$, dissolved in 70 ml of THF was added dropwise with exclusion of moisture under N_2 to NaH (8.24 g, 0.343 mol) suspended in 80 ml of THF. The suspension was refluxed for 30 min, and after cooling, $BrCH_2COONa$ (27.6 g, 0.17 mol) suspended in 70 ml of THF was added within 15 min under vigorous stirring. Then refluxing and stirring under N_2 was continued for 2 h. To the cold (basic) solution 300 ml of H_2O was added dropwise, the mixture extracted 10 times with a total of 500 ml of $CHCl_3$, acidified with 6 M HCl to pH 2 and finally extracted with 600 ml of EtOAc in 6 portions. The org. phase was washed 4 times with 50 ml portions of 0.1 M HCl and H_2O and dried with Na_2SO_4. Dicyclohexylamine was then added to pH 7, and after standing overnight at 4°, the colorless, virtually scentless needles were filtered off, washed with EtOAc and dried *in vacuo:* 38.5 g (61%) of **7a**, m.p. 214–217°.

$C_{23}H_{34}N_2O_2$ (370.5) Calc. C 74.60 H 9.24 N 7.60% Found C 74.86 H 8.95 N 7.64%

Preparation of (2-methyl-1-indolyl)acetic acid (**7**). The free acid was obtained by extracting a solution of **7a** in CH_2Cl_2 with $KHSO_4$-solution according to [11]. Removal of CH_2Cl_2 *in vacuo* left **7** as a crystalline material, m.p. 206–208°. TLC. (A): Rf 0.32, homogeneous; (C): Rf 0.44, homogeneous. – UV. (EtOH): 220 (27700), 274 (7200), 280 (7300), 289 (5800). – ^{1}H-NMR.: 7.51–7.21 (*m*, 2 H, H–C(5), H–C(8)); 7.20–6.91 (*m*, 2 H, H–C(6), H–C(7)); 6.25 (*d*×*qa*, 1 H, H–C(3)); 5.88 (br. *s*, COOH, H_2O); 4.94 (*s*, 2 H, $CH_2N(1)$); 2.39 (*s*, 3 H, H_3C–C(2)).

Preparation of (2-methyl-3-(2-nitrophenylthio)-1-indolyl)acetic acid (**8**). Nps-Gly-OH dicyclohexylammonium salt (410 mg, 1.0 mmol) dissolved in 30 ml of CH_2Cl_2, 4 ml of CH_3OH and 4 ml of CH_3COOH was stirred under Ar in the dark with 152 mg (2.0 mmol) of NH_4SCN and 180 mg (0.95 mmol) of **7** for 1 h. The solution was diluted with 300 ml of CH_2Cl_2 and extracted in a spray column extractor [6] with 1 l of 0.1 M HCl, 0.3 l of H_2O and 1.5 l of 0.2 M K_2CO_3. The K_2CO_3-extract was mixed with 100 ml of EtOAc and acidified under efficient stirring with hydrochloric acid to pH 2. The AcOEt layer was dried with Na_2SO_4 and evaporated *in vacuo:* 458 mg (92%) of **8** as orange powder, m.p. 202–204°. TLC. (A): Rf 0.18, homogeneous; (C): Rf 0.29, homogeneous. – UV. (EtOH): 222 (35000), 280 (12200), 289 (10200), 371 (3500). – ^{1}H-NMR.: 8.34–8.24 (*m*, 2 H, H–C(3′), H–C(5′)); 7.64–7.03 (*m*, 4 H, H–C(5), H–C(6), H–C(7), H–C(8)); 6.96–6.84 (*m*, 2 H, H–C(4′), H–C(6′)); 5.22 (*s*, 2 H, $CH_2N(1)$); 2.53 (*s*, 3 H, H_3C–C(2)).

Preparative removal of Nps from Nps-Lys(Boc)-[Lys(Boc)]$_6$-Lys(Boc)-OSuco. The protected peptide (5.56 g, 2.15 mmol) dissolved in 80 ml of $CH_2Cl_2/CH_3OH/CH_3COOH$ 15:2:2 was stirred with 4.30 mmol of NH_4SCN and 4.30 mmol of **7a** at r.t. in the dark for 70 min. After dilution with 300 ml of CH_2Cl_2 the solution was extracted in a spray column extractor [6] with 1 l of 0.1M HCl, 0.3 l of H_2O, 2 l of 0.2M K_2CO_3 and 0.5 l of H_2O. Removal of the solvent and drying *in vacuo* gave a colorless residue: 5.18 g (99%) of H-Lys(Boc)-[Lys(Boc)]$_6$-Lys(Boc)-OSuco. TLC. (A): Rf 0.30, homogeneous; (B): Rf 0.18, homogeneous.

Removal of Nps from Nps-Lys(Boc)-O-resin. To Nps-Lys(Boc)-O-resin (50 mg) stirred at r.t. in 19 ml of $CH_2Cl_2/CH_3OH/CH_3COOH$ 15:2:2, 0.50 mmol of NH_4SCN was added, followed by 0.57 mmol of **7a** after 5 min. After a total of 18 min, the resin was filtered off and washed with solvent to give a colorless product.

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