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2-(4-Nitrophenyl)sulfonylethoxycarbonyl (Nsc) Group As a Base-Labile α-Amino Protection for Solid Phase Peptide Synthesis

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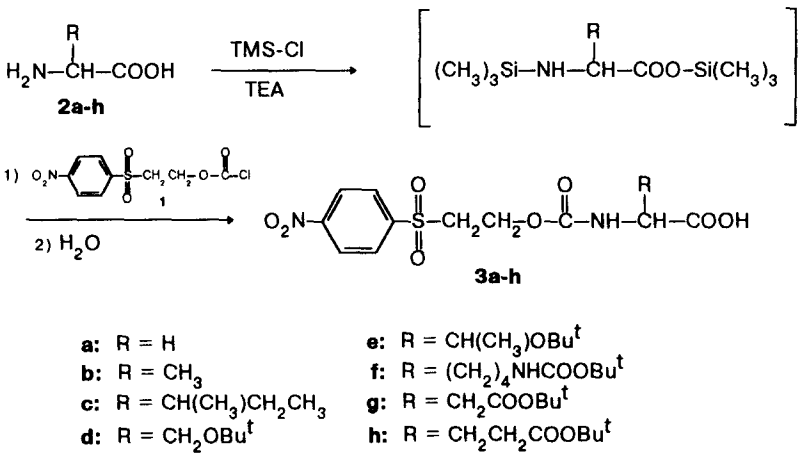
Abstract: Base-labile 2-(4-nitrophenylsulfonyl)ethoxycarbonyl (Nsc) group is proposed for a temporary α-amino protection in the solid phase peptide synthesis. Nsc-Group is cleaved by organic bases in aprotic solvents under mild conditions similar to that used for Fmoc-group. Several Nα-Nsc amino acids are prepared and used in the solid phase synthesis of the fragment 307-318 of S-protein from bovine eye retina.

For the protection of amino and carboxyl functions in peptide synthesis a wide variety of base-labile protective groups cleaved by β-elimination had been proposed, among them there were several groups on the basis of substituted 2-sulfonylethanols[1]. Cleavage of the groups of this type usually involved a short-term treatment with a strong inorganic base in an aqueous or mixed solution. Solubility problems which were often encountered during such deprotection procedure and the risk of side reactions appeared to be obstacles to more wide application of these protective groups in peptide chemistry. On the other hand, 9-fluorenylmethoxycarbonyl (Fmoc) amino-protective group[2] which is easily removed under mild non-hydrolytic conditions by secondary aliphatic amines in aprotic solvents, became very popular in solid phase peptide synthesis and in other synthetic applications.

Earlier we have found that ester and urethane derivatives of substituted 2-sulfonylethanols can also be cleaved by strong organic bases, e.g. 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU), 1,1,3,3-tetramethylguanidine, and piperidine, in aprotic solvents and that the rates of the cleavage are strongly dependent on the nature of a substituent at the sulfonyl group[3]. Considering the data of kinetic measurements performed with a series of substituted 2-sulfonylethyl urethanes we have supposed that 2-(4-nitrophenylsulfonyl)ethoxycarbonyl group may well be an appropriate temporary protection for α-amino groups in solid phase peptide synthesis[3c]. As compared to the Fmoc, this group has close, albeit slower, rates of cleavage by organic bases in DMF (except for 1,1,3,3-tetramethylguanidine) and is more stable in neutral solutions. Following to Verhard and Tesser[4] we have accepted for this group the designation Nsc.

In this communication we wish to report on the synthesis of several Nα-Nsc protected L-amino acids **3** and make an attempt to prove their usefulness in the solid phase synthesis of peptides. At first, we examined the acylation of amino acids with Nsc-Cl (**1**)[4] under usual Schotten-Baumann conditions. The treatment of ice-cooled solutions of amino acids **2** and 20% molar excess of K_2CO_3 in water-dioxane (4:1) with equimolar amount of the chloroformate **1** in dioxane-acetonitrile (1:1) gave the corresponding Nsc derivatives **3** in low to moderate yields. Due to the concomitant partial decomposition of the chloroformate **1** the acylation did not come to completion, and the resulting Nα-Nsc amino acids **3** could be isolated in a pure form only by chromatography on a silica gel column. The acylation of trimethylsilyl derivatives of amino acids in non-aqueous solutions according to the method of Bolin et al.[5] (Scheme 1) led to markedly better results.



Scheme 1. Synthesis of Nα-Nsc amino acids via trimethylsilyl derivatives

In a typical procedure, to a stirred suspension of finely powdered amino acid **2** (20 mmol) in 30 ml of dichloromethane 40 mmol of triethylamine or diisopropylethylamine was added. Then 40 mmol of trimethylchlorosilane was added dropwise with vigorous stirring and the resulting mixture was refluxed for 1.5 h. The mixture was then cooled in an ice bath, and 17 mmol of Nsc-Cl (**1**) was added. After stirring in ice for 30 min the mixture was allowed to warm to room temperature for 2 h, then evaporated and distributed between ether and 5% $NaHCO_3$. The aqueous layer was acidified to pH 2-2.5 with 2N H_2SO_4 and extracted with ethyl acetate, the extract was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was recrystallized from appropriate solvent (usually ethyl acetate/petroleum) to give compound **3** (Table 1). All products were found to be homogeneous by TLC and reversed phase HPLC.

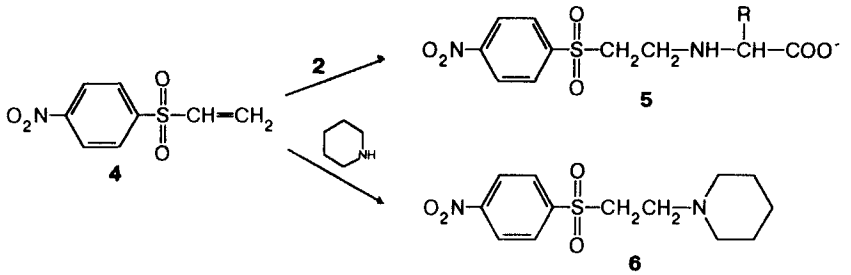
Table 1. Preparation of Nα-Nsc Amino Acids[a)]

Entry	Amino acid	Yield, %	mp °C[b)]	$[\alpha]_D$[c)]	R_f[d)]
3a	Gly	85	152-154	-	0.21
3b	Ala	89	134-135	-24.7°	0.35
3c	Ile	80	120-121	-12.7°	0.52
3d	Ser(^{t}Bu)	82	109-111	+3.3°	0.46
3e	Thr(^{t}Bu)	78	64-66	-9.0°	0.50
3f	Lys(Boc)	83	110-112	-11.3°	0.34
3g	Asp(O^{t}Bu)	83	73-75	-12.7°	0.38
3h	Glu(O^{t}Bu)	90	94-96	-17.0°	0.38

a)Elemental analyses are given in ref. 6; b)Uncorrected; c)c 1, DMF, 25°C; d)TLC on silica gel 60 F_{254} aluminium sheets (Merck, FRG), benzene-acetone-CH_3COOH (100:50:3)

Nα-Nsc amino acids **3a-h** are stable crystalline compounds and can be stored for months even at room temperature without any sign of decomposition. The protected amino acids are stable in DMF solutions for at least several hours, but are cleaved within 1-2 min on the treatment with 3-fold molar excess of DBU, affording free amino acid **2**, 4-nitrophenyl vinyl sulfone **4** and CO_2. Upon further standing a substantial accumulation of

side products is observed which arise from the reaction of the vinyl sulfone **4** with liberated α-amino group (product **5**) and, possibly, with DBU in the absense of a scavenger. In DMF-piperidine mixture (3:1), the cleavage of the Nsc-group proceeds completely within 6-8 min. At the same time, a large molar excess of piperidine serves as an effective trap for vinyl sulfone **4**, so that the only non-volatile products formed are free amino acid **2** and N-[2-(4-nitrophenyl)sulfonyl]ethylpiperidine (**6**).



Scheme 2. Reactions of 4-nitrophenyl vinyl sulfone

Prepared Nα-Nsc amino acids **3a-h** were used in the synthesis of the dodecapeptide Ala-Ser-Ser-Thr-Ile-Ile-Lys-Glu-Gly-Ile-Asp-Lys, a fragment 307-318 of S-protein from bovine eye retina. Synthesis was performed on aminomethylated 1% divinylbenzene-styrene copolymer (1.01 meq NH_2/g) modified with 4-hydroxymethylphenoxyprorionic acid[7], 5 ml disposable polypropylene syringe with inserted polyropylene porous disc was used as a reactor. The resin (150 mg) was placed into the reactor and esterified with C-terminal amino acid [0.6 mmol Nsc-Lys(Boc)-OH (**3f**), 0.6 mmol dicyclohexylcarbodiimide, 0.06 mmol 4-dimethylaminopyridine, 3 ml 1,2-dichloroethane/N-methylpyrrolidone (2:1), 12 h, rt]. After careful washings ($CHCl_3$, DMF) the synthesis proceeded according to the following protocol:

1) Prewash: 33% piperidine/DMF, 4 ml, 0.5 min.

2) Deblocking: 33% piperidine/DMF, 4 ml, 15 min.

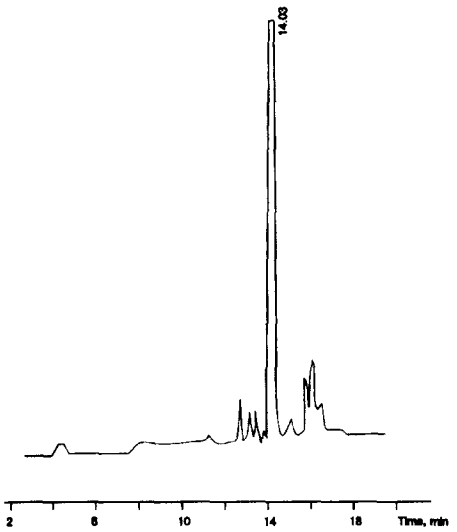
3) Wash: DMF, 6 x (4 ml, 0.5 min).

4) Coupling: Nsc-amino acid, 0.5 mmol; benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP), 0.5 mmol; 1-hydroxybenzotriazole, 0.5 mmol; N-methylmorpholine, 0.75 mmol; DMF, 2 ml; 60 min (for Nsc-Ile-OH 90 min).

5) Wash: DMF, 5 x (4 ml, 0.5 min).

Residual amino groups were not monitored after each coupling, however, the progress of the synthesis was controlled by analytical HPLC after couplings 4 and 8. Samples of the peptidyl resin (2-3 mg) were withdrawn, washed and treated with 50% trifuoroacetic acid (TFA)/1,2-dichloroethane (DCE), the cleaved peptides were then analysed by chromatography.

After the completion of the peptide assembly the peptidyl-resin was thoroughly washed with $CHCl_3$, dried and then treated with 50% TFA/DCE (60 min, rt) for the cleavage of the peptide from the resin. The resin was filtered off and washed with 50% TFA/DCE, combined washings were then diluted with cold dry ether, The precipitate formed was collected by filtration, washed with ether and dried affording 170 mg (90%) of the crude dodecapeptide. HPLC analysis[8] of the product (Fig. 1) revealed a major peak (70% of total area) corresponding to the target peptide.



The peptide was dissolved in 6% AcOH/water and purified by gel-permeation chromatography on TSK HW-40F column (1.5 x 70 cm) equilibrated with the same buffer. Fractions containing pure peptide were pooled and liophilyzed yielding 104 mg (41%) of the product which had >95% purity by HPLC and net peptide content 75% by quantitative amino acid analysis[9].

In conclusion, Nsc-amino acids appeared to be suitable intermediates for the solid phase peptide synthesis under conditions very similar to that used for Fmoc derivatives. As a temporary base-labile α-amino protective group, Nsc can be a viable and, probably, less expensive alternative to Fmoc for the purposes of practical synthesis.

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4. Verhart, C.G.J.; Tesser, G.I. *Rec. Trav. Chim. Pays-Bas.* **1988**, *107,* 621-626. These authors studied several para-substituted 2-arylsulfonylethoxycarbonyl groups (4-Br, 4-NO_2, 4-CH_3SO_2) as an α-amino protection and recommended the 2-[4-(methylsulfonyl)phenylsulfonyl]ethoxycarbonyl (Msc) group as an alternative to Fmoc for solution peptide synthesis.
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6. **3a**: $C_{11}H_{12}N_2O_8S$ (332.29); calcd C 39.76, H 3.64, N 8.43; found C 39.64, H 3.57, N 8.27; **3b**: $C_{12}H_{14}N_2O_8S$ (346.31); calcd C 41.62, H 4.07, N 8.09; found C 41.57, H 4.11, N 8.04; **3c**: $C_{15}H_{20}N_2O_8S$ (388.40); calcd C 46.39, H 5.19, N 7.21; found C 46.33, H 4.99, N 7.14; **3d**: $C_{16}H_{22}N_2O_9S$ (418.42); calcd C 45.93, H 5.30, N 6.70; found C 45.79, H 5.33, N 6.35; **3e**: $C_{17}H_{24}N_2O_9S$ (432.45); calcd C 47.22, H 5.59, N 6.48; found C 47.58, H 5.49, N 6.32; **3f**: $C_{20}H_{29}N_3O_{10}S$ (503.53); calcd C 47.71, H 5.81, N 8.35; found C 47.52, H 5.83, N 8.24; **3g**: $C_{17}H_{22}N_2O_{10}S$ (446.44); calcd C 45.74, H 4.97, N 6.28; found C 45.46, H 5.10, N 5.98; **3h**: $C_{18}H_{24}N_2O_{10}S$ (460.46); calcd C 46.95, H 5.25, N 6.08; found C 47.10, H 5.38, N 6.03.
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8. LiChrosphere 100 RP18 (5 mcm) column 4.6 x 125 mm; gradient from 0 to 60% MeCN/water (0.1% TFA) for 22 min; flow rate 1.1 ml/min; UV-detector at λ = 226 nm, 0.16 AUFS.
9. Amino acid composition after hydrolysis (6N HCl, 110°C, 24 and 48 h): Asp, 1.02 (1); Ser, 1.84 (2); Thr, 0.93 (1); Glu, 0.94 (1); Gly, 1.03 (1); Ala, 1 (1); Ile, 2.78 (3); Lys, 2.04 (2). Analysis was performed on Biotronik LC 5001 device.

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