Utilization of 2-(4-Nitrophenylsulfonyl)ethoxycarbonyl (Nsc) as a Substitute for 9*H*-Fluoren-9-ylmethoxycarbonyl (Fmoc) in Liquid Phase Chemistry

Thomas C. Maier, Joachim Podlech*

Institut für Organische Chemie der Universität Karlsruhe (TH), Fritz-Haber-Weg 6, 76131 Karlsruhe, Germany
Fax: (+49)-721-608-7652, e-mail: joachim.podlech@ioc.uka.de

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Dedicated to Joe Richmond on the occasion of his 60th birthday.

Abstract: 2-(4-Nitrophenylsulfonyl)ethoxycarbonyl (Nsc) is a useful substitute for the Fmoc group. It is easily removed not only with secondary amines but with tris(aminoethyl)amine (TAEA) and with resin-bound TAEA, thus allowing for a simplified work-up: the side products of the deprotection are removed either by extraction with phosphate buffer or by filtration.

Keywords: amino acids; polymer-bound Lewis bases; protecting groups

First reported by Carpino and Han[1] in 1970, the 9*H*-fluoren-9-ylmethoxycarbonyl group (Fmoc) has become one of the most frequently used α-amino protecting groups in solid-phase peptide synthesis. Its usefulness was immediately recognized with the observation that Fmoc cleavage is readily achieved by amines like piperidine.[2] The real break-through of this type of N^{α}-protection occurred with its application in solid-phase synthesis by Chang and Meienhofer[3] and Atherton et al.[4] The relatively mild, versatile deprotection procedure with amines is operationally simple, liberating the free amino function and the by-product dibenzofulvene (DBF), which is easily removed from the solid support by repeated washings. The chemistry and usefulness of this protecting group have been extensively reviewed.[5,6] While in solid phase synthesis using base-promoted Fmoc cleavage, DBF and its amine-adducts are simply removed by filtration and washings, the lack of volatility and the reactivity of the DBF by-product cause difficulties in solution phase synthesis rendering the Fmoc group less attractive for solution synthesis (Scheme 1).[6,7] This communication summarizes our efforts in solving this specific problem.

During deprotection studies of Fmoc-protected substrate **1** we encountered significant problems arising from the side products DBF, its gelatinous oligomer and the respective amine adducts. A purification of the substrate was neither possible with conventional nor with reverse-phase chromatography and purification by crystallization led to heavy loss of material. The DBF oligomer is hardly removable even by repeated filtrations and the amine adduct is not separable by extraction since it shows similar basicity as compared to the deprotected product. As Carpino et al. have shown, the latter problem can be faced by the use of tris(aminoethyl)amine (TAEA) as cleaving base (50 equivs., 1.5 M in CH_2Cl_2) leading to an amine adduct which can be effectively extracted with phosphate buffer (pH 5.5).[8] Nevertheless, with this, the formation of DBF oligomers is still a significant obstacle.

We found that the 2-(4-nitrophenylsulfonyl)ethoxycarbonyl group (Nsc) which has been occasionally used in solid-phase peptide synthesis[5d,9] and in nucleoside chemistry[10] is especially useful for peptide synthesis in solution. Nsc-protected amino acids are commercially available or easily prepared in four steps from 4-nitrochlorobenzene.[6,11,12] Like Fmoc, the Nsc group is cleaved under basic conditions *via* an E1cb mechanism, but – unlike dibenzofulvene – the primary product of Nsc cleavage, 4-nitrophenyl vinyl sulfone, does not tend to polymerize and is trapped virtually irreversibly by piperidine and other amines (Scheme 1).

We tested the applicability of the Nsc group by investigating the behavior of the dipeptide Nsc-Leu-Phe-OMe under varying reaction conditions: We could confirm that this protecting group is cleavable by mild bases (Table 1) with no formation of interfering polymeric material and with irreversible formation of the vinyl sulfone-amine adduct. When TAEA was used as cleaving base only a small excess was necessary to achieve complete deprotection. With three equivalents of TAEA only small traces of starting material were left (Entry 4) while full conversion was achieved with higher excess of the amine (Entry 3). Excess base and the vinyl sulfone-amine adduct could be removed by washing with phosphate buffer. Additionally we tested a solid phase-supported TAEA (®–TAEA)[13] as a cleaving base which is commercially available but has not previously been

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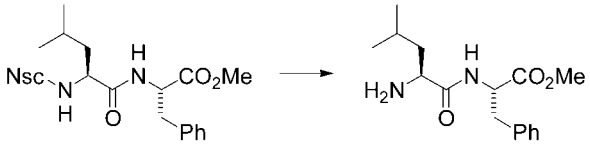
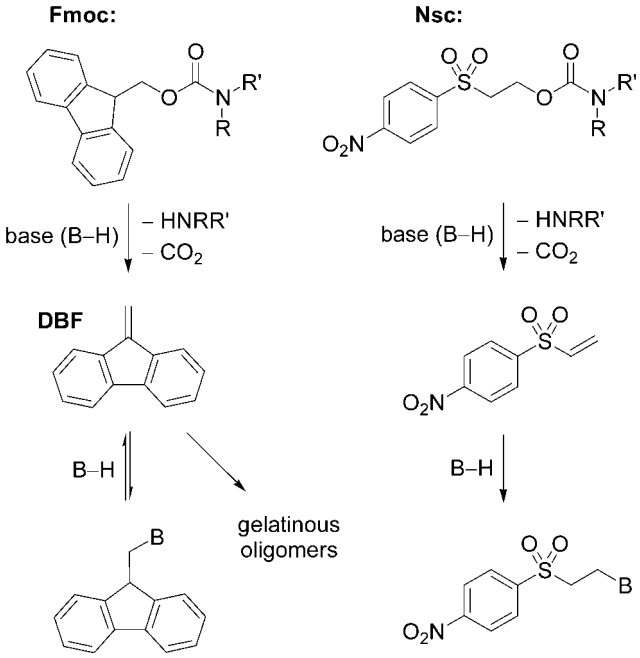
Table 1. Deprotection of an Nsc-protected dipeptide derivative.



Entry	Base	Equivalents (concentration)	Solvent	Time	Result
1	Et_2NH	50 (1.0 M)	CH_2Cl_2	12 h	complete conversion
2	TAEA	50 (1.5 M)	CH_2Cl_2	0.5 h	complete conversion
3	TAEA	50 (1.5 M)	MeOH	0.5 h	complete conversion
4	TAEA	3 (0.06 M)	CH_2Cl_2	15 h	traces of starting material
5	TAEA	2 (0.04 M)	CH_2Cl_2	15 h	starting material left
6	TAEA	1 (0.02 M)	CH_2Cl_2	15 h	starting material left
7	®-TAEA	8 (–)	CH_2Cl_2	50 h	complete conversion



Scheme 1.

used as cleaving base for this or similar protecting groups to the best of our knowledge. It was similarly applied and after use separated together with the corresponding vinyl sulfone adduct (irreversibly bound to the solid phase) simply by filtration. An excess of about eight equivalents led to a complete deprotection of the Nsc group (Entry 7). Both methods led to deprotected products which contained no significant amounts of impurities.

We applied comparable reaction conditions to more complex substrates and found that Nsc is again a useful alternative to the Fmoc group. Proline, a secondary amine is – mostly, but not only for reasons of steric hindrance – usually more difficult to deprotect, which often results in a somewhat longer reaction time (Table 2). Nsc-protected substrates bearing a proline moiety as *N*-terminal amino acid could in our case be deprotected quantitatively with TAEA within a few minutes (Entry 1). However, even after prolonged reaction times (95 h) with polymer-supported TAEA deprotection was not complete, though only traces of starting material could be detected by TLC (Entries 6 and 7). It is very likely that the required reaction times and/or the required excess of immobilized TAEA strongly depend on the quality/age of the cleaving agent. We tested aminomethyl-polystyrene resin (®-CH_2NH_2) as a somewhat cheaper reagent but found that cleavage rates were very low with this reagent (Entry 8).

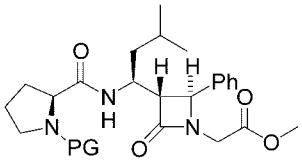
In conclusion, the Nsc group together with the herein presented deprotection methods (TAEA and supported TAEA, respectively) fulfills perfectly the prerequisites for a useful protecting group in liquid peptide synthesis. It is simply introduced, it is resistant towards usually applied reaction conditions and it is removed without traces simply by washing or filtration procedures. With these features it is similarly useful as the acid-labile *tert*-butoxycarbonyl group (Boc) to which it is completely orthogonal. Since Nsc-protected amino acids are now commercially available, this protecting group is a useful substitute for Fmoc and other groups in specific cases.

Experimental Section

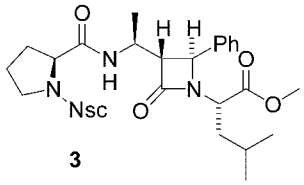
General Remarks

Nsc-protected amino acids were purchased from Hyundai Pharm (Bucheon, Korea) or were prepared from 2-(nitrophenyl)sulfonyl]ethyl chloroformate (Nsc-Cl)[6,11] by standard procedures.[6,11] Tris(2-aminoethyl)amine (TAEA, Aldrich), tris(2-aminoethyl)amine resin (®-TAEA, Advanced ChemTech) and aminomethyl-polystyrene resin (®-CH_2NH_2, Bachem) were commercially available.

Table 2. Deprotection of peptidomimetics bearing an *N*-terminal praline.



PG = Fmoc: **1**
PG = Nsc: **2**



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Entry	Substrate	Base	Equivalents (concentration)	Solvent	Time	Result
1	**3**	TAEA	50 (1.5 M)	CH_2Cl_2	0.2 h	complete conversion
2	**3**	TAEA	20 (0.6 M)	CH_2Cl_2	0.5 h	complete conversion
3	**2**	Et_2NH	30 (0.2 M)	CH_2Cl_2	15 h	complete conversion
4	**2**	piperidine	1.1 (0.02 M)	THF	50 h	starting material left
5	**2**	®-TAEA	8 (−)	CH_2Cl_2	50 h	starting material left
6	**2**	®-TAEA	15 (−)	CH_2Cl_2	95 h	traces of starting material
7	**2**	®-TAEA	15 (−)	MeCN	95 h	traces of starting material
8	**2**	®-CH_2NH_2	8 (−)	CH_2Cl_2	80 h	essentially starting material

General Procedure for the Deprotection of Fmoc- or Nsc-Protected Amino Acids or Oligopeptides with TAEA

To the compound to be deprotected (0.50 mmol) in CH_2Cl_2 (5 mL) TAEA (25.0 mmol) was added at room temperature and the solution was stirred for about 10 min (as monitored by TLC). The mixture was diluted with CH_2Cl_2, washed successively with brine, phosphate buffer (3×, pH 5.5: 6.54 g Na_2HPO_4 and 20.3 g $NaH_2PO_4 \cdot 2\,H_2O$ in 100 mL H_2O) and with brine, dried ($MgSO_4$) and evaporated at the rotary evaporator (40 °C, 100 mbar). The resulting product is usually pure enough for further reactions.

General Procedure for the Deprotection of Fmoc- or Nsc-Protected Amino Acids or Oligopeptides with immobilized TAEA

The compound to be deprotected (0.35–0.45 mmol) in CH_2Cl_2 (10 mL) was added to polymer-bound TAEA (1 g, loading *ca.* 0.70 mmol/g) and stirred at room temperature. The reaction was monitored by TLC; after about 70 h no starting material was detected (reaction time depends on the excess of immobilized TAEA and on its quality). The solid material was removed *via* a glass sinter and the solvents were removed at the rotary evaporator. The resulting product is usually pure enough for further reactions.

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