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S-2-(2,4-DINITROPHENYL)ETHYL-L-CYSTEINE: A NEW DERIVATIVE FOR SOLID-PHASE PEPTIDE SYNTHESIS[1,2]

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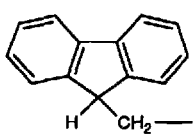
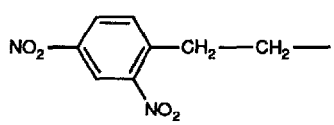
Key words: cysteine-protection, disulfide bridges, β-elimination, solid-phase peptide synthesis

Abstract: *A new acid stable protecting group for the sulfhydryl function of cysteine is described. The S-2-(2,4-dinitrophenyl)ethyl-L-cysteine derivative is fully compatible with the Boc/Bzl solid-phase peptide synyhesis strategy and can be smoothly and quantitatively removed to give the free thiol or a disulfide bridge.*

The synthesis of peptides with all cysteine residues as free thiols or with one or more disulfide bridges formed in a controlled fashion is tied to the availability of appropriate readily and orthogonal[5] removable protecting groups for the sulfhydryl function of cysteine.[6] Recently, we have described the use of *S*-9-fluorenylmethyl group (Fm)[7] for the protection of cysteine residues in the stepwise solid-phase synthesis of peptides.[8] This protecting group is easily removed under basic conditions and is stable to strong acid used to cleave the peptides from the resin. This orthogonal feature allows the isolation and purification of the *S*-cysteine(Fm)-protected peptides without the complications arising from the presence of the free thiol groups. However, the low solubility of some of these protected peptides in polar media[9] and the low HF cleavage yield observed when the *C*-terminal amino acid is Cys(Fm)[10] represent important drawbacks in the general usefulness of this protecting group. To overcome these complications, we focussed our attention on the 2-(2,4-dinitrophenyl)ethyl group (Dnpe) for the protection of the thiol function.



Fm

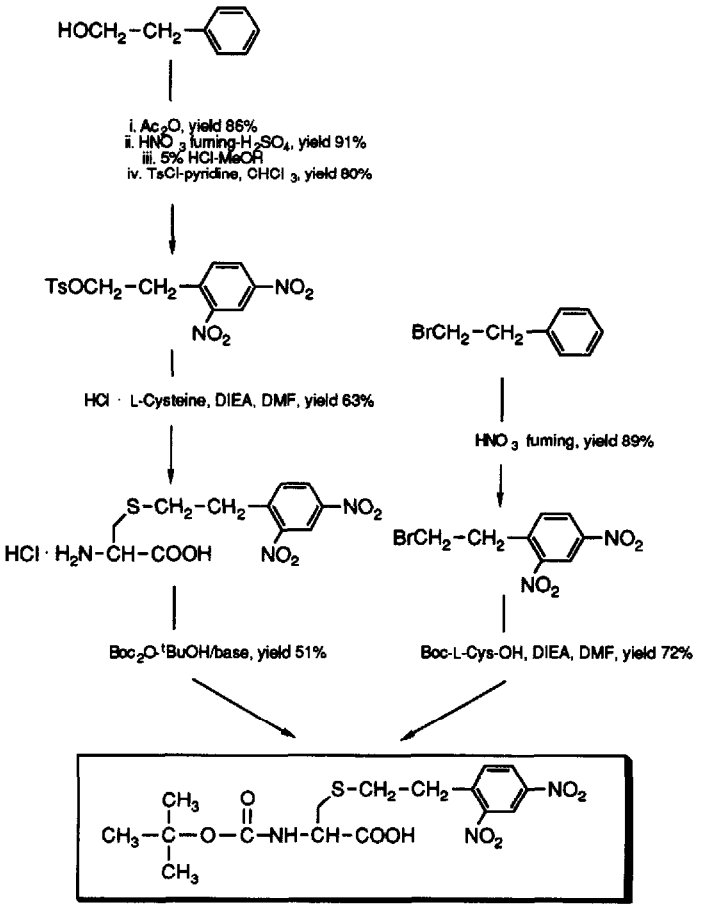


Dnpe

This type of protecting group has been introduced in oligonucleotide chemistry by Pfleiderer *et al* for the phosphate protection in the phosphotriester approach[11] and recently for the selective protection of 5'-hydroxy groups in deoxyribonucleotide synthesis.[12] The presence of electron withdrawing groups in the *ortho* and *para* positions confers similar properties to those of the Fm group (acid stability and deprotection with base through a β-elimination reaction). Moreover, the presence of the nitro groups will enhance the solubility of the protected peptides in polar media.

The N^{α}-*tert*-butyloxycarbonyl-*S*-2-(2,4-dinitrophenyl)ethyl-L-cysteine [Boc-L-Cys(Dnpe)-OH] was prepared by two alternative routes.[13] The first involved the incorporation of the Dnpe group into L-cysteine by treatment with *p*-toluensulfonate of 2-(2,4-dinitrophenyl)ethanol, which has been prepared in four steps from the

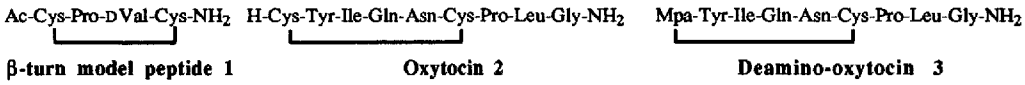
commercially available 2-phenylethanol. The final product was obtained by reaction of the corresponding protected derivative with di-*tert*-butyl dicarbonate (Boc_2O) according to the method of Moroder et *al.*[14] Alternatively, Boc-L-Cys-OH[15] was treated with 2-(2,4-dinitrophenyl)ethyl bromide, which has been obtained by direct nitration of 2-phenylethyl bromide. The second approach involves fewer steps and shows substantially improved purity of the final product.[16]



The Cys(Dnpe) derivative was quantitatively converted into cystine by treatment with piperidine–DMF (1:1 or 1:4) in 30 min and 2 h respectively, while the Cys(Fm) derivative required 2 and 15 h to complete the reaction under the same conditions. When the deprotection was made under argon in the presence of 2% of β-mercaptoethanol, the free thiol function was obtained after 1 h. In order to test the compatibility of this protecting group with a Boc/Bzl solid-phase peptide strategy, we have checked the stability of Cys(Dnpe) under several representative conditions used in that methodology. Thus, it was stable to DIEA–CH_2Cl_2 (1:19) for 2 h, TFA–CH_2Cl_2 (4:6) for 24 h, and HF–*p*-cresol or anisole (9:1), 1 h at 0 °C, and TFMSA–TFA–*p*-cresol (1:10:3), 2 h at 25 °C, reagents commonly used to cleave the peptide from the resin and deprotect the Bzl type protecting groups. Furthermore, Dnpe is also stable to oxidative conditions like $Tl(TFA)_3$ in TFA or I_2 in 80% aqueous HOAc used to form disulfides from Cys(Acm)-containing peptides, so it is orthogonal with *p*-methylbenzyl, acetamidomethyl and *tert*-butyl sulfide groups. These properties are similar to those of the Fm, however, the

Dnpe is less hindered than Fm, allowing better yields of the HF cleavage reaction when the *C*-terminal amino acid is cysteine.

Extending these studies to the syntheses of cysteine containing peptides, the Boc-Cys(Dnpe)-OH was applied to the stepwise solid-phase syntheses of several model peptides.



β-turn model peptide 1 **Oxytocin 2** **Deamino-oxytocin 3**

All peptide syntheses were carried out manually starting with an MBHA-resin, using a conventional synthetic program.[17] The tyrosine was protected as its *o*-chlorobenzyloxycarbonyl ether, while side-chain unprotected asparagine and glutamine were incorporated in presence of HOBt. Peptides were cleaved from resins in good yields (> 70 %) with anhydrous HF-anisole (95:5), 1 h, 0 °C. On the other hand, acidolytic cleavage with HF of the bis(Fm)-protected β-turn model peptide from the same MBHA-resin proceeded poorly in 20% yield. This result shows clearly the steric effect of the Fm group near the *C*-terminus. Bis(Dnpe)-protected peptides show good solubility properties in polar media and, if it is necessary, can be easily purified by conventional medium-pressure liquid chromatography. Unpurified protected peptides (> 95% purity, HPLC) were treated with piperidine–DMF (1:1) (10^{-4} M solutions) for 30 min at 25 °C and the cyclic peptides were obtained with good yields (90% for **1**, 57% for **2**, and 59% for **3**).[18]

Another variation in the syntheses of the above peptides involved the formation of disulfide bonds while peptide chains remain anchored on the solid-support.[19] This technique takes advantage of the solid-phase principle, thus excess of reactives (piperidine) and side-products (piperidine-adduct) can be removed easily by simple filtration and washing. Treatment of bis(Dnpe) peptide-resins with piperidine–DMF (1:1), 1 h, 25° C, provided the target peptides with similar yields to the ones obtained when cyclizations were carried out in solution.

In conclusion, a new protecting group for the thiol function of cysteine or 3-mercaptopropionic acid compatible with the Boc/Bzl solid-phase peptide synthesis strategy has been described. Our results indicate that *S*-Dnpe is a very useful alternative to the *S*-Fm group, since i) it is conveniently prepared by reaction of the corresponding thiol containing compound with 2-(2,4-dinitrophenyl)ethyl bromide, ii) it is safely removed by treatment with piperidine to give directly the disulfide bridge or in presence of β-mercaptoethanol the free thiol product, iii) it is less hindered facilitating the HF reaction when the *C*-terminal amino acid is Cys, and iv) the presence of the two nitro groups confers to peptides better solubility properties. Furthermore, a proper combination of Dnpe protecting group with Acm and/or *p*-MeBzl may facilitate orthogonal synthesis of target peptides containing two or more disulfide bridges. Finally, the cyclization reaction can be carried out before the HF cleavage, while peptide chains remain attached to the support.

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1) Taken in part from the Ph.D. Thesis of C.G.-E., University of Barcelona, 1990.

2) Abbreviations used are as follows: Acm, acetamidomethyl; Ac_2O, acetic anhydride; Boc, *tert*-butyloxycarbonyl; Boc_2O, di-*tert*-butyl dicarbonate; ^{t}BuOH, *tert*-butanol; Bzl, benzyl; DCC, *N,N'*-dicyclohexylcarbodiimide; DIEA, *N,N*-diisopropylethylamine; DMF, *N,N*-dimethylformamide; Dnpe, 2-(2,4-dinitrophenyl)ethyl; Fm, 9-fluorenylmethyl; HOBt, 1-hydroxybenzotriazole; HOAc, acetic acid; MBHA, *p*-

methylbenzhydrylamine; *p*-MeBzl, *p*-methylbenzyl; Mpa, 3-mercaptopropionic acid; TFA, trifluoroacetic acid; TFMSA, trifluoromethanesulfonic acid; Tl(TFA)3, thallium(III) trifluoroacetate; Ts, tosyl. Amino acid symbols denote the L-configuration unless indicated otherwise.

3) Present address: School of Pharmacy, University of Wisconsin-Madison, Madison, WI 53706, USA.

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12) Pfleiderer, W.; Schwarz, M.; Schirmeister, H. *Chem. Scripta* **1986**, 26, 147-154.

13) The corresponding derivative of 3-mercaptopropionic acid was obtained following the second strategy, with an overall yield of 67% from the 3-mercaptopropionic acid.

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16) The Dnpe derivatives of Boc-Cys-OH and 3-mercaptopropionic acid were obtained like a foam and a solid respectively, showed single spots in two different systems of TLC and correct IR, NMR, and MS spectra.

17) Cycles for incorporation of Boc-amino acids comprised deprotection with TFA–CH_2Cl_2 (3:7), neutralization with DIEA–CH_2Cl_2 (1:19), and a single coupling (2.5-fold excess) mediated by DCC in CH_2Cl_2. All couplings were ninhydrin [Kaiser, E.; Colescott, R.L.; Bossinger, C.D.; Cook, P.I. *Anal Biochem.* **1970**, 34, 595-598] or chloranil [Christensen, T. Acta Chem. Scan. **1973**, B33, 763-766] negative within 2 h.

18) These yields were calculated from amino acid analyses of peptide solutions before and after deprotection and cyclization reactions, and ascertained by comparison of the HPLC peak areas with those from a pure peptide sample of known concentration.

19) For a detailed report on techniques for resin-bound cyclization and related issues, see reference 10 and García-Echeverría, C.; Albericio, F.; Pons, M.; Barany, G.; Giralt, E. *Tetrahedron Lett.* **1988**, 30, 3845-3848.

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