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Photochemical desulfurization of L-cysteine derivatives *

Asensio González [a] and Gregorio Valencia [b]

[a] *Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, 08028 Barcelona, Spain*
[b] *Unit for Glycoconjugate Chemistry, C.I.D.-C.S.I.C., Jordi Girona 18-26, 08034 Barcelona, Spain*

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Abstract

Thiol groups can be reductively eliminated at room temperature by a photochemical method which makes use of triethylboron, triethylphosphite and visible light. Thus, after treating L-Cys **1a**, D-Pen **1b**, L-Cys-OMe **1c** and glutathione (γ-L-Glu-L-Cys-Gly) **3**, the corresponding desulfurized compounds L-Ala **2a**, D-Val **2b**, L-Ala-OMe **2c** and γ-L-Glu-L-Ala-Gly **4**, respectively, are prepared in high yields and with retention of configuration.

1. Introduction

The selective chemical modification of amino acid side chains in proteins is a subject of current interest.[1] The presence of free thiols and disulfide bridges in many proteins is of paramount importance in biological systems.[2] In this connection, the reductive desulfurization of the thiol function of L-cysteine in a protein, to convert the amino acid to L-alanine, is a chemical modification with many implications from the biochemical and structural point of view.

Only a few methods are known for thiol group elimination. Early work by Hoffmann on the conversion of alkanethiols to the corresponding alkanes used triethyl phosphite under thermal and photochemical conditions.[3] Improved procedures later proposed by Walling also relied on thermal treatment coupled to radical chain reactions initiated by catalytic amounts of azobisisobutyronitrile (AIBN).[4]

Owing to the low thermal stability of many biological substances, these harsh conditions are of little value in, for example, the selective chemical modification of amino acid side chains such as free thiol and disulfide bridges which play very important roles in maintaining the active conformation of many proteins and peptides. With the aim of developing new chemical tools for the study of sulfur related structural features of proteins, different attempts have been made at the reductive desulfurization of L-cysteine derivatives and L-cysteine containing peptides and proteins. However, up to now, the reaction

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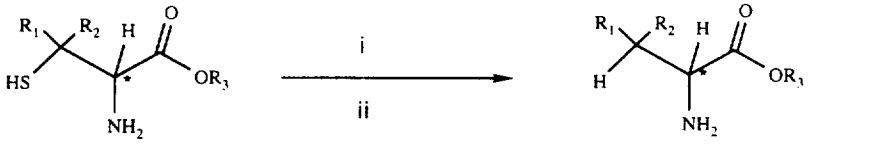
conditions assayed: i.e. Raney nickel,[5] palladium catalysis,[6] phosphorous acid at 100°C for 8 h[7] and triethyl phosphite at 110°C for 8 h,[8] do not guarantee the integrity of protein substrates since these reagents may also promote, for example, unwanted side chain hydrogenations, cleavage of peptide bonds and difficult separations of the modified proteins from metal based reagents.

2. Results and discussion

In trying to improve some of these methods, we have treated L-Cys **1a** with triethylphosphite under thermal conditions. However, L-Ala **2a** was not found among the reaction mixture which was always a phosphorus containing viscous oil that was not further characterized. At the same time, photochemical experiments by means of a 300 W visible light bulb, were conducted on L-Cys **1a** either in acetonitrile (ACN) or water solutions rendering unchanged L-Cys **1a**. Since these results could be partly explained by the limited solubility of L-Cys in ACN, a protective group of L-Cys was sought to improve the homogeneity of the reaction systems.

Organoboranes have received a great deal of attention.[9] In particular, Zwanenburg has described the use of different organoboranes for the simultaneous protection of α-amino and carboxyl groups of amino acids.[10] These derivatives allow the solubilization of amino acids in organic solvents and offer a number of advantages such as very easy and mild deprotection procedures. Although their applications are not widespread in amino acid chemistry,[11] the potential of triethylboron as an initiator of radical chain reactions[12] was considered a very suitable feature for the purposes of this work. Accordingly, new experiments were conducted by treating L-Cys **1a** in ACN with a *stoichiometric* amount of triethylboron for 1.5 h under an argon atmosphere to form the corresponding protected amino acid. After addition of triethylphosphite and irradiation with a light bulb for 6.5 h, the reaction mixture was quenched with 6 M HCl affording L-Ala hydrochloride **2a**·HCl in quantitative yield. When D-penicillamine **1b** was processed under identical conditions, D-Val·HCl **2b**·HCl was obtained in 95% yield (Scheme 1).



1a L- **2a** $R_1=R_2=R_3=H$ L-

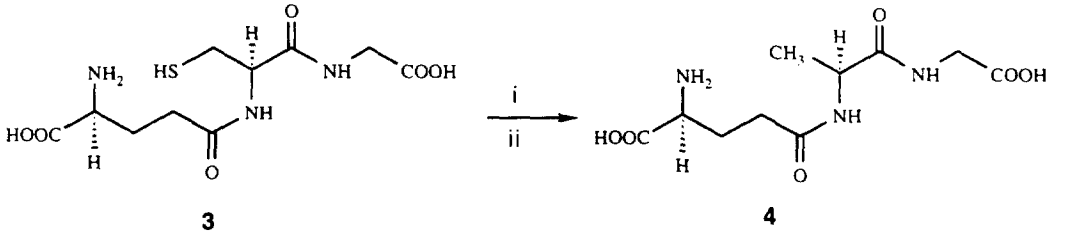
1b D- **2b** $R_1=R_2=CH_3$; $R_3=H$ D-

1c L- **2c** $R_1=R_2=H$; $R_3=CH_3$ L-

Scheme 1. (i) Et_3B/CH_3CN; (ii) $(EtO)_3P/h\nu$

The crucial role of triethylboron as radical initiator is evident in accomplishing this reductive desulfurization which is also supported by a number of recent kinetic mechanism studies of free-radical chain reactions of alkylthiyl radicals with organoboron and organophosphorous substrates.[13] A further argument in favor of the free-radical initiator hypothesis, rather than the protecting group solubilizing effect, was obtained by performing experiments with a readily soluble L-Cys derivative. Thus, when L-Cys-OMe·HCl **1c**·HCl containing triethylphosphite was irradiated as before in ACN, the starting compound was recovered. However, when the same experiment was conducted in the presence of *catalytic* amounts of triethylboron, L-Ala-OMe·HCl **2c**·HCl was obtained in high yield (93%).

Finally, from similar experiments performed on L-Ser, this sulfur elimination method was probed to be specific for thiol functions since L-Ser was always recovered as the exclusive reaction product. Moreover, this reaction system was successfully applied to a simple peptide model glutathione (γ-L-Glu-L-Cys-Gly) **3**, which was fully converted to the tripeptide, γ-L-Glu-L-Ala-Gly **4**,[14] in an acetonitrile/water mixture after 8 h of irradiation (Scheme 2).



3 **4**

Scheme 2. (i) Et_3B/CH_3CN; (ii) $(EtO)_3P/h\nu$

In conclusion, we propose a new method for the efficient removal of the thiol function from L-Cys and related molecules.[15] The procedure relies on a photochemical method that may follow a free-radical chain reaction mechanism initiated by triethylboron. Features such as mild reaction conditions (room temperature), high yields, operational simplicity, lack of side reactions (hydroxyl groups not affected) and compatibility of the reagents with the presence of water, makes this method an ideal procedure for the elimination of thiol groups from peptides. Studies aimed at the application of this process in protein chemistry are currently underway in our laboratories.

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References

1. Lundblad, R. L., *Techniques in Protein Modification*; CRC Press: Boca Raton, 1995.
2. (a) Stryer, L., *Biochemistry*, 4th Edn; W. H. Freeman & Co.: New York, 1995; (b) *Comprehensive Medicinal Chemistry*, Vol. 5; Sammes, P. G., Taylor, J. B., Ed.; Pergamon Press: Oxford, 1990; chapter 5; (c) Friedman, M., *The Chemistry and Biochemistry of the Sulfhydryl Group in Amino Acids, Peptides and Proteins*; Pergamon Press: Oxford, 1973; reviews on reductive desulfurization: (d) Caubère, P., Coutrot, P. In *Comprehensive Organic Synthesis*, Vol. 8; Trost, B. M., Fleming I., Eds; Pergamon Press: Oxford, 1991; chapter 4.3.1, pp. 835–847; (e) Cadogan, J. I. G., *Organophosphorus Reagents in Organic Synthesis*; Academic Press: London, 1979; (f) Motherwell, W. B., Crich, D., *Free Radical Chain Reactions in Organic Synthesis*; Academic Press: London, 1992; pp. 71–78.
3. Hoffmann, F. W., Ess, R. J., Simmons, T. C., Hanzel, R. S., *J. Am. Chem. Soc.*, **1956**, *78*, 6414.
4. (a) Walling, C., Rabinowitz, R., *J. Am. Chem. Soc.*, **1957**, *79*, 5326; (b) Walling, C., Rabinowitz, R., *J. Am. Chem. Soc.*, **1959**, *81*, 1243–1249; (c) Walling, C., Basedow, O. H., Savas, E. S., *J. Am. Chem. Soc.*, **1960**, *82*, 2181–2184; (d) Walling, C., Pearson, M. S., *J. Am. Chem. Soc.*, **1964**, *86*, 2262–2266.
5. Perstein, M. T., Atassi, M. Z., Cheng, S. H., *Biochem. Biophys. Acta*, **1971**, *236*, 174
6. Pham, P. In *Peptides 1992*, Schneider, C. H., Eberle, A. N., Eds; ESCOM: Leiden, 1993; pp. 183–184.
7. Ivanov, C., Ivanov, O. C., *Dokl. Bulg. Akad. Nauk*, **1970**, *23*, 1365–1367.
8. Ivanov, C., Ivanov, O. C., *Dokl. Bulg. Akad. Nauk*, **1969**, *22*, 49–52.
9. (a) *Comprehensive Organic Chemistry*, Vol. 3; Barton, D., Ollis, W. D., Eds; Pergamon Press: Oxford, 1979; pp. 687–940; (b) *Comprehensive Organometallic Chemistry*, Vol. 1; Wilkinson, G., Ed.; Pergamon Press: Oxford, 1982; chapter 5.2; (c) *Comprehensive Coordination Chemistry*, Vol. 3; Wilkinson, G., Ed.; Pergamon Press: Oxford, 1987; chapter 24; (d) *Comprehensive Organometallic Chemistry*, Vol. 7; Wilkinson, G., Ed.; Pergamon Press: Oxford, 1982; chapter 45; (e) *Current Topics in the Chemistry of Boron*; Kabalka, G. W., Ed.; The Royal Society of Chemistry: Cambridge, 1994.

10. (a) Nefkens, G. H. L., Zwanenburg, B., *Tetrahedron*, **1983**, *39*, 2995–2998; (b) Baum, G., *J. Organomet. Chem.*, **1970**, *22*, 269–271.
11. (a) Garrigues, B., Mulliez, M., Raharinirina, A., *J. Organomet. Chem.*, **1986**, *302*, 153–158; (b) Garrigues, B., Mulliez, M., *J. Organomet. Chem.*, **1986**, *314*, 19–24; (c) Brown, H. C.; Gupta, A. K., *J. Organomet. Chem.*, **1988**, *341*, 73–81; (d) Robles, J., Pedroso, E., Grandas, A., *Synthesis*, **1993**, 1261–1266; (e) Yang, L., Weber, A. E., Greenlee, W. J., Patchett, A. A., *Tetrahedron Lett.*, **1993**, 7035–7038; (f) Acher, F., Azerad, R., *Tetrahedron: Asymmetry*, **1994**, *5*, 731–744; (g) Vedejs, E.; Fields, S. C.; Lin, S.; Schrimpf, M. R., *J. Org. Chem.*, **1995**, *60*, 3028–3034.
12. (a) Nozaki, K., Oshima, K., Utimoto, K., *Tetrahedron Lett.*, **1988**, 1041–1044; (b) Nozaki, K., Oshima, K., Utimoto, K., *Tetrahedron*, **1989**, *45*, 923–933; (c) Barton, D. H. R., Jang, D. O., Jaszberenyi, J. C., *Tetrahedron Lett.*, **1990**, 4681–4684; (d) Nozaki, K., Oshima, K., Utimoto, K., *Bull. Chem. Soc. Jpn*, **1991**, *64*, 403–409; (e) Baciocchi, E., Muraglia, E., *Tetrahedron Lett.*, **1993**, 5015–5018; (f) Czernecki, S., Ayadi, E., Xie, J., *Tetrahedron Lett.*, **1996**, 9193–9194; (g) Brown H. C., Midland M. M., *Angew. Chem. Int. Ed. Engl.*, **1972**, *11*, 692–700; (h) Ghosez, A., Giese, B., Zipse, H., Houben-Weil E19a/Teil 2, 1989; chapter 9.2.3., pp. 753–765.
13. (a) Griller, D., Ingold, K. U., Patterson, L. K., Scaiano, J. C., Small, R. D., *J. Am. Chem. Soc.*, **1979**, *101*, 3780–3785; (b) Franz, J. A., Bushaw, B. A., Alnajjar, M. S., *J. Am. Chem. Soc.*, **1989**, *111*, 268–275; (c) McPhee, D. J., Campredon, M., Lesage, M., Griller, D., *J. Am. Chem. Soc.*, **1989**, *111*, 7563–7567.
14. Chen, W. J., Boehlert, C. C., Rider, K., Armstrong, R. N., *Biochem. Biophys. Res. Commun.*, **1985**, *128*, 233–240.
15. *Typical experiment*: A 1 M solution of Et_3B in tetrahydrofuran (10.5 mL, 10.5 mmol) was added to L-Cys **1a** (1.21 g, 10 mmol) in 20 mL of acetonitrile. The reaction mixture was stirred at room temperature under argon for 90 min. Triethylphosphite (5.6 mL, 33 mmol) was added and the resulting mixture was irradiated with a 300 W visible light bulb located about 20 cm from the flask for 6.5 h in an open system (Dimroth refrigerant). The organic solvent was evaporated under reduced pressure. 6 M HCl (6 mL) was added, the mixture was stirred for 5 min and then was extracted with CH_2Cl_2 (3×2 mL). Evaporation of the acidic fraction under high vacuum gave L-alanine hydrochloride **2a**·HCl (1.2 g, 96% yield) identical in all respects with the authentic product. *Selected spectral data*: **2a**·HCl: $[\alpha]_D^{22}$=+14.2 (c=10, 6 N HCl), lit.: $[\alpha]_D^{20}$=+14.5 (c=10, 6 N HCl); ^{13}C NMR (D_2O, 50 MHz) δ 175.3 (C=O), 51.4 (CH), 18.0 (CH_3). **2b**·HCl: $[\alpha]_D^{22}$=−27.1 (c=3.4, 6 N HCl), lit.: $[\alpha]_D^{23}$=−27.4 (c=3.4, 6 N HCl); ^{13}C NMR (D_2O, 50 MHz) δ 174.3 (C=O), 61.1 (CH), 31.8 (CH), 20.2 (CH_3), 19.7 (CH3). **2c**·HCl: $[\alpha]_D^{22}$=+6.8 (c=1.6, CH_3OH), lit.: $[\alpha]_D^{25}$=+7.0 (c=1.6, CH_3OH); ^{13}C NMR (D_2O, 50 MHz) δ 174.1 (C=O), 56.4 (CH_3), 51.6 (CH), 18.0 (CH_3). Glutathione (γ-L-Glu-L-Cys-Gly) **3** treated in acetonitrile/water with triethylboron for 2 min and irradiated with triethylphosphite for 8 h was converted into γ-L-Glu-L-Ala-Gly **4**.[14] The reaction was checked by RP-HPLC and MALDI-TOF-MS. Glutathione **3**, [M+H]=308.12, [M+Na]=331.11. Corresponding alanyl tripeptide **4**, [M+H]=276.10. ^{13}C NMR (D_2O, 50 MHz) δ 177.1, 175.6, 175.3, 174.1, 54.8 (CH), 52.5 (CH), 43.9 (CH_2), 33.7 (CH_2), 28.1 (CH_2), 19.3 (CH_3).