

Novel Conversion of Thiols into Disulfides, *via* S-Nitrosothiol Intermediates using Trichloronitromethane

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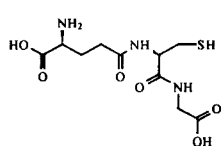
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Abstract: An efficient oxidative coupling of thiols to give disulfides *via* thionitrite (S-nitrosothiol) intermediate is described using trichloronitromethane as an efficient reagent in organic solvents and water. Cysteine and glutathione are converted into the corresponding disulfides in water in high yields. © 1999 Elsevier Science Ltd. All rights reserved.

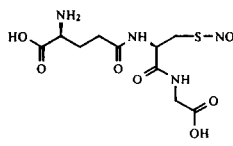
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INTRODUCTION

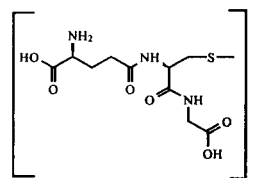
The vital role of thiols and disulfides in living systems has focused on their interconversion reactions. Oxidative coupling of thiols to give disulfides under neutral and mild conditions is important from biological and practical points of view,¹ and has been widely studied with different reagents.² There are a limited number of reagents, which could serve as candidates for the oxidation of thiol to disulfide *in vivo*. The formation of disulfides *via* thionitrite (S-nitrosothiol) intermediates is one of the methods, which also works under mild conditions. Thionitrites are the sulfur analogs of the much more well known alkyl nitrites. Intense interest in the chemistry of thionitrites has been generated in connection with the newly discovered remarkable physiological roles of nitric oxide, including particularly vasodilation and cytotoxic action of macrophages. Thiols and S-nitrosothiols are believed to be involved in *in vivo* processes, possibly in the mechanism of NO transfer reactions.^{3a}



glutathione



S-nitroso-glutathione



glutathione disulfide

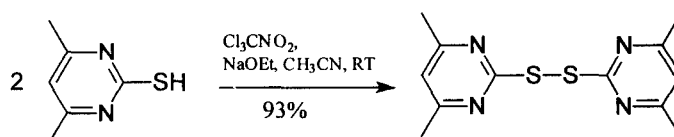
Using a model transnitrosation reaction between S-nitroso-glutathione (GSNO) and glutathione, Tannenbaum *et al.*^{3b} have shown that the S-nitroso-glutathione (GSNO) was decomposed to oxidized glutathione, nitrite, nitrous oxide, and ammonia. As early as 1840, a red color was observed when thiols were treated with nitrous acid. Much later Tasker and Jones⁴ reported that unstable red colored phenyl thionitrite was formed when thiophenol was treated with nitrosyl chloride. During this reaction thionitrite was found to decompose rapidly to give diphenyl disulfide and nitrogen oxide. Later, several thionitrites were prepared by treatment of thiols with nitrosyl chloride, alkyl nitrites, dinitrogen tetroxide, dinitrogen trioxide, nitrogen dioxide, and nitrous acid.⁵

Thiols are coupled, oxidatively, into dithiols by high-valent transition metal ions. In this process clay-supported ferric nitrate (“clayfen”) or cupric nitrate (“claycop”) act as oxidants and the reaction goes through thionitrite intermediates.⁶ A catalytic oxidative coupling of thiols to disulfides is described using $\text{Cu}(\text{NO}_3)_2 \cdot \text{N}_2\text{O}_4$ as a nitrosating agent in acetone. The NO^+ nitrosonium ion is supplied by the clay-based reagent. Both “clayfen” and “claycop” act as sources or reservoirs of NO^+ ions.⁶

Ongoing research by our group has included investigations into the use of trichloronitromethane in nitrosation reaction and syntheses. One of the observations was the mild and easy conversion of secondary amines into nitrosamines.⁷ The decomposition of trichloronitromethane was found to give phosgene and nitrosyl chloride⁸ (Scheme 2a). The nitrosyl chloride acts as a nitrosation reagent. Later investigations of reactions of trichloronitromethane with various thiols found that this compound might be a good general-purpose nitrosating agent. Attractive features included their efficiency, ease of preparation and handling.

We are pleased to report formation of the symmetrical RSSR disulfides with excellent yields (Table 1).

RESULTS AND DISCUSSIONS



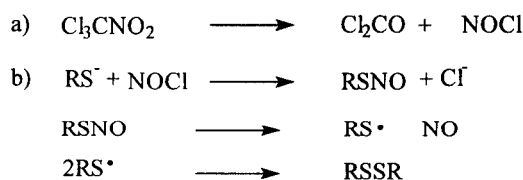
Scheme 1

In an initial reaction shown in Scheme 1 trichloronitromethane was added to a solution of 4,6-dimethylpyrimidine thiol (**1a**) and sodium ethoxide (0.1 mol/mol of thiol) in acetonitrile (the solution turned red). This was stirred at RT and the reaction was monitored by TLC. The reaction afforded pure 4,6,4',6'-tetramethyl-2,2'-disulfanediy-bis-pyrimidine (**2a**) in 93% yield after recrystallisation of crude product from

methanol (white needle like crystals, mp 168-170°C). Using the same procedure, different disulfides were prepared in high yield as summarized in Table 1. The reaction of thiophenol in organic solvents, aqueous suspension or aqueous ethanolic solution with trichloronitromethane at pH ranges 6-9 results in almost quantitative formation of disulfide. The reaction of thiophenol with trichloronitromethane without solvent also furnished the disulfide in 98% yield. Cysteine is converted to cystine by trichloronitromethane at pH 7 in water (yield 70%), and the rate of conversion is increased at pH 9 (yield 92%). During this conversion nitrosamine formation is not observed. Glutathione, a tripeptide, which is present in all living cells and plays an important role in biological redox systems,⁹ was also treated with trichloronitromethane in water at pH 7 and oxidized glutathione disulfide in 90% yield. Satisfactory spectral (NMR, IR) data were obtained. An important feature of these reactions, apart from their high yields, is the freedom from byproducts or products of over oxidation such as sulfoxides, sulfones, *etc.* that are formed by other oxidations. In addition, the reaction can be carried out in organic solvents, or in aqueous solution, and the workup of the reaction is extremely easy.

Attempts to prepare an unsymmetrical disulfide selectively starting from different thiols failed. The reaction of 2-thiopyridine with thiophenol in acetonitrile gave all three possible disulfides. Optimization experiments for the formation of unsymmetrical disulfide as major product were not successful.

The oxidative coupling of thiols into disulfides may occur by a thionitrite type pathway as shown in Scheme 2b.¹⁰ Our results may be explained by assuming a reaction of thiophenolate with NO⁺ to give thionitrite (red color of the solution) then formation of the radical followed by radical coupling giving the product. This is followed by a sudden evolution of nitric oxide with concomitant fading of the red color. All attempts to isolate and characterise the S-nitrosoglutathione failed.








Scheme 2

In conclusion, trichloronitromethane was shown to be a good general reagent for preparing symmetrical disulfides from thiols in high yield. This method, as compared with nitric oxide or nitrogen dioxide oxidation or clay-catalyzed oxidation, has the advantage of not requiring gaseous nitrogen dioxide or catalysts as reagents and is a novel way of activating thiols into thionitrites, using extremely mild reaction conditions. The reactions can be carried out without solvent, in organic solvents or aqueous solutions and ambient temperature.

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Table 1. Synthesis of disulfides from thiols

Thiols 1	Solvent	pH ^a	Reaction Time (h)	Disulfides ^b 2 Yield (%) mp or bp(torr) ^o C
 a	CH ₃ CN	Alkaline	2	93 mp 168-170 (166-168) ¹¹
 b	CH ₃ CN	Alkaline	3	91 mp 113-114 (113) ¹²
 c	CH ₃ CN	Alkaline	3	94 bp 90-92 (0.5) (90, (0.5)) ¹³
 d	CH ₃ CN	Alkaline	1	91 mp 128-130 (127-129) ^{2b}
 e	CH ₃ CN	Alkaline	2	92 mp 56-57 (57-58) ¹⁴
n-C₄H₉SH f	CH ₃ CN H ₂ O/EtOH (1:4)	Alkaline 9	3 1	93 bp 116-118(20) 112-115(18) ^{2b} 95
C₆H₅SH g	-	-	1	98
	CH ₃ CN H ₂ O H ₂ O/EtOH (1:4) H ₂ O/EtOH (1:4)	Alkaline 6 6 9	2 4 4 1	96 94 95 96
4-Cl-C₆H₄SH h	CH ₃ CN	Alkaline	3	91 mp 71-72 (71) ¹⁶
Cysteine i	H ₂ O H ₂ O/EtOH (1:4) H ₂ O	7 7 9	6 8 1	70 72 90
Glutathione j	H ₂ O	7	3	92 mp 178-180 (178-180) ¹⁷

a) Alkaline signifies addition of 0.1 mol of sodium ethoxide/mol of thiol. b) Products were isolated and characterized by physical and spectral properties. The spectroscopic and physical properties are identical in all respects with the reported values.

EXPERIMENTAL

All reagents were of commercial quality, and reagent quality solvents were used without further

purification. IR spectra were determined on a Philips model PU9700 spectrometer. ^1H NMR spectra were determined on a Bruker AC 80 MHz FT, AC 200 MHz and Bruker DPX 400 MHz FT spectrometers. GC analyses were determined on a HP 5890 gas chromatograph.

Synthesis of 4,6,4',6'-tetramethyl-2,2'-disulfanediyl-bis-pyrimidine (2a). Typical procedure: Trichloronitromethane (3.28 g, 20 mmol) was added to a solution of 4,6-dimethylpyrimidine thiol (1a) (0.6 g, 5 mmol) and sodium methoxide (0.1 mol/mol of thiol) in 30 ml of dry acetonitrile. This was stirred at RT 2 hours and the reaction was monitored by TLC (Silica gel, hexane: EtOAc 20:1). Then the mixture was evaporated to dryness and dissolved in 100 ml of chloroform. The chloroform solution was washed with brine (30 ml), dried over magnesium sulfate and the solvent was evaporated. The remaining solid material was recrystallized twice from methanol to afford 1.03 g (93%) of 4,6,4',6'-tetramethyl-2,2'-disulfanediyl-bis-pyrimidine (2a) as white needle like crystals (mp 168-170°C, lit.¹¹ 166-168°C). NMR, IR data are identical with the reported values.¹¹

Synthesis of diphenyldisulfide (2g) without solvent: Trichloronitromethane (6.56 g, 40 mmol) and thiophenol (1.05 g, 10 mmol) were stirred at RT for 1h and the (solid) formed was filtered off, the mother liquor was dissolved in 200 mL CHCl_3 , washed with water, and combined with the solid part. The chloroform solution was washed with brine (30 ml), dried over Na_2SO_4 . Evaporation of the solvent gave 2.13 g (98%) pure diphenyldisulfide (mp 58-60°C, lit.¹⁵ 60°C). NMR, IR data are identical with the reported values.¹⁵

Cystine (2i): Trichloronitromethane (3.28 g, 20 mmol) was added to a solution of cysteine (0.60 g, 5 mmol) in 25 ml of water and the pH was adjusted with 1M NaOH. The solution was stirred at RT and the reaction was monitored by TLC (Silica gel, MeOH). Then the mixture was evaporated to dryness and recrystallized from water to afford 0.76 g (92 %) of cystine (m.p. 257-259°C, lit.¹⁷ 260°C). NMR, IR data are identical with an authentic sample.

Glutathione disulfide (2j): Trichloronitromethane (3.28 g, 20 mmol) was added to a solution of glutathione (0.75 g, 2.5 mmol) in 20 ml of water. This was stirred at RT and the reaction was monitored by TLC (Silica gel, MeOH). Then the mixture was evaporated to dryness and recrystallization from aq. EtOH afforded 1.35 g (90%) of glutathione disulfide (m.p. 178-180°C, lit.¹⁷ 178-180°C). NMR, IR data are identical with an authentic sample.

REFERENCES AND NOTES

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1. (a) Jocelyn, P. C. "Biochemistry of the thiol groups" Academic Press, New York, 1977. (b) Rice, W. G.; Turpin, J. A.; Schaeffer, C. A.; Graham, L.; Clanton, D.; Buckheit, Jr. R. W.; Zaharevitz, D.; Summers, M. F.; Wallqvist, A.; Covell, D. G. *J. Med. Chem.* **1996**, *39*, 3606. (c) For a general

- review see: Friedman, M. "The Chemistry and Biochemistry of the Sulfhydryl group in Amino acids, Peptides and Proteins"; Pergamon Press: Oxford, 1973.
2. (a) Baird, C. P.; Rayner, C. M. *J. Chem. Soc., Perkin Trans. 1*, **1998**, 1973. (b) Iranpoor, N.; Firouzabadi, H.; Zolfigol, M. A. *Synthetic Commun.* **1998**, 28(2) 367. (c) Barton, D. R. H.; Chen, C.; Wall, G. M. *Tetrahedron*, **1991**, 47, 6127. (d) Brzezinska, E.; Ternay, Jr. A. L. *J. Org. Chem.* **1994**, 59, 8239. (e) McKillop, A.; Koyuncu, D. *Tetrahedron Lett.* **1990**, 31, 5010. (f) Dick, A. P.; Swift, H. R.; Williams, D. L. H.; Butler, A. R.; Al'Sa'doni, H. H., Cox, B. G. *J. Chem. Soc. Perkin Trans. 2*, **1996**, 481.
 3. (a) Askew, S. C.; Barnett, D. J.; McAninly, J. Williams, D.L. H. *J. Chem. Soc. Perkin Trans 2* **1995**. (b) Singh, S. P.; Wishnok, J. S.; Keshive, M.; Deen, W. M.; Tannenbaum, S. R. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, 93, 14428.
 4. Tasker, H. S.; Jones, H. O. *J. Chem. Soc.* **1909**, 95, 1917.
 5. Oae, S.; Shinhama, K. Review, *Organic Prep. Proced. Int.* **1983** 167.
 6. (a) Cornelis, A.; Depaye, N.; Gerstmans A.; Laszlo, P. *Tetrahedron Lett.* **1983**, 24, 3103. (b) Cornelis, A.; Laszlo, P. *Synlett* **1994**, 155.
 7. Demir, A. S.; Mahasneh, A. S.; Aksoy, H.; Gercek, Z. *Synthetic Commun.* **1992**, 22(18), 2607.
 8. Gardner, J. A.; Fox, F. W. *J. Chem. Soc.* **1919**, 115, 1188.
 9. (a) Kosmrlj, J.; Kocevar, M.; Polanc, S. *J. Chem. Soc. Perkin 1*, **1998**, 3917. (b) *Glutathione: Chemical, Biochemical, and Medical Aspects*, Eds. Dolphin, D.; Poulson, R.; Avramovic, O. Wiley-Interscience. New York, 1989. Part A and part B.
 10. Pryor, W. A.; Church, D. F.; Govindan, C. K.; Crank, G. *J. Org. Chem.* **1982**, 47, 156.
 11. Stoyanov, S.; Petkov, I.; Antonov, L.; Stoyanova, T.; Karagiannidis, P. *Can. J. Chem.* **1990**, 68, 1482.
 12. Goyal, R. N.; Verma, M. S. *Indian J. Chem. Sec. A* **1996**, 35, 281.
 13. Huber, U. A.; Bergamin, D. *Helv. Chim. Acta* **1993**, 76, 2528.
 14. Sisler, H. H.; Kotia, N. K., Highsmith, R. E. *J. Org. Chem.* **1970**, 35, 1742.
 15. Ogura, F. Yamaguchi, H.; Otsubo, T.; Tanaka, H. *Bull. Chem. Soc. Jpn.* **1982**, 55, 641.
 16. Seebach, D.; Beck, A. K. *Chem. Ber.* **1972**, 105, 3892.
 17. From commercial available compounds.