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# A SYNTHETIC METHOD FOR UNSYMMETRICAL DISULFIDES OF CYSTEINE: THE BIS-CYSTEINE DISULFIDE OF *meso*-2,3-DIMERCAPTOSUCCINIC ACID.

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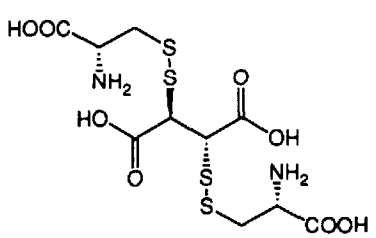
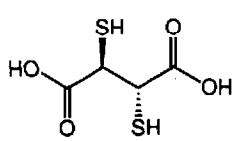
***Summary:*** *meso-Dimercaptosuccinic acid (DMSA) is the drug of choice for the treatment of lead-poisoning. DMSA is excreted in the urine of lead-poisoned rabbits as a conjugate with two molecules of cysteine. To confirm this, and to examine the hypothesis that DMSA may be acting as a pro-drug, we have synthesized the DMSA-bis-disulfide using novel methodology based on the use of sulfenimides derived from cysteine.*

*meso*-Dimercaptosuccinic acid (**DMSA**, **2a**) is a metal-binding agent,[1] which has been shown to be effective in the treatment of intoxication by lead, mercury, and arsenic.[2] DMSA was classified as an "orphan drug" and has recently been approved for the treatment of heavy metal poisoning by the U.S. Food and Drug Administration.[3] It has been suggested that DMSA is present *in vivo* as a mixed disulfide with cysteine, bound either to plasma proteins,[4] or glutathione.[5] It has also been postulated that most orally administered DMSA is excreted through the kidneys in the form of a *bis*-disulfide with two molecules of cysteine.[4a] In order to confirm this hypothesis we have synthesized the bis-disulfide **1**.



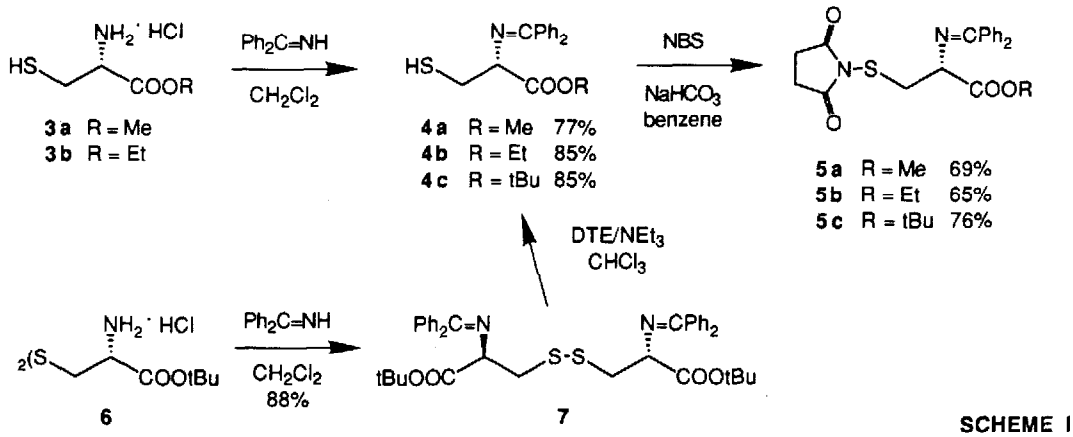

**1**




**(DMSA)**

**2a**

In spite of the numerous methods available for the synthesis of mixed disulfides,[6] the production of these compounds remains problematic. Disulfide interchange has been shown to be extremely fast, and is catalysed by free sulfhydryl groups, by amines, and by other bases.[7] This has proven to be a major difficulty in the synthesis, and synthetic use of unsymmetrical disulfides, particularly in the area of peptide synthesis.[8] The introduction of N-alkylthio-imides (sulfenimides) as organothio-group transfer reagents[9,10] seemed to be a particularly attractive solution for the preparation of cysteine disulfides, but appropriate N-protection still presented a serious synthetic problem.[11,8c] Since the N-diphenylmethylene group (-N=CPh$_2$) can be removed with dilute aqueous acid at 0°C,[12] we have explored its use in the synthesis of compound **1** .

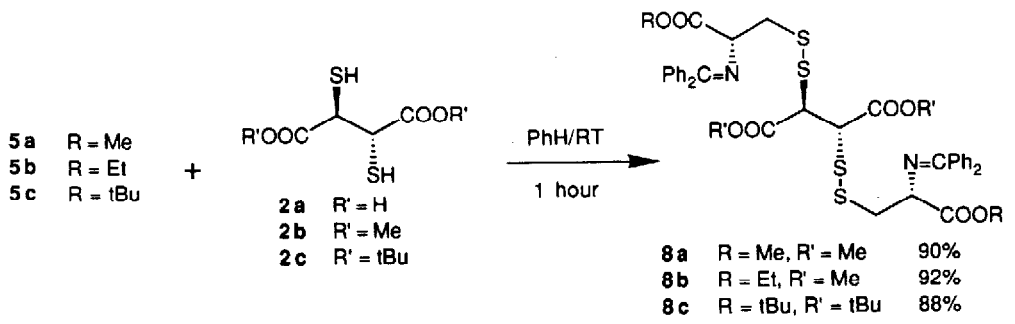



**SCHEME I**

The Schiff base esters **4a** and **4b** were synthesized from the commercially available L-cysteine ester hydrochlorides **3a** and **3b** using the procedure of O'Donnell[12] (**Scheme** I). The hindered *tert*-butyl ester Schiff base **4c** was prepared from the corresponding dimeric L-cystine derivative **7** by disulfide interchange with dithioerythritol (DTE).[13] Schiff base 7 was synthesized from L-cystine di-t-butyl ester·2HCl, **6**,[14] using the standard methodolgy used for **4a** and **4b**. Electrophilic activation of the free sulfides **4a—c** was accomplished by treatment with N-bromosuccinimide (NBS) in benzene for 30 minutes in the presence of solid $NaHCO_3$. Surprisingly, although Harpp's published stanylation procedure[10d] was satisfactory (79% for **4a**), treatment of the resulting stanylsulfide with NBS was not as effective (38% yield of **5a**) as the direct route. Perhaps more surprisingly, the order of addition was not important for this reaction— probably due to the fact that the acyclic Schiff bases **4a—c** are only minor species in solution, being tautomeric with the more stable thiazolidine heterocycles (**Scheme II**). Thus, regardless of the order of addition, the concentration of free sulfhydryl groups is low at any particular moment, and the expected oxidative disulfide formation is not observed. It is not clear if it is the cyclic thiazolidine form which reacts with NBS or the Schiff base form.

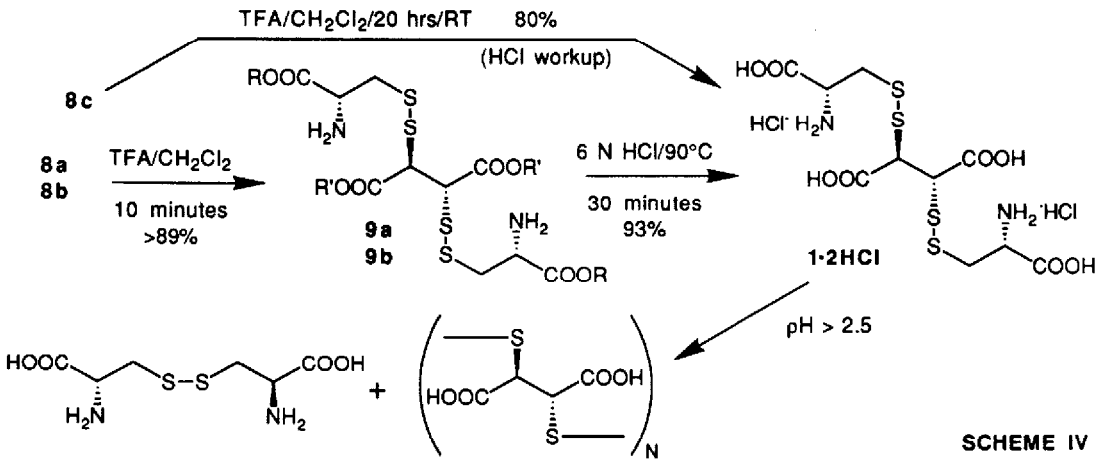

**SCHEME II**

The cysteinyl thiosuccinimides **5a—c** were purified on silica gel using flash chromatography,[15] and could be reacted with either *meso*-dimercaptosuccinic acid, **2a**, or the corresponding dimethylester, **2b**, and di-*tert*-butyl ester, **2c**, using Harpp's[10c] methodology (**Scheme** III). Although the yield of disulfide for the coupling reactions were similar, the less polar, fully protected tetra-ester intermediates **8a—c** were much easier to purify via silica gel chromatography than the corresponding diacid-bis-disulfides.




**SCHEME III**

Hydrolysis of compounds **8a-8b** could be accomplished (**Scheme IV**) in a stepwise procedure by removing the Schiff base first with 10% TFA in moist $CH_2Cl_2$ or THF for 10 minutes, followed by aqueous hydrolysis of the ester groups, or by simultaneous N- and C-deprotection with aqueous acid. Although hydrolysis of the DMSA -OR' ester groups proceeded quickly under a variety of conditions, the amino acid ester groups -OMe and -OEt were more recalcitrant, requiring temperatures of 60-90°C for complete hydrolysis. Compound **8c** could be hydrolysed easily in 20% TFA in $CH_2Cl_2$ to provide **1** in 80% yield after 20 hours at room temperature. The product **1** was completely stable only under acidic conditions (pH < 2.5), and was most stable in a 1.0 N HCl solution. Attempts to raise the pH of solutions of **1** above pH 2.5 resulted in rapid disulfide interchange to form cystine, and precipitation of polymeric disulfides of DMSA. Lyophilization of acidic aqueous solutions of **1** provided the pure substance as an off-white powder **1·2HCl**.[16]




**SCHEME IV**

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16. Compound **1·2HCl** was obtained as follows: To a stirred suspension of NBS (7.60 g, 43 mmol) and $NaHCO_3$ (1.90 g, 22 mmol) in 76 ml benzene was added cysteine methyl ester Schiff base **4a**[12] (4.60 g, 15.4 mmol) at 0-5°C. Stirring was continued for 30 minutes before the reaction was poured into ice water, extracted with benzene, washed with 5% $Na_2S_2O_3$, saturated $NaHCO_3$, dried ($MgSO_4$), and evaporated to yield a crude oil. Silica gel chromatography[15] (eluant - 40% EtOAc/hexanes) of this oil provided 4.20 g (10.6 mmol, 69%) crystalline **5a** (m.p. 116-117.5°, Calc. for $C_{21}H_{20}N_2O_4S$: $C_{63.62}H_{5.08}N_{7.06}$, Found: $C_{63.56}H_{5.06}N_{7.07}$). A solution of *meso*-dimercaptosuccinic acid dimethyl ester, **2b** (706.7 mg, 3.07 mmol), in 23 ml of benzene was added to a solution of sulfenimide **5a** (3.65 g, 9.2 mmol) in 42 ml benzene at room temperature with stirring. After 1 hour the reaction was filtered and evaporated to give a dark oil. Silica gel chromatography[15] (eluant - 30% EtOAc/hexanes) of this oil provided 2.27 g (2.76 mmol, 90%) of desired bis-disulfide **8a** (m.p. 115-117°). Compound **8a** (2.00 g, 2.42 mmol) was suspended in 100 ml 6 N HCl and immersed in an oil bath at 95°C with vigorous stirring. After 30 minutes the reaction was cooled, washed with $Et_2O$ (3 X 50 ml) to remove $Ph_2C{=}O$, and filtered with charcoal. The aqueous solution was then lyophilized to dryness providing 1.12 g of the bis-disulfide **1·2HCl** (2.27 mmol, 93%) as an off-white powder (m.p. 168-170° dec., Calc. for $C_{10}H_{18}N_2O_8S_4Cl_2$: $C_{24.34}H_{3.68}N_{5.68}$, Found: $C_{24.04}H_{3.76}N_{5.39}$).


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