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L-Cysteine as a water-soluble cation scavenger in the removal of the 2,4,6-trimethoxybenzyl group from thiols

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Abstract—L-Cysteine was used as a water-soluble cation scavenger in the acid-catalyzed removal of the 2,4,6-trimethoxybenzyl (Tmob) group from thiols. After aqueous extraction, the product thiols were isolated in good yields. For most substrates, trifluoroacetic acid (TFA) is the reagent of choice. However, for the deprotection of acid-sensitive compounds, formic acid with an extended reaction time is appropriate.

During the last decade nitric oxide (NO) has been identified as an important signaling molecule associated with neurotransmission, smooth muscle relaxation, platelet inhibition, and immune system regulation.[1] NitroMed is committed to the development of nitric oxide enhanced (NitRx) medicines in which NO complements the biological activity of existing drugs by increasing the safety and/or efficacy of the parent molecules. The nitrosothiol moiety is one of the NO-donor groups used by NitroMed for the preparation of NitRx medicines and frequently, the preparation of nitrosothiol containing compounds requires protection of an intermediate thiol.

The 2,4,6-trimethoxybenzyl (Tmob) group was originally developed as a side-chain protecting group for cysteine in the N^{α}-9-fluorenylmethoxycarbonyl (Fmoc) strategy of solid-phase peptide synthesis (SPPS).[2] Deprotection of the Tmob group in SPPS was done with trifluoroacetic acid (TFA)-methylene chloride in the presence of phenol, thioanisole and water or in the presence of triethylsilane or triisopropylsilane. When employing the synthesis on solid support strategy, removal of the byproducts and excess cation scavenger can be simply done by filtration.

We have utilized the Tmob protecting group in the course of preparing tertiary nitrosothiols via conventional solution phase methods. Deprotection of the intermediate Tmob protected thiols was successful with TFA in the presence of phenol, anisole and water, however the desired products were always contaminated with several trimethoxybenzyl-containing byproducts as well as the excess reagents. Consequently, isolation of the desired product thiols often required a tedious silica gel chromatographic separation.

Herein we wish to report the successful use of L-cysteine as a water-soluble cation scavenger in the removal of the Tmob group. The Tmob protected thiols were treated with 10 equiv. of L-cysteine in 50% TFA for 10 min. Following a simple aqueous work up the thiols were obtained in good yields. Eight examples (**2**–**9**) are shown in Fig. 1 along with the isolated yields of the thiols, except compound **3** whose yield was determined by quantitative mass spectra analysis. In each instance, the resulting thiol was further purified by recrystalization, elution through a pad of silica gel or was of sufficient purity to carry forward in the reaction sequence.

For the preparation of Tmob protected thiols, 2,4,6-trimethoxybenzyl alcohol **1** was first prepared from 2,4,6-trimethoxybenzaldehyde according to the literature in 97% yield.[2] Protected thiols **2**–**5** were prepared by reacting the corresponding thiols with **1** in methylene chloride with 5% TFA. For the preparation of compounds **6**–**9**, the thiol containing acid **10**,[3] was first converted into its S-Tmob derivative **11** with 2,4,6-trimethxybenzyl alcohol. This was then coupled (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride) to the corresponding thiol or alcohol to give **6**–**9**.

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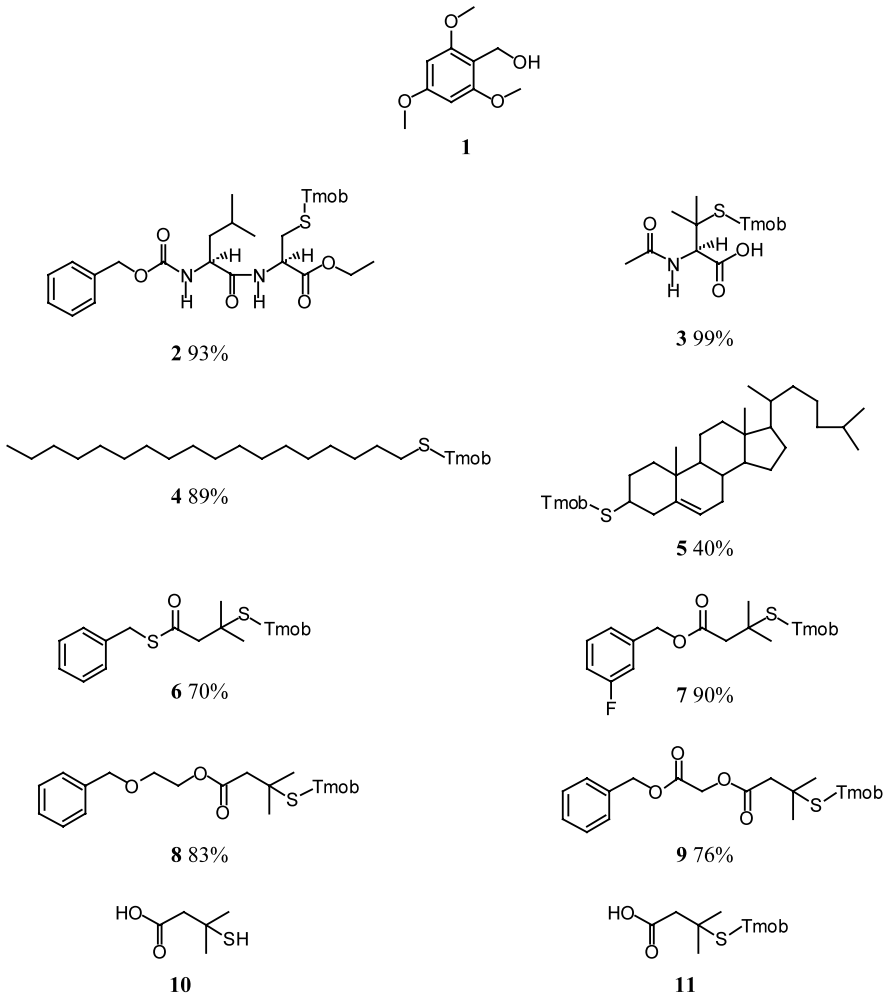
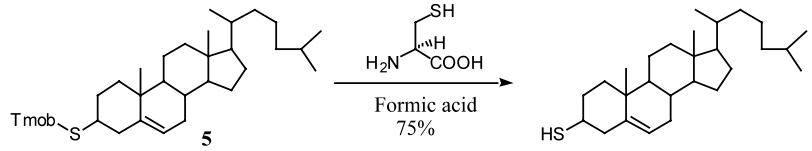


Figure 1.

For the deprotection reactions, good yields were obtained for all compounds examined except compound **5**, which was isolated in moderate yield. This lower yield was due to the formation of other sterol related side products in TFA. We therefore explored the use of a weaker acid as a catalyst for the deprotection process. Substituting formic acid for TFA in the deprotection of compound **5** improved the isolated yield of the product thiol to 75% (Eq. (1)).



(1)

To better define the reaction kinetics, the concentration of compounds **2** and **3** and the corresponding thiols at the equilibrium state using TFA and 10 equiv. of L-cysteine were measured by quantitative mass spectra analysis. The equilibrium constants were determined to be 2.3 and 14 for the primary thiol **2** and the tertiary thiol **3**, respectively. The somewhat bigger equilibrium constant for removing the Tmob group from the tertiary thiol indicated that the tertiary thiol has a lower affinity for the Tmob cation. Therefore, it is somewhat more favorable to remove the Tmob group from a tertiary thiol than from a primary thiol. When formic acid was used, the reaction time for achieving the steady state equilibrium under these conditions from a tertiary thiol was 40 min; from a secondary thiol, 5 h; from a primary thiol, 30 h.

In summary, we have found that formic acid can serve as an effective substitute for TFA in the removal of Tmob groups from acid sensitive compounds. In addition, using L-cysteine as a water soluble cation scavenger for Tmob deprotection (1) facilitates the isolation of the desired product; (2) improves the overall yield; and (3) presents itself as a practical alternative when

contemplating larger scale synthetic applications employing conventional solution phase methodology.

Experimental

Preparation of Tmob protected thiols as illustrated by the synthesis of dipeptide 2. The thiol dipeptide (281.2 mg, 0.7092 mmol, which was prepared from L-cysteine ethyl ester and *N*-cbz-L-leucine with EDC coupling in 55% yield) and 2,4,6-trimethoxybenzyl alcohol (182.9 mg, 0.9227 mmol) were dissolved in methylene chloride (2.0 mL). Trifluoroacetic acid (100 μL) was added dropwise to give a pale yellow solution. This was stirred at room temperature for 10 min, concentrated to dryness, treated with ethyl acetate and concentrated to dryness. The crude product was dissolved in hot ethyl acetate (1.1 mL) and hexane (3.3 mL) was added to give a crystalline product. After cooling with ice-water, the crystals were collected, washed with hexane, and dried in vacuum to give compound **2** (276.6 mg, 0.4796 mmol, 68%, mp = 124–125°C). The mother liquid was concentrated and purified by chromatography (silica gel, ethyl acetate/hexane: 20%/80% then 25%/75%) to give more compound **2** (88.2 mg, 0.153 mmol, 22%). ^{1}H NMR ($CDCl_3$) δ 7.32 (s, 5H), 6.65 (d, J = 7.2 Hz, 1H), 6.12 (s, 2H), 5.15 (m, 1H), 5.10 (s, 2H), 4.80 (m, 1H), 4.20 (m, 3H), 3.79 (m, 11H), 2.95 (m, 2H), 1.70 (m, 2H), 1.51 (m, 1H), 1.25 (t, J = 7.0 Hz, 3H), 0.96 (m, 6H). ^{13}C NMR ($CDCl_3$) δ 171.9, 170.7, 160.5, 158.8, 156.0, 136.3, 128.5, 128.1, 128.0, 107.7, 90.6, 67.0, 61.5, 55.7, 55.3, 53.4, 52.1, 41.9, 33.6, 24.6, 24.3, 22.9, 22.0, 14.1. LRMS (APIMS) m/z 577 ($M+H^+$), 594 ($M+NH_4^+$), 599 ($M+Na^+$).

Removal of the Tmob group with TFA as illustrated by deprotection of dipeptide 2. L-Cysteine (491.1 mg, 4.052 mmol) was dissolved in trifluoroacetic acid (10 mL). The Tmob protected dipeptide **2** (233.7 mg, 0.4052 mmol) was dissolved in methylene chloride (10 mL) and the resulting solution was added to the cysteine solution. The resulting pale yellow solution was stirred at room temperature for 10 min, concentrated to dryness, treated with ethyl acetate and concentrated to dryness three times. The resulting yellow gum was treated with ethyl acetate and washed with sodium bicarbonate solution and brine. The organic phase was dried (sodium sulfate) and concentrated to give a crude product (174.5 mg; expected weight for 100% yield is 160.7 mg). A HPLC analysis showed a 93% yield and a quantitative mass spectra analysis showed a 95% yield. The crude product was purified by column chromatography (silica gel, ethyl acetate/hexane: 15%/85%, then 20%/80%, and then 25%/75%) to give the free thiol (149.9 mg, 0.3780 mmol, 93.3%, mp = 77–79°C) and unreacted compound 2 (15.7 mg, 0.0272 mmol, 6.7%). ^{1}H NMR ($CDCl_3$) δ 7.31 (s, 5H), 7.19 (d, J = 7.2 Hz, 1H), 5.68 (d, J = 7.2 Hz, 1H), 5.10 (s, 2H), 4.82 (m, 1H), 4.33 (m, 1H), 4.22 (m, 2H), 2.95 (m, 2H), 1.66 (m, 2H), 1.53 (m, 2H), 1.27 (t, J = 7.1 Hz, 3H), 0.92 (m, 6H). ^{13}C NMR ($CDCl_3$) δ 172.3, 169.7, 156.1, 136.1, 128.4, 128.0, 127.9, 66.9, 61.8, 53.5, 41.2, 26.5, 24.5, 22.7, 21.9, 14.0. LRMS (APIMS) m/z 397 ($M+H^+$), 414 ($M+NH_4^+$), 419 ($M+Na^+$), 793 ($2M+H^+$), 810 ($2M+NH_4^+$), 815 ($2M+Na^+$).

Removal of the Tmob group with formic acid as illustrated by deprotection of compound 5. A solution of compound **5** (83 mg, 0.142 mmol) in methylene chloride (8 mL) was added dropwise rapidly to a solution of cysteine (171 mg, 1.42 mmol) in formic acid (8 mL). The resultant solution was stirred at room temperature for 5 h and the volatile material removed in vacuo. The residue was neutralized with sodium bicarbonate solution and extracted with methylene chloride. The organic phase was washed with more sodium bicarbonate solution, dried (sodium sulfate), filtered and concentrated to give the product (54 mg, 95%) which was further purified by chromatography on silica gel (ethyl acetate:hexane 5:95) to give the thiol (43 mg, 75%).

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