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A Facile, Inexpensive and Scalable Route to Thiol Protected α-Methyl Cysteine**

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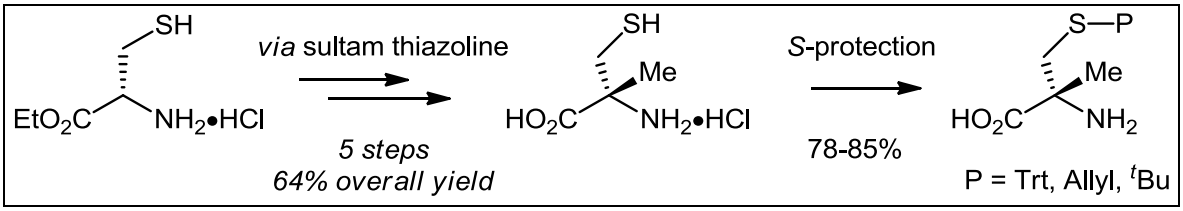
Supporting information:
Supporting information including the preparation of α-methyl cysteine **10**, and ^{1}H and ^{13}C spectra for all compounds is available online at http://www.thieme-connect.com/ejournals/toc/synlett

Graphical abstract:



Keywords:
natural products, α-methyl cysteine, solid phase peptide synthesis, camphor sultam auxiliary, P2-Et.

Abstract

A facile, scalable synthesis of α-methyl cysteine with three alternate thiol protecting groups (Trt, Allyl and ^{t}Bu) is described. The thiol protected amino acids are obtained in 6 steps from L-cysteine ethyl ester and are ideally suited for a range of natural product and solid phase peptide synthesis applications.

Introduction

Both the (*R*)- and (*S*)-enantiomers of α-methyl cysteine occur in Nature and they are often found incorporated within a thiazoline ring.[1] Many α-methyl cysteine containing natural products exhibit interesting pharmaceutical properties, *e.g.* bisebromoamide **1** (Figure 1) has anticancer activity at nanomolar levels against a wide range of cancer lines;[2] thiangazole **2** has anti-HIV activity and is highly selective for HIV-1 over HIV-2;[3] desferrithiocin **3** is an iron chelator with antineoplastic activity[4] and didehydromirabazole A **4** is an anticancer agent which exhibits selectivity for solid tumours.[5] α-Methyl cysteine is also frequently used in the field of peptide mimetics where quaternization of the α-centre: (i) prevents racemization, which otherwise is comparatively facile under both acidic and basic conditions;[6] (ii) restricts rotation about the N-C^{α} (φ) and C^{α}-C(O) (ψ) bonds, which can stabilize a preferred peptide conformation;[7] and (iii) enhances stability towards enzymatic degradation, thus increasing biological half-life.[8]

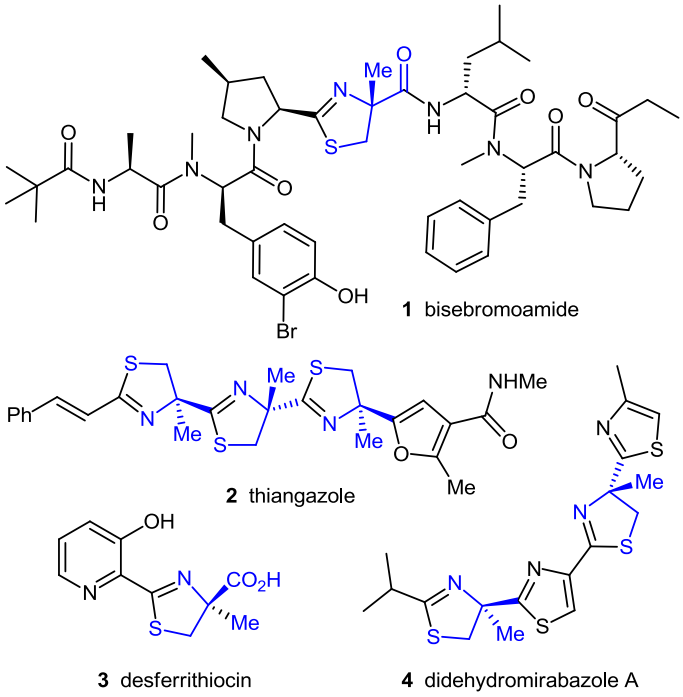
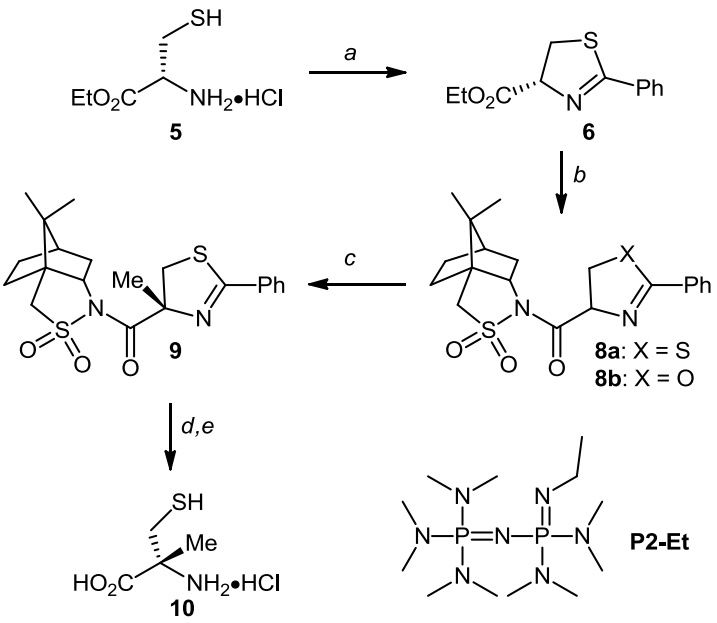


Figure 1. α-Methyl cysteine containing natural products.

A comprehensive review of approaches to the asymmetric synthesis of α-methyl cysteine was published by Singh in 2004 and identifies five common strategies: thiolation of a bromomethyl bislactam ether; regioselective ring opening of a chiral aziridine or β-lactone; utilization of Seebach's "self-regeneration of chirality" approach; enzymatic resolution; and use of a camphorsultam chiral auxiliary to direct methylation of a thiazoline.[6] Alternate routes have since been published, but these often require either expensive, non-commercial phase transfer catalysts,[9] utilize time-consuming enzymatic resolution,[7,10] or employ microwave technologies.[8] Our goal was to optimize a reliable, inexpensive, scalable route to optically pure α-methyl cysteine for solid phase peptide synthesis (SPPS), peptoid mimetic and natural product synthesis applications. Three thiol protecting groups [*S*-trityl (Trt), *S*-allyl (Allyl) and *S*-*tert*-butyl (^{t}Bu)] were targeted to provide appropriate oxidation-resistant, bench-stabile thiol-protected derivatives.

Whilst the asymmetric allylation, or benzylation, of appropriately functionalized thiazolines may be achieved using both cinchona and tertiary ammonium salt based PTCs;[9] the yields and % ee's obtained for aliphatic alkylation are generally poor. Thus in selecting a scalable synthetic route to α-methyl cysteine we were attracted to the precedented use of camphorsultam to direct the alkylation of thiazolines; not least due to the excellent handling properties which this auxiliary conveys (including the generation of crystalline intermediates, and its ease of removal and recycling) which we believed would outweigh the disadvantages of an auxiliary-based approach when working on a gram scale.[11] The required thiazoline precursor **6** (Scheme 1), was readily formed by reacting (*R*)-cysteine ethyl ester hydrochloride **5** and ethyl benzimidate hydrochloride in the presence of a tertiary amine base. Subsequent coupling to (1*S*)-(−)-2,10-camphorsultam **7** was achieved using $AlMe_3$; but while the reaction itself was not particularly problematic, purification proved troublesome. The desired product **8a** is described in the literature as colourless and crystalline,[11] however purification by column chromatography using a range of solvent systems rarely provided pure product and recrystallization also proved unsuccessful. The optimized purification method developed involved gentle heating of the crude product in diethyl ether followed by filtration.[12] Using this method, a colourless crystalline product **8a** was obtained in good yield (as an ~1:1 epimeric mixture in the thiazoline ring) which could be used directly in the subsequent step.



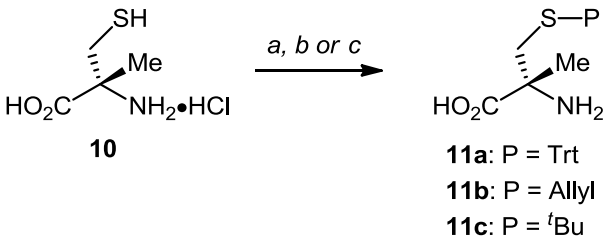
Scheme 1. Synthesis of α-methyl cysteine. *Reagents and Conditions:* (a) PhC(=NH)OEt•HCl, MeOH, Et_3N, rt, 24 h (95%); (b) (1*S*)-(−)-2,10-camphorsultam **7**, Me_3Al, $PhCH_3$, 55 °C, 24 h (76%); (c) P2-Et, MeI, TBAB, CH_2Cl_2, -78 °C, 1 h (99%); (d) LiOH (1 M aq), THF, rt, 30 min; (e) HCl (6 M aq), reflux, 24 h (89% over 2 steps).

Removal of the enolisable α–H in **8a** gives a planar intermediate,[11] the faces of which may be readily discriminated in the presence of the bulky camphorsultam, allowing for highly diastereoselective methylation. Pennington *et al.* have used BuLi in the presence of HMPA to effect this transformation,[11] but the yields obtained were modest and the use of HMPA on scale is not desirable.[13] In contrast, phosphazene bases provide an attractive alternative as extremely strong, non-ionic, non-charged nitrogen bases which generate highly reactive "naked" enolates.[14] Phosphazene bases also exhibit very low nucleophilicity and hence are comparatively inert towards the electrophilic component of a reaction. We opted to use the phosphazene base P2-Et,[15] as methylation of the oxazoline **8b** has been reported to proceed in high yield with excellent diastereoselectivity in the presence of this base.[15] The reaction of **8a** was found to proceed in high yield with 1 equivalent of P2-Et in 1 hour in the presence of the phase transfer catalyst TBAB. Attempts to reduce the loading of P2-Et to sub-stoichiometric levels (*e.g.* 10 mol%) were unsuccessful, resulting in sluggish reaction times and poor yields of product (~50%). The product was purified by column chromatography to give a colourless solid, methylated thiazoline **9**, as a single diastereomer. While the mass spectrometric and $[\alpha]_D$ values of this compound matched those of the literature, there was a significant shift in the δ value which we observed in the ^{1}H NMR for the CH_3 of the newly-introduced methyl group (δ 1.69 *vs* δ 2.50[11]). However, comparison of our NMR data to that of the oxazoline

reported by S. Jew *et al*.,[15] and other similar thiazolines[1,16] gave us full confidence in the outcome of this methylation reaction.

A two-step procedure for the removal of the auxiliary and hydrolysis of the thiazoline ring was found to provide optimum yields. The auxiliary was efficiently removed with LiOH (1 M aq); the (1*S*)-(−)-2,10-camphorsultam **7** was recovered in 89% yield and could be recycled following recrystallization from ethanol. The thiazoline ring was then cleaved using HCl (6 M aq) to liberate α-methyl cysteine. Purification of the α-methyl cysteine from the benzoic acid by-product was attempted using DOWEX 50WX8-200 resin with an initial eluant of pH 3 citrate buffer followed by 5% ammonium hydroxide solution to liberate the amino acid. Whilst removal of the by-product was successful, NMR analysis indicated the presence of citric acid (which acts as a stabilizer for the reduced cysteine). Any attempts to further purify **10** using this process resulted in dimerization to the corresponding cystine; a process which could be readily followed by NMR (cysteine $CH_2 - CH_AH_B$ δ 2.91, CH_AH_B δ 3.18, *vs* cystine $CH_2 - CH_AH_B$ δ 3.20, CH_AH_B δ 3.58). Fortunately, an alternative strategy employing azeotropic removal of residual water from the crude product mixture using toluene[17] caused the hydrochloride salt of α-methyl cysteine **10** to precipitate allowing its facile separation from the reaction by-product. Using this method, the hydrochloride salt **10** was isolated in excellent yield (89% over 2 steps).

To provide derivatives of α-methyl cysteine with extended bench-stability, three different sulfur protecting groups were targeted: the *S*-trityl (Trt), *S*-allyl (Allyl) and *S*-*tert*-butyl (^{t}Bu) groups (**11a-c**, Scheme 2). Whilst all three protecting groups are widely employed in SPPS strategies only the Trt and ^{t}Bu protected derivatives of (*R*)-**10** have previously been reported.



Scheme 2. Thiol-protection of α-methyl cysteine. *Reagents and Conditions:* (a) H_3PO_4 (85% aq), Ph_3COH, $PhCH_3$, reflux, 1 h (92%); (b) $H_2C{=}CHCH_2Br$, NH_4OH (2 M aq), rt, 18 h (78%); (c) ^{t}BuOH, HCl (37 % aq), 50 °C, 18 h (81%).

Trityl protection was achieved using a method developed by M. Yus *et al*.[18] Phosphoric acid (85 %, aq) was added to a solution of **10** and triphenyl methanol in toluene. Reflux for 1 hour gave the salt of

11a in high yield as a colourless crystalline solid. Introduction of the allyl group to give **11b** was achieved using allyl bromide and NH_4OH (2 M aq) following a procedure reported by Y. Tsantrizos *et al*.[19] Finally, the *tert*-butyl group was introduced following a slightly modified version of the procedure described by Chimiak *et al.*;[20] thus **10** was dissolved in HCl (37 % aq) and heated at 50 °C in the presence of *tert*-butyl alcohol to produce **11c**.

In conclusion, we report a simple, inexpensive, scalable and repeatable synthesis of α-methyl cysteine (5 steps from commercial cysteine ethyl ester hydrochloride, 64% overall yield). This route can be easily adapted to incorporate alternate sulfur protecting groups, which has been illustrated by the synthesis of three different species. It is anticipated that these products will find application in SPPS as their Fmoc derivatives, incorporation into the synthesis of a range of natural products and, in the case of **11b**, might provide an elimination-resistant modified cysteine with potential for RCM peptide stapling,[21,22] and bioorthogonal protein modification[23] through cross-metathesis[24] and the thiol-ene click (TEC) reaction.[25]

Experimental section

(2*R*)-2-Amino-2-methyl-3-[(triphenylmethyl)-sulfanyl]propanoic acid phosphate salt[11]: Phosphoric acid (0.2 mL; 85 % aq) was added to a stirred solution of α-methyl cysteine hydrochloride **10** (100 mg, 0.58 mmol) and trityl alcohol (0.15 g, 0.58 mmol) in toluene (5 mL) at rt. The reaction mixture was heated at reflux for 1 h, cooled to rt and then the reaction mixture was concentrated *in vacuo*. Water (5 mL) was added and the crude product was stirred for a further 30 min, then filtered and recrystallized from methanol to give the desired product **11a** as a colourless solid (202 mg, 92 %)**.** $\mathbf{R_f}$ (MeOH:DCM, 1:9) = 0.21; $\mathbf{[\alpha]_D}$ = +30.0 (c 1.00, MeOH); **mp** 178-180 °C, lit.[11] mp 179-180 ºC; **IR** (neat, cm^{-1}) 3471 (N–H), 3600–2580 (O–H), 1730 (C=O), 1630 (Ar), 1593 (Ar), 1512 (Ar); **^{1}H NMR** δ (500 MHz, DMSO) 7.38–7.24 (15H, m, Ar*H*), 3.50 (2H, br s, NH_2), 2.45 (1H, d, *J* = 11.7 Hz, CH_ACH$_B$), 2.39 (1H, d, *J* = 11.7 Hz, CH$_A$CH_B), 1.22 (3H, s, CCH_3); **^{13}C NMR** δ (126 MHz, DMSO) 171.12 (C), 143.96 (3 × C), 129.08 (6 × CH), 128.11 (6 × CH), 126.88 (3 × CH), 65.99 (C), 58.67 (CH_2), 21.96 (CH_3), trityl C absent; ***m/z*** (ESI+, MeOH) 378 ([M+H]$^+$, 24%), 243 (100), 179 (10). ^{1}H and ^{13}C spectroscopic data in good agreement with literature.[11]

(2*R*)-2-Amino-2-methyl-3-[(prop-2-en-1-yl)sulfanyl]-propanoic acid[26]: Allyl bromide (0.08 mL, 0.87 mmol) was added to a stirred solution of α-methyl cysteine hydrochloride **10** (100 mg, 0.58 mmol) in NH_4OH (2 mL, 2 M aq) at rt. The reaction mixture was stirred at rt for 18 h then the product was concentrated *in vacuo*. The crude product was then recrystallized from EtOH to give the desired product **11b** as a colourless solid (79.6 mg, 78%). $\mathbf{R_f}$ (MeOH:DCM, 1:9) = 0.45; $\mathbf{[\alpha]_D}$ = +25.0 (c 0.40, H_2O); **mp** 257-259 °C, lit.[23] mp 260 ºC; **IR** (neat, cm^{-1}) 3454 (N–H), 3419 (N–H), 3230–2700 (O–H),

1738 (C=O), 1605 (C=C), 1597 (COO^-); **^{1}H NMR** δ (400 MHz, D_2O) 5.83–5.69 (1H, m, C*H*=CH_2), 5.20–5.08 (2H, m, CH=CH_2), 3.22–3.08 (2H, m, CH_2), 3.05 (1H, d, *J* = 14.5 Hz, C$H_A$$CH_B$), 2.71 (1H, d, *J* = 14.5 Hz, CH_ACH_B), 1.45 (3H, s, CCH_3).; **^{13}C NMR** δ (126 MHz, D_2O) 175.30 (C), 133.72 (CH), 118.33 (CH_2), 61.14 (C), 36.85 (CH_2), 34.94 (CH_2), 22.20 (CH_3); ***m/z*** (ESI+, MeOH/DCM) 176 ($[M+H]^+$, 29%), 159 (25), 144 (24), 136 (30), 114 (27), 110 (40). IR data in good agreement with literature.[26]

(2*R*)-2-Amino-3-(*tert*-butylsulfanyl)-2-methyl-propanoic acid hydrochloride salt[17b]: Hydrochloric acid (2.5 mL; 37 % aq) was added to a stirred solution of α-methyl cysteine hydrochloride **10** (100 mg, 0.58 mmol) in *tert*-butanol (0.56 g, 5.8 mmol) at rt. The reaction mixture was heated to 50 °C and stirred for ~18 h at the same temperature. Once the reaction was judged to be complete, the reaction mixture was concentrated *in vacuo* until most of the solvent had been removed. On standing overnight at rt colourless crystals formed. The crystals were collected by filtration to give the desired product **11c** as a colourless solid (90.3 mg, 81%). **R_f** (MeOH:DCM, 1:9) = 0.10; **$[\alpha]_D$** = −50.0 (c 0.40, H_2O); **mp** 272-274 °C, lit.[17b] mp 272-275 ºC; **IR** (neat, cm^{-1}) 3389 (N–H), 3333 (N–H), 3100–2750 (O–H), 1734 (C=O); **^{1}H NMR** δ (500 MHz, D_2O) 3.13 (1H, d, *J* = 13.4 Hz, C$H_A$$H_B$S), 2.93 (1H, d, *J* = 13.4 Hz, $CH_A$$H_B$S), 1.56 (3H, s, CC$H_3$), 1.26 (9H, s, C(C$H_3$)$_3$); **^{13}C NMR** δ (126 MHz, D_2O) 173.31 (C), 60.07 (C), 43.51 (C), 34.09 (CH_2), 29.74 (3 × CH_3), 21.79 (CH_3); (ESI+, MeOH/DCM) 192 ($[M+H]^+$, 58%), 137 (72), 136 (100), 119 (88), 101 (81), 90 (72). ^{1}H and ^{13}C spectroscopic data in good agreement with literature.[17b]

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