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Research Article

# Synthesis of isotopically labelled thiol volatiles and cysteine conjugates for quantification of Sauvignon Blanc wine

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**Abstract:** The thiols 4-mercapto-4-methylpentan-2-ol (4MMPOH), 3-mercaptohexan-1-ol (3MH), and 3-mercaptohexyl acetate (3MHA), which contribute to the aroma profile of Sauvignon Blanc, as well as other varieties of wine, have been synthesized with deuterium labels. The cysteinylated precursors of the thiols, 4-(4-methylpentan-2-one)-L-cysteine (4MMP-cys) and 4-(4-methylpentan-2-ol)-L-cysteine (4MMPOH-cys), found in grape must were also synthesized with deuterium labels. These deuterated compounds provide useful internal standards for the quantification of these thiols in wine using LCMS. Copyright © 2007 John Wiley & Sons, Ltd.

**Keywords:** isotope labelling; internal standard; thiol; Sauvignon Blanc

## Introduction

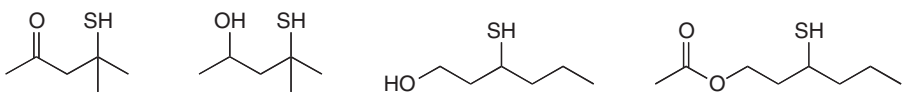
The identification of traces of the highly odorous compounds 4-mercapto-4-methylpentan-2-one **1** (4MMP),[1] 4-mercapto-4-methylpentan-2-ol **2** (4MMPOH), 3-mercaptohexan-1-ol **3** (3MH)[2] and 3-mercaptohexyl acetate **4** (3MHA)[3] in the Sauvignon Blanc wines allowed wine scientists to explain the varietal aroma characters of these wines associated with descriptors such as cat's urine, broom, grapefruit and passionfruit (Scheme 1). The presence of these volatile thiols was also later reported in wines made from Riesling, Gewurztraminer,[4] Petit and Gros Manseng,[4] Cabernet Sauvignon, and Merlot[5,6] thereby confirming the importance of these compounds for the varietal aroma of wine. These volatile compounds are almost totally absent from grape must and are released into the wine from their cysteinylated precursors, *S*-4-(4-methylpentan-2-one)-L-cysteine, *S*-4-(4-methylpentan-2-ol)-L-cysteine, and *S*-3-(hexan-1-ol)-L-cysteine, during alcoholic fermentation.[7]

The low levels of these thiols in complex wine and juice matrices make them particularly hard to quantify. Several quantification methods are available for the assessment of the varietal volatile thiols[8–10] and their cysteinylated precursors[11,12] mostly using GCMS. All these methods involve complex sample preparations which finally translate in low recoveries and high variability. The detection by MS allows the utilization of deuterated internal standards and has proved to be one of the best ways to improve the performance of the analytical quantification.

## Results and discussion

### Synthesis of the volatile thiols

The synthesis of several of the volatile thiols has been reported previously with and without deuterium labelling.[13–16] However, previous syntheses have either required the use of hydrogen sulfide gas which is both




1 2 3 4

**Scheme 1** The highly odorous thiol volatiles 4MMP (1), 4MMPOH (2), 3MH (3) and 3MHA (4).

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highly toxic and low yielding, or the existing synthetic methods reported were found to be unreliable in our hands. Consequently, a more robust synthesis of the thiols 3MH **3**, 3MHA **4**, 4MMP **1** and 4MMPOH **2** with deuterium labels was sought. The use of thioacetic acid to introduce the sulfur group was found to be cheap and less hazardous than using hydrogen sulfide gas and it also provided a protecting group for the highly pungent thiol group.

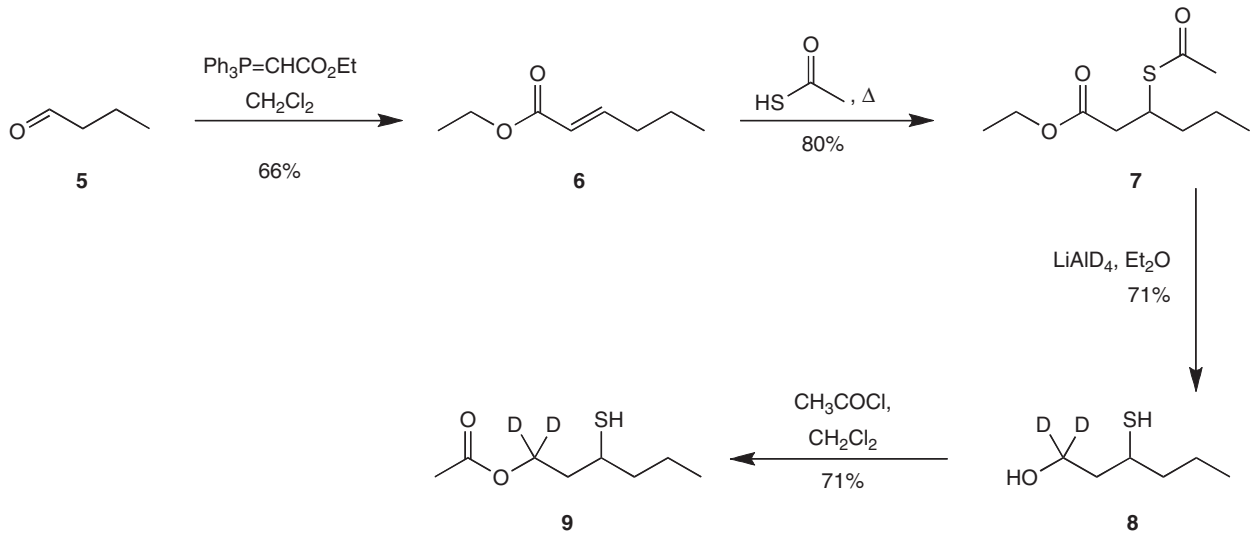
The synthesis of [1-$^2H_2$]3MH **8** was achieved by Wittig olefination of butyraldehyde **5** followed by Michael addition of thioacetic to introduce the sulfur functionality (Scheme 2). The 66% yield for the Wittig reaction is reflective of difficulties experienced with the isolation of the volatile product rather than the extremely clean reaction. The use of lithium aluminium deuteride effected the reduction of both ester and thioester allowing placement of the required deuterium labels to give [1-$^2H_2$]3MH **8**. Acetylation of [1-$^2H_2$]3MH **8** proceeded smoothly to afford [1-$^2H_2$]3MHA **9** with no acetylation of the thiol group observed.

Synthesis of [2-$^2H_1$]4MMPOH **12** followed a similar strategy via Michael addition of the thioacetic acid to mesityl oxide **10** followed by concomitant reduction of the ketone and thioester and introduction of the deuterium label using lithium aluminium deuteride (Scheme 3). The facile two-step synthesis proceeded in an excellent 70% overall yield.

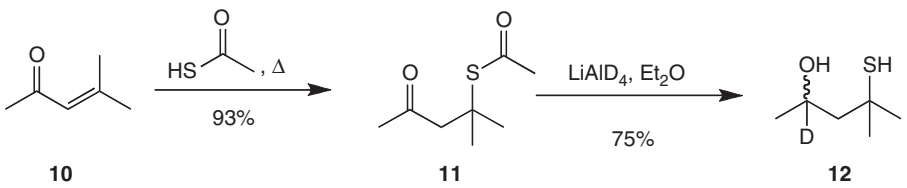
## Synthesis of the cysteine conjugates

The cysteine conjugate of 4MMPOH **15** was prepared by Michael addition of L-cysteine onto mesityl oxide **10** followed by *in situ* Boc protection of the amino group to give ketone **13** (Scheme 4). The Boc protection simply allows easier handling of the product. Reduction of the ketone **13** using sodium borodeuteride was sluggish and required heating over 3 days with several equivalents of deuteride to effect complete reaction. Alcohol **14** was obtained as a mixture of diastereoisomers in a 0.45:0.65 ratio. Finally, the Boc group was removed using hydrochloric acid in 1,4-dioxane to afford the desired cysteine conjugate **15** containing one deuterium label.

[1,1′-$^2H_6$]4MMP-Cys **18** was prepared by Michael addition of cysteine to [$^2H_{10}$]mesityl oxide **17** (Scheme 5). In this case the requirement for a ketone functionality rather than an alcohol as in all the other compounds precluded the use of a simple reduction




**Scheme 2** Synthesis of [1-$^2H_2$]3MH and [1-$^2H_2$]3MHA.




**Scheme 3** Synthesis of [2-$^2H_1$]4MMPOH.



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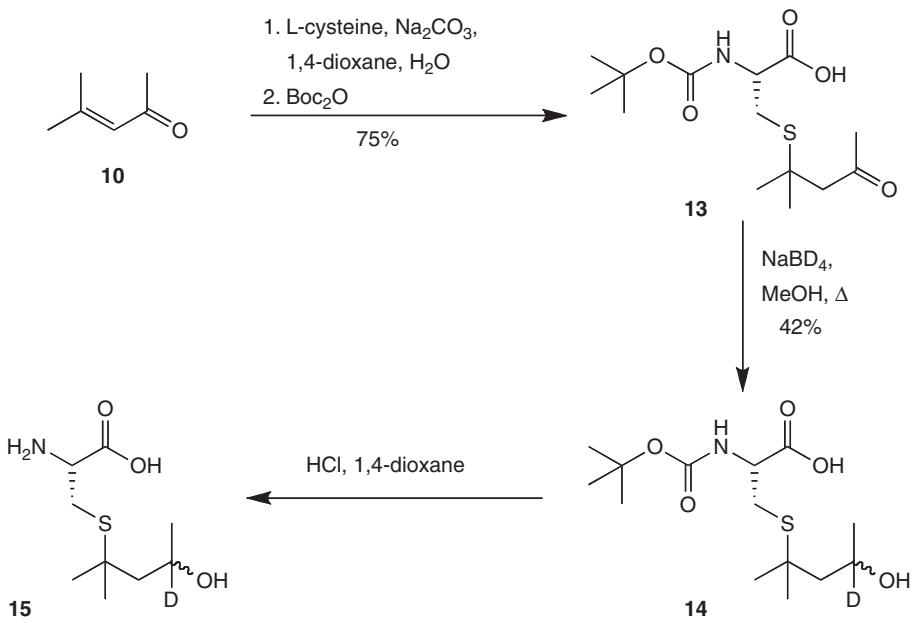
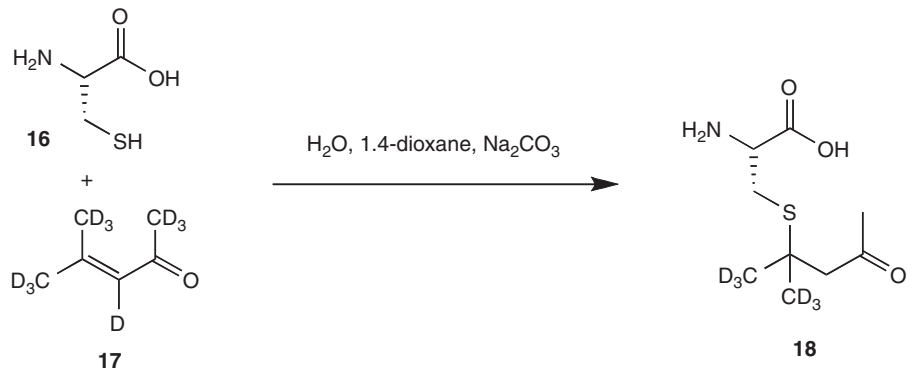



**Scheme 4** Synthesis of [4-$^2H_1$]4MMPOH-Cys.




**Scheme 5** Synthesis of [1,1′-$^2H_6$]4MMP-Cys.

to introduce the deuterium label hence the deuterium label was introduced into the Michael acceptor **17**. [$^2H_{10}$]Mesityl oxide was prepared via aldol condensation of readily available deuterated acetone using the classical method reported by Conant and Tuttle[17] followed by dehydration using a catalytic quantity of *p*-toluenesulfonic acid.[18] During the Michael addition that occurs in basic aqueous solution the deuterium labels at the enolisable position are lost. However, the more stable deuterium labels on the methyl groups remain intact with only limited scrambling observed affording [1,1′-$^2H_6$]4MMP-Cys **18** with 91% deuterium enhancement at the six equivalent positions.

## Conclusion

In summary, deuterated volatile thiols [1-$^2H_2$]3MH (3-mercapto[1-$^2H_2$]hexan-1-ol), [1-$^2H_2$]3MHA (3-mercapto [1-$^2H_2$]hexyl acetate), and [2-$^2H_1$]4MMPOH (4-mercapto-4-methyl[2-$^2H_1$]pentan-2-ol) have been prepared in good overall yield. These compounds are useful as internal standards for the quantification of the naturally occurring thiols found in wine using GC/MS. The cysteinylated precursors of the thiols, [1,1′-$^2H_6$]4MMP-Cys {4-(4-methyl[1,1′-$^2H_6$]pentan-2-one)-L-cysteine} and [4-$^2H_1$]4MMPOH-Cys {4-(4-methyl[4-$^2H_1$]pentan-2-ol)-L-cysteine} were also synthesized for use in studies on the fermentation process.



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## Experimental

### General

(Ethoxycarbonylmethylene)triphenylphosphorane was synthesized in two steps using the methods reported by Popaj and Heese[19] and Meschkat *et al.*[20] Sodium borodeuteride and lithium aluminium deuteride were both purchased from Aldrich with 98 atom % deuterium. All other chemicals used were obtained from commercial sources. Diethyl ether was dried over sodium/benzophenone and distilled prior to use and reactions were carried out using flame or oven-dried glassware under a dry nitrogen atmosphere. Flash chromatography was performed using Scharlau silica gel 60 (230–400 mesh) with the indicated solvents. Thin-layer chromatography (TLC) was carried out on precoated silica plates (Merck Kieselgel $60F_{254}$) and compounds were visualized by UV fluorescence or by heating the plates dipped in alkaline potassium permanganate solution. Infrared spectra were recorded with a Perkin Elmer 1600 series Fourier-transform infrared spectrometer as thin films between sodium chloride plates. Absorption maxima are expressed in wavenumbers ($cm^{-1}$). NMR spectra were recorded on a Bruker AVANCE DRX400 ($^1H$, 400 MHz; $^{13}C$, 100 MHz) or a Bruker AVANCE 300 ($^1H$, 300 MHz, $^{13}C$, 75 MHz) at 298 K. Chemical shifts are reported relative to internal trimethylsilane in $CDCl_3$ or the hydrogenated residue of the deuterated solvent in all other cases. The asterisk* denotes resonances assigned to the minor isomer. High-resolution mass spectra were recorded using a VG70-SE spectrometer.

### (E)-Ethyl hex-2-enoate 6

A solution of (ethoxycarbonylmethylene)triphenylphosphorane (3.86 g, 11.1 mmol) in dry dichloromethane (15 mL) was added to a solution of butyraldehyde **5** (1.0 mL, 11.1 mmol) in dry dichloromethane (15 mL). The reaction was stirred under nitrogen for 18 h, then the solvent was removed *in vacuo* (without application of heat). The resulting slurry was purified by flash chromatography (silica gel, pentane: diethyl ether, 95:5) to afford the title compound (1.04 g, 66%) as a colorless oil: $\nu_{max}$(film)/$cm^{-1}$ 1718 (CO); $^1H$ NMR (400 MHz; $CDCl_3$) $\delta = 0.94$ (3H, t, $J = 7.6$ Hz, CH═CHCH$_2$CH$_2$C$\underline{H}_3$), 1.29 (3H, t, $J = 7.2$ Hz, OCH$_2$C$\underline{H}_3$), 1.45–1.52 (2H, m, C$\underline{H}_2$CH$_3$), 2.15–2.21 (2H, m, CH═CHC$\underline{H}_2$), 4.18 (2H, q, $J = 7.2$ Hz, OC$\underline{H}_2$CH$_3$), 5.81 (1H, dt, $J =$ 15.6 and 1.6 Hz, C═OCH), 6.96 (1H, dt, $J = 15.6$ and 6.8 Hz, C═OCHC$\underline{H}$). $^{13}C$ NMR (100 MHz; $CDCl_3$) $\delta =$ 13.6 (CH$_2$CH$_2$C$\underline{H}_3$), 14.3 (OCH$_2$C$\underline{H}_3$), 21.3 (C$\underline{H}_2$CH$_3$), 34.2 (C$\underline{H}_2$CH$_2$CH$_3$), 60.1 (OC$\underline{H}_2$CH$_3$), 121.4 (C═OC$\underline{H}$), 149.2 (C═OCHC$\underline{H}$), 166.8 (CO). $m/z$ (EI+) 142.0983 ($M^+$ $C_8H_{14}O_2$ requires 142.0994).

### Ethyl 3-(acetylthio)hexanoate 7

Thioacetic acid (2.37 mL, 33.3 mmol) was added to (*E*)-ethyl hex-2-enoate **6** (2.37 g, 16.7 mmol) and the solution was heated at 150°C under nitrogen for 18 h. Diethyl ether (20 mL) and hydrochloric acid (2 M, 20 mL) were added to the cooled solution. The layers were separated and the organic layer was washed with saturated aqueous sodium bicarbonate solution, then dried over sodium sulfate and the solvent removed *in vacuo*. The oil was purified by flash chromatography (silica gel, pentane: diethyl ether, 95:5 to 90:10) to afford the title compound (2.90 g, 80%) as a yellow oil: $\nu_{max}$(film)/$cm^{-1}$ 1736 (CO) and 1689 (CO); $^1H$ NMR (400 MHz; $CDCl_3$) $\delta = 0.91$ (3H, t, $J = 7.3$ Hz, CH$_2$CH$_2$C$\underline{H}_3$), 1.26 (3H, t, $J = 7.1$ Hz, OCH$_2$C$\underline{H}_3$), 1.39 (2H, m, CH$_2$C$\underline{H}_2$CH$_3$), 1.59–1.72 (2H, m, C$\underline{H}_2$CH$_2$CH$_3$), 2.31 (3H, s, SC═OCH$_3$), 2.63 (2H, dd, $J = 6.7$ and 5.3 Hz, C═OCH$_2$CS), 3.86 (1H, m, SCH), 4.14 (2H, q, $J =$ 7.1 Hz, OC$\underline{H}_2$CH$_3$). $^{13}C$ NMR (100 MHz; $CDCl_3$) $\delta = 13.7$ (CH$_2$CH$_2$C$\underline{H}_3$), 14.1 (OCH$_2$C$\underline{H}_3$), 20.1 (CH$_2$C$\underline{H}_2$CH$_3$), 30.6 (SC═OC$\underline{H}_3$), 36.3 (C$\underline{H}_2$CH$_2$CH$_3$), 39.8 (C═OC$\underline{H}_2$CHS), 40.1 (CHS), 60.5 (OC$\underline{H}_2$CH$_3$), 170.9 (OCO), 195.2 (SCO). $m/z$ (FAB+) 219.1063 ($MH^+$ $C_{10}H_{19}O_3S$ requires 219.1055).

### 3-Mercapto(1-$^2H_2$)hexan-1-ol 8

A solution of ethyl 3-(acetylthio)hexanoate **7** (0.564 g, 2.59 mmol) in dry diethyl ether (10 mL) was added dropwise to a slurry of lithium aluminum deuteride (0.217 g, 5.17 mmol) in dry diethyl ether (10 mL) under an atmosphere of dry nitrogen. The reaction was stirred at room temperature under nitrogen for 2 h, then wet diethyl ether (2 mL) was added cautiously. Hydrochloric acid (2 M, 15 mL) was added to the slurry and the mixture was allowed to stir until the lithium salts had dissolved. The organic layer was separated and the aqueous layer was extracted twice with diethyl ether ($2 \times 20$ mL). The combined organic fractions were dried over sodium sulfate and the solvent was removed by distillation. The oil was purified by flash chromatography (silica gel, pentane: diethyl ether, 70:30 to 50:50) to give the title compound (0.248 g, 71%) as a colorless oil: $\nu_{max}$(film)/$cm^{-1}$ 3362 (OH) and 2101 (CD); $^1H$ NMR (300 MHz; $CDCl_3$) $\delta = 0.92$ (3H, t, $J = 7.1$ Hz, CH$_2$CH$_2$C$\underline{H}_3$), 1.38 (1H, d, $J = 7.4$ Hz, SH), 1.41–1.68 (5H, m, CH$_A\underline{H}_B$CH(S)C$\underline{H}_2$C$\underline{H}_2$CH$_3$), 1.96 (1H, dd, $J = 14.2$ and 4.3 Hz, CD$_2$C$\underline{H}_A$CH$_B$), 2.95 (1H, m, CH(S)). $^{13}C$ NMR (100 MHz; $CDCl_3$) $\delta = 13.6$ (CH$_2$CH$_2$C$\underline{H}_3$), 20.0 (C$\underline{H}_2$CH$_3$),



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37.5 (CH(S)), 41.0 ($CH_2CH_2CH_3$), 41.5 ($CD_2CH_2CH$(S)), 59.7 (q, $J = 21.5$ Hz, $CD_2$). *m/z* (EI+) 136.0893 ($M^+$ $C_6H_{12}D_2OS$ requires 136.0891).

### 3-mercapto(1-$^2H_2$)hexyl Acetate 9

Acetyl chloride (0.132 mL, 1.85 mmol) was added dropwise to a solution of [$^2H_2$]-3-mercaptohexan-1-ol **8** (0.168 g, 1.24 mmol) in dry dichloromethane (1 mL). The reaction was allowed to stir for 18 h under nitrogen. Water (10 mL) was added to the reaction then the aqueous layer was extracted with portions of dichloromethane ($2 \times 10$ mL). The combined organic fractions were dried over sodium sulfate, then the solvent was removed by distillation. The resulting oil was purified by flash chromatography (silica gel, pentane: diethyl ether, 80:20) to give the title compound (0.157 g, 71% yield) as a colorless oil: $\nu_{max}$(film)/$cm^{-1}$ 1736 (CO); $^1$H NMR (400 MHz; $CDCl_3$) $\delta = 0.92$ (3H, t, $J = 7.1$ Hz, $CH_2CH_3$), 1.38 (1H, d, $J = 7.3$ Hz, SH), 1.42–1.65 (4H, m, $CH_2CH_2CH_3$), 1.71 (1H, dd, $J =$ 14.3 and 9.2 Hz, $CD_2CH_AH_B$), 2.00 (1H, dd, $J = 14.3$ and 4.5 Hz, $CD_2CH_AH_B$), 2.05 (3H, s, $C(O)CH_3$), 2.88 (1H, m, CH(SH)). $^{13}$C NMR (100 MHz; $CDCl_3$) $\delta = 13.6$ ($CH_2CH_3$), 20.0 ($CH_2CH_3$), 20.8 ($C(O)CH_3$), 37.2 (CH(SH)), 37.5 ($CH_2CH_2CH_3$), 41.1 ($CD_2CH_2$), 61.5 (q, $J = 22.3$ Hz, $CD_2$), 170.9 (CO). *m/z* (EI+) 178.0999 ($M^+$ $C_8H_{14}D_2O_2S$ requires 178.0997).

### 2-Methyl-4-oxopentan-2-yl ethanethioate 11

Thioacetic acid (2.48 mL, 34.8 mmol) was added to mesityl oxide **10** (2 mL, 17.4 mmol) and left to stir under nitrogen for 18 h. Diethyl ether (20 mL) was added to the reaction followed by hydrochloric acid (2 M, 20 mL). The organic layer was separated from the aqueous then washed with saturated aqueous sodium bicarbonate solution (20 mL). The organic layer was dried over sodium sulfate and the solvent removed *in vacuo*. The resulting yellow oil was purified by flash chromatography (silica gel, pentane: diethyl ether, 80:20 to 60:40) to give the title compound (2.80 g, 93% yield) as a yellow oil: $\nu_{max}$(film)/$cm^{-1}$ 1710 (CO) and 1682 (CO); $^1$H NMR (300 MHz; $CDCl_3$) $\delta = 1.52$ (6H, s, $C(CH_3)_2$), 2.13 (3H, s, $SC(O)CH_3$), 2.24 (3H, s, $CH_3C(O)$), 3.09 (2H, s, $CH_2$). $^{13}$C NMR (75 MHz; $CDCl_3$) $\delta = 27.4$ ($C(CH_3)_2$), 31.2, ($CH_3C(O)$), 31.6 ($SC(O)CH_3$), 48.6 ($C(CH_3)_2$), 51.8 ($CH_2$), 196.9 (CO), 206.2 (SCO). *m/z* (EI+) 174.0711 ($M^+$ $C_8H_{14}O_2S$ requires 174.0715).

### 4-mercapto-4-methyl(2-$^2H_1$)pentan-2-ol 12

A solution of 2-methyl-4-oxopentan-2-yl ethanethioate **11** (0.61 g, 3.49 mmol) in dry diethyl ether (5 mL) was added dropwise to a slurry of lithium aluminum deuteride (0.29 g, 6.99 mmol) in dry diethyl ether (10 mL) under an atmosphere of dry nitrogen. The reaction was allowed to stir at room temperature for 2 h, then wet diethyl ether was added cautiously. Once fizzing had ceased hydrochloric acid (aq. 2 M, 10 mL) and diethyl ether (10 mL) were added to the reaction and the mixture was allowed to stir until the lithium salts had dissolved. The organic layer was separated and the aqueous layer was extracted with diethyl ether ($2 \times 10$ mL). The combined organic fractions were dried over sodium sulfate and the solvent was removed by distillation. The resulting oil was purified by flash chromatography (silica gel, pentane:diethyl ether, 60:40) to give the title compound as a colorless oil: $\nu_{max}$(film)/$cm^{-1}$ 3398 (OH), 2144 (CD); $^1$H NMR (400 MHz; $CDCl_3$) $\delta = 1.20$ (3H, s, $CH_3CD(OH)$), 1.42 (3H, s, $C(CH_3)_2$), 1.47 (3H, s, $C(CH_3)_2$), 1.67 (1H, d, $J = 14.8$ Hz, $CH_AH_B$), 1.80 (1H, d, $J = 14.8$ Hz, $CH_AH_B$), 1.86 (1H, s, SH), 2.77 (1H, bs, OH). $^{13}$C NMR (100 MHz; $CDCl_3$) $\delta = 24.6$ ($CH_3CD$), 31.7 ($C(CH_3)_2$), 34.9 ($C(CH_3)_2$), 43.6 ($C(CH_3)_2$), 54.1 ($CH_2$), 65.1 (t, $J = 21.5$ Hz, CD). *m/z* (EI+) 135.0829 ($M^+$ $C_6H_{13}DOS$ requires 135.0828).

### (R)-2-(tert-Butoxycarbonylamino)-3-(2-methyl-4-oxopentan-2-ylthio)propanoic acid 13

L-Cysteine **16** (3.63 g, 29.9 mmol) was dissolved in a mixture of 1,4-dioxane (30 mL) and water (30 mL). Sodium carbonate (0.63 g, 5.99 mmol) and mesityl oxide **10** (3.45 mL, 29.9 mmol) were then added to the reaction. After stirring the mixture for three days, di-*tert*-butyldicarbonate (6.55 g, 29.9 mmol) and 1,4-dioxane (10 mL) were added and the reaction was allowed to stir for 1 day after which the solution was acidified to pH 2 with 2 M hydrochloric acid. The mixture was extracted with ethyl acetate ($3 \times 40$ mL) and the combined organic fractions were dried over sodium sulfate. The solvent was removed *in vacuo* to give the title compound (7.14 g, 75%) as a clear oil: $\nu_{max}$(film)/$cm^{-1}$ 1712 (CO); $^1$H NMR (400 MHz; $CD_3OD$) $\delta = 1.39$ (6H, s, $C(CH_3)_2$), 1.44 (9H, s, $C(CH_3)_3$), 2.16 (3H, s, $C(O)CH_3$), 2.76 (2H, s, $CH_2C(O)CH_3$), 2.90 (1H, dd, $J = 12.8$ and 7.6 Hz, $SCH_AH_B$), 3.03 (1H, dd, $J = 12.8$ and 5.0 Hz, $SCH_AH_B$), 4.28 (1H, dd, $J = 7.6$ and 5.0 Hz, $SCH_2CH$). $^{13}$C NMR (100 MHz; $CD_3OD$) $\delta = 28.7$ ($C(CH_3)_3$), 28.9 ($C(CH_3)_2$), 31.2 ($CH_2C(O)$), 32.3 ($C(O)CH_3$), 44.7 ($C(CH_3)_2$), 55.1 ($SCH_2$), 55.2 (CHNH), 80.8 ($C(CH_3)_3$), 157.8 (NCO), 174.1 (C(O)OH), 209.4 ($C(O)CH_3$). *m/z* (FAB+) 320.1533 ($MH^+$ $C_{14}H_{26}NO_5S$ requires 320.1532).




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### (R)-2-(tert-Butoxycarbonylamino)-3-(4-hydroxy-2-methyl(4-$^2H_1$)pentan-2-ylthio) propanoic acid 14

(*R*)-2-(*tert*-Butoxycarbonylamino)-3-(2-methyl-4-oxopentan-2-ylthio)-propanoic acid **13** (0.242 g, 0.76 mmol) was dissolved in methanol (2 mL). Sodium borodeuteride (0.03 g, 0.76 mmol) was added portionwise, then the mixture was heated at reflux for 3 days. After cooling to room temperature, the reaction was adjusted to pH 2 by the addition of a few drops of 2 M hydrochloric acid. Water (10 mL) was added and the aqueous layer was extracted three times with ethyl acetate ($3 \times 10$ mL). The combined organic fractions were dried over sodium sulfate and the solvent removed *in vacuo* to give the title compound (0.103 g, 42%) as a pale yellow oil: $\nu_{max}$ (film)/cm$^{-1}$ 3333 (OH), 1710 (CO); $^1$H NMR (400 MHz; $CD_3OD$) $\delta = 1.17$ (3H, s, CD(OH)C$\underline{H}_3$), 1.34 (6H, s, C(C$\underline{H}_3$)$_2$), 1.44 (9H, s, C(C$\underline{H}_3$)$_3$), 1.62 (1H, d, $J = 14.8$ Hz, C$\underline{H}_A$H$_B$CD), 1.69 (1H, d, $J = 14.8$ Hz, CH$_A\underline{H}_B$CD), 2.85 (1H, m, SC$\underline{H}_A$H$_B$), 2.96 (1H, m, SCH$_A\underline{H}_B$), 4.27 (1H, m, NHCH). $^{13}$C NMR (100 MHz; $CD_3OD$) $\delta = 25.6$ CD$\underline{C}$H$_3$), 28.8 (C($\underline{C}$H$_3$)$_3$), 29.3 (C($\underline{C}$H$_3$)$_2$), 29.3* (C($\underline{C}$H$_3$)$_2$), 30.2* (C($\underline{C}$H$_3$)$_2$), 30.3 (C($\underline{C}$H$_3$)$_2$), 31.0 ($\underline{C}$H$_2$CD), 46.1 ($\underline{C}$(CH$_3$)$_2$), 51.5 (SCH$_2$), 55.2 (SCH$_2\underline{C}$H), 65.8 (t, $J = 21.6$ Hz, CD), 80.7 ($\underline{C}$(CH$_3$)$_3$), 157.7 (NCO), 174.4 (C(O)OH). *m/z* (FAB+) 323.1743 (MH$^+$ $C_{14}H_{27}DNO_5S$ requires 323.1751).

### (R)-2-Amino-3-(4-hydroxy-2-methyl(4-$^2H_1$)pentan-2-ylthio)propanoic acid hydrochloric salt 15

Hydrochloric acid (2 mL, 4 M solution in 1,4-dioxane) was added to *d*-(*R*)-2-(*tert*-butoxycarbonylamino)-3-(4-hydroxy-2-methylpentan-2-ylthio)propanoic acid **14** (0.09 g, 0.028 mmol) and the solution was left to stir for 30 min. The 1,4-dioxane was then removed *in vacuo*. The residue was passed though a short column of Li Chroprep (40–63 μm) with water. The fractions were freeze-dried to give the title compound (0.115 g) as a white highly hydroscopic paste: $\nu_{max}$(film)/cm$^{-1}$ 3347 (OH), 1736 (CO); $^1$H NMR (400 MHz; $CD_3OD$) $\delta = 1.19$ (3H, s, CD(OH)C$\underline{H}_3$), 1.36* (1.35H, s, C(C$\underline{H}_3$)$_A$(CH$_3$)$_B$), 1.37 (1.65H, s, C(C$\underline{H}_3$)$_A$(CH$_3$)$_B$), 1.38 (3H, s, C(CH$_3$)$_A$(C$\underline{H}_3$)$_B$), 1.64 (0.55H, d, $J = 14.9$ Hz, C$\underline{H}_A$H$_B$CD(OH)), 1.65* (0.45H, d, $J = 14.9$ Hz, C$\underline{H}_A$H$_B$CD(OH)), 1.74 (1H, d, $J = 14.9$ Hz, CH$_A\underline{H}_B$CD(OH)), 3.02* (0.45H, dd, $J = 13.9$ and 7.4 Hz, SC$\underline{H}_A$H$_B$), 3.03 (0.55H, dd, $J = 13.9$ and 7.0 Hz, SC$\underline{H}_A$H$_B$), 3.16 (1H, dd, $J = 4.2$ and 13.9 Hz, SCH$_A\underline{H}_B$), 4.15* (0.45H, dd, $J = 4.2$ and 7.4 Hz, C$\underline{H}$NH$_2$), 4.21 (0.55H, dd, $J = 4.2$ and 7.0 Hz, C$\underline{H}$NH$_2$). $^{13}$C NMR (100 MHz; $CD_3OD$) $\delta = 25.6$* ($\underline{C}$H$_3$CD(OH), 25.7 ($\underline{C}$H$_3$CH(OH), 29.1* (C($\underline{C}$H$_3$)$_2$), 29.3 (C($\underline{C}$H$_3$)$_2$), 29.5 (C($\underline{C}$H$_3$)$_2$), 29.8 ($\underline{C}$H$_2$CD(OH)), 46.9* ($\underline{C}$(CH$_3$)$_2$), 47.1 ($\underline{C}$(CH$_3$)$_2$), 51.4 (S$\underline{C}$H$_2$), 51.6* (S$\underline{C}$H$_2$), 53.9 (CHNH$_2$), 54.1* (CHNH$_2$), 65.7 (t, $J = 21.0$ Hz, CD), 170.2 (CO). *m/z* (FAB+) 223.1225 (MH$^+$ $C_9H_{19}DNO_3S$ requires 223.1227).

### (R)-2-Amino-3-(2-methyl-4-oxo(1,1′-$^2H_6$)pentan-2-ylthio)propanoic acid hydrochloric salt 18

L-Cysteine **16** (0.107 g, 0.88 mmol) and sodium carbonate-decahydrate (0.05 g, 0.18 mmol) were dissolved in a mixture of water (5 mL) and 1,4-dioxane (2 mL). [$^2H_{10}$]Mesityl oxide **17** (0.11 g, 0.10 mmol) was added and the reaction mixture was left to stir for 18 h. The solution was acidified to pH 2 by the addition of a few drops of 2 M hydrochloric acid and water was added (10 mL). The aqueous solution was washed with diethyl ether (10 mL), then concentrated *in vacuo* to give a mixture of the title compound and cysteine in the ratio 0.7:1; $^1$H NMR (300 MHz; $D_2O$) $\delta = 2.31$ (3H, s, C(O)C$\underline{H}_3$), 2.89 (1H, d, $J = 15.6$ Hz, C$\underline{H}_A$H$_B$), 2.96 (1H, d, $J = 15.6$ Hz, CH$_A\underline{H}_B$), 3.21 (1H, dd, $J = 7.0$ and 13.9 Hz, SC$\underline{H}_A$H$_B$), 3.32 (1H, dd, $J = 4.6$ and 13.9 Hz, SCH$_A\underline{H}_B$), 4.36 (1H, dd, $J = 4.6$ and 7.0 Hz, H$_2$NC$\underline{H}$). *m/z* (FAB+) 226.1380 (MH$^+$ $C_9H_{12}D_6NO_3S$ requires 226.1384).

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