A stereoselective synthesis of the urinary metabolite *N*-acetyl-*S*-(3,4-dihydroxybutyl)cysteine

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Abstract: On exposure to the potential carcinogen 1,3-butadiene, the major urinary metabolite in humans is *N*-acetyl-*S*-(3,4-dihydroxybutyl)cysteine. A novel, stereoselective synthesis of this cysteine-butadiene metabolite has been developed that is suitable for the production of either diastereomer for use in occupational exposure analysis. L-cysteine and 4-bromo-1-butene are coupled via an S_N2 reaction to give the core structure. A Sharpless asymmetric dihydroxylation using the DHQD ligand provided the terminal 1,2-diol with the 3-hydroxyl group in the *R* configuration.

Keywords: 1,3-butadiene, urinary metabolite, stereoselective synthesis, dihydroxylation

The volatile compound 1,3-butadiene used in the manufacture of rubber has been shown to cause cancer in mice and rats and has been labelled a probable carcinogen to humans.[1, 2] Although environmental levels of this compound are low, workers in butadiene manufacturing plants and rubber factories can be exposed to high levels of this compound.[3] The metabolism of 1,3-butadiene in mice and rats has been the subject of many studies with the first step being an epoxidation with Cytochrome P450 dependent enzymes and then a variety of metabolites are produced depending on the activity of the epoxide hydrolases and the glutathione transferases.[4, 5, 6] The first metabolites to be identified were the cysteine derivatives MI and MII.[7] Subsequently the regio and stereoisomers of MII have been separated and identified[8] and the metabolites have been shown to arise predominantly from the *R*-butadiene monoxide.[4] The MII metabolites are the major urinary metabolite in rats and mice, but

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the MI metabolite proved to be the major metabolite in monkeys.[7] A more recent study of the metabolites from low level exposure (1-20 ppm) of rats and mice to 1,3-butadiene by inhalation, showed that MI and new metabolites MIII were the dominant urinary metabolites in these animals at concentrations encountered in occupational exposure situations.[6] A thorough investigation by Albertini monitoring the urine of humans working in the production or the polymerisation of butadiene showed that MI is the major urinary metabolite in humans[3] and as such it represents a useful biomarker for monitoring the occupational exposure of factory workers to the potential carcinogen.[9]

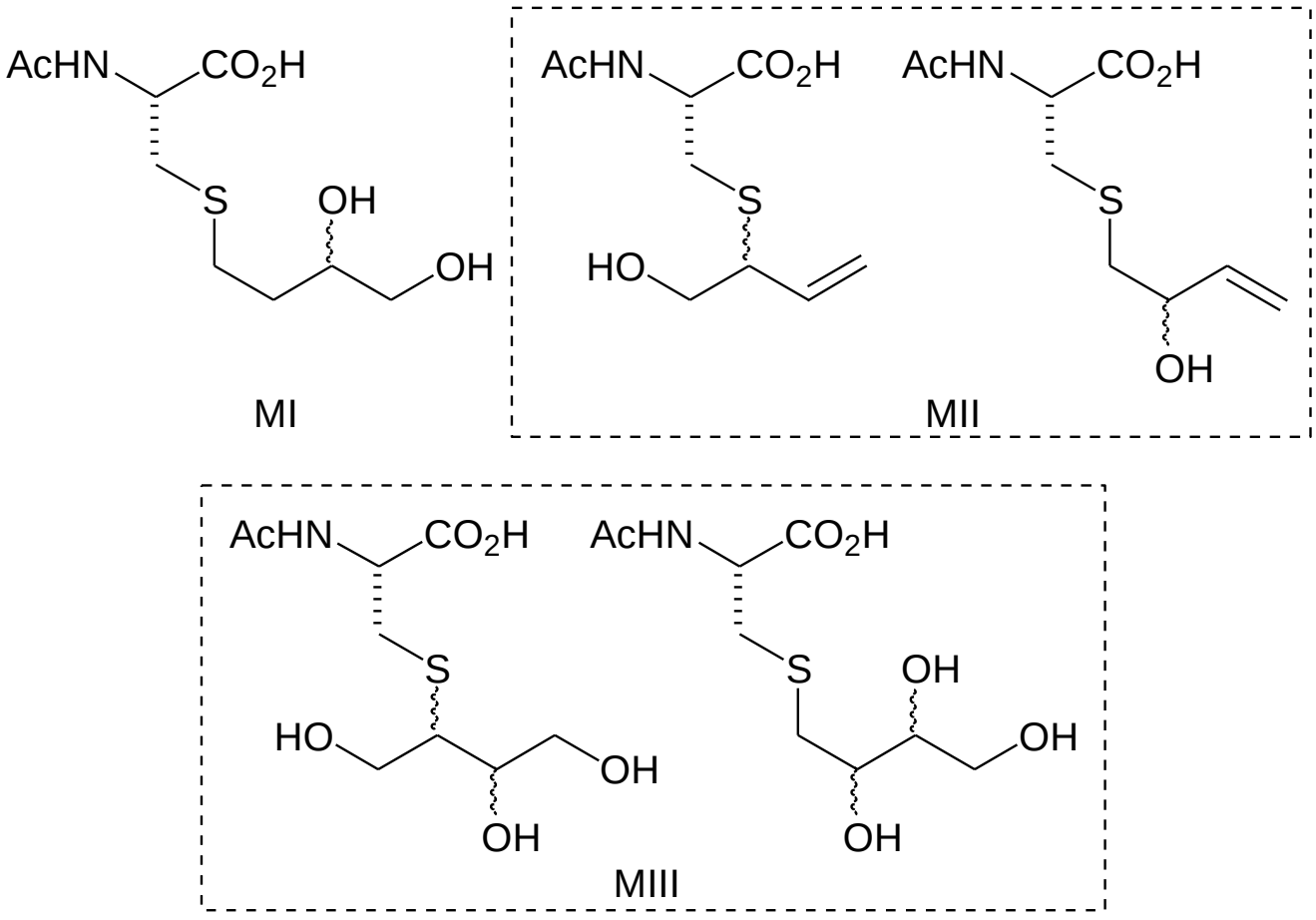
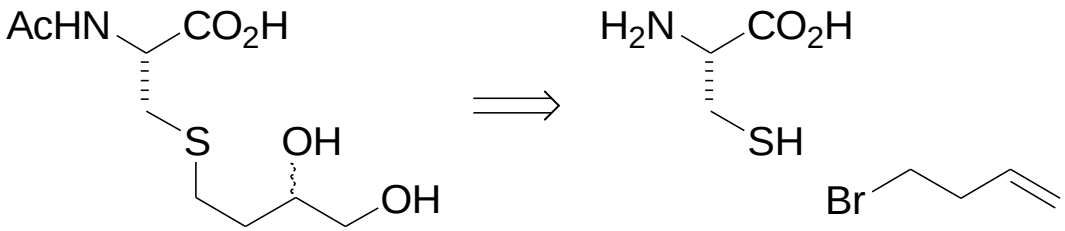


Figure 1. 1,3-Butadiene-cysteine urinary metabolites.

To support the structural characterisation of the urinary metabolites, synthetic standards of the cysteine derivatives have been prepared. Sabourin first reported the synthesis of MI from acetyl cysteine and acetoxy methyl vinyl ketone as a mixture of diastereomers.[7] where the butadiene component was prepared from commercially available 2-butyne-1,4-diol.[10] The four MII metabolites were first

prepared by Elfarra from acetyl cysteine and butadiene monoxide in a single reaction. The four regio/stereoisomers and a fifth product from an S_N2' reaction could be separated by high performance liquid chromatography (HPLC).[8] Subsequently Richardson has performed a stereospecific synthesis of the MII metabolites using *S*-butadiene monoxide and has demonstrated that the predominant urinary metabolites in mice and rats arise from the *R*-butadiene monoxide.[4] The metabolites MIII were similarly prepared by reaction of acetyl cysteine with 1,2-dihydroxy-3,4-epoxybutane.[4]

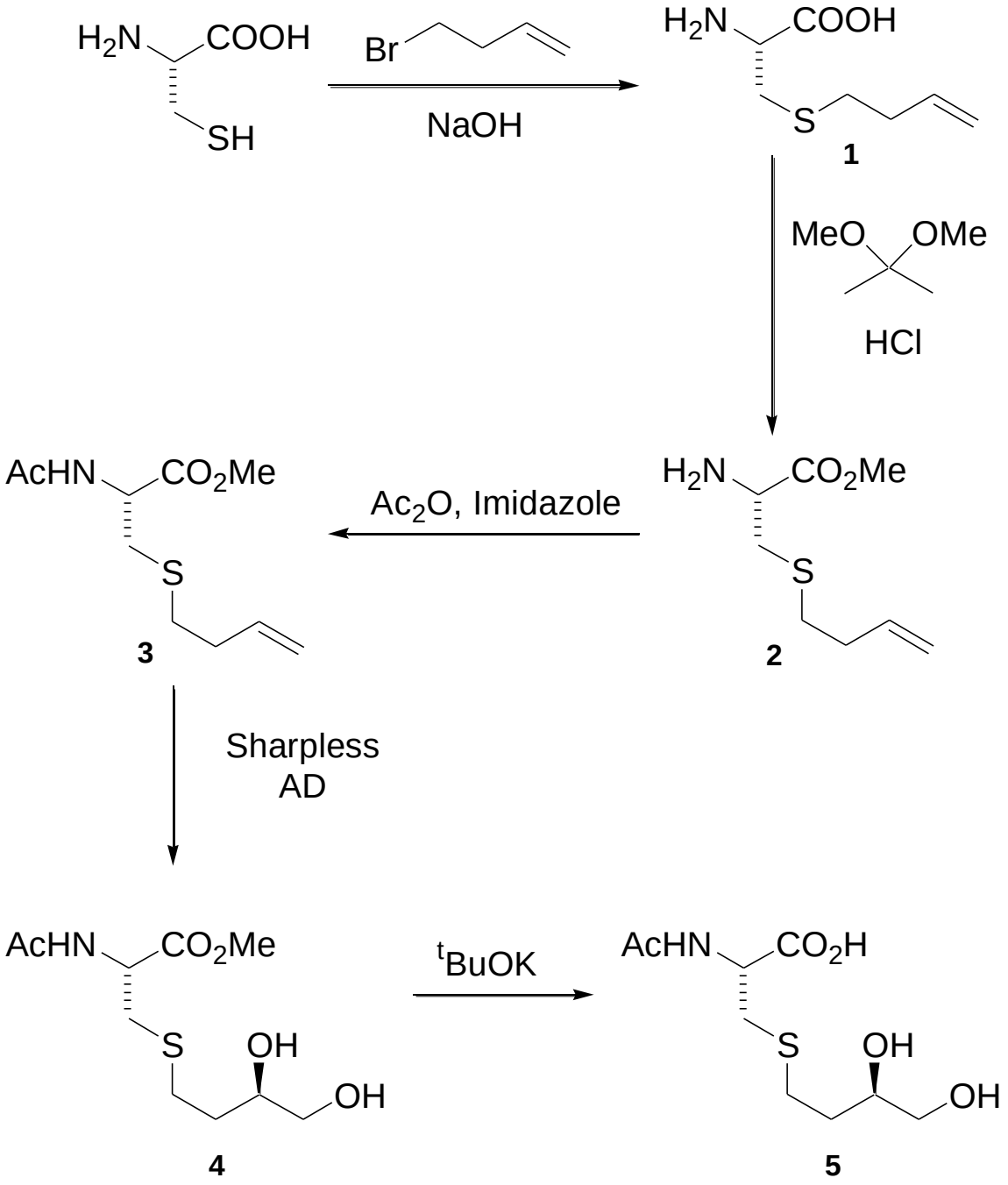
With the aim of developing a convenient analytical method to monitor occupational exposure to 1,3-butadiene in the South African context, our aim was to prepare a synthetic standard of the compound that was readily amenable to preparing an isotopically labelled substance that differed by 2 to 3 mass units. As a further consideration, the synthesis should be compatible with the production of either diastereomer as the stereochemistry of the metabolite is not known for the 3-hydroxyl group. The synthesis by Sabourin, et al. would give the title compound as a mixture of diastereomers.[7] We planned to use a Sharpless asymmetric dihydroxylation to enable us to produce either configuration at the 3-hydroxyl group and we planned to use L-cysteine and the four carbon equivalent 4-bromo-1-butene as our starting compounds..



Scheme 1 Retrosynthetic analysis of metabolite MI.

To prevent oxidation of the thiol to give cystine, the first step in the reaction was the formation of a thioether derivative of cysteine via a stereotypical S_N2 coupling between the cysteine dianion and the alkyl bromide according to the method of Armstrong[11]. The sulfide anion is much more nucleophilic than the carboxylate and the conversion is complete. The product **1** was isolated by crystallisation from hot water in 80% yield and a portion could be recrystallised to obtain a higher purity sample. As

reported for the saturated butyl analogue,[11] the compound is not very soluble in water at room temperature and the optical rotation appeared to vary greatly depending on the conditions under which measured. The sulfide **1** was also poorly soluble in organic solvents.



Scheme 2 Synthesis of metabolite MI.

Initially the aim of the project was to produce the methyl ester of the metabolite as a synthetic standard for the development of an occupational exposure assay, thus the carboxyl group was protected as the

methyl ester in the second step of the synthesis. The methyl esterification protocol of Rachele developed specifically for the preparation of hydrochloride methyl esters of amino acids proved to be a convenient and facile procedure.[12] The reagent 2,2-dimethoxypropane is the reaction solvent and also serves as the source of the methoxy group. The reaction is promoted by concentrated hydrochloric acid and it is not necessary to use hydrogen chloride gas as the 2,2-dimethoxypropane also acts as a dehydrating reagent as the acetal hydrolyses to methanol and acetone. The methyl ester **2** was isolated as the hydrochloride salt in 81% yield following recrystallisation from methanol and diethyl ether. This yield compared well with published yields in spite of improved organic solubility from the S-alkyl group.

Acetylation with acetic anhydride and imidazole gave the *N*-acetate **3** in 72% yield following purification by flash chromatography. This reaction is suitable to the introduction of the labelled acetate-d_3 for use as an internal standard.

Previous syntheses have produced the diol as a mixture of diastereomers[7, 4] and we initially sought to conduct an unselective dihydroxylation using catalytic osmium tetroxide and *N*-methylmorpholine *N*-oxide (NMO). However, the sulfide is prone to oxidation under these conditions and we found the the Sharpless Asymmetric Dihydroxylation was more suitable, not because the co-oxidant does not oxidise the sulfide, but because the reation is biphasic and the cysteine derivative remains in the organic phase while the co-oxidant remains in the aqueous phase.[13] We were able to obtain the product diol in 46% yield. This product is highly polar and it is suspected that the reason for the low yield is that some of the product partitioned into the aqueous phase and the sulfide was oxidised. No attempt was made to isolate the byproduct. The Sharpless dihydroxylation works best for more substituted alkenes and is known to give more variable results for terminal alkenes. Using the ligands we had on hand with a phthalazine (PHAL) core, we were able to achieve a selectivity of 4:1 in favour of the major diastereomer as measured by ^{1}H NMR integration of the NH resonance at 6.64 ppm and the α-H resonance at 4.8 ppm. When the ligands with a diphenylpyrimidine (PYR) core, reportedly best for terminal alkenes were used, an improved selectivity of 5:1 was obtained. Either diastereomer

could be produced by using the dihydroquinine (DHQ) or the dihydroquinidine (DHQD) ligands attached to the heterocyclic spacer with the stereochemistry predicted by the Sharpless mnemonic. The Jacobson method[14, 15] for obtaining a diol from a terminal alkene via preparation of the racemic epoxide was not considered because of the susceptibility of the sulfide to oxidation during the epoxidation reaction.

The final step in the synthesis was a deprotection of the methyl ester to reveal the acid. Ester hydrolysis was achieved using potassium *t*-butoxide in aqueous THF to give the target metabolite. Purification by silica gel chromatography gave this highly polar compound in moderate yield (63%). Use of preperative reverse phase HPLC should furnish the title compound with an improved yield,[4] but the product appears to be thermally unstable and care should be taken not to warm the sample. The ^{1}H NMR spectrum compares well with the published data reported for a mixture of diastereomers[4] apart from the α-proton which appears to occur at variable shift values, possible being concentration or pH dependent. Apart from the ^{1}H NMR spectrum and the mass spectral data of a trimethylsilyl derivative, no further characterisation data has been reported for the title compound. The ^{1}H NMR spectrum does not show resolution of the two diastereomers present, however the ^{13}C NMR spectrum does reveal the presence of the minor diastereomer in the doubling of the three methylene (CH_2) resonances.

EXPERIMENTAL

Commercially available reagents were purchased and used as supplied, unless stated otherwise. NMR spectra were recorded on a Bruker advance DRX-500 spectrometer or Bruker AC-300 spectrometer. Analytical thin layer chromatography (TLC) was performed on Merck Kieselgel 60 F254 plates. Flash column chromatography was performed using Merck 9385 silica gel 60 (230-400 mesh). Infra-red spectra were recorded on a Perkin Elmer BX-1 spectrometer. Optical rotations were measured on a Perkin Elmer model 341 polarimeter. High and low resolution mass spectra were obtained from the

University of the North West or the University of the Witwatersrand MS service centres. Melting points were recorded on a Reichert hot stage microscope and are uncorrected.

***S*-(3-butenyl)-L-cysteine (1).** Cysteine (2 g, 16 mmol) was dissolved in a solution of sodium hydroxide (20 mL, 2M aq.) and ethanol (28 mL) that had been deoxygenated by bubbling through a stream of nitrogen gas. A solution of 4-bromo-1-butene (3.4 mL, 33 mmol) in diethylether (3 mL) was added to the stirred reaction flask and stirring continued at room temperature overnight. The solution was adjusted to pH 5 by dropwise addition of concentrated hydrochloric acid giving a white precipitate. Recrystallisation from hot water gave a white crystalline product **1** (2.3 g, 13 mmol) in high yield (80%). This compound is poorly soluble in water and the NMR spectra were run at 35 °C. **^{1}H NMR** (300 MHz, D_2O) δ 6.08-5.92 (m, 1H), 5.32-5.18 (m, 2H), 4.04 (dd, *J* = 4.4, 7.5 Hz, 1H), 3.26 (dd, *J* = 4.4, 14.8 Hz, 1H), 3.15 (dd, *J* = 7.5, 14.8 Hz, 1H), 2.82 (t, *J* = 7.0 Hz, 2H), 2.54-2.44 (m, 2H); **^{13}C NMR** (75 MHz, D_2O) □ 172.8, 137.1, 116.3, 53.7, 32.8, 32.0, 30.7; **IR** (KBr pellet) 2978, 1621, 1581, 1488, 1417, 1342, 1303, 915, 849 cm^{-1}; **HRMS** (FAB) calc for $C_7H_{13}NO_2S$ [M+H]+ 176.0745, found 176.0743; **M.p.** 208-211 °C (dec.).

Methyl *S*-(3-butenyl)-L-cysteinate (2). To a suspension of the alkyl cysteine **1** (1.2 g, 6.7 mmol) in 2,2-dimethoxypropane (34 mL) was added conc. HCl (3.4 mL, 32% aq.) dropwise. The reaction mixture was stirred at room temperature overnight during which time the substrate dissolved. The products were concentrated *in vacuo* to give a dark viscous oil. Washing with ethyl acetate removed the majority of byproducts leaving the product as a precipitate. Careful crystallisation from methanol-diethyl ether mixtures gave the product as white needles that could be washed with a cold 10% solution of methanol in ether. The title compound **2** was obtained in high yield (1.4 g, 81%). $[\alpha]_D^{20}$ = +4.74 (*c* 1.0, $CHCl_3$); **^{1}H NMR** (300 MHz, $CDCl_3$) □ 8.7 (br-s, 2.4H), 5.86-5.71 (m, 1H), 5.13-4.97 (m, 2H), 4.43 (br-t, *J* = 5.4 Hz, 1H), 3.82 (s, 3H), 3.27 (br-d, *J* = 5.4 Hz, 2H), 2.67 (t, *J* = 7.0 Hz, 2H), 2.38-2.28 (m, 2H); **^{13}C NMR** (75 MHz, $CDCl_3$) □ 168.6, 136.3, 116.3, 53.5, 53.1, 33.6, 32.0,

32.0; **IR** (KBr pellet) 2847, 1738, 1494, 1434, 1328, 1245, 1056, 993, 925 cm^{-1}; **HRMS** (FAB) calc for $C_8H_{16}NO_2S$ [M+H]+ 190.0902, found 190.0901; **M.p.** 182-185 °C.

Methyl *N*-acetyl-*S*-(3-butenyl)-L-cysteinate (3). Compound **2** (1.3 g, 6.7 mmol) was dissolved in chloroform (4.5 mL) at 60 °C. Imidazole (0.4 g, 6 mmol) was added, followed by distilled acetic anhydride (4.5 mL, 48 mmol). After reflux for 1 hour under a calcium chloride drying tube, reaction was complete. Quenching with sodium bicarbonate, extraction, concentration and purification by chromatography (40-80% ethyl acetate in hexane) gave the acetate **3** as a colourless oil (1.1 g, 4.8 mmol, 72%). $\mathbf{R_f}$ 0.4 (80% ethyl acetate in hexane); $[\alpha]_D^{20}$ = +47.3 (*c* 1.0, $CHCl_3$); **^{1}H NMR** (500 MHz, $CDCl_3$) □ 6.50 (br-d, *J* = 6.8 Hz, 1H), 5.77-5.68 (m, 1H), 5.04-4.95 (m, 2H), 4.79-4.73 (m, 1H), 3.70 (s, 3H), 2.96 (dd, *J* = 5.0, 13.9 Hz, 1H), 2.91 (dd, *J* = 5.5, 13.9 Hz, 1H), 2.52 (t, *J* = 7.3 Hz, 2H), 2.28-2.22 (m, 2H), 1.99 (s, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$) □ 171.3, 169.8, 136.1, 116.1, 52.5, 51.7, 34.0, 33.5, 31.8, 22.9; **IR** (film) 3315, 1747, 1660, 1537, 1436, 1377, 1214, 954 cm^{-1}; **HRMS** (EI) calc for $C_{10}H_{17}NO_3S$ [M]+ 231.0929, found 231.0936; **m/z** (EI) 231 (M+, 1.4), 172 [(M-$CO_2Me)^+$, 39], 86 (100), 34 (95).

Methyl *N*-acetyl-*S*-(3,4-dihydroxybutyl)-L-cysteinate (4). The Sharpless procedure for asymmetric dihydroxylation was followed such that the alkene **3** (160 mg, 0.69 mmol), $(DHQD)_2PYR$ (6 mg, 1 mol%), $K_2OsO_2(OH)_4$ (1 mL, 0.5 mg/mL aq., 0.2 mol%), $K_3Fe(CN)_6$ (688 mg, 2.1 mmol) and K_2CO_3 (281 mg) were dissolved in *t*-butanol/water (7 mL, 1:1). The reaction mixture was immediately cooled to 0 °C and stirred for 9 hours, then at room temperature overnight. The excess oxidants were quenched with Na_2SO_3 (3 eq) and stirred at room temperature for 90 minutes. Extraction of this highly polar product with ethyl acetate, then isopropanol and purification by chromatography (gradient elution in 100% ethyl acetate to 15% methanol in ethyl acetate) gave the diol **4** (84.5 mg, 46%). $\mathbf{R_f}$ 0.6 (20% methanol in ethyl acetate); $[\alpha]_D^{20}$ = +43.6 (*c* 1.0, $CHCl_3$); **^{1}H NMR** (500 MHz, $CDCl_3$) □ 6.64 (br-d, *J* = 7.7 Hz, 1H), 4.79 (dt, *J* = 5.3, 7.9 Hz, 1H), 3.83-3.75 (m, 1H), 3.76 (s, 3H), 3.60 (dd, *J* = 3.3, 11.2 Hz, 1H), 3.44 (dd, *J* = 7.1, 11.2 Hz, 1H), 3.00 (dd, *J* = 5.2, 13.9 Hz, 1H), 2.92 (dd, *J* =

5.8, 13.9 Hz, 1H), 2.78 (br-s, 2H), 2.74-2.65 (m, 1H), 2.65-2.56 (m, 1H), 2.037 (s, 3H), 1.72-1.59 (m, 2H); **^{13}C NMR** (125 MHz, $CDCl_3$) □ 171.4, 170.2, 70.8, 66.5, 52.7, 52.0, 34.2, 32.7, 28.9, 23.1; **IR** (film) 3367, 3298, 1741, 1657 1547, 1438, 1375, 1218, 1038 cm^{-1}; **HRMS** (FAB) calc for $C_{10}H_{20}NO_5S$ [M+H]+ 266.10622, found 266.10620; **m/z** (EI-TOF) 247 [$(M-H_2O)^+$, 0.1], 229 [$(M-2H_2O)^+$, 6], 206 (28), 188 (82), 43 (100). **Minor diastereomer**: Identifiable resonances **^{1}H NMR** (500 MHz, $CDCl_3$) □ 6.68 (br-d, *J* = 7.7 Hz, 1H), 4.82 (ddd, *J* = 4.8, 6.4, 8.1 Hz, 1H), 2.87 (dd, *J* = 6.4, 13.9 Hz, 1H), 2.044 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) □ 170.9, 70.3, 51.8, 34.6, 32.4, 28.7; Integration of the ^{1}H NMR signals at □ 6.68 and 4.82 (minor isomer) and □ 6.64 and 4.79 (major isomer) gave a diastereomeric ratio of 83:17.

***N*-Acetyl-*S*-((*R*)-3,4-dihydroxybutyl)-L-cysteine (5)[4, 7].** To a solution of methyl ester **4** (116 mg, 0.44 mmol) in water (3 mL) and THF (9 mL) at room temperature was added potassium t-butoxide (198 mg, 1.8 mmol) and the reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure and the residue was purified by flash chromatography on silica gel. The product eluted with 30% methanol in ethyl acetate. However, the target metabolite proved to be thermally unstable and much degradation occurred during the purification to give the title compound **5** in moderate yield (70 mg, 63%). **R_f** 0.5 (50% methanol in ethyl acetate); $[\alpha]_D^{20}$ = +4.7 (*c* 1.0, CH_3OH); **^{1}H NMR** (300 MHz, D_2O) □ 4.34 (dd, *J* = 4.3, 8.2 Hz, 1H), 3.75-3.84 (m, 1H), 3.58 (dd, *J* = 4.0, 11.7 Hz, 1H), 3.47 (dd, *J* = 6.8, 11.7 Hz, 1H), 3.04 (dd, *J* = 4.3, 13.9 Hz, 1H), 2.86 (ddd, *J* = 2.8, 8.3, 13.9 Hz, 1H), 2.58-2.78 (m, 2H), 2.04 (s, 3H), 1.59-1.82 (m, 2H); **^{13}C NMR** (75 MHz, D_2O) □ 178.0, 174.6, 71.4, 66.1, 55.5, 34.7, 33.2, 28.7, 22.8; **IR** (film) 3313, 2923, 2852, 1601, 1395, 1040 cm^{-1}; **HRMS** (FAB) calc for $C_9H_{18}NO_5S$ [M+H]+ 252.09057, found 252.09062. **Minor diastereomer**: There were no identifiable resonances from the minor diastereomer in the ^{1}H NMR spectrum, but identifiable resonances in the ^{13}C NMR spectrum were as follows: **^{13}C NMR** (75 MHz, D_2O) □ 34.5, 33.0, 28.6; Analysis of the ^{13}C NMR spectrum suggested that there was no substantial change in the diastereomeric ratio from the methyl ester **4**.

ACKNOWLEDGMENTS

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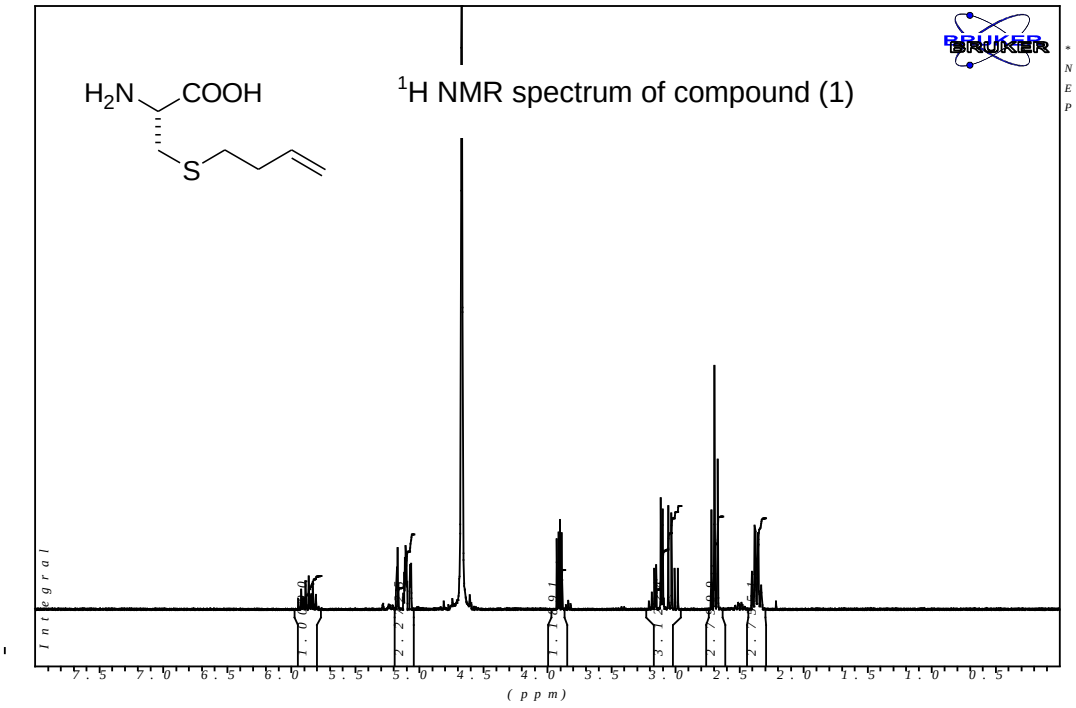
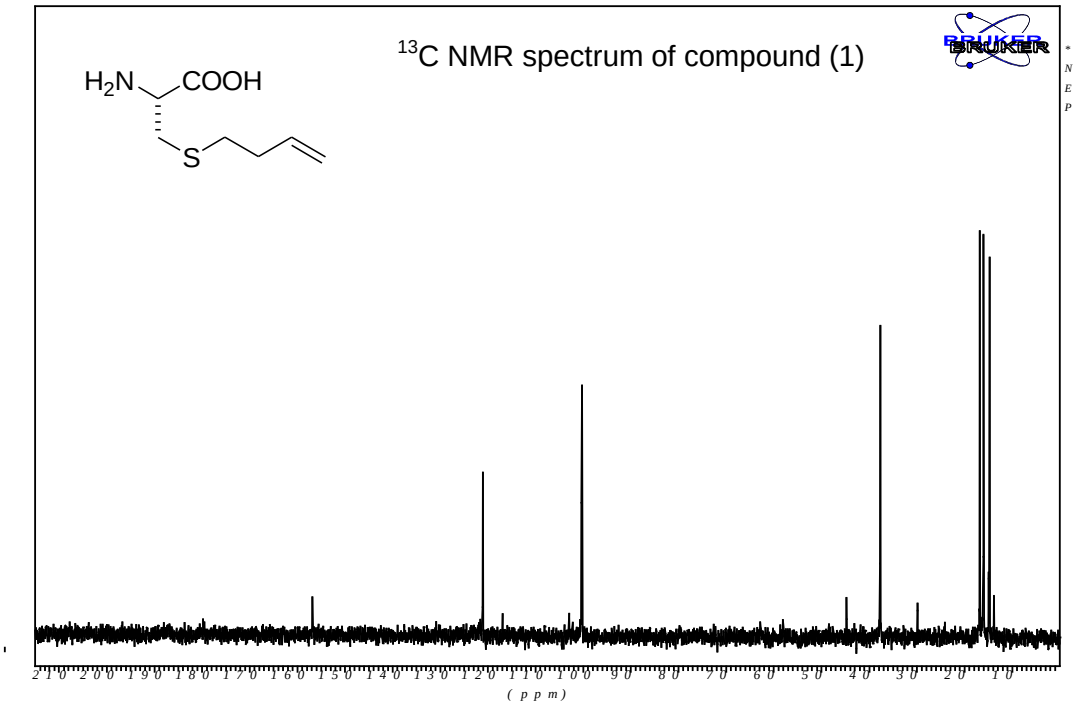
SUPPORTING INFORMATION

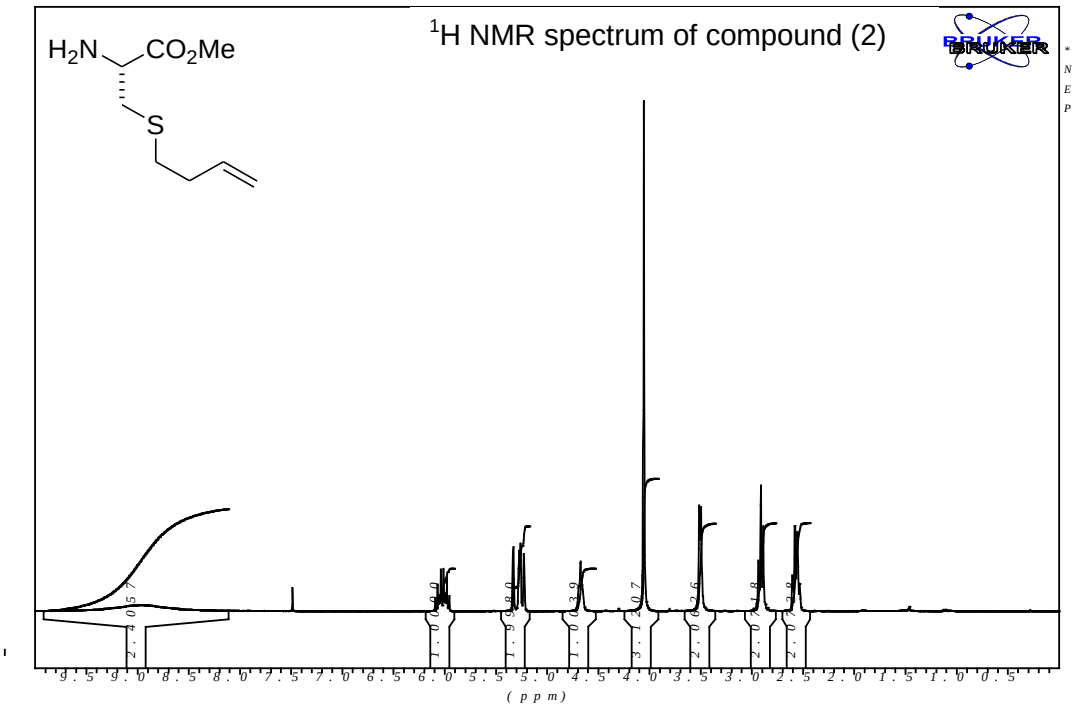
^{13}C and ^{1}H NMR spectra for compounds **1** – **5**

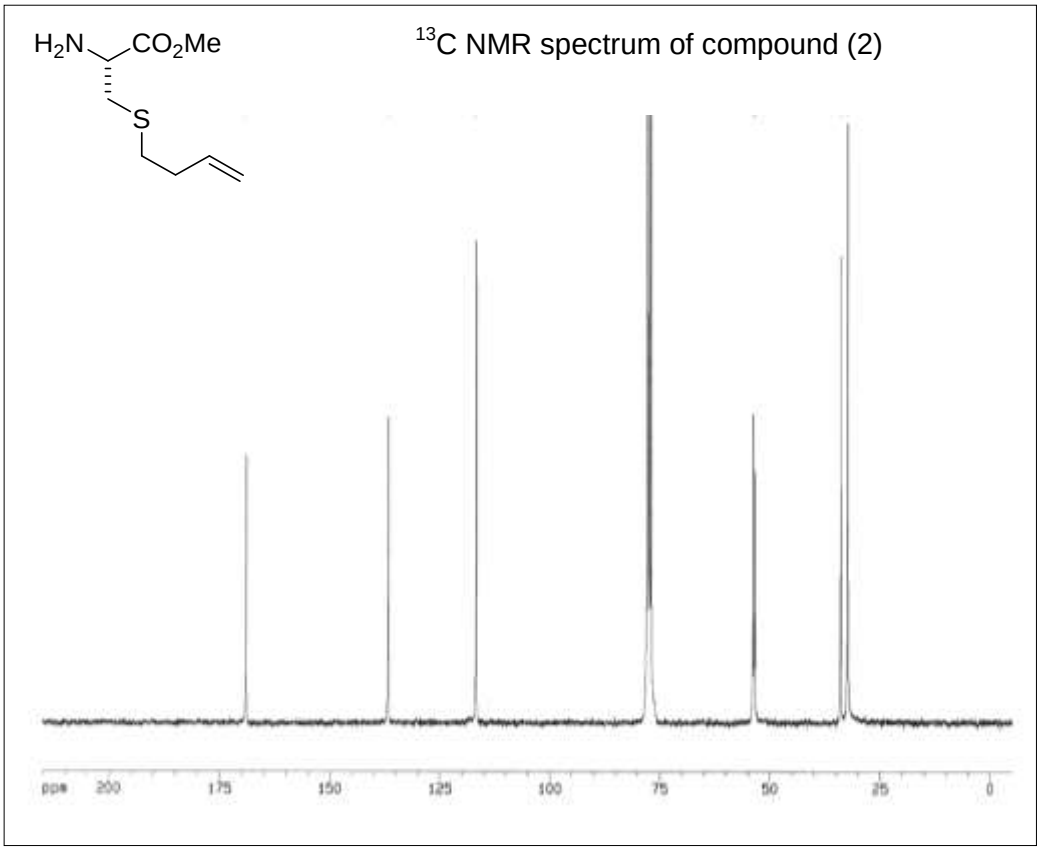
***S*-(3-butenyl)-L-cysteine (1) in D_2O**





Methyl *S*-(3-butenyl)-L-cysteinate (2) in $CDCl_3$





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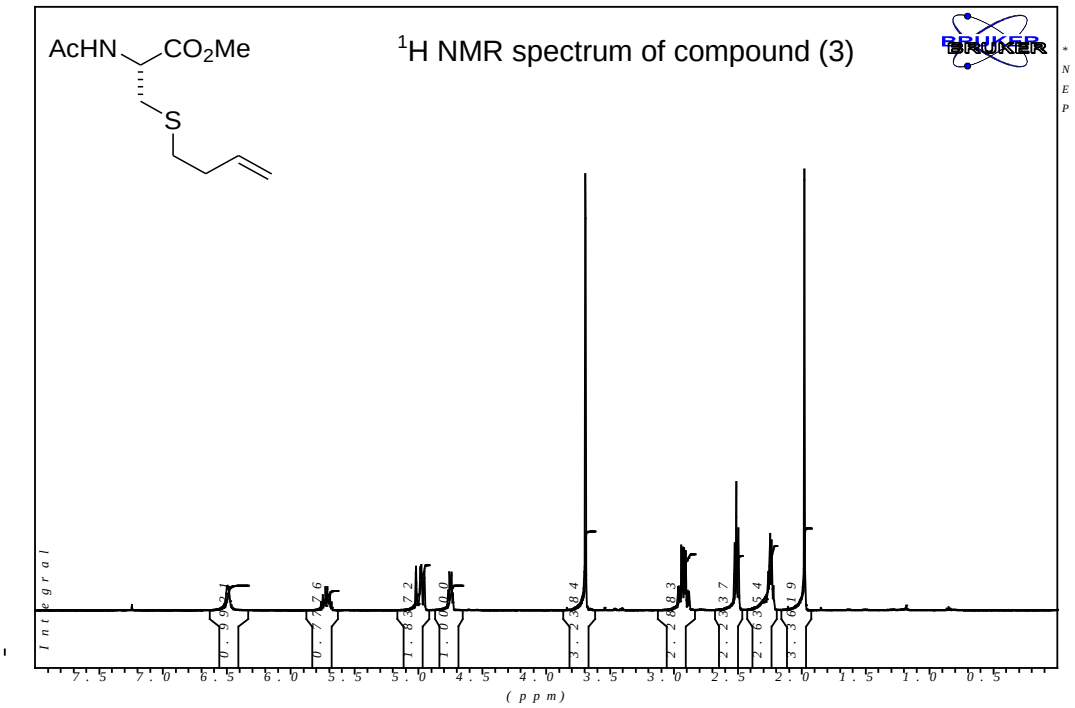
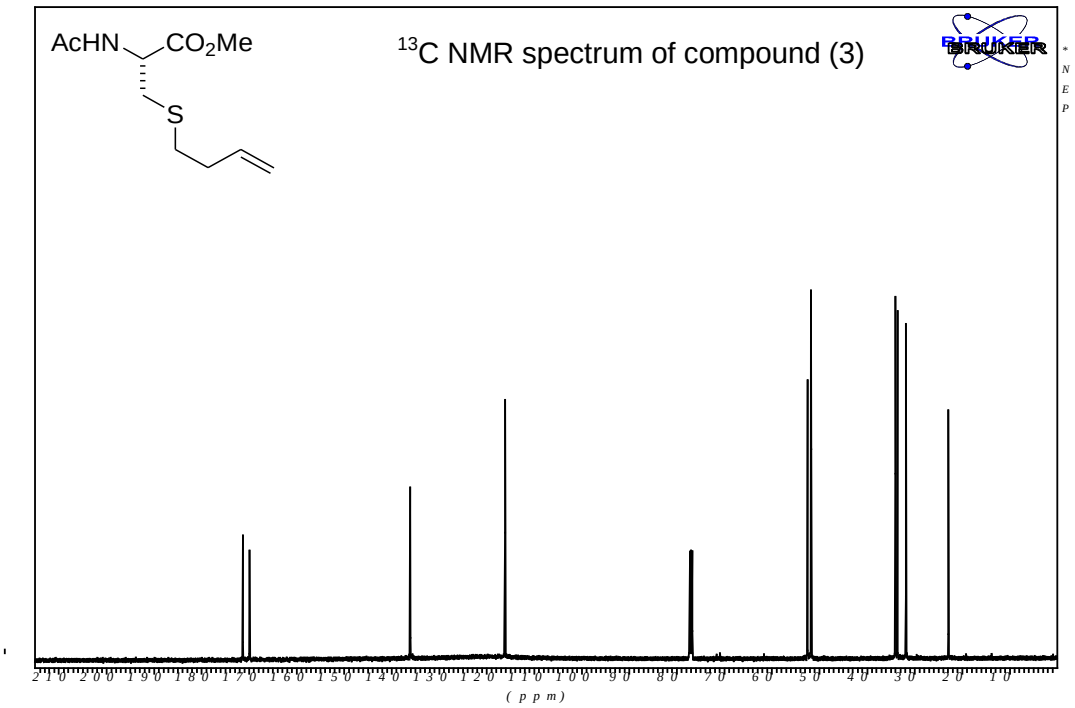
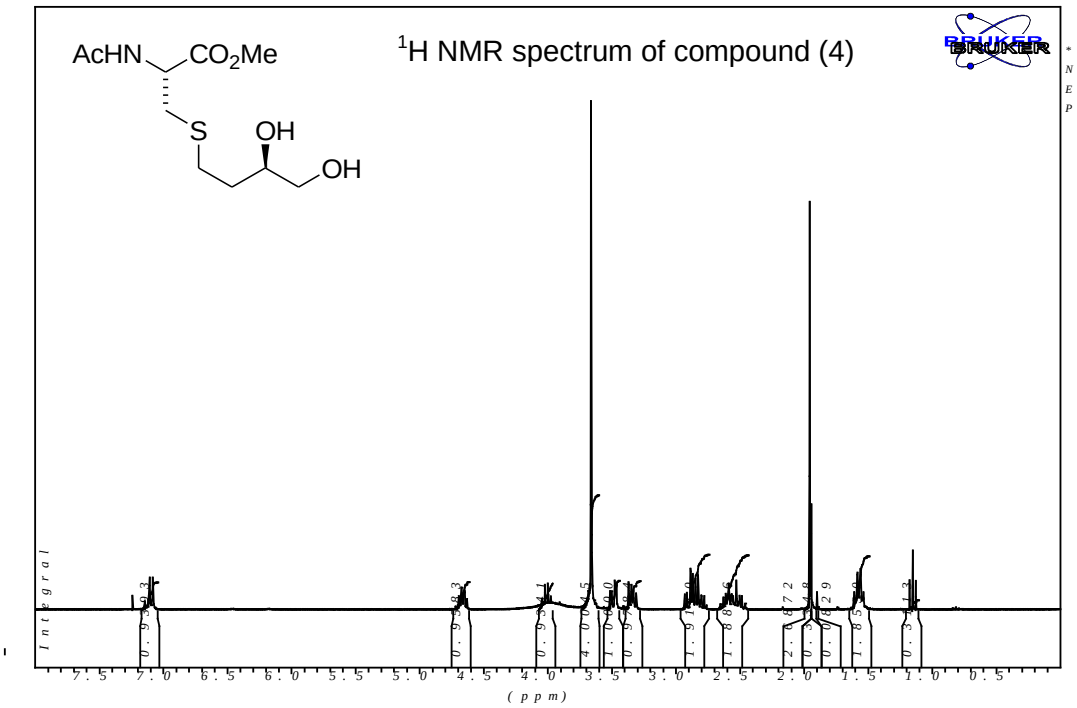
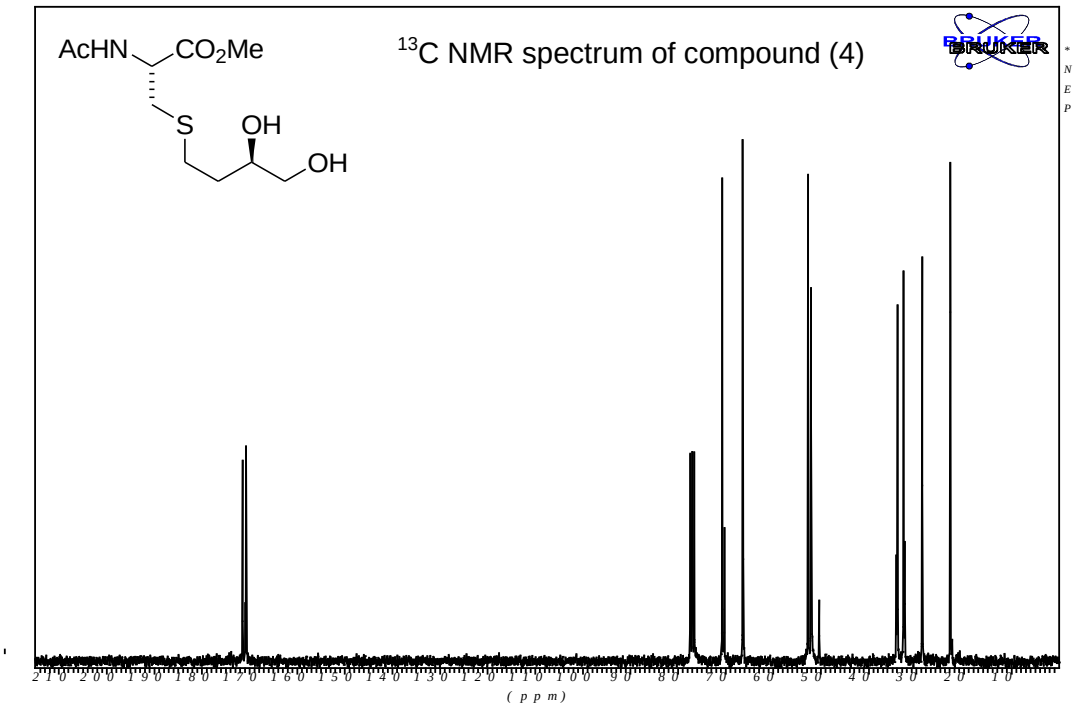




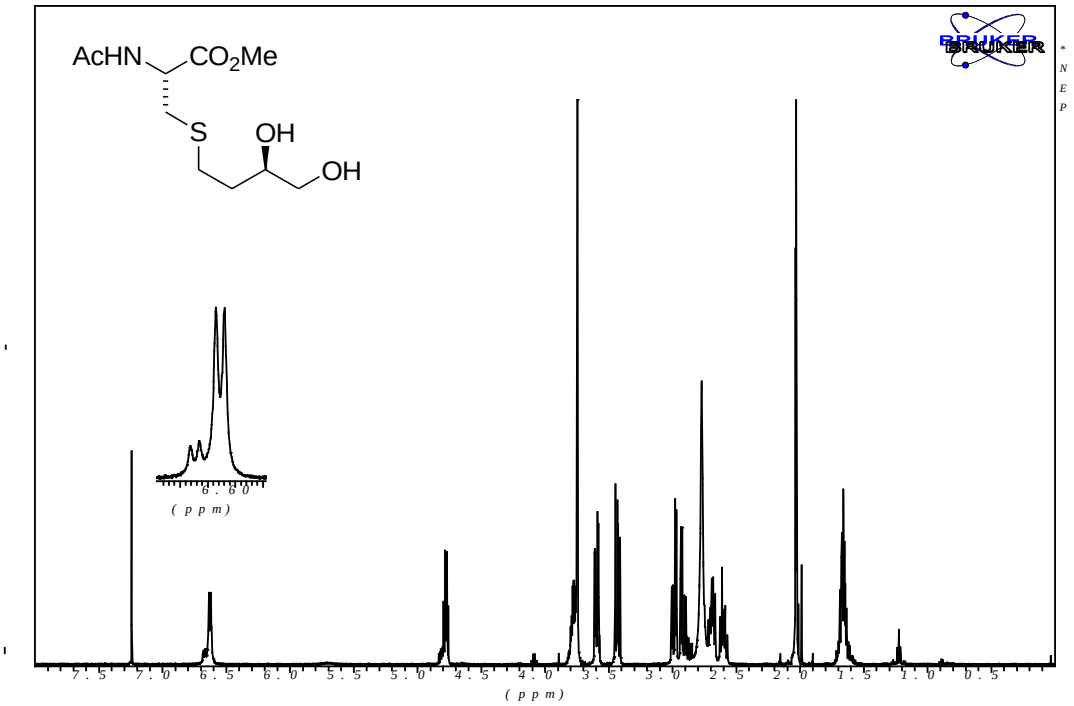
Methyl *N*-acetyl-*S*-(3,4-dihydroxybutyl)-L-cysteinate (4) in $CDCl_3$



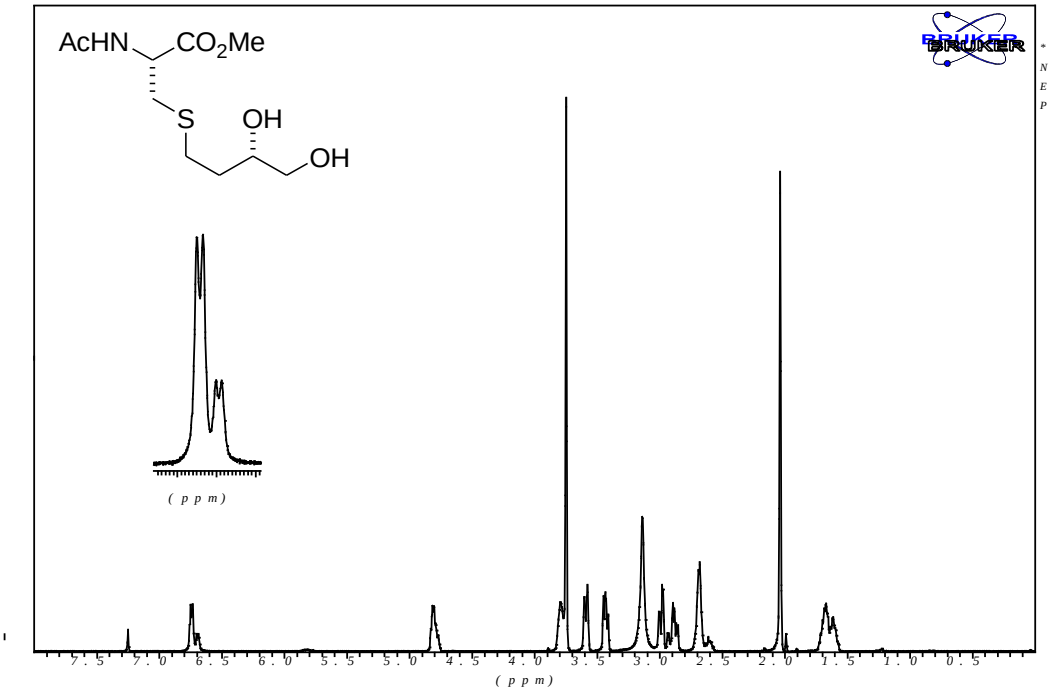


The ^{13}C NMR spectrum shows some duplication of chemical shifts at a lower intensity due to the presence of the minor diastereomer.

Product of the Sharpless dihydroxylation reaction using the $(DHQD)_2PYR$ ligand in the chiral catalyst.



Product of the Sharpless dihydroxylation reaction using the $(DHQ)_2PHAL$ ligand in the chiral catalyst showing reversal of the diastereoselectivity.



_N_-Acetyl-_S_-(_R_)-3,4-dihydroxybutyl)-L-cysteine (5) in D_2O