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ACYL-MELDRUM'S ACID IN REGIOSPECIFIC SYNTHESIS OF ISOTOPICALLY LABELLED COMPOUNDS FOR POLYKETIDE BIOSYNTHETIC STUDIES

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ABSTRACT. Synthesis of ^{2}H or ^{13}C labelled ß-keto esters/thioesters was accomplished by decarboxylative esterification/thioesterification of ^{2}H or ^{13}C labelled acetyl-Meldrum's acid, 5-acetyl-2,2-dimethyl-1,3-dioxane-4,6-dione. The compounds synthesised include, N-acetyl-S-([2,3-$^{13}C_2$]acetoacetyl)cysteamine, N-acetyl-S-([4,4,4-^{2}H]acetoacetyl)cysteamine, N-acetyl-S-([4,4,4-$^{2}H_3$]-3-hydroxybutyryl)cysteamine, N-acetyl-S-([3-^{13}C]-3-hydroxybutyryl)cysteamine and N-acetyl-S-([2,3-$^{13}C_2$]-3-hydroxybutyryl)cysteamine.

INTRODUCTION

Biosynthesis of non-aromatic polyketides, in both bacteria and fungi, is believed to occur by the processive mechanism [1] (a process in which the enzyme mediated functional group transformations of the growing polyketide chain take place before subsequent condensation with the next malonate unit). Such intermediates are usually activated as Co-enzyme A (CoA) or acylprotein (ACP) thioesters. In such a biosynthetic mechanism, acetoacetic (**1**), 3-hydroxybutyric acid (**2**) thioesters, the corresponding 2-methyl analogs, 3-oxo-pentanoic, 3-hydroxypentanoic acid thioesters and their 2-methyl analogs are believed to be among the first early intermediates depending on the biosynthetic compound. Due to the costs involved in the synthesis of isotopically labelled precursors and their inherent degradation by various polyketide producing micro-organisms, there are only a few isolated reports on their intermediacy. Successful incorporation of such intermediates has been reported in four polyketides, three propionate derived (tylosin, erythromycin-A and nargenicin) [2] and one acetate derived (aspyrone) [3,4]. Isolation of various intermediates [5] corresponding to advanced precursors of tylosin, protomycinolide and mycinamicin has further confirmed the processive mechanism.

During our biosynthetic investigation on an acetate derived polyketide [6], palitantin (**3**), we required putative advanced precursors labelled regiospecifically with ^{2}H or ^{13}C and activated as N-acetylcysteamine (NAC) thioesters (a model for acyl-CoA or acyl-ACP). Because of the high costs of labelled reagents, synthesis of these precursors require methods which give high yields in every step. Reports in the literature revealed that ß-keto esters [7] and thioesters [8] could be synthesised quantitatively from Meldrum's acid. We report here

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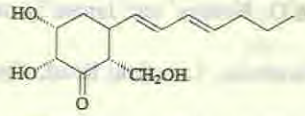
a convenient method for the synthesis of regiospecifically ^{2}H or ^{13}C labelled acetoacetic and 3-hydroxybutyric acid thioesters from ^{2}H or ^{13}C labelled acetic and malonic acid [9].



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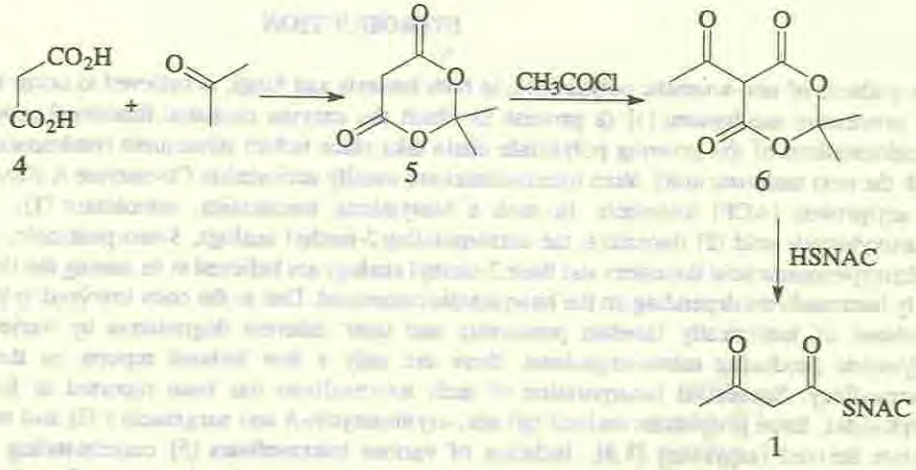
RESULTS AND DISCUSSION

Meldrum's acid (**5**) was prepared quantitatively from malonic acid (**4**). Acylation of Meldrum's acid with acetyl chloride [10] gave acetyl-Meldrum's acid (**6**) quantitatively. Decarboxylative thioesterification of acetyl-Meldrum's acid with N-acetylcysteamine afforded acetoacetyl cysteamine (**1**) in good yield (Scheme 1).



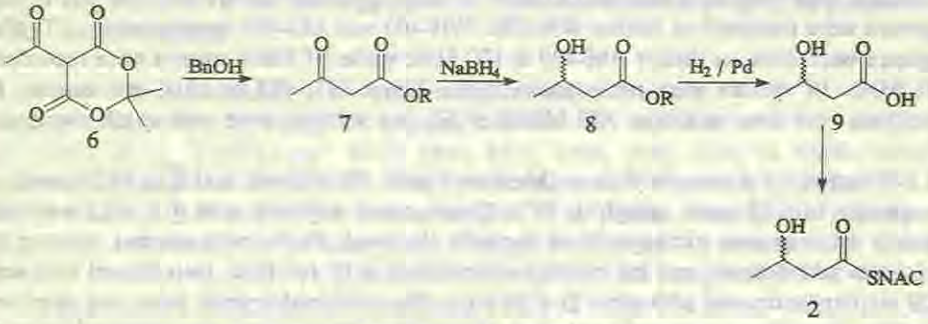
Scheme 1

[5-^{13}C]Meldrum's acid (**5c**) was prepared from [2-^{13}C]malonic acid (**4c**) and acetone. Acylation of [5-^{13}C]Meldrum's acid with [1-^{13}C] acetyl chloride gave 5-([1'-^{13}C]acetyl)-[5-^{13}C]Meldrum's acid (**6c**). Decarboxylative thioesterification of 5-(1'-^{13}C]acetyl)-[5-^{13}C]Meldrum's acid with N-acetylcysteamine afforded N-acetyl-S-([2,3-$^{13}C_2$]acetoacetyl)cysteamine (**1c**). This compound showed enhanced ^{13}C resonances at δ 59.94 (*d*, J = 36 Hz) and 199.84 (*d*, J = 36 Hz) for the keto form and at δ 99.81 (*d*, J = 70 Hz) and 173.79 (*d*, J = 70 Hz) for the enol form which confirmed the presence of two adjacent ^{13}C labels in the compound.

Acylation of Meldrum's acid (**5**) with [2,2,2-$^{2}H_3$]acetyl chloride gave 5-([2',2',2'-$^{2}H_3$]acetyl)Meldrum's acid (**6a**). Decarboxylative thioesterification of this compound with N-acetylcysteamine afforded N-acetyl-S-([4,4,4-$^{2}H_3$]acetoacetyl)cysteamine (**1a**) which showed

enhanced ^2H resonances at δ 2.24 and 1.96 for the keto and enol forms, respectively. ^1H NMR revealed only residual peaks at δ 2.24 and 1.96 for the keto and enol forms of the compound.

Synthesis of N-acetyl-S-(3-hydroxybutyryl)cysteamine (**2**) was started from 5-acetyl-Meldrum's acid (**6**). Decarboxylative esterification of this compound with benzyl alcohol gave benzyl acetoacetate in good yield. Selective reduction of benzyl acetoacetate (**7**) with sodium borohydride gave the corresponding hydroxy ester (**8**) in high yield. Hydrogenolysis of the benzyl ester gave 3-hydroxybutanoic acid (**9**) quantitatively. Thioesterification [11] of the hydroxy acid N-acetylcysteamine gave N-acetyl-S-(3-hydroxybutyryl)cysteamine (**2**) in reasonable yield (Scheme 2).



Scheme 2

Decarboxylative esterification of 5-([2',2',2'-$^2H_3$]acetyl)Meldrum's acid (**6a**) with benzyl alcohol gave benzyl[4,4,4-$^2H_3$]acetate (**7b**). Reduction of the benzyl ester with sodium borohydride gave benzyl[4,4,4-$^2H_3$]-3-hydroxybutanoate (**8a**). Hydrogenolysis of the hydroxy ester gave [4,4,4-$^2H_3$]-3-hydroxybutanoic acid (**9a**) which was thioesterified with N-acetylcysteamine to N-acetyl-S-([4,4,4,-$^2H_3$]-3-hydroxybutyryl)cysteamine(**2a**). The thioester showed only a single enhanced ^2H resonance at δ 1.22 and residual resonance at the same place in its ^1H NMR spectrum.

Similarly, decarboxylative esterification of 5-([1'-^{13}C]acetyl)Meldrum's acid (**6b**) with benzyl alcohol gave benzyl[3-^{13}C]acetoacetate (**7b**) which was reduced with sodium borohydride to give benzyl[3-^{13}C]-3-hydroxybutanoate (**8b**). Hydrogenolysis of the hydroxy ester gave [3-^{13}C]-3-hydroxybutanoic acid (**9b**). Thioesterification of the acid with N-acetylcysteamine gave N-acetyl-S-([3-^{13}C]-3-hydroxybutyryl)cysteamine(**2b**). This compound showed an enhanced ^{13}C resonance at δ 65.0 (C-3).

The reaction between 5-([1'-^{13}C]acetyl)-[5-^{13}C]Meldrum's acid (**6c**) and benzyl alcohol gave benzyl [2,3-$^{13}C_2$]acetoacetate (**7c**). This was reduced with sodium borohydride and the alcohol (**8c**) hydrogenolised to give [2,3-$^{13}C_2$]-3-hydroxybutanoic acid (**9c**). Thioesterification of the acid with N-acetylcysteamine gave N-acetyl-S-(2,3-$^{13}C_2$]-3-hydroxybutyryl)cysteamine (**2c**). The thioester showed enhanced ^{13}C resonances at δ 64.99 (*d*, J = 37 Hz, C-3) and 52.49 (*d*, J = 37 Hz, C-2) which confirmed the presence of two adjacent ^{13}C labels in the compound.

This approach to syntheses of labelled precursors offers an easy and reliable method of labelling acetoacetic acid, its analogs and derivatives at any desired carbon with ^{13}C, ^2H or

both. The importance of this method in universal ^{13}C labelling of acetoacetic acid, its analogs and derivatives needs no emphasis. With the availability of asymmetric methods [12] for the reduction of ß-keto carboxylic acid esters, preparation of enantiomers of 3-hydroxybutanoic acid thioester specifically labelled with ^{2}H or ^{13}C should be possible. Such compounds would be useful in investigations on stereochemical details of biosynthesis of non-aromatic polyketides.

EXPERIMENTAL

General. Mps were were done on a Koefler hot stage apparatue and are uncorrected. ^{1}H NMR spectra were recorded on Bruker WM-250, WH-400 and AM-400 spectrometers. ^{13}C NMR spectra were done on Bruker AM-400 at 100 MHz while ^{2}H NMR spectra were recorded at 61 MHz. IR spectra were recorded on Perkin-Elmer 297, 983 or 1310 instruments. MS analyses were done on Kratos AEI MS 30 or 50. Dry solvents were used in alll reactions.

2,2-Dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) (5). Malonic acid (2 g, 19.2 mmol) was suspended in cold acetic anhydride (0°). Concentrated sulphuric acid (0.2 mL) was added slowly with vigorous stirring until all the solid dissolved. To the cold solution, acetone (2.5 mL) was added slowly and the reaction mixture kept at 0° for 18 h, then diluted with water (20 mL) and extracted with ether (5 x 50 mL). The combined organic layer was dried with Na_2SO_4, the solvent removed to give an oily residue. The residue was cleaned by column chromatography (ether) to give 2.78 g (91%) which was further purified by recrystallisation to give 1.86 g (70%) of pure crystalline Meldrum's acid. Found 145.0491 ($M^+ + 1$); MS *m/z*: 145, 130, 129 (100%); m.p 94-96°; IR ν_{max} ($CHCl_3$) cm^{-1}: 3520 (w), 2800, 1780, 1745, 1480, 1300, 1070, and 1005; ^{1}H NMR ($CDCl_3$): δ 1.82 (6H, *s*, $-CH_3$), 3.65 (2H, *s*, $-CH_2-$). This procedure was used for the preparation of the corresponding labelled compound, [5-^{13}C]-2,2-dimethyl-1,3-dioxane-4,6-dione (**5c**).

5-Acetyl-2,2-dimethyl-1,3-dioxane-4,6-dione (Acetyl-Meldrum's acid) (6). Meldrum's acid (4.2 g, 28.7 mmol) was dissolved in a mixture of dry CH_2Cl_2 (15 mL) and dry C_5H_5N (2.3 mL) and the resulting solution cooled to 0°. To the solution, acetyl chloride (2.34 g, 28.7 mmol) in dry CH_2Cl_2 (5 mL) was added and the reaction mixture stirred for 1 h then for 3 h at 25°. The mixture was diluted with water, acidified with 3 M HCl and extracted with CH_2Cl_2(5 x 80 mL). The combined extract was dried and the solvent removed to give 4.22 g of acetyl-Meldrum's acid (**6**) as a dark brown solid. Attempted purification led to heavy loss of the product hence further reaction were done with the crude material. Found 186.0524 (M^+), $C_8H_{10}O_5$ requires 186.0528. MS *m/z*: 186, 129, 128, 85, 69, 59 (100%), 58; IR ν_{max} ($CHCl_3$) cm^{-1}: 1779, 1738, 1667, 1638, 1576, 1557; ^{1}H NMR ($CDCl_3$): δ 2.27 (3H, *s*, CH_3CO-), 1.80 (6H, *s*, $-CH_3$). This procedure was used in the synthesis of the labelled analogs, 5-([2',2',2'-$^{2}H_3$]acetyl)-2,2-dimethyl-1,3-dioxane-4,6-dione (**6a**), 5-(1'-^{13}C]acetyl)-2,2-dimethyl-1,3-dioxane-4,6-dione (**6b**) and 5-([1'-^{13}C]acetyl)-[5-^{13}C]-2,2-dimethyl-1,3-dioxane-4,6-dione (**6c**).

3-Oxobutanethioicacid-S-[(2-acetylamino)ethyl]ester(N-acetyl-S-(acetoacetyl)cysteamine)(1). N-acetylaminoethanethiol (0.13 g, 1.1 mmol) was dissolved in benzene (20 mL) and crude 5-acetyl-Meldrum's acid (**6**) added. The reaction mixture was refluxed for 18 h, the solvent removed and the oily residue purified by flash column chromatography (1-7% methanol in

ethyl acetate) to give 0.22 g (80%) of the thioester (1). Found 203.0628 (M^+), $C_8H_{13}NO_3S$ requires 203.0616. MS *m/z*: 203, 193, 161, 119 (100%), 60(100%); IR ν_{max} ($CHCl_3$) cm^{-1}: 3500, 3400,1700, 1650 and 1600; ^1H NMR (for the keto form) ($CDCl_3$): δ 5.97 (1H, *br s*, -NH-), 3.70 (2H, *s*, $-COCH_2CO-$), 3.46 (2H, *q*, J = 6 Hz, $-CH_2N-$), 3.08 (2H, *t*, J = 6.3 Hz, $-CH_2S-$), 2.26 (3H, *s*, CH_3CO-), 1.96 (3H, *s*, CH_3CON-); ^{13}C NMR (for the keto form) ($CDCl_3$): δ 199.91 (C-3), 199.20 (C-1), 170.52 (-CON-), 57.97 (C-2), 39.10 ($-CH_2N-$), 30.04 (C-4), 29.60 ($-CH^2S-$), 23.13 (CH_3CON-); ^1H NMR (for the enol form) ($CDCl_3$): δ 12.56 (1H, *s*, -OH), 5.97 (1H, *br s*, -NH-), 5.45 (1H, *s*, =CH-), 3.46 (2H, *q*, J = 6 Hz, $-CH_2N-$), 3.08 (2H, *t*, J = 6.3 Hz, $-CH_2S-$), 1.95 (2H, *s*, CH_3CON-), 1.94 (3H, *s*, CH_3-); ^{13}C NMR (for the enol form) ($CDCl_3$): δ 192.24 (C-1), 174.02 (C-3), 99.86 (C-2), 39.82 ($-CH_2N-$), 27.72 ($-CH_2S-$), 23.17 (CH_3CON-), 21.02 (C-4). This procedure was used for the preparation of the labelled analogs.

[4,4,4-$^2H_3$]-3-oxobutanethioic acid-S-[(2-acetylamino)ethyl] ester (1a).
Found 206.0799 (M^+), $C_8{}^2H_3H_{10}NO_3$ requires 206.0804. MS *m/z*: 206, 178, 173, 156, 119, 60 (100%); IR ν_{max} ($CHCl_3$) cm^{-1}: 3410, 3360, 3315, 1700, 1645, 1600; ^1H NMR (for the keto form) ($CDCl_3$): δ 5.95 (1H, *br s*, -NH-) , 3.67 (2H, *s*, $-COCH_2CO-$), 3.40 (2H, *q*, J = 6.0 Hz, $-CH_2N-$), 3.05 (3H, *t*, J = 6.3 Hz, $-CH_2S-$), 2.25 (res, *m*, CD_2HCO-), 1.95 (3H, *s*, CH_3CON-); ^{13}C NMR (for the keto form) ($CDCl_3$): δ 199.85 (C-3), 199.20 (C-1), 170.37 (-CON-), 57.92 (C-2), 39.05 ($-CH_2N-$), 29.96 ($-CH_2S-$), 23.04 (CH_3CON-); δ ^2H ($CHCl_3$): 2.24 (CD_3CO-); ^1H NMR (for the enol form) ($CDCl_3$): δ 13.60 (1H, *br s*, -OH), 5.95 (1H, *br s*, -NH-), 5.45 (1H, *s*, -CH=), 3.40 (2H, *q*, J = 6.0 Hz, $-CH_2N-$), 3.05 (2H, *s*, $-CH_2S-$), 1.95 (3H, *s*, CH_3CON-), 1.90 (res. $CD_2HC(OH)=$); ^{13}C NMR (for the enol form) ($CDCl_3$): δ 192.15 (C-1). 174.0 (C-3), 170.23 (-CON-), 99.81 (C-2), 39.78 ($-CH_2N-$), 27.68 ($-CH_2S-$), 23.10 (CH_2CON-); $\delta^2{}_H$ ($CHCl_3$): 1.90 ($CH_2C(OH)=$).

[2,3-$^{13}C_2$]-3-oxobutanethioic acid-S-[(2-acetylamino)ethyl] ester (1c).
Found 205.0683 (M^+), $C_6{}^{13}C_2H_{13}NO_3S$ requires 205.0683. MS *m/z*: 205, 177, 172, 157, 119 (100%), 60 (100%). ^1H NMR (for the keto form) ($CDCl_3$): δ 6.09 (1H, *br s*, -NH-), 3.70 (2H, *dd*, J_{CH} = 131.3 Hz, $^2J_{CH}$ = 6.1 Hz, $-O^{13}C^{13}CH_2-$), 3.43 (2H, *q*, J = 6.0 Hz, $-CH_2NH$), 3.04 2H, *t*, J = 6.3 Hz, $-CH_2S-$), 2.22 (3H, *d*, $^2J_{CH}$ = 5.7 Hz, $CH_3{}^{13}CO-$), 1.96 (3H, *s*, CH_3CON-); ^{13}C NMR ($CDCl_3$): δ 199.84 (*d*, J = 36 Hz, C-3), 199.20 (*d*, J = 50 Hz, C-1), 170.29 (-CON-), 57.94 (*d*, J = 36 Hz, C-2)), 39.08 ($-CH_2N-$), 30.04 (*d*, J = 40 Hz, C-4), 29.96 ($-CH_2S-$), 23.22 (CH_3CON-); ^1H NMR (for the enol form) ($CDCl_3$): δ 9.95 (1H, *br s*, -OH), 6.09 (1H, *br s*, -NH-), 5.44 (1H, *dd*, J_{CH} = 168.7 Hz, $^2J_{CH}$ = 4.4 Hz, $-(OH)^{13}C={}^{13}C-$), 3.43 (2H, *q*, J = 6.0 Hz, $-CH_2NH-$), 3.04 (2H, *t*, J = 6.3 Hz, $-CH_2S-$), 1.97 (3H, *d*, $^2J_{CH}$ 7.7 Hz, $CH_3{}^{13}CO-$), 1.95 (3H, *s*, CH_3CON-); ^{13}C NMR (for the enol form) ($CDCl_3$): δ 192.26 (C-1), 173.26 (*d*, J = 70.01 Hz, C-3), 170.29 (-CON-), 99.81 (*d*, J = 70.01 Hz, C-2), 39.81 ($-CH_2N-$), 27.96, ($-CH_2S-$), 21.01 (C-4), 23.14 (CH_3CON-).

Benzyl 3-oxobutanoate (7). 5-Acetyl-Meldrum's acid (0.45 g, 2.4 mmol) was suspended in benzene (20 mL), benzyl alcohol (2 mL) added and the reaction mixture refluxed for 18 h. The solvent was removed and the oily residue purified by flash chromatography to give 0.26 g (60%) of the benzyl ester (7) as a colourless oil. Found 192.0785 (M^+), $C_{11}H_{12}O_3$ requires 192.0786. MS *m/z*: 192, 164, 108, 91 (100%); IR ν_{max} ($CHCl_3$) cm^{-1}: 3400 (*w*), 3050, 1730, 1705 1680, 1620, 1590; ^1H NMR ($CDCl_3$): δ 7.45 (5H, ArH), 5.25 (2H, *s*, $-OCH_2-$), 3.60 (2H, *s*, $-COCH_2CO_2-$), 2.30 (3H, *s*, CH_3-). This procedure was used for the syntheses of the

labelled analogs, benzyl [4,4,4-$^{2}H_3$]-3-oxobutanoate (**7a**), benzyl [2-^{13}C]-3-oxobutanoate (**7b**) and benzyl [2,3-$^{13}C_2$]-3-oxobutanoate (**7c**).

Benzyl 3-hydroxybutanoate (8). Benzyl acetoacetate (0.1 g, 0.56 mmol) was dissolved in 6 mL of THF-water (5:1), the solution was cooled to 0° and sodium borohydride (7 mg, 0.33 eq.) added. The reaction mixture was stirred for 1 h, diluted with water, filtered through celite and the filtrate extracted with ether (5 x 20 mL). The ether extract was dried with Na_2SO_4 and the solvent removed to give an oily residue. Purification of the oil with flash column chromatography (petrol-ether 7:3) gave 80 mg (80%) of the β-hydroxy ester (**8**) as a colourless oil. Found 194.0926 (M^+), $C_{11}H_{14}O_3$ requires 194.0942. MS *m/z*: 194, 166, 149, 127, 108, 91 (100%); IR ν_{max} ($CHCl_3$) cm^{-1}: 3510 (*br*), 3060, 1710, 1580; ^{1}H NMR ($CDCl_3$): δ 7.40 (5H, *s*, ArH), 5.16 (2H, *s*, -OCH_2), 4.2 (1H, *m*, -CH(OH)-), 2.70-2.45 (3H, *m*, -OH and -CH_2CO_2-), 1.20 (3H, *d*, *J* = 7 Hz, CH_3-). This general procedure was used in the preparation of labelled analogs, benzyl [4,4,4-$^{2}H_3$]-3-hydroxybutanoate (**8a**), benzyl [3-^{13}C]-3-hydroxybutanoate (**8b**) and benzyl [2,3-$^{13}C_2$]-3-hydroxybutanoate (**8c**).

3-Hydroxybutanoic acid (9). Benzyl 3-hydroxybutanoate (55 mg, 0.28 mmol) was dissolved in methanol (10 mL) and palladium on charcoal (5 mg) added. Hydrogen gas (1 L) was slowly passed through the reaction vessel and stirring continued until all the gas was consumed (1 h). The reaction mixture was diluted with ether (20 mL), filtered through celite and the solvent removed under vacuum to afford 28.4 mg (97%) of 3-hydroxybutanoic acid (**9**). Found 104.0472 (M^+), $C_4H_8O_3$ requires 104.0473. MS *m/z*: 104, 87, 60 (100%); IR ν_{max} ($CHCl_3$) cm^{-1}: 3500-2500 (*br*), 1690; ^{1}H NMR ($CDCl_3$): δ 6.5 (2H, *br s*, -OH and -CO_2H), 4.15 (1H, *m*, -CH(OH)-), 2.50 (2H, *m*, -CH_2CO_2-), 1.22 (3H, *d*, *J* = 7 Hz, CH_3-); ^{13}C NMR ($CDCl_3$): δ 177.29 (C-1), 62.32 (C-3), 42.48 (C-2), 22.30 (C-4). This procedure was followed in the preparation of the labelled analogs, [4,4,4-$^{2}H_3$]-3-hydroxybutanoic acid (**9a**), [3-^{13}C]-3-hydroxybutanoic acid (**9b**) and [2,3-$^{13}C_2$]-hydroxybutanoic acid (**9c**).

3-Hydroxybutanethioic acid-S-[2-(acetylamino)ethyl] ester (3). β-Hydroxybutanoic acid (53 mg, 0.51 mmol) was dissolved in dry CH_2Cl_2 (5 mL), the solution cooled to 0° and DCC (0.31 g, 2 eq.) in dry CH_2Cl_2 (10 mL) added. To the reaction mixture, a catalytic amount of DMAP was added followed by a solution of N-acetylaminoethanethiol (73 mg, 0.61 mmol) in dry CH_2Cl_2. The reaction mixture was stirred for 18 h at 25°, then filtered and solvent removed to give an oily residue. The residue was purified by column chromatography (0-8% methanol in ethyl acetate) to give 0.1 g (50%) of the thioester (**3**) as a colourless oil. Found 187.0653 (M^+ - H_2O), $C_8H_{13}NO_2S$ requires 187.0667. MS *m/z*: 187, 119, 118, 60 (100%); IR ν_{max} ($CHCl_3$) cm^{-1}: 3400, 1640; ^{1}H NMR ($CDCl_3$): δ 6.0 (1H, *br s*, -NH-), 4.25 (1H, *m*, -CH(OH)-), 3.45 (2H, *q*, *J* = 6.0 Hz, -CH_2N-), 3.07 (2H, *t*, *J* = 6.3 Hz, -CH_2S-), 2.95 (1H, *br s*, -OH), 2.70 (2H, *m*, -CH_2COS-), 1.90 (3H, *s*, CH_3CON-), 1.20 (3H, *d*, *J* = 7 Hz, CH_3-); ^{13}C NMR ($CDCl_3$): δ 199.21 (C-1), 170.67 (-CON-), 64.96 (C-3), 52.46 (C-2), 39.18 (-CH_2N-), 28.70 (-CH_2S-), 23.07 (CH_3CON-), 22.70 (C-4). The procedure was used for the syntheses of the labelled analogs.

[4,4,4-$^{2}H_3$]-3-Hydroxybutanethioic acid-S-[2-(acetylamino)ethyl] ester (3a). Found 190.0841 (M^+ - H_2O), $C_8{}^2H_3H_{10}NO_3S$ requires 190.0855. MS *m/z*: 190, 119, 118, 60 (100%); ^{1}H NMR ($CDCl_3$): δ 6.0 (1H, *br s*, -NH-), 4.25 (1H, *br m*, -CH(OH)-), 3.45 (2H, *q*, *J* = 6.0 Hz, CH_2N-), 3.07 (2H, *t*, *J* = 6.3 Hz, -CH_2S-), 2.95 (1H, *br s*, -OH), 2.70 (2H, *m*, -CH_2COS-), 1.90 (3H *s*, CH_3CON-), 1.20 (res, *m*, CD_2H-); δ ^{2}H ($CHCl_3$): 1.22 (CD_3-).

[3-^{13}C]-3-Hydroxybutanethioic acid-S-[2-(acetylamino)ethyl] ester (3b). Found 206.0813 (M^+), $C_7{}^{13}CH_{15}NO_3S$ requires 206.0806. MS *m/z*: 206, 205, 191, 160, 119 (100%), 118, 60; ^{1}H NMR ($CDCl_3$): δ 6.1 (1H, *br s*, -NH-), 4.23 (1H, *dm*, J_{CH} = 147 Hz, -^{13}CH(OH)-), 3.42 (2H, *q*, J = 6.0 Hz, -CH_2NH-), 3.05 (2H, *t*, J = 6.3 Hz, -CH_2S-), 2.93 (1H, *br s*, -OH), 2.70 (2H, *m*, -CH_2COS-), 1.95 (3H, *s*, CH_3CON-), 1.21 (3H, *dd*, J = 6.3 Hz, $^2J_{CH}$ = 4.50 Hz, $CH_3{}^{13}$CH(OH)-); ^{13}C NMR ($CDCl_3$): δ 199.21 (C-1), 170.67 (-CON-), 64.96 (C-3), 52.45 (*d*, J = 37 Hz, C-2), 39.18 (-CH_2N-), 28.76 (-CH_2S-), 23.14 (CH_3CON-), 22.96 (*d*, J = 39 Hz, C-4)

[2,3-$^{13}C_2$]-3-Hydroxybutanethioic acid-S-[2-(acetylamino)ethyl) ester (3c). Found 207.0893 (M^+) $C_6{}^{13}C_2H_{15}NO_3S$, requires 207.0847. MS *m/z*: 207, 192, 165, 119 (100%), 118, 60; ^{1}H NMR ($CDCl_3$): δ 6.10 (1H, *br s*, -NH-), 4.23 (1H, *dm*, J_{CH} = 147 Hz, -^{13}CH(OH)-), 3.43 (2H, *q*, J = 6.0 Hz, -CH_2NH-), 3.04 (2H, *t*, J = 6.3 Hz, -CH2S-), 2.80 (1H, *br s*, -OH), 2.70 (2H, *dm*, J_{CH} = 133 Hz, -$^{13}CH_2$COS-), 1.94 (3H, *s*, CH_3CON-), 1.20 (3H, *dd*, J = 6.2 Hz, $^2J_{CH}$ = 4.6 Hz, $CH_3{}^{13}$CH(OH)-); ^{13}C NMR ($CDCl_3$): δ 199.25, (*d*, J = 46 Hz, C-1), 170.62 (-CON-), 64.99 (*d*, J = 37 Hz, C-3), 52.49 (*d*, J = 37 Hz, C-2), 39.21 (-CH_2N-), 28.76 (-CH_2S-), 23.14 (CH_3CON-), 22.72 (*d*, J = 39 Hz, C-4).

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REFERENCES

1. O'Hagan, D. *Nat. Prod. Reports* **1993**, 106, 593.
2. Cane, D.E.; Yang, C. *J. Am. Chem. Soc.* **1987**, 109, 1253; Yue, S.; Duncanson, J. S.; Hutchinson, C.R. *J. Am. Chem. Soc.* **1987**, 109, 1255; Cane, D.E.; Ott, W.R.; *J. Am. Chem. Soc.* **1988**, 110, 4840.
3. Staunton, J.; Sutkowski, A.C. *J. Chem. Soc. Chem. Commun.* **1991**, 1110.
4. Jacobs, A.; Staunton, J.; Sutkowski, A.C. *J. Chem. Soc. Chem. Commun.* **1991**, 1113.
5. Kinoshita, K.; Takenaka, S.; Hayashi, M. *J. Chem. Soc. Chem. Commun.* **1988**, 943; *J. Chem. Soc. Perkin Trans I* **1991**, 2547; Hubber, M.L.; Pascal, J.W.; Leeds, J.P.; Kirst, H.A.; Wind, J.A.; Millar, F.D.; Turner, J.R. *Antimicrob. Agents Chemother.* **1990**, 34, 1535.
6. Demetriadou, A.K.; Laue, E.D.; Staunton, J. *J. Chem. Soc., Perkin Trans. I* **1988**, 733.
7. Oikawa, Y.; Sugano, K.; Yonemitsu, O. *J. Org. Chem.* **1978**, 43, 2087.
8. Ley, S.V.; Woodward, P.R. *Tetrahedron Letters* **1987**, 28, 345.
9. Labelled reagents were bought from Aldrich Chemical Company.
10. Davidson, D.; Bernhard, S.A. *J. Am. Chem. Soc.* **1948**, 70, 3426; Philhaja, K.; Sielo, M. *Acta Chem. Scand.* **1968**, 22, 3053.
11. Baig, M.V.A.; Owen, L.N. *J. Chem. Soc. [C]* **1966**, 540; Neises, B.; Steglich, W. *Angew. Chem. Int. Ed.* **1978**, 17, 522.
12. Noyori, R.; Okhuma, T.; Kitamara, M.; Takaya, M.; Sayo, N.; Kumobayashi, H.; Akutagawa, M. *J. Am. Chem. Soc.* **1987**, 109, 5856.