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SYNTHESIS OF β-KETO AND α,β-UNSATURATED N-ACETYLCYSTEAMINE THIOESTERS.

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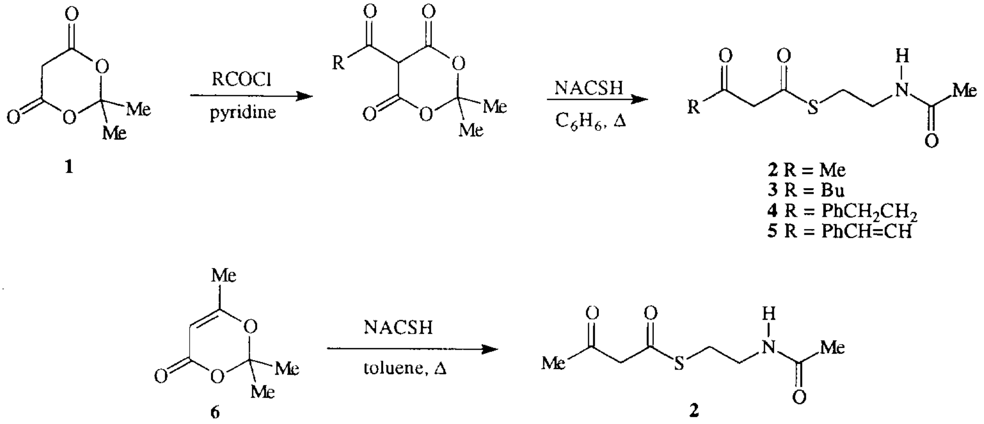
Abstract: Methods for the synthesis of N-acetylcysteamine thioesters of β-keto and α,β-unsaturated acids which avoid problems associated with methods requiring the intermediacy of the free acids are described, along with a convenient method for the removal of excess thiol from reaction mixtures using copper sulphate impregnated silica.

Studies to establish the exact sequence of events during the processive mode[1] of polyketide chain assembly require the synthesis of the putative intermediates in the form of their N-acetylcysteamine (NAC) thioesters which, in a number of studies[2], have been shown to be recognised and processed by the polyketide biosynthetic enzymes. The range of functionality present in these often inherently unstable intermediates requires a range of methods to be developed for their efficient synthesis. N-Acetylcysteamine thioesters have been successfully synthesised by a number of methods, the most generally applicable being the condensation of the corresponding carboxylic acid with N-acetylcysteamine in the presence of dicyclocarbodiimide (DCC) catalysed with DMAP[3]. Other valuable methods include the use of acyl imidazolides[4] and acyl azides prepared using diphenylphosphoryl azide[5]. However, these methods prove difficult with ß-keto esters due to the ease of decarboxylation of the free ß-ketoacids. In the case of αβ-unsaturated acids, problems may arise due to conjugate addition competing with thioester formation[6]. Simple methods to circumvent these problems are described below giving access to a range of valuable N-acetylcysteamine thioesters for biosynthetic studies.

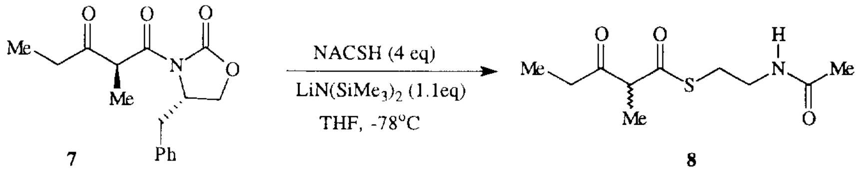
β-Keto N-acetylcysteamine thioesters.

Meldrum's acid **1** which is readily prepared from malonic acid and acetone, can be acylated by treatment with the appropriate acyl chloride[7]. Subsequent treatment with N-acetylcysteamine in refluxing benzene gives good yields of the corresponding thioesters **2** - **5** as indicated in Scheme 1[8,9]. The readily available 2,2,6-trimethyl-1,3-dioxin-4-one **6** on treatment with N-acetylcysteamine gives still better yields of the thioester **2** of acetoacetic acid[10]. Work to extend this to further ß-ketothioester derivatives via alkylation and acylation of the derived carbanion[11] is in progress and will be reported in full elsewhere.



Scheme 1

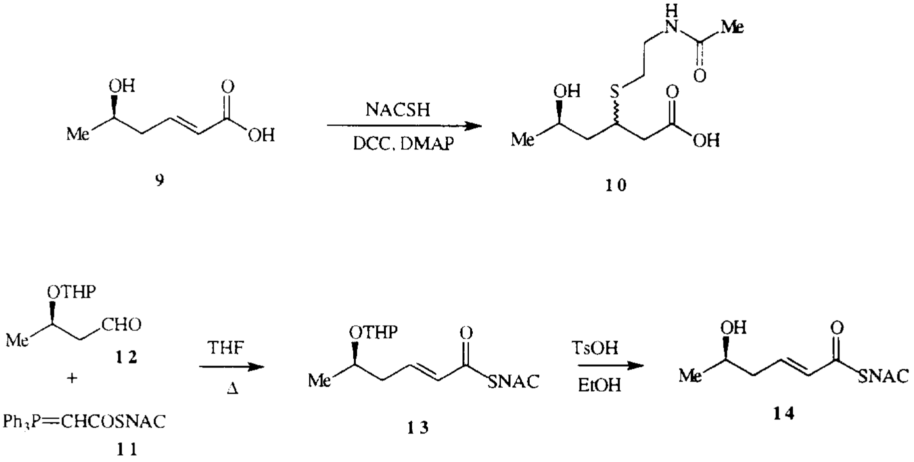
Polyketide chain assembly intermediates can be readily prepared in homochiral form via the enolates of acylated oxazolidinones[12]. The oxazolidinone may be converted to a thioester either directly[13] or by hydrolysis to release the free acid which can then be converted to the NAC thioesters by DCC/DMAP coupling. In the case of the β-keto derivative **7** this latter method fails due to facile decarboxylation of the intermediate β-keto acid. We have developed a procedure for the direct conversion of the acylated oxazolidinone **7** to the N-acetylcysteamine thioester (**8**, Scheme 2) by the addition of **7** to a solution of an excess of N-acetycysteamine deprotonated with lithium bis(trimethylsilylamide). During purification of the product by flash chromatography it was found that the excess N-acetylcysteamine co-ran with the thioester **8**. Several methods were examined to remove the excess reagent including treatment of the reaction mixture with either iron (III) chloride or copper sulphate solution. However in both cases there was a poor recovery of product. Hydrogen peroxide removed the N-acetylcysteamine but appeared to react with the thioester. It was eventually discovered that the most successful method to remove the reagent from the required product was to purify the reaction mixture by flash chromatography using a normal column but with a 3 cm layer of copper sulphate impregnated silica on the top[10]. The copper sulphate reacts with the excess N-acetylcysteamine to form a yellow layer which remains at the top of the column[14]. Using this approach, the β-keto NAC thioester **8** was prepared in 60% yield from the oxazolidinone 7. As expected racemisation of the methyl group at C-2 occurred since **8** undergoes rapid keto-enol tautomerism. We believe that this will be a general method for the removal of unwanted thiols.



Scheme 2

α,β-Unsaturated N-acetylcysteamine thioesters.

Thioester (**14**, Scheme 3) corresponds to the proposed C_6 -precursor for the macrodiolide colletodiol[15]. While the corresponding acid **9** was readily prepared by standard methods, attempted conversion to the NAC thioester by DCC/DMAP coupling gave only the acid **10** arising from conjugate addition of the thiol. This problem was overcome by Wittig chemistry. The ylid **11** was readily formed via DCC/DMAP coupling of bromoacetic acid with N-acetylcysteamine, and treatment of the thioester with triphenylphosphine followed by sodium hydroxide[16]. The resulting ylid **11** was reacted with the protected aldehyde **12**. Deprotection of the THP derivative **13** gave the desired product **14** in 55% overall yield. Analogous reaction of the ylid **11** with benzaldehyde and 4-bromobenzaldehyde gave the corresponding cinnamyl thioesters and in yields of 87 and 66% respectively.



Scheme 3

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References and Notes

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6 The reaction of diphenylphosphine anhydrides of α,β-unsaturated acids by the thallous salt of N-acetylcysteamine to overcome problems of conjugate addition has also been reported: Schwab, J.

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8 All new compounds gave satisfactory spectroscopic and analytical data.

9 The synthesis of the ethyl thioester of 4-bromoacetoacetate from Meldrum's acid has been reported: Ley, S.V. and Woodward, P.R., *Tetrahedron Lett.*, 1987, **28**, 345., and a report of a similar method of preparation of the NAC thioester of acetoacetate has appeared[2b].

10 *Typical experimental procedures for the synthesis of β-keto thioesters:*

Method 1. A solution of N-acetylcysteamine (1.91mmol) in distilled benzene (4ml) was added to a stirred solution of the butanoyl derivative of Meldrum's acid (2.87 mmol) in benzene (16 ml) and the reaction mixture was heated to reflux, under a nitrogen atmosphere, for 7 h. After chromatography to give the NAC thioester **3** of 3-oxohexanoic acid as a crystalline solid (64%).

Method 2. Lithiium bis(trimethylsilylamide) (0.45ml of 1.0M solution in THF, 0.45mmol, 1.1 equiv.) was added dropwise to a solution of N-acetylcysteamine (0.174g, 1.5mmol, 4 equiv.) in dry THF (3ml) under argon at -78°C. The mixture was stirred for 5 minutes at -78°C and then for 10 minutes at 0°C. It was then cooled to -78°C and the acylated oxazolidinone **7** (0.40 mmol) was added dropwise in dry THF (2ml). The reaction was slowly warmed to 0°C and stirred until no starting material could be detected by tlc. The reaction mixture was then loaded directly onto the flash column with 15 cm of normal flash silica and 3cm of copper sulphate impregnated silica on the top (prepared by stirring flash silica with saturated aqueous copper sulphate solution for 10 minutes then drying *in vacuo* until it was a free flowing blue powder). The copper sulphate reacts with excess N-acetylcysteamine to form a yellow layer which remains at the top of the column[11]

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14 In control experiments we found that copper sulphate impregnated silica completely absorbed N-acetylcysteamine, but acylated N-acetylcysteamine was recovered in 99% yield from the column.

15 O'Neill, J.A., Simpson, T.J., and Willis, C.L., *J.Chem. Soc. Chem. Commun.*, 1993, 738.

16 *Typical experimental procedure for the synthesis of α,β-unsaturated thioesters:*

(Carbo(N-acetylcysteamino)methyl)triphenylphosphonium bromide (5.98 mmol) was dissolved in water (50 ml) and the pH adjusted to 9.0 with 1M sodium hydroxide. The solution was extracted with dichloromethane (5 x 50ml), dried ($MgSO_4$) and concentrated *in vacuo* to give the ylid **11** as a solid (81%). The ylid (4 mmol) and (3*R*)-3-(tetrahydropyranyloxy)butanal **12** (4 mmol) were heated to reflux in dry tetrahydrofuran (20 ml) for 3 days. The reaction mixture was cooled, filtered through celite, and concentrated *in vacuo*. The N-acetylcysteamine thioester **13** of (2*E*,5*R*)-5-tetrahydropyranyloxyhex-2-enoic acid was recovered as an oil (55%) after flash chromatography.

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