Regioselective Cleavage of the Dithiolane S–S Bond of (±)-α-Lipoic Acid by Carbon and Phosphorus Nucleophiles

Edward H. Smith
Department of Chemistry, Imperial College, South Kensington, London SW7 2AY

Methyl-lithium and lithium dimethylcuprate react with (±)-α-lipoic acid (**1a**) to give in low yield a mixture of ring-opened isomers (8 : 1) resulting from nucleophilic attack at the C-8 and C-6 sulphur atoms respectively of the dithiolane ring. Methylmagnesium iodide, dimethyl sodiomalonate, and 1-pyrrolidin-1-ylcyclohexene fail to open the ring of (**1a**). In contrast, trimethyl phosphite cleaves the S–S bond efficiently and regioselectively at the C-8 sulphur atom; the resultant product is degraded by way of a new conversion of phosphorothioate into thioether.

The coenzyme (*R*)-α-lipoic acid, (*R*)-(**1a**), is ubiquitous in nature.[1] One of its more prominent roles is to mediate in the oxidative decarboxylation of α-keto acids to thioesters [equation (1)], a process catalysed *in vivo* by the α-keto-dehydrogenase enzyme complex.[2]

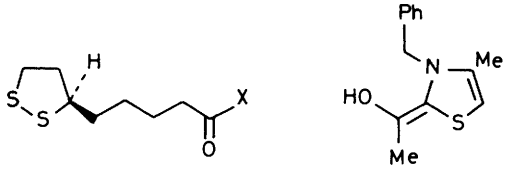
Of the three mechanisms (Schemes 1[3] and 2[4]) proposed for this process, two involve an initial nucleophilic attack by the carbanion equivalent (**2**) at the S–S bond of enzyme-bound α-lipoic acid (**1b**) (Scheme 1),[3] followed by subsequent cleavage of either a C–C or a C–S bond. A recent attempt to mimic the S–S cleavage process in the laboratory using the close analogue (**3**) of the carbanion equivalent (**2**) in a reaction with methyl α-lipoate (**1c**) was unsuccessful.[5]

Two facets of the proposed mechanism which we felt deserved close scrutiny, and which should be reproduced in any chemical model system, are that the opening of the dithiolane ring by a carbon nucleophile should be efficient and that it should occur with the regioselectivity shown (Scheme 1) in which the less hindered C-8 sulphur is released as thiol(ate). The regiochemical aspect had not been specifically addressed in the earlier work.

To this end we report in this paper on the intrinsic reactivity of α-lipoic acid (**1a**)† towards attack by a range of simple carbon and phosphorus nucleophiles.

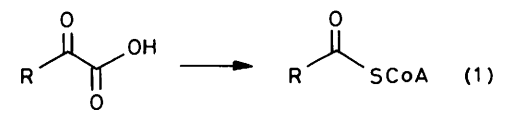
Results and Discussion

Reaction with Carbon Nucleophiles.—For simplicity and convenience the methyl anion in its various organometallic guises was chosen as the major carbon nucleophile. Reaction of α-lipoic acid (**1a**) with methyl-lithium (3 equiv.) in the dark in ether followed by quenching of any released thiolate anion with an excess of acetic anhydride led to a low yield (9.8%) of ring-opened products after silica-gel chromatography (Scheme 3). The major by-product was a yellow oil which rapidly polymerised in light, indicating the presence of the dithiolane ring, and which showed anhydride bands at 1 830 and 1 769 cm^{-1} in the i.r. spectrum. The similarity of its ^1H n.m.r. spectrum to that of (**1a**), and its partial conversion into the acid (**1a**) on silica, showed that it was α-lipoic acid anhydride (**4**). In addition, during the reaction with methyl-lithium and prior to quench-



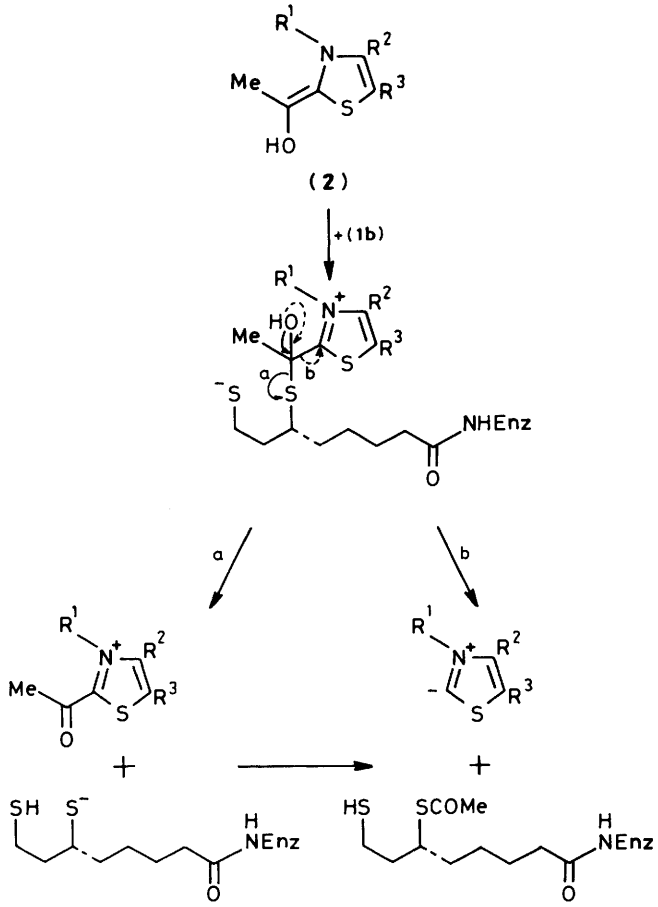
(**1**) **a**; X = OH
b; X = NHEnz
c; X = OMe

(**3**)

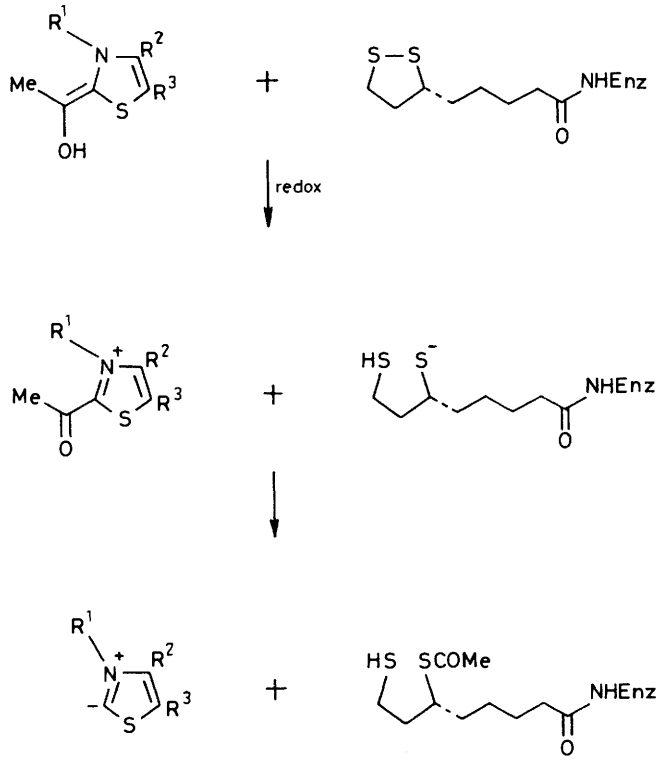


R = Me or $HO_2C{\cdot}CH_2CH_2$, HSCoA = coenzyme-A

† Racemic α-lipoic acid was used throughout.



Scheme 1. R^1 = 4-Amino-2-methylpyrimidin-5-ylmethyl, R^2 = Me, R^3 = $CH_2CH_2OP_2O_6H_3$



Scheme 2.

ing a small amount of insoluble, hygroscopic polymer was produced which was removed on work-up by filtration through a glass-wool plug. Since thiyl radical species are known to induce polymerisation of dithiolane derivatives[6] we assume that one-electron transfer from the methyl-lithium[7] caused this polymer formation.

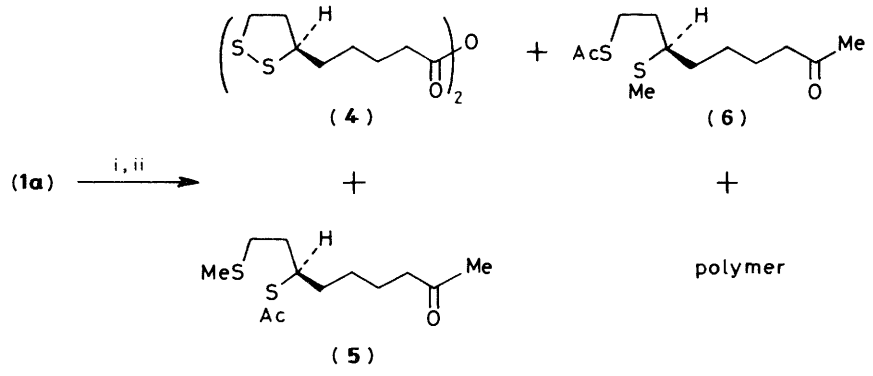
The ^{1}H n.m.r. spectrum of the crude reaction mixture before chromatography showed a complex pattern of sharp, singlet peaks in the 2.00—2.35 p.p.m. region due to methyl proton resonances in the ring-opened products. This pattern was reproduced in the spectrum of the combined early fractions from the silica-gel column, thus indicating that this purification procedure had not resulted in the preferential loss of one of the ring-opened products. Careful chromatography using a large absorbent to compound ratio (200 : 1) resulted in partial separation of two compounds. The major, slower-running component was clearly the regioisomer (**5**) from its ^{1}H n.m.r. (250 MHz) spectrum in which a low-field multiplet at 3.60 (C*H*SAc), a multiplet at 2.52 (CH_2SMe), and a triplet at 2.42 p.p.m. (CH_2CO) could be discerned as well as three singlet methyl peaks at 2.31, 2.13, and 2.10 p.p.m. The close similarity of this spectrum to that of the methyl ester (**12**) was noticeable.

The faster-running component was not so clearly identifiable since it was isolated as a mixture with the slow component in which the latter predominated (*ca.* 2 : 1). However, a multiplet at 3.00 p.p.m. (CH_2SAc) and three singlet methyl peaks at 2.32, 2.14, and 2.11 p.p.m. led us to assign the isomeric structure (**6**) to this product. The ratio of isomers (**5**) and (**6**) was estimated to be 8 : 1.

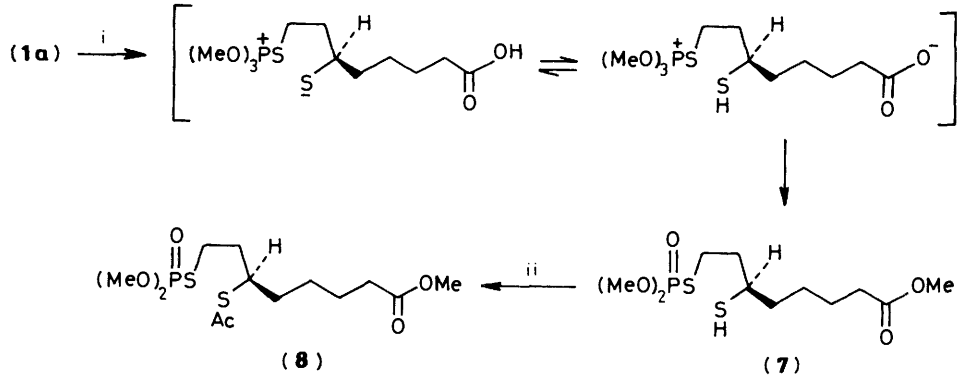
An increase in the amount of methyl-lithium to 11 equiv. gave a marginally better yield (11.4%) of ring-opened products. However, little advantage was gained by using lithium dimethylcuprate (5 equiv.; 12.2% yield) in ether, despite its normally better nucleophilicity towards soft electrophiles. The pattern of methyl singlets in the crude product from each of these attempts was very similar to that obtained in the methyl-lithium experiment. This suggests that the ratio of regioisomers produced in these reactions reflects the intrinsic electrophilicity of the S–S bond of α-lipoic acid rather than any differences in the formation or trapping of products of the one-electron reduction since Liotta and his co-workers have shown that methyl-lithium and lithium dimethylcuprate give widely differing proportions of products, albeit with a carbonyl compound as electrophile.[7] The addition of 20% hexamethylphosphoramide to the reaction with cuprate resulted in a reduction in yield to 1.5%. In both cuprate reactions slightly more of the insoluble polymer was produced than in the reaction with methyl-lithium which is consistent with the greater electron-transferring ability of cuprates.[7] Methylmagnesium iodide was ineffective in opening the ring of the acid (**1a**), the latter being recovered almost quantitatively. Methyl α-lipoate (**1c**) gave no identifiable ring-opened products with lithium dimethylcuprate and the ester (**1c**) thus appears to be a poor substrate for this type of reaction, a conclusion which is in line with Rastetter and Adams' observations.[5]

Although stable carbanions may cleave the S–S bond of diaryl disulphides,[8] α-lipoic acid (**1a**) proved resistant to nucleophilic attack by dimethyl sodiomalonate (2 equiv.) in ethanol. Likewise, 1-pyrrolidin-1-ylcyclohexene (2 equiv.) failed to open the dithiolane ring of (**1a**).

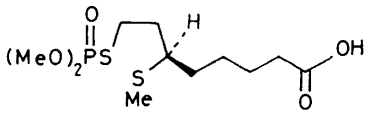
A report that isocyanides insert into the S–N bond of sulphenamides[9] led us to attempt the insertion of cyclohexyl isocyanide into the S–S bond of compound (**1a**). No cleavage of this bond was observed under a number of conditions (thermal, photochemical, copper-, and mercury-salt catalysis) in agreement with results obtained for the analogous reaction with diphenyl disulphide.[10]



Scheme 3. *Reagents:* i, MeLi, LiBr, Et_2O, 0 °C→room temp.; ii, Ac_2O, room temp.



Scheme 4. *Reagents:* i, $(MeO)_3P$, C_6H_6, reflux; ii, Ac_2O, pyridine, room temp.



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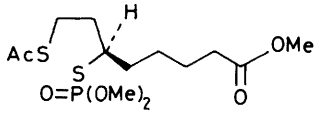

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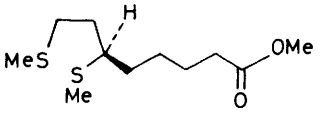


(11)

Reaction with Phosphorus Nucleophiles.—In contrast to its reaction with methyl-lithium, α-lipoic acid (**1a**) is cleanly and regioselectively converted into a single phosphorothioate on being heated with trimethyl phosphite in benzene for 20 h (Scheme 4). Both i.r. and ^1H n.m.r. spectral data indicated that the product did not contain a carboxylic acid group, and a weak SH bond could be seen in the i.r. spectrum (2 545 cm^{-1}). Acetylation of the crude product provided a phosphorothioate thioacetate (**8**) (66% for the two steps) which was stable enough to survive silica-gel chromatography. That only one regiosomer had been formed was indicated by the ^{13}C n.m.r. spectrum (13 peaks) and the lack of doubling of the peak due to the carboxymethyl protons in the ^1H n.m.r. (250 MHz) spectrum. None of the phosphorothioate thioether (**9**), which might have been expected from such a reaction,[11] could be detected. This result is not unexpected in view of the relative basicities of thiolate and carboxylate anions.

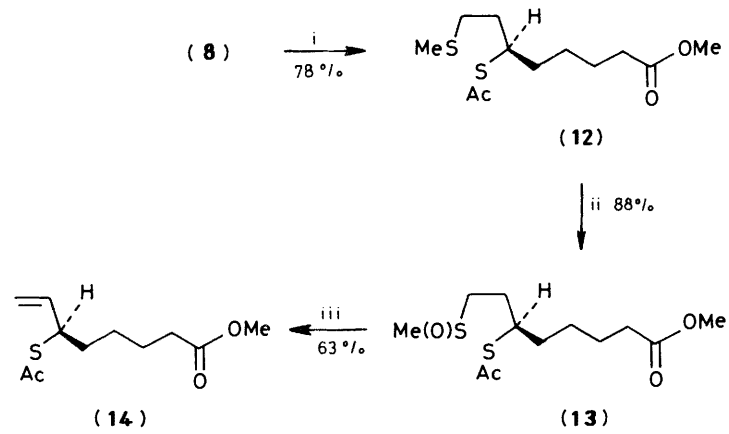
A 1 H multiplet at 3.60 and a 2 H multiplet at 2.88 p.p.m. in the ^1H n.m.r. spectrum of the product could be assigned to the protons on the sulphur-bearing carbons. However, differentiation between compound (**8**) and its isomer (**10**) could not be made because the deshielding influences of the phosphoryl and acetyl groups are similar.[12] In order to put two groups with very different deshielding characteristics onto the sulphur atoms an attempt was made to methylate the intermediate thiol (**7**) from the phosphite reaction using methyl iodide and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).[13] To our surprise the product isolated was not the anticipated methyl ester of the acid (**9**), but rather the bis(methylthio) ether (**11**) (45%). Although Cadogan and his co-workers had shown that phosphorothioates react as nucleophiles towards methyl iodide giving ultimately the sulphonium salt amongst other products,[14] the clean alkylative dephosphorylation that we had observed had not been reported previously. The application of this reaction to the thioester (**8**) [or (**10**)] provided a stringent test of its chemoselectivity which it passed admirably. Thus, the thioether (**12**) was obtained in good yield (78%) and without loss of either the acetyl group from the C-6 sulphur atom or the methyl group of the ester (Scheme 5). The ^1H n.m.r. (250 MHz) spectrum of the thioether (**12**) showed a 1 H multiplet at 3.60 (C*H*SAc) and a 2 H multiplet at 2.52 p.p.m. (CH_2SMe), both identical in coupling pattern with those in the spectrum of the ketone (**5**) isolated from the methyl-lithium reaction, as well as a triplet at 2.30 (CH_2CO_2Me) and three methyl singlet peaks at 2.10, 2.33, and 3.68 p.p.m.

Confirmation of the structure was provided by degradation, through the sulphoxide (**13**), to the olefin (**14**) (Scheme 5). The ^1H n.m.r. (250 MHz) spectrum clearly showed that (**14**) was the terminal olefin. A tiny multiplet at 5.40 p.p.m. may have been due to the olefinic protons in the internal isomers (**15**) and (**16**). It seems unlikely that the olefin (**14**) is derived by a [1,3]thioallylic shift from (**15**) since it probably represents the contrathermodynamic isomer.[15] Thus structure (**8**), rather than (**10**), is indicated for the phosphorothioate formed by opening of the dithiolane ring of (**1a**).

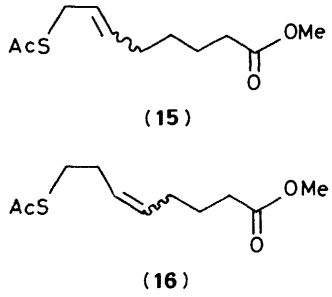
Conclusions

Our studies have shown that the intrinsic reactivity of α-lipoic acid (**1a**) towards anionic and neutral carbon nucleophiles is poor, in agreement with the results of Rastetter and Adams who used methyl α-lipoate (1b) as substrate.[5] Moreover, when ring opening does occur it proceeds with predominant attack at the less hindered terminal sulphur atom. The same regioselectivity is observed with the soft nucleophile trimethyl phosphite and also previously in reactions thought to involve radical species generated photochemically[16] or thermally.[17]

It is generally recognised that enzymes enhance intrinsic reactivities rather than create totally new pathways. To this extent our observation that nucleophilic attack by a carbanion at the internal C-6 sulphur atom of α-lipoic acid (**1a**) is possible, albeit at a very low level, could be construed as support for the proposed mechanism shown in Scheme 1. In this respect, our other results would then be simply the consequences of very imperfect models. We feel, however, that the case for the alternative mechanistic proposal (Scheme 2) should also be explored. The foundations would then be laid for iterative refinements of each model to be proposed and tested so as to



Scheme 5. *Reagents*: i, MeI, DBU, C_6H_6, reflux; ii, *m*-CPBA, CH_2Cl_2, room temp.; iii, decalin, $CaCO_3$, reflux, 1 h



provide a knowledge of the kinetic constraints imposed by the enzyme on the substrate. Given sufficient data of this sort one may dare to assume what is happening on the enzyme.

Experimental

M.p.s were determined using a Gallenkamp apparatus and are uncorrected. I.r. spectra were recorded (as thin films or in $CHCl_3$ solution) on a Perkin-Elmer 298 spectrometer; n.m.r. spectra were recorded on Varian EM 360A (60 MHz) and Bruker WM250 (250 MHz) machines for solutions in $CDCl_3$ using Me_4Si as internal standard. Mass spectra were recorded on A.E.I. MS9 or VG Micromass 7070 instruments. Ether and tetrahydrofuran (THF) were distilled from potassium diphenylketyl. All other solvents and reagents were purified by standard methods. (±)-α-Lipoic acid (**1a**), purchased from Sigma, was stored in the dark in a desiccator until required. Methyl-lithium was purchased from Aldrich as a 1.8M-solution in ether containing lithium bromide. All operations in which α-lipoic acid was used in solution were carried out in vessels protected from light. Silica gel (No. 9385) for column chromatography (gravity) was obtained from Merck. Light petroleum refers to that fraction with b.p. 60—80 °C. Ether refers to diethyl ether.

Reaction of α-Lipoic Acid with Methyl-lithium.—A solution of α-lipoic acid (432 mg, 2 mmol) in dry ether (3 ml) was added during 2 min to an ice-cooled, stirred solution of methyl-lithium in ether (4.2 ml, 5.95 mmol) under argon. An immediate pale yellow precipitate formed and the suspension was stirred with cooling for 15 min. The mixture was allowed to warm to room temperature and stirred for a further 3 h. Acetic anhydride (0.56 ml, 608 mg, 5.93 mmol) was added dropwise during 3 min and the suspension was stirred for a further 12 min. The mixture was quenched by the addition of 0.25M-sulphuric acid until the aqueous layer remained acidic (*ca.* 5 ml), diluted with water (20 ml), and gravity filtered through a plug of glass-wool to remove the hygroscopic, pale yellow polymeric material. The two layers of the filtrate were separated and the aqueous layer was extracted with ether (4 ×). The combined ethereal solution was washed with water until neutral (2 ×), dried ($MgSO_4$), and evaporated to a yellow oil (385.5 mg). T.l.c. and i.r. spectroscopy showed the absence of α-lipoic acid in this material. The oil, which deposited a colourless, jelly-like material on exposure to light, was chromatographed on a column of silica gel (20 g) using ethyl acetate–light petroleum (2 : 1) as eluant. When the rapidly moving yellow band was half-way down the column, fractions (0.5 ml) were collected. Fractions 6—9 were concentrated to a yellow oil (49.2 mg), $\nu_{max.}$ 1 830 and 1 760 cm^{-1}; δ 3.6 (1 H, m, CHS), 3.1 (2 H, t, CH_2S), 2.2—2.6 (4 H, m, dithiolane-CH_2 + CH_2CO), 1.4—1.8 (6 H, m). Fractions 11—13 were concentrated to a pale yellow oil (51.4 mg, 9.8%), δ (250 MHz) 3.6 (m), 2.9—3.2 (m), 2.4—2.6 (m), 2.32 (s), 2.31 (s), 2.14 (s), 2.13 (s), 2.11 (s), 2.10 (s), 2.00 (s), and 1.25—1.95 (m). Fractions 14—24 gave a yellow oil (84.7 mg) on concentration whose ^1H n.m.r. (60 MHz) and i.r. spectral data and t.l.c. properties were identical with those of α-lipoic acid.

Fractions 11—13 were passed through a second column of silica gel (10 g) using light petroleum–ethyl acetate (2 : 1) as eluant. Again fractions (0.5 ml) were collected when the yellow band had reached half elution. Fractions 12′—13′ were concentrated to a colourless oil (4 mg), δ (250 MHz) 3.6 (m), 3.0 (m, CH_2SAc), 2.4—2.7 (m), 2.32 (s), 2.31 (s), 2.14 (s), 2.13 (s), 2.11 (s), 2.10 (s), and 1.25—1.90 (m). Fractions 14′—20′ were concentrated to a colourless oil (28.4 mg), $\nu_{max.}$ 1 710 (CO_2Me) and 1 690 cm^{-1} (SAc); δ (250 MHz) 3.60 (1 H, m, CHSAc), 2.53 (2 H, m, CH_2SMe), 2.42 (2 H, t, CH_2CO), 2.31 (3 H, s, SMe), 2.13 (3 H, s, COMe or SCOMe), 2.10 (3 H, s, COMe or SCOMe), 1.85 (2 H, m), 1.58 (6 H, m), and 1.38 (2 H, m) (Found: M^+ 262.1071. $C_{12}H_{22}O_2{}^{32}S_2$ requires M^+ 262.1061).

S-(3-*Acetylthio*-7-*methoxycarbonylheptyl*) O,O-*Dimethyl Phosphorothioate* (**8**).—α-Lipoic acid (633 mg, 3.06 mmol) was dissolved in dry benzene (deoxygenated under argon by heating it to reflux for 0.5 h and cooling; 12 ml) and the solution was treated with trimethyl phosphite (reagent grade) (800.1 mg, 6.45 mmol). The yellow solution was heated at 90 °C (bath temperature) under argon for 24 h. The light yellow solution was cooled and the solvent and excess of phosphite were removed by evaporation under reduced pressure. The residual light yellow oil, $\nu_{max.}$ 2 525 (SH), 1 740 (C=O), 1 250 (P=O), and 1 025 cm^{-1} (P–O); δ 3.90 (3 H, s, POMe), 3.70 (3 H, s, POMe), 3.68 (3 H, s, CO_2Me), 3.30—2.60 (3 H, m, CH_2S + CHS), 2.20 (2 H, m, CH_2CO), and 1.90—1.30 (8 H, m), was dissolved in dry pyridine (6 ml) and treated with acetic anhydride (0.84 ml, 906

mg, 8.88 mmol) under argon. The solution was stirred at room temperature for 24 h and then treated with ice–water (20 ml). The suspension was stirred for 10 min, then acidified with concentrated hydrochloric acid, extracted with ether (4 ×), and the extracts washed with water (3 ×) and brine (1 ×) and dried ($MgSO_4$) to give a pale yellow solution from which the crude phosphorothioate could be obtained on evaporation as a yellow oil (1.05 g). The oil was chromatographed on a column of silica gel (24 g) using ethyl acetate–light petroleum (2 : 1) as eluant. Fractions (5 ml) were collected when the yellow band first reached the bottom of the column. Fractions 7—17 were concentrated to a colourless, viscous oil (754.5 mg, 66%), $\nu_{max.}$ 1 735 (CO_2Me), 1 690 (SAc), 1 260 (P=O), and 1 020 cm^{-1} (P–O); δ_H (250 MHz) 3.82 (3 H, s, POMe), 3.78 (3 H, s, POMe), 3.68 (3 H, s, CO_2Me), 3.60 (1 H, m, CHS), 2.88 (2 H, m, CH_2S), 2.33 (3 H, s, CH_3COS), 2.30 (2 H, t, CH_2CO), and 2.10—1.30 (8 H, m); δ_C 195.0, 173.6, 53.9, 53.7, 51.4, 43.4, 36.3, 34.5, 33.8, 30.7, 28.2, 26.3, and 24.7; *m/z* 372 (M^+, 3%), 341 (3, M^+ − OMe), 329 (10, M^+ − Ac), 298 [17, M^+ − MeO(HO)C=CH_2. McLafferty], 267 (28), 220 (62), and 143 (100) (Found: C, 41.7; H, 6.8. $C_{13}H_{25}O_6PS_2$ requires C, 41.92; H, 6.77%).

Methyl 6-Acetylthio-8-methylthio-octanoate (**12**).—A solution of the phosphorothioate (**8**) (257 mg, 1.22 mmol) in dry benzene (8 ml) was treated with DBU (202 mg, 1.32 mmol) and methyl iodide (188 mg, 1.32 mmol) under argon. A white precipitate formed immediately. The solution was heated under reflux under argon for 6.5 h. The cooled mixture was diluted with ethyl acetate (40 ml) and the resultant yellow solution was washed with water (4 ×) and dried ($MgSO_4$). Evaporation of the solvent under vacuum left a yellow oil (322 mg). This oil was chromatographed on a column of silica gel (18 g) using ethyl acetate–light petroleum (2 : 1) as eluant. Fractions (5 ml) were collected immediately after application of the sample to the column. Fractions 7—10 were concentrated to a colourless oil (264.6 mg, 78%), $\nu_{max.}$ 1 738 (CO_2Me) and 1 695 cm^{-1} (SAc); δ_H (250 MHz) 3.67 (3 H, s, CO_2Me), 3.60 (1 H, m, C*H*SAc), 2.52 (2 H, m, CH_2SMe), 2.33 (3 H, s, SMe), 2.31 (2 H, t, CH_2CO), 2.1 (3 H, s, SCOMe), and 1.95—1.30 (8 H, m); δ_C 195.1, 173.7, 51.3, 43.7, 34.6, 34.5, 33.9, 31.6, 30.7, 26.3, 24.7, and 15.5; *m/z* 278 (M^+, 5%), 235 (100, M^+ − Ac), 205 (14), and 122 (22) (Found: C, 51.6; H, 8.0. $C_{12}H_{22}O_3S_2$ requires C, 51.77; H, 7.96%).

Methyl 6-Acetylthio-8-methylsulphinyloctanoate (**13**).—The thioether (**12**) (264.6 mg, 0.94 mmol) was dissolved in dry dichloromethane (9 ml) and the solution was treated with *m*-chloroperbenzoic acid (85% purity; 190.8 mg, 0.94 mmol). The mixture was stirred at room temperature for 15 min, then diluted with ethyl acetate (60 ml). The resultant solution was washed with saturated sodium hydrogen carbonate (4 ×), water (3 ×), and dried ($MgSO_4$). Evaporation of the solvent under reduced pressure left a viscous, pale yellow oil (242.3 mg, 87.7%), $\nu_{max.}$ 1 735 (CO_2Me), 1 695 (SAc), and 1 045 cm^{-1} (S=O); δ (60 MHz) 3.60 (3 H, s, CO_2Me), 2.60 (3 H, s, SOMe), and 2.35 (3 H, s, SCOMe).

Attempted chromatographic purification of this oil on silica gel (15 g) led to the isolation from the column of the corresponding *sulphone* as a cream, waxy solid, m.p. 43—46 °C (23 mg), $\nu_{max.}$ 1 735 (CO_2Me), 1 695 (SAc), 1 300 (S=O), and 1 130 cm^{-1} (S=O); δ (60 MHz) 3.65 (3 H, s, CO_2Me), 2.90 (3 H, s, SO_2Me), and 2.35 (3 H, s, SCOMe) (Found: C, 46.8; H, 7.5. $C_{12}H_{22}O_5S_2$ requires C, 46.43; H, 7.14%).

Methyl 6-Acetylthio-oct-7-enoate (**14**).—The sulphoxide (**13**) (150 mg, 0.51 mmol) was suspended in decalin (2 ml) containing calcium carbonate (100 mg, 1 mmol) and the mixture was heated at 190—195 °C (bath temperature) with exclusion of moisture for 1 h. The cooled mixture was diluted with light petroleum–ethyl acetate (4 : 1) (10 ml), filtered and concentrated to a red-brown liquid. The decalin was distilled from this liquid in a Kugelrohr apparatus at 70—80 °C/0.015 mmHg to leave a red-brown oil (143.6 mg). This oil was chromatographed on a column of silica gel (15 g) using light petroleum–ethyl acetate (2 : 1) as eluant. After a forerun (20 ml), fractions (1 ml) were collected. Fractions 13—14 were concentrated to a light yellow oil (44.9 mg), $\nu_{max.}$ 1 745 (CO_2Me), 1 695 (SAc), and 1 640 cm^{-1} (C=C); δ (250 MHz) 5.75 (1 H, ddd, H_b, J_{ba} 8 Hz, J_{bc} 10 Hz, J_{bd} 16.5 Hz), 5.22 (1 H, ddd, H_d, J_{da} 1.25 Hz, J_{db} 16.5 Hz, J_{dc} 1.25 Hz), 5.08 (1 H, ddd, H_c, J_{ca} 1 Hz, J_{cb} 10 Hz, J_{cd} 1.25 Hz), 4.02 (1 H, q, H_a, J_{ab} 8 Hz, J_{ac} 1 Hz, J_{ad} 1.25 Hz), 3.65 (3 H, s, CO_2Me), 2.30 (3 H, s, SCOMe), and 1.70—1.30 (8 H, m); *m/z* 230 (M^+ 1.4%), 212 (20), 188 (33, M^+ − CH_2CO) 157 (40), 155 (43), 154 (30, M^+ − HSCOMe), 123 (73), 96 (87), and 81 (100) (Found: M^+ 230.0986. $C_{11}H_{18}O_3{}^{32}S$ requires M^+ 230.0977).

Fractions 15—19 were concentrated to a light brown oil (35.3 mg), the ^1H n.m.r. spectrum of which showed that it consisted of a mixture of the above olefin and the thioether (**12**) (ratio 4.4 : 1). Therefore the total yield of olefin is 73.6 mg (63%).

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