Studies on the Biosynthesis of the Fungal Metabolite Oudenone. 2. Synthesis and Enzymatic Cyclization of an α-Diketone, Open-Chain Precursor into Oudenone in Cultures of *Oudemansiella radicata*

Youla S. Tsantrizos*,†,‡,§ Xianshu Yang,† and Andrew McClory†,||

Department of Chemistry & Biochemistry, Concordia University, Montreal, Quebec H3G 1M8, Canada, Department of Chemistry, Boehringer Ingelheim (Canada) Ltd., Bio-Méga Research Division, Laval, Quebec H7S 2G5, Canada, and Department of Chemistry, McGill University, Montreal, Quebec H3A 2K6, Canada

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The α-diketone **4** was shown to be the open-chain biosynthetic precursor of the fungal metabolite oudenone (**1a** and **1b**). Intact incorporation of **4** into **1** was achieved upon incubation of a ^{2}H-labeled, *N*-acetylcysteamine thioester derivative of **4** with growing cultures of *Oudemansiella radicata*. A biosynthetic scheme for the formation of the hexaketide **4** and its enzymatic cyclization into oudenone (**1**), consistent with the experimental data, is described. The proposed mechanism for the cyclization of **4** to **1** is analogous to the "polyepoxide cascade" model, which has been previously implicated in the biosynthesis of polyether antibiotics.

Introduction

Over the last 10 years, polyketide synthase (PKS) enzymes associated with the biosynthesis of macrolides (bacterial PKS Type I; e.g., erythromycin A,[1] niddamycin,[2] FR-008,[3] rapamycin,[4] FK506[5]), and aromatic polyketides (bacterial PKS Type II; e.g., tetracenomycin C,[6] actinorhodin,[7] frenolicin,[8] and fungal PKS Type I; e.g., aflatoxins[9]) have been identified and studied extensively. Furthermore, major advances in the field of microbial genetics have illuminated our knowledge and ability to manipulate the gene clusters encoding for these enzymes.[10]

Although it is generally believed that the biosynthetic steps associated with the skeletal construction of polyether antibiotics are catalyzed by modular, multifunctional PKS type I enzymes analogous to those known in the cases of macrolides,[1−5] little is yet reported on the specific enzymes and genetics associated with this family of natural products.

An idea that was initially proposed by Westley for the biogenesis of lasalocid,[11] and later modified by Cane, Celmer, and Westley as a unified hypothesis for the biosynthesis of all polyether polyketides [e.g., monesin A **(8)**], is the "polyepoxide cascade" mechanism (Scheme 1, path A).[12] In this three-step process, the open-chain oligoketide precursor **9** is formed from acetate, propionate, and butyrate *via* repeated decarboxylative Claisen condensations and subsequent modifications of the *β*-keto group; all of these steps are believed to be catalyzed by the multifunctional PKS enzyme(s). The second step was proposed to be an enzymatic polyepoxidation reaction of the acyclic hydroxypolyene precursor **9**, followed by a cascade of intramolecular nucleophilic attacks on the keto and epoxide moieties of intermediate **10**, eventually leading to the formation of the tetrahydrofuran and tetrahydropyran rings of the final product **8** (Scheme 1, path A).

More recently, the alternative mechanism of "*syn*-oxidative polycyclization" of the hydroxypolyene precursor **9** was proposed by Townsend, Basak, and McDonald (Scheme 1, path B).[13] In this later model, an alkoxy-bound oxo metal derivative **11** was proposed to undergo a series of consecutive intramolecular [2 + 2] cycloadditions with the double bonds to give the corresponding metallaoxetane intermediate **12**, followed by reductive elimina-

* To whom correspondence should be addressed at Boehringer Ingelheim (Canada) Ltd.: Tel: (450) 682-4640. Fax: (450) 682-4189. E-mail: ytsantrizos@lav.boehringer-ingelheim.com.
† Department of Chemistry & Biochemistry, Concordia University.
‡ Department of Chemistry, Boehringer Ingelheim (Canada) Ltd.
§ Department of Chemistry, McGill University.
|| Undergraduate Research Participant.

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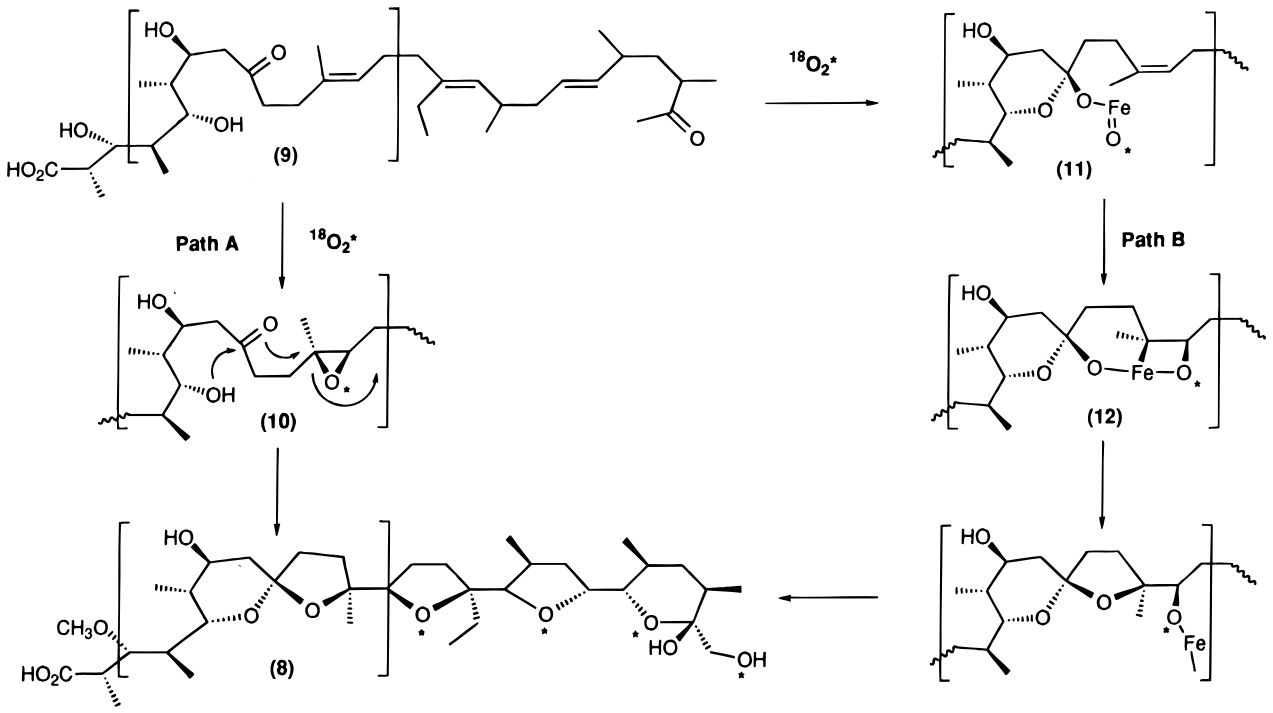
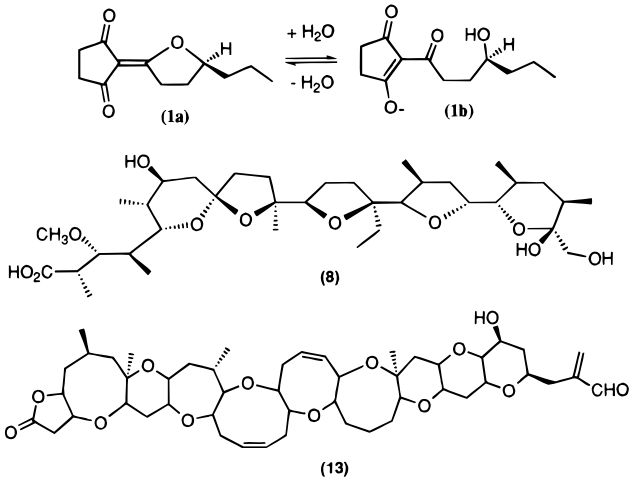
Scheme 1. Models for the Biogenesis of Tetrahydrofuranyl and Tetrahydropyranyl Moieties in Monensin A (11)



tion of the metal and closure of the tetrahydrofuran or tetrahydropyran ring (Scheme 1, Path B).

In addition to the polyether antibiotics, both of these elegant models may also be relevant in the biogenesis of some marine natural products that share common structural features with the polyether antibiotics. Among these metabolites, the best known examples are the marine toxins typified by brevetoxin A (**13**). Nonetheless, to date both of these proposals remain unproven, mainly due to the chemical complexity and instability of the precursors and intermediates associated with the biosynthesis of such structurally complex molecules as monensin A (**8**) and brevetoxin A (**13**).



In this paper, we report the progress of our ongoing investigations into the biosynthesis and enzymology of polyether-type microbial metabolites. Recently, we reported our studies on the biosynthesis of the fungal metabolite oudenone (**1a** and **1b**),[14] a structurally unique hexaketide characterized by a tetrahydrofuran and a 1,3-cyclopentadione moiety (**1a**).[15] We speculated that due to the small molecular structure of **1**, this metabolite could serve as a model for our exploration into the general biogenesis of tetrahydrofuranyl and tetrahydropyranyl moieties of polyether-type polyketides. In our previous report, we showed that the *N*-acetylcysteamine thioester derivative of (5*S*)-5-hydroxyoctanoic acid (**2**) can serve as a substrate of the PKS enzyme(s) catalyzing the biosynthesis of **1** in cultures of *Oudemansiella radicata*. Furthermore, the enzymatic incorporation of **2** into the tetrahydrofuran moiety of **1** was shown to proceed without any change in the absolute stereochemistry of the C9 chiral center. On the basis of these results, plausible hexaketide open-chain precursors and cyclization mechanisms analogous to the "polyepoxide cascade" and the "oxidative polycyclization" models were proposed.[14] In this paper, we present the synthesis and *in vivo* transformation of the α-diketone **4** into oudenone (**1**), strongly suggesting that the cyclization mechanism leading to the formation of **1** is very likely analogous to the "polyepoxide cascade" model (Scheme 2).

Results and Discussion

In our initial studies on the biosynthesis of oudenone (**1**), we showed that its carbon skeleton is derived from six acetate units, and we proposed that the α-diketone **4** was the most likely open-chain hexaketide intermediate (Scheme 2).[14] To test this hypothesis and further probe the mechanism of cyclization, the synthesis of the deuterium labeled **4** as its *N*-acetylcysteamine (NAC) thioester derivative **20** was undertaken (Scheme 3). Racemic 3-propyl-*δ*-valerolactol (**15**) was obtained from

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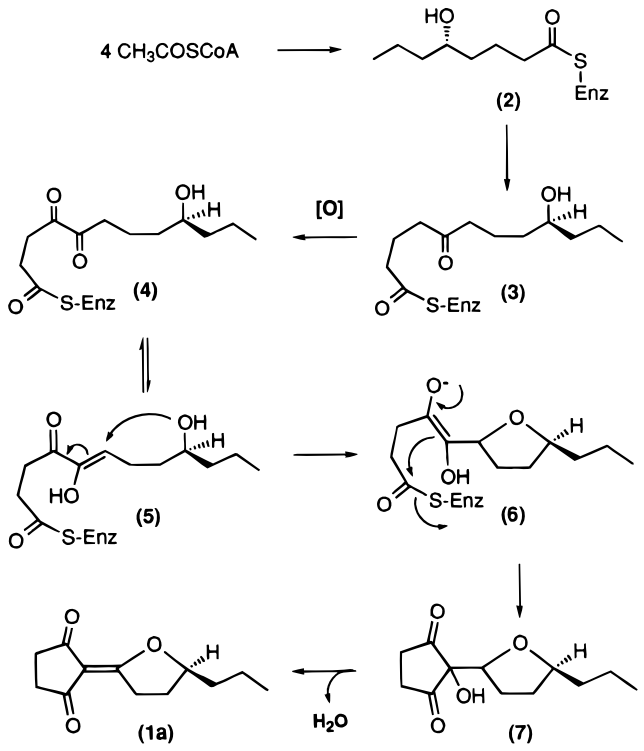
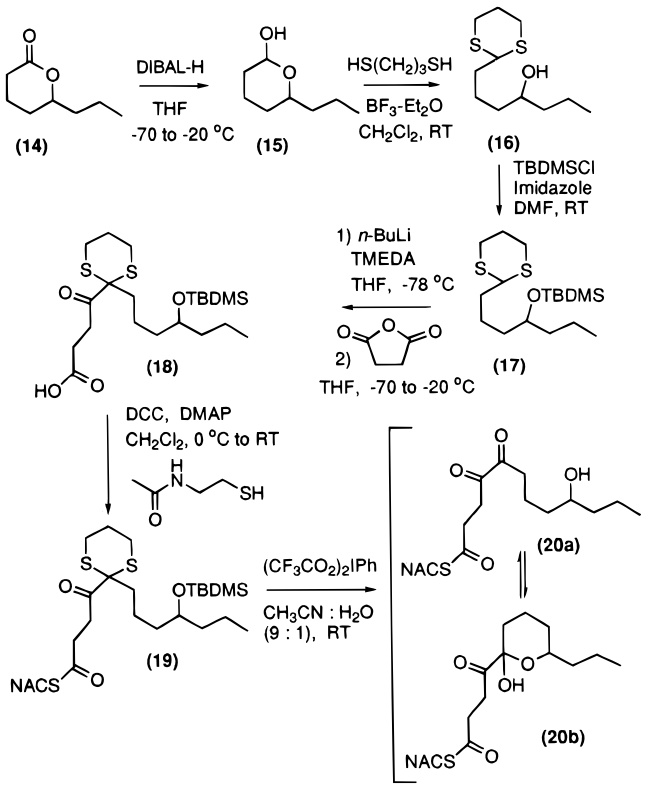
(15) The structure of oudenone in anhydrous organic solvents is that of **1a**; however, in aqueous solvents **1a** is in dynamic equilibrium with the *β*-trione anion **1b**, *via* the simple addition of H_2O (pH′ = 4.1).

Scheme 2. Plausible Mechanisms for the Biogenesis of Oudenone (1a)



Scheme 3. Synthesis of α-Diketone NAC Thioester 23



the DIBAL-H reduction of the corresponding lactone **14**;[16] the preparation of **14** from commercially available ethyl-2-oxocyclopentanecarboxylate was described previously.[14] Lactol **15** was subsequently reacted with 1,3-propanedithiol in the presence of BF_3-etherate in order to protect the masked aldehyde,[17] and the 4-hydroxy group of the crude dithiane **16** was converted to the *tert*-butyldimethylsilyl ether **17** in greater than 90% overall yield (Scheme 3).

Deprotonation of compound **17** (at C1) was carried out with the careful addition of 1 equiv of *n*-butyllithium at −78 to −20 °C.[18] To avoid the addition of excess base, extra care was taken to introduce the solution of *n*-butyllithium into the reaction mixture in small aliquots. The progress of the reaction was monitored by the ^1H NMR of the crude reaction mixture after quenching with D_2O. Once the deprotonation was complete, the anionic solution of **17** was transferred through a precooled cannula at −78 °C to an anhydrous solution of succinic anhydride (or [2,3-$^2H_4$]succinic anhydride) at the same temperature. Both the deuterium-labeled and unlabeled compounds **18** were prepared using the same protocol and fully characterized by ^1H, COSY, DEPT, HETCOR, ^{13}C and ^2H NMR, and MS. The yield of this condensation ranged between 35 and 55%, based on the isolated pure compound **18** (>80% based on the recovery of unreacted compound **17**, however, the unreacted [2,3-$^2H_4$]succinic anhydride could not be recovered). It is worth mentioning that any attempt to remove the 1,3-dithiane protecting group of **18** using standard literature procedures led primarily to decomposition of the starting material and the isolation of the TBDMS silyl ether of 4,5-dioxo-9-hydroxydodecanoic acid in less than 15% yield. Thus, we coupled the protected carboxylic acid **18** with *N*-acetylcysteamine in the presence of DCC and DMAP to obtain the thioester **19** in 50% yield after chromatography.

Finally, the removal of both the 1,3-dithiane and the silyl ether protecting groups were achieved in one step with [bis(trifluoroacetoxy)iodo]benzene following Stork's methodology.[19] The final product **20** (**20a** and **20b**) was isolated in 40% yield after chromatography and, not surprisingly, was found to be very chemically unstable. Therefore, feeding experiments had to be timed carefully so that the labeled final product, [2,3-$^2H_4$]-α-diketone NAC thioster **20**, could be used as soon as it was prepared.

The production of oudenone (**1**) in growing cultures of *O. radicata*, as well as the appropriate timing for the feeding of labeled **20**, were monitored by the changes in UV absorption of the liquid culture, as described previously.[14] Initially, unlabeled **20** was fed to cultures of *O. radicata* at various concentrations in order to establish the maximun amount tolerated by the culture without substantially inhibiting the production of oudenone (**1**). Pulse feeding of [2,3-$^2H_4$]-α-diketone NAC thioster **20** to three cultures of *O. radicata*, in the presence of the *β*-oxidase inhibitor 3-(tetradecylthio)propanoic acid,[20] led to the isolation of metabolite **1**, labeled with deuterium at C2/C3 as predicted (Figure 1). All three samples of labeled oudenone (**1**) were purified by semipreparative C18 reversed-phase HPLC and analyzed by analytical HPLC and NMR.

In one of the feeding experiments, deuterium incorporation (albeit with very low % incorporation) was ob-

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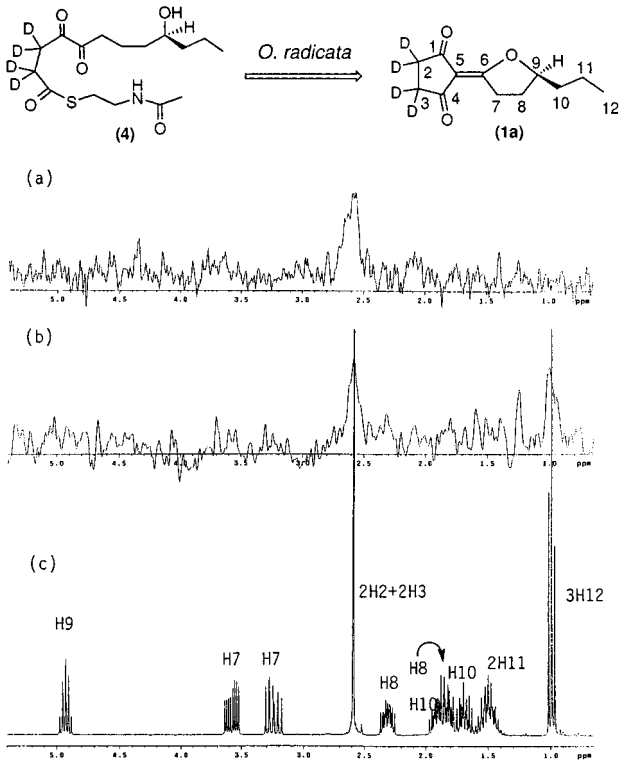

Figure 1. (a) 46.6 MHz ^{2}H NMR spectrum of oudenone (**1a**) derived from the intact incorporation of ^{2}H-labeled compound **20**; feeding experiment I. (b) 46.6 MHz ^{2}H NMR spectrum of oudenone (**1a**) derived from the feeding of ^{2}H-labeled compound **20** to cultures of *O. radicata*; experiments II and III. (c) 300 MHz ^{1}H NMR spectrum of oudenone (**1a**).

served exclusively at the characteristic chemical shift of the H2/H3 protons (δ 2.55, Figure 1a). In the other two cases (Figure 1b), deuterium incorporation was also observed at C12 due to the inevitable degradation of compound **20** to [2-^{2}H$_2$]acetate by *in vivo* β-oxidation.[21] However, the level of deuterium incorporation at C2/C3 was at least 2−4-fold higher than at C12;[21] on the basis of our previous results, the extent of incorporation expected exclusively from the *in situ* production of [2-^{2}H$_2$]-acetate should be significantly lower at C2/C3, C8, and C10 than at C12.[14,21] Therefore, these results strongly suggest that the α-diketone **4** is the open-chain precursor of oudenone (**1**). Consequently, it is reasonable to believe that the cyclization of **4** proceeds *via* an intramolecular β-addition, followed by a Claisen-type intramolecular condensation and dehydration to give **1** (Scheme 2). This type of mechanism bears many similarities to the "polyepoxide cascade" model, as well as the catalytic mechanism leading to the formation of the 2-amino-3-hydroxycyclopent-2-enone, a unique structural moiety found in several antibiotics including reductiomycin,[22] asukamycin,[23] moenomycin,[24] and L-155,175.[25] A pyridoxal phosphate-dependent cyclization mechanism leading to the formation of this moiety was proposed by Floss in the biosynthesis of reductiomycin.[22]

Summary

The results presented in this study provide strong evidence for the proposed pathway and mechanism of cyclization of the open-chain hexaketide precursor **4** into oudenone (Scheme 2). The formation of the tetrahydrofuran and 1,3-cyclopentadione moieties of **1** is consistent with the mechanisms previously proposed for microbial polyether metabolites[12] and natural products characterized by the 2-amino-3-hydroxycyclopent-2-enone structural unit,[22] respectively. To further explore the biosynthesis of **1**, we have now focused our efforts on the isolation of the gene cluster encoding for the enzymes associated with its formation. Our working hypothesis is that the enzyme(s) catalyzing the biosynthesis of **1** are most likely PKS type I enzymes. The details of the gene(s) and enzyme(s) associated with the biosynthesis of oudenone (**1**) are being sought in experiments currently in progress in our laboratory.

Experimental Section

General Procedures. NMR spectra were obtained at 20−22 °C. ^{1}H, ^{2}H, and ^{13}C NMR chemical shifts are given in ppm and are referenced to the internal deuterated solvent (^{2}H NMR spectra were recorded in $CHCl_3$). All reactions carried out under anhydrous conditions were performed under a nitrogen atmosphere using oven-dried syringes and glassware. THF was distilled from sodium/benzophenone ketyl, CH_2Cl_2 was distilled from P_2O_5, MeOH was distilled from Mg turnings, toluene was distilled from CaH_2, and DMF was distilled from CaO. Reagents and solvents were purchased from Aldrich Chemical Co. and VWR Scientific of Canada, respectively. Isotopically labeled reagents were purchased from Cambridge Isotope Laboratories. Stock cultures of *O. radicata* ATCC 20295 were maintained on PDY (ATCC medium No 337) with 1.5% agar in slant tubes at 4 °C. All culture media and glassware were autoclaved prior to use, and all biological manipulations were conducted in a sterile hood. *O. radicata* cultures were grown at 26.5 °C and 140 rpm, in a rotary shaker. Flash column chromatography was carried out on Merck Kieselgel 60, 230−400 mesh. Reversed-phase flash column chromatography was carried out on silica gel reacted with *n*-octadecyltrichlorosilane, following previously reported procedures.[26]

Fermentation of *O. radicata* ATCC 20295, Precursor Feeding, and Isolation of Oudenone. Cultures of *O. radicata* were grown on both solid and liquid media as previously described.[14] Oudenone (**1**) production, before and after feeding of labeled precursors, was monitored by UV.[14] In all cases, ^{2}H-labeled compound **20** was dissolved in absolute ethanol (~1.5 mg sample/100 μL of ethanol) and administered in 100 μL aliquots, every 6−12 h (3×/day) for a period of 48 h, to growing cultures of *O. radicata* (100 mL of fermentation broths). Between feedings, the ethanolic solution of compound **20** was stored at −85 °C to prevent decomposition. At the same time, the β-oxidase inhibitor 3-(tetradecylthio)propanoic acid (1.5 mg in 30 μL of ethanol) was also administered in order to suppress degradation of compound **20**.[20] All cultures were

(21) The *in vivo* production of [2-^{2}H$_2$]acetate would be expected to result in the incorporation of ^{2}H at C2, C8, C10, and C12 of metabolite **1**. However, we believe that the level of ^{2}H incorporation at C8 and C10 was signifficantly lower than at C12 and below detection limits.[14] Furthermore, the labeling pattern in the structure of **1** was scrambled between C2 and C3 due to the dynamic equilibrium of **1a** and **1b** in the growing cultures of *O. radicata* at the pH of the fermentation broth (pH = 4.5−5.5).

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harvested 24 h after the last feeding, and the labeled metabolite **1** was isolated following the purification protocol developed previously.[14] All final samples of ^{2}H-labeled oudenone (**1**) were further purified by C_{18} reversed-phase HPLC to ensure greater than 99% purity before they were analyzed by ^{2}H NMR.

3-Propyl-δ-valerolactol (15). An anhydrous solution of 3-propyl-δ-valerolactone (**14**)[14] (12.51 g, 88.06 mml) in THF was cooled to −70 °C for ∼30 min before a solution of DIBAL-H (176.2 mL of 1 M solution in anhydrous THF, 2 equiv) was added over a period of 30 min via a dropping funnel and under nitrogen. The reaction mixture was stirred at −70 °C for 1 h and then at −20 °C for an additional period of 5 h. The reaction was quenched by pouring the solution (slowly) into a mixture of 10 g of ice and 10 mL of acetic acid. The mixture was then extracted with diethyl ether (3 × 200 mL). The organic layers were combined, washed with brine (150 mL), saturated aqueous $NaHCO_3$ (3 × 150 mL), and brine (2 × 150 mL), and dried over anhydrous $MgSO_4$, and the solvents were evaporated to obtain the crude product as an oil. The pure lactol **15** (7.998 g) was isolated in 63% yield after purification by flash column chromatography, using a solvent mixture of hexanes−EtOAc (linear gradient from 15:1 to 10:1).

TLC [silica, hexane/EtOAc (4:1)]: $R_f = 0.22$.

^{1}H NMR (mixture of lactol conformers, $CDCl_3$, 270 MHz) δ: 0.80 (t, $J = 6.1$ Hz, 3H), 1.15−1.48 [m, 10H (several overlapping signals)], 3.29 and 3.85 (2m, 0.67H & 0.36, H3), 4.55 (dd, $J = 1.7$ Hz, 0.67H, $H1_{ax}$), 5.15 (bs, 0.36H, $H1_{eq}$).

HRFAB$^+$ MS (glycerol/ KCl at rt) *m/z*: 145.12290, calcd mass for $C_8H_{16}O_2 + H^+ = 145.122855$.

4-Hydroxyoctanal-1-(1,3)-dithiane (16). To a solution of **15** (7.95 g, 55.2 mmol) in anhydrous CH_2Cl_2 (50 mL) was added 8.32 mL of 1,3-propanedithiol (82.8 mmol, 1.5 equiv), and 3 mL of BF_3-etherate (3.46 g, 24.4 mmol, 0.44 equiv) were added via a syringe. The reaction mixture was allowed to stir at rt under nitrogen for 24 h. The crude reaction was subsequently washed with equal volumes of H_2O, 10% aqueous KOH, and again with H_2O. After drying of the organic layer over K_2CO_3 and evaporation of the solvent, the dithiane derivative **16** was obtained in 90% yield and in high enough purity to be used as such in the subsequent reaction.

TLC [silica, hexane/EtOAc (4:1)]: $R_f = 0.23$.

^{1}H NMR ($CDCl_3$, 270 MHz) δ: 0.8 (t, $J = 6.1$ Hz, 3H), 1.15−2.03 [m, 12H (several overlapping signals)], 2.64−2.87 (m, 4H), 3.55 (m, 1H), 4.00 (t, $J = 6.1$ Hz, 1H).

^{13}C NMR ($CDCl_3$, 67.5 MHz) δ: 14.2, 18.9, 22.8, 26.1, 30.5, 35.5, 37.0, 39.7, 47.6, 71.5.

HRFAB$^+$ MS (glycerol/ KCl at rt) *m/z*: 235.11894, calcd mass for $C_{11}H_{22}OS_2 + H^+ = 235.119034$.

4-*tert*-Butyldimethylsilyl Ether Derivative (17). A mixture of *tert*-butyldimethylsilyl chloride (17.0 g, 112.6 mmol, 4.5 equiv), imidazole (17.1 g, 250.5 mmol, 10 equiv), and dithiane derivative **16** (6.0 g, 50.1 mmol) was stirred in anhydrous DMF (2 mL) under nitrogen at rt for 24 h. The reaction was subsequently quenched with the addition of Et_2O (15 mL) and brine (15 mL). The aqueous layer was further extracted with Et_2O (2 × 50 mL), and the combined ether layers were dried over $MgSO_4$ and concentrated under reduced pressure to give the crude product **17** as an oil. Pure compound **17** was obtained in quantitative yields (>98%) after purification by flash column chromatography using hexane/EtOAc (25:1) as the eluting solvent.

TLC [silica, hexane/EtOAc (19:1)]: $R_f = 0.66$.

^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.01 (s, 6H), 0.85 (overlapping s and t, 12H), 1.15−2.03 (overlapping m 12H), 2.61−2.87 (m, 4H), 3.55 (m, 1H), 4.00 (t, $J = 6.7$ Hz, 1H).

^{13}C NMR ($CDCl_3$, 75 MHz) δ: −4.3, −4.2, 14.5, 18.3, 18.7, 22.5, 26.1, 26.2, 30.6, 35.9, 36.9, 39.4, 47.8, 71.9.

HRFAB$^+$ MS (glycerol at rt) *m/z*: 349.20553, calcd mass for $C_{17}H_{36}OS_2Si + H^+ = 349.205513$.

9-(*tert*-Butyldimethylsilyloxy)-5-(1,3-dithiane)-4-oxolauric Acid (18). To a cooled (−78 °C) solution of **17** (3.72 g, 10.7 mmol) in anhydrous THF (10 mL) was added a solution of *n*-butyllithium (1.6 M solution in hexanes, 16.0 mmol, 1.5 equiv), followed by tetramethylethylenediamine (2.4 mL, 1.86 g, 16 mmol, 1.5 equiv). The reaction mixture was allowed to warm to −20 °C and stirred at that temperature for ∼1 h. An aliquot of the reaction mixture was quenched with D_2O and analyzed by ^{1}H NMR to confirm complete deprotonation of the starting material before continuing with the addition of the electrophile. Succinic anhydride (1.6 g, 16.0 mmol, 1.5 equiv) was dissolved in anhydrous THF (18 mL) and cooled to −78 °C. The anionic solution of **17** was then cooled to −78 °C before it was transferred through a precooled cannula to the solution of the deuterated succinic anhydride. Once again, the mixture was allowed to warm to −20 °C and stirred at that temperature for an additional 5 h. Finally, the reaction was quenched with the addition of acidified water (50 mL, pH = ∼3). The crude product was extracted into EtOAc (3 × 150 mL), the organic layers were dried over $MgSO_4$, and the EtOAc was evaporated to dryness. The pure lauric acid derivative **18** was isolated after flash column chromatography (2.65 g, 82% yield based of the recovery of unreacted starting material) using a mixture of hexane/EtOAc (25:1) containing 0.3% acetic acid as the eluent.

TLC [silica, hexane/EtOAc (1:1) plus 0.3% AcOH]: $R_f = 0.37$.

^{1}H NMR ($CDCl_3$, 300 MHz) δ: −0.01 (s, 6H), 0.85 (overlapping s and t, 12H), 1.21−1.55 (m, 8H), 1.70−2.10 (m, 4H), 2.59−2.64 (m, 4H), 2.92−3.07 (m, 4H), 3.55 (m, 1H).

^{13}C NMR ($CDCl_3$, 75 MHz) δ: −4.4, −4.3, 14.2, 18.0, 18.6, 20.1, 24.8, 25.9, 27.8, 28.6 30.7, 37.3, 39.0, 39.2, 60.8, 71.6, 178.7, 202.7.

HRFAB$^+$ MS (glycerol at rt) *m/z*: 449.22158, calcd mass for $C_{21}H_{40}O_4S_2Si + H^+ = 449.221557$.

[2,3-$^2H_4$]-9-(*tert*-Butyldimethylsilyloxy)-5-(1,3-dithiane)-4-oxolauric Acid (18). The deuterium-labeled analogue **18** was obtained following the same synthetic procedure as described for the unlabeled compound **18**, with the exception that the anion of **17** was coupled with [2,3-$^2H_4$]succinic anhydride.

^{1}H NMR ($CDCl_3$, 300 MHz) δ: −0.01 (s, 6H), 0.85 (overlapping s and t, 12H), 1.21−1.55 (m, 8H), 1.70−2.10 (m, 4H), 2.66 (dt, $J = 3.6$, 1.2 Hz, 2H), 2.97−3.07 (m, 2H), 3.55 (m, 1H).

^{2}H NMR ($CDCl_3$, 46.6 MHz) δ: 3.06 (s), 2.62 (s) ∼1:1 ratio.

^{13}C NMR ($CDCl_3$, 75 MHz) δ: −4.3, −4.2, 14.5, 18.3, 18.8, 20.3, 24.9, 26.1, 27.9, (resonance at δ 28.6 could not be clearly observed),[27] 30.5 (m),[2] 37.5, 39.1, 39.4, 60.9, 71.8, 178.3, 202.8.

HRFAB$^+$ MS (glycerol at rt) *m/z*: 453.24656, calcd mass for $C_{21}H_{26}O_4S_2SiD_4 + H^+ = 453.246664$.

9-(*tert*-Butyldimethylsilyloxy)-5-(1,3-dithiane)-4-oxolauryl NAC Thioester (19). A solution of **18** (134 mg, 0.3 mmol) in anhydrous CH_2Cl_2 (4 mL) was cooled to 0 °C under nitrogen. 1,3-Dicyclohexylcarbodiimide (1 mL, 86 mg, 0.42 mmol, 1.4 equiv) and 4-(dimethylamino)pyridine (7.3 g, 0.06 mmol, 0.2 equiv, dissolved in 0.5 mL of anhydrous CH_2Cl_2) were added, followed by a solution of *N*-acetylcysteamine (142 mg, 1.2 mmol, 4 equiv, dissolved in 7 mL of anhydrous CH_2Cl_2). The reaction mixture was stirred at 0 °C for 30 min before it was allowed to warm to rt and stirred for an additional 2 h. The precipitated urea was subsequently removed by filtration, the solid material was rinsed with EtOAc, and the combined organic solvents were evaporated to dryness. The crude residue was chromatographed on silica gel using EtOAc/hexane (1:1) as the eluent providing pure NAC-derivative **19** (128 mg) in 78% yield.

TLC [silica, EtOAc]: $R_f = 0.17$.

^{1}H NMR ($CDCl_3$, 300 MHz) δ: −0.01 (s, 6H), 0.85 (overlapping s and t, 12H), 1.2−1.5 (m, 8H), 1.7−2.1 (m, 4H), 1.97 (s, 3H), 2.6 (dt, $J = 3.6$, 1.2 Hz, 2H),[27] 2.84 (t, $J = 5.2$ Hz, 2H, H3),[27] 2.92 (d, $J = 12.6$ Hz, 2H), 3.04 (t, $J = 6.0$ Hz, 2H, H2), 3.08 (t, $J = 5.2$ Hz, 2H, H3), 3.38 (q, $J = 5.7$ Hz, 2H), 3.58 (m, 1H), 5.95 (bs, NH).

^{13}C NMR ($CDCl_3$, 75 MHz) δ: −4.3, −4.2, 14.5, 18.3, 18.8, 20.3, 23.4, 24.9, 26.1, 27.9, 28.8, 31.4 (C2),[27] 37.4, 38.5 (C3),[27] 39.1, 39.4, 39.7, 61.0, 71.8, 170.5, 198.6, 202.7.

HRFAB$^+$ MS (glycerol at rt) *m/z*: 550.25174, calcd mass for $C_{25}H_{47}NO_4S_3Si + H^+ = 550.251478$.

(27) ^{1}H and ^{13}C resonances at C2 and C3 were assigned from the combined ^{1}H, ^{13}C, COSY, HETCOR, and DEPT NMR data of the unlabeled and labeled compound.

[2,3-$^2H_4$] 9-(*tert*-Butyldimethylsilyloxy)-5-(1,3-dithiane)-4-oxolauryl NAC Thioester (19). [2,3-$^2H_4$]-9-(*tert*-Butyldimethylsilyloxy)-5-(1,3-dithiane)-4-oxolauric acid (**18**) was converted to the deuterium-labeled NAS-derivative **19** following the same synthetic procedure as described for the unlabeled compound **19**.

^{1}H NMR ($CDCl_3$, 300 MHz) δ: −0.01 (s, 6H), 0.85 (overlapping s and t, 12H), 1.2−1.5 (m, 8H), 1.7−2.1 (m, 4H), 1.97 (s, 3H), 2.66 (dt, J = 3.6, 1.2 Hz, 2H), 2.92 (d, J = 12.6 Hz, 2H), 3.04 (t, J = 6.3 Hz, 2H), 3.38 (q, J = 6.3 Hz, 2H), 3.58 (m, 1H), 5.95 (s, NH).

^{2}H NMR ($CDCl_3$, 46.6 MHz) δ: 2.87 (s), 3.14 (s) ∼1:1 ratio.

^{13}C NMR ($CDCl_3$, 75 MHz) δ: −4.3, −4.2, 14.5, 18.3, 18.8, 20.3, 23.4, 24.9, 26.1, 27.9, 28.8, 31.4 (m, C2),[27] 37.4, (resonance at δ 38.5 could not be clearly observed, C3),[27] 39.1, 39.4, 39.7, 61.0, 71.8, 170.5, 198.6, 202.7.

HRFAB$^+$ MS (glycerol at rt) m/z: 554.27674, calcd mass for $C_{25}H_{43}NO_4S_3SiD_4 + H^+ = 554.276585$.

α-Diketone NAC Thioester 20 (a and b). To a solution of compound **19** (360 mg, 0.65 mmol) in CH_3CN/H_2O (9:1 ratio, 10 mL) was added [bis(trifluoroacetoxy)iodo]benzene (566 mg, 1.3 mmol, 2 equiv), and the reaction mixture was stirred at rt for 1.5 h. Most of the solvents were subsequently removed under high vacuum, and the crude reaction mixture was mixed with saturated aqueous $NaHCO_3$ (10 mL). After extraction of the aqueous layer with EtOAc (3 × 100 mL), the organic layer was dried over $MgSO_4$ and evaporated to dryness. Pure α-diketone **20** was obtained after flash column chromatography using 25% hexane in EtOAc in 40% yield (∼90 mg). ^{1}H and ^{13}C NMR indicated that the predominant form in solution was the hemiketal **20b**.

TLC [silica, EtOAc]: R_f = 0.12.

^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.85 (t, J = 6.9 Hz, 3H), 1.2−2.0 (overlapping m, 10H), 1.97 (s, 3H), 2.75−3.15 (m, 6H), 3.38 (q, J = 6.3 Hz, 2H), 3.58 (m, 1H), 5.95 (bs, NH).

^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.3, 18.6, 18.7, 23.4, 28.9, 30.1 (C2),[27] 30.8, 31.2, 37.6 (C3),[27] 38.5, 39.7, 71.0, 96.7, 170.6, 198.9, 207.3.

HRFAB$^+$ MS (glycerol at rt) m/z: 346.16867, calcd mass for $C_{16}H_{27}NO_5S + H^+ = 346.168820$.

[2,3-$^2H_4$]-α-Diketone NAC Thioester 20. Deprotection of [2,3-$^2H_4$]-9-(*tert*-butyldimethylsilyloxy)-5-(1,3-dithiane)-4-oxolauryl NAC thioester (**19**) was carried out using the same procedure as described for the unlabeled compound.

^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.85 (t, J = 6.9 Hz, 3H), 1.2−2.0 (overlapping m, 10H), 1.97 (s, 3H), 3.04 (dt, J = 6.3, 1.5 Hz, 2H), 3.38 (q, J = 6.3 Hz, 2H), 3.58 (m, 1H), 5.95 (bs, NH).

^{2}H NMR ($CDCl_3$, 46.6 MHz) δ: 2.9 (bs).

^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.3, 18.6, 18.7, 23.4, 28.9, 30.1 (m, C2), 30.8, 31.2, (resonance at δ 37.6 could not be clearly observed, C3)[27] 38.5, 39.7, 71.0, 96.7, 170.6, 198.9, 207.3.

HRFAB$^+$ MS (glycerol at rt) m/z: 350.19392, calcd mass for $C_{16}H_{23}NO_5SD_4 + H^+ = 350.193927$.

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Supporting Information Available: Copies of ^{1}H, ^{2}H, ^{13}C, COSY, DEPT, and HETCOR NMR data for key synthetic compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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