One-Pot Synthesis of β-Keto Esters and Preparation of 3-Ketopalmitoyl-CoA

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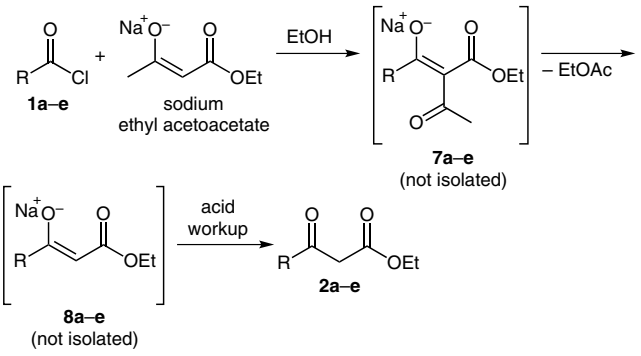
Abstract: β-Keto esters were synthesized from acyl chlorides and sodium ethyl acetoacetate in EtOH using a simple 'one-pot, one-step' method. The deacetylation of α-acetyl β-keto ester to β-keto ester was performed simply, by heating the reaction mixture at reflux for 12 hours, without the addition of additional reagents (e.g., NH_4Cl, NH_3, MeOH, or NaOH). One of the β-keto esters prepared using this method was ethyl 3-oxohexadecanoate, a key intermediate in the synthesis of 3-ketopalmitoyl coenzyme A (3-oxohexadecanoyl coenzyme A), a potential substrate of the biologically important enzyme 17β-hydroxysteroid dehydrogenase type 12.

Key words: β-keto esters, one-pot reaction, deacetylation, 3-ketopalmitoyl coenzyme A, 17β-hydroxysteroid dehydrogenase type 12

17β-Hydroxysteroid dehydrogenase type 12 (17β-HSD12) is a member of the 17β-HSD that catalyze the reduction of 17-ketosteroids or the oxidation of 17β-hydroxysteroids.[1] 17β-HSD12 has an important role in estrone formation, by selectively and efficiently catalyzing the transformation of the weak estrogen estron into the potent estrogen estradiol.[2] This enzyme also has ketoacyl-coenzyme A reductase activity and it is involved in lipid metabolism through participation in fatty-acid elongation.[3] 3-Ketopalmitoyl coenzyme A is hypothesized to be a substrate of this enzyme.[3] To investigate this hypothesis, milligram quantities of 3-ketopalmitoyl coenzyme A were required, and so a total synthesis is needed to be developed.

To date, a number of syntheses for β-keto esters have been reported.[4] Acylation of acetoacetic esters at the C2 carbon followed by deacetylation of α-acetyl β-keto esters to β-keto esters is a well-known method. Yuasa and Tsuruta[4a] discussed and solved a number of problems of previously reported methods. In their procedure, deacetylation of α-acetyl β-keto ester to β-keto ester is still performed by adding an additional reagent (MeOH) to the reaction mixture. To avoid this additional step, we developed a general 'one-pot, one-step' process for the synthesis of β-keto esters from acyl chlorides and sodium ethyl acetoacetate in EtOH, and applied this to the synthesis of 3-ketopalmitoyl coenzyme A. The main advantage of this procedure is that it is very simple, and it gives reasonably good yields.

Aliphatic or aromatic acyl chlorides **1a**–**e** (Table 1, entries 1–5) were reacted with a solution of sodium ethyl acetoacetate in EtOH at 0 °C, to form the α-acetyl β-keto esters **7a**–**e** (Scheme 1). These were further deacetylated simply by heating the reaction mixture at reflux for 12 hours. After filtration of the precipitated NaCl, solvent evaporation, and acid workup, the β-keto esters **2a**–**e** were purified by flash column chromatography. A series of β-keto esters **2a**–**e** were thus obtained in a one-pot procedure from acyl chlorides without isolation of any intermediates (Table 1). Overall yields for these three-step preparations of purified β-keto esters were between 30% and almost 50%, which is comparable to yields in analogous methods.[4]



Scheme 1 Reaction pathway

β-Keto ester **2b** was further used for coupling with CoASH. As coupling of β-ketopalmitoic acid and different thioles with no protection on the β-keto functionality proved to be unsuccessful, we first introduced the 1,3-dioxolane protecting group onto the β-keto group. β-Keto ester **2b** was treated with ethylene glycol in the presence of a catalytic amount of PTSA·H_2O. The ethyl ester **3** was prepared with 60% yield. Compound **3** was then hydrolyzed using 1 M aqueous LiOH solution in a THF–H_2O mixture to provide, after acidic treatment, the carboxylic acid **4** with 92% yield. This was followed by the preparation of the activated esters with the use of known activating agents, such as *N*-hydroxysuccinimide[5] or *N*-hydroxyphthalimide[5a] and dicyclohexylcarbodiimide. These activated derivatives reacted poorly with methyl thioglycolate, a model thiol reagent that was used to probe the reaction before the use of the more expensive CoASH. Compound **4** was then finally converted into a mixed anhydride using ethyl chloroformate in the presence of *N*-methylmorpholine in tetrahydrofuran (THF) at –5 °C and reacted in situ with an aqueous solution of the trilithium salt of CoASH. The pH of the reaction was 8–9 and tetra-*n*-butylammonium chloride was used as a phase-transfer catalyst to provide the protected thioester **5**. This was followed with one of the most demanding steps, the deprot-

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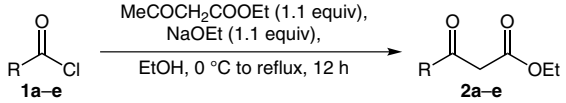
ection of the 1,3-dioxolane protecting group on the β-keto functionality. We found that the best way to deprotect intermediate **5** was by using a mixture of 4 M aqueous HCl and a small amount of THF at 40 °C. After alkaline work-up, the thioester **6** was purified by automated reverse-phase flash column chromatography (Scheme 2). The yield over the last two steps was 36%, calculated to the trilithium salt of CoASH. Other methods for the removal of 1,3-dioxolane were also tried; for example, using a catalytic amount of PTSA in water, a method described by Kurosawa et al.,[6] and a mixture of 2 M HCl and THF; however, they were not efficient in this case.

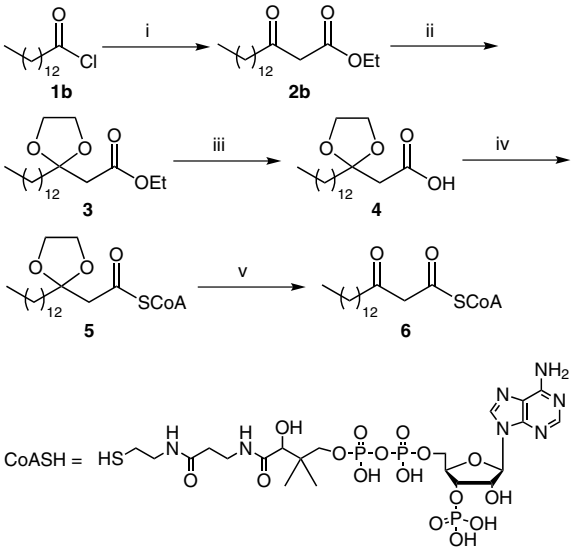
Table 1 One-Pot Synthesis of β-Keto Esters



Entry[a]	Acyl chloride	Product	Yield (%)[b]
1	**1a** R = $Me(CH_2)_4$	**2a** R = $Me(CH_2)_4$	30
2	**1b** R = $Me(CH_2)_{12}$	**2b** R = $Me(CH_2)_{12}$	33
3	**1c** R = Ph	**2c** R = Ph	47
4[c]	**1d** R = 4-$O_2NC_6H_4$	**2d** R = 4-$O_2NC_6H_4$	35
5	**1e** R = 4-ClC_6H_4	**2e** R = 4-ClC_6H_4	32

[a] Conditions: acyl chlorides (35.91 mmol), ethyl acetoacetate (5.0 ml, 39.50 mmol, 1.1 equiv), Na (0.908 g, 39.50 mmol, 1.1 equiv), EtOH (55 mL).
[b] Isolated yield.
[c] Solid acyl chloride was dissolved in THF (10 mL).

To conclude, we successfully synthesized a complex molecule, 3-ketopalmitoyl-CoA, which is a potential substrate for the biologically relevant 17β-HSD12 enzyme. Additionally, a previously unknown 'one-pot, one-step' method for the synthesis of β-keto esters from acyl chlorides was developed.



Scheme 2 *Reagents and conditions*: i. $MeCOCH_2COOEt$, NaOEt, EtOH, 0 °C to reflux, 12 h, 33%; ii. $HOCH_2CH_2OH$, PTSA·H_2O (cat.), PhMe, reflux, 16 h, 60%; iii. (a) 1 M LiOH aq, THF–H_2O, 0 °C to r.t., 24 h, (b) 2 M HCl aq, 0 °C, 92%; iv. (a) ClCOOEt, NMM, THF, –5 °C, 90 min, under argon; (b) Li_3CoASH, 1 M LiOH aq, H_2O, TBAC (cat.), 0 °C to r.t., pH 8–9, 45 min, under argon; (c) 1 M HCl aq (to pH 6–7), 0 °C; v. (a) 4 M HCl aq, THF, 40 °C, 90 min; (b) 1 M LiOH aq (to pH 6–7), 0 °C, 36% (for steps iv and v, calcd to Li_3CoASH).

Experimental Section

^1H NMR, ^{13}C NMR, and ^{31}P NMR were recorded on a Bruker Avance III NMR spectrometer at 400, 100, and 162 MHz respectively. The chemical shifts (δ) are reported in parts per million (ppm) and are referenced to either the internal standard TMS (when $CDCl_3$ was used) or the deuterated solvent (D_2O) used. The coupling constants (*J*) are reported in Hz, and the splitting patterns are indicated as: s (singlet), br s (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), br t (broad triplet), dt (doublet of triplets), q (quartet), and m (multiplet). IR spectra were recorded on a Perkin-Elmer FT-IR System Spectrum BX. Microanalyses were performed on a PerkinElmer C, H, N Analyzer 240 C. The analyses are indicated by the symbols of the elements and they were within ±0.4% of the theoretical values. Centrifugation was performed on a Tehtnica Centric 150. Analytical reversed-phase HPLC was performed on an Agilent 1100 LC modular system equipped with an autosampler, a quarternary pump system, a photodiode array detector, a thermostatted column compartment, and a ChemStation data system. The detector was set to 210.8, 254.16, and 280.16 nm. The column used for method A and method B was a Zorbax Eclipse Plus C18 analytical 150 × 4.6 mm column, at 5 micron (Agilent). The column used for method C was a Luna 5u C18(2) 100A 250 × 4.6 mm column, at 5 micron (Phenomenex). HPLC Guard Cartridge systems were used on both columns, as a Security Guard Cartridge C18 CODS, octadecyl 4 mm × 3.0 mm ID (Phenomenex). The HPLC columns were thermostatted at 25 °C.

Method A: 20 μL of sample solution was injected and eluted at a flow rate of 0.7 mL/min, using a linear gradient of mobile phase A (MeCN) and mobile phase B (aq phosphate buffer: 20 mM, pH 4.95). Gradient for method A: from 3% to 70% mobile phase A in 20 min, then 6 min at 70% mobile phase A, and back to 3% mobile phase A in 6 min.

Method B: 20 μL of sample solution was injected and eluted at a flow rate of 0.7 mL/min, using a linear gradient of mobile phase A (MeCN) and mobile phase B [aq phosphate buffer (20 mM, pH 4.95) with 3% MeCN]. Gradient for method B: from 0% to 67% mobile phase A in 20 min, then 6 min at 67% mobile phase A, and back to 0% mobile phase A in 6 min. Method C: 20 μL of sample solution was injected and eluted at a flow rate of 1.5 mL/min, using a linear gradient of mobile phase A (MeCN) and mobile phase B (aq phosphate buffer: 25 mM, pH 5.0). Gradient for method C: 3 min at 5% mobile phase A, then in 12 min to 70% mobile phase A, then 15 min at 70% mobile phase A, and back to 3% mobile phase A in 10 min. Automated reverse-phase flash column chromatography was carried out with a Biotage Isolera One System. The cartridge used was a 30 g Biotage SNAP Cartridge KP-C18-HS.

Method D: the sample was eluted at a flow rate of 22 mL/min using a linear gradient of mobile phase A (MeCN) and mobile phase B (double-distilled water). Gradient for method D: 5 column volumes of 0% mobile phase A, then in 8 column volumes to 30% mobile phase A. The detector was set at 254 and 210 nm.

MS were recorded on a VG-Analytical AutoSpec Q Micromass mass spectrometer. Melting points were determined on a Leica hot stage microscope and are uncorrected. Evaporation of solvents was

performed at reduced pressure. Reagents and solvents were purchased from Acros Organics, Aldrich, Carlo Erba, Euriso-Top, Fluka, Janssen Chimica, J.T. Baker, Kemika, Merck, Panreac, Riedel-de Haën, Sigma, and Sigma-Aldrich, and were used without further purification, unless otherwise stated. The trilithium salt of coenzyme A was from yeast at ≥93% purity, as purchased from Sigma. Double-distilled water was obtained using a Millipore Advantage A10 system. THF was refluxed under argon over sodium and benzophenone for 2 h, then fractionally distilled under argon through a helices-packed column prior to use. Ethylene glycol was dried with NaOH and distilled under vacuum prior to use. Ethyl chloroformate was washed with H_2O and sat. brine solution, then fractionally distilled under argon through a 20 cm Vigreux column prior to use. *N*-Methylmorpholine was refluxed under argon over sodium for 3 h, then fractionally distilled under argon through a 20 cm Vigreux column prior to use. Tetra-*n*-butylammonium chloride was crystallized from acetone by addition of Et_2O, then dried in vacuo at r.t. in the presence of NaOH, P_2O_5, and silica gel prior to use. Flash column chromatography was performed on silica gel 60 (0.040–0.063 mm) for column chromatography (particle size, 230–400 mesh). Analytical TLC was performed on silica gel Merck 60 F_{254} aluminium sheets (0.20 mm), using visualization with ultraviolet light and/or visualization reagents.

General Procedure for the Synthesis of β-Keto Esters 2a–f

A solution of sodium (1.1 equiv) in commercial absolute EtOH was cooled to 0 °C and ethyl acetoacetate (1.1 equiv) was added dropwise. Acyl chloride **1a–e** (1.0 equiv) was added dropwise (solid acyl chloride **1c** was dissolved in a minimal amount of THF and then added dropwise). The resulting suspension was refluxed for 12 h, then diluted with 96% EtOH, filtered through a pad of Celite, and evaporated. Toluene and H_2O were added to the residue, and the mixture was adjusted to pH 1–2 with 2 M aq HCl. The organic phase was washed with sat. brine solution, dried over anhyd Na_2SO_4, and evaporated. The residue was purified by flash column chromatography to produce the β-keto esters **2a–e**.

Ethyl 3-Oxooctanoate (2a)

Purified by flash column chromatography using Et_2O–PE (1:10) as the eluent, to produce β-keto ester **2a** as a colorless liquid (30% yield). R_f = 0.52 (*n*-hexane–EtOAc = 4:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.89 (3 H, t, *J* = 7.00 Hz), 1.23–1.35 (7 H, m), 1.56–1.64 (2 H, m), 2.54 (2 H, t, *J* = 7.40 Hz), 3.44 (1.84 H, keto, s), 4.19 (0.16 H, enol, q, *J* = 7.15 Hz), 4.20 (1.84 H, keto, q, *J* = 7.15 Hz), 4.98 (0.08 H, enol, s), 12.12 (0.08 H, enol, s).

Ethyl 3-Oxohexadecanoate (2b)

Purified by flash column chromatography using Et_2O–PE (1:15) as the eluent to produce the β-keto ester **2b** as a slightly golden oil that solidified into a white solid after cooling (33% yield). R_f = 0.56 (*n*-hexane–EtOAc = 4:1); mp 27–30 °C. IR (KBr): 3652, 2925, 2853, 2362, 1744, 1718, 1468, 1318, 1233, 1032, 945, 721, 591 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.90 (3 H, t, *J* = 6.8 Hz), 1.06–1.37 (23 H, m), 1.53–1.63 (2 H, m), 2.54 (2 H, t, *J* = 7.35 Hz), 3.44 (1.92 H, keto, s), 4.20 (0.08 H, enol, q, *J* = 7.16 Hz), 4.21 (1.92 H, keto, q, *J* = 7.16 Hz), 4.99 (0.04 H, enol, s), 12.11 (0.04 H, enol, s). HRMS (ESI$^+$): *m/z* calcd for $C_{18}H_{35}O_3$: 299.2586; found: 299.2596. Anal. Calcd for $C_{18}H_{34}O_3$: C, 72.44; H, 11,48. Found: C, 72.76; H, 11.87.

Ethyl 3-Oxo-3-phenylpropanoate (2c)

Purified by flash column chromatography using Et_2O–PE (1:7) as the eluent to produce the β-keto ester **2d** as a slightly pink liquid (47% yield). R_f = 0.47 (*n*-hexane–EtOAc = 5:1). IR (NaCl): 3647, 2984, 1741, 1688, 1625, 1450, 1268, 1198, 1037, 943, 757, 690, 593 cm^{-1}.^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.26 (2.52 H, keto, t, *J* = 7.10 Hz), 1.34 (0.48 H, enol, t, *J* = 7.10 Hz), 4.0 (1.68 H, keto, s), 4.22 (1.68 H, keto, q, *J* = 7.11 Hz), 4.27 (0.32 H, enol, q, *J* = 7.03 Hz), 5.67 (0.16 H, enol, s), 7.40-7.97 (5 H, m), 12.59 (0.16 H, enol, s).

Ethyl 3-(4-Nitrophenyl)-3-oxopropanoate (2d)

Purified by flash column chromatography using Et_2O–PE (4:21) as the eluent to produce the β-keto ester **2e** as a slightly yellow solid (35% yield). R_f = 0.45 (*n*-hexane–EtOAc = 4:1); mp 63–67 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.27 (1.32 H, keto, t, *J* = 7.15 Hz), 1.36 (1.68 H, enol, t, *J* = 7.15 Hz), 4.04 (0.88 H, keto, s), 4.23 (0.88 H, keto, q, *J* = 7.15 Hz), 4.30 (1.12 H, enol, q, *J* = 7.05 Hz), 5.77 (0.56 H, enol, s), 7.94 (1.12 H, enol, dt, J_1 = 4.89 Hz, J_2 = 2.20 Hz), 8.12 (0.88 H, keto, dt, J_1 = 4.89 Hz, J_2 = 2.13 Hz), 8.28 (1.12 H, enol, dt, J_1 = 4.89 Hz, J_2 = 2.20 Hz), 8.34 (0.88 H, keto, dt, J_1 = 4.89 Hz, J_2 = 2.16 Hz), 12.57 (0.56 H, enol, s).

Ethyl 3-(4-Chlorophenyl)-3-oxopropanoate (2e)

Purified by flash column chromatography using Et_2O–PE (1:8) as the eluent to produce the β-keto ester **2f** as a slightly pink liquid (32% yield). R_f = 0.48 (*n*-hexane–EtOAc = 4:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.26 (2.31 H, keto, t, *J* = 7.15 Hz), 1.34 (0.69 H, enol, t, *J* = 7.15 Hz), 3.97 (1.54 H, keto, s), 4.22 (1.54 H, keto, q, *J* = 7.11 Hz), 4.27 (0.46 H, enol, q, *J* = 7.19 Hz), 5.64 (0.23 H, enol, s), 7.38–8.11 (4 H, m), 12.59 (0.23 H, enol, s).

Ethyl 2-(2-Tridecyl-1,3-dioxolan-2-yl)acetate (3)

Ester **2b** (3.361 g, 11.26 mmol, 1.0 equiv) was dissolved in toluene (50 mL). Ethylene glycol (1.884 mL, 33.78 mmol, 3.0 equiv) and PTSA·H_2O (214.21 mg, 1.126 mmol, 0.1 equiv) were added, and the mixture was refluxed for 16 h. During the reaction, the toluene–H_2O azeotrope was removed using a Dean–Stark apparatus. The reaction mixture was washed with sat. aq $NaHCO_3$ solution (50 mL) followed by sat. brine solution (50 mL), dried over anhyd Na_2SO_4, and evaporated. The residue was purified by flash column chromatography using Et_2O–PE (1:5) as the eluent to produce 2.303 g of **3** as a colorless oil (60% yield). R_f = 0.63 (*n*-hexane–EtOAc = 1:3). IR (NaCl): 3650, 2925, 2854, 1739, 1466, 1369, 1304, 1219, 1040, 949, 842, 722 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.90 (3 H, t, *J* = 6.89 Hz), 1.05–1.52 (25 H, m), 1.78–1.84 (2 H, m), 2.66 (2 H, s), 3.94–4.05 (4 H, m), 4.17 (2 H, q, *J* = 7.10 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.13, 14.19, 22.70, 23.54, 29.37, 29.58, 29.66, 29.70, 29.72, 31.93, 37.79, 42.59, 60.50, 65.09, 109.49, 169.62. HRMS (ESI$^+$): *m/z* calcd for $C_{20}H_{39}O_4$: 343.2848; found: 343.2842. Anal. Calcd for $C_{20}H_{38}O_4$: C, 70.13; H, 11.18. Found: C, 70.35; H, 11.41.

2-(2-Tridecyl-1,3-dioxolan-2-yl)acetic acid (4)

A solution of 1 M aq LiOH (67.24 mL, 67.24 mmol, 10 equiv) was added dropwise to a solution of ester **3** (2.303 g, 6.724 mmol, 1.0 equiv) in a mixture of THF (40 mL) and H_2O (15 mL) at 0 °C. The reaction mixture was allowed to warm to r.t. and then stirred for 24 h. The reaction mixture was cooled to 0 °C and adjusted to pH 2 with 2 M aq HCl, and extracted with EtOAc (3 × 75 mL). The combined organic phases were washed with sat. brine solution (75 mL), dried over anhyd Na_2SO_4, and evaporated. The residue was crystallized from EtOAc by adding *n*-hexane to produce 1.974 g of **4** as white crystals (92% yield). R_f = 0.54 (EtOAc–*n*-hexane–AcOH = 1:2:0.25); mp 65–68 °C. IR (KBr): 2917, 2848, 1715, 1466, 1430, 1326, 1224, 1140, 1049, 940, 751, 724, 646, 562 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.90 (3 H, t, *J* = 6.90 Hz), 1.12–1.42 (22 H, m), 1.78-1.83 (2 H, m), 2.72 (2 H, s), 4.00–4.08 (2 H, m), 10.91 (1 H, br s). HRMS (ESI$^-$): *m/z* calcd for $C_{18}H_{33}O_4$: 313.2379; found: 313.2387. Anal. Calcd for $C_{18}H_{34}O_4$: C, 68.75; H, 10.90. Found: C, 69.08; H, 11.29.

2-(2-Tridecyl-1,3-dioxolan-2-yl)acetic Acid Coenzyme A Thioester (5)

To a 25 mL round-bottomed flask equipped with a magnetic stirring bar was added **3** (400.0 mg, 1.272 mmol, 1.0 equiv) under an argon atmosphere. THF (10 mL) was added with a glass syringe, and the resulting solution was cooled to –5 °C. *N*-Methylmorpholine (140.0 μL, 1.272 mmol, 1.0 equiv) was added dropwise with a glass syringe, and the solution was stirred for 15 min before ethyl chloroformate (121.6 μL, 1.272 mmol, 1.0 equiv) was added dropwise with a

glass syringe. The resulting suspension was stirred for 90 min at –5 °C, then the stirring was stopped, and the precipitate was allowed to settle to the bottom of the flask. Then 1.25 mL of the supernatant was transferred with a glass syringe into a 25 mL round-bottomed flask equipped with a magnetic stirring bar under an atmosphere of argon. THF (ca. 9 mL) was added with a double-tipped needle, followed by TBAC (3.54 mg). Solution A (see below) was then added dropwise with a double-tipped needle, and the resulting solution was stirred for 45 min. At this time, the nitroprusside test[7] of the reaction mixture was negative for the presence of thiols. During the reaction, this was maintained at pH 8–9 by adding 1 M aq LiOH (prepared under argon by dissolving solid LiOH in double-distilled water agitated with a stream of argon) with a glass syringe. The reaction mixture was then cooled to 0 °C and opened to the air. The solution was adjusted to pH 6–7 with 1 M aq HCl. The solution was transferred to a 50 mL round-bottomed flask, and THF was evaporated with the temperature of the water bath of the rotary evaporator kept at 30 °C. The residue was frozen and lyophilized, which produced 151.5 mg of a white solid. This product was used in the next step without further purification. R_f = 0.49 (*n*-BuOH–AcOH–H_2O = 5:2:3). HRMS (ESI$^-$): *m/z* calcd for $C_{39}H_{67}N_7O_{19}P_3S$: 1062.3425; found: 1062.3452. HPLC purity, 85% at 254.16 nm (method A, t_R = 20.63 min; method C, t_R = 16.76 min).

Preparation of solution A: Double-distilled H_2O (5 mL) was stirred with a stream of argon and was added with a glass syringe into the container with the trilithium salt of coenzyme A (100 mg, 0.1273 mmol). The solution was transferred with a glass syringe into a 10 mL round-bottomed flask equipped with a magnetic stirring bar under an argon gas atmosphere. The solution was cooled to 0 °C and adjusted to pH 8–9 by adding 1 M aq LiOH (prepared under argon by dissolving solid LiOH in double-distilled H_2O agitated with a stream of argon) with a glass syringe.

3-Oxohexadecanoic Acid Coenzyme A Thioester (6)

The raw thioester **5** (100 mg) was suspended in 4 M aq HCl (8 mL) and heated to 40 °C. Enough THF (ca. 2 mL) was added dropwise to produce a clear solution from the suspension. The reaction was monitored by HPLC using method B. After stirring for 90 min at 40 °C, the THF was removed by agitating the reaction mixture with a stream of nitrogen gas. The resulting suspension was transferred into a 14 mL centrifuge tube and cooled to 0 °C. Double-distilled H_2O (3.5 mL) was added, and the suspension was left at 0 °C for 10 min. The suspension was centrifuged (3 × 1.5 min at 5,000 rpm), and the supernatant was removed. HPLC analysis (method B) of the supernatant showed no presence of thioester **6**. Double-distilled H_2O (1 mL) was added to the residue, and the resulting suspension was cooled to 0 °C. This was adjusted to pH 6–7 with 1 M aq LiOH. The resulting solution was allowed to warm to r.t. and then purified by automated reverse-phase flash column chromatography using method D. The fractions containing thioester **6** were pooled, and the MeCN was evaporated with the temperature of the water bath of the rotary evaporator kept at 30 °C. The residue was frozen and lyophilized to produce 47.8 mg of **6** as a white solid (36% yield for the last two steps, calculated to the trilithium salt of coenzyme A). R_f = 0.53 (*n*-BuOH–AcOH–H_2O = 5:2:3); mp 185–190 °C. IR (KBr): 3402, 2925, 2854, 1652, 1244, 1083, 946, 518 cm^{-1}. ^{1}H NMR (400 MHz, D_2O): δ = 0.61 (3 H, s), 0.69–0.77 (6 H, m), 0.84–1.14 (20 H, m), 1.39 (2 H, br t), 2.32 (2 H, t, *J* = 6.90 Hz), 2.45 (2 H, t, *J* = 7.28 Hz), 2.59 (1.24 H, keto, s), 2.93 (2 H, t, *J* = 6.21 Hz), 3.24 (2 H, t, *J* = 6.40 Hz), 3.33 (2 H, t, *J* = 6.90), 3.42 (1 H, dd, J_1 = 9.73 Hz, J_2 = 4.83 Hz), 3.56 (1 H, dd, J_1 = 9.54 Hz, J_2 = 5.14 Hz), 3.91 (1 H, s), 4.12 (2 H, br s), 4.27 (0.38 H, enol, br s), 4.46 (0.62 H, keto, br t), 4.51 (0.38 H, enol, t, *J* = 4.58 Hz), 4.86–4.92 (0.38 H, enol, m), 6.02 (0.62 H, keto, d, *J* = 6.27 Hz), 6.09 (0.38 H, enol, d, *J* = 5.27 Hz), 8.69–8.15 (1 H, m), 8.38–8.44 (1 H, m). ^{31}P NMR (162 MHz, D_2O): δ = –11.25 (d, *J* = 19.51 Hz), –10.77 (d, *J* = 19.51 Hz), 1.93–2.10 (m, enol), 2.36–2.62 (m, keto). HRMS (ESI$^-$): *m/z* calcd for $C_{37}H_{63}N_7O_{18}P_3S$: 1018.3163; found: 1018.3150. HPLC purity, 95% at 254.16 nm (method B, t_R = 20.55 min; method C, t_R = 16.57 min).

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(7) The nitropruside test was carried out by adding a sample of the reaction mixture to a mixture of an aqueous solution of sodium nitroprusside and dilute aqueous ammonia solution.

