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A Highly Convergent Synthesis of Myristoyl-carba(dethia)-coenzyme A

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Co-translational myristoylation of the N-terminal glycine residues of diverse signalling proteins is required for membrane attachment and proper function of these molecules. The transfer of myristate from myristoyl-coenzyme A (myr-CoA) is catalysed by the enzyme *N*-myristoyltransferase (Nmt). Nmt has been implicated in a number of human diseases, including cancer and epilepsy, as well as in pathogenic mechanisms such as fungal and virus infections, including HIV and hepatitis B. Rational design has led to the development of potent competitive inhibitors, including several non-hydrolysable acyl-CoA substrate analogues. Linear synthetic strategies, following the route of the original CoA synthesis, however, generate such analogues in very low overall yields that typically are not sufficient for in vivo studies. Here we present a new, highly convergent synthesis of myristoyl-carba(dethia)-coenzyme A (**1**) that allows this substrate analogue to be obtained in a yield 11 times higher than that of the reported linear synthesis. In addition, enzymatic cleavage of the adenosine-2′,3′-cyclophosphate in the last step of the synthesis proved to be an efficient way to obtain the isomerically pure 3′-phosphate **1** free of the 2′-phosphate **13**.

Introduction

Post- or co-translational lipidation is required for the proper functioning of many proteins involved in signal transduction and cellular growth control.[1] The co-translational transfer of a myristoyl group from myristoyl-coenzyme A (myr-CoA) to an N-terminal glycine residue of a substrate protein is catalysed by the enzyme *N*-myristoyltransferase (Nmt; EC 2.1.3.97).[2] Nmt recognises the amino-terminal motif $G(X)_3(S/T/A/C/N)$ of the nascent peptide, after the initiating methionine has been removed by methionine aminopeptidase during translation. Known human *N*-myristoylated proteins include kinases such as the Src family kinases and the catalytic subunit of cAMP-dependent protein kinase, as well as phosphatases such as calcineurin.[3] Nmt has been implicated in human diseases such as colon cancer, in which *N*-myristoylation of the oncogene product p60*c-src* is required for its malign effect,[4] or epilepsy, in which myristoylation of calcineurin is involved in neuronal apoptosis.[5] Myristoylated proteins also play important roles in several pathogens such as *Cryptococcus neoformans*, the cause of a fungal infection,[6] or in viruses such as HIV-I,[7,8] polio virus[9] and hepatitis B virus.[10] The assembly of HIV infectious virions, for instance, is dependent on the myristoylation of gag, gag-pol and nef viral polyprotein precursors by cellular Nmt.[7] Nmt is thus regarded as a potential drug target for anticancer, antiepilepsy, antifungal and antiviral agents.

Inhibitors of Nmt have been reported (reviewed in ref.[11–13]) and include substrate analogues such as peptides, peptidomimetics and myr-CoA analogues, as well as several reported small molecules, including benzofurans and benzothiazole derivatives, showing promising antifungal activity in vivo.[14,15] Rational design has led to the development of several non-hydrolysable acyl-coenzyme A analogues as potent competitive inhibitors of Nmt, including *S*-(2-oxopentadecyl)-CoA,[16] *S*-(3-oxohexadecyl)-CoA and myristoyl-carba(dethia)-CoA.[17] One major drawback, however, is the limited amounts of such compounds accessible through existing synthetic strategies. Myristoyl-carba-(dethia)-CoA (**1**, Figure 1), in which the sulfur atom of the myr-CoA thioester bond is replaced by a methylene group, for example, was generated by a linear synthesis[17] that applies the route of Moffatt and Khorana's original coenzyme A synthesis.[18] The low overall yield of 1.7% (with regard to myristic acid) does not offer any easy means to obtain **1** or similar molecules in quantities necessary for in vivo studies. Here we present a novel and highly convergent strategy for easy synthesis of myristoyl-carba(dethia)-CoA (**1**) and other carba(dethia)-CoA derivatives as single isomers in high overall yields.

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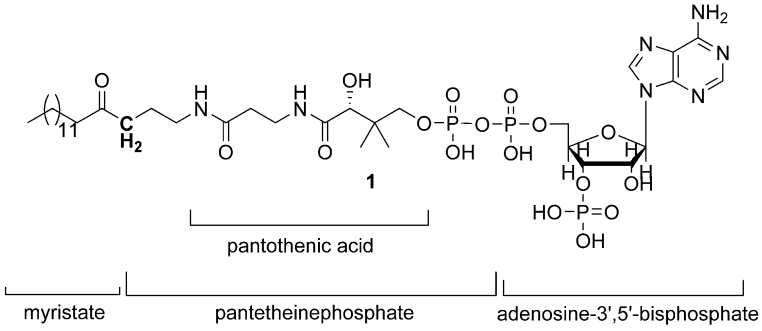
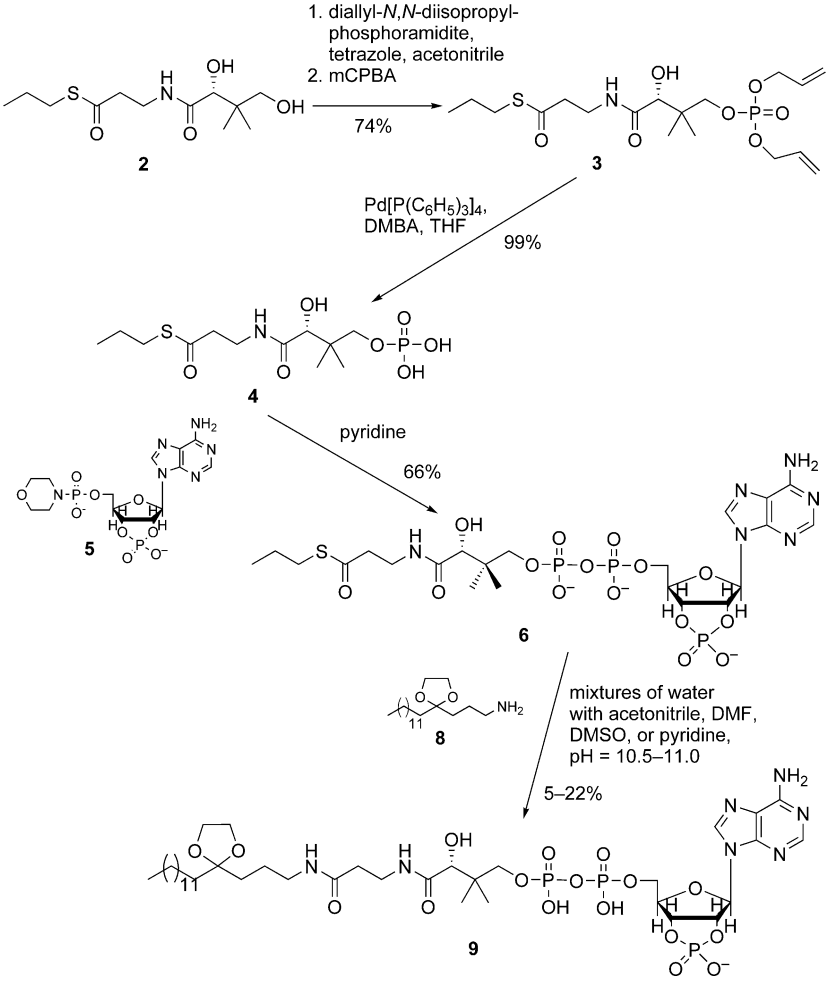


Figure 1. Myristoyl-carba(dethia)-CoA (**1**). The methylene group that replaces the sulfur atom of the thioester bond in myr-CoA is highlighted in bold.

Results and Discussion

Our goal of an efficient synthesis of myristoyl-carba-(dethia)-CoA (**1**) prompted us to explore more convergent synthetic opportunities to access this non-hydrolysable substrate analogue and potent inhibitor of Nmt. Because the modest overall yield of the linear synthesis[17] is primarily due to the very low levels of conversion during the phosphorylation reaction of myristoyl-carba(dethia)-pantetheine in the penultimate step (ca. 30% reported,[17] 10% in our hands), we sought a strategy to introduce the phosphate group earlier in the synthesis. In nature, the biosynthesis of coenzyme A starts with the phosphorylation of pantothenic acid (vitamin B_5).[19] Accordingly, Martin et al.[20] reported a method for the generation of acetyl-carba(dethia)-CoA, in which the pantothenic acid moiety is first phosphorylated, then coupled to adenosinephosphate, and in the last step coupled with the acetate moiety through aminolysis

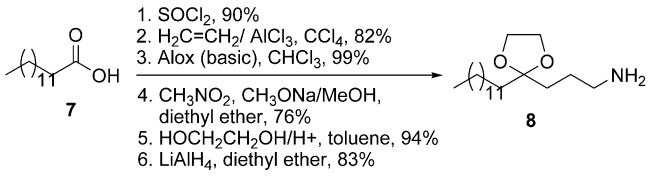


Scheme 1. Convergent strategy applied from Martin et al.'s acetyl-carba(dethia)-CoA synthesis,[20] involving an aminolysis reaction of amine **8** and thioester **6** in the penultimate step.

of the thioester-activated pantothenic acid. Inspired by the convergent strategy, we tested whether this route would also be suitable for effectively generating myristoyl-carba(dethia)-CoA (**1**).

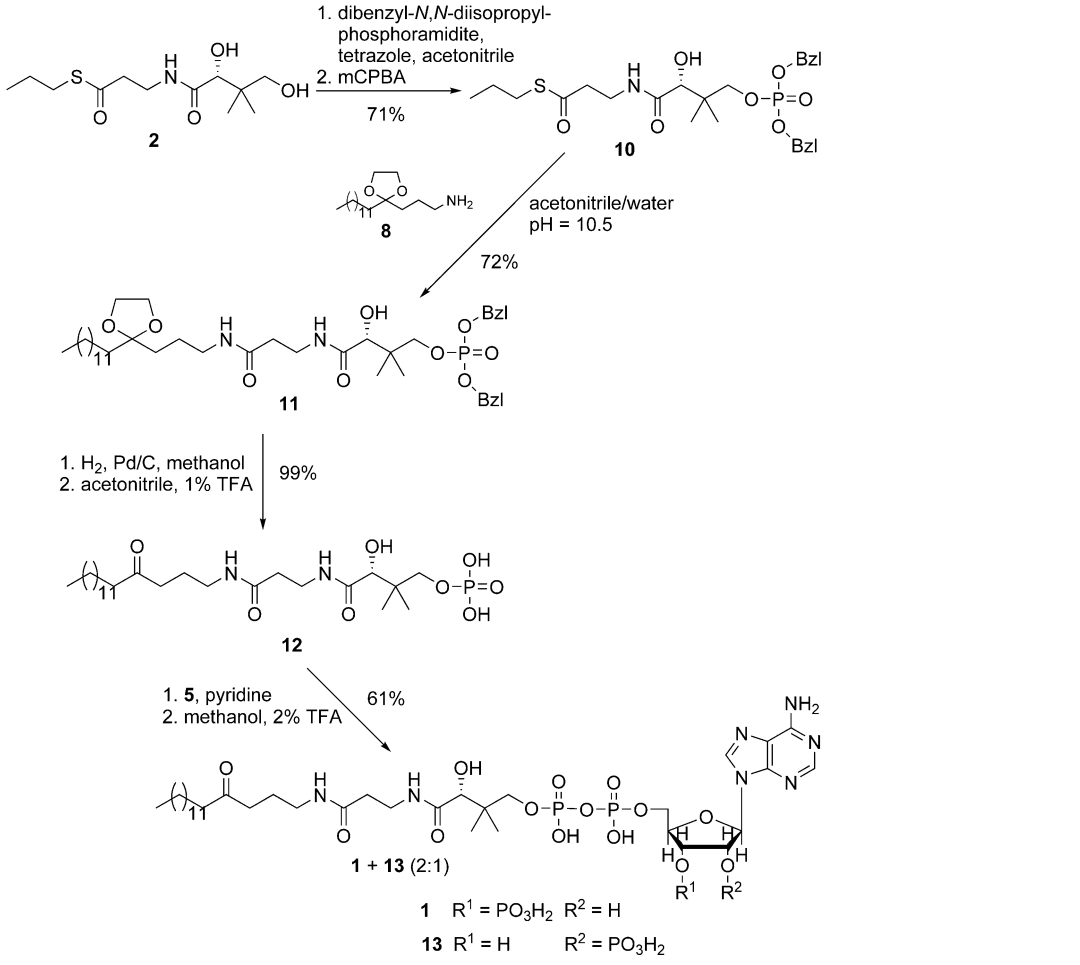
The *S*-propyl thiopantothenate (**2**) (Scheme 1) was synthesised as reported by Martin et al.[20] Unlike in that synthesis, however, phosphorylation of **2** was then accomplished by application of a phosphoramidite method based on P^{III} chemistry.[21] Deprotection of the allyl phosphate **3** afforded the phosphate **4** with an overall yield of 74% in the phosphorylation step, representing a significant improvement over the reported 17% obtained with dimethyl phosphorochloridate.[20] Compound **4** was then treated with the morpholidate **5**[22] in dry pyridine, with formation of a pyrophosphate bond to yield the thioester **6**.

The amine **8** was generated from myristic acid (**7**) as described,[22,23] with slight variations and yield improvements (Scheme 2 and Supporting Information). The subsequent aminolysis of **6** with **8** (Scheme 1) constituted the key reaction of this convergent strategy. Martin et al.[20] reported a 90% conversion to the amide in phosphate buffer at pH = 10.5 within 20 h to generate the acetyl-CoA analogue. In our case, however, finding a common solvent or solvent mixture for **6** and **8** turned out to be problematic. We used different mixtures of water/buffer with acetonitrile, DMF, DMSO or pyridine, at room temperature or 50 °C, with use of NaOH or DMAP to adjust the pH between 10.5 and 11. Although product **9** was always formed, yields were found to be extremely low (between 5 and 22%).



Scheme 2. Synthesis of amine **8**, starting from myristic acid (**7**).

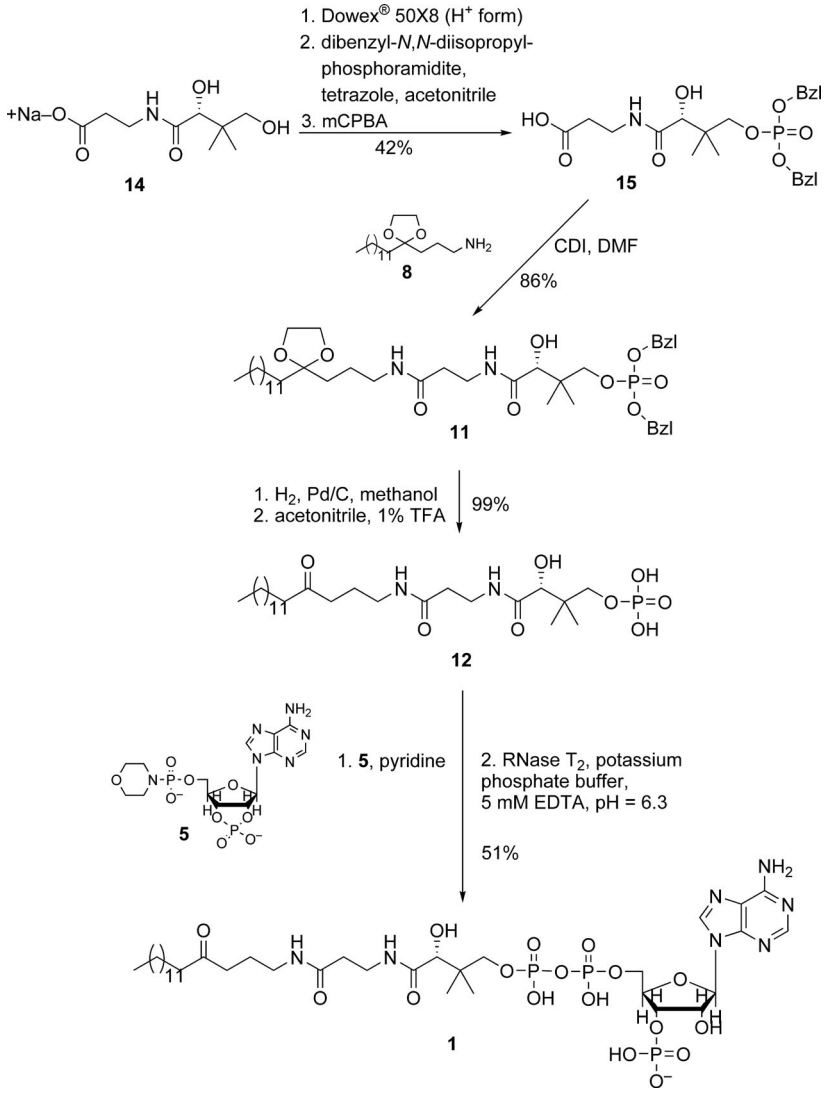
To overcome the solubility problems, we changed the synthetic strategy to that effect that the aminolysis reaction was carried out before formation of the pyrophosphate (Scheme 3). The thioester **10** was therefore generated and then used in the aminolysis reaction. Both **8** and **10** could readily be dissolved in acetonitrile/water at pH = 10.5. The reaction was monitored by HPLC, during which the pH



Scheme 3. Improved strategy with application of the aminolysis reaction earlier in the synthesis; acidic hydrolysis of the adenosine-2′,3′-cyclophosphate results in a mixture of the phosphate isomers **1** and **13**.

was kept at 10.5. After 4 d, the dibenzyl phosphate **11** was isolated in 72% yield. After cleavage of the benzyl groups and acidic hydrolysis of the ketal, **12** could be coupled with the morpholidate **5**. Subsequent acidic hydrolysis of the cyclophosphate yielded myristoyl-carba(dethia)-CoA (**1**) and its 2′-phosphate isomer myristoyl-carba(dethia)-isoCoA (**13**) in a ratio of 2:1, according to ^{1}H and ^{31}P NMR spectroscopy (Figures S1 and S2 in the Supporting Information). The overall yield of the final product **1**, with regard to myristic acid, was therefore 13%. However, attempts to separate the two isomers by ion-exchange chromatography with a DEAE-cellulose column as described for the coenzyme A synthesis,[18] or by use of Dowex® 1×8 (Cl^- form), were not successful, most probably because of the high surface activities of these compounds. Use of HPLC on a C18 reversed-phase column as described[17] was also not capable of separating the isomers, prompting us to search for an alternative strategy.

In addition to the failure to separate the two phosphate isomers, we also saw scope for improvement and simplification of the synthesis. The aminolysis conditions favoured by Martin et al. allowed the formation of the amide bond in the presence of the unprotected phosphate group. Because we had already modified the synthetic route such that a side reaction with the free phosphate group of the adenosine moiety was no longer a concern, we were now also able to use peptide chemistry to couple amine **8** with the pantothenic acid phosphate entity more efficiently (Scheme 4). In addition to the expected higher yields, relative to the aminolysis reaction, there was also no need for the two extra steps to generate the thioester. We first generated D-pantothenic acid 4′-dibenzyl phosphate (**15**) by applying the phosphoramidite method developed by Bannwarth and Trzeciak,[21] which had been shown to be superior to other phosphorylation strategies. Free D-pantothenic acid was obtained from its sodium salt **14** upon elu-



Scheme 4. Final route to generate isomerically pure myristoyl-carba(dethia)-CoA (**1**). The key intermediate **12** was obtained through the use of peptide chemistry to coupling of amine **8** with pantothenic acid phosphate **15**. The final step of the synthesis involves an enzymatic cleavage of the adenosine-2′,3′-cyclophosphate with RNase T_2, which exclusively generated the desired product **1**.

tion from a Dowex® 50×8 (H^+ form) column and removal of water by lyophilisation, followed by drying in high vacuum. Under water-free conditions, in the presence of the weak base tetrazole, the primary hydroxy group of D-pantothenic acid was selectively phosphitylated with dibenzyl-*N*,*N*-diisopropylphosphoramidite. Subsequent oxidation with *m*-chloroperbenzoic acid (mCPBA) led to the benzyl-ester-protected phosphate **15**. In the key reaction of this new synthetic strategy, the carboxy group of **15** was first activated with 1,1′-carbonyldiimidazole (CDI) to form the corresponding *N*,*N*-dimethylformamide. The amine **8** was then added, and the ketal-protected dibenzyl phosphate **11** was obtained in 86% yield (in comparison with 72% by aminolysis). Cleavage of the benzyl groups with hydrogen in the presence of palladium on charcoal, and acidic hydrolysis of the ketal were achieved quantitatively to yield the phosphate **12**. Adenosine-2′,3′-cyclophosphate 5′-phosphomorpholidate (**5**) was generated as reported[22] and was coupled with **12** in dry pyridine as described above.

Because attempts to separate the two phosphate isomers myristoyl-carba(dethia)-CoA (**1**) and myristoyl-carba(dethia)-isoCoA (**13**) had not been successful before, we sought a way to generate only the desired product **1**. Utilisation of the enzyme ribonuclease T_2 (RNase T_2) turned out to be an elegant solution, providing the pure isomer **1** through regioselective opening of the cyclophosphate. First used by Keep et al.,[24] RNase T_2 cleaves nucleoside-2′,3′-cyclophosphates specifically to afford the 3′-phosphates. The enzymatic reaction was performed in potassium phosphate buffer at 30 °C, and the pH was optimised to 6.3. Use of lower pH values as described in the original report[24] led to increased acidic hydrolysis, and consequently to formation of the 2′-phosphate isomer. After 24 h, over 90% (according to analytical HPLC) of the adenosine-2′,3′-cyclophosphate had been converted. The product was purified by preparative HPLC with use of a C18 reversed-phase column and an acetonitrile/potassium phosphate gradient. The desired myristoyl-carba(dethia)-CoA (**1**) was isolated in 51% yield with regard to phosphate **12**, and 19% with regard to myristic acid (**7**). More importantly, **1** was generated isomerically pure without any further need for separation from its 2′-phosphate isomer. ^1H and ^{31}P NMR spectroscopy (Figures S3 and S4 in the Supporting Information) clearly demonstrated the presence of only one isomer.

Conclusions

We have developed a new convergent synthesis that allows the potent Nmt inhibitor myristoyl-carba(dethia)-CoA (**1**) to be generated in a total of 14 steps and a very good overall yield of 19% (with regard to myristic acid). In relation to the linear synthesis, based on Moffatt's and Khorana's original method for generating coenzyme A,[18] this constitutes an 11-fold increase in yield, while the number of synthetic steps has been reduced by three. An effective new route to obtain the key intermediate myristoyl-carba(dethia)-pantethein-4′-phosphate (**12**), using peptide chemistry to link the readily accessible amine **8** and pantothenic acid phosphate **15**, together with the specific enzymatic cleavage of the adenosine-2′,3′-cyclophosphate with RNase T_2, resulted in a significant improvement over and simplification of existing methods. The use of peptide chemistry saved two synthetic steps relative to the modified aminolysis-based route[20] and should be easily applicable to the synthesis of any acyl-carba(dethia)-CoA analogue, regardless of the nature of the acyl group. Syntheses of analogues containing hydrophobic acyl groups should thus in particular benefit from our new strategy. Conveniently, the key reactant pantothenic acid phosphate can be easily obtained in good yield by application of the phosphoramidite method, which proved to be far superior to the reported phosphorylation of pantothenic acid with methyl phosphorochloridate.[20] Most importantly, the use of RNase T_2 in the last step of the synthesis exclusively generates the desired 3′-phosphate isomer, eliminating the need (and challenge) to separate the final product from its 2′-phosphate isomer.

Experimental Section

General: All reactions sensitive to air or moisture were carried out under argon in oven-dried glassware unless otherwise noted. Anhydrous solvents were purchased from Fluka and used without further drying. All other reagents were purchased from Acros Organics, Fluka, or Sigma-Aldrich, and were used as supplied unless otherwise stated. Analytical thin-layer chromatography (TLC) was performed by using Polygram Sil G/UV254 silica gel plates (Macherey-Nagel). For column chromatography, Merck silica gel 60 (40–63 μm) was used. Reversed-phase (RP) column chromatography was performed with an HP 1050 HPLC system with automated liquid sampler and diodearray detector (Hewlett-Packard). For analytical HPLC, a Grom column (250×4 mm) with Grom-Sil 100 ODS-0 AB (5 μm) was used. For preparative HPLC, a Macherey-Nagel column (250×20 mm) with Nucleosil 100 C18 (7 μm) was used. ^1H and ^{13}C NMR spectra were recorded with Bruker AM 400 or Bruker DRX 500 spectrometers. Chemical shifts are reported in ppm from tetramethylsilane with reference to internal residual solvent. The following abbreviations are used to designate the signal multiplicities: s = singlet, d = doublet, dd = doublet of doublet, t = triplet, and m = multiplet. Coupling constants are reported in Hertz (Hz). High-resolution mass spectrometry (HRMS-EI and HRMS-FAB) and MS-FAB spectra were were carried ot with a Finnigan MAT 90 spectrometer. The relative intensities (in %) are reported in reference to the base peak. Melting points were obtained with a Büchi B-535 apparatus.

***S*-Propyl 3-{(2*R*)-4-[Bis(allyloxy)phosphoryloxy]-2-hydroxy-3,3-dimethylbutyrylamino}thiopropionate (3):** Compound **2** (540 mg, 1.95 mmol) and diallyl-*N*,*N*-diisopropylphosphoramidite (514 μL, 1.95 mmol) were dissolved in dry acetonitrile (5 mL) under an inert gas in a round flask (100 mL) fitted with a septum. A tetrazole solution in acetonitrile (0.45 M, 4.33 mL, 1.95 mmol) was added, and the mixture was stirred at room temperature for 5 min. mCPBA (722 mg of a mixture of 70% mCPBA, 10% 3-chlorobenzoic acid and 20% water, 505 mg, 2.92 mmol) was then added, and the solution was stirred at room temperature for 2 h. After evaporation of the solvent and drying in high vacuum, the product was purified by silica gel flash chromatography [cyclohexane/ethyl acetate, 10:1, 2:1, 1:1, 1:5, 0:1; R_f(cyclohexane/ethyl acetate 1:1) = 0.06]. Compound **3** was obtained as a colourless oil. Yield: 629 mg (74%). ^1H NMR (CD_3OD, 500 MHz): δ = 0.95 (s, 3 H, -C-CH_3),

0.97 (t, J = 7.4 Hz, 3 H, CH_3-CH_2-), 1.01 (s, 3 H, -C-CH_3), 1.59 (m, 2 H, CH_3-CH_2-), 2.82 (t, J = 6.7 Hz, 2 H, -SCO-CH_2-), 2.87 (m, 2 H, -CH_2-SCO-), 3.50 (m, 2 H, CH_2-NHCO-), 3.86 (s, 1 H, -C*H*OH-), 3.96 (ABX, J_1 = 9.3, J_2 = 4.5 Hz, 2 H, -CH_2-OH), 4.58 (m, 4 H, 2 -CH_2-CH=CH_2), 5.34 (ABX, 4 H, 2 -CH_2-CH=CH_2), 5.99 (m, 2 H, 2 -CH_2-CH=CH_2) ppm. ^{31}P NMR (CD_3OD, 500 MHz): δ = 0.05 (1 P) ppm. HRMS (70 eV): calcd. 437.1637; found 437.1632.

***S*-Propyl 3-[(2*R*)-2-Hydroxy-3,3-dimethyl-4-phosphonooxybutyryl-amino]thiopropionate (4):** Compound **3** (630 mg, 1.44 mmol), $Pd[P(C_6H_5)_3]_4$ (167 mg, 5 mol-% per allyl group) and dimethylbarbituric acid (225 mg, 1.46 mmol) were dissolved in dry THF (7 mL) under an inert gas in a round flask (50 mL). After the mixture had been stirred at room temperature overnight, the solvent was evaporated, and the residue was dried in high vacuum, after which the product was purified by preparative HPLC on a C18 reversed-phase column with use of a water/acetonitrile gradient (A: H_2O + 0.1% TFA; B: acetonitrile + 0.1% TFA). Compound **4** eluted between 25% and 35% B. Yield: 505 mg (98%). ^{1}H NMR (CD_3OD, 500 MHz): δ = 0.94 (s, 3 H, -C-CH_3), 0.96 (t, J = 7.4 Hz, 3 H, CH_3-CH_2-), 1.00 (s, 3 H, -C-CH_3), 1.59 (m, 2 H, CH_3-CH_2-), 2.82 (t, J = 6.7 Hz, 2 H, -SCO-CH_2-), 2.87 (m, 2 H, -CH_2-SCO-), 3.50 (m, 2 H, CH_2-NHCO-), 3.84 (ABX, J_1 = 9.5, J_2 = 5.1 Hz, 2 H, -CH_2-OH), 3.91 (s, 1 H, -C*H*OH-) ppm. ^{31}P NMR (CD_3OD, 500 MHz): δ = 1.58 (1 P) ppm. MS-FAB (glycerol): m/z (%) = 358.2 (92) $[M + H]^+$, 282.1 (2.5) $[M - C_3H_7S]^+$, 260.2 (100) $[M - H_2PO_4]^+$.

***S*-Propyl 4′-(Adenosine-2′,3′-cyclophosphate-5′-pyrophosphate)thiopantothenate (6):** The bis(*N*,*N*′-dicyclohexyl-4-morpholinecarboxamidium) salt of adenosine-2′,3′-cyclophosphate-5′-phosphomorpholidate (**5**, 462 mg, 0.373 mmol) and compound **4** (134 mg, 0.373 mmol) were each repeatedly dissolved in dry pyridine (5 mL), concentrated through evaporation and dried in high vacuum. The reactants were then transferred to a round flask (100 mL) and dissolved in dry pyridine (5 mL), before being stirred under an inert gas at room temperature for 2 d. After evaporation of the solvent, the residue was washed with diethyl ether and dried in high vacuum. The product was then purified by preparative HPLC on a C18 reversed-phase column with a potassium phosphate buffer/acetonitrile gradient [A: 10 mM KPH (pH = 6.0); B: acetonitrile/10 mM KPH (pH = 6.0), 80:20]. Compound **6** eluted between 20 and 40% B. After concentration of the eluate by lyophilisation, buffer salts were removed by HPLC on a C18 reversed-phase column with use of a water/acetonitrile gradient. Compound **6** was obtained as its tripotassium salt. Yield: 213 mg (66%). ^{1}H NMR (D_2O, 500 MHz): δ = 0.63 (s, 3 H, -C-CH_3), 0.74 (s, 3 H, -C-CH_3), 0.77 (t, J = 7.4 Hz, 3 H, CH_3-CH_2-), 1.39 (m, 2 H, CH_3-CH_2-), 2.69 (m, 4 H, -CH_2-SCO-CH_2-), 3.38 (m, 2 H, CH_2-NHCO-), 3.56 (ABX, J_1 = 9.8, J_2 = 4.8 Hz, 2 H, -CH_2-OH), 3.88 (s, 1 H, -C*H*OH-), 4.16 (m, 2 H, 5′-position ribose), 4.53 (m, 1 H, 4′-position ribose), 5.14 (m, 1 H, 3′-position ribose), 5.33 (m, 1 H, 2′-position ribose), 6.24 (d, J = 4.1 Hz, 1 H, 1′-position ribose), 8.16 (s, 1 H, adenine ring C-2 position), 8.34 (s, 1 H, adenine ring C-8 position) ppm. ^{31}P NMR (D_2O, 500 MHz): δ = –10.67 to –9.90 (2 P, pyrophosphate), 20.41 (1 P, cyclophosphate) ppm. MS-FAB (glycerol): m/z (%) = 862.9 (44) $[M + 3 K - 2 H]^+$, 825.0 (100), $[M + 2 K - H]^+$, 787.0 (33) $[M + K]^+$, 749.3 (0.2) $[M + H]^+$, 748.0 (0.08) $[M]^+$.

Adenosine-2′,3′-cyclophosphate of 2-Methyl-2-tridecyl-1,3-dioxolan-dethio-CoA (9): Compounds **6** (66 mg, 0.076 mmol) and **8** (24 mg, 0.076 mmol) were heated to 50 °C in pyridine (2 mL) in a round flask (25 mL) and dissolved completely by brief sonication with a sonicator bath. The pH was then adjusted to 11.0 with NaOH solution (100 mM), and the mixture was stirred at room temperature for 4 d. During this time, the pH value was checked several times and adjusted to 11.0. The solution was then neutralised with HCl (1 M), and the solvent was removed by lyophilisation. The product was purified by preparative HPLC on a C18 reversed-phase column with a potassium phosphate buffer/acetonitrile gradient [A: 10 mM KPH (pH = 6.0); B: acetonitrile/10 mM KPH (pH = 6.0), 80:20]. Compound **9** eluted between 60 and 70% B. After concentration of the eluate by lyophilisation, buffer salts were removed by HPLC on a C18 reversed-phase column with a water/acetonitrile gradient, and **9** was obtained as its potassium salt. Yield: 18 mg (22%). ^{1}H NMR (D_2O, 500 MHz): δ = 0.74 (s, 3 H, -C-CH_3), 0.83 (t, J = 6.9 Hz, 3 H, CH_3-CH_2-), 0.88 (s, 3 H, -C-CH_3), 1.19 [s, 22 H, -$(CH_2)_{11}$-], 1.48 [m, 2 H, -C$(OCH_2)_2$-CH_2-CH_2-], 1.59 [m, 4 H, -CH_2-C$(OCH_2)_2$-CH_2-], 2.44 (m, 2 H, -NHCO-CH_2-), 3.12 (m, 2 H, CH_2-NHCO-), 3.45 (m, 2 H, -NHCO-CH_2-CH_2-), 3.67 (ABX, J_1 = 9.4, J_2 = 4.9 Hz, 2 H, -CH_2-OH), 3.96 [s, 4 H, -C$(OCH_2)_2$-], 4.00 (s, 1 H, -C*H*OH-), 4.25 (m, 2 H, 5′-position ribose), 4.62 (m, 1 H, 4′-position ribose), 5.23 (m, 1 H, 3′-position ribose), 5.40 (m, 1 H, 2′-position ribose), 6.33 (d, J = 4.1 Hz, 1 H, 1′-position ribose), 8.25 (s, 1 H, adenine ring C-2 position), 8.45 (s, 1 H, adenine ring C-8 position) ppm. ^{31}P NMR (D_2O, 500 MHz): δ = –11.18 to –10.41 (2 P, pyrophosphate), 20.13 (1 P, cyclophosphate) ppm. MS-FAB (glycerol): m/z (%) = 1068.9 (91), 1051.1 (20) [M + 3 Na – 3 H]$^+$, 1025.3 (100) $[M + K + H]^+$, 1007.4 (26) [M + Na – H]$^+$, 986.4 (1.5) $[M + H]^+$, 985.4 (4) $[M]^+$, 981.4 (94), 937.4 (77).

***S*-Propyl 3-{(2*R*)-4-[Bis(benzyloxy)phosphoryloxy]-2-hydroxy-3,3-dimethylbutyrylamino}thiopropionate (10):** Compound **2** (540 mg, 1.95 mmol) and dibenzyl-*N*,*N*-diisopropylphosphoramidite (641 μL, 1.95 mmol) were dissolved in dry acetonitrile (5 mL) under an inert gas in a round flask (100 mL) with a septum. A tetrazole solution in acetonitrile (0.45 M, 4.33 mL, 1.95 mmol) was added, and the mixture was stirred at room temperature for 5 min. mCPBA (722 mg of a mixture of 70% mCPBA, 10% 3-chlorobenzoic acid and 20% water, 505 mg, 2.92 mmol) was then added, and the solution was stirred at room temperature for 2 h. After evaporation of the solvent and drying in vacuo, the product was purified by silica gel flash chromatography [cyclohexane/ethyl acetate, 10:1, 2:1, 1:1, 1:5, 0:1; R_f(cyclohexane/ethyl acetate, 1:1) = 0.015]. Compound **10** was obtained as a colourless oil. Yield: 748 mg (71%). ^{1}H NMR ($CDCl_3$, 250 MHz): δ = 0.77 (s, 3 H, -C-CH_3), 0.95 (t, J = 7.4 Hz, 3 H, CH_3-CH_2-), 1.06 (s, 3 H, -C-CH_3), 1.58 (m, 2 H, CH_3-CH_2-), 2.78 (t, J = 6.3 Hz, 2 H, -SCO-CH_2-), 2.84 (m, 2 H, -CH_2-SCO-), 3.54 (m, 2 H, -CH_2-NHCO-), 3.80 (ABX, J_1 = 8.1, J_2 = 10.1 Hz, 2 H, -CH_2-O-), 3.91 (s, 1 H, -C*H*OH-), 5.03 (m, 4 H, 2 -CH_2-C_6H_5), 7.33 (m, 10 H, 2 -C_6H_5) ppm. ^{31}P NMR ($CDCl_3$, 500 MHz): δ = 1.04 (1 P) ppm. MS-FAB (3-NBA): m/z (%) = 560.2 (6) $[M + Na]^+$, 538.2 (28) $[M + H]^+$, 260.2 (56) [M – dibenzyl phosphate], 180.3 (100) [M – dibenzyl phosphate – {C(O)-$SCH_2CH_2CH_3$} + Na]$^+$.

Dibenzyl (3*R*)-3-Hydroxy-2,2-dimethyl-4-oxo-4-{3-oxo-3-[3-(2-tridecyl-1,3-dioxolan-2-yl)propylamino]propylamino}butyl Phosphate (11) by Aminolysis: Compounds **10** (100 mg, 0.186 mmol) and **8** (58 mg, 0.186 mmol) were dissolved in acetonitrile/water (1:1, 6 mL) in a round flask (25 mL). The pH was adjusted to 11.0 with an NaOH solution (100 mM), and the mixture was stirred at room temperature for 4 d. During this time, the pH value was checked several times and adjusted to 11.0. The solution was then neutralised with HCl (1 M), and the solvent was removed by lyophilisation. The product was purified by preparative HPLC on a C18 reversed-phase column with a water/acetonitrile gradient (A: H_2O; B: acetonitrile). Compound **11** eluted between 94 and 100% B. After concentration of the eluate by lyophilisation, compound **11** was ob-

tained as a white solid. Yield: 103 mg (72%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 0.80 (s, 3 H, CH_3- pantetheine), 0.87 (t, J = 7.0 Hz, 3 H, CH_3-CH_2-), 1.05 (s, 3 H, CH_3- pantetheine), 1.24 [s, 20 H, -$(CH_2)_{10}$-], 1.29 [m, 2 H, -CH_2-CH_2-$C(OCH_2)_2$-], 1.55 [m, 4 H, -CH_2-$C(OCH_2)_2$-CH_2-CH_2-], 1.62 [m, 2 H, -$C(OCH_2)_2$-CH_2-], 2.41 (m, 2 H, -NHCO-CH_2-), 3.21 (m, 2 H, -NHCO-CH_2-CH_2-), 3.55 [m, 2 H, -$C(OCH_2)_2$-CH_2-CH_2-CH_2-], 3.78 (ABX, J_1 = 10.1, J_2 = 7.4 Hz, 2 H, -CH_2-O-), 3.88 (s, 1 H, -C*H*OH-), 3.90 [s, 4 H, -$C(OCH_2)_2$-], 5.03 (m, 4 H, 2 -CH_2-C_6H_5), 6.21 (m, 1 H, -NH-), 7.28 (m, 1 H, -NH-), 7.34 (m, 10 H, 2 -C_6H_5) ppm. ^{31}P NMR ($CDCl_3$, 500 MHz): δ = 0.72 (1 P) ppm. MS-FAB (3-NBA): *m/z* (%) = 813.4 (2.5) $[M + K]^+$, 799.4 (11), 798.4 (45), 797.4 (100) $[M + Na]^+$, 776.4 (25), 775.4 (55) $[M + H]^+$, 774.4 (3) $[M]^+$.

(3*R*)-3-Hydroxy-2,2-dimethyl-4-oxo-4-[3-oxo-3-(4-oxoheptadecylamino)propylamino]butyl Dihydrogen Phosphate (12): Compound **11** (100 mg, 0.129 mmol) and palladium black (10%, ca. 10 mg) were added to dry methanol (5 mL) in a two-necked flask (50 mL) fitted with septum and stopcock. After purging of the flask several times with argon and then with hydrogen, the reaction mixture was stirred under hydrogen at room temperature overnight. The catalyst was then removed by filtration, and the solvent was evaporated. Remaining benzyl alcohol was removed in high vacuum. For hydrolysis of the ketal protecting group, the colourless oil was dissolved in ethanol (80%) and applied to a column (25 × 300 mm) with Dowex® 50×8 (H^+ form). The product was eluted with ethanol (80%). The eluate was then concentrated, and the residue was dissolved in ethanol (10 mL) and, after addition of toluene (2 mL), concentrated. After drying in high vacuum, compound **12** was obtained as a colourless oil. Yield: 76 mg (99%). ^{1}H NMR ([D_6]-DMSO, 500 MHz): δ = 0.78 (s, 3 H, CH_3- pantetheine), 0.83 (t, J = 6.9 Hz, 3 H, CH_3-), 0.86 (s, 3 H, CH_3- pantetheine), 1.21 [s, 20 H, -$(CH_2)_{10}$-], 1.41 (m, 2 H, -CH_2-CH_2-CO-), 1.54 (m, 2 H, -CO-CH_2-CH_2-), 2.22 (m, 2 H, -NHCO-CH_2-), 2.36 (m, 4 H, -CH_2-CO-CH_2-), 2.95 (m, 2 H, -CO-CH_2-CH_2-CH_2-), 3.25 (m, 2 H, -NHCO-CH_2-CH_2-), 3.63 (ABX, J_1 = 9.7, J_2 = 5.8 Hz, 2 H, -CH_2-O-), 3.67 (s, 1 H, -C*H*OH-), 7.70 (t, J = 5.8 Hz, 1 H, -NH-), 7.83 (t, J = 6.8 Hz, 1 H, -NH-) ppm. ^{13}C NMR ([D_6]DMSO, 500 MHz): δ = 14.38 (CH_3-CH_2), 19.91 (CH_3-), 21.23 (CH_3-), 22.54 (CH_2), 23.66 (CH_2), 23.76 (CH_2), 29.06 (CH_2), 29.15 (CH_2), 29.33 (CH_2), 29.36 (CH_2), 29.45 (4 CH_2), 31.74 (CH_2), 35.29 (CH_2), 35.56 (CH_2), 38.29 (CH_2), 39.03 [-$C(CH_3)_2$-], 39.55 (CH_2), 42.32 (CH_2), 71.65 (CH_2), 74.32 [-CH(OH)-], 170.84 (-HN-CO-), 172.53 (-HN-CO-), 210.56 (-CO-) ppm. ^{31}P NMR ([D_6]DMSO, 500 MHz): δ = 0.29 (1 P) ppm. MS-FAB (3-NBA): *m/z* (%) = 596.4 (8) $[M + 2 Na]^+$, 595.4 (22), 589.4 (5) $[M + K]^+$, 575.4 (9), 574.4 (34), 573.4 (100) $[M + Na]^+$, 571.4 (9), 551.4 (13) $[M + H]^+$, 550.4 (1) $[M]^+$.

Myristoyl-carba(dethia)-CoA (1) and Myristoyl-carba(dethia)-iso-CoA (13), 2′-Phosphate Isomer: To remove traces of water, compounds **5** (130 mg, 0.105 mmol) and **12** (36 mg, 0.065 mmol) were dissolved in dry pyridine (5 mL) and the mixtures concentrated three times. After drying in high vacuum, the reactants were dissolved in dry pyridine (5 mL) and stirred at room temperature under argon for 2 d. After evaporation of the solvent, the residue was washed with diethyl ether and dried in high vacuum. The residue was purified by preparative HPLC on a C18 reversed-phase column with a potassium phosphate buffer/acetonitrile gradient [A: 10 mM potassium phosphate (pH = 6.0); B: acetonitrile/10 mM potassium phosphate (pH = 6.0), 80:20]. The adenosine-2′,3′-cyclophosphate eluted between 55 and 70% buffer B. After concentration of the eluate by lyophilisation, the residue was stirred in methanol with TFA (2%) at room temperature overnight to open the cyclophosphate. Preparative HPLC on a C18 reversed-phase column with a water/acetonitrile gradient yielded a mixture of the two isomers **1** and **13** as a white solid. Yield: 38 mg (61%). ^{1}H NMR (CD_3OD, 500 MHz): δ = 0.83 (s, 3 H, CH_3- pantetheine), 0.89 (t, J = 7.1 Hz, 3 H, CH_3-CH_2-), 1.06 (s, 3 H, CH_3- pantetheine), 1.27 [s, 20 H, -$(CH_2)_{10}$-], 1.52 (m, 2 H, -CH_2-CH_2-CO-), 1.70 (m, 2 H, -CO-CH_2-CH_2-), 2.44 (m, 6 H, -NHCO-CH_2-, -CH_2-CO-CH_2-), 3.13 (m, 2 H, -CO-CH_2-CH_2-CH_2-), 3.45 (m, 2 H, -NHCO-CH_2-CH_2-), 3.78 (ABX, J_1 = 9.7, J_2 = 4.8 Hz, 2 H, -CH_2-O-), 4.07 (s, 1 H, -C*H*OH-), 4.21 (m, 2 H, ribose 5′-position, 2′-isomer), 4.27 (m, 2 H, ribose 5′-position, 3′-isomer), 4.49 (m, 1 H, ribose 4′-position, 2′-isomer), 4.62 (m, 1 H, ribose 4′-position, 3′-isomer), 4.72 (m, 1 H, ribose 3′-position, 2′-isomer), 5.15 (m, 1 H, ribose 2′-position, 2′-isomer), 6.13 (d, J = 5.6 Hz, 1 H, ribose 1′-position, 3′-isomer), 6.28 (d, J = 5.6 Hz, 1 H, ribose 1′-position, 2′-isomer), 8.19 (s, 1 H, adenine C-2 position), 8.57 (s, 1 H, adenine C-8 position) ppm. ^{31}P NMR (CD_3OD, 500 MHz): δ = –9.26 to –8.67 (m, 2 P, pyrophosphate), 1.91/2.18 (s, 1 P, 2′-/3′-position) ppm.

3-[4-(Bis-benzyloxy-phosphoryloxy)-2(*R*)-hydroxy-3,3-dimethyl-butyrylamino]propionate (15): D-Pantothenic acid sodium salt (**14**, 1 g, 4.15 mmol) was dissolved in water and applied to a column (25 × 300 mm) with Dowex® 50X8 (H^+ form). The free acid was eluted with water (250 mL), and the water was removed by lyophilisation. The residue was dried in high vacuum. The free acid (900 mg, 4.11 mmol) and dibenzyl-*N*,*N*-diisopropylphosphoramidite (1.352 mL, 4.11 mmol) were dissolved in dry acetonitrile (5 mL) under inert gas in a round flask (100 mL) with septum. A tetrazole solution in acetonitrile (0.45 M, 9.133 mL, 4.11 mmol) was added, and the solution was stirred for 15 min at room temperature. mCPBA (1.52 g of a mixture of 70% mCPBA, 10% 3-chlorobenzoic acid and 20% water, 1.064 g, 6.16 mmol) was then added, and the mixture was stirred for another 2 h at room temperature. The solvent was evaporated, and the residue was purified by silica gel flash chromatography (ethyl acetate, methanol). Compound **15** was obtained as a colourless oil. Yield: 836 mg (42%). ^{1}H NMR (CD_3OD, 500 MHz): δ = 0.89 (s, 3 H, -C-CH_3), 0.95 (s, 3 H, -C-CH_3), 2.51 (t, J = 6.6 Hz, 2 H, HOOC-CH_2-), 3.46 (m, 2 H, HOOC-CH_2-CH_2-), 3.84 (s, 1 H, -C*H*OH-), 3.92 (ABX, J_1 = 9.4, J_2 = 4.5 Hz, 2 H, -CH_2-O-), 5.04 (s, 2 H, -CH_2-C_6H_5), 5.06 (s, 2 H, -CH_2-C_6H_5), 7.36 (m, 10 H, 2 -C_6H_5) ppm. ^{13}C NMR (CD_3OD, 500 MHz): δ = 18.64 (CH_3-), 19.91 (CH_3-), 33.31 (HOOC-CH_2-), 34.38 (HOOC-CH_2-CH_2-), 38.65 [-$C(CH_3)_2$-], 69.34 (-CH_2-C_6H_5), 69.38 (-CH_2-C_6H_5), 73.41 (-CH_2-O-), 73.95 (-CHOH-), 127.75 (4 aromat. tert.), 128.27 (6 aromat. tert.), 135.81 (2 aromat. quart.), 173.80 (-NHCO-), 174.06 (HOOC-) ppm. ^{31}P NMR (CD_3OD, 500 MHz): δ = 0.16 (1 P) ppm. MS-FAB (3-NBA): *m/z* (%) = 504.2 (3), 503.2 (20) $[M + H + Na]^+$, 502.2 (59) $[M + Na]^+$, 482.2 (8), 481.2 (28), 480.2 (100) $[M + H]^+$, 479.3 (3) $[M]^+$.

Dibenzyl (3*R*)-3-Hydroxy-2,2-dimethyl-4-oxo-4-{3-oxo-3-[3-(2-tridecyl-1,3-dioxolan-2-yl)propylamino]propylamino}butyl Phosphate (11) by Peptide Chemistry: Compound **15** (150 mg, 0.312 mmol) was dissolved in dry DMF (6 mL) under an inert gas in a round flask (50 mL) fitted with septum and bubble counter and heated to 40 °C. CDI (100 mg, 0.624 mmol) in dry DMF (4 mL) was added, and the mixture was stirred for 90 min, until gas formation had stopped. The amine **8** (98 mg, 0.312 mmol), dissolved in dry DMF (3 mL), was then added, and the reaction mixture was stirred at 40 °C overnight. The solvent was evaporated, and the residue was purified by preparative HPLC on a C18 reversed-phase column with a water/acetonitrile gradient (A: H_2O; B: acetonitrile). Compound **11** eluted between 94 and 100% B. After concentration of the eluate by lyophilisation, compound **11** was obtained as a white solid. Yield: 208 mg (86%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 0.80 (s, 3 H, CH_3- pantetheine), 0.87 (t, J = 7.0 Hz, 3 H, CH_3-CH_2-), 1.05 (s, 3 H, CH_3- pantetheine), 1.24 [s, 20 H, -$(CH_2)_{10}$-], 1.29 [m,

2 H, -CH_2-CH_2-$C(OCH_2)_2$-], 1.55 [m, 4 H, -CH_2-$C(OCH_2)_2$-CH_2-CH_2-], 1.62 [m, 2 H, -$C(OCH_2)_2$-CH_2-], 2.41 (m, 2 H, -NHCO-CH_2-), 3.21 (m, 2 H, -NHCO-CH_2-CH_2-), 3.55 [m, 2 H, -$C(OCH_2)_2$-CH_2-CH_2-CH_2-], 3.78 (ABX, J_1 = 10.1, J_2 = 7.4 Hz, 2 H, -CH_2-O-), 3.88 (s, 1 H, -C*H*OH-), 3.90 [s, 4 H, -$C(OCH_2)_2$-], 5.03 (m, 4 H, 2 -CH_2-C_6H_5) 6.21 (m, 1 H, -NH-), 7.28 (m, 1 H, -NH-), 7.34 (m, 10 H, 2 -C_6H_5) ppm. ^{31}P NMR ($CDCl_3$, 500 MHz): δ = 0.72 (1 P) ppm. MS-FAB (3-NBA): *m*/*z* (%) = 813.4 (2.5) [M + K]$^+$, 799.4 (11), 798.4 (45), 797.4 (100) [M + Na]$^+$, 776.4 (25), 775.4 (55) [M$^+$+H]$^+$, 774.4 (3) [M]$^+$.

Myristoyl-carba(dethia)-CoA (1) by Enzymatic Cleavage of Cyclophosphate: To remove traces of water, compounds **5** (130 mg, 0.105 mmol) and **12** (36 mg, 0.065 mmol) were dissolved in dry pyridine (5 mL) and the mixtures concentrated three times. After drying in high vacuum, the reactants were dissolved in dry pyridine (5 mL) under argon and stirred at room temperature for 2 d. After evaporation of the solvent, the residue was washed with diethyl ether and dried in high vacuum. The residue was purified by preparative HPLC on a C18 reversed-phase column with a potassium phosphate buffer/acetonitrile gradient [A: 10 mM potassium phosphate (pH = 6.0); B: acetonitrile/10 mM potassium phosphate (pH = 6.0), 80:20]. The adenosine-2′,3′-cyclophosphate eluted between 55 and 70% buffer B. After concentration of the eluate by lyophilisation, the residue was dissolved in EDTA/water (5 mM, 1 mL), the pH was adjusted to 6.3, and ribonuclease T_2 (150 U) was added. The solution was incubated at 30 °C for 24 h, and the reaction was monitored by analytical HPLC. The enzyme was then denatured by a short incubation at 80 °C and could be removed by centrifugation. After sterile filtration, the mixture was separated by preparative HPLC on a C18 reversed-phase column with a potassium phosphate/acetonitrile gradient [A: 10 mM potassium phosphate (pH = 6.0); B: acetonitrile/potassium phosphate, 80:20]. Product **1** eluted between 44 and 49% B within 24 min. The combined product fractions were concentrated by lyophilisation, and buffer salts were removed by preparative HPLC on a C18 reversed-phase column with a water/acetonitrile gradient (A: H_2O; B: acetonitrile). After lyophilisation and drying in high vacuum, the potassium salt of **1** was obtained as a white solid. Yield: 33 mg (51%). ^{1}H NMR (CD_3OD, 500 MHz): δ = 0.86 (s, 3 H, CH_3- pantetheine), 0.89 (t, J = 7.0 Hz, 3 H, CH_3-CH_2-), 1.06 (s, 3 H, CH_3- pantetheine), 1.27 [s, 20 H, -$(CH_2)_{10}$-], 1.52 (m, 2 H, -CH_2-CH_2-CO-), 1.70 (m, 2 H, -CO-CH_2-CH_2-), 2.44 (m, 6 H, -NHCO-CH_2-, -CH_2-CO-CH_2-), 3.13 (m, 2 H, -CO-CH_2-CH_2-CH_2-), 3.45 (m, 2 H, -NHCO-CH_2-CH_2-), 3.81 (ABX, J_1 = 9.4, J_2 = 4.5 Hz, 2 H, -CH_2-O-), 4.06 (s, 1 H, -C*H*OH-), 4.35 (m, 2 H, ribose 5′-position), 4.50 (m, 1 H, ribose 4′-position), 4.75 (m, 1 H, ribose 2′-position), 6.09 (d, J = 5.2 Hz, 1 H, ribose 1′-position), 8.26 (s, 1 H, adenine C-2 position), 8.68 (s, 1 H, adenine C-8 position) ppm. ^{13}C NMR (CD_3OD, 500 MHz): δ = 13.45 (CH_3-CH_2), 21.15 (CH_3-), 22.74 (CH_2), 23.20 (CH_3-), 23.50 (CH_2), 23.85 (CH_2), 23.86 (CH_2), 29.33 (CH_2), 29.49 (CH_2), 29.60 (CH_2), 29.65 (CH_2), 29.67 (CH), 29.75 (CH_2), 29.76 (CH_2), 29.78 (CH_2), 29.80 (CH_2), 32.08 (CH_2), 35.48 (CH_2), 35.70 (CH_2), 38.73 (CH_2), 39.12 [-$C(CH_3)_2$-], 39.54 (CH_2), 42.53 (CH_2), 72.36 (CH_2), 74.09 [-CH(OH)-], 74.96 (CH), 83.28 (CH), 88.41 (CH), 88.43 (C), 110.00 (C), 172.82 (-HN-CO-), 174.61 (-HN-CO-), 212.31 (-CO-) ppm. ^{31}P NMR (CD_3OD, 500 MHz): δ = –9.57 (m, 1 P, pyrophosphate), –9.25 (m, 1 P, pyrophosphate), 1.41 (s, 1 P, 3′-position ribose) ppm. MS-FAB (glycerol): *m*/*z* (%) = 1035.9 (100) [M – 2 H + 2 K]$^+$, 998.3 (88) [M + K]$^+$, 959.3 (8) [M]$^+$. HRMS-FAB (glycerol): calcd. for $C_{36}H_{64}N_7O_{17}P_3K$ 998.3209; found 998.3249.

Supporting Information (see footnote on the first page of this article): Figures S1–S4, showing ^{1}H NMR and ^{31}P NMR spectra of the isomeric mixture of myristoyl-carba(dethia)-CoA (**1**) and myristoyl-carba(dethia)-isoCoA (**13**) and of the pure isomer myristoyl-carba(dethia)-CoA (**1**); details for the synthesis of 3-(2-tridecyl-1,3-dioxolan-2-yl)propan-1-amine (**8**).

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