Communications to the Editor

Combined Chemical and Enzymatic Synthesis of Coenzyme A Analogs

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Coenzyme A (CoA, **1a**) is involved in a wide variety of metabolic functions, including glycolysis, fatty acid β-oxidation, and biosynthetic pathways utilizing acetyl-CoA.[1] It has been estimated that about 4% of the known enzymes require CoA or a CoA ester as substrate.[2] These include enzymes of pharmaceutical importance such as HMG-CoA reductase, which catalyzes the first committed step in cholesterol biosynthesis and is the prime target for cholesterol-lowering drugs.[3]

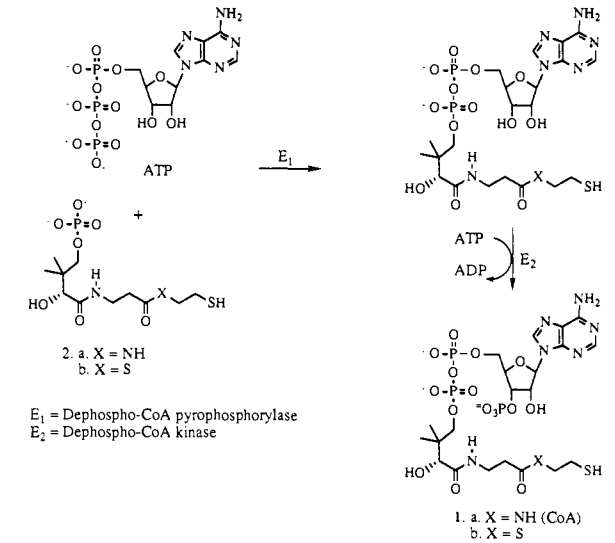
The study of this important class of enzymes has in a few cases been facilitated by CoA or CoA ester analogs, especially analogs which possess the binding properties of CoA but the altered reactivity of the thiol or thioester.[4] However, while CoA dithioesters[5] and dethio CoA[6] have been prepared directly from CoA, the preparation of most CoA analogs currently depends on a quite difficult chemical assembly of the CoA moiety.[4,7] We envisioned that the enzymes of CoA biosynthesis could provide a more convenient route to CoA analogs and report here a novel combined chemical and enzymatic approach to this class of compounds.

CoA biosynthesis has been studied in both mammalian and microbial species, and the final two steps are shown in Scheme I.[8-10] As the coupling of the adenylate and phosphopantetheine moieties and regiospecific phosphorylation of the 3′-phosphate are the two primary challenges in the chemical synthesis of CoA and its analogs, the enzymes catalyzing these last two steps in CoA biosynthesis have been previously employed synthetically.[11-13]

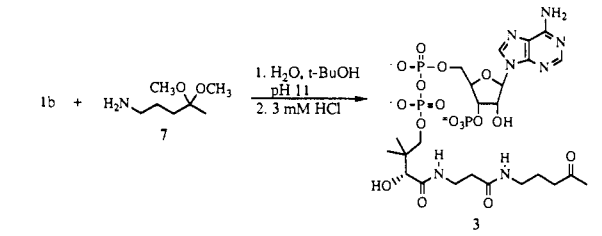
A potential obstacle in the use of enzymes as synthetic catalysts is the limitation of substrate specificity. Such limitations would likely become severe in the simple enzymatic conversion of various pantetheine phosphate analogs to the corresponding CoA analogs. For this reason, a more flexible approach to this problem was undertaken involving enzymatic synthesis of an easily functionalized thioester analog of CoA (**1b**) from the pantetheine phosphate analog **2b**. It was expected that **2b**, differing from the natural substrate only by replacement of an amide bond by a thioester, should serve as an efficient substrate for the enzymes of CoA biosynthesis. Aminolysis of the thioester bond of compound **1b** with an appropriate amine would form a CoA ester analog such as the dethio (carba) analog of acetyl CoA (acetonyldethio CoA, **3**), as shown in Scheme II. By aminolysis with a variety of amines, **1b** could serve as a versatile synthon for an unlimited number of CoA and CoA ester analogs.

For enzymatic preparation of CoA analog **1b**, the phosphopantetheine analog **2b** was prepared chemically as shown in Scheme III. Pantothenic acid (**4**) was first converted to the acylimidazole with concurrent trifluoroacetylation of the hydroxyl groups. Reaction of the acylimidazole with thiophenol followed by selective hydrolysis of the trifluoroacetate esters gave compound **5b**. Phosphorylation was accomplished by conversion to the dimethyl phosphate ester **6a** followed by removal of the methyl groups by procedures reported for the deprotection of dimethyl

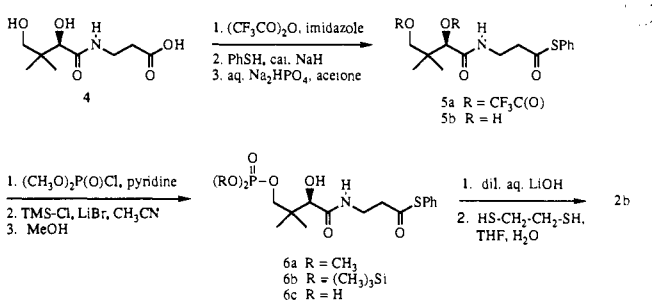
Scheme I



Scheme II



Scheme III



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phosphonates.[14] Thiol exchange with ethanedithiol in an aqueous tetrahydrofuran solution resulted in formation of compound **2b**.

Brevibacterium ammoniagenes was chosen as the enzyme source. While whole cells have been used previously in CoA synthesis,[11] it was expected that for the nucleophile-sensitive thioester analog a partially purified enzyme preparation would be necessary. *Brevibacterium ammoniagenes* was grown following a literature procedure.[11] The two enzymes were initially separated by ion exchange chromatography on DEAE Sepharose.[15] Dephospho-CoA kinase was further purified by dye ligand chromatography on red A-argarose.[16] The enzymes were coimmobilized in polyacrylamide gel.[17]

In preparative enzyme reactions, substrate **2b** was not isolated but was generated from **6c** and ethanedithiol just prior to use and analyzed via HPLC. Reactions were performed by combining compound **2b** (0.42 mmol, 20 mM final concentration), ethanedithiol (10 μL), magnesium chloride (0.5 mL, 0.5 M), ATP (10 mL, 0.1 M in 0.1 M HEPES buffer, pH adjusted to ~6 with LiOH), and inorganic pyrophosphatase (~5 units) in HEPES buffer (11 mL, 0.1 M, pH 7.5) followed by addition of the immobilized enzymes E_1 and E_2 (approximately 0.2 unit of each enzyme). The reaction mixture was stirred at room temperature under nitrogen to minimize oxidation of the thiols. Reaction progress was monitored by HPLC and was judged complete when ATP consumption stopped (2 days). No difference in rates was observed in the enzymatic conversion of **2b** to **1b** versus the rate determinations with the natural substrate. The crude enzyme product was reacted with the amine nucleophile **7** at alkaline pH. Adsorption/desorption on acidified charcoal and ECTEOLA cellulose chromatography[18] followed by C-8 reversed-phase chromatography provided compound **3** (45 mg, 12% from **6a**). The product **3** was characterized by ^1H, ^{31}P, and ^{13}C NMR spectroscopy, elemental analysis, and high-resolution mass spectrometry.[19] This methodology is now being employed in the synthesis of a range of novel CoA analogs for a variety of applications.

In addition to providing convenient access to an unlimited range of CoA analogs, the methodology described here represents a novel concept in utilizing enzymes as catalysts in organic synthesis. This enzymatic synthesis of an easily derivatized analog, followed by introduction of the functionality of interest in a final chemical step, minimizes substrate specificity limitations.[20] We expect that this concept may find similar applications in other classes of compounds.

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Registry No. 1b, 142611-88-9; **2b**, 142611-87-8; **3**, 66442-95-3; **4**, 79-83-4; **5a**, 142611-89-0; **5b**, 142611-90-3; **6a**, 142611-91-4; **6b**, 142611-92-5; **6c**, 142611-93-6; **7**, 142611-94-7; ATP, 56-65-5; PhSH, 4985-62-0; $(CH_3O)_2P(O)Cl$, 813-77-4; $HSCH_2CH_2SH$, 540-63-6; dephospho-CoA kinase, 9026-83-9; dephospho-CoA pyrophosphorylase, 9026-99-7.

Supplementary Material Available: Experimental details and characterization data for compounds **1b**, **2b**, **3**, and **5a-7** (6 pages). Ordering information is given on any current masthead page.

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(15) While a bifunctional enzyme catalyzing these two reactions has been purified from pork liver,[10] the enzyme(s) of *Brevibacterium ammoniagenes* and other microbial species had not to our knowledge been characterized previously. The two reactions were found to be catalyzed by separate enzymes easily separated on ion exchange chromatography. Details of the enzyme isolation are included in the supplementary material.

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(19) Data available in supplementary material.

(20) In parallel reactions, direct enzymatic synthesis of compound **3** from the corresponding acetonyldethiophosphopantetheine analog (see ref 13) was found to proceed at a rate less than 5% that of the rate of conversion of **2b** to **1b**.

Double C-H Activation at the α-Carbon of Cyclic Ethers by $Tp^*Ir(C_2H_4)_2$

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The activation of C-H bonds by pyrazolyl borate complexes of rhodium and iridium is receiving increased attention.[1,2] We recently demonstrated[3] that the complex $Tp^*Ir(H)(CH{=}CH_2)(C_2H_4)$ (**2**) ($Tp^* = HB(3,5\text{-}Me_2\text{-}pz)_3$) undergoes intramolecular coupling of the vinyl and ethylene ligands with formation of the allylic complex $Tp^*Ir(H)(\eta^3\text{-}CH_2CHCHMe)$ (**3**). Now we show that the hydride-vinyl **2** is also capable of regioselectively activating the two C-H bonds of the O-bearing methylene groups of cyclic ethers (e.g., tetrahydrofuran (THF)) with formation of Fischer-type carbene derivatives, which also contain an Ir-H and an Ir-butyl functionality.

Heating a THF solution of the bis(ethylene) complex **1** (60 °C, 8 h) quantitatively leads to a mixture of two complexes in a 1:1 ratio. One of them is the already mentioned allyl **3**, while for the other, **4**, analytical and spectroscopic studies (including 2D ^1H-^1H and ^1H-^{13}C NMR experiments) suggest the formulation shown in Scheme I. This has been confirmed by X-ray studies,[4] whose results are shown in Figure 1.

Formation of **4** constitutes an unprecedented double dehydrogenation of one of the α-methylene groups of tetrahydrofuran.[5]

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(4) Crystal data for $Tp^*Ir(H)(n\text{-}C_4H_9)(=\overline{C(CH_2)_3O})$: triclinic, $P\bar{1}$; a = 10.435 (8) Å, b = 10.809 (4) Å, c = 13.888 (2) Å, α = 81.54 (2)°, β = 68.43 (4)°, γ = 63.02 (3)°, V = 1298.8 (6) Å^3, Z = 2, ρ_{calcd} = 1.58 g cm^{-3}; λ (Mo Kα) = 0.710 69 Å (graphite monochromator); final R = 0.026, R_w = 0.029. The metal-bound hydrogen atom, H(1), was located in a final difference Fourier synthesis as the highest peak in the map.

(5) Radical C-H activation of THF by transition metal compounds is a known process. See: Bevan, P. C.; Chatt, J.; Diamantis, A. A.; Head, R. A.; Heath, G. A.; Leigh, G. J. *J. Chem. Soc., Dalton Trans.* **1977**, 1711. Setsune, J.; Ishimaru, Y.; Moriyama, T.; Kitao, T. *J. Chem. Soc., Chem. Commun.* **1991**, 556. Double α-C-H abstractions from phosphines[6a,b] and amines[6c] are also known. We are not aware, however, of such an activation of ethers, although very recently a 1,1-elimination of molecular hydrogen from 1,3-dimethoxypropane by a bare transition metal ion in the gas phase was reported: Prüsse, T.; Fiedler, A.; Schwarz, H. *J. Am. Chem. Soc.* **1991**, *113*, 8335. Dehydrogenation of one of the α-methylene groups of tetrahydrofuran with formation of lactone is selectively effected by the powerful oxidant RuO_4. See: Berkowitz, L. M.; Rylander, P. N. *J. Am. Chem. Soc.* **1958**, *80*, 6682. Raychandhuri, S. R.; Ghosh, S.; Salomon, R. G. *J. Am. Chem. Soc.* **1982**, *104*, 6841.

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