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Isolation and Synthesis of One of the Most Central Cofactors in Metabolism: Coenzyme A

Louis M. M. Mouterde* and Jon D. Stewart[†]

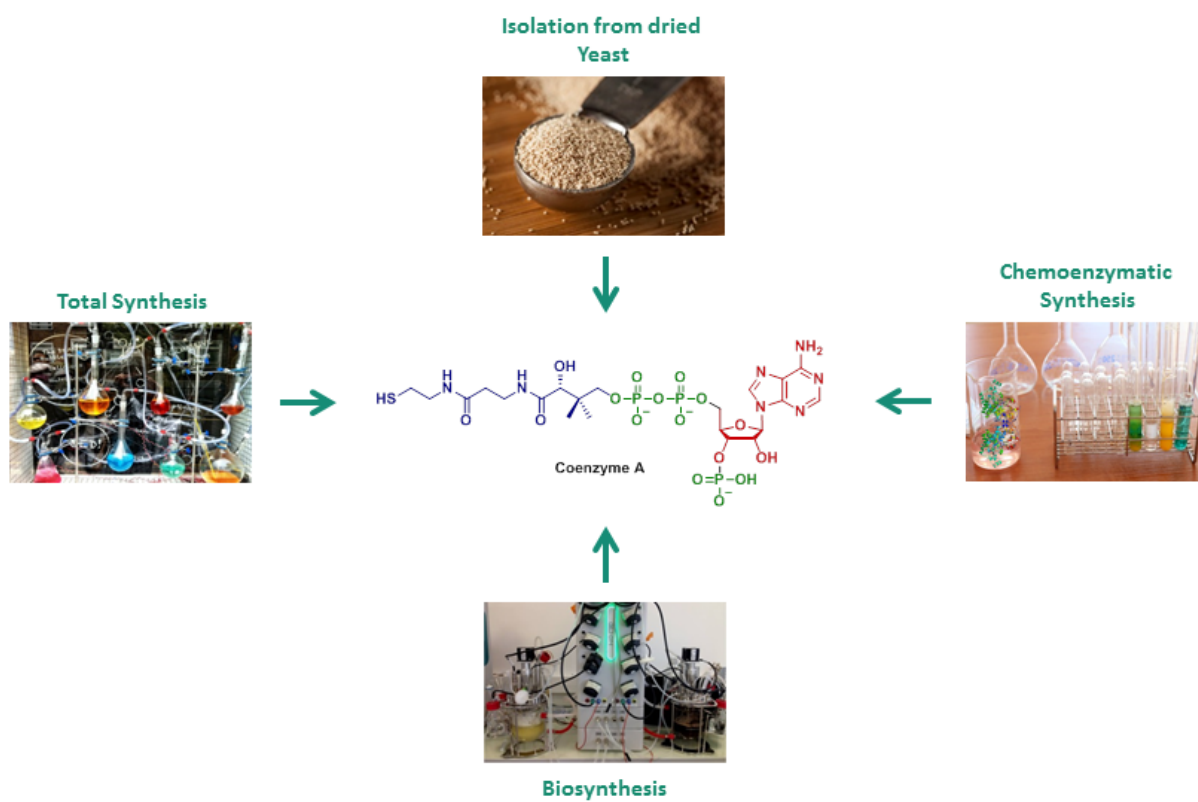
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Abstract

The isolation and synthesis of Coenzyme A (CoA) has been an important field since this cofactor was discovered in 1947. CoA plays a central role in human metabolism and is vital in several metabolic pathways including fatty acid transport and degradation as well as the biosynthesis of a wide variety of compounds including fatty acids. The high cost of commercially-available CoA (\$2600 / g with >85% purity) has motivated several research groups to find alternatives for its production. The variety of strategies that have been investigated for CoA production can be divided in three categories: isolation from microorganisms, total chemical synthesis and chemoenzymatic synthesis. These approaches provide access to CoA with different efficiencies. For example, direct isolation yields of ~25 mg/kg from dried yeast have been obtained. A variety of microorganisms such as *Pseudomonas alkalytica*, *Sarcina lutea* and *Brevibacterium ammoniagenes* accumulate CoA in their cultures at levels ranging from 0.03 to 115 mg/mL. Total chemical synthesis yields have ranged between 25 and 54% and chemoenzymatic approaches have provided overall yields of *ca.* 73%. This review covers all published for producing CoA in order to compare their efficiencies, scalabilities and convenience.

Keywords: Coenzyme A, Isolation, Total Synthesis, Chemo-Enzymatic Synthesis.

Introduction

The biosynthesis of CoA from vitamin B5 (pantothenic acid **1**) is universal in prokaryotes and eukaryotes; however, there was initially some controversy with regard to the actual sequence of steps (Figure 1). Hoagland and Novelli suggested that **1** reacts with cysteine to form pantothenoylcysteine **6**, which is then decarboxylated to D-pantetheine **7**. Subsequent phosphorylation of the primary alcohol by pantothenate kinase (PanK) was proposed to form phospho-D-pantetheine **4** followed by sequential additions of adenosine monophosphate (to form dephospho-CoA **5**) and a 3'-phosphate to afford CoA.¹ Brown *et al.* challenged this proposal and suggested an alternative route in which phosphorylation of **1** occurred first, yielding phosphopantothenate **2**. Subsequent condensation and decarboxylation would provide phosphopantothenoylcysteine **3**, followed by **4**.² The final two steps were thought to be the same as those proposed by Hoagland and Novelli (Figure 1).

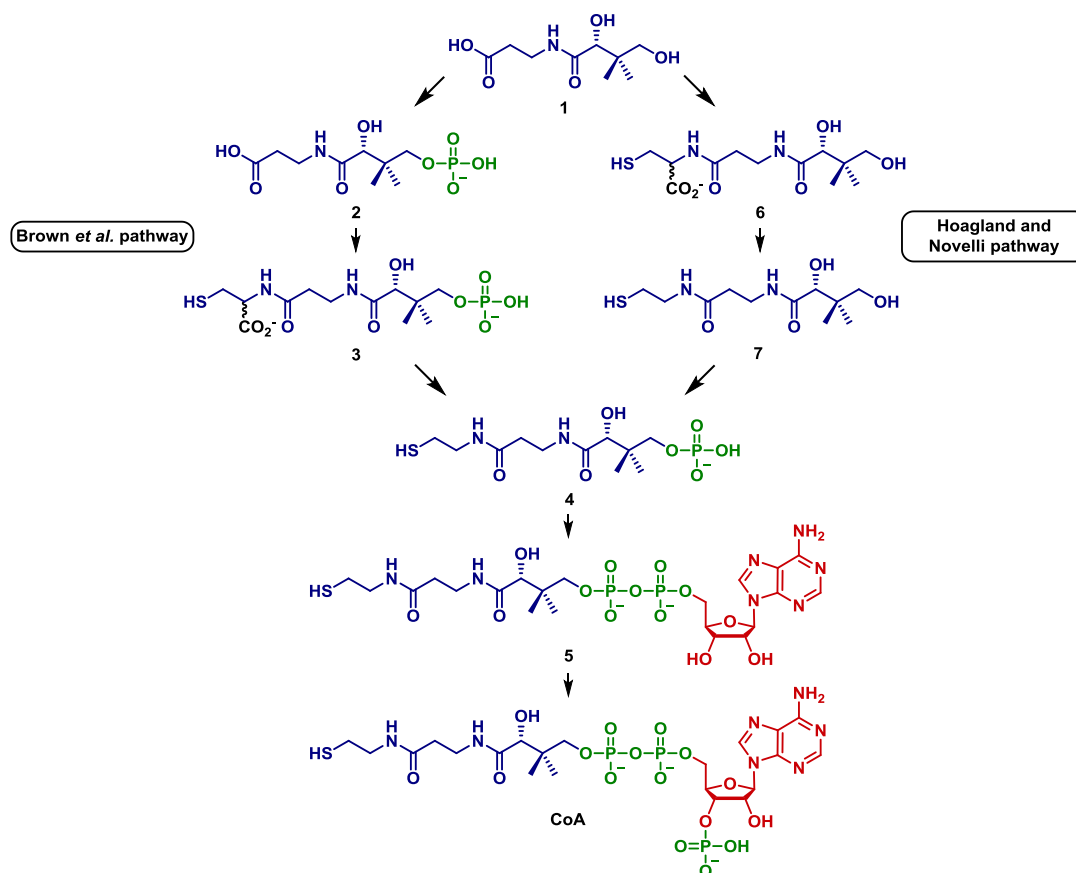


Figure 1. Two possible biosynthetic pathways proposed for CoA.

To determine the actual biosynthetic route, Abiko investigated substrate selectivity of both PanK and phosphopantothenoylcysteine synthetase (PPCS). By purifying and separating the two enzymes from rat liver, he showed that PPCS did *not* catalyze the condensation of pantothenate **1** with cysteine in presence of ATP; however, it did accept phosphopantothenate **2**. Furthermore, he showed that PanK catalyzed the ATP-dependent phosphorylation of pantothenate **1**. These two observations strongly suggested that the first steps of CoA biosynthesis involved the phosphorylation of pantothenate **1**, followed by a condensation with cysteine to provide **3**, as initially proposed by Brown *et al.*

Abiko also made two other observations that greatly influenced later work on CoA synthesis. He noted that PanK – in addition to its normal role in phosphorylating **1** – also catalyzed the phosphorylation of both D-pantetheine **7** and *N*-pantothenoyl-cysteine **6**. This suggested that PanK may also be part of a pathway that recycles intracellular CoA degradation products. This information also offered the possibility for a "shortcut" in synthesizing CoA by using D-pantetheine **7** as a precursor, thereby skipping the enzyme-catalyzed steps required to synthesize **7**.³

Since then, the CoA biosynthetic route proposed by Brown has been generally accepted and all the enzymes involved in the pathway have been characterized. The initial phosphorylation step is catalyzed by PanK. This is followed by condensation with cysteine (using PPCS), then decarboxylation by phosphopantothenoylcysteine decarboxylase (PPCDC). The last two steps are catalyzed by phosphopantetheine adenylyl transferase (PPAT) and dephospho-CoA kinase (DPCK) respectively. These five enzymes are commonly called CoaA, CoaB, CoaC, CoaD and CoaE (Figure 2).

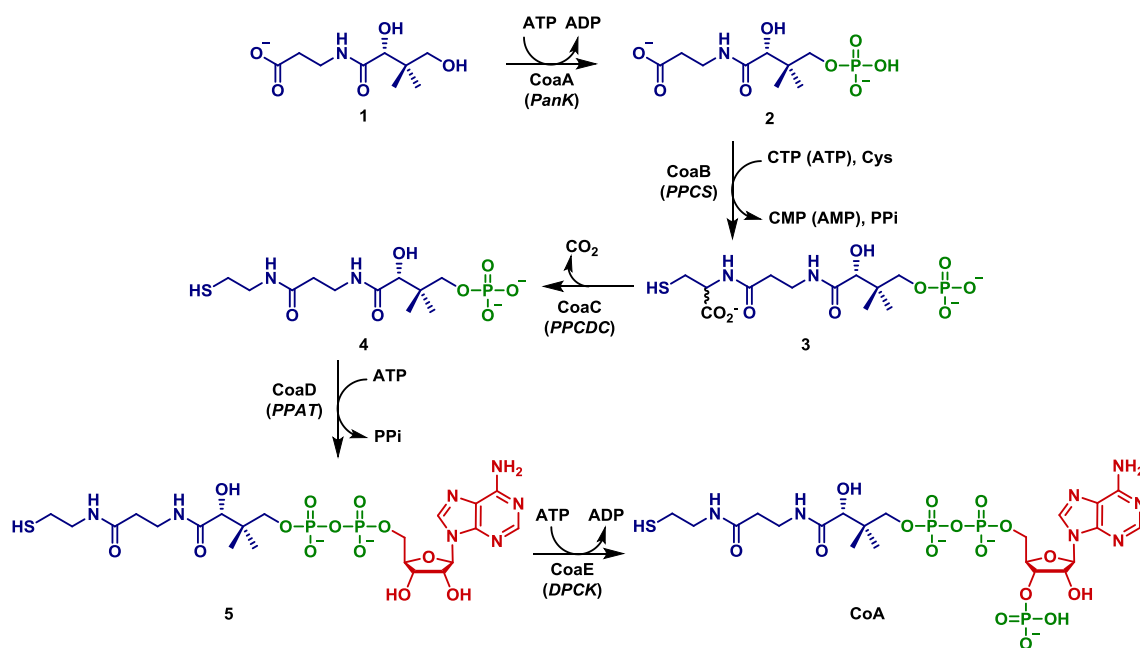


Figure 2. Biosynthesis of Coenzyme A

Many animals, as well as some microbes, cannot synthesize pantothenate, which makes them dependent on the uptake of exogenous vitamin B5. On the other hand, many bacteria (including *Escherichia coli*) and yeast (including *Saccharomyces cerevisiae*) can synthesize this precursor. Interestingly, *E. coli* produces 15 times more pantothenate than required for the biosynthesis of CoA.⁴ Several research teams have taken advantage of this ability to biosynthesize pantothenate **1** for the synthesis of CoA.

Because of its central role in metabolism, CoA has been intensively studied and several reviews have been published to date. These focus on various aspects of CoA chemistry and biology, including its roles specific organisms or CoA analogues and derivatives. Jackowski as well as Strauss focused on CoA biosynthesis and its implication for different metabolic pathways.^{4, 5} Saliba assessed the antimicrobial potential of CoA.⁶ With regard to CoA derivatives, two reviews have been published – by Drueckhammer and by Strauss – that describe the production of CoA and its non-natural analogs produced *via* enzymatic or non-enzymatic strategies.^{7, 8} While previously-published reviews cover important topics with regard to CoA, to the best of our knowledge, no review focused on

methods to obtain CoA and summarizing the yields and purities obtained has been published to date.

Isolation of Coenzyme A from Microorganisms

After its discovery in 1947,^{9, 10} several different strategies to obtain CoA from natural sources have been developed. The first work in this area occurred in the early 1950's when Lipmann *et al.* and Buyske *et al.* isolated CoA from animal tissues such as hog liver.^{11, 12} While these processes gave access to CoA in some degree of purity, they suffered from poor yields. In parallel, these two teams also showed that CoA could be produced and isolated from microbial sources such as *Streptomyces fradiae* or yeast.^{13, 14} These observations represented an important advance for the study and production of CoA because obtaining the starting materials could be readily obtained (since the microorganisms could be grown easily in short time frames). Buyske *et al.* improved their process dramatically when they discovered that CoA could be co-precipitated with glutathione in presence of cuprous sulfate.¹⁵ Using this strategy, they isolated 133 mg of CoA from 6 kilograms of dried yeast with a purity of *ca.* 26%.

In the same year, Stadtman and Kornberg reported a straightforward, two-step purification strategy for CoA from dried yeast. By using charcoal absorption followed by anion exchange chromatography (Dowex 1 \times 2), it was possible to purify 76 mg of CoA from 3 kilograms of dried yeast with a purity of *ca.* 25%. This method gave a similar yield and purity as the Buyske *et al.* method, but required fewer purification steps. Unfortunately, the Stadtman and Kornberg method would be difficult to scale up because of massive quantity of anion exchange resin required (2.3 kg) to provide a relatively small amount of CoA (75 mg).¹⁶

Crook and coworkers improved the process for isolating CoA from yeast, particularly with regard to purity. After 14 different steps of extraction and purification, they isolated 300 mg of CoA with a purity of approximately 90%. Unfortunately, even this improved procedure

was still limited by poor yield (30%) and substantial working volumes (*ca.* 700 L of various solvents were used).¹⁷

Production of Coenzyme A using Engineered Microorganisms

Nakao *et al.* discovered that microbial *n*-paraffin assimilators such as *Pseudomonas alkanolytica* Pd 192, *Alcaligenes marshallii* Ah 197 and *Achromobacter nucleacidives* Ba 108 all accumulated CoA at levels higher than those in organisms that did not assimilate *n*-paraffins. CoA-accumulating organisms yielded around 30 µg of CoA per mL of culture. This allowed them to isolate CoA with a purity of 90% and yield of 13%.¹⁸ A year later, Nishimura reported that *Sarcina lutea* IAM 1099 accumulated CoA in its reduced form with a concentration of *ca.* 600 µg / mL. They produced CoA in batches of 380 mg with yields over 33% (based on amounts present in culture broth) and with a purity of 96%.¹⁹

Shimizu and coworkers published a series of papers from 1970 to 1984 that described the isolation of high purity CoA from a microorganism that was capable of a surprisingly high accumulation capacity. They established that *Brevibacterium ammoniagenes* IFO 12071 synthesized CoA from pantothenic acid, and that it was possible to reach 3.0 to 5.5 mg / mL of CoA in its disulfide form (disulfide CoA) in the culture broth. The method used by the authors allowed an extracellularly accumulation of the desired product.²⁰ Combining this discovery with a clever purification sequence composed of Duolite S-30 resin, charcoal absorption and anion exchange chromatography (Dowex 1 × 2), they isolated approximately 1 g batches of disulfide-CoA with a purity of *ca.* 80% from only 1 liter of culture broth.²¹⁻²³ A simple reduction by 2-mercaptoethanol gave CoA in its reduced form with no loss in activity.

After this initial work, improved methods aimed at reaching an even higher degree of purity were explored. Pantothenic acid, cysteine and ATP were added to dried cells of *B. ammoniagenes* that contained the enzyme cocktail necessary for CoA synthesis. This allowed scaling down the reaction volume from 1,000 to 300 mL and also reduced the reaction time

from 6 days to 8 hours. While maximum accumulation levels were somewhat lower than in the fermentation method (2 to 3 mg / mL), this disadvantage was offset by better recovery (67%).

The methodology was further improved by using immobilized cells trapped in a polyacrylamide gel to improve enzyme stability.²⁴ While this provided lower CoA accumulation levels (0.5 to 1.2 mg / mL), the recovery was similar (65%) and final product purity was greater (91%). It should be noted that both dried cell-based methods gave CoA directly in its reduced form while the first approach provided CoA in its oxidized form. A comparison of the different methods is shown in Table 1.^{25, 26}

Table 1. Enzymatic activities comparison between different cells sources

	Cell fermentation	Dried cells	Immobilized cells
Accumulation (mg / mL)	3-5	2-3	0.5-1.2
Form of CoA produced	Oxidized form	Reduced form	Reduced form
Purity (%)	83-87	85	91
Recovery (%)	34	67	65

One drawback of isolating CoA from *B. ammoniagenes* was that the target accumulated to a maximum level of 5 mg / mL. The authors suspected that PanK might be feedback inhibited by CoA.^{27, 28} This was overcome by replacing the enzymatic phosphorylation step by chemical phosphorylation. The latter allowed cultures to accumulate CoA at a very high level of (33 mg / mL), yielding 1.13 g batches of CoA with a purity of 92%. A second route using D-pantetheine **7** as the precursor provided even better results (accumulation of 115 mg / mL in the culture medium and 8 g of CoA isolated with a purity of 93%).²⁹

A more elegant solution to the feedback inhibition problem was based on a mutant of *B. ammoniagenes* IFO127071, selected for resistance to oxy-pantetheine, an antimetabolite of D-pantetheine **7**. Stewart and Ball established that 4'-oxyphosphopantetheine was converted to the corresponding oxy-analog of CoA (oxy-CoA), which appears to be an inhibitor of

phosphotransacetylase.³⁰ It was therefore proposed that inhibition by oxypantetheine is due to a competition between oxy-CoA and CoA, which causes an up-regulation of CoA production in an attempt to overcome antimetabolite effect. Using the best mutant of *B. ammoniagenes* IFO127071 from strain improvement mutagenesis, the culture broth gave 9.3 mg of CoA per mL from pantothenic acid and 11.5 mg of CoA per mL from D-pantetheine **7**.³¹

Chemical Synthesis of Coenzyme A

Rather than isolating CoA from a microorganism, strategies for synthesizing CoA using both classical chemical methodologies as well as enzyme-assisted strategies have also been explored. For example, CoA can be obtained using a supernatant from disrupted liver cells from several organisms (pigeon, hog or rat) using pantothenic acid **1** as precursor. Dephospho-CoA pyrophosphatase (also called dephospho-CoA synthase) and dephospho-CoA kinase are both present in the livers of these preparations and produce CoA from phosphopantetheine. It was also found that pantothenic acid **1** could be converted to D-pantetheine **7** in presence of cysteine using the same liver cell supernatant. Finally, PanK was also detected in these supernatants, which converts D-pantetheine **7** to phospho-pantetheine, the missing link to synthesizing CoA. With the complete biosynthetic route found in the liver preparations, 10.4 Units of CoA were synthesized, which corresponds to *ca.* 7.42 μg .^{1, 32, 33} This was the first *in vitro* enzymatic synthesis of CoA reported; unfortunately, the yield of the coenzyme was too low for large scale production.

Several total syntheses of CoA were described in the 1960's. First, Khorana *et al.*, inspired by the work of Baddiley and Thain³⁴ reported a convergent 9 step synthetic strategy that started from D-pantolactone **8** and adenosine **11** that yielded CoA with an overall yield of *ca.* 30% (Figure 3).^{35, 36} The first branch of this scheme involved condensation of **8** with β -alanine **9**, followed by a second condensation with *S*-benzylcysteamine which yielded, after global deprotection, phospho-D-pantetheine **4**. The second branch of the synthetic strategy

began with phosphorylation of adenosine **11**, followed by formation of the 2',3'-cyclic phosphate **14**. Condensation of **4** and **14** under acidic conditions yielded CoA and iso-CoA, the 2'-phosphate isomer of CoA, in 98 mg batches with a purity of 65%.

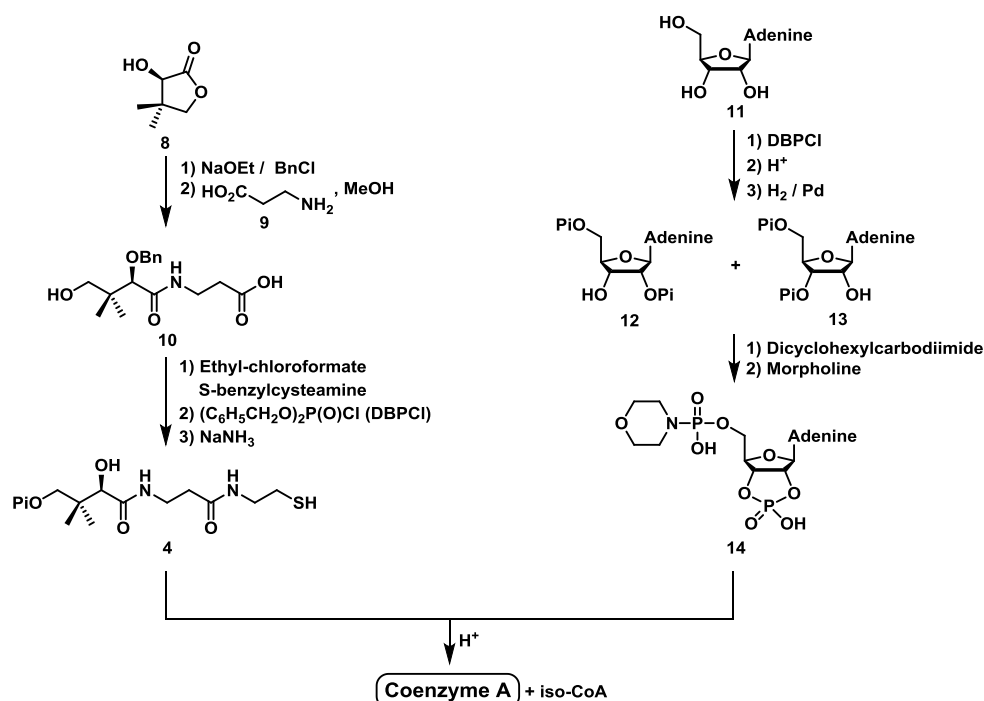


Figure 3. Khorana *et al.* chemical synthesis of CoA

Gruber *et al.* also devised a convergent synthetic strategy for synthesizing CoA, using cysteamine **16**, β -alanine **9** and 3'-adenylic acid **20** (isolated from yeast) as precursors (Figure 4). The authors judiciously used orthogonal protecting groups to mask the amine of **9** and the thiol of **16** with benzyl chloroformate (CbzCl) and benzyl chloride (BnCl), respectively. The resulting intermediates, **15** and **17**, were condensed to yield **18**. Selective deprotection of the amine function of **18** by sodium in liquid ammonia allowed condensation with 2'-*O*-methoxymethyl-pantolactone **19**. This was followed by deprotection of the thiol by sodium amalgam and its immediate reprotection using benzoyl chloride (BzCl) yielding **20**. Pyrophosphate tetrachloride was condensed with **20** and the resulting intermediate was reacted with 3'-phosphoadenosine, then hydrolyzed under acidic conditions to give benzoyl-CoA **22**. The final thiol deprotection used sodium hydroxide, providing CoA in 9 steps with a

purity of *ca.* 70% and an overall yield of approximately 25%.³⁷ Unfortunately, the authors noted that they were able to synthesize CoA in only small quantities using this strategy.

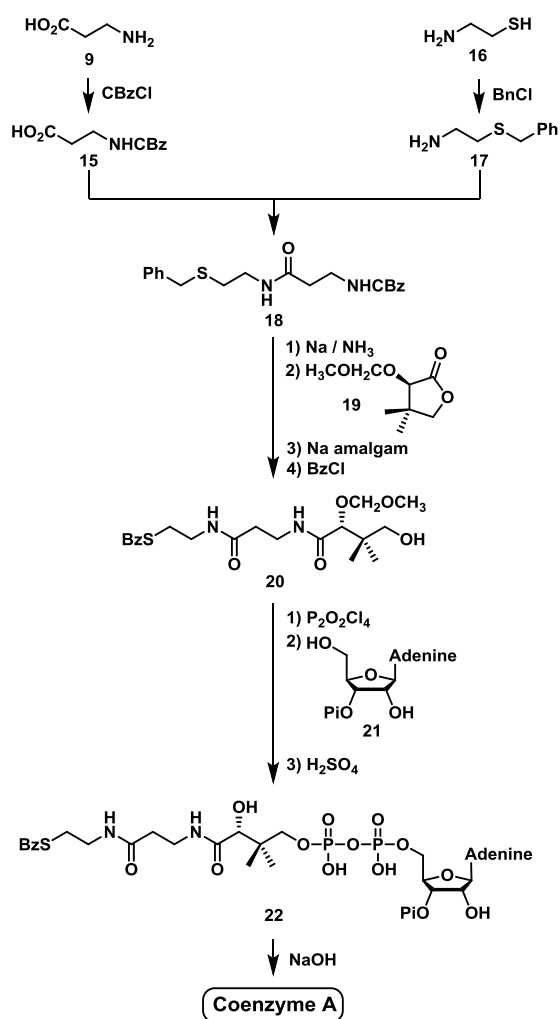


Figure 4. Gruber *et al.* route to Coenzyme A

Michelson suggested using the disulfide of pantetheine (pantethine) as the precursor for CoA since the disulfide itself would act as a thiol protecting group without introducing extraneous molecular structure. This route used the same methods as Khorana for phosphorylation of 2',3'-cyclic-adenosine **23**, 3',5'-adenosine diphosphates **24** and D-pantethine **46**. Condensation of phospho-D-pantethine with **25** yielded 2',3'-cyclic-disulfide-CoA **26**. Finally, treatment with Ribonuclease T₂ (RNase T₂), which opened the ring exclusively to adenosine-3'-phosphate, and β-mercaptoethanol gave access to the final

product. Michelson prepared batches of 800 mg of CoA with an overall yield of 63% and a purity of approximately 75% through a linear 5 step synthetic strategy (Figure 5).³⁸

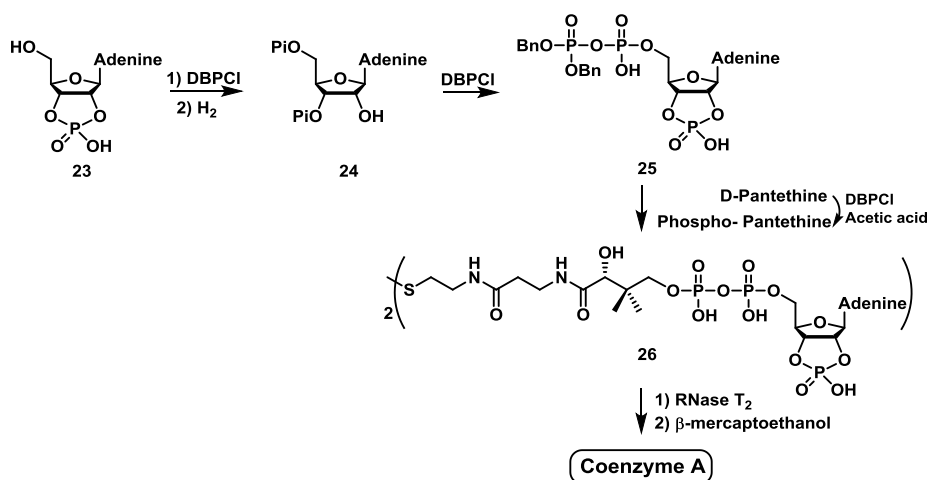


Figure 5. Michelson route to CoA

Shimizu *et al.* developed a clever synthesis of CoA from D-pantothenonitrile **27** and 2',3'-cyclic phosphate-5'-phosphoromorpholidate **14**, which were condensed in the presence of RNase T₂. Subsequent addition of cysteamine **16** gave thiazoline intermediate **28**. Hydrolysis under acidic conditions gave CoA (Figure 6). This linear 4 step synthetic strategy provided 50 mg batches of the target with an overall yield of approximately 30% and a purity of *ca.* 80%.^{39, 40}

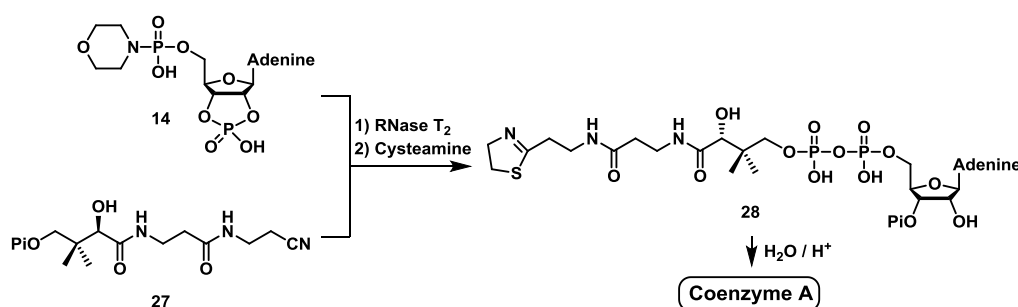


Figure 6. Shimizu route to CoA

Two additional synthetic routes to CoA were described in the 1970's. Mukaiyama and Hashimoto developed an approach that was inspired by the work of Shimizu. This approach combined the synthesis of adenosine 2',3'-cyclicphosphate-5'-phosphoromorpholidate **14**,

described by Khorana, with the use of RNase T₂ to avoid forming *iso*-CoA (Figure 7).⁴¹ Phospho-D-pantetheine **4** was obtained by condensation of pantothenic acid **1** with cystamine, followed by formation of dibenzyl phosphate intermediate **30**. This yielded phospho-D-pantetheine **4** when treated sequentially with acetic acid, solid sodium in ammonia and β-mercaptoethanol. The 9 step synthetic strategy afforded CoA with an overall yield of 36% and high purity (98%).⁴²

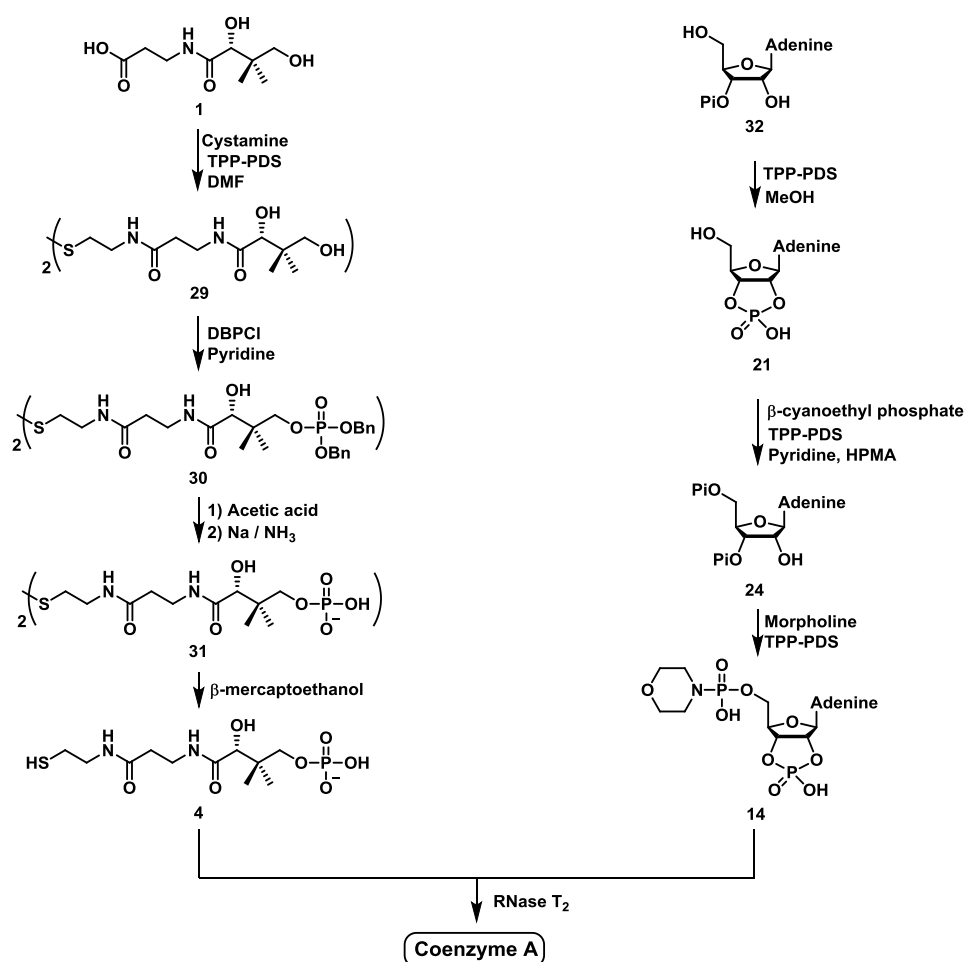


Figure 7. Mukaiyama and Hashimoto synthesis of CoA

A few years later, Mushika *et al.* published their synthesis of 2',3'-cyclic CoA **35** (oxidized form; Figure 8). The first step of this synthetic strategy involved phosphorylating adenosine **12** using *N,N'*-dicyclohexylcarbodiimide (DCC) and 2-dimethylamino-4-nitrophenyl phosphate **33**. The resulting intermediate **34** was then condensed with phospho-D-

pantethine to give 2',3'-cyclic disulfide-CoA **35**. They confirmed their target structure by hydrolyzing **35** with HCl and reducing the disulfide bond with 2-mercaptoethanol; this gave a mixture of CoA and *iso*-CoA, as expected. This convergent, 3-step synthetic strategy provided high purity 2',3'-cyclic CoA **35** in batches of 770 mg and an overall yield of 54%. Unfortunately, the two hydrolysis products, CoA and *iso*-CoA, formed an inseparable mixture, which greatly diminished the practical value of this route..⁴³

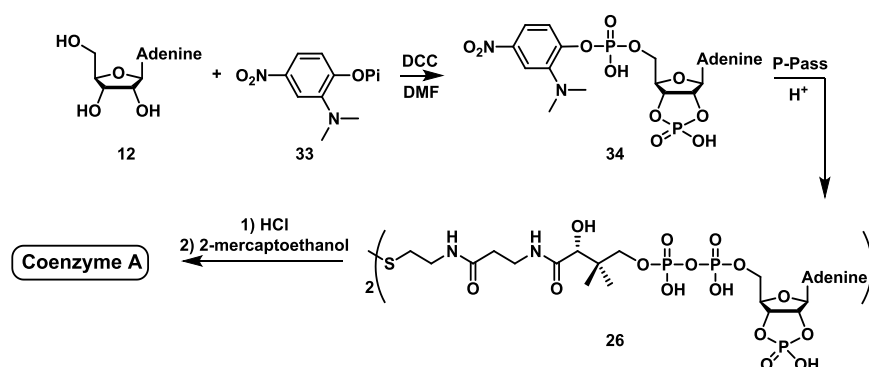


Figure 8. Mushika route to CoA

While majority of the abovementioned syntheses gave CoA at higher degrees of purity than in previous reports, all involved chromatographic purifications of intermediates, relatively complex organic synthesis methods and poor yields that conspired to make scale up difficult.

Chemoenzymatic Synthesis of Coenzyme A

In addition to isolating CoA from microorganisms or its total chemical synthesis, a third strategy has also been explored: combining chemical synthesis and enzyme-catalyzed steps. Cloning, sequencing and overexpression of the CoA biosynthetic enzymes PanK⁴⁴, PPAT⁴⁵ and DPCK⁴⁶ from *E. coli* facilitated greatly this approach.⁴⁷⁻⁵² Strauss, Begley and Ratnam *et al.* further simplified their production by fusing PanK, PPAT and DPCK with an *N*-terminal hexa-histidine tag for one-step purification after overexpression.^{53, 54} The method described by these authors – combined with the earlier discovery of Hoagland and Novelli on substrate promiscuity of PanK – suggested that D-pantetheine **7** might be a viable basis for a

new chemoenzymatic process for CoA. PPAT and DPCK also tolerated substrate modifications, accepting pendant groups that included thioesters to a *tert*-butyl ester. It was hypothesized by Stewart *et al.* that PanK, PPAT and DPCK could all accept substrates with more radical alterations and that D-pantethine **46** would be an ideal starting material. This would allow the synthesis of CoA in its oxidized form, which would prevent sulfur oxidation, the principal source of CoA degradation. For this reason, synthesis of these two targets has been a priority for all chemoenzymatic routes to CoA.

There are many different routes described in literature that yield D-pantetheine **7** and D-pantethine **46**. The first reports were published in the 1950's using different starting materials. The first report of Baddiley and Thain was motivated by needing access to D-pantetheine **7**, not only for biological studies but also for synthetic purposes. They therefore developed a convergent synthetic strategy that yielded D-pantetheine **7** with an overall yield of 70% (Figure 9). Inspired by the work of Sifferd and du Vigneaud,⁵⁵ the first branch of this synthetic strategy employed an activated acyl azide derivative of carbobenzoxy- β -alanine **37**. Azide **39** was prepared by treating **37** sequentially with PCl₅ and hydrazine to give **38**. A final treatment with sodium nitrite under acidic condition yielded azide **39**. In the second branch, *S*-benzyl-protected cysteamine was formed by reacting 2-bromoethylamine **35** with benzyl mercaptan **36**. The two intermediates formed from the two branches of this approach, **36** and **39**, were coupled to form **40**, which was then treated with ammonia to remove protecting groups from both the thiol and carboxyl ends of **40**, thereby affording aletheine **41**. Condensation with D-pantolactone **8** provided D-pantetheine **7**. The authors reported that they were able to produce 3.75 g batches of pure product **7**.⁵⁶

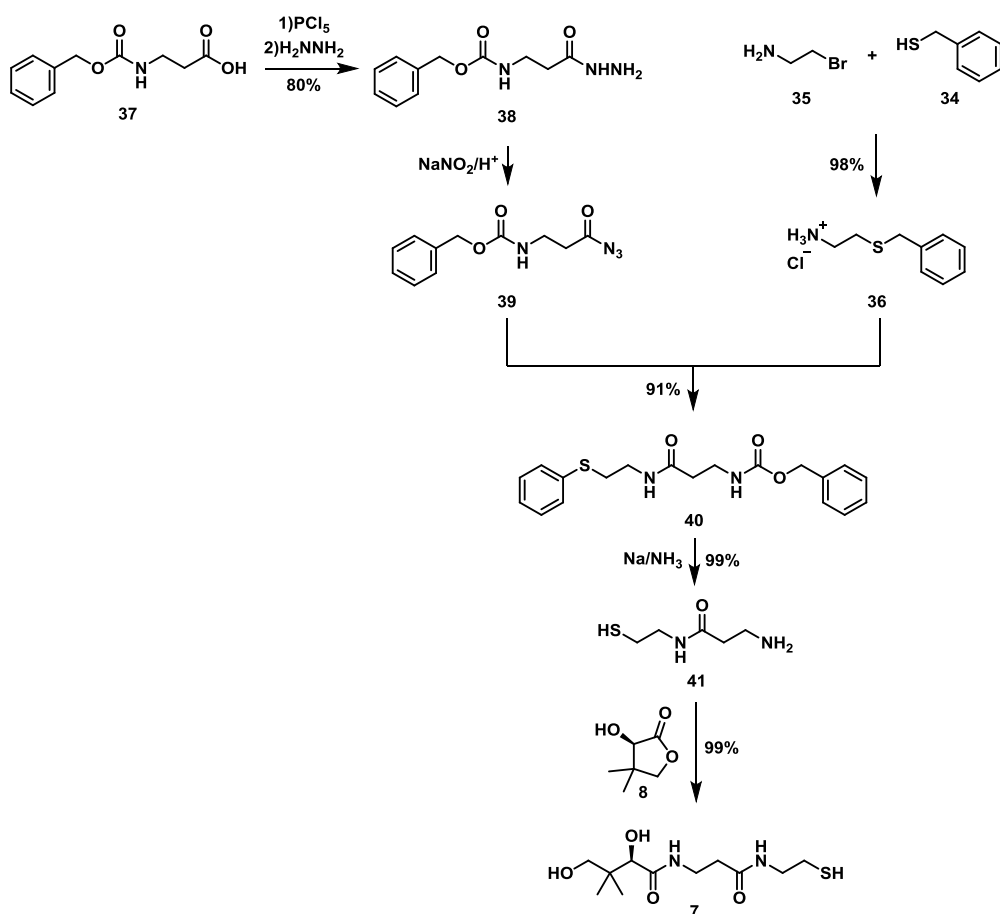


Figure 9. Baddiley and Thain scheme to D-pantetheine

A few months later, Brown and Snell described a "crossed" synthetic strategy that gave access to both D-pantetheine **7** and D-pantethine **46** (Figure 10). Three strategies were suggested. An "ester process" involved condensing methyl or ethyl pantothenate with cysteamine **16** or cystamine **45**, giving D-pantetheine **7** and D-pantethine **46** respectively. An "azide process" was based on the same principle as described above, in which cysteamine **16** or cystamine **45** was reacted with pantothenate azide **44**. The latter was obtained by reacting methyl or ethyl pantothenate with hydrazine followed by sodium nitrite under acidic conditions. These two strategies are thus linked to one another and can give either D-pantetheine **7** or D-pantethine **46**. The overall yield of the "ester process" was 20-30% while the "azide process" gave 30-40% yields. In a third strategy, D-pantolactone **8** was condensed with either aletheine **41** or its disulfide analog **47**, which gave results similar to those reported

by Baddiley and Thain with yields between 80-90%. Using this approach, the authors obtained 200 mg batches of D-pantetheine **7** and 900 mg batches of D-pantethine **46** by the "D-pantolactone condensation process".⁵⁷

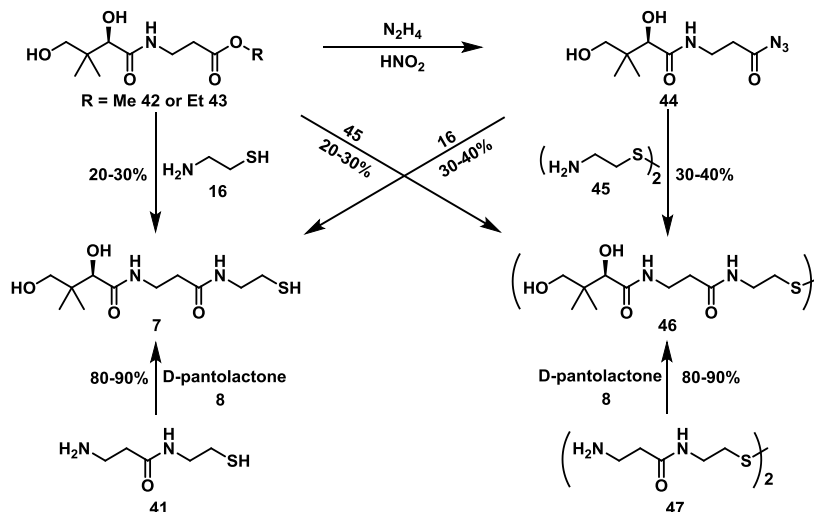


Figure 10. Brown and Snell "crossed" route to D-pantetheine and D-pantethine

In the same year, King *et al.* reported a linear, 6-step synthetic strategy starting from β -alanine **9** (Figure 11). After Cbz-protection of the amine moiety, the corresponding acyl chloride was prepared by treatment with thionyl chloride. This was followed by condensation with cystamine **45** and amine deprotection by sodium in liquid ammonia. The resulting intermediate formed a complex with oxalic acid. The oxalate ion was then trapped by sodium ethoxide in order to liberate the amine for its condensation with D-pantolactone that formed D-pantetheine **7**. The overall yield of **7** in 200 mg batches was 23%.⁵⁸

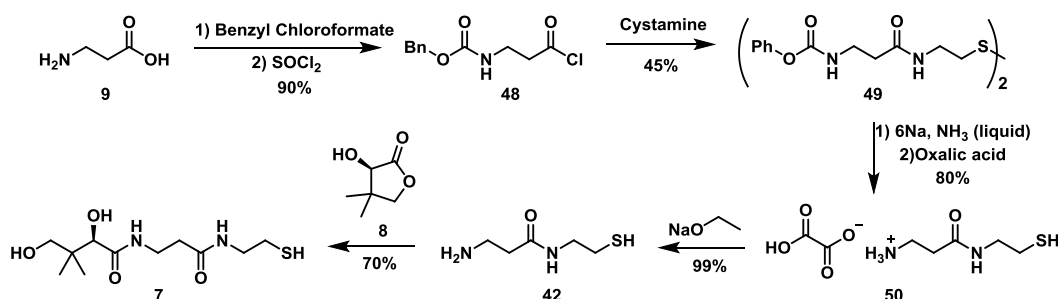


Figure 11. King *et al.* route to D-pantetheine

Ten years later, Hosokawa *et al.* proposed a novel synthesis of the targets using 3-aminopropionitrile as starting material (Figure 12). One noteworthy aspect of the authors' approach was that they proposed three different routes in which almost all intermediates are linked to one another. The principal route involved condensing 3-aminopropionitrile **51**, *N*-(2-cyanoethyl)-formamide **52** or *N*-(2-cyanoethyl)-acetamide **53** with D-pantolactone **8**. The resulting product was then reacted with cysteamine **16** to yield the corresponding thiazoline ring intermediate **64**, which was then hydrolyzed under acidic conditions to give D-pantetheine **7**. Conversion to D-pantethine **46** was done by oxidation using hydrogen peroxide. Using this principal route, the authors accessed both D-pantetheine **7** and D-pantethine **46** in a 70% overall yield and in 32 g batches.⁵⁹

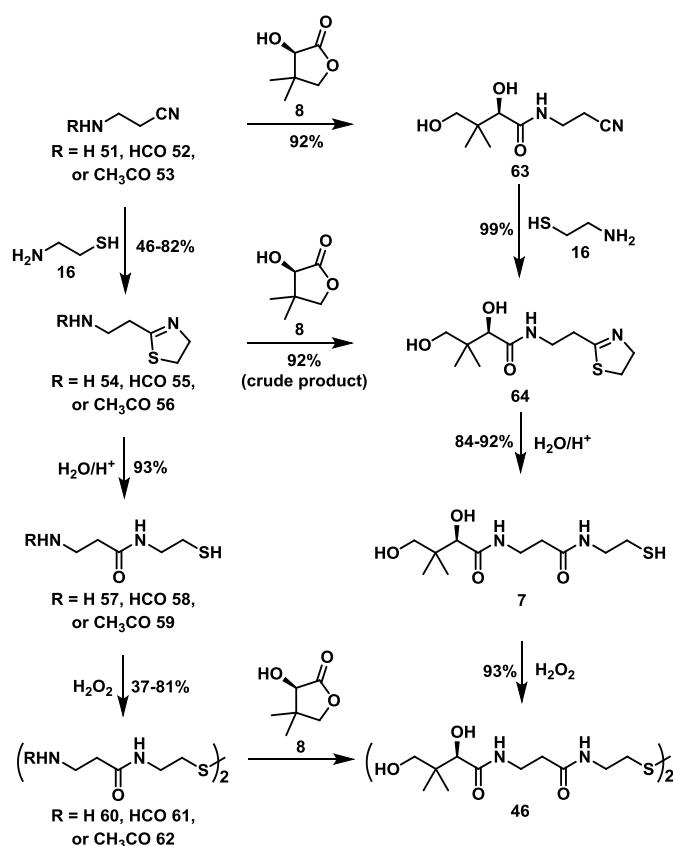


Figure 12. Hosokawa *et al.* scheme to D-pantetheine and D-pantethine

In 2004, Burkart and coworkers published a new convergent synthetic strategy that yielded D-pantetheine **7** (Figure 13). After reducing D-pantolactone **8** by LiAlH_4 , two of the

hydroxyls of triol **69** were protected as an acetal using *p*-(dimethoxy-methyl)-anisole in the presence of camphorsulfonic acid. This was followed by Swern oxidation of remaining hydroxyl and a subsequent mild chlorite (Pinnick) oxidation to yield the corresponding carboxylic acid **71**. In the other branch of the synthetic strategy, the authors protected the amine function of cysteamine **16** with triphenylmethyl chloride, then condensed **65** with Fmoc- β -alanine **66** to form amide **67**, from which the Fmoc group was cleaved by piperidine. The two key intermediates (**68** and **71**) were joined by an EDCI coupling to yield **72**. Global deprotection by iodine yielded D-pantetheine **7**, which was produced in 5 mg batches and with an overall yield of 25%.^{60, 61}

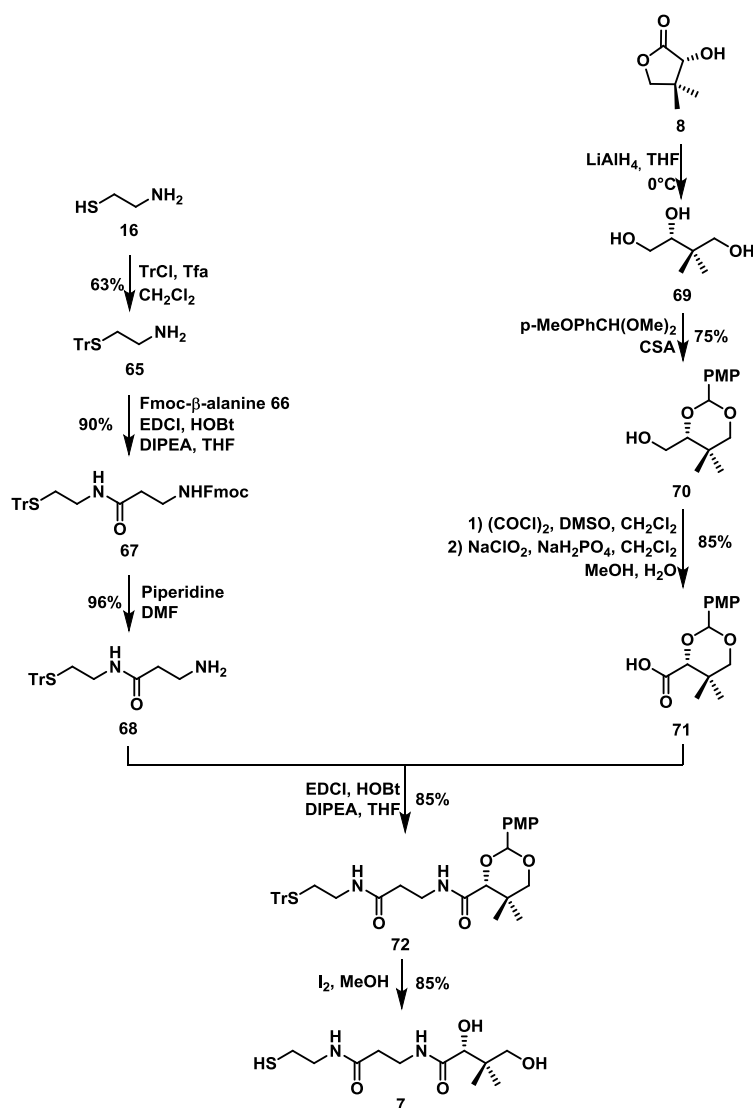


Figure 13. Burkart *et al.* scheme to D-pantetheine

One year later, the Burkart group proposed an alternative pathway to D-pantetheine **7** in a publication mainly concerned with carrier protein labeling (Figure 14). Using pantothenic acid **1** as the starting material, the authors employed the same acetal protecting and EDCI coupling strategies to form **73**. This was condensed with cysteamine **16** and deprotected with HCl to yield D-pantetheine **7** as well as its analogues in a linear, 4-step route. Unfortunately, no yields or batch sizes were indicated for this route.⁶²

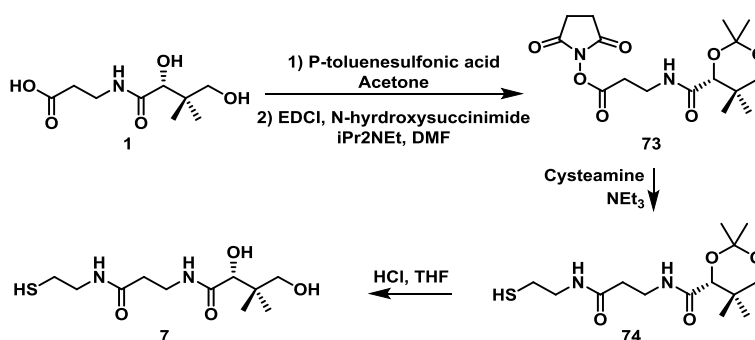


Figure 14. Burkart's alternative route to D-pantetheine

Another route to D-pantetheine **7** was described by Kandula in 2014 (Figure 15). The first 5 steps of this synthetic strategy were based on earlier the work of Marquez *et al.*, who focused on the synthesis of pantothenic acid **1**.⁶³ After opening D-pantolactone **8** with liquid ammonia and using the same acetal protecting strategy as described by Burkart, the resulting amide intermediate **76** was reacted with 1*H*-benzotriazole-1-carbaldehyde and *n*-butyl-lithium to give the corresponding formamide **77**. This was followed by a Wittig reaction and alkene hydrogenation in the presence of Pd/C. A second amide bond was formed with cysteamine **16** in the presence of HATU and triethylamine and this was followed by acetal deprotection using BiCl_3 to give the final product. Unfortunately, the overall yield of this process remains unknown because data were only reported for only three of the seven steps. However Kandula did report that 800 mg batches of D-pantetheine **7** were produced.⁶⁴

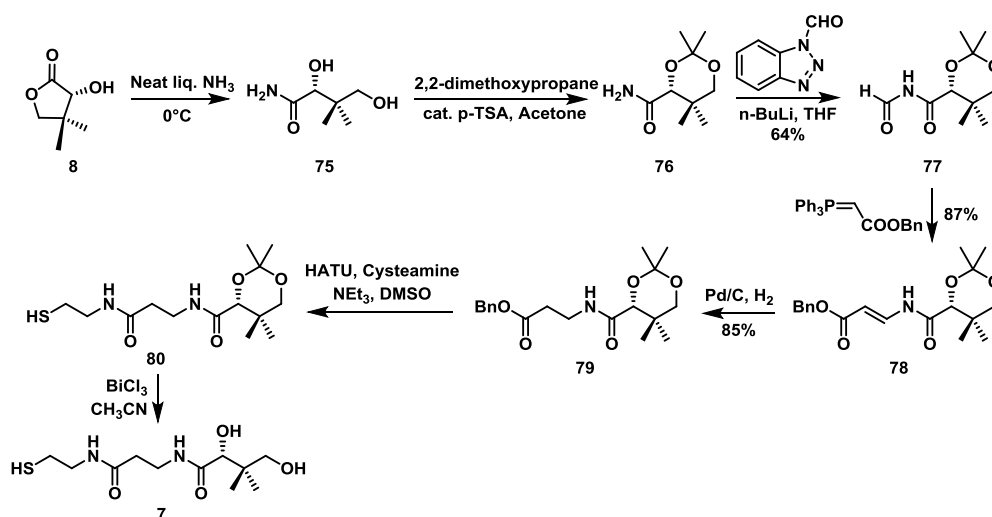


Figure 15. Kandula scheme to D-pantetheine

The last synthesis of D-pantetheine **7** and D-pantethine **46** reported in the literature was described by Stewart *et al.* in 2016 (Figure 16). This linear synthetic strategy involved the initial Boc protection of β -alanine **9** to yield **81**, which was condensed with cystamine using EDCI and HOBt to yield Boc-alethine **82**. As in previous approaches, the disulfide derivative acted both as a protecting group for the nucleophilic thiol as well as preventing undesired sulfur oxidation. After Boc deprotection using trifluoroacetic acid, alethine **83** was reacted with D-pantolactone **8** yielding D-pantethine **46**. Reduction of the disulfide bond using TCEP gave access to D-pantetheine **7**. Using this linear synthetic strategy, Stewart *et al.* accessed both D-pantetheine **7** and D-pantethine **46** in a 76% overall yield and in 5 g batches. The authors also reported that this route did not require any chromatographic purification steps, which facilitated greatly scale up of this method.⁶⁵

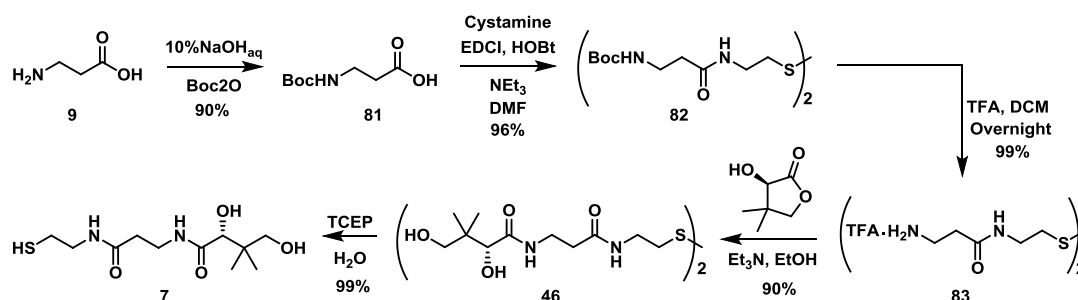


Figure 16. Stewart *et al.* scheme to D-pantethine and D-pantetheine

While all of these synthetic strategies yielded D-pantetheine **7** and/or D-pantethine **46**, they differed in regard to yield, scalability some contain one or more synthetically challenging steps. Three principal reagents that are common to nearly all synthetic strategies are D-pantolactone **8**, cysteamine **16** and β -alanine **9** (or their analogues). All data that were published for these different routes are summarized in Table 2.

Table 2. Summary of the different syntheses of D-pantetheine **7** and D-pantethine **46**

Method	Overall yield	Batch quantity	Chromatography required
Baddiley and Thain	70%	3.75 g	Yes
Brown and Snell	<i>ca.</i> 85%	200-900 mg	Yes
King <i>et al.</i>	23%	200 mg	Yes
Hosokawa <i>et al.</i>	70%	32 g	Yes
Burkart <i>et al.</i> (2004)	25%	5 mg	Yes
Burkart <i>et al.</i> (2005)	N/A	N/A	Yes
Kandula	47%	800 mg	Yes
Stewart and Mouterde	76%	5.04 g	No

Stewart *et al.* combined their synthetic method for D-pantetheine **7** and D-pantethine **46** with the observations made by Abiko ³ to develop a chemoenzymatic pathway to CoA. The catalytic enzymes activities of the three purified enzymes (PanK, PPAT and DPCK) were first verified using their natural substrate (D-pantetheine **7**). To overcome the issue of product inhibition, a sequential enzyme addition strategy was chosen, rather than a one-pot approach. Each enzymatic step proceeded with essentially complete conversion according to HPLC. Because these enzymes also accepted pantetheine analogs, the same sequence of enzymatic steps were applied to the disulfide derivative, pantethine **46**. These reactions were equally successful, and both ends of the dimer reacted completely. This conversion was carried out on a gram scale (using up to 720 mg of D-pantethine **46**) to yield 1.89 g of disulfide-CoA. The disulfide could be easily reduced with TCEP to yield free CoA with an 82% purity. The overall yield from β -alanine was 73% (Figure 17).⁶⁵ It should also be noted that this strategy was also used by Moore *et al.* in order to access acyl-CoAs.⁶⁶ The much greater chemical

stability of disulfide CoA and its precursors is a key advantage, allowing storage indefinitely at room temperature in the presence of oxygen.

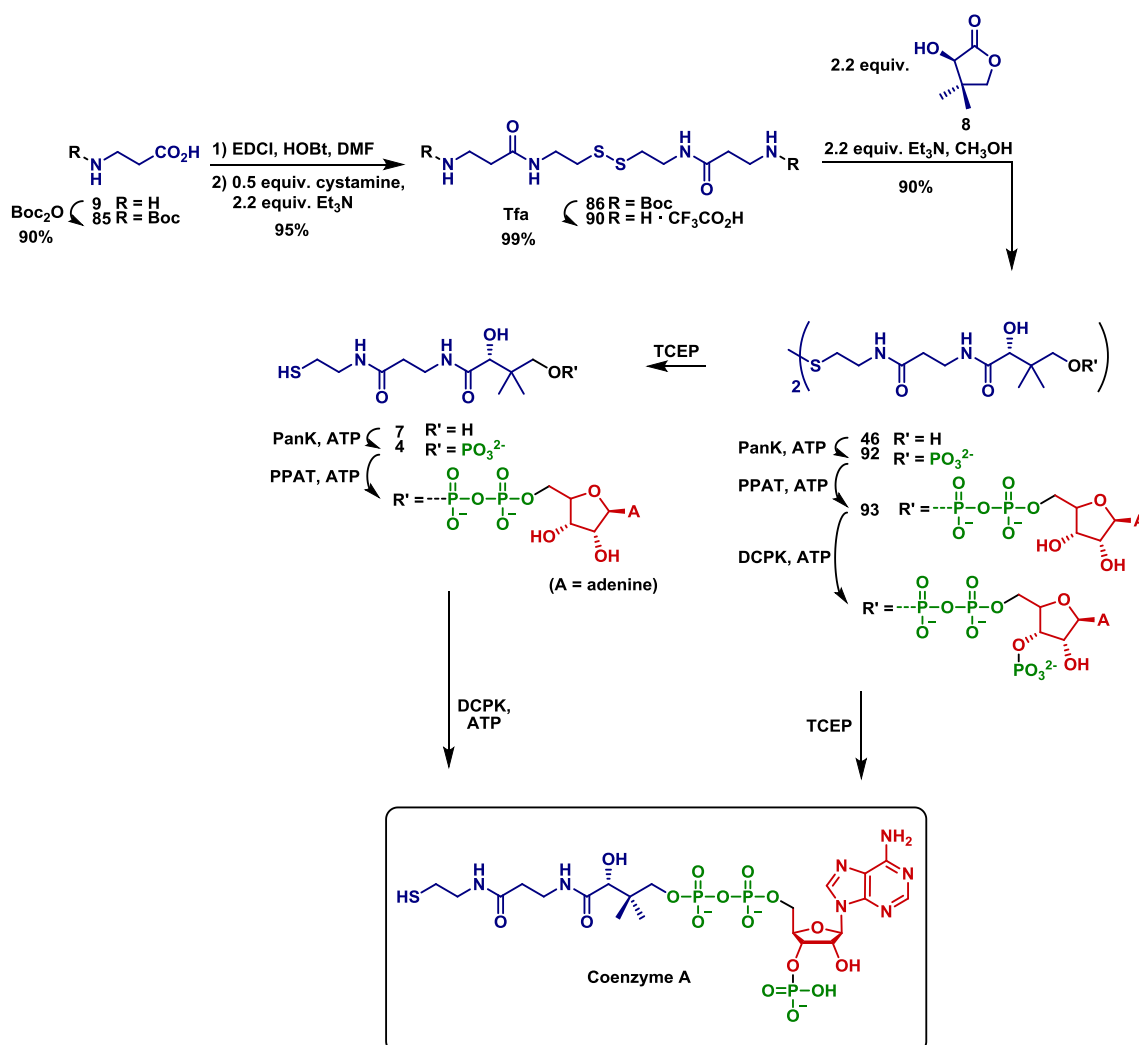


Figure 17. Stewart *et al.* chemoenzymatic scheme to CoA and its disulfide

Conclusion

Coenzyme A is an essential acyl carrier and co-factor for many biochemical processes. In addition to selectivity for the acyl moiety of CoA derivatives, enzymes also commonly interact with the cofactor portion and generally have little tolerance for structural perturbations. Because the CoA moiety is covalently joined, this cofactor is required in stoichiometric quantities. On small scales, *e.g.*, in biochemical assays, this is not a serious

problem; however, if acyl-CoA-requiring enzymes are to be used for preparative synthesis, the high cost of commercial CoA (\$2600 / g with >85% purity) becomes prohibitive.

Therefore, convenient, large-scale access to CoA has been the goal for numerous research teams over the past 70 years. A variety of methods have been described for CoA production, ranging from fermentation to total synthesis (including chemoenzymatic synthesis). This review has collected all methods known to be published to date, and these are summarized below in Table 3.

Table 3. Summary of the different pathways yielding to CoA

Author(s)	Method	Concentration	Yield (%)	Purity (%)
Buyske <i>et al.</i>	Isolation	22 mg/kg	-	26
Stadtman and Kornberg	Isolation	25 mg/kg	-	25
Crook <i>et al.</i>	Isolation	12 mg/kg	30	90
Nakao <i>et al.</i>	Isolation	30 µg/mL	13	90
Nishimura <i>et al.</i>	Isolation	600 µg/mL	33	96
Shimizu <i>et al.</i> (1973)	Isolation	3-5.5 mg/mL	34	80
Shimizu <i>et al.</i> (1974)	Isolation	2-3 mg/mL	67	85
Shimizu <i>et al.</i> (1979)	Isolation	0.5-1.2 mg/mL	65	91
Shimizu <i>et al.</i> (1983)	Isolation	115 mg/mL	68	93
Shimizu <i>et al.</i> (1984)	Isolation	11.5 mg/mL	65	91
Khorana <i>et al.</i>	Total synthesis	-	30	65
Gruber <i>et al.</i>	Total synthesis	-	25	70
Michelson	Total synthesis	-	63	75
Shimizu <i>et al.</i> (1965)	Total synthesis	-	30	80
Mukaiyama <i>et al.</i>	Total synthesis	-	36	98
Mushika <i>et al.</i>	Total synthesis	-	54	-
Stewart <i>et al.</i>	Chemoenzymatic	19 mg/mL	76	82

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References

1. Hoagland, M. B.; David Novelli, G., Biosynthesis of Coenzyme A from Phosphopantetheine and of Pantetheine from Pantothenate. *J. Biol. Chem.* **1954**, *207*, 767-773.
2. Brown, G. M., The Metabolism of Pantothenic Acid. *J. Biol. Chem.* **1958**, *234*, 370-378.
3. Abiko, Y., Investigations on Pantothenic Acid and its Related Compounds. *J. Biochem.* **1967**, *61*, 290-299.
4. Leonardi, R.; Zhang, Y.-M.; Rock, C. O.; Jackowski, S., Coenzyme A: Back in Action. *Prog. Lipid Res.* **2005**, *44*, 125-153.
5. Strauss, E., Coenzyme A Biosynthesis and Enzymology. In *Comprehensive of Natural Products II: Chemistry and Biology*, Elsevier, Ed.2010; pp 351-410.
6. Spry, C.; Kirk, K.; Saliba, K. J., Coenzyme A Biosynthesis: an Antimicrobial Drug Target. *FEMS Microbiol. Rev.* **2008**, *32*, 56-106.
7. Strauss, E.; de Villiers, M.; Rootman, I., Biocatalytic Production of Coenzymes A Analogues *ChemCatChem* **2010**, *2*, 929-937.
8. Mishra, P. K.; Drucehammer, D. G., Coenzyme A Analogues and Derivatives: Synthesis and Applications as Mechanistic Probes of Coenzyme A Ester-Utilizing Enzymes *Chem. Rev.* **2000**, *100*, 3283-3309.
9. Lipmann, F.; Kaplan, N. O.; Novelli, G. D.; Tuttle, C. L.; Guirard, B. M., Coenzyme for Acetylation, a Pantothenic Acid Derivative. *J. Biol. Chem.* **1947**, *167*, 869-870.
10. Kaplan, N. O.; Lipmann, F., The Assay and Distribution of Coenzyme A. *J. Biol. Chem.* **1948**, *174*, 37-44.
11. Lipmann, F.; Kaplan, N. O.; Novelli, G. D.; Tuttle, C. L., Isolation of Coenzyme A. *J. Biol. Chem.* **1950**, *186*, 235-242.
12. Buyske, D. M.; Handschumacher, R. E.; Higgins, H.; King, T. E.; Strong, F. M.; Cheldelin, V. H.; Teply, L. J.; Mueller, G. C., Preparation and Purification of Coenzyme A Concentrates. *J. Biol. Chem.* **1951**, *193*, 307-316.
13. DeVries, W. H.; Grover, W. M.; Evans, J. S.; Gregory, J. D.; Novelli, G. D.; Soodak, M.; Lipmann, F., Purification of Coenzyme A from Fermentation Sources and its further Partial Identification. *J. Am. Chem. Soc.* **1950**, *72*, 4838.
14. Beinert, H.; Von Korff, R. W.; Green, D. E.; Buyske, D. A.; Handschumacher, R. E.; Higgins, H.; Strong, F. M., A Method for Purification of Coenzyme A. *J. Am. Chem. Soc.* **1951**, *74*, 854-855.
15. Beinert, H.; Von Korff, R. W.; Green, D. E.; Buyske, D. A.; Handschumacher, R. E.; Higgins, H.; Strong, F. M., A Method for the Purification of Coenzyme A from Yeast. *J. Biol. Chem.* **1953**, *200*, 385-400.
16. Stadtman, E. R.; Kornberg, A., The Purification of Coenzyme A by Ion Exchange Chromatography. *J. Biol. Chem.* **1953**, *203*, 47-54.
17. Reece, M. C.; Donald, M. B.; Crook, E. M., The Evaluation of a Process for the Preparation of Coenzyme A from Yeast. *J. Biochem. Microbiol. Technol. Eng.* **1959**, *1*, 217-228.
18. Kuno, M.; Kikuchi, M.; Nakao, Y., Production of Coenzyme A by *n*-Paraffins-assimilating Microorganisms. *Agric. Biol. Chem.* **1973**, *37*, 313-319.
19. Nishimura, N.; Shibatani, T.; Kakimoto, T.; Chibata, I., Production of Coenzyme A by *Sarcina lutea*. *Appl. Microbiol.* **1974**, *28*, 117-123.
20. Shimizu, S.; Mityata, K.; Tani, Y.; Ogata, K., An Improved Method for the Fermentative Production of Coenzyme A from Pantothenic Acid, Cysteine, and 5'-AMP. *Agric. Biol. Chem.* **1973**, *37*, 607-613.

21. Ogata, K.; Shimizu, M.; Tani, Y., A New Preparation Method of Coenzyme A. *Agric. Biol. Chem.* **1970**, *34*, 1757-1759.
22. Shimizu, M.; Miyata, K.; Tani, Y.; Ogata, K., A New Process for the Production of Coenzyme A and its Intermediates with a Microorganism. *Biochim. Biophys. Acta* **1972**, *279*, 583-586.
23. Shimizu, S.; Miyata, K.; Tani, Y.; Ogata, K., A New Process for the Production of Coenzyme A. *Agric. Biol. Chem.* **1973**, *37*, 615-619.
24. Chibata, I.; Tosa, T.; Sato, T., Immobilized Aspartase-Containing Microbial Cells: Preparation and Enzymatic Properties. *Appl. Microbiol.* **1974**, *27*, 878.
25. Shimizu, S.; Tani, Y.; Ogata, K., Synthesis of Coenzyme A and Its Biosynthetic Intermediates by Microbial Processes. *Meth. in Enzymol.* **1979**, *62*, 236-245.
26. Shimizu, M.; Tani, Y.; Hideaki, Y., Synthesis of Coenzyme A by Immobilized Bacterial Cells. *ACS Symp. Ser.* **1979**, *106*, 87-100.
27. Shimizu, M.; Kubo, K.; Morioka, H.; Tani, Y.; Ogata, K., Some Aspects of the Enzyme Activities Involved in Coenzyme A Biosynthesis in Various Microorganisms. *Agric. Biol. Chem.* **1974**, *38*, 1015-1021.
28. Shimizu, M.; Kubo, K.; Tani, Y.; Ogata, K., Purification and Properties of Pantothenate Kinase from *Brevibacterium ammoniagenes* IFO 12071. *Agric. Biol. Chem.* **1973**, *37*, 2863-2870.
29. Shimizu, M.; Komaki, R.; Tani, Y.; Yamada, H., A High Yield Method for the Preparative Synthesis of Coenzyme A by Combination of Chemical and Enzymic Reactions. *FEBS Lett.* **1983**, *151*, 303-306.
30. Stewart, C. J.; Ball, W. J., Coenzyme A analogs. II. Enzymatic conversion of D-oxypantetheine 4'-phosphate to oxy-coenzyme A. *Biochemistry* **1966**, *5*, 3883-3886.
31. Shimizu, M.; Esumi, A.; Komaki, R.; Yamada, H., Production of Coenzyme A by a Mutant of *Brevibacterium ammoniagenes* Resistant to Oxypantetheine. *Appl. Environ. Microbiol.* **1984**, *48*, 1118-1122.
32. Novelli, G. D.; Schmetz, F. J.; Kaplan, N. O., Enzymatic Degradation and Resynthesis of Coenzyme A. *J. Biol. Chem.* **1953**, *206*, 533-545.
33. Levintow, L.; Novelli, G. D., The Synthesis of Coenzyme A from Pantetheine: Preparation and Properties of Pantetheine Kinase. *J. Biol. Chem.* **1954**, *207*, 761-765.
34. Baddiley, J.; Thain, E. M., Coenzyme A. Part VIII. The Synthesis of Pantetheine 4'-Phosphate (Acetobacter Stimulatory Factor), a Degradation Product of the Coenzyme. *J. Chem. Soc.* **1953**, 1610-1615.
35. Moffatt, J. G.; Khorana, H. G., The Total Synthesis of Coenzyme A. *J. Am. Chem. Soc.* **1959**, *81*, 1265-1265.
36. Moffatt, J. G.; Khorana, H. G., Nucleoside Polyphosphates. XII.1 The Total Synthesis of Coenzyme A. *J. Am. Chem. Soc.* **1961**, *83*, 663-675.
37. Gruber, W.; Lynen, F., Dinucleotidsynthesen Mit Pyrophosphoryltetrachlorid. Eine Synthese von Coenzym A. *Eur. J. Org. Chem.* **1962**, *659*, 139-156.
38. Michelson, A. M., Synthesis of Coenzyme A. *Biochim. Biophys. Acta* **1964**, *93*, 71-77.
39. Shimizu, M.; Nagase, O.; Okada, S.; Hosokawa, Y.; Tagawa, H., A Total Synthesis of Coenzyme A via Thiazoline Intermediate. *Chem. Pharm. Bull.* **1965**, *13*, 1142-1144.
40. Shimizu, M.; Nagase, O.; Okada, S.; Hosokawa, Y.; Tagawa, H.; Abiko, Y.; Suzuki, T., Investigations on Pantothenic Acid and its Related Compounds.V. Chemical Studies. A total Synthesis of Coenzyme A via Thiazoline Intermediate. *Chem. Pharm. Bull.* **1967**, *15*, 655-662.
41. Naoi-Tada, M.; Sato-Asano, K.; Egami, F., Ribonuclease in Taka-Diastase. III. Purification and Properties of Ribonuclease T2. *J. Biochem.* **1959**, *46*, 757-764.

42. Hashimoto, M.; Mukaiyama, T., A Total Synthesis of Coenzyme A by Oxidation-Reduction Condensation. *Chem. Lett.* **1972**, *1*, 595-598.
43. Taguchi, Y.; Nishimura, N.; Kakimoto, T.; Mushika, Y., Synthetic Studies on Phosphorylating Reagent. V. A Convenient Synthesis of 2',3'-Cyclic Coenzyme A. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 1122-1125.
44. Song, W.-J.; Jackowski, S., Cloning, Sequencing, and Expression of the Pantothenate Kinase (*coaA*) Gene of *Escherichia coli*. *J. Bacteriol.* **1992**, *174*, 6411-6417.
45. Geerlof, A.; Lewendon, A.; Shaw, W. V., Purification and Characterization of Phosphopantetheine Adenylyltransferase from *Escherichia coli*. *J. Biol. Chem.* **1999**, *274*, 27105-27111.
46. Mishra, P. K.; Park, P. K.; Drueckhammer, D. G., Identification of *yacE* (*coaE*) as the Structural Gene for Dephosphocoenzyme A Kinase in *Escherichia coli* K-12. *J. Bacteriol.* **2001**, *183*, 2774-2778.
47. Billhart, U.; Stein, P.; Whitesides, G. M., Enzymatic Methods for the Preparation of Acetyl-CoA and Analogs. *Bioorg. Chem.* **1989**, *17*, 1-12.
48. Martin, D. P.; Bibart, R. T.; Drueckhammer, D. G., Synthesis of Novel Analogs of Acetyl Coenzyme A: Mimics of Enzyme Reaction Intermediates *J. Am. Chem. Soc.* **1993**, *116*, 4660-4668.
49. Vogel, K. W.; Drueckhammer, D. G., A Reversed Thioester Analogue of Acetyl-Coenzyme A: an Inhibitor of Thiolase and a Synthron for other Acyl-CoA Analogues. *J. Am. Chem. Soc.* **1998**, *120*, 3275-3283.
50. Bibart, R. T.; Vogel, K. W.; Drueckhammer, D. G., Development of a Second Generation Coenzyme A Analogue Synthron. *J. Org. Chem.* **1999**, *64*, 2903-2909.
51. Vogel, K. W.; Stark, L. M.; Mishra, P. K.; Yang, W.; Drueckhammer, D. G., Investigating the Role of the Geminal Dimethyl Groups of Coenzyme A: Synthesis and Studies of a Didemethyl Analogue. *Bioorg. Med. Chem.* **2000**, *8*, 2451-2460.
52. Rootman, I.; de Villiers, M.; Brand, L. A.; Strauss, E., Creating CBD-Fusions of the CoA Biosynthetic Enzymes to Enable Reactor-Based Biotransformations. *ChemCatChem* **2010**, *2*, 1239-1251.
53. Strauss, E.; Begley, T. P., The Antibiotic Activity of N-Pentylpantothenamide Results from Its Conversion to Ethyldethia-Coenzyme A, A Coenzyme A Antimetabolite. *J. Biol. Chem.* **2002**, *277*, 48205-48209.
54. Choudry, A. E.; Mandichak, T. L.; Broskey, J. P.; Egolf, R. W.; Kinsland, C.; Begley, T. P.; Seefeld, M. A.; Ku, T. W.; Brown, J. R.; Zalacain, M.; Ratnam, K., Inhibitors of Pantothenate Kinase: Novel Antibiotics for Staphylococcal Infections. *Antimicrob. Agents Chemother.* **2003**, *47*, 2051-2055.
55. Sifferd, R. H.; du Vigneau, V., A New Synthesis of Carnosine, with some Observations on the Splitting of the Benzyl Group from Carbobenzoxo Derivatives and from Benzylthio Ethers. *J. Biol. Chem.* **1934**, *108*.
56. Baddiley, J.; Thain, E. M., A new and Convenient Synthesis of Pantetheine (Lactobacillus bulgaricus Factor). *J. Chem. Soc.* **1952**, 800-803.
57. Wittle, E. L.; Moore, J. A.; Stipek, R. W.; Peterson, F. E.; McGlohon, V. M.; Bird, O. D.; Brown, G. M.; Snell, E. E., The Synthesis of Pantetheine-Pantethine. *J. Am. Chem. Soc.* **1953**, *75*, 1694-1700.
58. King, T. E.; Stewart, C. J.; Cheldelin, V. H., β -Aletheine and Pantetheine. *J. Am. Chem. Soc.* **1953**, *75*, 1290-1292.
59. Shimizu, M.; Ohta, G.; Nagase, O.; Okada, S.; Hosokawa, Y., A Novel Synthesis of Pantethine. *Chem. Pharm. Bull.* **1965**, *13*, 180-188.
60. Mandel, A. L.; La Clair, J. J.; Burkart, M. D., Modular Synthesis of Pantetheine and Phosphopantetheine. *Org. Lett.* **2004**, *6*, 4801-4803.

61. Burkart, A. S.; Laclair, J. Analysis and Manipulation of Enzymes in Biosynthetic Proteomes Using Labeled Carrier Proteins. WO2005003307, 2005.
62. Burkart, A. S.; Worthington, M., D., One-Pot Chemo-Enzymatic Synthesis of Reporter-Modified Proteins. *Org. Biomol. Chem.* **2006**, *4*, 44-46.
63. Sewell, A. L.; Villa, V. J.; Matheson, M.; Whittingham, W. G.; Marquez, R., Fast and Flexible Synthesis of Pantothenic Acid and CJ-15,801. *Org. Lett.* **2011**, *13*, 800-803.
64. Kandula, M. Compositions and Methods for the Treatment of Inflammation and Lipid Disorder. WO2014037834 2014.
65. Mouterde, L. M. M.; Stewart, J. D., An Efficient Chemoenzymatic Synthesis of Coenzyme A and Its Disulfide. *Org. Process Res. Dev.* **2016**, *20*, 954-959.
66. Agarwal, V.; Diethelm, S.; Ray, L.; Garg, N.; Awakawa, T.; Dorrestein, P. C.; Moore, B. S., Chemoenzymatic Synthesis of Acyl Coenzyme A Substrates Enables in Situ Labeling of Small Molecules and Proteins. *Org. Lett.* **2015**, *17*, 4452-4455.