DESIGN AND SYNTHESIS OF MECHANISTIC PROBES FOR POLYHYDROXYBUTYRATE SYNTHASES

by

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ABSTRACT

Biodegradable polyhydroxybutyrates (PHBs) produced by a wide range of bacteria have been considered as an ideal alternative to petroleum-based plastics. Two types of mechanistic probes have been synthesized in order to understand the mechanism of PHB synthases (PhaCs). The first type is oxo analogs in which the sulfur in the coenzyme A (CoA) thioester has been replaced with an oxygen atom. A series of 3-*R*-hydroxybutyryl oxo CoA analogs, $(HB)_nOCoA$ (n = 1, 2 and 3), were synthesized chemoenzymatically in good yields. Two models involving covalent catalysis with Cys have been proposed for the chain elongation catalyzed by PhaCs. The first involves an active site composed of two monomers in which the growing hydroxybutyrate (HB) chain alternates between Cys on each monomer. The second involves noncovalent intermediates $(HB)_nCoA$ ($n \geq 2$). Here the substrate analog HBOCoA was successfully employed to trap the noncovalent intermediates in the reactions catalyzed by class III PhaC from *Allochromatium Vinosum*, which supports our preferred second mechanistic model. Furthermore, it is also the first time that a wild-type (*wt*) synthase was used to investigate the chain elongation models.

The other type of mechanistic probes is 3-*R*-hydroxyalkyl CoA that was used to investigate the substrate specificity of PhaCs from different classes. Substrate availability has been a challenge to study PHB synthases *in vitro*. Starting with commercially available dimethyl *S*-malate, the intermediate *S*-ethyl 2-(oxiran-2-yl) acetate **23** was synthesized *via* a ring-opening reaction involving lactone **21** and

trimethylsilyl iodide followed by an oxidation reaction involving silver oxide. The regiospecific ring-opening reaction of epoxide **23** with different organometallic reagents afforded a straightforward access to ethyl 3-*R*-hydroxybutanoates attached with a variety of side chains. The final CoA compounds were obtained through the thiotransesterification reaction between corresponding benzenethioesters and the thiol group in CoA. This synthetic approach provides a new avenue to modifications of alkyl groups in 3-*R*-hydroxyalkyl CoA in an efficient manner.

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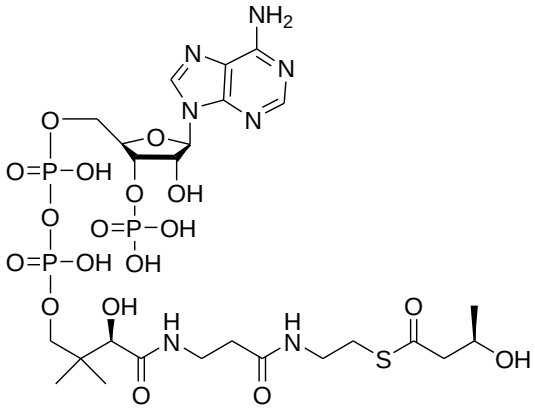

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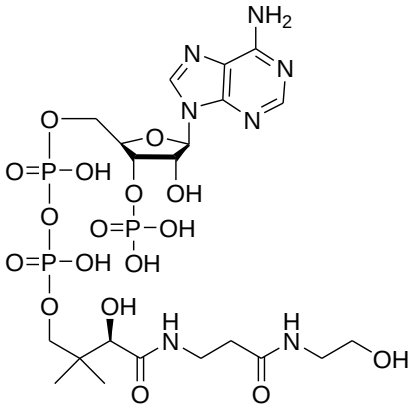

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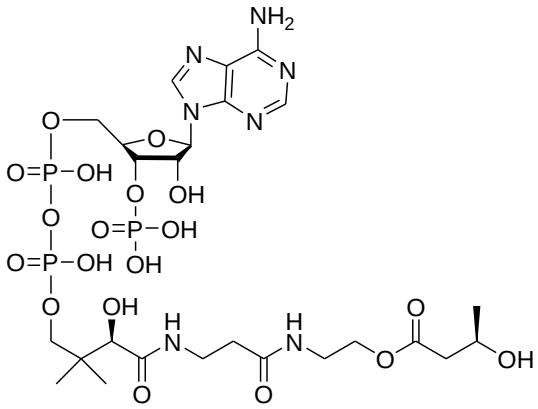

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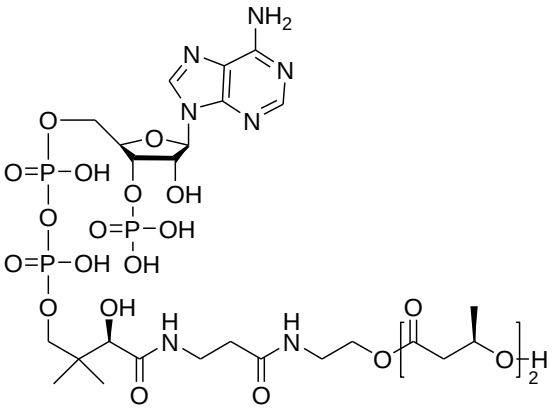

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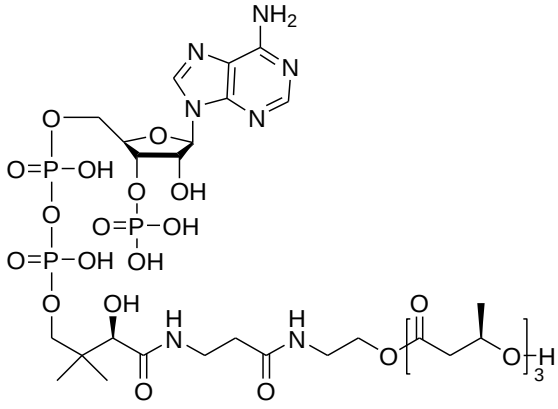

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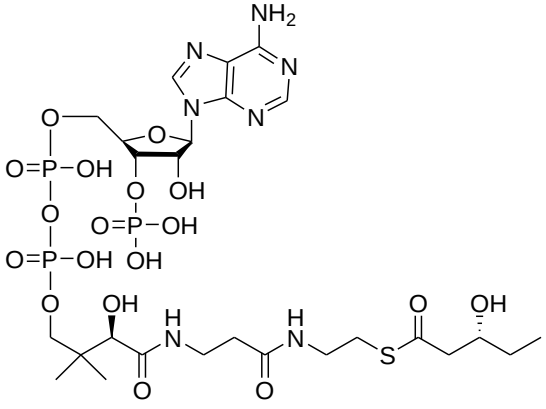

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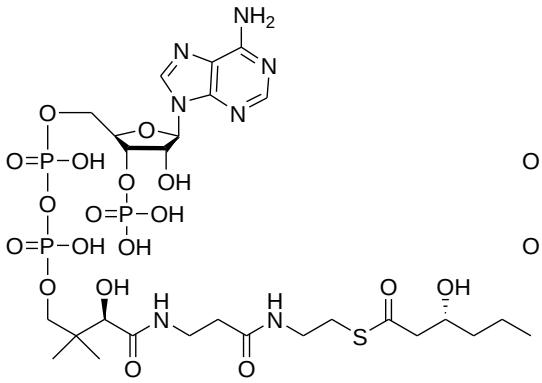

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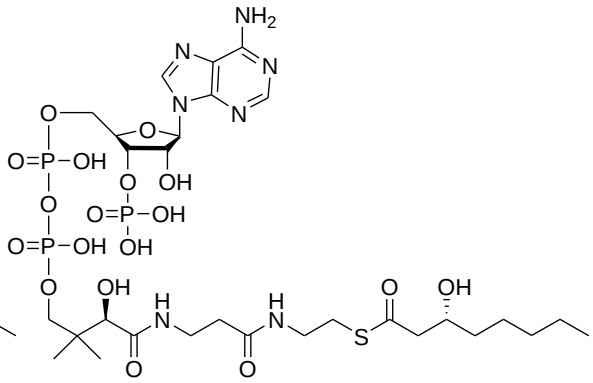

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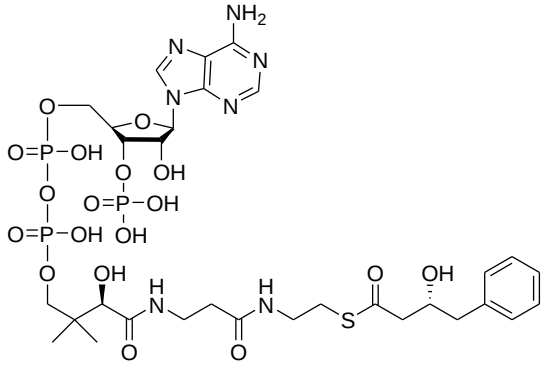

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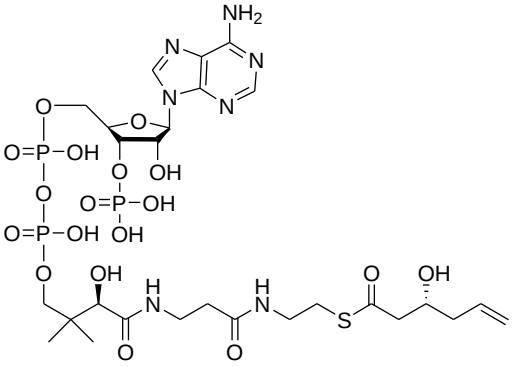

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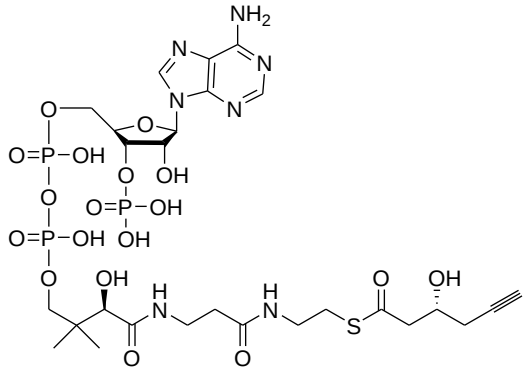

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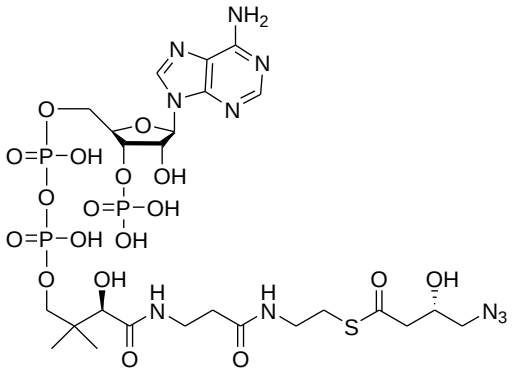

CoA analog 65

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Finally, let me thank my parents and my wife for their endless love and constant encouragement throughout these years.

CHAPTER 1 - Design and Synthesis of Mechanistic Probes for Trapping Noncovalent Intermediates in *wt*-PhaEC$_{AV}$-Catalyzed Polymerizations

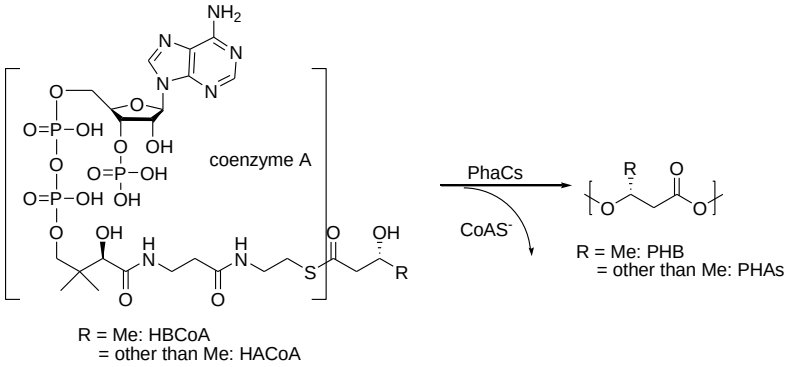
The paper published in the journal of *ACS Chemical Biology* (ASAP, **DOI:** 10.1021/cb5009958) includes this work.

1.1 Introduction

Petroleum-based plastics have led to environmental problems because they are non-biodegradable and environmentally hazardous. A large amount of plastic waste accumulates in the oceans and has adverse effects on marine ecosystems. Furthermore, combustion of plastic wastes is a common disposal method, resulting in the release of harmful pollution-causing wastes into the air.[1]

The advent of polyhydroxyalkanoates (PHAs) as promising alternatives to traditional plastics has drawn much attention because these materials are biodegradable and can be produced by a wide range of bacteria and plants.[2-3] Recently, the commercialization of PHAs has been considerably improved by Metabolix, Inc., a biological products company, that employs plants such as switchgrass and tobacco to produce a large volume of PHAs in the manner that is economically competitive with traditional plastics. As a result of its important contribution to the commercialization of PHAs, Metabolix, Inc. received the Presidential Green Chemistry Challenge Award

in 2005 and a 6 million-dollar grant from the U.S. Department of Energy in 2011.[4]



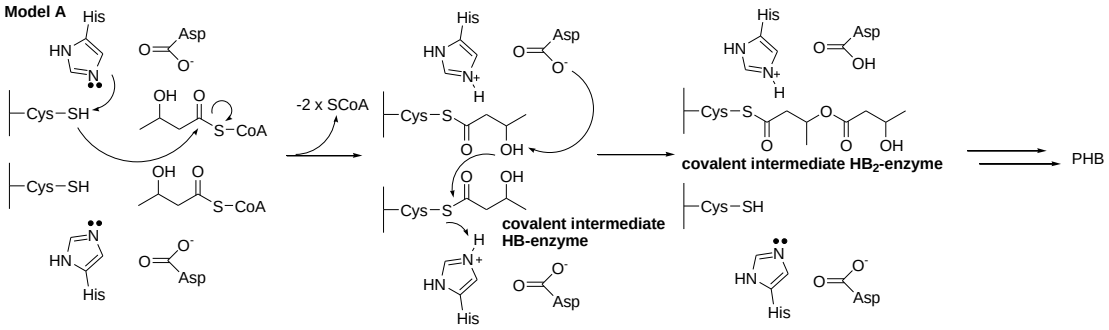
Scheme 1- 1 Structures of CoA analog and PHAs synthesis by PhaCs

As described in Scheme **1 - 1**, polymerizations of 3-*R*-hydroxyalkanoate-coenzyme A (HACoA) are catalyzed by PhaCs, which are representatives of a large number of polymerases found in bacteria and can be classified into four categories based on their different subunits. $PhaEC_{AV}$ investigated in our lab is obtained from *Allochromatium vinous*. This synthase is a class III enzyme composed of two subunits, PhaC and PhaE, whose molecular weights are 39 and 40k Da, respectively.[5-7]

PhaCs utilize HACoA to generate polyoxoesters, PHAs. Among all substrates, 3-*R*-hydroxybutyryl-coenzyme A (HBCoA) bears the simplest chemical structure and its synthesis is also relatively simple.[8] These advantages make HBCoA an ideal prototype with which to design mechanistic probes for $PhaEC_{AV}$-catalyzed polymerizations.

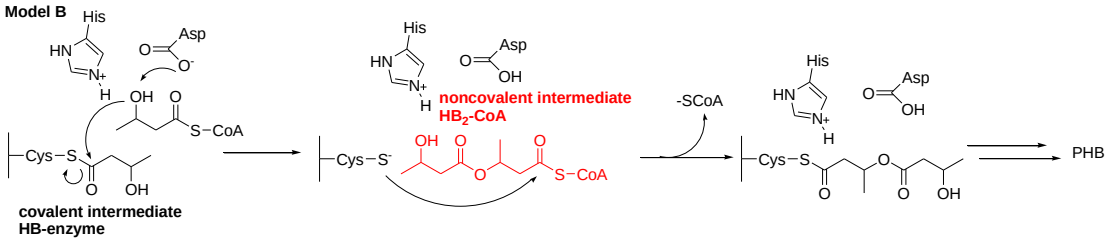
In 2003, Stubbe and coworkers proposed two mechanistic models for polymerization reactions of HBCoA catalyzed by PhaCs.[6] Model **A** was based on an analogy to fatty

acid biosynthesis. As shown in Scheme **1 – 2**, histidine (His) initially deprotonates cysteine (Cys) for nucleophilic attacks to thioester bonds in HBCoA, resulting in formation of covalent intermediates (HB-PhaCs) as well as releases of CoA. An aspartate, a general base catalyst in PhaCs, activates one of HB-PhaCs for a nucleophilic attack to another. As a result, a growing $(HB)_n$ chain is always covalently bound to PhaCs and alternates between two active sites.



Scheme 1- 2 Proposed mechanistic Model A for formation of PHB

This model requires two active sites to be close to the PhaCs surface. However, the lipases crystal structure and the molecular docking of $PhaEC_{AV}$ revealed that these active sites are deeply buried, rather than close to the surface.[9-10]



Scheme 1- 3 Proposed mechanistic Model B for formation of PHB

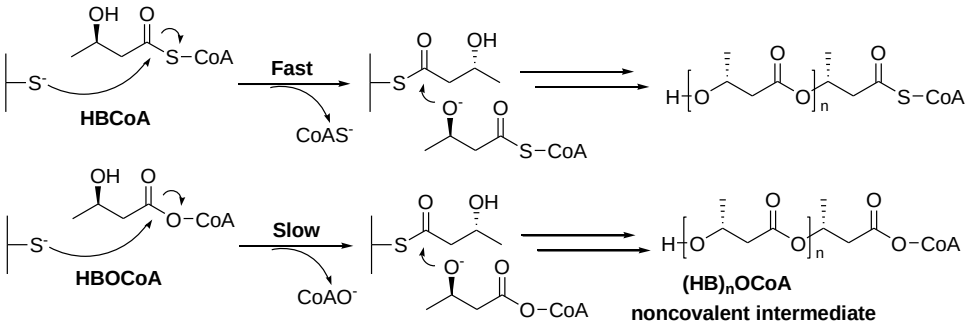
Therefore, Model **B** was proposed as described in Scheme **1 - 3**. After formation of

the covalent intermediate HB-PhaCs, an aspartate deprotonates the hydroxyl group in HBCoA, rather than the hydroxyl group in HB-PhaCs, for a nucleophilic attack to HB-PhaCs. As a result, a noncovalent intermediate $(HB)_2CoA$ (highlighted in the box) is obtained which subsequently reacylates Cys for the further growth of $(HB)_n$ chain.

According to Model **B**, noncovalent intermediates $(HB)_nCoA$ ($n \geq 2$) form which in contrast are absent in Model **A**. It seemed straightforward to distinguish between these models by detecting the existence of $(HB)_nCoA$ in the progress of the polymerization reactions. Unfortunately, previous attempts to trap those intermediates were not successful, even if a rapid flow-quench apparatus was employed. It is because the polymerization rate of HBCoA is much faster than that of initiation.[11] Therefore, the mechanism for PhaCs remains unclear. Herein, a series of 3-*R*-hydroxybutyryl oxo CoA analogs $(HB)_nOCoA$ (n = 1, 2 and 3) were designed and synthesized to decrease the polymerization rate, making those attempts successful.

1.2 Results and Discussion

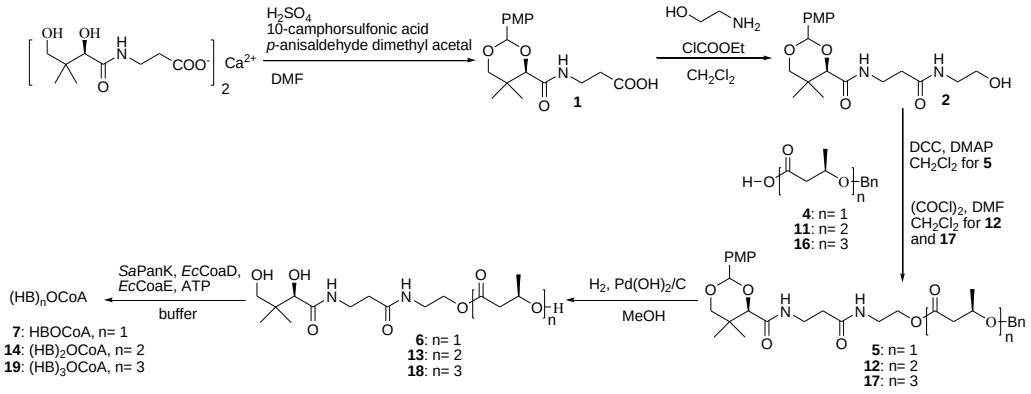
1.2.1 Design of HBOCoA as a Mechanistic Probe



Scheme 1- 4 HBCoA and HBOCoA loading onto $PhaEC_{AV}$ via Model B

Compared to a native substrate HBCoA, substitution of a sulfur atom in HBCoA with an oxygen atom in HBOCoA may greatly decrease the $PhaEC_{AV}$-catalyzed polymerization rate because the conversion of oxo esters to thioesters in enzymatic (re)acylation steps is predicted to be thermodynamically unfavorable.[12]

1.2.2 Chemoenzymatic Synthesis of $(HB)_nOCoA$ (n = 1, 2 and 3)



Scheme 1- 5 Chemoenzymatic synthesis of (HB)nOCoA

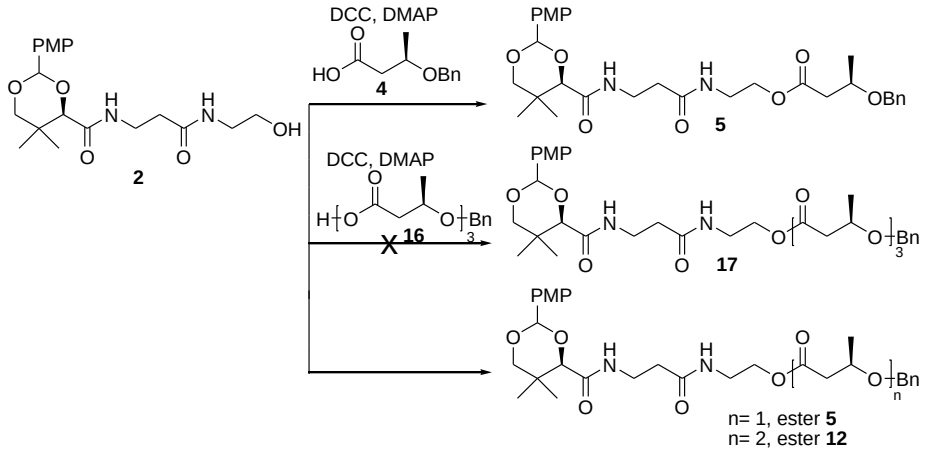
The synthetic route to $(HB)_nOCoA$ (n = 1, 2 and 3) is described in Scheme **1** - **5**. Starting with D-calcium pantothenate, the 1, 3-diol group in pantothenate was first protected with a *p*-methoxyphenyl group (PMP) to generate acid **1**. An amidation of carboxylic acid **1** with 2-aminoethanol was accomplished in the presence of ethyl chloroformate to generate amide **2**. Coupled with carboxylic acids **4**, **11** and **16**, respectively, amide **2** was converted to PMP-protected esters **5**, **12** and **17**. Depending on chemical structures of $(HB)_n$-carboxylic acids **4**, **11** and **16**, different coupling reagents were employed for formation of the corresponding esters. After a removal of PMP and benzyl (Bn) protecting groups from these esters in a single step, enzymatic precursors **6**, **13** and **18** were obtained which were subsequently converted to

$(HB)_nOCoA$ (n= 1, 2 and 3) using a chemoenzymatic approach involved a combination of three enzymes. Since this thesis mainly focuses on the synthetic route to CoA analogs, experimental details are discussed herein.

Although Burkart and coworkers previously synthesized carboxylic acid **1**, three steps were involved: an acidification of D-calcium pantothenate to the corresponding carboxylic acid using the ion exchange resin, a removal of aqueous solution with lyophilization and the protection of the residue with a PMP group.[13] Following this approach, compound **1** was first prepared in our lab. However, it was found that its yield (≤ 30%) was very low because a small amount of water in the residue could decrease the yield and it was difficult to dry it completely. To simplify the experimental procedure and increase the yield, we first treated a solution of D-calcium pantothenate in dry DMF with concentrated H_2SO_4 followed by the addition of *p*-anisaldehyde dimethyl acetal to generate carboxylic acid **1**. This modification increased the yield up to 65%. The choice of the PMP rather than a dimethyl acetal group as the protecting group to 1, 3 diol was because in the presence of $Pd(OH)_2/C$ and H_2, removals of both of Bn and PMP groups to afford $(HB)_nOCoA$ precursors, were readily accomplished in a single step with excellent yields.

To obtain amide **2**, the first approach seemed straightforward. The amidation of carboxylic acid **1** with 2-(benzyloxy) ethanamine was accomplished using coupling reagents, 1-ethyl-3-(3- dimethylaminopropyl) carbodiimide (EDCI) and

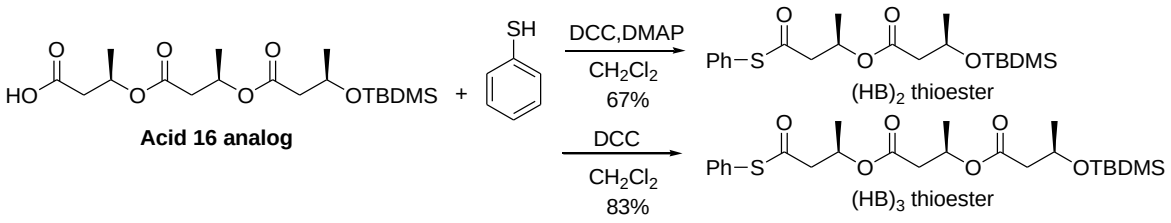
hydroxybenzotriazole (HOBt). The Bn group was then removed in the presence of Pd/C and H_2. However, it was found that a hydrogenation with Pd/C and H_2 led to a partial cleavage of the PMP group. To overcome this problem and simplify the synthetic route, we employed an alternative approach. Treated with ethyl chloroformate, carboxylic acid **1** was first converted to the corresponding acid anhydride. The unprotected 2-aminoethanol then reacted with the acid anhydride to generate thermodynamically stable amide **2** with a good yield (76%). Since 2-aminoethanol contains OH and NH_2 groups, concerns of regioselectivity were raised for formation of amide **2**. It was noted that no ester products were isolated by silica gel chromatography. Consequently, the hydrogenation was not required.



Scheme 1- 6 Formation of esters 5 and 12 catalyzed by DCC and DMAP

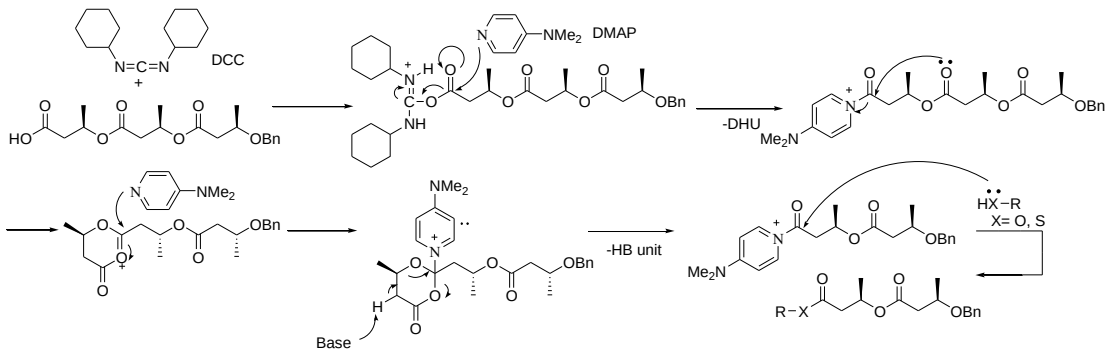
The key step was the esterification between a hydroxyl group in **2** and carboxylic acid in $(HB)_n$ units (n = 1, 2, and 3). Formation of ester **5** between hydroxyl **2** and carboxylic acid **4** was successfully accomplished using N, N-dicyclohexyl-carbodiimide (DCC) and 4-dimethylamino pyridine (DMAP). Unfortunately, a combination of DCC and DMAP gave rise to unexpected products when they were

employed for formation of esters **12** and **17**, respectively. For example, esters **5** and **12** was isolated, when $(HB)_3$ acid **16** was treated with DCC and DMAP followed by the addition of hydroxyl **2**. No desired ester **17** was observed after purification by silica gel chromatography. To obtain desired compounds **12** and **17**, corresponding acids were first converted to acyl chlorides using oxalyl chloride and a catalytic amount of dimethylformamide (DMF) and subsequently treated with hydroxyl **2**. This method successfully generated the desired esters **12** and **17** with good yields.



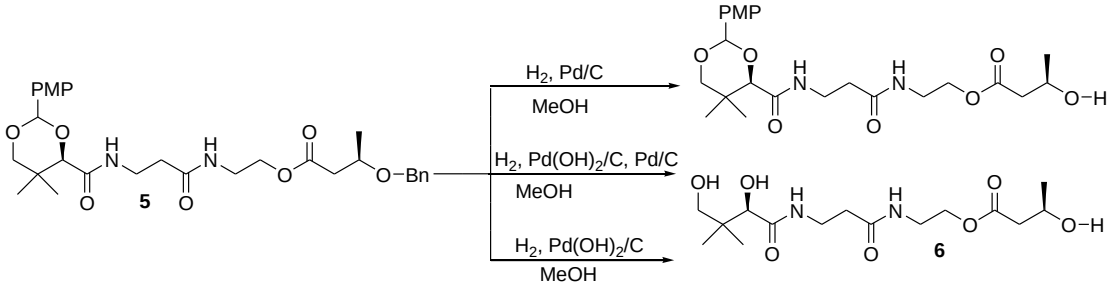
Scheme 1- 7 Formation of unexpected thioesters using DCC and DMAP

The similar observation is described in Scheme **1 - 7**. We were unsuccessful in synthesizing $(HB)_3$ thioester when acid **16** analog was treated with DCC and DMAP followed by addition of benzenethiol. Purification by silica gel chromatography revealed that $(HB)_2$ thioester was a major product rather than $(HB)_3$ thioester. Interestingly, in the absence of DMAP, DCC promoted thioester bond formation to afford the desired product, $(HB)_3$ thioester. This result is due to the fact that the thiol group is more nucleophilic than the hydroxyl group.



Scheme 1- 8 Proposed mechanism for formation of the ester (thioester)

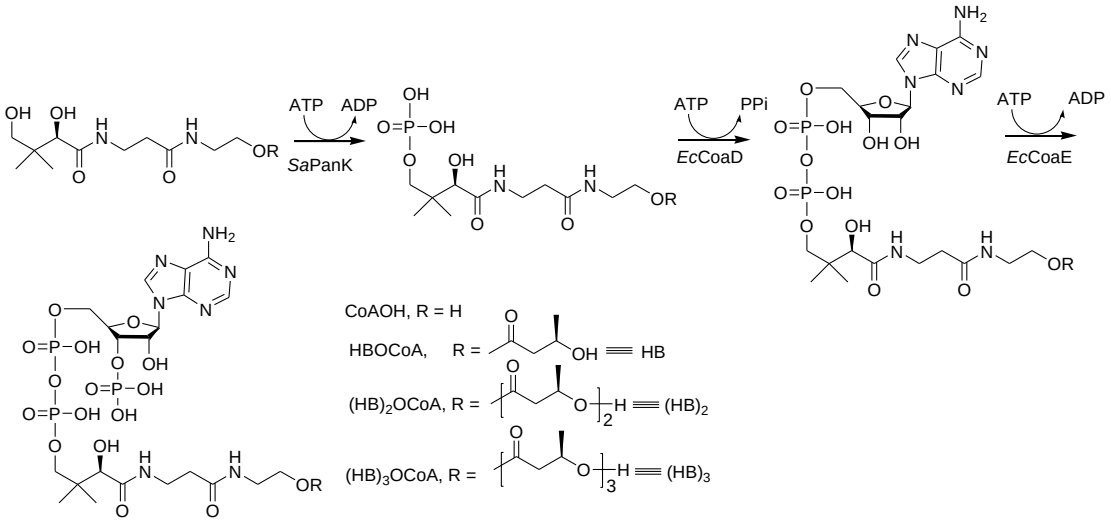
Based on these experimental results as well as those of previous work by our lab,[10] I proposed a mechanism for formation of esters (or thioester) with one or two HB units missing. As described in Scheme **1 – 8**, DCC activates carboxylic acid via formation of a reactive *O*-acylisourea intermediate to which DMAP then nucleophilically attacks to generate a reactive amide. Subsequently an intramolecular cyclization promoted by the next ester bond generates a six-member ring. Under a basic condition, an elimination reaction generates a reactive intermediate, $(HB)_2$-DMAP amide, leading to a cleavage of one HB unit. The further cyclization may occur to lead to a loss of the second HB unit, giving HB-DMAP amide. However, in this case we were not able to isolate released HB units due to complicated TLC patterns.



Scheme 1- 9 Hydrogenation of 5 with different palladium catalysts

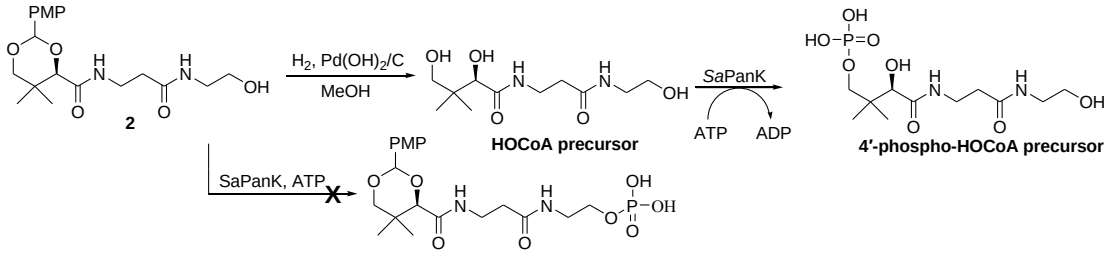
The next step was the hydrogenation of esters **2**, **5**, **12** and **17** respectively, to afford

enzymatic precursors. The first attempt to remove both Bn and PMP groups in one pot was successful by using a combination of palladium catalysts Pd/C and $Pd(OH)_2/C$, because Pd/C is generally used to remove a Bn group while $Pd(OH)_2/C$ is for a removal of a PMP group. This successful experience inspired us to think about if one of palladium catalyts could achieve this goal. As described in Scheme **1 - 9**, the hydrogenation carried out in the presence of H_2 and Pd/C for up to 2 days could not completely remove the PMP group from compound **5**, whereas the Bn group was reduced to toluene and removed. In contrast, PMP and Bn groups were quickly removed from compound **5** with an excellent yield in the presence of $Pd(OH)_2/C$ and H_2. Notably, the choice of solvents played an important role in determining the hydrogenation rate. When this hydrogenation was performed in ethyl acetate, the conversion of **5** to enzymatic precursor **6** was not complete even after 48 hr. In contrast, methanol could promote this reaction so that it was completed within 12 hr. Therefore, we synthesized all enzymatic precursors **6**, **13** and **18** in the same manner.



Scheme 1- 10 Chemoenzymatic synthesis of oxo coenzyme A analogs

Generation of oxo CoA analogs (HOCoA and $(HB)_nCoA$) from enzymatic precursors was achieved by three enzymes involved in CoA biosynthesis: a pantothenate kinase from *Staphylococcus aureus* (*Sa*PanK), a phosphopantetheine adenylyltransferase (*Ec*CoaD) and a dephospho CoA kinase (*Ec*CoaE) from *Escherichia coli*. Various CoA analogs have been prepared via this approach and applied as inhibitors to PhaCs.[14] To an aqueous solution of enzymatic precursors and adenosine triphosphate (ATP) was added *Sa*Pank followed by *Ec*CoaD and *Ec*CoaE. Reactions were slowly stirred at room temperature. To make sure that all enzymatic precursors were converted to $(HB)_nCoA$ (n = 1, 2 and 3), HPLC running condition was established and employed to monitor the progress of enzymatic reactions. The reaction was quenched by heating the reaction mixture in a 95 ^oC water bath for 5 min and the precipitated protein was removed by centrifugation. Oxo CoA analogs were purified by reverse-phase HPLC with reasonable yields (~50%) and fully characterized by ^1H, and ^{31}P NMR, gCOSY and HRMS.

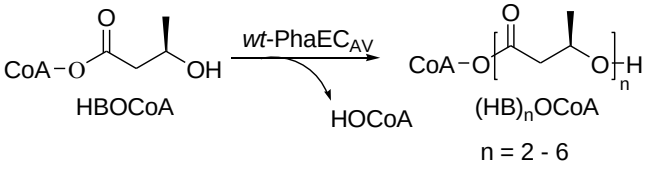


Scheme 1- 11 Regioselectivity of SaPanK to the HOCoA precursor

It should be noted that since HOCoA precursor contains two primary OH groups at both ends, concerns of regioselectivity were raised for the enzymatic conversion. To make sure that the phosphate was installed at the desired hydroxyl group by *Sa*PanK,

amide **2** in which 1, 3-diol was protected by PMP and there was only one primary OH group available, was subjected to phosphorylation using *Sa*Pank and ATP. HPLC profiles revealed that no reactions occurred in amide **2**. In contrast, *Sa*Pank was able to convert HOCoA precursor to the corresponding phosphate product. These experiments demonstrated that *Sa*Pank could accept the primary hydroxyl group at the pantothenate end rather than that at the aminoethanol end.

1.2.3 Detection of Noncovalent Intermediates $(HB)_nOCoA$ (n = 2 - 6) in Polymerizations with HBOCoA Catalyzed by wt-PhaEC$_{AV}$



Scheme 1- 12 Formation of noncovalent intermediates by PhaEC$_{AV}$ via Model B

Based on our hypothesis that the substitution of a sulfur atom with an oxygen atom in HBOCoA could decrease the polymerization rate, HBOCoA may serve an informative mechanistic probe to study. To detect $(HB)_nOCoA$ intermediates in polymerization reactions, we incubated *wt*-PhaEC$_{AV}$ (0.1 nM) and HBOCoA (20.0 mM) at 30 ^oC and monitored the progress of the reaction by analytical HPLC at the UV wavelength of 260 nm. As discussed above, according to Model **B**, noncovalent intermediates $(HB)_nOCoA$ ($n \geq 2$) and HOCoA could form and be detected in the reaction.

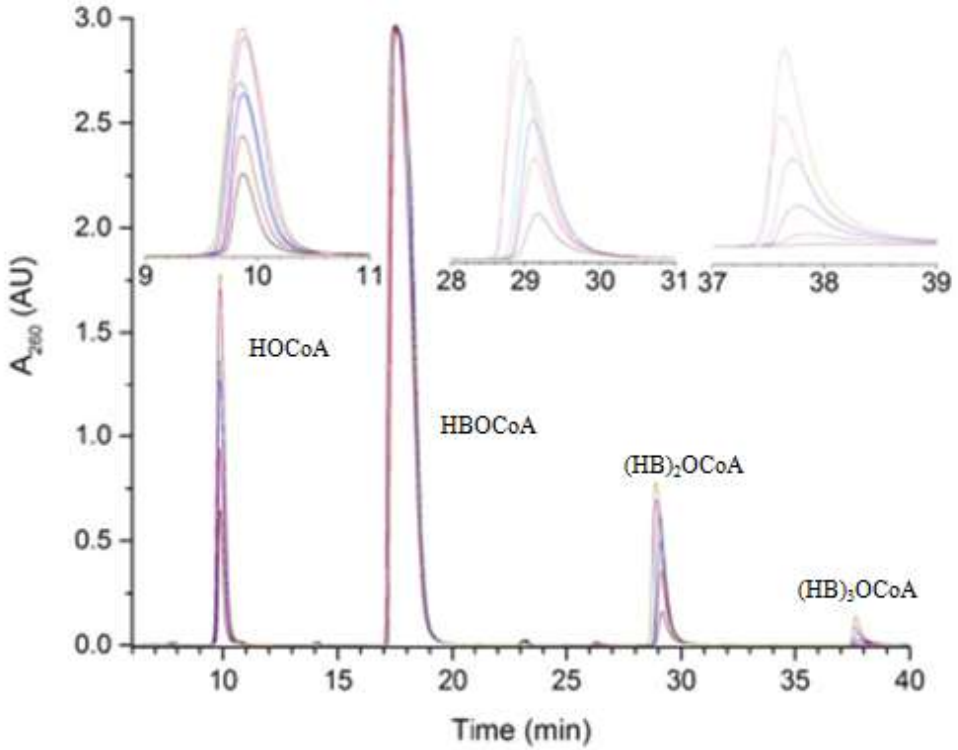


Figure 1- 1 Formation of HOCoA and (HB)nOCoA during incubation for 1 (black), 2 (red), 3 (green), 4 (blue), 5 (orange), and 6 hr (pink)

According to time-dependent HPLC profile, the concentration of HBOCoA decreased as three new peaks at 9.8, 29.0 and 37.7 min grew. Because HOCoA and $(HB)_nOCoA$ (n = 2 and 3) were synthesized, their retention times were measured and were identical to those of peaks. For the further confirmation of chemical structures of HOCoA and $(HB)_nOCoA$ (n = 2 and 3), these species from the reaction were isolated by HPLC, dried and analyzed by MALDI-TOF MS. Mass spectra confirmed our previous assumption that HOCoA, $(HB)_2OCoA$ and $(HB)_3OCoA$ formed in this reaction.

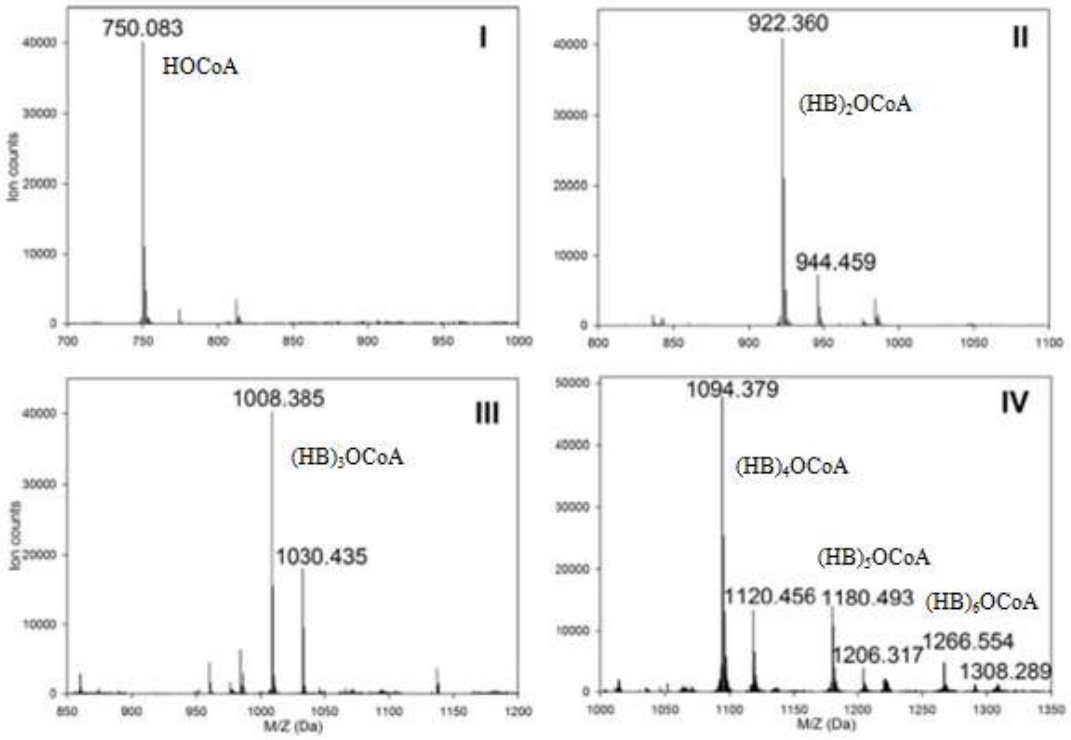


Figure 1- 2 MALDI-TOF MS of species at 9.80 (I), 29.0 (II), 37.7 (III) and 40.0-60.0 min (IV)

(MALDI-TOF MS was measured by Mr. Benjamin B. Katz and Prof. John M. Tomich)

Consequently, we continued to look for noncovalent intermediates containing more HB units. As the HB unit is hydrophobic, these intermediates in HPLC, if there were, were predicted to appear after $(HB)_3OCoA$. We pooled, dried and analyzed samples eluted at 38.0-60.0 min by MALDI-TOF MS, although no obvious peaks were observed at this period. Mass spectra revealed intermediates consisting of up to six HB units. MALDI-TOF results and the assignment of corresponding peaks are shown in **Fig**. 1 - 2 and **Table** 1 - 3.

	Intermediate	MW		Assignment
		Calcd.	Obsvd.	
I	CoAOH	750.131	750.083	$[M–H]^-$
II	$(HB)_2OCoA$	922.204	922.360	$[M–H]^-$
		944.186	944.459	$[M–2H+Na]^-$
III	$(HB)_3OCoA$	1008.241	1008.385	$[M–H]^-$
		1030.223	1030.435	$[M–2H+Na]^-$
IV	$(HB)_4OCoA$	1094.278	1094.379	$[M–H]^-$
	$(HB)_5OCoA$	1180.315	1180.493	$[M–H]^-$
	$(HB)_6OCoA$	1266.351	1266.554	$[M–H]^-$

Table 1- 1 Assignment of corresponding peaks in MALDI-TOF MS spectra

1.2.4 Extraction of PHB from Polymerizations with CoA Analogs Catalyzed by wt-PhaEC$_{AV}$

Based on our successful detection of noncovalent intermediates as well as our hypothesis that *wt*-PhaEC$_{AV}$ may utilize HBOCoA to produce PHB, extraction of PHB produced from HBOCoA catalyzed by *wt*-PhaEC$_{AV}$ *in vitro* was carried out using $CDCl_3$.

It is well known that *wt*-PhaEC$_{AV}$ utilizes HBCoA to produce PHB. Therefore, HBCoA was first employed in a control experiment to establish an extraction condition. Polymerization reactions were initiated by adding *wt*-PhaEC$_{AV}$ (5 μM) to a buffer containing KPi (pH = 7.8) and HBCoA (10 mM). The mixture was incubated overnight at 30 ^{o}C. Soluble and insoluble fractions were lyophilized to give a white powder to which $CDCl_3$ (0.5 mL) was added. The subsequent suspension was heated to reflux for 7 days. Vigorously stirring was necessary because extraction happened in two phases. During this period, more $CDCl_3$ was added to the suspension to make sure its total volume was about 0.5 mL. After being cooled to room temperature, the suspension was centrifuged and the supernatant was directly tested by ^{1}H NMR. ^{1}H NMR spectrum showed three peaks which corresponded to the PHB structure.

The polymerization reaction and extraction were performed in the same way as HBCoA using HBOCoA (10 mM) and PhaEC$_{AV}$ (100 μM). However, no PHB was observed in ^{1}H NMR spectra, as shown in **Fig.** 1 - 3 (top).

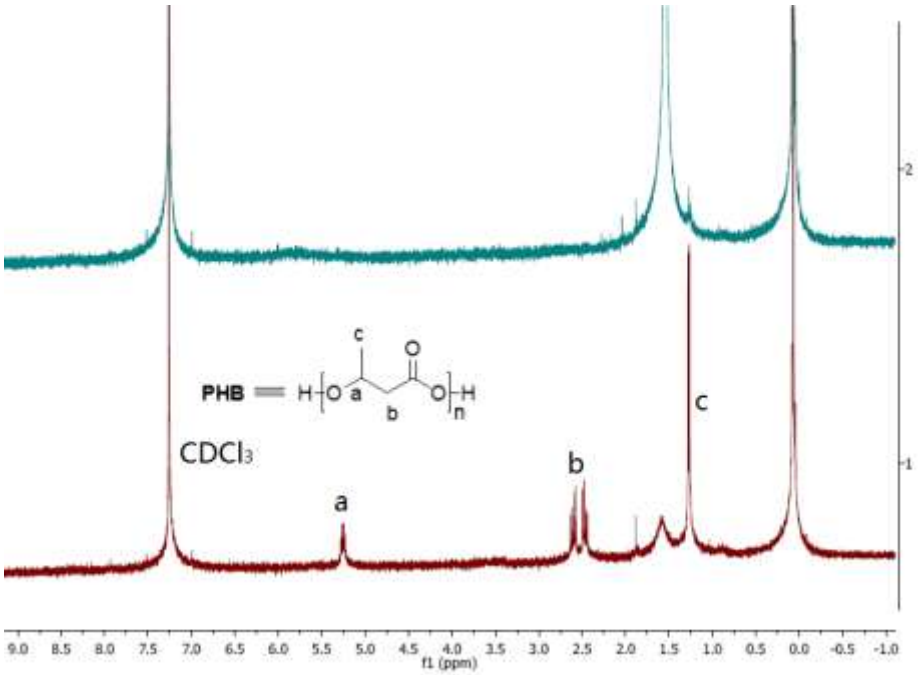


Figure 1- 3 ^{1}H NMR spectra of PHB isolated from in vitro polymerization reactions using HBOCoA (top one) and HBCoA (bottom one)

Other attempts to look for generation of PHB from HBOCoA via MALDI-TOF MS and gel permeation chromatography (GPC) were unsuccessful. These unsuccessful experiments may be due to enzyme-catalyzed hydrolysis of $(HB)_nOCoA$.

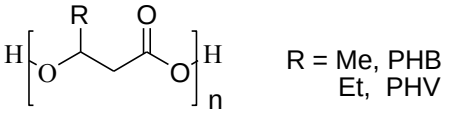
1.3 Conclusion

Through chemoenzymatic approaches with several modifications, the first type of mechanistic probes, $(HB)_nOCoA$ was synthesized with good yields. Their structures were fully characterized by ^{1}H, gCOSY and HRMS. The substitution of a sulfur atom in HBCoA with an oxygen atom in HBOCoA greatly decreased the polymerization rate. This decreased rate enabled us to successfully detect formation of noncovalent intermediates in reactions catalyzed by *wt*-$PhaEC_{AV}$. These results strongly supported our preferred mechanistic model. Furthermore, it is also the first time that a wild-type

synthase was used to investigate the chain elongation models.

CHAPTER 2 - Synthetic Studies of CoA Analogs for Substrate Specificity Studies

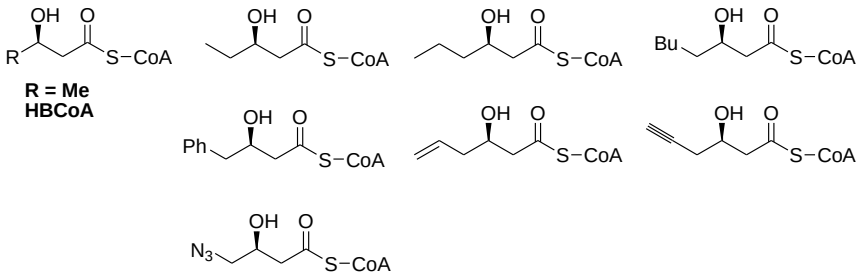
2.1 Introduction



Scheme 2- 1 Chemical structures of PHAs

Based on the exciting results described in Chapter **1**, our preferred mechanistic model for *wt*-PhaEC$_{AV}$-catalyzed polymerizations has been confirmed. In the following project, we extended the library of synthetic CoA analogs containing varied chain length and functional groups for polymerizations catalyzed by class I and III PHA synthases. Through modification of R groups in PHAs, polymer properties can be modified for specialty applications. It is well known that 125 different hydroxyalkanoic acids can be used to produce polyoxoesters through a variety of organisms.[15] For example, poly (3-hydroxybutyrate-co- 3-hydroxy- valerate) (PHBV) produced by *Ralstonia eutropha* under certain conditions has linear thermoplastic and aliphatic properties.[16] Functional groups-modified CoA analogs are also required for mechanistic purposes. In this project, we also introduced ethynyl and azide groups to CoA analogs that could load onto PHA synthases. When exposed to UV light, the onset of a click Huisgen cycloaddtion reaction occurs between these functional groups which in turn covalently couples synthases involved. As substrate specificity studies are in progress and this thesis mainly focuses on synthetic approaches, details of the

organic synthesis involved are discussed herein.

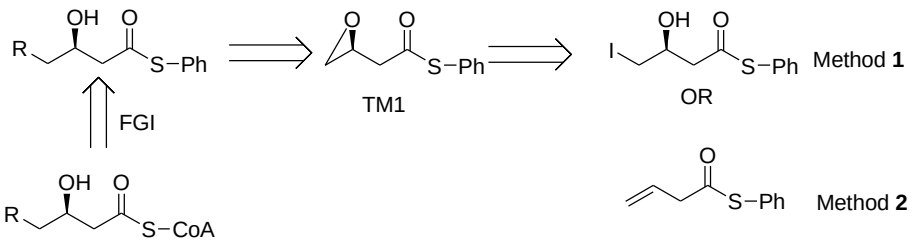


Scheme 2- 2 Chemical structures of CoA analogs synthesized in this project

Because class I and III PHA synthases utilize HBCoA as a native substrate to generate PHB, it is logical to consider HBCoA an ideal prototype to design a series of CoA analogs. According to our study about mechanistic Model **B**, a coenzyme A in HBCoA is necessary to load HB units onto active sites in PhaCs. A hydroxyl group in HBCoA is also required for the HB chain growth in polymerization reactions. The only part in HBCoA that can be modified is the R group. In this project, we designed and evaluated several synthetic routes to introduce various R groups into CoA analogs.

2.2 Results and Discussion

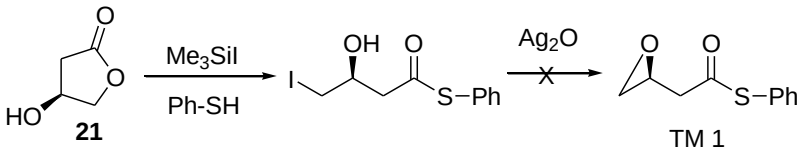
2.2.1 Design and Synthesis of (S)-S-phenyl 2-(oxiran-2-yl) Ethanethioate as a Key Synthetic Intermediate



Scheme 2- 3 Retrosynthetic analysis of CoA analogs

A retrosynthetic analysis of CoA analogs is described in Scheme **2 - 3**. Our previous experience of HBCoA synthesis demonstrated that thiotransesterification of 3-*R*-hydroxy thioesters with a thiol group in CoA provides efficient access to synthesis of CoA analogs. Since 3-*R*-hydroxyesters are commonly prepared by the ring-opening reaction of optically pure epoxyesters with organometallic reagents,[17] it was anticipated that a ring-opening reaction of an epoxythioester (TM 1) performed in the same manner as epoxyesters may also afford an access to 3-*R*-hydroxythioesters.

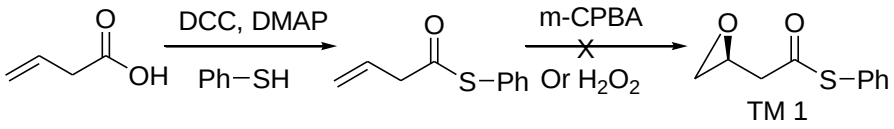
To generate TM 1, we designed and investigated two methods. The first method was based on publications in which oxidation reactions of optically pure iodohydrins with Ag_2O were able to provide enantiomerically pure epoxyesters.[17] The second one was deduced from well-established experimental procedures in which an oxidation of carbon-carbon double bonds with oxidizers like *meta*-chloroperoxybenzoic acid (*m*-CPBA) and H_2O_2 followed by a hydrolytic kinetic resolution using the Jacobsen's salen Co (III) catalyst was able to provide enantiomerically pure epoxyesters.[18] Unfortunately, it was found that neither of these methods afforded the desired products.



Scheme 2- 4 Synthesis of TM 1 via Method 1

To a solution of benzenethiol and hydroxylactone **21** in dry CH_2Cl_2 was slowly added Me_3SiI. The mixture was stirred overnight at room temperature followed by a

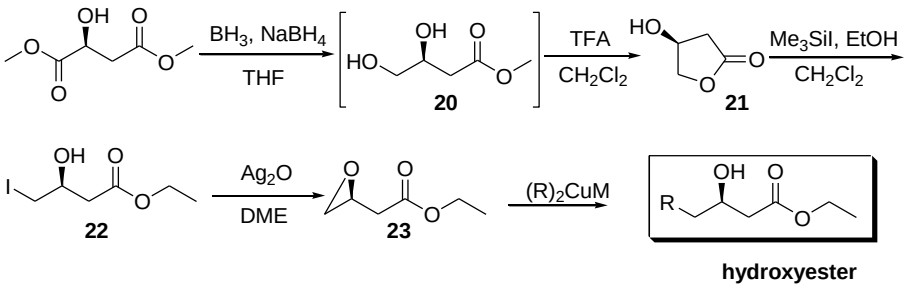
work-up that successfully afforded the synthetic intermediate iodothioester although its isolated yield was about 30%. Unfortunately, Ag_2O was unable to convert the iodothioester to the corresponding epoxide (TM 1). This is probably due to the fact that a sulfur atom in it may poison Ag_2O.[19]



Scheme 2- 5 Synthesis of TM 1 via Method 2

In the second method, formation of a thioester between but-3-enoic acid and benzenethiol was easily accomplished in the presence of DCC and DMAP as coupling reagents. However, the oxidation of a carbon-carbon double bond with *m*-CPBA or H_2O_2 led to complicated results (checked with TLC plates). This complexity may be because these oxidizers also oxidized thioester to sulfinyl or sulfonyl groups.[20] We did not test other peroxide compounds (CF_3CO_3H, *t*-BuOOH, etc.) known to be able to oxidize carbon-carbon double bonds to generate epoxides.

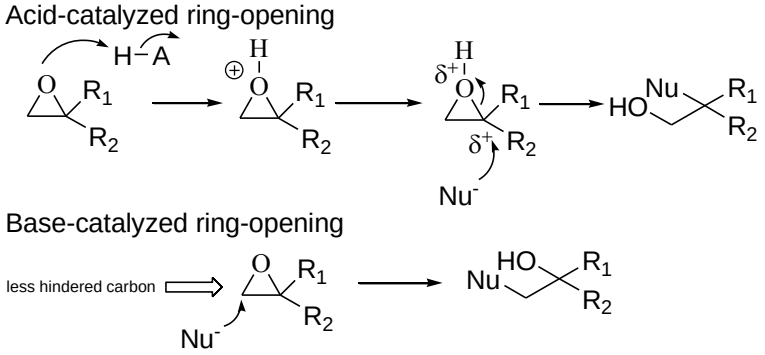
2.2.2 Design and Synthesis of (S)-ethyl 2-(oxiran-2-yl) Acetate as a Key Synthetic Intermediate



Scheme 2- 6 Synthesis of epoxide 23

Due to unsuccessful attempts to prepare TM 1, epoxide **23** was designed and synthesized as an alternative synthetic intermediate to TM 1, as shown in Scheme **2 - 6**. It is well known that a ring opening reaction of epoxide **23** with organometallic reagents was able to introduce various R groups to hydroxyesters.[21] As enantiomerically pure hydroxyesters (highlighted in the box) are important synthetic intermediates, a variety of methodologies for their generation were developed. Unfortunately, these methods require expensive catalysts and specific instruments. For example, the enantioselective Reformastsky reaction using aldehydes and α-halogenated esters is able to afford hydroxyesters, but catalysts involved are not commercially available.[22] Enantioselective hydrogenation of *β*-keto esters with H_2 and $RuCl_3$ (*R*)-SYNPHOS must be performed under the high pressure (10 - 50 bar).[23] These disadvantages limit these methodologies available within certain organic labs. Therefore, an efficient synthetic route is still required. Starting with dimethyl (*s*)-malate, a regioselective reduction reaction of the dimethyl ester to 1, 2-diol product **20** was accomplished using borane-dimethyl sulfide complex (BH_3·Me_2S) and a catalytic amount of sodium tetrahydroborate ($NaBH_4$).[24] Addition of $NaBH_4$ greatly increased the reduction rate, although BH_3·Me_2S could also afford the desired product. After the reaction was quenched with methanol, all solvents were removed to give crude product **20** which was used in the second step without further purification. The lactonization of **20** was carried out with a catalytic amount of trifluoroacetic acid (TFA) to afford hydroxylactone **21**. It should be noted that the onset of polymerization of **21** may occur and lead to a low yield, if this reaction was performed at 35 ℃ or

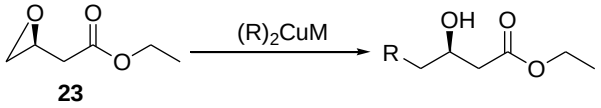
higher temperature. However, ^{1}H NMR result demonstrated that starting material **20** was still present even when this reaction was stirred at 20 ℃ for 48 hr. Therefore, the reaction temperature should be carefully controlled at a range of 24-28 ℃ to obtain a good yield. Upon treating **21** with absolute ethanol and Me_3SiI, iodohydrin **22** was obtained with a high yield (95%, based on **21**). We did not observe the presence of any TMS-protecting products after the isolation via silica gel chromatography, although Me_3SiI is prone to introducing a trimethylsilyl group to a hydroxyl group. The conversion of iodohydrin **22** to epoxide **23** was accomplished with Ag_2O upon refluxing for 4 hr. Interestingly, a suspension of **22** and Ag_2O in dimethoxyethane (DME) was able to afford the desired epoxide **23** while the same reaction preformed in acetonitrile (MeCN) failed to generate the epoxide. This failure is likely because DME has a higher boiling point than that of MeCN.



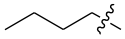
Scheme 2- 7 Regioselectivity of the ring-opening epoxide reaction

The synthesis of enantiomerically pure hydroxyesters was accomplished via the ring-opening epoxide reaction. This reaction is subjected to S_N1 or S_N2 mechanisms, depending on reaction conditions.[25] If the chemical structure of an epoxide, like compound **23**, is asymmetric, concerns of regioselectivity are raised for the

ring-opening reaction. Under acidic reaction conditions, the acid-catalyzed ring-opening reaction mainly undergoes an S_N1 mechanism to give more substituted products. Protonation of the oxygen in the epoxide by acids creates a good leaving group. The partially positive charge positions on the more substituted carbon which is subsequently attacked by nucleophiles. In contrast, the ring-opening reaction occurs via an S_N2 mechanism under basic reaction conditions, and the nucleophile attacks the less substituted carbon to open the epoxide. Because organometallic reagents are nucleophilic, ring-opening epoxide reactions with them affords access to the less substituted addition products.



23

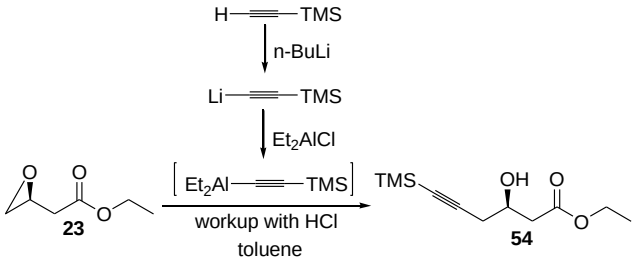
No.	M	R	Solvent	Yield%	$[\alpha]_D^{20}$(MeOH)	lit.$[\alpha]_D^{20}$
24	Mg	CH_3	THF/ether	67	-6.4^o	-16.6^o ($CHCl_3$)
30	Mg		THF/ether	65	-7.3^o	-6.0^o (MeOH)
36	Li		THF/ether	56	-11.4^o	-2.45^o ($CHCl_3$)
42	Mg	Ph-	THF/ether	45	-15.4^o	-14.5^o (MeOH)
48	Mg		THF/ether	54	-9.9^o	-22.5^o ($CHCl_3$)

Scheme 2- 8 Synthesis of enantiomerically pure hydroxyesters via organocuprates [26]

Preparation of enantiomerically pure hydroxyesters with epoxide **23** by way of ring-opening epoxide reactions using organocuprates is described in scheme **2 - 8**. As epoxide **23** contains an ethyl ester bond subjected to an attack by lithium or Grignard reagents, these metallic reagents should first be converted to organocuprates which are able to open epoxides to obtain desired hydroxyesters in which ethyl ester bonds

retain. This reaction occurs as a result of the relative reactivity of organocuprates to various functional groups (RCOCl > RCHO > epoxides >> ketones > esters).[21]

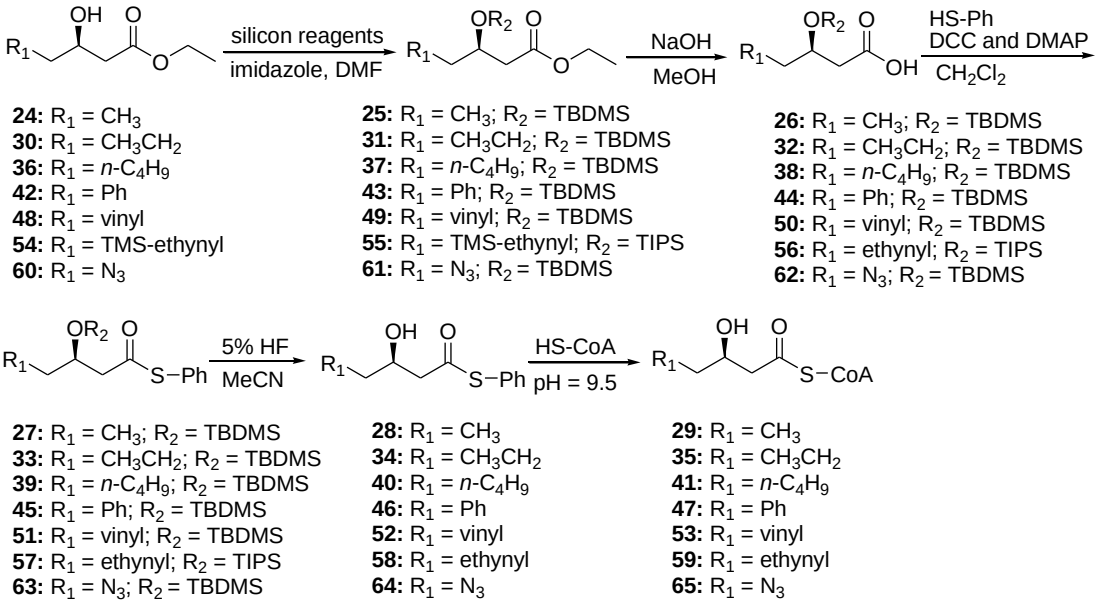
Generation of the corresponding cuprates from a copper bromide dimethyl sulfide complex (CuBr·Me_2S) was accomplished using lithium and Grignard reagents, respectively. At -60 ℃, to a suspension of CuBr·Me_2S (1.1 eq) in dry THF/ether (v/v = 1:2) were added lithium or magnesium reagents (2.2 eq). To make sure that lithium or Grignard reagents were completely consumed, the reaction was warmed to -25 - -30 ℃ and the mixture was stirred vigorously for 1 h. If the conversion of metallic reagents to their corresponding cuprates was not complete, the remaining of metallic reagents may attack ethyl ester bonds, leading to formation of ketones. Epoxide **23** was then added and the mixture was stirred at -25 - -30 ℃ for 4 hr. Finally, the reaction was quenched at 0 ^{o}C by adding saturated ammonium chloride (NH_4Cl) solution when epoxide **23** was completely consumed (checked with TLC plates).



Scheme 2- 9 Synthesis of enantiomerically pure hydroxyester 54

It is well known that regiospecific addition of alkynylaluminum reagents to epoxides is able to provide less substituted-carbon addition products.[27] To obtain an ethynyl aluminum reagent, ethynyl trimethylsilane was treated with *n*-butyllithium at - 45 ℃

to afford the corresponding lithium reagent, which subsequently reacted with diethylaluminum chloride. The mixture was stirred vigorously at 0 ℃ for 1 h to convert ethynyl lithium to an ethynylaluminum reagent. Epoxide **23** was then added and the mixture was stirred at 0 ℃ for 4 hr. The reaction was quenched by adding of diluted HCl followed by saturated NH_4Cl when epoxide **23** was completely consumed. The chemical structure of **54** was fully characterized with ^{1}H NMR, ^{13}C NMR and gCOSY.



Scheme 2- 10 Synthesis of CoA analogs

After formation of carbon-carbon bonds between organometallic reagents and the epoxide via ring-opening reactions, following steps to generate CoA analogs were the same as the synthetic route to HBCoA.[8] Under basic conditions, TBDMS- (TIPS- for ethynyl CoA analog) groups were employed to protect the hydroxyl groups in hydroxyesters. Hydrolysis of corresponding ethyl esters with NaOH followed by dilute HCl was able to afford carboxylic acids, which were subsequently coupled with

benzenethiol to obtain thioesters in the presence of DCC and DMAP. Diluted hydrofluoric acid (5% HF in MeCN) was utilized to quickly remove TBDMS- (TIPS-) protecting groups to give CoA precursors. To synthesize desired CoA analogs, we added CoA precursors (5 - 10 eq) to a solution of coenzyme A CoA (1 eq) in a basic buffer (Kpi, 50 Mm; pH = 9.5). Excess precursors were able to push the equilibrium of the reaction to products.[28] After 95% of CoA was converted to products (checked with analytical HPLC), we extracted excess CoA precursors as well as benzenethiol by adding ether. Further purification of final CoA analogs was accomplished with a semi-preparative HPLC column. By the way, CoA analog **65** consisting of an azide group was also obtained in the same manner. The structures of final compounds were fully characterized by ^{1}H NMR, ^{31}P NMR and MALDI-TOF MS. For more information, see Experimental Procedure.

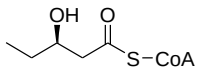
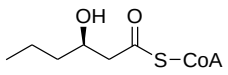
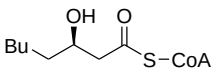
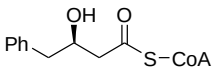
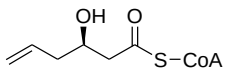
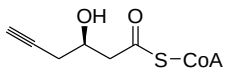
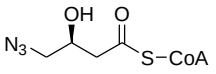
CoA analog	Calcd. MW	Obsvd. MW	Assignment
	867.65	866.188	$[M-H]^-$
	881.68	880.224	$[M-H]^-$
	909.73	908.229	$[M-H]^-$
	929.72	928.174	$[M-H]^-$
	879.66	878.807	$[M-H]^-$
	877.15	876.119	$[M-H]^-$
	894.64	893.015	$[M-H]^-$

Table 2- 1 MALDI-TOF MS analysis of CoA analogs

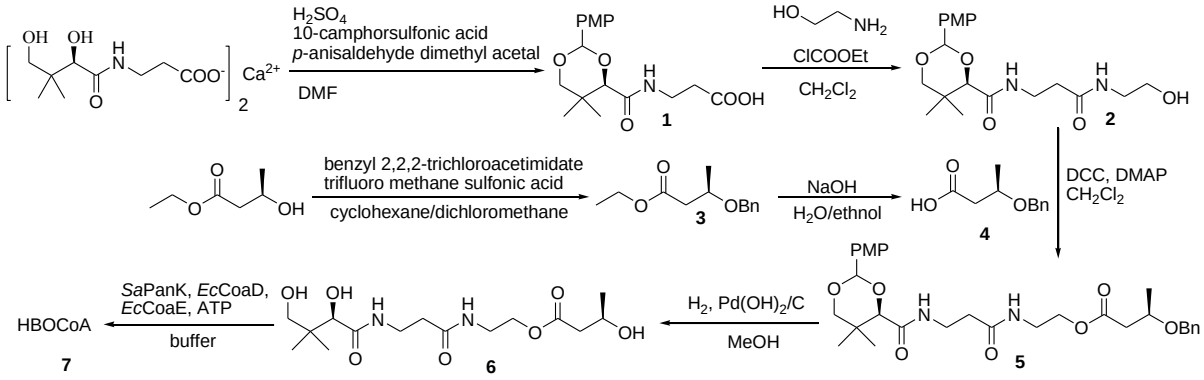
(MALDI-TOF MS was measured by Mr. Benjamin B. Katz and Prof. John M. Tomich)

2.3 Conclusion

Herein, we established a novel synthetic route to CoA analogs. Through the regiospecific ring-opening reaction of (S)-ethyl 2-(oxiran-2-yl)acetate with organometallic reagents, introduction of various functional groups to enantiomerically pure hydroxyesters was accomplished using organometallic reagents. The overall yield of this method was reasonable (CoA analog **29** for instance, 21% for 10 steps). Substrates specificity studies of the PHA synthases are in progress and will be reported in due course.

Experimental Procedure

General Method: All chemicals were purchased at the highest purity grade. All solvents were anhydrous. All reactions were performed under argon atmosphere unless otherwise specified. Thin layer chromatography (TLC) was performed using 60 mesh silica gel plates and visualization was performed using short wavelength UV light (254 nm) and basic $KMnO_4$ staining. NMR spectra were recorded on a Varian 400 MHz spectrometer. Chemical shifts of proton (^{1}H NMR) were reported in ppm relative to the residual solvent peaks ($CDCl_3$, 7.26 ppm; D_2O, 4.80 ppm). Chemical shifts of phosphorus (^{31}P NMR) were reported in ppm relative to the external reference of 85% H_3PO_4 (0.00 ppm). MALDI-TOF and high-resolution mass spectroscopies were recorded on Bruker Ultra Flex II and Q-Star Elite spectrometers, respectively.



Scheme **3 - 1**. Chemoenzymatic synthesis of HBOCoA **7**

3-((4R)-2-(4-methoxyphenyl)-5,5-dimethyl-1,3-dioxane-4-carboxamido)-propanoic acid (1). D-Calcium pantothenate (10 g, 38.7 mmol) in dry DMF (250 mL)

was treated with con. H_2SO_4 (2.22 mL). After 30 min, 10-camphorsulfonic acid (0.5 g, 2.1 mmol) and *p*-anisaldehyde dimethyl acetal (7.3 mL, 42.8 mmol) were added and the reaction was stirred overnight at room temperature. Concentration was accomplished under a reduced pressure. The residue was dissolved in ethyl acetate. The organic layer was washed with brine, dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc= 6/1 to 1/1) to afford the pure product **1** as a white solid (8.4 g, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.41 (d, 2H, *J* = 8.0 Hz), 7.02 (t, 1H, *J* = 12.0 Hz), 6.91 (d, 2H, *J* = 8.0 Hz), 5.46 (s, 1H), 4.09 (s, 1H), 3.81 (s, 3H), 3.68 (dt, 2H, *J* = 12.0, 8.0 Hz), 3.53 (m, 2H), 2.61 (t, 2H, *J* = 8.0 Hz), 1.10 (s, 3H), 1.09 (s, 3H).

(4R)-N-(3-((2-hydroxyethyl)amino)-3-oxopropyl)-2-(4-methoxyphenyl)-5,5-dimet hyl-1,3-dioxane-4- carboxamide (2). A solution of PMP-group protected D-pantothenic acid **1** (4.0 g, 11.8 mmol) in dry CH_2Cl_2 (150 mL) was treated with ethyl chloroformate (1.42 g, 13.0 mmol) for 10 min. Then 2-amino ethanol (0.87 g, 14.2 mmol) was added followed by pyridine (1.62 g, 16.5 mmol) and the mixture was stirred overnight at room temperature. The organic layer was washed with brine, dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc = 6/1 to 3/1) to afford the pure product **2** as a white solid (3.40 g, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.43 (d, 2H, *J* = 8.0 Hz), 7.03 (br, 1H), 6.93 (d, 2H, *J* = 8.0 Hz), 6.47 (br, 1H), 5.46 (s, 1H), 4.08 (s, 1H), 3.82 (s, 3H), 3.72 (d, 1H, *J* = 12.0 Hz), 3.69 (d, 1H, *J* = 12.0 Hz), 3.64 (m, 2H), 3.55 (dt,

2H, *J* = 12.0, 8.0 Hz), 3.40 - 3.34 (m, 2H), 2.85 (t, 1H, *J* = 4.0 Hz), 2.45 (t, 2H, *J* = 4.0 Hz), 1.11 (s, 3H), 1.09 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.91, 170.01, 160.43, 130.21, 127.67, 113.91, 101.50, 83.95, 78.57, 62.15, 55.48, 42.58, 36.45, 35.23, 33.23, 21.98, 19.26.

(R)-ethyl 3-(benzyloxy)-butanoate (3). To a solution of (R)-ethyl 3-hydroxybutanoate (17.0g, 0.13 mol) and benzyl 2, 2, 2-trichloroacetimidate (39.0 g, 0.15 mol) in cyclohexane/dichloromethane (170 mL / 85 mL) was added trifluoro methane sulfonic acid (1.7 mL) at 0 ℃. The reaction was stirred overnight at room temperature. After filtration, the organic layer was washed with saturated $NaHCO_3$ solution and brine, dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (Hexane/ EtOAc = 40/1) to afford the pure product **3** (17.0 g, 60%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.33 (m, 5H), 4.54 (dd, 2H, *J* = 12.0 Hz), 4.15 (m, 2H), 4.02 (m, 1H), 2.64 (dd, 1H, *J* = 16.0 Hz, 8.0 Hz), 2.42 (dd, 1H, *J* = 16.0, 4.0 Hz), 1.27 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.7, 138.8, 128.6, 127.7, 72.3, 71.0, 60.6, 42.4, 20.1, 14.5.

(R)-3-(benzyloxy)butanoic acid (4). To a solution of compound **3** (17.2 g, 77.3 mmol) in ethanol (230 mL) was added NaOH aqueous (120 mL, 386.9 mmol). The mixture was stirred at 50 ℃ for 2 h. Ethanol was removed under a reduced pressure. The pH value of aqueous residue was adjusted to 3.0 by adding HCl. Then water residue was extracted with dichloromethane. The combined organic layers was washed with brine,

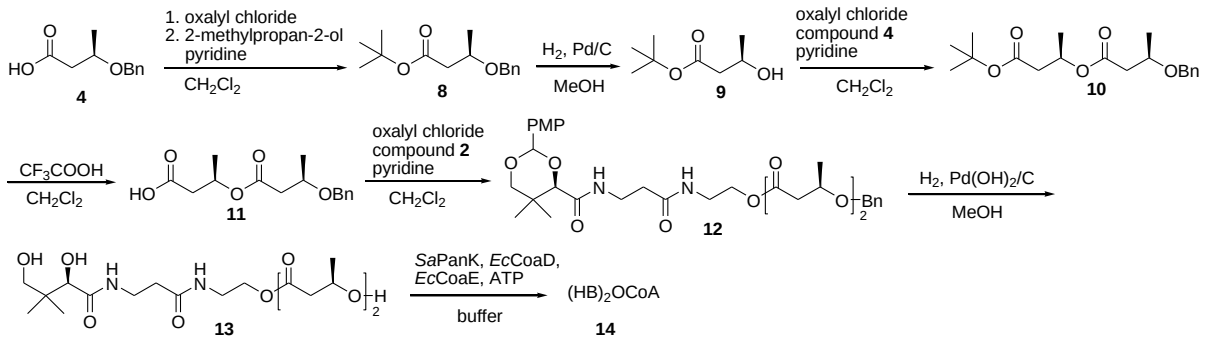
dried with Na_2SO_4 and concentrated under reduced pressure to afford the pure product **4** (14.9 g, 99%) as a colorless oil which was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.38 (m, 5H), 4.61 (dd, 2H, *J* = 12.0 Hz), 4.07 (m, 1H), 2.72 (dd, 1H, *J* = 12.0 Hz, 4.0 Hz), 2.57 (dd, 1H, *J* = 16.0 Hz, 8.0 Hz), 1.35 (d, 3H, *J* = 8.0 Hz).

2-(3-((4R)-2-(4-methoxyphenyl)-5,5-dimethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl(3R)-3-(benzyloxy)butanoate (5). To a solution of carboxylic acid **4** (0.60 g, 3.09 mmol) in dry CH_2Cl_2 (25 mL) were added DCC (0.93 g, 4.63 mmol) and catalytic amount of DMAP. After 10 min, alcohol **2** (1.17g, 3.09 mmol) in dry CH_2Cl_2 (5 mL) was added and the mixture was stirred overnight at room temperature. After filtration, the filtrate was washed with brine, dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (CH_2Cl_2/MeOH = 50/1 to 20/1) to afford the pure product **5** as a colorless oil (1.46 g, 84.9%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.42 (d, 2H, *J* = 8.0 Hz), 7.30 (m, 5H), 7.01 (t, 1H, *J* = 4.0 Hz), 6.92 (d, 2H, *J* = 8.0 Hz), 6.01 (t, 1H, *J* = 4.0 Hz), 5.45 (s, 1H), 4.58 (d, 1H, *J* = 12.0 Hz), 4.46 (d, 1H, *J* = 12.0 Hz), 4.13 (m, 2H), 4.11 (s, 1H), 4.06 (m, 1H), 3.81 (s, 3H), 3.68 (dt, 2H, *J* = 24.0, 12.0 Hz), 3.45 (m, 4H), 2.61 (m, 1H), 2.50 (m, 1H), 2.25 (m, 2H), 1.28 (d, 3H, *J* = 8.0 Hz), 1.09 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.48, 171.08, 169.53, 160.35, 138.40, 130.29, 128.56, 127.87, 127.81, 127.62, 113.86, 101.43, 83.95, 78.61, 72.21, 71.93, 63.33, 55.45, 41.94, 38.61, 35.85, 34.92, 33.19, 21.97, 19.81, 19.25.

(R)-4-(2-(3-((R)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethoxy)-4-oxobutan-2-yl(R)-3-hydroxybutanoate (6). In the presence of H_2 and $Pd(OH)_2/C$ (20 mg), a solution of ester **5** (0.20 g, 0.36 mmol) in MeOH (10 mL) was stirred vigorously overnight at room temperature. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel chromatography (CH_2Cl_2/MeOH= 20/1 to 10/1) to afford the pure product **6** as a colorless oil (0.12 g, 95.7%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.41 (t, 1H, *J* = 4.0 Hz), 6.78 (t, 1H, *J* = 4.0 Hz), 4.34 (d, 1H, *J* = 4.0 Hz), 4.32 - 4.23 (m, 2H), 4.17 (dq, 1H, *J* = 12.0, 4.0 Hz), 4.00 (d, 1H, *J* = 4.0 Hz), 3.93 (d, 1H, *J* = 4.0 Hz), 3.72 - 3.56 (m, 3H), 3.52 - 3.44 (m, 3H), 3.40 - 3.33 (m, 1H), 3.99 (d, 1H, *J* = 4.0 Hz), 3.93 (s, 1H), 3.72 - 3.56 (m, 3H), 3.52 - 3.43 (m 2H), 3.40 - 3.32 (m, 1H), 2.53 - 2.36 (m, 4H), 1.26 (d, 3H, *J* = 8.0 Hz), 1.01 (s, 3H), 0.91 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.56, 172.58, 172.09, 77.72, 70.98, 65.01, 63.43, 43.97, 39.47, 38.76, 36.05, 35.61, 23.23, 21.77, 20.48.

Enzymatic Synthesis of HBOCoA (7). A 2-mL reaction mixture consisted of precursor **6** (20.0 mM), ATP (50.0 mM), $MgCl_2$ (10.0 mM), *Sa*CoaA (80.0 μg), *Ec*CoaD (80.0 μg) and *Ec*CoaE (80.0 μg) in 100 mM Tris-HCl (pH = 7.60). The reaction was initiated by addition of the enzymes and incubated at 37 ℃ for 3 h. The reaction was quenched by heating the reaction mixture in a 95 ℃ water bath for 5 min, and the precipitated protein was removed by centrifugation (14,000 rpm x 5 min). The supernatant was loaded onto a semi-preparative HPLC column (Luna C18-2, 5 μm, 10

mm × 250 mm) that was eluted at 3.00 mL/min using a linear gradient from 5 to 90% methanol in 10.0 mM ammonium acetate (pH = 5.00) over 60 min. The fractions containing the product were pooled, concentrated, and lyophilized to give HBOCoA (**7**) as a white powder (17 mg, 50.8% yield based on UV absorbance, HPLC: t_R = 15.1 min); ^{1}H NMR (400MHz, D_2O) δ: 8.56 (s, 1H), 8.28 (s, 1H), 6.20 (d, 2H, *J* = 8.0 Hz), 4.62 (m, 1H), 4.28 - 4.23 (m, 3H), 4.21 (t, 2H, *J* = 8.0 Hz), 4.03 (s, 1H), 3.86 (d, 1H, *J* = 8.0 Hz), 3.60 (d, 1H, *J* = 8.0 Hz), 3.53 - 3.46 (m, 4H), 2.64 - 2.55 (m, 2H), 2.50 (t, 2H, *J* = 8.0 Hz), 1.26 (d, 3H, *J* = 4.0 Hz), 0.91 (s, 3H), 0.78 (s, 3H); ^{13}C NMR (100 MHz, D_2O) δ: 174.84, 174.26, 174.03, 155.64, 152.93, 149.43, 140.04, 118.76, 86.72, 83.68, 74.30, 74.01, 72.02, 65.65, 64.71, 63.56, 43.27, 38.41, 35.60, 35.48, 22.15, 20.98, 18.33; ^{31}P NMR (161 MHz, D_2O) δ: 2.13 (s, 1P), −11.31 (d, 1P, *J* = 19.3 Hz), −11.88 (d, 1P, *J* = 19.3 Hz); HRMS: calc. for $C_{25}H_{41}N_7O_{19}P_3^-$ $[M-H]^-$: 836.1676, found: 836.1710.



Scheme **3 - 2**. Chemoenzymatic synthesis of $(HB)_2$OCoA **14**

(R)-tert-butyl-3-(benzyloxy)butanoate (8). To a solution of (R)-3-(benzyloxy) butanoic acid **4** (0.6 g, 3.08 mmol) in dry CH_2Cl_2 (20 mL) was added oxalyl chloride (1.17g, 9.24 mmol) at room temperature. After 10 min, a drop of DMF was added as

an initiator. The mixture was stirred for 2 hours and concentrated under reduced pressure. Then the yellow residue was diluted in CH_2Cl_2 (5 mL) and added slowly to a solution of 2-methylpropan-2-ol (0.27 g, 3.60 mmol) and pyridine (0.7 g, 9.24 mmol) in dry CH_2Cl_2 (20 mL) at 0 ℃. The reaction was stirred overnight at room temperature. The reaction mixture was washed with brine, dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc = 50/1 to 20/1) to afford the pure product **8** as a colorless oil (0.37 g, 48%).^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.34 - 7.32 (m, 5H), 4.54 (m, 2H), 3.98 (m, 1H), 2.44 (m, 2H), 1.45 (s, 9H), 1.25 (d, 3H, *J* = 8.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.1, 138.8, 128.5, 127.9, 127.7, 80.6, 72.6, 71.1, 43.5, 28.3, 20.0.

(R)-tert-butyl 3-hydroxybutanoate (9). In the presence of H_2 and Pd/C (20 mg), a solution of ester **8** (0.15 g, 0.6 mmol) in MeOH (5 mL) was vigorously stirred overnight at room temperature. Pd/C was removed via filtration and the filtrate was concentrated under reduced pressure to afford the product **9** (94 mg, 98%) as a colorless oil which was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.13 (m, 1H), 3.22 (d, 1H, *J* = 4.0 Hz), 2.34 (m, 2H), 1.45 (s, 9H), 1.19 (d, 3H, *J* = 8.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 172.7, 81.5, 64.5, 43.9, 28.3, 22.5.

(R)-((R)-4-tert-butoxy-4-oxobutan-2-yl) 3-(benzyloxy)butanoate (10). To a

solution of acid **4** in dry CH_2Cl_2 (0.18g, 0.93 mmol) was added oxalyl chloride (0.37 g, 1.48 mmol) at room temperature. After 10 min, a drop of DMF was added as an initiator. The mixture was stirred for 2 h and concentrated under reduced pressure. Then the yellow residue was diluted in dry CH_2Cl_2 (5 mL) added slowly to a solution of hydroxyl **9** (0.16 g, 1.0 mmol) and pyridine (250 mg, 3.16 mmol) in dry CH_2Cl_2 (20 mL) at 0 ℃. The reaction was stirred overnight at room temperature. The organic solution was washed with brine, dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc = 30/1 to 10/1) to afford the pure product **10** as a colorless oil (0.18 g, 57%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.33 - 7.27 (m, 5H), 5.27 (m, 1H), 4.52 (m, 2H), 3.99 (m, 1H), 2.66 - 2.37 (m, 4H), 1.42 (s, 9H), 1.28 - 1.25 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.7, 169.6, 138.7, 128.5, 127.8, 127.7, 81.07, 72.1, 71.0, 67.9, 42.4, 42.3, 28.25, 20.1, 20.0.

(R)-3-((R)-3-(benzyloxy)butanoyloxy)butanoic acid (11). To a solution of ester **10** (0.16 g, 0.48 mmol) in CH_2Cl_2 (25 mL) was added trifluoro acetic acid (TFA) (2.7 g, 24 mmol) at room temperature. TLC plates were used to check the progress of reaction. After 20 min, the reaction mixture was washed with brine, dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc = 5/1 to 1/1) to afford the pure product **11** as a colorless oil (0.13 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.33 - 7.26 (m, 5H), 5.27 (m, 1H), 4.53 (m, 2H), 3.99 (m, 1H), 2.71 - 2.39 (m, 4H), 1.30 (d, 3H, *J* = 8.0 Hz),

1.25 (d, 3H, *J* = 8.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.8, 138.6, 128.5, 127.8, 127.7, 72.2, 71.0, 67.3, 42.3, 40.5, 20.0.

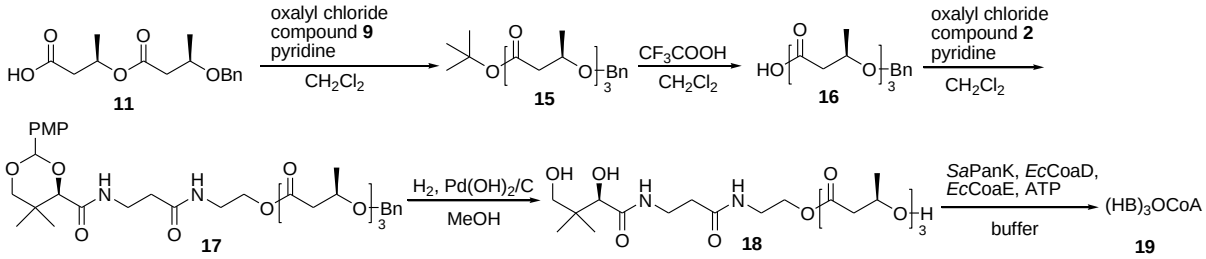
(2R)-4-(2-(3-((4R)-2-(4-methoxyphenyl)-5,5-dimethyl-1,3-dioxane-4-carboxamido) propanamido) ethoxy)-4-oxobutan-2-yl (3R)-3-(benzyloxy)butanoate (12). To a solution of acid **11** (0.13 g, 0.48 mmol) in dry CH_2Cl_2 (20 mL) was added oxalyl chloride (0.12 g, 0.96 mmol) at room temperature. After 10 min, a drop of DMF was added to initiate the reaction. The mixture was stirred for 2 hr and then concentrated under reduced pressure. The residue was diluted in dry CH_2Cl_2 (5 mL) and added to a solution of alcohol **2** (0.21 g, 0.55 mmol) and pyridine (0.11 g, 1.40 mmol) in dry CH_2Cl_2 (20 mL) at 0 ℃. The reaction was stirred overnight at room temperature. The reaction mixture was washed with brine, dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (CH_2Cl_2/MeOH = 100/1 to 20/1) to give the pure compound **12** as a white solid (0.28 g, 90.8%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.43 (d, 2H, *J* = 8.0 Hz), 7.32 - 7.27 (m, 5H), 7.06 (t, 1H, *J* = 4.0 Hz), 6.92 (d, 2H, *J* = 12.0 Hz), 6.30 (t, 1H, *J* = 4.0 Hz), 5.45 (s, 1H), 5.34 (m, 1H), 4.56 (d, 1H, *J* = 12.0 Hz), 4.47 (d, 1H, *J* = 12.0 Hz), 4.09 (m, 1H), 4.07 (s, 1H), 4.02 - 3.98 (m, 2H), 3.82 (s, 3H), 3.68 (dd, 2H, *J* = 24.0, 12.0 Hz), 3.52 (m, 2H), 3.34 (m, 2H), 2.61 - 2.51 (m, 3H), 2.45 - 2.39 (m, 3H), 1.27 (d, 3H, *J* = 8.0 Hz), 1.26 (d, 3H, *J* = 8.0 Hz), 1.10 (s, 3H), 1.09 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.00, 170.47, 169.74, 169.02, 159.85, 138.03, 129.89, 128.05, 127.40, 127.32, 127.25, 113.37, 100.89, 83.46, 78.04, 71.64, 70.48, 67.09, 63.00, 54.97, 41.79,

40.55, 37.95, 35.18, 34.59, 32.72, 21.58, 19.77, 19.39, 18.8.

(R)-4-(2-(3-((R)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethoxy)-4-oxobutan-2-yl (R)- 3-hydroxybutanoate (13). The reaction and silica gel chromatography were performed in the same way as **6** using ester **12** (0.12 g, 0.19 mmol) to yield the pure product **13** as a colorless oil (0.07 g, 92.0%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.43 (t, 1H, *J* = 4.0 Hz), 6.89 (t, 1H, *J* = 4.0 Hz), 5.36 (m, 1H), 4.36 (d, 1H, *J* = 8.0 Hz), 4.21 (m, 1H), 4.19 - 4.15 (m, 2H), 3.98 (d, 1H, *J* = 4.0 Hz), 3.82 (t, 1H, *J* = 8.0 Hz), 3.69 (d, 1H, *J* = 4.0 Hz), 3.57 - 3.52 (m, 2H), 3.49 - 3.41 (m, 4H), 2.58 (m, 2H), 2.46 (t, 2H, *J* = 8.0 Hz), 2.42 (m, 2H), 1.31 (d, 3H, *J* = 4.0 Hz), 1.23 (d, 3H, *J* = 8.0 Hz), 0.99 (s, 3H), 0.91 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.08, 171.97, 171.57, 170.33, 76.82, 70.27, 67.37, 64.28, 63.14, 43.54, 40.75, 39.10, 38.38, 35.47, 35.25, 22.80, 20.93, 20.41, 19.93.

Enzymatic Synthesis of $(HB)_2$OCoA (14). The reaction and HPLC were performed in the same way as **HBOCoA** (**7)** using precursor **13** to give the product $(HB)_2$OCoA (**14**) (18 mg, 48.7% yield based on UV absorbance, t_R = 26.4 min) as a white solid. ^{1}H NMR (400 MHz, D_2O) δ: 8.54, (s, 1H), 8.26 (d, 1H, *J* = 4.0 Hz), 6.18 (d, 1H, *J* = 8.0 Hz), 5.27 (dq, 2H, *J* = 12.0, 4.0 Hz), 4.87 (q, 1H, *J* = 4.0 Hz), 4.61 (m, 1H), 4.27 (m, 2H), 4.21 (m, 1H), 4.16 (t, 2H, *J* = 4.0 Hz) 4.02 (s, 1H), 3.86 (d, 1H, *J* = 8.0 Hz), 3.59 (d, 1H, *J* = 8.0 Hz), 3.48 (q, 2H, *J* = 8.0 Hz), 3.44 (t, 2H, *J* = 8.0 Hz), 2.71 (d, 2H, *J* = 8.0 Hz), 2.56 - 2.45 (m, 4H), 1.30 (d, 3H, *J* = 4.0 Hz), 1.22 (d, 3H, *J* = 4.0 Hz), 0.91 (s,

3H), 0.77 (s, 3H); ^{13}C NMR (100 MHz, D_2O) δ: 174.86, 174.21, 173.35, 172.98, 155.21, 152.39, 149.31, 140.14, 118.69, 86.67, 83.59, 83.54, 74.28, 74.23, 74.19, 73.94, 73.89, 72.08, 72.02, 68.79, 65.51, 64.78, 64.65, 63.71, 43.58, 40.37, 38.51, 38.43, 38.34, 35.58, 35.47, 22.08, 20.98, 19.11, 18.30; ^{31}P NMR (161 MHz, D_2O) δ: 0.51 (s, 1P), - 10.71 (d, 1P, *J* = 25.8 Hz), - 11.36 (d, 1P, *J* = 24.2 Hz); HRMS: calc. for $C_{29}H_{47}N_7O_{21}P_3^-$ $[M-H]^-$: 922.2043, found: 922.2076.



Scheme **3 - 3**. Chemoenzymatic synthesis of $(HB)_3$OCoA **19**

(R)-((R)-4-tert-butoxy-4-oxobutan-2-yl) 3-((R)-3-(benzyloxy)butanoyloxy) butane ate (15). To a solution of acid **11** (0.9 g, 3.2 mmol) in CH_2Cl_2 (15 mL) was added oxalyl chloride (0.81 g, 6.4 mmol) at room temperature. After 10 min, a drop of DMF was added as an initiator of reaction. The reaction was stirred for 2 h and concentrated under reduced pressure. Then the yellow residue was diluted in CH_2Cl_2 (5 mL) added slowly to a solution of (R)-tert-butyl 3-hydroxybutanoate **9** (0.56 g, 3.5 mmol) and pyridine (0.77 g, 9.6 mmol) in dry CH_2Cl_2 (15 mL) at 0 ℃. The reaction was stirred overnight at room temperature. The reaction mixture was washed with brine, dried with Na_2SO_4 and concentrated under reduced pressure. Further purification was accomplished with silica gel chromatography (hexane/EtOAc = 10/1 to 5/1) to give the pure product **15** (1.16 g, 83%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ:

7.33 - 7.27 (m, 5H), 5.26 (m, 2H), 4.52 (m, 2H), 3.98 (m, 1H), 2.63 - 2.42 (m, 6H), 1.43 (s, 9H), 1.28 - 1.19 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.7, 169.5, 169.4, 138.7, 128.5, 127.8, 127.7, 81.1, 72.1, 71.0, 68.1, 76.5, 42.3, 42.2, 41.1, 28.2, 20.1, 20.0, 19.9.

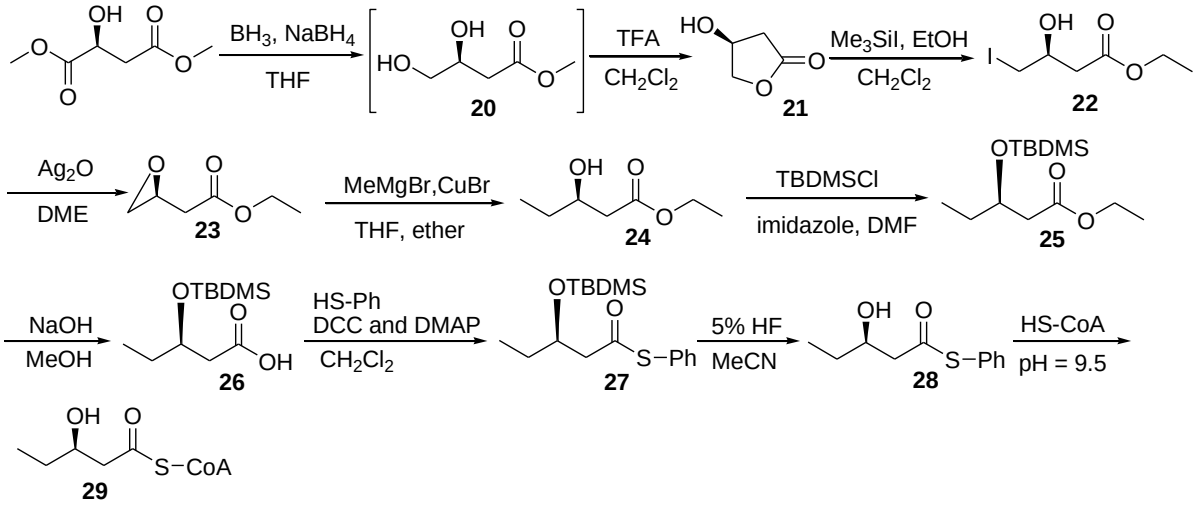
(R)-3-((R)-3-((R)-3-(benzyloxy)butanoyloxy)butanoyloxy)butanoic acid (16). To a solution of ester **15** (0.5 g, 1.18 mmol) in CH_2Cl_2 (10 mL) was added TFA (0.67 g, 5.9 mmol) at room temperature. The progress of the reaction was checked by TLC. After 20 min, the reaction mixture was washed with brine, dried with Na_2SO_4 and concentrated under reduced pressure. Further purification was accomplished with silica gel chromatography (CH_2Cl_2/MeOH = 100/1 to 20/1) to afford the pure product **16** (0.41 g, 94%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.33 (m, 5H), 5.30 (m, 2H), 4.56 (m, 2H), 4.13 - 4.03 (m, 1H), 2.73 - 2.04 (m, 6H), 1.28 - 1.23 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.6, 170.7, 169.7, 138.2, 128.5, 128.0, 127.8, 72.3, 71.2, 68.0, 67.8, 42.1, 41.3, 40.7, 20.1, 20.0, 19.9.

(12R,16R)-1-((4R)-2-(4-methoxyphenyl)-5,5-dimethyl-1,3-dioxan-4-yl)-12-methyl -1,5,10,14-tetraoxo- 9,13-dioxa-2,6-diazaheptadecan-16-yl (3R)-3-(benzyloxy) butanoate (17). The reaction and silica gel chromatography were performed in the same way as **12** using acid **16** (0.30 g, 0.82 mmol) and alcohol **2** (0.32 g, 0.84 mmol) to yield the product **17** as a white solid (0.49 g, 82.0%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.43 (d, 2H, *J* = 8.0 Hz), 7.32 - 7.27 (m, 5H), 7.07 (t, 1H, *J* = 4.0 Hz), 6.92 (d, 2H,

J = 8.0 Hz), 6.38 (t, 1H, *J* = 4.0 Hz), 5.46 (s, 1H), 5.32 (m, 1H), 5.25 (q, 1H, *J* = 8.0 Hz), 4.56 (d, 1H, *J* = 12.0 Hz), 4.49 (d, 1H, *J* = 12.0 Hz), 4.11 (m, 2H), 4.07 (s, 1H), 3.99 (q, 1H, *J* = 8.0 Hz), 3.81 (s, 3H), 3.68 (q, 2H, *J* = 12.0 Hz), 3.57 - 3.52 (m, 2H), 3.50 - 3.44 (m, 2H), 2.64 - 2.55 (m, 3H), 2.51 - 2.38 (m, 5H), 1.27 (d, 3H, *J* = 4.0 Hz), 1.25 (d, 3H, *J* = 4.0 Hz), 1.24 (d, 3H, *J* = 4.0 Hz), 1.10 (s, 3H), 1.09 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.22, 170.62, 170.00, 169.64, 169.37, 160.17, 138.42, 130.14, 128.33, 127.62, 127.56, 127.50, 113.70, 101.25, 83.80, 78.44, 71.86, 70.77, 67.69, 67.30, 63.51, 55.32, 42.07, 40.98, 40.89, 38.35, 35.66, 34.83, 33.06, 21.86, 20.07, 19.84, 19.79, 19.14.

(3R,15R,19R)-1,3-dihydroxy-2,2,15-trimethyl-4,8,13,17-tetraoxo-12,16-dioxa-5,9-diazaicosan-19-yl (R)-3-hydroxybutanoate (18). The reaction and silica gel chromatography were performed in the same way as **6** using ester **17** (0.15 g, 0.20 mmol) to yield the pure product **18** as a colorless oil (0.10 g, 96.1%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.47 (t, 1H, *J* = 4.0 Hz), 6.92 (t, 1H, *J* = 4.0 Hz), 5.28 (m, 2H), 4.49 (d, 1H, *J* = 4.0 Hz), 4.18 (nonet, 1H, *J* = 8.0 Hz), 4.16 - 4.13 (m, 2H), 3.96 (d, 1H, *J* = 4.0 Hz), 3.67 (br, 1H), 3.54 (q, 2H, *J* = 4.0 Hz), 3.48 - 3.40 (m, 4H), 2.63 - 2.57 (m, 2H), 2.52 (dd, 2H, *J* = 12.0, 4.0 Hz), 2.45 (t, 2H, *J* = 8.0 Hz), 2.40 (t, 2H, *J* = 4.0 Hz), 1.29 (d, 3H, *J* = 4.0 Hz), 1.28 (d, 3H, *J* = 4.0 Hz), 1.21 (d, 3H, *J* = 4.0 Hz), 0.97 (s, 3H), 0.89 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.13, 172.05, 171.81, 170.31, 170.06, 77.51, 70.62, 67.93, 67.53, 64.40, 63.37, 43.54, 40.90, 39.29, 38.47, 35.65, 35.32, 22.77, 21.27, 20.50, 20.08, 19.95.

Enzymatic Synthesis of $(HB)_3$OCoA (19). The reaction and HPLC were performed in the same way as **7** using precursor **18** to give the product $(HB)_3$OCoA (**19**) (19 mg, 47.1% yield based on UV absorbance, t_R = 36.5 min. ^{1}H NMR (400 MHz, D_2O) δ: 8.57 (s, 1H), 8.26 (s, 1H), 6.17 (d, 1H, *J* = 4.0 Hz), 5.26 (dq, 2H, *J* = 12.0, 4.0 Hz), 4.87 (m, 1H), 4.62 (m, 1H), 4.28 (m, 2H), 4.22 (m, 1H), 4.17 (t, 2H, *J* = 4.0 Hz) 4.03 (s, 1H), 3.86 (d, 1H, *J* = 8.0 Hz), 3.60 (d, 1H, *J* = 8.0 Hz), 3.49 (m, 2H), 3.45 (t, 1H, *J* = 4.0 Hz), 2.74 - 2.66 (m, 4H), 2.54 - 2.47 (m, 4H), 1.27 (d, 6H, *J* = 4.0 Hz), 1.24 (d, 3H, *J* = 8.0 Hz), 0.92 (s, 3H), 0.78 (s, 3H); ^{13}C NMR (100 MHz, D_2O) δ: 174.99, 174.31, 173.40, 173.01, 172.42, 155.32, 152.48, 140.38, 86.89, 83.71, 74.43, 74.36, 74.12, 72.22, 69.18, 68.85, 65.66, 64.74, 63.85, 43.72, 40.80, 40.43, 38.59, 38.50, 35.74, 35.64, 22.28, 21.15, 19.25, 19.22, 18.47; ^{31}P NMR (161 MHz, D_2O) δ: 0.85 (s, 1P), −10.06 (d, 1P, *J* = 17.7 Hz), −10.66 (d, 1P, *J* = 17.7 Hz); HRMS: calc. for $C_{33}H_{53}N_7O_{23}P_3^-$ $[M-H]^-$: 1008.2411, found: 1008.2398.



Scheme **3 - 4**. Synthesis of CoA analog **29**

(S)-4-hydroxy-dihydrofuran-2(3H)-one (21). To a solution of (S)-dimethyl

2-hydroxysuccinate (38.8 g, 0.24 mol) in 500 mL of dry THF was added slowly borane-dimethylsulfide complex (244 mL, 0.244 mol) for 10 min and the mixture was stirred at room temperature for 50 min. The reaction was cooled to 0 ℃. $NaBH_4$ (0.4 g, 12 mmol) was added and the mixture was stirred for 30 min. The reaction was warmed to room temperature again and quenched by slow addition of methanol (154 mL). When no air babble formed in the reaction, all solvents were removed under reduced pressure. The crude product **20** was used in the next step without further purification. To a solution of compound **20** in dry CH_2Cl_2 (300 mL) was added trifluoroacetic acid (6 mL). The reaction was stirred overnight at room temperature. TLC plate was used to check the progress of the reaction. The solvent was removed under reduced pressure and residue was purified by silica gel chromatography (hexane/EtOAc = 1/1 to 1/3) to afford the pure product **21** (21.5 g, 87%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.69 (s, 1H), 4.44 - 4.28 (m, 2H), 2.78 - 2.50 (m, 2H), 2.61 (s, 1H).

(S)-ethyl 3-hydroxy-4-iodobutanoate (22). To a solution of **21** (15.0 g, 0.148 mol) and absolute EtOH (26 mL, 0.444 mmol) in dry CH_2Cl_2 (500 mL) was added slowly Me_3SiI (30 mL, 0.22 mmol) within 1 h. The color of the reaction changed to a dark brown. The reaction was stirred overnight at room temperature. All solvents were removed under a reduced pressure and the brown residue was dissolved again in ether (500 mL). The organic layer was washed with 5% aqueous $Na_2S_2O_3$ solution (3 x 10 mL), dried with Na_2SO_4, and concentrated under reduced pressure. The residue was

purified by gel silica chromatography (hexane/EtOAc = 5/1 to 3/1) to afford the pure product **22** as a pale yellow oil (37.2 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.18 (q, 2H, *J* = 8.0 Hz,), 4.03 - 3.96 (m, 1H), 3.32 (m, 2H), 3.18 (d, 1H, *J* = 4.0 Hz), 2.70 - 2.57 (m, 2H), 1.28 (t, 3H, *J* = 8.0 Hz).

(S)-ethyl 2-(oxiran-2-yl)acetate (23). A solution of compound **22** (15.0 g, 58.2 mmol) and Ag_2O (16.2 g, 69.6 mmol) in DME (100 mL) was heated to reflux for 4 hr. After filtration, a volume of filtrate was concentrated down to 25 mL under reduced pressure. Further concentration may lead to a loss of the product. The concentrated solution was purified by gel silica chromatography (hexane/ether = 5/1 to 4/1) to afford the pure epoxide **23** (6.95 g, 92%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.18(q, 2H, *J* = 8.0 Hz), 3.30 - 3.28 (m, 1H), 2.83 (d, 1H, *J* = 4.0 Hz), 2.57 - 2.56 (m, 3H), 1.28(t, 3H, *J* = 8.0 Hz).

(R)-ethyl 3-hydroxypentanoate (24). To a suspension of copper bromide dimethyl sulfide complex (1.89 g, 9.21 mmol) in mixed solvents of dry THF (60 mL) and dry ether (120 mL) was added at -60 ℃ a solution of methylmagnesium bromide (6.14 mL, 18.4 mmol) in ether. The reaction temperature increased from -60 to -30 ^{o}C and the reaction was stirred vigorously for 1 h. A black suspension was given. Then, epoxide **23** (1.0 g, 7.68 mmol) was added and the mixture was stirred at -30 ℃. TLC plates were used to monitor the progress of the reaction. After 4 hr, the reaction was quenched by addition of an aqueous solution of saturated NH_4Cl and a deep blue

aqueous solution was given. The organic layer was washed with saturated NH_4Cl, dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified by gel silica chromatography (hexane/EtOAc = 10/1 to 5/1) to afford the pure product **24** (751 mg, 67%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.17 (q, 2H, *J* = 8.0 Hz), 3.96 - 3.90 (m, 1H), 3.00 (br, 1H), 2.53 - 2.40 (m, 2H), 1.52 (m, 2H), 1.28 (t, 3H, *J* = 4.0 Hz), 0.96 (t, 3H, *J* = 8.0 Hz).

(R)-ethyl 3-(tert-butyldimethylsilyloxy)pentanoate (25). To a solution of compound **24** (680 mg, 4.65 mmol) and imidazole (633 mg, 9.31 mmol) in dry DMF (5 mL) was added TBDMSCl (1.05 g, 6.97 mmol). The mixture was stirred overnight at room temperature. Then the reaction was quenched by addition of H_2O and extracted with ether. The organic layer was washed with brine, dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified by gel silica chromatography (hexane/EtOAc = 100/1 to 50/1) to afford the pure product **25** (1.14 g, 94%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.15 - 4.05 (m, 3H), 2.46 - 2.37 (m, 2H), 1.51 (m, 2H), 1.26 (t, 3H, *J* = 4.0 Hz), 0.88 (t, 3H, *J* = 4.0 Hz), 0.86 (s, 9H), 0.06 (s, 3H), 0.03 (s, 3H).

(R)-3-(tert-butyldimethylsilyloxy)pentanoic acid (26). To a solution of ester **25** (1.0 g, 3.84 mmol) in MeOH (10 mL) was added NaOH solution (10 mL, 19.2 mmol). The reaction was stirred at 50 ℃ for 3 hr and MeOH was removed under reduced pressure. The pH value of residue was adjusted to 4.0 with dilute hydrochloric acid (1 M) and a

white suspension was given. The suspension was extracted with ether. The combined organic solution was washed with brine, dried with Na_2SO_4 and concentrated under reduced pressure to afford the pure product **26** (780 mg, 88%) as a colorless oil which was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.10 - 4.05 (m, 1H), 2.51 - 2.49 (m, 2H), 1.58 - 1.53 (m, 2H), 0.91 (t, 3H, *J* = 6.0 Hz), 0.89 (s, 9H), 0.09 (s, 3H), 0.07 (s, 3H).

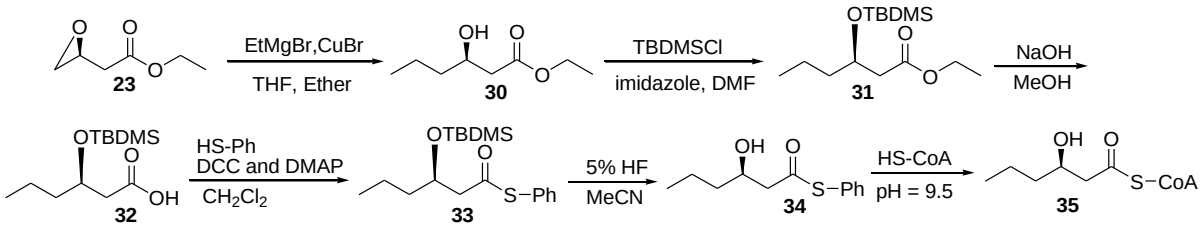
(R)-S-phenyl 3-(tert-butyldimethylsilyloxy)pentanethioate (27). To a solution of acid **26** (650 mg, 2.80 mmol), DCC (692 mg, 3.36 mmol) and catalytic amount of DMAP (10 mg) in dry CH_2Cl_2 (15 mL) was added freshly distilled benzenethiol (338 mg, 3.08 mmol). The reaction was stirred overnight at room temperature. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by gel silica chromatography (hexane/EtOAc = 50/1 to 20/1) to afford the pure product **27** (624 mg, 69%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.40 (s, 5H), 4.18 - 4.12 (m, 1H), 2.78 - 2.71 (m, 2H), 1.58 - 1.53 (m, 2H), 0.90 (t, 3H, *J* = 6.0 Hz), 0.89 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H).

(R)-S-phenyl 3-hydroxypentanethioate (28). The removal of TBDMS-protecting group in thiolester **27** (510 mg, 1.57 mmol) was readily accomplished with 5% HF in MeCN (7 mL). The mixture was stirred at room temperature. TLC plates were used to check the progress of the reaction. After 4 hr, saturated $NaHCO_3$ was added and the mixture was extracted with ether. The combined organic solution was washed with

brine, dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified by gel silica chromatography (hexane/EtOAc = 5/1 to 3/1) to afford the pure product **28** (303 mg, 92%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.43 (s, 5H), 4.06 - 3.99 (m, 1H), 2.90 - 2.77 (m, 2H), 2.66 (d, 1H, *J* = 4.0 Hz), 1.59 - 1.54 (m, 2H), 0.89 (t, 3H, *J* = 6.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 198.13, 134.75, 129.88, 129.11, 127.43, 70.09, 50.16, 29.69, 10.09.

Synthesis of CoA analog (29). To a solution of CoA (80 mg, 0.1 mmol) in KPi buffer (3 mL, 50mM, pH = 9.5) was added (R)-S-phenyl 3-hydroxypentanethioate **28** (0.5 mmol) which was dissolved in 0.3 mL of acetonitrile. The reaction was saturated with argon and stirred vigorously overnight at room temperature. The analytic HPLC was used to check the progress of the reaction. After 24 hr, excess **28** and byproduct benzenethiol were extracted with ether. The pH value of the aqueous solution was adjusted to 4 - 5 by addition of HCl (1 M). The solution was loaded onto a semi-preparative HPLC column (Luna C18-2, 5 μm, 10 mm × 250 mm) that was eluted at 3.00 mL/min using a linear gradient from 5 to 95% methanol in 10.0 mM ammonium acetate (pH = 4.00) over 70 min. The fractions containing the product were pooled, concentrated, and lyophilized to give CoA analog **29** a white powder (62 mg, 71% yield based on UV absorbance, HPLC: t_R = 22.0 min). NMR (400 MHz, D_2O) δ: 8.65 (s, 1H), 8.41 (s, 1H), 6.18 (d, 1H, *J* = 4.0 Hz), 4.58 (s, 1H), 4.25 (s, 2H), 4.01 (s, 1H), 3.96 (m, 1H), 3.84 (m, 1H), 3.58 (m, 1H), 3.43 (t, 2H, *J* = 8.0 Hz), 3.32(t, 2H, *J* = 8.0 Hz), 2.99 (m, 2H), 2.82 - 2.67 (m, 2H), 2.41 (t, 2H, *J* = 8.0 Hz), 1.49 -

1.42 (m, 2H), 0.92 (s, 3H), 0.86 (t, 3H, *J* = 8.0 Hz), 0.79 (s, 3H). ^{31}P NMR (161 MHz, D_2O) δ: 1.55 (s, 1H), -9.77 (d, 1H, *J* = 20.9 Hz), -10.34 (d, 1H, *J* = 20.9 Hz).



Scheme **3 - 5**. Synthesis of CoA analog **35**

(R)-ethyl 3-hydroxyhexanoate (30). The reaction and silica gel chromatography were performed in the same way as **24** using epoxide **23** (1.0 g, 7.68 mmol) and ethylmagnesium bromide solution (18.4 mL, 18.4 mmol) to afford the pure product **30** (798 mg, 65%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.16 (q, 2H, *J* = 8.0 Hz), 4.04 - 3.99 (m, 1H), 2.52 - 2.36 (m, 2H), 1.55 - 1.36 (m, 4H), 1.27 (t, 3H, *J* = 6.0 Hz), 0.93 (t, 3H, *J* = 6.0 Hz).

(R)-ethyl 3-(tert-butyldimethylsilyloxy)hexanoate (31). The reaction and silica gel chromatography were performed in the same way as **25** using alcohol **30** (650 mg, 4.1 mmol) to afford the pure product **31** (1.05 g, 94%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.14 - 4.08 (m, 3H), 2.43 - 2.40 (m, 2H), 1.55 - 1.37 (m, 4H), 1.26 (t, 3H, *J* = 4.0 Hz), 0.88 (t, 3H, *J* = 4.0 Hz), 0.86 (s, 9H), 0.06 (s, 3H), 0.03 (s, 3H).

(R)-3-(tert-butyldimethylsilyloxy)hexanoic acid (32). The reaction was performed in the same way as **26** using TBDMS-protected ester **31** (920 mg, 3.35 mmol) to

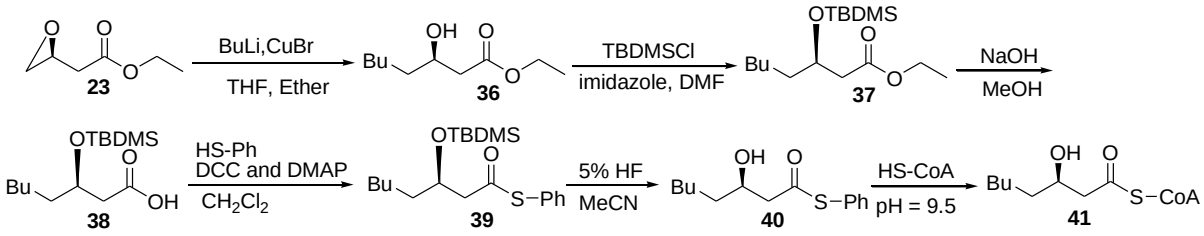
afford the pure product **32** (750 mg, 91%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.12 - 4.08 (m, 1H), 2.53 - 2.46 (m, 2H), 1.50 (m, 2H), 1.34 (m, 2H), 0.90 (t, 3H, *J* = 4.0 Hz), 0.86 (s, 9H), 0.10 (s, 3H), 0.08 (s, 3H).

(R)-S-phenyl 3-(tert-butyldimethylsilyloxy)hexanethioate (33). The reaction and silica gel chromatography were performed in the same way as **27** using TBDMS-protected acid **32** (650 mg, 2.64 mmol) to afford the pure product **33** (642 mg, 72%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.40 (s, 5H), 4.21 - 4.16 (m, 1H), 2.84 - 2.70 (m, 2H), 1.50 (m, 2H), 1.35 (m, 2H), 0.90 (t, 3H, *J* = 4.0 Hz), 0.86 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H).

(R)-S-phenyl 3-hydroxyhexanethioate (34). The reaction and silica gel chromatography were performed in the same way as **28** using thiolester **33** (520 mg, 1.53 mmol) to afford the pure product **34** (315 mg, 94%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.43 (s, 5H), 4.12 - 4.08 (m, 1H), 2.89 - 2.78 (m, 2H), 2.62 (d, 1H, *J* = 4.0 Hz), 1.52 - 1.38 (m, 4H), 0.94 (t, 3H, *J* = 8.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 197.62, 134.73, 129.79, 129.50, 127.65, 68.47, 50.85, 39.20, 18.96, 14.30.

Synthsis of CoA analog (35). The reaction and purification were performed in the same way as **29** using (R)-S-phenyl 3-hydroxyhexanethioate **34** (0.8 mmol) and HSCoA (80 mg, 0.1 mmol) to afford the CoA analog **35** as a white solid (57 mg, 64% yield based on UV absorbance, HPLC: t_R = 25.5 min). NMR (400 MHz, D_2O) δ: 8.52

(s, 1H), 8.24 (s, 1H), 6.15 (d, 1H, *J* = 4.0 Hz), 4.56 (s, 1H), 4.21 (s, 1H), 4.03 (m, 1H), 3.98 (s, 1H), 3.79 (m, 1H), 3.54 (m, 1H), 3.42 (t, 2H, *J* = 8.0 Hz), 3.30 (t, 2H, *J* = 8.0 Hz), 2.97 (m, 2H), 2.76 - 2.65 (m, 2H), 2.39 (t, 2H, *J* = 8.0 Hz), 1.42 - 1.27 (m, 4H), 0.86 (s, 3H), 0.84 (t, 3H, *J* = 8.0 Hz), 0.73 (s, 3H). ^{31}P NMR (161 MHz, D_2O) δ: 1.36 (s, 1H), -9.72 (m, 1H), -10.26 (m, 1H).



Scheme **3 - 6**. Synthesis of CoA analog **41**

(R)-ethyl 3-hydroxyoctanoate (36). The reaction and silica gel chromatography were performed in the same way as **24** using epoxide **23** (1.0 g, 7.68 mmol) and *n*-butyllithium solution (11.5 mL, 18.4 mmol) to afford the pure product **36** (808 mg, 56%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.16 (q, 2H, *J* = 8.0 Hz), 4.01 - 3.96 (m, 1H), 2.99 (d, 1H, *J* = 4.0 Hz), 2.51 - 2.39 (m, 2H), 1.48 - 1.24 (m, 12H), 0.88 (t, 3H, *J* = 6.0 Hz).

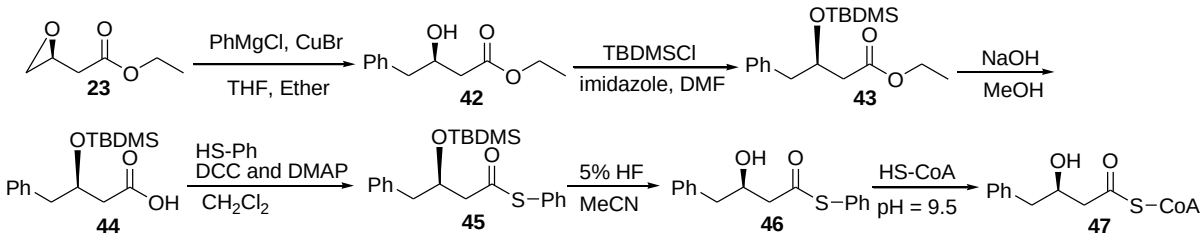
(R)-ethyl 3-(tert-butyldimethylsilyloxy)octanoate (37). The reaction and silica gel chromatography were performed in the same way as **25** using alcohol **36** (720 mg, 3.83 mmol) to afford the pure product **37** (1.06 g, 92%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.14 - 4.08 (m, 3H), 2.43 - 2.41 (m, 2H), 1.52 - 1.24 (m, 11H), 0.88 (t, 3H, *J* = 4.0 Hz), 0.85 (s, 9H), 0.06 (s, 3H), 0.03 (s, 3H).

(R)-3-(tert-butyldimethylsilyloxy)octanoic acid (38). The reaction was performed in the same way as **26** using protected ester **37** (920 mg, 3.05 mmol) to afford the pure product **38** (710 mg, 85%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.13 - 4.06 (m, 1H), 2.56 - 2.47 (m, 2H), 1.52 - 1.24 (m, 8H), 0.88 (t, 3H, *J* = 4.0 Hz), 0.89 (s, 9H), 0.10 (s, 3H), 0.09 (s, 3H).

(R)-S-phenyl 3-(tert-butyldimethylsilyloxy)octane thioate (39). The reaction and silica gel chromatography were performed in the same way as **27** using TBDMS-acid **38** (550 mg, 2.0 mmol) to afford the pure product **39** (361 mg, 66%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.40 (s, 5H), 4.22 - 4.16 (m, 1H), 2.84 - 2.70 (m, 2H), 1.56 (m, 2H), 1.31 (m, 6H), 0.89 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 196.00, 129.56, 134.58, 129.37, 129.29, 127.69, 127.37, 69.80, 51.54, 37.77, 32.06, 26.09, 24.84, 22.84, 18.30, 14.26, -4.29, -4.49.

(R)-S-phenyl 3-hydroxyoctane thioate (40). The reaction and silica gel chromatography were performed in the same way as **28** using TBDMS-thiolester **39** (290 mg, 0.86 mmol) to afford the pure product **40** (197 mg, 91%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.43 (s, 5H), 4.13 - 4.08 (m, 1H), 2.89 - 2.76 (m, 2H), 2.63 (d, 1H, *J* = 4.0 Hz), 1.56 - 1.29 (m, 8H), 0.89 (t, 3H, *J* = 6.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 198.36, 134.74, 129.91, 129.53, 127.32, 68.85, 50.45, 36.68, 31.92, 25.35, 22.82, 14.27.

Synthesis of CoA analog (41). The reaction and purification were performed in the same way as **29** using (R)-S-phenyl 3-hydroxyoctane thioate **40** (0.7 mmol) and HSCoA (80 mg, 0.1 mmol) to afford the CoA analog **41** as a white solid (45 mg, 49% yield based on UV absorbance, HPLC: t_R = 35.5 min). NMR (400 MHz, D_2O) δ: 8.52 (s, 1H), 8.22 (s, 1H), 6.14 (d, 1H, *J* = 4.0 Hz), 4.56 (s, 1H), 4.21 (s, 2H), 4.00 - 3.99 (m, 2H), 3.80 (m, 1H), 3.54 (m, 1H), 3.42 (t, 2H, *J* = 8.0 Hz), 3.30 (t, 2H, *J* = 8.0 Hz), 2.99 - 2.96 (m, 2H), 2.76 - 2.68 (m, 2H), 2.40 (t, 2H, *J* = 8.0 Hz), 1.42 - 1.19 (m, 8H), 0.86 (s, 3H), 0.80 (t, 3H, *J* = 8.0 Hz), 0.73 (s, 3H). ^{31}P NMR (161 MHz, D_2O) δ: 1.58 (s, 1H), -9.71 (m, 1H), -10.17 (m, 1H).



Scheme **3 - 7**. Synthesis of CoA analog **47**

(R)-ethyl 3-hydroxy-4-phenyl butanoate (42). The reaction and silica gel chromatography were performed in the same way as **24** using epoxide **23** (1.0 g, 7.68 mmol) and phenylmagnesium chloride solution (9.2 mL, 18.4 mmol) in THF to afford the pure product **42** (719 mg, 45%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.33 - 7.22 (m, 5H), 4.28 - 4.25 (m, 1H), 4.15 (q, 2H, *J* = 8.0 Hz), 3.00 (d, 1H, *J* = 4.0 Hz), 2.89 - 2.75 (m, 2H), 2.52 - 2.40 (m, 2H), 1.26 (t, 3H, *J* = 8.0 Hz).

(R)-ethyl 3-(tert-butyldimethylsilyloxy)-4-phenyl butanoate (43). The reaction and

silica gel chromatography were performed in the same way as **25** using alcohol **42** (620 mg, 2.98 mmol) to afford the pure product **43** (911 mg, 95%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.30 - 7.17 (m, 5H), 4.31 (q, 2H, *J* = 8.0 Hz), 4.11 - 4.06 (m, 1H), 2.81 - 2.79 (m, 2H), 2.42 (d, 2H, *J* = 8.0 Hz), 1.25 (t, 3H, *J* = 8.0 Hz), 0.83 (s, 9H), -0.02 (s, 3H), -0.14 (s, 3H).

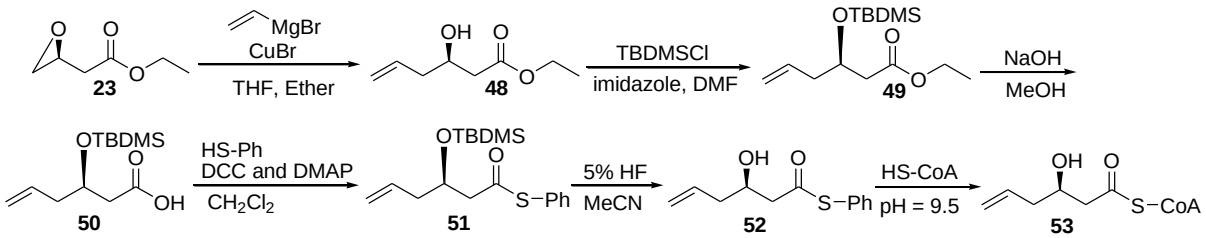
(R)-3-(tert-butyldimethylsilyloxy)-4-phenyl butanoic acid (44). The reaction was performed in the same way as **26** using TBDMS-protected ester **43** (800 mg, 2.48 mmol) to afford the pure product **44** (650 mg, 89%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.31 - 7.17 (m, 5H), 4.32 - 4.26 (m, 1H), 2.85 - 2.77 (m, 2H), 2.53 - 2.44 (m, 2H), 0.85 (s, 9H), 0.01 (s, 3H), -0.11 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 176.67, 137.92, 129.93, 128.65, 126.81, 70.95, 44.13, 41.74, 25.96, 18.18, 4.72, -4.89.

(R)-S-phenyl 3-(tert-butyldimethylsilyloxy)-4-phenyl butanethioate (45). The reaction and silica gel chromatography were performed in the same way as **27** using TBDMS-acid **44** (500 mg, 1.70 mmol) to afford the pure product **45** (486 mg, 74%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.41 - 7.18 (m, 10H), 4.39 - 4.35 (m, 1H), 2.84 - 2.70 (m, 4H), 0.87 (s, 9H), 0.00 (s, 3H), -0.13 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 195.89, 138.16, 134.60, 130.04, 129.63, 129.41, 128.56, 128.02, 126.69, 71.20, 50.79, 44.33, 26.09, 18.28, -4.69, -4.75.

(R)-S-phenyl 3-hydroxy-4-phenyl butanethioate (46). The reaction and silica gel

chromatography were performed in the same way as **28** using thiolester **45** (360 mg, 0.93 mmol) to afford the pure product **46** (238 mg, 94%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.44 - 7.22 (m, 10H), 4.38 - 4.31 (m, 1H), 2.91 - 2.63 (m, 4H), 2.63 (d, 1H, *J* = 4.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 197.78, 137.71, 134.79, 129.95, 129.77, 129.77, 129.70, 129.59, 128.90, 127.38, 127.02, 69.75, 49.67, 43.19.

Synthesis of CoA analog (47). The reaction and purification were performed in the same way as **29** using (R)-S-phenyl 3-hydroxy-4-phenyl butanethioate **46** (0.2 mmol) and HSCoA (40 mg, 0.05 mmol) to afford the CoA analog **47** as a white solid (20 mg, 44% yield based on UV absorbance, HPLC: t_R = 33.1 min). NMR (400 MHz, D_2O) δ 8.50 (s, 1H), 8.19 (s, 1H), 7.31 - 7.19 (m, 6H), 6.12 (d, 1H, *J* = 4.0 Hz), 4.56 (s, 1H), 4.29 (m, 1H), 4.21 (s, 1H), 3.98 (s, 1H), 3.80 (m, 1H), 3.53 (m, 1H), 3.40 (t, 2H, *J* = 4.0 Hz), 3.29 (d, 1H, *J* = 4.0 Hz), 2.99 - 2.94 (m, 2H), 2.76 (m, 3H), 2.38 (t, 2H, *J* = 6.0 Hz), 0.85 (s, 3H), 0.71 (s, 3H). ^{31}P NMR (161 MHz, D_2O) δ: 2.63 (s, 1H), -9.62 (m, 1H), -10.13 (m, 1H).



Scheme **3 - 8**. Synthesis of CoA analog **53**

(R)-ethyl 3-hydroxyhex-5-enoate (48). The reaction and silica gel chromatography were performed in the same way as **24** using epoxide **23** (1.0 g, 7.68 mmol) and

vinylmagnesium bromide solution in THF (18.4 mL, 18.4 mmol) to afford the pure product **48** (655 mg, 54%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.87 - 5.76 (m, 1H), 5.14 - 5.10 (m, 2H), 4.16 (q, 2H, *J* = 8.0 Hz), 4.10 - 4.05 (m, 1H), 2.98 (d, 1H, *J* = 4 Hz), 2.52 - 2.22 (m, 2H), 2.30 - 2.22 (m, 2H), 1.26 (t, 3H, *J* = 8.0 Hz).

(R)-ethyl 3-(tert-butyldimethylsilyloxy) hex-5-enoate (49). The reaction and silica gel chromatography were performed in the same way as **25** using alcohol **48** (540 mg, 3.43 mmol) to afford the pure product **49** (850 mg, 91%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.85 - 5.75 (m, 1H), 5.08 - 5.04 (m, 2H), 4.23 - 4.07 (m, 3H), 2.43 - 2.41 (m, 2H), 2.27 (t, 2H, *J* = 8.0 Hz), 0.86 (s, 9H), 0.07 (s, 3H), 0.04 (s, 3H).

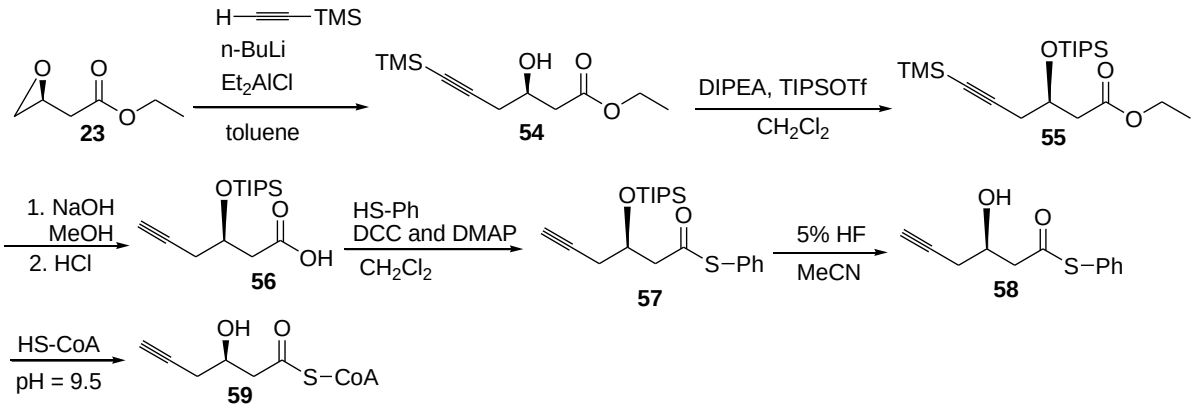
(R)-3-(tert-butyldimethylsilyloxy) hex-5-enoic acid (50). The reaction was performed in the same way as **26** using TBDMS-protected ester **49** (740 mg, 2.72 mmol) to afford the pure product **50** (604 mg, 91%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.83 - 5.73 (m, 1H), 5.12 - 5.07 (m, 2H), 4.21 - 4.15 (m, 1H), 2.57 - 2.45 (m, 2H), 2.30 (t, 2H, *J* = 6.0 Hz), 0.88 (s, 9H), 0.10 (s, 3H), 0.08 (s, 3H).

(R)-S-phenyl 3-(tert-butyldimethylsilyloxy) hex-5-ene thioate (51). The reaction and silica gel chromatography were performed in the same way as **27** using TBDMS-protected acid **50** (420 mg, 1.72 mmol) to afford the pure product **51** (440 mg, 76%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.40 (s, 5H), 5.84 - 5.77 (m, 1H), 5.12 - 5.08 (m, 2H), 4.30 - 4.24 (m, 1H), 2.82 - 2.72 (m, 2H), 2.32 - 2.28 (m,

2H), 0.89 (s, 9H), 0.07 (s, 3H), 0.05 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 195.94, 134.58, 134.10, 129.60, 129.39, 128.10, 118.33, 69.25, 50.95, 42.33, 26.07, 18.31, -4.26, -4.60.

(R)-S-phenyl 3-hydroxyhex-5-ene thioate (52). The reaction and silica gel chromatography were performed in the same way as **28** using TBDMS-protected thiolester **51** (280 mg, 0.83 mmol) to afford the pure product **52** (172 mg, 93%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.43 (s, 5H), 5.86 - 5.79 (m, 1H), 5.18 - 5.14 (m, 2H), 4.20 - 4.15 (m, 1H), 2.91 - 2.78 (m, 2H), 2.68 (d, 1H, *J* = 4.0 Hz), 2.31 (t, 2H, *J* = 8.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 197.98, 135.82, 133.91, 129.93, 129.54, 127. 27, 118.83, 68.02, 49.66, 41.14.

Synthesis of CoA analog (53). The reaction and purification were performed in the same way as **29** using (R)-S-phenyl 3-hydroxyhex-5-ene thioate **52** (0.3 mmol) and HSCoA (40 mg, 0.05 mmol) to afford the CoA analog **53** as a white solid (23 mg, 53% yield based on UV absorbance, HPLC: t_R = 23.2 min). NMR (400 MHz, D_2O) δ: 8.52 (s, 1H), 8.24 (s, 1H), 6.14 (d, 1H, *J* = 4.0 Hz), 5.82 – 5.75 (m, 1H), 5.13 - 5.09 (m, 2H), 4.56 (s, 1H), 4.21 (s, 2H), 4.12 (m, 1H), 3.99 (s, 1H), 3.80 (m, 1H), 3.55 (m, 1H), 3.42 (t, 2H, *J* = 8.0 Hz), 3.30 (t, 2H, *J* = 8.0 Hz), 2.99 (m, 2H), 2.82 - 2.66 (m, 2H), 2.40 (t, 2H, *J* = 8.0 Hz), 2.25 - 2.21 (m, 2H), 0.86 (s, 3H), 0.73 (s, 3H). ^{31}P NMR (161 MHz, D_2O) δ: 1.82 (s, 1H), -9.73 (m, 1H), -10.06 (m, 1H).



Scheme **3 - 9**. Synthesis of CoA analog **59**

(R)-ethyl 3-hydroxy-6-(trimethylsilyl) hex-5-ynoate (54). To a solution of trimethylsilyl acetylene (1.28 mL, 9.21 mmol) in dry toluene (20 mL) at - 45 ℃ was added *n*-BuLi (3.68 mL, 9.21 mmol). After 10 min, the mixture was warmed to 0 ℃ and vigorously stirred for 1 h. Then, diethylaluminum chloride (9.21 mL, 9.21 mmol) at 0 ℃ was added and the mixture was stirred for 1 h. Epoxide **23** (1.0 g, 7.68 mmol) in toluene (5 mL) was added and the reaction was stirred at 0 ^{o}C. TLC plates were used to check the progress of the reaction. After 4 hr, the reaction was quenched by adding saturated NH_4Cl dropwise. After filtration, the organic layer was washed with brine, dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc = 50/ to 10/1) to afford the pure product **54** (822 mg, 47%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.21 - 4.16 (m, 3H), 3.03 (d, 1H, *J* = 4.0 Hz), 2.68 - 2.65 (m, 1H), 2.56 - 2.45 (m, 3H), 1.28 (t, 3H, *J* = 6.0 Hz), 0.15 (s, 9H).

(R)-ethyl 3-(triisopropylsilyloxy)-6-(trimethylsilyl) hex-5-ynoate (55). To a

solution of alcohol **54** (710 mg, 3.11 mmol) and diisopropylethylamine (802 mg, 6.22 mmol) in dry CH_2Cl_2 (20 mL) at 0 ℃ was added triisopropylsilyl trifluoromethanesulfonate (1.43 g, 4.66 mmol). The reaction was stirred for 3 hr at 0 ℃ and was quenched with H_2O. The organic layer was washed with brine, dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane to hexane/EtOAc = 100/1) to afford the pure product **55** (931 mg, 78%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.43 - 4.37 (m, 1H), 4.13 (t, 2H, *J* = 8.0 Hz), 2.73 - 2.68 (m, 1H), 2.58 - 2.50 (m, 3H), 1.24 (q, 3H, *J* = 8.0 Hz), 1.09 - 1.05 (m, 21H), 0.13 (s, 9H).

(R)-3-(triisopropylsilyloxy)hex-5-ynoic acid (56). To a solution of compound **55** (850 mg, 2.21 mmol) in MeOH (10 mL) was added an aqueous solution of NaOH (10 mL, 11.0 mmol). The reaction was stirred at 50 ℃ for 7 hr. Then, MeOH was removed under reduced pressure. The residue was acidified (pH = 4.0) with dilute HCl and extracted with ether. The combined organic layer was washed with brine, dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc = 10/1 to 5/1) to afford the pure product **56** (514 mg, 82%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.45 - 4.37 (m, 1H), 2.83 - 2.64 (m, 2H), 2.53 - 2.51 (m, 2H), 2.04 (t, 1H, *J* = 4.0 Hz), 1.12 - 1.06 (m, 21H).

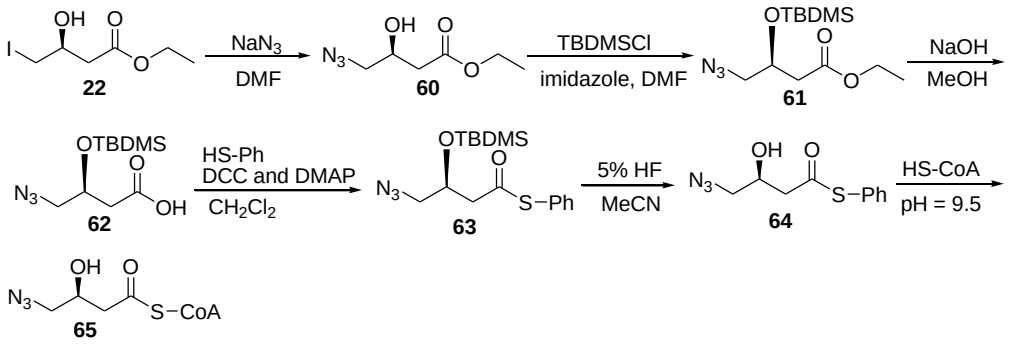
(R)-S-phenyl 3-(triisopropylsilyloxy)hex-5-yne thioate (57). The reaction and silica

gel chromatography were performed in the same way as **27** using TIPS-protected acid **56** (440 mg, 1.55 mmol) to afford the pure product **57** (379 mg, 65%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.41 (s, 5H), 4.51 - 4.45 (m, 1H), 3.13 - 2.94 (m, 2H), 2.50 (d, 2H, *J* = 4.0 Hz), 2.05 (t, 1H, *J* = 4.0 Hz), 10.7 (s, 21H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 195.49, 134.63, 129.66, 129.41, 127.94, 80.42, 71.41, 68.18, 50.49, 27.76, 18.35, 18.32, 12.67.

(R)-S-phenyl 3-hydroxyhex-5-yne thioate (58). The reaction and silica gel chromatography were performed in the same way as **28** using thiolester **57** (310 mg, 0.82 mmol) to afford the pure product **58** (164 mg, 91%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.43 (s, 5H), 4.31 - 4.25 (m, 1H), 3.08 - 2.84 (m, 2H), 2.59 (br, 1H), 2.49 - 2.46 (m, 2H), 2.11 (t, 1H, *J* = 4.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 197.70, 134.75, 130.01, 129.58, 127.09, 79.89, 71.71, 67.07, 48.93, 26.55.

Synthesis of CoA analog (59). The reaction and purification were performed in the same way as **29** using (R)-S-phenyl 3-hydroxyhex-5-yne thioate **58** (0.2 mmol) and HSCoA (40 mg, 0.05 mmol) to afford the CoA analog **59** as a white solid (24 mg, 56% yield based on UV absorbance, HPLC: t_R = 20.2 min). NMR (400 MHz, D_2O) δ: 8.53 (s, 1H), 8.25 (s, 1H), 6.16 (d, 1H, *J* = 4.0 Hz), 4.58 (s, 1H), 4.22 (m, 4H), 3.99 (s, 1H), 3.82 (m, 1H), 3.56 (m, 2H), 3.43 (t, 2H, *J* = 8.0 Hz), 3.32 (t, 2H, *J* = 8.0 Hz), 2.99 (t, 2H, *J* = 8.0 Hz), 2.89 - 2.85 (m, 2H), 2.44 - 2.40 (m, 4H), 1.17 (t, 1H, *J* = 4.0 Hz), 0.87 (s, 3H), 0.74 (s, 3H). ^{31}P NMR (161 MHz, D_2O) δ: 1.96 (s, 1H), -9.57 (m, 1H),

-10.10 (m, 1H).



Scheme **3 - 10**. Synthesis of CoA analog **65**

(S)-ethyl 4-azido-3-hydroxy butanoate (60). A solution of sodium azide (1.52 g, 233 mmol) and (S)-ethyl 3-hydroxy-4-iodobutanoate **22** (4.0 g, 15.6 mmol) in dry DMF (10 mL) was heated to 100 ℃ for 2 h. After cooling to room temperature, the solution was dissolved in ether (50 mL). The mixture was washed with brine, dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc = 2/1) to afford the pure product **60** (2.6 g, 95%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.19 (q, 2H, *J* = 6.0 Hz), 3.39 - 3.31 (m, 2H), 3.21 (br, 1H), 2.55 - 2.53 (m, 2H), 1.28 (t, 3H, *J* = 8.0 Hz).

(S)-ethyl 4-azido-3-(tert-butyldimethylsilyloxy) butanoate (61). The reaction and silica gel chromatography were performed in the same way as **25** using alcohol **60** (2.2 g, 12.7 mmol) to afford the pure product **61** (3.17 g, 87%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.27 - 4.16 (m, 1H), 4.15 - 4.09 (t, 2H, *J* = 8.0 Hz), 3.38 - 3.23 (m, 2H), 2.55 - 2.52 (m, 2H), 1.26 (q, 3H, *J* = 8.0 Hz), 0.88 (s, 9H), 0.12 (s, 3H), 0.08 (s, 3H).

(S)-4-azido-3-(tert-butyldimethylsilyloxy) butanoic acid (62). The reaction was performed in the same way as **26** using TBDMS-protected ester **61** (2.0 g, 6.97 mmol) to afford the pure product **62** (1.59 g, 88%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.27 - 4.23 (m, 1H), 3.40 - 3.27 (m, 2H), 2.66 - 2.54 (m, 2H), 0.89 (s, 9H), 0.13 (s, 3H), 0.10 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 177.74, 68.67, 56.44, 40.14, 25.86, 18.13, -4.47, -4.84.

(S)-S-phenyl 4-azido-3-(tert-butyldimethylsilyloxy) butanethioate (63). The reaction and silica gel chromatography were performed in the same way as **27** using TBDMS-protected acid **62** (1.22 g, 4.71 mmol) to afford the pure product **63** (1.27 g, 77%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ:7.41 (s, 5H), 4.33 - 4.29 (m, 1H), 3.41 - 3.21 (m, 2H), 2.90 - 2.88 (m, 2H), 0.91 (s, 9H), 0.12 (s, 3H), 0.10 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 195.17, 134.62, 134.46, 129.84, 129.70, 129.51, 127.65, 69.03, 56.57, 48.79, 26.00, 18.23, -4.44, -4.68.

(S)-S-phenyl 4-azido-3-hydroxybutane thioate (64). The reaction and silica gel chromatography were performed in the same way as **28** using TBDMS-protected thiolester **63** (1.05 g, 2.99 mmol) to afford the pure product **64** (673 mg, 95%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.44 (s, 5H), 4.31 - 4.25 (m, 1H), 3.42 - 3.34 (m, 2H), 2.97 - 2.85 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 197.42, 135.66, 130.12, 129.63, 126.89, 67.99, 55.69, 47.25.

Synthesis of CoA analog (65). The reaction and purification were performed in the same way as **29** using (S)-S-phenyl 4-azido-3-hydroxybutane thioate **64** (1.0 mmol) and HSCoA (80 mg, 0.1 mmol) to afford the CoA analog **65** as a white solid (59 mg, 66% yield based on UV absorbance, HPLC: t_R = 17.8 min). NMR (400 MHz, D_2O) δ: 8.53 (s, 1H), 8.25 (s, 1H), 6.16 (d, 1H, *J* = 4.0 Hz), 4.57 (s, 1H), 4.22 (m, 3H), 3.99 (s, 1H), 3.80 (m, 1H), 3.54 (m, 1H), 3.43 (t, 2H, *J* = 4.0 Hz), 3.39 (d, 1H, *J* = 4.0 Hz), 3.31 (t, 2H, *J* = 8.0 Hz), 2.99 (t, 2H, *J* = 8.0 Hz), 2.80 (m, 2H), 2.41 (t, 2H, *J* = 4.0 Hz), 0.87 (s, 3H), 0.74 (s, 3H). ^{31}P NMR (161 MHz, D_2O) δ: 1.70 (s, 1H), -9.72 (m, 1H), -10.22 (m, 1H).

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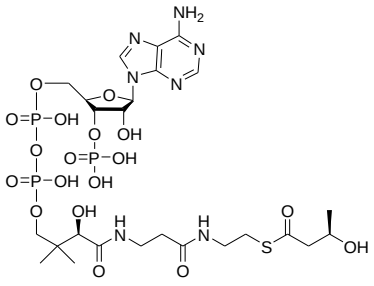
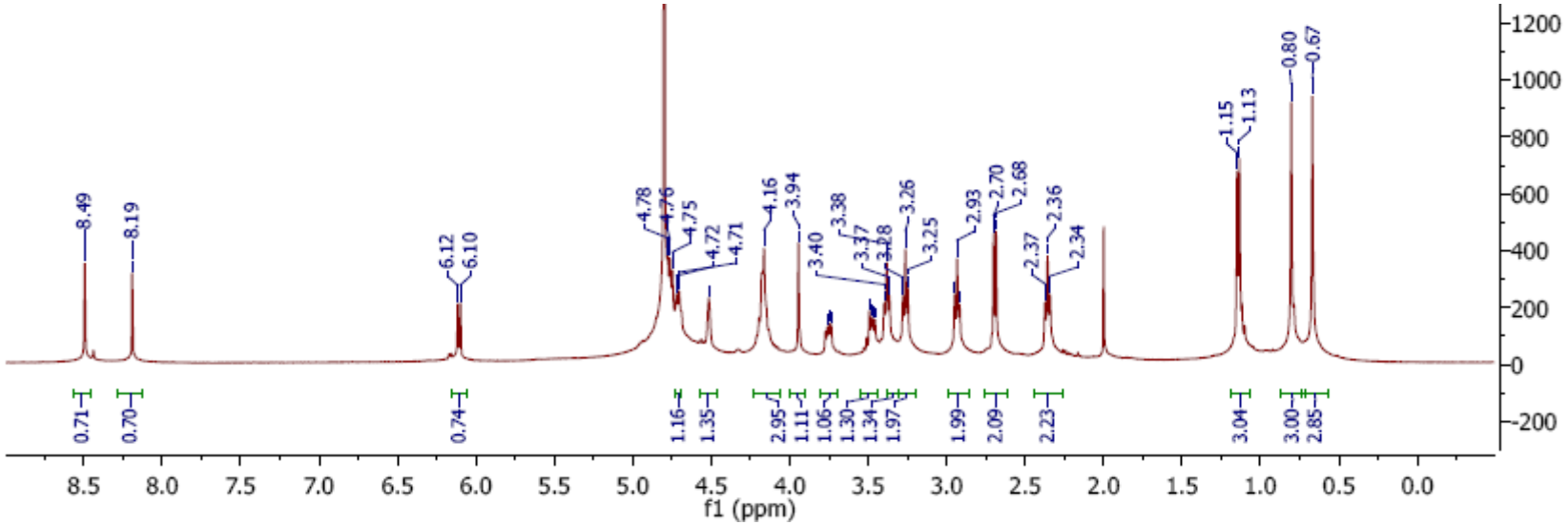
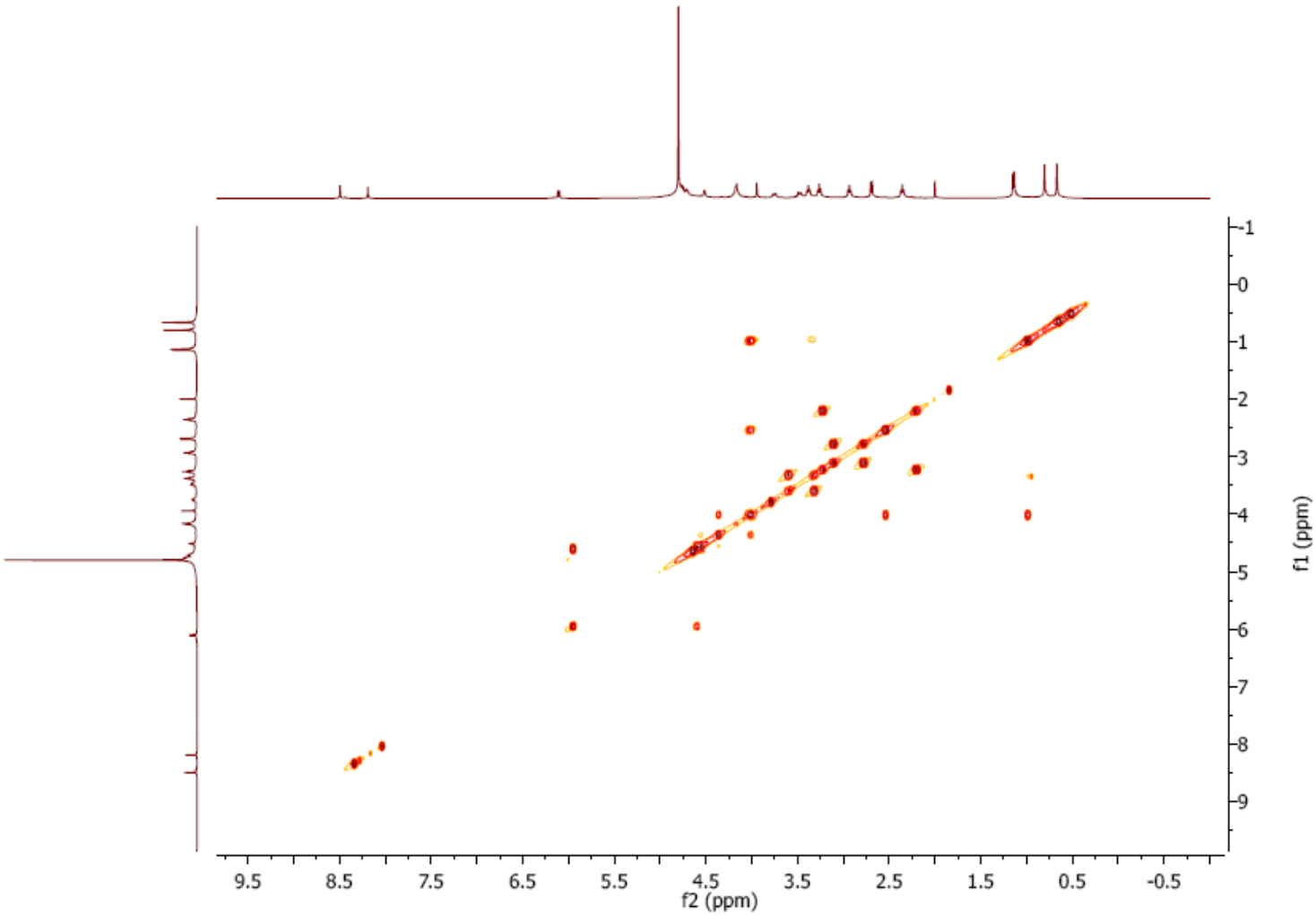
Appendix: ^{1}H NMR, gCOSY and ^{31}P NMR of CoA Analogs



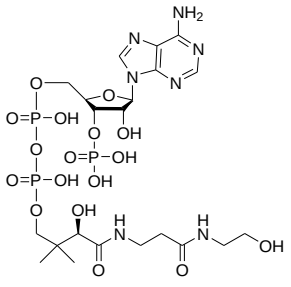
HBCoA



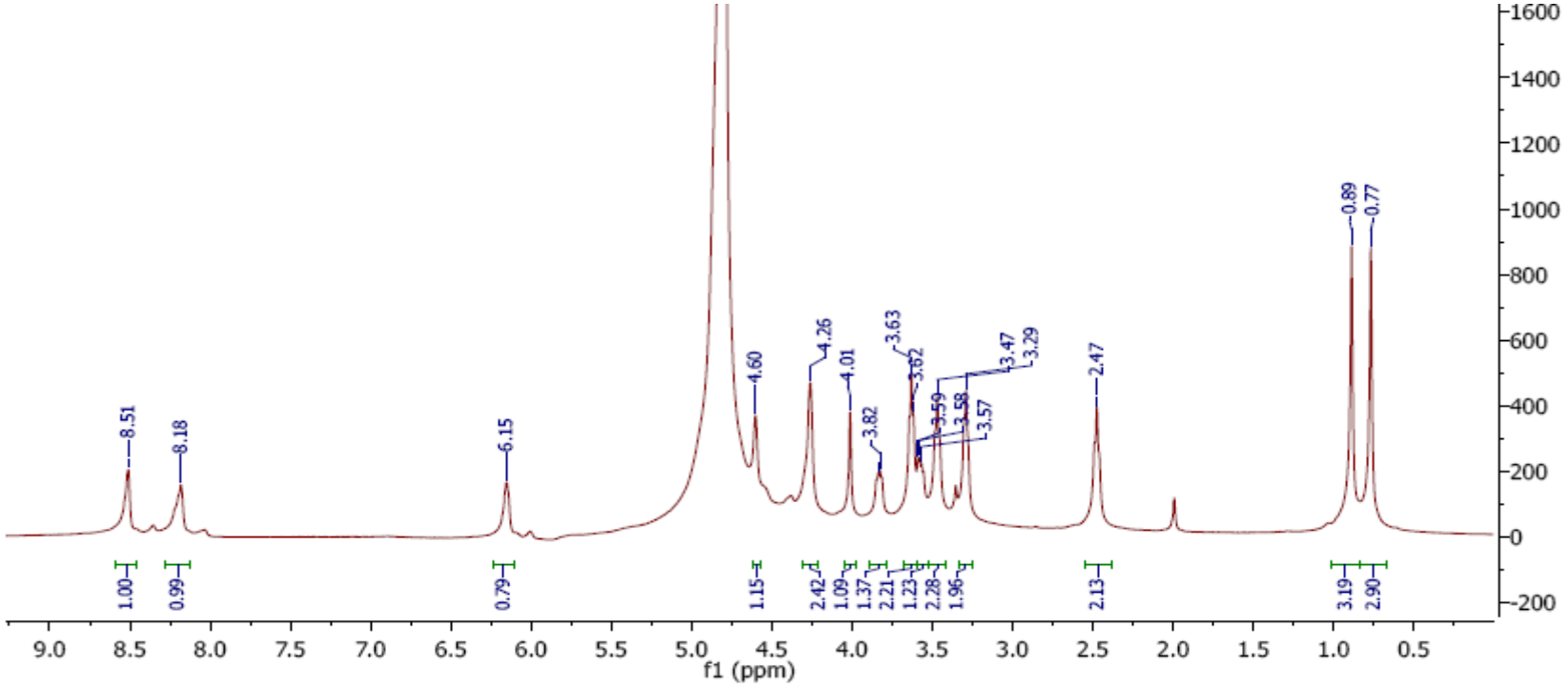
^{1}H NMR of HBCoA



gCOSY NMR of HBCoA



HOCoA



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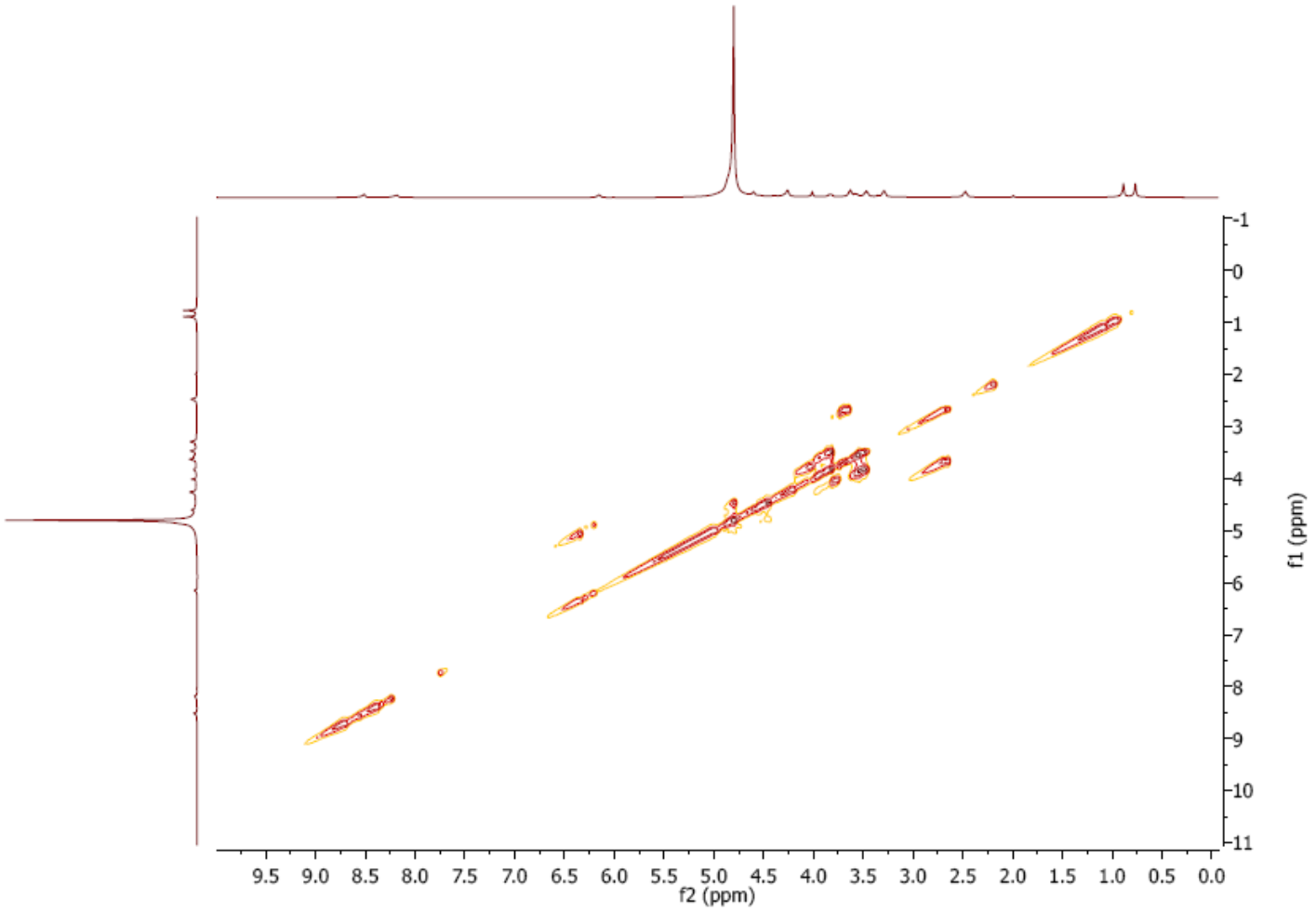
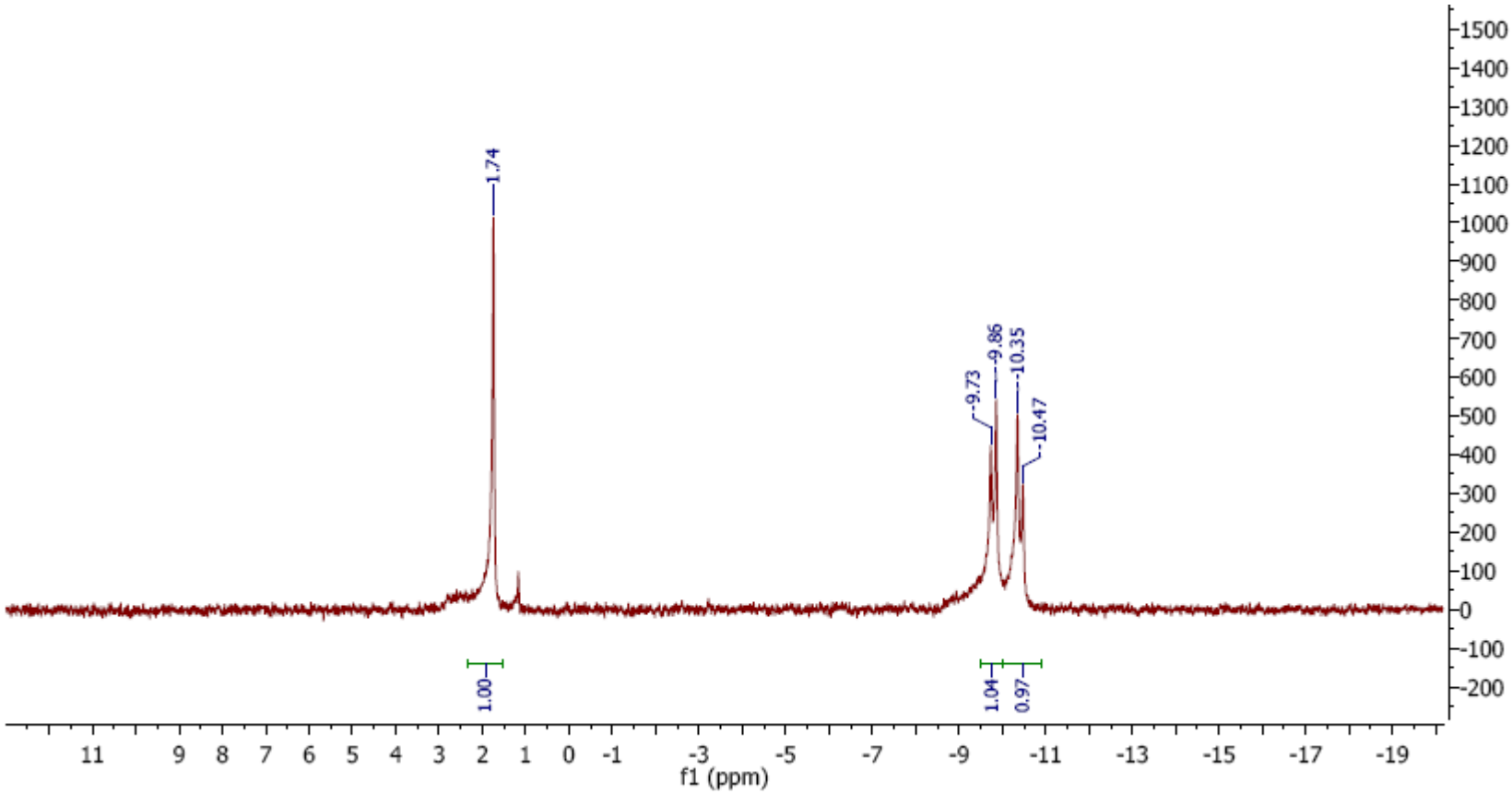
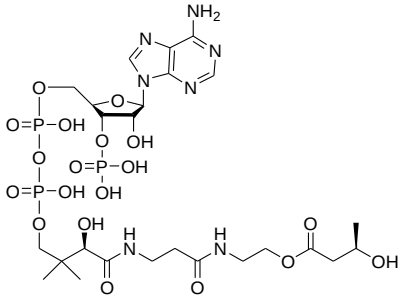


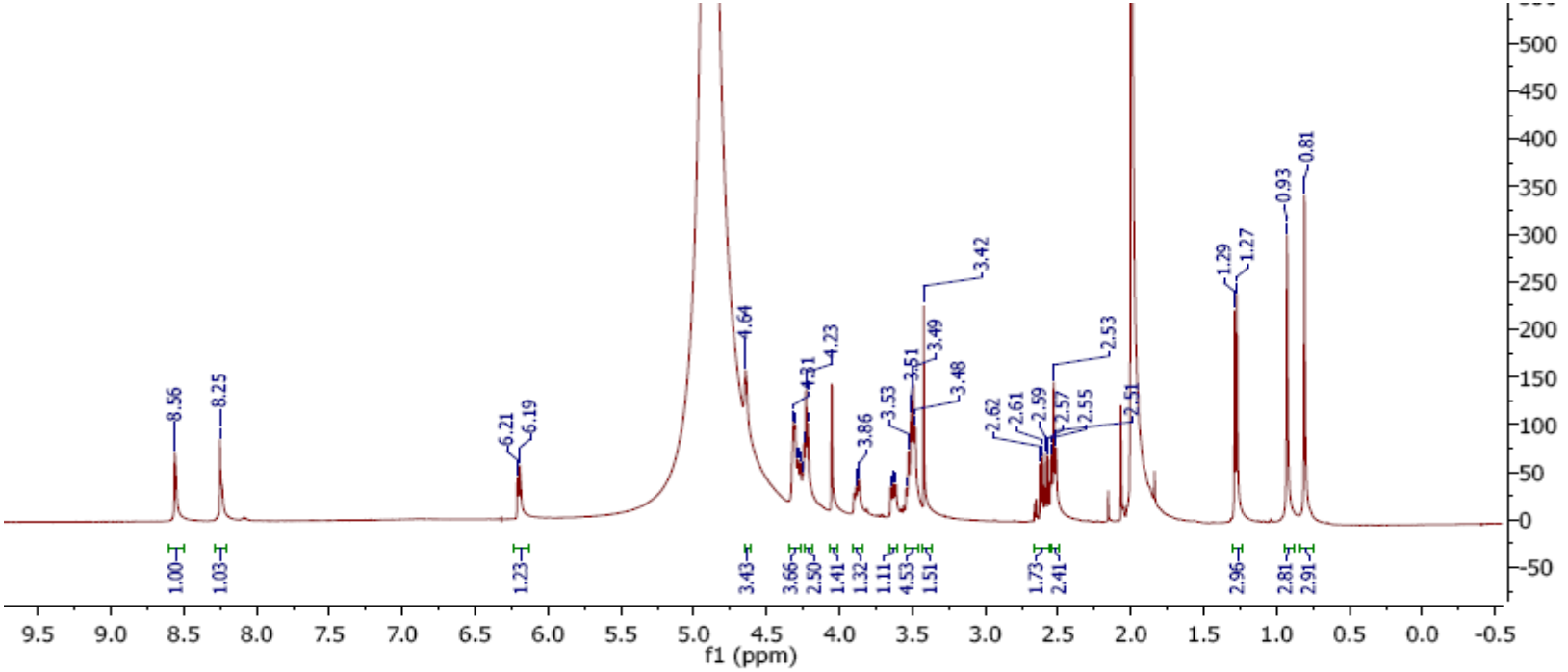
gCOSY NMR of HOCoA



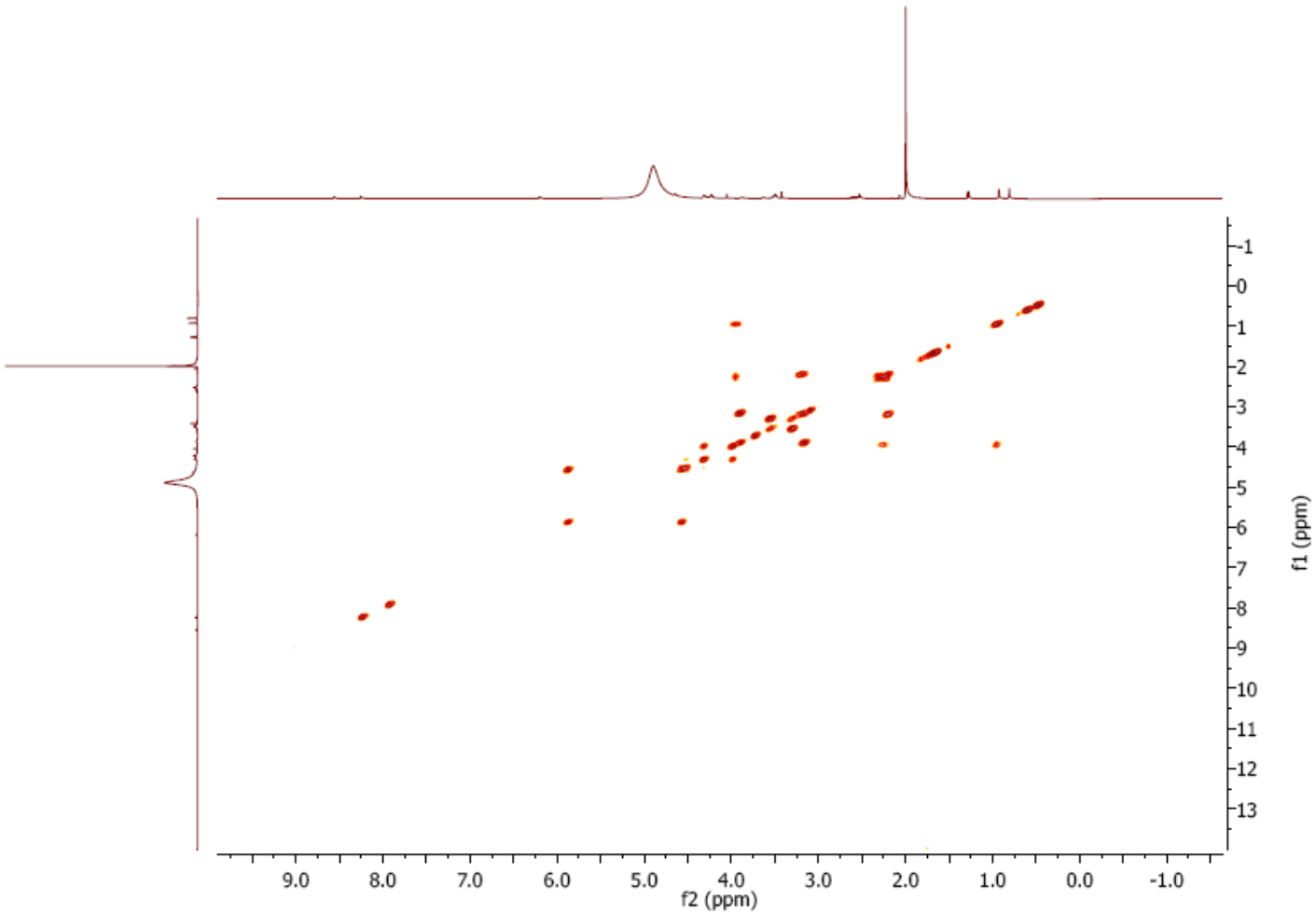
^{31}P NMR of HOCoA



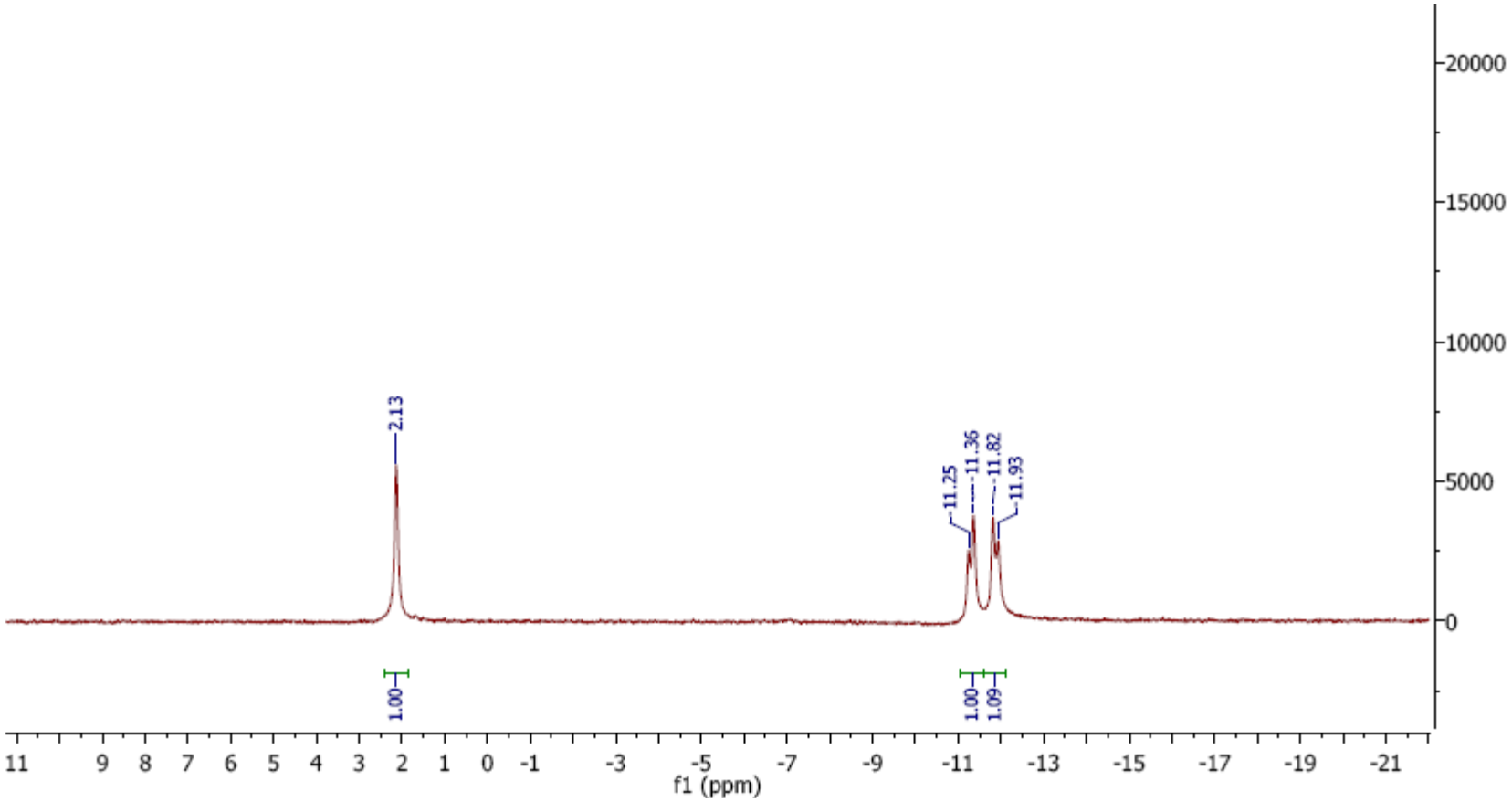
HBOCoA



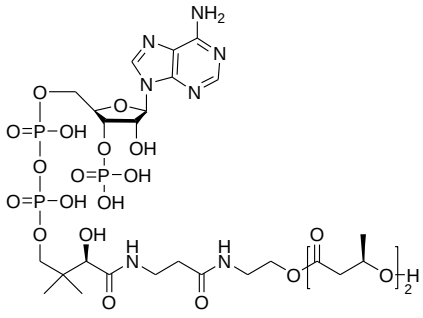
^{1}H NMR of HBOCoA



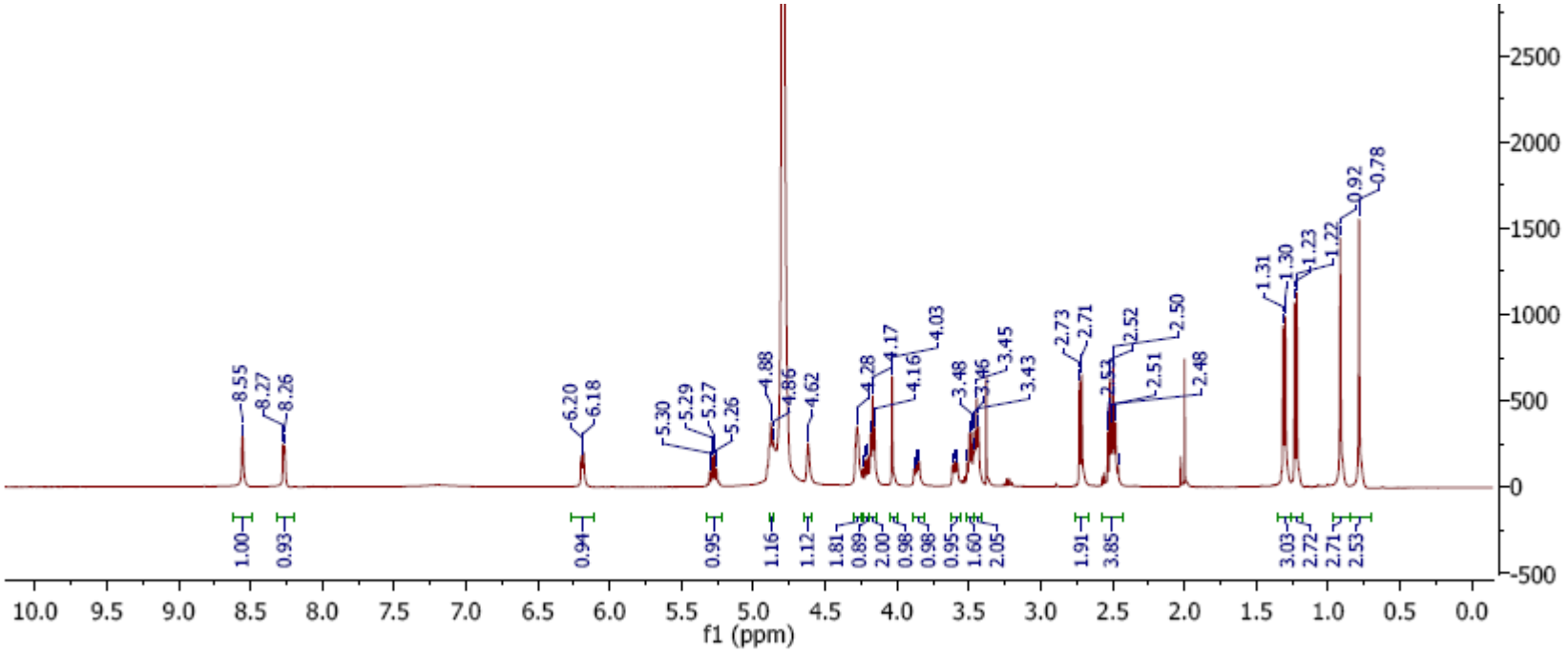
gCOSY NMR of HBOCoA



^{31}P NMR of HBOCoA



$(HB)_2OCoA$



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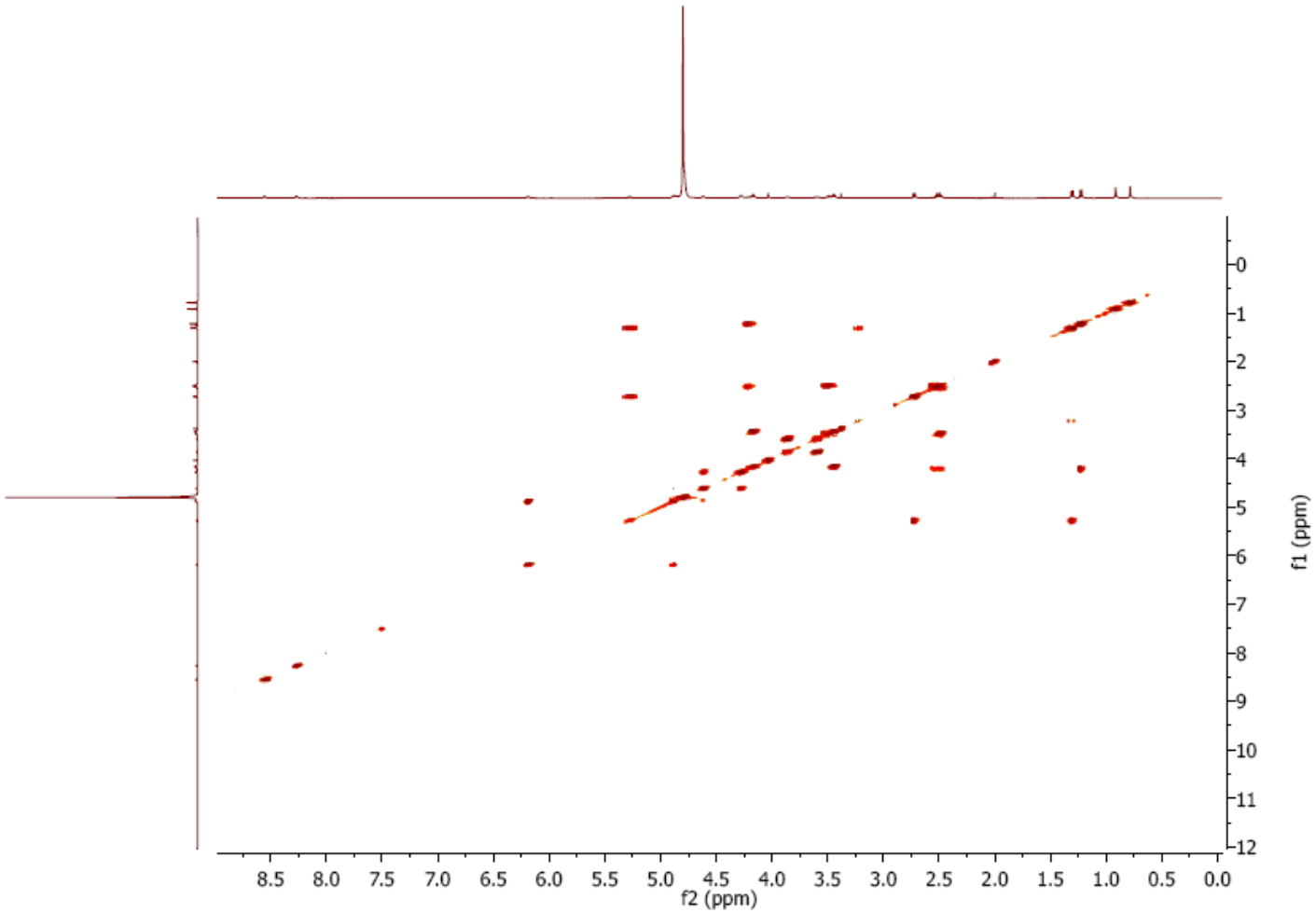

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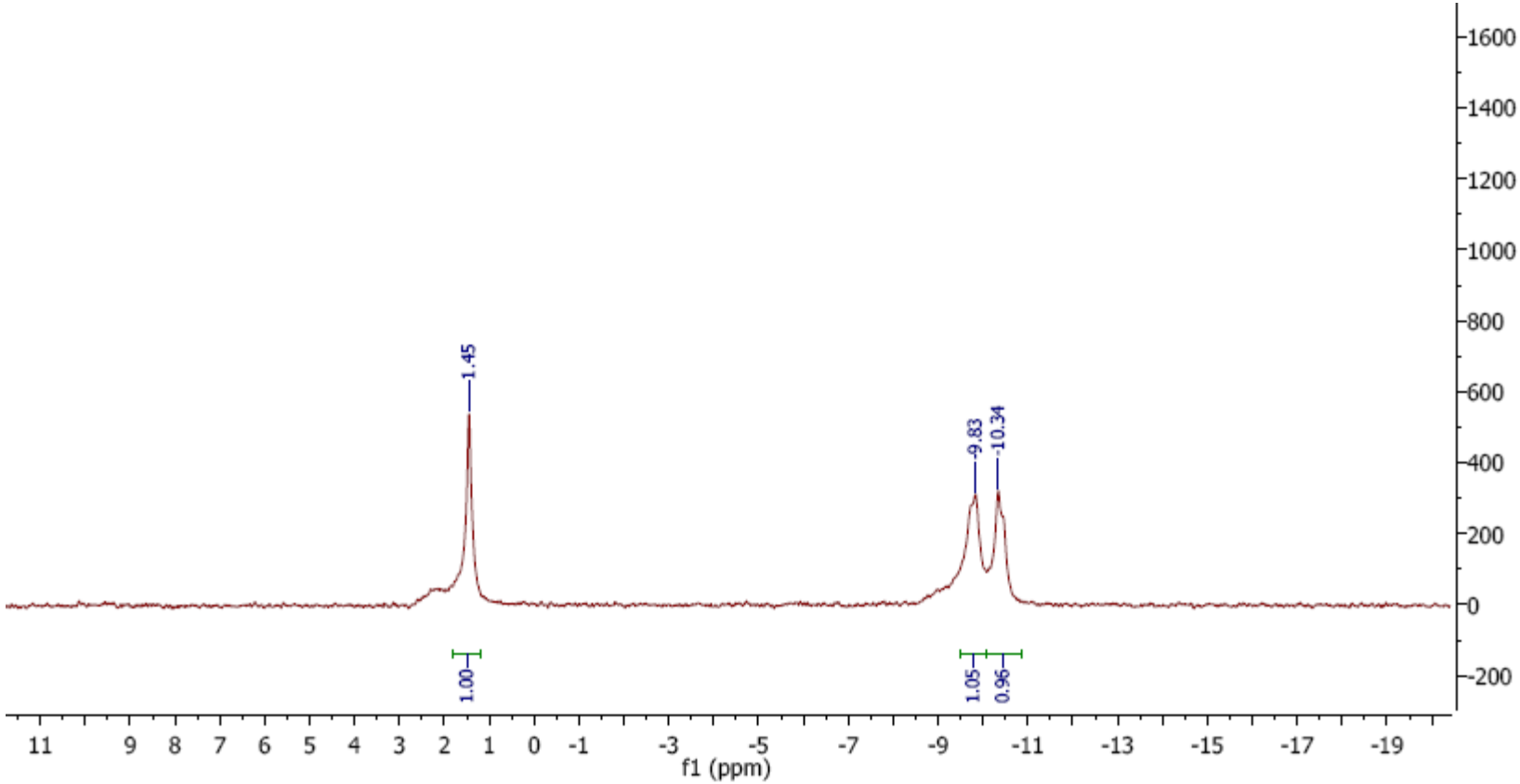

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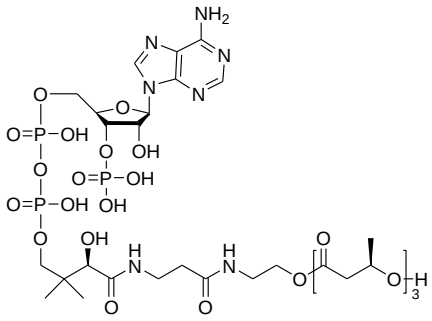
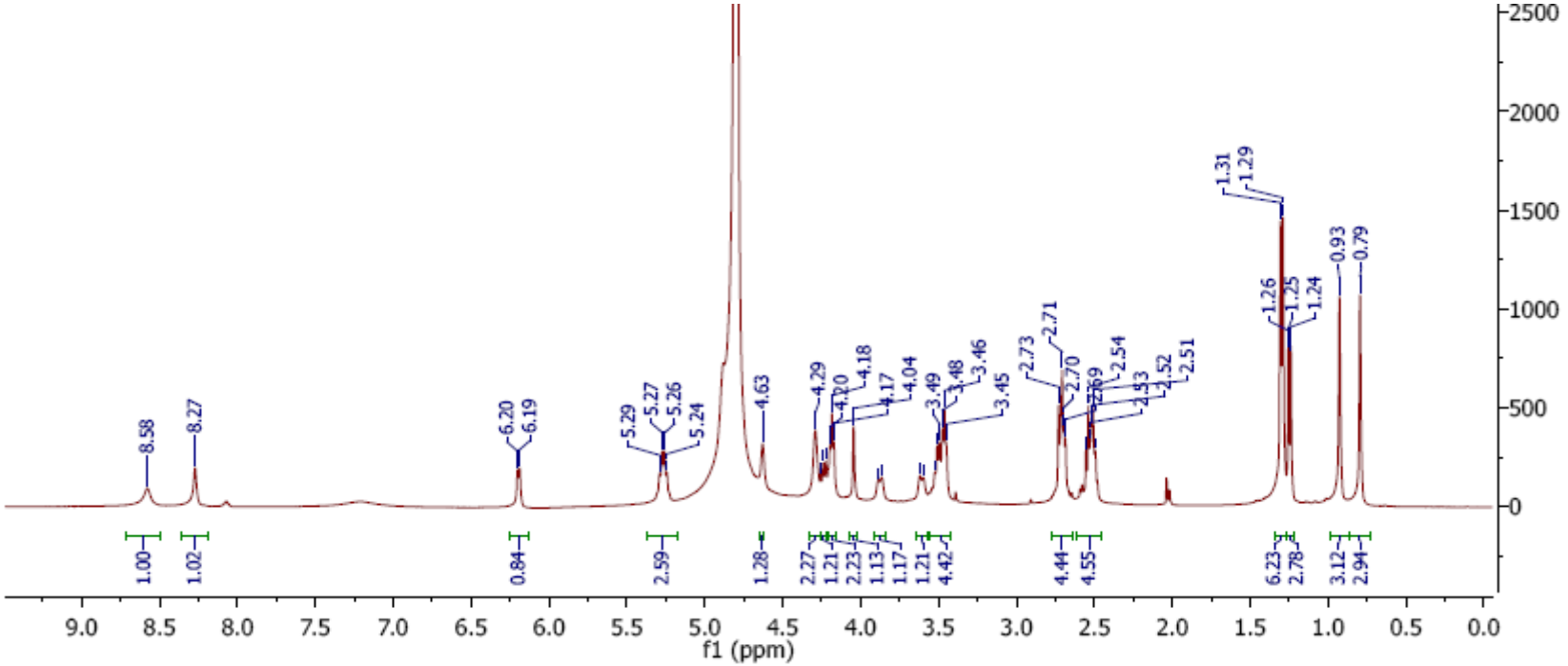
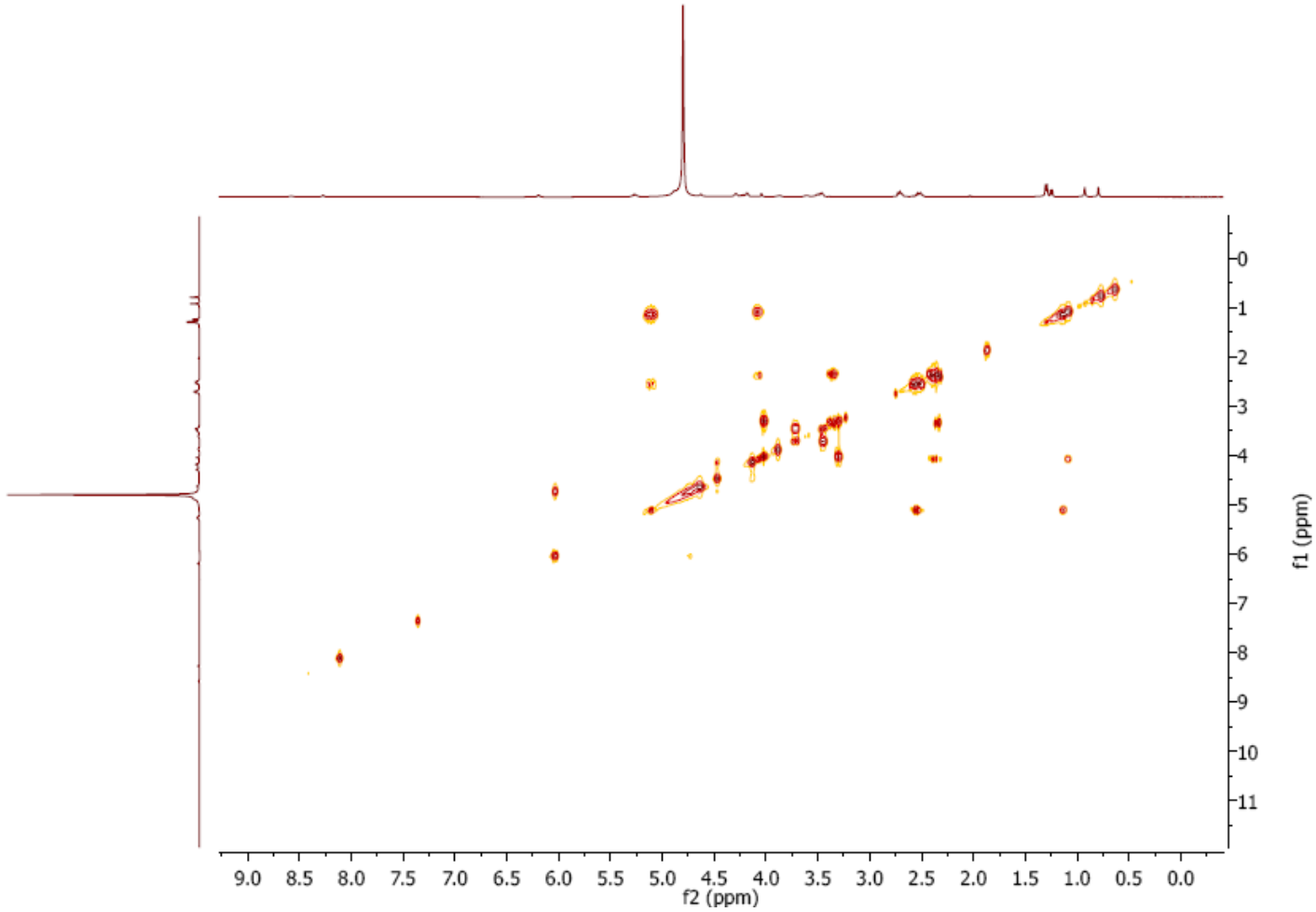


$(HB)_3OCoA$



^1H NMR of $(HB)_3OCoA$



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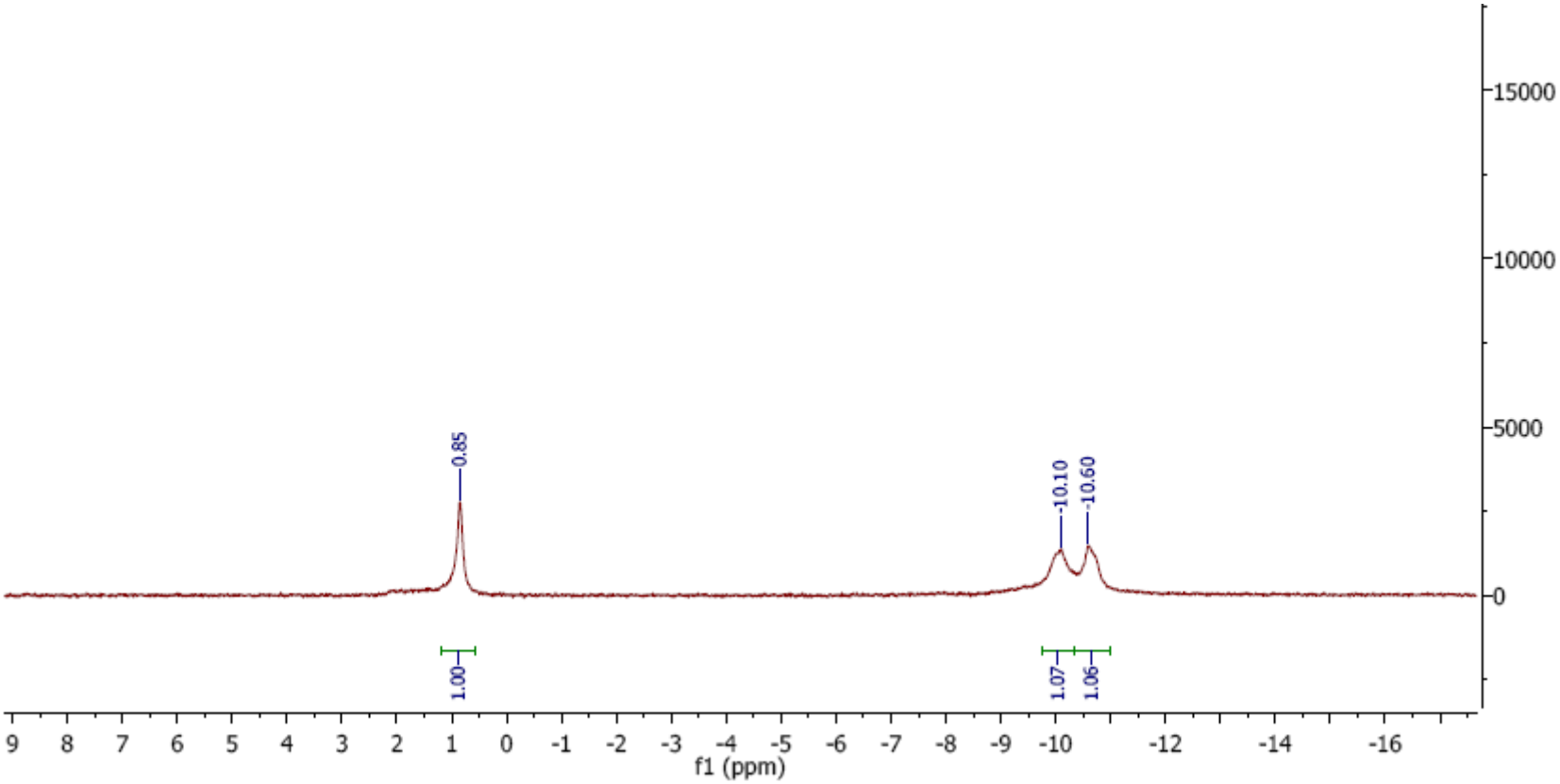

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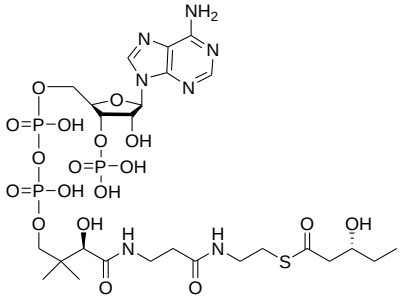
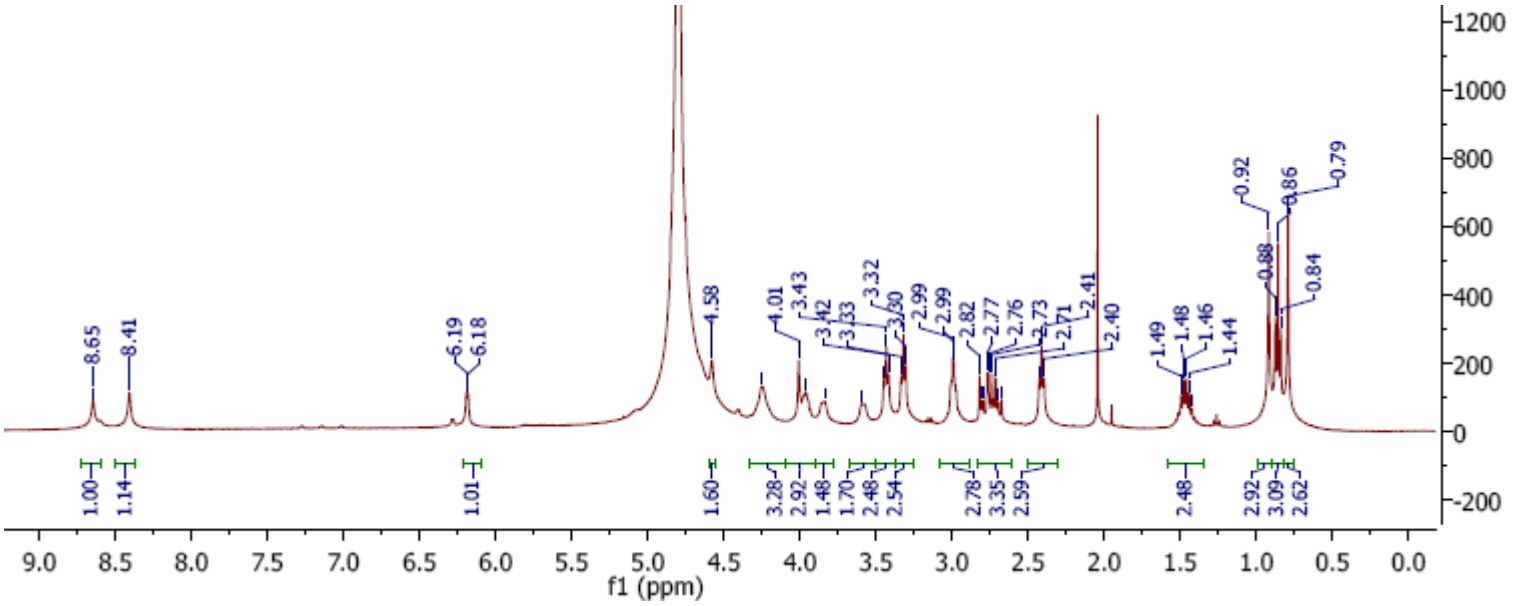
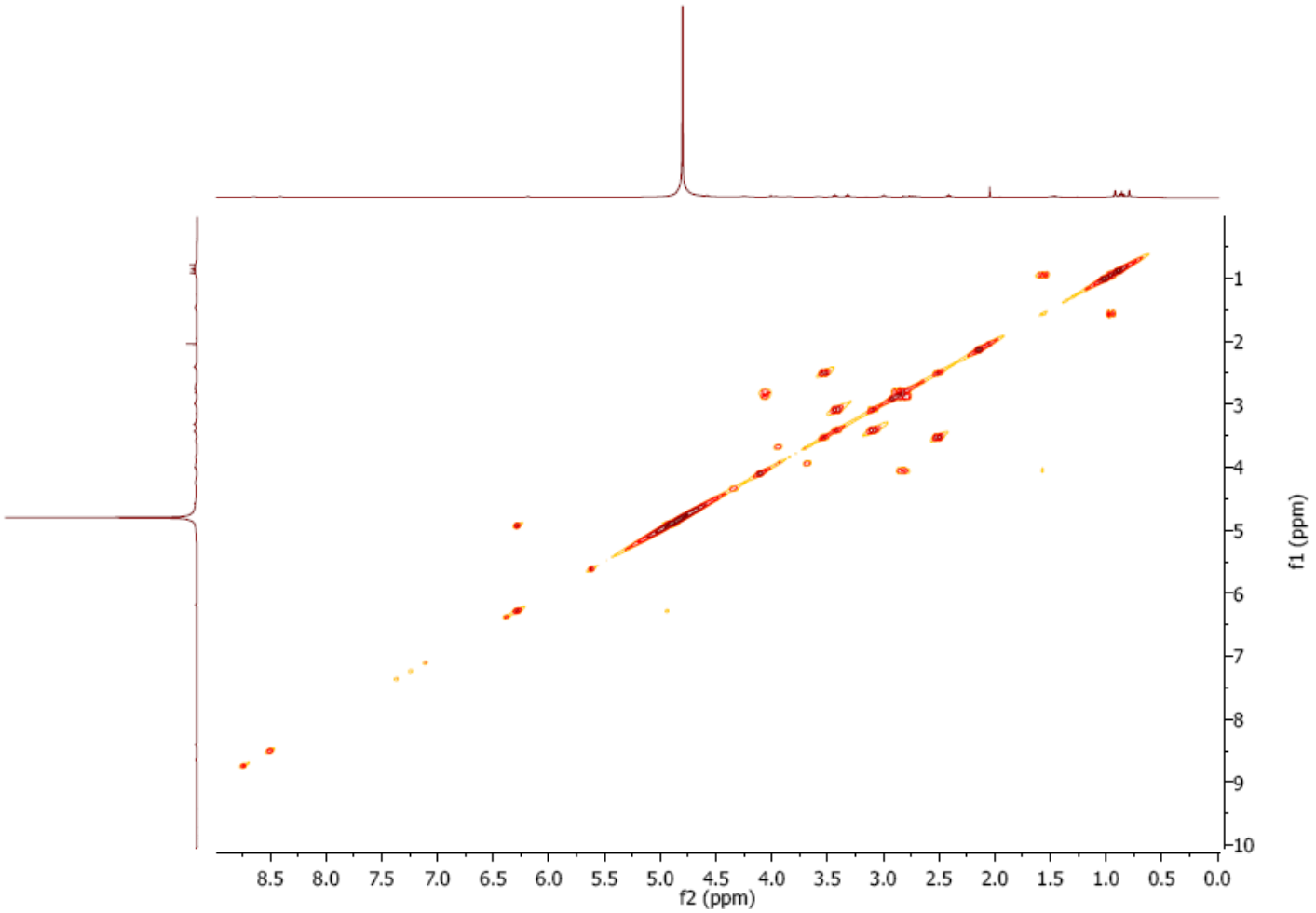


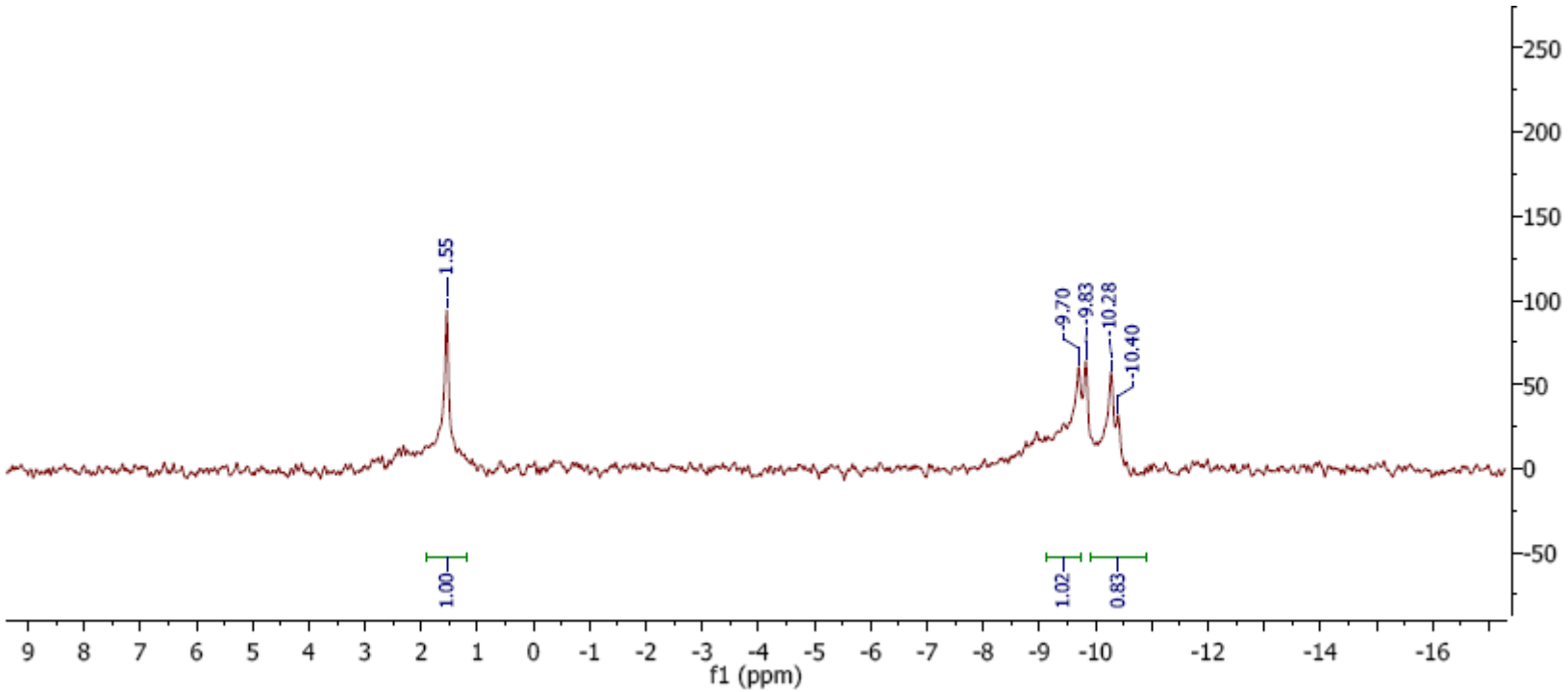
CoA analog 29



^{1}H NMR of CoA analog **29**



gCOSY NMR of CoA analog **29**



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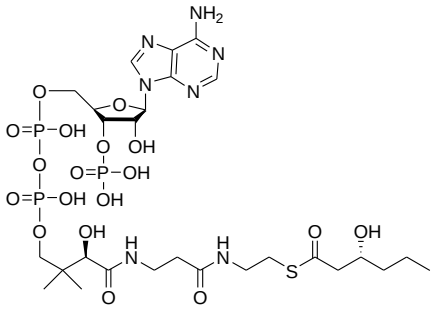
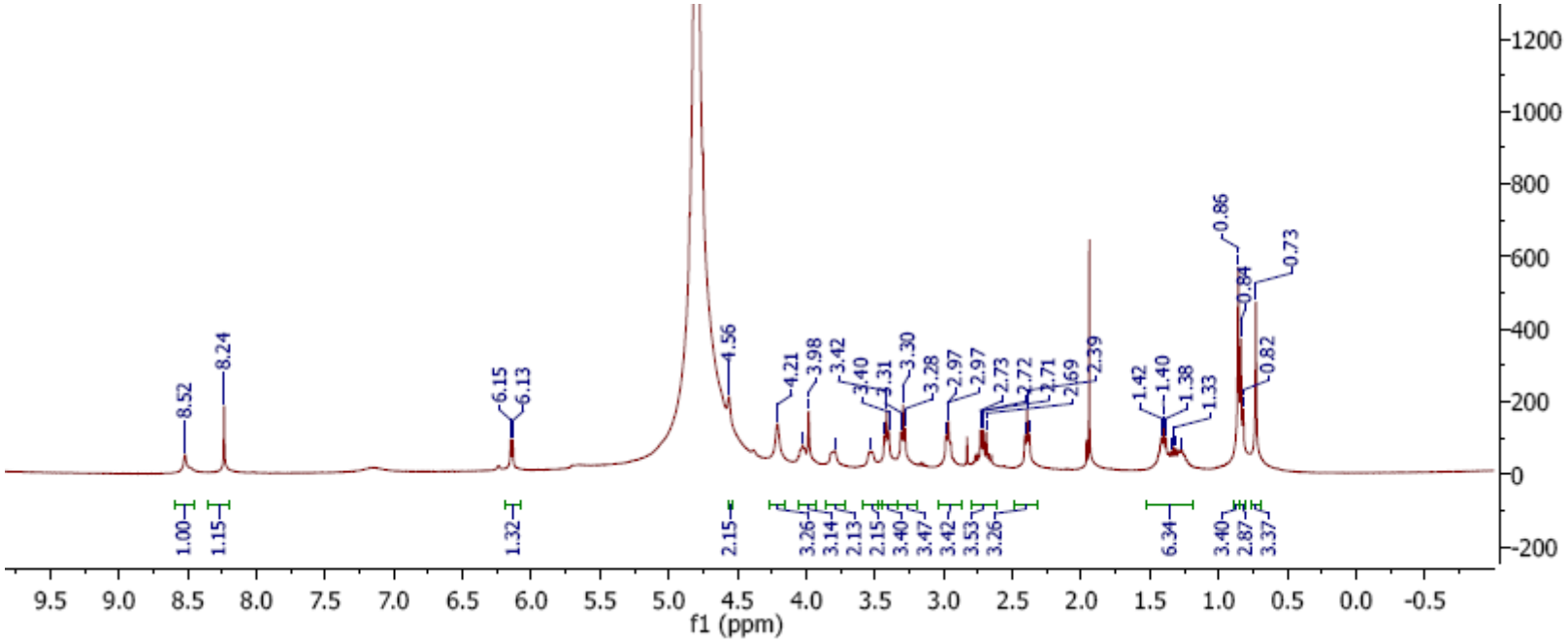


CoA analog 35



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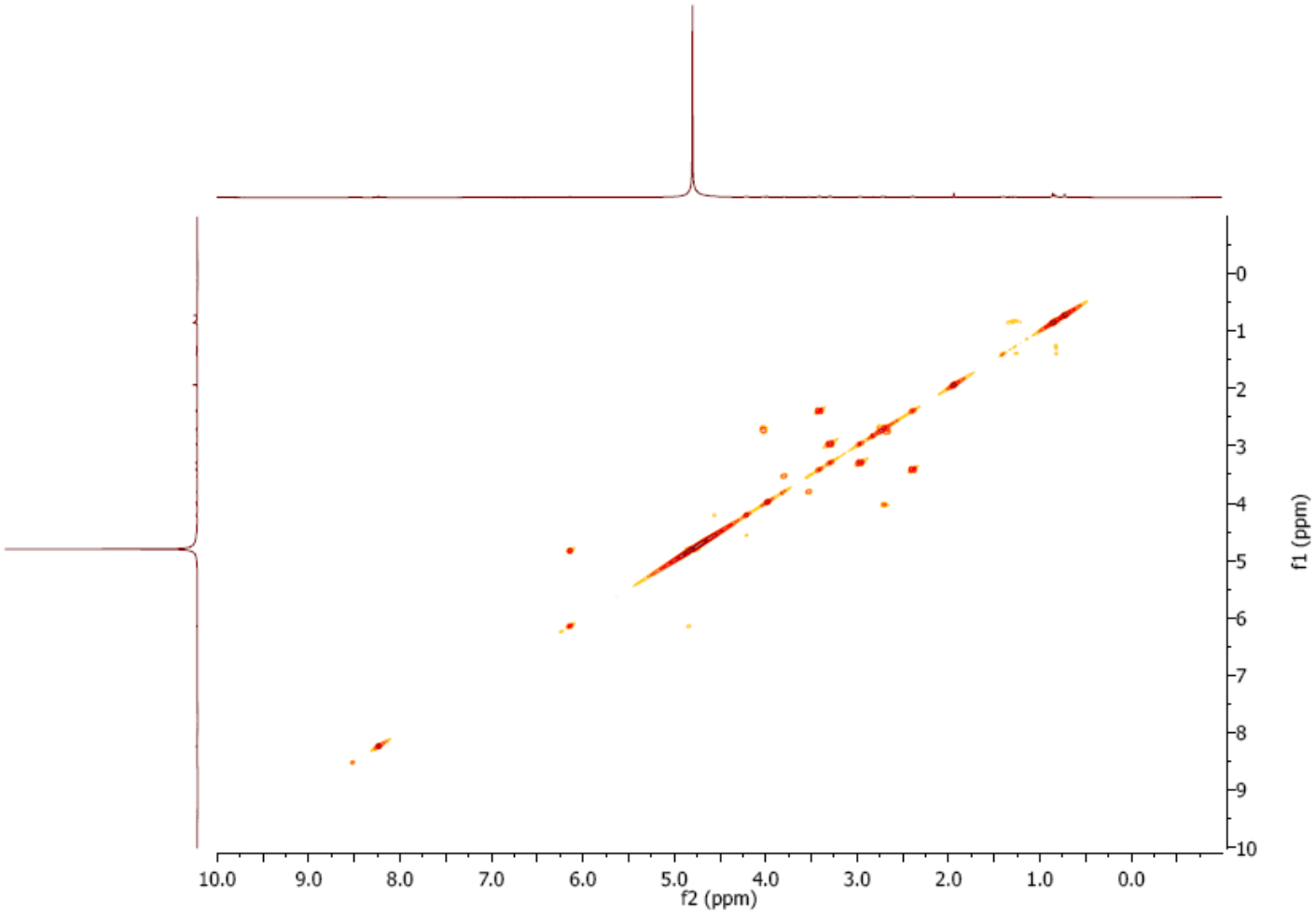
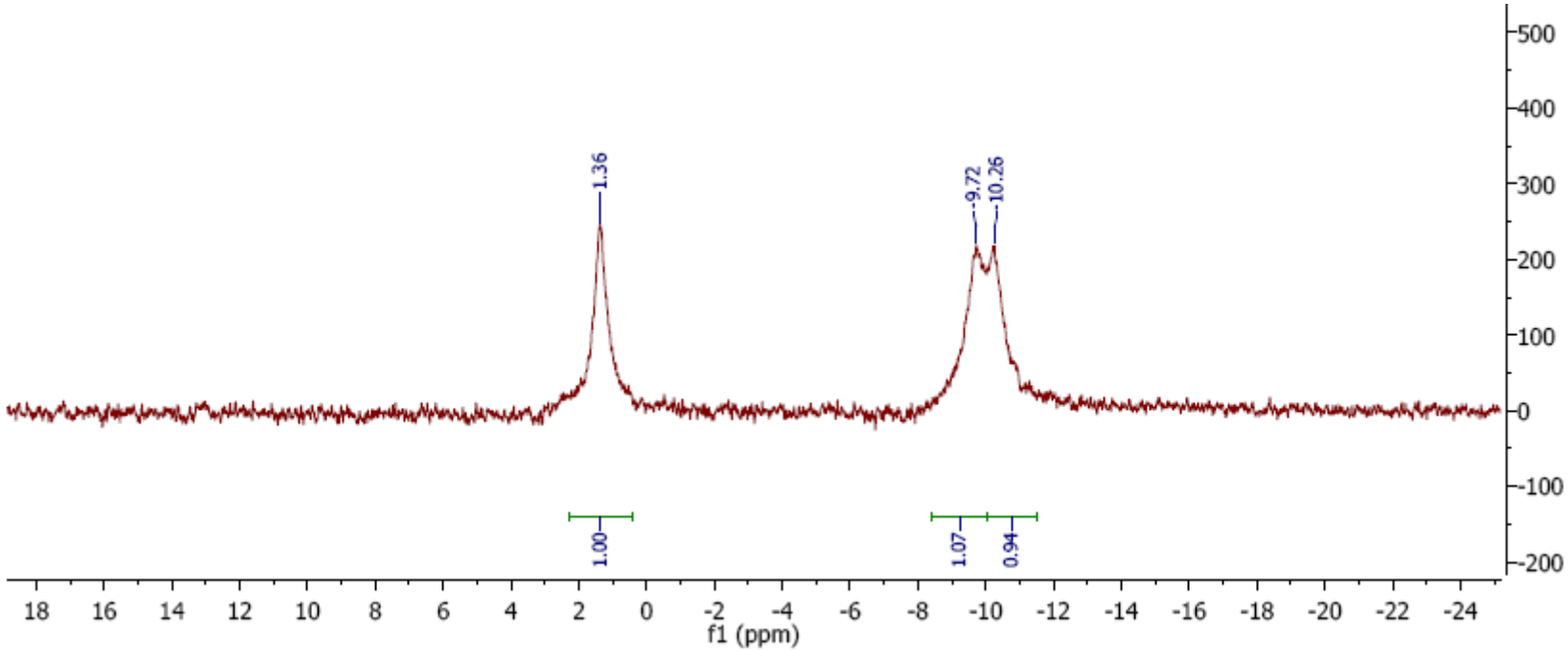


gCOSY NMR of CoA analog **35**



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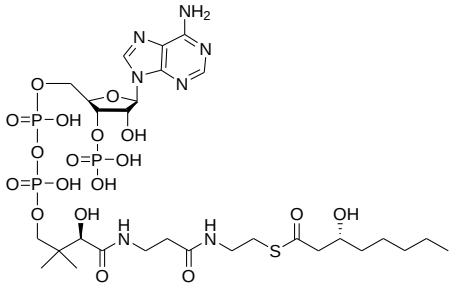
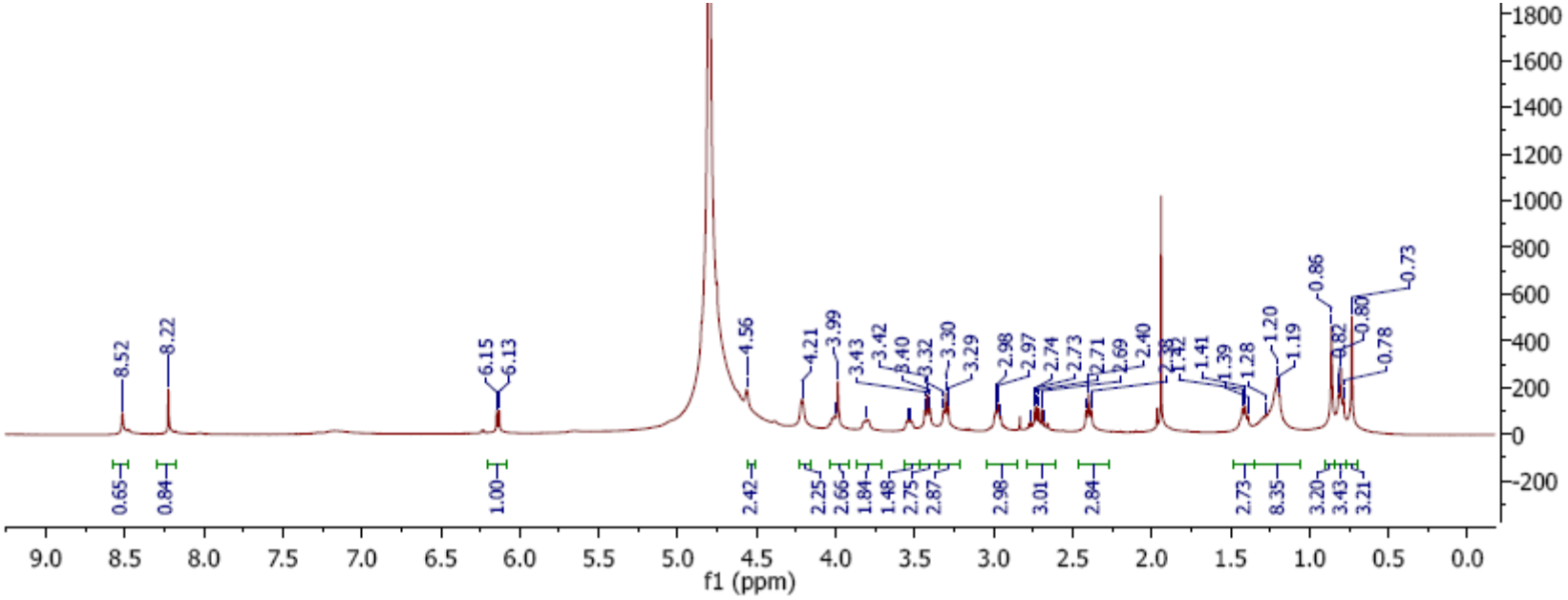


CoA analog 41



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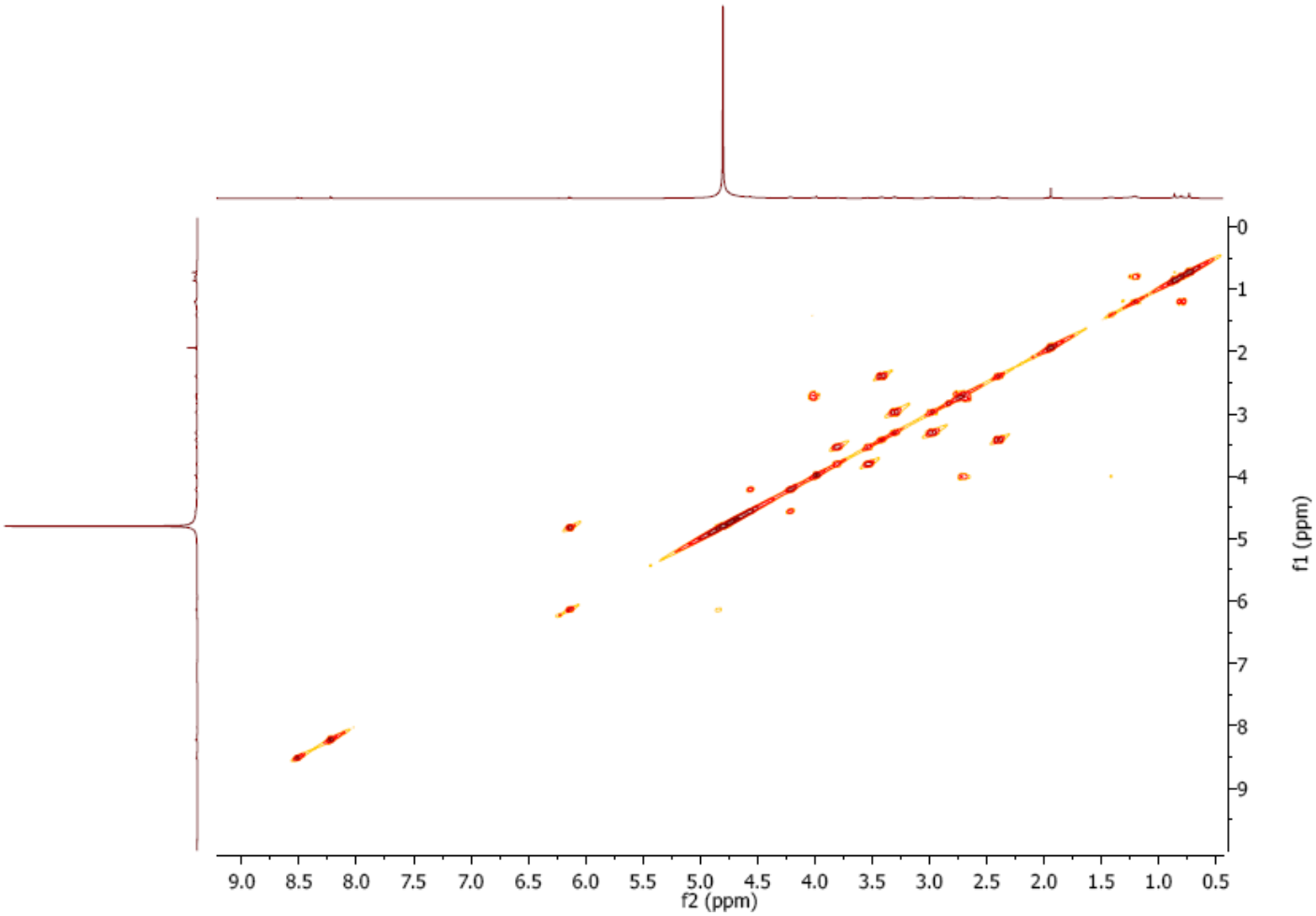
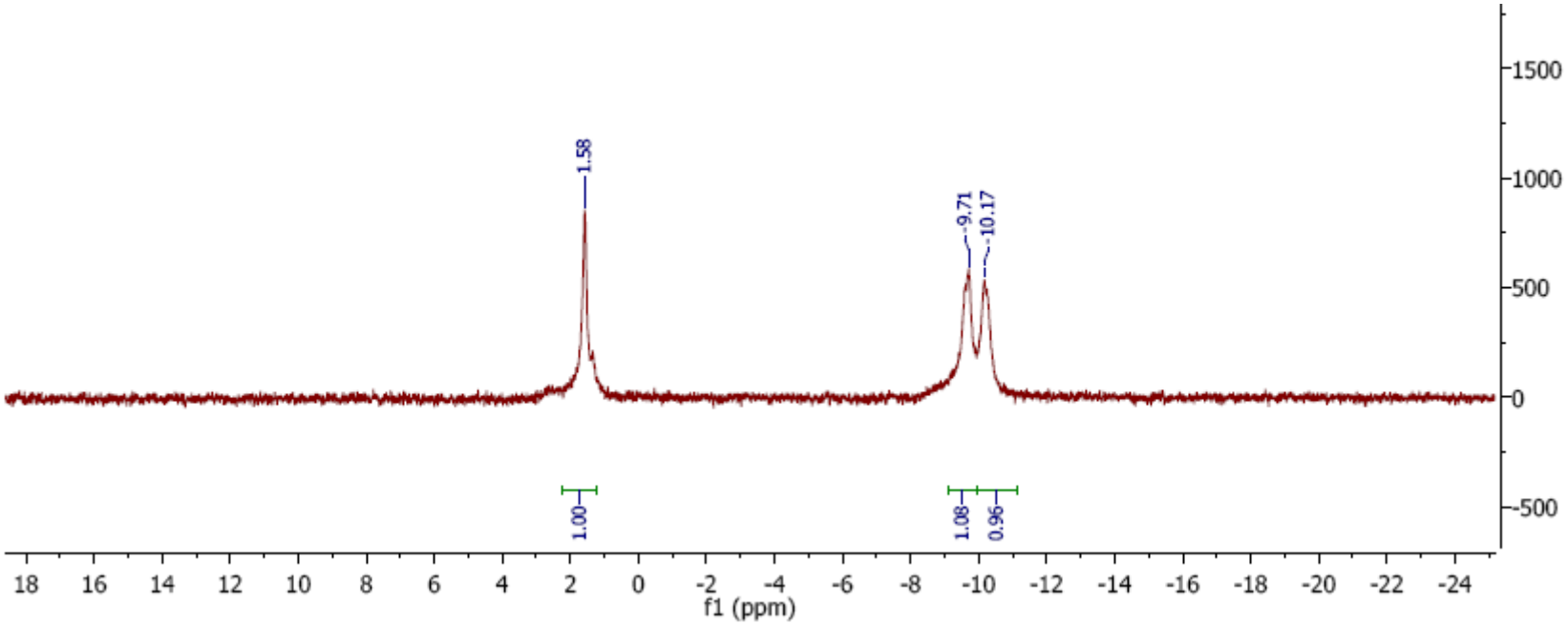
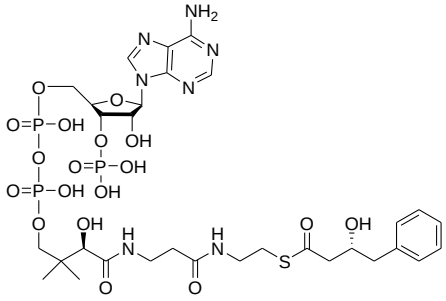


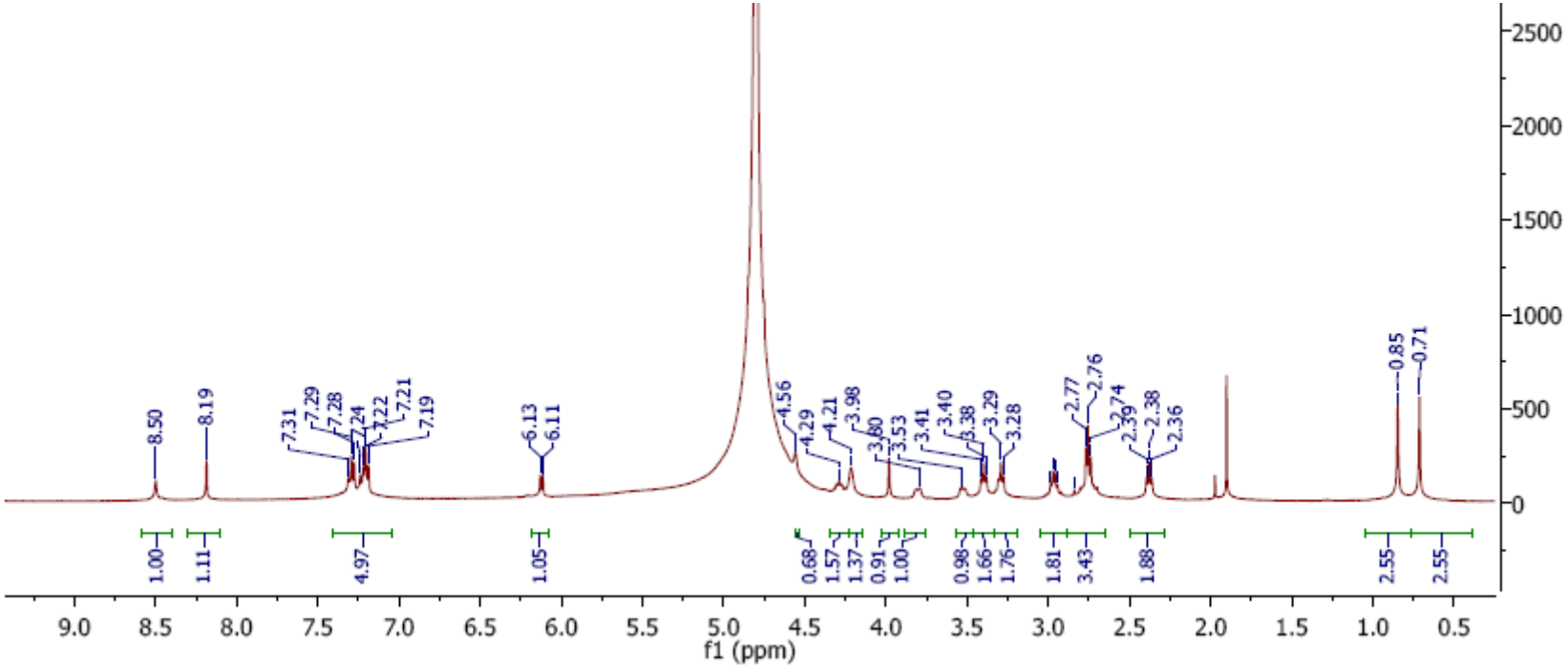
gCOSY NMR of CoA analog **41**



^{31}P NMR of CoA analog **41**



CoA analog 47



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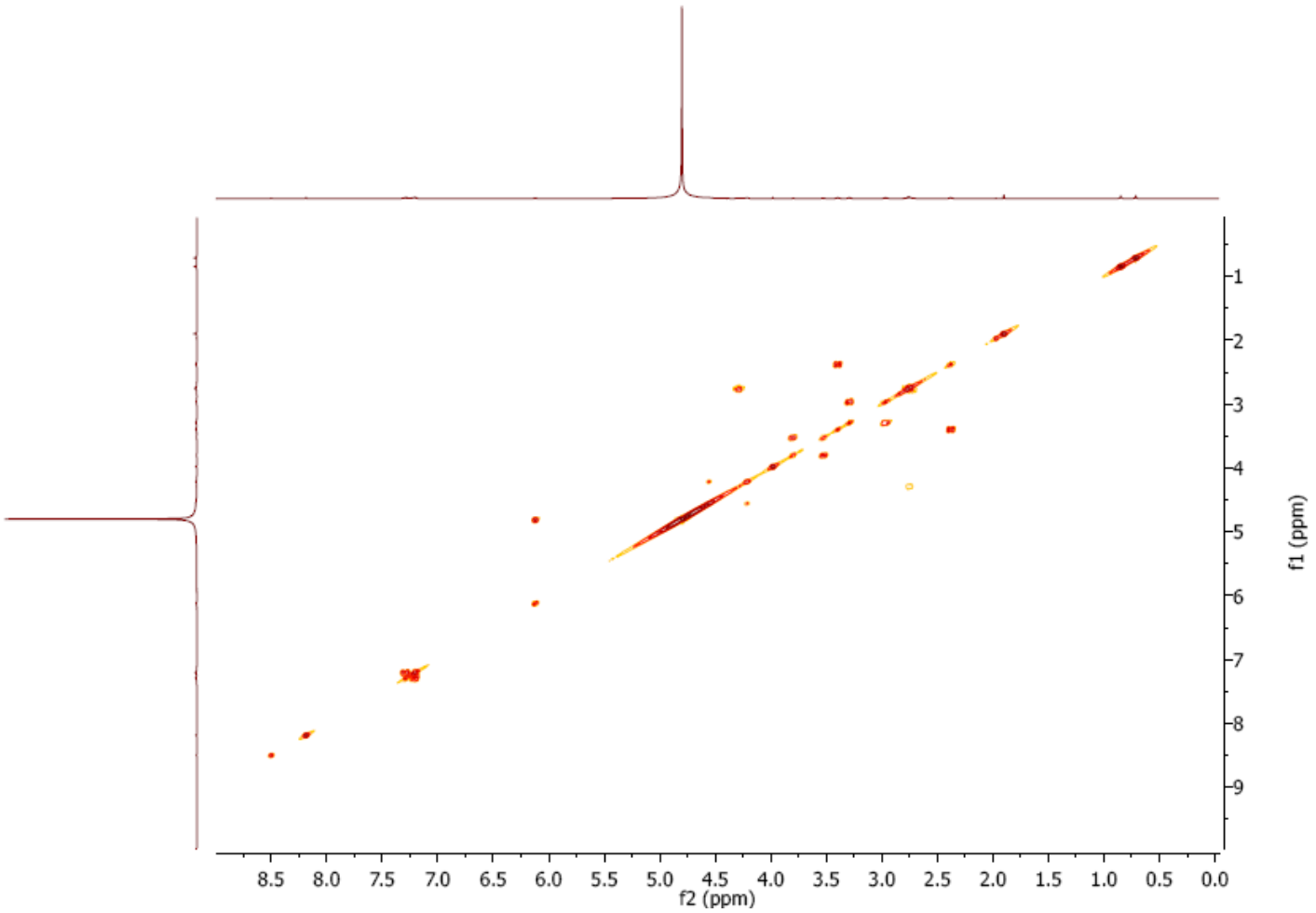

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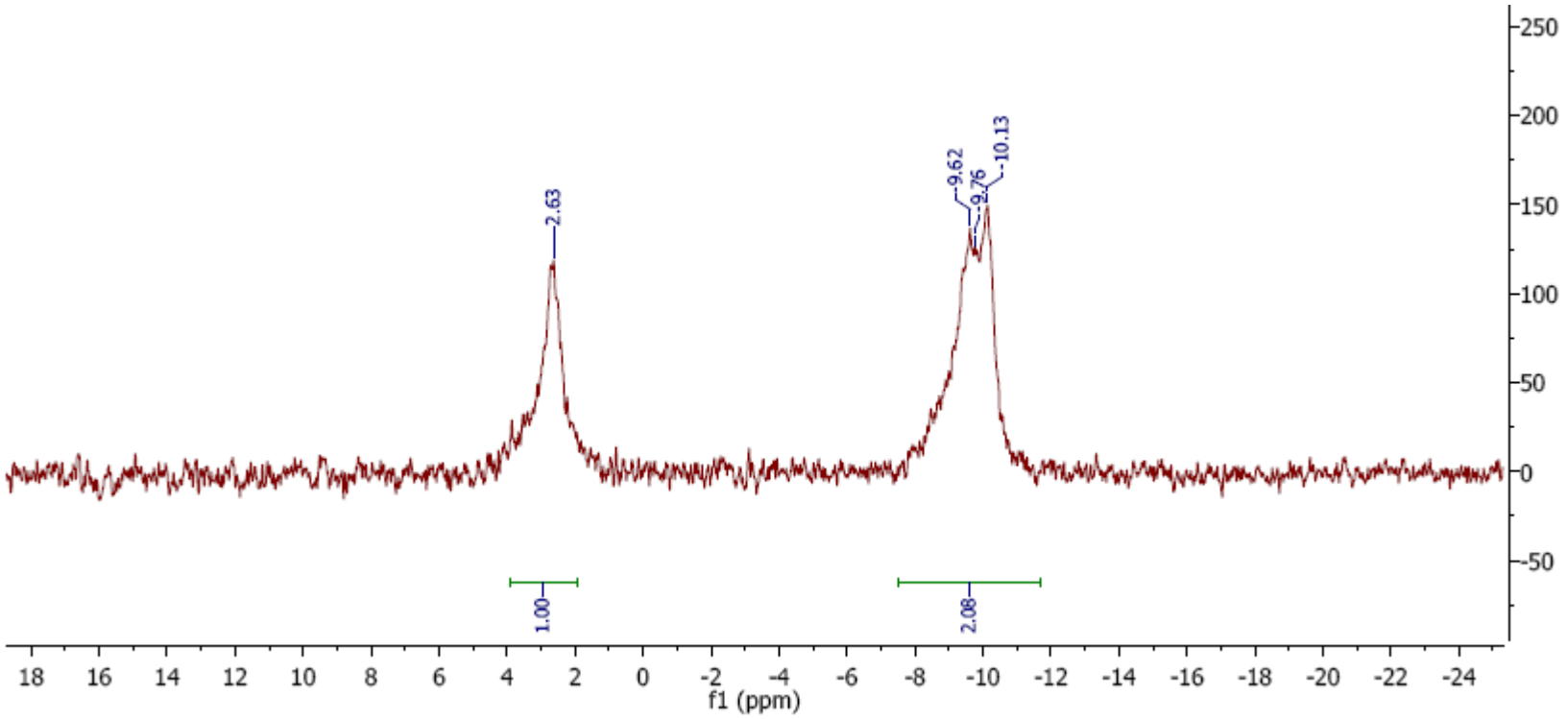
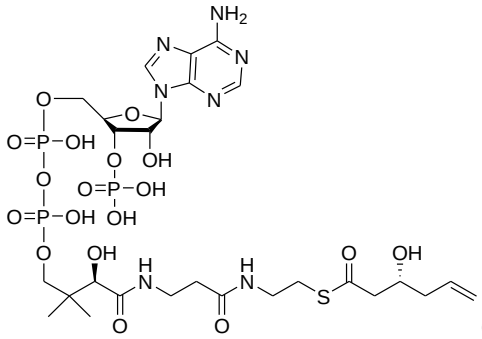
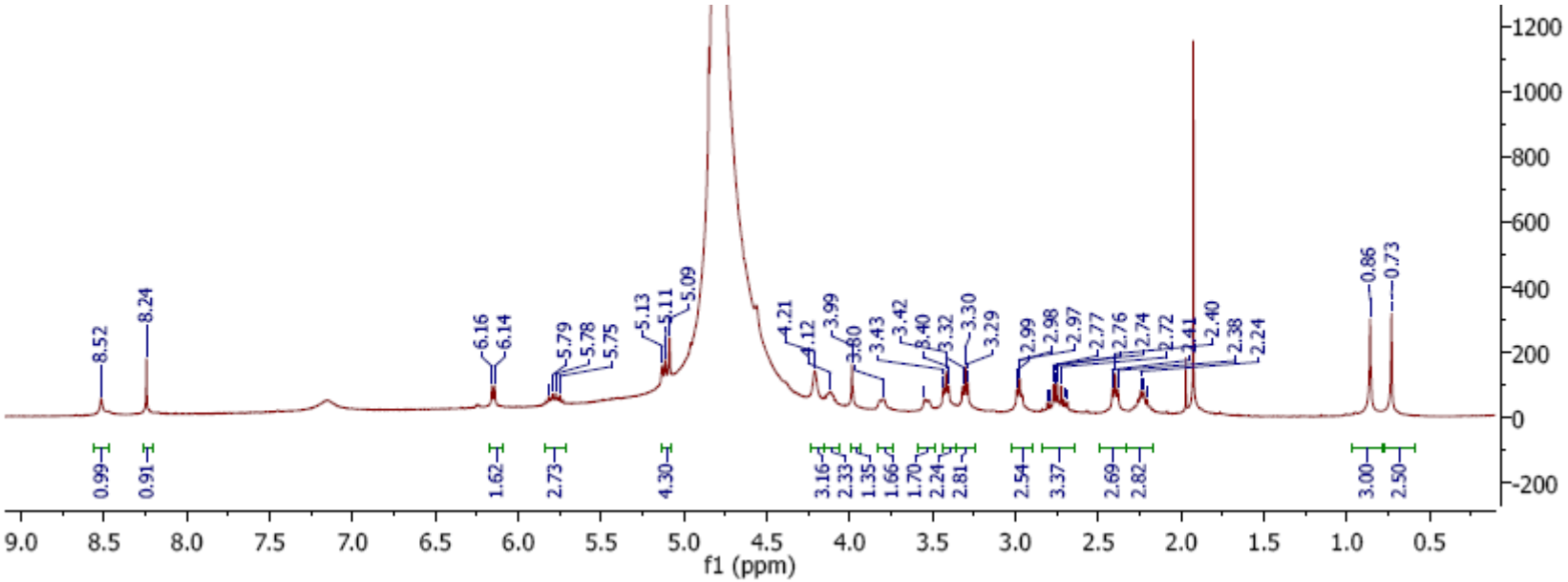


^{31}P NMR of CoA analog **47**



CoA analog 53



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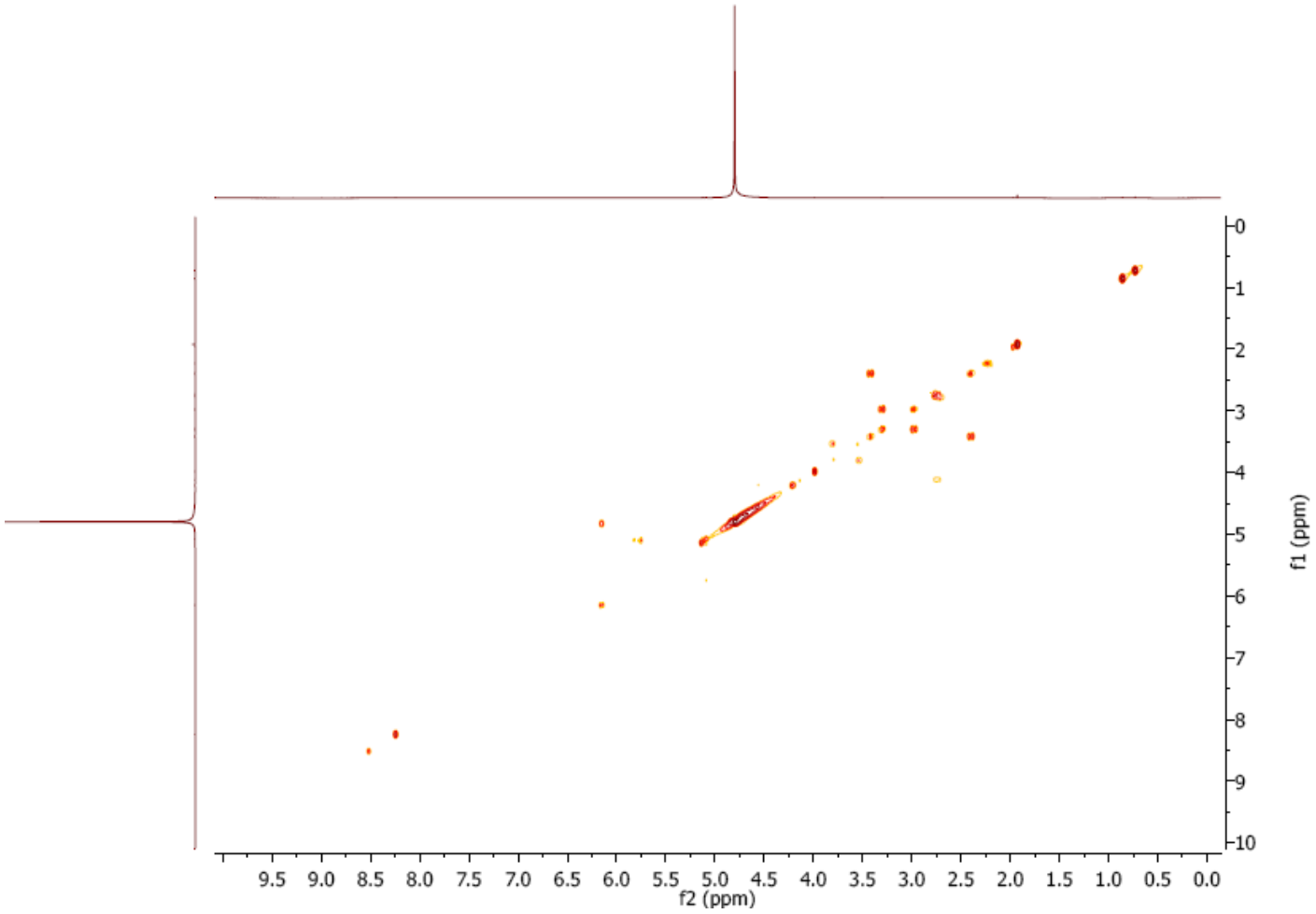
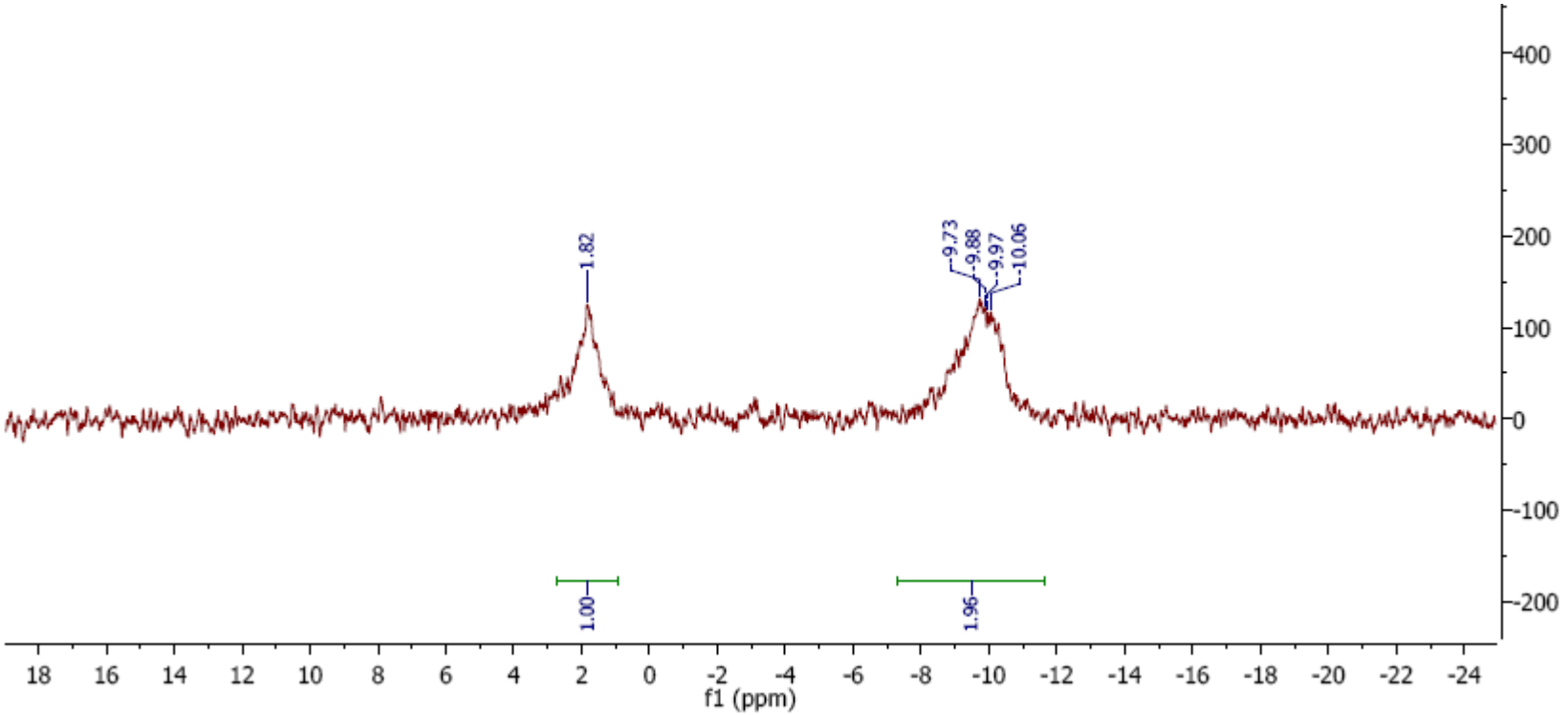
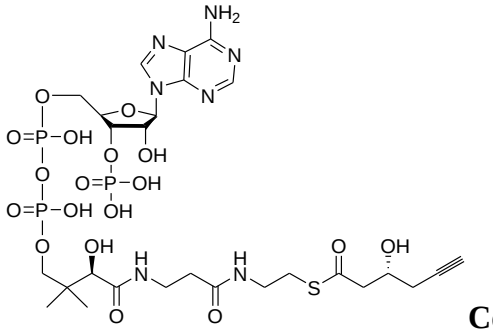


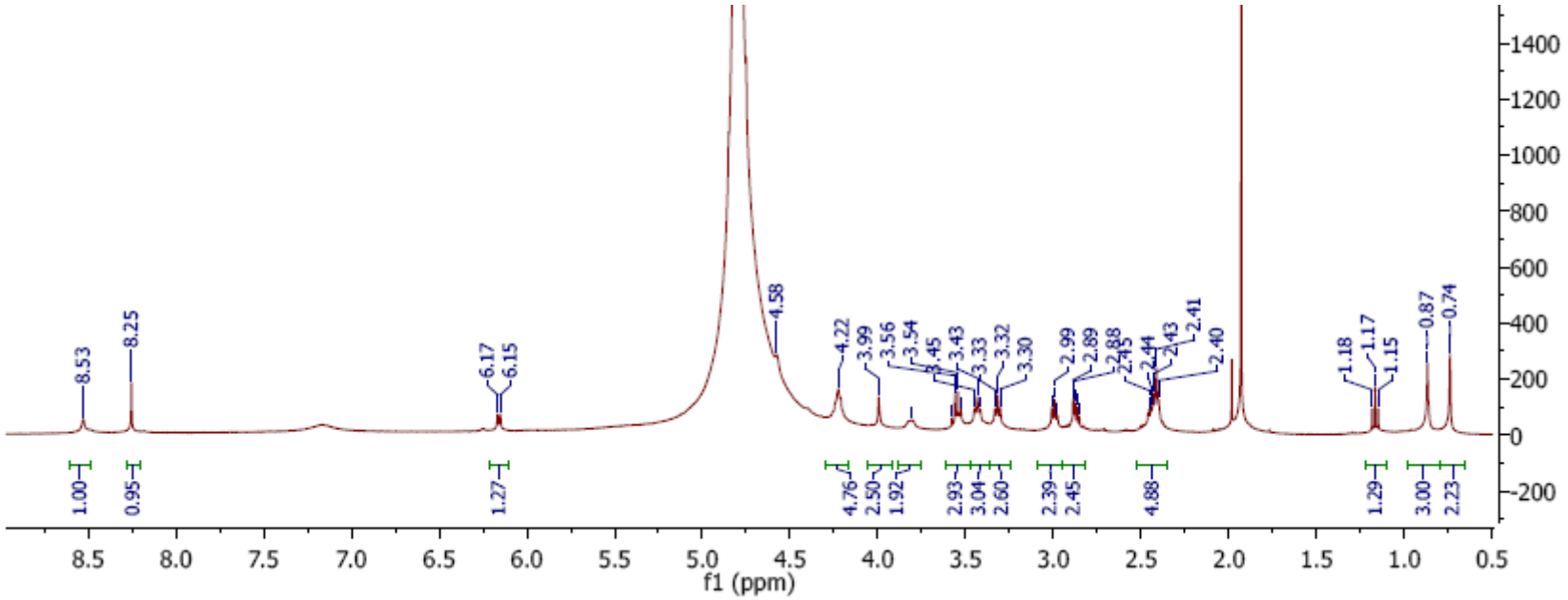
gCOSY NMR of CoA analog **53**



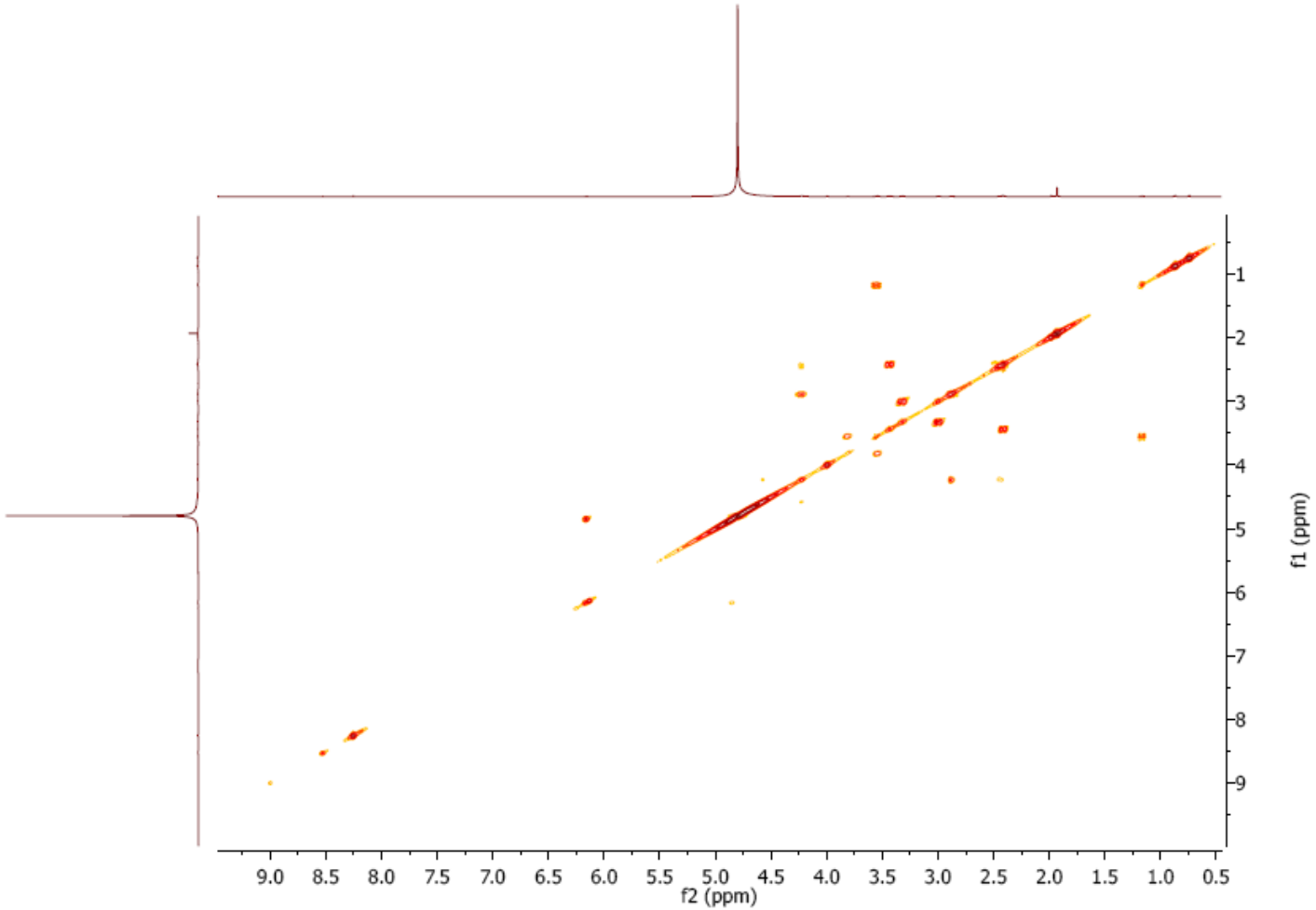
^{31}P NMR of CoA analog **53**



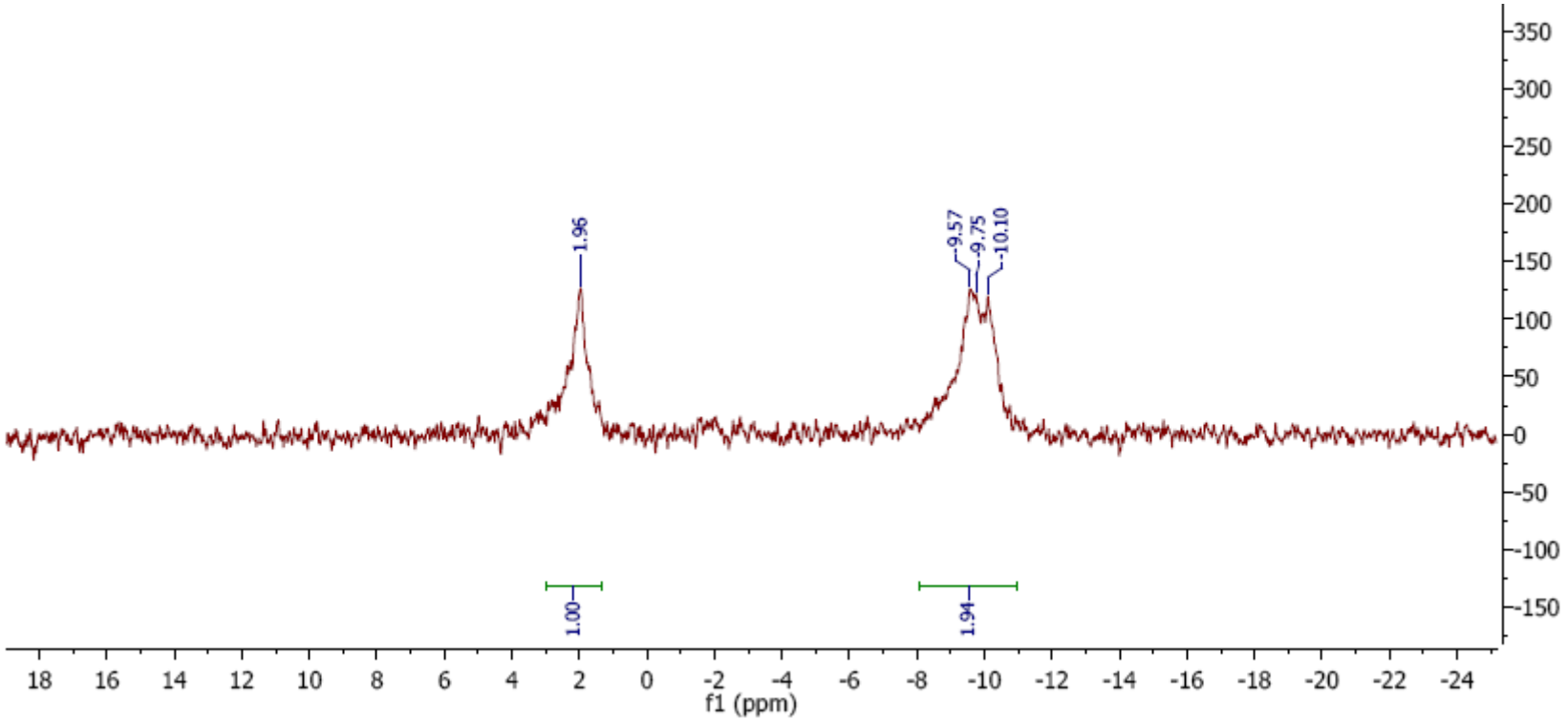
CoA analog 59



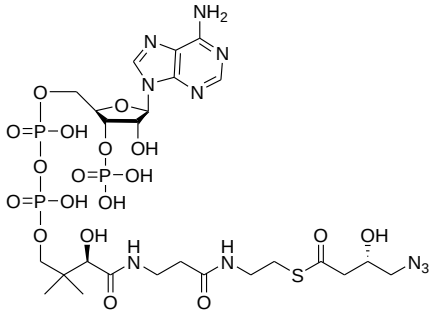
^{1}H NMR of CoA analog **59**



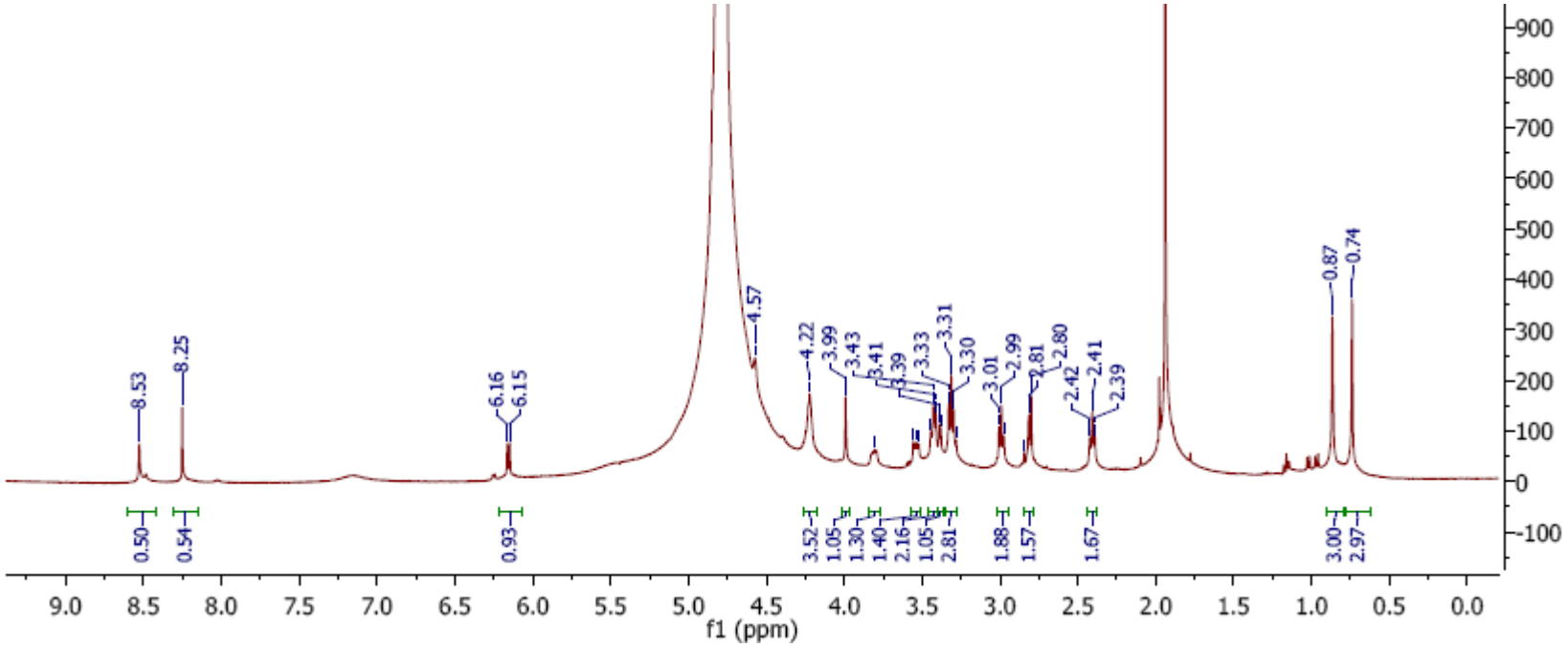
gCOSY NMR of CoA analog **59**



^{31}P NMR of CoA analog **59**



CoA analog 65



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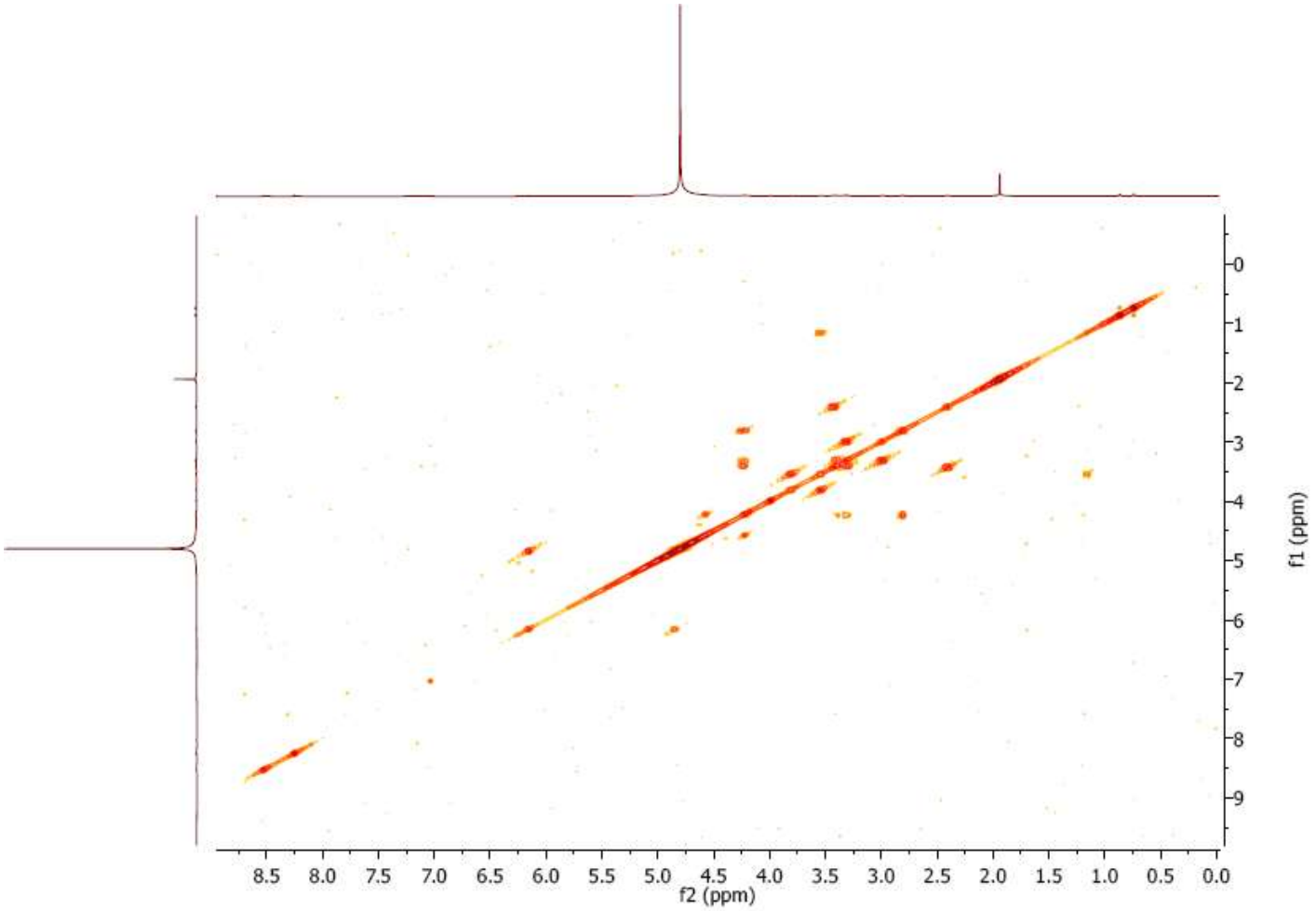
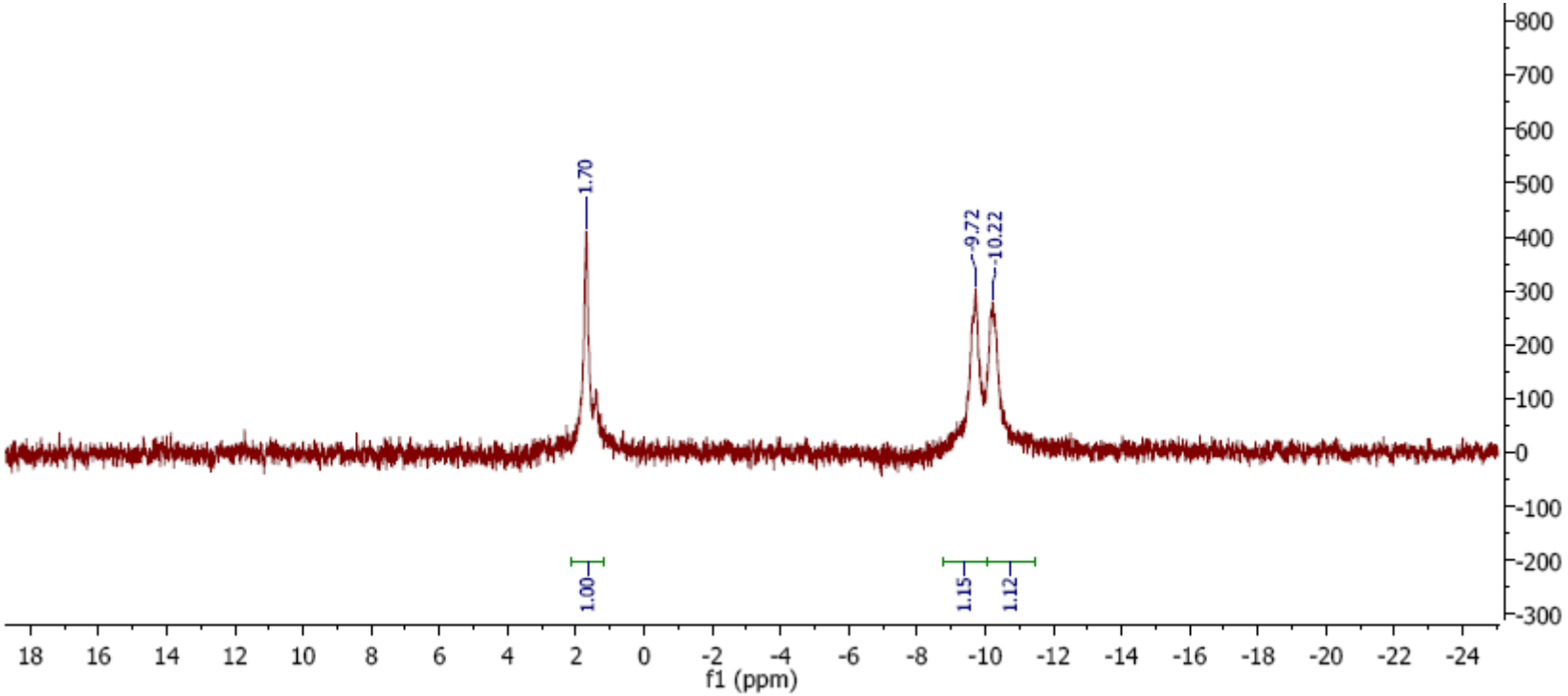


gCOSY NMR of CoA analog **65**



^{31}P NMR of CoA analog **65**