Organic LETTERS

Letter

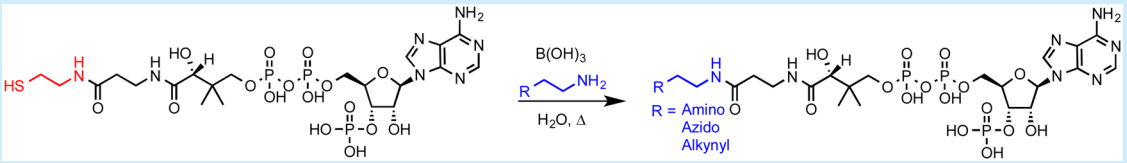
pubs.acs.org/OrgLett

One-Step Transformation of Coenzyme A into Analogues by Transamidation

Randy Sanichar and John C. Vederas*

Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2

Supporting Information

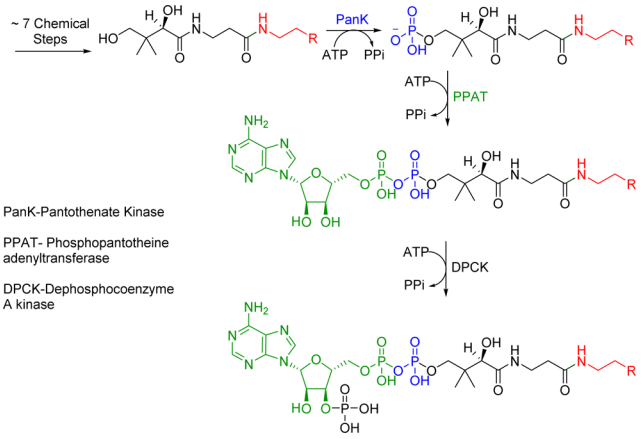


ABSTRACT: Several coenzyme A (CoA) analogues are made in a single step under mild conditions via transamidation reactions catalyzed by boric acid in water. This approach offers rapid access to compounds useful for the study of a wide spectrum of enzyme catalyzed reactions, especially processes involving acyl carrier proteins (ACP) of polyketide synthases (PKS), fatty acid synthases (FAS), and nonribosomal peptide synthetases (NRPS). The CoA analogues presented are readily elaborated or extended by precedented reactions for specific applications that may be required.

Coenzyme A (CoA) is a cofactor that is utilized in the activation and transfer of acyl groups in over 4% of known enzymatic reactions.[1,2] Some of these involve fatty acid synthesis and degradation, polyketide assembly, nonribosomal peptide biosynthesis, oxidation of pyruvate in the citric acid cycle, and a number of other diverse metabolic pathways.[1,3−7] Although CoA is highly functionalized, many of its functional groups serve primarily as recognition elements for binding to specific enzymes.[1] One key reactive feature of CoA is its nucleophilic thiol group that is readily acylated in water and subsequently allows facile transfer of the acyl moiety to other nucleophiles. A second important property is its ability to donate its phosphopantetheinyl group to acyl carrier proteins (ACP) that covalently bind growing acyl chains in fatty acid synthases (FAS), polyketide synthases (PKS), and nonribosomal peptide synthetases (NRPS).[8] Replacement of the reactive thioester moiety of acyl CoA with more chemically robust functionality to block acyl transfer has received considerable attention, and the amide bond has emerged as a good isostere that is physiologically stable to cleavage.[8]

A number of different strategies have been developed to synthesize CoA and its analogues, with considerable work following the original synthesis of CoA by Khorana et al. in 1959.[9] Their approach involved joining the adenosine bridge between the pyrophosphate and previously synthesized pantetheine portions, followed by the regioselective phosphorylation of the 3′ hydroxyl group on the ribose. Michelson later used addition of the adenosine diphosphate with subsequent enzymatic hydrolysis of the 2′,3′-cyclic phosphate to form CoA.[10] The groups of Wright,[11] Burkart,[12,13] and Bruner[8a,14] employed chemoenzymatic methods for preparation of CoA analogues. In these approaches, modified pantetheine moieties are synthesized and then further elaborated by three enzymes: pantethenate kinase (PanK); phosphopantetheine adenylyltransferase (PPAT); and dephosphocoezyme A kinase (DPCK), to produce modified CoA derivatives (Scheme 1).[8,11,12] In particular, the Bruner group used these enzymes to synthesize "amino CoA" (CoA-NH_2) in which the sulfhydryl is replaced with a primary amine, which could then be acylated with an amino acid.[8a,14]

Scheme 1. Final Enzymatic Steps for the Synthesis of CoA Analogues from Chemically Synthesized Pantetheine Derivatives[a]



[a]For CoA, R = SH whereas in "amino-CoA" (CoA-NH_2) R = NH_2.

It is mentioned in a review that, with the exception of desulfurized CoA, unnatural analogues cannot be made readily from CoA itself, due to its sensitivity to acid and high temperature.[15] Herein, we report the single-step chemical

Received: January 26, 2017

DOI: 10.1021/acs.orglett.7b00291
Org. Lett. XXXX, XXX, XXX−XXX

synthesis of CoA analogues directly from CoA by transamidation, using a green approach with mild aqueous conditions.

We initially used *N*-acetyl cysteamine (SNAC) as a CoA mimic to develop a method. The first approach was to transform the free thiol into a leaving group to be displaced by a nucleophile. We attempted the direct displacement of sulfur via the corresponding thiophosphonium salts following the work of Krafft and Siddall,[16] but unfortunately the displacement did not work in our hands, and the intact thiophosphonium salt was recovered. We next attempted a cyanogen bromide activation[17] of the sulfide prepared from reaction of SNAC with 1-fluoro-2,4-dinitrobenzene (Sanger's reagent).[18] Unfortunately, starting material was generally recovered, possibly due to the electron-withdrawing effect of the two nitro groups reducing sulfur nucleophilicity. Reduction of the nitro groups in this sulfide to amines[19] resulted in cyanogen bromide forming cyanamides and dicyanamides without sulfur displacement. A variety of other common approaches to improve the leaving ability of sulfur also failed.

We next considered an N to S acyl transfer, with the idea that the thiol could be displaced from the resulting thioester by an external nucleophile present in excess, essentially a reverse chemical ligation.[20] We also considered transamidation with boric acid as a possible catalyst, as was done in the studies of Al-Mourabit and co-workers on simple amides in nonpolar organic solvents (e.g., *p*-xylene) at higher temperature (e.g., 120−160 °C).[21] We switched to aqueous conditions and optimized to a lower temperature (55 °C) because CoA is primarily water-soluble and decomposes on heating much above that temperature. An attempt to transamidate acetamide with ethylenediamine (10 equiv) in water at 55 °C showed that no measurable transformation to the desired product after 48 h, in accord with the literature report[21] (Table 1). However, upon addition of 1.0 equiv of $B(OH)_3$ under the same conditions, the reaction gave 9% conversion to the targeted product, monoacetylated ethylenediamine, thereby supporting the beneficial effect of water as a solvent. To test the impact of potential intramolecular N to S acyl migration,[20] SNAC was used as the amide in water at 55 °C with ethylenediamine (10 equiv) in the absence of $B(OH)_3$. This reaction produced 23% of monoacetylated ethylenediamine. To confirm this was dependent on an intramolecular thiol transacylation, the acetamide reaction was repeated with $B(OH)_3$ using cysteamine as an external nucleophilic additive. However, the transamidation yield was only 9%, as seen earlier with acetamide in the absence of the additive. Combining the approaches into a single reaction using SNAC as the amide with 1.0 equiv of $B(OH)_3$ gave an increase to 35% conversion. This indicates a synergistic effect from combined use of boric acid catalysis accompanied by an intramolecular N to S acyl migration (Scheme 2). Attempts to improve the conversion by increasing the amount of $B(OH)_3$ or the amount of amine did not succeed and reduced the overall conversion significantly. To possibly improve the reaction, a number metal salts and other catalysts known to facilitate high temperature transamidations[21−24] were screened for activity with the optimized system. Five of the potential catalysts inhibited the transformation, including $ZrCl_3$, $CeCl_3$, $SnCl_2$, $Eu(NO_3)_3$, and $NH_2OH{\cdot}HCl$. However, boric acid showed significant activity, even after correcting for any background reaction with these agents. Boric acid was 10-fold more effective than $FeCl_3$, 20-fold more effective than $Fe(NO_3)_3$, and 100-fold more than $Fe_2(SO_4)_3$.

As mentioned above and described in the literature,[15] CoA dissolves readily in water and is quite insoluble in most organic solvents. It is also relatively unstable to acid and displays rapid decomposition under alkaline conditions at higher temperatures. With the conditions developed in the SNAC model system, we were able to prepare the "amino CoA (**8**)"[8] (CoA−NH_2, R = NH_2 in Scheme 1), as well as unpublished "azido CoA (**9**)" (CoA-N_3, R = N_3) and previously unreported "alkynyl CoA (**10**)" in single steps from commercially available CoA with reasonable yields, given both the complexity of the molecule and its sensitive nature (Table 2). The conditions were optimized to minimize degradation of CoA, which is sensitive and decomposes into complex mixtures upon more prolonged heating (>48 h) or more elevated temperatures. The addition of boric acid was essential for the reactions to proceed. Monitoring of these reactions in D_2O by NMR spectrometry showed relatively clean conversion with most of the remaining material as CoA and its disulfide (also recovered by HPLC purification).

Table 1. Screening of Reaction Conditions for Transamidation Using Ethylenediamine in Water at 55 °C for 48 h

entry	amide	amine equiv	$B(OH)_3$ equiv	conversion %
1		10	-	0
2		10	1	9
3[a]		10	1	9
4		10	-	23
5		10	1	35
6		10	30	23
7		40	1	26

[a]Cysteamine as an additive. See Supporting Information for complete procedures.

Scheme 2. Possible Mechanism for the Boric Acid Catalyzed N to S Acyl Migration, Followed by Nucleophilic Displacement of the Thiol by an External Nucleophile, Ethylenediamine

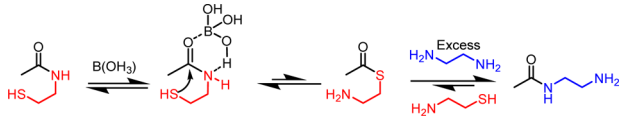
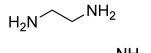
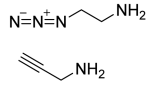
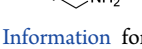


Table 2. One-Step Preparation of Amino CoA (8), Azido CoA (9), and alkynyl CoA (10) Analogues by Transamidation in Water at 55 °C Using $B(OH)_3$ for 48 h[a]

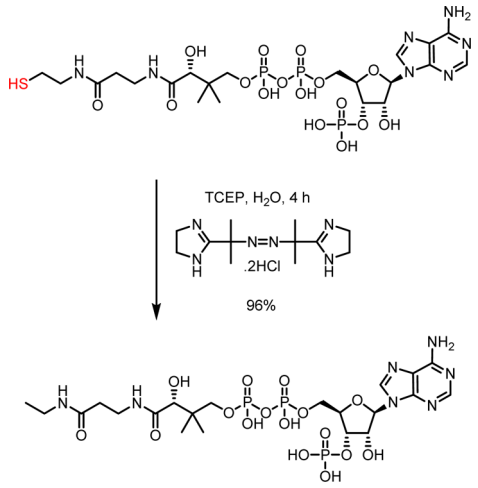
entry	amine	amine equiv	yield %
8		10	30
9		20	28
10		20	28

[a]See Supporting Information for complete procedures, purifications and characterizations.

DOI: 10.1021/acs.orglett.7b00291
Org. Lett. XXXX, XXX, XXX−XXX

The formation of CoA disulfide could not be completely suppressed even though attempts were made to exclude oxygen. Since the free thiol plays an important role in the transformation, water-soluble disulfide reducing agents were added to enhance the process. These included 1,4-dithiothreitol (DTT) and (tris(2-carboxyethyl)phosphine (TCEP). Although both are good reducing agents, TCEP was much faster at breaking the disulfide bond at room temperature. However, TCEP gives a side reaction with the free thiols at more elevated temperature (55 °C), namely radical desulfurization of CoA to form a small amount of desulfurized CoA. Given that the "desulfo" (des-thio) CoA is also an important compound used in crystallography and as an inhibitor in a number of cases,[10,25−28] we decided to examine this desulfurization reaction. We found that upon the addition of the commercially available water-soluble radical initiator, 2,2′-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride, we were able to transform CoA using TECP in water at 55 °C into desulfo CoA (**11**) in 96% yield in 4 h. (Scheme 3).

Scheme 3. Aqueous Radical Desulfurization of CoA for Rapid Preparation of Desulfo CoA (11)

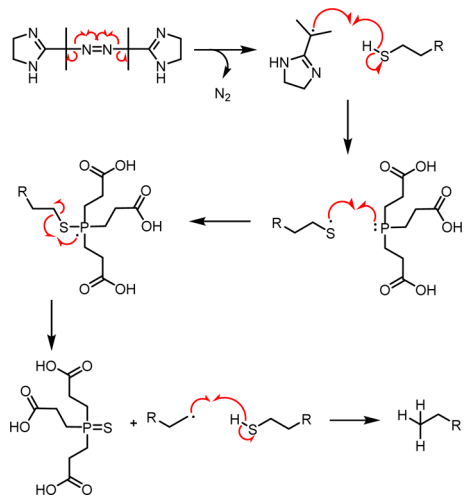


As shown earlier by Danishefsky and co-workers, this radical-based approach can also be used in the post-ligation desulfurization of cysteines in proteins, likewise almost quantitatively.[29]

Even though it had been proposed that the TCEP (phosphine) species is responsible for the desulfurization process,[29] we further probed the reaction to confirm that this was the sole phosphorus species present in the reaction mixture that can effect this radical process. This was accomplished by replacing TCEP with TCEP oxide and TCEP sulfide, respectively, under the same reaction conditions using SNAC as the thiol. The results showed that neither TCEP oxide nor TCEP sulfide led to any observed desulfurized product. This confirmed that the TCEP species is required for the formation of the thio-phosphine adduct, which then undergoes β-scission to produce the primary alkyl radical and TCEP sulfide as a byproduct. An intermolecular radical hydrogen transfer from nearby thiols presumably quenches the carbon radical forming the desulfurized product and propagates another thiyl radical (Scheme 4).

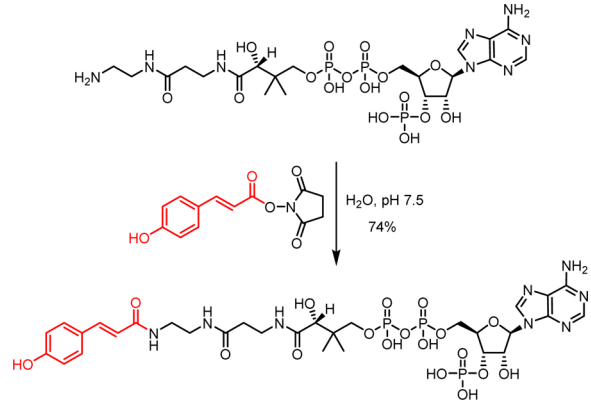
Thioester replacement in acyl CoA by an amide functionality has been commonly used in numerous studies on PKS, FAS, and NRPS enzymes. Usually this is accomplished by synthesis of the corresponding acylated pantetheine derivative followed by enzymatic transformation[30] as shown above in Scheme 1.

Scheme 4. Proposed Mechanism of the TCEP, Radical Desulfurization Reaction



However, the Bruner group has demonstrated acylation of amino CoA in water with an activated amino acid.[8a] Given its potential widespread application, we briefly examined the ease with which amino CoA may be elaborated by aqueous attachment to an activated acyl group. *p*-Coumaroyl CoA amide (**14**) could be readily made by the reaction of amino CoA and *p*-coumaric acid *N*-hydroxysuccinimide (NHS) ester in 74% isolated yield. The reaction was conducted at room temperature in phosphate buffer at pH 7.5 and was completed in 14 h, quite mild conditions (Scheme 5). As *p*-coumaroyl CoA (**14**) is a key precursor for type III polyketide synthases,[31] the corresponding amide analogue should be a useful probe for biochemical and structural studies.

Scheme 5. Preparation of *p*-Coumaroyl CoA Amide (14), from Amino CoA and *p*-Coumaric Acid NHS Ester



In summary, we have provided a novel transamidation approach for the one-step preparation of several useful CoA analogues. These compounds were prepared using a green method, under mild conditions with catalysis by boric acid in water. The CoA analogues presented are readily elaborated or extended by well precedented reactions for the specific applications that may be required. It offers rapid access to compounds that are useful for the study of a wide spectrum of enzyme catalyzed reactions, especially processes involving acyl carrier proteins (ACP) of polyketide synthases (PKS), fatty acid synthases (FAS), and nonribosomal peptide synthetases (NRPS).

DOI: 10.1021/acs.orglett.7b00291
Org. Lett. XXXX, XXX, XXX−XXX

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b00291.

Experimental procedures, product characterization, and copies of the ^{1}H and ^{13}C NMR spectra (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: john.vederas@ualberta.ca.

ORCID

John C. Vederas: 0000-0002-2996-0326

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Bela Reiz of the University of Alberta Mass Spectrometry facility and Mark Miskolzie of the NMR facility for their assistance in data acquisition. This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC).

■ REFERENCES

(1) (a) Mishra, P. K.; Drueckhammer, D. G. Coenzyme A Analogues and Derivatives: Synthesis and Applications as Mechanistic Probes of Coenzyme A Ester-Utilizing Enzymes. *Chem. Rev.* **2000**, *100*, 3283−3310. (b) Peter, D. M.; Vögeli, B.; Cortina, N. S.; Erb, T. J. A Chemo-Enzymatic Road Map to the Synthesis of CoA Esters. *Molecules* **2016**, *21*, 517.

(2) Lee, C.; Chen, A. F. Chapter 6 - Immobilized Coenzymes and Derivatives. In *The Pyridine Nucleotide Coenzymes*; Everse, J., Anderson, B., You, K., Eds.; Academic Press: 1982; pp 189−224.

(3) Dewick, P. M. The Acetate Pathway: Fatty Acids and Polyketides. In *Medicinal Natural Products*; John Wiley & Sons, Ltd: 2009; pp 39−135.

(4) Engel, C.; Wierenga, R. The diverse world of coenzyme A binding proteins. *Curr. Opin. Struct. Biol.* **1996**, *6*, 790−797.

(5) Knudsen, J.; Jensen, M. V.; Faergeman, N. J.; Neergaard, T. B. F.; Gaigg, B. Role of acyl CoA binding protein in acyl CoA transport, metabolism and cell signaling. *Mol. Cell. Biochem.* **1999**, *192*, 95−103.

(6) Brownell, J. E.; Allis, C. D. Special HATs for special occasions: linking histone acetylation to chromatin assembly and gene activation. *Curr. Opin. Genet. Dev.* **1996**, *6*, 176−184.

(7) Mandel, A. L.; La Clair, J. J.; Burkart, M. D. Modular Synthesis of Pantetheine and Phosphopantetheine. *Org. Lett.* **2004**, *6*, 4801−4803.

(8) (a) Liu, Y.; Bruner, S. D. Rational Manipulation of Carrier-Domain Geometry in Nonribosomal Peptide Synthetases. *ChemBioChem* **2007**, *8*, 617−621. (b) Li, Y.; Fiers, W. D.; Bernard, S. M.; Smith, J. L.; Aldrich, C. C.; Fecik, R. A. Polyketide Intermediate Mimics as Probes for Revealing Cryptic Stereochemistry of Ketoreductase Domains. *ACS Chem. Biol.* **2014**, *9*, 2914−2922.

(9) Moffatt, J. G.; Khorana, H. G. Nucleoside Polyphosphates. XII.1 The Total Synthesis of Coenzyme A2. *J. Am. Chem. Soc.* **1961**, *83*, 663−675.

(10) Michelson, A. M. Synthesis of Coenzyme A. *Biochim. Biophys. Acta, Gen. Subj.* **1964**, *93*, 71−77.

(11) Nazi, I.; Koteva, K. P.; Wright, G. D. One-pot chemoenzymatic preparation of coenzyme A analogues. *Anal. Biochem.* **2004**, *324*, 100−105.

(12) Meier, J. L.; Mercer, A. C.; Rivera, H.; Burkart, M. D. Synthesis and Evaluation of Bioorthogonal Pantetheine Analogues for in Vivo Protein Modification. *J. Am. Chem. Soc.* **2006**, *128*, 12174−12184.

(13) Worthington, A. S.; Burkart, M. D. One-pot chemo-enzymatic synthesis of reporter-modified proteins. *Org. Biomol. Chem.* **2006**, *4*, 44−46.

(14) Liu, Y.; Zheng, T.; Bruner, S. D. Structural basis for phosphopantetheinyl carrier domain interactions in the terminal module of nonribosomal peptide synthetases. *Chem. Biol.* **2011**, *18*, 1482−1488.

(15) Buyske, D. A.; Handschumacher, R. E.; Schilling, E. D.; Strong, F. M. The Stability of Coenzyme A1. *J. Am. Chem. Soc.* **1954**, *76*, 3575−3577.

(16) Krafft, G. A.; Siddall, T. L. Stereospecific displacement of sulfur from chiral centers. activation via thiaphosphonium salts. *Tetrahedron Lett.* **1985**, *26*, 4867−4870.

(17) Crimmins, D. L.; Mische, S. M.; Denslow, N. D. Chemical cleavage of proteins in solution. *Current Protocols in Protein Science*; John Wiley & Sons: Hoboken, NJ, 2005, *Chapter 11*, Unit 11.4.

(18) Carrot, G.; Hilborn, J. G.; Trollsås, M.; Hedrick, J. L. Two General Methods for the Synthesis of Thiol-Functional Polycaprolactones. *Macromolecules* **1999**, *32*, 5264−5269.

(19) Bellamy, F. D.; Ou, K. Selective reduction of aromatic nitro compounds with stannous chloride in non acidic and non aqueous medium. *Tetrahedron Lett.* **1984**, *25*, 839−842.

(20) Macmillan, D.; Adams, A.; Premdjee, B. Shifting Native Chemical Ligation into Reverse through N→S Acyl Transfer. *Isr. J. Chem.* **2011**, *51*, 885−899.

(21) Nguyen, T. B.; Sorres, J.; Tran, M. Q.; Ermolenko, L.; Al-Mourabit, A. Boric Acid: A Highly Efficient Catalyst for Transamidation of Carboxamides with Amines. *Org. Lett.* **2012**, *14*, 3202−3205.

(22) Becerra-Figueroa, L.; Ojeda-Porras, A.; Gamba-Sánchez, D. Transamidation of Carboxamides Catalyzed by Fe(III) and Water. *J. Org. Chem.* **2014**, *79*, 4544−4552.

(23) Allen, C. L.; Atkinson, B. N.; Williams, J. M. J. Transamidation of Primary Amides with Amines Using Hydroxylamine Hydrochloride as an Inorganic Catalyst. *Angew. Chem., Int. Ed.* **2012**, *51*, 1383−1386.

(24) Stephenson, N. A.; Zhu, J.; Gellman, S. H.; Stahl, S. S. Catalytic Transamidation Reactions Compatible with Tertiary Amide Metathesis under Ambient Conditions. *J. Am. Chem. Soc.* **2009**, *131*, 10003−10008.

(25) Foyn, H.; Jones, J. E.; Lewallen, D.; Narawane, R.; Varhaug, J. E.; Thompson, P. R.; Arnesen, T. Design, Synthesis, and Kinetic Characterization of Protein N-Terminal Acetyltransferase Inhibitors. *ACS Chem. Biol.* **2013**, *8*, 1121−1127.

(26) Stewart, C. J.; Thomas, J. O.; Ball, W. J.; Aguirre, A. R. Coenzyme A analogs. III. Chemical synthesis of desulfopantetheine 4′-phosphate and its enzymic conversion to desulfo-coenzyme A. *J. Am. Chem. Soc.* **1968**, *90*, 5000−5004.

(27) Montgomery, D. C.; Sorum, A. W.; Guasch, L.; Nicklaus, M. C.; Meier, J. L. Metabolic Regulation of Histone Acetyltransferases by Endogenous Acyl-CoA Cofactors. *Chem. Biol.* **2015**, *22*, 1030−1039.

(28) Chase, J. F. A.; Middleton, B.; Tubbs, P. K. A Coenzyme A analogue, Desulpho-CoA; Preparation and effects on various enzymes. *Biochem. Biophys. Res. Commun.* **1966**, *23*, 208−213.

(29) Wan, Q.; Danishefsky, S. Free-Radical-Based, Specific Desulfurization of Cysteine: A Powerful Advance in the Synthesis of Polypeptides and Glycopolypeptides. *Angew. Chem., Int. Ed.* **2007**, *46*, 9248−9252.

(30) Agarwal, V.; Diethelm, S.; Ray, L.; Garg, N.; Awakawa, T.; Dorrestein, P. C.; Moore, B. S. Chemoenzymatic Synthesis of Acyl Coenzyme A Substrates Enables in Situ Labeling of Small Molecules and Proteins. *Org. Lett.* **2015**, *17*, 4452−4455.

(31) Lim, Y. P.; Go, M. K.; Yew, W. S. Exploiting the Biosynthetic Potential of Type III Polyketide Synthases. *Molecules* **2016**, *21*, 806.

DOI: 10.1021/acs.orglett.7b00291
Org. Lett. XXXX, XXX, XXX−XXX