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A general scheme for synthesis of substrate-based polyketide labels for acyl carrier proteins

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ABSTRACT

A general strategy to enzymatically label acyl carrier proteins (ACPs) of polyketide synthases has been developed. Incorporation of a chloromethyl ketone or vinyl ketone moiety into polyketide chain elongation intermediate mimics allows for the synthesis of CoA adducts. These CoA adducts undergo enzymatic reaction with Sfp, a phosphopantetheinyl transferase, to afford labeled CurB carrier proteins.

The engineering of natural product biosynthetic pathways through combinatorial biosynthesis holds great promise for the discovery of new biologically active lead compounds. Polyketide natural products continue to be important for drug discovery, and >10% of the top 200 drugs by prescriptions dispensed in the United States were of polyketide origin.[1,2] Modular polyketide synthases (PKSs) are well suited for combinatorial biosynthesis because of the rich chemical and biological diversity of polyketides. However, little is known about the structural basis for substrate specificity and stereochemical outcome for the individual enzymatic reactions of PKSs. We previously demonstrated the power of affinity labels that mimic the substrates of pikromycin thioesterase (Pik TE) to determine its mechanism of macrolactonization.[3,4] Labels for acyl carrier protein (ACP) domains could be effective tools for study of substrate specificity and stereochemistry of other PKS catalytic domains because ACP domains are the natural delivery systems for PKS substrates.

PKSs make polyketides in a manner similar to fatty acid construction by human fatty acid synthase (FAS). The polyketide chain elongation intermediate is linked to the phosphopantetheine group of an ACP. Each intermediate is extended by a two- or three-carbon building block, and then modified by other enzymatic domains. Whereas FAS operates through an iterative process, type I PKSs are modular and have individual enzymatic domains for each reaction of the pathway.[5] Each module catalyzes the condensation and modification of one ketide building 'block' and contains at minimum a ketosynthase (KS), acyltransferase (AT), and ACP domain. Additional β-processing domains may be present, including ketoreductase (KR), dehydratase (DH), and enoyl reductase (ER). Each ACP of a type I PKS system passes the chain elongation intermediate to the KS domain of the next module. A thioesterase (TE) domain releases the final intermediate from the PKS.

Lack of information about the structural determinants of substrate specificity for nearly all catalytic domains of PKSs is a crucial barrier for combinatorial biosynthesis. Three-dimensional structures of domains from modular PKSs have only recently been reported. These include two KS–AT didomains,[6,7] two KR domains,[8,9] five DH domains,[10,11] two TE domains,[3,4,12,13] an ACP domain,[14] a pair of fused docking domains,[15] and two non-classical domains.[16,17] Among these, only Pik TE has an analogs of a chain elongation intermediate bound in the active site[3,4]: incorporation of diphenylphosphonate groups led to covalent modification of the active site Ser residue. These affinity labeled structures mimicked the covalent reaction intermediates, providing unprecedented insights to the mechanism and substrate specificity of Pik TE.

The covalent coupling of polyketide chain elongation intermediates to tethered ACP domains ensures a high effective concentration of these substrates with respect to the catalytic domains and, conversely, decreases the pressure to ensure a low 'natural' K_m. In addition, some substrate specificity of catalytic domains may be due to interactions with ACP domains although origins of

Abbreviations: ACP, acyl carrier protein; AT, acyltransferase; CoA/CoASH, coenzyme A; DEBS, 6-deoxyerythronolid B synthase; DH, dehydratase; ER, enoyl reductase; FAS, fatty acid synthase; KR, ketoreductase; KS, ketosynthase; NRPS, nonribosomal peptide synthetase; Pik TE, pikromycin thioesterase; PKS, polyketide synthase; PPTase, phosphopantetheinyl transferase; TE, thioesterase.

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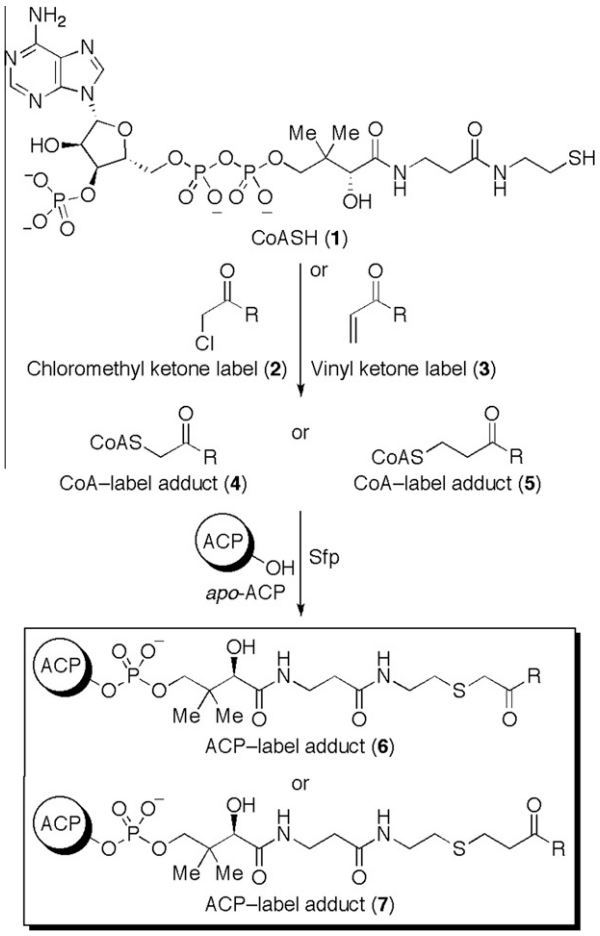
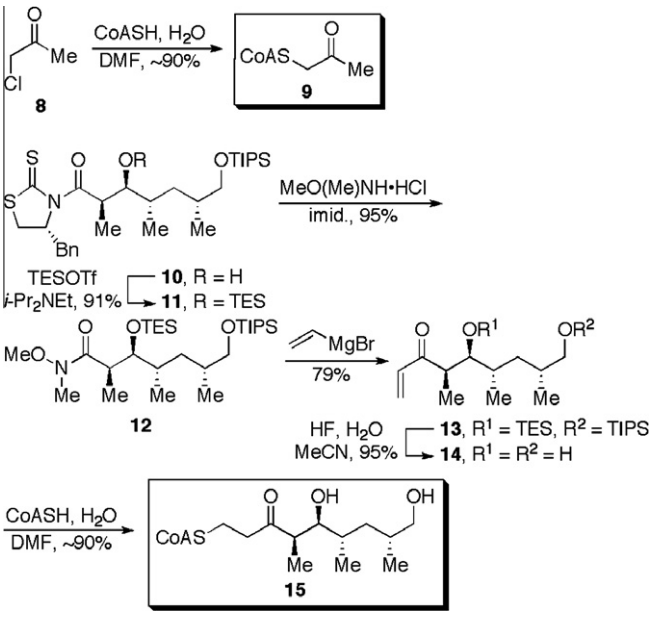
specificity are not clear.[18,19] Thus, solution techniques may be ineffective for trapping substrates or analogs within catalytic domains. PKS labels can help to overcome these obstacles. The capture of substrate mimics delivered to virtually all PKS domains is possible using ACP-bound labels. Electrophilic CoASH derivatives have been reported by others to be useful in labeling carrier protein domains for crosslinking to TE[20] and KS[21–23] domains. Liu and Bruner coupled amino-CoA with chloroacetic acid to give a CoA derivative that inhibited the catalytic activity of the TE domain of EntF.[20] Burkart and coworkers have used the chemoenzymatic synthesis of amino-CoA analogs with electrophilic groups (primarily vinyl chlorides) to crosslink ACP and KS domains of the 6-deoxyerythonolide B synthase (DEBS) PKS, type II FAS, and type II PKS systems.[21–23] This approach is useful for trapping and studying protein–protein interactions between ACP and catalytic domains. The crosslinking systems, however, lack incorporation of PKS-like substrates targeted to the active sites of the domain they modify, and the degree of crosslinking varies widely.[23] A method for the acylation of ACPs with simple β-lactones was recently reported, however, at the large excess concentration of β-lactone required for >90% ACP loading (50 equiv) the selectivity for ACP domains versus a KS–AT didomain is low (~1.5:1).[24] Additionally, the loading of a β-lactone with a *C*2-methyl group present in many polyketide chain elongation intermediates onto ACPs was low (~30%).[24] Here we describe the design and synthesis of two new classes of polyketide-based ACP labels, halomethyl ketones and vinyl ketones, that can be readily incorporated into mimics of polyketide chain elongation intermediates. A similar chloromethyl ketone-derived CoA analog was used to trap a biosynthetic intermediate in a type III stilbene synthase pathway.[25] These compounds were designed as labels for ACP domains with a substrate recognition element for delivery to the catalytic sites of other PKS domains. We show that both classes of labels react with CoASH, and we demonstrate enzymatic labeling of a model ACP, CurB.

Scheme 1 depicts our general strategy for the labeling of ACPs. A phosphopantetheine linker derived from CoASH serves as the point of attachment for polyketide chain elongation intermediates. The sulfhydryl group of CoASH (**1**) is modified by S_N2 reaction with a chloromethyl ketone affinity label (**2**) or by Michael addition to a vinyl ketone affinity label (**3**) to give stable, non-hydrolyzable CoA derivatives **4** or **5**. The labels are mimics of the substrates and products of a particular PKS catalytic domain. Similar Michael reactions of CoASH are known,[26] and modifications of the sulfhydryl group of CoASH have also been reported.[27,28] Each CoA derivative (**4** or **5**) is then attached enzymatically to a non-phosphopantetheinylated ACP (*apo*-form) by the promiscuous phosphopantetheinyl transferase (PPTase) Sfp.[29]



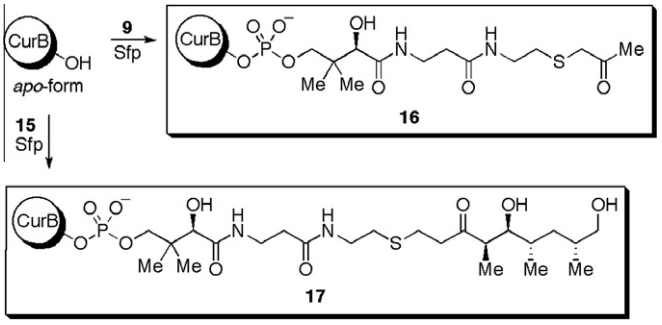
Scheme 1. General strategy for covalent modification of ACP domains by polyketide-based labels.

We sought to establish this approach through the synthesis of two model labels and CoA adducts (Scheme 2). The model chloromethyl ketone label we chose was chloroacetone (**8**). Chloroacetone smoothly reacted with the sulfhydryl group of CoASH to give CoA adduct **9**. A more complex model system was employed for the vinyl ketone labels. Synthesis of the second model label began with aldol product **10**, an intermediate used in our synthesis of Pik TE affinity labels.[4] The secondary alcohol was protected as silyl ether **11**, followed by displacement of the thiazolidinethione chiral auxiliary to afford Weinreb amide **12**. Ketone formation with vinylmagnesium bromide and deprotection gave vinyl ketone **14**. Attempts to form vinyl ketone **13** directly from silyl ether **11** were unsuccessful. Michael addition with CoASH completed the synthesis of CoA adduct **15**. Compound **15** mimics the C1–C7 segment of the hexaketide chain elongation intermediate of pikromycin.



Scheme 2. Synthesis of CoA adducts **9** and **15**.

We next examined the ability of Sfp, a non-specific PPTase from *Bacillus subtilis*, to modify the *apo*-form of a representative ACP. We validated this strategy with *Lyngbya majuscula* CurB, a stand-alone ACP from the curacin biosynthetic pathway.[30] CurB was quantitatively modified by both CoA adducts as determined by mass spectrometry to give CurB adducts **16** and **17** (Scheme 3 and Figs. 1, S1, S2). No unlabeled CurB was detected. The mass spectra of both



Scheme 3. Enzymatic labeling of the ACP CurB with CoA adducts **9** and **15**.

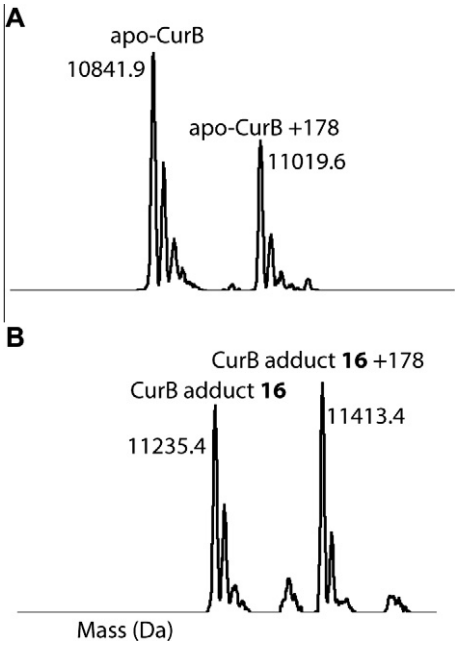


Figure 1. Deconvoluted mass spectra of *apo*-CurB (A) and of CurB adduct **16** (B). Unlabeled protein is observed with masses of 10841.9 Da for CurB (calculated mass 10842.5 Da) and 11019.6 Da for α-*N*-gluconyl-CurB (calculated mass 11020.6 Da). The PPTase reaction catalyzed by Sfp quantitatively labeled both forms of CurB, with mass shifts of 393.5 Da for CurB and 393.8 Da for α-*N*-gluconyl-CurB (calculated shift 395.4 Da).

unmodified and modified CurB showed two peaks in the labeling experiment with CoA adduct **9**: one corresponding to the protein and another corresponding to the protein +178 Da. This additional mass is likely due to α-*N*-gluconyl posttranslational modification of the N-terminal hexahistidine-tag. Others have noted similar posttranslational modification of several His-tagged proteins expressed in *Escherichia coli*.[31–33] The CurB protein used for the labeling experiment with CoA adduct **15** showed no posttranslational modification (Fig. S2). This sample was obtained from a separate purification experiment, and the reason for differences in posttranslational modification between CurB samples is not known. In our experiments, CurB in both its α-*N*-gluconyl posttranslationally modified and unmodified forms was quantitatively labeled with CoASH analogs **9** and **15**.

In summary, we have developed a general strategy for the design and synthesis of labels for ACP domains of PKS systems. Incorporation of a chloromethyl ketone or vinyl ketone group into a polyketide chain elongation intermediate mimic allows for reaction with CoASH. The resulting CoA adducts then undergo enzymatic reaction with Sfp to modify *apo*-CurB, a typical ACP. These CurB derivatives contain a phosphopantetheine group labeled with a non-hydrolyzable polyketide chain elongation intermediate mimic. Key features are the incorporation of stable chain elongation intermediate mimics into ACP and the relative ease of synthesis of the required vinyl ketone precursors.

We expect this labeling method to be broadly useful for the modification of more complex PKS systems, such as full PKS modules. Labeled constructs could be a powerful means to trap ACP-bound chain elongation intermediate mimics in the active sites of catalytic domains. The use of these and similar ACP labels in the study of ACP-containing PKS systems will be the subject of future investigations. This labeling technique also can be adapted readily to the study of mechanistically similar FAS and nonribosomal peptide synthetases (NRPSs) to expand their utility for a large number of primary and secondary metabolic pathways important for diseases and for combinatorial biosynthesis.

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Supplementary data

Experimental procedures and spectral data for all new compounds and CurB production and labeling, and copies of ^{1}H and ^{13}C NMR spectra for compounds **11**–**14**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.05.089.

References and notes

1. Butler, M. S. *Nat. Prod. Rep.* **2008**, *25*, 475.
2. Lamb, E. *Pharm. Times* **2009**, *75*, 26.
3. Giraldes, J. W.; Akey, D. L.; Kittendorf, J. D.; Sherman, D. H.; Smith, J. L.; Fecik, R. A. *Nat. Chem. Biol.* **2006**, *2*, 531.
4. Akey, D. L.; Kittendorf, J. D.; Giraldes, J. W.; Fecik, R. A.; Sherman, D. H.; Smith, J. L. *Nat. Chem. Biol.* **2006**, *2*, 537.
5. McDaniel, R.; Welch, M.; Hutchinson, C. R. *Chem. Rev.* **2005**, *105*, 543. and references therein.
6. Tang, Y.; Kim, C.-Y.; Mathews, I. I.; Cane, D. E.; Khosla, C. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 11124.
7. Tang, Y.; Chen, A. Y.; Kim, C.-Y.; Cane, D. E.; Khosla, C. *Chem. Biol.* **2007**, *14*, 931.
8. Keatinge-Clay, A. T.; Stroud, R. M. *Structure* **2006**, *14*, 737.
9. Keatinge-Clay, A. T. *Chem. Biol.* **2007**, *14*, 898.
10. Keatinge-Clay, A. *J. Mol. Biol.* **2008**, *384*, 941.
11. Akey, D. L.; Razelun, J. R.; Tehranisa, J.; Sherman, D. H.; Gerwick, W. H.; Smith, J. L. *Structure* **2010**, *18*, 94.
12. Tsai, S.-C.; Miercke, L. J. W.; Krucinski, J.; Gokhale, R.; Chen, J. C.-H.; Foster, P. G.; Cane, D. E.; Khosla, C.; Stroud, R. M. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 14808.
13. Tsai, S.-C.; Lu, H.; Cane, D. E.; Khosla, C.; Stroud, R. M. *Biochemistry* **2002**, *41*, 12598.
14. Alekseyev, V. Y.; Liu, C. W.; Cane, D. E.; Puglisi, J. D.; Khosla, C. *Protein Sci.* **2007**, *16*, 2093.
15. Broadhurst, R. W.; Nietlispach, D.; Wheatcroft, M. P.; Leadley, P. F.; Weissman, K. J. *Chem. Biol.* **2003**, *10*, 723.
16. Gu, L.; Geders, T. W.; Wang, B.; Gerwick, W. H.; Hakansson, K.; Smith, J. L.; Sherman, D. H. *Science* **2007**, *318*, 970.
17. Geders, T. W.; Gu, L.; Mowers, J. C.; Liu, H.; Gerwick, W. H.; Hakansson, K.; Sherman, D. H.; Smith, J. L. *J. Biol. Chem.* **2007**, *282*, 35954.
18. Castonguay, R.; He, W.; Chen, A. Y.; Khosla, C.; Cane, D. E. *J. Am. Chem. Soc.* **2007**, *129*, 13758.
19. Chen, A. Y.; Cane, D. E.; Khosla, C. *Chem. Biol.* **2007**, *14*, 784.
20. Liu, Y.; Bruner, S. D. *ChemBioChem* **2007**, *8*, 617.
21. Worthington, A. S.; Rivera, H., Jr.; Torpey, J. W.; Alexander, M. D.; Burkart, M. D. *ACS Chem. Biol.* **2006**, *1*, 687.
22. Kapur, S.; Worthington, A.; Tang, Y.; Cane, D. E.; Burkart, M. D.; Khosla, C. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3034.
23. Worthington, A. S.; Hur, G. H.; Meier, J. L.; Cheng, Q.; Moore, B. S.; Burkart, M. D. *ChemBioChem* **2008**, *9*, 2096.

24. Amoroso, J. W.; Borketey, L. S.; Prasad, G.; Schnarr, N. A. *Org. Lett.* **2010**, *12*, 2330.
25. Spiteller, D.; Waterman, C. L.; Spencer, J. B. *Angew. Chem., Int. Ed.* **2005**, *44*, 7079.
26. La Clair, J. J.; Foley, T. L.; Schegg, T. R.; Regan, C. M.; Burkart, M. D. *Chem. Biol.* **2004**, *11*, 195.
27. Yin, J.; Straight, P. D.; Hrvatin, S.; Dorrestein, P. C.; Bumpus, S. B.; Jao, C.; Kelleher, N. L.; Kolter, R.; Walsh, C. T. *Chem. Biol.* **2007**, *14*, 303.
28. Belshaw, P. J.; Walsh, C. T.; Stachelhaus, T. *Science* **1999**, *284*, 486.
29. Lambalot, R. H.; Gehring, A. M.; Flugel, R. S.; Zuber, P.; LaCelle, M.; Marahiel, M. A.; Reid, R.; Khosla, C.; Walsh, C. T. *Chem. Biol.* **1996**, *3*, 923. and references therein.
30. Gu, L.; Jia, J.; Liu, H.; Håkansson, K.; Gerwick, W. H.; Sherman, D. H. *J. Am. Chem. Soc.* **2006**, *128*, 9014.
31. Geoghegan, K. F.; Dixon, H. B. F.; Rosner, P. J.; Hoth, L. R.; Lanzetti, A. J.; Borzilleri, K. A.; Marr, E. S.; Pezzullo, L. H.; Martin, L. B.; LeMotte, P. K.; McColl, A. S.; Kamath, A. V.; Stroh, J. G. *Anal. Biochem.* **1999**, *267*, 169.
32. Dorrestein, P. C.; Van Lanen, S. G.; Li, W.; Zhao, C.; Deng, Z.; Shen, B.; Kelleher, N. L. *J. Am. Chem. Soc.* **2006**, *128*, 10386.
33. Sun, Y.; Hong, H.; Gillies, F.; Spencer, J. B.; Leadlay, P. F. *ChemBioChem* **2008**, *9*, 150.