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Modular Synthesis of Pantetheine and Phosphopantetheine

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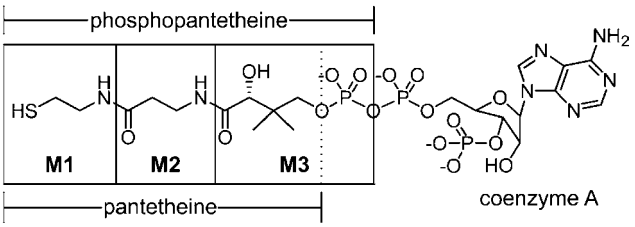
ABSTRACT



D-Pantetheine and D-phosphopantetheine, precursors to coenzyme A, have been synthesized though a linear sequence from three modules (M1–M3) in 9 and 10 steps, respectively. These routes provide access to analogues of coenzyme A containing modified cystamines, β-alanines, and pantoic acid residues. All three modules were joined using conventional methods of peptide synthesis. The chiral component, M3, was derived from D-pantolactone.

Coenzyme A (CoA), one of the most utilized cofactors in Nature, mediates the activation and transfer of acyl groups in fatty acid biosynthesis, secondary metabolite biosynthesis, primary metabolic pathways, and regulatory processes.[1−4] CoA plays an important role in cellular development, aging, and cancer.[5] Studies directed at the biosynthesis and processing of CoA remain vital to the understanding of metabolic disease.

CoA can be dissected into four modules as outlined in Figure 1. The first module, cystamine (**M1**), presents a terminal thiol that serves as the linkage point for acyl transport. The cystamine moiety is connected via an amide linkage to β-alanine (**M2**) that is further joined through an amide bond to pantoic acid (**M3**). In CoA, pantetheine, **M1**−**M3**, is joined to a 3′-adenosyl phosphate (**M4**) through a 5′-pyrophosphate bridge.

The first synthesis of CoA was unveiled in 1961, through the coupling of pantethenic acid, cystamine, and a 5′-phosphoromorpholidate.[6] Further modifications by Michelson provided an enhanced route to **M4** through addition of an adenosine diphosphate.[7] Both syntheses use pantolactone, the highly stable product of pantethenic acid hydrolysis, to increase versatility. However, low yields, racemization at the α-carbon of pantolactone, and unusual side reactions have limited the viability of schemes beginning with pantolactone. As a result, derivatives varying at the β-alanine moiety have generally been avoided,[4] and the β-alanine (**M2**) and pantoic acid (**M3**) modules are installed from pantothenate. Here we describe a modular route that permits individual derivatization at each module (Figure 1).

While pantolactone (**4**) can be coupled directly onto β-alanine to afford the necessary pantethenic acid analogue, such strategies depend solely on the nucleophilicity of the β-alaninylamine. Several disadvantages such as adverse effects from protecting groups and low-yielding reactions discourage variations that must be installed in this early stage

(1) Dewick, P. *Medicinal Natural Products: A Biosynthetic Approach*; Wiley: West Sussex, England, 2001; Chapter 1.
(2) Engel, C.; Wierenga, R *Curr. Opin. Struct. Biol.* **1996**, *6*, 790−797.
(3) Knudsen, J.; Jensen, M. V.; Hansen, J. K.; Faergeman, N. J.; Neergaard, T. B. F.; Gaigg B. *Mol. Cell. Biochem.* **1999**, *192*, 95−103.
(4) Mishra, P. K.; Drueckhammer, D. G. *Chem Rev.* **2000**, *100*, 3283−3309.
(5) Brownell, J. E.; Allis, C. D. *Curr. Opin. Gene. Dev.* **1996**, *6*, 176−184.
(6) Moffatt, J. G.; Khorana, H. G. *J. Am. Chem.. Soc.* **1961**, *83*, 663−675.
(7) Michelson, A. M. *Biochim. Biophys. Acta* **1964**, *93*, 71−77.

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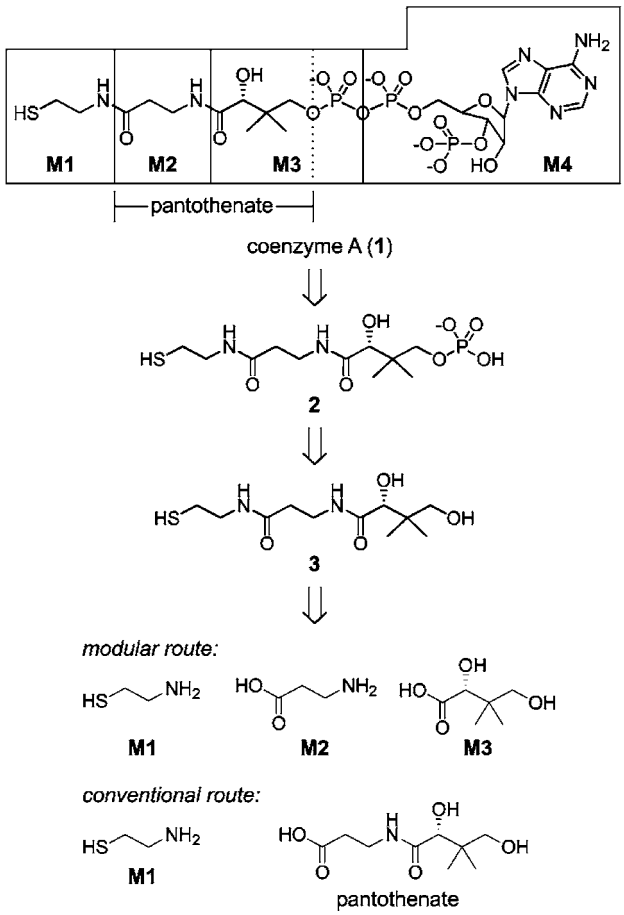
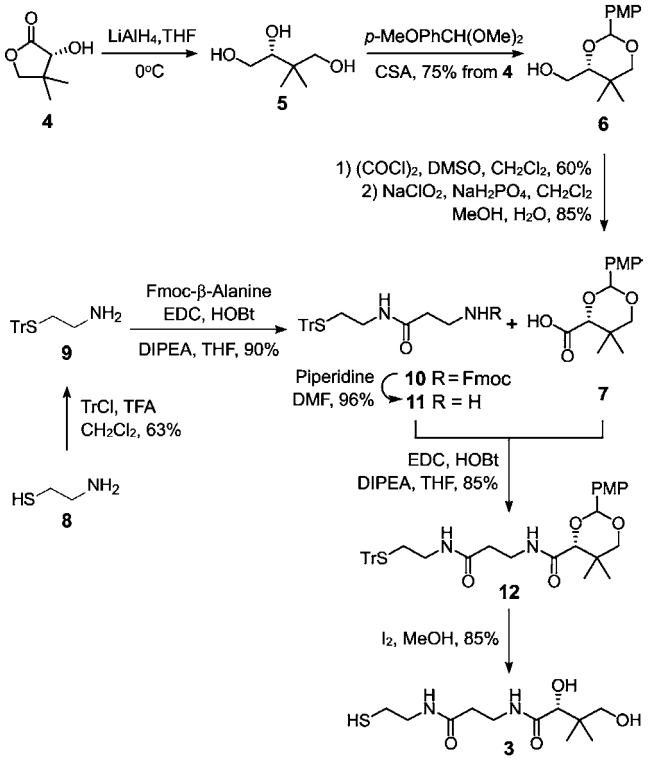


Figure 1. A modular view of coenzyme A, including a retrosynthetic breakdown to 4′-phosphopantetheine and pantetheine.

of the synthesis. In addition, pantolactone is extremely stable to hydrolysis and contains a sensitive α-hydroxyl group that enhances the potentional for α-carbon racemization under acidic or basic conditions.[8] Accordingly, a scheme for opening pantolactone was developed (Scheme 1).[9] D-Pantolactone **4** was reduced with lithium aluminum hydride to afford triol **5** in high yield. Subsequent conversion to *p*-anisaldehyde acetal **6** selectively protected the 1,3-diol, permitting Swern oxidation followed by mild chlorite oxidation to protected pantoic acid **7**.

In parallel, cystamine (**8**) was S-protected with trityl chloride under acidic conditions and then neutralized with sodium hydroxide to amine **9** (Scheme 1). Subsequent coupling with Fmoc-*β*-alanine provided amide **10** through standard carbodiimide conditions. The protected **M1**−**M2** block **10** was deprotected with piperidine and immediately coupled with **7** to provide **12**. Global deprotection was achieved with iodine in methanol to afford pantetheine (**3**) as a disulfide in 41% overall yield from cystamine or 28% from D-pantolactone (**4**).

Scheme 1

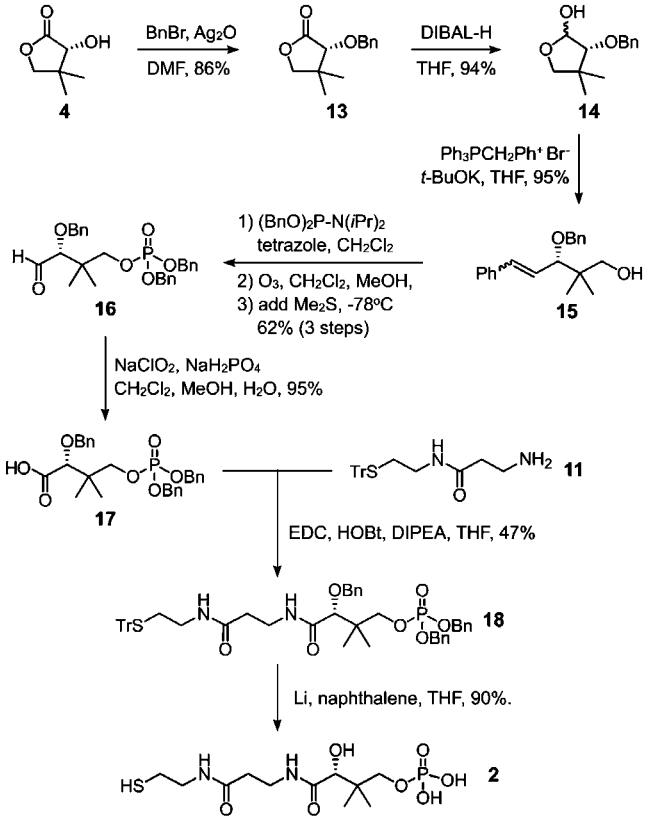


While both chemical and chemoenzymatic methods exist to convert pantetheine (**3**) to CoA, established procedures require 4′-phosphopantetheine (**2**).[6,7,10,11] Chemical phosphorylation of pantetheine (**3**) has been reported to give **2**, albeit in poor yield. Some alterations in the pantethiene backbone such as reversing the pantoic acid stereocenter from *R* to *S*[12] will hinder the biosynthetic pathway to CoA. For access to a wider range of CoA derivatives, it would be useful to build CoA chemically. Therefore, we developed a route to directly couple a 4′-phosphorylated pantoic acid to amide **11** (Scheme 2).

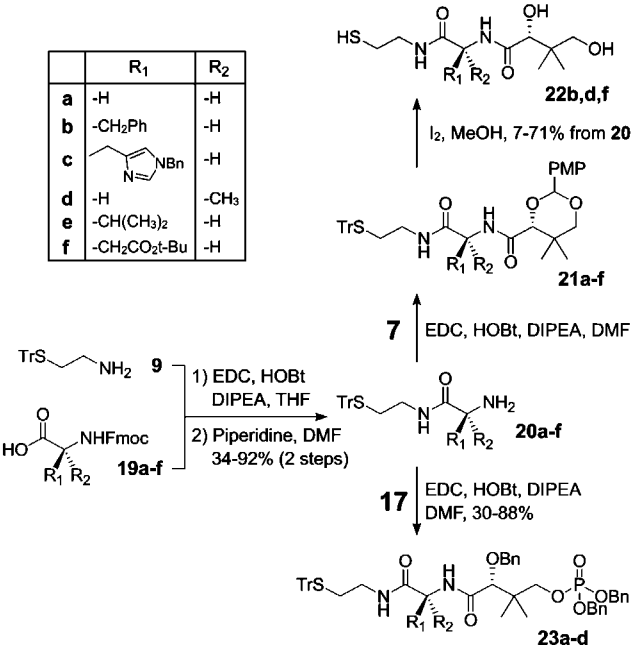
The α-hydroxyl of D-pantolactone (**4**) was protected by benzylation under mildly basic conditions. Careful control of pH is necessary to prevent epimerization. Benzyl pantolactone **13** was reduced to lactol **14** with DIBAL-H. Several conditions were attempted to open the lactol, but it was found that masking the aldehyde as olefin **15** using Wittig conditions[13,14] provided a facile means to open the ring. A sample of **15** was esterified with (*R*)-2-phenylbutyric acid and verified that enantiomeric purity had been maintained.[15] From this point, the phosphate was added by incubation with

(8) Dueno, E. E.; Chu, F.; Kim, S.; Jung, K. W. *Tetrahedron Lett.* **1999**, *40*, 1843−1846.
(9) Shiina, I.; Shibata, J.; Ibuka, R.; Imai, Y.; Mukaiyama, T. *Bull. Chem. Soc. Jpn.* **2001**, *74*, 113−122.
(10) Strauss, E.; Begley, T. *J. Biol. Chem.* **2002**, *277*, 48205−48209.
(11) Nazi, I.; Koteva, K. P.; Wright, G. D. *Anal. Biochem.* **2004**, *324*, 100−105.
(12) Bibart, R. T.; Vogel, K. W.; Drueckhammer, D. G. *J. Org. Chem.* **1999**, *64*, 2903−2909.
(13) Hart, D. J.; Patterson, S.; Zakarian, A. *Heterocycles* **2000**, *52*, 1025−1028.
(14) Miyaoka, H.; Sagawa, S.; Nagaoka, H.; Yamada, Y. *Tetrahedron: Asymmetry* **1995**, *6*, 587−594.
(15) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1968**, *90*, 3732−3738. See Supporting Information for more details.

Scheme 2



Scheme 3



dibenzyl-*N*,*N*-diisopropylphosphoramidite followed by immediate ozonolysis to provide aldehyde **16** in 62% yield from **15**.[16]

As in Scheme 1, mild chlorite oxidation of **16** completes the synthesis. After isolation, **17** was coupled to **11** using standard peptide coupling conditions to provide **18**. Final conversion to phosphopantetheine (**2**) was achieved through reduction with lithium naphthalenide.[17] The ^{1}H NMR spectrum of **2** matched the published spectra[18] with the exception of a downfield shift in the methylene group adjacent to the thiol ($\delta = 2.83$ found vs $\delta = 2.64$ reported by Sarma). This was attributed to a different state of thiol oxidation, as we suspect that Sarma examined the disulfide of **2**.

Both **7** and **17** were used to generate a set of pantetheine and phosphopantetheine analogues bearing an internal amino acid residue in place of β-alanine (Scheme 3). Using methods established in Scheme 1, *S*-trityl cystamine was coupled to Fmoc-protected amino acids **19a**−**f** to provide **20a**−**f** in yields ranging from 30 to 88%. Low yields in couplings of **19e** and **19f** were due to extended reaction times. Alternate coupling reagents could be screened to increase the yield of these reactions. Amides **20a**−**f** provided access to analogues of pantetheine and phosphopantetheine through conversion to **21a**−**f** or **23a**−**d**. Once coupled, **21a**−**f** could be deprotected with a variety of conditions.[19,20,21] As shown in Scheme 3, the deprotection of **21b**,**d**,**f** with iodine in methanol offered **22b**,**d**,**f** in unoptimized yields of 7, 71, and 11%, respectively, from **19b**,**d**,**f**. Comparable schemes were also available for the modification of **23a**−**d**.[15,22]

Schemes 1−3 provide access to the three-component modular synthesis of pantetheine, 4′-phosphopantetheine, and analogues therein. With this approach, modifications may be introduced at any of three peptoid modules **M1**, **M2**, and **M3**, accessing full synthetic flexibility within the pantetheine structure. We are currently following various avenues of parallel chemical and chemoenzymatic synthesis to investigate novel analogues of CoA in primary and secondary metabolic pathways.

Supporting Information Available: Experimental procedures and spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) Variations of this scheme can also be used to isolate the dibenzyl phosphite of **15**. Partial ozonolysis or oxidation with oxygen can also be used to generate the dibenzyl phosphate of olefin **15**. The phosphate oxidizes easily in the presence of ozone, whereas olefin cleavage takes several minutes of exposure.

(17) Liu, H.; Yip, J. *Tetrahedron Lett.* **1997**, *38*, 2253−2256.
(18) Lee, C.; Sarma, R. H. *J. Am. Chem. Soc.* **1975**, *97*, 1225−1235.
(19) Szarek, W. A.; Zamojski, A.; Tiwari, K. N.; Ison, E. R. *Tetrahedron Lett.* **1986**, *33*, 3827−3830.
(20) Zervas, L.; Photaki, I. *J. Am. Chem. Soc.* **1962**, *84*, 3887−3897.
(21) Riley, A. M.; Guedat, P.; Schlewer, G.; Spiess, B.; Potter, B. V. L. *J. Org. Chem.* **1998**, *63*, 295.
(22) Kitas, E. A.; Knorr, A.; Trzeciak, A.; Bannwarth, W. *Helv. Chim. Acta* **1991**, *74*, 1314.