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Synthesis and Analysis of Potential Prodrugs of Coenzyme A Analogues for the Inhibition of the Histone Acetyltransferase p300

Marek Cebrat,[a,b] Cheol M. Kim,[a] Paul R. Thompson,[a] Matthew Daugherty[c] and Philip A. Cole[a,*]

[a]*Department of Pharmacology and Molecular Sciences, Johns Hopkins University, School of Medicine, 725 N. Wolfe St., Baltimore, MD 21205, USA*
[b]*Faculty of Chemistry, University of Wroclaw, 50-383 Wroclaw, Poland*
[c]*Integrated Genomics, Incorporated, Chicago, IL 60612, USA*

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Abstract—Lys-CoA (**1**) is a selective inhibitor of p300 histone acetyltransferase (HAT) but shows poor pharmacokinetic properties because of its multiply charged phosphates. In an effort to overcome this limitation, truncated derivatives of **1** were designed, synthesized and tested as p300HAT inhibitors as well as substrates for the CoA biosynthetic bifunctional enzyme phosphopantetheine adenylyltransferase-dephospho-CoA kinase (PPAT/DPCK). Lys-pantetheine (**3**) and Lys-phosphopantetheine (**2**) showed no detectable p300HAT inhibition whereas 3′-dephospho-Lys-CoA (**5**) was a modest p300 inhibitor with IC_{50} of 1.6 μM (compared to IC_{50} of ~50 nM for **1** blocking p300). Compound **2** was shown to be an efficient substrate for PPAT whereas **5** was a very poor DPCK substrate. Further analysis with 3′-dephospho-Me-SCoA (**7**) indicated that DPCK shows relatively narrow capacity to accept substrates with sulfur substitution. While these results suggest that truncated derivatives of **1** will be of limited value as lead agents for p300 blockade in vivo, they augur well for prodrug versions of CoA analogues that do not require 3′-phosphate substitution for efficient binding to their targets, such as the GCN-5 related *N*-acetyltransferases.

Introduction

CoA derivatives can be powerful and selective enzyme inhibitors.[1–5] However, since the CoA moiety contains three phosphate groups which are negatively charged at neutral pH, CoA containing compounds tend to have poor pharmacokinetic performance in vivo. Our lab has recently developed several bisubstrate inhibitors of the acetyl-CoA-dependent histone acetyltransferases which contain CoA moieties.[5] Histone acetyltransferases (HATs) catalyze the transfer of an acetyl group from acetyl-CoA to the ε-amino group of lysines in histones and other proteins (Fig. 1) and are important in the regulation of chromatin remodeling and gene expression.[6] One compound in particular, Lys-CoA (**1**) (Fig. 2), has proved useful for blocking the HAT activity of transcriptional coactivator p300.[5] While it has been employed for in vitro transcription studies[7] and in cells via microinjection or with the use of cell permeabilizing agents,[8] compound **1** has generally been ineffective by simple addition to cell culture media.

A theoretical approach[9] to overcoming this problem is to develop truncated versions of **1** (compounds **2**–**5**) which are either p300 inhibitors on their own or could be capable of being bio-converted into Lys-CoA (**1**) inside cells containing the target. The biosynthetic pathway of the conversion of pantetheine to CoASH has long been known[10] and recently the protein responsible for the final two steps in CoASH production in human cells was isolated and characterized.[11] This bifunctional protein, phosphopantetheine adenylyltransferase-dephospho-CoA kinase (PPAT/DPCK), converts phosphopantetheine to CoASH (Fig. 3) but its ability to process phosphopantetheine analogues has not yet been addressed. In this study, we describe the design and synthesis of

*Corresponding author. Tel.: +1-410-614-8849; fax: +1-410-614-7717; e-mail: pcole@jhmi.edu

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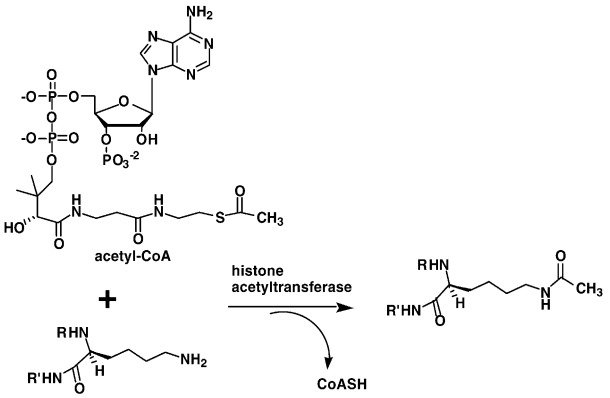

Figure 1. Histone acetyltransferase reaction catalyzed by p300.

several sub-structural analogues (compounds **2**–**5**) related to Lys-CoA (**1**), their action against p300HAT activity, and their properties as substrates of the human CoASH biosynthetic bifunctional enzyme PPAT/DPCK.

Results

Synthesis of Lys-pantetheine (3) and analogues

Fmoc-Lys containing Dde (dimethyldioxocyclohexylidene) protection on the ε-amino group was first immobilized on Rink amide resin via standard amide bond formation. Removal of the Fmoc group with piperidine was followed by treatment with acetic anhydride, affording the resin bound α-acetamide (Fig. 4). The Dde group was then removed with hydrazine and the free ε-amino group was reacted with bromoacetic acid. The derivatized Lys-bromacetyl compound (**8**) was then cleaved from the resin with trifluoroacetic acid and purified by HPLC. The purified compound **8** was treated with freshly prepared D-pantetheine and the product Lys-pantetheine (**3**) purified by HPLC. Phosphorylation of **3** was carried out in three steps:[4b] first regio-selective modification of the less hindered hydroxy group with dibenzyl isopropyl phospoamidate, oxidation to the dibenzyl phosphate triester (**9**), and then acidolysis of the benzyl groups with trifluoroacetic acid yielded the target compound Lys-phosphopantetheine (**2**).

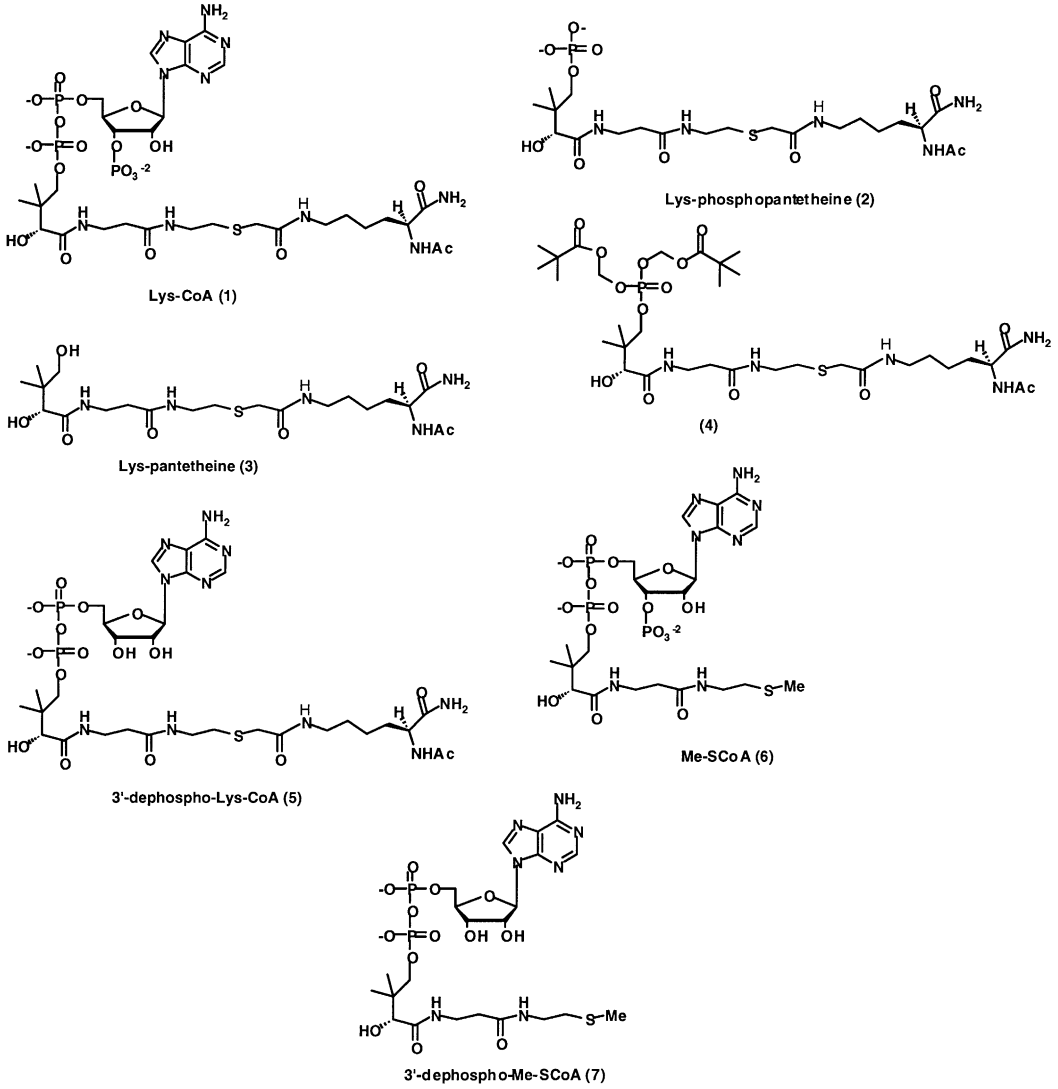

Figure 2. Lys-CoA (**1**) and analogues.

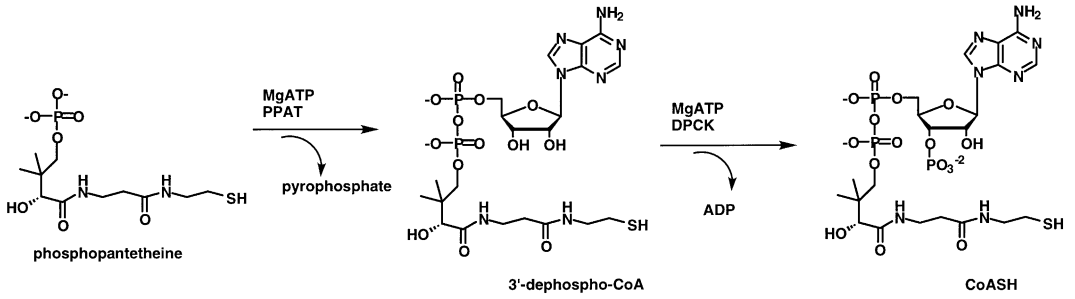

Figure 3. PPAT/DPCK catalyzed conversion of phosphopantetheine to CoASH.

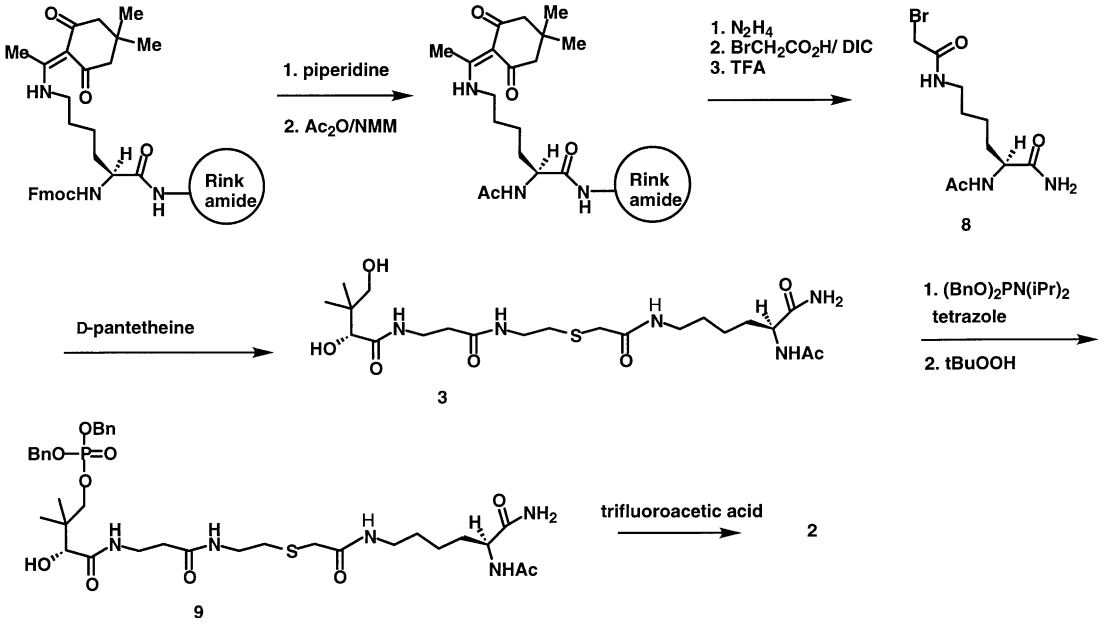

Figure 4. Synthetic approach to Lys-phosphopantetheine (**2**).

A variety of strategies have been used to protect organophosphate compounds in a fashion that allows them to be cell permeable and for efficient liberation of the phosphate moiety inside cells by nonspecific esterases.[12] We turned our attention to the synthesis of derivative **4** because bis[(pivaloyloxy)methyl] (POM) protection of phosphate moieties has been effective in enhancing the action of several published prodrugs.[13] Of the several approaches that we tested, two synthetic methods proved feasible, although both methods proceeded in low yields (5.0–7.5%). The first of them utilized the reaction of Lys-phosphopantetheine (**2**) with tributyltin methoxide and iodomethyl pivaloate. The other involved the reaction of Lys-pantetheine (**3**) with bis[(pivaloyloxy)methyl] hydrogen phosphate and diisopropylcarbodiimide (DIC). Poor yields of the POM derivative **4** may have resulted from the fact that the secondary hydroxy group of pantetheine could form an intramolecular interaction with the phosphoester system. Though low-yielding, these methods may provide a general solution to preparing bio-cleavable CoA precursors for cellular studies.

An additional compound that we synthesized was 3′-dephospho-Lys-CoA (**5**) which was prepared simply by reacting **8** with 3′-dephospho-CoASH. Compound **5** was synthesized both to evaluate how much the 3′-phosphate of **1** contributes to p300 inhibition as well as to determine the effects of lysyl-substitution on PPAT/DPCK biotransformation. Finally, other control compounds for the PPAT/DPCK biotransformation studies that were prepared in straightforward fashion include Me-SCoA (**6**) and 3′-dephospho-Me-SCoA (**7**).

p300HAT inhibition studies

The Lys-pantetheine analogues **2**, **3**, and **5** were evaluated as p300HAT inhibitors using recombinant p300HAT domain, radiolabelled acetyl-CoA, and H4-derived 20 amino acid peptide. Under these conditions, the compound Lys-CoA (**1**) displayed an IC_{50} ~50 nM. Both Lys-pantetheine (**3**) and Lys-phosphopantetheine (**2**) showed IC_{50} values $>>6.4$ μM (<10% inhibition at 6.4 μM) indicating a dramatic loss of inhibition. Interestingly, 3′-dephospho-Lys-CoA (**5**), while still reasonably potent at blocking p300 with IC_{50} of 1.6 μM, was about 33-fold weaker at blocking p300 compared to Lys-CoA (**1**) itself. This behavior stands in contrast to a bisubstrate analogue inhibitor of serotonin *N*-acetyltransferase where the 3′-phosphate of the compound was found to be dispensable.[4b]

PPAT/DPCK biotransformation studies

Since it became clear that substructures related to Lys-CoA (**1**) show significantly weakened potency in the blockade of p300 compared to Lys-CoA (**1**) itself, it was important to investigate the potential for Lys-phosphopantetheine (**2**) and 3′-dephospho-Lys-CoA (**5**) to be enzymatically processed by PPAT/DPCK. An HPLC assay was developed that cleanly separated each of these three compounds from each other as well as other reagents in the buffer (Fig. 5). Studies of the PPAT reaction with Lys-phosphopantheine (**2**) revealed a relatively rapid conversion to 3′-dephospho-Lys-CoA (**5**) with a pseudo first-order rate constant of 0.02 s^{-1}. Compared to published values, this was calculated to be within 20-fold of the rate measured for the underivatized phosphopantethetheine itself being processed by PPAT.[11,14]

In contrast, in the second step, conversion of 3′-dephospho-Lys-CoA (**5**) to Lys-CoA (**1**) by the DPCK activity was found to be very slow, with only 5% conversion after 17.5 h in the presence of 2 μM enzyme. To rule out that the DPCK activity was damaged in our preparation, we tested this enzyme activity with its natural substrate, 3′-dephospho-CoA. As expected, 3′-dephospho-CoASH was transformed to CoASH very efficiently, about 53,000-fold faster than the rate of conversion of 3′-dephospho-Lys-CoA (**5**) to Lys-CoA (**1**) under the same conditions, and at a rate in approximate agreement with the literature value.[11,14] It was formally possible that 3′-dephospho-Lys-CoA (**5**) was acting primarily as a competitive inhibitor rather than an efficient DPCK substrate. To test this, compound **5** was added to the reaction containing 3′-dephospho-CoASH and DPCK. However, compound **5** did not significantly block the conversion of 3′-dephospho-CoASH to CoASH in the concentration (500 μM) tested (data not shown).

Thus DPCK appeared to be poorly able to accept substrates with lysyl substitution of the thiol moiety of 3′-dephospho-CoASH. To further address this, 3′-dephospho-Me-SCoA (**7**) was examined as a DPCK substrate. This compound was found to be about 15-fold reduced as a DPCK substrate compared to 3′-dephospho-CoASH, again highlighting the sensitivity of DPCK to substrates containing structural substitution on sulfur. In summary, the PPAT component shows relatively large tolerance of pantetheinyl sulfur substitution in substrates whereas DPCK has quite strict substrate requirements even at this remote sulfur site.

Discussion

Although we were successful in the synthesis of a variety of Lys-CoA (**1**) derivatives, it was found that an intact CoA moiety in **1** is required for its potent blockade of p300HAT. That removal of the 3′-phosphate from **1** results in an apparent 33-fold reduction in affinity suggests that the p300 active site makes specific interactions with the 3′-phosphate within the natural substrate acetyl-CoA. This is interesting because other known HATs such as the GCN-5 related *N*-acetyltransferases do not appear to show a requirement for 3′-phosphate in molecular recognition.[4,15] Thus, in addition to known mechanistic differences with the GNAT superfamily,[5c] p300 appears to have a distinct strategy for binding to the acetyl-CoA substrate. Since CoA binding represents the most conserved feature of this diverse superfamily,[16] this result provides further evidence against a prior proposal suggesting close structural resemblance of p300 to the GNATs.[17]

It was our design that Lys-phosphopantetheine POM derivative (**4**) which was prepared here could be biotransformed inside cells, first to the free phosphate and then ultimately to Lys-CoA (**1**). While Lys-phosphopantetheine (**2**) proved to be a good PPAT substrate, the next step in the conversion catalyzed by DPCK was extremely inefficient. Since 3′-dephospho-Lys-CoA (**5**) is not a very strong p300 inhibitor, these results suggest that a Lys-CoA prodrug approach will probably not lead to the most potent p300 inhibitors in human cells. For the purposes of cell biology research it is possible

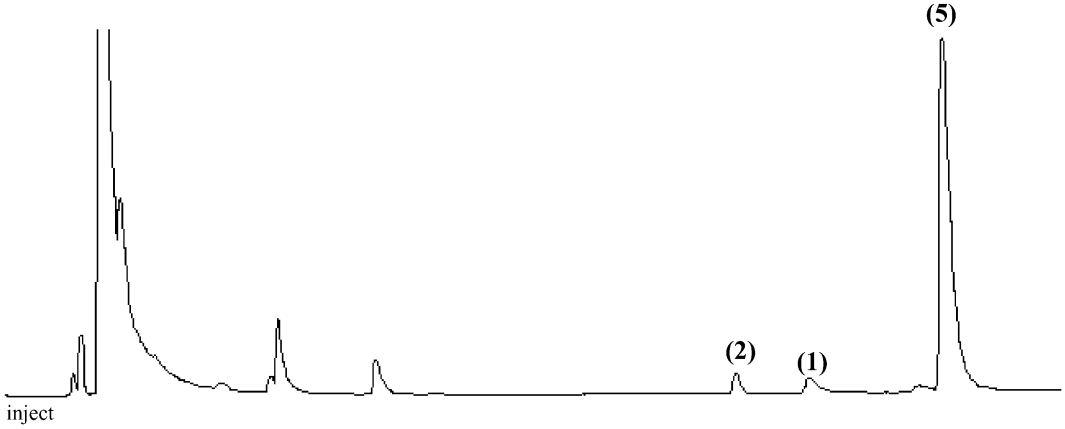

Figure 5. HPLC trace showing separation of Lys-phosphopantetheine (**2**) and products 3′-dephospho-Lys-CoA (**5**) and Lys-CoA (**1**). HPLC conditions: C-18 analytical column, flow rate of 1 mL/min and gradient of potassium phosphate pH 4.5 (A) and methanol (B) as follows, 0–5 min 100% A, 5–55 min linear gradient to 80% A, UV detection at 214 nm. Retention times are 42, 46, and 54 min for **2**, **1**, and **5**, respectively. See Experimental for further details.

that overexpression of PPAT/DPCK in cells of interest could be used to permit an efficient conversion of the desired reaction. On the other hand, if the lysyl moiety of **5** could be further modified to enhance inhibitory potency, it is conceivable that one could sacrifice the added affinity associated with the DPCK-catalyzed phosphorylation reaction.

More generally, since many CoA enzymes such as the GNAT superfamily members do not require the 3′-phosphate in substrates or inhibitors to achieve high affinity, this prodrug strategy may be best directed at such enzymes. For example, serotonin *N*-acetyltransferase is a potential drug target for disorders of circadian rhythm and is potently blocked by bisubstrate analogues containing a CoA moiety.[4] Removal of the 3′-phosphate from such a bisubstrate analogue only shows a 2-fold effect on diminishing inhibitory potency.[4b] Thus, appropriately designed prodrug analogues of these bisubstrate serotonin *N*-acetyltransferase inhibitors may well show promise at blocking melatonin production in vivo.

Experimental

General

The bifunctional human PPAT/DPCK enzyme and the p300 catalytic domain (N-terminal His tag, residues 1284–1673) were prepared as recombinant proteins as described.[11,18] Bis[(pivaloyloxy)methyl] hydrogen phosphate and iodomethyl pivaloate were synthesized following procedures described elsewhere.[13c] All other reagents were commercially available unless otherwise described. NMR spectra were recorded on a Varian Mercury 400 MHz instrument, signals are reported in ppm from tetramethylsilane (s, d, m, b for singlet, doublet, multiplet and broad, respectively). Electrospray ionization MS was carried out on a Psciex single quadrupole machine. FABHRMS were obtained at the UC Riverside MS facility.

Ac-Lys(AcBr)-NH_2 (8). 2.0 g of Rink Amide AM resin (0.63 mmol/g) was deprotected by 20% piperidine solution in DMF and treated with 3 equiv of Fmoc-Lys(Dde)-OH in the presence of 3 equiv BOP (benzotriazole - 1 - yl - oxy - tris - (dimethylamino) - phosphoniumhexafluorophosphate) and 3 equiv of HOBt (*N*-hydroxybenzotriazole) in a minimal volume of 0.4 M solution of *N*-methylmorpholine (NMM) in DMF for 3 h at room temperature. The Fmoc group was removed by standard piperidine treatment and the N-terminal amino group was acetylated by treatment with excess 50% acetic anhydride and 0.4 M NMM (DMF) over 1 h at room temperature. The Dde group was removed by reaction with 2% hydrazine (DMF) for 2 h and the ε-amino group was treated with 5 equiv of bromoacetic acid and 5 equiv diisopropylcarbodiimide (DIC) in a minimal volume of DMF overnight at room temperature. The product was cleaved from the resin by treatment with a mixture of 95% TFA/2.5% water/2.5% triisopropylsilane for 2 h at room temperature Crude compound was precipitated from the cleavage mixture with ice-cold diethyl ether, dissolved in water, lyophilized and purified by reversed phase HPLC using a water/acetonitrile gradient (0.05% trifluoroacetic acid) with a C-18 column. The yield was 300 mg of the pure compound **8** obtained after lyophilization. ESI-MS calcd for $C_{10}H_{18}N_3O_3Br$ 308.1, found *m*/*z* 330.0, 332.0 (Na^+ salts, bromine isotopes). ^1H NMR (400 MHz, CD_3OD) δ 1.41 (m, 2H), 1.54 (m, 2H), 1.65 (m, 1H), 1.80 (m 1H), 1.99 (s, 3H), 3.20 (m, 2H), 4.28 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 21.30, 22.96, 27.62, 28.59, 31.54, 39.38, 53.23, 168.32, 172.20, 175.98.

Lys-pantetheine (3). D-Pantetheine (0.46 g) was dissolved in 5 mL dioxane:water (4:1) mixture and 0.24 g of triphenylphosphine was added. The pH was adjusted to 2 by adding 6 M HCl and the mixture was stirred for 3 h at room temperature Ac-Lys(AcBr)-NH_2 (**8**) (285 mg) dissolved in 5 mL dioxane/water mixture (4:1) was added, and the pH was adjusted to 9.8 by adding triethylamine. The reaction mixture was stirred for 2.5 h at room temperature and then lyophilized. The crude mixture was dissolved in water, the insoluble material removed by filtration, and the solution was subjected to reversed phase HPLC purification affording 460 mg pure **3** after lyophilization. FABHRMS (M + H +) calcd for C21H40N5O7S 506.2648, found *m*/*z* 506.2649. ^1H NMR (400 MHz, CD_3OD) δ 0.91 (s, 6H), 1.40 (m, 2H), 1.54 (m, 2H), 1.66 (m, 1H), 1.80 (m, 1H), 1.99 (s, 3H), 2.43 (m, 2H), 2.69 (m, 2H), 3.21 (m, 4H), 3.36–3.49 (bm, 6H), 3.89 (s, 1H), 4.28 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 19.74, 20.19, 21.36, 23.06, 28.80, 31.58, 31.72, 34.74, 35.22, 35.30, 38.50, 39.20, 53.29, 69.15, 76.08, 171.28, 172.20, 172.70, 174.87, 176.00.

Lys-phosphopantetheine (2). Dibenzyl diisopropyl phosphoamidate (105 μL, 1 equiv) was added slowly to ice-cold, stirred solution of Lys-pantetheine (**3**) (150 mg) and 1*H*-tetrazole (22 mg, 1.1 equiv) in 3 mL DMF. After allowing gradual warming to room temperature, the mixture was stirred for 1 h. The reaction mixture was cooled down again to 0 °C and 2 equiv of 6 M *t*-butyl hydroperoxide solution in isooctane was added. After 2 h, the mixture was concentrated in vacuo and then purified by reversed phase HPLC to give 120 mg of pure Lys-phosphopantetheine dibenzyl ester (**9**). FABHRMS ($M + H^+$) calcd for $C_{35}H_{53}N_5O_{10}P_1S$ 766.3251, found *m*/*z* 766.3242. ^1H NMR (400 MHz, CD_3OD) δ 0.89 (s, 3H), 0.95 (s, 3H), 1.39 (m, 2H), 1.52 (m, 2H), 1.64 (m, 1H), 1.79 (m, 1H), 1.98 (s, 3H), 2.41 (m, 2H), 2.68 (m, 2H), 3.19 (m, 4H), 3.37 (m, 2H), 3.46 (m, 2H), 3.84 (m, 2H), 4.00 (m, 1H), 4.28 (m, 1H), 5.05 (d, 4H), 7.36 (s, 10H).

Benzyl protection was removed by treatment with 95% TFA over 3 h at room temperature. The mixture was concentrated in vacuo and then purified by reversed phase HPLC to afford 54 mg of the pure product **2**. FABHRMS ($M + H^+$) calcd for $C_{21}H_{41}N_5O_{10}$ 586.2312, found *m*/*z* 586.2334. ^1H NMR (400 MHz, D_2O) δ 0.86 (s, 3H), 0.93 (s, 3H), 1.35 (m, 2H), 1.50 (m, 2H), 1.68 (m, 1H), 1.77 (m, 1H), 1.99 (s, 3H), 2.46 (m, 2H), 2.66 (m, 2H), 3.18 (m, 2H), 3.23 (s, 2H), 3.35 (m,

2H), 3.47 (m, 2H), 3.60 (m, 1H), 3.79 (m, 1H), 3.99 (s, 1H), 4.16 (m, 1H).

Lys-CoA (1). Compound **8** was dissolved in a small volume of 0.5 M triethylammonium bicarbonate buffer, pH 8.2, and treated with 2 equiv coenzyme A dilithium salt (Sigma), overnight at room temperature. The mixture was diluted with water, lyophilized and purified by reversed phase HPLC in water/acetonitrile solvent system. Analytical data for the compound agreed with those measured previously. ESI-MS ($M+H^+$) calcd for $C_{31}H_{53}N_{10}O_{19}P_3S$ 994.8, found *m/z* 996.0. ^{1}H NMR (400 MHz, D_2O) δ 0.65 (s, 3H), 0.75 (s, 3H), 1.18 (m, 2H), 1.32 (m, 2H), 1.49 (m, 1H), 1.57 (m, 1H), 1.82 (s, 3H), 2.27 (m, 2H), 2.47 (m, 2H), 3.05 (s, 2H), 3.16 (m, 2H), 3.28 (m, 2H), 3.47 (m, 1H), 3.70 (m, 1H), 3.82 (s, 1H), 3.98 (m, 1H), 4.10 (m, 2H), 4.41 (s, 1H), 4.72 (m, 2H), 5.99 (d, 1H), 8.23 (s, 1H), 8.31 (s, 1H); ^{13}C NMR (100 MHz, D_2O) δ 179.82, 177.37, 177.01, 176.58, 175.02, 152.44, 151.11, 147.44, 145.07, 121.11, 90.13, 85.88, 77.06, 76.84, 76.47, 74.85, 71.83, 67.82, 56.31, 49.29, 42.02, 40.92, 38.13, 37.99, 37.44, 33.83, 33.25, 30.51, 25.12, 24.32, 23.47, 21.01.

3′-Dephospho-Lys-CoA (5). It was synthesized following the same procedure as for **1** but employed 3′-dephospho-CoASH in place of CoASH. HRMS ($M+H^+$) calcd for $C_{31}H_{53}N_{10}O_{16}P_2S_1$ 915.2837, found *m/z* 915.2810. ^{1}H NMR (400 MHz CD_3OD) δ 0.89 (s, 3H), 1.05 (s, 3H), 1.40 (m, 2H), 1.53 (m, 2H), 1.66 (m, 1H), 1.79 (m, 1H), 1.99 (s, 3H), 2.43 (m, 2H), 2.68 (m, 2H), 3.20 (m, 4H), 3.37 (m, 2H), 3.47 (m, 2H), 3.79 (m, 1H), 3.98 (s, 1H), 4.09 (d, 1H), 4.30 (m, 3H), 4.38 (m, 1H), 4.45 (m, 1H), 4.64 (m, 1H), 6.10 (d, 1H), 8.38 (s, 1H), 8.67 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 176.06, 174.26, 172.75, 172.27, 171.34, 158.48, 150.36, 144.48, 142.61, 118.76, 88.61, 84.38, 75.23, 74.38, 74.01, 73.14, 70.59, 65.96, 53.34, 39.20, 38.50, 35.33, 35.24, 34.72, 31.67, 31.55, 28.78, 23.06, 21.35, 20.63, 18.62.

Lys-phosphopantetheine bis[(pivaloyloxy)methyl)] ester (4). Method 1. Two equivalents of tributyltin methoxide was added to **2** dissolved in a minimal volume of methanol and the mixture was stirred for 30 min at room temperature. The mixture was concentrated in vacuo, dissolved in a similar volume of acetonitrile, and 2 equiv of tetrabutylammonium bromide and 10 equiv of iodomethyl pivaloate were added, and the mixture was stirred for 3 h at 40 °C. The mixture was concentrated in vacuo, dissolved in 50% methanol/water and purified by reversed phase HPLC using a water-acetonitrile gradient without the addition of TFA. The yield of purified **4** was ca. 7.5%.

Method 2. Compound **3** was reacted with 2 equiv of bis[(pivaloyloxy)methyl] hydrogen phosphate and 2 equiv of DIC in a minimal volume of DMF at room temperature overnight. Following the addition of 2 equiv more DIC, stirring was continued for another 2 h and then the precipitate was removed by filtration and the product purified by reversed-phase HPLC in a water/acetonitrile gradient without the addition of TFA. Reaction yield of purified **4** was ca. 5%. HRMS ($M+H^+$) calcd for $C_{33}H_{61}N_5O_{14}P_1S_1$ 814.3673, found *m/z* 814.3651. ^{1}H NMR (400 MHz, C_2D_5OD) δ 0.95 (s, 3H), 1.01 (s, 1H), 1.22 (s, 18H), 1.39 (m, 2H), 1.54 (m, 2H), 1.66 (m, 1H), 1.79 (m 1H), 1.98 (s, 3H), 2.43 (m, 2H), 2.69 (m, 2H), 3.21 (m, 4H), 3.38 (m, 2H), 3.48 (m, 2H), 4.32 (m, 1H), 5.64 (s, 2H), 5.67 (s, 2H), 7.91 (m, 1H), 8.06 (m, 1H), 8.22 (m, 1H), 8.30 (m, 1H); ^{31}P NMR (160 MHz, C_2D_5OD) −3.68 (s).

Methyl-SCoA (6). Gasous methyl bromide was bubbled through a solution of CoASH dilithium salt in 0.5 M triethylammonium bicarbonate buffer, pH 8.1, for 30 min. The reaction mixture was stirred for another 1.5 h, flash-frozen and lyophilized. Compound **6** and *S*-dimethyl-coenzyme A formed during the reaction were easily separated by reversed-phase HPLC in water/acetonitrile system containing 0.05% TFA and the known[19] compound **6** was obtained in high purity. ESI-MS ($M+H^+$) calcd for $C_{22}H_{38}N_7O_{16}P_3S$ 781.1, found *m/z* 782.0. ^{1}H NMR (400 MHz, D_2O) δ 0.65 (s, 3H, NOE coupling with s at 0.78 and s at 3.86), 0.78 (s, 3H), 1.91 (s, 3H, NOE coupling with m at 2.45, m at 3.20, and m at 3.31), 2.31 (m, 2H, NOE coupling with m at 3.31), 2.45 (m, 2H, NOE coupling with m at 3.20), 3.20 (m, 2H), 3.31 (m, 2H), 3.45 (d, 1H), 3.71 (d, 1H), 3.86 (s, 1H), 4.10 (m, H), 4.44 (s, 1H), 4.73 (m, 2H), 6.06 (d, 1H), 8.28 (s, 1H), 8.51 (s, 1H).

3′-Dephospho-Me-SCoA (7). This compound was synthesized and purified similarly to **6** except that 3′-dephospho-CoASH was used in place of CoASH. FABHRMS ($M+H^+$) calcd for $C_{22}H_{38}N_7O_{13}P_2S_1$ 702.1724, found *m/z* 702.1744. ^{1}H NMR (400 MHz, D_2O) δ 0.67 (s, 3H), 0.80 (s, 3H), 1.93 (s, 3H), 2.32 (m, 2H), 2.47 (m, 2H), 3.21 (m, 2H), 3.33 (m, 2H), 3.46 (d, 1H), 3.72 (d, 1H), 3.88 (s, 1H), 4.10 (m, 2H), 4.26 (m, 1H), 4.40 (m, 1H), 6.03 (d, 1H), 8.29 (s, 1H), 8.52 (s, 1H).

PPAT/DPCK assay

A modified procedure adopted from Daugherty et al. was used.[11] The reaction mixture contained 50 mM Tris (pH 8.0), 10 mM $MgCl_2$, 20 mM KCl, 1 mM dithiothreitol, 50 μg/mL bovine serum albumine, 5 mM ATP, 0.5 mM of the tested compound and 2 nM to 2 μM purified recombinant PPAT/DPCK enzyme. Reaction mixtures were incubated at 37 °C and timed aliquots (75 μL) samples were removed and quenched by the addition of 6 μL 0.5 M EDTA. The quenched solutions were passed through Microcon filters (10 kDa-MW cutoff, Millipore Corp.) and analyzed by reversed-phase HPLC using a gradient of 50 mM KH2PO4 (pH 4.5) in water and methanol with UV detection at 260 and/or 214 nm. Assignment of the HPLC peaks were based on co-injected standards as well as mass spec identification of each isolated compound from peak collection. Conversion rates were determined by comparison of relative peak areas.

Histone acetyltransferase p300 inhibition assay

The procedure was adapted from previously described methods.[5b] Briefly, the concentrations of [^{14}C]acetyl-coenzyme A (Amersham Pharmacia) and

peptide substrate (N-terminal 20-amino acid fragment of histone H_4) were fixed at 20 and 50 μM, respectively; buffer conditions included 50 mM Tris–HCl, pH 8, 1 mM dithiothreitol, 0.1 mM EDTA, and 50 μg/mL acetylated bovine serum albumin. Reactions employed purified recombinant human p300HAT domain enzyme (residues 1284–1673)[18] at a concentration of 5 nM. Assays were carried out in 0.5-mL plastic tubes at 30 °C, and the reaction volumes were 30 μL. Reactions were initiated with [^{14}C]acetyl-coenzyme A after allowing the other components to equilibrate at 30 °C for 10 min, and quenched after 5–10 min with 6 μL 6×Tris–Tricine gel loading buffer as described previously.[5b] Mixtures were run out on 16% SDS Tris–Tricine polyacrylamide gels, dried, and radioactivity quantified by phosphorimage analysis (Molecular Dynamics) by comparison to known quantities of ^{14}C-labeled bovine serum albumin standard (Amersham Pharmacia). Readings for background reactions carried out in the absence of p300 were subtracted from the total signal to net that used for calculation of enzyme rates. All assays were performed at least twice and duplicates agreed within 20%. For inhibition assays, the specified amount of inhibitors in all cases were at least 5-fold greater than the enzyme concentration employed.

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