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Bisubstrate analogue structure–activity relationships for p300 histone acetyltransferase inhibitors

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Abstract—p300 and CBP are important histone acetyltransferases (HATs) that regulate gene expression and may be anti-cancer drug targets. Based on a previous lead compound, Lys-CoA, we have used solid phase synthesis to generate a series of 11 new analogues and evaluated these compounds as HAT inhibitors. Increased spacing between the CoA moiety and the lysyl moiety generally decreases inhibitory potency. We have found two substituted derivatives that show about 4-fold increased potency compared to the parent compound Lys-CoA. These structure–activity studies allow for a greater understanding of the optimal requirements for potent inhibition of HAT enzymes and pave the way for a novel class of anti-cancer therapeutics.

1. Introduction

Histone acetyltransferases (HATs) catalyze acetyl transfer from acetyl-CoA to the ε-amino group of lysine side chains within histones and other proteins (Fig. 1). Along with other post-translational modifications, they have been shown to regulate gene expression in a variety of different physiological contexts.[1] While a number of different HATs have been identified, the highly related transcriptional co-activators p300 and CBP (p300/CBP) have been shown to be major regulators of gene expression via their HAT function.[2] Since these proteins possess a variety of modular domains that mediate protein–protein interaction, the role of the HAT activity of p300/CBP in gene regulation can be difficult to assign. Selective p300/CBP HAT inhibitors can be especially useful in this regard. Moreover, p300/CBP HAT activity appears to be enhanced in certain cancers and thus selective p300/CBP HAT inhibitors may have utility as lead therapeutic agents.[3–5]

Recently, several peptide-CoA conjugate bisubstrate analogues were disclosed and shown to be selective inhibitors of PCAF and p300.[6–8] Somewhat surprisingly, the very simple structure Lys-CoA (**1**, *N*-acetyllysinamide-CoA, Fig. 2) was found to be a potent and selective p300/CBP inhibitor. However, the more complex peptide CoA conjugates such as H3-CoA-7 (Fig. 2) showed reduced potency against p300/CBP. Part of the explanation for this finding may be related to the ping-pong kinetic mechanism for p300/CBP,[8] which argues against the presence of a ternary complex. Thus, the extra amino acids of H3-CoA-7 may undergo repulsive interactions with the enzyme active site but there may be sufficient binding space for a lysyl moiety. However, relatively little is known about the structural basis for inhibition. Sub-structures of the CoA moiety of Lys-CoA were recently generated and these studies revealed that the intact CoA moiety is critical.[9] Even deletion of the 3′-phosphate led to greatly decreased (40-fold) inhibition.[9] However, no systematic study of the lysyl or linker moieties of Lys-CoA has been reported. Here we describe the synthesis of a series of Lys-CoA analogues in which the lysyl and linker moieties have been varied and describe the effects of these substitutions on p300 HAT inhibition.

2. Results

2.1. Synthesis of Lys-CoA analogues

The general strategy for production of Lys-CoA analogues **2**–**11** (Fig. 2) involved solid phase synthesis starting with the dually protected lysine residue attached

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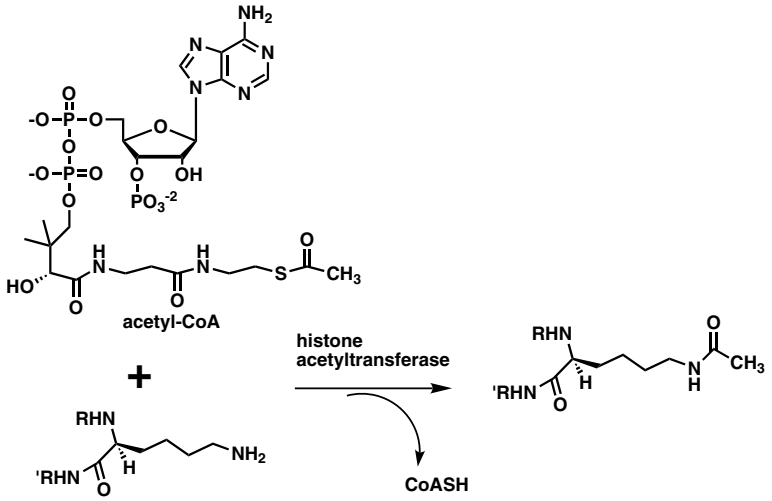

Figure 1. Histone acetyltransferase reaction.

to Rink amide resin (Fig. 3A). Removal of the Fmoc group with piperidine was followed by coupling with a series of carboxylic acids. These intermediates were then reacted with hydrazine to deblock the ε-amino group and then the amines were reacted with excess bromocarboxylic acids. The bromocarboxamide conjugates were cleaved from the resin and then purified by reversed phase HPLC. Reaction of the bromocarboxamides **2a**–**11a** with CoASH led to the target compounds. In this way, compounds **2**–**11** were readily prepared. Note that compound **2** is likely to be a 1:1 epimeric mixture based on previous studies on reactions between a primary amine and bromoisopropionic acid. However, we have been unable to separate these epimers by chromatographic methods as was the case in previous studies on related compounds.[10,11]

To produce compound **12**, we considered using an approach recently described for a related compound involving 1-bromo-3-chloropropane.[12] However, this multi-step procedure was relatively low yielding and thus we pursued a reductive amination strategy (Fig. 3B). Thus, CoASH was treated with 1,1-dimethoxy-3-bromopropane followed by acidolysis of **15** to generate the CoAS-aldehyde that was reacted with lysine analogue and then reduced with sodium cyanoborohydride, affording the desired compound **12**.

2.2. p300 HAT inhibition studies

The CoA conjugates were assayed against the catalytic domain of p300 in a system containing acetyl-CoA (250 μM), and a peptide substrate (H4–12 derived from histone H4, 250 μM) using a spectrophotometric assay in which CoASH formation was monitored by reaction with DTNB. This assay has been validated by comparison with a direct, radioactive assay.[13] The advantages of this spectrophotometric assay include its simplicity, avoidance of radioactive substrates, and ease of use with high substrate concentrations. Because previous studies showed that Lys-CoA has a slow dissociation rate from p300 ($t_{1/2}$ 4.5 min),[8] we carried out the inhibition studies over a relatively long time (40 min) to attempt to achieve an inhibitory effect under steady-state kinetic conditions. Thus, the inhibition here by Lys-CoA ($IC_{50} = 3.2\,\mu M$) is about 8-fold weaker than that reported earlier[6] where a lower concentration of acetyl-CoA was used, so that all compounds need to be referenced to Lys-CoA to compare these studies. Furthermore, we have recently discovered that the p300 HAT domain is activated by autoacetylation at several sites[13] and this heterogeneity limits our ability to carry out detailed kinetic measurements and measure meaningful K_i values for the synthesized CoA conjugates. However, it is highly likely, based on previous studies,[8,9] that the CoA moiety of the inhibitors **1**–**12** has an overlapping binding site with the acetyl-CoA substrate.

As can be seen in Table 1, most compounds showed IC_{50} values that were equivalent to, or worse than, Lys-CoA. In particular, all efforts to modify the linker with phenyl substitution or a longer alkyl chain reduced inhibitory potency. Likewise, deletion of the carbonyl group from the linker led to reduced inhibitory potency. The two compounds that showed enhanced potency compared to Lys-CoA were the benzoyl analogue **6** and the methyl-substituted analogue **2** which both had IC_{50} of 700–800 nM, approximately 4-fold lower than Lys-CoA. In contrast, the doubly modified compound **7** was similarly potent ($IC_{50} = 2.5\,\mu M$) to Lys-CoA, indicating a cancellation of the two affinity-enhancing effects of each substitution.

3. Discussion

The HAT activity of p300 appears to play a critical role in the regulation of gene expression and the selective inhibitor Lys-CoA (**1**) has been a useful reagent to analyze the contributions of p300 catalytic activity in a variety of pathways.[14–22] Despite its utility, the structural

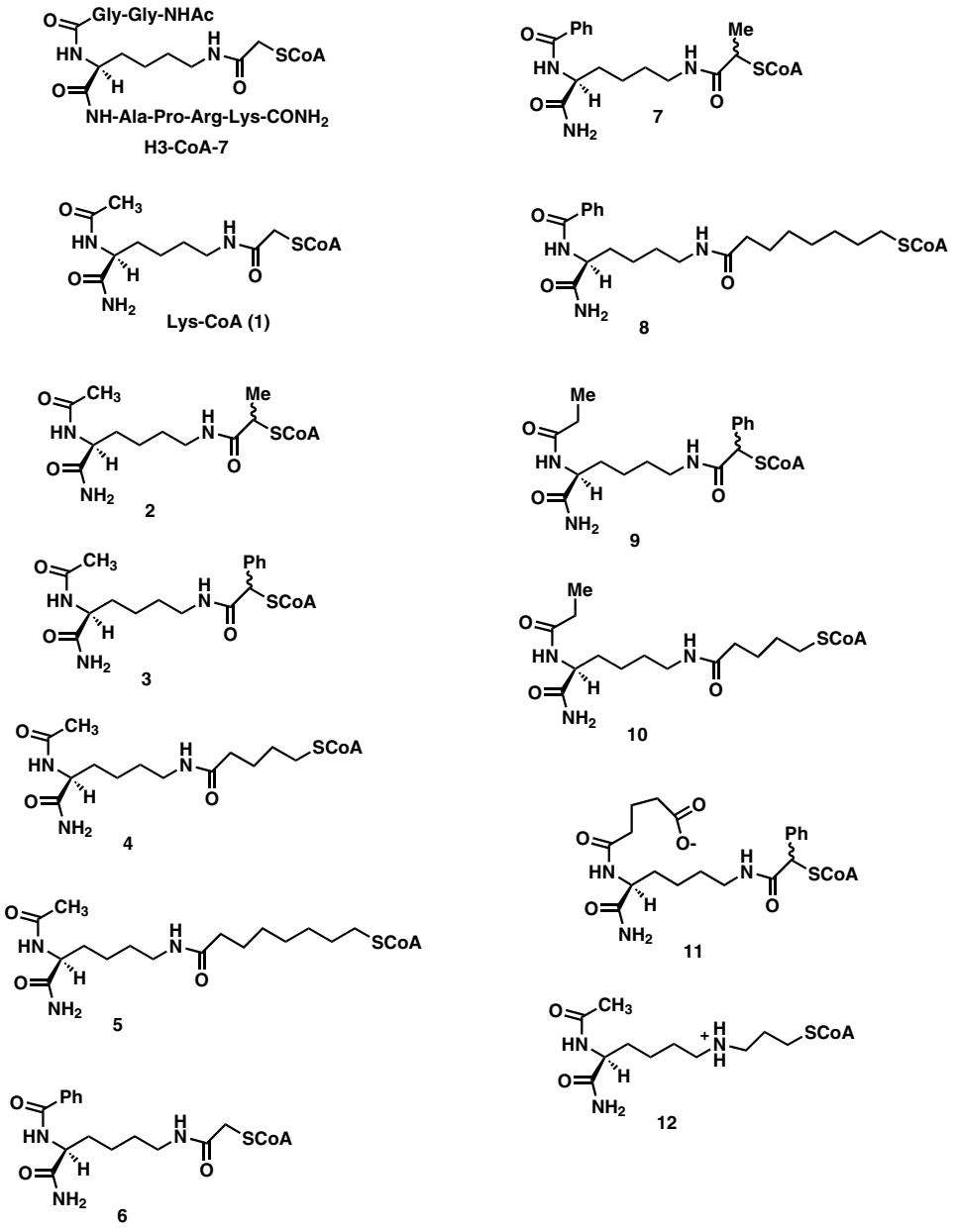

Figure 2. Lys-CoA analogues explored in this work.

basis of its potency and selectivity is not understood. The peculiar notion that a 'partial' bisubstrate analogue can potently inhibit an enzyme with a ping-pong mechanism has not been satisfactorily explained. In the absence of high resolution structural information, synthetic analogues of Lys-CoA can provide indirect information about active site interactions. By adopting the modular, solid-phase synthetic approach to analogue generation reported here, it is clear that a diverse library can readily be generated.

Based on the analogues studied here, the optimal spacing between CoA and the lysyl moiety for p300 inhibition appears to involve a short, 2-carbon linker. Although the 3- and 4-carbon amide-linker containing compounds were not examined, the fact that compound **2** is more potent than compound **1** argues that conformational restriction may be important for potency. Since compounds with longer linkers would tend to increase conformational flexibility, it would be expected that such compounds would show reduced potency as observed with **4** and **5**.

It is quite interesting that methyl substitution in compound **2** (isopropionyl linker) showed enhanced inhibitory potency compared to the parent agent **1**. This linker can enhance rotational restriction and may allow for the stabilization of some energetically favorable conformations for binding to p300. Since this compound is almost certainly a 1:1 epimeric mixture (inseparable by HPLC), it is likely that one of the two isomers actually has an 8-fold enhanced affinity compared to Lys-CoA (**1**). The finding that **2** shows enhanced potency compared to **1** is similar to the behavior of two other acetyltransferases.[10,11] The similarity may be coincidental since these other enzymes show sequential rather than ping-pong

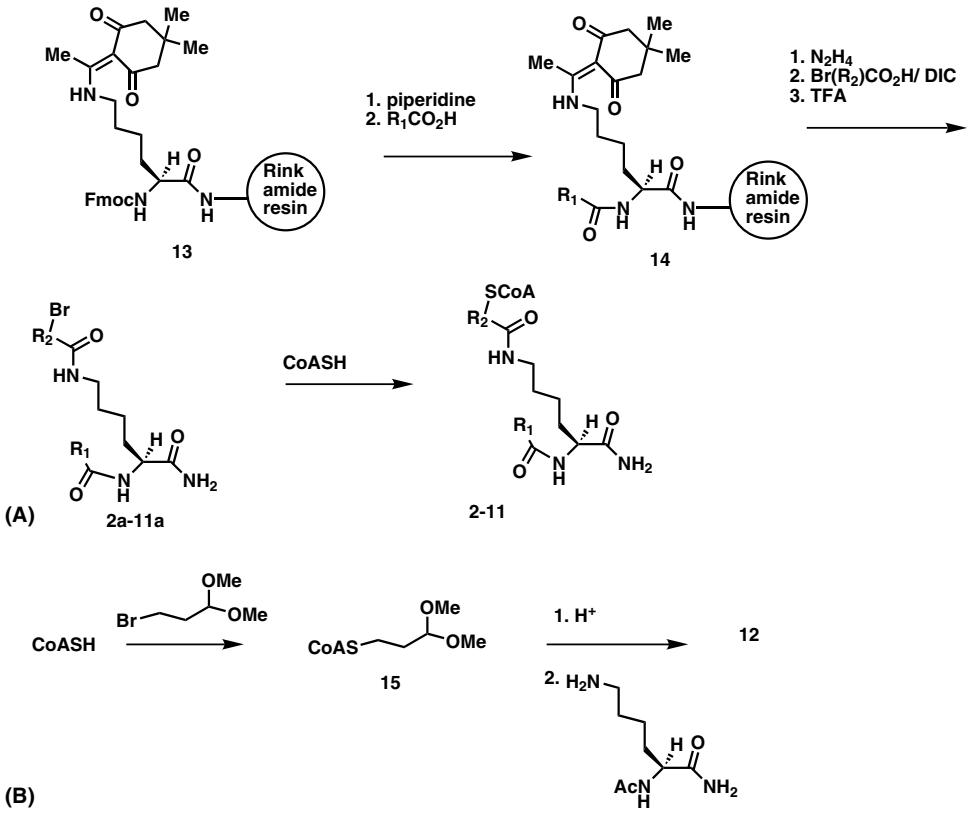

Figure 3. Synthetic schemes to compounds **2**–**11** (A) and **12** (B).

Table 1. Inhibitory values for compounds **1**–**12** against the p300 HAT domain

Compound	IC_{50} (μM)
Lys-CoA **(1)**	3.2 ± 0.4
2	0.8 ± 0.2
3	>6.4
4	>6.4
5	>6.4
6	0.7 ± 0.2
7	2.5 ± 0.5
8	5 ± 1
9	>6.4
10	>6.4
11	>6.4
12	>6.4

These were measured as described in Section 4 in the presence of 250 μM acetyl-CoA. Values are shown ± standard errors.

kinetic mechanisms or it may be a highly conserved feature of acetyltransferases that are otherwise rather divergent.[23,24]

The enhanced inhibitory potency of compound **6** compared to **1** suggests that a hydrophobic pocket can be filled by the rather bulky phenyl substituent. It was significant however that compound **7**, rather than showing enhanced potency compared to **2** or **6**, was actually less potent than either one. That these structural features apparently influence each other in binding to the enzyme suggests that further optimization may best be done in a combinatorial fashion. The solid phase synthetic approach outlined here will be amenable to such an approach.

The lack of potent inhibition by **12** is also a significant negative result. The linker amine in **12** will likely be positively charged at neutral pH, mimicking the free ε-amino group of the substrate and potentially generating such an electrostatic interaction. However, such an electrostatic interaction if formed does not appear to enhance overall affinity. This behavior is reminiscent of that seen with the GNAT superfamily member serotonin N-acetyltransferase,[12] again consistent with a weak conservation between these enzymes.[23,24]

Not investigated in this study was the α-carboxylic functionality of Lys-CoA. While somewhat more challenging to target because of the synthetic approach used here, it will be important to address its contributions in ultimately developing the most potent inhibitor. Moreover, it is hoped that it will be possible to obtain an X-ray structure of p300 HAT domain in complex with **1**, **2**, or **6** to learn more about how the best selectivity may be achieved. Enhancing cell permeability of these analogues remains an important challenge, and in the short run, conjugation of these compounds to cell penetrating motifs[25] may be the best strategy.

In summary, this study represents the first SAR elaboration on the p300-selective inhibitor Lys-CoA. The range of compounds developed will allow for a more

precise pharmacologic test of the role of p300 in chromatin remodeling and gene regulation. Findings reported here also pave the way toward a complete understanding of the structural basis for inhibition of p300 by Lys-CoA. Moreover, it is hoped that these studies may lead to a novel class of HAT inhibitors as therapeutic agents.

4. Experimental

4.1. General

The chemicals used in the synthetic methods and HAT assays were obtained as reagent grade quality from Novabiochem, Aldrich, and Sigma, and used without further purification. NMR spectra were obtained with a Varian Mercury 400 spectrometer, and chemical shift values (δ) were expressed as parts per million (ppm) relative to the respective deuterated solvent. Electrospray ionization mass (ESI-MS) spectra were obtained with API 150EX (PE Biosystems). High resolution mass (HRMS) spectra were recorded on a MALDI DE-STR machine at the mass spectrometry facility of the University of California, Riverside. HPLCs (analytical, semi-preparative, and preparative) were carried out on reversed phase Dynamax C-18 columns (Varian). Spectrophotometric analysis was carried out on a Beckman DU 640 instrument.

4.2. Preparation of bromo compound precursors, 2a–11a

The Fmoc and Dde orthogonally protected Rink Amide resin (0.06–0.13 mmol) was swelled with two washes of DMF, each for 10 min. The Fmoc protecting group was removed by three treatments with 20% piperidine/DMF, 5 min each. The resin was then washed five times with DMF and then 5 equiv of BOP, HOBT, and orthogonally protected Fmoc Dde-lysine were dissolved in 0.4 M *N*-methylmorpholine in DMF and mixed with the resin for 2 h. The resin was then washed eight times with DMF and the Fmoc protecting group was removed by three treatments with 20% piperidine/DMF, 5 min each. The resin was then washed in the following sequence: seven times with DMF, three times with dichloromethane, three times with methanol, and three times with dichloromethane. The resin was then vacuum dried for 2 h and partitioned into four portions. Each portion of resin was swelled with two washes of DMF for 10 min each. The resin portions were reacted with excess benzoic anhydride, glutaric anhydride, acetic anhydride, and propionic acid/DIC, respectively, in the presence of 0.9 M *N*-methylmorpholine. The Dde protecting group was removed from each portion by treatment with 3%hydrazine/DMF for 2 h. The four resin portions were then washed and dried as described above. The four resins were then vacuum dried for 2 h to complete dryness. Each was treated with one or more of the following acids (present in excess): bromooctanoic acid, 2-bromopropionic acid, bromoacetic, bromo-valeric acid, and α-bromophenylacetic acid in the presence of DIC in DMF overnight. The 10 resins were washed and dried as described above. The 10 resins were then mixed with 95% TFA/H_2O for 2 h. The 10 soluble phases were precipitated with diethyl ether and washed twice with diethyl ether to remove excess TFA. The 10 compounds **2a–11a** were lyophilized to dryness and purified using RP HPLC using a mobile phase of H_2O/0.05% TFA and acetonitrile/0.05% TFA. The calculated yields based on the starting lysyl derivative were 5–10%. Spectroscopic data are shown below.

4.2.1. Compound 2a. ^{1}H NMR (D_2O) δ 4.34 (q, $J = 4.0$ Hz, 1H), 4.03 (dd, $J = 8.0$, 4.0 Hz, 1H), 3.05 (m, 2H), 1.86 (s, 3H), 1.57 (d, $J = 8.0$ Hz, 3H), 1.71–1.48 (m, 2H), 1.46–1.30 (m, 2H), 1.31–1.13 (m, 2H); ^{13}C NMR (D_2O) δ 179.93, 177.10, 175.52, 56.26, 46.02, 41.92, 33.25, 30.28, 24.96, 24.35, 23.90; ESI MS calcd for $C_{11}H_{21}O_3N_3Br$ ($[M+H]^+$), found m/z 322.

4.2.2. Compound 3a. ^{1}H NMR (CD_3OD) δ 7.55 (m, 2H), 7.34 (m, 3H), 5.47 (s, 1H), 4.26 (dd, $J = 8.0$, 4.0 Hz, 1H), 3.21 (m, 2H), 1.97 (s, 3H), 1.85–1.73(m, 1H), 1.69–1.47 (m, 3H), 1.46–1.29 (m, 2H); ^{13}C NMR (CD_3OD) δ 176.00, 172.19, 169.02, 137.57, 128.80, 128.51, 128.40, 53.20, 49.17, 39.52, 31.51, 28.61, 22.98, 21.31; ESI MS calcd for $C_{16}H_{23}O_3N_3Br$ ($[M+H]^+$), found m/z 384.

4.2.3. Compound 4a. ^{1}H NMR (CD_3OD) δ 4.28 (dd, $J = 8.0$, 4.0 Hz, 1H), 3.45 (t, $J = 6$ Hz, 2H), 3.17 (t, $J = 6$ Hz, 2H), 2.20 (t, $J = 8.0$ Hz, 2H), 1.99 (s, 3H), 1.91–1.59 (m, 6H), 1.58–1.31 (m, 4H); ^{13}C NMR (CD_3OD) δ 179.96, 178.38, 176.12, 57.20, 42.80, 38.81, 36.52, 36.11, 35.51, 32.80, 28.34, 27.05, 25.24; ESI MS calcd for $C_{13}H_{25}O_3N_3Br$ ($[M+H]^+$), found m/z 350.

4.2.4. Compound 5a. ^{1}H NMR (CD_3OD) δ 4.28 (dd, $J = 8.0$, 4.0 Hz, 1H), 3.45 (t, $J = 6$ Hz, 2H), 3.17 (t, $J = 6$ Hz, 2H), 2.17 (t, $J = 8.0$ Hz, 2H), 1.99 (s, 3H), 1.89–1.75 (m, 3H), 1.70–1.27 (m, 13H); ^{13}C NMR (CD_3OD) δ 174.61, 172.58, 169.83, 52.92, 38.91, 36.07, 35.89, 32.91, 32.38, 29.64, 29.19, 28.51, 28.12, 25.88, 23.56, 23.22; ESI MS calcd for $C_{16}H_{31}O_3N_3Br$ ($[M+H]^+$), found m/z 392.

4.2.5. Compound 6a. ^{1}H NMR (CD_3OD) δ 7.90–7.85 (m, 2H), 7.58–7.43 (m, 3H), 4.54 (dd, $J = 8.0$, 4.0 Hz, 1H), 4.40 (q, $J = 8.0$ Hz, 1H), 3.29–3.13 (m, 2H), 1.98–1.77 (m, 2H), 1.63–1.39 (m, 4H), 1.71 (d, $J = 4.0$ Hz, 3H); ^{13}C NMR (CD_3OD) δ 179.98, 175.23, 173.05, 138.01, 135.63, 132.30, 131.33, 57.73, 46.69, 43.03, 35.53, 32.58, 27.11, 25.05; ESI MS calcd for $C_{15}H_{21}O_3N_3Br$ ($[M+H]^+$), found m/z 370.

4.2.6. Compound 7a. ^{1}H NMR (CD_3OD) δ 7.73 (d, $J = 8.0$ Hz, 2H), 7.37 (m, 3H), 4.41 (dd, $J = 8.0$, 4.0 Hz, 1H), 3.64 (s, 2H), 3.08 (t, $J = 8.0$ Hz, 2H), 1.63 (m, 1H), 1.53 (m, 1H), 1.37 (m, 2H), 1.23 (m, 2H); ^{13}C NMR

(CD_3OD) δ 174.60, 167.54, 166.80, 132.51, 130.12, 126.80, 125.81, 52.21, 37.80, 30.00, 27.05, 26.02, 21.61; ESI MS calcd for $C_{16}H_{23}O_3N_3Br$ ($[M+H]^+$), found m/z 384.

4.2.7. Compound 8a. ^{1}H NMR (CD_3OD) δ 7.89 (m, 2H), 7.87 (m, 1H), 7.59 (t, $J = 8.0$ Hz, 2H), 4.54 (dd, $J = 8.0$, 4.0 Hz, 1H), 3.42 (t, $J = 8.0$ Hz, 2H), 3.18 (t, $J = 8.0$ Hz, 2H), 2.14 (t, $J = 6.0$ Hz, 2H), 2.00–1.76 (m, 3H), 1.63–1.18 (m, 13H); ^{13}C NMR (CD_3OD) δ 177.25, 176.29, 170.28, 135.27, 132.91, 129.57, 128.58, 54.99, 39.95, 37.07, 35.37, 33.93, 32.78, 30.08, 29.47, 29.02, 26.95, 24.45; ESI MS calcd for $C_{21}H_{33}O_3N_3Br$ ($[M+H]^+$), found m/z 454.

4.2.8. Compound 9a. ^{1}H NMR (CD_3OD) δ 7,55 (m, 2H), 7.34 (m, 3H), 5.47 (s, 1H), 4.26 (dd, $J = 8.0$, 4.0 Hz, 1H), 3.21 (m, 2H), 2.24 (qd, $J = 4.0$, 4.0 Hz, 2H), 1.85–1.73 (m, 1H), 1.70–1.48 (m, 3H), 1.44–1.27 (m, 2H), 1.11 (t, $J = 8.0$ Hz, 3H); ^{13}C NMR (CD_3OD) δ 176.07, 175.89, 169.04, 137.60, 128.81, 128.52, 128.41, 53.06, 49.17, 39.55, 39.48, 31.57, 28.70, 28.61, 23, 9.11; ESI MS calcd for $C_{17}H_{25}O_3N_3Br$ ($[M+H]^+$), found m/z 398.

4.2.9. Compound 10a. ^{1}H NMR (CD_3OD) δ 4.04 (dd, $J = 6$ Hz, 1H), 3.32 (t, $J = 8$ Hz, 2H), 3.01 (t, $J = 8$ Hz, 2H), 2.17–2.04 (m, 5H), 1.71–1.47 (m, 6H), 1.38–1.15 (m, 4H), 0.93 (t, $J = 8$ Hz, 3H); ^{13}C NMR (CD_3OD) δ 178.38, 177.46, 176.12, 53.62, 39.26, 35.00, 34.10, 31.40, 30.69, 28.88, 27.93, 24.20, 22.60, 9.49; ESI MS calcd for $C_{14}H_{27}O_3N_3Br$ ($[M+H]^+$), found m/z 364.

4.2.10. Compound 11a. ^{1}H NMR (DMSO-d_6) δ 8.36 (br s, 1H), 7.77 (d, $J = 8.0$ Hz, 1H), 7.48 (d, $J = 8.0$ Hz, 2H), 7.33–7.22 (m, 3H), 6.88 (br s, 2H), 5.50 (s, 1H), 4.06 (m, 1H), 2.98 (m, 2H), 2.13 (t, $J = 8.0$ Hz, 2H), 2.08 (t, $J = 8.0$ Hz, 2H), 1.69–1.08 (m, 8H); ^{13}C NMR (DMSO-d_6) δ 179.71, 179.39, 177.10, 172.08, 142.93, 134.17, 133.96, 133.92, 60.34, 57.52, 54.91, 39.70, 38.53, 37.09, 33.85, 28.42, 26.13; ESI MS calcd for $C_{19}H_{26}O_5N_3Br$ ($[M+H]^+$), found m/z 455.

4.3. Preparation of CoA conjugates (2–11)

Bromo analogues **2a**–**11a** (5–10 mg) were treated with 2 equiv of CoASH in 1 mL of 0.5 M trimethylammonium bicarbonate buffer, pH 8.0 at room temperature. Bromo analogues containing the bromine atom alpha to the carbonyl were allowed to react for 24 h whereas the others were left to react for 48 h. The reaction mixtures were concentrated in vacuo and the desired compounds were purified using reversed phase HPLC and a water/acetonitrile/0.05% TFA gradient elution. The desired compounds **2**–**11** obtained in 70–80% yield from the bromo analogues appeared >95% pure as assessed via HPLC and NMR spectra. Spectroscopic data are shown below.

4.3.1. Compound 2. ^{1}H NMR (CD_3OD) δ 8.45 (s, 1H), 8.24 (s, 1H), 6.01 (d, $J = 4$ Hz, 1H), 4.43 (m, 1H), 4.11 (m, 2H), 3.99 (dd, $J = 8.0$, 4.0 Hz, 1H), 3.83 (s, 1H), 3.71 (dd, $J = 8.0$, 4.0 Hz,1H), 3.47 (dd, $J = 8.0$, 4.0 Hz, 1H), 3.30 (t, $J = 6.0$ Hz, 2H), 3.15 (t, $J = 6.0$ Hz, 2H), 3.00 (m, 3H), 2.47 (t, $J = 6.0$ Hz, 2H), 2.28 (t, $J = 8.0$ Hz, 2H), 1.83 (s, 3H), 1.69–1.40 (m, 2H), 1.40–1.11 (m, 7H), 0.76 (s, 3H), 0.65 (s, 3H) ; ^{13}C NMR (CD_3OD) δ 177.27, 175.74, 174.83, 174.47, 174.01, 149.92, 148.56, 144.84, 142.54, 118.58, 87.57, 83.37, 74.47, 74.27, 73.92, 72.27, 65.27, 53.75, 46.74, 43.27, 39.31, 38.64, 35.58, 35.43, 30.68, 30.45, 28.01, 22.56, 21.74, 20.92, 18.43, 17.37. HRMS calcd for $C_{32}H_{52}N_{10}O_{19}Na_4P_3S$ ($[M-3H+4Na]^+$) 1097.1929; found 1097.1946.

4.3.2. Compound 3. ^{1}H NMR (CD_3OD) δ 8.48 (s, 1H), 8.22 (s, 1H), 7.19 (m, 5H), 6.01 (d, $J = 8$ Hz, 1H), 4.57 (s, 1H), 4.43 (m, 1H), 4.11 (m, 2H), 3.91 (dd, $J = 8.0$, 4.0 Hz), 3.83 (s, 1H), 3.69 (dd, $J = 8.0$, 4.0 Hz,1H), 3.45 (dd, $J = 8.0$, 4.0 Hz, 1H), 3.30 (t, $J = 6.0$ Hz, 2H), 3.14 (t, $J = 6.0$ Hz, 2H), 3.02 (t, $J = 6.0$ Hz, 2H), 2.43 (t, $J = 8.0$ Hz, 2H), 2.26 (t, $J = 8.0$ Hz, 2H), 1.82 (s, 3H), 1.60–1.24 (m, 4H), 1.23–0.98 (m, 2H), 0.78 (s, 3H), 0.65 (s, 3H) ; ^{13}C NMR (CD_3OD) δ 178.33, 177.33, 174.85, 174.03, 172.61, 149.91, 148.64, 144.81, 142.60, 136.71, 129.25, 128.66, 128.02, 118.69, 87.65, 74.53, 74.33, 73.95, 72.24, 65.24, 53.67, 39.48, 38.39, 35.59, 35.47, 31.07, 30.62, 28.81, 27.94, 22.54, 20.89, 18.47. HRMS calcd for $C_{37}H_{58}N_{10}O_{19}P_3S$ ($[M+H]^+$) 1071.2808; found 1071.2795.

4.3.3. Compound 4. ^{1}H NMR (CD_3OD) δ 8.44 (s, 1H), 8.24 (s, 1H), 6.02 (d, $J = 8$ Hz, 1H), 4.42 (m, 1H), 4.10 (m, 2H), 3.99 (dd, $J = 8.0$, 4.0 Hz, 1H), 3.82 (s, 1H), 3.70 (dd, $J = 12.0$, 4.0 Hz,1H), 3.47 (dd, $J = 8.0$, 4.0 Hz, 1H), 3.28 (t, $J = 6.0$ Hz, 2H), 3.15 (t, $J = 6.0$ Hz, 2H), 2.96 (t, $J = 6.0$ Hz, 2H), 2.44 (t, $J = 6.0$ Hz, 2H), 2.34 (t, $J = 6.0$ Hz, 2H), 2.28 (t, $J = 6.0$ Hz, 2H), 2.03 (t, $J = 6.0$ Hz, 2H), 1.83 (s, 3H), 1.67–1.08 (m, 10H), 0.76 (s, 3H), 0.65 (s, 3H) ; ^{13}C NMR (CD_3OD) δ 177.30, 176.61, 174.83, 174.46, 173.98, 149.96, 148.59, 144.83, 142.60, 118.71, 87.62, 83.32, 74.52, 74.28, 73.89, 72.32, 65.30, 53.74, 39.06, 38.73, 38.43, 35.58, 35.43, 35.33, 30.68, 30.52, 30.32, 28.18, 27.95, 24.65, 22.55, 21.74, 20.88, 18.42. HRMS calcd for $C_{34}H_{60}N_{10}O_{19}P_3S$ ($[M+H]^+$) 1037.2964; found 1037.3022.

4.3.4. Compound 5. ^{1}H NMR (CD_3OD) δ 8.46 (s, 1H), 8.25 (s, 1H), 6.02 (d, $J = 8$ Hz, 1H), 4.42 (m, 1H), 4.10 (m, 2H), 3.99 (dd, $J = 8.0$, 4.0 Hz, 1H), 3.83 (s, 1H), 3.71 (dd, $J = 8.0$, 4.0 Hz,1H), 3.47 (dd, $J = 8.0$, 4.0 Hz, 1H), 3.29 (t, $J = 8.0$ Hz, 2H), 3.15 (t, $J = 8.0$ Hz, 2H), 2.45 (t, $J = 6.0$ Hz, 2H), 2.34 (t, $J = 6.0$ Hz, 2H), 2.28 (t, $J = 6.0$ Hz, 2H), 2.01 (t, $J = 6.0$ Hz, 2H), 1.84 (s, 3H), 1.68–1.42 (m, 4H), 1.42–1.28(m, 7H), 1.27–1.03 (m, 13H), 1.07 (t, $J = 6.0$ Hz, 2H), 0.76 (s, 3H), 0.65 (s, 3H) ; ^{13}C NMR (CD_3OD) δ 177.28, 177.17, 174.81, 174.44, 173.95, 149.94, 148.63, 144.81, 142.55, 118.72, 87.67, 83.32, 74.60, 74.28, 73.96, 72.30, 65.25, 53.78, 46.77, 39.05, 38.81, 38.50, 35.80, 35.61, 35.48, 31.05, 30.76,

30.38, 28.65, 27.98, 27.85, 27.73, 25.43, 22.60, 21.78, 20.95, 18.42. HRMS calcd for $C_{37}H_{62}N_{10}O_{19}Na_4P_3S$ ([M−3H+4Na]$^+$) 1167.2712; found 1167.2614.

4.3.5. Compound 6. ^{1}H NMR (CD_3OD) δ 8.42 (s, 1H), 8.20 (s, 1H), 7.60–7.50 (m, 2H), 7.43–7.24 (m, 3H), 5.99 (d, $J = 8.0$ Hz, 1H), 4.42 (m, 1H), 4.24 (dd, $J = 8.0$, 4.0 Hz, 1H), 4.11 (m, 2H), 3.82 (s, 1H), 3.70 (dd, $J = 8.0$, 4.0 Hz,1H), 3.46 (dd, $J = 8.0$, 4.0 Hz, 1H), 3.26 (t, $J = 6.0$ Hz, 2H), 3.06 (t, $J = 8.0$ Hz, 2H), 3.02 (t, $J = 8.0$ Hz, 2H), 2.99 (s, 2H), 2.38 (t, $J = 6.0$ Hz, 2H), 2.24 (t, $J = 6.0$ Hz, 2H), 1.80–1.60 (m, 2H), 1.45–1.18 (m, 4H), 0.75 (s, 3H), 0.64 (s, 3H);^{13}C NMR (CD_3OD) δ 177.27, 174.79, 173.94, 172.46, 170.87, 149.80, 148.50, 144.75, 142.50, 132.81, 132.54, 128.82, 127.32, 118.60, 87.59, 81.60, 74.54, 74.25, 73.86, 72.35, 65.28, 54.20, 39.42, 38.46, 38.26, 35.54, 35.38, 34.81, 31.21, 30.69, 27.91, 22.78, 20.88, 18.43. HRMS calcd for $C_{36}H_{56}N_{10}O_{19}P_3S$ ([M+H]$^+$) 1057.2651; found 1057.2723.

4.3.6. Compound 7. ^{1}H NMR (CD_3OD) δ 8.48 (s, 1H), 8.24 (s, 1H), 7.64–7.56 (m, 2H), 7.45–7.26 (m, 3H), 6.03 (d, $J = 8.0$ Hz, 1H), 4.43 (m, 1H), 4.29 (dd, $J = 8.0$, 4.0 Hz, 1H), 4.10 (m, 2H), 3.84 (s, 1H), 3.70 (dd, $J = 8.0$, 4.0 Hz,1H), 3.45 (dd, $J = 8.0$, 4.0 Hz, 1H), 3.29 (t, $J = 6.0$ Hz, 2H), 3.14–2.96 (m, 5H), 2.39 (t, $J = 6.0$ Hz, 2H), 2.25 (t, $J = 6.0$ Hz, 2H), 1.80–1.60 (m, 2H), 1.45–1.20 (m, 4H), 1.10 (d, $J = 6.8$ Hz, 3H), 0.76 (s, 3H), 0.64 (s, 3H); ^{13}C NMR (CD_3OD) δ 175.93, 174.38, 173.46, 172.56, 169.70, 148.60, 147.20, 143.36, 141.21, 131.50, 131.20, 127.48, 125.99, 117.30, 86.23, 82.10, 72.89, 72.59, 70.80, 63.90, 52.87, 41.83, 37.91, 37.20, 34.18, 34.04, 29.35, 29.02, 28.95, 26.63, 21.45, 19.58, 17.03, 15.91. HRMS calcd for $C_{37}H_{58}N_{10}O_{19}P_3S$ ([M+H]$^+$) 1071.2808; found 1071.2897.

4.3.7. Compound 8. ^{1}H NMR (CD_3OD) δ 8.50 (s, 1H), 8.25 (s, 1H), 7.60 (m, 2H), 7.43 (m, 1H), 7.33 (m, 2H), 6.04 (d, $J = 8$ Hz, 1H), 4.43 (m, 1H), 4.28 (dd, $J = 8.0$, 4.0 Hz, 1H), 4.10 (m, 2H), 3.86 (s, 1H), 3.71 (dd, $J = 8.0$, 4.0 Hz,1H), 3.45 (dd, $J = 8.0$, 4.0 Hz, 1H), 3.30 (t, $J = 6.0$ Hz, 2H), 3.14 (t, $J = 8.0$ Hz, 2H), 3.02 (t, $J = 6.0$ Hz, 2H), 2.43 (t, $J = 8.0$ Hz, 2H), 2.29 (m, 4H), 1.97 (t, $J = 6.0$ Hz, 2H), 1.49–1.20 (m, 9H), 1.11–0.95 (m, 7H), 0.76 (s, 3H), 0.65 (s, 3H) ; ^{13}C NMR (CD_3OD) δ 177.39, 177.23, 174.87, 173.95, 170.99, 149.99, 148.62, 144.79, 142.64, 132.90, 132.62, 128.90, 127.39, 118.67, 87.60, 83.72, 83.62, 74.39, 74.24, 74.07, 72.15, 65.21, 54.26, 38.89, 38.80, 38.53, 38.45, 35.79, 35.59, 35.48, 31.01, 30.72, 30.33, 28.63, 27.94, 27.79, 25.38, 22.79, 21, 18.37. HRMS calcd for $C_{42}H_{64}N_{10}O_{19}Na_4P_3S$ ([M−H + 2Na]$^+$) 1185.3217; found 1185.3155.

4.3.8. Compound 9. ^{1}H NMR (CD_3OD) δ 8.46 (s, 1H), 8.22 (s, 1H), 7.19 (m, 5H), 6.01 (d, $J = 8$ Hz, 1H), 4.52 (s, 1H), 4.43 (m, 1H), 4.11 (m, 2H), 3.91 (m, 1H), 3.82 (s, 1H), 3.71 (dd, $J = 8.0$, 4.0 Hz, 1H), 3.45 (dd, $J = 8.0$, 4.0 Hz, 1H), 3.30 (t, $J = 6.0$ Hz, 2H), 3.14 (t, $J = 6.0$ Hz, 2H), 3.02 (m, 2H), 2.43 (t, $J = 8.0$ Hz, 2H), 2.29 (t, $J = 8.0$ Hz, 2H), 2.07 (q, $J = 8.0$ Hz, 2H), 1.18–0.98 (m, 2H), 1.37–1.23 (m, 2H), 0.78 (s, 3H), 0.65 (s, 3H) ; ^{13}C NMR (CD_3OD) δ 178.33, 177.33, 174.85, 174.03, 172.61, 149.91, 148.64, 144.81, 142.60, 136.71, 129.25, 128.66, 128.02, 118.69, 87.65, 74.53, 74.40, 74.33, 73.95, 72.24, 65.24, 53.67, 53.57, 39.48, 38.39, 35.59, 35.47, 31.07, 30.62, 28.81, 27.94, 22.54, 20.89, 18.47, 9.47. HRMS calcd for $C_{38}H_{60}N_{10}O_{19}P_3S$ ([M+H]$^+$) 1085.2964; found 1085.3056.

4.3.9. Compound 10. ^{1}H NMR (CD_3OD) δ 8.53 (s, 1H), 8.28 (s, 1H), 6.07 (d, $J = 4$ Hz, 1H), 4.44 (m, 1H), 4.10 (m, 2H), 4.03 (dd, $J = 8.0$, 4.0 Hz, 1H), 3.87 (s, 1H), 3.70 (dd, $J = 8.0$, 4.0 Hz,1H), 3.43 (dd, $J = 8.0$, 4.0 Hz, 1H), 3.31 (t, $J = 8.0$ Hz, 2H), 3.18 (t, $J = 6.0$ Hz, 2H), 2.48 (t, $J = 6.0$ Hz, 2H), 2.39 (t, $J = 8.0$ Hz, 2H), 2.30 (t, $J = 6.0$ Hz, 2H), 2.12 (q, $J = 8.0$ Hz, 2H), 2.06 (t, $J = 6.0$ Hz, 3H), 1.68–1.13 (m, 10H), 0.92 (t, $J = 8.0$ Hz, 3H), 0.78 (s, 3H), 0.65 (s, 3H) ; ^{13}C NMR (CD_3OD) δ 178.40, 177.45, 176.67, 174.90, 174.04, 150.10, 148.70, 144.87, 142.66, 119.00, 87.61, 74.33, 74.28, 72.05, 65.25, 53.69, 48.96, 39.06, 38.79, 38.53, 35.58, 35.49, 35.39, 30.70, 30.54, 30.34, 28.88, 28.20, 27.99, 24.68, 22.61, 21.04, 18.32, 9.51. HRMS calcd for $C_{35}H_{62}N_{10}O_{19}P_3S$ ([M+H]$^+$) 1051.3121; found 1051.3205.

4.3.10. Compound 11. ^{1}H NMR (D_2O) δ 8.51 (s, 1H), 8.26 (s, 1H), 7.23 (m, 5H), 6.06 (d, $J = 8$ Hz, 1H), 4.13 (m, 1H), 3.95 (m, 2H), 3.86 (s, 1H), 3.72 (dd, $J = 8.0$, 4.0 Hz, 1H), 3.47 (dd, $J = 8.0$, 4.0 Hz, 1H), 3.31 (t, $J = 6.0$ Hz, 2H), 3.18 (t, $J = 6.0$ Hz, 2H), 3.05 (m, 2H), 2.51 (m, 2H), 2.29 (t, $J = 6.0$ Hz, 2H), 2.22 (t, $J = 8.0$ Hz, 2H), 2.16 (t, $J = 8.0$ Hz, 2H), 1.70 (m, 2H), 1.62–1.42 (m, 2H), 1.40–1.25 (m, 2H), 1.22–1.02 (m, 2H), 0.78 (s, 3H), 0.65 (s, 3H); ^{13}C NMR (D_2O) (176.52, 175.84, 174.80, 173.47, 172.68, 171.26, 148.61, 147.24, 143.40, 141.19, 135.26, 127.83, 127.28, 126.67, 117.28, 86.23, 82.64, 82.23, 72.94, 72.69, 70.75, 63.87, 52.36, 52.24, 38.10, 37.15, 37.05, 34.23, 34.09, 33.01, 31.55, 29.72, 29.24, 26.55, 21.24, 19.60, 19.22, 17.11. HRMS calcd for $C_{40}H_{62}N_{10}O_{21}P_3S$ ([M+H]$^+$) 1143.3019; found 1143.2975.

4.4. Preparation of compound 12

CoASH was reacted with 3-bromo-dimethoxy-propane as described for **2**–**11** to afford the CoA analogue **15**, purified by RPHPLC with a potassium phosphate (50 mM, pH 4.5)/methanol gradient and desalted with water/methanol as before.[12] ^{1}H NMR (D_2O) δ 8.39 (s, 1H), 8.11 (s, 1H), 6.00 (d, $J = 5.2$ Hz, 1H), 4.06 (m, 2H), 3.84 (s, 1H), 3.65 (dd, $J = 8.0$, 4.0 Hz, 1H), 3.37 (dd, $J = 8.0$, 4.0 Hz, 1H), 3.29 (t, $J = 6.0$ Hz, 2H), 3.25–3.10 (m, 8H), 2.47 (t, $J = 7.2$ Hz, 2H), 2.37 (t, $J = 7.2$ Hz, 2H), 2.28 (t, $J = 7.2$ Hz, 2H), 1.71–1.65 (m, 2H), 0.71 (s, 3H), 0.59 (s, 3H). ESI-MS calcd for $C_{26}H_{47}N_7O_{18}P_3S$ ([M+H]$^+$), m/z 870. Compound **15** (4 mg, 0.0046 mmol) was dissolved in 300 μL of water and cooled with an ice-water bath and 300 μL of trifluoroacetic acid was added

dropwise over a period of 5 min. After 40 min, triethylamine (~0.7 mL) was added dropwise to the solution until the solution reached pH 6.0. The mixture was concentrated to dryness and redissolved in 0.1 mL, 500 mM sodium phosphate buffer, pH 6.0. N^{α}-acetyl-carboxamido-lysine (1 equiv) was then added and after 10 min, 10 equiv of $NaCNBH_3$ (dissolved in 0.7 mL methanol) was added dropwise to the reaction vessel and the mixture was stirred at room temperature for 48 h. The mixture was then purified using reverse phase HPLC with a water/acetonitrile/0.05% TFA gradient to afford 1 mg (15% yield assessed by UV) of the desired product. Spectroscopic data were as follows: ^{1}H NMR (CD_3OD) δ 8.39 (s, 1H), 8.09 (s, 1H), 5.99 (d, $J = 8$ Hz, 1H), 4.40 (m, 1H), 4.05 (m, 2H), 4.00 (dd, $J = 8.0$, 4.0 Hz, 1H), 3.84 (s, 1H), 3.64 (dd, $J = 8.0$, 4.0 Hz, 1H), 3.37 (dd, $J = 8.0$, 4.0 Hz, 1H), 3.31 (t, $J = 6.0$ Hz, 2H), 3.29 (t, $J = 6.0$ Hz, 2H), 3.15 (t, $J = 6.0$ Hz, 2H), 2.91 (t, $J = 8.0$ Hz, 2H), 2.83 (t, $J = 8.0$ Hz, 2H), 2.46 (t, $J = 6.0$ Hz, 2H), 2.43 (t, $J = 6.0$ Hz, 2H), 1.85 (s, 3H), 1.84–1.72 (m, 2H), 1.66–1.44 (m, 4H), 1.33–1.16 (m, 2H), 0.70 (s, 3H), 0.59 (s, 3H). HRMS calcd for $C_{32}H_{58}N_{10}O_{18}P_3S$ ($[M+H]^+$) 995.2859; found 995.2909.

4.4.1. p300 HAT domain preparation. The expression and purification of the p300 HAT domain (N-terminally His-tagged 1284–1673) was carried out as described in Thompson et al.[13]

4.4.2. HAT assays. These assays were carried out essentially as described by Thompson et al.[13] The assay conditions employed 250 μM acetyl-CoA, 250 μM H4–12 peptide, 16 nM p300 catalytic domain pH 8.0, 30 °C for 40 min. The reactions were quenched with guanidinium and then mixed with DTNB and absorptions measured at 412 nm as previously described.[13] Rates were shown to be linear versus time and enzyme concentration in this range.

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