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Biomimetic artificial epigenetic code for targeted acetylation of histones

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ABSTRACT: While the central role of locus-specific acetylation of histone proteins in eukaryotic gene expression is well established, the availability of designer tools to regulate acetylation at particular nucleosome sites remains limited. Here, we develop a unique strategy to introduce acetylation by constructing a bifunctional molecule designated Bi-PIP. Bi-PIP has a P300/CBP-selective bromodomain inhibitor (Bi) as a P300/CBP recruiter and a pyrrole-imidazole polyamide (PIP) as a sequence-selective DNA binder. Biochemical assays verified that Bi-PIPs recruit P300 to the nucleosomes having their target DNA sequences and extensively accelerate acetylation. Bi-PIPs also activated transcription of genes that have corresponding cognate DNA sequences inside living cells. Our results demonstrate that Bi-PIPs could act as a synthetic programmable histone code of acetylation, which emulates the bromodomain-mediated natural propagation system of histone acetylation to activate gene expression in a sequence-selective manner.

■ INTRODUCTION

Posttranslational modifications (PTMs) on histone proteins play significant roles in epigenetic regulation of eukaryotic chromatin. Acetylation of epsilon-amine on lysine residues is one of the major PTMs on histones, and is strongly correlated with transcriptional activation. The code governing histone acetylation is regulated by histone acetyltransferases (HATs) as writers and by histone deacetylases (HDACs) as erasers. Acetylated histones are known to operate *via* two mechanisms. One is to decrease the positive charge of histone proteins, resulting in weakened interaction between histones and DNA, which in turn open up the chromatin structure.¹ The second is mediated by bromodomain (BD)-containing proteins, which selectively bind to acetylated lysine residues.^{2,3}

Although locus-specific (i.e., DNA sequence-specific) regulation of histone acetylation has been shown to be essential in biological processes, techniques for controlling histone acetylation at any region of interest remain limited. Recently, programmable DNA-binding module proteins such as transcription activator-like effectors (TALEs) and clustered regularly interspaced short palindromic repeat (CRISPR)/Cas systems were fused to the catalytic domain of histone acetyltransferase P300 for sequence-selective histone acetylation.⁴ Although targeted acetylation was demonstrated using the dCas9-P300 Core fusion protein, the transfection-based system could limit their clinical applications. Moreover, such protein-based molecules are

amenable to enzymatic degradation in the cellular environment. In these contexts, the development of chemically synthesized sequence-selective molecules is a promising alternative for locus-specific acetylation of histones, avoiding the need of transfection and enzymatic degradation.

Pyrrole-imidazole polyamides (PIPs) are a class of synthetic molecules that bind to DNA in a sequence-selective manner.⁵ In the anti-parallel hairpin structure, a pair of pyrrole and pyrrole (Py/Py) recognizes A·T or T·A base pair, while a pair of imidazole and pyrrole (Im/Py) does G·C base pair.⁶ It is known that the aliphatic β -alanine can substitute and partake the selective binding functionality of the Py in the PIP chemical architecture.⁷ Also, β -alanine could confer the PIPs with better flexibility and improved binding affinity. By conjugating PIP with a HDAC inhibitor SAHA, our group previously developed a class of designer molecule called SAHA-PIP for sequence-selective histone acetylation.⁸ SAHA-PIPs with different sequence-specificity activated silenced genes including pluripotency genes,^{9,10} germ cell-associated genes,¹¹ retinal genes,¹² neural genes,¹³ and key therapeutically important genes¹⁴ in human dermal fibroblasts. Despite the proclaimed potential of SAHA-PIPs as epigenetic drugs, the level of gene activation was still inconsistent. One reason for this observation could be the fact that induction of histone acetylation by inhibiting HDACs is an indirect outcome and acetylation will not occur if HATs are absent in the region. As an alternative approach, a P300 activator *N*-(4-chloro-3-trifluoro-

Figure 1. Strategy of targeted histone acetylation utilizing the bromodomain (BD)-mediated propagation of acetylation. (a) Structure of P300/CBP coactivator proteins. NRID, nuclear receptor interacting domain; TAZ1, transcriptional adaptor zinc finger 1; KIX, KID-interacting; BD, bromodomain; RING, really interesting new gene; PHD, plant homeodomain; HAT, histone acetyltransferase; ZZ, ZZ-type zinc finger; TAZ2, transcriptional adaptor zinc finger 2; IBiD, interferon-binding domain. (b) BD-mediated propagation of histone acetylation by P300/CBP. Existing acetyl lysine (yellow) is recognized by BD of P300/CBP, and *de novo* acetyl modification (green) is introduced by HAT domain. (c) Design of artificial epigenetic code of acetylation named 'Bi-PIP' (left) and model of targeted histone acetylation by Bi-PIP (right). Bi mimics acetyl lysine to recruit P300/CBP through its BD.

methyl-phenyl)-2-ethoxy-benzamide (CTB)¹⁵ was conjugated to PIPs.¹⁶ However, the mechanism by which CTB operates is yet to be clarified and there is no clear evidence to substantiate the interaction of CTB with P300.¹⁷ Therefore, there is a need for (1) a more reliable and robust molecular design, and (2) proof-of-concept experiments of the molecular mechanism, to achieve sequence-selective histone acetylation in a similar manner to that observed in natural cellular environment.

Interestingly, HAT domain and BD sometimes appear in the same protein. In humans, GCN5 (KAT2A), PCAF (KAT2B), P300 (KAT3B), CBP (KAT3A), TAF₁ (KAT4), and BRD4 contain both HAT and BD in their structures (Figure 1a).^{18–21} This is thought to contribute to the robust maintenance of highly acetylated chromatin; one acetyl mark causes acetylation of neighboring sites via BD-mediated recruitment of these proteins (Figure 1b).^{18,22,23} This natural system inspired us to develop a novel approach based on the BD-mediated acetylation to acetylate histones in targeted nucleosome.

Small molecules targeting the BD-containing proteins have been identified and developed in recent years.^{24–28} These BD inhibitors specifically bind to the pockets of BD to prevent binding with an acetylated lysine residue. Therefore, BD inhibitors could be used as a mimetic of acetylated lysine to probe BDs.

We hypothesized that locus-specific acetylation by a HAT-BD protein such as P300 could be achieved when a BD inhibitor is localized to a specific DNA sequence (Figure 1c). Herein, we report the development of a novel molecule named 'Bi-PIP' by conjugating a BD inhibitor (Bi) and PIP. We demonstrate a proof-of-concept that Bi-PIP can enhance histone acetylation by P300 in a sequence-selective manner and clarify their mode of action.

RESULTS

Design and synthesis of Bi-PIP as a sequence-specific artificial histone code of acetylation. We selected the coactivator P300/CBP family of proteins as the target for the artificial histone code (Figure 1a). P300/CBP plays a central role in transcriptional activation in eukaryotic cells.²⁹ Given that P300/CBP has both HAT and BD, BD-mediated propagation of histone acetylation was proposed (Figure 1b).¹⁸ A P300/CBP-selective BD inhibitor (CBP30) having 5-isoxazolyl-benzimidazole was previously developed by Hay *et al.* (Supplementary Figure 1).²⁵ X-ray crystal structure analyses revealed that the 3,5-dimethylisoxazole moiety in CBP30 enters the pocket in the BD of CBP and P300, mimicking acetylamine of epsilon-acetylated lysine residue.^{25,30} The other two domains, terminal aryl group and morpholine moiety, are located outside of the pocket, suggesting that they could be sites for conjugation with PIPs (Supplementary Figure 1).³⁰ For synthetic accessibility of building blocks, we chose one of the CBP30 derivatives (1 in Figure 2, named 'Bi' in this study), which showed a moderate binding affinity to the BD of CBP (Figure 2, Supplementary Figure 2) and attempted to attach a linker domain on the methoxy group.²⁵ A BD inhibitor unit (Bi Unit) with primary amine was then designed and synthesized (Supplementary Scheme 1). By coupling with PIPs, which has a carboxylic acid at the N-terminus, two conjugates, termed Bi-PIP₁ (2) and Bi-PIP₂ (4) targeting 5'-WWCWGWCW-3' and 5'-WGCCGCCW-3', respectively, were synthesized (W = A or T, Figure 2).

Bi-PIPs promote histone acetylation on nucleosomes that possess their target DNA sequences. To test sequence-selective histone acetylation by Bi-PIP, we sought to establish a biochemical assay by combining HAT reaction followed by immunoprecipitation using reconstituted nucleosomes reported previously²² and quantitative polymerase chain reaction (qPCR). We reconstituted mononucleosomes using a salt-dialysis method³¹ from a histone octamer and DNA templates that each have a Widom 601 nucleosome positioning sequence³² and five PIP-binding sequences: 5'-AACAGTCA-3' for Bi-PIP₁ and 5'-AGCCGCCA-3' for Bi-PIP₂ (Figure 3a). Gel electrophore-

Figure 2. Chemical structures of Bi (1), Bi-PIP1 conjugate (2), PIP1 monomer (3), Bi-PIP2 conjugate (4), and PIP2 monomer (5).

sis confirmed that the majority of DNA templates were successfully formed on the nucleosome (**Supplementary Figure 3**). Two types of nucleosomes, one with five match sequences for Bi-PIP1 (Nuc1) and the second for Bi-PIP2 (Nuc2), were mixed and incubated with recombinant human P300 (amino acid 965–1810) including the BD and HAT with or without Bi-PIP1. The reaction mixture was then immunoprecipitated using an antibody for pan-acetylated H3 (acetylation at K9, K14, K18, K23, K27), followed by qPCR analysis to measure the amount of each nucleosome (**Figure 3b**).

In the absence of the Bi-PIP, the level of acetylation of the nucleosomes got slightly increased only in the presence of 100 nM of P300. On the other hand, addition of 100 nM Bi-PIP1 induced intensive acetylation on its target Nuc1 but not on Nuc2 (**Figure 3c**). The increase in the level of acetylation was dependent on the concentration of P300. Next, to clarify the importance of the Bi and PIP domains, each monomer molecule was tested in the assay. The PIP monomer alone (3), Bi monomer alone (1), and even a mixture of them could not promote the acetylation (**Figure 3d**). The result clearly indicates that the Nuc1-selective acetylation by Bi-PIP1 was achieved by sequence-selective binding of the PIP domain to its target DNA sequence and P300 recruitment mediated by a BD–Bi interaction (**Figure 1c**). To examine the programmability of Bi-PIP, we tested Bi-PIP2 designed to target Nuc2. Bi-PIP2 caused selective acetylation of Nuc2, indicating that the target sequence of Bi-PIP is programmable (**Figure 3e**).

Taken together, we established that HAT reaction followed by *in vitro* ChIP-qPCR could be used to quantify the acetylation of individual nucleosomes, thereby demonstrating a proof-of-concept of sequence-selective acetylation with Bi-PIP using the assay.

Next, to investigate the effect of linker length of Bi-PIP, Bi-PIP1 with a shorter linker (Bi-PIP1-short, 6) and one with a longer linker (Bi-PIP1-long, 7) were synthesized (**Supplementary Figure 4a**) and compared with the original Bi-PIP1 in the assay. Acetylation of Nuc1 was enhanced at a similar level in Bi-PIP1-short, Bi-PIP1, and Bi-PIP1-long (**Supplementary Figure 4b**). The result suggests that all three Bi-PIPs have a sufficiently long and flexible linker for the effective acetylation of H3. Indeed, the N-terminal tail region of H3 is known to be located near the linker DNA,³³ where the binding sites for PIPs are located.

To clarify the binding site(s) essential for the acetylation, we carried out studies using five additional templates that have a single binding site for Bi-PIP1 at different positions (Nuc3–7). Among the five nucleosomes, Nuc3, which has a binding site at the closest position to the nucleosome core particle, showed the highest level of acetylation (**Supplementary Figure 5**). Thus, this is the most effective position for the acetylation. However, to our surprise, the acetylation level of Nuc3 was much lower than that of Nuc1 (**Supplementary Figure 5**). Moreover, even the sum of acetylation levels of Nuc3–7 did not reach the value for Nuc1, suggesting the existence of synergistic effects of multiple P300s on a single nucleosome (**Supplementary Figure 5**). Although this phenomenon could be explained by

Figure 3. Bi-PIPs promote histone acetylation on nucleosomes that possess their target DNA sequences. **(a)** Schematic of DNA templates used for the assay. Blue and red rectangles represent binding sites for Bi-PIP₁ and Bi-PIP₂, respectively. **(b)** Schematic of HAT reaction-*in vitro* ChIP-qPCR. **(c)** A HAT reaction-*in vitro* ChIP-qPCR was performed with a series of P300 concentration (0, 3, 10, 30, 100 nM) with or without 100 nM of Bi-PIP₁. Positive control represents a nucleosome with synthetic acetylated histone H₃ (K4ac, K9ac, K14ac, K18ac, K23ac). **(d)** A HAT reaction-*in vitro* ChIP-qPCR was performed with 10 nM of P300. Each compound was applied at a concentration of 100 nM. **(e)** A HAT reaction-*in vitro* ChIP-qPCR was performed Bi-PIP₁ (100 nM) or Bi-PIP₂ (100 nM) with 10 nM of P300.

the previously reported intermolecular auto-acetylation of P300,^{34,35} further studies is required to investigate the mechanism in detail.

Finally, histone acetylation with Bi-PIP was directly confirmed by mass spectrometry analysis. After HAT reaction, the histones were processed through propionylation of unmodified lysine, tryptic digestion, and propionylation of terminal amine.^{36,37} The resulting peptides were analyzed by liquid chromatography–tandem mass spectrometry (LC-MS/MS). Consistent with the antibody-based assays shown above, an increase in the signal intensity was observed for peptides containing acetyl lysine at the N-terminal tail region of H3, such as H3K14ac, H3K18ac, H3K23ac, H3K27ac, and H3K36ac (**Supplementary Figure 6a, 6c**). It was also found that acetylation was promoted in the N-terminal region of histone H4, including H4K5ac, H4K8ac, and H4K12ac (**Supplementary Figure 6b, 6d**). The acetylation at N-terminal tail region of H3 and H4 by P300 is consistent with previously reported results, where P300 gets recruited to reconstituted chromatin by DNA-binding activator proteins.³⁸

Bi-PIPs epigenetically activate selective gene expression inside living cells. We then evaluated if Bi-PIPs could function in a cellular environment. As an initial experiment, we performed a HAT reaction-*in vitro* ChIP-qPCR assay using HeLa nuclear extract as the HAT resource instead of recombinant P300. The nuclear extract containing not only P300/CBP but also other HATs and HDACs, provided a cell-like environment than that observed with only recombinant P300. Although the basal level of acetylation without Bi-PIP1 increased, Bi-PIP1 enhanced acetylation of Nuc1 but not Nuc2 (**Supplementary Figure 7**), suggesting the possibility of applying Bi-PIPs to living cells.

A bottleneck in the utility of Bi-PIPs is the potential of Bi to impair the natural function of P300/CBP such as the propagation of histone acetylation inside the living cells. In this regard, we evaluated the effect of increasing amount of Bi on the BD-mediated histone acetylation. Interestingly, even a ten-fold higher concentration of Bi than that of Bi-PIP1 did not affect the BD-mediated acetylation induced by Bi-PIP1 (**Supplementary Figure 8**). It is reasonable to assume that the binding of BD and Bi could be in equilibrium, which provides the BD with the opportunity to interact with Bi-PIP1 even with the high doses of Bi. The rapid dissociation of CBP30 from P300 further supports this notion.³⁹

We then investigated the cellular activity of Bi-PIPs using the HEK293T cell line. The cell permeability of PIPs is known to vary depending on the chemical structures and cell lines.^{39,40} To ensure that all the compounds enter the cells, we used a delivery reagent, Endo-Porter.⁴¹ We found that the inclusion of Endo-Porter greatly improved cellular uptake of a fluorescent PIP whose permeability is poor without the reagent (**Supplementary Figure 9**).

Considering the fundamental role of P300/CBP as the transcriptional coactivator to activate gene expression, we performed a transcriptome analysis of total RNA extracted

from cells treated with the compounds. The criteria of >2 or <-2 fold change and <0.05 P value was applied for detecting differentially expressed transcripts. The Bi monomer showed only a minimal change in gene expression (2 upregulated transcripts and 4 downregulated transcripts). The PIP1 and PIP2 monomers caused a moderate change in global gene expression (32 up- and 31 downregulated by PIP1, and 143 up- and 63 downregulated by PIP2). On the other hand, Bi-PIP1 and Bi-PIP2 conjugates gave greater transcriptome changes, mainly for activation, rather than repression (473 up- and 258 downregulated by Bi-PIP1, and 446 up- and 137 downregulated by Bi-PIP2, **Supplementary Figure 10**). The results, therefore, reflect the recruitment of P300/CBP onto the chromatin by Bi-PIPs, which cannot occur by Bi monomer or PIP monomers.

We then evaluated if the difference in DNA sequence-selectivity could cause a dissimilar change in gene expression. In contrast to the *in vitro* ChIP assay shown above, genes activated by the two Bi-PIPs were not exclusive, and 247 transcripts were commonly upregulated by both Bi-PIP1 and Bi-PIP2 (**Supplementary Figure 11**). This is reasonable because the binding sites of Bi-PIP1 and Bi-PIP2 are not exclusively distributed throughout the genome; thus, some genes could be activated by both Bi-PIP1 and Bi-PIP2. To check whether the sequence-selective activity attributes to the cellular function of Bi-PIPs, ChIP-sequencing (ChIP-seq) was performed using the antibody for pan-acetylated H3. Mapped reads were processed by One-stage DifferNtial peak caller (ODIN)⁴² to detect differential peaks between Bi-PIP1 and Bi-PIP2-treated cells. Analysis of the target sequences in the region of differential peaks revealed that the target sequence of Bi-PIP1 appeared more frequently in the loci where Bi-PIP1 showed higher peak signal than that in Bi-PIP2 (**Figure 4a, left**). Conversely, the target sequence of Bi-PIP2 got enriched in the loci where Bi-PIP2 showed higher peak signal than that in Bi-PIP1 (**Figure 4a, right**). The enrichment of the target sequence for Bi-PIP1 was relatively lower than that for Bi-PIP2, which could be attributed to the more extensive sequence match sites for the Bi-PIP1 than Bi-PIP2 (8 fold in theory because it has three more W recognition). Therefore, it is reasonable to assume that the Bi-PIP1 could distribute more broadly and the binding sequence might be often found in the differential peak region for Bi-PIP2. Taken together, the observed epigenome changes in acetylation verifies the sequence-selective bioactivity of Bi-PIPs.

Although the result above showed that some genes were activated by both Bi-PIP1 and Bi-PIP2, differentially activated transcripts were also identified in the microarray data by applying criteria of >1.5 or <-1.5 fold change (**Figure 4b, Supplementary Table 4, 5**). Among these uniquely activated transcripts, the most upregulated protein-coding genes with Bi-PIP1 and Bi-PIP2 were *NTS* (neurotensin) and *IER5L* (immediate early response 5-like), respectively (**Figure 4b, Supplementary Table 4, 5**). We verified the expression of these two transcripts using RT-qPCR (**Figure 4c**). To gain insights into the mechanism of activation, we searched for the target sequences of Bi-PIP1

Figure 4. Bi-PIPs epigenetically activate selective gene expression inside living cells. **(a)** Motif search analysis. After ChIP-seq for acetylated H₃, differential peaks between Bi-PIP1 and Bi-PIP2-treated cells were detected by ODIN. Each differential peak region was searched for the match sites of Bi-PIP1 (5'-WWCWGW CW-3', left) and Bi-PIP2 (5'-WGCCGCCW-3', right). **(b)** Volcano plots of transcriptome comparison of Bi-PIP1 vs. vehicle (left) and Bi-PIP2 vs. vehicle (right). Uniquely upregulated transcripts by individual Bi-PIP (>1.5 fold change) are indicated as colored dots. **(c)** Expression of *NTS* and *IER5L* was confirmed by RT-qPCR. Error bars represent standard deviation of data from two culture wells. **(d)** Genomic loci of *NTS* (upper) and *IER5L* (lower). Match sites for Bi-PIP1 (5'-WWCWGW CW-3') and Bi-PIP2 (5'-WGCCGCCW-3') are indicated as blue and red diamonds, respectively.

(5'-WWCWGW CW-3') and Bi-PIP2 (5'-WGCCGCCW-3') in each gene locus. Interestingly, the genomic sequence of the *NTS* locus contains the binding sites for Bi-PIP1 but not for Bi-PIP2 (**Figure 4d**). Likewise, the *IER5L* locus possesses the binding sites for Bi-PIP2 but not for Bi-PIP1 (**Figure 4d**). Furthermore, the differential peak analysis of ChIP-seq data between each Bi-PIP and vehicle-treated cells revealed that the acetylation at *IER5L* locus increased upon the Bi-PIP2 treatment and was detected as significant peaks by ODIN (**Supplementary Figure 12**). On the other hand, ODIN did not detect substantial peaks in the *NTS* locus. However, there was a notable change in the shape of the peak in the data (**Supplementary Figure 12**). This observation possibly reflects the chromatin remodeling or nucleosome eviction after Bi-PIP1 induced acetylation at the locus. These results suggest that *NTS* and *IER5L* are putative target genes of Bi-PIP1 and Bi-PIP2, respectively.

DISCUSSION

Acetylated histones function by neutralizing the positive charge of lysine to loosen the chromatin structure, and by being recognized by BD-containing proteins. BD-containing proteins are diverse nuclear proteins including HATs, histone methyltransferases, and ATP-dependent chromatin remodeling complexes.⁴³ Recruiting these proteins at acetyl lysine sites thus contributes to gene activation by remodeling chromatin,⁴⁴ introducing methylation at lysine 4 in histone H₃ (H₃K₄me), or introducing *de novo* acetylation near the site. In this study, we sought to take

advantage of the BD-mediated natural propagation system of histone acetylation for the control of targeted acetylation. With the reliable and robust molecular design, we successfully developed 'Bi-PIP,' which recruits P300/CBP to selective DNA sequences. Based on biochemical assays, Bi-PIP-mediated histone acetylation at target DNA sequences was demonstrated as a proof-of-concept of the molecular mechanism. Moreover, cellular experiments showed the sequence-selective histone acetylation and transcriptional activation in endogenous genome by Bi-PIPs, indicating their applicability in living cells.

Recently Ansari's group reported a conjugate called Syn-TEF by coupling a PIP with another bromodomain inhibitor (+)-JQ1, selective to the bromodomain and extraterminal (BET) protein family.⁴⁵ Syn-TEF1 targeting expanded GAA repeats in the frataxin (*FXN*) gene successfully recruited BET protein BRD4 (bromodomain 4) to the gene locus in Friedreich's ataxia (FRDA) patient-derived cells, resulting in transcriptional elongation of *FXN*. However, Syn-TEF and Bi-PIP target different members of the BD protein family, and therefore is demonstrated to cause different outcomes i.e., while Syn-TEF causes BRD4-dependent transcriptional elongation, Bi-PIP causes P300-dependent histone acetylation. Thus, Bi-PIP and Syn-TEF can be used for different purposes and this make to expand the range of potential applications of the synthetic epigenetic regulators.

Despite the successful demonstration of the potential of Bi-PIP as biomimetic artificial epigenetic code, there is still

a need to improve the sequence-selective capacity of the Bi-PIPs inside the living cells. The reason is that the ChIP-seq analysis of the Bi-PIP-treated cells implied that the acetylated loci by each Bi-PIP could contain not only its target sequences but also the other possible binding sites. Also, the microarray studies suggested that although some genes were selectively activated by each Bi-PIP, others were activated by both Bi-PIPs. The modest selectivity of the current version of Bi-PIPs could be attributed to the non-exclusive distribution of the two target sequences on the genome-wide scale. Nevertheless, the next-generation Bi-PIPs with extended recognition capacity⁴⁶ or those that are designed to have a cooperative binding effect⁴⁷ could target the longer DNA sequences and overcome the non-selective outcome. Furthermore, a comprehensive evaluation of the binding affinity could facilitate the design of advanced Bi-PIPs with better specificity towards the loci of interest.⁴⁸ Besides, the designer PIPs can also have an alternate application as the guiding factor for the BD inhibitor to target multiple short sequence motifs in the genome and epigenetically activate the desired gene network.⁴⁹

So far, we have developed a variety of PIP-based sequence-selective epigenetic regulators by employing small molecules that modulate epigenetic writers or erasers, including HDAC inhibitor (SAHA),⁸ HAT activator (CTB),¹⁶ and HAT inhibitor (C646).⁵⁰ In this study, we expanded the utility of PIP-based sequence-selective epigenetic regulators to target epigenetic readers. Given that histone codes fundamentally function by being recognized by epigenetic readers, sequence-selective inhibitors of epigenetic readers such as Bi-PIP work as readable artificial histone codes while operating similarly as the natural ones. In the future, other inhibitors of epigenetic readers will also be employed to achieve diverse outcomes such as the introduction of other histone PTMs, removing histone PTMs, and chromatin remodeling.

■ EXPERIMENTAL SECTION

All experimental procedures are provided in the Supporting Information.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge via the Internet at <http://pubs.acs.org>.

Synthesis and characterization of compounds
Experimental procedures
Sequence of DNA templates used for the nucleosomes
Supplementary Figures 1–12
Supplementary Tables 1–7

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Notes

The authors declare no competing financial interest.

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