Supporting Information

A synthetic transcription factor pair mimic for precise recruitment of an epigenetic modifier to the targeted DNA locus

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MATERIALS AND METHODS

General

The reagents for polyamide syntheses such as Fmoc-Py-OH, Fmoc-Im-OH, Fmoc-Py-Im-OH, and Im-CCl_3, solid supports (Fmoc-Py-oxime resin and Fmoc-β Ala-Wang resin), *O*-(1*H*-6-chlorobenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HCTU) and benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) were from HiPep Laboratories (Kyoto, Japan). Trifluoroacetic acid (TFA), 3,3'-diamino-*N*-methyldipropylamine, *N*,*N*-diisopropylethylamine (DIEA), dichloromethane (DCM), methanol, acetic acid (AcOH), 1-methyl-2-prrrolidone (NMP), and *N*,*N*-dimethylformamide (DMF) were obtained from Nacalai Tesque (Kyoto, Japan). Fmoc-D-Dab (Boc)-OH and Fmoc-NH-$dPEG_3$-COOH were obtained from Iris Biotech GmbH (Marktredwitz, Deutschland). Polyamide-chain assembly was performed on an automated synthesizer, PSSM-8 (Shimadzu, Kyoto, Japan). HPLC grade acetonitrile (Nacalai tesque) was used for both analytical and preparative HPLC. Water was prepared by a Milli-Q apparatus (Millipore, Tokyo, Japan). All chemicals were used as received. Analyses by reversed-phase RP-HPLC were carried out online LCMS (Agilent 1100 ion-trap mass spectrometer, HCT ultra, Bruker Daltonics, Yokohama, Japan), with analytical RP-HPLC columns, UV spectra were measured on a NanoDrop 2000c (Thermo Fisher Scientific).

Polyamide Fmoc coupling procedure

Polyamides were prepared using PSSM-8 peptide synthesizer (Shimadzu, Kyoto) with a computer-assisted operation system at 43 mg of Fmoc-Pyrrol-oxime resin and β Ala-Wang resin (ca. 0.42 mmol/g, 100~200 mesh) by Fmoc solid-phase chemistry[1]. Reaction cycles were as follows: deblocking step for 4 min x 2, 20% piperidine in DMF; coupling step for 60 min, corresponding carboxylic acids, HCTU (88 mg), diisopropylethylamine (DIEA) (36 μL), 1-methyl-2-pyrrolidone (NMP); washing step for 1 min x 5, DMF. Each coupling reagents in steps were prepared in NMP solution of Fmoc-Py-COOH (77 mg), Fmoc-Im-COOH (77 mg), Fmoc-Py-Im-COOH (70 mg), Fmoc-β-COOH (66 mg), Fmoc-γ-COOH (69 mg) and Fmoc-mini PEG-COOH (69 mg). All other couplings were carried out with single-couple cycles with stirred by N_2 gas bubbling. Typically, resin (40 mg) was swollen in 1 mL of NMP in a 2.5-mL plastic reaction vessel for 30 min. 2-mL plastic centrifuge tubes with loading Fmoc-monomers with HCTU in NMP 1 mL were placed in programmed position. All lines were washed with NMP after solution transfers. After the completion of the synthesis by the peptide synthesizer, the resin was washed with DMF (1 mL × 2), methanol (1 mL × 2), and dried in a desiccator at room temperature *in vacuo*.

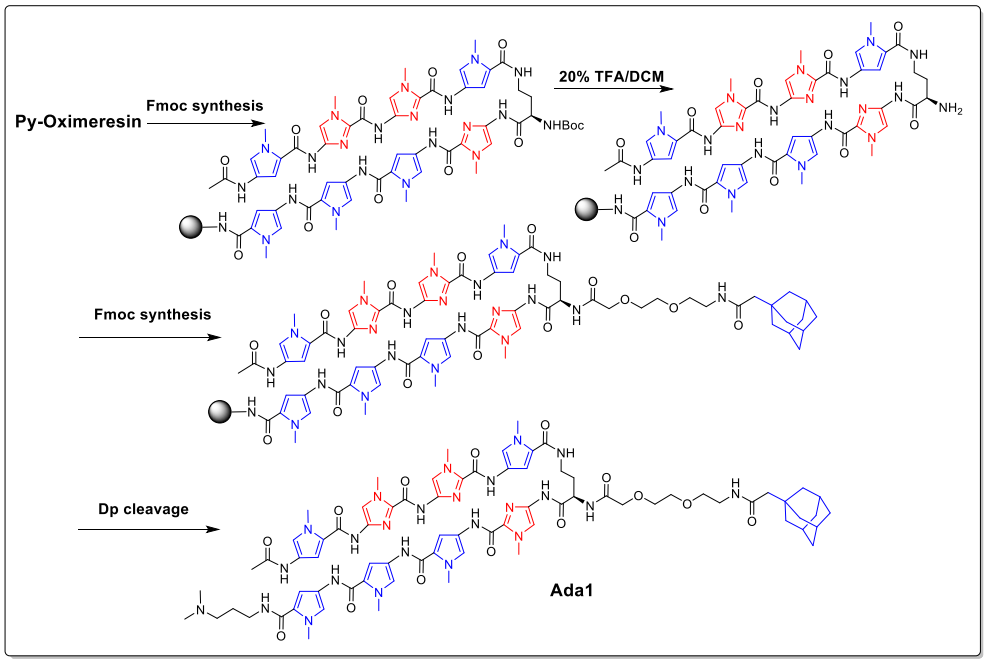
Resin cleavage and purification procedure

The resulting polyamide-oxime resin was cleaved from the solid support with *N*,*N*-dimethyl-1,3-propyldiamine for 3 h at 45 °C. Polyamide-β Ala-Wang resin was cleaved from the solid support with 95% TFA, 2.5% triisopropylsilane, and 2.5% water for 30 min at room temperature. Resin was filtered off, and the resulting liquor was treated with diethyl ether. The

precipitated crude polyamide was washed three times with diethyl ether and analyzed by HPLC. Crude polyamides were purified on a medium size HPLC column at 40°C. The purified peptides were assessed by the MALDI TOF-MS system.

Synthesis of Ada1

The synthetic procedure of **Ada1** and **Ada2** has been described previously[2]. The condition of Boc- deprotection is 20% TFA in DCM, 30 min at room temperature. The resin was washed by DMF, DCM, and dried in vacuum. Then Fmoc chemistry was conducted for the coupling of mini-PEG linker. After deprotection of Fmoc group, 1-Adamantaneacetic acid (40 mg) was coupled by Fmoc method. The resin cleavage and compound purification procedure have been described above. **Ada1** was obtained as a white powder. MALDI-TOF MS: *m/z* calcd for $C_{74}H_{97}N_{24}O_{14}^{+}$ $[M+H]^{+}$: 1545.7616; found: 1545.107.. HPLC: t_R=12.667 min (0.1 % TFA/MeCN, linear gradient 0–100 %, 0–20 min). (Mass data was attached in the bottom)



Synthesis of Ada3 (positive charge at NH+)

The synthetic procedure was same as **Ada1**, except replacing mini-PEG linker with an adipic acid linker. Adamantyl ethylenediamine was used as guest molecule. **Ada3** was obtained as a white powder. MALDI-TOF MS: *m/z* calcd for $C_{74}H_{97}N_{25}O_{12}^{+}$ $[M+H]^{+}$: 1528.775; found: 1528.217. HPLC: t_R=17.750 min (0.1 % TFA/MeCN, linear gradient 0–100 %, 0–40 min).

Synthesis of Ada3-biotin (positive charge at NH+)

The synthetic procedure was same as **Ada1**, except replacing mini-PEG linker with an adipic acid linker. Adamantyl ethylenediamine was used as guest molecule. **Ada3-biotin** was obtained as a white powder. MALDI-TOF MS: *m/z* calcd for $C_{113}H_{170}N_{29}O_{27}S^{+}$ $[M+H]^{+}$: 2397.254; found: 2399.222. HPLC: t_R=18.650 min (0.1 % TFA/MeCN, linear gradient 0–100 %, 0–40 min).

Synthesis of Ada4 (positive charge at NH+ and longer linker)

The synthetic procedure was same as **Ada1**. Adamantyl ethylenediamine was used as guest

molecule. **Ada4** was obtained as a white powder. MALDI-TOF MS: *m*/*z* calcd for $C_{80}H_{109}N_{26}O_{15}^{+}$ $[M+H]^{+}$: 1673.857; found: 1674.487. HPLC: t_R=17.233 min (0.1 % TFA/MeCN, linear gradient 0–100 %, 0–40 min).

Synthesis of Ada5 (positive chargeat NH+)

The synthetic procedure was same as **Ada1**. 1-(1-Adamantyl)piperazine was used as guest molecule. **Ada5** was obtained as a white powder. MALDI-TOF MS: *m*/*z* calcd for $C_{76}H_{100}N_{25}O_{12}^{+}$ $[M+H]^{+}$: 1554.798; found: 1554.639. HPLC: t_R=16.442 min (0.1 % TFA/MeCN, linear gradient 0–100 %, 0–40 min).

Synthesis of Ada6 (flexible linker; positive charge at NH+)

The synthetic procedure was same as **Ada1**. Adamantyl ethylenediamine was used as guest molecule. **Ada6** was obtained as a white powder. MALDI-TOF MS: *m*/*z* calcd for $C_{74}H_{98}N_{25}O_{14}^{+}$ $[M+H]^{+}$: 1560.773; found: 1560.914. HPLC: t_R=14.942 min (0.1 % TFA/MeCN, linear gradient 0–100 %, 0–40 min).

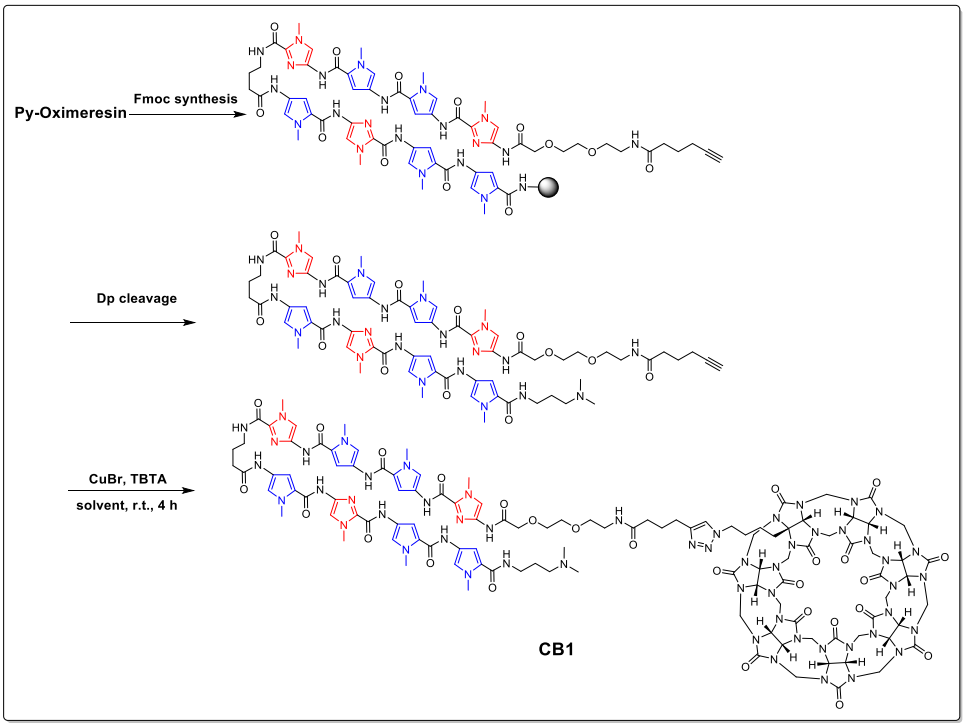
Synthesis of Ada7 (Longer PIP sequence)

The synthetic procedure was same as **Ada1**. Adamantyl ethylenediamine was used as guest molecule. **Ada7** was obtained as a white powder. MALDI-TOF MS: *m*/*z* calcd for $C_{92}H_{120}N_{31}O_{16}^{+}$ $[M+H]^{+}$: 1914.953; found: 1916.592. HPLC: t_R=17.992 min (0.1 % TFA/MeCN, linear gradient 0–100 %, 0–40 min).

Synthesis of Ada1_Bi

The purified **Ada8**-NH2 was conjugated with Bi catalyzed by HCTU. **Ada1_Bi** was obtained as a white powder. MALDI-TOF MS: *m*/*z* calcd for $C_{129}H_{177}N_{35}NaO_{27}^{+}$ $[M+H]^{+}$: 2671.345; found: 2673.178. HPLC: t_R=17.808 min (0.1 % TFA/MeCN, linear gradient 0–100 %, 0–40 min).

Synthesis of CB7-PIP conjugates



Polyamide synthetic procedures have been described above. 5-Hexynoic acid was used as the final coupling component. Crude polyamides were purified on a preparative column, HiPep-Intrada, at 40 °C. PIP-alkyne reacted with CB7-N3 through click chemistry assisted by CuBr/TBTA at room temperature[3]. After 4 h, the solution was washed by Et_2O and the crude powder was purified by HPLC system with medium size column. Purified compounds was characterized by MALDI TOF MASS and HPLC.

Synthesis of Cuc1

The purified PIPs was conjugated with CB7 catalyzed by CuBr/TBTA. **Cuc1** was obtained as a white powder. MALDI-TOF MS: *m/z* calcd for $C_{113}H_{135}N_{54}O_{27}^+$ $[M+H]^+$: 2680.085; found: 2679.977. HPLC: t_R=17.258 min (0.1 % TFA/MeCN, linear gradient 0–100 %, 0–40 min).

Synthesis of Cuc2

The synthetic procedure was same as **Cuc1**. **Cuc2** was obtained as a white powder. MALDI-TOF MS: *m/z* calcd for $C_{121}H_{148}N_{57}O_{30}^+$ $[M+H]^+$: 2879.181; found: 2879.120. HPLC: t_R=16.733 min (0.1 % TFA/MeCN, linear gradient 0–100 %, 0–40 min). (Mass data was attached in the bottom)

Synthesis of Cuc2_Bi

Cuc2_Bi was obtained as a white powder. MALDI-TOF MS: *m/z* calcd for $C_{164}H_{207}N_{64}O_{40}^+$ $[M+H]^+$: 3712.613; found: 3710.156. HPLC: t_R=16.800 min (0.1 % TFA/MeCN, linear gradient 0–100 %, 0–40 min).

Thermal Denaturation Analyses

Thermal denaturation analyses of the polyamide–DNA complex were performed on a V-650 spectrophotometer (JASCO) having a cell path length of 1 cm equipped with a thermocontrolled

PAC-743R cell changer (JASCO) and a refrigerated and heated circulator F25-ED (Julabo) as described[1a]. The sequences of the DNAs used were purchased from Sigma-Aldrich. The analysis buffer is as follows: the aqueous solution of 10 mM sodium chloride and 10 mM sodium cacodylate at pH 7.0 containing 0.25% v/v DMSO. The final concentrations of polyamides and dsDNA were 7.5 μM and 2.5 μM, respectively (3:1 stoichiometry). Denaturation profiles were recorded at λ = 260 nm from 25 to 95 °C at a rate of 1.0 °C/min, and melting temperatures were measured as the maximum of the first derivative of the profiles. Reported values were the averages of at least three measurements.

Electrophoretic mobility shift assay (EMSA)

Preparation loading mixture[4]. The sequences of the DNAs used were purchased from Sigma-Aldrich. The analysis buffer is as follows: the aqueous solution of 10 mM sodium chloride and 10 mM sodium cacodylate at pH 7.0 containing 0.25% v/v DMSO. The final concentrations of polyamides and dsDNA were clarified in the manuscript. Gel Loading Dye, Purple (6X no SDS B7025S, New England Biolab).

Preparation of gels. In a clean glass beaker, the following reagents were mixture in the given order (10 ml system, reagent volume doubled for 20 ml system). 5.25 mL MiliQ, 1 mL 10× TBE, and 3.75 mL of 40% Acrylamide/Bis Solution (29 : 1), followed by gas-removing to ensure the removal of all air bubbles. Then 90 μL APS (10% w/w in MiliQ) and 100 μL TEMED (10% v/v in MiliQ) were then added to the mixture and mixed properly before pouring it gently along parallel glass plates. Sufficient time was given for polymerization (20 min).

Electrophoresis. A pre-run of the gels was performed prior to loading. Care was taken to see that the gel was properly immersed in 1× Tris-Borate-EDTA buffer (TBE buffer) and the loading wells were free from any air bubbles. The wells were washed after the pre-run. Instrument settings: 120 V for 30 min at 4°C. 4 μL of the loading mixture was then loaded onto the wells. Pre-run again at 120 V for 30 minutes at 4°C. Then gel running as the instrument settings: 180 V for 160 min at 4°C.

Analysis of gels. The bands were stained with SYBR gold (10000× concentration in DMSO, from Thermofisher) and quantified with a FujiFilm FLA-3000G fluorescent imaging analyzer.

In vitro HAT assay

DNA template preparation

The DNA templates for nucleosome reconstruction was prepared from plasmid[5]. First, the plasmid was prepared as follow. The desired DNA sequences containing PIP binding sites were inserted just after the Widom 601 sequence in pGEM-3z/601 plasmid using NotI (GGCC/GC) and PstI (C/TGCA) as described below. pGEM-3z/601 plasmid digested with NotI and PstI and purified by agarose gel with purification kit Wizard SV gel and PCR clean up system. 8 insert DNA from Macrogene were annealed and ligated with digested plasmid using Quick ligation kit. Then the ligated plasmid was transformed to JM109 competent cells and plated the mixture on LB agar plates containing Ampicillin. The plasmid was extracted using a kit GenElute Plasmid Miniprep Kit and the sequence was checked using a primer such as T7 primer. Second, the preparation of DNA templates for nucleosome reconstitution. Amplify the DNA fragment from the master template

by PCR and purify the DNA fragment using a kit such as QIAquick PCR Purification Kit. Lastly, the purity was checked by gel electrophoresis.

Nucleosome reconstitution

Human histone proteins (H2A type1-B/E, H2B type1-K, H3.1 and H4) were expressed in *E. coli.* and purified by AKTA pure 25 protein purification system (GE Healthcare) as previously mentioned[6]. A mixture of template DNA (400 nM) and histone octamer (440 nM) in a 20 mM HEPES-KOH buffer (pH 7.5) was dialyzed in 2 M NaCl for 2 hours, then in 0 M overnight at 4°C. After the dialysis, concentration of nucleosome was determined by measuring the absorbance of DNA at the wave length of 260 nm. The reconstituted nucleosomes were analyzed by a polyacrylamide gel electrophoresis (6 % DNA retardation gel; Figure S6).

***In vitro* Histone acetyltransferase reaction (*In vitro* HAT reaction)**

HAT reaction was conducted as described previously with minor modifications[5]. Briefly, a mixture of reconstituted nucleosomes (25 nM each) in 15 μL of HAT buffer (50 mM Tris-HCl, 0.1 mM EDTA, 10% glycerol, 1 mM DTT, pH 8.0) was incubated with compounds at the indicated concentrations and recombinant human P300 (BPS Bioscience, catalog number: 50071, GenBank Accession No. NM_001429, a.a. 965-1810, with N-terminal His-GST-tag, MW=125 kDa, expressed in Sf9 cells via a baculovirus expression system.) in the presence of 10 μM of acetyl-CoA (Wako) and assay compounds indicated in the main text for 1 hour at 30 °C.

***In vitro* chromatin immunoprecipitation to quantitative polymerase chain reaction (*In vitro* ChIP-qPCR)**

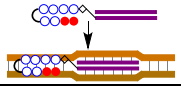
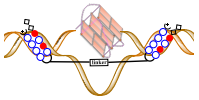
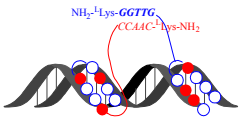
To a HAT reaction (15 μL), 85 μL of AB buffer (20 mM Tris-HCl, pH 7.5, 50 mM NaCl, 5 mM EDTA) containing 1/500 volume of anti–acetylated H3 (Abcam) was added and rotated for 1 hour at room temperature. 40 μL of Dynabead Protein G (Thermo Fisher Scientific) in 100 μL of AB buffer was then added to the mixture and further rotated for 1 hour at room temperature. The beads were washed three times with 200 μL of AB buffer and 100 μL of elution buffer (100 mM Tris-HCl, pH 7.5, 1 M NaCl) was added. The precipitated DNA was eluted by shaking at 90 °C for 5 minutes. The DNA solution was diluted and quantified by qPCR using SYBR FAST qPCR Kit (Kapa Bioscience) on LightCycler 480 (Roche Diagnostics GmbH). C_p values were determined by the 2nd derivative maximum method and relative RNA amount was calculated by the $\Delta\Delta C_p$ method. The sequences of the primers are listed in **Table S2**.

Statistical analysis

Results for continuous variables were presented as the mean ± standard error. Two-group differences in continuous variables were assessed by the unpaired t-test. Statistical analysis was performed by comparing treated samples with untreated controls. The statistical analyses were performed using GraphPad Prism 5.

SUPPORTING TABLES AND FIGURES

Table S1 Summary of representative covalent and non-covalent DNA binding systems

Covalent system	**Property**	**Ref.**
PIP covalent dimer 	Bind 10 bp with Ka of 7.5×10^{10} M^{-1}; 4-8 folds selectivity to one bp mismatch DNA; Difficulty for cell uptake; Not flexible binding modes;	[7]
PIP-Hoechst 	Up to 9 bp binding length; Anticancer and antivirus study; Hoechst targeting A/T rich sites;	[8]
PIP-peptide 	Simultaneously bind DNA major and minor groove; Enhance peptide binding affinity to 60 folds; Synthetic difficulty; Difficulty for cellular study;	[9]
PIP-PNA 	PNA binds dsDNA via strand invasion; Synthetically labour-consuming;Require double strand PNA;	[10]
PIP-based G4 stabilizer 	Enhance G4 formation in vitro; Difficulty for cellular study;	[11]
Non-covalent system	**Property**	**Ref.**
Host guest for PIP-Peptide	Noncovalent synergic interaction; Major and minor DNA groove binding; Need to be tested in cells;	[12]
PIP-HoGu system 	Small molecule synergic DNA binding; Proved efficacy in cells; Need to improve host-guest interaction;	[2]
PIP-NaCo system 	Cooperation is tunable; Orthogonality that cannot bind right handed DNA/RNA; Synthetically labour consuming;	[13]
2nd PIP-HoGu system 	Ultratight host-guest interaction; Higher cooperation;	In this study
ePIP-HoGu system	Epigenetically active synergistically DNA binding; Efficiently modify local nucleosomes with matched DNA binding sites;	In this study

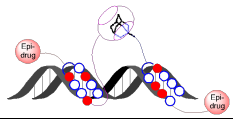


Table S2. DNA oligomers (ODNs) of Mode A and B

Gap distance (n)	Positive binding mode (Mode A) ODNs	Spacing	5'-AACTTAGGCTAATGACGTATATG-3' (Ac, Dp, Dp) 3'-TTGAATCCGATTACTGCATATAC-5'	Negative binding mode (Mode B) ODNs	Spacing	5'-AACTTGACGTAATAGGCTATATG-3' (Ac, Dp, Dp) 3'-TTGAACTGCATTATCCGATATAC-5'
-1	1'P	-1	5'-AAACTTAGGCTGACGTATATAT-3'	1'N	11	5'-AAACTTGACG-TAGGCTATATAT-3'
0	0P	0	5'-AAACTTAGGCTTGACGTATATA-3'	0N	12	5'-AAACTTGACGTTAGGCTATATA-3'
1	1P	1	5'-AACTTAGGCTATGACGTATATA-3'	1N	13	5'-AACTTGACGTATAGGCTATATA-3'
2	2P	2	5'-AACTTAGGCTAATGACGTATAT-3'	2N	14	5'-AACTTGACGTAATAGGCTATAT-3'
3	3P	3	5'-AACTTAGGCTAAATGACGTATAT-3'	3N	15	5'-AACTTGACGTAAATAGGCTATAT-3'
4	4P	4	5'-AACTTAGGCTATTATGACGTATAT-3'	4N	16	5'-AACTTGACGTATTATAGGCTATAT-3'
5	5P	5	5'-AATTAGGCTATTAATGACGTATAT-3'	5N	17	5'-AATTGACGTATTAATAGGCTATAT-3'
6	6P	6	5'-AATTAGGCTAATTAATGACGTATA-3'	6N	18	5'-AATTGACGTAATTAATAGGCTATA-3'
8	8P	8	5'-AATTAGGCTGGAATTCCTGACGTATA-3'	8N	19	5'-AATTGACGTGGAATTCCTAGGCTATA-3'

*DNA oligomers (ODNs) used in this research, including positive (Mode A, ODN1′P–ODN8P) and negative (Mode B, ODN1′N–ODN8N) binding sequences. The gap distance (green) is the number of base pairs between the binding sites of Ada-PIP (blue) and CB7-PIP (red). Spacing is the distance between host and guest conjugation sites, i.e., spacing equals to gap distance in Mode A, but in Mode B, it equals to gap distance plus two PIP-binding sites. The upper chart shows only the forward DNA strand and omits the complementary DNA strand.

Table S3. T_m assay of Cyd1– and Cuc1–guest conjugates with ODNs containing 2 bp spacing

Host \ Guest	Ada1	Ada3	Ada5	Ada6
Cyd1	63.2 ± 0.3	63.5 ± 0.2	63.1 ± 0.3	62.9 ± 0.4
Cuc1	64.5 ± 0.2	65.6 ± 0.1	63.6 ± 0.4	64.7 ± 0.1
ΔT_m	1.3 ± 0.5	2.2 ± 0.3	0.6 ± 0.7	1.8 ± 0.5

$\Delta T_m = T_{m\ (ODNs/\mathbf{Cuc1}/Ada\text{-}PIP)} - T_{m\ (ODNs/\mathbf{Cyd1}/Ada\text{-}PIP)}$. ODNs (2P) forward strand is 5'-AACTTAGGCTAATGACGTATAT-3'. Error bars are ranging from 0.1–0.7 °C indicating standard deviation of three replicates.

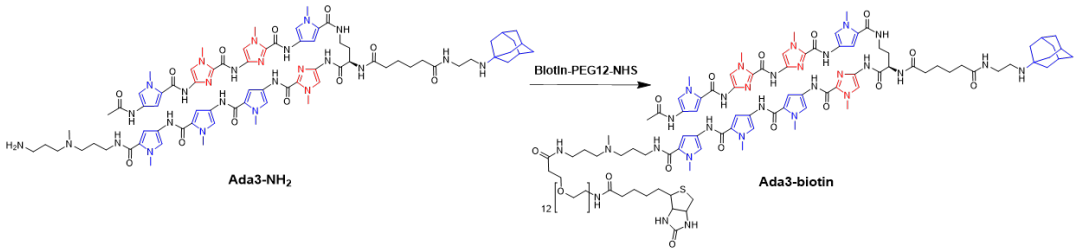
Table S4 Primers for in vitro ChIP-qPCR

Target	Forward / Reverse	Sequence (5′–3′)
Nuc1	F	TGCATGCCTGCAGTCACATAC
Nuc2	F	GCATGCCTGCAGTGAGTCAC
Nuc3	F	CATGCCTGCAGGCCACATC
Nuc4	F	CATGCCTGCAGACTCCGCA

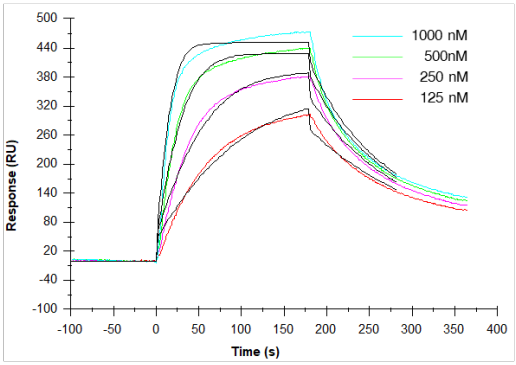
Nuc1–4 (common)	R	GCACCGGGATTCTCCAG

Figure S1. SPR assay to evaluate host-guest interaction in the absence of DNA

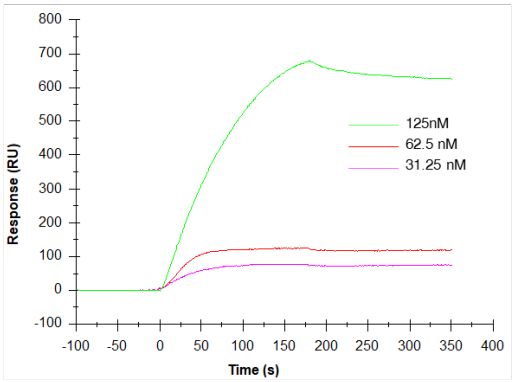
A. Synthetic route of **Ada3-biotin**



B. SPR sensorgram of **Cyd1**



C. SPR sensorgram of **Cuc1**



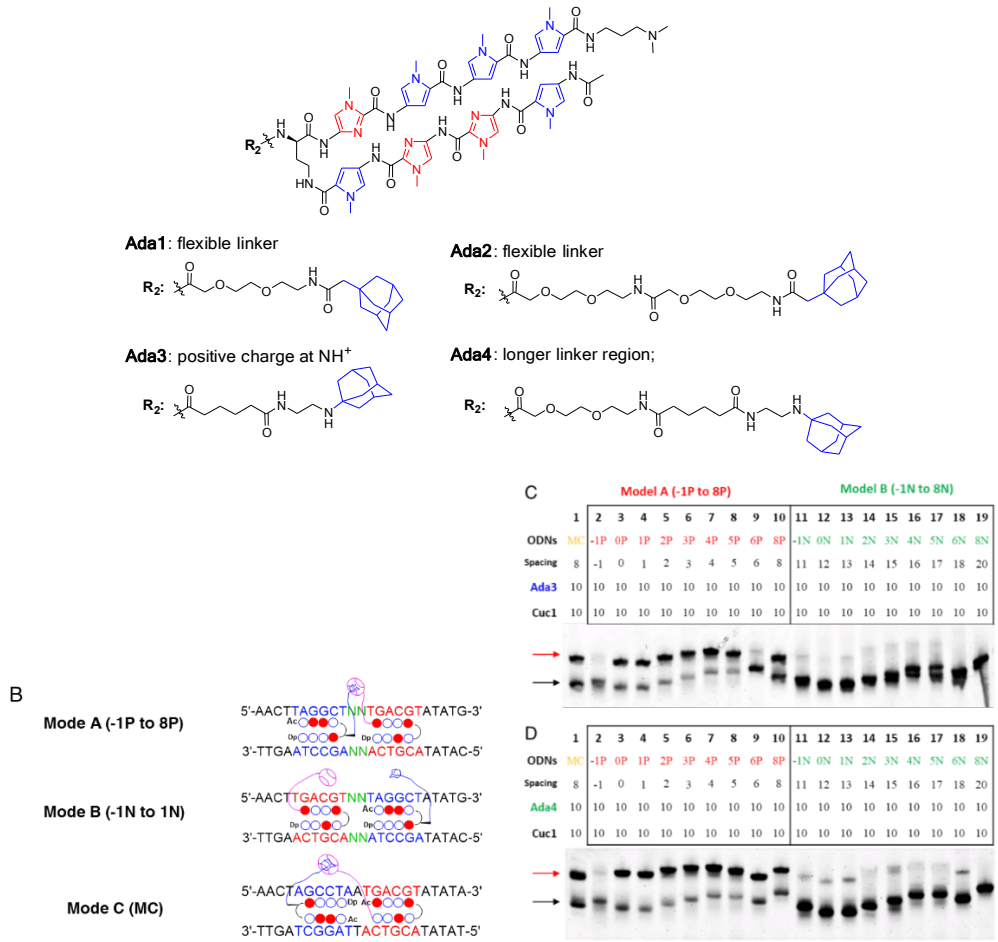
D. SPR data in summary

Compound solution	k_a [$M^{-1}s^{-1}$]	k_b [s^{-1}]	K_D [M]
Cyd1	1.40×10^{5}	1.47×10^{-3}	1.05×10^{-7}
Cuc1	4.09×10^{5}	$< 7.49\times10^{-6}$	$< 1.83\times10^{-11}$

*Determined by fitting with a 1:1 binding model with mass transfer.

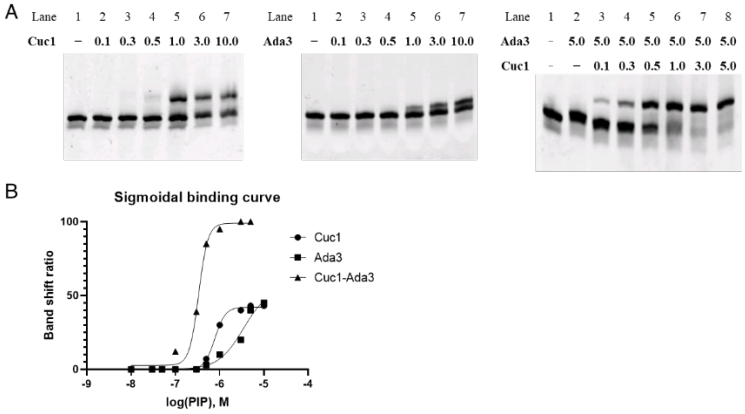
(A) Synthetic route of **Ada3-biotin**. (B) SPR sensorgram of **Cyd1**. (C) SPR sensorgram of **Cuc1**. The sensorgram were normalized to zero at the start point of injection, even though the interaction is irreversible. Thus, the accurate k_a of **Cuc1** can not be detected. (D) SPR data in summary. k_b of Cuc1 was calculated based on a single injection (125 nM **Cuc1**) in a new chip. The concentrations were showed in figure. Extensive concentrations of **Cyd1** and **Cuc1** were dissolved in HBS-EP buffer (10 mM HEPES, pH 7.4, 150 mM NaCl, 3 mM EDTA, and 0.005 % surfactant P20) with 0.1% DMSO. These solutions were passed over a **Ada3**-biointylated chip, in the absence of targeting DNA, immobilized on a sensor chip through a biotin-avidin system. Kinetic constants were calculated from the surface plasmon resonance sensorgrams for the interaction of guest conjugate Ada-PIP with host Cyd-PIP or CB7-PIP.

Figure S2. Chemical structures of Ada2 and Ada4 and EMSA results on Cuc1-Ada4



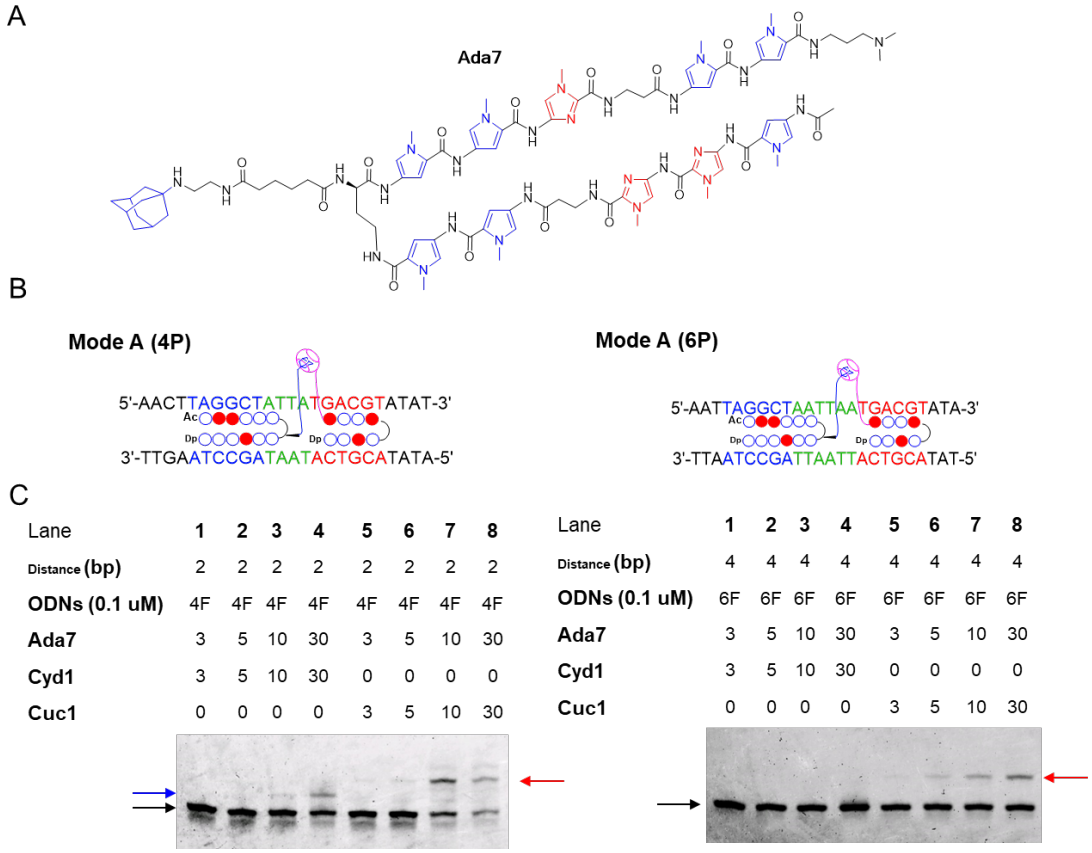
(A) Chemical structures of **Ada2** and **Ada4**. (B) Illustration of three binding modes, Mode A, B and C. (C) The gel-shift behavior of ODNs in Mode A, B and C with **Ada3–Cuc1**. (D) The gel-shift behavior of ODNs in Mode A, B and C with **Ada4–Cuc1**. Compound concentrations: 10.0 μM. ODNs concentration: 1.0 μM. Black arrow: ODNs; red arrow: ODNs/**Cuc1**/**Ada4**.

Figure S3. EMSA results of Cuc1 and Ada3 with ODNs 2P



(A) The gel-shift behavior of ODNs 2P with **Cuc1** or **Ada3**. (B) Sigmoidal dose-dependent binding curves after quantitative analysis of gel-shift study. ODNs concentration: 0.1 μM. Compound concentrations were shown in figure.

Figure S4. Comparative studies of two Pip–HoGu systems with longer guest conjugate Ada7



(A) Chemical structure of **Ada7**. (B) Schematic illustration of MoDe A-4P and Mode B-6P. (C) EMSA results. ODNs concentration was 100 nM. Black arrow indicates ODN2P duplex. Blue arrow shows ODNs/**Cyd1**/**Ada7**, while red one indicates ODNs/**Cuc1**/**Ada7**.

At a longer spacing of 4 bp in EMSA, **Cuc1**–**Ada7** showed significant gel-shift band potency with K_D value of 6.1 ×10^6 M^{-1}; however, no complex formation was observed at the excess amount of **Cyd1**–**Ada7**

Figure S5. Chemical structures of conjugates used in HAT assay

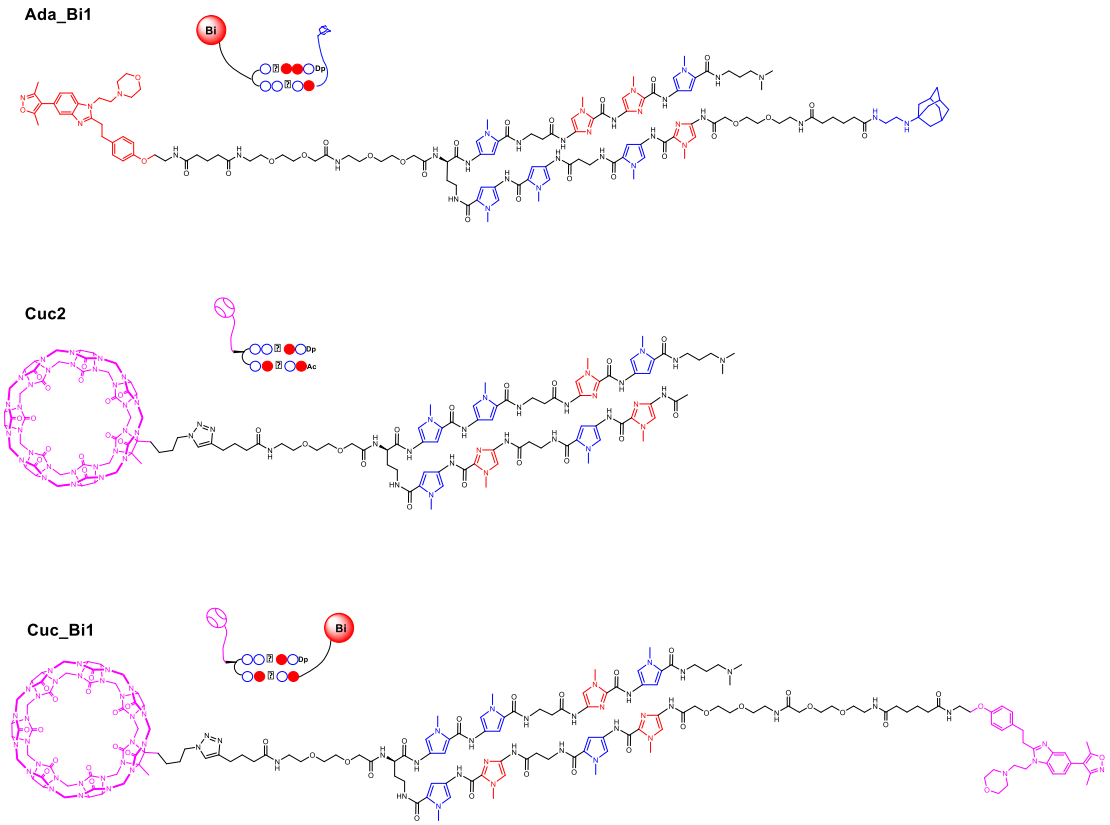
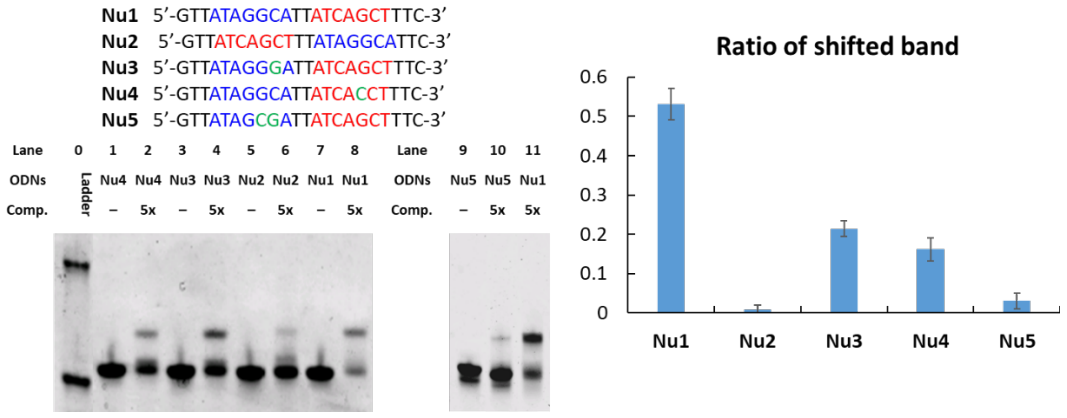
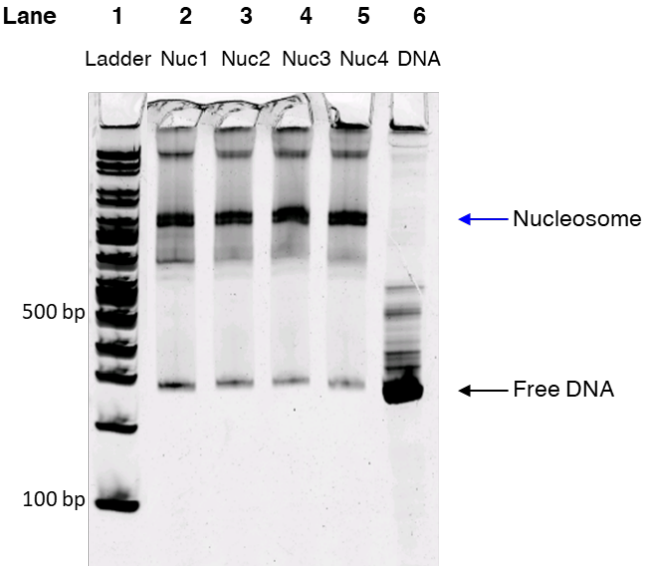


Figure S6. EMSA results of Ada_Bi1 + Cuc2 with four short dsDNA



The gel-shift behavior of **Ada_Bi1** + **Cuc2** with four kinds of ODNs: Nu1 to Nu4. ODNs concentration: 0.5 μM. Compound concentration is 2.5 μM. These short ODNs were inserted into nucleosome DNA strands. The ratio was calculated based on the equation of the intensity of shifted band ÷ (un-shifted band + shifted band). ODNs show only the forward DNA strand and omits the complementary DNA strand.

Figure S7. Gel electrophoresis of reconstituted nucleosomes



Reconstituted nucleosomes were analyzed by a polyacrylamide gel electrophoresis (6 % DNA retardation gel). Gel running setting is 100 V for 95 min. Gel was stained with SYBR gold for 15 min and visualized with a FujiFilm FLA-3000G fluorescent imaging analyzer.

Additional discussion of *in vitro* HAT-qPCR assay:

- Initially, the authors wanted to keep the insert sequence shorter (binding site 1 to 4) at Nuc 601 sequence as the two more extended insert DNA sequences may result in di-nucleosome formation. As pointed by the reviewer, we realized the issue after obtaining experimental results and have clarified it now in the manuscript. Because of the DNA length limitation of the insert DNA sequence, the ePIP-HoGu binding sites of Nuc2 contains partially (one or two pairs) cooperative binding sites in the center of the sequence (site 2 and 3). *(Nuc2 has the potential synergic binding partially between site 2 and 3, because of the short distance between them).*
- In the case of Nuc3 **Ada_Bi1**, Cuc2 could stabilize the complexes binding to DNA sequence even when there is a mismatch. However, the recruitment of p300 by **Cuc2_Bi** will decrease the overall stabilization of complexes-DNA. Meanwhile, the decrease with **Cuc2_Bi** is less significant because of the overall low acetylation level. The decrease in Nuc4 was not observed possibly owing to the higher DNA binding affinity of PIP-Cuc than PIP-Ada (Supplementary Fig S6).

DNA templates used for nucleosome reconstitution

Binding sequence for PIP conjugates binding

Widom 601 nucleosome positioning sequence

Forward PCR primer to prepare DNA template for nucleosome reconstitution

Primer for in vitro ChIP-PCR

Reverse primer sequence for amplification (common)

601-insert1_full (Nuc1)

*caagcttgcatgcctgcaG*TCACATACATAGGCATTATCAGCTTTATAGGCATTATCAGCTTTATAGGCATT
ATCAGCTTTATAGGCATTATCAGCTT*GC*GGCCGCCCTGGAGAATCCCGGTGCCGAGGCCGCTCAATT
GGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTACGCGCTGTCCCCCGCGTTTTAACCGCCAAG
GGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATACATCCTGT

601-insert2_full (Nuc2)

*caagcttgcatgcctgcaG*TGAGTCACATAGGCATTATAGGCATTATAGGCATTATAGGCATTATCAGCTT
TATCAGCTTTATCAGCTTTATCAGCTT*GC*GGCCGCCCTGGAGAATCCCGGTGCCGAGGCCGCTCAATT
GGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTACGCGCTGTCCCCCGCGTTTTAACCGCCAAG
GGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATACATCCTGT

601-insert3_full (Nuc3)

*caagcttgcatgcctgcaG*GCCACATCATAGGGTTTATCAGCTTTATAGGGTTTATCAGCTTTATAGGGTTT
ATCAGCTTTATAGGGTTTATCAGCTT*GC*GGCCGCCCTGGAGAATCCCGGTGCCGAGGCCGCTCAATT
GGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTACGCGCTGTCCCCCGCGTTTTAACCGCCAAG
GGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATACATCCTGT

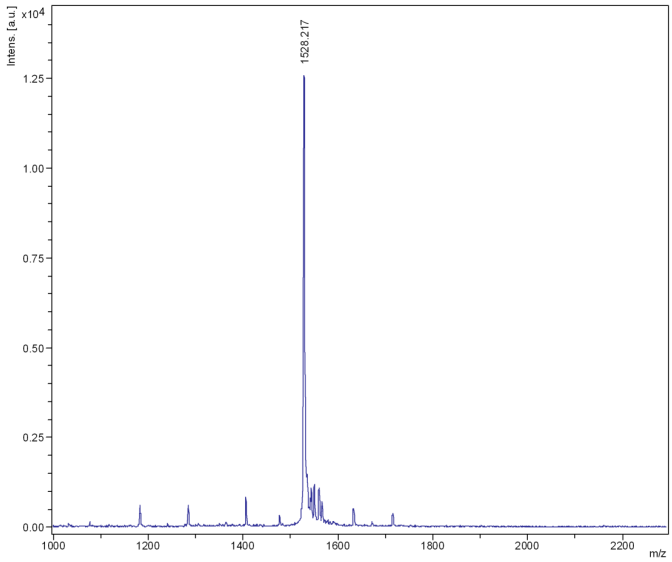
601-insert4_full (Nuc4)

*caagcttgcatgcctgcaG*ACTCCGCAATAGGCATTATCACCTTTATAGGCATTATCACCTTTATAGGCATT
ATCACCTTTATAGGCATTATCACCTT*GC*GGCCGCCCTGGAGAATCCCGGTGCCGAGGCCGCTCAATT
GGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTACGCGCTGTCCCCCGCGTTTTAACCGCCAAG
GGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATACATCCTGT

MS data

Synthesis of Ada3 (positive charge at NH+)

The synthetic procedure was same as **Ada1**, except replacing mini-PEG linker with an adipic acid linker. Adamantyl ethylenediamine was used as guest molecule. **Ada3** was obtained as a white powder. MALDI-TOF MS: *m/z* calcd for $C_{74}H_{97}N_{25}O_{12}^{+}$ $[M+H]^{+}$: 1528.775; found: 1528.217. HPLC: t_R=17.750 min (0.1 % TFA/MeCN, linear gradient 0–100 %, 0–40 min).



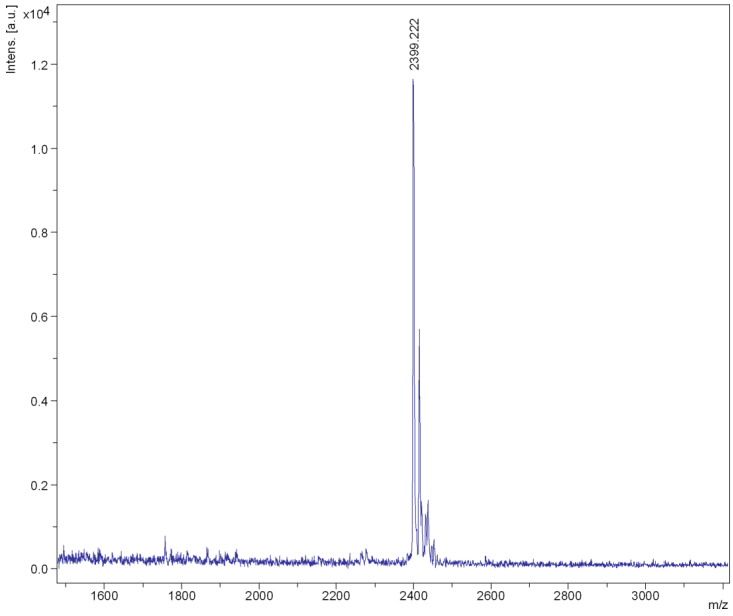
-- % CALCULATION RESULT --

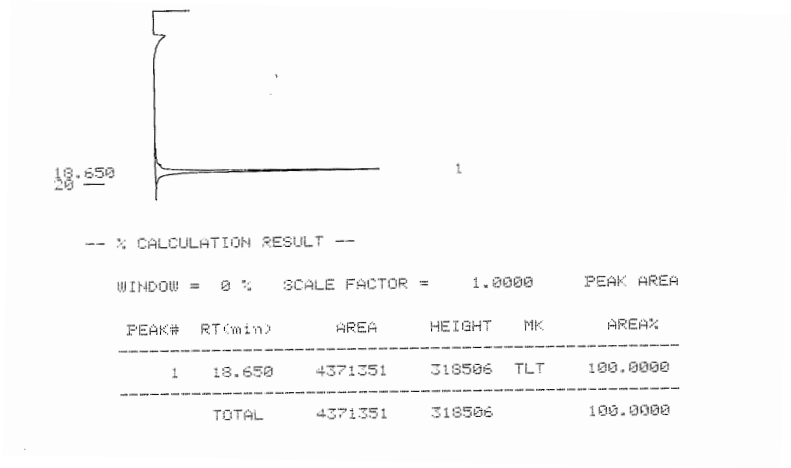
WINDOW = 0 % SCALE FACTOR = 1.0000 PEAK AREA

PEAK#	RT(min)	AREA	HEIGHT	MK	AREA%
1	17.750	16043274	1047078	EEE	100.0000
	TOTAL	16043274	1047078		100.0000

Synthesis of Ada3-biotin (positive charge at NH+)

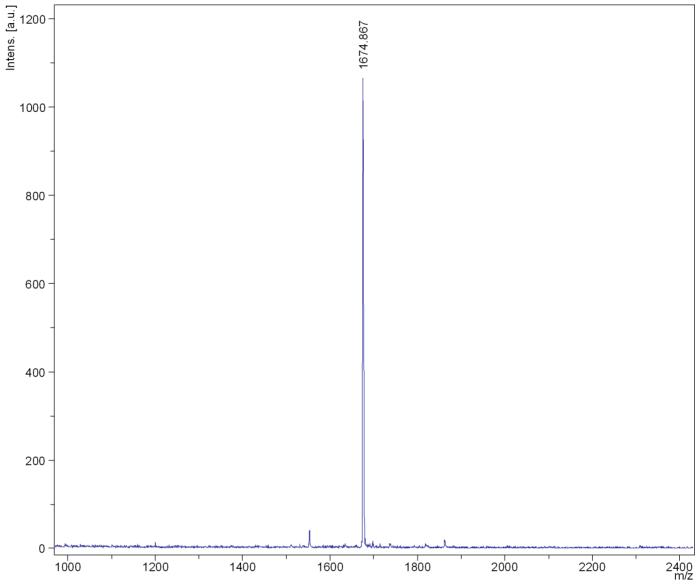
The synthetic procedure was same as **Ada1**, except replacing mini-PEG linker with an adipic acid linker. Adamantyl ethylenediamine was used as guest molecule. **Ada3-biotin** was obtained as a white powder. MALDI-TOF MS: *m*/*z* calcd for $C_{113}H_{170}N_{29}O_{27}S^+$ $[M+H]^+$: 2397.254; found: 2399.222. HPLC: t_R=18.650 min (0.1 % TFA/MeCN, linear gradient 0–100 %, 0–40 min).





Synthesis of Ada4 (positive charge at NH+ and longer linker)

The synthetic procedure was same as **Ada1**. Adamantyl ethylenediamine was used as guest molecule. **Ada4** was obtained as a white powder. MALDI-TOF MS: *m*/*z* calcd for $C_{80}H_{109}N_{26}O_{15}^{+}$ $[M+H]^{+}$: 1673.857; found: 1674.487. HPLC: t_R=17.233 min (0.1 % TFA/MeCN, linear gradient 0–100 %, 0–40 min).

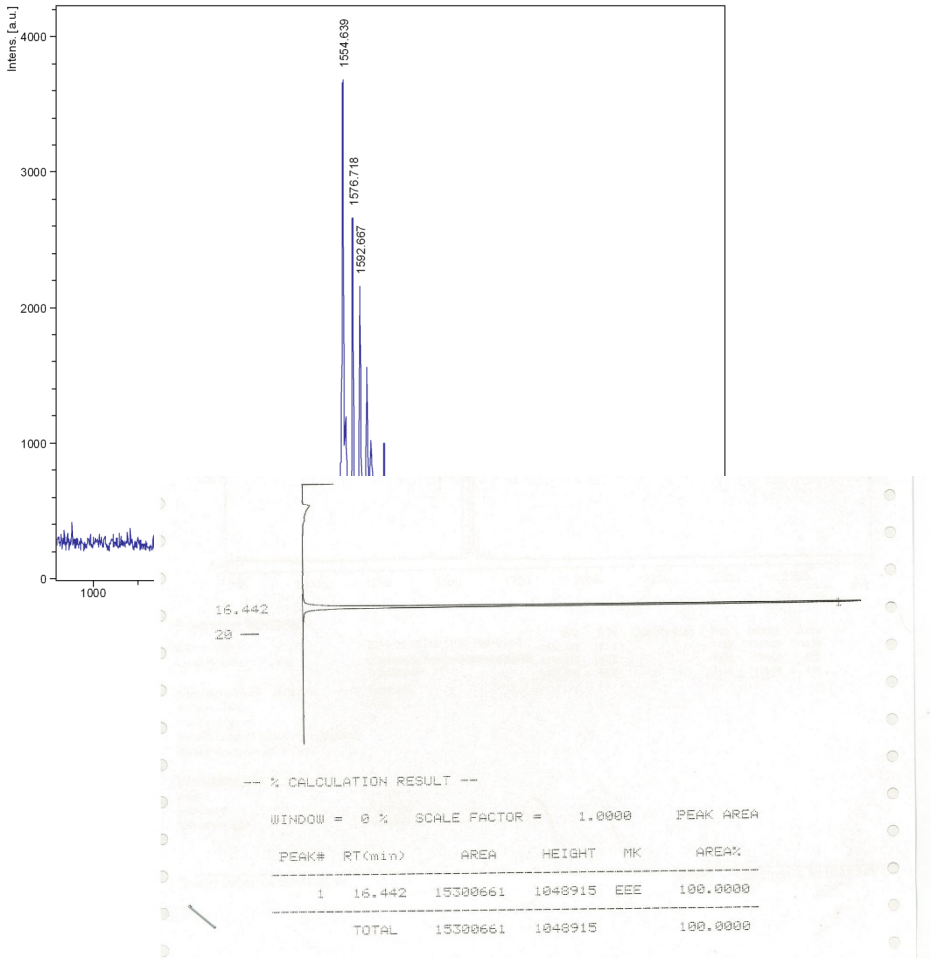


WINDOW = 0 % SCALE FACTOR = 1.0000 PEAK AREA

PEAK#	RT(min)	AREA	HEIGHT	MK	AREA%
1	17.233	2238660	204783	TLT	100.0000
	TOTAL	2238660	204783		100.0000

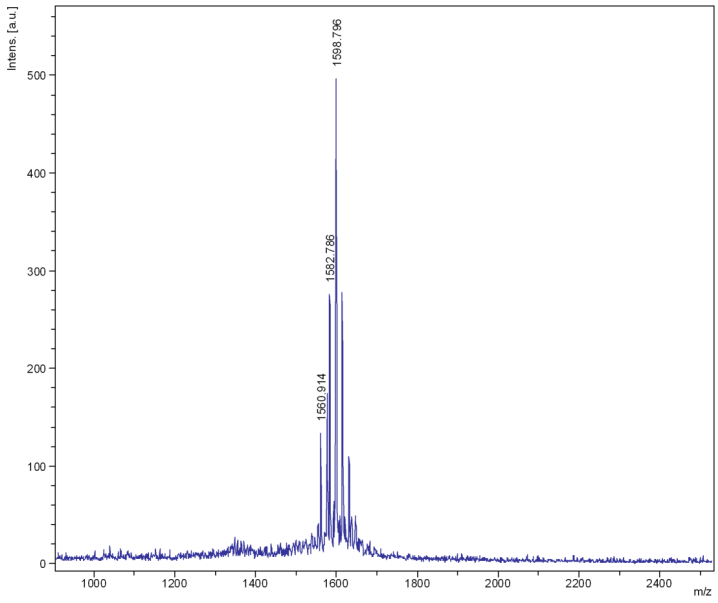
Synthesis of Ada5 (positive charge at NH+)

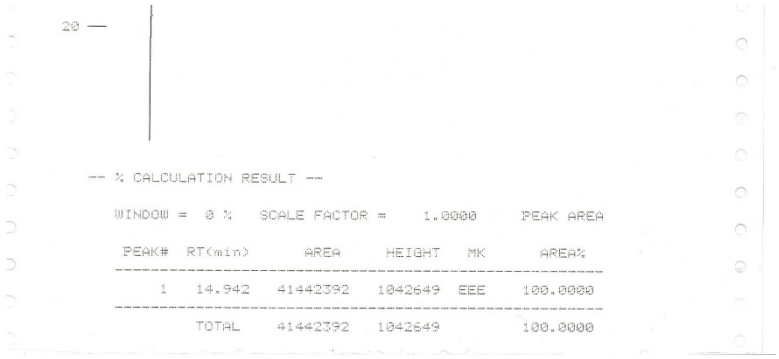
The synthetic procedure was same as **Ada1**. 1-(1-Adamantyl)piperazine was used as guest molecule. **Ada5** was obtained as a white powder. MALDI-TOF MS: *m*/*z* calcd for $C_{76}H_{100}N_{25}O_{12}^{+}$ $[M+H]^{+}$: 1554.798; found: 1554.639. HPLC: t_R=16.442 min (0.1 % TFA/MeCN, linear gradient 0–100 %, 0–40 min).



Synthesis of Ada6 (flexible linker; positive charge at NH+)

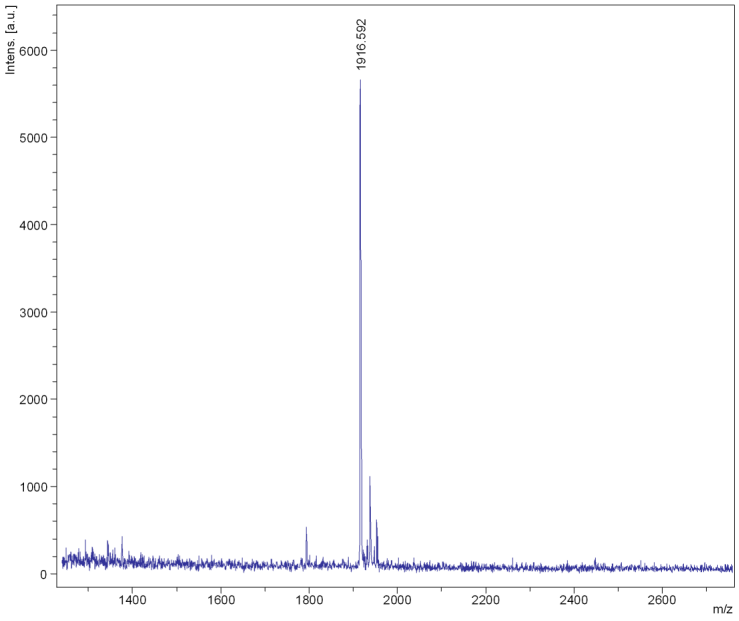
The synthetic procedure was same as **Ada1**. Adamantyl ethylenediamine was used as guest molecule. **Ada6** was obtained as a white powder. MALDI-TOF MS: *m*/*z* calcd for $C_{74}H_{98}N_{25}O_{14}^{+}$ $[M+H]^{+}$: 1560.773; found: 1560.914. HPLC: t_R=14.942 min (0.1 % TFA/MeCN, linear gradient 0–100 %, 0–40 min).

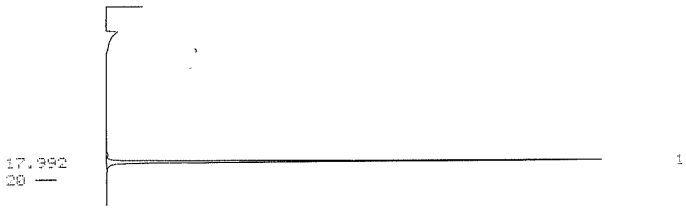




Synthesis of Ada7 (Longer PIP sequence)

The synthetic procedure was same as **Ada1**. Adamantyl ethylenediamine was used as guest molecule. **Ada7** was obtained as a white powder. MALDI-TOF MS: *m*/*z* calcd for $C_{92}H_{120}N_{31}O_{16}^{+}$ $[M+H]^{+}$: 1914.953; found: 1916.592. HPLC: t_R=17.992 min (0.1 % TFA/MeCN, linear gradient 0–100 %, 0–40 min).





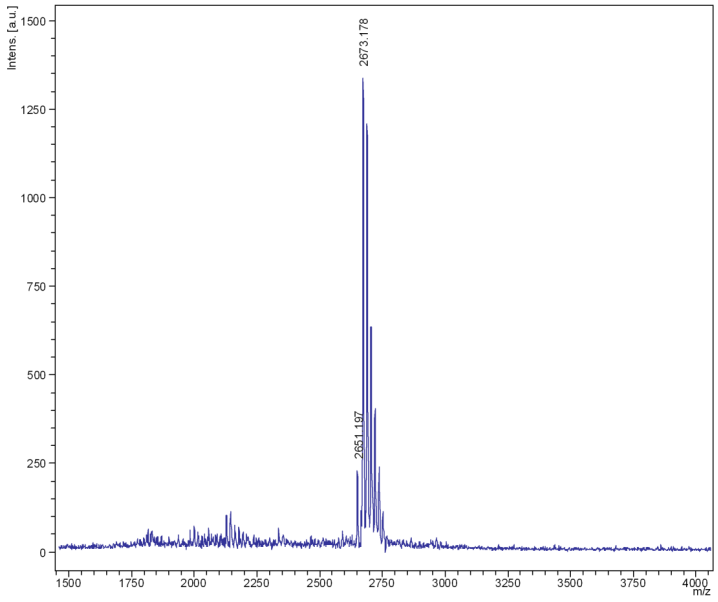
-- % CALCULATION RESULT --

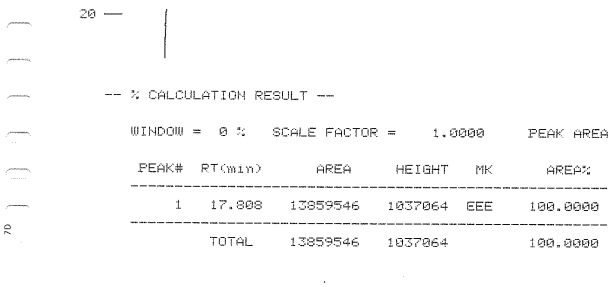
WINDOW = 0 % SCALE FACTOR = 1.0000 PEAK AREA

PEAK#	RT(min)	AREA	HEIGHT	MK	AREA%
1	17.992	6913668	705212	LLL	100.0000
	TOTAL	6913668	705212		100.0000

Synthesis of Ada1_Bi

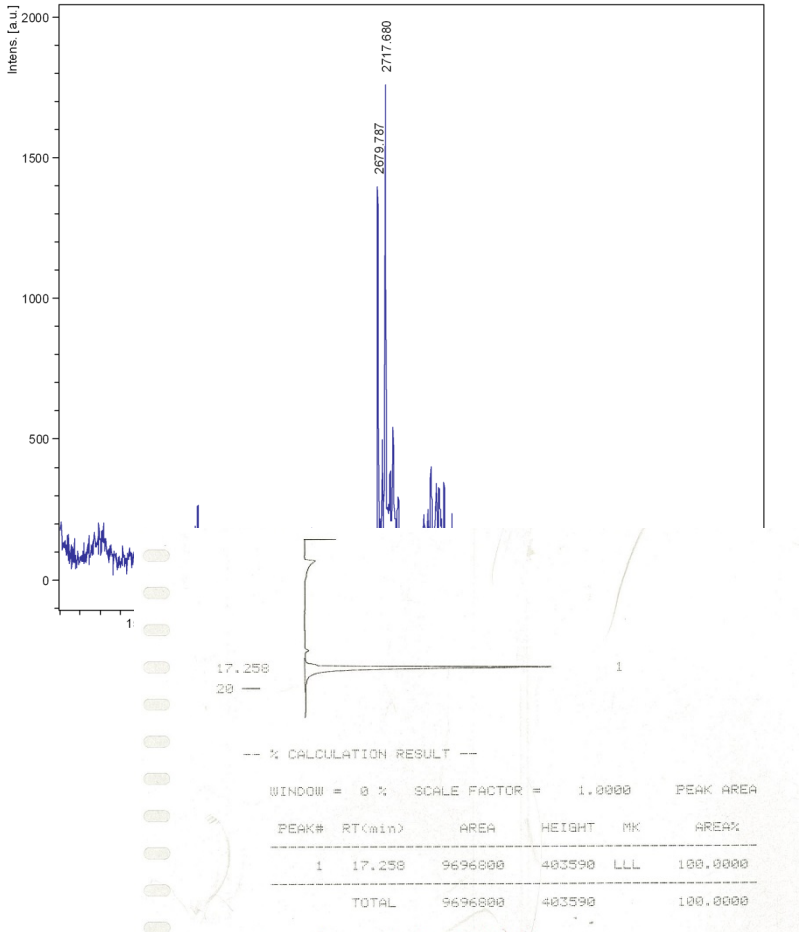
The purified **Ada8**-NH2 was conjugated with Bi catalyzed by HCTU. **Ada1_Bi** was obtained as a white powder. MALDI-TOF MS: *m/z* calcd for $C_{129}H_{177}N_{35}NaO_{27}^{+}$ $[M+H]^{+}$: 2671.345; found: 2673.178. HPLC: t_R=17.808 min (0.1 % TFA/MeCN, linear gradient 0–100 %, 0–40 min).





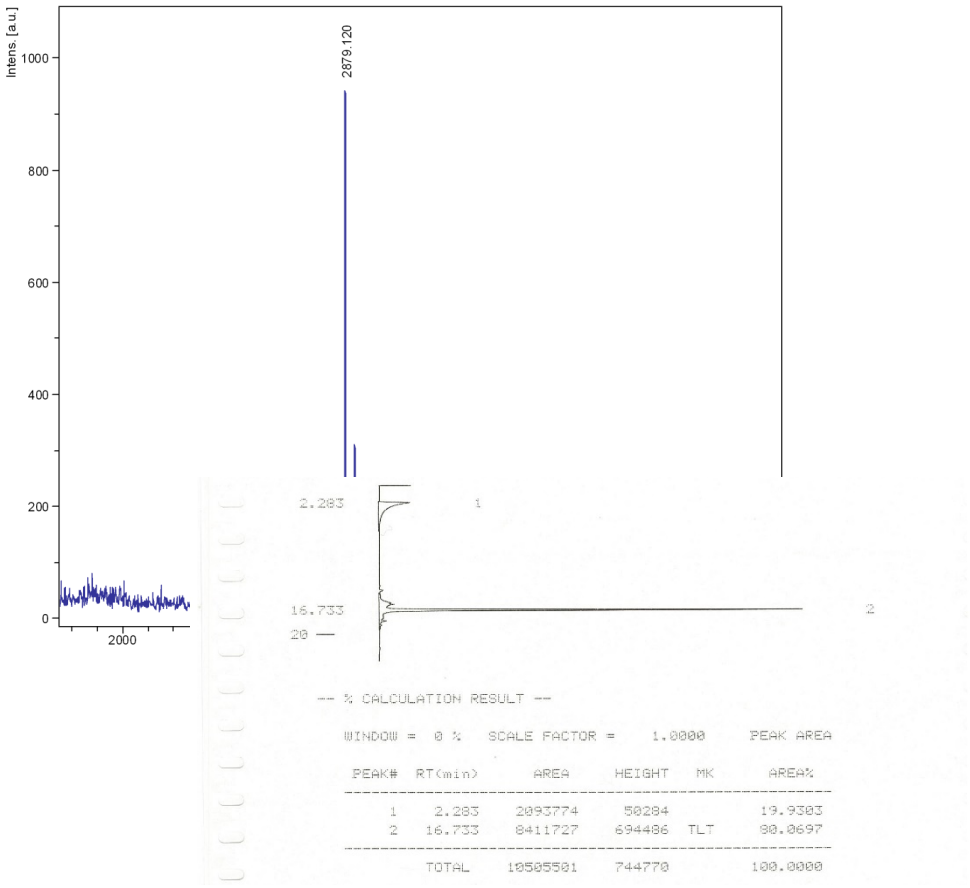
Synthesis of Cuc1

The purified PIPs was conjugated with CB7 catalyzed by CuBr/TBTA. **Cuc1** was obtained as a white powder. MALDI-TOF MS: *m*/*z* calcd for $C_{113}H_{135}N_{54}O_{27}^{+}$ $[M+H]^{+}$: 2680.085; found: 2679.977. HPLC: t_R=17.258 min (0.1 % TFA/MeCN, linear gradient 0–100 %, 0–40 min).



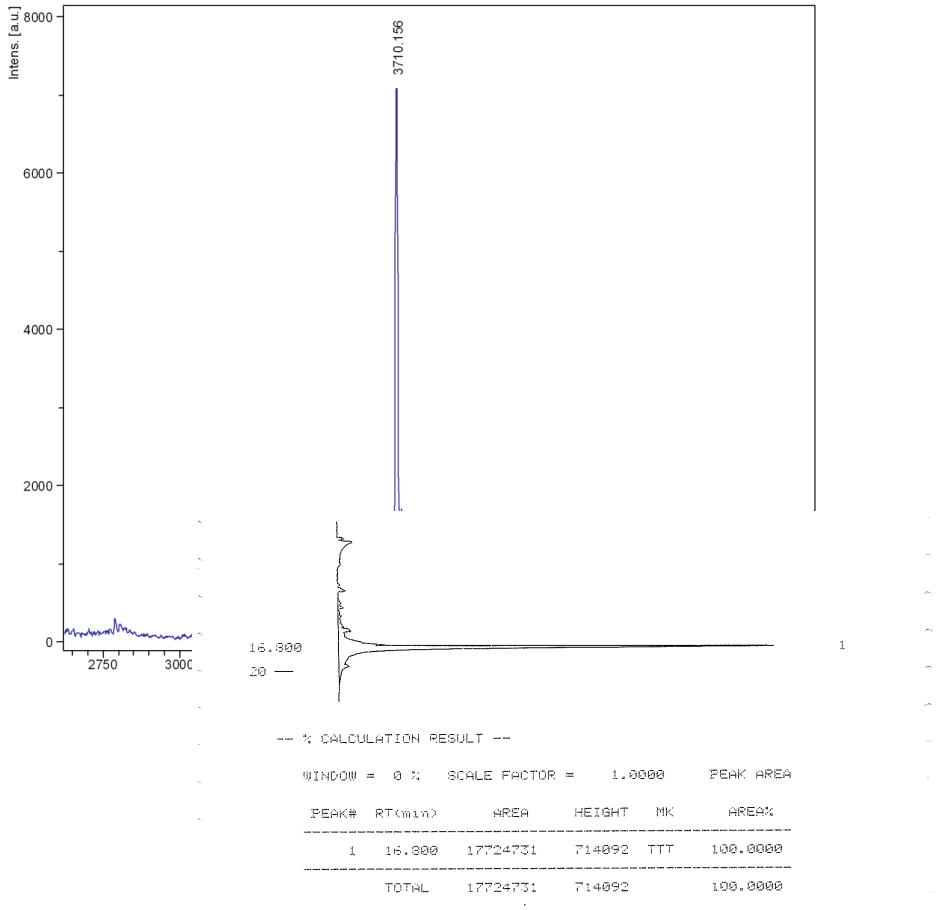
Synthesis of Cuc2

The synthetic procedure was same as **Cuc1**. **Cuc2** was obtained as a white powder. MALDI-TOF MS: *m*/*z* calcd for $C_{121}H_{148}N_{57}O_{30}^{+}$ $[M+H]^{+}$: 2879.181; found: 2879.120. HPLC: t_R=16.733 min (0.1 % TFA/MeCN, linear gradient 0–100 %, 0–40 min). (Mass data was attached in the bottom)



Synthesis of Cuc2_Bi

Cuc2_Bi was obtained as a white powder. MALDI-TOF MS: *m*/*z* calcd for $C_{164}H_{207}N_{64}O_{40}^{+}$ $[M+H]^{+}$: 3712.613; found: 3710.156. HPLC: t_R=16.800 min (0.1 % TFA/MeCN, linear gradient 0–100 %, 0–40 min).



-- % CALCULATION RESULT --

WINDOW = 0 % SCALE FACTOR = 1.0000 PEAK AREA

PEAK#	RT(min)	AREA	HEIGHT	MK	AREA%
1	16.800	17724731	714092	TTT	100.0000
TOTAL		17724731	714092		100.0000

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[1] aZ. Yu, J. Taniguchi, Y. Wei, G. N. Pandian, K. Hashiya, T. Bando, H. Sugiyama, *Eur. J. Med. Chem.* **2017**, *138*, 320-327; bC. Guo, Y. Kawamoto, S. Asamitsu, Y. Sawatani, K. Hashiya, T. Bando, H. Sugiyama, *Bioorg. Med. Chem.* **2015**, *23*, 855-860.

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