Supplementary Information

Pregnenolone activates CLIP-170 to promote microtubule growth and cell migration

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This file includes Supplementary Results (Supplementary Figures/Legends 1 to 18), Supplementary Notes 1 to 7, and Supplementary References 1 to 4.

Supplementary Results

Supplementary Figure 1: P5 regulates cell motility. (a) 3-hour-migration track recording of Y1 cells infected with control shluc or shSCC#4 lentivirus in the presence or absence of P5. Each colored line represents the migration path of one cell. P5 rescued the (b) total migration distance, (c) net migration distance, and (d) directionality of SCC-depleted cells. P5: pregnenolone.
Supplementary Figure 2: Inhibition of P5 production by AG does not affect MT formation but reduces MT growth rate. (a) Analysis of new MT formation. EB1-GFP was transiently expressed in human adrenocortical H295 cells. In the absence (CTR) or presence of SCC inhibitor AG, the emerging numbers of EB1-GFP comets from centrosome were counted. Data shown are means±SEM. (b) Analysis of MT growth by detecting EB1-GFP locations at various time points in the absence (CTR) or presence of AG. Arrows point locations of a particular EB1-GFP comet at different time points.
**Supplementary Figure 3**: P5 promotes MT polymerization requiring the participation of other molecules. (a) P5 does not promote MT polymerization from purified tubulin. Analysis of MT polymerization from purified tubulin in the presence of P5, taxol, H$_2$O, or DMSO. The amounts of polymerized MTs were measured by its absorbance. Taxol is used as a positive control. (b) Effects of P5 metabolites in MT polymerization are tested. The levels of *in vitro* polymerized MTs from tubulin supplemented with zebrafish embryo lysate are shown in immunoblot analysis. The numbers under the panel are the relative MT levels. 7-OH-P5: 7α-hydroxypregnenolone. P5S: pregnenolone sulfate. 17-OH-P5: 17α-hydroxypregnenolone. 17-OH-P4: 17α-hydroxyprogesterone.
Supplementary Figure 4: Summary of synthetic diaminobenzophenone (NBPN)-containing compounds. (a) Schematic illustration of pregnenolone (P5) photoaffinity probes that label their target covalently after irradiation. (b) Chemical structures of synthetic NBPN-containing photoaffinity probes, which contain a ligand linked to NBPN and biotin moieties. The ligand used here are P5 derivatives, in which P5-NBPN (1) and P5β-NBPN (2) have NBPN linked to P5 at C7 with α and β configuration, respectively. P5s-NBPN (3) has a shorter linker connecting P5 to NBPN. Detailed synthesis and characterization of photoaffinity probes is in Supplementary Notes.
Supplementary Figure 5: Identification of Clip1a as the P5-binding candidate. (a) The flowchart for the isolation of P5-associated proteins. Proteins in zebrafish embryo lysate were labeled by P5-NBPN after UV irradiation, enriched by precipitation together with additional MT. The labeled proteins were pulled down by the streptavidin (SA) beads, eluted and subject to gel electrophoresis. MTs: microtubules. Sup: supernatant. Ultra-cfg: ultra-centrifugation. SA: streptavidin. (b) Gel electrophoresis of proteins pulled down by streptavidin beads after proteins in embryo lysates were reacted with DMSO or P5-NBPN. Candidate protein bands were cut from SDS-PAGE and identified by LC-MS/MS. Vg: vitellogenin. (c) Summary of mass spectrometric data for the identification of clip1a. (d) Sequences of the Clip1a peptides identified by mass analysis (shown in red). Zebrafish clip1a is composed of 1411 amino acids.
Supplementary Figure 6: Characterization of P5-NBPN photoaffinity labeling efficiency and specificity. (a) Dose-dependent labeling of purified F-CLIP-170 by P5-NBPN. Each dot in the curve represents mean ± SEM from five independent experiments. (b) Labeling efficiency of P5-NBPN. The left panel is the chemiluminescence detection of known amounts of biotinylated IgG used as a standard for the amount of biotin. The right panel is the detection of biotin moiety in P5-NBPN in triplicate being retained to the membrane by F-CLIP-170 using biotin-based chemiluminescence. (c) Quantification of the amount of F-CLIP-170 labeled by P5-NBPN. P5-NBPN-bound F-CLIP-170 was purified by streptavidin-conjugated beads and visualized by gel silver staining. Different amounts of F-CLIP-170 proteins were used as the protein concentration standard. (d) Labeling of purified F-CLIP-170 by DMSO (-) or NBPN-linked hydroxyl group (OH), P5 or cholesterol (Cho). (e) Competition of P5-NBPN labeling to F-CLIP-170 by DMSO (-), P5, 7-OH-P5, or biotin. Detailed synthesis and characterization of 7-OH-P5 is in Supplementary Note 7.
Supplementary Figure 7: P5-NBPN cannot label MAP2c and tau in whole cell-extract.

(a) Labeling of FLAG tagged tau (F-tau) in whole cell extracts (WCL) by DMSO (-), P5-NBPN (P5), or Cho-NBPN (Cho) before immunoprecipitation of F-tau by FLAG antibody. Input: immunoblot with FLAG without immunoprecipitation. (b) Purification of F-MAP2c from 293T cells. Purified proteins were visualized by SDS-PAGE followed by silver staining. (c) MAP2c was labeled by P5-NBPN and Cho-NBPN as a purified protein (d) but not in whole cell-extract.
Supplementary Figure 8: CLIP-115 is not specifically activated by P5. (a) Visualization of purified F-CLIP-115 by SDS-PAGE followed by silver staining. (b) P5 does not enhance CLIP-115-dependent MT polymerization. (c) Purified CLIP-115 is labeled by both P5-NBPN and Cho-NBPN.
**Supplementary Figure 9:** The middle region of the coiled-coil domain in CLIP-170 is required and sufficient for P5 binding. Detection of P5-binding regions in CLIP-170. Purified various CLIP-170 deletion mutants (Fig. 3a) and FLAG-tagged GFP proteins were reacted with DMSO (-), P5-PBPN (P5), or Cho-NBPN (Cho) in labeling experiments. The labels were detected by streptavidin-HRP (the upper panel in all figures), and the amount of the protein was detected by the FLAG antibody (the lower panel in all figures). MT-binding: the microtubule-binding domain. Zn: zinc knuckle. Cho: cholesterol.
Supplementary Figure 10: Study of CLIP-170 conformation by TEM. TEM images of the purified wildtype F-CLIP-170, F-CLIP-170 + OA, and phospho-mimetic F-CLIP-170-S311D in the presence of P5 or P4. (a) larger view at low magnification. Bar, 200 nm. (b) Individual Y-like and bent molecules, also observed as described before, are defined as open conformation. Bar, 50 nm. (c) Galleries of the open and folded molecules at all conditions.
Supplementary Figure 11: P5 activates CLIP-170 and increases its binding to MTs, p150, and LIS1. (a) Detection of the association of microtubules with F-CLIP-170-S311D in 293T whole cell extract (WCL) after microtubules were precipitated and detected by immunoblotting. S, supernatant; P, pellet. Tub: tubulin staining. The numbers under the panel are the relative CLIP levels of CLIP-170. (b,c) P5 increases binding of wildtype F-CLIP-170 and phospho-mimetic CLIP-170-S311D to p150 and LIS1. Immunoblots show the amount of p150 or LIS1 being precipitated by FLAG antibody after expression of (b) F-CLIP-170 or (c) F-CLIP-170-S311D in cells. The asterisk refers to nonspecific cross-reacting material.
Supplementary Figure 12: P5 is sufficient to increase lengths of CLIP-170-S311D comets. (a) GFP images of U2OS cells expressing EGFP-CLIP-170-S311D in the absence (CTR) or presence P5. The lower figures show enlargement of the boxed areas. The right panel shows quantification of comet lengths. Data shown are means±SEM. Bar, 10 μm. (b) CLIP-170 is required for MT assembly after nocodazole treatment. After nocodazole treatment, MTs were allowed to regrow. MT morphology was monitored by immunostaining. Bar, 10 μm.
Supplementary Figure 13: SCC or Clip-170 depletion reduces the number of EB1 comets, but not EB1 expression. (a) P5 controls MT assembly through CLIP-170. EB1 comets were visualized after immunostaining in control, SCC, or Clip-170 deficient cells. The lower figures show enlargement of the boxed areas. Bar, 10 μm. (b) Y1 Cells were infected with lentivirus harboring luc control, SCC- or Clip-170-targeting shRNA. Immunoblotting shows EB1 protein levels.
Supplementary Figure 14: Genomic structures, protein alignment, and expression pattern of zebrafish clip1a and clip1b. (a) Shaded and open boxes indicate predicted coding and non-coding regions of exons, respectively. Arrows indicate transcriptional start sites. The recognition sites of clip1a MO1 and MO2 are indicated. (b) Comparison of zebrafish and mammalian CLIP-170. The numbers within the domains indicate the degree of amino acid identity to zebrafish clip1a. (c) Phylogenetic tree of zebrafish and mammalian CLIP family. (d) Expression pattern of clip1a, clip1b, and actin at 8-cell (1.25-hpf), 1k-cell (3-hpf), sphere (4-hpf), 30%-epiboly (4.7-hpf), 60%-epiboly (6-hpf), bud (10-hpf), 24-hpf and 48-hpf stages by RT-PCR. hpf: hour post fertilization. CTRL: no cDNA control.
Supplementary Figure 15: clip1a antisense morpholino oligonucleotide targets clip1a and causes embryonic movement delay. (a) A part of gene sequence of clip1a. The translation start site (ATG) is in the parenthesis. The recognition sequences of clip1a MO1 and MO2 are indicated. (b) The structure of GFP fusion mutant (clip1a-GFP) which contains recognition sequences of clip1a MO1 and MO2 in the 5’-UTR. (c,d) Both suboptimal and optimal dosages of clip1a MO1 blocks expression of clip1a-GFP in the embryos injected with 100 pg clip1a-GFP RNA. Expression levels of clip1a-GFP are detected by (c) imaging GFP signals and (d) by immunoblotting. (e) The degree of embryonic cell migration is categorized as normal, mild or severe epiboly delay at 9 hours post-fertilization (hpf).
**Supplementary Figure 16: CLIP-170 is required for P5-promoted MT polymerization.**
P5 enhances MT polymerization after embryo lysates were injected with control (CTR) but not clip1a MO1. Immunoblot analysis of the levels of MT and total tubulin (Total Tub) after *in vitro* MT polymerization assay.
Supplementary Figure 17: Full blot images for cropped gels. The boxed regions are used in figures.
Supplementary Figure 18: Full blot images for cropped gels. The boxed regions are used in figures.
Supplementary Note 1

General materials and methods for organic synthesis. All chemicals were purchased from commercial suppliers (Acros, Aldrich, Sigma and Merck) and used without further purification. Dry tetrahydrofuran (THF) and dry CH$_2$Cl$_2$ were distilled from Na/benzophenone and CaH$_2$, respectively, under N$_2$ prior to use. $^1$H (400 MHz) and $^{13}$C (100 MHz) nuclear magnetic resonance (NMR) spectra were either recorded on a Bruker AVANCE III 400 or a Varian Mercury Plus 400 NMR spectrometer. Chemical shifts were reported in δ (ppm) and calibrated against the deuterated solvents. $^1$H NMR data were reported in the following order: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constants in Hertz, number of protons. High resolution mass spectra were obtained either from a WATERS LCT Premier Xe or a Bruker micrOTOF-QII spectrometer equipped with an electrospray ionization (ESI) system. Infrared spectra were taken on a Varian-640-IR spectrometer. Melting points were determined by a Fargo MP-1D melting point apparatus without correction.
Supplementary Note 2

Synthesis and characterization of 8

**Synthetic scheme of 8**

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N-[4-(4-Aminobenzoyl)phenyl]-2-[2-(2-azidoethoxy)ethoxy]acetamide (6)
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A 25-mL round-bottom flask was charged with 2-[2-(2-azidoethoxy)ethoxy]acetic acid (757 mg, 4.00 mmol), 1-hydroxybenzotriazole (HOBt) (613 mg, 4.00 mmol) and DMF (10 mL) under argon atmosphere with stirring. To the solution was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (777 mg, 4.00 mmol), followed by the addition of 4,4’-diaminobenzophenone (425 mg, 2.00 mmol) after 10 min. After stirring the mixture for 5 h, EtOAc (50 mL) and water (50 mL) were added. Layers were separated. The organic layer was collected and further washed with saturated aqueous solution of NaHCO₃ (2×50 mL), 10% aqueous citric acid (2×50 mL), dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was then purified by flash column chromatography on silica gel with MeOH/CHCl₃ (1:18) as an eluent to yield 6 (391 mg, 1.02 mmol) as a brown-yellowish oil in 51% yield. TLC (MeOH:CHCl₃, 1:18 v/v); Rf = 0.28; \(^1\)H NMR (400 MHz, CDCl₃): \(\delta\) 8.74 (s, 1H), 7.73-7.71 (m, 2H), 7.67-7.64 (m, 4H), 6.68-6.60 (m, 2H), 4.20 (s, 2H), 4.10 (s, 2H), 3.76-3.68 (m, 6H), 3.52 (t, \(J = 4.8\) Hz, 2H); \(^{13}\)C NMR (100 MHz, CDCl₃): \(\delta\) 194.1, 168.2, 150.9, 140.3, 134.6, 132.8, 131.0, 127.5, 119.1, 113.7, 71.2, 70.8, 70.3, 50.7; IR (KBr): 3359 (v N-H) cm\(^{-1}\), 2111 (v N₃) cm\(^{-1}\); ESI-HRMS (m/z): [M+H]\(^+\) calcd. for C₁₉H₂₂N₅O₄, 384.1666; found, 384.1666.

**N-[4-(4-{2-[2-(2-Azidoethoxy)ethoxy]acetamide}benzoyl)phenyl]-2-bromoacetamide (7)**

A flame-dried 25-mL round-bottom flask was charged with 6 (330 mg, 0.86 mmol), Et₃N (0.3 mL, 2.16 mmol), and anhydrous CH₂Cl₂ (5 mL) under argon atmosphere with stirring. The
mixture was then cooled in an ice bath to 0 °C and added a solution of bromoacetyl bromide (0.2 mL, 2.29 mmol) dissolved in dry CH2Cl2 (5 mL) dropwise over 10 min. After the complete addition, the mixture was further stirred at 0 °C for 4 h. Distilled water was added and the layers were separated. The organic layer was collected and washed with saturated aqueous solution of NaHCO3 (2×20 mL), brine (2×20 mL), dried with MgSO4, filtered, and concentrated under reduced pressure. The residue was then purified by flash column chromatography on silica gel with EtOAc/Hexane (2:1) as an eluent to yield 7 (391 mg, 0.65 mmol) as a brown-yellowish oil in 72% yield. TLC (EtOAc:Hexane, 2:1 v/v): Rf = 0.30; 1H NMR (400 MHz, CDCl3): δ 8.81 (s, 1H), 8.73 (s, 1H), 7.80-7.60 (m, 8H), 4.13 (s, 2H), 3.99 (s, 2H), 3.79-3.71 (m, 6H), 3.42 (t, J = 4.8 Hz, 2H); 13C NMR (100 MHz, CDCl3): δ 194.1, 168.4, 164.2, 141.1, 141.0, 133.9, 133.3, 131.4 (2x), 119.3, 119.2, 71.2, 70.7, 70.3 (2x), 50.7, 29.6; IR (KBr): 3318 (ν N-H) cm⁻¹, 2106 (ν N3) cm⁻¹, 1695 (ν C=O) cm⁻¹; ESI-HRMS (m/z): [M-H]⁻ calcd. for C21H21BrN5O5, 502.0732; found, 502.0730.

N-[4-{4-[[2-(2-Azidoethoxy)ethoxy]acetamide]benzoyl}phenyl]-2-aminoacetamide (8)

A 100-mL round-bottom flask was charged with 7 N NH3 in MeOH (30 mL) and cooled in an ice bath to 0 °C with stirring. To this solution was added a solution of 7 (391 mg, 0.65 mmol) dissolved in THF (1 mL) and MeOH (9 mL) dropwise over 30 min at 0 °C. After stirring at 0 °C for additional 30 min, the reaction was warmed up to room temperature and stirred for 11 h. The resulting mixture was concentrated under reduced pressure and then purified by flash column chromatography on silica gel with MeOH/CHCl3 (1:9) as an eluent to afford 8 (261 mg, 0.59 mmol) as a light yellow oil in 91% yield. TLC (MeOH:CHCl3, 1:9 v/v): Rf = 0.20; 1H NMR (400 MHz, CDCl3): δ 9.66 (s, 1H), 8.76 (s, 1H), 7.80-7.67 (m, 8H), 4.12 (s, 2H), 3.78-3.71 (m, 6H), 3.47 (s, 2H), 3.42 (t, J = 4.8 Hz, 2H); 13C NMR (100 MHz, CDCl3): δ 194.4, 171.1, 168.2, 141.4, 141.0, 133.6, 133.1, 131.5, 131.4, 119.1, 118.6, 71.3, 70.8, 70.3 (2x), 50.7, 45.4; IR (KBr): 3359 (ν N-H) cm⁻¹, 2106 (ν N3) cm⁻¹; ESI-HRMS (m/z): [M+H]⁺ calcd. for C21H25N6O5, 441.1881; found, 441.1884.
Supplementary Note 3

Synthesis and characterization of P5-NBPN (1) and P5β-NBPN (2)

1. Bromatin, Cyclohexane, 70 °C, 2 h
2. K₂CO₃, KI, THF, rt, 60 h

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\text{P5-NBPN, 1: 53%} \\
\text{P5β-NBPN, 2: 61%}
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Supplementary Figure 20: Synthetic scheme of P5-NBPN (1) and P5β-NBPN (2)

Methyl 6-[[3β-O-(tert-butyldimethylsilyl)pregn-5-en-7α-yl-20-one]oxy]hexanoate (10a)
Compound 9a was prepared from pregnenalone as previously reported. A suspension of 9a (5.00 g, 11.61 mmol) and 1,3-dibromo-5,5-dimethylhydantoin (Bromatin) (2.16 g, 7.55 mmol) in cyclohexane (60 mL) in a 150-mL two-necked round-bottom flask with stirring was degassed by a vigorous argon sparge, and then heated to 70 °C for 2 h under argon atmosphere. The mixture was filtered immediately while it was hot. The filtrate was concentrated under reduced pressure to yield granular yellow powder, which was taken up in ether (15 mL) and kept at -10 °C for 12 h. The precipitate was then collected by vacuum filtration and washed with cold ether (3 mL) to obtain the brominated compound as a white

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solid. The brominated compound (3.21 g, 6.30 mmol), anhydrous THF (10 mL), K₂CO₃ (3.48 g, 25.18 mmol), and KI (209 mg, 1.26 mmol) were placed in a 100-mL round-bottom flask with stirring and subsequently added methyl 6-hydroxyhexanoate (18.42 g, 126.00 mmol) under argon atmosphere. The solution was allowed to stir at ambient temperature for 60 h. The resulting mixture was filtered, concentrated under reduced pressure, and purified by flash column chromatography on silica gel with EtOAc/Hexane (1:8) as an eluent to yield compound 10a (1.96 g, 3.41 mmol, in α form) as a colorless gelatinous liquid in 30% total yield over two steps. TLC (EtOAc:Hexane, 1:8 v/v): Rᵢ = 0.20; ¹H NMR (400 MHz, CDCl₃): δ 5.61-5.59 (m, 1H), 3.64-3.60 (m, 1H), 3.63 (s, 3H), 3.60-3.50 (m, 1H), 3.40-3.30 (m, 1H), 3.30-3.20 (m, 1H), 2.57 (t, J = 9.2 Hz, 1H), 2.28 (t, J = 8.0 Hz, 2H), 2.27-1.90 (m, 4H), 2.10 (s, 3H), 1.80-1.00 (m, 19H), 0.93 (s, 3H), 0.86 (s, 9H), 0.57 (s, 3H), 0.04 (s, 6H); ¹³C NMR (100 MHz, CDCl₃): δ 209.6, 174.1, 146.3, 121.0, 72.6, 72.4, 69.0, 63.8, 51.7, 49.6, 44.1, 43.1, 42.8, 38.5, 37.7, 37.5, 37.1, 34.4, 32.2, 31.9, 30.3, 26.3, 26.2, 25.1, 24.8, 23.2, 21.1, 18.5, 13.2, -4.2(2×); IR (KBr): 2932 (v C-H) cm⁻¹, 1741 (v C=O) cm⁻¹, 1705 (v C=O) cm⁻¹, 1093 (v C-O) cm⁻¹; ESI-HRMS (m/z): [M+Na]⁺ calcd. for C₃₄H₅₈NaO₅Si, 597.3946; found, 597.3962.

Methyl 6-[(3β-O-acetyl-pregn-5-en-7-yl-20-one)oxy]hexanoate (10b and 10b’)

Starting with 9b instead of 9a, 10b (β form) and 10b’ (α form), which are epimers only differing in the configuration at C7 position, could be obtained by the similar procedure used to prepare 10a. The reaction crude mixture was purified by flash column chromatography on silica gel with EtOAc/Hexane (1:5) as an eluent to yield 10b (610 mg, 1.22 mmol) in 16% yield and 10b’ (920 mg, 1.83 mmol) in 24% yield over two steps as colorless gelatinous liquids. 10b TLC (EtOAc:Hexane, 1:5 v/v): Rᵢ = 0.25; ¹H NMR (400 MHz, CDCl₃): δ 5.42 (s, 1H), 4.60-4.57 (m, 1H), 3.67 (s, 3H), 3.53-3.49 (m, 1H), 3.43-3.41 (m, 1H), 3.24-3.19 (m, 1H), 2.47 (t, J = 9.2 Hz, 1H), 2.40-2.35 (m, 2H), 2.30 (t, J = 7.6 Hz, 2H), 2.10 (s, 3H), 2.03 (s, 3H), 2.00-1.07 (m, 21H), 2.02 (s, 3H), 0.63 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 209.4, 174.1, 146.3, 122.8, 80.6, 73.5, 67.6, 63.2, 56.4, 51.5, 48.2, 44.5, 38.7, 37.9, 37.4, 36.9, 36.7, 34.1, 31.7, 30.1, 27.8, 26.3, 26.0, 24.9, 23.4, 21.5, 21.2, 19.1, 13.4; IR (KBr): 2944 (v C-H) cm⁻¹, 1736 (v C=O) cm⁻¹, 1704 (v C=O) cm⁻¹, 1094 (v C-O) cm⁻¹; ESI-HRMS (m/z): [M+Na]⁺ calcd. for C₃₀H₄₆NaO₆, 525.3187; found, 525.3192. 10b’ TLC (EtOAc:Hexane, 1:5 v/v): Rᵢ = 0.27; ¹H NMR (400 MHz, CDCl₃): δ 5.65-5.62 (m, 1H), 4.65-4.60 (m, 1H), 3.64 (s, 3H), 3.62-3.58 (m, 1H), 3.48-3.46 (m, 1H), 3.31-3.19 (m, 1H), 2.58 (t, J = 9.2 Hz, 1H), 2.40-2.30 (m, 4H), 2.20-2.10 (m, 3H), 2.02 (s, 3H), 2.00-1.07 (m, 21H), 0.96 (s, 3H), 0.59 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 209.7,174.1, 170.4, 144.5, 122.5, 73.7, 72.1, 68.8, 63.8, 51.7, 49.5, 44.1, 42.7, 38.4, 37.8, 37.5, 36.7, 34.4, 31.9, 30.3, 27.8, 26.3, 25.1, 24.8, 23.2, 21.7, 21.1, 18.5, 13.2; ESI-HRMS (m/z): [M+Na]⁺ calcd. for C₃₀H₄₆NaO₆, 525.3187; found, 525.3196.
2,5-Dioxopyrrolidin-1-yl 6-[(3β-O-(tert-butyldimethylsilyl)pregn-5-en-7β-yl-20-one)oxy]hexanoate (11a)

To a stirred solution of 10a (1.20 g, 2.09 mmol) in THF (2 mL) and MeOH (10 mL) was added 1 M aqueous LiOH (2.52 mL) at 0 °C. After stirring at 0 °C for 20 min, the reaction was warmed up to room temperature and stirred for additional 12 h. The mixture was neutralized by Doxex-50 ion-exchange resin and filtered. The filtrate was concentrated under reduced pressure and the residue was then dissolved in EtOAc (30 mL), washed with brine (2×20 mL), dried over MgSO₄, filtered, and concentrated under reduced pressure to yield the crude hydrolyzed product. A 25-mL round-bottom flask was charged with the crude hydrolyzed compound, N,N'-dicyclohexylcarbodiimide (DCC) (650 mg, 3.15 mmol), N-hydroxysuccinimide (NHS) (725 mg, 6.30 mmol), and anhydrous CH₂Cl₂ (15 mL) with stirring. Under argon atmosphere, the reaction mixture was stirred for 10 h. The resulting mixture was filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel with EtOAc/Hexane (1:2) as an eluent to yield compound 11a (981 mg, 1.49 mmol) as a colorless gelatinous liquid in 71% yield over two steps. TLC (EtOAc:Hexane, 1:2 v/v): Rf = 0.32; ¹H NMR (400 MHz, CDCl₃): δ 5.61-5.58 (m, 1H), 3.63-3.60 (m, 1H), 3.60-3.52 (m, 1H), 3.40-3.35 (m, 1H), 3.30-3.20 (m, 1H), 2.80 (s, 4H), 2.61-2.50 (m, 3H), 2.40-2.10 (m, 3H), 2.10 (s, 3H), 1.98-1.92 (m, 1H), 1.80-1.30 (m, 16H), 1.30-1.00 (m, 3H), 0.94 (s, 3H), 0.87 (s, 9H), 0.58 (s, 6H); ¹³C NMR (100 MHz, CDCl₃): δ 209.7, 169.1, 168.5, 146.3, 121.0, 72.6, 72.3, 68.8, 63.7, 49.5, 44.1, 43.1, 42.8, 38.4, 37.7, 37.5, 37.0, 32.1, 31.9, 31.2, 30.1, 26.2, 26.0, 25.9, 24.8 (2×), 23.2, 21.1, 18.5(2×), 13.2, -4.2 (2×); IR (KBr): 2931 (v C-H) cm⁻¹, 1743 (v C=O) cm⁻¹, 1703 (v C=O) cm⁻¹, 1090 (v C-O) cm⁻¹; ESI-HRMS (m/z): [M+Na]⁺ calcd. for C₃₇H₅₉NNaO₇Si, 680.3953; found, 680.3983.

2,5-Dioxopyrrolidin-1-yl 6-[(pregn-3β-ol-5-en-7β-yl-20-one)oxy]hexanoate (11b)

Starting with 10b, compound 11b was prepared by the similar procedure used for the synthesis of 11a. The crude product was purified by flash column chromatography on silica gel with EtOAc/Hexane (7:4) as an eluent to yield compound 11b (271 mg, 0.50 mmol) as a colorless gelatinous liquid in 60% yield over two steps. TLC (EtOAc:Hexane, 7:4 v/v): Rf = 0.20; ¹H NMR (400 MHz, CDCl₃): δ 5.36 (s, 1H), 3.54-3.40 (m, 3H), 3.23-3.19 (m, 1H), 2.80 (s, 4H), 2.57 (t, J = 7.2 Hz, 2H), 2.44 (t, J = 9.2 Hz, 1H), 2.40-2.12 (m, 3H), 2.08 (s, 3H), 2.01-1.02 (m, 21H), 0.99 (s, 3H), 0.60 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 209.5, 168.9, 168.3, 143.3, 121.8, 80.5, 71.3, 67.1, 63.1, 56.3, 48.2, 44.4, 41.9, 38.7, 37.2, 37.0, 36.5, 31.6, 31.5, 30.9, 29.8, 26.2, 25.6 (2×), 24.5, 23.3, 21.2, 19.1, 13.3; IR (KBr): 3510 (v O-H) cm⁻¹, 2931 (v C-H) cm⁻¹, 1743 (v C=O) cm⁻¹, 1703 (v C=O) cm⁻¹, 1090 (v C-O) cm⁻¹; ESI-HRMS: [M+Na]⁺ calcd. for C₃₇H₄₅NNaO₇, 566.3088; found, 566.3098.
**N-[4-{4-[2-{2-Azidoethoxy)ethoxy]acetamido}benzoyl]phenyl]-2-({6-[3β-O-(tert-butyl dimethylsilyl)pregn-5-en-7α-yl-20-one]oxy}hexanamido)acetamide (12a)**

A 25-mL round-bottom flask was charged with 11a (323 mg, 0.49 mmol) and anhydrous DMF (5 mL) with stirring under argon atmosphere. To this stirred solution were added 8 (261 mg, 0.59 mmol) and Et$_3$N (137 μL, 0.98 mmol). The reaction mixture was allowed to stir at ambient temperature for 10 h. The resulting mixture was concentrated under reduced pressure, and then purified by flash column chromatography on silica gel with EtOAc/Hexane (3:1) as an eluent to yield compound 12a (390 mg, 0.40 mmol) as a light yellow oil in 81% yield. TLC (EtOAc:Hexane, 3:1 v/v): R$_f$ = 0.20; $^1$H NMR (400 MHz, CDCl$_3$): δ 9.59 (s, 1H), 8.77 (s, 1H), 7.76-7.65 (m, 8H), 6.82-6.75 (m, 1H), 5.60-5.55 (m, 1H), 4.22-4.20 (m, 2H), 4.12 (s, 2H), 3.80-3.70 (m, 6H), 3.65-3.60 (m, 1H), 3.58-3.52 (m, 1H), 3.48-3.40 (m, 2H), 3.38-3.30 (m, 1H), 3.25-3.20 (m, 1H), 2.54 (t, J = 9.2 Hz, 1H), 2.32 (t, J = 8.0 Hz, 2H), 2.30-2.10 (m, 4H), 2.11 (s, 3H), 1.98-1.92 (m, 1H), 1.80-1.00 (m, 18H), 0.91 (s, 3H), 0.84 (s, 9H), 0.55 (s, 3H), 0.02 (s, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 209.8, 194.3, 174.1, 168.3, 167.4, 146.3, 141.7, 141.0, 133.5, 133.2, 131.3, 120.9, 119.2, 118.9, 72.5, 72.3, 71.2, 70.7, 70.3, 68.9, 63.8, 50.7, 49.5, 44.7, 44.1, 43.0, 42.8, 38.4, 37.7, 37.4, 37.0, 36.6, 32.1, 31.8, 30.4, 26.3, 26.2, 26.0, 25.8, 24.8, 23.1, 21.0, 18.5 (2x), 13.2, -4.1, -4.2; IR (KBr): 3343 (ν N-H) cm$^{-1}$, 2105 (ν N$_3$) cm$^{-1}$, 1700 (ν C=O) cm$^{-1}$, 1093 (ν C-O) cm$^{-1}$; ESI-HRMS (m/z): [M-H]$^-$ calcd. for C$_{54}$H$_{77}$N$_6$O$_9$Si, 981.5527; found, 981.5516.

**N-[4-{4-[2-{2-Azidoethoxy)ethoxy]acetamido}benzoyl]phenyl]-2-{{6-(pregn-3β-ol-5-en-7β-yl-20-one)oxy}hexanamido}acetamide (12b)**

Compound 12b was prepared by the similar procedure used for the synthesis of 12a. The crude product was purified by flash column chromatography on silica gel with MeOH/CHCl$_3$ (1:15) as an eluent to yield 12b (288 mg, 0.33 mmol) as a light yellow oil in 90% yield. TLC (MeOH:CHCl$_3$, 1:15 v:v): R$_f$ = 0.15; $^1$H NMR (400 MHz, CDCl$_3$): δ 9.63 (s, 1H), 8.79 (s, 1H), 7.75-7.61 (m, 8H), 6.94-6.91 (m, 1H), 5.30 (s, 1H), 4.17-4.14 (m, 2H), 4.12 (s, 2H), 3.77-3.70 (m, 6H), 3.40-3.38 (m, 2H), 3.36-3.31 (m, 1H), 3.20-3.15 (m, 1H), 2.60 (s, 1H), 2.43 (t, J = 9.2 Hz, 1H), 2.40-2.10 (m, 6H), 2.08 (s, 3H), 1.99-1.96 (m, 1H), 1.80-1.00 (m, 18H), 0.97 (s, 3H), 0.58 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 209.6, 194.1, 174.1, 168.1, 167.4, 143.3, 141.6, 140.7, 133.2, 132.9, 131.1, 121.7, 119.0, 118.7, 80.5, 71.2, 71.0, 70.5, 70.0 (2x), 67.2, 63.1, 56.3, 50.4, 48.1, 44.4, 41.9, 38.6, 37.2, 37.0, 36.5, 36.2, 31.6, 31.4, 30.0, 30.3, 26.2, 25.9, 25.5, 23.3, 21.1, 19.1, 13.3; IR (KBr): 3328 (ν N-H) cm$^{-1}$, 2934 (ν C-H) cm$^{-1}$, 2104 (ν N$_3$) cm$^{-1}$, 1700 (ν C=O) cm$^{-1}$; ESI-HRMS (m/z): [M+Na]$^+$ calcd. for C$_{48}$H$_{64}$N$_6$O$_9$Na, 891.4627; found, 891.4634.
A solution of 12a (390 mg, 0.40 mmol) in THF (5 mL) was added 1 M TBAF in THF (1.2 mL, 1.2 mmol) with stirring. The reaction mixture was allowed to stir at ambient temperature for 14 h. To the solution was then added EtOAc (20 mL), washed with distilled water (2×20 mL), dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was then purified by flash column chromatography on silica gel with MeOH/CHCl₃ (1:15) as an eluent to yield compound 13 (316 mg, 0.36 mmol) as a light-yellow oil in 91% yield. TLC (MeOH:CHCl₃, 1:15 v/v): Rᵣ = 0.15; ¹H NMR (400 MHz, CDCl₃): δ 9.67 (s, 1H), 8.80 (s, 1H), 7.76-7.30 (m, 8H), 6.92-6.89 (m, 1H), 5.60-5.52 (m, 1H), 4.17-4.12 (m, 2H), 4.12 (s, 2H), 3.76-3.68 (m, 6H), 3.60-3.50 (m, 2H), 3.41 (t, J = 4.4 Hz, 2H), 3.35-3.30 (m, 1H), 3.23-3.20 (m, 1H), 2.73 (s, 1H), 2.53 (t, J = 8.8 Hz, 1H), 2.40-2.10 (m, 6H), 2.11 (s, 3H), 1.98-1.92 (m, 1H), 1.80-1.00 (m, 18H), 0.87 (s, 3H), 0.55 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 209.9, 194.4, 174.2, 168.3, 167.6, 145.6, 141.8, 141.0, 133.5, 133.2, 131.4, 131.3, 121.4, 119.2, 119.0, 76.9, 72.3, 71.5, 71.2, 70.7, 70.3, 68.7, 63.7, 50.7, 49.5, 44.6, 44.1, 42.8, 42.5, 38.4, 37.6, 37.4, 37.0, 36.6, 31.9, 31.5, 30.3, 26.2, 25.7, 24.7, 23.1, 21.1, 18.5, 13.2; IR (KBr): 3338 (v N-H) cm⁻¹, 2108 (v N₃) cm⁻¹, 1790 (v C=O) cm⁻¹, 1088 (v C-O) cm⁻¹; ESI-HRMS (m/z): [M+Na⁺]⁺ calcd. for C₄₈H₆₄N₆NaO₉, 891.4627; found, 891.4639.

A solution of 13 (316 mg, 0.36 mmol) in THF (3 mL) and distilled H₂O (0.5 mL) was added PPh₃ (113 mg, 0.43 mmol) with stirring. The reaction mixture was allowed to stir at ambient temperature for 18 h. The resulting mixture was concentrated under reduced pressure. The residue was then dissolved in anhydrous DMF (2 mL) in a 25-mL round-bound flask with stirring under argon atmosphere. To the stirred solution were added Biotin-OSu (147 mg, 0.43 mmol) and Et₃N (100 μL, 0.72 mmol). The reaction mixture was allowed to stir at ambient temperature for 10 h. After that, the mixture was then concentrated under reduced pressure and purified by flash column chromatography on silica gel with MeOH/CHCl₃ (1:8) as an eluent to yield compound P5-NBPN (1) (203 mg, 0.19 mmol) as a light yellow oil in 53% yield over two steps. TLC (MeOH:CHCl₃, 1:8 v/v): Rᵣ = 0.20; ¹H NMR (400 MHz, CD₃OD): δ 7.70-7.90 (m, 8H), 5.75-5.72 (m, 1H), 4.50-4.40 (m, 1H), 4.25-4.20 (m, 1H), 4.18 (s, 2H), 4.04 (s, 2H), 3.80-3.60 (m, 7H), 3.55-3.38 (m, 4H), 3.38-3.30 (m, 2H), 3.20-3.10 (m, 1H), 2.90-2.80 (m, 1H), 2.70-2.60 (m, 4H), 2.20-2.10 (m, 3H), 2.10 (s, 3H), 1.98-1.92 (m, 1H), 1.80-1.00 (m, 26H), 0.94 (s, 3H), 0.60 (s, 3H); ¹³C NMR (100 MHz, CD₃OD): δ 212.2, 196.1, 176.5, 176.0, 171.1, 169.9, 147.2, 143.9, 143.1, 134.5, 133.9, 133.2, 132.2, 122.3, 120.5, 120.0, 73.7, 72.2, 71.1, 70.7, 69.9, 64.8, 63.4, 61.7, 57.1, 50.8, 45.1, 44.3, 44.2, 43.2, 41.2, 40.3, 39.6, 38.7, 38.3, 37.0, 36.9, 32.3, 32.0, 31.3, 30.0, 29.7, 27.4, 27.0, 26.8, 25.8, 24.0,
22.2, 19.0, 13.6; IR (KBr): 3428 (ν N-H) cm⁻¹, 1694 (ν C=O) cm⁻¹; ESI-HRMS (m/z): [M+Na]^+ calcd. for C₅₈H₈₀N₆NaO₁₁S, 1091.5498; found, 1091.5503.


Compound P5β-NBPN (2) was prepared by the similar procedure used for the synthesis of P5-NBPN (1). The crude product was purified by flash column chromatography on silica gel with MeOH/CHCl₃ (1:8) as an eluent to yield P5β-NBPN (2) (261 mg, 0.24 mmol) as a light yellow oil in 61% yield over two steps. TLC (MeOH:CHCl₃, 1:8 v/v): Rᵣ = 0.20; ¹H NMR (400 MHz, CD₃OD): δ 7.82-7.76 (m, 8H), 5.42 (s, 1H), 4.48-4.45 (m, 1H), 4.27-4.24 (m, 1H), 4.20 (s, 2H), 4.06 (s, 2H), 3.80-3.72 (m, 4H), 3.63 (t, J = 5.6 Hz, 2H), 3.60-3.56 (m, 1H), 3.49-3.39 (m, 4H), 3.29-3.25 (m, 2H), 3.15-3.10 (m, 1H), 2.90-2.85 (m, 1H), 2.69-2.54 (m, 2H), 2.34-2.17 (m, 6H), 2.12 (s, 3H), 2.01-2.03 (m, 1H), 1.90-1.10 (m, 25H), 1.03 (s, 3H), 0.60 (s, 3H); ¹³C NMR (100 MHz, CD₃OD): δ 212.2, 196.1, 176.6, 175.9, 171.1, 169.9, 165.9, 145.2, 143.9, 143.0, 134.5, 133.9, 132.3, 132.2, 122.7, 120.5, 120.0, 82.0, 72.2, 72.1, 71.7, 71.1, 70.7, 68.5, 64.2, 63.4, 61.7, 57.6, 57.1, 45.6, 44.3, 42.8, 41.2, 40.3, 39.8, 38.6, 38.3, 37.8, 36.9 (2×), 32.4, 32.0, 31.2, 30.0, 29.6, 27.6, 27.1, 27.0, 26.9, 24.4, 22.5, 19.6, 13.9; IR (KBr): 3270 (ν N-H) cm⁻¹, 1693 (ν C=O) cm⁻¹; ESI-HRMS: [M+H]^+ calcd. for C₅₈H₈₁N₆O₁₁S, 1069.5679; found, 1069.5688.
Supplementary Note 4

Synthesis and characterization of P5s-NBPN (3)

1. 1 M LiOH(aq), THF, MeOH, rt, 12 h
2. 4,4'-diaminobenzophenone, EDCI, HOBt, DMF, rt, 8 h, 56%

Bromoacetyl bromide, Et3N

CH2Cl2, 0 °C, 4 h, 67%

7 N NH3 in MeOH

MeOH, THF, 0 °C to rt

11 h, 80%

DCC, DMAP, CH2Cl2, rt, 3 h, 69%

TBAF, THF

50 °C, 10 h, 90%

1. PPh3, THF
   H2O, rt, 13 h
2. Biotin-OSu, Et3N
   DMF, rt, 6 h, 63%

Supplementary Figure 21: Synthetic scheme of P5s-NBPN (3)
Starting with compound 10a (540 mg, 0.94 mmol), saponification was effected as the procedure described in the preparation of 11a. The hydrolyzed compound was then dissolved in DMF (10 mL) under argon atmosphere. To the stirring mixture was added EDC (217 mg, 1.13 mmol), HOBt (173 mg, 1.13 mmol), and 4,4’-diaminobenzophenone (240 mg, 1.13 mmol). After stirring the reaction mixture for 8 h at ambient temperature, ETOAc (50 mL) was added. The combined organic layer was washed with saturated aqueous solution of NaHCO₃ (2×50 mL) and 10% aqueous citric acid (2×50 mL), dried over MgSO₄, filtered, concentrated under reduced pressure and then purified by flash column chromatography on silica gel with EtOAc/Hexane (1:1) as an eluent to yield 14 (397 mg, 0.53 mmol) as a light-yellow oil in 56% yield over two steps. TLC (EtOAc:Hexane, 1:1 v/v): Rf = 0.30; ¹H NMR (400 MHz, CDCl₃): δ 7.83 (s, 1H), 7.70-7.55 (m, 6H), 6.63 (d, J = 8.8 Hz, 2H), 5.62-5.58 (m, 1H), 4.18 (s, 2H), 3.65-3.60 (m, 1H), 3.60-3.48 (m, 1H), 3.36-3.30 (m, 1H), 3.30-3.18 (m, 1H), 2.54 (t, J = 9.1 Hz, 1H), 2.37 (t, J = 8.0 Hz, 2H), 2.32-2.21 (m, 1H), 2.21-2.08 (m, 2H), 2.07 (s, 3H), 1.96-1.90 (m, 1H), 1.80-1.00 (m, 19H), 0.93 (s, 3H), 0.86 (s, 9H), 0.56 (s, 3H), 0.03 (s, 6H); ¹³C NMR (100 MHz, CDCl₃): δ 209.9, 194.3, 171.6, 150.9, 146.3, 141.2, 134.1, 132.7, 131.0, 127.4, 120.8, 118.6, 113.6, 72.4, 72.1, 68.8, 63.6, 49.3, 43.8, 42.8, 42.6, 38.2, 37.6, 37.4, 37.2, 36.7, 31.5, 30.1, 26.0, 25.9, 25.3, 24.5, 22.8, 20.8, 18.2, 18.1, 12.8, -4.6(2×); IR (NaCl): 3354 (μ C=H) cm⁻¹, 2934 (μ C=H) cm⁻¹, 1700 (μ C=O) cm⁻¹, 1596 (μ C=O) cm⁻¹, 1596 (μ C=O) cm⁻¹, 1090 (μ C=O) cm⁻¹; ESI-HRMS (m/z): [M+Na]+ calcd. for C₄₆H₆₆N₂NaO₅Si, 777.4633; found, 777.4659.

Compound 15 was prepared by the similar procedure used for the synthesis of 7. The crude product was purified by flash column chromatography on silica gel with EtOAc/Hexane (7:4) as an eluent to yield compound 15 (250 mg, 0.29 mmol) as a light-yellow oil in 67% yield. TLC (EtOAc:Hexane, 7:4 v/v): Rf = 0.33; ¹H NMR (400 MHz, CDCl₃): δ 8.53 (s, 1H), 7.80-7.70 (m, 5H), 7.63 (t, J = 8.4 Hz, 4H), 5.62-5.58 (m, 1H), 4.01 (s, 2H), 3.70-3.60 (m, 1H), 3.60-3.48 (m, 1H), 3.36-3.30 (m, 1H), 3.30-3.18 (m, 1H), 2.54 (t, J = 9.2 Hz, 1H), 2.39 (t, J = 7.2 Hz, 2H), 2.32-2.21 (m, 1H), 2.21-2.08 (m, 2H), 2.08 (s, 3H), 1.96-1.90 (m, 1H), 1.80-1.00 (m, 19H), 0.93 (s, 3H), 0.86 (s, 9H), 0.56 (s, 3H), 0.03 (s, 6H); ¹³C NMR (100 MHz, CDCl₃): δ 210.0, 194.4, 171.6, 163.9, 146.4, 142.0, 140.7, 134.1, 132.9, 131.4, 131.3, 120.8, 119.1, 118.7, 72.1, 68.8, 63.6, 49.3, 43.9, 42.8, 42.6, 38.2, 37.7, 37.4, 37.2, 36.8, 31.8, 31.6, 30.1, 29.3, 26.0, 25.9, 25.3, 24.5, 22.8, 20.8, 18.2(2×), 12.9, -4.5, -4.6; IR (NaCl): 3410 (μ C-H) cm⁻¹, 2934 (μ C-H) cm⁻¹, 1698 (μ C=O) cm⁻¹, 1596 (μ C=O) cm⁻¹, 1528 (μ C=O) cm⁻¹, 1092 (μ C=O) cm⁻¹; ESI-HRMS (m/z): [M+Na]+ calcd. for C₄₆H₆₆BrₓNaO₅Si, 897.3844;
**N-{4-[4-(2-Aminoacetamido)benzoyl]phenyl}-6-{[3β-O-(tert-butyldimethylsilyl)pregn-5-en-7α-yl-20-one]oxy}hexanamide (16)**

Compound 16 was prepared by the similar procedure used for the synthesis of 8. The crude product was purified by flash column chromatography on silica gel with MeOH/CHCl₃ (1:9) as an eluent to yield compound 16 (150 mg, 0.18 mmol) as a pale-yellow oil in 80% yield. TLC (MeOH:CHCl₃, 1:9 v/v): Rf = 0.30; ¹H NMR (400 MHz, CDCl₃): δ 9.67 (s, 1H), 7.80-7.58 (m, 9H), 5.62-5.58 (m, 1H), 3.70-3.60 (m, 1H), 3.60-3.48 (m, 1H), 3.49 (s, 2H), 3.36-3.30 (m, 1H), 3.30-3.18 (m, 1H), 2.54 (t, J = 8.8 Hz, 1H), 2.39 (t, J = 7.2 Hz, 2H), 2.32-2.21 (m, 1H), 2.21-2.08 (m, 2H), 2.07 (s, 3H), 1.96-1.90 (m, 1H), 1.80-1.00 (m, 21H), 0.86 (s, 9H), 0.56 (s, 3H), 0.03 (s, 6H); ¹³C NMR (100 MHz, CDCl₃): δ 209.9, 194.5, 171.5, 171.1, 146.3, 141.8, 141.3, 133.2, 131.4(2×), 120.8, 118.7, 118.5, 72.4, 72.1, 68.8, 63.6, 49.3, 45.1, 43.8, 42.8, 42.6, 38.2, 37.7, 37.4, 37.2, 36.8, 31.8, 31.6, 30.1, 26.0, 25.9, 25.3, 24.5, 22.8, 20.8, 18.2(2×), 12.9, -4.4, -4.6; IR (NaCl): 3516 (ν N-H) cm⁻¹, 2934 (ν C-H) cm⁻¹, 1697 (ν C=O) cm⁻¹, 1596 (ν C=O) cm⁻¹, 1526 (ν C=O) cm⁻¹, 1092 (ν C-O) cm⁻¹; ESI-HRMS (m/z): [M+H]+ calcd. for C₄₈H₇₀N₃O₆Si, 812.5028; found, 812.5012.

**N-{4-[4-[2-{2-[2-(2-Azidoethoxy)ethoxy]acetamido}acetamido]benzoyl]phenyl}-6-{[3β-O-(tert-butyldimethylsilyl)pregn-5-en-7α-yl-20-one]oxy}hexanamide (17)**

A 10-mL round-bottom flask was charged with 16 (120 mg, 0.15 mmol), DCC (47 mg, 0.23 mmol), 4-dimethylaminopyridine (DMAP) (2 mg, 0.02 mmol), and anhydrous CH₂Cl₂ (3 mL) with stirring. Under argon atmosphere, the reaction mixture was stirred for 3 h. The resulting mixture was filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel with MeOH/CHCl₃ (1:19) as an eluent to yield compound 17 (98 mg, 0.10 mmol) as a colorless gelatinous liquid in 69% yield. TLC (MeOH:CHCl₃, 1:19 v/v): Rf = 0.31; ¹H NMR (400 MHz, CDCl₃): δ 9.16 (s, 1H), 7.91 (s, 1H), 7.75-7.58 (m, 9H), 5.62-5.58 (m, 1H), 4.16 (d, J = 5.6 Hz, 2H), 4.09 (s, 2H), 3.76-3.60 (m, 7H), 3.58-3.40 (m, 1H), 3.40 (t, J = 4.8 Hz, 2H), 3.36-3.30 (m, 1H), 3.28-3.16 (m, 1H), 2.53 (t, J = 9.2 Hz, 1H), 2.38 (t, J = 7.6 Hz, 2H), 2.32-2.21 (m, 1H), 2.21-2.08 (m, 2H), 2.06 (s, 3H), 1.96-1.90 (m, 1H), 1.80-1.00 (m, 19H), 0.92 (s, 3H), 0.85 (s, 9H), 0.55 (s, 3H), 0.03 (s, 6H); ¹³C NMR (100 MHz, CDCl₃): δ 210.1, 194.5, 171.6, 171.3, 167.2, 146.3, 141.9, 141.6, 134.3, 132.9, 131.3(2×), 120.8, 118.9, 118.7, 72.4, 72.1, 71.1, 70.3, 70.2, 70.0, 68.8, 63.6, 50.6, 49.3, 43.9, 43.8, 42.8, 42.5, 38.1, 37.6, 37.4, 37.2, 36.7, 31.8, 31.5, 30.1, 26.0, 25.8, 25.2, 24.5, 22.8, 20.7, 18.1(2×), 12.8, -4.6(2×); IR (NaCl): 3516 (ν N-H) cm⁻¹, 2932 (ν C-H) cm⁻¹, 2104 (ν N₃) cm⁻¹, 1700 (ν C=O) cm⁻¹, 1595 (ν C=O) cm⁻¹, 1528 (ν C=O) cm⁻¹, 1092 (ν C-O) cm⁻¹; ESI-HRMS (m/z): [M+Na]+ calcd. for C₅₄H₇₈N₆NaO₉Si, 1005.5492; found, 1005.5529.
A solution of 18 (80 mg, 0.08 mmol) in THF (2 mL) was added 1 M TBAF in THF (0.24 mL, 0.24 mmol) with stirring. The reaction mixture was allowed to stir at 50 °C for 8 h. To the solution was then added ETOAc (20 mL), washed with distilled water (2×20 mL), dried over MgSO4, filtered, concentrated under reduced pressure, and then purified by flash column chromatography on silica gel with MeOH/CHCl3 (1:19) as an eluent to yield compound 18 (62 mg, 0.07 mmol) as a light-yellow oil in 90% yield. TLC (MeOH:CHCl3, 1:19 v/v): Rf = 0.22; 1H NMR (400 MHz, CDCl3): δ 9.12 (s, 1H), 8.11 (s, 1H), 7.76-7.66 (m, 5H), 7.62 (t, J = 8.4 Hz, 4H), 5.65-5.60 (m, 1H), 4.15 (d, J = 6.0 Hz, 2H), 4.09 (s, 2H), 3.75-3.70 (m, 2H), 3.70-3.50 (m, 6H), 3.40 (t, J = 5.2 Hz, 2H), 3.37-3.32 (m, 1H), 3.29-3.20 (m, 1H), 2.53 (t, J = 8.8 Hz, 1H), 2.42-2.32 (m, 3H), 2.32-2.20 (m, 2H), 2.18-2.09 (m, 1H), 2.06 (s, 3H), 1.94-1.85 (m, 2H), 1.80-1.00 (m, 18H), 0.93 (s, 3H), 0.55 (s, 3H); 13C NMR (100 MHz, CDCl3): δ 210.0, 194.5, 171.9, 171.4, 167.3, 145.7, 142.0, 141.6, 133.2, 133.0, 121.3, 121.3, 118.9, 118.7, 72.4, 71.2, 71.1, 70.3, 70.2, 70.1, 68.8, 63.5, 50.6, 49.3, 44.1, 43.8, 42.6, 42.3, 38.1, 37.5, 37.3, 37.2, 36.8, 31.6, 31.2, 29.9, 25.8, 25.3, 24.5, 22.8, 20.8, 18.2, 12.9; IR (NaCl): 3523 (ν N-H) cm⁻¹, 2934 (ν C-H) cm⁻¹, 2107 (ν N3) cm⁻¹, 1696 (ν C=O) cm⁻¹, 1595 (ν C=O) cm⁻¹, 1529 (ν C=O) cm⁻¹, 1114 (ν C-O) cm⁻¹; ESI-HRMS (m/z): [M+Na]+ calcd. for C₄₈H₆₄N₆NaO₉, 891.4627; found, 891.4657.


Compound P5s-NBPN (3) was prepared by the similar procedure used for the synthesis of P5-NBPN (1). The crude product was purified by flash column chromatography on silica gel with MeOH/CHCl3 (1:8) as an eluent to yield P5s-NBPN (3) (41 mg, 0.04 mmol) as a light yellow oil in 63% yield over two steps. TLC (MeOH:CHCl3, 1:8 v/v): Rf = 0.22; 1H NMR (400 MHz, CD3OD): δ 7.80-7.70 (m, 8H), 5.75-5.70 (m, 1H), 4.56 (s, 2H), 4.50-4.40 (m, 1H), 4.30-4.20 (m, 1H), 4.16 (s, 2H), 4.11 (s, 2H), 3.80-3.66 (m, 5H), 3.59 (t, J = 5.2 Hz, 2H), 3.52-3.35 (m, 4H), 3.18-3.10 (m, 1H), 2.92-2.85 (m, 1H), 2.70-2.64 (m, 1H), 2.55 (t, J = 9.2 Hz, 1H), 2.50-2.40 (m, 2H), 2.35-2.24 (m, 2H), 2.20 (t, J = 7.6 Hz, 2H), 2.14-2.05 (m, 1H), 2.05 (s, 3H), 2.02-1.95 (m, 1H), 1.85-1.00 (m, 25H), 0.98 (s, 3H), 0.58 (s, 3H); 13C NMR (100 MHz, CD3OD): δ 212.5, 196.6, 176.4, 175.0, 173.6, 169.8, 166.2, 147.6, 144.5, 143.9, 134.5, 134.1, 132.5(2×), 122.4, 120.3, 120.2, 73.7, 72.3, 71.4, 70.9, 69.9, 64.8, 63.5, 61.8, 57.1, 50.8, 45.0, 44.2, 43.7, 43.2, 41.2, 40.5, 39.6, 38.8, 38.7, 38.2(2×), 36.9, 32.2, 31.9, 31.4, 29.9, 29.6, 27.5, 27.0, 26.7, 25.7, 23.9, 22.1, 18.9, 13.4; IR (NaCl): 3425 (ν N-H) cm⁻¹, 2934 (ν C-H) cm⁻¹, 1649 (ν C-O) cm⁻¹, 1095 (ν C-O) cm⁻¹; ESI-HRMS (m/z): [M+Na]+ calcd. for C₅₈H₇₀N₆NaO₁₁S, 1091.5498; found, 1091.5516.
Supplementary Note 5

Synthesis and characterization of Cho-NBPN (4)

1. Bromatin, Cyclohexane, 70 °C, 2 h
2. K₂CO₃, KI. THF, rt, 60 h, 23%

Supplementary Figure 22: Synthetic scheme of Cho-NBPN (4)

Methyl 6-[(3β-O-acetyl-cholest-5-en-7α-yl)oxy]hexanoate (19)

Compound 19 was prepared by the similar procedure used for the synthesis of 10a. The crude product was purified by flash column chromatography on silica gel with EtOAc/Hexane (1:4) as an eluent to furnish 19 (1.32 g, 2.31 mmol) as colorless oil in 23% yield over two steps. TLC (EtOAc:Hexane, 1:4 v/v): Rₚ = 0.45; ¹H NMR (400 MHz, CDCl₃): δ 5.68-5.62 (m, 1H), 4.70-4.58 (m, 1H), 3.63 (s, 3H), 3.62-3.54 (m, 1H), 3.35-3.28 (m, 1H), 3.25-3.18 (m, 1H), 2.38-2.28 (m, 2H), 2.29 (t, J = 7.6 Hz, 2H), 2.00 (s, 3H), 1.97-1.88 (m, 1H), 1.88-1.74 (m, 3H), 1.70-1.26 (m, 17H), 1.26-0.90 (m, 9H), 0.98 (s, 3H), 0.89 (d, J = 6.4 Hz, 3H), 0.87-0.80 (m, 6H), 0.62 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 173.9, 170.1, 144.2, 122.5, 73.5, 72.1, 68.6, 55.9, 51.4, 48.9, 42.5, 42.1, 39.5, 39.1, 38.2, 37.5, 37.3, 36.5, 36.3, 35.9, 34.2, 30.0, 28.3, 28.1, 27.6, 26.0, 24.9, 24.4, 24.0, 22.9, 22.6, 21.5, 20.8, 18.8, 18.2, 11.6; IR (KBr): 2946 (ν C-H) cm⁻¹, 1738 (ν C=O) cm⁻¹, 1240 (ν C=O) cm⁻¹; ESI-HRMS (m/z): [M+Na]⁺ calcd. for
C\textsubscript{36}H\textsubscript{60}NaO\textsubscript{5}, 595.4333; found, 595.4336.

2,5-Dioxopyrrolidin-1-yl 6-[(cholest-3β-ol-5-en-7α-yl)oxy]hexanoate (20)
Compound 20 was prepared by the similar procedure used for the synthesis of 11a. The crude product was purified by flash column chromatography on silica gel with EtOAc/Hexane (7:3) as an eluent to furnish 20 (954 mg, 1.42 mmol) as a white solid in 71% yield over two steps. mp 138-140 °C; TLC (EtOAc:Hexane, 7:3 v/v) R\textsubscript{i} = 0.50; \(^1\)H NMR (400 MHz, CDCl\textsubscript{3}): \(\delta\) 5.68-5.60 (m, 1H), 3.64-3.50 (m, 2H), 3.38-3.31 (m, 1H), 3.31-3.22 (m, 1H), 2.85-2.76 (m, 4H), 2.58 (t, \(J = 8.0\) Hz, 2H), 2.34-2.20 (m, 2H), 2.00-1.88 (m, 2H), 1.88-1.68 (m, 5H), 1.64-1.26 (m, 15H), 1.26-0.90 (m, 9H), 0.95 (s, 3H), 0.89 (d, \(J = 6.8\) Hz, 3H), 0.87-0.80 (m, 6H), 0.63 (s, 3H); \(^{13}\)C NMR (100 MHz, CDCl\textsubscript{3}): \(\delta\) 169.0, 168.3, 145.4, 121.5, 72.3, 71.3, 68.3, 55.9, 49.0, 42.6, 42.3, 42.1, 39.5, 39.1, 37.4, 37.3, 36.8, 36.2, 35.9, 31.4, 31.0, 29.8, 28.3, 28.0, 25.7, 25.6, 24.4, 24.0, 22.9, 22.6, 20.9, 18.8, 18.3, 11.6; IR (KBr): 3509 (\(\nu_{O-H}\)) cm\(^{-1}\), 2934 (\(\nu_{C-H}\)) cm\(^{-1}\), 1814 (\(\nu_{C=O}\)) cm\(^{-1}\), 1783 (\(\nu_{C=O}\)) cm\(^{-1}\); ESI-HRMS (m/z): [M-H]\(^-\) calcd. for C\textsubscript{37}H\textsubscript{58}NO\textsubscript{6}, 612.4270; found, 612.4263.

\(N\)-[4-[4-\{2-(2-Azidoethoxy)ethoxy\}acetamido]benzoyl]phenyl]-2-\{6-[(cholest-3β-ol-5-en-7α-yl)oxy]hexanamido\}acetamide (21)
Compound 21 was prepared by the similar procedure used for the synthesis of 12a. The crude product was purified by flash column chromatography on silica gel with MeOH/CHCl\textsubscript{3} (1:9) as an eluent to furnish 21 (332 mg, 0.35 mmol) as a light yellow oil in 72% yield. TLC (MeOH:CHCl\textsubscript{3}, 1:9 v/v): R\textsubscript{i} = 0.35; \(^1\)H NMR (400 MHz, CDCl\textsubscript{3}): \(\delta\) 9.60 (bs, 1H), 8.79 (bs, 1H), 7.82-7.70 (m, 4H), 7.70-7.62 (m, 4H), 6.82-6.75 (m, 1H), 5.65-5.56 (m, 1H), 4.19 (d, \(J = 4.8\) Hz, 2H), 4.14 (s, 2H), 3.82-3.76 (m, 2H), 3.76-3.68 (m, 4H), 3.60-3.48 (m, 2H), 3.43 (t, \(J = 4.8\) Hz, 2H), 3.36-3.22 (m, 2H), 2.49 (bs, 1H), 2.38-2.22 (m, 4H), 1.98-1.88 (m, 1H), 1.88-1.64 (m, 5H), 1.64-1.20 (m, 16H), 1.20-0.90 (m, 8H), 0.93 (s, 3H), 0.88 (d, \(J = 6.4\) Hz, 3H), 0.90-0.80 (m, 6H), 0.62 (s, 3H); \(^{13}\)C NMR (100 MHz, CDCl\textsubscript{3}): \(\delta\) 194.2, 174.1, 168.1, 167.3, 145.4, 141.6, 140.7, 133.3, 133.0, 131.2, 121.5, 119.0, 118.8, 72.3, 71.4, 71.0, 70.5, 70.1 (x2), 68.5, 56.0, 50.5, 49.0, 44.5, 42.6, 42.3, 42.1, 39.5, 39.1, 37.4, 37.3, 36.8, 36.3 (x2), 35.9, 31.3, 30.1, 28.3, 28.1, 26.0, 25.5, 24.4, 24.1, 22.9, 22.6, 20.9, 18.8, 18.3, 11.6; IR (KBr): 3321 (\(\nu_{N-H}\)) cm\(^{-1}\), 2932 (\(\nu_{C-H}\)) cm\(^{-1}\), 2104 (\(\nu_{N_3}\)) cm\(^{-1}\), 1695 (\(\nu_{C=O}\)) cm\(^{-1}\), 1650 (\(\nu_{C=O}\)) cm\(^{-1}\); ESI-HRMS (m/z): [M+Na]\(^+\) calcd. for C\textsubscript{54}H\textsubscript{78}N\textsubscript{6}NaO\textsubscript{8}, 961.5773; found, 961.5791.

\(N\)-[4-\{2-[2-(2-Biotin-amido-ethoxy)ethoxy]acetamido\}benzoyl]phenyl]-2-\{6-[(cholest-3β-ol-5-en-7α-yl)oxy]hexanamido\}acetamide (Cho-NBPN, 4)
Compound Cho-NBPN (4) was prepared by the similar procedure used for the synthesis of P5-NBPN (1). The crude product was purified by flash column chromatography on silica gel with MeOH/CHCl\textsubscript{3} (3:17) as an eluent to furnish Cho-NBPN (4) (173 mg, 0.15 mmol) as a
light yellow oil in 53% yield over two steps. TLC (MeOH:CHCl₃, 3:17 v/v): Rᶠ = 0.32; ¹H NMR (400 MHz, CD₃OD): δ 7.85-7.68 (m, 8H), 5.72-5.65 (m, 1H), 4.48-4.40 (m, 1H), 4.28-4.20 (m, 1H), 4.18 (s, 2H), 4.05 (s, 2H), 3.80-3.75 (m, 2H), 3.75-3.68 (m, 2H), 3.68-3.64 (m, 1H), 3.61 (t, J = 5.2 Hz, 2H), 3.52-3.42 (m, 1H), 3.42-3.35 (m, 3H), 3.35-3.25 (m, 2H), 3.15-3.08 (m, 1H), 2.90-2.82 (m, 1H), 2.35-2.22 (m, 4H), 2.18 (t, J = 7.2 Hz, 2H), 2.02-1.92 (m, 1H), 1.92-1.74 (m, 3H), 1.74-1.30 (m, 24H), 1.30-1.00 (m, 9H), 0.97 (s, 3H), 0.93 (d, J = 6.8 Hz, 3H), 0.88-0.82 (m, 6H), 0.67 (s, 3H); ¹³C NMR (100 MHz, CD₃OD): δ 196.4, 176.7, 176.1, 171.2, 170.0, 166.0, 147.3, 143.9, 143.1, 134.6, 134.0, 132.4(×2), 122.5, 120.5, 120.1, 73.9, 72.2, 71.7, 71.1, 70.7, 70.0, 63.3, 61.6, 57.5, 57.0, 50.4, 44.3, 44.1, 43.3, 41.2, 40.7, 40.6, 40.2, 38.7, 38.6, 38.1, 37.4, 37.2, 36.9, 36.8, 32.2, 31.2, 29.8, 29.5, 29.4, 27.2, 26.8, 26.7, 25.5, 25.1, 23.4, 23.1, 22.0, 19.5, 18.9, 12.2; IR (KBr): 3314 (v N-H) cm⁻¹, 2930 (v C-H) cm⁻¹, 1691 (v C=O) cm⁻¹; ESI-HRMS (m/z): [M+Na]⁺ calcd. for C₆₄H₉₄N₆NaO₁₀S, 1161.6644; found, 1161.6655.
Supplementary Note 6

Synthesis and characterization of OH-NBPN (5)

\[ \text{HO} \quad \text{N} \quad \text{O} \quad \text{N} \quad \text{O} \quad \text{N} \quad \text{N3} \]

1. PPh3, THF
   H2O, rt, 11 h
2. Biotin-OSu, Et3N
   DMF, rt, 8 h, 85%

OH-NBPN, 5

Supplementary Figure 23: Synthetic scheme of OH-NBPN (5)

\[ \text{N-[4-{4-[2-(2-Azidoethoxy)ethoxy]acetamido}benzoyl]phenyl]-2-(6-hydroxylhexanamido)acetamide (22)} \]

To a stirred solution of 6-hydroxyhexanoic acid (322 mg, 2.44 mmol) and 8 (894 mg, 2.03 mmol) in anhydrous CH2Cl2 (10 mL) was added DCC (503 mg, 2.44 mmol) and allowed to stir at ambient temperature under argon atmosphere for 8 h. The resulting mixture was filtered before concentrated under reduced pressure. The residue was then purified by flash column chromatography on silica gel with MeOH/CHCl3 (1:11) as an eluent to yield compound 22 (1.01 g, 1.83 mmol) as a light yellow oil in 90% yield. TLC (MeOH:CHCl3, 1:11 v/v): Rf = 0.23; 1H NMR (400 MHz, CDCl3): \( \delta \) 9.60 (bs, 1H), 8.81 (bs, 1H), 7.82-7.56 (m, 8H), 7.12-7.05 (m, 1H), 4.17-4.10 (m, 4H), 3.79-3.74 (m, 2H), 3.74-3.68 (m, 4H), 3.58 (t, \( J = 6.0 \) Hz, 2H), 3.41 (t, \( J = 5.2 \) Hz, 2H), 2.82 (bs, 1H), 2.28 (t, \( J = 7.2 \) Hz, 2H), 1.70-1.59 (m, 2H), 1.59-1.46 (m, 2H), 1.44-1.32 (m, 2H); 13C NMR (100 MHz, CDCl3): \( \delta \) 194.3, 174.3, 168.2, 167.7, 141.6, 140.8, 133.3, 133.0, 131.2 (\( \times 2 \)), 119.1, 118.8, 71.0, 70.6, 70.1, 62.3, 50.5, 44.4, 36.1, 32.2, 25.4, 25.3; IR (KBr): 3322 (\( \nu \) N-H) cm\(^{-1}\), 2927 (\( \nu \) C-H) cm\(^{-1}\), 2104 (\( \nu \) N3) cm\(^{-1}\), 1692 (\( \nu \) C=O) cm\(^{-1}\), 1650 (\( \nu \) C=O) cm\(^{-1}\); ESI-HRMS (m/z): [M-H]\(^{-}\) calcd. for C\(_{27}\)H\(_{33}\)N\(_{6}\)O\(_{7}\), 533.2416; found, 533.2403.

\[ \text{N-[4-{4-[2-(2-Biotin-amido-ethoxy)ethoxy]acetamido}benzoyl]phenyl]-2-(6-hydroxylhexanamido)acetamide (OH-NBPN, 5)} \]

OH-NBPN (5) was prepared by the similar procedure used for the synthesis of P5-NBPN (1). The crude product was purified by flash column chromatography on silica gel with MeOH/CHCl3 (1:5) as an eluent to yield OH-NBPN (5) (255 mg, 0.34 mmol) as a light yellow oil in 85% yield over two steps. TLC (MeOH:CHCl3, 1:5 v/v): Rf = 0.25; 1H NMR (400 MHz, CD\(_{3}\)OD): \( \delta \) 7.80-7.65 (m, 8H), 4.48-4.42 (m, 1H), 4.26-4.21 (m, 1H), 4.19 (s, 2H), 4.04 (s,
2H), 3.81-3.76 (m, 2H), 3.76-3.70 (m, 2H), 3.62 (t, $J = 5.6$ Hz, 2H), 3.56 (t, $J = 6.4$ Hz, 2H), 3.40 (t, $J = 5.6$ Hz, 2H), 3.16-3.08 (m, 1H), 2.92-2.84 (m, 1H), 2.70-2.62 (m, 1H), 2.33 (t, $J = 7.6$ Hz, 2H), 2.18 (t, $J = 7.2$ Hz, 2H), 1.75-1.63 (m, 4H), 1.63-1.50 (m, 4H), 1.50-1.34 (m, 4H);

$^{13}$C NMR (100 MHz, CD$_3$OD): $\delta$ 196.6, 177.0, 176.3, 171.5, 170.3, 166.2, 144.1, 143.3, 134.8, 134.2, 132.5, 132.4, 120.7, 120.3, 72.3, 71.8, 71.2, 70.8, 63.5, 62.9, 61.7, 57.1, 44.3, 41.2, 40.3, 36.9 ($\times$2), 33.5, 29.9, 29.6, 27.0, 26.8, 26.7; IR (KBr): 3304 ($\nu$ N-H) cm$^{-1}$, 2926 ($\nu$ C-H) cm$^{-1}$, 2858 ($\nu$ C-H) cm$^{-1}$, 1693 ($\nu$ C=O) cm$^{-1}$, 1650 ($\nu$ C=O) cm$^{-1}$; ESI-HRMS ($m/z$): [M+H]$^+$ calcd. for C$_{37}$H$_{51}$N$_6$O$_9$S, 755.3433; found, 755.3459.
**Supplementary Note 7**

**Synthesis and characterization of 7-OH-P5**

\[ \text{9a} \quad \rightarrow \]

1. Bromatin, Cyclohexane, 70 °C, 2 h
2. CaCO₃, KI, THF/H₂O, rt, 18 h
3. TBAF, THF, rt, 4 h, 35%

**Supplementary Figure 24:** Synthetic scheme of 7-OH-P5

3β, 7α-Dihydroxypregn-5-en-20-one (7-OH-P5)

9a (431 mg, 1.00 mmol) was brominated as previous procedure and used without further purification. The crude brominated compound was dissolved in THF (3 mL) in a 25-mL round-bottom flask with stirring and subsequently added CaCO₃ (500 mg, 5.00 mmol) and H₂O (2 mL). The solution was allowed to stir at ambient temperature for 18 h. The resulting mixture was filtered, concentrated under reduced pressure and preliminary separation by running short plug of silica with EtOAc/Hexane (1:5) as an eluent. Then, the residue dissolved in THF was added 1 M TBAF in THF (0.6 mL, 0.60 mmol) with stirring. The reaction mixture was allowed to stir at ambient temperature for 4 h. To the solution was then added EtOAc (15 mL), washed with distilled water (2×15 mL), dried over MgSO₄, filtered, concentrated under reduced pressure, and then purified by flash column chromatography on silica gel with MeOH/CHCl₃ (1:15) as an eluent to yield 7-OH-P5 (115 mg, 0.35 mmol) as a white powder in 35% yield (over three steps). mp: 178-179 °C (lit.° 173-178 °C); TLC (MeOH:CHCl₃, 1:15 v/v): Rₜ = 0.25; ¹H NMR (400 MHz, CDCl₃): δ 5.61-5.50 (m, 1H), 3.82 (t, J = 2.4 Hz, 1H), 3.60-3.46 (m, 1H), 2.53 (t, J = 9.2 Hz, 1H), 2.35-2.10 (m, 3H), 2.09 (s, 3H), 2.04-1.95 (m, 1H), 1.80-1.00 (m, 15H), 0.95 (s, 3H), 0.59 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 209.7, 146.2, 123.6, 71.1, 65.1, 63.4, 49.6, 43.7, 42.1, 41.9, 38.2, 37.4, 37.3, 37.0, 31.5, 31.2, 24.3, 22.9, 20.6, 18.2, 12.9.
Supplementary References


