Supplementary Materials for Manuscript

Novel bile acid–mediated cysteine modification regulates bacterial virulence

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Supplementary Tables S1-S3

Table S1: List of peptides with modifications by LCA-Galk and its analogue probes as identified by TOP-ABPP from *E. coli* and *S.* Typhimurium. (see the attached excel file)

Table S2: List of LCA-modified cysteines with competition ratios quantified by competitive rdTOP-ABPP profiling using the IA-alkyne probe in *E. coli* with LCA treatment. (see the attached excel file)

Table S3: List of proteins in *S.* Typhimurium that are quantified with localization changes upon LCA treatment. (see the attached excel file)

Supplementary Figures

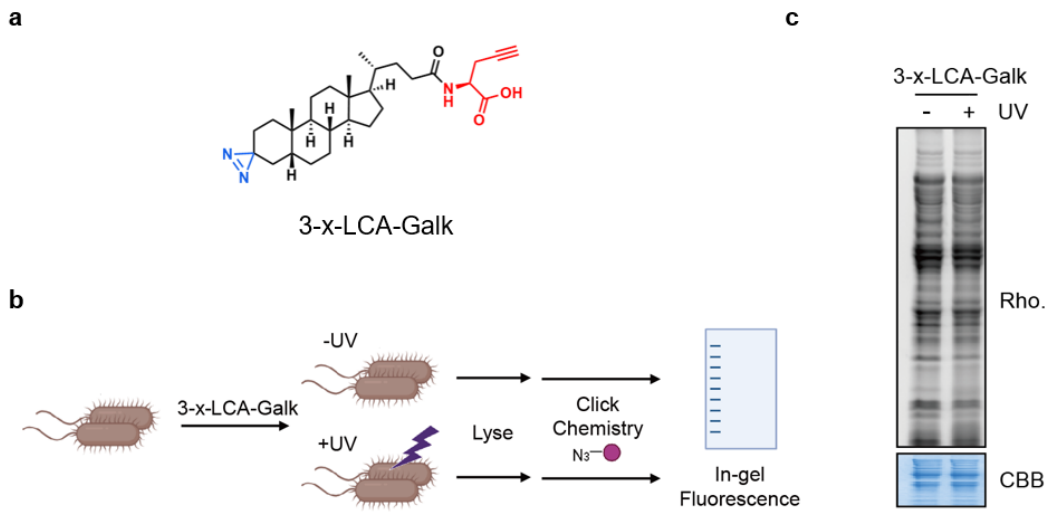


Fig. S1. The UV-independent labeling of 3-x-LCA-Galk. a, Structure of the photo-affinity LCA probe, 3-x-LCA-Galk. **b,** Workflow of the *in situ* probe labeling and in-gel fluorescence. *E. coli* was incubated with 3-x-LCA-Galk in dark, subjected to UV irradiation and lysed for in-gel fluorescence procedures. **c,** Comparison of the labeling efficiency of 3-x-LCA-Galk with or without UV irradiation.

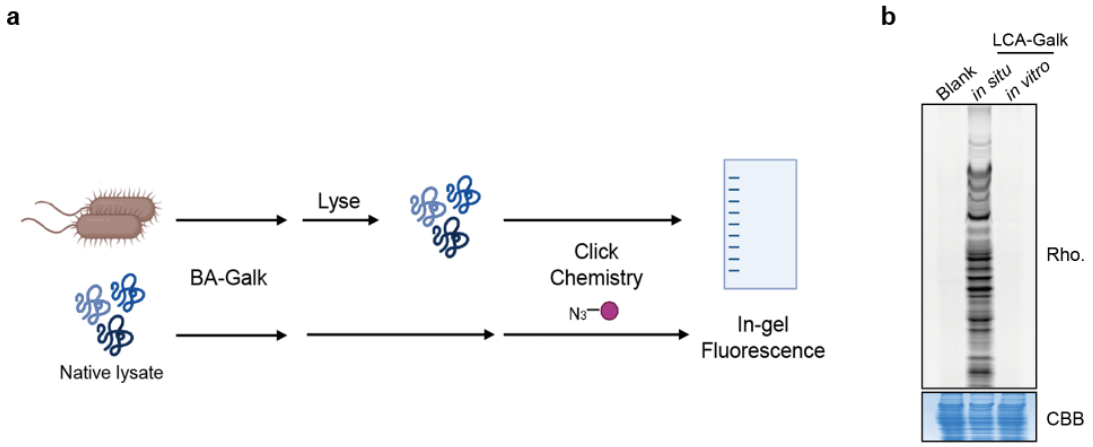


Fig. S2. Comparison of the *in situ* and *in vitro* labeling efficiency of LCA-Galk. a, Workflow of the *in situ* and *in vitro* probe labeling. **b,** Comparison of the labeling efficiency of LCA-Galk under *in situ* and *in vitro* conditions by in-gel fluorescence.

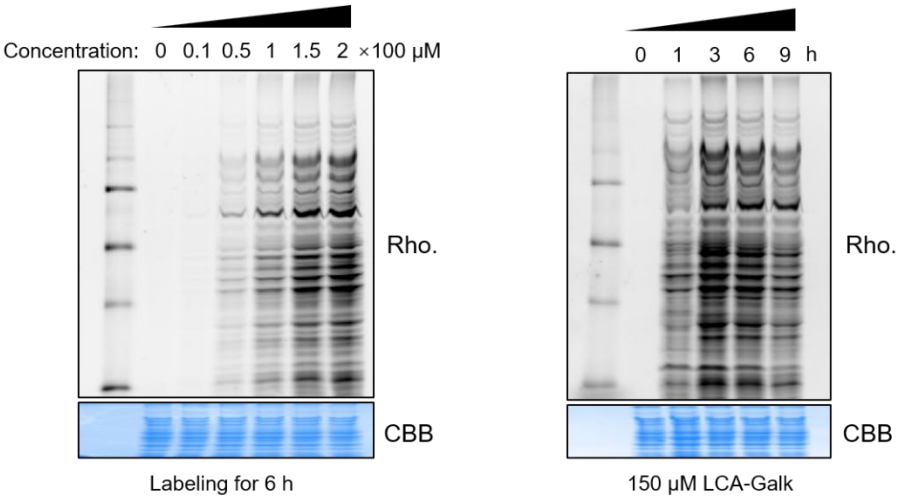


Fig. S3. Optimization of LCA-Galk's labeling by time and concentration. LCA-Galk's labeling is dose- and time-dependent and reached saturation at 150 μM for 6 h.

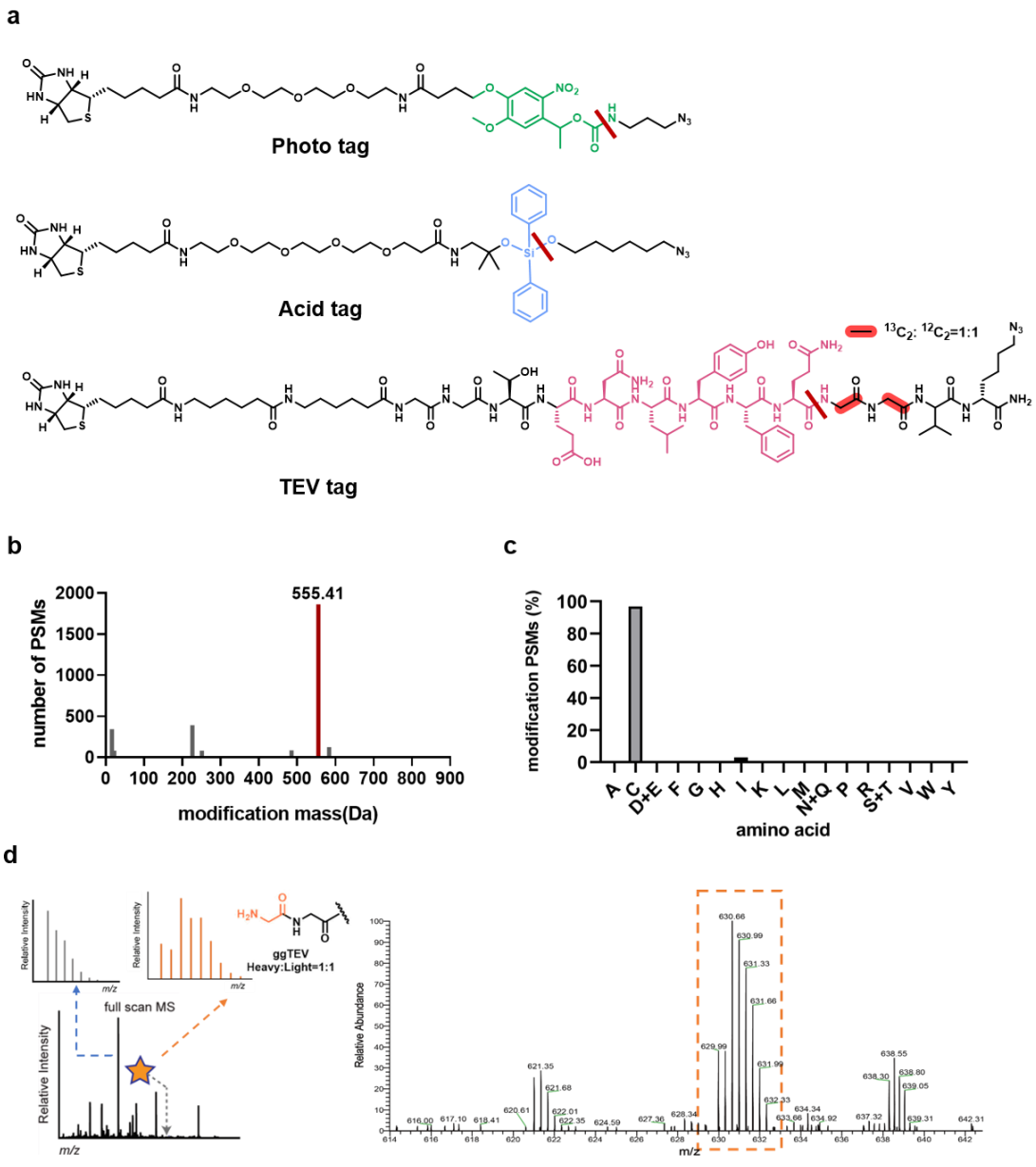
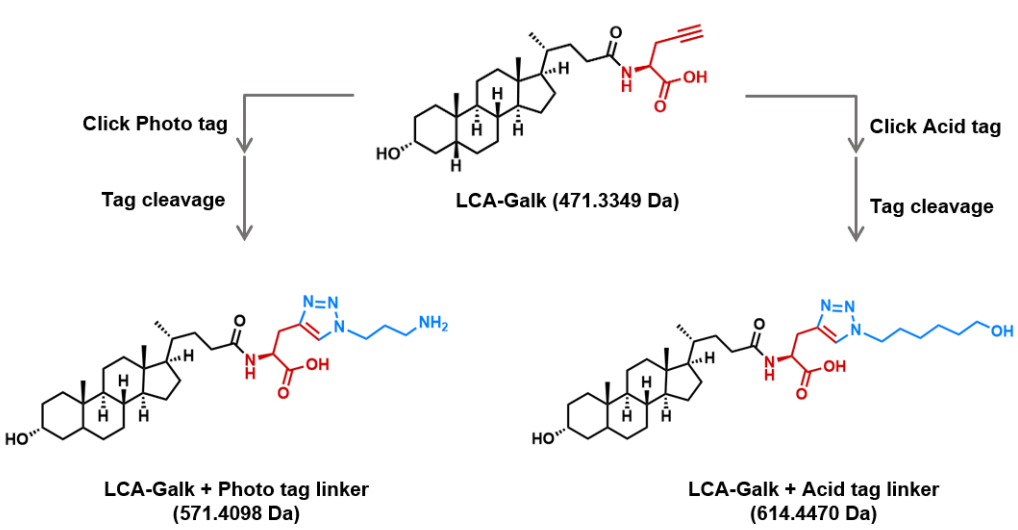


Fig. S4. The open-search results and MS1 pattern of LCA-Galk labeling with different tags. a, Structures of photo-cleavable, acid-cleavable and isotopically labeled TEV-cleavable azido-biotin tags. Cleavage sites are indicated by red lines. **b-c,** Modification mass and sites of LCA-Galk with the acid-cleavable tag. **d,** Introduction of two isotopically labeled glycines into the TEV-cleavable azido-biotin tags ("ggTEV tag") resulted in a distinct isotopic pattern in the representative MS1 spectrum of an LCA-Galk modified peptide.

a



b

Delta mass = Detected mass from open search + Carbamidomethyl - LCAGalk with tag linker

	Photo tag	Acid tag
Detected mass (Open Search)	512.3680 Da	555.4054 Da
Modification mass (plus Carbamidomethyl)	569.3880 Da	612.4254 Da
LCA-Galk with tag linker	571.4098 Da	614.4470 Da
Delta mass	2.0218 Da	2.0153 Da

Fig. S5. Calculation of the modification mass of LCA-Galk using two different cleavable tags. a, Chemical structures of the LCA-Galk-tag adducts after either photo or acid cleavage. **b,** Calculation of the modification masses based on the formula revealed a delta mass of 2 Da between the observed LCA-Galk modification and the theoretical mass of LCA-Galk (mass error < 20 ppm), indicating two hydrogens were lost during the modification process.

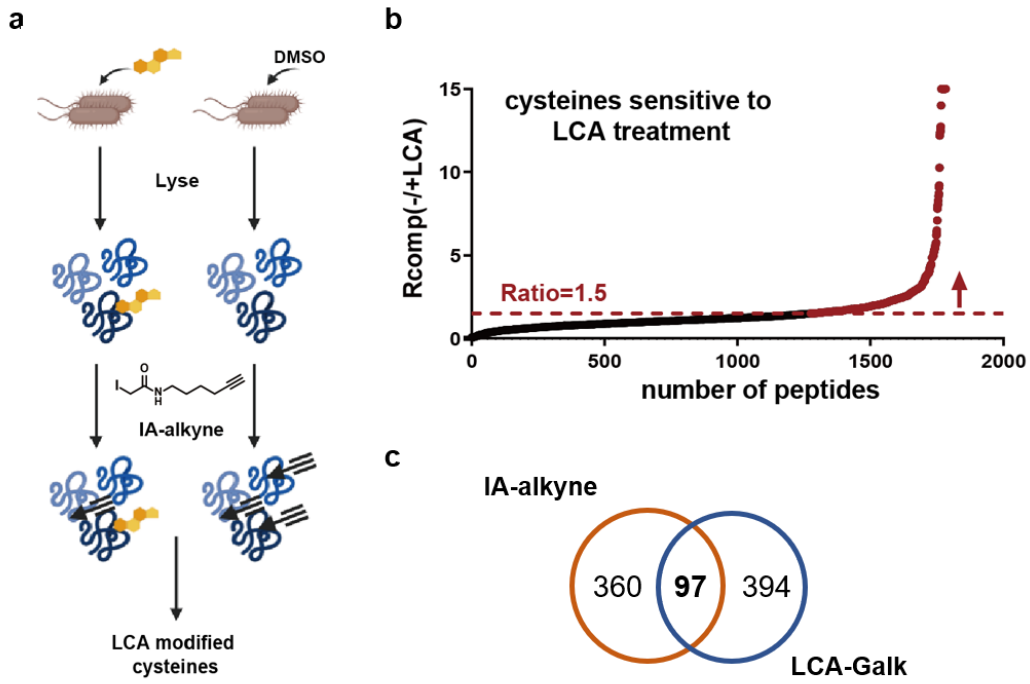


Fig. S6. Competitive isoTOP-ABPP analysis of the LCA-modified cysteines. a, Workflow of the competitive isoTOP-ABPP with a cysteine-reactive IA-alkyne probe. *E. coli* was treated by 150 μM LCA as the experiment group or DMSO as the control group, respectively. The lysates were treated with 100 μM IA-alkyne for 1 h and subjected to the rest of competitive isoTOP-ABPP procedures to quantify LCA-sensitive cysteines in proteomes. **b,** Distribution of the competitive isoTOP-ABPP ratios quantified from *E. coli* pre-treated with LCA. Peptides with ratio over 1.5 was colored in red. **c,** Comparison of the numbers of the LCA-modified cysteines profiled by the competitive isoTOP-ABPP using IA-alkyne and by the direct TOP-ABPP using LCA-Galk.

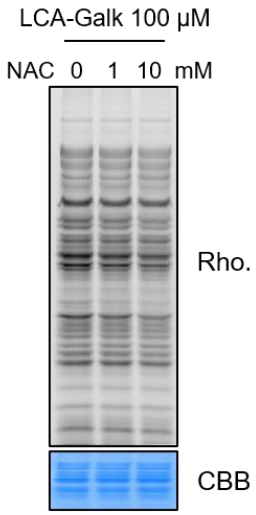


Fig. S7. N-acetylcysteine (NAC) cotreatment competed LCA modification. *E. coli* was treated by 100 μM LCA-Galk following the incubation of 0, 1 or 10mM of NAC for 6 h. The labeling of LCA-Galk was visualized by in-gel fluorescence and NAC treatment was able to compete the labeling of LCA-Galk.

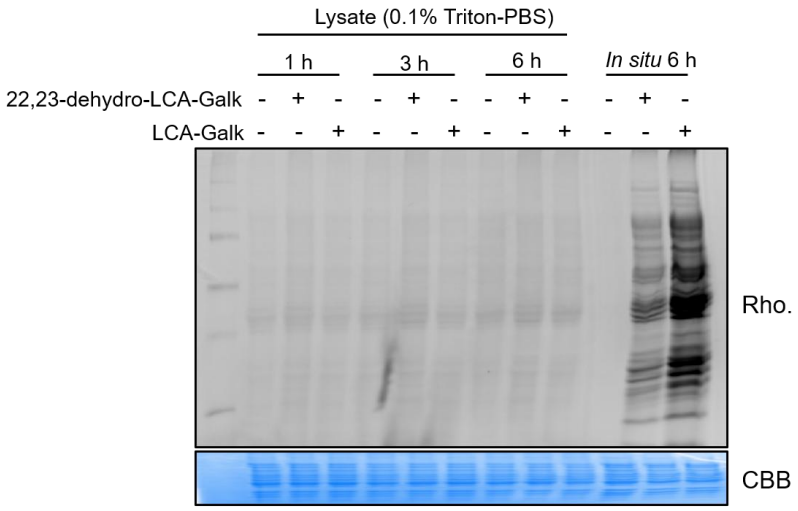


Fig. S8. The probe of 22,23-Dehydro-LCA-Galk failed to label bacterial lysates. Comparison of 22,23-dehydro-LCA-Galk labeling under *in situ* and *in vitro* conditions by in-gel fluorescence. For *in vitro* labeling, lysates of *E. coli* were incubated with 150 μM 22,23-dehydro-LCA-Galk for 1, 3 or 6 h, followed by the standard in-gel fluorescence workflow.

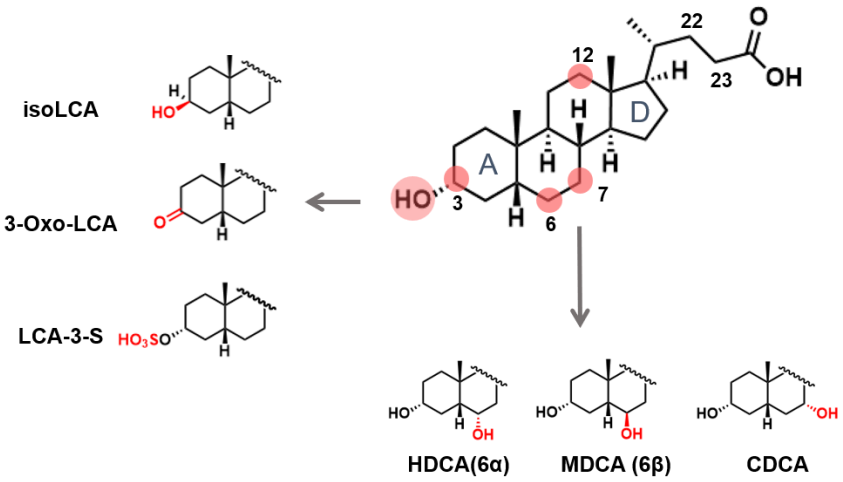


Fig. S9. Structures of representative LCA-derived metabolites in human. isoLCA and 3-oxoLCA could be generated through bacterial modification of the 3-hydroxyl group. Lithocholic acid-3-sulfate (LCA-3-S) could be formed by sulfotransferase-mediated sulfation of the 3-hydroxyl group[1]. HDCA, MDCA and CDCA could arise from P450-mediated modification at C6 or C7 of the steroid scaffold during reabsorption.

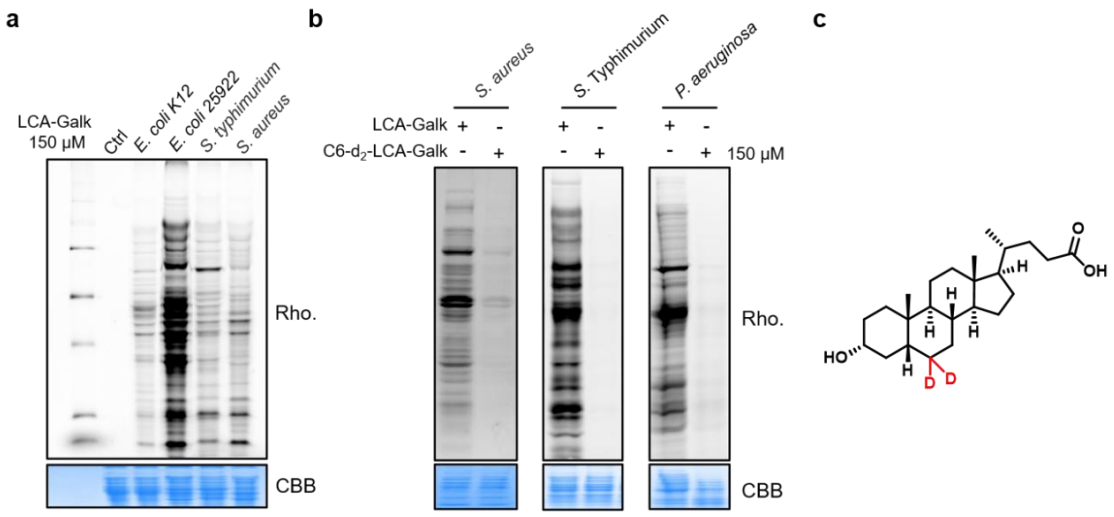


Fig. S10. LCA modification is conserved across multiple bacteria species. a, Comparison of the labeling efficiency of LCA-Galk in different bacteria species by in-gel fluorescence. **b,** C6-d_2-LCA-Galk failed to label the proteomes in multiple pathogenic bacteria, which is consistent with the phenomenon observed in *E. coli*. **c.** Structure of C6-d_2-LCA.

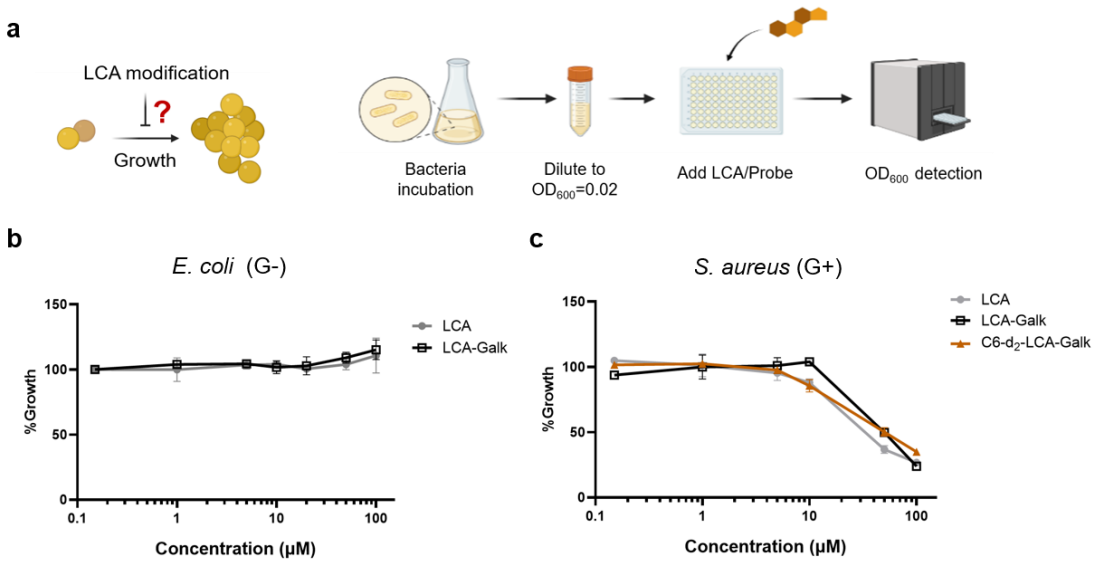


Fig. S11. Growth inhibition activity of LCA-Galk and C6-d_2-LCA-Galk in *S. aureus*. a, Workflow of the growth inhibition assay. **b,** Comparison of the growth inhibition activity between LCA and LCA-Galk in *E. coli*. **c,** Comparison of the growth inhibition activity between LCA, LCA-Galk and C6-d_2-LCA-Galk in *S. aureus*. Data are presented as mean ± SD (n = 3 biological replicates).

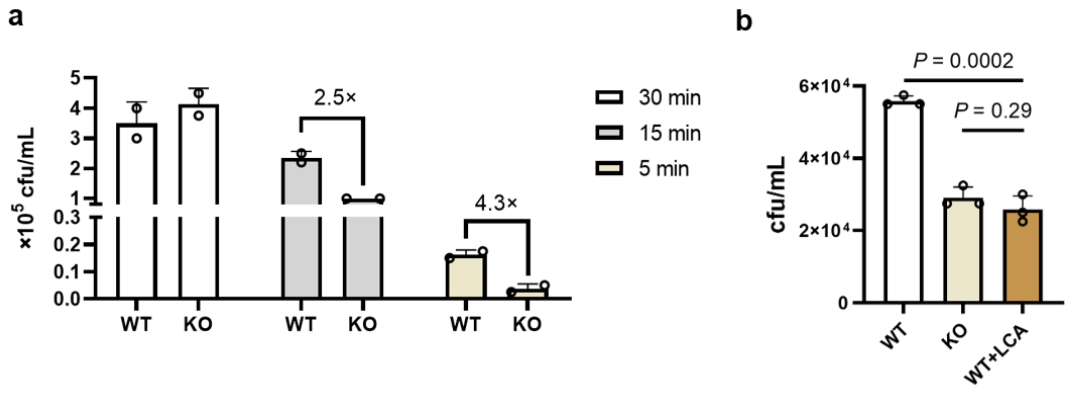


Fig. S12. Evaluation of S. Typhimurium infection with *sipA* knockout or LCA treatment. **a,** Comparison of the infection ability between the wide-type strain and the *sipA* knockout (KO) strain. Knockout of *sipA* impaired *S.* Typhimurium infection at 5 min and 10 min. Data are presented as mean ± SD (n = 2 biological replicates) **b,** LCA treatment impairs the infection of *S.* Typhimurium, which is consistent with the phenotype of *sipA* knockout strain. Data are presented as mean ± SD (n = 3 biological replicates). P value was determined via two-tailed Student's t-test.

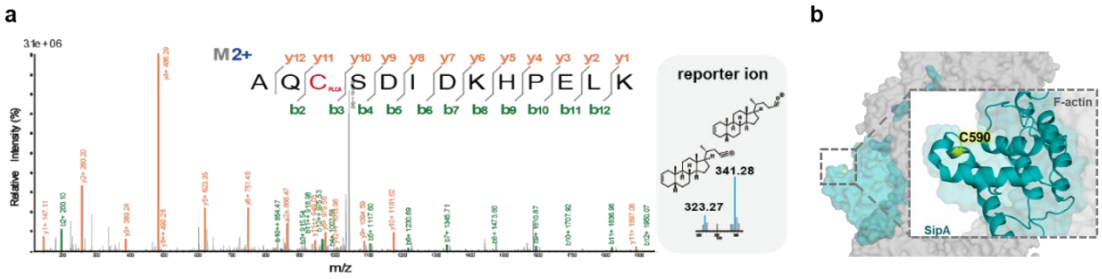


Fig. S13. Cys590 as a LCA modification site in SipA. a, MS/MS spectrum of the LCA-Galk-modified peptide containing the peptide sequence is shown with the modified cysteine marked in red. The b and y ions are marked with those from the LCA-Galk probe fragmentations highlighted in blue. **b,** Structural location of Cys590 within the C-terminal domain of SipA (teal) in complex with F-actin (grey) (PDB: 8UEE).

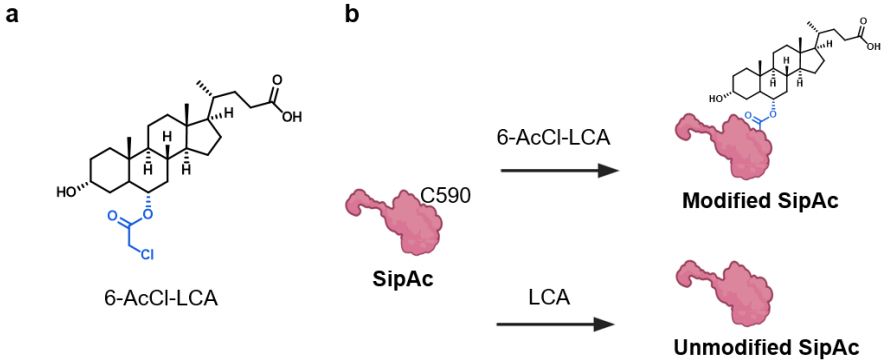


Fig. S14. Construction of the LCA-mimicking modification at Cys590 of SipA. a, Structure of 6-AcCl-LCA, which contains a cysteine-reactive chloroacetamide group. **b,** Workflow for introducing the LCA-mimicking modification on Cys590 of SipA. The recombinant C-terminal domain of the wild-type SipA was incubated with 500 μM of 6-AcCl-LCA or LCA as a control. After incubation, small molecules were removed by ultrafiltration, and the samples were subsequently incubated with HeLa cell lysate for co-immunoprecipitation assays.

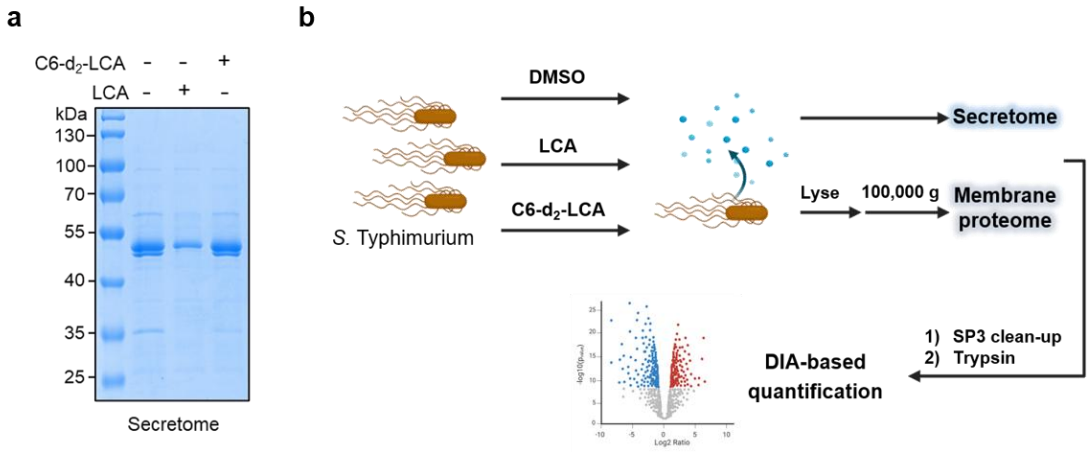


Fig. S15. Profiling of the protein localization changes in *S.* Typhimurium mediated by LCA modification. a, Comparison of the secretomes after the treatment of LCA or C6-d_2-LCA by Coomassie brilliant blue staining. LCA treatment induced a significant loss of secreted proteome in S. Typhimurium. **b,** Workflow for extracting the secreted and membrane proteomes. The samples were then cleaned up by SP3 and digested for DIA-based quantitative proteomics analysis.

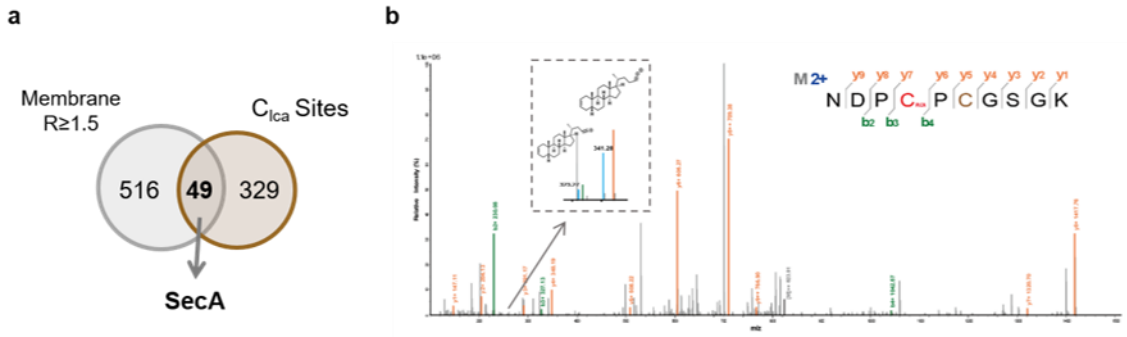


Fig. S16. Identification of SecA as an LCA-modified protein. a, Comparison between the proteins undergoing the localization change and those modified by LCA-Galk. SecA was selected as a key component of the bacterial protein translocase complex. **b,** MS/MS spectrum of the LCA-Galk-modified peptide from SecA. The peptide sequence is shown with the modified cysteine marked in red. The b and y ions are marked with those from the LCA-Galk probe fragmentations highlighted in blue.

Supplementary Methods

Bacteria. *E. coli* ATCC25922, *E. coli* K12, *S.* Typhimurium SL1344, *S. aureus* strain NCTC 8325 and *P*. aeruginosa PAO1 strains were used in this study. *E. coli* and *P*. aeruginosa were cultured with Luria-Bertani (LB) medium, *S.* Typhimurium SL1344 was cultured in LB and SPI-1-inducing LB medium (10 g liter^{-1} tryptone, 5 g liter^{-1} yeast extract and 300 mM NaCl), *S. aureus* NCTC 8325 was cultured in Tryptic Soy Broth (TSB) medium. All strains were grown at 37℃ on plates of their respective medium with 1% agar. Overnight cultures and subcultures were grown in media at 37℃ with shaking.

Cell culture. HeLa cells were cultured in DMEM (Thermo Fisher Scientific, Cat# C11995500CP) supplemented with 10% FBS (Gibco, Cat# 10099-141C) and 1% P/S (Gibco, Cat# 15140122) at 37℃ in a 5% CO_2 incubator.

Chemical synthesis of BA-Galk probes, LCA-Galk probe derivatives and LCA-6(α)-Cys standard. Detailed synthesis methods and characterization data are included in Supplementary Information.

***In situ* and *in vitro* labeling of BA-Galk probes and derivatives.** For *in situ* labeling, bacteria were sub-cultured into fresh LB medium at a 1:20 ratio and incubated at 37°C until it reached the logarithmic phase (OD > 1). For metabolic labeling, the culture was diluted to an OD of 0.1 and treated with the probe. After incubation, the culture was harvested by centrifugation (4000 g, 15 min, 4°C), and the bacterial pellet was subsequently washed with pre-chilled PBS buffer. The washed pellets were resuspended with 200 μL of 0.4% Sodium dodecyl sulfate (SDS) in PBS and lysed by sonication. The lysate was centrifuged (20,000 g, 10 min, 16°C) and normalized to 2 mg/mL 50 μL.

For *in vitro* labeling, the untreated bacteria were lysed with 200 μL of pre-chilled PBS buffer, and centrifuged at 20,000 g for 30 min. After the lysate was normalized to 2 mg/mL, the probe was added into 50 μL lysate and incubated on the Thermomixer (600 rpm, 37℃) for 6 h.

In-gel fluorescence analysis of probe labeling. For 50 μL of cell lysate prepared as described above, copper(I)-catalyzed azide-alkyne cycloaddition (‘‘click chemistry’’) was performed with 5.3 μL of Click reagent mix (3 μL of TBTA ligand (1.667 μL tris (benzyltriazolylmethyl)amine (Strem) in 80% tBuOH and 20% DMSO), 1 μL of 50 mM $CuSO_4$ (Sigma), 1 μL of freshly prepared 50 mM TCEP (Sigma) and 0.3 μL of 20 mM azide-TAMRA (Invitrogen)) and incubated on the Thermomixer (1,200 rpm, 1 h, 29°C). The reaction was stopped by the addition of 16 μL of Loading Buffer (Bio-Rad), mixed thoroughly and boiled on the Thermomixer (10 min, 95°C). Then, the reacted samples were immediately chilled on the ice, shortly centrifuged, vigorously mixed and resolved with 10% SDS-PAGE gel. Fluorescent bands were visualized by a ChemiDoc XRS+ (Bio-Rad) with a 560DF50 filter to monitor Rhodamine signal. Subsequently, total protein band was stained by Coomassie brilliant blue as loading controls.

TOP-ABPP for discovery and profiling of the LCA modification. The cell lysate was reacted with an acid-cleavable tag (Click Chemistry Tools, 1330) or a photo-cleavable tag (Click Chemistry Tools, 1119) and then precipitated by methanol-chloroform-water (4:1:3) and centrifuged at 4,000 g for 10 min; the precipitated proteins were washed and centrifuged at 20,000 g for 8 min at 4 °C with 1 mL of cold methanol and resuspended in 1 mL PBS containing 1.2% SDS. 100 μL streptavidin beads (Thermo Fisher Scientific) were washed for three times with 1 mL PBS, and resuspended in 5 mL PBS, which was added to the protein solution. The resulting solution was incubated for 4 h at 29 °C, followed by washing with 5 mL PBS for three times, and 5 mL distilled water for three times. The resulting beads were resuspended in 500 μL PBS containing 6 M urea and 10 mM DTT and incubated for 30 min at 37 °C, followed by addition of 20 mM iodoacetamide for 30 min at 35 °C in the dark. The beads were then collected by centrifugation and resuspended in 200 μL of 100 mM triethylammonium bicarbonate (TEAB) buffer (Sigma) and 2 μg of trypsin (Promega), and incubated in Thermomixer (1150 rpm, 37°C, 16 h). On the next morning, the beads were first washed with 1 mL of PBS for three times and 1 mL of distilled water for four times. For the photo cleavable tag, 1 mL of methanol-H_2O (7/3,

v/v) was added to the beads, and the suspensions were transferred to thin-walled glass tubes before irradiation with UV 365 nm (5000 × 100 μJ/cm^2) for 1 h on ice to release the adducted peptides. For the acid-cleavable tag, the samples were cleaved with two sequential one-hour treatments of 2% formic acid/ H_2O (200 μL) at 25 °C with 1150 rpm shaking. The eluents from all three cleavage cycles were collected and combined. Afterward, the beads were washed with 50% acetonitrile-H_2O+1% formic acid (2 × 200 μL), and the washes were combined with the eluents to form the cleavage fraction. The cleavage fraction was concentrated using a vacuum centrifuge. The resulting samples were analyzed by LC-MS/MS using Orbitrap.

Competitive rdTOP-ABPP analysis of LCA-modified cysteines. For the identification of LCA-modified cysteines by IA-alkyne, LCA or DMSO treated *E. coli* were resuspended in pre-chilled PBS with 0.1% Triton X-100 and lysed by sonication on ice. After centrifugation (20,000 g, 10 min, 4°C), the supernatant was collected and diluted to 2 mg/mL and incubated with 100 μM IA-alkyne at 29 °C for 1 h. The resulting lysates were precipitated by methanol-chloroform-water (4:1:3) and centrifuged at 4,000 g for 10 min; the precipitated proteins were washed and centrifuged at 20,000 g for 8 min at 4 °C with 1 mL of cold methanol. After being resuspended in 1 mL PBS containing 0.4% SDS, all samples were reacted with the acid-cleavable tag, subjected for streptavidin enrichment and on-bead trypsin digestion as described above. Then, each sample were suspended in 100 μL of 100 mM TEAB buffer (Sigma), and 8 μL of 4% HCHO (Sigma) and 4% D^{13}CDO (Sigma) were added to the DMSO and LCA-treated samples, respectively, and 8 μL of 0.6 M $NaBH_3CN$ (Sigma) was added to each sample on ice. After labeling for 2 h at 29 °C, samples were washed by 100 mM TEAB buffer twice, then mixed and washed with 2 × 1 mL H_2O, followed by on-beads cleavage. The resulting samples were analyzed by LC-MS/MS using Orbitrap.

Exhaustive proteome hydrolysis of LCA-treated bacterial proteome. LCA-treated *E. coli* was suspended in PBS containing 1.2% SDS and lysed by sonication at room temperature. After centrifugation at 20,000 g for 10 min at 16 °C, the proteome was collected and precipitated by methanol-chloroform-water (4:1:3) to remove the metabolites. The precipitated proteins were

washed and resuspended in PBS (pH 8.0) with proteinase K (50:1, Promega), then incubated in Thermomixer (1,000 rpm, 55°C, 48 h). Once the total digestion procedure was completed, reaction mixtures were filtered through 3 kDa molecular weight cutoff filters (Millipore). The resulting samples were analyzed by LC-MS/MS using Orbitrap.

Extraction of secreted and membrane proteomes. *S.* Typhimurium was cultured in LB overnight and diluted into 3 mL of SPI-1-inducing LB (OD_{600}=0.2) with 100 μM LCA/C6-d_2-LCA for 4 h at 37 °C with 220 rpm shaking. Bacterial culture supernatants were separated by centrifugation at 4,000 g for 20 min, and filtered through a 0.22 μm syringe filter (Millipore). The secretome was extracted by methanol-chloroform-water (4:1:3). For the extraction of membrane proteome, bacteria pellets were re-suspended in pre-chilled PBS, sonicated on ice, and separated by ultracentrifugation at 100,000 g for 45 min at 4 °C. Secreted and membrane proteomes were resuspended in PBS with 1.2% SDS respectively, and subjected to reductive alkylation, $SP3^2$ (Cytiva) clean-up and trypsin digestion. The resulting samples were analyzed by LC-MS/MS using TOF.

LC-MS/MS analysis using Orbitrap. For ABPP experiments and PRM analysis, a Q-Exactive plus Orbitrap mass spectrometer (Thermo Fisher Scientific) coupled with Ultimate 3000 LC system was used. In general, mobile phase A was 0.1% formic acid in H_2O, and mobile phase B was 0.1% formic acid, 80% acetonitrile in H_2O. Flow rate was 3 μL/min for loading and 0.3 μL/min for eluting.

For data-dependent mode and peptide analysis, Samples were chromatographically separated using a 5-min gradient from 8% to 13% solvent B, followed by a 50-min gradient from 13% to 45% B, a 10-min gradient from 45% to 60% B, and a 5-min gradient from 60% to 95% B. The total acquisition time per sample was 85 min. full-scan mass spectra were acquired over the *m/z* range from 350 to 1800 using the Orbitrap mass analyzer with a mass resolution of 70,000 and the 20 most intense ions are selected for MS/MS analysis a resolution of 17,500 using collision mode of HCD. Other important mass parameters: isolation window, 1.6 *m/z* units; default charge, 2+; normalized collision energy, 28%; maximum IT, 100 ms; dynamic exclusion, 20.0s.

For PRM analysis of the LCA-cysteine adduct, full-scan mass spectra were acquired over an *m/z* range of 350-1,400 using the Orbitrap mass analyzer. The maximum MS2 injection time was set to 100 ms with an isolation window of 1.5 *m/z*. Other parameters were set to default values. PRM data were acquired using an inclusion list targeting the precursor ion at *m/z* 496.3091.

LC-MS/MS analysis using TOF. For DIA-based quantitative proteomic analysis, peptides were analyzed on a timsTOF Pro 2 mass spectrometer (Bruker) coupled to a nanoElute2 UHPLC system. Chromatographic separation was performed using a single-column setup with a 25 cm × 75 μm analytical column packed with 1.9 μm C18 particles. Peptides were separated over a 60-min gradient at a constant flow rate of 0.3 μL/min. Mobile phase A consisted of 0.1% formic acid in H_2O, and mobile phase B consisted of 0.1% formic acid in acetonitrile.

For mass spectrometric analysis, data-dependent acquisition with parallel accumulation–serial fragmentation (ddaPASEF) was first performed. Standard ion-source parameters were used. The ion mobility range was set to 0.75–1.30 Vs/cm², with both the ramp and accumulation times set to 100 ms. The MS scan range was set to *m/z* 100-1,700. Each PASEF cycle comprised 10 MS/MS scans, with a target intensity of 10,000 and an intensity threshold of 2,500. Precursors with charge states of 2-4 were selected for fragmentation. The isolation width was set to 2.0 *m/z* for precursors below *m/z* 700 and 3.0 *m/z* for precursors above *m/z* 800. Collision energy was linearly ramped from 20 to 59 eV over an ion mobility range of 0.60-1.60 Vs/cm². Subsequently, data-independent acquisition with parallel accumulation-serial fragmentation (diaPASEF) was performed using an acquisition scheme established on the basis of the ddaPASEF data obtained from the DMSO-treated group. The final precursor mass range was *m/z* 325.0-1,203.0, with an ion mobility range of 0.75–1.29 Vs/cm² and a predicted cycle time of 1.1 s.

MS data analysis. Open search was performed by using FragPipe GUI v22.0 with MSFragger3 (version 3.3), Philosopher4 (version 4.0.0), and IonQuant5, (version 1.7.5). Precursor mass tolerance was set as 0-500 ppm. Missed cleavages were allowed up to 2. Peptide length was set

7 to 50, and peptide mass range was set 500 to 5000. Cysteine carbamidomethyl (+57.02146 Da) was set as fixed modification.

For data analysis of the competitive isoTOP-ABPP experiments, MS/MS spectra were extracted from the raw file using RAW Xtractor into an "ms2" format, which were searched using the ProLuCID[3] with a reverse concatenated, nonredundant variant of the *E. coli* (strain ATCC25922) UniProt database. Cysteine carbamidomethylation (+57.0215 Da) was chosen as static modification. N-terminus dimethyl labeling, and lysine residue dimethyl labeling were chosen as static modifications with tag masses of +28.03130 Da (light), + 34.06312 Da (heavy), respectively. IA-alkyne modification (322.23688 Da) was set as variable modifications on cysteines. Methionine oxidation (15.9949 Da) was set as variable modification on methionine. The searched results are filtered by DTASelect[4] and peptides are also restricted to be fully tryptic with a defined peptide false positive rate of 1%. The ratios of reductive dimethylation are quantified by the CIMAGE software as previously described[5].

For quantitative proteomics analysis based on diaPASEF, raw data files were processed collectively using DIA-NN (v 1.8.12) software[6] in the library-free mode for protein and peptide identification and quantification. Trypsin/P with maximum 3 missed cleavage was set. Variable modifications were set to carbamidomethylation on cysteine (+57.02146 Da), oxidation on methionine (+15.99491 Da), and acetylation on the protein N-terminus (+42.010564 Da). Cross-run normalization was enabled by default. For protein-level quantification, protein intensities from DIA-NN output were used directly for subsequent analysis.

Growth inhibition assay. The overnight-cultured *S. aureus* and *E. coli* were diluted to an OD_{600} of 0.05 and added to 96-well plates with 2× pre-reduced LCA or probes in LB for a total volume of 200 μL/well (75 μL culture + 75 μL 2× compound in LB). OD_{600} was read in an Epoch 2 plate reader (Thermo Fisher Scientific). Percent growth inhibition was calculated from the OD_{600} relative to DMSO at after incubation (4 h, 37°C). Two biological replicates from independent starter cultures were used per experiment.

***S.* Typhimurium infection and quantification of intracellular bacteria.** *S.* Typhimurium was cultured in LB overnight and diluted into of SPI-1-inducing LB (OD_{600}=0.2) with 100 μM of LCA or derivatives for 4 h at 37 °C with 220 rpm. shaking. HeLa cells were seeded in 24-well plates (0.5×10^6 cells/well) and cultured for 12 h at 37℃ in a 5% CO_2 incubator. Bacterial culture was added onto cells at MOI = 50 for 5-30 min incubation at 37℃. Cells were washed with PBS three times to remove extracellular bacteria and incubated in fresh DMEM containing 100 mg/mL gentamycin (Sigma) for another 1 h. For intracellular bacteria quantification, HeLa cells were lysed in cold PBS containing 0.1% Triton X-100, and colony-forming units (CFU) was determined by serial-dilution plating on LB plates containing 100 mg/mL streptomycin (Sigma) and cultured at 37 °C for 8 h.

Immunofluorescence and confocal microscopy. To visualize the infection of *S.* Typhimurium, HeLa cells were grown to 80% confluency in an 8-well chamber (Thermo Fisher Scientific) and infected by DMSO/LCA/C6-d_2-LCA treated *S.* Typhimurium with two replicates each. These cells were then permeabilized with 0.2% Triton X-100 for 30 min and blocked with 5% bovine serum albumin (BSA) for 30 min. Immunostaining was performed with anti-Salmonella (ab35156, abcam) overnight at 4°C. After being washed with TBST, cells were incubated with fluorescent secondary antibodies, Phalloidin-iFluor 647 (ab176759, abcam) and Hoechst 33342 (MCE) in TBST for 1 h at room temperature. Images were acquired using a Zeiss LSM 900 confocal microscope (Carl Zeiss MicroImaging GmbH, 07440, Jena, Germany).

Knock-out of *sipA* in *S.* Typhimurium. Bacterial gene deletion was performed as previously described[7]. Briefly, the target gene in the bacterial chromosome was replaced with a kanamycin-resistance cassette by homologous recombination using the following primers:
sipA-KO-forward: 5'-ATGGTTACAAGTGTAAGGACTCAGCCCCCCGTCATAATGCCAG GTATGCAGACCGAGATCATTCCGGGGATCCGTCGACC-3'
sipA-KO-reverse: 5'-TTAACGCTGCATGTGCAAGCCATCAACGGTAGTAATAACCCGA TCCACGCCAGGTTTATTGTGTAGGCTGGAGCTGCTTCG-3'

Protein purification and chemical modification of SipA. The C-terminal domain of SipA (SipAc, residues 425–685) was cloned into the pET28a vector to generate an N-terminal His-tagged construct. The recombinant protein was subsequently expressed in *E. coli* BL21(DE3) cells (Alpalifebio) upon induction with 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) overnight at 16°C and purified using Ni-NTA Beads 6FF (Smart-Lifesciences). For chemically mimicking the LCA modification, 10 μg of $SipA_C$ was incubated with 500 μM of 6-AcCl-LCA in 30 μL of PBS with 0.1% Triton X-100 for two hours at 29 ℃. After incubation, the small molecule was removed by ultrafiltration and the modified protein was subjected to immunoprecipitation.

Immunoprecipitation. HeLa cells were lysed by 0.1% Triton X-100 in PBS (with protease inhibitor) and diluted to 1 mg/mL 300 μL. Then, 5 μg of native SipAc or modified SipAc were added and incubated for 2 h at room temperature. After incubation, the anti-Myc magnetic beads (Selleck) were washed with 0.1% Triton X-100 in PBS three times and added into the mixture (10 μL each sample) for incubation over night at 4℃. After enrichment, the beads were washed three times by PBS with 0.1% Triton X-100 and 300 mM NaCl. The proteins were eluted by 30 μL 2×Loading buffer at 95℃ for western blot analysis.

Western blotting. Cell lysates were prepared as mentioned above and separated by SDS-PAGE. Proteins were transferred on to a poly(vinylidene fluoride) (Sigma) membrane. The membranes were blocked with 5% nonfat milk (Cell Signaling Technology) in tris-buffered saline with Tween-20 (TBST) buffer for 1 h at room temperature and incubated with primary antibody in TBST with 5% BSA overnight at 4 °C. The membranes were then washed with TBST three times, followed by incubation with secondary antibody in 5% nonfat milk and TBST buffer for 1 h at room temperature. After washing three times with TBST, the membranes were visualized by ChemiDoc XRS+ (Bio-Rad). Primary antibodies used for immunoblotting including anti-Flag tag antibody (66008, Proteintech), anti-Myc tag antibody (16286, Proteintech) and anti-Actin antibody (66009, Proteintech).

Data visualization. Venn diagrams were generated on https://bioinfogp.cnb.csic.es/tools/venny

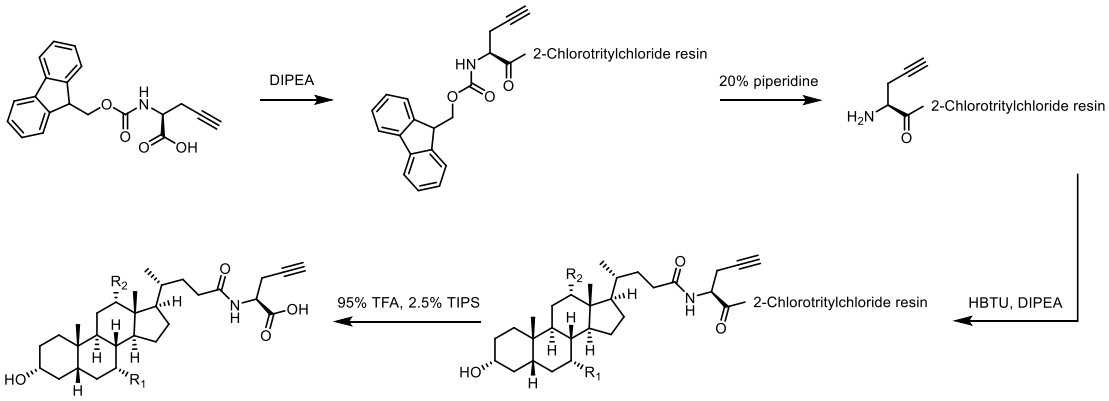
/index.html. Heat maps were generated with https://www.bioinformatics.com.cn using normalized and scale abundance.

Procedure of Synthesis and NMR data

1. General information

All chemicals were purchased from commercial suppliers and used without further purification unless otherwise noted. All purification procedures of products were carried out using reagent grade solvents. Thin layer chromatography (TLC) was performed on TLC Silica Gel 60 F254 (Merck). TLC visualized using ceric ammonium nitrate (CAN). Flash column chromatography was performed on Silica Gel 60 (40-64 m, Fluka, Canada). NMR spectra were measured on Bruker AM 400, Agilent 500 or 600 MHz NMR spectrometer at 25 ^{o}C. ^{1}H and ^{13}C NMR signals were calibrated to the residual proton and carbon resonance of the solvent (chloroform-*d*: δ_H = 7.26 ppm; δ_C = 77.16 ppm. methanol-d_4: δH = 3.31 ppm; δ_C = 49.00 ppm. dimethyl sulfoxide-d_6: δ_H = 2.50 ppm; δ_C = 39.52 ppm). Multiplicities are recorded as: s = singlet, d = doublet, t = triplet, dd = doublet of doublets, td = triplet of doublets, brs = broad singlet, m = multiplet. High-resolution mass spectra were recorded with Thermo Scientific Q Exactive Orbitrap Mass Spectrometers.

2. Synthesis of BA-Galk probes



2-Chlorotrityl chloride resin (1.0 g, loading capacity of approximately 1.0 mmol) was washed with dichloromethane (DCM) and subsequently swollen in DCM for 10 min with gentle agitation. The resin was then washed with *N*,*N*-dimethylformamide (DMF), followed by the addition of Fmoc-Gly-alk (1.0 mmol) and N,N-diisopropylethylamine (DIPEA; 2.0 mmol). The reaction mixture was agitated at room temperature for 1 h. After coupling, the resin was washed with DMF and treated with 20% piperidine in DMF for 45 min to remove the Fmoc protecting group. CA/CDCA/DCA/LCA (1.5 mmol), HBTU (1.5 mmol), and DIPEA (3.0 mmol) were then added to the resin at a molar ratio of 1:1:2, and the coupling reaction was allowed to proceed for 2 h at room temperature with agitation. Upon completion of the reaction, the resin was sequentially washed with DMF, methanol, and DCM. The product was cleaved from the resin by a cleavage cocktail consisting of trifluoroacetic acid (TFA)/triisopropylsilane (TIPS)/H_2O (95:2.5:2.5, v/v/v) for 30 min with agitation and concentrated under reduced pressure. The residues were purified by silica gel column chromatography (DCM/MeOH = 20:1).

CAGalk: ^{1}H NMR (400 MHz, Methanol-d_4) δ 4.52 (dd, J = 7.3, 5.2 Hz, 1H), 3.95 (t, J = 2.9 Hz, 1H), 3.79 (q, J = 3.0 Hz, 1H), 2.79 – 2.60 (m, 2H), 2.41 – 2.14 (m, 5H), 2.09 – 1.22 (m, 22H), 1.11 (dd, J = 12.2, 5.5 Hz, 1H), 1.04 (d, J = 6.5 Hz, 3H), 0.91 (s, 3H), 0.71 (s, 3H).

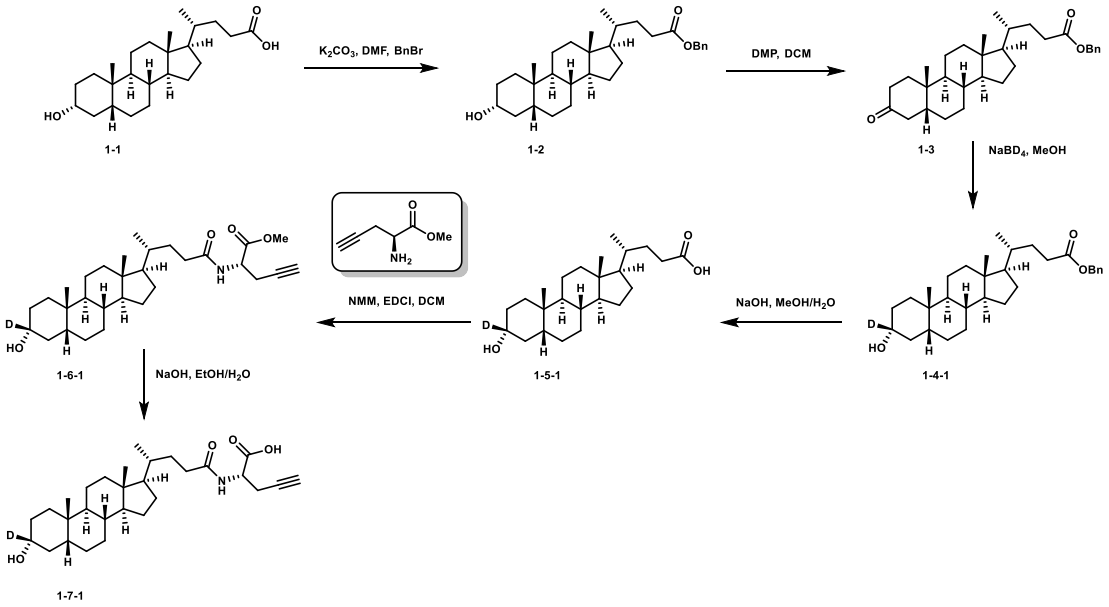
CDCAGalk: ^{1}H NMR (400 MHz, Methanol-d_4) δ 4.41 (s, 1H), 3.79 (s, 1H), 3.49 (q, J = 7.1 Hz, 3H), 2.83 – 2.58 (m, 3H), 2.39 – 2.15 (m, 5H), 2.05 – 1.75 (m, 8H), 1.75 – 1.40 (m, 11H), 1.31 (d, J = 20.1 Hz, 9H), 1.18 (t, J = 7.1 Hz, 7H), 0.98 (d, J = 6.4 Hz,

4H), 0.92 (s, 4H).

DCAGalk: ^{1}H NMR (400 MHz, Methanol-d_4) δ 4.52 (t, J = 6.2 Hz, 1H), 3.96 (q, J = 2.7 Hz, 1H), 3.52 (ddd, J = 15.6, 10.7, 4.5 Hz, 1H), 2.79 – 2.61 (m, 2H), 2.38 – 2.28 (m, 2H), 2.25 – 2.12 (m, 1H), 2.02 – 1.73 (m, 8H), 1.69 – 1.22 (m, 16H), 1.17 – 0.85 (m, 10H), 0.71 (d, J = 2.2 Hz, 3H).

LCAGalk: ^{1}H NMR (400 MHz, Methanol-d_4) δ 4.43 (d, J = 6.4 Hz, 1H), 3.59 – 3.45 (m, 1H), 3.34 (s, 1H), 2.70 (qdd, J = 16.9, 5.9, 2.6 Hz, 2H), 2.38 – 2.31 (m, 1H), 2.30 (t, J = 2.6 Hz, 1H), 2.25 – 2.10 (m, 1H), 2.02 (d, J = 11.8 Hz, 1H), 1.98 – 1.68 (m, 5H), 1.61 (s, 2H), 1.51 – 1.07 (m, 19H), 0.99 – 0.92 (m, 6H), 0.69 (s, 3H).

3. Synthesis of compound 1-7-1.



A 25 mL Schlenk-tube equipped with a magnetic stir bar were charged with **1-1** (367 mg, 1.0 mmol) and potassium carbonate (276 mg, 2.0 mmol) and *N*,*N*-dimethylformamide (2 mL). benzyl bromide (0.178 mL, 1.5 mmol) was added, and the mixture was stirred at room temperature for 12 h. The reaction was quenched by the addition of H_2O (15 mL), followed by extraction with ethyl acetate (10 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 5:1) to afford **1-2** as a white solid (440

mg, 94%).

To a solution of **1-2** (100 mg, 0.21 mmol) in dichloromethane was added Dess-Martin periodinane (91 mg, 0.42 mmol), and the mixture was stirred at room temperature for 12 h. The insoluble solid was removed by filtration. The filtrate was washed with saturated $NaHCO_3$ solution (5 mL) and extracted with dichloromethane (5 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by column chromatography (petroleum ether/ethyl acetate = 5:1) afforded compound **1-3** as a white solid (92 mg, 94%).

NMR Data of 1-3：

^{1}H NMR (500 MHz, chloroform-*d*) δ 7.40 – 7.30 (m, 5H), 5.16 – 5.06 (m, 2H), 2.45 – 2.34 (m, 1H), 2.34 – 2.24 (m, 2H), 1.01 (s, 3H), 0.91 (d, *J* = 6.4 Hz, 3H), 0.66 (s, 3H).

^{13}C NMR (125 MHz, chloroform-*d*) δ 213.6, 174.2, 136.2, 128.7, 128.4, 128.3, 66.2, 56.5, 56.1, 44.5, 42.9, 42.5, 40.8, 40.2, 37.4, 37.1, 35.6, 35.4, 35.0, 31.4, 31.1, 28.3, 26.7, 25.9, 24.3, 22.8, 21.3, 18.4, 12.2.

To a solution of **1-3** (238 mg, 0.51 mmol) in methanol (5 mL) was added sodium borodeuteride (32 mg, 0.77 mmol), and the mixture was stirred at room temperature for 2 h. The reaction was quenched by the addition of saturated NH_4Cl solution (5 mL) and extracted with dichloromethane (5 mL×3). The organic layers were combined, washed with saturated brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by column chromatography (petroleum ether/ethyl acetate = 5:1) afforded compound **1-4-1** as a white solid (171 mg, 70%).

NMR Data of 1-4-1：

^{1}H NMR (500 MHz, chloroform-*d*) δ 7.40 – 7.28 (m, 5H), 5.16 – 5.06 (m, 2H), 2.40 (ddd, J = 15.1, 9.9, 5.0 Hz, 1H), 2.27 (ddd, J = 15.7, 9.4, 6.7 Hz, 1H), 0.91 (s, 3H), 0.90 (d, J = 6.4 Hz, 3H), 0.61 (s, 3H).

^{13}C NMR (125 MHz, chloroform-*d*) δ 174.3, 136.2, 128.7, 128.4, 128.3, 66.2, 56.6, 56.1, 42.8, 42.2, 40.5, 40.3, 36.5, 36.0, 35.5, 35.4, 34.7, 31.4, 31.1, 30.5, 28.3, 27.3, 26.5, 24.3, 23.5, 20.9, 18.4, 12.1.

To a solution of **1-4-1** (172 mg, 0.36 mmol) in methanol/H_2O (10 mL/2.5 mL) was added sodium hydroxide (144 mg, 3.6 mmol), and the mixture was stirred at room temperature for 12 h. The reaction was quenched by the addition of 2 M hydrochloric acid (20 mL), then extracted with dichloromethane (30 mL×3). The organic layers were combined, washed with saturated brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dried under vacuum to afford crude product **1-5-1**, which was used directly in the next step without further purification.

A 25 mL Schlenk-tube equipped with a magnetic stir bar were charged with **1-5-1** (17 mg, 0.045 mmol), methyl *L*-propargylglycinate (17 mg, 0.135 mmol), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (26 mg, 0.135 mmol) and *N*,*N*-dimethylformamide (2 mL). *N*-methylmorpholine (25 μL, 0.225 mmol) was added, and the mixture was stirred at room temperature for 12 h. The reaction was quenched by the addition of H_2O (10 mL), followed by extraction with ethyl acetate (10 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (dichloromethane/methanol = 50:1) to afford **1-6-1** as a white solid (15 mg, 70%).

NMR Data of 1-6-1：

^{1}H NMR (500 MHz, chloroform-*d*) δ 6.27 (d, J = 7.8 Hz, 1H), 4.76 (dt, J = 8.5, 4.7 Hz, 1H), 3.79 (s, 3H), 2.77 (dt, J = 4.4, 2.2 Hz, 2H), 2.32 (ddd, J = 15.1, 10.4, 5.0 Hz, 1H), 2.16 (ddd, J = 15.1, 10.0, 6.4 Hz, 1H), 2.03 (t, J = 2.7 Hz, 1H), 0.93 (d, J = 6.5 Hz, 3H), 0.91 (s, 3H), 0.64 (s, 3H).

^{13}C NMR (125 MHz, chloroform-*d*) δ 173.4, 171.2, 78.7, 71.7, 56.6, 56.1, 52.9, 50.6, 42.9, 42.2, 40.5, 40.3, 36.5, 36.0, 35.6, 35.5, 34.7, 33.5, 31.6, 30.6, 28.4, 27.3, 26.5, 24.3, 23.5, 22.6, 21.0, 18.5, 12.2.

To a solution of **1-6-1** (13 mg, 0.027 mmol) in ethanol/H_2O (1 mL/0.5 mL) was added sodium hydroxide (10.8 mg, 0.27 mmol), and the mixture was stirred at room temperature for 1 h. The reaction was quenched by the addition of 2 M hydrochloric acid (15 mL). The precipitated solid was collected by filtration and dried under vacuum to afford compound **1-7-1** as a white solid (9 mg, 73%).

NMR Data of 1-7-1：

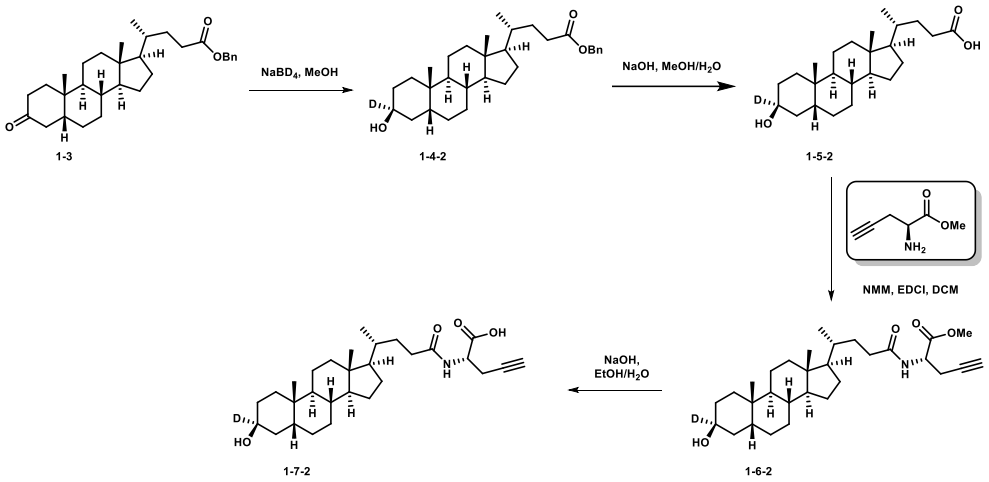
^{1}H NMR (600 MHz, dimethyl sulfoxide-d_6) δ 8.16 (d, J = 7.9 Hz, 1H), 2.85 (t, J = 2.6 Hz, 1H), 2.16 (ddd, J = 14.4, 9.6, 5.1 Hz, 1H), 2.04 (ddd, J = 14.1, 9.5, 6.8 Hz, 1H), 0.88 (d, J = 6.7 Hz, 3H), 0.87 (s, 3H), 0.60 (s, 3H).

^{13}C NMR (150 MHz, dimethyl sulfoxide-d_6) δ 172.7, 171.9, 80.6, 72.9, 56.1, 55.6, 50.9, 42.3, 41.5, 40.1, 40.0, 36.2, 35.4, 35.2, 34.9, 34.2, 32.0, 31.5, 30.3, 27.7, 26.9, 26.2, 23.9, 23.3, 21.1, 20.4, 18.3, 11.9.

HRMS data of 1-7-1：

HRMS (ESI): calcd for $C_{29}H_{45}DNO_4$ $[M+H]^+$ 473.3484, found: 473.3492.

4. Synthesis of compound 1-7-2.



To a solution of **1-3** (238 mg, 0.51 mmol) in methanol (5 mL) was added sodium borodeuteride (32 mg, 0.77 mmol), and the mixture was stirred at room temperature for 2 h. The reaction was quenched by the addition of saturated NH_4Cl solution (5 mL) and extracted with dichloromethane (5 mL×3). The organic layers were combined, washed with saturated brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by column chromatography (petroleum ether/ethyl acetate = 5:1) afforded compound **1-4-2** as a white solid (48 mg, 20%).

NMR Data of 1-4-2：

^{1}H NMR (500 MHz, chloroform-*d*) δ 7.40 – 7.30 (m, 5H), 5.18 – 5.06 (m, 2H), 2.40 (ddd, *J* = 15.2, 10.0, 5.1 Hz, 1H), 2.27 (ddd, *J* = 15.7, 9.5, 6.7 Hz, 1H), 0.95 (s, 3H), 0.90 (d, *J* = 6.5 Hz, 3H), 0.62 (s, 3H).

^{13}C NMR (100 MHz, chloroform-*d*) δ 174.3, 136.3, 128.7, 128.7, 128.4, 128.3, 66.2, 56.8, 56.1, 42.9, 40.4, 39.9, 36.7, 35.8, 35.5, 35.3, 33.5, 31.4, 31.1, 30.1, 29.8, 28.3, 27.9, 26.8, 26.4, 24.3, 24.0, 21.2, 18.4, 12.2.

To a solution of **1-4-2** (46 mg, 0.1 mmol) in methanol/H_2O (3 mL/0.6 mL) was added sodium hydroxide (40 mg, 1.0 mmol), and the mixture was stirred at room temperature for 12 h. The reaction was quenched by the addition of 2 M hydrochloric acid (20 mL), then extracted with dichloromethane (10 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dried under vacuum to afford crude product **1-5-2**, which was used directly in the next step without further purification.

A 25 mL Schlenk-tube equipped with a magnetic stir bar were charged with **1-5-2** (17 mg, 0.045 mmol), methyl *L*-propargylglycinate (17 mg, 0.135 mmol), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (26 mg, 0.135 mmol) and *N*,*N*-dimethylformamide (2 mL). *N*-methylmorpholine (25 μL, 0.225 mmol) was added, and the mixture was stirred at room temperature for 12 h. The reaction was quenched by the addition of H_2O (10 mL), followed by extraction with ethyl acetate (10 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (dichloromethane/methanol = 50:1) to afford **1-6-2** as a white solid (13 mg, 60%).

NMR Data of 1-6-2：

^{1}H NMR (400 MHz, chloroform-*d*) δ 6.26 (d, *J* = 7.9 Hz, 1H), 4.75 (dt, *J* = 8.4, 4.6 Hz, 1H), 3.79 (s, 3H), 2.80 – 2.74 (m, 2H), 2.31 (ddd, *J* = 15.0, 10.3, 5.1 Hz, 1H), 2.15 (ddd, *J* = 14.7, 9.8, 6.3 Hz, 1H), 0.95 (s, 3H), 0.93 (d, *J* = 6.4 Hz, 3H), 0.64 (s, 3H).

^{13}C NMR (100 MHz, chloroform-*d*) δ 173.4, 171.2, 78.7, 71.7, 56.8, 56.2, 52.9, 50.6, 42.9, 40.4, 39.9, 36.7, 35.8, 35.6, 35.3, 33.54, 33.52, 31.7, 30.1, 28.4, 27.8, 26.8, 26.4, 24.3, 24.0, 22.6, 21.2, 18.5, 12.2.

To a solution of **1-6-2** (13 mg, 0.027 mmol) in ethanol/H_2O (1 mL/0.5 mL) was added sodium hydroxide (10.8 mg, 0.27 mmol), and the mixture was stirred at room temperature for 1 h. The reaction was quenched by the addition of 2 M hydrochloric acid (15 mL), The precipitated solid was collected by filtration and dried under vacuum to afford compound **1-7-2** as a white solid (12 mg, 94%).

NMR Data of 1-7-2：

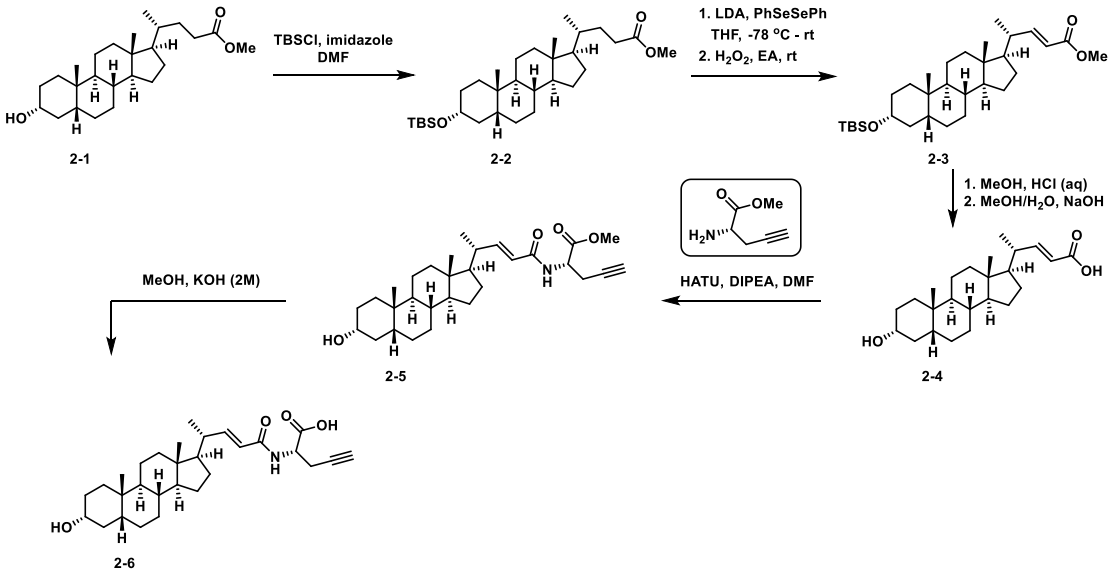
^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ 12.79 (s, 1H), 8.18 (d, J = 7.9 Hz, 1H), 4.31 (td, J = 7.7, 5.5 Hz, 1H), 4.16 (s, 1H), 2.87 (t, J = 2.6 Hz, 1H), 2.16 (ddd, *J* = 14.4, 9.5, 5.1 Hz, 1H), 2.04 (ddd, *J* = 14.2, 8.9, 6.6 Hz, 1H), 0.92 – 0.84 (m, 6H), 0.61 (s, 3H).

^{13}C NMR (100 MHz, dimethyl sulfoxide-d_6) δ 172.7, 171.9, 80.5, 72.9, 56.1, 55.6, 50.8, 42.3, 36.0, 35.2, 34.9, 34.7, 33.2, 32.0, 31.5, 29.7, 27.7, 27.4, 26.4, 26.0, 23.8, 21.1, 20.7, 18.3, 11.9.

HRMS data of 1-7-2：

HRMS (ESI): calcd for $C_{29}H_{45}DNO_4$ $[M+H]^+$ 473.3484, found: 473.3492.

5. Synthesis of compound 2-6.



A 50 mL Schlenk-tube equipped with a magnetic stir bar were charged with **2-1** (1.0 g, 2.56 mmol), imidazole (348 mg, 5.12 mmol) and *N*,*N*-dimethylformamide (10

mL). After cooling to 0 °C, tert-butyldimethylsilyl chloride (578 mg, 3.84 mmol) was added while stirring. The mixture was warmed to room temperature and stirred for 6 h. The reaction was quenched by the addition of saturated NH_4Cl solution (30 mL), followed by extraction with ethyl acetate (50 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 30:1) to afford **2-2** as a white solid (1.12 g, 86%).

NMR Data of 2-2：

^{1}H NMR (500 MHz, chloroform-*d*) δ 3.66 (s, 3H), 3.57 (tt, *J* = 10.5, 4.6 Hz, 1H), 2.35 (ddd, *J* = 15.3, 10.2, 5.1 Hz, 1H), 2.21 (ddd, *J* = 15.6, 9.8, 6.5 Hz, 1H), 0.89 (d, *J* = 2.2 Hz, 15H), 0.63 (s, 3H), 0.06 (s, 6H).

^{13}C NMR (100 MHz, chloroform-*d*) δ 174.9, 73.0, 56.5, 56.1, 51.6, 42.9, 42.4, 40.4, 40.3, 37.1, 36.0, 35.7, 35.5, 34.7, 31.21, 31.18, 31.16, 28.3, 27.4, 26.5, 26.1, 24.3, 23.5, 21.0, 18.5, 18.4, 12.2, -4.4.

Under argon atmosphere, a 50 mL Schlenk-tube equipped with a magnetic stir bar were charged with **2-2** (50 mg, 0.1 mmol) and anhydrous tetrahydrofuran (2 mL). After cooling to -78 °C, lithium diisopropylamide (75 μL, 0.15 mmol) was added dropwise, the mixture was stirred at -78 °C for 0.5 h. Then a solution of Ph_2Se_2 (62 mg, 0.2 mmol) in tetrahydrofuran (0.5 mL) was slowly added, followed by stirring at -78 ºC for 2 h. The reaction was quenched with saturated NH_4Cl solution (10 mL) and extracted with ethyl acetate(10 mL×3). The organic layers were combined, washed with 1 M hydrochloric acid and saturated aqueous $NaHCO_3$ solution sequentially, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by column chromatography (petroleum ether/ethyl acetate = 50:1) afforded a white solid. The product was dispersed in ethyl acetate (2 mL), and the reaction mixture was cooled to 0 ºC. hydrogen peroxide (30 wt% solution in water, 50 μL, 0.6 mmol) was added, and the mixture was gradually warmed to room temperature and stirred for 40 min. The reaction was quenched with brine (5 mL) and extracted with ethyl acetate (5 mL×3). The organic layers were combined, washed with saturated brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by column chromatography (petroleum ether/ethyl acetate = 50:1) afforded compound **2-3** as a white solid (20 mg, 40%).

NMR Data of 2-3：

^{1}H NMR (500 MHz, chloroform-*d*) δ 6.84 (dd, *J* = 15.6, 9.0 Hz, 1H), 5.73 (dd, *J* = 15.6, 0.9 Hz, 1H), 3.72 (s, 3H), 3.58 (tt, *J* = 10.6, 4.6 Hz, 1H), 2.25 (q, *J* = 8.2 Hz, 1H), 1.93 (d, *J* = 11.0 Hz, 1H), 0.90 (s, 3H), 0.89 (s, 9H), 0.67 (s, 3H), 0.06 (s, 6H).

^{13}C NMR (100 MHz, chloroform-*d*) δ 167.6, 155.3, 118.6, 72.9, 56.4, 55.2, 51.5, 43.2, 42.4, 40.4, 40.1, 39.9, 37.1, 36.0, 35.7, 34.7, 31.2, 28.3, 27.4, 26.5, 26.1, 24.4, 23.5, 20.9, 19.3, 18.5, 12.4, -4.4.

To a solution of **2-3** (20 mg, 0.04 mmol) in methanol/dichloromethane (1 mL/0.5 mL) was added concentrated hydrochloric acid (10 μL), and the mixture was stirred at room temperature for 1 h. The reaction was quenched with saturated $NaHCO_3$ (4 mL) and extracted with dichloromethane (5 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dissolved in methanol/dichloromethane/H_2O (1 mL/0.5 mL/0.5 mL), sodium hydroxide (16 mg, 0.4 mmol) was added, and then the resulting mixture was stirred at 60 ºC for 2 h. After that, the reaction mixture was cooled to room temperature, quenched with 2 M hydrochloric acid (5 mL), and extracted with dichloromethane (5 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dried under vacuum to afford crude product **2-4**, which was used directly in the next step without further purification.

LRMS data of 2-4：

LRMS (ESI): calcd for $C_{24}H_{37}O_3$ $[M-H]^-$ 373.2, found: 373.0.

A 25 mL Schlenk-tube equipped with a magnetic stir bar were charged with **2-4** (15 mg, 0.04 mmol), methyl *L*-propargylglycinate (15 mg, 0.12 mmol), *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (45 mg, 0.12 mmol) and *N*,*N*-dimethylformamide (1 mL). *N*,*N*-diisopropylethylamine (35 μL, 0.2 mmol) was added, and the mixture was stirred at room temperature for 12 h. The reaction was quenched by the addition of H_2O (10 mL), followed by extraction with ethyl acetate (10 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue

was dried under vacuum to afford crude product **2-5**, which was used directly in the next step without further purification.

To a solution of **2-5** in methanol (1 mL) was added 2 M sodium hydroxide (0.5 mL), and the mixture was stirred at room temperature for 1 h. The reaction was quenched by the addition of 2 M hydrochloric acid (20 mL), The precipitated solid was collected by filtration and dried under vacuum to afford compound **2-6** as a white solid (12 mg, 64% over three steps).

NMR Data of 2-6:

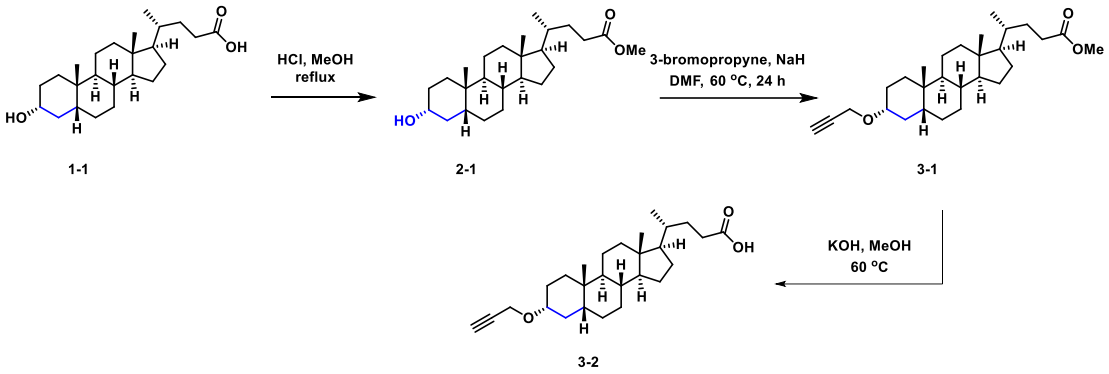
^{1}H NMR (400 MHz, methanol-d_4) δ 6.62 (dd, J = 15.3, 9.2 Hz, 1H), 5.92 (dd, J = 25.1, 15.2 Hz, 1H), 3.64 – 3.47 (m, 1H), 1.09 (d, J = 7.0 Hz, 3H), 0.95 (s, 3H), 0.73 (s, 3H).

^{13}C NMR (100 MHz, methanol-d_4) δ 151.2, 122.6, 72.4, 57.8, 56.7, 47.3, 44.1, 43.5, 43.5, 41.9, 41.3, 40.9, 37.24, 37.17, 36.5, 35.7, 35.4, 31.2, 29.9, 29.3, 28.3, 27.6, 25.3, 23.9, 21.9, 20.0, 12.7, 11.6.

HRMS data of 2-6:

HRMS (ESI): calcd for $C_{29}H_{44}NO_4$ $[M+H]^+$ 470.3265, found: 470.3275.

6. Synthesis of compound 3-2.



A 250 mL Schlenk-tube equipped with a magnetic stir bar were charged with **1-1** (1.88 g, 5.0 mmol) and methanol (50 mL), concentrated hydrochloric acid (0.2 mL) was added. The mixture was heated under reflux with stirring for 8 h. After that, the reaction mixture was cooled to room temperature, quenched with saturated $NaHCO_3$ (100 mL),

and extracted with ethyl acetate (100 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dried under vacuum to afford crude product **2-1**, which was used directly in the next step without further purification.

Under argon atmosphere, a 25 mL Schlenk-tube equipped with a magnetic stir bar were charged with **2-1** (195 mg, 0.5 mmol) and anhydrous tetrahydrofuran (2 mL), sodium hydride (40 mg, 1.0 mmol) was added. The mixture was stirred at room temperature for 0.5 h. Subsequently, 3-bromopropyne (0.37 mL, 0.75 mmol) was added, and the reaction mixture was stirred at room temperature overnight. After that, the reaction mixture was quenched with saturated NH_4Cl solution (10 mL), and extracted with ethyl acetate (10 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 40:1) to afford **3-1** as a white solid (14 mg, 6%).

NMR Data of 3-1：

^{1}H NMR (400 MHz, chloroform-*d*) δ 4.18 (d, *J* = 2.4 Hz, 2H), 3.66 (s, 3H), 3.48 (ddt, *J* = 15.6, 10.8, 4.6 Hz, 1H), 2.39 (t, *J* = 2.5 Hz, 1H), 2.34 (td, *J* = 10.2, 5.0 Hz, 1H), 2.21 (ddd, *J* = 15.6, 9.6, 6.5 Hz, 1H), 0.90 (d, *J* = 7.8 Hz, 6H), 0.63 (s, 2H).

^{13}C NMR (100 MHz, chloroform-*d*) δ 174.9, 80.7, 78.4, 73.8, 56.6, 56.0, 55.1, 51.6, 42.9, 42.2, 40.4, 40.3, 36.0, 35.5, 35.4, 35.0, 32.9, 31.2, 31.1, 28.3, 27.4, 27.0, 26.5, 24.3, 23.5, 20.9, 18.4, 12.2.

To a solution of **3-1** (14 mg, 0.032 mmol) in ethanol (2.0 mL) was added 2 M sodium hydroxide (0.5 mL), and the mixture was stirred at 60 ºC for 1 h. After that, the reaction mixture was cooled to room temperature, quenched with 2 M hydrochloric acid (15 mL). The precipitated solid was collected by filtration and dried under vacuum to afford compound **3-2** as a white solid (12 mg, 90%).

NMR Data of 3-2：

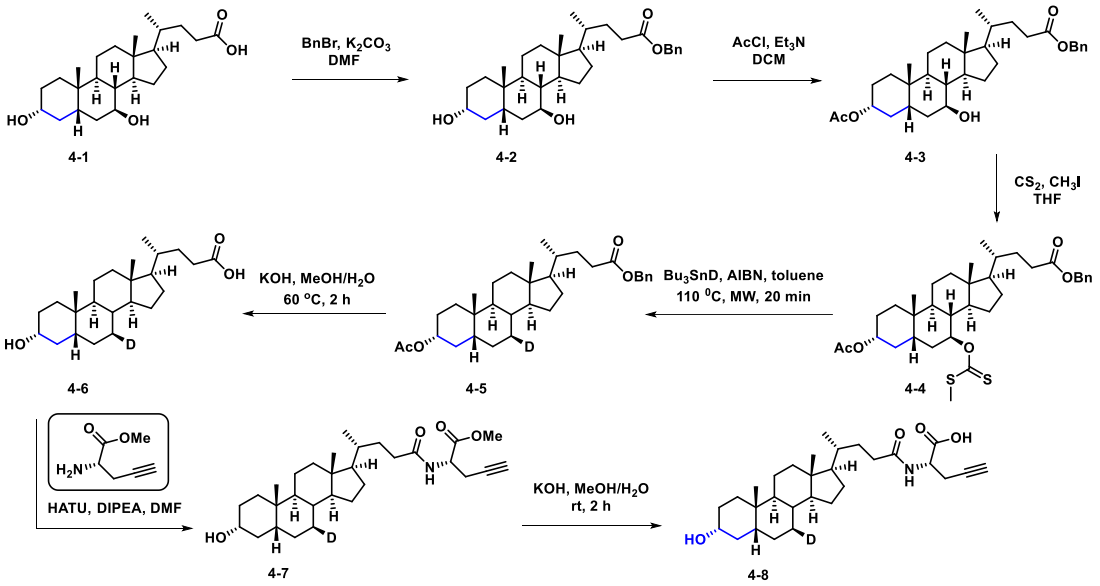
^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ 11.96 (s, 1H), 4.12 (d, *J* = 2.4 Hz, 2H), 2.22 (ddd, *J* = 15.2, 9.6, 5.2 Hz, 1H), 2.09 (ddd, *J* = 15.7, 9.3, 6.8 Hz, 1H), 0.88 (s, 3H), 0.86 (d, *J* = 6.7 Hz, 3H), 0.61 (s, 3H).

^{13}C NMR (100 MHz, dimethyl sulfoxide-d_6) δ 174.9, 81.2, 77.2, 76.3, 55.9, 55.5, 54.3, 42.3, 41.4, 35.3, 34.84, 34.79, 34.4, 32.4, 30.7, 30.7, 27.7, 26.8, 26.6, 26.0, 23.8, 23.2, 20.4, 18.1, 11.9.

HRMS data of 3-2：

HRMS (ESI): calcd for $C_{27}H_{42}NaO_3$ $[M+Na]^+$ 437.3026, found: 437.3030.

7. Synthesis of compound 4-8.



A 100 mL Schlenk-tube equipped with a magnetic stir bar were charged with **4-1** (2.35 g, 6.0 mmol) and potassium carbonate (1.67 g, 9.0 mmol) and *N*,*N*-dimethylformamide (20 mL). benzyl bromide (1.0 mL, 1.5 mmol) was added, and the mixture was stirred at room temperature for 12 h. The reaction was quenched by the addition of H_2O (50 mL), followed by extraction with ethyl acetate (100 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (dichloromethane/methanol = 10:1) to afford **4-2** as a white solid (2.8 g, 97%).

A 25 mL Schlenk-tube equipped with a magnetic stir bar were charged with **4-2** (1.40 g, 3.0 mmol), triethylamine (0.49 mL, 3.6 mmol) and dichloromethane (30 mL).

The reaction mixture was cooled to 0 °C, acetyl chloride (0.23 mL, 3.3 mmol) was added, and the mixture was stirred at room temperature overnight. The reaction was quenched by the addition of H_2O (30 mL), followed by extraction with ethyl acetate (60 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 5:1) to afford **4-3** as a white solid (508 mg, 32%).

NMR Data of 4-3：

^{1}H NMR (400 MHz, chloroform-*d*) δ 7.40 – 7.28 (m, 5H), 5.23 – 4.99 (m, 2H), 4.71 – 4.61 (m, 1H), 3.63 – 3.50 (m, 1H), 2.40 (ddd, *J* = 14.9, 9.9, 4.8 Hz, 1H), 2.27 (ddd, *J* = 15.5, 9.7, 6.4 Hz, 1H), 0.95 (s, 3H), 0.91 (d, *J* = 6.2 Hz, 3H), 0.65 (s, 3H).

^{13}C NMR (100 MHz, chloroform-*d*) δ 174.1, 170.7, 136.2, 128.7, 128.6, 128.34, 128.29, 73.9, 71.3, 66.2, 55.8, 55.0, 43.8, 43.8, 42.4, 40.2, 39.3, 36.8, 35.9, 34.7, 34.2, 33.3, 31.4, 31.1, 28.7, 26.9, 26.6, 23.4, 21.5, 21.3, 18.5, 12.2.

Under argon atmosphere, a 25 mL Schlenk-tube equipped with a magnetic stir bar were charged with **4-3** (300 mg, 0.57 mmol) and anhydrous tetrahydrofuran (5 mL), sodium hydride (34 mg, 0.855 mmol) was added. The mixture was stirred at room temperature for 1 h. Subsequently, carbon disulfide (136 μL, 2.25 mmol), and the reaction mixture was stirred at room temperature overnight. After that, iodomethane (53 μL, 0.855 mmol) was added, the mixture was stirred at room temperature for 2 h. The mixture was quenched with saturated NH_4Cl solution (10 mL), and extracted with ethyl acetate (10 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 10:1) to afford **4-4** as a yellow oil (30 mg, 8%).

NMR Data of 4-4：

^{1}H NMR (400 MHz, chloroform-*d*) δ 7.40 – 7.29 (m, 5H), 5.61 (td, *J* = 10.9, 5.2 Hz, 1H), 5.19 – 5.04 (m, 2H), 4.67 (tt, *J* = 10.7, 5.1 Hz, 1H), 2.55 (s, 3H), 2.39 (ddd, *J* = 14.8, 9.6, 4.9 Hz, 1H), 2.26 (ddd, *J* = 15.5, 8.9, 6.8 Hz, 1H), 2.03 (s, 3H), 1.00 (s, 3H), 0.91 (d, *J* = 6.2 Hz, 3H), 0.67 (s, 3H).

^{13}C NMR (100 MHz, chloroform-*d*) δ 215.2, 174.1, 170.7, 136.2, 128.7, 128.4, 128.3, 84.4, 73.6, 66.2, 55.2, 55.1, 43.7, 42.4, 40.3, 40.0, 39.8, 35.3, 34.5, 34.2, 32.7, 31.5, 31.4, 31.1, 28.6, 26.6, 25.7, 23.4, 21.6, 21.4, 19.1, 18.5, 12.2.

Under argon atmosphere, a 25 mL Schlenk-tube equipped with a magnetic stir bar were charged with **4-4** (18 mg, 0.028 mmol), tributyldeuteriostannane[8] (15.6 μL, 0.058 mmol), 2,2'-azobis(2-methylpropionitrile (6.2 mg, 0.038 mmol) and toluene (2 mL). The mixture was then heated to 110 °C under microwave irradiation for 20 min. After cooling to room temperature, the solvent was removed under reduced pressure, and the crude residue was purified by column chromatography (petroleum ether/ethyl acetate = 20:1) to afford **4-5** as a white solid (10 mg, 68%).

NMR Data of 4-5：

^{1}H NMR (600 MHz, chloroform-*d*) δ 7.39 – 7.29 (m, 5H), 5.15 – 5.06 (m, 2H), 4.72 (tt, *J* = 11.4, 4.7 Hz, 1H), 2.40 (ddd, *J* = 15.2, 10.0, 5.1 Hz, 1H), 2.27 (ddd, *J* = 15.7, 9.5, 6.7 Hz, 1H), 2.03 (s, 3H), 0.92 (s, 3H), 0.90 (d, *J* = 6.5 Hz, 3H), 0.62 (s, 3H).

^{13}C NMR (125 MHz, chloroform-*d*) δ 174.3, 170.8, 136.3, 128.7, 128.4, 128.3, 74.5, 66.2, 56.6, 56.1, 42.9, 42.0, 40.5, 40.3, 35.8, 35.4, 35.2, 34.7, 32.4, 31.4, 31.1, 28.3, 27.1, 26.8, 24.3, 23.5, 21.6, 21.0, 18.4, 12.1.

To a solution of **4-5** (10 mg, 0.02 mmol) in methanol (2.0 mL) was added 2 M potassium hydroxide (1.0 mL), and the mixture was stirred at 60 ºC for 2 h. After that, the reaction mixture was cooled to room temperature, quenched with 2 M hydrochloric acid (15 mL) and extracted with dichloromethane (10 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dried under vacuum to afford crude product **4-6**, which was used directly in the next step without further purification.

LRMS data of 4-6：

LRMS (ESI): calcd for $C_{24}DH_{28}O_3$ $[M-H]^-$ 376.2, found: 376.1.

A 25 mL Schlenk-tube equipped with a magnetic stir bar were charged with **4-6**, methyl *L*-propargylglycinate (7.6 mg, 0.06 mmol), *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (22.8 mg, 0.06) and *N*,*N*-dimethylformamide (1 mL). *N*,*N*-diisopropylethylamine (17.8 μL, 0.1 mmol) was

added, and the mixture was stirred at room temperature for 3 h. The reaction was quenched by the addition of H_2O (10 mL), followed by extraction with ethyl acetate (10 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (dichloromethane/methanol = 50:1) to afford **4-7** as a white solid (7 mg, 70%).

NMR Data of 4-7：

^{1}H NMR (500 MHz, chloroform-*d*) δ 6.26 (d, *J* = 7.8 Hz, 1H), 4.76 (dt, *J* = 8.6, 4.6 Hz, 1H), 3.80 (s, 3H), 3.62 (tt, *J* = 10.6, 4.7 Hz, 1H), 2.79 – 2.75 (m, 2H), 2.32 (ddd, *J* = 15.1, 10.2, 4.9 Hz, 1H), 2.16 (ddd, *J* = 15.1, 9.9, 6.3 Hz, 1H), 2.03 (t, *J* = 2.6 Hz, 1H), 0.93 (d, *J* = 6.5 Hz, 3H), 0.91 (s, 3H), 0.64 (s, 3H).

To a solution of **4-7** (7 mg, 0.014 mmol) in methanol (1.0 mL) was added 2 M potassium hydroxide (0.5 mL), and the mixture was stirred at room temperature for 1 h. After that, the reaction mixture was cooled to room temperature, quenched with 2 M hydrochloric acid (15 mL). The precipitated solid was collected by filtration and dried under vacuum to afford compound **4-8** as a white solid (6 mg, 91%).

NMR Data of 4-8：

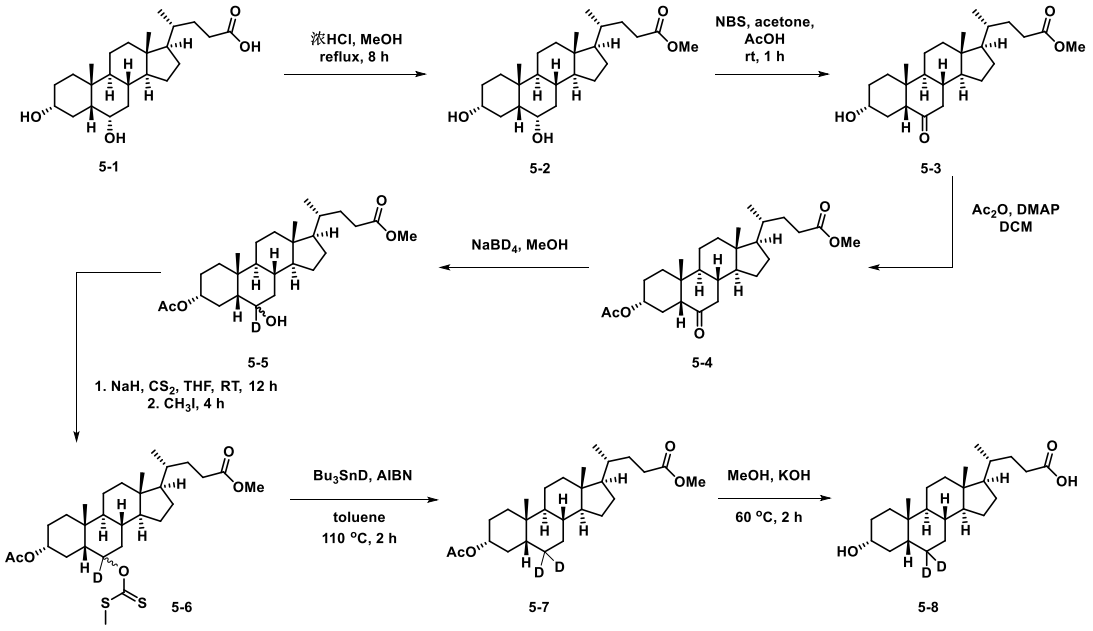
^{1}H NMR (600 MHz, methanol-d_4) δ 4.53 (dd, *J* = 7.5, 5.1 Hz, 1H), 3.57 – 3.50 (m, 1H), 2.78 – 2.64 (m, 2H), 2.35 (t, *J* = 2.7 Hz, 1H), 2.34 – 2.29 (m, 1H), 2.19 (ddd, *J* = 14.1, 9.5, 7.0 Hz, 1H), 0.97 (d, *J* = 6.6 Hz, 3H), 0.95 (s, 3H), 0.69 (s, 3H).

^{13}C NMR (150 MHz, methanol-d_4) δ 176.7, 173.4, 80.3, 72.4, 72.0, 57.9, 57.5, 52.5, 43.9, 43.55, 43.53, 41.9, 41.8, 41.5, 37.18, 37.16, 36.8, 36.5, 35.7, 33.8, 33.2, 31.2, 29.3, 28.3, 25.3, 23.9, 22.3, 21.9, 18.9, 12.5.

HRMS data of 4-8：

HRMS (ESI): calcd for $C_{29}H_{45}DNO_4$ $[M+H]^+$ 473.3484, found: 473.3492.

8. Synthesis of compound 5-8.



A 250 mL Schlenk-tube equipped with a magnetic stir bar were charged with **5-1** (5.4 g, 13.7 mmol) and methanol (81 mL), concentrated hydrochloric acid (0.57 mL) was added. The mixture was heated under reflux with stirring for 8 h. After that, the reaction mixture was cooled to room temperature, quenched with saturated $NaHCO_3$ solution (100 mL), and extracted with ethyl acetate (100 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dried under vacuum to afford crude product **5-2**, which was used directly in the next step without further purification.

A 500 mL Schlenk-tube equipped with a magnetic stir bar were charged with **5-2** (5.58 g, 13.7 mmol), acetic acid (0.94 mL, 16.5 mmol) and acetone/H_2O (200 mL/10 mL). The mixture was cooled to 0 °C, *N*-bromosuccinimide（4.89 g, 27.0 mmol）was added in portions, then the mixture was stirred at room temperature for 1 h. The reaction was quenched by the addition of saturated Na_2SO_3 solution (100 mL), followed by extraction with ethyl acetate (200 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 3:1) to afford **5-3** as a white solid[9] (4.02 g, 72% over two steps).

Under argon atmosphere, a 250 mL Schlenk-tube equipped with a magnetic stir bar were charged with **5-3** (4.02 g, 9.93 mmol), 4-dimethylaminopyridine (182 mg, 1.49

mmol) and dichloromethane (20 mL), acetic anhydride (1.4 mL, 14.9 mmol) was added. The mixture was stirred at room temperature for 2 h. The reaction was quenched by the addition of saturated $NaHCO_3$ solution (40 mL), followed by extraction with dichloromethane (40 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 5:1) to afford **5-4** as a white solid (4.52 g, 99%).

NMR Data of 5-4：

^{1}H NMR (400 MHz, chloroform-*d*) δ 5.15 (dt, *J* = 12.2, 4.8 Hz, 1H), 3.65 (s, 3H), 2.47 (dd, *J* = 15.4, 13.3 Hz, 1H), 1.99 (s, 3H), 1.05 (s, 3H), 0.91 (d, *J* = 6.5 Hz, 3H), 0.66 (s, 3H).

^{13}C NMR (100 MHz, chloroform-*d*) δ 212.1, 174.8, 170.4, 70.7, 56.2, 56.0, 51.6, 47.2, 43.0, 40.4, 39.9, 37.03, 36.98, 36.9, 36.4, 35.4, 34.5, 31.1, 31.03, 31.01, 28.2, 24.2, 22.8, 21.4, 21.1, 18.4, 12.1.

Under argon atmosphere, a 100 mL Schlenk-tube equipped with a magnetic stir bar were charged with **5-4** (1.0 g, 2.15 mmol) and methanol (20 mL), sodium borodeuteride (135 mg, 3.22 mmol) was added. The mixture was stirred at room temperature for 1 h. The reaction was quenched by the addition of saturated NH_4Cl solution (20 mL), followed by extraction with ethyl acetate (50 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 2:1) to afford **5-5** as a white solid (936 mg, 93%).

NMR Data of 5-5：

^{1}H NMR (500 MHz, chloroform-*d*) δ 5.12 (dt, *J* = 12.3, 4.8 Hz, 1H), 3.65 (s, 3H), 2.34 (ddd, *J* = 15.4, 10.1, 5.2 Hz, 1H), 2.21 (ddd, *J* = 15.7, 9.7, 6.5 Hz, 1H), 2.01 (s, 3H), 0.95 (s, 3H), 0.90 (d, *J* = 6.4 Hz, 3H), 0.63 (s, 3H).

^{13}C NMR (125 MHz, chloroform-*d*) δ 174.9, 170.7, 71.4, 56.3, 56.0, 51.6, 45.6, 43.0, 40.02, 39.98, 36.2, 35.5, 35.4, 34.8, 31.5, 31.2 31.1, 30.3, 30.1, 28.2, 24.2, 23.4, 21.5, 20.8, 18.4, 12.1.

Under argon atmosphere, a 25 mL Schlenk-tube equipped with a magnetic stir bar were charged with **5-5** (150 mg, 0.33 mmol) and anhydrous tetrahydrofuran (2.5 mL), sodium hydride (40 mg, 1.0 mmol) was added. The mixture was stirred at room temperature for 1 h. Subsequently, carbon disulfide (0.2 mL, 3.3 mmol) was added, and the reaction mixture was stirred at room temperature overnight. After that, iodomethane (0.2 mL, 3.2 mmol) was added, and then reaction mixture was stirred at room temperature for 2 h. The mixture was quenched with saturated NH_4Cl solution (10 mL), and extracted with ethyl acetate (10 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 15:1) to afford **5-6** as a yellow oil (91 mg, 51%).

NMR Data of 5-6：

^{1}H NMR (400 MHz, chloroform-*d*) δ 5.27 – 5.10 (m, 1H), 3.66 (s, 3H), 2.55 (s, 3H), 2.35 (ddd, *J* = 15.2, 10.1, 5.1 Hz, 1H), 2.21 (ddd, *J* = 15.7, 9.6, 6.5 Hz, 1H), 2.01 (s, 3H), 0.98 (s, 3H), 0.91 (d, *J* = 6.4 Hz, 3H), 0.64 (s, 3H).

^{13}C NMR (100 MHz, chloroform-*d*) δ 215.3, 174.8, 170.6, 71.0, 56.2, 56.0, 51.6, 45.5, 43.0, 43.0, 40.00, 39.96, 36.2, 35.4, 35.0, 34.8, 31.4, 31.2, 31.1, 29.8, 28.2, 25.8, 25.7, 24.2, 23.3, 21.5, 20.8, 19.1, 18.4, 12.1.

Under argon atmosphere, a 25 mL Schlenk-tube equipped with a magnetic stir bar were charged with **5-6** (30 mg, 0.056 mmol), tributyldeuteriostannane (38 μL, 0.14 mmol), 2,2'-azobis(2-methylpropionitrile (13.8 mg, 0.084 mmol) and toluene (2 mL). The mixture was then heated to 110 °C for 2 h. After cooling to room temperature, the solvent was removed under reduced pressure, and the residue was purified by column chromatography (petroleum ether/ethyl acetate = 15:1) to afford **5-7** as a white solid (23 mg, 94%).

NMR Data of 5-7：

^{1}H NMR (600 MHz, chloroform-*d*) δ 5.17 (dt, *J* = 12.4, 4.8 Hz, 1H), 3.66 (s, 3H), 2.35 (ddd, *J* = 15.4, 10.3, 5.2 Hz, 1H), 2.21 (ddd, *J* = 15.4, 9.9, 6.5 Hz, 1H), 2.01 (s, 3H), 0.96 (s, 3H), 0.91 (d, *J* = 6.6 Hz, 3H), 0.64 (s, 3H).

^{13}C NMR (150 MHz, chloroform-*d*) δ 174.9, 170.8, 72.3, 56.4, 56.1, 51.6, 47.2, 43.1, 40.2, 40.1, 37.6, 36.9, 35.5, 34.8, 31.5, 31.2, 31.1, 28.3, 24.30, 24.27, 21.6, 20.9, 20.8, 20.4, 18.4, 12.2.

To a solution of **5-7** (20 mg, 0.02 mmol) in methanol/H_2O (2.0 mL/0.4 mL) was added potassium hydroxide (26 mg, 0.47 mmol), and the mixture was stirred at 60°C for 2 h. After that, the reaction mixture was cooled to room temperature, quenched with 2 M hydrochloric acid (15 mL) and extracted with dichloromethane (10 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure, The residue was purified by column chromatography (dichloromethane/methanol = 30:1) to afford **5-8** as a white solid (17 mg, 95%).

NMR Data of 5-8：

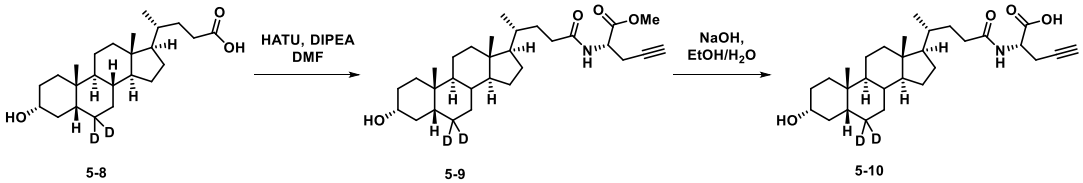
^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ 11.92 (s, 1H), 4.20 – 4.15 (m, 1H), 3.87 (dd, J = 11.5, 5.9 Hz, 1H), 2.31 – 2.16 (m, 1H), 2.15 – 2.03 (m, 1H), 0.87 (d, *J* = 6.8 Hz, 6H), 0.60 (s, 3H).

^{13}C NMR (100 MHz, dimethyl sulfoxide-d_6) δ 174.9, 66.1, 55.8, 55.5, 49.8, 37.4, 36.1, 34.9, 34.8, 34.3, 30.7, 30.7, 27.7, 24.3, 23.9, 20.6, 20.4, 19.0, 18.1, 11.9.

HRMS data of 5-8：

HRMS (ESI): calcd for $C_{24}H_{38}D_2NaO_3$ $[M+Na]^+$ 401.2995, found: 401.2999.

9. Synthesis of compound 5-10.



A 25 mL Schlenk-tube equipped with a magnetic stir bar were charged with **5-8** (9.0 mg, 0.024 mmol), methyl *L*-propargylglycinate (9.1 mg, 0.072 mmol), *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (27 mg, 0.072 mmol) and *N*,*N*-dimethylformamide (1 mL). *N*,*N*-diisopropylethylamine (21 μL, 0.12 mmol) was added, and the mixture was stirred at room temperature for 4 h. The

reaction was quenched by the addition of H_2O (10 mL), followed by extraction with ethyl acetate (10 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dried under vacuum to afford crude product **5-9**, which was used directly in the next step without further purification.

To a solution of **5-9** in ethanol/H_2O (1.5 mL/0.3 mL) was added sodium hydroxide (4.8 mg, 0.12 mmol), and the mixture was stirred at room temperature for 1 h. After that, the reaction mixture was cooled to room temperature, quenched with 2 M hydrochloric acid (15 mL). The precipitated solid was collected by filtration and dried under vacuum to afford compound **5-10** as a white solid (4 mg, 35% for two steps).

NMR Data of 5-10：

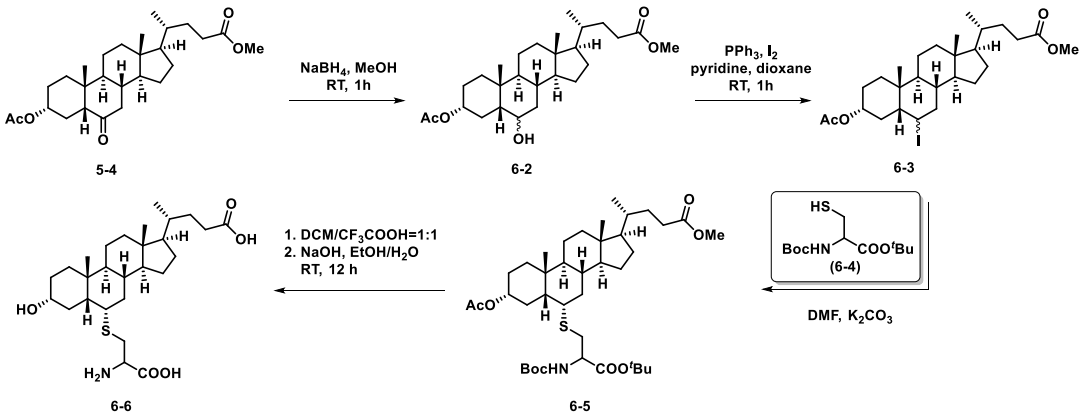
^{1}H NMR (600 MHz, methanol-d_4) δ 4.53 (dd, J = 7.5, 5.1 Hz, 1H), 4.04 (dt, J = 12.2, 4.7 Hz, 1H), 2.77 – 2.63 (m, 2H), 2.35 (t, J = 2.7 Hz, 1H), 0.98 (d, J = 6.6 Hz, 3H), 0.93 (s, 3H), 0.69 (s, 3H).

^{13}C NMR (150 MHz, methanol-d_4) δ 176.7, 173.4, 80.3, 72.0, 69.2, 57.7, 57.5, 52.5, 51.5, 44.0, 41.4, 41.3, 38.9, 37.6, 36.8, 36.2, 35.6, 33.8, 33.1, 30.8, 29.2, 25.3, 24.9, 22.3, 22.0, 21.9, 20.4, 18.9, 12.5.

HRMS data of 5-10：

HRMS (ESI): calcd for $C_{29}H_{44}D_2NO_4$ $[M+H]^+$ 474.3547, found: 474.3552。

10. Synthesis of compound 6-6.



Under argon atmosphere, a 100 mL Schlenk-tube equipped with a magnetic stir bar were charged with **5-4** (4.6 g, 10.0 mmol) and methanol/dichloromethane (80 mL/8 mL), sodium borohydride (571 mg, 15.0 mmol) was added. The mixture was stirred at room temperature for 1 h. The reaction was quenched by the addition of saturated NH_4Cl solution (160 mL), followed by extraction with ethyl acetate (160 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 2:1) to afford **6-2** as a white solid (3.84 g, 86%).

NMR Data of 6-2：

^{1}H MR (400 MHz, chloroform-*d*) δ 5.11 (dt, *J* = 12.4, 4.8 Hz, 1H), 3.64 (s, 3H), 2.33 (ddd, *J* = 15.2, 10.0, 5.1 Hz, 1H), 2.20 (ddd, *J* = 15.5, 9.5, 6.4 Hz, 1H), 2.00 (s, 3H), 0.94 (s, 3H), 0.89 (d, *J* = 6.4 Hz, 3H), 0.62 (d, *J* = 2.7 Hz, 3H).

^{13}C NMR (100 MHz, chloroform-*d*) δ 174.84, 170.65, 71.41, 71.37, 56.24, 55.98, 51.61, 45.54, 42.97, 39.99, 39.95, 36.15, 35.46, 35.42, 34.75, 31.44, 31.13, 31.05, 30.39, 30.13, 28.20, 24.20, 23.39, 21.51, 20.77, 18.35, 12.11.

A 50 mL Schlenk-tube equipped with a magnetic stir bar were charged with **6-2** (1.0 g, 2.22 mmol), triphenylphosphine (1.74 g, 6.66 mmol), and pyridine/dioxane (4 mL/4 mL). Iodine (1.69 g, 6.66 mmol) was added, and the mixture was stirred at room temperature for 6 h. The reaction was quenched by the addition of H_2O (20 mL), followed by extraction with ethyl acetate (20 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 10:1) to afford **6-3** as a white solid (1.17 g, 94%).

NMR Data of 6-3：

^{1}H NMR (400 MHz, chloroform-*d*) δ 5.26 – 5.02 (m, 1H), 3.68 – 3.61 (m, 3H), 2.39 – 2.28 (m, 1H), 2.04 – 1.97 (m, 3H), 1.04 (s, 3H), 0.95 – 0.87 (m, 3H), 0.63 (d, *J* = 1.6 Hz, 3H).

^{13}C NMR (100 MHz, chloroform-*d*) δ 174.8, 170.6, 170.5, 71.0, 70.7, 56.3, 56.1, 56.03, 55.97, 51.6, 49.6, 43.1, 43.0, 41.2, 40.05, 40.00, 39.8, 39.4, 37.1, 36.9, 36.0, 35.4, 35.2, 34.7, 34.6, 32.7, 32.1, 31.4, 31.1, 31.1, 31.0, 29.9, 28.2, 24.2, 24.1, 23.8, 23.7, 21.53, 21.51, 20.81, 20.77, 18.4, 12.1.

A 25 mL Schlenk-tube equipped with a magnetic stir bar were charged with **6-3** (200 mg, 0.36 mmol), **6-4**[10] (200 mg, 0.72 mmol), potassium carbonate (99 mg, 0.72 mmol) and *N*,*N*-dimethylformamide (2 mL). The reaction mixture was stirred at room temperature for 12 h. After that, the reaction was quenched by the addition of H_2O (20 mL), followed by extraction with ethyl acetate (20 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 10:1) to afford **6-5** as a white solid (226 mg, 87%).

NMR Data of 6-5：

^{1}H NMR (400 MHz, chloroform-*d*) δ 5.38 – 5.25 (m, 1H), 5.22 – 5.09 (m, 1H), 4.62 – 4.29 (m, 1H), 3.65 (s, 3H), 2.34 (ddd, *J* = 15.3, 10.1, 5.1 Hz, 1H), 2.20 (ddd, *J* = 15.6, 9.6, 6.4 Hz, 1H), 2.06 – 1.99 (m, 3H), 1.47 (s, 9H), 1.44 (s, 9H), 0.96 (d, *J* = 6.4 Hz, 3H), 0.90 (d, *J* = 6.4 Hz, 3H), 0.63 (s, 3H).

^{13}C NMR (100 MHz, chloroform-*d*) δ 174.8, 170.7, 170.6, 170.2, 169.8, 155.3, 82.8, 82.6, 82.5, 80.1, 79.9, 71.2, 56.4, 56.1, 51.6, 43.0, 43.0, 41.4, 40.1, 39.8, 36.9, 36.4, 35.4, 34.7, 31.5, 31.2, 31.1, 28.5, 28.2, 28.1, 28.1, 24.2, 23.9, 23.7, 21.6, 21.5, 20.9, 18.4, 12.1.

LRMS data of 6-5：

LRMS (ESI): calcd for $C_{39}H_{65}NNaO_8S$ $[M+Na]^+$ 730.4, found: 730.4.

To a solution of **6-5** (100 mg, 0.14 mmol) in dichloromethane (2 mL) was added trifluoroacetic acid (2 mL), and the mixture was stirred at room temperature overnight. The solvent was removed in vacuo. The residue was dissolved in ethanol/H_2O (2 mL/0.5 mL/0.5 mL), sodium hydroxide (56 mg, 0.14 mmol) was added, and then the resulting mixture was stirred at room temperature for 12 h. After that, the reaction mixture was quenched with 2 M hydrochloric acid (5 mL), and concentrated under

reduced pressure. The residue was purified by reversed-phase column chromatography (methanol/H_2O = 3:1) to afford **6-6** as a white solid (50 mg, 72%).

NMR Data of 6-6:

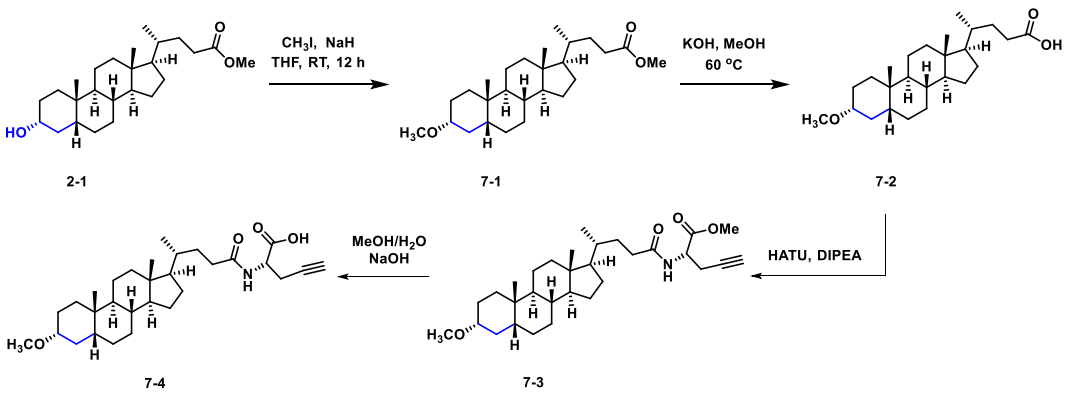
^{1}H NMR (400 MHz, methanol-d_4) δ 4.06 (dt, J = 12.2, 4.7 Hz, 1H), 3.67 (dd, J = 8.7, 3.8 Hz, 1H), 3.39 (s, 1H), 3.14 (dd, J = 14.3, 3.8 Hz, 1H), 2.88 (dd, J = 14.2, 8.7 Hz, 1H), 2.30 (ddd, J = 15.1, 10.0, 5.2 Hz, 1H), 2.16 (ddd, J = 15.3, 9.6, 6.6 Hz, 1H), 0.98 – 0.92 (m, 6H), 0.69 (s, 3H).

^{13}C NMR (100 MHz, methanol-d_4) δ 179.6, 172.9, 68.6, 57.6, 57.5, 55.4, 45.2, 44.2, 44.0, 41.3, 41.0, 37.7, 36.8, 36.1, 35.7, 33.5, 33.2, 32.8, 32.6, 29.2, 26.9, 25.3, 25.0, 24.5, 22.0, 18.8, 12.5.

HRMS data of 6-6:

HRMS (ESI): calcd for $C_{27}H_{46}NO_5S$ $[M+H]^+$ 496.3091 found: 496.3098.

11. Synthesis of compound 7-4.



Under argon atmosphere, a 25 mL Schlenk-tube equipped with a magnetic stir bar were charged with **2-1** (195 mg, 0.5 mmol) and anhydrous tetrahydrofuran (3 mL), sodium hydride (30 mg, 0.75 mmol) was added. The mixture was stirred at room temperature for 0.5 h. Subsequently, iodomethane (62 μL, 1.0 mmol) was added, and the reaction mixture was stirred at room temperature overnight. After that, the reaction mixture was quenched with saturated NH_4Cl solution (10 mL), and extracted with ethyl acetate (10 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was

purified by column chromatography (petroleum ether/ethyl acetate = 30:1) to afford **7-1** as a white solid (27 mg, 13%).

NMR Data of 7-1：

^{1}H NMR (400 MHz, chloroform-*d*) δ 3.66 (s, 1H), 3.34 (s, 3H), 3.15 (tt, *J* = 10.6, 4.5 Hz, 1H), 2.35 (ddd, *J* = 15.2, 10.1, 5.0 Hz, 1H), 2.21 (ddd, *J* = 15.6, 9.7, 6.5 Hz, 1H), 0.90 (d, *J* = 7.1 Hz, 6H), 0.63 (s, 3H).

^{13}C NMR (100 MHz, chloroform-*d*) δ 174.9, 80.5, 56.6, 56.0, 55.7, 51.6, 42.9, 42.2, 40.4, 40.3, 36.0, 35.5, 35.4, 35.0, 32.9, 31.2, 31.1, 28.3, 27.5, 26.9, 26.5, 24.3, 23.5, 20.9, 18.4, 12.2.

To a solution of **7-1** (25 mg, 0.061 mmol) in methanol (5.0 mL) was added 2 M sodium hydroxide (1.0 mL), and the mixture was stirred at 60 ºC for 2 h. After that, the reaction mixture was cooled to room temperature, quenched with 2 M hydrochloric acid (15 mL) and extracted with ethyl acetate (10 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dried under vacuum to afford crude product **7-2**, which was used directly in the next step without further purification.

A 25 mL Schlenk-tube equipped with a magnetic stir bar were charged with **7-2**, methyl *L*-propargylglycinate (24 mg, 0.19 mmol), *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (15 mg, 0.19 mmol) and *N*,*N*-dimethylformamide (1 mL). *N*,*N*-diisopropylethylamine (55 μL, 0.12 mmol) was added, and the reaction mixture was stirred at room temperature overnight. The reaction was quenched by the addition of saturated NH_4Cl solution (10 mL), followed by extraction with ethyl acetate (10 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 2:1) to afford **7-3** as a white solid (11 mg, 33% over two steps).

NMR Data of 7-3：

^{1}H NMR (400 MHz, chloroform-*d*) δ 6.26 (d, *J* = 7.9 Hz, 1H), 4.76 (dt, *J* = 8.0, 4.6 Hz, 1H), 3.79 (s, 3H), 3.35 (s, 3H), 3.15 (tt, *J* = 10.8, 4.5 Hz, 1H), 2.83 – 2.71 (m, 2H), 2.32

(ddd, J = 15.0, 10.2, 5.0 Hz, 1H), 2.16 (ddd, J = 14.7, 9.8, 6.3 Hz, 1H), 2.03 (q, J = 2.9 Hz, 1H), 0.94 – 0.90 (m, 6H), 0.63 (s, 3H).

^{13}C NMR (100 MHz, chloroform-*d*) δ 173.4, 171.1, 80.6, 78.6, 71.7, 56.6, 56.1, 55.7, 52.9, 50.6, 42.9, 42.2, 40.4, 40.3, 36.0, 35.5, 35.4, 35.0, 33.5, 32.9, 31.6, 29.8, 28.4, 27.4, 26.9, 26.5, 24.3, 23.5, 22.6, 20.9, 18.5, 12.2.

To a solution of **7-3** (11 mg, 0.022 mmol) in ethanol (2.0 mL) was added 2 M sodium hydroxide (0.4 mL), and the mixture was stirred at room temperature for 2 h. After that, the reaction mixture was quenched with 2 M hydrochloric acid (15 mL). The precipitated solid was collected by filtration and dried under vacuum to afford compound **7-4** as a white solid (9 mg, 84%).

NMR Data of 7-4：

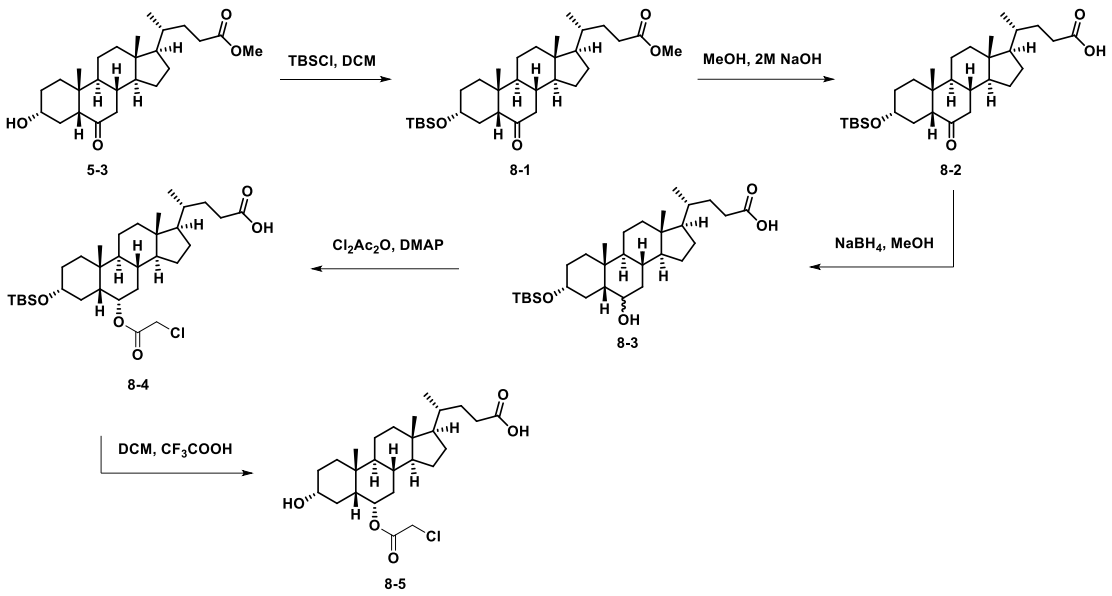
^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ 12.76 (s, 1H), 8.17 (d, J = 7.9 Hz, 1H), 4.31 (td, J = 7.6, 5.4 Hz, 1H), 3.21 (s, 3H), 3.15 – 3.04 (m, 1H), 2.86 (t, J = 2.6 Hz, 1H), 2.23 – 2.12 (m, 1H), 2.09 – 1.97 (m, 1H), 0.88 (d, J = 5.9 Hz, 6H), 0.61 (s, 3H).

^{13}C NMR (100 MHz, dimethyl sulfoxide-d_6) δ 172.7, 171.9, 80.5, 79.5, 72.9, 56.0, 55.6, 54.8, 50.8, 42.3, 41.3, 35.3, 34.9, 34.8, 34.4, 32.4, 32.0, 31.5, 27.7, 26.8, 26.5, 26.1, 23.8, 23.2, 21.1, 20.4, 18.3, 11.9.

HRMS data of 7-4：

HRMS (ESI): calcd for $C_{30}H_{48}NO_4$ $[M+H]^+$ 486.3578, found: 486.3588.

12. Synthesis of compound 8-6.



A 50 mL Schlenk-tube equipped with a magnetic stir bar were charged with **5-3** (480 mg, 1.18 mmol), imidazole (156 mg, 2.3 mmol) and dichloromethane (15 mL). After cooling to 0 °C, tert-butyldimethylsilyl chloride (268 mg, 1.78 mmol) was added while stirring. The mixture was warmed to room temperature and stirred for 6 h. The reaction was quenched by the addition of saturated NH_4Cl solution (15 mL), followed by extraction with dichloromethane (15 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 30:1) to afford **8-1** as a white solid (446 mg, 73%).

To a solution of **8-1** (346 mg, 0.02 mmol) in methanol (2.0 mL) was added 2 M potassium hydroxide (2.0 mL), and the mixture was stirred at room temperature for 2 h. After that, the reaction mixture was quenched with 2 M hydrochloric acid (15 mL) and extracted with ethyl acetate (25 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dried under vacuum to afford crude product **8-2**, which was used directly in the next step without further purification.

Under argon atmosphere, a 50 mL Schlenk-tube equipped with a magnetic stir bar were charged with **8-2** (146 mg, 0.289 mmol) and methanol (5 mL), sodium borohydride (21.7 mg, 0.57 mmol) was added. The mixture was stirred at room temperature for 2 h. The reaction was quenched by the addition of saturated NH_4Cl

solution (10 mL), followed by extraction with ethyl acetate (10 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dried under vacuum to afford crude product **8-3**, which was used directly in the next step without further purification.

A 50 mL Schlenk-tube equipped with a magnetic stir bar were charged with **8-3**, 4-dimethylaminopyridine (3.6 mg, 1.49 mmol) and dichloromethane (10 mL), chloroacetic anhydride (97 mg, 0.57 mmol) was added. The mixture was stirred at room temperature for 2 h. The reaction was quenched by the addition of saturated $NaHCO_3$ solution (20 mL), followed by extraction with dichloromethane (20 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (dichloromethane/methanol = 30:1) to afford **8-4** as a white solid (116 mg, 30% for two steps).

NMR Data of 8-4：

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.79 (dd, J = 10.4, 6.1 Hz, 1H), 4.04 (s, 2H), 3.99 (d, J = 3.2 Hz, 1H), 2.40 (ddd, J = 15.4, 10.2, 5.1 Hz, 1H), 2.26 (ddd, J = 15.8, 9.7, 6.4 Hz, 1H), 0.95 – 0.90 (m, 6H), 0.87 (s, 9H), 0.65 (s, 3H), 0.03 (s, 6H).

To a solution of **8-4** (18.6 mg, 0.14 mmol) in dichloromethane (2.5 mL) was added trifluoroacetic acid (2.5 mL), and the mixture was stirred at room temperature overnight. The solvent was removed in vacuo. The residue was purified by column chromatography (dichloromethane/methanol = 30:1) to afford **8-5** as a white solid (11 mg, 65%).

NMR Data of 8-5：

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.90 – 4.75 (m, 1H), 4.12 – 4.05 (m, 1H), 4.03 (s, 2H), 2.39 (ddd, J = 15.3, 10.0, 5.1 Hz, 1H), 2.26 (ddd, J = 15.6, 9.5, 6.5 Hz, 1H), 0.96 – 0.89 (m, 6H), 0.65 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 178.0, 166.9, 68.0, 56.3, 56.1, 48.4, 43.0, 41.3, 40.0, 40.0, 36.1, 35.4, 35.3, 35.0, 34.9, 33.9, 30.9, 30.8, 29.8, 28.2, 26.5, 25.7, 25.3, 25.0, 24.3, 23.5, 20.9, 18.4, 12.2.

Supplementary References

1 D'Agostino, G. D. *et al.* Host-Microbiome Orchestration of the Sulfated Metabolome. *Nat. Chem. Biol.* **20**, 410-421 (2024).

2 Hughes, C. S. *et al.* Single-Pot, Solid-Phase-Enhanced Sample Preparation for Proteomics Experiments. *Nat. Protoc.* **14**, 68-85 (2019).

3 Xu, T. *et al.* Prolucid: An Improved Sequest-Like Algorithm with Enhanced Sensitivity and Specificity. *J. Proteomics* **129**, 16-24 (2015).

4 Tabb, D. L. *et al.* Dtaselect and Contrast: Tools for Assembling and Comparing Protein Identifications from Shotgun Proteomics. *J Proteome Res* **1**, 21-26 (2002).

5 Gao, J. *et al.* Cimage2.0: An Expanded Tool for Quantitative Analysis of Activity-Based Protein Profiling (Abpp) Data. *J Proteome Res* **20**, 4893-4900 (2021).

6 Demichev, V. *et al.* Dia-Nn: Neural Networks and Interference Correction Enable Deep Proteome Coverage in High Throughput. *Nat. Methods* **17**, 41-44 (2020).

7 Datsenko, K. A. & Wanner, B. L. One-Step Inactivation of Chromosomal Genes in Escherichia Coli K-12 Using Pcr Products. *Proc. Natl. Acad. Sci. U. S. A.* **97**, 6640-6645 (2000).

8 Greene, F. D. & Lowry, N. Stereoselectivity in Hydrogen Atom Transfer. Reduction of Cis- and Trans-9-Chlorodecalins with Organotin Hydrides. *The Journal of Organic Chemistry* **32**, 882-885 (1967).

9 Dou, Q. & Jiang, Z. A Facile Route to Ursodeoxycholic Acid Based on Stereocontrolled Conversion and Aggregation Behavior Research. *Synthesis* **48**, 588-594 (2016).

10 Shen, F. *et al.* Hydrazine-Sensitive Thiol Protecting Group for Peptide and Protein Chemistry. *Org. Lett.* **13**, 568-571 (2011).