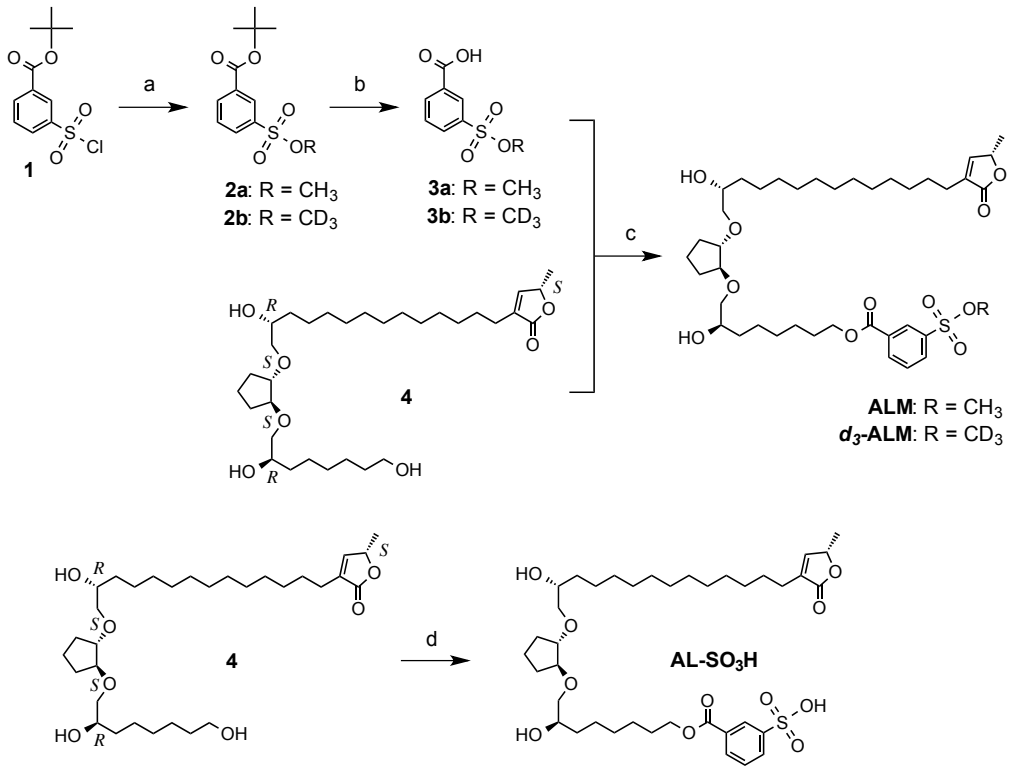
Supporting Information

Specific Methylation of Asp160 (49 kDa subunit) Located Inside the Quinone Binding Cavity of Bovine Mitochondrial Complex I

Masatoshi Murai, Hiroyuki Inaoka, Takahiro Masuya, Shunsuke Aburaya, Wataru Aoki, and Hideto Miyoshi

Scheme 1[a]



[a]*Reagents and conditions*: (a) MeOH (MeOH-d_4 for **2b**), aqueous NaOH, THF, 0˚C, 2.5 h; (b) trifluoroacetic acid (TFA), CH_2Cl_2, rt, 2 h; (c) 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), *N*,*N*-dimethyl-4-aminopyridine (DMAP), CH_2Cl_2, 0˚C, 1 h; (d) 3-(chlorosulfonyl)benzoyl chloride, pyridine, DMAP, CH_2Cl_2, rt, 2 h.

General synthetic methods

All moisture- and air-sensitive reactions were performed in oven-dried glassware under argon atmosphere with dry solvents under anhydrous conditions using standard syringe septum techniques. ^{1}H-NMR spectra were recorded at 500 or 400 MHz with Bruker AVANCE III 500 or AVANCE III 400 spectrometers, respectively, using tetramethylsilane (TMS) as the internal standard. ^{13}C-NMR spectra were recorded at 125 or 100 MHz. Chemical shifts (δ) are given in ppm relative to TMS with coupling constants (*J*) in Hz. Thin-layer chromatography (TLC) was performed on Merk TLC plate silica-gel 60F^{254}, and the spot was detected by iodine, anis, phosphomolybdic acid or UV absorbance. Dry solvents were either used as purchased or freshly distilled using common practices where appropriate.

*Synthesis of compound **1***

Compound **1** was synthesized according to the procedure described in ref. 1: ^{1}H NMR (500 MHz; $CDCl_3$) δ 8.61 (dd, *J* = 1.7, 1.7 Hz, 1H), 8.36 (ddd, *J* = 1.3, 1.3, 7.8 Hz, 1H), 8.19 (ddd, *J* = 1.2, 1.8, 8.0 Hz, 1H), 7.71 (dd, *J* = 7.9, 7.9 Hz, 1H), 1.63 (s, 9H); ^{13}C NMR (125 MHz; $CDCl_3$) δ 163.37, 144.89, 136.04, 134.20, 130.49, 130.03, 128.13, 83.17, 28.33 (3C).

*Synthesis of compound **2a***

To an ice-cooled mixture of THF (0.5 mL) and 4.6 M aqueous NaOH (0.196 mL, 0.90 mmol), methanol (10 mg, 0.30 mmol) was added. The solution of **1** in THF (0.5 mL) was then added dropwise, and the mixture was stirred for 2.5 h at 0˚C. The reaction mixture was extracted with EtOAc, washed with brine and dried over anhydrous $MgSO_4$. The crude product was purified by silica gel column chromatography (Wako gel® C-200, 20% EtOAc/*n*-hexane) to provide **2a** as a colorless oil (59 mg, 0.22 mmol, 72%): ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.47 (t, *J* = 1.6 Hz, 1H), 8.26 (dt, *J* = 7.8 Hz, 1.4 Hz, 1H), 8.05 (dt, *J* = 7.8 Hz, 1.6 Hz, 1H), 7.63 (t, *J* = 7.8 Hz, 1H), 3.78 (s, 3H), 1.63 (s, 9H); ^{13}C NMR (100 MHz; $CDCl_3$) δ 163.72, 134.58 (2C), 133.50, 131.46, 129.38, 128.88, 82.51, 56.54, 28.11 (3C); ESI-MS (*m/z*) 311.0 $[M+K]^+$.

*Synthesis of compound **2b***

Compound **2b** was prepared according to the procedure described for **2a** using methanol-d_4 (yield: 47%): ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.46 (t, *J* = 1.6 Hz, 1H), 8.26 (dt, *J* = 7.8 Hz, 1.4 Hz, 1H), 8.04 (dt, *J* = 7.8 Hz, 1.5 Hz, 1H), 7.63 (t, *J* = 7.8 Hz, 1H), 1.63 (s, 9H); ^{13}C NMR (100 MHz; $CDCl_3$) δ 163.72, 135.77, 134.58, 133.49, 131.45, 129.39, 128.86, 82.51, 55.83 (a weak signal derived from d_3-methyl group), 28.11 (3C); ESI-MS (*m/z*) 257.0 $[M-CD_3]^-$.

*Synthesis of compound **3a***

To a solution of **2a** (59 mg, 0.22 mmol) in anhydrous CH_2Cl_2 (3 mL), TFA (1 mL) was carefully added at room temperature. After stirring for 2 h at room temperature, the solvent was removed *in vacuo*, leaving a yellow oily residue. TFA remained in the residue was further co-evaporated with toluene to give **3a** as a slightly yellow oil (46 mg, 0.21 mmol, 96%); ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.65 (t, *J* = 1.6 Hz, 1H), 8.40 (dt, *J* = 7.9 Hz, 1.5 Hz, 1H), 8.17 (dt, *J* = 7.8 Hz, 1.6 Hz, 1H), 7.73 (t, *J* = 7.9 Hz, 1H), 3.83 (s, 3H); ^{13}C NMR (100 MHz; $CDCl_3$) δ 170.05, 136.35, 135.24, 132.86, 130.68, 129.87, 129.76, 56.68; ESI-MS (*m/z*) 215.1 $[M-H]^-$.

*Synthesis of compound **3b***

Compound **3b** was prepared from **2b** according to the procedure described for **3a** (yield: 58%): ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.48 (t, *J* = 1.6 Hz, 1H), 8.34 (dt, *J* = 7.8 Hz, 1.4 Hz, 1H), 8.11 (dt, *J* = 7.9 Hz, 1.5 Hz, 1H), 7.28 (t, *J* = 7.8 Hz, 1H); ^{13}C NMR (100 MHz; $CDCl_3$) δ 167.31, 137.42, 136.11, 133.05, 131.26, 130.16, 129.34, 57.803 (a weak signal derived from d_3-methyl group); ESI-MS (*m/z*) 218.1 $[M-H]^-$.

*Synthesis of compound **4***

Compound **4** was synthesized according to the procedure described in refs. 1 and 2: ^{1}H NMR (400 MHz; $CDCl_3$) δ 6.99 (m, 1H), 5.00 (dq, *J* = 1.7, 6.8 Hz, 1H), 3.80-3.77 (m, 2H), 3.75-3.72 (m, 2H), 3.64 (t, *J* = 6.6 Hz, 2H), 3.54 (dd, *J* = 2.9, 9.5 Hz, 2H), 3.28 (dd, *J* = 3.0, 9.6 Hz, 1H), 3.26 (dd, *J* = 3.1, 9.6 Hz, 1H), 2.30-2.23 (m, 2H), 1.96-1.85 (m, 2H), 1.71-1.59 (m, 2H), 1.58-1.53 (m, 4H), 1.45-1.26 (m, 28H), 1.41 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz; $CDCl_3$) δ 174.14, 149.08, 134.55, 85.88, 77.63, 77.43, 74.27, 74.20, 70.89, 70.80, 63.13, 33.30, 33.15, 32.86, 29.89 (2C), 29.80 (3C), 29.76 (2C), 29.72, 29.55, 29.51, 29.39, 27.61, 25.81, 25.74, 25.65, 25.38, 20.97, 19.42.

*Synthesis of **ALM***

To a stirred solution of **3a** (9.3 mg, 0.043 mmol) and **4** (20 mg, 0.036 mmol) in anhydrous CH_2Cl_2, EDC (14 mg, 0.072 mmol) and DMAP (4 mg, 0.04 mmol) were added at 0˚C under N_2 atmosphere. The mixture was stirred for an hour at 0˚C, then the reaction was quenched with saturated NH_4Cl, extracted with CH_2Cl_2, and dried over anhydrous $MgSO_4$. The crude product was purified by silica gel column chromatography (Wako gel® C-200, 70-100% EtOAc/*n*-hexane) to provide **ALM** as a colorless oil (5.2 mg, 6.9 μmol, 19%): ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.55 (t, *J* = 1.6 Hz, 1H), 8.33 (dt, *J* = 7.8 Hz, 1.6 Hz, 1H), 8.09 (dt, *J* = 7.9 Hz, 1.5 Hz, 1H), 7.67 (t, *J* = 7.8 Hz, 1H), 6.98 (d, *J* = 1.6 Hz, 1H), 4.99 (qd, *J* = 5.1 Hz, 1.7 Hz, 1H), 4.36 (t, *J* = 6.7 Hz, 2H), 3.80 (s, 3H), 3.80-3.72 (m, 4H), 3.52 (dd, *J* = 6.9 Hz, 2.6 Hz, 2H), 3.29-3.23 (m, 2H), 2.28-2.23 (m, 2H), 1.94-1.89 (m, 2H), 1.79 (quint, *J* = 7.3 Hz, 2H), 1.67 (quint, *J* = 7.4 Hz, 2H) 1.60-1.24 (m, 32H), 1.40 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz; $CDCl_3$) δ 173.90, 164.73, 148.85, 136.02, 134.69, 134.36, 131.99, 131.85, 129.57, 129.05, 85.67, 85.65, 77.40, 74.05, 74.02, 70.67, 70.56, 65.95, 56.56, 37.05, 33.10, 32.95, 30.34, 29.72, 29.68, 29.60, 29.57, 29.52, 29.31, 29.27, 29.19, 28.57, 27.41, 25.90, 25.55, 25.42, 25.18, 21.02, 20.82, 19.23; ESI-MS (*m/z*) 775.5 $[M+Na]^+$.

Synthesis of ***d_3-ALM***

d_3*-ALM** was prepared from **3b** and **4** according to the procedure described for **ALM** (yield: 31%): ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.55 (t, *J* = 1.6 Hz, 1H), 8.32 (dt, *J* = 7.8 Hz, 1.5 Hz, 1H), 8.09 (dt, *J* = 7.9 Hz, 1.5 Hz, 1H), 7.67 (t, *J* = 7.8 Hz, 1H), 6.98 (d, *J* = 1.6 Hz, 1H), 4.99 (qd, *J* = 5.1 Hz, 1.7 Hz, 1H), 4.36 (t, *J* = 6.7 Hz, 2H), 3.80-3.71 (m, 4H), 3.52 (dd, *J* = 9.5 Hz, 2.6 Hz, 2H), 3.28-3.23 (m, 2H), 2.28-2.23 (m, 2H), 1.94-1.89 (m, 2H), 1.77 (quint, *J* = 7.1 Hz, 2H), 1.67 (quint, *J* = 7.4 Hz, 2H) 1.61-1.25 (m, 32H), 1.40 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (125 MHz; $CDCl_3$) δ 173.87, 164.74, 148.84, 136.15, 134.67, 134.39, 131.83, 130.37, 129.57, 129.04, 85.68, 85.34, 77.39, 75.07, 74.07, 70.70, 70.59, 65.96, 33.15, 33.10, 30.37, 29.74, 29.70, 29.60, 29.57, 29.52, 29.44, 29.32, 29.28, 29.20, 28.59, 27.44, 25.91, 25.56, 25.43, 25.20, 21.04, 20.83, 19.23; ESI-MS (*m/z*) 778.5 $[M+Na]^+$. The signal derived from d_3-methyl group (-OC***D_3) was not observed in ^{13}C-NMR.

Synthesis of ***AL-SO_3H***

To an ice-cooled solution of **4** (15 mg, 0.027 mmol) in anhydrous CH_2Cl_2 (0.5 mL), anhydrous pyridine (4.4 μL, 0.054 mmol) and DMAP (2 mg, 0.001 mmol) were added at 0˚C and the mixture was stirred for 5 min. Then, 3-(chlorosulfonyl)benzoyl chloride (10 mg, 0.04 mmol) in anhydrous CH_2Cl_2 (0.5 mL) was carefully added to the mixture and the stirring was continued for 15 min at 0˚C, and for another 2 h at room temperature. The solvents were removed *in vacuo*, and the residue was purified by silica gel column chromatography (Wako gel® C-200, 50-100% EtOAc/*n*-hexane), and further purified using 0-20% MeOH/$CHCl_3$ as an another eluent to provide **AL-SO_3H** as a colorless oil (4.5 mg, 6.1 μmol, 23%): ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.45 (m, 1H), 8.09 (m, 1H), 8.07 (m, 1H), 7.45 (t, *J* = 7.7 Hz, 1H), 6.99 (d, *J* = 1.4 Hz, 1H), 5.00 (qd, *J* = 5.1 Hz, 1.7 Hz, 1H), 4.33-4.28 (m, 2H), 3.99 (m, 2H), 3.88 (m, 2H), 3.56-3.49 (m, 4H), 2.26 (t, *J* = 7.5 Hz, 2H), 2.06-1.23 (m, 40H), 1.40 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (125 MHz; $CDCl_3$) δ 173.85, 165.72, 148.86, 144.54, 134.33, 131.52, 130.96, 130.62, 128.48, 126.97, 77.56, 75.05, 73.50 (2C), 71.33 (2C), 65.55, 65.34, 41.51, 39.35, 37.04, 32.63, 32.31, 30.34, 29.68, 29.62, 29.52, 29.42, 29.30, 29.19, 28.46, 28.32, 27.43, 27.36, 25.67, 25.46, 25.18, 24.66, 19.20; ESI-MS (*m/z*) 737.4 $[M-H]^-$. The HMBC spectra of AL-SO_3H showed a cross peak between methylene proton (-C***H_2***O-) and ester carbonyl carbon (-***C***O-Ph-SO_3H) at 4.30 and 165.72, respectively.

Reference

1. Masuya, T., Murai, M., Ifuku, K., Morisaka, H., and Miyoshi, H. (2014) Site specific chemical labeling of mitochondrial respiratory complex I through ligand-directed tosylate chemistry, *Biochemistry* 53, 2307-2317.
2. Fujita, D., Ichimaru, N., Abe, M., Murai, M., Hamada, T., Nishioka, T., and Miyoshi, H. (2005) Synthesis of non-THF analogs of acetogenin toward simplified mimics, *Tetrahedron Lett*. 46, 5775-5779.

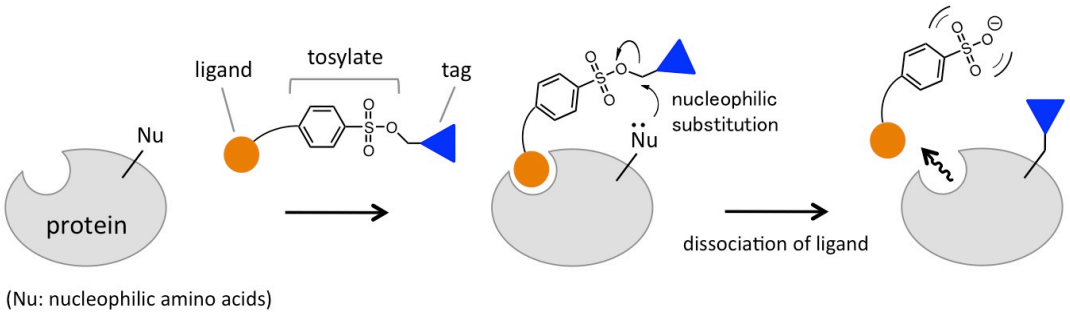


Figure S1.

Schematic representation of LDT chemistry technique.

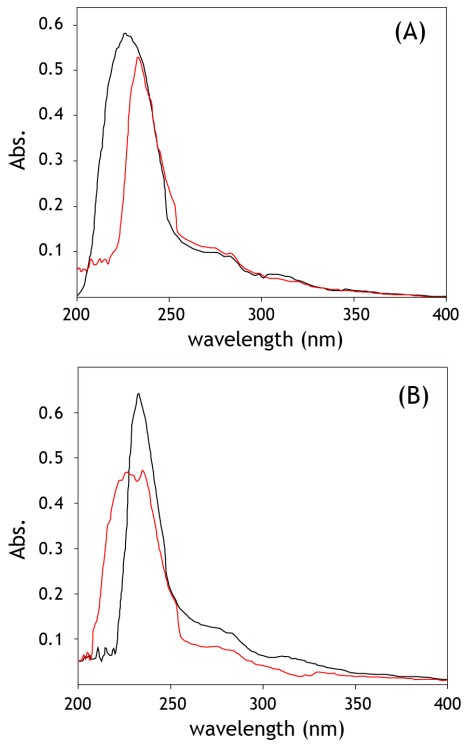


Figure S2.

UV-visible spectra of ALM in the presence of histidine or cysteine in water. (A) ALM (50 μM) and histidine (5.0 mM) were incubated in a buffer (2.5 mL) containing 250 mM sucrose and 50 mM KPi (pH 7.4) at 30˚C: time point zero (black line), after 24 h incubation (red line). (B) ALM (50 μM) and cysteine (5.0 mM) were incubated in a buffer (2.5 mL) containing 250 mM sucrose and 50 mM KPi (pH 7.4) at 30˚C: time point zero (black line), after 24 h incubation (red line).

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  1 ARQWQPDVEW AEQYGGAVMY PTKETAHWKP PPWNDVDPPK DTLVSNLTLN FGPQHPAAHG
 61 VLRLVMELSG EMVRKCDPHI GLLHRGTEKL IEYKTYLQAL PYFDRLDYVS MMCNEQAYSL
121 AVEKLLNIQP PPRAQWIRVL FGEITRLLNH IMAVTTHALD IGAMTPFFWM FEEREKMFEF
181 YERVSGARMH AAYVRPGGVH QDLPLGLMDD IYEFSKNFSL RIDELEEMLT NNRIWRNRTV
241 DIGIVTAEDA LNYGFSGVML RGSGIQWDLR KTQPYDVYDQ VEFDVPIGSR GDCYDRYLCR
301 VEEMRQSIRI ISQCLNKMPP GEIKVDDAKV SPPKRAEMKT SMESLIHHFK LYTEGYQVPP
361 GATYTAIEAP KGEFGVYLVS DGSSRPYRCK IKAPGFAHLA GLDKMSKGHM LADVVAIIGT
421 QDIVFGEVDR
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Figure S3

Characterization of the 49 kDa subunit of bovine complex I by LC-MS. The sequences of the tryptic digests of the 49 kDa subunit were analyzed by an Orbitrap mass spectrometer, and the identified sequences are shown in *red*. The sequences matched with "high-confidence" in two independent experiments are *underlined*. The methylated residue is highlighted in *orange*. Total 29 peptides were detected and the sequence coverage was 89.3 %. The residue number refers to the mature sequence of the bovine 49 kDa subunit (P17694).