Electronic Supplementary Information

Charged Chiral Derivatization for Enantioselective Imaging of D-, L-2-Hydroxyglutaric Acid Using Ion Mobility Spectrometry/Mass Spectrometry

Eiji Sugiyama,[a] Yuki Nishiya,[a] Kenji Yamashita,[a] Ryo Hirokawa,[a] Yoshiteru Iinuma,[b] Takashi Nirasawa,[c] Hajime Mizuno,[a,d] Yoshitaka Hamashima[a] and Kenichiro Todoroki*[a]

Laboratory of Analytical and Bio-Analytical Chemistry, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka, 422-8526, Japan

a: School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka, 422-8526, Japan.
b: Okinawa Institute of Science and Technology Graduate University, 1919-1 Tancha, Onna-son, Kunigami-gun, Okinawa, Japan 904-0495.
c: Daltonics division, Bruker Japan K.K., Moriyacho Kanagawa-ku, Yokohama-shi, Kanagawa, Japan 221-0022
d: Laboratory of Analytical Chemistry, Faculty of Pharmacy, Meijo University, 150 Yagotoyama, Tempaku, Nagoya, 468-8503, Japan
*: Corresponding Author; Postal address: 52–1 Yada, Suruga-ku, Shizuoka 422–8526, Japan; Telephone number: +81-54-264-5654; e-mail address: todoroki@u-shizuoka-ken.ac.jp; ORCID iD: 0000-0002-9714-2659

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Experimental methods

Materials

D-2-HG, L-2-HG, and DL-2-HG were purchased from Cayman Chemical (MI, USA). $^{13}C_5$-DL-2-HG (stable isotope) was purchased from Cambridge Isotope Laboratories (MA, USA). 1,3,5-trimethoxybenzene, diethyl carbonate, magnesium sulfate, triphenylphosphine (TPP), 2,2'-dipyridyl-disulfide (DPDS), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC), 3*H*-1,2,3-triazolo[4,5-*b*]pyridine-3-ol (HOAt), (3*S*)-(-)-3-(*tert*-butoxy-carbonylamino)pyrrolidine were obtained from Tokyo Chemical Industry (Tokyo, Japan). *n*-butyllithium solution in hexane, dichloromethane, methanol (LC-MS grade), hexane, trifluoroacetic acid (TFA), silica gel for flash chromatography (particle size: 40–100 μm) were purchased from Kanto Chemical (Tokyo, Japan). Acetonitrile (LC-MS grade) was purchased from Thermo Fisher Scientific (MA, USA). Tetrafluoroboric acid aqueous solution and ammonium acetate were purchased from Fujifilm Wako Pure Chemical Industries (Osaka, Japan). 2,5-Dihydroxybenzoic acid (DHB) and conductive glass slides were purchased from Bruker (MA, USA). DMT(*S*)A was synthesized as described in a previous study (T. Takayama, T. Kuwabara, T. Maeda, I. Noge, Y. Kitagawa, K. Inoue, K. Todoroki, J. Z. Min, T. Toyo'oka, *Anal. Bioanal. Chem.*, 2015, **407**, 1003–1014.).

Synthetic procedure for tris(2,4,6-trimethoxyphenyl)carbenium tetrafluoroborate (1).

To a solution of 1,3,5-trimethoxybenzene (1.68 g, 10 mmol) in diethyl ether (3 mL), *n*-butyllithium solution in hexane (2.3 M, 4.9 mL, 11.3 mmol) was added dropwise under an argon atmosphere. The reaction mixture was stirred at 0 ºC for 3 h, and diethyl carbonate (0.4 mL, 3.3 mmol) was added. The resulting mixture was stirred under reflux for 3 days and then cooled to 0 °C, and the reaction was quenched by adding 1M NaOH aqueous solution. The aqueous phase was extracted with diethyl ether 4 times. The combined organic phase was dried over $MgSO_4$ and filtered, yielding a clear yellow solution. Addition of aqueous HBF_4 solution (3.27 mol/L, 1.1 mL, 3.6 mmol) resulted in immediate precipitation of the deep blue carbenium salt. The precipitate was filtered off, washed thoroughly with *n*-hexane, and reprecipitation by addition of dichloromethane gave the compound in 7% overall yield. Spectral data of ^{1}H NMR (500 MHz, $CDCl_3$) and HRMS (ESI^+) for **1** were consistent with those reported by Laursen *et al.* (B. W. Laursen, F. K. Krebs, M. F. Nielsen, K. Bechgaard, J.B. Christensen, N. Harrit, *J. Am. Chem. Soc.*, 1998, **120**, 12255–12263).

Synthetic procedure for (*S*)-1-(4-(bis(2,4,6-trimethoxyphenyl)methyliumyl)-3,5-dimethoxy-phenyl)pyrrolidine-3-amine (BTMD(*S*)A)

To a solution of **1** (24 mg, 0.04 mmol) in dichloromethane, a solution of (3*S*)-(-)-3-(*tert*-butoxy-carbonylamino)pyrrolidine (11 mg, 0.06 mmol) in dichloromethane was added. The reaction mixture

was stirred at room temperature for 3 h, and then the volatiles were removed *in vacuo*. The residue was purified by silica gel column chromatography (eluent: *n*-hexane:dichloromethane:MeOH = 5:4:3) to afford (*S*)-3-(*tert*-butoxycarbonylamino)-1-(4-(bis(2,4,6-trimethoxyphenyl)methyliumyl)-3,5-dimethoxyphenyl)-pyrrolidine as a red-purple precipitation. The resulting precipitate was dissolved in dichloromethane, and then the Boc group was deprotected by adding TFA. Diethyl ether was added to the solution, and the volatiles were removed *in vacuo* to afford BTMD(*S*)A as the salt of tetrafluoroborate and TFA as a black-purple crystal (2.1 mg, 8% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.000 (TMS), 3.780 (1H, m), 2.549 (2H, m), 4.130 (2H, m), 4.130-4.246 (2H, m), 5.619 (2H, s), 6.029 (4H, s), 3.835 (6H, s), 3.533 (18H, s), 8.693 (NH_3^+); HRMS (MALDI$^+$) calculated for $C_{31}H_{39}N_2O_8^+$ $[M]^+$ 567.2701, found 567.2716; UV–vis λ_{max} (Abs) in ACN: 212 (0.972), 268 (0.176), 350 (0.11), 543 nm (0.293).

Chiral derivatization for D-, L-, and DL-2-HG in solutions

The derivatization using BTMD(*S*)A, EDC, and HOAt was performed as follows: 10 μL of 1 mM D-, L-, or DL-2HG solution in 90% ACN aqueous (aq.), 10 μL of a solution containing 5 mM BTMD(*S*)A or DMT(*S*)A in 90% ACN aq., 10 μL of 30 mM EDC solution in 90% ACN aq., and 10 μL of 30 mM HOAt solution in 90% ACN aq. were mixed in a 1.5 mL polypropylene tube. The mixtures were placed at room temperature and were allowed to react for 0, 1, 3, 6, or 12 h. The derivatization using BTMD(*S*)A, TPP, and DPDS was performed as follows: 20 μL of 1 mM D-, L-, or DL-2HG solution in 90% ACN aq., 200 μL of a solution containing 5 mM BTMD(*S*)A in ACN, 200 μL of 10 mM TPP solution in ACN, 200 μL of 10 mM DPDS solution in ACN were mixed in a 1.5 mL polypropylene tube. The mixtures were placed at 60 ℃ and were allowed to react for 3 h. After drying the mixture under vacuum, 10 μL of 90% ACN aq. was added to dissolve the residue.

Animal experiments and preparation of tissue slices and the dried droplets of D-, L-, and DL-2-HG

The animal experiments were approved by the Experimental Animal Committee of the University of Shizuoka (approved No. 216534) based on the following provisions in Japan: the Act on Welfare and Management of Animals (Act No. 105, 1973); Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain (Notice No. 88 of the Ministry of the Environment, 2006); Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions (Notice No. 71 of the Ministry of Education, Culture, Sports, Science and Technology, 2006); Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan, 2006).

Eight-weeks-old C57BL/6J male mice were purchased from CLEA Japan (Tokyo, Japan), and the mice were anesthetized with 3% isoflurane. After harvesting the testis and immediately freezing it by

powdered dry ice, the mice were killed by decapitation. The frozen testis was stored at −80 ℃ until use. The testis slices at 10 μm-thickness were prepared at −15℃ using a cryostat (CM1860, Leica), and they were thaw-mounted on ITO-coated conductive slide glass (Bruker). Dried droplets were prepared by spotting and drying 1 μL of 1 mM D-, L-, or DL-2HG solution in 90% ACN aq. on the testis slices.

On-tissue chiral derivatization

Dried droplets on the mouse testis were prepared using 1 μL of 0, 10, 100, and 1000 μM $^{13}C_5$-DL-2HG solution. The reaction solution containing 1.5 mM BTMD(*S*)A, 30 mM EDC, and 30 mM HOAt in 90% ACN aq. was sprayed to the dried droplets using an airbrush (PS270, GSI Creos, Tokyo, Japan). The samples were then placed on a paper wet by 1.0 mL of 90% ACN aq. in a glass dish at 25 ℃ for 2 h.

Matrix coating

DHB solution (50 mg/mL in 50% MeOH aq.) was sprayed on the samples by an automatic sprayer (HTX M5 Sprayer, HTX Technologies) with a following setting: Temperature: 80℃; Passes: 12, 24 (only for the spray-coated samples); Flow rate: 0.04 mL/ min; Velocity: 1200 mm/min; Track spacing: 3 mm; Pressure: 10 psi; Gas flow rate: 3 L/min.

Mass spectrometry

ESI/MS data was obtained by flow injection using an ACQUITY UPLC I-class system and a Xevo G2-XS QTof (Waters, Japan). The parameters were as follows: mobile phase: 50%ACN aq.; flow rate: 0.2 mL/min; Ion mode: ESI positive; Capillary voltage: 3.0 kV; Desolvation temperature: 500℃; Desolvation gas flow: 800 L/h; Cone voltage: 30 V. MALDI/MS was performed using an autoflex maX (Bruker, MA, USA) with the following parameters: Ion mode: MALDI positive; Laser power: 80 %; Laser frequency: 2,000 Hz; Smartbeam Parameter set: small; Shots per pixel: 500; Random walk shots at raster spot: 50; Mass range: *m/z* 200–1200; Detector gain: 2150 V; Scan pitch for imaging: 125 μm × 125 μm. MALDI/IMS/MS was performed using a timsTOF fleX (Bruker, MA, USA) with the following parameters: Ion mode: MALDI positive; Laser Power: 50 %; Laser frequency: 10,000 Hz; Shots per pixel: 200; Mass range: *m/z* 300–2000, $1/K_0$ Start: 0.80 V·s/cm^2; $1/K_0$ End: 1.45 V·s/cm^2; Ramp time: 1100 ms (for confirming the resolution), 300 ms (for imaging); Accumulation time: 31.0 ms; Duty cycle: 10.33%; Ramp rate: 3.27 Hz; Scan pitch for imaging: 90 μm × 90 μm (for dried droplets) or 20 μm × 20 μm (for testis). Data were analysed using flexImaging and SCiLS Lab (Bruker).

UV-Vis and ^{1}H-NMR spectroscopy

Spectrum of UV-Vis absorbance for BTMD(*S*)A in ACN was obtained using U-2010 Spectrophotometer (HITACHI) with the following setting; scan range: 190–1100 nm; scan speed: 800 nm/min; sampling interval: 1.0 nm; Wavelength for ramp switching: 370.0 nm; Cell length: 10.0 mm. ^{1}H-NMR spectrum of BTMD(*S*)A in CD_3Cl was obtained using JNM-ECA-500 (JEOL) with the following setting: Field strength: 500 MHz: Relaxation Delay: 1.5 s; Temperature: 21.7℃.

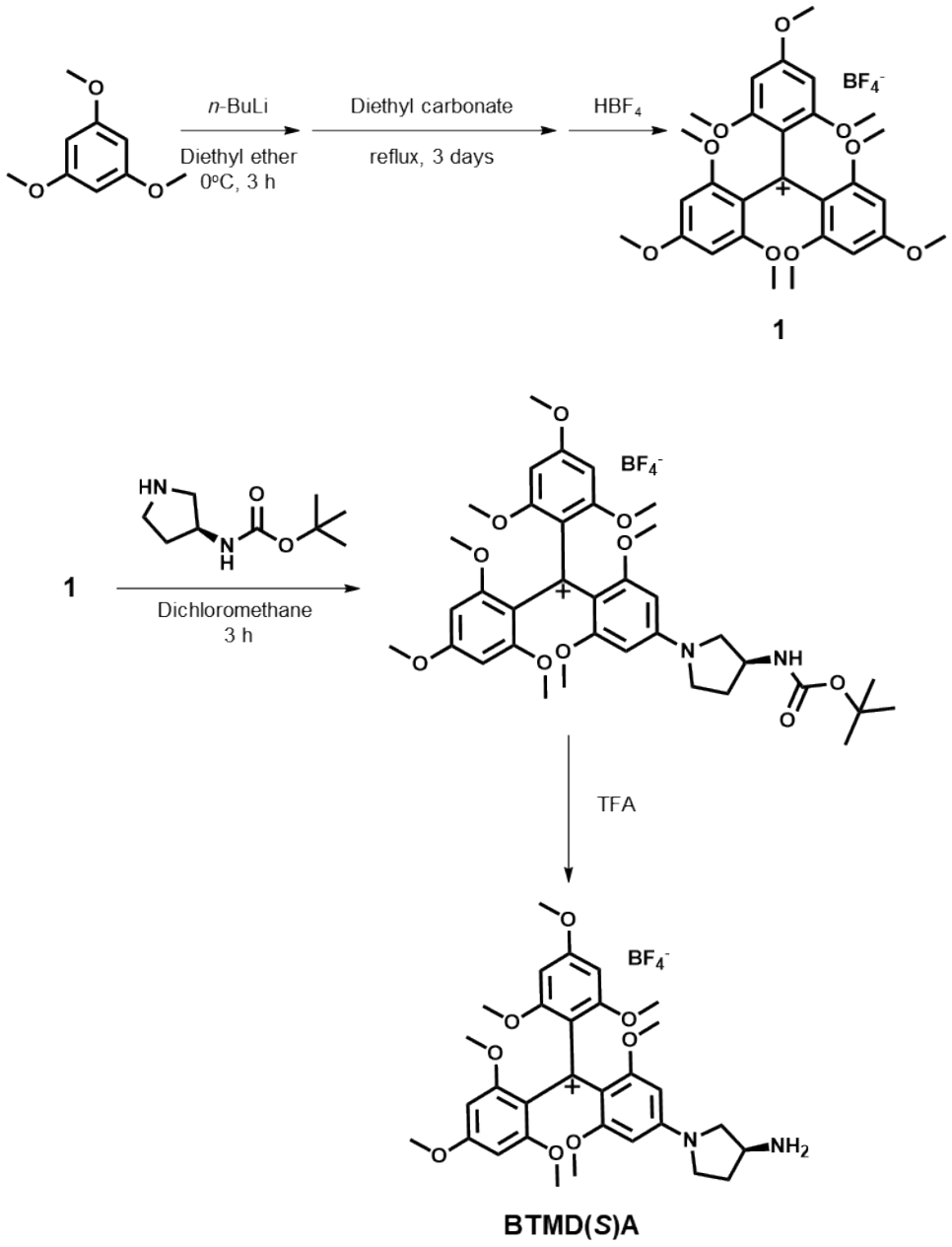

Fig. S1: Synthetic scheme of BTMD(*S*)A.

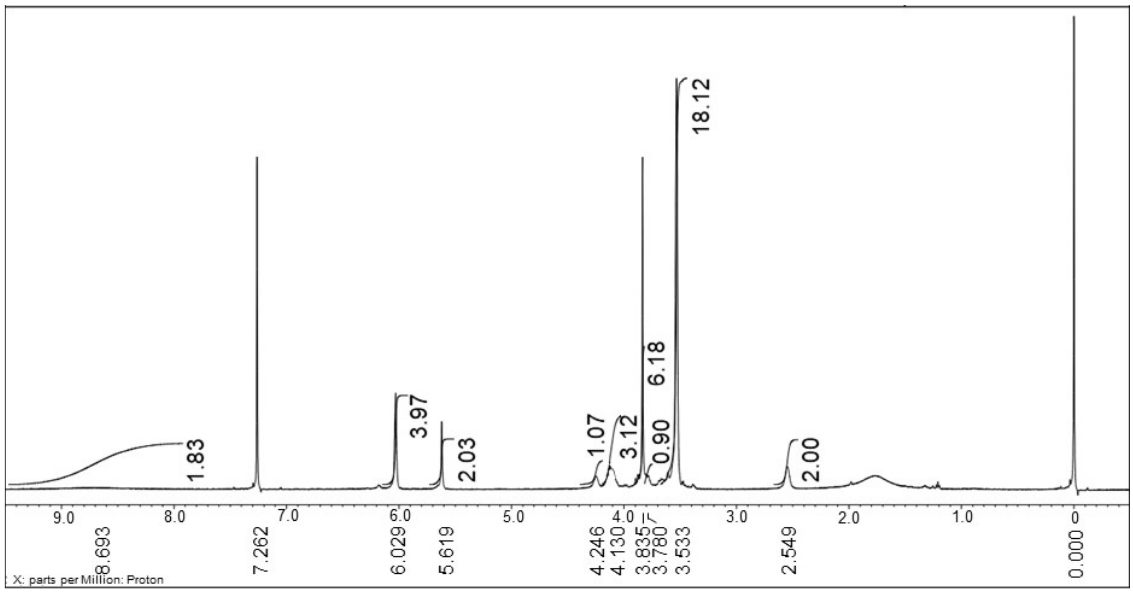

Fig. S2: ^{1}H NMR spectrum (500 MHz) of BTMD(*S*)A in $CDCl_3$.

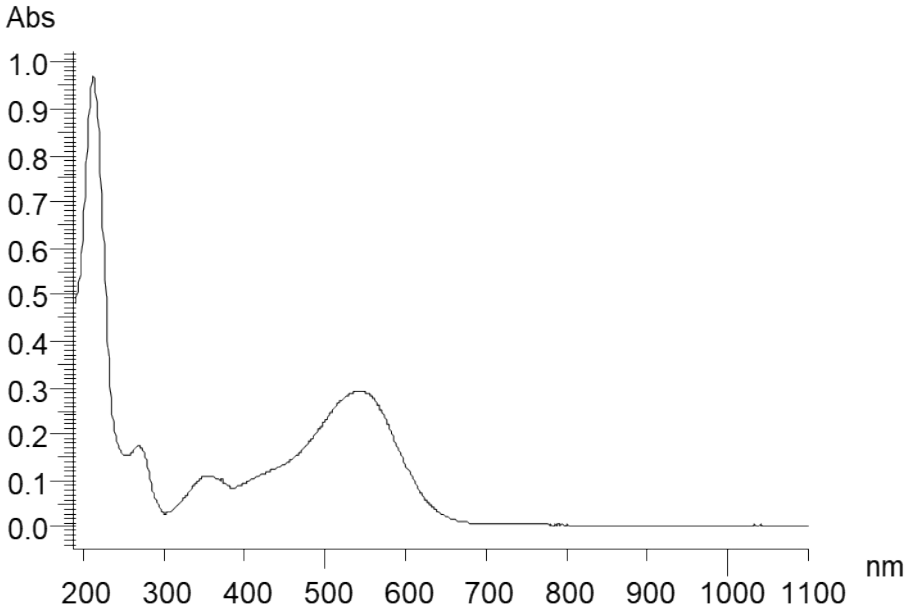

Fig. S3: UV–Vis spectrum of BTMD(*S*)A.

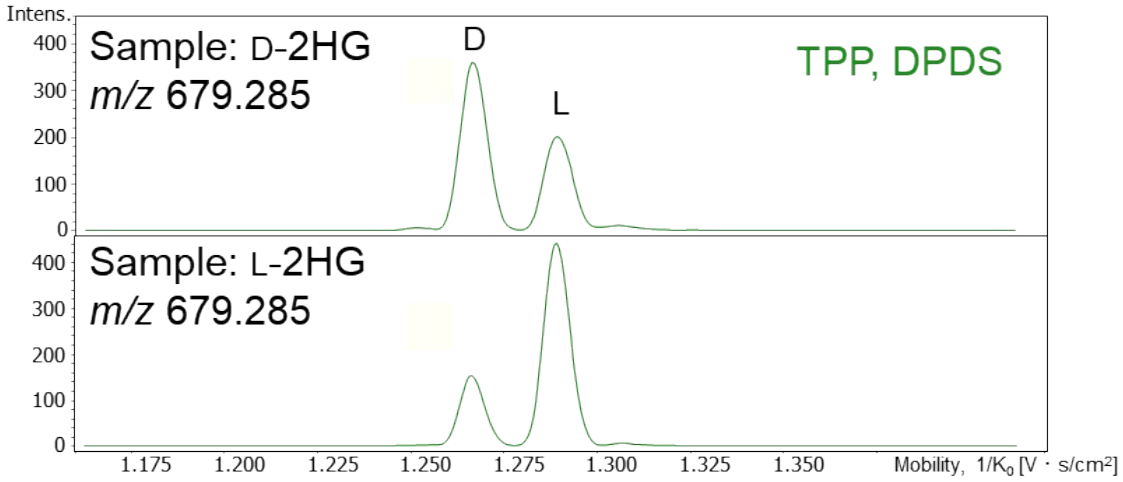

Fig. S4: Mobilogram obtained by the BTMD(*S*)A derivatization of D-2HG or L-2HG with TPP and DPDS. Upper: mobilogram obtained with D-2HG; **Lower**: mobilogram obtained with L-2HG. Note that significant isomerization occurred during the derivatization.

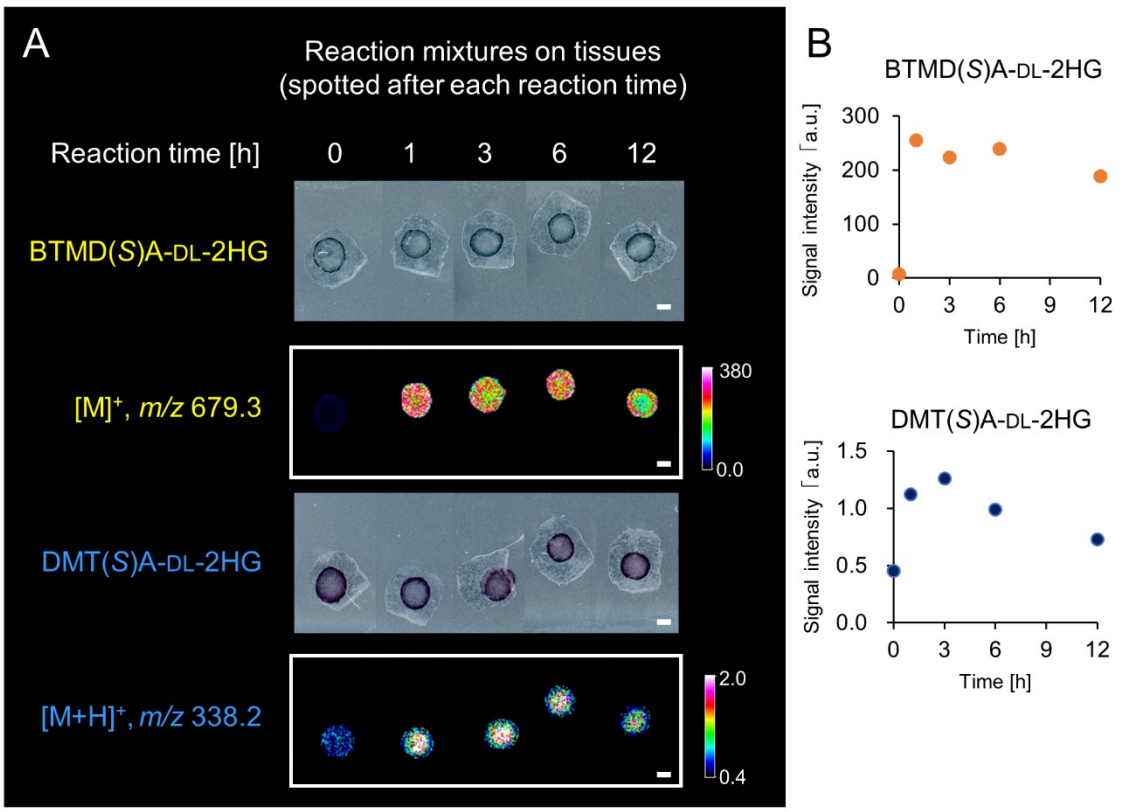

Fig. S5: MALDI/MS analysis for dried droplets of the reaction solutions after a series of reaction time. The reaction solutions containing BTMD(*S*)A or DMT(*S*)A in addition to DL-2HG, EDC, and HOAt were spotted on the mouse testis. The data was obtained using an instrument with a relatively low mass resolution (autoflex maX). **A**: MS images showing the signal intensity of BTMD(*S*)A-DL-2HG or DMT(*S*)A-DL-2HG. The images were prepared with a mass window of 0.6 Da. **B**: Plots of averaged signal intensities of each spot against reaction time. Scale bar = 1 mm.

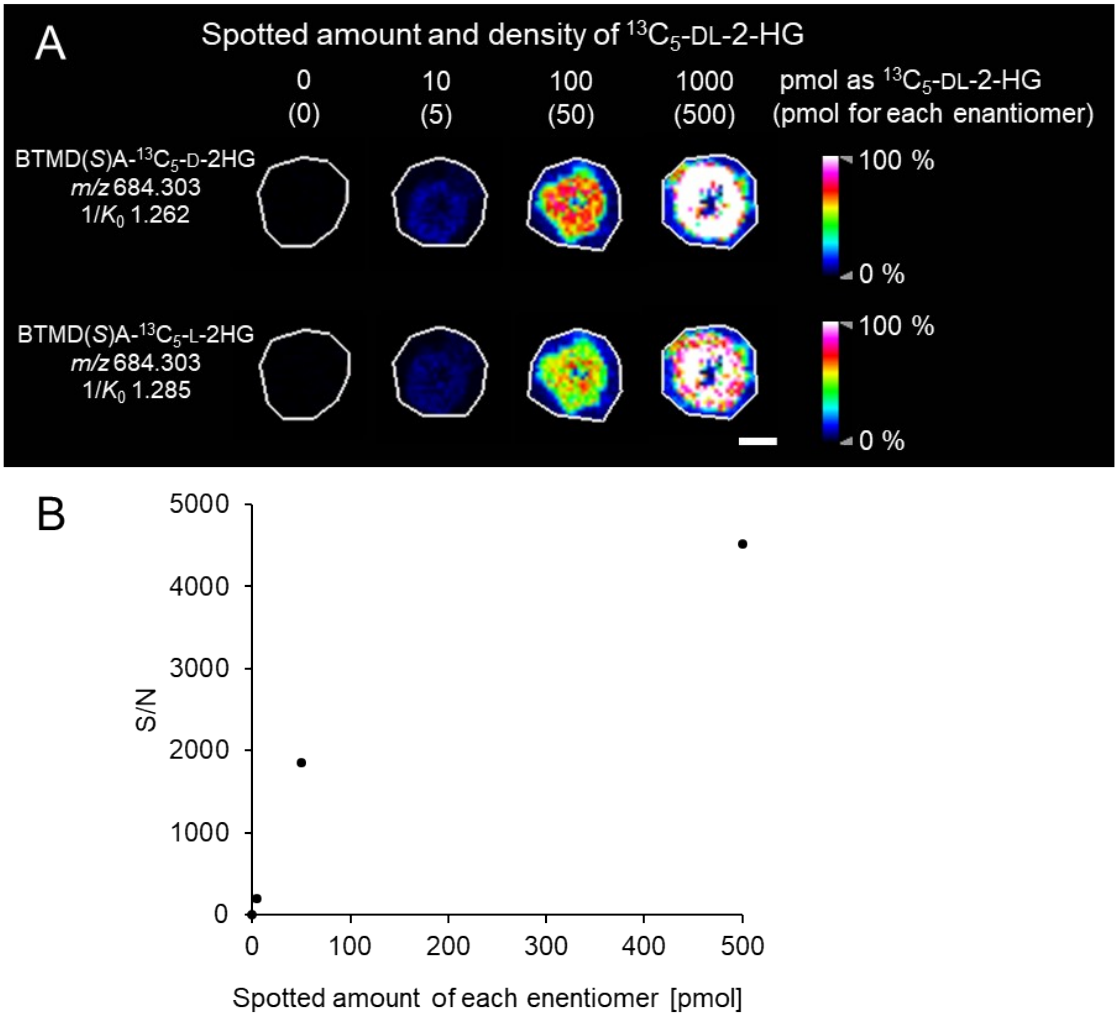

Fig. S6: Concentration-dependent increase of the signal of BTMD(*S*)A derivatives by on-tissue chiral derivatization. The white encloses show the measured regions covering area of dried droplets of $^{13}C_5$-DL-2HG (racemate) on the mouse testis. The on-tissue chiral derivatization was performed by spraying a solution containing BTMD(*S*)A, EDC, and HOAt and the following incubation for 2 h. The images were prepared with a mass window of 0.01 Da and a 1/K_0 window of 0.01 [V·S/cm^2]. Scale bar = 1 mm.