Electronic Supplementary Information

Photocyclization of Photoswitches with High Enantioselectivity in Human Serum Albumin under an Artificial Environment

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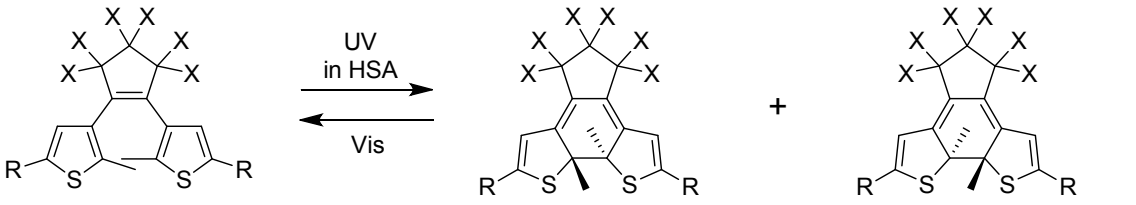


1o R = CH_2OH, X = F
2o R = CO_2H, X = H
3o R = CO_2CH_3, X = H
4o R = CO_2H, X = F
5o R = CO_2CH_3, X = F

(R,R)-**1c** R = CH_2OH, X = F
(R,R)-**2c** R = CO_2H, X = H
(R,R)-**3c** R = CO_2CH_3, X = H
(R,R)-**4c** R = CO_2H, X = F
(R,R)-**5c** R = CO_2CH_3, X = F

(S,S)-**1c** R = CH_2OH, X = F
(S,S)-**2c** R = CO_2H, X = H
(S,S)-**3c** R = CO_2CH_3, X = H
(S,S)-**4c** R = CO_2H, X = F
(S,S)-**5c** R = CO_2CH_3, X = F

1. Experimental Procedures

Materials: HSA (Sigma, fatty-acid free, lot # 068K7538v, molecular weight: 66,500), phosphate buffer powder (Wako, 1/15 mol/dm^3, ph 6.8, composition: Na_2HPO_4 4.7g, KH_2PO_4 4.5g, 1 packet for 1L solution), acetonitrile (Wako, spectrosol, purity: 99.8%) were used as received. Distilled water was purified by Milli Pore (>18MΩ cm, model: Simpli Lab), Merck) and was used for all aqueous sample solutions.

Measurement instruments: ^{1}H NMR Spectra were recorded in $CDCl_3$ with Bruker DRX300 (300 MHz) or DRX500 (500 MHz) NMR spectrometers. The *J* values are expressed in Hz and chemical shifts in ppm. The coupling patterns are indicated as s, singlet; d, doublet; t, triplet; q: quartet, quint: quintet, m; multiplet. Infrared spectra (IR) were recorded on a JASCO FT/IR-4100 spectrometer. Mass spectra were measured by the electron impact ionization using a JEOL JMS-AX-600 mass spectrometer. Ultraviolet (UV) and visible (vis) spectra were recorded on a JASCO V-550 spectrophotometer. CD spectra were recorded on a JASCO J-725 spectrometer at 25℃.

Diarylethenes: Diarylethenes **1o**[1], **2o**[2] and **4o**[3] were prepared according to the literature procedures. Diarylethene **3o**, a new compound, was also prepared by the methylation of **2o** with trimethylsilyldiazomethane in toluene and methanol as shown below. Diarylethene **5o**[4], known in literature, was prepared by the methylation of **4o** with trimethylsilyldiazomethane in toluene and methanol with the similar method to the preparation of **3o**.

3o: To a stirred solution of **2o** (50.1 mg, 0.144 mmol) in the mixture of methanol (2 mL) and toluene (7 mL) was added an ether solution of trimethylsilyldiazomethane (2.0 mol dm^{-3}, 0.2 mL, 0.4 mmol, 1.4 eq for each carboxy group) and the mixture was stirred for 1.5 h at r.t. The solvent was removed, and the residual (brown oil) was purified with silica gel column chromatography (15% ethyl acetate/hexane) to give 52.2 mg (0.139 mmol) of **3o** as a viscous oil in 96% yield.

^{1}H NMR (500 MHz, $CDCl_3$, TMS as the internal standard): δ/ppm 1.91 (6H, s), 2.07 (2H, quint, J/Hz = 7.5 Hz), 2.79 (4H, t, J /Hz =7.6), 3.84 (6H, s), 7.51 (2H, s).

^{13}C NMR (125 MHz, $CDCl_3$, TMS as the internal standard): δ/ppm 14.79, 22.83, 38.64, 52.02, 129.23, 134.44, 134.76, 136.59, 142.76, 162.58.

IR (neat, ν/cm^{-1}): 2951, 2843, 1706, 1246, 1080, 750.

LRMS (EI, 70 eV): 376 (M^+, 100), 361 (13), 345 (14), 301 (20), 257 (8), 157 (15).

HRMS (ESI, positive) Found: 377.0899. Calcd for $C_{19}H_{21}O_4S_2$: 377.0876 $(M+H)^+$.

[1] Y. Yokoyama, N. Hosoda, Y. T. Osano, C. Sasaki, *Chem. Lett*., 1998, **27**, 1093-1094.

[2] L. N. Lucas, J. J. D. de Jong, J. H. van Esch, R. M. Kellogg, B. L. Feringa, *Eur. J. Org. Chem.* **2003**, 155-166.

[3] A. J. Myles, N. R. Branda, *Macromolecules*, 2003, **36**, 298-303.

[4] S. Hiroto, K. Suzuki, H. Kamiya, H. Shinokubo, *Chem. Commun.*, 2011, **47**, 7149-7151.

Sample preparation for photochromic reaction: The buffer solution at pH 6.8 was prepared by adding 1 packet of phosphate buffer powder into 1 dm^3 of pure water. Stock solutions of diarylethenes (4 x 10^{-3} mol dm^{-3}) were prepared by acetonitrile.

A solution of a diarylethene and HSA in acetonitrile-buffer was prepared as follows: weigh appropriate amount of HSA into a volumetric flask and then adding a few mLs of buffer solution. The solution was set aside until the HSA was completely dissolved. Then the appropriate amount of the diarylethene stock solution in acetonitrile was injected into the solution, the designated amount of acetonitrile was added, and the flask was filled to the mark with buffer solution.

The solutions thus prepared were kept at 25℃ or at -4 °C to reach the equilibrium for 24 h.

Photoreaction and HPLC analysis: Photochemical reactions were all carried out in a 10-mm path length quartz cell. Photoirradiation with 313-nm light was carried out using a 500-W high-pressure mercury lamp, separated by filters (a 5-cm water filter, a UV-31 glass filter, a UVD-33S glass filter, a 5-cm aqueous $NiSO_4$ $6H_2O$ solution, a 1-cm aqueous K_2CrO_4 solution, and a 1-cm aqueous potassium hydrogen phthalate solution). Photoirradiation with 506-nm light was carried out using a 500-W xenon lamp which was separated by filters (a 5-cm water filter, a Y-47 glass filter, and a KL-50 glass filter). During the photoreactions, the solutions in the cell were stirred continuously.

Diarylethenes in the irradiated solutions were separated from HSA by ether extraction and then used for HPLC analysis.

High-performance liquid chromatography HPLC on a Shimadzu LC-6AD system or a JASCO X-LC system equipped with a UV/Vis detector and a column (Daicel OD-H, 4.6 mm x 250 cm for **1c**, **3c** and **4c**, Daicel OD-3, 2.1 mm x 150 mm for **5c**) was used to determine the enantiomer excess values of the compounds. Ee values were determined by peak area on the HPLC chromatogram

detected by the absorbance at **1o**: 510 or 555 nm, **3o**: 580 nm, **4o**: 587 nm, and **5o**: 585 nm.

2. Effect of Acetonitrile on HSA examined by CD Spectra

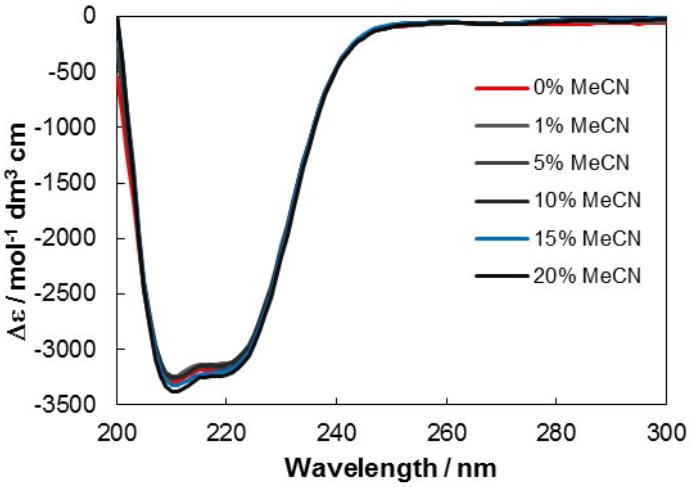


Fig. ESI-1. CD spectral change of HSA in buffer solution with different amount of acetonitrile added.

HSA: 4.90 x 10^{-7} mol dm^{-3}.
Solvent: Phosphate buffer solution (pH 6.8) with acetonitrile.
Cell length: 1 cm.

3. Effect of Amount of Acetonitrile on Ee Values of Closed Forms

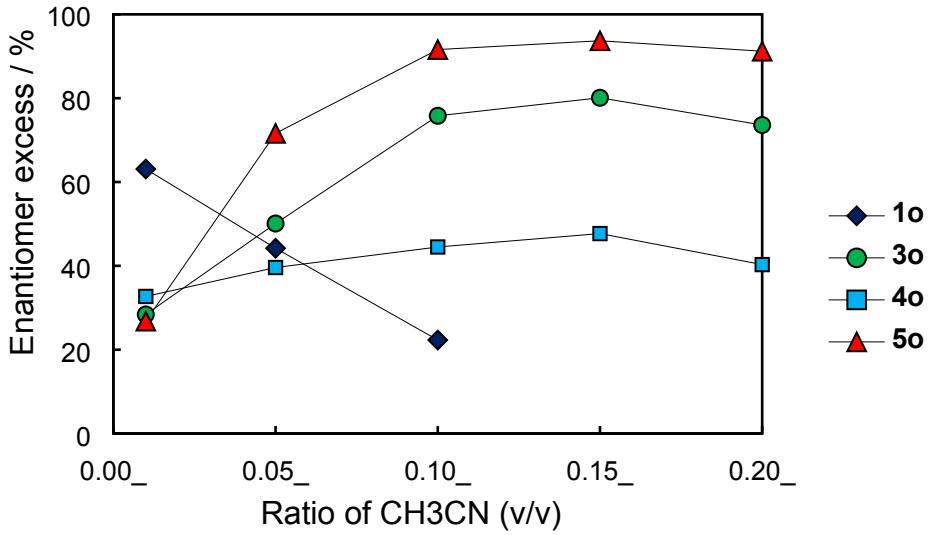


Fig. ESI-2. Relationship between the ee values of closed forms and amount of acetonitrile in buffer solution.

Reaction conditions: In ref. 19 in the text except for the amount of acetonitrile.

4. Effect of Acetonitrile on ^{19}F NMR Spectra of **5o** with HSA

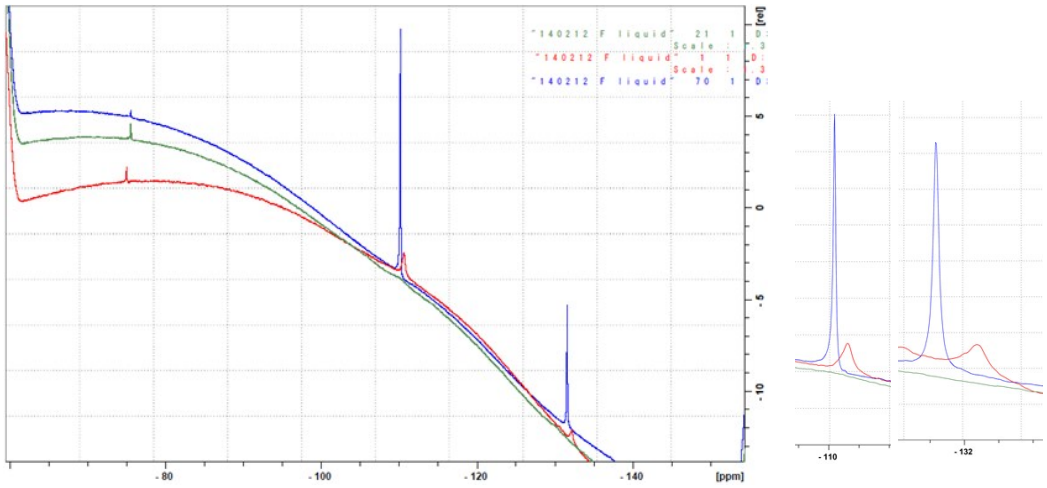
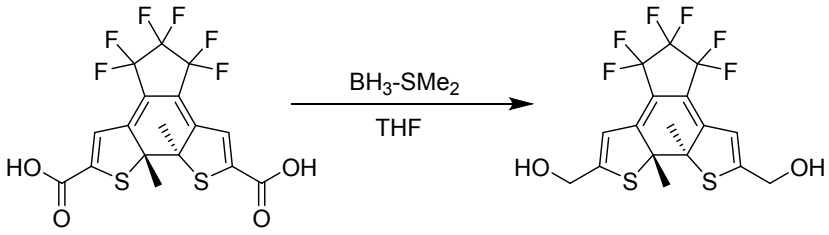


Fig. ESI-3. ^{19}F NMR of **5o**.

Blue: In acetonitrile without HSA at r.t.
Green: In 10% acetonitrile-buffer with two eq HSA at r.t.
Red: In 10% acetonitrile-buffer with two eq HSA at 50 °C.

5. Transformation of **4c** to **1c**

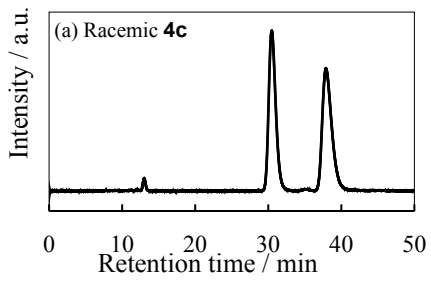


(R,R)-**4c** (**4c-f**)
Major enantiomer in HSA

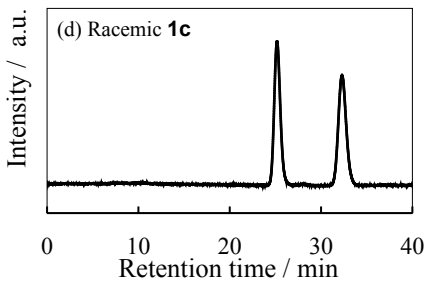
(R,R)-**1c** (**1c-s**)
Minor enantiomer in HSA

To a solution of optically resolved *(R,R)*-**4c** (faster moving enantiomer of **4c** (**4c-f**) on Daicel OD-H. 21.1mg, 0.046 mmol, 1.0 eq) in THF (3 ml) was added a THF solution of boran-dimethylsulfide complex (DMSB) (2.0 mol dm^{-3} in THF) (0.23 ml, 0.46 mmol, 10.0 eq) at -78 ^{O}C. The resulting solution was stirred for one hour at -78 ^{O}C, and the reaction was quenched by adding water. The resultant mixture was extracted with ether three times. The combined organic layer was dried over anhydrous Na_2SO_4, the drying agent filtered off, and the solvent evaporated. The residue was purified by silica gel column chromatography using 40% ethyl acetate/hexane as the eluent, to give *(R,R)*-**1c** (7.34 mg, 0.017 mmol) in 37 % yield, which was spectroscopically identical with **1c** photochemically generated from **1o**. On Daicel OD-H it is identical with the faster moving enantiomer of **1c** (**1c-f**), which is known to be *(R,R)*-**1c**, the minor enantiomer generated in HSA from **1o** by 313 nm light irradiation.

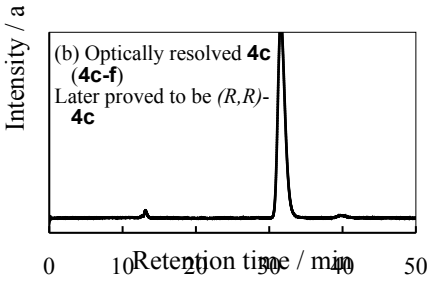
^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ/ppm 1.97 (2H, s), 2.07 (6H, s), 4.54 (4H, s), 6.26 (2H, s).



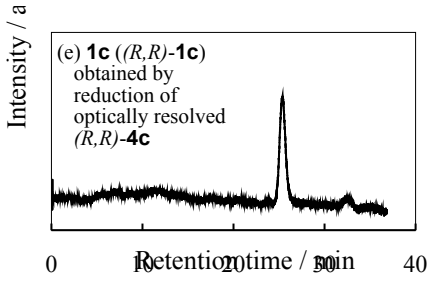
(a) HPLC: X-LC (Double pump device)
Column: Daicel OD-H
Eluent: 3% 2-propanol/hexane + 0.5% CF_3CO_2H
Flow rate: 0.5 mL / min
Detection: 587 nm



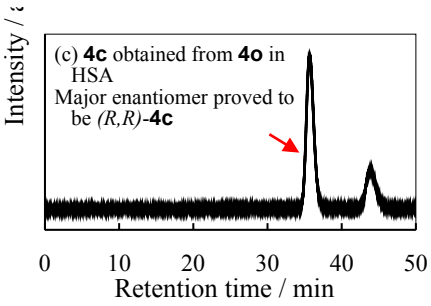
(d) HPLC: X-LC
Column: Daicel OD-H
Eluent: 10% 2-propanol/hexane
Flow rate: 0.5 mL / min
Detection: 510 nm



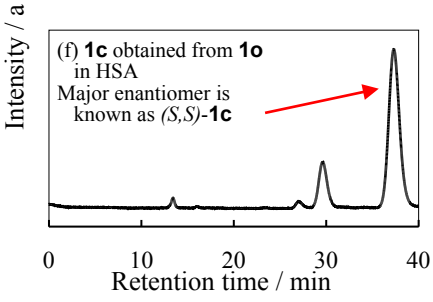
(b) HPLC: X-LC (Double pump device)
Column: Daicel OD-H
Eluent: 3% 2-propanol/hexane + 0.5% CF_3CO_2H
Flow rate: 0.5 mL / min
Detection: 587 nm



(e) HPLC: X-LC
Column: Daicel OD-H
Eluent: 10% 2-propanol/hexane
Flow rate: 0.5 mL / min
Detection: 510 nm



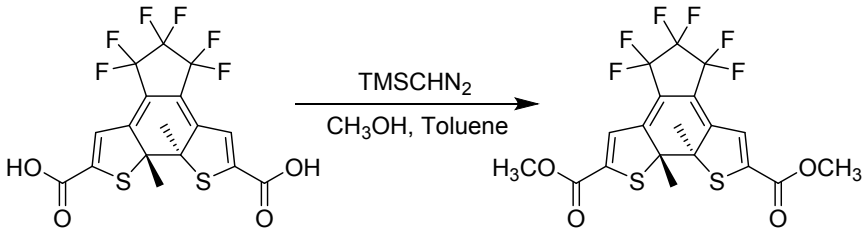
(c) HPLC: X-LC (Single pump device)
Column: Daicel OD-H
Eluent: 3% 2-propanol/hexane + 0.5% CF_3CO_2H
Flow rate: 0.5 mL / min
Detection: 587 nm



(f) HPLC: LC-6AD
Column: Daicel OD-H
Eluent: 10% 2-propanol/hexane
Flow rate: 0.5 mL / min
Detection: 555 nm

Fig. ESI-4. Synthesis of *(R,R)*-**1c** from optically resolved **4c-f** (*(R,R)*-**4c**).

6. Transformation of **4c** to **5c**

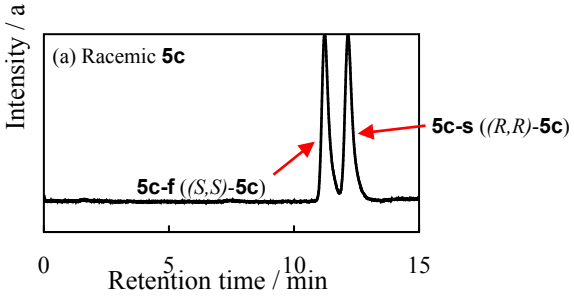


(R,R)-**4c** (**4c-f**)
Major enantiomer in HSA

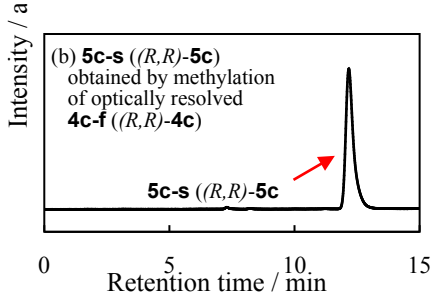
(R,R)-**5c** (**5c-s**)
Minor enantiomer in HSA

To a solution of optically resolved *(R,R)*-**4c** (faster moving enantiomer of **4c** (**4c-f**) on Daicel OD-H. 20.6 mg, 0.045 mmol, 1.0 eq) in toluene (3.5 ml) and methanol (1.0 ml) was added an ether solution of trimethylsilyldiazomethane ($TMSCHN_2$) (2.0 mol dm^{-3}) (0.1 ml, 0.22 mmol, 5.0 eq). The resulting solution was stirred for overnight at room temperature. The reaction was quenched by adding water. The resultant mixture was extracted with ether three times. The combined organic layer was dried over anhydrous Na_2SO_4, the drying agent filtered off, and the solvent evaporated. The residue was purified by silica gel column chromatography using 10% ethyl acetate/hexane as the eluent to give **5c** (8.6 mg, 0.018 mmol) in 40 % yield, which was spectroscopically identical with **5c** photochemically generated from **5o**.

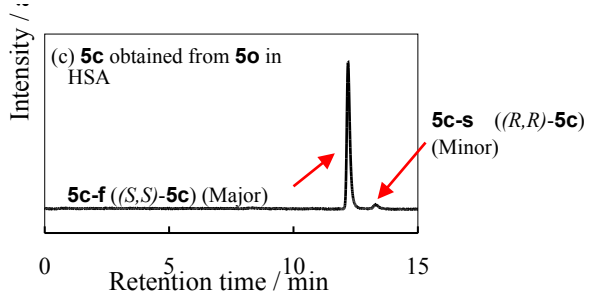
^1H NMR (300 MHz, $CDCl_3$, TMS) δ/ppm 2.21 (6H, s), 3.88 (6H, s), 6.93(2H, s).



(a) HPLC: X-LC
Column: Daicel OD-3
Eluent: 0.5% 2-propanol/hexane
Flow rate: 0.5 mL / min
Detection: 585 nm



(b) HPLC: X-LC
Column: Daicel OD-3
Eluent: 0.5% 2-propanol/hexane
Flow rate: 0.5 mL / min
Detection: 585 nm



(c) HPLC: X-LC
Column: Daicel OD-3
Eluent: 0.5% 2-propanol/hexane
Flow rate: 0.5 mL / min
Detection: 585 nm

Fig. ESI-5. Synthesis of *(R,R)*-**5c** from optically resolved **4c-f** (*(R,R)*-**4c**).

7. Competitive Incorporation Experiments of **4o** and **5o** in HSA

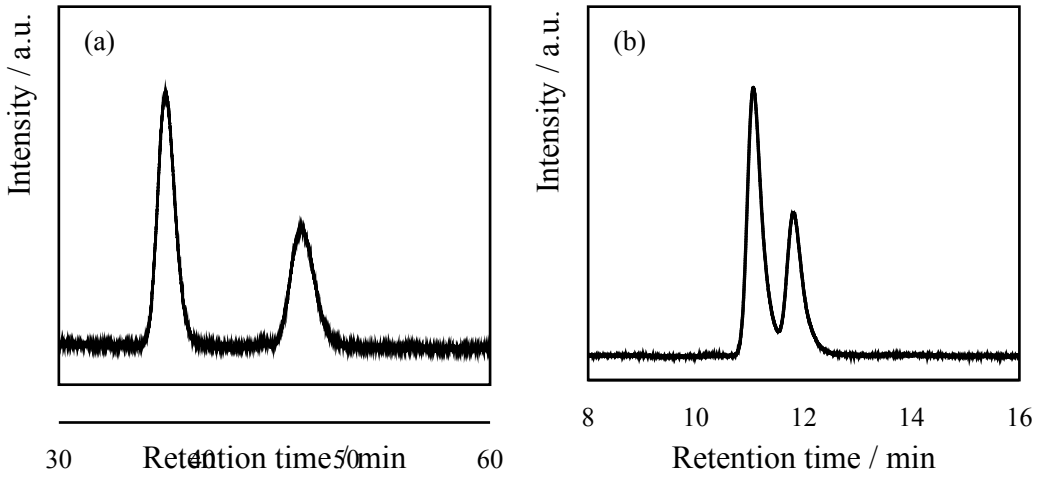


Fig. ESI-6. HPLC chromatograms of enantioselectivity of competition experiments of **4o** and **5o** in HSA (1:1:1) in 15% acetonitrile – buffer solution.
(a) **4c**. Column: Daicel OD-H. Eluent: 3% 2-propanol/hexane + 0.5% CF_3CO_2H. Flow rate: 0.5 mL/min. Detection wavelength: 587 nm.
(b) **5c**. Column: Daicel OD-3. Eluent: 1% 2-propanol/hexane. Flow rate: 0.5 mL/min. Detection wavelength: 585 nm.

8. Competitive Incorporation Experiments of **5o** and Warfarin in HSA

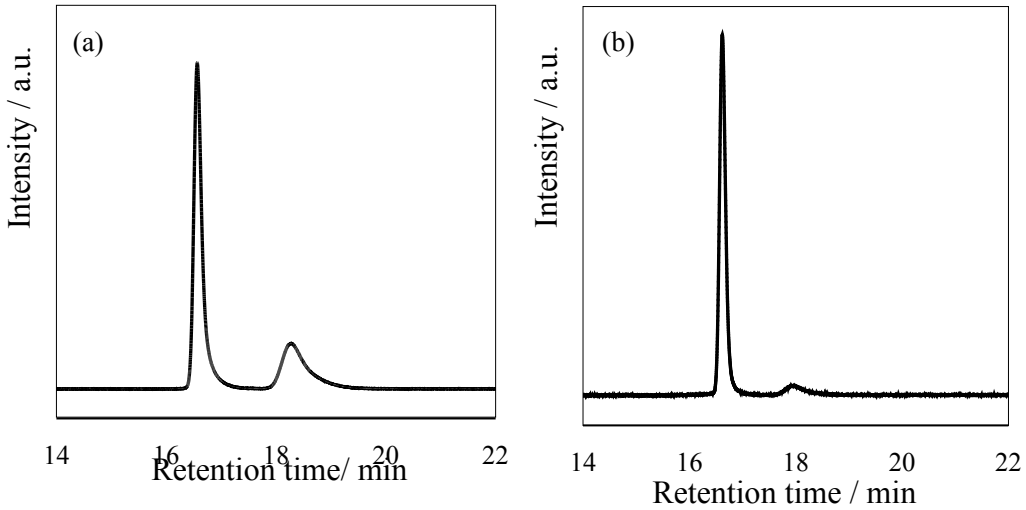
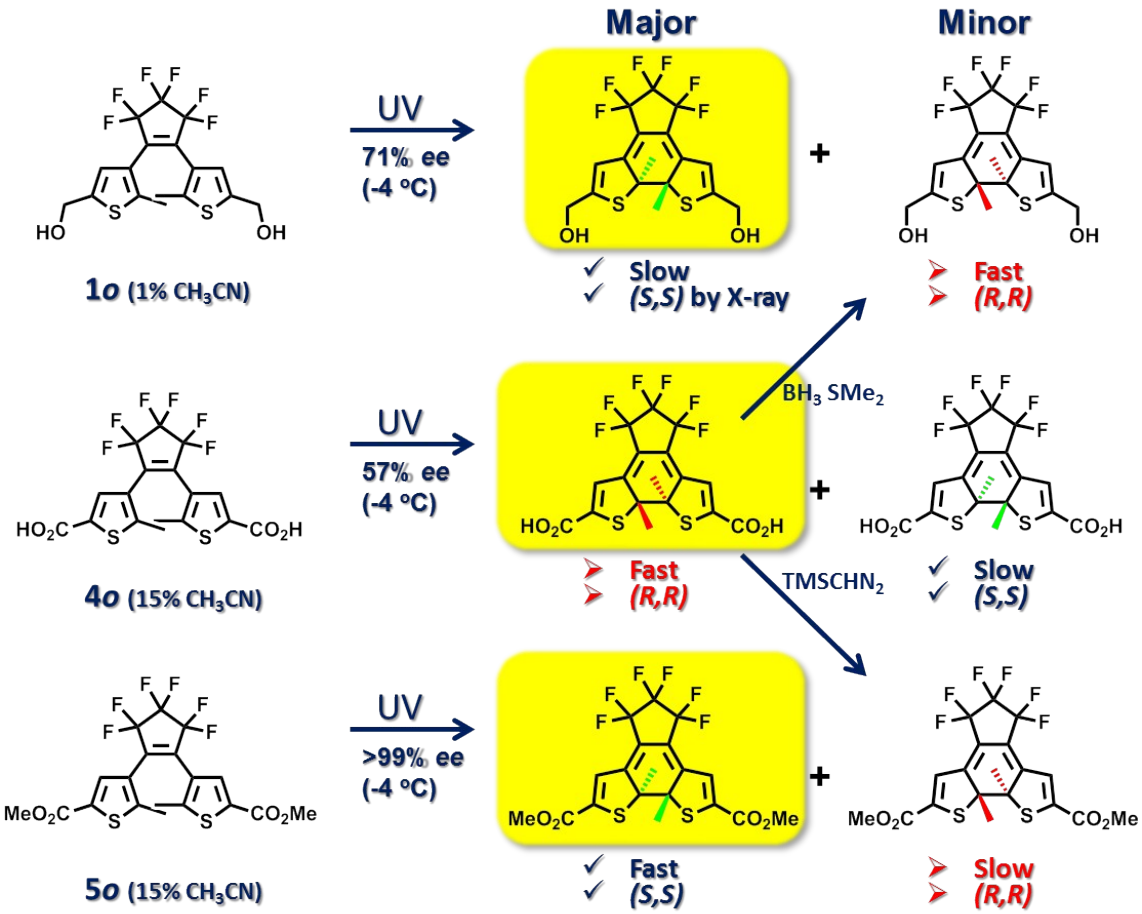


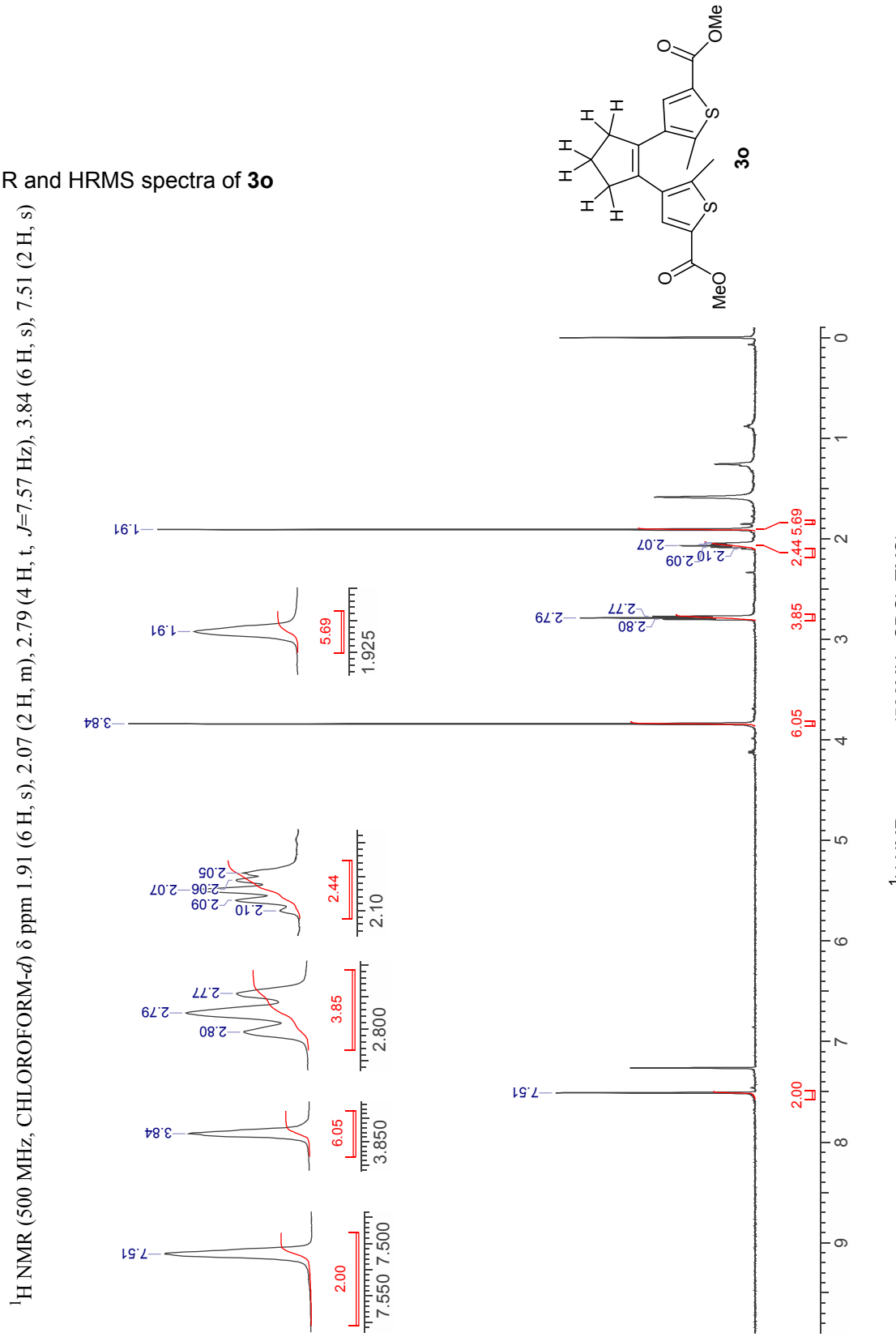
Fig. ESI-7. HPLC chromatograms of enantioselectivity of competition experiments of **5o** and warfarin in HSA in 15% acetonitrile – buffer solution.
Column: Daicel OD-3. Eluent: 0.5% 2-propanol/hexane. Flow rate: 0.5 mL/min. Detection wavelength: 585 nm.
(a) **5c** from **5o**:warfarin:HSA = 1:1:1. (b) **5c** from **5o**:warfarin:HSA = 1:10:10.

9. Correlation diagram of enantiomers obtained in HSA and the derivatives obtained from the optically resolved*(R,R)*- **4c**

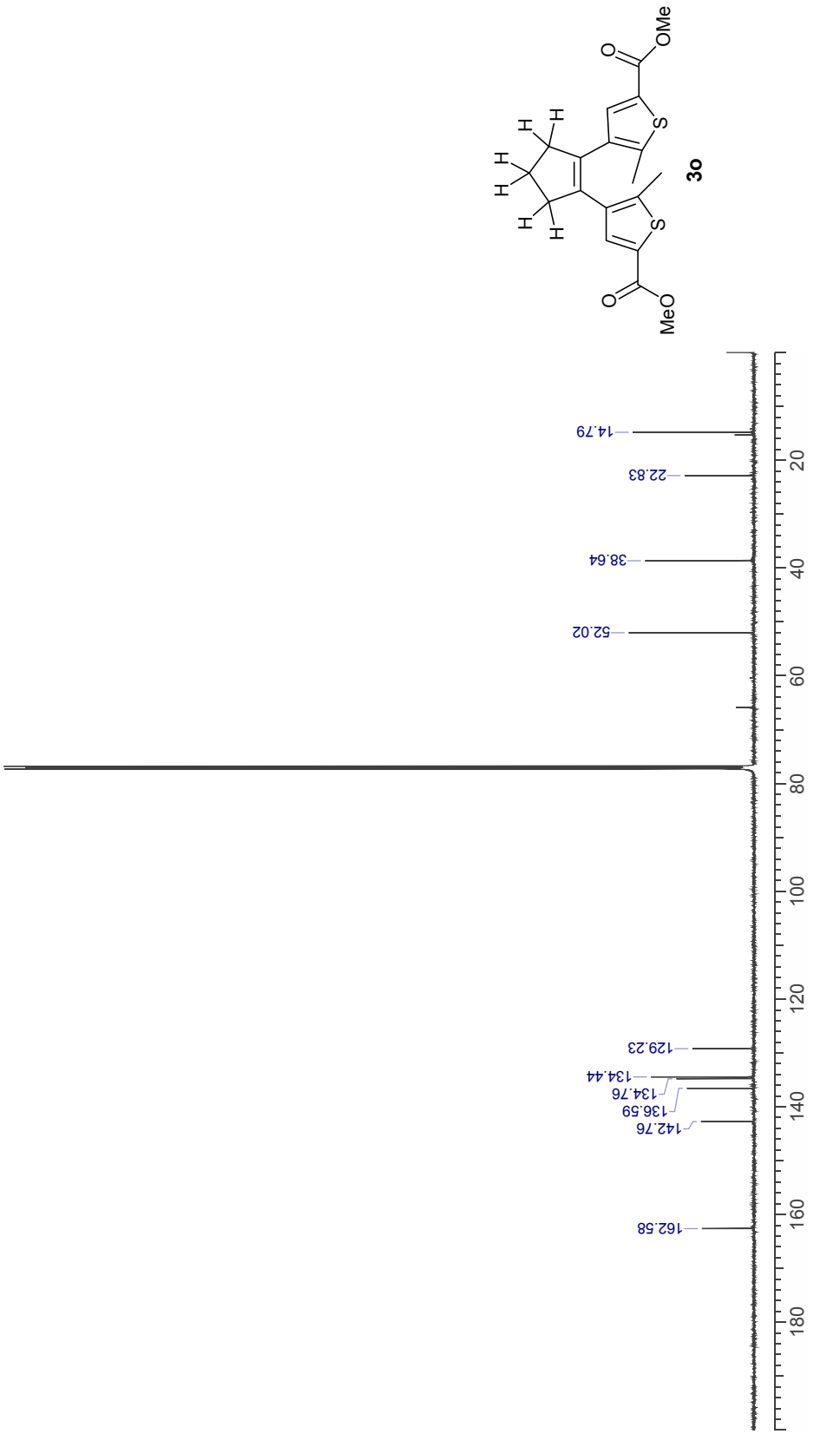


10. ^{1}H NMR, ^{13}C NMR and HRMS spectra of **3o**

^{1}H NMR



^{13}C NMR



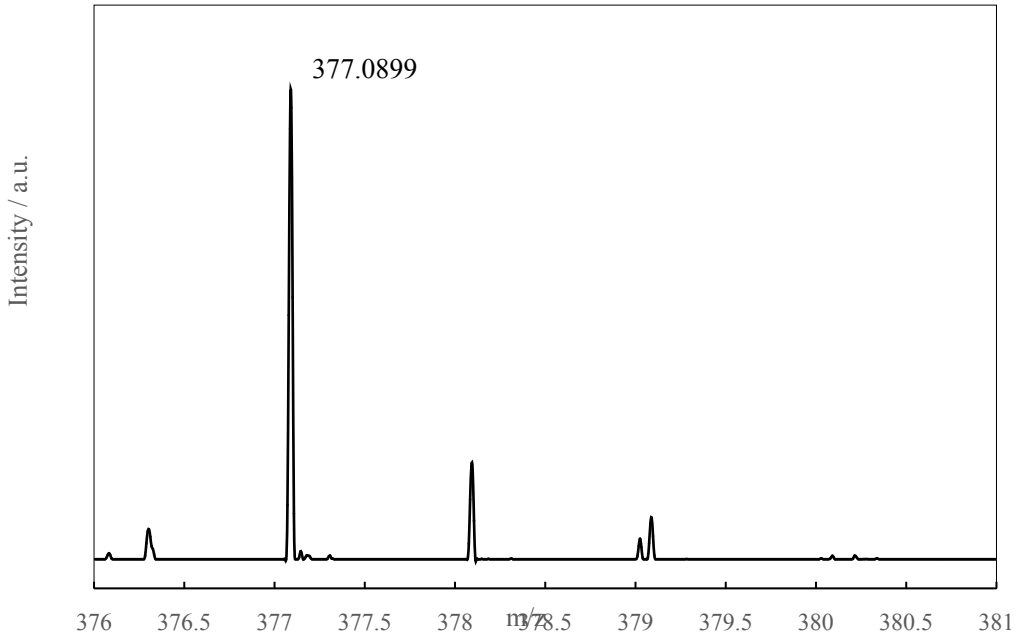
No.	(ppm)	(Hz)	Height
1	14.79	1859.5	0.1616
2	22.83	2871.2	0.0926
3	38.64	4859.0	0.1451

No.	(ppm)	(Hz)	Height
4	52.02	6542.5	0.1670
5	129.23	16252.2	0.0820
6	134.44	16907.5	0.1374

No.	(ppm)	(Hz)	Height
7	134.76	16946.9	0.1033
8	136.59	17176.9	0.0896
9	142.76	17953.1	0.0704

No.	(ppm)	(Hz)	Height
10	162.58	20445.9	0.0695

HRMS



HRMS (ESI, positive) Found: 377.0899. Calcd for $C_{19}H_{21}O_4S_2$: 377.0876 $(M+H)^+$.