Design, synthesis, and biological evaluation of C7-functionalized DMXAA derivatives as potential human-STING agonists

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Supplementary Information

General Procedures

All reactions were performed in oven-dried flasks. Unless otherwise noted, the flasks were fitted with rubber septa and reactions were conducted under a positive pressure of argon. Stainless steel syringes or cannula were used to transfer air- and moisture-sensitive liquids. Flash column chromatography was performed as described by Still et al. using silica gel (60-Å pore size, 40–63 μm, 4-6% H_2O content, Merck).[1] Analytical thin–layer chromatography (TLC) was performed using glass plates pre-coated with 0.25 mm silica gel impregnated with a fluorescent indicator (254 nm). Thin layer chromatography plates were visualized by exposure to ultraviolet light, an aqueous solution of ceric ammonium molybdate (CAM).

Materials and Instrumentations

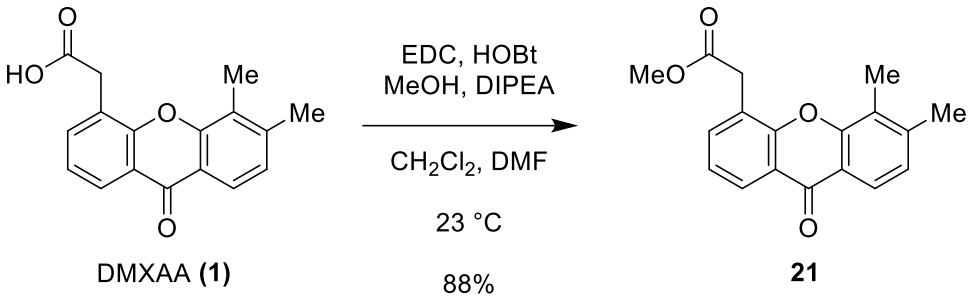
Unless otherwise stated, all commercial reagents and solvents were used without additional purification with the following exceptions: dichloromethane and tetrahydrofuran were purchased from Merck and Daejung Inc., respectively and were purified by the method of Grubbs et al. under positive argon pressure.[2] 5,6-dimethylxanthenone-4-acetic acid (**1**) was purchased from Ark Pharm.

Proton and carbon nuclear magnetic resonance spectra were recorded with Bruker Ascend 400 (400 MHz), Agilent Technologies DD2 (600 MHz). Proton nuclear magnetic resonance spectra are referenced from the residual protium in the NMR solvent ($CDCl_3$: δ 7.24 ($CHCl_3$) or DMSO-d_6: δ 2.50 (DMSO-d_5)). Data are reported in the following manners: chemical shift in ppm [multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet,

[1] W. C. Still, M. Kahn and A. Mitra, *J. Org. Chem*., 1978, **43**, 2923.

[2] A. B. Pangborn, M. A. Giardello, R. H. Grubbs, R. K. Rosen and F. J. Timmers, *Organometallics,* 1996, **15**, 1518.

app = apparent, br = broad), coupling constant(s) in Hertz, integration]. Carbon-13 nuclear magnetic resonance spectra are referenced from the carbon resonances of the solvent ($CDCl_3$: δ 77.23, DMSO-d_6: δ 39.51). Data are reported in the following manners: chemical shift in ppm. High resolution mass spectra were obtained from KAIST Research Analysis Center (Daejeon) by using an ESI ionization method.



Methyl 2-(5,6-dimethyl-9-oxo-9*H*-xanthen-4-yl)acetate (21):

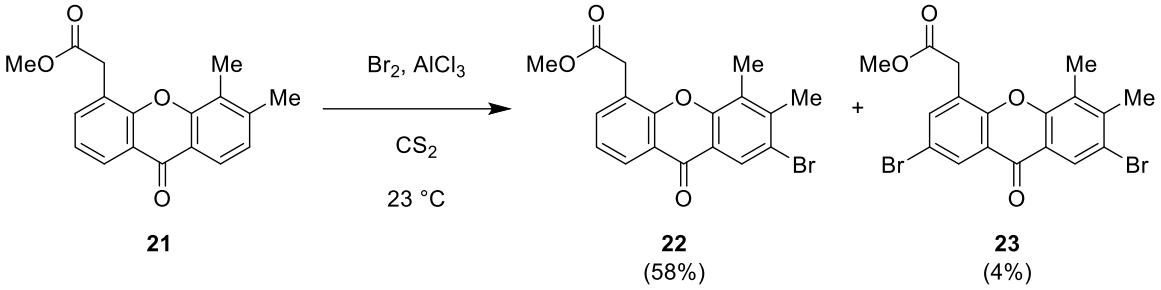
To a stirred solution of **1** (200 mg, 0.71 mmol, 1 equiv) in dichloromethane (5 mL) and *N*,*N*-dimethylformamide (0.5 mL) were added methanol (3 mL), *N*,*N*-diisopropylethylamine (DIPEA, 0.2 mL), 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC·HCl, 203 mg, 1.06 mmol, 1.50 equiv) and 1-hydroxybenzotriazole hydrate (HOBt, 143 mg, 1,06 mmol, 1.50 equiv) at 23 °C. After 3 h, distilled water (15 mL) was added to the reaction mixture, and the layers were separated. The aqueous layer was extracted with dichloromethane (3 × 10 mL), and the combined organic layers were washed with saturated aqueous sodium bicarbonate solution (2 × 30 mL) and aqueous citric acid solution (2 × 30 mL). The washed solution was dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The resulting crude residue was purified by flash column chromatography (silica gel: diam. 3 cm, ht. 14 cm; eluent: ethyl acetate : hexanes = 1 : 3) to afford **21** (184 mg, 88%) as a white solid.

^{1}H NMR (599.1 MHz, $CDCl_3$): δ 8.26 (dd, *J* = 8.0, 1.5 Hz, 1H), 8.06 (d, *J* = 8.1 Hz, 1H), 7.61 (dd, *J* = 7.2, 1.5 Hz, 1H), 7.32 (app-t, *J* = 7.6 Hz, 1H), 7.17 (d, *J* = 8.1 Hz, 1H), 3.97 (s, 2H), 3.71 (s, 3H), 2.43 (s, 3H), 2.42 (s, 3H).

^{13}C NMR (150.7 MHz, $CDCl_3$): δ 177.7, 171.3, 154.4, 154.3, 144.7, 136.1, 126.3, 126.2, 125.4, 123.9, 123.7, 123.7, 121.8, 119.9, 52.4, 36.1, 20.9, 11.7.

HRMS (ESI): Calculated for $C_{18}H_{16}O_4$ $[M+Na]^+$: 319.0941, found: 319.0971.

TLC (ethyl acetate : hexanes = 1 : 3) R*f*: 0.34 (UV).



Methyl 2-(7-bromo-5,6-dimethyl-9-oxo-9*H*-xanthen-4-yl)acetate (22) and Methyl 2-(2,7-dibromo-5,6-dimethyl-9-oxo-9*H*-xanthen-4-yl)acetate (23):

To a stirred solution of **21** (97.2 mg, 0.328 mmol, 1 equiv) and aluminum trichloride ($AlCl_3$, 131 mg, 0.984 mmol, 3.00 equiv) in carbon disulfide (CS_2, 11.5 mL) was added bromine (Br_2, 0.336 mL, 6.56 mmol, 20.0 equiv) dropwise at 23 °C. After 56 h, saturated aqueous sodium thiosulfate solution (10 mL) was added to the reaction mixture, and the reaction mixture was extracted with diethyl ether (2 × 20 mL). The combined organic layers were washed with brine (2 × 40 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The resulting crude residue was purified by flash column chromatography (silica gel: diam. 3 cm, ht. 7 cm; eluent: dichloromethane) to afford **22** (68 mg, 58%) and **23** (6.3 mg, 4%) as a white solid.

Methyl 2-(7-bromo-5,6-dimethyl-9-oxo-9*H*-xanthen-4-yl)acetate (22)

^{1}H NMR (599.1 MHz, $CDCl_3$): δ 8.36 (s, 1H), 8.24 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.63 (dd, *J* = 7.3, 1.7 Hz, 1H), 7.33 (app-t, *J* = 7.6 Hz, 1H), 3.96 (s, 2H), 3.71 (s, 3H), 2.52 (s, 3H), 2.50 (s, 3H).

^{13}C NMR (150.7 MHz, $CDCl_3$): δ 176.5, 171.1, 154.4, 153.1, 143.8, 136.5, 127.7, 127.3, 126.4, 124.0, 124.0, 121.7, 121.2, 120.7, 52.5, 36.1, 20.9, 13.1.

HRMS (ESI): Calculated for $C_{18}H_{15}BrO_4$ $[M+H]^+$: 375.0226, found: 375.0220.

TLC (dichloromethane) R*f*: 0.23 (UV).

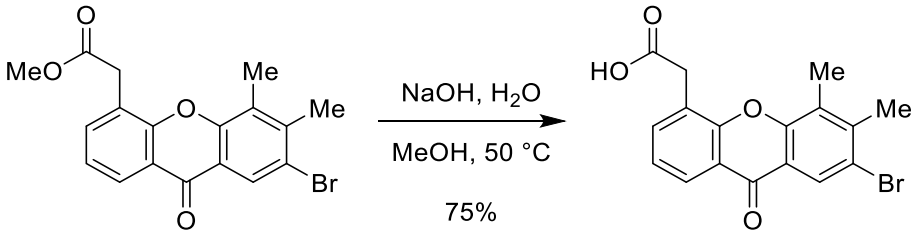
Methyl 2-(2,7-dibromo-5,6-dimethyl-9-oxo-9*H*-xanthen-4-yl)acetate (23)

^{1}H NMR (599.1 MHz, $CDCl_3$): δ 8.36–8.30 (m, 2H), 7.73 (d, *J* = 2.2 Hz, 1H), 3.93 (s, 2H), 3.72 (s, 3H), 2.52 (s, 3H), 2.48 (s, 3H).

^{13}C NMR (150.7 MHz, $CDCl_3$): δ 175.3, 170.5, 153.2, 153.0, 144.4, 139.1, 128.8, 127.7, 127.4, 126.4, 122.8, 121.6, 120.4, 117.1, 52.6, 35.7, 21.0, 13.1.

HRMS (ESI): Calculated for $C_{18}H_{14}Br_2O_4$ $[M+Na]^+$: 474.9151, found: 474.9140.

TLC (dichloromethane) R*f*: 0.50 (UV).



22 **20**

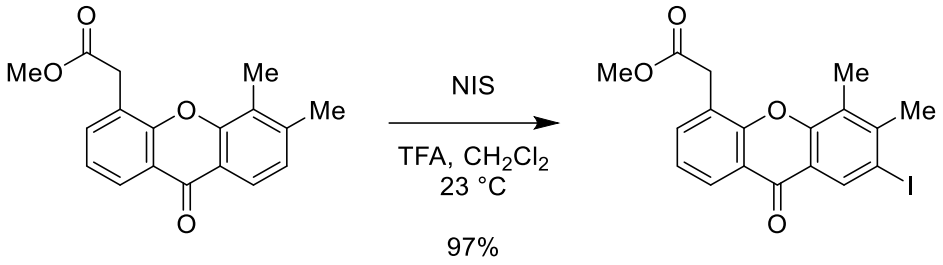
2-(7-bromo-5,6-dimethyl-9-oxo-9*H*-xanthen-4-yl)acetic acid (20):

To a stirred solution of **22** (11 mg, 0.0293 mmol, 1 equiv) in methanol (0.8 mL) and H_2O (2 mL) were added sodium hydroxide (3.5 mg, 0.879 mmol, 3.00 equiv) at 50 °C. After 10 h, 1M aqueous hydrochloric acid solution (3mL) was added to the reaction mixture, and the reaction mixture was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine (2 × 30 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resulting crude residue was purified by flash column chromatography (silica gel: diam. 1.5 cm, ht. 10 cm; eluent: ethyl acetate : acetic acid = 100 :1) to afford **20** (7.9 mg, 75%) as a white solid.

^{1}H NMR (599.1 MHz, $(CD_3)_2SO$) δ 12.80 (br, 1H), 8.12 (s, 1H), 8.07 (d, *J* = 8.0 Hz, 1H), 7.81 (d, *J* = 7.2 Hz, 1H), 7.43 (app-t, *J* = 7.6 Hz, 1H), 3.96 (s, 2H), 2.48 (s, 3H), 2.45 (s, 3H).

^{13}C NMR (150.7 MHz, $(CD_3)_2SO$) δ 175.1, 171.7, 153.7, 152.2, 143.3, 137.0, 128.0, 125.7, 125.6, 124.5, 124.0, 120.3, 120.1, 119.7, 35.6, 20.3, 12.5.

HRMS (ESI): Calculated for $C_{17}H_{13}BrO_4$ $[M-H]^-$: 358.9924, found: 358.9907.



21 **24**

Methyl 2-(7-iodo-5,6-dimethyl-9-oxo-9*H*-xanthene-4-yl)acetate (24):

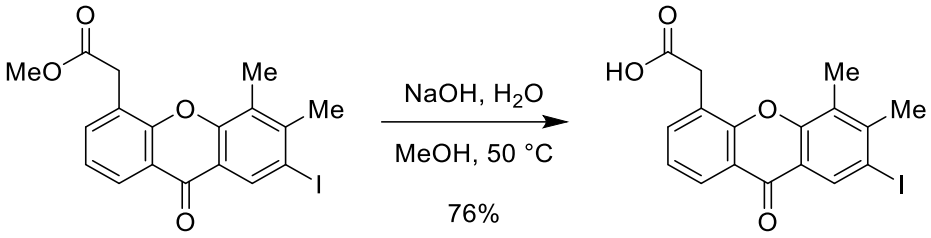
To a stirred solution of **21** (221 mg, 0.75 mmol, 1 equiv) in dichloromethane (7.5 mL) and trifluoroacetic acid (TFA, 7.5 mL) was added *N*-iodosuccinimide (NIS, 185 mg, 0.82 mmol, 1.10 equiv) at 23 °C. After 4 h, saturated aqueous sodium thiosulfate solution (15 mL) was added to the reaction mixture, and the layers were separated. The aqueous layer was extracted with dichloromethane (3 × 15 mL), and the combined organic layers were washed with saturated aqueous sodium bicarbonate solution (3 × 45 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resulting crude residue was purified by flash column chromatography (silica gel: diam. 3 cm, ht. 15 cm; eluent: ethyl acetate : hexanes = 1 : 3) to afford **24** (307 mg, 97%) as a pale yellow solid.

^{1}H NMR (599.1 MHz, $CDCl_3$) δ 8.62 (s, 1H), 8.22 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.62 (dd, *J* = 7.3, 1.4 Hz, 1H), 7.31 (app-t, *J* = 7.6 Hz, 1H), 3.95 (s, 2H), 3.70 (s, 3H), 2.56 (s, 3H), 2.51 (s, 3H).

^{13}C NMR (150.7 MHz, $CDCl_3$) δ 176.3, 171.1, 154.3, 153.9, 146.6, 136.5, 134.3, 126.7, 126.3, 124.0, 124.0, 121.7, 121.1, 96.5, 52.4, 36.0, 26.5, 13.5.

HRMS (ESI): Calculated for $C_{18}H_{15}IO_4$ $[M+Na]^+$: 444.9907, found: 444.9937.

TLC (ethyl acetate : hexanes = 1 : 3) R*f*: 0.36 (UV).



24 **25**

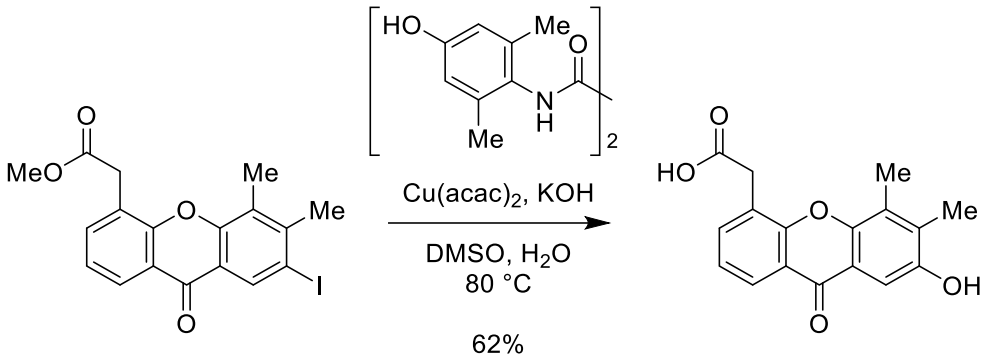
2-(7-iodo-5,6-dimethyl-9-oxo-9*H*-xanthen-4-yl)acetic acid (25):

To a stirred solution of **24** (25.6 mg, 0.0606 mmol, 1 equiv) in methanol (2 mL) and H_2O (5 mL) were added sodium hydroxide (7.3 mg, 0.182 mmol, 3.00 equiv) at 50 °C. After 7 h, 1M aqueous hydrochloric acid solution (5mL) was added to the reaction mixture, and the reaction mixture was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine (2 × 30 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resulting crude residue was purified by flash column chromatography (silica gel: diam. 1.5 cm, ht. 12 cm; eluent: ethyl acetate : acetic acid = 100 :1) to afford **25** (19.0 mg, 76%) as a pale yellow solid.

^{1}H NMR (599.1 MHz, $(CD_3)_2SO$) δ 12.71 (br, 1H), 8.35 (s, 1H), 8.05 (d, *J* = 8.0 Hz, 1H), 7.78 (d, *J* = 7.1 Hz, 1H), 7.40 (app-t, *J* = 7.7 Hz, 1H), 3.93 (s, 2H), 2.50 (s, 3H), 2.43 (s, 3H).

^{13}C NMR (150.7 MHz, $(CD_3)_2SO$) 174.9, 171.7, 153.7, 152.9, 146.2, 136.9, 132.6, 126.7, 125.5, 124.5, 123.9, 120.3, 120.1, 96.6, 35.5, 25.8, 12.8.

HRMS (ESI): Calculated for $C_{17}H_{13}IO_4$ $[M+Na]^+$: 430.9751, found: 430.9767.



24 **2**

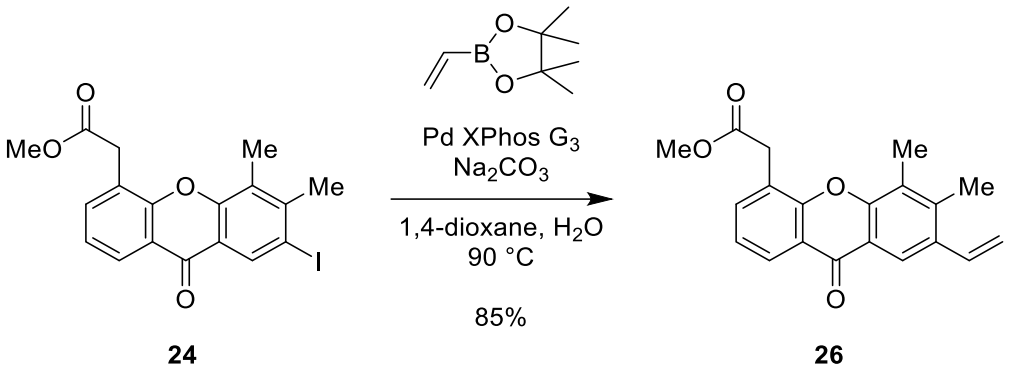
2-(7-hydroxy-5,6-dimethyl-9-oxo-9*H*-xanthen-4-yl)acetic acid (2):

To a stirred solution of **24** (37 mg, 0.088 mmol, 1 equiv) in DMSO (0.8 mL) and H_2O (0.2 mL) were added copper acetate ($Cu(acac)_2$, 0.8 mg, 0.0044 mmol, 0.05 equiv), potassium hydroxide (KOH, 14.8 mg, 0.264 mmol, 3.0 equiv), and *N,N′*-bis(4-hydroxyl-2,6-dimethylphenyl)oxalamide (BHMPO, 3.2 mg 0.0088 mmol, 0.1 equiv) at 23 °C. The reaction mixture was heated to 80 °C. After 17 h, the reaction mixture was cooled to 23 °C, and 1M aqueous hydrochloric acid solution (1 mL) was added to the reaction mixture. The reaction mixture was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine (3 × 30 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The resulting crude residue was purified by flash column chromatography (silica gel: diam. 1.5 cm, ht. 14 cm; eluent: ethyl acetate : acetic acid = 100 : 1) to afford **2** (16.3 mg, 62%) as a pale yellow solid.

^{1}H NMR (599.1 MHz, $(CD_3)_2SO$) δ 12.55 (br, 1H), 9.88 (br, 1H), 8.05 (d, *J* = 8.0 Hz, 1H), 7.74 (d, *J* = 7.2 Hz, 1H), 7.39 (s, 1H), 7.37 (app-t, *J* = 7.6 Hz, 1H), 3.95 (s, 2H), 2.39 (s, 3H), 2.25 (s, 3H).

^{13}C NMR (150.7 MHz, $(CD_3)_2SO$) δ 175.9, 171.8, 153.7, 151.9, 147.3, 136.0, 133.3, 126.4, 125.2, 124.4, 123.1, 120.1, 118.8, 105.0, 35.5, 12.8, 11.6.

HRMS (ESI): Calculated for $C_{17}H_{14}O_5$ $[M+H]^+$: 299.0914, found: 299.0942.



24 **26**

Methyl 2-(5,6-dimethyl-9-oxo-7-vinyl-9*H*-xanthene-4-yl)acetate (26):

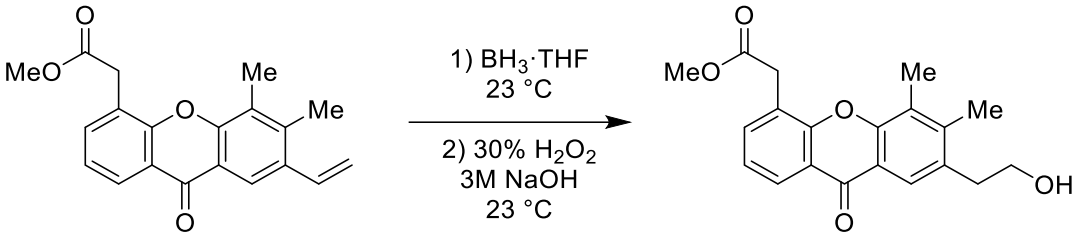
To a stirred solution of **24** (327 mg, 0.774 mmol, 1 equiv) in 1,4-dioxane (9 mL) and H_2O (1 mL) were added Pd XPhos G_3 (65.5 mg, 0.0774 mmol, 0.1 equiv), sodium carbonate (Na_2CO_3, 164 mg, 1.55 mmol, 2.0 equiv), and vinylboronic acid pinacol ester (0.390 mL, 2.32 mmol, 3.0 equiv) at 23 °C. The reaction mixture was heated to 90 °C. After 4 h, the reaction mixture was cooled to 23 °C, and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine (3 × 30 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The resulting crude residue was purified by flash column chromatography (silica gel: diam. 3 cm, ht. 13 cm; eluent: ethyl acetate : hexanes = 1 : 4) to afford **26** (212 mg, 85%) as a white solid.

^{1}H NMR (599.1 MHz, $CDCl_3$) δ 8.26 (dd, *J* = 7.9, 1.6 Hz, 1H), 8.22 (s, 1H), 7.60 (dd, *J* = 7.1, 1.6 Hz, 1H), 7.31 (app-t, *J* = 7.6 Hz, 1H), 6.96 (dd, *J* = 17.3, 10.8 Hz, 1H), 5.71 (dd, *J* = 17.3, 1.5 Hz, 1H), 5.36 (dd, *J* = 10.8, 1.5 Hz, 1H), 3.96 (s, 2H), 3.71 (s, 3H), 2.44 (s, 3H), 2.38 (s, 3H).

^{13}C NMR (150.7 MHz, $CDCl_3$) δ 177.6, 171.3, 154.4, 153.6, 142.3, 136.1, 134.8, 134.5, 126.3, 125.5, 123.9, 123.6, 121.8, 121.2, 119.6, 117.4, 52.4, 36.1, 16.8, 12.1.

HRMS (ESI): Calculated for $C_{20}H_{18}O_4$ $[M+Na]^+$: 345.1097, found: 345.1127.

TLC (ethyl acetate : hexanes = 1 : 3) R*f*: 0.38 (UV).



26 **27**

Methyl 2-(7-(2-hydroxyethyl)-5,6-dimethyl-9-oxo-9*H*-xanthene-4-yl)acetate (27):

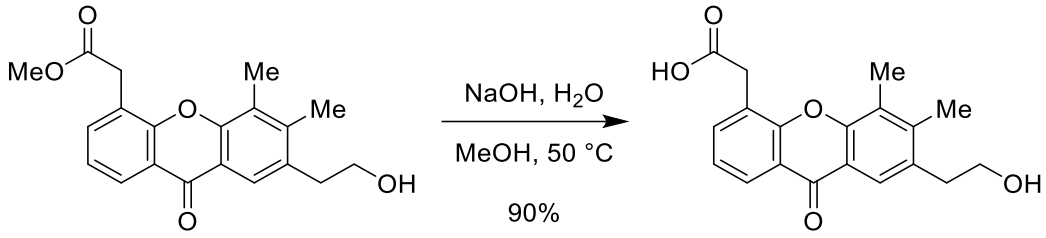
To a stirred solution of **26** (50 mg, 0.155 mmol, 1 equiv) in THF (4 mL) was added 1M BH_3·THF solution (1M in THF, 0.465 mL, 0.465 mmol, 3.0 equiv) at 23 °C. After 1 h, 3M aqueous sodium hydroxide solution (0.258 mL, 0.775 mmol, 5.0 equiv) and 30% aqueous H_2O_2 solution (0.158 mL, 1.55 mmol, 10.0 equiv) were added to the reaction mixture. After 30 min, saturated aqueous sodium thiosulfate (5mL) was added to the reaction mixture, and the reaction mixture was extracted with ethyl acetate (3 × 7 mL). The combined organic layers were washed with saturated aqueous ammonium chloride solution (2 × 20 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resulting crude residue was purified by flash column chromatography (silica gel: diam. 1.5 cm, ht. 12 cm; eluent: ethyl acetate : hexanes = 1 : 1) to afford **27** (21.5 mg, 41%) as a white solid.

^{1}H NMR (599.1 MHz, $CDCl_3$) δ 8.24 (dd, *J* = 8.1, 1.7 Hz, 1H), 7.97 (s, 1H), 7.61 (dd, *J* = 7.3, 1.7 Hz, 1H), 7.31 (app-t, *J* = 7.6 Hz, 1H), 3.96 (s, 2H), 3.89 (t, *J* = 6.9 Hz, 2H), 3.70 (s, 3H), 3.02 (t, *J* = 6.9 Hz, 2H), 2.43 (s, 3H), 2.39 (s, 3H).

^{13}C NMR (150.7 MHz, $CDCl_3$) δ 177.6, 171.3, 154.4, 153.1, 143.8, 136.1, 133.2, 126.3, 126.0, 124.3, 123.9, 123.6, 121.9, 119.4, 63.0, 52.4, 37.0, 36.1, 16.7, 12.3.

HRMS (ESI): Calculated for $C_{20}H_{20}O_5$ $[M+Na]^+$: 363.1203, found: 363.1205.

TLC (ethyl acetate) R*f*: 0.62 (UV).



27 **4**

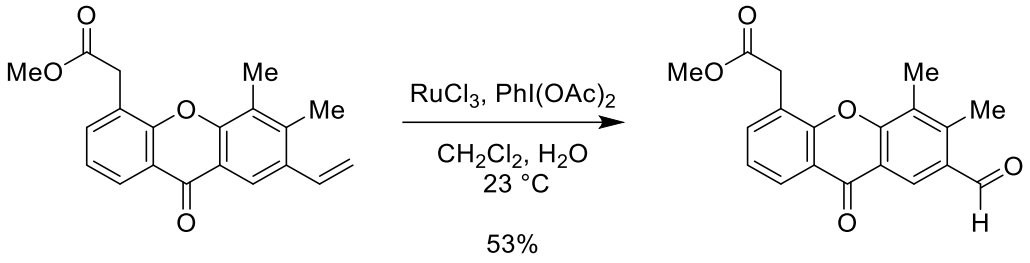
2-(7-(2-hydroxyethyl)-5,6-dimethyl-9-oxo-9*H*-xanthene-4-yl)acetic acid (4):

To a stirred solution of **27** (16.6 mg, 0.0486mmol, 1 equiv) in methanol (1.6 mL) and H_2O (4 mL) were added sodium hydroxide (5.8 mg, 0.146 mmol, 3.00 equiv) at 50 °C. After 1 h, 1M aqueous hydrochloric acid solution (5mL) was added to the reaction mixture, and the reaction mixture was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine (2 × 30 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resulting crude residue was purified by flash column chromatography (silica gel: diam. 1.5 cm, ht. 10 cm; eluent: ethyl acetate : acetic acid = 100 :1) to afford **4** (14.4 mg, 90%) as a white solid.

^{1}H NMR (599.1 MHz, $(CD_3)_2SO$) δ 8.07 (d, *J* = 7.9 Hz, 1H), 7.80 (s, 1H), 7.76 (d, *J* = 7.2 Hz, 1H), 7.38 (app-t, *J* = 7.4 Hz, 1H), 4.70 (br, 1H), 3.94 (s, 2H), 3.61 (t, *J* = 6.7 Hz, 2H), 2.88 (t, *J* = 6.7 Hz, 2H), 2.38 (s, 3H), 2.34 (s, 3H).

^{13}C NMR (150.7 MHz, $(CD_3)_2SO$) δ 176.1, 171.8, 153.8, 151.9, 143.8, 136.4, 134.1, 125.3, 125.2, 124.6, 123.5, 123.2, 120.6, 118.2, 61.1, 36.7, 35.5, 16.1, 11.7.

HRMS (ESI): Calculated for $C_{19}H_{18}O_5$ $[M+Na]^+$: 349.1046, found: 349.1056.



26 **28**

Methyl 2-(7-formyl-5,6-dimethyl-9-oxo-9*H*-xanthen-4-yl)acetate (28):

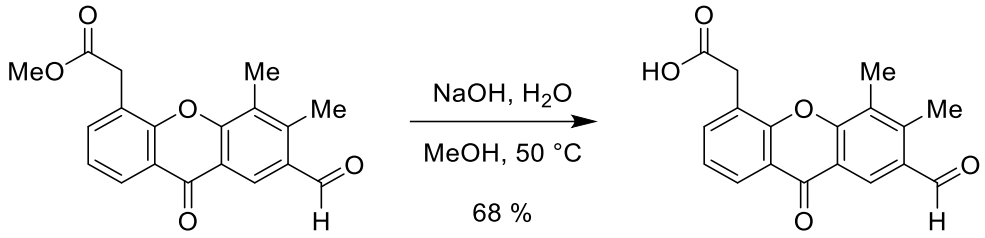
To a stirred solution of **26** (78 mg, 0.242 mmol, 1 equiv) in dichloromethane (4 mL) and H_2O (1 mL) were added ruthenium trichloride ($RuCl_3$, 2.5 mg, 0.012 mmol, 0.05 equiv) and [bis(acetoxy)iodo]benzene ($PhI(OAc)_2$, 234mg, 0.726 mmol, 3 equiv) at 23 °C. After 3 h, saturated aqueous sodium bicarbonate solution (5 mL) was added to the reaction mixture, and the reaction mixture was extracted with dichloromethane (3 × 7 mL). The combined organic layers were washed with brine (2 × 20 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resulting crude residue was purified by flash column chromatography (silica gel: diam. 2.5 cm, ht. 11 cm; eluent: ethyl acetate : hexanes = 1 : 2) to afford **28** (42 mg, 53%) as a white solid.

^{1}H NMR (599.1 MHz, $CDCl_3$) δ 10.23 (s, 1H), 8.59 (s, 1H), 8.27 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.66 (dd, *J* = 7.2, 1.5 Hz, 1H), 7.38 (app-t, *J* = 7.6 Hz, 1H), 3.99 (s, 2H), 3.72 (s, 3H), 2.75 (s, 3H), 2.48 (s, 3H).

^{13}C NMR (150.7 MHz, $CDCl_3$) δ 192.5, 176.8, 171.0, 156.7, 154.3, 145.8, 136.9, 132.1, 131.1, 127.9, 126.4, 124.6, 124.2, 121.9, 119.5, 52.5, 36.0, 16.2, 11.7.

HRMS (ESI): Calculated for $C_{19}H_{16}O_5$ $[M+H]^+$: 325.1071, found: 325.1061.

TLC (ethyl acetate : hexanes = 1 : 2) R*f*: 0.34 (UV).



28 **29**

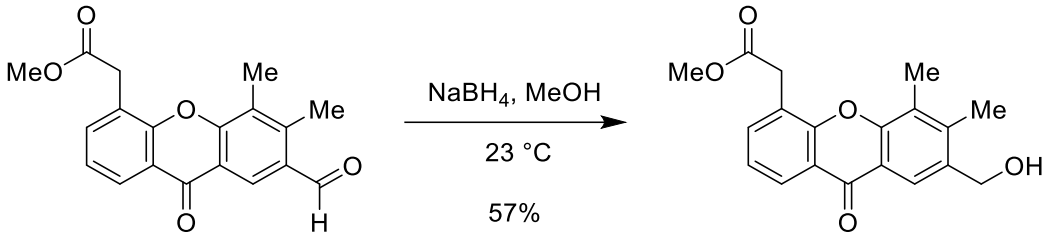
2-(7-formyl-5,6-dimethyl-9-oxo-9H-xanthen-4-yl)acetic acid (29):

To a stirred solution of **28** (13.5 mg, 0.042 mmol, 1 equiv) in methanol (1.6 mL) and H_2O (4 mL) were added sodium hydroxide (5 mg, 0.126 mmol, 3.00 equiv) at 50 °C. After 3 h, 1M aqueous hydrochloric acid solution (3mL) was added to the reaction mixture, and the reaction mixture was extracted with ethyl acetate (3 × 8 mL). The combined organic layers were washed with brine (2 × 25 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resulting crude residue was purified by flash column chromatography (silica gel: diam. 1.5 cm, ht. 8 cm; eluent: ethyl acetate : acetic acid = 100 :1) to afford **29** (8.8 mg, 68 %) as a white solid.

^{1}H NMR (599.1 MHz, $(CD_3)_2SO$) δ 12.68 (br, 1H), 10.30 (s, 1H), 8.49 (s, 1H), 8.10 (d, *J* = 8.0 Hz, 1H), 7.83 (d, *J* = 7.3 Hz, 1H), 7.46 (app-t, *J* = 7.5 Hz, 1H), 3.99 (s, 2H), 2.70 (s, 3H), 2.44 (s, 3H).

^{13}C NMR (100.6 MHz, $(CD_3)_2SO$) δ 192.9, 175.8, 171.8, 155.8, 153.8, 145.6, 137.3, 130.5, 128.7, 127.5, 125.7, 124.7, 124.4, 120.6, 118.5, 35.6, 15.3, 11.1.

HRMS (ESI): Calculated for $C_{18}H_{14}O_5$ $[M+H]^+$: 311.0914, found: 311.0901.



28 **30**

Methyl 2-(7-(hydroxymethyl)-5,6-dimethyl-9-oxo-9*H*-xanthen-4-yl)acetate (30):

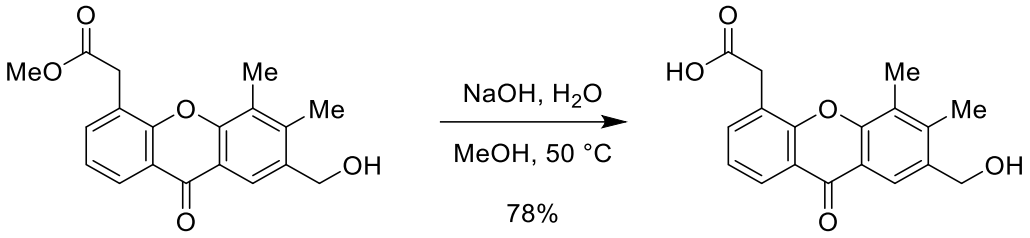
To a stirred solution of **28** (30 mg, 0.092 mmol, 1 equiv) in methanol (3 mL) was added sodium borohydride ($NaBH_4$, 3.8 mg, 0.10 mmol, 1.1 equiv) at 23 °C. After 1 h, 1M aqueous hydrochloric acid solution (3 mL) was added to the reaction mixture, and the reaction mixture was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine (2 × 30 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resulting crude residue was purified by flash column chromatography (silica gel: diam. 2.5 cm, ht. 12 cm; eluent: ethyl acetate : hexanes = 1 : 1) to afford **30** (17.3 mg, 57%) as a white solid.

^{1}H NMR (599.1 MHz, $CDCl_3$) δ 8.22 (dd, *J* = 7.9, 1.4 Hz, 1H), 8.06 (s, 1H), 7.61 (dd, *J* = 7.2, 1.4 Hz, 1H), 7.32 (app-t, *J* = 7.6 Hz, 1H), 4.75 (s, 2H), 3.96 (s, 2H), 3.71 (s, 3H), 2.37 (s, 3H), 2.36 (s, 3H), 2.19 (br, 1H).

^{13}C NMR (150.7 MHz, $CDCl_3$) δ 177.5, 171.2, 154.2, 153.6, 143.8, 136.1, 135.5, 126.2, 126.1, 123.9, 123.7, 123.1, 121.7, 118.9, 64.1, 52.4, 35.9, 16.0, 11.7.

HRMS (ESI): Calculated for $C_{19}H_{18}O_5$ $[M+Na]^+$: 349.1046, found: 349.1045.

TLC (ethyl acetate) R*f*: 0.60 (UV).



30 **3**

2-(7-(hydroxymethyl)-5,6-dimethyl-9-oxo-9*H*-xanthen-4-yl)acetic acid (3):

To a stirred solution of **30** (15 mg, 0.046 mmol, 1 equiv) in methanol (1.6 mL) and H_2O (4 mL) were added sodium hydroxide (5.5 mg, 0.138 mmol, 3.00 equiv) at 50 °C. After 1 h, 1M aqueous hydrochloric acid solution (5mL) was added to the reaction mixture, and the reaction mixture was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine (2 × 30 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resulting crude residue was purified by flash column chromatography (silica gel: diam. 1.5 cm, ht. 10 cm; eluent: ethyl acetate : acetic acid = 100 :1) to afford **3** (11.4 mg, 78%) as a white solid.

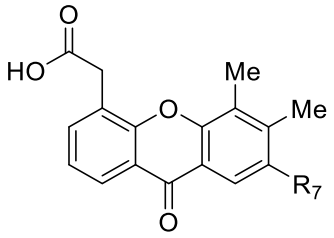
^{1}H NMR (599.1 MHz, $(CD_3)_2SO$) δ 12.59 (br, 1H), 8.09 (dd, *J* = 8.0, 1.7 Hz, 1H), 8.05 (s, 1H), 7.78 (dd, *J* = 7.4, 1.7 Hz, 1H), 7.40 (app-t, *J* = 7.6 Hz, 1H), 5.33 (br, 1H), 4.60 (s, 2H), 3.97 (s, 2H), 2.42 (s, 3H), 2.32 (s, 3H).

^{13}C NMR (150.7 MHz, $(CD_3)_2SO$) δ 176.2, 171.8, 153.8, 152.4, 142.6, 136.7, 136.4, 125.3, 125.1, 124.6, 123.5, 120.6, 120.6, 118.0, 61.2, 35.5, 15.1, 11.3.

HRMS (ESI): Calculated for $C_{18}H_{16}O_5$ $[M+Na]^+$: 335.0890, found: 335.0871.

Molecular Docking

By using AutoDock 4.2[3], docking study was performed. Ligands were drawn in MarvinSketch ver 18.15 (ChemAxon)[4] and converted to 3D structure through Clean3D. The energy-minimized structures of ligands were saved in PDB format. Then each molecule was loaded on to AutoDock Tools, torsions were set and saved it in PDBQT format. Based on 4QXR.pdb (hSTING mutant S162A, G230I, Q266I), 4QXP.pdb (hSTING mutant G230I), 4QXQ.pdb (hSTING mutant S162A, Q266I), human STING closed form was generated and converted it in PDBQT format. All calculations for protein-ligand docking were performed using the Lamarckian Genetic Algorithm (LGA) method. A grid box with the points of X: 70, Y: 80, Z: 80 and the grid center coordinates are X: 10.663, -77.551, -58.929, with a default grid spacing of 0.375 Å was used. The best conformation was chosen with the lowest docked energy, after the docking search was completed.



Name	R7	ΔG (kcal/mol)
1	H	–9.04
2	OH	–9.51
3	CH_2OH	–9.18
4	C_2H_4OH	–9.88

Table S1. Gibbs free energy of the formation of ligand + hSTING complex calculated by molecular docking

[3] Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S. and Olson, A. J. *J. Computational Chemistry* 2009, **16**: 2785-91.

[4] MarvinSketch (version 18.15), calculation module developed by ChemAxon, http://www.chemaxon.com/products/marvin/marvinsketch/, 2018

Expression and purification of STING-CTD

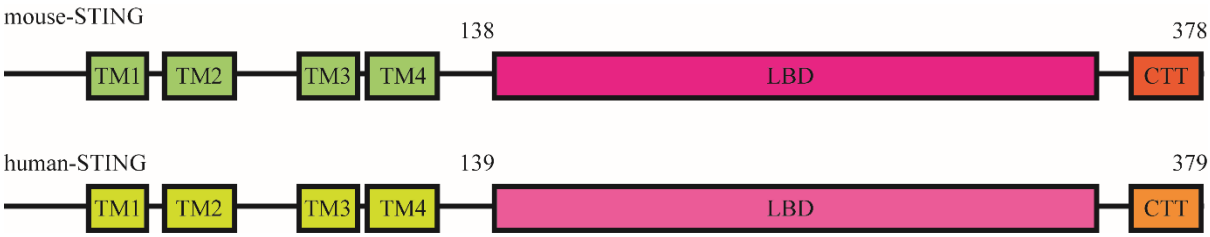


Fig. S1 Domains of mouse-STING and human-STING

The gene encoding mouse STING-CTD (residues 138-378) was cloned into pET15b vector using NdeI and BamHI restriction sites. E. coli BL21(DE3) harboring pET15b-mSTING-CTD were induced with 0.4 mM IPTG for 5 hours at 30℃. Cultures were pelleted and resuspended in lysis buffer (50 mM Sodium phosphate buffer (pH 8.0), 300 mM NaCl, 10 mM imidazole). The suspension was lysed by sonication. After centrifugation, the supernatant was loaded onto a Ni-NTA column (Qiagen) and washed with washing buffer (50 mM Sodium phosphate buffer (pH 8.0), 300 mM NaCl, 20 mM imidazole). The protein was eluted by elution buffer (50 mM Sodium phosphate buffer (pH 8.0), 300 mM NaCl, 250 mM imidazole) and dialyzed using 20mM Tris-Cl (pH 7.5) buffer. The protein sample was loaded onto Q-HP column(GE Healthcare) equipped in AKTA machine. The peak fraction was eluted by a 0-1 M linear gradient of NaCl. The protein was further purified by Superdex-75 gel filtration chromatography using final buffer (20 mM Tris-Cl (pH 7.5), 50 mM NaCl). The purity and homogeneity of the sample were assessed by using SDS-PAGE.

C-terminal domain of human STING (residues 139-379) was cloned into 2S-T vector by ligation-independent cloning. This expression plasmid was transformed into the BL21(DE3)RIL strain. Cells were grown in LB medium at 37℃ and induced with 0.4mM IPTG when OD_{600} reached 0.6. Sixteen hours incubation at 18℃, the cells were harvested by centrifugation. Cell pellet was resuspended in the buffer containing 50 mM Sodium phosphate buffer (pH 8.0), 300 mM NaCl, 10 mM imidazole. After sonication and centrifugation, the protein was purified using His-Bind resin (Qiagen). The SUMO-tag was then removed by digesting the proteins using TEV protease at 37℃, 2 hours. Ion exchange chromatography and gel filtration chromatography was performed as described for mouse STING-CTD.

Differential scanning fluorimetry

Thermal shift assays were performed using a CFX 96 Real-Time System (Bio-Rad). Fluorescence signal as a function of temperature was recoded with the excitation and emission wavelengths of 470 and 605nm, respectively. The temperature gradient was set from 18℃ to 80℃, with an increment of 0.5℃ and incubation steps of 15 seconds. Each 20 μL reaction (buffer : 20mM Tris-Cl (pH 7.5), 150 mM NaCl), with or without 2 mM of chemicals contained 1mg/ml of STING-CTD and a dilution of 1:500 of SYPRO Orange dye (Sigma).

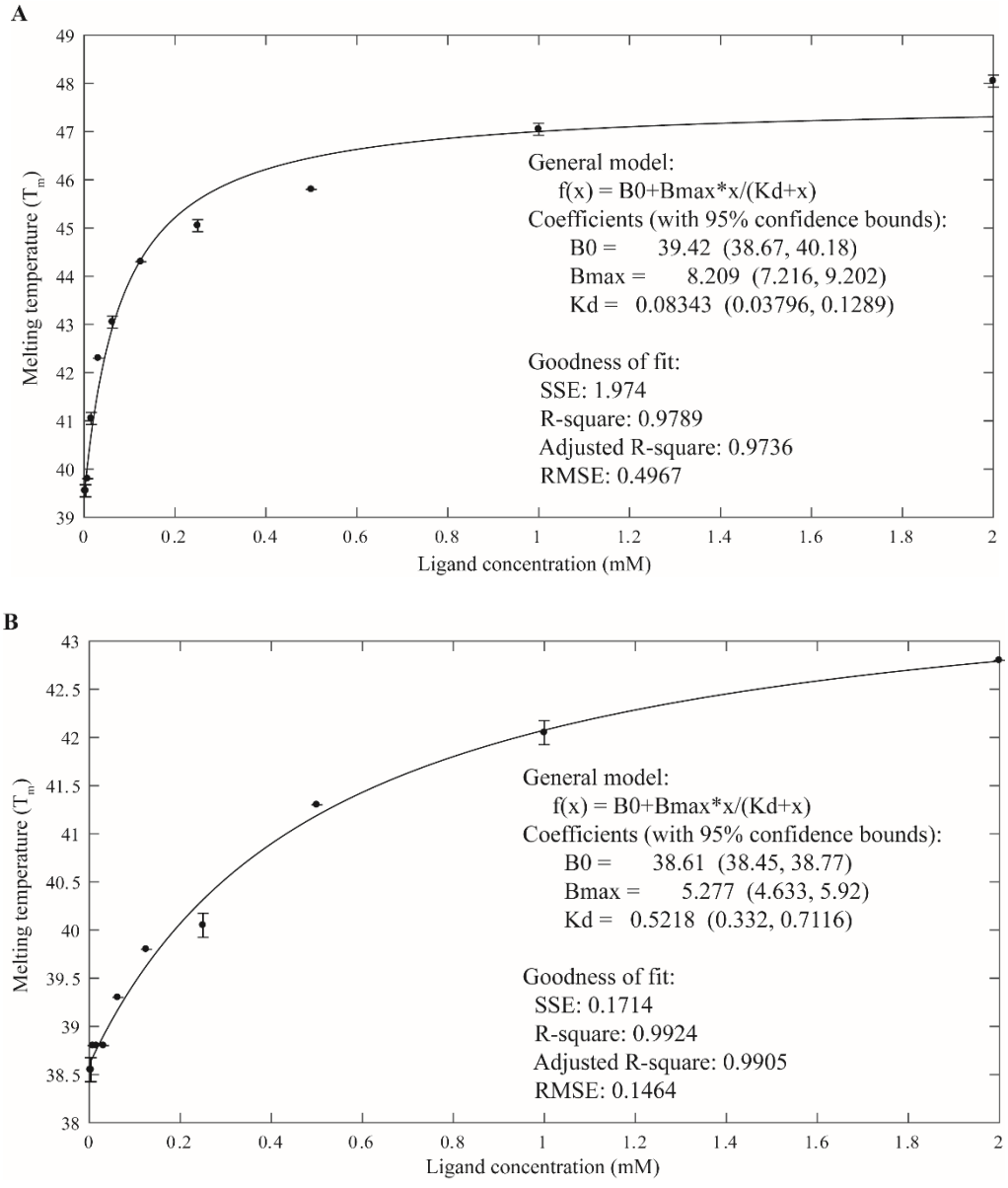
	ΔTm(℃)
cGAMP	13.50
c-di-GMP	5.83
c-di-AMP	9.50
1	8.67
2	3.33
3	1.00
4	-1.00
20	8.67
24	-0.83
25	-12.83
26	-5.83
27	-2.83
29	-1.17

Table S2. Melting temperature of mouse-STING with various ligands

The dissociation constant (Kd) for the interactions between mouse-STING and selected DMXAA derivatives was determined by using the following equation:

$$y=B0+Bmax*x/(Kd+x)$$

where B0 and Bmax are melting temperature of mouse-STING in the absence and presence of DMXAA derivatives, respectively, and x is ligand concentration (mM).[5]



[5] Vivoli, M., Novak, H.R., Littlechild, J.A., Harmer, N.J. *J. Vis. Exp.* 2014, **91**, e51809

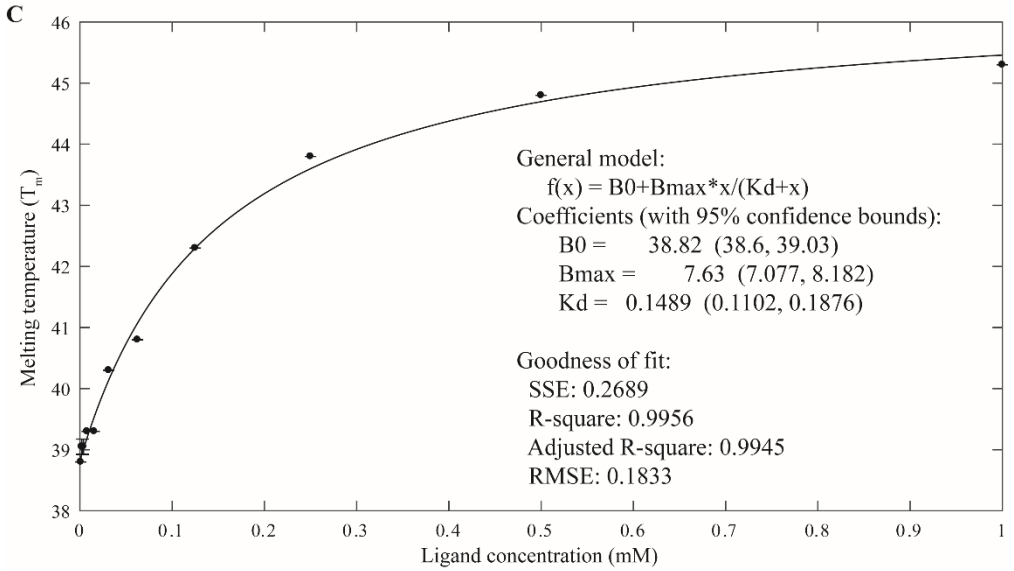


Fig. S2 Differential scanning fluorimetry of mouse-STING as a function of (A) compound **1** (DMXAA), (B) compound **2** (7-hydroxy-DMXAA), and (C) compound **20** (7-bromo-DMXAA) concentrations

Isothermal Calorimetry

Isothermal Calorimetry between STING and DMXAA derivatives were measured using an VP-ITC calorimeter (MicroCal). Titrations of STING with the selected chemicals were carried out at 298 K in assay buffer consisting of 20 mM Tris-Cl (pH 7.5), 150 mM NaCl. The concentration of STING in the cell was 20 μM and that of chemicals in the syringe was 200 μM. 10 μL of the chemicals was injected into the cell a total of 30 times, with a time lag of 240 seconds between each injection. Data analysis was performed with Origin 7.0 (MicroCal).

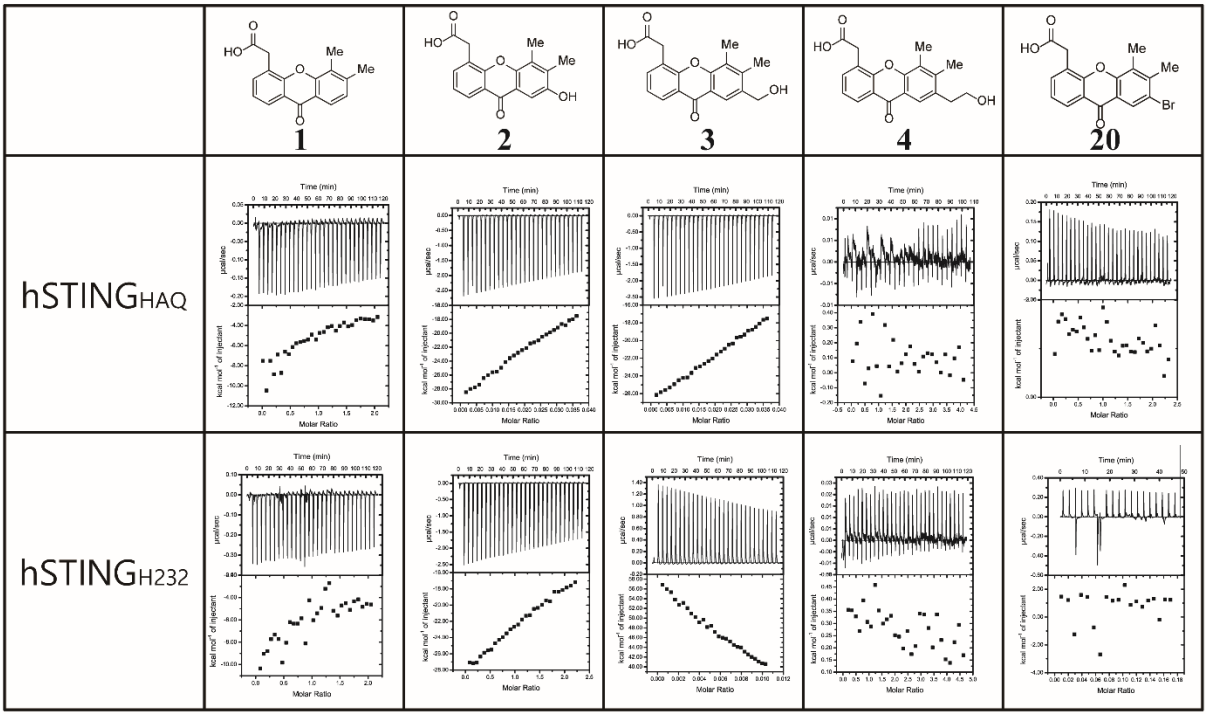
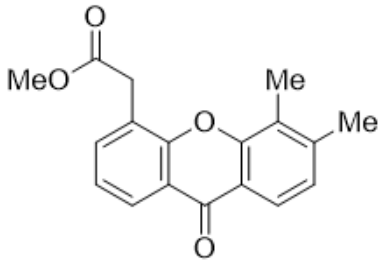


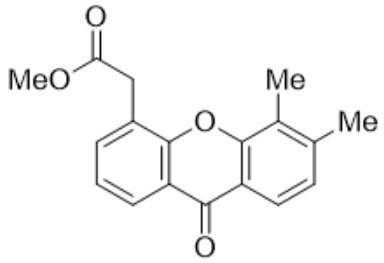
Fig. S3 ITC results of human STING and DMXAA derivatives

	Parameter	Value
1	Origin	Varian
2	Instrument	vnmrs
3	Solvent	cdcl3
4	Temperature	25.0
5	Pulse Sequence	s2pul
6	Experiment	1D
7	Probe	oneprobe
8	Number of Scans	16
9	Receiver Gain	18
10	Relaxation Delay	1.0000
11	Pulse Width	3.6000
12	Acquisition Time	1.7039
13	Spectrometer Frequency	599.13
14	Spectral Width	9615.4
15	Lowest Frequency	-1212.9
16	Nucleus	1H
17	Acquired Size	16384
18	Spectral Size	65536



21

Parameter	Value
1 Origin	Varian
2 Instrument	vnmrs
3 Solvent	cdcl3
4 Temperature	25.0
5 Pulse Sequence	s2pul
6 Experiment	1D
7 Probe	oneprobe
8 Number of Scans	512
9 Receiver Gain	42
10 Relaxation Delay	2.0000
11 Pulse Width	3.6250
12 Acquisition Time	0.8651
13 Spectrometer Frequency	150.67
14 Spectral Width	37878.8
15 Lowest Frequency	-2332.5
16 Nucleus	13C
17 Acquired Size	32768
18 Spectral Size	65536



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	Parameter	Value
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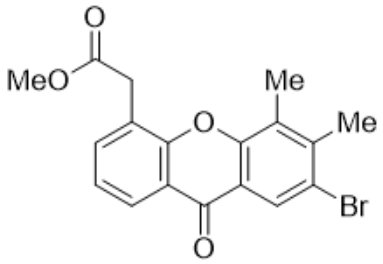
2	Instrument	vnmrs
3	Solvent	cdcl3
4	Temperature	25.0
5	Pulse Sequence	s2pul
6	Experiment	1D
7	Probe	oneprobe
8	Number of Scans	16
9	Receiver Gain	18
10	Relaxation Delay	1.0000
11	Pulse Width	3.6000
12	Acquisition Time	1.7039
13	Spectrometer Frequency	599.13
14	Spectral Width	9615.4
15	Lowest Frequency	-1223.5
16	Nucleus	1H
17	Acquired Size	16384
18	Spectral Size	65536



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	Parameter	Value
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6	Experiment	1D
7	Probe	oneprobe
8	Number of Scans	512
9	Receiver Gain	60
10	Relaxation Delay	2.0000
11	Pulse Width	3.6250
12	Acquisition Time	0.8651
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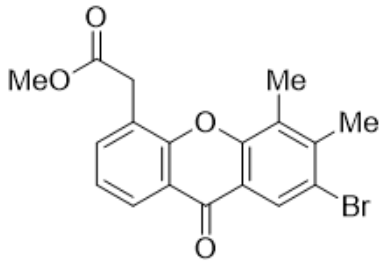
15	Lowest Frequency	-2367.8
16	Nucleus	13C
17	Acquired Size	32768
18	Spectral Size	65536



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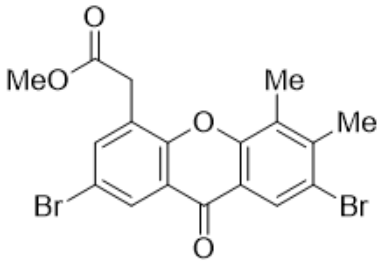
	Parameter	Value
1	Origin	Varian
2	Instrument	vnmrs
3	Solvent	cdcl3
4	Temperature	25.0
5	Pulse Sequence	s2pul
6	Experiment	1D
7	Probe	oneprobe
8	Number of Scans	16
9	Receiver Gain	18
10	Relaxation Delay	1.0000
11	Pulse Width	3.6000
12	Acquisition Time	1.7039
13	Spectrometer Frequency	599.13
14	Spectral Width	9615.4
15	Lowest Frequency	-1223.9
16	Nucleus	1H
17	Acquired Size	16384
18	Spectral Size	65536



23

175.33
170.45
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152.96
144.36
139.08
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127.73
127.40
126.36
122.83
121.61
120.43
117.09
52.62
35.72
20.95
13.09

	Parameter	Value
1	Origin	Varian
2	Instrument	vnmrs
3	Solvent	cdcl3
4	Temperature	25.0
5	Pulse Sequence	s2pul
6	Experiment	1D
7	Probe	oneprobe
8	Number of Scans	2500
9	Receiver Gain	60
10	Relaxation Delay	2.0000
11	Pulse Width	3.6250
12	Acquisition Time	0.8651
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15	Lowest Frequency	-2367.8
16	Nucleus	13C
17	Acquired Size	32768
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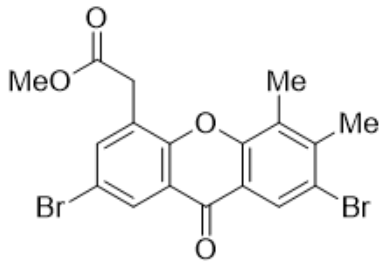

23

	Parameter	Value
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7	Probe	oneprobe
8	Number of Scans	16
9	Receiver Gain	18
10	Relaxation Delay	1.0000
11	Pulse Width	3.6000
12	Acquisition Time	1.7039
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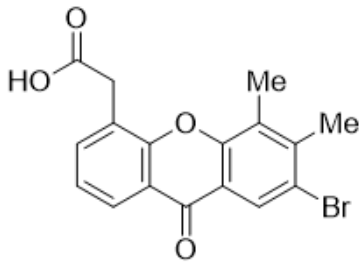

20

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152.18
143.31
136.96
128.02
125.68
125.61
124.54
123.96
120.29
120.06
119.74
35.60
20.32
12.48

	Parameter	Value
1	Origin	Varian
2	Instrument	vnmrs
3	Solvent	dmso
4	Temperature	25.0
5	Pulse Sequence	s2pul
6	Experiment	1D
7	Probe	oneprobe
8	Number of Scans	2500
9	Receiver Gain	60
10	Relaxation Delay	2.0000
11	Pulse Width	3.6250
12	Acquisition Time	0.8651
13	Spectrometer Frequency	150.67
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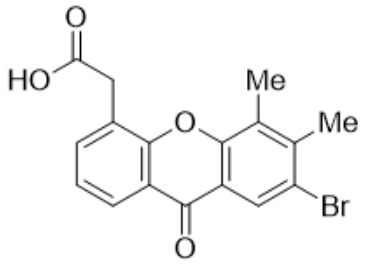
15	Lowest Frequency	-2439.8
16	Nucleus	13C
17	Acquired Size	32768
18	Spectral Size	65536



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	Parameter	Value
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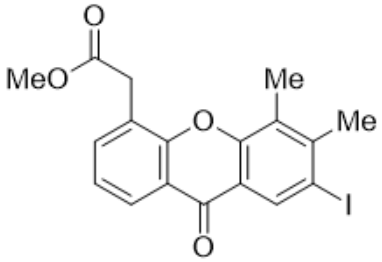
5	Pulse Sequence	s2pul
6	Experiment	1D
7	Probe	oneprobe
8	Number of Scans	16
9	Receiver Gain	18
10	Relaxation Delay	1.0000
11	Pulse Width	3.6000
12	Acquisition Time	1.7039
13	Spectrometer Frequency	599.13
14	Spectral Width	9615.4
15	Lowest Frequency	-1212.9
16	Nucleus	1H
17	Acquired Size	16384
18	Spectral Size	65536



24

176.30 171.11 154.30 153.94 146.59 136.50 134.28 126.73 126.34 124.03 123.96 121.68 121.08 96.49 52.44 36.03 26.51 13.50

	Parameter	Value
1	Origin	Varian
2	Instrument	vnmrs
3	Solvent	cdcl3
4	Temperature	25.0
5	Pulse Sequence	s2pul
6	Experiment	1D
7	Probe	oneprobe
8	Number of Scans	512
9	Receiver Gain	60
10	Relaxation Delay	2.0000
11	Pulse Width	3.6250
12	Acquisition Time	0.8651
13	Spectrometer Frequency	150.67
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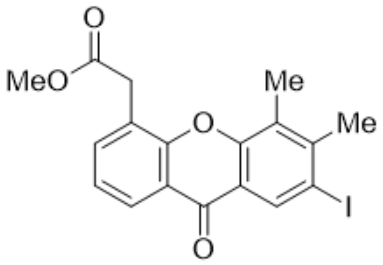
15	Lowest Frequency	-2332.5
16	Nucleus	13C
17	Acquired Size	32768
18	Spectral Size	65536



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	Parameter	Value
1	Origin	Varian
2	Instrument	vnmrs
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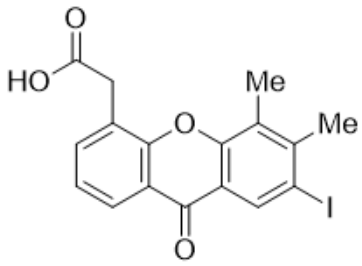
4	Temperature	25.0
5	Pulse Sequence	s2pul
6	Experiment	1D
7	Probe	oneprobe
8	Number of Scans	6
9	Receiver Gain	18
10	Relaxation Delay	1.0000
11	Pulse Width	3.6000
12	Acquisition Time	1.7039
13	Spectrometer Frequency	599.14
14	Spectral Width	9615.4
15	Lowest Frequency	-1198.6
16	Nucleus	1H
17	Acquired Size	16384
18	Spectral Size	65536



25

Parameter	Value
1 Origin	Varian
2 Instrument	vnmrs
3 Solvent	dmso
4 Temperature	25.0
5 Pulse Sequence	s2pul
6 Experiment	1D
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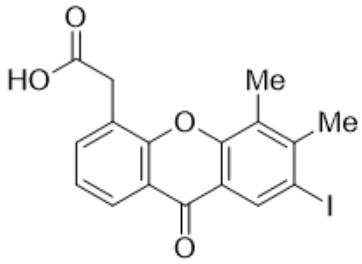
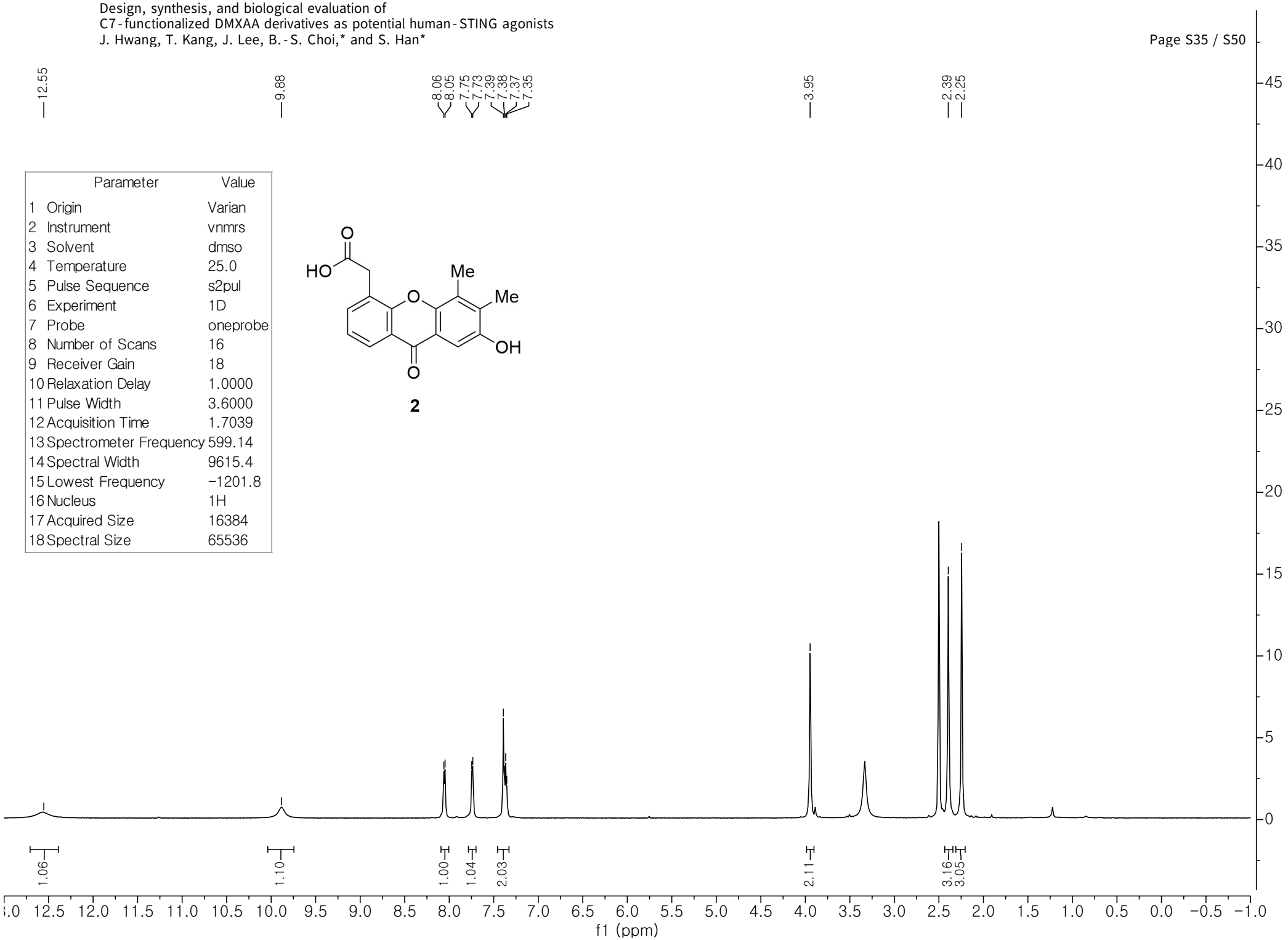
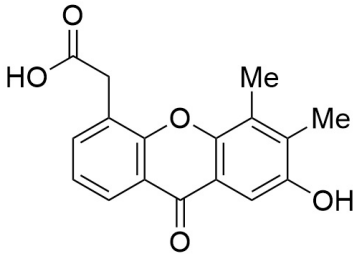
8 Number of Scans	512
9 Receiver Gain	60
10 Relaxation Delay	2.0000
11 Pulse Width	3.6250
12 Acquisition Time	0.8651
13 Spectrometer Frequency	150.67
14 Spectral Width	37878.8
15 Lowest Frequency	-2443.4
16 Nucleus	13C
17 Acquired Size	32768
18 Spectral Size	65536



25

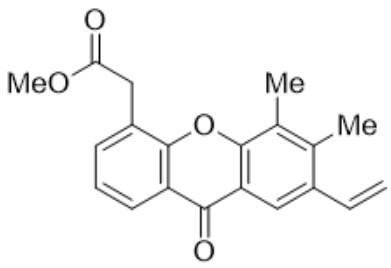

12.55
9.88
8.06
8.05
7.75
7.73
7.39
7.38
7.37
7.35
3.95
2.39
2.25
Parameter Value
1 Origin Varian
2 Instrument vnmrs
3 Solvent dmso
4 Temperature 25.0
5 Pulse Sequence s2pul
6 Experiment 1D
7 Probe oneprobe
8 Number of Scans 16
9 Receiver Gain 18
10 Relaxation Delay 1.0000
11 Pulse Width 3.6000
12 Acquisition Time 1.7039
13 Spectrometer Frequency 599.14
14 Spectral Width 9615.4
15 Lowest Frequency -1201.8
16 Nucleus 1H
17 Acquired Size 16384
18 Spectral Size 65536
HO
O
Me
Me
O
OH
O
2
1.06
1.10
1.00
1.04
2.03
2.11
3.16
3.05
f1 (ppm)

	Parameter	Value
1	Origin	Varian
2	Instrument	vnmrs
3	Solvent	dmso
4	Temperature	25.0
5	Pulse Sequence	s2pul
6	Experiment	1D
7	Probe	oneprobe
8	Number of Scans	512
9	Receiver Gain	60
10	Relaxation Delay	2.0000
11	Pulse Width	3.6250
12	Acquisition Time	0.8651
13	Spectrometer Frequency	150.67
14	Spectral Width	37878.8
15	Lowest Frequency	-2441.2
16	Nucleus	13C
17	Acquired Size	32768
18	Spectral Size	65536



2

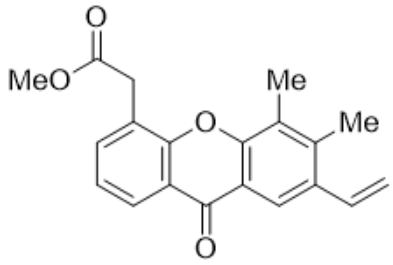
	Parameter	Value
1	Origin	Varian
2	Instrument	vnmrs
3	Solvent	cdcl3
4	Temperature	25.0
5	Pulse Sequence	s2pul
6	Experiment	1D
7	Probe	oneprobe
8	Number of Scans	16
9	Receiver Gain	18
10	Relaxation Delay	1.0000
11	Pulse Width	3.6000
12	Acquisition Time	1.7039
13	Spectrometer Frequency	599.13
14	Spectral Width	9615.4
15	Lowest Frequency	-1223.8
16	Nucleus	1H
17	Acquired Size	16384
18	Spectral Size	65536



26

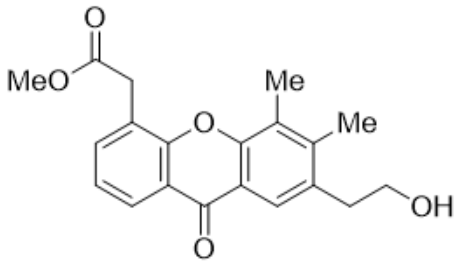
177.62 171.20 154.35 153.68 142.28 136.10 134.84 134.52 126.31 125.48 123.92 123.64 121.79 121.23 119.56 117.41 52.40 36.05 16.82 12.13

	Parameter	Value
1	Origin	Varian
2	Instrument	vnmrs
3	Solvent	cdcl3
4	Temperature	25.0
5	Pulse Sequence	s2pul
6	Experiment	1D
7	Probe	oneprobe
8	Number of Scans	512
9	Receiver Gain	48
10	Relaxation Delay	2.0000
11	Pulse Width	3.6250
12	Acquisition Time	0.8651
13	Spectrometer Frequency	150.67
14	Spectral Width	37878.8
15	Lowest Frequency	-2332.5
16	Nucleus	13C
17	Acquired Size	32768
18	Spectral Size	65536



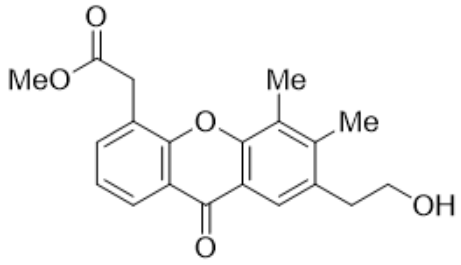
26

	Parameter	Value
1	Origin	Varian
2	Instrument	vnmrs
3	Solvent	cdcl3
4	Temperature	25.0
5	Pulse Sequence	s2pul
6	Experiment	1D
7	Probe	oneprobe
8	Number of Scans	16
9	Receiver Gain	18
10	Relaxation Delay	1.0000
11	Pulse Width	3.6000
12	Acquisition Time	1.7039
13	Spectrometer Frequency	599.13
14	Spectral Width	9615.4
15	Lowest Frequency	-1224.3
16	Nucleus	1H
17	Acquired Size	16384
18	Spectral Size	65536



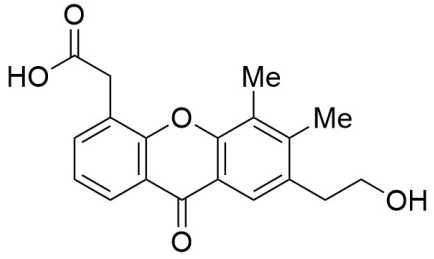
27

	Parameter	Value
1	Origin	Varian
2	Instrument	vnmrs
3	Solvent	cdcl3
4	Temperature	25.0
5	Pulse Sequence	s2pul
6	Experiment	1D
7	Probe	oneprobe
8	Number of Scans	512
9	Receiver Gain	60
10	Relaxation Delay	2.0000
11	Pulse Width	3.6250
12	Acquisition Time	0.8651
13	Spectrometer Frequency	150.67
14	Spectral Width	37878.8
15	Lowest Frequency	-2367.8
16	Nucleus	13C
17	Acquired Size	32768
18	Spectral Size	65536



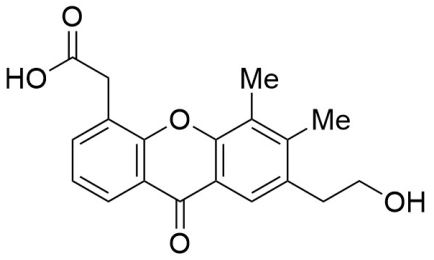
27

	Parameter	Value
1	Origin	Varian
2	Instrument	vnmrs
3	Solvent	dmso
4	Temperature	25.0
5	Pulse Sequence	s2pul
6	Experiment	1D
7	Probe	oneprobe
8	Number of Scans	16
9	Receiver Gain	18
10	Relaxation Delay	1.0000
11	Pulse Width	3.6000
12	Acquisition Time	1.7039
13	Spectrometer Frequency	599.14
14	Spectral Width	9615.4
15	Lowest Frequency	-1212.9
16	Nucleus	1H
17	Acquired Size	16384
18	Spectral Size	65536



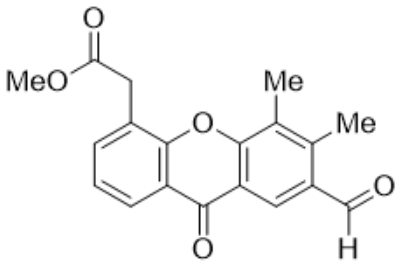
4

	Parameter	Value
1	Origin	Varian
2	Instrument	vnmrs
3	Solvent	dmso
4	Temperature	25.0
5	Pulse Sequence	s2pul
6	Experiment	1D
7	Probe	oneprobe
8	Number of Scans	500
9	Receiver Gain	54
10	Relaxation Delay	2.0000
11	Pulse Width	3.6250
12	Acquisition Time	0.8651
13	Spectrometer Frequency	150.67
14	Spectral Width	37878.8
15	Lowest Frequency	-2439.0
16	Nucleus	13C
17	Acquired Size	32768
18	Spectral Size	65536



4

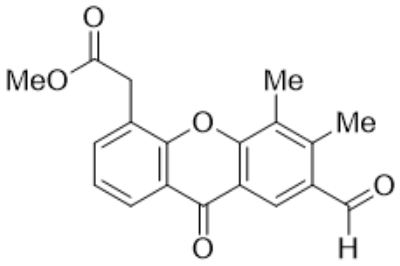
Parameter	Value
1 Origin	Varian
2 Instrument	vnmrs
3 Solvent	cdcl3
4 Temperature	25.0
5 Pulse Sequence	s2pul
6 Experiment	1D
7 Probe	oneprobe
8 Number of Scans	16
9 Receiver Gain	18
10 Relaxation Delay	1.0000
11 Pulse Width	3.6000
12 Acquisition Time	1.7039
13 Spectrometer Frequency	599.13
14 Spectral Width	9615.4
15 Lowest Frequency	-1223.9
16 Nucleus	1H
17 Acquired Size	16384
18 Spectral Size	65536



28

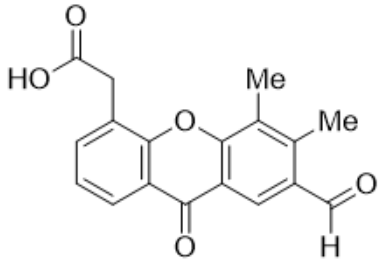
192.48 176.80 171.01 156.69 154.30 145.79 136.88 132.08 131.10 127.93 126.44 124.59 124.18 121.87 119.49 52.51 36.00 16.24 11.69

	Parameter	Value
1	Origin	Varian
2	Instrument	vnmrs
3	Solvent	cdcl3
4	Temperature	25.0
5	Pulse Sequence	s2pul
6	Experiment	1D
7	Probe	oneprobe
8	Number of Scans	512
9	Receiver Gain	60
10	Relaxation Delay	2.0000
11	Pulse Width	3.6250
12	Acquisition Time	0.8651
13	Spectrometer Frequency	150.67
14	Spectral Width	37878.8
15	Lowest Frequency	-2367.8
16	Nucleus	13C
17	Acquired Size	32768
18	Spectral Size	65536



28

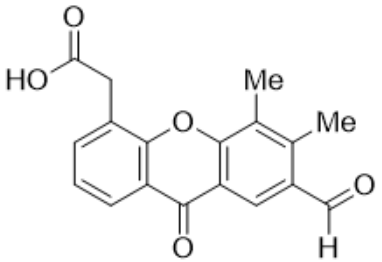
	Parameter	Value
1	Origin	Varian
2	Instrument	vnmrs
3	Solvent	dmso
4	Temperature	25.0
5	Pulse Sequence	s2pul
6	Experiment	1D
7	Probe	oneprobe
8	Number of Scans	16
9	Receiver Gain	18
10	Relaxation Delay	1.0000
11	Pulse Width	3.6000
12	Acquisition Time	1.7039
13	Spectrometer Frequency	599.13
14	Spectral Width	9615.4
15	Lowest Frequency	-1418.2
16	Nucleus	1H
17	Acquired Size	16384
18	Spectral Size	65536



29

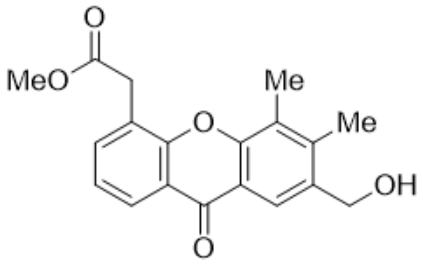
192.87, 175.83, 171.77, 155.75, 153.78, 145.56, 137.26, 130.46, 128.72, 127.49, 125.69, 124.71, 124.44, 120.61, 118.52, 35.55, 15.28, 11.14

Parameter	Value
1 Origin	Bruker BioSpin GmbH
2 Instrument	spect
3 Solvent	DMSO
4 Temperature	298.0
5 Pulse Sequence	zgpg30
6 Experiment	1D
7 Probe	Z116098_0402 (PA BBO 400S1 BBF-H-D-05 Z SP)
8 Number of Scans	4700
9 Receiver Gain	207.1
10 Relaxation Delay	2.0000
11 Pulse Width	10.0000
12 Acquisition Time	1.3631
13 Spectrometer Frequency	100.62
14 Spectral Width	24038.5
15 Lowest Frequency	-1958.2
16 Nucleus	13C
17 Acquired Size	32768
18 Spectral Size	65536



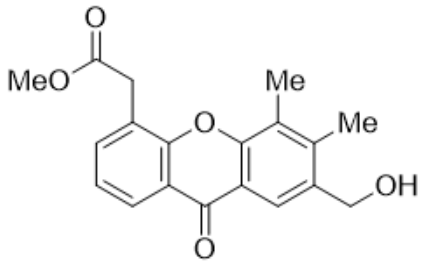
29

	Parameter	Value
1	Origin	Varian
2	Instrument	vnmrs
3	Solvent	cdcl3
4	Temperature	25.0
5	Pulse Sequence	s2pul
6	Experiment	1D
7	Probe	oneprobe
8	Number of Scans	16
9	Receiver Gain	18
10	Relaxation Delay	1.0000
11	Pulse Width	3.6000
12	Acquisition Time	1.7039
13	Spectrometer Frequency	599.13
14	Spectral Width	9615.4
15	Lowest Frequency	-1223.7
16	Nucleus	1H
17	Acquired Size	16384
18	Spectral Size	65536



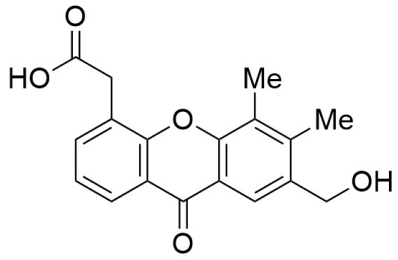
30

	Parameter	Value
1	Origin	Varian
2	Instrument	vnmrs
3	Solvent	cdcl3
4	Temperature	25.0
5	Pulse Sequence	s2pul
6	Experiment	1D
7	Probe	oneprobe
8	Number of Scans	204
9	Receiver Gain	60
10	Relaxation Delay	2.0000
11	Pulse Width	3.6250
12	Acquisition Time	0.8651
13	Spectrometer Frequency	150.67
14	Spectral Width	37878.8
15	Lowest Frequency	-2367.8
16	Nucleus	13C
17	Acquired Size	32768
18	Spectral Size	65536



30

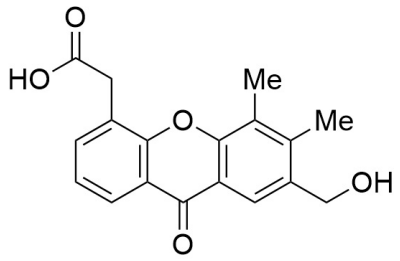
	Parameter	Value
1	Origin	Varian
2	Instrument	vnmrs
3	Solvent	dmso
4	Temperature	25.0
5	Pulse Sequence	s2pul
6	Experiment	1D
7	Probe	oneprobe
8	Number of Scans	16
9	Receiver Gain	18
10	Relaxation Delay	1.0000
11	Pulse Width	3.6000
12	Acquisition Time	1.7039
13	Spectrometer Frequency	599.14
14	Spectral Width	9615.4
15	Lowest Frequency	-1202.0
16	Nucleus	1H
17	Acquired Size	16384
18	Spectral Size	65536



3

176.17
171.83
153.79
152.38
142.60
136.72
136.42
125.33
125.06
124.58
123.52
120.64
120.61
118.02
61.21
35.50
15.11
11.27

	Parameter	Value
1	Origin	Varian
2	Instrument	vnmrs
3	Solvent	dmso
4	Temperature	25.0
5	Pulse Sequence	s2pul
6	Experiment	1D
7	Probe	oneprobe
8	Number of Scans	400
9	Receiver Gain	60
10	Relaxation Delay	2.0000
11	Pulse Width	3.6250
12	Acquisition Time	0.8651
13	Spectrometer Frequency	150.67
14	Spectral Width	37878.8
15	Lowest Frequency	-2439.7
16	Nucleus	13C
17	Acquired Size	32768
18	Spectral Size	65536



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