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Chemistry & Drug Discovery

Supporting Information

Supporting Information

Importance of interaction between C1 domain and lipids in protein kinase Cα activation: Hydrophobic side chain direction in isobenzofuranone ligands controls enzyme activation level

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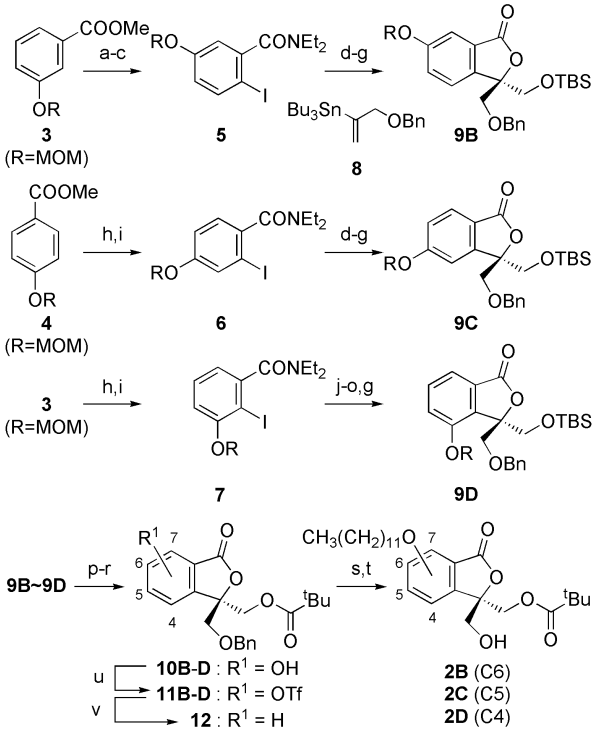
Chemistry

General: NMR spectra were recorded on a JEOL JNM-LA400 and a JEOL JNM-AL270 spectrometer, operating at 400 MHz for ^{1}H-NMR, 100.4 MHz and 67.8 MHz for ^{13}C-NMR. Chemical shifts were reported in the scale relative to $CDCl_3$ as an internal reference. MALDI-TOF/MS was taken on Bruker Daltonics Reflex III with matrix dimer and angiotensin I as internal standards. Optical rotations were measured on a JASCO DIP-370 polarimeter. IR was measured on Thermo Nicolet AVATAR 370 FT-IR. Column chromatography was performed with silica gel 60 (40-100 μm) purchased from KANTO CHEMICAL Co. Dehydrated stabilizer-free tetrahydrofuran (THF) was purchased from KANTO CHEMICAL Co., and was directly used.

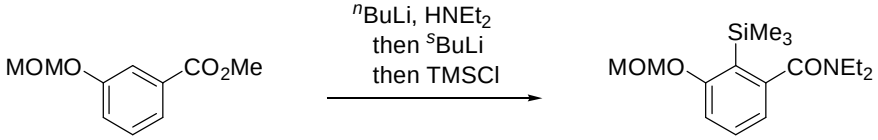
Synthesis of isobenzofuranone derivatives



Preparation of Type B-D derivatives. a) ^{n}BuLi, $HNEt_2$, THF, 0 °C, 1 h then ^{s}BuLi, -78 °C, 1 h then TMSCl, RT, 30 min; b) ^{s}BuLi, TMEDA, THF, -78 °C then I_2; c) TBAF, DMPU, 60 °C, 1 h (56%, 3 steps); d) $PdCl_2(PPh_3)_2$, LiCl, **8**, DMF, 90 °C; e) OsO_4, NMO, ^{t}BuOH, H_2O, RT; f) TBSCl, imidazole, DMF, RT (for **5**, 73%, 3 steps; for **6**, 73%, 3 steps); g) separation by HPLC (CHIRALPAK AD-H, hexane/^{i}PrOH = 95/5); h) ^{n}BuLi, $HNEt_2$, THF, 0 °C, 1 h; i) ^{s}BuLi, THF, -92 °C, 2 h then I_2 (for **4**, 67%, 2 steps; for **3**, 82%, 2 steps); j) $PdCl_2(PPh_3)_2$, LiCl, **8**, DMF, 90 °C; k) conc.HCl aq. MeOH, 40 °C, 18 h (38%, 2 steps); l) OsO_4, NMO, ^{t}BuOH, H_2O, RT; m) TBSCl, imidazole, DMF, RT (39%, 2 steps); n) K_2CO_3, MeOH, RT, 4 h; o) MOMCl, $^{i}Pr_2NEt$, CH_2Cl_2, RT, 1 h (90%, 2 steps); p) TBAF, THF, RT; q) ^{t}BuCOCl, DMAP, CH_2Cl_2, RT; r) HCl, MeOH, RT (for **10B**, 74%, 3 steps; for **10C**, 96%, 3 steps; for **10D**, 77%, 3 steps); s) $CH_3(CH_2)_{11}OH$, PPh_3, DEAD, THF, rt; t) $Pd(OH)_2$, H_2, AcOEt, RT (for **2B**, 79%, 2 steps; for **2C**, 97%, 2 steps; for **2D**,

52%, 2 steps); u) Tf_2O, Et_3N, CH_2Cl_2, -78 °C; v) $Pd(OAc)_2$, DPPB, Et_3N, HCOOH, 90 °C (from **10B**, 93%, 2 steps; from **10C**, 78%, 2 steps; from **10D**, 89%, 2 steps).

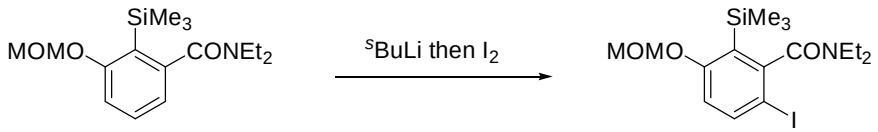
N,N-Diethyl-3-methoxymethoxy-2-trimethylsilylbenzamide (S1)



3 **S1**

To a solution of diethylamine (5.13 g, 70.2 mmol) in THF (150 mL) was added ^{n}BuLi (1.56 M in hexane, 41.5 mL, 64.7 mmol) at -78 °C. After stirring for 30 min at 0 °C, **3** (10.6 g, 54.0 mmol) in THF (20 mL) was added at -78 °C. After stirring for 1 h at 0 °C, ^{s}BuLi (0.99 M in hexane, 82 mL, 81.2 mmol) was added at -78 °C. After stirring for 1 h at -78 °C, TMSCl (17.6 g, 162 mmol) was added. After stirring for 30 min at room temperature, the reaction mixture was diluted with hexane / ethyl acetate (2:1), and a saturated aqueous solution of $NaHCO_3$. The organic layer was separated and washed with a saturated aqueous solution of NH_4Cl and brine. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. Further purification was carried out by silica gel column chromatography (eluent: hexane / ethyl acetate = 10 / 1) to give **S1** (12.6 g, 75 %) as a brown oil. IR (neat, cm^{-1}) ν 2978, 2896, 1631, 1555, 1428, 1286, 1237, 1153, 1028, 999, 842, 797, 758; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.27 (s, 9H), 1.07 (t, *J* = 7.3 Hz, 3H), 1.25 (t, *J* = 7.3 Hz, 3H), 3.1-3.7 (m, 4H), 3.48 (s, 3H), 5.18 (s, 2H), 6.78 (dd, *J* = 1.0 and 7.6 Hz, 1H), 7.05 (dd, *J* = 1.0 and 8.3 Hz, 1H), 7.29 (dd, *J* = 7.6 and 8.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 0.5 (3C), 12.8, 13.6, 38.9, 43.3, 56.1, 94.1, 112.5, 119.7, 124.6, 130.5, 144.6, 162.7, 171.7; MALDI-TOF/MS (positive ion, α-cyano-4-hydroxycinnamic acid) calcd. for $C_{16}H_{27}NO_3SiNa$ ($M+Na^+$) 332.17; found 332.21.

N,N-Diethyl-6-iodo-3-methoxymethoxy-2-trimethylsilylbenzamide (S2)

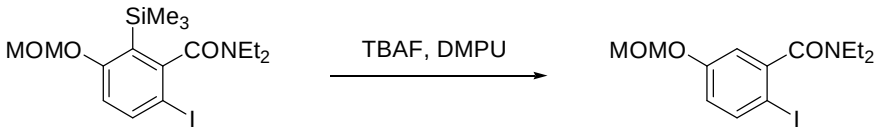


S1 **S2**

To a solution of TMEDA (457 mg, 3.93 mmol) and ^{s}BuLi (0.96 M in hexane, 4.1 mL, 3.94 mmol) in THF (5 mL) was added a solution of **S1** (811 mg, 2.62 mmol) in THF (5 mL) at -78 °C. After stirring for 1 h at -78 °C, iodine (1.33 g, 5.24 mmol) was added. After stirring for 4 h at room temperature, the reaction mixture was diluted with ether, and a saturated aqueous solution of $Na_2S_2O_3$ was added. The organic layer was separated and washed with brine. The organic layer was dried over $MgSO_4$ and concentrated under

reduced pressure. Further purification was carried out by silica gel column chromatography (eluent: hexane / ethyl acetate = 10 / 1) to give **S2** (852 mg, 75%) as a pale yellow solid. IR (neat, cm^{-1}) ν 2962, 2900, 1624, 1555, 1419, 1234, 1155, 1079, 1057, 1020, 991, 905, 838, 811, 767; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.27 (s, 9H), 1.12 (t, *J* = 7.3 Hz, 3H), 1.31 (t, *J* = 7.3 Hz, 3H), 3.0-3.2 (m, 2H), 3.22 (dq, *J* = 7.3 and 14.4 Hz, 1H), 3.46 (s, 3H), 3.94 (dq, *J* = 7.3 and 14.4 Hz, 1H), 5.15 (s, 2H), 6.81 (d, *J* = 8.8 Hz, 1H), 7.71 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 0.5 (3C), 12.0, 13.1, 38.9, 43.0, 56.2, 85.6, 94.2, 114.7, 127.6, 141.3, 147.6, 162.7, 170.1; MALDI-TOF/MS (positive ion, α-cyano-4-hydroxycinnamic acid) calcd. for $C_{16}H_{26}INO_3SiNa$ ($M+Na^+$) 458.06; found 458.03.

***N,N*-Diethyl-2-iodo-5-(methoxymethoxy)benzamide (5)**



S2 **5**

To a solution of **S2** (329 mg, 0.756 mmol) in DMPU (3.5 mL) was added a solution of 1 M TBAF in THF (3.8 mL, 3.78 mmol) at room temperature. After stirring for 1 h at 60 °C, the reaction mixture was diluted with ether, and water was added. The organic layer was separated and washed with water 3 times. The combined aqueous layers were extracted with ethyl acetate, and the combined organic layers were washed with water. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. Further purification was carried out by silica gel column chromatography (eluent: hexane / ethyl acetate = 5 / 1) to give **5** (300 mg, quant.) as a yellow oil; FT-IR (neat, cm^{-1}) ν 2970, 2920, 2896, 1629, 1458, 1434, 1292, 1268, 1228, 1153, 1079, 1025, 990, 921, 824; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.08 (t, *J* = 7.1 Hz, 3H), 1.28 (t, *J* = 7.1 Hz, 3H), 3.1-3.3 (m, 3H), 3.44 (s, 3H), 3.85 (m, 1H), 5.11 (d, *J* = 6.6 Hz, 1H), 5.18 (d, *J* = 6.6 Hz, 1H), 6.77 (dd, *J* = 2.9 and 8.5 Hz, 1H), 6.92 (d, *J* = 2.9 Hz, 1H) 7.66 (d, *J* = 8.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.4, 13.8, 38.9, 42.7, 56.0, 82.6, 94.3, 115.1, 118.3, 139.9, 143.7, 157.5, 169.6; MALDI-TOF/MS (positive ion, α-cyano-4-hydroxycinnamic acid) calcd. for $C_{13}H_{18}INO_3Na$ ($M+Na^+$) 386.02; found 386.00.

(1-(Benzyloxy)prop-2-en-2-yl)tributylstannane (8)

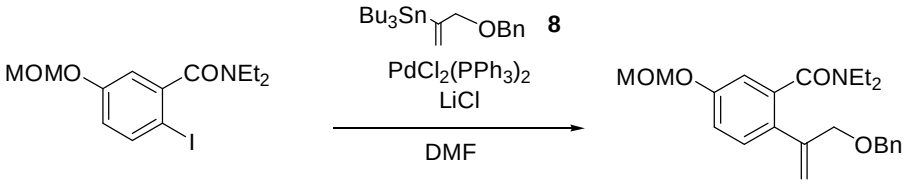
To a THF (300 mL) solution of methyl propiolate (7.9 g, 93.6 mmol) and $PdCl_2(PPh_3)_2$ (1.31 g, 1.87 mmol) was added tributyltin hydride (30 g, 103 mmol) dropwise with a syringe. After stirring for 4.5 h, solvent was removed under reduced pressure. Further purification was carried out by silica gel column chromatography (eluent: hexane / ethyl acetate = 100 / 1) to give methyl 2-tributylstannanylacrylate (24.7

g, 70%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.8-1.7 (m, 27H), 3.74 (s, 3H), 5.92 (d, *J* = 2.7 Hz, 1H), 6.89 (d, *J* = 2.7 Hz, 1H).

To a solution of methyl 2-tributylstannanylacrylate (24.7 g, 65.8 mmol) in hexane (300 mL) was added diisobutylaluminium hydride (0.93 M in hexane, 145 mL, 145 mmol) slowly at –100 °C under nitrogen atmosphere. After stirring for 3 h at –100 °C, the reaction mixture was diluted with ethyl acetate, and a saturated aqueous solution of potassium sodium tartrate was added. The resulting solution was extracted with hexane/ethyl acetate (3:1). The aqueous layer was extracted with hexane/ethyl acetate (3:1). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. Further purification was carried out by silica gel column chromatography (eluent: hexane / ethyl acetate = 80 / 1) to give 2-tributylstannanyl-3-propen-1-ol (13.4 g, 59%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.6-1.6 (m, 27H), 4.27 (brs, 2H), 5.24 (dt, *J* = 2.0 and 2.0 Hz, 1H), 5.87 (dt, *J* = 2.0 and 2.0 Hz, 1H).

To a solution of 2-tributylstannanyl-3-propen-1-ol (5.72 g, 16.5 mmol) and BnBr (5.64 g, 33 mmol) in THF / DMF (72 mL, 5:1, v/v) was added NaH (871 mg, 36.3 mmol) at 0 °C. After stirring for 2.5 h at room temperature, water and tetrabutylammonium iodide (609 mg 14.7 mmol) was added. The resulting solution was extracted with ether. The combined organic layers were washed with water, dried over $MgSO_4$, and concentrated under reduced pressure. Further purification was carried out by silica gel column chromatography (eluent: hexane) to give **8** (6.39 g, 88%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.8-1.6 (m, 27H), 4.15 (t, *J* = 2.0 Hz, 2H), 4.49 (s, 2H), 5.28 (dd, *J* = 2.0 and 2.0 Hz, 1H), 5.89 (dt, *J* = 2.0 and 2.0 Hz, 1H), 7.2-7.4 (m, 5H).

2-(1-Benzyloxyprop-2-en-2-yl)- *N*,*N*-diethyl-5-(methoxymethoxy)benzamide (S3)

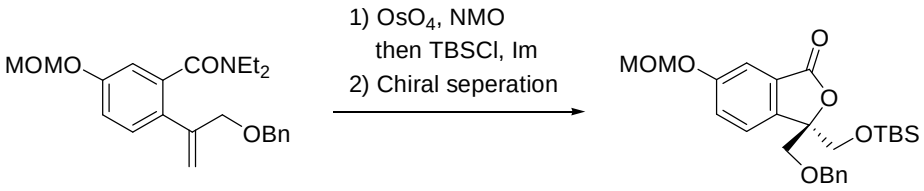


5 **S3**

After azeotropic evaporation of **5** (5.06 g, 13.9 mmol) and **8** (8.2 g, 18.7 mmol) with toluene, mixture was dissolved in DMF (20 mL). The solution was degassed by the four freeze-pump-thaw cycles, then under nitrogen atmosphere $PdCl_2(PPh_3)_2$ (1.47 g, 2.09 mmol) and LiCl (886 mg, 20.9 mmol) were added in one portion at room temperature. After stirring for 6.5 h at 90 °C, the reaction mixture was diluted with ether, and a saturated aqueous solution of $NaHCO_3$ was added. The organic layer was separated and washed with water. The combined aqueous layers were extracted with ether twice. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. Further purification was carried out by silica gel column chromatography (eluent: hexane / ethyl acetate = 5 / 1) to give **S3** (3.19 g,

mixture) as a brown oil. MALDI-TOF/MS (positive ion, α-cyano-4-hydroxycinnamic acid) calcd. for $C_{23}H_{29}NO_4Na$ ($M+Na^+$) 406.20; found 406.21.

3-Benzyloxymethyl-3-(*t*-butyldimethylsiloxymethyl)-6-methoxymethoxy-*3H*-isobenzofuran-1-one (9B)



S3 **9B**

To a solution of **S3** (3.19 g) and 50 wt% aqueous solution of NMO (5.9 mL, 25 mmol) in ^{t}BuOH / H_2O (1:1, 40 mL) was added a 20 mM solution of OsO_4 in ^{t}BuOH (7.5 mL, 0.15 mmol) at room temperature. After stirring for 59 h at room temperature, the reaction mixture was diluted with ethyl acetate, and solid $Na_2S_2O_3$ was added. The mixture was stirred at room temperature for a further 30 min. The mixture was extracted with ethyl acetate 3 times. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was dissolved in DMF, and imidazole (2.83 g, 41.6 mmol) and TBSCl (2.50 g, 16.6 mmol) were added at room temperature. After monitored by TLC, TBSCl (1.25 g, 8.8 mmol) was added furthermore. After stirring for 2.5 h at room temperature, the reaction mixture was diluted with MeOH and ether, and water was added. The organic layer was separated and washed with water. The combined aqueous layers were extracted with ether. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. Further purification was carried out by silica gel column chromatography (eluent: hexane / ethyl acetate = 15 / 1) to give *rac*-**9B** (1.73 g, 27 % in 3 steps) as a colorless oil. IR (neat, cm^{-1}) ν 2958, 2929, 2858, 1766, 1274, 1254, 1153, 1079, 1026, 992, 835, 778, 751, 698; ^{1}H NMR (400 MHz, $CDCl_3$) δ -0.01 (s, 3H), 0.00 (s, 3H), 0.81 (s, 9H), 3.48 (s, 3H), 3.77 (d, *J* = 10.2 Hz, 1H), 3.82 (d, *J* = 10.2 Hz, 1H), 3.82 (d, *J* = 10.2 Hz, 1H), 4.01 (d, *J* = 10.2 Hz, 1H), 4.55 (d, *J* = 12.2 Hz, 1H), 4.58 (d, *J* = 12.2 Hz, 1H), 5.22 (s, 2H), 7.2-7.4 (m, 6H), 7.42 (d, *J* = 8.3 Hz, 1H), 7.51 (d, *J* = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.64, -5.57, 18.1, 25.7 (3C), 56.1, 64.8, 70.8, 73.7, 87.9, 94.6, 111.4, 123.2, 124.0, 127.5 (2C), 127.7, 128.3, 128.4 (2C), 137.7, 142.5, 158.4, 169.6; MALDI-TOF/MS (positive ion, α-cyano-4-hydroxycinnamic acid) calcd. for $C_{25}H_{34}O_6SiNa$ ($M+Na^+$) 481.20; found 481.17.

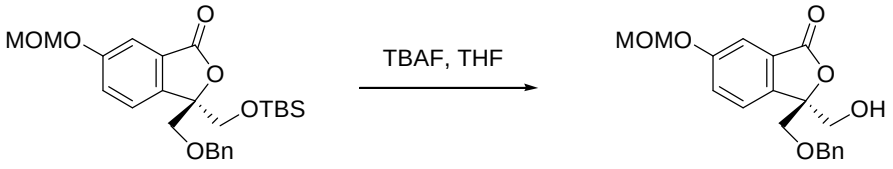
Separation of enantiomers (**9B (*R*)**, *ent*-**9B (*S*)**)

rac-**9B** (149 mg) was separated by HPLC (CHIEALPAK AD-H (ϕ 2 cm x 25 cm), flow rate 6.0 mL / min, hexane / ^{i}PrOH = 50 / 1) to give **9B** and *ent*-**9B**

9B (68.7 mg), colorless oil; $[\alpha]_D^{27}$ = +4.1 (c = 1.2, $CHCl_3$), t_r = 19 min (CHIRALPAK AD-H, flow late 1.0 mL / min, hexane / ^{i}PrOH = 99 / 1)

ent-**9B** (59.8 mg), colorless oil; $[\alpha]_D^{29}$ = –1.4 (c = 1.3, $CHCl_3$), t_r = 22 min (CHIRALPAK AD-H, flow late 1.0 mL / min, hexane / ^{i}PrOH = 99 / 1)

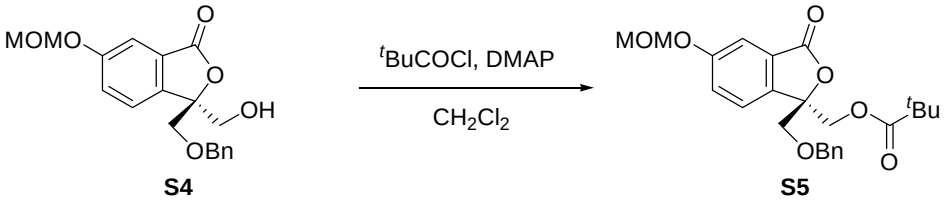
(*S*)-(+)-3-Benzyloxymethyl-3-hydroxymethyl-6-methoxymethoxy-*3H*-isobenzofuran-1-one (S4)



9B **S4**

To a solution of **9B** (68.9 mg, 0.123 mmol) in THF (0.5 mL) was added a 1 M solution of TBAF in THF (0.225 mL, 0.225 mmol) at room temperature. After stirring for 10 min at room temperature, the reaction mixture was diluted with ethyl acetate, and water was added. The organic layer was separated and washed with brine. The combined aqueous layers were extracted with ethyl acetate, and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. Further purification was carried out by silica gel column chromatography (eluent: hexane / ethyl acetate = 2 / 1) to give **S4** (51.5 mg, quant.) as a colorless oil. $[\alpha]_D^{30}$ = +12.1 (c = 0.455, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.04 (t, *J* = 6.4 Hz, 1H), 3.48 (s, 3H), 3.70 (d, *J* = 9.8 Hz, 1H), 3.92 (d, *J* = 9.8 Hz, 1H), 3.93 (dd, *J* = 6.4 and 12.4 Hz, 1H), 4.03 (dd, *J* = 6.4 and 12.4 Hz, 1H), 4.55 (d, *J* = 12.2 Hz, 1H), 4.60 (d, *J* = 12.2 Hz, 1H), 5.22 (s, 2H), 7.2-7.4 (m, 6H), 7.45 (d, *J* = 8.3 Hz, 1H), 7.53 (d, *J* = 2.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.2, 64.9, 71.1, 73.8, 87.7, 94.6, 111.7, 123.6, 123.7, 127.6 (2C), 127.9, 128.0, 128.5 (2C), 137.4, 142.0, 158.6, 169.4; MALDI-TOF/MS (positive ion, α-cyano-4-hydroxycinnamic acid) calcd. for $C_{19}H_{20}O_6Na$ ($M+Na^+$) 367.12; found 367.09.

(*R*)-(+)-3-Benzyloxymethyl-3-(2,2-dimethylpropionyloxymethyl)-6-methoxymethoxy-*3H*-isobenzofuran-1-one (S5)

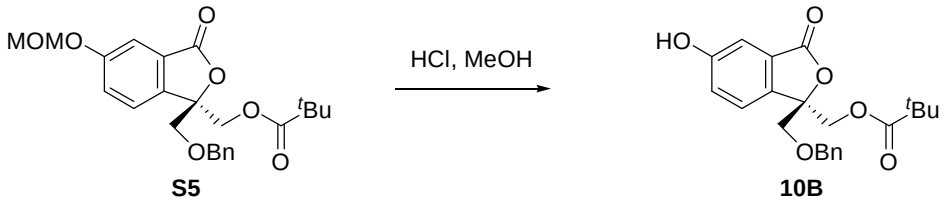


S4 **S5**

To a solution of **S4** (9.1 mg, 0.0264 mmol) and 4-DMAP (6.5 mg, 0.0528 mmol) in CH_2Cl_2 (0.3 mL) was added pivaloyl chloride (6.4 mg, 0.0528 mmol) at room temperature. After stirring for 30 min at room temperature, the reaction mixture was diluted with ether, and a saturated aqueous solution of $NaHCO_3$ was

added. The organic layer was separated and washed with brine. The aqueous layer was extracted with ether. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. Further purification was carried out by silica gel column chromatography (eluent: hexane / ethyl acetate = 30 / 1) to give **S5** (9.5 mg, 84%) as a pale yellow oil. $[\alpha]_D^{28}$ = +5.1 (c = 0.475, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.98 (s, 9H), 3.47 (s, 3H), 3.67 (d, *J* = 10.0 Hz, 1H), 3.85 (d, *J* = 10.0 Hz, 1H), 4.40 (d, *J* = 12.0 Hz, 1H), 4.55 (d, *J* = 12.2 Hz, 1H), 4.59 (d, *J* = 12.2 Hz, 1H), 4.68 (d, *J* = 12.0 Hz, 1H), 5.22 (s, 2H), 7.2-7.4 (m, 6H), 7.43 (d, *J* = 8.8 Hz, 1H), 7.53 (d, *J* = 2.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.8 (3C), 38.7, 56.1, 64.1, 71.3, 73.9, 86.3, 94.5, 111.5, 123.5, 123.9, 127.6 (2C), 127.9, 128.3, 128.5 (2C), 137.2, 141.0, 158.7, 169.1, 177.5; MALDI-TOF/MS (positive ion, α-cyano-4-hydroxycinnamic acid) calcd. for $C_{24}H_{28}O_7Na$ ($M+Na^+$) 451.17; found 451.07.

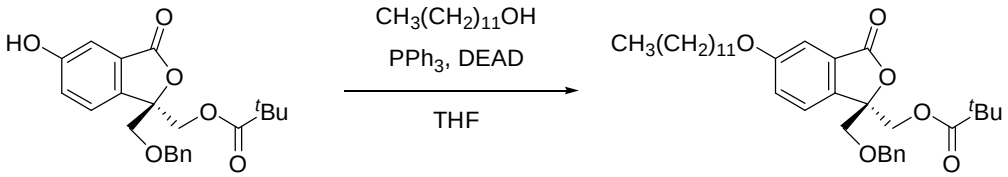
(*R*)-(+)-Benzyloxymethyl-3-(2,2-dimethylpropionyloxymethyl)-6-hydroxy-*3H*-isobenzofuran-1-one (10B)



S5 **10B**

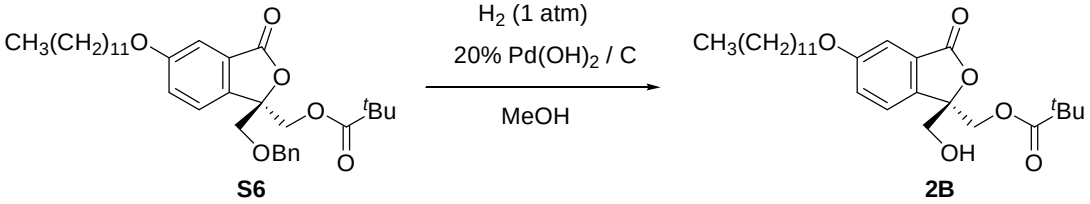
To a solution of **S5** (63.5 mg, 0.148 mmol) in MeOH (1.5 mL) was added a 12N aqueous hydrochloric acid solution (1 drop) at room temperature. After being stirred for 12 h at 40 °C and concentration of the solution under reduced pressure, water was added to the mixture. The mixture was extracted with ether, and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 2) to give **10B** (50.2 mg, 88%) as a colorless oil. $[\alpha]_D^{28}$ = +5.9 (c = 0.35, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.98 (s, 9H), 3.68 (d, *J* = 10.0 Hz, 1H), 3.84 (d, *J* = 10.0 Hz, 1H), 4.42 (d, *J* = 12.0 Hz, 1H), 4.55 (d, *J* = 12.0 Hz, 1H), 4.59 (d, *J* = 12.0 Hz, 1H), 4.68 (d, *J* = 12.0 Hz, 1H), 6.21 (s, 1H), 7.14 (dd, *J* = 1.7 and 8.3 Hz, 1H), 7.2-7.4 (m, 6H), 7.38 (d, *J* = 8.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.9 (3C), 38.8, 64.2, 71.3, 73.9, 86.6, 110.9, 122.4, 124.1, 127.7 (2C), 128.0, 128.4, 128.5 (2C), 137.2, 139.8, 157.6, 169.4, 177.7; MALDI-TOF/MS (positive ion, α-cyano-4-hydroxycinnamic acid) calcd. for $C_{22}H_{24}O_6Na$ ($M+Na^+$) 407.15; found 407.11.

(*R*)-3-Benzyloxymethyl-3-(2,2-dimethylpropionyloxymethyl)-6-dodecyloxy-*3H*-isobenzofuran-1-one (S6)



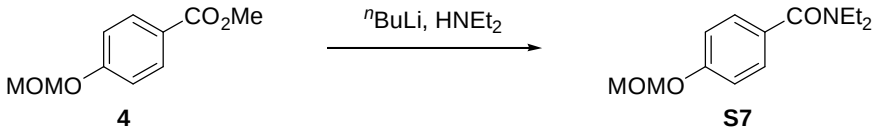
To a solution of **10B** (7.0 mg, 0.0182 mmol), 1-dodecanol (5.1 mg, 0.0273 mmol), and triphenylphosphine (14.3 mg, 0.0546 mmol) in THF (0.3 mL) was added diethyl azodicarboxylate (9.5 mg, 0.0546 mmol) at room temperature. After stirring for 30 min at room temperature, 1-dodecanol (10.2 mg, 0.0546 mmol), triphenylphosphine (57.2 mg,, 0.218 mmol), and diethylazodicarboxylate (38.0 mg, 0.218 mmol) was added furthermore. After stirring for 2.5 h at room temperature, the reaction mixture was diluted with ether, and brine was added. The aqueous layer was extracted with ether. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. Further purification was carried out by silica gel column chromatography (eluent: hexane / ethyl acetate = 100 / 1) to give **S6** (15.8 mg, mixture) as a pale yellow oil. MALDI-TOF/MS (positive ion, α-cyano-4-hydroxycinnamic acid) calcd. for $C_{34}H_{48}O_6Na$ ($M+Na^+$) 575.34; found 575.27.

(*R*)-(-)-3-(2,2-Dimethylpropionyloxymethyl)-6-dodecyloxy-3-hydroxymethyl-*3H*-isobenzofuran-1-one (2B)



To a solution of **S6** (15.8 mg) in methanol (0.3 mL) was added $Pd(OH)_2/C$ (20 wt%, 3 mg) at room temperature. After stirring for 1.5 h under hydrogen atmosphere at room temperature, the reaction mixture was filtrated through Celite. The filtrate was concentrated under reduced pressure. This protocol was repeated twice until starting material was consumed. Further purification was carried out by silica gel column chromatography (eluent: hexane / ethyl acetate = 5 / 1) to give **2B** (6.7 mg, 79% 2 steps) as a colorless amorphous. $[\alpha]_D^{27}$ = -10.0 (c = 0.335, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.3, 3H), 1.07 (s, 9H), 1.2-1.6 (m, 18H), 1.80 (quintet, *J* = 6.6 Hz, 2H), 2.17-2.25 (br, 1H), 3.88-3.98 (br s, 2H), 4.00 (t, *J* = 6.6 Hz, 2H), 4.47 (d, *J* = 12.0 Hz, 1H), 4.56 (d, *J* = 12.0 Hz, 1H), 7.22 (dd, *J* = 2.2 and 8.3 Hz, 1H), 7.32 (d, *J* = 2.2 Hz, 1H), 7.40 (d, *J* = 8.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 25.9, 26.9 (3C), 29.0, 29.3, 29.57 (2C), 29.61, 29.64 (2C), 31.9, 38.9, 63.8, 64.5, 68.8, 87.3, 108.6, 123.3(2C), 128.2, 139.3, 160.9, 169.3, 178.1; ESI-TOF/MS (positive ion) calcd. for $C_{27}H_{46}NO_6$ ($M+NH_4^+$) 480.33; found 480.97.

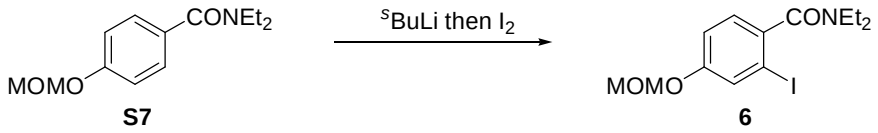
***N*,*N*-Diethyl-4-(methoxymethoxy)benzamide (S7).**



4 **S7**

To a solution of diethylamine (10.3 mL, 98.0 mmol) in THF (50 mL) was added ^{n}BuLi (1.56 M in hexane, 48.0 mL, 74.5 mmol) slowly under the nitrogen atmosphere at –78 °C, and the mixture was stirred for 0.5 h at 0 °C. The solution was cooled to -78 °C, and a solution of **4** (9.63 g, 49.0 mmol) in THF (19 mL) was added slowly. After being stirred for 20 min at 0 °C, a saturated aqueous solution of $NaHCO_3$ was added. The aqueous layer was extracted with ether and ethyl acetate, and the combined organic layers were washed with brine. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 1) to give **S7** (11.5 g, 99%) as a pale yellow solid: IR (neat, cm^{-1}) ν 2971, 2927, 2896, 1713, 1621, 1578, 1490, 1429, 1377, 1311, 1289, 1236, 1145, 1101, 1075, 1009, 982,7, 824, 790, 649; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.05-1.38 (br, 6H), 3.22-3.47 (br, 4H), 3.48 (s, 3H), 5.19 (s, 2H), 7.03 (d, *J* = 8.6 Hz, 2H), 7.33 (d, *J* = 8.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.8, 14.0, 39.2, 43.2, 55.9, 94.1, 115.7 (2C), 127.9 (2C), 130.5, 157.7, 170.9; MALDI-TOF/MS (positive ion, α-cyano-4-hydroxycinnamic acid) calcd. for $C_{13}H_{20}NNaO_3$ ($M+Na^+$) 260.13; found 260.24.

***N*,*N*-Diethyl-2-iodo-4-(methoxymethoxy)benzamide (6).**

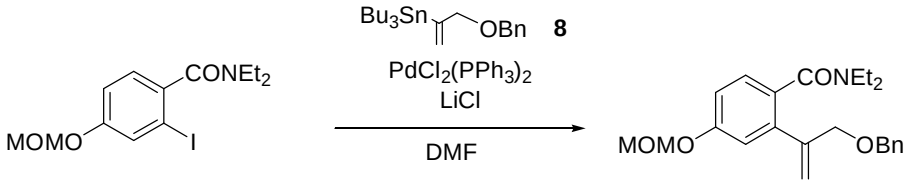


S7 **6**

After azeotropic evaporation of **S7** (2.65 g, 11.2 mmol) with toluene, **S7** was dissolved in THF (56 mL). To this solution was added ^{s}BuLi (0.96 M in hexane, 17.5 mL, 16.8 mmol) slowly under nitrogen atmosphere at –92 °C. The reaction mixture was stirred for 1 h at –92 °C, and iodine (5.65 g, 22.4 mmol) was added. The reaction mixture was stirred for 30 min at room temperature, and then a saturated aqueous solution of $NaHCO_3$ and a saturated aqueous solution of $Na_2S_2O_3$ were added. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were washed with brine. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 2) to give **6** (3.39 g, 83%) as a yellow oil: IR (neat, cm^{-1}) ν 2971, 2923, 2896, 2855, 1617, 1596,1565, 1495, 1451, 1420, 1377, 1307, 1285, 1219, 1285, 1188, 1149, 1075, 991, 921, 860, 820, 737, 693; ^{1}H NMR .400 MHz, $CDCl_3$) δ 1.06 (t, *J* = 7.1 Hz , 3H),

1.28 (t, J = 7.1 Hz , 3H), 3.15 (q, J = 7.1 Hz , 2H), 3.20-3.41 (br s, 1H) 3.47 (s, 3H), 3.71-3.95 (br, 1H), 5.15 (s, 2H), 7.03 (dd, J = 2.4 and 8.6 Hz, 1H), 7.10 (d, J = 8.6 Hz, 1H), 7.50 (d, J = 2.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.5, 14.0, 38.9, 42.8, 56.2, 92.9, 94.4, 116.2, 126.6, 127.4, 136.5, 157.2, 170.9; MALDI-TOF/MS (positive ion, α-cyano-4-hydroxycinnamic acid) calcd. for $C_{13}H_{18}INNaO_3$ ($M+Na^+$) 386.02; found 386.00.

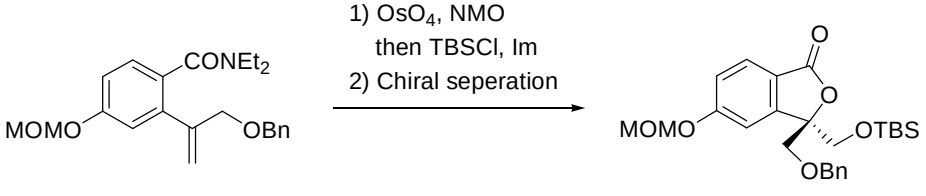
2-(1-Benzyloxyprop-2-en-2-yl)-*N*,*N*-diethyl-4-(methoxymethoxy)benzamide (S8).



6 **S8**

After azeotropic evaporation of **6** (4.90 g, 13.5 mmol) and **8** (8.88 g, 20.3 mmol) with toluene, mixture was dissolved in DMF (45 mL). The solution was degassed by the four freeze-pump-thaw cycles, then under the nitrogen atmosphere $PdCl_2(PPh_3)_2$ (1.90 g, 2.70 mmol) and LiCl (0.861 g, 20.3 mmol) were added in one portion at room temperature. After being stirred for 20 h at 90 °C, the reaction mixture was diluted with ethyl acetate at room temperature, and a saturated aqueous solution of $NaHCO_3$ was added. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 2) to give **S8** (3.82 g, 74%) as an orange oil: IR (neat, cm^{-1}) ν 2966, 2936, 1618, 1556, 1473, 1429, 1381, 1311, 1289, 1250, 1153, 1123, 1074, 1026, 987, 899, 789, 715; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.99 (t, J = 7.3 Hz, 3H), 1.16 (t, J = 7.3 Hz, 3H), 2.96-3.19 (br, 2H), 3.46 (s, 3H), 3.47-3.50 (br, 2H), 4.26 (s, 2H), 4.56 (s, 2H), 5.16 (s, 2H), 5.29 (s, 1H), 5.44 (s, 1H), 6.96 (d, J = 8.6 Hz, 1H), 6.98 (s, 1H), 7.15 (d, J = 8.6 Hz, 1H), 7.21-7.39 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.3, 13.8, 38.5, 42.8, 56.0, 72.3, 72.7, 94.3, 114.9, 116.4, 116.5, 127.5 (2C), 127.6, 128.0, 128.3 (2C), 129.9, 138.3, 138.8, 144.0, 157.1, 170.5; MALDI-TOF/MS (positive ion, α-cyano-4-hydroxycinnamic acid) calcd. for $C_{23}H_{29}NNaO_4$ ($M+Na^+$) 406.20; found 406.45.

(*R*)-3-Benzyloxymethyl-3-(*t*-butyldimethylsiloxymethyl)-5-methoxymethoxy-*3H*-isobenzofuran-1-one (9C).



S8 **9C**

To a solution of **S8** (1.57 g, 4.11 mmol) in ^{t}BuOH (13 mL) and H_2O (13 mL) were added a 50 wt% aqueous solution of NMO (2.8 mL, 12.3 mmol) and a 20 mM solution of OsO_4 in ^{t}BuOH (10.0 mL, 0.200 mmol). After being stirred for 48 h at room temperature, the reaction mixture was added solid $Na_2S_2O_3$. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was dissolved in DMF (10 mL), and imidazole (0.550 g, 8.12 mmol) and TBSCl (0.740 g, 4.90 mmol) were added at 0 °C. After being stirred for 2 h at room temperature, the reaction mixture was diluted with ether, and brine was added. The aqueous layer was extracted with ether, and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 9) to give *rac*-**9C** (1.37 g, 73% 2 steps) as a colorless oil: IR (neat, cm^{-1}) ν 2953, 2927, 2892, 2853 1762, 1604, 1482, 1460, 1302, 1250, 1149, 1096, 1057, 1018, 974, 917, 838, 772, 689; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.00 (s, 3H), 0.02 (s, 3H), 0.82 (s, 9H), 3.48 (s, 3H) , 3.80 (d, *J* = 10.2 Hz, 1H), 3.84 (d, *J* = 10.2 Hz, 1H), 3.84 (d, *J* = 10.5 Hz, 1H), 4.05 (d, *J* = 10.5 Hz, 1H), 4.58 (s, 2H), 5.23 (s, 2H), 7.14 (dd, *J* = 2.2 and 8.5 Hz, 1H), 7.16 (d, *J* = 2.2 Hz, 1H), 7.22-7.40 (m, 5H), 7.79 (d, *J* = 8.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.7, -5.6, 18.0, 25.6 (3C), 56.2, 64.6, 70.8 73.7, 87.4, 94.2, 109.4, 118.3, 120.1, 126.9, 127.5 (2C), 127.6, 128.3 (2C), 137.7, 151.6, 161.8, 169.4; MALDI-TOF/MS (positive ion, α-cyano-4-hydroxycinnamic acid) calcd. for $C_{25}H_{34}NaO_6Si$ ($M+Na^+$) 481.20; found 481.07.

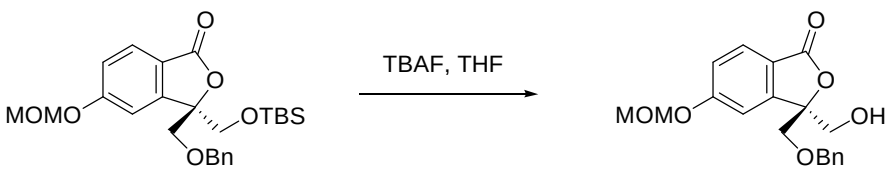
Separation of enantiomers (9C (*R*), *ent*-9C (*S*)).

rac-**9C** (200 mg, 0.436 mmol) was separated by HPLC (CHIERALPAK AD-H (ϕ 2 cm x 25 cm), flow rate 6.0 mL / mim, hexane / ^{i}PrOH = 50 / 1) to give **9C (**89.1 mg, 0.194 mmol**)** and *ent*-**9C** (88.9 mg, 0.193 mmol**).**

9C (89.1 mg), colorless oil; $[\alpha]_D^{28}$ = –13.0 (c = 1.00, $CHCl_3$), t_r = 11.3 min (CHIRALPAK AD-H, flow late 1.0 mL / min, hexane / ^{i}PrOH = 99 / 1)

ent-**9C** (88.9 mg), colorless oil; $[\alpha]_D^{29}$ = +11.5 (c = 1.40, $CHCl_3$), t_r = 12.3 min (CHIRALPAK AD-H, flow late 1.0 mL / min, hexane / ^{i}PrOH = 99 / 1)

(*S*)-(+)-3-Benzyloxymethyl-3-hydroxymethyl-5-methoxymethoxy-*3H*-isobenzofuran-1-one (S9).

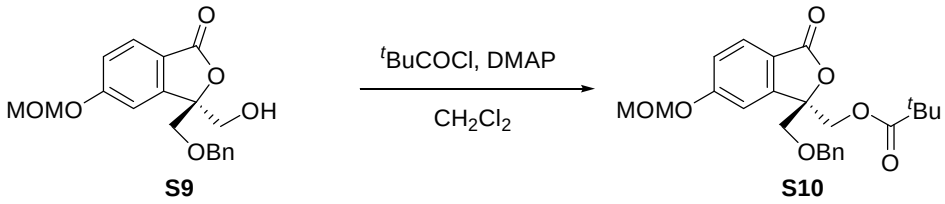


9C **S9**

To a solution of **9C** (66.6 mg, 0.145 mmol) in THF (0.22 mL) was added a 1 M solution of TBAF in THF

(0.290 mL, 0.290 mmol) at room temperature. After being stirred for 15 min, a saturated aqueous solution of NaCl was added to the reaction mixture. The aqueous layer was extracted with ether, and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 1) to give **S9** (50.8 mg, 100%) as a colorless amorphous: $[\alpha]_D^{28}$ = +8.0 (c = 0.70, $CHCl_3$); IR (neat, cm^{-1}) ν 3431, 2953, 2927, 2848, 1753, 1600, 1491, 1455, 1399, 1337, 1289, 1241, 1153, 1066, 978, 921, 741, 693; ^1H NMR (400 MHz, $CDCl_3$) δ 2.13 (t, *J* = 6.8 Hz, 1H), 3.49 (s, 3H), 3.72 (d, *J* = 10.0 Hz, 1H), 3.91 (d, *J* = 10.0 Hz, 1H), 3.93 (dd, *J* = 6.8 and 12.2 Hz, 1H), 4.03 (dd, *J* = 6.8 and 12.2 Hz, 1H), 4.55 (d, *J* = 12.2 Hz, 1H), 4.60 (d, *J* = 12.2 Hz, 1H), 5.21 (d, *J* = 6.8 Hz, 1H), 5.25 (d, *J* = 6.8 Hz, 1H), 7.15 (d, *J* = 2.2 Hz, 1H), 7.16 (dd, *J* = 2.2 and 6.8 Hz, 1H), 7.25-7.43 (m, 5H), 7.78 (d, *J* = 6.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.4, 64.7, 70.9, 73.8, 87.4, 94.3, 109.2, 118.5, 119.7, 127.3, 127.6 (2C), 127.8, 128.4 (2C), 137.4, 151.2, 162.2, 169.4; MALDI-TOF/MS (positive ion, α-cyano-4-hydroxycinnamic acid) calcd. for $C_{19}H_{21}NaO_6$ ($M+Na^+$) 367.12; found 367.05.

(*R*)-(+)-3-Benzyloxymethyl-3-(2,2-dimethylpropionyloxymethyl)-5-methoxymethoxy-*3H*-isobenzofuran-1-one (S10).

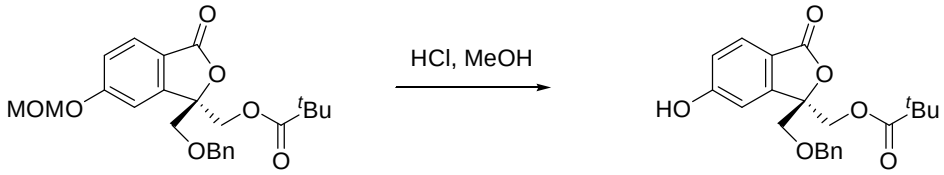


S9 **S10**

To a solution of **S9** (41.2 mg, 0.120 mmol) and 4-DMAP (44.0 mg, 0.360 mmol) in CH_2Cl_2 (0.60 mL) was added pivaloyl chloride (44.0 mg, 0. 360 mmol) at room temperature. After being stirred for 5 min, to the reaction mixture was added a saturated aqueous solution of $NaHCO_3$. The aqueous layer was extracted with ether, and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 2) to give **S10** (49.3 mg, 97%) as a colorless oil: $[\alpha]_D^{28}$ = +10.6 (c = 0.10, $CHCl_3$); IR (neat, cm^{-1}) ν 2958, 2905, 2861, 1766, 1736, 1604, 1482, 1451, 1399, 1364, 1337, 1276, 1241, 1149, 1083, 1061, 1022, 978, 925, 693; ^1H NMR (400 MHz, $CDCl_3$) δ 1.00 (s, 9H), 3.48 (s, 3H), 3.70 (d, *J* = 10.0 Hz, 1H), 3.87 (d, *J* = 10.0 Hz, 1H), 4.41 (d, *J* = 11.7 Hz, 1H), 4.55 (d, *J* = 12.2 Hz, 1H), 4.61 (d, *J* = 12.2 Hz, 1H), 4.67 (d, *J* = 11.7 Hz, 1H), 5.21 (d, *J* = 7.0 Hz, 1H), 5.24 (d, *J* = 7.0 Hz, 1H), 7.16 (d, *J* = 0.96 Hz, 1H), 7.17 (dd, *J* = 0.96 and 8.3 Hz, 1H), 7.22-7.42 (m, 5H), 7.81 (d, *J* = 8.3 Hz, 1H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 26.9 (3C), 38.8, 56.3, 64.2, 71.1, 73.8, 85.6, 94.3, 109.4, 118.6, 119.9, 127.0, 127.5 (2C), 127.8, 128.3 (2C), 137.1, 150.2, 162.0, 168.7, 177.2; MALDI-TOF/MS (positive ion, α-cyano-4-hydroxycinnamic acid)

calcd. for $C_{24}H_{28}NaO_7$ ($M+Na^+$) 451.17; found 451.21.

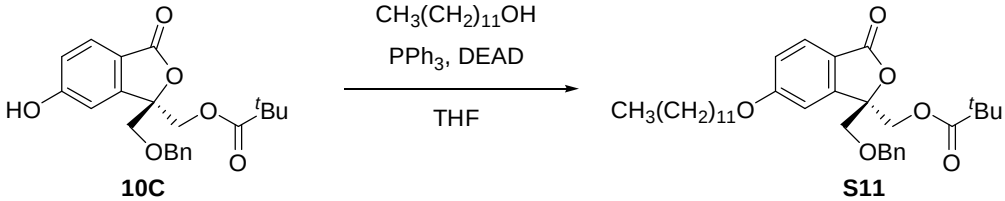
(*R*)-(+)-3-Benzyloxymethyl-3-(2,2-dimethylpropionyloxymethyl)-5-hydroxy-*3H*-isobenzofuran-1-one (10C).



S10 **10C**

To a solution of **S10** (49.3 mg, 0.115 mmol) in MeOH (1.2 mL) was added a 12N aqueous hydrochloric acid solution (1 drop) at room temperature. After being stirred for 12 h at 40 °C, the mixture was concentrated under reduced pressure, water was added. The mixture was extracted with ether, and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 2) to give **10C** (43.8 mg, 99%) as a colorless oil: $[\alpha]_D^{29}$ = +15.6 (c = 0.99, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.99 (s, 9H), 3.65 (d, *J* = 9.9 Hz, 1H), 3.86 (d, *J* = 9.9 Hz, 1H), 4.42 (d, *J* = 11.8 Hz, 1H), 4.55 (d, *J* = 13.0 Hz, 1H), 4.60 (d, *J* = 13.0 Hz, 1H), 4.67 (d, *J* = 11.8 Hz, 1H), 6.76 (bds, 1H), 6.95 (d, *J* = 1.1 Hz, 1H), 7.01 (dd, *J* = 1.1 and 8.0 Hz, 1H), 7.16-7.42 (m, 5H), 7.77 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 26.8 (3C), 38.9, 64.2, 71.1, 73.9, 86.1, 109.2, 118.1, 118.1, 127.4, 127.6 (2C), 127.9, 128.4 (2C), 137.0, 150.5, 162.1, 169.8, 177.9; MALDI-TOF/MS (positive ion, α-cyano-4-hydroxycinnamic acid) calcd for $C_{23}H_{29}NNaO_4$ ($M+Na^+$) 406.20; found 406.45.

(*R*)-(+)-3-Benzyloxymethyl-3-(2,2-dimethylpropionyloxymethyl)-5-dodecyloxy-*3H*-isobenzofuran-1-one (S11).

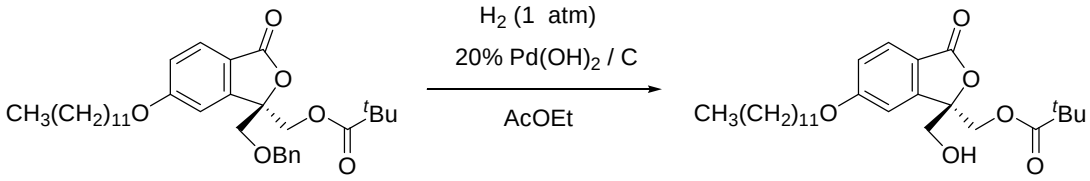


10C **S11**

After azeotropic evaporation of **10C** (19.8 mg, 0.052 mmol), 1-dodecanol (17.2 μL, 0.077 mmol) and triphenylphosphine (40.2 mg, 0.154 mmol) with toluene (twice), the mixture was dissolved in THF (0.51 mL). A 40 wt% toluene solution of diethyl azidocarboxylate (24.2 μL, 0.154 mmol) was added to the mixture under the nitrogen atmosphere at room temperature. After being stirred for 1 h, the mixture was added a saturated aqueous solution of NaCl. The aqueous layer was extracted with ether, and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified

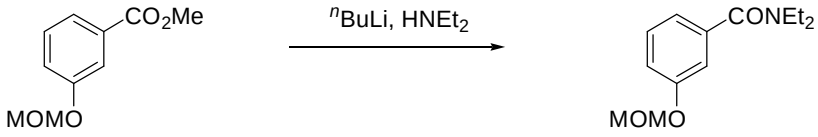
by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 9) to give **S11** (28.3 mg, 100%) as a colorless oil: $[\alpha]_D^{29}$ +11.0 (c = 1.40, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.8 Hz, 3H), 0.99 (s, 9H), 1.11-1.55 (m, 18H), 1.80 (quintet, *J* = 6.5 Hz, 2H), 3.68 (d, *J* = 10.0 Hz, 1H), 3.87 (d, *J* = 10.0 Hz, 1H), 3.98 (t, *J* = 6.5 Hz, 2H), 4.42 (d, *J* = 11.7 Hz, 1H), 4.55 (d, *J* = 11.9 Hz, 1H), 4.60 (d, *J* = 11.9 Hz, 1H), 4.66 (d, *J* = 11.7 Hz, 1H), 6.93 (d, *J* = 2.0 Hz, 1H), 7.03 (dd, *J* = 2.0 and 8.6 Hz, 1H), 7.17-7.43 (m, 5H), 7.77 (d, *J* = 8.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 25.9, 26.8 (3C), 28.9, 29.3, 29.3, 29.5, 29.6, 29.6, 29.6, 31.9, 38.7, 64.2, 68.8, 71.3, 73.9, 85.5, 107.3, 117.7, 118.7, 127.0, 127.6 (2C), 127.9, 128.4 (2C), 137.2, 150.6, 164.1, 169.1, 177.5.

(*R*)-(+)-3-(2,2-Dimethylpropionyloxymethyl)-5-dodecyloxy-3-hydoxymethyl-*3H*-isobenzofuran-1-one (2C).



To a solution of **S11** (28.3 mg, 0.051 mmol) in methanol (0.51 mL) was added 20 wt% $Pd(OH)_2$/C (5.7 mg) at room temperature. After being stirred for 30 min under hydrogen atmosphere, the mixture was filtrated through Celite. The filtrate was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 9) to give **2C** (22.9 mg, 97%) as a colorless oil: $[\alpha]_D^{28}$ = +7.6 (c = 1.1, $CHCl_3$); IR (neat, cm^{-1}) ν 3443, 2953, 2923, 2848, 17762, 1740, 1604, 1495, 1482, 1460, 1337, 1302, 1280, 1241, 1193, 1140, 1079, 1048, 1013, 916, 781, 689; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.8 Hz, 3H), 1.05 (s, 9H), 1.14-1.52 (m, 18H), 1.80 (quintet, *J* = 6.6 Hz, 2H), 2.56 (t, *J* = 6.6 Hz, 1H), 3.90 (d, *J* = 6.6 Hz, 2H), 4.00 (td, *J* = 6.6 and 8.9 Hz, 1H), 4.04 (td, *J* = 6.6 and 8.9 Hz, 1H), 4.48 (d, *J* = 11.9 Hz, 1H), 4.57 (d, *J* = 11.9 Hz, 1H), 6.93 (d, *J* = 2.0 Hz, 1H), 7.04 (dd, *J* = 2.0 and 8.3 Hz, 1H), 7.78 (d, *J* = 8.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 25.9, 26.9 (3C), 29.0, 29.3, 29.3, 29.5, 29.6, 29.6, 29.6, 31.9, 38.8, 63.8, 64.4, 68.9, 86.8, 107.0, 117.6, 118.6, 127.4, 150.1, 164.4, 169.2, 178.07; MALDI-TOF/MS (positive ion, α-cyano-4-hydroxycinnamic acid) calcd. for $C_{27}H_{42}NaO_6$ ($M+Na^+$) 485.29; found 485.25.; Anal. Calcd for $C_{27}H_{42}O_6$: C, 70.1; H, 9.15. Found C, 70.2; H, 9.17.

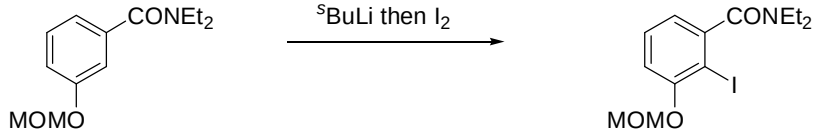
***N*,*N*-Diethyl-3-(methoxymethoxy)benzamide (S12).**



3 **S12**

To a solution of diethylamine (2.2 mL, 30 mmol) in THF (20 mL) was added ^{n}BuLi (1.56 M in hexane, 17 mL, 26 mmol) slowly under the nitrogen atmosphere at –78 °C, and the mixture was stirred for 20 min at 0 °C. The solution was cooled to -78 °C, and a solution of **3** (3.90 g, 6.50 mmol) in THF (10 mL) was added slowly. After being stirred for 1 h at 0 °C, a saturated aqueous solution of $NaHCO_3$ was added. The aqueous layer was extracted with ether and ethyl acetate, and the combined organic layers were washed with brine. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 1) to give **S12** (4.60 g, 96%) as a pale yellow oil: IR (neat, cm^{-1}) ν 2971, 2927, 2901, 1718, 1622, 1578, 1495, 1486, 1473, 1464, 1433, 1376, 1315, 1289, 1236, 1214, 1149, 1074, 1013, 982, 916, 785, 746; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.98-1.13 (br, 3H), 1.13-1.28 (br, 3H), 3.11-3.28 (br, 2H), 3.38-3.59 (br, 2H), 3.44 (s, 3H), 5.15 (s, 2H), 6.96 (td, *J* = 1.2 and 7.5 Hz, 1H), 6.98-7.05 (m, 2H), 7.24 (d, *J* = 1.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.76, 14.05, 39.08, 43.13, 55.59, 94.29, 114.06, 116.81, 119.50, 129.48, 138.49, 157.06, 170.67.

***N*,*N*-Diethyl-2-iodo-3-(methoxymethoxy)benzamide (7).**

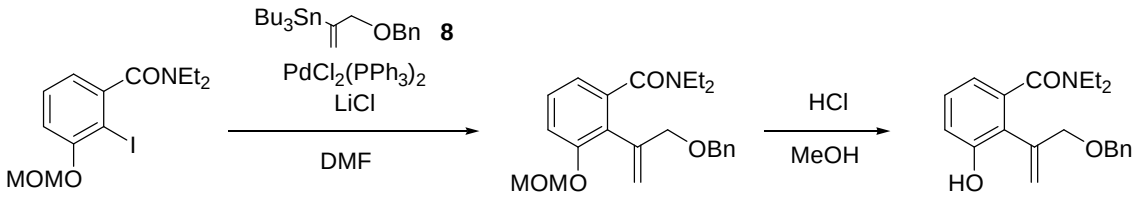


S12 **7**

After azeotropic evaporation of **S12** (1.92 g, 8.09 mmol) with toluene, to a solution of **S12** in THF (40 mL) was added a 0.96 M hexane solution of ^{s}BuLi (13.0 mL, 12.1 mmol) slowly under the nitrogen atmosphere at –92 °C. The reaction mixture was stirred for 4 h at –92 °C, and iodine (4.08 g, 16.2 mmol) was added. The reaction mixture was stirred for 30 min at room temperature, and then a saturated aqueous solution of $NaHCO_3$ and a saturated aqueous solution of $Na_2S_2O_3$ were added. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were washed with brine. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 2) to give **7** (2.07 g, 70%) as an orange oil: IR (neat, cm^{-1}) ν 2970, 2927, 1622, 1561, 1473, 1425, 1381, 1311, 1289, 1254, 1149, 1074, 1022, 982, 925, 904, 785, 750, 715; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.06 (t, *J* = 6.8 Hz , 3H), 1.29 (t, *J* = 6.8 Hz , 3H), 3.11

(qd, *J* = 6.8 and 7.1 Hz, 1H), 3.15 (qd, *J* = 6.8 and 7.1 Hz, 1H), 3.29 (qd, *J* = 6.8 and 7.1 Hz, 1H), 3.51 (s, 3H), 3.87 (qd, *J* = 6.8 and 7.1 Hz, 1H), 5.23 (d, *J* = 6.8 Hz, 1H), 5.27 (d, *J* = 6.8 Hz, 1H), 6.85 (dd, *J* = 1.2 and 9.6 Hz, 1H), 7.02 (dd, *J* = 1.2 and 8.3 Hz, 1H), 7.30 (dd, *J* = 8.3 and 9.6 Hz, 1H),; ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.4, 13.9, 38.8, 42.7, 56.5, 86.3, 95.0, 114.1, 120.2, 129.8, 144.8, 156.2, 169.9; MALDI-TOF/MS (positive ion, α-cyano-4-hydroxycinnamic acid) calcd. for $C_{13}H_{19}INNaO_3$ ($M+Na^+$) 386.02; found 386.05.

2-(1-Benzyloxyprop-2-en-2-yl)-*N*,*N*-diethyl-3-hydroxybenzamide (S14).

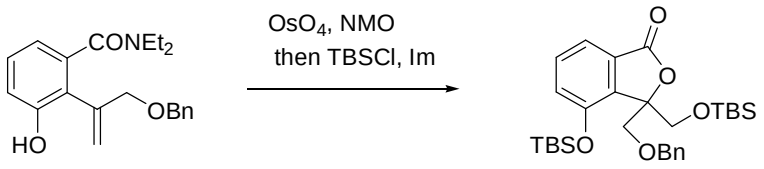


After azeotropic evaporation of **7** (2.07 g, 5.69 mmol) and **8** (3.73 g, 8.54 mmol) with toluene, the mixture was dissolved in DMF (14 mL). The solution was degassed by the four freeze-pump-thaw cycles, then under the nitrogen atmosphere $PdCl_2(PPh_3)_2$ (0.800 g, 1.14 mmol) and LiCl (0.360 g, 8.54 mmol) were added in one portion at room temperature. After being stirred for 18 h at 90 °C, the reaction mixture was diluted with ethyl acetate at room temperature, and a saturated aqueous solution of $NaHCO_3$ was added. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 2) to give crude **S13** (5.50 g): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.04 (t, *J* = 7.3 Hz, 3H), 1.16 (t, *J* = 7.3 Hz, 3H), 2.88-3.33 (br, 3H), 3.38 (s, 3H), 3.73-3.92 (br, 1H), 4.29 (dd, *J* = 1.4 and 1.4 Hz, 2H), 4.58 (d, *J* = 12.3 Hz, 1H), 4.65 (d, *J* = 12.3 Hz, 1H), 5.10 (dd, *J* = 1.4 and 1.4 Hz, 1H), 5.11 (s, 2H), 5.56 (dd, *J* = 1.4 and 1.4 Hz, 1H), 6.88 (d, *J* = 7.5 Hz, 1H), 7.09 (d, *J* = 7.5 Hz, 1H), 7.23 (dd, *J* = 7.5 and 7.5 Hz, 1H), 7.24-7.40 (m, 5H).

To a solution of crude **S13** (5.50 g) in MeOH (20 mL) was added a 12N aqueous hydrochloric acid solution (2 drops) at room temperature. After being stirred for 18 h at 40 °C, the mixture was concentrated under reduced pressure, and then water was added. The mixture was extracted with ether, and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 2) to give **S14** (0.73 g, 38% from **7**) as a colorless oil: IR (neat, cm^{-1}) ν 3181, 2975, 2913, 2844, 1591, 1569, 1490, 1447, 1433, 1376, 1363, 1298, 1280, 1223, 1123, 1083, 1057, 1031, 965, 934, 912, 746, 697; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.08 (t, *J* = 7.3 Hz, 3H), 1.11 (t, *J* = 7.3 Hz, 3H), 2.84-3.98 (br, 4H), 4.24 (s, 3H), 4.62 (s, 2H),

5.23 (br s, 1H), 5.55 (br s, 1H), 6.75 (d, *J* = 7.5 Hz, 1H), 6.92 (d, *J* = 7.5 Hz, 1H), 7.19 (dd, *J* = 7.5 and 7.5 Hz, 1H), 7.24-7.40 (m, 5H), 7.90 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.2, 13.8, 38.2, 42.8, 73.4, 75.2, 117.0, 117.2, 122.5, 125.0, 128.0 (2C), 128.0, 128.5 (2C), 129.0, 136.8, 137.7, 140.4, 154.6, 170.2; MALDI-TOF/MS (positive ion, α-cyano-4-hydroxycinnamic acid) calcd. for $C_{21}H_{25}NNaO_3$ ($M+Na^+$) 362.17; found 362.26.

3-Benzyloxymethyl-4-*t*-butyldimethylsiloxy-3-(*t*-butyldimethylsiloxymethyl)-*3H*-isobenzofuran-1-one (S15).

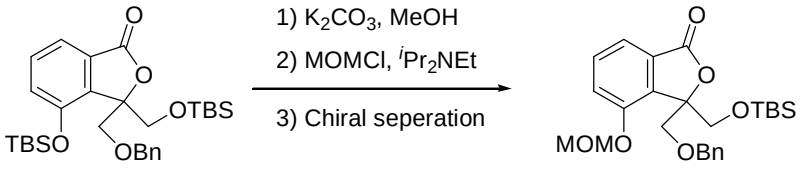


S14 **S15**

To a solution of **S14** (0.690 g, 2.04 mmol) in ^{t}BuOH (5.0 mL) and H_2O (3.0 mL) were added a 50 wt% aqueous solution of NMO (2.8 mL, 12.2 mmol) and a 20 mM solution of OsO_4 in ^{t}BuOH (15.3 mL, 0.312 mmol). After being stirred for 72 h at 60 °C, the reaction mixture was added a saturated aqueous $Na_2S_2O_3$ solution. The aqueous layer was extracted with ether, and the combined organic layers were dried over $MgSO_4$. Concentration under reduced pressure gave the crude product, which was used for the next step without purfication.

To a mixture of the crude product and imidazole (0.922 g, 8.16 mmol) in DMF (4.0 mL) was added TBSCl (0.555g, 6.12 mmol) at 0 °C. After being stirred for 1 h at room temperature, the reaction mixture was diluted with ether, and a saturated aqueous solution of NaCl was added. The aqueous layer was extracted with ether, and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 10) to give **S15** (0.42 g, 39%) as a colorless oil: IR (neat, cm^{-1}) ν 2953, 2922, 2853, 1771, 1604, 1486, 1469, 1285, 1258, 1166, 1114, 1074, 1000, 916, 829, 781, 755, 693; ^{1}H NMR (400 MHz, $CDCl_3$) δ -0.08 (s, 3H), -0.04 (s, 3H), 0.28 (s, 3H), 0.29 (s, 3H), 0.66 (s, 9H), 0.97 (s, 9H), 3.81 (d, *J* = 10.9 Hz, 1H), 4.07 (d, *J* = 10.9 Hz, 1H), 4.15 (d, *J* = 10.9 Hz, 1H), 4.26 (d, *J* = 10.9 Hz, 1H), 4.50 (d, *J* = 12.1 Hz, 1H), 4.59 (d, *J* = 12.1 Hz, 1H), 7.00 (dd, *J* = 1.5 and 8.0 Hz, 1H), 7.18-7.32 (m, 5H), 7.35 (dd, *J* = 7.5 and 8.0 Hz, 1H), 7.46 (dd, *J* = 1.5 and 7.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.6, -4.1 (2C), -4.0, 17.8, 18.3, 25.4 (3C), 25.8 (3C), 62.8, 70.0, 73.7, 90.7, 117.6, 122.1, 127.5 (2C), 127.6, 128.3 (2C), 130.6, 130.7, 136.8, 139.1, 150.6, 170.1; MALDI-TOF/MS (positive ion, α-cyano-4-hydroxycinnamic acid) calcd. for $C_{29}H_{44}NaO_5Si_2$ ($M+Na^+$) 551.26; found 551.53.

3-Benzyloxymethyl-3-(*t*-butyldimethylsiloxymethyl)-4-methoxymethoxy-*3H*-isobenzofuran-1-one (9D).



S15 **9D**

To a solution of **S15** (0.41 g, 0.78 mmol) in MeOH (2.6 mL) was added potassium carbonate (11 mg, 0.078 mmol). After stirring for 4 h at room temperature and concentration of the reaction mixture under reduced pressure, 1N HCl solution was added. The aqueous layer was extracted with ether, and the combined organic layers were dried over $MgSO_4$. Concentration under reduced pressure gave the crude product, which was used for the next step without purfication.

To a mixture of the crude product and diisopropylethylamine (0.67 mL, 3.9 mmol) in CH_2Cl_2 (2.6 mL) was added MOMCl (0.18 mL, 2.3 mmol) at 0 °C. After being stirred for 1 h at room temperature, the reaction mixture was added a saturated aqueous solution of NaCl. The aqueous layer was extracted with ether, and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 10 – 1 /4) to give *rac*-**9D** (0.32 g, 90% overall) as a colorless oil: IR (neat, cm^{-1}) ν 2949, 2927, 2848, 1762, 1604, 1491, 1464, 1250, 1149, 1088, 1057, 1022, 978, 917, 834, 777, 742, 689; ^1H NMR (400 MHz, $CDCl_3$) δ -0.05 (s, 3H), 0.00 (s, 3H), 0.90 (s, 9H), 3.45 (s, 3H), 3.91 (d, *J* = 11.1 Hz, 1H), 4.09 (d, *J* = 10.8 Hz, 1H), 4.18 (d, *J* = 11.1 Hz, 1H), 4.27 (d, *J* = 10.8 Hz, 1H), 4.59 (s, 2H), 5.20 (d, *J* = 6.8 Hz, 1H), 5.23 (d, *J* = 6.8 Hz, 1H), 7.23 (d, *J* = 6.8 Hz, 1H), 7.24-7.46 (m, 5H), 7.46 (dd, *J* = 6.8 and 7.5 Hz, 1H), 7.55 (d, *J* = 7.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.8, -5.6, 17.8, 25.5 (3C), 56.2, 62.9, 69.5, 73.6, 90.6, 94.1, 118.0, 118.2, 127.5 (2C), 127.6, 128.3, 130.2, 131.0, 135.5, 137.8, 151.6, 169.8.

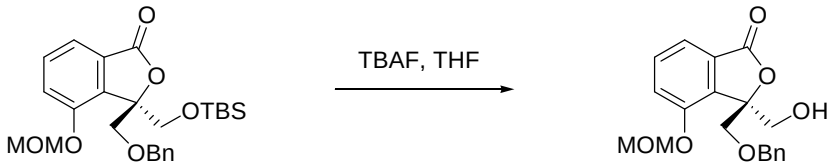
Separation of enantiomers (9D (*R*), *ent*-9D (*S*)).

rac-**9D** (464 mg, 1.01 mmol) was separated by HPLC (CHIERALPAK AD-H (ϕ 2 cm x 25 cm), flow rate 6.0 mL / mim, hexane / ^{i}PrOH = 50 / 1) to give **9D** and *ent*-**9D.**

9D (221 mg), colorless oil; $[\alpha]_D^{28}$ = +3.50 (c = 1.00, $CHCl_3$), t_r = 12.1 min (CHIRALPAK AD-H, flow late 1.0 mL / min, hexane / ^{i}PrOH = 98 / 2)

ent-**9D** (206 mg), colorless oil; $[\alpha]_D^{29}$ = -4.30 (c = 1.60, $CHCl_3$), t_r = 20.1 min (CHIRALPAK AD-H, flow late 1.0 mL / min, hexane / ^{i}PrOH = 98 / 2)

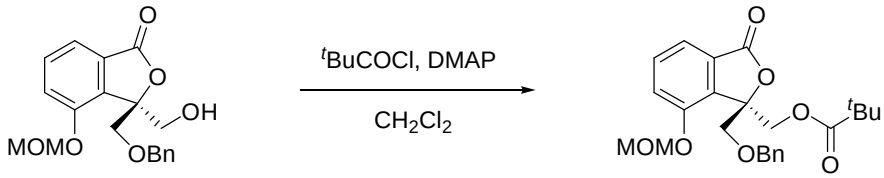
(*S*)-(-)-3-Benzyloxymethyl-3-hydroxymethyl-4-methoxymethoxy-*3H*-isobenzofuran-1-one (S16).



9D **S16**

To a solution of **9D** (21.0 mg, 0.045 mmol) in THF (0.36 mL) was added a 1 M solution of TBAF in THF (0.090 mL, 0.090 mmol) at room temperature. After being stirred for 15 min, a saturated aqueous solution of NaCl was added to the reaction mixture. The aqueous layer was extracted with ether, and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 1) to give **S16** (15.2 mg, 98%) as a colorless amorphous: $[\alpha]_D^{28}$ = -22.3 (c = 0.76, $CHCl_3$); IR (neat, cm^{-1}) ν 3418, 2931, 2897, 1758, 1609, 1486, 1446, 1298, 1236, 1148, 1066, 982, 917, 745, 689; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.13 (t, *J* = 6.0 Hz, 1H), 3.41 (s, 3H), 3.92 (d, *J* = 10.9 Hz, 1H), 4.00 (dd, *J* = 6.0 and 11.0 Hz, 1H), 4.16 (d, *J* = 10.9 Hz, 1H), 4.25 (dd, *J* = 6.0 and 11.0 Hz, 1H), 4.50 (d, *J* = 12.2 Hz, 1H), 4.55 (d, *J* = 12.2 Hz, 1H), 5.18 (d, *J* = 6.8 Hz, 1H), 5.21 (d, *J* = 6.8 Hz, 1H), 7.17 (d, *J* = 7.1 Hz, 1H), 7.20-7.40 (m, 5H), 7.46 (dd, *J* = 7.1 and 7.5 Hz, 1H), 7.54 (d, *J* = 7.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.3, 63.5, 69.6, 73.6, 90.1, 94.1, 118.6, 118.6, 127.5 (2C), 127.7, 128.3 (2C), 129.6, 131.5, 135.2, 137.6, 151.6, 169.8; MALDI-TOF/MS (positive ion, α-cyano-4-hydroxycinnamic acid) calcd. for $C_{19}H_{20}NaO_6$ ($M+Na^+$) 367.12; found 367.09.

(*R*)-(-)-3-Benzyloxymethyl-3-(2,2-dimethylpropionyloxymethyl)-4-methoxymethoxy-*3H*-isobenzofuran-1-one (S17).

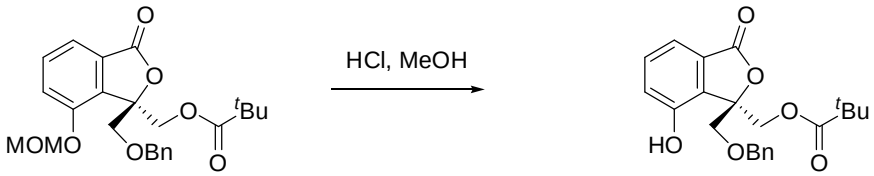


S16 **S17**

To a solution of **S16** (14.1 mg, 0.041 mmol) and 4-DMAP (15.0 mg, 0.123 mmol) in CH_2Cl_2 (0.40 mL) was added pivaloyl chloride (15 μL, 0.123 mmol) at room temperature. After being stirred for 10 min, a saturated aqueous solution of $NaHCO_3$ was added to the reaction mixture. The aqueous layer was extracted with ether, and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 4) to give **S17** (15.9 mg, 90%) as a colorless oil: $[\alpha]_D^{30}$ = -18.2 (c = 0.80, $CHCl_3$); IR (neat, cm^{-1}) ν 2958, 2905, 2861, 1766, 1727, 1600, 1477, 1451, 1328, 12756, 1153, 1100, 1065, 1021, 986, 925, 693; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90 (s, 9H), 3.41 (s, 3H), 3.86 (d, *J* = 11.0 Hz, 1H), 4.16 (d, *J* = 11.0 Hz, 1H), 4.55 (d, *J* = 12.2 Hz, 1H), 4.59 (d, *J* = 11.9 Hz, 1H), 4.60 (d, *J* = 12.2 Hz, 1H), 4.80 (d, *J* = 11.9

Hz, 1H), 5.15 (d, *J* = 7.1 Hz, 1H), 5.22 (d, *J* = 7.1 Hz, 1H), 7.22 (dd, *J* = 1.6 and 7.6 Hz, 1H), 7.23-7.41 (m, 5H), 7.47 (dd, *J* = 7.6 and 8.0 Hz, 1H), 7.53 (d, *J* = 1.6 and 8.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.8 (3C), 38.7, 56.3, 62.5, 69.6, 73.7, 88.2, 94.3, 118.3, 118.4, 127.6 (2C), 127.8, 128.4 (2C), 129.7, 131.7, 134.2, 137.5, 151.9, 163.3, 169.3, 177.5; MALDI-TOF/MS (positive ion, α-cyano-4-hydroxycinnamic acid) calcd. for $C_{24}H_{28}NaO_7$ ($M+Na^+$) 451.17; found 451.25.

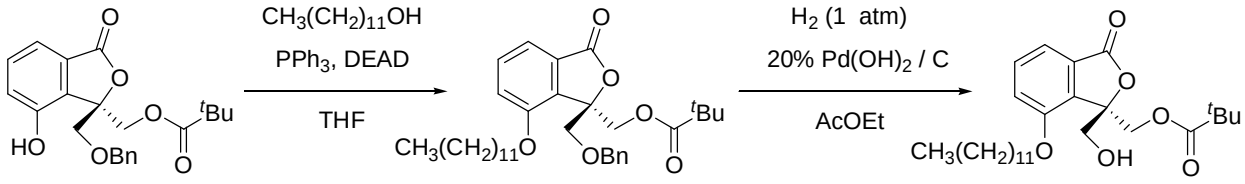
(*R*)-(+)-3-Benzyloxymethyl-3-(2,2-dimethylpropionyloxymethyl)-4-hydroxy-*3H*-isobenzofuran-1-one (10D).



S17 **10D**

To a solution of **S17** (15.9 mg, 0.037 mmol) in MeOH (0.37 mL) was added a 12N aqueous hydrochloric acid solution (1 drop) at room temperature. After being stirred for 8 h at 50 °C, the mixture was concentrated under reduced pressure, and water was added. The mixture was extracted with ether, and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 8 – 1 / 2) to give **10D** (12.4 mg, 87%) as a colorless oil: $[\alpha]_D^{30}$ = +64.5 (c = 0.62, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.99 (s, 9H), 3.71 (d, *J* = 8.5 Hz, 1H), 4.17 (d, *J* = 8.5 Hz, 1H), 4.52 (d, *J* = 12.0 Hz, 1H), 4.60 (d, *J* = 12.0 Hz, 1H), 4.69 (d, *J* = 11.5 Hz, 1H), 4.73 (d, *J* = 11.5 Hz, 1H), 7.10-7.21 (m, 1H), 7.29-7.51 (m, 7H), 8.10 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.8 (3C), 38.8, 63.2, 71.6, 74.9, 84.4, 117.6, 122.7, 128.0, 128.4 (2C), 129.0 (2C), 129.1, 132.0, 132.9, 134.9, 151.9, 168.8, 177.4.

(*R*)-(+)-3-(2,2-Dimethylpropionyloxymethyl)-4-dodecyloxy-3-hydoxymethyl-*3H*-isobenzofuran-1-one (2D).



10D **S18** **2D**

After azeotropic evaporation of **10D** (8.8 mg, 0.026 mmol), 1-dodecanol (8.7 μL, 0.039 mmol) and triphenylphosphine (27.2 mg, 0.104 mmol) with toluene (twice), the mixture was dissolved in THF (0.26 mL). A 40 wt% toluene solution of diethyl azodicarboxylate (48 μL, 0.104 mmol) was added to the mixture under the nitrogen atmosphere at room temperature. After being stirred for 3 h, a saturated

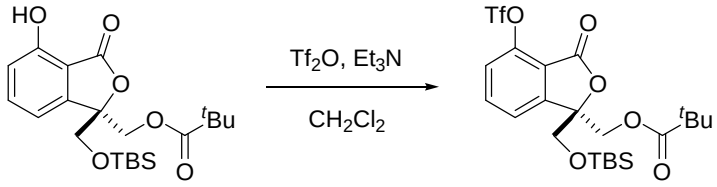
aqueous solution of NaCl was added to the mixture. The aqueous layer was extracted with ether, and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 9) to give crude **S18** as a yellow oil.

To a solution of crude **S18** in methanol (0.52 mL) was added 20 wt% $Pd(OH)_2/C$ (4.0 mg) at room temperature. After being stirred for 15 min under hydrogen atmosphere, the mixture was filtrated through Celite. The filtrate was concentrated under reduced pressure, and purified by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 9 – 1 / 2) to give **2D** (6.2 mg, 52% in 2 steps) as a colorless oil: $[\alpha]_D^{29}$ = +16.9 (c = 0.31, $CHCl_3$); IR (neat, cm^{-1}) ν 3470, 2958, 2918, 2848, 1762, 1735, 1604, 1491, 1482, 1460, 1337, 1272, 1241, 1193, 1140, 1079, 1035, 1013, 917, 697; ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.8 Hz, 3H), 0.92 (s, 9H), 1.14-1.52 (m, 18H), 1.83 (quintet, *J* = 6.6 Hz, 2H), 2.14 (t, *J* = 6.6 Hz, 1H), 4.00 (dd, *J* = 6.6 and 12.2 Hz, 1H), 4.00 (td, *J* = 6.6 and 8.9 Hz, 1H), 4.10 (td, *J* = 6.6 and 8.9 Hz, 1H), 4.19 (dd, *J* = 6.6 and 12.2 Hz, 1H), 4.60 (d, *J* = 11.9 Hz, 1H), 4.76 (d, *J* = 11.9 Hz, 1H), 7.08 (dd, *J* = 2.0 and 6.8 Hz, 1H), 7.45 (dd, *J* = 2.0 and 7.1 Hz, 1H), 7.45 (dd, *J* = 6.8 and 7.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 26.0, 26.8 (3C), 29.0, 29.3, 29.3, 29.5, 29.6, 29.6 (2C), 31.9, 38.7, 62.4, 64.5, 68.8, 86.6, 116.1, 117.2, 129.4, 131.8, 134.0, 153.8, 169.4, 177.4; MALDI-TOF/MS (positive ion, α-cyano-4-hydroxycinnamic acid) calcd for $C_{27}H_{42}NaO_6$ ($M+Na^+$) 485.29; found 485.44.; Anal. Calcd for $C_{27}H_{42}O_6$: C, 70.1; H, 9.15. Found C, 69.9; H, 9.14.

Determination of absolute stereochemistry of 10B, 10C, and 10D:

Synthesis of authentic sample S22 from S19.

(*S*)-(-)-3-(*tert*-Butyldimethylsiloxymethyl)-3-(2,2-dimethylpropionyloxymethyl)-7-trifluoromethanesulfonyloxy-*3H*-isobenzofuran-1-one (S20).

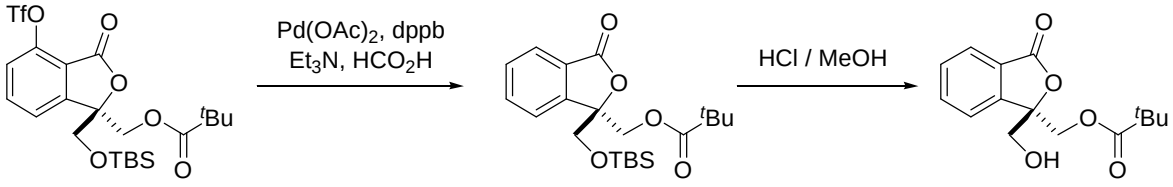


S19 **S20**

To a solution of **S19**[11a] (11 mg, 0.026 mmol) and triethylamine (36 μL, 0.26 mmol) in CH_2Cl_2 (0.52 mL) was added trifluoromethanesulfonic anhydride (17.6 μL, 0.104 mmol) at -78 °C. After being stirred for 30 min, a saturated aqueous solution of $NaHCO_3$ was added to the reaction mixture. The aqueous layer was extracted with ether, and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 9) to give **S20** (14 mg, 99%) as a yellow oil: $[\alpha]_D^{28}$ = -9.3 (c = 0.70, $CHCl_3$); IR (neat, cm^{-1}) ν

2953, 2927, 2892, 2848, 1775, 1736, 1473, 1429, 1214, 1135, 1035, 951, 838, 820, 776; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.02 (s, 3H), 0.04 (s, 3H), 0.85 (s, 9H), 0.97 (s, 9H), 3.80 (d, *J* = 10.4 Hz, 1H), 4.05 (d, *J* = 10.4 Hz, 1H), 4.40 (d, *J* = 12.1 Hz, 1H), 4.77 (d, *J* = 12.1 Hz, 1H), 7.40 (d, *J* = 8.2 Hz, 1H), 7.57 (d, *J* = 7.7 Hz, 1H), 7.74 (dd, *J* = 7.7 and 8.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.7, -5.6, 18.1, 25.6 (3C), 26.8 (3C), 38.7, 63.5, 64.8 87.0, 119.9, 122.8, 123.0, 135.7 (2C), 145.8, 150.8, 164.5, 177.5; MALDI-TOF/MS (positive ion, α-cyano-4-hydroxycinnamic acid) calcd. for $C_{22}H_{31}F_3NaO_8SSi$ ($M+Na^+$) 563.14; found 563.26.

(*R*)-(-)-3-(2,2-Dimethylpropionyloxymethyl)-3-hydroxymethyl-*3H*-isobenzofuran-1-one (S22).



S20 **S21** **S22**

To a solution of $Pd(OAc)_2$ (5.8 mg, 0.026 mmol) in dimethylacetamide (0.50 mL) was added 1,2-bis(diphenylphosphino)butane (33 mg, 0.104 mmol) at room temperature, and the mixture was stirred for 30 min affording a 0.52 M dimethylacetamide solution of $Pd(dppb)(OAc)_2$.

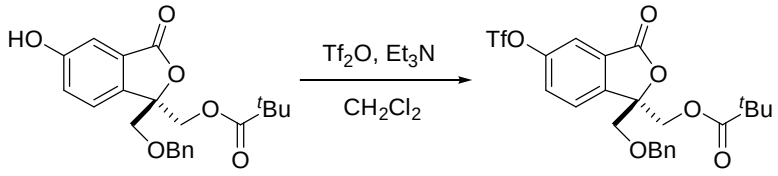
To a solution of **S20** (14.0 mg, 0.026 mmol) and triethylamine (18.0 μL, 0.13 mmol) in dimethylacetamide (0.26 mL) were added a 0.52 M solution of $Pd(dppb)(OAc)_2$ in dimethylacetamide (50 μL, 0.0026 mmol) and formic acid (4.9 μL, 0.13 mmol) at room temperature. After being stirred for 30 min at 90 °C, brine was added to the reaction mixture. The aqueous layer was extracted with ether, and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 2) to give **S21** (13.2 mg, mixture) as a yellow oil.

To a solution of **S21** (13.2 mg, mixture) in methanol (0.52 mL) was added a 12N aqueous hydrochloric acid solution (1 drop) at room temperature. After being stirred for 3 h at 40 °C, the reaction mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 1) to give **S22** (4.9 mg, 68% from **S20**) as an amorphous: $[\alpha]_D^{27}$ = -20 (c = 0.25, $CHCl_3$); IR (neat, cm^{-1}) ν 3461, 2966, 2870, 1766, 1731, 1608, 1596, 1477, 1455, 1385, 1364, 1280, 1227, 1145, 1123, 1070, 1035, 916, 798, 759, 693; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.03 (s, 9H), 2.23 (t, *J* = 7.3 Hz, 1H), 3.94 (d, *J* = 7.3 Hz, 2H), 4.50 (d, *J* = 12.2 Hz, 1H), 4.63 (d, *J* = 12.2 Hz, 1H), 7.54 (dd, *J* = 0.95 and 7.5 Hz, 1H), 7.58 (ddd, *J* = 0.95, 7.5 and 7.5 Hz, 1H), 7.70 (dd, *J* = 1.2 and 7.5 Hz, 1H), 7.92 (ddd, *J* = 1.2, 7.5 and 7.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.9 (3C), 38.8, 63.7, 64.4, 87.6, 122.4, 125.9, 126.9, 130.1, 134.3, 147.3, 169.3, 178.0; MALDI-TOF/MS (positive ion, α-cyano-4-

hydroxycinnamic acid) calcd. for $C_{15}H_{18}NaO_5$ ($M+Na^+$) 301.11; found 301.12.

Transformation to 12 from 10B

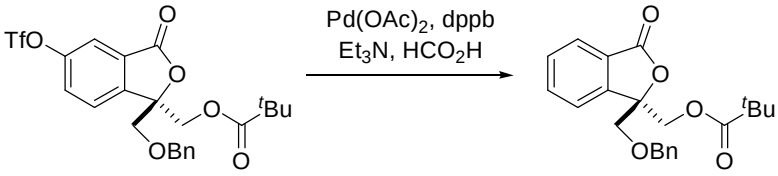
(*R*)-(+)-3-Benzyloxymethyl-3-(2,2-dimethylpropionyloxymethyl)-6-trifluoromethanesulfonyloxy-*3H*-isobenzofuran-1-one (11B).



10B **11B**

To a solution of **10B** (50.2 mg, 0.13 mmol) and triethylamine (0.18 mL, 1.3 mmol) in CH_2Cl_2 (0.65 mL) was added trifluoromethanesulfonic anhydride (88 μL, 0.52 mmol) at -78 °C. After being stirred for 15 min, a saturated aqueous solution of $NaHCO_3$ was added to the reaction mixture. The aqueous layer was extracted with ether, and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 9) to give **11B** (64 mg, 95%) as a yellow oil: $[\alpha]_D^{28}$ = +5.6 (c = 3.3, $CHCl_3$); IR (neat, cm^{-1}) ν 2975, 2870, 1780, 1731, 1477, 1424, 1280, 1205, 1135, 1092, 1031, 904, 837, 816, 745, 697, 605; ^1H NMR (400 MHz, $CDCl_3$) δ 0.92 (s, 9H), 3.67 (d, *J* = 9.7 Hz, 1H), 3.91 (d, *J* = 9.7 Hz, 1H), 4.41 (d, *J* = 11.8 Hz, 1H), 4.57 (s, 2H), 4.80 (d, *J* = 11.8 Hz, 1H), 7.17-7.41 (m, 5H), 7.56 (dd, *J* = 2.4 and 8.7 Hz, 1H), 7.66 (d, *J* = 8.7 Hz, 1H), 7.78 (d, *J* = 2.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.7 (3C), 38.7, 63.7, 70.6, 73.9, 86.6, 117.0, 118.4, 120.2, 125.4, 127.2, 127.7 (2C), 128.2, 128.6 (2C), 129.4, 136.7, 147.5, 150.4, 167.0, 177.3; MALDI-TOF/MS (positive ion, α-cyano-4-hydroxycinnamic acid) calcd. for $C_{23}H_{23}F_3NaO_8S$ ($M+Na^+$) 539.10; found 539.22.

(*R*)-(+)-3-Benzyloxymethyl-3-(2,2-dimethylpropionyloxymethyl)-*3H*-isobenzofuran-1-one (12).

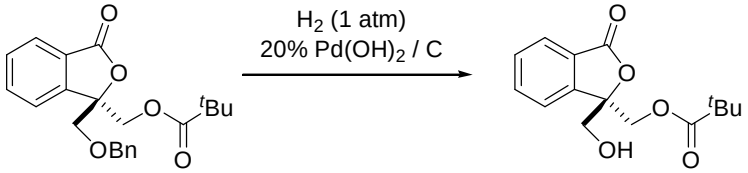


11B **12**

To a solution of **11B** (26 mg, 0.050 mmol) and triethylamine (34 μL, 0.25 mmol) in dimethylacetamide (0.26 mL) were added a 0.52 M solution of $Pd(dppb)(OAc)_2$ in dimethylacetamide (96 μL, 0.0050 mmol) and formic acid (9.4 μL, 0.25mmol) at room temperature. After being stirred for 40 min at 90 °C, brine was added to the reaction mixture. The aqueous layer was extracted with ether, and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica

gel column chromatography (eluent: ethyl acetate / hexane = 1 / 9) to give **12** (18 mg, 98%) as a colorless oil. $[\alpha]_D^{28}$ = +7.4 (c = 0.90, $CHCl_3$); IR (neat, cm^{-1}) ν 2971, 2874, 1775, 1731, 1477, 1455, 1284, 1149, 1118, 1070, 1022, 759, 693; ^1H NMR (400 MHz, $CDCl_3$) δ 0.95 (s, 9H), 3.71 (d, *J* = 9.9 Hz, 1H), 3.88 (d, *J* = 9.9 Hz, 1H), 4.44 (d, *J* = 11.8 Hz, 1H), 4.55 (d, *J* = 11.8 Hz, 1H), 4.60 (d, *J* = 11.8 Hz, 1H), 4.73 (d, *J* = 11.8 Hz, 1H), 7.20-7.41 (m, 5H), 7.53 (dd, *J* = 0.94 and 7.5 Hz, 1H), 7.56 (dd, *J* = 1.2 and 7.5 Hz, 1H), 7.65 (dt, *J* = 1.2 and 7.5 Hz, 1H), 7.90 (dt, *J* = 0.94 and 7.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.8 (3C), 38.7, 64.1, 71.2, 73.9, 86.5, 122.9, 125.6, 126.9, 127.6 (2C), 127.9, 128.5 (2C), 129.9, 133.9, 137.1, 147.8, 169.3, 177.4; MALDI-TOF/MS (positive ion, α-cyano-4-hydroxycinnamic acid) calcd. for $C_{22}H_{24}NaO_5$ ($M+Na^+$) 391.15; found 391.20.

(*R*)-(-)-3-(2,2-Dimethylpropionyloxymethyl)-3-hydroxymethyl-*3H*-isobenzofuran-1-one (S22).

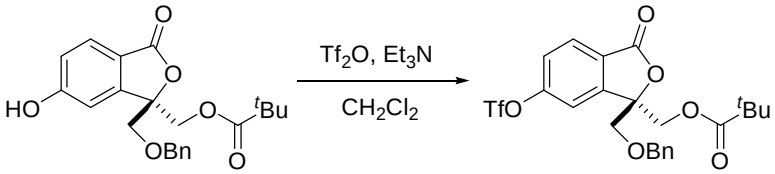


12 **S22**

To a solution of **12** (18 mg, 0.049 mmol) in methanol (0.49 mL) was added 20 wt% $Pd(OH)_2/C$ (3.6 mg) at room temperature. After being stirred for 30 min under hydrogen atmosphere, the mixture was filtrated through Celite. The filtrate was concentrated under reduced pressure, and purified by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 9 – 1 / 1) to give **S22** (14 mg, 100%) as a colorless amorphous: $[\alpha]_D^{27}$ = -22 (c = 0.68, $CHCl_3$).

Transformation to 12 from 10C

(*R*)-(-)-3-Benzyloxymethyl-3-(2,2-dimethylpropionyloxymethyl)-5-trifluoromethanesulfonyloxy-*3H*-isobenzofuran-1-one (11C).

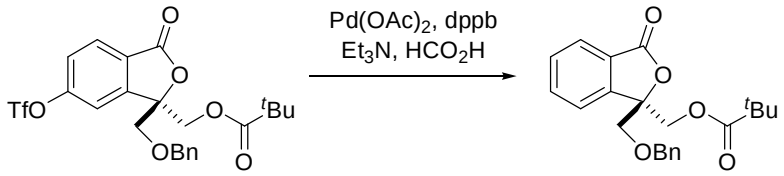


10C **11C**

To a solution of **10C** (44 mg, 0.11 mmol) and triethylamine (0.16 mL, 1.1 mmol) in CH_2Cl_2 (0.57 mL) was added trifluoromethanesulfonic anhydride (76 μL, 0.46 mmol) at -78 °C. After being stirred for 20 min, a saturated aqueous solution of $NaHCO_3$ was added to the reaction mixture. The aqueous layer was extracted with ether, and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: ethyl acetate /

hexane = 1 / 9) to give **11C** (52 mg, 87%) as a yellow oil: $[\alpha]_D^{28}$ = -1.3 (c = 2.50, $CHCl_3$); IR (neat, cm^{-1}) ν 2970, 2866, 1775, 1731, 1617, 1595, 1472, 1455, 1420, 1271, 1210, 1131, 1092, 1070, 1026, 908, 846, 825, 737, 697, 614; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.95 (s, 9H), 3.64 (d, *J* = 9.4 Hz, 1H), 3.91 (d, *J* = 9.4 Hz, 1H), 4.41 (d, *J* = 12.1 Hz, 1H), 4.56 (s, 2H), 4.75 (d, *J* = 12.1 Hz, 1H), 7.18-7.40 (m, 5H), 7.47 (dd, *J* = 2.2 and 8.2 Hz, 1H), 7.50 (d, *J* = 2.2 Hz, 1H), 7.99 (d, *J* = 8.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.7 (3C), 38.7, 63.8, 70.5, 74.0, 86.0, 116.9, 117.0, 120.2, 123.5, 127.7 (2C), 127.8, 128.2, 128.6 (2C), 136.6, 150.4, 152.9, 167.2, 177.2; MALDI-TOF/MS (positive ion, α-cyano-4-hydroxycinnamic acid) calcd. for $C_{23}H_{23}F_3NaO_8S$ ($M+Na^+$) 539.10; found 539.10.

(*R*)-(+)-3-Benzyloxymethyl-3-(2,2-dimethylpropionyloxymethyl)-*3H*-isobenzofuran-1-one (12).

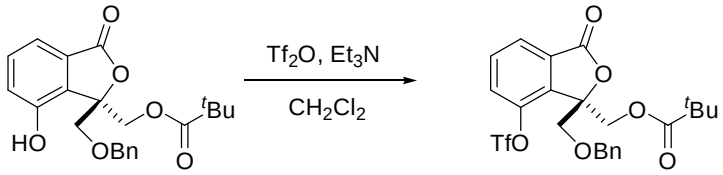


11C **12**

To a solution of **11C** (26 mg, 0.050 mmol) and triethylamine (34 μL, 0.25 mmol) in dimethylacetamide (0.26 mL) were added a 0.52 M solution of $Pd(dppb)(OAc)_2$ in dimethylacetamide (96 μL, 0.0050 mmol) and formic acid (9.4 μL, 0.25 mmol) at room temperature. After being stirred for 4 h at 90 °C, brine was added to the reaction mixture. The aqueous layer was extracted with ether, and the combined organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 9) to give **12** (17 mg, 90%) as a colorless oil. $[\alpha]_D^{28}$ = +7.7 (c = 0.83, $CHCl_3$).

Transformation to 12 from 10D

(*R*)-(-)-3-Benzyloxymethyl-3-(2,2-dimethylpropionyloxymethyl)-4-trifluoromethanesulfonyloxy-*3H*-isobenzofuran-1-one (11D).

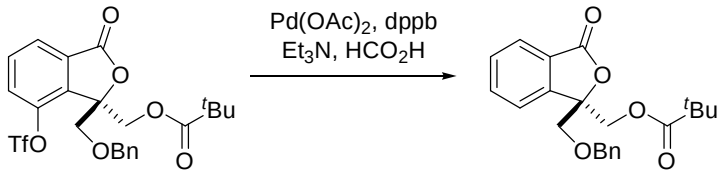


10D **11D**

To a solution of **10D** (13 mg, 0.033 mmol) and triethylamine (46 μL, 0.33 mmol) in CH_2Cl_2 (0.33 mL) was added trifluoromethanesulfonic anhydride (22 μL, 0.13 mmol) at -78 °C. After being stirred for 20 min, a saturated aqueous solution of $NaHCO_3$ was added to the reaction mixture. The aqueous layer was extracted with ether, and the combined organic layers were dried over $MgSO_4$ and concentrated under

reduced pressure. The residue was purified by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 9) to give **11D** (17 mg, 100%) as a yellow oil: $[\alpha]_D^{28}$ = -27 (c = 0.85, $CHCl_3$); IR (neat, cm^{-1}) ν 2971, 2909, 2861, 1775, 1736, 1591, 1473, 1451, 1420, 1359, 1333, 1276, 1215, 1162, 1136, 1061, 1035, 965, 912, 816, 755, 693; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92 (s, 9H), 3.93 (d, *J* = 10.6 Hz, 1H), 3.98 (d, *J* = 10.6 Hz, 1H), 4.54 (s, 2H), 4.56 (d, *J* = 12.1 Hz, 1H), 4.65 (d, *J* = 12.1 Hz, 1H), 7.10-7.38 (m, 5H), 7.61 (d, *J* = 8.2 Hz, 1H), 7.68 (dd, *J* = 7.5 and 8.2 Hz, 1H), 7.95 (d, *J* = 7.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.7 (3C), 38.7, 62.8, 69.2, 73.7, 87.7, 116.7, 119.8, 125.3, 125.7, 127.7 (2C), 128.0, 128.4 (2C), 131.4, 132.3, 136.8, 138.0, 143.7, 167.1, 177.0; MALDI-TOF/MS (positive ion, α-cyano-4-hydroxycinnamic acid) calcd. for $C_{23}H_{23}F_3NaO_8S$ ($M+Na^+$) 539.10; found 539.23.

(*R*)-(+)-3-Benzyloxymethyl-3-(2,2-dimethylpropionyloxymethyl)-*3H*-isobenzofuran-1-one (12).



11D **12**

To a solution of **11D** (15 mg, 0.030 mmol) and triethylamine (22 μL, 0.15 mmol) in dimethylacetamide (0.15 mL) were added a 0.52 M solution of $Pd(dppb)(OAc)_2$ in dimethylacetamide (58 μL, 0.0030 mmol) and formic acid (5.9 μL, 0.15 mmol) at room temperature. After being stirred for 4 h at 90 °C, brine was added to the reaction mixture. The aqueous layer was extracted with ether, and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: ethyl acetate / hexane = 1 / 9) to give **12** (10 mg, 89%) as a colorless oil: $[\alpha]_D^{28}$ = +7.4 (c = 0.52, $CHCl_3$).

Assay

Inhibition of [^{3}H]PDBu Binding to PKCa

Briefly, plastic test tubes of each assay mixture (300 μL) contained 50 mM Tris-HCl (pH 7.5), 4 mM $CaCl_2$, 100 μg/mL 1,2-di-(*cis*-9-octadecenoyl)-*sn*-glycero-3-phospho-L-serine sodium salt (L-PS, from SIGMA), 4 mg/mL BSA, 10 nM [^{3}H]PDBu, 4.3-4.8 nM protein kinase Cα (human, recombinant, *Spodoptera frugiperda* from CALBIOCHEM), and each concentration of the synthetic compound or TPA. L-PS was sonicated in 50 mM Tris-HCl at 0 °C prior to use. After incubation at 0 °C for 2 h, the mixture was diluted with cold 0.5 % DMSO (2.5 mL) then filtered through a glass-fiber filter (Whatman GF/B) which had been pretreated with 0.3% polyethyleneimine for 1 h. The filter was washed four times with 2

mL of cold 0.5% DMSO. The radioactivity of each filter was counted in a scintillation vial with 5 mL of scintillator (Clear-sol I from NACALAI TESQUE) using a liquid scintillation counter. The count for the tube with 10 μM TPA was taken as the background (100% inhibition) and subtracted from the count of each tube. Data points are averages of triplicate or quadruplicate values.

2A	conc. (μM)	inhibition	SD	**2C**	conc. (μM)	inhibition	SD
	100.0	99.6	0.6		100.0	84.7	4.4
	10.0	70.2	4.7		10.0	21.7	2.7
	1.0	26.6	4.3		1.0	6.3	4.8
	0.1	10.5	2.2		0.1	2.2	3.2
2B	conc. (μM)	inhibition	SD	**2D**	conc. (μM)	inhibition	SD
	100.0	99.6	0.6		100.0	70.4	8.3
	10.0	50.9	8.6		10.0	24.7	2.9
	1.0	19.8	7.3		1.0	10.1	3.7
	0.1	5.7	3.8		0.1	3.2	2.9

The K_d value of [^{3}H]PDBu under these assay conditions was determined to be 0.19 nM by Scatchard plot analysis. K_i value was determined according to the reported equation: $K_i = IC_{50}/(1+L/K_d)$ (Sharkey, N. A.; Blumberg, P. M. *Cancer Res.* **1985**, *45*, 19-24). K_i value of PMA was determined to be 0.5 nM under the assay conditions as follows[11a]. IC_{50} value was determined from dose-dependent inhibition curve. Concentration of free [^{3}H]PDBu at the IC_{50} (L) was calculated from the counts of the free [^{3}H]PDBu (dpm), which was determined by subtracting the counts of bound [^{3}H]PDBu from that of total [^{3}H]PDBu, according to the calibration curve.

IC_{50} values of the isobenzofuranone derivatives were determined from dose-dependent inhibition curves. K_i values of the isobenzofuranone derivatives were calculated by the same equation using the L/K_d value determined for PMA.

Measurements of PKCα **Activity**

PKCα activity was determined by measuring the rate of phosphate incorporation into a PepTag® C1 peptide (Promega). Briefly, the assay (21 μL) consisted of 20 mM HEPES (pH = 7.4), 10 μM $CaCl_2$, 10 mM $MgCl_2$, 4 μg/mL L-PS (SIGMA), 1 mM ATP, 1 mM DTT, 350 μM PepTag® C1 peptide, and PMA (10 μM) or test compound (as indicated). After thermal equilibration to 30 °C for 2 min, assays were initiated by addition of 4 μL PKCα (final 6.5 nM, PANVERA) and terminated after 40 min by heating at 95 °C for 10 min. Phosphorylated and nonphosphorylated PepTag® C1 peptide were separated by agarose gel electrophoresis (50 mM Tris·HCl (pH = 8.0), 50 V, 45 min). The intact agarose gel was analyzed in a scanning by fluorescence and quantified by densitometry. The results are shown as the relative activity with respect to the controls (phosphorylation level in the absence of activator = 0%; that in the presence of

PMA 10 μM = 100%). Data points are averages of triplicate or quadruplicate values.

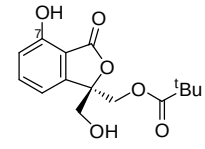
2A	conc. (μM)	activity	SD	**2C**	conc. (μM)	activity	SD
	100.0	127.1	7.8		100.0	54.2	6.3
	10.0	105.2	6.7		10.0	35.4	1.6
	1.0	31.8	15.6		1.0	10.9	3.8
	0.1	6.3	12.2		0.1	7.0	6.3
2B	conc. (μM)	activity	SD	**2D**	conc. (μM)	activity	SD
	100.0	99.0	8.9		100.0	-8.6	7.0
	10.0	79.7	7.8		10.0	1.9	2.0
	1.0	53.5	6.4		1.0	-0.5	1.0
	0.1	22.0	13.2		0.1	0.6	1.3

Construction of Binding Model of PKCαC1B Domain in Complex with Phorbol Ester or Isobenzofuranone Derivatives

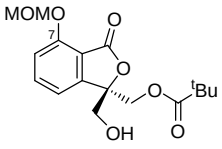
A homology model of the PKCαC1B domain was generated using DS modeling 1.1 (Accelrys) based on the PKCδC1B domain complexed with phorbol 13-acetate (PDB code:1PTR). Complexes of PKCαC1B domain with the compounds were constructed by DS modeling 1.2 (Accelrys). The preliminary binding models were generated by docking simulation. These structures were then subjected to energy minimization based on molecular dynamics. **2A'** ~ **2D'** having a propyl group instead of a dodecyl group was used for molecular modeling. For DAG analogs two types of possible binding mode, *sn*-1 mode and *sn*-2 mode, have been discussed (see, Marquez, V. E. and Blumberg, P. M. *Acc. Chem. Res.* **2003**, 36, 434-443.). Similar discussion would be applicable to the isobenzofuranone derivatives. But in this paper we focused on *sn*-1 mode binding.

Additional Discussions on the Binding Model

We have synthesized various isobenzofuranone derivatives including type B, type C, and type D derivatives and evaluated their binding ability to PKCα. The SAR data of the selected type A derivatives were already reported in the reference 11a. In addition, we found that the compounds **S23** and **S24**, having the same pivaloyl group at C3 position but no hydrophobic chain at C7 position, showed very little inhibition of [^{3}H]PDBu binding to PKCα (18% and 16% inhibition respectively even at 100 μM), while **2A** almost completely inhibited the binding of [^{3}H]PDBu to PKCα at the same concentration. These facts indicate that interaction of the hydrophobic side chain at C7 position with the lipid membrane significantly contributes to not only activation but also binding of the isobenzofuranone derivatives. The importance of the hydrophobic side



S23



S24

chain for the PKC binding was also reported for other PKC ligands (See, reference 7; Bertolini *et al. J. Org. Chem.* **2003**, *68*, 5028-2036; Yamatsugu, *et al. Chem. Asian J.* **2006**, *1*, 314-321). Since **2B** ~ **2D** showed significant inhibition of [^{3}H]PDBu binding to PKCα at 100 μM, the complexes of PKCα with these compounds should associate with lipid membrane. Actually, due to the highly lipophilic nature of the structures (CLogP = 8.78), **2A** ~ **2D** should be partitioned into the lipid membrane rather than aqueous phase even in the absence of PKCα.

Figure S1 shows hypothetical models of the interaction of **2A**- or **2D**-PKCαC1B domain with the lipid membrane. It is likely that the top part of the **2A**-PKC complex (containing hydrophobic residues such as Leu121 and 125) is inserted into the PS membrane (Figure S1A), in a similar manner to the PMA-PKC complex (See, Zhang *et al. Cell*, **1995**, *81*, 917-924; Hritz *et al. J. Med. Chem.* **2004**, *47*, 6547-6555). The reported PS-dependent binding of the PKCαC1 domain to phospholipids vesicle (Bittova *et al. J. Biol. Chem.* **2001**, *276*, 4218-4226) indicates the cationic residues of PKCαC1 domain would have positive interaction with the anionic head group of PS (Figure S1A). Such strong interaction stabilizes the active conformer. In contrast, **2D** may prefer the different type of interaction with the lipid membrane (Figure S1B), because the position of the side chain is totaly different. The results of the activation assays suggest that the latter type of interaction stabilizes the inactive conformer. In fact, weak inhibition of PKCα by **2D** was observed. When 10 μM and 10 nM of PMA was used as an activator, **2D** (100 μM) showed 7.3 ± 4.4% and 26.0 ± 9.7% inhibition respectively. The inhibitory activity of **2D** was weak because the affinity of **2D** to PKC is not strong enough to compete with PMA; however, the new concept on the importance of the hydrophobic side chain direction discussed in this manuscript should be useful for designing a novel type of inhibitor. Studies for the development of better inhibitor based on this new concept are currently underway.

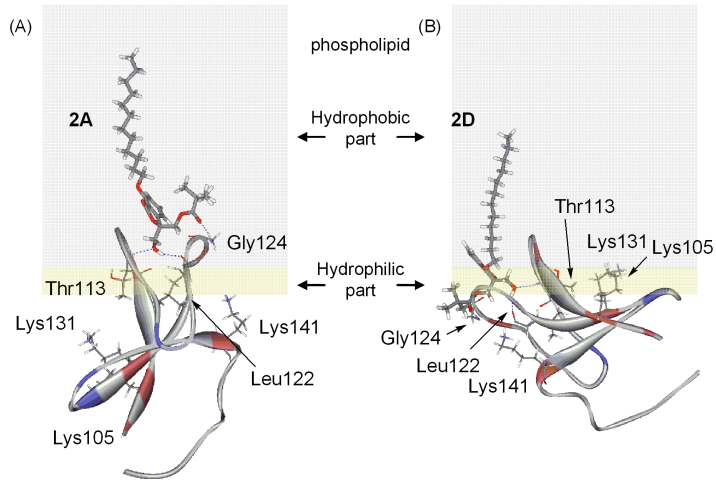
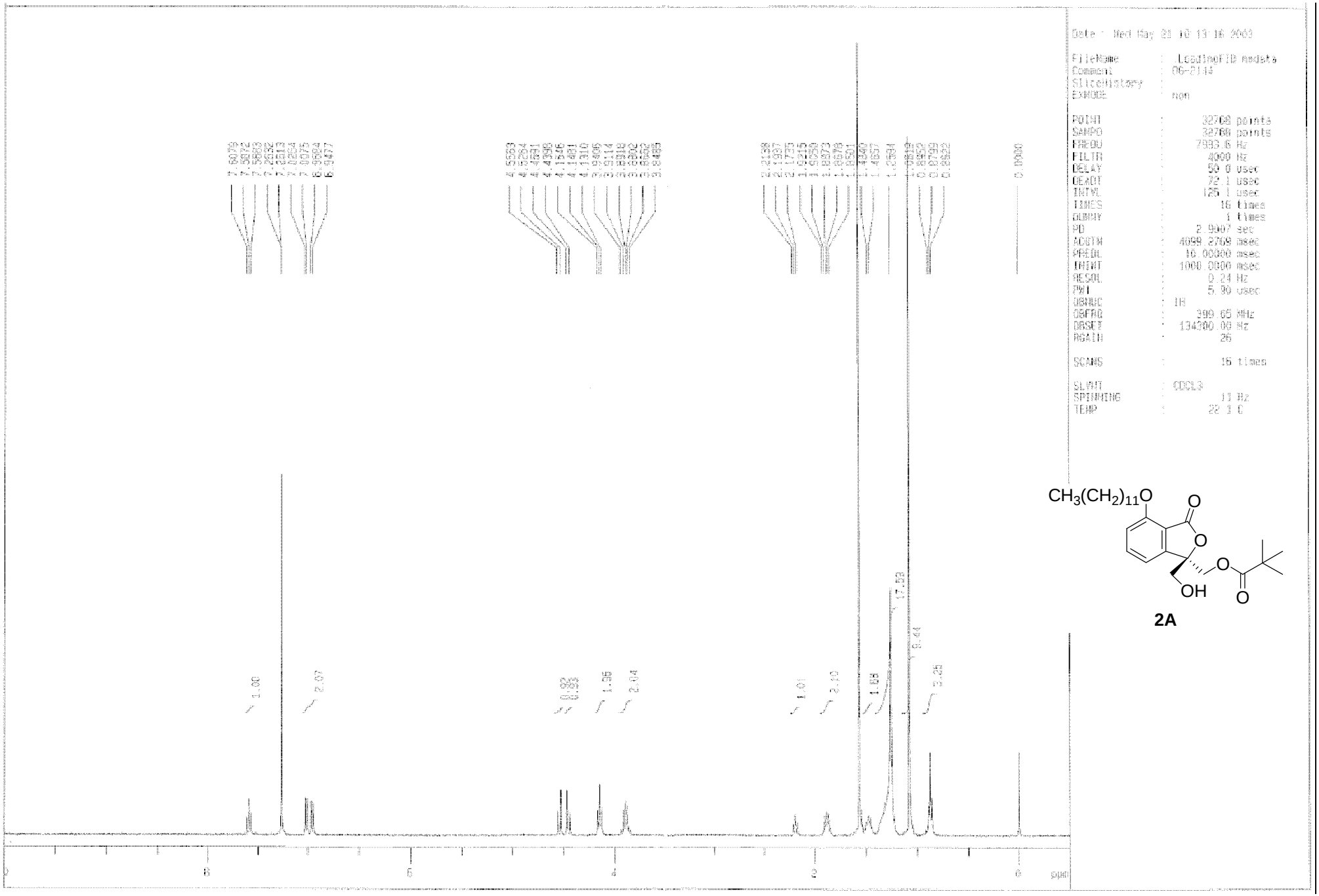


Figure S1. Hypothetical models of the interaction of **2A**- or **2D**-PKCαC1B domain with the lipid membrane. Amino acid residues that form hydrogen bondings with the ligand (Thr113, Leu122, and Gly124) and that may interact with PS (Lys105, Lys131, and Lys141) are shown.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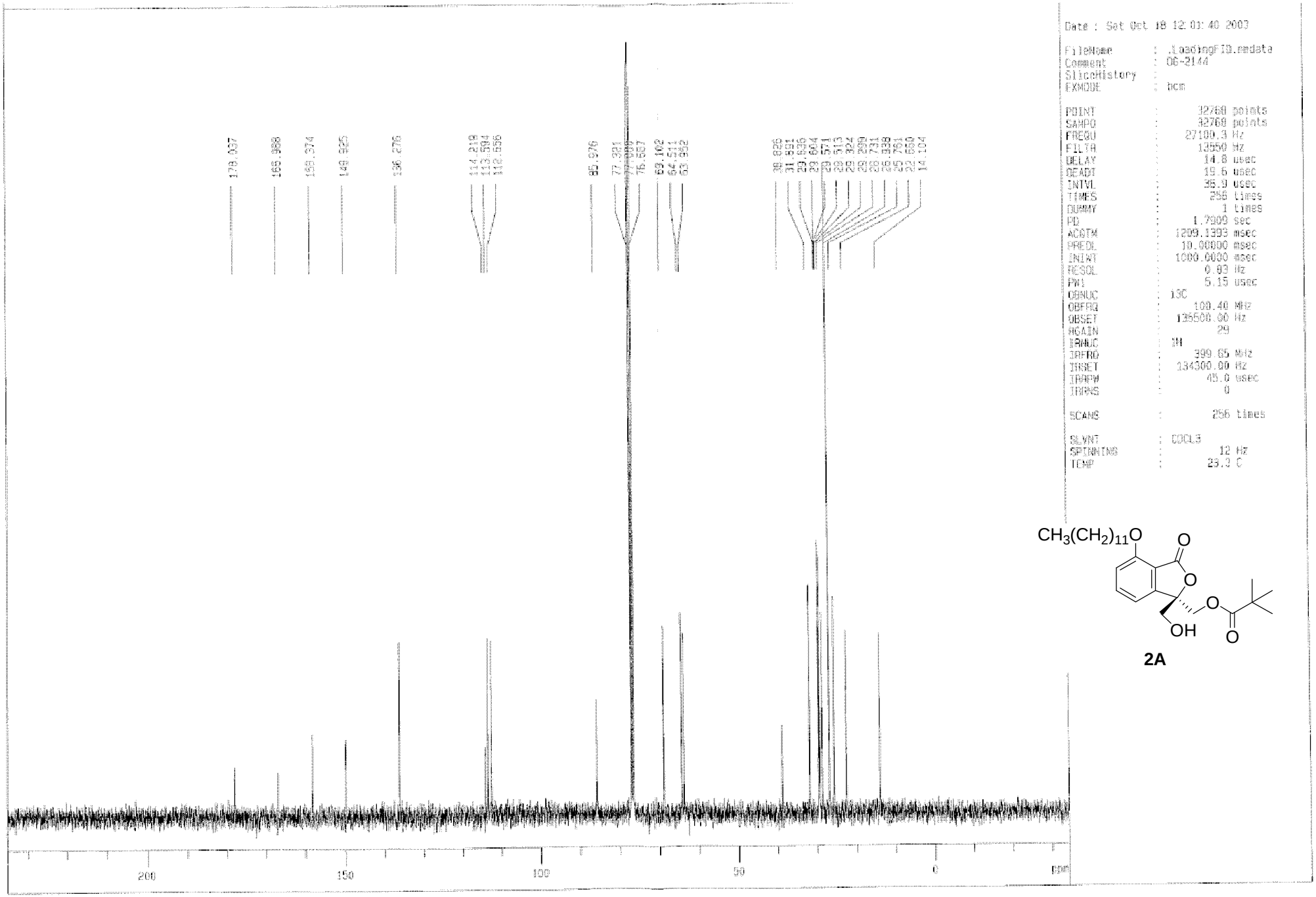

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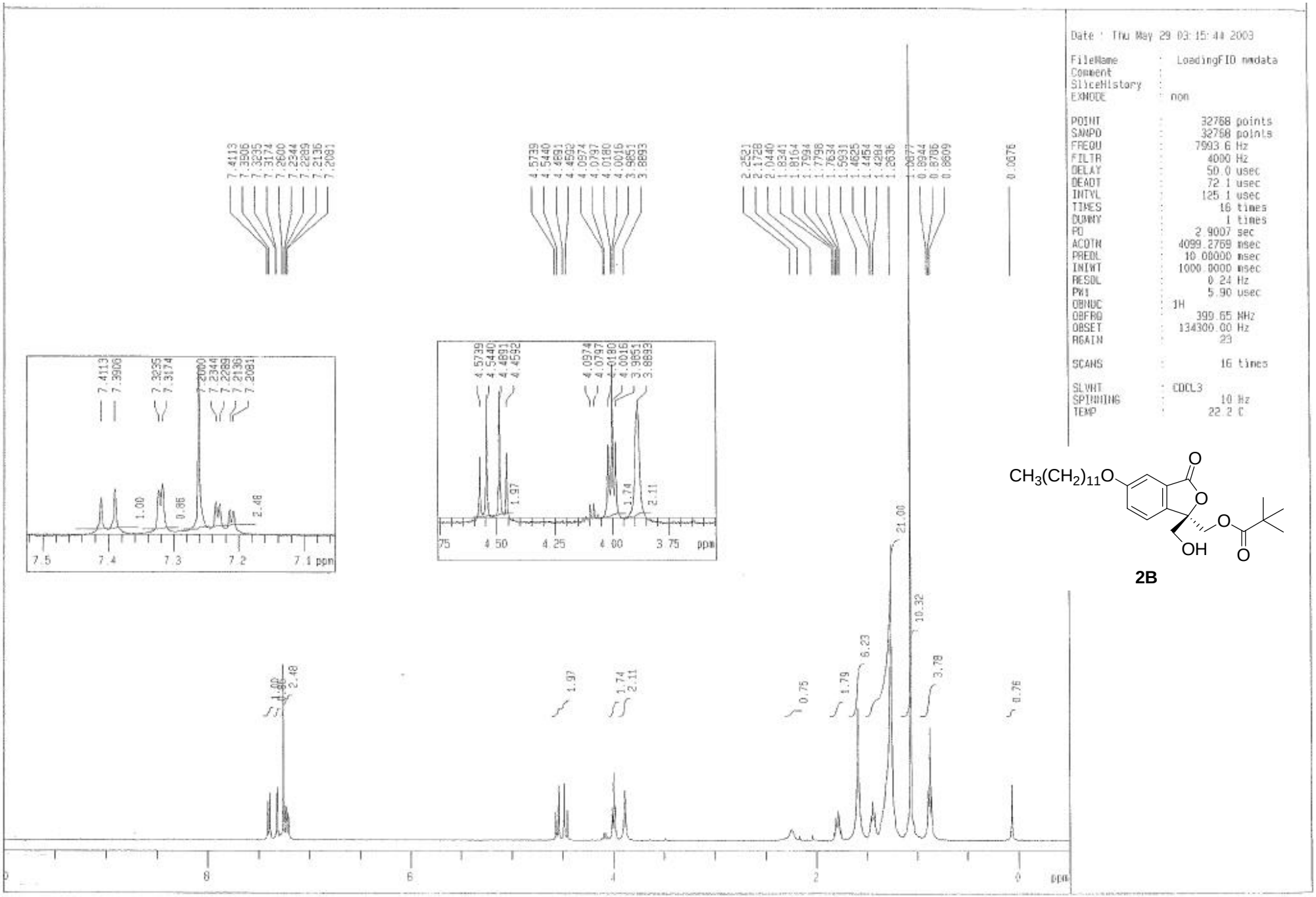
2A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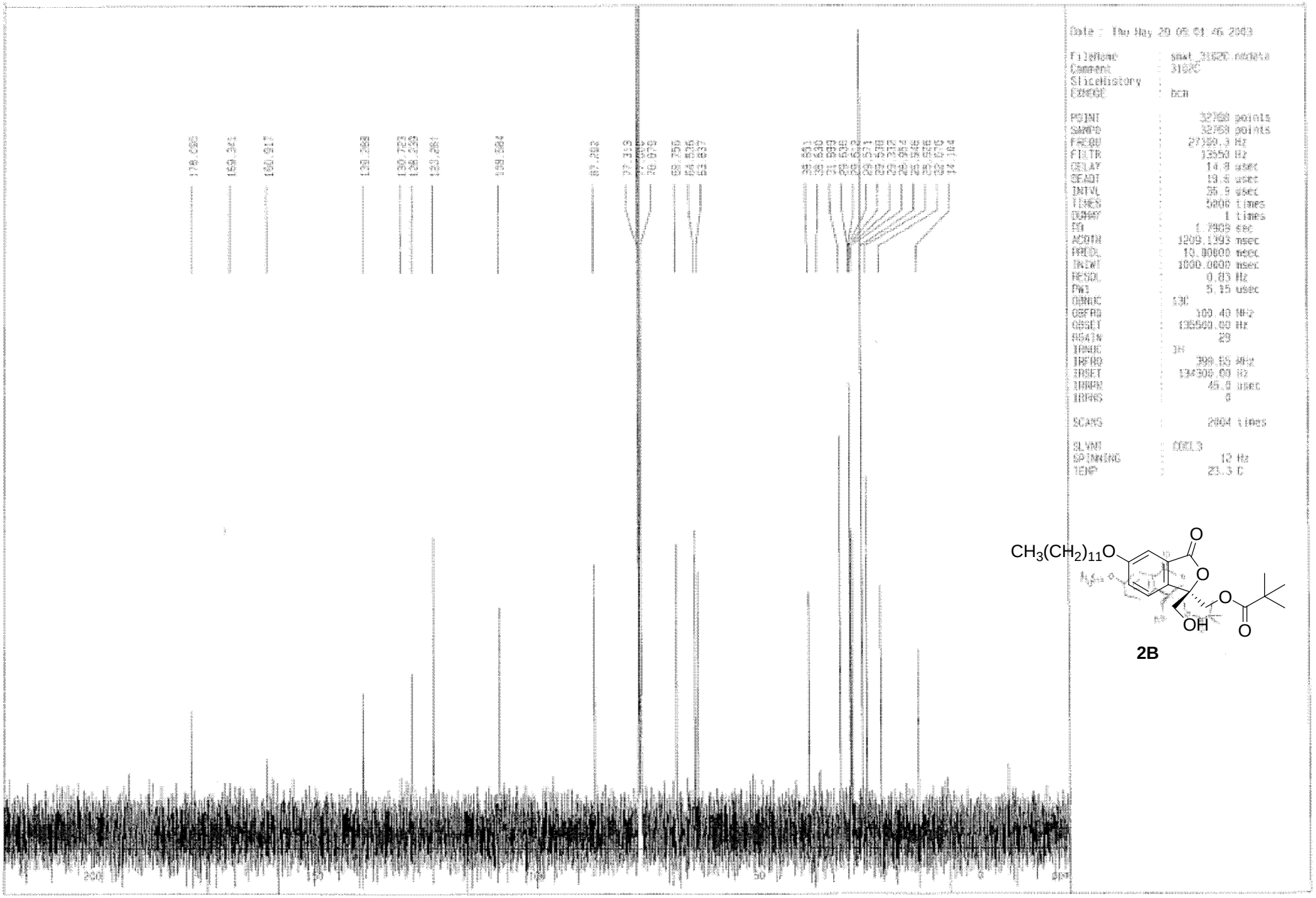
2B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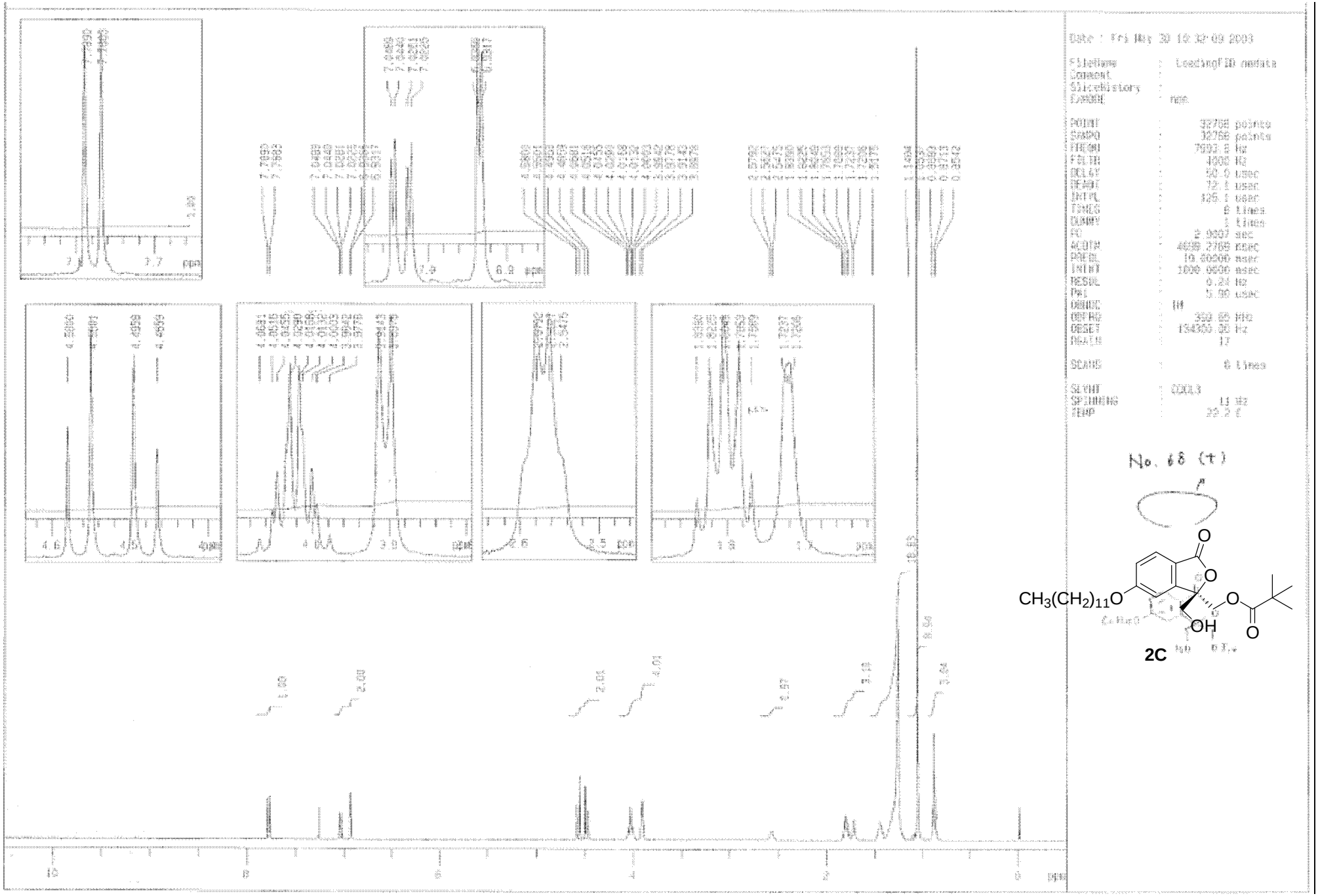
O
O
O
OH
2B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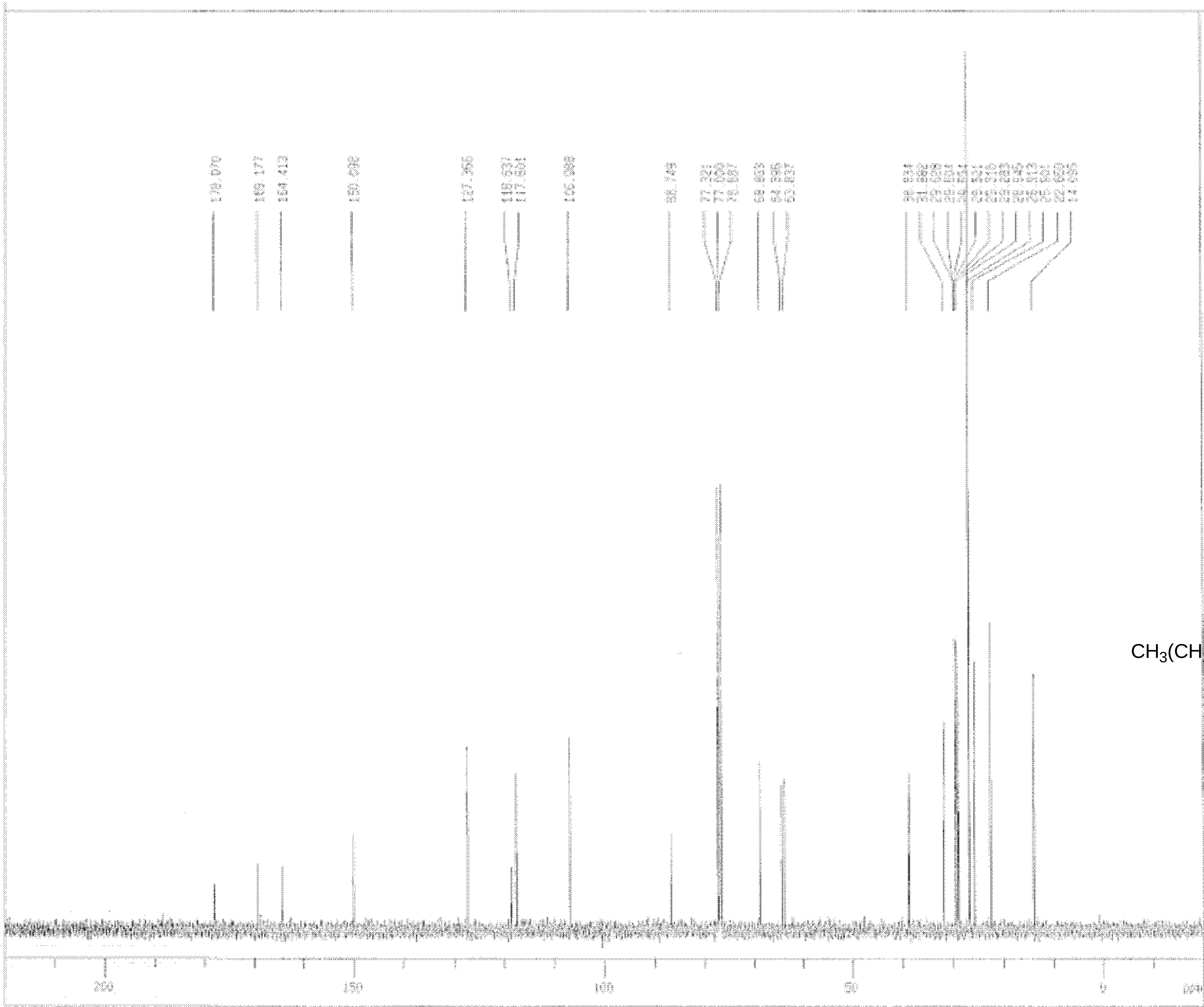
O
2C



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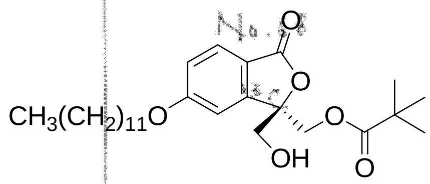

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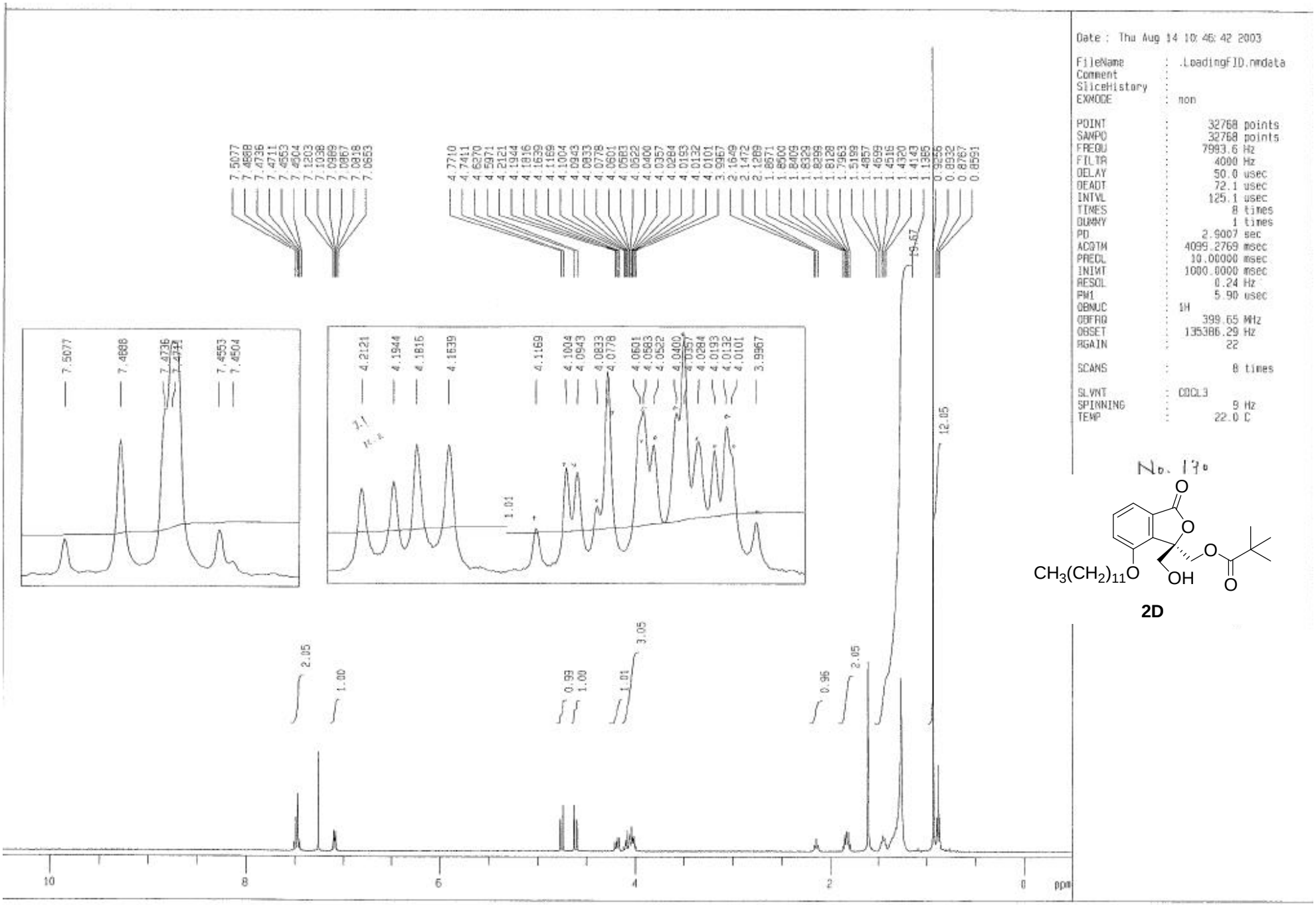
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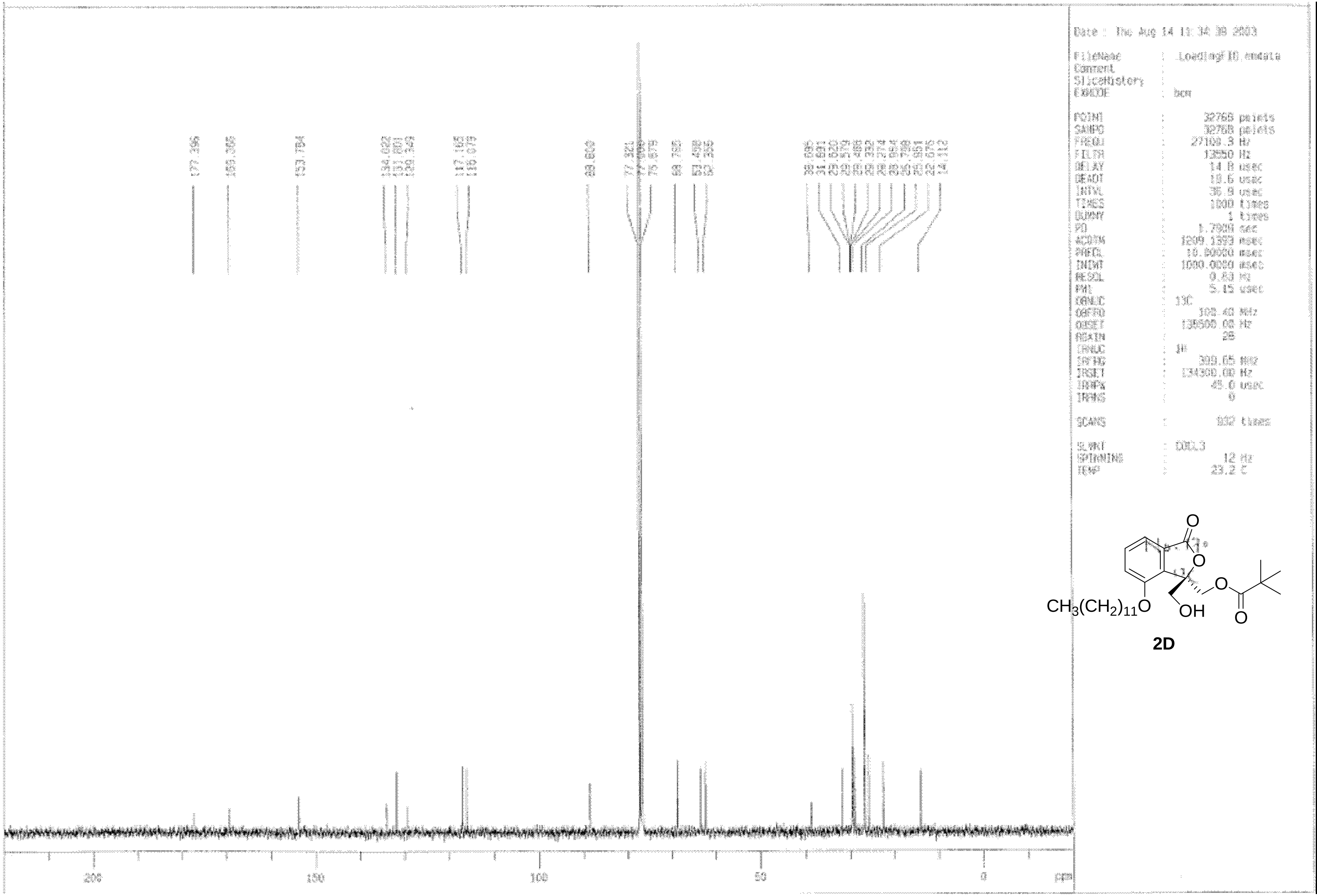

2D