Supplementary Information

Total Synthesis and Characterization of Thielocin B1 as a Protein–Protein Interaction Inhibitor of PAC3 Homodimer

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Experimental Section

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General methods for synthesis.

All commercially available reagents were used as received. Dry THF and CH_2Cl_2 (Kanto Chemical Co.) were obtained by passing commercially available pre-dried, oxygen-free formulations. All reactions were monitored by thin-layer chromatography carried out on 0.2 mm E. Merck silica gel plates (60F-254) with UV light, and visualized by *p*-anisaldehyde H_2SO_4-ethanol solution. Column chromatography and flash column chromatography were carried out with silica gel 60 N (Kanto Chemical Co. 100–210 μm) and silica gel 60 N (Kanto Chemical Co. 40–50 μm), respectively. ^{1}H NMR spectra (400 MHz and 500 MHz) and ^{13}C NMR spectra (100 MHz and 125 MHz) were recorded on JEOL JNM-AL400 and JEOL ECP-500 spectrometers, respectively, in the indicated solvent. Chemical shifts (δ) are reported in units parts per million (ppm) relative to the signal for internal tetramethylsilane (0.00 ppm for ^{1}H) for solutions in chloroform-*d*. NMR spectral data are reported as follows: chloroform-*d* (77.0 ppm for ^{13}C), methanol-d_4 (3.30 ppm for ^{1}H and 49.0 ppm for ^{13}C) when internal standard is not indicated. Multiplicities are reported by the following abbreviations: s (singlet), d (doublet), m (multiplet), brs (broad singlet), *J* (coupling constants in Hertz). EI mass spectra and EI high-resolution mass spectra, FAB mass spectra, and ESI mass spectra were measured on JEOL JMS-DX303, JEOL JMS-700, and SYNAPT G2 HDMS instruments, respectively. IR spectra were recorded on a JASCO FT/IR-4100. Only the strongest and/or structurally important absorption are reported as the IR data afforded in cm^{-1}. Melting points were measured with Yazawa Micro Melting Point BY-2 or Round Science RFS-10 and are uncorrected. Microwave irradiation was performed with Biotage Initiator^{TM}. Reversed-phase HPLC was performed by a Waters LC/MS system ZQ-2000 with XBridge^{TM} C18-3.5 μm, 4.6 x 7.5 mm (Waters), and peak area was detected at UV 254 nm. Gradient method is as follows: 10% of B (0.0 min), 10-95% of B (0.0-4.0 min), 95% of B (4.0-11.0 min), 95-10% of B (11.0-15.0 min), 10% of B (15.0 min) (A: 0.1% HCOOH/H_2O, B: 0.1% HCOOH/MeOH).

Synthetic procedures and characterization for products

Methyl 3-hydroxy-8-methoxy-1,4,6,9-tetramethyl-11-oxo-11*H*-dibenzo-[*b*,*e*][1,4]-dioxepine-7-carboxylate (6).[1] To a suspension of phenol **5** (2.70, 7.21 mmol) in water (350 mL) was added K_2CO_3 (19.9 g, 144 mmol, 20 equiv) and $K_3[Fe(CN)_6]$ (5.22 g, 15.9 mmol, 2.2 equiv) in H_2O (150 mL) at room temperature. After being stirred at the same temperature for 4.5 h, the reaction mixture was diluted with EtOAc and was quenched with 6 M HCl. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried with $MgSO_4$, and filtered. The filtrate was concentrated in vacuo and the resulting residue was recrystallized from CH_2Cl_2/hexane to afford lactone **6** (2.21 g, 5.94 mmol, 82%) as a yellow solid. M.p. 227-228 °C [lit.[1] M.p. 220-221 °C]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.51 (3H, s), 5.42 (1H, s), 3.92 (3H, s), 3.74 (3H, s), 2.43 (3H, s), 2.37 (3H, s), 2.34 (3H, s), 2.28 (3H, s); ^{13}C NMR (100 MHz, CD_3OD) δ 169.1, 164.8, 162.5, 161.9, 153.9, 147.6, 146.0, 143.4, 127.7, 126.5, 123.0, 115.7, 114.5, 112.9, 62.6, 52.9, 21.2, 14.4, 10.0, 9.8; IR (Neat) 3330, 2930, 1729, 1699, 1611, 1590, 1459, 1413, 1343, 1272, 1135 cm^{-1}; HREIMS: calcd for $C_{20}H_{20}O_7$: 372.1209, found: 372.1208.

Methyl 3,8-di(methoxymethoxy)-1,4,6,9-tetramethyl-11-oxo-11*H*-dibenzo-[*b,e*][1,4]-dioxepine-7-carboxylate (S1). To a suspension of depsidone derivative **6** (3.50 g, 9.40 mmol) in dry CH_2Cl_2 (30 mL) was added BCl_3 (1.0 M in CH_2Cl_2, 28.2 mL, 28.2 mmol, 3.0 equiv) at 0 °C under an argon atmosphere. After being stirred at room temperature for 4.5 h, the reaction mixture was diluted with EtOAc and quenched with water at 0 °C. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried with $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give phenol as a yellow solid. The crude phenol was used for next reaction without further purification.

To a solution of the crude phenol in DMF (40 mL) was added DIPEA (16.4 mL, 94.0 mmol, 10 equiv) and methoxymethyl (MOM) chloride (3.57 mL, 47.0 mmol, 5.0 equiv) at room temperature under an argon atmosphere. After being stirred at the same temperature for 1 h, the reaction mixture was diluted with EtOAc and quenched with 3 M HCl. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with brine twice, saturated aqueous $NaHCO_3$ and brine, dried with $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 3:1) to afford diMOM ether **S1** (3.58 g, 8.01 mmol, 85% for 2 steps) as a white solid. M.p. 118–119 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.80 (1H, s), 5.23 (2H, s), 4.93 (2H, s), 3.90 (3H, s), 3.53 (3H, s), 3.47 (3H, s), 2.47 (3H, s), 2.39 (3H, s), 2.33 (3H, s), 2.30 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.5, 162.9, 160.4, 158.9, 150.1, 146.5, 144.7, 142.1, 126.5, 125.6, 122.5, 115.8, 114.7, 113.1, 100.5, 94.2, 57.5, 56.3, 52.3, 21.3, 14.5, 10.5, 10.0; IR (Neat) 2953, 1735, 1730, 1607, 1566, 1458, 1342, 1281, 1127, 977, 940, 759 cm^{-1}; HREIMS calcd for $C_{23}H_{26}O_9$ 446.1577, found 446.1559.

Methyl 8-(4-chlorobenzyloxy)-3-methoxymethoxy-1,4,6,9-tetramethyl-11-oxo-11*H*-dibenzo-[*b,e*][1,4]-dioxepine-7-carboxylate (7). To a suspension of the diMOM ether **S1** (3.20 g, 7.17 mmol) in MeOH (72 mL) was added iodine (717 mg, 1 wt/vol% iodine/MeOH) at room temperature. After being stirred at 45 °C for 16 h, the reaction mixture was cooled to room temperature, diluted with EtOAc and quenched with saturated aqueous $Na_2S_2O_3$. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried with $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give phenol as a white solid. The crude phenol was used for next reaction without further purification.

To a solution of crude phenol in DMF (70 mL) was added K_2CO_3 (4.95 g, 35.9 mmol, 5.0 equiv) and 4-chlorobenzyl chloride (2.30 g, 14.3 mmol, 2.0 equiv) at room temperature under an argon atmosphere. After being stirred at the same temperature for 11 h, the reaction mixture was filtered through a pad of Celite®. The filtrate was diluted with EtOAc and acidified with 3 M HCl. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with brine twice, saturated aqueous $NaHCO_3$ and brine, dried with $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 9:1) to afford benzyl ether **7** (2.40 g, 4.55 mmol, 63% for 2 steps) as a white solid. M.p. 129–130 °C; ^{1}H NMR (400

MHz, $CDCl_3$) δ 7.35 (2H, d, J =8.8 Hz), 7.32 (2H, d, J = 8.8 Hz), 6.81 (1H, s), 5.23 (2H, s), 4.82 (2H, s), 3.80 (3H, s), 3.47 (3H, s), 2.48 (3H, s), 2.39 (3H, s), 2.34 (3H, s), 2.29 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.6, 162.8, 160.4, 158.9, 151.1, 146.4, 144.7, 142.1, 135.2, 134.0, 129.1, 128.6, 126.4, 125.6, 122.2, 115.8, 114.6, 113.1, 94.1, 75.6, 56.3, 52.4, 21.3, 14.4, 10.2, 10.0; IR (Neat) 2952, 1735, 1606, 1454, 1342, 1282, 1204, 1130, 1063, 980, 759 cm^{-1}; HREIMS calcd for $C_{28}H_{27}ClO_8$ 526.1394, found 526.1380.

Methyl 2-(4-chlorobenzyloxy)-4-hydroxy-5-(2-hydroxymethyl-5-methoxymethoxy-3,6-dimethyl-phenoxy)-3,6-dimethylbenzoate (8). To a solution of lactone **7** (150 mg, 0.285 mmol) in dry THF (31.7 mL) was added $NaBH_4$ (323 mg, 8.54 mmol, 30 equiv) at 0 °C under an argon atmosphere. After being stirred at the same temperature for 90 h, the reaction mixture was diluted with EtOAc and quenched with 3 M HCl at 0 °C. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried with $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 9:1) to afford benzyl alcohol **8** (99.8 mg, 0.188 mmol, 66%) as a white solid. M.p. 159–160 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (4H, s), 6.71 (1H, s), 5.17 (1H, d, J = 6.8 Hz), 5.12 (1H, d, J = 6.8 Hz), 5.00 (1H, d, J = 10.8 Hz), 4.85 (1H, d, J = 10.8 Hz), 4.81 (1H, d, J = 10.8 Hz), 4.67 (1H, d, J = 10.8 Hz), 3.78 (3H, s), 3.45 (3H, s), 2.37 (3H, s), 2.13 (3H, s), 2.09 (3H, s), 1.76 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.6, 156.1, 155.2, 150.3, 147.7, 140.8, 136.1, 135.7, 133.7, 129.2, 128.5, 124.4, 121.9, 121.3, 118.7, 115.6, 111.7, 94.6, 75.5, 57.2, 56.1, 52.1, 19.3, 13.8, 9.4, 8.9; IR (Neat) 3422, 2952, 2927, 1726, 1611, 1586, 1492, 1460, 1289, 1209, 1113, 1089, 1057, 980, 755 cm^{-1}; HRESIMS calcd for $C_{28}H_{31}ClO_8Na$ $[M+Na]^+$ 553.1605, found 553.1606.

Methyl 4-benzyloxy-2-(4-chlorobenzyloxy)-5-(3-hydroxy-2,5,6-trimethylphenoxy)-3,6-dimethylbenzoate (9). To a solution of phenol **8** (530 mg, 0.998 mmol) in acetone (4.0 mL) was added K_2CO_3 (552 mg, 3.99 mmol, 4.0 equiv) and BnBr (0.154 mL, 1.30 mmol, 1.3 equiv) at room temperature under an argon atmosphere. After being stirred at the same temperature for 5 h, the reaction mixture was diluted with EtOAc and quenched with 1 M HCl. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried with $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 9:1) to afford Methyl 4-benzyloxy-2-(4-chlorobenzyloxy)-5-(2-hydroxymethyl-5-methoxymethoxy-3,6-dimethylphenoxy)-3,6-dimeth ylbenzoate (**S2**) (572 mg, 0.921 mmol, 92%) as a white solid. M.p. 108–109 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (4H, s), 7.24-7.27 (3H, m), 7.01-7.04 (2H, m), 6.69 (1H, s), 5.17 (1H, d, J = 7.0 Hz), 5.14 (1H, d, J = 7.0 Hz), 4.86 (2H, s), 4.68 (1H, d, J = 11.6 Hz), 4.55 (1H, d, J = 7.0 Hz), 4.52 (1H, d, J = 7.0 Hz), 4.12 (1H, d, J = 11.6 Hz), 3.84 (3H, s), 3.45 (3H, s), 2.26 (3H, s), 2.24 (3H, s), 2.14 (3H, s), 1.98 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.2, 155.7, 155.4, 149.4, 148.4, 146.1, 137.0, 136.2, 135.6, 133.9, 129.1, 128.6, 128.2, 127.9, 127.3, 126.4, 124.9, 124.8, 124.2, 115.4, 111.8, 94.6, 75.5, 74.8, 57.1, 56.0, 52.3, 19.1, 13.9, 10.4, 9.5; IR (Neat) 3522, 2952, 1731, 1610, 1580, 1492, 1444, 1367, 1337, 1284, 1207, 1115, 1091, 1059, 983, 753 cm^{-1}; HREIMS calcd

for $C_{35}H_{37}ClO_8$ 620.2177, found 620.2150.

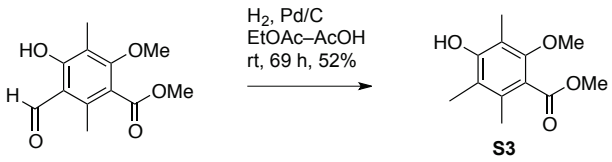
To a solution of benzyl ether **S2** (460 mg, 0.741 mmol) in dry CH_2Cl_2 (7.6 mL) was added trifluoroacetic acid (0.4 mL) and triethylsilane (2.32 mL, 14.8 mmol, 20 equiv) at −30 °C under an argon atmosphere and the mixture was stirred at 0 °C for 30 min. To the reaction mixture was added MeOH (8.0 mL) and 6 M HCl (1.6 mL) at 0 °C. After being stirred at 50 °C for 19 h, the reaction mixture was cooled to room temperature, dulited with EtOAc and quenched with saturated aqueous $NaHCO_3$. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with brine, dried with $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 5:1) to afford phenol **9** (367 mg, 6.54 mmol, 88%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (4H, s), 7.25-7.28 (3H, m), 7.06-7.09 (2H, m), 6.36 (1H, s), 4.85 (2H, s), 4.67 (1H, brs), 4.58 (1H, d, J = 11.2 Hz), 4.49 (1H, d, J = 11.2 Hz), 3.82 (3H, s), 2.14 (3H, s), 2.10 (3H, s), 2.08 (3H, s), 1.98 (3H, s), 1.93 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.6, 155.0, 151.9, 149.1, 148.9, 146.2, 137.0, 135.7, 135.5, 133.8, 129.1, 128.6, 128.1, 127.7, 127.3, 125.9, 124.6, 124.4, 119.4, 112.3, 111.8, 75.4, 74.5, 52.3, 19.9, 13.9, 12.5, 10.2, 9.4; IR (Neat) 3443, 2950, 2925, 1729, 1618, 1598, 1454, 1407, 1338, 1285, 1212, 1099, 755 cm^{-1}; HREIMS calcd for $C_{33}H_{33}ClO_6$ 560.1966, found 560.1942.

Methyl 4-benzyloxy-2-(4-chlorobenzyloxy)-5-(3-methoxy-2,5,6-trimethylphenoxy)-3,6-dimethylbenzoate (10). To a solution of phenol **9** (500 mg, 0.891 mmol) in DMF (4.0 mL) was added K_2CO_3 (690 mg, 4.99 mmol, 5.6 equiv) and MeI (0.128 mL, 2.05 mmol, 2.3 equiv) at room temperature under an argon atmosphere. After being stirred at the same temperature for 8 h, the reaction mixture was diluted with EtOAc and quenched with 1 M HCl. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with brine twice, saturated aqueous $NaHCO_3$ and brine, dried with $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 9:1) to afford methyl ether **10** (469 mg, 0.815 mmol, 91%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (4H, s), 7.24-7.26 (3H, m), 7.05-7.07 (2H, m), 6.41 (1H, s), 4.85 (2H, s), 4.57 (1H, d, J = 11.2 Hz), 4.47 (1H, d, J = 11.2 Hz), 3.82 (3H, s), 3.74 (3H, s), 2.15 (3H, s), 2.13 (3H, s), 2.09 (3H, s), 2.00 (s, 3H, s), 1.93 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.5, 155.9, 154.9, 149.0, 148.8, 146.2, 137.1, 135.7, 135.0, 133.8, 129.1, 128.6, 128.1, 127.6, 127.3, 125.9, 124.5, 124.4, 119.6, 114.4, 107.7, 75.4, 74.4, 55.7, 52.2, 20.3, 13.9, 12.6, 10.2, 9.4; IR (Neat) 2950, 2927, 1731, 1613, 1599, 1583, 1492, 1338, 1283, 1206, 1125, 754 cm^{-1}; HREIMS calcd for $C_{34}H_{35}ClO_6$ 574.2122, found 574.2133.

4-Benzyloxy-2-(4-chlorobenzyloxy)-5-(3-methoxy-2,5,6-trimethylphenoxy)-3,6-dimethyl-benzoic acid (2b). To a solution of methyl ether **10** (145 mg, 0.252 mmol) in dioxane (1.0 mL) was added 2 M aqueous NaOH (2.0 mL, 4.0 mmol, 16 equiv) and ethylene glycol (0.5 mL) at room temperature. After being stirred at 200 °C under microwave irradiation for 40 min, the reaction mixture was cooled to room temperature, diluted with EtOAc and quenched with 3 M HCl. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with brine, dried with $MgSO_4$ and filtered. The filtrate was

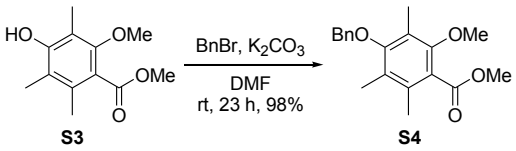
concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/ EtOAc = 4:1) to afford carboxylic acid **2b** (118 mg, 211 mmol, 84%) as an orange oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23-7.32 (7H, m), 7.03-7.05 (2H, m), 6.41 (1H, s), 4.85 (2H, s), 4.54 (1H, d, J = 11.2 Hz), 4.44 (1H, d, J = 11.2 Hz), 3.74 (3H, s), 2.26 (3H, s), 2.14 (3H, s), 2.06 (3H, s), 1.99 (3H, s), 1.92 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 155.8, 154.9, 149.1, 148.9, 146.3, 137.0, 135.2, 135.0, 133.9, 129.5, 128.6, 128.0, 127.6, 127.2, 125.5, 124.9, 124.5, 119.5, 114.4, 107.7, 75.6, 74.3, 55.7, 20.3, 14.0, 12.6, 10.2, 9.4; IR (Neat) 3380, 2926, 1725, 1699, 1614, 1599, 1580, 1492, 1455, 1365, 1219, 1126, 1092 cm^{-1}; HREIMS calcd for $C_{33}H_{33}ClO_6$ 560.1966, found 560.1941.

Methyl 4-hydroxy-2-methoxy-3,5,6-trimethylbenzoate (S3).



To a solution of methyl 3-formyl-4-hydroxy-5-methoxy-2,5-dimethylbenzoate[1] (200 mg, 0.839 mmol) in EtOAc (10 mL) and AcOH (1.0 mL) was added 10% palladium on carbon (300 mg, 150 wt%) at room temperature, and the flask was purged with hydrogen 7 times. After being stirred at the same temperature for 69 h, the reaction mixture was filtered through a pad of Celite®. The filtrate was concentrated in vacuo, and the resulting residue was recrystallized from CH_2Cl_2/hexane to afford trimethylbenzene **S3** (97.5 mg, 0.435 mmol, 52%) as a white solid. M.p. 81–82 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.79 (1H, s), 3.90 (3H, s), 3.74 (3H, s), 2.17 (3H, s), 2.16 (3H, s), 2.13 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.6, 153.73, 153.71, 132.3, 121.6, 118.2, 114.1, 62.0, 52.1, 16.7, 11.6, 8.8; IR (Neat) 3458, 2949, 1728, 1713, 1583, 1467, 1294, 1202, 1179, 1108 cm^{-1}; HREIMS calcd for $C_{12}H_{16}O_4$ 224.1049 ,found 224.1059.

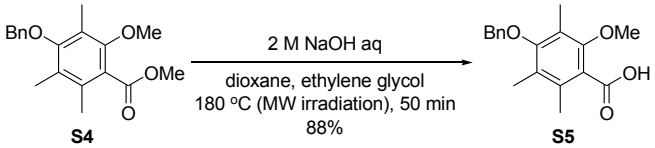
Methyl 4-benzyloxy-2-methoxy-3,5,6-trimethylbenzoate (S4).



To a solution of phenol **S3** (780 mg, 3.48 mmol) in DMF (15 mL) was added K_2CO_3 (1.44 g, 10.4 mmol, 3.0 equiv) and BnBr (0.50 mL, 4.2 mmol, 1.2 equiv) at room temperature under an argon atmosphere. After being stirred at the same temperature for 23 h, the reaction mixture was filtered through a pad of Celite®. The filtrate was diluted with EtOAc and acidified with 3 M HCl. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with brine twice, saturated aqueous $NaHCO_3$ and brine, dried with $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 5:1) to afford benzyl ether **S4** (1.08 g, 3.42 mmol, 98%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-7.48 (5H, m), 4.75 (2H,

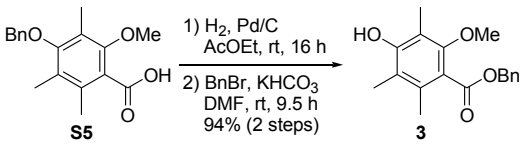
s), 3.93 (3H, s), 3.75 (3H, s), 2.21 (3H, s), 2.18 (3H, s), 2.17 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.1, 156.9, 153.7, 137.0, 132.3, 128.2, 127.8, 127.5, 125.9, 125.2, 121.9, 74.2, 61.4, 51.8, 16.4, 12.3, 9.4; IR (Neat) 2948, 1731, 1577, 1455, 1327, 1284, 1200, 1177, 1105, 1005 cm^{-1}; HREIMS calcd for $C_{19}H_{22}O_4$ 314.1518, found 314.1499.

4-Benzyloxy-2-methoxy-3,5,6-trimethylbenzoic acid (S5)



To a solution of methyl ester **S4** (300 mg, 0.954 mmol) in dioxane (1.2 mL) was added 2 M aqueous NaOH (2.4 mL, 4.80 mmol, 5.0 equiv) and ethylene glycol (0.8 mL) at room temperature. After being stirred at 180 °C under microwave irradiation for 50 min, the reaction mixture was cooled to room temperature, diluted with EtOAc and quenched with 3 M HCl. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with brine, dried with $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 1:1) to afford carboxylic acid **S5** (251 mg, 0.836 mmol, 88%) as a white solid. M.p. 124–125 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36-7.47 (5H, m), 4.77 (2H, s), 3.81 (3H, s), 2.34 (3H, s), 2.23 (3H, s), 2.22 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0, 157.7, 154.2, 137.1, 133.6, 128.6, 128.2, 127.8, 126.7, 123.9, 122.4, 74.5, 62.1, 16.9, 12.7, 9.8; IR (Neat) 2934, 1699, 1578, 1455, 1322, 1221,1106, 696 cm^{-1}; HREIMS calcd for $C_{18}H_{20}O_4$ 300.1362, found 300.1347.

Benzyl 4-hydroxy-2-methoxy-3,5,6-trimethylbenzoate (3)

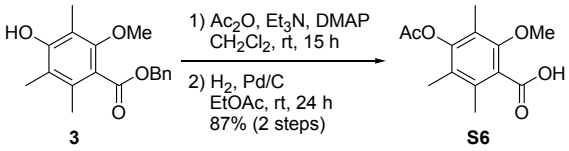


To a solution of carboxylic acid **S5** (3.50 g, 11.7 mmol) in EtOAc (30 mL) was added 5% palladium on carbon (2.0 g, 57 wt%) at room temperature, and the flask was purged with hydrogen 7 times. After being stirred at the same temperature for 16 h, the reaction mixture was filtered through a pad of Celite®. The filtrate was concentrated in vacuo to give phenol as a white solid. The crude phenol was used for next reaction without further purification.

To a solution of crude phenol in DMF (30 mL) was added $KHCO_3$ (3.21 g, 32.1 mmol, 2.7 equiv) and BnBr (1.53 mL, 12.8 mmol, 1.09 equiv) at room temperature under an argon atmosphere. After being stirred at the same temperature for 9.5 h, the reaction mixture was filtered through a pad of Celite®. The filtrate was diluted with EtOAc and acidified with 3 M HCl. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with brine twice, saturated aqueous $NaHCO_3$ and brine, dried with $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the resulting residue was

purified by column chromatography on silica gel (eluted with hexane/EtOAc = 5:1) to afford benzyl ester **3** (3.29 g, 10.9 mmol, 94% for 2 steps) as an orange solid. M.p. 61–62 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31-7.45 (5H, m), 5.36 (2H, s), 4.78 (1H, s), 3.64 (3H, s), 2.14 (6H, s), 2.11 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.0, 153.8, 153.7, 135.8, 132.3, 128.5, 128.4, 128.2, 121.5, 118.3, 114.9, 66.9, 61.9, 16.6, 11.6, 8.8; IR (Neat) 3466, 2943, 1721, 1584, 1456, 1291, 1190, 1108 cm^{-1}; HREIMS calcd for $C_{18}H_{20}O_4$ 300.1362, found 300.1369.

4-Acetoxy-2-methoxy-3,5,6-trimethylbenzoic acid (S6)

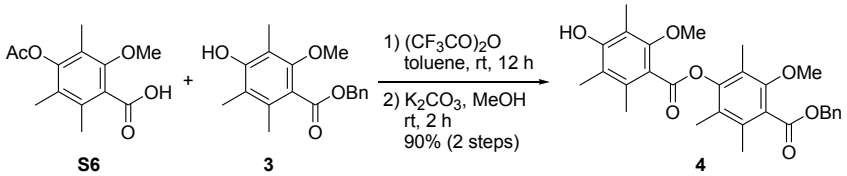


3 **S6**

To a solution of benzyl ester **3** (1.50 g, 4.99 mmol) in dry CH_2Cl_2 (10 mL) was added acetic anhydride (0.567 mL, 5.99 mmol, 1.2 equiv), triethylamine (2.09 mL, 15.0 mmol, 3.0 equiv) and DMAP (61.0 mg, 0.499 mmol, 0.10 equiv) at room temperature under an argon atmosphere. After being stirred at the same temperature for 15 h, the reaction mixture was diluted with EtOAc and quenched with 3 M HCl. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried with $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give acetate as a white solid. The crude acetate was used for next reaction without further purification.

To a solution of crude acetate in EtOAc (30 mL) was added 5% palladium on carbon (800 mg, 53 wt%) at room temperature, and the flask was purged with hydrogen 7 times. After being stirred at room temperature for 24 h, the reaction mixture was filtered through a pad of Celite®. The filtrate was concentrated in vacuo, and the resulting residue was recrystallized from CH_2Cl_2/hexane to afford carboxylic acid **S6** (1.09 g, 4.33 mmol, 87% for 2 steps) as a white solid. M.p.158–159 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.81 (3H, s), 2.37 (3H, s), 2.31 (3H,s), 2.09 (3H, s), 2.06 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 168.6, 153.6, 149.7, 132.9, 126.3, 125.5, 121.7, 62.3, 20.4, 16.8, 12.7, 9.7; IR (Neat) 3265, 2942, 1754, 1737, 1579, 1462, 1370, 1200, 1097 cm^{-1}; HREIMS calcd for $C_{13}H_{16}O_5$ 252.0998, found 252.0997.

4-Benzyloxycarbonyl-3-methyl-2,5,6-trimethylphenyl 4-hydroxy-2-methoxy-3,5,6-trimethyl-benzoate (4)



S6 **3** **4**

To a solution of carboxylic acid **S6** (1.09 g, 0.432 mmol, 1.2 equiv) in dry toluene (30 mL) was added phenol **3** (1.08 g, 3.60 mmol, 1.0 equiv) and trifluoroacetic anhydride (2.00 mL, 14.4 mmol, 4.0 equiv) at room temperature under an argon atmosphere. After being stirred at the same temperature for 12 h, the reaction mixture was quenched with 2 M aqueous NaOH. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with brine, dried with $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give ester as a colorless oil. The crude ester was used for next reaction without further purification.

To a solution of crude ester in MeOH (10 mL, 2.8 mL/mmol) was added K_2CO_3 (746 mg, 5.40 mmol, 1.5 equiv) at room temperature. After being stirred at the same temperature for 2 h, the reaction mixture was diluted with EtOAc and quenched with 1 M HCl. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried with $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the resulting residue was recrystallized from CH_2Cl_2/hexane to afford the phenol **4** (1.59 g, 3.23 mmol, 90% for 2 steps) as a white solid. M.p. 172–173 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33-7.47 (5H, m), 5.39 (2H, s), 4.90 (1H, s), 3.79 (3H, s), 3.70 (3H, s), 2.36 (3H, s), 2.23 (3H, s), 2.22 (3H, s), 2.193 (3H, s), 2.188 (3H, s), 2.17 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4, 166.8, 154.6, 154.5, 153.5, 149.7, 135.6, 133.3, 132.6, 128.50, 128.48, 128.3, 127.1, 125.7, 122.1, 120.4, 118.7, 114.5, 67.0, 62.1, 61.9, 17.1, 16.7, 12.9, 11.8, 10.1, 9.0; IR (Neat) 3481, 2942, 1736, 1733, 1576, 1463, 1456, 1286, 1159, 1097, 1078 cm^{-1}; HRFABMS calcd for $C_{29}H_{32}O_7Na$ $[M+Na]^+$ 515.2046, found 515.2076.

Preparation of Ester 11. To a solution of carboxylic acid **2b** (70.0 mg, 0.125 mmol, 1.2 equiv) in dry toluene (2.0 mL) was added phenol **4** (51.2 mg, 0.104 mmol) and trifluoroacetic anhydride (0.579 mL, 4.16 mmol, 40 equiv) at room temperature under an argon atmosphere. After being stirred at 80 °C for 9 h, the reaction mixture was cooled at room temperature and quenched with 2 M aqueous NaOH. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with brine, dried with $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by flash column chromatography on silica gel (eluted with hexane/EtOAc = 9:1) to afford ester **11** (105 mg, 0.101 mmol, 97%) as a white solid. M.p. 85–86 °C;^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46-7.48 (2H, m), 7.26-7.40 (10H, m), 7.06-7.08 (2H, m), 6.44 (1H, s), 5.40 (2H, s), 4.94 (2H, s), 4.60 (1H, d, J = 11.0 Hz), 4.49 (1H, d, J = 11.0 Hz), 3.78 (3H, s), 3.76 (3H, s), 3.71 (3H, s), 2.41 (3H, s), 2.35 (3H, s), 2.25 (3H, s), 2.21 (3H, s), 2.18 (3H, s), 2.171 (3H, s), 2.168 (3H, s), 2.14 (3H, s), 2.10 (3H, s), 2.05 (3H, s), 1.98 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.3, 166.2, 165.8, 155.9, 154.8, 154.3, 153.6, 150.0, 149.8, 149.5, 149.4, 146.4, 136.9, 135.7, 135.4, 135.1, 133.8, 133.4, 132.7, 129.2, 128.54, 128.50, 128.3, 128.1, 127.7, 127.4, 127.2, 126.5, 126.1, 125.7, 125.2, 124.9, 124.7, 122.5, 122.2, 119.5, 114.4, 107.7, 75.7, 74.4, 67.0, 62.1, 62.0, 55.7, 20.4, 17.3, 16.7, 14.5, 13.5, 13.0, 12.6, 10.6, 10.4, 10.2, 9.5; IR (Neat) 2939, 1738, 1578, 1463, 1322, 1278, 1150, 1122, 1095, 1076 cm^{-1}; HRFABMS calcd for $C_{62}H_{63}ClO_{12}Na$ $[M+Na]^+$ 1057.3906, found 1057.3905.

Preparation of aldehyde 12. To a solution of ester **11** (260 mg, 0.251 mmol) in dry CH_2Cl_2 (3.0 mL) was added Cl_2CHOMe (0.225 mL, 2.51 mmol, 10 equiv) and AgOTf (142 mg, 0.552 mmol, 2.2 equiv) at −40 °C under an argon atmosphere. After being stirred at 0 °C for 30 min, the reaction mixture was poured into saturated aqueous $NaHCO_3$ at 0 °C. After being stirred at room temperature for 30 min, the reaction mixture was filtered through a pad of Celite®. The organic layer of the filtrate was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with brine, dried with $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give desired aldehyde **12** and deprotected phenol. The crude mixture was used for next reaction without further purification.

To a solution of crude mixture of aldehyde **12** and deprotected phenol in DMF (3.0 mL) was added K_2CO_3 (104 mg, 0.753 mmol, 3.0 equiv) and 4-chlorobenzyl chloride (60.6 mg, 0.377 mmol, 1.5 equiv) at room temperature under an argon atmosphere. After being stirred at the same temperature for 14 h, the reaction mixture was diluted with EtOAc and quenched with 1 M HCl. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with brine twice, saturated aqueous $NaHCO_3$ and brine, dried with $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by flash column chromatography on silica gel (eluted with hexane/EtOAc = 9:1) to afford aldehyde **12** (144 mg, 0.136 mmol, 54%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.4 (1H, s), 7.46-7.47 (2H, m), 7.31-7.40 (8H, m), 7.23-7.24 (2H, m), 6.96-6.98 (2H, m), 5.40 (2H, s), 4.97 (2H, s), 4.60 (1H, d, J = 11.4 Hz), 4.55 (1H, d, J = 11.4 Hz), 3.79 (3H, s), 3.71 (3H, s), 3.65 (3H, s), 2.47 (3H, s), 2.39 (3H, s), 2.36 (3H, s), 2.25 (3H, s), 2.22 (3H, s), 2.20 (3H, s), 2.18 (3H, s), 2.15 (3H, s), 2.14 (3H, s), 2.11 (3H, s), 1.99 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.4, 168.3, 166.1, 165.5, 162.2, 159.0, 154.3, 153.7, 150.4, 150.0, 149.4, 149.0, 145.5, 138.9, 136.3, 135.7, 135.3, 134.0, 133.5, 132.7, 129.2, 128.59, 128.56, 128.53, 128.3, 128.2, 127.9, 127.4, 126.6, 126.5, 126.1, 125.7, 125.29, 125.25, 124.79, 124.75, 123.8, 122.4, 122.2, 119.3, 75.8, 74.2, 67.1, 63.0, 62.1, 62.0, 17.3, 16.7, 15.9, 14.5, 13.5, 13.0, 12.9, 10.7, 10.4, 10.2, 9.8; IR (Neat) 2932, 1740, 1684, 1573, 1460, 1323, 1278, 1150, 1111, 755 cm^{-1}; HRESIMS calcd for $C_{63}H_{63}ClO_{13}Na$ $[M+Na]^+$ 1085.3855, found 1085.3860.

Preparation Carboxylic acid 13. To a solution of aldehyde **12** (26.0 mg, 24.4 μmol) in *t*BuOH (1.0 mL) and water (1.0 mL) was added 2-methyl-2-butene (0.5 mL), $NaClO_2$ (22.1 mg, 244 μmol, 10 equiv) and NaH_2PO_4•$2H_2O$ (38.1 mg, 244 μmol, 10 equiv) at room temperature. After being stirred at the same temperature for 4 h, the reaction mixture was diluted with EtOAc. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with brine, dried with $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by flash column chromatography on silica gel (eluted with hexane/EtOAc = 1:1) to afford carboxylic acid **13** (21.9 mg, 20.3 μmol, 83%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46-7.48 (2H, m), 7.28-7.40 (10H, m), 6.99-7.01 (2H, m), 5.40 (2H, s), 4.97 (2H, s), 4.62 (1H, d, J = 11.4 Hz), 4.51 (1H, d, J = 11.4 Hz), 3.79 (3H, s), 3.71 (3H, s), 3.70 (3H, s), 2.46 (3H, s), 2.37 (3H, s), 2.26 (3H, s), 2.22 (6H, s), 2.19 (3H, s), 2.18 (3H, s), 2.16 (3H, s), 2.12 (6H, s), 2.01 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 168.3, 166.2, 165.6, 156.3, 154.4, 153.7, 150.2, 150.0, 149.5, 149.2, 145.7, 136.4, 135.7, 135.3, 134.2, 134.0, 133.5, 132.7, 129.2, 128.6, 128.5, 128.34, 128.32, 127.9, 127.4, 126.7, 126.6, 126.1, 125.7, 125.3, 125.2, 124.8, 124.3, 122.9, 122.4, 122.2, 119.4, 75.8, 74.3, 67.1, 62.1, 62.0, 17.3, 16.9, 16.7, 14.5, 13.5, 13.2, 13.0, 10.7, 10.4, 10.24, 10.17; IR (Neat) 2939, 1738, 1733, 1600, 1575, 1462, 1323, 1279, 1152, 1097, 755 cm^{-1}; HRESIMS calcd for $C_{63}H_{63}ClO_{14}Na$ $[M+Na]^+$ 1101.3804, found 1101.3816.

Preparation of Ester 14. To a solution of carboxylic acid **13** (110 mg, 0.102 mmol) in dry toluene (5.0 mL) was added phenol **3** (36.7 mg, 0.122 mmol, 1.2 equiv) and trifluoroacetic anhydride (0.568 mL, 4.08 mmol, 40 equiv) at room temperature under an argon atmosphere. After being stirred at 80 °C for 11 h, the reaction mixture was cooled at room temperature and quenched with 2 M aqueous NaOH. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with brine, dried with

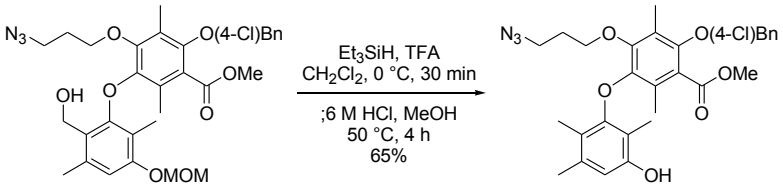
$MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by flash column chromatography on silica gel (eluted with hexane/EtOAc = 6:1) to afford ester **14** (105 mg, 0.0773 mmol, 76%) as a white solid. M.p. 106–107 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46-7.48 (4H, m), 7.21-7.40 (13H, m), 7.01-7.02 (2H, m), 5.402 (2H, s), 5.398 (2H, s), 4.97 (2H, s), 4.63 (1H, d, J = 11.2 Hz), 4.52 (1H, d, J = 11.2 Hz), 3.79 (3H, s), 3.72 (3H, s), 3.71 (3H, s), 3.68 (3H, s), 2.48 (3H, s), 2.37 (3H, s), 2.26 (3H, s), 2.25 (3H, s), 2.23 (3H, s), 2.22 (6H, s), 2.21 (3H, s), 2.19 (3H, s), 2.18 (3H, s), 2.16 (3H, s), 2.15 (3H, s), 2.12 (3H, s), 2.05 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.33, 168.30, 166.4, 166.2, 165.6, 156.2, 154.7, 154.4, 153.7, 150.2, 150.0, 149.6, 149.5, 149.2, 145.7, 136.4, 135.7, 135.3, 134.0, 133.53, 133.49, 132.71, 132.69, 129.2, 128.59, 128.58, 128.55, 128.4, 128.3, 127.9, 127.43, 127.36, 126.8, 126.6, 126.1, 125.71, 125.69, 125.31, 125.26, 124.8, 124.1, 123.8, 122.4, 122.20, 122.19, 119.7, 75.8, 74.4, 67.1, 62.1, 62.0, 61.9, 17.3, 17.2, 16.8, 16.7, 14.5, 13.5, 13.2, 13.01, 12.99, 10.7, 10.44, 10.38, 10.3; IR (Neat) 3011, 2940, 1735, 1600, 1576, 1461, 1323, 1280, 1149, 1096, 1077, 754 cm^{-1}; HRESIMS calcd for $C_{81}H_{81}ClO_{17}Na$ $[M+Na]^+$ 1383.5060, found 1383.5070.

Synthesis of thielocin B1 (1). To a solution of ester **14** (5.0 mg, 3.7 μmol) in EtOH (0.5 mL) and EtOAc (0.5 mL) was added 10% palladium on carbon (10 mg, 200 wt%) at room temperature, and the flask was purged with hydrogen 7 times. After being stirred at the same temperature for 15 min, the reaction mixture was filtered through a pad of Celite®. The filtrate was concentrated in vacuo, and the resulting residue was purified by preparative TLC (eluted with $CHCl_3$/MeOH = 3:1) to afford thielocin B1 (**1**) (1.9 mg, 2.0 μmol, 54%) as a white solid. M.p. >300 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 3.832 (3H, s), 3.825 (3H, s), 3.80 (3H, s), 3.73 (3H, s), 2.39 (3H, s), 2.38 (3H, s), 2.36 (3H, s), 2.284 (3H, s) , 2.276 (3H, s), 2.22 (3H, s), 2.21 (3H, s), 2.20 (6H, s), 2.18 (3H, s), 2.14 (6H, s), 2.11 (3H, brs), 2.01 (3H, s); ^{13}C NMR (100 MHz, CD_3OD) δ 171.3, 168.4, 167.8, 161.0, 158.4, 156.2, 155.7, 153.23, 153.22, 150.9, 148.5, 148.4, 140.1, 136.7, 136.6, 134.50, 134.47, 131.63, 131.58, 128.5, 128.1, 127.1, 125.9, 125.1, 124.8, 123.3, 122.3, 120.2, 111.9, 62.8, 62.6, 62.01, 61.99, 17.6, 17.4, 17.1, 16.5, 13.4, 13.3, 10.6, 10.54, 10.51, 10.4, 8.6; IR (Neat) 3430, 2918, 1736, 1656, 1572, 1460, 1160, 1093, 1074 cm^{-1}; HRESIMS calcd for $C_{53}H_{59}O_{17}$ $[M+H]^+$ 967.3752, found 967.3751.

Methyl 4-(3-azidopropyloxy)-2-(4-chlorobenzyloxy)-5-(2-hydroxymethyl-5-methoxymethoxy-3,6-dimethyl phenoxy)-3,6-dimethylbenzoate (16). To a solution of phenol **8** (290 mg, 546 μmol) in DMF (1.0 mL) was added K_2CO_3 (302 mg, 2.18 mmol, 4.0 equiv) and 1-azido-3-iodopropane[2] (207 mg, 983 μmol, 1.8 equiv) in DMF (1.0 mL) at room temperature under an argon atmosphere. After being stirred at the same temperature for 8 h, the reaction mixture was diluted with EtOAc, and quenched with 1 M aqueous HCl. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine twice, saturated aqueous $NaHCO_3$ and brine, dried with Na_2SO_4, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 4:1) to afford 3-azidopropyl ether **16** (291 mg, 474 μmol, 87%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (4H, s), 6.76 (1H, s), 5.19 (1H, d, J = 6.6 Hz), 5.17 (1H, d, J = 6.6 Hz), 4.85 (2H, s), 4.78 (1H, d, J = 11.2 Hz), 4.54 (1H, d, J = 11.2 Hz), 3.83 (3H, s), 3.65 (1H, dd, J = 15.0, 7.4 Hz), 3.47 (3H, s), 3.11-3.24 (3H, m), 2.43 (3H, s), 2.21 (3H, s), 2.13 (3H, s), 1.92 (3H, s), 1.43-1.55 (2H, m); ^{13}C NMR (100 MHz,

$CDCl_3$) δ 168.2. 155.7. 155.4. 149.3. 148.0. 145.8. 136.8. 135.5. 133.9. 129.1. 128.6. 126.5. 124.9. 124.5. 124.2. 115.5. 111.8. 94.7. 75.5. 70.0. 57.1. 56.1. 52.3. 47.9. 28.7. 19.2. 13.9. 10.1. 9.4; IR (Neat) 3520, 2952, 2930, 2098, 1732, 1610, 1580, 1284, 1208, 1115, 1091, 1059 cm^{-1}; HREIMS calcd for $C_{31}H_{36}ClN_3O_8$ 613.2191, found 613.2195.

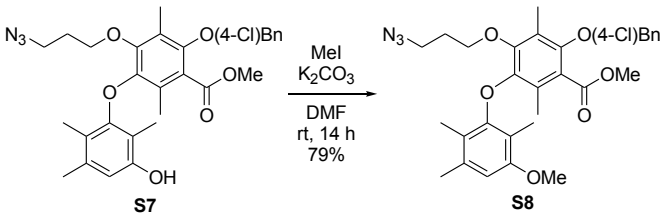
Methyl 4-(3-azidopropyloxy)-2-(4-chlorobenzyloxy)-5-(3-hydroxy-2,5,6-trimethylphenoxy)-3,6-dimethyl benzoate (S7).



16 **S7**

To a solution of benzyl alcohol **16** (290 mg, 472 μmol) in dry CH_2Cl_2 (6.0 mL) was added TFA (0.3 mL) and Et_3SiH (1.51 mL, 9.44 mmol, 20 equiv) at 0 °C under an argon atmosphere, and the mixture was stirred at the same temperature for 30 min. To the reaction mixture was added MeOH (6.0 mL) and 6 M aqueous HCl (1.2 mL) at 0 °C. After being stirred at 50 °C for 4 h, the reaction mixture was cooled to room temperature, diluted with EtOAc, and quenched with saturated aqueous $NaHCO_3$. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine, dried with Na_2SO_4, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 9:1) to afford phenol **S7** (170 mg, 307 μmol, 65%) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 7.35 (4H, s), 6.44 (1H, s), 4.85 (2H, s), 4.56 (1H, brs), 3.82 (3H, s), 3.55 (2H, t, J = 6.2 Hz), 3.20 (2H, t, J = 6.8 Hz), 2.20 (3H, s), 2.14 (3H, s), 2.12 (3H, s), 1.98 (3H, s), 1.94 (3H, s), 1.51-1.60 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.5, 154.9, 151.9, 148.9, 148.5, 145.8, 135.6, 135.2, 133.8, 129.1, 128.6, 125.9, 124.4, 124.0, 119.3, 112.5, 111.8, 75.5, 69.5, 52.3, 48.1, 28.9, 20.0, 13.8, 12.5, 10.0, 9.4; IR (Neat) 3429, 2951., 2926, 2097, 1730, 1616, 1593, 1337, 1285, 1210, 1102 cm^{-1}; HREIMS calcd for $C_{29}H_{32}ClN_3O_6$ 553.1980, found 553.1991.

Methyl 4-(3-azidopropyloxy)-2-(4-chlorobenzyloxy)-5-(3-methoxy-2,5,6-trimethylphenoxy)-3,6-dimethyl benzoate (S8).

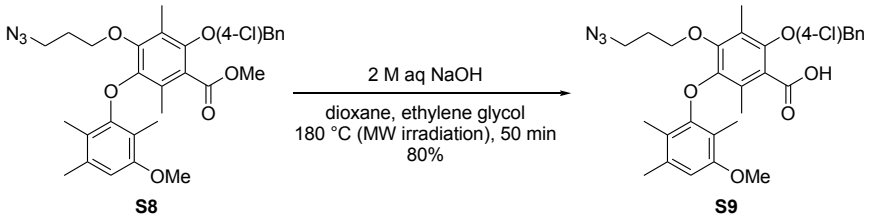


S7 **S8**

To a solution of phenol **S7** (170 mg, 307 μmol) in DMF (2.0 mL) was added K_2CO_3 (149 mg, 1.07 mmol, 3.5 equiv) and MeI (22.3 μL, 491 μmol, 1.6 equiv) at room temperature under an argon atmosphere. After being stirred at the same temperature for 14 h, the reaction mixture was diluted with EtOAc, and quenched with 1 M aqueous HCl. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine twice, saturated aqueous $NaHCO_3$ and brine, dried with

Na_2SO_4, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by flash column chromatography on silica gel (eluted with hexane/EtOAc = 9:1) to afford methyl ether **S8** (137 mg, 241 μmol, 79%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (4H, s), 6.49 (1H, s), 4.85 (2H, s), 3.81 (3H, s), 3.80 (3H, s), 3.48-3.59 (2H, m), 3.17 (2H, t, J = 6.6 Hz, n), 2.26 (3H, s), 2.13 (3H, s), 2.11 (3H, s), 2.00 (3H, s), 1.91 (3H, s), 1.54 (2H, quint, J = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.5, 155.8, 154.7, 148.9, 148.5, 145.8, 135.7, 134.8, 133.8, 129.1, 128.6, 125.9, 124.4, 124.0, 119.5, 114.5, 107.5, 75.4, 69.4, 55.8, 52.2, 48.1, 28.9, 20.4, 13.8, 12.5, 10.0, 9.4; IR (Neat) 2925, 2097, 1731, 1613, 1283, 1125 cm^{-1}; HREIMS calcd for $C_{30}H_{34}ClN_3O_6$ 567.2136, found 574.2108.

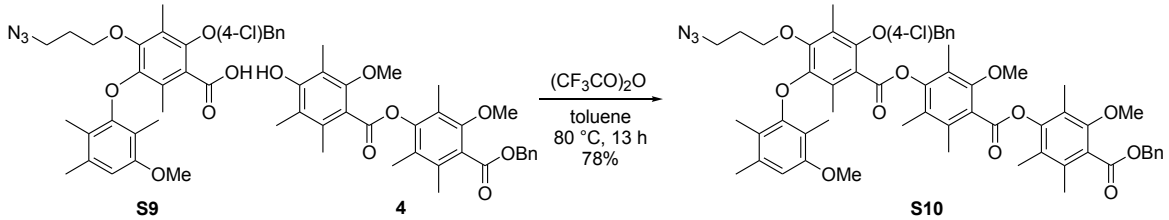
4-(3-Azidopropyloxy)-2-(4-chlorobenzyloxy)-5-(3-methoxy-2,5,6-trimethylphenoxy)-3,6-dimethylbenzoic acid (S9).



S8 **S9**

To a solution of methyl ester **S8** (580 mg, 1.02 mmol) in dioxane (5.0 mL) was added 2 M aqueous NaOH (10 mL, 20.0 mmol, 20 equiv) and ethylene glycol (2.5 mL) at room temperature. After being stirred at 180 °C under microwave irradiation for 50 min, the reaction mixture was cooled to room temperature, diluted with EtOAc, and quenched with 3 M HCl. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine, dried with Na_2SO_4, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/ EtOAc = 4:1) to afford carboxylic acid **S9** (455 mg, 821 μmol, 80%) as an orange oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (2H, d, J = 8.0 Hz), 7.32 (2H, d, J = 8.0 Hz), 6.50 (1H, s), 4.87 (2H, s), 3.80 (3H, s), 3.49-3.60 (2H, m), 3.18 (2H, t, J = 6.4 Hz), 2.27 (3H, s), 2.25 (3H, s), 2.15 (3H, s), 2.01 (3H, s), 1.91 (3H, s), 1.55 (2H, quint, J = 6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 155.9, 154.7, 149.3, 149.2, 146.1, 135.1, 134.9, 134.1, 129.4, 128.7, 125.7, 124.1, 119.5, 114.4, 107.6, 75.8, 69.5, 55.8, 48.1, 28.9, 20.5, 14.0, 12.6, 10.0, 9.4; IR (Neat) 3316, 2926, 2097, 1703, 1613, 1465, 1370, 1125, 1092 cm^{-1}; HREIMS calcd for $C_{29}H_{32}ClN_3O_6$ 553.1980, found 553.1972.

Preparation of Ester S10.

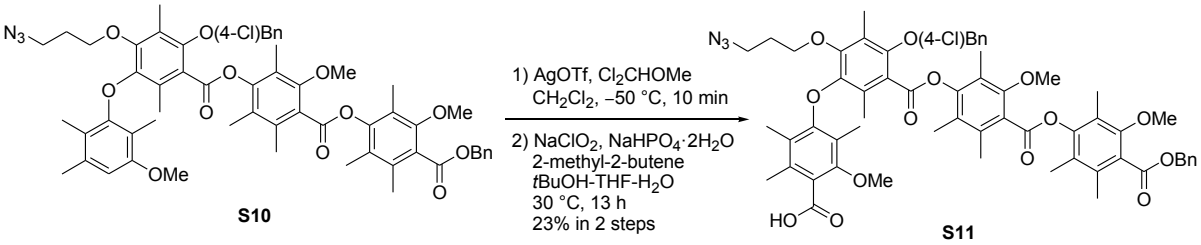


S9 **4** **S10**

To a solution of carboxylic acid **S9** (450 mg, 812 μmol) in dry toluene (10 mL) was added phenol **4** (480 mg, 975 μmol, 1.2 equiv) and trifluoroacetic anhydride (3.95 mL, 28.4 mmol, 35 equiv) at room temperature under

an argon atmosphere. After being stirred at 80 °C for 13 h, the reaction mixture was cooled at room temperature, and quenched with 2 M aqueous NaOH. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine, dried with Na_2SO_4, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by flash column chromatography on silica gel (eluted with hexane/EtOAc = 9:1) to afford ester **S10** (651 mg, 633 μmol, 78%) as a white solid. M.p. 92–93 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46-7.47 (2H, m), 7.30-7.40 (7H, m), 6.52 (1H, s), 5.40 (2H, s), 4.94 (2H, s), 3.82 (3H, s), 3.78 (3H, s), 3.71 (3H, s), 3.53-3.61 (2H, m), 3.19 (2H, t, J = 6.8 Hz), 2.39 (3H, s), 2.35 (3H, s), 2.25 (3H, s), 2.21 (6H, s), 2.18 (3H, s), 2.14 (3H, s), 2.10 (3H, s), 2.05 (3H, s), 1.96 (3H, s), 1.56 (2H, quint, J = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.3, 166.2, 165.8, 155.9, 154.7, 154.2, 153.6, 150.0, 149.6, 149.4, 149.2, 146.0, 135.6, 135.4, 134.9, 133.8, 133.4, 132.7, 129.2, 128.5, 128.3, 127.4, 126.5, 126.1, 125.7, 125.1, 124.9, 124.3, 122.4, 122.2, 119.5, 114.5, 107.6, 75.7, 69.5, 67.0, 62.1, 62.0, 55.8, 48.1, 28.9, 20.5, 17.3, 16.8, 14.5, 13.4, 13.0, 12.6, 10.6, 10.2, 9.5; IR (Neat) 2926, 2097, 1735, 1578, 1461, 1278, 1150, 1122 cm^{-1}; HRESITOFMS calcd for $C_{58}H_{62}ClN_3O_{12}Na$ $[M+Na]^+$ 1050.3920, found 1050.3896.

Preparation of Carboxylic acid S11.

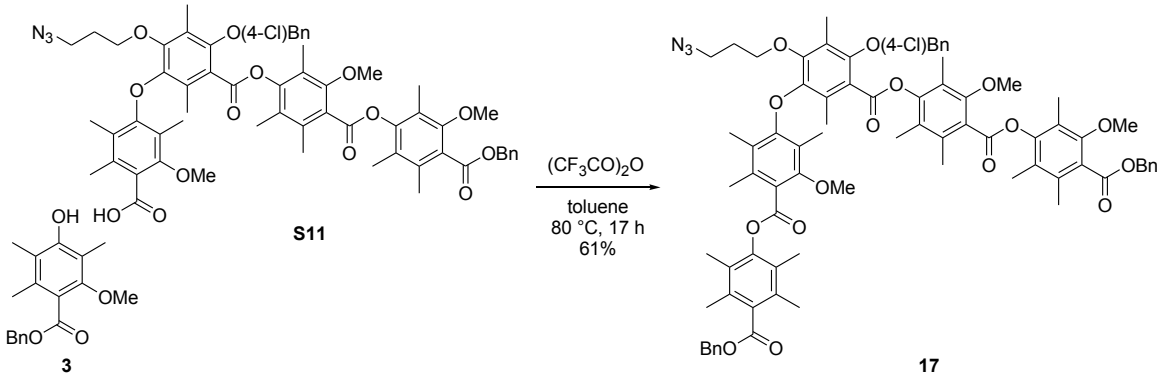


To a solution of ester **S10** (200 mg, 194 μmol) and AgOTf (250 mg, 972 μmol, 5.0 equiv) in dry CH_2Cl_2 (12 mL) was added Cl_2CHOMe (88.0 μL, 972 mmol, 5.0 equiv) in dry CH_2Cl_2 (4.0 mL) at −78 °C under an argon atmosphere. After being stirred at −50 °C for 10 min, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ at 0 °C. After being stirred at room temperature for 30 min, the reaction mixture was filtered through a pad of Celite®. The organic layer of the filtrate was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine, dried with Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to give the mixture of desired aldehyde and ester **S10** as a yellowish oil. The crude mixture was used for next reaction without further purification.

To a solution of crude aldehyde and ester **S10** in *t*BuOH (2.0 mL), THF (1.0 mL) and water (3.0 mL) was added 2-methyl-2-butene (2.0 mL), $NaClO_2$ (87.7 mg, 970 μmol, 5.0 equiv) and NaH_2PO_4•$2H_2O$ (151 mg, 970 μmol, 5.0 equiv) at 0 °C. After being stirred at 30 °C for 13 h, the organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine, dried with Na_2SO_4, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by flash column chromatography on silica gel (eluted with $CHCl_3$/MeOH = 9:1) to afford carboxylic acid **S11** (47.8 mg, 44.6 μmol, 23% in 2 steps) as a yellowish oil. Ester **S10** was recovered (69.2 mg, 67.2 μmol, 35% in 2 steps) as a yellowish oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45-7.47 (2H, m), 7.31-7.40 (7H, m), 5.40 (2H, s), 4.96 (2H, s), 3.79 (3H, s), 3.78 (3H, s), 3.71 (3H, s), 3.53-3.61 (2H, m), 3.14 (2H, t, J = 6.6 Hz), 2.40 (3H, s), 2.36 (3H, s), 2.34 (3H, s), 2.25 (3H, s), 2.22 (3H, s), 2.21 (3H, s), 2.18 (3H, s), 2.15 (3H, s), 2.14 (3H, s), 2.10 (3H, s), 2.02

(3H, s), 1.56 (2H, quint, J = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 168.3, 166.2, 165.6, 156.0, 154.3, 154.1, 153.7, 150.1, 150.0, 149.4, 149.2, 145.4, 135.7, 135.3, 133.9, 133.5, 133.3, 132.7, 129.2, 128.57, 128.55, 128.53, 128.3, 127.4, 126.6, 126.1, 125.7, 125.1, 124.5, 124.1, 122.4, 122.2, 119.7, 75.8, 69.6, 67.1, 62.2, 62.1, 62.0, 47.9, 28.9, 17.3, 16.9, 16.7, 14.5, 13.4, 13.1, 13.0, 10.6, 10.3, 10.2, 10.1; IR (Neat) 3444, 2931, 2098, 1739, 1733, 1601, 1575, 1463, 1279, 1150, 755 cm^{-1}; HRESITOFMS calcd for $C_{59}H_{62}N_3ClO_{14}Na$ $[M+Na]^+$ 1094.3818, found 1094.3801.

Preparation of Ester 17.



To a solution of carboxylic acid **S11** (169 mg, 158 μmol) in dry toluene (10 mL) was added phenol **3** (56.8 mg, 189 μmol, 1.2 equiv) and trifluoroacetic anhydride (660 μL, 4.74 mmol, 30 equiv) at room temperature under an argon atmosphere. After being stirred at 80 °C for 17 h, the reaction mixture was cooled at room temperature, and quenched with 2 M aqueous NaOH. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine, dried with $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by flash column chromatography on silica gel (eluted with hexane/EtOAc = 5:1) to afford ester **17** (131 mg, 96.8 μmol, 61%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46-7.48 (4H, m), 7.33-7.40 (10H, m), 5.40 (4H, s), 4.96 (2H, s), 3.78 (6H, s), 3.71 (3H, s), 3.70 (3H, s), 3.58-3.66 (2H, m), 3.14 (2H, dt, J = 6.4, 2.0 Hz), 2.41 (3H, s), 2.40 (3H, s), 2.36 (3H, s), 2.25 (6H, s), 2.24 (3H, s), 2.21 (6H, s), 2.18 (9H, s), 2.14 (3H, s), 2.10 (3H, s), 2.06 (3H, s), 1.59 (2H, quint, J = 6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.30, 168.28, 166.3, 166.2, 165.6, 156.2, 154.5, 154.3, 153.6, 150.2, 149.9, 149.5, 149.4, 149.2, 145.4, 135.6, 135.3, 133.9, 133.5, 133.3, 132.7, 129.2, 128.57, 128.55, 128.53, 128.3, 127.40, 127.37, 126.6, 126.1, 125.7, 125.6, 125.12, 125.08, 124.5, 124.1, 123.8, 122.4, 122.2, 119.9, 75.8, 69.6, 67.07, 67.06, 62.10, 62.06, 62.0, 47.9, 28.9, 17.3, 17.2, 16.8, 14.5, 13.5, 13.2, 12.97, 12.95, 10.6, 10.34, 10.29, 10.22, 10.21; IR (Neat) 2940, 2097, 1734, 1576, 1457, 1279, 1148 cm^{-1}; HRESITOFMS calcd for $C_{77}H_{80}ClN_3O_{17}Na$ $[M+Na]^+$ 1376.5074, found 1376.5076.

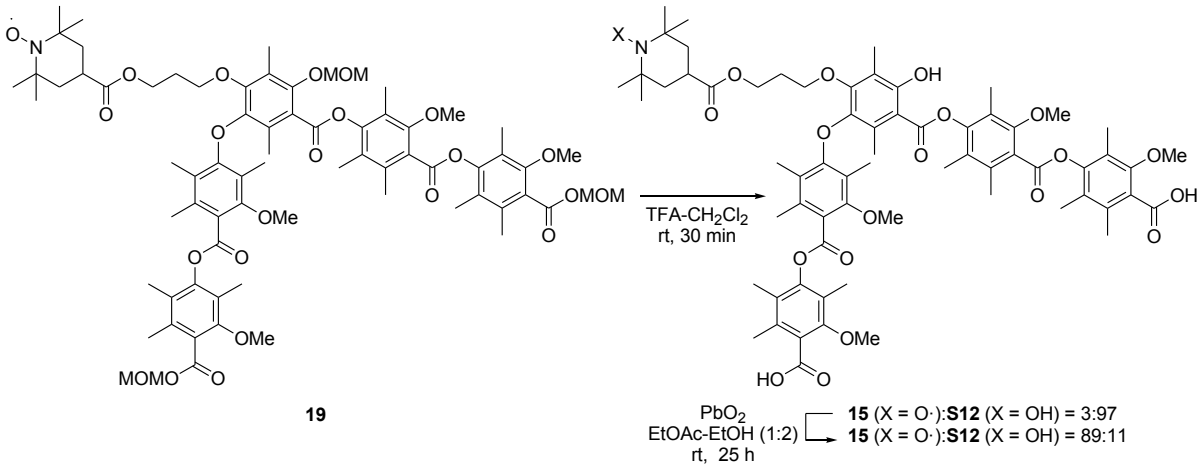
Preparation of MOM ester 18. To a solution of I_2 (42.0 mg, 166 μmol, 15 equiv) in dry CH_2Cl_2 (2.4 mL) was added hexamethyldisilane (33.4 μL, 166 μmol, 15 equiv) at room temperature under an argon atmosphere. After being stirred at 40 °C for 30 min, the reaction mixture was cooled at room temperature. To the resulting brown solution was added benzyl ester **17** (15.0 mg, 11.1 μmol) in dry CH_2Cl_2 (0.6 mL) dropwise at −78 °C under an argon atmosphere. After being stirred at −40 °C for 9 min, the reaction mixture was quenched with 1 M aqueous

HCl at −40 °C, and stirred for 15 min at room temperature. The mixture was concentrated in vacuo to give carboxylic acid. The crude carboxylic acid was used for next reaction without further purification.

To a solution of the crude carboxylic acid in DMF (1.0 mL) was added NaH (50-72% in oil, 6.5 mg, 166 μmol, 15 equiv) and MOMCl (12.6 μL, 166 μmol, 15 equiv) at 0 °C under an argon atmosphere. After being stirred at the same temperature for 15 min, the reaction mixture was diluted with EtOAc, and quenched with 1M aqueous HCl. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine twice, saturated aqueous $NaHCO_3$ and brine, dried with Na_2SO_4, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by flash column chromatography on silica gel (eluted with hexane/EtOAc = 3:1) to afford MOM ester **18** (7.7 mg, 60.8 μmol, 55%) as an orange oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.50 (4H, s), 5.06 (2H, s), 3.85 (3H, s), 3.82 (6H, s), 3.79 (3H, s), 3.58 (9H, s), 3.56-3.64 (2H, m), 3.10 (2H, t, J = 6.8 Hz), 2.42 (3H, s), 2.41 (6H, s), 2.33 (3H, s), 2.32 (3H, s), 2.28 (9H, s), 2.27 (6H, s), 2.25 (6H, s), 2.18 (3H, s), 2.05 (3H, s), 1.82 (2H, quint, J = 6.8 Hz, z); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.1, 168.0, 166.4, 166.2, 165.4, 156.2, 154.6, 154.4, 153.6, 150.0, 149.6, 149.5, 149.3, 148.9, 145.4, 133.6, 133.3, 132.57, 132.55, 128.5, 127.3, 127.2, 126.6, 126.1, 125.8, 125.2, 125.1, 125.0, 124.1, 123.8, 122.4, 122.2, 120.0, 101.3, 91.1, 72.4, 62.2, 62.11, 62.07, 57.9, 57.8, 33.3, 17.34, 17.29, 16.7, 14.5, 13.4, 13.2, 13.1, 13.0, 11.1, 10.7, 10.4, 10.3, 10.2; IR (Neat) 2940, 1743, 1678, 1575, 1463, 1323, 1279, 1146, 1100, 1077 cm^{-1}; HRESITOFMS calcd for $C_{62}H_{75}IO_{20}Na$ $[M+Na]^+$ 1289.3794, found 1279.3789.

Preparation of Ester 19. To a solution of iodide **18** (45.0 mg, 35.5 μmol) in DMF (2.0 mL) was added CsF (27.0 mg, 178 μmol, 5.0 equiv) and 4-Carboxyl-TEMPO (13.0 mg, 64.9 μmol, 1.8 equiv) at room temperature under an argon atmosphere. After being stirred at the same temperature for 17 h, the reaction mixture was diluted with EtOAc, and quenched with saturated aq $NaHCO_3$. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate twice. The combined organic layers were washed with brine, dried with Na_2SO_4, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 6:1) to afford ester **19** (39.5 mg, 29.5 μmol, 83%) as an orange solid. M.p. 87–88 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.50 (4H, s), 5.06 (2H, s), 4.17 (2H, brs), 3.85 (3H, s), 3.82 (6H, s), 3.79 (3H, brs), 3.58 (11H, s), 2.42 (6H, s), 2.41 (3H, s), 2.32 (3H, s), 2.28 (9H, s), 2.27 (9H, s), 2.25 (6H, s), 2.20 (3H, brs), 2.07 (3H, brs), 2.05 (3H, s), 1.69 (2H, brs); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.7, 166.6, 165.0, 164.8, 164.1, 154.9, 153.2, 153.0, 152.30, 152.26, 148.6, 148.24, 148.16, 148.1, 147.6, 144.0, 132.2, 131.9, 131.3, 131.2, 126.0, 125.9, 125.3, 124.7, 124.41, 124.37, 123.8, 123.7, 123.6, 122.8, 122.5, 121.0, 120.9, 118.6, 100.2, 89.9, 89.8, 67.9, 61.1, 60.9, 60.8, 60.4, 57.1, 57.0, 56.6, 27.5, 16.0, 15.7, 15.4, 13.2, 12.1, 12.0, 11.9, 11.7, 9.4, 9.3, 9.1, 9.0, 8.9; IR (Neat) 2940, 1739, 1576, 1462, 1279, 1146 cm^{-1}; HRESITOFMS calcd for $C_{72}H_{92}NO_{23}Na$ $[M+Na]^+$ 1361.5958, found 1361.5952.

Preparation of spin-labeled derivative of thielocin B1 15.



19

To a solution of MOM ester (10.0 mg, 7.5 μmol) in dry CH_2Cl_2 (1.0 mL) was added TFA (0.2 mL) at room temperature. After being stirred at the same temperature for 30 min, the reaction mixture was concentrated in vacuo. The resulting residue was solved in MeOH (1 mL), and the solution was concentrated in vacuo to give the mixture of hydroxylamine **S12** and desired nitroxyl radical **15** as a white solid. The ratio of **S12** and **15** was determined by reversed-phase HPLC (**S12**:**15** = 97:3). The crude mixture was used for next reaction without further purification.

To the solution of crude mixture of hydroxylamine **S12** and desired nitroxyl radical **15** in EtOAc (1.0 mL) and EtOH (2.0 mL) was added PbO_2 (8.9 mg, 37.4 μmol, 5.0 equiv) at room temperature. After being stirred at the same temperature for 25 h, the reaction mixture was filtered through a pad of Celite®. The filtrate was concentrated in vacuo, and the resulting residue was washed with $CDCl_3$. The supernatant was removed, and the resulting residue was dried in vacuo to give a mixture of desired nitroxyl radical **15** and hydroxylamine **S12** as a white solid. The ratio of **15** and **S12** was determined by reversed-phase HPLC (**15**:**S12** = 89:11). The mixture (7.6 mg) was used for NMR experiment without further purification. **S12**: M.p. > 300 °C; ^{1}H NMR (600 MHz, CD_3OD) δ 3.96-4.05 (2H, m), 3.83 (3H, s), 3.803 (3H, s), 3.799 (3H, s), 3.76 (3H, s), 3.66-3.69 (1H, m), 3.56-3.61 (1H, m), 2.92-2.96 (1H, m), 2.68 (3H, s), 2.42 (3H, s), 2.39 (3H, s), 2.273 (3H, s) , 2.265 (3H, s), 2.26 (3H, s), 2.25 (6H, s), 2.24 (3H, s), 2.21(6H, s), 2.18 (3H, s), 2.16 (3H, s), 2.10-2.12 (2H, m), 2.05 (3H, s), 1.82 (2H, t, J = 13.8 Hz), 1.61-1.69 (2H, m), 1.384 (6H, s), 1.379 (6H, s); ^{13}C NMR (125 MHz, CD_3OD) δ 174.0, 172.1, 172.0, 170.3, 168.1, 167.6, 160.4, 158.1, 156.0, 155.8, 154.5, 154.1, 151.1, 150.5, 150.4, 143.6, 134.7, 134.5, 133.2, 129.9, 129.3, 127.9, 127.2, 126.9, 125.1, 124.7, 123.4, 123.3, 120.6, 119.7, 118.0, 116.0, 122.5, 111.0, 70.7, 69.0, 68.9, 63.2, 62.9, 62.7, 62.5, 40.0, 34.3, 29.9, 28.1, 26.5, 19.9, 17.63, 17.56, 16.9, 16.12, 16.09, 13.4, 10.7, 9.9; IR (Neat) 3349, 2923, 2853, 1739, 1676, 1593, 1459, 1413, 1154 cm^{-1}; HPLC retention time: 9.0 min; HRESITOFMS calcd for $C_{66}H_{82}NO_{20}$ $[M+H]^+$ 1208.5430, found 1208.5430. **15**: M.p. > 300 °C; ^{1}H NMR (600 MHz, CD_3OD) δ 4.08 (2H, brs), 3.83 (3H, s), 3.82 (6H, s), 3.77 (3H, s), 3.63 (2H, m), 2.68 (3H, brs), 2.41 (3H, s), 2.39 (3H, brs), 2.28 (6H, s) , 2.25 (6H, s), 2.23 (6H, s), 2.22 (6H, s), 2.18 (6H, s), 2.05 (3H, brs), 1.69 (2H, brs); ^{13}C NMR δ 170.3, 168.1, 167.6, 163.2, 162.9, 158.1, 156.1, 155.7, 154.2, 154.1, 154.0, 151.0, 149.8, 149.6, 143.6, 134.7, 134.5, 132.7, 132.6, 129.3, 128.1, 127.1, 126.5, 125.1, 124.8, 123.4, 122.9, 121.6, 119.6, 112.0, 70.8, 63.0, 62.9, 62.8, 62.3, 17.8, 17.6, 17.0, 16.1, 13.54, 13.45, 13.4, 11.0, 10.8, 10.6, 9.9; IR (Neat) 3385, 2935, 1685, 1577, 1458, 1309, 1208, 1187, 11474 cm^{-1}; HPLC retention time: 10.7 min, HRESITOFMS calcd

for $C_{66}H_{81}NO_{20}$ $[M+H]^+$1207.5352, found 1207.5388.

Docking study and molecular dynamics simulation

Thielocin B1 and thielocin B3 were docked into the X-ray structure of monomeric PAC3 (PDB code: 2Z5E_A) [3] using Glide 5.0 (SP mode) (Schrödinger, Portland, OR). The grid box for the docking calculation was defined around the small concavity in the PAC3 homodimer interface. Concavity and protein–ligand interaction fingerprint analyses were performed using the SiteFinder and PLIF modules of MOE, respectively (Chemical Computing Group Inc., Montreal, Quebec, Canada). Spin-labeled derivative **15** was docked into the X-ray structure of dimeric PAC3 (PDB code: 2Z5E) using MOE. Visualization was generated by PyMOL (Schrödinger, Portland, OR). An MD simulation was carried out using the AMBER9 package with the ff99SB force field. The protein/compound was surrounded with a 15 Å layer of TIP3PBOX water molecules, and its electrostatic charge was neutralized by adding counter ions using the LeaP module of AMBER9. After minimization, heating, and equilibration, the production of the MD phase was carried out at 300 K for 20 ns, with a time step of 1 fs, using the constant volume and temperature (NVT) ensemble and the PME algorithm. All simulations were performed on an Intel Xeon(R) 5160 3.00 GHz HP workstation with 112 computing nodes.

Protein expression and purification

The cDNA encoding PAC3 was cloned into the pRSF-Duet1 vector (Novagen) to express PAC3 with an N-terminal hexahistidine tag, followed by an enterokinase cleavage site. Using this construct, the protein was expressed in *Escherichia coli* BL21 (DE3) Codon-Plus cells, which were grown in M9 minimal medium containing [^{15}N]NH_4Cl (1 g/L) using a previously described protocol.[4] The uniformly ^{15}N-labeled protein was purified using a Ni Sepharose 6 Fast Flow (GE Healthcare Bio-Sciences). Subsequently, the histidine tag was cleaved by enterokinase treatment, followed by anion exchange chromatography using a Resource Q column (GE Healthcare).

NMR titration

^{15}N-labeled PAC3 (0.2 mM) sample, dissolved in PBS (pH 6.8) containing 10% D_2O (v/v), 1 mM DTT, 1 mM EDTA, and 0.01% NaN_3, was titrated with a 10 mM methanol-d_4 solution of **1** (0.1, 0.2, 0.4, or 0.8 mM) or the solvent alone as a reference control. All NMR data were acquired at 303 K using a Bruker DMX500 spectrometer equipped with a cryogenic probe. For ^{1}H–^{15}N HSQC measurements, spectra were recorded at a ^{1}H observation frequency of 500 MHz with 128 (t_1) × 1024 (t_2) complex points and 8 scans per t_1 increment. ^{1}H chemical shifts were referenced to DSS (0 ppm), while ^{15}N chemical shifts were indirectly referenced to DSS using the absolute frequency ratios. Chemical shift changes were quantified as $[(\Delta\delta_H)^2 + (0.2\ \Delta\delta_N)^2]^{1/2}$, where $\Delta\delta_H$ and $\Delta\delta_N$ are the observed chemical shift changes for ^{1}H and ^{15}N, respectively. The data were processed using NMRPipe[5] software and analyzed with SPARKY 3[6] and CCPNMR[7] software. Conventional 3D NMR

experiments[8] were carried out for assignments of the HSQC peaks originating from the PAC3 homodimer.

NMR study for paramagnetic relaxation enhancement (PRE) effects

^{15}N-labeled PAC3 was dissolved at a concentration of 0.3 mM in PBS (pH 6.8) containing 10% D_2O (v/v), 1 mM EDTA, and 0.01% NaN_3 and a 10 mM methanol-d_4 solution of **15** was added to this solution. PRE effects were measured from the peak intensity ratios between two ^{1}H-^{15}N HSQC spectra of ^{15}N-labeled PAC3 with **15** (0.3, 0.6, or 0.9 mM) in presence and absence of L-(+)-ascorbic acid (3, 6, or 9 mM) for radical quenching. All NMR data were acquired at 303 K using a Bruker AVANCE800 spectrometer equipped with a cryogenic probe. For ^{1}H–^{15}N HSQC measurements, spectra were recorded at a ^{1}H observation frequency of 800 MHz with 256 (t_1) × 2048 (t_2) complex points and 16 scans per t_1 increment. The data were processed using Bruker TopSpin 2.1 software and analyzed with SPARKY 3[6] software.

References

[1] T. Sala, M. V. Sargent, *J. Chem. Soc. Perkin Trans. 1,* 1981, 877.

[2] L. Yao, L. T. Smith, J. Aube, *J. Org. Chem.* 2004, **69**, 1720.

[3] H. Yashiroda, T. Mizushima, K. Okamoto, T. Kameyama, H. Hayashi, T. Kishimoto, S. Niwa, M. Kasahara, E. Kurimoto, E. Sakata, K. Takagi, A. Suzuki, Y. Hirano, S. Murata, K. Kato, T. Yamane, K. Tanaka, *Nat. Struct. Mol. Biol.,* 2008, **15**, 228.

[4] M. Yagi-Utsumi, K. Matsuo, K. Yanagisawa, K. Gekko, K. Kato, *Int. J. Alzheimers Dis.,* 2011, 925073, doi:10.4061/2011/925073.

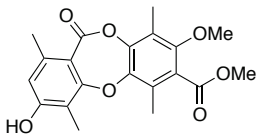
[5] F. Delaglio, S. Grzesiek, G. W. Vuister, G. Zhu, J. Pfeifer, A. Bax, *J. Biomol. NMR,* 1995, **6**, 277.

[6] T. Goddard, D. Kneller, University of California, San Francisco.

[7] W. F. Vranken, W. Boucher, T. J. Stevens, R. H. Fogh, A. Pajon, M. Llinas, E. L. Ulrich, J. L. Markley, J. Ionides, E. D. Laue, *Protein,* 2005, **59**, 687.

[8] Y. Uekusa, S. Mimura, H. Sasakawa, E. Kurimoto, E. Sakata, Serve O.; Yagi, H.; Tokunaga, F.; Iwai, K.; Kato, K. *Biomol. NMR Assign.,* 2012, **6**, 177.

Copies of NMR spectra



6
^{1}H NMR
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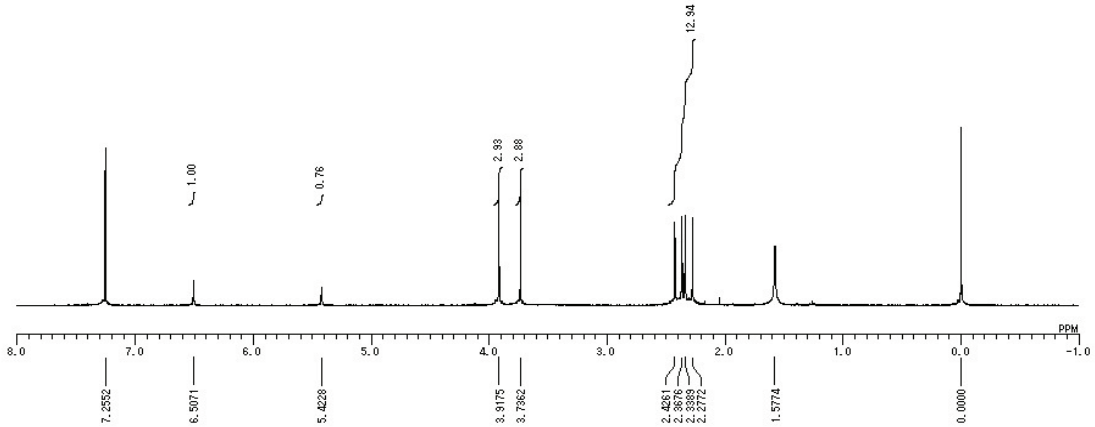

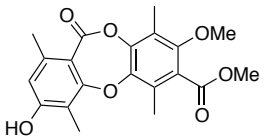

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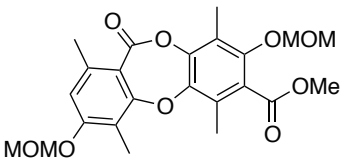

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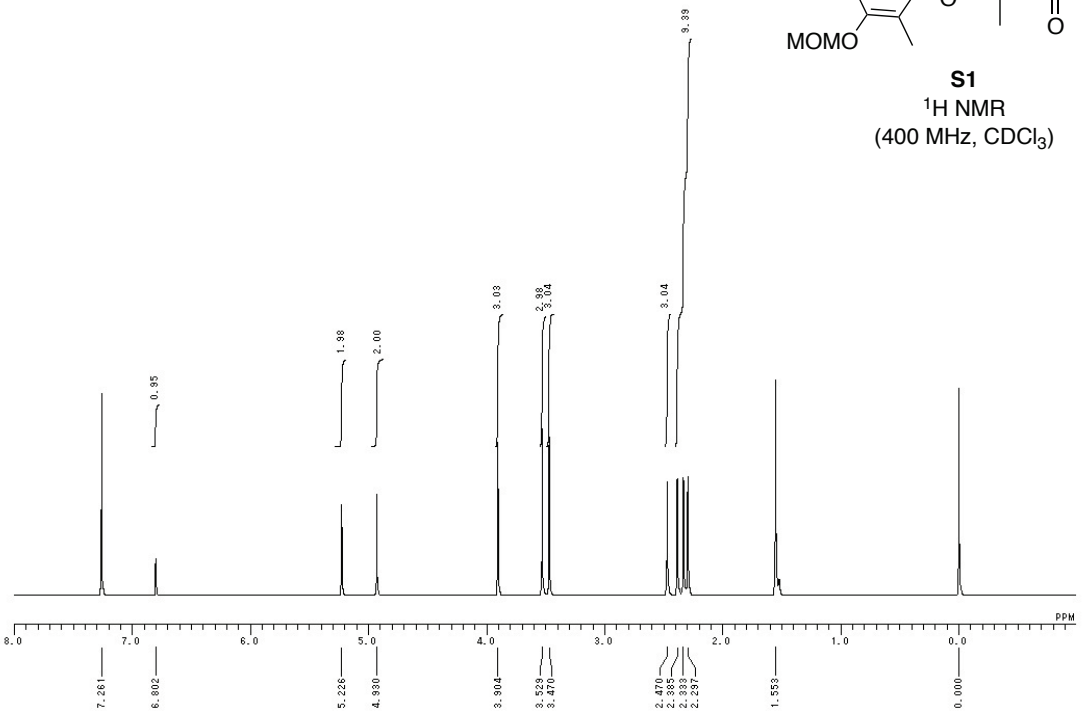

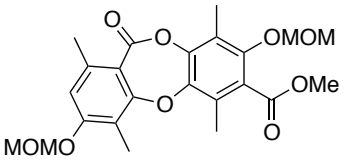

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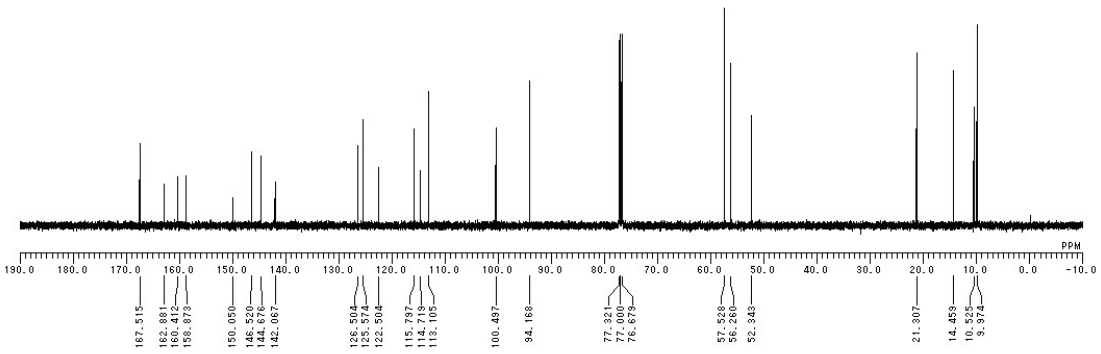

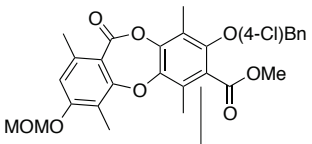

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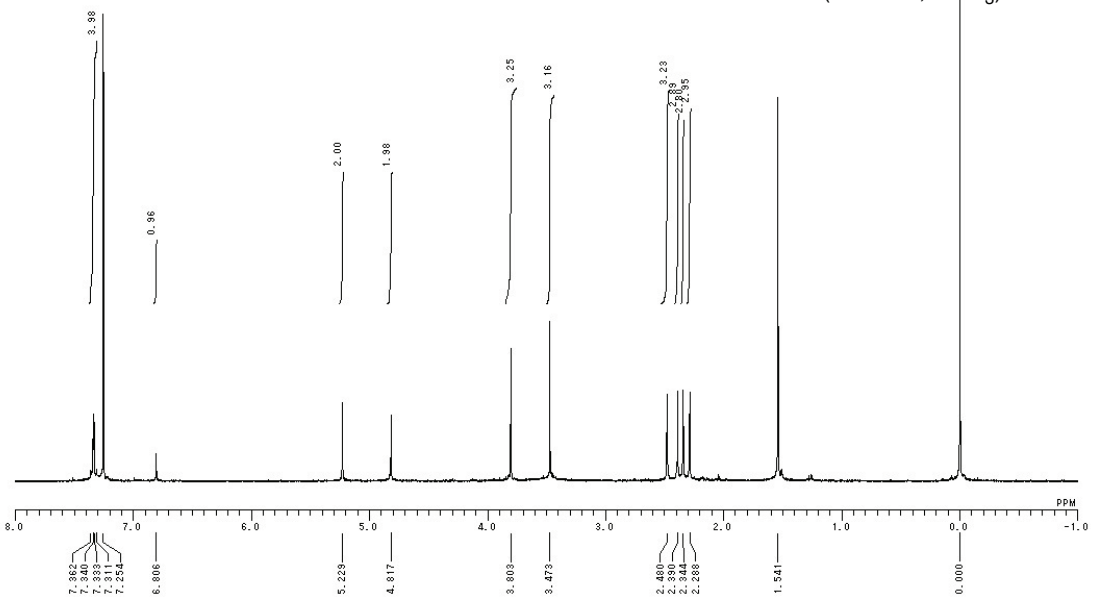

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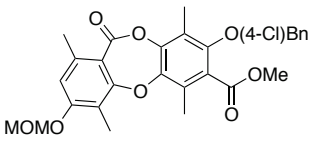

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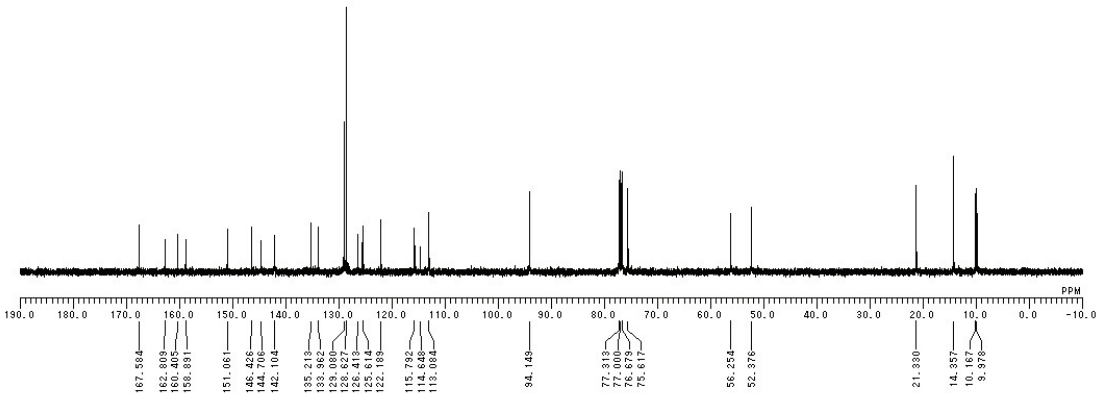

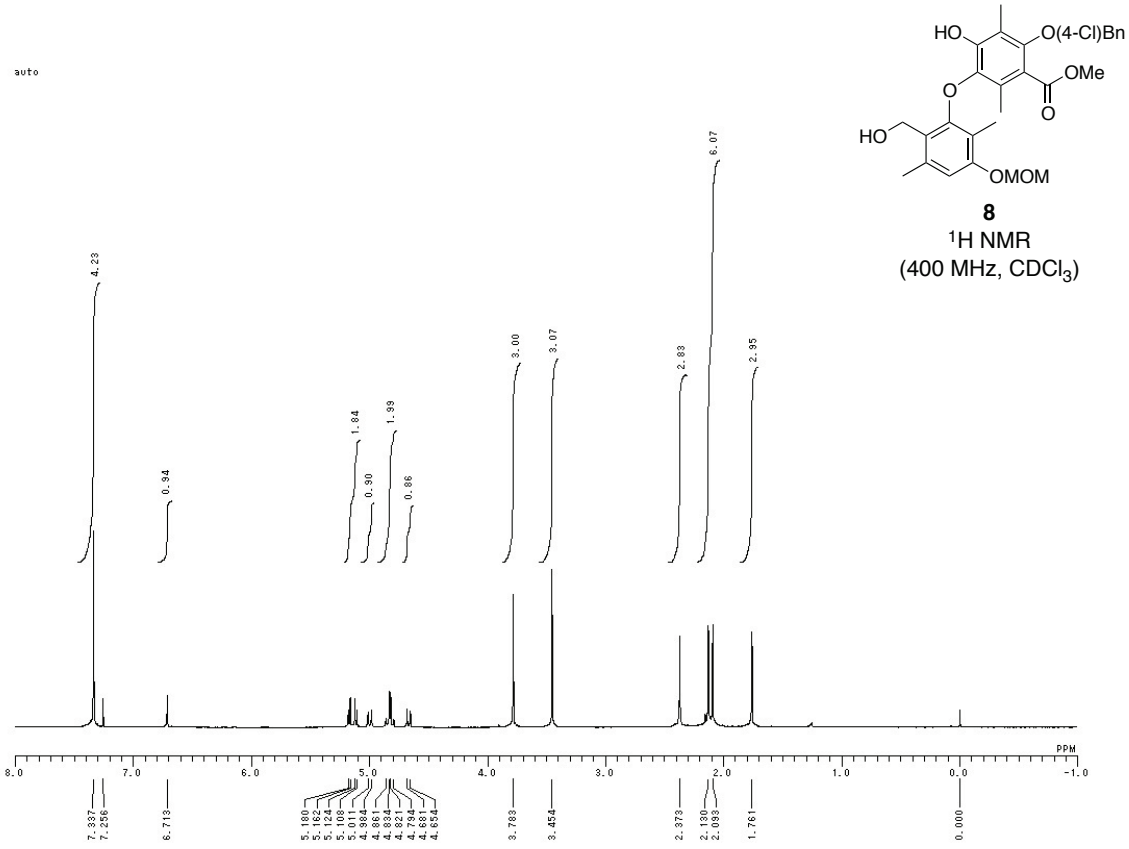

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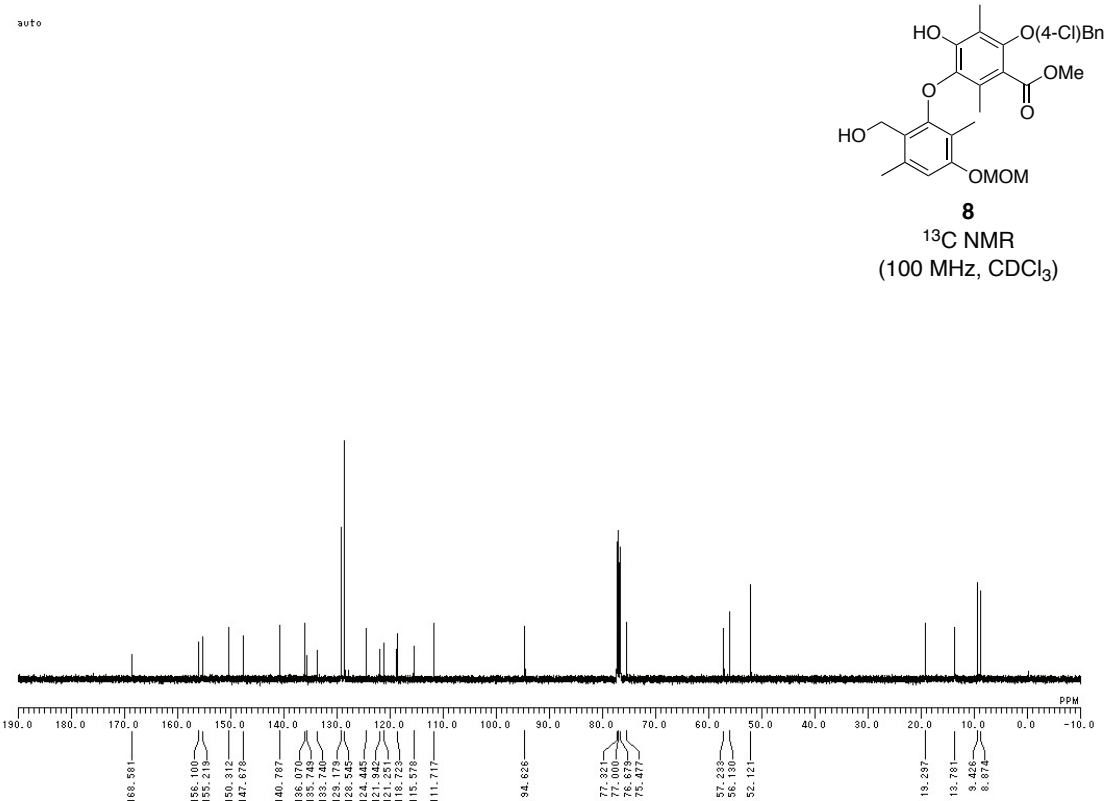

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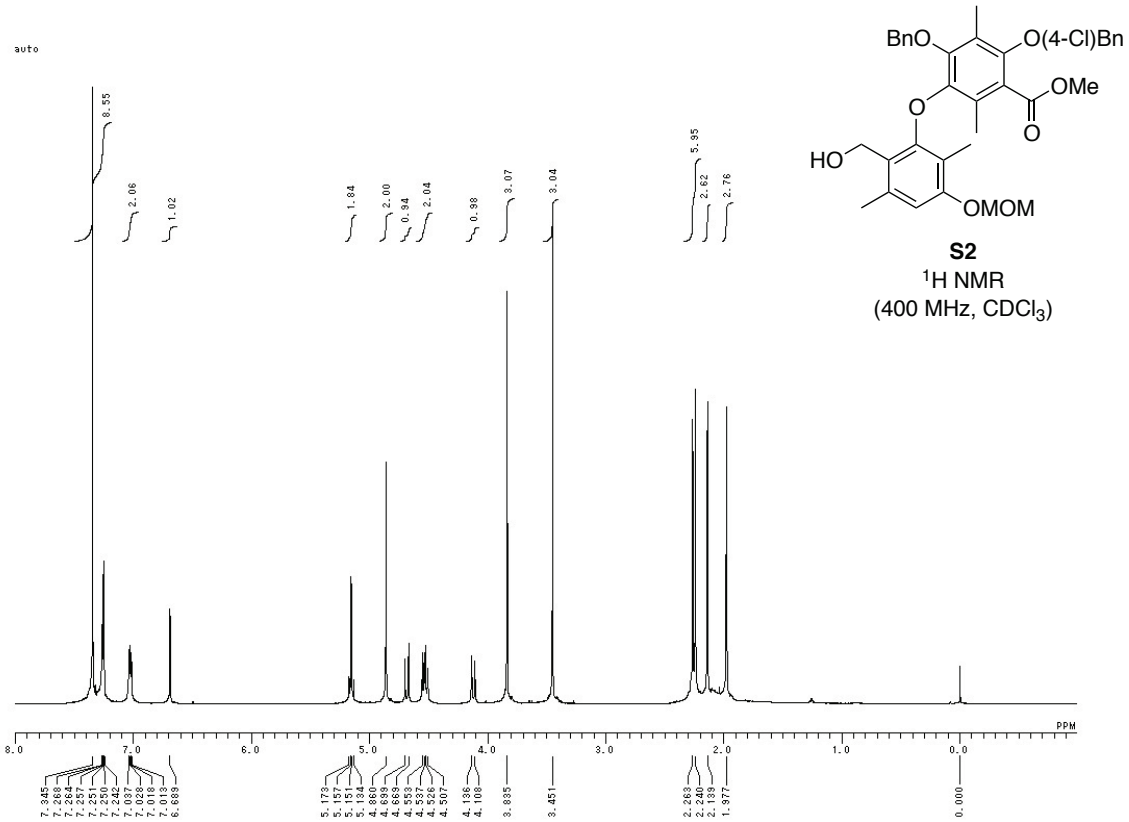

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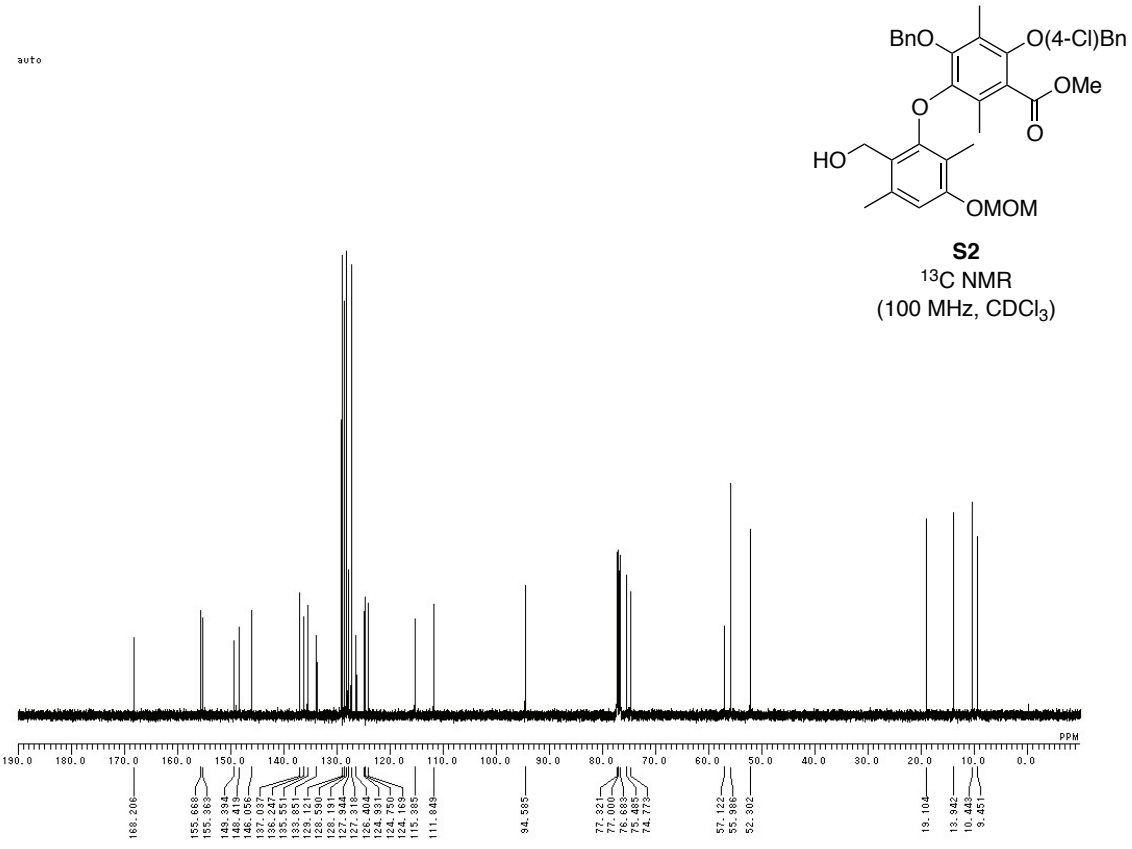

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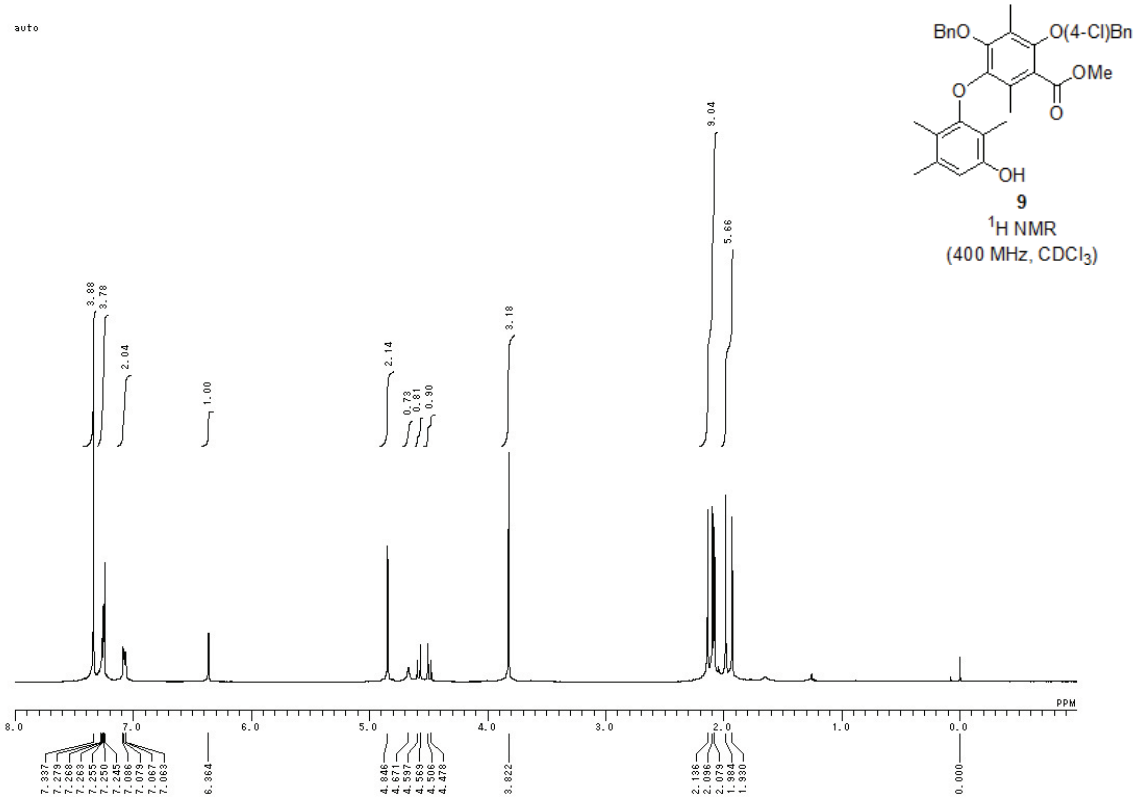
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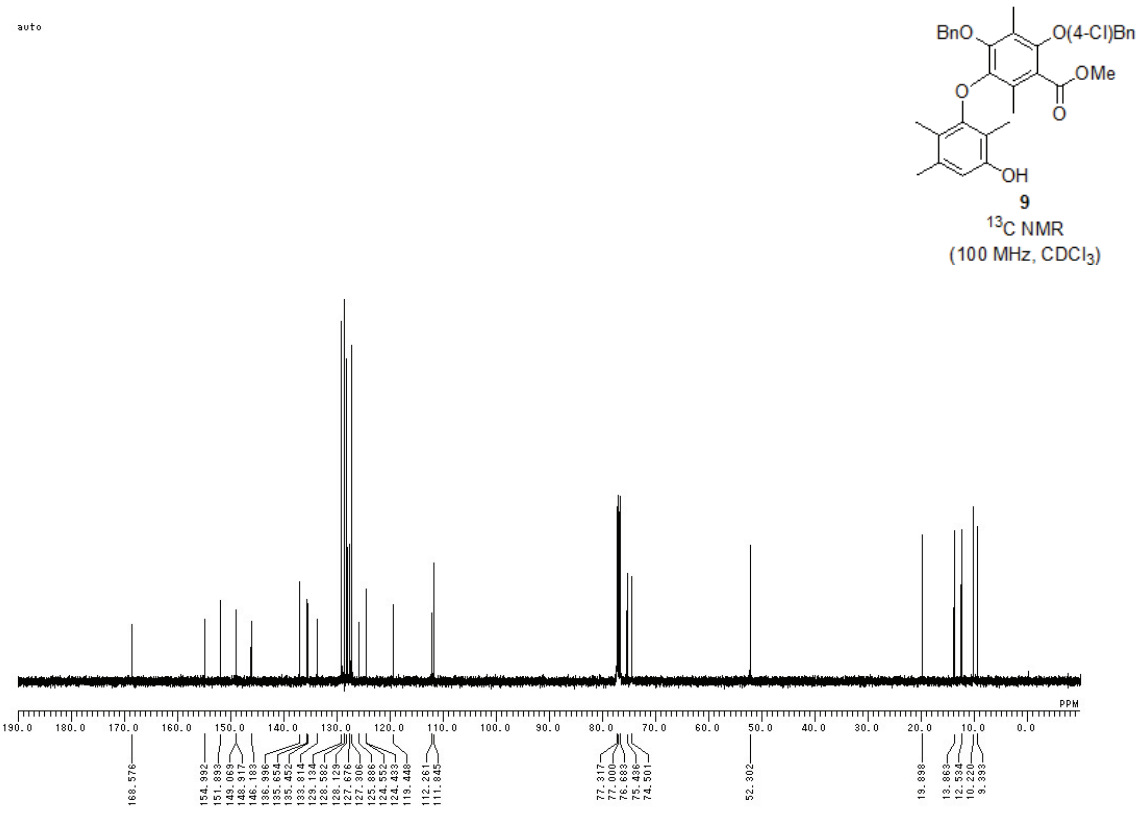
5.66
PPM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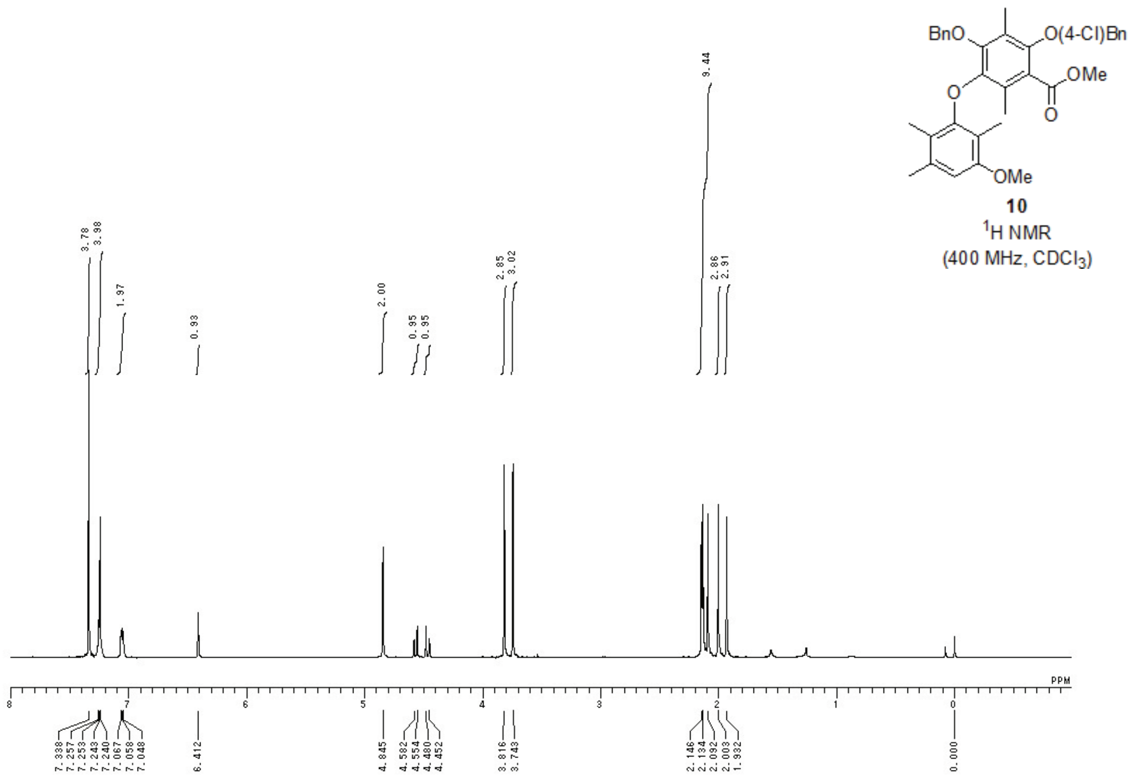
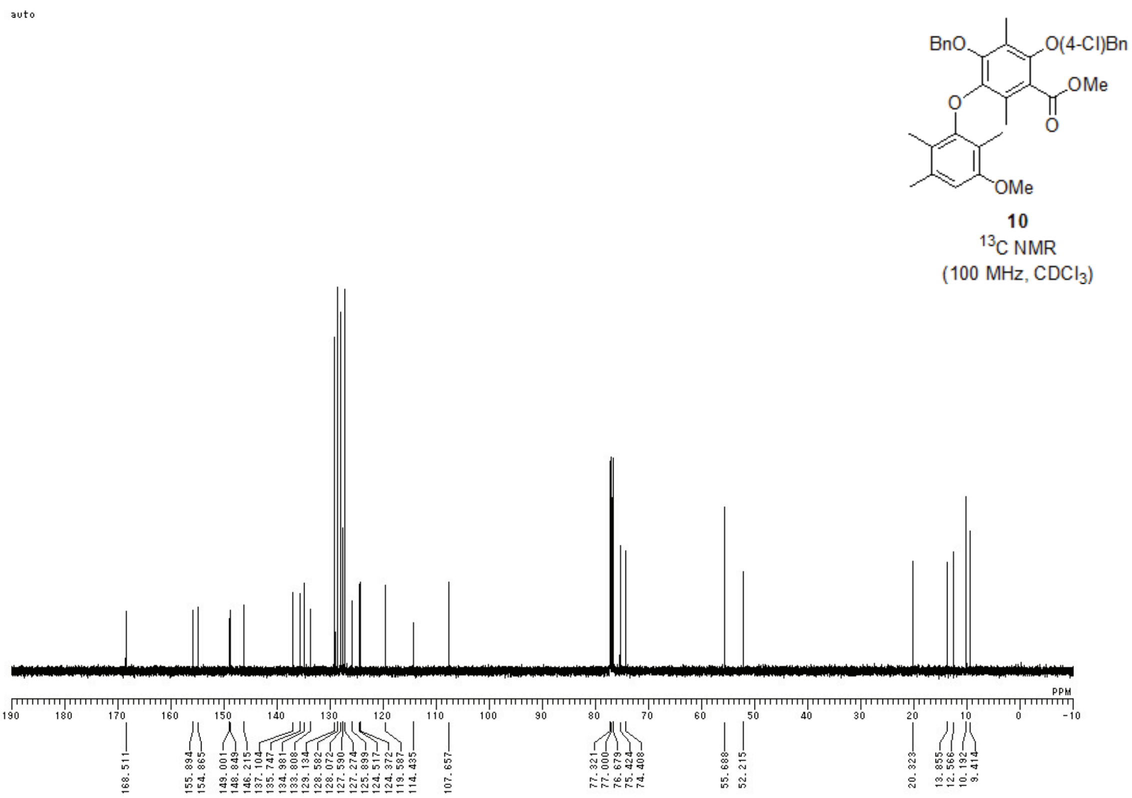
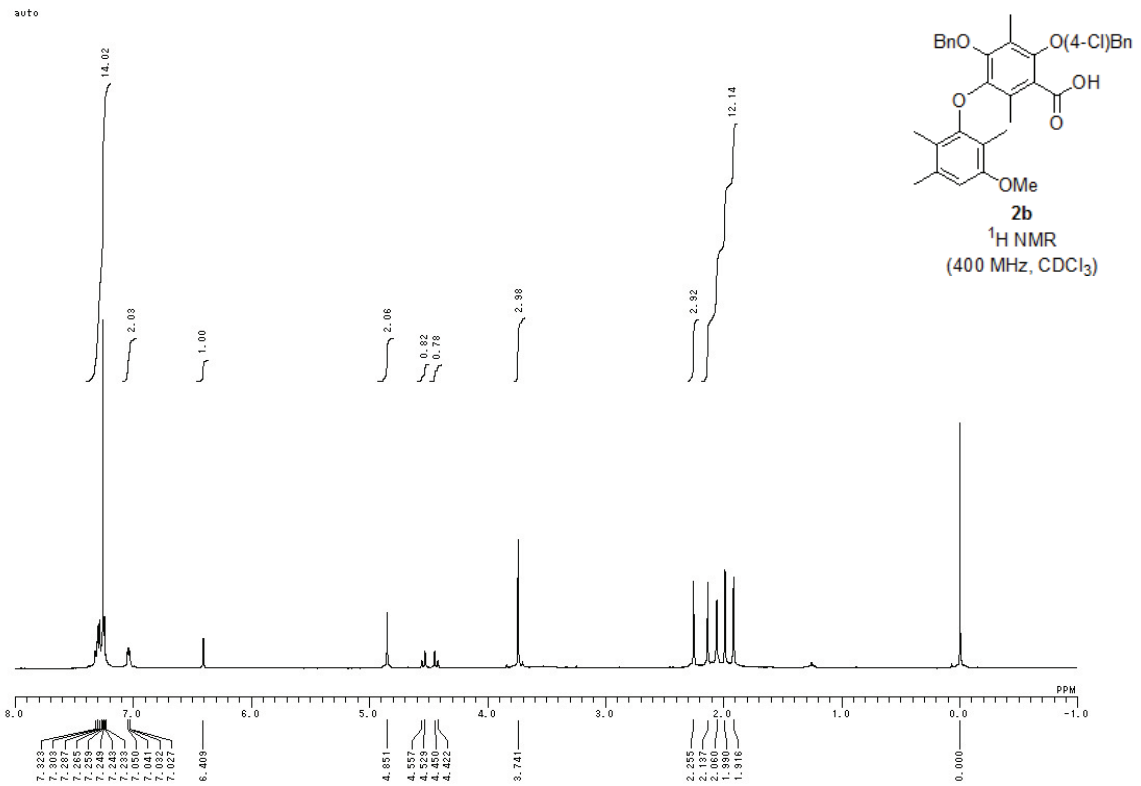
Electronic Supplementary Material (ESI) for Chemical Science



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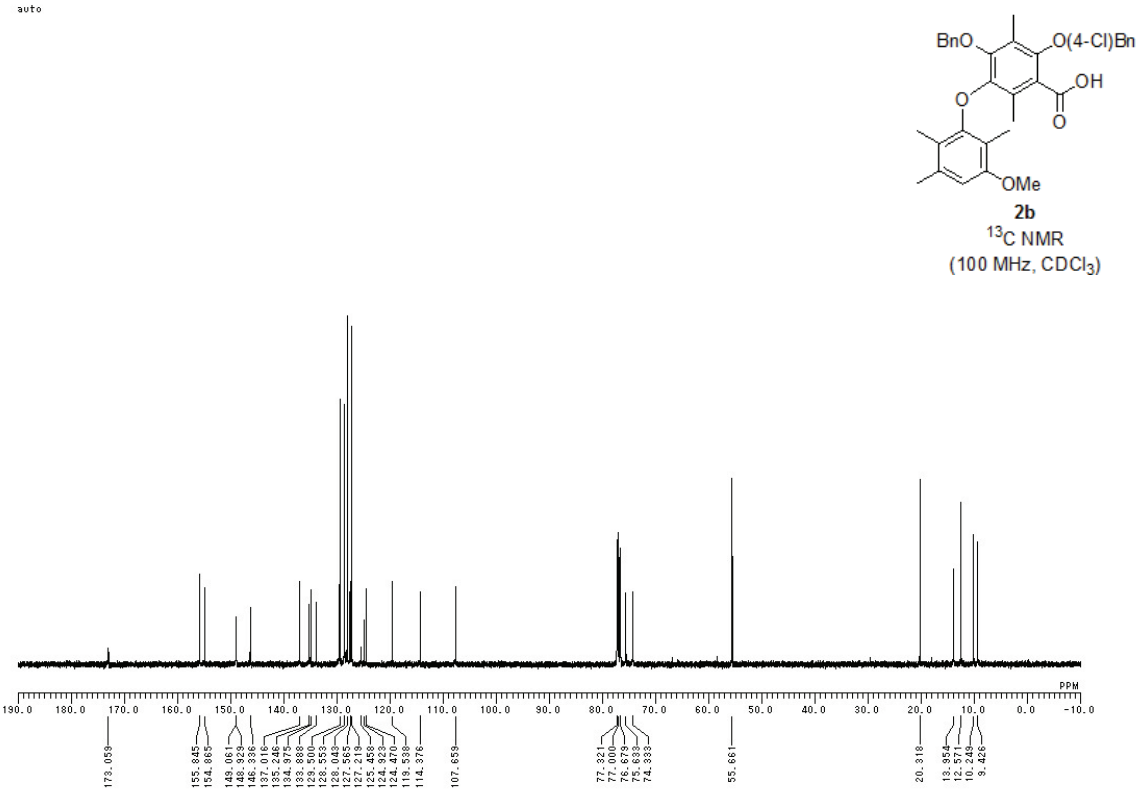
PPM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2b
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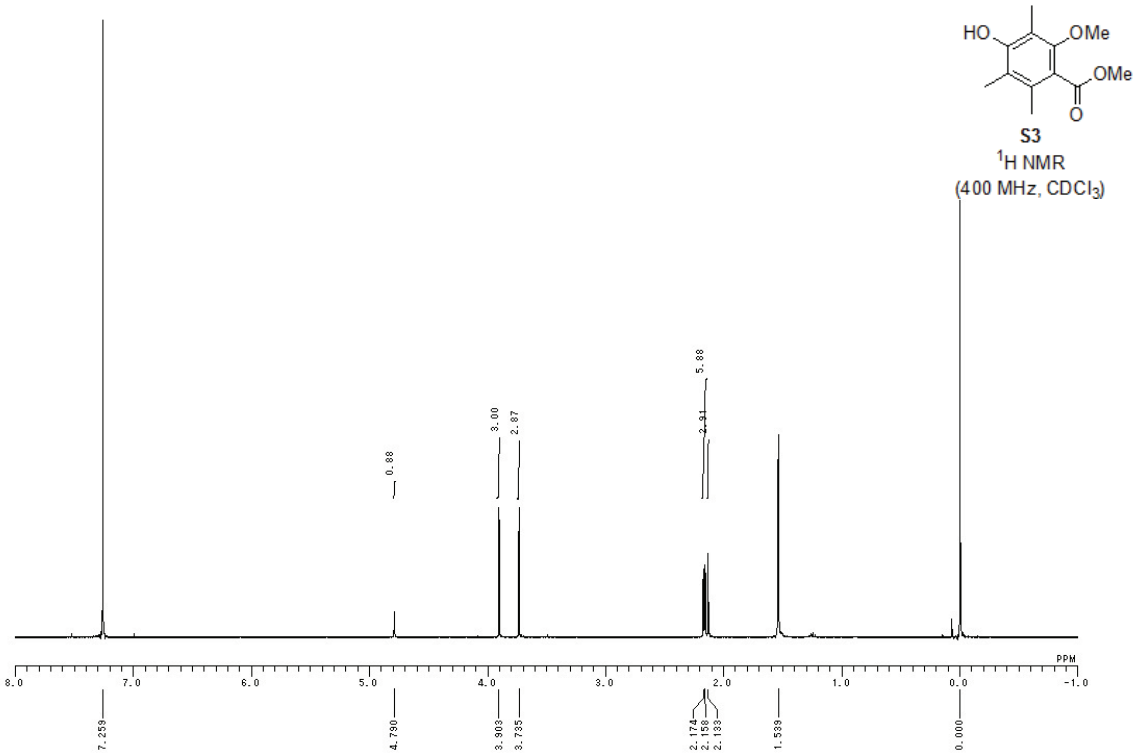
PPM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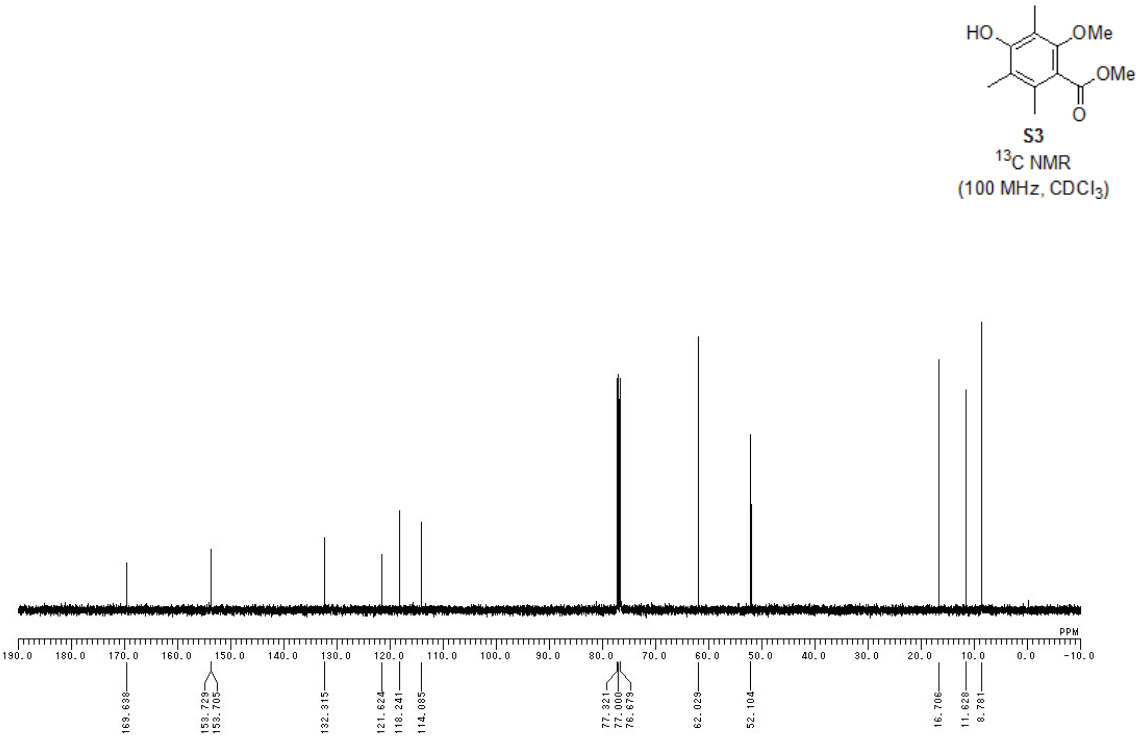
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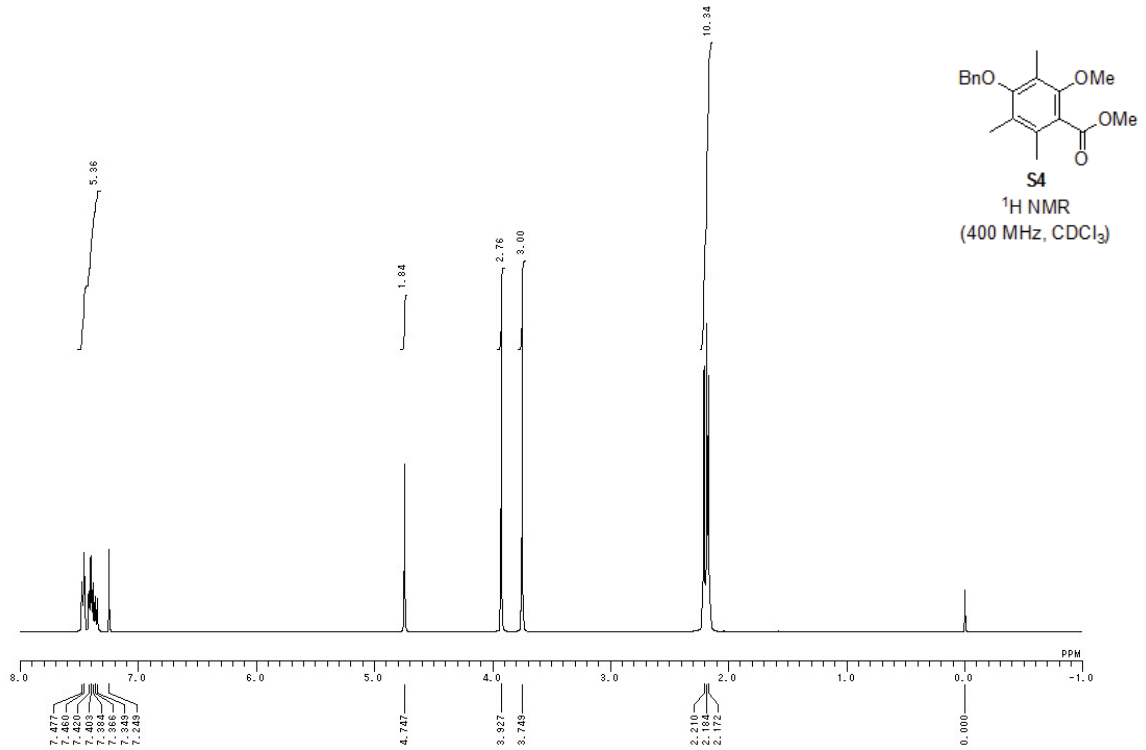
8.781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3.00
PPM
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7.460
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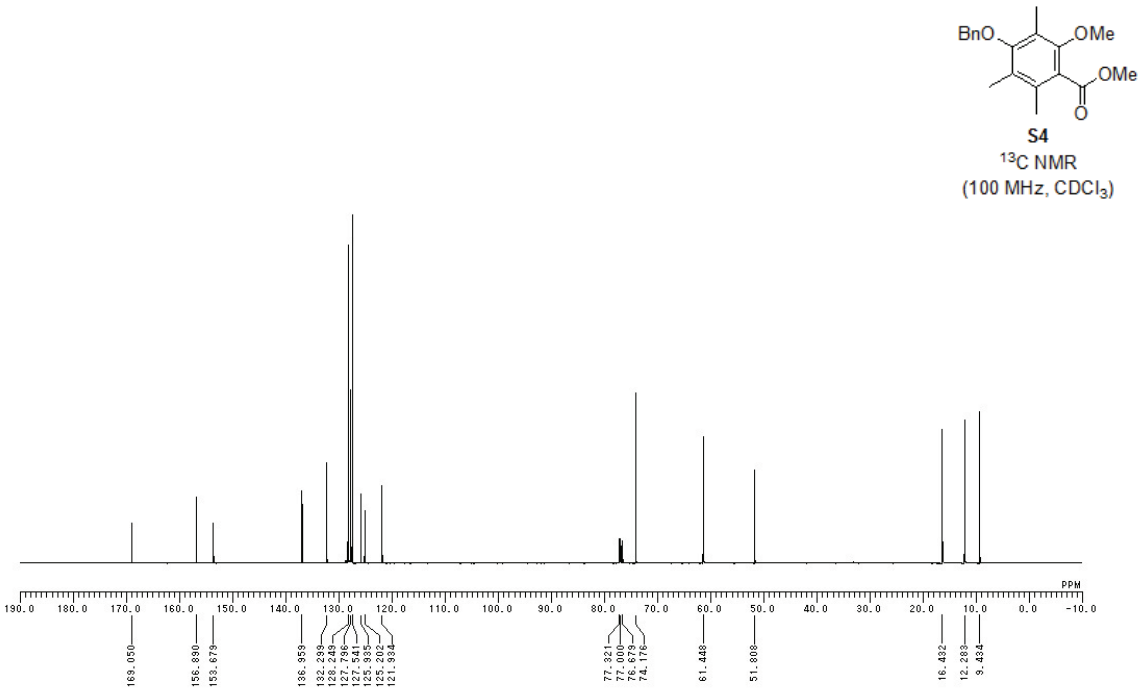
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OMe
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O
S4
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(100 MHz, $CDCl_3$)
PPM
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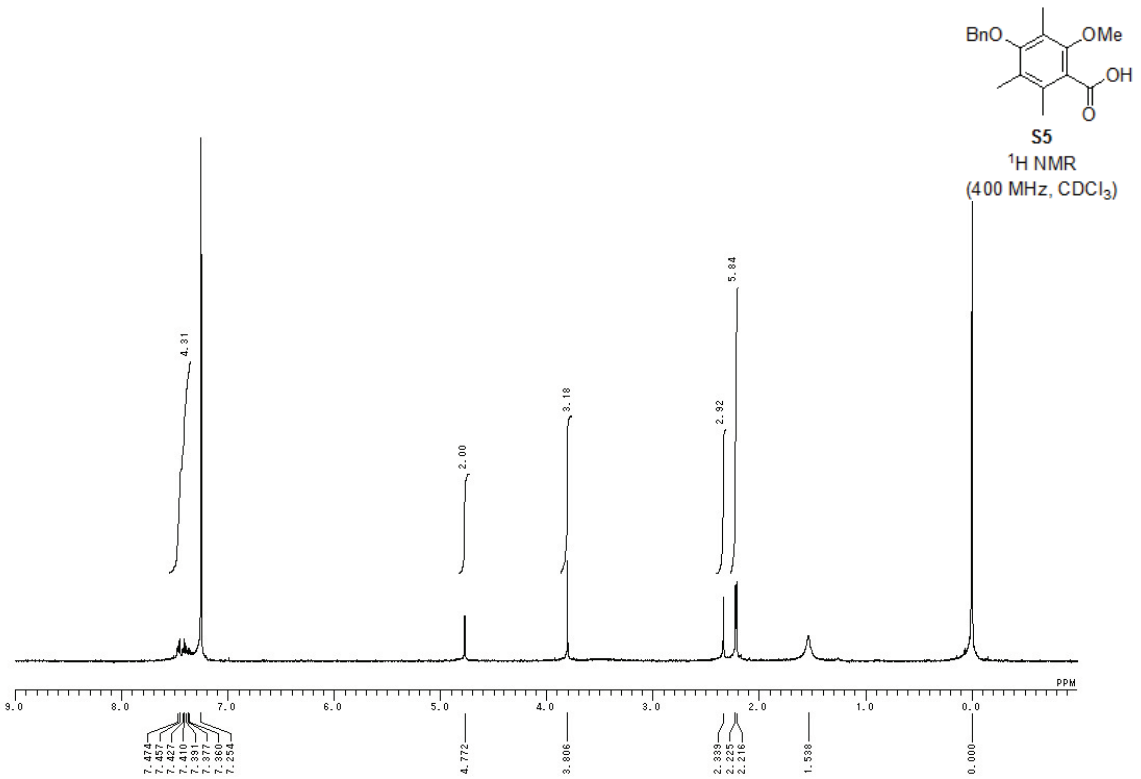
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5.84
PPM
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
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7.457
7.427
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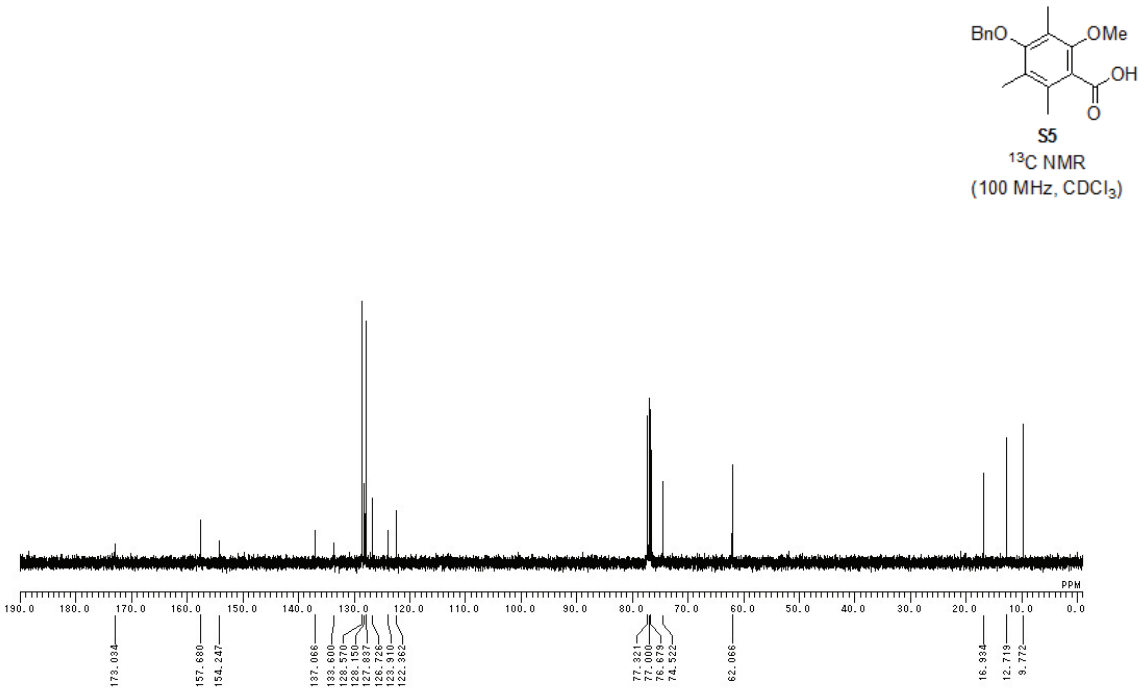
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OMe
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S5
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(100 MHz, $CDCl_3$)
PPM
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150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
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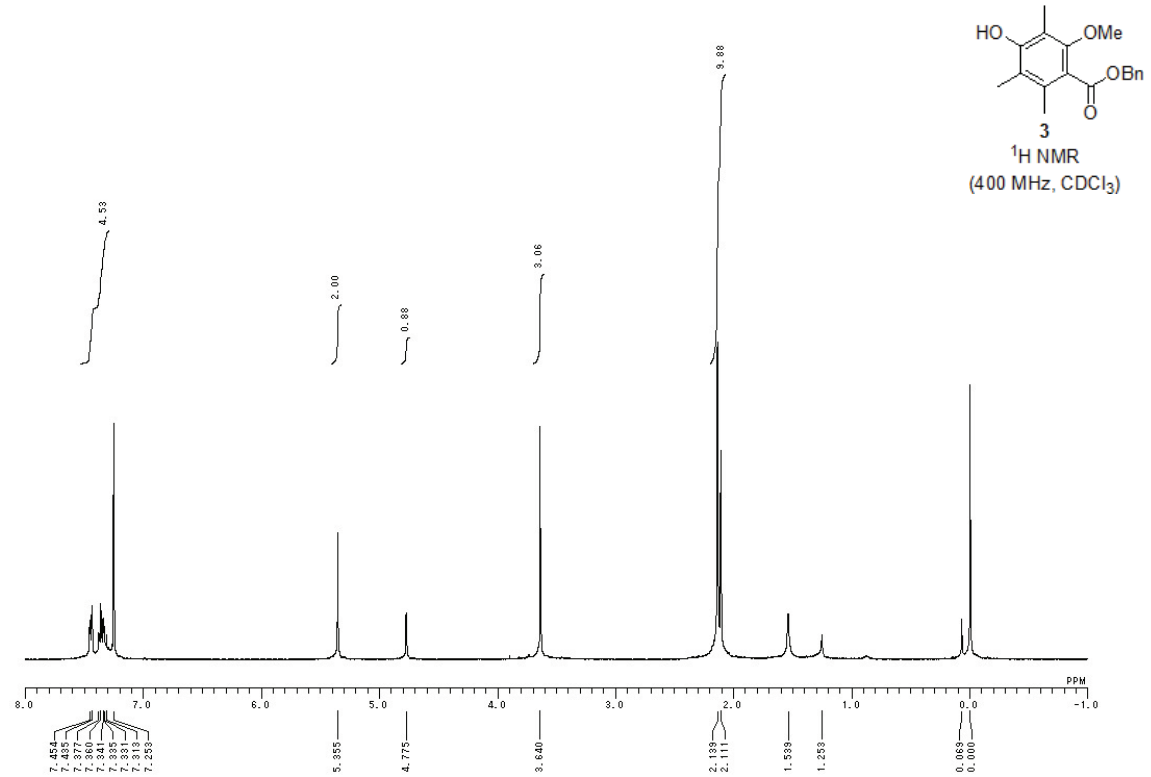
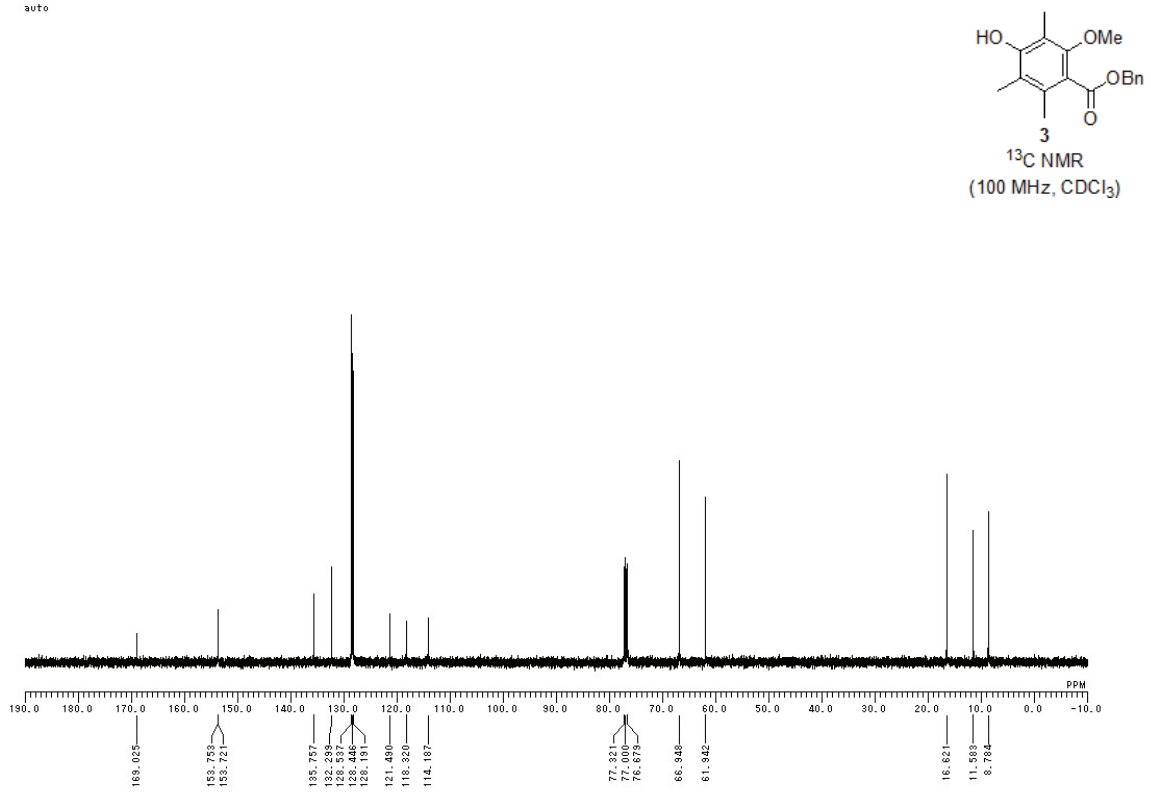
20.0
10.0
0.0
173.034
157.680
154.247
137.066
128.600
128.370
128.159
127.837
126.728
123.910
122.362
77.321
77.000
76.679
74.522
62.066
16.934
12.719
9.772


HO
OMe
OBn
O
3
1H NMR
(400 MHz, CDCl3)
4.53
2.00
0.88
3.06
9.88
PPM
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
-1.0
7.454
7.435
7.377
7.360
7.341
7.335
7.313
7.253
5.355
4.775
3.640
2.139
2.111
1.539
1.253
0.069
0.000


auto
HO
OMe
OBn
O
3
13C NMR
(100 MHz, CDCl3)
PPM
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
-10.0
169.025
153.758
153.721
135.757
132.239
128.537
128.446
128.191
121.490
118.220
114.187
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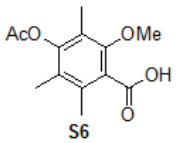
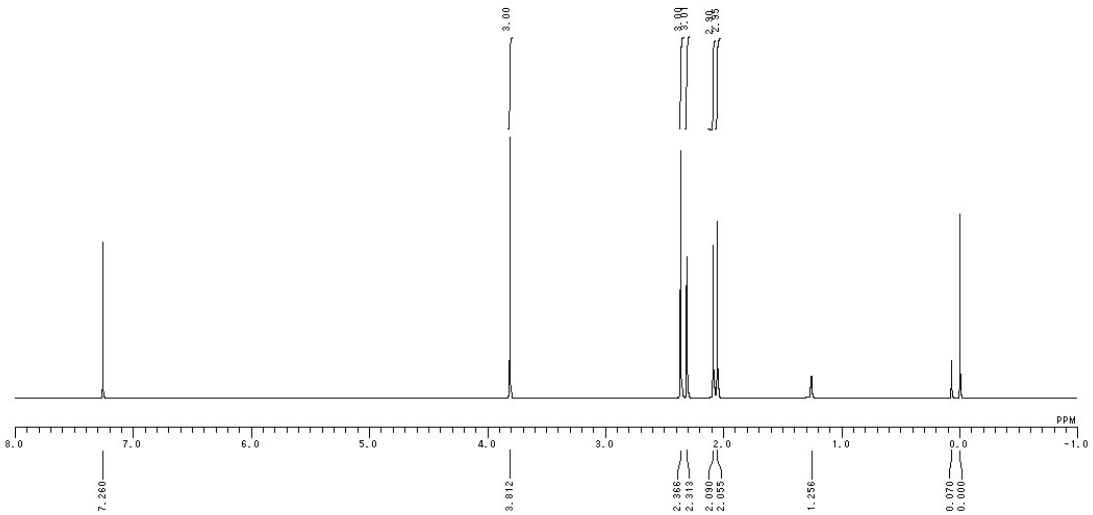
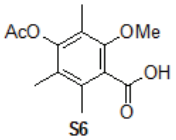
76.679
66.948
61.942
16.621
11.583
8.784



S6

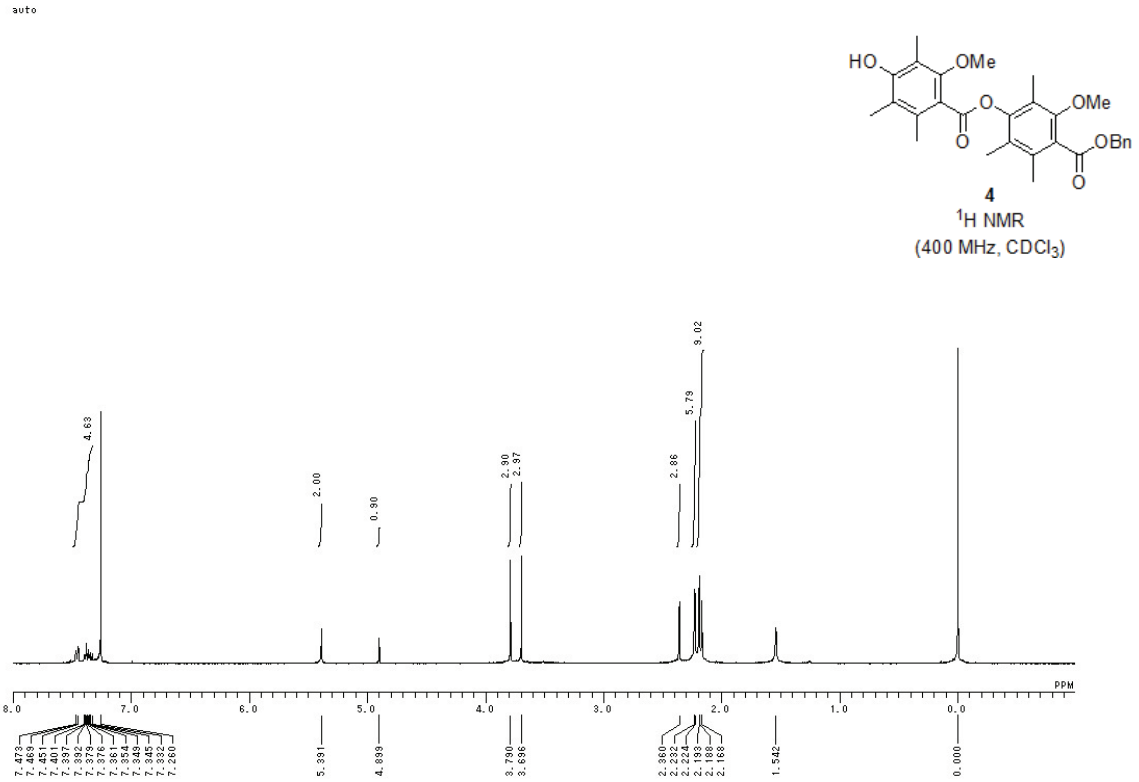
^{1}H NMR
(400 MHz, $CDCl_3$)

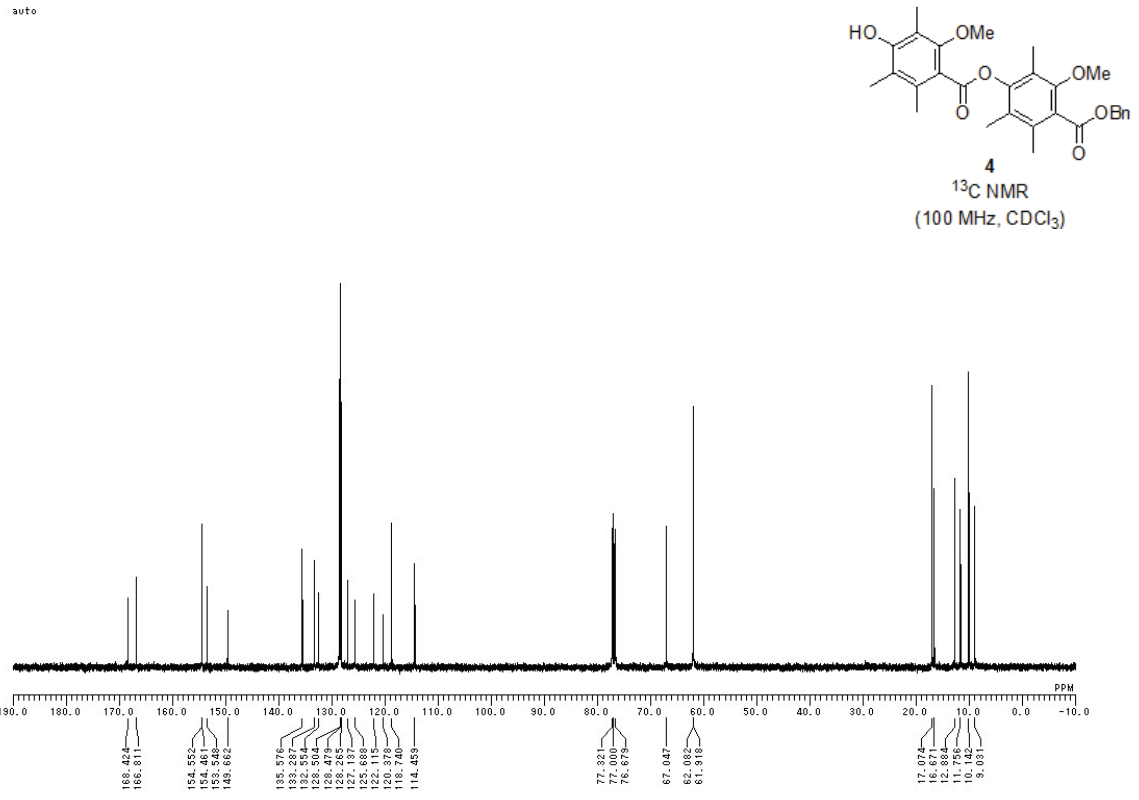


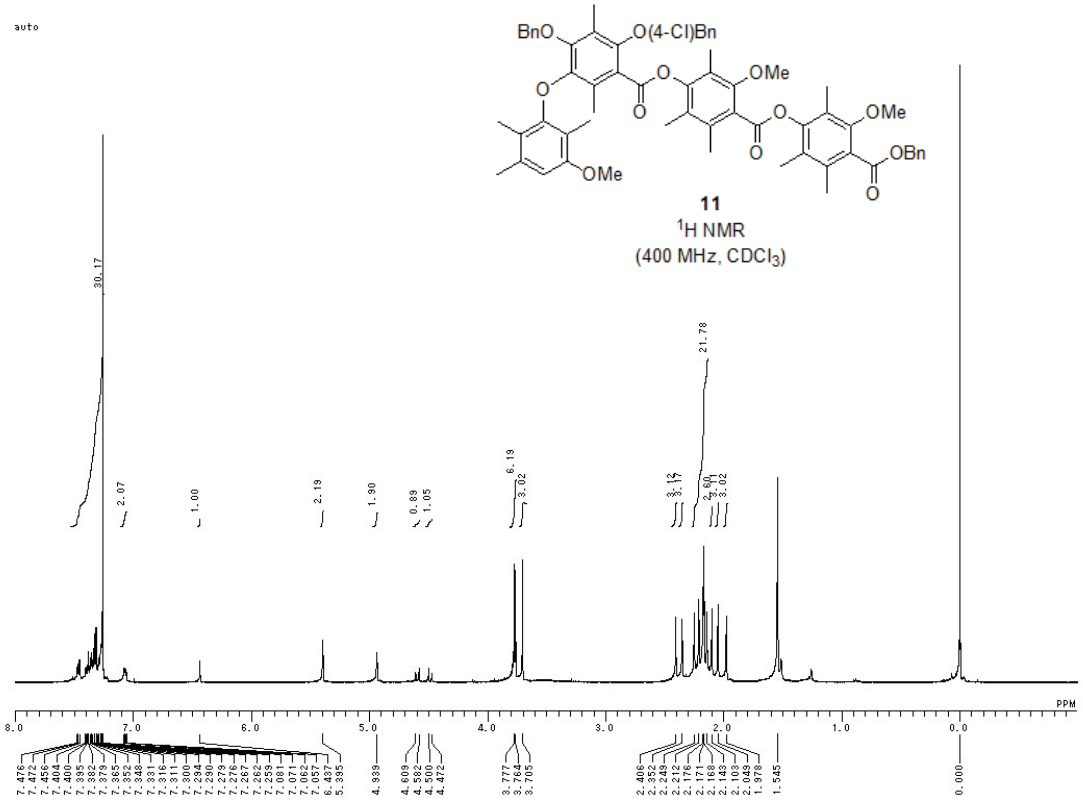


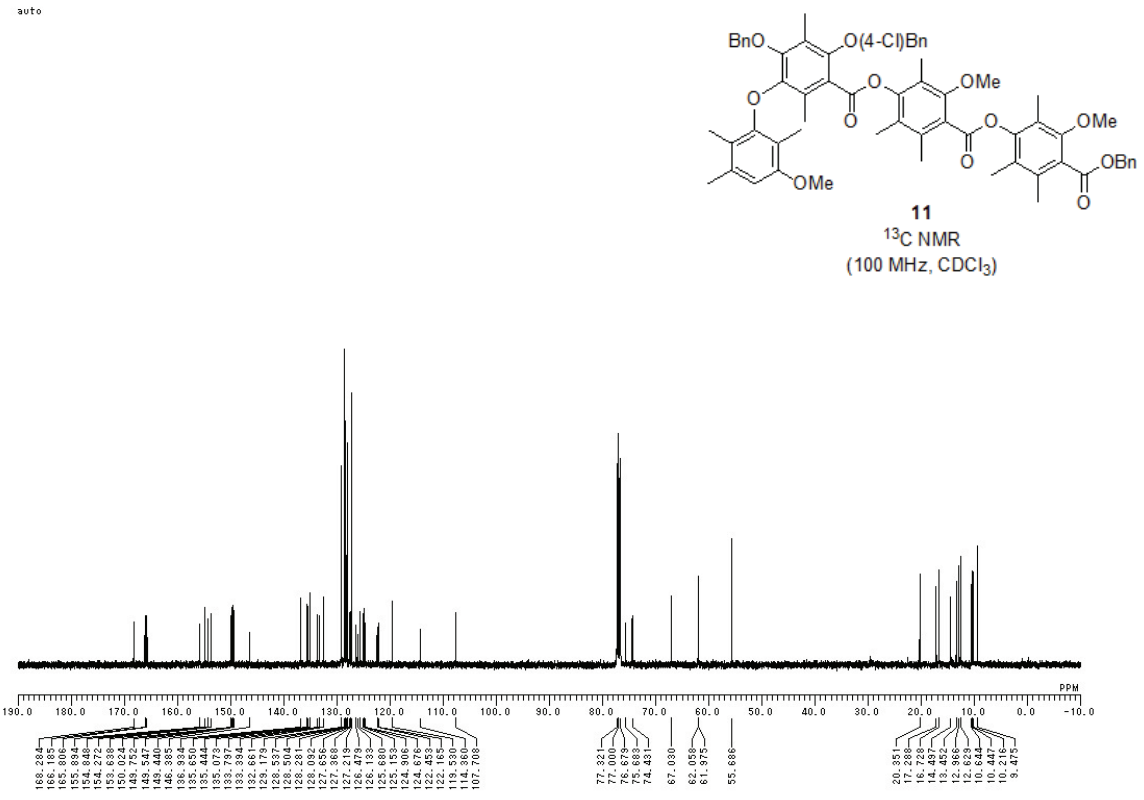
S6

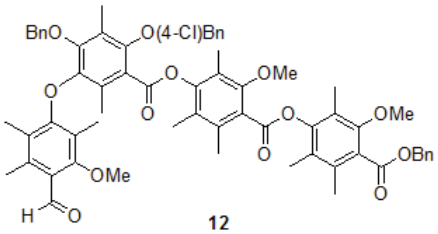
^{13}C NMR
(100 MHz, $CDCl_3$)


auto
HO
OMe
O
O
OMe
OBn
O
4
1H NMR
(400 MHz, CDCl3)
4.63
2.00
0.90
2.90
2.87
2.86
5.79
9.02
PPM


auto
HO
OMe
O
O
OMe
OBn
O
4
13C NMR
(100 MHz, CDCl3)
PPM


auto
BnO
O(4-Cl)Bn
OMe
OMe
OBn
OMe
11
1H NMR
(400 MHz, CDCl3)
PPM

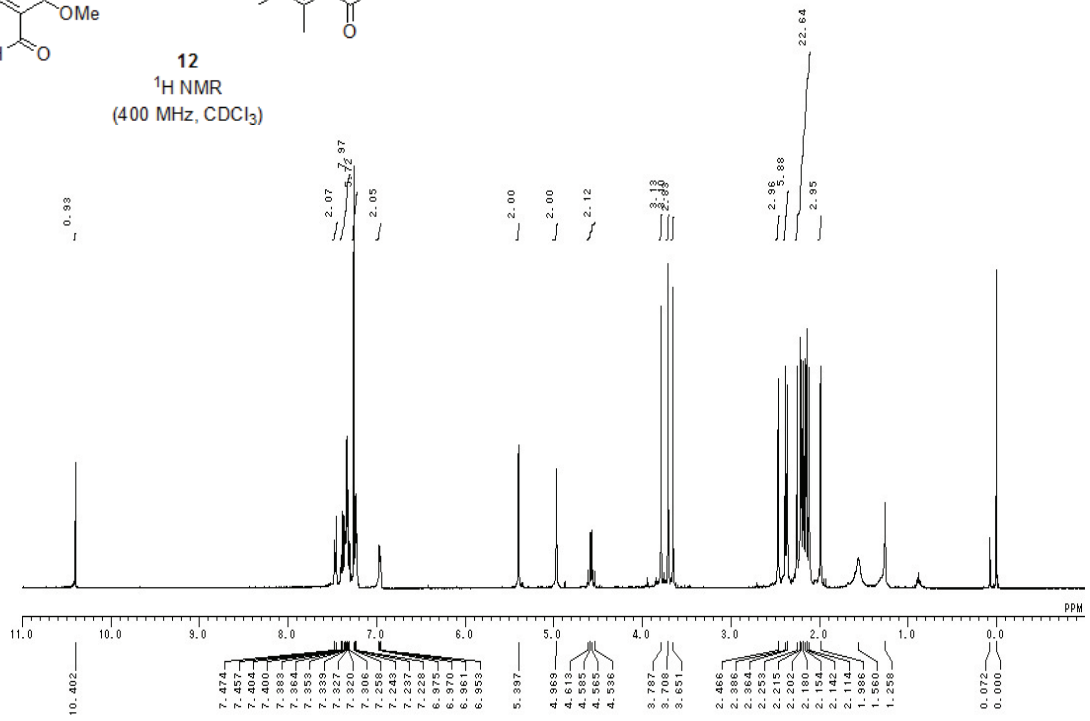

auto
BnO
O(4-Cl)Bn
OMe
OMe
OBn
OMe
11
13C NMR
(100 MHz, CDCl3)
PPM

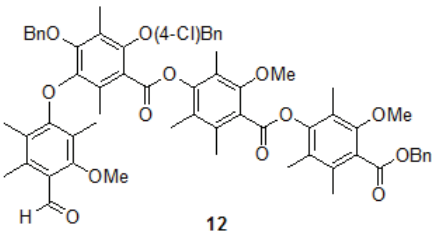


12

^{1}H NMR

(400 MHz, $CDCl_3$)

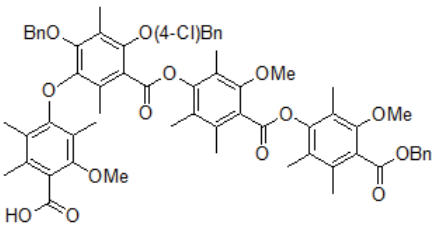




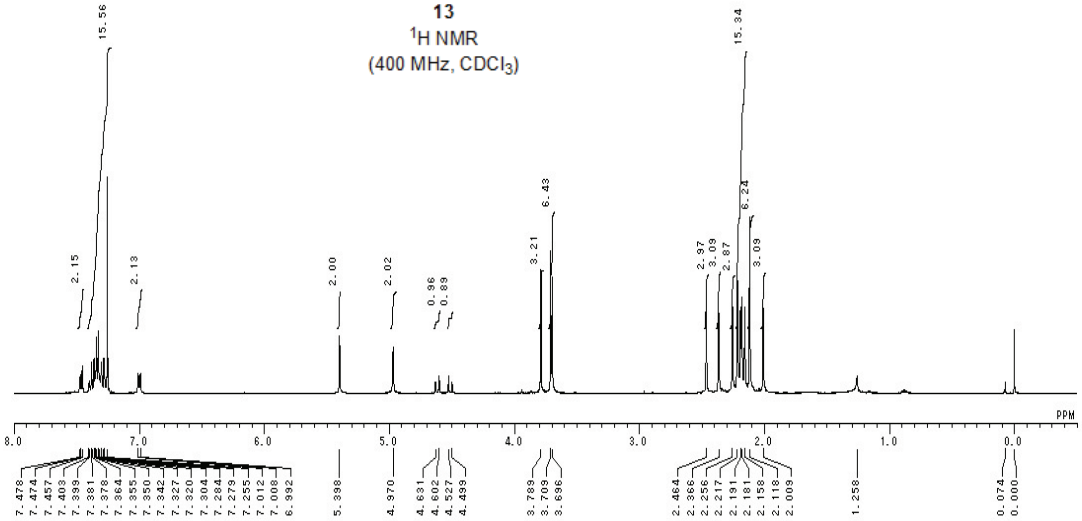
12

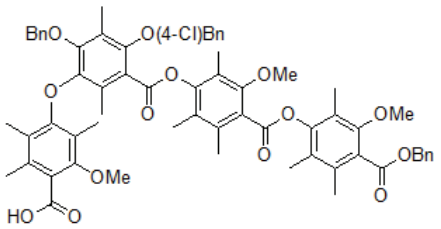
^{13}C NMR

(100 MHz, $CDCl_3$)

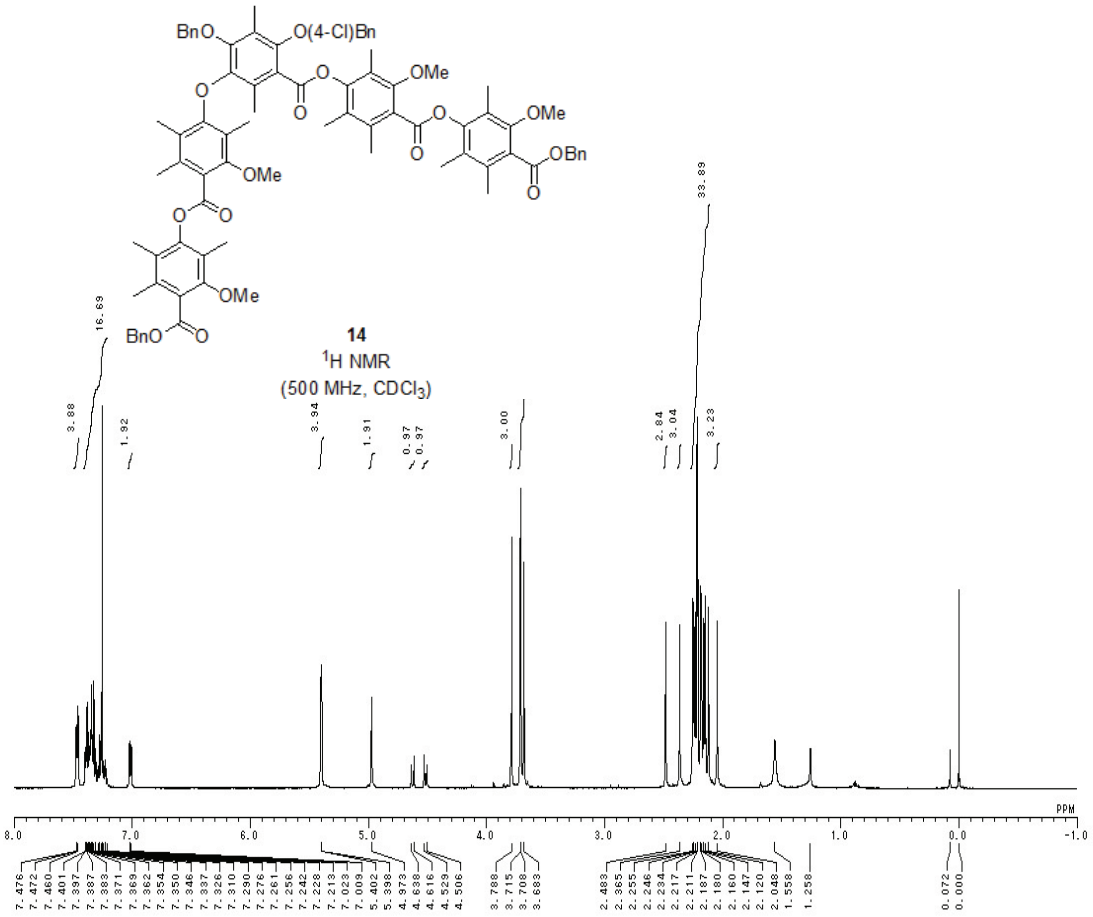


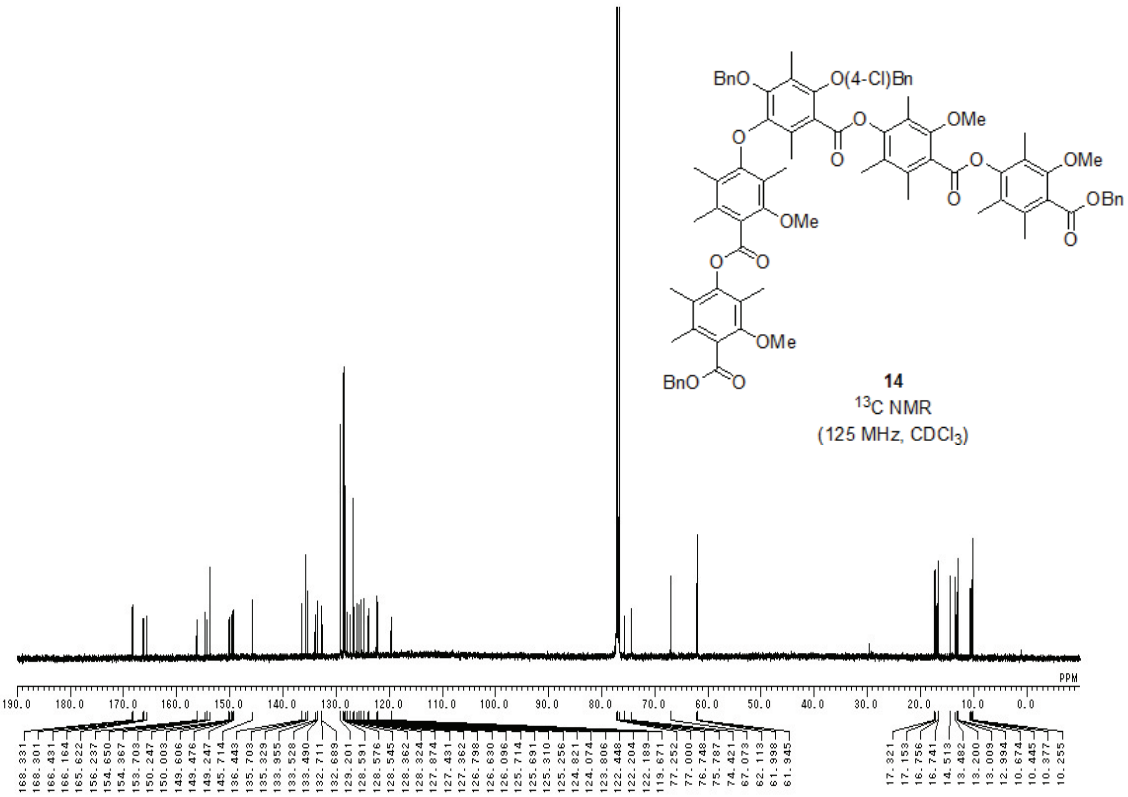
13
^{1}H NMR
(400 MHz, $CDCl_3$)

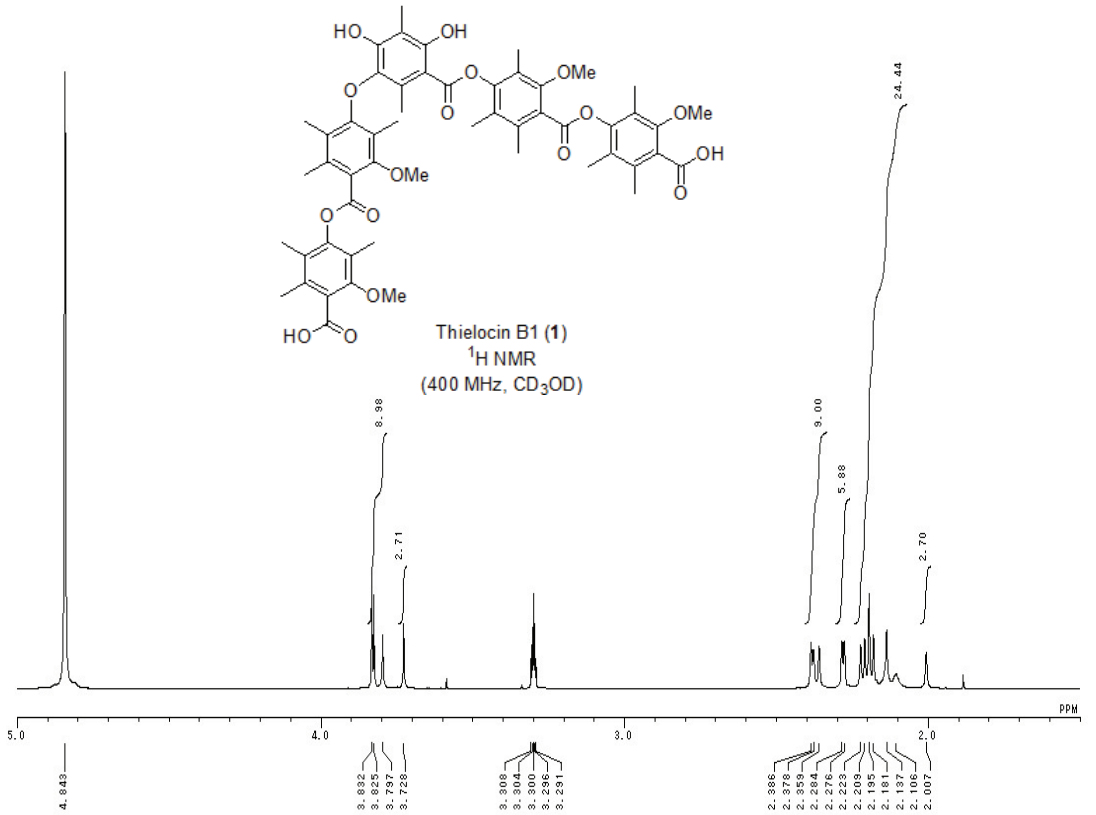


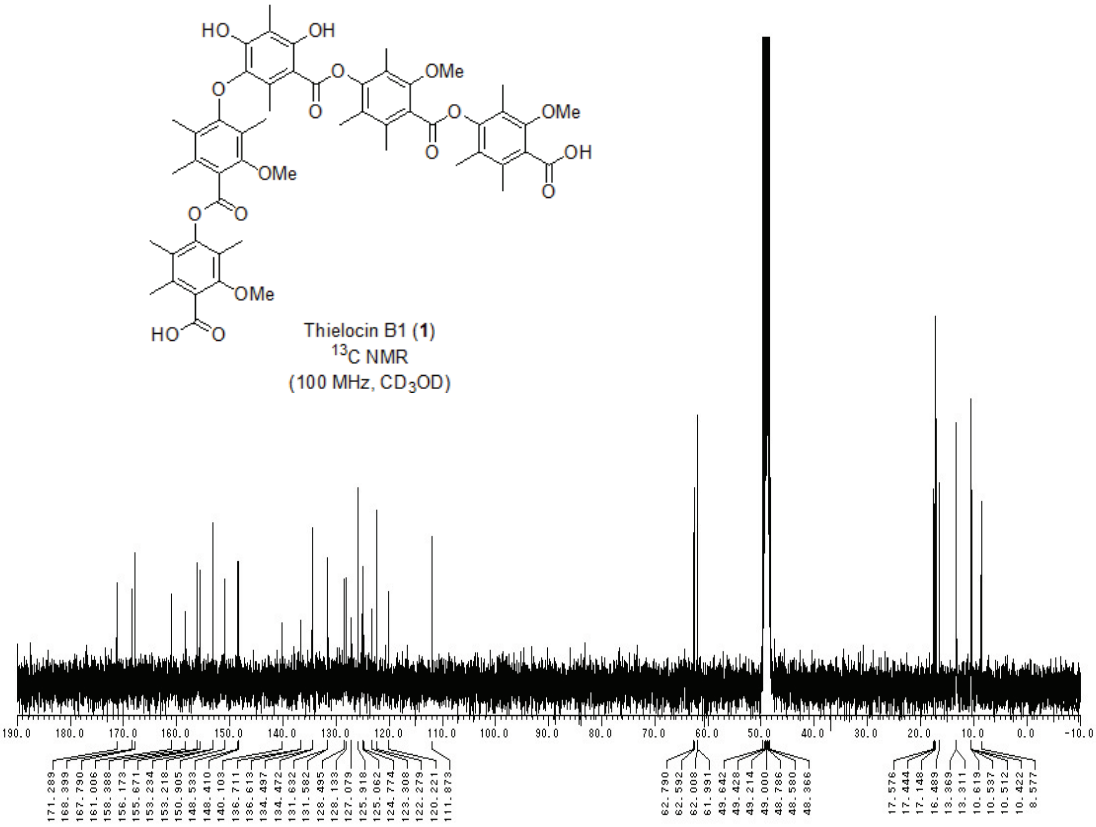


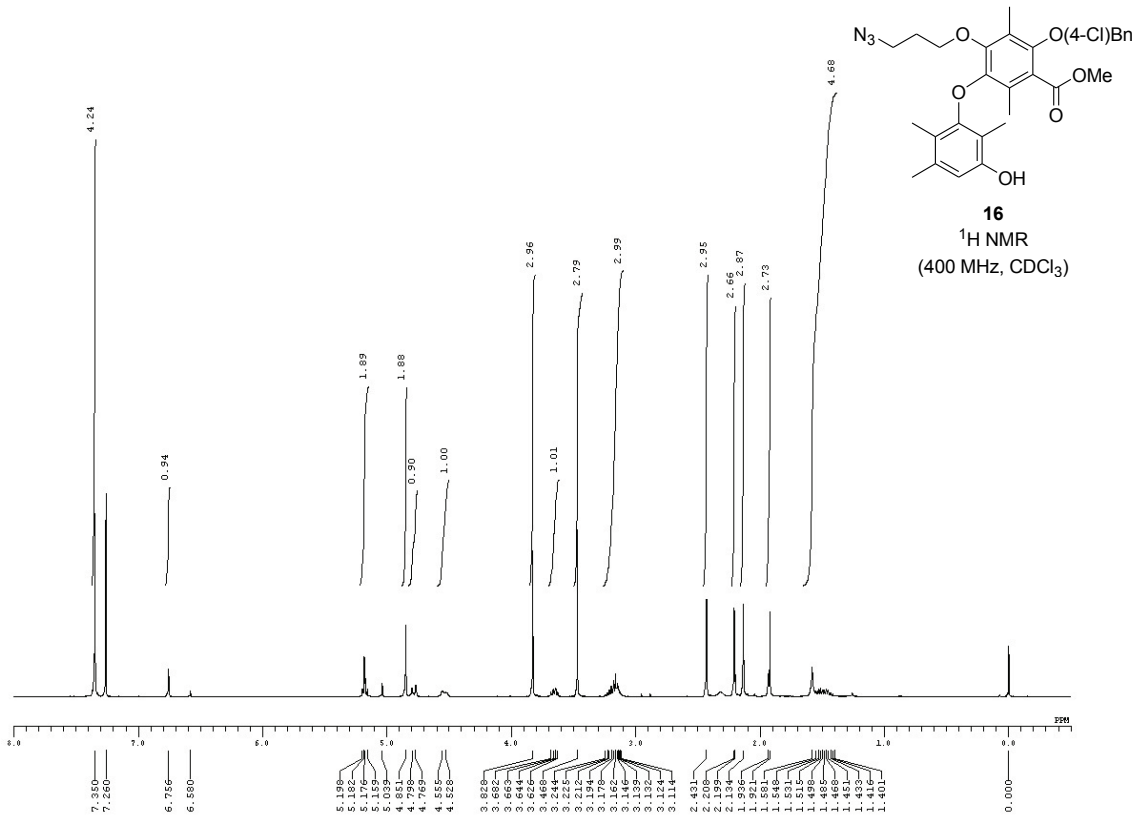
13
^{13}C NMR
(100 MHz, $CDCl_3$)


BnO
O(4-Cl)Bn
OMe
OBn
14
1H NMR
(500 MHz, CDCl3)
PPM

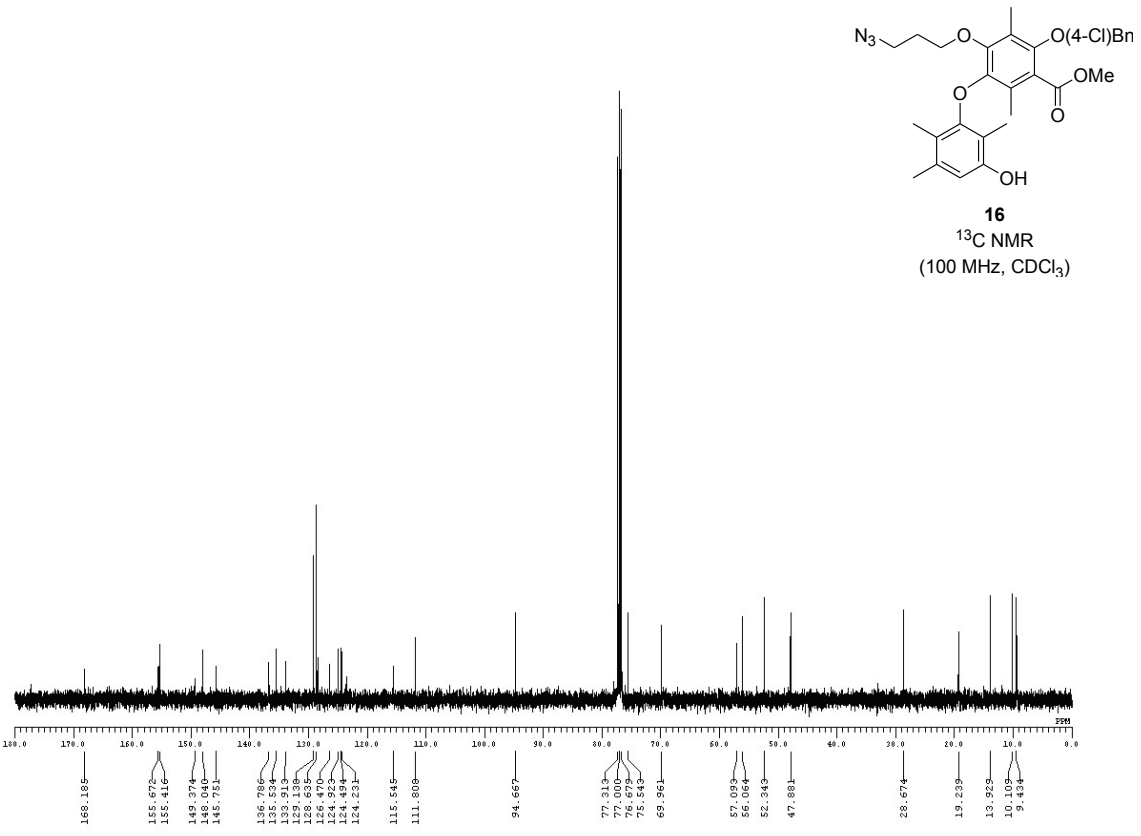

BnO
O(4-Cl)Bn
OMe
OBn
14
13C NMR
(125 MHz, CDCl3)
PPM


Thielocin B1 (1)
1H NMR
(400 MHz, CD3OD)
PPM

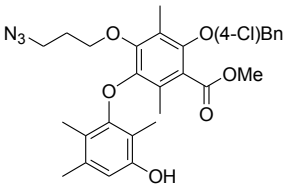

Thielocin B1 (1)
13C NMR
(100 MHz, CD3OD)



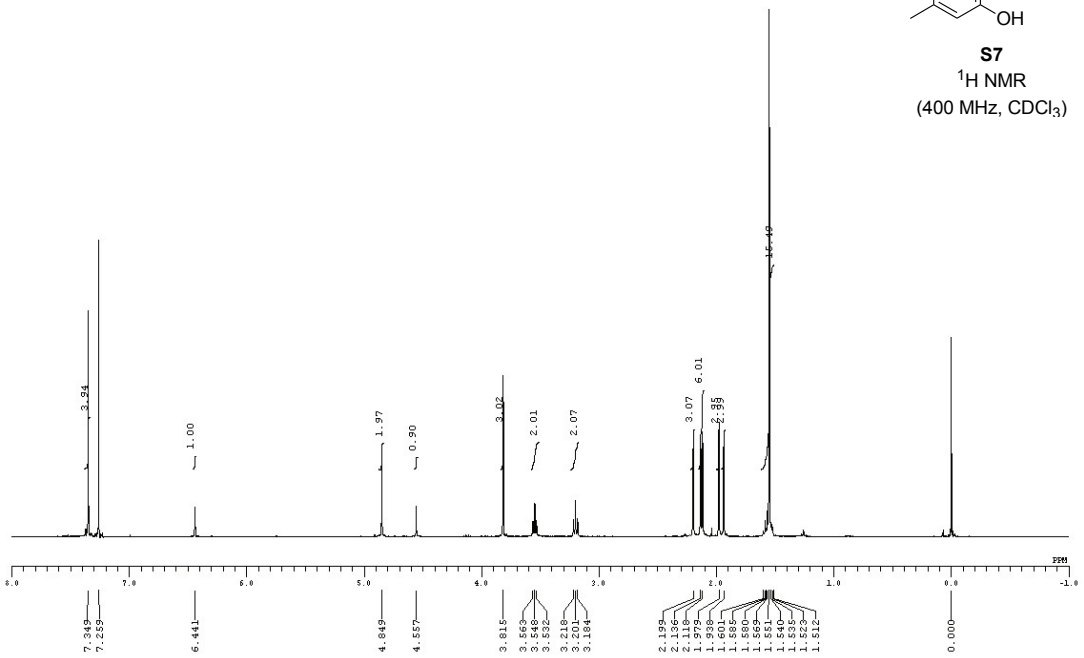
16
^{1}H NMR
(400 MHz, $CDCl_3$)

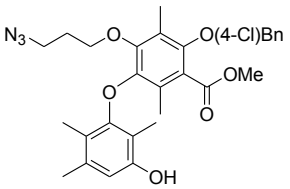


16
^{13}C NMR
(100 MHz, $CDCl_3$)

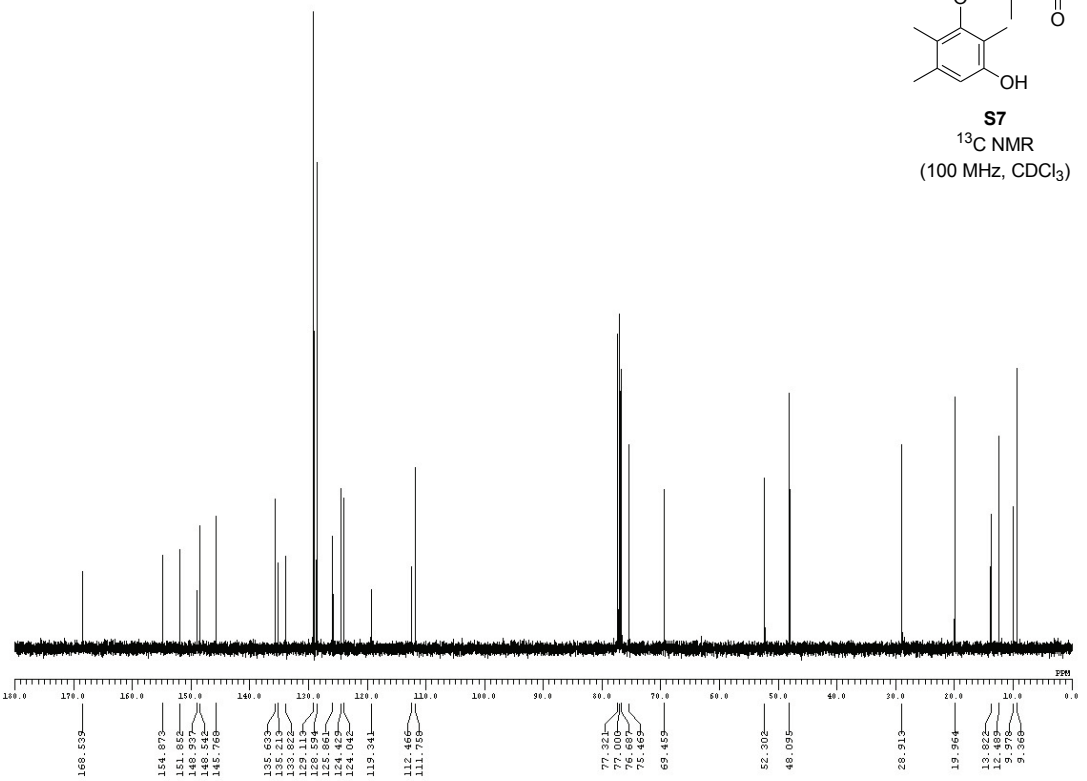


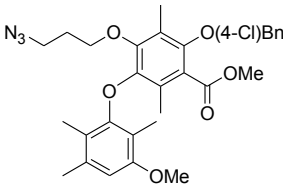
S7
^{1}H NMR
(400 MHz, $CDCl_3$)



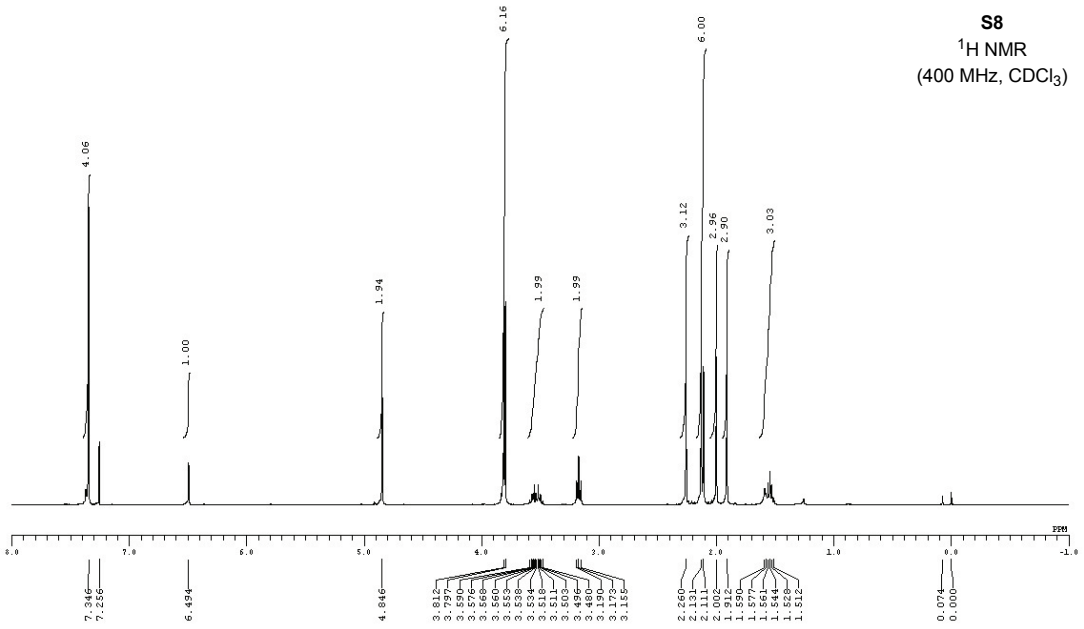


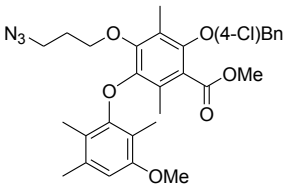
S7
^{13}C NMR
(100 MHz, $CDCl_3$)



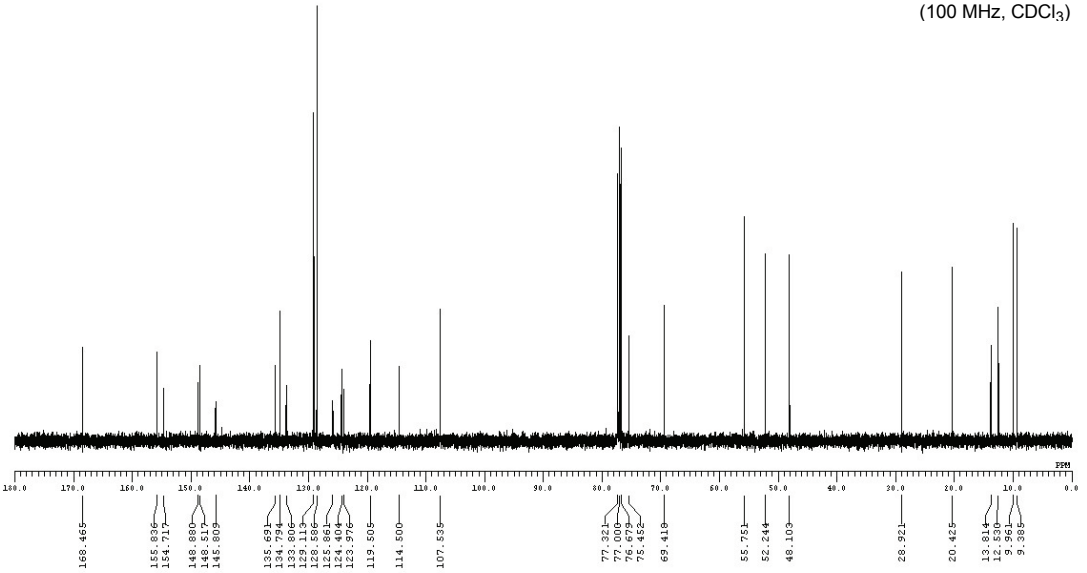


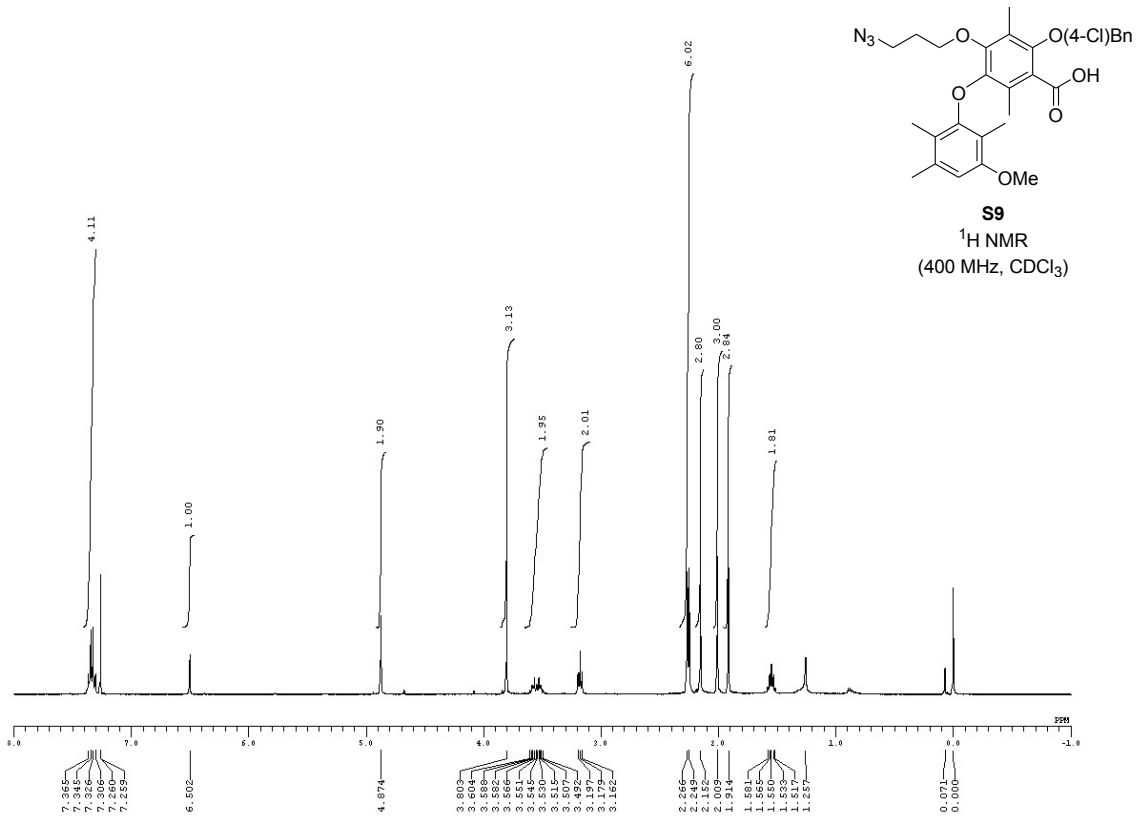
S8
^{1}H NMR
(400 MHz, $CDCl_3$)



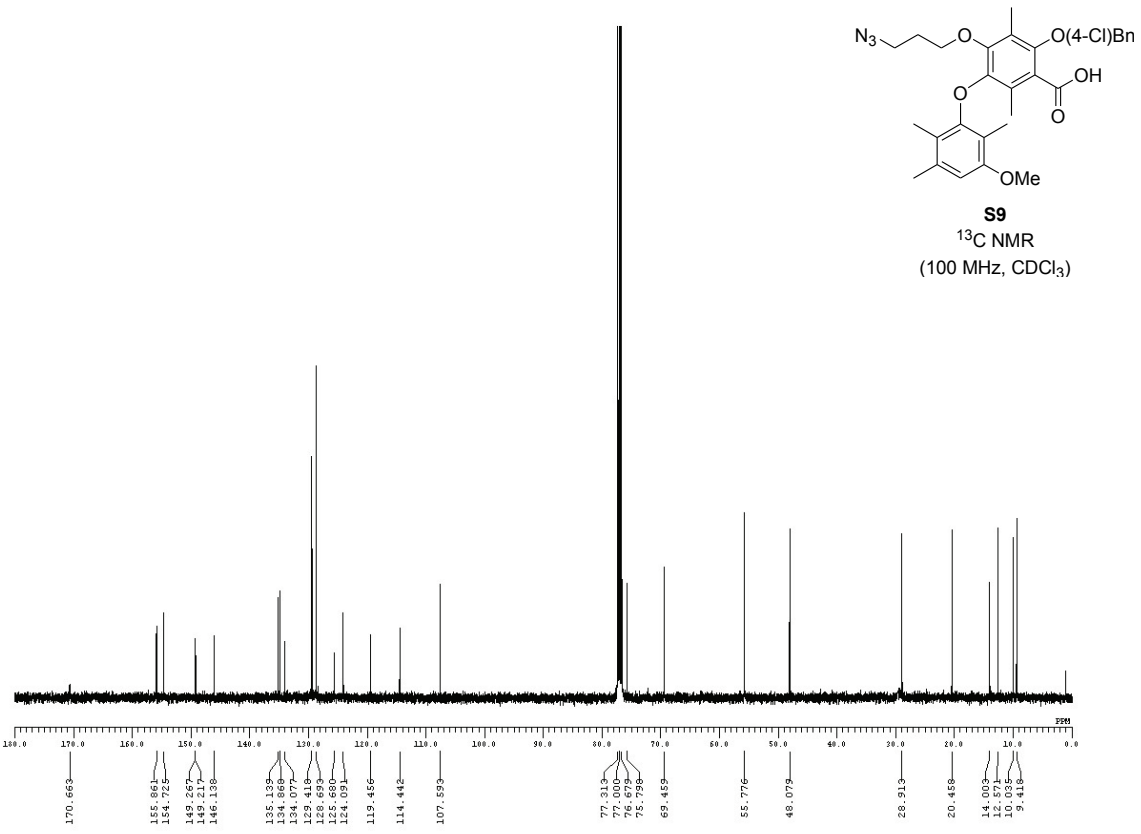


S8
^{13}C NMR
(100 MHz, $CDCl_3$)

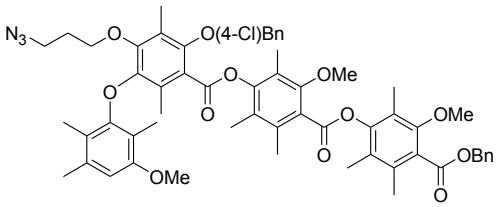




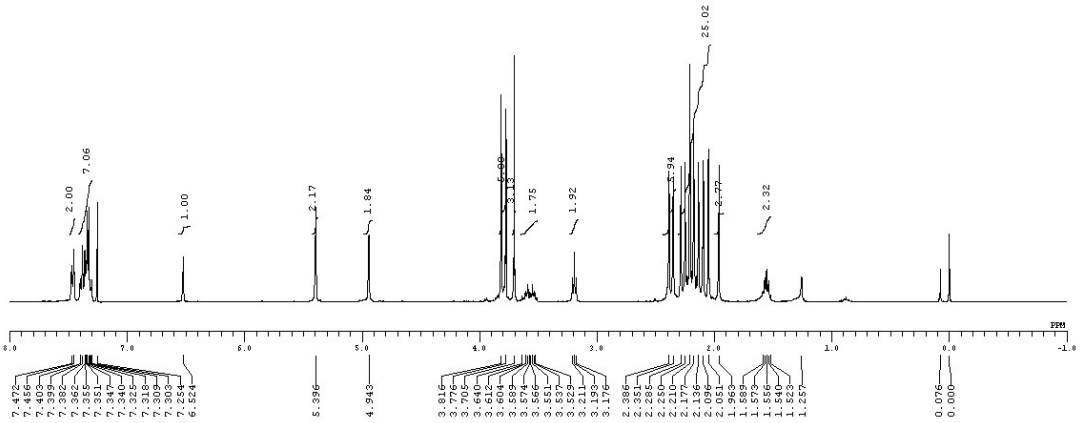
S9
^{1}H NMR
(400 MHz, $CDCl_3$)

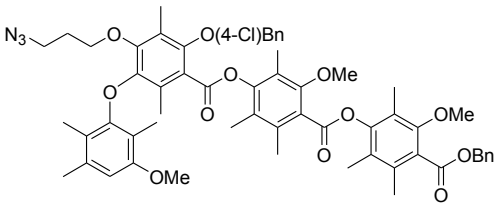


S9
^{13}C NMR
(100 MHz, $CDCl_3$)

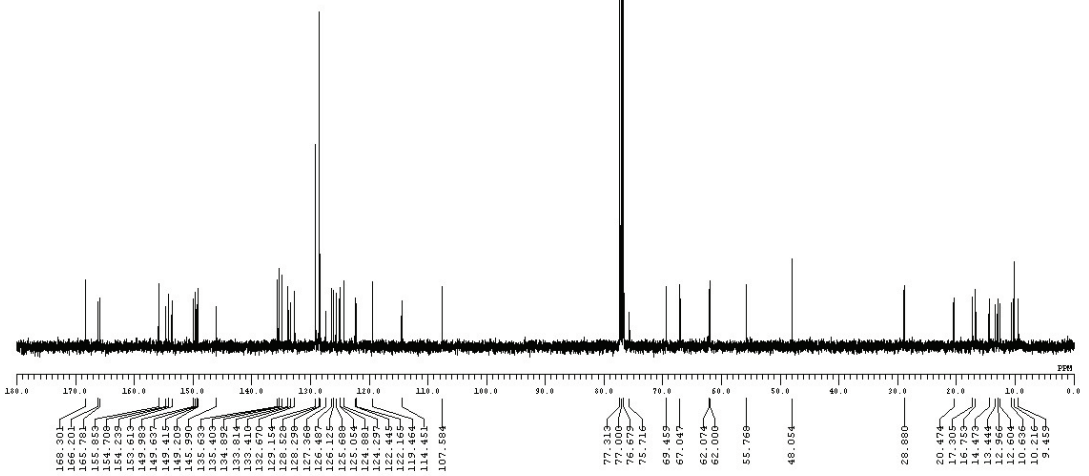


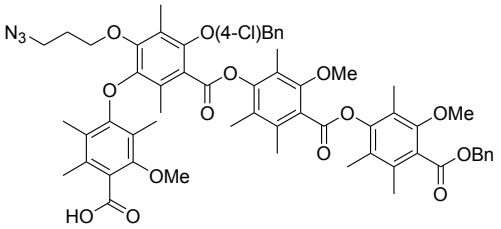
S10
^{1}H NMR
(400 MHz, $CDCl_3$)



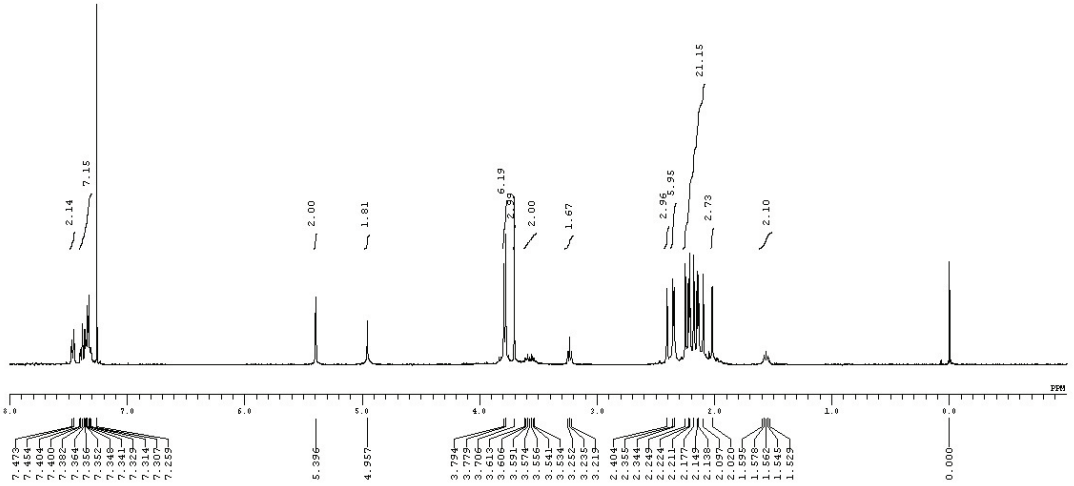


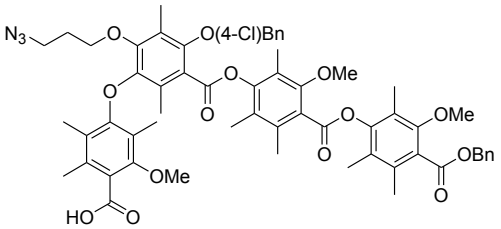
S10
^{13}C NMR
(100 MHz, $CDCl_3$)



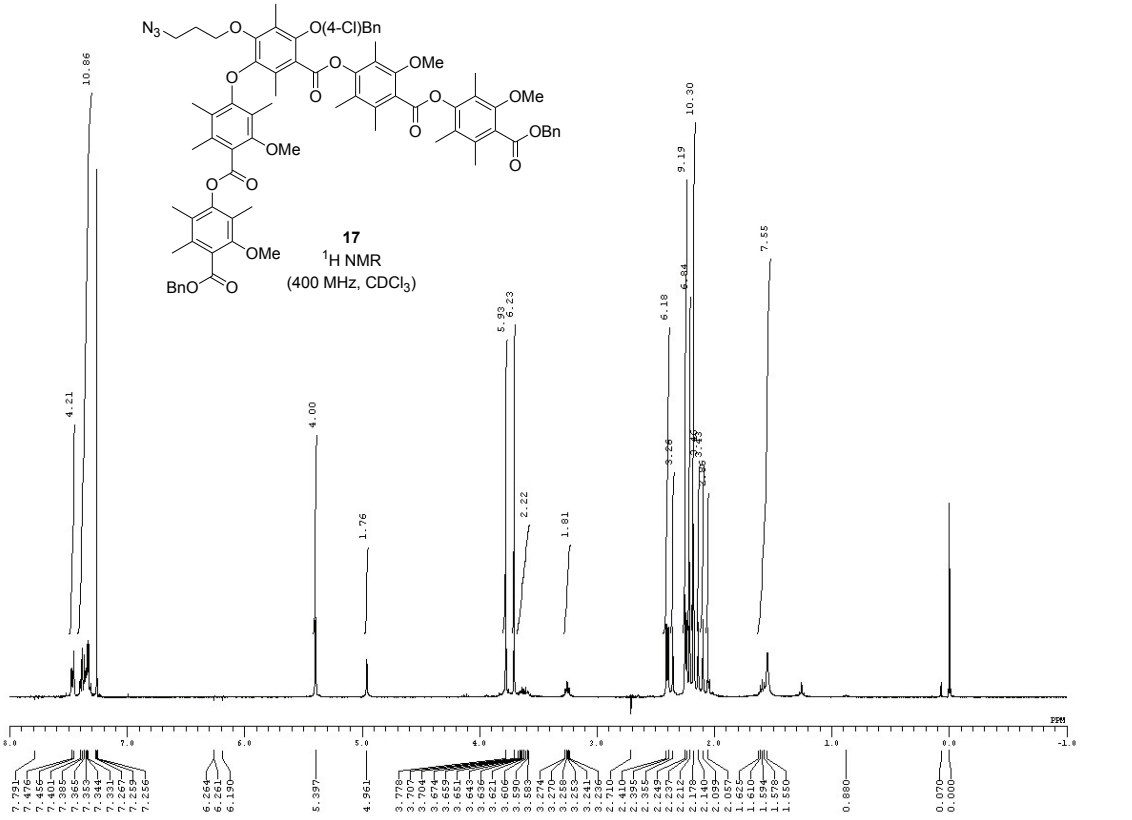


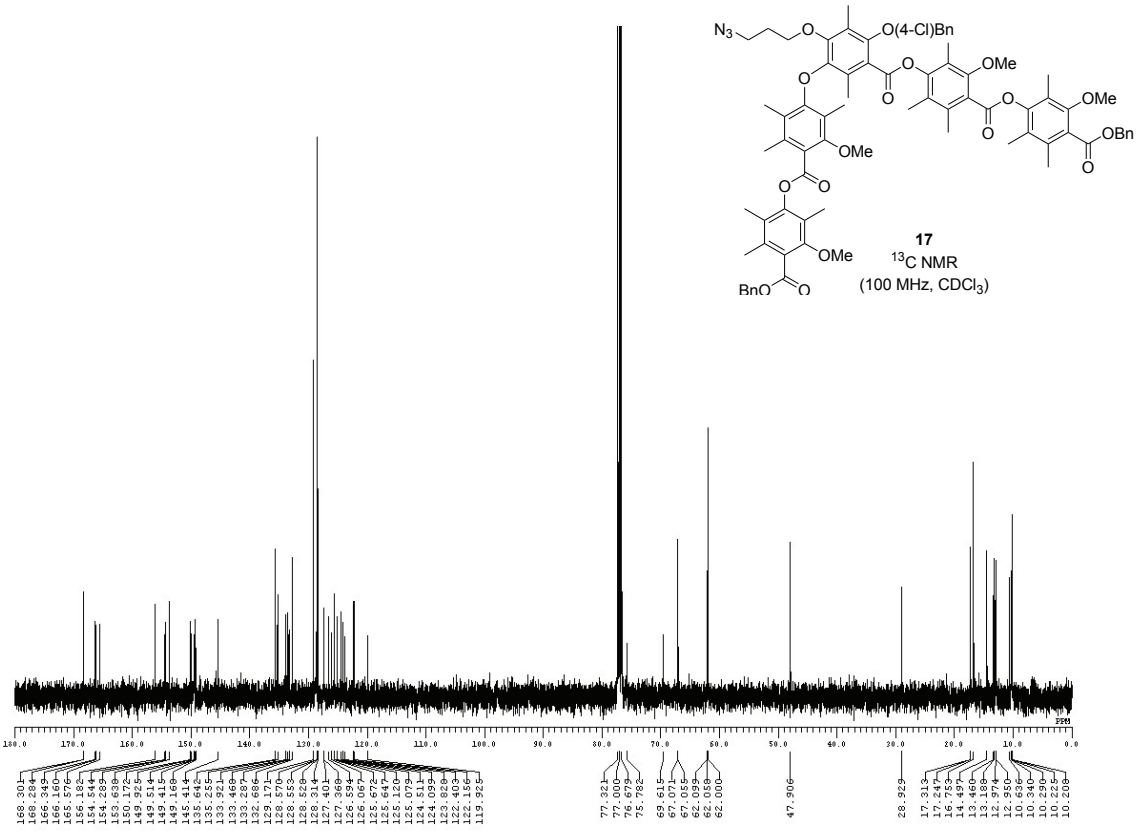
S11
^{1}H NMR
(400 MHz, $CDCl_3$)

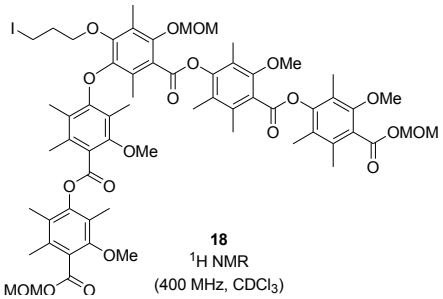




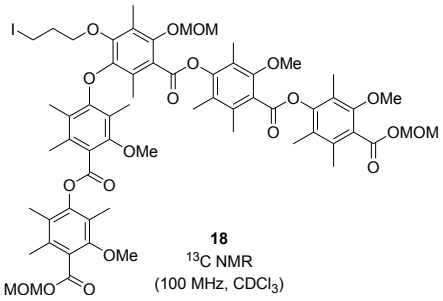
S11
^{13}C NMR
(100 MHz, $CDCl_3$)


N3
O(4-Cl)Bn
OMe
OBn
BnO
17
1H NMR
(400 MHz, CDCl3)

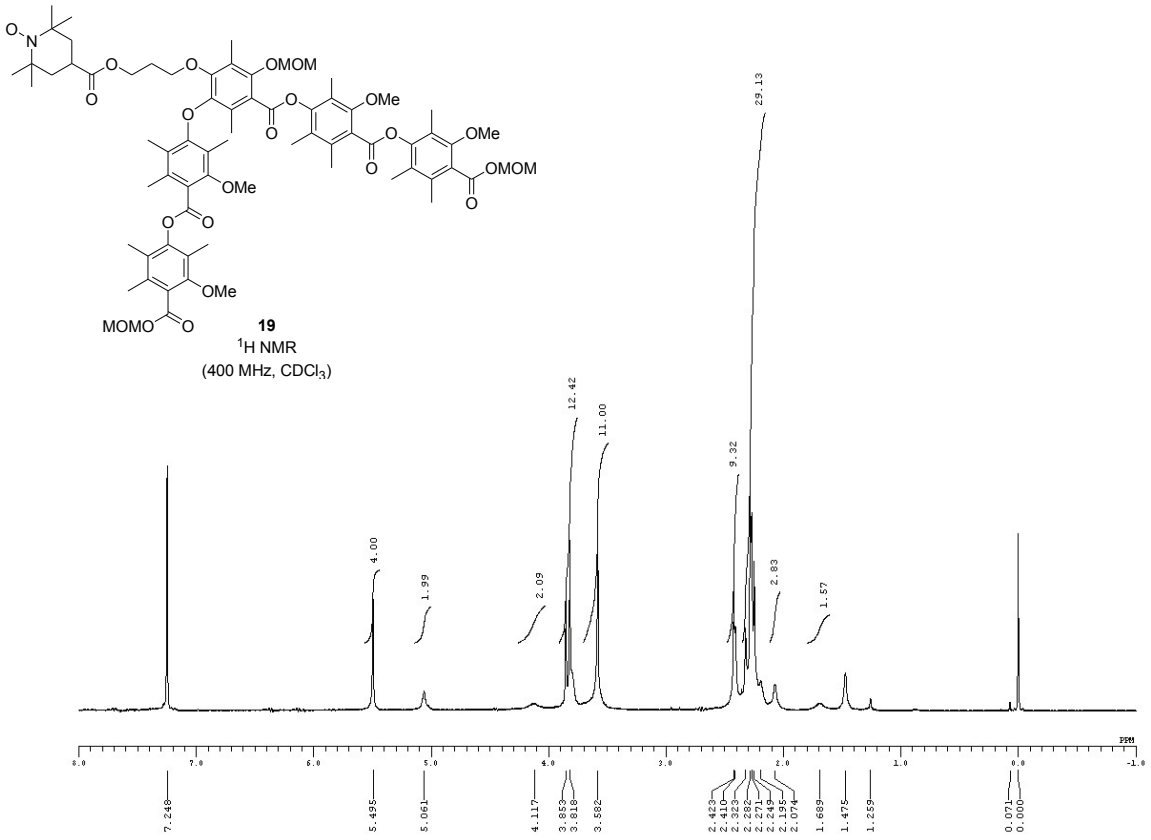

N3
O(4-Cl)Bn
OMe
OBn
BnO
17
13C NMR
(100 MHz, CDCl3)



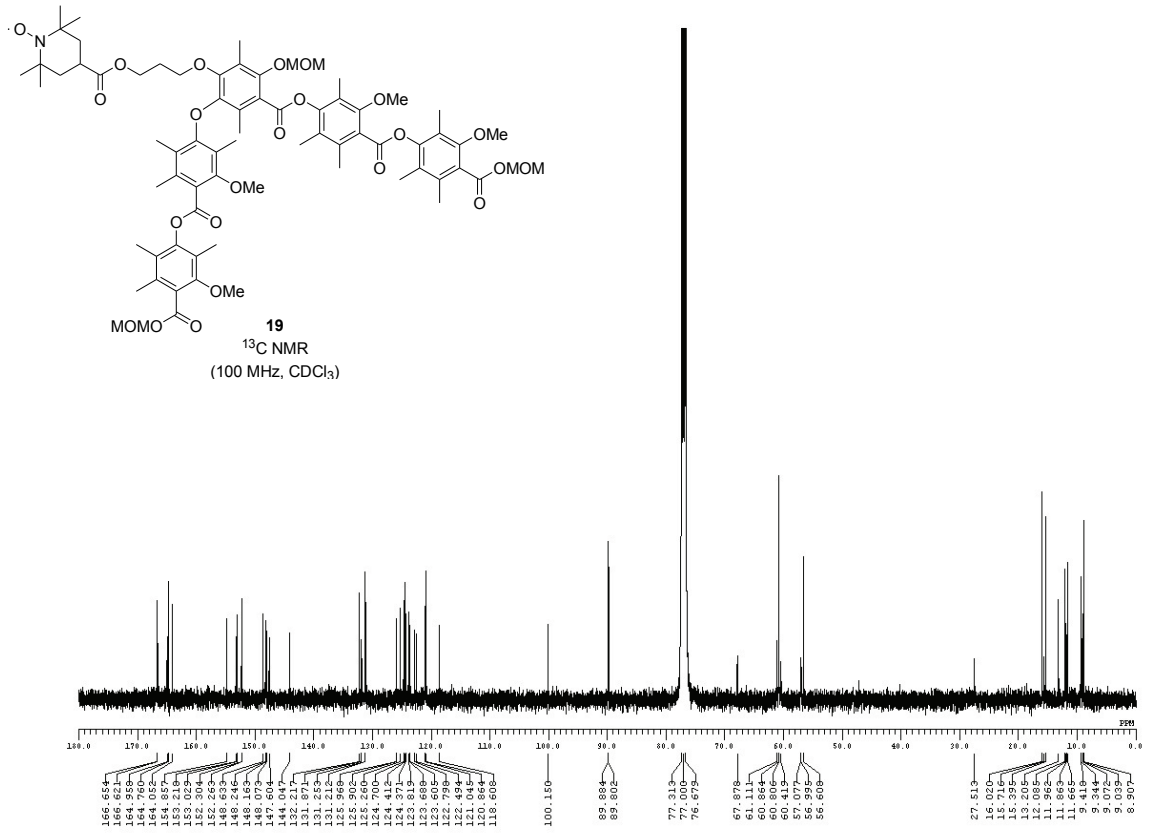
18
^{1}H NMR
(400 MHz, $CDCl_3$)



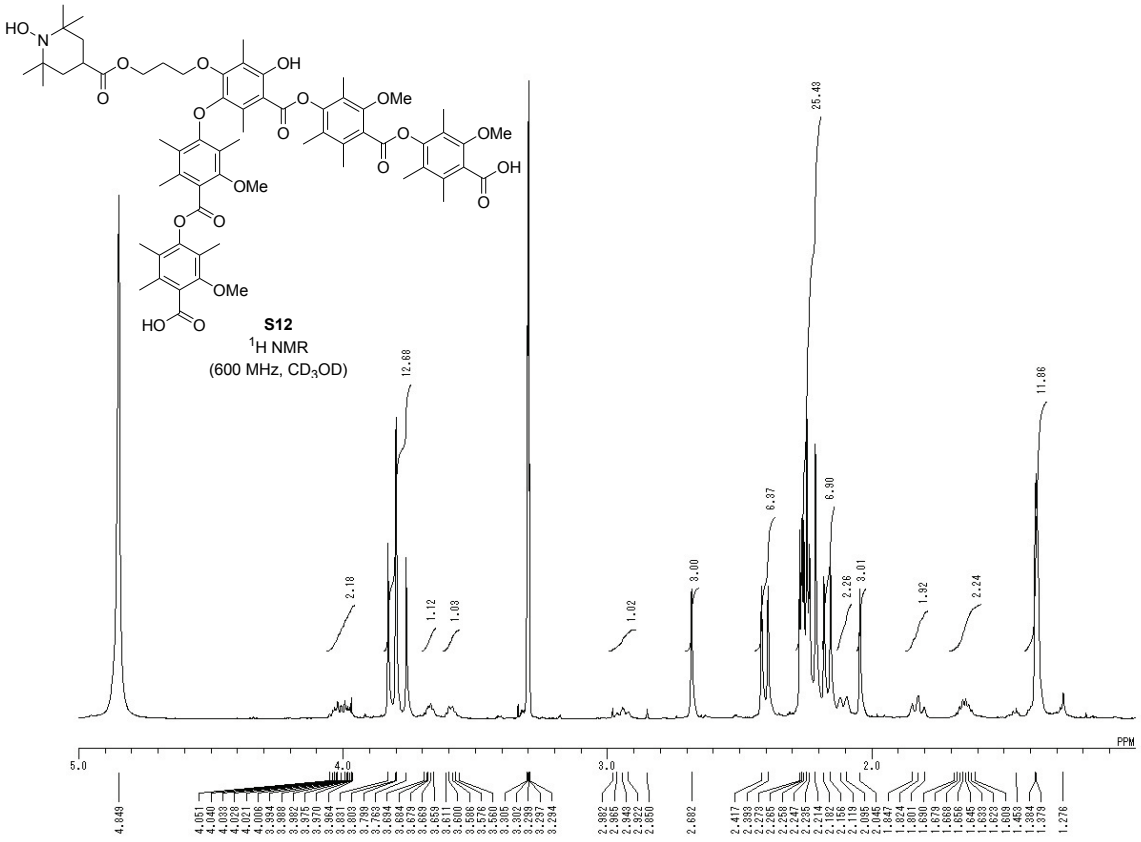
18
^{13}C NMR
(100 MHz, $CDCl_3$)



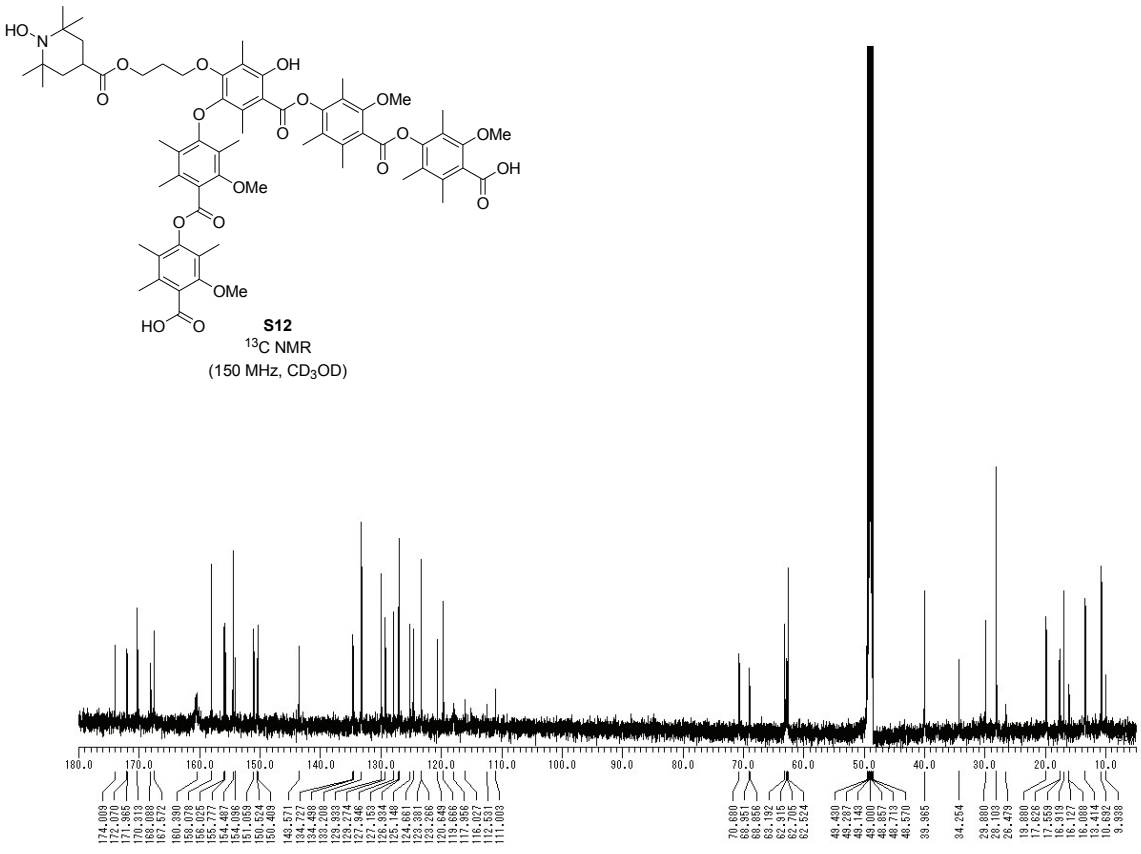
19
^{1}H NMR
(400 MHz, $CDCl_3$)



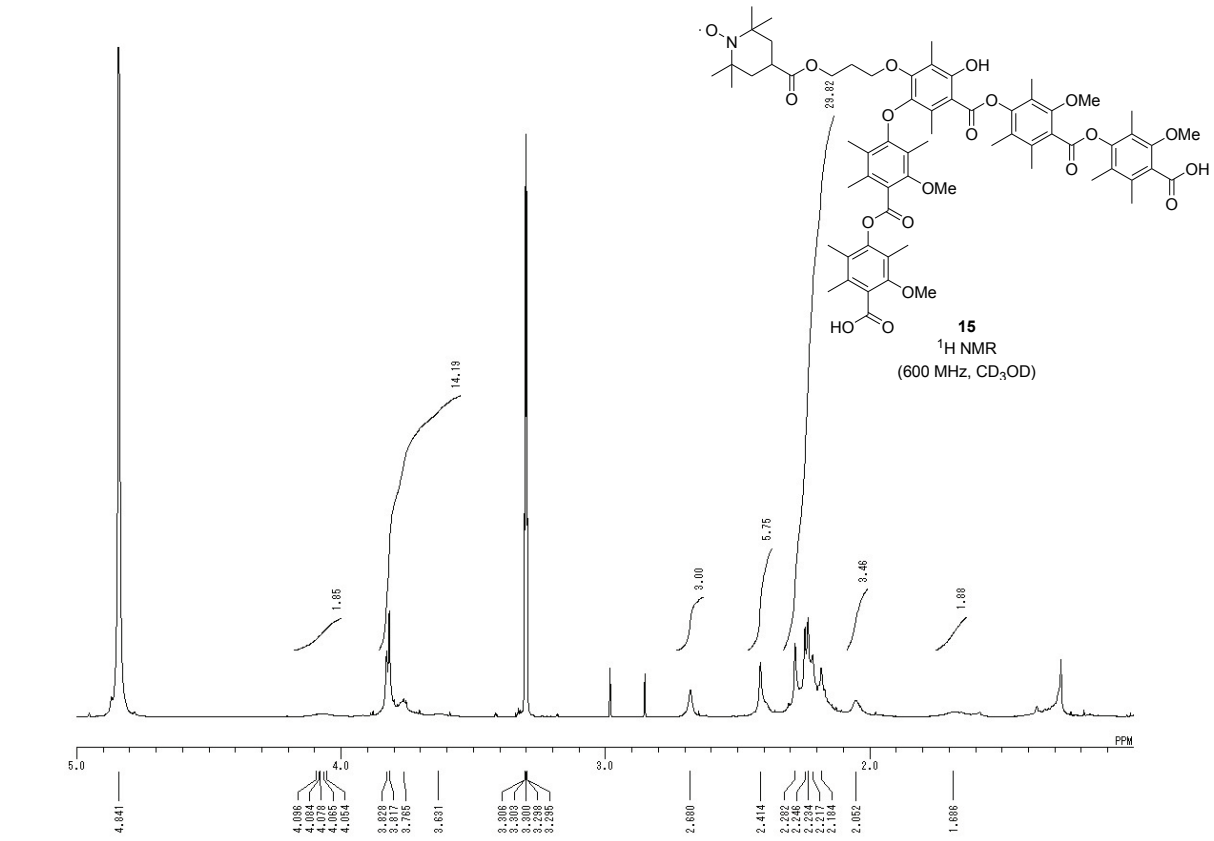
19
^{13}C NMR
(100 MHz, $CDCl_3$)

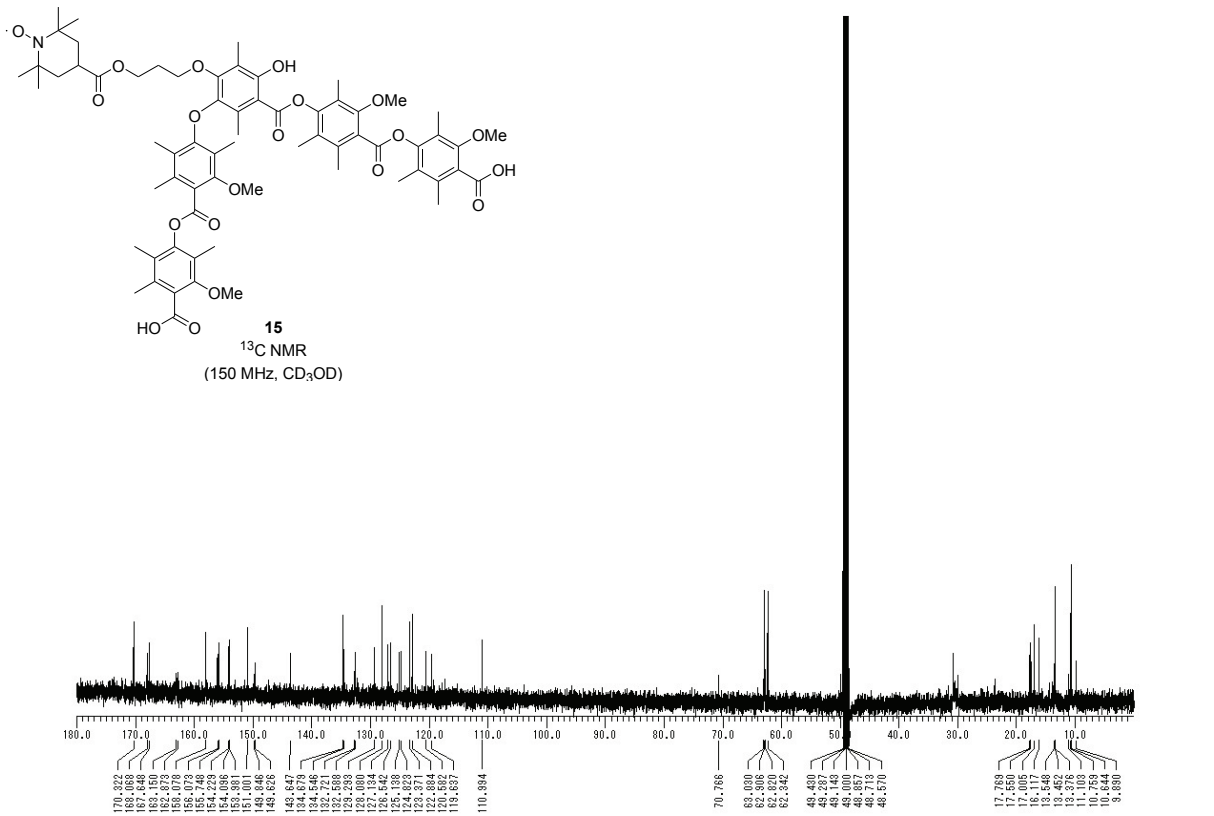


S12
^{1}H NMR
(600 MHz, CD_3OD)



S12
^{13}C NMR
(150 MHz, CD_3OD)


15
1H NMR
(600 MHz, CD3OD)
PPM
4.841
4.096
4.084
4.078
4.065
4.054
3.828
3.817
3.765
3.631
3.308
3.303
3.300
3.298
3.295
2.680
2.414
2.282
2.246
2.214
2.197
2.184
2.052
1.886


15
13C NMR
(150 MHz, CD3OD)
170.322
168.068
167.648
163.150
162.873
159.078
156.073
155.748
154.223
154.096
153.881
151.001
149.846
149.826
149.647
134.674
134.546
132.721
132.588
129.288
129.080
127.334
126.542
125.138
124.823
123.971
122.884
120.593
119.637
110.884
70.766
63.030
62.306
62.820
62.342
49.430
49.287
49.143
49.000
48.857
48.713
48.570
17.769
17.550
17.005
16.117
13.548
13.452
13.376
11.103
10.759
10.644
9.880