CHEMBIOCHEM

Supporting Information

CHEMBIOCHEM

Supporting Information

for

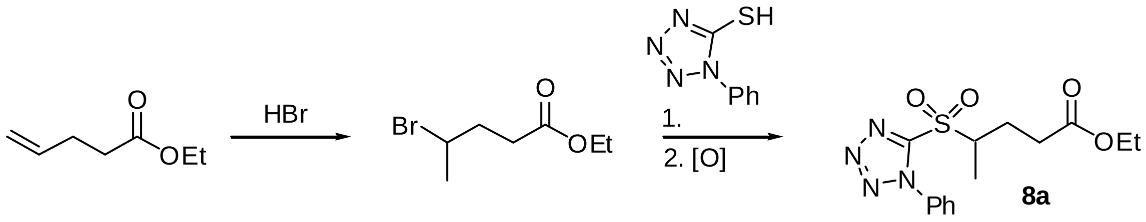
Chemoenzymatic Total Synthesis of the Antiproliferative Polyketide (+)-R-Aureothin

Martina Werneburg and Christian Hertweck*

Experimental Section

General information: Unless otherwise noted, all reagents were purchased from commercial suppliers and used without further purification. Melting points were measured on a Büchi melting point B-540 apparatus. Optical rotation was determined with a JASCO P-1020 polarimeter supplemented with a thermostat. NMR spectra (in $CDCl_3$) were measured on Bruker Avance DRX 300 and DPX 500 instruments. Chemical shifts are reported in ppm. The NMR numbering system refers to the general structure shown in Scheme 1. HR-MS (ESI) was recorded on a triple quadrupole mass spectrometer TSQ Quantum Ultra AM (Thermo Electron). LC-MS experiments were performed on a LCQ mass spectrometer equipped with an ESI source and an ion trap (Thermo Electron). Further analytical and preparative HPLC experiments were carried out on a Gilson machine. IR spectra were measured using a JASCO FTIR-4100 spectrometer supplemented with the ATR system. Substances were applied as solids or thin films (highly concentrated solutions in volatile solvents). Unless otherwise noted, all reaction mixtures were magnetically stirred in oven-dried glassware under an nitrogen or argon atmosphere. External bath temperatures were used to record all reaction mixture temperatures. Analytical thin layer chromatography was carried out on Merck silica gel 60 F_{254} TLC plates. TLC visualization was accomplished using 254/366 nm UV light or charring solutions of molybdatophosphoric acid. Preparative column chromatography was performed on (0.04-0.063 mm, 230-400 mesh, normal phase) silica gel. All solvents were destilled immediately prior to use. Tetrahydrofuran (THF), benzene, dimethyl ethoxide (DME) and toluene were destilled from Na/benzophenone. Acetone was dried over diphoshorpentoxide (P_2O_5) for 1 h and distilled. Dichloromethane (CH_2Cl_2) was distilled from CaH_2. *n*-Butyl lithium (*n*-BuLi) was titrated using diphenylacetic acid in THF. 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was distilled from KOH. CuI was purchased on pure metal basis and treated with heat under reduced pressure immediately prior to use. Extracts were dried over anhydrous Na_2SO_4. Representative procedures are demonstrated below.

Preparation of PT-sulfone (8a)



Ethyl 4-pentenoate (0.078 mol, 10 g, 11.1 mL) was slowly dropped into a solution of hydrogen bromide (45%, 0.434 mol, 78.12 g, 55 mL) in glacial acetic acid at 0 °C within 0.5 h. Then the reaction mixture was stirred for further 4 h. Afterwards the reaction mixture was added to ice water (120 g) and stirred for 15 min. The aqueous layer was extracted with Et_2O (3 x 75 mL). The combined organic layers were washed with H_2O (40 mL), saturated aqueous $NaHCO_3$ (2 x 40 mL) and saturated aqueous NaCl (2 x 40 mL). After drying over Na_2SO_4 the organic solution was concentrated under reduced pressure. A colourless liquid was obtained by destillation. Yield: 64%.

Ethyl 4-bromopentanoate.[1] b.p. (15 mbar) 88-90 °C; ^1H NMR (300 MHz, $CDCl_3$): *d* = 1.22 (t, *J* = 7.14 Hz; 3H, CH_2-C$\mathbf{H_3}$), 1.69 (d, *J* = 6.68 Hz, 3H, C$\mathbf{H_3}$-CHBr), 2.07 (m, 2H, CHBr-C$\mathbf{H_2}$), 2.49 (m, 2H, C$\mathbf{H_2}$-COOEt), 4.13 ppm (m, 3H, C**H**-Br/C$\mathbf{H_2}$-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): *d* = 14.16 (1C, CH_2-**C**H_3), 26.37 (1C, **C**H_3-CHBr), 32.5 (1C, **C**H_2-COOEt), 35.87 (1C, CHBr-**C**H_2), 50.27 (1C, **C**H-Br), 60.48 (1C, **C**H_2-CH_3), 172.7 ppm (1C, **C**OOEt); IR: ν 2981/2925/2871 (m-w, CH_3, CH_2), 1777 (m-w, C=O) 1731 (vs, C=O), 1444/1420/1376/1338 (m-w, CH_3, CH_2), 1270 (m, C-O) 1186/1165 (s, C-O), 1120/1094/1025 (m), 941/855/826/785 (w) cm^{-1}.

[1] E.J. Lease, S. M. McElvain, *J. Am. Chem. Soc.* **1933**, 55, 806-808. M.S. Kharsch, P.S. Skell, P. Fisher, *J. Am. Chem. Soc.* **1948**, 70, 1055-1059. No NMR data were given.

1-Phenyl-1*H*-tetrazole-5-yl thiol (0.017 mol, 3.03 g) was dissolved in dry acetone (35 mL), and K_2CO_3 (0.026 mol, 3.59 g) was added After refluxing for 1 h the reaction mixture was cooled to room temperature. Ethyl 4-bromopentanoate (0.0175 mol, 3.66 g) was added and then the mixture was refluxed for 18 h. Then the yellow suspension was cooled to room temperature and water (30 mL) as well as Et_2O (30 mL) were added. The aqueous layer was further extracted with Et_2O (3 x 20 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (2 x 35 mL) and saturated aqueous NaCl (35 mL), dried over Na_2SO_4 and concentrated under reduced pressure. A light-yellow oily product was obtained that was used without any further purification. Yield: 97%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.2 (t, *J* = 7.13 Hz, 3H, CH_2-$C\mathbf{H_3}$), 1.5 (d, *J* = 6.86 Hz, 3H, $C\mathbf{H_3}$-CH), 2.04 (m, 2H, CH-$C\mathbf{H_2}$), 2.44 (t, *J* = 7.32/ 7.97 Hz, 2H, $C\mathbf{H_2}$-COOEt), 4.06 (m, 3H, C**H**-S/$C\mathbf{H_2}$-CH_3), 7.5 ppm (br s, 5H, aromatic hydrogens); ^{13}C NMR (75 MHz, $CDCl_3$): d = 14.11 (1C, $\mathbf{C}H_3$-CH), 21.34 (1C, CH_2-$\mathbf{C}H_3$); 31.54/31.64 (2C, CH-$\mathbf{C}H_2$/$\mathbf{C}H_2$-COOEt), 44.15 (1C, **C**H-S), 60.57 (1C, $\mathbf{C}H_2$-CH_3), 123.98 (2C, aromatic carbons near C-N), 129.68 (2C, aromatic carbons), 130.07 (1C, aromatic carbon), 133.67 (1C, aromatic C-N), 153.6 (1C, N=**C**-S), 172.7 ppm (1C, **C**OOEt); IR: n = 3064 (vw, =C-H), 2979/2930/2871 (m-w, CH_3, CH_2), 1771 (vw, C=O), 1729 (vs, C=O), 1596 (m, C=N), 1498 (s, C=C), 1458/1448/1411/1386 (m-s, CH_3, CH_2), 1274/1238 (m, C-N), 1173 (br s, C-O), 1090/1013 (m), 915/854 (vw, =C-H), 761 (s, =C-H), 693 cm^{-1} (s, C-S); HR-MS (ESI) calcd. for $C_{14}H_{19}O_2N_4S$: 307.1223, observed: 307.1197.

Ammonium molybdate tetrahydrate (1.65 mmol, 2.04 g) in H_2O_2 (30%, 0.175 mol, 19.54 g, 17.5 mL) was slowly dropped to a solution of thioether (0.016 mol, 4.92 g) in EtOH (87 mL). The emerging dark yellow solution was stirred at room temperature for 18 h. Then H_2O (150 mL) and Et_2O (50 mL) were added to the reaction mixture and the aqueous layer was extracted with Et_2O (4 x 50 mL). The combined organic layers were washed with H_2O (55 mL) and saturated aqueous $NaHCO_3$ (55 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel, Et_2O:hexane = 2:1) to yield a colourless solid (91%).

8a. m.p. 59.4 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22 (t, *J* = 7.16 Hz, 3H, CH_2-C**H_3**), 1.47 (d, *J* = 6.97 Hz, 3H, C**H_3**-CH), 1.97 (m, 1H, CH-C**H_2**), 2.45 (m, 3H, C**H_2**-COOEt/ CH-C**H_2**), 3.91 (m, 1H, C**H**-SO_2), 4.1 (q, *J* = 7.1 Hz, 1H, C**H_2**-CH_3), 7.58 ppm (m, 5H, aromatic hydrogens); ^{13}C NMR (75 MHz, $CDCl_3$): *d* = 12.81 (1C, **C**H_3-CH), 14.07 (1C, CH_2-**C**H_3), 24.26 (1C, CH-**C**H_2), 30.59 (1C, **C**H_2-COOEt), 60.23 (1C, **C**H-SO_2), 60.81 (1C, **C**H_2-CH_3), 125.4 (2C, aromatic carbons near C-N), 129.5 (2C, aromatic carbons), 131.39 (1C, aromatic carbon), 133.02 (1C, aromatic C-N), 152.59 (1C, N=**C**-SO_2), 171.74 ppm (1C, **C**OOEt); IR: ν 3066 (w, =C-H), 2992/2975/2926/2870 (m-vw, CH_3, CH_2), 1729 (vs, C=O), 1593 (m-w, C=N), 1497 (s, C=C), 1460/1440/1414/1375 (m, CH_3, CH_2), 1296/1238 (m, C-N), 1336 (v, S=O s), 1110 (s, S=O), 1195 (s, C-O), 1152 (vs, C-O), 1077/1044/1016 (m), 934 (m, =C-H), 761/743 (s-m, =C-H), 823 (m, C-S), 694 (s-m, C-S) cm^{-1}; HR-MS (ESI) calcd. for $C_{14}H_{19}O_4N_4S$: 339.1108, obs: 339.1122.

9 (*E,E* isomer): PT-sulfone **8a** (0.052 mol, 17.69 g) was dissolved in dry DME (200 mL) and (*E*)-3-iodo-2-methylpropenal **7** (0.04 mol, 7.86 g) was added to the solution. The reaction mixture was cooled to –60 °C and a solution of KHMDS (0.059 mol, 118 mL, 0.5 M in toluene) was slowly added within 1 h. After stirring for another 2 h the reaction mixture was quenched with H_2O (24 mL) and diluted with Et_2O (90 mL) and H_2O (45 mL). The aqueous layer was extracted with Et_2O (3 x 40 mL). The combined organic layers were washed with H_2O (100 mL) and saturated aqueous NaCl (100 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was loaded onto silica and purified by open column chromatography (silica gel, Et_2O/hexane=1:99) to yield a yellow liquid (48%).

^{1}H NMR (300 MHz, $CDCl_3$): *d* = 1.22 (t, *J* = 7.15 Hz, 3H, CH_2-C**H_3**), 1.7 (d, *J* = 1.29 Hz, 3H, CH=C(C**H_3**)), 1.86 (d, *J* = 0.39 Hz, 3H, ICH=C(C**H_3**)), 2.32 (m, 2H, C(CH_3)-C**H_2**), 2.41 (m, 2H, C**H_2**-COOEt), 4.09 (q, *J* = 7.12 Hz, 2H, C**H_2**-CH_3), 5.61 (br s, 1H, C**H**=C(CH_3)), 5.93 ppm (br s, 1H, I-C**H**); ^{13}C NMR (75 MHz, $CDCl_3$): *d* = 14.23 (1C, CH_2-**C**H_3), 17.79 (1C, CH=C(**C**H_3)), 25.07 (1C, ICH=C(**C**H_3)), 32.91 (1C, **C**H_2-COOEt), 34.91 (1C, C(CH_3)-**C**H_2), 60.34 (1C, **C**H_2-CH_3), 79.21 (1C, I-**C**H), 126.32 (1C, **C**H=C(CH_3)), 137.03 (1C, **C**(CH_3)-CH_2), 144.68 (1C, ICH=**C**(CH_3)), 172.92 ppm (1C, **C**OOEt); IR: ν 3067 (vw, =C-H), 2979/2931/2912/2885 (m, CH_3, CH_2), 1732 (vs, C=O), 1645/1595 (vw, C=C), 1443 (br m), 1371 (m, CH_3, CH_2), 1342 (w), 1290 (m-w, C-O), 1256 (m, C-O), 1159 (br s, C-O), 1095/1053/1031 (m-w), 857 (m-w, =C-H), 766/672 (w, =C-H) cm^{-1}.

10: Ethyl ester **9** (0.017 mol, 5.4 g) was dissolved in dry toluene (75 mL). After cooling to –78 °C a solution of DIBAH (0.023 mol, 23 mL, 1 M in *n*-hexane) was slowly added over 1 h. The reaction mixture was stirred for further 3 h at –78 °C. Then an acidic methanolic solution (MeOH/1 N HCl = 2:1) was slowly added until no further bubbles appear. The solution was then diluted with H_2O (10 mL) and Et_2O (10 mL), and the aqueous layer was extracted with Et_2O (3 x 20 mL). The combined organic layers were washed with saturated aqueous NaCl (40 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by open column chromatography (silica gel, Et_2O/hexane = 1:2) to yield a yellow liquid (88%). ^1H NMR (500 MHz, $CDCl_3$): *d* = 1.71 (s, 3H, CH=C(C**H$_3$**)), 1.86 (s, 3H, ICH=C(C**H$_3$**)), 2.35 (br t, *J* =7.51 Hz, 2H, C(CH_3)-C**H$_2$**), 2.54 (br t, *J* = 7.48 Hz, 2H, C**H$_2$**-CHO), 5.62 (br s, 1H, C**H**=C(CH_3)), 5.94 (br s, 1H, I-C**H**), 9.75 ppm (s, 1H, C**H**O); ^{13}C NMR (125 MHz, $CDCl_3$): *d* = 17.96 (1C, CH=C(**C**H$_3$)), 25.07 (1C, ICH=C(**C**H$_3$)), 31.98 (1C, C(CH_3)-**C**H$_2$), 41.96 (1C, **C**H$_2$-CHO), 79.43 (1C, I-**C**H), 126.52 (1C, **C**H=C(CH_3)), 136.68 (1C, **C**(CH_3)-CH_2), 144.54 (1C, ICH=**C**(CH_3)), 201.58 ppm (1C, **C**HO); IR: *n* = 3065 (vw, =C-H), 2978/2934/2910/2850/2819 (m-w, CH_3, CH_2), 2718 (m, C-H(O)), 1721 (vs, C=O), 1645/1594 (w, C=C), 1439/1409/1374 (m, CH_3, CH_2), 1295/1159 (m), 1068/1004 (w), 866 (br w, =C-H), 766/677 (w, =C-H) cm^{-1}.

12: 2-methyl-3-oxopentanoic acid methyl ester **11** (11.4 mmol, 1.64 g) was added to a suspension of NaH (12.1 mmol, 291 mg) in dry THF (70 mL) at 0 °C. After stirring for 10 min the solution cleared off bubbles. Then a solution of *n*-BuLi (12.3 mmol, 8.7 mL, 1.6 M in *n*-hexane) was added dropwise. After stirring for 10 min a solution of aldehyde **10** (7.6 mmol, 2 g) in dry THF (7 mL) was added and the reaction mixture was stirred overnight at 0 °C. The reaction mixture was quenched with saturated aqueous NH_4Cl (100 mL). The aqueous layer was extracted with Et_2O (3 x 40 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by open column chromatography (silica gel, Et_2O:hexane = 1:2) to yield a mixture of diastereomers as yellow oil (55%).

A mixture of diasteromers and enantiomers as well as keto-enole tautomerism were observed. An attribution of protons was done, but no ratio of the different isomers was determined. ^{1}H NMR (500 MHz, CDCl$_3$): δ 1.13 (d, *J* = 7.19 Hz, CH(OH)-CH(C$\mathbf{H_3}$)-CO), 1.134 (d, *J* = 7.06 Hz, CH(OH)-CH(C$\mathbf{H_3}$)-CO), 1.31 (d, *J* = 7.09 Hz, CH(C$\mathbf{H_3}$)-COOMe), 1.33 (d, *J* = 7.03 Hz, CH(C$\mathbf{H_3}$)-COOMe), 1.44 (m, C$\mathbf{H_2}$-CH(OH)), 1.59 (m, C$\mathbf{H_2}$-CH(OH)), 1.7 (s, 3H, CH=C(C$\mathbf{H_3}$)), 1.87 (s, ICH=C(C$\mathbf{H_3}$)), 2.04 (m, C(CH$_3$)-C$\mathbf{H_2}$), 2.2 (m, C(CH$_3$)-C$\mathbf{H_2}$), 2.38 (br s, CH-O**H),** 2.6 (br s, CH-O**H**), 2.79 (m, CH(OH)-C**H**(CH$_3$)-CO), 3.7/3.705 (2 s, OMe), 3.71 (m, CO-C**H**(CH$_3$)-CO), 3.83 (dt, *J* = 3.7/8.71 Hz, C**H**-OH), 3.91 (dt, *J* = 3.59/8.67 Hz, C**H**-OH), 5.63 (br s, C**H**=C(CH$_3$)), 5.93 ppm (br s, I-C**H**); ^{13}C NMR (125 MHz, CDCl$_3$): δ 10.11/10.29 (CH(OH)-CH(**C**H$_3$)-CO), 12.76/12.95 (CH(**C**H$_3$)-COOMe), 17.94 (CH=C(**C**H$_3$)), 25.16 (ICH=C(**C**H$_3$), 32.06/32.08 (**C**H$_2$-CH(OH)), 36.4/36.47 (C(CH$_3$)-**C**H$_2$), 49.79/49.91 (CH(OH)-**C**H-(CH$_3$)-CO), 51.27/52.05 (CO-**C**H(CH$_3$)-CO), 52.43/52.5 (OMe), 70.04/70.49 (**C**H- OH), 79.03/79.13 (I-**C**H), 126.04/126.15 (**C**H=C(CH$_3$)), 138.24/138.36 (**C**(CH$_3$)-CH$_2$), 144.78/144.85 (ICH=**C**(CH$_3$), 170.69 (**C**OOMe), 210.25/210.63 ppm (**C**=O); IR: *n* = 3354 (br m, O-H), 3062 (vw, =C-H), 2975/2940/2913/2877 (m-w, CH$_3$, CH$_2$), 1742 (s, C=O), 1709 (br vs, C=O), 1690 (br s, C=O), 1454/1435/1376 (br m, CH$_3$, CH$_2$), 1297/ 1258/1201/1152 (m, C-O), 1111 (m), 1052 (s-m, C-OH), 1024 (vs, C-OH), 1003 (s, C-OH), 860 (vw, =C-H), 820 (w, =C-H), 757 (w-vw, =C-H), 671 (br w, =C-H) cm^{-1}; HR-MS (ESI) calcd. for $C_{16}H_{25}O_4INa$: 431.0695, obs: 431.0669.

13: Solid Dess–Martin periodinane (7.8 mmol, 3.3 g) was added to the solution of **12** (7.1 mmol, 2.88 g) in dry CH_2Cl_2 (65 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1.5 h and then quenched with a solution of saturated aqueous $Na_2S_2O_3$, saturated aqueous $NaHCO_3$ and H_2O (1:1:1, total 90 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 30 mL). The combined organic layers were washed with H_2O (50 mL) and saturated aqueous NaCl (50 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by open column chromatography (silica gel, Et_2O:hexane = 1:1) to yield a mixture of diastereomers as orange liquids (46%).

A mixture of diasteromers and enantiomers as well as keto-enole tautomerism were observed. An attribution of protons was done, but no ratio of the different isomers was determined. ^{1}H NMR (500 MHz, CDCl$_3$): *d* = 1.31 (m, CO-CH(C$\mathbf{H_3}$)-CO), 1.37 (d, *J* = 7.1 Hz, CH(C$\mathbf{H_3}$)-COOMe), 1.7 (m, CH=C(C$\mathbf{H_3}$)), 1.86/1.869 (2 x s, ICH=C(C$\mathbf{H_3}$)), 2.27 (m, C(CH$_3$)-C$\mathbf{H_2}$), 2.35 (m, C(CH$_3$)-C$\mathbf{H_2}$), 2.53 (m, C$\mathbf{H_2}$-CO), 2.62 (m, C$\mathbf{H_2}$-CO), 3.68/3.69/3.7 (3 x s, OMe), 3.69 (m, C**H**(CH$_3$)-COOMe), 3.86 (q, *J* = 7.13 Hz, CO-C**H**(CH$_3$)-CO), 3.93 (q, J = 7.07 Hz, CO-C**H**(CH$_3$)-CO), 5.59 (m, C**H**=C(CH$_3$)), 5.93

(m, I-C**H**), 9.75 (br s, C(O**H**)=C), 12.64 (br s, C(O**H**)=C), 16.39 ppm (br s, C(O**H**)=C); ^{13}C NMR (125 MHz, CDCl$_3$): d = 12.05/12.72/12.87/12.94/13.11/13.6 (CO-CH(**C**H$_3$)-CO, CH(**C**H$_3$)-COOMe, C(OH)=C(**C**H$_3$)), 17.97/17.99 (CH=C(**C**H$_3$)), 25.09 (ICH=C(**C**H$_3$)), 33.3/33.32 (C(CH$_3$)-**C**H$_2$), 34.72/35.0/39.28/39.31 (**C**H$_2$-CO), 45.1/51.43/51.67 (CO-**C**H(CH$_3$)-COOMe), 52.33/52.4/52.52/52.55 (OMe), 59.48/59.67 (CO-**C**H-(CH$_3$)-CO), 79.26/79.3/79.36 (I-**C**H), 104.1 (C(OH)=**C**(CH$_3$)), 126.27/126.41 (**C**H=C(CH$_3$)), 136.96/137.15/137.29 (**C**(CH$_3$)-CH$_2$), 144.59/144.67 (ICH=**C**(CH$_3$)), 170.47/170.55/171.36 (**C**OOMe), 188.82/193.54 (**C**(OH)=C), 202.22/203.03 (CH(CH$_3$)-**C**=O), 205.36/206.4 ppm (CH$_2$-**C**=O); IR: ν 3065 (vw, =C-H), 2981/2940/2914/2858 (m-w, CH$_3$, CH$_2$), 1745 (s, C=O), 1722 (vs, C=O), 1613/1594 (br m, C=C), 1450/1435/1375 (m, CH$_3$, CH$_2$), 1258/1201/1175/1151 (s-m, C-O), 1126/1085/1023/996 (m), 969/857 (m, =C-H), 755 (w, =C-H), 669 (m, =C-H) cm^{-1}; HR-MS (ESI) calcd. for $C_{16}H_{24}O_4I$: 407.0714, obs: 407.0703.

14: DBU (4.2 mmol, 642 mg, 640 µL) was added to a solution of **13** (3 mmol, 1.22 g) in dry benzene (30 mL). The reaction mixture was refluxed at 80 °C for 4 h, then cooled to room temperature and concentrated under reduced pressure. The crude product was purified by open column chromatography (silica gel, CHCl$_3$:MeOH/AcOH = 50:1:1) to yield a light-yellow solid (62%). m.p. 132.2 °C; ^{1}H NMR (500 MHz, CDCl$_3$): d = 1.72 (d, J = 1 Hz, 3H, 9a-CH$_3$), 1.84 (br s, 3H, 11a-CH$_3$), 1.94 (s, 3H, 5a-CH$_3$), 1.97 (s, 3H, 3a-CH$_3$), 2.31 (br t, J = 7.75 Hz, 2H, 8-CH$_2$), 2.62 (br t, J = 7.75 Hz, 2H, 7-CH$_2$), 5.54 (br s, 1H, 10-CH), 5.92 ppm (s, 1H, 12-CH); ^{13}C NMR (75 MHz, CDCl$_3$): d = 8.5 (1C, 3a-CH$_3$), 9.81 (1C, 5a-CH$_3$), 17.85 (1C, 9a-CH$_3$), 25.05 (1C, 11a-CH$_3$), 29.54 (1C, 7-CH$_2$), 37.27 (1C, 8-CH$_2$), 79.43 (1C, 12-CH), 98.34 (1C, 3-C), 107.09 (1C, 5-C), 126.97 (1C, 10-CH), 136.75 (1C, 9-C), 144.6 (1C, 11-C), 158.04 (1C, 6-C), 164.74 (1C, 4-C-OH), 166.03 ppm (1C, 2-C=O); IR: ν 3060 (vw, =C-H), 2980/2932/2911/2850 (br m, CH$_3$, CH$_2$, O-H), 1682 (s, C=O), 1658 (vs, C=O), 1631 (s, C=O), 1543 (br s, C=C), 1429 (br)/1381/1371 (m, CH$_3$, CH$_2$), 1215/1185/1192 (s, C-OH), 1144/1105/1081/1028 (s-m, C-O-C), 868/841 (w, =C-H), 752 (m, =C-H), 670 (w, =C-H) cm^{-1}; HR-MS (ESI) calcd. for $C_{15}H_{19}O_3INa$: 397.0277, obs: 397.0305.

15: MeOSO$_2$F (1.37 mmol, 152.5 mg, 108 µL) was slowly added to a solution of a-pyrone **14** (0.27 mmol, 100 mg) in dry CH$_2$Cl$_2$ (8 mL) at 0 °C. The reaction mixture was stirred for 8-9 h at room temperature and then concentrated under reduced pressure carefully avoiding exposure to the volatile and toxic fluoro reagent. The residue was taken up in CH_2Cl_2 (5 mL) and concentrated three more times to remove remaining $MeOSO_2F$. The residue was taken up in CH_2Cl_2 (5 mL) and treated with 1 N aqueous NaOH (4.6 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL) and the combined organic layers were washed with saturated aqueous NaCl (10 mL). The aqueous layer was reextracted with CH_2Cl_2 (2 x 10 mL). After drying over Na_2SO_4 the organic solution was concetrated under reduced pressure. A light yellow oil was obtained that could be used without further purification. Yield: 80%. ^1H NMR (300 MHz, $CDCl_3$): d = 1.75 (s, 3H, 9a-CH_3), 1.81 (s, 3H, 3a-CH_3), 1.84 (s, 3H, 11a-CH_3), 1.91 (s, 3H, 5a-CH_3), 2.33 (br t, J = 7.6 Hz, 2H, 8-CH_2), 2.7 (br t, J = 7.6 Hz, 2H, 7-CH_2), 3.91 (s, 3H, OMe), 5.59 (br s, 1H, 10-CH), 5.94 ppm (br s, 1H, 12-CH); ^{13}C NMR (75 MHz, $CDCl_3$): d = 6.82 (1C, 3a-CH_3), 9.9 (1C, 5a-CH_3), 17.83 (1C, 9a-CH_3), 25.03 (1C, 11a-CH_3), 29.43 (1C, 7-CH_2), 37.0 (1C, 8-CH_2), 55.29 (1C, OMe), 79.54 (1C, 12-CH), 99.54 (1C, 3-C), 118.57 (1C, 5-C), 127.1 (1C, 10-CH), 136.41 (1C, 9-C), 144.43 (1C, 11-C), 157.32 (1C, 6-C), 162.05 (1C, 2-C), 180.82 ppm (1C, 4-C=O); IR: ν 2952/2924/2855 (m, CH_3, CH_2), 1667 (vs, C=O), 1596 (vs, C=C), 1538 (vw, C=C), 1460/1409/1377 (m, CH_3, CH_2), 1318/1247/1164/1033/984 (m-w, C-O-C), 768/679 (w, =C-H) cm^{-1}; HR-MS (ESI) calcd. for $C_{16}H_{22}O_3I$: 389.0614, obs: 389.0610.

Enzymatic methylation: 50 mL of Soy 2G/fine medium (15 g soy flour, 15 g glucose, 5 g NaCl, 1 g $CaCO_3$, 0.3 g KH_2PO_4, 1000 mL purified water, pH 6.3-6.5) or J medium (10 g yeast extract, 100 g sucrose, 30 g tryptone soya broth, 10 g $MgCl_2{\bullet}6H_2O$, 1000 mL purified water), respectively, supplemented with thiostreptone (c = 5 µg mL^{-1}) was inoculated with 50 µL spore suspension (*S. lividans* ZX1/pHJ95 or *S. albus*/ pHJ95, respectively) and incubated at 30 °C for 2-3 days with orbital shaking (200 rpm). An inoculum (2.5 mL) of the preculture was transferred into 50 mL fresh M10 medium (4 g glucose, 4 g yeast extract, 10 g malt extract, 1000 mL purified water, pH 7.3) and continuously incubated at 30 °C. After 2 days **14** (1.3 µmol dissolved in DMSO, c = 1 mg 25 $µL^{-1}$) was administered to the fermentation, which was incubated for another 5 days on a rotary shaker. Mycelia and culture filtrate were extracted with EtOAc (3 x 80 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in 1 mL MeOH and analyzed by HPLC (Phenomenex Luna C18(2) 10 µm, 250 x 4.6 mm; eluent ACN/ H_2O: 0-10 min from 20:80 to 100:0, 10-25 min at 100/0, flow rate 1 mL min^{-1}).

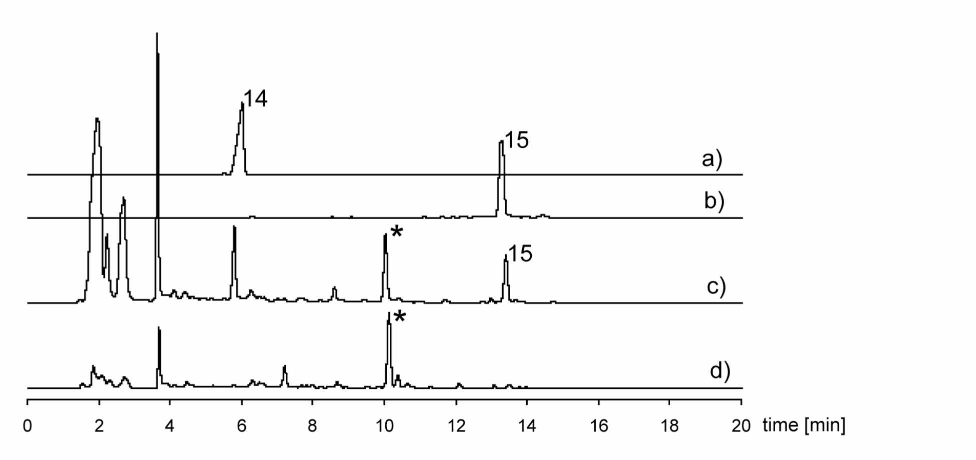


Figure S1. HPLC profile: in vivo biotransformation of α-pyrone **14** with O-methyltransferase AurI (marked peak: * unidentified side product; a) reference of α-pyrone **14**, b) reference of **15**, c) extract of *S. albus*/pHJ95 culture + **14**, d) extract of *S. lividans* ZX1/pHJ95 culture + **14**).

Stille Coupling

Deoxyaureothin (16): Methylated *g*-pyrone **15** (0.192 mmol, 74.5 mg) was dissolved in dry THF (5 mL) and hexamethylditin (0.29 mmol, 94.4 mg, 59.7 µL) as well as $Pd(PPh_3)_4$ (9 µmol, 11.1 mg) were added to the solution. The reaction mixture was refluxed for 4 h. After cooling to room temperature the reaction mixture was stirred with KF on Celite® (50/50 wt) for 10 min, filtered through a pad of Celite® and the filtrate was concentrated under reduced pressure. The crude product was purified by open column chromatography (short pad of basic alumina Al_2O_3, Et_2O/hexane 2:1) to yield a light-yellow oil (85%). ^{1}H NMR (300 MHz, $CDCl_3$): *d* = 0.05 (s, 3H, $Sn(CH_3)_3$), 0.15 (s, 6H, $Sn(CH_3)_3$), 1.81 (m, 9H, 3a, 9a, 11a-CH_3), 1.92 (s, 3H, 5a-CH_3), 2.32 (br t, *J* = 7.54 Hz, 2H, 8-CH_2), 2.7 (t, *J* = 7.61 Hz, 2H, 7-CH_2), 3.91 (s, 3H, OMe), 5.56 (s, 1H, 12-CH), 5.72 ppm (br s, 1H, 10-CH); ^{13}C NMR (75 MHz, $CDCl_3$): *d* = - 8.78/1.0 (3C, $Sn(CH_3)_3$), 6.84 (1C, 3a-CH_3), 9.88 (1C, 5a-CH_3), 17.84 (1C, 9a-CH_3), 25.01 (1C, 11a-CH_3), 29.69 (1C, 7-CH_2), 37.59 (1C, 8-CH_2), 55.26 (1C, OMe), 99.45 (1C, 3-C), 118.47 (1C, 5-C), 129.44 (1C, 12-CH), 131.06 (1C, 10-CH), 134.01 (1C, 9-C), 150.48 (1C, 11-C), 157.72 (1C, 6-C), 162.07 (1C, 2-C), 180.93 ppm (1C, 4-C=O); IR: *n* = 2958/2923/2854 (s-m, CH_3, CH_2), 1667 (vs, C=O), 1600 (vs, C=C), 1539 (vw, C=C), 1459/1408/1376 (m, CH_3, CH_2), 1317/1247/1163/1092/1026 (br)/987 (m-w, C-O-C), 871 (w, =C-H), 768 (br s, =C-H) cm^{-1}.

Solutions of vinyl stannane (18 µmol, 7.6 mg) in dry THF (1 mL) and $Pd_2(dba)_3$ (9 µmol, 8.2 mg) in dry THF (2 mL) were added to a solution of 1-iodo-4-nitrobenzene (27 µmol, 6.7 mg) and CuI (18 µmol, 3.4 mg) in dry THF (1 mL) and the reaction mixture was refluxed for 4 h. After cooling to room temperature the mixture was stirred with KF on Celite® (50/50 wt) for 2 h, filtered through a pad of Celite® and the filtrate was concentrated under reduced pressure. The crude product was purified by semi-preparative HPLC (Macherey Nagel Nucleosil C18 100 5 µm, 250 x 10 mm, using an ACN/H_2O gradient: 0-25 min from 20/80 to 100/0, 25-35 min 100/0, flow rate 5 mL min^{-1}) to yield a yellow solid (82%). The physicochemical data were identical with those reported in ref 10b.

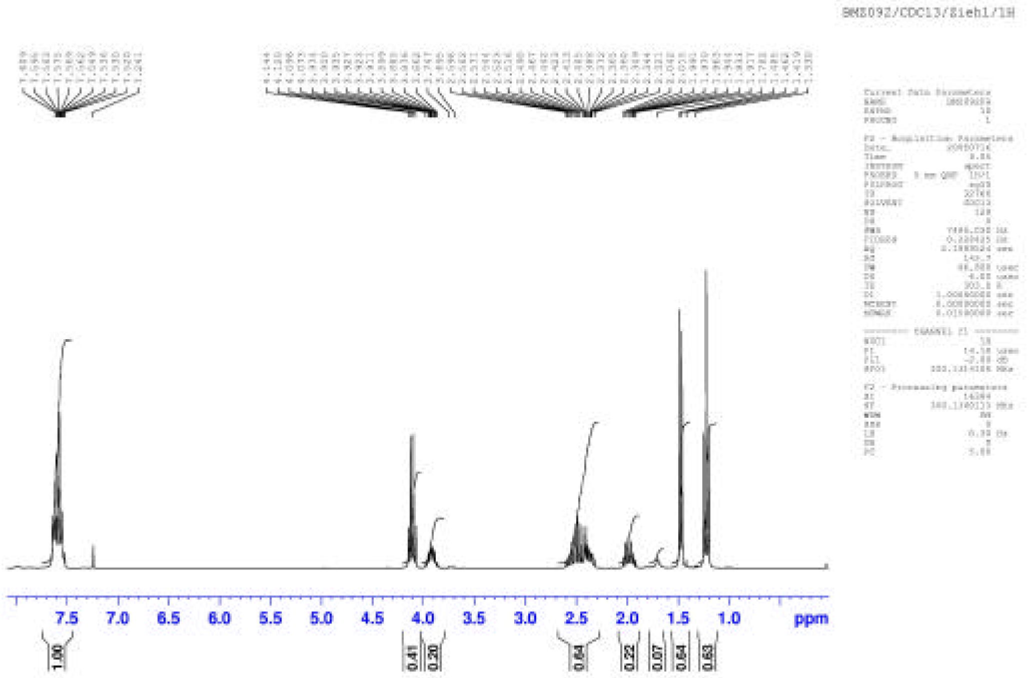
(+)-*R*-Aureothin (1): Based on vinyl iodide **18** stannane forming as well as Stille coupling was performed in analogy to deoxyaureothin (see above) and racemic aureothin.[5, 7b]

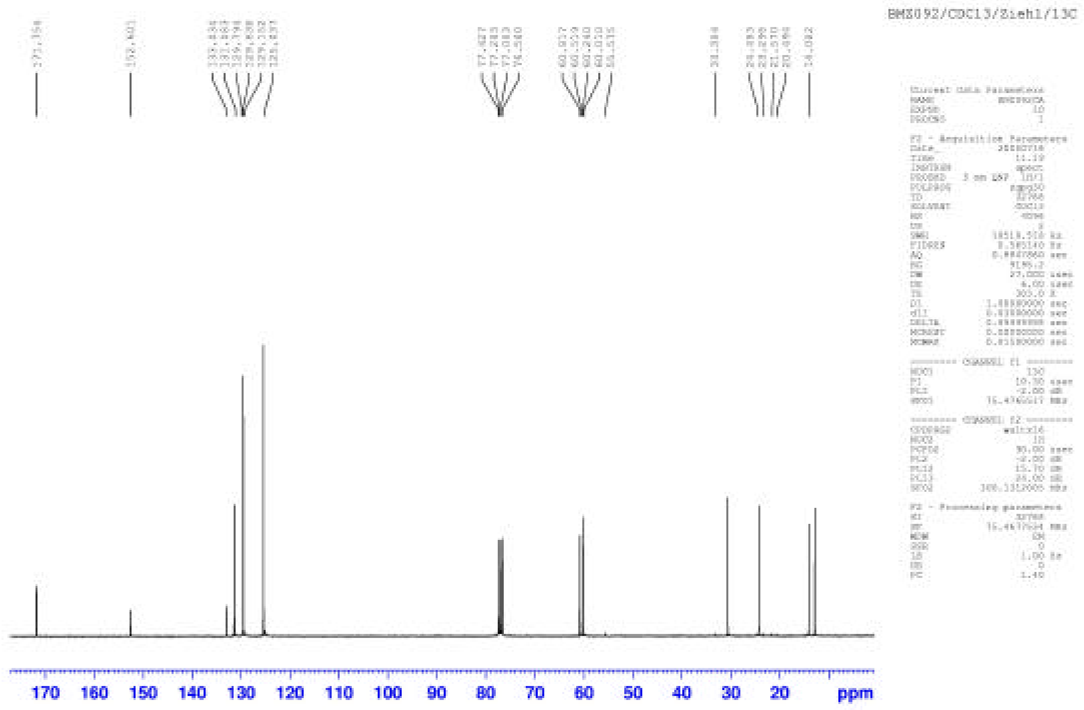
General enzymatic oxygenation: 50 mL of Soy 2G/fine medium (15 g soy flour, 15 g glucose, 5 g NaCl, 1 g $CaCO_3$, 0.3 g KH_2PO_4, 1000 mL purified water, pH 6.3-6.5) supplemented with thiostreptone (c = 5 µg mL^{-1}) was inoculated with 50 µL spore suspension (*S. lividans* ZX1/pHJ110) and incubated at 30 °C for 3 days with orbital shaking (200 rpm). An inoculum (2.5 mL) of the preculture was transferred into 50 mL fresh M10 medium (4 g glucose, 4 g yeast extract, 10 g malt extract, 1000 mL purified water, pH 7.3) and continuously incubated at 30 °C. After 2 days **15** or **16** (1.3 µmol dissolved in DMSO, c = 1 mg 25 $µL^{-1}$) was administered to the fermentation, which was incubated for another 3-5 days on a rotary shaker. Mycelia and culture filtrate were extracted with EtOAc (3 x 80 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in 1 mL MeOH and analyzed by HPLC (Phenomenex Luna C18(2) 10 µm, 250 x 4.6 mm; eluent ACN/H_2O: 0-10 min from 20/80 to 100/0, 10-25 min at 100/0, flow rate 1 mL min^{-1}). The crude extracts from a preparative scale biotransformation were subjected to open column chromatography on silca gel using a $CHCl_3$/MeOH gradient as eluent. All fractions containing the desired product were combined and further purified by semipreparative RP-HPLC (Macherey Nagel Nucleosil C18 100 5 µm, 250 x 10 mm; eluent ACN/H_2O: 0-25 min from 20/80 to 100/0, until 35 min at 100/0, flow rate 5 mL min^{-1}).

17. $[a]_D^{20}$ = + 4.1 (c = 1.07 mg mL^{-1} in MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): *d* = 1.82 (s, 3H, 3a-CH_3), 1.95 (s, 3H, 11a-CH_3), 1.99 (s, 3H, 5a-CH_3), 2.8 (br dd, *J* = 5.76/6.33/ 15.97 Hz, 2H, 8-CH_AH_B), 2.92 (br dd, *J* = 6.41/7.13/16.16 Hz, 2H, 8-CH_AH_B), 3.89 (s, 3H, OMe), 4.54 (br d, *J* = 13.79 Hz, 2H, 9a- CH_AH_B), 4.67 (br d, *J* = 13.98 Hz, 2H, 9a-CH_AH_B), 5.1 (t, *J* = 6.87 Hz, 1H, 7-CH_2), 6.05 (br s, 1H, 10-CH), 6.1 ppm (br s, 1H, 12-CH); ^{13}C NMR (125 MHz, $CDCl_3$): *d* = 6.89 (1C, 3a-CH_3), 9.4 (1C, 5a-CH_3), 23.96 (1C, 11a-CH_3), 37.76 (1C, 8-CH_2), 55.24 (1C, OMe), 70.04 (1C, 9a-CH_2), 73.38 (1C, 7-CH), 81.85 (1C, 12-CH), 100.05 (1C, 3-C), 120.08 (1C, 5-C), 122.41 (1C, 10-CH), 139.41 (1C, 9-C), 144.14 (1C, 11-C), 154.73 (1C, 6-C), 162.06 (1C, 2-C), 180.55 ppm (1C, 4-C=O); IR: *n* = 3052 (vw, =C-H), 2955/2922/2852 (m-w, CH_3, CH_2), 1715 (br vw, C=O), 1661 (s, C=O), 1630 (w, C=C), 1595 (vs, C=C), 1450/1414/1371 (m-w, CH_3, CH_2), 1322/1256/1158/1050/1030/975 (s-m, C-O-C), 962/918/800 (m-w, =C-H), 768 (s, =C-H), 731/669 (m, =C-H) cm^{-1}; HR-MS (ESI) calcd. for $C_{16}H_{20}O_4I$: 403.0401, observed: 403.0405.

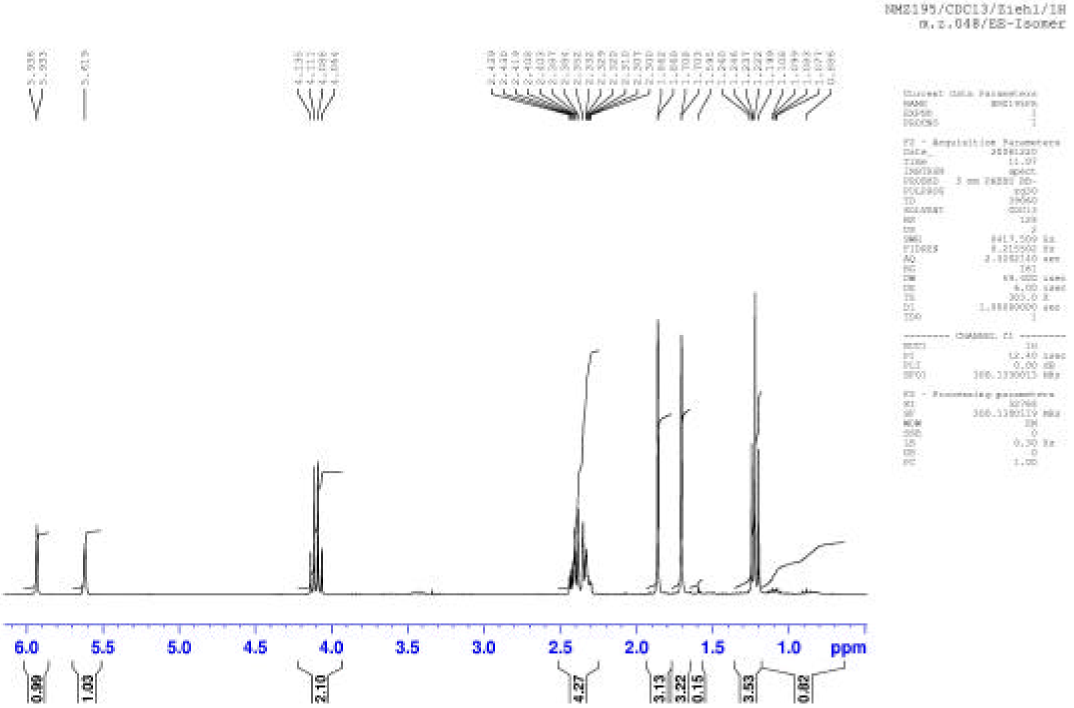
^{1}H and ^{13}C Spectra of New Compounds

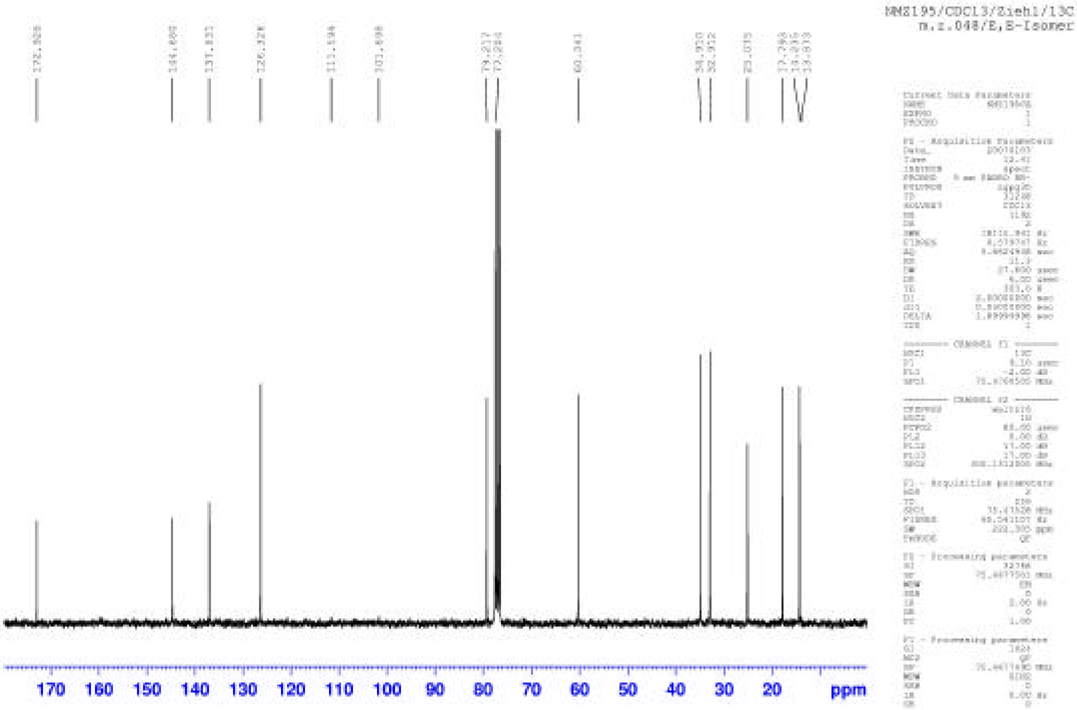
Compound 8a



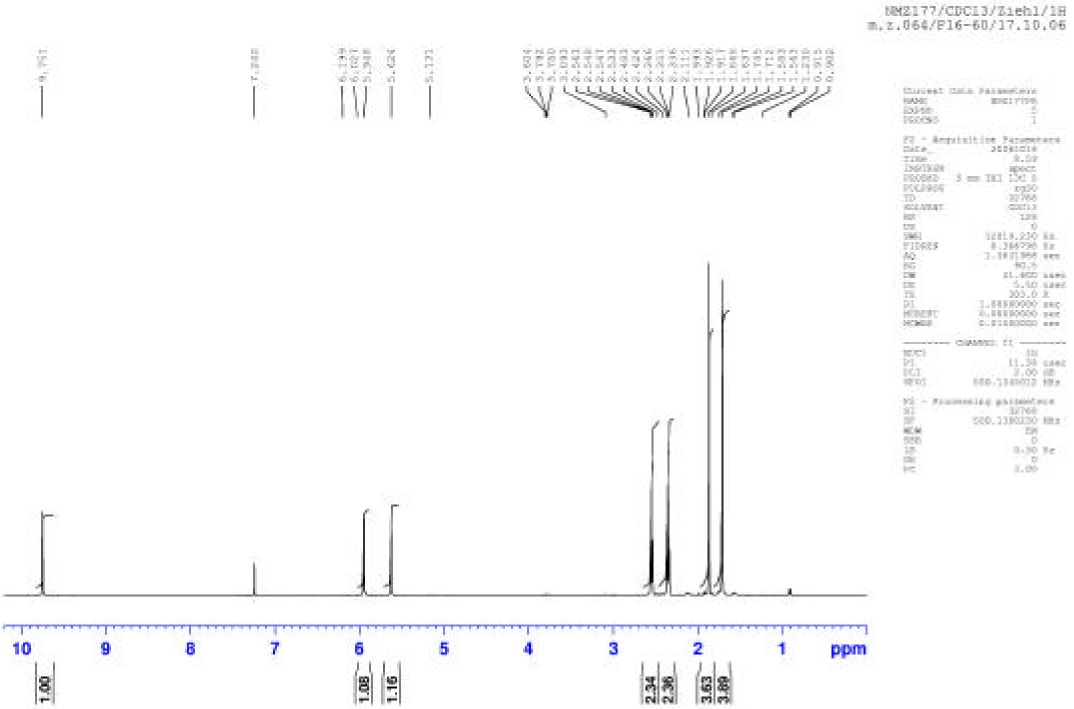


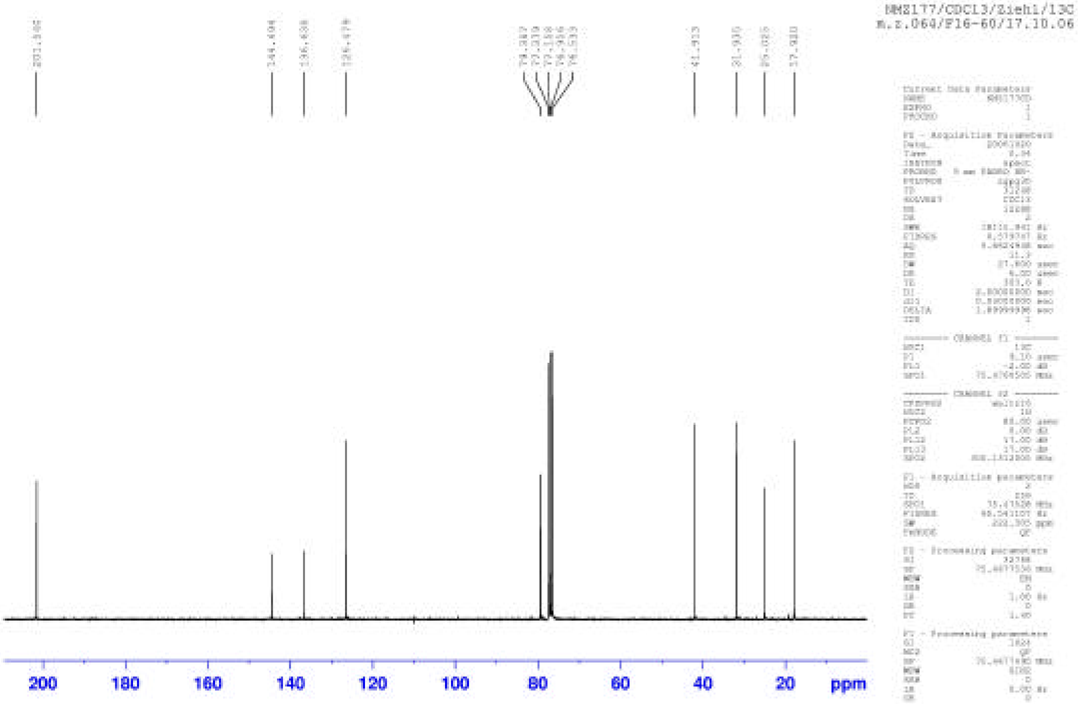
Compound 9


NMZ195/CDCl3/Ziehl/1H
m.z.048/EE-Isomer
5.936
5.933
5.619
4.135
4.111
4.088
4.064
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
0.99
1.03
2.10
4.27
3.13
3.22
0.15
3.53
0.82

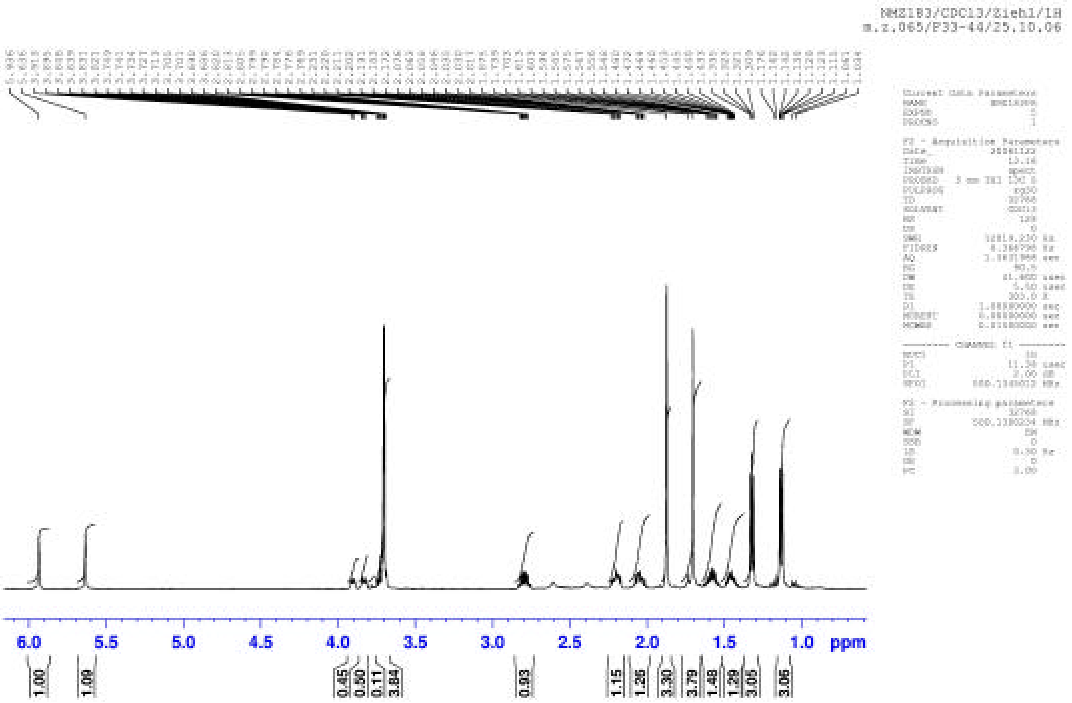

NMZ195/CDCl3/Ziehl/13C
m.z.048/E,E-Isomer
172.928
144.889
136.851
126.328
111.594
101.698
79.217
77.284
60.341
34.920
32.912
25.035
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

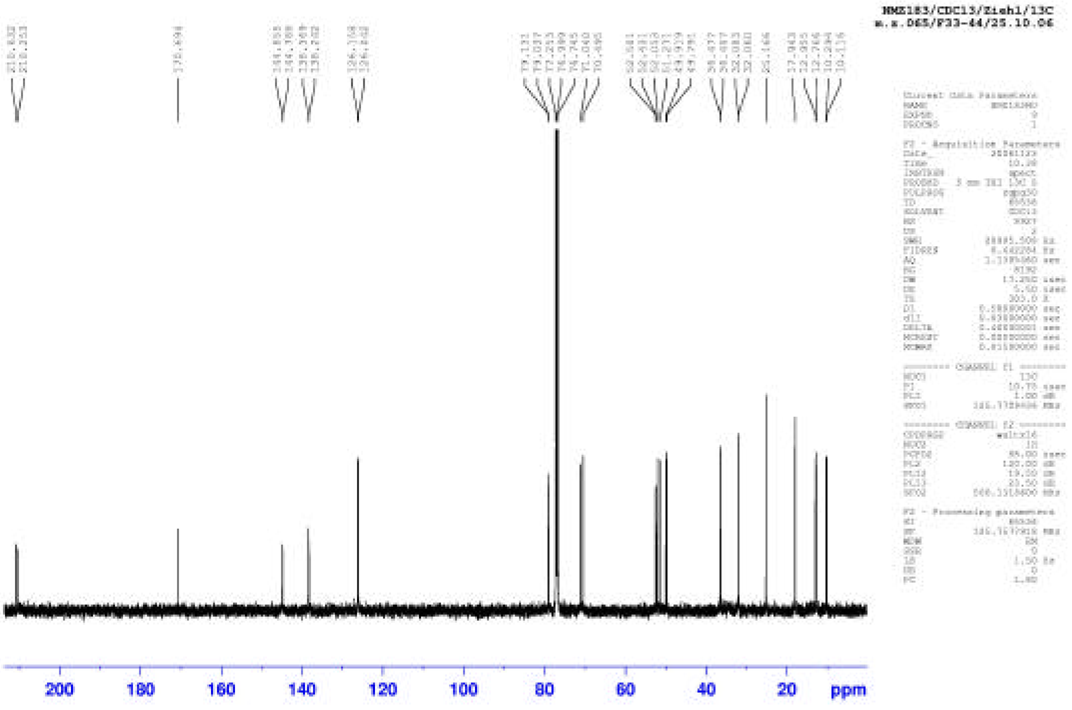
Compound 10


NMZ177/CDCl3/Ziehl/1H
m.z.064/F16-60/17.10.06
9.751
7.260
6.139
6.027
5.948
5.624
5.171
10
9
8
7
6
5
4
3
2
1
ppm
1.00
1.08
1.16
2.34
2.36
3.63
3.89

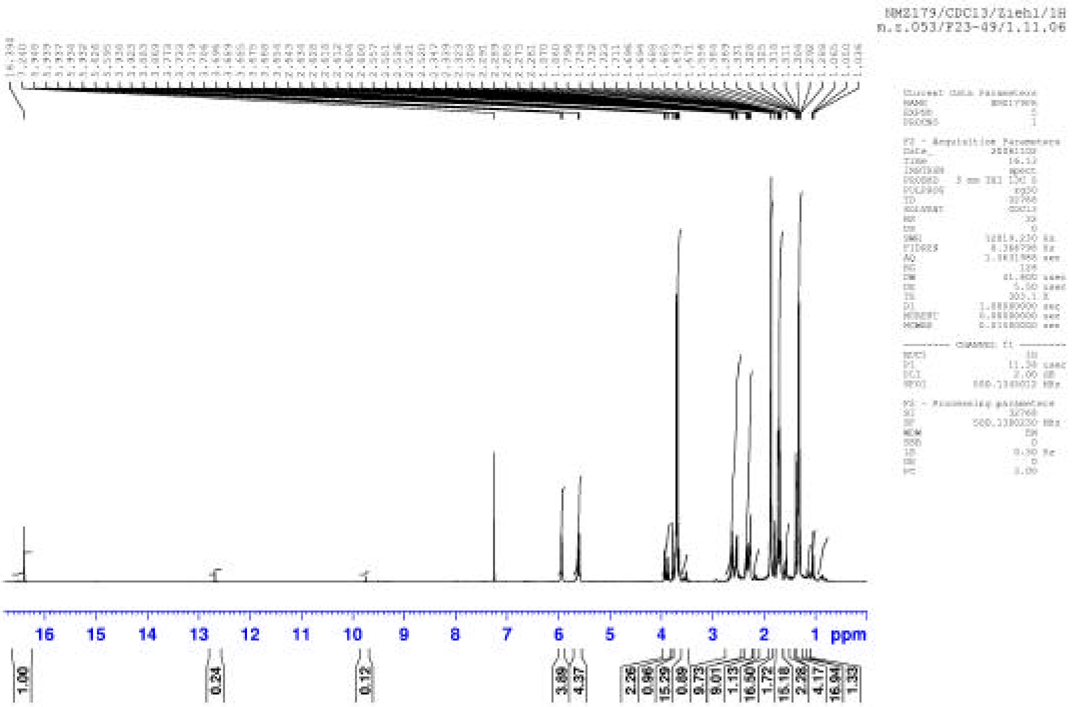

NMZ177/CDCl3/Ziehl/13C
m.z.064/F16-60/17.10.06
201.546
144.494
136.658
126.479
41.913
200
180
160
140
120
100
80
60
40
20
ppm

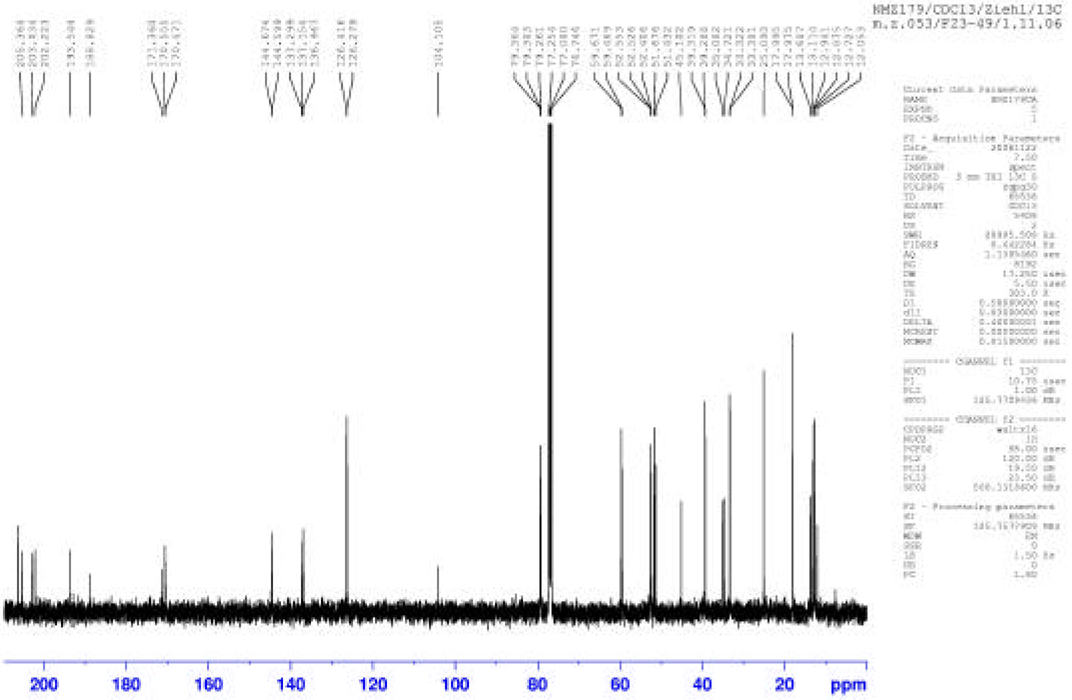
Compound 12


NMZ183/CDCl3/Ziehl/1H
m.z.065/F33-44/25.10.06
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
1.00
1.09
0.45
0.50
0.11
3.84
0.93
1.15
1.26
3.30
3.79
1.48
1.29
3.05
3.06

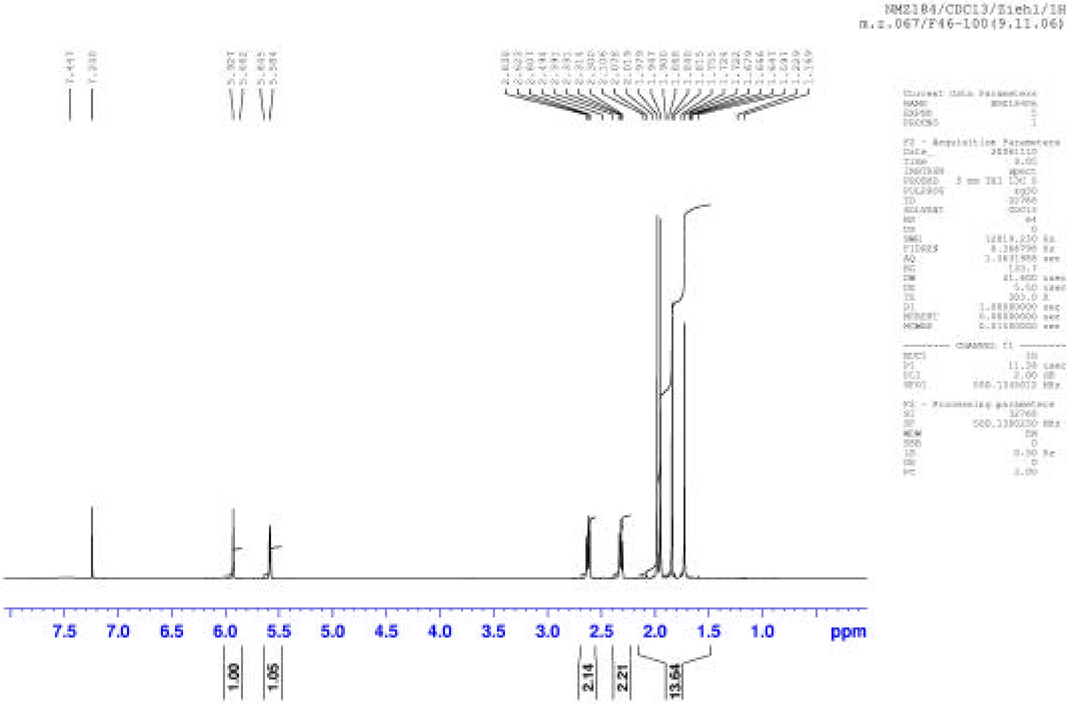

NMZ183/CDCl3/Ziehl/13C
m.z.065/F33-44/25.10.06
200
180
160
140
120
100
80
60
40
20
ppm

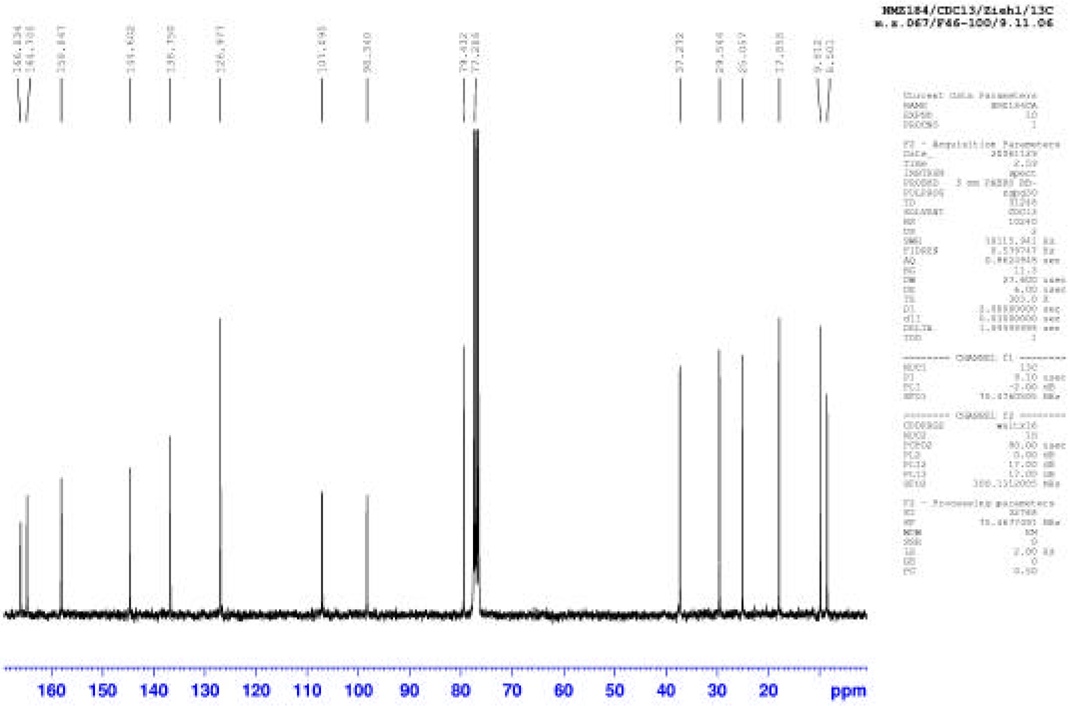
Compound 13


NMZ179/CDCl3/Ziehl/1H
n.z.053/F23-49/1.11.06
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.00
0.24
0.12
3.89
4.37
2.26
0.96
15.29
0.89
9.73
9.01
1.13
16.50
1.72
15.18
2.28
4.17
16.94
1.33

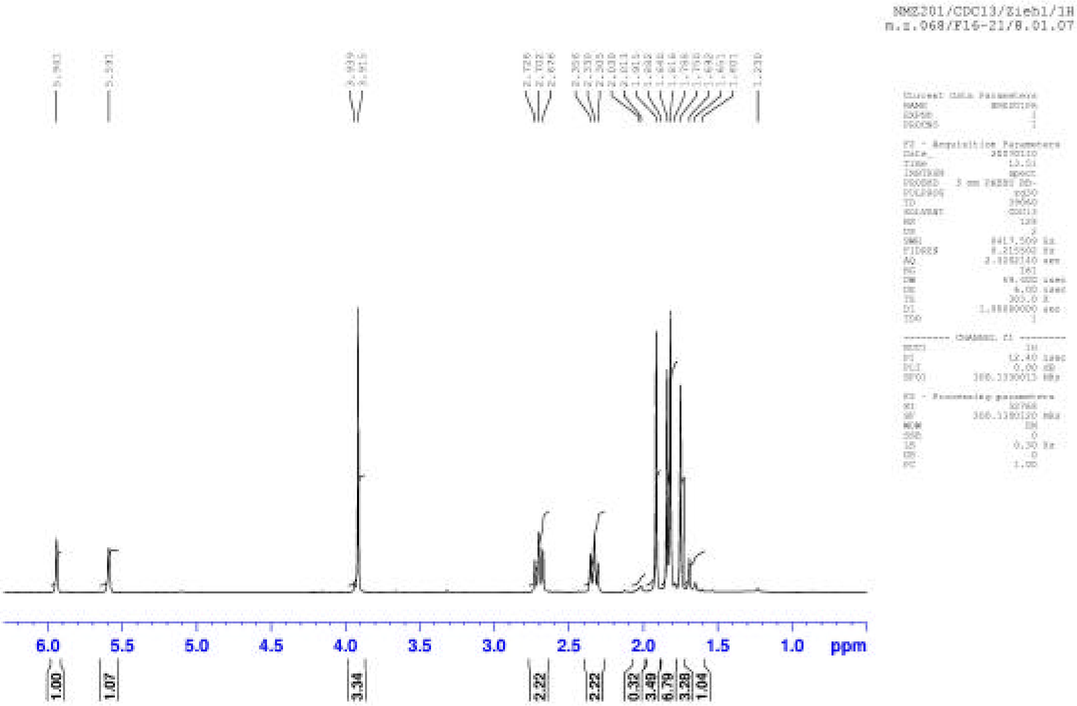

NMZ179/CDCl3/Ziehl/13C
n.z.053/F23-49/1.11.06
200
180
160
140
120
100
80
60
40
20
ppm

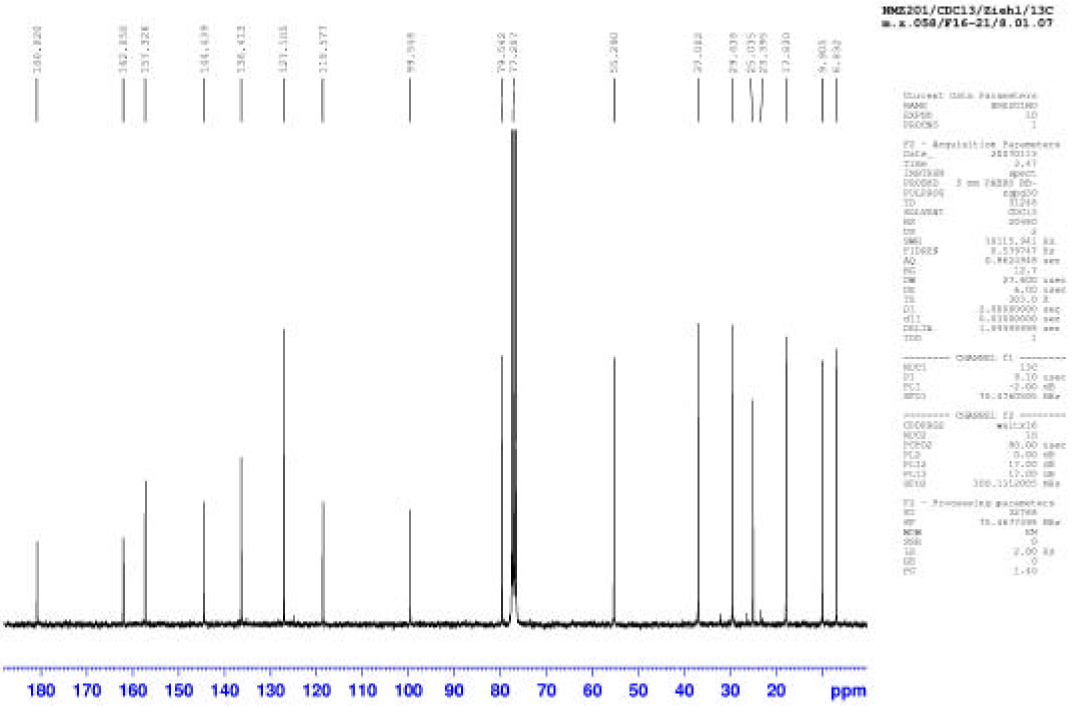
Compound 14


NMZ184/CDCl3/Ziehl/1H
m.z.067/F46-100(9.11.06)
7.447
7.260
5.927
5.882
5.645
5.584
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
1.00
1.05
2.14
2.21
13.64

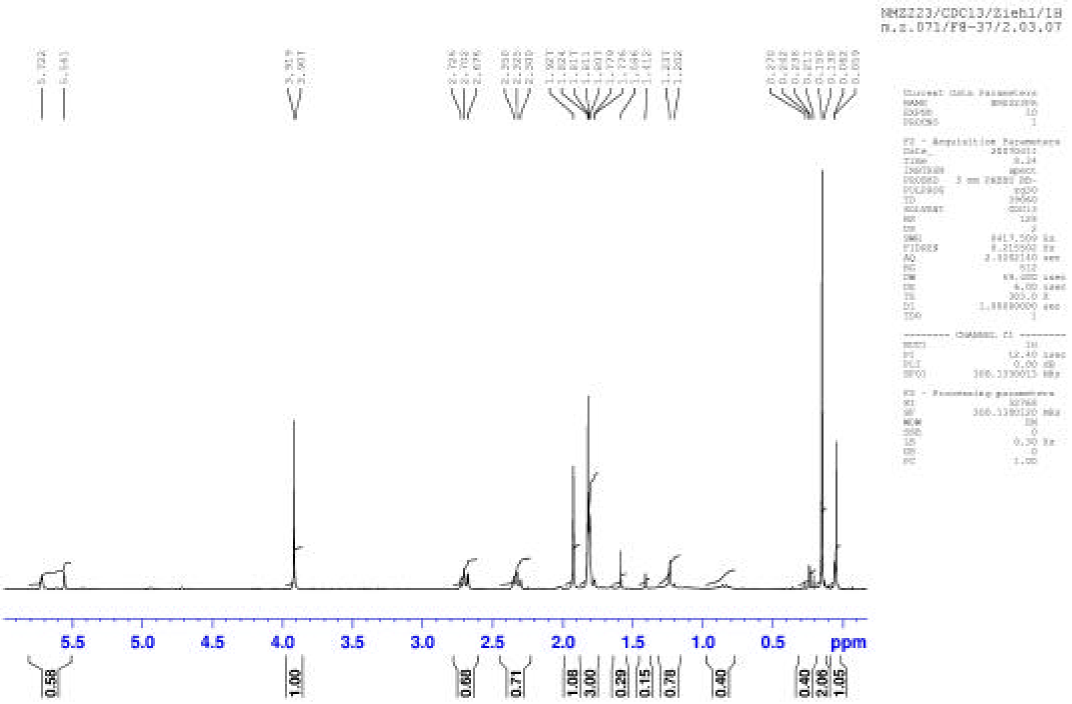

NMZ184/CDCl3/Ziehl/13C
m.z.067/F46-100/9.11.06
166.234
164.708
158.847
144.602
136.758
126.977
107.498
98.340
79.432
77.286
37.272
29.544
25.057
17.858
9.812
8.503
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

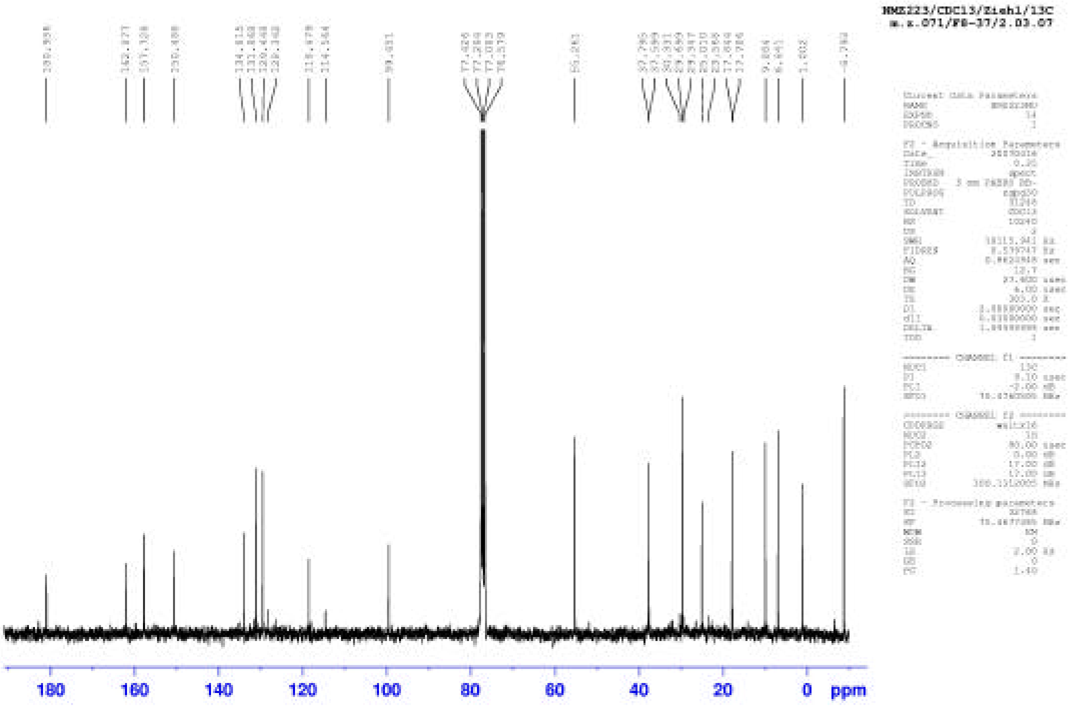
Compound 15


NMZ201/CDCl3/Ziehl/1H
m.z.068/F16-21/8.01.07
5.941
5.591
3.939
3.915
2.726
2.702
2.676
2.356
2.330
2.305
2.030
2.011
1.915
1.882
1.846
1.816
1.788
1.750
1.692
1.661
1.601
1.230
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
1.00
1.07
3.34
2.22
2.22
0.32
3.49
6.79
3.28
1.04

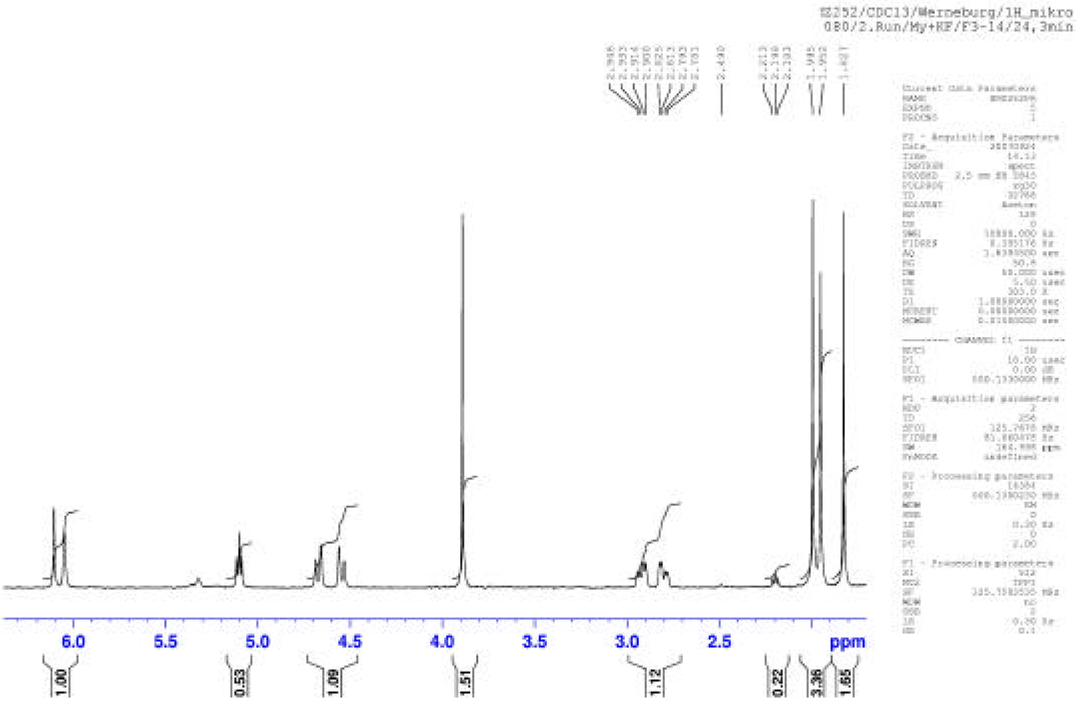

NMZ201/CDCl3/Ziehl/13C
m.z.058/F16-21/8.01.07
180.820
162.858
157.328
144.438
136.413
127.505
118.577
99.598
79.542
77.287
55.280
37.083
29.638
25.035
23.395
17.830
9.905
6.832
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

Stannane derived from 15

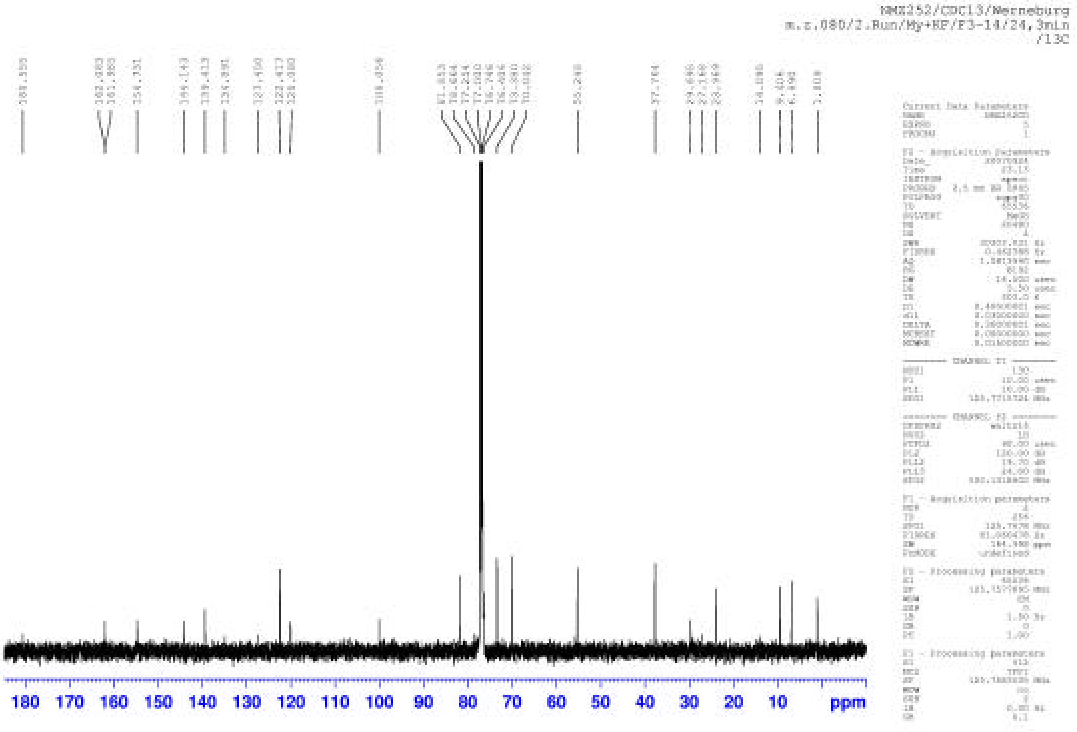




Compound 17

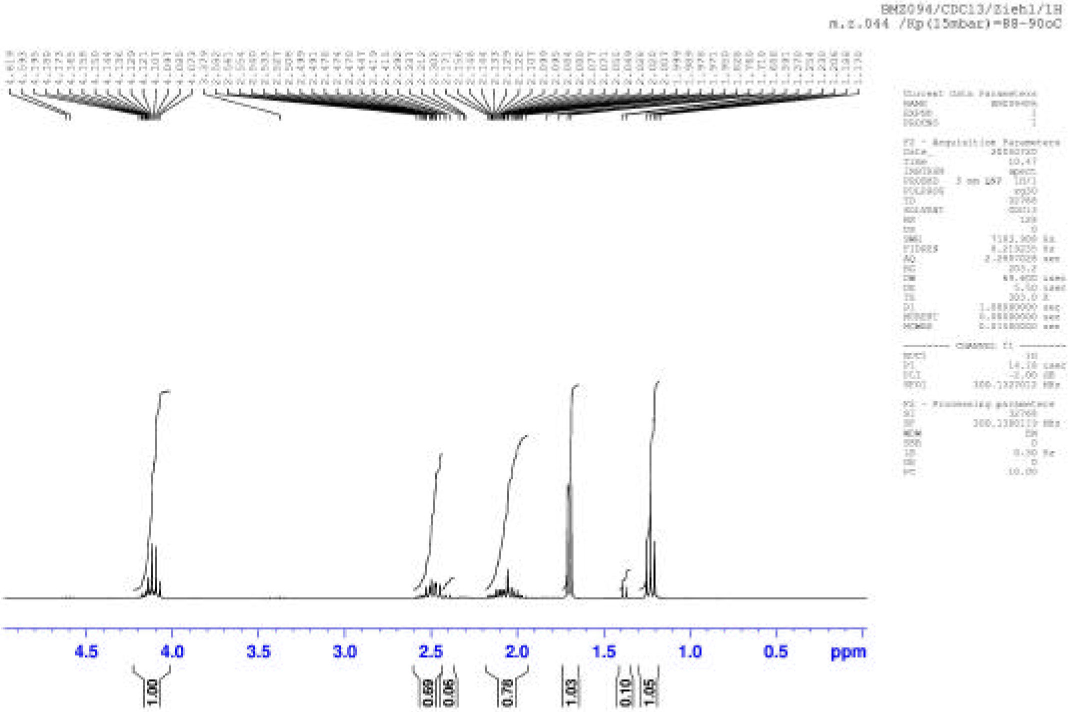


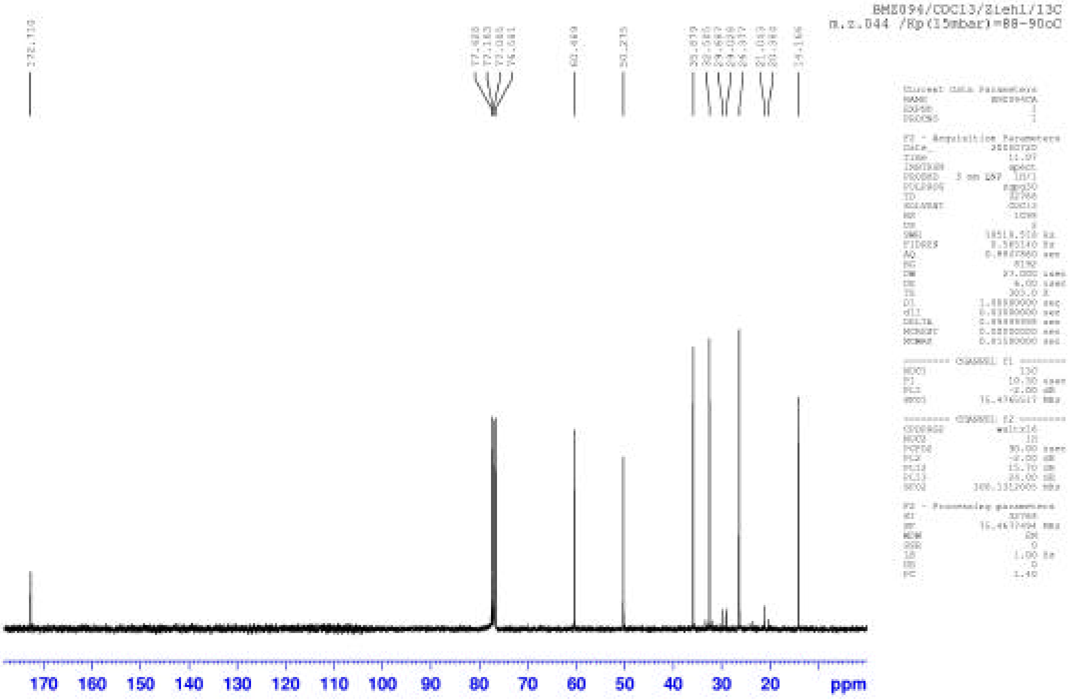
NMZ252/CDCl3/Werneburg/1H_mikro
080/2.Run/My+KF/F3-14/24,3min
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
ppm
1.00
0.53
1.09
1.51
1.12
0.22
3.36
1.65



NMZ252/CDCl3/Werneburg
m.z.080/2.Run/My+KF/F3-14/24,3min
/13C
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

Ethyl 4-bromopentanoate





PT-thioether