Supporting Information

for

Highly Sensitive, Operationally Simple, and Cost/Time Effective Detection of the Mycolactones from the Human Pathogen *Mycobacterium Ulcerans*

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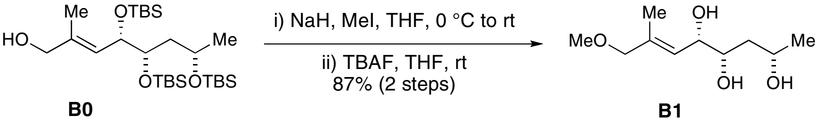
1. General procedures and methods

Reactions were carried out under nitrogen or argon atmosphere in flame-dried glassware. Reagents and ^{1}H NMR spectra were recorded on a Varian Inova 600, Varian Inova 500, and/or Varian 400 spectrometers. ^{13}C NMR spectra were recorded at 100 or 125 MHz. Chemical shifts were reported in parts per million (ppm). For ^{1}H and ^{13}C NMR spectra, the central residual solvent peak (chloroform, acetone) was used as the internal reference (7.26 and 2.05 ppm for ^{1}H NMR and 77.16 and 29.84 ppm for ^{13}C NMR). Electrospray ionization experiments were performed on Micromass Inc. Platform II Atmospheric Pressure Ionization Mass Spectrometer. Analytical thin layer chromatography (TLC) was performed with E. Merck precoated TLC plates, silica gel 60F-254, layer thickness 0.25 mm and visualized by using either $KMnO_4$ or *p*-anisaldehyde stain. Flash chromatography separations were performed on E. Merck kieselgel 60 (230-400 mesh) silica gel. Solvents are of commercial grade and were used as supplied.

Fluorescent dye-free TLC plates [silica gel 60 (5721-7) 250 µM, batch HX822461] were purchased form EMD chemical Inc and were sized to 5 x 2 cm. The UV lamps used were purchased from UVP Inc. [UVLM-28 EL Series UV lamp 365/302 nm (8 Watt) and UVGL-25 254/365 nm (4 Watt)]. The heating plate was purchased from Thermoline Scientific (∅ 9 cm).

2. Synthesis of models for boronate ring size determination

(2*S*,4*S*,5*S*,*E*)-8-methoxy-7-methyloct-6-ene-2,4,5-triol (B1)

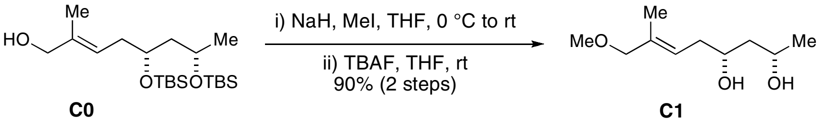


For the preparation of **B0**, see: (a) F. Song, S. Fidanze, A. B. Benowitz, Y. Kishi, *Org. Lett.*, 2002, **4**, 647; (b) F. Song, S. Fidanze, A. B. Benowitz, Y. Kishi, *Tetrahedron*, 2007, **63**, 5739.

To a 0 °C suspension of sodium hydride (60% in mineral oil, 6.5 mg, 0.157 mmol) in anhydrous THF (300 μL) was added a solution of **B0** (30 mg, 56.2 μmol) in THF (120 μL) dropwise. H_2 evolution was observed, and the mixture was allowed to warm to room temperature. After 30 min, methyl iodide (18 μL, 0.281 mmol) was added and the reaction was stirred overnight while protected from light. Saturated aqueous NH_4Cl was added, and the mixture was extracted with Et_2O (3 × 10 mL). The combined organic layer was washed with brine and dried over $MgSO_4$. The solvents were removed *in vacuo* to provide the desired methyl ether as a colorless oil which was used for the next step without further purification. R_f=0.77 (*n*-pentane/Et_2O 7/3). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.44 (dd, *J*=9.1, 1.1 Hz, 1H), 4.33 (dd, *J*=9.1, 3.8 Hz, 1H), 3.90 (m, 1H), 3.59 (dt, *J*=8.5, 3.8 Hz, 1H), 3.24 (s, 3H), 1.82 (ddd, *J*=13.4, 8.7, 3.6 Hz, 1H), 1.67 (d, *J*=1.2 Hz, 3H), 1.61 (ddd, *J*=13.3, 8.6, 5.0 Hz, 1H), 1.13 (d, *J*=6.0 Hz, 3H), 0.875 (s, 9H), 0.870 (s, 9H), 0.86 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H), 0.038 (s, 3H), 0.035 (s, 3H), 0.02 (s, 3H), -0.02 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$): δ 133.71 (Cquat), 128.25 (CH), 78.62 (CH_2), 73.71 (CH), 71.03 (CH), 66.22 (CH_3), 42.52 (CH_2), 26.08 (*t*-Bu), 26.03 (*t*-Bu), 25.94 (*t*-Bu), 23.44 (CH_3), 18.34 (Cquat), 18.19 (Cquat), 18.06 (Cquat), 14.71 (CH_3), -3.94 (CH_3), -4.06 (CH_3), -4.25 (CH_3), -4.38 (CH_3), -4.52 (CH_3), -4.58 (CH_3).

To a room temperature solution of methyl ether (crude from the preceding step) in anhydrous THF (300 μL) was added TBAF (1.0 M solution in THF, 393 μL). The resultant mixture was stirred for 3 h, and the solvent was removed *in vacuo*. The crude residue was purified by silica gel chromatography (100% EtOAc) to yield triol **B1** (10 mg, 87% over 2 steps). R_f=0.21 (100% EtOAc). **^{1}H NMR** (600 MHz, acetone-D_6): δ 5.43 (dq, *J*=9.0, 1.3 Hz, 1H), 4.16 (d, *J*=7.5 Hz, 1H), 4.14 (d, *J*=6.9 Hz, 1H), 4.03 (brs, 1H), 3.97 (m, 1H), 3.81 (brs, 1H), 3.77 (d, *J*=3.9 Hz, 2H), 3.61 (t, *J*=7.9 Hz, 1H), 1.67 (d, *J*=1.2 Hz, 3H), 1.54 (ddd, *J*=13.9, 3.8, 3.0 Hz, 1H), 1.47 (m, 1H), 1.11 (d, *J*=6.1 Hz, 3H). **^{13}C NMR** (125 MHz, acetone-D_6): δ 136.36 (Cquat), 128.02 (CH), 78.31 (CH_2), 75.82 (CH), 72.07 (CH), 67.72 (CH), 57.58 (CH), 41.86 (CH_2), 24.20 (CH_3), 14.56 (CH_3). **HRMS** (ESI positive): found $[M+Na]^+$ 227.1307, calculated $[M+Na]^+$ 227.1254.

(2*S*,4*R*,*E*)-8-methoxy-7-methyloct-6-ene-2,4-diol (C1)

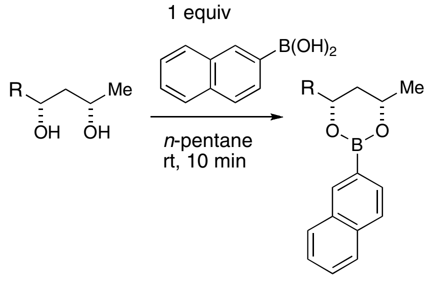


For preparation of **C0**, see: (a) T. C. Judd, A. Bischoff, Y. Kishi, S. Adusumilli, P. L. C. Small, *Org. Lett.*, 2004, **6**, 4901; (b) H.-J. Kim, Y. Kishi, *J. Am. Chem. Soc.,* 2008, **130**, 1842.

To a 0 °C suspension of sodium hydride (60% in mineral oil, 7 mg, 0.174 mmol) in anhydrous THF (300 μL) was added a solution of **C0** (25 mg, 62.1 μmol) in THF (120 μL) dropwise. H_2 evolution was observed, and the mixture was allowed to warm to room temperature. After 30 min, methyl iodide (19 μL, 0.310 mmol) was added and the reaction was stirred overnight while protected from light. Saturated aqueous NH_4Cl was added, and the mixture was extracted with Et_2O (3 × 10 mL). The combined organic layer was washed with brine and dried over $MgSO_4$. The solvents were removed *in vacuo* to provide the desired methyl ether as a colorless oil which was used for the next step without further purification. R_f=0.70 (*n*-pentane/Et_2O 7/3). ^{1}**H NMR** (400 MHz, $CDCl_3$): δ 5.44 (t, *J*=7.1 Hz, 1H), 3.90 (m, 1H), 3.80 (s, 2H), 3.78 (m, 1H), 3.26 (s, 3H), 2.22 (m, 2H), 1.67 (m, 1H), 1.63 (s, 3H), 1.49 (m, 1H), 1.12 (d, *J*=6.0 Hz, 3H), 0.88 (s, 18H), 0.05 (s, 3H), 0.04 (s, 8H). ^{13}**C NMR** (100 MHz, $CDCl_3$): δ 133.66 (Cquat), 124.58 (CH), 78.86 (CH_2), 69.68 (CH), 66.07 (CH), 57.42 (CH_3), 47.38 (CH_2), 35.83 (CH_2), 26.04 (*t*-Bu), 26.00 (*t*-Bu), 23.90 (CH_3), 18.27 (Cquat), 18.19 (Cquat), 14.17 (CH_3), -4.17 (CH_3), -4.46 (CH_3), -4.63 (CH_3).

To a room temperature solution of methyl ether (crude from the preceding step) in anhydrous THF (300 μL) was added TBAF (1.0 M solution in THF, 311 μL). The resultant mixture was stirred for 3 h, and the solvent was removed *in vacuo*. The crude residue was purified by silica gel chromatography (100% EtOAc) to yield the triol **C1** (11 mg, 90%, over 2 steps). R_f=0.40 (100% EtOAc). ^{1}**H NMR** (600 MHz, acetone-D_6): δ 5.50 (td, *J*=7.2, 1.0 Hz, 1H), 4.19 (brd, *J*=2.7 Hz, 1H), 4.11 (brd, *J*=2.2 Hz, 1H), 3.96 (m, 1H), 3.83 (m, 1H), 3.76 (s, 2H), 3.20 (s, 3H), 2.20 (brt, *J*=6.7 Hz, 2H), 1.60 (s, 3H), 1.55 (dt, *J*=14.1, 3.2 Hz, 1H), 1.46 (m, 1H), 1.12 (d, *J*=6.2 Hz, 3H). ^{13}**C NMR** (125 MHz, acetone-D_6): δ 134.65 (Cquat), 124.60 (CH), 78.90 (CH_2), 72.23 (CH), 68.29 (CH), 57.28 (CH), 45.68 (CH_2), 37.14 (CH_2), 24.48 (CH_3), 14.06 (CH_3). **HRMS** (ESI positive): found $[M+Na]^+$ 211.1322, calculated $[M+Na]^+$ 211.1355.

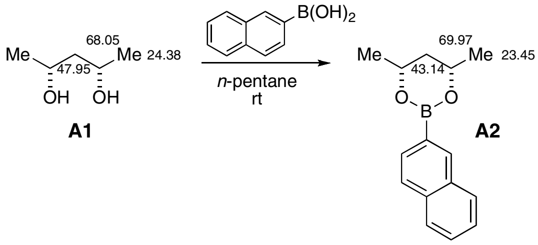
3. General procedure for preparation of boronic esters from corresponding diols



To a solution of diol (1 equiv) in *n*-pentane (1M) was added the boronic acid (1 equiv). The resultant mixture was stirred vigorously for 10 min. The solvent was removed *in vacuo*, and the resultant residue was dried under high vacuum (0.1 mm Hg) for 1 h.

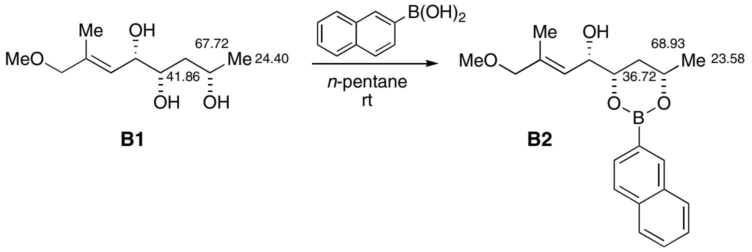
rac-(4*S*,6*R*)-4,6-dimethyl-2-(naphthalen-2-yl)-1,3,2-dioxaborinane (A2)

For preparation of cis-2,4-pentanediol (**A1**), see: L. Bonner, S. Frescas, D. E. Nichols, *Synth. Commun.*, 2004, **34**, 2767.



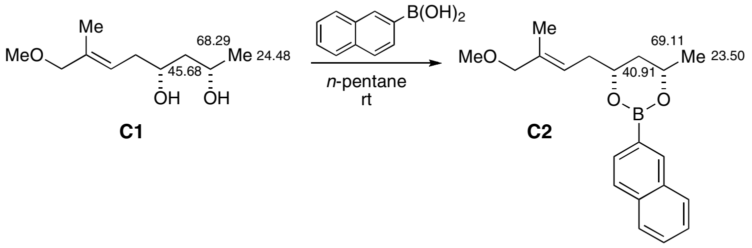
^{1}H NMR (500 MHz, acetone-D_6): δ 8.32 (s, 1H), 7.92 (brd, *J*=7.8 Hz, 1H), 7.87 (brd, *J*=7.8 Hz, 1H), 7.82 (s, 2H), 7.49 (m, 2H), 4.36 (ddd, *J*=12.5, 6.1, 2.7 Hz, 1H), 4.31 (ddd, *J*=12.5, 6.1, 2.7 Hz, 1H), 2.11 (dt, *J*=13.7, 2.5 Hz, 1H), 1.44 (brq, *J*=12.2 Hz, 1H), 1.34 (d, *J*=6.2 Hz, 6H). **^{13}C NMR** (125 MHz, acetone-D_6): δ 134.84 (Cquat), 134.66 (CH), 132.94 (CH), 128.42 (CH), 127.55 (CH), 126.51 (CH), 126.42 (CH), 125.58 (CH), 68.17 (2 × CH), 42.24 (CH_2), 22.51 (2 × CH_3).

(*S*,*E*)-4-methoxy-3-methyl-1-((4*S*,6*S*)-6-methyl-2-(naphthalen-2-yl)-1,3,2-dioxaborinan-4-yl)but-2-en-1-ol (B2)



^{1}H NMR (600 MHz, acetone-D$_6$): δ 8.40 (s, 1H), 7.93-7.86 (3H), 7.83 (d, *J*=7.3 Hz, 1H), 7.51 (m, 1H), 5.63 (dq, *J*=9.0, 1.4 Hz, 1H), 4.47 (dd, *J*=8.6, 5.9 Hz, 1H), 4.37 (m, 1H), 4.18 (ddd, *J*=11.5, 5.8, 2.9 Hz, 1H), 3.82 (d, *J*=6.4 Hz, 2H), 3.24 (s, 3H), 5.29-2.07 (m, 1H), 1.77 (d, *J*=1.1 Hz, 3H), 1.63 (ddd, *J*=13.1, 11.8, 11.8 Hz, 1H), 1.37 (d, *J*=6.2 Hz, 3H). **^{13}C NMR** (125 MHz, acetone-D$_6$): δ 135.86 (CH), 133.86 (Cquat), 131.04 (CH), 129.35 (CH), 128.47 (CH), 128.43 (Cquat), 127.46 (CH), 127.30 (CH), 126.93 (Cquat), 126.48 (CH), 78.29 (CH$_2$), 76.62 (CH), 71.42 (CH), 68.93 (CH), 57.63 (CH), 36.72 (CH$_2$), 23.58 (CH$_3$), 14.74 (CH$_3$).

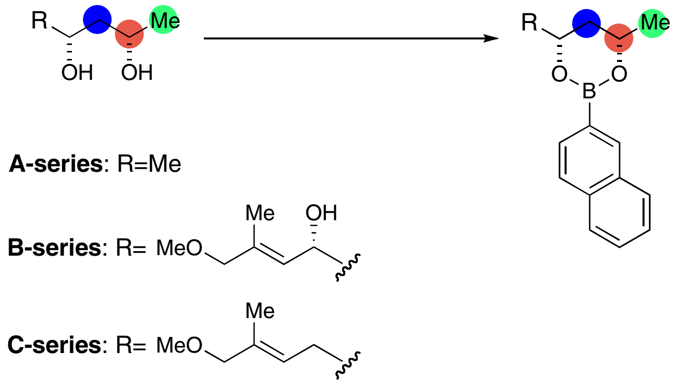
(4*R*,6*S*)-4-((*E*)-4-methoxy-3-methylbut-2-enyl)-6-methyl-2-(naphthalen-2-yl)-1,3,2-dioxaborinane (C2)

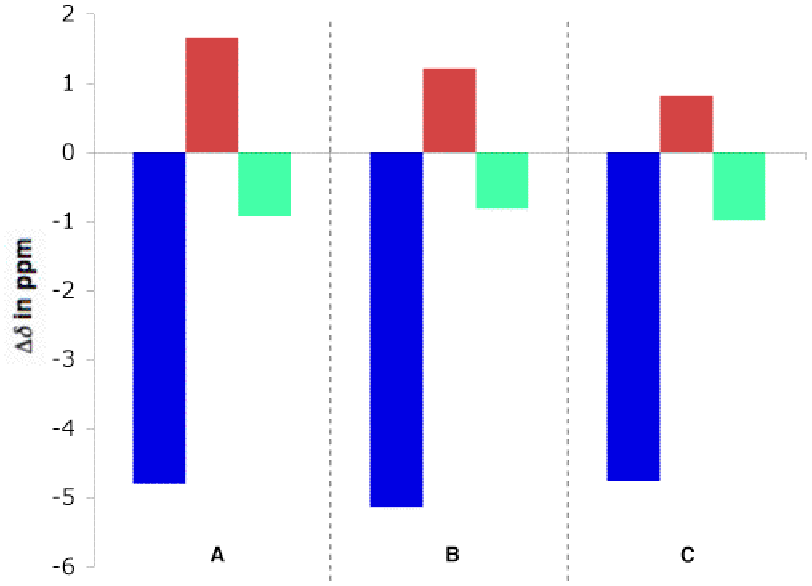


^{1}H NMR (500 MHz, acetone-D$_6$): δ 8.31 (brs, 1H), 7.92 (brd, *J*=6.8 Hz, 1H), 7.88 (brd, *J*=7.9 Hz, 1H), 7.84 (s, 2H), 7.15 (m, 2H), 5.64 (td, *J*=7.4, 1.5 Hz, 1H), 4.36 (m, 1H), 4.28 (m, 1H), 3.83 (s, 2H), 3.24 (s, 3H), 2.46 (m, 2H), 2.15 (dt, *J*=13.8, 2.7 Hz, 1H), 1.72 (s, 3H), 1.51 (q, *J*=12.2 Hz, 1H), 1.36 (d, *J*=6.2 Hz, 3H). **^{13}C NMR** (125 MHz, acetone-D$_6$): δ 135.65 (CH), 133.86 (Cquat), 131.35 (Cquat), 130.91 (CH), 129.34 (CH), 128.48 (CH), 127.45 (CH), 127.36 (CH), 126.51 (CH), 123.21 (CH), 78.78 (CH$_2$), 72.69 (CH), 69.11 (CH), 57.36 (CH), 40.91 (CH$_2$), 36.09 (CH$_2$), 23.50 (CH$_3$), 14.19 (CH$_3$).

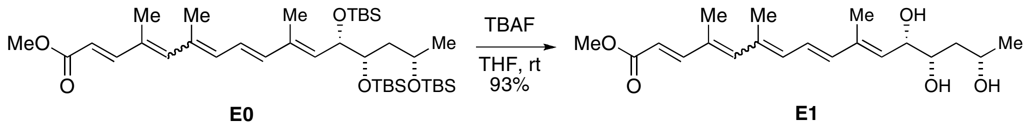
4. NMR analyses

Carbon chemical shift changes observed in transformation from 1,3-diols to the corresponding 2-naphthylboronates [$\Delta\delta$(acetone-D_6), blue=CH_2, red=CH, green=CH_3]





5. Preparation of (2*E*,4*E*,6*E*,8*E*,10*E*,12*S*,13*S*,15*S*)-methyl 12,13,15-trihydroxy-4,6,10-trimethylhexadeca-2,4,6,8,10-pentaenoate (E1)



For the preparation of **E0**, see: (a) F. Song, S. Fidanze, A. B. Benowitz, Y. Kishi, *Org. Lett.*, 2002, **4**, 647; (b) F. Song, S. Fidanze, A. B. Benowitz, Y. Kishi, *Tetrahedron*, 2007, **63**, 5739.

To a room temperature solution of **E0** (33 mg, 47.6 μmol) in anhydrous THF (0.5 mL) was added TBAF (1.0 M solution in THF, 0.290 mL), and the resultant mixture was stirred for 4 h. Following the general workup procedure of Kaburagi and Kishi,[1] $CaCO_3$ (59 mg) was added. After 5 min DOWEX-50WX8-400 (178 mg) and MeOH (200 μL) were added, and the resultant mixture was diluted with EtOAc and filtered over a pad of Celite®. The solvents were removed *in vacuo* to yield **E1** as a yellow oil (15.5 mg, 93%). R_f= 0.70 (100% acetone). $[\alpha]^{23}_{D}$=+37.0 (*c*=1, $CHCl_3$). This ester exists mainly as a 3:2 mixture of 4'*Z*:*E* isomers, but contains trace amounts other minor isomers as well. **^{1}H NMR** ($CDCl_3$, 500 MHz, 4'*Z* isomer) δ 7.37 (d, *J*=15.6 Hz, 1H), 6.56 (d, *J*=15.1, 11.4 Hz, 1H), 6.35 (s, 1H), 6.23 (brd, *J*=10.6 Hz, 1H), 5.87 (d, *J*=15.6 Hz, 1H), 5.50 (d, *J*=9.0 Hz, 1H), 4.29 (dd, *J*=8.7, 7.3 Hz, 1H), 7.07 (m, 1H), 3.76 (s, 3H), 3.79-3.72 (m, 1H), 3.60 (brs, 1H), 2.96 (brs, 1H), 2.36 (brs, 1H), 2.05 (s, 3H), 2.02 (s, 3H), 1.93 (m, 1H), 1.91 (s, 3H), 1.55 (m, 1H), 1.20 (d, *J*=6.1 Hz, 3H). **^{13}C NMR** (125 MHz, $CDCl_3$): δ 168.08 (CO), 150.99 (CH), 143.68 (CH), 138.67 (Cquat), 138.48 (CH), 135.32 (Cquat), 134.69 (CH), 132.77 (Cquat), 131.14 (CH), 125.38 (CH), 116.15 (CH), 75.98 (CH), 72.28 (CH), 68.73 (CH), 51.66 (CH_3), 40.57 (CH_2), 24.30 (CH_3), 17.31 (CH_3), 14.32 (CH_3), 13.51 (CH_3). **HRMS** (ESI positive): found $[M+Na]^+$ 373.1985, calculated $[M+Na]^+$ 373.1965. UV (EtOAc) λ_{max} 349 nm (ε log=4.363).

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[1] Y. Kaburagi, Y. Kishi, *Org. Lett.*, 2007, **9**, 723.

6. Table of attempted boronic acids

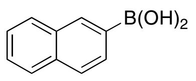
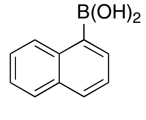
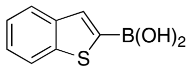
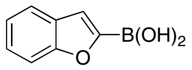
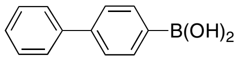
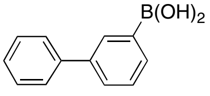
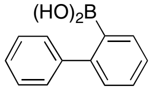
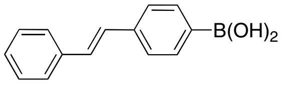
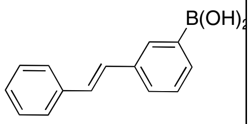
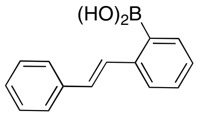
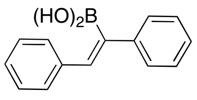
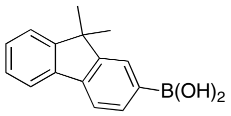
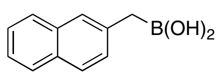
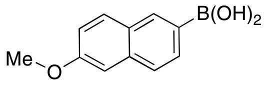
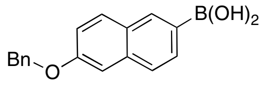
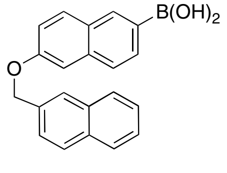
Experimental protocol:

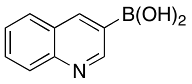
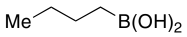
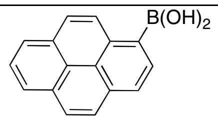
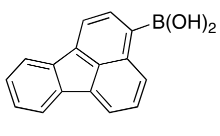
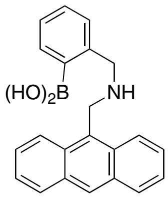
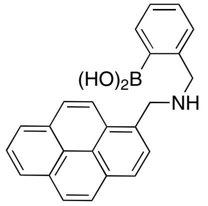
1. 2 µL of a solution of mycolactone A/B in acetone (0.02 mg/mL) is applied to a dye-free silica gel C60 TLC plate and eluted with 90/9/1 $CHCl_3/MeOH/H_2O$ as the mobile phase. In the of case of the C1’-C16’ side chain, 100% acetone was used as a mobile phase.

2. The eluted TLC plate is briefly warmed on a hot plate to evaporate the solvents, and while warm, quickly immersed into a 0.1 M acetone solution of the boronic acid.

3. The TLC plate is then heated to 100 °C for 5~10 seconds.

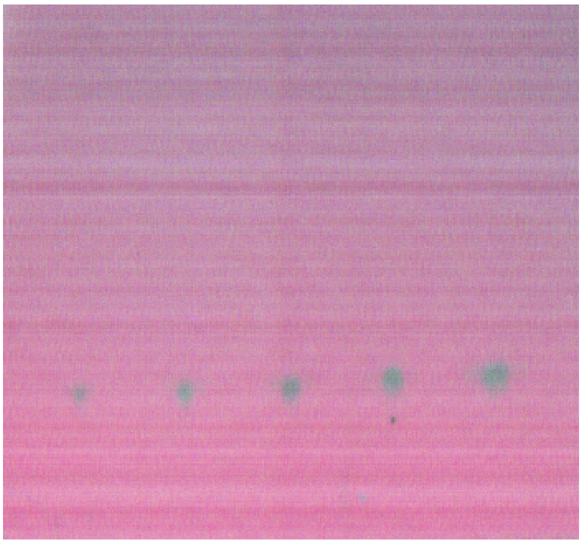
4. The glass side of the TLC plate is wiped cleaned using acetone, then the TLC plate is irradiated with a 8 W hand-held UV lamp with a 365 nm filter. Mycolactone A/B is detected as a green-yellow fluorescent spot.

Boronic acids for which fluorescence was observed on a dye-free silica gel TLC C60 ranked by intensity			
			
			
			
			

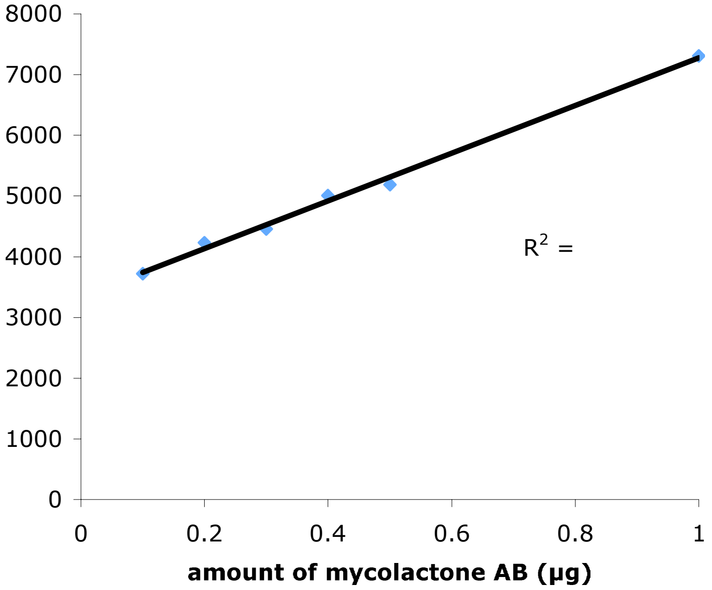
Boronic acid for which fluorescence quenching or no fluorescence was observed		
		
		

7. Quantitative TLC analyses using the flatbed scanner

The developed TLC plate (using the general procedure with 2-naphthyl boronic acid)[2] was scanned with the TLC-2400A scanner (BioDit Technology Co., Ltd) using the 366 nm wavelength (power 3.2 W, 1200 dpi). After acquisition, the image was analyzed with ImageDecipher-TLC© using the red channel (setup: brightness 59, contrast 46) of the TLC plate image to yield peak area.



Digitized TLC under 366 nm irradiation with eluted amounts of mycolactone A/B from 0.1, 0.2, 0.3, 0.4, 0.5, 1 µg respectively



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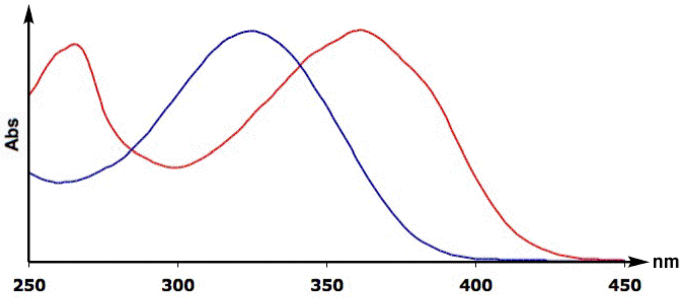
[2] Purchased from Alfa-Aesar.

8. UV spectra of mycolactone A/B, C, F and E

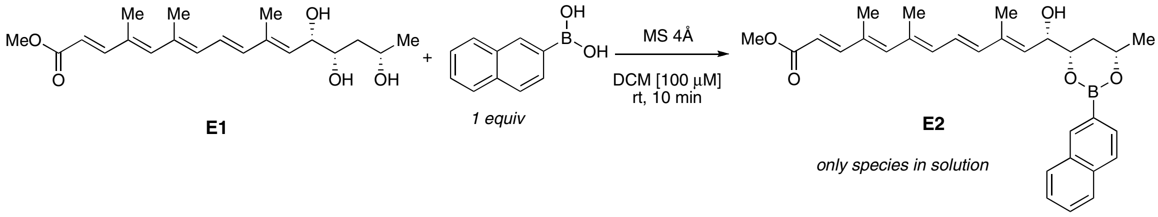


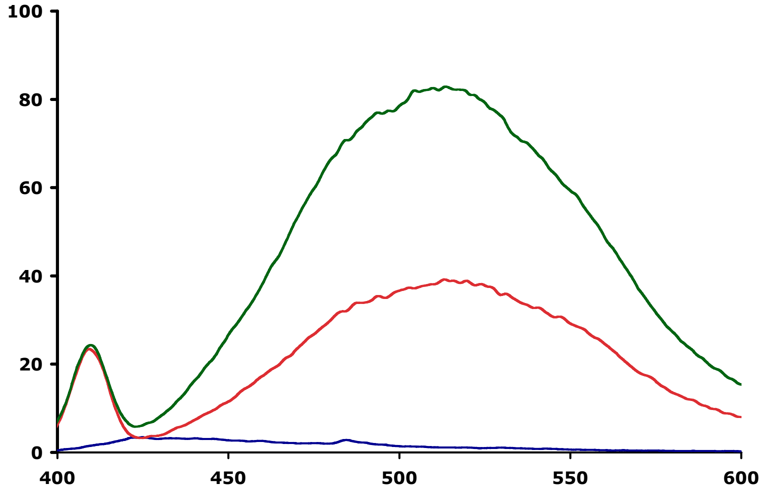
Red: mycolactone A/B in MeOH (λ_{max} 362 nm, log $\varepsilon = 4.29$) and mycolactone C in MeOH (λ_{max} 365 nm, log $\varepsilon = 4.69$)

Blue: mycolactone F in MeOH (λ_{max} 323.5 nm, log $\varepsilon = 4.12$) and mycolactone E in MeOH (λ_{max} 323 nm, log $\varepsilon = 4.19$)

9. Fluorescence experiments

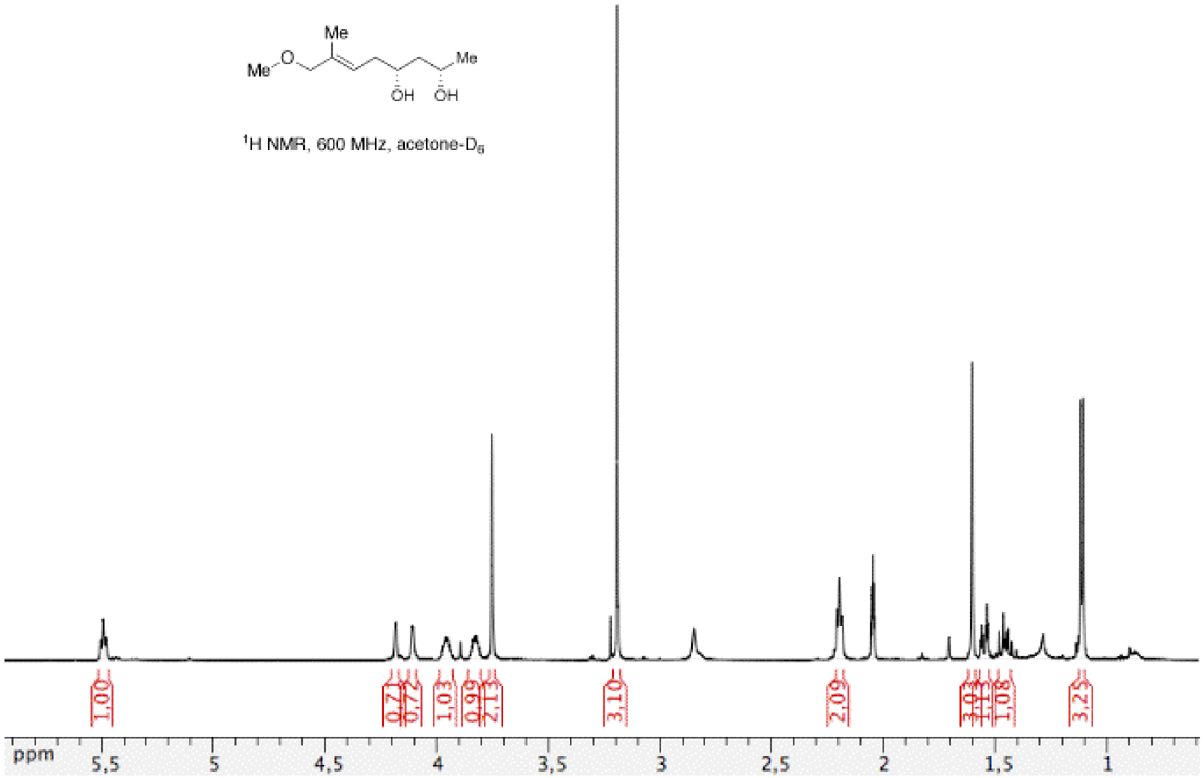
To a vial was introduced **E1** (0.125 mg, 0.36 µmol), 2-naphthylboronic acid (100 µL, 3.6 µM in DCM), and 4Å molecular sieves (2 mg). The resultant mixture was stirred for 10 min at room temperature and used as stock solution.

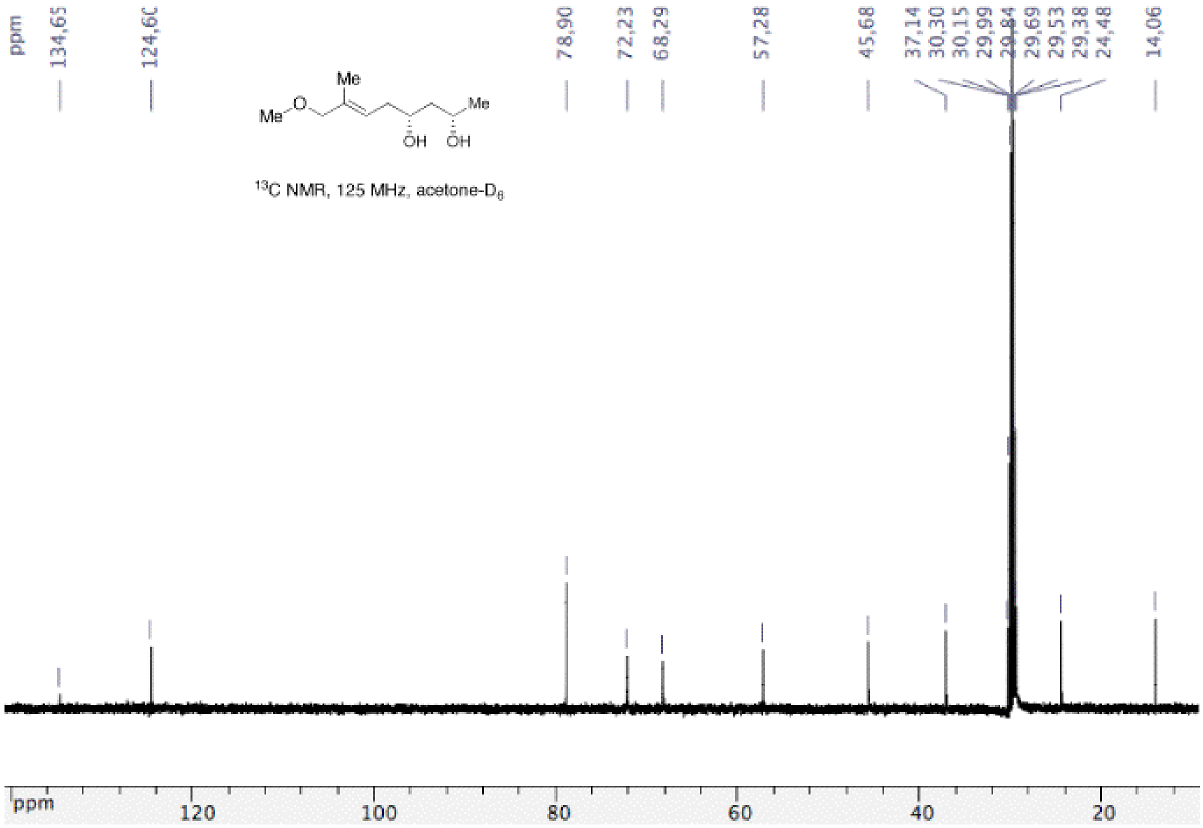


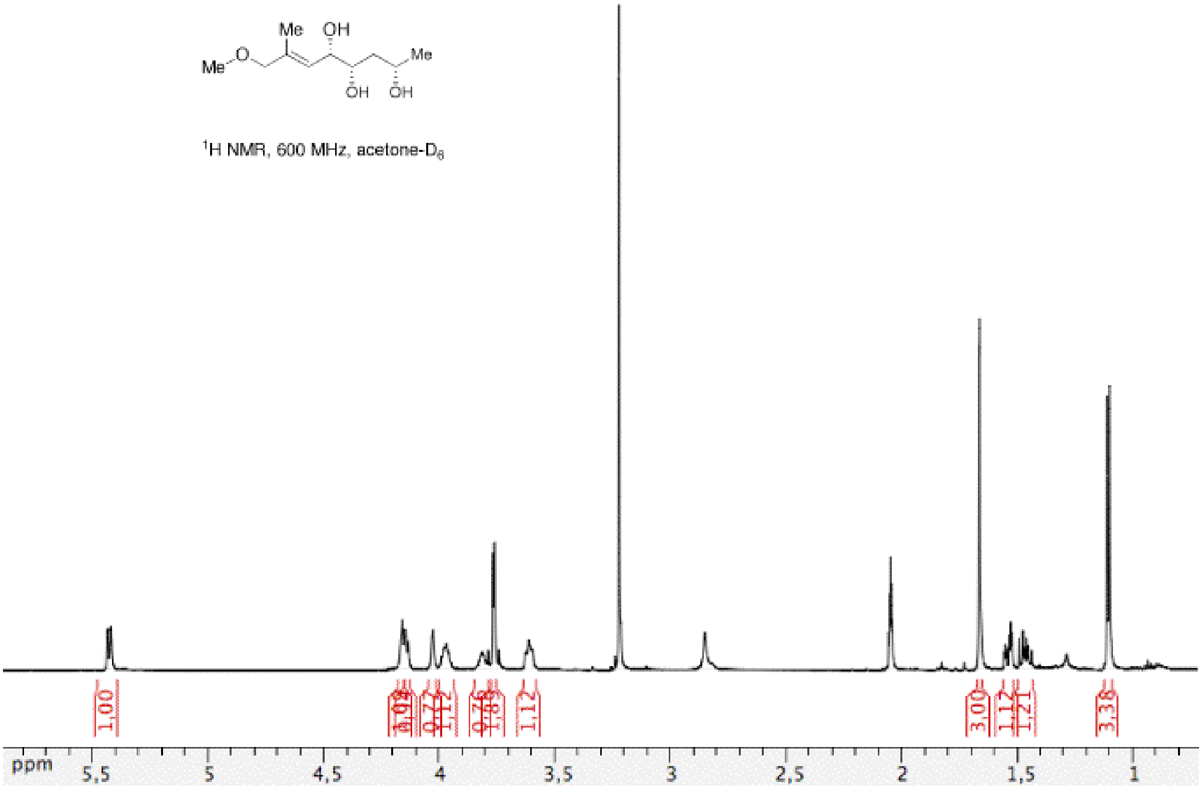


Fluorescence emission spectra were recorded at room temperature with a Perkin Elmer Luminescence Spectrometer L50. Excitation wavelength 365 nm (excitation slit width 5 nm, emission slit width 10 nm). Red: **E1** at 3 µM in *cyclohexane*, λ_{max} 520 nm: Green: **E2** at 3 µM in *cyclohexane*, λ_{max} 520 nm. Blue: **E1** at 3 µM in *methanol*, λ_{max} 425 nm.

10. Selected NMR spectra






Me
OH
Me
O
Me
OH
OH
¹H NMR, 600 MHz, acetone-D6
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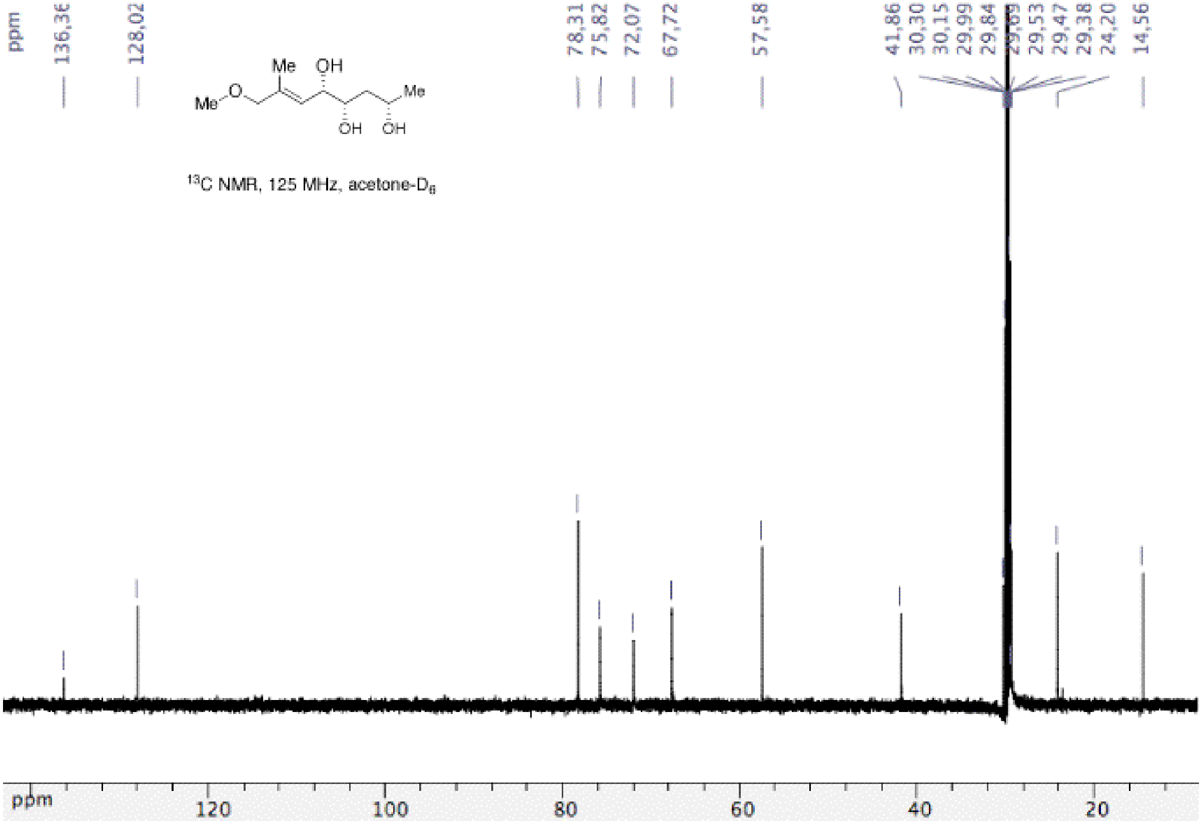
0,72
1,12
0,76
1,80
1,12
3,00
1,12
1,21
3,38
ppm
5,5
5
4,5
4
3,5
3
2,5
2
1,5
1


ppm
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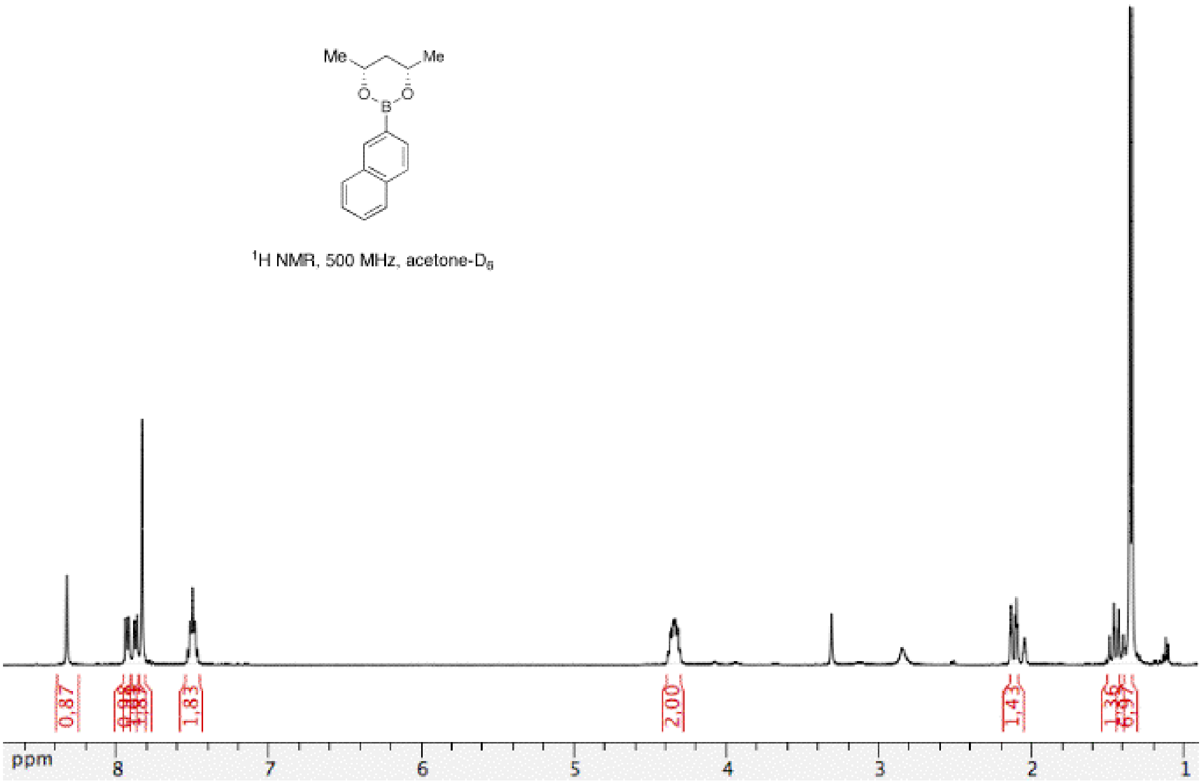
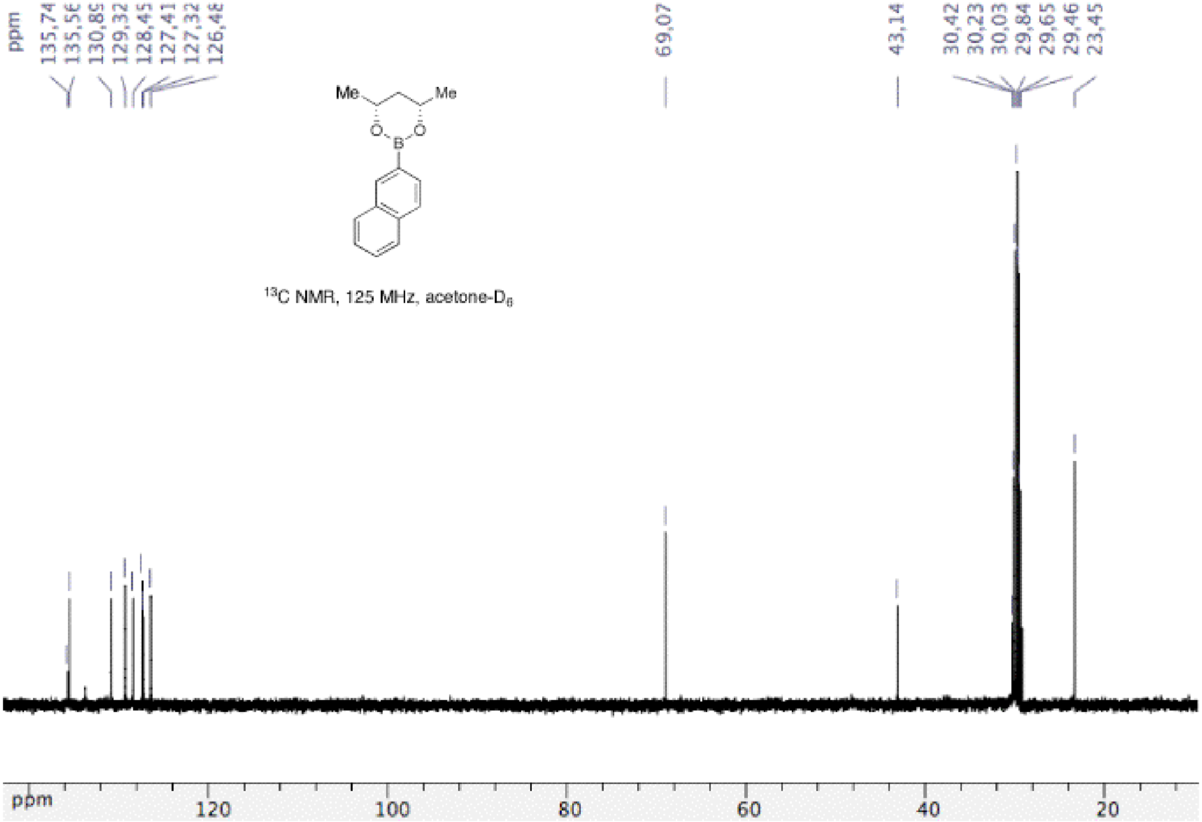
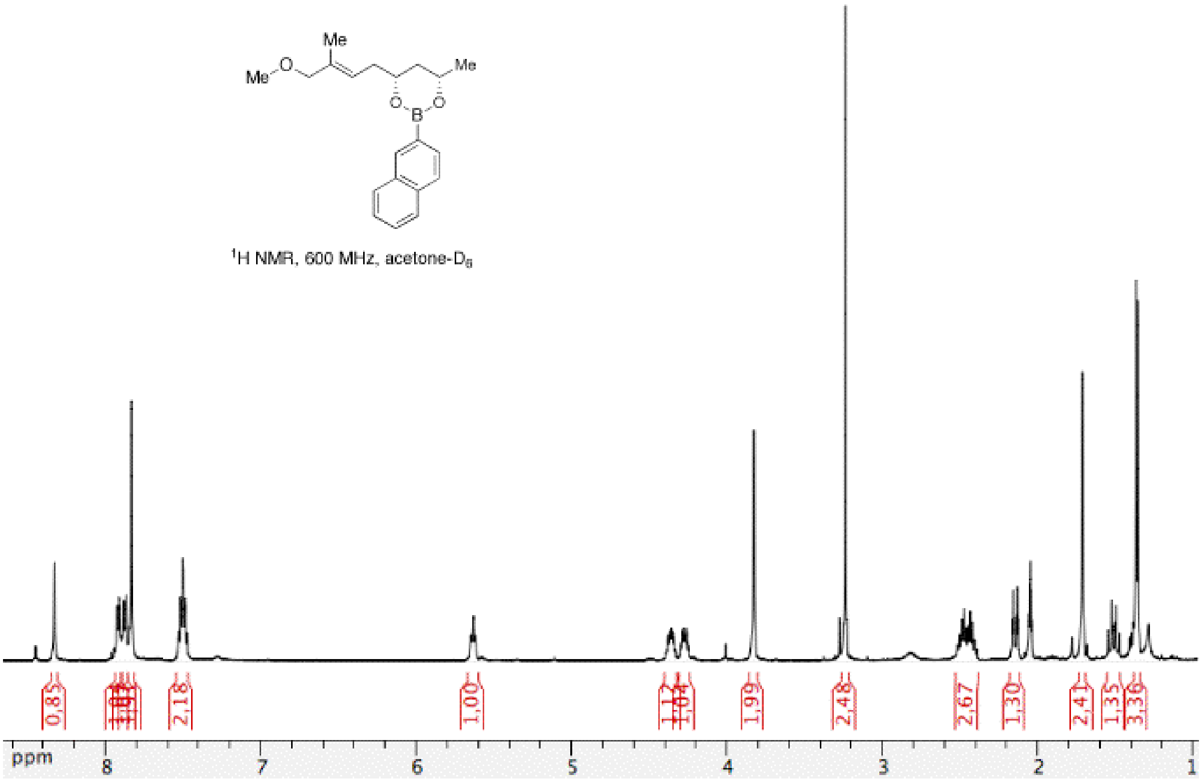
78,31
75,82
72,07
67,72
57,58
41,86
30,30
30,15
29,99
29,84
29,69
29,53
29,47
29,38
24,20
14,56
Me
OH
Me
O
Me
OH
OH
¹³C NMR, 125 MHz, acetone-D6
ppm
120
100
80
60
40
20



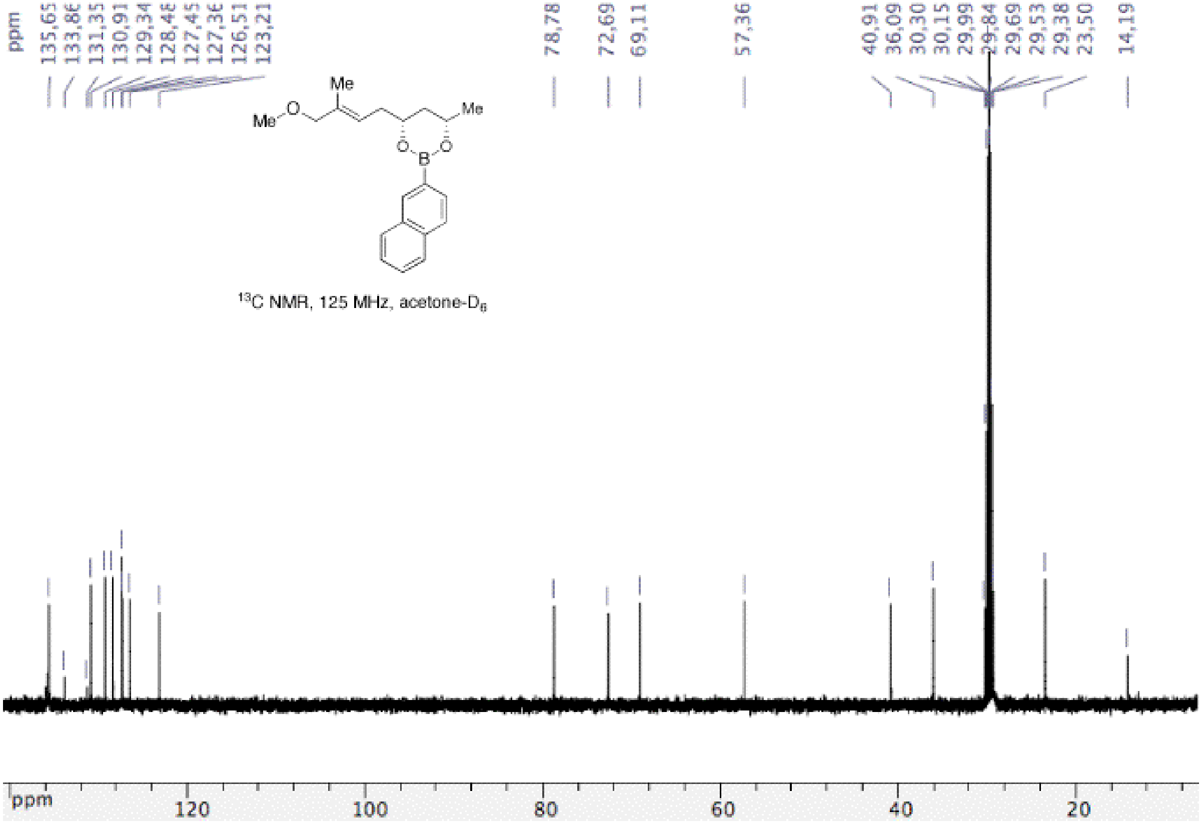
^{1}H NMR, 500 MHz, acetone-D_6



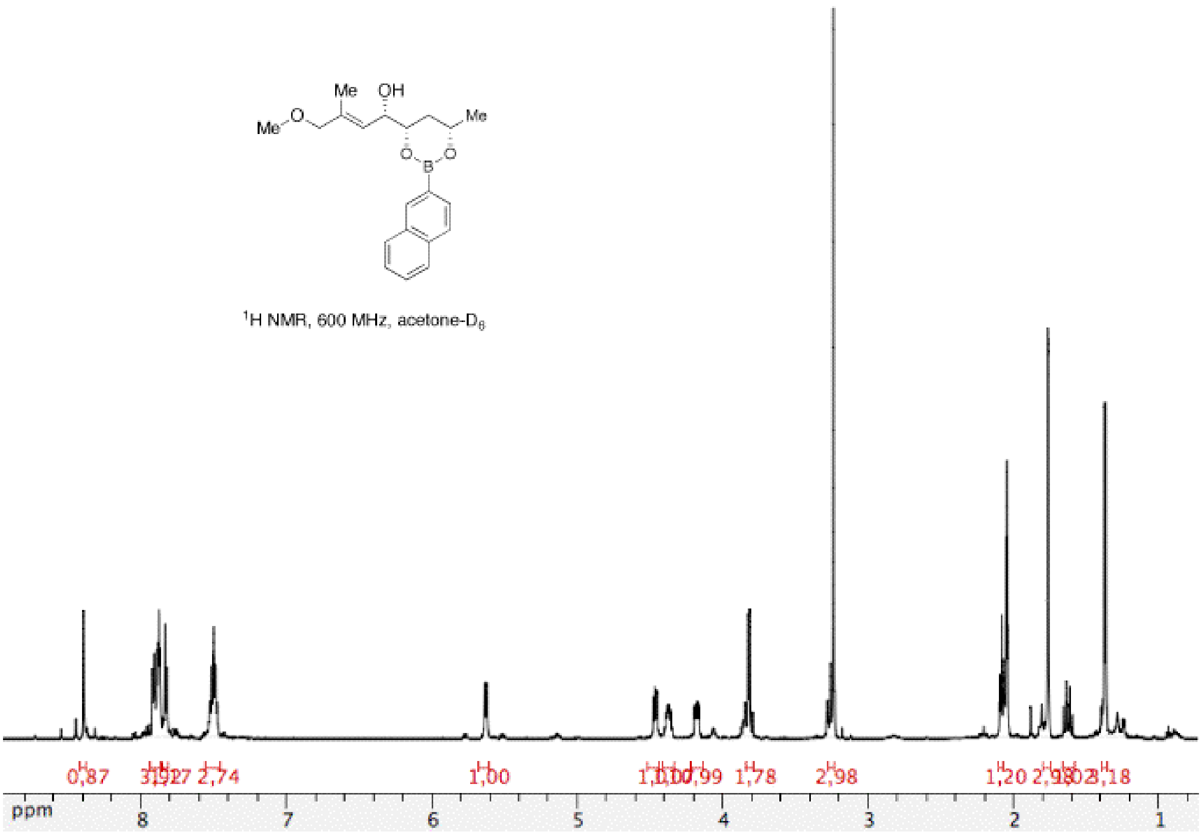
^{13}C NMR, 125 MHz, acetone-D_6

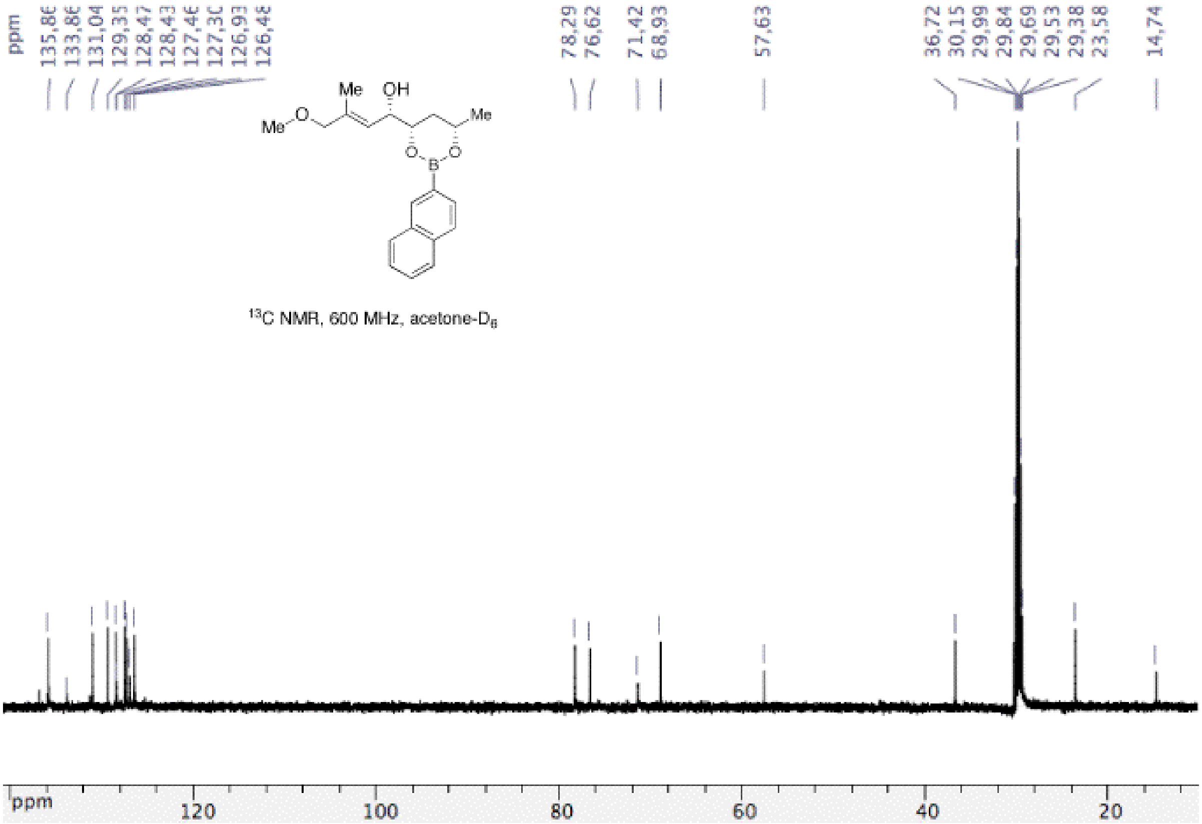


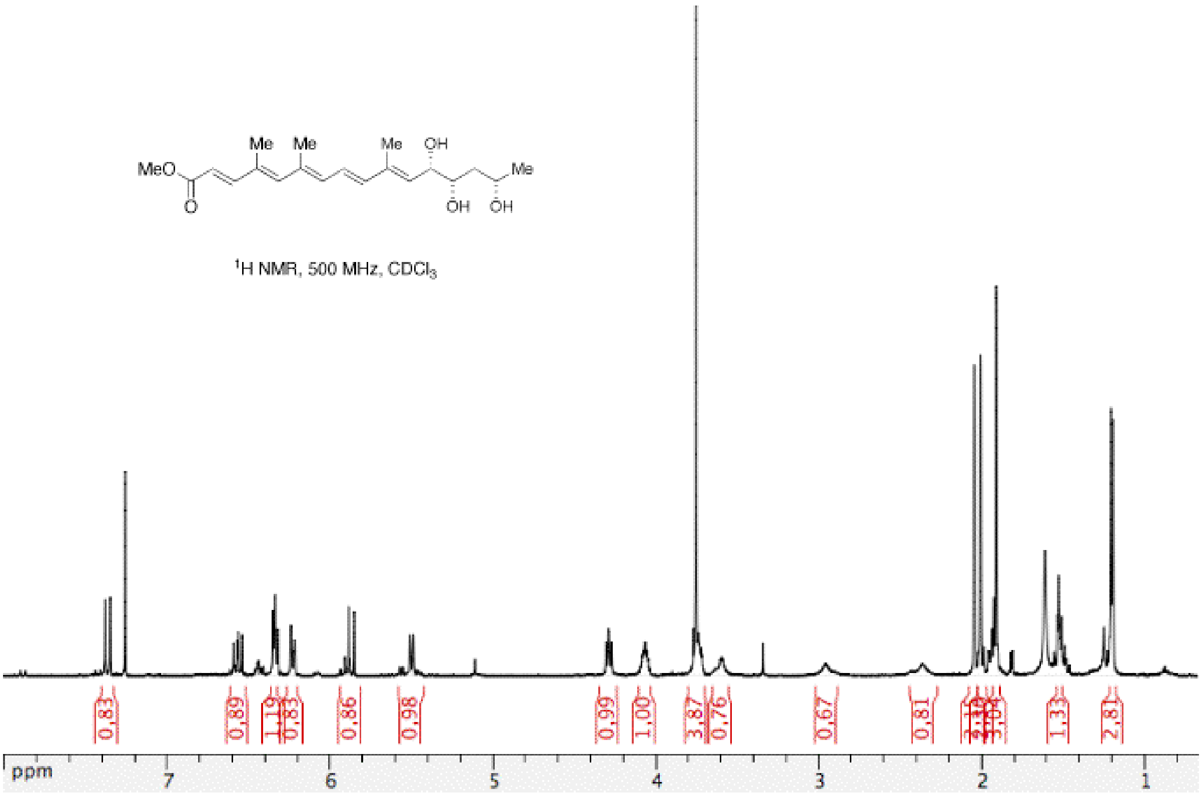
^{1}H NMR, 600 MHz, acetone-D$_6$

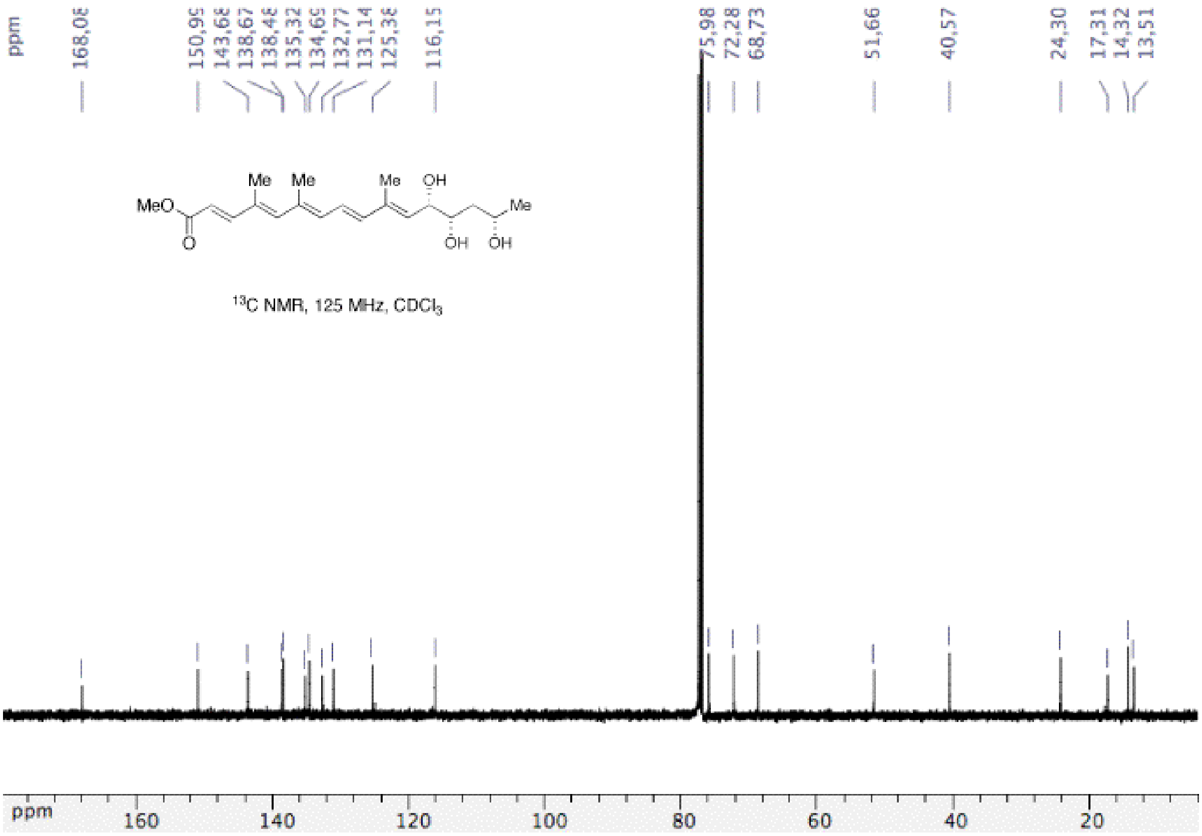


^{13}C NMR, 125 MHz, acetone-D$_6$


^{1}H NMR, 600 MHz, acetone-D$_6$


^{13}C NMR, 600 MHz, acetone-D$_6$


Me
Me
Me
OH
MeO
Me
O
OH
OH
¹H NMR, 500 MHz, CDCl₃
0,83
0,89
1,19
0,83
0,86
0,98
0,99
1,00
3,87
0,76
0,67
0,81
3,04
1,3
2,8
ppm
7
6
5
4
3
2
1


ppm
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143,68
138,67
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132,77
131,14
125,38
116,15
75,98
72,28
68,73
51,66
40,57
24,30
17,31
14,32
13,51
Me
Me
Me
OH
MeO
Me
O
OH
OH
¹³C NMR, 125 MHz, CDCl₃
ppm
160
140
120
100
80
60
40
20