# Supplementary information

## Synthesis of new carolacton derivatives and their activity against biofilms of oral bacteria

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## 1. Supporting information (Chemical Syntheses)

### 1.1 General Information

$^{1}$H NMR spectra were recorded at 400 MHz with a Bruker Avance-400 spectrometer at room temperature. $^{13}$C NMR spectra were recorded at 100 MHz with a Bruker AvanceS-400 spectrometer. Chemical shift values of $^{1}$H and $^{13}$C NMR spectra are reported as values in ppm relative to (residual non deuterated) solvent signal as internal standard.[S1] Multiplicities for $^{1}$H NMR signals are described using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet; where appropriate with the addition of *br* = broad or *p* = pseudo. Coupling constants (*J*) are stated in Hertz (Hz) and were determined after Gaussian multiplication. Multiplicities for $^{13}$C NMR signals refer to the resonances as they would appear in the non-proton-decoupled spectra. Multiplicities are reported using the following abbreviations: s = singlet (due to quaternary carbon), d = doublet (methine), t = triplet (methylene), q = quartet (methyl). Analytical thin-layer chromatography was performed by using precoated silica gel 60 F254 plates (Merck, Darmstadt), and the spots were visualised with UV light at 254 nm or alternatively stained with potassium permanganate solution. Commercially available reagents were used as supplied. Column chromatography was performed with J. T. Baker brand silica gel (40 -60 μm, 60 Å pores). Eluents used for chromatography were distilled prior to use. All reagents were purchased from Aldrich or Acros.

Carolacton derivative **5** was reported by Reck et al. 2011 [S1] and advanced intermediates **8** and **9** were reported before (Schmidt, 2012)[S2]. Carolacton derivative **6** was obtained according to Schmidt 2009 [S3]. Ligand **10** was prepared according to Kishi et al. 2002 [S4] Carolacton derivative **7** was first reported by Jansen **2010** [S5] while derivatives **11a**,**b**, and **12** can be found in Schmidt 2012 [S2].

### 1.2. Analytical data of ketone 12

$[\alpha]_D^{25}$ = -15.1° (c = 0.65, $CH_2Cl_2$); $R_f$ = 0.33 (petroleum ether/ethyl acetate = 3:1); **HRMS** (ESI): *m/z* für $C_{41}H_{64}O_{10}Na$ $[M+Na]^+$: calculated 739.4397, found 739.4377; **$^{1}$H-NMR** ($C_6D_6$, 400 MHz, $C_6D_5H$ = 7.16 ppm); **$^{13}$C-NMR** ($C_6D_6$, 100 MHz, $C_6D_6$ = 128.06 ppm)

| δ-C [ppm] | # | mult. | δ –H [ppm] | mult. | *J* [Hz] |
|---|---|---|---|---|---|
| 204.9 | 9 | s | - | - | - |
| 171.1 | 1 | s | - | - | - |
| 170.1 | 19 | s | - | - | - |
| 159.8 | Ar-PMB | s | - | - | - |
| 143.4 | 7 | d | 6.72 | dd | 10.0, 1.3 |
| 141.6 | 15 | d | 5.60 | ddd | 15.4, 7.5, 1.0 |
| 137.1 | 8 | s | - | - | - |
| 131.4 | Ar-PMB | s | - | - | - |
| 129.5 (2C) | Ar-PMB | d | 7.29 | d, 2H | 8.5 |
| 123.2 | 16 | d | 5.38 | ddd | 15.4, 7.3 |
| 114.2 (2C) | Ar-PMB | d | 6.83 | d, 2H | 8.5 |
| 111.2 | acetonide | s | - | - | - |
| 83.3 | 5 | d | 3.44 | dd | 6.0, 4.2 |
| 80.1 | *tert*-butyl | s | - | - | - |
| 79.8 | 3 | d | 3.74 | ddd | 6.9, 6.5, 4.6 |
| 79.2 | 17 | d | 4.58 | ddd | 7.3, 7.1, 1.0 |
| 78.5 | 18 | d | 4.50 | d | 7.1 |
| 75.1 | PMB-$CH_2$ | t | 4.51 ($H_a$) | d | 10.6 |
| | | | 4.47 ($H_b$) | d | 10.6 |
| 56.7 | 20-OMe | q | 3.27 | s | - |
| 54.8 | PMB-Me | q | 3.31 | s | - |
| 51.0 | Me-ester | q | 3.34 | s | - |
| 40.0 | 4 | d | 2.09-2.02 | m | - |
| 39.3 | 10 | d | 3.14 | q | 6.7 |
| 38.0 | 2 | t | 2.45 ($H_a$) | dd | 15.5, 6.9 |
| | | | 2.39 ($H_b$) | dd | 15.5, 4.6 |
| 37.9 | 6 | d | 2.86 | ddq | 10.0, 6.5, 6.0 |
| 37.1 | 13 | t | 1.20-1.05 | m, 2H | - |
| 36.3 | 14 | d | 1.97-1.89 | m | - |
| 34.6 | 11 | t | 1.85-1.76 ($H_a$) | m | - |
| | | | 1.25-1.15 ($H_b$) | m | - |
| 28.2 | *tert*-butyl | q | 1.42 | s, 9H | - |
| 27.4 | acetonide | q | 1.74 | s | - |
| 25.9 | acetonide | q | 1.28 | s | - |
| 25.5 | 12 | t | 1.25-1.10 | m, 2H | - |
| 20.2 | 25-Me | q | 0.83 | d | 6.8 |
| 18.4 | 24-Me | q | 1.08 | d | 6.7 |
| 17.3 | 22-Me | q | 0.93 | d | 6.5 |
| 12.4 | 23-Me | q | 2.04 | d | 1.3 |
| 10.2 | 21-Me | q | 1.02 | d | 7.2 |

### 1.3 Analytical data of lactone 14

$[\alpha]_D^{19}$ = +1.0° (c = 2.0, $CH_2Cl_2$); $R_f$ = 0.27 (petroleum ether : ethyl acetate = 5:1); **HRMS** (ESI): *m/z* for $C_{40}H_{63}O_9$ $[M+H]^+$: calculated 687.4472, found 687.4476; **$^1$H-NMR** ($C_6D_6$, 400 MHz, $C_6D_5H$ = 7.16 ppm); **$^{13}$C-NMR** ($C_6D_6$, 100 MHz, $C_6D_6$ = 128.06 ppm)

| δ-C [ppm] | # | mult. | δ –H [ppm] | mult. | *J* [Hz] |
|---|---|---|---|---|---|
| 171.43 | 1 | s | - | - | - |
| 170.08 | 19 | s | - | - | - |
| 159.51 | Ar-PMB | s | - | - | - |
| 135.90 | 15 | d | 5.83 | ddd | 15.2, 10.4, 1.7 |
| 132.64 | 8 | s | - | - | - |
| 132.21 | Ar-PMB | s | - | - | - |
| 129.04 (2C) | Ar-PMB | d | 7.41 | d, 2H | 8.7 |
| 127.82 | 7 | d | 5.67 | dd | 9.6, 1.4 |
| 122.30 | 16 | d | 5.41 | dd | 15.2, 1.8 |
| 114.04 (2C) | Ar-PMB | d | 6.90 | d, 2H | 8.7 |
| 111.26 | acetonide | s | - | - | - |
| 83.80 | 5 | d | 3.40 | dd | 5.8, 4.1 |
| 80.26 | 3 | d | 3.83 | ddd | 6.5, 6.5, 5.1 |
| 80.24 | *tert*-butyl | s | - | - | - |
| 79.78 | 18 | d | 4.63 | d | 7.9 |
| 77.16 | 9 | d | 5.51 | *p*s | - |
| 76.34 | 17 | d | 4.60 | ddd | 7.9, 1.8, 1.7 |
| 74.18 | PMB-$CH_2$ | t | 4.72 ($H_a$) | d | 10.9 |
| | | | 4.50 ($H_b$) | d | 10.9 |
| 56.80 | 20-OMe | q | 3.35 | s | - |
| 54.89 | PMB-Me | q | 3.31 | s | - |
| 41.16 | 14 | d | 2.00-1.91 | m | - |
| 39.25 | 4 | d | 2.20-2.10 | m | - |
| 38.05 | 2 | t | 2.47 ($H_a$) | d | 6.5 |
| | | | 2.46 ($H_b$) | d | 5.1 |
| 36.27 | 6 | d | 2.83 | ddq | 9.6, 5.8, 6.7 |
| 35.88 | 10 | d | 1.67-1.58 | m | - |
| 35.85 | 11 | t | 1.63-1.53 ($H_a$) | m | - |
| | | | 1.10-1.00 ($H_b$) | m | - |
| 35.74 | 13 | t | 1.05-0.95 ($H_a$) | m | - |
| | | | 0.78-0.68 ($H_b$) | m | - |
| 28.18 | *tert*-butyl | q | 1.41 | s, 9H | - |
| 27.04 | acetonide | q | 1.66 | s | - |
| 25.31 | acetonide | q | 1.20 | s | - |
| 24.48 | 12 | t | 1.18-0.95 | m, 2H | - |
| 22.16 | 25-Me | | 0.92 | d | 6.8 |
| 18.15 | 22-Me | q | 0.99 | d | 6.7 |
| 16.37 | 24-Me | q | 0.96 | d | 6.5 |
| 14.38 | 23-Me | q | 1.65 | d | 1.4 |
| 10.16 | 21-Me | q | 1.01 | d | 6.8 |

### 1.4 Analytical data of lactone 15

$[\alpha]_D^{19}$ = -17.9° (c = 0.34, $CH_2Cl_2$); $R_f$ = 0.28 (petroleum ether : ethyl acetate = 5:1); **HRMS** (ESI): *m/z* für $C_{40}H_{63}O_9$ $[M+H]^+$: calculated 687.4472, found 687.4476; **$^1$H-NMR** ($C_6D_6$, 400 MHz, $C_6D_5H$ = 7.16 ppm); **$^{13}$C-NMR** ($C_6D_6$, 100 MHz, $C_6D_6$ = 128.06 ppm)

| δ-C [ppm] | # | Mult. | δ –H [ppm] | Mult. | *J* [Hz] |
|---|---|---|---|---|---|
| 171.76 | 1 | s | - | - | - |
| 169.03 | 19 | s | - | - | - |
| 159.69 | Ar-PMB | s | - | - | - |
| 140.69 | 9 | d | 5.46 | ddd | 15.5, 6.1, 1.1 |
| 140.61 | 15 | d | 5.88 | ddd | 15.5, 7.0, 1.4 |
| 131.31 | Ar-PMB | s | - | - | - |
| 130.79 | 8 | s | - | - | - |
| 129.20 (2C) | Ar-PMB | d | 7.25 | d, 2H | 8.9 |
| 121.43 | 16 | d | 5.43 | ddd | 9.9, 1.4 |
| 114.15 (2C) | Ar-PMB | d | 6.80 | d, 2H | 8.9 |
| 111.15 | acetonide | s | - | - | - |
| 83.13 | 7 | d | 5.57 | d | 10.5 |
| 81.94 | 3 | d | 3.75 | ddd | 7.9, 5.1, 3.8 |
| 79.72 | *tert*-butyl | s | - | - | - |
| 79.31 | 17 | d | 4.65 | ddd | 6.4, 6.1, 1.4 |
| 78.58 | 5 | d | 3.40 | dd | 4.4, 1.4 |
| 78.21 | 18 | d | 4.53 | d | 6.4 |
| 71.45 | PMB-$CH_2$ | t | 4.48 ($H_a$) | d | 11.1 |
| | | | 4.23 ($H_b$) | d | 11.1 |
| 57.07 | 20-OMe | q | 3.28 | s | - |
| 54.78 | PMB-Me | q | 3.30 | s | - |
| 38.20 | 2 | t | 2.58 ($H_a$) | dd | 15.7, 3.8 |
| | | | 2.52 ($H_b$) | dd | 15.7, 7.9 |
| 37.85 | 11 | t | 1.52-1.45 ($H_a$) | m | - |
| | | | 1.05-0.98 ($H_b$) | m | - |
| 37.07 | 14 | d | 2.02-1.94 | m | - |
| 36.98 | 6 | d | 2.55-2.48 | m | - |
| 35.94 | 4 | d | 2.19-2.12 | m | - |
| 35.28 | 13 | t | 1.35-1.20 | m, 2H | - |
| 32.48 | 10 | d | 2.37-2.28 | m | - |
| 28.17 | *tert*-butyl | q | 1.40 | s, 9H | - |
| 27.38 | acetonide | q | 1.82 | s | - |
| 26.28 | acetonide | q | 1.34 | s | - |
| 24.99 | 12 | t | 1.52-1.45 ($H_a$) | m | - |
| | | | 1.05-0.98 ($H_b$) | m | - |
| 21.88 | 25-Me | | 0.95 | d | 6.8 |
| 21.49 | 24-Me | q | 1.06 | d | 6.5 |
| 12.18 | 23-Me | q | 1.64 | d | 1.4 |
| 11.86 | 22-Me | q | 1.09 | d | 6.8 |
| 9.66 | 21-Me | q | 1.08 | d | 7.0 |

### 1.5 Analytical data of alcohol S1

$[\alpha]_D^{25}$ = -5.8° (c= 1.2, $CH_2Cl_2$); $R_f$ = 0.14 (petroleum ether : ethyl acetate = 5:1); **HRMS** (ESI): *m/z* for $C_{32}H_{54}O_8Na$ $[M+Na]^+$: calculated 589.3716, found 589.3712; **$^1$H-NMR** ($C_6D_6$, 400 MHz, $C_6D_5H$ = 7.16 ppm); **$^{13}$C-NMR** ($C_6D_6$, 100 MHz, $C_6D_6$ = 128.06 ppm)

| δ-C [ppm] | # | mult. | δ –H [ppm] | mult. | *J* [Hz] |
|---|---|---|---|---|---|
| 171.16 | 1 | s | - | - | - |
| 168.97 | 19 | s | - | - | - |
| 140.62 | 15 | d | 5.85 | ddd | 15.6, 7.2, 0.9 |
| 139.10 | 9 | d | 5.37 | dd | 10.4. 1.0 |
| 133.19 | 8 | s | - | - | - |
| 121.58 | 16 | d | 5.40 | ddd | 15.6, 5.9, 0.4 |
| 110.99 | acetonide | s | - | - | - |
| 83.57 | 7 | d | 5.55 | d | 8.9 |
| 82.94 | 3 | d | 3.76-3.72 | m | - |
| 79.96 | *tert*-butyl | s | - | - | - |
| 79.28 | 17 | d | 4.62 | ddd | 6.8, 5.9, 0.9 |
| 78.14 | 18 | d | 4.49 | d | 6.8 |
| 71.46 | 5 | d | 3.79-3.74 | m | - |
| - | 5-OH | - | 2.64 | d | 2.4 |
| 58.36 | 20-OMe | q | 3.17 | s | - |
| 39.93 | 6 | d | 2.25-2.17 | m | - |
| 39.30 | 2 | t | 2.45 ($H_a$) | dd | 15.9, 7.3 |
| | | | 2.40 ($H_b$) | dd | 15.9, 5.3 |
| 38.15 | 4 | d | 1.78-1.72 | m | - |
| 37.65 | 11 | t | 1.38-1.33 ($H_a$) | m | - |
| | | | 0.90-0.85 ($H_b$) | m | - |
| 36.90 | 14 | d | 2.00-1.92 | m | - |
| 35.25 | 13 | t | 1.22-1.07 | m, 2H | - |
| 32.25 | 10 | d | 2.30-2.21 | m | - |
| 28.13 | *tert*-butyl | q | 1.39 | s, 9H | - |
| 27.42 | acetonide | q | 1.79 | s | - |
| 26.23 | acetonide | q | 1.32 | s | - |
| 24.79 | 12 | t | 0.98-0.85 | m, 2H | - |
| 21.59 | 25-Me | q | 0.93 | d | 7.2 |
| 21.20 | 24-Me | q | 0.89 | d | 6.5 |
| 12.83 | 22-Me | q | 0.91 | d | 7.2 |
| 12.66 | 23-Me | q | 1.66 | d | 1.0 |
| 10.45 | 21-Me | q | 0.97 | d | 6.8 |

### 1.6 Analytical data of ketone S2

$[\alpha]_D^{27}$ = -55.5° (c = 0.75, $CH_2Cl_2$); $R_f$ = 0.26 (petroleum ether : ethyl acetate = 5:1); **HRMS** (ESI): *m/z* for $C_{32}H_{52}O_8Na$ $[M+Na]^+$: calculated 587.3560, found 587.3569; **$^1$H-NMR** ($C_6D_6$, 400 MHz, $C_6D_5H$ = 7.16 ppm); **$^{13}$C-NMR** ($C_6D_6$, 100 MHz, $C_6D_6$ = 128.06 ppm).

| δ-C [ppm] | # | mult. | δ –H [ppm] | mult. | *J* [Hz] |
|---|---|---|---|---|---|
| 212.41 | 5 | s | - | - | - |
| 170.38 | 1 | s | - | - | - |
| 168.56 | 19 | s | - | - | - |
| 140.77 | 15 | d | 5.87 | ddd | 15.6, 7.1, 1.2 |
| 140.68 | 9 | d | 5.34 | dd | 9.9, 1.4 |
| 129.43 | 8 | s | - | - | - |
| 121.43 | 16 | d | 5.38 | ddd | 15.6, 6.1, 1.1 |
| 111.10 | acetonide | s | - | - | - |
| 82.03 | 7 | d | 5.83 | d | 10.4 |
| 80.16 | *tert*-butyl | s | - | - | - |
| 80.04 | 3 | d | 3.85 | ddd | 7.3, 6.5, 4.0 |
| 79.23 | 17 | d | 4.63 | ddd | 6.4, 6.1, 1.2 |
| 78.07 | 18 | d | 4.51 | d | 6.4 |
| 57.80 | 20-OMe | q | 3.15 | s | - |
| 50.46 | 4 | d | 2.89 | dq | 7.3, 6.9 |
| 49.08 | 6 | d | 3.19-3.14 | m | - |
| 37.77 | 2 | t | 2.37 ($H_a$) | dd | 15.3, 4.0 |
| | | | 2.26 ($H_b$) | dd | 15.3, 6.5 |
| 37.38 | 11 | t | 1.28-1.22 ($H_a$) | m | - |
| | | | 0.82-0.76 ($H_b$) | m | - |
| 37.13 | 14 | d | 1.99-1.90 | m | - |
| 35.20 | 13 | t | 1.15-1.05 | m, 2H | - |
| 32.38 | 10 | d | 2.19-2.10 | m | - |
| 28.10 | *tert*-butyl | q | 1.38 | s, 9H | - |
| 27.40 | acetonide | q | 1.80 | s | - |
| 26.29 | acetonide | q | 1.33 | s | - |
| 24.93 | 12 | t | 1.22-1.17 ($H_a$) | m | - |
| | | | 0.91-0.85 ($H_b$) | m | - |
| 21.39 | 25-Me | q | 0.93 | d | 6.9 |
| 21.06 | 24-Me | q | 0.82 | d | 6.5 |
| 13.67 | 22-Me | q | 1.17 | d | 6.9 |
| 12.15 | 21-Me | q | 0.89 | d | 6.9 |
| 12.05 | 23-Me | q | 1.58 | d | 1.4 |

### 1.7 Analytical data of Carolacton derivative 2

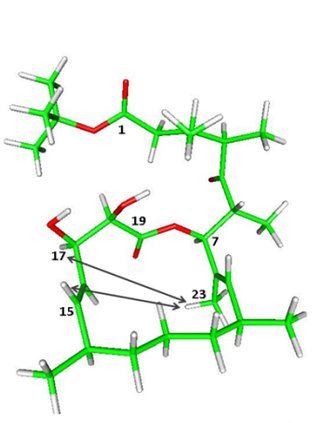
$[\alpha]_D^{25}$ = -24.3° (c = 0.21, $CH_2Cl_2$); $R_f$ = 0.16 (petroleum ether : ethyl acetate = 2:1); **HRMS** (ESI): *m/z* for $C_{29}H_{48}O_8Na$ $[M+Na]^+$: calculated 547.3247, found 547.3248; **$^1$H-NMR** ($C_6D_6$, 400 MHz, $C_6D_5H$ = 7.16 ppm); **$^{13}$C-NMR** ($C_6D_6$, 100 MHz, $C_6D_6$ = 128.06 ppm)

| δ-C [ppm] | # | mult. | δ –H [ppm] | mult. | *J* [Hz] |
|---|---|---|---|---|---|
| 212.27 | 5 | s | - | - | - |
| 171.85 | 19 | s | - | - | - |
| 170.33 | 1 | s | - | - | - |
| 141.10 | 9 | d | 5.20 | dd | 10.3, 1.4 |
| 139.80 | 15 | d | 5.54 | ddd | 15.7, 5.5, 0.5 |
| 128.61 | 8 | s | - | - | - |
| 124.60 | 16 | d | 5.34 | ddd | 15.7, 7.5, 1.4 |
| 83.38 | 7 | d | 5.68 | d | 10.8 |
| 80.25 | *tert*-butyl | s | - | - | - |
| 79.99 | 3 | d | 3.84 | ddd | 7.7, 6.5, 4.2 |
| 74.87 | 17 | d | 4.32-4.25 | m | - |
| 73.88 | 18 | d | 4.36-4.31 | m | - |
| 57.77 | 20-OMe | q | 3.17 | s | - |
| 50.61 | 4 | d | 2.87 | dq | 7.7, 7.0 |
| 48.46 | 6 | d | 3.07 | dq | 10.8, 6.7 |
| 37.63 | 2 | t | 2.35 ($H_a$) | dd | 15.4, 4.2 |
| | | | 2.25 ($H_b$) | dd | 15.4, 6.5 |
| 36.47 | 11 | t | 1.16-1.10 ($H_a$) | m | - |
| | | | 0.98-0.89 ($H_b$) | m | - |
| 35.25 | 14 | d | 2.16-2.08 | m | - |
| 34.35 | 13 | t | 1.27-1.17 ($H_b$) | m | - |
| | | | 1.02-0.94 ($H_b$) | m | - |
| 30.93 | 10 | d | 2.25-2.15 | m | - |
| 28.10 | *tert*-butyl | q | 1.39 | s, 9H | - |
| 21.63 | 12 | t | 1.08-0.99 ($H_a$) | m | - |
| | | | 0.85-0.79 ($H_b$) | m | - |
| 21.04 | 24-Me | q | 0.78 | d | 6.5 |
| 17.23 | 25-Me | q | 0.92 | d | 6.8 |
| 13.69 | 22-Me | q | 1.08 | d | 6.7 |
| 12.16 | 21-Me | q | 0.87 | d | 7.0 |
| 11.79 | 23-Me | q | 1.46 | d | 1.4 |

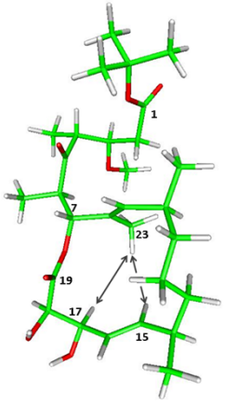
The key stereogenic center at C7 in carolacton derivative **2** was generated by macrolactonisation under Mitsunobu conditions. To verify the configuration at C7, NOE-experiments were carried out and data were compared with the inter-atomic distances of the relevant groups of both possible diastereomers. The most stable conformations of both diastereomers were obtained by molecular modeling. NMR-measurements showed NOE-contacts of protons 17-H and 15-H with 23-Me. These results indicate, that the stereogenic center at C7 is (*R*)-configured because the interatomic distances of the above mentioned protons is significantly shorter than 4Å, compared to > 4Å determined for the (*S*)-diastereomer. **$^1$H-NMR** ($CDCl_3$, 500 MHz, $CHCl_3$ = 7.26 ppm):

| # | δ –H [ppm] | mult. | *J* [Hz] | NOE |
|---|---|---|---|---|
| 1 | - | - | - | |
| 2 | 2.44 ($H_a$) | dd | 15.3, 4.1 | |
| | 2.31 ($H_b$) | dd | 15.3, 6.8 | |
| 3 | 3.76 | ddd | 7.7, 6.8, 4.1 | |
| 4 | 2.91 | dq | 7.7, 7.0 | |
| 5 | - | - | - | |
| 6 | 3.25 | dq | 10.8, 6.7 | |
| 7 | 5.42 | d | 10.8 | |
| 8 | - | - | - | |
| 9 | 5.17 | d | 10.5, 1.0 | |
| 10 | 3.84 | ddd | 7.7, 6.5, 4.2 | |
| 11 | 1.35-1.28 ($H_a$) | m | - | |
| | 1.16-1.10 ($H_b$) | m | - | |
| 12 | 1.19-1.12 ($H_a$) | m | - | |
| | 0.82-0.73 ($H_b$) | m | - | |
| 13 | 1.40-1.32 ($H_a$) | m | - | |
| | 1.21-1.13 ($H_b$) | m | - | |
| 14 | 2.91 | dq | 7.7, 7.0 | |
| 15 | 5.65 | dd | 15.7, 5.1 | 23 |
| 16 | 5.35 | dd | 15.7, 7.9 | |
| 17 | 4.27 | dd | 7.9, 2.6 | |
| 18 | 4.39 | d | 2.6 | |
| 18-OH | 3.02 | s | - | |
| 19 | - | - | - | |
| 20-OMe | 3.31 | s | - | |
| 21-Me | 0.90 | d | 7.0 | |
| 22-Me | 1.09 | d | 6.7 | |
| 23-Me | 1.66 | d | 1.0 | 15, 17 |
| 24-Me | 0.84 | d | 6.6 | |
| 25-Me | 0.94 | d | 6.9 | |
| t-Bu | 1.46 | s, 9H | - | |

| NOE contacts | distances in Å | |
|---|---|---|
| | (*R*)-epimer | (*S*)-epimer |
| 17-H <-> 23-Me | 2.99 | >4.0 |
| 15-H <-> 23-Me | 2.24 | >4.0 |




*(7S)*-diastereomer




*(7R)*-diastereomer

### 1.8 Analytical data of of ketone 16

$[\alpha]_D^{25}$ = -67.3° (c = 0.15, $CH_2Cl_2$); $R_f$ = 0.14 (petroleum ether : ethyl acetate = 20:1); **HRMS** (ESI): *m/z* for $C_{28}H_{44}O_8Na$ $[M+Na]^+$: calculated 531.2934, found 531.2939; **$^1$H-NMR** ($CDCl_3$, 500 MHz, $CHCl_3$ = 7.26 ppm); **$^{13}$C-NMR** ($CDCl_3$, 125 MHz, $CDCl_3$ = 77.16 ppm)

| δ-C [ppm] | # | mult. | δ –H [ppm] | mult. | *J* [Hz] |
|---|---|---|---|---|---|
| 213.63 | 5 | s | - | - | - |
| 172.60 | 1 | s | - | - | - |
| 170.26 | 19 | s | - | - | - |
| 136.18 | 15 | d | 5.66 | ddd | 15.2, 10.4, 1.9 |
| 135.80 | 8 | s | - | - | - |
| 122.20 | 16 | d | 5.38 | dd | 15.2, 2.1 |
| 121.51 | 7 | d | 5.06 | d | 10.1 |
| 111.49 | acetonide | s | - | - | - |
| 80.23 | 3 | d | 3.75 | ddd | 8.8 , 5.5, 4.1 |
| 79.74 | 9 | d | 5.23 | *ps* | - |
| 76.78 | 18 | d | 4.73 | d | 8.0 |
| 76.05 | 17 | d | 4.95 | dd | 8.0, 2.1 |
| 58.34 | 20-OMe | q | 3.33 | s | - |
| 47.84 | 6 | d | 3.48 | dq | 10.1, 6.7 |
| 46.94 | 4 | d | 3.04 | dq | 8.4, 6.9 |
| 40.92 | 14 | d | 2.10-2.01 | m | - |
| 35.40[a] | 10 | d | 1.76-1.66 | m | - |
| 35.35[a] | 13 | d | 1.70-1.60 ($H_a$) | m | - |
| | | | 0.88-0.80 ($H_b$) | m | - |
| 35.30[a] | 11 | t | 1.66-1.24 | m, 2H | - |
| 34.68 | 2 | t | 2.69 ($H_a$) | dd | 15.9, 4.1 |
| | | | 2.46 ($H_b$) | dd | 15.9, 5.5 |
| 26.77 | acetonide | q | 1.61 | s | - |
| 25.33 | acetonide | q | 1.42 | s | - |
| 24.25 | 12 | t | 1.60-1.00 | m, 2H | - |
| 22.10 | 25-Me | | 0.96 | d | 6.4 |
| 15.60 | 22-Me | q | 1.08 | d | 6.7 |
| 15.49 | 24-Me | q | 0.78 | d | 6.9 |
| 14.80 | 23-Me | q | 1.69 | *ps* | - |
| 13.11 | 21-Me | q | 1.95 | d | 6.9 |

a) The assignment of protons at 10-H, 13-H and 11-H was not possible.

**1.9 Analytical data of** bislactone **17**

$[\alpha]_D^{20}$ = -40.0° (c = 0.13, $CH_2Cl_2$); $R_f$ = 0.66 (petroleum ether : ethyl acetate = 20:1); **HRMS** (ESI): *m/z* for $C_{28}H_{44}O_7Na$ $[M+Na]^+$: calculated 515.2985, found 515.2990; **$^1$H-NMR** ($C_6D_6$, 500 MHz, $C_6D_5H$ = 7.16 ppm); **$^{13}$C-NMR** ($C_6D_6$, 125 MHz, $C_6D_6$ = 128.06 ppm)

| δ-C [ppm][a] | # | mult. | δ –H [ppm] | mult. | *J* [Hz] |
|---|---|---|---|---|---|
| 168.40 | 1 | s | - | - | - |
| 168.00 | 19 | s | - | - | - |
| 141.27 | 15 | d | 6.00 | dd | 15.2, 10.6 |
| 133.35 | 8 | s | - | - | - |
| 125.89 | 7 | d | 5.25 | d | 9.6 |
| 123.37 | 16 | d | 5.64 | dd | 15.2 |
| 114.25 | acetonide | s | - | - | - |
| 112.05 | 18 | s | - | - | - |
| 84.05 | 17 | d | 5.03 | d | 2.0 |
| 83.08 | 5 | d | 3.12 | dd | 10.0, 1.7 |
| 77.37 | 9 | d | 5.46 | *br*s | - |
| 75.99 | 3 | d | 2.80 | ddd | 10.5, 6.9, 4.3 |
| 55.28 | 20-OMe | q | 2.82 | s | - |
| 40.74 | 14 | d | 1.99-1.89 | m | - |
| 35.75 | 11 | t | 1.47-1.41 ($H_a$) | m | - |
| | | | 1.10-1.01 ($H_b$) | m | - |
| 35.38 | 13 | t | 1.56-1.50 ($H_a$) | m | - |
| | | | 0.74-0.67 ($H_b$) | m | - |
| 34.37 | 10 | d | 1.71-1.65 | m | - |
| 33.93 | 6 | d | 2.62-2.54 | m | - |
| 33.37 | 2 | t | 2.55 ($H_a$) | dd | 18.0, 6.9 |
| | | | 2.24 ($H_b$) | dd | 18.0, 10.5 |
| 31.05 | 4 | d | 1.85-1.79 | m | - |
| 28.13 | acetonide | q | 1.64 | s | - |
| 27.30 | acetonide | q | 1.47 | s | - |
| 24.17 | 12 | t | 1.20-1.12 ($H_a$) | m | - |
| | | | 1.10-1.01 ($H_b$) | m | - |
| 22.04 | 25-Me | q | 0.90 | d | 6.6 |
| 15.99 | 22-Me | q | 0.60 | d | 6.6 |
| 15.61 | 24-Me | q | 1.26 | d | 6.9 |
| 15.08 | 23-Me | q | 1.61 | s | - |
| 3.48 | 21-Me | q | 0.61 | d | 7.0 |

### 1.10 Analytical data of 9-*epi*-Carolacton 3

$[\alpha]_D^{19}$ = -43.0° (c = 0.03, $CH_2Cl_2$); $R_f$ = 0.36 ($CH_2Cl_2$ : MeOH = 10:1); **HRMS** (ESI): *m/z* for $C_{25}H_{40}O_8Na$ $[M+Na]^+$: calculated 491.2621, found 491.2618; **$^1$H-NMR** ($CDCl_3$, 500 MHz, $CHCl_3$ = 7.26 ppm)

| δ-C [ppm][a] | # | mult. | δ –H [ppm] | mult. | *J* [Hz] |
|---|---|---|---|---|---|
| 213.3 | 5 | s | - | - | - |
| 171.8 | 1 | s | - | - | - |
| 169.1 | 19 | s | - | - | - |
| 134.5 | 8 | s | - | - | - |
| 138.3 | 15 | d | 5.75 | dd | 15.8, 7.0 |
| 124.2 | 16 | d | 5.50 | dd | 15.8, 5.9 |
| 124.1 | 7 | d | 5.14 | *p*d | 10.2 |
| 80.0 | 3 | d | 3.73 | ddd | 9.0, 4.7, 4.3 |
| 79.8 | 9 | d | 5.20 | d | 2.9 |
| 75.5 | 18 | d | 4.52 | s | - |
| 73.8 | 17 | d | 4.52 | d | 5.9 |
| 58.0 | 20-OMe | q | 3.34 | s | - |
| 47.6 | 6 | d | 3.49 | dq | 10.2, 6.7 |
| 46.3 | 4 | d | 3.13 | dq | 9.0, 7.0 |
| 35.8 | 14 | d | 2.36-2.30 | m | - |
| 34.7 | 2 | d | 2.71 ($H_a$) | dd | 15.6, 4.3 |
| | | | 2.47 ($H_b$) | dd | 15.6, 4.7 |
| 34.6 | 10 | d | 1.88-1.82 | m | - |
| 33.1 | 11 | t | 1.48-1.19 | m, 2H | - |
| 33.1 | 13 | t | 1.71-1.62 ($H_a$) | m | - |
| | | | 1.18-1.12 ($H_b$) | m | - |
| 23.6 | 12 | t | 1.39-1.14 | m, 2H | - |
| 20.1 | 25-Me | q | 1.06 | d | 6.9 |
| 15.7 | 22-Me | q | 1.11 | d | 6.7 |
| 15.6 | 24-Me | q | 0.80 | d | 7.0 |
| 14.5 | 23-Me | q | 1.71 | *p*s | - |
| 13.4 | 21-Me | q | 0.98 | d | 7.0 |

a) Due to the small amount of material available, the chemical shifts of carbon atoms were determined by HSQC and HMBC experiments.

### 1.11 Analytical data of δ-lactone **4**

$[\alpha]_D^{20}$ = -8.8° (c = 0.08, $CH_2Cl_2$); $R_f$ = 0.15 (petroleum ether : ethyl acetate = 1:1); **HRMS** (ESI): *m/z* for $C_{25}H_{40}O_7Na$ $[M+Na]^+$: calculated 475.2672, found 475.2660; **$^1$H-NMR** ($C_6D_6$, 500 MHz, $C_6D_5H$ = 7.16 ppm); **$^{13}$C-NMR** ($C_6D_6$, 125 MHz, $C_6D_6$ = 128.06 ppm)

| δ-C [ppm][a] | # | mult. | δ –H [ppm] | mult. | *J* [Hz] |
|---|---|---|---|---|---|
| 171.99 | 19 | s | - | - | - |
| 168.53 | 1 | s | - | - | - |
| 134.30 | 8 | s | - | - | - |
| 133.96 | 15 | d | 5.48 | ddd | 15.3, 10.3, 2.1 |
| 133.08 | 7 | d | 5.41 | *p*d | 9.5 |
| 126.60 | 16 | d | 5.25 | dd | 15.3, 2.2 |
| 83.93 | 9 | d | 5.00 | d | 11.4 |
| 83.32 | 5 | d | 3.20 | dd | 9.9, 1.9 |
| 75.89 | 3 | d | 2.82 | ddd | 10.6, 6.7, 4.0 |
| 73.91 | 18 | d | 4.09 | *br*s | - |
| - | 18-OH | - | 3.65 | *br*s | - |
| 73.26 | 17 | d | 4.36 | *br*s | - |
| - | 17-OH | - | 2.33 | *br*s | - |
| 55.28 | 20-OMe | q | 2.80 | s | - |
| 35.99 | 14 | d | 2.23-2.16 | m | - |
| 34.96 | 13 | t | 1.28-1.10 | m, 2H | - |
| 34.00 | 6 | d | 2.58-2.50 | m | - |
| 33.32 | 10 | d | 2.13-2.04 | m | - |
| 33.19 | 2 | t | 2.50 ($H_a$) | dd | 18.1, 6.7 |
| | | | 2.19 ($H_b$) | dd | 18.1, 10.6 |
| 31.18 | 4 | d | 1.82-1.88 | m | - |
| 28.67 | 11 | t | 1.79-1.71 ($H_a$) | m | - |
| | | | 0.91-0.84 ($H_b$) | m | - |
| 22.03 | 25-Me | q | 0.95 | d | 6.5 |
| 19.10 | 12 | t | 1.28-1.19 ($H_a$) | m | - |
| | | | 1.07-0.99 ($H_b$) | m | - |
| 15.80 | 22-Me | q | 0.64 | d | 6.8 |
| 15.70 | 24-Me | q | 1.02 | d | 7.0 |
| 12.61 | 23-Me | q | 1.68 | d | 1.0 |
| 3.61 | 21-Me | q | 0.59 | d | 7.0 |

### 1.12 Analytical data of methyl ester 20

$[\alpha]_D^{20}$ = -40.0° (c = 0.05, $CH_2Cl_2$); $R_f$ = 0.21 (petroleum ether : ethyl acetate = 1:1); **HRMS** (ESI): *m/z* for $C_{26}H_{44}O_8Na$ $[M+Na]^+$: calculated 507.2943, found 507.2945; **$^1$H-NMR** ($C_6D_6$, 500 MHz, $C_6D_5H$ = 7.16 ppm); **$^{13}$C-NMR** ($C_6D_6$, 125 MHz, $C_6D_6$ = 128.06 ppm)

| δ-C [ppm][a] | # | mult. | δ –H [ppm] | mult. | *J* [Hz] |
|---|---|---|---|---|---|
| 171.18 | 1 | s | - | - | - |
| 171.14 | 19 | s | - | - | - |
| 134.86 | 7 | d | 5.53 | dd | 9.6, 1.0 |
| 133.96 | 15 | d | 5.44 | ddd | 15.3, 10.3, 1.0 |
| 133.48 | 8 | s | - | - | - |
| 126.52 | 16 | d | 5.22 | dd | 15.3, 2.3 |
| 84.08 | 9 | d | 4.90 | d | 11.4 |
| 82.29 | 3 | d | 3.78 | ddd | 7.2, 5.0, 4.7 |
| 74.96 | 5 | d | 3.48 | ddd | 8.4, 2.1, 1.9 |
| - | 5-OH | - | 2.19 | d | 1.9 |
| 73.85 | 18 | d | 4.00 | dd | 9.1, 3.6 |
| - | 18-OH | - | 3.33 | d | 9.1 |
| 73.27 | 17 | d | 4.29 | ddd | 8.0, 3.6, 2.3 |
| - | 17-OH | - | 2.21 | d | 8.0 |
| 55.16 | 20-OMe | q | 3.15 | s | - |
| 51.19 | Me-ester | q | 3.35 | s | - |
| 37.94 | 4 | d | 1.77 | ddq | 7.0, 4.7, 2.1 |
| 37.27 | 2 | t | 2.50 ($H_a$) | dd | 15.6, 7.2 |
| | | | 2.45 ($H_b$) | dd | 15.5, 5.0 |
| 36.35 | 6 | d | 2.57-2.48 | m | - |
| 36.03 | 14 | d | 2.20-2.14 | m | - |
| 34.94 | 13 | t | 1.28-1.19 ($H_a$) | m | - |
| | | | 1.17-1.08 ($H_b$) | m | - |
| 33.34 | 10 | d | 2.01-1.93 | m | - |
| 28.72 | 11 | t | 1.74-1.66 ($H_a$) | m | - |
| | | | 0.90-0.83 ($H_b$) | m | - |
| 22.01 | 25-Me | q | 0.94 | d | 6.6 |
| 19.19 | 12 | t | 1.17-1.08 ($H_a$) | m | - |
| | | | 1.01-0.94 ($H_b$) | m | - |
| 16.87 | 22-Me | q | 0.81 | d | 6.9 |
| 15.70 | 24-Me | q | 0.79 | d | 7.1 |
| 12.56 | 23-Me | q | 1.64 | d | 1.0 |
| 9.57 | 21-Me | q | 0.97 | d | 7.0 |

### 1.13 Analytical data of methyl ester 5

$[\alpha]_D^{20}$ = -171.4° (c = 0.5, $CH_2Cl_2$); $R_f$ = 0.57 ($CH_2Cl_2$ : MeOH = 10:1); **HRMS** (ESI): *m/z* for $C_{26}H_{42}O_8Na$ $[M+Na]^+$: calculated 505.2777, found 505.2773; **$^1$H-NMR** ($C_6D_6$, 400 MHz, $C_6D_5H$ = 7.16 ppm); **$^{13}$C-NMR** ($C_6D_6$, 100 MHz, $C_6D_6$ = 128.06 ppm)

| δ-C [ppm][a] | # | mult. | δ –H [ppm] | mult. | *J* [Hz] |
|---|---|---|---|---|---|
| 211.9 | 5 | s | - | - | - |
| 172.0 | 19 | s | - | - | - |
| 171.4 | 1 | s | - | - | - |
| 136.4 | 8 | s | - | - | - |
| 134.0 | 15 | d | 5.48 | ddd | 15.4, 10.2, 2.2 |
| 129.8 | 7 | d | 5.44 | dd | 10.2, 1.4 |
| 126.5 | 16 | d | 5.23 | dd | 15.4, 2.3 |
| 83.1 | 9 | d | 4.74 | d | 11.6 |
| 80.6 | 3 | d | 3.82 | ddd | 8.3, 6.1, 4.3 |
| 73.9 | 18 | d | 3.96 | d | 3.4 |
| 73.3 | 17 | d | 4.32 | dd | 3.4, 2.3 |
| 57.8 | 20-OMe | q | 3.15 | s | - |
| 51.3 | Me-ester | q | 3.35 | s | - |
| 47.8 | 6 | d | 3.52 | dq | 10.2, 6.7 |
| 47.6 | 4 | d | 3.03 | dq | 8.3, 6.9 |
| 36.1 | 2 | t | 2.43 ($H_a$) | dd | 15.4, 4.3 |
| | | | 2.26 ($H_b$) | dd | 15.4, 6.1 |
| 36.0 | 14 | d | 2.20-2.10 | m | - |
| 34.9 | 13 | t | 2.28-1.18 ($H_a$) | m | - |
| | | | 1.18-1.08 ($H_b$) | m | - |
| 34.0 | 10 | d | 1.94-1.83 | m | - |
| 28.8 | 11 | t | 1.74-1.65 ($H_a$) | m | - |
| | | | 0.88-0.78 ($H_b$) | m | - |
| 22.0 | 25-Me | q | 0.96 | d | 6.1 |
| 19.2 | 12 | d | 1.10-1.00 ($H_a$) | m | - |
| | | | 0.95-0.85 ($H_b$) | m | - |
| 15.6 | 22-Me | q | 1.23 | d | 6.7 |
| 15.5 | 24-Me | q | 0.66 | d | 7.5 |
| 13.2 | 23-Me | q | 1.65 | d | 1.4 |
| 12.9 | 21-Me | q | 0.94 | d | 6.9 |

### 1.14 Analytical data of ketone 6

$[\alpha]_D^{20}$ = -159.8° (c = 0.4, $CH_2Cl_2$); $R_f$ = 0.52 (petroleum ether : ethyl acetate = 1:1); **HRMS** (ESI): *m/z* for $C_{26}H_{40}O_8Na$ $[M+Na]^+$: calculated 503.2620, found 503.2621; **$^1$H-NMR** ($C_6D_6$, 400 MHz, $C_6D_5H$ = 7.16 ppm); **$^{13}$C-NMR** ($C_6D_6$, 100 MHz, $C_6D_6$ = 128.06 ppm)

| δ-C [ppm][a] | # | mult. | δ –H [ppm] | mult. | *J* [Hz] |
|---|---|---|---|---|---|
| 211.8 | 5 | s | - | - | - |
| 193.3 | 17 | s | - | - | - |
| 171.3 | 1 | s | - | - | - |
| 168.0 | 19 | s | - | - | - |
| 153.0 | 15 | d | 6.26 | dd | 15.9, 9.1 |
| 135.5 | 8 | s | - | - | - |
| 130.5 | 7 | d | 5.45 | dd | 10.2, 1.2 |
| 126.0 | 16 | d | 5.97 | d | 15.9 |
| 84.4 | 9 | d | 4.65 | d | 11.2 |
| 80.6 | 3 | d | 3.84 | ddd | 8.2, 6.3, 4.3 |
| 77.4 | 18 | d | 4.75 | d | 8.9 |
| - | 18-OH | | 4.30 | d | 8.9 |
| 57.9 | 20-OMe | q | 3.16 | s | - |
| 51.2 | Me-ester | q | 3.34 | s | - |
| 47.8 | 6 | d | 3.52 | dq | 10.2, 6.7 |
| 47.7 | 4 | d | 3.04 | dq | 8.2, 7.0 |
| 36.2[a] | 14 | d | 2.05-1.95 | m | - |
| 36.1[a] | 2 | t | 2.44 ($H_a$) | dd | 15.4, 4.3 |
| | | | 2.28 ($H_b$) | dd | 15.5, 6.3 |
| 34.2 | 10 | d | 1.87-1.75 | m | - |
| 33.8 | 13 | t | 1.94-1.83 ($H_a$) | m | - |
| | | | 0.93-0.85 ($H_b$) | m | - |
| 29.8 | 11 | t | 1.74-1.65 ($H_a$) | m | - |
| | | | 0.86-0.76 ($H_b$) | m | - |
| 20.3 | 12 | t | 0.93-0.66 | m | - |
| 18.9 | 25-Me | q | 0.71 | d | 6.5 |
| 15.9 | 24-Me | q | 0.63 | d | 6.8 |
| 15.6 | 22-Me | q | 1.21 | d | 6.7 |
| 13.0 | 21-Me | q | 0.94 | d | 7.0 |
| 12.8 | 23-Me | q | 1.65 | d | 1.2 |

a) The assignment of protons at 2-H and 14-H was not possible.

## 2. Supporting information (Biological Test Procedures)

### 2.1 Bacterial cultivation and biofilm formation

The bacterial strains *Streptococcus mutans* ATCC 700610 (identical to *S. mutans* UA159), *Streptococcus gordonii* DSM 20568, *Streptococcus oralis* DSM 20627 and *Aggregatibacter actinomycetemcomitans* DSM 11123 were routinely propagated in Bacto™ Todd Hewitt Broth (THB, Becton Dickinson, Heidelberg, Germany) under anaerobic conditions (10% $H_2$, 10 $CO_2$, 80% $N_2$) at 37°C. To induce biofilm formation, fresh overnight cultures were diluted to an $OD_{600}$ of 0.01 – 0.03 in THB supplemented with 0.5% sucrose (THBS). 195 µl aliquots of this inoculum were transferred to a 96-well plate (Nunc™ MicroWell™, Thermo, Roskilde, Denmark). 5 µl of a DMSO solution of carolacton or its derivatives were added to give a final concentration of 5.3 µM. As control, an equal volume of DMSO was added to the bacterial suspension. The bacteria were cultivated for 24 hours under anaerobic conditions without shaking. All experiments were run in triplicate.

### 2.2 Quantitative evaluation of membrane damage

The biofilms were stained live/dead with the BacLight™ Bacterial Viability Kit (Life Technologies, Carlsbad, USA). This stain is based on the two dyes propidium iodide (PI) and Syto 9. PI is only capable of entering cells if membranes are damaged. In contrast, Syto 9 penetrates all cells without regard to their vitality. As a consequence, live and dead cells are stained differently; dead or damaged cells fluoresce red and live cells green. Biofilms were washed with 200 µl PBS buffer (Biochrom, Berlin, Germany) before staining with 200 µl of a 1:1000 dilution of BacLight™ dye. Staining was performed for 15 min at room temperature in the dark. Green (535nm) and red fluorescence (595 nm) were measured with an Infinite 200 plate reader (Tecan, Männedorf, Switzerland) using appropriate filter sets. The ratio of green/red fluorescence was determined and biofilm damage quantified as described elsewhere (Kunze et al. 2010). The experiments were run in triplicate.

### 2.3 Quantitative evaluation of biofilm biomass

Biofilms were grown in 96-well culture plates as described above. The biofilms were washed with 200 µl PBS (Biochrom, Berlin, Germany) to remove non-adherent cells, and stained for 20 min with 0.1% (w/v) crystal violet solution (Roth, Karlsruhe Germany). After destaining with 99% ethanol (Büfa, Hude, Germany) crystal violet in the supernatant was determined by measuring the absorbance at 630 nm in a Synergy 2 plate reader (BioTek, Bad Friedrichshall, Germany). All experiments were run in triplicate.

### 2.4 Confocal Laser Scanning Microscopy

For the observation of live/dead stained biofilms, a Leica DM LFSA upright fixed stage microscope connected to a TCS SP2 AOBS scan head was used. Biofilms were grown as described above, with the exception that the experiments were conducted in 35 mm petri dishes (Sarstedt, Nümbrecht, Germany). A 488 nm argon-laser was used as excitation source for PI and Syto 9. Appropriate filter sets were chosen to measure green and red fluorescence. The acquired stacks were processed using the IMARES (Bitplane AG, Zurich, Switzerland) and Leica AF Lite (Leica, Wetzlar, Germany) software packages.

### 2.5 Extraction of carolacton and its derivatives after incubation with *S. mutans* biofilms

*S. mutans* UA159 was used for this analysis. The strain was cultivated in 24-well polystyrene microtiter plates (Thermo Scientific, Waltham, USA) in THBS at 37°C under aerobic conditions with 5% $CO_2$ for 20 h as described earlier (Reck et al., 2011). Each experiment consisted of two plates: Plate A contained 800 µl of the sterile cultivation medium in each well (control), and plate B contained 800 µl of the *S. mutans* biofilm culture in each well. Prior to inoculation of the plates with the bacterium, 10 µl of the test compound solved in methanol or DMSO was added to the wells and the solvent was allowed to evaporate. The final concentration of each compound in the test wells was 5 µM.

After 20 h of biofilm growth, the supernatants from each plate were pooled separately into labelled glass bottles. Controls and biofilm plates were extracted with methanol (800 µl and 300 µl per well, respectively) by incubation on a shaker at 350 rpm for 30 min. The supernatants were pooled separately into labelled glass bottles. To ensure complete removal of the biofilm, the above procedure was repeated by scraping the biofilm from the microtiter plate using a pipette tip.

The pooled fractions were added onto a fixed glass extraction flask using a funnel. Equal volumes of ethyl acetate were added into the flask for extraction. The flask was stoppered and shaken vigorously. The stopper was opened at intervals to release the pressure inside. The flask was allowed to stand undisturbed until the liquid in the flask separated into a clear upper organic solvent phase and a lower water phase. The top phase was collected into a round bottom flask for further evaporation and drying. The bottom phase was collected into respective glass bottles to repeat the extraction. The entire procedure was carried out thrice in order to achieve an efficient extraction of the compounds.

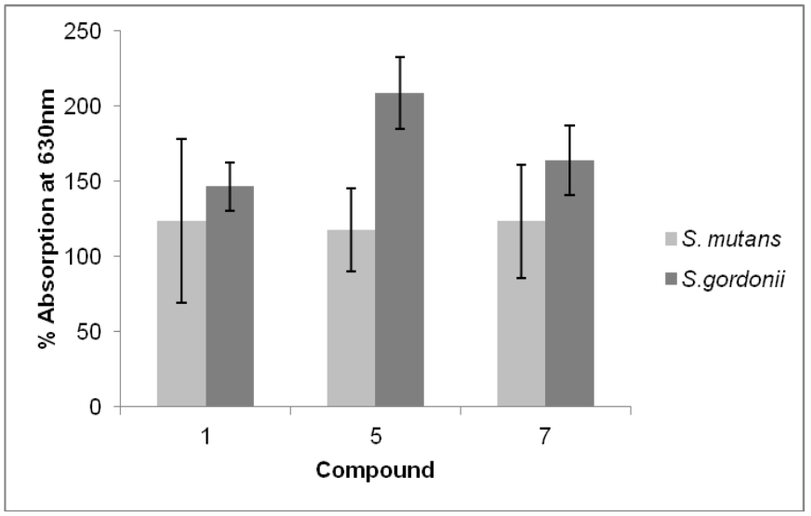
The samples were evaporated at 200 mbar. To ensure complete removal of any residual water, the pressure was reduced to 0 mbar in the final stage of evaporation. The crude material was taken up and dissolved with 1-2 ml of methanol and the samples were pooled. Drying was performed in labelled glass vials. If necessary, the solutions were filtered to remove any particles in order to obtain a clear solution for the HPLC analysis. The vials were placed on a heating block at 38˚C with an inflow of $N_2$ gas for 30 min or until the liquid in the vials had evaporated completely. The samples were stored at -20˚C before RP-HPLC MS analysis.

### 2.6 RP-HPLC-MS analysis

The vials were thawed to room temperature. Methanol (50 µl) was added to dissolve the concentrated samples. The procedure was repeated twice and samples were pooled into a special glass vial used for MS analysis to a final volume of 150 µl. Analytical RP HPLC was carried out with an Agilent 1260 RRLC system equipped with a diode-array UV detector (DAD) and a Corona Ultra detector (Dionex) or a Maxis ESI TOF mass spectrometer (Bruker Daltonics). The following conditions were used to perform HPLC. Waters Acquity $C_{18}$ BEH column (50×2 mm, 1.7 µm) was used at 40°C. The separation was carried out using solvent A: Water, 0.1% HCOOH and solvent B: acetonitrile, 0.1% HCOOH. 5% of solvent B was used as a gradient at a flow rate of 0.6 mL/min with an initial time period of 1 min, and later increased to 95% of solvent B in 20 min. The HRESIMS data were recorded on a Maxis ESI TOF mass spectrometer (Bruker Daltonics) and the molecular formulae including the isotopic pattern were calculated using the Smart Formula algorithm.

**2.7 Quantitative evaluation of the influence of carolacton 1 and derivatives 5 and 7 on biofilm formation of *S. gordonii* and *S. mutans.***

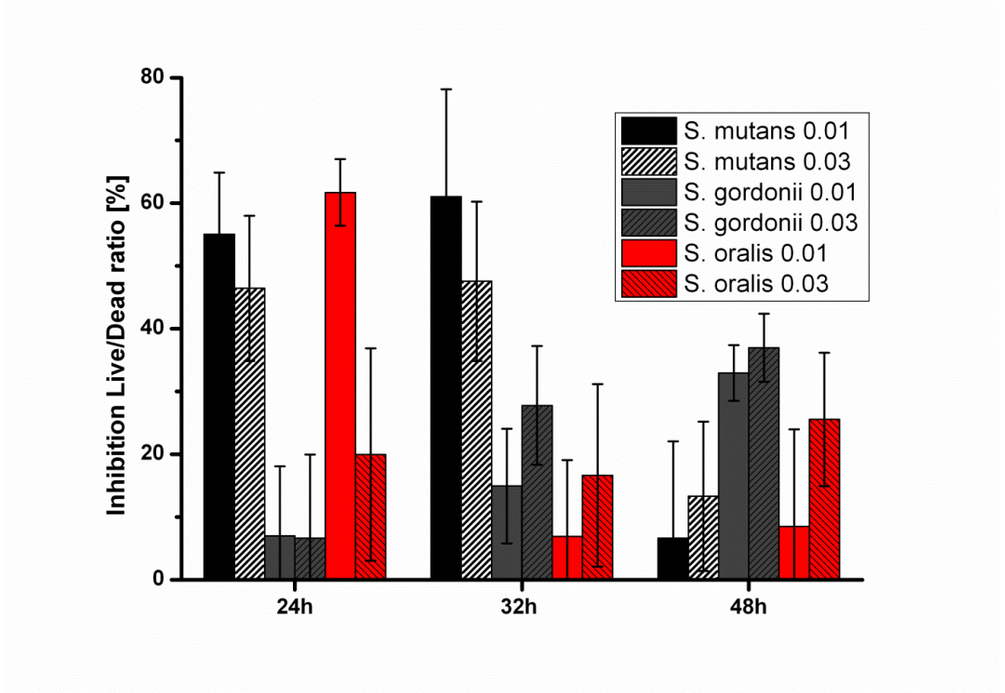
**Figure S1.** Biofilms were grown for 24 h and stained with crystal violet dye. The absorption of crystal violet is expressed relative to the untreated control, taken as 100%.

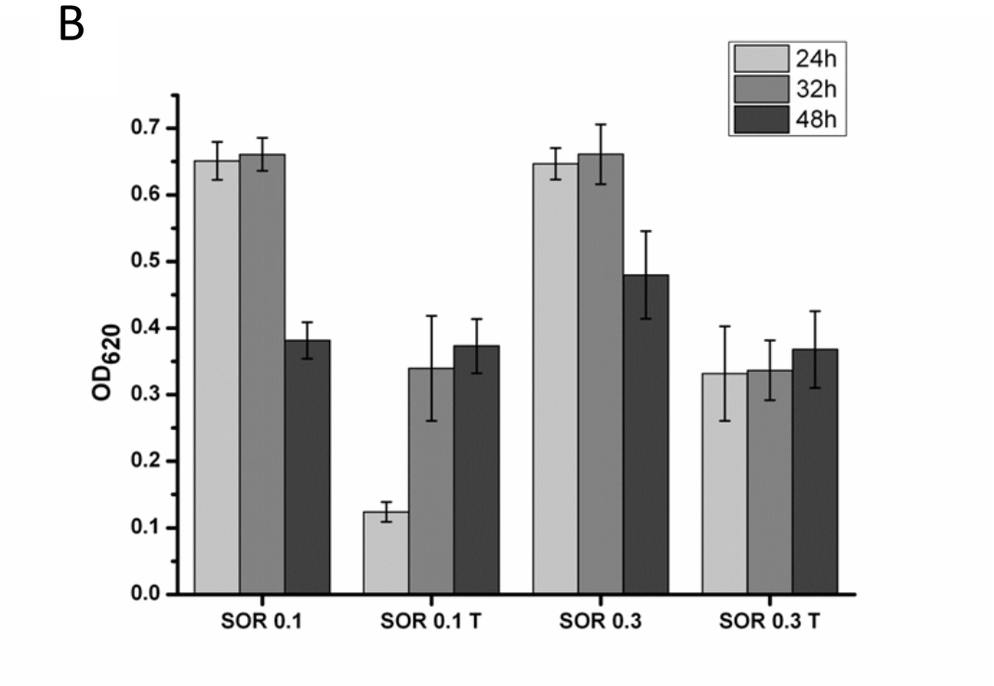



**2.8. Effect of carolacton on membrane damage of *S. mutans*, *S. gordonii* and *S. oralis* biofilms and on growth of *S. oralis* biofilms.**

**Figure S2. *S. mutans*, *S. gordonii* and *S. oralis* (A) and growth of *S. oralis* (B).**

A






For A, biofilms were cultivated as described, treated with 5.3 µM carolacton, and stained with the Live/Dead BacLight Viability kit. The ratio of green/red fluorescence was used as a measure of viability, and the difference in viability between untreated and treated biofilms is shown here as % inhibition (see methods for details). Two different optical densities of the inoculum cultures were tested ($OD_{600}$ 0.01 or 0.03). For B, biofilms were cultivated as described and $OD_{620}$ was determined after 24 h, 32 h and 48 h. Two different optical densities of the inoculum cultures were tested (0.01 or 0.03). T = treated with 5.3 µM carolacton.

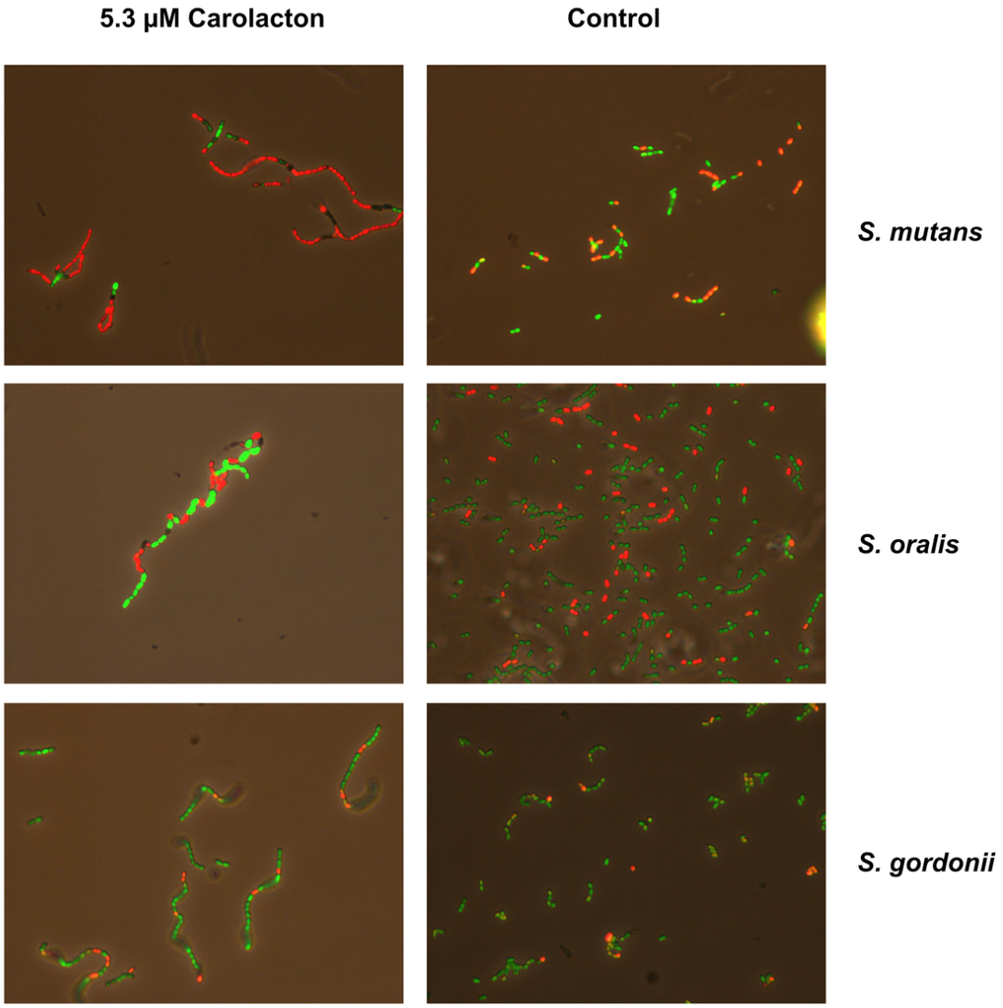
Fig. S2A shows that in *S. mutans*, carolacton causes roughly 60 % inhibition of biofilm viability after 24 h and 32 of biofilm cultivation at both inoculation densities. At 48 h, a large fraction of the cells are dead and there is no difference between carolacton treated biofilms and untreated controls.

In *S. gordonii*, no membrane damage is detectable after 24 h of treatment of biofilms with carolacton for both inoculation densities, in accordance with the CLSM pictures. Biofilms cultivated for 32 h show 15 – 25 % reduction of the life/dead fluorescence ratio. After 48 h, the biofilms of *S. gordonii* treated with carolacton show 30% inhibition of viability.

In *S. oralis*, 60 % membrane damage of the biofilms by carolacton is observed at the low inoculum density ($OD_{620}$ = 0.01) and only in 24 h old biofilms. In *S. oralis*, carolacton causes a strong inhibition of growth (Fig. S5B, and also seen on the CLSM pictures). The biofilms are therefore very thin, and the fluorescence measurements are prone to error. Older biofilms inoculated with $OD_{620}$ = 0.01 show ~10 % reduction of the life/dead fluorescence ratio, and those inoculated with an $OD_{620}$=0.03 show approximately 20 % reduction of the life/dead fluorescence ratio.

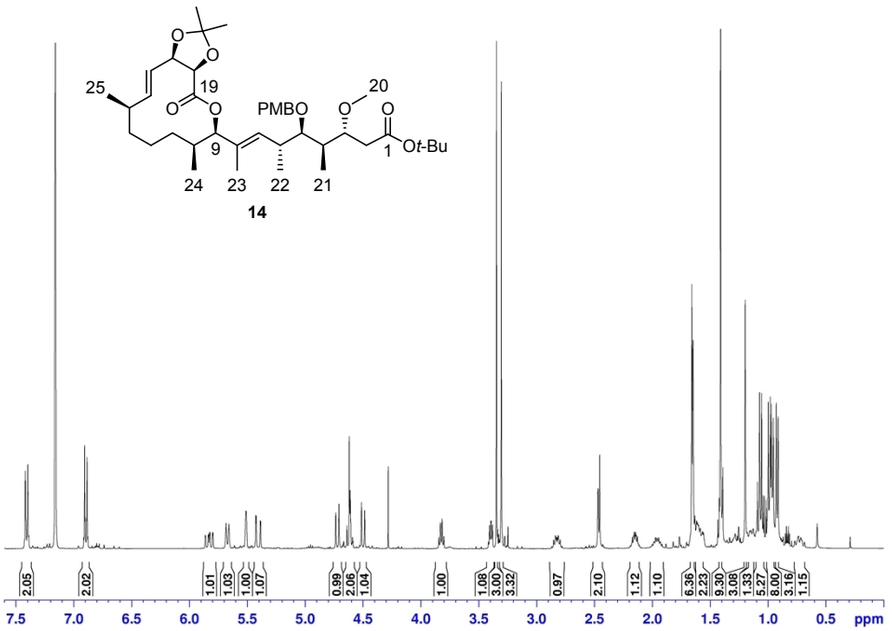
**2.9 Carolacton causes elongated cell chains.**

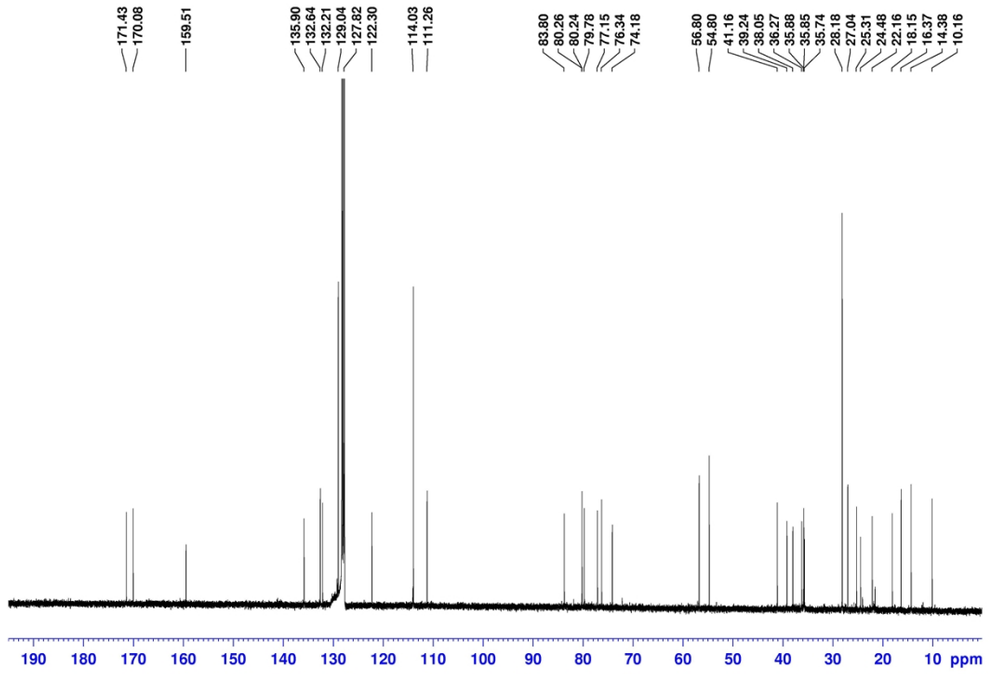
**Figure S3.** Biofilms of *S. gordonii*, *S. oralis* and *S. mutans* were grown as described w/o 5.3 µM carolacton and stained using the live/dead BacLightTM Bacterial Viability Kit.

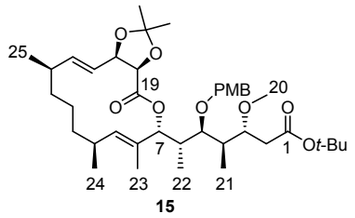


## 3. Copies of NMR and MS spectra

NMR and MS spectra of carolacton intermediates **14**, **15**, **S1**, **S2**, **S4**, **17** and **20** and derivatives **2**-**6**.

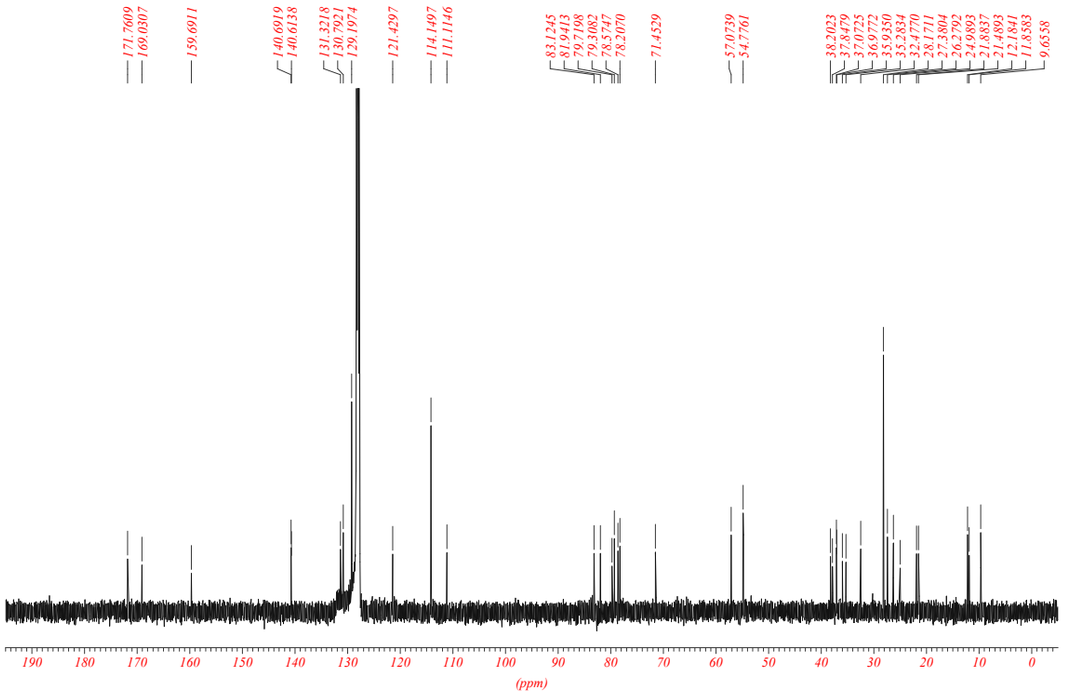


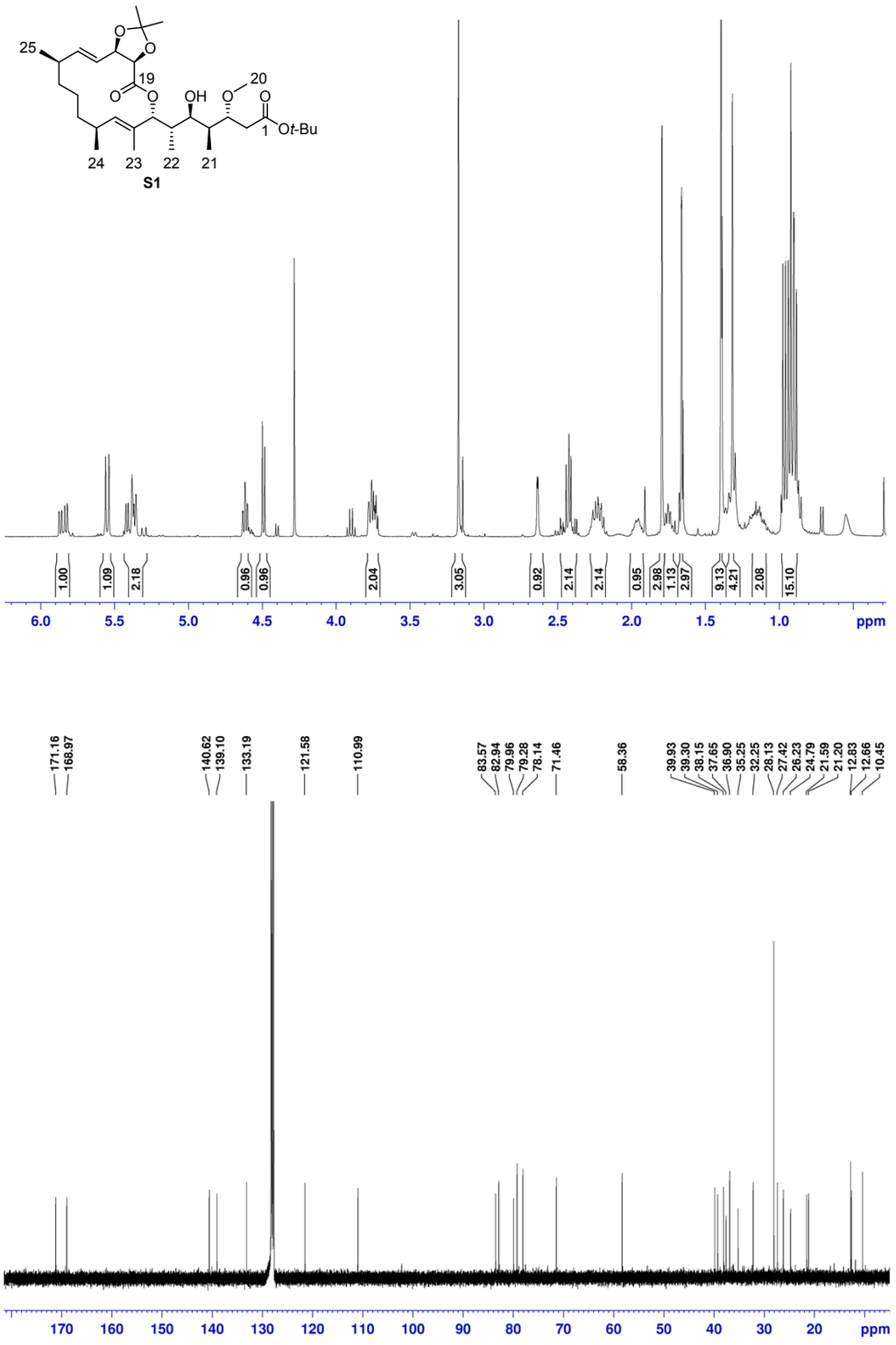



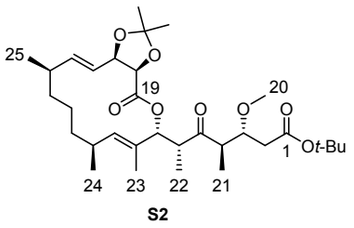

25
19
PMB
20
7
1
Ot-Bu
24
23
22
21

**15**


Integral
2.0895
2.0134
0.9991
0.9679
1.9087
1.0000
1.9999
0.9588
1.0046
0.9558
3.0475
3.0273
2.9328
0.9545
1.0030
0.9843
2.9503
2.9877
9.0955
1.1823
3.1296
2.3533
8.4263
4.2042
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
0.5
0.0
(ppm)

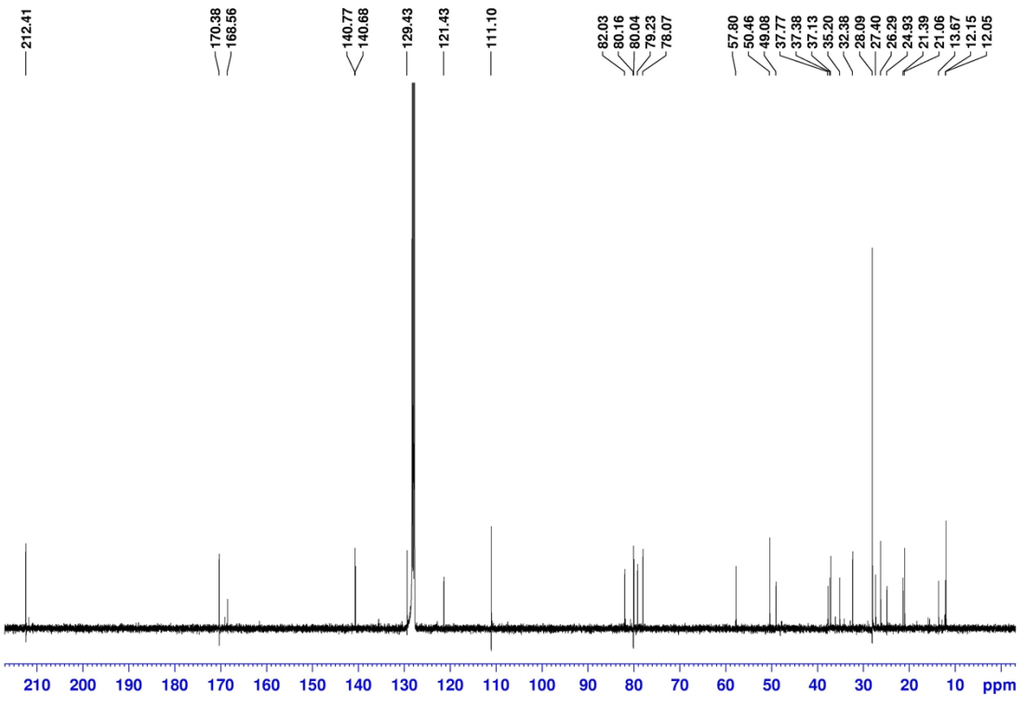

171.7609
169.0307
159.6911
140.6919
140.6138
131.3218
130.7921
129.1974
121.4297
114.1497
111.1146
83.1245
81.9413
79.7198
79.3082
78.5747
78.2070
71.4529
57.0739
34.7761
38.2023
37.8479
37.0725
36.9772
35.9350
35.2834
32.4770
28.1711
27.3804
26.2792
24.9893
21.8837
21.4493
12.1841
11.8383
9.6558
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

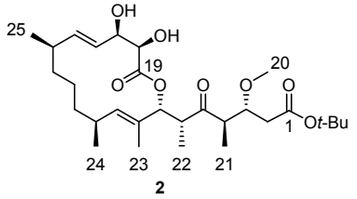

25
19
20
OH
1
Ot-Bu
24
23
22
21
S1
1.00
1.09
2.18
0.96
0.96
2.04
3.05
0.92
2.14
2.14
0.95
2.98
1.13
2.97
9.13
4.21
2.08
15.10
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
171.16
168.97
140.62
139.10
133.19
121.58
110.99
83.57
82.94
79.96
79.28
78.14
71.46
58.36
39.93
39.30
38.15
37.65
36.90
35.25
32.25
28.13
27.42
26.23
24.79
21.59
21.20
12.83
12.66
10.45
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm


25
19
20
1
Ot-Bu
24
23
22
21

**S2**


2.10
2.15
1.08
1.00
1.11
4.03
1.05
1.10
1.19
1.05
1.07
3.12
3.01
9.12
3.03
2.06
3.06
2.34
7.07
4.00
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm


212.41
170.38
168.56
140.77
140.68
129.43
121.43
111.10
82.03
80.16
80.04
79.23
78.07
57.80
50.46
49.08
37.77
37.38
37.13
35.20
32.38
28.09
27.40
26.29
24.93
21.39
21.06
13.67
12.15
12.05
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


OH
25
OH
19
O
O
O
O
20
O
O
1
Ot-Bu
24
23
22
21

**2**


1.00
1.01
1.00
1.02
2.02
1.02
3.01
1.09
0.89
1.07
0.91
1.05
1.12
1.95
3.04
9.37
1.99
0.73
3.18
1.90
3.39
2.96
0.77
3.06
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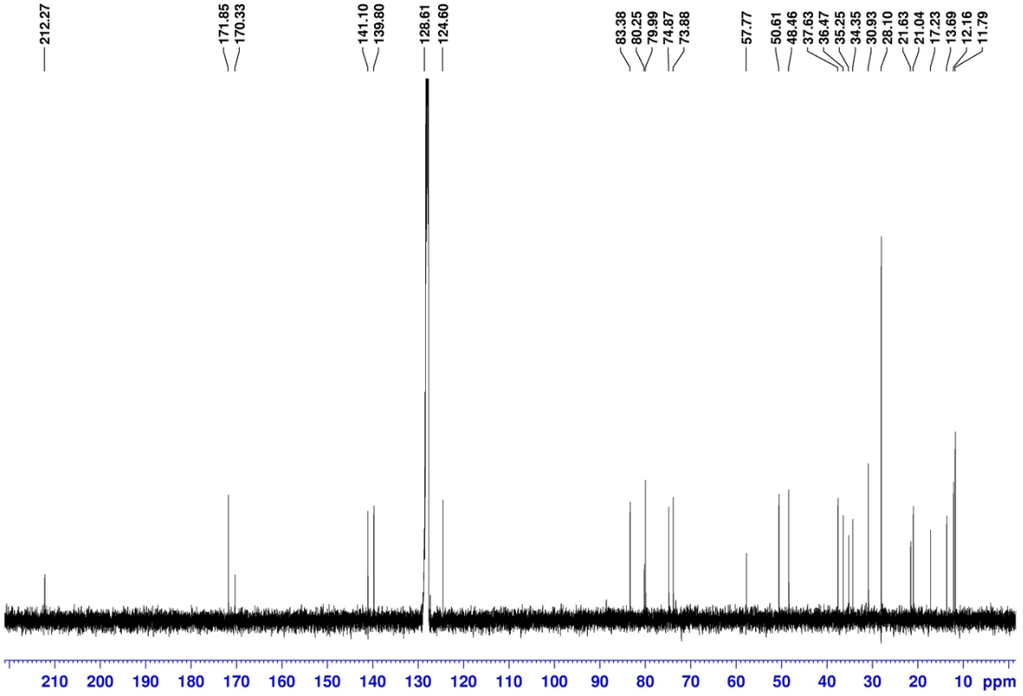
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm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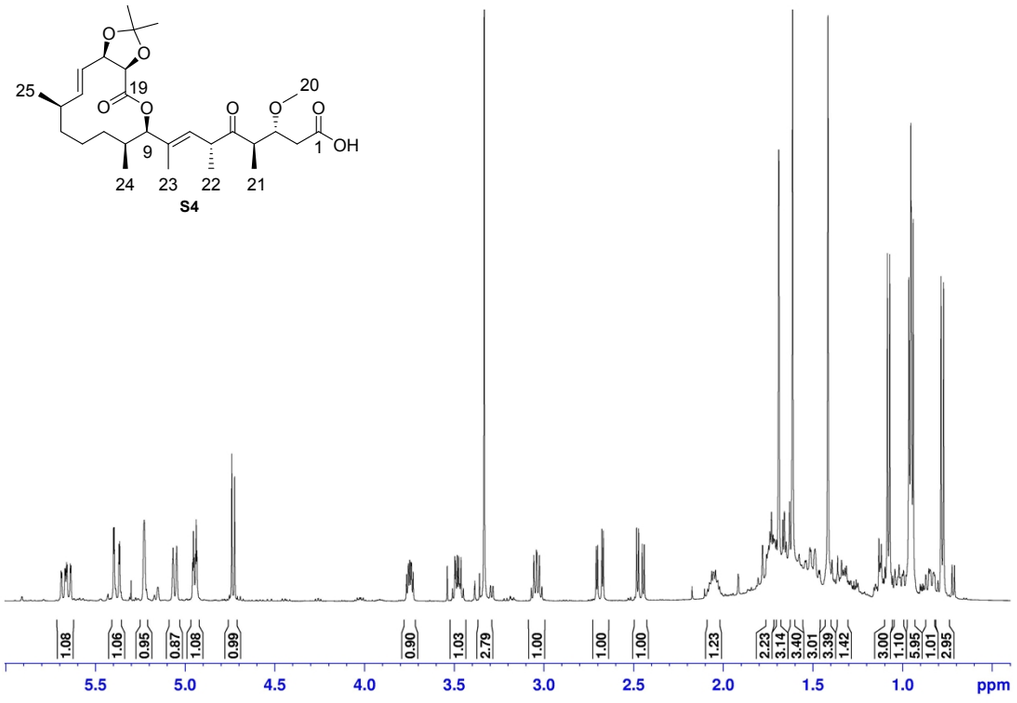
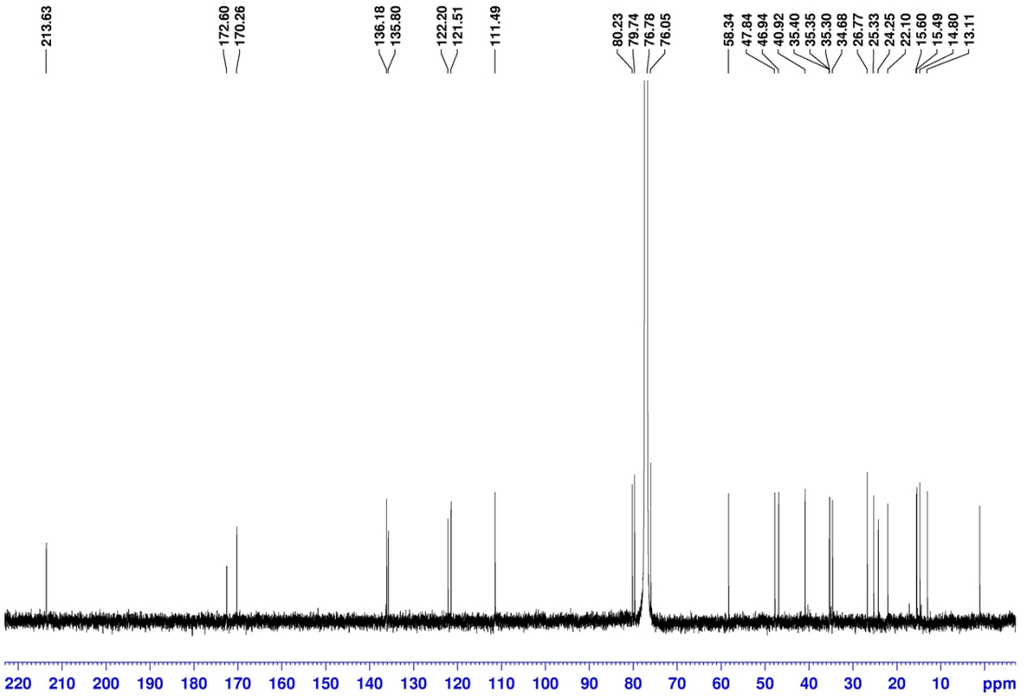
170.33
141.10
139.80
128.61
124.60
83.38
80.25
79.99
74.87
73.88
57.77
50.61
48.46
37.63
36.47
35.25
34.35
30.93
28.10
21.63
21.04
17.23
13.89
12.16
11.79
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


25
19
20
9
1
OH
24
23
22
21
S4
1.08
1.06
0.95
0.87
1.08
0.99
0.90
1.03
2.79
1.00
1.00
1.00
1.23
2.23
3.14
3.40
3.01
3.39
1.42
3.00
1.10
5.95
1.01
2.95
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm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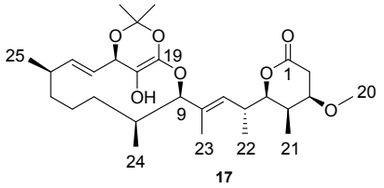
172.60
170.26
136.18
135.80
122.20
121.51
111.49
80.23
79.74
76.78
76.05
58.34
47.84
46.94
40.92
35.40
35.35
35.30
34.68
26.77
25.33
24.25
22.10
15.60
15.49
14.80
13.11
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


25
19
OH
9
24
23
22
21
1
20

17


0.91
0.88
1.00
0.91
0.68
0.95
3.86
2.03
1.00
1.01
1.05
4.30
3.16
1.23
3.11
1.12
1.30
3.30
1.47
2.39
3.17
1.09
6.22
6.0
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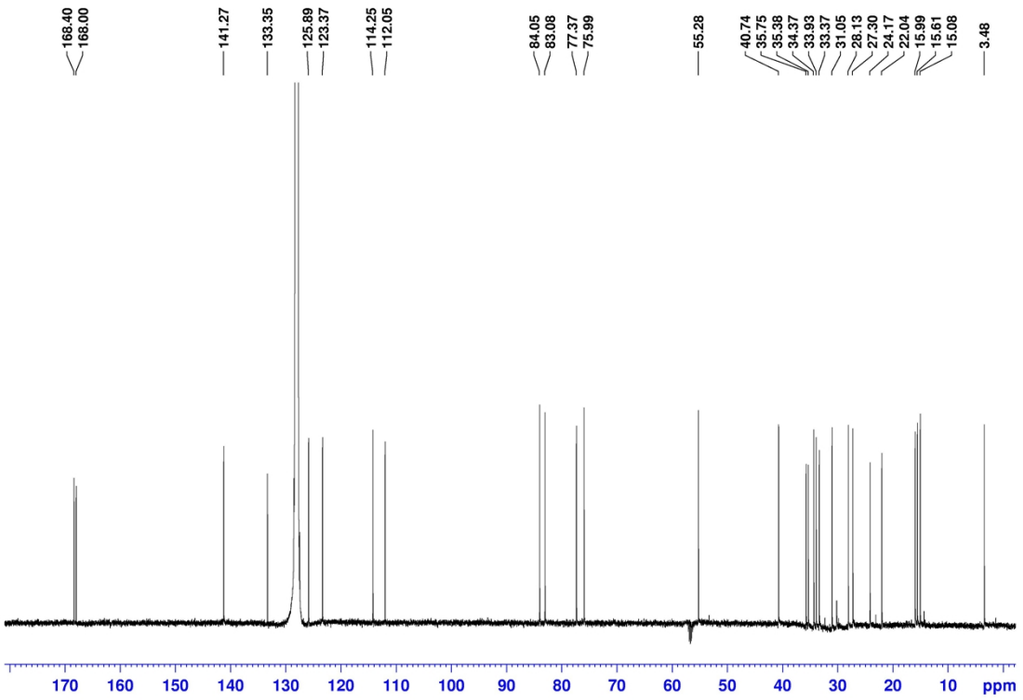
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm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168.00
141.27
133.35
125.89
123.37
114.25
112.05
84.05
83.08
77.37
75.99
55.28
40.74
35.75
35.38
34.37
33.93
33.37
31.05
28.13
27.30
24.17
22.04
15.99
15.61
15.08
3.48
170
160
150
140
130
120
110
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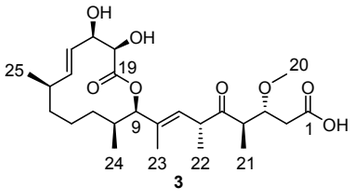
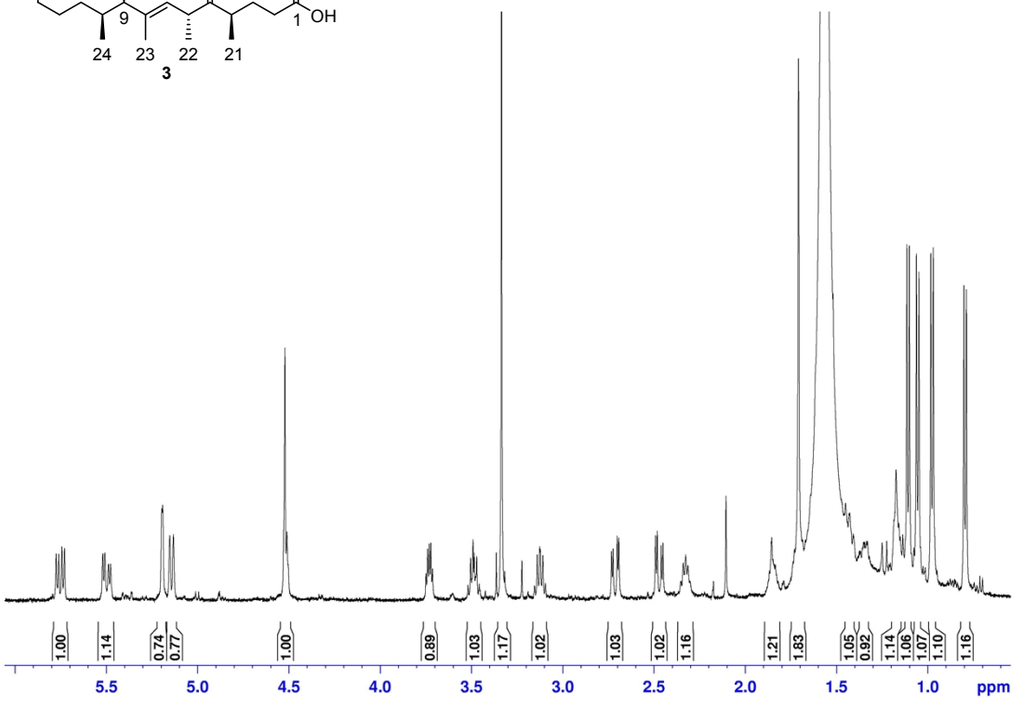
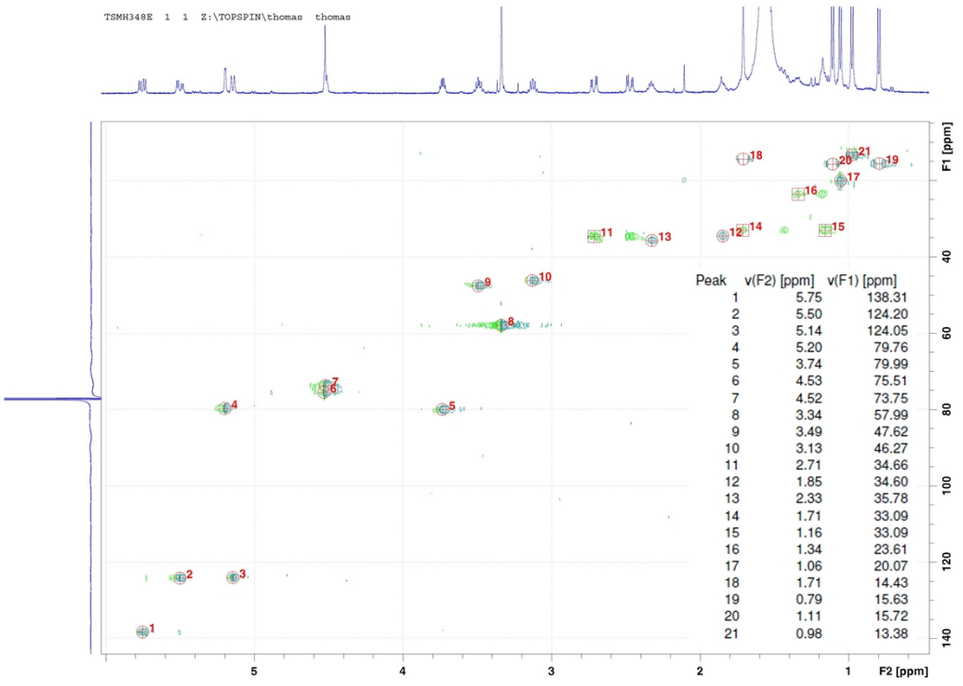
90
80
70
60
50
40
30
20
10
ppm




**3**

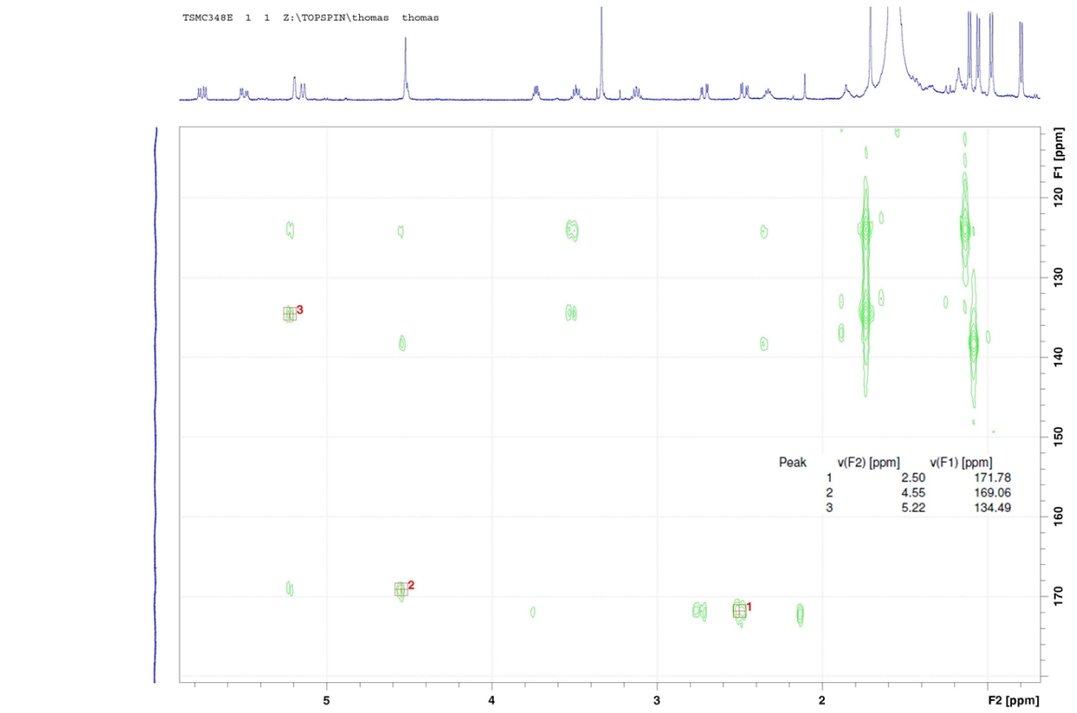


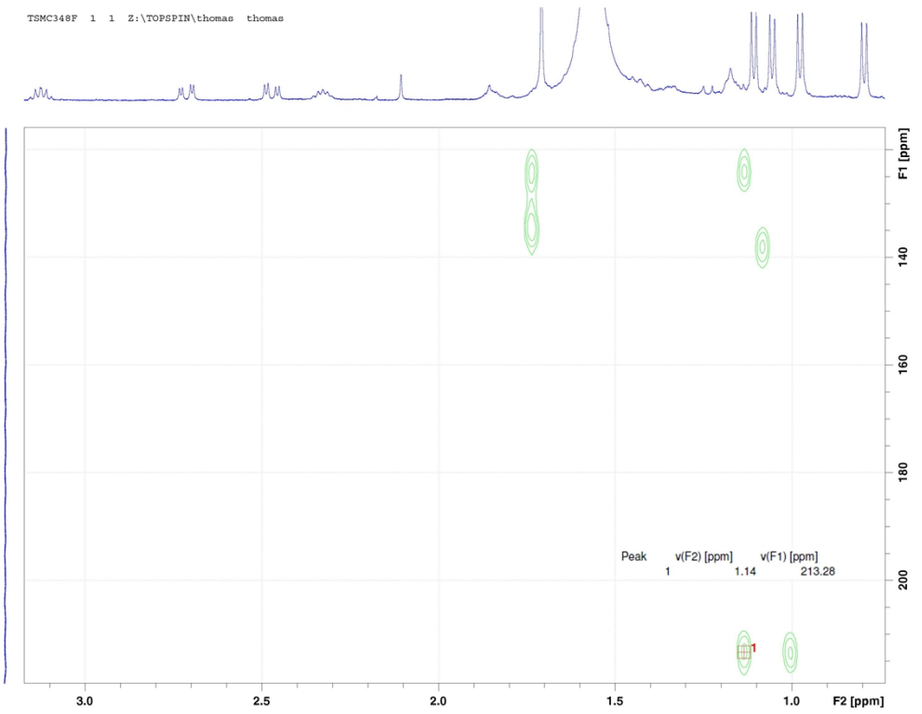

Indirect assignment of carbon chemical shifts via HSQC

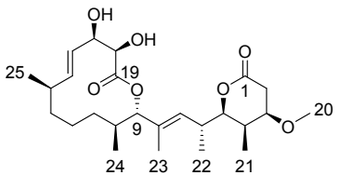



| Peak | v(F2) [ppm] | v(F1) [ppm] |
|---|---|---|
| 1 | 5.75 | 138.31 |
| 2 | 5.50 | 124.20 |
| 3 | 5.14 | 124.05 |
| 4 | 5.20 | 79.76 |
| 5 | 3.74 | 79.99 |
| 6 | 4.53 | 75.51 |
| 7 | 4.52 | 73.75 |
| 8 | 3.34 | 57.99 |
| 9 | 3.49 | 47.62 |
| 10 | 3.13 | 46.27 |
| 11 | 2.71 | 34.66 |
| 12 | 1.85 | 34.60 |
| 13 | 2.33 | 35.78 |
| 14 | 1.71 | 33.09 |
| 15 | 1.16 | 33.09 |
| 16 | 1.34 | 23.61 |
| 17 | 1.06 | 20.07 |
| 18 | 1.71 | 14.43 |
| 19 | 0.79 | 15.63 |
| 20 | 1.11 | 15.72 |
| 21 | 0.98 | 13.38 |

Indirect assignment of carbon chemical shifts via HMBC

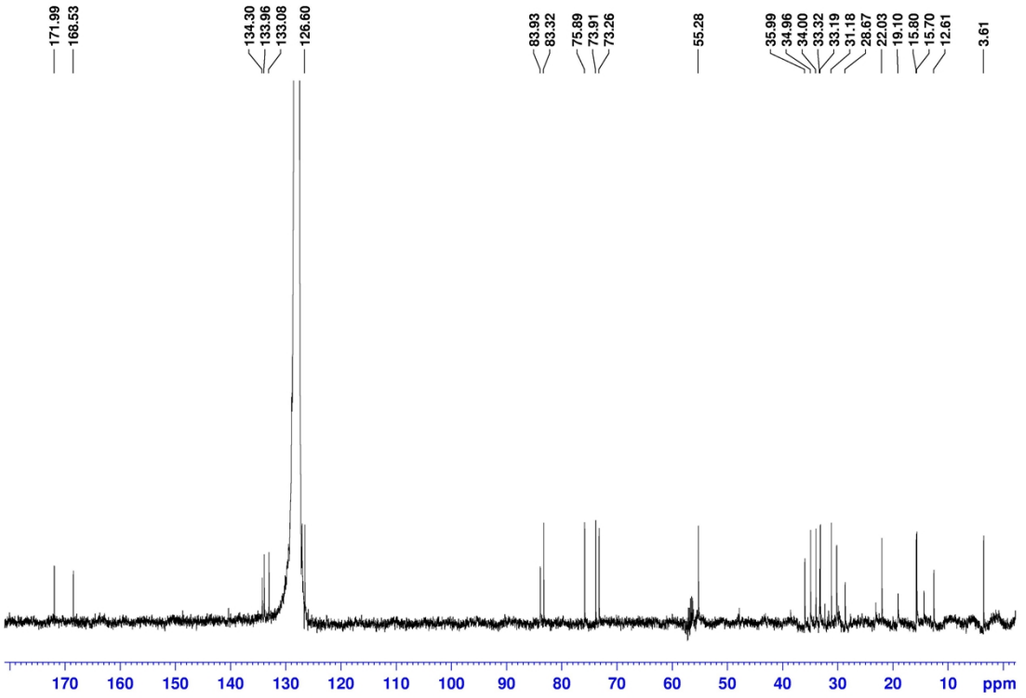



OH
OH
25
19
O
O
O
1
O
20
9
24
23
22
21

**4**


1.07
0.96
1.07
0.98
1.09
1.08
0.72
1.00
3.39
2.06
0.64
2.07
1.05
1.12
1.13
2.94
3.72
4.03
3.20
0.99
2.75
2.86
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm


171.99
168.53
134.30
133.96
133.08
126.60
83.93
83.32
75.89
73.91
73.26
55.28
35.99
34.96
34.00
33.32
33.19
31.18
28.67
22.03
19.10
15.80
15.70
12.61
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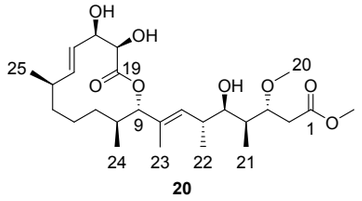
90
80
70
60
50
40
30
20
10
ppm


OH
OH
25
19
O
O
OH
O
20
O
9
1
O
24
23
22
21

**20**


1.01
0.98
0.97
0.96
0.99
0.95
0.96
1.00
2.59
0.66
2.77
3.00
2.41
1.06
1.07
1.09
3.11
1.27
2.21
7.33
1.12
6.00
5.5
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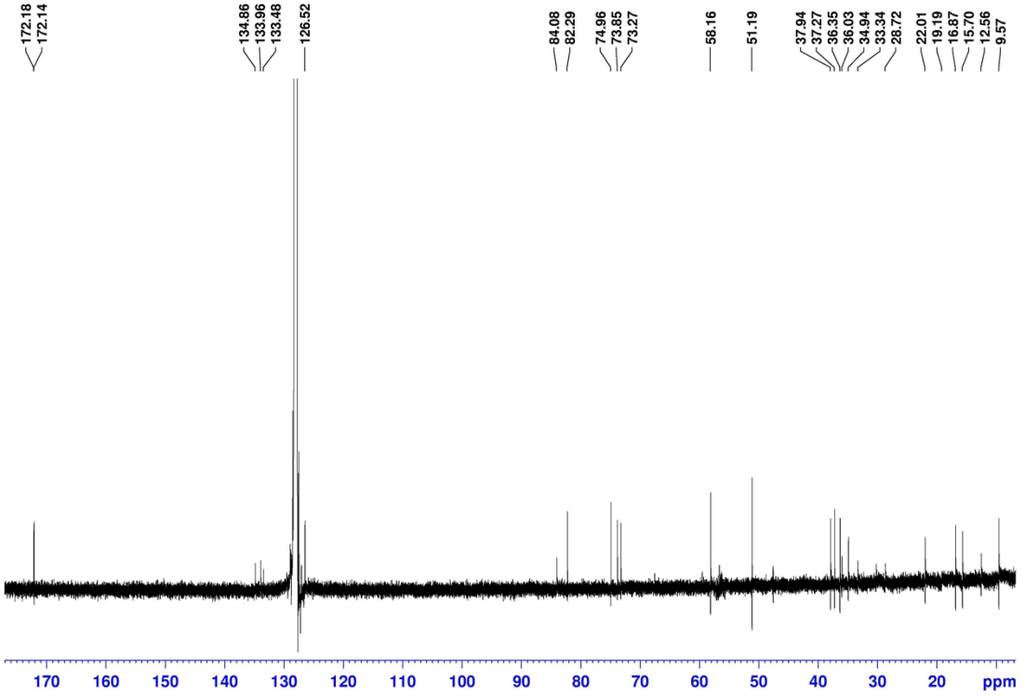
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm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172.14
134.86
133.96
133.48
126.52
84.08
82.29
74.96
73.85
73.27
58.16
51.19
37.94
37.27
36.35
36.03
34.94
33.34
28.72
22.01
19.19
16.87
15.70
12.56
9.57
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm


OH
OH
25
19
20
O
O
O
O
O
9
1
O
24
23
22
21
5
Integral
2.0452
1.0000
0.9622
0.9765
0.9458
0.9655
1.2439
3.0819
2.9212
0.9987
1.1971
1.1279
1.0712
0.9809
3.9758
4.0857
2.2909
6.3034
2.0139
2.9911
7.0
6.5
6.0
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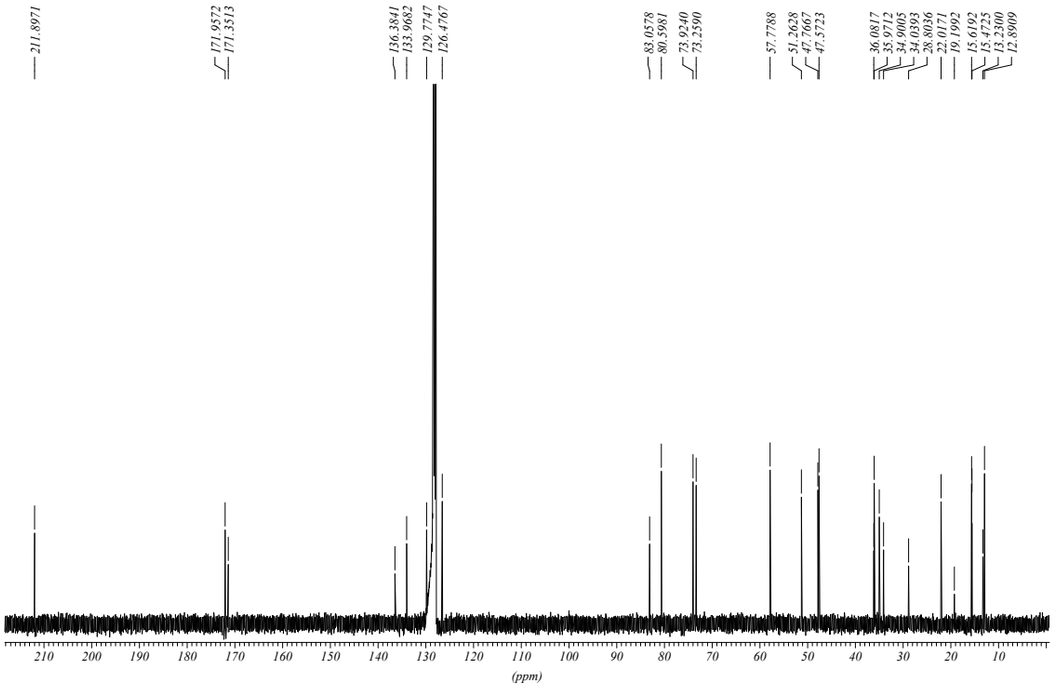
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
(ppm)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171.9572
171.3513
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133.9682
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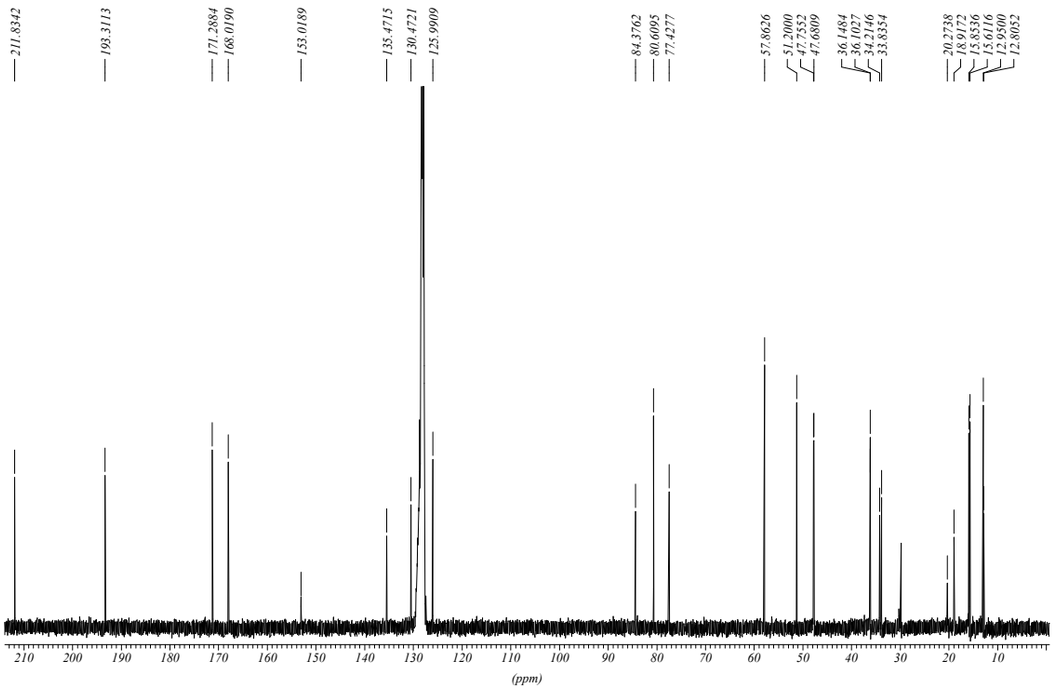
73.2590
57.7788
51.2628
47.7667
47.5723
36.0817
35.9712
34.9005
34.0393
28.8036
22.0171
19.1992
15.6192
15.4725
13.2300
12.8909
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)


O
OH
19
25
O
O
20
O
O
9
1
O
24
23
22
21
6
Integral
1.0000
1.0075
1.0158
0.9891
0.9884
0.9810
0.9867
1.0240
2.9622
2.9802
1.0451
1.0802
1.0702
1.1805
1.1810
3.0824
1.3019
3.1815
1.2682
7.2527
4.0265
3.0166
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
0.5
0.0
(ppm)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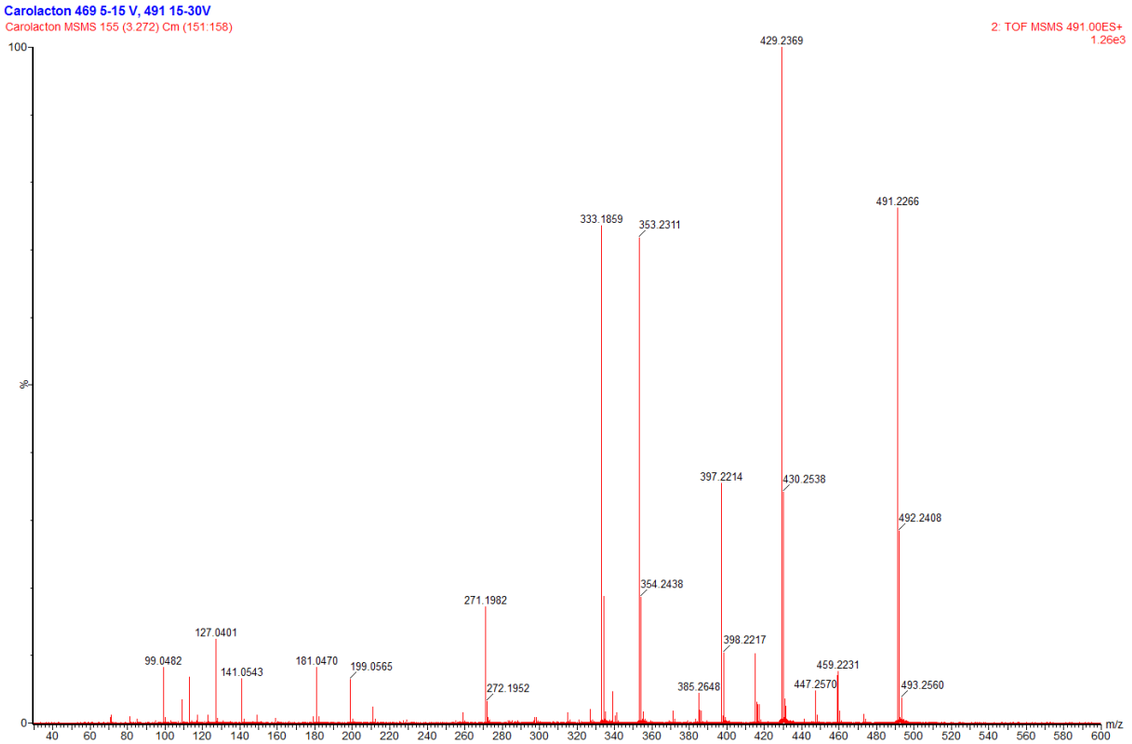
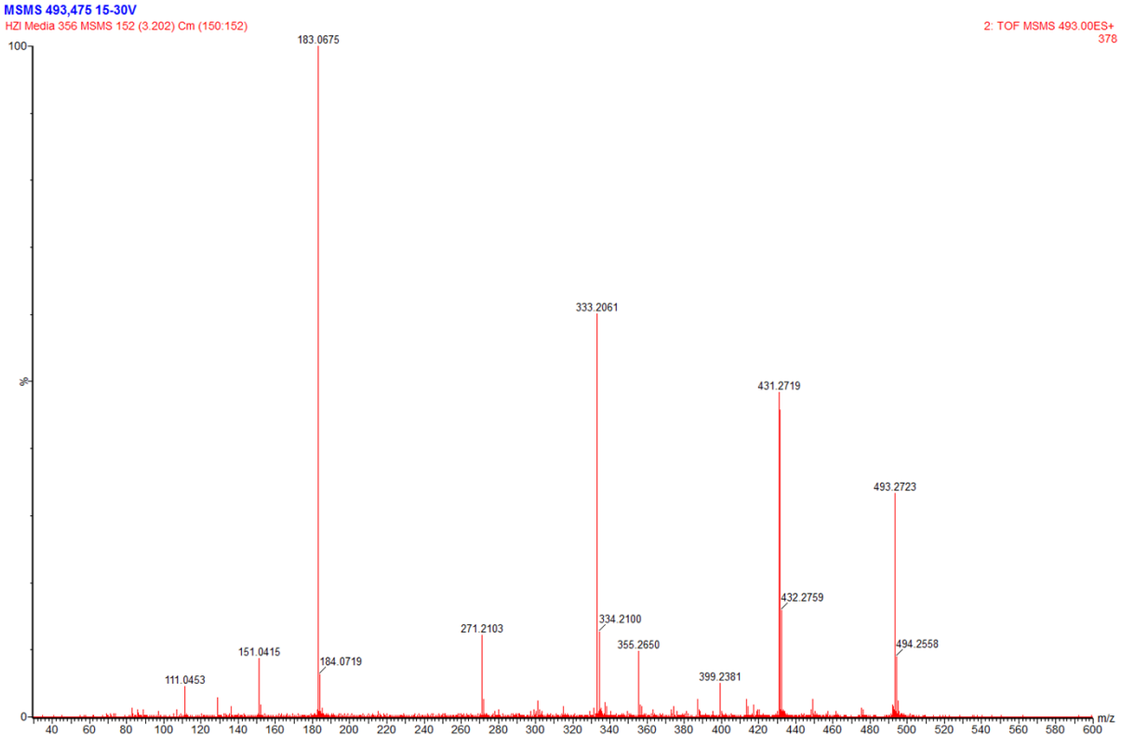
171.2884
168.0190
153.0189
135.4715
130.4721
125.9909
84.3762
80.6095
77.4277
57.8626
51.2000
47.7552
47.6809
36.1484
36.1027
34.2146
33.8334
20.2738
18.9172
15.8536
15.6116
12.9500
12.8052
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

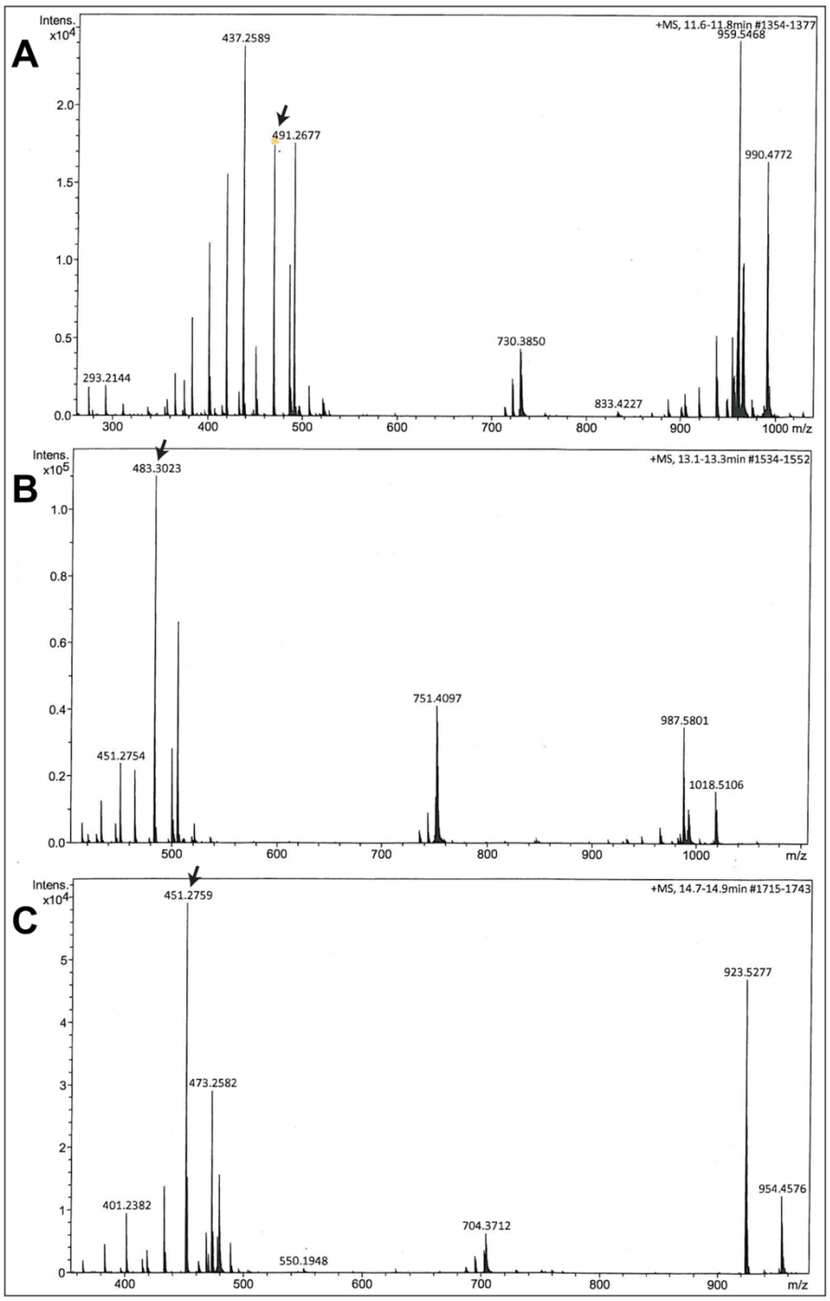
MS-MS spectrum carolacton **1** [precursor ion: $M+Na^+$ (491 Da); collision energy: 15-30 eV].



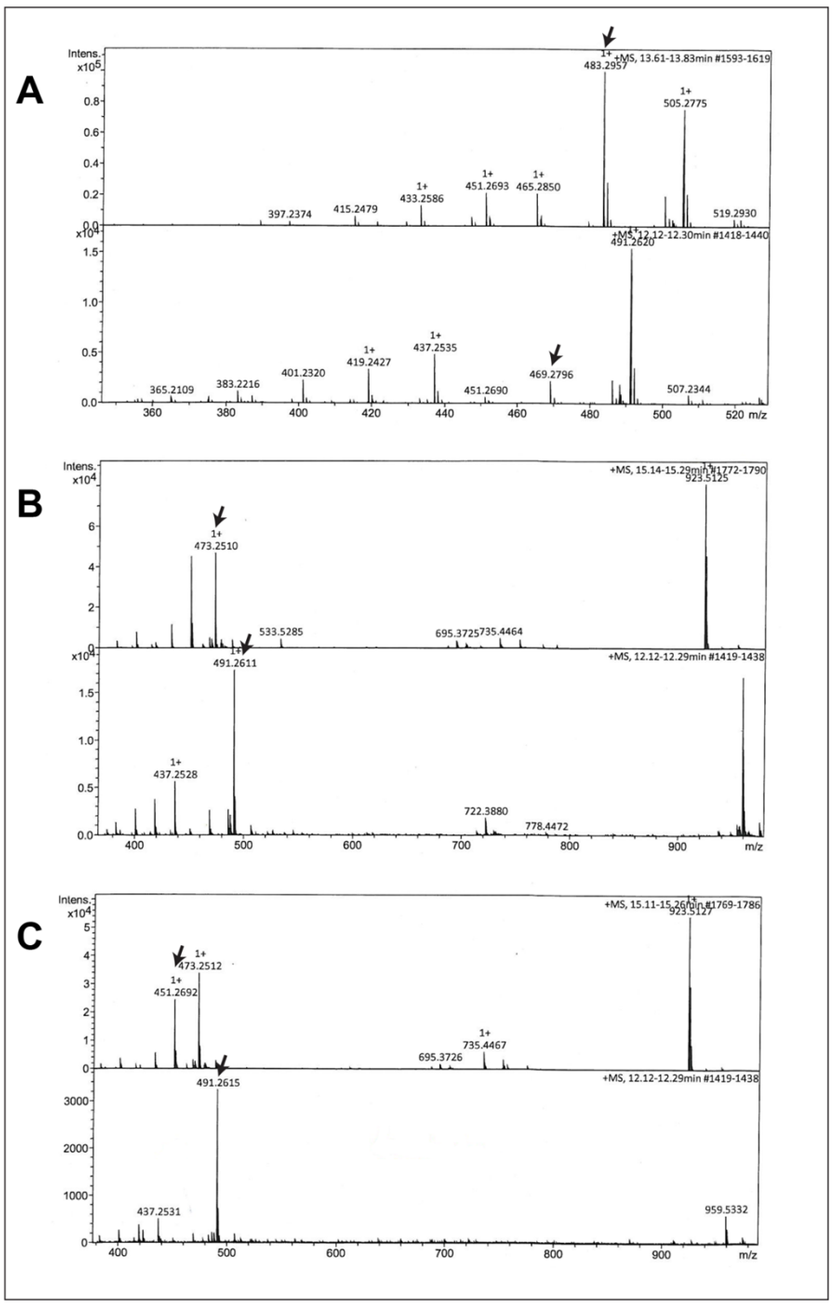

MS-MS spectrum of derivative **4**/media-supernatant [precursor ion: $M+Na^+$ (493 Da); collision energy: 15-30 eV].



**Mass spectrometric data of the reference sample of carolacton 1 and its derivatives 5 and 7 (A) and after re-extraction from the bioassay (B): A** The m/z peaks of the reference samples corresponding to carolacton **1** at a mass of 469.27, **5** at 483.30 and **7** at 451.27 are shown in panel A, B & C. The masses refer to the $[M+H^+]$ and are indicated by arrows. Some of the peaks include those corresponding to $[2M+H^+]$, $[M+Na^+]$ or $[M-H_2O]$. The rest of the peaks were analyzed and either generated as a result of the ionisation while the rest are not found to be related to the samples under study.



**Mass spectrometric data of carolacton 1 and derivatives 5 and 7 re-extracted from the biofilm:** Panels A&B show the m/z analysis from the supernatant of the biofilm treated with **5** and **7**, respectively, and panel C shows the m/z analysis from the biofilm treated with **7**. The peaks corresponding to **5** and **7** are indicated in the top panels of all figures. The hydrolyzed carolacton **1** detected in the assay is denoted in all bottom panels and indicated by the arrows at mass 469.27 [M+H$^+$] in panel A and 491.26 [M+Na$^+$] in B&C, respectively.



## 4. References (Supporting Information)

[S1] Reck, Rutz, K., Kunze, B., Tomasch, J., Surapaneni, S.K., Schulz, S., and Wagner-Döbler, I. (2011). The biofilm inhibitor carolacton disturbs membrane integrity and cell division of Streptococcus mutans through the serine/threonine protein kinase PknB. Journal of bacteriology 193, 5692-5706.

[S2] Schmidt, T., and Kirschning, A. (2012). Totalsynthese von Carolacton, einem hochwirksamen Inhibitor von Biofilmen. Angewandte Chemie 124, 1087-1091; Total Synthesis of Carolacton, a Highly Potent Biofilm Inhibitor", Angewandte Chemie International Edition 51, 1063-1066.

[S3] Schmidt, T. (2009). Untersuchungen zur Struturaufklärung von Carolacton, Diploma thesis, Hannover.

[S4] Wan, Z.-K.; Choi, H.-w.; Kang, F. A.; Nakajima, K.; Demeke, D.; Kishi, Y. (2002). Asymmetric Ni(II)/Cr(II)-Mediated Coupling Reaction: Stoichiometric Process, Organic Letters 4, 4431-4434.

[S5] Jansen, R., Irschik, H., Huch, V., Schummer, D., Steinmetz, H., Bock, M., Schmidt, T., Kirschning, A., and Müller, R. (2010). Carolacton – A Macrolide Ketocarbonic Acid that Reduces Biofilm Formation by the Caries- and Endocarditis-Associated Bacterium Streptococcus mutans. European Journal of Organic Chemistry 2010, 1284-1289.