Supplementary information

The lactam strategy in question: Synthesis and biological evaluation of "carolactam"

Jonas Ammermann[a], Thomas Schmidt[a], Jannik Donner[b], Michael Reck[b], Marly Dalton[c], Nico Stumpp[c], Meike Stiesch[c], Irene Wagner-Döbler[b] and Andreas Kirschning[a]*

---

1. Supporting information (Chemical Syntheses)

1.1. General Information

All reactions were performed in oven dried glassware under an atmosphere of nitrogen gas unless otherwise stated. ^{1}H NMR spectra were recorded at 400 MHz with a Bruker Avance-400 or at 500 MHz with a Bruker DRX-500 spectrometer at room temperature. ^{13}C NMR spectra were recorded at 100 MHz with a Bruker Avance-400 and at 125 MHz with a BRUKER DRX-500 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. 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 *c* = centered. 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). For interpretation of spectra, supporting ^{1}H-^{1}H correlation (COSY) and ^{1}H-^{13}C correlation (phase-sensitive HSQC, HMBC) experiments were performed. High resolution mass spectra are obtained with a Micromass LCT via loop-mode injection from a Waters (Alliance 2695) HPLC system. Alternatively a Micromass Q-TOF in combination with a Waters Aquity Ultraperformance LC system is employed. Ionization is achieved by ESI. Modes of ionization, calculated and found mass are given. Analytical thin-layer chromatography was performed using precoated ALUGRAM SIL G/UV254 plates (Macherey-Nagel) and the spots were visualized with UV light at 254 nm or alternatively by staining with permanganate or 4-methoxybenzaldehyde solutions. Commercially available reagents were used as supplied. Column chromatography was performed with silica gel (40-60 μm) from Macherey-Nagel. Eluents used for chromatography were distilled prior to use. All reagents were purchased from Aldrich or Acros.

Isolation of azides **11a/11b** was achieved by preparative high performance liquid chromatography using a Gilson HPLC system (pump 331/332) with additional Merck Hitachi split pump (pump L-6200A, UV/VIS detector L-4250) with a column from Macherey-Nagel: Nucleodur C18 ISIS 5 µm, 250 × 21 mm, with guard column, 40 × 21 mm. Operating conditions and retention times (t_R) are reported in the experimental part. Isolation of "carolactam" (**5**) was achieved by semipreparative high performance liquid chromatography using a Waters Alliance 2695 HPLC system (diode array detector, λ= 200-350 nm) with a column from Macherey-Nagel: Nucleodur C18 HTec 5 µm, 250 × 8 mm. Operating conditions and retention times (t_R) are reported in the experimental part. Optical rotations [α] were measured on a Polarimeter 341 (Perkin Elmer) at a wavelength of 589 nm and are given in 10^{-1} cm^2 g^{-1}. Carolacton derivative **4** was reported by Stumpp et al. 2011 [S1] and advanced intermediates **6**, **7**, **9a**, **9b** and **10** were reported before (Schmidt, 2012) [S2]. Ligand **8** was prepared according to Kishi et al. 2002 [S3].

1.2. Analytical data of azide 11a

$[\alpha]_D^{25}$ = -27.4° (c = 0.70, CH_2Cl_2); R_f = 0.64 (petroleum ether : ethyl acetate = 3:1); **HRMS** (ESI): *m/z* for $C_{41}H_{65}N_3O_9Na$ $[M+Na]^+$: calculated 766.4619, found 766.4617; **^{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]
171.30	1	s	-	-	-
170.08	19	s	-	-	-
159.76	Ar-PMB	s	-	-	-
141.52	15	d	5.63	ddd	15.4, 7.6, 0.5
133.55	7	d	5.54	d_{br}	9.2
132.62	8	s	-	-	-
131.77	Ar-PMB	s	-	-	-
129.47 (2C)	Ar-PMB	d	7.39	d, 2H	8.6
123.50	16	d	5.44	ddd	15.4, 7.3, 0.6
114.14 (2C)	Ar-PMB	d	6.86	d, 2H	8.6
111.23	acetonide	s	-	-	-
83.69	5	d	3.36-3.33	m	-
79.98	3	d	3.83	ddd	7.4, 6.1, 4.0
79.83	*tert*-butyl	s	-	-	-
79.21	17	d	4.63-4.60	m	-
78.50	18	d	4.52	d	7.0
77.25	9	d	3.29	d	9.5
75.10	PMB-CH_2	t	4.60 (H_a)	d	10.6
			4.50 (H_b)	d	10.6
56.92	20-OMe	q	3.35	s, 3H	-
54.82	PMB-OMe	q	3.32	s, 3H	-
51.01	Me-ester	q	3.34	s, 3H	-
39.84	4	d	2.19	m_c	-
37.93	2	t	2.50 (H_a)	dd	15.3, 7.4
			2.40 (H_b)	dd	15.3, 4.0
37.20	13	t	1.19-1.14 (H_a)	m	-
			1.10-1.05 (H_b)	m	-

36.75	14	d	2.03-1.95	m	-
36.67	6	d	2.77	m_c	-
34.98	10	d	1.55-1.47	m	-
33.51	11	t	1.19-1.14 (H_a)	m	-
			0.85-0.77 (H_b)	m	-
28.19	*tert*-butyl	q	1.43	s, 9H	-
27.38	acetonide	q	1.74	s, 3H	-
25.89	acetonide	q	1.27	s, 3H	-
24.37	12	t	1.19-1.14 (H_a)	m	-
			1.10-1.05 (H_b)	m	-
20.50	25-Me	q	0.89	d, 3H	6.8
18.49	22-Me	q	1.00	d, 3H	6.8
16.57	24-Me	q	0.94	d, 3H	6.5
12.21	23-Me	q	1.64	d, 3H	1.0
10.65	21-Me	q	1.12	d, 3H	6.9

1.3. Analytical data of azide 11b

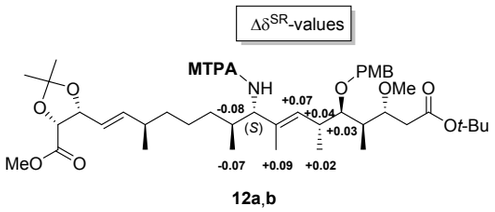
$[\alpha]_D^{24}$ = -21.3° (c = 2.24, CH_2Cl_2); R_f = 0.65 (petroleum ether : ethyl acetate = 3:1); **HRMS** (ESI): *m/z* $C_{41}H_{65}N_3O_9Na$ $[M+Na]^+$: calculated 766.4619, found 766.4617; **^{1}H-NMR** (C_6D_6, 400 MHz, C_6D_5H = 7.16 ppm); **^{13}C-NMR** (C_6D_6, 100 MHz, C_6D_6 = 128.06 ppm)

δ-C [ppm]	#	mult.	δ –H [ppm]	mult.	*J* [Hz]
171.27	1	s	-	-	-
170.12	19	s	-	-	-
159.69	Ar-PMB	s	-	-	-
141.64	15	d	5.64	ddd	15.5, 7.7, 0.6
135.09	8	s	-	-	-
131.95	Ar-PMB	s	-	-	-
131.76	7	d	5.54	dd	9.8, 0.9
129.16 (2C)	Ar-PMB	d	7.32	d, 2H	8.6
123.49	16	d	5.46	ddd	15.5, 7.3, 0.8
114.11 (2C)	Ar-PMB	d	6.84	d, 2H	8.6
111.20	acetonide	s	-	-	-
83.52	5	d	3.37-3.33	m	-
79.98	3	d	3.76	td	6.9, 4.2
79.94	*tert*-butyl	s	-	-	-
79.28	17	d	4.61	td	7.3, 0.6
78.51	18	d	4.52	d	7.3
77.06	9	d	3.30	d	7.3
74.71	PMB-CH_2	t	4.55 (H_a)	d	10.8
			4.46 (H_b)	d	10.8
56.79	20-OMe	q	3.27	s, 3H	-
54.82	PMB-OMe	q	3.31	s, 3H	-
51.03	Me-ester	q	3.34	s, 3H	-
39.86	4	d	2.11-2.05	m	-
37.87	2	t	2.46 (H_a)	dd	15.4, 6.9

			2.39 (H_b)	dd	15.4, 4.2
37.27	13	t	1.25-1.18	m, 2H	-
36.82	6	d	2.76	m_c	-
36.73	14	d	2.06-1.95	m	-
34.68	10	d	1.52-1.46	m	-
33.56	11	t	1.67-1.62 (H_a)	m	-
			0.97-0.92 (H_b)	m	-
28.17	*tert*-butyl	q	1.42	s, 9H	-
27.39	acetonide	q	1.73	s, 3H	-
25.90	acetonide	q	1.27	s, 3H	-
24.64	12	t	1.18-1.12 (H_a)	m	-
			1.04-0.99 (H_b)	m	-
20.66	25-Me	q	0.91	d, 3H	6.7
19.36	22-Me	q	1.08	d, 3H	6.9
16.64	24-Me	q	0.67	d, 3H	6.7
11.75	23-Me	q	1.60	d, 3H	0.9
10.48	21-Me	q	1.08	d, 3H	6.9

1.4. Analytical data of mosher amides 12a and 12b

12a: R_f = 0.45 (petroleum ether : ethyl acetate = 3:1); **HRMS** (ESI): *m/z* for $C_{51}H_{74}F_3NO_{11}Na$ $[M+Na]^+$: calculated 956.5112, found 956.5111; **12b**: R_f = 0.46 (petroleum ether : ethyl acetate = 3:1); **HRMS** (ESI): *m/z* for $C_{51}H_{74}F_3NO_{11}Na$ $[M+Na]^+$: calculated 956.5112, found 956.5113.



12a,**b**

Selected ^{1}H-NMR data used for the determination of the absolute configuration at C9 [S4]:

$CDCl_3$	(*R*)-MTPA-amide **12b**	(*S*)-MTPA-amide **12a**	
#	δ_H [ppm]	δ_H [ppm]	$\Delta\delta^{SR}$
C5	3.29	3.32	+0.03
C6	2.64	2.68	+0.04
C7	5.41	5.48	+0.07
C22	0.95	0.97	+0.02
C23	1.54	1.63	+0.09
C10	1.70	1.62	-0.08
C24	0.86	0.79	-0.07

1.5. Analytical data of lactam 13

$[\alpha]_D^{23}$ = -34.9° (c = 0.35, CH_2Cl_2); R_f = 0.17 (petroleum ether : ethyl acetate = 4:1); **HRMS** (ESI): *m/z* for $C_{40}H_{63}NO_8Na$ $[M+Na]^+$: calculated 708.4451, found 708.4457; **^{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]
171.51	1	s	-	-	-
167.89	19	s	-	-	-
159.64	Ar-PMB	s	-	-	-
136.16	8	s	-	-	-
135.42	15	d	5.85	ddd	15.3, 9.9, 1.6
131.88	Ar-PMB	s	-	-	-
130.16	7	d	5.53	dd	9.4, 1.1
129.31 (2C)	Ar-PMB	d	7.32	d, 2H	8.7
123.24	16	d	5.44	dd	15.3, 3.8
114.08 (2C)	Ar-PMB	d	6.83	d, 2H	8.7
110.50	acetonide	s	-	-	-
83.65	5	d	3.32-3.29	m	-
79.99/79.98[a]	3	d	3.77	ddd	7.6, 6.0, 3.9
79.99/79.98[a]	*tert*-butyl	s	-	-	-
79.79	18	d	3.98	d	6.9
77.35	17	d	4.62	ddd	6.9, 3.8, 1.6
74.43	PMB-CH_2	t	4.53 (H_a)	dd	10.9
			4.44 (H_b)	d	10.9
61.23	9	d	3.86	m_c	-
56.96	20-OMe	q	3.30	s, 3H	-
54.78	PMB-OMe	q	3.30	s, 3H	-
39.30	4	d	2.14-2.06	m	-
37.89	2	t	2.46 (H_a)	dd	15.2, 7.6
			2.37 (H_b)	dd	15.2, 3.9
37.30	14	d	2.25-2.18	m	-
36.18	6	d	2.76	dqd	9.4, 6.9, 4.0
34.99	13	t	1.38-1.30 (H_a)	m	-
			1.25-1.18 (H_b)	m	-
32.89	10	d	2.18-2.13	m	-
30.91	11	t	1.38-1.30 (H_a)	m	-
			1.25-1.18 (H_b)	m	-
28.16	*tert*-butyl	q	1.41	s, 9H	-
27.14	acetonide	q	1.80	s, 3H	-
25.58	acetonide	q	1.27	s, 3H	-
22.06	25-Me	q	0.99	d, 3H	6.6
20.52	12	t	1.38-1.30 (H_a)	m	-
			1.25-1.18 (H_b)	m	-
17.91	22-Me	q	0.98	d, 3H	6.9
17.76	24-Me	q	0.84	d, 3H	6.8
14.95	23-Me	q	1.80	d, 3H	1.1
10.59	21-Me	q	1.09	d, 3H	7.0
-	NH	-	5.19	d_{br}	7.3

a) The assignment of these carbon atoms was not possible.

1.6. Analytical data of ketone 14

$[\alpha]_D^{27}$ = -174.0° (c = 0.44, CH_2Cl_2); R_f = 0.33 (petroleum ether : ethyl acetate = 2:1); HRMS (ESI): *m/z* for $C_{32}H_{53}NO_7Na$ $[M+Na]^+$: calculated 586.3720, found 586.3718; **^{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]
212.23	5	s	-	-	-
170.50	1	s	-	-	-
168.06	19	s	-	-	-
138.63	8	s	-	-	-
135.38	15	d	5.83	ddd	15.3, 9.9, 1.7
127.32	7	d	5.18	dd	10.1, 1.1
123.38	16	d	5.47	dd	15.3, 3.7
110.37	acetonide	s	-	-	-
80.71	3	d	3.89	ddd	8.0, 6.6, 4.2
80.15	*tert*-butyl	s	-	-	-
79.68	18	d	4.04	d	7.1
77.24	17	d	4.62	ddd	7.1, 3.7, 1.7
62.51	9	d	3.86	m_c	-
57.90	20-OMe	q	3.20	s, 3H	-
47.88	4	d	3.03	dq	8.0, 6.9
47.60	6	d	3.53	dq	10.1, 6.7
37.83	2	t	2.42 (H_a)	dd	15.4, 4.2
			2.28 (H_b)	dd	15.4, 6.6
37.62	14	d	2.27-2.15	m	-
34.97	13	t	1.35-1.30 (H_a)	m	-
			1.21-1.13 (H_b)	m	-
32.31	10	d	2.27-2.15	m	-
31.02	11	t	1.35-1.30 (H_a)	m	-
			1.21-1.13 (H_b)	m	-
28.12	*tert*-butyl	q	1.38	s, 9H	-
27.10	acetonide	q	1.74	s, 3H	-
25.30	acetonide	q	1.25	s, 3H	-
22.10	25-Me	q	0.99	d, 3H	6.6
21.02	12	t	1.35-1.30 (H_a)	m	-
			1.21-1.13 (H_b)	m	-
17.61	24-Me	q	0.78	d, 3H	6.9
15.95	22-Me	q	1.15	d, 3H	6.7
13.82	23-Me	q	1.75	d, 3H	1.1
12.89	21-Me	q	0.96	d, 3H	6.9
-	NH	-	5.27	d_{br}	6.1

1.7. Analytical data of "carolactam" (5)

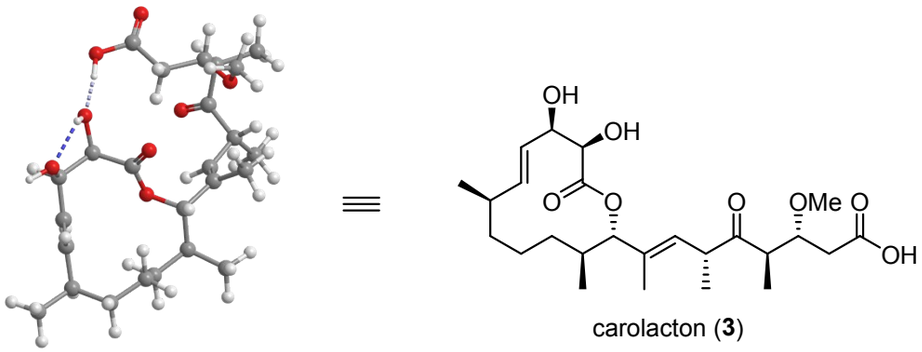
$[\alpha]_D^{23}$ = -164.4° (c = 0.25, CH_2Cl_2); R_f = 0.20 (CH_2Cl_2 : MeOH = 10:1); **HRMS** (ESI): *m/z* for $C_{25}H_{41}NO_7Na$ $[M+Na]^+$: calculated 490.2781, found 490.2780; **^{1}H-NMR** ($CDCl_3$, 500 MHz, $CHCl_3$ = 7.26 ppm); **^{13}C-NMR** ($CDCl_3$, 125 MHz, $CDCl_3$ = 77.16 ppm)

δ-C [ppm]	#	mult.	δ –H [ppm]	mult.	*J* [Hz]
214.28	5	s	-	-	-
173.52	1	s	-	-	-
171.23	19	s	-	-	-
137.18	8	s	-	-	-
136.10	15	d	5.54	dd	15.2, 9.7
126.69	7	d	5.29	d_{br}	10.1
125.60	16	d	5.46	d	15.2
80.46	3	d	3.66	dt	9.0, 4.5
73.49	18	d	4.11	s_{br}	7.1
73.33	17	d	4.45	s_{br}	-
58.78	9	d	4.03	m_c	-
57.78	20-OMe	q	3.31	s, 3H	-
47.54	6	d	3.44	dq	10.1, 6.9
46.14	4	d	3.15	dq	9.0, 6.8
36.05	14	d	2.34-2.26	m	-
35.13	13	t	1.43-1.28	m, 2H	-
34.69	2	t	2.73 (H_a)	dddd	15.0, 4.5
			2.41 (H_b)	dd	15.0, 4.5
33.26	10	d	1.96-1.88	m	-
29.63	11	t	1.68-1.58 (H_a)	m	-
			1.10-1.04 (H_b)	m	-
22.11	25-Me	q	0.98	d, 3H	6.6
19.34	12	t	1.28-1.22 (H_a)	m	-
			1.10-1.04 (H_b)	m	-
16.71	24-Me	q	0.85	d, 3H	6.7
16.04	22-Me	q	1.13	d, 3H	6.8
14.90	23-Me	q	1.19	s_{br}	-
13.28	21-Me	q	0.96	d, 3H	6.9
-	NH	-	5.82	s_{br}	-

2. Supporting information (Molecular modeling)

Molecular modeling studies were performed using Macromodel/Maestro (version 9.8) and the OPLS2005 force field together with mixed *low-frequency-mode conformational search* (42 000 steps) using chloroform as solvent background. Structures were subjected to a minimization procedure to the nearest local minimum prior to the generation of new local energy conformers.

Lowest energy conformer of carolacton (**3**)



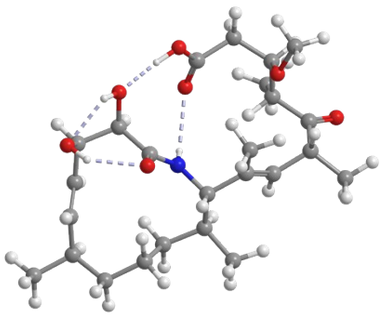
carolacton (**3**)

```
@<TRIPOS>MOLECULE
carolacton
73   73    1
SMALL
USER_CHARGES


@<TRIPOS>ATOM
      1 C1          -3.6541   -3.5607   -5.1752 C.3       1 UNK        -0.0600
      2 C2          -4.8635   -2.5989   -5.1229 C.3       1 UNK        -0.1200
      3 C3          -5.0843   -1.7351   -6.3819 C.3       1 UNK        -0.1200
      4 C4          -3.8923   -0.8223   -6.7393 C.3       1 UNK        -0.1200
      5 C5          -3.6347   -4.3864   -6.4503 C.2       1 UNK        -0.1150
      6 C6          -4.0503   -0.0134   -8.0496 C.3       1 UNK        -0.0600
      7 C7          -4.3371   -0.9022   -9.2899 C.3       1 UNK         0.2890
      8 O8          -3.4393   -2.0077   -9.3015 O.3       1 UNK        -0.3300
      9 C9          -3.7542   -3.1250   -9.9820 C.2       1 UNK         0.5100
     10 C10         -2.6277   -4.1783   -9.9537 C.3       1 UNK         0.2050
     11 C11         -2.6070   -5.0826   -8.6905 C.3       1 UNK         0.2050
     12 C12         -2.6597   -4.3179   -7.3760 C.2       1 UNK        -0.1150
     13 C13         -4.2412   -0.1391  -10.6093 C.2       1 UNK        -0.0690
     14 C14         -3.0418    0.1981  -11.1288 C.2       1 UNK        -0.1150
     15 C15         -2.7512    0.8515  -12.4711 C.3       1 UNK        -0.0600
     16 C16         -1.8351   -0.0913  -13.2674 C.2       1 UNK         0.4700
     17 C17         -2.3180   -0.6575  -14.6135 C.3       1 UNK        -0.0600
     18 C18         -3.4005   -1.7665  -14.4941 C.3       1 UNK         0.1700
     19 C19         -3.0085   -2.9779  -13.6019 C.3       1 UNK        -0.1200
     20 C20         -1.8869   -3.8527  -14.1733 C.2       1 UNK         0.5200
     21 O21         -0.7271   -0.4015  -12.8269 O.2       1 UNK        -0.4700
     22 C22         -2.0996    2.2307  -12.2814 C.3       1 UNK        -0.1800
     23 C23         -2.7215    0.4587  -15.5959 C.3       1 UNK        -0.1800
     24 O24         -1.3775   -3.6673  -15.2760 O.2       1 UNK        -0.4400
     25 O25         -1.5370   -4.8649  -13.3301 O.3       1 UNK        -0.5300
     26 C26         -5.5808    0.1404  -11.2709 C.3       1 UNK        -0.1800
     27 O27         -2.7875   -5.0907  -11.0267 O.3       1 UNK        -0.6830
     28 O28         -3.6476   -6.0520   -8.7873 O.3       1 UNK        -0.6830
     29 O29         -4.8001   -3.3134  -10.6088 O.2       1 UNK        -0.4300
     30 C30         -3.6191   -4.4757   -3.9436 C.3       1 UNK        -0.1800
     31 C31         -5.0909    1.1092   -7.8820 C.3       1 UNK        -0.1800
     32 O32         -4.5988   -1.1591  -14.0156 O.3       1 UNK        -0.4000
```

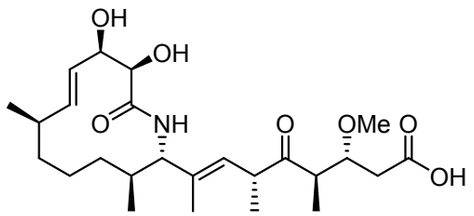
```
      33 C33        -5.7996   -1.8081  -14.4113 C.3       1 UNK         0.1100
      34 H34        -2.7436   -2.9606   -5.1488 H         1 UNK         0.0600
      35 H35        -5.7714   -3.1784   -4.9488 H         1 UNK         0.0600
      36 H36        -4.7624   -1.9447   -4.2556 H         1 UNK         0.0600
      37 H37        -5.9727   -1.1222   -6.2278 H         1 UNK         0.0600
      38 H38        -5.3243   -2.3917   -7.2177 H         1 UNK         0.0600
      39 H39        -2.9874   -1.4220   -6.8250 H         1 UNK         0.0600
      40 H40        -3.7017   -0.1394   -5.9105 H         1 UNK         0.0600
      41 H41        -4.4886   -5.0301   -6.6078 H         1 UNK         0.1150
      42 H42        -3.0892    0.4756   -8.2160 H         1 UNK         0.0600
      43 H43        -5.3538   -1.2891   -9.2005 H         1 UNK         0.0300
      44 H44        -1.6773   -3.6528  -10.0577 H         1 UNK         0.0600
      45 H45        -1.6637   -5.6318   -8.6856 H         1 UNK         0.0600
      46 H46        -1.8273   -3.6496   -7.2036 H         1 UNK         0.1150
      47 H47        -2.1576   -0.0427  -10.5540 H         1 UNK         0.1150
      48 H48        -3.6713    0.9759  -13.0351 H         1 UNK         0.0600
      49 H49        -1.4320   -1.1234  -15.0450 H         1 UNK         0.0600
      50 H50        -3.5837   -2.1328  -15.5065 H         1 UNK         0.0300
      51 H51        -2.7175   -2.6353  -12.6112 H         1 UNK         0.0600
      52 H52        -3.8682   -3.6296  -13.4543 H         1 UNK         0.0600
      53 H53        -1.1610    2.1589  -11.7297 H         1 UNK         0.0600
      54 H54        -1.8845    2.6994  -13.2422 H         1 UNK         0.0600
      55 H55        -2.7599    2.8999  -11.7296 H         1 UNK         0.0600
      56 H56        -2.8269    0.0615  -16.6053 H         1 UNK         0.0600
      57 H57        -1.9702    1.2484  -15.6329 H         1 UNK         0.0600
      58 H58        -3.6736    0.9147  -15.3234 H         1 UNK         0.0600
      59 H59        -2.0380   -4.8978  -12.5077 H         1 UNK         0.4500
      60 H60        -6.2525    0.6373  -10.5715 H         1 UNK         0.0600
      61 H61        -6.0439   -0.8012  -11.5669 H         1 UNK         0.0600
      62 H62        -5.5088    0.7705  -12.1541 H         1 UNK         0.0600
      63 H63        -3.4610   -5.6724  -10.6680 H         1 UNK         0.4180
      64 H64        -3.5450   -6.6686   -8.0745 H         1 UNK         0.4180
      65 H65        -4.4997   -5.1175   -3.8962 H         1 UNK         0.0600
      66 H66        -2.7392   -5.1200   -3.9563 H         1 UNK         0.0600
      67 H67        -3.5838   -3.8935   -3.0222 H         1 UNK         0.0600
      68 H68        -5.0816    1.7887   -8.7345 H         1 UNK         0.0600
      69 H69        -6.1008    0.7093   -7.7897 H         1 UNK         0.0600
      70 H70        -4.8870    1.7084   -6.9944 H         1 UNK         0.0600
      71 H71        -5.8881   -1.8508  -15.4976 H         1 UNK         0.0300
      72 H72        -5.8703   -2.8200  -14.0135 H         1 UNK         0.0300
      73 H73        -6.6539   -1.2470  -14.0328 H         1 UNK         0.0300

@<TRIPOS>BOND
     1    1    2 1
     2    1    5 1
     3    1   30 1
     4    1   34 1
     5    2    3 1
     6    2   35 1
     7    2   36 1
     8    3    4 1
     9    3   37 1
    10    3   38 1
    11    4    6 1
    12    4   39 1
    13    4   40 1
    14    5   12 2
    15    5   41 1
    16    6    7 1
    17    6   31 1
    18    6   42 1
    19    7    8 1
    20    7   13 1
    21    7   43 1
    22    8    9 1
    23    9   10 1
    24    9   29 2
```

```
    25   10   11 1
    26   10   27 1
    27   10   44 1
    28   11   12 1
    29   11   28 1
    30   11   45 1
    31   12   46 1
    32   13   14 2
    33   13   26 1
    34   14   15 1
    35   14   47 1
    36   15   16 1
    37   15   22 1
    38   15   48 1
    39   16   17 1
    40   16   21 2
    41   17   18 1
    42   17   23 1
    43   17   49 1
    44   18   19 1
    45   18   32 1
    46   18   50 1
    47   19   20 1
    48   19   51 1
    49   19   52 1
    50   20   24 2
    51   20   25 1
    52   22   53 1
    53   22   54 1
    54   22   55 1
    55   23   56 1
    56   23   57 1
    57   23   58 1
    58   25   59 1
    59   26   60 1
    60   26   61 1
    61   26   62 1
    62   27   63 1
    63   28   64 1
    64   30   65 1
    65   30   66 1
    66   30   67 1
    67   31   68 1
    68   31   69 1
    69   31   70 1
    70   32   33 1
    71   33   71 1
    72   33   72 1
    73   33   73 1
@<TRIPOS>SUBSTRUCTURE
     1 UNK         1 GROUP            0       ****    0 ROOT
```

Lowest energy conformer of “carolactam” (**5**)



≡



carolactam (**5**)

```
@<TRIPOS>MOLECULE
carolactam
74   74    1
SMALL
USER_CHARGES


@<TRIPOS>ATOM
      1 C1          -2.8206   -0.8612   -3.5149 C.3       1 UNK         -0.0600
      2 C2          -1.7491    0.1973   -3.1648 C.3       1 UNK         -0.1200
      3 C3          -0.2994   -0.1200   -3.5974 C.3       1 UNK         -0.1200
      4 C4           0.2913   -1.4150   -2.9963 C.3       1 UNK         -0.1200
      5 C5          -2.7238   -1.3079   -4.9620 C.2       1 UNK         -0.1150
      6 C6           1.7850   -1.6916   -3.3311 C.3       1 UNK         -0.0600
      7 C7           2.0846   -1.8402   -4.8574 C.3       1 UNK          0.1400
      8 N8           1.1460   -2.7717   -5.4798 N.am      1 UNK         -0.5000
      9 C9           0.2862   -2.4763   -6.4615 C.2       1 UNK          0.5000
     10 C10         -0.7340   -3.5617   -6.8639 C.3       1 UNK          0.2050
     11 C11         -2.2015   -3.0803   -6.7372 C.3       1 UNK          0.2050
     12 C12         -2.4873   -2.5794   -5.3291 C.2       1 UNK         -0.1150
     13 C13          3.4949   -2.2921   -5.2541 C.2       1 UNK          0.0000
     14 C14          4.3458   -2.9297   -4.4176 C.2       1 UNK         -0.1150
     15 C15          5.7135   -3.5116   -4.7570 C.3       1 UNK         -0.0600
     16 C16          5.6659   -5.0509   -4.6415 C.2       1 UNK          0.4700
     17 C17          4.4776   -5.8048   -5.2779 C.3       1 UNK         -0.0600
     18 C18          4.6518   -6.0294   -6.8068 C.3       1 UNK          0.1700
     19 C19          3.4228   -6.6825   -7.5035 C.3       1 UNK         -0.1200
     20 C20          2.1038   -5.9134   -7.3447 C.2       1 UNK          0.5200
     21 O21          6.5585   -5.6665   -4.0543 O.2       1 UNK         -0.4700
     22 C22          6.8096   -2.8818   -3.8778 C.3       1 UNK         -0.1800
     23 C23          4.2165   -7.1282   -4.5247 C.3       1 UNK         -0.1800
     24 O24          1.5491   -5.7350   -6.2614 O.2       1 UNK         -0.4400
     25 O25          1.6181   -5.4744   -8.5378 O.3       1 UNK         -0.5300
     26 C26          3.8274   -1.9768   -6.7073 C.3       1 UNK         -0.1800
     27 O27         -0.5399   -3.9165   -8.2192 O.3       1 UNK         -0.6830
     28 O28         -2.4488   -2.0901   -7.7291 O.3       1 UNK         -0.6830
     29 O29          0.2589   -1.3849   -7.0341 O.2       1 UNK         -0.5000
     30 C30         -4.2334   -0.3505   -3.2018 C.3       1 UNK         -0.1800
     31 C31          2.6855   -0.6323   -2.6611 C.3       1 UNK         -0.1800
     32 O32          4.9245   -4.7836   -7.4426 O.3       1 UNK         -0.4000
     33 C33          5.6905   -4.8703   -8.6364 C.3       1 UNK          0.1100
     34 H34         -2.6456   -1.7328   -2.8822 H         1 UNK          0.0600
     35 H35         -2.0309    1.1467   -3.6230 H         1 UNK          0.0600
     36 H36         -1.7665    0.3773   -2.0890 H         1 UNK          0.0600
     37 H37          0.3201    0.7276   -3.3058 H         1 UNK          0.0600
     38 H38         -0.2472   -0.1521   -4.6847 H         1 UNK          0.0600
     39 H39         -0.3106   -2.2661   -3.3126 H         1 UNK          0.0600
     40 H40          0.1767   -1.3788   -1.9122 H         1 UNK          0.0600
     41 H41         -2.7791   -0.5275   -5.7077 H         1 UNK          0.1150
     42 H42          2.0024   -2.6465   -2.8521 H         1 UNK          0.0600
     43 H43          1.9546   -0.8444   -5.2862 H         1 UNK          0.0600
     44 H44          1.1704   -3.7325   -5.1661 H         1 UNK          0.3000
     45 H45         -0.6005   -4.4427   -6.2361 H         1 UNK          0.0600
     46 H46         -2.8773   -3.9158   -6.9269 H         1 UNK          0.0600
```

```
      47 H47        -2.4115   -3.3325   -4.5574 H         1 UNK         0.1150
      48 H48         4.0516   -3.1072   -3.3952 H         1 UNK         0.1150
      49 H49         5.9697   -3.3025   -5.7911 H         1 UNK         0.0600
      50 H50         3.6049   -5.1785   -5.1189 H         1 UNK         0.0600
      51 H51         5.5116   -6.6897   -6.9379 H         1 UNK         0.0300
      52 H52         3.2645   -7.6839   -7.1049 H         1 UNK         0.0600
      53 H53         3.6228   -6.8333   -8.5636 H         1 UNK         0.0600
      54 H54         6.8381   -1.7995   -4.0050 H         1 UNK         0.0600
      55 H55         7.7955   -3.2674   -4.1406 H         1 UNK         0.0600
      56 H56         6.6439   -3.0907   -2.8201 H         1 UNK         0.0600
      57 H57         4.2226   -6.9783   -3.4442 H         1 UNK         0.0600
      58 H58         3.2430   -7.5466   -4.7775 H         1 UNK         0.0600
      59 H59         4.9765   -7.8752   -4.7568 H         1 UNK         0.0600
      60 H60         0.8092   -4.9655   -8.4287 H         1 UNK         0.4500
      61 H61         4.8839   -2.0587   -6.9478 H         1 UNK         0.0600
      62 H62         3.2781   -2.6448   -7.3713 H         1 UNK         0.0600
      63 H63         3.5336   -0.9555   -6.9498 H         1 UNK         0.0600
      64 H64        -1.0083   -3.2301   -8.6900 H         1 UNK         0.4180
      65 H65        -1.7886   -1.4102   -7.6186 H         1 UNK         0.4180
      66 H66        -4.4849    0.5237   -3.8033 H         1 UNK         0.0600
      67 H67        -4.9822   -1.1174   -3.4036 H         1 UNK         0.0600
      68 H68        -4.3271   -0.0696   -2.1523 H         1 UNK         0.0600
      69 H69         3.7421   -0.8893   -2.7190 H         1 UNK         0.0600
      70 H70         2.5724    0.3452   -3.1296 H         1 UNK         0.0600
      71 H71         2.4445   -0.5217   -1.6035 H         1 UNK         0.0600
      72 H72         5.1816   -5.4437   -9.4106 H         1 UNK         0.0300
      73 H73         5.8604   -3.8673   -9.0280 H         1 UNK         0.0300
      74 H74         6.6652   -5.3223   -8.4482 H         1 UNK         0.0300

@<TRIPOS>BOND
     1    1    2 1
     2    1    5 1
     3    1   30 1
     4    1   34 1
     5    2    3 1
     6    2   35 1
     7    2   36 1
     8    3    4 1
     9    3   37 1
    10    3   38 1
    11    4    6 1
    12    4   39 1
    13    4   40 1
    14    5   12 2
    15    5   41 1
    16    6    7 1
    17    6   31 1
    18    6   42 1
    19    7    8 1
    20    7   13 1
    21    7   43 1
    22    8    9 am   BACKBONE|DICT|INTERRES
    23    8   44 1
    24    9   10 1
    25    9   29 2
    26   10   11 1
    27   10   27 1
    28   10   45 1
    29   11   12 1
    30   11   28 1
    31   11   46 1
    32   12   47 1
    33   13   14 2
    34   13   26 1
    35   14   15 1
    36   14   48 1
    37   15   16 1
```

```
    38   15   22 1
    39   15   49 1
    40   16   17 1
    41   16   21 2
    42   17   18 1
    43   17   23 1
    44   17   50 1
    45   18   19 1
    46   18   32 1
    47   18   51 1
    48   19   20 1
    49   19   52 1
    50   19   53 1
    51   20   24 2
    52   20   25 1
    53   22   54 1
    54   22   55 1
    55   22   56 1
    56   23   57 1
    57   23   58 1
    58   23   59 1
    59   25   60 1
    60   26   61 1
    61   26   62 1
    62   26   63 1
    63   27   64 1
    64   28   65 1
    65   30   66 1
    66   30   67 1
    67   30   68 1
    68   31   69 1
    69   31   70 1
    70   31   71 1
    71   32   33 1
    72   33   72 1
    73   33   73 1
    74   33   74 1
@<TRIPOS>SUBSTRUCTURE
     1 UNK         1 GROUP             0       ****    0 ROOT
```


3. Supporting information (Biological Test Procedures)

3.1. Bacterial strains and culture conditions

S. mutans wild-type strain UA159 (ATCC 700610) was maintained routinely on Todd-Hewitt (TH) agar plates (Difco) and liquid cultures were grown in Todd-Hewitt broth Bacto™(THB). For biofilm growth, THB was supplemented with 0.5% sucrose (THBS). Incubation was at 37°C without agitation under aerobic (with 10% CO_2) conditions. 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). The bacteria were cultivated for 24 hours under aerobic conditions(with 10% CO_2) without shaking. All experiments were run in triplicate.

S. pneumoniae TIGR4 serotype 4 (ATCC BAA-334), used for growth experiments in liquid medium, was routinely grown on Columbia blood agar plates containing 5% sheep blood (BD Biosciences) overnight at 37 °C and 5% CO_2. Cells were then scraped from the plate and used to inoculate Todd-Hewitt-Broth + 1% (w/v) yeast extract (THBY, BD Biosciences) liquid medium, starting from an optical density at 600 nm (OD_{600}) of 0.1. After the initial culture had reached an OD_{600} of 0.15, it was split into equal volumes and Carolacton was added to a final concentration of

0.25 µg/ml. Growth was determined by measuring optical density at 600 nm (OD_{600}). Growth inhibition was calculated as follows:

$$\text{growth inhibition (\%)} = \left(\frac{(\text{OD600 Control} - \text{OD600 Treated})}{\text{OD600 Control}}\right)100\% \qquad (1)$$

Carolacton was solved in methanol and stored 1000-fold concentrated (5.3 mM) in small aliquots in glass vials at −20 °C in the dark.

3.2. Quantitative evaluation of membrane damage

Biofilms were analysed using the LIVE/DEAD *Bac* Light bacterial viability staining kit L13152 (Invitrogen, Molecular Probes, Inc. Eugene, OR, USA) according to the manufacturer's instructions. The kit consists of two stains, propidium iodide and SYTO9, which both stain nucleic acids. When used alone, green fluorescing SYTO9 generally labels all bacteria in a population, whereas red fluorescing propidium iodide only penetrates bacteria with damaged membranes, causing a reduction in the SYTO9 stain fluorescence. Thus with an appropriate mixture of the SYTO9 and Propidium iodide stains, bacteria with intact membranes stain fluorescent green, and bacteria with damaged membranes stain fluorescent red. Staining of biofilms was usually carried out for 15 min in the dark at room temperature with 100 µl of a 1:1 mixture of the two dye components. To remove planktonic and loosely bound bacteria the biofilms were carefully washed before staining with 100 µl of 0.85% NaCl. Fluorescence was measured in a microtiter plate reader (Wallac Victor3™1420 Multilabel Counter, Perkin-Elmer Life Sciences) equipped with detectors and filter sets for monitoring red (630 nm) and green (535 nm) fluorescence. Results are expressed as reduction of the ratio of green/red fluorescence compared to untreated controls. The experiments were run in triplicate.

3.3. Bacterial cultivation and biofilm formation

The bacterial strain *Streptococcus mutans* ATCC 700610 (identical to *S. mutans* UA159) was 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% saccharose (THBS). 1995µl of this inoculum were transferred to a 6-well plate (Cellstar®, Greiner bio-one, Kremsmuenster, Austria). 5.3 µl of a 2 mM DMSO solution of "carolactam" (**5**) or carolacton (**3**) 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. Experiments were run in duplicate.

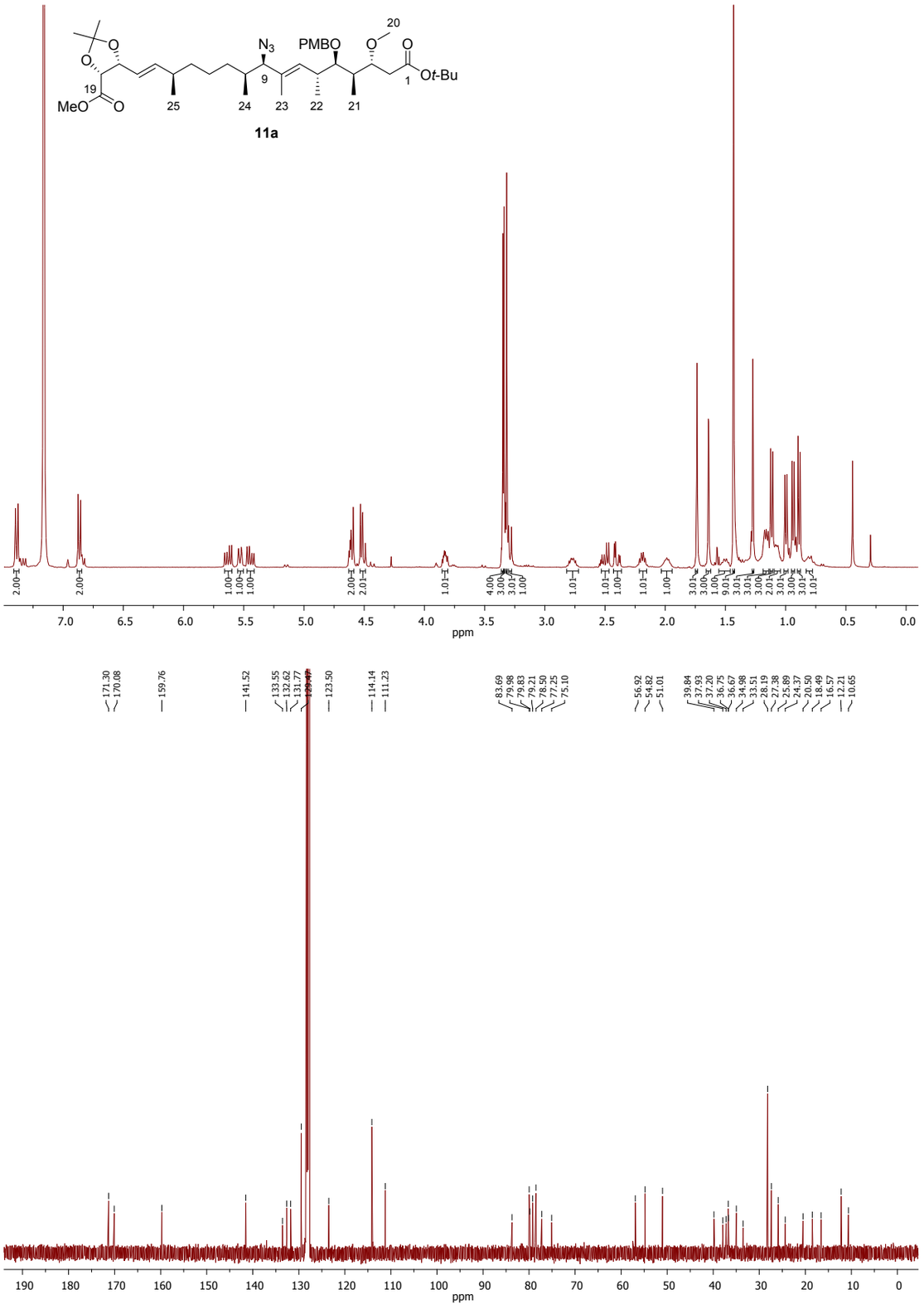
3.4. Live/Dead staining and Confocal Laser Scanning Microscopy

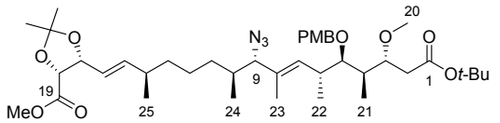
Biofilms were grown as described above (see 3.1). Prior to microscopic observation biofilms were stained live/dead using the BacLight™ Bacterial Viability Kit (Life Technologies, Carlsbad, USA). Biofilms were washed twice with 2 ml PBS buffer (Biochrom, Berlin, Germany) before staining with 2 ml of a 1:1000 dilution of BacLight™ dye. The stain is based on the dyes propidium iodide (PI) and Syto 9. The latter dye enters bacterial cells by diffusion whereas PI is not capable of penetrating through intact cell membranes. As a consequence, live and dead cells are stained differently; dead or damaged cells fluoresce red, live cells green. Staining was performed for 30 min at room temperature in the dark. Subsequently biofilms were washed twice with PBS buffer (Biochrom, Berlin, Germany) and fixed with 2.5% glutardialdehyde solution (Roth, Karlsruhe, Germany). A final washing step was performed with

PBS buffer (Biochrom, Berlin, Germany) prior to microscopic analysis. Biofilms were observed by confocal laser scanning microscopy (CLSM, Leica DM LFSA, TCS SP2 AOBS scan head, objective: HCX APO L 63.0x0.90). 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 Leica AF Lite (v 2.6.3, Leica, Wetzlar, Germany) software package.

4. Copies of NMR spectra

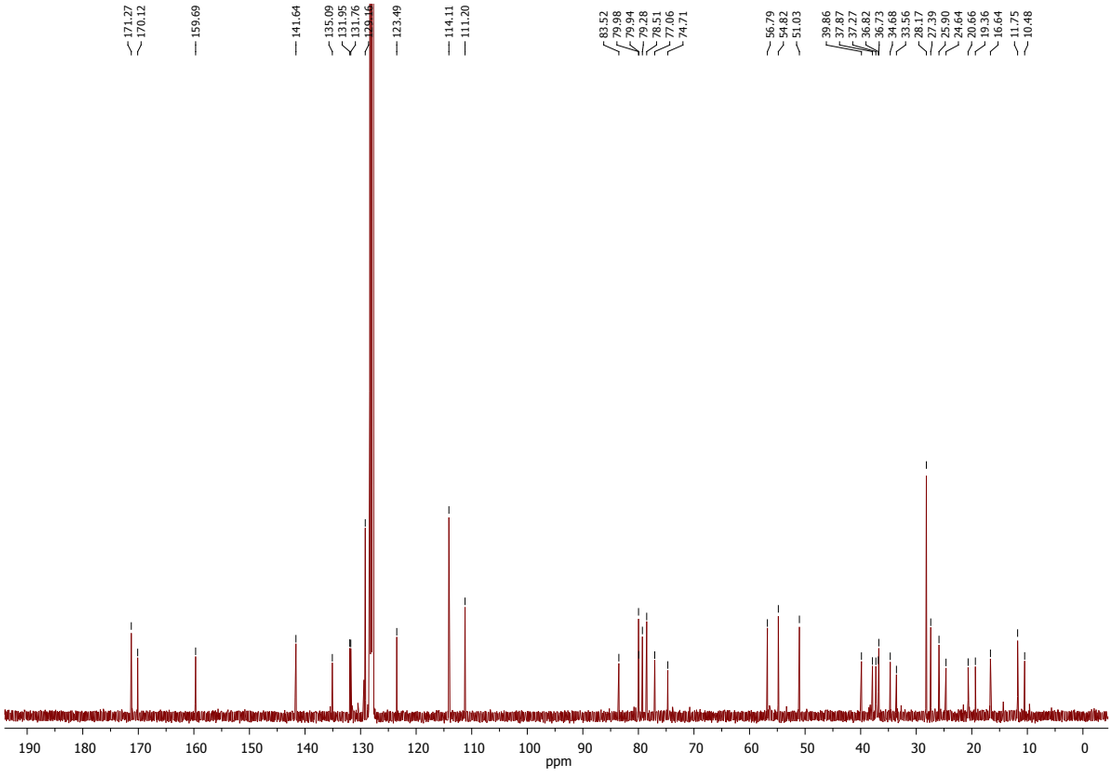
NMR and of carolacton intermediates **11a**, **11b**, **13**, **14**, and "carolactam" (**5**).

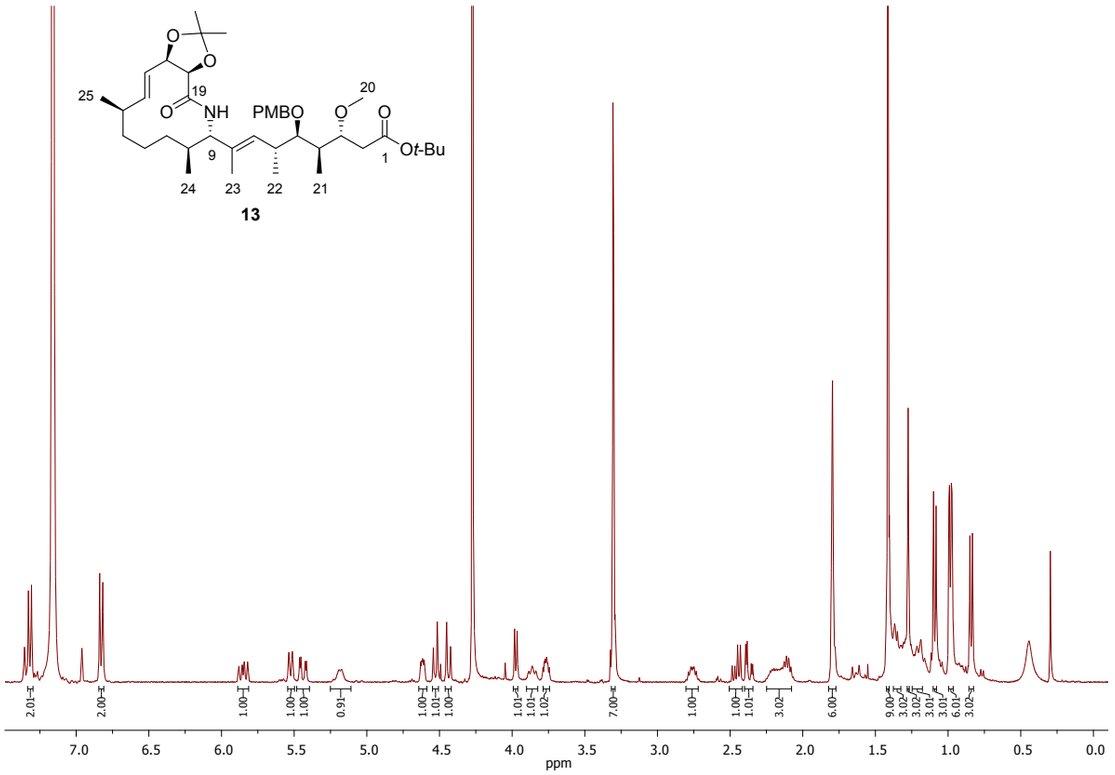


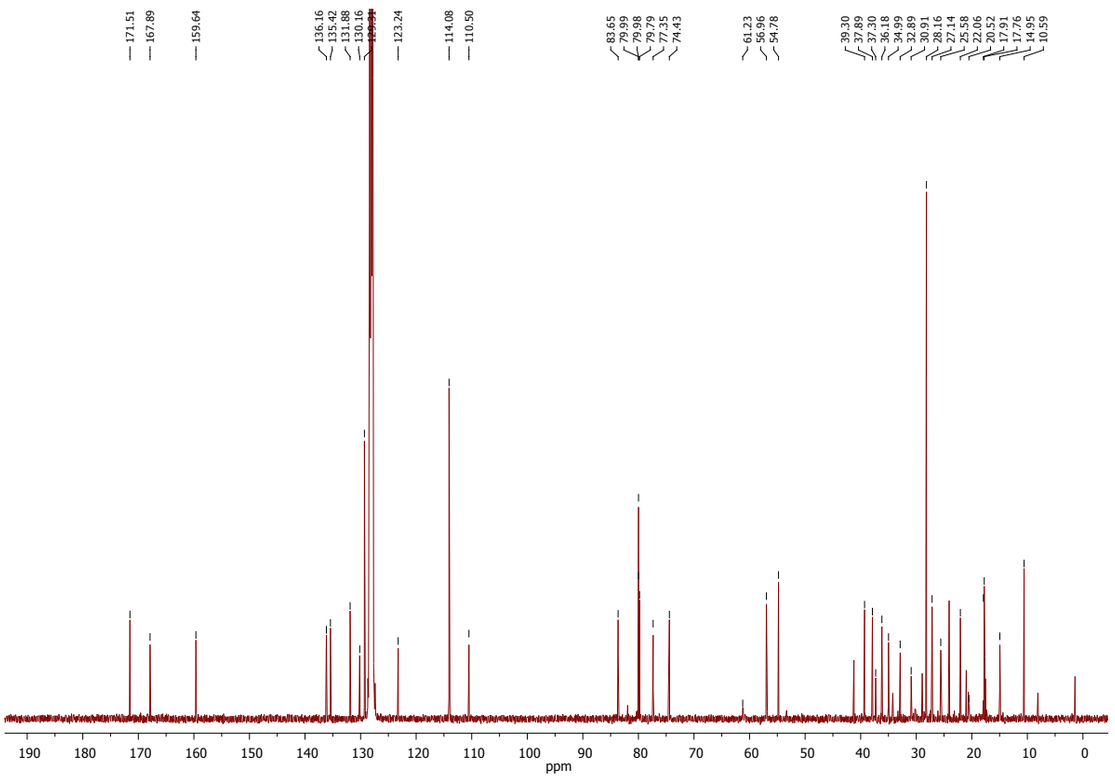

20
O
O
N3
PMBO
O
O
19
9
1
Ot-Bu
MeO
O
25
24
23
22
21

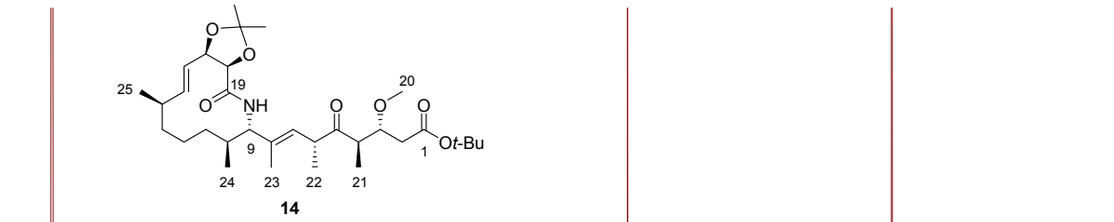
11b

2.01
2.00
1.00
1.00
1.02
1.00
1.01
1.00
1.01
1.01
4.01
3.01
1.00
3.02
1.00
1.01
1.02
1.01
1.01
3.00
1.00
3.00
1.01
9.05
3.02
2.00
1.02
6.01
1.03
1.01
3.00
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


171.27
170.12
159.69
141.64
135.09
131.95
131.76
129.16
123.49
114.11
111.20
83.52
80.98
79.94
79.28
78.51
77.06
74.71
56.79
54.82
51.03
39.86
37.87
37.27
36.82
36.73
34.68
33.56
28.17
27.39
25.90
24.64
20.66
19.36
16.64
11.75
10.48
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

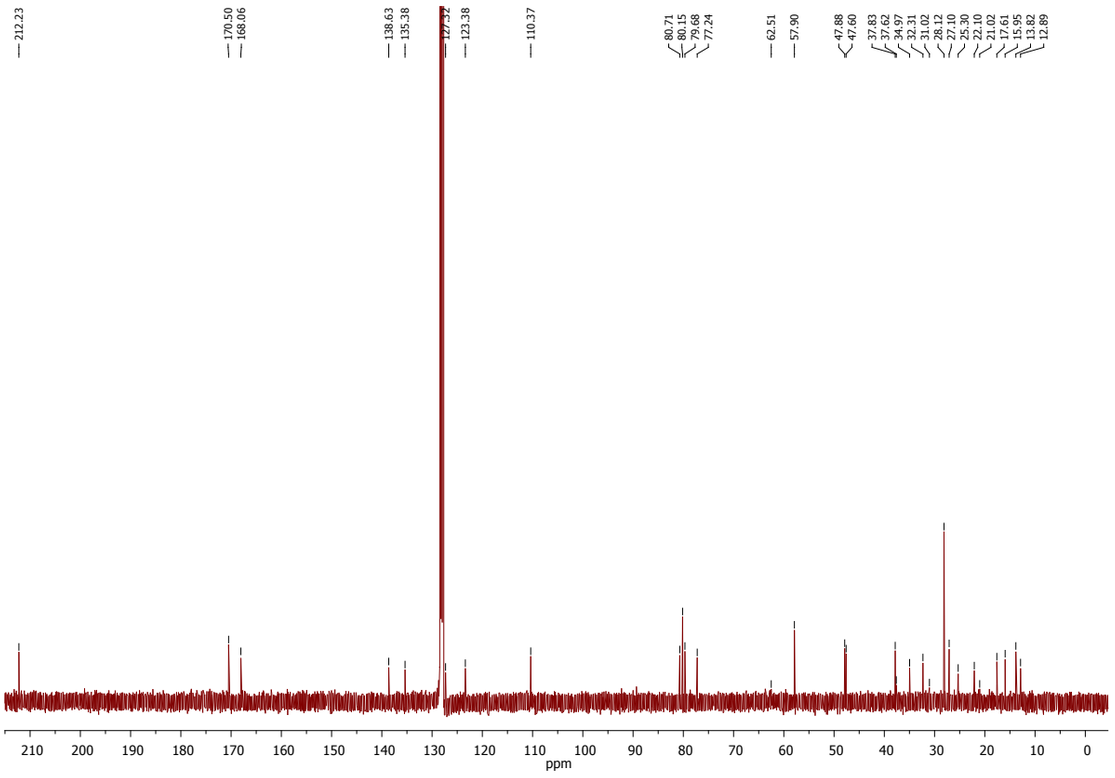

O
O
19
25
O
NH
PMBO
O
20
O
9
1
Ot-Bu
24
23
22
21
13
2.01
2.00
1.00
1.00
1.00
0.91
1.00
1.01
1.00
1.01
1.01
1.02
7.00
1.00
1.00
1.01
3.02
6.00
9.00
3.02
3.02
3.01
3.01
6.01
3.02
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm

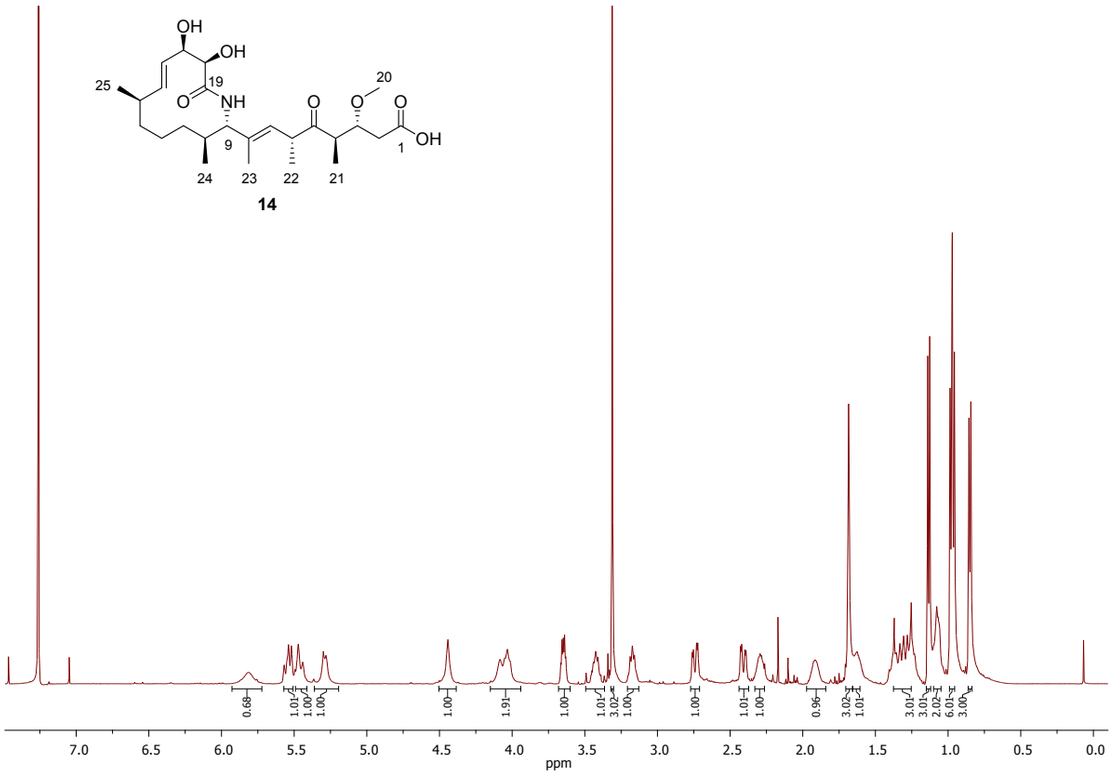

171.51
167.89
159.64
136.16
135.42
131.88
130.16
123.24
114.08
110.50
83.65
79.99
79.98
79.79
77.35
74.43
61.23
56.96
54.78
39.30
37.89
37.30
36.18
34.99
32.89
30.91
28.16
27.14
25.58
22.06
20.52
17.91
17.76
14.95
10.59
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm


O
O
19
25
O
NH
O
O
20
O
1
Ot-Bu
9
24
23
22
21

14

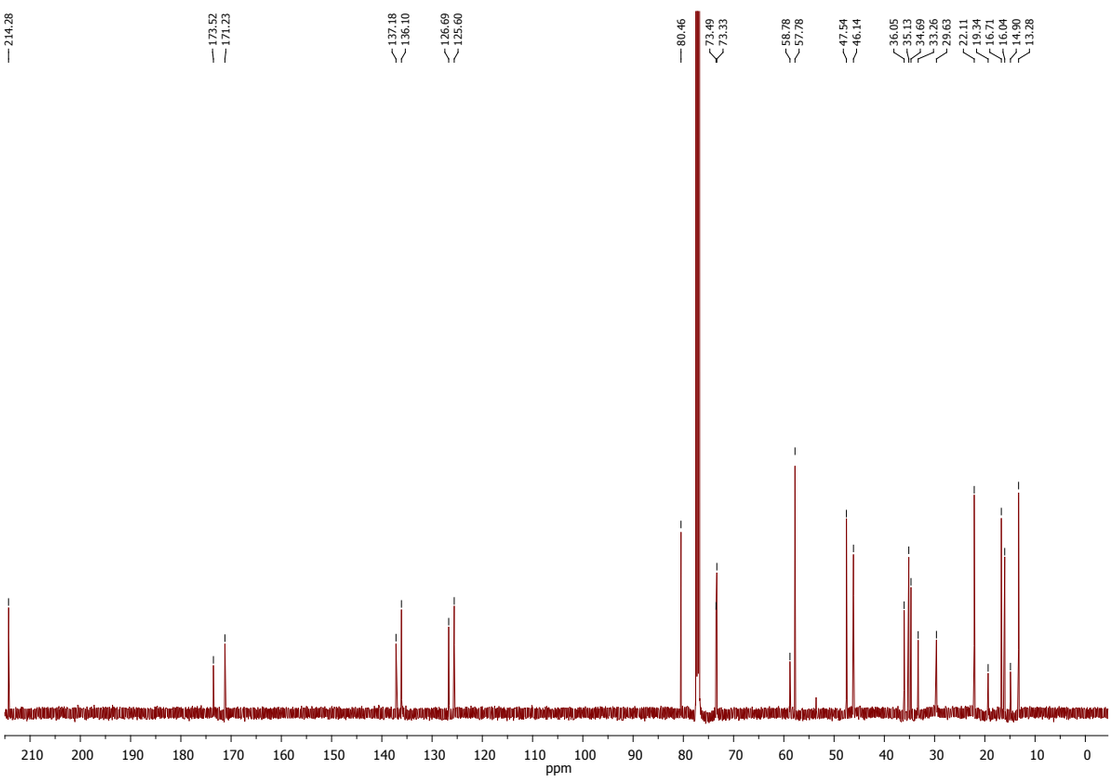
1.00
0.97
1.00
1.00
1.00
0.99
1.01
0.90
1.00
3.01
1.00
1.00
1.05
2.00
6.00
9.01
3.00
3.01
6.01
3.00
3.01
3.00
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm


212.23
170.50
168.06
138.63
135.38
127.32
123.38
110.37
80.71
80.15
79.68
77.24
62.51
57.90
47.88
47.60
37.83
37.62
34.97
32.31
31.02
28.12
27.10
25.30
22.10
21.02
17.61
15.95
13.82
12.89
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm


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

14

0.68
1.01
1.00
1.00
1.00
1.91
1.00
1.01
3.02
1.00
1.00
1.01
1.00
0.96
3.02
1.01
3.01
3.01
2.02
6.01
3.00
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


214.28
173.52
171.23
137.18
136.10
126.69
125.60
80.46
73.49
73.33
58.78
57.78
47.54
46.14
36.05
35.13
34.69
33.26
29.63
22.11
19.34
16.71
16.04
14.90
13.28
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

5. References (Supporting Information)

[S1] Stumpp, N, Premnath, P., Schmidt, T., Ammermann, J., Dräger, G., Reck, M., Jansen, R., Stiesch, M., Wagner-Döbler, I. and Kirschning, A. (2015). Synthesis of new carolacton derivatives and their activity against biofilms of oral bacteria. Organic & Biomolecular Chemistry 13, 5765-5775.

[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] 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.

[S4] Dale, J. A., Dull, D. L., Mosher, H. S. (1969). α-Methoxy-α-trifluoromethylphenylacetic Acid, a Versatile Reagent for the Determination of Enantiomeric Composition of Alcohols and Amines. Journal of Organic Chemistry 34, 2543-2549.