Total Synthesis

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Total Synthesis of Carolacton, a Highly Potent Biofilm Inhibitor**

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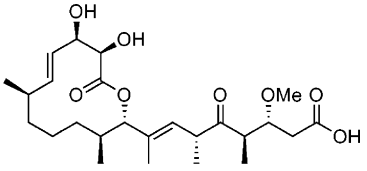
Recently, we discovered and isolated carolacton (**1**) from the extracts of *Sorangium cellulosum* strain So ce96.[1] Subsequent screening revealed unique biological properties of carolacton (**1**): it drastically reduces the number of viable cells



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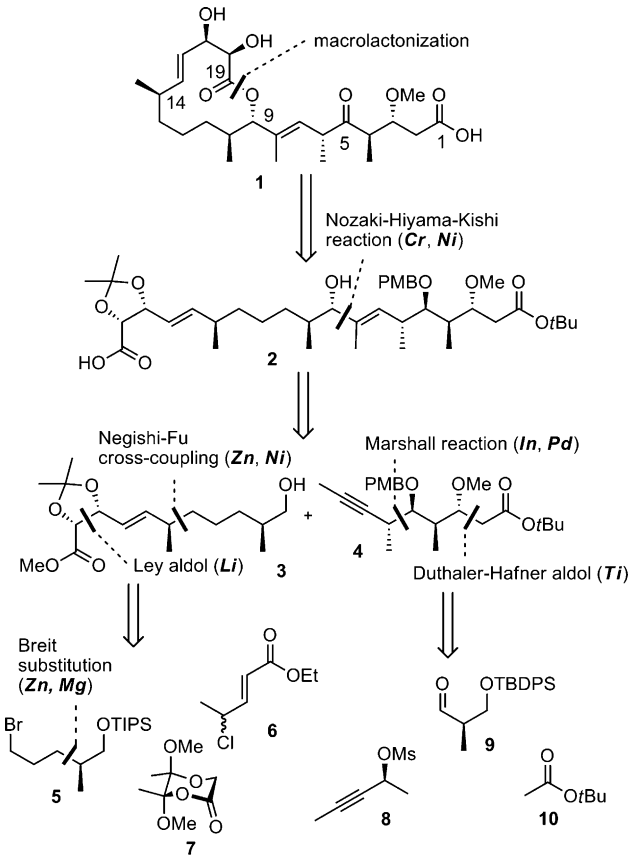
in biofilms at nanomolar concentration.[2] In particular, biofilms containing the caries- and endocarditis-associated bacterium *Streptococcus mutans* were sensitive to a solution of **1** at a concentration of 0.005 $\mu g\,mL^{-1}$, at which approximately 35% of the cells within the biofilm die. Importantly, planktonic cultures were nearly insensitive to carolacton (**1**). Bacterial biofilms have become a major concern in clinical treatment,[3] as they evade host defenses and are inherently resistant to antimicrobial agents whether they grow on natural tissues or medical devices and implants. Importantly, the antibiotic resistance of bacteria in biofilms is about 1000-fold higher than in planktonic form.[4]

Our first report on carolacton (**1**) also covered a concise structure elucidation that included the assignment of all stereogenic centers. Carolacton (**1**) features a polyketide-type backbone which forms a 12-membered macrolactone with a 1,2-diol moiety with *trans* orientation in the open-chain conformation. Remarkably, the other terminus is a carboxylic acid, a terminal group rarely found in polyketides.

The exceptional inhibitory effects of carolacton (**1**) led us to initiate a synthetic program that would also be conducive to the study of structure–activity relationships. Our highly convergent approach is depicted in Scheme 1 and relies on a series of metal-mediated or metal-catalyzed C–C coupling reactions. First, the complete polyketide backbone is prepared in the form of seco acid **2**, which is macrocyclized. Advanced intermediate **2** is further dissected into western fragment **3** and eastern fragment **4**. Fragment **3** was planned to be prepared from building blocks **5**–**7** by an aldol reaction according to Ley[5] and an asymmetric Negishi reaction between racemic allyl chloride **6** and bromide **5** following Fu's method.[6] The eastern fragment was to originate from propargylic mesylate **8**[7,8] and aldehyde **9**,[8] which are merged by a Marshall reaction[9] followed by the Duthaler–Hafner aldol reaction.[10]



Scheme 1. Retrosynthetic analysis of carolacton (**1**); metals involved in key transformations are given in parenthesis. Ms = mesyl, PMB = *p*-methoxybenzyl , TIPS = triisopropylsilyl, TBDPS = *tert*-butyldiphenylbenzylsilyl.

The synthesis of the western fragment **3** commenced with lactic acid **11**, which was transformed into bromide **5**[11] by means of Breit's S_N2-type zinc-catalyzed alkylation (Scheme 2).[13] The next C–C coupling step was a key step in our synthesis and relied on the asymmetric cross-coupling reaction between racemic allylic chloride **6**[6b,8] and aliphatic bromide **5** according to the method of Fu et al.[6] Ester **12** was generated in the presence of the (*S*,*S*)-pybox ligand **14** under

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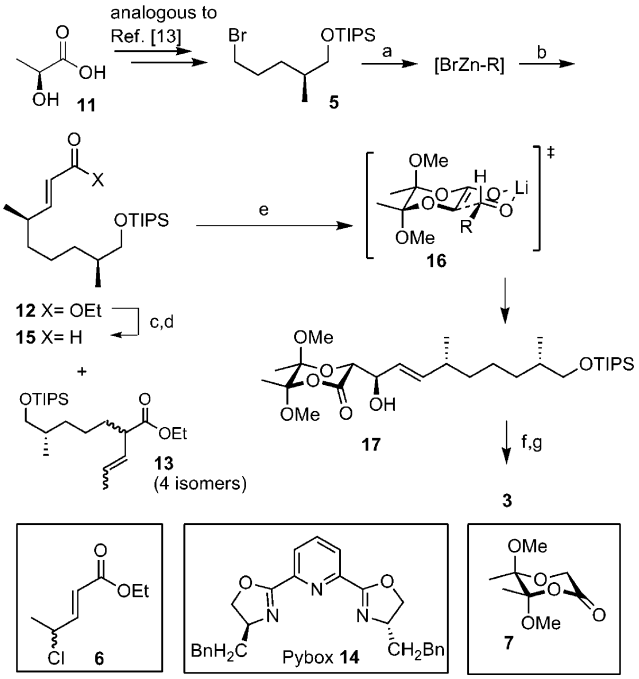


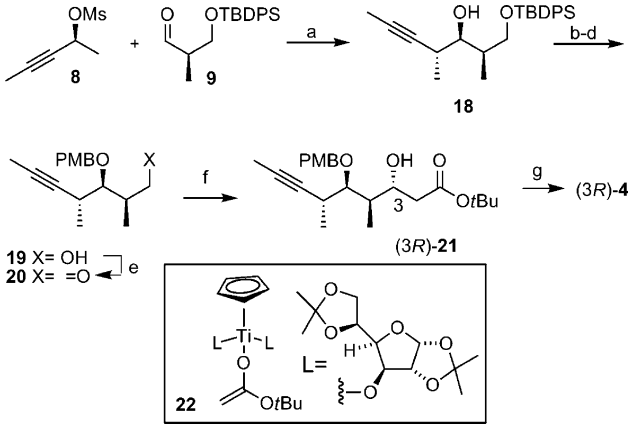
Scheme 2. Preparation of western fragment **3**. Reagents and conditions: a) Zn, I_2 (3 mol%), DMA, 70°C, 12 h; b) **6**, **14**, $NiCl_2$·glyme, NaCl, DMF/DMA, −10°C, 24 h, 82% (d.r. > 10:1) and **13** (ca. 3%); c) DIBAL-H, CH_2Cl_2, −60°C, 45 min, 94%; d) MnO_2, CH_2Cl_2, 0°C–RT, 2 h; e) 1. **7**, LiHMDS, THF, −78°C, 10 min, 2. **15**, −78°C, 6 min, 57% for two steps (d.r. > 20:1); f) CSA, MeOH, RT, 18 h, 74%, g) 2,2-dimethoxypropane, PPTS, CH_2Cl_2, RT, 1 h, 87%. CSA = camphorsulfonic acid, DMA = dimethylacetamide, glyme = 1,2-dimethoxyethane, DIBAL-H = diisobutylaluminum hydride, LiHMDS = lithium hexamethyldisilazide, PPTS = pyridimium *p*-toluenesulfonic acid.

nickel catalysis.[14] However, this crucial reaction step succeeded only after optimization,[15] because initially we obtained regioisomer **13** as a mixture of diastereomers. We found that the added sodium chloride must be ground first in order to generate the cross-coupling product **12** in good yield and high diastereomeric ratio (d.r. < 10:1).

Next, the *trans*-1,2-diol unit at C17–C18 was introduced employing Ley's butane-2,3-diacetal-protected (BDA) desymmetrized glycolic acid building block **7**.[5,16] Formation of the lithium enolate **16** and reaction with aldehyde **15**, which was obtained from ester **12** after a reduction/oxidation sequence, furnished aldol adduct **17** in good yield and with excellent diastereocontrol. The synthesis of the C9–C19 fragment **3** of carolacton (**1**) was completed after transesterification of the butane-2,3-diacetal unit, which was accompanied by TIPS ether cleavage, followed by acetonide protection of the intermediate diol.

Synthesis of the eastern fragment **4** commenced with the formation of the (*R*)-Roche ester derived aldehyde **9** (Scheme 3). The subsequent Marshall reaction[9] with mesylate (*S*)-**8** yielded adduct **18** with excellent diastereocontrol. Alkyne **18** was further elaborated to alcohol **19** by a set of standard protecting-group manipulations. After oxidation, aldehyde **20** was subjected to different aldol protocols.[17]

Efforts to prepare the desired anti-Felkin aldol product (3*R*)-**21** by exploiting 1,2-, 1,3-substrate control[18] using

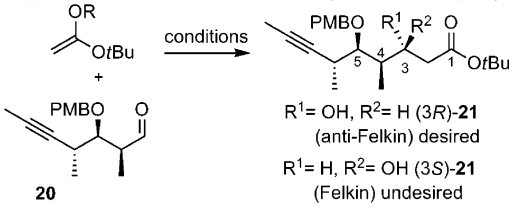


Scheme 3. Preparation of eastern fragment **4**. Reagents and conditions: a) InI, $[Pd(dppf)Cl_2]$, THF/HMPA, 0°C–RT, 17 h, 87% single diastereomer (+ 5% of diastereomeric mixture of side products); b) TBAF, THF, 0°C–RT, 13 h, 98%; c) $(MeO)_2CHPh$-OMe, PPTS, MS 3 Å, CH_2Cl_2, 0°C, 1 h; d) DIBAL-H, CH_2Cl_2, −78°C to RT, 3 h, 85% for 2 steps; e) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78°C to −40°C, 1 h; f) **20**, Et_2O, −105°C added to **22** (formed from **10** at −105°C according to Ref. [10]) warmed up to −78°C over 1.5 h, then −78°C for 12 h, 88% for two steps (d.r. = 5.5:1); g) OMe_3BF_4, proton sponge, CH_2Cl_2, RT, 12 h, 90%. dppf = 1,1′-bis(diphenylphosphino)ferrocene, HMPA = hexamethylphosphoramide, TBAF = tetra-*n*-butylammonium fluoride, MS = molecular sieves, DMSO = dimethyl sulfoxide, Cp = cyclopentadienyl, proton sponge = 1,8-bis(dimethylamino)naphthalene.

different Lewis acids preferentially delivered the undesired diastereomer (3*S*)-**21** (Table 1, entries 1–3). In fact, in the case of the Mukaiyama-type aldol reaction (entry 2) this asymmetric induction was excellent (d.r. 3*R*/3*S* = 1:20).

Discarding the idea of substrate-controlled asymmetric induction, we turned to the Duthaler–Hafner aldol reaction,

Table 1: Optimization of acetaldol reaction (selected examples).

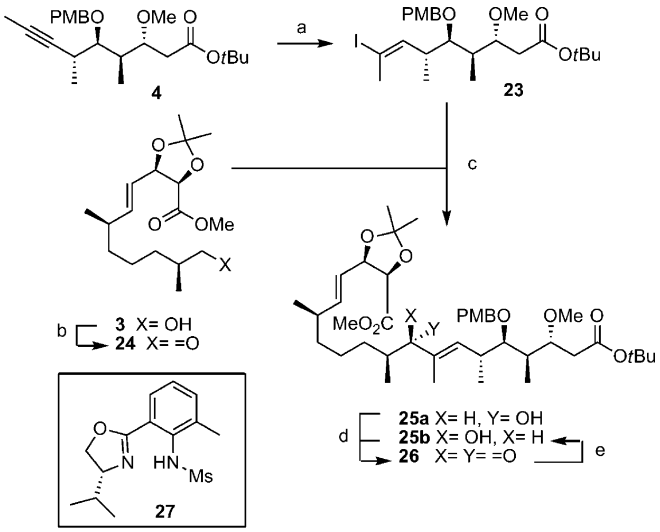


Entry	R	Conditions	3*R*/3*S*	Yield[a]
1[a]	TMS	$TiCl_4/Ti(OiPr)_4$ (1:1), CH_2Cl_2, −78°C, 30 min	1:20	66%
2[a]	TMS	$TiCl_4/Ti(OiPr)_4$ (3:1), CH_2Cl_2, −78°C, 30 min	1:20	73%
3[a]	TMS	BF_3·OEt_2, toluene, −78°C, 30 min	1:4.5	79%
4	Li	LDA, THF, −78°C, 20 min	1:1	61%
5	"Ti"	$TiCl_4$, NEt_3, CH_2Cl_2, −78°C, 30 min	–	–
6	Ti[b,c]	Ti,[c] Et_2O, RT, Et_2O, −78°C, 12 h	2.1:1	92%
7	Ti[b,c]	Ti,[c] Et_2O, −78°C, Et_2O, −90°C, 12 h	3.4:1	93%
8	Ti[b,c]	Ti,[c] Et_2O, −105°C, Et_2O, −105°C, 12 h	5.5:1	88%

[a] Reactions were conducted with 2 equiv of ketene silyl acetal; Lewis acids were added 30 s prior to addition of ketene silyl acetal for chelation. [b] Chiral titanium complex given in Ref. [10]. [c] A cooled solution of the titanium enolate was added to the cooled reaction mixture.

which relies on titanium enolate **22** generated from *tert*-butyl ester **10**. Inexpensive diacetoxy-D-glucose served as the chiral ligand. In our case the mismatched aldol reaction gave the two separable diastereomers (3*R*)-**21** and (3*S*)-**21** at −78 °C in excellent yield (92 %), with moderate stereoselectivity in favor of the desired 3*R* diastereomer (d.r. = 2:1).[10] The stereoselectivity could be improved by lowering the reaction temperature to −105 °C (88 %; d.r. = 5.5:1) (Table 1, entries 6–8). Attempts to convert aldol adduct (3*S*)-**21** into the desired 3*R* diastereomer by means of the Mitsunobu reaction failed and resulted mainly in the formation of the elimination product. Finally, O-methylation of (3*R*)-**21** furnished the eastern fragment **4**.

Next, both fragments **3** and **4** had to be appropriately refunctionalized. Alcohol **3** was oxidized under Swern conditions to give aldehyde **24** (Scheme 4). Alkyne **4** was transformed into vinyl iodide **23** by hydrozirconation and electrophilic trapping of the intermediate vinylzirconium species with iodine.[19]

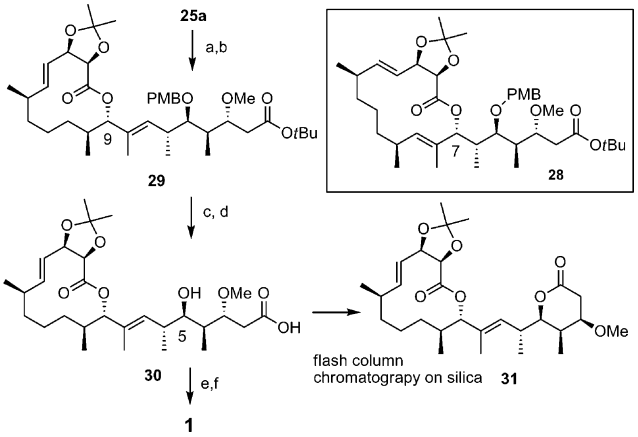


Scheme 4. Preparation of the complete backbone of carolacton. Reagents and conditions: a) 1. **4**, [Cp_2ClHZr], benzene, 1 h, 50 °C, then CH_2Cl_2, 2. I_2, benzene/CH_2Cl_2 2:1, −15 °C, 73 %; b) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C to −40 °C, 1 h; c) 1. (*R*)-**27**, $CrCl_2$, proton sponge, MeCN, 2 h, RT, 2. **23**, **24** (1.8 equiv), [$NiCl_2$(dppp)], MeCN, 16 h, RT, 96 % for two steps (**25a**/**25b** = 4.7:1); d) Dess–Martin periodinane, CH_2Cl_2, $NaHCO_3$, 0 °C to RT, 2.5 h, 70 %; e) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, CH_2Cl_2, −50 to −30 °C, 2 h, 95 % (**25a**/**25b** = 1:10). dppp = 1,3-bis(diphenylphosphino)propane.

The Nozaki–Hiyama–Kishi reaction between aldehyde **24** and vinyliodide **23** yielded carbinols **25a** and **25b** (94 %, d.r. = 1.5:1) containing the complete carbon backbone of carolacton (**1**).[20–22] The diastereomeric mixture **25a**,**b** was preferentially transformed into **25b** by Dess–Martin oxidation followed by Luche reduction of **26**. The absolute configuration of the newly formed stereogenic center at C9 was assigned by transforming **25b** into the corresponding (*R*)- and (*S*)-Mosher esters.[12] Macrolactonization of **25b** under Mitsunobu conditions ($P(Ph_3)_3$, DIAD, THF, 0 °C to RT, 6 h) did not provide the desired macrolactone with inversion of configuration at C9 but proceeded through an S_N2' process to afford the rearranged macrocycle **28** in 57 % yield (confirmed by HSQC and HMBC NMR measurements).[15] Therefore, we aimed for improving the yield of diastereomer **25a**. To our delight, the asymmetric Nozaki–Hiyama–Kishi reaction that utilizes ligand **27**[21] provided carbinol **25a** with good stereoselectivity (d.r. = 4.7:1) and in almost quantitative yield.

At this point we note that differentiating the two carboxylate groups is one key issue for the endgame towards carolacton (**1**). The chosen *tert*-butyl ester is essential because it can be orthogonally manipulated in the presence of the methyl ester and the lactone moiety. Additionally, we found that the methyl ester that corresponds to **4** was reduced in the presence of [Cp_2ClHZr] (see Scheme 4).

Thus, chemoselective hydrolysis of the ester **25a** was followed by ring closure using Shiina's protocol to yield macrolactone **29** (Scheme 5).[23] To our surprise epimer **25b** did not cyclize under these conditions. Importantly, hydrolysis of the *tert*-butyl ester is conducted before oxidative removal of the PMB group and oxidation of C5. In fact, oxidation was conducted as late as possible in the synthesis in order to avoid epimerization adjacent to the keto group, which could occur under the acidic conditions required for the deprotection of the *tert*-butyl group.



Scheme 5. Completion of the total synthesis of carolacton (**1**). Reagents and conditions: a) LiOH (1 M), THF, H_2O, RT, 13 h; b) MNBA, DMAP, toluene, 50 °C, 12 h, 76 % for two steps; c) 1. TESOTf, lutidine, CH_2Cl_2, 0 °C to RT, 12 h; 2. TBAF, 10 min, 72 %; d) DDQ, CH_2Cl_2, pH 7 phosphate buffer, 0 °C to RT, 45 min; e) Dess–Martin periodinane, CH_2Cl_2, $NaHCO_3$, RT, 1.5 h, 88 % for 2 steps; f) PPTS, *i*PrOH/H_2O 3:1, 6 d, 68 °C, 87 % (96 % based on recovered starting material). MNBA = 2-methyl-6-nitrobenzoic anhydride, TBAF = tetrabutylammonium fluoride, TESOTf = triethylsilyl trifluoromethanesulfonate, DDQ = 2,3-dichloro-5,6-dicyanobenzoquinone, DIAD = diisopropyl azodicarboxylate).

Thus, in the final sequence, removal of the *tert*-butyl group was achieved by mild transesterification with TESOTf.[24] PMB removal yielded acid **30** which had to be worked up rapidly, and the crude product was directly oxidized at C5. This protocol was essential because flash chromatography of **30** led to the formation of lactone **31**. Finally, cleavage of the acetonide moiety under mild acidic conditions provided

carolacton (**1**) in good yield when bulky isopropanol in water was used as the solvent system. Otherwise, acetonide cleavage was accompanied by methyl ester formation when methanol was used. ^{1}H and ^{13}C NMR spectra of synthetically prepared and natural carolacton (**1**) were identical; a mixture of both showed only one set of signals.[15]

In conclusion, we described the total synthesis of the highly potent biofilm inhibitor carolacton (**1**) in 4.3% overall yield (22 steps in the longest linear sequence starting from commercially available (*S*)-(−)-2-acetoxypropionic acid (**S1**)). The synthesis is based on several metal-mediated key transformations such as the Ley and Duthaler–Hafner aldol reactions, the Marshall reaction, and Breit's substitution as well as the asymmetric Nozaki–Hiyama–Kishi and asymmetric Negishi–Fu C–C coupling reactions.

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