Natural Product Synthesis

DOI: 10.1002/anie.201402255

α- and β-Lipomycin: Total Syntheses by Sequential Stille Couplings and Assignment of the Absolute Configuration of All Stereogenic Centers **

*Max L. Hofferberth and Reinhard Brückner**

Dedicated to Professor Axel Zeeck on the occasion of his 75th birthday

Abstract: *40 years ago spectroscopy, derivatization, and degradation revealed the structures of α-lipomycin and its aglycon β-lipomycin except for the configurations of their side-chain stereocenters. We synthesized all relevant β-lipomycin candidates: the (12R,13S) isomer has the same specific rotational value as the natural product. By the same criterion the (12R,13S)-configured* D*-digitoxide is identical to α-lipomycin. We double-checked our assignments by degrading α- and β-lipomycin to the diesters* ***33*** *and* ***34*** *and proving their 3D structures synthetically.*

Tetramic acids[1] are weakly acidic heterocycles **1** (Figure 1; $R_x = H$: $pK_a = 6.4$[2]). 3-Acyltetramic acids (**2**), which consist mostly of enols (*Z*)-**2** and to a lesser extent their stereoisomers (*E*)-**2**,[3,4] are more acidic than acetic acid (3-R′ = 3-acetyl, $R_x = 5$-*s*Bu: $pK_a = 3.35$[5]). There are at least 118 naturally occurring 3-acyltetramic acids **2** in the REAXYS database.[6] Of these, 59 have the enol structure (*Z*)-**2a**; this means that they are derived from saturated acyl substituents. Twelve naturally occurring 3-acyltetramic acids are dienol tautomers (*Z*)-**2b** of 3-enoyl tetramic acids. Fourteen naturally occurring 3-dienoyl tetramic acids are known as well which correspond to trienols (*Z*)-**2c**. 3-Polyenoyl tetramic acids from nature are known, too. Their number, at present 33,[6] has been growing steadily.[7] They are vinylogues, bis(vinylogues), and tris(vinylogues) of the trienols (*Z*)-**2c**.

Naturally occurring tetramic acids, the configurations of which were recently (claimed to be) fully elucidated by total synthesis, are penicillenol A_2,[8] penicillenol C_1,[9] epicoccarine A,[10] and virginenone[11] (Figure 2). The structurally most complex 3-acyltetramic acids isolated from natural sources to date include aflastatin A,[12] which comprises 28 acyclic stereocenters, and aurantoside A,[13] which is an *N*-glycoside of a trisaccharide.[14]

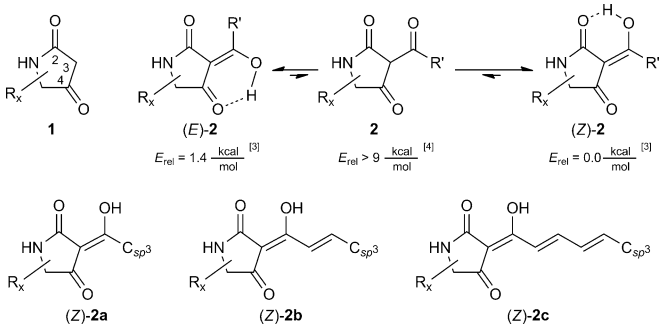


Figure 1. Tetramic acids (**1**), 3-acyl tetramic acids (**2**; preferred tautomers shown), and their subclasses **2a**–**c** (major isomer shown).

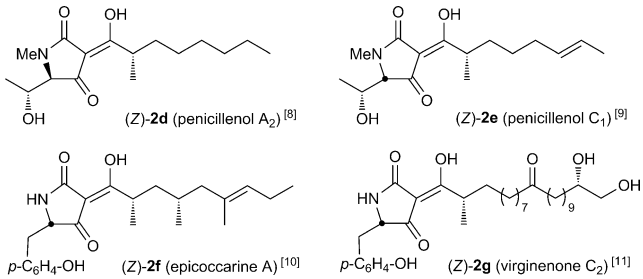


Figure 2. Naturally occurring tetramic acids, the configurations of which were recently fully elucidated by total synthesis.

The 3-polyenoyltetramic acid natural product α-lipomycin (**3**) and its aglycon β-lipomycin (**8**) were isolated from *Streptomyces aureofaciens* by Zeeck et al. 40 years ago (Scheme 1).[15] Neither **3** nor **8** was crystalline. As a consequence, structure elucidation was based upon mass spectrometry and IR, UV/Vis, and 100 MHz ^{1}H NMR spectroscopy in conjunction with chemical degradation studies.[15–17] Only the configurations of the stereocenters in the polyenoyl chain could not be determined. In 2006 the gene cluster that encodes its biosynthesis was characterized.[18] A recent comparison of the amino acid sequence of the ketoreductase, which this gene cluster encodes and which establishes the mentioned stereocenters, with 78 other ketoreductase sequences read from 38 other polyketide synthase gene clusters revealed clues for predicting the configurations of the

[*] Dr. M. L. Hofferberth, Prof. Dr. R. Brückner
Institut für Organische Chemie, Albert-Ludwigs-Universität Freiburg
Albertstrasse 21, 79104 Freiburg (Germany)
E-mail: reinhard.brueckner@ocbc.uni-freiburg.de
Homepage: http://www.brueckner.uni-freiburg.de

[**] We are grateful to Prof. Dr. Andreas Bechthold and to Dipl.-Biol. E. Welle (both Institut für Pharmazeutische Wissenschaften, Albert-Ludwigs-Universität Freiburg) for an authentic sample of α-lipomycin and for assisting in growing and extracting *Streptomyces aureofaciens* (Tü 117) to obtain α- and β-lipomycin. Financial support for this study by the DFG (IRTG 1038) is gratefully acknowledged.

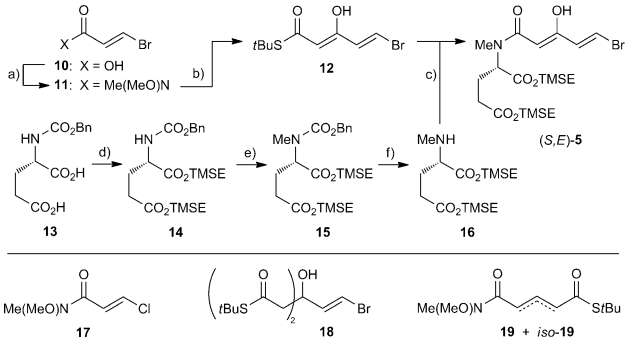
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201402255.

stereocenters in the corresponding gene products in general and in β-lipomycin (**8**) in particular.[19,20]

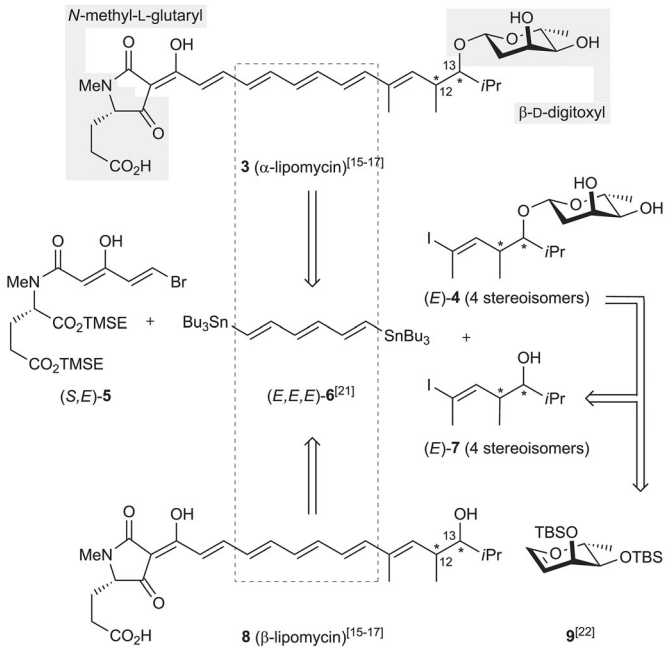
We have now established the configurations of all of the stereocenters of α-lipomycin (**3**) and β-lipomycin (**8**) by total synthesis. Retrosynthetically we dissected the conceivable candidate structures—four diastereomers per target molecule—into three building blocks each (Scheme 1). The distannane (*E*,*E*,*E*)-**6** was meant to provide the all-*E*-configured triene core of **3** and **8** by stepwise couplings of the two C(sp^2)–Sn moieties; this distannane had been introduced and a related biscoupling strategy successfully exploited in earlier work from our group.[21] The coupling "on the left" should engage the bromoalkene building block (*S*,*E*)-**5**, the coupling "on the right" one of the four iodoalkene diastereomers (*E*)-**7** or one of their glycosides (*E*)-**4**; the latter were unknown yet looked accessible from additions of the former to the bis(*tert*-butyldimethylsilylether) **9**[22] of D-digitoxal.

The iodoalkenes (*E*)-**7** of Scheme 1 had not been not reported previously in the literature. Syntheses of the benzyl and *tert*-butyldimethylsilyl ether of the racemic *syn*-alcohol [(4,5*ul*,*E*)-**7**] were the closest precedence.[23] There was an enantioselective synthesis of the same unprotected homopropargyl alcohol, which we used as a precursor of the iodoalkene isomer (4*R*,5*R*,*E*)-**7**.[24] However, as will be shown in Scheme 3 we accessed this alcohol differently. The bromoalkene (*S*,*E*)-**5** of Scheme 1 contains an enolized β-ketoamide and an ester group. These entities should make it possible to establish the 3-acyltetramic acid motif by an intramolecular acylation known as the Lacey–Dieckmann condensation.[25] Building block (*S*,*E*)-**5** is polarity-reversed relative to all previously used substrates for cross-coupling/Lacey–Dieckmann cyclocondensation routes to tetramic acids.[26] Since (*S*,*E*)-**5** reacted as envisaged it represents a worthwhile addition to the synthetic arsenal.

Our first goal was the synthesis of β-ketothioester **12** (Scheme 2). Isomerically pure *trans*-bromoacrylic acid (**10**) was activated as the chloride or mixed anhydride. Surprisingly, lithiated *t*BuSAc reacted beyond the desired mono-



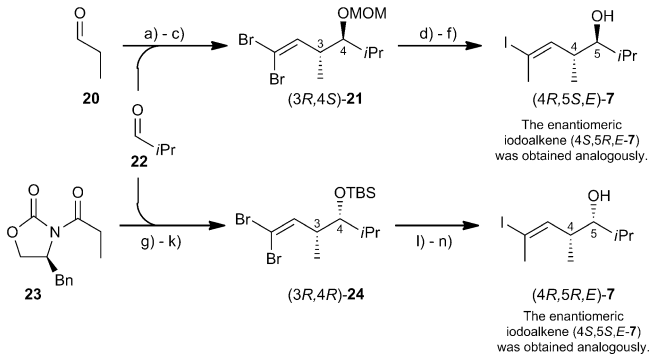
Scheme 2. Preparation of building block (*S*,*E*)-**5** by aminolysis of the β-ketothioester **12**: a) T3P, Me(MeO)NH, NMP, RT, 25 min; 86%. b) Solution from *n*BuLi (in hexane) and HN[Si(Me_3)]$_2$ in THF; addition of *t*BuSAc, THF, −78°C, 1 h; $MgBr_2{\cdot}OEt_2$, 1 h; addition of **11** (1.0 equiv), 2 h; 83%. c) β-Ketothioester **12**, AgO_2CCF_3 (1.1 equiv), MS 4 Å, THF, 0°C, 1 h; 83% over the 2 steps. d) $Me_3Si(CH_2)_2OH$, DMAP, CH_2Cl_2/DMF (10:1), 0°C, dropwise addition of DCC in CH_2Cl_2, →RT, 15 h; 82%. e) MeI, Ag_2O, DMF, RT, 18 h; 93%. f) Pd (10% on C), H_2 (ca. 1.3 bar), EtOAc, 30 min; the crude product was carried on without purification. DCC = dicyclohexylcarbodiimide, MS = molecular sieves, T3P = propylphosphonic anhydride, TMSE = 2-(trimethylsilyl)ethyl.



Scheme 1. The stereochemically incompletely characterized[15–17] polyenoyltetramic acids α-lipomycin (**3**) and β-lipomycin (**8**). Tracing back the isomeric candidates to the building blocks (*S*,*E*)-**5** (novel), (*E*,*E*,*E*)-**6**,[21] (*E*)-**4** or (*E*)-**7**, and to the disilylether **9**[22] of D-digitoxal. TBS = *tert*-butyldimethylsilyl, TMSE = 2-(trimethylsilyl)ethyl.

(thioester) stage (**12**) giving some bis(thioester) **18**. As a bypass *trans*-bromoacrylic acid (**10**) was converted into the Weinreb amide[27] **11** although we were unaware of the acylation of thioester enolates by Weinreb amides prior to our work. Reaction with DCC, cat. DMAP, and Me(MeO)NH·HCl[28] provided **11** in up to 65% yield in a mixture with the analogous chloride (**17**, 3%). Bromoacrylic acid **10**, propylphosphonic anhydride (T3P), *N*-methylmorpholine,[29] and Me(MeO)NH gave 86% Weinreb amide **11** selectively. Compound **11** and lithiated *t*BuSAc afforded not only the desired acylation product **12** (40% yield) but also a 68:32 mixture (19% yield) of the 1,4-addition/β-elimination products **19** and *iso*-**19**. Gratifyingly, transmetalation of lithio-*t*BuSAc with 2.2 equiv of $MgBr_2{\cdot}OEt_2$ before addition of the Weinreb amide favored selective acylation. The β-ketothioester **12** resulted without by-products according to the 300 MHz ^{1}H NMR spectrum. It was isolated in 83% yield by flash chromatograpy over a pad of silica gel.[30]

The bis[(trimethylsilyl)ethyl] ester **16** of L-*N*-methylglutamic acid, not previously described, was prepared next (Scheme 2). It was designed to undergo a deprotection under mild conditions with Bu_4NF at the polyenoyltetramic acid stage.[31] Accordingly, *N*-(benzyloxycarbonyl)glutamic acid (**13**) was esterified in 82% yield with 2-(trimethylsilyl)ethanol and DCC.[32] Treatment with MeI and Ag_2O[33] in DMF—as described more generally by Olsen[34] and applied to a diester of L-*N*-(benzyloxycarbonyl)aspartic acid by de Meijere

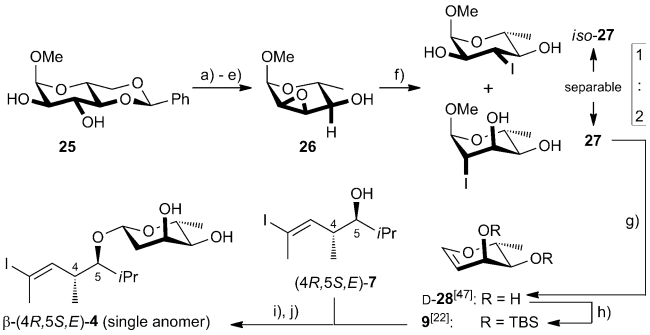


Scheme 3. Preparation of the four stereoisomers of iodoalkene (*E*)-**7** as pure enantiomers: a) Aldehyde **20**, L-proline, DMF, 4 °C; addition of aldehyde **22** in DMF over 30 h; 4 °C, 15 h; 41 %. b) CBr_4, PPh_3, CH_2Cl_2, 0 °C, 10 min; addition of product from step (a), −78 °C, 1 h; 73 %, 99 % *ee* (GC). c) CH_3OCH_2Cl, iPr_2NEt, CH_2Cl_2, 50 °C sealed tube, 4 h; 84 %. d) *n*BuLi (in hexane), THF, −78 °C, 1 h; MeI, →RT, 1 h; 87 %. e) [Cp_2ZrHCl], THF, −10 °C; →40 °C, 45 min; addition of solution of I_2 in THF, 0 °C, 5 min; 75 %. f) HCl_{conc}, MeOH, 60 °C, 30 min; 87 %. g) Bu_2BOTf, NEt_3, CH_2Cl_2, 0 °C, 15 min; −78 °C; aldehyde **22**, 1 h; → 0 °C; addition of H_2O_2 (30 % in H_2O), MeOH, phosphate buffer (pH 7), 1.5 h; 91 % (Ref. [39]: 99 %), d.r. > 95:5 (^{1}H NMR, 400 MHz, $CDCl_3$). h) Me(MeO)NH·HCl, $AlMe_3$, CH_2Cl_2, −15 °C→RT, 3 h; 88 % (Ref. [39]: 88 %). i) TBSOTf, 2,6-lutidine, CH_2Cl_2, RT, 20 min; 97 % (Ref. [39]: 99 %). j) $(iBu)_2AlH$, THF, −78 °C, 1.5 h; the crude product was processed further without purification. k) PPh_3, CBr_4, CH_2Cl_2, 0 °C, 30 min; 80 % over the 2 steps. l) Same as (d) but 90 %. m) [Cp_2ZrHCl], MS 4 Å, THF, −10 °C→RT, 1.5 h; I_2, 0 °C, 5 min; 79 %. n) $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0 °C→RT, 2 h; 95 %. MOM = methoxymethyl, MS = molecular sieves, TBSOTf = tBuMe$_2$SiO$_3$SCF$_3$.

et al.[35]—furnished the *N*-methylated diester **15** in 93 % yield and reliably with 97–98 % *ee*. The benzyloxycarbonyl group was removed by hydrogenolysis within 30 min. The resulting aminodiester **16** lactamized at room temperature within 2 days.[36] In order to circumvent this, **16** had to be engaged without delay in the aminolysis of β-ketothioester **12**. We used AgO_2CCF_3 as a promoter as described for related reactants[37] and the β-ketoamide (*S*,*E*)-**5** was prepared in 83 % yield over the two steps.

The four stereoisomeric iodoalkenes (*E*)-**7** were obtained by known diastereo- and enantioselective aldol additions to isobutanal (Scheme 3). L- and D-Proline-catalyzed crossed additions of propionaldehyde to isobutanal provided the *anti*-aldols with (2*R*,3*S*) and (2*S*,3*R*) configuration, respectively (step a[38]), albeit in lower yields than previously reported. Additions of the boron enolate of Evans' propionyl oxazolidinone **23** and of its enantiomer to isobutanal delivered the expected *syn*-hydroxyimides with (2*R*,3*R*) and (2*S*,3*S*) configuration, respectively (step g[39]). Without protection of the OH group[40] each of the *anti*-aldols was C_1-elongated by the Wittig reagent formed from CBr_4 and PPh_3 to give the corresponding *gem*-dibromoalkene (step b[41]). The latter was MOM-protected, which furnished the *anti*-configured ethers (3*R*,4*S*)-**21** and (3*S*,4*R*)-**21**, respectively. The Evans-type *syn*-aldols were processed further through known transformations (Weinreb amide formation; *tert*-butyldimethylsilylation of the OH group; DIBAH reduction; steps h–j[39]) and *gem*-dibromomethylenation as before (step k[41]). This furnished the *syn*-configured ethers (3*R*,4*R*)-**24** and (3*S*,4*S*)-**24**, respectively. Each of the four dibromoalkenes was subjected separately to Br→Li exchange, which induced a Fritsch–Buttenberg–Wiechell rearrangement.[42] The resulting lithioalkynes were *C*-methylated in situ.[43] Hydrozirconation, iodolysis, and removal of the respective protecting group afforded the *anti*-configured iodoalkenes (4*R*,5*S*,*E*)- and (4*S*,5*R*,*E*)-**7** via steps d–f and their *syn* diastereomers (4*R*,5*R*,*E*)- and (4*S*,5*S*,*E*)-**7** via steps l–n.

At a later stage of our work we learnt that synthesizing naturally configured α-lipomycin (**3**) most likely required including the *anti*-configured iodoalkene enantiomer (4*R*,5*S*,*E*)-**7** as a β-digitoxide in our route. With this goal in mind we synthesized the digitoxide β-(4*R*,5*S*,*E*)-**4** from the digitoxyl donor D-**28** (Scheme 4), which had been synthesized once (in 9 steps).[22] We made D-**28** differently, though, namely by silylating the underlying diol **9**, for which a 7-step synthesis



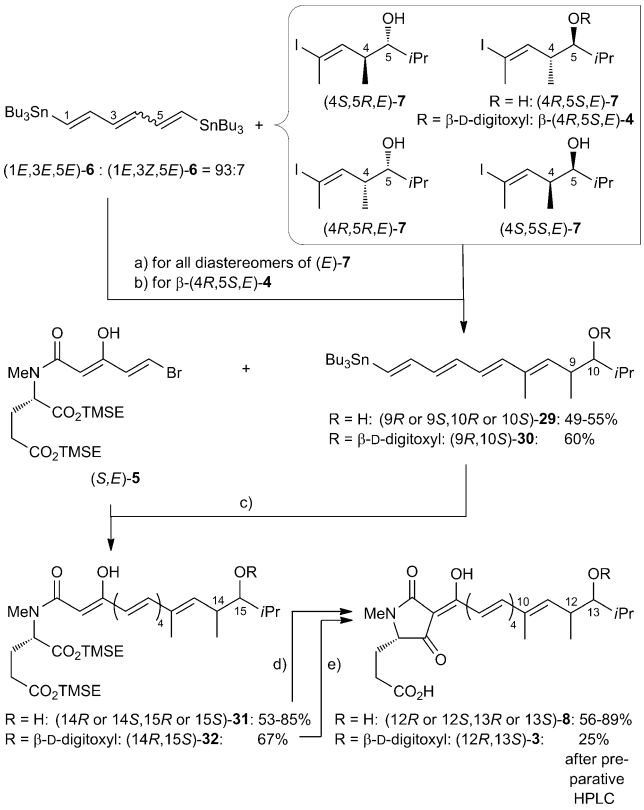
Scheme 4. Preparation of D-digitoxal (D-**28**) by literature procedures,[44–47] by a silylation (→**9**) in analogy to Ref. [47], and by the β-selective digitoxylation of iodoalkene (4*R*,5*S*)-**7**: a) *p*TsCl, pyridine, 55 °C, 4 d; 95 % (Ref. [44]: 78 %). b) NaOMe, CH_2Cl_2/MeOH (6:1), 0 °C, 2 d; RT, 1 d; 86 % (Ref. [45]: 85 %). c) NBS, AIBN, benzene, reflux, 15 min; again AIBN, reflux, 15 min; 84 % [Ref. [46] published no yield for a related procedure employing $(PhCO_2)_2$ instead of AIBN and 30 min reaction time]. d) NaOMe, MeOH, RT, 20 min; 92 % (77 % over the 2 steps; Ref. [46] "overnight": 80 % over the 2 steps). e) $NaBH_4$, $NiCl_2{\cdot}6H_2O$, H_2O/EtOH (3:1), reflux, 50 min; addition of more $NaBH_4$ in H_2O, reflux, 40 min; 84 % (Ref. [46]: 95 %). f) $LiI{\cdot}(H_2O)_{1.5-3.0}$, HOAc, $CHCl_3$, RT, 2 h; 40 °C, 1.5 h; 61 % (Ref. [47]: 52 %). g) *n*BuLi (in hexane), THF, −15 °C, 30 min; 40 °C, 1 h; 80 % of the crude product (Ref. [47] with MeLi instead of *n*BuLi: 67 %). h) TBSOTf, imidazole, DMAP, DMF, 55 °C, 2 h; 63 %. i) $PPh_3{\cdot}HBr$, 50 °C, 3 d; 80 % (4*R*,5*S*,*E*)-**4**. j) $Bu_4N^+F^-$, THF, RT, 5 h; 73 %. AIBN = azobis(isobutyronitrile), DMAP = 4-(*N*,*N*-dimethylamino)pyridine, NBS = *N*-bromosuccinimide, pTs = *para*-toluenesulfonyl.

existed. First, the commercially available methyl α-D-glucoside **25** was tosylated (step a[44]). Epoxide formation in the presence of base (step b[45]) and defunctionalization of C-6 ensued (steps c–e[46]). The resulting epoxide **26** was ring-opened with lithium iodide with a 2:1 bias (Ref.[47]: 4:1) for the Fürst–Plattner product **27** (step f). The latter was separated from the minor ring-opening product *iso*-**27** by flash chromatography on silica gel.[30] Though it is an iodohydrin, when compound **27** was treated with BuLi it did not re-form epoxide **26** but underwent I→Li exchange. Thereupon elimination of LiOMe afforded D-digitoxal (D-**28**; step g[47]). The latter was bis(*tert*-butyldimethylsilylated)[48] giving glycal **9** (step h, 63 % yield).

Glycal **28** and 1 mol% PPh_3·HBr had been shown to effect the β-selective (97:3) digitoxylation of a sterically demanding secondary alcohol at room temperature in 88% yield.[22] An analogous digitoxylation of our secondary alcohol ("iodoalkene") (4*R*,5*S*,*E*)-**7** proceeded much more slowly. Complete conversion required more catalyst (11 mol% PPh_3·HBr), higher temperature (50°C), and an extended reaction time (3 d). Gratifyingly, a single digitoxide was formed (80% yield). Its anomeric center was β-configured as evidenced by the magnitudes of the vicinal coupling constants in the ^{1}H NMR spectrum (400.1 MHz, $CDCl_3$).[49] Deprotection with $Bu_4N^+F^-$ afforded the desired building block (4*R*,5*S*,*E*)-**4** in 73% yield.

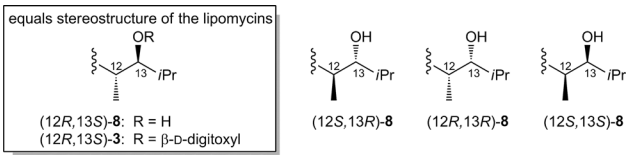
Having prepared the six building blocks identified by the retrosynthetic analysis of Scheme 1 for the assembly of four diastereomers of β-lipomycin (**8**), including the one from nature, we proceeded to the Stille couplings (Scheme 5). We obtained the best overall yields when any iodoalkene stereoisomer **7** was first coupled with the distannane (*E*,*E*,*E*)-**6**[21] (step a; 49–55% yield). Secondly, each of the resulting monostannanes **29** was Stille-coupled with the bromovinylated β-ketoamide (*S*,*E*)-**5** (step c; 53–85% yield). The resulting pentaenes **31** contained the full carbon backbone of β-lipomycin (**8**).[50] Each pentaene **31** underwent a Lacey–Dieckmann cyclization[25] in the presence of tetramethylguanidine; tetrabutylammonium fluoride was then added to remove the TMSE group (one-pot transformation; step d, 56–89% yield). This provided the desired set of four diastereomers with the constitution of β-lipomycin (**8**). None of their ^{1}H or ^{13}C NMR spectra in $CDCl_3$ revealed a sizeable amount of isomers and the spectra of (12*R*,13S)-**8** showed no isomers at all. In each isomer of **8** the disubstituted C=C bonds were *E*-configured; this followed from the corresponding $^3J_{CH=CH}$ values. In compound (12*R*,13*S*)-**8** the trisubstituted C=C bond was also *E*-configured, as shown by a NOE between 12-H and 10-CH_3.

Methanol solutions of (12*R*,13*S*)-, (12*S*,13*R*)-, (12*R*,13*R*)-, and (12*S*,13*S*)-**8** were uniformly levorotatory. The specific rotation of (12*R*,13*S*)-**8** ($[\alpha]_D^{20}=-180$) matched the value reported for natural β-lipomycin (**8**; −176[15]) closely whereas the specific rotations of the other diastereomers (−45, −136, and −120, respectively) differered substantially (Table 1). Unless contaminants contributed to these data one could conclude that the side-chain of β-lipomycin (**8**) is (12*R*,13*S*)-configured. This analysis was corroborated by completing the synthesis of diastereomer (12*R*,13*S*)-**3** of α-lipomycin (Scheme 5). This compound possessed the same side-chain configuration as that deduced for natural β-lipomycin, (12*R*,13*S*)-**8**, and was reached from the digitoxylated iodoalkene building block β-(4*R*,5*S*,*E*)-**4** by the analogous transformations: 1) Stille coupling with distannane (*E*,*E*,*E*)-**6**[21] (step b; 60% yield); 2) Stille coupling of the resulting monostannane (9*R*,10*S*)-**30** with the bromovinylated β-ketoamide (*S*,*E*)-**5** (step c; 67% yield); 3) Lacey–Dieckmann cyclization[25]/removal of the TMSE group (step e). A 67:33 mixture of α-lipomycin [(12*R*,13*S*)-**3**] and β-lipomycin [(12*R*,13*S*)-**8**] resulted at first. Separation by preparative HPLC afforded (12*R*,13*S*)-**3** in 25% yield. The ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of this compound showed very



Scheme 5. Five stepwise Stille biscouplings of the bisstannane (*E*,*E*,*E*)-**6**,[21] each setting the stage for a terminating Lacey–Dieckmann condensation[25] (**31**→**8**; **32**→**3**)/desilylation sequence: a) **6**, $Bu_4N^+Ph_2PO_2^-$, $AsPh_3$, CuI, $[Pd(dba)_2]$, molecular sieves (4 Å), NMP, RT, 0.25–2 h; 49–55%.[+] b) Same as (a) but using β-(4*R*,5*S*,*E*)-**4**, NMP/THF (10:1), 30 min; 60%. c) (*S*,*E*)-**5**, $AsPh_3$, $[Pd(dba)_2]$, NMP, 1.25–3 h; 53–85%.[++] d) *N*,*N*,*N*′,*N*′-Tetramethylguanidine, THF, RT, 2–3 h; addition of $Bu_4N^+F^-$, 40–50°C, 1–3 h; 56–89%. e) *N*,*N*,*N*′,*N*′-Tetramethylguanidine, THF, RT, 30 min; addition of $Bu_4N^+F^-$, 50°C, 1 h; mixture of 49% (12*R*,13*S*)-**3** and 24% (12*R*,13*S*)-**8**; after preparative HPLC 25% (12*R*,13*S*)-**3**. BHT = Di-*tert*-butylated *para*-hydroxytoluene, dba = dibenzylidenacetone, NMP = *N*-methylpyrrolidone. [+] Isomer (9*S*,10*S*)-**29** was prepared by replacing $Bu_4N^+Ph_2PO_2^-$ and the molecular sieves by BHT (2 mol%). [++] For preparing isomers (14*S*,15*S*)- and (14*R*,15*S*)-**31** we employed $Bu_4N^+Ph_2PO_2^-$ (1.5 equiv).

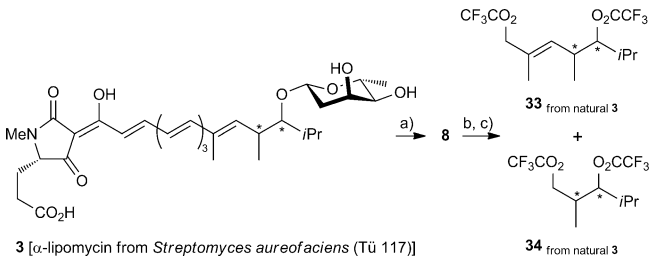
Table 1: Specific rotations of synthetic β-lipomycin [(12*R*,13*S*)-**8**], its diastereomers (12*S*,13*R*)-, (12*R*,13*R*)-, and (12*S*,13*S*)-**8**, natural β-lipomycin (**8**),[15] synthetic α-lipomycin [(12*R*,13*S*)-**3**], and natural α-lipomycin (**3**).[15]



Tetramic acid	$[\alpha]_D^{20}$ in MeOH	Natural lipomycins: $[\alpha]_D^{20}$ in MeOH[15]
(12*R*,13*S*)-**8**	−180 (*c* = 0.080)	−176 (*c* = 0.09)
(12*S*,13*R*)-**8**	−45 (*c* = 0.031)	
(12*R*,13*R*)-**8**	−136 (*c* = 0.035)	
(12*S*,13*S*)-**8**	−120 (*c* = 0.045)	
(12*R*,13*S*)-**3**	−237 (*c* = 0.10)	−229 (*c* = 0.10)

broad signals. Broadened ^{1}H and weak ^{13}C NMR resonances, which we observed for (12*R*,13*S*)-**3**, too, are known in acyltetramic acids.[3,51] The phenomenon was explained by the fact that these compounds sequester Mg^{2+}, Ca^{2+}, and Fe^{2+} contaminants of silica gel.[51] Accordingly, we considered the NMR spectra of (12*R*,13*S*)-**3** to be normal and did not resort to any countermeasures (cf. Ref. [3]). Synthetic (12*R*,13*S*)-**3** exhibited $[\alpha]_D^{20}=-237$ in methanol solution (Table 1). This was only 3.5% off the value $[\alpha]_D^{20}=-229$ for natural α-lipomycin (**3**[15]).

As likely as it appeared that we had identified configurationally and achieved to synthesize both α- (**3**) and β-lipomycin (**8**), we were hesitant to base our analysis on $[\alpha]_D^{20}$ values alone. The non-existence of high-field NMR data for the natural products[15–17] thwarted ^{1}H and ^{13}C NMR comparisons as identity proofs. We therefore sought independent evidence for the lipomycin side-chain configurations by isolating/preparing β-lipomycin (**8**) from the natural source (*Streptomyces aureofaciens* Tü 177; Scheme 6). We extracted

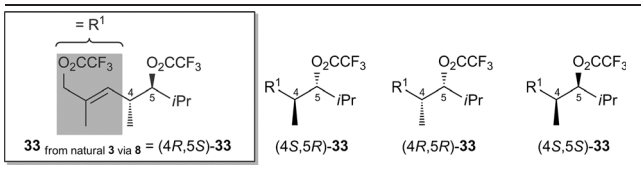


Scheme 6. Chemical degradation of α-lipomycin (**3**) via β-lipomycin (**8**); yields were not determined. a) Aq. HCl (10%)/H_2O/MeCN (4:32:64 v/v/v), 5 h; preparative HPLC [XBridge, MeOH/H_2O (45:55 v/v) + NH_3 (10 mM), 16 mL min^{-1}, $\lambda_{UV/vis}=300$ nm, $t_{r,8}=8.0$ min]. b) $NaIO_4$ (13.2 equiv), $K_2OsO_4{\cdot}2H_2O$ (30 mol%), EtOH/H_2O (1:1 v/v), RT, 1.5 h; $NaBH_4$ (120 equiv), 1 h. c) $(CF_3CO)_2NMe$ (50 equiv), Et_2O, RT, 2 h. The resulting **33**/**34** mixture (ca. 1:1) was analyzed by GC (Table 2).

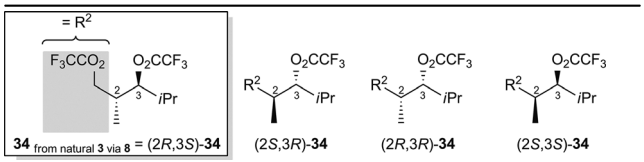
4.4 L of the corresponding culture broth with an equal volume of ethyl acetate, evaporated the solvent, purified the residue by HPLC, and dissolved the isolate in acetonitrile/H_2O (2:1). We added HCl for deglycosylating α-lipomycin (**3**) to obtain β-lipomycin (**8**). Extractive isolation with *t*BuOMe after saturation with solid NaCl and final purification by HPLC delivered 7.8 mg of β-lipomycin (**8**) admixed with 0.9 mg of a presumed (*Z*)-isomer. The 400 MHz ^{1}H NMR spectrum ($CDCl_3$) of this sample of β-lipomycin (**8**) from nature resembled the ^{1}H NMR spectra of (12*R*,13*S*)-**8** and (12*S*,13*R*)-**8** (i.e., the *ul* isomers) in all respects but differed from the spectra of (12*R*,13*R*)-**8** and 12*S*,13*S*)-**8** (i.e., the *lk* isomers) in two regards: 1) the three CH-CH_3 groups gave rise to two doublets in natural **8** and in *ul*-**8** while they caused three doublets in *lk*-**8**; 2) the CMe=C*H* resonance of natural **8** and *ul*-**8** was at $\delta=5.60$ ppm whereas it appeared at $\delta=5.48$ ppm in *lk*-**8**. These coincidences and discrepancies proved unambiguously that the side-chains of α- (**3**) and β-lipomycin (**8**) were *anti*-configured in accordance with our earlier conclusions.

We subjected the above-mentioned 7.8:0.9 mixture of β-lipomycin (**8**) and a presumed isomer with a (*Z*)-configured disubstituted C=C bond to a Lemieux–Johnson cleavage of (most of) the C=C bonds. We reduced the expected carbonyl compounds by $NaBH_4$ and isolated a mixture wherein we identified one C=C-containing 1,5-diol and one C=C-free 1,3-diol by GC–MS analysis. This mixture was esterified with bis(trifluoroacet)imide[52] giving the corresponding bis(trifluoroacetates) **33** and **34** (Table 2). Comprehensive sets of all

Table 2: Proving the 3D structure of the side-chains of α-lipomycin (**3**) and β-lipomycin (**8**) by GC comparisons of their trifluoroacetylated degradation products $\mathbf{33}_{\text{from 3 via 8}}$ and $\mathbf{34}_{\text{from 3 via 8}}$ with two comprehensive sets of synthetic reference compounds.



Degradation product of lipomycin	GC retention time of degradation product[a]	Synthetic bis(trifluoroacetate) for reference	GC retention time[a]
$\mathbf{33}_{\text{from 3 via 8}}$	45.5 min	(4*R*,5*S*)-**33**	45.7 min
		(4*S*,5*R*)-**33**	43.4 min
		(4*R*,5*R*)-**33**	60.1 min
		(4*S*,5*S*)-**33**	59.7 min



Degradation product of lipomycin	GC retention time of degradation product[b]	Synthetic bis(trifluoroacetate) for reference	GC retention time[b]
$\mathbf{34}_{\text{from 3 via 8}}$	38.9 min	(2*R*,3*S*)-**34**	38.9 min
		(2*S*,3*R*)-**34**	38.0 min
		(2*R*,3*R*)-**34**	37.1 min
		(2*S*,3*S*)-**34**	36.1 min

[a] GC conditions: Astec Chiraldex G-TA, column length 30 m, column diameter 0.25 mm, coating thickness 0.12 μm, $T_{injector}=200$ °C, $T_{column}=85$ °C (isocratic), flame ionization detector. [b] GC conditions: FS-Cyclodex beta-I/P, column length 50 m, column diameter 0.32 mm, coating thickness not published on manufacturer's website (http://www.cs-chromatographie.de) and accordingly unknown, $T_{injector}=250$ °C, $T_{column}=80$ °C (isocratic), flame ionization detector.

stereoisomers of both bis(trifluoroacetates) were synthesized for comparison.[53] GC analyses of each bis(trifluoroacetate) from the degradation of lipomycin and of the pertinent reference compounds from synthesis allowed the identification of bis(trifluoroacetate) $\mathbf{33}_{\text{from 3 via 8}}$ as compound (4*R*,5*S*)-**33** and bis(trifluoroacetate) $\mathbf{34}_{\text{from 3 via 8}}$ as compound (2*R*,3*S*)-**34** by their pairwise matching retention times (Table 2). The two identities proved that the sterocenters in the side-chain of α-lipomycin (**3**) and β-lipomycin (**8**) were configured 12*R* and 13*S*. This result coincided with what we had concluded from our synthetic endeavour.

Our synthesis of β-lipomycin [(12*R*,13*S*-**8**)] required 16 steps, comprised 9 steps in the longest linear sequence, and gave an overall yield of 6%. The following Communication describes an independent different synthesis of β-lipomycin (12*R*,13*S*-**8**).[20] It was realized after a comprehensive analysis of the polyketide synthase genome,[19] by which the configurations of C-12 and C-13 were predicted correctly. Whatever concerns one might have held against "assignments derived from synthesis and analysis", there are none against the identical "assignments from chemical degradation", which we provided.

Altamycin[54] and oleficin[55] are 3-polyenoyl tetramic acids, which possess the constitution of a lower and a higher vinylogue, respectively, of α-lipomycin (**3**). This similarity leaves one wondering whether their unexplored side-chain stereocenters have the same configurations as those of α-lipomycin (**3**), in other words, whether altamycin is bisnor-α-lipomycin and whether oleficin is bishomo-α-lipomycin. Our conditions for degrading α-lipomycin (**3**) to the bis(trifluoroacetates) **33** and **34** and our pool of reference compounds[53] for assessing their configurations should reveal the 3D structures of altamycin and oleficin readily. Moreover the retrosynthetic disconnection on which we based our total synthesis of α-lipomycin (**3**) should be applicable to the syntheses of altamycin, oleficin, and other 3-(polyenoyl)tetramic acids as well.

Received: February 17, 2014
Published online: June 4, 2014

Keywords: configuration determination · cross-coupling · natural products · polyenes · tetramic acids

[1] Reviews: a) H.-G. Henning, A. Gelbin, *Adv. Heterocycl. Chem.* **1993**, *57*, 139 – 185; b) B. J. L. Royles, *Chem. Rev.* **1995**, *95*, 1981 – 2001; c) E. L. Ghisalberti in *Studies in Natural Product Chemistry*, *Vol. 28* (Ed.: Atta-ur-Rahman), Elsevier, Dordrecht, **2003**, pp. 109 – 163; d) R. Schobert, A. Schlenk, *Bioorg. Med. Chem.* **2008**, *16*, 4203 – 4221; e) Y.-C. Jeong, M. Anwar, Z. Bikadi, E. Hazai, M. G. Moloney, *Chem. Sci.* **2013**, *4*, 1008 – 1015.

[2] L. Jurd, *J. Heterocycl. Chem.* **1996**, *33*, 1227 – 1232.

[3] Y.-C. Jeong, M. G. Moloney, *J. Org. Chem.* **2011**, *76*, 1342 – 1354.

[4] V. V. Gromak, V. G. Avakyan, O. F. Lakhvich, *J. Appl. Spectrosc.* **2000**, *67*, 205 – 215.

[5] C. E. Stickings, *Biochem. J.* **1959**, *72*, 332 – 340.

[6] January 29, 2013: The search items were tautomers **1** (125 hits) and their enols (90 hits); altogether they comprised 177 different hits.

[7] For example: F. Vinale, G. Flematti, K. Sivasithamparam, M. Lorito, R. Marra, B. W. Skelton, E. L. Ghisalberti, *J. Nat. Prod.* **2009**, *72*, 2032 – 2035; b) J. C. Carlson, S. Li, D. A. Burr, D. H. Sherman, *J. Nat. Prod.* **2009**, *72*, 2076 – 2079; c) S. Cao, J. A. V. Blodgett, J. Clardy, *Org. Lett.* **2010**, *12*, 4652 – 4654; d) M. Isaka, P. Chinthanom, S. Supothina, P. Tobwor, N. L. Hywel-Jones, *J. Nat. Prod.* **2010**, *73*, 2057 – 2060; e) Z. Yu, S. Vodanovic-Jankovic, N. Ledeboer, S.-X. Huang, S. R. Rajski, M. Kron, B. Shen, *Org. Lett.* **2011**, *13*, 2034 – 2037; f) R. Kumar, R. Subramani, K.-D. Feussner, W. Aalbersberg, *Mar. Drugs* **2012**, *10*, 200 – 208; g) M. E. Rateb, Z. Yu, Y. Yan, D. Yang, T. Huang, S. Vodanovic-Jankovic, M. A. Kron, B. Shen, *J. Antibiot.* **2014**, *67*, 127 – 132.

[8] T. Sengoku, Y. Nagae, Y. Ujihara, M. Takahashi, H. Yoda, *J. Org. Chem.* **2012**, *77*, 4391 – 4401.

[9] K. Kempf, A. Raja, F. Sasse, R. Schobert, *J. Org. Chem.* **2013**, *78*, 2455 – 2461.

[10] Y. Ujihara, K. Nakayama, T. Sengoku, M. Takahashi, H. Yoda, *Org. Lett.* **2012**, *14*, 5142 – 5145.

[11] A. Yajima, C. Ida, K. Taniguchi, S. Murata, R. Katsuta, T. Nukada, *Tetrahedron Lett.* **2013**, *54*, 2497 – 2501.

[12] H. Ikeda, N. Matsumori, M. Ono, A. Suzuki, A. Isogai, H. Nagasawa, S. Sakuda, *J. Org. Chem.* **2000**, *65*, 438 – 444.

[13] S. Matsunaga, N. Fusetani, Y. Kato, *J. Am. Chem. Soc.* **1991**, *113*, 9690 – 9692.

[14] Bicyclic tetramic acids represent another field of recent interest. See: a) C. A. Holloway, C. J. Matthews, Y.-C. Jeong, M. G. Moloney, C. F. Roberts, M. Yaqoob, *Chem. Biol. Drug Des.* **2011**, *78*, 229 – 235; b) Y.-C. Jeong, M. Anwar, T. M. Nguyen, B. S. W. Tan, C. L. L. Chai, M. G. Moloney, *Org. Biomol. Chem.* **2011**, *9*, 6663 – 6669; c) M. Anwar, M. G. Moloney, *Chem. Biol. Drug Des.* **2013**, *80*, 645 – 649.

[15] B. Kunze, K. Schabacher, H. Zähner, A. Zeeck, *Arch. Mikrobiol.* **1972**, *86*, 147 – 174.

[16] K. Schabacher, A. Zeeck, *Tetrahedron Lett.* **1973**, *14*, 2691 – 2694.

[17] A. Zeeck, *Justus Liebigs Ann. Chem.* **1975**, 2079 – 2088.

[18] A. Bihlmaier, E. Welle, C. Hofmann, K. Welzel, A. Vente, E. Breitling, M. Müller, S. Glaser, A. Bechthold, *Antimicrob. Agents Chemother.* **2006**, *50*, 2113 – 2121.

[19] A. Kitsche, M. Kalesse, *ChemBioChem* **2013**, *14*, 851 – 861.

[20] This insight set the stage for an independent second total synthesis of **8** (O. Hartmann, M. Kalesse, *Angew. Chem.* **2014**, DOI: 10.1002/ange.201402259; *Angew. Chem. Int. Ed.* **2014**, DOI: 10.1002/anie.201402259).

[21] a) R. Brückner, K. Siegel, A. Sorg in *Strategies and Tactics in Organic Synthesis*, *Vol. 5* (Ed.: M. Harmata), Elsevier, Amsterdam, **2004**, pp. 437 – 473; b) A. Sorg, R. Brückner, *Angew. Chem.* **2004**, *116*, 4623 – 4626; *Angew. Chem. Int. Ed.* **2004**, *43*, 4523 – 4526; c) A. Sorg, K. Siegel, R. Brückner, *Chem. Eur. J.* **2005**, *11*, 1610 – 1624; d) J. Burghart, R. Brückner, *Eur. J. Org. Chem.* **2011**, 150 – 165.

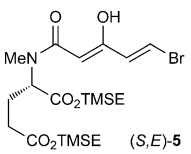
[22] Bis(*tert*-butyldimethylsilyl)-protected D-digitoxal (**9**) as a glycosylating agent: a) F. E. McDonald, K. Subba Reddy, Y. Diaz, *J. Am. Chem. Soc.* **2000**, *122*, 4304 – 4309; b) F. E. McDonald, K. Subba Reddy, *Angew. Chem. Int. Ed.* **2001**, *40*, 3653 – 3655; *Angew. Chem.* **2001**, *113*, 3765 – 3767.

[23] M. J. C. Buckle, I. Fleming, S. Gil, K. L. C. Pang, *Org. Biomol. Chem.* **2004**, *2*, 749 – 769.

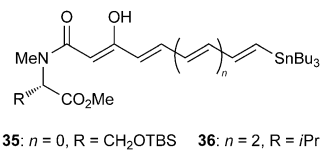
[24] S. C. Archibald, D. J. Barden, J. F. Y. Bazin, I. Fleming, C. F. Foster, A. K. Mandal, A. K. Mandal, D. Parker, K. Takaki, A. C. Ware, A. R. B. Williams, A. B. Zwicky, *Org. Biomol. Chem.* **2004**, *2*, 1051 – 1064.

[25] R. N. Lacey, *J. Chem. Soc.* **1954**, 850 – 854.

[26] Earlier substrates of cross-coupling/Lacey – Dieckmann cyclocondensation[25] routes to tetramic acids were the stannylated β-ketoamide esters **35** (D. J. Dixon, S. V. Ley, T. Gracza, P. Szolcsanyi, *J. Chem. Soc. Perkin Trans. 1* **1999**, 839 – 841) and **36** (D. A. Longbottom, A. J. Morrison, D. J. Dixon, S. V. Ley, *Angew. Chem. Int. Ed.* **2002**, *41*, 2786 – 2790; *Angew. Chem.* **2002**, *114*, 2910 – 2914; D. A. Longbottom, A. J. Morrison, D. J. Dixon, S. V. Ley, *Tetrahedron* **2003**, *59*, 6955 – 6966). In contrast to our β-ketoamide ester **5**, **35** and **36** act as nucleophiles in their cross-couplings.



(*S*,*E*)-**5**



35: *n* = 0, R = CH_2OTBS **36**: *n* = 2, R = *i*Pr

[27] S. Nahm, S. M. Weinreb, *Tetrahedron Lett.* **1981**, *22*, 3815 – 3818.
[28] Method: R. H. Munday, R. M. Denton, J. C. Anderson, *J. Org. Chem.* **2008**, *73*, 8033 – 8038.
[29] Procedure: http://www.euticals.com/attachments/082_t3p.pdf.
[30] W. C. Still, M. Kahn, A. Mitra, *J. Org. Chem.* **1978**, *43*, 2923 – 2925.
[31] P. Sieber, *Helv. Chim. Acta* **1977**, *60*, 2711 – 2716.
[32] Method: A. J. Pearson, M. V. Chelliah, *J. Org. Chem.* **1998**, *63*, 3087 – 3098.
[33] Ag_2O was prepared as described by D. E. Janssen, C. V. Wilson, *Org. Synth.* **1956**, *36*, 46 – 47.
[34] R. K. Olsen, *J. Org. Chem.* **1970**, *35*, 1912 – 1915.
[35] V. N. Belov, M. Brands, S. Raddatz, J. Krüger, S. Nikolskaya, V. Sokolov, A. de Meijere, *Tetrahedron* **2004**, *60*, 7579 – 7589.
[36] For details see the Supporting Information.
[37] S. V. Ley, S. C. Smith, P. R. Woodward, *Tetrahedron* **1992**, *48*, 1145 – 1174.
[38] a) A. B. Northrup, D. W. C. MacMillan, *J. Am. Chem. Soc.* **2002**, *124*, 6798 – 6799; b) P. M. Pihko, A. Erkkliä, *Tetrahedron Lett.* **2003**, *44*, 7607 – 7609; c) A. Córdova, *Tetrahedron Lett.* **2004**, *45*, 3949 – 3952.
[39] D. A. Evans, B. D. Allison, M. G. Yang, C. E. Masse, *J. Am. Chem. Soc.* **2001**, *123*, 10840 – 10852.
[40] Dibromomethylenations of unprotected β-hydroxyaldehydes with CBr_4/PPh_3: a) M. J. Robins, S. F. Wnuk, X. Yang, C.-S. Yuan, R. T. Borchardt, J. Balzarini, E. De Clerq, *J. Med. Chem.* **1998**, *41*, 3857 – 3864; b) N. F. Langille, J. S. Panek, *Org. Lett.* **2004**, *6*, 3203 – 3206.
[41] Method: F. Ramirez, N. B. Desai, N. McKelvie, *J. Am. Chem. Soc.* **1962**, *84*, 1745 – 1747.
[42] Method: G. Köbrich, H. Trapp, K. Flory, W. Drischel, *Chem. Ber.* **1966**, *99*, 689 – 697.
[43] Such dibromomethylenation / Br,Li exchange / rearrangement sequences, possibly followed by a C-alkylation, were introduced by E. J. Corey, P. L. Fuchs, *Tetrahedron Lett.* **1972**, *13*, 3769 – 3972.
[44] V. P. Miller, D.-y. Yang, T. M. Weigel, O. Han, H.-w. Liu, *J. Org. Chem.* **1989**, *54*, 4175 – 4188.
[45] P.-E. Sum, L. Weiler, *Can. J. Chem.* **1982**, *60*, 327 – 334.
[46] H. Paulsen, V. Sinnwell, *Chem. Ber.* **1978**, *111*, 879 – 889.
[47] J. Thiem, P. Ossowski, J. Schwentner, *Chem. Ber.* **1980**, *113*, 955 – 969.
[48] Procedure: J. Ma, Y. Zhao, S. Ng, J. Zhang, J. Zeng, A. Than, P. Chen, X.-W. Liu, *Chem. Eur. J.* **2010**, *16*, 4533 – 4540.
[49] For details see the Supporting Information.
[50] We did not assess either the C=C bond configurations or the stereochemical homogeneity of the monostannanes **29** or the pentaenes **31** because of signal overlap (**29**, **31**) and the interference of satellite peaks caused by $^{1}H,^{117}Sn$, $^{1}H,^{119}Sn$, $^{13}C,^{117}Sn$, and $^{13}C,^{119}Sn$ couplings. Each isomer of **31** was a mixture of the keto and the enol tautomers of both the (*Z*)- and the (*E*)-configured amide "rotamers". In contrast, the isomers of compound **8** represented 9:1 mixtures of (*Z*)- and (*E*)-configured enols.
[51] B. Barnickel, R. Schobert, *J. Org. Chem.* **2010**, *75*, 6716 – 6719.
[52] M. Donike, *J. Chromatogr. A* **1973**, *78*, 273 – 279.
[53] For syntheses see the Supporting Information.
[54] Y. D. Shenin, *Antibiot. Med. Biotekhnol.* **1986**, 835 [*Chem. Abstr.* **1987**, *106*, 66971].
[55] a) G. Horváth, J. Gyimesi, Z. Méhesfalvi-Vajna, *Tetrahedron Lett.* **1973**, *14*, 3643 – 3648; b) Structure revision: J. Gyimesi, Z. Méhesfalvi-Vajna, G. Horváth, *J. Antibiot.* **1978**, *31*, 626 – 627.

