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## Sigillin A, a unique polychlorinated arthropod deterrent from the snow flea *Ceratophysella sigillata*\*\*

Witali Schmidt, Thies M. Schulze, Gregor Brasse, Edyta Nagrodzka, Michael Maczka, Jürg Zettel, Peter G. Jones, Jörg Grunenberg, Monika Hilker, Ute Trauer-Kizilelma, Ute Braun, and Stefan Schulz\*

**Abstract:** The snow flea Ceratophysella sigillata, a winteractive species of springtail, produces unique polychlorinated octahydroisocoumarins to repel predators. The structure of the major compound, sigillin A, was elucidated through isolation, spectroscopic analysis, and X-ray crystallography. Sigillin A showed high repellent activity in a bioassay with predatory ants. A promising approach for the total synthesis of members of this new class of natural compounds was also developed.

he snow flea Ceratophysella sigillata (Hypogastruridae) is a member of the taxon Collembola (springtails), which are wingless, soil-inhabiting hexapods. The feeding-active individuals of C. sigillata can be found from December to March in forests, especially north of the alps; they feed at temperatures down to -2°C on microorganisms, with a strong preference for coccal algae.<sup>[1]</sup> In February and March, the springtails may form big colonies with millions of individuals migrating like superorganisms on snow or litter.<sup>[2,3]</sup> Although C. sigillata can escape arthropod predators by using its furca to propel itself away from them in a manner typical for collembola, they also show a second line of defense that relies on chemical substances. Several arthropod predators are repelled, including spiders (Neriene peltata), pseudoscorpions (Neobisium muscorum), centipedes (Lithobius forficatus), predatory insects (Stenus sp., Microvelia reticulata), and mites (Pergamasus barbarus), although tolerance has also been observed.<sup>[2]</sup> We became interested in deciphering the chemical nature of this avoidance behavior. Previous work has shown that other Collembola successfully use chemical defense to repel predators. C. denticulata produces syringic and 3-hydroxy-4,5-dimethoxy benzoic acids<sup>[4]</sup> to ward off attacks. Other known defense compounds from collembola include 2-aminophenol from *Neanura muscorum*,<sup>[5]</sup> and unique pyrido[2,3-b]pyrazines contained in the pseudocelluar fluid of *Tetrodontophora bielanensis*<sup>[6]</sup> and also produced by *Onychiurus scotarius* and *O. circulans*.<sup>[7]</sup>

To identify the repellent principle of *C. sigillata*, the Collembola were collected during the major winter activity period and extracted and analyzed, but none of the known compounds were detected. The distinct odor that Collembola release when handled could be identified by headspace analysis to be (-)-geosmin, a known sesquiterpenoid released by many actinobacteria, cyanobacteria, and myxobacteria.<sup>[8]</sup> Nevertheless, geosmin proved to be ineffective in repelling predators.

Therefore, whole-body methyl acetate extracts were analyzed by GC–MS. Besides common compounds such as fatty acids, cholesterol, and cuticular hydrocarbons, a major compound **A** with an unusual mass spectrum was detected (Figure 1). GC–HRMS revealed its molecular formula to be  $C_{14}H_{15}Cl_5O_6$  (m/z obs. 453.9315, calc. 453.9311), with five double-bond equivalents. The isotope pattern of five Cl atoms can be recognized in the otherwise mostly uncharacteristic mass spectrum. The base peak at m/z 43 and the ion [M–60] hinted at the presence of an acetoxy group. The rigorous exclusion of any chlorine source during work-up excluded the possibility that compound **A** was a work-up artefact.

By using preparative HPLC, one mg of compound A was isolated from about 10 g springtails with 78% purity. The gasphase infrared spectrum showed significant absorptions at 3554 cm<sup>-1</sup> and at 3465 cm<sup>-1</sup>, which indicate the presence of two hydroxy groups. Absorptions at 1764 cm<sup>-1</sup> and 1220 cm<sup>-1</sup> hinted at an ester or lactone group. Several microscale derivatization reactions were performed to obtain more information on the functional groups present in A. No reaction was observed with diazomethane, thus excluding the presence of an acid functionality. Reaction with N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) led to a monosilylated compound. IR spectroscopy of this product showed that only one of the two hydroxy groups was silvlated, thus indicating a hindered OH group. Saponification of the natural sample with sodium methoxide followed by silylation with MSTFA led to two major compounds with molecular masses of 660 and 588. These values can only be explained by the opening of a lactone ring to a methyl ester, cleavage of an acetate group, and finally silvlation of two or all three OH groups now present. It followed that compound A should be a lactone with three additional OH groups, of which one is

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<sup>[\*]</sup> Dr. W. Schmidt, Dr. T. M. Schulze, Dr. G. Brasse, Dr. E. Nagrodzka, M. Maczka, Prof. Dr. J. Grunenberg, Prof. Dr. S. Schulz Institut für Organische Chemie, TU Braunschweig Hagenring 30, 38106 Braunschweig (Germany) E-mail: stefan.schulz@tu-bs.de Homepage: http://www.oc.tu-bs.de/schulz/index.html Prof. Dr. P. G. Jones Institut für Analytische und Anorganische Chemie TU Braunschweig, Hagenring 30, 38106 Braunschweig (Germany) Prof. Dr. I. Zettel Speichergasse 8, 3150 Schwarzenburg (Switzerland) Prof. Dr. M. Hilker, U. Trauer-Kizilelma, U. Braun Institut für Biologie, Freie Universität Berlin Haderslebener Strasse 9, 12163 Berlin (Germany) [\*\*] We thank the Deutsche Forschungsgemeinschaft for financial support by a grant, DFG Schu984/8-1.

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Figure 1. a) EI mass spectrum of unknown compound A, identified to be sigillin A (1). b) Total ion chromatogram of a methyl acetate extract of *Ceratophysella sigillata*. Fatty acids (FA), cholesterol (CH), hydrocarbons (HC), and minor sigillin derivatives (B–I) of major compound A (1, sigillin A), are present in the extract. The structures of sigillins B–I (B–I; compounds 5–12) are shown in Scheme 2.

acetylated; five chlorine atoms; and two additional doublebond equivalents. No similar compound is known from the literature.

The <sup>13</sup>C NMR and <sup>1</sup>H NMR spectra revealed a total of 14 carbon atoms and 15 hydrogen atoms in agreement with the molecular formula (see Table S1 in the Supporting Information). The <sup>13</sup>C NMR resonances were assigned to two carbonyl groups ( $\delta$  170.9, 168.4 ppm), two sp<sup>2</sup> quaternary carbons ( $\delta$  130.5, 121.0 ppm), three sp<sup>3</sup> quaternary carbons ( $\delta$  130.5, 121.0 ppm), three sp<sup>3</sup> quaternary carbons ( $\delta$  99.3, 73.4, 50.0 ppm), one sp<sup>3</sup> methine ( $\delta$  82.7 ppm), two sp<sup>3</sup> oxomethine ( $\delta$  74.5, 73.0 ppm), two sp<sup>3</sup> methylene ( $\delta$  31.9, 27.0 ppm), and two sp<sup>3</sup> methyl ( $\delta$  20.3, 14.9 ppm) carbon atoms. The remaining two double bond equivalents showed the presence of an additional ring and one double bond in **A**. Signals for two protons ( $\delta$  5.14, 3.4 ppm) could be eliminated from the <sup>1</sup>H NMR spectrum by adding [D<sub>4</sub>]MeOH and were thus assigned to two hydroxy groups.

Two-dimensional NMR experiments ( ${}^{1}$ H, ${}^{1}$ H-COSY,  ${}^{1}$ H, ${}^{1}$ H-COSY-long range,  ${}^{1}$ H, ${}^{1}$ SC-HMBC and  ${}^{1}$ H, ${}^{1}$ SC-HSQC) were performed to determine the connectivity. A CCl<sub>3</sub>-CH(O)-CH<sub>2</sub>-CH(C)(O)-CH<sub>2</sub>-C= spin system was deduced but it remained unclear whether the adjacent, obviously chlorine-substituted double bond was endo- or exocyclic. No reliable  ${}^{13}$ C NMR data are available for similar structural elements because they have rarely been synthesized or isolated. Furthermore, the trichloromethyl group and the two hydroxyl groups were located on the same side, as indicated by NOE NMR experiments, while the orientation of the acetyloxy group could not be clarified. In conclusion, the four structures **1**–**4** (relative configurations, Scheme 1) seemed to be in accord with the spectroscopic data.



**Scheme 1.** Possible structures of natural compound **A** that are consistent with the obtained NMR and MS data. The correct structure (1) is highlighted.

DFT (density functional theory) calculations of the gas phase IR spectra of compounds **1–4** were performed using the B3LYP hybrid functional in combination with a standard 6-31(d) basis set (Figure S16 in the Supporting Information). The sum of the difference of the nine important lines of the calculated versus natural spectrum was markedly lower for **1** compared to all of the others, thus pointing to structure **1** as the one that most likely represents the natural product.

At this stage, a new work-up procedure, including extraction of live colonies and immediate work-up, was developed (see the Supporting Information), which resulted in an increase of the yield of **A** and related metabolites to about 20 mg per 10 g fresh weight. Evidently, the concentration of **A** in *C. sigillata* is very high (0.2% or higher of fresh weight). From this material, a single crystal suitable for X-ray analysis was obtained. The result confirmed the proposed structure (Figure 2) and established the absolute configura-



*Figure 2.* Structure of sigillin A as determined by X-ray crystallography. H atoms are omitted for clarity in the structure on the right.

tion to be 3R, 5R, 6R, 9S, 10R. The large trichloromethyl group prefers a quasi-equatorial position, thereby fixing the conformation of the lactone ring into a twisted boat conformation typical for  $\delta$ -lactones,<sup>[9]</sup> while the cyclohexane ring remains in the common chair conformation. We propose the name sigillin A for the natural compound **A**.

Careful analysis of the natural extract revealed the presence of eight additional sigillin derivatives, named sigillins B–I (Figure 1 and Scheme 2). The structures were tentatively deduced from their mass spectra (see the Supporting Information). The level of chlorination, easily detectable

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**Scheme 2.** The sigillins A–I (1, 5-12). The percentages shown describe the peak area relative to that of 1.

by the distinctive isotopic pattern of chlorine, differs between the sigillins, with values of between four and six detected. Deacetylated compounds accompany the acetates, usually in lower amounts. Despite their relatively high numbers of alcohol groups, the sigillins can be investigated by GC. The presence of additional more polar derivatives was ruled out using HPLC–UV/MS in ESI negative mode (see the Supporting Information). No additional derivatives were found, while most of the sigillins were detected. Derivatives containing a dichloromethylidene group proved to be UV active, while the others were not, thus further supporting the structural assignments. Samples from different years and regions always showed the presence of sigillins, although the content varied.

The biosynthetic origin of the sigillin family is unknown. The compounds are probably polyketides because they show the appropriate arrangement of methyl and hydroxy groups (Figure S17). The octahydrobenzopyranone ring system could be formed from two malonate and three methylmalonate units through ring closure. The C6 acetyloxy group requires an additional oxidation. The chlorine atoms are probably introduced by radical halogenation. A sequence via a dichloromethyl group that is transformed through halogenation into the trichloromethyl group, followed by elimination to the dichloromethenyl group seems to take place; all these structural elements are found in the sigillins. There are known examples of radical formation of a trichloromethyl group. Unactivated methyl groups have been shown to be radically chlorinated by non-heme iron halogenases in a reaction requiring  $\alpha$ -ketoglutarate and oxygen.<sup>[10]</sup> These enzymes have been investigated in detail in the biosynthesis of barbamide,<sup>[11]</sup> armentomycin, and 2-amino-4,4-dichlorobutanoic acid.<sup>[12]</sup> A similar enzyme might mediate a radical chlorination in the biosynthesis of the sigillins. Because no polyketide synthases are known from insects or other arthropods, it seems likely that sigillin is produced by symbiotic bacteria or taken up from the algal food. The release of geosmin further supports an insect/bacterial symbiosis. Nevertheless, the large amounts of sigillins present in C. sigillata are striking and might indicate biosynthesis by the animal itself.

The ecological effects of sigillin A were tested in a bioassay with the ant Myrmica rubra. This ant species is a generalist predatory model insect that has been used in many bioassays for testing the feeding deterrent activity of natural products identified in hexapods.<sup>[13,14]</sup> Collembola are potential prey of *M. rubra*,<sup>[15]</sup> which forages both on soil and in vegetation for food. An aqueous suspension of crushed larvae of Drosophila melanogaster containing 0.2% sigillin A was prepared to simulate the natural concentration of **1** in the Collembola. This preparation and a control sample consisting of crushed D. melanogaster alone were offered simultaneously in an arena containing 15 ants. The feeding behavior at test and control samples was recorded for a period of 10 min. First encounters of workers with the test and control samples occurred randomly. When an ant encountered a test sample, it first took a taste and started briefly to feed upon it. Hence, the ant behavior did not indicate that test or control samples had any repellent activity via the gas phase.

However, soon after tasting the test samples, the ants moved away from them. Significantly fewer ants fed upon the test samples than upon the controls over the entire bioassay period (Figure 3). Ant individuals started to feed at different times during the assay. The bioassay showed that sigillin A acts as strong feeding deterrent against a generalist predatory insect. Therefore, the predator reaction described by Zettel et al.<sup>[2]</sup> is most likely caused by sigillin.



**Figure 3.** Bioassay with the ant Myrmica rubra feeding on crushed Drosophila melanogaster larvae without (black bar) or with (white bar) 0.2% sigillin A (1). The numbers of ants feeding on the two samples were recorded every minute for ten minutes. Twelve arenas with 15 ants each were used. N: number of feeding ants; \*\*  $P \le 0.01$  (Wilcoxon signed-rank test for paired differences).

The unique structural features of sigillin A make it a challenging target for total synthesis. We were interested in obtaining derivatives for testing in various biological assays to evaluate the activity profile of these halogenated octahydroisocoumarines. As a start, the dideoxy derivative **20** of sigillin A served as synthetic target because it contains the isocoumarin backbone, as well as the two chlorinated functional groups.

The synthesis started from key lactone **13**, which was obtained from chloral by building on a strategy introduced by

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Shimizu et al.,<sup>[16]</sup> (see the Supporting Information) but can alternatively be obtained through a ring-closing metathesis (RCM) approach.<sup>[17]</sup> It served as Michael acceptor in a copper-catalyzed conjugated 1,4-addition reaction with the use of 3-butenylmagnesium bromide and a copper bromide dimethylsulfide complex<sup>[18]</sup> to furnish lactone **14** (Scheme 3).



Scheme 3. Synthesis of didesoxysigillin 20.

In the next step, a vinyl group had to be attached next to the carbonyl group. The use of acetaldehyde as a vinyl cation synthon proved to be unsuccessful. While the addition is uneventful, the following elimination reaction, even when using a good leaving group such as mesylate, results in concomitant loss of HCl and a low yield. This problem was circumvented through the addition of phenylselenoacetaldehyde to form the alcohol **15** and subsequent elimination under mild basic conditions to arrive at diene **16**.<sup>[19]</sup> Only the desired 2,3-*anti*-diastereomer was formed. Diene lactone **16** was then subjected to RCM by using a second-generation Grubbs catalyst<sup>[20]</sup> to obtain the *trans*-fused bicyclic lactone **17** in good yield.

Only moderate yields of acyloin **19** were obtained through ketohydroxylation with in situ generated ruthenium tetroxide,<sup>[21]</sup> and overoxidation products were observed. By changing to a two-step synthesis, the yield was improved through stepwise dihydroxylation reaction with AD-mix,<sup>[22]</sup> followed by Parikh–Doering oxidation<sup>[23]</sup> of the better accessible hydroxy group of **18** to give **19** in 67% overall yield (Scheme 3). In the last step, the keto group needed to be transformed into the dichloromethylidene unit found in sigillin A. This transformation was achieved, but with limited success. Although model compounds such as 2-hydroxy- or 2acetyloxycyclohexanone could be converted with the standard method using CCl<sub>4</sub>/PPh<sub>3</sub>,<sup>[24]</sup> this reaction completely failed with 19. The transformation of 19 or analogues with a protected hydroxy group by various other methods, e.g., the Takeda method of dichloromethenylation with a titanocene<sup>[25]</sup> or a magnesium/titanium catalyst,<sup>[26]</sup> also proved unsuccessful. Finally, the synthesis of **20** was achieved in very low yield by treating 19 with hydrazine and subsequent addition of CBrCl<sub>3</sub>, ammonia, and catalytic amounts of CuCl.<sup>[27]</sup> Nevertheless, the target compound didesoxysigillin A (20) was isolated and a crystal structure was obtained (see the Supporting Information). The relative stereochemistry is identical to that of **1**.

While marine organisms are well known producers of a wide variety of halogenated compounds, fewer complex halogenated secondary metabolites are found in terrestrial organisms.<sup>[28]</sup> Most are produced by bacteria and fungi, only a few are produced by more complex organisms such as plants or arthropods.<sup>[28]</sup> Trichloromethylcarbinol or its ester and dichloromethylidene functional groups are rare structural elements in natural products. They occur in Muironolide A,<sup>[29]</sup> a marine sponge metabolite, citreochlorol, which is produced by *Penicillium* sp.,<sup>[30]</sup> and the fungal products pinicoloform<sup>[31]</sup> and KS-504 compounds.<sup>[32]</sup>

In conclusion, sigillin A and its congeners represent a new class of natural products. They function as strong feeding deterrents against ants and seem to play an important role in the ecology of springtails. A synthesis of the parent chlorinated ring system was developed, which allows the synthesis of structural analogues. Currently, the bacteria associated with Collembola are under investigation to establish the biosynthetic origin of the sigillins. Furthermore, biological testing and chemical synthesis of sigillin analogues are under way.

**Keywords:** insect repellents  $\cdot$  isocoumarine  $\cdot$  natural products  $\cdot$  organohalogen compounds  $\cdot \alpha$ -vinylation

- J. Zettel, U. Zettel, C. Suter, S. Streich, B. Egger, *Pedobiologia* 2002, 46, 404–413.
- [2] J. Zettel, U. Zettel, Mitt. Naturforsch. Ges. Bern 2008, 65, 79– 110.
- [3] E. Christian, E. Meyer, Ber. Natwiss.-Med. Ver. Innsbruck 1997, 84, 315–320.
- [4] C. Bitzer, G. Brasse, K. Dettner, S. Schulz, J. Chem. Ecol. 2004, 30, 1591–1602.
- [5] C. Messer, J. Walther, K. Dettner, S. Schulz, *Pediobiologia* 2000, 44, 210–220.
- [6] K. Dettner, A. Scheuerlein, P. Fabian, S. Schulz, W. Francke, J. Chem. Ecol. 1996, 22, 1051–1074.
- [7] E. Nilsson, G. Bengtsson, J. Chem. Ecol. 2004, 30, 1431-1443.
- [8] a) N. N. Gerber, *Tetrahedron Lett.* 1968, 25, 2971–2974; b) S. Schulz, J. S. Dickschat, *Nat. Prod. Rep.* 2007, 24, 814–842; c) J. S. Dickschat, H. B. Bode, T. Mahmud, R. Müller, S. Schulz, *J. Org. Chem.* 2005, 70, 5174–5182.

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- [9] H. Wolf, Tetrahedron Lett. 1966, 7, 5151-5156.
- [10] a) D. P. Galonić, F. H. Vaillancourt, C. T. Walsh, J. Am. Chem. Soc. 2006, 128, 3900-3901; b) C. S. Neumann, D. G. Fujimori, C. T. Walsh, Chem. Biol. 2008, 15, 99-109; c) M. Ueki, D. P. Galonić, F. H. Vaillancourt, S. Garneau-Tsodikova, E. Yeh, D. A. Vosburg, F. C. Schroeder, H. Osada, C. T. Walsh, Chem. Biol. 2006, 13, 1183-1191; d) F. H. Vaillancourt, J. Yin, C. T. Walsh, Proc. Natl. Acad. Sci. USA 2005, 102, 10111-10116.
- [11] J. Orjala, W. H. Gerwick, J. Nat. Prod. 1996, 59, 427-430.
- [12] A. D. Argoudelis, R. R. Herr, D. J. Mason, T. R. Pyke, J. F. Zieserl, *Biochemistry* 1967, 6, 165–170.
- [13] M. Hilker, S. Schulz, J. Chem. Ecol. 1991, 17, 2323-2332.
- [14] M. Hilker, C. Häberlein, U. Trauer, M. Bünnige, M.-O. Vicentini, S. Schulz, *ChemBioChem* **2010**, *11*, 1720–1726.
- [15] Z. I. Reznikova, S. N. Panteleeva, Dokl. Biol. Sci. 2001, 380, 475-477.
- [16] M. Shimizu, K. Ishii, T. Fujisawa, Chem. Lett. 1997, 765-766.
- [17] E. Roulland, Angew. Chem. Int. Ed. 2008, 47, 3762–3765; Angew. Chem. 2008, 120, 3822–3825.
- [18] M. Kanai, Y. Nakagawa, K. Tomioka, *Tetrahedron* 1999, 55, 3843–3854.
- [19] a) D. L. J. Clive, C. G. Russell, J. Chem. Soc. Chem. Commun.
  1981, 434–436; b) C. J. Kowalski, J.-S. Dung, J. Am. Chem. Soc.
  1980, 102, 7950–7951.
- [20] M. Scholl, S. Ding, C. W. Lee, R. H. Grubbs, Org. Lett. 1999, 1, 953–956.
- [21] B. Plietker, J. Org. Chem. 2003, 68, 7123-7125.
- [22] a) V. VanRheenen, R. C. Kelly, D. Y. Cha, *Tetrahedron Lett.* **1976**, *17*, 1973–1976; b) G. A. Molander, R. Figueroa, J. Org. Chem. **2006**, *71*, 6135–6140.

- [23] J. R. Parikh, W. E. Doering, J. Am. Chem. Soc. 1967, 89, 5505-5507.
- [24] a) R. Appel, Angew. Chem. Int. Ed. Engl. 1975, 14, 801-811;
  Angew. Chem. 1975, 87, 863-874; b) N. S. Isaacs, D. Kirkpatrick,
  J. Chem. Soc. Chem. Commun. 1972, 443-444; c) G. Burton, J. S. Elder, S. C. M. Fell, A. V. Stachulski, Tetrahedron Lett. 1988, 29, 3003-3006.
- [25] T. Takeda, Y. Endo, A. S. Reddy, R. Sasaki, T. Fujiwara, *Tetrahedron* 1999, 55, 2475–2486.
- [26] C.-T. Chien, C.-C. Tsai, C.-H. Tsai, T.-Y. Chang, P.-K. Tsai, Y.-C. Wang, T.-H. Yan, J. Org. Chem. 2006, 71, 4324–4327.
- [27] V. G. Nenajdenko, A. V. Shastin, V. M. Muzalevskii, E. S. Balenkova, *Russ. Chem. Bull. Int. Ed.* **2004**, *53*, 2647–2649.
- [28] G. W. Gribble, Naturally Occurring Organohalogen Compounds—A Comprehensive Update, Springer, Wien, 2010.
- [29] D. S. Dalisay, B. I. Morinaka, C. K. Skepper, T. F. Molinski, J. Am. Chem. Soc. 2009, 131, 7552–7553.
- [30] S. Lai, Y. Shizuri, S. Yamamura, K. Kawai, H. Furukawa, *Heterocycles* 1991, 32, 297–305.
- [31] U. Becker, A. Timm, O. Sterner, Z. Naturforsch. C 1994, 49, 772– 774.
- [32] S. Nakanishi, K. Ando, I. Kawamoto, T. Yasuzawa, H. Sano, H. Kase, J. Antibiot. 1989, 42, 1775–1783.

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## **Communications**

**Chlorinated Repellents** 

Sigillin A, a unique polychlorinated arthropod deterrent from the snow flea *Ceratophysella sigillata* 



**Inedible**: The snow flea *Ceratophysella sigillata* produces unique polychlorinated octahydroisocoumarins to repel predators. The structure of the major representative, sigillin A, was elucidated through isolation, spectroscopic analysis, and X-ray crystallography. A promising synthetic approach for the total synthesis of this class of compounds was developed, in which  $\alpha$ -vinylation of a lactone and ring-closing metathesis are they key steps. (Photo F. Kohl)

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