Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 2823–2833

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis and Antimicrobial Activity of Tetrodecamycin Partial Structures

Franz F. Paintner,[a,*] Lars Allmendinger,[a] Gerd Bauschke,[a] Caroline Berns[a] and Peter Heisig[b]

[a]*Department Pharmazie-Zentrum für Pharmaforschung, Ludwig-Maximilians-Universität München, Butenandtstraße 5-13, Haus C, D-81377 München, Germany*

[b]*Institut für Pharmazie der Universität Hamburg, Abteilung für Pharmazeutische Biologie und Mikrobiologie, Bundesstraße 45, D-20146 Hamburg, Germany*

Received 17 January 2003; accepted 25 March 2003

Abstract—An efficient synthetic approach to the core structure **5** of the novel polyketide antibiotic tetrodecamycin (**1**) was developed. This approach features the acid-catalyzed cyclization of a *tert*-butyldimethylsilyl protected methyl α-(γ-hydroxyacyl) tetronate, leading to the novel tricyclic ring skeleton exhibited by **5**, and an efficient strategy for the parallel introduction of the *cis*-diol and *exo*-methylene function. In addition to **5**, diastereomer **26**, analogue **6** and several derivatives (**16**, **27**–**29**) were prepared and evaluated for their antibacterial activities against *Staphylococcus aureus* (including MRSA) and *Enterococcus faecalis* and for their cytotoxic activities against human leukemia cell lines (HL-60, Jurkat T-cells). While compound **5** did not inhibit the growth of the Gram-positive pathogens (MICs $>128\,\mu g\,mL^{-1}$), analogue **6** and 2-naphthoyl derivative **27** showed promising antibacterial activities with MICs of 4–16 $\mu g\,mL^{-1}$. Remarkably, the antibacterial activity of these compounds was paralleled by cytotoxicity (IC_{50} 10–23 μM). The reactive *exo*-methylene moiety was shown to be crucial, but not sufficient by its own, for both the antibacterial and the cytotoxic activities.

Introduction

New classes of antibacterial agents with novel mechanisms of action are urgently needed, due to the worldwide emergence of infections caused by multidrug resistant bacteria.[1] In particular, resistances to Gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant coagulase negative staphylococci (MRCNS) as well as vancomycin-resistant *Enterococcus faecalis* and *E. faecium* (VRE) give rise to significant problems amongst hospitalized patients.[2]

Tetrodecamycin (**1**), a novel polyketide antibiotic isolated from the culture broth of *Streptomyces nashvillensis* MJ885-mF8, has been shown to have promising antibacterial activity against Gram-positive bacteria including *Staphylococcus aureus* (MIC 6.25–12.5 $\mu g\,mL^{-1}$) as well as *Bacillus anthracis*.[3] Markedly enhanced activity was observed for several 14-*O* substituted derivatives (e.g., 2-naphthoyl derivative **2** with a MIC of 0.78–3.12 $\mu g\,mL^{-1}$ against *S. aureus* in vitro).[4] On the other hand, dihydrotetrodecamycin (**3**), which has been isolated from the Streptomyces strain, too, is virtually inactive, revealing the crucial role of the *exo*-methylene moiety in **1** and **2** for biological activity.

Although the mechanism of action of these antibiotics is still unknown, one can speculate, that the biological activities of **1** and **2** may be attributed to their reactivity with the cystein residues of functional proteins forming covalent bonds via Michael type addition. Indeed, thiols like *N*-acetylcysteamine were shown to add smoothly across the *exo*-methylene moiety of **1**, leading to the respective *S*-alkylation products (e.g., **4**).[4] However, unlike **3**, these derivatives still displayed marked antibacterial activity in vitro. This may be due to retro-Michael reactions regenerating tetrodecamycin (**1**) as the biological active compound (Fig. 1).

*Corresponding author. Fax +49-89-2180-7247; e-mail: franz.paintner@cup.uni-muenchen.de

0968-0896/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0968-0896(03)00221-9

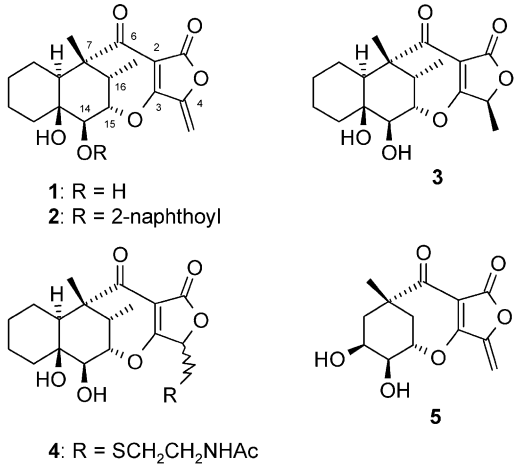


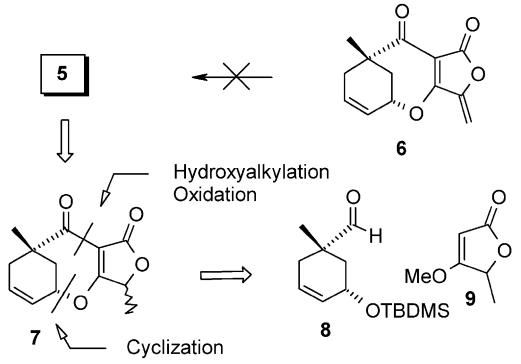
Figure 1.

We now report the synthesis of a tricyclic partial structure **5** of tetrodecamycin (**1**) comprising the unique anhydro α-(γ-hydroxy-acyl) tetronic acid derived core structure of the parent antibiotic.[5] The antibacterial activity of **5** as well as of a series of derivatives and analogues thereof was evaluated in vitro in an attempt to elucidate minimum structural requirements for antimicrobial potency. In addition, partial structures exhibiting an *exo*-methylene moiety were screened against human cell cultures in vitro in an effort to gauge the cytotoxicity of this class of compounds.

Results and Discussion

Synthetic plan

Our synthetic strategy is outlined in Scheme 1. Tetrodecamycin partial structure **5** was envisioned to arise from tricyclic intermediate **7**. This building block should be readily accessible from two key transformations: (a) regioselective 3-hydroxyalkylation of 4-*O*-methyl tetronate **9** with aldehyde **8** followed by an oxidation step to afford the respective 3-acyl-4-*O*-methyl tetronate[6] and (b) subsequent acid catalyzed cyclization of this precursor. In a previous communication we reported a short approach to tricyclic substructure **6**, containing the crucial *exo*-methylene moiety.[7] In the course of further investigations, however, it became clear, that final



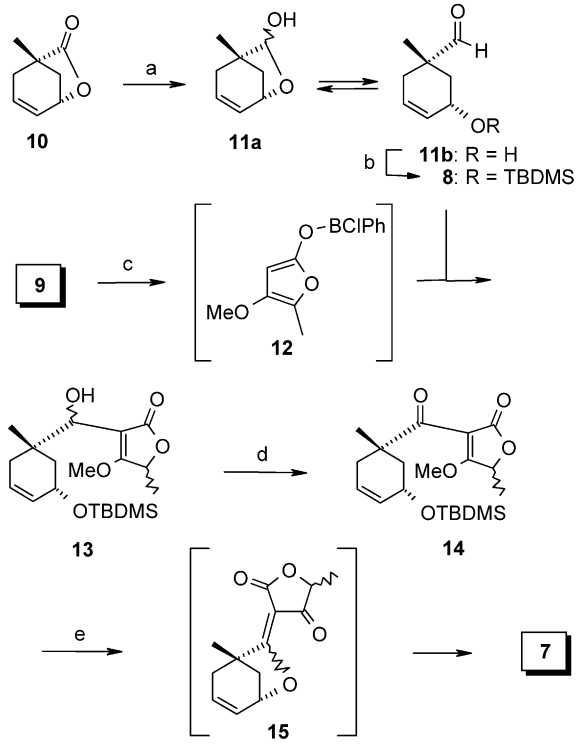
Scheme 1.

osmium tetroxide promoted *cis*-dihydroxylation of **6** to obtain partial structure **5** is unfeasible, due to an exclusive attack of the reagent on the *exo*-methylene group. Accordingly to obtain **5** from **7** it was obvious to introduce the *syn* diol group prior to the generation of the exocyclic C–C double bond.

Synthesis of key building block 7

As depicted in Scheme 2, key building block **7** could be readily prepared in five steps from the known bicyclic lactone **10**.[8] First, **10** was treated with DIBAL in dichloromethane at −90 °C to give reduction product **11** in 86% yield. Selective silylation of the ring opened hydroxy aldehyde form **11b**, which in dichloromethane solution prevails in an equilibrium with the corresponding bicyclic lactol **11a** [**11a**/**11b** ~1:3, as determined by ^{1}H NMR (CD_2Cl_2, rt)], was accomplished on treatment of **11** with TBDMSOTf in the presence of 2,6-lutidine at low temperature (CH_2Cl_2, −78 °C). Thus, aldehyde **8** was obtained in high yield (94%) in addition to small amounts of the respective silylated lactol. Cyclization precursor **14** (1:1 mixture of diastereomers) was obtained in 78% overall yield by highly regioselective 3-hydroxyalkylation of in situ generated boron furanolate **12**,[6] with aldehyde **8** and subsequent oxidation of the intermediate alcohol **13** (mixture of diastereomers) with 2-iodoxybenzoic acid (IBX).

To effect the desired cyclization, 3-acyl tetronate **14** was treated with catalytic amounts of concentrated H_2SO_4 in dichloromethane at room temperature, thus affording



Scheme 2. (a) DIBAL, CH_2Cl_2, −90 °C (87%); (b) TBDMSOTf, lutidine, CH_2Cl_2, −78 °C (94%); (c) (i) **9**, $PhBCl_2$, *i*-Pr_2NEt, CH_2Cl_2, −78 °C; (ii) **8**, −78 °C to rt (86%); (d) IBX, DMSO, rt (91%); (e) cat concd H_2SO_4, CH_2Cl_2, rt (89%).

7 in high yield (89%). This acid-catalyzed ring closure step comprises the kinetically controlled initial formation of tetrahydrofuranylidene tetrahydrofuran-2,4-dione **15** (mixture of isomers), which slowly isomerizes to the thermodynamically favored product **7**.[9] The structure assigned for **7** was confirmed by IR and 2D NMR correlation with the respective 4-unsubstituted derivative, whose structure was unambiguously proven by X-ray diffraction analysis.[5]

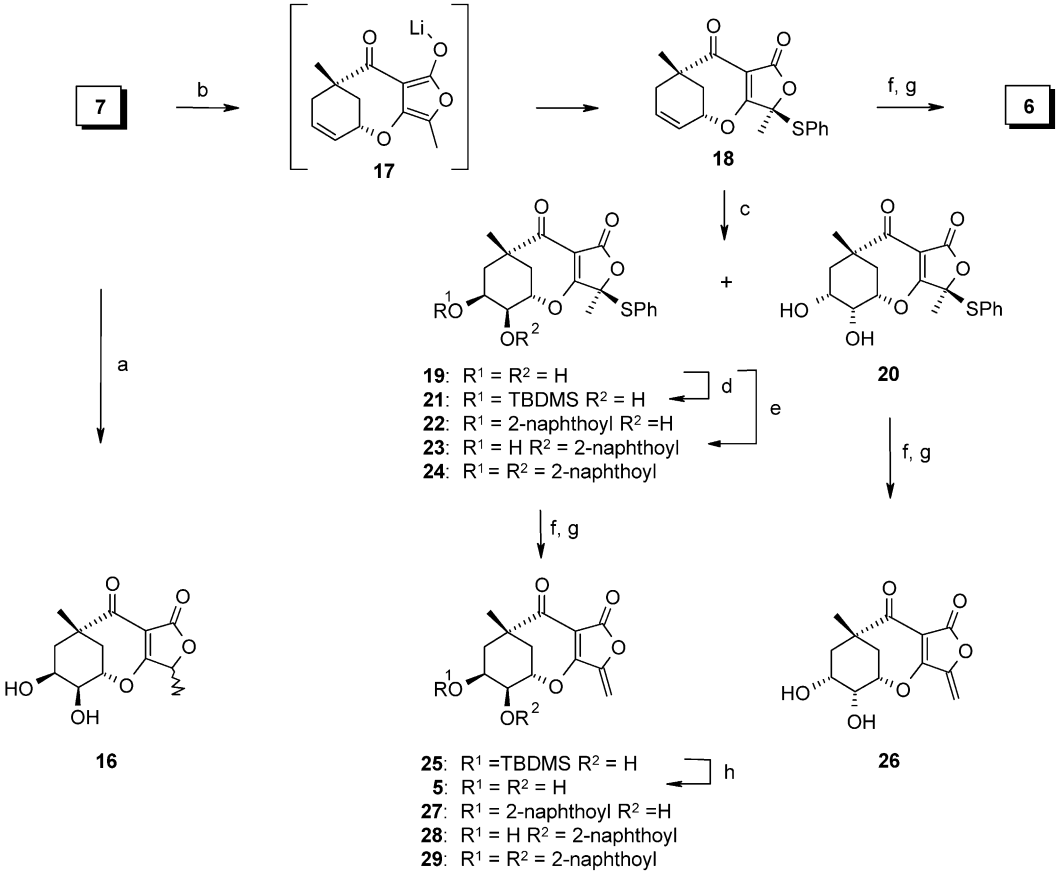
Synthesis of tetrodecamycin partial structure 5

Our initial efforts to dihydroxylate building block **7** were disappointing. Ruthenium-catalyzed *cis*-dihydroxylation according to Shing et al.[10] [$RuCl_3$ (0.07 equiv), $NaIO_4$ (1.5 equiv), MeCN, EtOAc, H_2O, 0 °C] led to the desired diol **16** (as an inseparable 1:1 mixture of C-4 epimers) with good stereoselectivity (d.s = 95:5), but only in poor yield (27%), probably as a result of concurrent glycole cleavage (Scheme 3). On the other hand, commonly employed osmium tetroxide[11] or potassium permanganate[12] based dihydroxylations [e.g., OsO_4 (0.02 equiv), *N*-methylmorpholine *N*-oxide (1.0 equiv), acetone, *t*-BuOH, H_2O, rt; $KMnO_4$, 18-crown-6, CH_2Cl_2, rt) did not succeed at all, due to preferred oxidation of the acidic γ-methine group of the tetronate by these reagents.

To overcome this obstacle, it was obvious to block the acidic γ-position with an appropriate substituent, which both has to be compatible with standard *cis*-dihydroxylation conditions and should serve as an elimination precursor, suitable for the generation of the exocyclic C–C double bond.

We focused on the phenylsulfanyl group, since it is known to withstand osmium tetroxide under certain reaction conditions.[13,14] Moreover, this group serves as *syn* elimination precursors upon oxidation to the corresponding sulfoxide.

Introduction of the phenylsulfanyl group at the γ-position was accomplished by converting **7** first into the corresponding lithio furanolate **17** (LHMDS, THF, −78 °C) and then by reacting it with *N*-phenylmercaptophthalimide (−78 °C to rt) to give **18** (9:1 mixture of diastereomers) in 87% yield. Subsequent dihydroxylation with osmium tetroxide under stoichiometric conditions (pyridine, CCl_4, rt) provided *cis*-diols **19** and **20** in high overall yield (87 and 6%, respectively) and with good diastereoselectivity (d.s. **19**:**20** = 93:7). The relative configurations of *cis*-diols **19** and **20** as well as that of **16** (vide supra) were determined on the basis of ^1H–^1H *J*-coupling values and NOE studies. Next diol **19** was monoprotected as a *tert*-butyldimethylsilyl ether



Scheme 3. (a) $RuCl_3$ (0.07 equiv), $NaIO_4$ (1.5 equiv), MeCN, EtOAc, H_2O, 0 °C, (27%); (b) (i) LHMDS, THF, −78 °C; (ii) *N*-phenylmercaptophthalimide, −78 °C to rt (87%); (c) (i) OsO_4, pyridine, CCl_4, rt; (ii) aq $NaHSO_3$ (**19**: 87%; **20**:%); (d) TBDMSOTf, lutidine, CH_2Cl_2, −78 °C (92%); (e) 2-naphthoylchloride; NEt_3, DMAP, CH_2Cl_2, rt (**22**: 35%; **23**: 18%; **24**: 15%); (f) MCPBA, CH_2Cl_2, −20 °C; (g) $BaCO_3$, benzene, 80 °C (**25**: 57% from **21**); (**26**: 22% from **20**); (**27**: 28% from **22**); (**28**: 40% from **23**); (**29**: 19% from **24**); (**6**: 51% from **18**); (h) HF, MeCN, 0 °C (100%).

(TBDMSOTf, lutidine, CH_2Cl_2, −78 °C) to form **21** regioselectively (92% yield). This additional step was necessary to enable isolation and purification of the subsequent products, which were hardly soluble in appropriate organic solvents when derived from **19**. Sulfide **21** was readily oxidized to the corresponding sulfoxide (mixture of diastereomers) by 3-chloroperoxybenzoic acid (CH_2Cl_2, −20 °C), which on pyrolysis ($BaCO_3$, benzene, 80 °C) gave *exo*-methylene derivative **25** in 57% overall yield. Finally, deprotection of the hydroxyl group (HF, MeCN, 0 °C) afforded tetrodecamycin partial structure **5** in quantitative yield.

In the same way, diol **20** as well as 2-naphthoyl derivatives **22**, **23** and **24** were converted into the corresponding *exo*-methylene compounds **26**, **27**, **28** and **29** (in 22, 28, 40, and 19% overall yields, respectively). Esters **22**, **23** and **24** had been obtained in 35, 18 and 15% yield, respectively, from **19** by acylation with 2-naphthoyl chloride in the presence of catalytic amounts of DMAP (NEt_3, CH_2Cl_2, rt).

Finally, key building block **18** was converted into tetrodecamycin substructure **6** (51% yield). Indeed, this current approach was more convenient than our previously reported.[7]

Evaluation of in vitro antibacterial activities

The in vitro antibacterial activities of compounds **5**–**7**, **16** and **26**–**29** were evaluated at concentrations from 0.5 to 128 μg mL^{-1} against strains of two Gram-positive bacterial species: *S. aureus* [ATCC 25923, ATCC 29213 (MRSA)] and *E. faecalis* (ATCC 29212). The observed minimum inhibitory concentrations are listed in Table 1.

Tetrodecamycin partial structure **5**, which lacks a methyl group and in particular one cyclohexane ring of the *trans*-decalin fragment of the parent antibiotic, did not show growth inhibitory activity against the tested strains of *S. aureus* and *E. faecalis*, even at concentrations as high as 128 μg mL^{-1}. This may be due to a loss of lipophilicity as compared with **1** or even more likely to a different geometry of the cyclohexane substructure included in both molecules. In case of **1** this ring, as part of the *trans*-decalin fragment, exists in the boat form, in case of partial structure **5**, however, it is chairlike. As a consequence the spatial orientation of the hydroxyl groups in both structures is also markedly different.

Table 1. In vitro antibacterial activity MIC[a] (μg mL^{-1})

Compd	*S. aureus* ATCC 25923	*S. aureus*[b] ATCC 29213	*E. faecalis* ATCC 29212
5	> 128	> 128	> 128
6	4	8	4
7	> 128	—	> 128
16	> 128	> 128	> 128
26	> 128	> 128	64
27	8	16	4
28	128	> 128	64
29	> 128	—	> 128

[a]Minimum inhibitory concentrations.
[b]MRSA.

Interestingly a weak activity against the *E. faecalis* strain, but not against the *S. aureus* strains, could be noticed for diastereomer **26**. As was to be expected no activity was observed for dihydrotetrodecamycin partial structure **16**.

In case of tetrodecamycin (**1**) a clear enhancement of antimicrobial activity has been reported for certain 14-*O* substituents, particularly for a 2-naphthoyl residue (derivative **2**, vide supra). Indeed, acylation of the respective hydroxyl group in **5** with 2-naphthalene carboxylic acid now led to weakly antibacterial active compound **28**. A 16-fold higher activity, however, could be observed for 2-naphthoyl derivative **27**, in which the neighbouring hydroxyl group has been acylated. This marked difference in activity may be due to the spatial orientation of the acyl residue, which in **27** is equatorial, just like for tetrodecamycin derivative **2**, but is axial in case of compound **28** (vide supra). Notably, diacyl derivative **29** is bare of antimicrobial activity, indicating that a free, particularly axial aligned hydroxyl group may be relevant for efficacy.

Surprisingly we also found distinct antibacterial activity for tetrodecamycin substructure **6**, which lacks the vicinal *syn* diol function. Thus **6** had MICs of 4–8 μg mL^{-1} against *S. aureus* and *E. faecalis* suggesting a promising antibacterial potential. On the other hand dihydro derivative **7** was found to be inactive emphasizing the crucial role of the *exo*-methylene moiety for antibacterial activity.

In vitro cytotoxicity of compounds 5–7 and 27

The cytotoxicity of tetrodecamycin partial structure **5** and of compounds **6**, **7** and **27** was assessed in vitro against human leukemia HL-60 cells and human T cell leukemia Jurkat cells. The observed IC_{50} values are listed in Table 2.

Remarkably, the cytotoxicity of these compounds parallels with their antimicrobial activity. Thus for **5** at concentrations as high as 100 μM (26.5 μg mL^{-1}) no cytotoxicity was observed. On the other hand distinct activity against the two tested cell lines (IC_{50} 10–23 μM) was found for compounds **6** and **27**. In contrast to **6** compound **7** proved to be inactive, evidently due to the absence of the *exo*-methylene moiety.

In conclusion, we have developed an efficient approach to the core structure **5** of the novel polyketide antibiotic

Table 2. Cytotoxicity of compounds **5**–**7** and **27**

Compd	IC_{50} (μM)[a]	
	HL-60	Jurkat
5	> 100	> 100
6	10	23
7	> 100	> 100
27	N.D.	22

[a]IC_{50}, 50% inhibitory concentration represents the mean from dose response curves of at least three experiments.

tetrodecamycin (**1**). In addition to **5**, diastereomer **26**, analogue **6** and several derivatives (**16**, **27**–**29**) have been prepared and evaluated for their antibacterial and cytotoxic activities. While tetrodecamycin partial structure **5** did not inhibit the growth of the tested Gram-positive pathogenes, 2-naphthoyl derivative **27** and analogue **6** showed promising antibacterial activities. However, these activities were paralleled by appreciable cytotoxicity. In summary the reactive *exo*-methylene moiety was shown to be crucial, but not sufficient by its own, for both antibacterial and cytotoxic activities. Further investigations, with the objective of determining whether the antimicrobial and cytotoxic activities could be detached from each other, are in progress in our laboratories.

Experimental

Chemistry

Unless otherwise noted, reactions were carried out in oven-dried glassware under an atmosphere of dry N_2. All reagents were used as commercially available. CH_2Cl_2 and *i*-Pr_2NEt were distilled from CaH_2 and THF from sodium metal immediately before use. Standard syringe techniques were applied for transferring anhydrous solvents. Flash chromatography: silica gel (Merck 60, 0.040–0.063 mm). Preparative HPLC: L-6000 pump, L-4000 UV/Vis, D-2000 Chromato Integrator (Merck-Hitachi), column, Hibar RT LiChrosorb® Si 60 (7 μm, 250 × 25 mm) (Merck). Melting points (mp, uncorrected values): Melting point apparatus according to Dr. Tottoli (Büchi no. 510). ^1H and ^{13}C NMR spectra: Eclipse 500 FTNMR spectrometer (Jeol), 500 and 125 MHz, respectively, chemical shifts (δ) are reported in ppm, TMS as internal standard. IR spectra: FT-IR spectrometer Paragon 1000 (Perkin–Elmer). Mass spectra: 5989 Mass spectrometer with 59980 B particle beam LC/MS interface (Hewlett Packard). High resolution mass spectrometry: MStation 700 (Jeol). Elemental analysis: CHN Rapid (Heraeus).

(1*RS*,5*RS*)-1-Methyl-6-oxabicyclo[3.2.1]oct-3-ene-7-ol (11a). (1*RS*,5*RS*)-5-Hydroxy-1-methylcyclohex-3-enecarbaldehyde (11b). DIBAL (1.0 M in CH_2Cl_2, 22.0 mL, 22.0 mmol) was added dropwise to a solution of **10** (2.763 g, 20.0 mmol) in CH_2Cl_2 (100 mL) at −90 °C. After stirring for 6 h at −90 °C the reaction was quenched by the addition of a saturated aqueous solution of potassium sodium tartrate (20 mL). The mixture was allowed to attain ambient temperature and was stirred at that temperature for 1.5 h. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (5 × 20 mL). The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by flash chromatography (*n*-hexane–Et_2O, 4:6) to give **11** (2.430 g, 86.7%) as a colorless oil.

IR (film) 3390, 3030, 2927, 1728, 1716, 1652 cm $^{-1}$; MS (CI, CH_5^+): *m*/*z* (%) = 141 (3) [M + H$^+$], 93 (100); ^1H NMR ($CDCl_3$) [In $CDCl_3$ the ring opened hydroxy aldehyde prevails in an equilibrium mixture with the corresponding diastereomeric tricyclic lactols. Hydroxy aldehyde/lactol (major isomer)/lactol (minor isomer) = 75:17:8]: δ = 1.18 (s, 0.75 × 3H, CH_3), 1.20 (s, 0.17 × 3H, CH_3), 1.22 (s, 0.08 × 3H, CH_3), 1.66 (d, 0.17 × 1H, *J* = 10.8 Hz, 8-H), 1.77–1.87 (m, 0.08 × 1H, 8-H), 1.80 (dd, 0.75 × 1H, *J* = 5.4, 14.2 Hz, 6-H), 1.84 (dd, 0.75 × 1H, *J* = 2.1, 17.5 Hz, 2-H), 1.96–2.05 (m, 0.08 × 2H, 2-H and 8-H), 2.00 (dd, 0.75 × 1H, *J* = 3.5, 14.2 Hz, 6-H), 2.01 (dd, 0.17 × 1H, *J* = 5.2, 10.8 Hz, 8-H), 2.18 (br d, 0.17 × 1H, *J* = 18.6 Hz, 2-H), 2.30 (dt, 0.17 × 1H, *J* = 2.9, 18.6 Hz, 2-H), 2.44 (dd, 0.75 × 1H, *J* = 2.7, 17.5 Hz, 2-H), 2.53 (d, 0.08 × 1H, *J* = 19.3 Hz, 2-H), 2.66 (br s, 0.75 × 1H, OH), 3.28 (br s, 0.17 × 1H, OH), 3.41 (br s, 0.08 × 1H, OH), 4.19 (br t, 0.75 × 1H, *J* = 5.4 Hz, 5-H), 4.49 (t, 0.17 × 1H, *J* = 5.2 Hz, 5-H), 4.71 (m, 0.08 × 1H, 5-H), 4.99 (s, 0.17 × 1H, 7-H), 5.18 (s, 0.08 × 1H, 7-H), 5.72 (m, 0.17 × 1H, 3-H), 5.75–5.79 (m, 0.75 × 2H, 3-H and 4H), 5.80–5.90 (m, 0.08 × 1H, 3-H), 6.16 (m, 0.08 + 0.17 × 1H, 4-H), 9.43 (s, 0.75 × 1H, CHO). ^{13}C NMR ($CDCl_3$) (only data for **11b** are shown): δ = 21.33 (CH_3), 31.21 (C-2), 38.19 (C-6), 44.64 (C-1), 63.73 (C-5), 126.90 (C-4), 129.56 (C-3), 205.20 (CHO). Anal. calcd for $C_8H_{12}O_2$ (140.18): C, 68.55; H, 8.63. Found: C, 68.58; H, 8.65.

(1*RS*,5*RS*)-5-(*tert*-Butyldimethylsilanyloxy)-1-methylcyclohex-3-enecarbaldehyde (8). TBDMSOTf (3.44 mL, 14.98 mmol) was added dropwise to a solution of **11** (2.00 g, 14.27 mmol) and 2,6-lutidine (1.91 g, 2.07 mL, 17.83 mmol) in CH_2Cl_2 (100 mL) at −78 °C. After stirring for 4 h at −78 °C the reaction mixture was poured into saturated aqueous sodium hydrogencarbonate (50 mL), and the resultant layers were separated. The aqueous layer was extracted with CH_2Cl_2 (5 × 50 mL), and the combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by flash chromatography (CH_2Cl_2) to give **8** (3.40 g, 93.6%) as a colorless oil.

IR (film) 3030, 2929, 1728 cm $^{-1}$; MS (CI, CH_5^+): *m*/*z* (%) = 255 (7) [M + H$^+$], 123 (100); ^1H NMR ($CDCl_3$): δ = 0.05 (s, 3H, $SiCH_3$), 0.06 (s, 3H, $SiCH_3$), 0.87 [s, 3H, $(CH_3)_3C$], 1.06 (s, 3H, CH_3), 1.77 (dd, 1H, *J* = 4.5, 13.5 Hz, 6-H), 1.78 (d, 1H, *J* = 18.2 Hz, 2-H), 1.95 (dd, 1H, *J* = 4.5, 13.5 Hz, 6-H), 2.52 (d, 1H, *J* = 18.2 Hz, 2-H), 4.23 (m, 1H, 5-H), 5.68 (m, 1H, 4-H), 5.77 (m, 1H, 3-H), 9.50 (s, 1H, CHO). ^{13}C NMR ($CDCl_3$): δ = −4.8 ($SiCH_3$), −4.5 ($SiCH_3$), 18.1 [$C(CH_3)_3$], 21.4 (CH_3), 26.9 [$C(CH_3)_3$], 30.7 (C-2), 39.7 (C-6), 44.3 (C-1), 64.4 (C-5), 127.0 (C-4), 129.2 (C-3), 204.0 (CHO). Anal. calcd for $C_{14}H_{26}O_2Si$ (254.45): C, 66.08; H, 10.30. Found: C, 65.94; H, 10.24.

(5*RS*)-3-[(1*RS*,5*RS*)-5-(*tert*-Butyldimethylsilanyloxy)-1-methylcyclohex-3-enecarbonyl]-4-methoxy-5-methyl-2(5*H*)-furanone and (5*SR*)-3-[(1*RS*,5*RS*)-5-(*tert*-butyldimethylsilanyloxy)-1-methylcyclohex-3-enecarbonyl]-4-methoxy-5-methyl-2(5*H*)-furanone (14). $PhBCl_2$ (2.49 g, 2.03 mL, 15.75 mmol) and then *i*-Pr_2NEt (3.87 g, 5.18 mL, 30.0 mmol) were added dropwise to a solution of **9**[15] (1.92 g, 15.0 mmol) in CH_2Cl_2 (60 mL) at −78 °C. After stirring for 1 h at −78 °C a solution of **8** (3.82 g, 15.0 mmol) in CH_2Cl_2 (15.0 mL) was added, and the

mixture was allowed to warm to room temperature and stirred for 1.5 h. Saturated aqueuos sodium hydrogencarbonate (30.0 mL) was added, and the mixture was extracted with CH_2Cl_2 (5 × 100 mL). The combined organic layers were dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by flash chromatography (*n*-hexane–Et_2O, 20:80) to give **13** (4.39 g, 85.8%) as an inseparable ~1:1:1:1 mixture of diastereomers. A solution of 2-iodoxybenzoic acid (IBX) (5.41 g, 19.30 mmol) in DMSO (20 mL) was added to this mixture of diastereomers. The mixture was stirred at room temperature until TLC control indicates complete conversion (~1.5 h). The resulting suspension was diluted with Et_2O (100 mL), applied on a silica gel column (~250 mL) and eluated with *n*-hexane–Et_2O (1:1) to give **14** [4.45 g, 78% (from **8**)] as colorless crystals (inseparable 1:1 mixture of diastereomers).

IR (KBr) 3041, 2952, 2929, 2858, 1750, 1681, 1629 cm^{-1}; MS (CI, CH_5^+): *m*/*z* (%) = 381 (1) [M + H$^+$], 249 (100); ^{1}H NMR ($CDCl_3$): diastereomer 1: δ = 0.08 (s, 3H, CH_3Si), 0.09 (s, 3H, $SiCH_3$), 0.89 [s, 3H, $(CH_3)_3C$], 1.38 [s, 3H, CH_3-(C-1′)], 1.49 [d, *J* = 6.8 Hz, 3H, CH_3-(C-5)], 1.83 (dd, *J* = 12.6/8.7 Hz, 1H, 6′-H), 2.09–2.22 (m, 2H, 6′-H and 2′-H), 2.45–2.55 (m, 1H, 2′-H), 3.85 (s, 3H, OCH_3), 4.35 (m, 1H, 5′-H), 4.82 (q, *J* = 6.8 Hz, 1H, 5-H), 5.63 (m, 1H, 4′-H), 5.68 (m, 1H, 3′-H); diastereomer 2: δ = 0.09 (s, 3H, CH_3Si), 0.10 (s, 3H, $SiCH_3$), 0.90 [s, 3H, $(CH_3)_3C$], 1.37 [s, 3H, CH_3-(C-1′)], 1.49 [d, *J* = 6.8 Hz, 3H, CH_3-(C-5)], 1.71 (dd, *J* = 12.6/8.7 Hz, 1H, 6′-H), 2.09–2.22 (m, 2H, 6′-H and 2′-H), 2.45–2.55 (m, 1H, 2′-H), 3.85 (s, 3H, OCH_3), 4.35 (m, 1H, 5′-H), 4.80 (q, *J* = 6.8 Hz, 1H, 5-H), 5.63 (m, 1H, 4′-H), 5.68 (m, 1H, 3′-H). Anal. calcd for $C_{20}H_{32}O_5Si$ (380.56): C, 63.12; H, 8.48. Found: C, 63.25; H, 8.65.

(1*RS*,4*RS*,9*RS*)-4,9-Dimethyl-2,5-dioxa-tricyclo[7.3.1.0^{3,7}]-trideca-3(7),11-diene-6,8-dione and (1*RS*,4*SR*,9*RS*)-4,9-dimethyl-2,5-dioxa-tricyclo[7.3.1.0^{3,7}]trideca-3(7),11-diene-6,8-dione (7). Sulphuric acid (96%, 1.64 g, 0.90 mL, 16.75 mmol) was added to a solution of **14** (2.55 g, 6.70 mmol) in CH_2Cl_2 (250 mL) at room temperature. After stirring for 1.5 h the reaction mixture was poured into H_2O (60 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (5 × 150 mL). The combined organic layers were washed with H_2O (2 × 50 mL), dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by flash chromatography (CH_2Cl_2–EtOAc, 80:20) to give **7** (1.398 g, 89.1%) as colorless crystals (inseparable 1:1 mixture of diastereomers).

IR (KBr) 3037, 2979, 2932, 1762, 1650, 1599 cm^{-1}; MS (CI, CH_5^+): *m*/*z* (%) = 235 (100) [M + H$^+$]; ^{1}H NMR ($CDCl_3$): diastereomer 1: δ = 1.21 [s, 3H, CH_3-(C-9)], 1.44 [d, *J* = 6.7 Hz, 3H, CH_3-(C-4)], 1.96 (d, *J* = 17.9 Hz, 1H, 10-H), 2.10 (dd, *J* = 16.4/6.4 Hz, 1H, 13-H), 2.46 (d, *J* = 16.4 Hz, 1H, 13-H), 2.73 (d, *J* = 17.9 Hz, 1H, 10-H), 4.80 (q, *J* = 6.7 Hz, 1H, 4-H), 5.29 (m, 1H, 1-H), 5.71 (m, 1H, 12-H), 6.17 (m, 1H, 11-H); diastereomer 2: δ = 1.22 [s, 3H, CH_3-(C-9)], 1.53 [d, *J* = 6.8 Hz, 3H, CH_3-(C-4)], 1.96 (d, *J* = 17.9 Hz, 1H, 10-H), 2.10 (dd, *J* = 16.4/6.4 Hz, 1H, 13-H), 2.46 (d, *J* = 16.4 Hz, 1H, 13-H), 2.73 (d, *J* = 17.9 Hz, 1H, 10-H), 4.69 (q, *J* = 6.8 Hz, 1H, 4-H), 5.30 (m, 1H, 1-H), 5.72 (m, 1H, 12-H), 6.17 (m, 1H, 11-H). Anal. calcd for $C_{13}H_{14}O_4$ (234.25): C, 66.66; H, 6.02. Found: C, 66.68; H, 5.93.

(1*RS*,4*RS*,9*RS*,11*SR*,12*SR*)-11,12-Dihydroxy-4,9-dimethyl-2,5-dioxatricyclo[7.3.1.O^{3,7}]trideca-3(7)-ene-6,8-dione and (1*RS*,4*SR*,9RS,11*SR*,12*SR*)-11,12-dihydroxy-4,9-dimethyl-2,5-dioxatricyclo[7.3.1.O ^{3,7}]trideca-3(7)-ene-6,8-dione (16). Ruthenium trichloride (0.6 mg, 2.3 μmol) and a solution of sodium periodate (10.7 mg, 0.05 mmol) in H_2O (66 μL) were added to a solution of **7** (7.8 mg, 0.033 mmol) in EtOAc–CH_3CN, 1:1 (0.4 mL) at 0 °C. The resulting brown solution was stirred at 0 °C for 3 min. Then the reaction was quenched by the addition of a 10% aqueous solution of sodium bisulfite (1.0 mL). The mixture was extracted with EtOAc (5 × 5 mL), and the combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure to obtain the crude product as a complex mixture of diastereomers (95:5 with respect to C11–C12 and 1:1 with respect C-4 in each case as determined by ^{1}H NMR). The crude product was purified by flash chromatography (*n*-hexane–EtOAc, 60:40) to obtain **16** (2.4 mg, 27.0%) as colorless crystals (1:1 mixture of C-4 epimers).

IR (KBr) 3491, 2923, 2847, 1754, 1648, 1603 cm^{-1}; MS (CI, CH_5^+) *m*/*z* (%) = 269 (100) [M + H$^+$]; ^{1}H NMR (Acetone-d_6): δ = 1.13 (s, 3H, 9-CH_3), 1.44 (d, 0.5 × 3H, *J* = 6.8 Hz, 4-CH_3), 1.45 (d, 0.5 × 3H, *J* = 6.8 Hz, 4-CH_3), 1.74–1.79 (m, 2H, 10-H), 2.12 (dd, 1H, *J* = 4.6, 16.4 Hz, 13-H), 2.33 (d, 0.5 × 1H, *J* = 16.4 Hz, 13-H), 2.35 (d, 0.5 × 1H *J* = 16.4 Hz, 13-H), 3.59 (m, 1H, 11-H); ^{13}C NMR (acetone-d_6): δ = 17.4, 17.6 (4-CH_3), 26.3, 26.4 (9-CH_3), 37.2, 37.3 (C-13), 39.8, 40.0 (C-10), 47.8, 47.9 (C-9), 65.75, 65.84 (C-11), 70.2, 70.3 (C-12), 72.6, 72.7 (C-5), 84.23, 84.24 (C-1); HRMS (EI): *m*/*z* calcd for $C_{13}H_{16}O_6$: 268.0947; Found: 268.0990.

(1*RS*,4*RS*,9*RS*)-4,9-Dimethyl-4-phenylsulfanyl-2,5-dioxatricyclo7.3.1.O^{3,7}trideca-3(7),11-dien-6,8-dione (18). A 1.0 M solution of LiHMDS in THF (6.14 mL, 6.14 mmol) was added dropwise to a suspension of **7** (1.370 g, 5.85 mmol) in THF (25 mL) at −78 °C. The resulting orange solution was stirred at −78 °C for 1.5 h before a solution of *N*-phenylmercaptophthalimide (1.57 g, 6.14 mmol) in THF (25 mL) was added dropwise. After stirring at −78 °C for 1 h the reaction mixture was allowed to attain room temperature before it was poured into potassium dihydrogenphosphate buffer pH 4.5 (50 mL). The mixture was extracted with Et_2O (5 × 100 mL). The combined organic layers were washed with saturated aqueous sodium hydrogencarbonate (2 × 50 mL), dried ($MgSO_4$) and evaporated under reduced pressure. The crude product (90:10 mixture of diastereomers as determined by ^{1}H NMR) was purified by flash chromatography (*n*-hexane–EtOAc, 60:40) and prep. HPLC (*n*-heptane–EtOAc, 60:40, 15 mL/min, 275 nm, R_t = 23.9 min) to give **18** (1.735 g, 86.7%) as colorless crystals; mp 176 °C.

IR (KBr) 3051, 2960, 2927, 1773, 1655, 1603 cm^{-1}; MS (CI, CH_5^+): *m*/*z* (%) = 343 (100) [M + H$^+$]; ^{1}H NMR

($CDCl_3$): δ = 0.88 [s, 3H, CH_3-(C-9)], 1.72–1.88 (m, 3H, 10-H and 13-H), 1.78 [s, 3H, CH_3-(C-4)], 2.59 (dd, 1H, J = 17.8/6.2 Hz, 10-H), 5.29 (m, 1H, 1-H), 5.67 (m, 1H, 12-H), 6.10 (m, 1H, 11-H), 7.31–7.41 (m, 3H, H_{arom}), 7.56–7.60 (m, 2H, H_{arom}); ^{13}C NMR ($CDCl_3$): δ = 23.5 [CH_3-(C-4)], 25.4 [CH_3-(C-9)], 34.1 (C-13), 37.2 (C-10), 44.6 (C-9), 76.4 (C-1), 88.6 (C-4), 102.9 (C-7), 121.4 (C-12), 128.4, 129.2, 130.3, 136.4 (C_{arom}), 135.5 (C-11), 165.4 (C-6), 177.1 (C-3), 198.0 (C-8). Anal. calcd for $C_{19}H_{18}O_4S$ (342.42): C, 66.65; H, 5.30; S, 9.36. Found: C, 66.66; H, 5.23; S, 9.30.

(1*RS*,4*RS*,9*RS*,11*SR*,12*SR*)-11,12-Dihydroxy-4,9-dimethyl-4-phenylsulfanyl-2,5-dioxa tricyclo[7.3.1.O ^{3,7}]tridec-3(7)-en-6,8-dione (19) and (1*RS*,4*RS*,9*RS*,11*RS*,12*RS*)-11,12-dihydroxy-4,9-dimethyl-4-phenylsulfanyl-2,5-dioxa tricyclo[7.3.1.O ^{3,7}]tridec-3(7)-en-6,8-dione (20). A solution of osmium tetroxide (1.134 g, 4.46 mmol) in CCl_4 (30 mL) was added to a solution of **18** (1.45 g, 4.25 mmol) in pyridine (90 mL) at room temperature. The resulting brown solution was stirred for 2 h before a 10% aqueous solution of sodium sulfite (100 mL) and pyridine (30 mL) were added. After stirring for 1 h the layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (5 × 120 mL). The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure to give a crude product as a mixture of diastereomers (**19**:**20** = 93:7 as determined by ^1H NMR). The crude product was purified by flash chromatography (CH_2Cl_2–EtOAc–2-propanol, 30:70:1) to give **19** (1.394 g, 87.1%) and **20** (91 mg, 5.7%) as colorless crystals; mp 149 and 229 °C, respectively.

19. IR (KBr) 3439, 2932, 1766, 1659, 1607 cm^{-1}; MS (CI, CH_5^+): m/z (%) = 377 (66) [M + H$^+$], 269 (100); ^1H NMR ($CDCl_3$): δ = 0.96 [s, 3H, CH_3-(C-9)], 1.39 (d, J = 16.6 Hz, 1H, 13-H), 1.66 (dd, J = 12.7/12.6 Hz, 1H, 10-H_{ax}), 1.79 (dd, J = 12.7/4.0 Hz, 1H, 10-H_{eq}), 1.84 [s, 3H, CH_3-(C-4)], 1.90 (dd, J = 16.6/4.6 Hz, 1H, 13-H), 2.71 (d, J = 4.5 Hz, 1H, OH), 2.80 (d, J = 2.0 Hz, 1H, OH), 3.58 (m, 1H, 11-H), 4.13 (m, 1H, 12-H), 5.09 (m, 1H, 1-H), 7.30–7.42 (m, 3H, H_{arom}), 7.50–7.54 (m, 2H, H_{arom}); ^{13}C NMR ($CDCl_3$): δ = 23.8 [CH_3-(C-4)], 26.4 [CH_3(C-9)], 29.2 (C-13), 39.3 (C-10), 47.6 (C-9), 66.1 (C-11), 70.4 (C-12), 83.3 (C-1), 89.5 (C-4), 102.5 (C-7), 128.3, 129.5, 130.7, 136.4 (C_{arom}), 165.6 (C-6), 179.2 (C-3), 197.9 (C-8); HRMS: Anal. calcd for $C_{19}H_{20}O_6$ (M$^+$): 376.0981; Found: 376.1114.

20. IR (KBr) 3461, 3302, 2927, 1754, 1648, 1616 cm^{-1}; MS (CI, CH_5^+): m/z (%) = 377 (28) [M + H$^+$], 111 (100); ^1H NMR (acetone-d_6): δ = 0.82 [s, 3H, CH_3-(C-9)], 1.60 (dd, J = 14.4/2.3 Hz, 1H, 10-H), 1.64 (dt, J = 16.5/2.9 Hz, 1H, 13-H), 1.75 [s, 3H, CH_3-(C-4)], 1.84 (dd, J = 16.5/3.8 Hz, 1H, 13-H), 2.18 (dt, J = 14.4/3.3 Hz, 1H, 10-H), 3.76 (dt, J = 8.8/3.9 Hz, 1H, 12-H), 3.92 (ddd, J = 3.9/3.3/2.3 Hz, 1H, 11-H), 3.99 [d, J = 2.9 Hz, 1H, OH-(C-12)], 4.02 [d, J = 8.8 Hz, 1H, OH-(C-11)], 5.14 (dd, J = 3.9/2.3 Hz, 1H, 4-H), 7.41 (m, 2H_{arom}), 7.46 (m, 1H, H_{arom}), 7.57 (m, 2H, H_{arom}). ^{13}C NMR (acetone-d_6): δ = 23.8 [(C-4)-CH_3], 26.6 [(C-9)-CH_3], 33.6 (C-13), 43.0 (C-10), 44.8 (C-9), 68.4 (C-11), 71.4 (C-12), 83.1 (C-1), 89.6 (C-4), 102.8 (C-7), 129.3, 129.7, 130.6, 136.7 (C_{arom}), 164.8 (C-6), 177.9 (C-3), 198.0 (C-8); HRMS: Anal. calcd for $C_{19}H_{20}O_6$ (M$^+$): 376.0981; Found: 376.1006.

(1*RS*,4*RS*,9*RS*,11*SR*,12*SR*)-11-(*tert*-Butyldimethylsilanyloxy)-12-hydroxy-4,9-dimethyl-4-phenylsulfanyl-2,5-dioxa-tricyclo[7.3.1.0^{3,7}]tridec-3(7)-ene-6,8-dione (21). *tert*-Butyldimethylsilyl trifluoromethanesulphonate (775 mg, 630 μL, 2.766 mmol) was added dropwise to a solution of **19** (496 mg, 1.317 mmol) and 2,6-lutidine (353 mg, 382 μL, 3.293 mmol) in CH_2Cl_2 (30 mL) at −78 °C. After stirring for 4 h at −78 °C the reaction mixture was poured into saturated aqueous sodium hydrogencarbonate (30 mL), and the resultant layers were separated. The aqueous layer was extracted with CH_2Cl_2 (5 × 50 mL), the combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by flash chromatography (*n*-hexane–Et_2O, 40:60) to give **21** (591 mg, 91.8%) as colorless crystals; mp 155 °C.

IR (KBr) 3485, 3061, 2952, 2931, 2855, 1792, 1767, 1669, 1607 cm^{-1}; MS (CI, CH_5^+): m/z (%) = 491 (100) [M + H$^+$]; ^1H NMR ($CDCl_3$): δ = −0.01 (s, 3H, CH_3Si), 0.00 (s, 3H, CH_3Si), 0.84 [s, 9H, $(CH_3)_3C$], 0.98 [s, 3H, CH_3-(C-9)], 1.40 (d, J = 16.5 Hz, 1H, 13-H), 1.62 (dd, J = 13.1/12.3 Hz, 1H, 10-H_{ax}), 1.80 (dd, J = 13.1/4.5 Hz, 1H, 10-H_{eq}), 1.81 [s, 3H, CH_3-(C-4)], 1.88 (dd, J = 16.5/4.6 Hz, 1H, 13-H), 2.56 (s_{br}, 1H, OH), 3.50 (ddd, J = 12.3/4.5/3.0 Hz, 1H, 11-H), 3.94 (m, 1H, 12-H), 5.09 (m, 1H, 1-H), 7.31–7.43 (m, 3H, H_{arom}), 7.51–7.57 (m, 2H, H_{arom}); ^{13}C NMR ($CDCl_3$): δ = −5.1 (CH_3Si), 4.6 (CH_3Si), 18.8 [$(CH_3)_3C$], 23.8 [CH_3-(C-4)], 25.6 [$(CH_3)_3C$], 26.4 [CH_3-(C-9)], 29.0 (C-13), 39.3 (C-10), 67.1 (C-11), 71.0 (C-12), 82.1 (C-1), 89.1 (C-4), 102.7 (C-7), 128.1, 129.3, 130.5, 136.2 (C_{arom}), 154.9 (C-6), 178.7 (C-3), 197.2 (C-9). Anal. calcd for $C_{25}H_{34}O_6SSi$ (490.70): C, 61.19; H, 6.98; S, 6.53; Found: C, 61.24; H, 7.08; S, 6.84.

2-Naphthalene-2-carboxylic acid (1*RS*,4*RS*,9*RS*,11*SR*,12*SR*)-12-hydroxy-4,9-dimethyl-6,8-dioxo-4-phenylsulfanyl-2,5-dioxatricyclo-[7.3.1.0^{3,7}tridec-3(7)-en-11-yl ester (22) and 2-naphthalene-2-carboxylic acid (1*RS*,4*RS*,9*RS*,11*SR*,12*SR*)-11-hydroxy-4,9-dimethyl-6,8-dioxo-4-phenylsulfanyl-2,5-dioxatricyclo-[7.3.1.0^{3,7}tridec-3(7)-en-12-yl ester (23) and 2-naphthalene-2-carboxylic acid (1*RS*,9*RS*,11*SR*,12*SR*)-4,9-dimethyl-12-(naphthalene-2-carbonyloxy)-6,8-dioxo-4-phenylsulfanyl-2,5-dioxatricyclo-[7.3.1.0^{3,7}-tridec-3(7)-en-11-yl ester (24). A solution of 2-naphthoyl chloride (76 mg, 0.4 mmol) in CH_2Cl_2 (1.5 mL) was added dropwise to a solution of **19** (125 mg, 0.333 mmol) and *N*-ethyldiisopropylamine (56 mg, 75 μL, 0.433 mmol) in CH_2Cl_2 (3 mL) at room temperature during 4 h. After stirring at room temperature for 12 h saturated aqueous sodium hydrogencarbonate (3 mL) was added, and the resultant layers were separated. The aqueous layer was extracted with Et_2O (5 × 15 mL). The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by flash chromatography

(CH_2Cl_2–EtOAc, 95:5) to give **22** (61.9 mg, 35.1%), **23** (31.6 mg, 17.9%) and **24** (35.1 mg, 15.4%) as colorless crystals; mp 258, 220–222 and 89 °C, respectively.

22. IR (KBr) 3480, 3055, 2934, 1763, 1721, 1603 cm^{-1}; ^1H NMR ($CDCl_3$): δ = 1.10 (s, 3H, 9-CH_3), 1.54 (m, 1H, 10-H), 1.94 (s, 3H, 4-CH_3), 2.04–2.13 (m, 3H, 10-H and 13-H), 2.34 (br. s, 1H, OH), 4.46 (m, 1H, 12-H), 5.00 (ddd, 1H, *J* = 2.6, 5.6, 11.8 Hz, 1-H), 5.15 (m, 1H, 11-H), 7.36 (m, 2H, H_{arom}), 7.41 (m, 1H, H_{arom}), 7.54–7.58 (m, 3H, H_{arom}), 7.62 (m, 1H, H_{arom}), 7.88 (m, 2H, H_{arom}), 7.94 (br. d, 1H, *J* = 8.4 Hz, H_{arom}), 7.96 (dd, 1H, *J* = 1.7, 8.7 Hz, H_{arom}), 8.53 (br. s, 1H, H_{arom}); ^{13}C NMR ($CDCl_3$): 23.8 (4-CH_3), 26.4 (9-CH_3), 29.0 (C-10), 35.4 (C-13), 47.5 (C-9), 68.6 (C-12), 69.3 (C-1), 82.8 (C-11), 89.3 (C-4), 102.9 (C-7), 124.9, 126.4, 127.0, 127.9, 128.3, 128.4, 128.8, 129.0, 129.4, 130.5, 131.4, 131.4, 132.5, 135.9, 136.3, 164.8 (C_{arom}), 165.4 (C-6), 178.7 (C-3), 196.2 (C-8); MS (CI, CH_5^+): *m*/*z* = 531 (100) [M + H^+]; HRMS (EI): *m*/*z* calcd for $C_{30}H_{26}O_7S$–C_6H_5S: 421.1287; Found: 421.1287.

23. IR (KBr) 3487, 3054, 2925, 1775, 1717, 1615 cm^{-1}; ^1H NMR ($CDCl_3$): δ = 1.08 (s, 3H, 8-CH_3), 1.53 (d, 1H, *J* = 16.8 Hz, 13-H), 1.80 (dd, 1H, *J* = 11.3, 13.5 Hz, 10-H), 1.88 (s, 3H, 4-CH_3), 1.95 (br. s, 1H, OH), 1.97 (dd, 1H, *J* = 5.0, 16.8 Hz, 13-H), 2.24 (dd, 1H, *J* = 5.0, 13.5 Hz, 10-H), 3.84 (m, 1H, 11-H), 5.24 (m, 1H, 1-H), 5.56 (m, 1H, 12-H), 7.31–7.36 (m, 2H, H_{arom}), 7.39 (m, 1H, H_{arom}), 7.52–7.56 (m, 2H, H_{arom}), 7.60 (t, 1H, *J* = 7.6 Hz, H_{arom}), 7.65 (t, 1H, *J* = 7.6 Hz, H_{arom}), 7.92 (d, 1H, *J* = 8.2 Hz, H_{arom}), 7.93 (d, 1H, *J* = 8.6 Hz, H_{arom}), 7.99 (d, 1H, *J* = 8.2 Hz, H_{arom}), 8.01 (dd, 1H, *J* = 1.7, 8.6 Hz, H_{arom}), 8.59 (br. s, 1H, H_{arom}); ^{13}C NMR ($CDCl_3$): δ = 24.0 (4-CH_3), 26.9 (9-CH_3), 30.6 (C-13), 40.9 (C-10), 47.4 (C-9), 65.6 (C-11), 71.7 (C-12), 81.3 (C-1), 89.6 (C-4), 103.6 (C-7), 125.1, 126.1, 126.1, 127.2, 128.0, 128.4, 128.4, 128.8, 129.0, 129.5, 129.5, 130.5, 131.7, 132.5, 136.0, 136.3, 165.0 (C_{arom}), 166.0 (C-6), 178.5 (C-3), 197.3 (C-8); MS (CI, CH_5^+); *m*/*z* (%) = 531 (9) [M + H^+], 249 (100); HRMS (EI): *m*/*z* calcd for $C_{30}H_{26}O_7S$–C_6H_5S: 421.1287; Found: 421.1287.

24. IR (KBr) 3061, 2925, 1784, 1720, 1610 cm^{-1}; ^1H NMR ($CDCl_3$): δ = 1.19 (s, 3H, 9-CH_3), 1.71 (d, 1H, *J* = 16.7 Hz, 13-H), 2.00 (s, 3H, 4-CH_3), 2.14 (dd, 1H, *J* = 4.8, 16.7 Hz, 13-H), 2.18 (dd, 1H, *J* = 12.1, 13.2 Hz, 10-H), 2.34 (dd, 1H, *J* = 4.6, 13.2, 10-H), 5.23 (ddd, 1H, *J* = 3.0, 4.6, 12.1 Hz, 11-H), 5.34 (m, 1H, 1-H), 5.89 (m, 1H, 12-H), 7.34–7.40 (m, 2H, H_{arom}), 7.41 (m, 1H, H_{arom}), 7.45 (m, 1H, H_{arom}), 7.54 (m, 1H, H_{arom}), 7.56–7.60 (m, 2H, H_{arom}), 7.59 (m, 1H, H_{arom}), 7.66 (m, 1H, H_{arom}), 7.70 (d, 1H, *J* = 8.2 Hz, H_{arom}), 7.77 (d, 1H, *J* = 8.7 Hz, H_{arom}), 7.81 (d, 1H, *J* = 8.2 Hz, H_{arom}), 7.86 (dd, 1H, *J* = 1.7, 8.7 Hz, H_{arom}), 7.91–7.98 (m, 3H, H_{arom}), 8.01 (dd, 1H, *J* = 1.7, 8.6 Hz, H_{arom}), 8.39 (br. s, 1H, H_{arom}), 8.58 (br. s, 1H, H_{arom}); ^{13}C NMR ($CDCl_3$): δ = 23.8 (4-CH_3), 26.7 (9-CH_3), 30.3 (C-10), 37.2 (C-13), 47.3 (C-9), 66.9 (C-11), 69.0 (C-12), 80.7 (C-1), 89.5 (C-4), 103.2 (C-7), 124.9, 125.0, 126.1, 126.5, 126.8, 127.2, 127.8, 128.0, 128.4, 128.4, 128.6, 128.8, 129.0, 129.4, 129.5, 129.5, 130.6, 131.5, 131.8, 132.4, 132.5, 135.7, 136.0, 136.4, 164.7, 165.1 (C_{arom}), 165.4 (C-6), 178.5 (C-3), 196.0 (C-8); MS (CI, CH_5^+): *m*/*z* (%) = 685 (100) [M + H^+]; HRMS (EI): *m*/*z* calcd for $C_{41}H_{32}O_8S$–C_6H_5S: 575.1735; Found: 575.1706.

Oxidative elimination of the phenylsulfanyl group—general procedure

A 0.1 M solution of 3-chloroperoxybenzoic acid in CH_2Cl_2 (2.50 mL, 0.25 mmol) was added dropwise to a solution of the respective sulfide (0.25 mmol) in CH_2Cl_2 (15 mL) at −20 °C. After stirring for 1–4 h at −20 °C (until TLC control indicates complete conversion) the reaction mixture was poured into saturated aqueous sodium hydrogencarbonate (25 mL), and the resultant layers were separated. The aqueous layer was extracted with Et_2O or CH_2Cl_2 (5 × 50 mL). The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure. A suspension of the resulting crude product and barium carbonate (245 mg, 1.25 mmol) in benzene (50 mL) was refluxed for 30 min. The suspension was filtered, and the filtrate was evaporated under reduced pressure. The crude product was purified by flash chromatography.

(1*RS*,9*RS*,11*RS*,12*RS*)-11-(*tert*-Butyldimethylsilanyloxy)-12-hydroxy-9-methyl-4-methylene-2,5-dioxatricyclo[7.3.1.0^{3,7}]tridec-3(7)-ene-6,8-dione (25). Prepared according to general procedure from **21** (24.5 mg, 0.05 mmol). Purified by flash chromatography (*n*-hexane–EtOAc, 70:30) to give **25** (11 mg, 57.7%) as colorless crystals; mp 155–158 °C.

IR (KBr) 3487, 2928, 2856, 1794, 1673, 1586 cm^{-1}; MS (CI,CH_5^+): *m*/*z* (%) = 381 (71) [M + H^+], 257 (100); ^1H NMR ($CDCl_3$): δ = 0.03 (s, 3H, CH_3Si), 0.04 (s, 3H, CH_3Si), 0.86 [s, 9H, $(CH_3)_3C$], 1.25 [s, 3H, CH_3-(C-9)], 1.75 (dd, *J* = 13.0/12.2 Hz, 1H, 10-H_{ax}), 1.93 (dd, *J* = 13.0/4.5 Hz, 1H, 10-H_{eq}), 2.17 (dd, *J* = 16.7/4.5 Hz, 1H, 13-H), 2.25 (d, *J* = 16.7 Hz, 1H, 13-H), 2.65 (s_{br}, 1H, OH), 3.64 (ddd, *J* = 12.2/4.5/3.1 Hz, 11-H), 4.01 (m, 1H, 12-H), 5.12 (m, 1H, 1-H), 5.30 [d, *J* = 2.8 Hz, 1H, (C-4)=CH_2], 5.38 [d, *J* = 2.8 Hz, 1H, (C-4)=CH_2]. Anal. calcd for: $C_{19}H_{28}O_6Si$ (380.52): C, 59.87; H, 7.42; Found: C, 60.14; H, 7.41.

(1*RS*,9*RS*,11*SR*,12*SR*)-11,12-Dihydroxy-9-methyl-4-methylene-2,5-dioxatricyclo-[7.3.1.0^{3,7}tridec-3(7)-ene-6,8-dione (5). A 0.1 M HF solution in acetonitrile (660 μL, 0.066 mmol) was added to a solution of **25** (8.4 mg, 0.022 mmol) in acetonitrile (1 mL) at 0 °C. After stirring at 0 °C for 21 h, a colorless precipitate has been formed, which was filtered off and was washed with pentane to give **5** (5.9 mg, 100%) as colorless crystals; mp > 370 °C.

IR (KBr) 3460, 2924, 1786, 1588 cm^{-1}; MS (CI, CH_5^+): *m*/*z* (%) = 267 (100) [M + H^+]; ^1H NMR (DMSO-d_6): δ = 1.07 [s, 3H, CH_3-(C-9)], 1.58 (dd, *J* = 12.4/3.7 Hz, 1H, 10-H_{eq}), 1.72 (t, *J* = 12.4 Hz, 1H, 10-H_{ax}), 1.99 (dd,

J = 16.4/4.5 Hz, 1H, 13-H), 2.31 (d, J = 16.4 Hz, 1H, 13-H), 3.39 (d, J = 12.4 Hz, 1H, 11-H), 3.89 (m, 1H, 12-H), 4.80 (d, J = 5.6 Hz, 1H, OH), 4.98 (m, 1H, 1-H), 5.39 [d, J = 2.8 Hz, 1H, (C-4)=CH_2], 5.41 [d, J = 2.8 Hz, 1H, (C-4)=CH_2], 5.43 (d, J = 4.5 Hz, 1H, OH); ^{13}C NMR (DMSO-d_6): δ = 26.3 [CH_3-(C-9)], 28.4 (C-13), 39.2 (C-10), 47.7 (C-9), 64.7 (C-11), 72.3 (C-12), 84.2 (C-1), 96.7 [(C-4)=CH_2], 101.5 (C-7), 147.6 (C-4), 163.4 (C-6), 165.6 (C-3), 198.3 (C-8); HRMS: m/z calcd for $C_{13}H_{14}O_6$ (M^+): 266.0790; Found: 266.0762.

2-Naphthalene-2-carboxylic acid (1*RS*,9*RS*,11*SR*,12*SR*) -12-hydroxy-9-methyl-4-methylene-6,8-dioxo-2,5-dioxatricyclo-[7.3.1.0^{3,7}tridec-3(7)-en-11-yl ester (27). Prepared according to general procedure from **22** (43.2 mg, 0.0814 mmol). Purified by flash chromatography (CH_2Cl_2–EtOAc, 95:5) to give **27** (9.5 mg, 27.8%) as colorless crystals; mp 226 °C.

IR (KBr) 3474, 3132, 3061, 2924, 1789, 1721, 1589 cm^{-1}; MS (CI, CH_5^+): m/z (%) = 421 (5) [M + H$^+$], 251 (100); ^{1}H NMR (CD_2Cl_2): δ = 1.30 (s, 3H, CH_3), 2.15 (d, 1H, J = 15.1 Hz, 10-H), 2.17 (d, 1H, J = 15.1 Hz, 10-H), 2.31 (dd, 1H, J = 3.9, 16.7 Hz, H-13), 3.35 (d, 1H, J = 16.7 Hz, 13-H), 2.51 (d, 1H, J = 3.0 Hz, OH), 4.50 (q, 1H, J = 3.0 Hz, 12-H), 5.07 (m, 1H, 11-H), 5.17 (m, 1H, 1-H), 5.31 (d, 1H, J = 2.8 Hz, CH_2), 5.42 (d, 1H, J = 2.8 Hz, CH_2), 7.57 (ddd, 1H, J = 1.3, 7.5, 8.2 Hz, H_{arom}), 7.62 (ddd, 1H, J = 1.3, 7.5, 8.2 Hz, H_{arom}), 7.90 (d, 2H, J = 8.7 Hz, H_{arom}), 7.97 (d, 1H, J = 8.2 Hz, H_{arom}), 7.99 (dd, 1H, J = 1.7, 8.7 Hz, H_{arom}), 8.57 (br. s, 1H, H_{arom}); ^{13}C NMR (CD_2Cl_2): δ = 26.7 (CH_3), 29.7 (C-13), 36.1 (C-10), 48.2 (C-9), 69.1 (C-12), 69.9 (C-11), 83.2 (C-1), 97.2 (CH_2), 102.7 (C-7), 125.0, 126.8, 127.0, 128.4, 128.7, 129.3, 131.2, 132.4, 135.8 (C_{arom}), 148.5 (C-4), 165.6 [CO_2(C-11)], 166.0 (C-3), 197.6 (C-8); HRMS (DEI+): m/z calcd for $C_{24}H_{20}O_7$: 420.1209; Found: 420.1208.

2-Naphthalene-2-carboxylic acid (1*RS*,9*RS*,11*SR*,12*SR*) -11-hydroxy-9-methyl-4-methylene-6,8-dioxo-2,5-dioxatricyclo-[7.3.1.0^{3,7}tridec-3(7)-en-12-yl ester (28). Prepared according to general procedure from **23** (12.0 mg, 0.023 mmol). Purified by flash chromatography (CH_2Cl_2–EtOAc, 80:20) to give **28** (3.8 mg, 40.0%) as colorless crystals; mp > 260 °C (decomp.).

IR (KBr) 3428, 3055, 2924, 2853, 1787, 1719, 1669, 1591 cm^{-1}; MS (CI, CH_5^+): m/z (%) = 421 (100) [M + H$^+$]; ^{1}H NMR ($CDCl_3$): δ = 1.38 (s, 3H, CH_3), 1.97 (t, 1H, J = 12.0 Hz, 10-H), 2.25 (dd, 1H, J = 4.7, 16.7 Hz, 13-H), 2.30 (br. d, 1H, J = 12.0 Hz, 10-H); 2.45 (br. d, 1H, J = 16.7 Hz, 13-H), 3.99 (br. d, 1H, J = 9.5 Hz, 11-H), 5.29 (m, 1H, 1-H), 5.34 (d, 1H, J = 2.9 Hz, C=CH_2), 5.44 (d, 1H, J = 2.9 Hz, C=CH_2), 5.66 (m, 1H, 12-H), 7.61 (d, 1H, J = 8.1 Hz, H_{arom}), 7.64 (dd, 1H, J = 1.4, 8.0 Hz, H_{arom}), 7.90–7.95 (m, 2H, H_{arom}), 8.00 (d, 1H, J = 8.1 Hz, H_{arom}), 8.03 (dd, 1H, J = 1.7, 8.6 Hz, H_{arom}), 8.60 (s, 1H, H_{arom}); ^{13}C NMR (100 MHz; $CDCl_3$): δ = 26.9 (CH_3), 30.6 (C-13), 40.7 (C-10), 47.9 (C-9), 65.2 (C-11), 71.8 (C-12), 80.6 (C-1), 97.6 (CH_2), 102.5 (C-7), 124.9, 125.0, 127.1, 127.9, 128.7, 128.7, 129.0, 129.4, 129.5, 131.7 (C_{arom}), 147.8 (C-4), 165.2 (C-6), 165.9 [CO_2(C-12)], 174.0 (C-3), 197.1 (C-8); HRMS (EI) m/z calcd for $C_{24}H_{20}O_7$: 420.1209; Found: 420.1161.

2-Naphthalene-2-carboxylic acid (1*RS*,9*RS*,11*SR*,12*SR*) -12-(naphthalene-2-carbonyloxy)-9-methyl-4-methylene-6,8-dioxo-2,5-dioxatricyclo-[7.3.1.0^{3,7}tridec-3(7)-en-11-yl ester (29). Prepared according to general procedure from **23** (40.6 mg, 0.059 mmol). Purified by flash chromatography (n-hexane–CH_2Cl_2–EtOAc, 50:40:10) to give **29** (6.4 mg, 18.8%) as colorless crystals; mp 255–260 °C (decomp.).

IR (KBr) 3132, 3055, 2924, 2847, 1786, 1725, 1669, 1583 cm^{-1}; MS (CI, CH_5^+): m/z (%) = 575 (34) [M + H$^+$], 235 (100); ^{1}H NMR: ($CDCl_3$): δ = 1.47 (s, 3H, CH_3), 2.31 (t, 1H, J = 12.8 Hz, 10-H), 2.41 (dd, 1H, J = 4.6, 16.7 Hz, 13-H), 2.47 (dd, 1H, J = 4.4, 12.8 Hz, 10-H), 2.58 (d, 1H, J = 16.7 Hz, 13-H), 5.34 (m, 1H, 11-H), 5.36 (m, 1H, 1-H), 5.40 (d, 1H, J = 3.0 Hz, C=CH_2), 5.50 (d, 1H, J = 3.0 Hz, C≒CH_2), 6.00 (m, 1H, 12-H), 7.43 (t, 1H, J = 7.5 Hz, H_{arom}), 7.54 (t, 1H, J = 7.5 Hz, H_{arom}), 7.61 (t, 1H, J = 7.5 Hz, H_{arom}), 7.64–7.69 (m, 2H, H_{arom}), 7.76 (d, 1H, J = 8.8 Hz, H_{arom}), 7.80 (d, 1H, J = 8.3 Hz, H_{arom}), 7.87 (dd, 1H, J = 1.7, 8.7 Hz, H_{arom}), 7.94 (d, 1H, J = 8.3 Hz, H_{arom}), 7.96 (d, 1H, J = 8.8 Hz, H_{arom}), 7.98 (d, 1H, J = 8.3 Hz, H_{arom}), 8.05 (dd, 1H, J = 1.7, 8.7 Hz, H_{arom}), 8.38 (s, 1H, H_{arom}), 8.62 (s, 1H, H_{arom}); ^{13}C NMR ($CDCl_3$): δ = 27.1 (CH_3), 30.7 (C-13), 37.4 (C-10), 47.8 (C-9), 66.9 (C-11), 69.2 (C-12), 80.4 (C-1), 97.9 (C=CH_2), 102.6 (C-7), 125.2, 125.3, 126.4, 126.7, 127.0, 127.4, 128.0, 128.2, 128.6, 128.8, 129.0, 129.3, 129.6, 129.8, 131.7, 132.0, 132.6, 132.8, 135.9, 136.2 (C_{arom}), 147.7 (C-4), 165.2 (C-6), 165.2, 165.3 [CO_2(C-11), CO_2(C-12)], 196.9 (C-8). HRMS (EI): m/z calcd for $C_{35}H_{26}O_8$: 574.1637; Found: 574.1628.

(1*RS*,9*RS*,11*RS*,12*RS*) - 11,12 - Dihydroxy - 9 - methyl - 4-methylene - 2,5 - dioxatricyclo - [7.3.1.0^{3,7}tridec - 3(7) - ene-6,8-dione (26). Prepared according to general procedure from **20** (6.4 mg, 0.017 mmol). Purified by flash chromatography (CH_2Cl_2–EtOAc—acetone–i-PrOH, 45:40:10:5) to give **26** (1.0 mg, 22.2%) as colorless crystals; mp > 280 °C (decomp.).

IR (KBr) 3441, 2924, 1778, 1648, 1594 cm^{-1}; MS (CI, CH_5^+): m/z (%) = 267 (62) [M + H$^+$], 242 (100); ^{1}H NMR (acetone-d_6): δ = 1.11 (s, 3H, CH_3), 1.74 (d, 1H, J = 14.2 Hz, CH_2), 2.13 (dd, 1H, J = 4.0, 16.6 Hz, CH_2), 2.31 (dt, 1H, J = 3.5, 14.2 Hz, CH_2), 2.68 (dt, 1H, J = 3.0, 16.2 Hz, CH_2), 3.84 (m, 1H, 12-H), 3.99 (m, 1H, 11-H), 4.08 (d, 1H, J = 3.0 Hz, OH), 4.15 (d, 1H, J = 9.0 Hz, OH), 5.13 (d, 1H, J = 2.6 Hz, C=CH_2), 5.15 (m, 1H, 1-H), 5.23 (d, 1H, J = 2.6 Hz, C=CH_2); ^{13}C NMR (acetone-d_6): δ = 27.1 (CH_3), 34.2 (C-13), 44.0 (C-10), 45.4 (C-9), 68.8 (C-12), 72.0 (C-11), 83.2 (C-1), 94.4 (=CH_2), 101.1 (C-7), 150.1 (C-4), 161.4 (C-6), 166.4 (C-3), 199.0 (C-8); HRMS (EI): m/z calcd for $C_{13}H_{14}O_6$: 266.0790; Found 266.0825.

(1*RS*,9*RS*)-9-Methyl-4-methylene-2,5-dioxatricyclo[7.3.1.O^{3,7}]trideca-3(7),11-diene-6,8-dione (6). Prepared according to general procedure from **18** (61.9 mg, 0.18 mmol).

Purified by flash chromatography (CH_2Cl_2–EtOAc, 97:3) to give **6** (21.4 mg, 50.9%) as colorless crystals; mp $>300\,^{\circ}C$ (decomp.).

IR (KBr) 3041, 2875, 1790, 1672, 1652, 1589 cm^{-1}; MS (CI; CH_5^+): $m/z = 233$ (100) $[M+H^+]$; ^1H NMR ($CDCl_3$): $\delta = 1.22$ (s, 3H, CH_3), 1.93 (dq, 1H, $J = 2,3$, 18.0 Hz, 10-H), 2.13 (dd, 1H, $J = 6.4$, 16.4 Hz, 13-H), 2.47 (d, 1H, $J = 16.4$ Hz, 13-H), 2.71 (dd, 1H, $J = 6.1$, 18.0 Hz, 10-H), 5.23 (d, 1H, $J = 2.6$ Hz, C=CH_2), 5.30 (d, 1H, $J = 2.6$ Hz, C=CH_2), 5.34 (m, 1H, 1-H), 5.75 (d_{br}, 1H, $J = 10.0$ Hz, 12-H), 6.18 (ddt, 1H, $J = 1.6$, 6.1, 10.0 Hz, 11-H); ^{13}C NMR ($CDCl_3$): $\delta = 25.9$ (CH_3), 34.4 (C-13), 37.4 (C-10), 44.8 (C-9), 77.0 (C-1), 96.2 (C=CH_2), 102.6 (C-7), 121.5 (C-12), 135.2 (C-11), 148.2 (C-4), 164.2 (C-3), 164.5 (C-6), 198.6 (C-8); HRMS (EI): m/z calcd for $C_{13}H_{12}O_4$: 232.0733; Found: 232.0736.

Antibacterial activities

The compounds were evaluated in vitro for their antimicrobial activities against Gram-positive [*S. aureus* ATCC 25923, *S. aureus* ATCC 29213 (MRSA) and *E. faecalis* ATCC 29212] bacteria at concentrations ranging from 0.5 to 128 μg/mL. Minimum inhibitory concentration (MIC) values were determined by a broth microdilution method carried out according to DIN 58940-8.[16] Each compound was dissolved in dimethyl sulfoxide to give a 2.56 mg/mL stock solution, which was serially diluted 2-fold with Mueller–Hinton broth. Overnight cultures of the test organisms were suspended in saline, and the cell density was adjusted to give an initial inoculum of 10^6 colony forming units per milliliter (CFU mL^{-1}) in Mueller–Hinton broth. To each spot of a 96-well micro-plates containing 100 μL of graded concentrations of each compound 100 μL of the bacterial suspension of (10^6 CFU mL^{-1}) was added to yield a final inoculum of 5×10^5 CFU mL^{-1}. After incubation at 37 °C for 18 h the MIC was defined as the minimum concentration of a test compound that resulted in no visible growth of bacteria, compared with the drug-free control. It should be noted that the concentration of dimethyl sulfoxide in the medium did not affect the growth of any of the microorganisms tested. The MIC of each compound was determined at least twice.

Cytotoxicity

The cytotoxicity of compounds **5**–**7** and **27** was determined using two cell lines: human acute myeolid leukemia HL-60 cells (DSMZ ACC 3) and human T cell leukemia Jurkat cells (DSMZ ACC 282). Impaired cell viability was measured using the MTT assay according to Mosmann, based on the ability of viable cells to reduce yellow MTT to blue formazan.[17] Solutions of the test compounds were freshly prepared from stock solutions in dimethyl sulfoxide, which were diluted to the required concentrations. Final DMSO concentrations were 1% (v/v). The test was carried out in 96-well plates with an inoculum of 9×10^5 cells mL^{-1}. Total assay volume was 101 μL. The cells were exposed to the test compounds at 37 °C for 24 h. Then they were incubated with methylthiazolyldiphenyl tetrazolium chloride (MTT, 0.5 mg/mL, 37 °C, 2 h) and subsequently solubilized in DMSO (200 μL) for at least 2 h in the dark. The extend of reduction of MTT was quantified by absorbance measurement ($A^{550\,nm}$, SLt Spectra, SLT Labinstruments, Crailsheim, Germany). For determination of the IC_{50} values, the optical density was plotted against the log concentration. At least four different concentrations have been tested. Every test was performed at least in triplicates, and all experiments have been repeated at least twice. Positive control measurements were performed with actinomycin.

Acknowledgements

We are greatly indebted to Prof. Dr. Klaus Th. Wanner for his generous support. We would also like to thank Prof. Dr. F. Bracher for his great support in performing the MTT assays and T. Claußen for technical assistance in performing the in vitro antibacterial assays.

References and Notes

1. Johnson, A. P.; Woodford, N. *J. Antimicrob. Chemother.* **2002**, *50*, 621 and references cited therein.
2. Vincent, J.-L. *Intensive Care Med.* **2000**, *26*, 83.
3. (a) Isolation and biological evaluation: Tsuchida, T.; Iinuma, H.; Nishida, C.; Kinoshita, N.; Sawa, T.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1995**, *48*, 1104. (b) Structure determination: Tsuchida, T.; Iinuma, H.; Sawa, R.; Takahashi, Y.; Nakamura, H.; Nakamura, K. T.; Sawa, T.; Naganawa, H.; Takeuchi, T. *J. Antibiot.* **1995**, *48*, 1110.
4. Tsuchida, T.; Iinuma, H.; Nakamura, K. T.; Nakamura, H.; Sawa, T.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1995**, *48*, 1330.
5. A part of this work has been communicated in preliminary form: Paintner, F. F.; Allmendinger, L.; Bauschke, G.; Polborn, K. *Synlett* **2002**, 1308.
6. Paintner, F. F.; Allmendinger, L.; Bauschke, G. *Synthesis* **2001**, 2113.
7. Paintner, F. F.; Bauschke, G.; Kestel, M. *Tetrahedron Lett.* **2000**, *41*, 9977.
8. Stork, G.; Logusch, E. W. *Tetrahedron Lett.* **1979**, 3361.
9. For a mechanistic discussion of this key ring closure step, see: ref 5.
10. Shing, T. K. M.; Tam, E. K. W.; Tai, V. W.-F.; Chung, I. H. F.; Jiang, Q. *Chem. Eur. J.* **1996**, *2*, 50.
11. VanRheenen, V.; Kelly, R. C.; Cha, D. Y. *Tetrahedron Lett.* **1976**, 1973.
12. Mukaiyama, T.; Tabusa, F.; Suzuki, K. *Chem. Lett.* **1983**, 173.
13. (a) The facile oxidation of sulfides to sulfones in the presence of C–C double bonds using catalytic osmium tetroxide and *N*-methylmorpholine-*N*-oxide or trimethylamine-*N*-oxide as co-oxidant has been reported: Kaldor, S. W.; Hammond, M. *Tetrahedron Lett.* **1991**, *32*, 5043. (b) Priebe, W.; Grynkiewicz, G. *Tetrahedron Lett.* **1991**, *32*, 7353.
14. (a) On the other hand, sulfides are known to be essentially inert to oxidation by osmium tetroxide under stoichiometric conditions: Stork, G.; van Tamelen, E. E.; Friedman, L. J.; Burgstahler, A. W. *J. Am. Chem. Soc.* **1953**, *75*, 384. (b) Djerassi, C.; Engle, R. R. *J. Am. Chem. Soc.* **1953**, *75*, 3838. (c)

Henbest, H. B.; Khan, S. A. *J. Chem. Soc., Chem. Commun.* **1968**, 1036. (d) For chemoselective oxidations of C–C double bonds in the presence of sulfides with catalytic OsO_4 and $K_3Fe(CN)_6$ as co-oxidant, see: Walsh, P. J.; Ho, P. T.; King, B.; Sharpless, K. B. *Tetrahedron Lett*. **1994**, *35*, 5129.

15. Pollet, P.; Gelin, S. *Tetrahedron* **1978**, *34*, 1453.
16. Methods for Susceptibility Testing of Bacterial Pathogens (except mycobacteria) against Chemotherapeutic Agents (DIN paperback); Beuth: Berlin, Vienna, Zurich, 2000; Vol. 222.
17. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.