Natural Products

International Edition: DOI: 10.1002/anie.201501526
German Edition: DOI: 10.1002/ange.201501526

The Synthesis and Biological Evaluation of Desepoxyisotedanolide and a Comparison with Desepoxytedanolide**

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Abstract: *The tedanolides are biologically active polyketides that exhibit a macrolactone constructed from a primary alcohol. Since polyketidal transformations only generate secondary alcohols, it has been hypothesized by Taylor that this unique lactone could arise from a postketidal transesterification. In order to probe this hypothesis and to investigate the biological profile of the putative precursor of all members of the tedanolide family, we embarked on the synthesis of desepoxyisotedanolide and its biological evaluation in comparison to desepoxytedanolide. The biological experiments unraveled a second target for desepoxytedanolide and provided evidence that the proposed transesterification indeed provides a survival advantage for the producing microorganism.*

Tedanolides are biologically active polyketides that have been isolated from various marine sponges at different locations. The isolation of these toxic metabolites first came to light in 1984 with the isolation of tedanolide from the marine sponge *Tedania ignis* by Schmitz et al.[1]. To date, several other molecules (13-deoxytedanolide (**2**),[2] tedanolide C (**3**),[3] candidaspongiolides (**4**, **5**)[4]) belonging to this family have been isolated (Figure 1). Based on studies performed with radioactively labeled derivatives of tedanolide, Fusetani confirmed that tedanolide (**1**) binds to the 60S ribosomal subunit.[2, 5] The structural complexity of tedanolides has attracted the attention of several research groups,[6] with total syntheses put forward by the groups of Smith,[7] Roush[8] and Kalesse.[9]

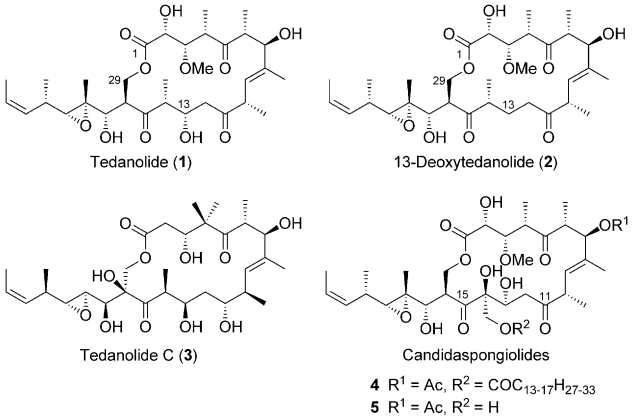

Figure 1. Structures of tedanolide (**1**) and related macrolides.

The significant structural characteristics of the tedanolides are an 18-membered macrolactone and an epoxide on the side chain. A more subtle but important structural feature is the fact that the tedanolides form the lactone bond with a primary hydroxy group. This unusual motif raises questions about the biosynthetic origin of tedanolide. In 2008, Taylor hypothesized that this unique lactone could arise from a postketidal transesterification (Figure 2).[10]

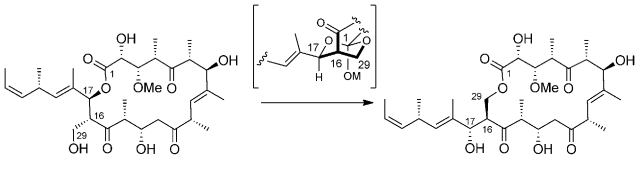

Figure 2. Putative biosynthetic precursors of tedanolide and the hypothetical transesterification.

In order to substantiate this hypothesis and to answer the question about the biological profile of the putative biosynthetic precursor, the synthesis of the 18-membered lactone desepoxyisotedanolide (**6**) was initiated. Our retrosynthetic analysis dissects desepoxyisotedanolide at the ester linkage

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[**] We thank Prof. Daniel Romo, Department of Chemistry, Texas A&M University for a generous gift of DMDA-pateamine A, Bettina Hinkelmann, HZI, for skilful technical assistance and gratefully acknowledge funding by the DFG (KA 913/17-1, SA 356/7-1). RET acknowledges funding support from the National Science Foundation (CHE-0924351).

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201501526.

and between C12 and C13. The resulting methyl ketone is the ketone Roush et al. used in their total synthesis and was also employed in our refined synthesis of tedanolide. The protecting-group pattern of the aldehyde fragment had to be altered in comparison to tedanolide synthesis, which proved to be nontrivial (Figure 3).

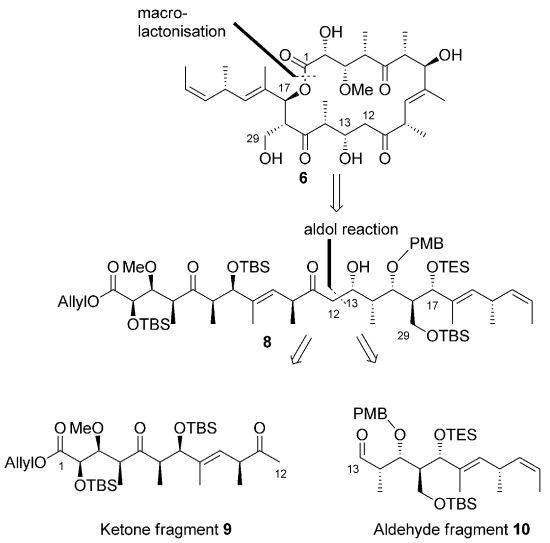
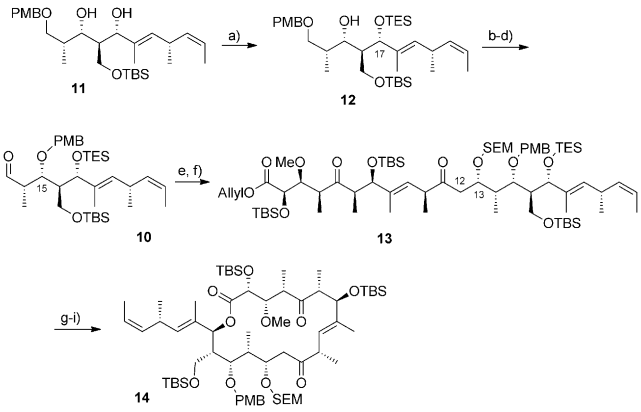


Figure 3. Retrosynthetic analysis for desepoxyisotedanolide (**6**).



Scheme 1. Synthesis of fragment **10**, aldol coupling, and macrocyclization. a) TESCl, 2,6-lutidine, DMAP, CH_2Cl_2, −78 °C, 3 h, 80%; b) DDQ, 4 Å MS, CH_2Cl_2, −10 °C, 84%; c) DIBAL-H, CH_2Cl_2, −78 °C; d) DMP, $NaHCO_3$, CH_2Cl_2, RT, 80% over 2 steps; e) **9**, LiHMDS, THF, −78 °C, 30 min, 50%, d.r. 2.5:1; f) SEMCl, DIPEA, TBAI, THF, 50 °C, 72 h, 0 to 50 °C, 90%; g) PPTS, MeOH, THF, RT, 60%, h) $Pd(PPh_3)_4$, nBu_3SnH, CH_2Cl_2; i) 2,6-Cl-C_6H_2COCl, Et_3N, DMAP, C_6H_6, RT, 24 h, 45% over 2 steps. TES = triethylsilyl, DMAP = 4-dimethylaminopyridine, DIBAL-H = diisobutylaluminum hydride, DMP = Dess-Martin periodinane, LiHMDS = lithium hexamethyldisilazanide, THF = tetrahydrofuran, SEM = trimethylsilylethoxymethyl, DIPEA = *N*,*N*-diisopropylethylamine, TBAI = tetra-*n*-butylammonium iodide, PPTS = pyridinium 4-toluenesulfonate.

For the synthesis of **6**, choosing the appropriate protecting group at C17 proved pivotal for the successful synthesis. After unsuccessful experimentation with various protecting groups (e.g., alloc, MMTr) our attention was caught by a method published by the Roush group, which reported the selective protection of the C17 hydroxy group as the TES ether. By following their protocol, selective protection at the C17 hydroxy group of **11**[8a,b] was accomplished through treatment with triethylsilyl chloride. Subsequent treatment with DDQ under anhydrous conditions generated the corresponding PMP acetal, followed by regioselective reductive acetal opening with DIBAL-H (Scheme 1). Dess–Martin oxidation then provided aldehyde **10**.

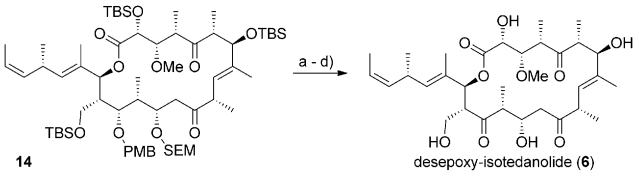
The aldol reaction between fragments **9** and **10** was achieved by using the conditions provided by Roush.[8] Ketone **9** was treated with LiHMDS at −78 °C and aldehyde **10** was added dropwise to the reaction mixture, which was quenched after 15 min with brine (Scheme 1). First, the aldol products **13** (d.r. 2.5:1), unreacted ketone **9**, and the aldehyde **10** were separated by column chromatography. A second separation by normal-phase HPLC with EtOAc and heptane as eluent was required to separate the diastereomeric aldol products. The observed selectivity of 2.5:1 can be rationalized by the competing effects of the Felkin-directing centre in the α position and the Felkin-opposing configuration at C15.

Initially, we tried to protect the newly formed hydroxy group at the C13 stereocenter as a TBS ether so that all the TBS groups could be removed in a single step at a later stage. However, we were not able to achieve this particular protection strategy. After looking at different protecting groups that would be resistant to the TES removal conditions, we came to the conclusion that the SEM group would fulfill all the requirements necessary for successful completion of the synthesis. Consequently, the C13 hydroxyl group was protected as an SEM ether.

The stage was then set for the macrolactonisation. The C17 TES group was removed selectively by using PPTS in methanol/THF. Since extended reaction times lead to deprotection of the primary TBS group, the reaction was quenched prior to completion and the starting material was recovered. The carboxylic acid was liberated by using $Pd(PPh_3)_4$ and tributyltinhydride (Scheme 1).[9a,b] The crude material was passed through a plug of silica gel and eluted with hexane to remove tin residues. Elution with ethyl acetate gave the acid, which was used immediately for macrolactonisation. After screening several conditions (Shiina, Evans–Mukaiyama, and Yamaguchi–Yonemitsu)[11] the highest yields were obtained by using a modified version of the Yamaguchi protocol in dry benzene.

The next steps towards completion of the synthesis of desepoxyisotedanolide (**6**) began with removal of the PMB group in compound **14** (Scheme 2). The reaction proceeded smoothly to completion and the resulting alcohol was oxidized to the corresponding ketone. Cleavage of the SEM ether at the C13 hydroxy group was performed with $MgBr_2$ and an excess of ethane thiol. Global deprotection of the TBS groups was achieved by using $HF{\cdot}Et_3N$ in CH_3CN. The reaction proceeded to completion over a period of 3 days to provide desepoxyisotedanolide (**6**) in good yields.

The pivotal question to be answered was whether the biological profile had changed and whether the target would remain the same. The antiproliferative activity of desepoxyisotedanolide (**6**) was tested with different mammalian cell



Scheme 2. Endgame synthesis of **6**. a) DDQ, CH_2Cl_2, pH7 buffer, RT; b) DMP, $NaHCO_3$, CH_2Cl_2, 80% (over 2 steps); c) $MgBr_2$, EtSH, Et_2O, RT, 80%; d) HF·Et_3N, Et_3N, CH_3CN, RT, 70%. DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.

lines in comparison with desepoxytedanolide (**7**) and tedanolide (**1**). As it can be seen from Table 1, these compounds showed different effectivities in inhibiting the proliferation of the cells. The most active one was tedanolide (**1**), which showed half maximal inhibitory concentration (IC_{50}) values in

Table 1: Antiproliferative activity [IC_{50} (nmol L^{-1})] of tedanolides **1**, **6**, **7** agains mammalian cells.

Cell line[a]	6	7	1
KB-3-1	42	0.6	0.33
L-929	109	1.6	0.35
PtK2	1008	1.8	–

[a] KB-3-1, human cervix carcinoma; L-929, mouse fibroblasts; PtK2, potoroo kidney.

the picomolar range, followed by **7**. The antiproliferative activity of desepoxyisotedanolide (**6**) was clearly reduced when compared to desepoxytedanolide (**7**).

The tedanolides have been shown to inhibit eukaryotic translation. We therefore measured the inhibitory effect of desepoxyisotedanolide (**6**) in in vitro translation assays in comparison with **7** (Figure 4). A rabbit reticulocyte lysate was

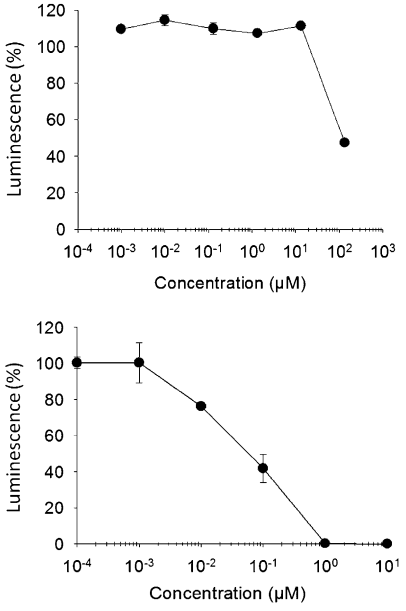


Figure 4. Translation inhibition by desepoxyisotedanolide (**6**; top) and desepoxytedanolide (**7**; bottom) in rabbit reticulocyte lysates.

supplemented with firefly luciferase mRNA and amino acids and incubated with different concentrations of the test compounds.

The resulting luminescence, which is directly proportional to the translation of the luciferase mRNA, was measured after 90 min of incubation. The IC_{50} values obtained were 0.01 μM for desepoxytedanolide (**7**) and 120 μM for desepoxyisotedanolide (**6**). The data reveals the different potential for translation inhibition of these compounds, which should be consistent with the different antiproliferative activities. However, the differences between **6** and **7** are extremely high and go beyond the differences measured in cell cultures.

With **7**, we have already shown that the mode of action resembles that of the candidaspongiolides rather than 13-deoxytedanolide.[12] As seen with candidaspongiolide,[4a] desepoxytedanolide (**7**) induces the phosphorylation of the eukaryotic initiation factor eIF2α and as a consequence the cap-dependent initiation of translation should be inhibited. To confirm this hypothesis, a bicistronic system, which consisted of firefly luciferase translated by a cap-dependant system and Renilla luciferase translated by a cap-independent cricket paralysis virus (CrPV) Internal Ribosome Entry Site (IRES) system, was used.

CrPV IRES does not require initiation factors for the formation of the 80S initiation complex. It was therefore hypothesised that the CrPV IRES translation would not be inhibited by desepoxytedanolide (**7**). KB 3–1 cells transfected with the CrPV bicistronic vector were treated with desepoxytedanolide, cycloheximide, a known inhibitor of elongation, and pateamine A, a known inhibitor of initiation. The luminescence generated from the two luciferases was measured. Unexpectedly, desepoxytedanolide (**7**) inhibited both of the translation reporter systems, i.e., the expression of both Renilla and firefly luciferase, as did cycloheximide (Figure 5). Pateamine A however, inhibited only the translation of the firefly luciferase. Translation of the Renilla luciferase encoded by the CrPV IRES was not inhibited. Des-methyl-desamino pateamine A (DMDA-pateamine A) however, inhibited only the translation of the firefly luciferase. The translation of Renilla luciferase encoded by the CrPV IRES was not inhibited. The results suggest that desepoxytedanolide (**7**) has an additional target in the eukaryotic translation system apart from eIF2α.

While searching for additional targets of **7**, we stained incubated cells for phosphorylated eEF2 (eukaryotic elongation factor 2) and observed clearly increased fluorescence compared to the control. This means that desepoxytedanolide induces the phosphorylation of eEF2 (Figure 6), which leads to inactivation of this elongation factor.

The only kinase known to phosphorylate eEF2 is eEF2K. For eEF2K to be active, it has to be dephosphorylated at Ser366. To check whether desepoxytedanolide induces eEF2K dephosphorylation, PtK2 cells were treated with 50 nM of desepoxytedanolide (**7**) and stained for phospho-eEF2K. As can be seen from Figure 7, desepoxytedanolide indeed reduces the phosphorylation state of eEF2K, thereby making it active.

After we had observed this dual mode of action of **7**, in which both translation initiation and translation elongation

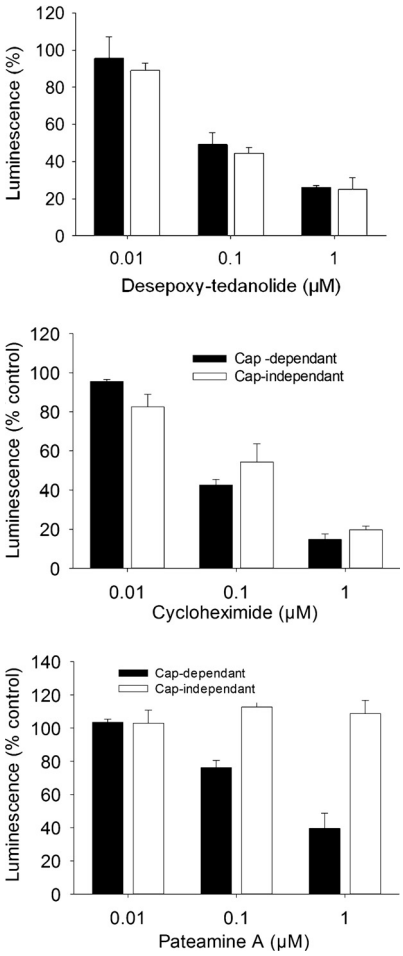


Figure 5. Desepoxytedanolide (**7**) does not only inhibit translation initiation. Desepoxytedanolide and cycloheximide inhibited both the cap-independent translation of Renilla luciferase mediated by the CrPV IRES (white bars) and the cap-dependent translation of firefly luciferase (black bars). DMDA-pateamine A, which targets eIF4A, inhibited the cap-dependant translation only.

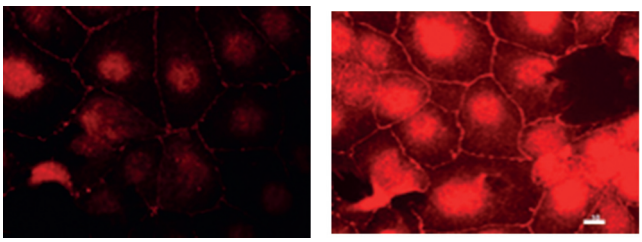


Figure 6. Desepoxytedanolide-induced phosphorylation of eEF2. PtK2 cells were treated either with methanol (left) or 50 nM of desepoxytedanolide (right) for four hours. The cells were then fixed and stained for phospho-eEF2. Scale bar: 10 μm.

were inhibited, we also tested whether desepoxyisotedanolide (**6**) acts the same way. Indeed, we also found induction of eEF2 phosphorylation and a slight reduction in the phosphorylation state of eEFK (Figure 8) but we did not observe enhanced phosphorylation of eIF2α (not shown). Induction of eEF2 phosphorylation has also been observed with myriaporone 3/4. Through drug affinity responsive target stability (DARTS) experiments, it could be shown that this was due to direct binding of myriaporone to eEF2 kinase.[13] With **7**, we did not observe any indication of direct binding.

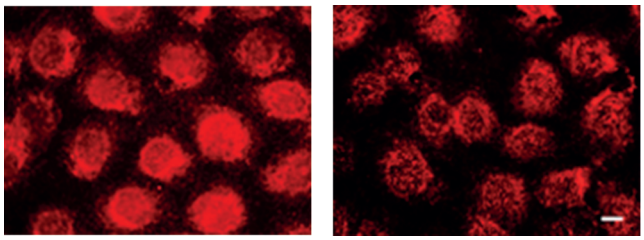


Figure 7. Desepoxy-tedanolide induced a dephosphorylation of the eEF2 kinase (eEF2K). PtK2 cells were treated with methanol (left) which served as control or desepoxy-tedanolide (right) for 4 h. The cells were fixed and stained for phospho-eEF2K (Ser 366). Scale bar: 10 μm.

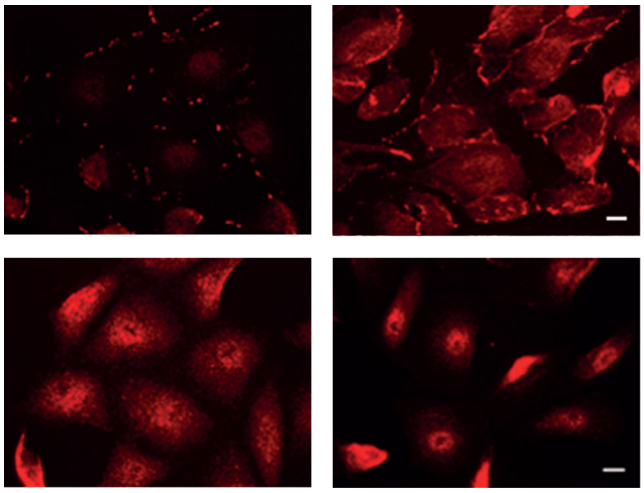


Figure 8. Desepoxyisotedanolide (**6**) induced phosphorylation of eEF2 (upper row) and reduced the phosphorylation state of eEF2 kinase (eEF2K; lower row). PtK2 cells were treated either with methanol (left) or 500 nM **6** (right) overnight and stained for phospho-eEF2 or phospho-eEF2K. Scale bar: 10 μm.

The fact that **7** inhibits translation at two separate sites of the translation machinery explain its high efficacy. Desepoxyisotedanolide (**6**), which was much less effective in blocking the translation process, obviously inhibits the elongation phase only, which makes it less efficacious than **7** (Figure 9). Our results are consistent with evolutionary optimization of the biological activity of the tedanolides. Even though we cannot exclude isomerization in vivo, our unsuccessful experiments to isomerize desepoxyisotedanolide under laboratory conditions indicate that this isomerization is not a spontaneous process. Through intramolecular transesterification from the putative precursor **6** to desepoxytedanolide (**7**), tedanolide can inhibit translation through two different mechanisms, which renders tedanolide significantly more potent than the hypothetical precursor. These biological data provide a rational for the existence of this very unusual polyketide family of macrolactones with primary alcohols as esters.

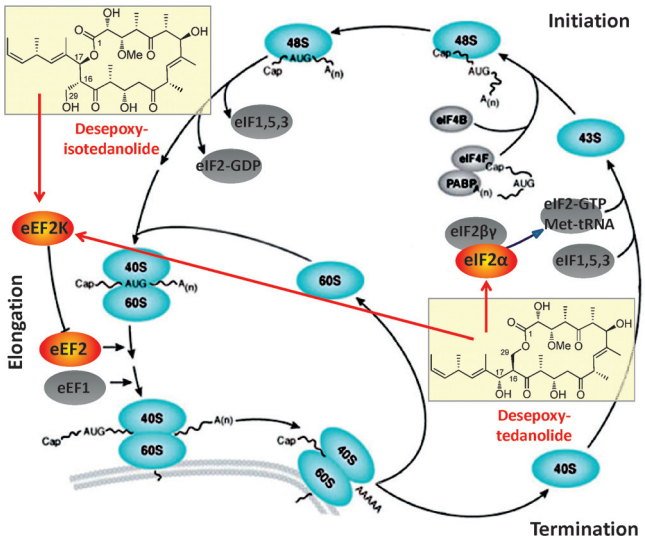


Figure 9. Schematic representation of the translation cycle and the molecular targets of desepoxytedanolide (**7**) and desepoxyisotedanolide (**6**).

Keywords: antitumor agents · macrolactones · natural products · polyketides · tedanolide

How to cite: *Angew. Chem. Int. Ed.* **2015**, *54*, 6935 – 6939
Angew. Chem. **2015**, *127*, 7039 – 7043

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Received: February 16, 2015
Published online: April 27, 2015

