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From the Total Synthesis of Semi–Viriditoxin, Semi–Viriditoxic Acid and Dimeric Naphthopyranones to their Biological Activities in Burkitt B Cell Lymphoma

Frederike Weber,[a] Anja Weber,[a] Laura Schmitt,[b] Ilka Lechtenberg,[b] Julian Greb,[a] Birgit Henßen,[a, c] Sebastian Wesselborg,[b] and Jörg Pietruszka*[a, c]

Dimeric naphthopyranones are known to be biologically active, however, for the corresponding monomeric naphthopyranones this information is still elusive. Here the first enantioselective total synthesis of semi-viriditoxic acid as well as the synthesis of semi–viriditoxin and derivatives is reported. The key intermediate in the synthesis of naphthopyranones is an α,β-unsaturated δ-lactone, which we synthesized in two different ways (*Ghosez*–cyclization and *Grubbs* ring–closing metathesis), while the domino–*Michael*–*Dieckmann* reaction of the α,β-unsaturated δ-lactone with an orsellinic acid derivative is the key reaction. A structure-activity relationship study was performed measuring the cytotoxicity in Burkitt B lymphoma cells (Ramos). The dimeric structure was found to be crucial for biological activity: Only the dimeric naphthopyranones showed cytotoxic and apoptotic activity, whereas the monomers did not display any activity at all.

Introduction

Naphthopyranones are aromatic tricycles containing a δ-lactone moiety. They represent a class of natural products produced by plants, fungi and lichens.[1] In nature, naphthopyranones are found as monomers and as C-6/C-6′ or C-8/C-8′ coupled biaryls. One example for a C-6/C-6′ coupled dimeric naphthopyranone is (–)-viriditoxin (**1**). Viriditoxin was first isolated in 1971 from *Aspergillus viridinutans* and assigned incorrectly as C-8/C-8′[2] and has later been revised to structure **1**.[3] Nowadays it is conveniently isolated from different fungal species (e.g., *Paecilomyces variotii*, *Cladosporium cladosporioides*, *Aspergillus fumigatus*)[4] and is also commercially available. Furthermore, viriditoxin (**1**) exhibits a broad–spectrum of antibiotic activities against Gram–positive pathogens, including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococci*, via inhibition of the bacterial cell division protein FtsZ,[2–3,5] as well as potent antitumor activities.[4a,6] In 1991, *Ayer et al.* isolated semi–viriditoxin (**2**) and semi–viriditoxic acid (**3**), the monomeric form of viriditoxin (**1**) and its corresponding acid, from the environmental mold *Paecilomyces variotii* (Figure 1).[7]

Concerning the monomeric natural products, no biological activities are known for the time being. *Shaw et al.* establish the total synthesis[8] for viriditoxin (**1**) and *Tan and Donner*[9] in 2009 for semi–viriditoxin (**2**), but no synthesis for semi–viriditoxic acid (**3**) was reported.[8–10] *Tan and Donner* synthesized (*S*)-semi–viriditoxin (**2**) in ten steps starting from (*S*)-aspartic acid with an overall yield of 7%.

In this article, we describe the first asymmetric total synthesis of semi–viriditoxic acid (**3**), as well as the enantioselective synthesis of substituted monomers and dimers of viriditoxin and semi–viriditoxin analogues, harboring simplified side chains and hydroxyl–group protection patterns. All monomers and dimers obtained were subjected to a study on the structure-activity relationships (see substitution pattern in Scheme 1) by comparing the cytotoxicity against Burkitt B lymphoma cells (Ramos) in a cell viability assay to assess their biological activities.

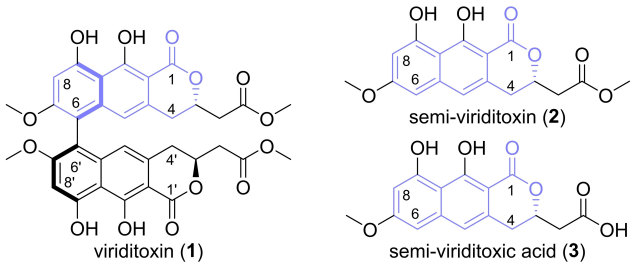

Figure 1. Structures of the natural products viriditoxin (**1**), semi–viriditoxin (**2**) and semi–viriditoxic acid (**3**), containing the characteristic naphthopyranone backbone (depicted in blue).

[a] *F. Weber, Dr. A. Weber, J. Greb, B. Henßen, Prof. Dr. J. Pietruszka*
Institute for Bioorganic Chemistry
Heinrich Heine University Düsseldorf in Forschungszentrum Jülich
52426 Jülich (Germany)
E-mail: j.pietruszka@fz-juelich.de

[b] *L. Schmitt, I. Lechtenberg, Prof. Dr. S. Wesselborg*
Institute for Molecular Medicine I
Heinrich Heine University Düsseldorf
40225 Düsseldorf (Germany)

[c] *B. Henßen, Prof. Dr. J. Pietruszka*
Institute of Bio- and Geosciences (IBG-1: Biotechnology)
Forschungszentrum Jülich
52426 Jülich (Germany)

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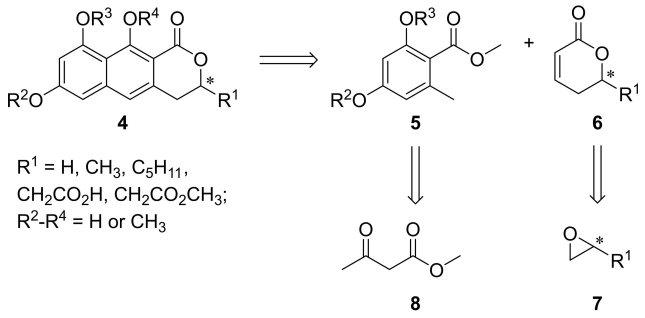


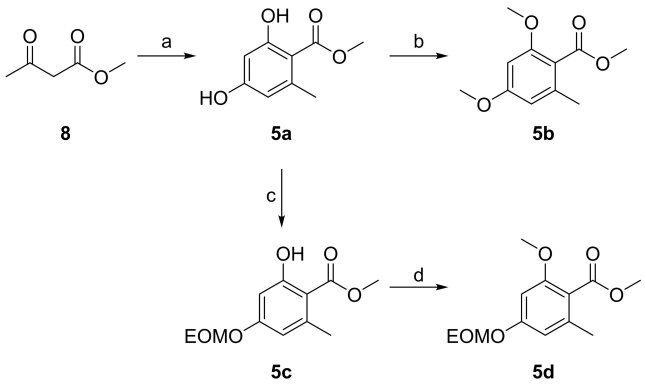
Scheme 1. Retrosynthetic analysis of naphthopyranones **4**.

Results and Discussion

The domino–*Michael–Dieckmann* reaction has been shown to be of great value to access the tricyclic carbon framework of naphthopyranones **4**.[8–11] Therefore, orsellinates **5** and lactones **6** can be recognized as key intermediates for the base–mediated annulation reaction that is followed by a consecutive oxidation reaction. While orsellinate **5** can be synthesized from commercially available methyl acetoacetate (**8**), chiral lactones are accessible from the corresponding enantiomerically pure oxiranes **7** (Scheme 1).[12]

Michael–Donor Synthesis

The orsellinic acid carbon framework **5a** was synthesized in 46% yield starting from methyl acetoacetate (**8**) following a procedure of *Barrett et al.* utilizing sodium hydride and *n*-butyl lithium (^{n}BuLi) (Scheme 2).[12] Prior to the application of the orsellinic acid derivatives in the domino–*Michael–Dieckmann* annulation reaction to build the naphthopyranone scaffold **4**, protection of the free hydroxyl groups is required. Depending on the desired natural product or derivative, different protection patterns and protecting groups were used. For the natural products, only the 2,4-bis-methoxy–protected orsellinic acid derivative **5b** was needed, whereas for the derivatives that have been used in the dimer formation only the ethoxymethyl (EOM) ether **5d** (protection in 4-position) was required. The protected orsellinic acid derivative **5b** was synthesized with 95% yield from unsubstituted orsellinic acid **5a**, according to a procedure of *Drochner et al.*[11f] The EOM ether **5c** was regioselectively formed in 4-position in 78% yield, following a procedure of *Park et al.*[8] The regioselective protection can be accounted to the reduced nucleophilicity of the phenolic 2-hydroxyl group due to coordination of the phenolic proton between the hydroxyl group and the carbonyl oxygen of the adjacent ester. In the third step, the EOM-protected orsellinic acid derivative **5c** was also converted into the methyl ether **5d** with 96% yield by protection in 6-position.



Scheme 2. Synthesis of methoxy- and EOM-protected orsellinic acid esters **5b** and **5d**. Reagents and conditions: (a) NaH, ^{n}BuLi, THF, 0 °C→ −78 °C→21 °C→70 °C→0 °C, 24 h, 46%; (b) $(CH_3O)_2SO_2$, K_2CO_3, TBAI, acetone, 70 °C, 95%; (c) EOMCl, K_2CO_3, acetone, 70 °C, 16 h, 78%; (d) $(CH_3O)_2SO_2$, K_2CO_3, TBAI, acetone, 70 °C, 96%.

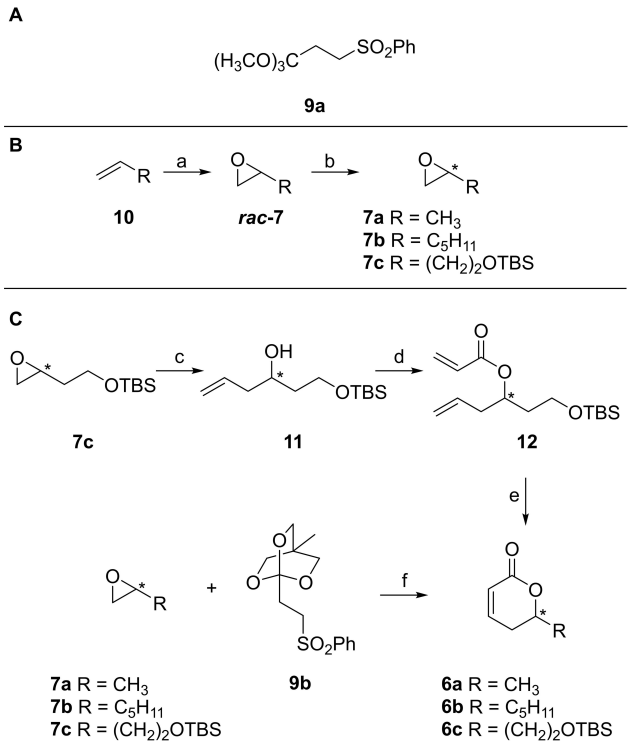
Lactone Synthesis

To synthesize α,β-unsaturated δ-lactones, a variety of synthetic routes are available;[13] uncommon but frequently used in natural product synthesis[13b,14] is the *Ghosez* cyclization.[13i,j] In this process, an oxirane **7** is reacted with the orthoester–carrying sulfone **9** to form the α,β-unsaturated δ-lactone **6**.[14b,15] In particular, the *Shaw* group established a practical alternative to the original *Ghosez* reagent **9a** and developed the 2,6,7-trioxabicyclo[2.2.2] octane (OBO) sulfonic ester **9b**, which carries a cyclic orthoester instead of a trimethyl orthoester (Scheme 3A).[14b,16]

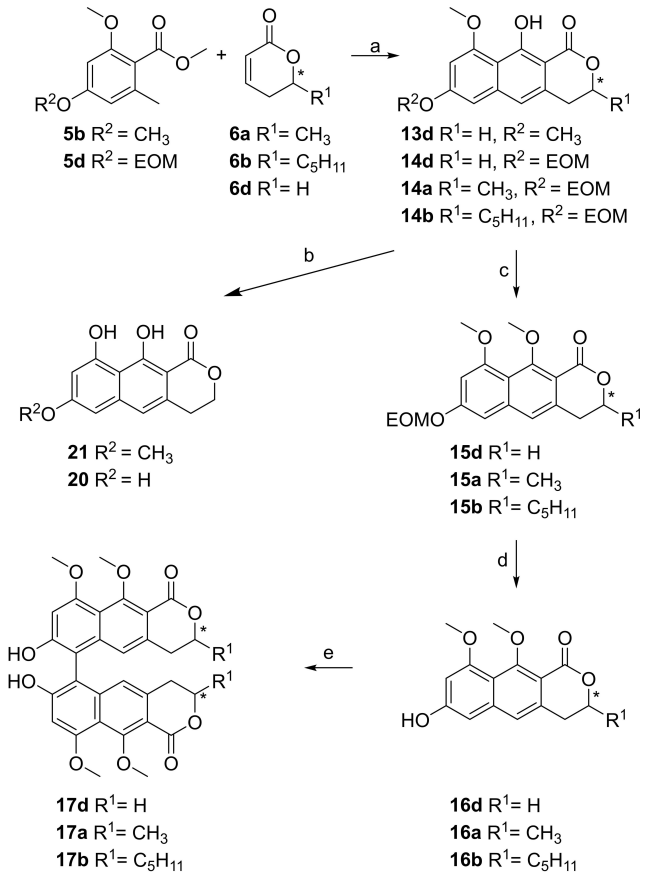
The second building blocks that are required to create chiral and enantiomerically pure α,β-unsaturated δ-lactones in a *Ghosez* cyclization are enantiomerically pure oxiranes **7** (Scheme 3B). In case they are not commercially available, they also have to be synthesized. Therefore, the corresponding alkenes **10** were converted into the oxiranes **7** in a *Prileschajew* reaction[17] with *meta*–chloroperbenzoic acid (*m*CPBA)[18] and then subjected to a kinetic resolution with the help of the *Jacobsen*–salen–cobalt catalysts to obtain the enantiomerically pure oxiranes in yields of 39–42%.[18a,19]

For the methyl- and pentyl–substituted lactone derivatives **6a** and **6b**, respectively, the *Ghosez* cyclization between the modified *Ghosez* reagent **9b** and the chiral oxiranes **7a** and **7b** works with yields between 51–71% and an enantiomeric excess (*ee*) of 97–>99% *ee* (Scheme 3C). Moderate yields could be explained by the volatility of the alkyl-substituted lactones **6a** and **6b**, even if great care was taken during solvent removal. Unfortunately, the *Ghosez* cyclization with the TBS-protected oxirane **7c** gave yields of 4 and 5%, only. Thus, this approach was discontinued for compound **7c**, despite the excellent enantiomeric excesses of (*S*)- and (*R*)-**7c** of >99%. The low yields can be explained by the fact that the addition of 3 M sulphuric acid leads to a deprotection of the primary alcohol.

Grubbs ring–closing metathesis,[20] which is a frequently used method in the synthesis of natural products,[21] was chosen as an alternative synthetic pathway for the synthesis of (*S*)- and (*R*)-**6c**, since the synthesized enantiomerically pure TBS-protected oxiranes (*S*)-**7c** and (*R*)-**7c** were already successfully employed as starting materials (Scheme 3C). According to a



Scheme 3. Lactone and reagent synthesis. (**A**) Original *Ghosez* reagent **9a**, (**B**) Oxirane synthesis. (a) *m*CPBA, CH_2Cl_2, 0 °C→21 °C, 14 h, 76–93 %; (b) (*R*,*R*)-(−)- or (*S*,*S*)-(+)-*N*,*N*′-bis-(3,5-di-tert-butylsalicylidene)-1,2-cyclohexane-diaminecobalt (II), CH_3COOH, H_2O, THF, 0 °C→21 °C, 15–18 h, 39–42 %, (**C**) Lactone synthesis. (c) vinylmagnesium bromide (1 M in THF), CuBr (25 mol %), THF, −30 °C, 30 min, 98 %–quantitative; (d) acryloyl chloride, diisopropylethylamine (DIPEA), CH_2Cl_2, 0 °C, 3 h, 95 %–quantitative; (e) Grubbs I, CH_2Cl_2, 40 °C, 4 h, 89–93 %; (f) i) *n*BuLi (2.5 M in *n*-hexane), *N*,*N*′-dimethylpropyleneurea (DMPU), 3 M H_2SO_4, THF, −78 °C→21 °C, 16–18 h, ii) *p*TsOH, Et_3N, 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU), CH_2Cl_2, −10 °C→21 °C, 48 h, 51–71 %.



Scheme 4. Synthesis of Naphthopyranone derivatives and oxidative biaryl coupling. Reagents and conditions: (a) i) diisopropylamine (DIPA), *n*BuLi (2.5 M in *n*-hexane), THF, −78 °C→21 °C; ii) DDQ, toluene, 21 °C, 13 h, 25–66 %; (b) BBr_3 (1 M in CH_2Cl_2), CH_2Cl_2, 0 °C→21 °C, 8 h, 60–78 %; (c) $(CH_3)_2SO_4$, K_2CO_3, acetone, 60 °C, 24 h, 87 %–quantitative; (d) HCl (1.25 M in EtOH), MeOH, 60 °C, 2 h, 62–95 %; (e) $VO(acac)_2$, CH_2Cl_2, O_2 overpressure, 21 °C, 16–24 h, 54–86 %.

procedure by *Kobayashi et al.*,[22] oxirane **7c** was subjected to a copper–catalyzed reaction[23] with vinylmagnesium bromide. The homoallylic alcohol **11** was obtained in 98 % to quantitative yield and can be used in the next reaction step without purification. Hence, acryloyl chloride was used to generate diene **12** according to a protocol by *Toneto Novaes et al.*, followed by Grubbs I catalysed ring–closing metathesis[20] to give the TBS-protected lactone **6c** in 87–89 % yield (over two steps) and enantiomeric excess of >99 % *ee*.[24] In total three different lactones **6a**–**c** have been synthesized, each in both their enantiomeric forms with excellent enantiomeric excesses of >99 % *ee* and scales of up to 2.9 g.

Naphthopyranone Synthesis and Oxidative Coupling

The key step in the synthesis of naphthopyranones is the domino–*Michael*–*Dieckmann* reaction between an α,β-unsaturated lactone **6** and an orsellinic acid derivative **5**. In order to optimize the reaction conditions for Michael donors **5b** or **5d** and the α,β-unsaturated lactone **6**, the commercially available, unsubstituted lactone **6d** was first used as a test substrate (Scheme 4, R^1 = H; for details see supporting information). Under modified *Shaw*[8] conditions, the protected naphthopyranones **13d** and **14a**,**b** + **d** were obtained after 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) oxidation with yields between 25–66 %.

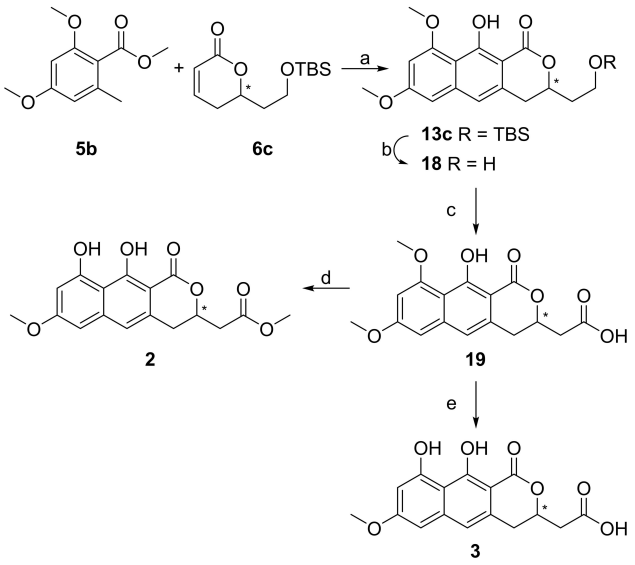
For the synthesis of derivatives **16a**,**b** + **d**, the methoxy- and EOM-protected donor **5d** was used in order to be able to carry out a dimerization after selective EOM-deprotection via an oxidative phenol coupling to give the naphthopyranone dimers **17a**,**b** + **d** (it should be noted that **17d** is a protected precursor of talaroderxine C[1e]). The first step after the domino–*Michael*–*Dieckmann* reaction is the protection of the free hydroxyl group in 10-position with dimethyl sulphate. The desired fully protected naphthopyranones **15a**,**b** + **d** were obtained with yields >87 %. In the next step, the EOM-protection group in 7-position was selectively cleaved with 1.25 M HCl in ethanol at 60 °C[14b] forming the naphthopyranones **16a**,**b** + **d** in 62–95 % yield.

Using the monomeric naphthopyranone **16d** (R^1=H) or the 3-alkyl derivatives **16a** and **16b**, dimerization to the axially chiral 6,6′-homo–dimers was approached. To test suitable reaction conditions for the oxidative coupling, the unsubsti-

tuted naphthopyranone **16d** was used. Initially a procedure by *Müller et al.* was applied using iron(III) chloride on silica gel for the dimerization of two phenol derivatives.[11d,e,25] However, here the approach failed and no conversion could be detected. A protocol by *Nakajima et al.* with catalytic amounts of copper (I) chloride and TMEDA did also not produce the desired dimerization product.[26] Furthermore, applying catalytic amounts of $FeCl_3$ and stoichiometric amounts of di-*tert*-butyl peroxide did not lead to any conversion.[27] The same was observed utilizing the laccase from *Agaricus bisporus*. Only the use of a vanadium(V) species, originating from $VO(acac)_2$ in an oxygen atmosphere was successful.[8] The dimers **17** were obtained in 54–86% yield and diastereomeric ratios between 1:1 and 1:3. An attempt was also made to synthesize dimeric naphthopyranones via a double domino–*Michael–Dieckmann* reaction on a previously reported 5,5′-linked orsellinic acid derivative.[28] However, only the monoadduct SI-**9** was obtained. After DDQ oxidation, the mixed dimer SI-**10** was again subjected to a domino–*Michael–Dieckmann* reaction (for reaction and reaction conditions see supporting information), but no further conversion could be observed.

Since the domino–*Michael–Dieckmann* reaction between Michael donor **5b** with the TBS-protected lactone **6c** already provides the complete carbon skeleton of semi–viriditoxin (**2**) and semi–viriditoxic acid (**3**) within the synthesized naphthopyranone **13c** (Scheme 5, 68–70% yield), only the functional groups had to be adapted to get access to the natural products. First, the TBS protecting group was cleaved off by exposure to aqueous HCl (10%). For purification, the alcohol **18** can either be recrystallized from acetone (40% yield) or can be subjected to column chromatography (81% yield). Subsequently, the alcohol **18** was oxidized with (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) to the corresponding aldehyde and then converted into the acid **19** in a *Pinnick* oxidation.[29] The addition of 2-methyl-2-butene as a hypochlorous acid scavenger is essential for the success of the reaction. The TBS-deprotection and oxidation were carried out according to a procedure of *Tan* and *Donner*.[9]

In the last step, the methoxy group in 9-position must be cleaved selectively and, in case of semi–viriditoxin (**2**), the acid must be transformed into the methyl ester. Applying the conditions reported by *Tan* and *Donner*[9] (BCl_3) was not successful and only starting material could be recovered. Again, the achiral naphthopyranone **13d** served as a test substrate (Scheme 4): BBr_3 was used instead of BCl_3. Temperature and number of equivalents proved to be essential: In the end, 0 °C was ideal for the reaction. With five equivalents of BBr_3 almost exclusively the globally deprotected naphthopyranone **20** was obtained, while for selective methyl ether cleavage in 9-position two equivalents were sufficient yielding 60% of the desired product **21**. Because BBr_3 can also coordinate to the acid at the sidechain within the lactone subunit, 2.5 equivalents BBr_3 were used in the total synthesis of semi–viriditoxin (**2**) and semi–viriditoxic acid (**3**) (Scheme 5). Whether the natural product with a free acid or the methyl ester substitution is obtained in the last step, depends mainly on the work-up of the BBr_3 deprotection: Quenching with methanol leads to semi–viriditoxin (**2**), while the viriditoxic acid (**3**) is obtained with water. After purification via column chromatography, both products still contained traces of a minor impurity. After HPLC, an overall yield between 9–11% for the methyl ester **2** and 5–7% for acid **3**, could be obtained over five steps starting from orsellinat **5b** and the TBS-protected lactone **6c**.



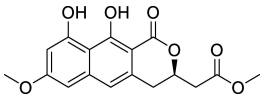
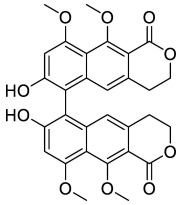
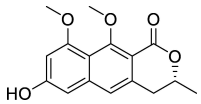
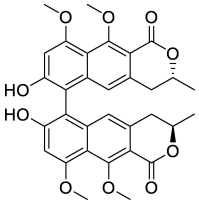
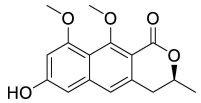
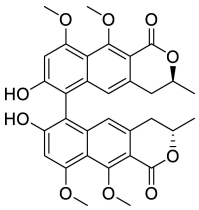
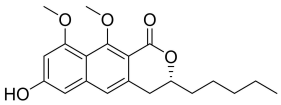
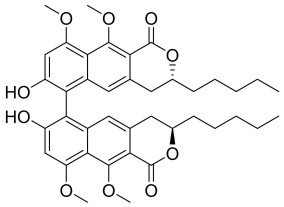
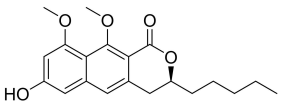
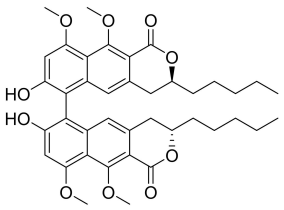
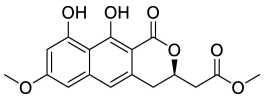
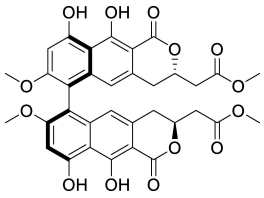
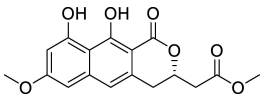
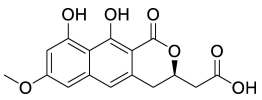
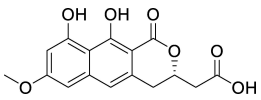
Scheme 5. Total synthesis of semi–viriditoxin (**2**) and semi–viriditoxic acid (**3**). Reagents and conditions: (a) i) DIPA, ^{n}BuLi (2.5 M in *n*-hexane), THF, −78 °C→21 °C; ii) DDQ, toluene, 21 °C, 12 h, 66–70%; (b) HCl, THF, 17 h, 70–81%; (c) i) $PhI(OAc)_2$, TEMPO, CH_2Cl_2, 21 h; ii) NaH_2PO_4, $NaClO_2$, 2-methyl-2-butene, acetone, ^{t}BuOH, H_2O, 21 °C, 3 h, 48–67%; (d) BBr_3 (1 M in CH_2Cl_2), CH_2Cl_2, 0 °C→21 °C, 6 h, workup: MeOH, 33–35%; (e) BBr_3 (1 M in CH_2Cl_2), CH_2Cl_2, 0 °C→21 °C, 6 h, workup: H_2O, 19–21%.

Biological Activity

In order to elucidate the biologically active parts of the naphthopyranone scaffold, the different synthesized monomeric and dimeric intermediates for the synthesis of semi–viriditoxin (**2**) or semi–viriditoxic acid (**3**) were analyzed concerning their cytotoxic activity against lymphoma cells. Therefore, Ramos cells were treated with increasing concentrations of all compounds in both enantiomeric forms and incubated for 24 h (supporting information Figure S1–Figure S5). Afterwards, the cell viability was determined using the resazurin based AlamarBlue® assay (Figure 2). The respective IC_{50} values (half–maximal inhibitory concentration) that have been determined from the dose–response fitted date tests are shown in Table 1.

The naphthopyranone dimers viriditoxin (**1**), **17d**, (3*R*,3′*R*)-**17b**, and (3*S*,3′*S*)-**17b** reduced the cell viability of lymphoma cells and displayed IC_{50} values of 0.02 μM, 20 μM, 5.27 μM and 8.58 μM, respectively, after 24 h treatment (Figure 2, Table 1). With a prolonged treatment duration of 72 h, the IC_{50} values can be reduced for the derivatives **17d**, (3*R*,3′*R*)-**17b**, and (3*S*,3′*S*)-**17b** to 9.9 μM, 3.58 μM and 3.57 μM (supporting information, Figure S1). In contrast, the 3-methyl-substituted

Table 1. Comparison of the IC_{50} values (after 24 h incubation) of the dimers viriditoxin (**1**) and **17a**,**b**+**d** and their corresponding monomeric naphthopyranones semi–viriditoxin (**2**), **16a**,**b**+**d** and semi–viriditoxic acid (**3**). n.c.: indicates that respective IC_{50} values could not be determined due to lacking convergence of the corresponding fit.

Compound	Structure	IC_{50} (24 h)	Compound	Structure	IC_{50}
16d		n.c.	**17d**		20 μM (24 h) 9.9 μM (72 h)
(*R*)-**16a**		n.c.	(3*R*,3′*R*)-**17a**		n.c. (24 h)
(*S*)-**16a**		n.c.	(3*S*,3′*S*)-**17a**		n.c. (24 h)
(*R*)-**16b**		n.c.	(3*R*,3′*R*)-**17b**		5.27 μM (24 h) 3.58 μM (72 h)
(*S*)-**16b**		n.c.	(3*S*,3′*S*)-**17b**		8.58 μM (24 h) 3.57 μM (72 h)
(*R*)-**2**		n.c.	**1**		Commercial VDT: 0.02 μM (24 h)
(*S*)-**2**		n.c.			
(*R*)-**3**		n.c.	(*S*)-**3**		n.c. (24 h)

dimers (3*R*,3′*R*)-**17a** and (3*S*,3′*S*)-**17a** did not show any biological activity. The monomeric naphthopyranones semi–viriditoxin (**2**) and **16a**–**d** did not show any effect on the cell viability, suggesting that a dimeric structure is crucial for biological activity. Compared to the commercially available viriditoxin (**1**), naphthopyranone (3*R*,3′*R*)-**17b** was most active with a low IC_{50} value of 5.27 μM. The structures of viriditoxin (**1**) and (3*R*,3′*R*)-**17b** differs in the sidechain as well as in the protection pattern. Whereas viriditoxin (**1**) has a polar sidechain due to the ester functionality, the sidechain of (3*R*,3′*R*)-**17b** is a non–polar aliphatic substituent. Viriditoxin (**1**) has methyl ether protected hydroxyl groups in 7- and 7′-position and free hydroxyl groups

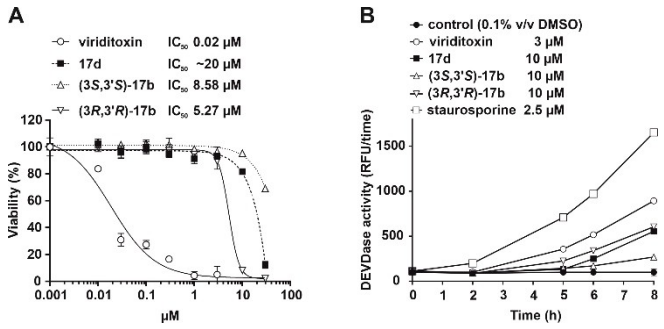


Figure 2. Comparison of viriditoxin (**1**) to dimeric derivatives **17 d**, (3*S*,3′*S*)-**17 b**, and (3*R*,3′*R*)-**17 b** in (**A**) cytotoxicity and (**B**) their inductive effect on caspase–3 activity (DEVDase activity) in Burkitt B cell lymphoma. (**A**) Cytotoxicity was determined in Ramos cells after 24 h treatment with increasing concentrations of viriditoxin (**1**), **17 d**, (3*S*,3′*S*)-**17 b**, and (3*R*,3′*R*)-**17 b** using AlamarBlue® viability assay. The viability is plotted against the concentration and a dose–response fit is applied to calculate the corresponding half–maximal inhibitory concentration (IC_{50}). The resulting IC_{50} values are given in the legend. Each graph shows the mean ± SD of one representative experiment performed in technical triplicates. (**B**) Ramos cells were treated with DMSO (0.1% v/v; negative control), viriditoxin (**1**) (3 µM), **17 d** (10 µM), (3*S*,3′*S*)-**17 b** (10 µM), (3*R*,3′*R*)-**17 b** (10 µM) and staurosporine (2.5 µM; positive control). Activation of caspase-3 was determined by adding the pro-fluorescent caspase-3 substrate Ac-DEVD-AMC and subsequent measurement of the increase in fluorescence of AMC, which reflects caspase-3 activity in Ramos cells. Each graph shows the mean ± SD of one representative experiment performed in technical duplicates.

in 9/9′- and 10/10′-position, in (3*R*,3′*R*)-**17 b** the protection pattern is exactly the other way around.

Additionally, viriditoxin (**1**) is a single atropisomer [(*M*)-isomer] and (3*R*,3′*R*)-**17 b** has not been synthesized atroposelectively, but as a 1 : 1 mixture of (*M*)- and (*P*)-dimer. It was demonstrated that the original viriditoxin (**1**) displays the highest cytotoxicity to lymphoma cells. The relevance of viriditoxin (**1**) as potential therapeutic for lymphoma and leukemia treatment was recently published.[6a] It was proven that it activates the intrinsic mitochondrial apoptosis pathway at low concentrations. Therefore, the potential of the dimers **17 d**, (3*S*,3′*S*)-**17 b**, and (3*R*,3′*R*)-**17 b** to induce activation of caspase-3 in Ramos cells was investigated by treatment with the compounds followed by enzyme activity measurement over time using the pro–fluorescent caspase-3 substrate Ac-DEVD-AMC (Figure 2B). The potent apoptosis inducer staurosporine was used as a positive control for caspase activation and DMSO as a negative control. It can be seen that high concentrations of 10 µM for the synthesized dimeric viriditoxin derivatives **17 d**, (3*S*,3′*S*)-**17 b**, and (3*R*,3′*R*)-**17 b** are needed to induce activation of caspase-3. In contrast, as little as 3 µM viriditoxin (**1**) are sufficient and induce a more pronounced caspase activation. Interestingly, (3*R*,3′*R*)-**17 b** seems to be again the most active dimer when taking its IC_{50} value with 5.27 µM and its level of caspase-3 activation into account. Regarding the mechanism of action, it was recently shown that viriditoxin (**1**) directly activates the mitochondrial apoptosis pathway, even in the presence of the antiapoptotic regulatory protein Bcl-2.[6a] The high mitochondrial toxicity was caused by a breakdown of the mitochondrial membrane potential, the inhibition of mitochondrial respiration, the mitochondrial release of proapoptotic cytochrome c, the generation of reactive oxygen, species and mitochondrial fragmentation.[6a] Thermal proteome profiling (TIPP) was performed as target identification approach in order to identify proteins that are thermally (de)stabilized by viriditoxin (**1**) treatment. The mitochondrial ribosomal proteins were identified as destabilized proteins, among others. Since viriditoxin (**1**) did not show a pronounced effect on the mitochondrial encoded protein synthesis,[6a] it is more likely that it affects the overall stability or association with the inner mitochondrial membrane. Therefore, the information provided by the comprehensive analysis of the cytotoxicity of the synthesized intermediates and derivatives provides meaningful insights to further study the biological mechanism of viriditoxin (**1**) in lymphoma.

Conclusions

In conclusion, we were able to synthesize the naphthopyranone semi–viriditoxin (**2**) and we report the first total synthesis of semi–viriditoxic acid (**3**). Based on the naphthopyranone lead structure of (semi−)viriditoxin, different monomeric and dimeric derivatives were generated. The key intermediate for the synthesis of naphthopyranones **13 c**,**d** as well as **14 a**,**b** + **d** are enantiomerically pure α,β-unsaturated lactones **6 a**–**d**, which could be obtained by performing a *Ghosez* cyclization[13i,j] or via a *Grubbs* ring–closing metathesis.[20] The stereogenic information originates from a kinetic resolution of the oxirane–intermediate **7 a**–**c** with the help of *Jacobsen*–salen–cobalt catalysts. The essential step in the synthesis of naphthopyranones is the domino–*Michael*–*Dieckmann* annulation reaction between an α,β-unsaturated lactone **6** and an orsellinic acid derivative **5**. In an oxidative coupling reaction with vanadyl acetoacetonate the naphthopyranones **16 a**,**b** + **d** were converted to the corresponding atropisomeric dimers **17 a**,**b** + **d**. The obtained monomers and axially chiral dimers were subjected to an analysis of the structure-activity relationships by comparing the cytotoxicity against Ramos cells in a cell viability assay (AlamarBlue®). In this assay only the dimers **1**, **17 d**, (3*R*,3′*R*)-**17 b**, and (3*S*,3′*S*)-**17 b** reduced the cell viability of lymphoma cells. The monomeric naphthopyranones and their intermediates did not show any effect on the cell viability. This leads to the conclusion that the dimeric structure is crucial for the biological activity and further research should focus on their synthesis.

Supporting Information

All synthetic procedures including optimizations are described together with the analytical data. AlamarBlue® assay results and NMR spectra are shown. Additional references cited within the supporting information.[30]

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: Biaryls · Cytotoxicity · Natural Product · Total synthesis · Naphthopyranone

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