Natural Products

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The Synthesis of Novel Disorazoles**

*Romy Schäckel, Bettina Hinkelmann, Florenz Sasse, and Markus Kalesse**

The disorazoles were first isolated by the groups of Reichenbach and Höfle in 1999.[1] As highly potent inhibitors of microtubule stabilization they have attracted considerable attention since they show biological activity at picomolar concentrations.[2] Recently, a new member of this family, disorazole Z (**1**), was reported.[3] This C_2-symmetrical compound exhibits the characteristic functionalities of the other disorazoles within a 26-membered macrocycle. The obvious differences compared to other disorazoles are the smaller ring size and the ester moiety which is placed at the site of the geminal dimethyl groups and consequently introduces a quaternary chiral center (Figure 1, configuration was not assigned). As a consequence of the remarkable biological activities, a variety of different synthetic approaches were put forward with only one synthesis completed by the Wipf group.[4] Most of the problems were associated with the construction of the conjugated polyene system and consecutive lactonization protocols. The synthetic challenge of the disorazoles can be described best by the fact that the lack of success by different research groups to complete the synthesis culminated in the speculation that the structure could have been missassigned. This synthetic challenge combined with our interest to abrogate the structure–activity relationship prompted us to initiate a "chemical editing"[5] program aimed at synthesizing the simplified disorazole **2** (Figure 1). The major difference was that disorazole **2** lacks one C2 unit on each hemisphere, just as in disorazole Z, and the characteristic germinal dimethyl groups, framed by an *anti* diol and the oxazole, are present. Additionally, the construction of symmetrical disorazoles from one precursor for each hemisphere should simplify the synthetic access.

Based on the existing disorazoles we decided to incorporate a *Z*,*E*,*E* pattern of the conjugated double bonds. Our retrosynthetic analysis placed one disconnection between C5 and C6, which could be established in the forward direction through a Wittig reaction (Scheme 1). The remaining two

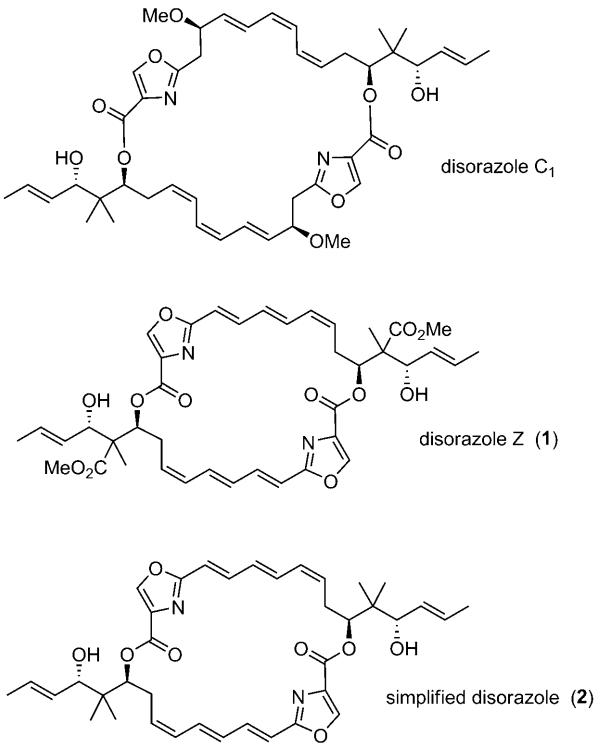
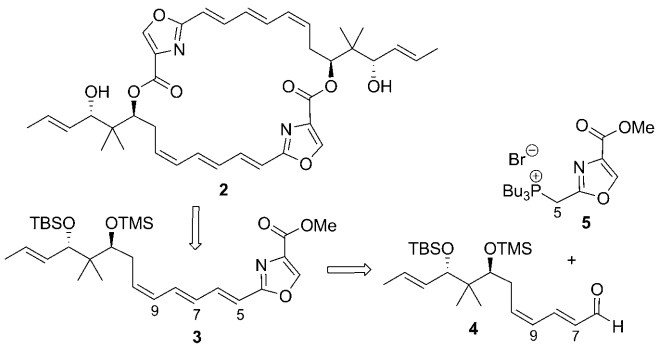


Figure 1. Disorazole C_1 and its truncated analogue.

double bonds of the triene moiety would be realized through a vinylogous aldol reaction and an *E*-selective olefination.

The synthesis begins with the known Kiyooka aldol[6] reaction to construct the geminal dimethyl group and the alyllic alcohol (**8**; Scheme 2). The developing cationic center at the ester moiety is concomitantly reduced during this step and provides the appropriate oxidation state for additional transformations. The aldehyde is liberated by migration of the TBS group to the newly generated secondary alcohol (**9**).[7] Next, the asymmetric vinylogous aldol reaction using the



Scheme 1. Retrosynthetic analysis of disorazole **2**. TBS = *tert*-butyldimethylsilyl, TMS = trimethylsilyl.

[*] R. Schäckel, Prof. Dr. M. Kalesse
Biomolekulares Wirkstoffzentrum (BMWZ), Leibniz Universität Hannover
Schneiderberg 1B, 30167 Hannover (Germany)
Fax: (+49) 511-762-3011
E-mail: markus.kalesse@oci.uni-hannover.de
and
Medizinische Chemie, Helmholtz Zentrum für Infektionsforschung
Inhoffenstraße 7, 38124 Braunschweig (Germany)
B. Hinkelmann, Dr. F. Sasse
Chemische Biologie, Helmholtz Zentrum für Infektionsforschung
Inhoffenstraße 7, 38124 Braunschweig (Germany)

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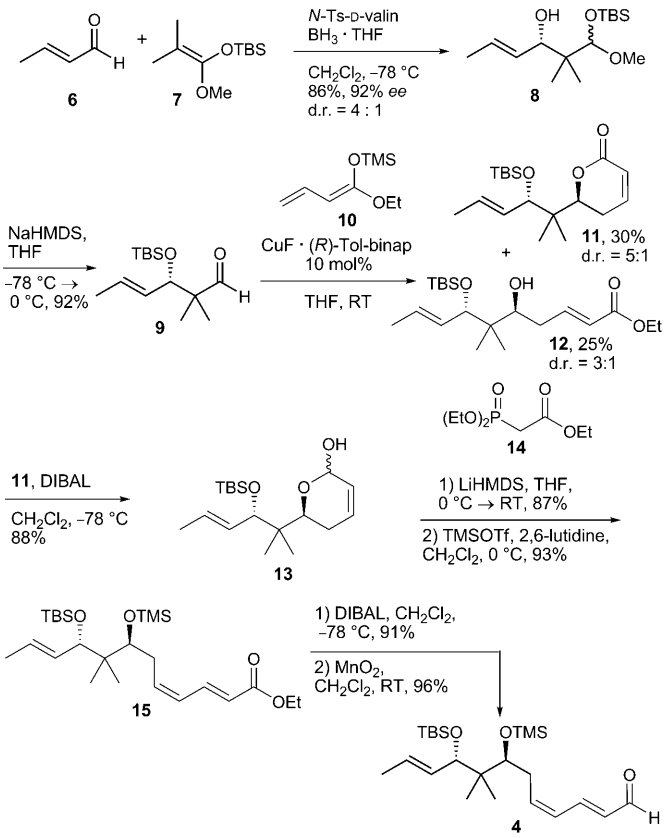
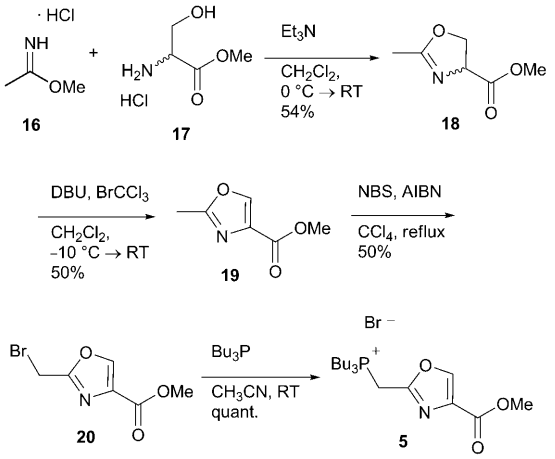


Scheme 2. Synthesis of *Z*,*E*-aldehyde **4**. binap = 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl, DIBAL-H = diisobutylaluminum hydride, HMDS = hexamethyldisilazide, Tf = triflouromethansulfonyl, THF = tetrahydrofuran, Ts = 4-toluenesulfonyl.

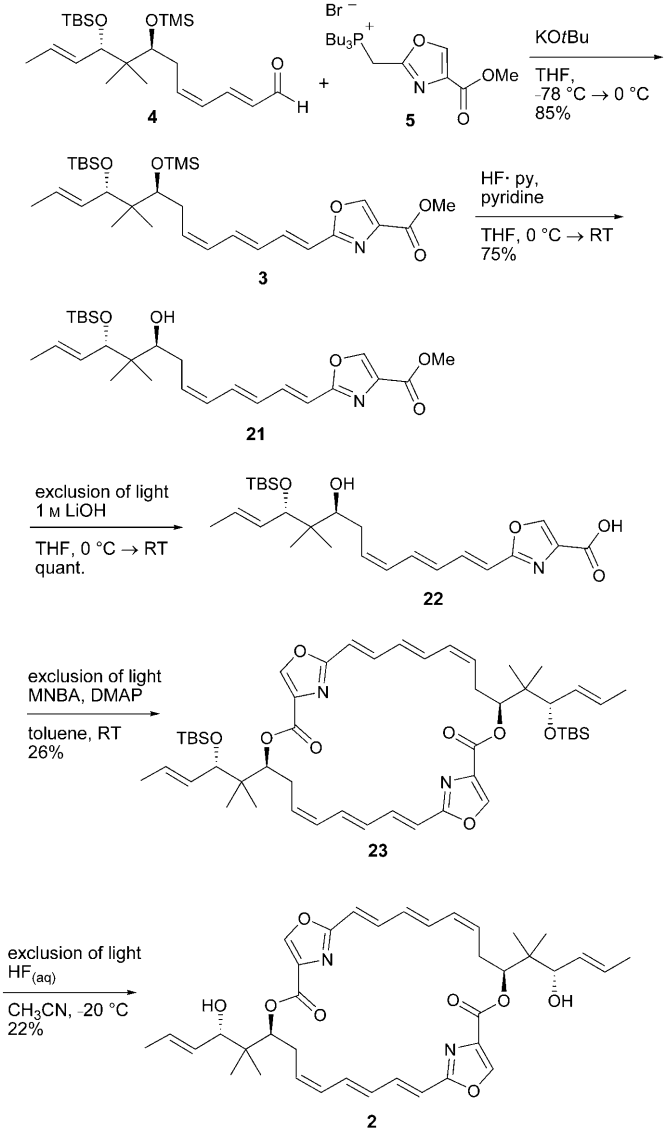
Carreira catalyst[8] and the Champagne protocol[9] provided the *Z*-configured double bond in unsaturated lactone **11**. During this transformation, we also observed 25% of the open chain *E*-isomer **12** which could be separated by chromatography and used for the synthesis of the analog **24** (see Figure 2).

The synthesis continues with reduction of **11** to lactol **13** and subsequent olefination using the Wittig–Horner protocol. During the process of identifying the appropriate protecting group which could be selectively removed at the end of the synthesis, we realized that the TMS ether would be sufficiently stable under the reaction conditions employed. An additional sequence of reduction and oxidation reactions then provided aldehyde **4**. The oxazole fragment required was obtained by a standard condensation between **16** and **17**[10] and subsequent elimination using DBU and $BrCCl_3$ (Scheme 3).[11] Functionalization of the methyl group was achieved by radical bromination and subsequent displacement with PBu_3 to provide **5** in good yield .

Selective construction of the triene system (**3**) was achieved using KO*t*Bu , and liberation of one secondary hydroxy group was accomplished by treatment with HF·py (Scheme 4). Next the acid had to be liberated upon saponification. This liberation and the subsequent transformation proved to be challenging since none of the established protocols provided neither clean **22** nor the dimeric product



Scheme 3. Synthesis of oxazole segment **5**. AIBN = azobis(isobutyronitrile), DBU = 1,8-diazabicyclo[5.4.0.]undec-7-ene, NBS = *N*-bromosuccinamide.



Scheme 4. Endgame of the disorazole synthesis. DMAP = 4-(dimethylamino)pyridine, MNBA = 4′-nitrobenzylidene-3-acetylamino-4-methoxyaniline, py = pyridine.

23 in a one-pot procedure. After extensive experimentation we realized that all problems encountered so far could be omitted by the exclusion of light.

Consequently, we used brown glassware and turned off all sources of light. These conditions allowed the isolation of the acid **22** and the protected disorazole **23** by using the Shiina protocol for dimerization.[12] Finally, removal of the silicon protecting groups with HF in acetonitrile provided the disorazole **2**. Taking advantage of *E*-stereoisomer **12** generated in the vinylogous aldol reaction (Scheme 2) and by using the above described route, the truncated disorazole **24**, exhibiting all *E*-configured double bonds, could be achieved (Figure 2, and see the Supporting Information). The so-obtained disorazoles were tested for their biological activity.

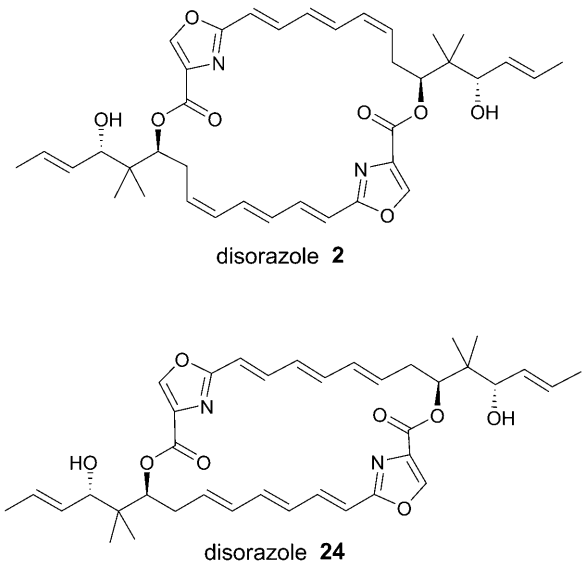


Figure 2. Novel disorazoles.

Table 1 shows a striking difference in the inhibitory activity of **2**, and **24** on the proliferation of L-929 mouse fibroblasts and different human cell lines. Compound **2**, having *Z*,*E*,*E*-configured double bonds, was in general approximately 50 times more active than compound **24**, which exhibited the *E*,*E*,*E* configuration. Interestingly, these analogs have substantially different cytotoxic behavior with mouse fibroblasts as compared to human cancer cells, which was not observed for natural disorazole A_1. Disorazole A_1 showed an IC_{50} value of 4 pM with L-929, and, 5 and 7 pM with SK-OV-3 and PC-3, respectively.[2d] Investigations with primary human umbilical vein endothelium cells, however, did not show a differential activity between propagating malignant and sane cells.

The influence of compound **2**, and **24** on tubulin polymerization was measured in vitro by experiments with purified microtubule protein from porcine brain (Figure 3).[13,14,15] As seen in experiments with cell cultures, compound **2** was much more active than **24**. Disorazole A_1 on the other hand is more active by several orders of magnitude. From these results it can be concluded that the different

Table 1: Antiproliferative activity of **2** and **24** on different mammalian cell lines.[a]

Cell line	Origin	**2** IC_{50} [nM]	**24** IC_{50} [nM]
L-929	Murine connective tissue fibroblast	4.9	290
KB-3-1	Human cervix carcinoma	0.97	49
A-431	Human epidermoid carcinoma	1.44	49
PC-3	Human prostate carcinoma	1.44	50
MCF-7	Human breast carcinoma	0.70	35
SK-OV-3	Human ovary adenocarcinoma	0.65	30
U-937	Human lymphoma	0.58	8.0
HUVEC	Human umbilical vein endothelium	1.3	29

[a] Cells were grown in presence of serial dilutions of the compounds for 5 d. Growth was determined with an 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

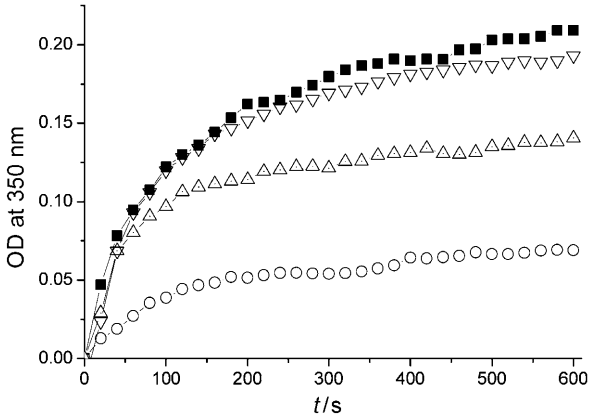


Figure 3. Influence of compound **2** and **24** on polymerization of purified porcine tubulin at 37 °C in comparison with disorazole A_1 (■ control; △ **2**; ▽ **24**; ○ disorazole A_1; microtubule protein 1 mg mL^{-1}; compound concentrations 5 μM mL^{-1}).

activities of compounds **2** and **24** in cell cultures are a result of different binding affinities to the target protein rather than different cellular uptake or detoxifying mechanisms.

In conclusion, the analogues described are simplified, active disorazoles which open access to synthetically more accessible derivatives of this kind of potent cytotoxic compounds. Additionally, these analogs exhibit selectivity between mouse fibroblast cells and tumor cell lines. It provides evidence that highly cytotoxic natural products are ideal starting points for altering the biological profile and thus introducing selectivity to anti-tumor compounds.

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