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Chemo-, regio-, and stereoselectivity of F-ring opening reactions in the cephalostatin series

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Dedicated to Professor Ekkehard Winterfeldt on his 75th birthday.

Abstract—In an effort to prepare unsymmetrical cephalostatin analogues with multi-functionality, we tried the route of selective opening of the spiroketal joining rings E and F. In this study, we have tested several borane complexes (like borane-9-BBN, borane-(*N*-tosyl)-D-valine, and borane–catechol) with some bis-steroidal pyrazine derivatives like **3**, **4**, and **16** aiming at opening ring-F at only one spiro-system of the dimer. Upon testing these borane reagents, satisfying results were obtained in the case of the keto-methylene **4** using the catechol–borane complex. The structures of the resulting mono-opened and also some double-opened spiro dimers have been completely confirmed. Some of the prepared compounds were tested against three cancer cell lines: HM02 (stomach cancer), HEP G2 (hepatocellular cancer), and MCF 7 (breast cancer).

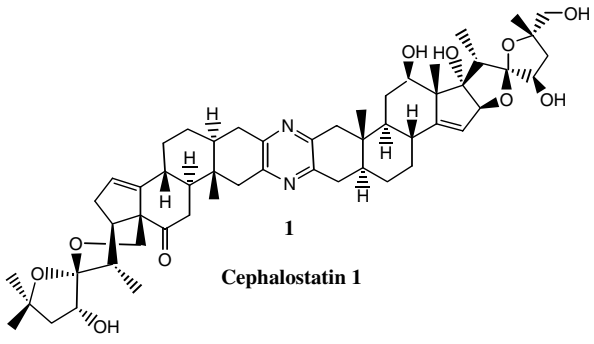
1. Introduction

Cephalostatin 1 **1** which was isolated by Pettit's group from *Cephalodiscus gilchristi*, a tube-inhabiting marine invertebrate worm collected from the Indian Ocean,[1] is one of the most potent anti-cancer natural products ever tested in the National Cancer Institute NCI.

In the last few years, various efforts have been published by different groups aiming at either the total synthesis of cephalostatins (e.g., cephalostatin 1 **1**) and, a structurally related, ritterazines[2] (e.g., ritterazine K **2**) as Fuchs's group[3] in the US or at the synthesis of analogues with high biological activity against different cancer cell lines as Winterfeldt's group[4] in Germany.

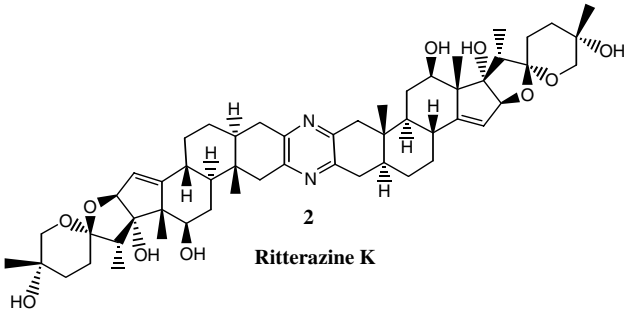
2. Results and discussion

From the beginning, Winterfeldt's group was concentrating on the synthesis of extremely active cephalostatin analogues using symmetrical[5] and asymmetrical[6] coupling pathways (see Scheme 1). To achieve this purpose,



1

Cephalostatin 1



2

Ritterazine K

Winterfeldt's group followed new routes; one of these routes was to reconstruct the spiroketal moiety at the terminal part of the steroid.[5b,7] The main challenge in

Keywords: Cephalostatin; F-ring opening; Mono-opened and double-opened spiroketal derivatives.
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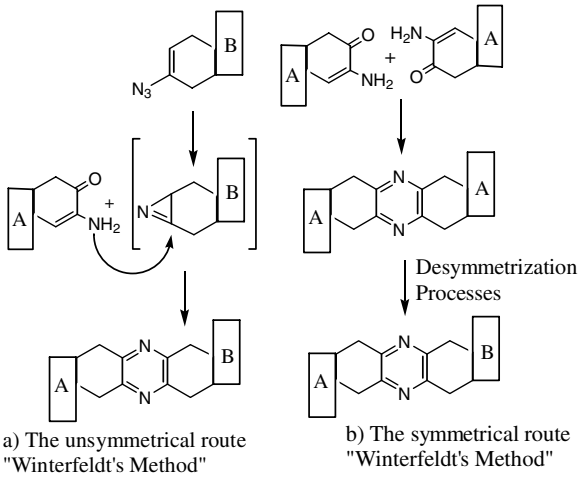
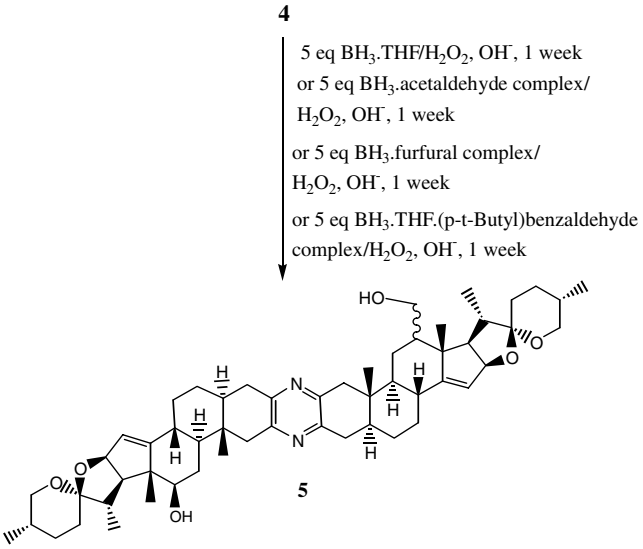


Scheme 1.

the symmetrical pathway was to open the spiroketal moiety in only one half of the dimer leaving the other half untouched. This would open the road to various transformations at one part of the dimer. Moreover, this could help in the evaluation of the necessity of the spiroketal moiety for the biological activity (in vitro) of these analogues.

Upon testing some borane complexes for diastereoselective hydroboration of the exocyclic double bond, we noticed that some of these complexes react with the spiroketal moiety. The work was directed to chemo- and stereoselective opening of the spiroketal moiety in the dimer. Previously, a diastereoselective ring opening of the spiroketal moiety in some steroids was reported.[7,8] In principle, this process is governed by two factors; the selection of the substrate and the selection of the borane complex. Because the functional group at position 12 plays a crucial role in this process, the substrates **3**, **4**, and **16** were selected to be tested with some borane–ether complexes. Compounds **4** and **16** were synthesized from compound **3**[4c,d] which was synthesized through a straightforward and well-established methodology by Winterfeldt's group[5] (Scheme 1). The borane complexes are borane–catechol, borane-(*N*-tosyl)-D-valine in addition to borane-(9-BBN) complexes.

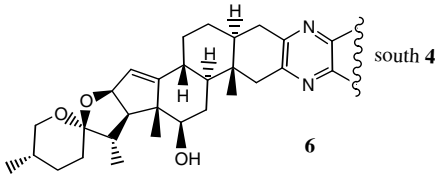
The space demand of the selected borane complex, in addition to the nature of the functional group at C-12 in the substrate, was considered to be the important variables which could control the ring-opening process.

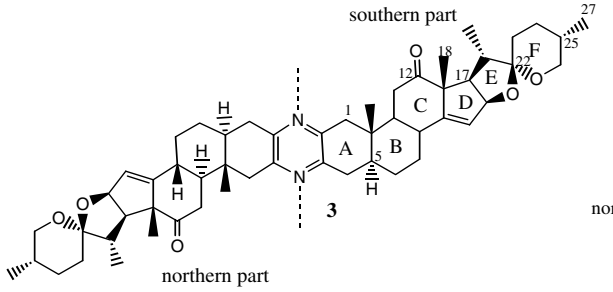


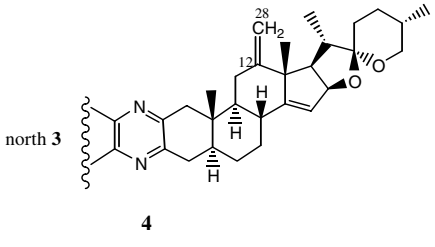
Scheme 2.

We started with the borane–ether complexes derived from aldehydes and having only one active hydride.[9] Treating an acetaldehyde solution (2 equiv) with BH_3·THF (1 equiv) followed by slow addition of compound **4** at 2 °C (one week), led after work-up with H_2O_2/OH^- to the hydroxymethyl derivative **5** resulting from hydroboration of the exocyclic double bond (see Scheme 2) instead of spiroketal opening products (F-ring opening). The same results were obtained upon the treatment of **4** with both *p*-(*tert*-butyl)-benzaldehyde and furfural–borane complexes.

However, reacting compound **4** with 5 equiv of 9-BBN under the same conditions led neither to hydroboration products nor to reductive opening of the spiroketal moiety. The only conversion noticed, even after one week, and after the work-up with H_2O_2/OH^-, was the reduction of the carbonyl group to give the β-hydroxy isomer **6** (selectively >95% de).







After these experiences, we decided to use borane complexes in which the boron atom is coordinated to a bidentate ligand. As a bulky bidentate candidate, boran-(*N*-tosyl)-D-valine complex **a** was chosen. *N*-Tosyl-D-valine was prepared as described in the literature.[10] Treating compound **4** with borane-(*N*-tosyl)-D-valine complex for one week followed by working-up the reaction, the highly polar product **7** (which is called for brevity double-opened spiro-methylene triol and in which ring-F was opened in both sides) and a side product were obtained, which were different from the hydroboration products described above (TLC-control) (Scheme 3).

Structural investigations of the major product **7** showed that the exocyclic double bond had survived. The ^{13}C NMR spectra did not show any of the very typical spiro-carbon signals. Moreover, the ^{1}H NMR spectra showed two new protons in the region between δ 3.22 and 3.55. The full assignment of the NMR data has been done on the basis of combination of different 2D NMR experiments as shown in Figure 1. The mass spectrum of this compound (**7**) showed an increase of only 6 mass units, while the acetylated derivative **8** showed an increase of 126 mass units corresponding to the presence of three new acetyl groups. Along with the low field shift for the two protons (22, 22′-H) ^{1}H NMR spectrum showed, unambiguously, the opening of the two spiroketal moieties. Besides this product, a small amount (~10%) of a less polar substance was isolated.

The spectral analysis of this side product showed that the spiroketal moiety (F-ring) was opened at one side only. Since the available data pointed at a selective opening of the spiroketal in the neighborhood of the exocyclic double bond, all efforts were directed towards experiments providing such an interesting compound as a major product. Selective opening of one spiroketal in the dimer could dramatically change the approach toward the synthesis of cephalostatin analogues. For this reason, we decided to look for a reagent which can differentiate between the two halves of the bis-steroids. Repeating the reaction shown in Scheme 3 by changing

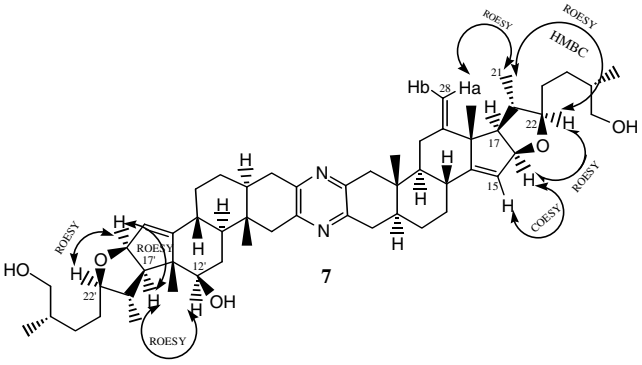
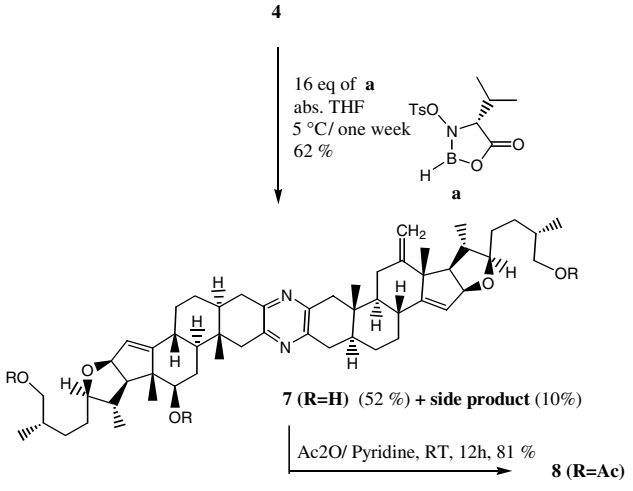


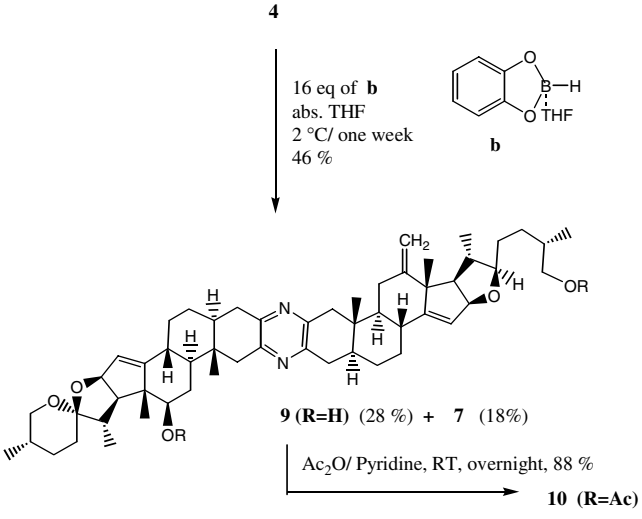
Figure 1.

some kinetic variables did not make a considerable change in the selectivity. Catechol–borane complex **b**, as bidentate ligand, was used with the intention to achieve either regioselective hydroboration of the exocyclic double bond or chemoselective ring opening or both in one single step. Treating the keto-methylene derivative **4** with complex **b** (16 equiv) at 2 °C for two weeks (Scheme 4) led, along with compound **7**, to the desired product **9** (identical to the side product obtained above) as the major product. In this compound, only one side of the dimer **4** was opened with marginal chemoselectivity but high regioselectivity at C-22 and without any hydroboration of the exocyclic double bond. Different spectroscopic methods were used to prove the structures of compounds **7** and **9** and their acetylated derivatives **8** and **10**, respectively.

The main issue in the sequel was to study the regio- and chemoselectivity of the ring opening process. To solve the first part of this problem, an intensive study of the spectroscopic data of compound **9**, especially 2D NMR, was done. From the 1D NMR alone it was impossible to determine the part (north or south) of the dimer which was opened. Therefore, the need arose to do different 2D NMR experiments (like HMBC, HMQC, ROESY, TOCSY, etc.). The new proton



Scheme 3.



Scheme 4.

H-22 resonates in the region between $\delta = 3.40–3.56$ together with four other protons (26a, 26b, 26′a, and 26′b-H) in the same region. This made the problem more difficult to solve. The combination of different types of 2D NMR, however, still enabled us to find a reasonable assignment for the structure. As a result, it was proven that the opening of the spiroketal took place in the neighborhood of the exocyclic double bond, since the correlation between 21-H and 28a-H was found which clearly confirmed the structure of compound **9** (Fig. 2). Because the intermediate complex **11** (Fig. 3) is a non-ionized intermediate, this emphasizes that the ring opening process is a stereospecific process.

The above results unambiguously establish that the ring opening process in this case is a chemoselective process which takes place in the region of the exocyclic double bond.

This selectivity can be rationalized by a two-step mechanism. The first step is the reduction of the keto group which is facile from the axial side leading to β-configuration of the C–O bond at C-12′ with high diastereoselectivity (>93%, NMR). The second step, which is much slower, is the attack of the borane catechol complex at the spiroketal namely the oxygen in the F-ring involving the hydride insertion on the spiro-carbon along with the breakage of the carbon–oxygen bond. This is accomplished with simultaneous formation of the oxygen–boron bond leading to the ring opening (Fig. 3). In the southern part of the starting material (i.e. the keto side), the attack of the reagent complex at the spiroketal is envisaged to be more difficult because the borane complex is still close to the oxygen atom at C-12′, shielding the spiroketal neighborhood from any further attack. This explains the favored ring-opening process in the methylene neighborhood rather than in the vicinity of the keto group. It is important to mention that, a mono-opened by-product at the side of the keto group only (e.g., **12**) was never isolated or noticed during these reactions. This emphasizes that the first step (the fast step) in this process is the reduction of the keto group, in which the ketal region is shielded by the borane complex (Fig. 3). However, the second step (the slow step) is the reductive opening of the spiroketal moiety beside the exocyclic double bond.

Using another reducing agent namely, sodium borohydride $NaBH_4$, the diketone **3** was reduced to give, in addition to the 12β-hydroxyketone **15**, small amount of the diol **13** (Scheme 5).

The question arose, by which factor this process is governed? To gather more information, the diketone **3** was

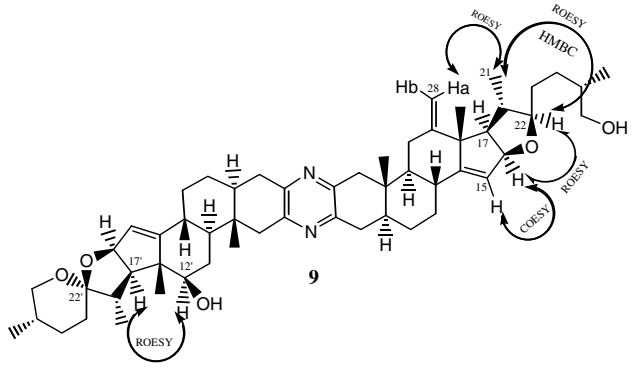


Figure 2.

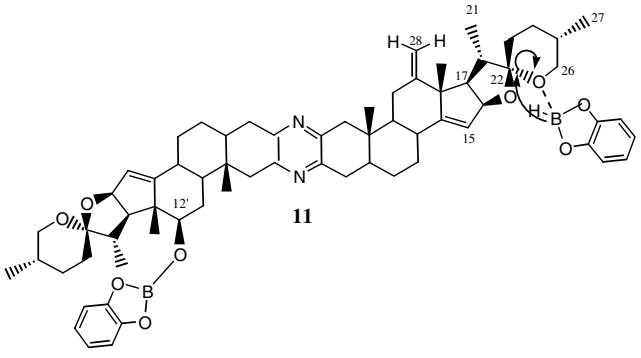
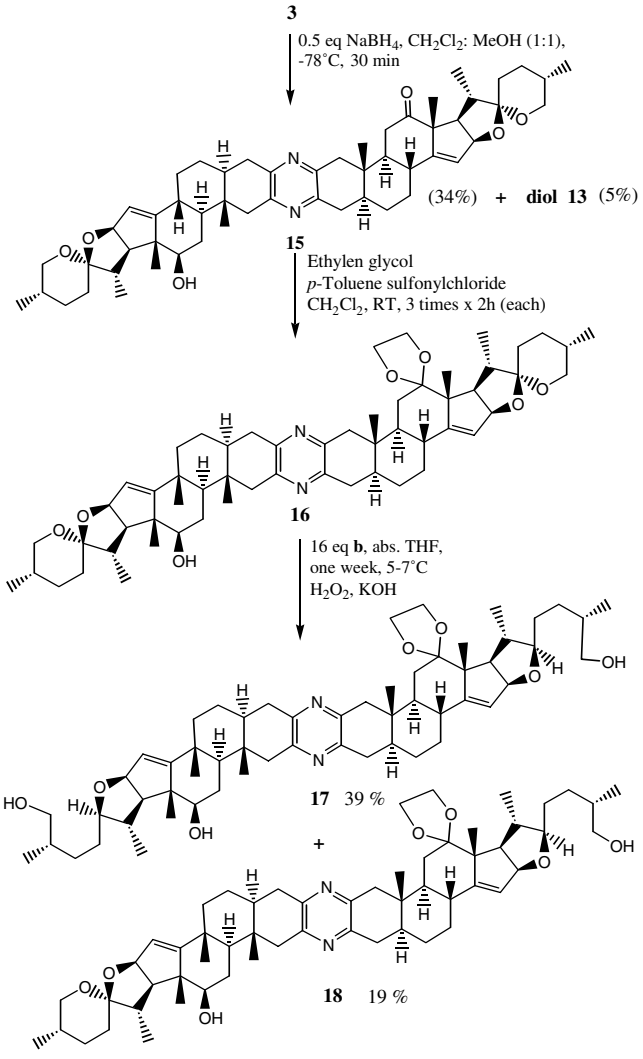
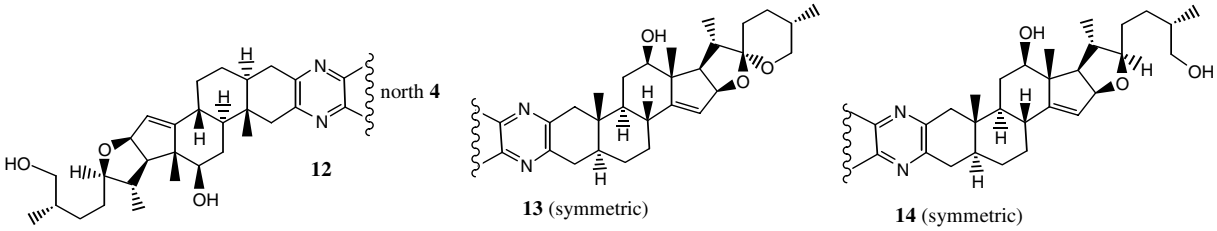


Figure 3.



Scheme 5.



treated with the same reagent complex (catechol–borane complex) and under the same conditions. The results showed that, even after 4 weeks, the major product isolated was the 12β, 12′β-diol **13** and traces of a more polar substance, which could be the tetraol **14**. This could again be rationalized by steric influence of the aromatic ring of the intermediate formed in the reduction of the carbonyl group, which shielded partially the spiroketal moiety from any further attack from the reagent. However, compound **14** was obtained in 96% yield upon treatment of compound **3** with the less space demanding $NaBH_3CN$ for one hour.[7] Opening of the spiroketal moiety in certain steroids using different method was also reported by Fuchs and LaCour.[8]

Repeating the reaction of compound **4** with the catechol–borane complex at slightly higher temperature (+7 °C) led to the loss of selectivity and compound **7** became the major product (~40%), while compound **9** became a minor product (~10%). This means that the temperature plays a key role in the selectivity.

As a result, one may conclude that the chemoselectivity of the spiroketal opening process could be directed by a steric factor which is controlled by both the nature of the borane complex chosen and the reaction temperature.

Compounds **7** and **9** are quite useful because both of them could lead to interesting types of compounds from which it is possible to gain knowledge about the necessity of the spiroketal for the biological activity. However, such compounds could also be important starting materials for further hydroboration processes. Unfortunately, performing this reaction by treating compound **7** with 5 equiv of BH_3·THF complex led, after oxidation of the resulting intermediates, to three different products each of which represents a mixture of isomers. In these products the hydroboration reaction took place first on the exocyclic double bond and then on $\Delta^{14,15}$ either in the southern part or northern part or both. This has been concluded from ^1H NMR and mass spectra. It was noticed from studying the nature of the products obtained from the hydroboration of compound **8** that the $\Delta^{14,15}$ near the opened spiroketal underwent hydroboration faster than the other double bond.

Concerning both issues selectivity and biological activity, we also studied another system. Starting from 12β-hydroxyketone **15**, compound **16** was prepared as mentioned in the literature.[11] Indeed, compound **15** was obtained along with **13** from selective reduction of compound **3** using $NaBH_4$.[5b] Compound **16**, in which the keto group was protected in a ketal form, was selected to be studied (second system). The hydroxy-ketal **16** underwent the ring-opening process with an excess (16 equiv) of the borane complex **b** to give compound **17** (the double-opened spiro) and **18** (the mono-opened spiro-derivative) in a ratio of 2:1 (Scheme 5) with no improvement of the chemoselectivity. Once more, no mono-opened side product from the alcohol side only was isolated or noticed. This led us to confirm that the steric factor of the bonded reagent, as well as the kinetic factor (reaction temperature), are responsible for controlling the chemoselectivity.

Since compound **16** is totally different from the keto-methylene **4** discussed before, determining the opening part (southern or northern) in the minor product was the most important challenge. Using different NMR techniques (1D and 2D), the two protons of **18**: 17-H and 17′-H were identified and further connection with the neighboring protons was found ending with a very clear peak which represents 22-H that resonates at $\delta = 3.33$ ppm.

As shown in Figure 4, the corresponding correlations between the protons and carbon atoms involved were established, the opening region became clear and was shown to be in the vicinity of the ketal moiety. Although the selectivity in this case is not high, it still reflects that the northern part is the preferable area for ring opening under these conditions. Indeed, the selectivity of the ring-opening process could be rationalized now more reliably as a consequence of steric aspects, which in this case resulted from the fast reaction between the non-protected hydroxyl group (as in **16**) or the keto group (as in **4**) and the borane complex. This conclusion is based on the fact that the ring-opening process (or F-

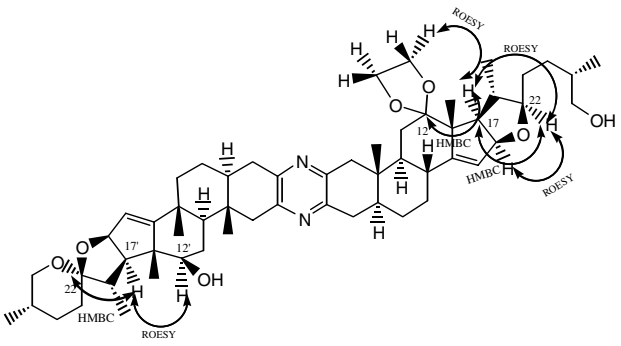


Figure 4.

ring opening) takes place smoothly, as long as the spiroketal is free from any *substantial* shielding effects. Moreover, the 12-C ketal group is apparently inert to reduction due to steric factor. No traces were observed or isolated of ring opening of only the southern spiroketal.

Improving the chemoselectivity through the substrate or the borane complex as well as the reaction conditions is under progress.

2.1. Biological study

The cytostatic activity of some of the synthesized compounds was evaluated by the National Cancer Institute (NCI) in the United States and by the Medizinische Hochschule Hannover (MHH) in Germany. In the NCI, these compounds were tested[12] in vitro against 60 cell lines representing all categories of cancer; leukemia, lung, colon, CNS, melanoma, renal, prostate, and breast cancer. The evaluation of the cytostatical activity is given in terms of GI_{50}, TGI, and LC_{50} for each cell line and for all cell lines together in terms of MG_MID (meangraph midpoint). The two compounds which were tested in the NCI are **4** and **5** (Table 1). Compound **5** was suggested by NCI to be tested in vivo in the Hollow Fiber Assay.

The biological activity of some compounds was tested in the MHH by Prof. W. Beil. These compounds were tested against the three cell lines: HM02 from the stomach cancer, HEP G2 from the hepatocellular cancer, and MCF 7 from the breast cancer using standard procedures.[13]

The results were tabulated concerning the cell line and how strongly it is affected by the tested compounds according to the following evaluations[14].

- Compounds with good activity are those which have $GI_{50} \leqslant 1$ μM and TGI $\leqslant 5$ μM.
- Moderately active compounds are those which have $GI_{50} \leqslant 1$ μM and TGI > 5μM.
- Weakly active compounds are those which have $1 \leqslant GI_{50} \leqslant 10$ μM.
- Inactive compounds are those with $GI_{50} > 10$ μM.

Compound **5**, which had been tested by both NCI and MHH, showed a good activity against different cell lines by NCI and moderate to good activity against the three cell lines by the MHH. Also, compound **4**, tested by both NCI and MHH, has shown a weak activity. This could tentatively imply a considerable agreement between the two measurements.

The concept which was studied here is the importance of the spiroketal moieties at C-22 and C-22′ in the keto-methylene **4** and the hydroxy-ketal **16** to the biological activity. In the first system, a remarkable enhancement in the biological activity of both mono- and double-opened spiroketals in **7** and **9**, respectively, compared to their precursor **4** was noticed as shown in Tables 2–4. However, in the second system (the hydroxy-ketal

Table 2. The biological activity data against the cell line HEP G2

Compound	GI_{50}	TGI	LC_{50}
13[a]	0.65	5.0	>10
7	1.0	>10	>10
17	1.0	>10	>10
16[a]	0.07	1.7	>10
3	4.6	>10	>10
5	6.9	10	>10
18	10	>10	>10
15	>10	>10	>10
4	>10	>10	>10

[a] Compounds with good activity against this cell line.

Table 3. The biological activity data against the cell line HM02

Compound	GI_{50}	TGI	LC_{50}
17[a]	0.63	1.0	>10
18[a]	0.95	3.1	>10
3	5.5	7.0	>10
15	3.0	>10	>10
13	2.6	7.0	>10
5	2.3	>10	>10
7	1.7	5.8	9.5
16	2.8	>10	>10
4	>10	>10	>10

[a] Compounds with good activity against this cell line.

Table 4. The biological activity data against the cell lines MCF 7

Compound	GI_{50}	TGI	LC_{50}
5	0.10	>10	>10
7	5.0	>10	>10
13	5.2	>10	>10
16	0.10	>10	>10
17	8.2	>10	>10
18	2.5	10	>10
3	>10	>10	>10
4	>10	>10	>10
15	>10	>10	>10

Table 1. The biological activity data of compounds **4**, **5** and other potent anti-cancer drugs tested in NCI (values are given in μM)

Compound	MG_MID GI_{50}	MG_MID TGI	MG_MID LC_{50}	NSC No.
5-Fluorouracil	<20	<585	>2500	19,893
Taxol	0.013	3.16	19.9	125,973
Cephalostatin 1	<0.0004	0.0063	>0.25	363979-N
5	12.6	63.1	>100	D-701224-J/1
4	95	100	100	D-701223-I/1

Note: The data of 5-fluorouracil, taxol, and cephalostatin 1 are according to Ref. 12.

16), the biological activity of the mono- and double-opened spiroketal derivatives; **17** and **18**, showed, generally, less activity compared to their precursor **16** (as shown in Tables 2–4).

Compound **17** seems to affect the two cell lines (HEP G2 and MCF 7) somewhat weaker than its precursor **16**, but it is considerably more active in the cell line HM02 case.

3. Experimental

3.1. The testing method used in the MHH

Cells were grown in 96-well microtiter plates of RPMI tissue culture medium supplemented with 10% fetal calf serum at 37 °C in a humidified atmosphere of 5% CO_2 in air. After 24 h of incubation, the tested compounds (0.1–10 μM/l) were added to the cells. Stock solutions of the test compounds were prepared in methanol or DMSO. After a 48-h incubation in the presence of the test drugs, the cells were fixed by addition of trichloroacetic acid and cell protein was assayed with sulforhodamine B.[13b] For each compound tested the GI_{50} (drug concentration causing 50% growth inhibition), TGI (drug concentration causing 100% growth inhibition), and LC_{50} values (drug concentration causing 50% reduction of the cell number present at 24 h) were determined (Tables 2–5).

^1H NMR spectra were obtained using the instruments WP 200 (200 MHz), AM 400 (400 MHz), AVS 400 (400 MHz, Avance), and AVS 500 (500 MHz, Avance), from the firm Brucker. The measurements were taken using duterated solvents and tetramethylsilane as a reference. In the case where no tetramethylsilane (TMS, $\delta = 0.00$) was used as a reference, the solvent peak was used as a reference (for $CDCl_3$, for example, $\delta = 7.27$ ppm). The chemical shifts are given in terms of δ and the coupling constants in Hz. The multiplicity of the peaks is abbreviated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. The integration of the peaks is given as number of protons. The region (0.7–2.0 ppm), which corresponds to the aliphatic region, will not be explained except for the methyl groups and in few cases 9-H.

Table 5. The biological activity data of some references used by the MHH

Substance	Cell line	MG_MID GI_{50}	MG_MID TGI	MG_MID LC_{50}
5-Fluorouracil	HM02	−5.9	−4.7	−4.3
5-Fluorouracil	HEP G2	−7.0	−4.6	−4.3
5-Fluorouracil	MCF 7	−4.3	>−4.3	>−4.3
Cisplatin	HM02	−6.8	−5.8	−4.4
Cisplatin	HEP G2	−6.3	−5.3	>−4.3
Cisplatin	MCF 7	−7.0	−5.0	>−4.3
Doxorubicin	HM02	<−7.0	−6.9	−6.4
Doxorubicin	HEP G2	−6.6	−6.0	>−4.3
Doxorubicin	MCF 7	<−7.0	−6.7	>−4.3

^{13}C NMR spectra were obtained using the instruments AM 400 (100 MHz), AVS 400 (100 MHz), and AVS 500 (125 MHz). The measurements were taken using duterated solvents and in cases where no tetramethylsilane was used as a reference, the solvent peak was used as a reference (for $CDCl_3$, for example, 77.0 ppm). The chemical shift is given in terms of δ and the peak multiplicity is given as s, singlet (C); d, doublet (CH); t, triplet (CH_2); q, quartet (CH_3).

IR-spectra were obtained using the spectrometer FT 1710 from the firm Perkin-Elmer. The frequency is given in cm^{-1}. The following abbreviations were used to indicate the strength of the peak: s, strong; m, middle; w, weak.

UV-spectra were obtained using a Beckman Spectrometer (model 3600) and in each case the solvent was given. The abbreviations used were s, strong; w, weak; m, middle; and sh, shoulder.

FAB-MS-spectra were obtained using the VG-Autospec in a low resolution measurement in which the Nitrobenzyl Alcohol Matrix (NBA-Matrix) was used.

HR-FAB-MS-spectra were obtained using the VG-Autospec in which the peak-matching method and the NBA-Matrix were used.

Thin layer chromatography (TLC) was carried out using aluminum plates coated with the silica-gel $60F_{254}$ from the firm Merck combined with the polygram® Alox N/UV_{254} from the firm Macherey–Nagel. The detection of charged substances over the TLC was done with the help of the UV-lamp ($\lambda = 254$ nm) and developed in Ce(IV) sulfate reagent.

Preparative column chromatography was carried out using the flash chromatography principle. Silica-gel used was from the firm Baker (0.03–0.06 mm). Elution was carried out under a light pressure achieved by use of a hand pump.

Solvents used are commercial solvents after fractional distillation. In other cases, absolute solvents were used.

Chemicals used have not been further purified. The organometallic reagents were handled under absolute conditions.

3.1.1. Preparation of the keto-methylene 4. In a dry 2-neck 150 ml RBF, 1.177 g (2.229 mmol, 1.32 equiv) of methyl-triphenylphosphonium bromide (98%, Acros) was poured and the reaction flask was evacuated and flashed with argon several times. The salt was suspended in 20 ml of abs THF. Dropwise, 1.83 ml (3.294 mmol, 1.32 equiv) of phenyl lithium (1.8 M solution in hexane, Aldrich) was added and stirred at rt for 1.5 h to give a yellowish brown solution. The resulting solution was slowly added to another flask containing a solution of 2.11 g (2.497 mmol, 1.0 equiv) of the diketone **3** in 20 ml of abs THF. The reaction solution was stirred under absolute conditions at rt for 4 h. After the end of the

reaction, 30 ml of diethyl ether was added and the reaction solution was extracted with brine. The aqueous phase was further extracted with dichloromethane and the combined organic phase was dried over Na_2SO_4. Filtration of the solution and removing solvent under reduced pressure gave a pale yellow crude substance which after chromatographic separation on silica-gel (elution with PE/EA (4:1)) provided 0.844 g of the keto-methylene **4** (33.8%) as a white solid, 0.232 g of the dimethylene derivative (9.3%) as a white crystalline material, and 0.945 g of starting diketone **3**.

IR: ($CHCl_3/\nu_{max}/cm^{-1}$); 2928 s (C–H), 1708 s (C=O), 1632 w (C=C), 1448 s (C–H), 1400 s (pyrazine), 1240 s (C–O).

FAB-MS: (NBA-Matrix) *m/z* (%); 841 ($[M-1]^+$, 100).

^1H NMR: (400 MHz, $CDCl_3$); δ = 5.48 (br s, 1H, 15′-H), 4.48 (dd, $J_{16'-17'}$ = 7.9 Hz, $J_{16'-15'}$ = 1.8 Hz, 1H, 16′-H), 4.80 (br s, 1H, 28a-H), 4.78 (dd, J_{16-17} = 8.3 Hz, J_{16-15} = 2.04 Hz, 1H, 16-H), 4.70 (br s, 1H, 28b-H), 3.34–3.56 (m, 5H, 26a/26b/26′a/26′b/17′-H), 2.22–2.99 (m, 14H, 1a/1b/1′a/1′b/4a/4b/4′a/4′b/11a/11b/11′a/11′b/8/17-H), 1.33 (s, 3H, 18′-H), 1.22 (s, 3H, 18-H), 1.09 (d, J_{21-20} = 6.8 Hz, 3H, 21-H), 1.05 (d, $J_{21'-20'}$ = 6.8 Hz, 3H, 21′-H), 0.93 (s, 3H, 19′-H), 0.88 (s, 3H, 19-H), 0.81 (d, $J_{27-25} = J_{27'-25'}$ = 6.4 Hz, 6H, 27/27′-H).

^{13}C NMR: (100 MHz, $CDCl_3$); δ = 210.66 (C, 12′-C), 158.78 (C, 14-C), 155.25 (C, 12-C), 154.27 (C, 14′-C), 148.84, 148.62, 148.04, 148.02 (all C, pyrazine–C), 121.56 (CH, 15′C), 117.81 (CH, 15-C), 107.44 (CH_2, 28-C), 107.03 (C, 22′-C), 106.04 (C, 22-C), 84.25 (CH, 16-C), 83.92 (CH, 16′-C), 67.10, 67.08 (both CH_2, 26/26′-C), 62.27 (C, 13′-C), 54.75 (9-C), 53.97 (CH, 17-C), 53.36 (CH), 53.09 (CH, 9′-C), 49.72 (CH, 17′-C) 45.59, 45.17 (both CH_2, 1/1′-C), 44.20, 44.16 (both CH, 20/20′-C), 41.31 (CH), 41.22 (CH), 37.18 (CH_2, 11′-C), 36.36, 36.01 (both C, 10/10′-C), 35.19 (2× CH_2, 4/4′-C), 34.63 (CH), 33.95 (CH), 32.33 (CH_2), 31.50 (CH_2), 31.26 (CH_2), 30.36 (CH), 30.30 (CH), 29.42 (CH_2), 29.13 (CH_2), 28.78 (CH_2), 28.76 (CH_2), 27.90 (CH_2), 27.86 (CH_2), 23.71 (CH_3, 18-C), 20.75 (CH_3, 18′-C), 17.17, 17.15 (both CH_3, 27/27′-C), 14.00 (CH_3, 21-C), 13.76 (CH_3, 21′-C), 11.67 (CH_3, 19-C), 11.51 (CH_3, 19′-C).

3.1.2. Preparation of methylene triol 7. In a 100 ml 2-neck RBF a borane-(*N*-tosyl)-D-valine complex solution (16 equiv) was prepared by dissolving 0.817 g of *N*-tosyl-D-valine in 10 ml abs THF at 0 °C followed by addition of 0.9 ml (1.6 M) BH_3·THF and the solution was stirred under argon for 30 min at 0 °C and 20 min at rt then it was cooled down to 0 °C. To this solution, 0.150 g of the keto-methylene **4** dissolved in 5 ml abs THF was slowly added and further stirred for 20 min before conducting the reaction at 5 °C for one week without stirring. The reaction solution was then quenched with a careful addition of 1 ml KOH solution (10%) at 0 °C. The turbid solution was warmed to room temperature and 5 ml of each EtOH and DEE was added followed by the addition of 2 ml H_2O_2 (35%) and the solution was vigorously stirred for 2 h. A saturated solution (1 ml) of NH_4Cl was added and the reaction solution was extracted with chloroform several times. The collected organic phase was washed with brine solution and dried over $MgSO_4$. After solvent removal under reduced pressure, the resulting crude material was dried under high vacuum and subjected to column chromatography eluted with 1% MeOH/chloroform. Compound **7** was obtained in a pure form as a white crystalline solid (78 mg, 52%) in addition to 16 mg of a less polar substance, which was later identified as compound **9** (10%).

IR: ($CHCl_3/\nu_{max}/cm^{-1}$); 3620, 3415 (both br, O–H), 3080 w (=C–H), 2931 s (C–H), 1648 w (C=C), 1634 w (C=C), 1400 s (pyrazine), 1235 m (C–O).

UV: $CHCl_3/\lambda_{max}$ (nm), 310 (sh), 291 (s).

FAB-MS: (NBA-Matrix) *m/z* (%); 849 (MH^+, 37).

^1H NMR: (400 MHz, $CDCl_3$); δ = 5.45 (br s, 1H, 15′-H), 5.39 (br s, 1H, 15-H), 4.78 (dd, $J_{16'-17'}$ = 8.2 Hz, $J_{16'-15'}$ = 1.5 Hz, 1H, 16′-H), 4.71 (br s, 1H, 28b-H), 4.69 (br s, 1H, 28a-H), 4.66 (dd, J_{16-17} = 7.5 Hz, J_{16-15} = 2.0 Hz, 1H, 16-H), 3.40–3.55 (m, 5H, 26a/26b/26′a/26′b/22-H), 3.23–3.35 (m, 2H, 22′/12′-H), 2.75–3.01 (m, 4H, 1a/1′a/4a/4′a-H), 2.46–2.72 (m, 5H, 1b/1′b/4b/4′b/17-H), 2.41 (m, 2H, 11a/11b-H), 2.33 (tr, $J_{17'-20'} = J_{17'-16'}$ = 8.0 Hz, 1H, 17′-H), 2.26 (m, 1H, 8-H), 1.23 (s, 3H, 18-H), 1.13 (d, J_{21-20} = 6.5 Hz, 3H, 21-H), 1.06 (d, $J_{21'-20'}$ = 8.0 Hz, 3H, 21′-H), 1.05 (s, 3H, 18′-H), 0.92 (2× d, $J_{27-26} = J_{27'-25'}$ = 8.0 Hz, 6H, 27/27′-H), 0.88 (s, 3H, 19′-H), 0.87 (s, 3H, 19-H).

^{13}C NMR: (100 MHz, $CDCl_3$); δ = 160.34 (C, 14′-C), 157.65 (C, 14-C), 155.79 (C, 12-C), 148.60, 148.59, 148.37, 148.36 (all C, pyrazine–C), 119.82 (CH, 15′-C), 117.44 (CH, 15-C), 106.31 (CH_2, 28-C),89.15 (CH, 22-C), 87.17 (CH, 22′-C), 86.01 (CH, 16′-C), 85.87 (CH, 16-C), 79.31 (CH, 12′-C), 68.11 (2× CH_2, 26/26′-C), 59.37 (CH, 17′-C), 58.49 (CH, 17-C), 54.16 (CH), 53.93 (C, 13-C), 52.93 (C, 13′-C), 51.95 (CH), 45.64 (2× CH_2, 1/1′-C), 41.43 (CH), 41.24 (CH), 41.19 (CH), 40.11 (CH), 35.98, 35.95 (both C, 10/10′-C), 35.83 (2× CH), 35.18, 35.14 (both CH_2, 4/4′-C), 34.87 (CH), 33.81 (CH), 32.35 (CH_2), 30.82 (2× CH_2), 30.41 (CH_2), 30.25 (CH_2), 3.18 (CH_2), 30.13 (CH_2), 29.70 (CH_2), 29.44 (CH_2), 29.22 (CH_2), 28.02 (CH_2), 27.86 (CH_2), 25.42 (CH_3, 18-C), 17.82 (CH_3, 21-C), 16.87, 16.63, 16.64 (all CH_3, 21′/27/27′-C), 13.98 (CH_3, 18′-C), 11.86, 11.68 (both CH_3, 19/19′-C).

3.1.3. Preparation of methylene diol 9. To a solution of catechol (1.413 g, 12.834 mmol) dissolved in 20 ml of abs THF in a dry 150 ml 2-neck RBF at 0 °C, 12.8 ml of BH_3·THF complex was slowly added under argon and stirred for 30 min (until no further evolution of H_2 gas was noticed). To the borane–catechol complex, a solution of **4** (0.676 g, 0.802 mmol, 1 equiv) in 10 ml abs THF was slowly added (in 15 min) and the yellowish reaction solution was stirred for 30 min at 0 °C. The homogeneous reaction was run in a cold room (at

2 °C) without stirring for two weeks. The reaction progress was monitored by TLC which showed that the major product is the desired one. The reaction was quenched with the careful addition of 2 ml of KOH solution (10%) at 0 °C. To the turbid solution, 10 ml of each EtOH and DEE was added and stirred for 20 min at rt followed by the addition of 5 ml of H_2O_2 (35% solution) and the reaction mixture was again vigorously stirred for 2 h. After the addition of 2 ml of a saturated solution of NH_4Cl, the reaction solution was partitioned between chloroform and brine and the aqueous phase was washed further with chloroform. The black organic phase was washed with 10% solution of KOH twice (to remove excess catechol) and dried over $MgSO_4$. Removing the solvent under reduced pressure gave a gray crude material. After drying the crude substance under high vacuum, the resulting material was subjected to column chromatography on silica-gel (1% MeOH/$CHCl_3$ elution) which gave 185 mg of the mono-opened spiro-derivative (methylene diol) **9** in addition to 124 mg of the double-opened spiro-derivative (methylene triol) **7** (overall yield 46%).

IR: ($CHCl_3/\nu_{max}/cm^{-1}$); 3617 br (O–H), 2931 s (C–H), 1649 w (C=C), 1634 w (C=C), 1458 s (C–H), 1400 s (pyrazine), 1242 s (C–O).

FAB-MS: (NBA-Matrix), *m/z* (%), 847 (MH^+, 100).

^{1}H NMR: (400 MHz, $CDCl_3$); δ = 5.54 (br s, 1H, 15′-H), 5.4 (br s, 1H, 15-H) 4.88 (dd, $J_{16'-17'}$ = 8.2 Hz, $J_{16'-15'}$ = 1.2 Hz, 1H, 16′-H), 4.71 (br s, 1H, 28b-H), 4.69 (br s, 1H, 28a-H), 4.67 (dd, J_{16-17} = 7.6 Hz, J_{16-15} = 2.1 Hz, 1H, 16-H), 3.40–3.56 (m, 5H, 26a/26b/26′a/26′b/22-H), 3.25 (dd, $J_{12'-11'a}$ = 10.6 Hz, $J_{12'-11'b}$ = 3.6 Hz, 1H, 12′-H), 2.80–3.05 (m, 4H, 1a/1′a/4a/4′a-H), 2.40–2.72 (m, 8H, 1b/1′b/4b/4′b/17/17′/11a/11b-H), 2.26 (m, 1H, 8-H), 1.23 (s, 3H, 18-H), 1.13 (d, J_{21-20} = 6.6 Hz, 3H, 21-H), 1.06 (d, $J_{21'-20'}$ = 6.9 Hz, 3H, 21′-H), 1.04 (s, 3H, 18′-H), 0.92 (d, J_{27-25} = 6.7 Hz, 3H, 27-H), 0.88 (s, 3H, 19′-H), 0.87 (s, 3H, 19-H), 0.81 (d, $J_{27'-25'}$ = 6.3 Hz, 3H, 27′-H).

^{13}C NMR: (100 MHz, $CDCl_3$), 160.35 (C, 14′-C), 157.21 (C, 14-C), 155.80 (C, 12-C), 148.60, 148.59, 148.37, 148.35 (all C, pyrazine–C), 119.77 (CH, 15′-C), 117.44 (CH, 15-C), 106.75 (CH_2, 28-C), 106.31 (C, 22′-C), 89.15 (CH, 22-C), 85.87 (CH, 16′-C), 84.55 (CH, 16-C), 79.01 (CH, 12′-C), 68.10 (CH_2, 26-C), 67.18 (CH_2, 26′-C), 58.49 (CH, 17-C), 56.08 (CH, 17′-C), 53.93 (C, 13-C), 52.72 (C, 13′-C), 52.14 (CH), 45.64, 45.55 (both CH_2, 1/1′-C), 44.45 (CH), 41.49 (CH), 41.20 (CH), 40.11 (CH), 36.04, 35.95 (both C, 10/10′-C), 35.83 (2× CH), 35.22, 35.14 (both CH_2, 4/4′-C), 34.87 (CH), 33.72 (CH), 32.35 (CH_2), 31.17 (CH_2), 30.83 (CH_2), 30.39 (CH), 30.26 (CH_2), 29.93 (CH_2), 29.44 (CH_2), 29.17 (CH_2), 28.75 (CH_2), 28.06 (CH_2), 27.85 (CH_2), 25.42 (CH_3, 18-C), 17.82 (CH_3, 21-C), 17.16 (CH_3, 27′-C), 16.64 (CH_3, 27-C), 13.87 (CH_3, 21′-C), 13.36 (CH_3, 18′-C), 11.83 (CH_3, 19′-C), 11.68 (CH_3, 19-C).

3.1.4. Preparation of hydroxy- ketal 16. To a solution of 12β-hydroxyketone **15** (0.339 g, 0.400 mmol) dissolved in 4 ml of DCM, 0.047 g of *p*-toluenesulfonylchloride monohydrate (cat.) and 2 ml of ethylene glycol were added, and the reaction mixture was stirred at rt overnight. The ethylene glycol/water phase was removed and the dichloromethane phase was dried over sodium sulfate, filtered, and concentrated to about 4 ml. To this solution, 0.08 ml of ethylene glycol and 0.047 g of *p*-toluenesulfonylchloride were added and the solution was stirred for 2 h. This process was repeated twice. The collected organic phase was dried over $MgSO_4$ and after removal of the solvent, the residue was chromatographed on silica-gel (elution system PE/EA, 1:1) to give 145 mg of the hydroxy-ketal derivative **16** (41% yield) and 116 mg of recovered starting material.

IR: ($CHCl_3/\nu_{max}/cm^{-1}$); 3681 br (O–H), 2931 s (C–H), 1650 w (C=C), 1602 w (C=C), 1458 m (C–H), 1400 (pyrazine), 1241 s (C–O).

FAB-MS: (NBA-Matrix) *m/z* (%); 891 (MH^+, 100).

^{1}H NMR: (400 MHz, $CDCl_3$); δ = 5.49 (br s, 1H, 15-H), 5.45 (br s, 1H, 15′-H), 4.88 (br d, J_{15-17} = $J_{15'-17'}$ = 8.2 Hz, 2H, 16/16′-H), 3.94–4.13 (m, 4H, 28/29-H), 3.40–3.55 (m, 4H, 26a/26b/26′a/26′b-H), 3.25 (m, 1H, 12′-H), 2.76–2.95 (m, 4H, 1a/1′a/4a/4′a-H), 2.48–2.68 (m, 6H, 1b/1′b/4b/4′b/17/17′-H), 1.21 (s, 3H, 18-H), 1.06 (d, $J_{21'-20'}$ = 6.8 Hz, 3H, 21′-H), 1.03 (s, 3H, 18′-H), 1.02 (d, J_{21-20} = 8.2 Hz, 21-H), 0.86 (s, 6H, 19/19′-H), 0.81 (d, J_{27-25} = $J_{27'-25'}$ = 6.3 Hz, 6H, 27/27′-H).

3.1.5. Preparation of ketal triol 17. To a cold solution (0 °C) of catechol (0.190 g, 1.727 mmol in 3 ml abs THF), 1.73 ml of BH_3·THF complex (1 M solution in THF) was added very slowly under argon atm and the solution was stirred until evolution of gas ceased (≈30 min). A solution of **16** (0.095 g, 0.107 mmol in 5 ml abs THF) was dropwise added to the catechol complex solution, which became light yellow, and stirred for 30 min at 0 °C. The reaction was conducted in refrigerator (+5 °C to +7 °C) for one week and its progress was followed by TLC. The reaction was quenched by careful addition of 2 ml of 10% KOH solution and then 2 ml of each H_2O, DEE, and EtOH was added. After stirring the solution for 20 min at rt, 3 ml of H_2O_2 (35% solution) was added and the reaction mixture was vigorously stirred for a further 4 h. The light pink solution was partitioned twice between chloroform and 5% solution of KOH (to remove excess catechol) and washed further with brine solution. The organic phase was dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The resulting material was dried under high vacuum and subjected to column chromatography (silica-gel, 1–2% MeOH:$CHCl_3$ elution) which then yielded 37 mg of double-opened spiro (ketal triol) **17** and 18 mg of mono-opened spiro (ketal diol) **18** (58% overall yield).

IR: ($CHCl_3/\nu_{max}/cm^{-1}$); 3682 br (O–H), 3619 br (O–H), 3417 br (O–H), 2931 s (C–H), 1649 w (C=C), 1602 w (C=C), 1400 s (pyrazine), 1230 s (C–O).

UV: $CHCl_3/\lambda_{max}$ (nm); 313 (sh), 291 (s).

FAB-MS: (NBA-Matrix) *m/z* (%); 896 (M^+, 100).

^{1}H NMR: (400 MHz, $CDCl_3$); δ = 5.48 (br s, 1H, 15-H), 5.45 (br s, 1H, 15′-H), 4.80 (m, 2H, 16/16′-H), 3.95–4.05 (m, 4H, 28a/28b/29a/29b-H), 3.42–3.53 (m, 4H, 26a/26b/26′a/26′b-H), 3.33 (m, 2H, 22/22′-H), 3.25 (br d, $J_{12'-11'a}$ = 9.9 Hz, 1H, 12′-H), 2.82–3.03 (m, 4H, 1a/1′a/4a/4′a-H), 2.51–2.69 (m, 4H, 1b/1′b/4b/4′b-H), 2.47 (tr, $J_{17-16/20}$ = 8.5 Hz, 1H, 17-H), 2.32 (tr, $J_{17'-16'/20'}$ = 8.0 Hz, 1H, 17′-H), 1.22 (s, 3H, 18-H), 1.16 (m, 1H, 9-H), 1.06 (d, $J_{21'-20'}$ = 6.6 Hz, 3H, 21′-H), 1.04 (s, 3H, 18′-H), 1.01 (d, J_{21-20} = 6.5 Hz, 21-H), 0.98 (m, 1H, 9′-H), 0.92 (d, J_{27-25} = $J_{27'-25'}$ = 6.6 Hz, 6H, 27/27′-H), 0.87 (s, 3H, 19′-H), 0.86 (s, 3H, 19-H).

^{13}C NMR: (100 MHz, $CDCl_3$); δ = 157.63 (C, 14′-C), 156.08 (C, 14-C), 148.39, 148.60, 148.47, 148.46 (all C, pyrazine–C), 120.42 (CH, 15-C), 119.82 (CH, 15′-C), 113.41 (C, 12-C), 87.17, 87.11 (both CH, 22/22′-C), 86.51 (CH, 16′-C), 86.01 (CH, 16-C), 79.82 (CH, 12′-C), 68.09 (2× CH_2, 26/26′-C), 65.18, 65.02 (both CH_2, 28/29-C), 59.37 (CH, 17′-C), 55.51 (C, 13-C), 53.53 (CH, 17-C), 52.93 (C, 13′-C), 51.94 (CH, 9′-C), 50.48 (CH, 9-C), 45.65 (2× CH_2, 1/1′-C), 41.45 (CH), 41.32 (CH), 41.24 (CH), 41.20 (CH), 35.98, 35.66 (both C, 10/10′-C), 35.85 (2× CH), 35.27, 35.13 (both CH_2, 4/4′-C), 33.81 (CH), 33.72 (CH), 30.41 (2× CH_2), 30.19 (CH_2), 30.13 (CH_2), 30.11 (CH_2), 29.77 (CH_2), 29.20 (CH_2), 29.04 (CH_2), 28.04 (CH_2), 27.94 (CH_2), 18.98 (CH_3, 18-C), 16.88 (CH_3), 16.66 (3× CH_3), 13.98 (CH_3, 21-C), 11.86 (2× CH_3, 19/19′-C).

3.1.6. Ketal diol 18. *IR:* ($CHCl_3$/ν_{max}/cm^{-1}): 3689 br (O–H), 3616 br (O–H), 2931 s (C–H), 1649 w (C=C), 1601 w (C=C), 1459 s (C–H), 1400 s (pyrazine), 1241 s (C–O).

FAB-MS: (NBA-Matrix) *m/z* (%); 894 (M^+, 73).

HR-FAB-MS: M = calculated 893.6044, found 893.5960.

^{1}H NMR: (400 MHz, $CDCl_3$); δ = 5.48 (br s, 1H, 15-H), 5.45 (br s, 1H, 15′-H), 4.88 (dd, 1H, $J_{16'-17'}$ = 6.7 Hz, $J_{16'-15'}$ = 1.4 Hz, 16′-H), 4.80 (dd, J_{16-17} = 7.3 Hz, J_{16-15} = 1.0 Hz, 1H, 16-H), 4.95–4.05 (m, 4H, 28a/28b/29a/29b-H), 3.41–3.56 (m, 4H, 26a/26b/26′a/26′b-H), 3.33 (m, 1H, 22-H), 2.25 (dd, $J_{12'-11'a}$ = 11.0 Hz, $J_{12'-11'b}$ = 4.4 Hz, 1H, 12′-H), 2.75–2.95 (m, 4H, 1a/1′a/4a/4′a-H), 2.40–2.66 (m, 6H, 1b/1′b/4b/4′b/17/17′-H), 1.22 (s, 3H, 18-H), 1.15 (m, 1H, 9-H), 1.06 (d, $J_{21'-20'}$ = 6.8 Hz, 3H, 21′-H), 1.03 (s, 3H, 18′-H), 1.01 (d, J_{21-20} = 6.4 Hz, 3H, 21-H), 0.95 (m, 1H, 9′-H), 0.92 (d, J_{27-25} = 6.8 Hz, 3H, 27-H), 0.87 (s, 3H, 19′-H), 0.86 (s, 3H, 19-H), 0.81 (d, $J_{27'-25'}$ = 6.3 Hz, 3H, 27′-H).

^{13}C NMR: (100 MHz, $CDCl_3$); δ = 157.15 (C, 14′-C), 156.07 (C, 14-C), 148.0 (4× C, pyrazine–C), 120.44 (CH, 15-C), 119.80 (CH, 15′-C), 113.41 (C, 12-C), 106.75 (C, 22-C), 87.12 (CH, 22-C), 86.52 (CH, 16-C), 84.54 (CH, 16′-C), 79.05 (CH, 12′-C), 68.17 (CH_2, 26-C), 67.19 (CH_2, 26′-C), 65.19, 65.03 (both CH_2, 28/29-C), 56.09 (CH, 17′-C), 55.56 (C), 53.56 (CH, 17-C), 52.72 (C), 52.13 (CH, 9′-C), 50.48 (CH, 9-C), 45.55, 45.53 (both CH_2, 1/1′-C), 44.45 (CH), 41.47 (CH), 41.30 (CH), 41.22 (CH), 36.03, 35.66 (both C, 10/10′-C), 35.87 (CH), 35.12, 35.10 (both CH_2, 4/4′-C), 33.72 (2× CH), 31.18 (CH_2), 30.45 (CH_2), 30.39 (CH), 30.14 (CH_2), 29.89 (CH_2), 29.77 (CH_2), 29.15 (CH_2), 29.03 (CH_2), 28.76 (CH_2), 28.06 (CH_2), 27.93 (CH_2), 18.98 (CH_3, 18-C), 17.16 (CH_3, 27′-C), 16.66 (2× CH_3, 27/18′-C), 13.87 (CH_3, 21-C), 13.36 (CH_3, 21′-C), 11.87, 11.84 (both CH_3, 19/19′-C).

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References and notes

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