Natural Products

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# A Synthesis-Driven Structure Revision of Berkelic Acid Methyl Ester**

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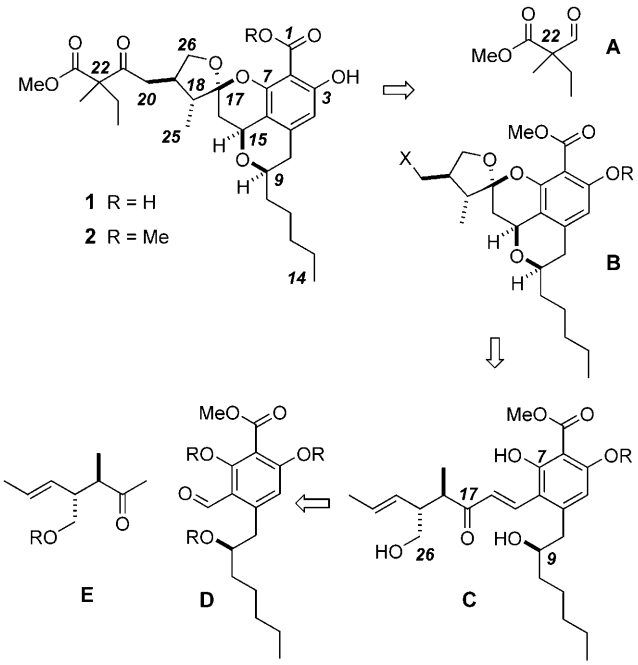
Matrix metalloproteinases (MMPs) are a family of zinc-containing endopeptidases involved in homeostasis of the extracellular matrix.[1] Abnormal activity of such enzymes is implicated in pathological processes that result in osteoarthritis, rheumathoid arthritis, and multiple sclerosis, and also plays a decisive role in tumor metastasis. Small-molecule inhibitors of the individual MMPs are therefore highly interesting as prospective complements to the current chemotherapeutic regimens used in clinical settings.[2]

A promising lead in this context is berkelic acid, which was isolated from a *Penicillium* species collected in the very hostile environment of Berkeley Pit Lake, a flooded former copper mine in Butte, Montana.[3] This particular extremophile has adapted to the waters of this lake, which are highly acidic (pH ≈ 2.5) and contain a cocktail of heavy-metal salts in remarkably high concentrations. Bioassay-guided fractionation of the $CHCl_3$ extracts of the fungus showed berkelic acid to be the major metabolite responsible for the pronounced inhibition of MMP-3 ($GI_{50} = 1.87$ μM).[3] Moreover, the compound exhibits selective and potent activity against the ovarian cancer cell line OVCAR-3 ($GI_{50} = 91$ nM).[3] As MMP-3 is upregulated in OVCAR-3 but not in other ovarian cancer cell lines, these preliminary activity and selectivity data are highly encouraging and suggest that berkelic acid and derivatives thereof deserve more intense scrutiny.[3,4]

The remarkable structural attributes of berkelic acid add further to the appeal of this compound. It was assigned the constitution and relative configuration **1** (Scheme 1) mainly on the basis of NMR experiments.[3] Recent model studies directed towards synthesizing berkelic acid appear to corroborate this proposed structure, even though they reached contradictory conclusions as to whether the acetalization that produces the conspicuous chromane spiroketal core is thermodynamically or kinetically controlled.[5–7] We now report our own investigations on berkelic acid methyl ester (**2**) which not only resolve this open question but also suggest that the original structure assignment needs to be revised.




***Scheme 1.*** Retrosynthetic analysis of the structure **1** attributed to berkelic acid.

Since the configuration at the lateral quaternary stereocenter C22 of berkelic acid is unknown, a convergent approach was adopted that should allow both possible isomers to be prepared by incorporating either enantiomer of synthon **A** at a late stage (Scheme 1). This route utilizes a Michael addition/spiroacetalization cascade intended to convert a linear precursor of type **C** into the tetracyclic core **B** of the target in one step. Compound **C**, in turn, should arise from an aldol condensation between the aromatic nucleus **D** and the polyketide segment **E**.

The preparation of the required building block **D** (Scheme 2) commenced with the copper-catalyzed opening of (*R*)-(+)-2-pentyloxirane (> 99% *ee*)[8] by the Grignard reagent derived from 3,5-bis(benzyloxy)-1-bromobenzene[9] to give **3**. Hydrogenolysis of the benzyl ethers followed by a regioselective Kolbe–Schmitt carboxylation of the resulting phenol **4** furnished acid **5**,[9] which was esterified by treatment with diazomethane prior to conversion into bis-TBS ether **8** by exhaustive silylation and selective mono-desilylation. Since the attempted direct formylation of this product was unrewarding,[10] we chose to introduce the required formyl

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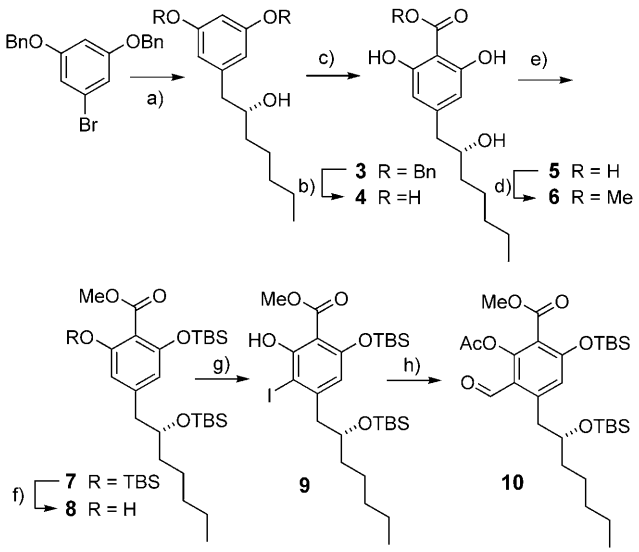
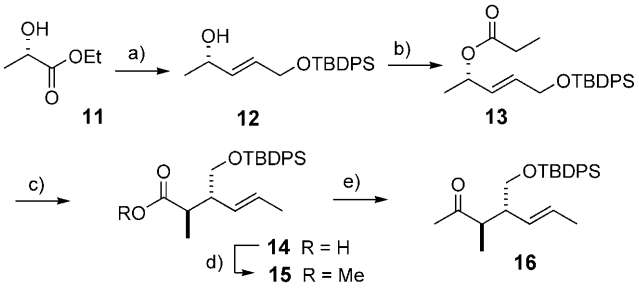



***Scheme 2.*** Reagents and conditions: a) 1. Mg, THF, reflux; 2. [CuCl(cod)] (10 mol%), (*R*)-2-pentyloxirane, −50 °C→RT, 74%; b) $H_2$, Pd/C (10% w/w), MeOH, quant.; c) $CO_2$ (1 atm), $KHCO_3$, glycerine, 150 °C; d) $TMSCHN_2$, MeOH, 66–77% (over both steps); e) TBSCl, imidazole, $CH_2Cl_2$, 96%; f) $K_2CO_3$, MeOH, 45 °C, 72%; g) *N*-iodosuccinimide, $CH_2Cl_2$, 98%; h) 1. MeLi, $Et_2O$, −78 °C; 2. *t*BuLi, −105 °C; 3. DMF, −105→−35 °C; 4. AcCl, −55→−25 °C, 71%. Bn = benzyl, cod = 1,5-cyclooctadiene, TMS = trimethylsilyl, TBS = *tert*-butyldimethylsilyl.

group by a high-yielding sequence of regioselective iodination (**8**→**9**), metal–halogen exchange, and trapping of the resulting organolithium reagent with DMF. The fact that the unprotected phenolate site in the resulting primary product could be acylated in situ to give acetate **10** turned out to be highly advantageous for the ensuing fragment coupling (see below).

An Ireland–Claisen rearrangement[11] of ester **13**, resulting from the lactate-derived alcohol **12**[12] and propionyl chloride, furnished the second building block (Scheme 3). The reaction was best performed with KHMDS in toluene,[13] and delivered the required *anti*-configured ester **15** in good yield and high diastereoselectivity (d.r. = 10.2:1, 91% *ee*).[14] After transformation into the corresponding Weinreb amide,[15] the




***Scheme 3.*** Reagents and conditions: a) Ref. [12]; b) propionyl chloride, pyridine, $CH_2Cl_2$, −20 °C, 98% (98% *ee*); c) KHMDS (1.5 equiv), TMSCl (2 equiv), toluene, −78 °C→RT; d) $TMSCHN_2$, MeOH, 77% (over both steps, *anti*/*syn* = 10.2:1, 91% *ee*); e) 1. Me(MeO)NH·HCl, *i*PrMgCl, THF, −18 °C, 78%; 2. MeMgBr, −18→0 °C, 93%. KHMDS = potassium hexamethyldisilazide.

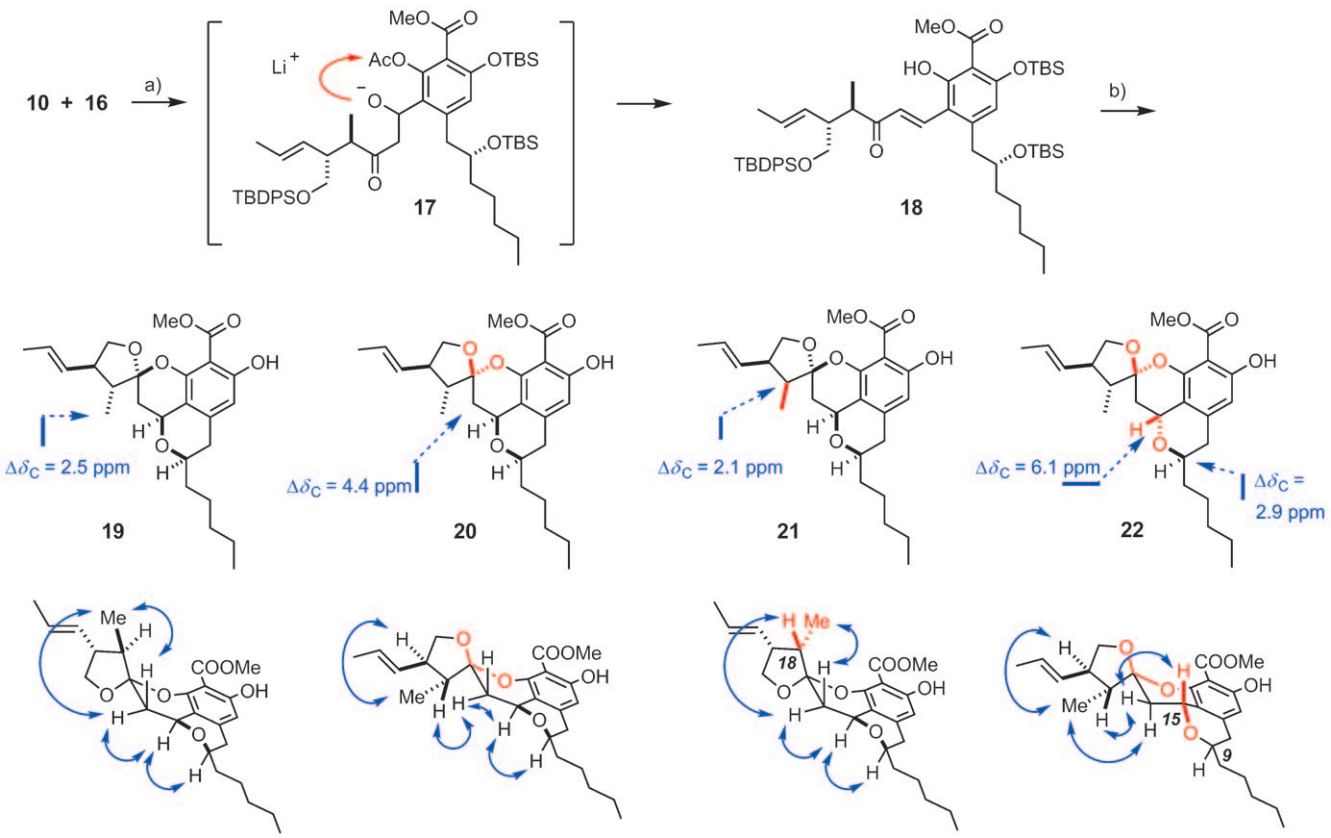
minor *syn* isomer was removed by flash chromatography before the pure *anti*-configured compound was converted into methyl ketone **16** by treatment with MeMgBr at low temperature.

The kinetic enolate of **16** was treated with aldehyde **10** to give enone **18** (Scheme 4). This transformation exploits the propensity of the acetyl group in intermediate **17** to migrate from the phenolic site to the more basic alkoxide generated in the aldol step. This transfer facilitates the subsequent elimination by turning this site into a good leaving group and deprotects the phenolic hydroxy group that is needed to participate in the subsequent Michael addition/spirocyclization cascade. Much to our surprise, however, treatment of **18** with HCl in MeOH did not deliver a single (or at least a major) spiroketal product; rather, an almost statistical mixture of four isomeric compounds (**19**–**22**, d.r. = 0.9:1:0.8:1.3) was formed, which could be separated by preparative HPLC. In contrast to the results of a previous yet simpler model study,[5] attempts to equilibrate the crude mixture by exposure to different acids were in vain. Likewise, treatment of any of the individual isomers with pyridinium *p*-toluenesulfonate (PPTS) rapidly regenerated the original product distribution.

Extensive NMR investigations allowed the stereostructure of each isomer to be established beyond doubt (for details see the Supporting Information). The assignment was independently confirmed when crystals of **19** were grown that were suitable for X-ray structure analysis (Figure 1).[16] Our fully consistent NMR data set also allowed for a highly informative comparison with the reported spectroscopic properties of berkelic acid,[3] even though **19**–**22** differ from the natural product in the lateral chain and by the presence of a methyl ester moiety. While an in-depth discussion must await a future full paper, a few comments are necessary. First, compound **19** — which features the structure attributed to berkelic acid — is the only isomer in which the $^{13}C$ NMR signal of the methyl branch C25 ($\delta = 14.38$ ppm) deviates considerably from the chemical shift of this group in the natural product ($\delta = 11.9$ ppm; Scheme 4). NOESY data as well as the solid-state structure of this compound (Figure 1) show an unfavorable *syn*-periplanar arrangement between the methyl substituent and the C16 methylene group of the adjacent tetrahydropyran ring (torsion angle C25-C18-C17-C16: 13°). All other isomers avoid such an eclipsed situation by orienting the methyl group away from the benzopyran unit. This is possible by either adopting the opposite configuration at the spiroacetal C17 (**20** and **22**) or, alternatively, by an isomerization of the stereocenter C18 which carries the methyl branch during the acetalization process (**21**). Collectively, these structural data suggest that the positioning of the methyl group has a strong impact on the stability of the core.[17] Moreover, the recorded $^{13}C$ NMR spectra show that the signal for at least one C atom in each of the isomers deviates significantly from the corresponding signal of berkelic acid (see Scheme 4 and the Supporting Information).

Further important information can be deduced from NOE data. Compound **22** is the only isomer in which H9 and H15 are *trans*-configured, and therefore cannot correspond to the natural product. Moreover, Stierle et al. reported that "irra-




***Scheme 4.*** Reagents and conditions: a) LDA, THF, −78 °C, then **10**, −78 °C→RT, 85 %; b) acetyl chloride, MeOH/$CH_2Cl_2$, 0 °C→RT, 91 % (**19**/**20**/**21**/**22** = 0.9:1:0.8:1.3). LDA = lithium diisopropylamide. The $\Delta\delta_C$ values refer to the differences in the chemical shifts between the indicated C atoms in the individual isomers and the corresponding resonances in berkelic acid (for the full data set, see the Supporting Information). The blue arrows indicate strong and characteristic NOE interactions.

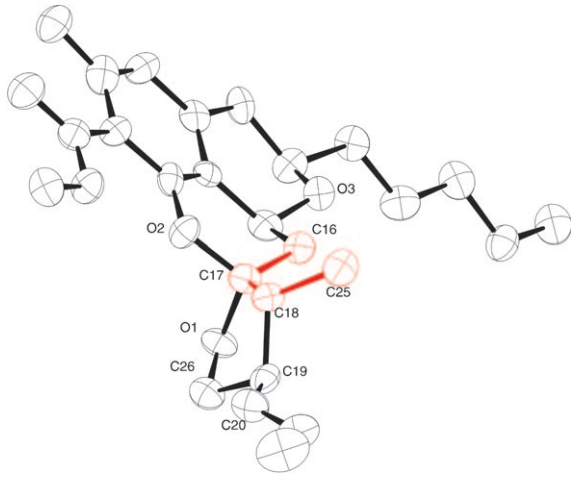
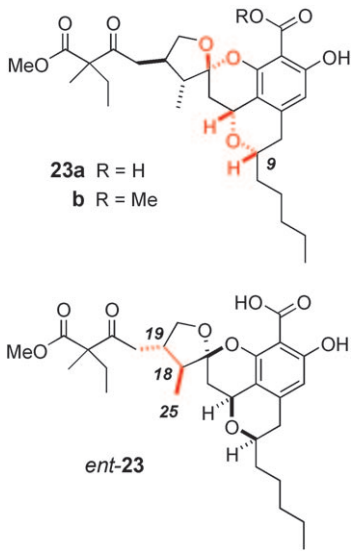



***Figure 1.*** Structure of **19** in the solid state (for additional information, see the Supporting Information).[16]

diation of methyl H-25 resulted in enhancement of H-16α and H-20".[3] However, when this experiment was repeated with **19**, the isomer corresponding to the supposed structure, unmistakable enhancements of not only H16α but also H16β signals were observed. Together with the analysis of the $^{13}C$ NMR data outlined above, this inconsistent pattern makes it clear that not only is the originally proposed stereostructure incorrect, but that none of the isomers produced in the acid-catalyzed cyclization of precursor **18** matches the core of the natural product (Scheme 4).

The conclusions drawn from this analysis let us envisage that structure **23** or its mirror image *ent*-**23** represents berkelic acid (Scheme 5). Inspection of a molecular model shows that the C25 methyl branch in these compounds is oriented away from the crowded core, and the NOE interactions are expected to be consistent with the reported ones. Spurred on by the prospect of finally solving this puzzle, we opted for the preparation of enantiomer **23** because its synthesis only requires readily accessible *ent*-**3**, which can then be elaborated into the cyclization precursor **24** by following the established route (Scheme 6). It was gratifying to see that **24** indeed cleanly converted into one major product on exposure to HCl/MeOH, whereas its 9-*epi*-analogue **18** had given an almost statistical mixture of four isomers under the same conditions. As expected, the NMR spectra of spiroketal **25** showed that this compound features the acetal configuration which allows the C25 methyl branch to reside in the unencumbered periphery; importantly, all NOE interactions are in accord with the characteristic pattern of berkelic acid. The structure assignment was corroborated by the X-ray analysis of iodide **27** (Figure 2) derived from **25**, which was prepared for the final fragment coupling.




***Scheme 5.*** Proposed structure revision for berkelic acid: depending on whether **23** or its enantiomer *ent*-**23** turns out to be the natural product, the differences relative to the original structure either originate from a misassignment of the configuration at C9 (which then formally propagates through the structure by the observed NOE interactions), or are caused by a misassignment of C18 and C19. The resulting deviations from the previously proposed structure **1** are highlighted in red.

To this end, iodide **27** was subjected to a metal–halogen exchange at low temperature, followed by addition of the resulting polyfunctional lithium species to aldehyde (*S*)-**28**; both enantiomers of this coupling partner can be obtained in high purity from malic acid by following a literature procedure (Scheme 6).[18] Oxidation of the resulting diastereomeric alcohols **29** furnished (22*S*)-**23b**. Even though this final oxidation turned out to be difficult and was plagued by formation of the corresponding methylthiomethyl adduct **30** (Scheme 7),[19] the final product could be obtained in sufficient quantity to allow for an unambiguous analysis. The match between the recorded data and the reported spectrum of berkelic acid methyl ester in $CDCl_3$ is excellent, and leaves no room for interpretation (see the Supporting Information). Therefore, we confidently reassign the relative configuration of this promising bioactive metabolite to that of **23** (or *ent*-**23**).[20,21]

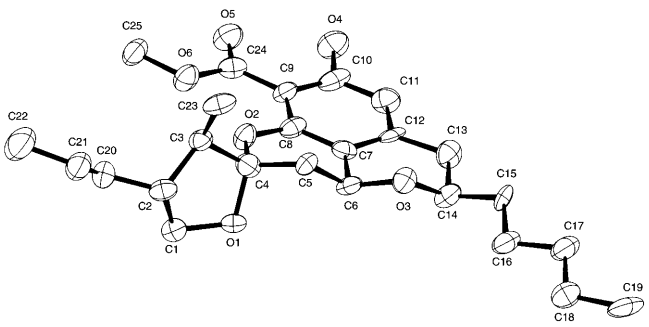
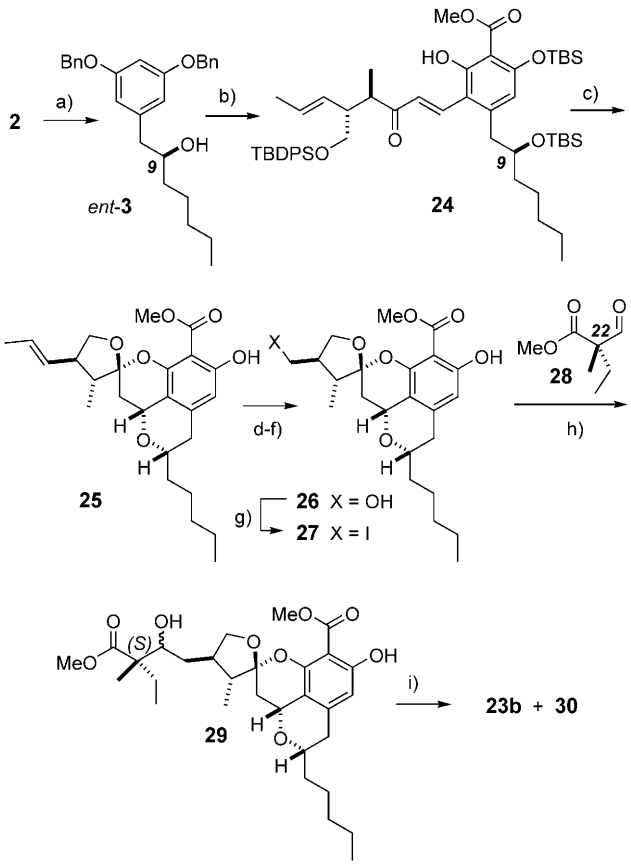
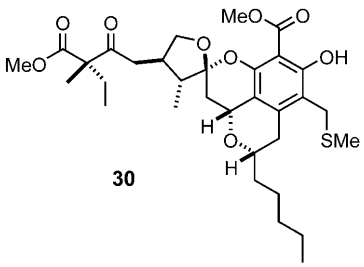


***Figure 2.*** Structure of **27** in the solid state.[16]




***Scheme 6.*** Reagents and conditions: a) 1. Mg, THF, reflux; 2. [CuCl(cod)] (10 mol%), (*S*)-(−)-2-pentyloxirane, −50°C, 74%; b) see Scheme 2 and step (a) in Scheme 4; c) acetyl chloride, MeOH/$CH_2Cl_2$, 0°C→RT, 94% (d.r. ≥ 12.5:1); d) $OsO_4$ (2 mol%), *N*-methylmorpholine-*N*-oxide, acetone; e) $Pb(OAc)_4$, $CH_2Cl_2$; f) $NaBH_4$, MeOH, 0°C, 59% (over three steps); g) $I_2$, $PPh_3$, imidazole, $Et_2O$/MeCN, 85%; h) 1. MeLi, $Et_2O$, −105°C; 2. *t*BuLi, then **28**; i) $(COCl)_2$, DMSO, $CH_2Cl_2$, then $Et_3N$, −78→0°C, 69% (**23b**/**30** = 1:1).




***Scheme 7.*** Structure of the by-product formed in the final Swern oxidation reaction.

The 22*R*-configured product was prepared analogously by using (*R*)-**28** as the reaction partner. The $^1H$ NMR spectrum of (22*R*)-**23b** in $CD_3OD$ recorded at 600 MHz is subtly different from that of (22*S*)-**23b**, but a confident assignment of the configuration of the lateral quaternary center at C22 mandates direct comparison with an authentic sample.[22] We are now in the process of refining the first generation total

synthesis outlined above and adjusting the protecting groups used such that free berkelic acid itself can also be reached.[23]

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[22] Only the $^1$H NMR spectrum in $CDCl_3$ has been recorded; we were informed that there is very little of the authentic sample left; supplying material is therefore currently not possible.
[23] Saponification of **23b** is unselective, with both methyl esters cleaved at similar rates.

  