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A Concise Synthesis of Berkelic Acid Inspired by Combining the Natural Products Spicifernin and Pulvilloric Acid

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Berkeley Pit Lake in Montana, a 30 billion gallon flooded copper mine and the largest superfund cleanup site in the United States, is an unlikely source of structurally novel natural products. Yet, this highly acidic, heavy-metal contaminated poisonous broth harbors microbial life, including an extremophilic *Penicilium* fungus that produces the unique tetracyclic chroman/isochroman spiroketal berkelic acid (Figure 1). This compound, isolated by Stierle et al. in 2006, was found to possess selective activity against the human ovarian cancer cell line OVCAR-3 (GI_{50} 91 nM) and moderate inhibitory activity against the matrix metalloproteinase MMP-3 (1.87 μM) and the cysteine protease caspase-1 (98 μM).[1]

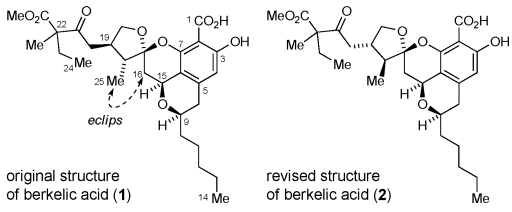
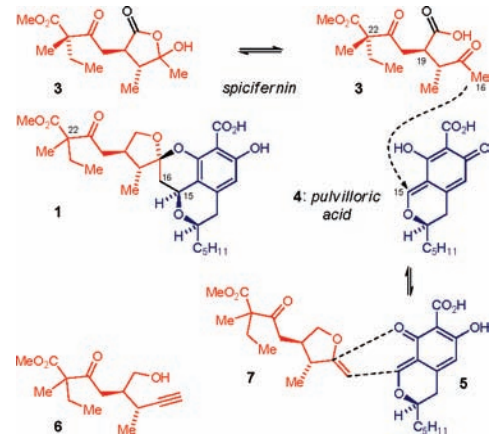


Figure 1. Original and revised structures of berkelic acid.

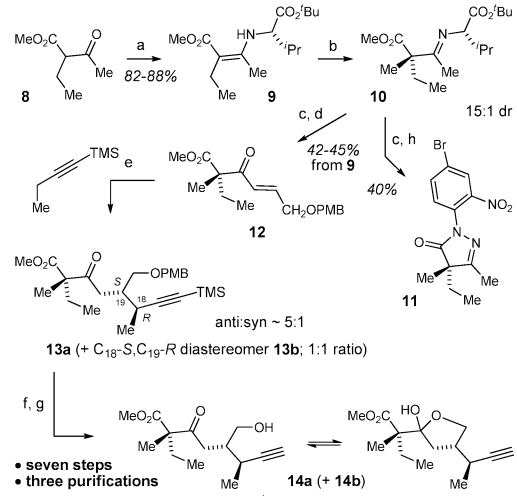
The stereochemistry of berkelic acid was originally assigned as shown in structure **1** on the basis of NMR experiments, although the configuration at the quaternary stereocenter C22 and absolute configuration were left undetermined. Recently, the Fürstner group reported studies leading to a revision of the relative stereochemistry of berkelic acid as shown in structure **2** through total synthesis of the corresponding methyl ester.[2] Through elegant synthetic, NMR, and crystallographic studies, they further revealed that the originally proposed relative stereochemistry does not represent a thermodynamic minimum because of a key *syn*-periplanar interaction between the C25 methyl substituent and C16 methylene group.[3] Subsequently, Snider and co-workers reported their total synthesis of berkelic acid, which established its absolute configuration as shown in **2**, and putatively assigned the stereochemistry at the quaternary center as C22-*S*.[4,5] Herein, we wish to report a concise synthesis of the two C22 epimers of berkelic acid (**2**) that fully corroborates the revised stereochemistry and unambiguously resolves the remaining issue of C22 stereochemistry.

Our approach was inspired by the recognition that the original assigned berkelic acid structure **1** represents a formal combination of the natural products spicifernin[6] (**3**) and pulvilloric acid[7] (**4**, Scheme 1).[8] Based on this notion, we developed a strategy that would emulate this hypothetical combination and designed a suitable spicifernin-like synthon such as enolether **7**,[9] available via metal-catalyzed cycloisomerization.[10] Participation of this material in a [4+2] cycloaddition with the *ortho*-quinone methide tautomer **5** of pulvilloric acid (**4**) would deliver spiroketal **1**. It did not escape our attention that this chemistry could potentially be implemented with minimal oxidation state adjustments.[11]

Scheme 1. Synthetic Strategy



Scheme 2. Synthesis of Alkyne Fragment[a]



[a] Reagents and conditions: (a) *L*-^{t}Bu-Val-NH_2, $BF_3 \cdot Et_2O$, PhH, reflux (82−88%); (b) LDA, PhMe, −78 °C, 1 h, THF (2.5 equiv), −78 °C, 3 h, MeI, −78 °C, 17 h; (c) 1 M aq. HCl/THF (1:1), rt, 1 h; (d) $TiCl_4$, THF, 4 Å MS, 0 °C, 30 min, NEt_3, −78 °C, 1 h, $PMBOCH_2CHO$, −78 °C, 1.5 h, rt 1.5 h (42−45%, 3 steps); (e) 1-trimethylsilyl-1-butyne, ^{s}BuLi, THF, −78 °C, 2 h, $CuBr \cdot SMe_2$, −78 °C, 1 h, then add **12**, −78 °C, 24 h; (f) K_2CO_3, MeOH, rt, 2 h; (g) DDQ, CH_2Cl_2/H_2O (7:1), rt (70%, 3 steps); (h) 4-Br-2-$NO_2PhNHNH_2 \cdot$HCl, EtOH, reflux, 2 d (40%, 2 steps).

Given the ambiguity related to the absolute stereochemistry at C22, we opted for a synthesis that would enable access to the two C22 epimers of fragment **6** (Scheme 2). Starting with commercially available methyl 2-ethyl-3-oxobutanoate (**8**), the corresponding (L)-^{t}Bu valinate-derived enamine **9** was prepared (82−88% yield) and alkylated with methyl iodide to afford the α-quaternary substituted imine derivative **10** with high stereoselectivity (>15:1 dr).[12] The

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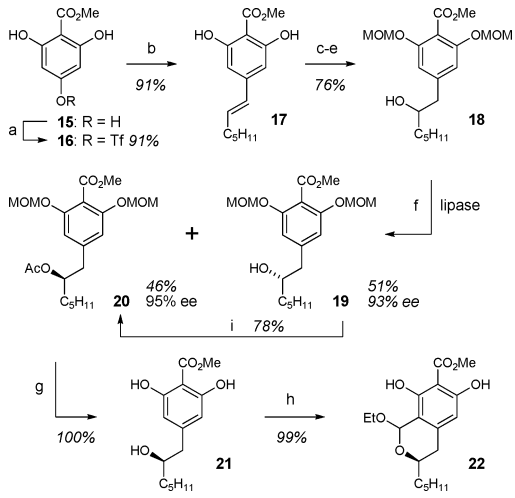
Scheme 3. Synthesis of Aromatic Fragment[a]

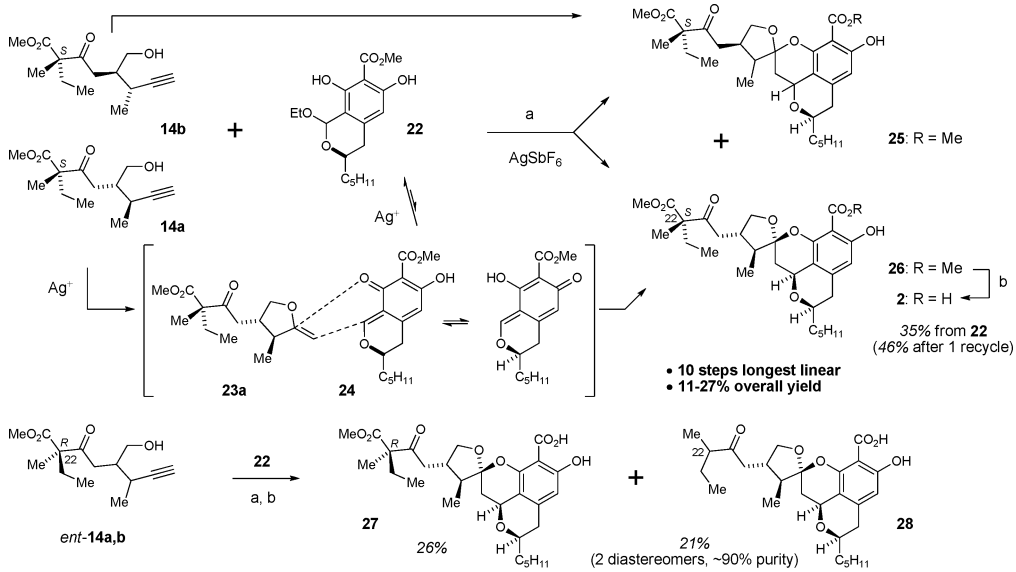


[a] Reagents and conditions: (a) Tf_2O, lutidine, CH_2Cl_2, 0 °C, 16 h (91%); (b) $^nC_5H_{11}CHCHB(OH)_2$, 5% Pd(dppf)Cl_2, K_2CO_3, THF/H_2O (10:1), Δ, 2.5 h (91%); (c) MOMCl, $^i Pr_2NEt$, CH_2Cl_2, 0 °C → rt, 18 h; (d) *m*CPBA, CH_2Cl_2, rt, 5 h (84%, 2 steps); (e) Pd/$CaCO_3$, H_2, MeOH, rt, 20 h (90%); (f) Lipase (*Alcaligenes* sp., lyophilized), MTBE, 4 ÅMS, vinyl acetate, rt, 7 d; (g) 0.25 M HCl in MeOH, rt, 15 h (100%); (h) $(EtO)_3CH$, TFA, rt, 15 h (99%); (i) PPh_3, DEAD, HOAc, PhMe, rt, 7 h (78%).

absolute stereochemistry at C22 was determined by a single crystal X-ray diffraction analysis of the cyclic 4-bromo-2-nitrophenylhydrazone derivative **11**.[13] Continuing with the synthesis, hydrolysis of crude imine **10** was followed by a titanium tetrachloride-mediated dehydrative aldol reaction with (4-methoxybenzyloxy)ethanal yielding enone **12** in 42−45% yield (3 steps) from enamine **9**. We explored various options to introduce the α-methyl-substituted propargyl unit and settled on an approach that entails a conjugate addition of a metalated propargyl/allenyl species to enone **12**. Although the stereoselective propargylation of aldehydes is well precedented, we could find only one example of the corresponding conjugate addition in the literature.[15] After substantial experimentation, we found that addition of enone **12** to a cold (−78 °C) dark red solution of a cuprate derived from adding (4-(trimethylsilyl)but-3-yn-2-yl)lithium to a suspension of CuBr·SMe_2 in THF (−78 °C) efficiently effected the desired conjugate propargylation.[16] Although the *anti*-selectivity was acceptable, the stereogenic quaternary center did not impart any facial selectivity, leading to an inseparable equimolar mixture of *R*,*S*- and *S*,*R*-diastereomers **13a** and **13b**.[17] As such, this crude mixture was carried forward by treatment with methanolic potassium carbonate, followed by oxidative deprotection to yield compounds **14a,b**. Proton NMR analysis of chromatographically homogeneous material (with correct elemental analysis),[13] isolated in 70% yield from enone **12**, indicated a complex mixture of equilibrating lactols and open-chain isomers. The corresponding mixture of enantiomers *ent*-**14a,b** was prepared from the (D)-^{t}Bu valinate-derived enamine *ent*-**9**, or cheaper, by switching the additive from THF to HMPA during the alkylation of (L)-^{t}Bu valinate-derived enamine **9**.[12]

A concise enantioconvergent synthesis of the precursor to pulvilloric acid **4** begins with a cross-coupling of triflate **16**, obtained from commercially available methyl 2,4,6-trihydroxybenzoate **15** in 91% yield, with 1-heptenylboronic acid to afford styrene derivative **17** (91% yield, Scheme 3). Installation of the homobenzylic alcohol was best achieved via oxidation with *m*CPBA of the MOM protected derivative of **17**, followed by benzylic epoxide reduction. Racemic alcohol **18** was thus obtained in 76% yield for the three-step sequence. Screening of a set of enzymes to mediate a kinetic resolution identified a lyophilized formulation of a lipase from *Alcaligenes* sp. to effect the transesterification (vinyl acetate) with high enantioselectivity at ∼50% conversion.[18] Alcohol **19** and acetate **20** were isolated in 51% and 46% isolated yield and 93% and 95% ee, respectively. Alcohol **19** was easily recycled to the desired acetate **20** via Mitsunobu esterification (78% yield). Simultaneous removal of the protecting groups was achieved *via* stirring in acidic methanol (quant.). Condensation of the resulting triol **21** with triethyl orthoformate according to an adapted procedure described for the synthesis of pulvilloric acid (**4**) yielded isochroman acetal **22** (99% yield), the precursor to pulvilloric acid methyl ester. Although it has been reported that the carboxylic acid corresponding to **22** will yield pulvilloric acid (**4**) upon removal of ethanol under ultrahigh vacuum,[7d] we opted to explore Lewis acid promoted in situ dearomatization of **22** as described below.

Scheme 4. Synthesis of Berkelic Acid (**2**) and C22-*R* Diastereomer **27**[a]



[a] Reagents and conditions: (a) **14a,b** or *ent*-**14a,b** (2.6 equiv), **22** (1 equiv), Et_2O, rt, 2 h; (b) $(Bu_3Sn)_2O$ (35 equiv), PhMe, Δ, 8 h for **2**, 14 h for **27**.

As noted above, we were intrigued by the possibility to effect in situ dearomatization of lactol **22** to pulvilloric acid methyl ester under conditions that would allow tandem C−C bond formation with spicifernin-like fragment **14**. We speculated that Ag^+ would have a proper balance of hard Lewis acidic properties to induce removal of ethanol from **22** and sufficient alkynophilic character to induce cycloisomerization of alkynol **14** to enolether **23** (Scheme 4).[19] Gratifyingly, stirring a solution of lactol **22** (1 equiv) and $AgSbF_6$ (3.5 equiv) in the presence of alkynols **14a,b** (2.6 equiv) resulted in the formation of methyl berkelate **26** (from **14a**) and four additional diastereomeric berkelates **25** (from **14b**)[20] in a ratio of ∼6:4, indicating a slight kinetic preference for the formation of **26**. We hypothesize that $AgSbF_6$ instigated a reaction cascade involving (1) in situ formation of *ortho*-quinone methide **24**,[21] (2) cycloisomerization of **14** to enolether **23**, and (3) coupling *via* [4+2] cycloaddition.[22]

Because the methyl berkelate diastereomers were not separable by chromatography, they were carried forward as a crude mixture. Although Fürstner and co-workers disclosed that they could not identify conditions for the selective deprotection of the methyl benzoate in the presence of the aliphatic methyl ester,[2] we found that $(Bu_3Sn)_2O$ in toluene accomplished the task when the reaction was interrupted at partial conversion.[23] Berkelic acid **2** was thus isolated in 35% isolated yield (from lactol **22**) at 70% conversion and 46% yield after one recycling (77% based on theoretical maximum yield). Prolonged reaction times resulted in the formation of decarboxylated product **28** (∼4:1 mixture of C22 diastereomers). The corresponding C22-*R* diastereomer **27** was prepared via an identical sequence from *ent*-**14a,b** and lactol **22** in 26% yield. Only C22-*S* diastereomer **2** displayed spectral data fully congruent with natural berkelic acid,[1] thus establishing the complete stereostructure of this unique natural product for the first time. The rotation of synthetic **2** ($[\alpha]_D = -76.7$, $c = 0.06$ in MeOH) agreed with those for natural ($[\alpha]_D = -83.5$, $c = 0.0113$ in MeOH)[1] and Snider's synthetic berkelic acid ($[\alpha]_D = -115.5$, $c = 0.55$ in MeOH).[4]

In conclusion, we have achieved a highly convergent and efficient synthesis of berkelic acid that fully establishes the stereochemistry at C22 in 10 steps and 11−27% overall yield from commercially available starting materials. Notably, we identified a unique Ag-catalyzed cascade dearomatization−cycloisomerization−cycloaddition sequence to couple two natural product inspired fragments and a potentially useful *anti*-selective conjugate propargylation reaction.

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Supporting Information Available: Experimental procedures and characterization data for new compounds (PDF, CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) This notion may or may not have biosynthetic relevance, a question that remains to be answered. It is interesting to note that spiciferone A was isolated alongside berkelic acid.[1] Spiciferone A was also isolated together with spicifernin from the phytopathogenic fungus *Cochliobolus spicifer* Nelson,[6] and both were shown to derive from a common hexaketide precursor. Hence, the genetic machinery to produce the common precursor to spiciferone A and spicifernin is also present in the *penicilium* species that produces berkelic acid. For the biosynthesis of spiciferone A and spicifernin, see: Nakajima, H.; Fujimoto, H.; Matsumoto, R.; Hamasaki, T. *J. Org. Chem.* **1993**, *58*, 4526–4528.
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(20) Reaction of **14b** leads to a berkelate with the original assigned stereochemistry, which exists in equilibrium with C15, C17, and C18 epimers.[2]
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