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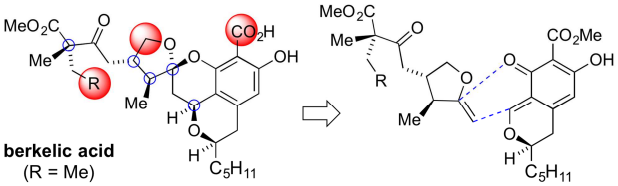

berkelic acid
(R = Me)

berkelic acid analogs (a) and stereoisomers (b)

A biosynthetically inspired synthesis of (–)-berkelic acid and analogs

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Abstract

We describe a complete account of our total synthesis and biological evaluation of (–)-berkelic acid and analogs. We delineate a synthetic strategy inspired by a potentially biomimetic union between the natural products spicifernin and pulvilloric acid. After defining optimal parameters, we executed a one-pot silver-mediated in situ dehydration of an isochroman lactol to methyl pulvillorate, the cycloisomerization of a spicifernin-like alkynol to the corresponding exocyclic enol ether, and a subsequent cycloaddition to deliver the tetracyclic core of berkelic acid. Our studies confirm that the original assigned berkelic acid structure is not stable and equilibrates into a mixture of 4 diastereomers, fully characterized by X-ray crystallography. In addition to berkelic acid, C22-*epi*-berkelic acid, and *nor*-berkelic acids, we synthesized C26-oxoberkelic acid analogs that were evaluated against human cancer cell lines. In contrast to data reported for natural berkelic acid, our synthetic material and analogs were found to be devoid of activity.

1. Introduction

In 2006 Stierle *et al.* disclosed the tetracyclic chroman/isochroman/spiroketal natural product (–)-berkelic acid (Figure 1) isolated from the fermentation broth of an extremophilic *Penicillium* fungus encountered in the Berkeley Pit Lake in Butte, Montana.[1] The Berkeley Pit Lake, which formed by groundwater seepage into an abandoned copper pit mine, is currently the United States' largest superfund cleanup site containing approximately 30 billion gallons of highly acidic (pH 2.5), heavy metal-contaminated (arsenic, copper, cadmium, cobalt, iron, manganese, and zinc) water.[2] Berkelic acid was found to be a moderate inhibitor of MMP-3 (1.87 μM) and the cysteine protease caspase-1 (98 μM), and, in testing against the NCI 60 cell line panel, was reported to possess selective activity against the human ovarian cancer cell line OVCAR-3 (GI_{50} 91 nM). However, a subsequent analysis of fully synthetic (–)-berkelic acid from the Snider group in the NCI 60 cell line panel indicated no activity against any of the cell lines (up to 10 μM) including OVCAR-3.[3]

The initially assigned structure of (–)-berkelic acid (**1**) was determined by NMR experiments, but did not address the configuration of the C22-quaternary stereocenter or the overall absolute configuration. Subsequent work of the Fürstner group led to a revised structure of berkelic acid (**2**) through an elegant synthetic, NMR, and crystallographic study culminating initially in the synthesis of both C22-epimers of the corresponding methyl esters of *ent*-**2**,[4] and subsequently (–)-berkelic acid (**2**).[5] These efforts established the absolute configuration of five stereocenters and a revision of the configuration at C18 and C19 in the original assigned structure. In fact, Fürstner noticed that an advanced tetracyclic intermediate with configuration reminiscent of the original structure could not be prepared as a single diastereomer. An unfavorable eclipsing interaction interaction between C16 and C25 instead provided a driving force for equilibration into a nearly statistical mixture of four diasteromers epimeric at C15, C17, and C18.[6,7] Unfortunately, insufficient spectroscopic differences between two synthetic methyl berkelates epimeric at C22, lack of an authentic sample, and the inability to selectively saponify the C1-methyl benzoate prevented an unambiguous assignment of the C22 stereocenter. Later, Snider and coworkers reported the first total synthesis of berkelic

acid, confirming Fürstner's structural revision and putatively assigning the quaternary stereocenter as C22-*S*.[3,8] As the C22 stereocenter was introduced *via* a non-selective Kiyooka aldol reaction,[9] the stereochemical assignment relied upon correlation of the resultant diastereomers to a model compound. Our subsequent synthesis of both C22-epimers of berkelic acid fully corroborated the Snider assignment.[10] Finally, a gram-scale total synthesis was reported in 2012 by Fañanás and Rodríguez that centered on a silver-catalyzed addition/cyclization cascade of appropriately substituted alkyne and aldehyde precursors to set four new chiral centers in a 2:1 diastereomeric ratio.[11,12,13]

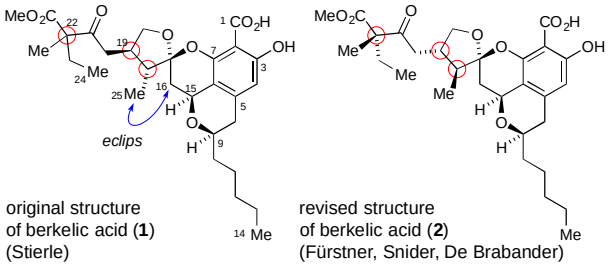
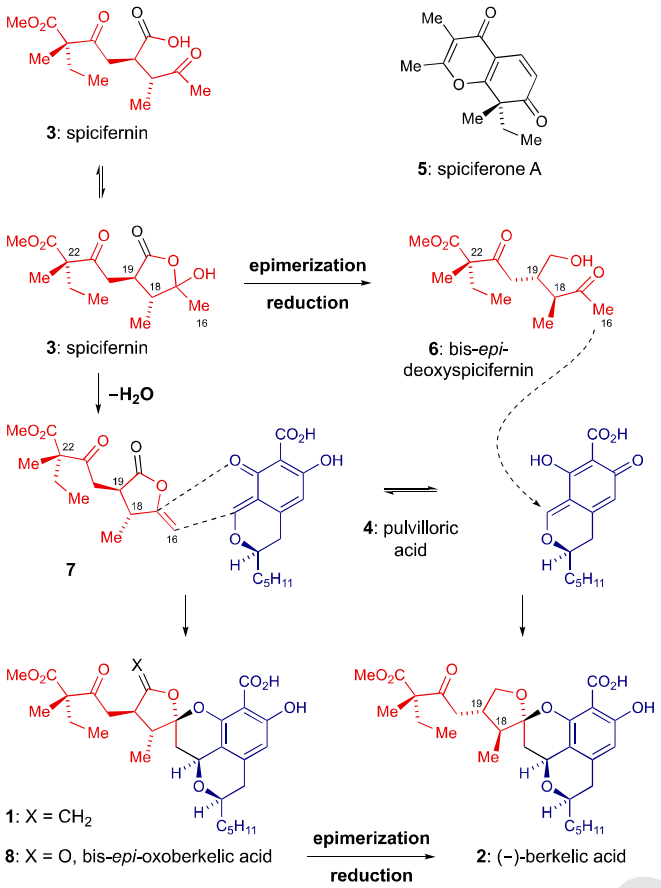


Figure 1. Original and revised structures of berkelic acid.

Constitutionally, the originally proposed structure of (–)-berkelic acid (**1**) emerges as an amalgamation of two other natural products, namely pulvilloric acid (**4**)[14] and spicifernin (**3**). The co-isolation of berkelic acid with spiciferone A (**5**),[1] which previously had been isolated alongside spicifernin (**3**) from a *Cochliobolus* fungus,[15] is indeed suggestive of a proposal that berkelic acid could be the end-product of such a biosynthetic merger (Scheme 1). Biosynthetic studies utilizing ^{13}C- and ^{2}H-labeling have specified that both spicifernin (**3**) and spiciferone A (**5**) are generated from a common hexaketide precursor.[15,16] Thus, the presence of spiciferone A (**5**) in the berkelic acid culture medium suggests that the Berkeley Pit Lake *Penicillium* sp. might also possess the biosynthetic machinery to produce spicifernin (**3**). To account for the stereochemical discrepancy between natural spicifernin (**3**) and (–)-berkelic acid (**2**), which display an epimeric relationship at C18 and C19 (berkelic acid numbering), Snider proposed that spicifernin (**3**) or an immediate biosynthetic precursor could undergo a double epimerization (C18 and C19) followed by a reduction to provide a correctly configured bis-*epi*-deoxyspicifernin (**6**) for addition to pulvilloric acid (**4**).[3,17] We proposed an alternative hypothesis where spicifernin (**3**) would be unified with pulvilloric acid (**4**) first, perhaps via its dehydrated enollactone **7** in a formal cycloaddition with the ortho-quinone methide tautomer of pulvilloric acid, followed by a double epimerization (at C18 and C19) and final reduction.[18,19]

Given berkelic acid's unique structural features, stereochemical questions, biological activity, and potential biosynthetic origin from combination of two other natural products, we embarked on a synthetic program that culminated in the total synthesis of (–)-berkelic acid (**2**) communicated several years ago.[10] Here, we present a full account of our extensive research program including the development of a novel silver-catalyzed dehydration/cycloisomerization/cycloaddition cascade to forge the tetracyclic chroman/isochroman/spiroketal structure embedded within berkelic acid, the synthesis and full characterization of diastereomers of the originally proposed structure, C22-geminal dimethyl substituted analogs, and C26-oxoberkelates to explore the proposed biosynthesis, and biological evaluation of synthetic berkelic acid and analogs.

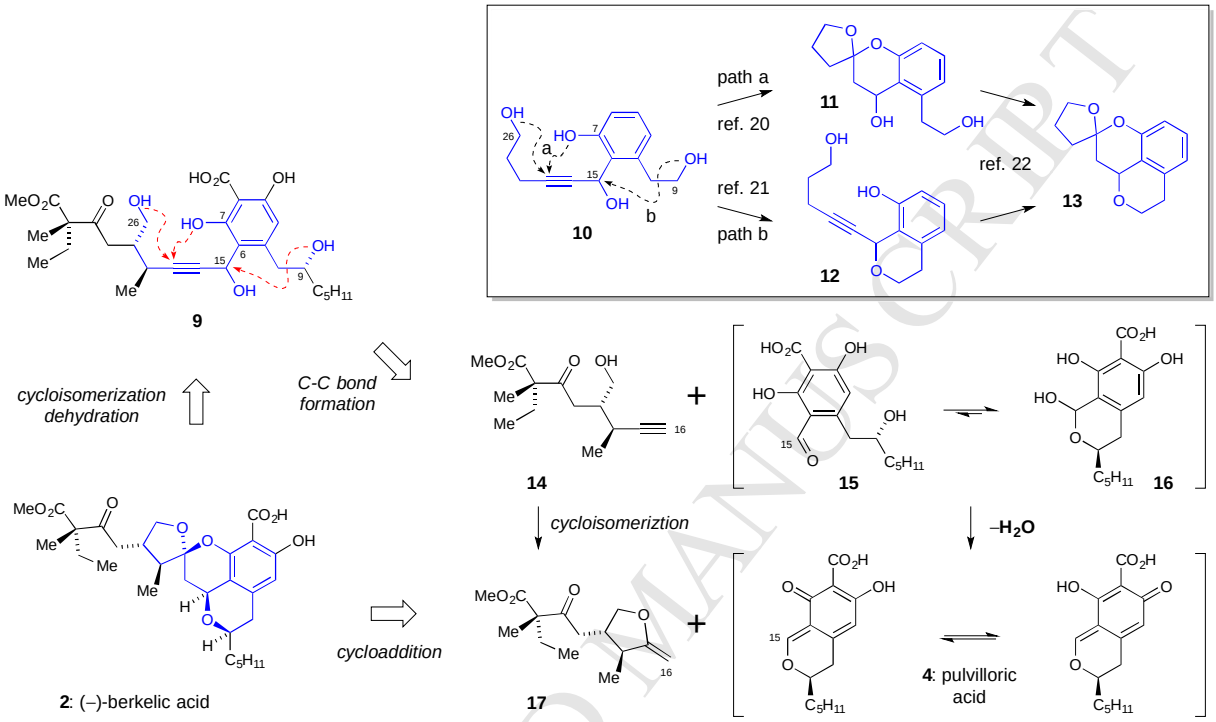


Scheme 1. Biosynthetic proposal for the synthesis of (–)-berkelic acid (**2**) from the natural products spicifernin (**3**) and pulvilloric acid (**4**).

2. Results and discussion

Initially, we became interested in a synthetic campaign toward berkelic acid due to a unique constellation of structural features that we perceived as a fruitful platform to implement methodology developed in our group. In our initial decisively non-biomimetic analysis, we thus perceived linear polyhydroxy alkyne **9** as a suitable direct precursor to berkelic acid (**2**) if conditions could be identified to forge a one-pot cycloisomerization / dehydrative cycloetherification as depicted in Scheme 2. The dividend pay-off would be even higher if thus identified reaction conditions could operate on a protecting group free substrate with all final functionality at the correct oxidation state (i.e. substrate **9**). Based on studies from our group, we had considerable confidence in executing such a strategy. Indeed, as shown in the box in Scheme 2, we developed methodology to exploit internal alkynes as a potential nucleus for metal-catalyzed remolding of linear substrates to cyclic/polycyclic structures. In 2006, we reported on the metal-catalyzed cycloisomerization of dihydroxy-alkynes to form spiroketals under mild conditions (relevant to path a, **10** → **11**)[20] followed by propargylic cycloetherification in 2008 (relevant to transformation **10** → **12**)[21] and a tandem combination that would yield **13** directly from **10** (via **12**; propargylic substitution preceding cycloisomerization).[22] En route to berkelic acid, we envisioned polyhydroxy-alkyne precursor **9** to be available from the addition of terminal alkyne **14** to aldehyde **15** (C15-C16 bond formation). However, if we were to implement a protecting group free synthesis, this aldehyde **15** would entirely exist as the corresponding non-electrophilic lactol **16**, leading us to contemplate the natural product pulvilloric acid (**4**, the dehydration product of **16**) as a viable electrophilic coupling partner instead. It is at this point that our synthetic analysis metamorphosed to one that appeared more reminiscent of the biosynthetic pathway proposed in Scheme 1 and contemplated that a metal-catalyzed 5-exo-dig hydroalkoxylation

of alkynol **14** should deliver enol **17**,[20] a material that would combine with the *ortho*-quinone methide tautomer of pulvilloric acid (**4**) via a formal cycloaddition to deliver directly the chroman/isochroman/spiroketal tetracycle of berkelic acid (i.e. bypassing proposed precursor **9**). Ideally, a one-pot operation would be identified that enables (1) the dehydration of lactol **16** to pulvilloric acid **4**, (2) the cycloisomerization of **14** to **17**, and (3) the final cycloaddition between **4** and **17** to deliver berkelic acid (**2**).[23]

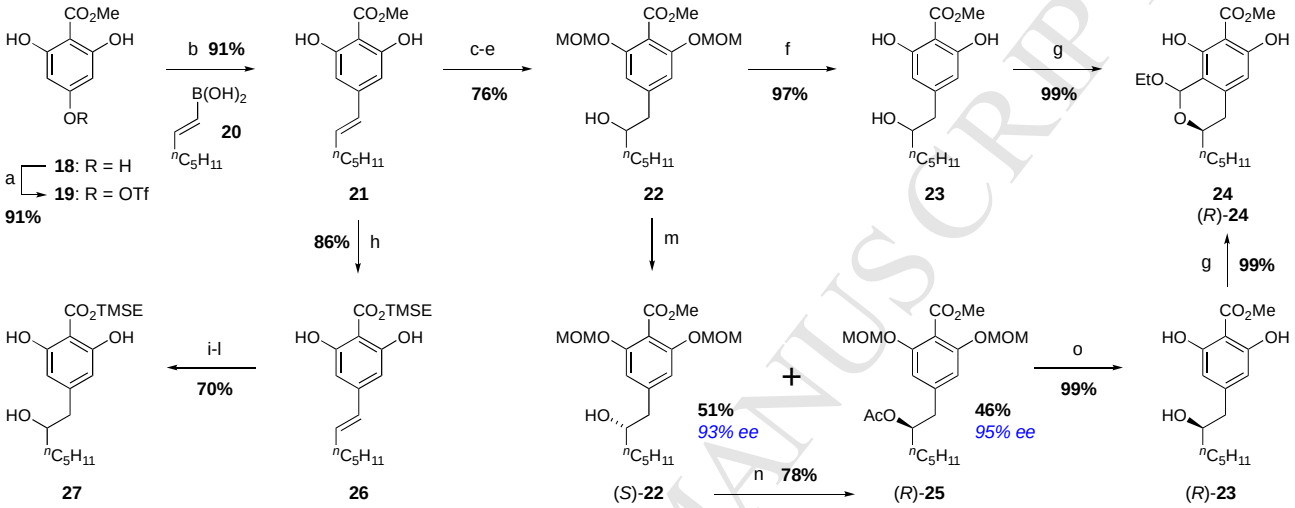


Scheme 2. Retrosynthetic analysis and strategy.

Our synthetic studies began with the development of a concise route to a lactol similar to **16** as a precursor to pulvilloric acid (**4**). As shown in Scheme 3, selective triflation of commercially available methyl 2,4,6-trihydroxybenzoate **18** provided triflate **19**, which readily underwent Suzuki-Miyaura cross coupling with known (*E*)-1-heptenylboronic acid (**20**)[24] to yield styrene derivative **21** in 83% yield from commercial **18**.[25] Installation of the homobenzylic alcohol was best accomplished via a three-step sequence from **21** including MOM protection of the free phenols, styrene epoxidation with *m*-CPBA, and benzylic hydrogenolysis providing alcohol **22** in 76% overall yield from **21**.[26] Methanolysis of the MOM protecting groups and methyl ester delivered alcohol **23** in 97% yield and was followed by a condensation with triethyl orthoformate *via* a procedure adapted from the synthesis of pulvilloric acid[14c] to deliver the ethanol adduct of methyl pulvillorate **24** in excellent yield.

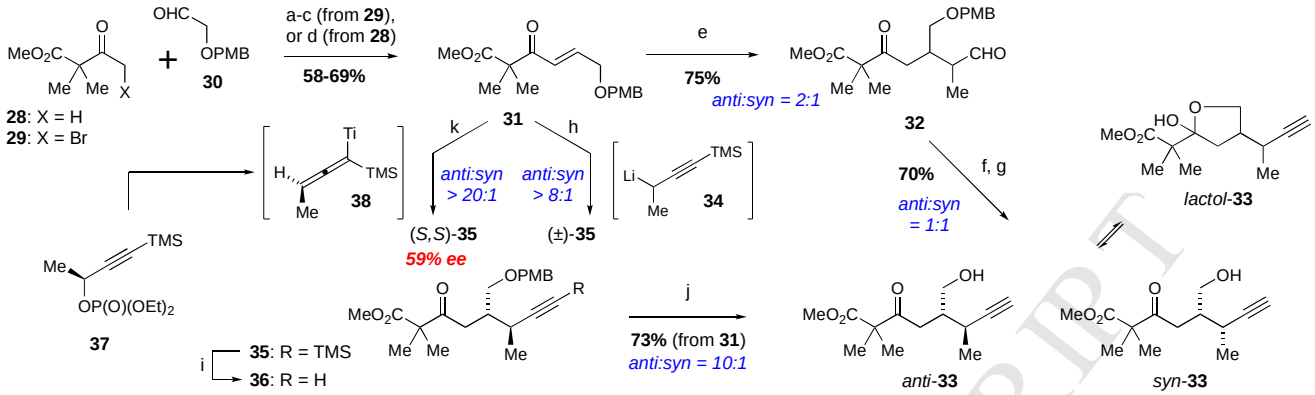
With a viable racemic route established, we next explored the possibility to intercept racemic homobenzylic alcohol **22** for a late-stage enzymatic resolution. We subjected (±)-**22** to a set of 21 different lipases for acetylation of the homobenzylic alcohol with vinyl acetate and identified three enzymes that provided the desired acetate **25** at a synthetically practical rate. Of these, a lyophilized formulation of lipase from *Alcaligenes* sp. exhibited high selectivity and scalability, allowing for the preparation of (*R*)-**25** and (*S*)-**22** in 95% and 93% ee, respectively.[27] As it was ultimately discovered that berkelic acid was of the C9-(*R*) configuration,[3-5] a Mitsunobu esterification of the enantioenriched homobenzylic alcohol (*S*)-**22** (93% ee) with acetic acid provided additional (*R*)-**25** in 78% yield, for an overall yield of 85% of (*R*)-**25** from (±)-**22**. Global deprotection of (*R*)-**25** with acidic MeOH gave (*R*)-**23** in quantitative yield. Treatment with triethylorthoformate as described above afforded (*R*)-**24**. Expecting potential difficulties with a selective late-stage methyl benzoate

saponification based on precedent by Fürstner and Snider,[4,8] we briefly explored alternative benzoate esters at this stage. For example, transesterification of **21** using catalytic Bu_2SnO afforded 2-(trimethylsilyl)ethyl (TMSE) benzoate **26** in good yield (Scheme 5).[28] Elaboration *via* the method described for **23** supplied racemic **27** for enzymatic resolution. However, screening of the same set of 21 lipases as above did not identify a single lipase to affect this transformation. Literature suggests this failure is likely due to the occupation of the lipase active site by the TMSE ester, preventing transesterification.[29] At this point, we decided to move on with methyl benzoate (*R*)-**24** and force ourselves to find a solution for its selective removal at a later stage.



Scheme 3. Synthesis of lactol **24**, a precursor to pulvilloric acid (**4**). Reagents and conditions: (a) Tf_2O, lutidine, CH_2Cl_2, 0 °C, 16 h (91%); (b) **20**, Pd(dppf)Cl_2 (5 mol%), K_2CO_3, THF/H_2O (10:1), reflux, 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 (1 atm), MeOH, rt, 20 h (90%); (f) 10-CSA, MeOH, rt, 1 d (97%); (g) $(EtO)_3CH$, TFA, rt, 18 h (99%); (h) Bu_2SnO (1 mol%), TMSE-OH, PhMe, 100 °C, 16 h (86%); (i) MOMCl, *i*-Pr_2NEt, CH_2Cl_2, 0 °C → rt, 24 h; (j) *m*-CPBA, CH_2Cl_2, rt, 4 h; (k) Pd/$CaCO_3$ (cat.), H_2 (1 atm), MeOH, rt, 24 h; (l) HCl/MeOH, rt, 16 h (70%, four steps); (m) *Alcaligenes* sp. lipases (10 weight%), vinyl acetate, MTBE, 4Å MS, rt, 7 d; (n) DEAD, PPh_3, AcOH, PhMe (0.1 M), 0 °C →rt (78%); (o) HCl/MeOH, rt, 16 h (99%).

At the time we started our synthetic studies in early 2007, the stereochemistry at the quaternary center (C22) was not assigned. In light of that, and our desire to explore a rather bold approach as outlined in Scheme 2, we decided to perform our exploratory chemistry toward a gem-dimethyl substituted berkelic acid analog (i.e. *nor*-berkelic acid). The synthesis of the required alkyne coupling partner analogous to **14** (Scheme 2) is depicted in Scheme 4. To this end, formation of the corresponding phosphonium bromide from commercially available α-bromo ketone **29**,[30] followed by ylide formation and Wittig olefination with known 2-(4-methoxybenzyloxy)acetaldehyde (**30**)[31] gave α,β-unsaturated ketone **31** in 58% yield over three steps. Alternatively, a more efficient one-step titanium-mediated dehydrative aldol condensation between commercially available methyl ketone **28** and aldehyde **30** yielded enone **31** in one step (69% yield). Next, a conjugate addition of the enamine derived in situ from propanal and diethylamine to enone **31** afforded aldehyde **32** in acceptable yield but poor diastereoselectivity (75%, dr = 2:1).[32,33] The *anti*/*syn* ratio further eroded to unity during the ensuing Seyferth-Gilbert homologation of aldehyde **32** with the Ohira-Bestmann reagent.[34] NMR analysis of the chromatographically homogeneous material obtained following oxidative deprotection with DDQ indicated that the product exists as a mixture of open-chain alkynols *syn*-**33** and *anti*-**33** (1:1) and **33**-lactol isomers (verified by gHMBC).[35] Samples of **33** enriched in each diastereomer (*anti*-**33**:*syn*-**33** - 14:1; *anti*-**33**:*syn*-**33** - 1:3.8) were obtained by repeated flash chromatography with arbitrary division of material and served to confirm *anti*-**33** as the less polar diastereomer.

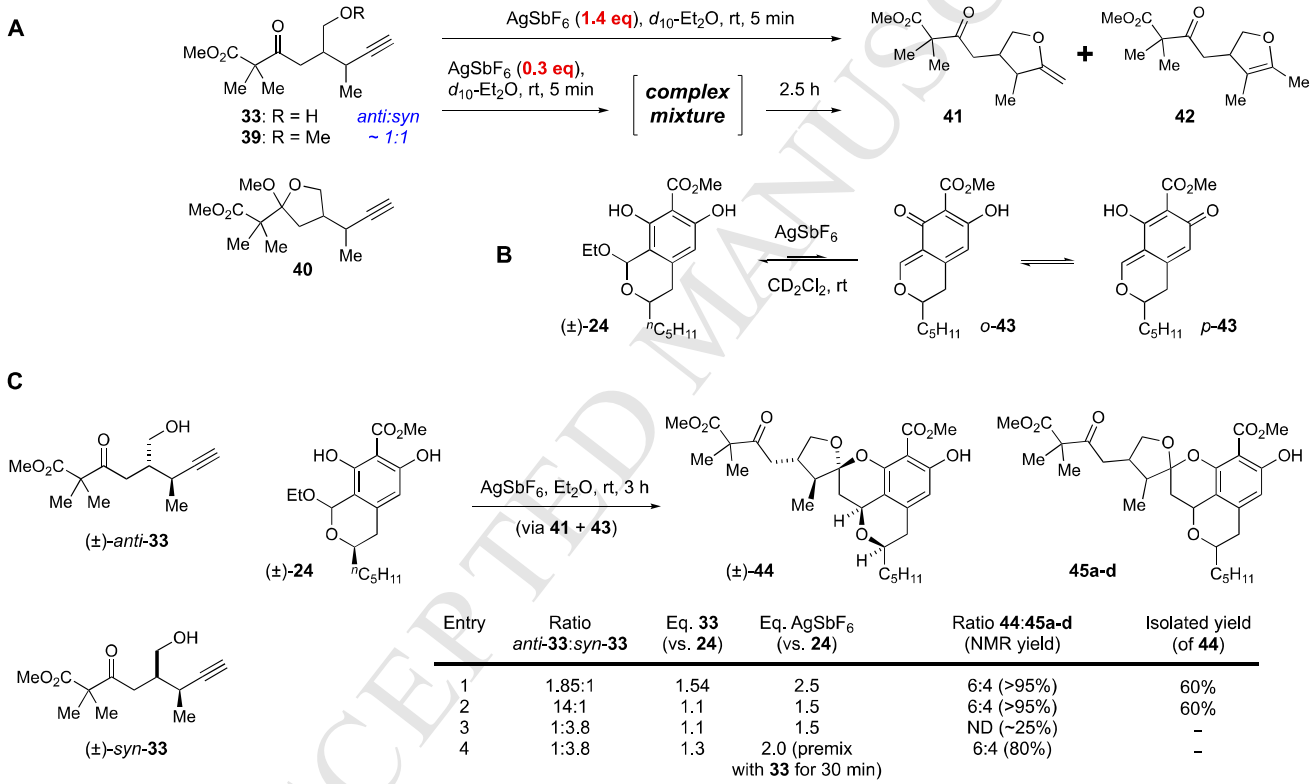


Scheme 4. Synthesis of gem-dimethyl substituted alkynol **33**. Reagents and conditions: (a) **29**, PPh_3, THF, reflux, 1 h (71%); (b) NaOH, MeOH, rt, 2 h (c) **30**, PhMe, reflux, 4 h (81%, 2 steps); (d) **28**, $TiCl_4$, THF, 0 °C, 30 min; Et_3N, –78 °C, 45 min; **30** (1.2 eq), –78 °C → rt, 2.5 h (69%); (e) EtCHO, Et_2NH, dioxane, rt, 21 h (75%, dr = 2:1); (f) Ohira-Bestmann reagent, K_2CO_3, MeOH, 0 °C, 30 min, rt, 17 h (78%, dr = 1:1); (g) DDQ, CH_2Cl_2:H_2O (10:1), rt, 3.5 h (90%, dr = 1:1); (h) preform cuprate with **34**, CuBr·DMS, THF, –78 °C, 1 h; then add to **31**, THF, –78 °C, 2 h; (i) K_2CO_3, MeOH, rt, 4 h; (j) DDQ, CH_2Cl_2, H_2O, rt, 2.5 h (73%, three steps); (k) **37**, Ti(O*i*-Pr)$_4$, *i*-PrMgCl, Et_2O, –45 °C, 2 h; then **31**, –45 °C, 15 min → –20 °C, 3 h (54%).

The above enamine-based route was designed with the prospect to eventually control diastereo- and facial selectivity using an enantioselective organocatalytic variant. However, the ease of epimerization of the aldehyde α-stereocenter during the subsequent alkynylation forced us to consider an alternative approach. Conceptually, 1,4-addition of a metalated propargyl/allenyl species to enone **31** should deliver an α-methyl substituted alkyne straightforwardly. Although initial exploration of reagents more commonly used for the propargylation of aldehydes (Marshall chemistry,[36] allenylstannanes,[37] allenylboronic acids,[38] allenylzinc species,[39] and allenyl/propargyl lithiates)[40] proved frustrating (no Michael addition products **35** or **36**), traction was gained when moving to cuprate chemistry. After substantial experimentation, we found that addition of 4 equivalents of a cuprate generated from lithiate **34** and CuBr·DMS to enone **31** in THF at –78 °C yielded racemic TMS-alkyne **35** with the desired *anti*-isomer dominating in a ratio of ~8-10:1.[41,42] In contemplating an enantioselective route, the synthesis of configurationally defined and stable allenyl/propargyl cuprate species from propargyllithium species **34** appeared daunting.[43] Ultimately, we encountered a manuscript disclosed by Sato and coworkers describing the conjugate addition of configurationally stable homochiral allenyltitanium species to alkylidenemalonates.[44] Using their conditions, we found that treatment of **31** with allenyltitanium reagent **38**, derived from **37** (>98% ee),[45] provided (*S*,*S*)-**35** in moderate yield and enantiomeric excess (54% yield; *ee* = 59%)[46] but excellent diastereoselectivity (*anti*:*syn* > 20:1).[47] Although **31** was completely consumed, a mixture of byproducts including the 1,2-addition product (~20%) was formed, leading to the diminished yield with respect to copper-mediated conjugate propargylation. Treatment of the crude racemic alkyne (±)-**35** with methanolic base to remove the TMS (→ **36**) was followed by oxidative PMB-removal to yield the final alkyne (±)-*anti*-**33** in 73% yield from enone **31** (dr = 10:1). Enantioenriched (*S*,*S*)-**35** on the other hand provided enantioenriched (*S*,*S*)-*anti*-**33** in 92% yield (2 steps; 59% ee; dr >20:1).

As noted above, our initial approach to berkelic acid entailed the addition of a spicifernin-like alkyne to an electrophilic pulvilloric acid-like synthon, followed by a final cyclopropargylation/ cycloisomerization of the thus formed linear alkyne coupled intermediate (Scheme 2, path a). As a prelude to explore this coupling reaction, we attempted to protect the free alcohol of model alkynol **33** (*anti*:*syn* = 1:1) as a methyl ether **39** (or acetal **40**) with Ag_2O and MeI (Scheme 5A).[48] Instead, we obtained a crude mixture containing compounds consistent with structure **41** (diastereomers) and some endocyclic enol ether **42** (crude NMR, not isolated, assignment tentative). It was this observation that led us to explore the possibility for a 1-pot metal-mediated cascade entailing: (1) cycloisomerization of an alkynol, (2) dearomatization of a lactol to a quinone methide, and (3) in situ cycloaddition to deliver the berkelic acid scaffold (Scheme 2, path b). After

a brief survey of transition metal sources,[49] we found that $AgSbF_6$ stroke an ideal balance in effecting both the alkynol cycloisomerization and the quinone methide formation from lactol and acetal precursors, respectively (vide infra). As shown in Scheme 5A, in situ NMR monitoring of a solution of **33** (*anti:syn* = 1.1:1) and $AgSbF_6$ (1.4 eq) in d_{10}-Et_2O showed 100% conversion to a mixture of compounds featuring resonances consistent with enol ether **41** within 5 min (no signals indicative of **42**). When using sub-stoichiometric $AgSbF_6$ (0.3 eq) a far more complex mixture was observed after 5 min that simplified over time (≤2.5 h) to a mixture similar to that observed with supra-stoichiometric $AgSbF_6$ but significant accumulation of endocyclic enol ether **42**. Although it has been reported that the carboxylic acid corresponding to **24** will yield the quinone methide pulvilloric acid (**4**) upon removal of ethanol under ultra high vacuum,[14c] we opted to explore the possibility of generating quinone methide **43** in situ via a Lewis acid catalyzed dearomatization.[50] Gratifyingly, through titration experiments in CD_2Cl_2 monitored by ^1H NMR, we found that the alkynophilic $AgSbF_6$, effective for the cycloisomerization of alkynol **33**, enjoyed sufficient hard Lewis acidity to promote a concentration-dependent equilibrium between (±)-**24** and quinone methide **43** (Scheme 5B).



Entry	Ratio *anti*-**33**:*syn*-**33**	Eq. **33** (vs. **24**)	Eq. $AgSbF_6$ (vs. **24**)	Ratio **44**:**45a-d** (NMR yield)	Isolated yield (of **44**)
1	1.85:1	1.54	2.5	6:4 (>95%)	60%
2	14:1	1.1	1.5	6:4 (>95%)	60%
3	1:3.8	1.1	1.5	ND (~25%)	–
4	1:3.8	1.3	2.0 (premix with **33** for 30 min)	6:4 (80%)	–

Scheme 5. One-pot Ag-catalyzed synthesis of *nor*-berkelic acid methyl esters (**44**/**45**) from alkynol (±)-**33** and lactol (±)-**24**.

With the above defined parameters in hand, we were more than pleased to observe that stirring an ethereal solution of racemic alkynol **33** (*anti*:*syn* = 1.85:1) and racemic lactol **24** in the presence of $AgSbF_6$ (2.5 eq.) at room temperature cleanly converted to a mixture of five methyl berkelates **44** (~60% of total) and **45a-d** (~40% of total, 2.5:1.0:1.5:1.2 ratio) comprising >95% of the aromatic containing products (Scheme 5C, entry 1).[51,52] The same results were obtained with an equimolar mixture of diastereomerically enriched (±)-*anti*-**33** (*anti*:*syn* = 14:1) and (±)-**24** (Scheme 5C, entry 2). Repeated semi-preparative HPLC yielded pure samples of (±)-**44** (60% isolated) and the four individual diastereomers **45a-d**, the structures of which were unambiguously assigned by X-ray crystallography (**44** and **45a-c**) and multidimensional NMR studies (**45d**; Figure 2). Diastereomer **44** corresponds to the revised structure of berkelic acid, whereas

diastereomers **45a-d** correspond to the equilibrating diastereomers identified by Fürstner when attempting to synthesize the originally assigned structure of berkelic acid.[4,5] In agreement, when individual diastereomer **45a** (originally assigned berkelate stereochemistry) was stirred at room temperature with HCl/MeOH, an equilibrium was established yielding a mixture of diastereomers **45a-d** in the same 2.5:1.0:1.5:1.2 ratio as observed by NMR of the crude reaction mixture obtained according to Scheme 5C.[53] These and other published data,[3-8] thus establish that the C15, C17 and C18 stereocenters can epimerize under the reaction conditions, and therefore, theoretically either *anti*- or *syn*-**33** could provide access to the correctly configured diastereomer **44**. To investigate this idea, a mixture of (±)-**24** (1.0 eq), $AgSbF_6$ (1.5 eq), and (±)-*syn*-**33** (1.1 eq, *syn*:*anti* = 3.8:1) in Et_2O was stirred at room temperature, which led to a complex mixture comprised of ~25% berkelates in addition to previously unobserved, non-berkelate byproducts (crude NMR, Scheme 5C, entry 3). However, pre-mixing an ethereal solution of *syn*-**33** (dr = 3.8:1, 1.3 eq) and $AgSbF_6$ (2.0 eq) for 30 min prior to the introduction of (±)-**24** (1.0 eq) generated a mixture similar (~6:4, ~80% of mixture, crude NMR) to that observed previously with alkynol *anti*-**33** (Scheme 5C, entry 4). These results are consistent with a 5-*exo*-dig hydroalkoxylation in which alkynol *anti*-**33** undergoes cyclization at a rate competitive with berkelate formation, while *syn*-**33** does not. This is likely to the result of a build-up of an unfavorable eclipsing interaction during the cycloisomerization of *syn*-**33** versus *anti*-**33**. Pre-incubating *syn*-**33** with $AgSbF_6$ for 30 min enables a more advanced conversion to *in situ* formed cyclic enol ether **41** prior to the addition of acetal **24**.

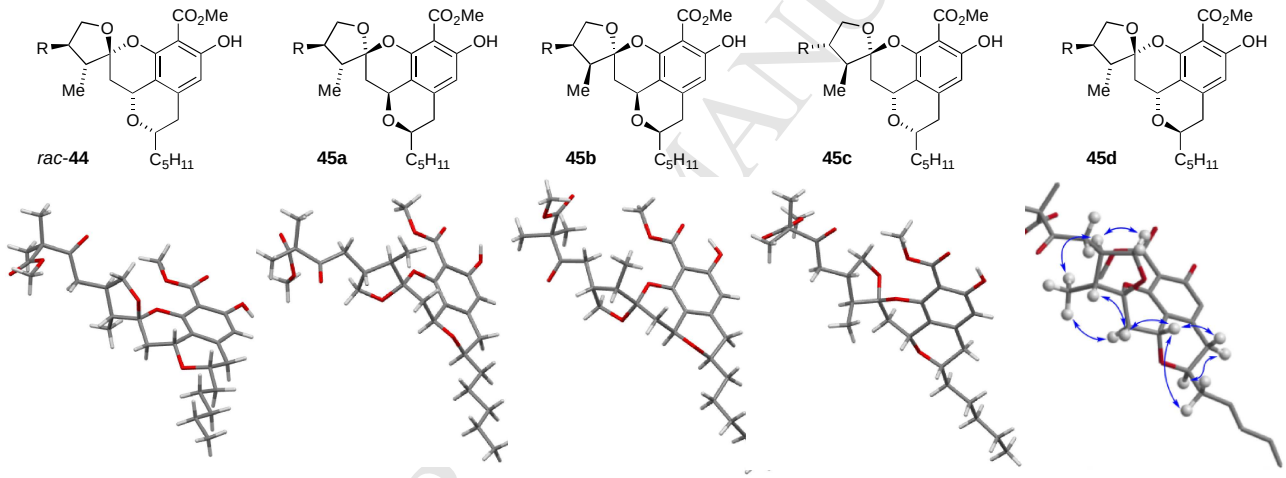
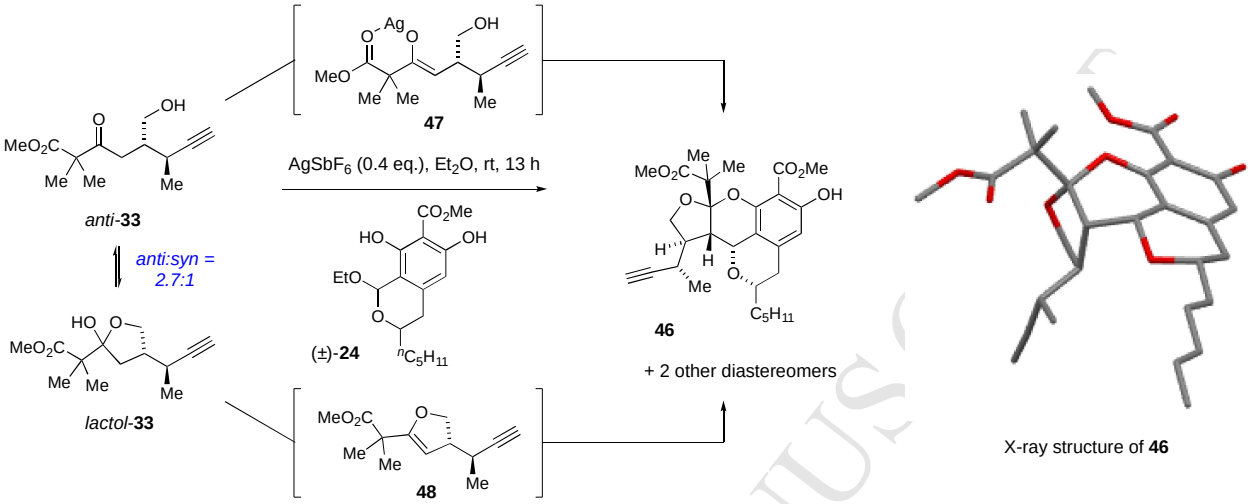


Figure 2. Representations of X-ray crystal structures of **44** and **45a-c** and NMR structure of **45d** (diagnostic nOe correlations are indicated with blue arrows).

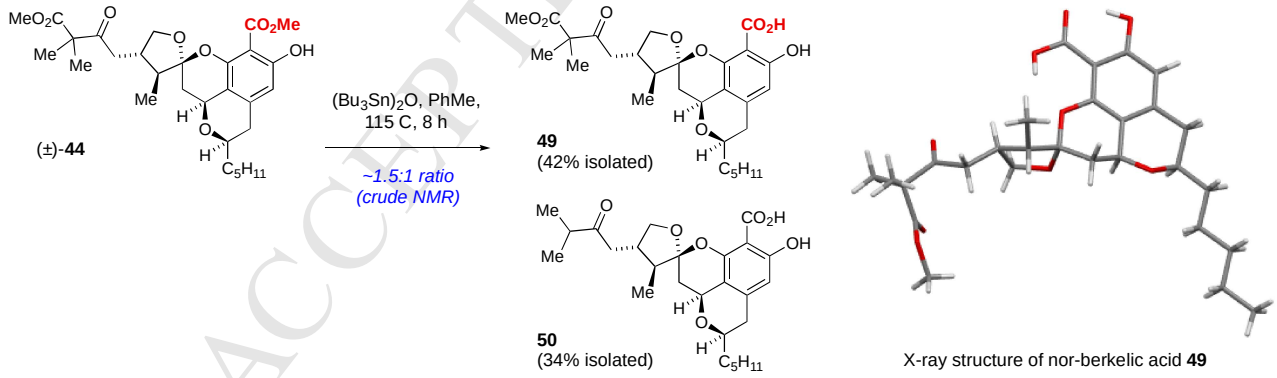
In an attempt to render the reaction catalytic, we were surprised to find that stirring alkynol (±)-**33** (*anti*:*syn* = 2.7:1; 1.2 eq.) and acetal (±)-**24** in the presence of a substoichiometric amount of $AgSbF_6$ (0.4 eq.) generated an inseparable mixture of 3 fused tetracyclic ring products (60% yield)[54] from which the major *endo*-isomer **46** was crystalized by slow evaporation from a EtOAc/hexanes/dichloromethane solution. X-ray crystallographic analysis unambiguously determined the structure to be the one shown in Scheme 6. Of the two conceivable pathways, interception of the quinone methide **43** derived from acetal **24** with either a silver enolate **47** derived from β-keto ester **33** or the endocyclic enol ether **48** derived from a silver-mediated dehydration of *lactol*-**33**, we prefer the silver enolate pathway (via **47**). Indeed, under otherwise identical conditions, suprastoichiometric $AgSbF_6$ provided spirocyclic structures (**44**/**45**) via **33**-derived exocyclic enol ether **41** (Scheme 5C), but the hydroxymethylene required for this cycloisomerization (**33** → **41**) would already be sequestered if the reaction proceeds through endocyclic enol **48**.[55] Moreover, given the bidentate nature of β-keto esters it is not unreasonable to envision that **33** would sequester the first equivalent of Ag(I) as the silver enolate **47**, leading to fused products (e.g. **46**) in reaction with quinone methide **43**. In agreement with our in situ NMR-studies (Scheme 5A) further cycloisomerization of the γ-hydroxy alkynol in **47** to an

exocyclic enol ether is slow with substoichiometric $AgSbF_6$ possible due to a decreased alkynophilicity of bidentate-sequestered Ag (cf. **47**). Longer incubation times or suprastoichiometric $AgSbF_6$ (Scheme 5A) are thus required to complete the cycloisomerization to exocyclic enol ether **41** en route to the spirocyclic berkelate products **44**/**45** (Scheme 5C).[56]



Scheme 6. Reaction of alkynol (±)-**33** and lactol (±)-**24** with catalytic $AgSbF_6$ produces fused instead of spirocyclic products.

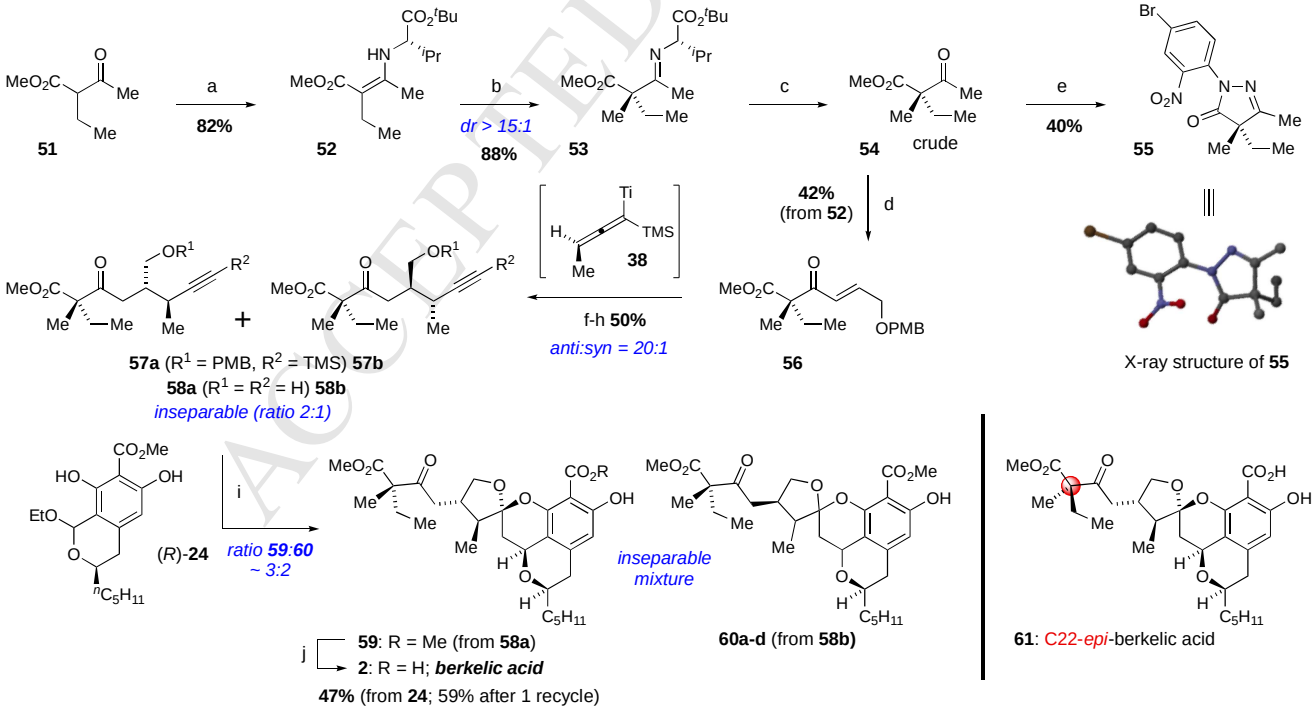
As noted in the introduction, attempts by Fürstner and coworkers to selectively hydrolyze the phenolic methyl ester of berkelic acid methyl ester were unsuccessful. They, and later Snider and coworkers, solved this issue by resorting to orthogonal ester protecting groups (phenolic benzyl and allyl ester, respectively).[3-8] We decided to reexamine the issue of selective demethylation and after some experimentation found that heating a solution of *nor*-berkelic acid methyl ester **44** with excess $(Bu_3Sn)_2O$ in toluene provided a mixture of the desired *nor*-berkelic acid **49** (42% isolated) and a decarboxylated byproduct **50** (34% isolated) in a ratio of ~1.5:1 (based on crude NMR, Scheme 7).[57] Byproduct **50** arises from double methyl ester cleavage and spontaneous decarboxylation at the C28 terminus.



Scheme 7. Synthesis of *nor*-berkelic acid **49** via chemoselective methyl ester cleavage.

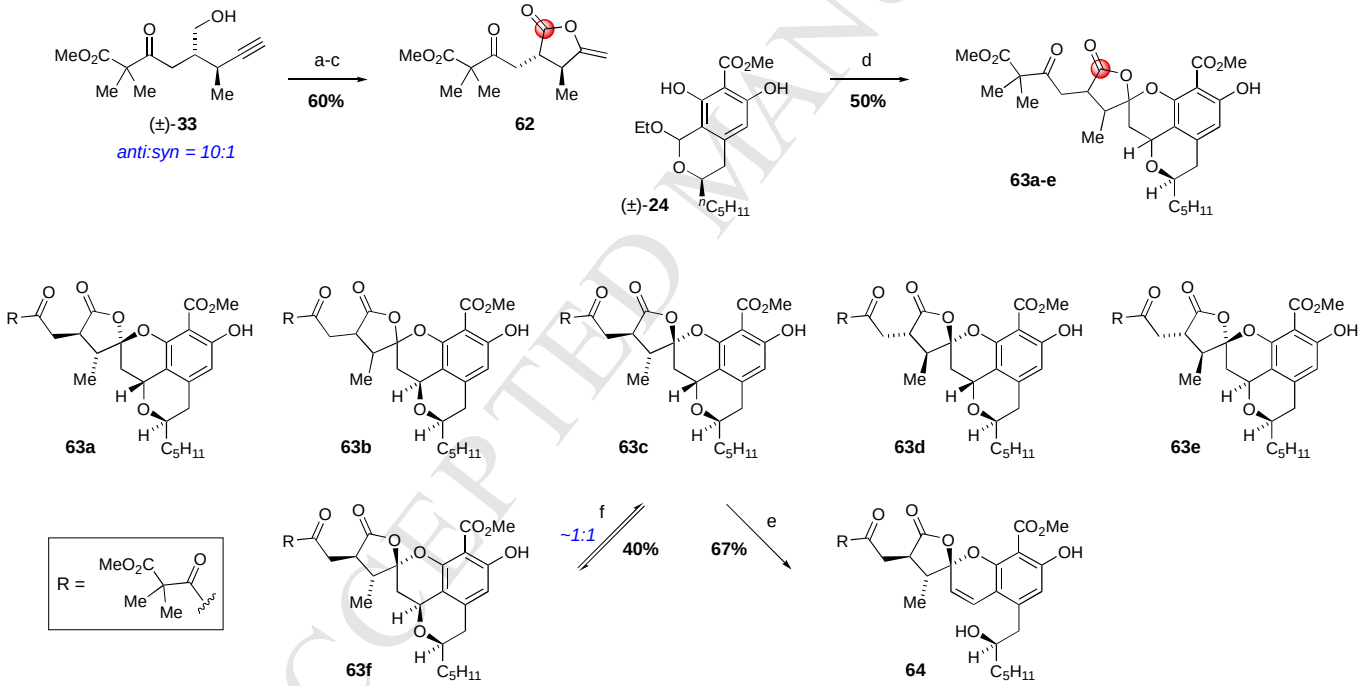
With a convergent streamlined synthetic strategy established, we set out to tackle the total synthesis of berkelic acid containing the quaternary C22-stereocenter. To that end, we decided to exploit the asymmetric enamine alkylation method developed by Koga and coworkers to install the C22-quaternary stereocenter.[58] Accordingly, valine-derived enamine **52** was prepared by Lewis acid-catalyzed condensation of commercially available 2-ethyl-3-oxobutanoate (**51**) and (*L*)-*t*-butyl valinate in 82% yield (Scheme 8). Sequential lithiation of a toluene solution of **52** with LDA in the presence of lithium-coordinating THF (2.6 eq), followed by alkylation with methyl iodide

provided α-quaternary substituted imine derivative **53** (88%, dr > 15:1). Hydrolysis of this material gave crude β-keto ester **54**, which was converted to bromine-containing cyclic hydrazone **55** (40% yield) for single crystal X-ray analysis, thus confirming the (*S*)-absolute configuration in agreement with the Koga model for stereoinduction.[58] Continuing with the synthesis, titanium-mediated dehydrative aldol condensation of β-keto ester **54** with aldehyde **30** yielded enone **56** in 42% overall yield from enamine **52** (three steps, no intermediate purifications).[59] Conjugate propargylation of **56** using the homochiral allenyl titanate (*R*)-**38** as described in Scheme 4 afforded an inseparable mixture of **57a,b** in a ratio of 2:1 (*anti*:*syn* > 20:1) and 52% yield.[60] Sequential TMS-removal and oxidative PMB-deprotection delivered alkynols **58a,b** in 91% yield (2:1 ratio, inseparable, in equilibrium with corresponding hemiketals). Stirring an ethereal mixture of alkynols **58a,b** (2 eq) and enantiopure lactol (*R*)-**24** (1 eq) in the presence of $AgSF_6$ (3 eq) provided a mixture of methyl berkelates **59** (from **58a**) and **60a-d** (from **58b**). Unlike the corresponding *nor*-berkelates **44** / **45a-d**, individual diastereomers could not be obtained from this inseparable mixture. Crude NMR-analysis indicated a ratio of berkelic acid methyl ester (**59**) versus four other diastereomers **60a-d** in a ratio of 3:2, similar to that observed for *nor*-berkelates **44** / **45a-d**.[61] Treatment of the crude mixture of **59** / **60a-d** with $(Bu_3Sn)_2O$ in toluene provided pure (–)-berkelic acid (**2**) in 47% isolated yield over the two steps from lactol (*R*)-**24**. The reaction was interrupted at partial conversion to prevent saponification and subsequent decarboxylation at the C28 terminus as was observed for *nor*-berkelic acid **49** (Scheme 7). The recycled material was subjected to another round of $(Bu_3Sn)_2O$ treatment to yield additional (–)-berkelic acid (**2**) for a total isolated yield of 59% from lactol (*R*)-**24** based on 1 recycle. Given that at the outset of our program, the absolute configuration at the quaternary C22-stereocenter remained in doubt, we also prepared the corresponding C22-*epi*-berkelic acid **61** from the enantiomeric alkynols *ent*-**58a,b** (53%, 70% after 1 recycle from (*R*)-**24**).[62] Only the C22-*S* diastereomer **2** displayed spectral data fully congruent with natural (–)-berkelic acid (**2**),[1] thus unambiguously establishing the complete stereochemical configuration of the natural product. The spectral data and optical rotation of this synthetic (–)-berkelic acid (**2**) is in full agreement with the data reported for natural and synthetic (–)-berkelic acid from the Snider, Fürstner, and Fañanàs groups.[1,3,5,11] The spectral data for C22-*epi*-berkelic acid (**61**) matched those reported for the same material synthesized by Snider and Fürstner.[3,5]



Scheme 8. Synthesis of (–)-berkelic acid (**2**). Reagents and conditions: (a) L-*t*-Bu-valinate (0.99 eq), $BF_3 \cdot OEt_2$ (5 mol%), PhH, 80 °C, 14 h; (b) LDA, PhMe, –78 °C, 1 h; then THF (2.6 eq), 1 h; then MeI, 16 h (88%); (c) HCl/H_2O, THF, rt, 1 h; (d) $TiCl_4$, Et_3N, 4Å MS, THF, –78 °C, 1.5 h; aldehyde **30** (1.2 eq) –78 °C → rt, 3 h (48% over two steps); (e) (4-bromo-2-nitrophenyl)hydrazine hydrochloride, EtOH, reflux, 2 d (40%); (f) (*R*)-**38** (1.2 eq), THF, –40 °C → –20 °C, 3 h (52%); (g) K_2CO_3, MeOH, rt, 4 h; (h) DDQ, CH_2Cl_2, H_2O, rt, 2.5 h (91%, two steps); (i) **58a,b** (dr 2:1, 2.0 eq.), (*R*)-**24** (1.0 eq), $AgSbF_6$ (3.0 eq), Et_2O, rt, 4 h; (j) $(Bu_3Sn)_2O$, PhMe, 115 °C, 8 h (47% over two steps; 59% after 1 recycle of recovered **59**/**60**).

As noted in the introduction, berkelic acid (**2**) appears to be comprised of two other natural products, namely spicifernin (**3**) and the quinone methide pulvilloric acid (**4**), with the caveat that the C18 and C19 stereocenters (berkelic acid numbering, see Fig. 1) of spicifernin (**3**) are of opposite configuration to those found in berkelic acid (**2**) and a lower oxidation state at C26. Snider had proposed the possibility of a biosynthetic transformation of spicifernin (**3**) to bis-*epi*-deoxyspicifernin (**6**, Scheme 1), followed by incorporation into berkelic acid (**2**). We offered the possibility of incorporating spicifernin (**3**) first into bis-*epi*-oxoberkelic acid (**8**), followed by epimerization (C18 and C19) and reduction at C26 (Scheme 1). We, and others had already demonstrated that C15, C17, and C18 are all epimerizable within the context of the berkelic acid tetracyclic scaffold.[5,10] The higher oxidation state at C26 (oxo) is thus expected to enable the required additional epimerization at C19 to complete the berkelic acid stereochemical constellation. To test this hypothesis, we embarked on the synthesis of C26-oxoberkelate analogs with C22-gem dimethyl-substituted quaternary carbon (**63**, Scheme 9) using the silver mediated cycloaddition strategy employed for berkelic acid synthesis (Schemes 5C and 8).



Scheme 9. Synthesis of oxoberkelates **63a-f**. Reagents and conditions: (a) DMP, CH_2Cl_2, rt, 1 h; (b) $NaH_2PO_4 \cdot H_2O$, $NaHClO_2 \cdot H_2O$, 2-methyl-2-butene, *t*-BuOH, H_2O, 0 °C → rt, 1 h (90%, two steps); (c) $NaAuCl_4 \cdot 2H_2O$ (5 mol%), MeCN, H_2O, rt, 2.5 h (67%); (d) (±)-**24** (1.0 eq), (±)-**62** (1.2 eq), $AgSbF_6$ (1.4 eq), Et_2O, rt, 3 h (50% **63a-e**); (e) HCl, Et_2O, CH_2Cl_2, rt, 24 h (67%); (f) $AgSbF_6$ (1 eq), CD_2Cl_2, rt, 3 h (40%, **63f**). The structure of **63b** could not be determined unambiguously.

The requisite spicifernin-like γ-lactone **62** was prepared from *nor*-berkelic acid precursor **33** (racemic) in 60% overall yield by double oxidation (Dess-Martin and Lindgren)[63,64] and gold-catalyzed cycloisomerization.[65] Treatment of racemic lactone **62** (1.2 eq) with racemic lactol **24** (1 eq) in the presence of $AgSbF_6$ (1.4 eq) at room temperature in ether for 3 hours produced a mixture containing at least six oxoberkelate diastereomers **63a-f** in 61% yield after flash chromatography. Normal phase semi-preparative HPLC purification with two different eluents yielded pure diastereomers **63a** (4% isolated), **63b** (4% isolated), **63c** (13% isolated), **63d** (9% isolated), and **63e** (2%

isolated).[66] Diastereomer **63e** has the same relative configuration as berkelic acid.[67] Of all the other diastereomers, only **63c** was obtained in quantities sufficient enough for equilibration studies. Unfortunately, attempts to equilibrate this diastereomer to **63e** (with berkelic acid configuration) have thus far been unsuccessful. For example, treatment of **63c** with Bronsted acid (HCl, CH_2Cl_2) – conditions that were effective for the equilibration of berkelate **45a** into the 4 berkelate diastereomers **45a-d** (Scheme 4) – resulted in isochroman ring-opening to yield elimination product **64**, whereas treatment with $AgSbF_6$ yielded a 1:1 equilibrium mixture of **63c** (configuration similar to **45d**) and **63f** (configuration similar to **45c**) from which **63f** could be isolated in 40% yield. No equilibration via epimerization at C17, C18, or C19 could be detected. While these initial results do not disprove our biosynthetic hypothesis involving oxoberkelic acid intermediates en route to berkelic acid, our inability to identify suitable epimerization conditions appears to bolster the proposal by Snider that epimerization at C18 and C19 occurs before incorporation of spicifernin into berkelic acid.

During the course of our synthetic campaign toward (–)-berkelic acid (**2**) described herein, we prepared a number of structural analogs – notably *nor*-berkelic acid (**49**), a decarboxylated *nor*-berkelic acid analog **50**, *nor*-berkelic acid methyl ester (**44**) and four diastereomers **45a-d**, C22-*epi*-berkelic acid **61**, and six diastereomeric oxoberkelic acid methyl ester analogs **63a-f**. In the original isolation paper, berkelic acid (**2**) was shown to be a selective inhibitor of the human ovarian cancer cell line OVCAR-3 with a GI_{50} of 91 nM.[1] Later, Snider reported that synthetic (–)-berkelic acid (**2**) and C22-*epi*-berkelic acid (**61**) failed to register any antiproliferative activity when tested at a10 μM single concentration in the NCI human disease-oriented 60-cell line panel, which includes the OVCAR-3 cell line.[3] Recently Rodríguez et al. reported similarly that their synthetic (–)-berkelic acid (**2**) and corresponding methyl ester **59** were devoid of activity against the OVCAR-3 cell line.[68] We therefore decided to test our synthetic (–)-berkelic acid (**2**), C22-*epi*-berkelic acid (**61**), *nor*-berkelic acid (**49**), decarboxylated *nor*-berkelic acid (**50**), *nor*-berkelic acid methyl ester (**44**) and diastereomer **45a**, and oxoberkelic acid methyl ester diastereomer **63c** against the HeLa (cervical cancer), A549 (adenocarcinomic human alveolar basal epithelial cells), and OVCAR-3 cancer cell lines. Unfortunately, none of these compounds demonstrated any activity when measured up to 10μM concentrations. These results in combination with previous literature observations indicate that the original sample of natural (–)-berkelic acid (**2**) was either contaminated with a minor potent metabolite, or that there was a methodological problem with the assay.[69]

3. Conclusion

In conclusion, we have developed a scalable, highly convergent synthesis of berkelic acid and analogs based on a key silver-mediated cascade involving the cycloisomerization of an alkynol to an exocyclic enol ether, quinone methide formation from an aromatic isochroman lactol, and subsequent cycloaddition to provide berkelic acid methyl ester in a one-pot operation. This highly efficient approach, inspired by the notion that berkelic acid constitutionally appeared as a combination of two other natural products spicifernin and pulvilloric acid, enabled the total synthesis of (–)-berkelic acid (**2**) in 10 steps overall longest linear sequence (LLS, 17 total steps) and 10% overall yield. This compares favorable to the total synthesis described by Fürstner (19 steps LLS, 26 total steps, 5% overall yield),[5] Snider (13 steps LLS, 20 total steps, 2% overall yield),[3] and Fañanás (10 steps LLS, 18 total steps, 12% overall yield).[11] Furthermore, we observed that substoichiometric amounts of $AgSbF_6$ during the key-step led to fused tetracyclic products instead of the tetracyclic spiroketal/chroman/isochroman ring system present in berkelic acid. In addition to berkelic acid and its C22-epimer, we also synthesized several analogs including *nor*-berkelic acid, a decarboxylated *nor*-berkelic acid analog, *nor*-berkelic acid methyl ester and four diastereomers, and six diastereomeric oxoberkelic acid analogs. The latter were prepared to explore an alternative to Snider's biosynthetic proposal to reconcile the opposite C18/C19 configuration of spicifernin to that found in berkelic acid.[3,18] Our studies with *nor*-berkelic acid analogs (quaternary gem-dimethyl at C22, versus quaternary Me, Et) enabled us to obtain X-ray quality crystal structures of naturally configured *nor*-berkelic acid and several diasteromeric methyl esters for the first time. These unambiguously confirm the observations by Fürstner[4] and Snider[3] that the originally assigned configuration of berkelic acid[1] is not a thermodynamically stable entity and readily

equilibrates into a more or less statistical mixture of four diastereomers. Finally, we tested berkelic acid, C22-*epi*-berkelic acid and several analogs for activity against several human cancer cell lines, and found that in agreement with previous observations, synthetic berkelic acid and the various analogs were inactive. This indicates that the natural sample of berkelic acid contained a minor potent contaminant, or a methodological problem with the bioassay.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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Appendix A – Supplementary Data

Supplementary data associated with this article can be found in the online version, at

CCDC 1571417 (**44**, C29H40O9), CCDC 1571415 (**45a**, C29H40O9), CCDC 1571419 (**45b**, C29H40O9), CCDC 1571416 (**45c**, C29H40O9), CCDC 1571420 (**46**, C29H38O8) and CCDC 1571418 (**49**, C28H38O9) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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[26] For a similar sequence, see ref. 25.

[27] For the use of this enzyme for the resolution of benzylic and homobenzylic alcohols, see: Naemura, K.; Murata, M.; Tanaka, R.; Yano, M.; Hirose, K.; Tobe, Y. *Tetrahedron: Asymmetry* **1996**, *7*, 3285–3295.

[28] Transesterification to an allyl benzoate was also successful, but would prove incompatible with later steps in the sequence.

[29] This is likely a result of the stereoelectronic similarities between TMSE esters and methoxyethyl esters, the latter being employed as a carboxylate protecting group when enzymatic hydrolysis is the desired means of removal. For a review of the enzymatic deprotection of methoxyethyl ester, see: Gewehr, M.; Kunz, H. *Synthesis* **1997**, 1499–1511.

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[31] Chenevert, R.; Dasser, M. *J. Org. Chem.* **2000**, *65*, 4529–4531.

[32] (a) Hagiwara, H.; Okabe, T.; Hakoda, K.; Hosji, T.; Ono, H.; Kamat, V. P.; Suzuki, T.; Ando, M. *Tetrahedron Lett.* **2001**, *42*, 2705–2707; (b) Hagiwara, H.; Okabe, T.; Ono, H.; Kamat, V. P.; Hosji, T.; Suzuki, T.; Ando, M. *J. Chem. Soc., Perkin Trans. 1* **2002**, 895–900.

[33] Monitoring of the reaction by ^{1}H NMR in d_8-dioxane indicated an initial dr of ~3:1, eroding to 2:1 at full conversion.

[34] (a) Ohira, S. *Synth. Commun.* **1989**, *19*, 561–564; (b) Mueller, S.; Liepold, B.; Roth, G. J.; Bestmann, H. J. *Synlett* **1996**, 521–522.

[35] See supporting information.

[36] For a review on the propargylation of aldehydes, see: Marshall, J. A. *J. Org. Chem.* **2007**, *72*, 8153–8166.

[37] (a) Marshall, J. A.; Wang, X. *J. Org. Chem.* **1992**, *57*, 1242–1252; (b) Marshall, J. A.; Perkins, J. F.; Wolf, M. A. *J. Org. Chem.* **1995**, *60*, 5556–5559.

[38] (a) Ikeda, N.; Arai, I.; Yamamoto, H. *J. Am. Chem. Soc.* **1986**, *108*, 483–486; (b) Corey, E. J.; Yo, C.-M.; Lee, D.-H. *J. Am. Chem. Soc.* **1990**, *112*, 878–879.

[39] (a) Marshall, J. A.; Adams, N. D. *Org. Lett.* **2000**, *2*, 2897–2900; (b) Marino, J. P.; McClure, M. S.; Holub, D. P.; Comasseto, J. V.; Tucci, F. C. *J. Am. Chem. Soc.* **2002**, *124*, 1664–1668.

[40] Huynh, C.; Linstrumelle, G. *J. Chem. Soc., Chem. Commun.* **1983**, 1133–1134.

[41] Poor regioselectivity (1,4 vs. 1,2) was obtained using CuI (2.5:1), CuBr (2.5:1) and CuCN (5.0:1), while use of CuBr·DMS afforded a >20:1 ratio of regioisomers as determined by ^{1}H NMR of the crude reaction product.

[42] For examples of 1,2-additions to aldehydes with similarly prepared cuprates, see: Alouane, N.; Vrancken, E.; Mangeney, P. *Synthesis* **2007**, 1261–1264.

[43] To the best of our knowledge, there are no reported examples of enantioselective additions with allenyl/propargyl cuprates (1,2- or 1,4). Our lab is currently exploring the use of chiral ligands for copper.

[44] Song, Y.; Okamoto, S.; Sato, F. *Org. Lett.* **2001**, *3*, 3543–3545.

[45] Phosphonate **37** (>98% ee) was prepared from (*S*)-4-(trimethylsilyl)-3-butyn-2-ol in one step according to Song et al.,[44] and is known to occur without erosion of enantiopurity. Chirality transfer of phosphonate **37** to allenyltitanium **38** is >97%.[44] For the synthesis of (*S*)- and (*R*)-4-(trimethylsilyl)-3-butyn-2-ol in multigram quantities *via* enzymatic resolution, see: Marshall, J. A.; Chobanian, H. *Org. Synth.* **2005**, *82*, 43–54.

[46] Using reagents **37**/**38** Sato reports yields of 82-96% and ee's >92% in Michael additions to alkyl- and aryl-substituted alkylidenemalonates.[44]

[47] As noted in the main text, we have had no success with other allenyl organometallic species (Sn, B, Zn, In) commonly used for the propargylic addition to aldehydes.[36-39]

[48] Paquette, L. A.; Hu, Y.; Luxenburger, A.; bishop, R. L. *J. Org. Chem*. **2007**, *72*, 209–222.

[49] Surveyed metals include $AuCl_3$, Ag_2CO_3, $AgSbF_6$, and $AgPF_6$.

[50] For a review of *o*-quinine methides, see: Van De Water, R. W.; Pettus, T. R. R. *Tetrahedron* **2002,** *58*, 5367–5405.

[51] Monitoring the reaction by NMR (d_{10}-Et_2O) indicated the initial formation (< 5 min) of a major diastereomer different than **44** and **45a-d**, which disappeared at full conversion. This indicates that at least part of the reaction funnels through a kinetic cycloaddition product that differs from the final observed diastereomers.

[52] Lowering the reaction temperature resulted in poorer conversion and increased by-product formation. A brief survey of solvents indicated that only benzene produced results comparable to Et_2O.

[53] Fürstner observed a slightly different 1.1: 1.0:1.3:1.6 ratio of diastereomers at equilibrium. His stereochemical assignments relied on NMR studies.[4,5]

[54] The crude ^{1}H NMR spectra show only minor variations suggesting that the three products are simply stereoisomers. The ratio of the compounds varies in $CDCl_3$ at room temperature, ultimately reaching a presumed thermodynamic mixture (ratio 1.4:1.1:1.0).

[55] It is hard to envision a pathway from endocyclic enol ether **48** to exocyclic enol ether **41**, merely by increasing the amount of $AgSbF_6$.

[56] Increasing amounts of $AgSbF_6$ would also shift the equilibrium from **33**-lactol (via open chain isomer **33**) to silver enolate **47**, thereby increasing the concentration of primary alcohol required for cycloisomerization to exocyclic enol ether **41**.

[57] Mata, E. G.; Mascaretti, O. A. *Tetrahedron Lett.* **1988**, *29*, 6893–6896.

[58] Ando, K.; Takemasa, Y.; Tomioka, K.; Koga, K. *Tetrahedron* **1993**, *49*, 1579–1588.

[59] Due to volatility, **54** was not isolated but used as a crude concentrated THF solution.

[60] This 2:1 ratio of **57a**:**57b** indicates a mismatch with the chiral quaternary center given that the same reaction with the achiral gem-dimethyl substituted substrate **31** delivered *anti*-**35** with 59% ee (a 4:1 ratio of enantiomers, Scheme 4).

Reaction of **56** with the achiral allenyl cuprate (as in Scheme 4) delivered a 1:1 ratio of **57a**:**57b** but with a lower *anti*:*syn* selectivity (5:1).

[61] Full structural assignment of diastereomers **60a-d** was impossible due to our inability to separate them. However, we presume that their relative stereochemistry represents that of the four *nor*-berkelate diastereomers **45a-d** by comparison of the ^{1}H NMR spectrum of the crude mixture of **59** / **60a-d** to the ^{1}H NMR spectra of pure **44** and **45a**-**d**. Specifically, correlation to the diagnostic phenolic and C15 ^{1}H resonances was used.

[62] Alkynols *ent*-**58a,b** were prepared in a manner similar to **58a,b**, but starting from D-valine derived enamine *ent*-**52**. Full experimental details can be found in the supporting information to reference 10.

[63] Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277–7287.

[64] Kraus, G. A.; Roth, B. *J. Org. Chem.* **1980**, *45*, 4825–4830.

[65] Genin, E.; Toullec, P. Y.; Antoniotti, S.; Brancour, C.; Genêt, J.-P.; Michelet, V. *J. Am. Chem. Soc.* **2006**, *128*, 3112–3113.

[66] A sixth diastereomer (**63f**) was observable in the ^{1}H NMR spectrum of the crude mixture but could not be isolated in pure form. Its identity was confirmed by comparison with the NMR spectrum of pure **63f** obtained from the equilibration of diastereomer **63c**.

[67] The structure of oxoberkelates **63a,c-f** was assigned via a combination of ^{1}H, ^{13}C, ^{1}H-^{1}H gCOSY, ^{1}H-^{13}C gHSQC, ^{1}H-^{13}C gHMBC NMR experiments and correlation to spectra obtained for the *nor*-berkelic acid methyl ester diastereomers **44** and **45a-d**. The structure of oxoberkelate diastereomer **62b** could not be assigned unambiguously beyond the relative C9-C15 *cis*-stereochemistry of the isochroman ring system.

[68] Arto, T.; Sáenz de Santa-María, I.; Chiara, M.-D.; Fañanás, F. J.; Rodríguez, F. *Eur. J. Org. Chem.* **2016**, 5876–5880.

[69] The original isolation paper mentions the testing of natural berkelic acid in the NCI 60-cell line panel with selective activity for the OVCAR-3 cell line. However, no actual data or description of the assay are included in the manuscript or associated supporting information file.[1]