Divergent Synthesis of Bioactive Dithiodiketopiperazine Natural Products Based on a Double C(sp^3)–H Activation Strategy

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Abstract: This article provides a detailed report of our efforts to synthesize the dithiodiketopiperazine (DTP) natural products (−)-epicoccin G and (−)-rostratin A using a double C(sp^3)–H activation strategy. The strategy's viability was first established on a model system lacking the C8/C8′ alcohols. Then, an efficient stereoselective route including an organocatalytic epoxidation was secured to access a key bis-triflate substrate. This bis-triflate served as the functional handles for the key transformation of the synthesis: a double C(sp^3)–H activation. The successful double activation opened access to a common intermediate for both natural products in high overall yield and on a multigram scale. After several unsuccessful attempts, this intermediate was efficiently converted to (−)-epicoccin G and to the more challenging (−)-rostratin A via suitable oxidation/reduction and protecting group sequences, and via a final sulfuration that occurred in good yield and high diastereoselectivity. These efforts culminated in the synthesis of (−)-epicoccin G and (−)-rostratin A in high overall yields (19.6% over 14 steps and 12.7% over 17 steps, respectively), with the latter being obtained on a 500 mg scale. Toxicity assessments of these natural products and several analogues (including the newly synthesized epicoccin K) in the leukemia cell line K562 confirmed the importance of the disulfide bridge for activity and identified dianhydrorostratin A as a 20x more potent analogue.

Introduction

Dithiodiketopiperazines (DTPs), namely 2,5-diketopiperazines (DKPs) containing sulfur atoms at positions 1,4 in bridged or open form, are an abundant family of natural products best known for their activity as anti-infective agents, among a large array of other biological properties.[1] In particular, (−)-epicoccin G (**1**), isolated from the fungus *Epicoccum nigrum*, has demonstrated antiplasmodial[2] and anti-HIV-1[3] activities while (−)-rostratin A (**2**), isolated from the fungus *Exserohilum rostratum*, was proven to be cytotoxic against HCT-116 cancer cells (Figure 1).[4]

In light of these interesting bioactivities, together with their intriguing sulfurated fused pentacyclic structure, this family of natural products has attracted the attention of synthetic chemists in recent years. However, DTPs featuring a fused pentacyclic diketopiperazine framework remain a major synthetic challenge, illustrated by the fact that only three research groups reported total syntheses of such compounds. Nicolaou and coworkers disclosed the first total synthesis of a DTP featuring a 6-5-6-5-6 system, namely (−)-epicoccin G (**1**) (Scheme 1).[5] This molecule was synthesized in 1.5% overall yield over 17 steps via the tetraene intermediate **9**, formed from dimerization of aminoester **7** (Scheme 1). The latter was obtained in 6 steps from commercially available *N*-Boc-L-tyrosine. Shortly after, the same group reported the collective synthesis of haematocin (**3**), emethallicin E and several other structurally related natural DTPs and analogues from the same chiral pool molecule.[2]

In the same year, the synthesis of the dihydrooxepine-containing 7-5-6-5-7 DTP acetylaranotin (**4**) was achieved by the

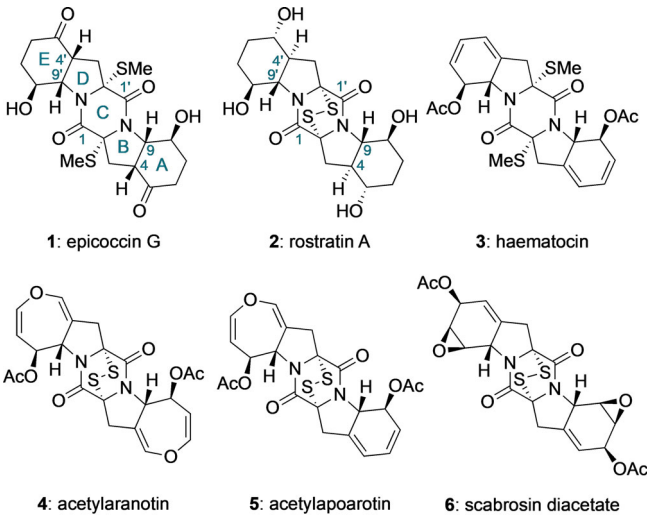

Figure 1. Selected natural DTPs with a pentacyclic framework.

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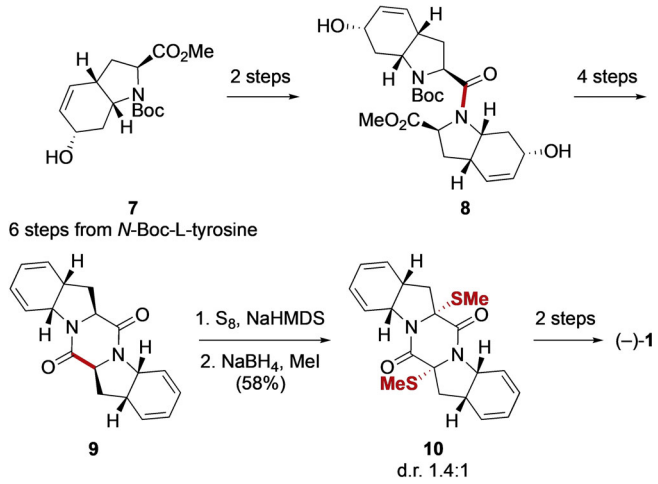
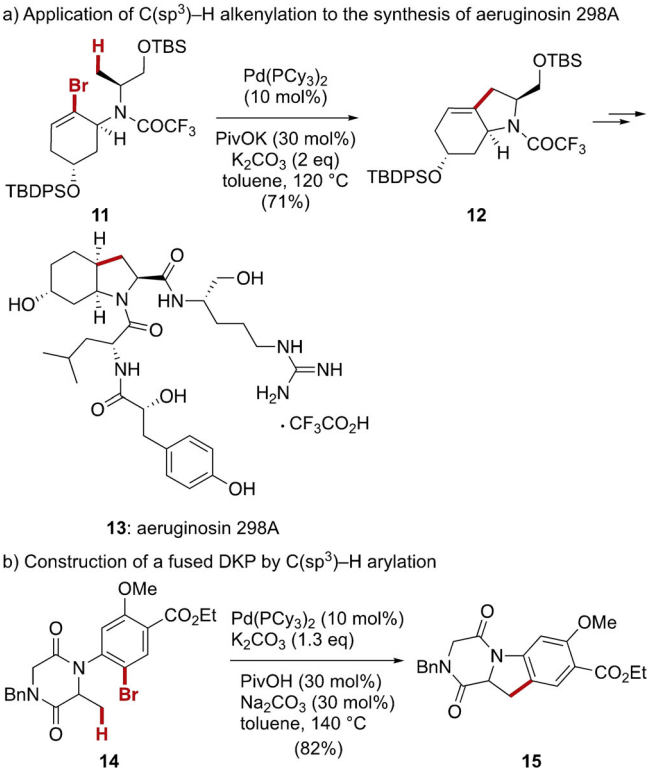


Scheme 1. Nicolaou's enantioselective total synthesis of (−)-epicoccin G (**1**).



Scheme 2. Precedents relevant to the current study.

groups of Reisman[6] and Tokuyama.[7] Reisman and co-workers further pursued their synthetic efforts toward DTPs and disclosed the synthesis of the challenging heterodimeric 7-5-6-5-6 DTP (−)-acetylapoaranotin (**5**).[8] Noteworthy, the strategies used in these DTP total syntheses are comparable, with the initial construction of the AB/DE bicyclic systems and the late-stage assembly of the central DKP ring C. In addition to these successfully completed total syntheses, a significant number of synthetic studies toward other DTPs were also reported.[9] Among them, unfruitful attempts from the Carreira, Diver, Metz and He groups to access the scabrosin esters,[10] such as scabrosin diacetate **6**, an epoxide-containing DTP with nanomolar in vitro activity against human breast MCF7 cancer cells,[11] highlight the challenges of total synthesis of natural products from this family.

In the past decades, C–H bond activation was successfully employed in total synthesis in order to simplify access to key intermediates, facilitate the end-game introduction of crucial functional groups and allow the use of easily available starting materials.[12] Overall, this implementation allows achieving higher overall yields with an improved step-economy. We and others contributed to this effort by developing methods based on Pd^0-catayzed C(sp^3)–H activation to build a large variety of carbo- and heterocyclic systems.[13] We demonstrated the applicability of these methods in natural product synthesis,[14] as illustrated with the total synthesis of aeruginosin 298A (**13**) (Scheme 2a).[15] In addition, our group reported a single case of intramolecular C(sp^3)–H arylation on a DKP scaffold, efficiently forming the tricyclic heterocycle **15** from aryl bromide **14** (Scheme 2b).[16]

In this article, we report in detail our investigations that led to the concise, scalable and divergent enantioselective total synthesis of two complex DTP natural products, (−)-epiccocin G (**1**) and (−)-rostratin A (**2**), which were synthesized for the second and first time, respectively.[17] This achievement was enabled by a new strategy based on an efficient double Pd^0-catalyzed C(sp^3)–H activation reaction as a key step. Structurally, these two natural products display distinctive oxidative degree at C5/C5′, different sulfurated functionalities at C2/C2′, and the presence of *cis* (**1**) or *trans* (**2**) A-B/D-E ring junctions. In particular, rostratin A contains unusual contra-thermodynamic *trans* ring junctions, adding supplementary challenges to its total synthesis. Indeed, DFT calculations indicated that the *cis,cis* 6-5-6-5-6 pentacyclic system is more stable than the *trans,trans* system by 5 kcal mol^{-1}.[17] To the best of our knowledge, no total synthesis of a *trans*-fused pentacyclic DTP natural product was reported prior to this study, thus underlining the difficulty to access these particular structures. The presence of four contiguous stereocenters makes this target even more arduous to synthesize. On a more technical note, the high polarity of **2** and related synthetic intermediates, conferred by the central DKP core along with the presence of four hydroxy groups, would likely make these compounds incompatible with common transformations requiring apolar and/or non-protic solvents, in addition to predictable purification issues requiring the exclusion of traditional aqueous workup.

The planned retrosynthetic analysis to access (−)-epicoccin G and (−)-rostratin A is depicted in Figure 2. We envisioned that the two targeted natural products could arise from a common intermediate **16** through redox manipulation of the C4-C5/C4′-C5′ double-bonds, allowing to install the correct oxidation states and missing stereocenters at these particular carbon atoms. This 'oxidase phase' would be followed by the late-stage introduction of the sulfur atoms at C2/C2′ in either dithioether or epidisulfide form, depending on the targeted DTP. Earlier studies already reported the sulfenylation of analogous DKP systems,[18,19] however this transformation was only

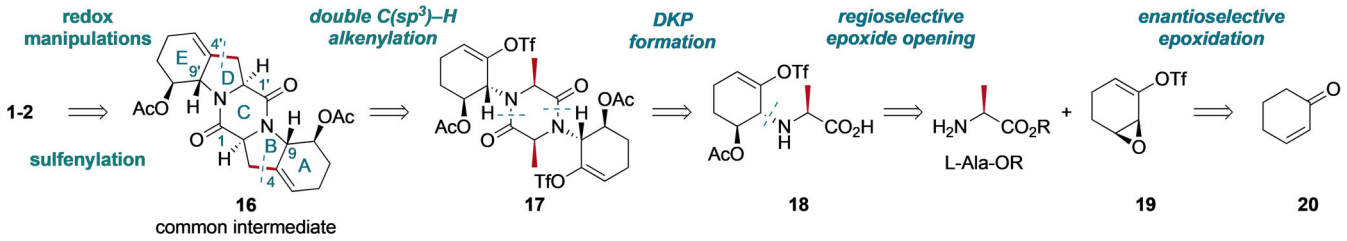


Figure 2. Retrosynthetic analysis.

rarely described on heavily functionalized substrates.[2,5–8] The common intermediate **16**, keystone of this divergent total synthesis, would be formed via a twofold application of our C(sp^3)–H alkenylation methodology, hence forging the C3-C4 and C3′-C4′ bonds in a single transformation from the divinyl triflate **17**. This double C(sp^3)–H activation reaction would be crucial to the success of the synthesis, and therefore would need to be efficient, high-yielding and easily scalable to allow substantial amounts of **16** to be quickly obtained, in order to explore different routes toward the DTP targets. Precedents for this type of intramolecular C(sp^3)–H alkenylation of cyclohexenyl bromides[20] and triflates[21] had been reported, but this transformation featuring a double C(sp^3)–H activation is unprecedented.[12g,22] The straightforward access to DKP **17** would be secured through cyclocondensation of amino acid **18**. The latter would be formed through the regioselective and stereospecific opening of the vinyl triflate-containing epoxide **19** by an L-alanine-derived ester. The opening of such an electron-poor epoxide was described with azide as the nucleophile,[23] but not with primary amines. Finally, epoxide **19** would be obtained from cyclohexanone **20** via enantioselective epoxidation. A major innovation in this retrosynthetic approach resides in the simultaneous construction of rings B and D from A-C-E precursor **17**, itself formed by early formation of the DKP ring C. Previous total syntheses of DTPs were rather based on the late-stage formation of ring C from AB/DE bicyclic systems via linear dimerization sequences,[2,5–8] with only rare approaches using a similar strategy.[10b,c] The current strategy should efficiently decrease the number of non-essential functional group manipulations, while significantly increasing the overall step-economy and efficiency for the synthesis of epicoccin G (**1**) and allowing a first access to the challenging rostratin A (**2**).

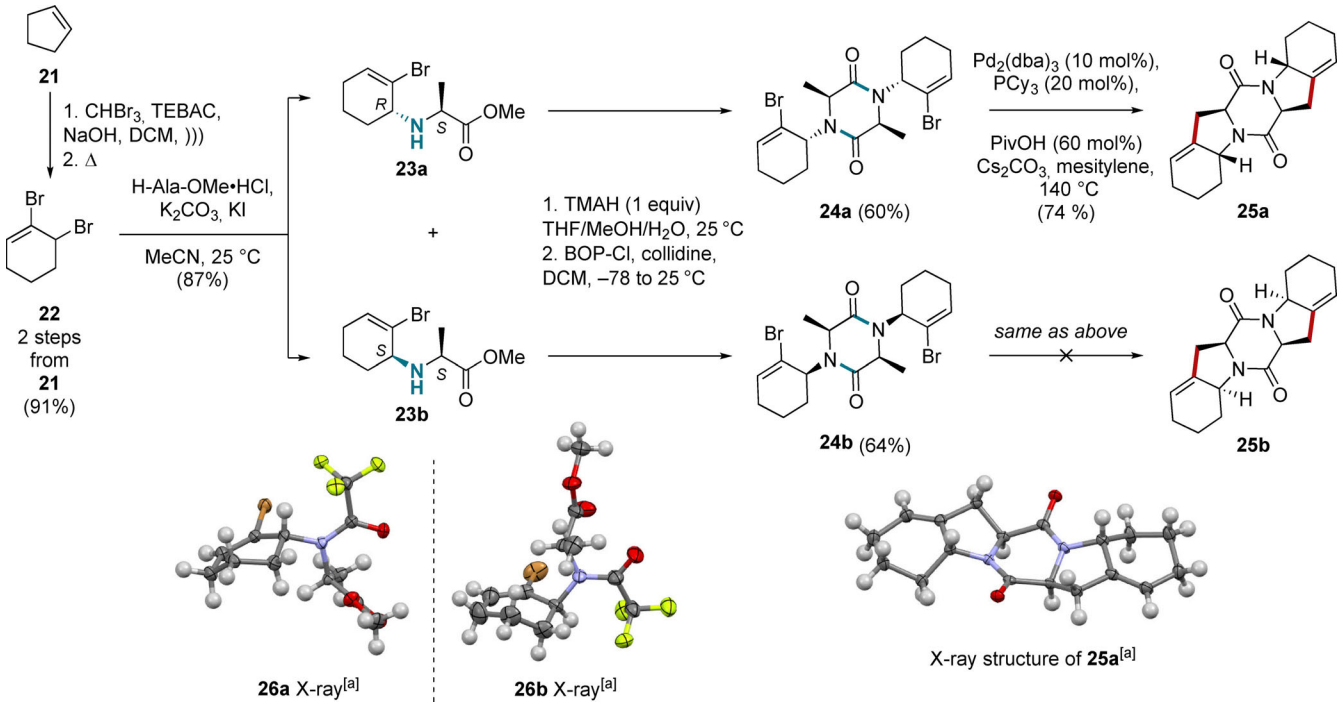
Results and Discussion

Model study

To test the feasibility of the key double C(sp^3)–H activation reaction, we decided to synthesize the model DKP substrate **24a**, which mimics compounds **17** but lacks the protected hydroxy groups at C8/C8′ (Scheme 3). To this purpose, we envisioned that the nucleophilic attack of methyl L-alaninate on dibromide **22** would allow a rapid access to the corresponding *N*-alkylated amino ester, both diastereoisomers of which could be separated and independently tested in the C–H activation step.

The synthesis of the model substrate started with the described dibromocyclopropanation of cyclopentene (**21**) and subsequent thermal electrocyclic ring-opening to yield dibromide **22** in 91 % yield over two steps, according to a method previously used in our group.[15,20,24] Bromoallyl bromide **22** was readily attacked by excess methyl L-alaninate in conjunction with potassium carbonate and catalytic potassium iodide to provide a 1:1 mixture of the two expected *N*-alkylated amino ester diastereoisomers **23a**,**b**, which were separable by column chromatography on silica gel. To assign their relative configurations, these diastereoisomers were readily derivatized as trifluoroacetamides **26a** and **26b**. Single-crystal X-ray crystallographic analysis of the latter allowed establishment of the (*R*,*S*) and (*S*,*S*) configuration, respectively. Therefore, amino ester **23a** possessed the same relative configuration as compound **17** (see Figure 2), but both compounds **23a,b** were further processed to compare the reactivity of the diastereoisomers **24a,b** in the C–H activation step.

We first tried to form the DKP ring through a classical linear sequence, as used for instance in the total synthesis of (−)-epicoccin G (see Scheme 1).[2,5] These attempts were unfruitful, largely due to the steric hindrance and lack of nucleophilicity of the nitrogen atom in **23a,b**, making the amide coupling and *N*-Boc protection at this position difficult. A more step-economical strategy, involving direct DKP formation from the free amino acid, as previously described by Reisman and co-workers in the total synthesis of acetylapoaranotin (**5**),[8] was then studied. Obtaining the neutral zwitterionic amino acids required for this cyclodimerization proved to be difficult, since they were too polar for a standard aqueous work-up. Attempts at isolating them as the lithium, sodium or potassium carboxylate salts resulted in the isolation of clean products, but in hydrated form, which was detrimental to the next coupling step. This issue was overcome by performing the cleavage of the methyl ester using one equivalent of tetramethylammonium hydroxide (TMAH). This delivered the TMA carboxylate salts in anhydrous form upon solvent removal, which were considerably more soluble in apolar solvents, essential to the subsequent dimerization step. However, these amino acid TMA salts failed to react with classic amide coupling reagent such as HATU, HBTU, BOP, DCC, pyBOP or EDCI, giving either no conversion or total decomposition. To our delight, treatment of the crude TMA carboxylates with BOP-Cl at low temperature yielded the desired DKPs **24a** and **24b**. Good yields (60–64 % for two steps) were achieved after a short optimization of this 2-step sequence, notably through the use of *sym*-collidine as a



Scheme 3. Model study. [a] Thermal ellipsoids shown at 50% probability.[25] TEBAC = benzyltriethylammonium chloride; TMAH = tetramethylammonium hydroxide; BOP-Cl = bis(2-oxo-3-oxazolidinyl)phosphinic chloride.

base in the cyclocondensation. At this point, we hoped to be able to transpose this cyclodimerization methodology to the synthesis of DTPs targeted in this study.

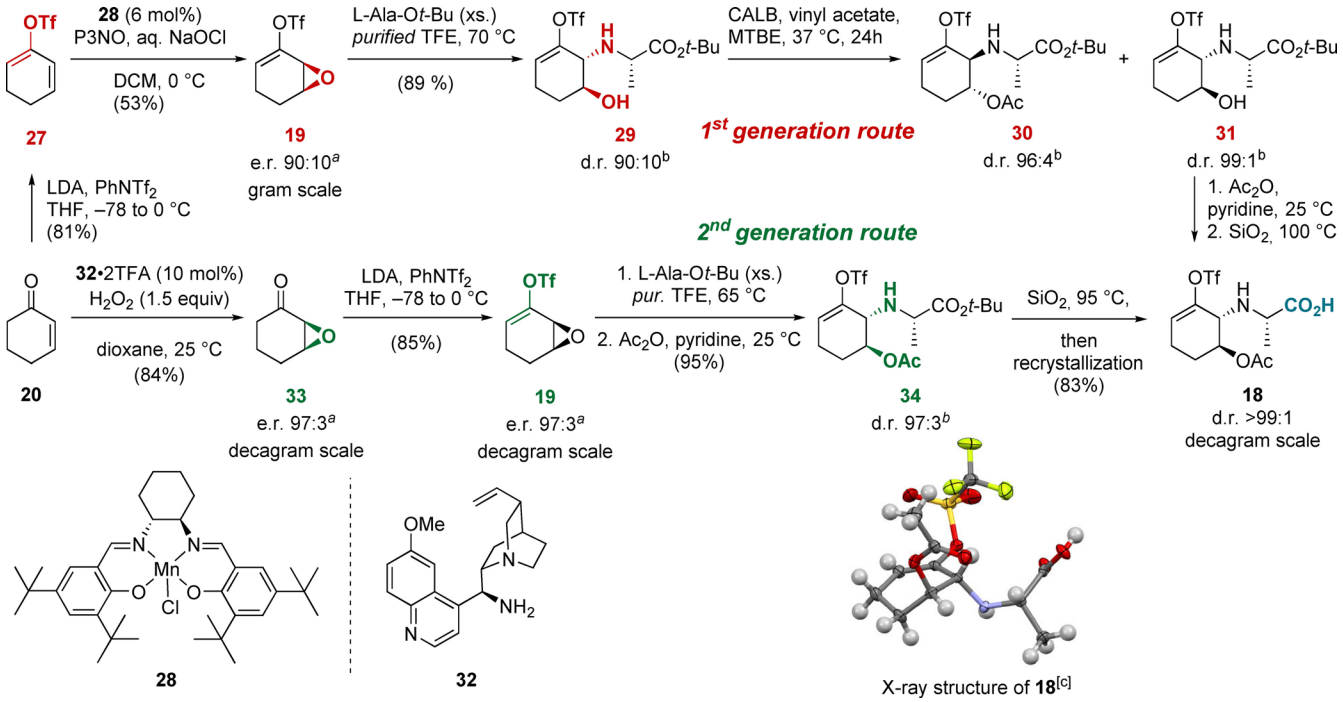
To complete this model study, the double C(sp^3)–H activation was attempted on both diastereoisomers **24a** and **24b** under conditions commonly used in such reactions,[13] employing Pd_2dba_3, PCy_3 and PivOH as catalysts. Interestingly, the double ring closure was only observed from **24a**, yielding the 6-5-6-5-6 pentacyclic product **25a** in 74% yield, the three-dimensional structure of which was confirmed by X-ray diffraction analysis. In contrast, the other diastereoisomer **24b** predominantly gave rise to the proto-dehalogenated product, along with various decomposition products, and the C–H alkenylation product **25b** was not detected. This interesting result could be explained by the higher ring strain to generate the pentacycle **25b** compared to **25a**, disfavoring the reductive elimination against other reaction pathways.[20] Fortunately, the diastereoisomer that underwent the double ring closure was the one possessing the appropriate configuration for the synthesis of DTP natural products.

In conclusion, by achieving the desired 6-5-6-5-6 DKP system **25a** in 37% over 6 steps, the model study established the feasibility of the key double C(sp^3)–H alkenylation. In addition, a new 2-step method was developed to access the required sterically hindered 2,5-DKP framework from the amino ester precursor via the TMA carboxylate salt under mild conditions.

Synthesis of the common intermediate

According to our divergent synthetic strategy, (−)-epicoccin G (**1**) and (−)-rostratin A (**2**) would be both obtained from the common intermediate **16**. The initial retrosynthesis, as depicted earlier (Figure 2), was akin to the model study, except for the early introduction of hydroxyl groups at C8/C8′, which would be enabled by regioselective and stereospecific opening of epoxide **19**. Similar to the model substrate **24a**, the C(sp^3)–H activation precursor **17** would be easily obtained from cyclodimerization of the corresponding amino acid unit **18**.

With this strategy in mind, we started to look for a scalable method to access epoxide **19** with high enantioselectivity. We first tested the Jacobsen epoxidation of dienyl triflate **27** (Scheme 4, top), in accord with previous work by Fuchs and co-workers,[23] using hydrogen peroxide as the stoichiometric oxidant and Na_3PO_4/NH_4BF_4 as additives. Dienyl triflate **27** was prepared in 81% yield from cyclohexanone **20** upon treatment with LDA and $PhNTf_2$. However, **27** was sensitive to oxygen, and quickly underwent decomposition and aromatization under air. Nevertheless, its enantioselective epoxidation using (*R*,*R*)-Jacobsen catalyst **28** under conditions described by Fuchs and co-workers allowed us to obtain the desired enantioenriched epoxide **19** in decent yield (typically 65–70%) but a disappointing *ee* of 60%. This result contrasts with the published result (86% yield, >91% *ee* described for this specific transformation),[23] which we failed to reproduce despite an extensive screening of conditions. Another Jacobsen catalyst-based methodology, also described by Fuchs and co-workers,[26] employed a combination of aqueous sodium hypochlorite and the pyridine-*N*-oxide P3NO, respectively as oxidant and co-cat-



Scheme 4. First and second generation routes toward the amino acid **18**. [a] Measured by GC on a chiral stationary phase. [b] Measured by ^{19}F NMR. [c] Thermal ellipsoids shown at 50% probability.[25] P3NO = 4-(3-phenylpropyl)pyridine *N*-oxide; CALB = Candida Antarctica lipase B; MTBE = methyl *tert*-butyl ether.

alyst. Once again, we failed to reproduce the reported enantioselectivity, but the vinylic epoxide **19** was nevertheless obtained in 58% yield and 80% *ee*, which was judged satisfactory enough to carry on with the planned synthesis.

The opening of epoxide **19** has previously been reported with azide, but never with less potent nucleophiles.[23] Performing this epoxide opening with an amino ester was deemed a significant challenge, both because of the reduced nucleophilic character of the amine compared to azide, but also because of the instability of epoxide **19** towards various conditions. Multiple conditions were screened, with only specific protic fluorinated solvent as HFIP and TFE providing encouraging results. The reaction of methyl L-alaninate with epoxide **19** gave, according to TLC-MS, the desired ring-opened product along with side-products arising from further nucleophilic attack at the electrophilic ester. These included the linear dipeptide, the corresponding DKP and the lactone arising from intramolecular cyclization of the alcohol (see the Supporting information for details). To solve this issue, we envisioned to replace L-alanine methyl ester with the bulkier *tert-butyl* ester, to shield the ester group from further nucleophilic attack. This strategy proved to be successful, and allowed exclusive access to the desired amino ester **29**. Of note, 15 equivalents of *tert*-butyl L-alaninate were necessary to reach complete conversion of the epoxide. Fortunately, when TFE was used as solvent instead of HFIP, 50–60% of this costly amino ester could be recovered upon completion of the reaction by simple distillation of the crude mixture. After optimization, the ring-opening occurred with complete regioselectivity and stereospecificity, providing amino-ester **29** as a 9:1 diastereoisomeric mixture, reflecting the initial enantiomeric ratio.

To resolve these two inseparable diastereoisomers, we turned our attention to the use of enzymes to selectively *O*-acetylate one of the two diastereoisomers, which would enable purification via classical chromatographic methods.[27] Upon screening, lipases AK Amano and from porcine pancreas were found to be inefficient for this transformation, but *Candida Antarctica* lipase B supported on acrylic resin (CALB), in the presence of vinyl acetate and using MTBE as solvent, conveniently delivered the *O*-acetylated minor diastereoisomer **30**, leaving the useful major diastereoisomer **31** untouched. To our delight, the secondary amine was not acetylated under these conditions. The successful conditions were adapted for scale-up to gram quantities by applying a mild vacuum (900 mbar) to the reaction mixture, thus allowing removal of the problematic acetaldehyde formed during the reaction. Upon simple column chromatography on silica gel, the desired free alcohol **31** was obtained in 99:1 d.r. and 92% theoretical yield. On the downside, this method suffered from reproducibility issues, especially on larger scale, and was highly dependent on the lipase batch used. The diastereomerically enriched secondary alcohol **31** was easily acetylated using simple treatment with acetic anhydride in pyridine, and subsequent facile cleavage of the *tert*-butyl ester occurred using silica gel under a nitrogen stream to furnish the key amino acid **18**. The latter was isolated as a single diastereoisomer, the absolute configuration of which was confirmed by Xray diffraction analysis. Of note, recrystallization attempts on the crude amino acid **18** possessing a d.r. of only 9:1 were not fruitful, making the enzyme-mediated resolution step mandatory.

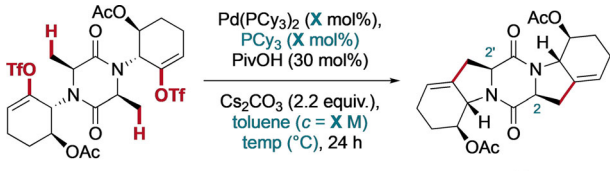
This first route to access key amino acid **18** (32% over 6 steps) was not judged sufficiently efficient, mainly due to the

low yields and enantioselectivity obtained in the first two steps to access epoxide **19**. To solve this issue, we searched for a better way to access **19** (Scheme 4, bottom). An inversion of reaction order and switching of epoxidation methods solved the problem. In particular, the use of List's organocatalytic epoxidation of cyclohexanone **20**, catalyzed by cinchona amine **32** and using aqueous hydrogen peroxide as the oxidant,[28] gave ketoepoxide **33** in excellent yield and enantioselectivity. Minor modifications of the initially described work-up were performed to make it safely scalable and to prevent volatility-related loss of product. This method was robust, easily scalable and routinely performed on a decagram scale. Next, the triflation of **33** was easily achieved via formation of the lithium enolate and treatment with phenyl triflimide to yield the desired alkenyl triflate **19**, with no loss of enantio-purity and in excellent yield over two steps (71%). In contrast to the Jacobsen epoxidation-based approach, the excellent enantiomeric ratio made the enzymatic resolution pointless, and following the same sequence as described earlier, the key amino acid **18** was obtained in 56% yield over 5 steps from **20** on a decagram scale.

With an efficient and scalable access to amino-acid **18** being secured, we turned our attention to the synthesis of diketopiperazine **17** (Scheme 5). As previously described in the model study, treatment of amino acid **18** with BOP-Cl and collidine efficiently triggered cyclodimerization and furnished the desired C_2-symmetric DKP **17** in excellent yield on a decagram scale. Noteworthy, as observed in the model system, all other coupling reagents tried (HATU, HBTU, EDCI, T3P, PyBOP) failed to provide any observable amount of DKP product.

With the bis-triflate **17** in hand, the double C(sp^3)–H activation was attempted. As expected from the model study, the desired cyclized product **16** was formed under similar conditions employing the well-defined $Pd(PCy_3)_2$ catalyst (Table 1). First, the optimal temperature was found to be 110 °C (entries 1–3), which is rather on the low end for Pd^0-catalyzed intramolecular C(sp^3)–H activation reactions. The reaction was also found to proceed efficiently at 100 °C (entry 2), albeit with much slower kinetics. Unfortunately, epimerization at the DKP was observed when operating at higher concentrations and lower catalyst loadings required for upscaling (entries 4–7). This problem could be due to the presence of undesired palladium species in the medium, which bind to the amide group of the DKP ring and increase the acidity at C2/C2'. In addition, the yield was found to be highly dependent of the quality of catalyst batch. In particular, premature catalyst decomposition led to unproductive mixtures including **16**, unreacted **17**, the mono C–H alkenylation product and proto-detriflated products. Adding free tricyclohexylphosphine (20 mol%) was found to be highly beneficial to solve these different issues, by completely suppressing epimerization and preventing catalyst decomposition. Moreover, this modification enabled us to increase the reaction concentration (up to 0.084 M) and to decrease the catalyst loading (down to 4 mol%) necessary to reach complete conversion (entries 8–13).

Table 1. Optimization of the double C(sp^3)–H alkenylation.

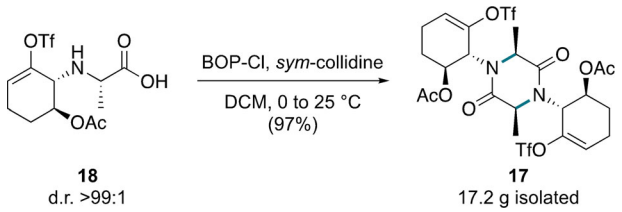


17 **16**

	$Pd(PCy_3)_2$ **X** [mol%]	PCy_3 **X** [mol%]	T [°C]	Conc. [mol L^{-1}]	Yield [%][a,b]
1	20	–	80	0.056	61 (0)
2	20	–	100	0.056	100[c] (2)
3	20	–	110	0.03	87[d] (3)
4	20	–	110	0.075	77[d] (18)
5	10	–	110	0.0125	99 (0)
6	10	–	110	0.05	100 (5)
7	10	–	110	0.1	95 (22)
8	10	20	110	0.025	100 (0)
9	10	20	110	0.075	100 (0)
10	5	20	110	0.084	100 (0)
11	**4**	**20**	**110**	**0.084**	**95[d] (0)**
12	3	20	110	0.084	89 (0)
13	1	20	110	0.084	41 (0)

[a] Determined by ^{1}H NMR spectroscopy using trichloroethylene as the internal standard. [b] Values in parentheses refer to the sum of other diastereoisomers determined by ^{1}H NMR. [c] After 48 h instead of 24 h. [d] Yield of the isolated product.

Thanks to this optimization, the double C–H alkenylation occurred in high yield and in a reproducible manner, and was gradually scaled-up to 17 g of substrate, furnishing almost 9 g of the key intermediate **16** in 93% yield, the structure and absolute configuration of which were confirmed by X-ray diffraction analysis (Scheme 6). Overall, the optimized route to this common intermediate was short and efficient (51% over 7 steps), thus securing an easy and scalable access and enabling further investigations toward the synthesis of DTP natural products.



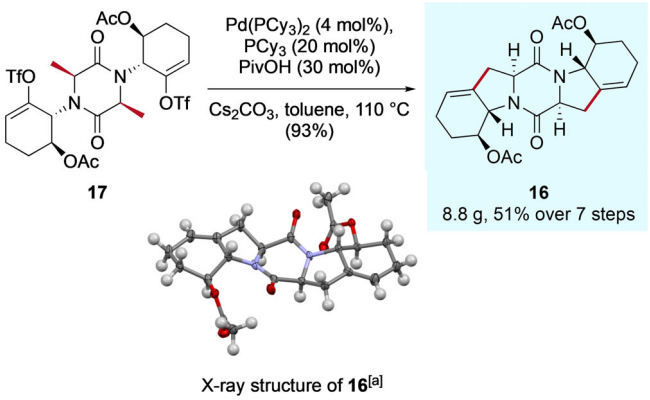
18
d.r. >99:1

17
17.2 g isolated

Scheme 5. Large-scale synthesis of DKP **17**.

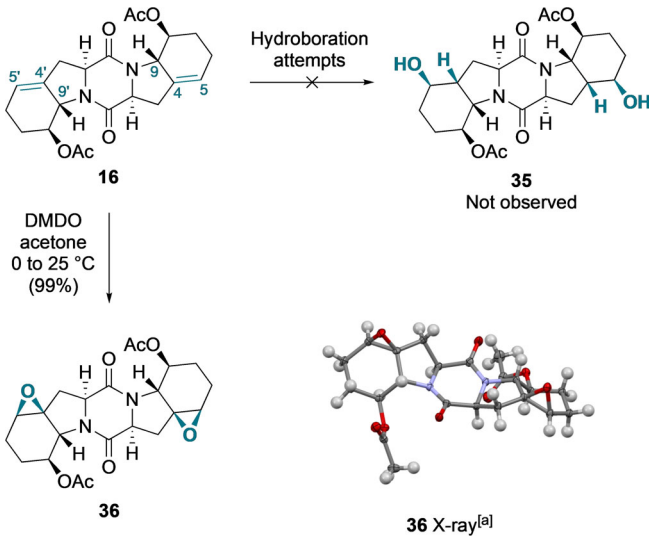
Synthesis of (−)-epicoccin G

(−)-Epicoccin G (**1**), already synthesized by Nicolaou and coworkers,[2,5] features a carbonyl group at C5/C5' along with *cis,cis* ring junctions between cycles A-B and D-E. The hydroxy groups at C8/C8' were already installed with the appropriate configuration in the common intermediate **16**. Therefore, the double bonds at C4-C5/C4'-C5' first needed to be transformed to install the C5/C5' ketones, followed by introduction of the methyl thioethers at C2/C2'.



Scheme 6. Scale-up of the key double C(sp^3)–H alkenylation. [a] Thermal ellipsoids shown at 50% probability.[25]

Our first investigations to convert the C4-C5/C4′-C5′ alkenes to ketones started with the classic hydroboration/oxidation strategy, as depicted in Scheme 7. Unfortunately, despite a thorough screening of different conditions including boranes, reaction time/temperature and oxidative work-ups, these attempts were unfruitful. Results varied from no conversion to the observation of the desired hydroxylation(s) together with concomitant reduction of the ester(s) and partial or total reduction of the DKP ring to piperazine. In these cases, the complex mixtures prevented any efficient purification to obtain the desired diol **35**, and this strategy was then abandoned. On the other hand, the epoxidation of intermediate **16** with a solution of DMDO in acetone gave bis-epoxide **36** in quantitative yield and complete *cis* diastereoselectivity with respect to the angular C9/C9′ hydrogen atoms, as confirmed by NOESY NMR and later by X-ray diffraction analysis. Noteworthy, other epoxidation methods such as peracid-mediated ones failed to provide complete conversion to the bis-epoxide.

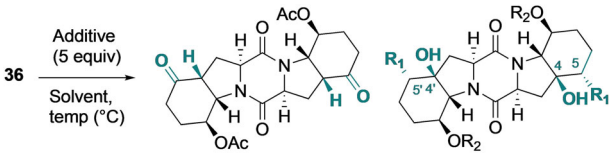


Scheme 7. Oxidation attempts on **16**. [a] Thermal ellipsoids shown at 50% probability.[25] DMDO = dimethyldioxirane.

With an efficient access to **36** being secured, and considering the known regioselective rearrangement of oxiranes to ketones,[29] compound **36** was reacted with a range of Lewis or Brønsted acids in the hope to generate the diketone **37** (Table 2). Unfortunately, the bis-epoxide was instead regioselectively and stereospecifically (as confirmed by NOESY NMR) opened at the C5/C5′ positions, despite the low nucleophilicity of the employed reagents and the use of acidic conditions which should favor opening at C4/C4′. Indeed, upon treatment with excess magnesium iodide (entry 3), indium chloride (entry 4), methanol (entry 7) or *p*TSA (entry 8), diiodo (**38a**), dichloro (**38b**), dimethoxy (**38c**)—with concomitant ester cleavage in this case—and disulfonyloxy (**38d**) -substituted products were obtained in good yields. Other conditions gave no conversion or total decomposition. Overall, the desired ketone **37** was never observed under the conditions tested, which might be explained by a particular epoxide geometry preventing this type of rearrangement.

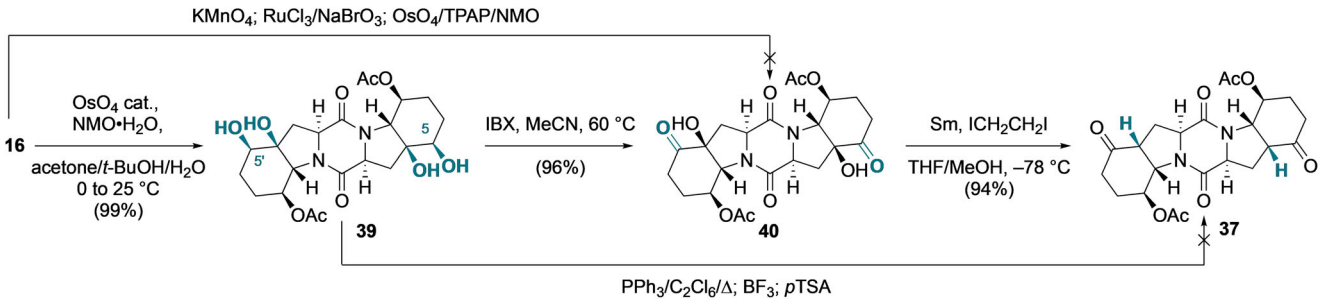
From these failures, a more indirect strategy was devised, going through bis-α-hydroxyketone **40**, which would undergo subsequent reduction to the desired diketone **37** (Scheme 8). The metal-mediated direct oxidation of **16** to the bis-ketol **40**, using potassium permanganate, ruthenium oxide, or the osmium tetroxide/TPAP system only gave poor conversions, along with reproducibility issues. A less direct approach was then designed via Upjohn dihydroxylation, which provided tetraol **39** in quantitative yield and, as previously observed for the epoxidation of **16**, complete *cis* diastereoselectivity. With tetraol **39** in hand, reported methodologies for the direct and regioselective transformation of vicinal diols to the corresponding ketones, including treatment with BF_3[30] or a Brønsted acid,[31] or via in situ formation of a cyclic phosphorane,[32] were attempted, but without success. Therefore, a two-step sequence was pursued, starting with the oxidation of secondary alcohols at C5/C5′ to provide the desired bis-α-hydroxyketone

Table 2. Attempts at rearranging bis-epoxide **36** to diketone **37**.



	Additive	Solvent	T [°C]	Yield **37**/**38** [%][a]	Structure **38x**/ observation
1	$LiClO_4$	MeCN	80	0/0	no conversion
2	InI_3	DCM	40	0/0	no conversion
3	MgI_2	MeCN	25	0/87	**38a**: R_1 = I, R_2 = Ac
4	$InCl_3$	THF	80	0/59	**38b**: R_1 = Cl, R_2 = Ac
5	$BF_3{\cdot}Et_2O$	DCM	25	0/0	decomposition
6	SiO_2[b]	–	200	0/0	decomposition
7	Amberlyst 15	MeOH	25	0/91	**38c**: R_1 = OMe, R_2 = H
8	*p*TSA	DCM	25	0/95	**38d**: R_1 = OTs, R_2 = Ac
9	TfOH	DCM	0	0/0	decomposition

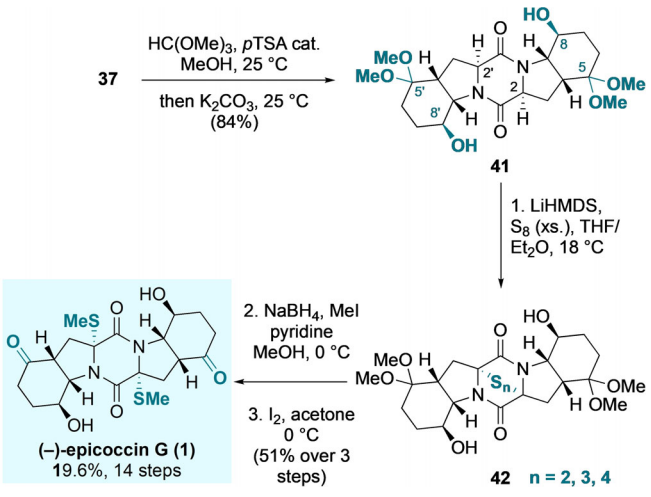
[a] Yield of the isolated product. [b] 50 equiv pTSA = p-toluenesulfonic acid; TsO = p-toluenesulfonate.



Scheme 8. Three-step access to diketone **37**.

40. Different conditions were tested, and IBX in hot acetonitrile was found to perform best (96% yield). The subsequent reduction of these α-hydroxyketones with freshly prepared samarium(II) iodide at low temperature in THF and with methanol as a protic co-solvent, as previously described by Molander and co-workers,[33] was found to be highly efficient. The desired diketone **37** was obtained in high yield (94%) and perfect diastereoselectivity, favoring as expected the thermodynamically favored *cis* ring junctions. Overall, the desired diketone **37** was obtained with an excellent yield (89%) over 3 steps from the common intermediate **16**.

Finally, with all stereocenters set on the pentacyclic scaffold, the last challenging step to complete our synthesis of epicoccin G was the introduction of the methyl thioether groups at C2/C2′ (Scheme 9). After reviewing the literature, we decided to use the method developed by Nicolaou and co-workers for the *cis*-sulfenylation of diketopiperazines,[18] which features mild conditions and was used in previous total syntheses of DTPs with good results. We sought to employ an adequate precursor for this transformation, which would not possess any enolizable position other than those on the DKP ring to avoid competitive sulfenylation. Furthermore, we hypothesized that the free hydroxy groups at C8/C8′ would provide a beneficial directing effect favoring the $C2_{\alpha}/C2'_{\alpha}$ diastereoisomer. Indeed,

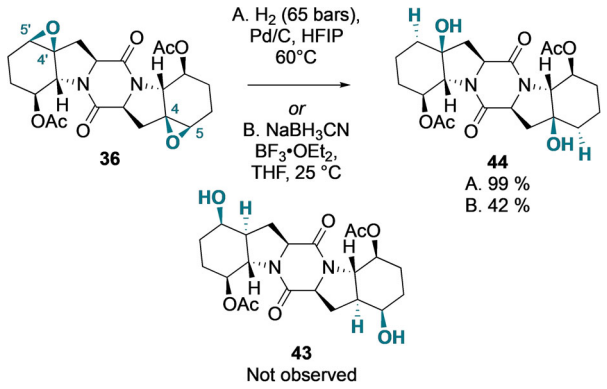


Scheme 9. Completion of the synthesis of (−)-epicoccin G. LiHMDS = lithium bis(trimethylsilyl)amide.

the C8 and C8′ hydroxy groups are, according to molecular models, spatially close to the C2′ and C2 positions, respectively. This analysis is consistent with previous examples for which diastereoselectivity was total for this type of transformation when free alcohols were present at these positions.[2,6–8] Moreover, the latter were absent during the sulfuration stage in Nicolaou's synthesis, which led to the formation of a 1.4:1 mixture of *cis* diastereoisomers epimeric at both C2 and C2′, as depicted in Scheme 1.[5] Following this analysis, **37** was first protected as a dimethyl ketal and deacetylated in a one-pot fashion. A mixture of methyl orthoformate and methanol was used in the presence of a catalytic amount of *p*TSA. Upon complete conversion to the diketal, addition of potassium carbonate effected the desired transesterication to give diol **41** in 84% yield. The sulfenylation of the latter was then performed, as planned previously, under conditions adapted from Nicolaou and co-workers. To our delight, a complete diastereoselectivity was observed for the corresponding mixture of epidi-, tri- and tetrasulfides **42**, as confirmed by the successful synthesis of **1**. This mixture was then reduced and methylated in a one-pot fashion to provide the C2/C2′ methyl thioethers. The last required transformation to access (−)-epicoccin G was the deprotection of the C5/C5′ ketals, which proved challenging due to the lability of thiomethyl ethers under conditions traditionally employed to deprotect dimethyl ketals, i. e. acidic hydrolysis or Brønsted acid-catalyzed transketalization. Gratifyingly, a mild treatment with catalytic iodine in acetone[34] for 2 min proved successful to access the desired natural product. Overall, (−)-epiccocin G (23 mg) was synthesized in 19.6% yield over 14 steps from cyclohexenone **20**, which constitutes a significant improvement compared to the first synthesis (1.5% over 17 steps).[2,5]

Synthesis of rostratin A

Rostratin A (**2**) possesses two stereocenters more than epicoccin G (**1**). In addition, its *trans-trans* ring junctions makes it arguably more challenging to access. The first strategy that we considered was the early installation of the *trans-trans* ring junctions. To this purpose, we decided to take advantage of the previously prepared bis-epoxide **36** by reducing the epoxides in a regioselective and stereospecific way at C4/C4′ to obtain the diol **43** with the desired configuration (Scheme 10). Upon screening, only a few conditions proved successful to re-



Scheme 10. Reductive opening of bis-epoxide **36**.

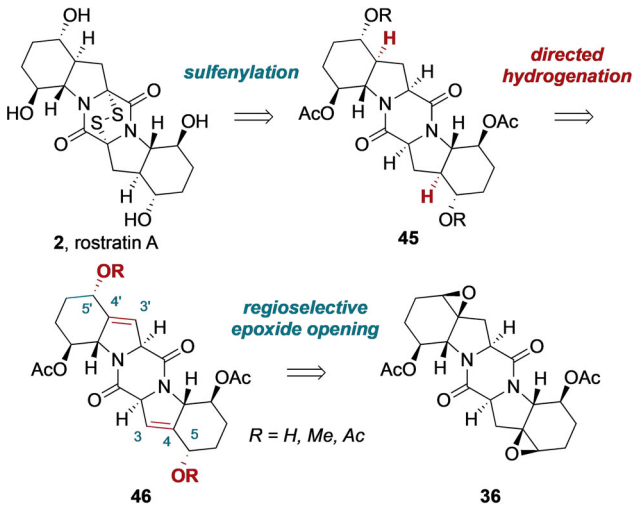
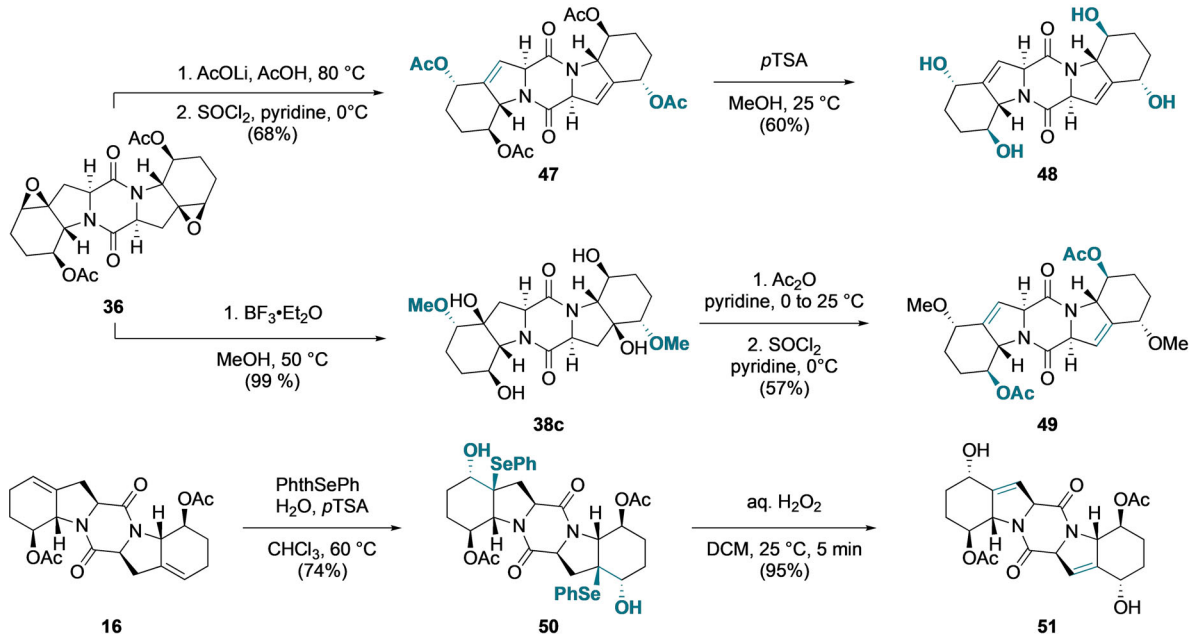


Figure 3. Retrosynthetic analysis based on directed hydrogenation.

ductively open the bis-epoxide. Hydride-mediated reductive epoxide opening using $NaBH_3CN/BF_3$ and Pd-catalyzed hydrogenation at 65 bars in HFIP failed to afford the desired product, but provided instead the tertiary alcohols (**44**) with complete regioselectivity for the opening by the hydride at C5/C5′, as determined by DEPT and NOESY NMR analysis. These observations were consistent with previous attempts to rearrange/open bis-epoxide **36** (see Table 2).

After these failures, we decided to take advantage of this undesired ring-opening regioselectivity. The new strategy, as depicted in Figure 3 would consist of performing directed hydrogenations of C3-C4/C3′-C4′ double-bonds from $C5_\alpha/C5'_\alpha$ free or substituted allylic hydroxy groups, as described by Crabtree and co-workers.[35] The C3-C4/C3′-C4′ double bonds of the corresponding hydrogenation substrates **46** would arise from the dehydration of C4/C4′ hydroxy groups, whereas the free or protected hydroxy groups at $C5_\alpha/C5'_\alpha$ would be obtained from the regioselective and stereospecific opening of bis-epoxide **36**.

Different precursors for this directed hydrogenation were then prepared, containing various directing groups at the $C5_\alpha/C5'_\alpha$ positions (Scheme 11). The tetraacetylated precursor **47** was easily obtained by regioselective opening of bis-epoxide **36** with excess lithium acetate in acetic acid and elimination of the resulting tertiary alcohols using $SOCl_2$/pyridine. The dimethoxy analogue **49** was prepared in a less direct fashion via epoxide opening of **36** with methanol in the presence of boron trifluoride etherate, with concomitant cleavage of the acetyl ester to provide tetraol **38c** in quantitative yield. The latter was then selectively reprotected at C8/C8′, and upon dehydration of hydroxy groups at C4/C4′ the dimethoxylated unsaturated precursor **49** was obtained (56% yield over 3 steps from **36**). According to previous reports, free hydroxy groups should be the most efficient to direct olefin hydrogenation at allylic



Scheme 11. Synthesis of the directed hydrogenation precursors.

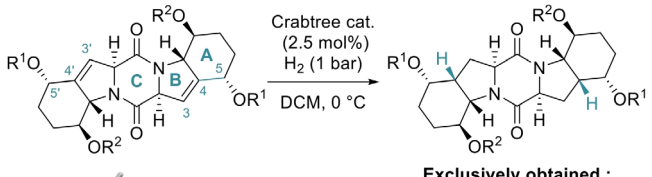
positions.[35] Tetraol **48** was thus synthesized by acidic transesterification of tetraacetate **47**. However, we expected its high polarity to be problematic for the Crabtree hydrogenation, which needs to be carried out in non-polar aprotic solvents. In contrast with **47, 48** and **49**, we were not able to obtain bis-allylic alcohol **51** from the bis-epoxide **36**, and a different approach was then used. Treating dialkene **16** with PhthSePh and water under acid catalysis formed diselenide **50**, which underwent oxidative elimination to provide the desired bis-allylic alcohol **51** in 71 % yield over two steps.

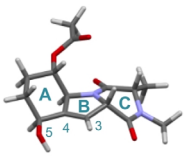
Unfortunately, subsequent hydrogenation attempts using Crabtree conditions and other related catalysts on these different substrates **47**, **49** and **51** yielded exclusively the undesired *cis,cis*-fused diastereoisomers, respectively **52**, **54** and **55**, in excellent yields, as confirmed by NOESY NMR (Scheme 12). Not even a trace of *trans,cis*-fused product was observed. Of note, tetraol **48** did not give any conversion toward **53** under these conditions, due to its poor solubility in dichloromethane. These disappointing results were rationalized upon examination of the DFT-optimized structure of an A-B-C truncated model **A** inspired from diol **51**. The dihedral angle C3(C3′)-C4(C4′)-C5(C5′)-O was indeed found to be close to 0°, meaning that the directing groups introduced at C5/C5′ may possess poor—if any—directing ability. In addition, cyclic tertiary amides are also known to direct the hydrogenation of alkenes under similar conditions,[36] and competitive binding of the iridium catalyst by the tertiary amides of the DKP would direct the hydrogenation toward formation of the undesired *cis*-fused rings. Another hypothesis is that with no competent directing groups available, the hydrogenation occurred on the convex face of the alkenes, thus providing the *cis,cis* diastereoisomer.

In a second attempt at using the directed alkene hydrogenation strategy to solve the *trans* ring fusion issue, we considered a C2/C2′ dihydroxylated DKP substrate. Such a compound was previously obtained by Reisman and co-workers during the synthesis of acetylaranotin (**4**).[6] The X-ray analysis performed in this study indicated that the hydroxy groups at the $C2_{\alpha}/C2'_{\alpha}$ positions would have the appropriate geometry to efficiently direct the hydrogenation of C3-C4/C3′-C4′ double-bonds with the desired diastereoselectivity. Bis-hydroxy-DKPs are easily accessible through oxidation at C2/C2′ thanks to the acidic nature of the C–H bonds at these positions, and would also serve as convenient precursors of the desired sulfurated groups at a later stage, as previously described.[37] To test this strategy, the previously obtained bis-allylic alcohol **51** (see Scheme 11) was protected with TBS groups, and the DKP ring was dihydroxylated through classic enolate oxidation method, i. e. deprotonation with *t*BuOK under oxygen atmosphere and with $P(OMe)_3$ as a reducing agent at low temperature (Scheme 13). To our delight, this two-step sequence furnished the desired dihydroxy-DKP **56** in good yield and with complete diastereoselectivity. However, when compound **56** was engaged in Crabtree's hydrogenation at different temperatures, no expected reduced product **57** was observed. Instead, we observed the appearance of a new bright spot under UV light during TLC analysis, which after complete characterization proved to be bis-pyrrole **58**. The latter was formed quantitatively, presumably via Ir^{I}-mediated dehydration.

These failures led us to switch again strategies, and to consider to employ a sterically controlled hydrogenation of C3-C4/C3′-C4′ alkenes in order to install the highly sought-after *trans,-trans* ring junctions. Indeed, careful examination of the DFT-optimized structure of an A-B-C truncated model **B** showed that the β face of the C3-C4/C3′-C4′ alkenes should be efficiently shielded by bulky silyloxy groups at $C5_{\beta}/C5'_{\beta}$ (Scheme 14). To test this hypothesis, an efficient access to dialkene **60** was first secured through a two-step sequence starting from previously obtained tetraol **39**. Bis-silyl ether protection via treatment by TBSOTf and collidine, and regioselective elimination of the tertiary alcohols using $SOCl_2$ and pyridine allowed synthesis of intermediate **60** on a multigram scale and in good yield (69 % over 2 steps).

An extensive screening of conditions for the diastereoselective hydrogenation of the C3-C4/C3′-C4′ alkenes was essential to achieve a satisfying level of diastereoselectivity toward the desired *trans,trans*-fused product **61**, together with scalability

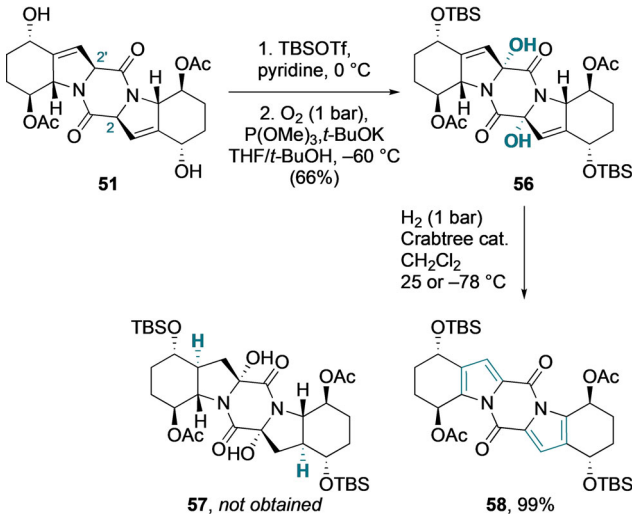




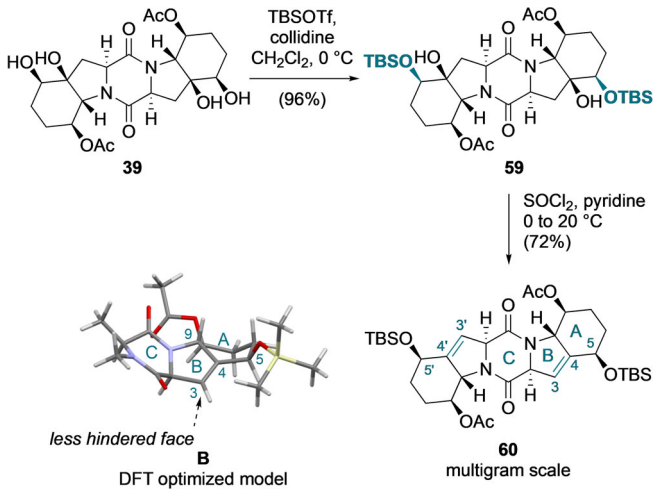
Crabtree cat. = $[Ir(cod)(PCy_3)(py)]PF_6$

Precursors	R^1	R^2	Products	Yield
47	Ac	Ac	**52**	95%
48	H	H	**53**	0%
49	Me	Ac	**54**	98%
51	H	Ac	**55**	92%

Scheme 12. Attempts at directed hydrogenation.



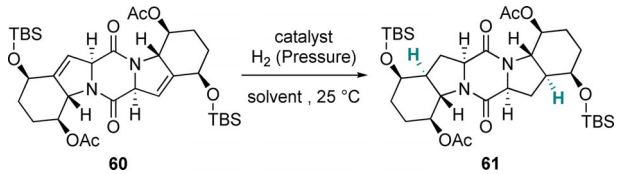
Scheme 13. Strategy employing a dihydroxy-DKP. TBS = *tert*-butyldimethylsilyl.



Scheme 14. Two-step access to dialkene **60** from tetraol **39**.

(Table 3). Palladium on charcoal was found to be the most competent catalyst for this challenging transformation, as Pt/C did not give any conversion (entry 1) and Rh/C readily deprotected the silyl ethers at C5/C5′, leading to low yields (entry 2). The solvent was also found to be a major parameter. Ethanol and ethyl acetate only provided low conversions and diastereoselectivities (entries 3–6), whereas the use of TFE or HFIP delivered the desired major diastereoisomer with a good conversion (entries 7–11). Of note, rigorous drying and neutralization of TFE via distillation over potassium carbonate was found to be essential to avoid premature TBS deprotection, presumably due to the presence of residual trifluoracetic acid in the commercial solvent. Moreover, a pressure of 45 bars of hydrogen was found to be optimal (entries 9, 11), since a lower pressure led to incomplete conversion (entry 8), and a higher pressure to partial or total reduction of the DKP ring (as observed by TLC-MS) alongside with a reduced diastereoselectivity (entry 10). The optimized conditions were used to perform the hydrogenation of **60** on scale (up to 4 g), providing an 81 % yield and a complete diastereoselectivity in favor of the *trans,-trans*-fused product, as determined by NOESY NMR, without any observable cleavage of the silyl groups.

Table 3. Optimization of the sterically-controlled hydrogenation of **60**.



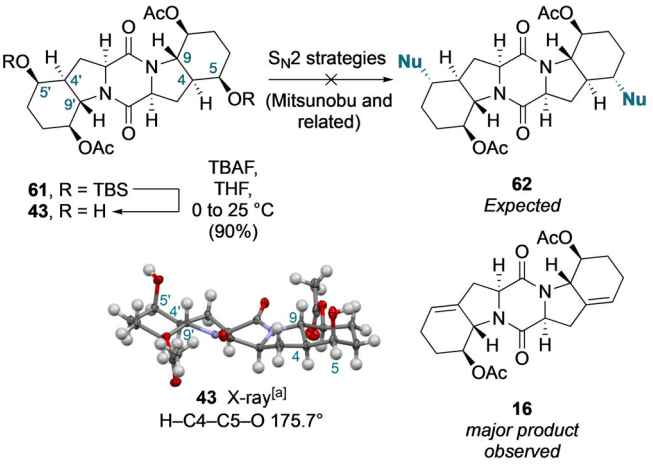
60 **61**

	Catalyst	Solvent	P_{H2} [bar]	Yield **61** [%][a]	d.r.[a]
1	Pt/C	AcOEt	20	0	–
2	Rh/C	AcOEt	20	6	–
3	Pd/C	AcOEt	1	0	–
4	Pd/C	AcOEt	10	26	2:1
5	Pd/C	AcOEt	50	48	8:5
6	Pd/C	EtOH	20	45	5:1
7	Pd/C	HFIP	3	61	10:1
8	Pd/C	TFE[b]	5	24	20:1
9	Pd/C	TFE[b]	50	79	>20:1
10	Pd/C	TFE[b]	100	51	8:1
11	**Pd/C**	**TFE[b]**	**45**	**81**[c]	**>20:1**

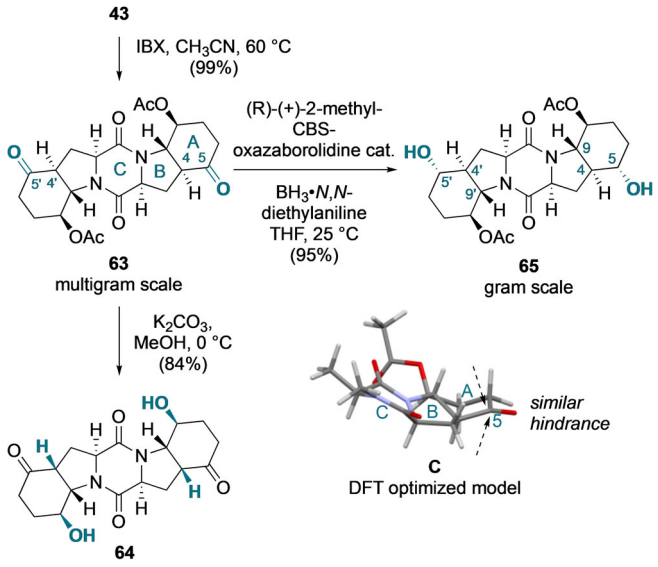
[a] Determined by ^{1}H NMR using trichloroethylene as an internal standard. Yields refer to the mixture of diastereoisomers. [b] Distilled over anhydrous K_2CO_3. [c] Yield of the isolated product. TFE = 2,2,2-trifluoroethanol.

With the bis-silyl ether **61** in hand, the TBAF-mediated deprotection to the corresponding diol **43** was readily executed, the X-ray diffraction analysis of which confirmed the *trans,trans* configuration set during the previous step (Scheme 15). Different approaches were then attempted to invert the configuration of the C5$_\beta$/C5′$_\beta$ alcohols and set the C5 $_\alpha$/C5′$_\alpha$ configurations present in rostratin A. Classic alcohol inversion strategies via S_N2 mechanisms such as the Mitsunobu or related reactions failed to accomplish this stereoinversion. Indeed, despite an extensive screening of conditions for the Mitsunobu reaction, in particular through modification of the pKa of the carboxylic acid partner by using *p*-nitrobenzoic or chloroacetic acid, no ester formation occurring with stereo-inversion was detected. Instead, the major product was invariably compound **16**, arising from double elimination, and regenerating the C4-C5/C4′-C5′ double-bonds. This result was rationalized upon examination of the X-ray structure of **43**, which revealed that the C5OH and C5′OH groups, which lie at axial positions of the chair-like rings A and E, are in nearly-perfect anti-periplanar relationship with the C4H and C4′H hydrogen atoms, respectively, with a mean dihedral angle H-C4(C4′)-C5(C5′)-O of 175.7°. This alignment makes *anti*-elimination a facile process upon activation of the hydroxy groups, efficiently favoring elimination over nucleophilic substitution.

To overcome this obstacle, we decided to rely on a less direct, but nevertheless classic oxidation/reduction strategy to invert the stereochemistry of secondary alcohols (Scheme 16). To avoid any epimerization at the presumably sensitive C4/C4′ positions in **43** during the oxidation process, the neutral oxidant IBX was utilized. For this oxidation, the freshness of the IBX reagent and a high dilution ($c < 0.044$ M) were found to be



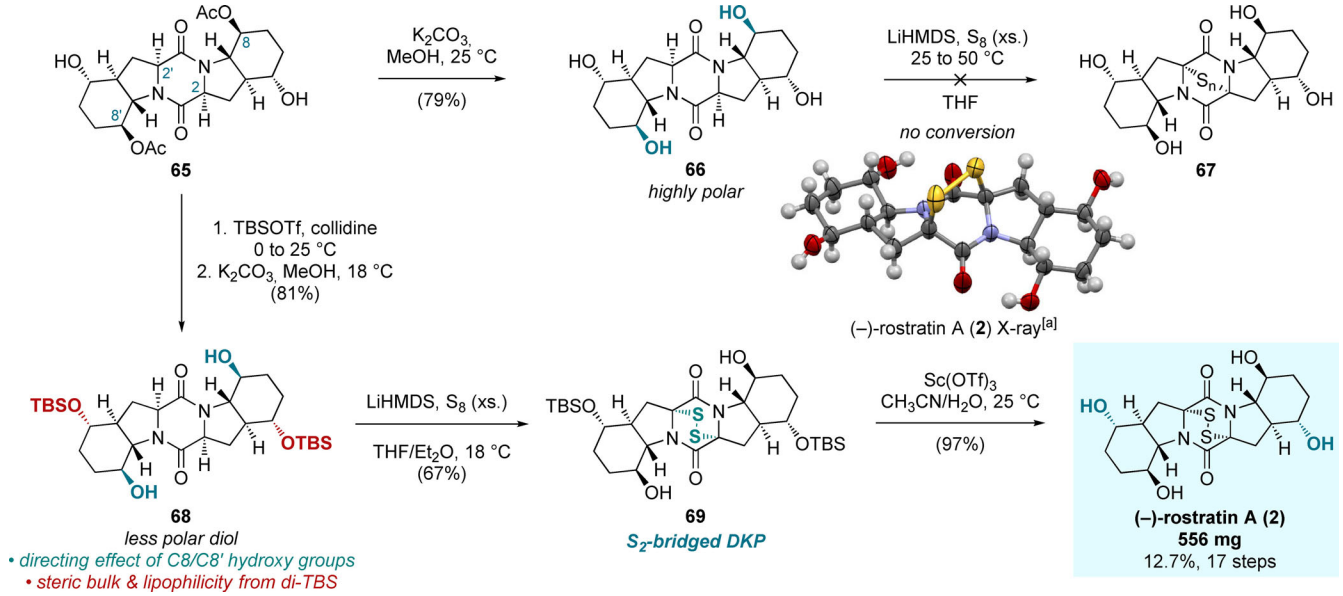
Scheme 15. Direct stereoinversion attempts on diol **43**. [a] Thermal ellipsoids shown at 50 % probability.[25] TBAF = nBu$_4$NF.



Scheme 16. Successful stereoinversion strategy based on oxidation and reduction.

essential to obtain consistent results. However, the purification proved to be difficult, the polarity of the resulting diketone **63** being too high to use a standard aqueous work up, and column chromatography on silica or alumina triggering epimerization at C4/C4', leading to the thermodynamically favored *cis-cis* ring junctions. This high propensity of diketone **63** to epimerize was proven by the facile formation of the *cis,cis*-fused product **64** upon deprotective transesterification of **63** despite the use of mild conditions. With these precautions, the oxidation with IBX furnished diketone **63** in quantitative yield on multigram scale upon simple filtration through Celite®. With diketone **63** in hand, we started investigations toward the diastereoselective reduction to diol **65**. The DFT-optimized truncated model **C** displayed no particular steric preference for the reduction of the C5 ketone toward formation of $C5_\alpha$. Hence, as expected, standard achiral metal hydride reagents such as sodium or lithium borohydride were inefficient, only yielding a complex and inseparable mixture of diastereoisomers. In addition, attempted ketone hydrogenations only gave poor conversions. To favor the formation of the desired diastereoisomer, it was envisioned to use a reagent-based stereochemical control, for example, with a chiral hydride reagent. However, different attempts using the (*R*)-(+)-2-methyl-CBS-oxazaborolidine together with catecholborane or $BH_3{\cdot}SMe_2$ under various conditions gave either decomposition or the undesired $C5_\beta/C5'_\beta$ diastereoisomer **43**. To our delight, employing conditions well-suited to aliphatic ketones,[38] i. e. a combination of CBS catalyst and borane·*N*,*N*-diethylaniline complex (DEANB), directly afforded the desired $C5_\alpha/C5'_\alpha$ diastereoisomer **65** with a diastereoisomeric ratio of 95:5. After optimization and transposition on a gram-scale, a slow addition of DEANB being essential on scale, diketone **63** was reduced to the desired $C5_\alpha/C5'_\alpha$ diastereoisomer **65** exclusively, in high yield (95 %) and without any epimerization at C4/C4'.

With all the required stereocenters in place, introducing the disulfide bridge was the last challenging step of this synthesis (Scheme 17). The most direct approach, i.e. deprotective transesterification to yield tetraol **66** and direct DKP sulfenylation according to Nicolaou's methodology, did not give any conversion to the desired epidisulfide **67**, even at higher temperature (50 °C). This result was expected, due to the significant polarity of tetraol **66** making it insoluble in the common aprotic solvents required for this transformation. To solve this problem, a more indirect strategy was envisioned. Diacetate **65** was subjected to TBS diprotection and subsequent acetate cleavage, which furnished the less polar diol **68** in high yield on a gram scale. To our delight, upon reaction optimization, submitting **68** to sulfuration using LiHMDS and elemental sulfur similar to



Scheme 17. Sulfenylation and completion of the synthesis of rostratin A (**2**). [a] Thermal ellipsoids shown at 50 % probability.[25]

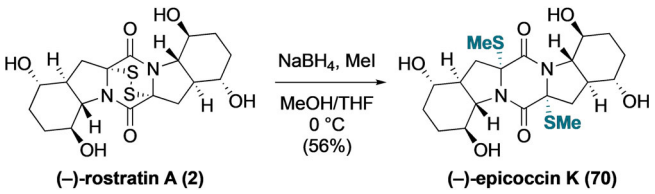
the synthesis of epicoccin G (see Scheme 9) provided predominantly the desired 2,2′-epidisulfide **69** in good yield. This result, in contrast to compound **41** which provided a mixture of C2/C2′ epipolysulfides **42** (see Scheme 9), was fortunate and suppressed the need for an additional reduction/reoxidation sequence. This interesting behavior might be explained by the steric hindrance brought by the TBS groups, disfavoring the formation of larger sulfur bridges (S_3 and S_4) that are otherwise typically observed. For the same reason, the reaction time was notably longer than for the sulfenylation of **41** during the synthesis of epicoccin G. Moreover, a reaction temperature of 18 °C was found to be essential to keep a correct balance between the open intermediate,[2] the desired product and decomposition products. Furthermore, the sulfuration of **68** occurred with exclusive $C2_\alpha/C2'_\alpha$ diastereoselectivity, as expected from the presence of free hydroxy groups at the C8/C8′ positions, as similarly observed during the synthesis of **1**. Last but not least, the deprotection of **69** to access **2** proved to be challenging. Indeed, disulfide bridges are known to be unstable under various conditions. Classical reagents known to cleave TBS groups such as fluorine-based ones (TBAF, HF·pyridine), Brønsted acids (HCO_2H, phosphomolybdic acid supported on silica, HCl) and oxidants (sodium periodate, cerium ammonium nitrate) led to either decomposition of **69** or gave no conversion at all. Only the mild scandium triflate-mediated[39] cleavage of silyl groups was successful, furnishing after a short optimization and scale-up rostratin A in excellent yield (97%). The physical properties of the synthetic material matched the reported data,[4] and a further structural proof was later provided by single-crystal X-ray diffraction analysis of the synthesized product. Rostratin A was synthesized for the first time, in 12.7% yield over 17 steps (see the Supporting Information for a complete Scheme from the common intermediate **16**). Upon extensive screening and validation of each step on a gram-scale, the synthetic sequence proved to be both reproducible and scalable, allowing the isolation of 556 mg of synthetic rostratin A in a single run.

Biological evaluation

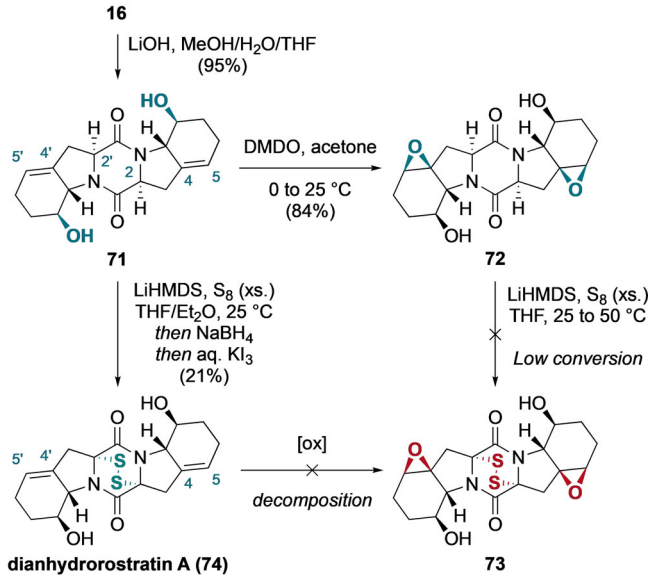
Previous reports suggested that the cytotoxicity of DTPs containing a disulfide bridge (epiDTPs) may stem from two general mechanisms.[1] First, a general scavenging of protective sulfur or selenium nucleophiles (i.e. thioredoxin or glutathione peroxidase), which leads to redox stress.[40] In a second mechanism, epiDTPs were shown to efficiently block the interaction between the transcriptional coactivator p300 and the hypoxia-inducible transcription factor-1α via extraction of a critical zinc ion in the transcriptional activation Zn-binding domain (TAZ) of p300. This results in a fast downregulation of hypoxia-inducible genes, which are critical for maintaining cell survival in the tumor microenvironment.[41]

With this in mind, and after having successfully completed the synthesis of two DTP natural products, we decided to evaluate the potential cytotoxicity of some intermediates synthesized during this study and to perform strategic modifications in order to look for more potent analogues. First, to further investigate the influence of the disulfide bridge in rostratin A on cytotoxicity, we synthesized (−)-epicoccin K (**70**) bearing methyl thioether moieties (Scheme 18). This transformation was easily achieved by one-pot reduction and methylation of the sulfur atoms of **2** using a previously described methodology.[2] The physical properties of synthetic (−)-epicoccin K matched the reported data.[3a]

Then, motivated by the reported nanomolar activity of scabrosins (Figure 1),[11] we targeted compound **73**, possessing an epidisulfide bridge and epoxides, which were deemed to be responsible for the high cytotoxicity of these compounds (Scheme 19). Taking advantage of the concise and efficient access to diene **16**, the latter was first hydrolyzed under basic conditions to provide diol **71** in excellent yield. Then, **71** was subjected to DMDO-mediated epoxidation, which efficiently provided bis-epoxide **72** in 84% yield. Unfortunately, application of sulfenylation conditions previously employed in this study to **72** only gave a low conversion and decomposition, with no desired C2/C2′ sulfenylation being observed. This result could be explained by the high polarity of this substrate (as previously observed for **66**), and by the sensitivity of the oxirane moieties to the reaction conditions. After this failure, we decided to change our strategy to access **73** by first introducing the sulfur bridge on **71** and then perform the epoxidation of double bonds at C4-C5/C4′-C5′. After a short optimiza-



Scheme 18. Synthesis of (−)-epicoccin K (**70**).



Scheme 19. Unsuccessful access to „scabrosin-like" compound **73** and synthesis of dianhydrorostratin A (**74**).

tion, the sulfenylation of **71** provided a mixture of C2/C2′ epipolysulfides, which was readily transformed to the pure epidisulfide **74**, termed dianhydrorostratin A, via a reduction/oxidation sequence in 21% yield over three steps. Unfortunately, different epoxidation conditions attempted on **74** only led to total decomposition and the formation of **73** was not observed.

With six compounds in hand that would interrogate various aspects of the cytotoxicity of the DTP core, we performed MTT assays in the leukemia cell line K562 (Figure 4). Importantly (and consistent with previous reports), all compounds lacking the key disulfide bridge (**1**, **66**, **70**) were completely inactive. The differences between the three active compounds revealed some important insights that warrant further study. The IC_{50} curve for rostratin A (shown in Figure 4A) indicated a modest activity of 42.9 μM. Interestingly, the bulky bis-TBS derivative **69** showed a significant improvement. The most potent variant, however, was the doubly-dehydrated molecule dianhydrorostratin A, with an IC_{50} of 2.0 μM. The result with this molecule is particularly intriguing in light of the fact that it resembles a dimeric version of another epiDTP natural product, gliotoxin. Gliotoxin is a potent cytotoxin because it interferes with thioredoxin redox cycling.[40] These results suggest several avenues of future work: Is thioredoxin inhibition the major cytotoxic mechanism of rostratins? Are rostratin-type epiDTPs covalently targeting the nucleophilic cysteine residues of thioredoxin? If the answer to these questions is yes, then it would be important to examine the toxicity of epiDTPs in cancer models susceptible to ferroptosis.[42] Our rapid synthetic access to this family of molecules should allow us to build pull-down probes for examining the protein targets of DTPs.

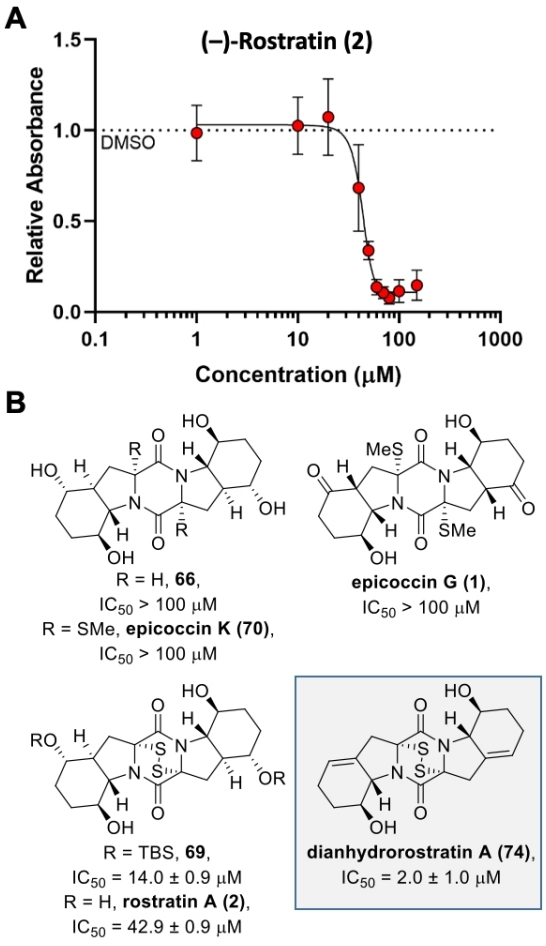


Figure 4. Cytotoxicity of DTPs in K562 cells. A) Representative IC_{50} curve with (−)-rostratin A showing clear dose-response behavior. B) Six DTPs tested for cytotoxicity and the corresponding IC_{50} values. Dianhydrorostratin A (**74**, blue box) was the most active in the series.

Conclusions

In summary, the enantioselective total synthesis of two dithiodiketopiperazine natural products was successfully achieved. (−)-Epicoccin G was synthesized for the second time in 19.6% overall yield over 14 steps, hence greatly improving the yield reported in the first total synthesis. (−)-Rostratin A was synthesized for the first time with a high overall yield of 12.7% over 17 steps. These two complex molecules were prepared from inexpensive starting materials via a novel strategy featuring a double C(sp^3)–H activation reaction, that allowed a rapid access to an advanced pentacyclic intermediate, which was the common precursor to both targeted molecules. The power of this strategy was further demonstrated by the isolation of more than 500 mg of the most challenging target, (−)-rostratin A, featuring two *trans* ring junctions, thanks to a careful structural analysis and the appropriate use of substrate- and reagent-based stereochemical control. The cytotoxicity of epicoccin G, rostratin A and four analogues, including epicoccin K, was evaluated on K-562 leukemia cells. This assay confirmed the importance of the disulfide bridge and allowed to identify dianhydrorostratin A as a 20x more potent analogue. As this molecule is the one that most closely resembles the monomeric epiDTP gliotoxin, these results suggest a study of rostratin derivatives in thioredoxin inhibition is important. Overall, our synthetic studies further illustrate the power of C–H activation to quickly access complex biologically active natural products with a high efficiency. Furthermore, the synthesis of other pentacyclic DTP natural products should be greatly facilitated by using a similar strategy.

Experimental Section

For full experimental procedures; spectroscopic and analytical data for all new compounds including copies of NMR spectra, see the Supporting Information. The Department of Chemistry of the University of Basel is registered at the BAFU (Bundesamt für Umwelt) with the registration number A120895 for conducting Class 1 activities.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: C–H activation · cytotoxicity · diketopiperazines · natural products · total synthesis

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