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A Biomimetic Strategy to Access the Silybins: Total Synthesis of (−)-Isosilybin A

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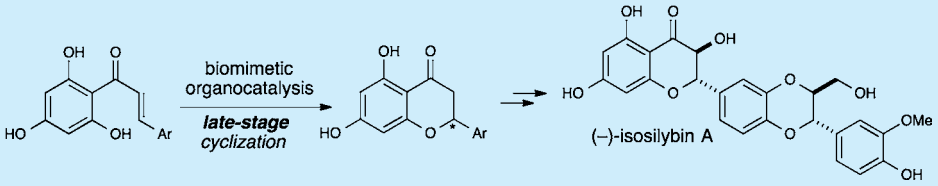
Supporting Information



ABSTRACT: We report the first asymmetric, total synthesis of (−)-isosilybin A. A late-stage catalytic biomimetic cyclization of a highly functionalized chalcone is employed to form the characteristic benzopyranone ring. A robust and flexible approach to this chalcone provides an entry to the preparation of the entire isomeric family of silybin natural products.

Milk thistle (*Silybum marianum*) has been used for centuries for the treatment of liver disorders and as a hepatoprotectant.[1] The extracts of the fruit (achenes) are known as silymarin and consist of a complex mixture of flavonolignans and flavonoids including silybin A, silybin B, isosilybin A, isosilybin B, silychristin, isosilychristin, silydianin, and dihydroquercetin, also known as taxifolin.[2] Our interest in these compounds is driven by the limited efficient asymmetric approaches for their construction,[3] their promising activity against prostate cancer,[4] and our ongoing translational interest in this area.[5] The members of the silybin family share a functionalized benzopyranone core, but differ in a benzodioxane core that is differentially substituted to produce regioisomers with complementary diastereomers (Figure 1).[6] While gram quantities of each isomer can be isolated via arduous processes,[7] no general and flexible stereoselective routes to these molecules exist, which prohibits a detailed, molecularly driven understanding of their promising pharmacology.[8] Flavanolignans such as the silybin isomers originate from the cyclization of a chalcone to a stereodefined flavanone by chalcone isomerase (CHI) in an estimated S/R ratio of 100 000:1 (ee = 99.998%).[9] In nature, these simple flavanones are then further processed and functionalized to produce a vast array of flavonoids.[10] Given that the diversity-generating biosynthetic pathway occurs after CHI cyclization, our initial interest was to explore a general synthesis strategy that could regio- and stereoselectively produce each silybin member. In contrast to a general route following an early cyclization to the flavanone core followed by functionalization (biosynthesis), we considered that a late-stage, biomimetic cyclization could enable the stereoselective synthesis of the silybin isomers (chemical synthesis).[11] A major challenge to our approach would be the limited stereoselective methods to access complex flavanones from 2′-hydroxy chalcones in a manner similar to CHI.[3,12]

Our unified strategy to construct each member of the silybin family follows a biomimetic approach inspired by CHI, specifically the cyclization of an appropriate 2′-hydroxy chalcone substrate to forge the characteristic benzopyranone.[13]

Of the two different regioisomeric pairs (silybins A, B and isosilybins A, B), we selected the isosilybins as initial targets to pursue due to their superior activity against prostate cancer. The chalcone precursor requires (1) an aldol condensation between a sterically congested 2′,4′,6′-trisubstituted acetophenone and a 1,4-benzodioxane aldehyde and (2) careful orchestration of the alcohol/ether stereocenters in the aldehyde fragment **3** (Figure 1). Subsequent chalcone cyclization studies by Hintermann[14] toward flavanones and additional investigations by our group[15] have also provided key information about the subtle yet crucial aspects of conformational and electronic factors of the 2′,4′,6′-trisubstituted aryl ring vs typical 2′-monosubstituted substrates.[3] Reported herein is the first asymmetric, total synthesis of (−)-isosilybin A.

The synthesis of the requisite benzodioxane fragment began from commercially available vanillin (**4**), which was elaborated to cinnamate **5** via Boc-protection and the Horner−Wadsworth−Emmons olefination in 86% yield over two steps (Scheme 1). A reduction with DIBAL-H and subsequent dihydroxylation with AD-mix β yielded enantioenriched triol **6** in 69% yield.[16] A regioselective mesylation followed by treatment with a mild base afforded multigram quantities of enantioenriched epoxide **7** in 58% yield and 99:1 er over two steps.[17] Aldehyde **8** was coupled with the epoxy alcohol via a robust Mitsunobu reaction in 91% yield (>20:1 dr) on gram scale.[18] We found that the protection of the phenol of **4** with an electron-withdrawing group (i.e., Boc) was essential for stereochemical transfer. The use of a benzyl protective group led to incomplete conversion and low diastereoselectivity (2.5:1), presumably via partial or complete

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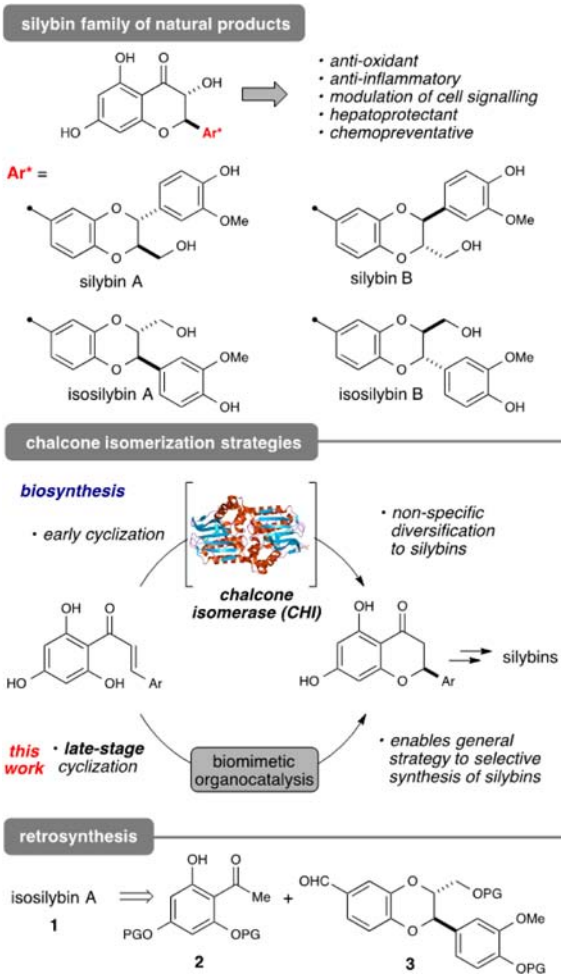
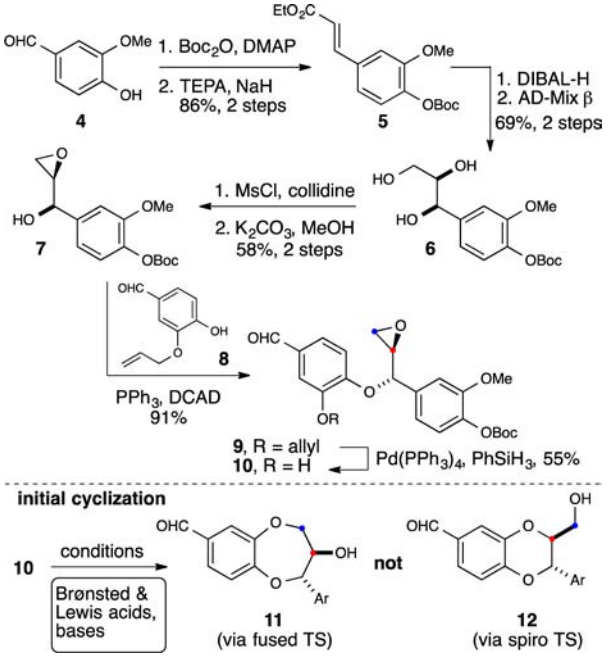


Figure 1. Synthetic strategy for the total synthesis of **1**.

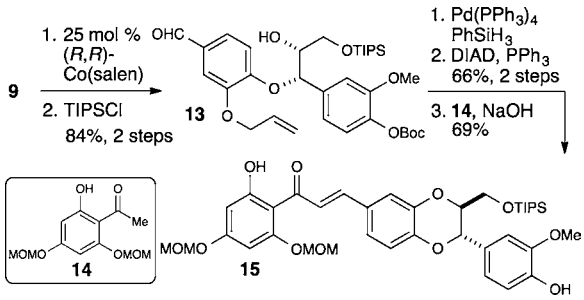
Scheme 1. Preparation of Epoxy Ether 10



formation of a *para*-quinone methide intermediate and subsequent S_N1-type addition of **8**.

With epoxy aldehyde **9** in hand, the removal of the allyl protective group under Pd(0) conditions occurred (55%, unoptimized) to provide a phenol that, upon subsequent treatment with potassium carbonate in MeOH, would yield the desired aldehyde **12** (Scheme 1). Unexpectedly, epoxide **10** underwent cyclization to provide preferentially the seven-membered benzodioxipine (**11**) vs the desired six-membered benzodioxane (**12**) under all conditions examined.[19] This challenging ring closure step was obviated by a Co(III)-catalyzed hydrolytic epoxide opening[20] of **9** and subsequent regioselective TIPS protection to provide secondary alcohol **13** on gram scale and in excellent yield (84% over two steps) (Scheme 2). A palladium(0)-catalyzed

Scheme 2. Generation of Chalcone 15



deallylation followed by a Mitsunobu ring closure provided the completed aldehyde fragment **3** in 66% yield over two steps. The subsequent aldol condensation with acetophenone **14** yielded the desired chalcone **15**.

With a robust route to the key chalcone substrate in place, attention was turned to the development of a catalytic system to promote formation of the benzopyranone ring with control at the newly established C-2 stereocenter. Based on previous reports[14,21] and our own knowledge with related cyclizations,[15] we envisioned bifunctional (thio)urea cinchona alkaloid catalysts would activate the carbonyl of the chalcone through hydrogen bonding, deprotonate the phenol with the quinuclidine nitrogen, and organize the chalcone complex, thereby allowing for asymmetric intramolecular conjugate addition of the phenol. Preliminary studies were carried out on the cyclization of naringenin dimethyl ether chalcone **16** (Table 1). Notably, the 2′- and 6′-phenolic oxygens must remain unprotected for successful cyclizations under these conditions.[22] Quinine and quinidine stereodivergently produced the desired flavanone with good stereoselectivity, but the overall process was extremely slow (Table 1, entries 1−2). The addition of Schreiner's thiourea[23] (**C**) accelerated reaction times, but resulted in lower stereoselectivity (Table 1, entries 3−4). Merging thiourea functionality and cinchona alkaloid scaffolds into a single compound based on Hiemstra's reports[24] (catalysts **D** and **E**) improved reaction times (<6 h). These pseudoenantiomeric catalysts[25] led to good diastereoselectivity (85:15) for the desired (*R*)-flavanolignan (with **F**) yet an almost racemic product (56:44) with **D** (Table 1, entries 5−6). Further studies to understand the stereochemical complexities of this cyclization (i.e., matched vs mismatched)[26] and the investigation of new catalysts to favor the (*S*) antipode in a manner that is complementary to selectivities observed with catalyst **F** are ongoing.[27]

Chalcone **17**, possessing an unsubstituted benzodioxane ring and unprotected 4′-phenol, was then explored as a simplified analog. Switching the solvent to MeCN allowed for a lower catalyst loading (15 mol %) without significant loss of

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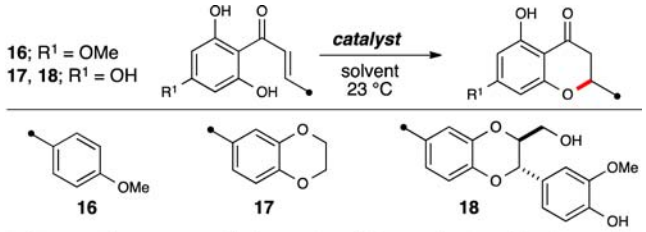
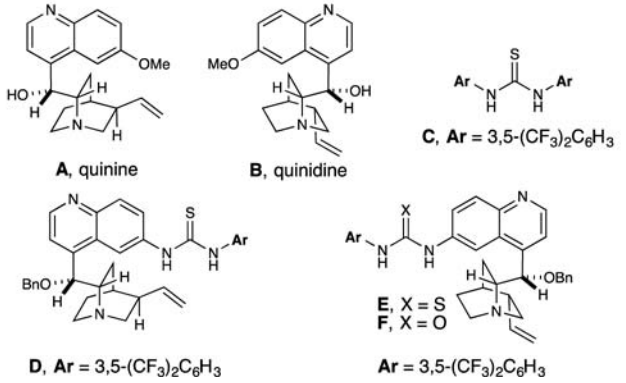
Table 1. Optimization of Biomimetic Chalcone Cyclization



entry	substrate	catalyst	cat. mol %	solvent	time[a]	*er/dr*
1	**16**	**A**	50	PhCl	9 d	80:20
2	**16**	**B**	50	PhCl	9 d	20:80
3[b]	**16**	**A + C**	50	PhCl	2 d	60:40
4[b]	**16**	**B + C**	50	PhCl	2 d	40:60
5	**16**	**D**	50	PhCl	<6 h	56:44
6	**16**	**E**	50	MeCN	<6 h	75:25
7	**17**	**D**	15	MeCN	48 h	65:35
8	**17**	**E**	15	MeCN	48 h	79:21
10	**18**	**C**	30	MeCN	–	50:50
11	**18**	**D**	30	MeCN	48 h	56:44
12	**18**	**E**	30	MeCN	48 h	83 :17
13	**18**	**F**	30	MeCN	36 h	85:15

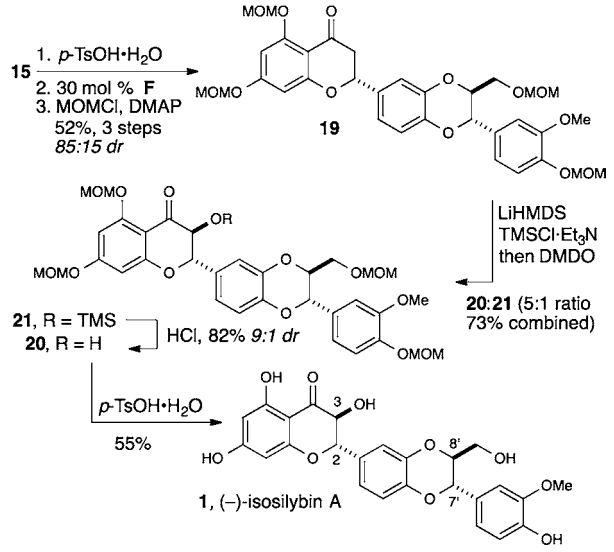


[a]Time required for complete consumption of starting material; yield not determined. [b]1:1 ratio of catalysts (50 mol % each).

stereoselectivity. The subjection of the fully elaborated substrate **18**[28] to the optimized conditions using catalyst **E** necessitated increasing the catalyst loading to 30 mol %, but afforded the product with 83:17 dr. Notably, the use of achiral thiourea **C** with chalcone **18** led to a 50:50 mixture of diastereomers, indicating the existing stereocenters in the benzodioxane portion do not exert stereocontrol over ring closure (Table 1, entry 10). Ultimately, urea analog **F** was determined to be the optimal catalyst with reduced reaction times (36 h vs 48 h) and a small improvement in stereoselectivity (entry 13).

Following our carefully optimized biomimetic cyclization, the free phenols and alcohols of the flavanone were globally protected for the Rubottom oxidation sequence.[29] While global Boc-protection was high-yielding, the resulting *tert*-butyl carbonates unfortunately impeded the oxidation of the resulting silyl enol ether. Presumably, these groups significantly decreased the nucleophilicity of the silyl enol ether and rendered it unreactive toward a wide variety of electrophilic oxidizing reagents (e.g., DMDO, MoOPh, *m*CPBA, and Davis oxaziridine). Thus, conditions were developed for an unusual global MOM-protection, which was challenging due to the stereoelectronic differences of the four free hydroxyl groups. Based on reports by Mander,[30] DMAP was a key additive to enable efficient global MOM-protection (72% yield, Scheme 3). At this point, flavonolignan **19** and its diastereomer were separated by semipreparative HPLC. The careful treatment of enantiopure MOM-protected **19** with $TMSCl/Et_3N$, followed by LiHMDS at −78 °C, provided the trimethylsilyl enol ether, which was treated

Scheme 3. Completion of the Total Synthesis of (−)-Isosilybin A



directly with DMDO to smoothly furnish a mixture of α-hydroxyl **20** and α-siloxy **21** in a combined 73% yield (5:1 ratio of **20**:**21**).

The α-siloxy flavanone (**21**) was cleanly converted to α-hydroxy flavanone **20** with treatment of dilute aqueous HCl. Unfortunately, minor erosion of the stereochemistry at the C2-position of **20** was observed, with enantiopure MOM-flavanone **19** yielding a 9:1 mixture of *trans* diastereomers. We attributed this minimal epimerization to ring opening during the enolization/silylation protocol. Unfortunately, exploration of soft enolization conditions resulted in complex mixtures of chalcone, flavanones, and silyl enol ether. Global MOM-deprotection was carried out using pTsOH·H_2O conditions to obtain isosilybin A (**1**).

Gratifyingly, our synthetic material matched data reported for the natural material by high-field NMR spectroscopy (^{1}H and ^{13}C).[31] Electronic circular dichroism (ECD) has been used extensively in the assignment of the absolute stereochemistry of flavanones[32] as well as the silybins.[2] Oberlies and co-workers have further validated the use of ECD to assign the stereochemistry of silybins via X-ray crystallography of a heavy atom analog of natural (+)-isosilybin A.[33] Through ECD and optical rotation measurements, synthetic **1** was determined to be the unnatural (−) antipode (see Supporting Information for this analysis). The application of this approach to other silybins is ongoing and ultimately should provide a general and unique method to fashion new silylbin analogs for structure−biological activity relationships.

In summary, the first synthesis of isosilybin A, a member of the silybin flavanolignans, was achieved in 16 steps (longest linear). The strategy employs a late-stage biomimetic cyclization of a chalcone to install the benzopyranone ring, which was inspired by the biosynthesis route employing chalcone isomerase (CHI). The applications of an asymmetric Sharpless dihydroxylation and Mitsunobu inversion transformations further provide a robust and flexible approach to selectively synthesize the benzodioxane ring systems of the silybin isomers. These stereoselective operations combined with our biomimetic cyclization to install the flavanone core ring system provide a general platform to access all silybins. This pursuit and the use of these molecules in biological investigations are ongoing and will be reported in due course.

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■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, characterization data, and spectra, including ^{1}H and ^{13}C NMR, ORD, and CD. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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