A diastereoselective oxa-Pictet-Spengler-based strategy for (+)-frenolicin B and *epi*-(+)-frenolicin synthesis

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Abstract
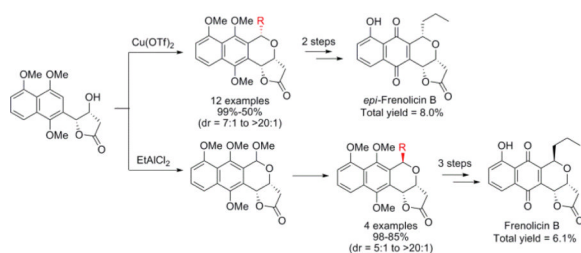
An efficient diastereoselective oxa-Pictet-Spengler reaction strategy was developed to construct benzoisochroman diastereomers. The utility of the reaction was demonstrated in the context of both the total synthesis of naturally-occurring pyranonaphthoquinones (+)-frenolicin B and *epi*-(+)-frenolicin B as well as a range of frenolicin precursor analogs. The method is versatile and offers exquisite stereocontrol and, as such, offers a synthetic advance for the synthesis of pyranonaphthoquinone analogs.

The pyranonaphthoquinone antibiotic (+)-frenolicin B (**1**) was isolated from a *Streptomyces roseofulvus* strain AM-3867.[1] Since this initial discovery, several members of the pyranonaphthoquinone family, including frenolicin B, have been explored in the context of anticoccidial, anti-cancer and anti-malarial lead development.[2] As a part of a new Appalachian-based natural product discovery initiative, [3] we recently isolated a bacterial strain (*Streptomyces* RM-4-15, isolated from soil samples near a thermal vent of the Ruth Mullins underground coal mine fire in eastern Kentucky) capable of producing a range of both known and new pyranonaphthoquinones, some of which displayed potent cancer cell line cytotoxicity *in vitro*. While such studies revealed new naturally-occurring bioactive chemical entities, low yields and variability in the production of pyranonaphthoquinone analogs via fermentation prompted the need for a complementary robust synthetic strategy to provide access to pyranonaphthoquinones of interest.

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Beginning with the first asymmetric total synthesis of (+)-frenolicin B (**1**) by Kraus and coworkers, [4] several alternative synthetic strategies have been put forth.[5] The most recent, by Fernandes *et al.*,[6] employed a Dotz benzannulation to construct the naphthquinone scaffold from Fisher carbene **3** and chiral alkyne **4** (Figure 1). While this method achieved the desired product, the approach was limited by the number of steps and, more notably, the lack of diastereoselectivity in the late stage oxa-Pictet-Spengler reaction (the degree to which is often dependent upon the substrate architecture).[7] To address the limitations of the prior study, herein we report the optimization and application of a diastereoselective oxa-Pictet-Spengler reaction to afford benzoisochroman **6** in good to excellent yields en route to the synthesis of frenolicin B, *epi*-frenolicin B and precursors for a diverse array of pyranonaphthoquinone analogs.[8]

Following prior precedent for the synthesis of intermediate **5** by Bruckner, bromonaphthlene **7** was initially synthesized from commercially available 1,5-dihydroxy- naphthlene via a slightly modified procedure (Scheme 1).[9] Heck coupling of **7** with isobutyl but-3-enoate afforded the desired ester **9** in good yield (84%). However, subsequent direct Sharpless asymmetric dihydroxylation of **9** failed to provide **5** in >30% yield. Suspecting steric infringement to be a limiting factor, simple isobutyl- to methyl-ester substitution (**9** → **10**) followed by Sharpless asymmetric dihydroxylation, afforded the desired alcohol **5** in good yield and excellent ee (>99.5%).[10]

With the core reaction established, we next explored optimization of the oxa-Pictet-Spengler reaction by first assessing the potential of the Lewis acid to favor the production of the α- or β-configured product. Among the Lewis acids tested, 50 mol % $Cu(OTf)_2$ offered the best yield and α-diastereoselectivity (Table 1, entries 2–7, see also Table S1 in Supporting Information).[11] Further investigation of solvents and reaction time in the context of the $Cu(OTf)_2$ reaction revealed a slight improvement in yield and dr ratio with overnight stirring in dichloromethane (Table 1, entries 8–10). In contrast, $FeCl_3$ was the only Lewis acid to favor the production β-configured product (2:1 β/α; Table 1, entry 13, and Supporting Information) and this poor diastereoselectivity could not be improved upon via further optimization (solvents, temperature and/or variant aldehyde source - data not shown).

Using the optimized conditions for α-configured benzoisochromane synthesis developed in Table 1, we next explored the scope of aldehyde substrates in the context of this reaction (Scheme 2). The aliphatic aldehydes tested led to the desired benzoisochromanes **6b– i** with equal or better diastereoselectivity than the model *n*-butyraldehyde reaction (**6a**) with one exception - isopropionaldehyde (leading to **6g**). Yields with the aliphatic set were also comparable to the model reaction (80%), with one exception – vinylacetaldehyde (leading to **6f**), possibly due to aldehyde decomposition.[12] For the aromatic aldehydes examined, the reaction afforded the desired products **6j–l** with comparative yields (86%) but varied diastereoselectivity with dramatic improvements in α-selectivity observed upon aromatic ring substitution. Neverthless, unlike most aliphatic counterparts, all the aromatic diastereomers were readily resolved via standard silica gel chromatography.

As previously indicated, $FeCl_3$ was the only Lewis acid to favor the production β-configured product (2:1 β/α; Table 1, entry 13 and Supporting Information). Thus, focus was next shifted to developing an orthogonal route to bias the production β-configured products. Considering that the oxocarbenium formed either by the oxidation of isochroman or by the reduction of isochroman acetal could be intercepted by a range of nucleophiles,[13] we envisioned the rigid lactone ring located on the *si* face of the corresponding oxocarbenium generated by isochroman acetal **8** to favor *re* face nucleophilic attack and thereby favor formation of β-diastereomer. Several C1 cation building block (formaldehyde, dimethoxymethane, methyl chloromethyl ether, methyl formate, and trimethyl

orthoformate)/Lewis acid combinations were examined for isochroman or isochroman acetal formation. Table 2 highlights optimization of the reaction containing **5** and trimethyl orthoformate ultimately affording isochroman acetal **8** in good isolated yield (70%) on reasonable scale (Table 2, entries 8–11). To test the feasibility of this strategy to access β-configured adducts, the reaction of acetal **8** with a small set of representative nucleophiles was subsequently examined (Scheme 3). As anticipated, both allyltrimethylsilane and cyanide led to β-configured products (**6f** and **12**, respectively) in 85% yield, the former of which could be hydrogenated to frenolicin precursor **6a**β. Acetophenone led to a 5:1 β/α product distribution, likely due to an influence of phenylnaphthalene π-stacking upon selectivity. Notably, compound **14** was inaccessible via the previously discussed oxa-Pictet-Spengler reaction with **5**.

Final maturation of **6a**β and **6a**α to the desired frenolicin B and *epi*-frenolicin B, respectively, followed a slight modification of previously reported strategies (Scheme 4).[6b] Specifically, while boron tribromide-mediated demethylation led to product epimerization in prior reports,[6b] we found this could be avoided by simply replacing boron tribromide with boron trichloride.

While **6a**β was previously reported to display notable cancer cell line cytotoxicity,[2d] the cytotoxicity of **6a**α was not previously reported. A comparison of the *in vitro* anticancer activities of **6a**α and **6a**β (Figure S1) revealed similar potencies against the colon cancer cell line HCT116 (IC_{50} of 109±24 nM and 215±21 nM, respectively) compared to a slight reduction (~5-fold) in potency for **6a**α against the non-small cell lung cancer cell line A549 (IC_{50} of 965±60 nM and 179±29 nM, respectively), suggesting the relative configuration of the C-ring to have a moderate influence upon cancer cell line cytotoxicity/specificity.

In summary, a diastereoselective oxa-Pictet-Spengler reaction has been established for the construction of naphthquinone analogs. The targeted strategy for the synthesis of α-configured congeners hinges upon the use of $Cu(OTf)_2$, while desired β-configured analogs were assembled via nucleophilic substitution upon a suitable isochroman acetal precursor. The route has been successfully applied in the synthesis of various frenolicin derivatives with good to excellent diastereoselectivity/yields. Further anticancer structure-activity assessments are ongoing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.
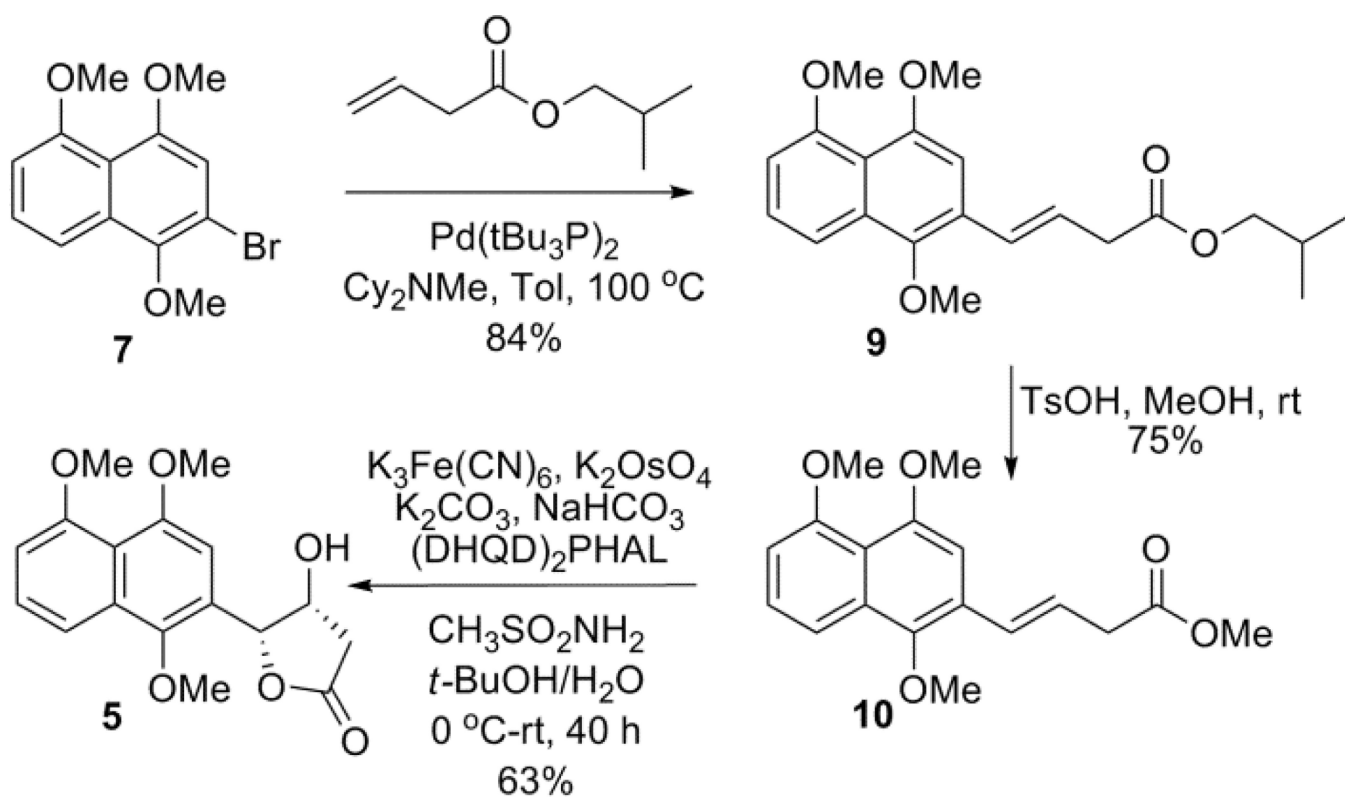
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8. All the relative configurations were determined by key NOE correlations (see Supporting Information).

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10. Enantioselectivity were determined by HPLC analysis [chiral IC column (Daciel Chemical Ind. Ltd.) 25.0×4.6 mm, 80/20 hexane/iPrOH, 0.6 mL/min, UV 254 nm, $t_{major} = 39.2$ min, $t_{minor} = 41.8$ min]. The observed ee value is consistent with the data reported in ref. 9a.

11. The epimers can be slightly isolated on a preparative reverse phase HPLC (Colunm: Supelco C18, 25 cm $\times$ 21.2 mm, 10 μm; eluent: gradient 35%–50% CH_3CN in water; rate: 10 mL/min; $t_\beta = 58.2$ min, $t_\alpha = 62.3$ min; loading amount: 5 mg).

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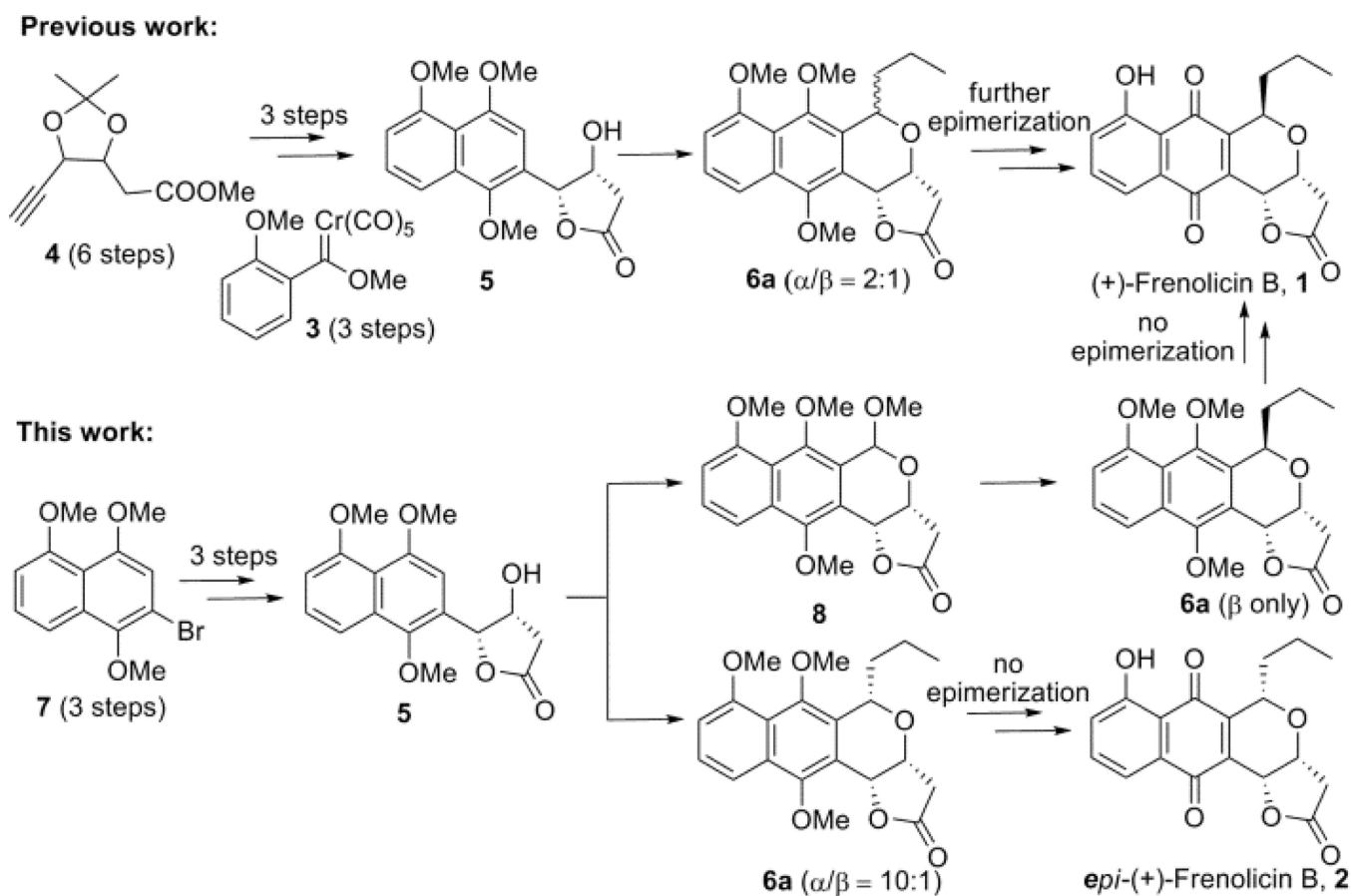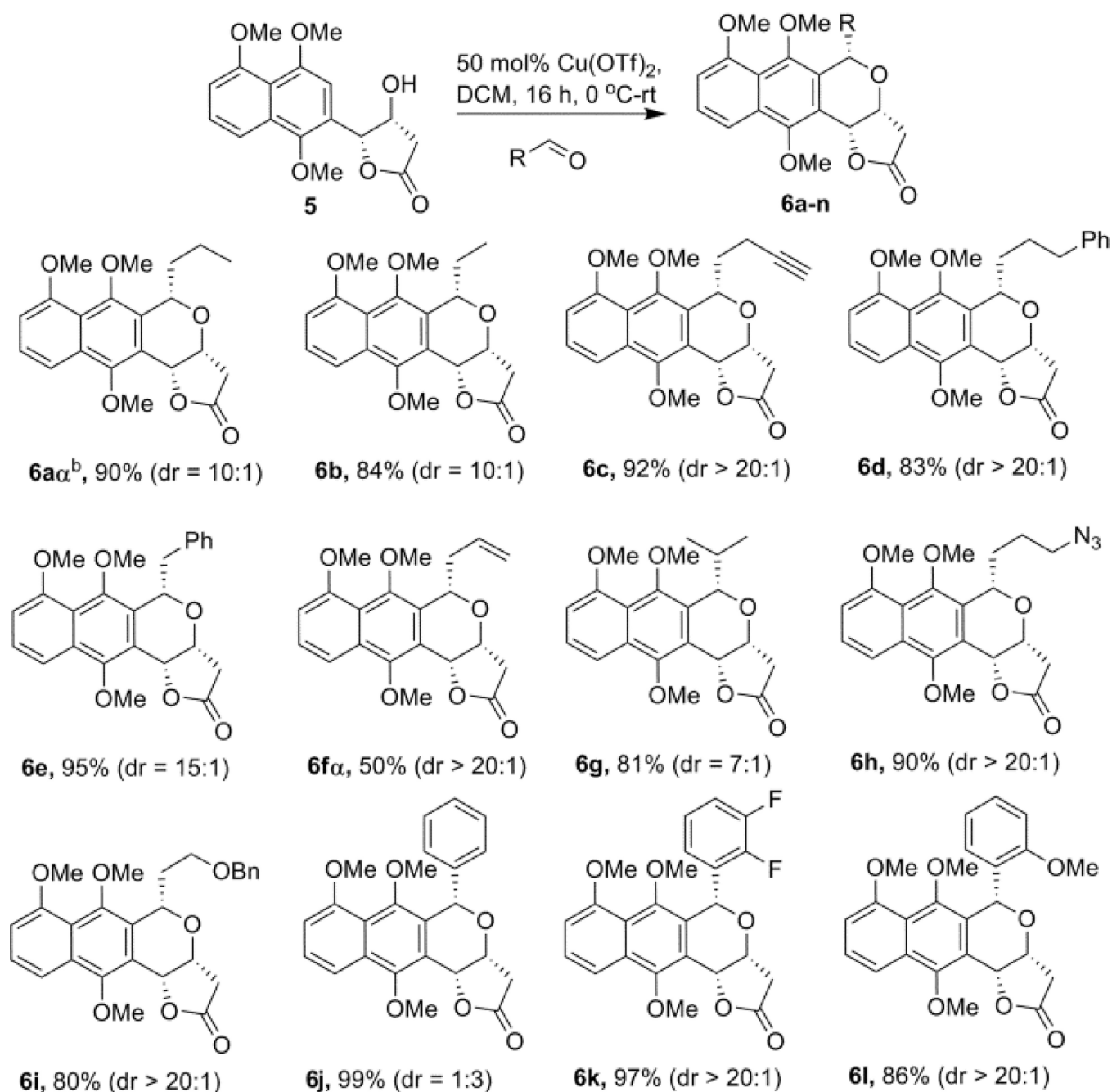
Figure 1.
A strategic comparsion of oxa-Pictet-Spengler-based approaches toward frenolicin B
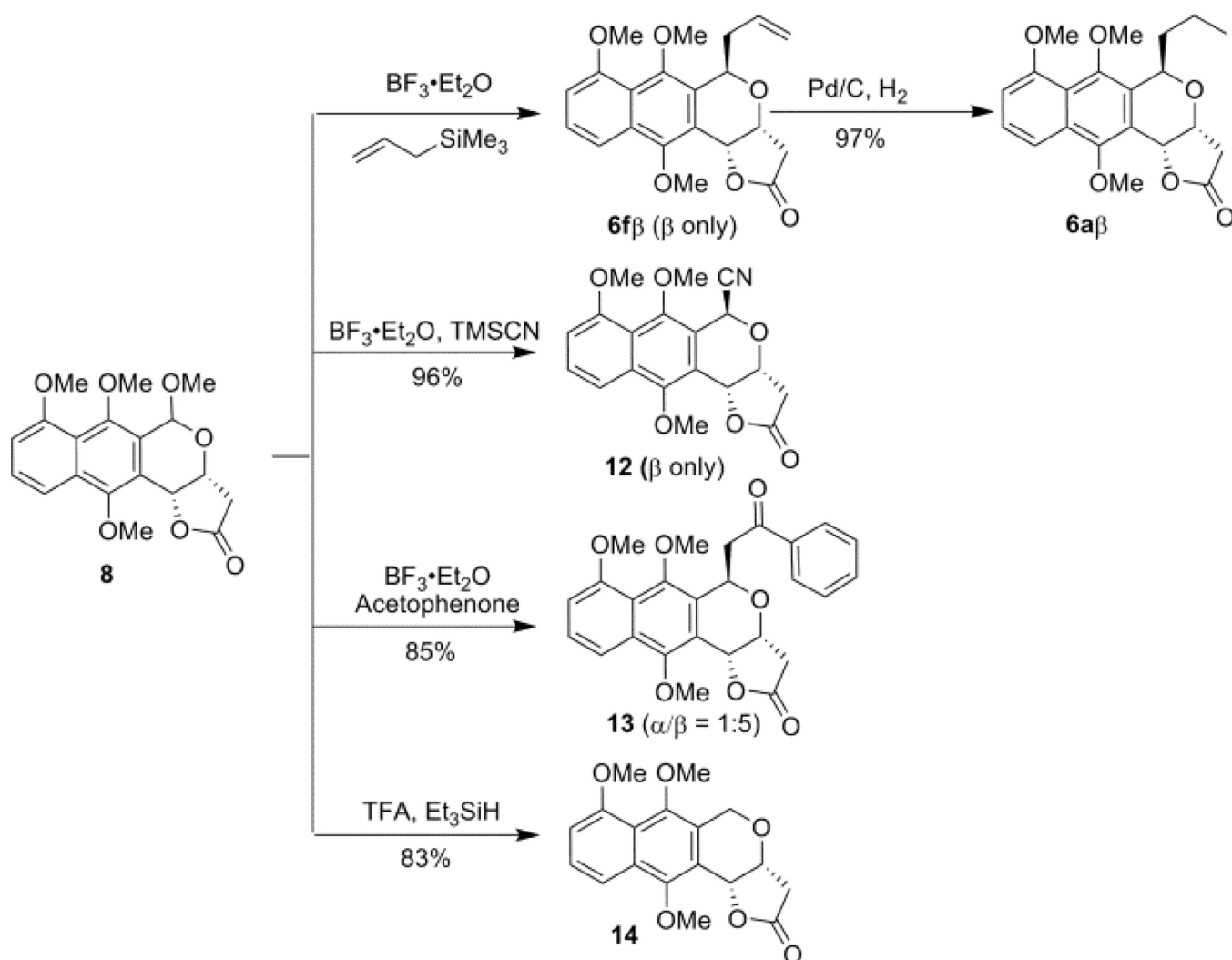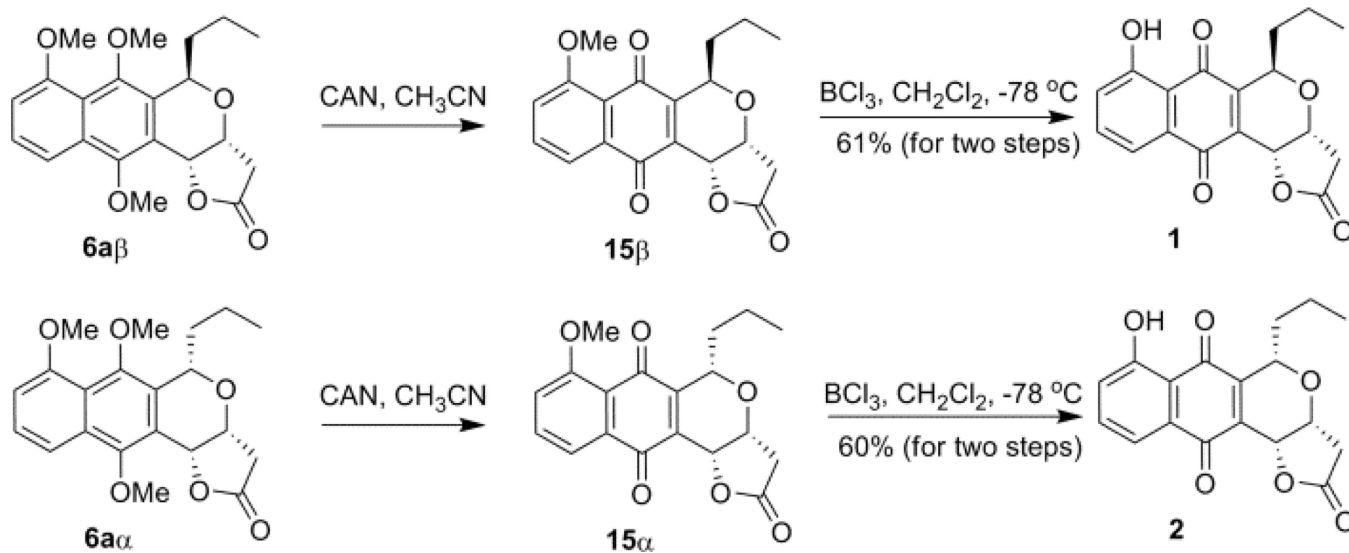synthesis.

Scheme 1.
Synthesis of compound **5**.

6aα[b], 90% (dr = 10:1)   6b, 84% (dr = 10:1)   6c, 92% (dr > 20:1)   6d, 83% (dr > 20:1)

6e, 95% (dr = 15:1)   6fα, 50% (dr > 20:1)   6g, 81% (dr = 7:1)   6h, 90% (dr > 20:1)

6i, 80% (dr > 20:1)   6j, 99% (dr = 1:3)   6k, 97% (dr > 20:1)   6l, 86% (dr > 20:1)

Scheme 2.
Scope of the α-reaction with varying R group.a
[a] Reaction was performed with 0.2 mmol **5**, 0.4 mmol aldehyde, and 50 mol % Cu(OTf)$_2$ at 0 °C and allowed the temp raise to room temp with overnight stirring. [b] Isolated yields after column chromatography; dr ratio (α/β) was determined by the proton NMR of crude products.
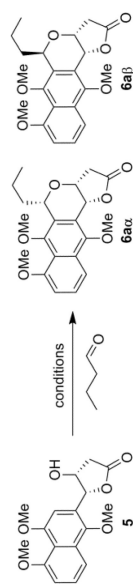
Scheme 3.
Scope of the β-oriented nucleophilic attack with different nucleophiles.

Scheme 4.
Synthesis of (+)-frenolicin B **1** and epi-(+)-frenolicin B **2**

Table 1

Optimization of the oxa-Pictet-Spengler reaction.[a]

entry	Lewis acids	solvent	temp (°C)	conversion (%)[b]	dr (6aα/6aβ)[c]
1	$BF_3 \cdot OEt_2$	CH_2Cl_2	0-rt	80(75)	66:34
2	$Yb(OTf)_3$	CH_2Cl_2	0-rt	10	80:20
3	$Y(OTf)_3$	CH_2Cl_2	0-rt	23	67:33
4[d]	$TiCl_4$	CH_2Cl_2	−78-rt	70	70:30
5	$SnCl_4$	CH_2Cl_2	0-rt	100	60:40
6	$FeCl_3$	CH_2Cl_2	0-rt	100	52:48
7	$Cu(OTf)_2$	CH_2Cl_2	0-rt	85	89:11
8[e]	$Cu(OTf)_2$	CH_2Cl_2	0-rt	95(90)	91:9
9	$Cu(OTf)_2$	DCE	0-rt	(64)	90:10
10	$Cu(OTf)_2$	$CHCl_3$	0-rt	(43)	94:6
11[f]	$Cu(OTf)_2$	CH_2Cl_2	0-rt	(32)	93:7
12[g]	$Cu(OTf)_2$	CH_2Cl_2	0-rt	(48)	91:9
13	$FeCl_3$	THF	0-rt	(50)	34:66

[a] Reaction was performed with 0.2 mmol **5**, 0.4 mmol aldehyde, and 50 mol % Lewis acid at 0 °C. The temperature was allowed to subsequently raise to rt over 4 h with stirring.

[b] Conversion was determined by HPLC analysis. The data in the parentheses are the isolated yields after column chromatography.

[c] dr ratio was determined by the proton NMR of crude products.

[d] 2 h reaction time.

[e] Overnight.

[f] Using 1,1-dimethoxybutane instead of butaldehyde.
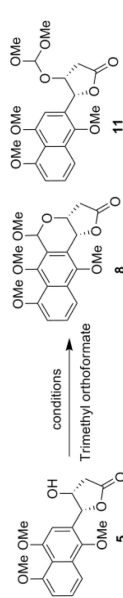
[g]Using 20 mol % Lewis acid.

Table 2

Optimization of the oxa-Pictet-Spengler reaction with trimethyl orthoformate.[a]

entry	Lewis acids	solvent	temp (°C)	time (h)	conversion (%)[b]
1	FeCl$_3$	CH$_2$Cl$_2$	0	1	40(31)
2	BF$_3$·OEt$_2$	CH$_2$Cl$_2$	0	1	20
3	Fe(OTf)$_3$	CH$_2$Cl$_2$	0	2	23
4	AlCl$_3$	CH$_2$Cl$_2$	0	1	<10
5	SnCl$_4$	CH$_2$Cl$_2$	0	1	45(33)
6	EtAlCl$_2$	CH$_2$Cl$_2$	0	1	60(51)
7	Et$_2$AlCl	CH$_2$Cl$_2$	0	2	<10[d]
8	EtAlCl$_2$	CH$_2$Cl$_2$	–40	16	45(36)
9	EtAlCl$_2$	CH$_2$Cl$_2$	–20	16	80(70)
10	EtAlCl$_2$	DCE	–20	16	74(62)
11[e]	EtAlCl$_2$	CH$_2$Cl$_2$	–20	16	78(65)

[a] Reaction was performed with 0.2 mmol **5**, 0.24 mmol trimethyl orthoformate, and 100 mol % Lewis acid at 0 °C with 1 h stirring.

[b] Conversion was determined by HPLC analysis. The data in the parentheses are the isolated yields after column chromatography.

[c] dr ratio was determined by the proton NMR of crude products.

[d] formation of the side product **11** in 60% yield.

[e] 2 mmol **5** was loaded.