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Synthesis of (+)-7,20-Diisocyanoadociane and Liver-Stage Antiplasmodial Activity of the Isocyanoterpene Class

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Supporting Information

ABSTRACT: 7,20-Diisocyanoadociane, a scarce marine metabolite with potent antimalarial activity, was synthesized as a single enantiomer in 13 steps from simple building blocks (17 linear steps). Chemical synthesis enabled identification of isocyanoterpene antiplasmodial activity against liver-stage parasites, which suggested that inhibition of heme detoxification does not exclusively underlie the mechanism of action of this class.

Isocyanoterpene (ICT) metabolites[1] are secreted from soft-bodied marine organisms and are thought to protect against predation or colonization.[2] ICTs also kill the malaria parasite *Plasmodium falciparum* at low-nanomolar concentrations with high selectivity over human (KB) cells.[3] Neither the mechanism of antiplasmodial activity nor the structural requirements for potency are fully understood.[1,4] One mechanism of action ascribed to the amphilectene and adociane ICT classes involves inhibition of heme detoxification (crystallization to hemazoin), for which computational models of binding have been reported.[3c,4a−c] Among the isonitrile metabolites screened for inhibition of heme crystallization in vitro, isocyanoamphilectene (**1**) and 7,20-diisocyanoadociane (**2**) were identified as the most effective members.[4c] Increased heme binding was correlated to higher antiplasmodial activity relative to their congeners [**1**: IC_{50} = 47 nM (D6), 423 nM (W2); **2**: IC_{50} = 5 nM (D6), 4 nM (W2)]. Further study of **1** and **2** has been explicitly impeded by scarcity of material.[4c] Here we describe an asymmetric chemical synthesis of (+)-**2**, whose previous syntheses have been 40 steps with stereocontrol[5] and 27 steps to produce a near-equimolar mixture of isonitrile diastereomers.[6] Access to **1** and **2** through synthesis enabled the demonstration that heme detoxification cannot be the exclusive mechanism of antiplasmodial activity for the amphilectene and adociane class, as recently claimed.[4c]

In 2012, we reported an approach to the amphilectane class of terpenes, which includes the pseudopterosins, pseudopteroxazoles, and isocyanoamphilectenes like **1**, by way of a polarized Danishefsky-type dendralene ([3]-Dd; Figure 1a).[7] Application of this overall strategy to an efficient synthesis of **2** required solutions to several problems: (1) simultaneous incorporation of two *tert*-alkyl isonitriles, one axial and one equatorial; (2) installation of the correct C1 stereochemistry opposite to that of amphilectene **1**; (3) stereoselective annulation to access the fourth ring; (4) chemoselective solutions to these problems to minimize functional group interconversions (FGIs);[8] and (5) full control of the relative and absolute stereochemistry from double dienophile **3** and a multiply substituted dendralene (**4**), a problem that appears to be simple but is deceptively complex.

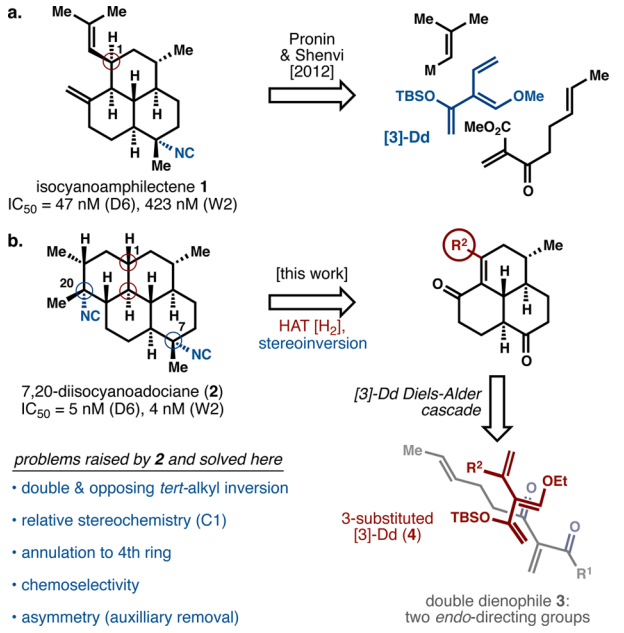

Figure 1. (a) Our approach to amphilectene (±)-**1** via Danishefsky dendralene [3]-Dd; (b) problems and solutions in the approach to (+)-**2**.

Access to the all-*trans*-fused scaffolds of the amphilectene, adociane, and kalihinol ICT classes has been challenged by the thermodynamic favorability of alternative *cis*-fused configurations in synthetic intermediates.[9] Control of the absolute stereochemistry is also complicated by the double dienophile targeted for cycloaddition. Two electron-withdrawing groups are necessary for an efficient Diels−Alder reaction, but both direct endo, rendering C_2-symmetric chiral catalysts ineffective[10] and preventing high diastereoselectivity in related systems.[11] After a screen of chiral auxiliaries, we identified

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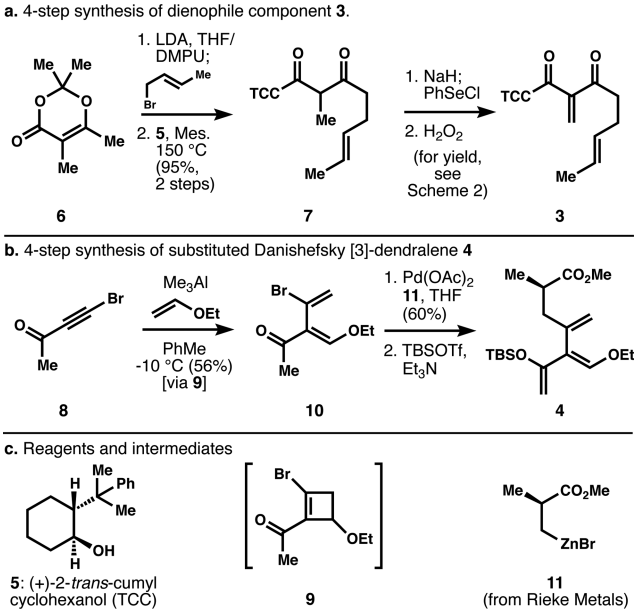
(+)-8-phenylmenthol or (+)-2-*trans*-cumylcyclohexanol (TCC, **5**)[12] as ideal controllers for three reasons. First, incorporation into the substrate was simple and even stabilized the reactive dienophile (Scheme 1a). Second, control of the cycloaddition stereoselectivity proved to be high (95:5 dr; Scheme 2). Third, we could not excise auxiliaries by saponification because of competing substrate reactivity, whereas **5** offered an alternative solution (see below).

Scheme 1. Synthesis of Building Blocks 3 and 4

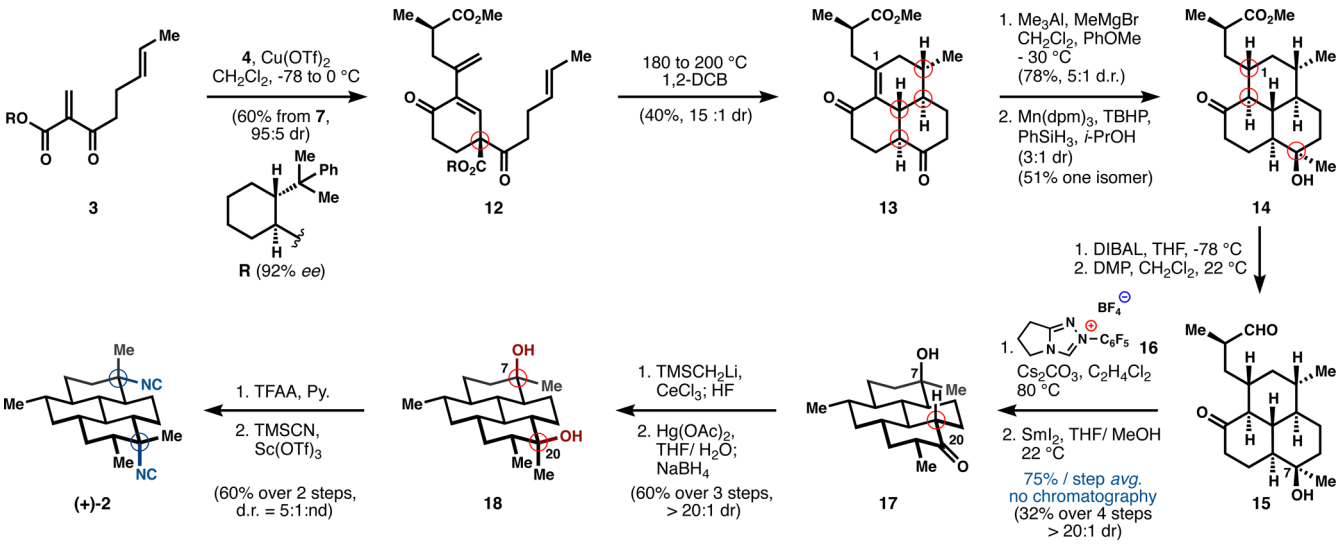


A single enantiomer of double dienophile **3** was synthesized from dioxenone **6** via γ-selective crotylation, acyl ketene generation and capture with **5**, and unsaturation via the selenoxide. This short sequence could generate large quantities of **3**, but its instability prevented its isolation and storage, so it was used crude and immediately. Highly substituted dendralene **4** proved to be more difficult to access,[13] so we turned to the tandem [2 + 2] cycloaddition/retroelectrocyclization reaction reported for electron-deficient alkynes[14] in the hope that this process could be applied to simple alkynones. In the absence of Lewis acid, 4-bromo-2-butynone (**8**) and ethyl vinyl ether do not react, but trimethylaluminum catalyzes their cycloaddition to form **9**,[15] and over time retroelectrocyclization occurs to deliver **10** in 56% yield as a single geometrical isomer. We anticipate this reaction to be a general route to many substituted Danishefsky-type dendralenes. After some exploration, we found that **10** could efficiently couple to commercially available (Rieke Metals) zinc bromide **11** (99% ee) using catalytic palladium(II) acetate, but only in the absence of phosphine ligands. Enol silane formation completed the synthesis of dendralene **4**.

The merger of dendralene **4** and double dienophile **3** occurred at −78 °C in the presence of copper(II) triflate using crude solutions of both components (yield calculated from **7**). The cycloaddition generated cyclohexenone **12** with 95:5 diastereoselectivity (relative to the auxiliary) after elimination of the ethyl silyl ether, which occurred in the presence of the Lewis acid. The second Diels−Alder reaction proceeded at 180 °C, and auxiliary removal via heteroretroene/decarboxylation took place when the temperature was increased to 200 °C.[16] Themolysis offered an excellent solution for auxiliary removal since standard nucleophilic substitution was outcompeted by both deconjugation of the strained cyclohexenone in **13** and retro-Dieckmann ring scission at the intermediate β-keto ester. The gross simplification of this disconnection and the overall yield of these two processes (40%, 15:1 dr; 63% per step) allowed us to push forward toward the target.

Conversion of **13** to target (+)-**2** was not straightforward. First, addition of standard methyl nucleophiles such as Grignard and organocerium reagents to the saturated ketone failed because of competitive addition to the enone. Eventually, tetramethylaluminum magnesium bromide in the presence of anisole was found to deliver the axial alcohol in good yield. Second, hydrogenation of the enone was problematic because of competitive deconjugation of the strained alkene using Pd, Pt, or Rh catalysis. This deconjugated alkene delivered the incorrect configuration at C1 upon hydrogenation. Instead, we turned to hydrogen atom transfer (HAT) hydrogenation,[17] which we thought would deliver the thermodynamic diastereomer independent of any competing isomerization. Under

Scheme 2. Synthesis of (+)-7,20-Diisocyanoadociane [(+)-2]



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HAT conditions,[18] ketone **14** was produced in good yield as the major diastereomer.[19]

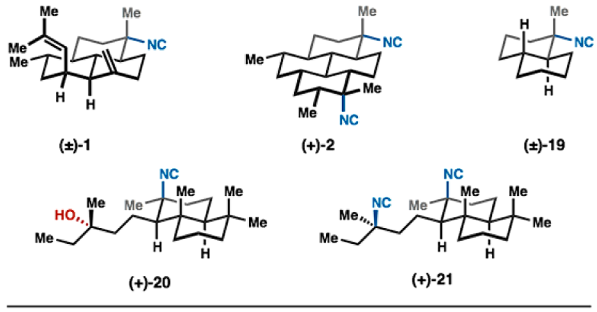
We intended to directly access ketone **17** by tandem acyloin cyclization and alkoxy ketone deoxygenation. Unfortunately, all methods for ketyl generation yielded complex mixtures, so we devised a workaround. Keto ester **14** was reduced with DIBAL and oxidized to keto aldehyde **15**. Cyclization catalyzed by the N-heterocyclic carbene generated in situ from triazolium salt **16** generated the expected α-hydroxy ketone, and samarium(II) iodide-mediated deoxygenation delivered ketone **17**.

In previous work, the *diaxial* diol corresponding to **18** has been converted by non-stereoselective displacement using $TMSCN/TiCl_4$ to (+)-**2** and its three other diastereomers, which were separated by HPLC.[6] We have previously shown that *tert*-alkyl trifluoroacetates can undergo stereoinversion via $Sc(OTf)_3$-catalyzed solvolysis with TMSCN.[20] For such a reaction to generate **2**, we required an axial alcohol at C7 but an equatorial alcohol at C20. Yamamoto's MAD reagent achieved this stereochemistry in model ketones but delivered only the axial alcohol from **17** using organolithium, -magnesium, or -cerium nucleophiles. Finally, we found that methylenation followed by oxymercuration cleanly generated the equatorial, axial diol **18** as a single diastereomer. Isocyanation according to our solvolysis protocol yielded a 5:1 mixture of two diastereomers in 60% yield, and the two other diastereomers were not detected.

Access to (±)-**1** and (+)-**2** allowed their antiplasmodial activities to be investigated. Strictly chemical experiments have shown that **1** and **2** can bind free heme in aqueous solution and prevent crystallization of β-hematin.[4a,c] The ability of several isonitriles to prevent crystallization were correlated to their ability to kill *P. falciparum* and taken as proof that inhibition of biocrystallization is the mechanism of ICT antiplasmodial activity.[4c]

The liver schizont of *Plasmodia* species does not rely on catabolism of host hemoglobin for nutrition and therefore does not rely on biocrystallization as a protective mechanism. Consequently, liver-stage assays have been used to identify the existence of alternative mechanisms of action for putative biocrystallization disruptors.[21] We assayed[22] the metabolites amphilectene (±)-**1**[7a] and (+)-**2** for activity against asexual-blood-stage (*P. falciparum*, Dd2 strain) and liver-stage (*Plasmodium berghei*) parasites in addition to the simple isonitriles **19**, **20**, and **21** synthesized in our lab (Figure 2).[20] The blood-stage inhibition values for **1** and **2** are comparable to those reported in the literature for related strains.[4] Remarkably, isonitriles **1**, **2**, **20**, and **21** are all active against liver-stage parasites with IC_{50} values near or below 1 μM. Therefore, a mechanism or mechanisms other than or in addition to heme detoxification inhibition may also underlie the activity of **1** and **2**, in contrast to prior theories about their activity.[4] The marked differences between the blood-stage and liver-stage potencies of the different isonitriles underscore the functional role of additional molecular architecture, e.g., (+)-**20** vs (+)-**21**. Abstraction of the presumed pharmacophore yields decalin **19**, which is completely inactive in both liver-stage and blood-stage assays. Clearly, hydrophobic isonitriles are not all equivalent, possibly because of differences in productive binding, cellular uptake, or intracellular pharmacokinetics, for example.

The related kalihinol class of ICTs has been shown to inhibit bacterial folate biosynthesis[23] and also to induce a copper-deficient phenotype in zebrafish embryos.[22b,24] Strategies to target folate metabolism are precedented in malaria therapeutics,[25] and intracellular copper chelation has been shown to arrest parasite maturation.[26] However, since multiple pathways may be targeted by the ICTs, an agnostic approach to target identification may be the best strategy for mechanistic study.[27]



μM values	(±)-1	(+)-2	(±)-19	(+)-20	(+)-21
ABS IC_{50}	0.992	0.016	>50	0.150	0.009
PbLuc IC_{50}	0.932	1.296	104.8	0.221	1.546
$HepG_2$ IC_{50}	27.96	24.13	>50	13.36	27.96

Figure 2. Asexual-blood-stage (ABS, *P. falciparum*) and liver-stage (PbLuc, *P. berghei*) values for **1**, **2**, and **19**–**21**.[22]

To summarize, we have reported a concise and fully stereocontrolled synthesis of (+)-**2**, a potent antimalarial metabolite.[28] Highlights of the synthesis include (1) a short route to substituted polarized dendralenes via Lewis acid-mediated [2 + 2] cyclization/retroelectrocyclization; (2) application of these reagents (which we termed Danishefsky-type dendralenes) to the synthesis of the carbon scaffold of the adocianes; (3) use of HAT hydrogenation to establish the correct C1 stereochemistry when all other methods failed; (4) stereocontrolled installation of the axial, equatorial bis-(isonitrile) of **2**; and (5) design of the reaction sequence to minimize FGIs,[8] which includes the absence of protecting groups. In contrast to other approaches to this family, our route through polarized dendralenes and *tert*-alkyl alcohol inversion is highly stereocontrolled. Access to isonitriles **1**, **2**, and **19**–**21** allowed us to demonstrate that heme detoxification cannot exclusively underlie the antiplasmodial activity of the ICT class and that other mechanisms must operate, even for potent heme crystallization disruptors like **1** and **2**.[29] Nevertheless, our data also indicate that the structure–activity relationships within this class are nebulous. We hope that these data may stimulate more interest in the community and perturb the impression that all hydrophobic isonitriles are functionally equivalent.[30] The identification of easily synthesized isonitriles (+)-**20** and (+)-**21** as potent liver- and blood-stage inhibitors, respectively, should significantly aid further studies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b03899.

Detailed experimental procedures, spectral data, and chromatograms (PDF)

Crystallographic data for (+)-**14** and (−)-**17** (PDF)

Crystallographic data for (−)-**17** (CIF)

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Notes

The authors declare no competing financial interest.

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