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

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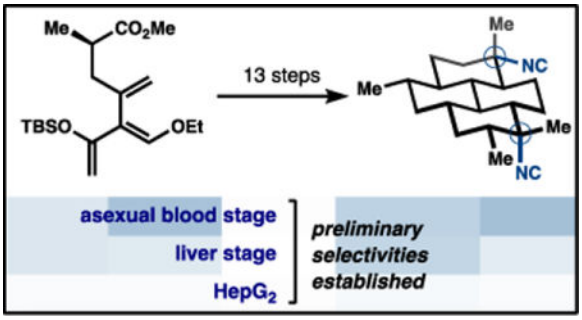
Abstract

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 ICT antiplasmodial activity against liver-stage parasites, which suggested heme detoxification does not exclusively underlie the mechanism of action of this class.

TOC image



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 *P. 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

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Supporting Information. Detailed experimental procedures, spectral data, chromatograms and x-ray crystallography. This material is available free of charge via the Internet at http://pubs.acs.org.

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 compared 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 enables 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, namely: 1. simultaneous incorporation of two *tert*-alkyl isonitriles: one axial, one equatorial; 2. installation of the correct C1 stereochemistry opposite to that of amphilectene **1**; 3. stereoselective annulation to access the 4th ring; 4. chemoselective solutions to these problems to minimize functional group interconversions (FGIs);[8] and 5. full control of relative and absolute stereochemistry from double-dienophile **3** and a multiply-substituted dendralene (**4**), a problem that appears simple, but is deceptively complex.

Access to the all *trans*-fused scaffolds of the amphilectenes, adocianes and kalihinol ICT classes has been challenged by the thermodynamic favorability of alternative *cis*-fused configurations in synthetic intermediates.[9] Control of 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 C2-symmetric chiral catalysts ineffective[10] and preventing high diastereoselectivity in related systems.[11] After a screen of chiral auxiliaries, we identified (+)-8-phenylmenthol or (+)-2-*trans*-cumyl-cyclohexanol (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 cycloaddition stereoselectivity proved to be high (95:5 dr, Scheme 2). Third, we could not excise auxiliaries by saponification due to competing substrate reactivity, whereas **5** offered an alternative solution (see below).

A single enantiomer of double dienophile **3** was synthesized from dioxenone **6** via γ-selective crotylation, acyl ketene generation and capture with **5**, followed by unsaturation via the selenoxide. This short sequence could generate large quantities of **3**, but its instability prevented isolation and storage, so it was used crude and immediately. Highly-substituted dendralene **4** proved more difficult to access,[13] so we turned to the tandem [2+2]/ retroelectrocyclizations reactions 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 trimethyl aluminium catalyzes their cycloaddition to **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. Enolsilane formation completed the synthesis of dendralene **4**.

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 hetero-retroene/decarboxylation took place upon increasing the temperature to 200°C.[16] Themolysis offered an excellent solution to 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-ketoester. 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 like Grignard and organocerium reagents to the saturated ketone failed due to 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 due to competitive deconjugation of the strained alkene using Pd, Pt or Rh catalysis. This deconjugated alkene delivered the incorrect configuration at C1 upon hydrogenation. So instead we turned to hydrogen atom transfer (HAT) hydrogenation,[17] which we thought would deliver the thermodynamic diastereomer independent of any competing isomerization. Under 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 alkoxyketone deoxygenation. Unfortunately, all methods for ketyl generation yielded complex mixtures, so we devised a work-around. Keto-ester **14** was reduced with DIBAL and oxidized to keto-aldehyde **15**. Cyclization catalyzed by *N*-heterocyclic carbene generated in situ from triazolium salt **16** generated the expected α-hydroxyketone, 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 only delivered 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 (FP) 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 biocrystalization 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 biocrystalization as a protective mechanism. Consequently, liver stage assays have been used to identify the existence of alternative mechanisms of action for putative biocrystalization disrupters.[21] We assayed[22] metabolites amphilectene (±)-**1**[7a] and (+)-**2** for activity against asexual blood stage (*P. falciparum*, Dd2 strain) and liver stage (*P. berghei*) parasites, in addition to the simple isonitriles **19**, **20** and **21** synthesized in our lab.[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}'s 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 in blood-stage and liver-stage potencies between the different isonitriles underscore the functional role of additional molecular architecture, e.g. (+)-**20** vs. (+)-**21**. Abstraction of the pharmacophore yields decalin **19**, which is completely inactive in both liver and blood-stage assays. Clearly hydrophobic isonitriles are not all equivalent, possibly due to 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 induce a copper-deficient phenotype in zebra fish 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] But since multiple pathways may be targeted by the ICTs, an agnostic approach to target identification may be the best strategy for mechanistic study.[27]

To summarize, we report 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]/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 functional group interconverions (FGI),[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 other mechanisms must operate, even for potent heme crystallization disrupters like **1** and **2**.[29] Nevertheless, our data also indicates that the structure-activity relationships within this class are nebulous. We hope this data may stimulate more interest in the community and perturb the impression that all hydrophobic

isonitriles are functionally equivalent.[30] Identification of easily-synthesized isonitriles (+)-**20** and (+)-**21** as potent liver- and blood-stage inhibitors, respectively, should significantly aid further studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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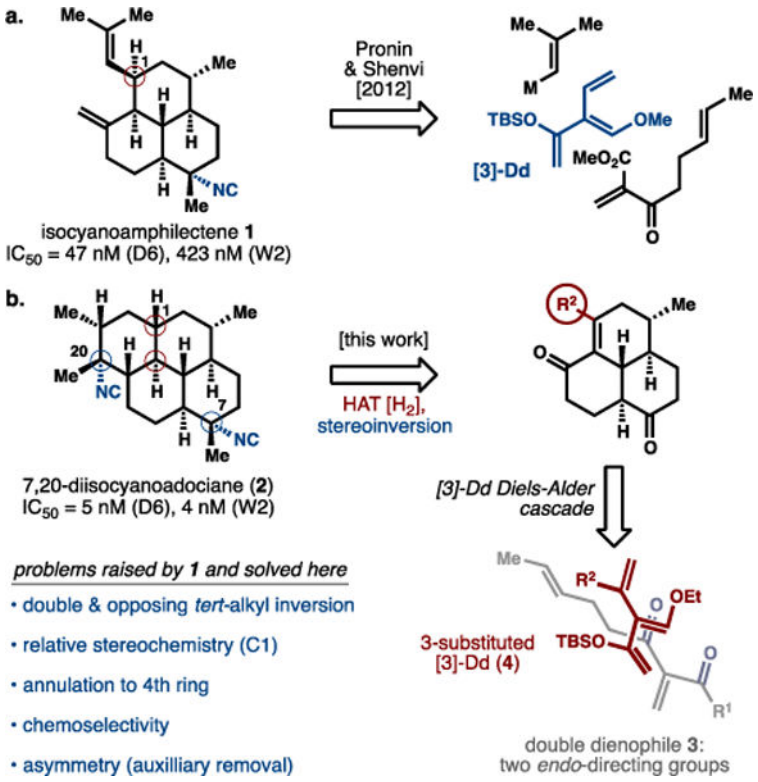
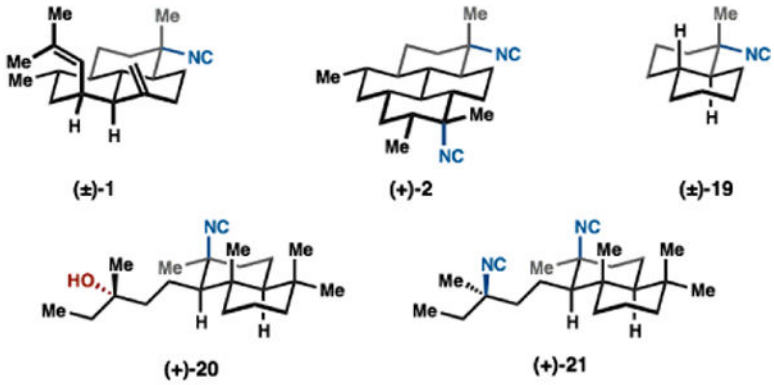


Figure 1.

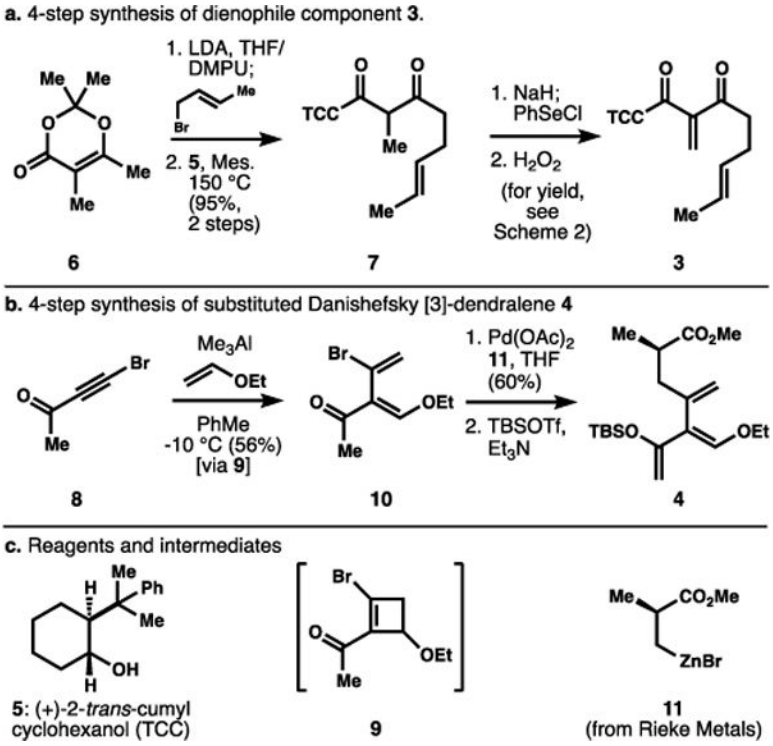
(a) Our approach to amphilectene (±)-**1** via a Danishefsky-dendralene, [3]-Dd; (b) problems and solutions in the approach to (+)-**2**.



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

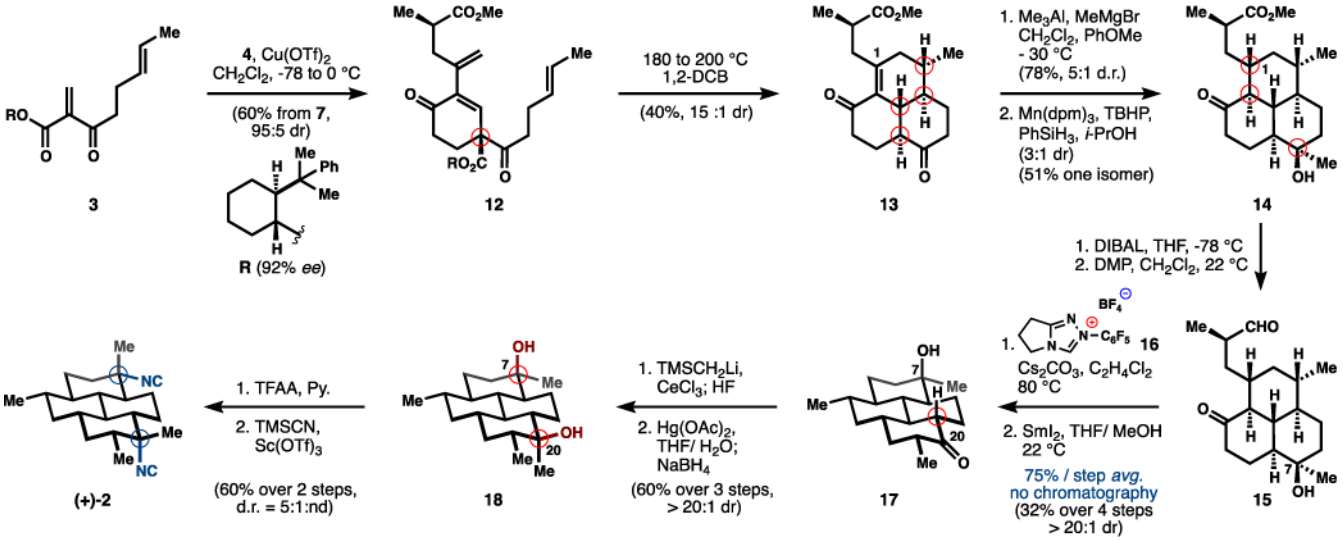
Figure 2.

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



Scheme 1.

Synthesis of building blocks **3** and **4**.



Scheme 2.

Synthesis of (+)-7,20-diisocyanoadociane [(+)-**2**)].