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Directed Synthesis of All Four Pure Stereoisomers of the *N,C*-Coupled Naphthylisoquinoline Alkaloid Ancistrocladinium A

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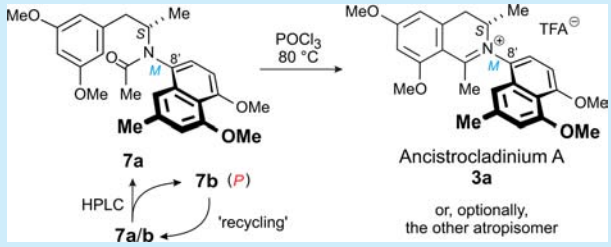
Supporting Information

ABSTRACT: The first preparation of the *N,C*-coupled naphthylisoquinoline alkaloid ancistrocladinium A and its likewise naturally occurring minor atropisomer, in an atropisomerically pure form, is described. The synthesis succeeded by resolution of the already rotationally hindered, and thus atropo-diastereomeric acetamide precursors, which were then, without major loss of stereochemical information, cyclized to the respective target molecules. The strategy was applied to the first synthesis of the regioisomeric product ancistrocladinium D, likewise in a stereochemically pure form.



Naphthylisoquinoline alkaloids[1] including korupensamine A (**1**)[2] and ancistrocladidine (**2**)[3] from tropical lianas consist of a naphthalene and an isoquinoline portion, linked via a biaryl axis, which, in most cases, is rotationally hindered and thus axially chiral (Figure 1). Depending on the individual structure, they show pronounced anti-infective activities against pathogens causing severe diseases[4] like malaria[5] or African sleeping sickness,[6] or against tumor cells,[7] while some of their dimers exhibit anti-HIV activities.[8] More recently, the first *N,C*-coupled representatives have been discovered in nature, among them ancistrocladinium A (**3**) and ancistrocladinium B (**4**) (Figure 1).[9] They possess an unprecedented chiral iminium-aryl axis, which is configurationally stable in the case of **3**, while it is rotationally semistable in the case of **4**, which, thus, occurs as a mixture of its two slowly interconverting atropo-diastereomers, **4a** and **4b**. These *N,C*-coupled naphthylisoquinolines are active, in particular against *Leishmania major*, the parasite causing cutaneous leishmaniasis.[9,10]

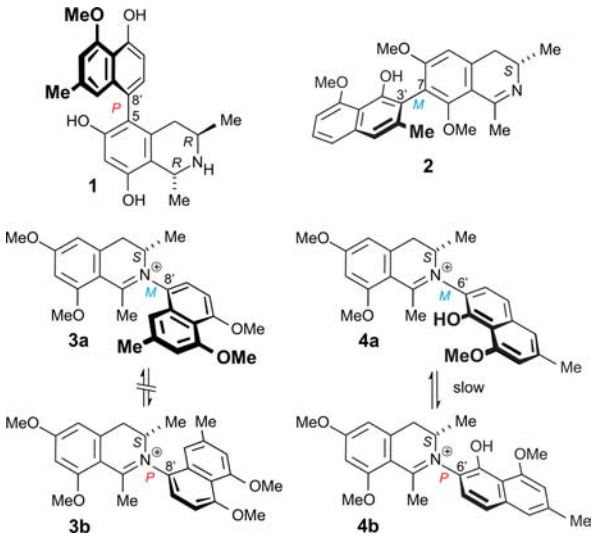


Figure 1. Structures of *C,C*- and *N,C*-coupled naphthylisoquinoline alkaloids.

Naphthylisoquinoline alkaloids are, thus, attractive and important synthetic targets. In the case of the *C,C*-coupled representatives, this goal has been achieved either by direct, intermolecular biaryl coupling or, with higher atroposelectivities, by first prefixing the two molecular halves via a chiral tether,[11] by using a chiral catalyst,[12] or by applying the lactone concept.[13] *N,C*-coupled naphthylisoquinoline alkaloids like **3** and **4** have previously been synthesized,[14] but not in an atropisomerically pure form. While this would not be necessary for ancistrocladinium B (**4**) (because it would equilibrate), it would be desirable for ancistrocladinium A (**3**), in order to test the bioactivities of the pure atropo-diastereomers separately. In nature, **3** does not occur in a stereochemically pure form, but as mixtures (*M*:*P* = 10:1) in *Ancistrocladus ikela*.[9] Unfortunately, resolution of its atropo-diastereomers, **3a** and **3b**, has not been successful, even on a chiral phase.[14] The first total synthesis of **3**, by Bischler-Napieralski cyclization of the acetamide **7** (available from Buchwald-Hartwig amination of **6** with **5**), led

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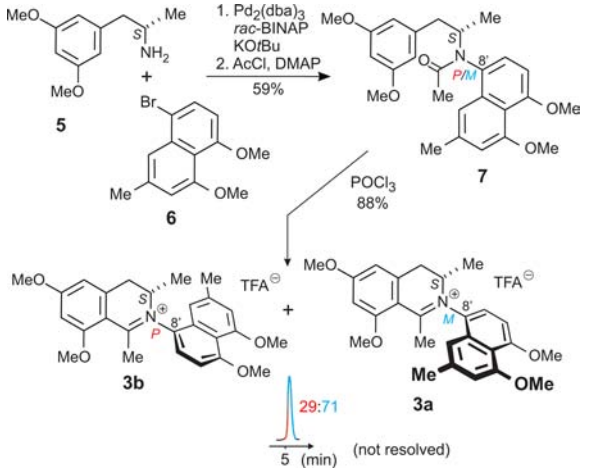
to atropisomeric ratios of up to 71:29 (*M*:*P*), as analyzed by NMR (Scheme 1).[14]

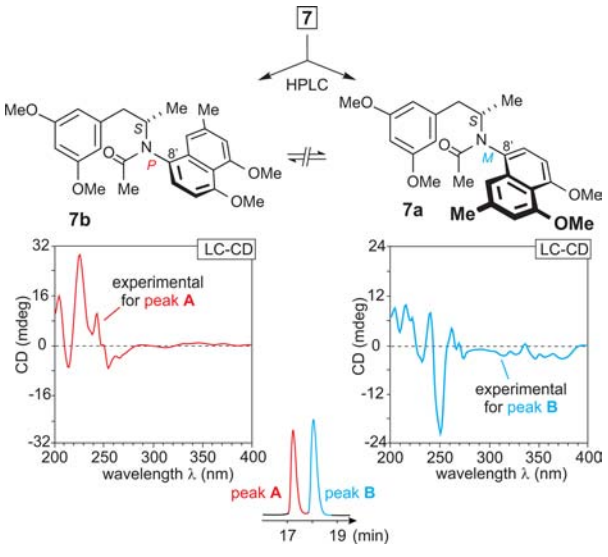
Scheme 1. Previous Total Synthesis of 3a/b[14]



In this paper, we describe the efficient first preparation of atropisomerically pure **3a** and **3b**, by chromatographic resolution of the acetamide precursors **7a** and **7b**, which are likewise rotationally hindered and, thus, atropo-diastereomeric to each other, their configurational assignment, and specific conversion to the respective ancistrocladinium A isomer. As a proof of concept, the developed strategy was also applied to the synthesis of the unnatural enantiomers, *ent*-**3a** and *ent*-**3b**, and to the preparation of the regioisomeric (as yet likewise unnatural) ancistrocladinium D (**9**).

In an attempt to further improve the above-mentioned atroposelectivity, we found that, according to NMR and HPLC, **7** occurs as a pair of diastereomers, despite the presence of only one stereogenic center, apparently due to the fact that the acetamide precursor **7** already displayed the phenomenon of atropisomerism at the later *N*,*C*-axis[15] of the target molecule. Since in this case, in contrast to **3a**/**b**, it was possible to efficiently resolve the diastereomers (Scheme 2), the circular-

Scheme 2. Atropisomeric Resolution of Amides 7a and 7b and Their CD Spectra Measured Online by LC−CD



dichroism (CD) spectra could be measured online, by HPLC−CD coupling. The nearly opposite CD curves of the two diastereomers indeed hinted at the presence of rotational isomers, with the expected chiroptical predominance of the *N*,*C*-axis over the stereogenic center at C-3.

Due to the baseline separation, a resolution of **7a** and **7b** succeeded even at a preparative scale, permitting additional spectroscopic investigations offline, on the pure isolated compounds. NOESY interactions of CH_3-3 with H-7′ in **7a** showed that both spin systems were on the same side of the molecule, which, in conjunction with the known *S*-configuration at C-3, revealed the *M*-configuration at the *N*,*C*-axis (Figure 2, right).

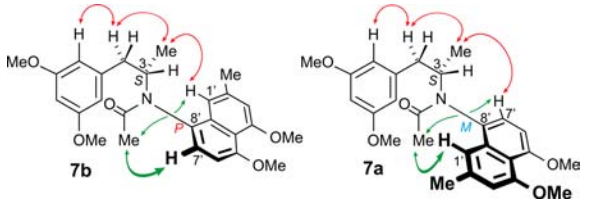
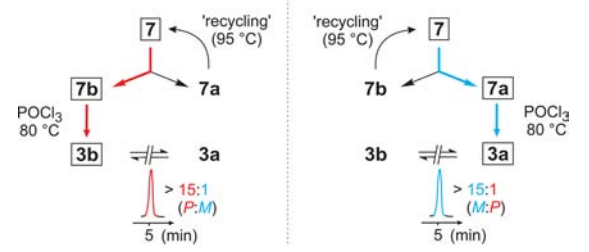


Figure 2. NOESY interactions indicative of the relative axial configurations of **7a** and **7b**.

In **7b**, by contrast, CH_3-3 interacted with H-1′, evidencing the opposite configuration in this case, i.e., *P* at the axis (Figure 2, left). These NMR investigations established the slower isomer (peak **B**) to be *M*-configured, i.e., possessing structure **7a**, and the faster one (peak **A**) to be the *P*-isomer, i.e. **7b**.

The two rotational isomers, **7a** and **7b**, were found to be configurationally stable at room temperature, but gradually interconverted at elevated temperature (above ca. 80 °C). Further robust proof for the above assignment of the axial configuration of the two atropisomers was achieved by the specific conversion of the *M*-isomer **7a** into the likewise *M*-configured target molecule **3a** (Scheme 3, right). Although the

Scheme 3. Specific Preparation of 3b from 7b with Recycling of 7a (Left) and Directed Synthesis of 3a from 7a with Recycling of 3b (Right)



cyclization was best done at 80 °C, no substantial isomerization at the *N*,*C*-axis was observed during the reaction. This unexpected configurational stability under the reaction conditions is presumably due to the reaction of the amide oxygen with $POCl_3$, converting the bond between the nitrogen and the carbonyl *C*-atom into a 'true' *N*-*C* double bond, which enhances the rotational barrier at the *N*,*C*-axis.

And even the−here undesired − *P*-atropisomeric acetamide **7b** is not lost, but can be recycled by briefly heating it up to 95 °C, thus equilibrating it to an *M*/*P* mixture, which can then again be resolved (Scheme 3, right). This opens the possibility to convert, in principle, the entire synthetic material of **7a**/**b**

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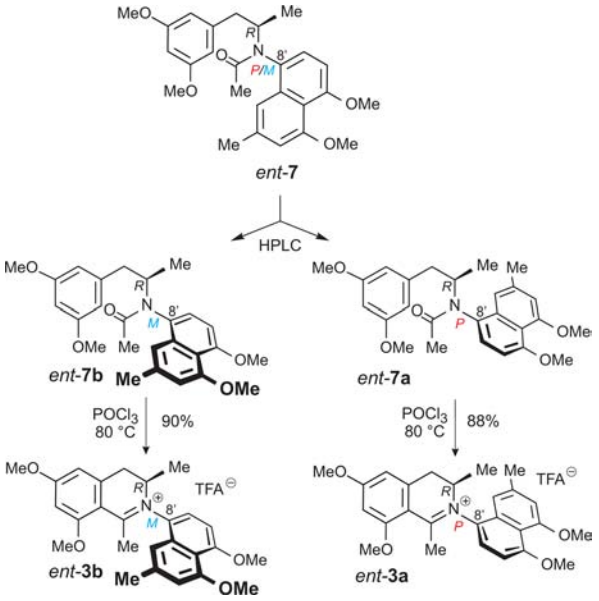
into **3a**. In a similar way, **3b**, the minor natural ancistrocladinium A atropisomer, can be prepared by cyclization of **7b** and thermal equilibration of the (in this case not required) *M*-isomer **7a** (Scheme 3, left).

In view of all unsuccessful previous attempts to resolve **3a** and **3b** chromatographically,[14] the described strategy provides the first access to stereochemically homogeneous material of ancistrocladinium A of any desired axial configuration—of **3a** (*M*) or, if required, of **3b** (*P*) – e.g. for bioactivity testing.

In order to show the efficiency of the methodology and to examine in more detail the influence of both stereogenic elements of **3** - the *N*,*C*-axis and the stereogenic center (C-3) - on the bioactivities, we applied the described strategy to the other enantiomeric series, i.e. with *S*-configuration at C-3 (Scheme 4). In our previous work, we had synthesized the

Scheme 4. Application of the Strategy to the First Synthesis of Pure *ent*-3a and *ent*-3b

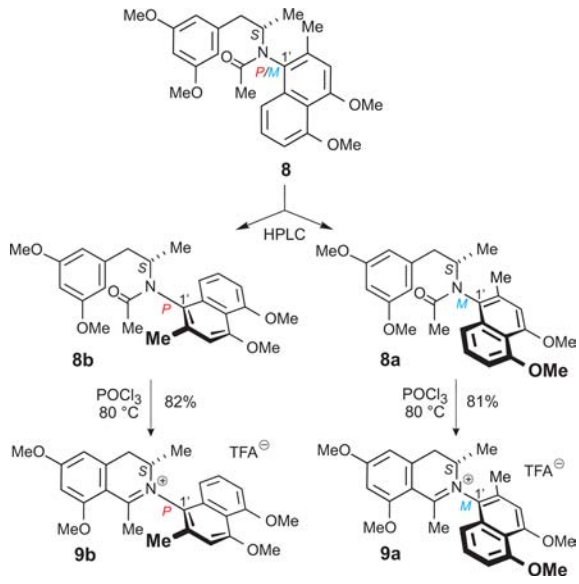


(unnatural) enantiomer of ancistrocladinium A, *ent*-**3**, starting from the—at that time nonresolved—acetamide *ent*-**7**.[14] As expected, resolution of this intermediate now led to pure *ent*-**7a** and *ent*-**7b**, which, again, were specifically converted into the target molecules *ent*-**3a** and *ent*-**3b**, respectively.

Additional evidence for the general applicability of the synthetic strategy described in this paper, was expected from the preparation of ancistrocladinium D (**9**) (Scheme 5).[14] Although this regioisomer of **3** had not yet been found in nature,[14] it was also a probable natural product. In the case of **9**, the methyl group on the naphthalene part was next to the *N*,*C*-axis, so that the steric hindrance around this axis was significantly higher than in the case of **3**. Resolution of the atropisomeric acetamides **8a** and **8b** and their specific conversion into **9a** and **9b**, respectively, showed that the method can even be applied to more hindered *N*,*C*-coupled naphthylisoquinoline alkaloids.

The known[9] antileishmanial activity (IC_{50} = 1.35 μM) of natural ancistrocladinium A (**3**), as a mixture of **3a** and **3b**, made the selective testing of its now available four stereoisomeric forms a rewarding task. Among the pure compounds (Table 1), the main isomer **3a** (*M*,*S*) of the natural product had the best activity against *Leishmania donovani* (IC_{50} = 0.081

Scheme 5. Analogous Synthesis of Atropisomerically Pure Ancistrocladinium D (9a and 9b)



μM), better than the standard miltefosine (IC_{50} = 0.174 μM). Due to its low cytotoxicity (IC_{50} = 36.4 μM), it reached a high selectivity index of 450. The likewise natural, but minor alkaloid **3b** (*P*,*S*), by contrast, had a significantly lower activity (IC_{50} = 0.401 μM). The unnatural isomers, *ent*-**3a** and *ent*-**3b**, with *R*-configuration at C3, were also active. In particular, *ent*-**3a** (*P*,*R*), the enantiomer of the major natural compound **3a**, i.e. with opposite configurations at both stereogenic elements, showed an IC_{50} of 0.233 μM. The much weaker antileishmanial activity of the region-isomers **9** demonstrated the strong influence of a seemingly small structural change - the position of the methyl group.

High selectivity indices were also observed for the activities against *Trypanosoma cruzi* and *T. brucei rhodesiense*, showing the same trend - that the natural *M*,*S*-isomer and its un-natural *P*,*R*-enantiomer exhibited the highest potency. A significant structure—activity relationship was also found in the case of *P. falciparum*, where again the natural *M*,*S*-isomer - and its mirror image *ent*-**3a** - were the most active stereoisomers. Again the regioisomer **9** was substantially less active, in both atropisomeric forms.

This work describes the first directed total synthesis of all four stereoisomeric forms of ancistrocladinium A and their specific biotesting. Recently, Kim and Cheon[16] have published a paper likewise leading to 'ancistrocladinium A', but consisting of a (nonseparable) mixture of all four possible stereoisomers (enantiomeric mixtures of the precursor already, and atropisomeric mixtures of the product), mainly with the non-natural 3*R*-configuration, and no pathway was presented to the natural 3*S*-isomer.[17] Our synthesis,[18] by contrast, relies on the preparation of enantiopure atropo-diastereomeric amides **7a**/**b** and/or *ent*-**7a**/**b**, their structural assignment, and their specific conversion into the respective target molecule of any desired configuration. Remarkable were the high antileishmanial activities of the four stereoisomers of ancistrocladinium A (**3**), of which **3a** (*M*,*S*) and *ent*-**3a** (*P*,*R*) were the most active ones.[19] The work paves the way for the directed synthesis of further natural and modified *N*,*C*-coupled naphthylisoquinolines of any desired configuration.

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Table 1. Bioactivities of Compounds 3 and 9 (IC_{50} Values in $\mu M \pm SD$[a] (SI[b]))

compd	*P. falciparum* NF54	*P. falciparum* K1	*T. cruzi*	*T. brucei rhodesiense*	*L. donovani*	*cytotoxicity* (L6 cells)
standard	0.006 ± 0.002[c]	0.364 ± 0.153[c]	3.21 ± 1.13[d]	0.010 ± 0[e]	0.174 ± 0.054[f]	0.012 ± 0.007[g]
3a (*M*,*S*)	0.582 ± 0.082 (63)	0.484 ± 0.172 (75)	0.99 ± 0.565 (37)	0.098 ± 0.062 (372)	0.081 ± 0.017 (452)	36.4 ± 17.1
3b (*P*,*S*)	2.35 ± 0.336 (48)	3.11 ± 1.12 (37)	4.49 ± 1.83 (23)	0.611 ± 0.485 (186)	0.401 ± 0.164 (284)	113.7 ± 52.5
ent-**3a** (*P*,*R*)	0.503 ± 0.093 (58)	0.520 ± 0.105 (56)	4.23 ± 1.23 (7)	0.067 ± 0.043 (439)	0.233 ± 0.025 (126)	29.3 ± 5.8
ent-**3b** (*M*,*R*)	1.05 ± 0.35 (69)	1.50 ± 0.35 (48)	9.91 ± 3.48 (7)	0.165 ± 0.093 (439)	0.588 ± 0.158 (123)	72.4 ± 26.6
9a (*M*,*S*)	3.74 (6.39)	NM[h]	0.73 (32.7)	0.93 (25.7)	7.43 (3.22)	23.9
9b (*P*,*S*)	6.81 (>14.7)	NM[h]	3.29 (>30.4)	2.86 (>35.0)	18.5 (>5.41)	>100

[a]Standard deviation. [b]Selectivity index. [c]Chloroquine. [d]Benznidazole. [e]Melarsoprol. [f]Miltefosine. [g]Podophyllotoxin. [h]Not measured.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b03480.

Experimental procedures, NMR data (PDF)

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Notes

The authors declare no competing financial interest.

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(17) The intermediate amides of that route, although resolved into the respective atropisomers *ent*-**7a**/**b**, were not convincingly assigned configurationally (only based on the optical rotation of a precursor, no NOE or other investigations) and were not specifically converted into the respective axially chiral products, since the cyclization led to atropisomeric mixtures.

(18) For the first preparation of pure **7a** and **7b**, their stereoanalysis, and their specific transformation into **3a** and **3b**, respectively, see: Amslinger, B. Ph.D. dissertation, Julius-Maximilians-Universität, Würzburg, 2013.

(19) For the specific bioactivities of the pure stereoisomers of the acetamides **7**, see the Supporting Information.

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