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# Ancistrocladinium A and B, the First N,C-Coupled Naphthyldihydroisoquinoline Alkaloids, from a Congolese *Ancistrocladus* Species¶

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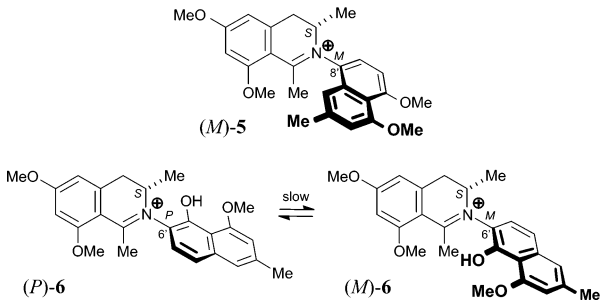


The isolation and structural elucidation of three novel-type naphthylisoquinoline alkaloids, ancistrocladinium A and B (the latter along with its atropisomer), from a Congolese *Ancistrocladus* species collected in the habitat Yeteto is reported. Their structures, including all stereochemical features, were elucidated by spectroscopic, chemical, and chiroptical methods. Ancistrocladinium A and B are the first N,C-coupled naphthyldihydroisoquinoline alkaloids found in nature, i.e., with an iminium−aryl axis. Although ancistrocladinium A, which is N,8′-coupled, is configurationally stable at this axis, ancistrocladinum B and its rotational isomer are based on a hitherto unprecedented N,6′-coupling type, with a slow rotation about the hetero biaryl axis at room temperature; they thus occur as a 46:54 mixture of two configurationally semistable atropo-diastereomers. For the isomerization of (*P*)-ancistrocladinium B to its (*M*)-diastereomer and for the opposite direction, the Gibbs free energies of activation were determined to be $\Delta G^{\ddagger}_1 = 105.8$ kJ mol$^{-1}$ and $\Delta G^{\ddagger}_2 = 105.7$ kJ mol$^{-1}$, respectively. In addition, the compounds were shown to have promising antileishmanial activities.

## Introduction

The naphthylisoquinoline alkaloids[1,2] constitute a rapidly growing class of structurally, biosynthetically, and pharmacologically unique naturally occurring biaryls.[3] About 120 alkaloids of this type have so far been isolated, all of them from plant species belonging to the Ancistrocladaceae and Dioncophyllaceae families, thus likewise serving as a phytochemical

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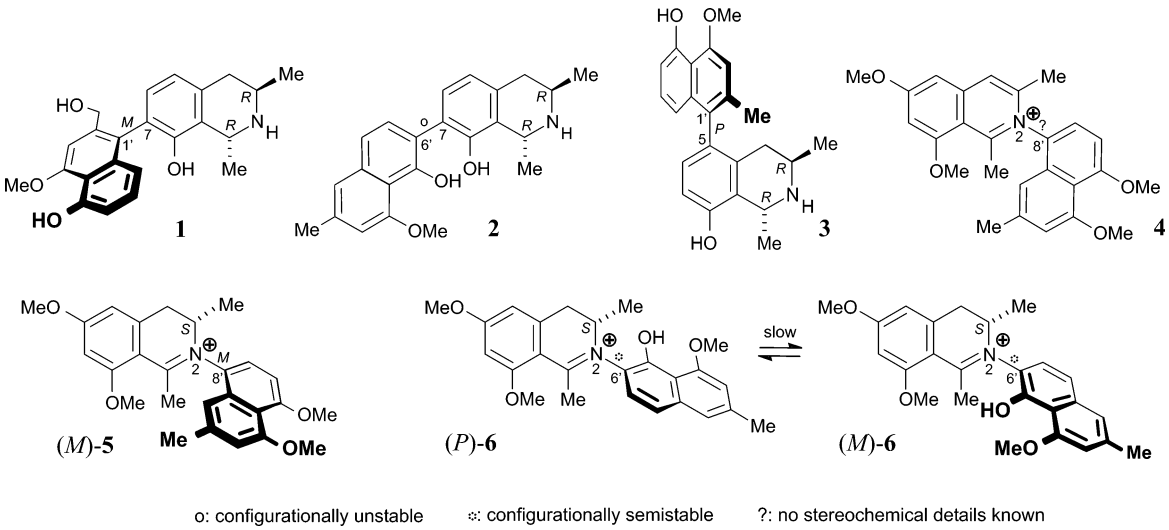


**FIGURE 1.** Structures of naphthylisoquinoline alkaloids of different coupling types, including the new alkaloids **5** and **6**.

marker for these two closely related tropical plant families. Some of the compounds display high antimalarial activities against *Plasmodium falciparum* both in vitro and in vivo, such as dioncopeltine A (**1**) and dioncophyllines B (**2**) and C (**3**),[4−6] whereas other representatives show remarkable activities against the pathogens of leishmaniasis,[7,8] Chagas' disease, and African sleeping sickness.[9,10] This makes it rewarding to search for further such alkaloids in other, phytochemically still unexplored *Ancistrocladus* species, even including new, botanically undescribed species. As an example, the new species *A. benomensis*,[11] reported by Rischer and G. Bringmann from the peninsula of Malaysia, has proven to be a rich source of novel-type naphthylisoquinoline alkaloids.[12,13]

The two molecular portions of naphthylisoquinoline alkaloids have been shown to be formed from six acetate units each, with subsequent regio- and stereoselective coupling via a phenol-oxidative mechanism.[14,15] This implies coupling positions ortho or para to the phenolic oxygen functions present in the isoquinoline and naphthalene moieties, resulting in eight possible coupling types (four in the naphthalene portion, C-1′, C-3′, C-6′, and C-8′, and two in the isoquinoline part, C-5 and C-7), of which seven have so far been identified in nature. With the recent discovery of ancisheynine (**4**),[16] the first N,C-coupled naphthylisoquinoline alkaloid, the spectrum of possible coupling types has been further enlarged.

In this paper, we report on the isolation, structural elucidation, and bioactivities of the three first N,C-coupled naphthyldihydroisoquinoline alkaloids, named ancistrocladinium A [(*M*)-**5**], ancistrocladinium B [(*P*)-**6**], and *N*-6′-*epi*-ancistrocladinium B [(*M*)-**6**], all of them being quaternary and thus cationic isoquinolinium metabolites, from the leaves of an *Ancistrocladus* species collected in the vicinity of Ikela. Although as of yet nothing is known about the stereochemical behavior of natural ancisheynine (**4**),[16,17] the rotational isomerism of the new compounds described here, **5** and **6**, has been closely investigated. Accordingly, ancistrocladinium A [(*M*)-**5**] has a stable axial *M*-configuration, whereas ancistrocladinium B [(*P*)-**6**] and *N*-6′-*epi*-ancistrocladinium B [(*M*)-**6**], the first N,6′-linked naphthylisoquinoline alkaloids, occur as a pair of configurationally semistable and thus slowly interconverting atropo-diastereomers. The configurations at the axes relative to the stereogenic centers were attributed by NOESY measurements, whereas the absolute axial configurations were assigned by a combination of experimental and computational CD investigations.

## Results and Discussion

Samples of a probably new *Ancistrocladus* species were collected in the Democratic Republic of Congo, in the swamp

(1) Bringmann, G.; Pokorny, F. In *The Alkaloids*; Cordell, G. A., Ed.; Academic Press: New York, 1995; Vol. 46, pp 127−271.
(2) Bringmann, G.; François, G.; Aké Assi, L.; Schlauer, J. *Chimia* **1998**, *52*, 18−28.
(3) Bringmann, G.; Günther, C.; Ochse, M.; Schupp, O.; Tasler, S. In *Prog. Chem. Org. Nat. Prod*; Herz, W., Falk, H., Kirby, G. W., Moore, R. E., Tamm, C., Eds.; Wien: 2001; Vol. 82, pp 1−293.
(4) François, G.; Timperman, G.; Eling, W.; Aké Assi, L.; Holenz, J.; Bringmann, G. *Antimicrob. Agents Chemother.* **1997**, *41*, 2533−2539.
(5) François, G.; Chimanuka, B.; Timperman, G.; Holenz, J.; Plaizier-Vercammen, J.; Aké Assi, L.; Bringmann, G. *Parasitol. Res.* **1999**, *85*, 935−941.
(6) Bringmann, G.; Feineis, D. *Act. Chim. Thérapeut.* **2000**, *26*, 151−171.
(7) Bringmann, G.; Hamm, A.; Günther, C.; Michel, M.; Brun, R.; Mudogo, V. *J. Nat. Prod.* **2000**, *63*, 1465−1470.
(8) Bringmann, G.; Dreyer, M.; Faber, J. H.; Dalsgaard, P. W.; Stærk, D.; Jaroszewski, J. W.; Ndangalasi, H.; Mbago, F.; Brun, P.; Reichert, M.; Maksimenka, K.; Christensen, S. B. *J. Nat. Prod.* **2003**, *66*, 1159−1165.
(9) Bringmann, G.; Holzgrabe, U.; Hoerr, V.; Stich, G. *Pharmazie* **2003**, *58*, 343−346.
(10) Bringmann, G. In *Guidelines and Issue for the Discovery and Drug Development against Tropical Diseases*; Vial, H., Fairlamb, A., Ridley, R., Eds.; World Health Organization: Geneva, 2003; pp 145−152.
(11) Rischer, H.; Heubl, G.; Meimberg, H.; Dreyer, M.; Hadi, A.; Bringmann, G. *Blumea* **2005**, *50*, 357−365.
(12) Bringmann, G.; Dreyer, M.; Kopff, H.; Rischer, H.; Wohlfarth, M.; Hadi, H. A.; Brun, R.; Meimberg, H.; Heubl, G. *J. Nat. Prod.* **2005**, *68*, 686−690.
(13) Bringmann, G.; Dreyer, M.; Michel, M.; Tayman, F. S. K.; Brun, R. *Phytochemistry* **2004**, *65*, 2903−2907.
(14) Bringmann, G.; Wohlfarth, M.; Rischer, H.; Grüne, M.; Schlauer, J. *Angew. Chem., Int. Ed.* **2000**, *39*, 1464−1466.
(15) Bringmann, G.; Feineis, D. *J. Exp. Bot.* **2001**, *52*, 2015−2022.
(16) Yang, L.-K.; Glover, R. P.; Yoganathan, K.; Sarnaik, J. P.; Godbole, A. J.; Soejarto, D. D.; Buss, A. D.; Butler, M. S. *Tetrahedron Lett.* **2003**, *44*, 5827−5829.
(17) For the first total synthesis of ancisheynine (**4**), including the resolution and stereochemical assignment of its atropo-enantiomers, see: Bringmann, G.; Gulder, T.; Reichert, M.; Meyer, F. *Org. Lett.* **2006**, *8*, 1037−1040.

region of the rainforest Yeteto near the town Ikela. From the Congo Basin, only three species—*A. congolensis*, *A. likoko*, and *A. ealaensis*, all described by J. Leonard[18]—are known so far.[19] Because of the lack of availability of species from this region and because most herbarium specimens lack flowers or fruits, it is difficult to estimate the variability of the species or to get a sound knowledge of the distribution range and ecological preferences. It is likely that more new species and more new records will come to light if fertile collections become available. In some, but not all, characters, the samples investigated from Ikela approach *A. ealaensis*, therefore, further investigations, including DNA analysis, are necessary to clarify the presumed species status.[20]

Air-dried and powdered leaves of the collected plant material were exhaustively extracted with $CH_2Cl_2$/MeOH (6:4). The extract was submitted to liquid−liquid separation, fast centrifugal partition chromatography (FCPC), and preparative HPLC, which permitted isolation of three alkaloids. Their UV profiles hinted at the presence of naphthylisoquinoline alkaloids, but the second maximum of each of the compounds was found to be shifted bathochromically by 30−40 nm in comparison to those previously reported for other naphthylisoquinoline alkaloids (typically ca. 306 nm).[8,12,13,21]

The first, relatively apolar compound was found to possess a cationic molecular formula of $C_{27}H_{34}NO_4^+$, as revealed by HRMS (ESI) and by the number of signals in the $^{13}C$ NMR spectrum. The presence of a naphthyl-1,3-dimethyldihydroisoquinoline alkaloid was deduced from the typical downfield shift of the methyl group at C-1 ($\delta$ 2.52) in the $^1H$ NMR spectrum, from its multiplicity (singlet) and from the chemical shift of the $^{13}C$ NMR signal of C-1 ($\delta$ 179.6). This was in agreement with the lack of the H-1 quartet, which normally appears around $\delta$ 4 for 1,3-dimethyltetrahydroisoquinolines.[1] The aromatic region showed the presence of six protons, one more than in previously reported naphthyldi- or naphthyltetrahydroisoquinolines containing four oxygen atoms.[1] Two of these protons ($\delta$ 6.97 and 7.46) were ortho-coupled, as revealed by their H,H−COSY interactions, giving rise to two doublets. Furthermore, four singlets ($\delta$ 6.74, 6.77, 6.98, and 7.08) were detected, indicating that the biaryl axis was located in the methyl-free ring of the naphthalene moiety, which was further confirmed by the normal-shifted signal of $CH_3$-2′ ($\delta$ 2.50). NOE correlations between the doublet of H-6′ ($\delta$ 6.97) and the protons of $OCH_3$-5′ ($\delta$ 4.01) left C-8′ as the only possible location of the biaryl axis in the naphthalene moiety. This attribution was confirmed by NOE interactions found in the series {H-1′−$CH_3$-2′−H-3′−$OCH_3$-4′} and by the fact that both H-1′ and H-6′ showed $^3J$ HMBC couplings with the quaternary carbon atom C-8′.

By NOE correlations in the series {$CH_3$-1−$OCH_3$-8−H-7−$OCH_3$-6−H-5−$H_{eq}$-4−$CH_3$-3}, the remaining two aromatic protons ($\delta$ 6.74 and 6.77) were deduced to be located in the isoquinoline moiety at C-7 and C-5, respectively, which was confirmed by HMBC effects of both $H_{eq}$-4 and H-7 to C-5 and, furthermore, by HMBC couplings of H-5 to C-7. This assignment unequivocally left N-2 as the only possible coupling position of the biaryl axis in the isoquinoline portion, thus

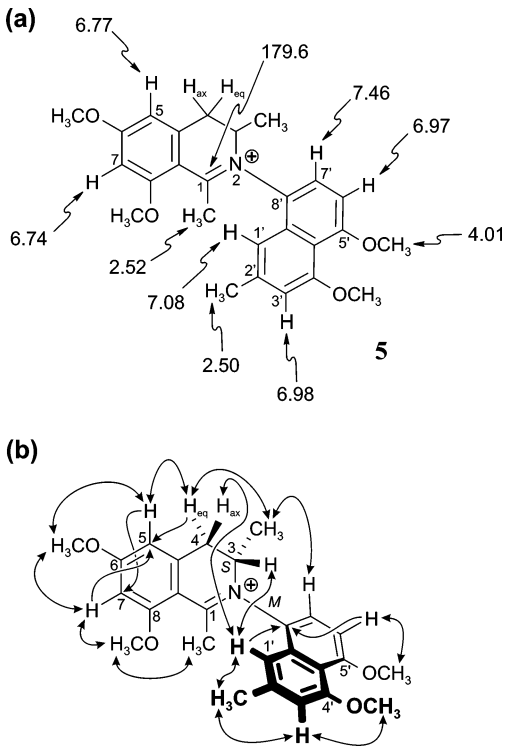


**FIGURE 2.** Selected NMR data of ancistrocladinium A [(*M*)-**5**]: $^1H$ and $^{13}C$ NMR shifts ($\delta$ in ppm) (a) and HMBC (single arrows) and NOE (double arrows) interactions for the determination of the relative configuration (b).

indicating the presence of a dihydroisoquinolinium cation. Because the isolation procedure gave rise to the respective trifluoroacetate, the identity of the genuine counterion of this quaternary alkaloid is as of yet unknown.

In conclusion, the compound was found to possess the constitution **5** shown in Figure 2a, with an N,8′-linkage and the structure of an N,C-coupled naphthyldihydroisoquinoline alkaloid. This novel compound was henceforth given the trivial name ancistrocladinium A. The only other known representative of the same coupling type, ancisheynine (**4**) from *A. heyneanus*,[16] has a fully dehydrogenated isoquinolinium moiety and hence no stereogenic center.

Ancistrocladinium A (**5**) has two different stereogenic elements: the stereocenter at C-3 and the rotationally stable hetero biaryl axis between the isoquinoline part and the naphthalene portion. The configuration at the axis relative to the stereogenic center at C-3 was deduced from NOE correlations between H-1′ and both H-3 and $H_{ax}$-4, which are above the isoquinoline plane, indicating a close spatial proximity of these atoms. This relative configuration was fully confirmed by a similar specific interaction below the plane, viz. between H-7′ and the protons of the methyl group at C-3.

The absolute configuration at C-3 of **5** was determined by ruthenium-mediated oxidative degradation[22] followed by stereochemical analysis of the degradation products using gas chromatography with mass selective detection (GC-MSD) after derivatization with the *R*-enantiomer of Mosher's chloride. The formation of (*S*)-3-aminobutyric acid clearly showed the alkaloid

(18) Léonard, J. *Bull. Soc. Bot. Belg.* **1949**, *82*, 27−40.
(19) Cheek, M. *Kew Bull.* **2000**, *55*, 871−882.
(20) The botanical description of the plant species is in progress.
(21) Bringmann, G.; Dreyer, M.; Faber, J. H.; Dalsgaard, P. W.; Stærk, D.; Jaroszewski, J. W.; Ndangalasi, H.; Mbago, F.; Brun, R.; Christensen, S. B. *J. Nat. Prod.* **2004**, *67*, 743−748.
(22) Bringmann, G.; God, R.; Schäffer, M. *Phytochemistry* **1996**, *43*, 1393−1403.

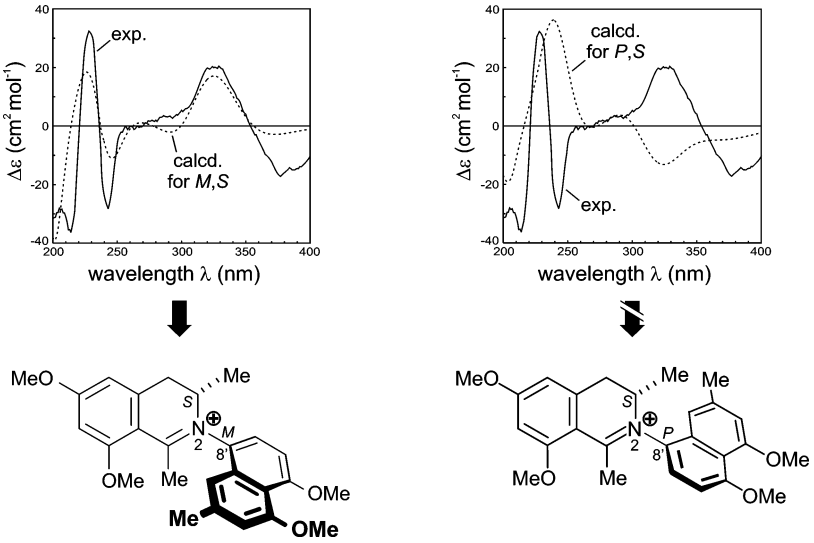


**FIGURE 3.** Attribution of the absolute axial configuration of ancistrocladinium A (**5**) as *M*, by comparison of its experimental CD curve with the spectra calculated for (*M*,3*S*)-**5** (left) and (*P*,3*S*)-**5** (right), using TDDFT (B3LYP/TZVP).

to be *S*-configured at C-3. From the configuration at the axis relative to the stereogenic center as determined by NMR (see above), together with the absolute *S*-configuration at C-3 assigned by degradation, the absolute configuration at the axis was unambiguously deduced as *M*, as shown in Figure 2b.

For a further confirmation of the absolute axial configuration of ancistrocladinium A (**5**), circular dichroism (CD) investigations were carried out. Because **5** constitutes the first N,8′-coupled naphthyldihydroisoquinoline alkaloid, an empirical comparison of the CD spectrum of **5** with that of a configurationally known similar alkaloid was not possible. Therefore, quantum chemical CD calculations[23−25] were performed. In two independent approaches, conformational analyses were carried out, each for the (*M*,3*S*)- and (*P*,3*S*)-atropo-diastereomers of **5**, by using the semiempirical PM3[26] method. The corresponding minimum geometries found were further optimized by DFT calculations at the B3LYP[27]/6-31G*[28] level, leading to four minimum structures in both cases. For these geometries, CD computations were performed by means of TDDFT (B3LYP/TZVP),[29] followed by a weighted summation of the calculated CD curves for (*M*,3*S*)- and (*P*,3*S*)-**5**, according to the Boltzmann statistics. The overall spectra thus obtained were subsequently UV corrected[25] and compared with the measured CD curve of **5**, resulting in good agreement between the spectrum calculated for the (*M*,3*S*)-atropo-diastereomer and the experiment (Figure 3, left) over the whole range of wavelength under investigation, whereas the spectrum simulated for the (*P*,3*S*)-atropo-diastereomer exhibited an opposite CD behavior in the wavelength range between approximately 250 and 350 nm, compared to the experimental CD curve (Figure 3, right). The absolute configuration of ancistrocladinium A (**5**) was thus assigned as *M*,3*S*, which fully confirmed the above attribution by the NOE correlations. Hence, ancistrocladinium A has the full absolute stereostructure **5** as shown in Figure 3, i.e., with an *M*,3*S*-configuration.

In the more apolar region of the analytical HPLC chromatogram, two baseline-separated peaks, A and B (retention times of 16.8 and 18.1 min, respectively), were detected in a ratio of 46:54.

Preparative isolation of the faster peak A again resulted in a mixture of peaks A and B, but now enriched in peak A with a 75:25 ratio. Immediate HPLC analysis of the freshly resolved single peaks revealed an equilibration of the two peaks by gradual interconversion even at room temperature. By HPLC-MS, a corresponding mass of $m/z = 406$ was obtained, for both peaks A and B. HRMS (ESI) again, as for ancistrocladinium A (**5**), showed that the two compounds were cationic, with a molecular formula of $C_{25}H_{28}NO_4^+$, i.e., smaller than **5** by 14 mass units. Even though pure fractions of peak A were obtained by HPLC, a renewed partial equilibration by atropo-diastereomerization could not be prevented during workup. $^1$H NMR spectra of pure isolated peak A were, however, obtained by LC-NMR. The online spectra showed a three-proton singlet at low field for C$H_3$-1 ($\delta$ 2.56), which in combination with the absence of a three-proton doublet at ca. $\delta$ 1.5 and an H-1 signal at ca. $\delta$ 4 (as typical of naphthyl-1,3-dimethyltetrahydroisoquinolines[1]) again hinted at the presence of a naphthyldihydroisoquinoline alkaloid. This assumption was confirmed by the chemical shift of the C-1 peak ($\delta$ 174.5) in the $^{13}$C NMR spectrum (taken offline, from a diastereotropic mixture, after isolation of the compound), to which the protons of C$H_3$-1 showed HMBC couplings. The presence of three methoxy groups was established by the signals resonating at $\delta$ 3.97, 4.00, and 4.04, each integrating for three protons. Two of them were assigned to be situated at C-6 and C-8 in the isoquinoline moiety, by NOE correlations in the series {C$H_3$-1−OC$H_3$-8−H-7−OC$H_3$-6−H-

(23) Bringmann, G.; Mühlbacher, J.; Reichert, M.; Dreyer, M.; Kolz, J.; Speicher, A. *J. Am. Chem. Soc.* **2004**, *126*, 9283−9290.

(24) Bracher, F.; Eisenreich, W. J.; Mühlbacher, J.; Dreyer, M.; Bringmann, G. *J. Org. Chem.* **2004**, *69*, 8602−8608.

(25) Bringmann, G.; Busemann, S. In *Natural Product Analysis*; Schreier, P., Herderich, M., Humpf, H. U., Schwab, W., Eds.; Vieweg: Wiesbaden, 1998; pp 195−212.

(26) Stewart, J. J. P. *J. Comput. Chem.* **1989**, *10*, 209−264.

(27) (a) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648−5652. (b) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785−789. (c) Stephens, P. J.; Devlin, F. J.; Chabalowski, C. F.; Frisch, M. J. *J. Phys. Chem.* **1994**, *98*, 11623−11627.

(28) Hariharan, P. C.; Pople, J. A. *Theor. Chim. Acta* **1973**, *28*, 213−222.

(29) (a) Schäfer, A.; Horn, H.; Ahlrichs, R. *J. Chem. Phys.* **1992**, *97*, 2571−2577. (b) Schäfer, A.; Huber, C.; Ahlrichs, R. *J. Chem. Phys.* **1994**, *100*, 5829−5835.

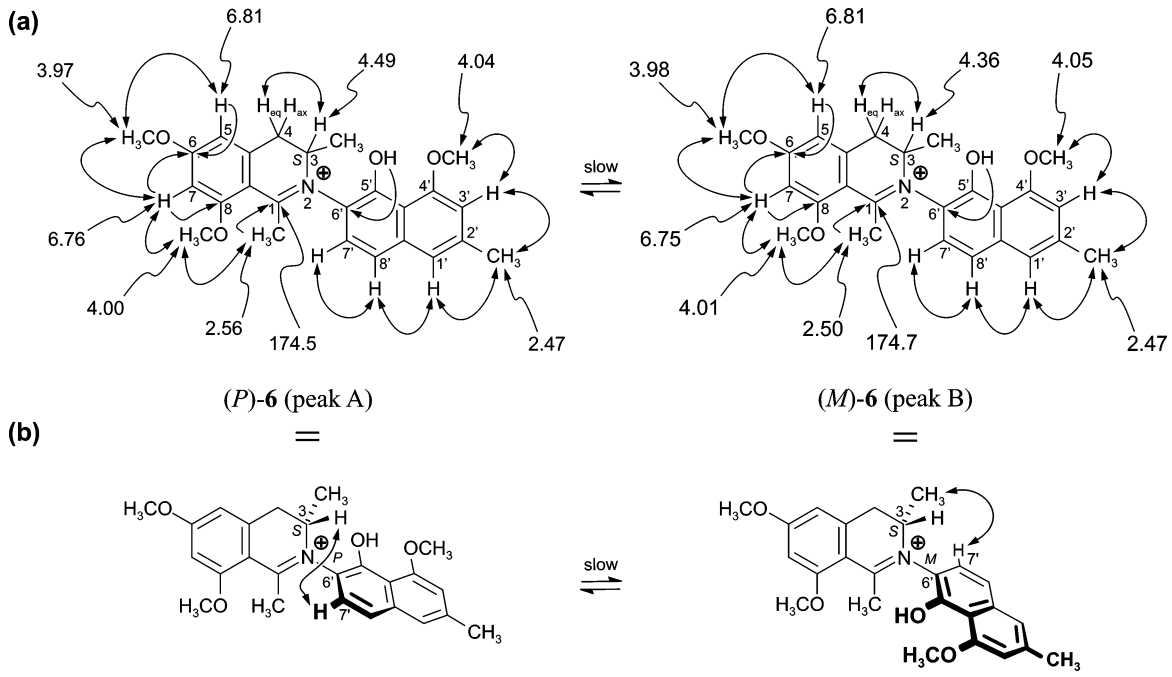


**FIGURE 4.** Ancistrocladinium B (*P*-**6**, peak A) and *N*,6′-*epi*-ancistrocladinium B (*M*-**6**, peak B): $^{1}$H and $^{13}$C NMR shifts ($\delta$ in ppm), HMBC (single arrows), and NOE (double arrows) interactions used for the determination of the constitution (a) and the configuration at the axis relative to the stereogenic center through NOE interactions (b).

5−$H_{eq}$-4−H-3}, which was further confirmed by HMBC couplings of the singlet signals of H-7 ($\delta$ 6.76) to both C-8 and C-6 and of H-5 ($\delta$ 6.81) to C-6. The remaining OMe group was established to be located at C-4′ by an NOE interaction with H-3′.

This assignment unambiguously left N-2 as the only possible coupling position of the biaryl to the isoquinoline moiety thus, as for ancistrocladinium A [(*M*)-**5**], hinting at the presence of a dihydroisoquinolinium cation. The quaternary alkaloid was isolated as its trifluoroacetic acid salt, but the identity of the authentic, natural counterion was not yet established.

In the naphthalene moiety, the aromatic spin pattern of two singlets and two doublets and the normal, not high-field shifted, signal of $CH_3$-2′ ($\delta$ 2.47) showed that C-6′ or C-8′ was the coupling position of the biaryl axis. The latter was excluded by an NOE correlation sequence in the series {H-7′−H-8′−H-1′−$CH_3$-2′−H-3′−$OCH_3$-4′}. This attribution was in agreement with HMBC long-range couplings, found crosswise from H-8′ to C-1′ and from H-1′ to C-8′ and between the signal of O*H*-5′ and the quaternary C-6′, thus unequivocally revealing the coupling position in the naphthalene moiety to be at C-6′.

In conclusion, peak A is an N,6′-coupled naphthylisoquinoline alkaloid with the constitution **6** shown in Figure 4a. This novel compound was henceforth named ancistrocladinium B.

Fortunately, the more slowly eluting peak B, likewise corresponding to $m/z$ = 406 and to a molecular formula of $C_{25}H_{28}NO_4^+$, was immediately obtained in a pure form in the separation mentioned above. Again, immediate HPLC analysis of the freshly resolved single peak B obtained by preparative HPLC showed a slow conversion to a mixture of peaks A and B at room temperature. The NMR data of peak B were found to be nearly identical to those of peak A, just slightly shifted for some of the signals. As an example, the $^{13}$C NMR peak for C-1 ($\delta$ 174.5 for peak A) was now observed at $\delta$ 174.7 and the $^{1}$H NMR shifts for C$H_3$-1 ($\delta$ 2.56 for peak A) were observed at $\delta$ 2.50; H-7 ($\delta$ 6.76 for peak A) occurred at $\delta$ 6.75. The signals of the three methoxy groups resonated now at $\delta$ 3.98, 4.01, and 4.05 ($\delta$ 3.97, 4.00, and 4.04 for peak A).

HMBC couplings and NOE correlations clearly showed that peak B had the same constitution (**6**) as peak A, leading to the conclusion that the slowly interconverting peaks A and B might be atropo-diastereomers (see Figure 4), rather than, e.g., epimers at C-3. This second isomer of **6** was thus given the name *N*,6′-*epi*-ancistrocladinium B.

Similar to that for (*M*)-**5**, the absolute configuration at C-3 was determined to be *S* for both atropisomers, again by ruthenium-mediated oxidative degradation,[22] performed here directly on a mixture of peaks A and B, affording exclusively the (*S*)-enantiomer of 3-aminobutyric acid, analyzed by GC-MS of its Mosher derivative.

By NOESY investigations on the pure atropisomers, the relative configuration at the axis for each of the two epimers was established. For the more rapid isomer (peak A), an atropisomer-specific NOE correlation was observed between H-3 and H-7′. In combination with the known *S*-configuration at C-3, this interaction revealed an absolute *P*-configuration at the chiral axis as shown in Figure 4b. A similar specific NOE interaction was observed between the protons of C$H_3$-3 and H-7′ for peak B, allowing us to attribute the absolute axial *M*-configuration to the slower atropisomer (see Figure 4b). This assignment further confirmed the above assumption that peaks A and B represent the slowly interconverting atropo-diastereomers (*P*)-**6** and (*M*)-**6** of a novel-type N-6′-coupled naphthylisoquinoline alkaloid, **6**.

The assumption that **6** consists of an atropo-diastereomeric mixture, was further corroborated by the individual CD spectra of the two atropisomers recorded online, by using the HPLC-

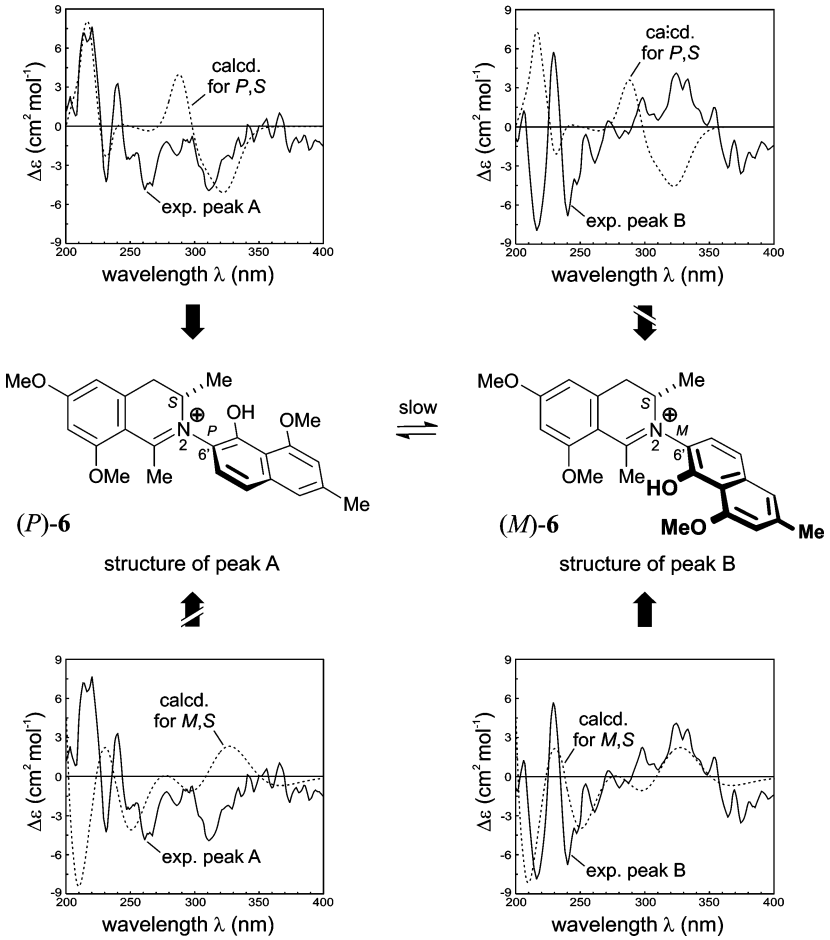


**FIGURE 5.** Assignment of the absolute configuration to ancistrocladinium B [(*P*)-**6**] and its atropo-diastereomer, (*M*)-**6**, by comparison of the experimental LC-CD spectra (stopped-flow mode) of peaks A (left) and B (right) with the spectra calculated for (*P*,3*S*)-**6** and (*M*,3*S*)-**6**, by using TDDFT (B3LYP/TZVP).

CD technique in the stopped-flow mode.[30] The two spectra were found to be virtually opposite, as anticipated from the fact that—like in conventional C,C-coupled cases[31,32]—the hetero biaryl system, with its particular dihedral angle at the axis between the chromophores, strongly dominates the CD curves. The presence of the new coupling type in **6** precluded a configurational assignment of the two atropisomers by empirical comparison of their experimental CD spectra with that of a configurationally known similar alkaloid. The attribution of the two peaks to the atropo-diastereomers of **6** was therefore achieved by quantum chemical CD calculations.[23−25] With the stereogenic center at C-3 assigned as *S* (see above), the computational efforts concentrated on the absolute configuration at the chiral axes. The two independent conformational analyses at the semiempirical PM3 level for the two atropo-diastereomers, (*P*,3*S*)-**6** and (*M*,3*S*)-**6**, resulted in five and six minimum geometries, respectively, which were further optimized using DFT (B3LYP/6-31G*). In both cases, this approach delivered four minimum structures, which were submitted to CD calculations by means of TDDFT (B3LYP/TZVP). The overall two CD curves were received as described for ancistrocladinium A (**5**) and compared with the experimental spectra (Figure 5).

The experimental CD spectrum of peak A (faster eluting in HPLC) agreed well with the one simulated for (*P*,3*S*)-**6** (Figure 5, top left), as did the CD curve of peak B (more slowly eluting in HPLC) with the one calculated for (*M*,3*S*)-**6** (Figure 5, bottom right). From this pairwise match, the absolute configurations of the two atropo-diastereomers were unambiguously determined, leading to the same results as those deduced from the observed NOE correlations for the two signal sets in $^1$H NMR (see Figure 4b).

Until now, only one other configurationally semistable biaryl axis has been reported: dioncophylline E, which is C,C-coupled (7,3′; structure not shown).[33] All other known representatives of this class of secondary metabolites form rotational isomers that—depending on the substitution pattern in the proximity of the biaryl linkage—either are configurationally fully stable, such as dioncopeltine A (**1**)[34] and dioncophylline C (**3**),[35] or rotate rapidly at room temperature, as in the case of dioncophylline B (**2**).[36]

(30) Bringmann, G.; Messer, K.; Wohlfarth, M.; Kraus, J.; Dumbuya, K.; Rückert, M. *Anal. Chem.* **1999**, *71*, 2678−2686.

(31) Bringmann, G.; Teltschik, F.; Michel, M.; Busemann, S.; Rückert, M.; Haller, R.; Bär, S.; Robertson, A.; Kaminsky, R. *Phytochemistry* **1999**, *52*, 321−332.

(32) Bringmann, G.; Gulden, K.-P.; Hallock, Y. F.; Manfredi, K. P.; Cardellina, J. H., II; Boyd, M. R.; Kramer, B.; Fleischhauer, J. *Tetrahedron* **1994**, *50*, 7807−7813.

(33) Bringmann, G.; Messer, K.; Wolf, K.; Mühlbacher, J.; Grüne, M.; Brun, R.; Louis, A. M. *Phytochemistry* **2002**, *60*, 389−397.

(34) Bringmann, G.; Rübenacker, M.; Vogt, P.; Busse, H.; Aké Assi, L.; Peters, K.; von Schnering, H. G. *Phytochemistry* **1991**, *30*, 1691−1696.

(35) Bringmann, G.; Rübenacker, M.; Weirich, R.; Aké Assi, L. *Phytochemistry* **1992**, *31*, 4019−4024.

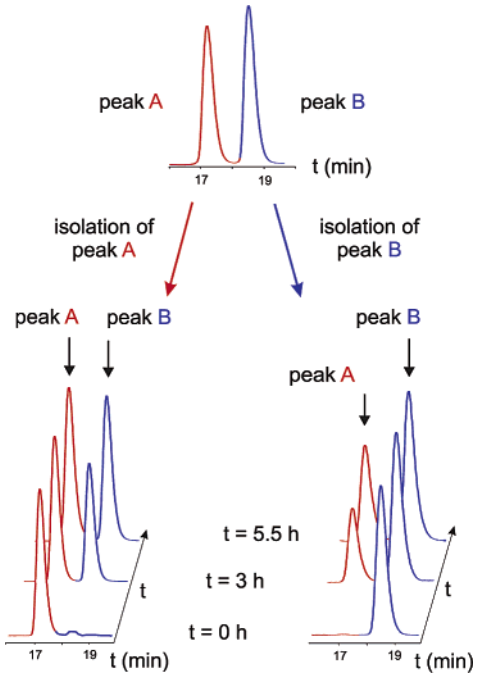


**FIGURE 6.** Determination of the atropisomerization rates of ancistrocladinium B [(*P*)-**6**] and its epimer (*M*)-**6**, by resolution of its two atropo-diastereomers (peaks A and B), followed by their separate thermal equilibration over time in MeOH/$H_2O$ (1:1) at 65 °C, analyzed by analytical HPLC on an achiral $C_{18}$ phase.

This configurational semistability of the axis of this pair of novel naphthylisoquinoline alkaloids, (*P*)-**6** and (*M*)-**6**, made it rewarding to measure its rotational barrier by investigating the temperature-dependent atropo-diastereomerization. The decrease of the diastereomeric excess of freshly resolved chromatographical fractions enriched in the respective (*P*)- or (*M*)-atropisomer was analyzed by HPLC-UV (see Figure 6). The diastereomerization rates of ancistrocladinium B and its atropisomer (**6**) were determined for both directions at different temperatures between 55 and 85 °C. The Gibbs free energy of activation obtained from the slopes of the Eyring plots (see Figure S1 in the Supporting Information) was found to be $\Delta G^{\ddagger}_1 = 105.8$ kJ mol$^{-1}$ and $\Delta G^{\ddagger}_2 = 105.7$ kJ mol$^{-1}$, for (*P*)-**6** and (*M*)-**6**, respectively. The obtained isomerization barriers are just above the defined energy barrier of well-separable stable isomers[37] and thus are in good accordance with the observed slow interconversion of the two atropo-diastereomers at room temperature.[38] A satisfying agreement was also found with the results from DFT calculations (B3LYP/6-31G*), leading to a value of $\Delta G^{\ddagger}_1 = 112.6$ kJ mol$^{-1}$ for the atropisomerization from (*P*)- to (*M*)-**6** and of $\Delta G^{\ddagger}_2 = 113.9$ kJ mol$^{-1}$ for the axial rotation from (*M*)- to (*P*)-**6**, thus even reproducing the slight preference for the *M*-atropo-diastereomer of 54:46 (*M*/*P*) as found experimentally in the atropisomeric equilibrium at room temperature. The differences between the observed Gibbs free energies of activation and the calculated ones can be explained by the fact that the computations were carried out in vacuo, while the solvents used will certainly affect the experimentally obtained values.

**TABLE 1. Bioactivities of Compounds (*M*)-5 and (*M*/*P*)-6**

| | $IC_{50}$ ($\mu$g mL$^{-1}$) | |
|---|---|---|
| | (*M*)-**5** | (*M*/*P*)-**6** |
| *P. falciparum* (strain: K1) | 0.25 | 0.862 |
| standard: chloroquine 0.041 | | |
| *T. cruzi* (strain: tulahuen C4) | >30 | 1.24 |
| standard: benznidazole 0.53 | | |
| *T. b. rhodesiense* (strain: STIB 900) | 0.075 | 0.39 |
| standard: melarsoprol 0.00046 | | |
| *L. donovani* (strain: MHOM-ET-67/L82) | 0.722 | 1.1 |
| standard: miltefosine 0.05 | | |
| cytotoxicity cells: | 25.0 | 2.01 |
| rat skeletal myoblast L-6 | | |
| *L. major* (strain: MHOM/IL/81/FE/BNI) | 3.08 | 2.69 |
| standard: miltefosine 13.00 | | |
| cytotoxicity cells: | 22.15 | 7.93 |
| J774.1 macrophages | | |

The discovery of these first N,C-coupled naphthyldihydroisoquinoline alkaloids and the availability of authentic reference material made it rewarding to screen for ancistrocladinium A (**5**) and B (**6**, both atropisomers) in other *Ancistrocladus* species, by using the analytical "triad" HPLC-MS, HPLC-NMR, and HPLC-CD. This preliminary analysis immediately led to the likewise successful identification of ancistrocladinium A (**5**) in the crude extracts of *A. cochinchinensis* and *A. tectorius* (both from Vietnam) and of ancistrocladinium B [(*P*)-**6** and its atropisomer, (*M*)-**6**] in *A. benomensis* and *A. heyneanus* (from Malaysia and India, respectively), showing N,C-coupled alkaloids of this type to be far more widespread than anticipated.

The pronounced antiprotozoal activities of naphthylisoquinoline alkaloids against pathogens belonging to the genera *Plasmodium*, *Leishmania*, and *Trypanosoma*[4−10] warranted a screening of ancistrocladinium A [(*M*)-**5**] and B (**6**, as the thermodynamical mixture) against these parasites (see Table 1). Both alkaloids exhibited moderate antiplasmodial activities against the K1 strain of *P. falciparum*, although they did not reach the splendid $IC_{50}$ values obtained for other naphthylisoquinoline alkaloids.[4−6] The results are, however, most useful for ongoing quantitative structure−activity relationship (QSAR) investigations[39,40] because both compounds are based on as of yet unscreened and permanently cationic coupling types. Ancistrocladinium B [(*M*/*P*)-**6**] exhibited moderate activity against the pathogens *T. cruzi* and *T. b. rhodesiense*, whereas ancistrocladinium A [(*M*)-**5**] displayed a 5-fold higher activity against the pathogen *T. rhodesiense* but was inactive against *T. cruzi*. The most significant activities were detected against Old World *Leishmania* species. Both ancistrocladinium A [(*M*)-**5**] and B [(*M*/*P*)-**6**] were highly active against *L. donovani*, the pathogen responsible for visceral leishmaniasis. Against *L. major*, a pathogen that causes cutaneous leishmaniasis, ancistrocladinium B **6** displayed an activity 15-fold higher than that for miltefosine, which was used as a reference compound and positive control. The compounds were highly toxic against L-6 myoblast cells and J774.1 macrophages, but the indexes describing the cytotoxicity of the compounds against the cell lines in relation to their activity against the parasites ($IC_{50}$ cytotoxicity/$IC_{50}$ activity), for ancistrocladinium A [(*M*)-**5**] against *L. donovani* and for ancistrocladinium B [(*M*/*P*)-**6**] against *L. major*, suggest pharmacological profiles better than that of miltefosine in

(36) Bringmann, G.; Rübenacker, M.; Geuder, T.; Aké Assi, L. *Phytochemistry* **1991**, *30*, 3845−3847.
(37) Clayden, J.; Greeves, N.; Warren, S.; Wothers, P. *Organic Chemistry*; Oxford University Press: Oxford, 2001; p 450.
(38) Boiadjiev, S. E.; Lightner, D. A. *Tetrahedron: Asymmetry* **2002**, *13*, 1721−1732.
(39) Bringmann, G.; Rummey, C. *J. Chem. Inf. Comput. Sci.* **2003**, *43*, 304−316.
(40) Stiefl, N.; Bringmann, G.; Rummey, C.; Baumann, K. *J. Comput.-Aided Mol. Des.* **2003**, *17*, 347−365.

experiments performed in parallel. Further characterization of these specific activities is in progress.

## Conclusion

The discovery of the novel-type N,C-coupled naphthylisoquinoline alkaloids described in this paper opens new perspectives in the chemistry of axially chiral natural products. After a first hint of the occurrence of an N,C-coupled naphthylisoquinoline alkaloid, ancisheynine, in the literature (yet without any stereochemical investigations) and after its recent total synthesis and stereochemical characterization by our group, the present paper clearly shows that N,C-coupled alkaloids of this type are more widespread than anticipated. The work moreover reveals that, besides the discovery of new coupling types, this interesting novel subclass of naphthylisoquinoline alkaloids occurs not only as fully dehydrogenated isoquinolinium salts but also as dihydroisoquinolinium salts, equipped with a fully or partially stereogenic hetero biaryl axis. The compounds described here show the unique combination of alkaloids possessing stereogenic centers and stable or semistable axial chirality at a rotationally hindered iminium salt derived *N*,*C*-aryl axis.

Work to further explore this intriguing novel type of alkaloids, including other coupling types in the naphthalene portion and also the respective N,C-coupled tetrahydroisoquinolines (with possibly likewise rotationally hindered *N*-($sp^3$)-*C*-aryl axes), their stereochemistry, and their pharmacology is presently in progress.

## Experimental Section

**Plant Material.** Voucher specimens of all *Ancistrocladus* species have been deposited at the Herbarium Bringmann, University of Würzburg, with an individual number (no.). Leaf material of the Congolese *Ancistrocladus* plant species (no. 62) was collected by B. Loleko and one of us (V.M.) in the swamp region of the rainforest Yeteto near the town Ikela, in the Democratic Republic of Congo in November 2003. Leaf material of *A. cochinchinensis* Gagnepain (no. 63) was collected by Dr. M. Dreyer, Dr. L. H. Ngoc, and C. Bräuchler in April 2004 in the area Ba Na, of the province Da Nang, and *A. tectorius* (no. 48) was from the area Cao Som and Da Bac, of the province Hoa Binh, in Vietnam. Fresh leaves of *A. heyneanus* (Wallich ex J. Graham, no. 4) were collected from a live specimen in the greenhouse of the Botanical Garden of Würzburg in 2005. Leaf material of *A. benomensis* (Rischer and G. Bringmann,[11] no. 61) was used from a collection reported previously.[12]

**General Extraction and Isolation Procedures.** Air-dried leaves from the Congolese *Ancistrocladus* species (200 g) were ground and extracted exhaustively with $CH_2Cl_2$/MeOH (6:4). The solvent was removed under reduced pressure to give a combined organic extract (10 g). The extract was partioned between *n*-hexane and $H_2O$/MeOH (1:9). Evaporation of the aqueous phase to dryness afforded a residue (4 g), which was subsequently submitted to FCPC using a two-phase solvent system consisting of $CHCl_3$/EtOAc/MeOH/$H_2O$ (5:3:5:3). The lower phase served as the stationary phase (flow rate 15 mL $min^{-1}$, rotational speed 900 $min^{-1}$, descending mode, retention rate 75%). After ca. 140 min, the stationary phase was flushed out in reversed mode by using MeOH and concentrated under reduced pressure to give 450 mg of a residue. By preparative HPLC on a SymmetryPrep $C_{18}$ column (19 × 300 mm, 5 $\mu$m, Waters) with a flow rate of 12.0 mL $min^{-1}$ and a solvent system consisting of (A) $H_2O$ (0.05% trifluoroacetic acid) and (B) ACN (0.05% trifluoroacetic acid), this residue was resolved to give 10 mg of compound **5** (retention time 9.7 min) and 12 mg of **6** (retention time 13.5 min). For the separation of the atropisomeric mixture of (*P*)-**6** and (*M*)-**6** (obtained in a ratio of 46:54) with retention times of 16.2 and 18.4 min, respectively, an isocratic solvent system consisting of 40% $H_2O$ (10% MeOH) and 60% MeOH (10% $H_2O$), both doped with 0.05% TFA, was used with a runtime of 25 min. The column (Symmetry C18 (4.6 × 250 mm, 5 $\mu$m, Waters)) was cooled down to 10 °C, and the flow rate was set to 0.8 mL $min^{-1}$.

The same chromatographical conditions were used for the HPLC-CD investigations and for the LC-NMR experiments performed with $D_2O$ (10% MeOH, 0.05% TFA) and MeOH (10% $D_2O$, 0.05% TFA) as the solvent system.

**Ancistrocladinium A [(*M*)-5].** The isolated compound gave pale yellow crystals in a purity of >96% determined by HPLC: mp ≥230 °C (dec); $[\alpha]_D^{20}$ −6 (*c* = 0.05, MeOH); IR (NaCl) $\nu_{max}$ 2955, 2925, 2848, 1682, 1609, 1585, 1458, 1438, 1417, 1312, 1278, 1259, 1204, 1176, 1130, 1040, 836, 801 $cm^{-1}$; UV−vis (MeOH) $\lambda_{max}$ 335 (log $\epsilon$ 1.65), 225 (log $\epsilon$ 2.69), 214 (log $\epsilon$ 2.73) nm; CD (MeOH) $\Delta e_{311}$ −4.4, $\Delta e_{238}$ +2.3, $\Delta e_{230}$ −3.5, $\Delta e_{214}$ +6.4; $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 1.30 (d, $^3J$ = 7.1 Hz, 3 H, $CH_3$-3), 2.50 (s, 3 H, $CH_3$-2′), 2.52 (s, 3 H, $CH_3$-1), 3.13 (dd, $^2J$ = 17.4 Hz, $^3J$ = 2.5 Hz, 1 H, $H_{eq}$-4), 3.83 (dd, $^2J$ = 17.4 Hz, $^3J$ = 6.2 Hz, 1 H, $H_{ax}$-4), 3.97 (s, 3 H, $OCH_3$-4′), 4.01 (s, 3 H, $OCH_3$-5′), 4.03 (s, 3 H, $OCH_3$-6), 4.04 (s, 3 H, $OCH_3$-8), 4.25 (m, 1 H, H-3), 6.74 (s, 1 H, H-7), 6.77 (s, 1 H, H-5), 6.97 (d, $^3J$ = 8.5 Hz, 1 H, H-6′), 6.98 (s, 1 H, H-3′), 7.08 (s, 1 H, H-1′), 7.46 (d, $^3J$ = 8.5 Hz, 1 H, H-7′) ppm; $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$ 17.2 ($CH_3$-3), 23.9 ($CH_3$-2′), 26.5 ($CH_3$-1), 36.7 (C-4), 58.6 ($OCH_3$-5′), 58.9 ($OCH_3$-4′), 58.8 ($OCH_3$-6), 58.9 ($OCH_3$-8), 61.3 (C-3), 100.7 (C-7), 106.6 (C-6′), 111.1 (C-5), 112.9 (C-3′), 113.8 (C-9), 114.8 (C-1′), 119.7 (C-8′), 128.9 (C-7′), 131.6 (C-10′), 133.8 (C-9′), 143.7 (C-2′), 143.9 (C-10), 161.5 (C-4′), 162.7 (C-5′), 168.1 (C-8), 172.4 (C-6), 179.6 (C-1) ppm; EIMS *m*/*z* (%) = 420.2 (12.3) [$M^+$], 419.2 (27.9) [$M - H^+$], 404.1 (100) [$M - CH_4^+$]; HRMS (ESI) calcd for $C_{26}H_{30}NO_4^+$ 420.21748; found 420.21750.

**Ancistrocladinium B [(*P*)-6]/*N*-6′-*epi*-Ancistrocladinium B [(*M*)-6].** This compound was obtained likewise as pale yellow crystals with a chemical purity of >99%, determined by HPLC, yet still as an atropisomeric mixture (46:54): mp ≥ 230 °C (dec); $[\alpha]_D^{20}$ −8 (*c* = 0.06, MeOH); IR (NaCl) $\nu_{max}$ 3330, 2925, 2852, 1690, 1612, 1580, 1555, 1460, 1380, 1310, 1280 1200, 1170, 1125, 1090, 960, 841, 800 $cm^{-1}$; UV−vis (MeOH) $\lambda_{max}$ 346 (log $\epsilon$ 0.34), 227 (log $\epsilon$ 0.94) nm; EIMS *m*/*z* (%) = 406 ($M^+$, 38), 390 ($M - CH_4^+$, 100); HRMS(ESI) calcd for $C_{25}H_{28}NO_4^+$ 406.20153, found 406.20183.

**Ancistrocladinium B [(*P*)-6].** The *P*-atropo-diastereomer of **6** was isolated in a purity of 98% determined by HPLC: $[\alpha]_D^{20}$ −11.3 (*c* = 0.04, MeOH); CD (MeOH−$H_2O$, recorded online, by LC-CD) $\Delta e_{311}$ −4.4, $\Delta e_{238}$ +2.3, $\Delta e_{230}$ −3.5, $\Delta e_{214}$ +6.4; $^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$ 1.11 (d, $^3J$ = 6.9 Hz, 3 H, $CH_3$-3), 2.47 (s, 3 H, $CH_3$-2′), 2.56 (s, 3 H, $CH_3$-1), 3.04 (dd, $^2J$ = 16.9 Hz, $^3J$ = 1.9 Hz, 1 H, $H_{eq}$-4), 3.71 (dd, $^2J$ = 16.9 Hz, $^3J$ = 6.1 Hz, 1 H, $H_{ax}$-4), 3.97 (s, 3 H, $OCH_3$-6), 4.00 (s, 3 H, $OCH_3$-8), 4.04 (s, 3 H, $OCH_3$-4′), 4.49 (ddq, 1 H, H-3), 6.76 (s, 1 H, H-7), 6.81 (s, 1 H, H-5), 6.99 (s, 1 H, H-3′), 7.36 (s, 1 H, H-1′), 7.51 (d, 1 H, $^3J$ = 8.9 Hz, H-8′), 7.62 (d, 1 H, $^3J$ = 8.9 Hz, H-7′), 10.16 (s, 1 H, OH-5′) ppm; $^{13}$C NMR (150 MHz, DMSO-$d_6$) $\delta$ 14.0 ($CH_3$-3), 21.6 ($CH_3$-2′), 22.9 ($CH_3$-1), 33.3 (C-4), 56.3 ($OCH_3$-4), 56.4 ($OCH_3$-6), 56.7 ($OCH_3$-8), 59.0 (C-3), 97.9 (C-7), 107.7 (C-5), 108.2 (C-3′), 109.2 (C-9), 112.3 (C-10′), 119.9 (C-8′), 120.0 (C-1′), 121.3 (C-6′), 124.5 (C-7′), 136.5 (C-9′), 138.8 (C-2′), 140.6 (C-10), 147.7 (C-5′), 156.3 (C-4′), 163.6 (C-8), 167.8 (C-6), 174.5 (C-1) ppm.

***N*-6′-*epi*-Ancistrocladinium B [(*M*)-6].** The *M*-atropo-diastereomer of **6** was obtained in a purity of 99% determined by HPLC: $[\alpha]_D^{20}$ −4.5 (*c* = 0.04, MeOH); CD (MeOH−$H_2O$, recorded online, by LC-CD) $\Delta e_{322}$ +3.6, $\Delta e_{239}$ −6.4, $\Delta e_{228}$ +5.2, $\Delta e_{215}$ −7.7; $^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$ 1.19 (d, $^3J$ = 6.9 Hz, 3 H, $CH_3$-3), 2.47 (s, 3 H, $CH_3$-2′), 2.50 (s, 3 H, $CH_3$-1), 3.12 (dd, $^2J$ = 16.9 Hz, $^3J$ = 1.9 Hz, 1 H, $H_{eq}$-4), 3.49 (dd, $^2J$ = 16.9 Hz, $^3J$ = 6.1 Hz,

1 H, $H_{ax}$-4), 3.98 (s, 3 H, $OCH_3$-6), 4.01 (s, 3 H, $OCH_3$-8), 4.05 (s, 3 H, $OCH_3$-4′), 4.36 (ddq, 1 H, H-3), 6.75 (s, 1 H, H-7), 6.81 (s, 1 H, H-5), 6.99 (s, 1 H, H-3′), 7.36 (s, 1 H, H-1′), 7.44 (d, 1 H, $_3J$ = 8.9 Hz, H-8′), 7.54 (d, 1 H, $_3J$ = 8.9 Hz, H-7′), 10.06 (s, 1 H, OH-5′) ppm; $^{13}$C NMR (150 MHz, DMSO-$d_6$) $\delta$ 14.6 ($CH_3$-3), 21.6 ($CH_3$-2′), 23.6 ($CH_3$-1), 33.3 (C-4), 56.4 ($OCH_3$-4′), 56.4 ($OCH_3$-6), 56.7 ($OCH_3$-8), 57.8 (C-3), 98.0 (C-7), 107.8 (C-5), 108.0 (C-3′), 109.3 (C-9), 113.1 (C-10′), 119.0 (C-8′), 120.0 (C-1′), 121.8 (C-6′), 124.7 (C-7′), 136.6 (C-9′), 138.6 (C-2′), 140.5 (C-10), 147.7 (C-5′), 156.3 (C-4′), 164.0 (C-8), 168.0 (C-6), 174.7 (C-1) ppm.

**Oxidative Degradation.** Ruthenium(III)-catalyzed periodate degradation, derivatization of the resulting amino acids with MeOH/HCl and then (*R*)-α-methoxy-α-trifluoromethyl-phenylacetyl chloride [(*R*)-MTPA-Cl] prepared from (*S*)-MTPA, and subsequent GC-MSD analysis were carried out as described previously.[22]

**Biological Experiments.** Antiparasitic activities against pathogens *P. falciparum*, *T. cruzi*, *T. brucei rhodesiense*, *L. donovani* (all tested in Basel), and *L. major* (tested in Würzburg) and cytotoxicities against host cells (rat skeletal myoblast L-6 cells, J774.1 macrophages) were assessed as described earlier.[41,42]

**Computational Methods.** Computational methods were carried out as described earlier;[17] see also the Supporting Information.

(41) Ponte-Sucre, A. I.; Campos, Y.; Fernández, M.; Moll, H.; Mendoza-Léon, A. *Exp. Parasitol.* **1998**, *88*, 11−16.
(42) Mikus, J.; Steverding, D. *Parasitol. Int.* **2000**, *48*, 265−269.

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**Supporting Information Available:** General experimental details, experimental procedures at full length, copies of the $^1$H and $^{13}$C NMR spectra for (*M*)-**5** and (*P*)- and (*M*)-**6**, chromatographical conditions for the resolution of the atropo-diastereomers (*P*)-**6** and (*M*)-**6** as well as details and data of the kinetic study of the atropisomerization of **6**, conditions for the hyphenated analysis of plant material, and detailed information concerning the computational methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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