Total Synthesis

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Total Syntheses and Biological Evaluation of Both Enantiomers of Several Hydroxylated Dimeric Nuphar Alkaloids**

Alexander Korotkov, Hui Li, Charles W. Chapman, Haoran Xue, John B. MacMillan, Alan Eastman,* and Jimmy Wu**

Abstract: *Herein, we describe the first total syntheses of five members of the dimeric nuphar alkaloids: (+)-6,6′-dihydroxythiobinupharidine (+)-**1a**, (+)-6-hydroxythiobinupharidine (+)-**1b**, (−)-6,6′-dihydroxythionuphlutine (−)-**2a**, (−)-6,6′-dihydroxyneothiobinupharidine (−)-**3a**, and (+)-6,6′-dihydroxyneothionuphlutine (+)-**4a**. The latter two have not been found in nature. We have also made each of their enantiomers (−)-**1a–b**, (+)-**2a**, (+)-**3a**, and (−)-**4a**. The key step in these syntheses was the dimerization of an α-aminonitrile (a hydrolytically stable surrogate for its corresponding hemiaminal) with chiral Lewis acid complexes. We have also reassigned the literature structures of (+)-**1a–1b**—for those instances in which the NMR spectra were obtained in CD_3OD—to their corresponding CD_3O-adducts. Our efforts provide for the first time apoptosis data for (−)-**3a**, (+)-**4a**, and all five non-natural enantiomers prepared. The data indicate high apoptotic activity regardless of the enantiomer or relative stereochemical configuration at C7 and C7′.*

In 2006, Yoshikawa and co-workers reported that 6-hydroxythiobinupharidine (+)-**1b** induces apoptosis in U937 human leukemia cells within 1 h (2.5–10 μM).[1] To their knowledge, this was the most rapid induction of apoptosis by a small molecule ever reported. Additional experiments based on incubation with 20 μM of specific caspase inhibitors implicated activation of caspases 8 and 3 (but not caspase 9). Gopas and co-workers subsequently reported that partially purified combination extracts of (+)-**1b** and (−)-**2b** inhibit NFκB signaling.[2] Despite the unprecedented speed with which the dimeric nuphar alkaloids are able to induce apoptosis, it is surprising that no other studies on their biological mechanism of action have been published. Previous reports also show that (+)-**1b** is an effective antibacterial,[3] antifungal,[4] and immunosuppressant agent.[5]

The dimeric nuphar alkaloids are structurally unique sulfur-containing triterpenoids isolated from the yellow water lilies, *Nuphar pumilum*, *Nuphar japonicum*, and *Nuphar lutea*, and were first described by Achmatowicz and co-workers (Figure 1).[6] Three series (i.e., thiobinupharidines (+)-**1a–d**, thionuphlutines (−)-**2a–d**, and neothiobinupharidines **3b–d**, whose structures differ in the relative stereochemical configurations at C7 and C7′, are known.[7] However, neither (−)-**3a** from the neothiobinupharidine series nor any of the members of the neothionuphlutine series **4a–d** have been found in nature.

LaLonde proposed a biosynthetic mechanism for the formation of the gross structures of the dimeric nuphar alkaloids beginning from elimination/oxidation of (−)-

[*] Dr. A. Korotkov, H. Li, Dr. H. Xue, Prof. J. Wu
Department of Chemistry
Dartmouth College
Hanover, New Hampshire 03755 (United States)
E-mail: jimmy.wu@dartmouth.edu
C. W. Chapman, Prof. A. Eastman
Department of Pharmacology and Toxicology
Geisel School of Medicine
Lebanon, New Hampshire 03756 (United States)
E-mail: alan.r.eastman@dartmouth.edu
Dr. H. Xue
Natural Products LINCHPIN Laboratory
Texas A&M University
College Station, Texas, 77840 (United States)
Prof. J. B. MacMillan
Department of Biochemistry
UT Southwestern Medical Center
Dallas, Texas, 75390 (United States)
E-mail: john.macmillan@utsouthwestern.edu

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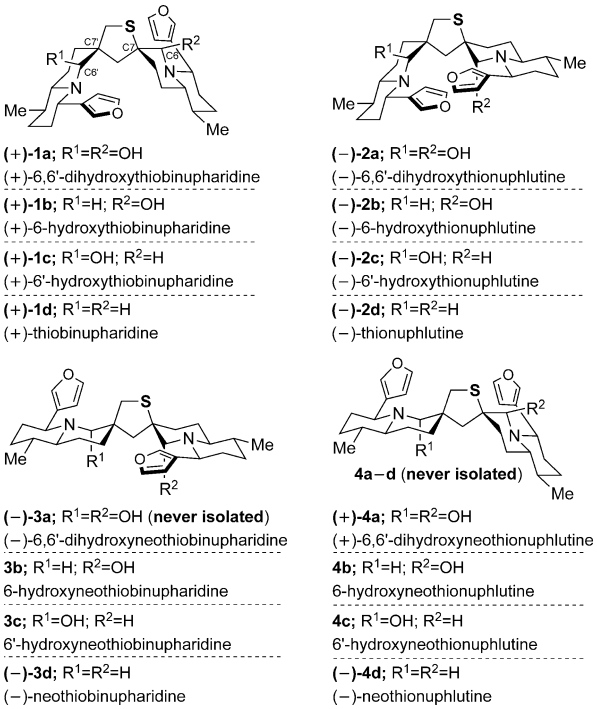
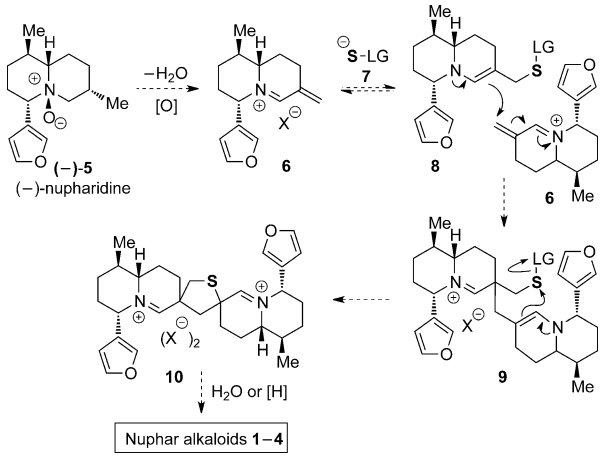


Figure 1. Dimeric nuphar alkaloids.

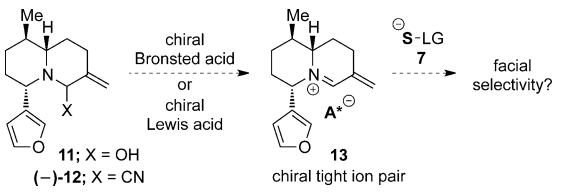


Scheme 1. LaLonde's proposed biosynthesis.

nupharidine (Scheme 1).[8] Attack of ene-iminium **6** with an ambiphilic sulfur reagent **7** would initiate dimerization through formation of the central thiaspirane ring. Hydration or reduction of bis-iminium **10** would result in compounds **1**–**4**. Shenvi and co-workers proved the feasibility of LaLonde's proposal by completing the first and only total synthesis of a member of the dimeric nuphar alkaloids, (−)-neothiobinupharidine (−)-**3d**,[9] which unfortunately is not active in apoptosis assays.[1] Yoshikawa reported that at least one hemiaminal is required to see substantial apoptotic activity.

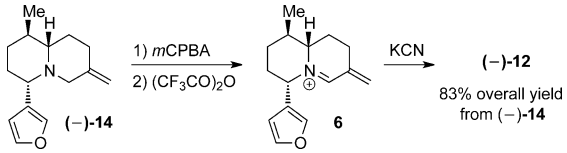
Considering 1) the unprecedented speed at which the dimeric nuphar alkaloids are able to induce apoptosis, 2) the lack of information regarding their biological mechanism of action, and 3) their complex and exquisite chemical structure, we decided to pursue the total syntheses of the biologically active hydroxylated dimeric nuphar alkaloids. In the absence of any catalysts or promoters, Shenvi reports that dimerization favors the formation of the stereochemical relationship corresponding to the neothiobinupharidine architecture (4:1 series **3** versus all others).[9] Thus, the principle challenge we faced was how to override the inherent diastereochemical outcome at C7 and C7′ during the formation of the thiaspirane ring.

Although the requisite ene-iminium **6** (Scheme 1) can be derived from hemiaminal **11** (Scheme 2), **11** is a hydrolytically labile compound that cannot be purified by chromatography and must be freshly made before each use. We therefore elected to pursue a strategy employing nitrile (−)-**12** as a "masked" hemiaminal that is both chromatographically and hydrolytically stable.[10–12] We further reasoned that promoting the dimerization by application of chiral Brønsted or Lewis acids to (−)-**12** would generate a chiral ion-pair intermediate **13** in which the stereochemical information of counterion **A***⁻ could assist in controlling the facial selectivity (Scheme 2).



Scheme 2. Strategy for controlling facial selectivity in thiaspirane formation.

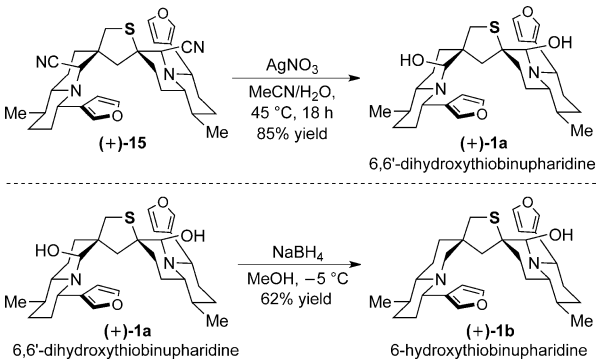
We prepared both enantiomers of **14** using known methods.[9,13,14] We then converted (−)-**14** to the nitrile (−)-**12** by oxidation to the *N*-oxide, followed by Polonovski rearrangement and quenching with KCN (Scheme 3).



Scheme 3. Synthesis of nitrile **14**.

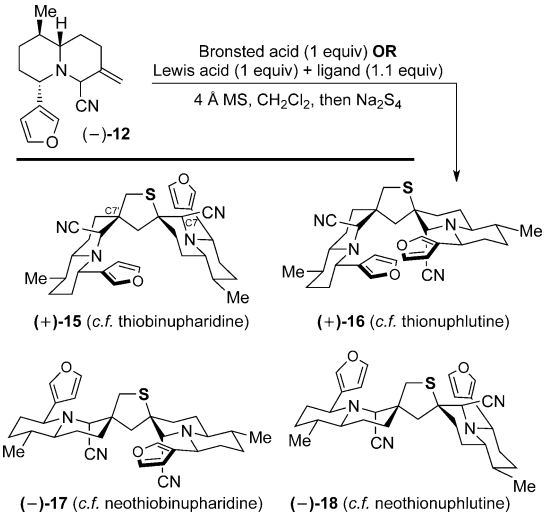
With (−)-**12** in hand, we surveyed an extensive set of chiral and achiral Brønsted acids and Lewis acid complexes for their ability to alter the intrinsic selectivity at C7/C7' in the dimerization reactions with Na_2S_4. These included chiral phosphoric acids and numerous permutations of Lewis acids and ligands. Table 1 is an abbreviated summary of these studies (for a complete list, see the Supporting Information, SI). The products obtained are bis-nitriles **15**–**18**. The assignment of the stereochemical configuration at C7/C7′ was accomplished by converting them to the corresponding fully reduced compounds **1d**, **2d**, **3d**, and **4d**, which were then compared with literature data. After considerable experimentation, we identified $In(OTf)_3$/(3*R*,8*S*)-**22** as the optimal Lewis acid–ligand[15–17] combination for generating (+)-**15** (corresponds to the thiobinupharidine configuration; entry 16), whereas $Cu(OTf)_2$ alone gave predominantly (−)-**17** (corresponds to the neothiobinupharidine configuration; entry 1). Under these conditions, we could obtain synthetically useful amounts of all stereoisomers **15**–**18**. The use of chiral phosphoric acids or catalytic amounts of Lewis acids was not optimal. That there was no pronounced matched–mismatched effect with opposite enantiomers of ligands suggests that the mechanism of dimerization may be more complex than simple substrate–ligand interaction.

We then converted bis-nitrile (+)-**15** to the corresponding bis-hemiaminal (+)-**1a** by treatment with $AgNO_3$ in MeCN/H_2O at 45 °C (85 % yield, Scheme 4).[18] The preparation of



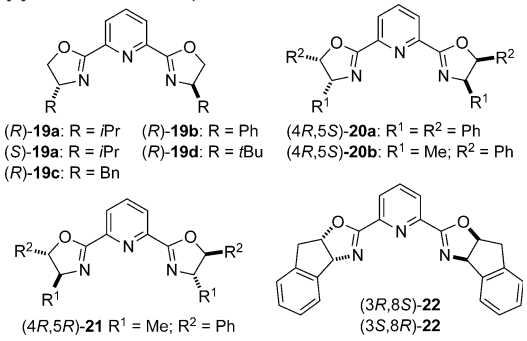
Scheme 4. Completion of synthesis.

Table 1: Optimization of dimerization.



Entry[a]	Metal	Ligand[b]	15/16/17/18
1	$Cu(OTf)_2$	none	0.2:0.2:1.0:0.3 7%:6%:51%:10%[c]
2	$Sc(OTf)_3$	none	0.3:0.3:1.0:0.2
3	$In(OTf)_3$	none	0.3:0.2:1.0:0.2
4	$Cu(OTf)_2$	(*S*)-**19a**	0.4:0.2:1.0:0.3
5	$Sc(OTf)_3$	(*S*)-**19a**	0.4:0.2:1.0:0.2
6	$In(OTf)_3$	(*S*)-**19a**	0.3:0.2:1.0:0.2
7	$Cu(OTf)_2$	(*R*)-**19a**	0.4:0.2:1.0:0.2
8	$In(OTf)_3$	(*R*)-**19a**	0.9:0.2:1.0:0.2
9	$In(OTf)_3$	(*R*)-**19b**	0.8:0.3:1.0:0.2
10	$In(OTf)_3$	(*R*)-**19c**	0.9:0.3:1.0:0.3
11	$In(OTf)_3$	(*R*)-**19d**	1.1:0.3:1.0:0.2
12	$In(OTf)_3$	(4*R*,5*S*)-**20a**	1.1:0.3:1.0:0.2
13	$In(OTf)_3$	(4*R*,5*S*)-**20c**	1.0:0.3:1.0:0.3
14	$In(OTf)_3$	(4*R*,5*R*)-**21**	1.0:0.3:1.0:0.2
15	$Cu(OTf)_2$	none	0.2:0.2:1.0:0.3
16	$In(OTf)_3$	(3*R*,8*S*)-**22**	1.9:0.4:1.0:0.3 42%:8%:23%:6%[c]
17	$In(OTf)_3$	(3*S*,8*R*)-**22**	1.6:0.3:1.0:0.3

[a] Yields of isolated products for selected entries.



2a–**4a** was carried out in a similar fashion and in comparable yields. Selective monoreduction of the C6' hemiaminal over the one at C6 in **1a** could be accomplished to give **1b**.[19] This type of chemoselective reduction appears limited to **1a** as similar attempts on **2a**--**4a** resulted in numerous reduction products. The absolute stereochemical configurations as depicted in Figure 1 are the naturally occurring ones, but we also completed the syntheses of the nonnatural (or predicted nonnatural) enantiomers (−)-**1a**–**b**, (+)-**2a**, (+)-**3a**, and (−)-**4a**.

The ^1H and ^{13}C NMR spectra of synthetic (+)-**1a**–**1b** in both CD_3OD and $CDCl_3$ were identical to both reported literature values and to the spectra of materials isolated from *Nuphar lutea* carried out by our group (see SI for isolation/purification details) on the condition that the NMR experiment in $CDCl_3$ occurred first.[20–23] If the data acquisition for the NMR spectrum in CD_3OD preceded that of the NMR experiment in $CDCl_3$, then noticeable differences between synthetic and literature values were apparent in the $CDCl_3$ ^1H NMR spectra, while no differences were observed in the CD_3OD spectrum. Under these circumstances, if we removed the CD_3OD in vacuo and allowed the residue to age in air, the material slowly converted back to (+)-**1a**–**1b** as judged by ^1H NMR spectroscopy in $CDCl_3$. The conversion was greatly facilitated if the residue was washed with saturated aq. $NaHCO_3$. We speculated that in CD_3OD, (+)-**1a**–**1b** is converted to the CD_3O-adducts **23a** and **24a** (Figure 2).

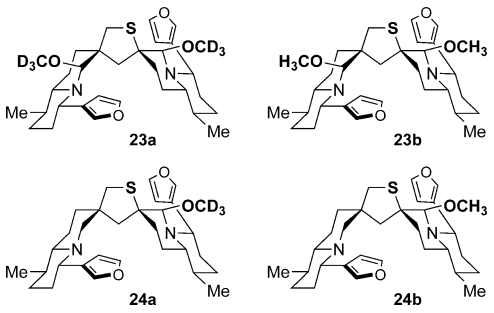


Figure 2. Revised structures.

Indeed, when we dissolved (+)-**1a**–**b** in CH_3OH, then removed the excess methanol and obtained the ^1H NMR spectra in $CDCl_3$, new singlet resonances integrating to 3H each were present. We assigned these resonances to the methoxy protons in **23b**–**24b**. From these data, we believe it necessary to revise those literature structures of (+)-**1a**–**b**—in which NMR experiments were carried out in CD_3OD—to the corresponding CD_3O-adducts **23a** and **24a**. It is likely that an analogous revision of **2a** is also warranted. The ethoxylated versions of some dimeric nuphar alkaloids have been noted, but there is some disagreement as to whether these were artifacts of the isolation/purification process.[24,25]

We then assayed both enantiomers of **1a**–**1b**, **2a**, **3a**, **4a**, as well as bis-nitriles **15**–**18** for their apoptotic properties against the human U937 cell line as judged by caspase cleavage of poly(ADP-ribose) polymerase (PARP), a marker of apoptosis (Table 2 and Figure 3). The activity of synthetic (+)-**1a**–**1b** and (−)-**2a** were similar to that of the corresponding materials we isolated from *Nuphar lutea* (see SI for full data set). Moreover, the mono- and dihydroxylated dimeric nuphar alkaloids all exhibited nearly the same activity, regardless of their absolute or relative stereochemical configurations. It also appears that (−)-**3a**, which is not found in nature, seems to be the most potent at the 1 h time point (entry 7). Bis-nitriles **16**–**18** exhibited no apoptotic activity.

Table 2: Apoptosis assays (PARP cleavage).

Entry	Compound	Enantiomer	1 h[a]	6 h[a]
1	(+)-**1a**	natural	–	5.0
2	(−)-**1a**	unnatural	–	2.5
3	(+)-**1b**	natural	–	5.0
4	(−)-**1b**	unnatural	–	5.0
5	(−)-**2a**	natural	–	2.5
6	(+)-**2a**	unnatural	–	5.0
7	(−)-**3a**	predicted natural	5	1.25
8	(+)-**3a**	predicted unnatural	10	2.5
9	(+)-**4a**	predicted natural	–	2.5
10	(−)-**4a**	predicted unnatural	–	2.5

[a] Minimum concentration (μM) at which at least 50% or greater of PARP cleavage is observed. – indicates that no PARP cleavages was observed up to 10 μM.

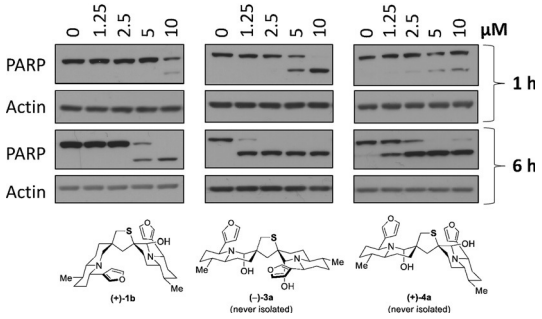


Figure 3. Representative western blots for PARP cleavage.

In conclusion, we have successfully completed the first syntheses of several hydroxylated nuphar alkaloids. This was accomplished by means of dimerization of nitrile (−)-**12**, promoted by chiral Lewis acid complexes. Chemoselective mono-reduction of (+)-6,6′-dihydroxythiobinupharidine (+)-**1a** furnished (+)-6-hydroxythiobinupharidine (+)-**1b**. Moreover, for the first time, apoptosis data is available for these compounds. PARP cleavage assays confirm their ability to induce very rapid apoptosis in human U937 cells (within 1 h). We anticipate that these compounds will serve as useful tools for dissecting an important, but as yet undefined step in the regulating apoptosis. Studies to clarify the biological mechanism by which they operate are ongoing.

Keywords: apoptosis · cancer · caspases · nuphar alkaloids · total synthesis

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