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Total Synthesis of Unsymmetrically Oxidized Nuphar Thioalkaloids via Copper-Catalyzed Thiolane Assembly

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Abstract

An asymmetric total synthesis of (+)-6-hydroxythiobinupharidine (**1b**) and (–)-6-hydroxythionuphlutine (**2b**), a set of hemiaminal containing dimeric sesquiterpenes isolated from yellow water lilies of the *Nuphar* genus, is described. The central bis-spirocyclic tetrahydrothiophene ring was forged through the Stevens rearrangement of a sulfonium ylide, generated in situ from the coupling of a copper-carbene with a spirocyclic thietane. This strategy diverges both from the proposed biosynthesis¹ and previous syntheses of this family of alkaloids,^{2,3} all of which employ dimerization of symmetric monomers to form the aforementioned thiaspirane. The coupling of unsymmetrical monomers allowed access to the unsymmetrically oxidized product **2b** for the first time.

The unusual bis(spirothiolane) structural signature of *Nuphar* alkaloids has incited innovative synthetic studies over the past two decades.⁴ This series of dimeric sesquiterpenes was first isolated from the yellow water lily *Nuphar lutea* by Achmatowicz in 1964,⁵ and the structure of neothiobinupharidine (**3c**) was determined by Birnbaum in 1965 using X-ray crystallography.⁶ Over the following decade, extensive structural studies by LaLonde conclusively characterized a number of alkaloids belonging to the thiobinupharidine, thionuphlutine, and neothiobinupharidine series, particularly those containing hemiaminal groups (Figure 1).⁷ Early investigations of the antifungal and antibacterial activity of these compounds were somewhat disappointing.⁸ However, recent reports by Yoshikawa in the early 2000s rekindled interest in the biological activity of these natural products. Seminal publications showed that (+)-6,6'-dihydroxythiobinupharidine (**1a**), (+)-6-hydroxythiobinupharidine (**1b**), (–)-6-hydroxythionuphlutine (**2b**), and (+)-6'-

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Supporting Information

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Experimental procedures and characterization (PDF)

Spectral data for all compounds (PDF)

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hydroxythionuphlutine (**2d**) displayed significant immunosuppressive activity in mouse splenocytes at 1 μM concentration.^{9,10} Remarkably, the monohydroxylated alkaloids **1b**, **2b**, and **2d** were not cytotoxic at this concentration, while (+)-6,6'-dihydroxythiobinupharidine (**1a**) showed minor cytotoxicity. Further studies by the same group demonstrated that (+)-6,6'-dihydroxythiobinupharidine (**1a**), (+)-6-hydroxythiobinupharidine (**1b**), and (–)-6-hydroxythionuphlutine (**2b**) inhibited invasion of B16 melanoma cells across collagen-coated filters with an IC_{50} value of 29–360 nM in vitro.¹¹ These same compounds also exhibited substantial cytotoxicity at a concentration of 10 μM against U937, B16F10, and HT1080 cell lines, with **1b** inducing apoptosis in cell line U937 within 1 h.¹² Dimeric alkaloids lacking a hydroxyl group or possessing only a 6'-rather than 6-hydroxyl group showed weak activity, leading the authors to conclude that the 6-hydroxyl moiety is essential to the observed apoptotic and antimetastatic activity. Recently it was discovered that semi-purified extracts of *Nuphar lutea* containing (+)-6-hydroxythiobinupharidine (**1b**) and (–)-6-hydroxythionuphlutine (**2b**) strongly inhibited nuclear factor κB (NF κB).¹³ Additionally, these extracts were shown to work synergistically with cisplatin and etoposide, demonstrating a potential for *Nuphar* alkaloids to act as sensitizers in chemotherapy.

Despite being known since the 1960s, the first total synthesis of a dimeric *Nuphar* alkaloid was not reported until Shenvi's pioneering synthesis of (–)-neothiobinupharidine (**3c**) in 2013.² Wu and co-workers later expanded upon this work and completed syntheses of five hydroxylated *Nuphar* alkaloids (**1a**, **1b**, **2a**, **3a**, and **4a**), along with their unnatural enantiomers.³ Biological evaluation of these synthetic products showed that both natural and unnatural enantiomers exhibited nearly the same activity against human cell line U937. In a subsequent study, Wu prepared a library of simplified monomeric analogues and analyzed their ability to induce apoptosis.¹⁴ These monomers were found to possess comparable or slightly more potent activity compared to the dimeric alkaloids. Monomers corresponding to both the natural and unnatural configurations displayed cytotoxicity, but greater potency was seen in the unnatural antipodes. It also appears that the C1 methyl group is not essential to the biological activity, as analogues lacking a C1 methyl group or with an epimeric configuration at C1 maintained activity.¹⁵ Furthermore, an anti relationship between the 6-hydroxyl group and the adjacent sulfide substituent (corresponding to the configurations of **2a–b** and **3a–b**) was found to produce more potent cytotoxicity than a syn-relationship (corresponding to the configurations of **1a–b** and **4a–b**).

Shenvi and co-workers have proposed that the apoptotic activity of 6,6'-dihydroxy *Nuphar* alkaloids arises from a retrodimerization event, triggered by nucleophilic attack of sulfur, to form an electrophilic ene-iminium species.¹⁶ To test this hypothesis, simplified monomeric and dimeric thiaspirane iminium analogues were prepared. The thioether moiety was indeed shown to be electrophilic, as treatment of these analogues with nucleophilic thiols resulted in disulfide formation.¹⁷ The dimeric analogues also proved to be cytotoxic against Jurkat and HT29 cells, whereas their monomeric counterparts exhibited no activity. However, as noted by the authors, this hypothesis cannot account for the apoptotic activity of 6-hydroxy *Nuphar* alkaloids nor the monomeric analogues prepared by Wu, neither of which can undergo retro-dimerization. Given that all 6-hydroxy containing diastereomeric members of the natural *Nuphar* alkaloids, in addition to their enantiomers and Wu's monomeric

analogues, possess similar apoptotic activity, it is likely that a common mechanism is at hand. It is unclear, however, what the exact mechanism is, or whether this mechanism can also account for the observed immunosuppressive activity, antimetastatic activity, or the inhibition of NF- κ B.

The elegant syntheses put forth by both Shenvi and Wu have paved the way for future biological investigations on the *Nuphar* alkaloids. Yet, a critical challenge that has remained unmet is the selective synthesis of several of the *unsymmetrically* oxidized, or 6-hydroxy, congeners. Wu et al. were able to access one such compound, (+)-6-hydroxythiobinupharidine (**1b**), through a reduction of (+)-6,6'-dihydroxythiobinupharidine (**1a**) based on innate selectivity. However, attempts to perform similar reductions on other 6,6'-dihydroxy members were met with little success,³ and as a result, **2b**, **3b**, and **4b** have remained inaccessible. Our interest was piqued by one compound in particular, namely (–)-6-hydroxythiobinupharidine (**2b**), which represents a particularly attractive synthetic target given the intriguing results observed in the biological assays described above. In addition to developing a general strategy for the synthesis of all *Nuphar* thioalkaloids, the goal of our synthesis was to gain access to **2b** and provide a platform for further exploration of its biological activity.

The basis of our synthesis design was the advanced fragment coupling by a stereodivergent metallocarbene insertion into the thietane C–S bond forming the central spirothiolane ring system of the alkaloids (**6** + **7**, Scheme 1). We envisaged this taking place through a sulfonium-ylide intermediate **5** which could undergo a Stevens-type ring expansion.¹⁸ In the latest iteration of the synthesis plan, we opted to use an acyclic diazo ester for the fragment coupling, as attempts to utilize a cyclic diazo lactam intermediate during thiolane formation were unsuccessful. It was thought that both the diazo ester and thietane components could be synthesized from a common piperidine intermediate **8**, for which we determined carbamate **9** to be a suitable precursor. In the forward sense, we imagined that direct, asymmetric alkylation of 3-furanacetic acid **10** with (3*R*)-1-iodo-3-methyl-4-pentene **11** would provide access to the requisite Troc carbamate **9**.

In accordance with this plan, the enantioselective synthesis of (3*R*)-1-iodo-3-methyl-4-pentene **11** was accomplished in 4 steps from 2-methylene- γ -butyrolactone **12** in 71% overall yield, 96% ee as shown in Scheme 2. Direct asymmetric alkylation of 3-furanacetic acid with **11** was accomplished in the presence of the dilithium amide of **13** by a protocol developed previously in our group, delivering **14** in 70% yield and >20:1 dr on >3g scale.¹⁹ Curtius rearrangement performed directly on the alkylation product **14** followed by exposure of the intermediate isocyanate to 2,2,2-trichloroethanol afforded carbamate **9** in 94% yield with full retention of configuration. A cross-metathesis of **9** with acrolein diethylacetal²⁰ followed by Wittig homologation of enal **15** gave conjugated dienyl ester **16**. Removal of Troc using conditions developed by Ciufolini and co-workers²¹ led directly to intramolecular 1,6-addition to form piperidine **17** in 65% yield along with its C10 epimer. Attempts to recycle the epimer to the more thermodynamically stable isomer **17** led mostly to double bond migration and formation of only minor amounts of **17**. The final step in the synthesis of amino ester **8** was a seemingly trivial olefin hydrogenation. Unfortunately,

subjection of **8** to numerous conditions using classic Pd hydrogenation catalysts (including deactivated catalysts such as Lindlar's) led to competitive hydrogenation of the 3-substituted furan. A literature survey revealed that we were not the first to experience this issue in the synthesis of *Nuphar* alkaloids. A similar problem was reported by Bates and co-workers during the synthesis of nupharamine, which they resolved through the use of Wilkinson's catalyst.²² Gratifyingly, hydrogenation of **17** in an autoclave at a pressure of 300 psi in the presence of Wilkinson's catalyst afforded the desired reduction product **8** in 88% yield.

The diazo ester (**20**) and thietane (**7**) coupling partners were then prepared from piperidine **8** in three and six steps, respectively (Scheme 3). After initial *N*-allyloxycarbonylation, enolization of ester **8** with $\text{KN}(\text{SiMe}_3)_2$ followed by acylation with methyl benzoate provided keto ester **19**. The use of LDA as a base in the acylation step led to rapid decomposition of the substrate, presumably due to competitive lithiation of the allyloxycarbonyl group. Keto ester **19** was converted to diazo ester **20** with 4-nitrobenzenesulfonyl azide in the presence of DBU in 87% yield.²³ A direct diazo transfer to ester **8** proved to be inefficient. For the synthesis of thietane **7**, piperidine **8** was first cyclized through treatment with acetic acid in refluxing toluene to give lactam **21**. A series of transformations involving bisacylation, reduction to a diol, mesylate formation, and displacement with sodium sulfide successfully installed the thietane ring. Quinolizidinone **23** was then reduced with *i*-Bu₂AlH to give spirocyclic thietane **7** in 90% yield.

Having gained access to sufficient quantities of coupling partners **7** and **20**, the stage was now set to assemble the central thiolane ring. Extensive screening using $\text{Rh}_2(\text{OAc})_4$, $\text{Rh}_2(\text{O}_2\text{CC}_3\text{F}_7)_4$, $\text{Rh}_2(\text{PTAD})_4$, $\text{Rh}_2(\text{esp})_2$, $\text{Cu}(\text{CH}_3\text{CN})_4 \text{BF}_4$, $\text{Cu}(\text{tfacac})_2$, and $\text{Cu}(\text{hfacac})_2$ catalysts²⁴ revealed that a combination of $\text{Cu}(\text{hfacac})_2$ and microwave irradiation was the most effective for **7** and **20**, and that the reaction was sensitive to seemingly minor structural variations of both coupling partners. Under optimized conditions, **7** and **20** were heated to 100 °C using microwave irradiation in the presence of $\text{Cu}(\text{hfacac})_2$ to give two products corresponding to the configurations of thiobinupharidine (**24**) and thionuphlutine (**25**). While this reaction has the potential to produce four diastereomeric products, only two are observed both by ¹H NMR analysis of the crude reaction mixture and after isolation by column chromatography. We believe the striking selectivity of this reaction can be rationalized through a mechanism that involves anchimeric assistance from the 5' nitrogen (Scheme 4). After formation of sulfonium ylide **i**, the axially oriented nitrogen lone pair²⁵ can open the thietane ring through an S_N2 displacement at the axial carbon. The final carbon–carbon bond of the thiolane ring is then formed through an enolate ring opening of the intermediate azetidinium species **ii**, which occurs exclusively from the bottom face in order to avoid a developing twist boat-like product conformation in the disfavored transition state. Given the approximately 1:1 dr observed at C7, there appears to be no facial bias with respect to the enolate.

To complete the synthesis, the allyloxycarbonyl groups were first removed using 5 mol % of $\text{Pd}(\text{PPh}_3)_4$ in the presence of phenylsilane to form **26** and **27** in 87% and 98% yields, respectively. Our initial plan called for lactamization of these amino esters and a reduction of the corresponding quinolizidinones to access the hemiaminal natural products. However, these substrates proved to be surprisingly resistant to lactamization under a variety of acidic

or basic conditions, including treatment with acetic acid, sulfuric acid, trimethylaluminum, DBU, and sodium methoxide. We reasoned that perhaps reversing the order of these steps could circumvent this obstacle. It was thought that careful reduction of the ester moiety would produce an intermediate amino-aldehyde, which should tautomerize to a hemiaminal.²⁶ Reduction of amino ester **26** with *i*-Bu₂AlH at -89 °C afforded hemiaminal (+)-6-hydroxythiobinupharidine (**1b**) in 38% isolated yield, along with 6% of the fully reduced product (-)-thiobinupharidine (**1c**). Shortening the reaction time and decreasing the amount of *i*-Bu₂AlH did not lead to an increase in yield. To our delight, submission of amino ester **27** to analogous conditions resulted in the formation of (-)-6-hydroxythionuphlutine (**2b**) in 60% yield, along with a 10% yield of (-)-thionuphlutine (**2c**). The thiaspirane stereochemistry in the products was determined by correlation of the ¹H NMR and ¹³C NMR data obtained for **1b**, **1c**, and **2c** to those reported by Lalonde²⁷ and by Wu,³ all of which were in full agreement.

In summary, an asymmetric total synthesis of two hemiaminal containing *Nuphar* thioalkaloids, (+)-6-hydroxythiobinupharidine **1b** and (-)-6-hydroxythionuphlutine **2b**, has been accomplished. The essential feature of the synthesis was assembly of the central bis-spirocyclic through a copper-catalyzed coupling of unsymmetric monomers. This biodivergent strategy was successful in providing chemoselective access to unsymmetrically oxidized hemiaminal alkaloids **1b** and **2b**, with **2b** being synthesized for the first time.

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Figure 1.
Nuphar sesquiterpene thioalkaloids.

Scheme 1.
Synthesis Plan

all reactions performed on >1g scale.

Scheme 2.
Synthesis of Piperidine Intermediate 8

Scheme 3.

Convergent Fragment Coupling by Cu-Mediated Stevens-Type Ring Expansion to Install the Central Thiolane

Scheme 4.
Mechanistic Hypothesis for Selective Thiolane Synthesis