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Synthesis of (−)-Neothiobinupharidine

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Supporting Information

ABSTRACT: An eight step, asymmetric synthesis of a dimeric thiaspirane nuphar alkaloid from 3-methyl-2-cyclopentenone is reported. The brevity of the route relies on a useful procedure for tandem reductive allylation of cyclopentenones, as well as the minimization of redox manipulations and other functional group interconversions. The distribution of products that arise from spontaneous dimerization points to a more complex biosynthesis.

The first of the nuphar dimers (**1**−**3**, Figure 1a) was isolated from the fresh water plant *Nuphar lutea* by Achmatowicz,[1] and neothiobinupharidine **3d** was definitively assigned in 1965 by Birnbaum via X-ray crystallographic analysis.[2] Extensive studies by LaLonde over the course of a decade established the stereochemistry of a family of 11 topologically complex alkaloids.[3] Initial bioassays were disappointing, as these molecules demonstrated only weak activity (80−200 μM) against pathogenic bacteria and fungi.[3] However, more recent studies on the effects of nuphar dimers on cancer cells has reinvigorated interest in these compounds. Yoshikawa found that in vitro invasion of murine (B16) melanoma cells across collagen fibers was inhibited by **1c**, **1a**, and **2c** at IC_{50} levels of 29, 87, and 360 nM, respectively. Remarkably, lung tumor formation in mice was inhibited by **1c** by >90% versus control at 10 days post injection of B16 melanoma cells.[4] These same compounds induced apoptosis of human leukemia U937 cells within only 1 h.[5] More recent studies by Golan-Goldhirsh and Gopas have found that a crude extract of *N. lutea* downregulates NFκB activity, and potentiates the cytotoxicity of cisplatin toward Hodgkin's lymphoma-derived cells (L428) at concentrations lower than 0.1 μg/mL.[6] Currently, the biological target of these alkaloids is unknown.

An insightful proposal for the biogenesis of the thiaspirane alkaloids' gross structure (Figure 1b) was put forth by LaLonde[7] but has never been investigated in vivo or reproduced in vitro. Notably, this proposed biosynthesis does not account for distribution of these stereoisomeric compounds in nature. For instance, monomeric enamines similar to **6** have been shown to react preferentially with electrophiles on their convex face (Figure 1c),[8−10] but this selectivity for the C−C (Mannich-type) and C−S (sulfenylation) bond forming reactions of **6** and **7** would lead to a stereochemical outcome which has never been observed (**4a**−**d**) in *Nuphar* sp.[3,11]

Here we report the shortest synthesis to date of the monomeric nuphar quinolizidine series in enantiomerically pure form,[12] and the first total synthesis of a thiaspirane nuphar dimer. We also show that the preferred product of sulfurizing dimerization of **5** in bulk solvent is not **4**, but the neothiobinupharidine series **3**. This outcome derives from the opposite stereoselectivity of bond formation as the major series

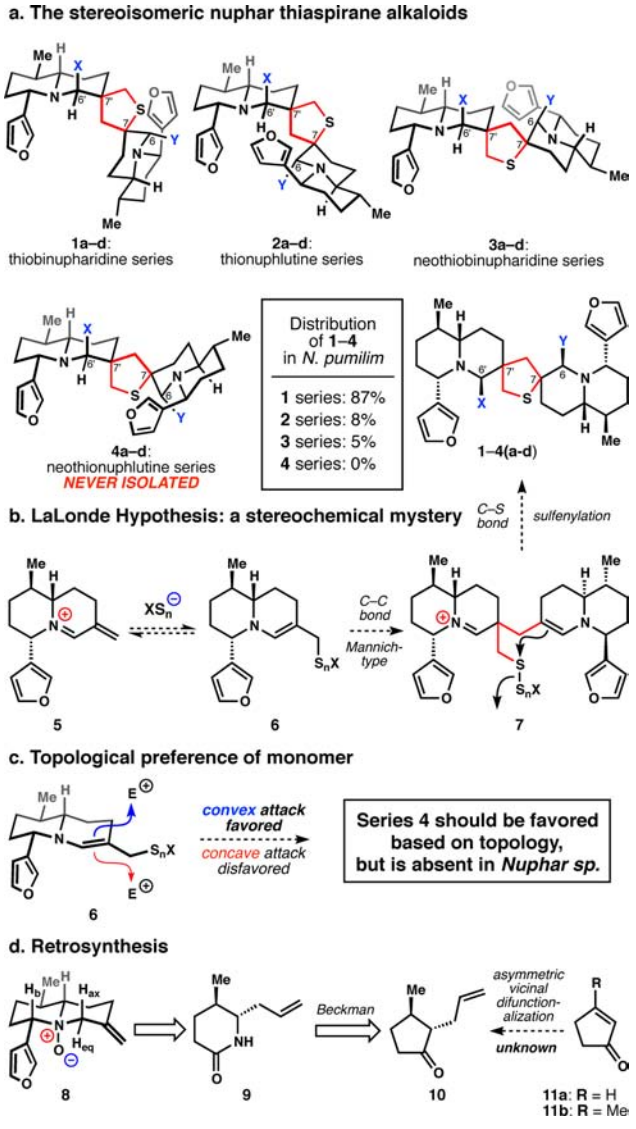

Figure 1. The nuphar thiaspirane dimers. (a) **a**: X,Y = OH, **b**: X = OH, Y = H, **c**: X = H, Y = OH, **d**. X,Y = H; (b) LaLonde's biosynthetic proposal; (c) stereochemical preferences of monomeric enamines; (d) retrosynthetic analysis.

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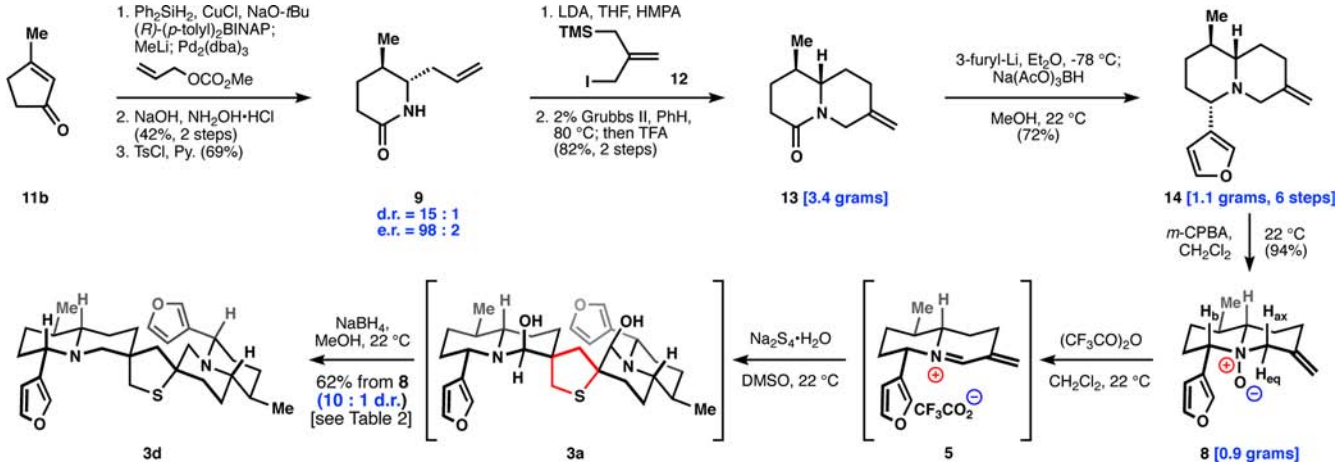
Scheme 1. Synthesis of (−)-Neothiobinupharidine in 8 Steps



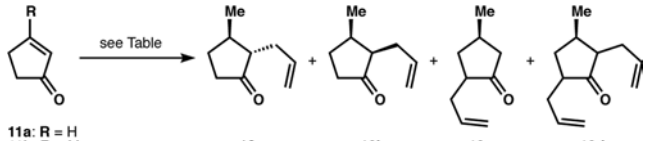
(**1**) found in *Nuphar* sp. and may suggest a more complex biosynthesis mechanism than spontaneous dimerization.[13]

We planned to access iminium **5** via *N*-epi-dehydronupharidine **8** in the anticipation that a Polonovsky reaction would only eliminate the equatorial proton (H_{eq}) *syn*- to the N-oxide (Figure 1d). The stereotetrad of **8** could arise through substrate controlled reactions from lactam **9**, and so rapid access to this stereodiad with absolute stereochemical control was explored. Honda synthesized **9** in 9 steps from a chiral pool starting material,[12b] but we thought that a Beckman rearrangement,[14] followed retrosynthetically by asymmetric vicinal difunctionalization of 2-cyclopentenone, may offer a more efficient entry to the synthesis. Unfortunately, there are no known methods that are capable of achieving a conjugate methyl addition and tandem allylation of cyclopentenones with good absolute or relative stereocontrol. Instead, the intermediate cyclopentenolates undergo proton exchange with the product ketones and yield diastereo- and regioisomers in addition to doubly allylated products.[15] For instance, methylzinc conjugate additions to cyclopentenone proceed with high levels of enantioselectivity, but allylation of these intermediates is problematic (Table 1, entries 1,[16] 2,[17] and 3[18]), in line with recent observations by Cook.[19]

Therefore, we designed our own procedure based on Buchwald's conjugate reduction reaction (entry 4),[20] in combination with a modified Tsuji-Trost allylation (entries 5 and 6). These new conditions lead to ketone **10a** in one pot with high diastereoselectivity (d.r. > 10:1), high enantioselectivity (e.r. = 97.5:2.5) and only minimal byproducts, although use of precise amounts of methyl lithium is crucial, and the isolated yield is still modest due to the volatility of **10a**. This new procedure for cyclopentenone vicinal difunctionalization forms the basis of a short synthesis of the nuphar alkaloids.

Ketone **10a** could be converted into the corresponding oxime (42% from **11b**) and engaged in a Beckman rearrangement to yield lactam **9** in 69% yield. To rapidly advance this monocycle to quinolizidine **14**, we employed Vanderwal's allylsilane-RCM methodology[21] to access the required exocyclic methylene. Alkylation of **9** with iodide **12** proceeded cleanly, as did ring-closing metathesis with Grubb's second generation catalyst.[12b] In situ proto-desilylation with trifluoroacetic acid delivers **13** in good yield. This short 5-step sequence allows **13** to be procured from 3-methyl-2-cyclopentenone (0.29 $/mmol) on multigram scale (3.4 g prepared). This

Table 1. Optimization of Vicinal Difunctionalization



entry	conditions	%10a[a]	%10b[a]	%10c[a]	%10d[a]	%int[a,b]
1	**11a**, CuOTf, **L***, Me_2Zn, PhMe; allyl-Br [Ref. 14]	32	17	0	0	51
2	**11a**, CuOTf, **L***, Me_2Zn; allyl-OAc, PPh_3, $Fe_2(CO)_9$, [Ref. 15]	0	0	0	0	100
3	**11a**, 6,6'-Br_2-BINOL, Me_2Zn, Cy_2NMe, CuSPh; allyl-Br, [Ref. 16]	0	0	0	0	100
4	**11b**, Ph_2SiH_2, (*R*)-(*p*-tolyl)$_2$BINAP, CuCl, NaO*t*-Bu; TBAT, allyl-Br [Ref. 18]	22	25	8	0	42
5	**11b**, Ph_2SiH_2, (*R*)-(*p*-tolyl)$_2$BINAP, CuCl, NaO*t*-Bu; 1.15 equiv. MeLi, $Pd_2(dba)_3$, allyl-Me-carbonate [this work]	59	2	6	30	2
6	**11b**, Ph_2SiH_2, (*R*)-(*p*-tolyl)$_2$BINAP, CuCl, NaO*t*-Bu; 1.03 equiv. MeLi, $Pd_2(dba)_3$, allyl-Me-carbonate [this work]	**82**	**1**	**1**	**0**	**15**

[a]Determined by GC. [b]Residual 3-methyl-cyclopentanone from unreacted cyclopentenolate.

quinolizidone could be elaborated to quinolizidine **14** following a variation of Fowler's procedure for introduction of the 3-furyl moiety.[12e] We found sodium triacetoxyborohydride to be superior to previously reported reducing agents in this reaction.[22]

Oxidation of **14** with *m*-CPBA proceeded uneventfully to provide the *trans*-quinolizidine *N*-oxide **8** (stereochemistry proven by NOE enhancement experiments between H_{ax}, H_{eq}, and H_b; see Supporting Information). Polonovsky elimination of naturally occurring nuphar quinolizidines like nupharidine (a *cis*-quinolizidine *N*-oxide) proceeds over 5 days using acetic anhydride and provides a maximum 60% yield of Δ^6-dehydrodeoxynupharidine, whereas trifluoroacetic anhydride is sluggish and gives decreased yields.[23] In contrast, we found that elimination of **8** is rapid with trifluoroacetic anhydride, and selectively produces α,β-unsaturated iminium ion **5** in less than 3 h. The production of **5** from **8** can be observed by ^{1}H NMR in $CDCl_3$ and this iminium is stable over 24 h at 22 °C.

Dimerization of **5** was not expected to be straightforward. Reineke reported the unexpected dimerization of chalcone in the presence of polysulfides to yield tetrahydrothiophanes,[24] but this reaction could only be effected with chalcones, despite attempts by LaLonde and Clardy to apply this reaction to

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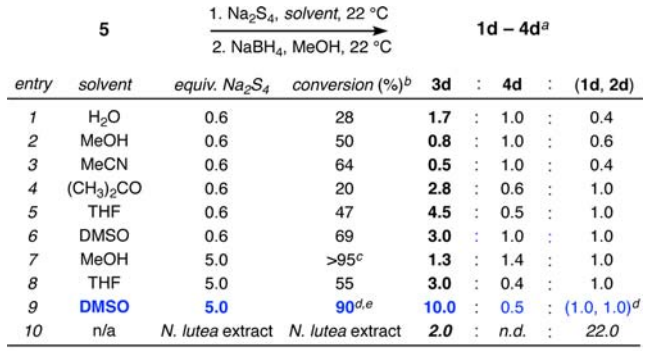
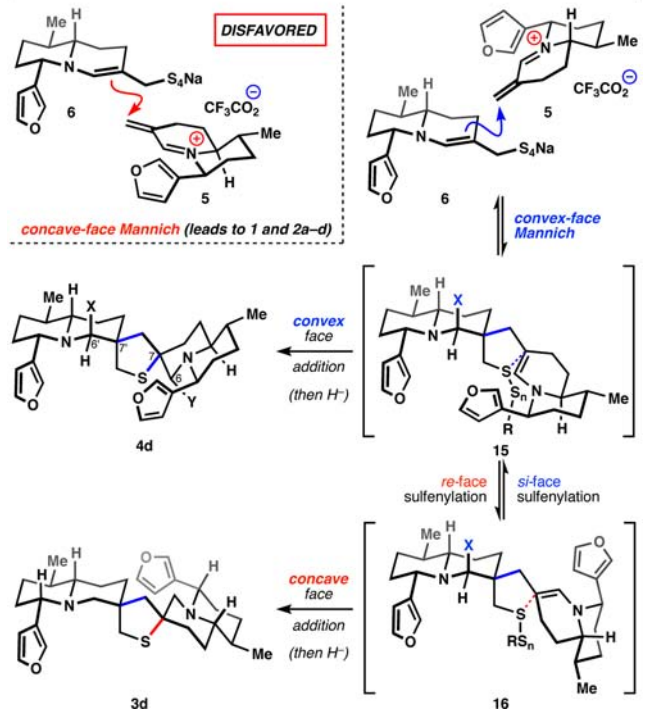
substrates relevant to the thiophane dimers.[25] Furthermore, there have been five syntheses of quinolizidine nuphar monomers,[8,12] but no reports of successful dimerizations to sulfur-containing binuphar alkaloids. Not surprisingly, we found that attempts to dimerize **5** according to the conditions of Reinecke gave complex mixtures and no dimers.

After extensive optimization, we found that addition of 0.6 equivalents of aqueous sodium tetrasulfide to a concentrated solution of **5** in water effected dimerization, albeit in low conversion and with poor stereoselectivity (entry 1, Table 2).

Table 2. Optimization of Dimerization



entry	solvent	equiv. Na_2S_4	conversion (%)[b]	3d	:	4d	:	(1d, 2d)
1	H_2O	0.6	28	**1.7**	:	1.0	:	0.4
2	MeOH	0.6	50	**0.8**	:	1.0	:	0.6
3	MeCN	0.6	64	**0.5**	:	1.0	:	0.4
4	$(CH_3)_2CO$	0.6	20	**2.8**	:	0.6	:	1.0
5	THF	0.6	47	**4.5**	:	0.5	:	1.0
6	DMSO	0.6	69	**3.0**	:	1.0	:	1.0
7	MeOH	5.0	>95[c]	**1.3**	:	1.4	:	1.0
8	THF	5.0	55	**3.0**	:	0.4	:	1.0
9	DMSO	5.0	90[d,e]	**10.0**	:	0.5	:	(1.0, 1.0)[d]
10	n/a	*N. lutea* extract	*N. lutea* extract	***2.0***	:	*n.d.*	:	***22.0***



[a]Ratios determined by HPLC and ^{1}H NMR. [b]According to HPLC. [c]Monomer not detected by HPLC. [d]Determined by ^{1}H NMR. [e]62% isolated yield of **3d**, see Scheme 1.

However, we found that both yield and stereoselection[26] could be modulated with solvent: water, methanol, and acetonitrile afforded modest yields, but poor selectivity (entries 1–3); whereas acetone induced good stereoselectivity with poor conversion (entry 4). Eventually, we found that THF and DMSO (entries 5 and 6) provided the highest levels of conversion and selectivity for a single stereoisomeric series, **3**. Increased equivalents of Na_2S_4 (entries 7–9) led to markedly higher levels of conversion, and in DMSO provided very high (10:1) diastereoselectivity. Reduction of the crude mixture with sodium borohydride allowed isolation of neothiobinupharidine in 62% yield (from *N*-oxide **8**), which we found remarkable given the complexity of the transformation.

Isomers **1**–**4d** were easily separated and their ^{1}H and ^{13}C NMR spectra were correlated to values reported in the literature. Isomer **4d** (named here 'neothionuphlutine'[27]) has never been isolated from *N. lutea*,[28] so its structure was assigned definitively by X-ray crystallographic analysis (see Figure 2).

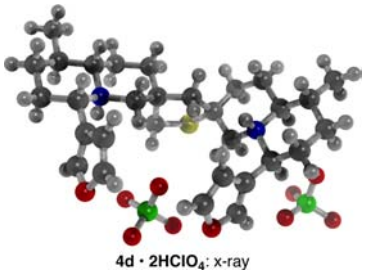


Figure 2. Stereoisomer **4d**: 'neothionuphlutine.'

The stereoselectivity of dimerization deserves some discussion. Nuphar quinolizidines have been shown to react stereoselectively on the convex face.[8−10] This facial selectivity is observed for the initial Mannich-type reaction between **5** and **6**, with an average 4.5:1 preference for convex versus concave Mannich (**3d**+**4d**:**1d**+**2d**, Table 2). However, the subsequent *concave*-face enamine sulfenylation (**16**) that must occur to form **3a** as the major product is more difficult to rationalize since the stereoselectivity of this process is dependent on both solvent choice and equivalents of polysulfide. The distribution of diastereomers does not appear to be a result of equilibration to a thermodynamic sink, since the ratio of products does not change over the course of the reaction.[29] Convex-face sulfenylation (**15**) may be disfavored due to a steric clash in the transition state between furyl groups, which are close in space in **4d** (see Figure 2). The role of polysulfide in affecting diastereoselectivity is challenging to explain since it can be involved at multiple points in the complex kinetic profile associated with enamine reactions,[30] a hypothesis which we are currently exploring.

More importantly, the distribution of dimers from spontaneous dimerization of **5** does not reflect the ratios of dimers isolated from *N. lutea* (entry 10, Table 2). Remarkably, the major stereoisomers **3** resulting from spontaneous dimerization derives from the *opposite stereoselectivity* of C–C and C–S bond formation than the major stereoisomers **1** in *Nuphar* sp. This data points to a more complex dimerization mechanism in vivo, which may involve enzymatic mediation or other chiral species in the cellular milieu. Thus, while LaLonde's insightful proposal for the origins of the nuphar dimers' gross structure is supported by our studies, the data also indicates that a more complex mechanism may be necessary to explain the predominance of **1** and **2** in nature.

In summary, a concise (8 steps from **11b**), asymmetric synthesis of (−)-neothiobinupharidine **3d** has been described. The approach relies on a new procedure for the reductive allylation of cyclopentenones, which accomplishes a challenging vicinal difunctionalization reaction. To the best of our knowledge, this is the only catalytic asymmetric approach to the nuphar quinolizidine family. Furthermore, this reaction enables an 'ideal' total synthesis, without recourse to fluctuating

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redox manipulation or protecting group interchange.[31] Access to **1**−**4** (**a**−**d**) in pure form will facilitate biological evaluation of these molecules. Efforts are currently underway to divert the inherent topological preferences of dimerization toward the thiobinupharidine series of dimers.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(26) Determined by integration of the ^{1}H NMR spectrum and LC chromatogram before purification.

(27) Nomenclature for dimers **1**−**4** is nonuniform in the literature. Compound **2d** was previously designated thionuphlutine B, but we have removed the 'B' since there is no unique compound 'thionuphlutine A,' a name that was incorrectly assigned to the known compound thiobinupharidine (**1d**). Compound **4d** had therefore been given the less systematic name 'thionuphlutine C' in ref. 28. The 'neo' prefix is more effective to differentiate series **1** from **3** and **2** from **4**, and refers to the stereochemistry of the C7′ quaternary carbon.

(28) Compound **4d** was produced in trace amounts by thermolysis of a sulfoxide derived from **3d** followed by reduction, but its structure was not unequivocally proven. See: LaLonde, R. T.; Wong, C. F. *Can. J. Chem.* **1978**, *56*, 56.

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■ NOTE ADDED AFTER ASAP PUBLICATION

Table 2 was incorrect in the version published ASAP January 11, 2013. The corrected version was re-posted on January 16, 2013.