Alkaloids

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# Enantioselective Formal Syntheses of 11 Nuphar Alkaloids and Discovery of Potent Apoptotic Monomeric Analogues

*Hui Li, Alexander Korotkov, Charles W. Chapman, Alan Eastman,* and Jimmy Wu**

***Abstract:*** *Concise, scalable, and enantioselective formal syntheses of eight dimeric and three monomeric nuphar alkaloids were achieved, along with the construction of a stereochemically diverse collection of the first known monomeric analogues having apoptotic activity. The syntheses involved the development of highly enantioselective Brønsted acid catalyzed vinylogous Mukaiyama–Mannich reactions, which feature the unprecedented use of a supersilyl group to control the regio-, enantio- and diastereoselectivity. Biological studies reveal that several of these novel nuphar analogues are even more potent than their dimeric natural product counterparts.*

The dimeric nuphar alkaloids were first isolated from the yellow water lily in 1962.[1] As exemplified by (+)-6-hydroxythiobinupharidine [(+)-**1a**; Figure 1], they are structurally unique sulfur-containing quinolizidine triterpenoids endowed with multiple biological profiles (antibacterial,[2] antifungal,[3] immnosuppressant,[4] etc.). In particular, (+)-**1a** induces apoptosis in U937 human leukemia cells within 1 hour (2.5–10 μM).[5] Such a rapid induction of apoptosis is the fastest reported for a small molecule.

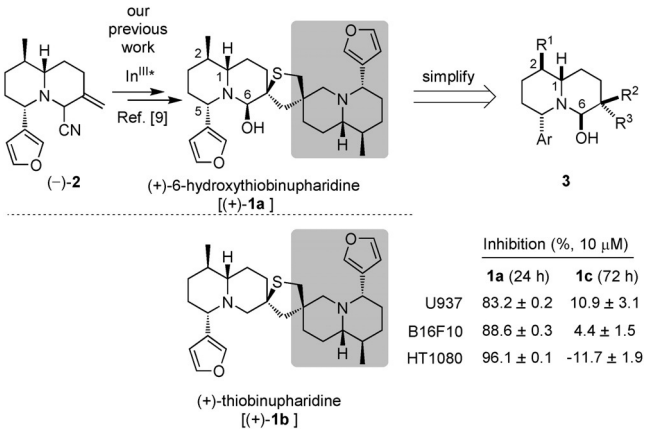


***Figure 1.*** Rational for simplification of (+)-**1a**.

Given their challenging architecture and potential as cancer chemotherapeutic agents, members of this family have caught the attention of the chemical[6] and biological[7] communities with the first synthesis of a dimeric nuphar alkaloid, (−)-neothiobinupharidine [(−)-**5b**; Figure 2],

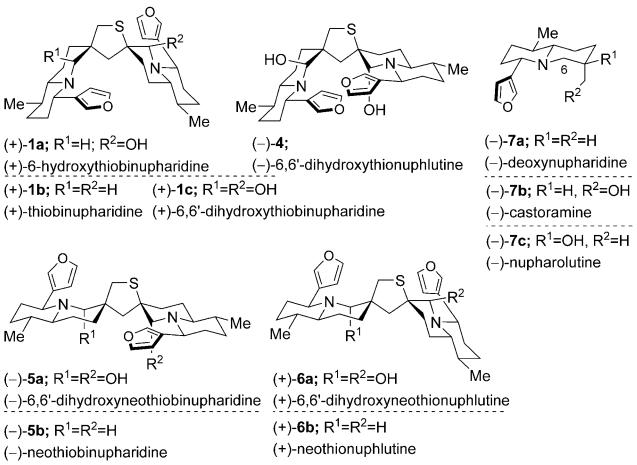


***Figure 2.*** Nuphar alkaloids.

reported by Shenvi and co-workers in 2013.[8] Very recently, our group disclosed the first total syntheses of both enantiomers of **1a** and four other hydroxylated congeners by the dimerization of enantiomerically pure monomeric the cyano quinolizidine **2** (Figure 1) using chiral indium(III) complexes.[9] However, stoichiometric amounts of the indium(III) complexes were used in the dimerization process, and their ability to control the diastereochemical outcome during the thiaspirane ring-formation is only moderate, thus resulting in the need for careful purification of each diastereomer. Such limitations render SAR studies and further attempts of using dimeric nuphar analogues to identify biological target(s) difficult in terms of practicality, synthetic economy, and efficiency.

Inspired by the previous successes of function/biology-oriented synthesis (FOS/BIOS),[10] we wondered whether a monomeric quinolizidine subunit of **1** might be sufficient to impart apoptotic properties with similar potencies as compared to their dimeric counterparts. We were intrigued by the report of Yoshikawa and co-workers[5] in which (+)-**1a** was considerably more cytotoxic than (+)-**1b**, with the only structural difference being the presence of the C6 aminal moiety in (+)-**1a** (Figure 1). We hypothesized that the aminal

[*] H. Li, Dr. A. Korotkov, Prof. J. Wu
Department of Chemistry, Dartmouth College
Hanover, NH 03755 (USA)
E-mail: jimmy.wu@dartmouth.edu
C. W. Chapman, Prof. A. Eastman
Department of Pharmacology and Toxicology, Geisel School of Medicine, Lebanon, NH 03756 (USA)
E-mail: alan.r.eastman@dartmouth.edu
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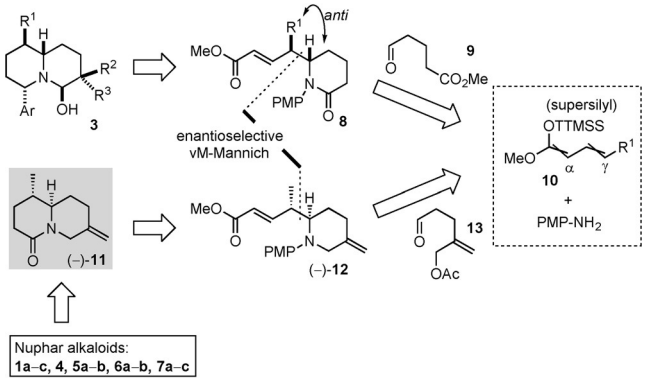
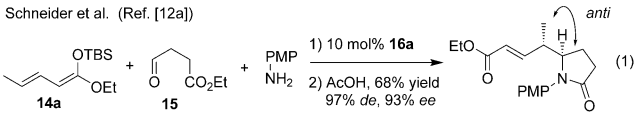
hydroxy group of (+)-**1a** is a critical feature for apoptotic activity. Because the highlighted portions (Figure 1) of (+)-**1a** and (+)-**1b** are identical, we anticipated that they may be deleted to reduce molecular complexity. Moreover we also proposed replacement of the C5 furyl group of (+)-**1a** (structural alert) with aryl groups of greater metabolic stability. As a result, the truncated structure **3** was conceived. Although the family of nuphar alkaloids contains many monomeric quinolizidines such as (−)-**7a–c**[6a] (Figure 1) and others, none of them possess the C6 hydroxy functionality. It is therefore not surprising that none of these naturally occurring compounds are known to be apoptotically active.[11]

We reasoned that **3** and related analogues may arise from the common intermediate **8** (Scheme 1). To access the *anti* relationship between $R^1$ and the piperidone ring of **8**, we were cognizant of chiral phosphoric acid catalyzed stereoselective vinylogous Mukaiyama–Mannich (vM-Mannich) reactions reported by Schneider et al. [Eq. (1); TBS = *tert*-butyldimethylsilyl].[12,13] By employing this protocol, we envisaged constructing diastereo- and enantiomerically enriched **8** from the aldehyde **9** by a three-component vM-Mannich reaction (Scheme 1).[14]




***Scheme 1.*** Retrosynthetic analysis. PMP = *para*-methoxyphenyl, TTMSS = tris(trimethylsilyl)silyl.




Moreover, we hypothesized that the above strategy may also allow the construction of the enantioenriched quinolizidine (−)-**11** (Scheme 1), an advanced precursor in: 1) our syntheses of dimeric nuphar alkaloids **1a–c**, **4**, **5a**, and **6a–b**,[9] 2) the synthesis of **5b** by Shenvi et al.,[8] and the syntheses of **7a–c**[6a] by Harrity et al. (Figure 2). We envisioned that (−)-**11** could be derived from the piperidine (−)-**12**. In analogy, (−)-**12** could be assembled stereoselectively from the aldehyde **13** by a vM-Mannich reaction between **10** and *p*-anisidine.

The groups of Yamamoto and Tilley, as well as our group and others have reported the use of supersilyl groups to control stereoselectivity in aldol, vinylogous Mukaiyama–Michael (vM-Michael) reactions, and other processes (Figure 3).[15,16] In this account, we describe the unprecedented use of supersilyl groups on vinylketene acetals, such as **10**, to influence the regiochemical outcome and the diastereo- and enantioselectivity of vM-Mannich reactions. The resulting enantiomerically pure δ-amino α,β-unsaturated carbonyl products have allowed us to carry out the concise and scalable formal syntheses of 11 nuphar alkaloids, as well as a library of biologically active truncated analogues of **1a**.

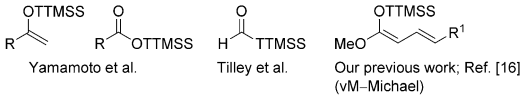
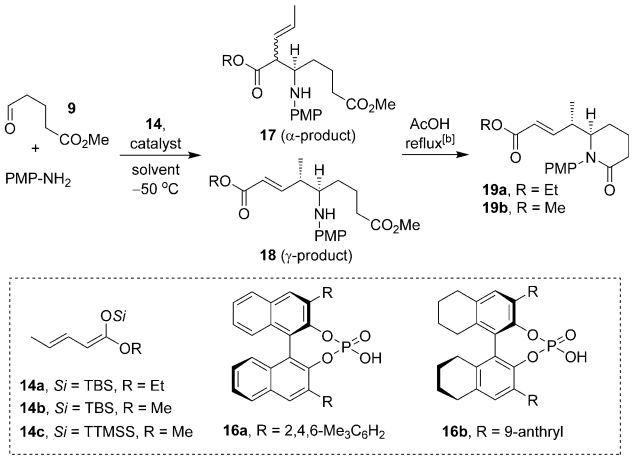



***Figure 3.*** Supersilyl groups.

We initiated our study by examining the vM-Mannich reaction between **9**, *p*-anisidine, and the TBS-based vinylketene acetal **14a** by adopting the optimal protocol reported by Schneider and co-workers (Table 1, entry 1). Surprisingly, the reaction yielded only moderate γ- versus-α regioselectivity and enantioselectivity, and were inferior to results obtained when starting with the aldehyde **15** [Eq. (1)]. As a positive control, we performed the reaction reported by Schneider et. al. in Equation (1) and were able to replicate the selectivities that they observed.

From these data, it was apparent that even a single extra methylene unit on **9**, as compared to **15**, leads to substantial

***Table 1:*** Selected optimization data.[a]




| Entry | **14** | Catalyst (mol%) | Solvent | *t* [h] | Yield [%][c] **18** (γ) | **17** (α) | d.r. (**18**)[d,g] | *ee* [%] (**19b**)[e] |
|---|---|---|---|---|---|---|---|---|
| 1 | **14a** | **16a** (10) | THF | 16 | 65 | 23 | 21:1 | 50[f] |
| 2 | **14b** | **16b** (10) | THF | 14 | 59 | 29 | 13.5:1 | 79 |
| 3 | **14c** | **16b** (10) | THF | 11 | 90 | <5 | 22:1 | 92 |

[a] **9** (1 equiv), *p*-anisidine (1 equiv) and **14** (2.0 equiv) and 0.1 M concentration. [b] Quantitative yield. [c] Yield of isolated product. [d] Determined by $^1$H NMR analysis of unpurified product. [e] Determined by chiral-phase HPLC analysis for **19b**. [f] *ee* value for **19a**. [g] Absolute configuration of **18** was assigned by analogy to Ref. [12]. THF = tetrahydrofuran.

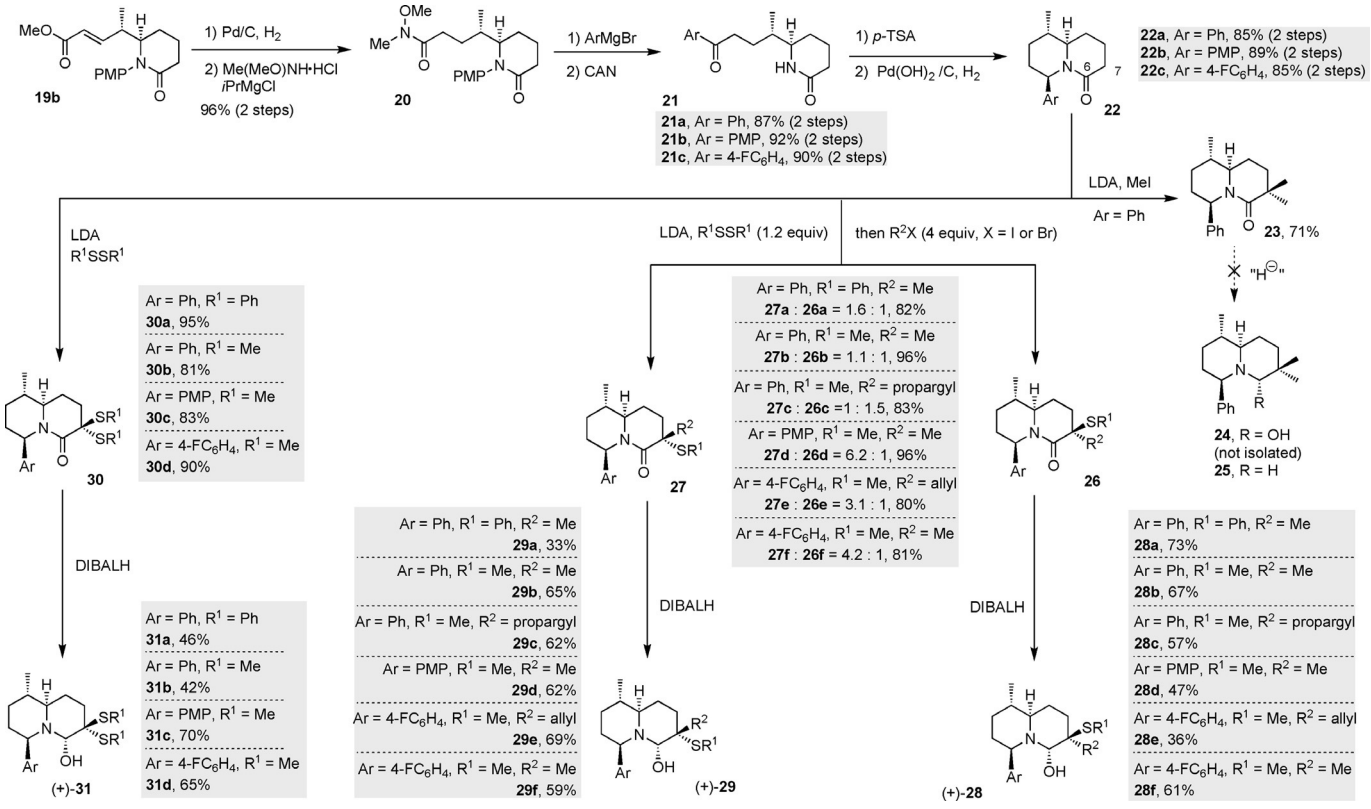
  

degradation of the regio-, diastereo-, and enantioselectivity. This observation prompted us to search for improved reaction conditions for our reaction system (see the Supporting Information for details). A survey of several BINOL- and H8-BINOL-based chiral phosphoric acids identified **16b** as a promising candidate, but still with substantial amounts of α alkylation (Table 1, entry 2). Inspired by our recent studies on using supersilyl groups to influence the regioselectivity of vM-Michael[16] reactions, as well as the pioneering work by Yamamoto et al.,[15] we elected to employ the vinylketene acetal **14c**, bearing the sterically demanding supersilyl group tris(trimethylsilyl)silyl (TTMSS), as the nucleophile. Gratifyingly, this transformation furnished **19b** with excellent selectivities (entry 3) and was also performed on gram-scale.

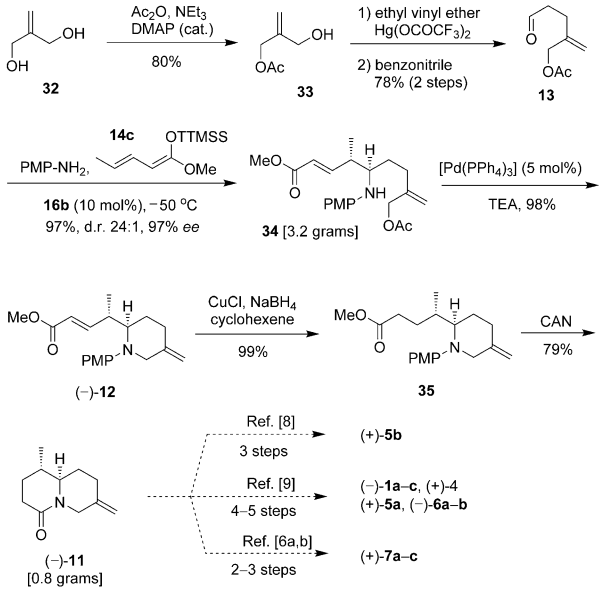
With an ample supply of **19b** in hand, we then proceeded to the syntheses of the target monomeric nuphar alkaloid analogues **3**. As shown in Scheme 2, **19b** was subjected to hydrogenation and subsequent Weinreb amide formation to give **20**, a compound that represents a versatile intermediate from which a range of aryl groups may be installed. For instance, treatment of **20** with PhMgBr and then removal of the PMP group provided the ketone **21a**, whose structure was further confirmed by single-crystal X-ray analysis. *p*-TsOH-mediated condensation of **21a** and subsequent stereoselective hydrogenation yielded the quinolizidine **22a** in 85% yield over the two steps. The late-stage intermediate **22a** could then be transformed into a variety of truncated nuphar analogues by two consecutive operations: 1) base-mediated difunctionalization of C7, and 2) stereoselective semi-reduction of the lactam moiety to install the C6 hydroxy group. Our attempts to reduce the dimethylated quinolizidine **23**, however, were unsuccessful despite surveying a variety of reductive conditions. These reactions invariably yielded the fully reduced quinolizidine compound **25**. In contrast the semi-reduction of the thioethers **26a** and **27a** with DIBAL-H successfully furnished **28a** and **29a**, respectively, bearing the desired C6 aminal hydroxy groups.[9,17]

Following the same strategy, we synthesized a range of structurally diverse enantioenriched monomeric quinolizidines, (+)-**28a–f**, (+)-**29a–f**, and (+)-**31a–d** (Scheme 2). To evaluate the effect that the specific antipode of these analogues has on their apoptotic activities, we also prepared the enantioenriched (−)-**28b** and (−)-**31b**, as well as racemic **28b**, **29a**, **29c**, and **31a–b**. The relative stereochemical relationship of monomeric nuphar analogues were assigned by 2D-NOESY experiments (see the Supporting Information).

We then took advantage of the vM-Mannich reactions developed above to construct the key monomeric quinolizidine intermediate (−)-**11** en route to the hydroxylated dimeric nuphar alkaloids as well as several monomeric nuphar alkaloid natural products. As shown in Scheme 3, our efforts began with the preparation of **13**, which could be derived from the commercially available **32** by: 1) monoacylation, 2) vinylation of the free allylic alcohol, and 3) Claisen rearrangement. Treatment of **13** with **14c** in the presence of 10 mol% of the catalyst **16b** smoothly afforded the desired γ-product **34** in 97% yield with complete γ-regioselectivity, and




***Scheme 2.*** Synthesis of monomeric nuphar alkaloid analogues. CAN = ceric ammonium nitrate, DIBALH = diisobutylaluminum hydride, LDA = lithium diisopropyl amide, *p*-TSA = *para*-toluenesulfonic acid.




***Scheme 3.*** Enantioselective formal syntheses of eight dimeric and three monomeric nuphar alkaloids. DMAP = 4-(*N*,*N*-dimethylamino)-pyridine, TEA = triethylamine.

excellent diastereo- (d.r. 24:1) and enantioselectivity (97% *ee*).[18] This sequence furnished 3.2 grams of **34**, which underwent intramolecular Tsuji–Trost allylation to give the piperidine (−)-**12**. Copper(I)-mediated chemoselective reduction of the enone alkene gave the product **35** which was subjected to CAN-promoted removal of the PMP group and spontaneous lactam formation to furnish (−)-**11**. This sequence is scalable and allowed 0.8 grams of (−)-**11** to be procured from **32** in only seven total steps, and is comparable to the current shortest route to (−)-**11** (eight total steps, five longest linear).[8] The quinolizidine (−)-**11** is a key late-stage intermediate in the previous syntheses of (−)-**1a** and several other dimeric and monomeric nuphar alkaloids.[6a,8,9]

We then assayed the monomeric analogues synthesized above 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 4). Rapid apoptosis of U937 cells (within 2 h) was observed in most cases. The majority of the synthetic monomeric nuphar analogues possessed more potent or comparable apoptotic activities to that of the parent dimeric nuphar alkaloid (+)-**1a**.[9] Notably, these compounds represent the first known examples of monomeric nuphar alkaloids which are biologically active for apoptosis. Among them (+)-**28b** seems to be the most potent at the 2 and 6 hour time points, and is thus eightfold more cytotoxic than (+)-**1a** (Figure 4). It is also noteworthy that enantiopure quinolizidines (+)-**28b** and (+)-**31b**, which correspond to the absolute configuration of unnatural (−)-**1a**, were more potent than their respective antipodes (−)-**28b** and (−)-**31b**. Moreover, the monomeric quinolizidines **28**, which feature an *anti* relationship between the hemiaminal hydroxy group and adjacent sulfide substituents, tended to exhibit slightly more potent apoptotic activities than their epimeric structures (**29;** e.g. entry 2 versus 3).

***Table 2:*** Apoptosis assays (PARP cleavage).

| Entry | Compound | 2 h[a] | 6 h[a] |
|---|---|---|---|
| 1 | (+)-**1a** (natural) | 10 | 5.0 |
| 2 | (+)-**28a** | 5.0 | 2.5 |
| 3 | (+)-**29a** | 10 | 5.0 |
| 4 | (±)-**29a** | 10 | 5.0 |
| 5 | (+)-**28b** | 1.25 | 0.625 |
| 6 | (−)-**28b** | 10 | 2.5 |
| 7 | (±)-**28b** | 4.0 | 3.0 |
| 8 | (+)-**29b** | 10 | 5.0 |
| 9 | (+)-**28c** | 5.0 | 2.5 |
| 10 | (+)-**29c** | 5.0 | 2.5 |
| 11 | (±)-**29c** | 10 | 5.0 |
| 12 | (+)-**28d** | 5.0 | 1.25 |
| 13 | (+)-**29d** | 5.0 | 5.0 |
| 14 | (+)-**28e** | 5.0 | 2.5 |
| 15 | (+)-**29e** | 10 | 5.0 |
| 16 | (+)-**28f** | 2.5 | 1.25 |
| 17 | (+)-**29f** | 10 | 5.0 |
| 18 | (+)-**31a** | –[b] | –[b] |
| 19 | (±)-**31a** | –[b] | –[b] |
| 20 | (+)-**31b** | 5.0 | 1.25 |
| 21 | (−)-**31b** | –[b] | 10 |
| 22 | (±)-**31b** | 10 | 5.0 |
| 23 | (+)-**31c** | 5.0 | 5.0 |
| 24 | (+)-**31d** | 5.0 | 5.0 |

[a] Minimum concentration (μM) at which 50% or greater PARP cleavage is observed. [b] No PARP cleavage was observed up to 10 μM at the indicated time.

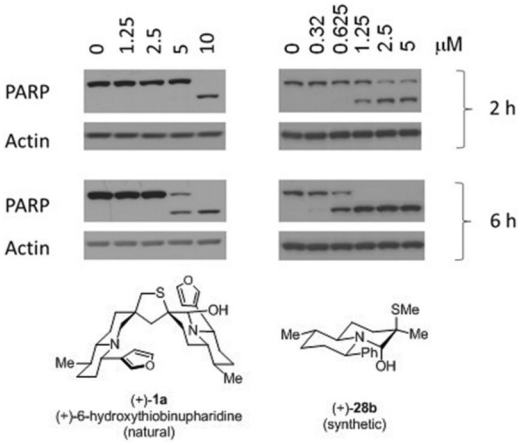



***Figure 4.*** Representative western blots for PARP cleavage.

In conclusion, we have accomplished the formal synthesis of (−)-6-hydroxythiobinupharidine and several other dimeric and monomeric nuphar alkaloids. We have also designed and constructed a diverse range of simplified monomeric quinolizidine analogues. The syntheses feature the development of vM-Mannich reactions which make use of the supersilyl group as a control element for regio-, diastereo-, and enantioselectivity. Biological assays of these truncated analogues identified the first known apoptotically active monomeric nuphar alkaloids.

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