Chemical Science

EDGE ARTICLE

A robust and scalable synthesis of the potent neuroprotective agent (−)-huperzine A†

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(−)-Huperzine A (**1**) is a tricyclic alkaloid that is produced in low yield by the Chinese herb *Huperzia serrata*. There is intense contemporary interest in clinical application of (−)-huperzine A (**1**) for treating neurodegenerative diseases and protecting against the lethal effects of chemical warfare agents, such as sarin and VX. We report a robust, scalable, and efficient synthesis of (−)-huperzine A (**1**) from (*R*)-4-methyl-cyclohex-2-ene-1-one (**5**). Our route proceeds in 35–45% overall yield, delivers (−)-huperzine A (**1**) in only eight steps from cyclohexenone **5**, requires only three chromatographic purifications, and can provide gram quantities of the target. This route represents a critical, enabling advance toward detailed evaluation of (−)-huperzine A (**1**) in clinical settings.
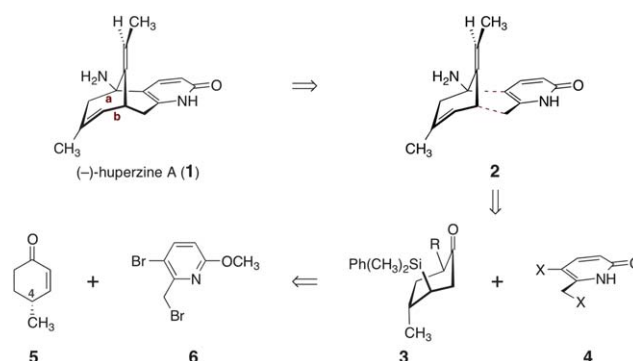
(−)-Huperzine A (**1**) is a tricyclic alkaloid produced by the Chinese herb *Huperzia serrata*.[1] (−)-Huperzine A (**1**) is a potent, selective, and reversible inhibitor of acetylcholine esterase (AChE, $K_i = 23$ nM).[2] Recent studies have established that this activity may be exploited to counteract organophosphate chemical warfare agents, such as sarin and VX, by inhibiting their covalent modification of peripheral and cerebral AChE.[3] A large body of evidence also suggests that (−)-huperzine A (**1**) may slow the progression of neurodegenerative diseases, including Alzheimer's disease.[4] (−)-Huperzine A (**1**) is well-tolerated in humans, even at doses well above those required clinically.[5] Consequently, clinical investigation of (−)-huperzine A (**1**) is a subject of intense research in the pharmaceutical and defense industries.

The primary obstacle to the clinical development of (−)-huperzine A (**1**) has been one of supply. Extraction from natural sources is low-yielding (average yield = 0.011% from the dried herb),[4a] and overharvesting has caused a rapid decline in the abundance of Huperziaceae.[6] Compounding these issues, the producing species requires nearly 20 years to reach maturity.[6]

Total synthesis offers an alternative potential source of huperzine. An enantioselective synthesis is highly desirable, because (+)-huperzine A is significantly less potent than the natural (−)-antipode (**1**).[7] The first total syntheses of (±)-huperzine A were reported by Kozikowski and Xia[8] and Qian and Ji.[9] A chiral auxiliary-based route was later developed by Kozikowski *et al.*[10] In the interceding years, several research groups have reported modifications to the Kozikowski route,[11] as

well as complete,[12] partial,[13] and formal[14] routes to huperzine. Nonetheless, Kozikowski's chiral controller-based route,[10] which proceeds in 16 steps and *ca.* 2.8% yield, remains the most efficient published pathway to synthetic (−)-huperzine A (**1**).[15]

The majority of approaches to huperzine A have relied on introduction of a four-carbon fragment to a bicyclic structure (retrosynthetic cleavage of bonds a and b in **1**, Scheme 1). We envisioned a distinct approach, wherein disconnection of two alternative bonds (see **2**) forms the ketone and pyridone-based synthons **3** and **4**, respectively. The former might be obtained from (*R*)-4-methyl-cyclohex-2-ene-1-one (**5**) while 3-bromo-2-(bromomethyl)-6-methoxypyridine (**6**) would serve as a functional equivalent to **4**. The C-4 stereocenter in **5** is used in our route to control relative and absolute stereochemistry in the target. Several convenient methods to prepare (*R*)-4-methyl-cyclohex-2-ene-1-one (**5**) have been reported.[16] We elected to use a straightforward four-step sequence starting from (+)-pulegone.[16a] Dihalopyridines such as **6** have found use in a distinct and significantly more lengthy route to (−)-huperzine A (**1**),[14c] as well as in the synthesis of other *Lycopodium* alkaloids.[17]

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Scheme 1 Retrosynthetic analysis of (−)-huperzine A (**1**).
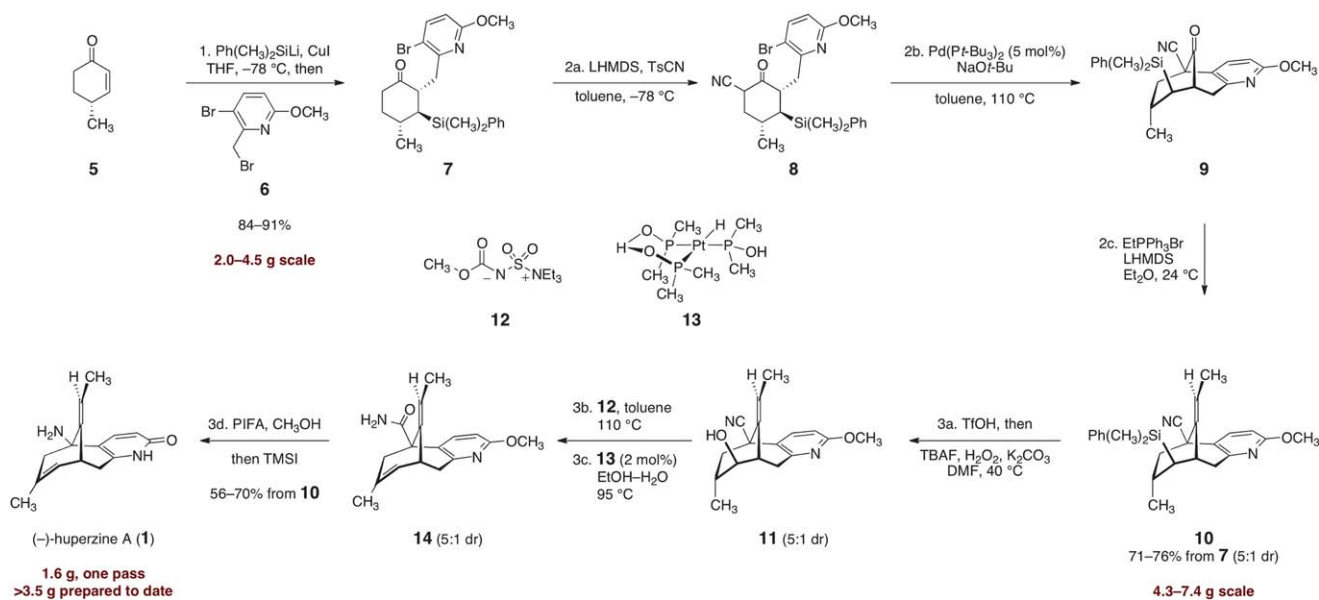
Scheme 2 Enantioselective synthesis of (−)-huperzine A (**1**). Reagents and conditions: (1) Ph(CH₃)₂SiLi, CuI, HMPA, THF, −78 → −23 → −78 °C, then **6**, −78 → −23 °C, 84–91%; (2a) LHMDS, *p*-TsCN, toluene, −78 °C; (2b) Pd(P*t*-Bu₃)₂ (5 mol%), NaO*t*-Bu, toluene, 110 °C; (2c) EtPPh₃Br, LHMDS, Et₂O, 24 °C, 71–76% from **7**, E : Z = 5 : 1; (3a) TfOH, DCM, 0 → 24 °C, then TBAF, H₂O₂, K₂CO₃, DMF, 40 °C, E : Z = 5 : 1; (3b) **12**, toluene, 110 °C, E : Z = 5 : 1; (3c) **13** (2 mol%), EtOH–H₂O (2 : 1), 95 °C, E : Z = 5 : 1; (3d) PIFA, CH₃OH, reflux, then TMSI, CHCl₃, reflux, then CH₃OH, reflux, 56–70% from **10**.

The successful implementation of this strategy is shown in Scheme 2. To render the route amenable to large-scale synthesis, we extensively optimized each step, and this allowed many transformations to be efficiently telescoped (the final synthetic route requires three chromatographic purification steps). Our work commenced with conjugate addition of lithium dimethylphenylsilylcuprate to (*R*)-4-methyl-cyclohex-2-ene-1-one (**5**). Alkylation of the incipient enolate with 3-bromo-2-(bromomethyl)-6-methoxypyridine (**6**) afforded the addition–alkylation product **7** as a single detectable diastereomer (¹H NMR analysis), isolated in 84–91% yield after purification (2.0–4.5 g scale).

Table 1 Optimization of the enolate heteroarylation.[a]
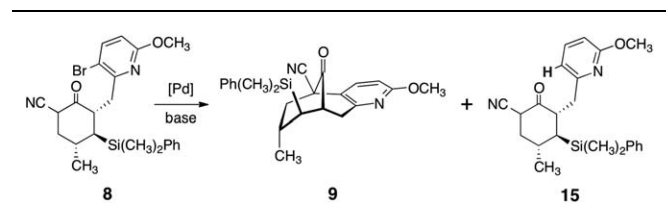
Entry	Base	mol% Pd	Yield 9[b]	Yield 15[c]	Dec.[c]
1	K₂CO₃	10	<1%	99%	—
2	Na₂CO₃	10	<1%	99%	—
3	NaH	10	50%	<1%	30%
4	KO*t*-Bu	10	64%	10%	20%
5	NaO*t*-Bu	5	>99%	<1%	<1%

[a] All reactions were conducted using Pd(P*t*-Bu₃)₂ as precatalyst in toluene at 110 °C for 3 h. [b] Isolated yield after purification by flash-column chromatography. [c] Estimated by ¹H NMR and LC/MS analysis of the unpurified reaction mixture. Dec. = decomposition.

Kinetically-controlled deprotonation of **7** and trapping of the resulting enolate with *para*-toluenesulfonyl cyanide,[18] followed by immediate work up of the product mixture, formed the α-cyanoketone **8** in high purity (est. >95%, ¹H NMR analysis). Rapid isolation of the product was critical, as the α-cyanoketone **8** underwent disproportionation to starting material (**7**) and an α,α-dicyanoketone (not shown) if the mixture was allowed to age.

The unpurified α-cyanoketone **8** was then subjected to a palladium-catalyzed intramolecular enolate heteroarylation.[19] Among several catalyst precursors examined, bis(tri-*tert*-butylphosphine)palladium (0), prepared by the method of Dai and Fu,[20] emerged as the most effective. A dramatic dependence on base was observed (Table 1). Thus, in the presence of carbonate bases (entries 1, 2), the protodebrominated product **15** predominated. Sodium hydride (entry 3) improved conversion to the cyclized product (**9**), although extensive decomposition also occurred. Ultimately, we identified sodium *tert*-butoxide (entry 5) as optimal, and using this base the product was obtained in essentially quantitative yield (¹H NMR analysis). The next step of the sequence called for the stereoselective olefination of the ketone function of **9**. Treatment of **9** with the lithium ylide derived from ethyltriphenylphosphonium bromide (ether, 24 °C) afforded the olefination product **10** in high yield. A clear trend between E : Z selectivity and concentration was observed (E/Z = 1.1, 1.8, 5 at 1.0, 0.1, and 0.01 M, respectively), which is consistent with a salt effect and suggests the desired E-isomer is the kinetically-favored product.[21] Under optimized conditions, the olefinated product **10** was isolated in 71–76% yield from **7** as a 5 : 1 mixture of E/Z isomers by flash-column chromatography (4.3–7.4 g scale). By this approach, the entire carbon skeleton of **1** was formed in high overall yield and in four steps on a multigram scale.

Treatment of the olefination product (**10**) with tri-fluoromethanesulfonic acid, followed by oxidative desilylation, provided the cyanoalcohol **11** in high purity (^{1}H NMR analysis). The unpurified cyanoalcohol **11** was efficiently dehydrated by heating with the Burgess reagent (**12**) in toluene. Thermolysis of the dehydrated product (not shown) in the presence of the platinum catalyst **13**[22] in aqueous ethanol afforded the amide **14**. Finally, Hofmann rearrangement [bis(trifluoroacetoxy)iodo-benzene], global deprotection, and purification by flash-column chromatography afforded separately (−)-huperzine A (**1**, 56–70% over four operations) and its olefin isomer (not shown, 11–14%). Synthetic (−)-huperzine A (**1**) was identical in all respects (^{1}H NMR, ^{13}C NMR, IR, three TLC solvent systems, LC/MS retention time, optical rotation) to an authentic sample. Batches of (−)-**1** as large as 1.6 g have been prepared.[23]

To date, over 3.5 g of (−)-huperzine A (**1**) have been prepared by the route delineated above. Our synthesis proceeds in 35–45% overall yield (16-fold more efficient than any other previously reported enantioselective route), and requires only three chro-matographic purifications. We envision that this chemistry will provide a reliable supply of synthetic (−)-huperzine A (**1**) and will greatly facilitate its clinical development for neuroprotective and anti-neurodegenerative applications.

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23 As a consequence of the potent activity and high skin permeability of **1**, we have not attempted to prepare larger batches in our laboratory.