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Development of a Large-Scale Synthetic Route to Manufacture (−)-Huperzine A

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ABSTRACT: A safe, practical and scalable process for manufacture of (−)-huperzine A has been developed and scaled up to manufacture several hundred grams of (−)-huperzine A with chemical and optical purity of >99%. The process consists of 11 chemical stages starting from commercially available materials with only nine isolation steps and no chromatography purification. This process provides a reliable and cost-effective source of synthetic (−)-huperzine A and its derivatives for pharmaceutical and nutraceutical markets.

INTRODUCTION

(−)-Huperzine A is a naturally occurring quinolizidine alkaloid extracted from *Huperzia serrata*,[1] a species of fir clubmoss. The plant grows mainly in southern China and has been used for centuries for the treatment of schizophrenia, fever, blood disorder, and loss of memory. In addition to its traditional use in Chinese medicine, it has been found that (−)-huperzine A is a potent and reversible acetylcholinesterase inhibitor,[2] and clinical studies have shown a significant improvement in memory deficit and cognitive performance in patients with Alzheimer's disease.[3] Alzheimer's disease is a largely unmet clinical need, with an estimated 20 million sufferers worldwide. However, supply of natural (−)-huperzine A from *Huperzia serrata* is limited, as the plant takes nearly 20 years to reach maturity,[4] and 100 kg of the dried plant is required to produce just 10 g of crude (−)-huperzine A.[5] A scalable and cost-effective synthetic route to provide kilogram quantities of (−)-huperzine A is therefore required to meet the current medical demand.

Two independent total syntheses of racemic (±)-huperzine A were reported in 1989,[6,7] both utilizing the same tetrahydroquinalone intermediate **1**. Xia and Kozikowski elaborated commercially available 1,4-cyclohexanedione monoethylene ketal in six steps to compound **1** in 35% overall yield (Scheme 1),[6] whereas Qian and Ji developed 5-ethoxycarbonyl-6-methyl-2-pyridone to compound **1** also in six steps with 39% overall yield (Scheme 2).[7] However, 5-ethoxycarbonyl-6-methyl-2-pyridone was itself synthesized in three steps and 33% yield from ethylacetoacetate. Both groups then utilized a similar strategy to convert intermediate **1** into racemic (±)-huperzine A. The main differences were that Xia and Kozikowski incorporated an isomerisation step using thiophenol and azobisisobutyronitrile (AIBN) to dramatically improve the yield of the desired *E*-isomer following the Wittig reaction, and Qian and Ji used a modified Curtius reaction to produce the urethane intermediate, which was deprotected in two steps, in contrast to the methylcarbamate intermediate of Xia and Kozikowski, which was deprotected in a single step.

Kozikowski subsequently reported an improved synthesis of a key intermediate, reducing the synthesis of compound **1** to four steps from commercially available materials and improving the yield from 35% to 48% with significantly reduced reagent costs (Scheme 3).[8,9] They further improved the route by replacing the tandem Michael-aldol reaction with palladium-catalysed bicycloannulation methodology,[10] originally developed by Gravel et al. using 1,3-diacetoxy-2-methylenepropane in a model system.[11] This not only improved the yield to huperzine A but also enabled the application of asymmetric catalysis to the bicycloannulation reaction. Terashima's group developed this approach and achieved a chiral induction of 64% ee using a modified Hayashi ligand.[12] Following recrystallisa-

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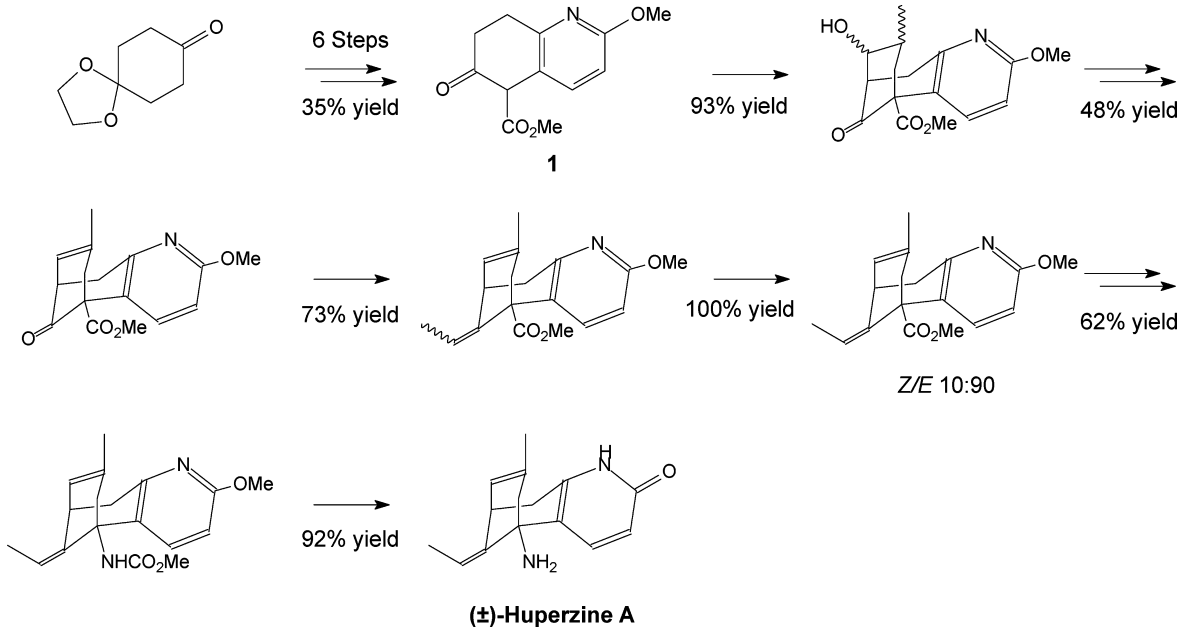
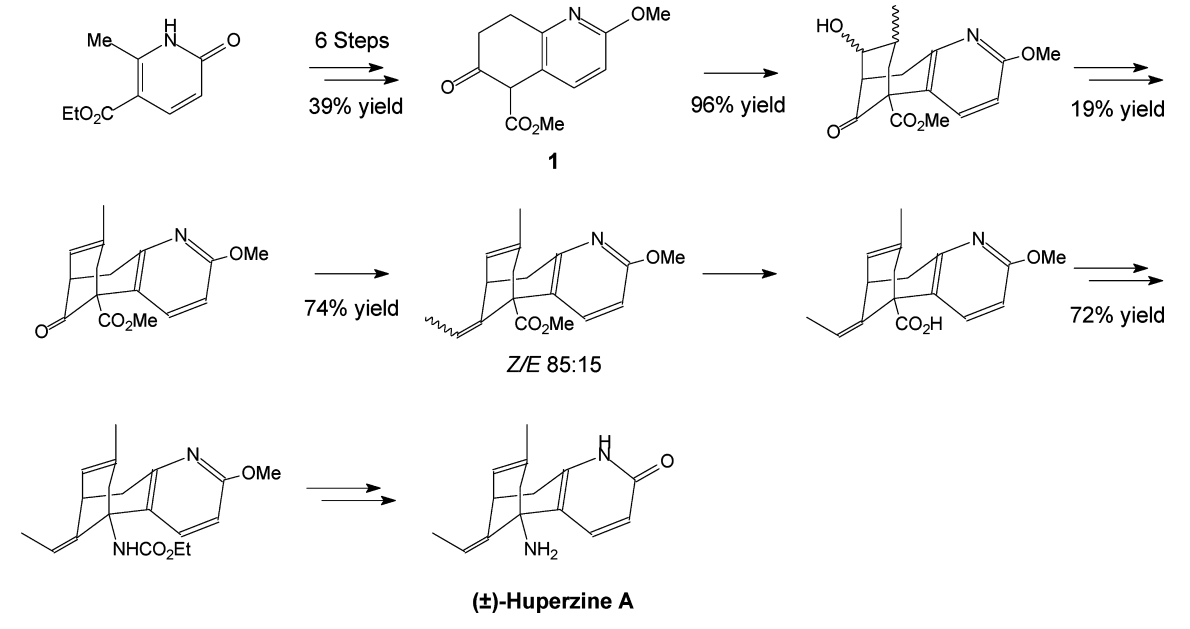
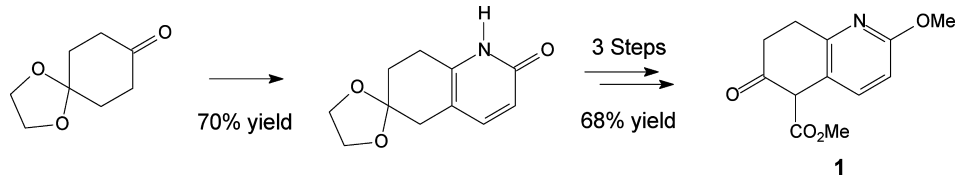
Scheme 1. Synthetic route to (±)-huperzine A by Xia and Kozikowski



Scheme 2. Synthetic route to (±)-huperzine A by Qian and Ji



Scheme 3. Improved route to compound 1 by Kozikowski et al.



tion, they obtained compound **2** with greater than 99% enantiomeric excess (ee) from compound **1** in 29% yield (Scheme 4).

An alternative approach to chiral synthesis utilised chiral auxiliary esters to influence the Michael-aldol reaction of **1** with methacrolein.[13,14] Kozikowski's group utilized the (−)-8-phenylmenthol ester, which gave 9:1 diastereomeric ratio after elimination. Following Wittig reaction, however, the thiophenol/AIBN-catalysed isomerisation was found to be less effective than when the methyl ester was present, giving only a 1.4:1 *E*/*Z* ratio after two repetitions. The desired *E*-isomer was isolated by column chromatography. Furthermore, the chiral auxiliary could not be hydrolysed by conventional methods and was removed by reduction to the corresponding alcohol

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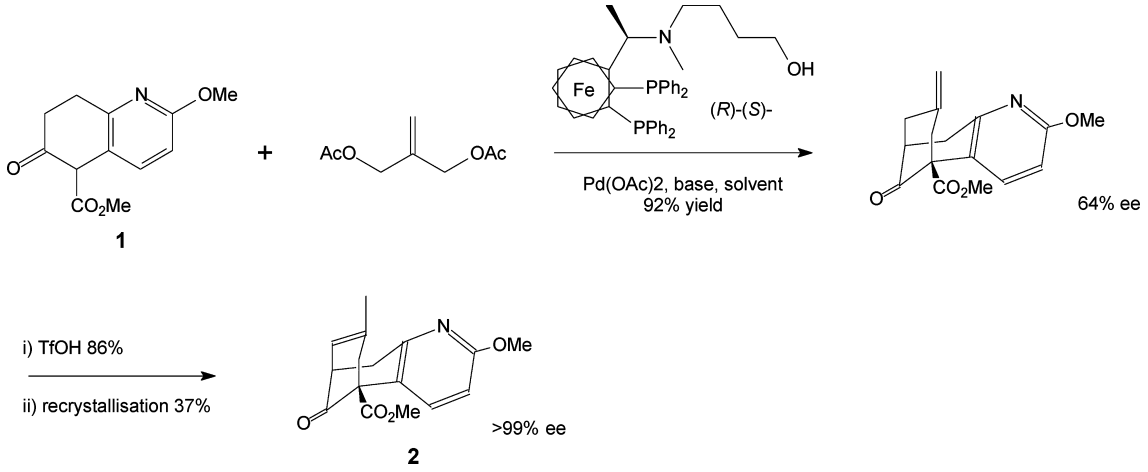
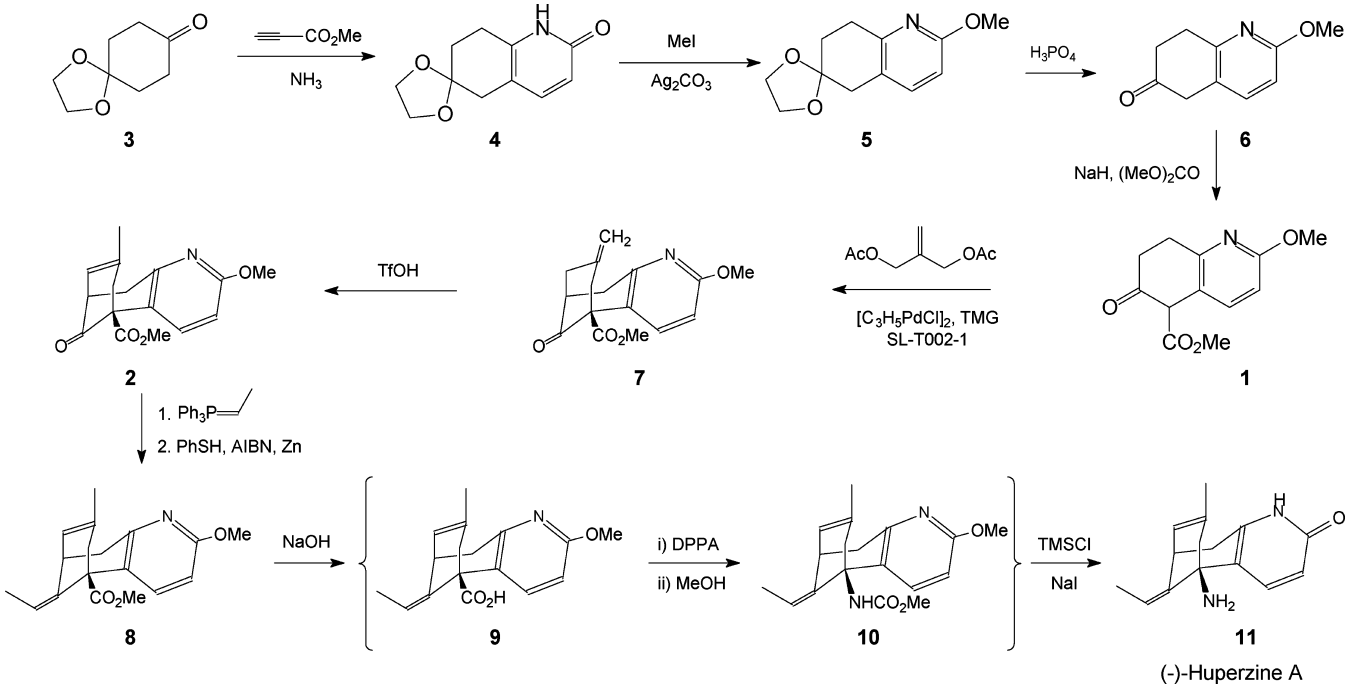
Scheme 4. Asymmetric palladium-catalysed bicycloannulation



Scheme 5. Synthetic route developed to (−)-huperzine A



followed by oxidation back to the acid.[13] Haudrechy et al. used the (1*R*,2*S*)-2-phenylcyclohexanol ester as chiral auxiliary and 1,3-diacetoxy-2-methylenepropane to perform the bicycloannulation. After further elaboration they determined the diastereomeric excess to be 92%. The chiral auxiliary was removed by reduction to give the alcohol with opposite stereochemistry to the natural huperzine configuration. Application of the (1*S*,2*R*)-2-phenylcyclohexanol ester chiral auxiliary should therefore lead to the desired alcohol intermediate.[14]

Primarily to facilitate the synthesis of huperzine A analogues, Camps et al. developed a different strategy for the synthesis of huperzine A, forming the bridged ring structure first, before elaborating the pyridine moiety.[15,16] However, this approach did not appear to offer any advantage in yield or potential for scale-up, and the separation of isomers proved difficult and required column chromatography.

To develop a process that could be performed safely and economically on an industrial scale we required a low-cost, high-yielding process, giving minimal byproduct or isomers so that bulk purification methods such as extraction and crystallization may be applied without the requirement for column chromatography. For this reason we decided to base the route on the improved procedure presented by Kozikowski,[10] combining the chiral bicycloannulation methodology of Terashima[12] and aspects of Qian's process, such as the modified Curtius reaction and ether cleavage methodology.[7] The process would require optimization and development to remove the column chromatography used in the literature.

■ RESULTS AND DISCUSSION

The chosen process consists of 11 chemical stages from commercially available raw materials as shown in Scheme 5. With minor modifications of Kozikowski's simplified procedure[8] for the preparation of the cyclohexylpyridinone **4**, we have performed this reaction in a 1400-L pressure vessel and obtained product of 98% purity without chromatography in

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43% yield with a simple workup involving distillation, filtration, and washing. The literature *O*-methylation method[9] utilised 2 equiv of silver carbonate and 10 equiv of methyl iodide in chloroform. Screening for improved conditions using more environmentally friendly solvents and methylating agents such as dimethyl sulfate tended to result in more *N*-methylated byproduct being formed. Alternative silver (oxide, acetate, trifluoroacetate, *p*-toluenesulfonate) and copper (oxide, (II) chloride) salts were also examined, but the results were not satisfactory. A phase-transfer method utilizing 1 equiv of silver carbonate, 3 equiv of methyl iodide in dichloromethane/aqueous sodium hydroxide, and 0.5 equiv of benzyltriethylammonium chloride as the phase transfer catalyst was found to be optimal,[17] giving 92% yield and 92% purity of compound **5** after separation of the organic layer and filtration through a bed of filter aid to remove the silver salts.

The ethylene ketal **5** was hydrolysed in dilute phosphoric acid to give, after neutralisation and extraction with ethyl acetate, the ketone **6** in 98% yield and 92% purity. Phosphoric acid was found to be superior for this hydrolysis compared to other acids investigated (hydrochloric, trifluoroacetic, sulphuric, nitric, acetic), giving a rapid hydrolysis without generation of significant impurities.[18]

Methoxycarbonylation of ketone **6** was conducted as described by Kozikowski,[9] with some improvements in economy. Dimethyl carbonate, used as solvent and reactant, was reduced from 43 to 30 volumes, and potassium hydride was substituted with sodium hydride and reduced from 4.0 to 1.2 equiv. Column chromatography was avoided in the workup, and the *β*-keto ester **1** was obtained in 65% yield and 95% purity by partitioning in hexane/ethyl acetate mixtures.

The asymmetric palladium-catalysed bicycloannulation of *β*-keto ester **1** to compound **7** was based on the work of Terashima.[12] In order to maximise the enantioselectivity of the reaction, a ligand screen was conducted by Solvias AG.[19] The Taniaphos ligand SL-T002-1 (Figure 1) was identified as a promising lead from a screen of 33 phosphine and diphosphine ligands. A secondary screen was then conducted using SL-T002-1 to optimise the reaction parameters of temperature, solvent, concentration, and catalyst loading. Using the optimised conditions, the desired annulation product **7** was obtained with 99% conversion and 84% ee, with a 20-fold reduction in catalyst and ligand loading compared to those of the published method. Chromatographic purification was again avoided in the workup, and recrystallisation from isopropyl alcohol furnished the desired product **7** in 73% overall yield and 99% ee.[20]

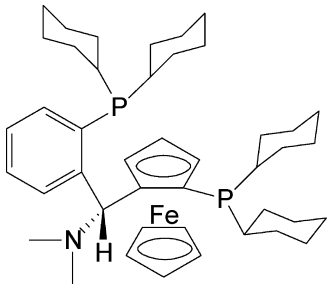

Figure 1. Taniaphos Ligand SL-T002-1.

The isomerisation of the olefin from exocyclic to endocyclic was conducted with trifluoromethanesulfonic acid in ethylene dichloride. Ethylene dichloride, although not considered to be a green solvent, was preferred for this reaction over 1,4-dioxane[10] as it gave a more facile isomerisation allowing the reaction to be conducted at room temperature over just 1 h, thus reducing emissions. The reaction in 1,4-dioxane required reflux temperature for an extended period of more than 5 h. The product was recovered from the organic layer after neutralisation with aqueous sodium bicarbonate and contained less than 0.1% of the exocyclic isomer. Recystallisation from heptanes gave the desired product **2** in 80% overall yield, with greater than 99% purity and greater than 99% ee. The process has been scaled to produce 2.7 kg of compound **2** of the aforementioned quality.

The Wittig reaction of ethylidenetriphenylphosphorane with compound (**8**) was conducted in dry THF at 0−2 °C. The workup, to avoid column chromatography, was to quench the reaction and solvent exchange the THF layer for toluene. The toluene solution was washed with water, extracted with hydrochloric acid, and the acidic extracts were neutralised to precipitate the crude product (**8**) free from phosphine impurities in 80% yield and 95% purity (as a mixture of *E*- and *Z*-isomers in a ∼1:3 ratio). Isomerisation of the crude product, to enhance the *E*-isomer ratio, was conducted in refluxing toluene in the presence of thiophenol and AIBN. During development, this reaction was found to give variable results, with thiophenol from some manufacturers being ineffective. It was discovered that addition of a catalytic quantity of fresh zinc dust initiated the isomerisation in all cases, even in reactions that had previously failed.[18] Zinc may be responsible for removing adventitious oxygen which would otherwise lead to oxidation of the thiol to disulfide, which in turn would inhibit alkene isomerisation. On completion of the reaction, the ratio of *E*- to *Z*-isomers was greater than 10. During the workup the toluene solution was washed with sodium hydroxide to remove thiophenol, and then the product was extracted with hydrochloric acid. The hydrochloric acid extract was washed with toluene and neutralised to precipitate the product. This was collected and crystallised from methanol to furnish the pure product **8** in 67% yield from intermediate **2** with an *E*- to *Z*-isomer ratio of greater than 100 and purity (*E* + *Z* isomers) of 99.4%.

The conversion of compound **8** into (−)-huperzine A (**11**) was conducted without isolation of the intermediates. The ester (**8**) was hydrolysed using aqueous sodium hydroxide in a mixture of methanol and tetrahydrofuran. The organic solvents were removed by vacuum distillation, and then the aqueous solution of the sodium salt of **9** was washed with dichloromethane to remove residual **8**. The batch was partially concentrated, acidified, and extracted with toluene to furnish a toluene solution of compound **9**. The toluene solution was dried by azeotropic distillation and converted to the azide by reaction with diphenylphosphorylazide (DPPA) in the presence of triethylamine. Curtius rearrangement to give the isocyanate was effected by heating, and upon completion, the residual DPPA was decomposed by addition of glacial acetic acid and brief reflux. The batch was then refluxed with methanol to convert the isocyanate to carbamate **10**. Methanol was removed by distillation, chasing with additional toluene, and then the batch was washed with aqueous sodium hydroxide and water (containing 5% methanol to prevent emulsion formation) to remove phosphate impurities. The excess water was removed by azeotropic distillation, and then the solvent was exchanged for acetonitrile by twice concentrating to the minimum stir level and diluting with acetonitrile. Trimethylsilyl iodide was

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generated in situ by addition of trimethylsilyl chloride and sodium iodide to the acetonitrile solution and the batch refluxed for 4 h to cleave the *O*-methyl group and furnish (−)-huperzine A (**11**). Workup was to cool, dilute with dichloromethane, and extract with dilute hydrochloric acid. The acid extract was adjusted to pH 9−10 by addition of sodium hydroxide and extracted with dichloromethane. The dichloromethane extracts were washed with dibasic sodium phosphate solution to remove silyl impurities and drummed up. Analysis of the dichloromethane solution indicated the desired product (**11**) was obtained in 82% physical yield from ester (**8**), with HPLC purity of 75%. The product contained one major impurity that was identified as *N*-methylated huperzine, with the methyl attached to the pyridone nitrogen. This had been observed as a minor impurity on small-scale reactions but increased unexpectedly upon scale-up. Due to the highly potent nature of huperzine A, the crystallisation and isolation of the final product was conducted in a glovebox in a dedicated 10-L reaction vessel and 1-L filter assembly. The dichloromethane solution of crude (−)-huperzine A was solvent exchanged to acetonitrile by twice concentrating to a low volume and dilution with acetonitrile. The product was crystallised from a 1:1 mixture of acetonitrile and distilled water, and repeated crystallisation gave (−)-huperzine A in 36% yield with 99.5% HPLC purity and greater than 99.9% ee.

After initial proof of concept of the route, several key areas have been improved in order to provide a safe and scalable process for multigram to kilogram quantities. During the course of the development we have increased concentrations to improve the throughput, replaced solvents and reagents with cheaper and safer alternatives where possible, and eliminated all column chromatography for isolation. Some stages were telescoped to improve the yield and therefore cost of the product. We also conducted Hazard Evaluation assessment of the use of azide chemistry for the Curtius reaction. This work consisted of thermal stability testing of DPPA, reaction calorimetry of the DPPA addition using a Mettler RC1 calorimeter, and thermal stability testing of the reaction mixture after the DPPA addition. In summary, DPPA was found to undergo strongly exothermic decomposition from temperatures of around 182 °C by differential scanning calorimetry (DSC), with an enthalpy value of 806 J g^{1-}. In further testing under more adiabatic conditions using an accelerating rate calorimeter (ARC) the onset temperature was determined as 163.1 °C, and the decomposition was shown to proceed with relatively high self-heat and pressure rates. As the batch reflux temperature is around 110 °C, the safety margin to the decomposition onset is approximately 50 °C, and this was felt to be sufficient to avoid being a major thermal hazard at the intended scale of operation. The addition of DPPA was studied in the RC1 and found to be only very mildly exothermic with a calculated adiabatic temperature rise of 1 °C. After the addition, batch thermal stability was tested by DSC, and two small exotherms were detected, the first of which occurred at 192.9 °C, and it was not thought likely that these would generate a major thermal hazard under the intended operating conditions.

■ CONCLUSION

A safe, scalable, and cost-effective process for manufacture of (−)-huperzine A has been developed. This process eliminates all of the chromatography isolation and telescopes stages where possible. A total of 275 g (−)-huperzine A of high chemical and optical purity has been manufactured in a cGMP environment. This process is capable of providing multikilograms of high-quality (−)-huperzine A for the pharmaceutical and nutraceutical markets.

■ EXPERIMENTAL SECTION

Instrumentation and Materials. *^{1}H NMR.* A Bruker 300 MHz instrument was used for compounds **1**−**7**, and a Jeol 400 MHz instrument was used for compounds **8**−**11**. Chemical shifts were reported as (δ) values in parts per million relative to tetramethylsilane. The following abbreviations were used: s, singlet; d, doublet; dd, doublet of doublets; m, multiplet; br s, broad signal.

HPLC Analytical Methods. *HPLC Method for Achiral Analysis for Compounds 1, 3, 4, 5, 6.* Column, Zorbax SB Phenyl (250 mm × 4.6 mm), 5 μm, wavelength 220 nm, flow rate 1.5 mL/min, column oven 40 °C; mobile phase A, 0.1% TFA/water; mobile phase B, 0.1% TFA/acetonitrile; run time 20 min; gradient profile (% mobile phase A given with the remainder mobile phase B), 90% with a linear gradient to 40% over 20 min and then a linear gradient to 90% over 2 min and re-equilibrate for 8 min. Retention times for the compounds observed were: **3**, t_R 5.0 min; **4**, t_R 5.9 min; **5**, t_R 5.3 min; **6**, t_R 4.0 min; **1**, t_R 9.4 min.

HPLC Method for Chiral Analysis for Compounds 1, 7, 2. Column, Chiralcel OD-H (250 mm × 4.6 mm), 5 μm, wavelength 230 nm, flow rate 1.0 mL/min, column oven 25 °C; mobile phase *n*-hexane/isopropanol, 97:3; run time 20 min. Retention times for the compounds observed were: **1**, t_R 4.7 min; **7** (undesired enantiomer), t_R 7.7 min; **7** (desired enantiomer), t_R 10.2 min; **2** (undesired enantiomer), t_R 6.8 min; **2** (desired enantiomer), t_R 8.7 min.

*HPLC Method for Achiral Analysis for Compound **8**.* Column, Phenomenex Luna C18 (150 mm × 3 mm), 3 μm, wavelength 220 nm, flow rate 1.0 mL/min, column oven 30 °C; mobile phase A, 0.1% TFA/water; mobile phase B, 0.1% TFA/acetonitrile; run time 25 min; gradient profile (% mobile phase A given with the remainder mobile phase B), 70% for 10 min followed by a linear gradient to 20% over 5 min, hold for 5 min and then change to 70% and re-equilibrate for 5 min. Retention times for the compounds observed were: **8 (*E*-isomer)**, t_R 9.9 min; **8 (*Z*-isomer)**, t_R 12.3 min.

*HPLC Method for Achiral Analysis for Compounds **8**−**11**.* Column, Phenomenex Luna C18 (50 mm × 2 mm), 3 μm, wavelength 220 nm, flow rate 1.0 mL/min, column oven 40 °C; mobile phase A, 0.1% TFA/water; mobile phase B, 0.1% TFA/acetonitrile; run time 25 min; gradient profile (% mobile phase A given with the remainder mobile phase B), 100% linear gradient to 5% over 8 min, then change to 100% and re-equilibrate for 2 min. Retention times for the compounds observed were: **8 (*E*-isomer)**, t_R 4.48 min; **9**, t_R 3.57 min; **10**, t_R 3.33 min; **11**, t_R 2.18 min.

*HPLC Method for Chiral Analysis for Compound **11**.* Column, Chiralcel OD-H (250 mm × 4.6 mm), 5 μm, wavelength 308 nm, flow rate 1.0 mL/min, column oven 30 °C; mobile phase *n*-heptane/ethanol/methanol, 85:13:2; run time 20 min. Retention times: **(+)-Huperzine A**, t_R 7.3 min; **(−)-Huperzine A**, t_R 9.5 min.

1′,5′,7′,8′-Tetrahydro-spiro[1,3-dioxolane-2,6′(2′*H*)-quinolin]-2′one (4). 1,4-Cyclohexanedione monoethylene ketal (**3**) (13.9 kg, 1.0 equiv), methyl propiolate (16.68 L, 2.1 equiva), isopropyl alcohol (222 L), and ammoniacal methanol (7−8 N, 146 L) were charged to a pressure vessel and sealed. The reaction mixture was heated to 135−140 °C

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with constant stirring and maintained for 9 h. The mass was allowed to cool to 20−25 °C, and the solvent was distilled under vacuum at 40−45 °C until 70−75% of the solvent was removed. The solution was cooled to 0−5 °C, stirred for 2 h, and filtered. The filtered solid was washed with portions of cold isopropyl alcohol (0−5 °C) and dried under vacuum to yield the pyridine of formula **4** (7.9 kg, 43% yield, HPLC purity >98%). ^{1}H NMR ($CDCl_3$) δ 1.90 (2H, t, J = 6.6 Hz), δ 2.67 (2H, s), δ 2.90 (2H, t, J = 6.6 Hz), δ 3.98 (4H, s), δ 6.35 (1H, d, J = 9.3 Hz), δ 7.12 (1H, d, J = 9.3 Hz).

7′,8′-Dihydro-2′methoxyspiro[1,3-dioxolane-2,6′(5′*H*)-quinoline] (5). The pyridone of formula **4** (17 kg, 1.0 equiv) was combined with dichloromethane (170 L), 1 M sodium hydroxide solution (90.6 L, 1.1 equiv) and benzyltriethylammonium chloride (9.35 kg, 0.5 equiv) and stirred at 20−25 °C for 15 min. Silver carbonate (22.6 kg, 1.0 equiv) was added followed by iodomethane (15.3 L, 3.0 equiv) at 20−25 °C and stirred for 5 h at the same temperature. An in-process analysis by HPLC showed <0.1% starting material (**4**). Workup was carried out by layer separation followed by filtration through filter aid and distillation of the dichloromethane layer to yield the *O*-methylated compound of formula **5** (16.5 kg, 92% yield, HPLC purity of 92%). ^{1}H NMR ($CDCl_3$) δ 2.01 (2H, t, J = 7.5 Hz), δ 2.90 (2H, s), δ 3.01 (2H, t, J = 7.5 Hz), δ 3.89 (3H, s), δ 4.04 (4H, s), δ 6.52 (1H, d, J = 6 Hz), δ 7.25 (1H, d, J = 6 Hz).

2-Methoxy-7,8-dihydro-5*H*-quinolin-6-one (6). The *O*-methylated compound **5** (16 kg) was combined with water (144 L) and phosphoric acid (88%, 64 L) and stirred at 20−25 °C for complete dissolution. The solution was slowly heated to 75−80 °C and maintained at that temperature for 3 h, whereupon HPLC analysis indicated <1% of the starting material (**5**) remained. The reaction mass was cooled to 5−10 °C and the pH adjusted to 7.0−7.5 by addition of 59% sodium hydroxide solution. The resulting solution was then extracted with ethyl acetate (three times with 64 L each time) and distilled to yield the ketone compound of formula **6** as a brown solid (12.64 kg, 98% yield, HPLC purity of 92%). ^{1}H NMR ($CDCl_3$) δ 2.65 (2H, t, J = 6 Hz), δ 3.15 (2H, t, J = 6 Hz), δ 3.51 (2H, s), δ 3.93 (3H, s), δ 6.61 (1H, d, J = 6 Hz), δ 7.31 (1H, d, J = 6 Hz).

5,6,7,8-Tetrahydro-2-methoxy-6-oxo-5-quinolinecarboxylic acid methyl ester (1). Sodium hydride (50%, 3.42 kg, 1.2 equivalents) and dimethyl carbonate (157.5 L) were heated to 85−90 °C under a nitrogen atmosphere, and the ketone of formula **6** (10.5 kg, 1.0 equivalent) diluted with dimethyl carbonate (157.5 L) was added over a period of 1.5 h. After addition, the reaction mixture was maintained at the same temperature for approximately 30 min. A sample for HPLC showed <1% of the ketone starting material (**6**) remained. Dimethyl carbonate was then distilled off completely under vacuum at 40−50 °C and the residue was cooled to 10−15 °C. Chilled water was added and the residue dissolved completely. The pH was adjusted to 2−3 by addition of 5 M HCl (~20 L) and extraction was performed with ethyl acetate (once with 42 L and then twice with 21 L). The solvent was distilled off completely to give the crude β-keto ester of formula **1**. The crude ester was dissolved in 105 L 5% ethyl acetate/hexane mixture by heating at 60−65 °C, and the resulting mixture was allowed to cool to 20−25 °C and filtered. The filtrate was evaporated to dryness under vacuum at 40−45 °C, and the resulting residue was stirred with 31.5 L hexane for 30 min at 20−25 °C. The product was collected by filtration, washed with portions of hexane, and dried under vacuum at 25−30 °C to give the desired product of formula **1** (9.06 kg, 65% yield, HPLC purity of 95%). ^{1}H NMR ($CDCl_3$) δ 2.63 (2H, t, J = 7.8 Hz), 2.94 (2H, t, J = 7.8 Hz), 3.90 (3H, s), 3.91 (3H, s), 6.57 (1H, d, J = 8.7 Hz), 7.90 (1H, d, J = 8.7 Hz), 13.16 (1H, s).

(+)-5-Methoxy-11-methylene-13-oxo-6-aza-tricyclo-[7.3.1.0^{2,7}]trideca-2(7),3,5-triene-1-carboxylic Acid Methyl Ester (7). The chiral ligand SL-T002-1 (42.6 g, 2 mol %), allyl palladium chloride dimer (11.2 g, 1 mol %), and acetone (2.8 L) were combined and stirred at 20−25 °C for 1 h under a nitrogen atmosphere. To the mixture was added 1,3-diacetoxy-2-methylenepropane (0.524 L, 1.0 equiv) and acetone (0.70 L), and the new mixture was stirred and maintained at the same temperature for 1 h. A mixture of the purified keto ester of formula **1** (0.70 kg, 1.0 equiv), 1,1,3,3-tetramethylguanidine (0.84 L, 2.2 equiv), and acetone (3.5 L) was added to the above solution in lots over a period of 30 min at 20−25 °C. The resulting mixture was then stirred at the same temperature for 1 h under a nitrogen atmosphere. At this time, a sample for HPLC indicated <1% of the starting material (**1**) remained. Acetone was then distilled off under vacuum at 40−45 °C, and the residues were passed through a bed of silica gel, eluting with hexane and ethyl acetate mixtures to remove the catalyst and ligand. The solvent was then distilled completely to give the crude product of formula (**7**) (0.70 kg, 82% yield and HPLC purity of 70%). This crude material (0.70 kg) was stirred with isopropyl alcohol (2.8 L) at 20−25 °C for 30 min. The resulting solid was filtered and washed with isopropyl alcohol (0.35 L) and the material dried under vacuum at 35−40 °C to give the pure product as a white solid (0.385 kg, 45% yield and HPLC purity of 99.8%). ^{1}H NMR ($CDCl_3$) δ 2.55−2.64 (2H, m), 2.77−2.83 (1H, m), 2.95−2.99 (1H, m), 3.08−3.20 (2H, m), 3.46 (1H, dd, J = 18.0, 6 Hz), 3.82 (3H, s), 3.89 (3H, s), 4.50 (1H, d, J = 3.0 Hz), 4.84 (1H, d, J = 3.0 Hz), 6.59 (1H, d, J = 9 Hz), 6.99 (1H, d, J = 9 Hz).

(+)-5-Methoxy-11-methyl-13-oxo-6-aza-tricyclo-[7.3.1.0^{2,7}]trideca-2(7),3,5,10-tetraene-1-carboxylic Acid Methyl Ester (2). To a solution of the purified compound of formula **7** (0.586 kg) in ethylene dichloride (5.86 L) was added trifluoromethanesulfonic acid (0.586 L) at 20−25 °C, and the solution was stirred for 1 h at the same temperature. An in-process analysis by HPLC showed <0.1% starting material (**7**) at this time. The reaction mixture was cooled to 10−15 °C and neutralised with 10% sodium bicarbonate solution (8.79 L). The layers were separated, and the aqueous layer was extracted with additional ethylene dichloride (1.81 L). The organic layers were combined, dried over anhydrous sodium sulphate, and distilled to dryness under vacuum at 40−45 °C to give the crude olefinic ester of formula **2**. The crude material was stirred with heptanes (mixed isomers, 0.525 L) at 90−95 °C for 30 min and hot filtered at 80−85 °C. After cooling to 20−25 °C, the solution was allowed to rest without agitation for 2 h. The supernatant liquid was decanted, and the product crystals were isolated by stirring with heptanes (0.586 L), followed by filtration and washing with portions of heptanes (0.586 L). The product was dried at 35−40 °C to give compound of formula **2** (0.469 kg, 80% yield, HPLC purity of >99%). ^{1}H NMR ($CDCl_3$) δ 1.63 (3H, s), 2.55 (1H, d, J = 17 Hz), 3.15−3.21 (2H, m), 3.36−3.44 (2H, m), 3.76 (3H, s), 3.91 (3H, s), 5.42−5.45 (1H, m), 6.63 (1H, d, J = 9 Hz), 7.12 (1H, d, J = 9 Hz).

(*E*)-(+)-11-Ethylidene-9,10-dihydro-2-methoxy-7-methyl-5,9-methanocyclo-octa[*b*]pyridine-5(6*H*)-carboxylic Acid Methyl Ester (8). Ethyltriphenylphosphonium

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bromide (2.9 kg, 3 equiv) was suspended in dry tetrahydrofuran (12.5 L) in a 50-L reaction vessel and stirred under nitrogen for 15 min at 20−25 °C. 2.5 M *n*-Hexyllithium (2.2 L, 2 equiv) was added over approximately 30 min, maintaining the batch temperature at 20−30 °C. The reaction mixture was maintained at this temperature and stirred for an additional 30 min before cooling to 2−5 °C. Compound **2** (0.75 kg, 1.0 equiv), dissolved in tetrahydrofuran (3 L), was added slowly to the ylide solution, maintaining the batch temperature at 2−5 °C, and was washed in with further tetrahydrofuran (4 L). The reaction mixture was stirred at 2−5 °C for 1 h, then warmed to 20−25 °C over 1 h, and maintained at that temperature for 4 h. The batch temperature was then reduced to 2−5 °C and the reaction quenched by slow addition of deionised water (10 L). The batch temperature was readjusted to 20−25 °C, the agitator stopped, and the lower, aqueous layer, removed. The batch was then reduced to ∼5 L by vacuum distillation and toluene (20 L) added to the reaction vessel. Distillation was continued until the batch volume was reduced to ∼15 L and toluene (20 L) added. The batch volume was again reduced to ∼15 L by distillation, and then the vacuum was released and deionised water (20 L) added. The batch was stirred, then allowed to settle, and the aqueous layer removed. The water wash was repeated two more times, and then the toluene layer was extracted with 6 M hydrochloric acid (first 8 L, then second with 7 L). The vessel was drained, and the acidic extracts were recharged, washing with fresh toluene (three times, each 15 L) to remove triphenylphosphine oxide. The purified acidic extracts were recharged to the vessel and neutralised to pH 6−8 by addition of 6 M sodium hydroxide solution (∼15 L), maintaining the batch temperature at 20−25 °C. The product slurry was stirred out at 20−25 °C for 3 h and then collected by filtration, washed with deionised water (12 L) and dried on the filter under a flow of air until the water content was less that 5% w/w. The crude product **8** was obtained as a white solid (0.63 kg, 80% yield, HPLC purity (*E* + *Z* isomers) 95%). Three batches of crude material were combined (1.80 kg), dissolved in toluene (20 L) and dried by azeotropic distillation at atmospheric pressure in a 50-L reaction vessel until the water content was <0.2% w/w. The solution was then cooled to 20−25 °C and fresh zinc dust (3.5 g, 0.01 equiv) added. Thiophenol (5.7 kg, 8.7 equiv) and toluene (∼1.5 L) were charged to the vessel header (combined volume 7 L), and the batch temperature was adjusted to 75−85 °C. A portion of the thiophenol solution (0.5 L) was charged to the vessel, together with a portion of AIBN (0.19 kg), via a seed-pot, and the reaction stirred for 1 h, maintaining the same temperature. The addition of portions of thiophenol solution (0.5 L) and AIBN (0.19 kg) was repeated 13 times in hourly intervals until all the thiophenol solution and AIBN (2.66 kg, 2.7 equiv) had been charged and the reaction stirred out for a final hour at 75−85 °C. The batch was cooled to 20−25 °C and washed with 3 M sodium hydroxide solution (three times, each 10 L). The product was then extracted from the toluene layer with 6 M hydrochloric acid (three times, each 8 L), and the vessel emptied. The acidic extracts were combined, returned to the vessel, and washed with toluene (three times, each 8 L). The acid extract was then neutralised (pH 6−8) by slow addition of 6 M hydrochloric acid (∼24 L), maintaining the batch temperature at 20−25 °C. The product slurry was stirred out at 20−25 °C for 3 h and then collected by filtration, washed with deionised water (12 L), and dried on the filter under a flow of air until the water content was less that 5% w/w. The crude product **8** was obtained as a white solid (1.4 kg, 78% yield, HPLC purity 85.3% *E*-isomer, 7.8% *Z*-isomer). The crude product (1.4 kg) was recharged to the vessel with methanol (14 L) and the mixture heated to reflux to give a clear solution. The solution was cooled to −5 to 5 °C over ∼2 h and that temperature maintained for a further 2 h to ensure complete crystallisation. The product was collected by vacuum filtration, washed with methanol (2 L), and dried to give compound **8** as a white solid (1.2 kg, 67% yield, HPLC purity 98.5% *E*-isomer; 0.9% *Z*-isomer). ^{1}H NMR ($CDCl_3$) δ 1.52 (3H, s), 1.68 (3H, d, J = 6.6 Hz), 2.13 (1H, d, J = 17.0 Hz), 2.83 (1H, dd, J = 17.0, 1.8 Hz), 3.04 (2H, dd, J = 17.0, 5.0 Hz), 3.57 (1H, m), 3.72 (3H, s), 3.86 (3H, s), 5.03 (1H, q, J = 6.6 Hz), 5.38 (1H, m), 6.51 (1H, d, J = 8.5 Hz), 7.06 (1H, d, J = 8.5 Hz).

(−)-Huperzine A (11). To a solution of compound **8** (1.2 kg, 1.0 equiv) in a mixture of tetrahydrofuran (7 L) and methanol (11 L) in a 50-L reaction vessel, was added a solution of 6 M sodium hydroxide (7 L). With agitation, the batch was brought to reflux (∼65 °C) and refluxed for 40 h, after which time there was less than 1% of compound **8** remaining. The batch was concentrated under reduced pressure (∼8 L residual volume) to remove the majority of the organic solvents and diluted with deionised water (24 L). The batch was then extracted with dichloromethane (twice, each 12 L) to remove residual starting material and then concentrated under vacuum again (∼20 L residual volume) to ensure removal of organic solvents. A solution of 6 M hydrochloric acid (∼7 L) was added at 20−25 °C to bring the pH to 2−5, and the mixture was extracted four times with toluene (two times with 10 L each, and two times with 8 L each). The combined toluene extracts were dried by azeotropic distillation until the water content was less than 0.1% w/w. To the toluene solution of compound **9** was charged diphenylphosphoryl azide (DPPA, 1.7 L) and triethylamine (1.5 L), and the reaction mixture stirred at 20−25 °C for 30 min before increasing the temperature to reflux and refluxing for 2 h to complete the Curtius rearrangement. The batch was cooled to 20−25 °C, acetic acid (0.3 L) charged to consume excess DPPA, and the batch reheated to reflux for 1 h to decompose any azides present. The batch was cooled again to 20−25 °C, and analysis confirmed the absence of DPPA. The batch was concentrated by vacuum distillation (∼18 L residual volume), diluted with methanol (18 L), and refluxed for 12 h to convert the isocyanate intermediate into the carbamate **10**. In-process analysis at this time indicated 1.5% isocyanate remaining. The batch was concentrated by vacuum distillation to remove methanol (∼18 L residual volume), diluted with toluene (18 L), and successively washed with 1.2 M sodium hydroxide solution (25 L) and deionised water containing 5% v/v methanol (twice, each 20 L) to give a toluene solution of carbamate **10** containing 6% phosphate impurities. The batch was then concentrated to near dryness (∼3 L residual volume) and diluted with acetonitrile (25 L). The distillation was repeated, concentrating to near dryness (∼3 L residual volume) and diluting with acetonitrile (25 L) to give a solution of carbamate **10** in acetonitrile. To this solution was charged sodium iodide (3.0 kg) and trimethylsilyl chloride (2.4 L), maintaining the batch temperature at 20−25 °C. The batch was then heated to reflux and refluxed for 4 h before cooling again to 20−25 °C. Dichloromethane (20 L) was charged to the reaction vessel, followed by slow addition of 1.5 M hydrochloric acid (8 L), maintaining the batch temperature at 20−25 °C. The dichloromethane layer was separated and the aqueous layer washed twice more with dichloromethane (each

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18 L). To the acidic solution containing (−)-huperzine A was then charged dichloromethane (15 L) and the batch was slowly basified to pH 9−10 by addition of 6 M sodium hydroxide solution (∼4.5 L). The dichloromethane layer was separated and the aqueous layer extracted twice more with dichloromethane (each 10 L), each time adjusting the aqueous phase pH to 9−10 if necessary by addition of 6 M hydrochloric acid or 6 M sodium hydroxide. The dichloromethane extracts were combined, washed twice with 10% dibasic sodium phosphate solution (each 15 L), and concentrated (residual volume ∼10 L) to give a solution of crude (−)-huperzine A (**11**) in dichloromethane (0.75 kg, 82% yield, HPLC purity 75%). The dichloromethane solutions of (−)-huperzine A were charged in portions via an inline 0.5 μm filter to a clean, dedicated, 10 L reaction vessel and concentrated by vacuum distillation (residual volume ∼2 L). Acetonitrile (4 L) was added via the in-line filter and the solution concentrated by vacuum distillation at atmospheric pressure (residual volume ∼2 L). To ensure removal of dichloromethane, this process was repeated, charging acetonitrile (4 L) via the in-line filter and concentrating by vacuum distillation at atmospheric pressure (residual volume ∼4 L). Deionised water (4 L) was charged to the vessel via the inline filter, and the mixture heated to reflux to give a clear solution. The solution was slowly cooled over 3 h to 0 ± 5 °C and maintained at that temperature for 2 h. The product was collected by vacuum filtration and deliquored, washing the vessel and filter cake with 1:1 acetonitrile/deionised water (1 L) and dried to give (−)-huperzine A (0.30 kg, 40% yield, HPLC purity 97.0%). The product was recrystallised in an identical manner from 1:1 acetonitrile/deionised water (4 L) to furnish (−)-huperzine A (0.275 kg, 37% yield, HPLC purity 99.5%, >99.5% ee (the opposite enantiomer was not detected)). ^{1}H NMR ($CDCl_3$) δ 1.35 (2H, br s), 1.55 (3H, s), 1.68 (3H, d, J = 6.7 Hz), 2.10 (1H, d, J = 17.0 Hz), 2.16 (1H, d, J = 17.0 Hz), 2.75 (1H, dd, J = 16.9, 1.5 Hz), 2.90 (1H, dd, J = 16.9, 5.1 Hz), 3.61 (1H, m), 5.41 (1H, d, J = 5.0 Hz), 5.49 (1H, q, J = 6.7 Hz), 6.42 (1H, d, J = 9.4 Hz), 7.90 (1H, d, J = 9.4 Hz).

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Notes

The authors declare no competing financial interest.

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