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Development of a Multi Kilogram-Scale, Tandem Cyclopropanation Ring-Expansion Reaction *En Route* to Hedgehog Antagonist IPI-926.

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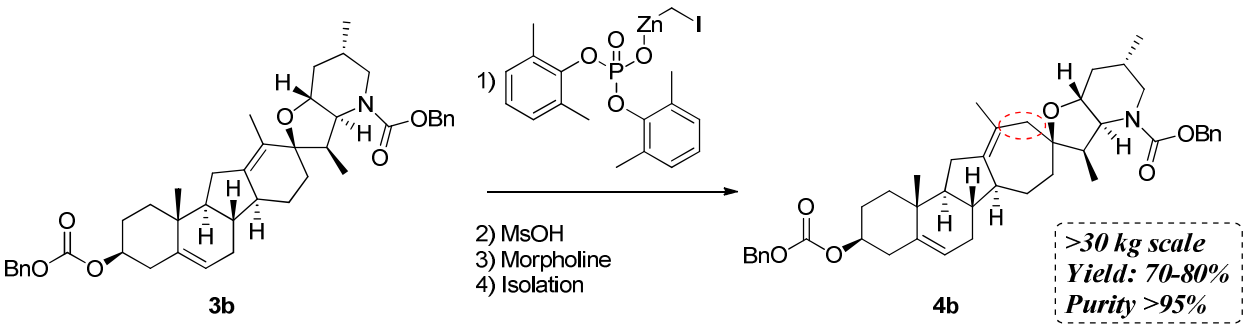

3b **4b**

Keyword: Cyclopropanation; Simmons-Smith; cyclopamine; cycloheptane; cycloheptene; ring expansion; carbenoid; homocyclopamine; carbocation rearrangement; iodomethylzinc phosphate saridegib; IPI-926

ABSTRACT

The formation of the D-homocyclopamine ring system in IPI-926 is the key step in its semi-synthesis and proceeds *via* a chemoselective cyclopropanation followed by a stereoselective acid-catalyzed carbocation rearrangement. In order to perform large scale cyclopropanation reactions, we developed new iodomethylzinc bis(aryl)phosphate reagents that were found to be both effective and safe. These soluble reagents can be prepared under mild conditions and are stable during the course of the reaction. Importantly, they have favorable energetics relative to other cyclopropanating agents such as $EtZnCH_2I$. Herein, we describe the process optimization studies that led to successful large scale production of the D-homocyclopamine core necessary for IPI-926.

INTRODUCTION

Malignant activation of the Hedgehog pathway is implicated in multiple cancer settings.[1] Cyclopamine (**1**), a plant steroidal *Veratrum* alkaloid, is an antagonist of the Hedgehog pathway and has been used as a biological tool to interrogate the pathway.[2] However, cyclopamine's modest inhibitory activity on the pathway and poor pharmaceutical properties make it subpar as a therapeutic agent. A few years ago, our group reported D-homo analogs of cyclopamine with improved stability, potency and/or solubility.[3] This work led to the identification of a novel, semi-synthetic analog of cyclopamine (IPI-926, **2**) with potent activity on the Hedgehog pathway, significant anti-tumor activity, and attractive pharmaceutical properties.[4] IPI-926 was evaluated in phase I and II clinical trials.

The clinical development of IPI-926 required the manufacture of multi-kilogram quantities of drug substance. The formation of the D-homocyclopamine ring system in IPI-926 is the crux in its semi-synthesis and proceeds *via* a chemoselective cyclopropanation followed by stereoselective acid-catalyzed rearrangement. In order to perform large scale cyclopropanation reactions, we developed new iodomethylzinc bis(aryl)phosphate reagents that were found to be both very effective and safe. Herein, we describe the process optimization studies that led to successful large scale production of the D-homocyclopamine core necessary for IPI-926.

METHODS AND RESULTS

The overall synthetic scheme for the production of IPI-926 is captured in Scheme 1.[5] Cyclopamine (**1**) is first converted to either bis-protected cyclopamine analog **3a** or **3b**, which can be used as substrates for the cyclopropanation ring-expansion chemistry to give the seven-

membered D-ring compounds **4a** or **4b**, respectively. After evaluation of various protecting groups, we found N-carbamates and O-esters/carbonates **3a** and **3b** to be superior for a number of reasons, including ease of synthesis/removal, crystallinity/stability of intermediates, and compatibility with Zn-carbenoid species. While **3a** was a substrate of choice during the early optimization phase of the cyclopropanation reaction, bis-Cbz substrate **3b** proved to be the preferred intermediate due to streamlined downstream processing to D-homocyclopamine **5**. Oppenauer oxidation of **5** into **6** sets the stage for stereoselective reduction of the enone,[6] methanesulfonamide installation,[7] and finally salting of the piperidine ring[8] to produce the active pharmaceutical ingredient **2**. This paper focuses primarily on the D-ring homologation chemistry whereas the optimization of the large-scale production of **2** from **6** will be the topics of other publications.

Early Optimization

The gram-scale synthesis of **6**, involving a two-step Simmons-Smith cyclopropanation and Lewis acid-mediated ring expansion, was reported by our discovery group.[9] At the time, the key Simmons-Smith cyclopropanation step suffered from multiple issues that hampered scalability: 1) requirement for large excess of Zn carbenoid (~10 eq) mostly due to decomposition of the reagents and subsequent work-up challenges; 2) decomposition of the starting material; 3) heterogeneous reaction mixture; and 4) moderate overall yield. Most importantly, there were considerable safety concerns with these types of Zn carbenoid species[10] with reports of explosions. We explored alternative cyclopropanating reagents such as Al carbenoids,[11] transition metal catalyzed diazomethane decomposition,[12] as well as haloform-derived carbenes.[13] However, none of these alternatives gave the desired transformation in any practical yield on our substrates. Consequently, various Zn carbenoids were evaluated, particularly

Furukawa's conditions (Et_2Zn, CH_2I_2),[14] Shi's conditions[15] (Et_2Zn, CH_2I_2, TFA), Merck's modifications of Shi's protocol (Et_2Zn, CH_2I_2, TCA),[16] and Charette's iodomethylzinc phosphate reagents.[17] All of these conditions converted substrate **3a** into **4a** relatively cleanly, but required >5 equivalents of reagent to obtain useful conversions. Substrates **3a** and **3b** proved to be quite sensitive to Lewis acidic conditions due to the cleavage of the spirotetrahydrofuran C-O bond and subsequent aromatization of the D-ring (Scheme 2).[18] Consequently, a delicate balance between substrate stability and reactivity of the cyclopropanating agent needed to be achieved. At the onset, two attractive features of the iodomethylzinc phosphate reagents sparked our interest in pursuing further optimizations: 1) relative stability of the reagent since iodomethylzinc phosphate could potentially be isolated as solid;[17] 2) ability to tune the reactivity and physicochemical properties of the Zn carbenoid by modifying the phosphate ligand. The major drawback of the known iodomethylzinc diphenylphosphate reagent was the poor solubility of the carbenoid in solvents which dissolve **3a** or **3b**, resulting in a heterogeneous mixtures. We pursued an optimization effort aiming to develop a volume efficient, homogeneous cyclopropanation conditions by modification of the phosphate portion of the reagent. A number of bis(aryl) and bis(alkyl) phosphoric acids were thus tested in the cyclopropanation reaction using **3a** as substrate (Table 1). Generally, iodomethylzinc bis(aryl)phosphates had better reactivity toward our substrate than iodomethylzinc bis(alkyl)phosphates. The nature of the substitution on the phenyl was explored to modulate the physicochemical properties such as a) solubility of both the phosphate and the resulting carbenoid in dichloromethane, b) the ease of removal after the reaction through basic aqueous washes, and c) crystalline properties of the phosphate raw material. Gratifyingly, all these criteria were met by bis(2,6-dimethylphenyl)phosphate (Table 1, entry 2), which was selected for further investigations.

Although not commercially available, bis(2,6-dimethylphenyl)phosphoric acid can be readily prepared on large scale from reaction of 2,6-dimethylphenol with phosphoryl chloride and recrystallized to high purity.

Both substrates **3a** and **3b** performed very well under these representative cyclopropanating conditions (see Experimental section for all details): neat Et_2Zn (3.05 eq) is charged to a cooled solution of DCM (5 volumes, below -10 °C *vide infra*), then the mixture is cooled to -20 °C before addition of a solution of bis(2,6-dimethylphenyl)phosphoric acid (3.1 eq) in DCM (11.5 volumes). The solution is then warmed to 0 °C before the addition of substrates **3a** or **3b** (1 eq) in DCM (3 volumes). Finally, CH_2I_2 (3.1 eq) in DCM (0.2 volume) is charged to initiate the formation of the carbenoid and cyclopropanation reaction. The homogenous mixture is stirred at 30 °C and typically proceeds in >90% conversion after 5 h.

Calorimetry and safety assessment

With optimized cyclopropanating conditions for substrates **3a** and **3b** in hand, we first measured the energetics of the various components of the reaction using differential scanning calorimetry (DSC). A solution of Et_2Zn in anhydrous DCM recorded an onset temperature of decomposition at 83 °C by DSC (ramp rate of 2 °C/min) releasing energy of 222 J/g of total mass. Solutions of Et_2Zn/DCM/bis(2,6-dimethylphenyl)phosphoric acid and Et_2Zn/DCM/ bis(2,6-dimethylphenyl)-phosphoric acid/CH_2I_2 recorded no significant decomposition event up to 160 °C and 120 °C, respectively. Based on these results, it was determined that the preparation of Et_2Zn/DCM solution could be safely operated at -10 °C which provides a safety window of 90 °C, whereas the cyclopropanating agent can be handled safely at room temperature.

We then measured the heat generation of the reaction performed under Furukawa's conditions compared with the optimized conditions using SuperCRC calorimeter (Omnical). For Furukawa's conditions, the order of addition was Et_2Zn, **3b**, and then CH_2I_2. As expected, the addition of CH_2I_2 to Et_2Zn generated a substantial exotherm ranging from 734 – 870 kJ/mol of of **3b** (N=2) (Figure 2A). Under adiabatic conditions, this exotherm would translate to an increase in the internal temperature averaging 85 °C. For the optimized conditions, the order of addition was Et_2Zn, bis(2,6-dimethylphenyl)phosphoric acid, **3b**, and then CH_2I_2. The addition of phosphate to diethylzinc generated an exotherm ranging from 489 – 537 kJ/mol of **3b** (N=2), whereas an exotherm (224 – 360 kJ/mol of **3b**, N=2) was observed during the addition of CH_2I_2 (Figure 2B). From a temperature control standpoint, these exotherms were deemed controllable since they would correspond to adiabatic temperature rise of 25 °C and 11 °C, respectively. The energy released during the generation of the optimized cyclopropanating reagent was not insignificant, but is broken down into two separate events. The acid/base reaction between diethylzinc and the phosphate was the most exothermic event, which could be controlled by the rate of phosphate addition. Of note, nitrogen sweeps were used to prevent accumulation of ethane, which was then carried for scrubbing, dilution, or burning. The energy associated with the alkyl transfer between $(ArO)_2P(O)OZnEt$ and CH_2I_2 was significantly lower compared to Et_2Zn. The formation of $EtZnCH_2I$ and $Zn(CH_2I)_2$ is typically accompanied with significant decomposition and side reactions, which could also pose a safety concern.[10a] Consequently, the iodomethylzinc bis(2,6-dimethylphenyl)phosphate carbenoids were deemed safer, less energetic, alternatives to the Furukawa process and thus chosen for scale-up.

One-pot ring expansion protocol

The initial gram-scale synthetic route involved a two-step procedure to convert **3a** into **4a**.[5, 9] After cyclopropanation, the cyclopropyl analog of **3a** was isolated and then treated with BF_3-OEt_2 to achieve the ring expansion to the seven-membered D-ring analog. After careful analysis of other products generated under various cyclopropanating conditions, it was determined that ring-expanded products were minor by-products under those conditions. This observation pointed to the possibility of a one-pot procedure in which either *in situ* generated ZnX_2 Lewis acids or organic acids could be used directly at the end of the cyclopropanation reaction to cause the desired ring expansion. To this end, we found the addition of $Zn(OTf)_2$, and various alkyl or aryl sulfonic acids to readily effect the ring expansion of **4a** or **4b** *in situ* after the cyclopropanation was complete. Ultimately, stoichiometric methanesulfonic acid (MsOH) was chosen based on conversion, purity profile, and ease of removal. Importantly, the addition of MsOH to the cyclopropanation reaction mixture generated only a modest exotherm (174 kJ/mol of **3b**). However, the reaction required cooling (-55 °C) during this addition to minimize the formation of a cyclic carbamate impurity (**8a** and **8b**, Scheme 3) when the F-ring nitrogen was protected with the preferred Cbz protecting group.

An isolation and purification procedure for **4a** and **4b** coming from the cyclopropanation ring expansion reaction was developed. A morpholine quench was implemented to mop up alkylating by-products (L-$ZnCH_2I$, CH_3I, EtI, and HI) and avoid occupational exposure to harmful and potentially genotoxic species. Zinc salts, the aryl phosphate, and MsOH were removed by aqueous washes (2N HCl followed by 4.8 wt% Na_2CO_3, 4.8 wt% Na_2SO_3 and 4.8 wt% NaCl). The reaction mixture was then concentrated and solvent exchanged for the crystallization of **4b** from MeOH/IPA.

Kilogram scale cyclopropanation ring expansion: reproducibility and key parameters

With an efficient and safe one-pot procedure in hand, a number of kilogram scale batches were run to assess the performance and reproducibility of the conditions (Figure 3, entries 1-6). From 1 kg to 10 kg, the cyclopropanation ring-expansion procedure proved to be very reproducible in terms of yield (70-80%) and purity (95-97%) of **4b**. Unfortunately, as the scale increased, the cyclopropanation step began to stall frequently (Figure 3, entries 7-14). Although the stalled reaction could be salvaged by charging fresh cyclopropanating reagent, this operation increased plant time and complexity of the fixed equipment set up considerably. Root cause analysis and follow-up experiments determined that an aging period after the phosphate addition was necessary to promote a more robust reagent preparation and a faster cyclopropanation reaction. Consequently, a two-hour hold period (before adding **3b**) was implemented for subsequent campaigns (Figure 3, entries 12-19). In these eight campaigns, the cyclopropanation step did not stall except for one instance when CH_2I_2 was undercharged due to a line priming issue. This issue was addressed with the use of a stainless steel tank fitted onto a balance to ensure the proper weight was delivered after the system had been primed and rinsed.

A tight operating range of ± 0.5 weight % of Et_2Zn, phosphate, and CH_2I_2 was preferred to ensure only the desired iodomethylzinc bis(aryl)phosphates reagent was formed and for consistent reactivity. This tight control was a challenge in metering of pyrophoric neat diethylzinc, and the order of addition (Et_2Zn, phosphate, **3b**, then CH_2I_2) exacerbated the problem since one has to carefully control the stoichiometry while the substrate is already committed to the reaction. We thus tested an alternative order of addition (Et_2Zn, phosphate, CH_2I_2, then **3b**) and found that the cyclopropanating reagent could be prepared ahead of time and used up to 19 hours later or charged directly into the substrate. Importantly, the new addition

order could withstand charge fluctuations of ± 5 wt% without adversely affecting the outcome of the cyclopropanation reaction (Figure 4). Employing the new order of addition, the cyclopropanation progressed with as little as 2.5 equivalents of active reagent whereas the reaction did not reach completion when 2.0 equivalents were used (Figure 5A). Interestingly, the rate of cyclopropanation under the new order of addition was significantly better than the old order even when suboptimal conditions (2.0 equivalents) were used (Figure 5B). Calorimetry measurements of the CH_2I_2 addition to the $(ArO)_2P(O)OZnEt$ in the absence of substrate **3b** demonstrated a comparable exotherm to earlier work in the presence of substrate.[19] The subsequent addition of **3b** in solution to the reaction mixture was minimally exothermic (0.37 kJ/mol of **3b**). Based on the results of the reaction calorimetry, the reverse addition (generation of the cyclopropanating reagent by addition of diiodomethane before the dosage of **3b**) was suitable to carry out the reaction in a safe manner. As a result, this process change was implemented and performed on 33 kg scale (Figure 3, entries 20-26). Overall, this last change improved the robustness of the one-pot cyclopropanation ring expansion process and offered greater flexibility in the plant.

CONCLUSION

In conclusion, a robust and scalable one-pot cyclopropanation ring expansion reaction applicable to the D-ring homologation of cyclopamine has been developed. We found that iodomethylzinc bis(aryl)phosphate carbenoids could be used safely and efficiently on multi-kilogram scale. Aside from typical key parameters such as oxygen/moisture sensitivity, quality of starting materials, and physical properties of the phosphate, achieving the right stoichiometry and order of addition of reagents were particularly important. Once the cyclopropanating agent

is formed with the right ratio of reagents, the stability is excellent and can support smooth conversion of structurally complex and very acid labile allylic ether **3b**.

The robustness of this reaction and the stability of the iodomethyl bis(2,6-dimethylphenyl)phosphate carbenoid allow for its use in radiosynthesis. To demonstrate this, substrate **3a** was successfully converted to ^{14}C-**4a** in acceptable yield and good purity using the cyclopropanation/ring expansion conditions described above (Scheme 4). Isotopically ^{14}C-labeled diiodomethane has been used for Eschenmoser salt formation[20] and dialkylation,[21] while Simmons-Smith cyclopropanation reactions have been reported using ^{13}C-labeled[22] and ^{3}H-diiodomethane.[23] To our knowledge, a cyclopropanation reaction using ^{14}C-CH_2I_2 has not been previously reported.

Although we have not looked at extending the scope of this reaction beyond cyclopamine substrates, these reagents have attractive properties (soluble, stable, safe, and easy to remove) and expand the pharmaceutical chemists' toolbox.

Experimental section

General methods. Commercial reagents and solvents were used as received without further purification or drying. All experiments involving water-sensitive compounds were carried out under argon and scrupulously dry conditions, using commercially available anhydrous solvents. Thin-layer chromatography was performed on glass-backed precoated Merck silica gel (60 F254) plates, and compounds were visualized using UV light, ceric ammonium molybdate or 2% aqueous potassium permanganate solution. Silica gel column chromatography was carried out

using Merck silica gel 60. Flash chromatography was run using silica gel (200-400 mesh) from Sorbent Technologies. The purity of tested compounds was determined by analytical liquid chromatography (HPLC) performed a number of different methods captured in the supporting information. ^{1}H-NMR spectra were recorded on a Bruker 400 spectrometer (400 MHz). Chemical shifts are reported in ppm with the solvent resonance as the internal standard ($CHCl_3$: δ 7.26). Data are reported as follows: chemical shift, integration, multiplicity (s=singlet, d=doublet, dd=doublet of doublet, ddd=doublet of doublet of doublet, t=triplet, q=quartet, br=broad, m=multiplet), and coupling constants (Hz). ^{13}C-NMR spectra were recorded on a Bruker 400 spectrometer (100 MHz) with complete proton decoupling. Chemical shifts are reported in ppm with the solvent as the internal reference ($CDCl_3$: δ 77.16). Electro-spray positive ionization mass spectrometry was performed on samples using a reversed phase HPLC-MS system. The system was equipped with a Diode Array UV detector, a single quadrupole mass spectrometer, and utilized the above mentioned reversed-phase HPLC gradient method.

Cyclopamine extraction and isolation: Cyclopamine was obtained from *Veratrum californicum* harvested in August. The samples were air-dried and milled to fine particles and extracted according to A) methods adapted from Keelers and co-workers,[24] B) supercritical fluid extraction ,[25] or C) liquid-liquid extraction.[26] Cyclopamine can be recrystallized to purity in hot MeOH (30-40 volumes). This protocol typically delivers 1-4 g of cyclopamine per kg of dry biomass. Typical RP-HPLC (Method A) purity of cyclopamine obtained in this process exceeds 95 a/a%.

Synthesis of Cbz-Obt (3-Cbz-benzotriazole-1-oxide): 1-Hydroxybenzotriazole hydrate (70.9 kg, 463.4 mol, 1 eq.), dichloromethane (876 kg), and triethylamine (55.6 kg, 549.5 mol, 1.2 eq)

are added to a reactor. The content of the reactor is cooled to 0 to 5 °C. Benzyl chloroformate (87.0 kg, 510 mol, 1.1 eq) is added. The reaction solution is stirred for 4 hours, after which, it is sampled for thin layer chromatography. Upon the completion of the reaction, water (192 kg) and dichloromethane (600 kg) are added and the reaction is warmed to 15 °C. The quenched reaction is settled and the aqueous layer is removed. The organic layer is washed twice with water (192 kg for each wash) to remove any triethylamine hydrochloride. The washed organic layer is concentrated to a slurry and solvent exchanged to ethyl acetate until the dichloromethane content in the distillate is below 5%. The material is then concentrated to a thick slurry and filtered. The product collected is washed with ethyl acetate and dried under vacuum. 100.9 kg of 3-Cbz-benzotriaole-1-oxide is produced at 81% yield. Mp = 107 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.20 (d, J = 8.4 Hz, 1H), 8.00 (dt, J = 8.4, 1.0 Hz, 1H), 7.75 (ddd, J = 8.4, 7.2, 1.1 Hz, 1H), 7.58 – 7.47 (m, 3H), 7.47 – 7.37 (m, 3H), 5.55 (s, 2H); ^{13}C NMR (101 MHz, CDCl3) δ 147.42, 133.86, 133.52, 132.87, 132.82, 129.35, 129.00, 128.91, 126.45, 115.89, 115.23, 70.70; LRMS: *m/z* = 270.4 $[M + H]^+$; HPLC purity (method B): 99.9 a/a%.

Synthesis of Bis(2,6-dimethylphenyl)phosphoric acid: To a 500-gallon reactor, pyridine (620 kg) and 2,6-dimethylphenol (256 kg, 2096 mol, 1.98 eq) are charged. Phosphoryl chloride (162 kg, 1057 mol, 1 eq) is added over 2-3 hours in order to maintain the internal temperature between 65 and 70 °C. The resulting suspension is stirred for 1-2 hours at the same temperature and then cooled to 8-12 °C. The reaction is sampled for in-process-control by HPLC (acceptance criterion: 2,6-dimethylphenol < 5%). Upon the reaction completion, the reaction mixture is filtered and the filter cake is washed with 23.5 kg of pyridine. The combined filtrate is stirred at 14-18 °C. Water (1249 L) is added over 1-2 hours and the resulting suspension is warmed to 20-

25 °C. Dichloromethane (420 kg) is then added. The mixture is settled and the phases are separated. The aqueous layer is washed twice with dichloromethane (550 kg for each wash). The combined organic layers are washed with aqueous HCl solution (1059 kg conc. HCl in 984 L of water) three times and water (519 L) once. Water (757 L) is then added to the organic layer, followed by 227 L of 20% aqueous NaOH solution to adjust the pH to 13-13.5. The mixture is stirred for about an hour and the phases are separated. The aqueous layer is acidified to pH 0.5-1.0 by the addition of 130-140 kg of 37% HCl solution. The resulting off-white suspension is stirred for 90 min. The supernatant is decanted and water (719 L) is added and stirred well for about an hour. The decanting, water addition, and stirring are repeated. After the final decanting of supernatant, wet white solid is left in the reactor for recrystallization. *First Recrystallization:* 2-Propanol (790.5 kg) is charged to the wet white solid in the reactor. Water (1500 L) is added and the whole mixture is transferred to a 2000-gallon reactor. The mixture is heated at 78-82 °C for 70 minutes to give a hazy solution. The solution is cooled to 8-12 °C in about 6 hours and stirred for 3-5 hours. The suspension is filtered in a centrifuge filter and the filter cake is washed with a 2:3 mixture of 2-propanol and water (382 L in two portions). The wet product is dried further to afford 245 kg of off-white solid of 99.96% purity by HPLC. *Second recrystallization:* To the product from the last recrystallization, 2018 kg of cyclohexane is added. The mixture is heated to 78-82 °C and a slightly hazy solution is formed. The solution is hot-filtered through a pressure filter with dicalite dish pack and heated with stream. The filtrate is cooled to 8-12 °C in about 6 hours. The resulting white slurry is held at the same temperature for at least 40 hours. The material is filtered in a centrifuge filter. The filter cake is washed with 150 kg of cold cyclohexane and then spun for 50-70 minutes. The wet product is dried further at 40-50 °C under vacuum for at least 24 hours. Crystallized bis(2,6-dimethylphenyl)phosphoric acid (230 kg) is

obtained at 72% yield. Mp = 143 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.88 (s, 1H), 6.97 (s, 6H), 2.18 (d, J = 1.1 Hz, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.25, 148.17, 130.52, 129.10, 129.08, 125.37, 125.35, 16.91; ^{31}P NMR (162 MHz, $CDCl_3$) δ -7.87; LRMS: *m/z* = 307.5 $[M + H]^+$; Purity method C: >99.9 a/a%

Compound 3a: Recrystallized cyclopamine **1** (14.1 g, 34.0 mmol, 1 eq) is dissolved in anhydrous DCM (70 mL) and anhydrous MeOH (29 mL). The clear solution is cooled, and triethylamine (10.4 g, 102.7 mmol, 3 eq) followed by benzyl chloroformate (6.20 g, 36.3 mmol, 1.1 eq) is added. After the addition is complete, the solution is stirred in the ice bath for 30 min. Three portions of benzyl chloroformate (3x0.35 g, 3.46 mmol, 0.03 eq) are added over the 3 h. The reaction is slowly quenched with water (71 mL), while maintaining the temperature below 20 °C. The mixture is stirred for 15 min before the layers are settled and separated. The organic layer is dried over sodium sulfate and filtered. The combined filtrate is buffered with anhydrous pyridine (30 mL), concentrated, and solvent exchanged with additional anhydrous pyridine (43 mL) and concentrated. The solution of the compound in pyridine (43 mL) is further diluted with additional anhydrous pyridine (85 mL). Trimethylacetyl chloride (8.3 g, 68.7 mmol, 2 eq) is added slowly to the reaction mixture, and the reaction is heated to 45 °C. The reaction is stirred at 45 °C for 30 min. The reaction is cooled and quenched by the addition of anhydrous MeOH (4.5 mL). The quenched reaction mixture is stirred at 23 °C for 40 min and then diluted with toluene (97 mL) and is treated sequentially with water (35 mL) and a 10 wt% aqueous sodium carbonate solution (100 mL). After vigorous stirring, the layers are separated and the organic layer is washed twice with water (2x 100 mL), dried over sodium sulfate, and filtered. The filter cake is rinsed with toluene (49 mL) and discarded. The combined filtrates are concentrated, and

solvent exchanged with concentration to toluene (145 mL) and further concentrating to dryness. The product is recrystallized from toluene and heptane. The crystalline product is isolated by suction filtration, washed with cold heptane and dried to a constant weight to afford 15.1 g of **3a**. ^{1}H NMR (400 MHz, Pyridine-d_5) δ 7.61 – 7.54 (m, 2H), 7.47 – 7.37 (m, 2H), 7.39 – 7.30 (m, 1H), 5.41 (s, 2H), 5.41 – 5.34 (m, 1H), 4.84 (ddd, J = 11.3, 11.1, 5.6 Hz, 1H), 3.79 (dd, J = 12.9, 4.2 Hz, 1H), 3.71 – 3.58 (m, 1H), 3.40 (dd, J = 10.3, 7.2 Hz, 1H), 3.15 (p, J = 7.2, 7.2, 7.0, 7.0 Hz, 1H), 2.96 (dd, J = 12.9, 8.3 Hz, 1H), 2.54 (ddd, J = 13.3, 4.9, 2.1 Hz, 1H), 2.40 (br t, J = 12.5 Hz, 1H), 2.26 – 2.02 (m, 4H), 2.01 –1.86 (m, 2H), 1.85 – 1.79 (m, 3H), 1.82 – 1.60 (m, 6H), 1.59 – 1.45 (m, 2H), 1.39 (ddd, J = 11.2, 10.8, 8.7 Hz, 1H), 1.29 (d, J = 1.5 Hz, 9H), 1.30 – 1.16 (m, 2H), 1.17 – 1.04 (m, 4H), 0.88 (d, J = 7.2 Hz, 3H), 0.87 (s, 3H); ^{13}C NMR (101 MHz, Pyridine-d_5) δ 178.02, 158.60, 142.84, 141.30, 138.08, 129.40 (2C), 128.93 (2C), 128.91, 127.88, 123.47, 85.10, 74.19, 73.50, 67.77, 64.43, 52.57, 51.98, 49.68, 42.64, 42.57, 39.20, 38.49, 38.38, 37.26, 36.13, 33.32, 31.74, 29.60, 29.20, 28.19, 27.74 (3C), 25.57, 20.65, 18.95, 14.35, 11.31; LRMS: m/z = 630.7 $[M + H]^+$

Compound 3b: A suitable sized jacketed reactor, equipped with a temperature control unit, distillation set-up, and a receiving vessel is flushed with nitrogen, then charged with cyclopamine (11 kg, 1 wt, 1 eq), n-propyl acetate (116.6 kg, 10.6 wt, 12 volumes), DMAP (1.63 kg, 0.148 wt, 0.5 eq) and triethylamine (8.12 kg, 0.738 wt or 1.016 volumes, 3 eq). The mixture is stirred at 20 ± 5 °C, then Cbz-Obt (16.56 kg, 1.505 wt, 2.3 equiv.) is charged in three equal portions (≈ 0.5 wt each) while maintaining the reaction at 20 ± 5 °C. After each charge, the reaction is held for a minimum of 30 minutes. Once the charge is complete, the reaction is stirred for a minimum of 3 hours at 20 ± 5 °C. Once the reaction is complete, the suspension is heated to

50 ± 5 °C to obtain a homogeneous solution. Methanol (139.7 kg, 12.7 wt, 16 volumes) is slowly added to the reaction while maintaining the reaction temperature at 50 ± 5 °C. The slurry is then cooled to 0 ± 5 °C over 5 hours, then held at 0 ± 5 °C for ≥ 12 hours. The solids are collected by vacuum filtration, followed by rinsing with methanol (49.5 kg, 4.5 wt, 5.7 volumes) and with heptane (33 kg, 3.0 wt, 4.4 volumes). The solid is dried with warm nitrogen (40 °C) for ≥ 12 hours, then under vacuum at 40 °C for ≥ 8 hours. This procedure delivered 14.7-16.6 kg of **3b** (N=10, 81.4%-91.8% yield). Mp = 152 °C; ^{1}H NMR (400 MHz, Pyridine-d_5) δ 7.56 (ddd, J = 15.7, 7.6, 1.5 Hz, 4H), 7.47 – 7.30 (m, 6H), 5.41 (s, 2H), 5.37 (s, 2H), 5.36 – 5.32 (m, 1H), 4.93 (d, J = 1.7 Hz, 2H), 4.71 (tt, J = 11.4, 4.7 Hz, 1H), 3.79 (dd, J = 13.0, 4.2 Hz, 1H), 3.63 (td, J = 10.9, 4.3 Hz, 1H), 3.39 (dd, J = 10.3, 7.2 Hz, 1H), 3.15 (t, J = 7.3 Hz, 1H), 2.95 (dd, J = 12.9, 8.2 Hz, 1H), 2.60 (ddd, J = 13.2, 5.0, 2.1 Hz, 1H), 2.48 – 2.37 (m, 1H), 2.24 – 1.92 (m, 6H), 1.82 (s, 3H), 1.80 – 1.45 (m, 7H), 1.36 (td, J = 11.2, 8.5 Hz, 1H), 1.28 – 1.04 (m, 5H), 0.88 (d, J = 6.8 Hz, 3H), 0.83 (s, 3H); ^{13}C NMR (101 MHz, Pyridine-d_5) δ 158.60, 155.47, 142.77, 140.88, 138.08, 136.93, 129.43, 129.40, 129.20, 129.10, 128.92, 127.91, 123.82, 85.08, 78.46, 73.49, 69.89, 67.77, 64.42, 52.50, 51.97, 49.68, 42.63, 42.50, 38.37, 38.29, 37.15, 36.12, 33.32, 31.70, 29.56, 29.19, 28.24, 25.54, 20.64, 18.83, 14.33, 11.30; LRMS: *m/z* = 680.7 $[M + H]^+$; HPLC purity (Method D) of 99.6-99.7 a/a %.

Compound 4a: Bis(2,6-dimethylphenyl)phosphoric acid (10.65 g, 34.8 mmol, 3.1 eq) is dried by concentration from anhydrous dichloromethane (42 mL) and held under a nitrogen atmosphere. The phosphoric acid is then redissolved in anhydrous dichloromethane (110 mL). In a separate flask, a solution of neat diethylzinc (4.17 g, 34.0 mmol, 3.0 eq) in anhydrous dichloromethane (35 mL) is prepared and cooled to -25 °C. The phosphate solution is slowly

transferred to the vessel containing the diethylzinc solution over 1 h, maintaining the temperature at or below -10 °C. The clear ethylzinc phosphate solution is warmed to 0 °C and stirred for 15 min. Diiodomethane (9.25 g, 34.5 mmoles, 3.0 eq) is slowly added to the ethylzinc phosphate solution, maintaining the reaction temperature between 0 and 5 °C. After the addition is complete, the zinc carbenoid solution is stirred for an additional 20 min. In a separate flask, compound **3a** (7.20 g, 11.4 mmol, 1 eq) is dissolved in anhydrous dichloromethane (36 mL) and transferred to the reaction flask. After the addition is complete, the ice bath is removed and the reaction mixture is allowed to warm to rt. After 6 h the contents of the flask are cooled to -53 °C. A solution of methanesulfonic acid (3.38 g, 35.2 mmol, 3.1 eq) in anhydrous dichloromethane (3 mL) is added, maintaining the reaction temperature below -45 °C. After 10 min, morpholine (20 g, 230 mmol, 20 eq) is added to the reaction mixture, maintaining the reaction temperature below -40 °C. The reaction is allowed to warm to rt overnight. The morpholine salts are removed by filtration and the filter cake rinsed with dichloromethane (22 mL). The combined filtrates are washed with 2N aqueous hydrochloric acid (2x140 mL), 5% aqueous sodium bicarbonate (140 mL), 5% aqueous sodium bicarbonate (70 mL) and 5% aqueous sodium bisulfite (70 mL), and brine (140 mL). The organic layer is dried over magnesium sulfate and filtered. Without going to dryness, the dichloromethane solution is concentrated and solvent exchanged with methanol (280 mL). The suspension are chilled with an ice bath and stirred for 40 minutes. The solids are isolated by filtration, washed twice with cold methanol (2x25 mL), and dried to a constant weight to afford 5.94 g of **4a**. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.41 – 7.27 (m, 5H), 5.40 (dd, J = 4.8, 2.2 Hz, 1H), 5.20 – 5.07 (m, 2H), 4.58 (dddd, J = 11.3, 11.3, 4.8, 4.8 Hz, 1H), 3.82 (dd, J = 12.9, 4.3 Hz, 1H), 3.63 (ddd, J = 11.2, 10.1, 4.2 Hz, 1H), 3.09 (dd, J = 10.2, 6.2 Hz, 1H), 2.69 – 2.53 (m, 2H), 2.47 (d, J = 14.0 Hz, 1H), 2.42 – 2.32 (m, 1H), 2.33 – 2.08 (m, 5H), 2.06 –

1.91 (m, 3H), 1.86 – 1.69 (m, 4H), 1.68 (s, 3H), 1.66 – 1.51 (m, 3H), 1.50 – 1.36 (m, 3H), 1.32 – 1.19 (m, 2H), 1.18 (s, 9H), 0.99 (s, 3H) 0.98 (d, J = 8.3 Hz, 3H), 0.91 (d, J = 7.0 Hz, 3H).

Compound 4b: *Equipment*: Reaction Vessel A 2500 L (for diethylzinc solution and reaction) is an appropriately sized, cryogenic capable jacketed reactor equipped with a temperature control unit, a nitrogen/vacuum port, a charge port, an overhead stirrer and condenser. Reagent Vessel B (for bis(2,6-dimethylphenyl)phosphoric acid [BDMPP] solution) is an appropriately sized jacketed reactor equipped with a temperature control unit, a nitrogen/ vacuum port, a charge port, an overhead stirrer and condenser. Reagent Vessel C (for starting material compound **3b** solution) is an appropriately sized jacketed reactor equipped with a temperature control unit, a nitrogen/ vacuum port, a charge port, an overhead stirrer and condenser. Reagent Vessel D (for methanesulfonic acid solution) is an appropriately sized jacketed reactor equipped with a temperature control unit, a nitrogen/ vacuum port, a charge port, an overhead stirrer and condenser. *Reaction set-up*: Vessel B is charged with bis(2,6-dimethylphenyl)phosphoric acid (45.46 kg, 1.39 wt, 3.10 eq), then sealed and pressurized with nitrogen to 1.7 bar. The pressure is released and a vacuum to 0.3 bar is pulled. This step is repeated twice, then the reactor is back filled with nitrogen to 1 bar. Using residual vacuum and utilizing anhydrous techniques, charge anhydrous dichloromethane (400 L, 13 volumes) to vessel B. The batch is agitated at 20 ± 5 °C until all the solid has dissolved. Vessel B is sealed and pressurized with nitrogen to 1.7 bar. The pressure is released to a minimum of 0.3 bar and held for ≥ 5 minutes, then nitrogen is back filled to 1 bar. This step is repeated three more times. The diethylzinc cylinder is connected to the transfer manifold and purged accordingly (see supporting information). The charge manifold is connected to vessel A via a transfer line employing Swagelok fittings, and also to a cylinder of

anhydrous dichloromethane, and a nitrogen source. A nitrogen sweep (2 – 5 psi) is applied through the charge manifold, transfer line and reaction vessel A for a minimum of 12 hours. Using residual vacuum and utilizing anhydrous techniques, anhydrous dichloromethane (500 L, 15 volumes) is charged to vessel A, which is then sealed and pressurized with nitrogen to 1.7 bar. The pressure is released and a vacuum is pulled to 0.3 bar. This step is repeated twice, then finished with nitrogen back fill to 1 bar. The batch is agitated under nitrogen, then cooled to -10 ± 5 °C. Using low pressure nitrogen, diethylzinc (18.03 kg, 0.55 wt, 3.05 eq) charged into vessel A. The manifold and transfer line are rinsed with anhydrous dichloromethane three times, the rinses (30 L) directed into the reaction (vessel A). After each rinse the lines are flushed with nitrogen for ≥ 2 minutes. The phosphate solution is slowly transfer from vessel B into the reaction (vessel A), while maintaining the temperature ≤ 5 °C under nitrogen. This transfer typically takes 4 hours and must not exceed 7 hours. Diiodomethane is charged (39.75 kg, 1.22 wt, 3.10 eq) to the reaction (vessel A), while maintaining the temperature ≤ -10 ± 5 °C. Vessel C is charged with compound **3b** (32.55 kg, 1 wt, 1.0 eq), then pressurized with nitrogen to 1.7 bar. The pressure is released and a vacuum is pulled to 0.3 bar. This step is repeated twice, then finished with a nitrogen back fill to 1 bar. Using residual vacuum and utilizing anhydrous techniques, anhydrous dichloromethane is charged (65 L, 2 volumes) to vessel C and the material is agitated at 20 ± 5 °C until it dissolves. The dichloromethane solution of compound **3b** is transferred from vessel C into the reaction (vessel A), while maintaining the temperature ≤ -10 ± 5 °C with stirring. Using residual vacuum and utilizing anhydrous techniques, vessel C is rinsed with anhydrous dichloromethane (32 L, 1.33 wt, 1 volume). Once the transfer is complete, the batch is warmed to 7.5 ± 2.5 °C and held with a nitrogen purge to the scrubber for 1 hour in order to allow dissolved ethane to vent off. The batch is then warmed to 30 ± 5 °C while

monitoring the internal pressure monitored every 5 °C. If the reactor pressure increases by 1 bar above atmospheric pressure, suspend the warm up and vent the reactor with a nitrogen purge to the scrubber over 1 hour. The batch is agitated at 30 ± 5 °C for ≥ 4 hours. After the reaction is found to be complete, the batch is cooled to - 60 ± 5 °C. *Ring-expansion:* Using residual vacuum methanesulfonic acid (13.9 kg, 0.424 wt, 3.0 eq) is charged to vessel D. Using residual vacuum and utilizing anhydrous techniques, anhydrous dichloromethane (55.6 kg, 1.72 wt, 1.3 volumes) is charged to vessel D. When the batch in vessel A reaches ≤ - 55 °C, the methanesulfonic acid solution in vessel D is transferred to the batch (vessel A), while maintaining the temperature at ≤ -50 °C. Using residual vacuum and utilizing anhydrous techniques, anhydrous dichloromethane is transferred (11.7 kg, 0.36 wt, 0.27 volumes) to vessel D, then the rinse is transferred to the batch (vessel A). *Quench*: Once the ring expansion reaction is complete, morpholine (42.1 kg, 1.28 wt, 1.29 volumes, 10 eq) is charged to the batch (vessel A), while maintaining the temperature at ≤ - 35 °C. The batch (vessel A) is then warmed to 20 °C and held for ≥ 1 hour. The batch is washed with an aqueous solution of 2N hydrochloric acid (336 kg, 10.3 wt, 10 volumes, 13.6 eq) for ≥ 60 min at 20 – 25 °C. The agitation is stopped and the organic layer (bottom layer) is separated from aqueous layer (top layer). The organic layer is transferred back into the reactor (vessel A) and the washing step with 2N hydrochloric acid (336 kg, 10.3 wt, 10 volumes, 13.6 eq) is repeated for ≥ 15 min at 20 – 25 °C. The organic layer is washed with an aqueous solution of 4.8 wt% sodium carbonate (338 kg, 10.5 wt, 10 volumes, 3.2 eq) for ≥ 15 min at 20 – 25 °C. The agitation is stopped, and the organic layer (bottom layer) is separated from aqueous layer (top layer). The organic layer is transferred back into the reactor (vessel A). The batch is washed with an aqueous solution of 4.8 wt% sodium sulfite (339.4 kg, 10.5 wt, 10 volumes, 2.7 eq) for ≥ 15 min at 20 – 25 °C. The agitation is stopped and the organic layer

(bottom layer) is separated from aqueous layer (top layer). The organic layer is transferred back into the reactor (vessel A). The batch is washed with purified water (326 kg, 10.5 wt, 10 volumes, 5.9 eq) for ≥ 15 min at 20 – 25 °C. The agitation is stopped, and the organic layer (bottom layer) is separated from aqueous layer (top layer). The organic layer is polished filtered and transferred into a 630 L reactor. The filtrate is distilled under vacuum to 2 ± 0.25 volumes, while maintaining the batch temperature at ≤ 35 °C during the distillation. To the concentrated filtrate is charged 2-propanol (97.7 kg, 3.0 wt, 3.82 volumes) and the batch is agitated at 20 ± 5 °C until the solution is homogeneous. Methanol (49.0 kg, 1.5 wt, 1.90 volumes) is then charged into the batch over ≥ 10 minutes at 20 ± 5 °C. The mixture is agitated for 30 ± 5 minutes at 20 ± 5 °C to initiate nucleation. Then, more methanol (179.2 kg, 5.5 wt, 6.95 volumes) is charged over 45 ± 15 minutes at 20 ± 5 °C. The batch is agitated at 20 ± 5 °C for ≥ 4 hours, then at 0 ± 5 °C for ≥ 4 hours. The solid is filtered off over a 30 micron nylon filter cloth and de-liquored with nitrogen. The solid is washed with methanol (130.4 kg, 4 wt, 5.1 volumes), soaked for ≥ 10 minutes, then de-liquored with nitrogen. The solid is washed with heptane (130 kg, 4 wt, 5.8 volumes), soaked for ≥ 10 minutes, then de-liquored with nitrogen. The solid is dried with nitrogen for ≥ 4 hours, then warm nitrogen (40 °C) for ≥ 12 hours while periodically stirring the filter cake. The solid is dried under vacuum at 40 °C for ≥ 8 hours while periodically stirring the filter cake. This procedure delivered 18.3-26.7 kg of **4b** (N=7 on 33 kg scale, range 55-80% yield). Mp = 148 °C; ^{1}H NMR (400 MHz, Pyridine-d_5) δ 7.63 – 7.50 (m, 4H), 7.47 – 7.30 (m, 6H), 5.48 – 5.32 (m, 5H), 4.93 (s, 3H), 4.69 (tt, J = 11.3, 4.7 Hz, 1H), 3.95 (dd, J = 12.8, 4.3 Hz, 1H), 3.64 (td, J = 10.8, 4.1 Hz, 1H), 3.24 (dd, J = 10.1, 6.1 Hz, 1H), 2.88 (m, 1H), 2.70 – 2.56 (m, 2H), 2.52 (d, J = 14.1 Hz, 1H), 2.42 (td, J = 12.4, 2.9 Hz, 1H), 2.23 (dtd, J = 14.5, 6.7, 6.1, 3.2 Hz, 2H), 2.16 – 1.92 (m, 5H), 1.90 – 1.53 (m, 7H), 1.47 – 1.28 (m, 2H), 1.26 – 1.00 (m, 6H),

0.90 – 0.77 (m, 6H); ^{13}C NMR (101 MHz, Pyridine-d_5) δ 158.20, 155.46, 141.67, 140.71, 138.10, 136.94, 129.43, 129.41, 129.20, 129.10, 129.02, 128.91, 124.58, 82.42, 78.53, 75.70, 69.87, 67.72, 63.06, 53.29, 52.75, 50.67, 50.46, 46.86, 44.37, 38.42, 38.38, 38.34, 38.14, 37.37, 32.42, 31.16, 29.37, 29.28, 28.24, 24.55, 19.81, 18.95, 11.18; LRMS: *m/z* = 694.7 $[M + H]^+$; HPLC purity (Method D) range of 95.6-97.2 a/a %.

Compound **5** from **4a**: In a round bottom flask, compound **4a** (11.67 g, 18.1 mmol, 1 eq) and 20% palladium hydroxide on wet carbon (2.40 g, 1.71 mmol, 0.09 eq) are placed under a nitrogen atmosphere and diluted with EtOAc (115 mL) and toluene (60 mL). The solution is degassed with nitrogen (3x) with evacuation/purge cycles, and the process is repeated for hydrogen. The suspension is vigorously stirred at 22 °C for 1.5 h. The hydrogen atmosphere is replaced with nitrogen. Ethylenediamine (0.57 g, 9.5 mmol, 0.52 eq) is added to the reaction, and the resulting mixture stirred for 20 min. The solution is filtered under nitrogen, and the filtrate is washed with a 2% (wt/wt) aqueous solution of ethylenediamine (125 mL) then water (130 mL), and then dried over sodium sulfate. The drying agent is removed by filtration and the filtrate is concentrated to dryness under vacuum. The remaining solids are chased with toluene (2x55 mL) on the rotary evaporator and the resulting material used without further purification in the next step. The material from the previous step is dissolved in anhydrous dichloromethane (26 mL). The resulting clear solution is added to a 1 M solution of DIBAL in dichloromethane (65 mL, 65 mmol, 3.6 eq) while maintaining the reaction temperature between -10 and -25 °C. After 30 min the reaction is quenched with acetone (13 mL), maintaining the reaction temperature at or below 0 °C. After stirring the quenched reaction mixture for 20 min, it is added in portions to a flask containing a cold, stirred solution of 20% (wt/wt) aqueous Rochelle salt (200 mL). The resulting

gelatinous suspension is stirred at 23 °C for 15 h. After stirring, the clean layers are separated and the aqueous layer back extracted with dichloromethane (30 mL). The combined organic layers are washed with water (60 mL) and dried over sodium sulfate. The drying agent is removed by filtration and discarded. The filtrate is concentrated under vacuum and solvent exchanged to toluene (225 mL added in portions). The resulting solution is further concentrated to a suspension (50 mL) and diluted with heptane (115 mL). The resulting mixture is heated until turning homogeneous (92 °C). The solution is cooled slowly over 12 h to 15 °C, and then held for an additional 16 h. The crystalline product is isolated by suction filtration, washed with heptane (2x75 mL) and dried to a constant weight to afford 7.70 g of **5**.

Compound **5** from **4b**: An size-appropriate reaction vessel, equipped with temperature controller unit, a condenser, a nitrogen/vacuum port, a paddle-type stirrer, and a hydrogen inlet, is flushed with nitrogen then charged with **4b** (25.3 kg, 1 wt, 1.0 eq) and 5% Pd/C (Johnson Matthey type A503038-5, (9.3 kg, 0.368 wt, 0.06 eq of Pd metal) 50% wet catalyst). The vessel is evacuated and back fill with nitrogen once. To the reaction vessel is charged tetrahydrofuran (263 kg, 11.7 volumes or 10.4 wt; HPLC solvent grade inhibited w/ BHT) and deionized water (32.9 kg, 1.3 volumes or 1.3 wt). The batch is stirred and the system is evacuated the system until solvent is seen dripping from the condenser (pressure should be about -0.8 bar), and then back fill with nitrogen. This process is repeated four times, then back fill with hydrogen. A hydrogen pressure of 1 ± 0.1 bar should be used. The heterogeneous mixture is stirred rapidly at 20 ± 5 °C. Once the reaction is complete, the vessel is evacuated to -0.8 bar and refill with nitrogen 3 times. To the reaction vessel is charged ethylenediamine (0.153 kg, 0.00605 wt, 0.07 eq) and Celite (9.3 kg, 0.368 wt). The suspension is stirred at 50 ± 2 °C for 15 min, then cooled

to 20 ± 2 °C. The batch is filtered under nitrogen, collecting the filtrate in a vessel appropriate for distillation. The Celite pad is rinsed with a pre-mixed combination of tetrahydrofuran (51 kg, 1.9 vol or 1.69 wt) and deionized water (3 kg, 0.1 vol or 0.1 wt), then acetone (100 kg, 5 vol or 3.96 wt). The solvents were atmospherically distilled off using a 62 °C internal temperature set point. Once the batch volume reaches ~5 volumes, additional acetone (539 kg, 19 vol or 15 wt) is charged to the batch. This charge is made slowly to maintain the batch at ~5 volumes. Once the acetone addition is complete, the distillation is continued until the internal temperature reaches 62 °C (jacket temperature set point 65 °C), or until the minimum stirable volume of 3-4 volumes is reached. At this point, the internal temperature set point is changed to 50 °C, and heptane (138 kg, 8 vol or 5.5 wt) is simultaneously added in slowly. The batch stirred at 50 ± 5 °C for a minimum of 1 hour, then cooled down to 20 ± 5 °C and held for a minimum of 2 hours. After the hold time is complete, the batch is filtered and the product cake is washed with 5 volumes of a pre-mixed combination of acetone (25 kg, 1.25 vol, 0.99 wt) and heptanes (65 kg, 3.75 vol, 2.56 wt). The solid is dried with nitrogen for ≥ 1 hour, warm nitrogen (40 °C) for ≥ 12 hours, then under vacuum at 40 °C for ≥ 8 hours. Mp = 248 °C; ^{1}H NMR (400 MHz, Pyridine-d_5) δ 6.17 (s, 1H), 5.46 (dt, J = 4.2, 1.9 Hz, 1H), 4.93 (s, 1H), 3.93 – 3.80 (m, 1H), 3.51 – 3.40 (m, 1H), 3.12 – 3.03 (m, 1H), 2.82 – 2.67 (m, 2H), 2.66 – 2.49 (m, 2H), 2.40 – 2.25 (m, 3H), 2.19 (dt, J = 11.4, 3.8 Hz, 1H), 2.13 – 1.75 (m, 6H), 1.71 (m, 2H), 1.62 – 1.36 (m, 3H), 1.36 – 1.13 (m, 7H), 1.02 (d, J = 7.3 Hz, 3H), 0.93 (s, 3H), 0.85 (t, J = 6.9 Hz, 2H), 0.82 (d, J = 6.6 Hz, 3H); ^{13}C NMR (101 MHz, Pyridine-d_5)) δ 143.14, 141.60, 124.80, 122.28, 82.93, 77.42, 71.84, 65.27, 55.79, 53.20, 51.02, 50.73, 45.86, 44.60, 43.50, 41.32, 39.35, 38.11, 37.58, 32.85, 32.60, 32.49, 32.11, 31.39, 29.67, 29.33, 24.70, 23.36, 19.56, 19.35, 14.71, 11.17; LRMS: *m/z* = 426.7 $[M + H]^+$; HPLC (Method A): 99.2 a/a%

Compound **6**: A size appropriate jacketed reactor is equipped with an overhead stirrer, thermocouple, temperature control unit, a condenser, and a nitrogen/vacuum port. The reactor is flushed with nitrogen for 15 minutes. To the reactor is charged 2-butanone (153 kg, 9.0 vol, 7.2 wt), compound **5** (21.1 kg, 1 wt, 1.0 eq) and aluminum isopropoxide (10.36 kg, 0.48 wt, 1.0 eq) under a nitrogen blanket. To the reactor is charged toluene (164.7 kg, 9.0 vol, 7.8 wt) and stirring is initiated. The reactor is degassed by first pulling a vacuum until solvent is seen dripping from the condenser and then back flushed with nitrogen. This process is repeated 4 times. The mixture is then warmed to an internal temp of 80 ± 5 °C and allowed to stir until completion of the reaction (>12 hours). Once the reaction is deemed complete, the jacket temperature is set to 45 °C, while simultaneously a solution of potassium sodium tartrate (Rochelle's Salt 20%) (300 kg) is added to the vessel. The reaction mixture is allowed to cool to an internal temperature of 45 ± 5 °C, and stirred for at least 2 hours from the time that the Rochelle's Salt solution was added to the reactor. After the hold time is complete, the reaction mix is cooled to 20 ± 5 °C. The stirring is stopped and the layers are allowed to separate. The bottom aqueous layer is removed and held in a separate container. The stirring is started, and a second solution of Rochelle's Salt 20% (300 kg), is charged to the reactor. The stir rate can be adjusted so that good mixing of the aqueous and organic phases is achieved. The reaction mixture is allowed to stir for a minimum of 30 min at an internal temperature of 20 ± 5 °C. The stirring is stopped and the layers are allowed to separate. The bottom aqueous layer is removed and held in a separate container. The stirring is started, and toluene (146 kg, 8 vol, 6.92 wt) followed by water (125 kg, 5.93 vol, 5.93 wt) is charged to the reactor. The stir rate can be adjusted so that good mixing of the aqueous and organic phases is achieved. The reaction

mixture is allowed to stir for a minimum of 30 min at an internal temperature of 20 ± 5 °C. The stirring is stopped and the layers are allowed to separate. The bottom aqueous layer is removed, while the remaining organic phase (batch) is removed from the reactor and charged to a receiving vessel. The reactor is cleaned with methanol and blown dry, then the batch is charged back to the reactor through a polish filter. The stirring is started and to the reactor is charged with ethanol (83 kg, 5 vol, 3.95 wt). The stir rate can be adjusted to insure vigorous mixing. The reactor is fitted with an addition funnel, and 31% aqueous citric acid solution (30.8 kg) is charged to the addition funnel. The reactor containing the batch is degassed by first pulling a vacuum until solvent is seen dripping from the condenser and then back filled with nitrogen. This process is repeated 3 times. The contents of the reactor are heated to 45 ± 5 °C. Once the batch reaches 45 ± 5 °C, approximately 50% of the citric acid solution is charged dropwise (18.5 kg). Once nucleation is observed, stop the addition and age the suspension for 30 - 45 minutes. After aging, the remaining citric acid solution (12.3 kg) is charged dropwise. The slurry is aged at 45 ± 5 °C for a minimum of 1 hour, then cooled to 20 ± 5 °C over an hour, and held at this temperature for a minimum of 2 hours. The slurry is then heated back to 45 ± 5 °C over the course of 1 hour and held at this temperature for a minimum of 1 hour. The slurry is cooled back to 20 ± 5 °C over the course of 1 hour, then held at this temperature for a minimum of 2 hours before filtration. The solids are filtered, and the wet cake is washed three times with 2-methyltetrahydrofuran (48 kg, 1.7 wt, 2 volumes each time). The solid is dried with nitrogen for 1 - 2 hours until no more solvent is seen exiting the filter, then with warm nitrogen (40 °C) for ≥ 12 hours while periodically stirring the filter cake. The solid is dried under vacuum at 40 °C for ≥ 8 hours. This procedure delivers the citrate salt of compound **6**. The corresponding free base is produced by the following method: a size appropriate jacketed reactor is equipped with an

overhead stirrer, thermocouple, temperature control unit, and a nitrogen/vacuum port is charged with tetrahydrofuran (107 kg, 2.9 wt, 3.2 volumes), **6**-citrate (36.85 kg, 1 wt, 1 eq), and then water (6.3 kg, 0.17 wt, 0.17 vol). The mixture is stirred at 20 °C, then a 20 wt% aqueous potassium carbonate solution (148.9 kg, 4 wt) is charged under a nitrogen blanket. The biphasic mixture is stirred in the reactor at 30 ± 5 °C for ≥ 30 minutes. At the end of this hold all solids should be dissolved. After the hold is complete and the internal batch temp is 30 ± 5 °C, the stirring is stopped and the phases are allowed to separate. The bottom aqueous phase is removed. To the reactor is charged with another portion of 20 wt% aqueous potassium carbonate solution (148.9 kg, 4 wt). The batch is allowed to mix at 30 ± 5 °C for ≥ 30 minutes. The stirrer is stopped and the layers are allowed to separate. The bottom aqueous phase is removed. The batch is agitated at 20 °C, then ethanol is charged (49, 4 kg, 1.7 vol, 1.32 wt) all at once. Water (348 kg, 10.2 vol, 10.2 wt) is then charged dropwise to the reactor. When nucleation is observed, the batch is held at 20 ± 5 °C for ≥ 30 minutes. The batch is filtered with 30 micron nylon filter cloth to collect solids. The reaction and wet cake is rinsed with at least 2-3 cake volumes of water (205 kg). The solid is dried with nitrogen for 1 - 2 hours until no more solvent is seen exiting the filter. The solid is dried with warm nitrogen (40 °C) for ≥ 12 hours, while periodically stirring the filter cake. The solid is dried under vacuum at 40 °C for ≥ 8 hours, while periodically stirring the filter cake. Mp = 199 °C; ^{1}H NMR (400 MHz, Pyridine-d_5) δ 5.89 (d, J = 1.9 Hz, 1H), 4.92 (s, 1H), 3.46 (ddd, J = 10.7, 9.5, 3.9 Hz, 1H), 3.07 (ddd, J = 12.2, 4.2, 1.5 Hz, 1H), 2.77 (dd, J = 9.6, 7.2 Hz, 1H), 2.58 – 2.12 (m, 8H), 2.01 (dddd, J = 28.6, 13.2, 7.5, 2.4 Hz, 5H), 1.90 – 1.61 (m, 8H), 1.53 (dddt, J = 13.7, 10.4, 6.9, 3.4 Hz, 1H), 1.40 (tdd, J = 12.5, 10.0, 3.8 Hz, 2H), 1.22 (q, J = 11.2 Hz, 1H), 1.17 – 1.05 (m, 2H), 1.02 (d, J = 7.3 Hz, 3H), 0.96 (s, 3H), 0.83 (d, J = 6.7 Hz, 3H); ^{13}C NMR (101 MHz, Pyridine-d_5) δ 198.72, 170.54, 140.42, 125.82, 125.40,

82.89, 77.47, 65.27, 55.79, 55.51, 50.67, 49.63, 47.04, 45.84, 41.31, 38.91, 37.92, 37.09, 34.69, 33.73, 32.10, 31.64, 30.83, 28.78, 24.66, 19.56, 16.39, 11.18; LRMS: *m/z* = 424.6 $[M + H]^+$; HPLC (Method A): 97.7 a/a%

Compound **^{14}C-4a**: A flame-dried 15 mL flask, purged under argon and placed in the dry box, was charged with neat diethyl zinc (Strem Chemicals, 120 mg, 0.95 mmol, 3 eq). The flask was sealed by a septum and taken out of the dry box. The flask was placed under atmosphere of argon and charged with 15 volumes of anhydrous dichloromethane (3 mL). The mixture was cooled to -12ºC in acetone/ice bath. In a dry pear-shaped flask, bis(2,6-dimethylphenyl)phosphoric acid (290 mg, 0.95 mmol, 3 eq) was azeotroped twice with warm anhydrous dichloromethane (6 mL, 30 volumes) than dissolved with 3 mL (15 volumes) of anhydrous dichloromethane. The phosphate solution was added via cannula over 5 min to the diethyl zinc solution. The flask was rinsed with 0.5 mL. The clear solution was allowed to stir at 0 ºC for 30 min under argon. ^{14}C-Diiodomethane (Moravek, 50 mCi with specific activity of 50 mCi/mmol packaged as a neat liquid) was dissolved in 1 mL of dry dichloromethane, dried with 300 mg (1 weight) of Na_2SO_4 and filtered through a 0.2 μm PTFE AcroDisc into a dried vial under argon. The resulting pink solution was transferred to the reaction vessel. The resulting pink solution was stirred at 0ºC for 1 h. In a dry flask, **3a** (200 mg, 0.32 mmol, 1 eq) was dissolved in 3 mL of dry dichloromethane and dried over $MgSO_4$ (500 mg, 3 weights) for 5 min then filtered into a dry pear-shaped flask under argon using a syringe equipped with a 0.2 μm PTFE AcroDisc. The resulting solution was transferred over 2 min to the reaction flask (0ºC) by cannula. The flask was rinsed with 0.5 mL. The ice bath was removed and the reaction was allowed to warm gradually at room temperature. After aging of 12 h, the clear solution was

cooled to -60ºC, then a solution of methanesulfonic acid in DCM (60 μL of MsOH (3 eq) and 60 μL of dichloromethane) was added dropwise. After 15-20 min, morpholine (600 μL) was added to the reaction mixture, which became colorless and clear. Gradually, a fine precipitate formed and the slurry was stirred for 7h at 25ºC. The mixture was diluted with 6 mL (30 volumes) of dichloromethane, then 30 volumes of 20% citric acid. The aqueous layer was back extracted once with 3 mL (15 volumes) of dichloromethane. The combined organic layers were washed once with 6 mL (30 volumes) of 20% citric acid, twice with 6 mL (30 volumes) of 5% $NaHCO_3$, once with 6 mL (30 volumes) of 5% Na_2SO_3, once with 6 mL (30 volumes) of water. The organic layer was dried over Na_2SO_4 and filtered. The solvent was removed using a stream of argon. The crude residue was dissolved in a minimum of EtOAc diluted with hexanes and loaded onto 17 g (80 weights) of SiO_2 (wet with hexanes). Elution with 100 mL hexanes, 250 mL hexanes/EtOAc (97:3), and 300 mL hexanes/EtOAc (95:5) gave **^{14}C-4a** (84 mg, 93.9 a/a% pure by UV 205 nm).

Compound **^{14}C-5**: To a solution of **^{14}C-4a** (84 mg, 0.130 mmol, 1 eq) in dry THF (3 mL) at -78 ºC under argon, 1.8 mL MeLi. LiBr (Aldrich, 1.5 M) was added dropwise. The mixture was stirred at this temperature for 30 min and was quenched by slow addition of methanol (150 μL). The mixture was stirred at -78 ºC for 15 min, then diluted with 6 mL of dichloromethane and 6 mL of water. The cooling bath was removed and the mixture was allowed to warm to room temperature. The phases were cut and the aqueous layer was back extracted with two portions of dichloromethane (2x5 mL). The combined organic layers were washed with water (5 mL), dried over Na_2SO_4 (5 weights) and the solvents were removed using a stream of argon. The crude material (87 mg) was dissolved in a minimum of dichloromethane and loaded onto 4.2 g (50

weights) of SiO_2 (wet with dichloromethane). Elution with 25 mL dichloromethane, 200 ml dichloromethane /MeOH (97:3), and 200 ml dichloromethane /MeOH (95:5) gave the desired material as a white solid (HPLC showed 89% 205 nm), which was dissolved in dichloromethane, filtered through 0.2 μm Acrodisc (to remove silica gel) and placed in 5 mL Weathon volumetric vial. The material was taken up in 600 μL of toluene and 600 μL of heptane. The slurry was stirred at 120 ºC for 15 min and the heat source was shut off. The material was stirred overnight at 22 ºC. A solid appeared, the material was transferred into polypropylene eppendorf and centrifuged for 30 sec at 9800 rpm. The supernatant was removed by a pipette and the pellet was resuspended in dichloromethane to transfer into a 5 mL Wheaton vial. Evaporation of the solvent using a stream of argon gave **^{14}C-5** as a white solid (38 mg, 98 a/a% pure by UV 205 nm).

Compound **^{14}C-6**: A 5 mL Wheaton vial containing **^{14}C-5** (38 mg, 0.089 mmol, 1 eq) and equipped with screw condenser, was charged with Al(O-*t*-Bu)$_3$ (Aldrich, tech. 36 mg, 0.120 mmol, 1.4 eq) and a mixture a toluene/2-butanone (1:1, 30 volumes, 1.5 mL; toluene anhydrous and 2-butanone Chromasolv). The heterogeneous mixture was stirred at 75-80ºC under atmosphere of argon. After 5h, the mixture was cooled to 25ºC and 20% (w/w) aqueous Rochelle's salt (1 mL) was added. The slurry was stirred overnight at room temperature. The layers were cut layers and a mixture of toluene/EtOAc (1:1) was used to back extract the aqueous layer. The combined organic layers was washed with 30 volumes (1 mL) of saturated Rochelle's salt twice, then 30 volumes (1 mL) of water. The organic layer was treated with 3 weights of Na_2SO_4, filtered, and concentrated to dryness under a stream of argon. The residue was loaded onto 3 g (80 weights) of SiO_2 (wet with dichloromethane). Elution with 25 mL dichloromethane,

75 mL dichloromethane /MeOH (97:3), 75 mL dichloromethane/MeOH (95:5) gave 30 mg of **^{14}C-6** as an off-white foam (specific activity 50 mCi/mmol, purity UV 205 nm 93 a/a% and radiopurity 91%).

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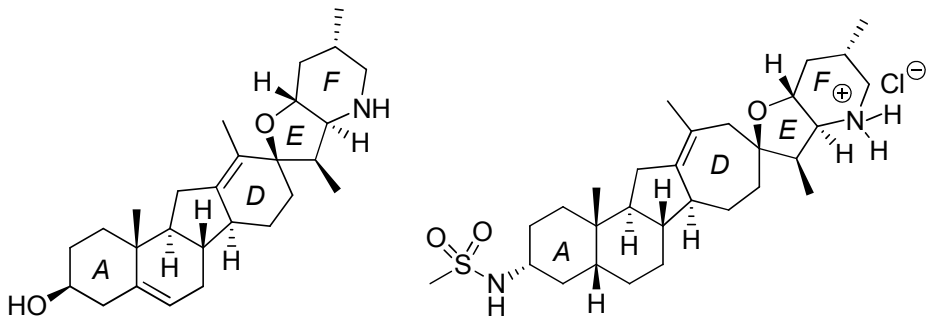


Figure 1. Structure of plant steroidal *Veratrum* alkaloid cyclopamine (**1**) and semi-synthetic drug candidate IPI-926 (**2**)

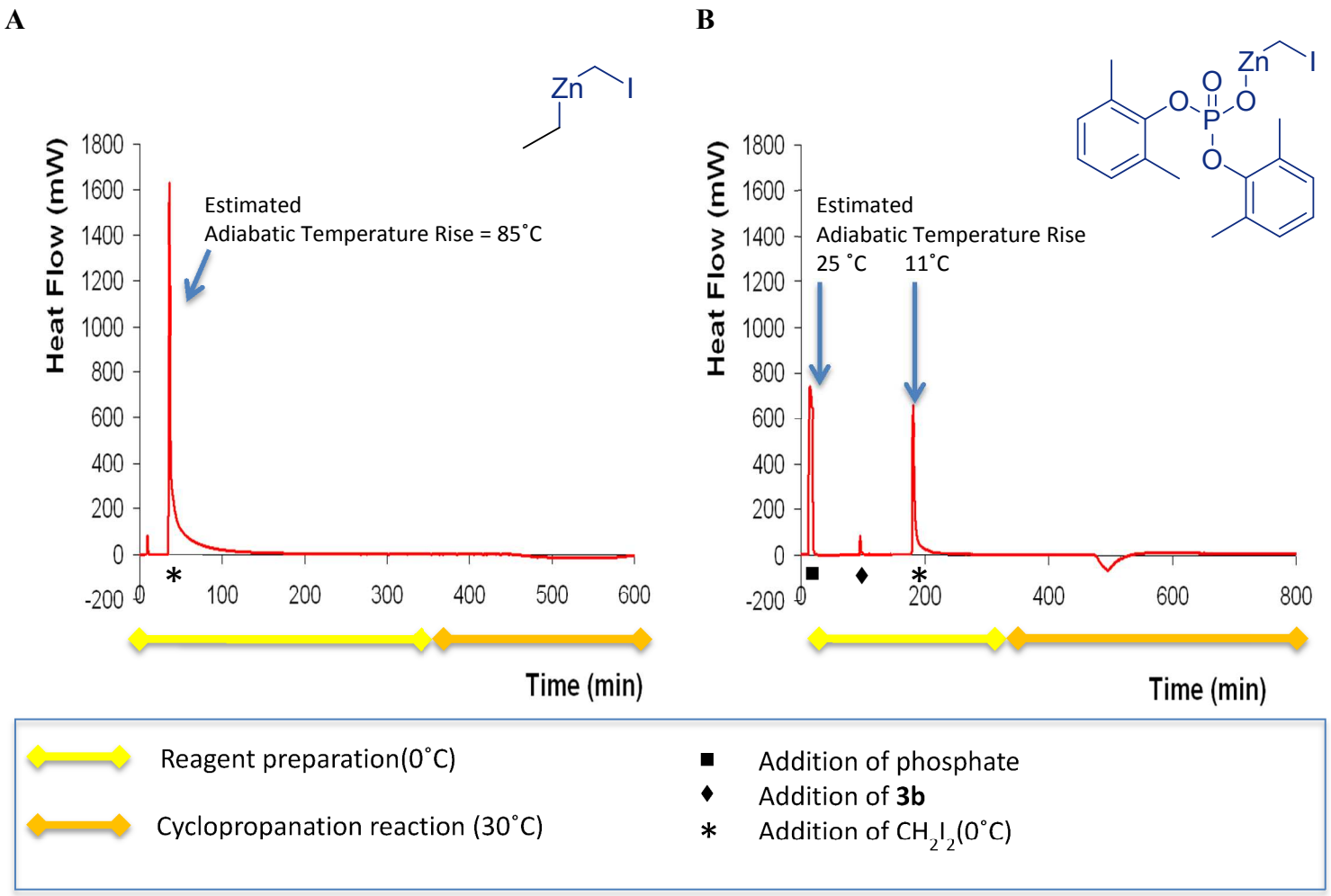


Figure 2. Comparative calorimetry between cyclopropanation of compound **3b** under a) Furukawa's conditions (Et_2Zn, CH_2I_2) or b) New conditions (Et_2Zn, Bis(2,6-dimethylphenyl)phosphoric acid, CH_2I_2).

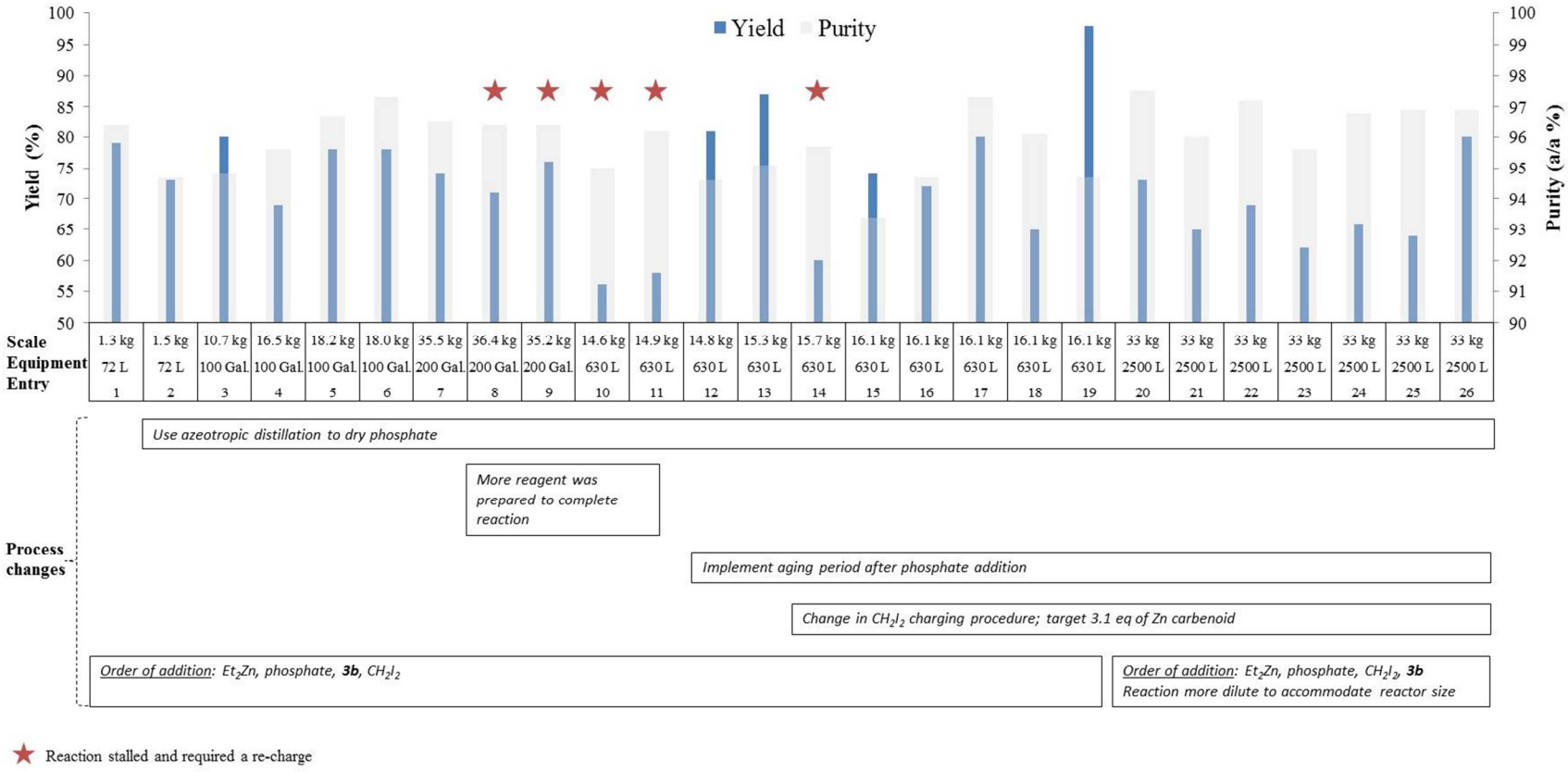
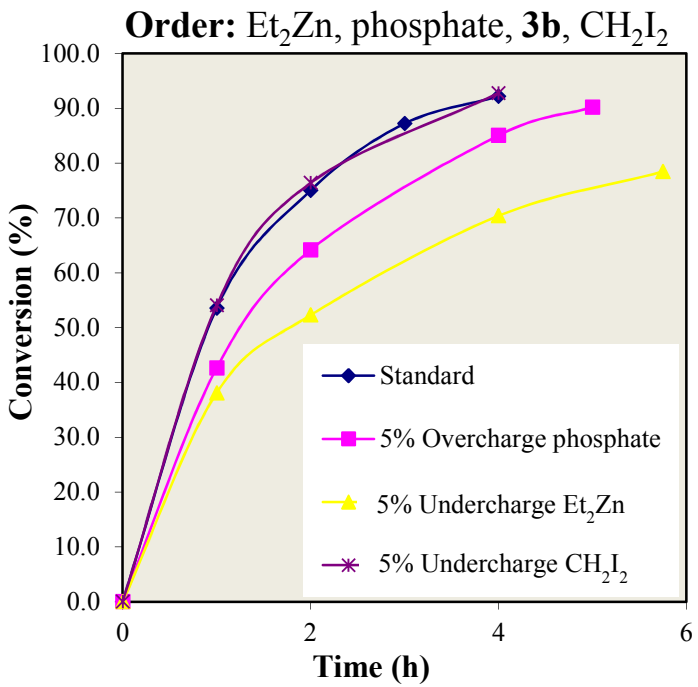


Figure 3. Kilo lab and pilot plant campaign summary for the conversion of **3b** to **4b**

A



B

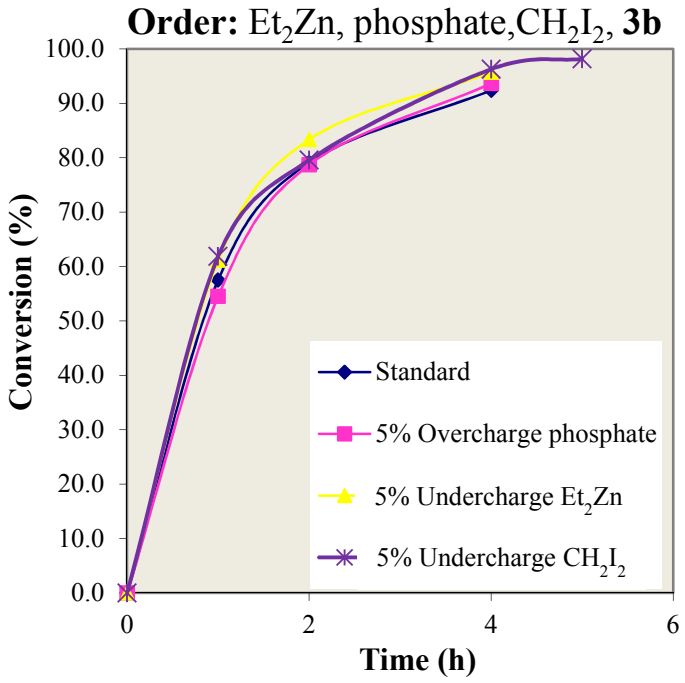


Figure 4 Effect of under or overcharging reagents by 5 % vs. order of addition (Old order: Et_2Zn, phosphate, substrate, CH_2I_2; new order: Et_2Zn, phosphate, CH_2I_2, substrate).

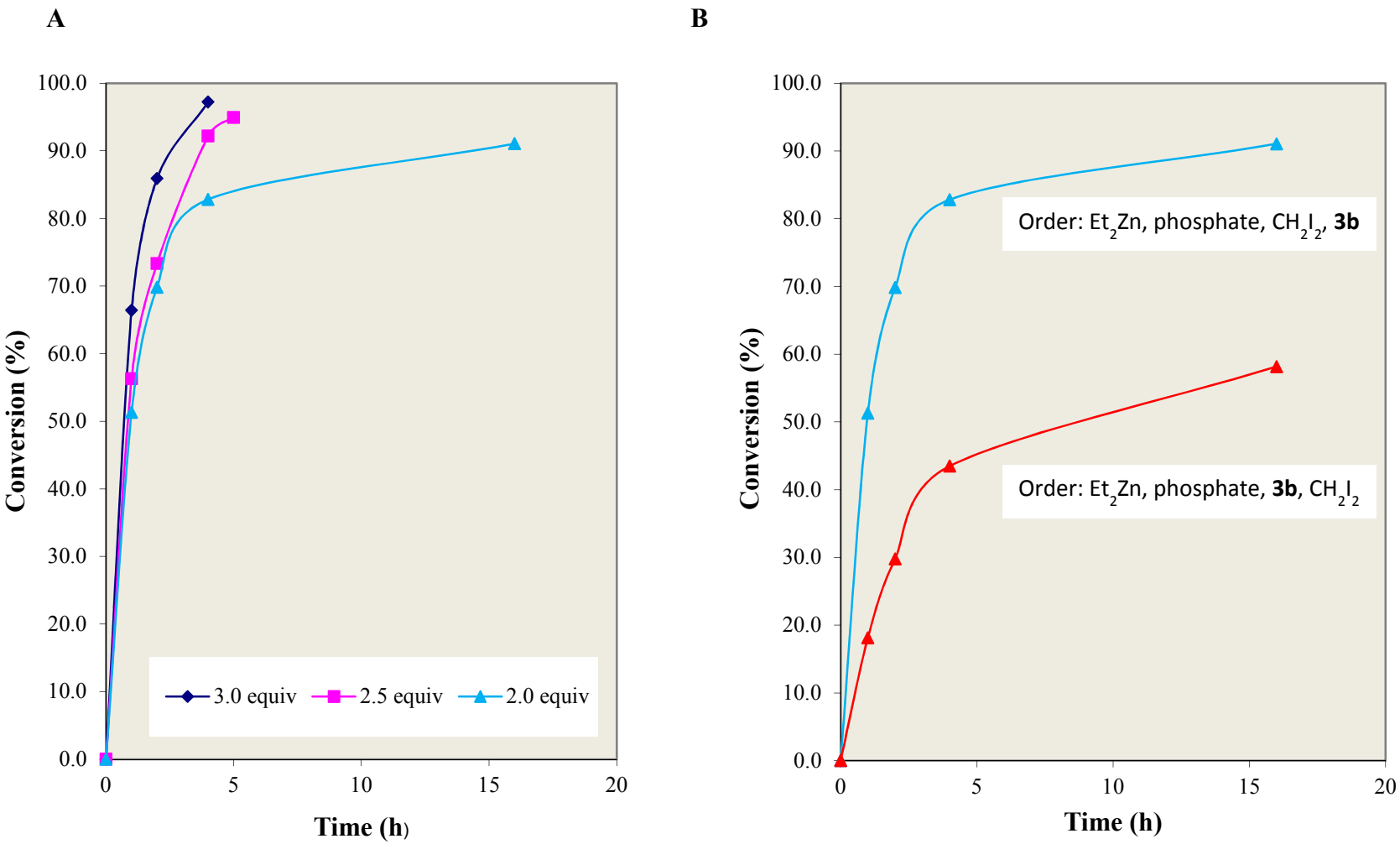
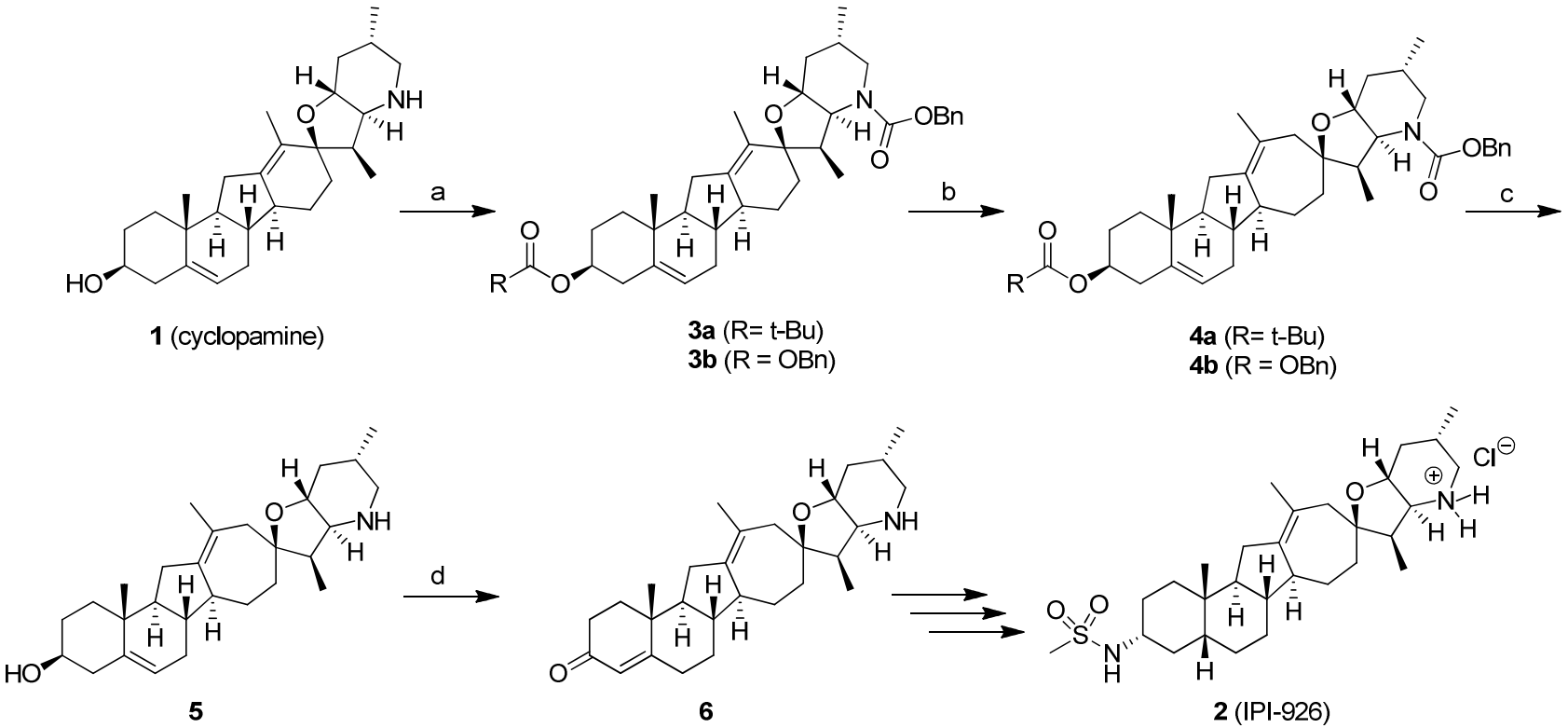
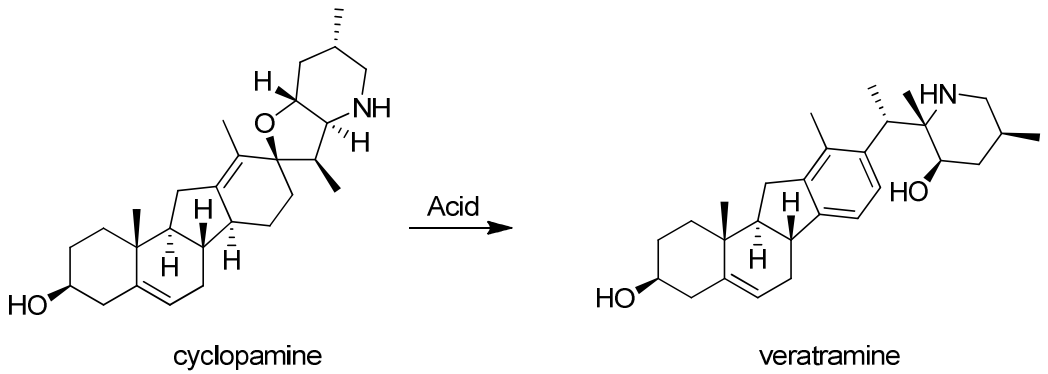


Figure 5: (A) Relationship between rate of cyclopropanation and equivalent of reagents under new order of addition (Et_2Zn, phosphate, CH_2I_2, **3b**; (B) Relationship between rate of cyclopropanation with suboptimal 2 equivalent of reagent and the order of addition.

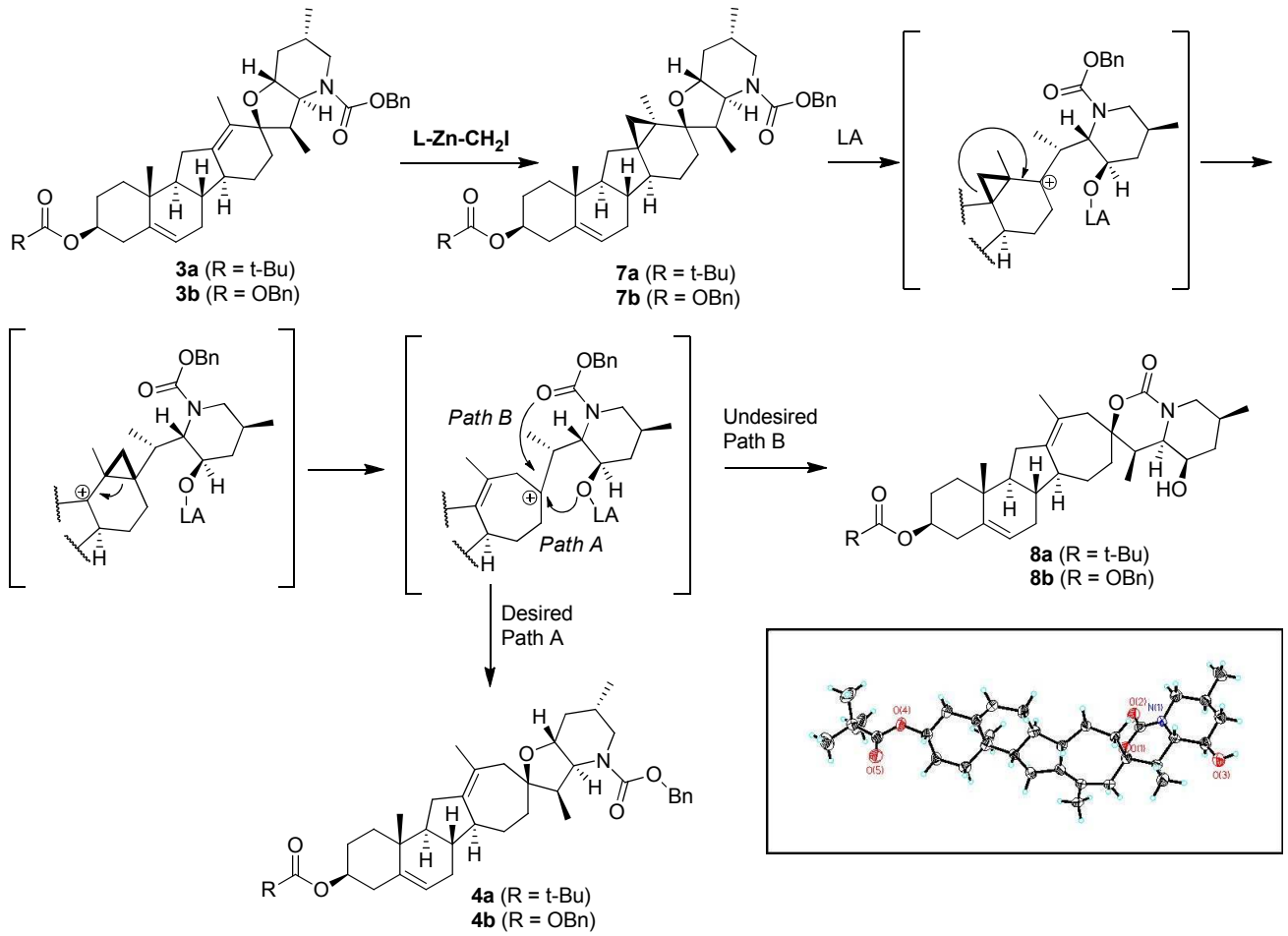


Scheme 1. Overall synthesis of IPI-926 (**2**) from cyclopamine (**1**). *Reagents and Conditions*: (a) **3a**: Cbz-Cl, Et_3N, CH_2Cl_2/MeOH, 25 °C; Piv-Cl, pyridine, 45-50 °C **3b**: Cbz-HOBt, Et_3N, DMAP, EtOAc, 40 ºC; (b) Et_2Zn, $(ArO)_2P(O)OH$, CH_2I_2, CH_2Cl_2, 27 ºC; MsOH, -45 ºC; (c) **4a**: H_2, $Pd(OH)_2$, EtOAc/toluene; DIBAL-H, CH_2Cl_2, -10 to -25 ºC; **4b**: H_2, Pd/C, toluene/IPA; (d) $Al(O\textit{i}\text{-}Pr)_3$, toluene/2-butanone, 80 ºC.

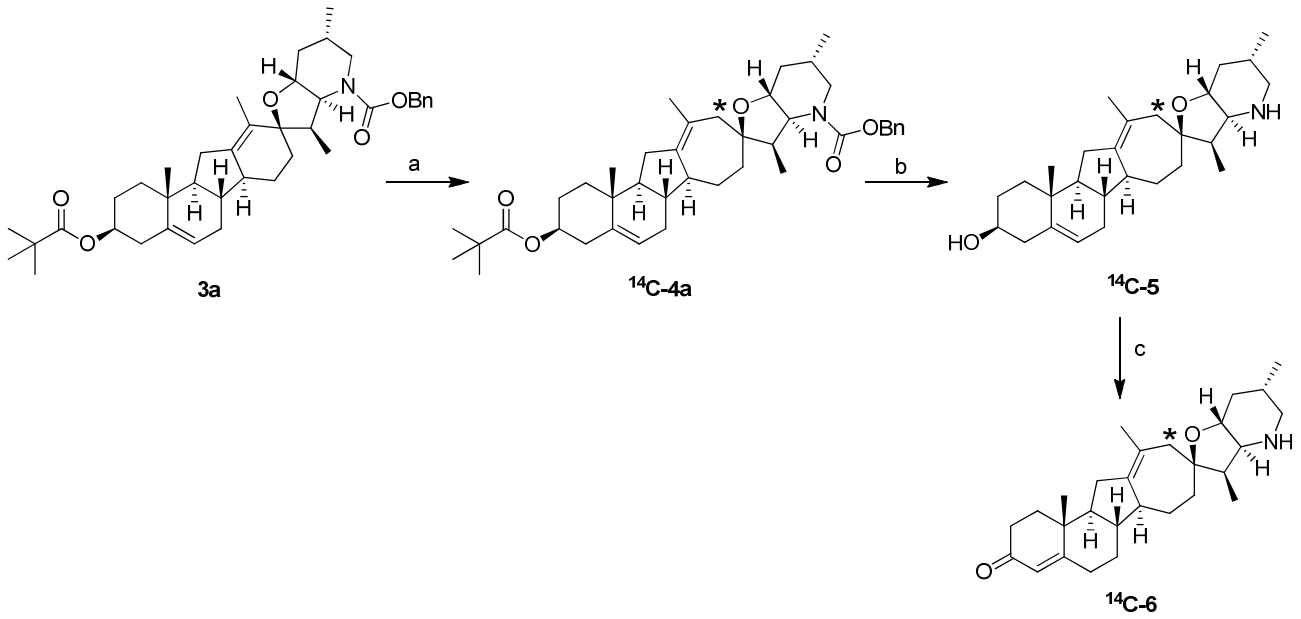


cyclopamine veratramine

Scheme 2. Cyclopamine converts to veratramine under acidic conditions. N-carbamates and O-esters/carbonates **3a** and **3b** are also susceptible to this transformation.

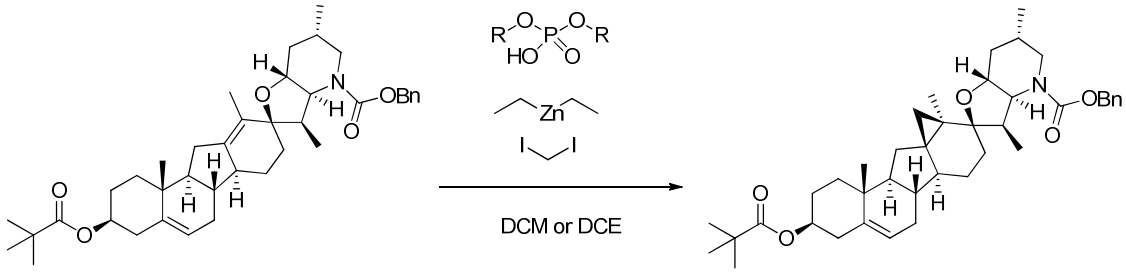


Scheme 3. Development of one-pot cyclopropanation ring-expansion reaction

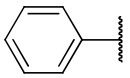
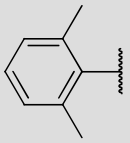
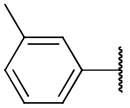
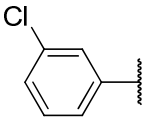
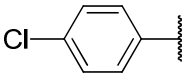
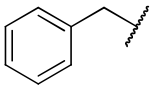
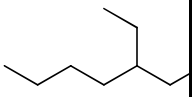
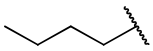


Scheme 4. Radiosynthesis of compound **6** using one-pot cyclopropanation/ring-expansion protocol. *Reagents and Conditions*: (a) 1. Et_2Zn, $(ArO)_2P(O)OH$, $^{14}CH_2I_2$, CH_2Cl_2, 25 °C; 2. MsOH, -78 °C; (b) MeLi-LiBr, THF, -78 °C; (c) $Al(O\text{-}tBu)_3$, 2-butanone, toluene, 80 °C.

Table 1. Optimization of the phosphate portion of the iodomethylzinc phosphate carbenoid



3a **7a**

Entry	Phosphate (R)	Physical state at 23 °C	Solubility in CH_2Cl_2 or $ClCH_2CH_2Cl$	Solubility in basic aqueous phase	Carbenoid Solubility in CH_2Cl_2 or $ClCH_2CH_2Cl$	Conversion
1		Solid	Medium	Yes	Low	High
2		Solid	High	Yes	High	High
3		Oil	High	Yes	High	High
4		Oil	High	Emulsion	High	Not determined
5		Solid	High	Emulsion	Low	Not determined
6		Solid	High	Low	High	Medium
7		Liquid	High	Emulsion	High	Low
8		Liquid	High	Emulsion	High	Very Low

Supporting Information

The Supporting Information, available free of charge on the ACS Publications website, contains analytical HPLC methods, structural characterization (^{1}H- and ^{13}C-NMR spectra, mass spectrometry data), and a pictrogram of the diethylzinc delivery system (PDF).

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