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Microwave assisted Westphal condensation and its application to synthesis of sempervirine and related compounds

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ABSTRACT

A concise synthesis of a potent lead in anticancer therapeutics, sempervirine, was achieved by one pot Westphal condensation, ester hydrolysis, and decarboxylation under microwave irradiation. The method was extended to the synthesis of several similar heterocycles.

Sempervirine, an alkaloid isolated[1a,b] from the roots of *Gelsemium sempervirens*, is known as an antiproliferative agent both in vitro[2,3] and in vivo.[4,5] Earlier, in a high throughput screening (HTS) campaign of natural products, sempervirine was discovered as a MDM2 E3 ubiquitin ligase inhibitor.[6,7] Sempervirine is known to stabilize p53 tumor suppressor protein levels[8] by blocking its proteasomal degradation[9] via a ubiquitin-dependent pathway. It inhibits both murine double minutes-2 (MDM2) dependent p53 ubiquitinylation and MDM2 auto-ubiquitinylation.[10] Thus cancer cells carrying wild-type p53 when treated with this compound induce stabilization of p53 leading to apoptosis.[10]Sempervirine is also known to intercalate DNA, and inhibits DNA topoisomerase I;[11]therefore, it is a potential lead in anticancer therapeutics.

The structure of sempervirine was reported by Woodward[1c] and Stevens[1d] as a resonance hybrid of **5A** and **B** (Fig. 1a). A concise synthesis of sempervirine methochloride was reported by Woodward et al. (Fig. 1b),[12] but the application of their method to the actual synthesis of sempervirine never appeared in the literature. Though many routes to the synthesis of sempervirine are known,[13–18] all but one route by Mattingly[16] involve a long synthetic sequence, while all reported methods suffer from low overall yield, and use starting materials which are difficult to prepare.

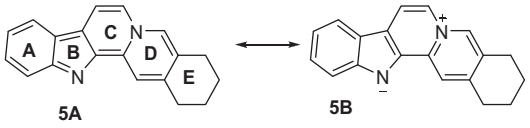

Figure 1a. Structure of semervirine.[1c,d]

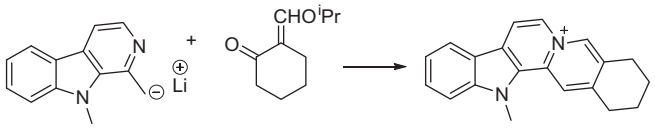

Figure 1b. Synthesis of sempervirine methochloride.[12]

We embarked on the synthesis of sempervirine, required by us in gram quantities for biological evaluation, following the route described by Lipińska.[13] We started with 3-(methylthio)-1,2,4-triazine, which was converted to 3-(1-(phenylsulfonyl)-1*H*-indol-2-yl)-5,6,7,8-tetrahydroisoquinoline in 7 steps in 1.5% overall yield. However, construction of C ring via Gribble's[15] C-lithiation strategy was not successful. The condensation of 1-methyl-2-ethoxycarbonylmethyl-9*H*-pyrido [3, 4–b] indolinium bromide **2** with cyclohexan-1,2-dione $\mathbf{D_1}$ as described by Pottsand Mattingly[16] (Scheme 1), afforded the cyclized compound **3** in ~10 % yield.

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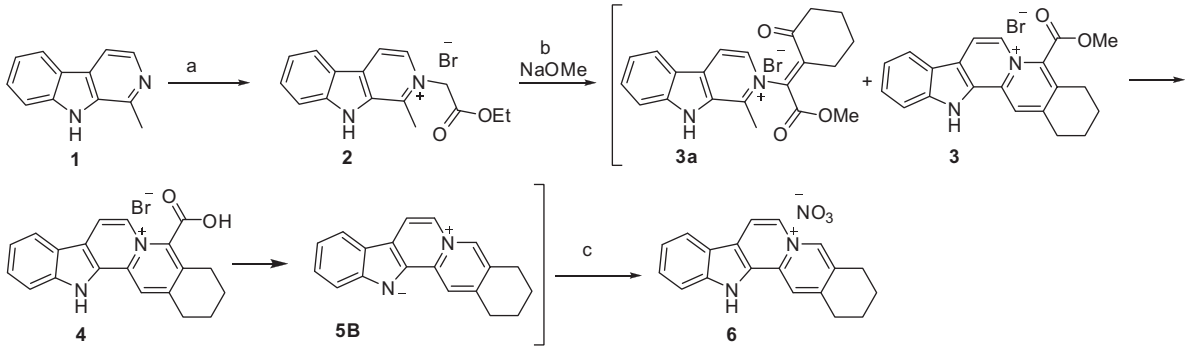

Reagents and Conditions: a) ethyl bromoacetate, acetone, reflux, 18 h, 90%; b) 1,2-cyclohexanedione, microwave, 95 °C, 1 h, 53 %; c) i)MeOH, 6N HCl, 2h, ii) MeOH, $NaNO_3$, 2h, 78 %;

Scheme 1. Synthesis of sempervirine via microwave heating, and its conversion to the nitrate salt.

Table 1

Entry	Base	Equiv	Heating	Temperature	Time	3[a]	3a[a]	4[a]	5[a]
1	NaOAc[20]	3 equiv	Oil bath	60 °C	24 h	—	—	—	—
2	NaOMe	2.5 equiv	Oil bath	70 °C	16 h	12%	60%	Nf	Nf
3	NaOMe	3 equiv	μwave	80 °C	10 min	30%	Nf	13%	10%
4	NaOMe	3 equiv	μwave	80 °C	30 min	15%	Nf	14%	14%
5	NaOMe	3 equiv	μwave	95 °C	40 min	30%	Nf	15%	30%
6	NaOMe	3 equiv	μwave	95 °C	60 min	10%	Nf	20%	45%
7	NaOMe	4 equiv	μwave	95 °C	60 min	Nf	Nf	Nf	75%

Nf: not formed.
[a] The reported numbers are percentage values from LCMS spectra, not the isolated yield.

The decarboxylation of **4** at 250 °C resulted in decomposition, and hence no product could be isolated. During further investigations on the synthesis of sempervirine by Westphal condensation reaction,[19] the idea of application of microwave technology was conceived by us. Sempervirine (**5B**) was obtained by the reaction of quaternary compound **2**[21] with 1,2-cyclohexanedione under microwave heating, accomplishing three sequential steps in one pot (Scheme 1).This methodology was also applied to other substrates, which has allowed us to gain access to compounds similar to sempervirine. The results of our initial investigations on the Westphal condensation reaction are summarized in Table 1.

When a mixture of **2** and 1, 2-cyclohexanedione was heated in methanol in the presence of sodium methoxide, formation of the condensation product **3a** (major) and cyclized ester **3** (minor) (entry 2) was observed. On further heating, there was slow conversion of **3a** to **3**, but **3a** remained as the major product even after 72 h owing to the preference for the *s-trans* conformation. In order to enhance the rate of cyclization, it was decided to carry out the condensation reaction under microwave heating. We were absolutely delighted to see the dramatic impact microwave heating had on the course of the reaction (entry 3). A mixture of **3**, **4,** and **5B** (sempervirine) was formed, with no detectable quantity of **3a**. Further optimization of the reaction conditions was done by varying the reaction temperature, time, and the quantity of base.

With an increase in the reaction temperature from 80 °C to 95 °C and a concomitant increase in reaction time, a gradual improvement in conversion of the reaction intermediates to sempervirine **5B** was observed (entry 3 to 7). The best conversion was obtained by heating the substrates in methanol with 4 equiv of sodium methoxide for 60 minutes at 95 °C (entry 7). Sempervirine **5B** was isolated in 48% overall yield from harmane **1**.[22] It was further transformed into nitrate via its hydrochloride salt.[23] Formation of the nitrate salt was also confirmed by overlapping IR spectra of an authentic sample.

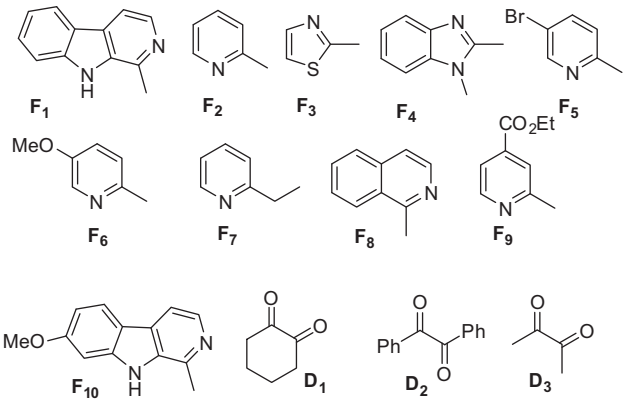

Figure 2. Heterocycle and diketone building blocks.

In order to explore the scope of this methodology several five- and six-membered heterocycles ($\mathbf{F_{1-10}}$) were (Fig. 2) reacted with ethyl bromoacetate in ethanol or acetone to afford the quaternized compounds ($\mathbf{Q_{1-10}}$) in 70–90% yield (Scheme 2).[21] The Westphal condensation reactions were carried out by heating a mixture of quaternized compound $\mathbf{Q_i}$ (1 mmol), diketone $\mathbf{D_j}$ (1.2 equiv), and sodium methoxide (2.5–4 equiv) in methanol between 95 °C and 105 °C for an hour.[24] 1–3 runs were done for each substrate, varying the quantity of base (2.5–4 equiv) and/or temperature (95–105 °C) and/or the dilution.

In most of the reactions carried out at 95 °C, the acid $\mathbf{A_{ij}}$ was formed as the minor product. In one of the cases, when a purified