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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.

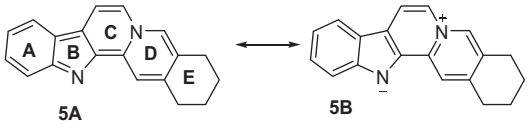

Figure 1a. Structure of semervirine.[1c,d]

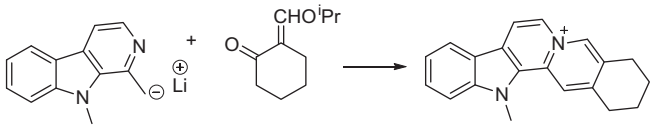

Figure 1b. Synthesis of sempervirine methochloride.[12]

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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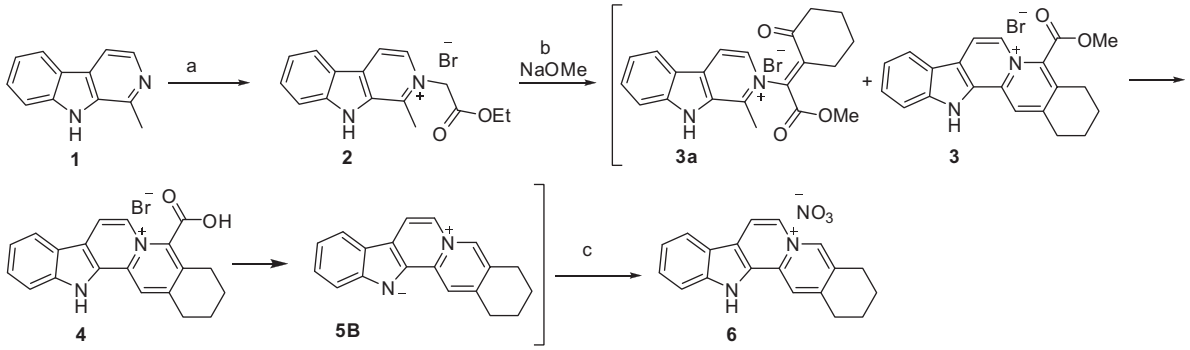


Reagents and Conditions: a) ethyl bromoacetate, acetone, reflux, 18 h, 90%; b) 1,2-cyclohexanedione, microwave, 95 °C, 1 h, 53 %; c) i)MeOH, 6N HCl, 2h, ii) MeOH, $NaNO_3$, 2h, 78 %;

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.

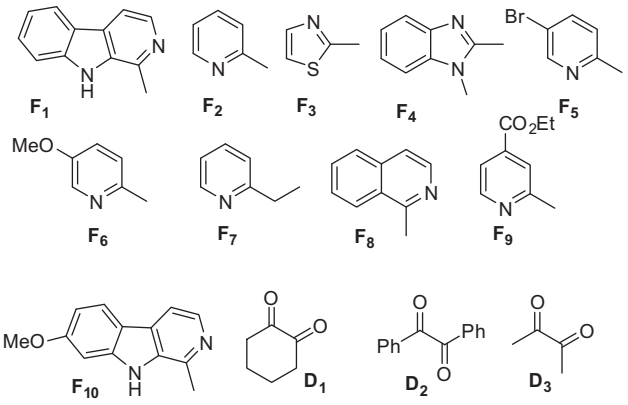
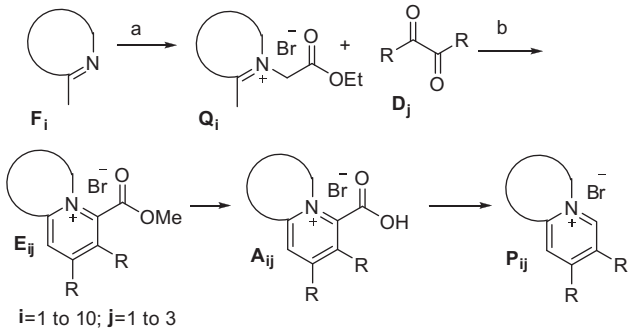


Figure 2. Heterocycle and diketone building blocks.

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



Reagents and Conditions: a) ethyl bromoacetate, acetone/EtOH, reflux, 18 h, 70-90%; b) NaOMe, μwave, 95 °C, 1 h;

Scheme 2. Synthesis of sempervirine and its analogues.

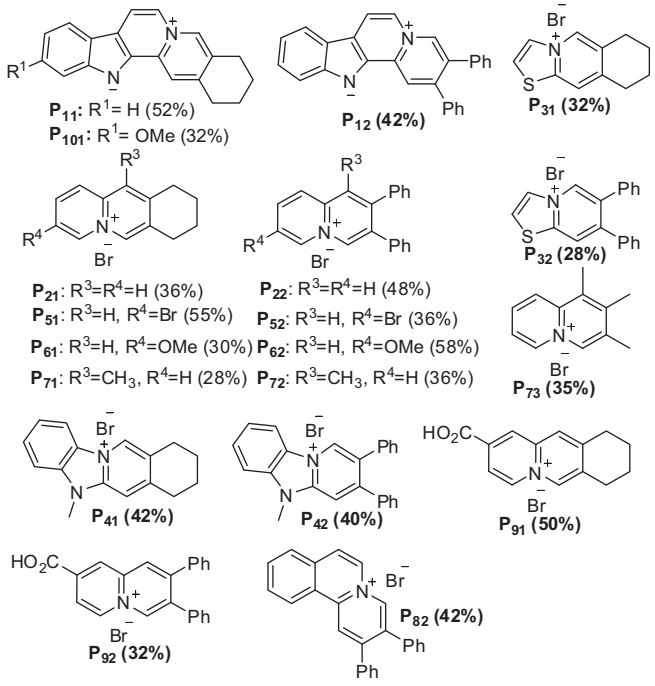


Figure 3. Compounds prepared by Westphal condensation. The number in brackets indicate isolated yield.

sample of 11-carboxy-5-methyl-7,8,9,10-tetrahydro-5*H*-benzo [4,5]imidazo [1,2-b]isoquinolin-12-ylium bromide (**A_{41}**) (Obtained from the reaction between **Q_4** and **D_1**) was heated in methanol in a microwave reactor at 100 °C for 30 minutes, no decarboxylation was observed. However, spontaneous decarboxylation occurred when the condensation reaction of **Q_4** and **D_1** was done at 105 °C with two fold increase in dilution. This suggests that the decarboxylation is base mediated and is also favored under higher dilution. In all cases, the required products were purified by column chromatography over silica-gel. The compounds prepared are shown in Figure 3.

In another case, cyclization of 5-bromo-1-(2-ethoxy-2-oxoethyl)-2-methylpyridinium bromide **Q_5** with cyclohexane-1,2-dione **D_1** and with benzil **D_2** resulted in the formation of side products by substitution of bromo group with methoxy group. The formation of this side product was eliminated by using a lesser quantity (2 equiv) of sodium methoxide.

To summarize, the products **P_{ij}** were obtained in modest yields ranging between 28 % and 58 %. All the compounds were obtained in >95 % chromatographic purity and were characterized by ^{1}H NMR and mass spectra.

Table 2

Entry	Base	P_{22} (%)
1	Triethylamine	36
2	DBU	46
3	Pyridine	40
4	Morpholine	25
5	*n*-Butylamine	Nil
6	*N*-Methylpiperazine	16
7	*Tri-n*-Butylamine	50

In order to explore the Westphal condensation reaction in the presence of various organic bases, 1-(2-ethoxy-2-oxoethyl)-2-methylpyridinium bromide **Q_2** and benzil **D_2** were chosen as substrates. The reactions were carried out using **Q_2** (1 mmol), benzil (1.2 mmol) and base (4 equiv) at 95 °C/1 h using methanol as solvent. The results are summarized in Table 2.

The results indicated that the condensation reaction worked quite well in the presence of tertiary bases: triethylamine, *tri-n*-butylamine, DBU, and pyridine; whereas *N*-methylpiperazine and morpholine were not so effective. Most importantly, no acid derivative was detected in the reaction mixtures, indicating occurrence of spontaneous decarboxylation under microwave heating.

The effect of solvent on Westphal condensation reaction was also explored using 1-(2-ethoxy-2-oxoethyl)-2-methylpyridinium bromide (**Q_2**, 1 mmol) and cyclohexane-1,2-dione (**D_1**, 1.2 eq) as substrates. The reactions were carried out at 95 °C/1 h using four solvents: ethanol, acetonitrile, 1,4-dioxane, and *N,N*-dimethylformamide. The reactions in acetonitrile and ethanol afforded the desired product **P_{21}** (Fig. 3) in 44 % and 46 % yield respectively. The reactions in DMF and dioxane were not clean (TLC) and hence the product was not isolated.

In conclusion, we have synthesized sempervirine by Westphal condensation reaction under microwave irradiation. We have also synthesized several known as well as new quinolizinium and pyridinium compounds by the same methodology. The usefulness of this one-pot method lies in the fact that the starting materials are very easy to prepare, and the method can be applied to a variety of starting materials bearing different functional groups. Initially, all the products were synthesized using sodium methoxide as the base. Further investigations revealed that tertiary amines like DBU, triethylamine, and di-*tert*-butylamines were superior compared to sodium methoxide and afforded cleaner reactions with improved yields.

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Supplementary data

Supplementary data (^{1}H, ^{13}C NMR and analytical data for Sempervirine free base **5** and nitrate salt **6** is available. For all other compounds ^{1}H NMR scans are available. LCMS scans of Table 1

entries are also available) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.11.059.

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21. *General procedure for quaternization:* To a solution of the heterocycle (10.0 mmol) in ethanol (20 ml) or in acetone (20 mL), ethyl bromoacetate (15.0 mmol, 1.5 equiv) was added at room temperature. The resulting reaction mixture was refluxed for 16 h. The progress of the reaction was monitored by TLC. The reaction mixture was cooled and concentrated under reduced pressure. The residue was crystallized in acetone (~100 ml) to give the desired quaternary ammonium salt.
22. *Preparation of sempervirine (**5**)*: To a stirred solution of **2** (6.0 g, 17.2 mmol) in methanol, 1,2-cyclohexanedione (2.5 g, 22.3 mM) and sodium methoxide (3.6 g, 66.9 mmol) were added at room temperature. The resulting reaction mixture was stirred in CEM-Microwave (Power-150 W, Pressure-250 psi) at 95 °C for 1 h after which it was cooled and acidified with dilute acetic acid. The mixture was concentrated, and the obtained residue was purified by flash column chromatography over silica-gel (230–400 mesh) using 0–10% MeOH in DCM as eluent, to afford 2.4 g (53% yield) of as yellow solid. ^{1}HNMR (DMSO-d_6, 400 MHz): δ 1.90 (m, 4H), 3.01 (m, 2H), 3.17 (m, 2H), 7.43 (t, 1H, J = 10 Hz), 7.67 (t, 1H, J = 10 Hz), 7.83 (d, 1H, J = 10.8 Hz), 8.38 (1H, d, J = 10.8 Hz), 8.68 (1H, d, J = 9.2 Hz), 8.76 (bs, 1H), 8.86 (1H, d, J = 9.2 Hz), 13.17 (bs, 1H). ^{13}C NMR (CD_3OD, 100 MHz) δ: 22.7, 22.8, 27.4, 30.4, 113.5, 116.9, 120.4, 122.5, 122.2, 123.0, 127.1, 130.3, 130.9, 132.2135.0, 135.9, 142.5, 151.4; MS (m/z): 273.18 (100%), 274.19 (13%); mp: 270–271 °C [Lit: 258–260 °C][14]; UV λ_{max}(MeOH): 388, 337, 295, 242 nm.
23. *Preparation of sempervirine nitrate (**6**)*: To a suspension of (1.5 g, 5.5 mmol) in methanol (10 ml), 6 N HCl (7.0 ml) was added at room temperature. The resulting clear solution was stirred for 2 h, and then concentrated to dryness. The residue was dissolved in methanol (10 ml); a solution of sodium nitrate (10 ml) was added to it. A solid precipitated out after some time. It was stirred for 2 h and filtered. The solid was washed with water, and air dried to yield 1.4 g of sempervirine nitrate as greenish yellow solid. ^{1}HNMR (DMSO-d_6, 400 MHz): δ 1.90 (m, 4H), 3.02 (m, 2H), 3.18 (m, 2H), 7.44 (t, 1H, J = 10 Hz), 7.68 (t, 1H, J = 9.6 Hz), 7.83 (d, 1H, J = 10.8 Hz), 8.39 (1H, d, J = 10.8 Hz), 8.69 (1H, d, J = 8.4 Hz), 8.86 (1H, d, J = 9.2 Hz), 9.27 (1H, bs), 13.17 (1H, bs). MS (m/z): 273.05 (100%), 274.06 (13%). HRMS: 273.1370 which corresponds to $[C_{19}H_{17}N_2]^+$; IR (cm^{-1}): 3400, 2937, 1646, 1632, 1524, 1469, 1383. mp: 280.5–282.1 °C [Lit: 267 °C, decomp][18]; ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 21.1, 21.1, 25.7, 28.7, 112.5, 115.7, 118.9, 120.4, 120.7, 121.4, 121.5, 125.7, 128.6, 129.2, 130.0, 132.7, 134.5, 140.4, 148.9.
24. *General procedure*: To a solution of quanternary ammonium bromide derivative (1.0 mmol) in methanol, the diketo compound (1.2 mmol, 1.2 equiv) and sodium methoxide (4.0 mmol, 4.0 equiv) were added at room temperature. The resulting reaction mixture was heated in CEM-Microwave (Explorer) between 95 °C and 105 °C (Power 150 W and Pressure 250 psi) temperature for 50 min. The reaction mixture was acidified with acetic acid and concentrated under reduced pressure. The crude product was purified by column chromatography over a silica-gel eluting with a mixture of methanol and dichloroemethane.