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Synthesis of New Substituted Quinolizidines as Potential Inhibitors of Ergosterol Biosynthesis.

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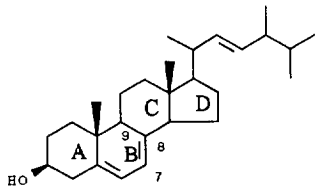
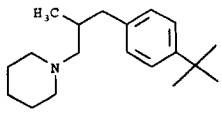
Abstract: Carbocationic species (High Energy Intermediates) have been postulated as intermediates in the course of enzymatic synthesis of ergosterol. Protonated aza-analogues of the sterol are thus potential inhibitors. The synthesis of substituted quinolizidines (**3**) is reported. 4-Methoxy-2,3-dimethylpyridine (**4**) was metalated and reacted with substituted 3-chloropropanal to build the 1-methyl-2-quinolizidinone. The lateral chain was prepared via a Wittig-Horner reaction.

Fungitoxicity of fenpropidin and other commercial compounds such as tridemorph and fenpropimorph appears to be due to their capacity to inhibit ergosterol biosynthesis.[1] Recently the inhibition of enzymatic cyclization of squalene and oxydosqualene have received increasing attention.[2]

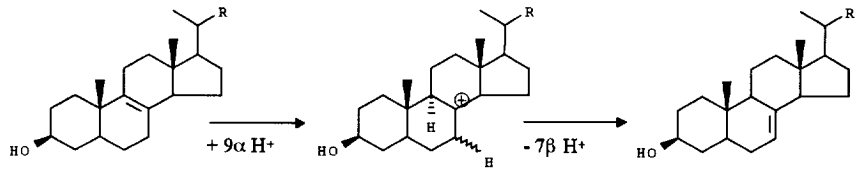


Ergosterol



Fenpropidin

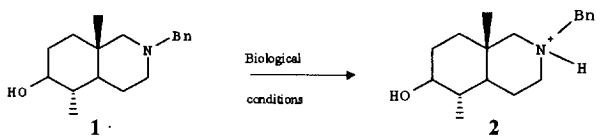
It was assumed twenty years ago that several enzymes implicated in sterol biosynthesis catalyzed reactions involving postulated carbocationic high energy intermediates (HEI).[3] For example, a key step in ergosterol biosynthesis, the transformation of fecosterol to episterol with $\Delta^8 \rightarrow \Delta^7$ isomerase, is rationalized by an intermediate as described in scheme 1.[1]



fecosterol **HEI** **episterol**

Scheme 1

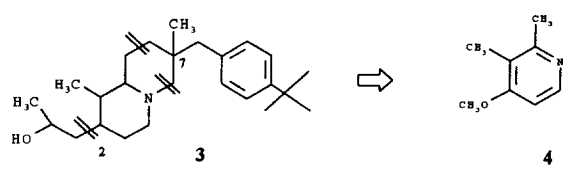
Thus, molecules possessing structural or electronic analogy with such intermediates (HEI) could be inhibitors of the corresponding reactions.[4] Benveniste[3] reported the preparation of azadecalines such as **1** (scheme 2) which have been shown to inhibit the action of the $\Delta^8 \rightarrow \Delta^7$ isomerase blocking the synthesis of the Δ^7-sterol. Under biological conditions, **1** presumably exists as the ammonium ion **2** in which the cationic nitrogen mimics the carbocation in HEI.



1 **2**

Scheme 2

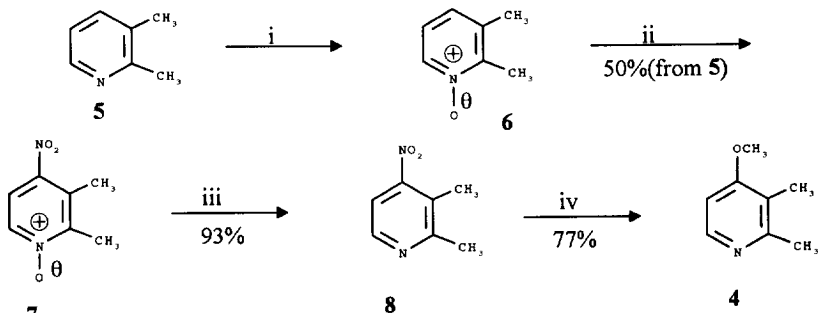
As part of a project aimed to the development of ergosterol biosynthesis inhibitors, we were interested in whether structural relatives of **1** could also inhibit isomerase activity. Our approach led us to the substituted quinolizidine **3**, which has an endocyclic nitrogen which could be protonated, an hydroxy propyl chain at C-2 which could mimic ring A of the sterols and finally a benzyl group at C-7 chosen for its lipophilicity.



Herein, we report the preparation and separation of several isomers of quinolizidine **3.**

RESULTS AND DISCUSSION

Our selected starting compound, 4-methoxy-2,3-dimethylpyridine (**4**) was prepared in four steps starting from 2,3-dimethylpyridine (**5**). Pyridine N-oxide **6** was prepared by oxidation of **5** as described by Ochiai.[5] Nitration of **6**, according to Evan's method[6] for dimethylpyridine N-oxides, afforded the nitro N-oxide (**7**). Reduction of **7** and nucleophilic substitution with methanol led to **4** in 36% overall yield (Scheme 3).

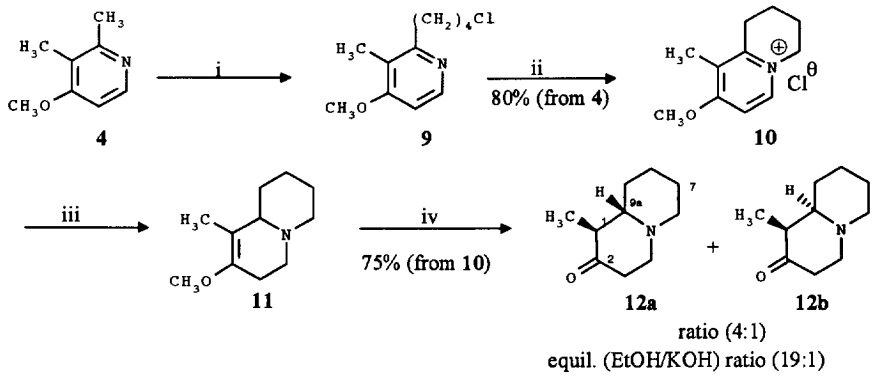


5 **6** **7** **8** **4**

(i) H_2O_2, AcOH, reflux; (ii) HNO_3, H_2SO_4, 95°C; (iii) PCl_3, CH_2Cl_2, -20°C
(iv) MeOH, K_2CO_3, reflux

Scheme 3

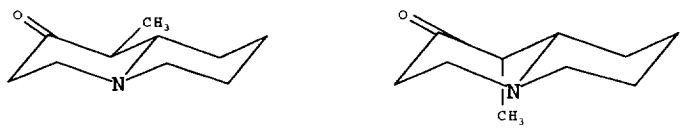
The intermediate picolyllithium obtained by reaction of **4** with n-butyllithium (scheme 4), according to Beumel's procedure,[7] was allowed to react with 1-bromo-3-chloropropane to give **9**, which then underwent cyclization in refluxing ethanol to afford the pyridinium salt **10**. Compound **10** was reduced by sodium borohydride in methanol to give the enol ether **11** which was then hydrolyzed to 1-methyl-2-quinolizidinone (**12**) by reaction with aqueous hydrochloric acid. An excellent overall yield (60% from **4**) was obtained.[8] ^1H and ^{13}C NMR analysis of the raw product indicated the presence of two stereoisomers of **12** in a ratio of 4:1.[9] The product mixture was then equilibrated with ethanolic potassium hydroxide to a 19 : 1 ratio as confirmed by vpc and ^{13}C NMR analysis. Isomers **12a** and **12b** could not be separated by column chromatography.



(i) a) n-BuLi, THF, -25°C, b) $Br(CH_2)_3Cl$, -30°C ; (ii) EtOH, reflux ; (iii) $NaBH_4$, MeOH, r.t. ; (iv) HCl 10%, r.t.

Scheme 4

If quinolizidines may exist in solution as an equilibrium, the predominance of the trans-fused conformation has been demonstrated by IR, ^1H NMR and ^{13}C NMR spectroscopy.[10] Compounds **12** shows Bohlman IR absorptions in the 2850-2700 cm^{-1} region, indicating the presence of the predominantly trans-fused conformer.

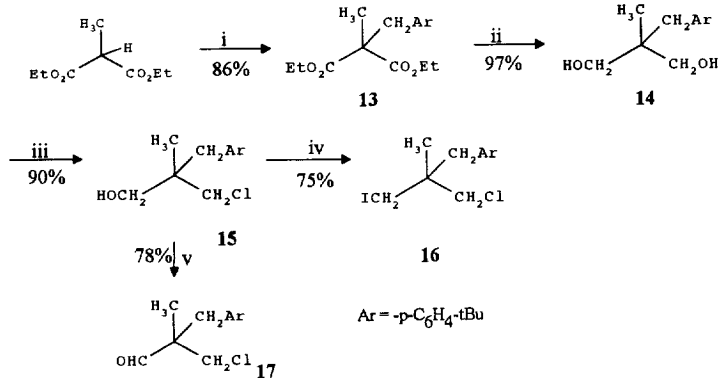


12a **12b**

As a consequence of the hydrolysis of the intermediate enol-ether **11**, the major isomer was assumed to have an equatorial methyl group. ^1H NMR spectra present a doublet for the methyl group at 1.02 ppm for the preponderant isomer and at 1.20 ppm for the other one. The ^{13}C NMR spectra show a signal at 9.5 ppm for the methyl group of the major isomer and at 12.0 ppm for the other one.

In view of our requirement for introducing methyl and p-*tert*-butylbenzyl groups at C-7 of compound **3** it was desirable to prepare a 1,3-dihalogeno-2-methyl-2-(p-*tert*-butylbenzyl) propane as bis electrophile. The suitably substituted 1-iodo-3-chloro propane was synthetized as follows (scheme 5).

Diethyl methylmalonate was alkylated with p-*tert*-butylbenzyl bromide and the resulting diester **13** reduced with lithium aluminium hydride to give the diol **14**. One hydroxyl group of **14** was replaced by a chlorine atom with a good yield as described by Castro.[11] The second hydroxyl group was then replaced by an iodine atom using the method described by Garegg.[12] The chloroiodopropane **16** was obtained in 56% overall yield from diethyl methylmalonate.



(i) a) EtONa, EtOH, r.t., b) $BrCH_2Ar$, reflux ; (ii) $LiAlH_4$, Et_2O, r.t. ; (iii) a) CCl_4, $P(NMe_2)_3$, THF, -30°C and r.t., b) DMF, 100°C ; (iv) PPh_3, Imidazole, I_2, toluene, reflux ; (v) PCC, CH_2Cl_2, r.t.

Scheme 5

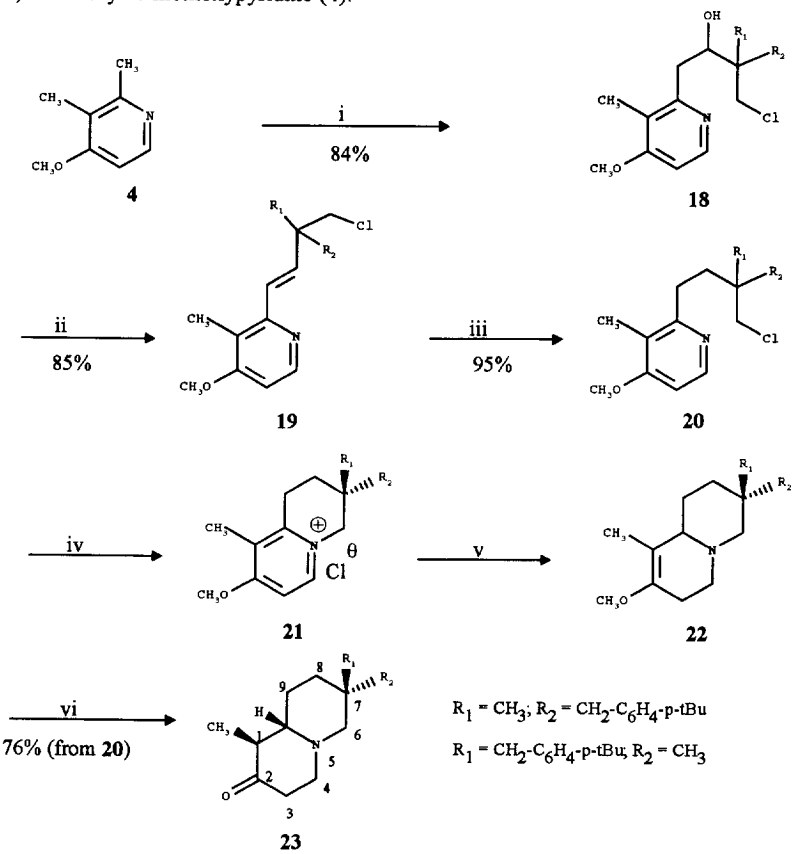
Unlike the parent example, this dihalogeno compound **16** proved totally unreactive with the lithiated derivative of **4** (see scheme 6). Obviously steric hindrance prevents nucleophilic substitution SN_2.

An alternative route for carbon-carbon bond formation would be to add the lithiated derivative of **4** to an appropriate aldehyde ; such additions are known to be less dependant on steric hindrance. The required aldehyde **17** was synthesized by oxidation of the chloropropanol **15** with PCC[13] (scheme 5).

The addition of picolyllithium obtained by metalation of **4** to aldehyde **17** afforded the expected alcohol **18** in good yield (84%) (scheme 6). Dehydration of the secondary alcohol **18** in the presence of triphenylphosphine and carbon tetrachloride[14] gave the alkene **19**. Hydrogenation of the double bond afforded compound **20** in an excellent yield (95%).

The pyridinium salt **21** was obtained by intramolecular quaternarization in refluxing ethanol. Reduction of **21** with sodium borohydride afforded the enol-ether **22**

which was then hydrolyzed into ketones **23**. The overall yield was 51% starting from 2,3-dimethyl-4-methoxypyridine (**4**).



4 **18** **19** **20** **21** **22** **23**

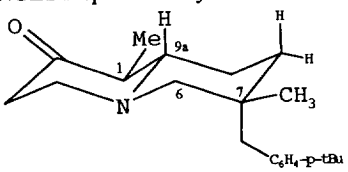
(i) a) n-BuLi, THF, -30°C, b) **17**, THF, -60°C ; (ii) PPh_3, CCl_4, CH_3CN, reflux ; (iii) H_2, Pd-C 10%, EtOH, AcOH ; (iv) EtOH, reflux ; (v) $NaBH_4$, MeOH, r.t. ; (vi) HCl 10%, r.t.

Scheme 6

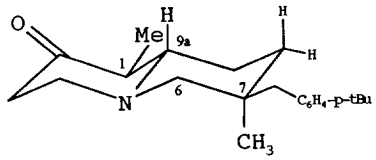
As shown by ^{13}C NMR analysis of crude **23**,[9] four diastereoisomers were formed in a 4:4:1:1 ratio . Flash chromatography on silicagel delivered two pure diastereoisomers, **23a** and **23b**, each one in a racemic mixture.

Because **23** presents four stereogenic centers, sixteen stereoisomers could be obtained. Nevertheless several features of the intermediates make the synthesis more selective. On one hand, it could be expected, as for compound **12**, that major stereoisomers bear the methyl group at C-1 in an equatorial position in a bicyclic

structure having a *trans*-junction. On the other hand, a new stereogenic center at C-7 is now present. Finally, four major stereoisomers were isolated as two racemates **23a** and **23b**(scheme 7), the structures of which were established by IR, ^1H NMR, COSY and NOESY spectra analysis.



cis **23a** 1S*, 7R*,9aS*

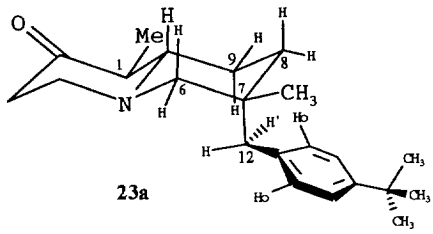


trans **23b** 1S*, 7S*,9aS*

Scheme 7

The main difference between the two stereoisomeric pairs is the relative position of benzyl and methyl groups at C-7. Structures having (C-7)-benzyl bond and (C-1)-(C-9a) bond in a cis position have been called cis and the others trans.

Trans-jonction of the two fused rings is confirmed by the presence of Bohlmann bands[15] in IR spectra of **23a** and **23b** at 2760 and 2800 cm^{-1}. ^1H NMR spectra of the two quinolizidinones **23a** and **23b** present a doublet at 0.98 ppm and 1.02 ppm respectively which can be attributed to the 1-Me group in an equatorial position as seen before for compound **12**. In the same manner ^{13}C NMR spectra show signals at 9.8 ppm for the equatorial 1-Me group. Moreover, ^1H NMR spectra of the two quinolizidones **23** show different features attributed to the methyl group and to the methylene of the benzyl group at C-7. On one hand, the signals of the methylenic hydrogen of the benzyl group are quite different. For compound **23b**, there is a singlet at 2.40 ppm and for compound **23a**, there is an AB pattern ($J = 13.0$ *Hz*) at 2.54 and 2.93 ppm. Finally, the NOESY spectra of compound **23a**, recorded on a Bruker Avance DMX 500 spectrometer in $CDCl_3$ revealed conclusive correlations for H_{8eq}/H_O and H_{6eq}/H_O, H_{12}/H_{9ax}, H_{12}/H_{6eq} and H'_{12}/H_{9ax}, H'_{12}/H_{8eq} (scheme 8).[16]

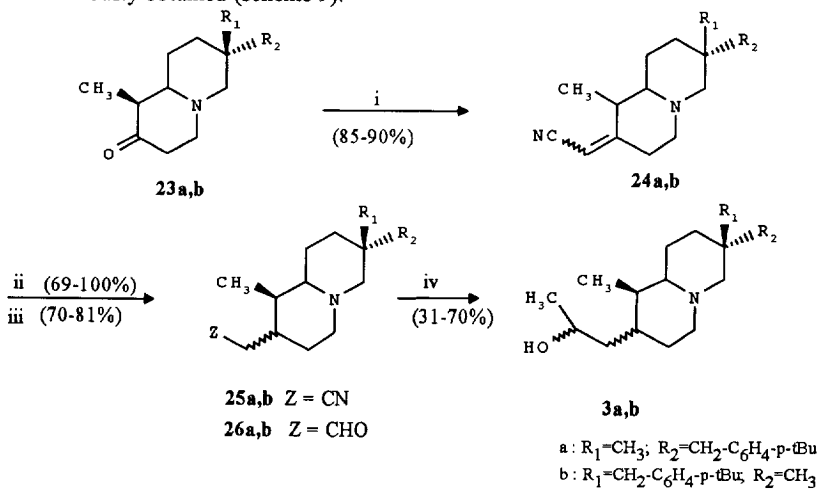


23a

Scheme 8

On the other hand, Moneyhan[17] has shown that for 2-methylquinolizidines , the axial methyl group close to the electron pair of nitrogen is deshielded (δ = 1.08 ppm) compared to the equatorial methyl group (δ = 0.82 ppm). In the same way, the singlet at 1.02 ppm was ascribed to the trans isomer **23b** in which the methyl group is in an axial position, the singlet at 0.71 ppm was ascribed to the equatorial methyl group of cis isomer **23a**.

Wittig-Horner reaction of quinolizidinones **23a** with diethyl cyanomethylphosphonate according to Corey's procedure[18] led to compounds **24a**. Sequential reductions of the double bond with H_2 and of the cyano group with diisobutylaluminium hydride[19] afforded the substituted aldehydes **26a**. Synthesis of compounds **3a** was performed by reaction of methyllithium in the presence of cerium trichloride to prevent enolization.[20] The C-7 epimeric mixture **23b** was converted to **3b** in a similar way. The stereochemistry of the new stereogenic centers at C-2 and at the C bearing the secondary alcohol were not controled and mixtures of stereoisomers were obviously obtained (scheme 9).



23a,b **24a,b**

25a,b Z = CN
26a,b Z = CHO

3a,b

a : R_1=CH_3; R_2=CH_2-C_6H_4-p-tBu
b : R_1=CH_2-C_6H_4-p-tBu; R_2=CH_3

(i) $(EtO_2)PO$-CH_2CN, NaH, DME, argon ; (ii) H_2, Pd-C 10%, AcOH ; (iii) DIBAH, Hexane, -70°C ; (iv) CH_3Li, $CeCl_3$, THF, -70°C, 12h.

Scheme 9

In conclusion, compounds **3a** and **3b** have been synthetized starting from 2,3-dimethylpyridine (**5**) in 1.15% and 4.65% overall yields, respectively. These compounds were tested for their activity on the $\Delta^8 \rightarrow \Delta^7$ sterol isomerase by using the cell-free system from *Saccharomyces cerevisiae* as described by Masner.[21] Isomer **3b** proved to be an inhibitor of $\Delta^8 \rightarrow \Delta^7$ isomerase as strong as fenpropidin used as standard whereas isomer **3a** was slightly less active (IC_{50} = 0.96 μM). The synthesis of further potential inhibitors of ergosterol biosynthesis by variation of substituents at C-7 of **3** can easily be achieved by use of other biselectrophiles in the metalation step (scheme 6).

EXPERIMENTAL PART

^1H NMR spectra were recorded in $CDCl_3$ on a VARIAN 360L (60 MHz) spectrometer, on a BRUCKER AC 200F (200 MHz) or on a BRUCKER AM 360 (360 MHz) spectrometer. Chemical shifts are in parts per million with respect to TMS in $CDCl_3$. Melting points were determined on a Kofler apparatus. Infrared spectra were recorded on a BECKMAN IR 4250 spectrometer. Flash chromatography was carried out on MERCK Silica gel 60, 0.063-0.200 mm (70-730 mesh ASTM). Microanalyses were performed on a CARLO ERBA 1106 apparatus. Mass spectral data were obtained on a JEOL JMS-AX 500 mass spectrometer.

2,3-Dimethyl-4-nitropyridine (**8**). A solution of **7** (46 g, 0.273 mol) in CH_2Cl_2 (180 ml) was cooled to -20°C. A solution of freshly distilled PCl_3 (30 ml) in CH_2Cl_2 (40 ml) was then added and the temperature maintained at -20°C for 15 min after addition. The solution was heated to r.t. and stirred for 15 min. After cooling to -20°C, cold water (30 ml) was added and the solution neutralized with diluted aqueous NaOH. The organic layer was washed with water, dried ($MgSO_4$) and evaporated to dryness to afford compound **8** (38.3 g, 92%) which was used in the next step without purification. ^1H NMR (60 MHz, $CDCl_3$) δ 2.4 (s, 3H), 2.7 (s, 3H), 7.4 (d, *J = 5.5 Hz*, 1H), 8.5 (d, *J = 5.5 Hz*, 1H).

2,3-Dimethyl-4-methoxypyridine (**4**). K_2CO_3 (70 g, 0.51 mol) was added to a stirred solution of **8** (38.85 g, 0.255 mol) in anhydrous methanol (510 ml). The mixture was refluxed for 20 h and solvent was evaporated. Water (50 ml) and ether (150 ml) were then added, the aqueous layer removed and organic layer washed with water (50 ml), dried ($MgSO_4$) and evaporated to dryness. Distillation under reduced pressure yielded 2,3-dimethyl-4-methoxypyridine (26.9 g, 77%) as a colorless oil; bp : 100°C (15 mmHg). ^1H NMR (60 MHz, $CDCl_3$) δ 2.1 (s, 3H), 2.45 (s, 3H), 3.8 (s, 3H), 6.6

(d, *J = 5.5 Hz,* 1H), 8.2 (d, *J = 5.5 Hz,* 1H).IR (film) ν 1585, 1480, 1420, 1290, 1130, 1095, 1010, 810 cm^{-1}. Anal. calcd for $C_8H_{11}NO$: C, 70.04; H, 8.08; N, 10.21. Found : C, 69.80; H, 7.99; N, 10.23.

8-Methoxy-9-methyl-1,2,3,4-tetrahydroquinolizinium chloride (10). n-Butyllithium (1.6M solution in hexane, 1.5 ml, 2.2 mmol) was added dropwise to a solution of **4** (274 mg, 2 mmol) in anhydrous THF (15 ml) cooled at -25°C under argon. The solution was stirred at -25°C for 1 h. 1-Bromo-3-chloropropane (340 µl, 3.4 mmol) was then added dropwise and the mixture stirred at -30°C for 1 h. After hydrolysis with saturated aqueous NH_4Cl (5 ml) at 0°C, CH_2Cl_2 (50 ml) was added. The usual workup (the aqueous layer was removed and the organic layer was washed with water, dried ($MgSO_4$), filtered and evaporated to dryness) afforded a crude product which was diluted with absolute ethanol (20 ml) and brought to reflux for 20 h. Washings with hexane (4x5 ml) of the residue obtained after evaporation of the ethanol afforded a white solid (342 mg, 80%) which was used in the next step without further purification; mp : 162-164°C. ^1H NMR (60 MHz, $CDCl_3$) δ 2.15 (m, 4H), 2.3 (s, 3H), 3.2 (m, 2H), 4.2 (s, 3H), 4.8 (m, 2H), 7.55 (d, *J = 7Hz,* 1H), 9.3 (d, J = *7Hz,* 1H). IR (film) ν 3446, 1630, 1498, 1308, 1107 cm^{-1}.

1-Methylquinolizidin-2-ones (12a and 12b). To a solution of **10** (1.5 g, 7 mmol) in absolute methanol (50 ml) was added sodium borohydride (2.66 g, 70 mmol) in several portions for 36 h at r.t.. Aqueous K_2CO_3 (10 ml) was added and methanol was evaporated. Extraction with CH_2Cl_2 and evaporation in vacuo afforded a crude enol-ether **11** which was dissolved in 10% aqueous HCl (20 ml) and stirred overnight at r.t.. After addition of K_2CO_3, until neutral pH, the aqueous layer was extracted with CH_2Cl_2 (50 ml). After usual workup, crude **12** was purified by distillation under reduced pressure to give a slightly yellow oil (937 mg, 80%); bp : 120°C (15 mm Hg). ^1H NMR (200 MHz, $CDCl_3$) δ 1.0 (d, *J = 6.7 Hz,* 2.4H), 1.18 (d, *J = 7.1 Hz,* 0.6H), 1.20-1.25 (m, 2H), 1.45-1.8 (m, 4H), 1.8-2.05 (m, 2H), 2.1-2.4 (m, 3H), 2.55-2.75 (m, 1H), 2.8-3.05 (m, 2H); ^{13}C NMR ($CDCl_3$) **12a** δ 9.5, 23.2, 25.1, 31.2, 41.1, 49.4, 55.4, 55.8, 67.9, 209.6; **12b** 12.0, 23.3, 24.9, 28.1, 37.8, 49.1, 55.6, 64.1, 212.5. IR (film) ν 2934, 2856, 2801, 2760, 1716, 1363, 1351, 1290 cm^{-1}. Anal. calcd for $C_{10}H_{17}NO$: C, 71.81; H, 10.25; N, 8.38. Found : C, 71.54; H, 10.40; N, 8.51.

Diethyl methyl-(p-*tert*-butylbenzyl)malonate (13). Sodium (1.8 g, 78.2 mmol) was added to absolute ethanol (60 ml). Then diethyl methylmalonate (8.6 ml, 50 mmol) was added dropwise and the mixture was stirred at r.t. for 2 h. The mixture was cooled at 0°C and p-*tert*-butylbenzyl bromide (13.8 ml, 75 mmol) was added. After

refluxing for 3 h and then cooling, water (20 ml) and CH_2Cl_2 (200 ml) were added. Usual workup gave the raw product which was purified by distillation under reduced pressure (13.8 g, 86%); bp : 145°C (1.5 mm Hg). ^1H NMR (60 MHz, $CDCl_3$) δ 1.28 (t, *J = 7Hz*, 6H), 1.33 (s, 9H), 1.38 (s, 3H), 3.2 (s, 2H), 4.2 (q, *J = 7Hz*, 4H), 7.07 (d, *J = 10Hz*, 2H), 7.31 (d, *J = 10Hz*, 2H). Anal. Calcd for $C_{19}H_{28}O_4$: C, 71.22; H, 8.81. Found : C, 71.01; H, 8.97.

2-Methyl-2-(p-*tert*-butylbenzyl)propan-1,3-diol (14). To a suspension of $LiAlH_4$ (2.34 g, 61.7 mmol) in dry ether (50 ml) was added dropwise a solution of **13** (13.15 g, 41.1 mmol) in dry ether (90 ml). The mixture was stirred for 4 h at r.t. and water (2.4 ml), 15 % aqueous NaOH (2.4 ml) and water (7.6 ml) were added. The precipitate was filtered off, washed with AcOEt and the organic layers concentrated in vacuo to give **14** (9.4 g, 97%). ^1H NMR (200 MHz, $CDCl_3$) δ 0.80 (s, 3H), 1.35 (s, 9H), 2.68 (s, 2H), 3.02 (s, 2H), 3.57 (s, 4H), 7.13 (d, *J = 10 Hz*, 2H), 7.32 (d, *J = 10 Hz*, 2H).

3-Chloro-2-methyl-2-(p-*tert*-butylbenzyl)propan-1-ol (15). To a solution of **14** (1.624 g, 6.88 mmol) in anhydrous THF (30 ml) cooled to -30°C under argon was added CCl_4 (1.92 ml, 19.9 mmol). Then freshly distilled hexamethylphosphorous triamide (1.25 ml, 6.88 mmol) was introduced dropwise within 0.5 h. The reaction mixture was stirred for 1.5 h at -30°C, then 3 h at r.t.. The solvent was then evaporated in vacuo, the crude salt was dissolved into freshly distilled DMF (9 ml) and the mixture was heated to 90-100°C for 16 h. DMF was then removed by distillation. A solution of the residue in ether was filtered on silicagel (eluent : ether). After removal of the solvent, the crude oil (1.57 g, 90 %) was used without further purification. ^1H NMR (60 MHz, $CDCl_3$) δ 0.9 (s, 3H), 1.3 (s, 9H), 2.3 (s, 1H), 2.6 (s, 2H), 3.45 (m, 4H), 7.1 (d, *J = 10 Hz*, 2H), 7.3 (d, *J = 10 Hz*, 2H).

1-Iodo-3-chloro-2-methyl-2-(p-*tert*-butylbenzyl)propane (16). To a stirred solution of **15** (514 mg, 2.02 mmol) in dry toluene (50 ml) were added in the following order : PPh_3 (2.098 g, 8 mmol), imidazole (545 mg, 8 mmol) and I_2 (1.52 g, 6 mmol). The mixture was refluxed for 18h, cooled and treated with aqueous $NaHCO_3$ (50 ml). After stirring for 5 min, a small quantity of I_2 was added to colorize the organic layer, the mixture stirred for 10 min and excess I_2 reduced with $Na_2S_2O_3$ until decolorization. The organic layer was washed with water (20 ml), dried ($MgSO_4$) and concentrated under reduced pressure. Crude **16** was purified by chromatography on silicagel (eluent : n-hexane) to give a pale yellow oil (550 mg, 75%). ^1H NMR (60 MHz, $CDCl_3$) δ 1.05 (s, 3H), 1.3 (s, 9H), 2.7 (s, 2H), 3.25 (s, 2H), 3.4 (s, 2H), 7.1 (d, *J = 10 Hz*, 2H), 7.3

(d, *J = 10 Hz*, 2H). Anal. calcd for $C_{15}H_{22}ClI$: C, 49.4; H, 6.08. Found : C, 49.5; H, 6.14.

3-Chloro-2-methyl-2-(p-*tert*-butylbenzyl)propanal (17). A solution of **15** (508 mg, 2 mmol) in dry CH_2Cl_2 was added to a suspension of pyridinium chlorochromate (647 mg, 3 mmol) in dry CH_2Cl_2 (4 ml) at r.t.. The mixture was stirred for 2 h and dry Et_2O (3 ml) introduced. The reaction mixture was filtered, the solid was washed with dry Et_2O and the combined organic filtrates filtered on Florisil and concentrated in vacuo. Purified product (colorless oil) was obtained by flash chromatography (eluent : ether/n-hexane, 1:1) (0,39 g, 78%). ^1H NMR (200 MHz, $CDCl_3$) δ 1.20 (s, 3H), 1.35 (s, 9H), 2.88 (d, *J = 13.8 Hz*, 1H), 3.02 (d, *J = 13.8 Hz*, 1H), 3.55 (d, *J = 11.4 Hz*, 1H), 3.64 (d, *J = 11.4 Hz*, 1H), 7.12 (d, *J = 8.4 Hz*, 2H), 7.35 (d, *J = 8.4 Hz*, 2H), 9.67 (s, 1H). MS (EI) : m/z 252 (M^+).

2-(4-Chloro-2-hydroxy-3-methyl-3-(p-*tert*-butylbenzyl)butyl)-4-methoxy-3-methylpyridine (18). To a solution of **4** (180 mg, 1.31 mmol) in anhydrous THF (10 ml) cooled at -30°C under argon, was added dropwise n-butyllithium (2.5 M solution in hexane, 640 µl, 1.6 mmol). The mixture was stirred at -30°C for 1 h and cooled to -60°C before introduction of a solution of **17** (383 mg, 1.6 mmol) in anhydrous THF (4 ml). After 3 h at -60°C, the reaction mixture was hydrolyzed at -10°C with saturated aqueous NH_4Cl (5 ml). Aqueous layer was extracted with AcOEt (50 ml) and the combined organic layers were concentrated in vacuo. The crude product was purified by flash chromatography on silicagel (eluent : AcOEt/n-hexane 7:3) to afford a white solid (0.43 g, 84%) as a mixture of two diastereomers : ^1H NMR (200 MHz, $CDCl_3$) δ 1.02 (s, 3H), 1.32 (s, 9H), 2.18 (s, 3H), 2.70-3.15 (m, 4H), 3.37 (d, *J = 11.4 Hz*, 0.5H), 3.42 (d, *J = 11.4 Hz*, 0.5H), 3.70 (d, *J = 11.4 Hz* ,0.5H), 3.78 (d, *J = 11.4 Hz*, 0.5H), 3.85 (s, 3H), 4.15 (m, 1H), 6.70 (d, *J = 6.2 Hz*, 1H), 7.3 (m, 4H), 8.25 (d, *J = 6.2 Hz*, 1H). IR (film) ν 3300, 2960, 2900, 2860, 1590, 1480, 1290, 1270, 1100 cm^{-1}.Anal. calc for $C_{23}H_{32}ClNO_2$: C, 70.84; H, 8.27; N, 3.59. Found : C, 70.48; H, 8.34; N, 3.45.

2-(4-Chloro-3-methyl-3-(p-*tert*-butylbenzyl)-(E)-but-1-enyl)-4-methoxy-3-methylpyridine (19). To a refluxed solution of **18** (113 mg, 0.29 mmol) and PPh_3 (91 mg, 0.35 mmol) in dry CH_3CN (2 ml) was added CCl_4 (28µl,0.29 mmol). After 20 min, the solvent was evaporated and water (3 ml) added. The aqueous layer was extracted with CH_2Cl_2 (20 ml), the organic layer was dried ($MgSO_4$) and concentrated in vacuo. The crude product was purified by flash chromatography on silicagel (eluent : AcOEt/n-hexane 6:4) to afford a colorless oil (92 mg, 85 %). ^1H NMR (200 MHz,

$CDCl_3$) δ 1.25 (s, 3H), 1.31 (s, 9H), 2.15 (s, 3H), 2.83 (d, *J = 13.4 Hz,* 1H), 2.93 (d, *J = 13.4 Hz,* 1H), 3.54 (s, 2H), 3.85 (s, 3H), 6.56 (d, *J = 15.8 Hz,* 1H), 6.65 (d, *J = 5.6 Hz,* 1H), 6.88 (d, *J = 15.8 Hz,* 1H), 7.15 (d, *J = 8.2 Hz,* 2H), 7.29 (d, *J = 8.2 Hz,* 2H), 8.35 (d, *J = 5.6 Hz,* 1H). IR (film) ν 2960, 2860, 1650, 1570, 1515, 1470, 1440, 1270, 1100, 975, 740 cm^{-1}. Anal.calcd for $C_{23}H_{30}ClNO$: C, 74.27; H, 8.13; N, 3.77. Found : C, 74.10; H, 8.11; N, 3.80.

2-(4-Chloro-3-methyl-3-(p-*tert*-butylbenzyl)butyl)-4-methoxy-3-methyl pyridine (20). To a solution of **19** (2.468 g, 6.64 mmol) in absolute ethanol (110 ml) was added 10% Pd on charcoal (245 mg) and acetic acid (760 ml, 15.3 mmol). The mixture was stirred under H_2 atmosphere. After the end of absorption, the reaction mixture was filtered on Celite and eluted with ethanol. The solvent was evaporated in vacuo. To the residue was added aqueous K_2CO_3 (5 ml) and the aqueous layer was extracted with CH_2Cl_2 (20 ml). The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The crude slightly yellow oil (2.35 g, 95 %) was used in the next step without further purification. ^1H NMR (200 MHz, $CDCl_3$) δ 1.04 (s, 3H), 1.31 (s, 9H), 1.77 (m, 2H), 2.20 (s, 3H), 2.62 (d, *J = 13.4 Hz,* 1H), 2.77 (d, *J = 13.4 Hz,* 1H), 2.84 (m, 2H), 3.41 (s, 2H), 3.85 (s, 3H), 6.64 (d, *J = 5.7 Hz,* 1H), 7.16 (d, *J = 8.3 Hz,* 2H), 7.3 (d, *J = 8.3 Hz,* 2H), 8.29 (d, *J = 5.7 Hz,* 1H). IR (film) :ν 2960, 2860, 1580, 1510, 1475, 1460, 1435, 1285, 1095, 740 cm^{-1}. MS (EI) : m/z 373 (M+).

3,9-Dimethyl-8-methoxy-3-(p-*tert*-butylbenzyl)-1,2,3,4-tetrahydro quinolizinium chloride (21). A solution of **20** (3.6 g, 9.6 mmol) in absolute ethanol (12 ml) was refluxed for 68 h. The ethanol was evaporated, a crude oil was obtained in a quantitative yield (3.6 g) and used in the next step without further purification. ^1H NMR (200 MHz, $CDCl_3$) δ 0.95 (s, 3H), 1.21 (s, 9H), 1.80 (m, 2H), 2.15 (s, 3H), 2.61 (m, 2H), 3.00 (m, 2H), 4.05 (s, 3H), 4.35 (s, 2H), 6.98 (d, *J = 8.3 Hz,* 2H), 7.23 (d, *J = 8.3 Hz,* 2H), 7.6 (d, *J = 7.2 Hz,* 1H), 9.21 (d, *J = 7.2 Hz,* 1H).

1,7-Dimethyl-2-methoxy-7-(p-*tert*-butylbenzyl)quinolizid-1-ene (22). Compound **21** (3.6 g, 9.6 mmol) was dissolved in absolute methanol (100 ml) and sodium borohydride (3.63 g, 96 mmol) was added by portions within 12 h. The mixture was stirred for 12 h and the solvent removed. K_2CO_3 (10 ml) and CH_2Cl_2 (50 ml) were added. The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The crude product (3.27 g) was used without further purification in the next step. ^1H NMR (200 MHz, $CDCl_3$) δ 0.8 (s, 3H), 1.05 (s, 3H), 1.3 (s, 9H), 1.65-3.05 (m, 13H), 3.55 (s, 3H), 7.1 (d, *J = 8.3 Hz,* 2H), 7.3 (d, *J = 8.3 Hz,* 2H).

1,7-Dimethyl-7-(p-*tert*-butylbenzyl)quinolizidin-2-ones (23). The crude compound **22** (3.27 g) was dissolved in 10% aqueous HCl (50 ml). The mixture was stirred overnight and then K_2CO_3 was added to bring the solution to pH 9. The aqueous layer was extracted with Et_2O (200 ml). The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The crude product was purified by flash chromatography on silicagel (eluent : n-hexane/ether 1:1) to afford two compounds **23a** and **23b**. Isomer **23a** : white solid (1.16 g, 37 % from **20**) : mp : 78°C. ^1H NMR (360 MHz, $CDCl_3$) δ 0.71 (s, 3H), 0.98 (d, *J = 6.6 Hz*, 3H), 1.04 (m, 1H), 1.26 (s, 9H), 1.50 (m, 1H), 1.65 (m, 3H), 1.77 (m, 1H), 2.23 (m, *J = 2.8, 10.6* and *11.4 Hz*, 1H), 2.32 (m, *J = 13.4 Hz*, 1H), 2.34 (m, 1H), 2.47 (dd, *J = 2.3* and *11.4 Hz*, 1H), 2.54 (d, *J = 13.0 Hz*, 1H), 2.73 (td, *J = 1.3* and *6.4 Hz*, 1H), 2.93 (d, *J = 13.0 Hz*, 1H), 2.96 (m, 1H), 7.05 (d, *J = 7.6 Hz*, 2H), 7.25 (d, *J = 7.6 Hz*, 2H). ^{13}C NMR ($CDCl_3$) δ 9.9, 26.1, 28.2, 31.4, 34.0, 34.6, 35.0, 41.2, 41.6, 49.7, 55.8, 64.7, 68.3, 124.5, 130.2, 136.2, 148.4, 210.6. IR (KBr) ν 2960, 2920, 2860, 2800, 2760, 1720, 1510, 1465, 1365 cm^{-1}. MS (EI) : m/z 327 (M+). Anal. calcd for $C_{22}H_{33}NO$: C, 80.68; H, 10.16; N, 4.28. Found : C, 80.52; H, 10.32; N, 4.06. Isomer **23b** : white solid (1.16 g, 37 % from **20**) : mp : 112°C. ^1H NMR (360 MHz, $CDCl_3$) δ 0.95 (d, *J = 6.6 Hz*, 3H), 1.02 (s, 3H), 1.15 (m, 1H), 1.26 (s, 9H), 1.4 (m, 2H), 1.63 (m,1H), 1.75 (m, 1H), 1.95 (d, *J = 11 Hz*, 1H), 2.25 (m, 3H), 2.40 (s, 2H), 2.50 (d, *J = 11 Hz*, 1H), 2.65 (m, 1H), 2.95 (m, 1H), 7.0 (d, *J = 7.6 Hz*, 2H), 7.2 (d, *J = 7.6 Hz*, 2H). IR (KBr) ν 2960, 2940, 2860, 2800, 2760, 1720, 1520, 1460, 1360 cm^{-1}. MS (EI) : m/z 327 (M+). Anal. calcd for $C_{22}H_{33}NO$: C, 80.68; H, 10.16; N, 4.28. Found : C, 80.55; H, 10.56; N, 3.98.

2-Cyanomethylene-1,7-dimethyl-7-(p-*tert*-butylbenzyl)quinolizidine (24a). To a suspension of NaH (60% in mineral oil, 444 mg, 11.1 mmol) in distilled DME (20 ml) under argon was added a solution of diethyl cyanomethylphosphonate (1.966 g, 11.1 mmol) in distilled DME (5 ml). The mixture was stirred for 5 min and a solution of **23a** (1.209 g, 3.7 mmol) in distilled DME (10 ml) was added. The mixture was stirred for 27 h at r.t. and poured into water (100 ml). The aqueous layer was extracted with CH_2Cl_2 (200 ml), the organic layer was dried ($MgSO_4$) and concentrated under vacuo. The residue was purified by flash chromatography on silicagel (eluent : n-hexane/ether 3:1) to give **24a** (1.10 g, 85%) : mp: 106°C. ^1H NMR (200 MHz, $CDCl_3$) δ 0.75 (s, 3H), 1.05 (d, *J = 6.6 Hz*, 3H), 1.25 (m, 1H), 1.35 (s, 9H), 1.4-1.75 (m, 5H), 1.8 (m, 1H), 2.1 (m, 1H), 2.3 (m, 1H), 2.5 (m, 3H), 2.95 (m, 2H), 5.05 (s, 1H), 7.05 (d, *J = 7.6 Hz*, 2H), 7.25 (d, *J = 7.6 Hz*, 2H). IR (KBr) ν 2960, 2900, 2860, 2800, 2760, 2365, 2220, 1635, 1460, 1365, 1110 cm^{-1}. Anal.calcd for $C_{24}H_{34}N_2$: C, 82.23; H, 9.78; N, 7.99. Found : C, 82.12; H, 10.12; N, 7.74.

2-Cyanomethylene-1,7-dimethyl-7-(p-*tert*-butylbenzyl)quinolizidine (24b). Isomer **24b** was prepared as described above and purified by flash chromatography on silicagel (eluent : ether) to afford a slightly yellow solid (1.165 g, 90%): mp: 142-144°C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.0 (m, 6H), 1.2 (m, 1H), 1.3 (s, 9H), 1.4 (m, 2H), 1.6-2.0 (m, 3H), 2.0-2.25 (m, 2H), 2.45 (s, 2H), 2.45 (m, 2H), 2.9 (m, 2H), 5.05 (s, 1H), 7.05 (d, *J = 7.6 Hz*, 2H), 7.25 (d, *J = 7.6 Hz*, 2H). IR (KBr) ν 2960, 2900, 2860, 2800, 2760, 2215, 1630, 1460, 1365, 1015 cm^{-1}. Anal. calcd for $C_{24}H_{34}N_2$: C, 82.23; H, 9.78; N, 7.99. Found : C, 82.32; H, 10.10; N, 7.79.

2-Cyanomethyl-1,7-dimethyl-7-(p-*tert*-butylbenzyl)quinolizidines (25a). To a solution of **24a** (1.17 g, 34 mmol) in AcOH (30 ml) was added 10% Pd-C (117 mg). The reaction mixture was stirred under H_2 atmosphere. When absorption was finished, the mixture was filtered on Celite and eluted with ethanol. The filtrate was concentrated in vacuo, and aqueous K_2CO_3 (15 ml) added to the residue. The aqueous solution was extracted with Et_2O (2x20 ml), the organic phase was dried ($MgSO_4$) and concentrated in vacuo. The mixture of diastereomers **25a** was isolated by flash chromatography on silicagel (eluent : n-hexane/ether 3:2) as a pale yellow oil (826 mg, 69%). ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.75 (s, 3H), 0.95 (d, *J = 6Hz*, 3H), 1.1 (m, 1H), 1.3 (s, 9H), 1.4-1.75 (m, 4H), 1.75-2.2 (m, 5H), 2.3-2.65 (m, 5H), 2.75-3.0 (m, 2H), 7.1 (d, *J = 7.6 Hz*, 2H), 7.3 (d, *J = 7.6 Hz*, 2H). Anal. calcd for $C_{24}H_{36}N_2$: C, 81.76; H, 10.29; N, 7.94. Found : C, 81.54; H, 10.22; N, 8.01.

2-Cyanomethyl-1,7-dimethyl-7-(p-*tert*-butylbenzyl)quinolizidines (25b). The mixture of diastereomers **25b** was prepared as described above and purified by flash chromatography on silicagel (eluent : ether/n-hexane 1:1) as a yellow solid (766 mg, 64%) : mp : 103°C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.9 (t, *J = 6Hz*, 3H), 1.0 (s, 3H), 1.2 (m, 1H), 1.3 (s, 9H), 1.4 (m, 2H), 1.6 (m, 1H), 1.65-2.2 (m, 6H), 2-2.5 (m, 5H), 2.55 (m, 1H), 2.75 (m, 1H), 7.05 (d, *J = 7.6 Hz*, 2H), 7.25 (d, *J = 7.6 Hz*, 2H). Anal. calcd for $C_{24}H_{36}N_2$: C, 81.76; H, 10.29; N, 7.94. Found : C, 82.02; H, 10.32; N, 7.64.

1,7-Dimethyl-2-formylmethyl-7-(p-*tert*-butylbenzyl)quinolizidines (26a). To a solution of **25a** (759 mg, 2.15 mmol) in distilled n-hexane (30 ml) cooled at -70°C under argon was added dropwise diisobutyl aluminium hydride (1M solution in hexane, 2.8 ml, 2.8 mmol) and the temperature was kept at -70°C for 4.5 h. The reaction mixture was hydrolyzed at -10°C with a saturated aqueous NH_4Cl solution (14 ml) and stirred for 15 min. Then a solution of sulfuric acid (333 µl) in water (7 ml) was added and the aqueous layer was extracted with Et_2O. Aqueous K_2CO_3 was added to bring

aqueous solution to pH 9 and extracted with AcOEt. The organic layers were dried ($MgSO_4$) and concentrated in vacuo. The mixture of diastereomers **26a** was isolated by flash chromatography on silicagel (eluent : n-hexane/ether 3/2) to yield a pale yellow oil (534 mg, 70%). ^1H NMR (200 MHz, $CDCl_3$) δ 0.75 (s, 3H), 0.95 (m, 3H), 1.2 (m, 1H), 1.3 (s, 9H), 1.4-2.2 (m, 9H), 2.3-2.8 (m, 6H), 2.9 (m, 1H), 7.1 (d, *J = 7.6 Hz,* 2H), 7.3 (d, *J = 7.6 Hz,* 2H), 9.8 (m, 1H). IR (film) ν 2960, 2900, 2800, 2755, 1715, 1465, 1365, 1125 cm^{-1}. Anal. calcd for $C_{24}H_{37}NO$: C, 81.07; H, 10.49; N, 3.94. Found : C, 81.29; H, 10.40; N, 3.81.

1,7-Dimethyl-2-formylmethyl-7-(p-*tert*-butylbenzyl)quinolizidines (26b). Compounds **26b** were prepared as described above starting from **25b** and were isolated by flash chromatography on silicagel (eluent : ether/n-hexane 1/1) to afford a yellow oil (618 mg, 81%).^1H NMR (200 MHz, $CDCl_3$) δ 0.95 (m, 3H), 1.0 (s, 3H), 1.2 (m, 1H), 1.3 (s, 9H), 1.4 (m, 2H), 1.5-2.0 (m, 6H), 2.2 (m, 1H), 2.4 (m, 5H), 2.7 (m, 1H), 2.75 (m, 1H), 7.05 (d, *J = 7.6 Hz,* 2H), 7.25 (d, *J = 7.6 Hz,* 2H), 9.8 (m, 1H). IR (film) ν 2960, 2900, 2800, 2760, 1725, 1465, 1365, 1125 cm^{-1}. Anal. calcd for $C_{24}H_{37}NO$: C, 81.07; H, 10.49; N, 3.94. Found : C, 80.77; H, 10.48; N, 3.81.

1,7-Dimethyl-2-(2-hydroxypropyl)-7-(p-*tert*-butylbenzyl)quinolizidines (3a). A suspension of $CeCl_3$ (468 mg, 1.89 mmol) in anhydrous THF (10 ml) was stirred under argon at r.t. for 2 h then cooled at -65°C. Methyllithium (1.6 M solution in ether, 1.27 ml, 2.04 mmol) was added dropwise. The mixture was stirred for 1 h and a solution of **26a** (520 mg, 1.46 mmol) in anhydrous THF (8 ml) was added. The mixture was stirred at -65°C for 4.5 h, saturated aqueous NH_4Cl was added at -10°C. The mixture was filtered on Celite and eluted with AcOEt. The aqueous phase was extracted with AcOEt, the organic layer was dried ($MgSO_4$) and concentrated in vacuo. Compounds **3a** were isolated by flash chromatography on silicagel as a mixture of diastereomers (eluent : n-hexane/ether 1/1) to give a pale yellow oil (168 mg, 31%). ^1H NMR (200 MHz, $CDCl_3$) δ 0.75 (s, 3H), 0.95 (m, 6H), 1.2 (m, 1H), 1.3 (s, 9H), 1.4-2.2 (m, 10H), 2.3-2.8 (m, 6H), 2.9 (m, 1H), 3.9 (m, 1H), 7.1 (d, *J = 7.6 Hz,* 2H), 7.3 (d, *J = 7.6 Hz,* 2H). MS (CI) : m/z 372 (MH^+). Anal. calcd for $C_{25}H_{41}NO$: C, 80.80; H, 11.12; N, 3.77. Found : C, 81.04; H, 11.04; N, 3.61.

1,7-Dimethyl-2-(2-hydroxypropyl)-7-(p-*tert*-butylbenzyl)quinolizidines (3b). Compounds **3b** were synthesized as described above. The only change was the stirring at -65°C overnight instead of 4.5 h. Compounds **3b** were isolated by flash chromatography on basic alumina (eluent : ether/n-hexane 2/3) to afford a yellow oil (379 mg, 70%). ^1H NMR (200 MHz, $CDCl_3$) δ 0.9 (m, 6H), 1.05 (s, 3H), 1.2 (m, 1H),

1.3 (s, 9H), 1.4 (m, 2H), 1.5-2.0 (m, 8H), 2.2 (m, 1H), 2.4 (m, 5H), 2.7 (m, 1H), 3.9 (m, 1H), 7.05 (d, *J = 7.6 Hz,* 2H), 7.25 (d, *J = 7.6 Hz,* 2H). MS (CI) : m/z 372 (MH^+) Anal. calcd for $C_{25}H_{41}NO$: C, 80.80; H, 11.12; N, 3.77. Found : C, 80.94; H, 11.41; N, 3.66.

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