HETEROCYCLES, Vol. 68, No. 2, 2006, pp. 257 - 270, © The Japan Institute of Heterocyclic Chemistry
Received, 25th October, 2005, Accepted, 12th December, 2005, Published online, 13th December, 2005. COM-05-10603

SYNTHESIS OF VINCA ALKALOIDS AND RELATED COMPOUNDS 103. RECOGNITION OF AN UNEXPECTED REACTION AND ITS APPLICATION IN BUILDING THE ASPIDOSPERMANE SKELETON. SIMPLE SYNTHESIS OF 15β-HYDROXYVINCADIFFORMINE

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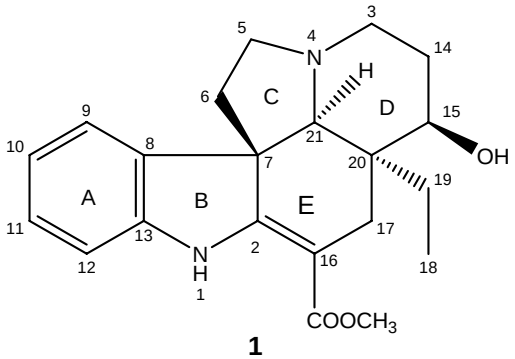
Abstract – Reaction of tryptamine derivative (**2b**) and acetate ester (**11**) built up from 2-(chloromethylene)butanal (**4**) resulted in enamino ketone (**14**). Dehydration of **14** and subsequent intramolecular [4+2] cycloaddition led to 15-oxovincadifformine (**15**). Regio- and stereoselective reduction of the latter molecule supplied 15β-hydroxyvincadifformine (**1**).

INTRODUCTION

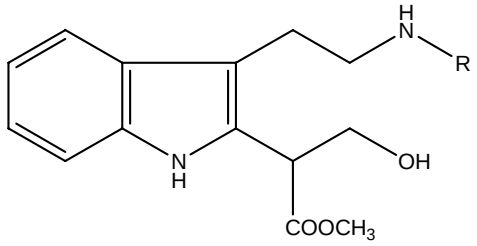
Atta-ur-Rahman and his co-workers were the first to isolate the title compound (**1**) from *Rhazya stricta*[1] in 1988. Research group of Li-Wei Guo found it also in *Melodinus hemsleyanus*[2] in 1993. Before its isolation, Kuehne *et al.* effected the total synthesis of 15β-hydroxyvincadifformine (**1**) when trying to produce another alkaloid [3].

Dedicated to Professor András Lipták on the occasion of his 70th birthday.



1

In our earlier publications we described an efficient convergent synthesis pathway to build up the aspidospermane skeleton, where we obtained structural units with the D-seco-aspidospermane skeleton from an N_b-benzyltryptamine derivative (**2a**) and appropriately arranged aldehydes (or aldahyde equivalents). As a result of the reactions synthese of numerous alkaloids or alkaloid-like molecules were effected by intramolecular acylation or alkylation [4-9].

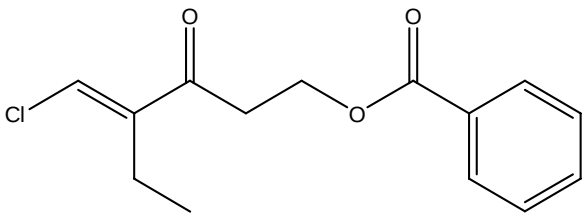


2a R: Bn
2b R: H

We also tried to build up **1**, however, we did not obtain the target alkaloid by the applied synthesis[10]. Analyzing the reaction pathways we set up a hypothesis by which we may obtain the alkaloid with the aspidospermane skeleton (**1**).

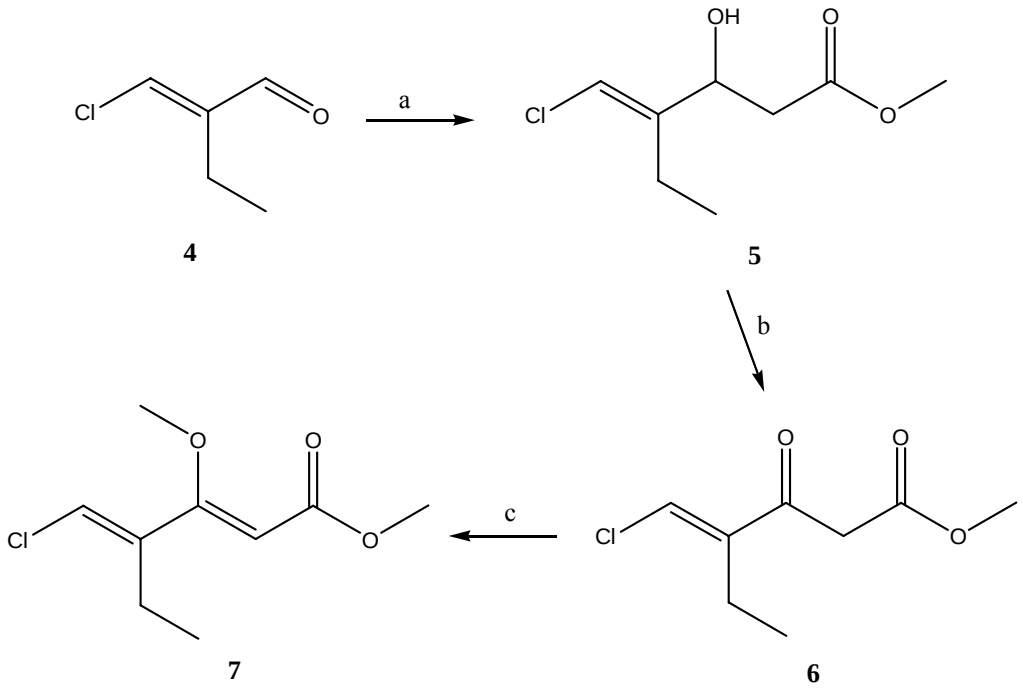
RESULTS AND DISCUSSION

As a substrate of the planned synthesis we selected **2a** proved to be effective previously, [4, 7] and we intended to apply as reaction partner the 4-chloromethylene-3-oxo-hexyl benzoate (**3**) being an aldehyde equivalent. In our opinion, by this reaction we can obtain a molecule with the D-seco-aspidospermane skeleton from which the target compound (**1**) can be built up by reduction after debenzylation and intramolecular alkylation.



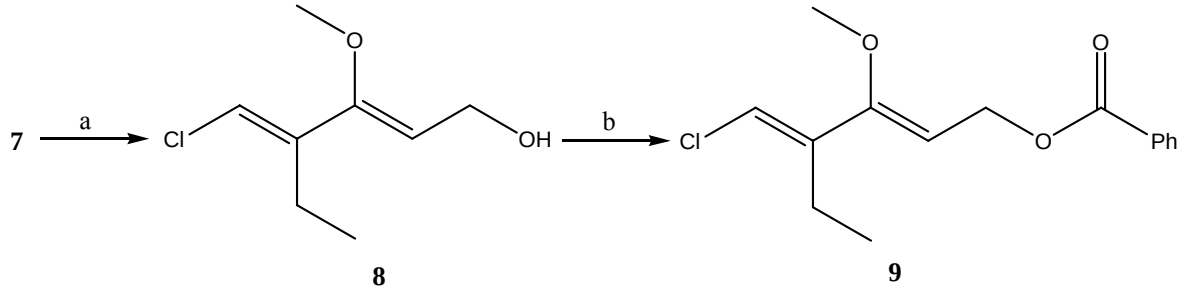
3

Starting from 2-(chloromethylene)butanal (**4**) [11] we produced **3**. Reformatsky reaction of **4** with methyl bromoacetate resulted in alcohol (**5**) in a good yield, from which we obtained **7** by Jones oxidation of **6**, followed by protection of the oxo group by dimethyl sulfate in the presence of sodium hydride (Scheme 1).



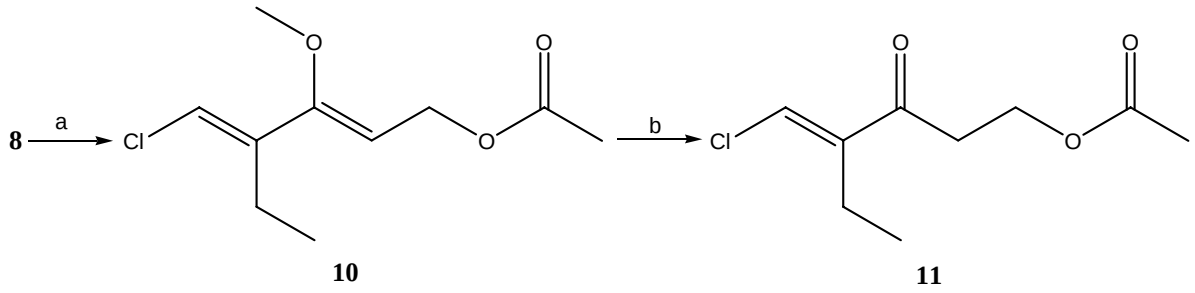
Scheme 1. Reagents and conditions: (a) $BrCH_2COOCH_3$, Zn, benzene (78%) , Δ; (b) CrO_3, H_2SO_4, acetone, rt (79%) ; (c) NaH, $(CH_3)_2SO_4$, HMPA, rt (70%).

Afterwards enol ether (**7**) was reduced with $LiAlH_4$ into alcohol (**8**), then acylated by benzoyl chloride (or benzoic acid). We could isolate the expected product (**9**) from the reaction mixture in a poor yield only, moreover the benzoate ester formed proved to be unstable (Scheme 2).



Scheme 2. Reagents and conditions: (a) $LiAlH_4$, ether, 0°C (85%); (b) PhCOCl, TEA, CH_2Cl_2, 0°C (20%) or PhCOOH, DCC, DMAP, CH_2Cl_2, rt (28%).

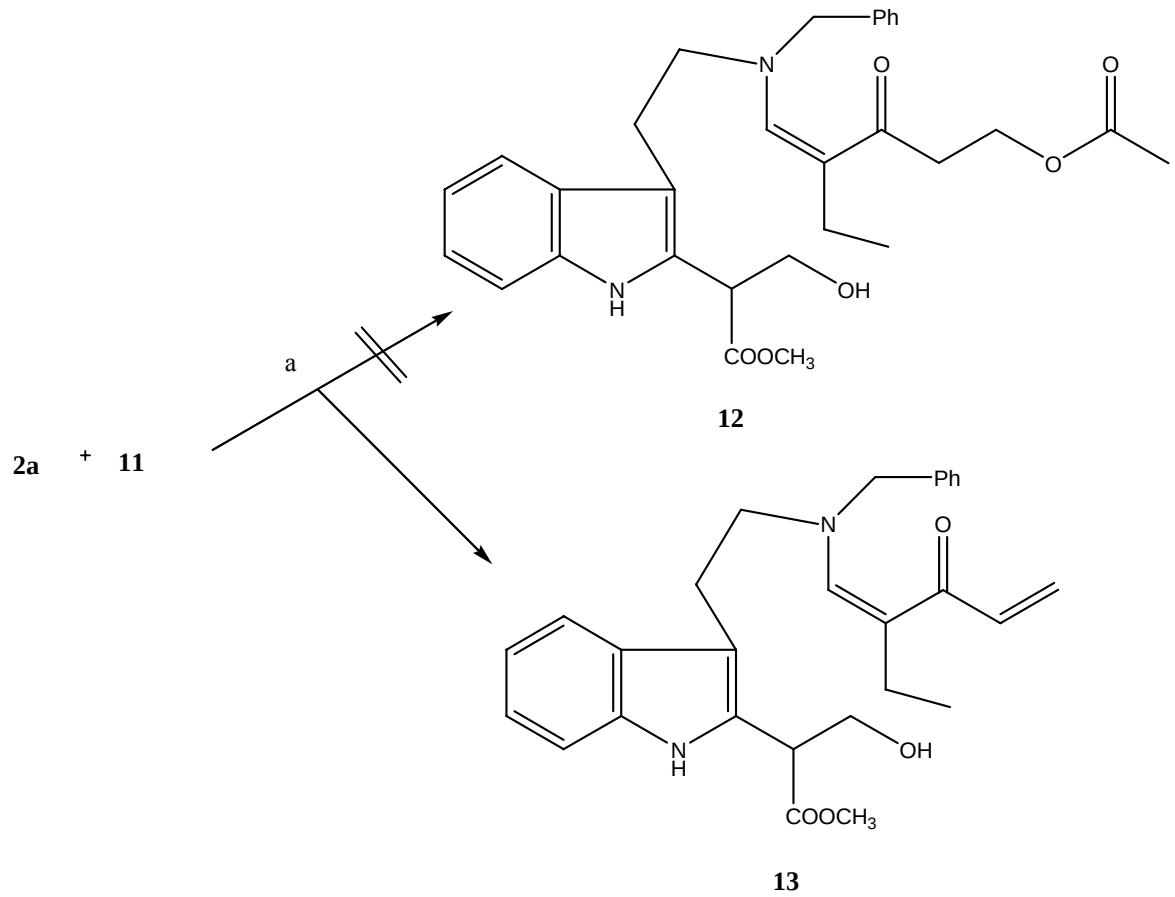
We found it obvious to study first the acetylation of molecule (**8**). Reaction of alcohol (**8**) with acetic anhydride resulted in a stable acetate (**10**) with a good yield. Compound (**11**) was obtained by boiling **10** in aqueous acetic acid, thus eliminating the protection group (Scheme 3).



10 **11**

Scheme 3. Reagents and conditions: (a) Ac_2O, TEA, CH_2Cl_2, rt (88%); (b) AcOH, H_2O, Δ (74%).

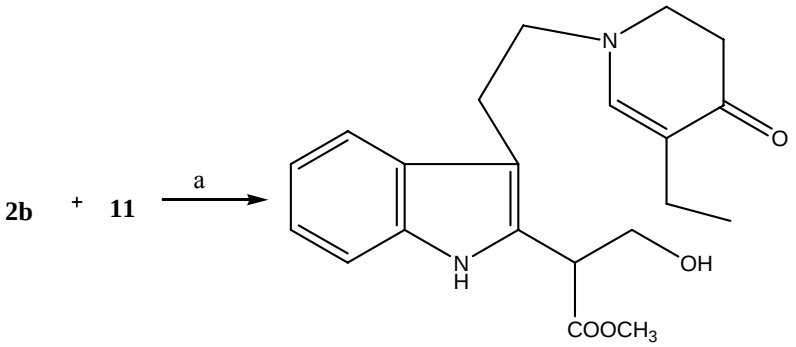
Then the secondary amine (**2a**) we allowed to react with activated vinyl chloride derivative (**11**) in methanol, in the presence of triethylamine. To our surprise, instead of the expected product (**12**) the enamino ketone with terminal (α, β) carbon-carbon double bond (**13**) was obtained by elimination of acetic acid (Scheme 4).



Scheme 4. Reagents and conditions: (a) CH_3OH, TEA, rt (65%).

In aware of this fact we reacted **11** with a tryptamine derivative containing primary amino group (**2b**)[10]. Supposing that the amino group of the substrate (**2b**) would first react with the activated vinyl halide derivative (**11**) also in this case, followed by intramolecular Michael addition to the conjugated carbon-carbon double bond formed after elimination of acetic acid would produce cyclic enamino ketone

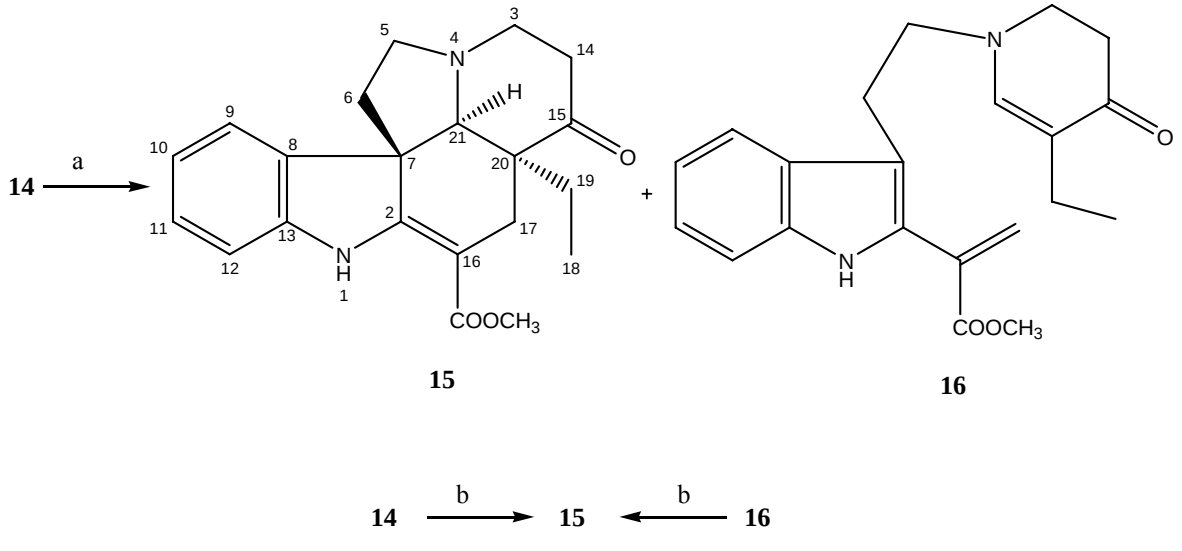
(**14**). We allowed **2b** to react with **11** in the presence of triethylamine and in accordance with our expectations, was obtained only **14** (Scheme 5).



14

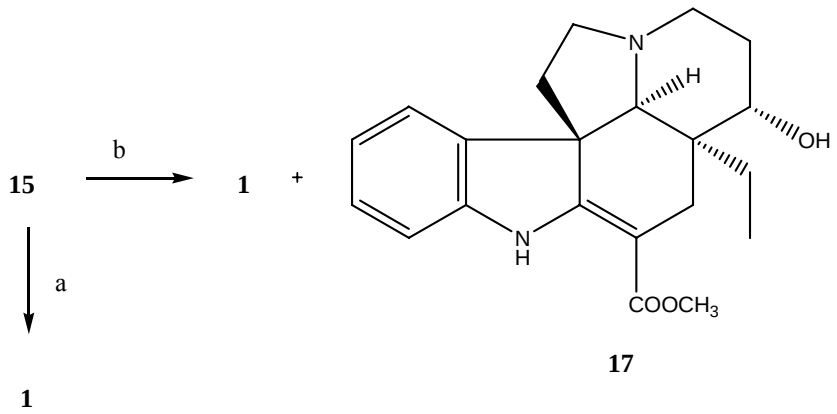
Scheme 5. Reagents and conditions: (a) CH_3OH, TEA, rt (74%).

Afterwards, the cyclic enamino ketone (**14**) was boiled in toluene in the presence of catalytic amounts of *p*-toluenesulfonic acid monohydrate yielding two products. On the one hand, we obtained the expected pentacyclic molecule with the aspidospermane skeleton (**15**)[3], on the other hand we succeeded in isolating **16**, the intermediary product of the Diels-Alder type cycloaddition reaction. Executing the above described reaction of **14** in xylene led to 15-oxovincadifformine (**15**)[3] exclusively (method I.). Reactoin of **16** in xylene supplied the same compound (method II.) (Scheme 6).



Scheme 6. Reagents and conditions: (a) $TsOH{\cdot}H_2O$, toluene, Δ (**15** (45%), **16** (23%)); (b) $TsOH{\cdot}H_2O$, xylene, Δ (method I. (68%), method II. (76%)).

After reduction of compound (**15**) by L-Selectride® we could isolate only one stereoisomer compound, the aimed isomer (**1**)[3]. Effecting the reduction in sodium borohydride, we obtained the mixture of both possible isomers (**1:17**=99:1 (by NMR spectrum)) (Scheme 7).



Scheme 7. Reagents and conditios: (a) L-Selectride®, THF, 0°C, (85%); (b) $NaBH_4$, CH_3OH, 0°C (86%).

CONCLUSION

Reaction of tryptamine derivative (**2a**) with benzoate ester (**3**), built up from 2-(chloromethylene)butanal (**4**), can not be realized due to the instability of the latter molecule. We obtained an unexpected product (**13**) in reaction of **2a** with acetate ester (**11**), and we utilized this observation advantageously through changing the substrate (**2b**) instead of **2a** (**2b**+**11**→**14**). After dehydration and intramolecular [4+2] cycloaddition of **14** we obtained 15-oxovincadifformine (**15**). The regio- and stereoselective reduction of **15** resulted in the 15β-hydroxyvincadifformine (**1**).

EXPERIMENTAL

Melting points were determined on a hot-stage microscope Boetius and are uncorrected. IR spectra were recorded on a Specord JR-75 spectrophotometer. NMR spectra were recorded on a Varian Unity INOVA-400 instrument at 400 MHz for ^1H and 100 MHz for ^{13}C. All NMR spectra were recorded at rt. J_{1r}, long range coupling constant. Chemical shifts are relative to Me_4Si (δ=0 ppm). Mutual ^1H-^1H couplings are given only once, at the first occurrences. MS spectra were recorded on a PE Sciex API 2000 triple-quadrupole mass spectrometer equipped with a Turbo Ion Spray source and VG ZAB2-SEQ tandem mass spectrometer (high resolution mass spectra). Preparative TLC analyses were performed on silica gel F_{254} plates, and column chomatography was carried out on Merck Kieselgel 60 (0.063-0.200 mm).

Methyl 4-(chloromethylene)-3-hydroxyhexanoate (5)

A 100 mL, 3-necked flask fitted with a condenser, mechanical stirrer, and 20 mL dropping funnel was flushed with nitrogen. Freshly activated zinc powder (1.2 g, 18 mmol), and dry benzene (10 mL) were placed into the flask. Methyl bromoacetate (2.3 g, 15 mmol), 2-(chloromethylene)butanal (**4**) (2.2 g, 18 mmol) and dry benzene (10 mL) were placed into the dropping funnel. Nitrogen was introduced into the apparatus *via* a septum on the condenser and released by a septum on the dropping funnel as outlet. Without stirring, the solution of methyl bromoacetate-aldehyde (2 mL) was added to the zinc suspension

and the mixture was cautiously brought to reflux. After *ca.* 10 min of gentle reflux the heating mantle was removed and the remined methyl bromoacetate and (**4**) aldehyde solution was dropped for maintaining the smooth reflux. After the addition the dark yellow reaction mixture was vigorously stirred and brought to reflux again by the heating mantle. After 1 h reflux, the reaction mixture became green and most of the zinc reacted. The reaction mixture was cooled and 10% aqueous solution of H_2SO_4 (15 mL), and ethyl acetate (15 mL) were added. The mixture was shaken well, and the two-phase system was filtered to remove remained zinc. The aqueous layer was extracted with ethyl acetate (3×15 mL). The combined organic extracts were washed with brine (2×20 mL), dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by column chromatography (eluting with acetone/hexane=1/5, R_f=0.45) to afford 2.7 g (78%) of the product **(5)** as yellow oil. IR (neat) ν_{max} 3472, 2976, 1720, 1632. HRMS (EI) calcd for $C_8H_{11}O_2Cl$ 174.0448, found for [M-H_2O^+] 174.0456. ^{1}H NMR δ_H ($CDCl_3$): 1.09 (3H, t, J_{vic}=7.5 Hz; C4-$CH_2C\underline{H}_3$), 2.16+2.32 (2×1H, 2×dq, J_{gem}=13.5 Hz; C4-C$\underline{H}_2CH_3$), 2.56 (1H, dd, J_{gem}=28 Hz, J_{vic}=16 Hz; 2-H_a), 2.59 (1H, dd, J_{gem}=32 Hz, J_{vic}=16 Hz; 2-H_b), 3.04 (1H, br; 3-OH), 3.73 (3H, s; OCH_3), 4.58 (1H, dd, J_{vic1}=9 Hz, J_{vic2}=3.2 Hz; 3-H), 6.21 (1H, br s; 5-H). ^{13}C NMR δ_C ($CDCl_3$): 12.23 (C4-$CH_2\underline{C}H_3$), 21.23 (C4-$\underline{C}H_2CH_3$), 40.07 (C2), 51.99 (OCH_3), 70.12 (C3), 116.10 (C5), 144.15 (C4), 172.65 (C1).

Methyl 4-(chloromethylene)-3-oxohexanoate (6)

Jones reagent was prepared by addition of concentrated H_2SO_4 (5 mL) to CrO_3 (5.6 g) followed by careful dilution with water (to get 42 mL of total solution). Then Jones reagent (18 mL, 18 mmol) was added dropwise to a stirred soluiton of **5** (2.7 g, 14 mmol) in acetone (70 mL) at 0^{o}C. After complete addition of the oxidizing agent, the mixture was allowed to warm up to rt and stirred for 12 h. Methanol (10 mL) was added to quench excess Jones reagent. The reaction mixture was extracted with ether (3×70 mL). The organic extracts were washed with water (3×70 mL) and then 5% aqueous solution of $NaHCO_3$ (50 mL). The combined organic phases were dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by column chromatography (eluting with ether/hexane=1:5, R_f=0.69) to afford 2.1 g (79%) of the product (**6**) as a yellow oil. IR (neat) ν_{max} 2976, 1744, 1680, 1620. HRMS (EI) calcd for $C_8H_{11}O_3Cl$ 190.0397, found for 190.0396. (oxo enol tautomers ~ 3:1) ^{1}H NMR δ_H ($CDCl_3$): oxo-form: 1.01 (3H, t, J_{vic}=7.5 Hz; C4-$CH_2C\underline{H}_3$), 2.50 (2H, q, J_{vic}=10 Hz; C4-C$\underline{H}_2CH_3$), 3.68 (2H, s; 2-H_2), 3.75 (3H, s; OCH_3), 7.23 (1H, s; 5-H); enol-form: 1.07 (3H, t, J_{vic}=7.5 Hz; C4-$CH_2C\underline{H}_3$), 2.41 (2H, q, J_{vic}=10 Hz; C4-C$\underline{H}_2CH_3$), 3.77 (3H, s; OCH_3), 5.28 (1H, s; =C2-H), 7.02 (1H, s; 5-H), 12.06 (1H, s; =C3-OH). ^{13}C NMR δ_C ($CDCl_3$): oxo-form: 12.01 (C4-$CH_2\underline{C}H_3$), 20.03 (C4-$\underline{C}H_2CH_3$), 45.40 (C2), 52.56 (OCH_3), 134.58 (C5), 145.37 (C4), 167.47 (C1), 189.93 (C3); enol-form: 12.46 (C-4-$CH_2\underline{C}H_3$), 20.30 (C4-$\underline{C}H_2CH_3$), 51.50 (OCH_3), 88.44 (C2), 125.32 (C5), 138.0 (C4), 168.89 (C1).

Methyl 4-(chloromethylene)-3-methoxyhex-2-enoate (7)

A solution of **6** (1.0 g, 5 mmol) in HMPA (5 mL) was added to NaH (60%, 0.23 g, 10 mmol) dissolved in HMPA (5 mL) over 15 min period at 0°C. After being stirred for 30 min a solution of dimethyl sulfate (1.26 g, 10 mmol) in HMPA (5 mL) was added at this temperature. After complete addition of the alkylation agent, the mixture was allowed to warm up to rt and stirred for 2 h. The brown mixture was diluted with water (5 mL), and the solution was extracted with ether (3×20 mL). The combined organic phases were washed with the solution of dimethylamine (2×10 mL) and water (3×10 mL), then dried ($MgSO_4$) and evaporated in vacuo. The main component was separated by column chromatography (eluting with ether/hexane=1:6, R_f=0.55) to yield **7** (0.72 g, 70%) as a yellow oil. IR (neat) ν_{max} 2952, 1724, 1616, 1224. HRMS (EI) calcd for $C_9H_{13}O_3Cl$ 204.0553, found for 204.0558. ^{1}H NMR δ_H ($CDCl_3$): 1.05 (3H, t, J_{vic}=7.5 Hz; 4-$CH_2C\underline{H}_3$), 2.40 (2H, q, J_{vic}=10 Hz; 4-$C\underline{H}_2CH_3$), 3.72+3.80 (2×3H, 2×s; 2×OCH_3), 5.34 (1H, s; 2-H), 6.62 (1H, s; 5-H). ^{13}C NMR δ_C ($CDCl_3$): 11.30 (C4-$CH_2\underline{C}H_3$), 23.09 (C4-$\underline{C}H_2CH_3$), 51.04 ($COO\underline{C}H_3$), 56.08 (C3-$O\underline{C}H_3$), 93.42 (C2), 120.53 (C5), 138.92 (C4), 166.81 (C1), 170.03 (C3).

4-(Chloromethylene)-3-methoxyhex-2-en-1-ol (8)

0.32 g (8 mmol) of $LiAlH_4$ was suspended in 20 mL of dry ether under argon atm. The suspension was cooled below 5°C with ice bath, then 1.0 g (5 mmol) of **7** in 20 mL of anhydrous ether was added droppwise. After the addition, the reaction mixture was allowed to warm up to rt, and was stirred for 1 h. It was cooled to 0°C, the excess of the $LiAlH_4$ was decomposed with slow addition of 5 mL of 2M sodium hydroxide to the reaction mixture. The inorganic salts were separated by filtration, and the filtrate was concentrated in vacuo. The residue was dissolved in dichloromethane (30 mL), washed with water (2×10 mL), dried ($MgSO_4$), concentrated in vavuo. The residue was purified by column chromatography (eluting with acetone/hexane=1:5, R_f=0.25) to afford 0.75 g (85%) of the product (**8**) as a yellow oil. IR (neat) ν_{max} 3344, 2968, 1644, 1208. HRMS (EI) calcd for $C_8H_{13}O_2Cl$ 176.0604, found for 176.0607. ^{1}H NMR δ_H ($CDCl_3$): 1.05 (3H, t, J_{vic}=7.5 Hz; 4-$CH_2C\underline{H}_3$), 1.8 (1H, br; OH), 2.36 (2H, q, J_{vic}=10 Hz; 4-$C\underline{H}_2CH_3$), 3.53 (3H, s; 3-OCH_3), 4.28 (2H, d, $J_{1,2}$=6.6 Hz; 1-H_2), 5.27 (1H, s; 5-H). ^{13}C NMR δ_C ($CDCl_3$): 11.95 (C4-$CH_2\underline{C}H_3$), 21.39 (C4-$\underline{C}H_2CH_3$), 57.43 (C1), 59.34 (C3-$O\underline{C}H_3$), 113.62 (C2), 118.40 (C5), 139.0 (C4), 155.3 (C3).

4-(Chloromethylene)-3-methoxyhex-2-enyl benzoate (9)

Method I.: 0.57 g (6 mmol) of triethylamine was added to a solution of 1.0 g (6 mmol) of **8** in dry dichloromethane (20 mL). The mixture was cooled to 0°C, and at this temperature 0.79 g (6 mmol) of benzoyl chloride was added dropwise. The mixture was allowed to warm up to rt and then stirred for 30 min. The suspension was extracted with 5% aqueous solution of $NaHCO_3$ (2×10 mL). The organic phase

was dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by column chromatography (eluting with acetone/hexane=1:5, R_f=0.67) to afford 0.34 g (20%) of the product **(9)** as a colorless oil. IR (neat) ν_{max} 2968, 1720, 1648, 1600. HRMS (EI) calcd for $C_{15}H_{17}O_3Cl$ 280.0866, found for 280.0848. ^{1}H NMR δ_H ($CDCl_3$): 1.05 (3H, t, J=7.5 Hz; 4-$CH_2C\underline{H}_3$), 2.36 (2H, q, J_{vic}=10 Hz; 4-$C\underline{H}_2CH_3$), 3.59 (3H, s; 3-OCH_3), 4.99 (2H, d, $J_{1,2}$=7.0 Hz; 1-H_2), 5.33 (1H, t, J_{vic}=8 Hz; 2-H), 6.41 (1-H, s; 5-H), 7.44 (2H, m; 3'-H+5'-H), 7.55 (1H, m; 4'-H), 8.05 (2H, m; 2'-H+6'-H). ^{13}C NMR δ_C ($CDCl_3$): 11.98 (C4-$CH_2\underline{C}H_3$), 21.42 (C4-$\underline{C}H_2CH_3$), 59.54 (C3-$O\underline{C}H_3$), 59.63 (C1), 108.73 (C2), 119.11 (C5), 128.36 (C3'+C5'), 129.64 (C2'+C6'), 130.4 (C1'), 132.93 (C4'), 139.00 (C4), 157.10 (C3), 166.68 (O$\underline{C}$OPh).

Method II.: 0.55 g (2.6 mmol) of 1,3-Dicyclohexylcarbodiimide and 32 mg (0.26 mmol) of 4-dimethylaminopyridine were added to a solution of 0.32 g (2.6 mmol) of benzoic acid in dry dichloromethane (10 mL). The mixture was cooled to 0^{o}C and at this temperature 0.47 g (2.6 mmol) of **8** in dry dichloromethane (10 mL) was added dropwise. The mixture was allowed to warm up to rt and then stirred for 3 h. The suspension was extracted with brine (2×10 mL). The organic phase was dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by column chromatography (eluting with acetone/hexane=1:5, R_f=0.67) to afford 0.21 g (28%) of the product **(9)** as a colorless oil. The analytical data were identified in the previous method.

4-(Chloromethylene)-3-methoxyhex-2-enyl acetate (10)

0.57 g (6 mmol) of triethylamine was added to a solution of 1.0 g (6 mmol) of **8** in dry dichloromethane (20 mL). The mixture was cooled to 0^{o}C, and at this temperature 0.58 g (6 mmol) of acetic anhidride was added dropwise. The mixture was allowed to warm up to rt and then stirred for 30 min. The suspension was extracted with 5% aqueous solution of $NaHCO_3$ (2×10 mL). The organic phase was dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by column chromatography (eluting with acetone/hexane=1:5, R_f=0.65) to afford 1.15 g (88%) of the product (**10**) as a colorless oil. IR (neat) ν_{max} 2976, 1744, 1664, 1640, 1232. HRMS (EI) calcd for $C_{10}H_{15}O_3Cl$ 218.0710, found for 218.0716. ^{1}H NMR δ_H ($CDCl_3$): 1.04 (3H, t, J_{vic}=7.5 Hz; 4-$CH_2C\underline{H}_3$), 2.06 (3H, s; $COCH_3$), 2.35 (2H, q, J_{vic}=10 Hz; 4-$C\underline{H}_2CH_3$), 3.54 (3H, s; 3-OCH_3), 4.72 (2H, d, $J_{1,2}$=7.1 Hz; 1-H_2), 5.19 (1H, t, J_{vic}=8 Hz; 2-H), 6.38 (1H, s; 5-H). ^{13}C NMR δ_C ($CDCl_3$): 11.93 (C4-$CH_2\underline{C}H_3$), 21.02 ($CO\underline{C}H_3$), 21.40 (C4-$\underline{C}H_2CH_3$), 59.14 (C1), 59.40 (C3-$O\underline{C}H_3$), 108.63 (C2), 119.06 (C5), 138.95 (C4), 156.93 (C3), 171.07 (O$\underline{C}$OCH_3).

4-(Chloromethylene)-3-oxohexyl acetate (11)

5M aqueous acetic acid solution (2 mL) was added to a solution of **10** (1.0 g, 5 mmol) in dichloromethane (20 mL), and the mixture was refluxed for 30 min. The reaction mixture was cooled to rt and extracted with 5% aqueous solution of $NaHCO_3$ (2×10 mL) and water (2×10 mL).The organic phase was dried ($MgSO_4$) and the solvent was evaporated in vacuo. The residue was purified by column chromatography

(eluting with acetone/hexane=1:5, R_f=0.5) to afford 0.76 g (74%) of the product (**11**) as a colorless oil. IR (neat) ν_{max} 2976, 1740, 1680, 1600, 1244. HRMS (EI) calcd for $C_9H_{13}O_3Cl$ 204.0553, found for 204.0554. ^{1}H NMR δ_H ($CDCl_3$): 1.01 (3H, t, J_{vic}=7.5 Hz; 4-$CH_2C\underline{H}_3$), 2.03 (3H, s; $COCH_3$), 2.49 (2H, q, J_{vic}=10 Hz; 4-$C\underline{H}_2CH_3$), 2.95 (2H, t, $J_{1,2}$=6.5 Hz; 3-H_2), 4.39 (2H, t, J_{vic}=7 Hz; 1-H_2), 7.23 (1H, s; 5-H). ^{13}C NMR δ_C ($CDCl_3$): 12.17 (C4-$CH_2\underline{C}H_3$), 19.92 (C4-$\underline{C}H_2CH_3$), 20.84 ($CO\underline{C}H_3$), 37.08 (C2), 59.64 (C1), 133.26 (C5), 145.65 (C4), 170.87 ($O\underline{C}OCH_3$), 194.71 (C3).

2-(3-{2-[Benzyl-(2-ethyl-3-oxo-penta-1,4-dienyl)amino]ethyl}-1*H*-indol-2-yl)-3-hydroxypropionic acid methyl ester (13)

A solution of **11** (60 mg, 0.3 mmol) in dry methanol (5 mL) was added to a mixture of **2a** (100 mg, 0.3 mmol) and triethylamine (61 mg, 0.6 mmol) in anhydrous methanol (5 mL) dropwise at rt. After being stirred for 48 h at rt the reaction mixture was concentrated in vacuo. The residue was purified by column chromatography (eluting with acetone/hexane=1:1, R_f=0.4) to afford 90 mg (65%) of the product (**13**) as a yelow oil. IR (neat) ν_{max} 3384, 2952, 1736, 1616, 1588. MS m/z (%) (rel inten) 460 (4, $[M]^+$) 334 (13) , 228 (98), 170 (11), 91 (100). HRMS (EI) calcd for $C_{28}H_{32}N_2O_4$ 460.2347, found for 460.2362. ^{1}H NMR δ_H ($CDCl_3$): 0.99 (3H, t, J_{vic}=7.4 Hz; 2'-$CH_2C\underline{H}_3$), 2.45 (2H, q, J_{vic}=7.4 Hz; 2'-$C\underline{H}_2CH_3$), 3.0 (1H, br; OH), 3.00+3.07 (2×1H, 2×dt, J_{gem}=14.6 Hz, J_{vic}=7.0 Hz; 3-CH_2), 3.52 (2H, m; 3-$CH_2C\underline{H}_2N$), 3.67 (3H, s; OCH_3), 3.96-4.14 (3H, m; 2-C<u>H</u>-C<u>H</u>$_2$OH), 4.41+4.45 (2×1H, 2×d, J_{gem}=15.8 Hz; $NC\underline{H}_2Ph$), 5.43+5.98 (2×1H, 2×d, J_{gem}=2.2 Hz, J_{cis}=10.5 Hz and J_{trans}=16.9 Hz; 5'-H_2), 6.57 (1H, dd, J_{cis}=10.5 Hz, J_{trans}=16.9 Hz; 4'-H), 7.10 (1H, ddd, $J_{4,5}$=7.8 Hz, $J_{5,6}$=7.0 Hz, $J_{5,7}$=1.0 Hz; 5-H), 7.15 (2H, m; 2''-H+6''-H), 7.19 (1H, ddd, $J_{5,6}$=7.0 Hz, $J_{6,7}$=8.0 Hz, $J_{4,6}$=1.2 Hz; 6-H), 7.27-7.37 (4H, m; 3''-H+4''-H+5''-H+7-H), 7.38 (1H, s; 1'-H), 7.43 (1H, dm; 4-H), 8.94 (1H, br s; indol-NH). ^{13}C NMR δ_C ($CDCl_3$): 15.70 (2'-$CH_2\underline{C}H_3$), 17.73 (2'-CH_2), 24.07 (3-CH_2), 44.40 (2-CH), 52.66 ($COO\underline{C}H_3$), 53.50 (3-$CH_2\underline{C}H_2N$), 57.05 ($N\underline{C}H_2Ph$), 64.07 (CH_2OH), 109.93 (C3), 111.36 (C7), 113.10 (C2'), 118.20 (C4), 119.84 (C5), 122.52 (C6), 124.37 (C5'), 126.99 (C2''+C6), 127.27 (C3a), 127.83 (C4''), 128.93 (C3''+C5''), 129.95 (C2), 133.39 (C4'), 135.76 (C7a), 137.29 (C1''), 150.92 (C1'), 172.65 ($\underline{C}OOCH_3$), 190.38 (C3').

2-(3-(2-5-Ethyl-3,4-dihydro-4-oxopyridin-1(2*H*)-yl)ethyl)-1*H*-indol-2-yl)-3-hydroxypropionic acid methyl ester(14)

A solution of **11** (80 mg, 0.4 mmol) in dry methanol (5 mL) was added to a mixture of **2b** (100 mg, 0.4 mmol) and triethylamine (81 mg, 0.8 mmol) in dry methanol (5 mL) dropwise at rt. After being stirred for 24 h at rt the reaction mixture was concentrated in vacuo. The residue was purified by column chromatography (eluting with acetone/hexane=7:3, R_f=0.6) to afford 110 mg (74%) of the product (**14**) as a yelow oil. IR (neat) ν_{max} 3296, 2960, 1736, 1624, 1560. MS m/z (%) (rel inten) 371 (11), 370 (42, $[M]^+$), 232 (100), 170 (40), 138 (76). HRMS (EI) calcd for $C_{21}H_{26}N_2O_4$ 370.1892, found for 370.1908. ^{1}H NMR

δ_H ($CDCl_3$): 0.85 (3H, t, J_{vic}=7.5 Hz; 3'-$CH_2C\underline{H}_3$), 2.02 (2H, q, J_{vic}=7.5 Hz; 2'-$C\underline{H}_2CH_3$),2.40 (2H, t, J_{vic}=7.7 Hz; 5'-H_2), 2.80 (1H, br s; OH), 3.00+3.04 (2×1H, 2×dt, J_{gem}=14.5, J_{vic}=7.0 Hz; 3-CH_2), 3.41 (2H, t, J_{vic}=7.2 Hz; 6'-H_2), 3.45 (2H, t, J_{vic}=8 Hz; 3-$CH_2C\underline{H}_2$-N), 3.73 (3H, s; $COOC\underline{H}_3$), 4.02-4.16 (3H, m; 2-C<u>H</u>-$C\underline{H}_2OH$), 6.65 (1H, s; 2'-H), 7.13 (1H, ddd, $J_{4,5}$=7.8, $J_{5,6}$=7.00, $J_{5,7}$=1.2 Hz; 5-H), 7.20 (1H, ddd, $J_{6,7}$=8.00, $J_{5,6}$=7.00 Hz, $J_{4,6}$=1.3 Hz; 6-H), 7.36 (1H, dm; 4-H), 8.98 (1H, br s; indol-NH). ^{13}C NMR δ_C ($CDCl_3$): 14.36 (3'-$CH_2\underline{C}H_3$), 20.25 (3'-$\underline{C}H_2CH_3$), 24.22 (3-CH_2), 36.02 (C5'), 44.59 (2-CH), 47.72 (C6'), 52.76 ($COO\underline{C}H_3$), 56.21 (3-$CH_2\underline{C}H_2$-N), 64.01 (2-CH-$\underline{C}H_2OH$), 109.99 (C3), 111.43 (C7), 111.48 (C3'), 118.17 (C4), 119.83 (C5), 122.56 (C6), 127.24 (C3a), 129.78 (C2), 135.84 (C7a), 151.96 (C2'), 172.60 ($\underline{C}OOCH_3$), 190.42 (C4').

15-Oxovincadifformine (15) and 15-oxo-$\Delta^{20(21)}$-secodine (16)

A solution of **14** (100 mg, 0.3 mmol) and *p*-toluenesulfonic acid monohydrate (5 mg, 0.03 mmol) in toluene (10 mL) was refluxed under argon for 24 h. The reaction mixture was extracted with brines (2×5 mL), and the combined aqueous phases were extracted with dichloromethane (2×10 mL). The combined organic layers were dried ($MgSO_4$) and evaporated in vacuo. The two main components were separated by column chomatography (eluent: acetone/hexane=3:7). The less polar compound (**15**, R_f=0.6) was obtained as white crystal after recrystallization from methanol; mp 166-168 ^oC (48 mg, 45%). IR (KBr) ν_{max} 2968, 1704, 1680, 1616. MS ($C_{21}H_{24}N_2O_3$) m/z (%) (rel inten) 352 (59, $[M]^+$), 321 (4, $[M-31]^+$), 214 (100), 154 (13), 138 (72), 57 (14), 45 (17). ^{1}H NMR δ_H ($CDCl_3$): 0.67 (3H, t, J_{vic}=7.5 Hz; 20-$CH_2C\underline{H}_3$), 1.06-1.20 (2×1H, m; 20-$C\underline{H}_2CH_3$), 1.81+2.15 (2×1H, 2×dd, J_{gem}=11.6 Hz, J_{vic}=4.5 Hz; 6-H_2), 2.41+2.96 (2×1H, d+dd, J_{gem}=15.3 Hz, $J_{17\alpha,21}$=1.9 Hz; 17-H_2), 2.56+2.70 (2×1H, 2×ddd, J_{gem}=15.2 Hz, J_{vic1}=9.2+4.3 Hz and J_{vic2}=9.0+5.5 Hz; 14-H_2), 2.75+3.05 (2×1H, 2×dd, J_{gem}=9.0 Hz, J_{vic}=5 Hz; 5-H_2), 3.06+3.37 (2×1H, 2×dt, J_{gem}=9.7 Hz, J_{vic}=6 Hz; 3-H2), 3.19 (1H, d; 21-H), 3.77 (3H, s; OCH_3), 6.83 (1H, dm, $J_{11,12}$=7.8 Hz; 12-H), 6.90 (1H, ddd, $J_{9,10}$=7.4 Hz, $J_{10,11}$=7.5 Hz, $J_{10,12}$=1.0Hz; 10-H), 7.17 (1H, ddd, $J_{10,11}$=7.5 Hz, $J_{11,12}$=7.8 Hz, $J_{9,11}$=1.2 Hz; 11-H), 7.24 (1H, br d; 9-H), 9.03 (1H, br s; N1-H). NOE: 3.19 (21-H_α): 1.10+1.17+0.67 (20-Et_α), 7.24 (9-H), 2.75 (5-H_α). ^{13}C NMR δ_C ($CDCl_3$): 8.64 (C18), 24.36 (C19), 24.59 (C17), 37.36 (C14), 44.11 (C6), 46.83 (C3), 51.03 ($COO\underline{C}H_3$), 51.48 (C5), 55.32 (C7), 56.37 (C20), 71.84 (C21), 91.21 (C16), 109.55 (C12), 120.76 (C10), 121.73 (C9), 128.14 (C11), 137.43 (C8), 143.22 (C13), 164.52 (C2), 168.96 ($\underline{C}OOCH_3$), 213.47 (C15). Anal. Calcd for $C_{21}H_{24}N_2O_3 \cdot 0.5H_2O$: C, 69.72; H, 6.92; N, 7.74. Found C, 69.39; H, 6.82; N, 7.61. The more polar compound (**16**, R_f=0.3) was obtained as a yellow oil (24 mg, 23%). The product is labile. IR (neat) ν_{max} 2950, 1724, 1592. MS m/z (%) (rel inten) 353 (20), 352 (48, $[M]^+$), 214 (85), 138 (100). HRMS (EI) calcd for $C_{21}H_{24}N_2O_3$ 352.1702, found for 352.1708. ^{1}H NMR δ_H ($CDCl_3$): 0.85+2.02 (3H+2H, t+q, J_{vic}=7.4 Hz; 3'-CH_2CH_3), 2.38 (2H, t, J_{vic}=7.8 Hz; 5'-H_2), 3.12 (2H, t, J=7.0 Hz; 3-CH_2), 3.36 (2H, t; 6'-H_2), 3.45 (2H, t; 3-CH_2CH_2N), 3.86 (3H, s; $COOCH_3$),

6.05+6.52 (2×1H, 2×d, J_{gem}=1.5 Hz; C=CH_2), 6.58 (1H, s; 2'-H), 7.14 (1H, ddd, $J_{4,5}$=8.0 Hz, $J_{5,6}$=7.0 Hz, $J_{5,7}$=1.1Hz; 5-H), 7.24 (1H, ddd, $J_{6,7}$=8.2 Hz, $J_{4,6}$=1.3 Hz; 6-H), 7.39 (1H, dm; 7-H), 7.56 (1H, dm; 4-H), 9.09 (1H, br s; indol-NH). ^{13}C NMR δ_C ($CDCl_3$): 14.36 (3'-$CH_2\underline{C}H_3$), 20.26 (3'-$\underline{C}H_2CH_3$), 24.32 (3-CH_2), 36.05 (C5'), 47.72 (C6'), 52.43 (COO$\underline{C}H_3$), 56.18 (3-$CH_2\underline{C}H_2$-N), 111.95 (C3), 111.48 (C7), 111.46 (C3'), 118.56 (C4), 119.84 (C5), 122.48 (C6), 127.37 (C3a), 129.17 (2-CH=$\underline{C}H_2$), 131.58 (2-$\underline{C}H$=CH_2), 132.43 (C2), 135.81 (C7a), 152.06 (C2'), 168.12 ($\underline{C}OOCH_3$), 190.45 (C4').

15-Oxovincadifformine (15)

Method I.: A solution of **14** (100 mg, 0.3 mmol) and *p*-toluenesulfonic acid monohydrate (5 mg, 0.03 mmol) in xylene (10 mL) was refluxed under argon for 24 h. The reaction mixture was extracted with brine (2×5 mL), and the combined aqueous phases were extracted with dichloromethane (2×10 mL). The combined organic layers were dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by column chromatography (eluting with acetone/hexane=3:7, R_f=0.6) to afford 72 mg (68%) of the product (**15**) as white recrystals after crystallization from methanol. The analytical data were identified in the previous method.

Method II.: A solution of **16** (100 mg, 0.3 mmol) and *p*-toluenesulfonic acid monohydrate (5 mg, 0.03 mmol) in xylene (10 mL) was refluxed under argon for 12 h. The reaction mixture was extracted with brines (2×5 mL), and the combined aqueous phases were extracted with dichloromethane (2×10 mL). The combined organic layers were dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by column chromatography (eluting with acetone/hexane=3:7, R_f=0.6) to afford 81 mg (76%) of the product (**15**) as white crystals after recrystallization from methanol. The analytical data were identified in the previous method.

15β-Hydroxyvincadifformine (1)

300 μL (57 mg, 0.3 mmol) 1M solution of L-Selectride® was added to a solution of **15** (100 mg, 0.3 mmol) in anhydrous methanol (10 mL) at 0°C. After the addition, the reaction mixture was allowed to warm up to rt, and was stirred for 1 h. It was then poured into brine (10 mL) and extracted with dichloromethane (2×10 mL). The combined organic layers were dried ($MgSO_4$) and the solvent was evaporated in vacuo. The residue was purified by column chromatography (eluting with acetone/hexane=1:2, R_f=0.4) to afford 90 mg (85%) of the product (**1**) as white crystals after recrystallization from methanol; mp 97-98 °C. IR (KBr) ν_{max} 3420, 2930, 1607. MS ($C_{21}H_{26}N_2O_3$) m/z (%) (rel inten) 355 (12), 35 (41, $[M]^+$), 140 (100), 57 (13). HRMS (EI) calcd for $C_{21}H_{26}N_2O_3$ 354.1943, found for 354.1940. ^{1}H NMR δ_H ($CDCl_3$): 0.68 (3H, t, J_{vic}=7.5 Hz ; 20-$CH_2C\underline{H}_3$), 0.95+1.08 (2×1H, 2×dq, J_{gem}=14.4 Hz; 20-C$\underline{H}_2CH_3$), 1.74+2.11 (2×1H, 2×ddd, J_{gem}=11.5 Hz, $J_{5,6}$=4.6+1 and 11.3+6.4 Hz; 6-H_2), 1.75 (1H, br; 15-OH), 1.83+1.94 (2×1H, 2×ddd, J_{gem}=12.5 Hz, $J_{3,14}$=4.4+3.8, 9.2+5.6 Hz, $J_{14,15}$=4.5 and

9.8 Hz; 14-H_2), 2.50-2.66 (5H, m; 3-H_A+5-H_A+17-H_2+21-H), 2.92 (1H, br dd, J_{gem}=8.5 Hz; 5-H_B), 3.14 (1H, ddd, J_{gem}=10.8 Hz;3-H_B), 3.76 (3H, s; OCH_3), 3.77 (1H, dd; 15-H), 6.80 (1H, dm; $J_{11,12}$=7.8 Hz; 12-H), 6.86 (1H, ddd, $J_{9,10}$=7.4, $J_{10,11}$=7.5, $J_{10,12}$=1.0 Hz; 10-H), 7.13 (1H, ddd, $J_{9,11}$=1.3 Hz; 11-H), 7.18 (1H, br d; 9-H), 8.95 (1H, br s; N1-H). ^{13}C NMR δ_C ($CDCl_3$): 8.50 (C18), 22.38 (C17), 26.26 (C19), 30.53 (C14), 43.78 (C20), 45.54 (C6), 47.51 (C3), 50.99 (COO<u>C</u>H_3), 51.38 (C5), 55.35 (C7), 70.50 (C21), 73.84 (C15), 92.21 (C16), 109.48 (C12), 120.56 (C10), 121.06 (C9), 127.67 (C11), 137.48 (C8), 143.33 (C13), 167.25 (C2), 169.28 (<u>C</u>OOCH$_3$). Anal. Calcd for $C_{21}H_{26}N_2O_3 \cdot 1.5CH_3OH$: C, 67.90; H, 6.91; N, 7.03. Found C, 67.61; H, 6.92; N, 6.91.

15α-Hydroxyvincadifformine (17) and 15β-Hydroxyvincadifformine (1)

15 mg (0.4 mmol) $NaBH_4$ was added to a solution of **15** (100 mg, 0.3 mmol) in dry methanol (10 mL) at 0^{o}C. After the addition, the reaction mixture was allowed to warm up to rt, and was stirred for 1 h. It was then poured into brine (10 mL) and extracted with dichloromethane (2×10 mL). The combined organic phases were dried ($MgSO_4$) and the solvent was evaporated in vacuo. The residue was purified by column chromatography (eluting with acetone/hexane=1:2, R_f=0.4) to afford 91 mg (86%) of the product (**1**) and (**17**) as white crystals after recrystallization from methanol; mp 97-98 ^{o}C. The analytical data were identified in the previous method.

ACKNOWLEDGEMENTS

The authors are grateful to the National Scientific Research Foundation (OTKA T046060) for financial support of this work.

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