Synthesis of metabolites of cis and trans apovincamine derivatives

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Dedicated to Professor Csaba Szántay on the occasion of his 80th birthday

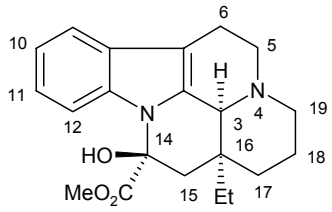
Abstract

Synthesis of oxidative metabolites of ethyl *cis*- and hydroxyethyl *trans*-apovincaminates have been described. For *cis*-metabolite **8** the functionalization of the 10-hydroxy group was established through key intermediate 8-methoxy-indolopyranoquinolizine **16**, for *trans*-metabolite **10** the crucial 19-oxo-intermediate **24** was constructed from methyl 4-oxo-octahydroindoloquinolizine propionate **21a**.

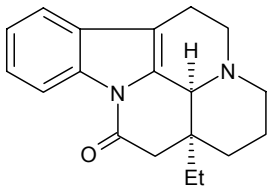
Keywords: Alkyl apovincaminates, oxidative metabolites, total synthesis

Introduction

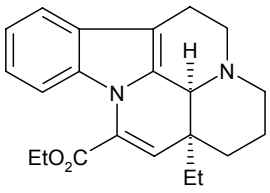
Over the past decades clinical and non-clinical research on vincamine and its semi-synthetic derivatives **1**–**3** has confirmed their beneficial cerebrovascular effect, including a neuroprotective action (Scheme 1).[1-3]



1 (+)-vincamine



2 (-)-eburnamonine

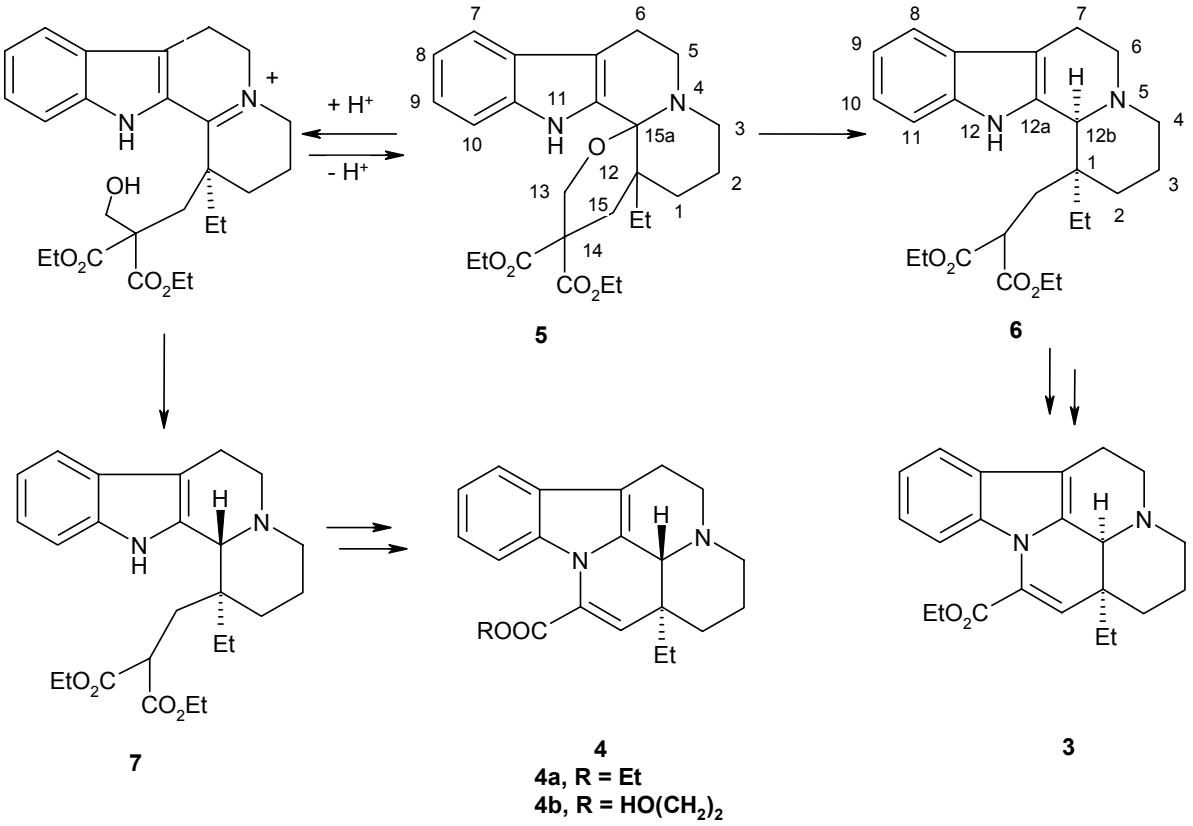


3 ethyl apovincaminate

Scheme 1

Previous SAR studies focused on structural modifications involving C-14 and the C/D/E ring junctions.[4,5] In order to find a potent antiamnesic agent, further changes in the structure of compound **3** have been considered. To that end, a new series of substituted-alkyl esters of (3*S*,16*R*)- and (3*R*,16*S*)-*trans*-apovincaminic acid was synthesized.[6] From the combined results of the data obtained from in vitro and in vivo tests and metabolism studies, 2'-hydroxyethyl (3*R*,16*S*)-apovincaminate (**4b, RGH-10885**) was identified as the most promising compound, owing to its potent neuroprotective and antiamnesic activities.[7]

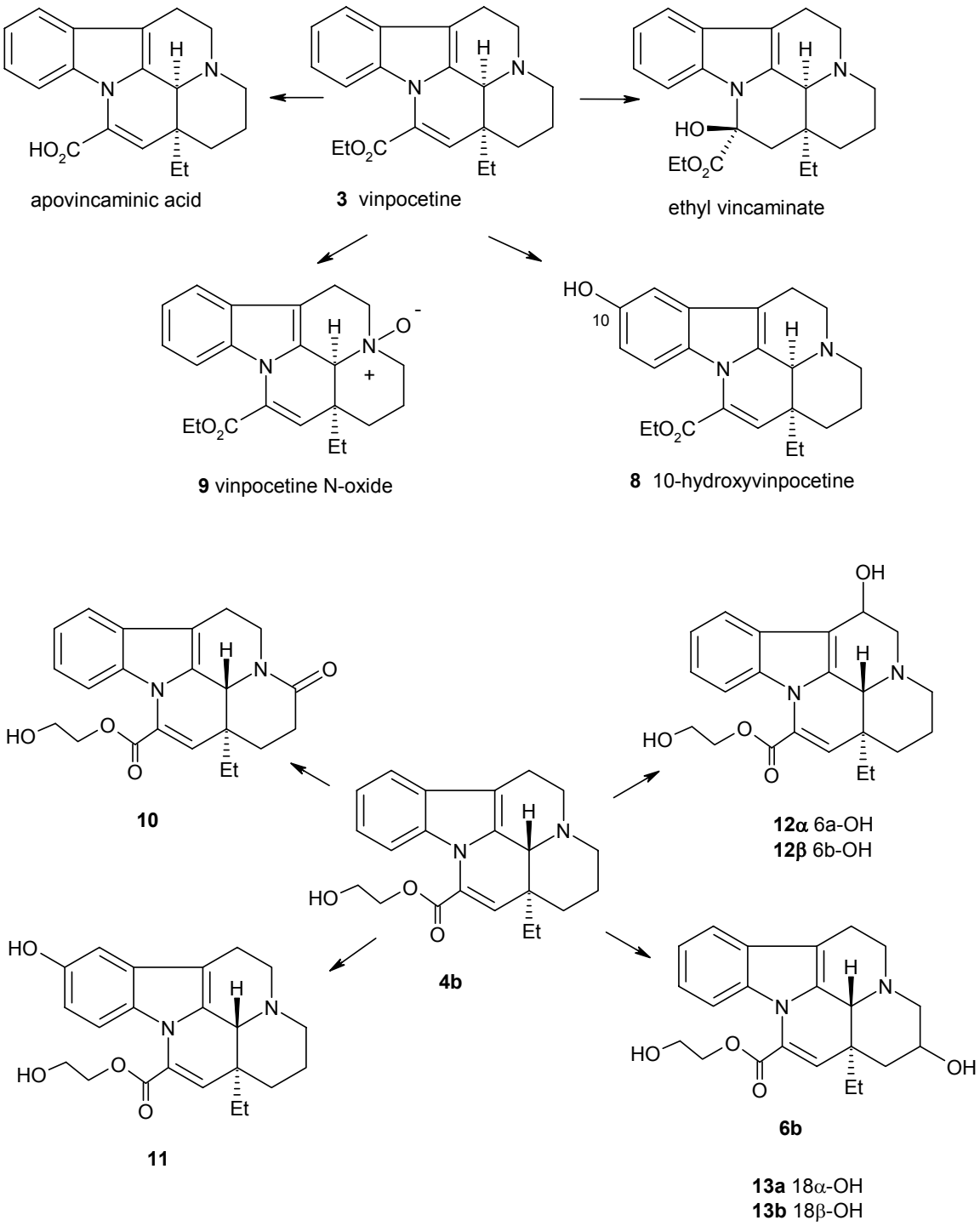
Compounds **3** and **4b** can be synthesized from the common intermediate **5**. The direction of stereoselectivity could be modified by variation of the reaction conditions: *cis*-diester **6**[8] or *trans*-diester **7**[7] were isolated from the reduction, wich was further transformed to the targeted endproducts (Scheme 2).



Scheme 2

The metabolism of compounds **1-3** has been established earlier. Apovincaminic acid is the major metabolite of vinpocetine (**3**). Ethyl vincaminate and a compound hydroxylated on the aromatic ring were also reported as being minor metabolites of **3**.[9] In recent studies the latter

compound was identified as 10-hydroxyvinpocetine **8**, and a new minor metabolite, vinpocetine-*N*-oxide **9**[10] was also isolated (Scheme 3).



Scheme 3. Metabolism of vinpocetine (**3**) and **4b**.

A quite different pattern of metabolism was observed for the *trans* hydroxyethylester **4b**. In contrast to the *cis* ethyl ester **3** and *trans* ethyl ester **4a**, **4b** does not appear to be affected by the esterase enzyme, but by the CYP enzymes. A series of oxidative metabolites were isolated. As a major metabolite the 19-oxo-derivative **10** was identified. Production of minor metabolites 10-hydroxy-**4b** (**11**), 6α- and 6β-hydroxy-**4b** (**12a,b**) as well as 18α- and 18β-hydroxy-**4b** (**13a,b**) were also observed (Scheme 3).[11]

We synthesized *cis* metabolite **8**, and the major metabolite of **4a** *trans*-apovincaminate, **10** for the purpose of structural confirmation and for further studies of the biological activity.

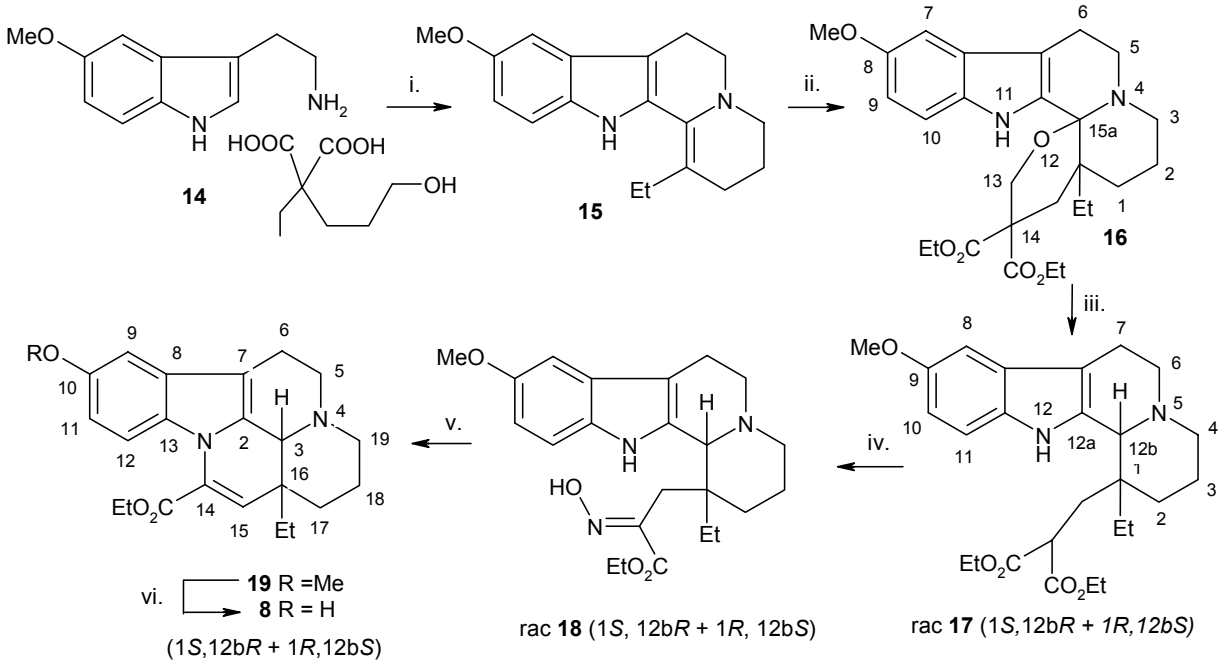
Results and Discussion

A simple transformation of **3** into N-oxide **9**[10] was accomplished with magnesium monoperoxy-phtalate in DMF.

The synthesis of the *cis*-metabolite **8** follows the route outlined in Scheme 4. Enamine **15**,[12] formed from 5-methoxytryptamine (**14**) was reacted with diethyl malonate and paraformaldehyde to obtain pyranoindoloquinolizine **16**. Stereoselective reduction, followed by deformylation afforded *cis*-diester **17**. Partial hydrolysis and reaction with sodium nitrite/acetic acid led to oximeester **18**. Ring closure/deoximation yielded ethyl 10-methoxy-apovincaminate (**19**). Demethylation of **19** with boron tribromide afforded rac. **8**(Scheme 4).

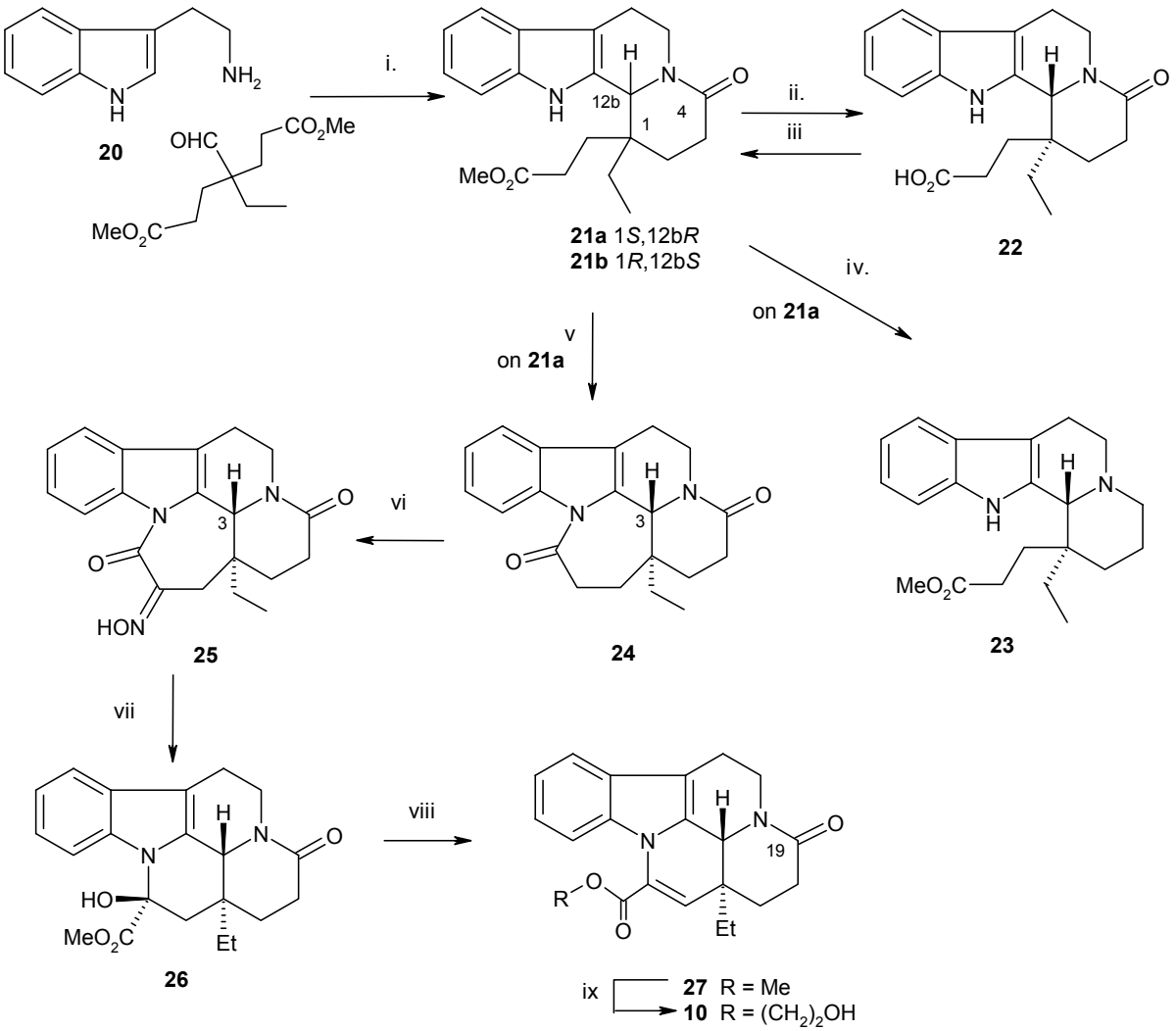
A multistep synthesis of *trans*-metabolite **10** was established from triptamine (**20**, Scheme 5).

Reaction with dimethyl formylpimelate was followed by the separation of *trans* isomers **21a,b**.[13] The desired (1*S*,12b*R*)-indoloquinolizinyl-propionate ester (**21a**) was isolated by means of optical resolution of acids by (–)-ephedrine. The so obtained 4-oxo-(1*S*,12b*R*)-propionic acid **22** was reesterified to **21a**. The correct stereochemistry of **21a** was proved by transformation to authentic **23** ester.[4] Ring closure to dilactam **24** followed by oximation led to dilactam oxime **25**. Hydrolysis and reaction of the so obtained trioxo compound with sodium methoxyde resulted in (3*R*,16*S*)-19-oxovincamine **26**. Dehydration to **27** followed by transesterification completed the synthesis of **10** (Scheme 5).



Scheme 4. *Reagents and conditions*: (i) (a) ClC_6H_5, 110 °C, (b) $POCl_3$, 120 °C, $NaOH/H_2O$; (ii) EtOH, diethyl malonate, paraformaldehyde, Et_3N, 50 °C; (iii) (a) H_2, Pd/C, then NH_4OH; (iv) (a) KOH/H_2O, EtOH, (b) $NaNO_2/H_2O$, AcOH; (v) pTsOH, toluene, 110 °C; (vi) BBr_3, $CHCl_3$.

In conclusion, different strategies were employed to synthesize oxidative metabolites of apovincaminic acid esters. The AB(C)D→ABCDE strategy ($C_{17}N_2$ + C_3) was applied by alkylation of the 9-methoxy-Wenkert-enamine **15** to prepare 10-hydroxy metabolite **8** of ethyl *cis*-apovincaminate. The skeleton of 19-oxo *trans*-metabolite **10** was constructed in a reaction of tryptamine and dimethyl formylpimelate ($C_{10}N$ + C_{10}, AB→ABCDE approach). The subsequent steps to the end-products was carried out by using standard methods.[15]



Scheme 5. *Reagents and conditions*: (i) (a) toluene, 110°C, then (b) AcOH, 120°C, (c) $NaOH/H_2O$, CH_2Cl_2 work-up, (d) EtOH; (ii) (a) $NaOH/H_2O$, MeOH, (b) HCl/H_2O, (–)-ephedrine, acetone, (d) $AcOH/H_2O$, EtOH; (iii) (a) KOH/MeOH, (b) MeI, DMF; (iv) (a) P_2S_5, THF, (b) Raney Ni, THF; (v) *t*BuOK, toluene; (vi) *t*BuONa, *t*BuONO, toluene; (vii) (a) pTsOH, CH_2O, AcOH, 100°C, (b) *t*BuOK, MeOH; (viii) AcOCl, HCOOH, CH_2Cl_2; (ix) *t*BuOK, $HO(CH_2)_2OH$, 100°C.

Experimental Section

General Procedures. General procedures followed during the course of the work detailed herein were similar to those reported elsewhere.[4,8] IR spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrometer using KBr pellets. NMR spectra were recorded on a Varian VNMRS-400 spectrometer (400 MHz for ^1H detection). 2D NMR experiments (COSY, HSQC,

HMBC, NOESY) experiments were recorded by using the standard spectrometer software package; 0.75 s mixing time was used in the NOESY experiments. Mass spectrometric (high-resolution /HRMS/) measurements were performed on a LTQ FT Ultra (Thermo Finnigan, San Jose, CA) system. The ionization method was ESI (275 °C was the ion transfer capillary temperature and 4.2 kV was the capillary voltage). For CID experiment helium was used as the collision gas, and normalized collision energy (expressed in percentage), which is a measure of the amplitude of the resonance excitation RF voltage applied to the endcaps of the linear ion trap, was used to bring about fragmentation.

(±)-Diethyl 15a-ethyl-2,3,6,11,15,15a-hexahydro-8-methoxy-1*H*,5*H*-indolo[2,3-*a*]pyrano [3,2-*i*]quinolizine-14,14-(13*H*)-dicarboxylate (16). The solution of 5-methoxy-tryptamine (**14**) (20 g, 0.105 mol) and ethyl 3-hydroxypropylmalonic acid[16] (23.2 g, 0.12 mol) in chlorobenzene (220 mL) was refluxed for 2 h, then 35 g (21 mL, 0.23 mol) phosphorus oxychloride was added at 50 °C and refluxed for 2 h. The reaction mixture was cooled to 70 °C and ethanol (40 mL) was added. The mixture was added dropwise to sodium hydroxide (31.2 g) in water (150 mL) at 70 °C. After stirring an additional 0.5 h, the separated organic phase was dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a red oil. It was solved in ethanol (50 mL), then diethyl malonate (24 g, 0.15 mol), paraformaldehyde (9 g, 0.3 mol) and triethylamine (1 mL) was charged. The reaction mixture was stirred for 2 h at 50 °C, then 2 h for 5 °C. The separated yellow crystals were filtered, washed with ethanol, to give 18.1 g (35 %) of the title **16**, mp 147-148 °C. IR (cm^{-1}): 3450, 2936, 1748, 1716, 1623, 1591, 1561, 1491, 1256, 1152, 888, 797; ^{1}H NMR (DMSO-d_6, 50 ^{o}C, δ_{TMS}=0.00 ppm): 0.77 (3H, t, $CH_2C\underline{H}_3$), 1.15 (1H, dq, $C\underline{H}_xH_yCH_3$), 1.21 (3H, t, $OCH_2C\underline{H}_3$), 1.26 (3H, t, $OCH_2C\underline{H}_3$), 1.30-1.44 (2H, m, H_x-2, H_x-1), 1.54-1.68 (2H, m, H_y-2, H_y-1), 1.83 (1H, dq, $CH_x\underline{H}_yCH_3$), 2.49-2.80 (6H, m, H_2-15, H_2-6, H_x-3, H_x-5), 2.89 (1H, m, H_y-3), 3.31 (1H, td, H_y-5), 3.77 (s, 3H, OMe), 3.83 (1H, d, H_x-13), 4.19 (2H, q, $OC\underline{H}_2CH_3$), 4.25 (2H, q, $OC\underline{H}_2CH_3$), 4.43 (1H, d, H_y-3), 6.77 (1H, dd, H-9), 6.92 (1H, d, H-7), 7.35 (1H, d, H-10), 9.62 (1H, brs, NH); MS (ESI, CID=18 %) m/z (rel. int. %): 485(/M+H/$^+$, 6), 455(64), 409(100), 283(14). Mass accuracy was between -0.87 and -0.21 ppm for the fragment ions. The protonated molecular ion peak can be detected at m/z 485.26455 (delta: -0.13 ppm), calculated value for $C_{27}H_{37}O_6N_2$: 485.26461.

(±)-*cis*-1-(2',2'-Diethoxycarbonylethyl)-1−ethyl-9-methoxy-1,2,3,4,6,7,12,12b-octahydro-indolo[2,3-*a*]quinolizine(17). A solution of **16** (30 g, 62 mmol) in DMF (60 mL) was hydrogenated over 10 % palladium on activated carbon (0.6 g) for 2 h. After filtration, 25% NH_4OH solution (12 mL) was added and the mixture was stirred for 1 h. EtOH (20 mL) then water (180 mL) was added. The precipitated crystals were separated, washed with water, then suspended in a mixture of EtOH/water 2:1 (110 mL) to yield 19.4 g (68 %) of **17**, mp 120-121 ^{o}C (recrystallization from EtOH/water). IR (cm^{-1}): 3435, 2940, 1756, 1728, 1627, 1588, 1461, 1271, 1224, 1025, 807; ^{1}H NMR (DMSO-d_6, 25 ^{o}C, δ_{TMS}=0.00 ppm): 1.01 (3H, t, $CH_2C\underline{H}_3$), 1.03 (3H, t, $OCH_2C\underline{H}_3$), 1.11 (3H, t, $OCH_2C\underline{H}_3$); 1.40-1.57 (4H, m, H_2-2, H_x-3, $C\underline{H}_xH_yCH_3$); 1.70 (1H, m, H_y-3); 1.76 (1H, dd, $C\underline{H}_xH_yCH(COOEt)_2$), 1.86 (1H, dq, $CH_x\underline{H}_yCH_3$), 2.28 (1H, m, H_x-4), 2.34

(1H, dd, $CH_x\underline{H}_yCH(COOEt)_2$), 2.42 (1H, td, H_x-6), 7.50 (1H, m, H_x-7), 2.72 (1H, m, H_y-7), 2.91-2.98 (2H, m, H_y-6, H_y-4), 3.24 (1H, t, $CH_xH_yC\underline{H}(COOEt)_2$), 3.27 (1H, s, H-12b), 3.73 (3H, s, OMe), 3.91-4.06 (4H, m, 2x($OC\underline{H}_2CH_3$)), 6.66 (1H, dd, H-10), 6.84 (1H, d, H-8), 7.33 (1H, d, H-11), 9.62 (1H, s, NH); MS (ESI, CID=19 %) m/z (rel. int. %): 457(/M+H/$^+$, 15), 440(1), 411(100), 365(5), 284(88), 238(6), 192(1). Mass accuracy was between -0.91 and -0.25 ppm for the fragment ions. The protonated molecular ion peak can be detected at m/z 457.26962 (delta: -0.17 ppm), calculated value for $C_{26}H_{37}O_5N_2$: 457.26970.

(±)-*cis*-Ethyl-1-ethyl-9-methoxy-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-*a*]quinolizine-1-(2'-hydroxyimino)-propionate (18). To **17** (6 g, 13 mmol) in EtOH (100 mL) a solution of potassium hydroxide (0.9 g, 16 mmol) in water (9 mL) was added and stirred for 3 h at 25-30 °C. The pH was adjusted to 7 with AcOH and the solution was evaporated *in vacuo*. The residue was dissolved in AcOH (18 ml). Nitrogen oxide was evolved from sodium nitrite (2.6 g) in water (7 mL) and concd. HCl (6 mL) and bubbled to the solution. for 0.5 h at 10-15 °C, then a solution of sodium nitrite (1.1g) in water (5 mL) was added. The pH was adjusted with 1:2 mixture of concd HCl and water to 3. The precipitated **18** HCl crystals were filtered, stirred in a mixture of ethanol (20 mL), dichloromethane (1 mL) and 25% NH_4OH (5 mL) to give **18 (**2.6 g, 48% yield), mp 178-180 °C (recrystallization from EtOH). IR (cm^{-1}): 3457, 2951, 1702, 1625, 1592, 1491, 1297, 1157, 1024, 800; ^{1}H NMR (DMSO-d_6, 25 °C, δ_{TMS}=0.00 ppm): 0.98 (3H, t, $CH_2C\underline{H}_3$), 1.05 (3H, t, $OCH_2C\underline{H}_3$), 1.30 (1H, m, H_x-2); 1.41 (2H, m, H_x-3, $C\underline{H}_xH_yCH_3$), 1.49 (1H, m, H_y-2), 2.07 (1H, dq, $CH_x\underline{H}_yCH_3$), 2.16 (1H, m, H_y-3), 2.22 (1H, d, $C\underline{H}_xH_yCH(COOEt)(NOH)$), 2.29 (1H, td, H_x-4), 2.42 (1H, td, H_x-6), 2.52 (1H, m, H_x-7), 2.74 (1H, m, H_y-7), 2.90-2.98 (2H, m, H_y-6, H_y-4), 3.08 (1H, d, $CH_x\underline{H}_yCH(COOEt)(NOH)$), 3.25 (1H, s, H-12b), 3.74 (3H, s, OMe), 4.01-4.16 (2H, m, $OC\underline{H}_2CH_3$), 6.66 (1H, dd, H-10), 6.85 (1H, d, H-8), 7.35 (1H, d, H-11), 9.68 (1H, s, NH), 11.97 (1H, s, OH); MS (ESI, CID=22 %) m/z (rel. int. %): 414(/M+H/$^+$, 12), 396(74), 381(9), 340(3), 324(39), 284(97), 253(14), 241(100). Mass accuracy was between -0.68 and -0.31 ppm for the fragment ions. The protonated molecular ion peak can be detected at m/z 414.23881 (delta: 0.18 ppm), calculated value for $C_{23}H_{32}O_4N_3$: 414.23873.

(±)-Ethyl 10-methoxy-apovincaminate (19). A mixture of *p*-toluenesulfonic acid hydrate (5.3 g, 27 mmol) **18** (4.6 g, 11 mmol), toluene (55 mL) and EtOH (5 ml) was stirred and distilled until the temperature of the overhead rose to 108 °C. The reaction mixture was refluxed for 2 h. The reaction mixture was cooled to 20 °C and washed with Na_2CO_3 7% solution (110 mL). The organic layer was dried over $MgSO_4$, clarified with Al_2O_3 (2 g), and evaporated. Crystallization of the residue from ethanol (15 mL) afforded ester **19** (3.2 g, 74%), mp 148-150 °C. IR (cm^{-1}): 2945, 1719, 1629, 1607, 1474, 1254, 1225, 1077, 830, 797; ^{1}H NMR (DMSO-d_6, 25 °C, δ_{TMS}=0.00 ppm): 0.80 (1H, td, H_{ax}-17), 0.93 (3H, t, H_3-21), 1.30 (3H, t, $OCH_2C\underline{H}_3$), 1.33 (1H, m, H_{eq}-18), 1.48 (1H, m, H_{eq}-17), 1.57 (1H, m, H_{ax}-18), 1.83 (2H, q, H_2-20), 2.39 (1H, m, H_{eq}-6), 2.41 (1H, m, H_{ax}-19), 2.52 (1H, m, H_{eq}-19), 3.00 (1H, m, H_{ax}-6), 3.08-3.24 (2H, m, H_2-5), 3.77 (3H, s, OMe), 4.05 (1H, s, H-3), 4.29-4.41 (2H, m, $OC\underline{H}_2CH_3$), 6.06 (1H, s, H-15), 6.72 (1H, dd, H-11), 6.94 (1H, d, H-9), 7.07 (1H, d, H-12); MS (ESI, CID=22 %) m/z (rel. int. %): 381(/M+H/$^+$, 62), 364(6), 353(87), 338(100), 324(44), 309(31), 296(6), 266(8). Mass accuracy

was between -0.45 and -0.23 ppm for the fragment ions. The protonated molecular ion peak can be detected at m/z 381.21736 (delta: 0.24 ppm), calculated value for $C_{23}H_{29}O_3N_2$: 381.21727.

(±)-Ethyl 10-hydroxy-apovincaminate (8). A solution of **19** (1 g, 2,6 mmol) in chloroform (50 mL) was treated with BBr_3 (2.4 mL) at 0-5 °C, under nitrogen. The mixture was warmed to 20 °C, then water (50 mL) was added and the pH was adjusted to 9 with NH_4OH 10% solution. The organic layer was separated, the aqueous was etracted with chloroform (20 ml). the combined organic solutions were evaporated, the residue was crystallized from methanol, to give **8** (0,6 g, 63%), mp 209-210 °C. IR (cm^{-1}): 3042, 2931, 2860, 1731, 1636, 1614, 1570, 1457, 1298, 1266, 1203, 1078, 1023, 782; ^{1}H NMR (DMSO-d_6, 25 °C, δ_{TMS}=0.00 ppm): 0.80 (1H, td, H_{ax}-17), 0.93 (3H, t, H_3-21), 1.30 (3H, t, $OCH_2C\underline{H}_3$), 1.33 (1H, m, H_{eq}-18), 1.46 (1H, m, H_{eq}-17), 1.56 (1H, m, H_{ax}-18), 1.81 (2H, q, H_2-20), 2.32 (1H, m, H_{eq}-6), 2.43 (1H, m, H_{ax}-19), 2.51 (1H, m, H_{eq}-19), 2.94 (1H, m, H_{ax}-6), 3.05-3.21 (2H, m, H_2-5), 4.02 (1H, s, H-3), 4.28-4.40 (2H, m, $OC\underline{H}_2CH_3$), 6.00 (1H, s, H-15), 6.58 (1H, dd, H-11), 6.74 (1H, d, H-9), 6.97 (1H, d, H-12), 8.89 (1H, brs, OH); MS (ESI, CID=20 %) m/z (rel. int. %): 367(/M+H/$^+$, 33), 350(8), 339(81), 324(100), 310(40), 295(31), 293(10), 282(5), 252(8). Mass accuracy was between -0.46 and -0.24 ppm for the fragment ions. The protonated molecular ion peak can be detected at m/z 367.20164 (delta: 0.06 ppm), calculated value for $C_{22}H_{27}O_3N_2$: 367.20162.

(±)-Methyl *trans*-1-ethyl-4-oxo-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-*a*]quinolizine-1-propionate ((±)-21). Tryptamine (**20**) (57 g, 0.35 mol) and dimethyl 3-ethyl-3-formyl-pimelate[14] (100 g, 0.41 mol) was refluxed in toluene (450 mL) for 2 h. After evaporation in vacuo, the residue was dissolved in acetic acid (200 mL) and refluxed for 2 h. Water (500 mL) and dichloromethane was added to the cooled reaction mixture and the pH was adjusted at 25 °C to 9 with 20% sodium hydroxide solution. The organic layer was washed with water (100 mL) and evaporated. The residue was refluxed in ethyl acetate (530 mL), then stirred for 2 h at r.t. The separated crystals of *cis* and *trans* (40:60 ratio, according to HPLC) methyl indoloquinolizine propionates (60 g) was refluxed in ethanol (600 mL) for 1 h, then stirred at 40 °C for 1 h to obtain 24 g (20%) of **(±)-21**, mp 199-200 °C. IR (cm^{-1}): 3300, 2920, 1443, 1628, 1467, 1304, 1168, 736; ^{1}H NMR ($CDCl_3$, 25 °C, δ_{TMS}=0.00 ppm): 0.72 (3H, t, $CH_2C\underline{H}_3$), 0.99 (1H, dq, $C\underline{H}_xH_yCH_3$), 1.48 (1H, dq, $CH_x\underline{H}_yCH_3$), 1.64 (1H, m, H_x-2), 1.78 (1H, m, H_y-2), 2.07 (1H, m, $C\underline{H}_xH_yCH_2COOMe$), 2.28 (1H, m, $C\underline{H}_xH_yCH_2COOMe$), 2.40-2.58 (2H, m, H_2-3), 2.54-2.66 (2H, m, $CH_2C\underline{H}_2COOMe$),2.70 (1H, m, H_{ax}-6), 2.72-2.80 (2H, m, H_2-7), 3.80 (1H, s, OMe), 4.77 (1H, s, H-12b), 5.14 (1H, m, H_{eq}-6), 7.19 (1H, td, H-9), 7.19 (1H, td, H-10), 7.43 (1H, d, H-11), 7.51 (1H, d, H-8), 9.28 (1H, brs, NH); MS (ESI, CID=19 %) m/z (rel. int. %): 355(/M+H/$^+$, 30); 337(23), 323(100), 305(5), 295(1), 244(3), 212(8), 144(2). Mass accuracy was between -0.96 and -0.32 ppm for the fragment ions. The protonated molecular ion peak can be detected at m/z 355.20165 (delta: 0.09 ppm), calculated value for $C_{21}H_{27}O_3N_2$: 355.20162.

(1*S*,12b*R*)-1-Ethyl-4-oxo-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-*a*]quinolizine-1-propionic acid (22). A solution of **(±)-21** (24 g, 67 mmol) in methanol (108 mL) and sodium hydroxide 20% solution (18.5 mL) was stirred for 2 h. The pH was adjusted to 6 with concd HCl solution, the solvent was evaporated in vacuo, then the solution was further acidified to pH 3. the

precipitated crystals were filtered to obtain 22.5 g of racem oxoacid, which was suspended in acetone (375 ml). (–)- Ephedrine (11 g, 67 mmol) in acetone (110 mL) was added and the mixture was stirred for 2.5 h, the separated (–)-oxoacid ephedrine salt was filtered and the filtrate was evaporated in vacuo, the residue was treated with water (190 mL) and acetic acid (7.5 mL). the precipitated crystals were refluxed in ethanol (110 mL), filtered, and evaporated to 15 mL, and stirred for 2 h at 0 °C to yield (+)-**22** (6 g, 26%), mp 191-192°C, $[\alpha]_D$ +145 (c 1, AcOH). IR (cm^{-1}): 3401, 2943, 1708, 1596, 1468, 1414, 1309, 1227, 741; ^{1}H NMR (DMSO-d_6, 25 ^{o}C, δ_{TMS}=0.00 ppm): 0.64 (3H, t, $CH_2C\underline{H}_3$), 0.89 (1H, dq, $C\underline{H}_xH_yCH_3$), 1.25 (1H, dq, $CH_x\underline{H}_yCH_3$), 1.49 (1H, m, H_x-2), 1.82 (1H, m, H_y-2), 1.93-2.10 (2H, m, $C\underline{H}_2CH_2COOMe$), 2.23 (1H, m, H_x-3), 2.32-2.44 (2H, m, H_y-3, $CH_2C\underline{H}_xH_yCOOMe$), 2.48-2.74 (4H, m, H_{ax}-6, H_2-7, $CH_2CH_x\underline{H}_yCOOMe$), 4.85 (1H, s, H-12b); 5.86 (1H, m, H_{eq}-6), 6.97 (1H, td, H-9), 7.06 (1H, td, H-10), 7.42 (2H, d, H-8, H-11), 7.51 (1H, d, H-8); 10.45 (1H, brs, NH); MS (ESI, CID=17 %) m/z (rel. int. %): 341(/M+H/$^+$, 15), 323(100), 305(1.1), 198(2.2), 144(1.4). Mass accuracy was between -0.82 and -0.25 ppm for the fragment ions. The protonated molecular ion peak can be detected at m/z 341.18597 (delta: 0 ppm), calculated value for $C_{20}H_{25}O_3N_2$: 341.18597.

(1*S*,12b*R*)-Methyl 1-ethyl-4-oxo-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-*a*]quinolizine-1-propionate (21a). A mixture of **22** (6 g, 17.6 mmol), methanol (50 mL), and KOH (1 g, 18 mmol) was stirred for 0.5 h, then evaporated in vacuo to dryness. The residue was dissolved in DMF (24 mL) and stirred with MeI (5 g, 35 mmol) for 3 h. The reaction mixture was diluted with water (120 ml), stirred for 1 h at 10 °C, the separated crystals were filtered to give **21a** (6.1 g ,97%), mp 190-191 °C (MeOH), $[\alpha]_D$ +131 (c 1, MeOH). IR (cm^{-1}): 3362, 2934, 1736, 1648, 1620, 1463, 1415, 1306, 1195, 739; ^{1}H NMR ($CDCl_3$, 25 ^{o}C, δ_{TMS}=0.00 ppm): 0.72 (3H, t, $CH_2C\underline{H}_3$), 0.99 (1H, dq, $C\underline{H}_xH_yCH_3$), 1.48 (1H, dq, $CH_x\underline{H}_yCH_3$), 1.64 (1H, m, H_x-2), 1.78 (1H, m, H_y-2), 2.07 (1H, m, $C\underline{H}_xH_yCH_2COOMe$), 2.28 (1H, m, $C\underline{H}_xH_yCH_2COOMe$), 2.40-2.58 (2H, m, H_2-3), 2.54-2.66 (2H, m, $CH_2C\underline{H}_2COOMe$), 2.70 (1H, m, H_{ax}-6), 2.72-2.80 (2H, m, H_2-7), 3.80 (1H, s, OMe), 4.77 (1H, s, H-12b), 5.14 (1H, m, H_{eq}-6), 7.19 (1H, td, H-9), 7.19 (1H, td, H-10), 7.43 (1H, d, H-11), 7.51 (1H, d, H-8), 9.28 (1H, brs, NH); MS (ESI, CID=19 %) m/z (rel. int. %): 355(/M+H/$^+$, 20), 337(22), 323(100), 305(3), 244(2), 212(43), 144(2). Mass accuracy was between -0.93 and -0.39 ppm for the fragment ions. The protonated molecular ion peak can be detected at m/z 355.20162 (delta: 0 ppm), calculated value for $C_{21}H_{27}O_3N_2$: 355.20162.

(1*S*,12b*R*)-Methyl 1-ethyl-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-*a*]quinolizine-1-propionate (23) from **(21a).** A solution of **21a** (1 g, 2.8 mmol) in THF (10 mL) was stirred with P_2S_5 (0.7 g, 1,6 mmol) for 1 h. The reaction mixture was filtered, the filtrate was added to Raney-Nickel (4 g) in THF (10 ml), the mixture was stirred for 1 h, filtered, evaporated in vacuo, the residue was crystallized from methanol, to obtain **23** (0.4 g, 42%), mp 106-107°C, $[\alpha]_D$ +65 (c 1, $CHCl_3$) {lit[4] mp 108-109°C, $[\alpha]_D$ +69)}

(3*R*,16*S*)-14,16-Dioxo-E-homoeburnane (24). To a solution of **21a** (6 g, 17 mmol) in toluene potassium *tert*-butoxide (3 g, 27 mmol) was charged at 30 °C, under nitrogen, and stirred for 1 h. The reaction mixture was treated with a solution of water (60 mL) and acetic acid (2 mL), filtered, the organic layer was evaporated and the residue was crystallized from methanol to give

24 (4.2 g, 77%) mp 146-148 °C, $[\alpha]_D$ +121 (c 1, DMF). IR (cm^{-1}): 3319, 2929, 1707, 1650, 1622, 1458, 1414, 1317, 1135, 759; ^1H NMR ($CDCl_3$, 25 °C, δ_{TMS}=0.00 ppm): 0.77 (3H, t, H_3-21), 0.91 (1H, dq, H_x-20), 1.38 (1H, dq, H_y-20), 1.70 (1H, dt, H_x-15), 1.78 (1H, td, H_{ax}-17), 1.89 (1H, ddd, H_{eq}-17), 2.12 (1H, td, H_y-15), 2.39 (1H, ddd, H_{ax}-18), 2.53 (1H, ddd, H_{eq}-18), 2.71-2.86 (4H, m, H_{eq}-14a, H_{ax}-5, H_2-6), 3.00 (1H, td, H_{ax}-14a), 4.95 (1H, s, H-3), 5.17 (1H, m, H_{eq}-5), 7.28-7.40 (2H, m, H-10, H-11), 7.47 (1H, d, H-9); 8.50 (1H, d, H-12); MS (ESI, CID=21 %) m/z (rel. int. %): 323(/M+H/$^+$, 14), 305(23), 295(100), 280(22), 266(53), 252(29), 238(11), 212(11), 184(4). Mass accuracy was between -0.72 and -0.47 ppm for the fragment ions. The protonated molecular ion peak can be detected at m/z 323.17545 (delta: 0.14 ppm), calculated value for $C_{20}H_{23}O_2N_2$: 323.17540.

(3*R*,16*S*)-14,16-Dioxo-15-hydroxyimino-E-homoeburnane (25). To a solution of **24** (4 g, 12.4 mmol) in toluene (60 mL) *tert*-butyl nitrite (2.1g, 20mmol) in toluene (5ml), then sodium *tert*-butoxide (1.9 g, 20 mmol) was added under nitrogen, at 28 °C, and stirred for 3 h. The reaction mixture was treated with a solution of water (30 mL) and acetic acid (3 mL), then diluted with water (150 mL). The separated crystals were filtered to yield **25** (2 g, 46%), mp 233-234 °C, $[\alpha]_D$ +119 (c 1, DMF). IR (cm^{-1}): 3159, 2928, 1698, 1614, 1457, 1324, 1259, 1008, 855, 759; ^1H NMR (DMSO-d_6, 25 °C, δ_{TMS}=0.00 ppm); the spectrum shows two signal sets owing to the slowly exchanging oxime isomers in a ca. 1.2 : 0.8 Z/E ratio; characteristic signals: 2.19 (d, H_x-15'), 2.39 (d, H_x-15), 2.82 (d, H_y-15'), 3.25 (d, H_y-15), 4.78-4.96 (m, H-3, H-3', H_y-5, H_y-5'), 7.58 (d, H-9, H-9'), 8.28 (d, H-12'), 8.40 (d, H-12), 11.79 (brs, NOH), 12.64 (brs, NOH'); MS (ESI, CID=20 %) m/z (rel. int. %): 352(/M+H/$^+$, 17), 335(40), 323(11), 307(100), 295(6), 266(13), 251(28), 239(47), 224(25), 211(5). Mass accuracy was between -0.91 and -0.76 ppm for the fragment ions. The protonated molecular ion peak can be detected at m/z 352.16558 (delta: 0.03 ppm), calculated value for $C_{20}H_{22}O_3N_3$: 352.16557.

(3*R*,16*S*,14*R*)-19-Oxovincamine (26). A solution of **25** (2 g, 5.7 mmol), *p*-toluenesulfonic acid hydrate (1.3 g, 6.7 mmol), and paraformaldehyde (1.24 g, 40 mmol) in acetic acid (38 mL) was stirred at 100 °C. After 4 h the reaction mixture was cooled, treated with water (120 mL) and extracted with dichloromethane (2×30 mL). The organic layer was washed with water (2×20 mL) and $NaHCO_3$ 1% solution (2×20 mL), dried ($MgSO_4$) and evaporated. The residue was dissolved in *ter*BuOK/methanol (0.2 g in 6 mL) and stirred under nitrogen for 10 h. The reaction mixture was treated with acetic acid (0.1mL), then filtered, the filtrate was evaporated to 5 mL and poured to water (20 mL) to obtain **26** (1.35 g, 64%), mp 133-134 °C (MeOH), $[\alpha]_D$ +99 (c 1, $CHCl_3$). IR (cm^{-1}): 3342, 2952, 1746, 1626, 1440, 1305, 1137, 1034, 744, ^1H NMR ($CDCl_3$, 25 °C, δ_{TMS}=0.00 ppm): 0.86 (3H, t, H_3-21), 1.20 (1H, dq, H_x-20), 1.37 (1H, dq, H_y-20), 1.56 (1H, td, H_{ax}-17), 2.00 (1H, ddd, H_{eq}-17), 2.18 (1H, d, H_x-15), 2.41 (1H, d, H_y-15), 2.46-2.60 (2H, m, H_2-18), 2.76-2.84 (2H, m, H_2-6), 3.06 (1H, m, H_{ax}-5), 3.88 (3H, s, OMe), 4.44 (1H, s, H-3), 4.93 (1H, m, H_{eq}-5), 7.10-7.20 (3H, m, H-10, H-11, H-12), 7.50 (1H, m, H-9); MS (ESI, CID=19 %) m/z (rel. int. %): 369(/M+H/$^+$, 6), 351(3), 337(34), 322(11), 309(100), 292(11), 250(8). Mass accuracy was between -0.53 and -0.24 ppm for the fragment ions. The protonated molecular ion

peak can be detected at m/z 369.18085 (delta: -0.09 ppm), calculated value for $C_{21}H_{25}O_4N_2$: 369.18088.

(3*R*,16*S*)-19-Oxoapovincamine (27). To vincamine **26** (1,3 g, 3.5 mmol) in a mixture of dichloromethane (25 mL) and formic acid (2 mL) acetyl chloride (1.2 mL, 16 mmol) was added and stirred for 2 h. the reaction mixture was treated with water (15 mL), the organic layer was washed with 10% $NaHCO_3$ solution (5 mL) and evaporated in vacuo. The residue was crystallized from methanol, to yield **27** (0.95 g, 77.5%), mp 119-120 °C (MeOH), $[\alpha]_D$ +36 (c 1, $CHCl_3$). IR (cm^{-1}): 3418, 2933, 1730, 1650, 1439, 1264, 1194, 1085, 745; ^1H NMR ($CDCl_3$, 25 ^oC, δ_{TMS}=0.00 ppm): 0.51 (1H, dq, H_x-20), 0.72 (3H, t, H_3-21), 1.43 (1H, dq, H_y-20), 1.90 (1H, m, H_{ax}-17), 2.16 (1H, m, H_{eq}-17), 2.52 (1H, ddd, H_{ax}-18), 2.63 (1H, dd, H_{eq}-18), 2.78-2.86 (2H, m, H_2-6), 3.10 (1H, m, H_{ax}-5), 3.98 (3H, s, OMe), 4.52 (1H, s, H-3); 4.98 (1H, m, H_{eq}-5), 6.39 (1H, s, H-15), 7.16-7.24 (2H, m, H-10, H-11), 7.34 (1H, m, H-12); 7.49 (1H, m, H-9); MS (ESI, CID=19 %) m/z (rel. int. %): 351(/M+H/$^+$, 52), 319(100), 291(20), 240(61), 208(26). Mass accuracy was between -0.67 and -0.48 ppm for the fragment ions. The protonated molecular ion peak can be detected at m/z 351.17035 (delta: 0.09 ppm), calculated value for $C_{21}H_{23}O_3N_2$: 351.17032.

(3*R*,16*S*)-2'Hydroxyethyl 19-oxo-apovincaminate (10). To the solution of **27** (0.9 g, 2.5 mmol) in ethylene glycol (9 mL) potassium *tert*-butoxide (0.05 g, 0.4 mmol) was added. The reaction mixture was stirred at 100 °C for 2 h. After cooling to r.t. the solution was treated with acetic acid/water (0.05ml/45 ml) to obtain 0.77 g (80% yield) of **10**, mp 115-117 °C (MeOH), $[\alpha]_D$ +25 (c 1, MeOH). IR (cm^{-1}): 3401, 2965, 1725, 1631, 1438, 1265, 1194, 1079, 746; ^1H NMR ($CDCl_3$, 25 ^oC, δ_{TMS}=0.00 ppm): 0.51 (1H, dq, H_x-20), 0.67 (3H, t, H_3-21), 1.37 (1H, dq, H_y-20), 1.98 (1H, m, H_{ax}-17), 2.08 (1H, m, H_{eq}-17), 2.32-2.52 (2H, m, H_2-18), 2.68 (1H, m, H_{ax}-6), 2.80 (1H, m, H_{eq}-6), 3.06 (1H, m, H_{ax}-15), 3.71 (2H, m, $CO_2CH_2C\underline{H_2}OH$), 4.37 (2H, m, $CO_2C\underline{H_2}CH_2OH$), 4.68 (1H, s, H-3), 4.76 (1H, dd, H_{eq}-5), 4.50 (1H, brs, OH), 6.55 (1H, s, H-15), 7.10-7.18 (2H, m, H-10, H-11), 7.35 (1H, m, H-12), 7.50 (1H, m, H-9); MS (ESI, CID=20 %) m/z (rel. int. %): 381(/M+H/$^+$, 100), 363(5), 352(8), 337(53), 319(96), 291(28), 270(33), 208(91). Mass accuracy was between -0.55 and -0.34 ppm for the fragment ions. The protonated molecular ion peak can be detected at m/z 381.18080 (delta: -0.22 ppm), calculated value for $C_{22}H_{25}O_4N_2$: 381.18088.

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