Pergamon

Bioorganic & Medicinal Chemistry 7 (1999) 2961–2969

BIOORGANIC & MEDICINAL CHEMISTRY

D-Ring Substituted Rhazinilam Analogues: Semisynthesis and Evaluation of Antitubulin Activity

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Received 20 April 1999; accepted 2 August 1999

Abstract—Novel (−)- and (+)-rhazinilam derivatives substituted on the D-ring (compounds **3**, **4**, **5** and **6**) have been prepared from (+)-vincadifformine **7** and (−)-tabersonine and evaluated against the disassembly of microtubules into tubulin. Along with this study, a reproducible 'one pot' semisynthesis of (−)-rhazinilam **1** from (+)-1,2-didehydroaspidospermidine **2** was performed allowing the easy preparation of these new compounds.

Introduction

Among the different classes of natural antimitotic compounds, (−)-rhazinilam **1** is a unique molecule due to its activity on tubulin. This compound induces spiralization of tubulin such as vinblastine and inhibits the cold-induced disassembly of microtubules (paclitaxel like activity).[1] Despite the apparent similarity between the effects of (−)-rhazinilam and paclitaxel on the microtubule skeleton, these two compounds possess two distinct mechanisms of action.[1] First isolated by Linde from *Melodinus australis*, in 1965,[2] the structure of rhazinilam **1** was established in 1972.[3,4] A total synthesis of (±)-rhazinilam was performed by Smith's group a year later along with a semisynthesis of (−)-**1** from (+)-1,2-didehydroaspidospermidine **2**.[5] The antitubulin activity of rhazinilam **1** was discovered in our laboratory during a tubulin assay based systematic screening of the Malaysian Flora.[6,7] Bioassay-guided purification of a crude extract of *Kopsia singapurensis* Ridl. led to the isolation of (−)-rhazinilam **1** as the main active product. Structure–activity relationships then have been studied from analogues obtained by chemical modifications of (−)-rhazinilam **1**, and by semisynthesis from (+)-1,2-didehydroaspidospermidine analogues.[8,9] Other phenyl–pyrrole[10,11] and biphenyl compounds.[12,13] showing a similar activity as natural (−)-rhazinilam **1** also have been synthesized. Most of the chemical modifications realised in these previous studies concerned the phenyl–pyrrole part and the amide function of the molecule. In this study, we focused our attention on chemical modifications of the D-ring, relatively unknown regarding interaction with the receptor. We thus report here the synthesis as well as the biological activity evaluation of new D-ring substituted rhazinilam analogues **3**, **4**, **5** and **6**.

Results and Discussion

D-Ring substituted rhazinilam analogues can be prepared rapidly by two different ways. The first method is the direct functionalization of rhazinilam **1**. The second one is the semisynthesis of (−)-rhazinilam analogues from (+)-1,2-didehydroaspidospermidine derivatives according to Smith's methodology.[5] The former method was not readily adapted to our purpose due to the instability of rhazinilam **1** under acid and basic conditions.

The synthetic strategy, using the semisynthetic pathway, starts from (+)-vincadifformine **7** affording easily (+)-1,2-didehydroaspidospermidine **2** after acid treatment. Our investigations in the preparation of (−)-rhazinilam analogues led us first to improve the semisynthesis of (−)-rhazinilam **1** from (+)-1,2-didehydroaspidospermidine **2** (Scheme 1). Indeed, the reactions previously described[5] were not always reproducible and poor yields were obtained due to work up difficulties. Two

Key words: Natural products; rhazinilam; tubulin; cytotoxicity; structure–activity relationships.

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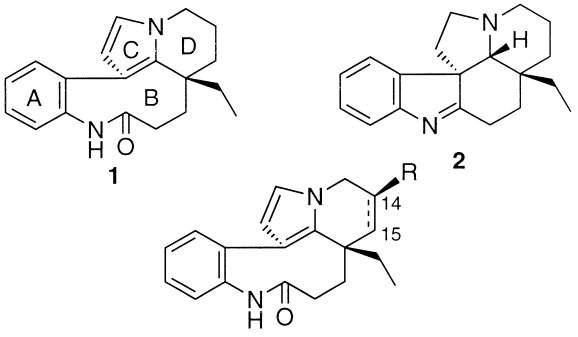
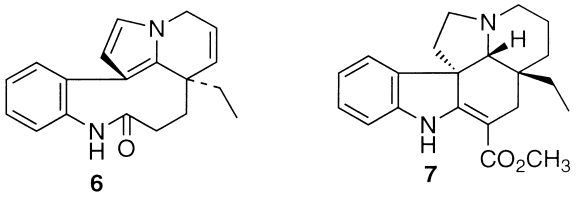
0968-0896/99/$ - see front matter
PII: S0968-0896(99)00241-2

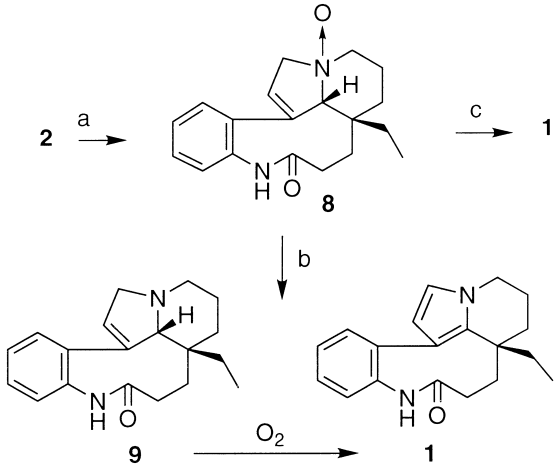
observations were made concerning the starting point of the synthetic modifications. On one hand, the reaction between **2** and *meta*-chloroperoxybenzoic acid led to a reaction mixture containing 5,21-dihydrorhazinilam *N*-oxide **8** as it was previously described.[8] Submitted to the action of Fe(II) salt, compound **8** afforded rhazinilam **1** (45% yield) along with 5,21-dihydrorhazinilam **9** (5% yield), in approximately 30 min (Scheme 1, pathway b). On the other hand, compound **9** led in 1 to 2 days to (−)-rhazinilam **1** by autoxidation. These two considerations were not consistent with the hypothesis of the only action of Fe(II) as reductive agent of the *N*-oxide function of **8** leading to **9** which gave rhazinilam **1** after autoxidation.[8] We thus considered that rhazinilam **1** could be formed directly from the *N*-oxide **8** through a Polonovski reaction involving the Fe(II)/Fe(III) redox reaction of iron. In order to avoid the work up worries due to the presence of iron ions, we submitted the *N*-oxide **8** to more classical Polonovski conditions (Ac_2O, Et_3N) to form a pyrrole group from an α,α′-dihydropyrrole *N*-oxide moiety.[14] Under these conditions compound **8** afforded directly (−)-rhazinilam **1** in 81% yield (Scheme 1, pathway c). Starting from (+)-1,2-didehydroaspidospermidine **2**, the 'one pot' reaction yielded as well (−)-rhazinilam **1** in reproducible yield (50%) (see Experimental). Recently, *seco*-rhazinilams were also prepared from *seco*-didehydroaspidospermidine derivatives by *meta*-chloroperoxybenzoic acid oxidation and thermolysis.[15]





In order to prepare (−)-rhazinilam analogues substituted on the D-ring, we aimed to obtain a lactam function at position 3 (Scheme 2).

(+)-Vincadifformine **7** was first protected by a Boc group to yield **10**. The oxidation of **10** was best performed with bromine in tetrahydrofuran:water according to Picot and Lusinchi.[16] No regioselectivity was observed and **10** afforded **11** (45%) along with **12** (43%). Compound **11** was then functionalized at position 14 using lithium diisopropylamide

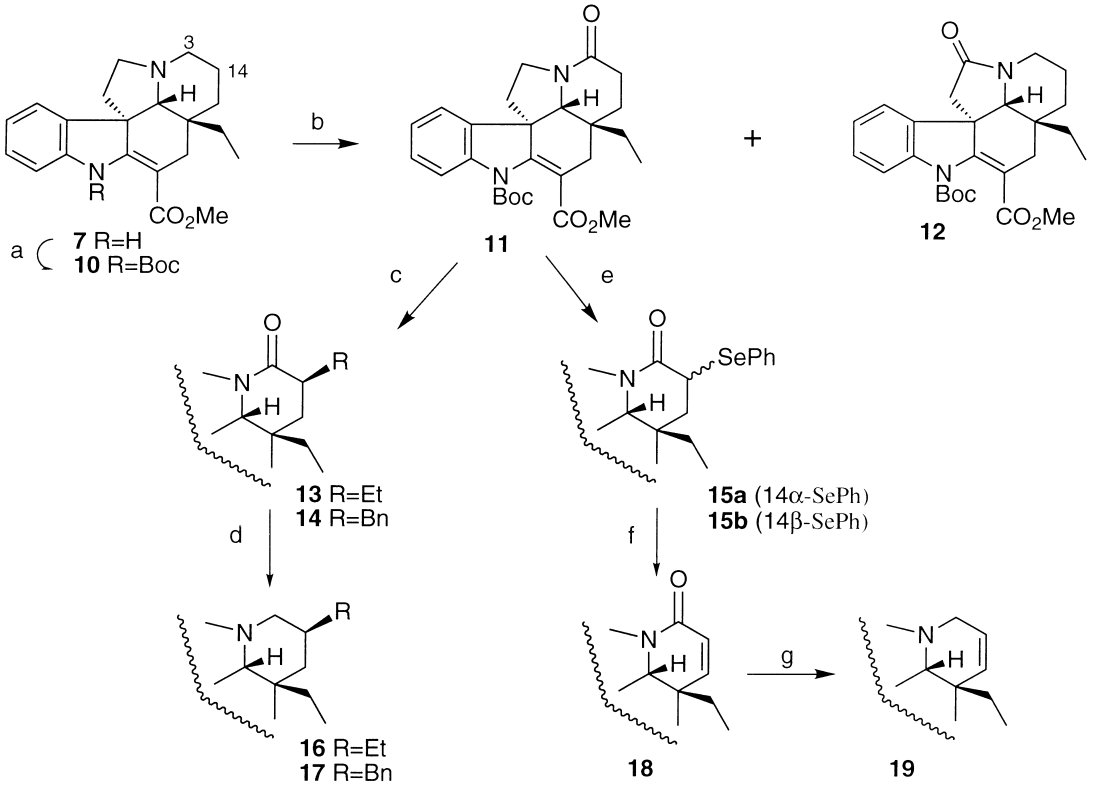


Scheme 1. (a) mCPBA, CH_2Cl_2, −20°C (**8** (65%)); (b) $FeSO_4$, H_2O, rt (**1** (45%), **9** (5%)); (c) Ac_2O, Et_3N, CH_2Cl_2, 0°C (**1** (81%)).

in tetrahydrofuran at −78°C. The reaction of the anion generated in situ with ethyl iodide and benzyl bromide afforded the kinetically favored compounds **13** and **14**, respectively, whereas phenylselenenyl chloride[17] led to the two epimers **15a** and **15b** due to the acidity of the hydrogen adjacent to the selenium. The β stereoselectivity observed in the case of **13** and **14** as well as the configuration of each product (α:β = 2:1) in the case of **15a** and **15b** were deduced from NMR spectra analysis (^{1}H, NOESY). Moreover, the β-position of the ethyl group at C-14 of compound **13** was confirmed by X-ray analysis (Fig. 1).

The selective reduction of **13** and **14** at position 3 was realized with borane–tetrahydrofuran complex in tetrahydrofuran at 0°C.[18] Under these conditions, no reactivity of the carbonate and ester groups was observed and compounds **16** and **17** were both obtained in quantitative yield. Concerning the 14,15 unsaturated series, compounds **15a** and **15b** were treated with *meta*-chloroperoxybenzoic acid in methylene chloride at −78°C to yield the corresponding selenoxide products. Elimination of the selenoxide group occurred smoothly at room temperature to afford **18** in quantitative yield. In the case of **15a**, addition of sodium hydroxide was necessary to aid elimination. Finally, 1,2 reduction of the α,β-unsaturated amide **18** was achieved selectively with diisobutylaluminum hydride[19] giving the N_a-protected derivative of (+)-tabersonine **19**.

Compounds **16**, **17** and **19** were then submitted to decarboxylation and Boc-deprotection with hydrochloric acid to afford 1,2-didehydroaspidospermidine derivatives **20**, **21** and **22** in high yields. No purification was used at this time since these compounds showed a great tendency to autooxidise. The 'one pot' semisynthesis was finally performed as described above to give (−)-14β-ethylrhazinilam **3**, (−)-14β-benzylrhazinilam **4** and (−)-14,15-didehydrorhazinilam **5** in 50% yield each from compounds **20**, **21** and **22**, respectively. In order to compare the effects of the enantiomer series ((+)-form) to that of natural (−)-rhazinilam series, we also prepared (+)-14,15-didehydrorhazinilam **6** from (−)-1,2,14,15-



Scheme 2. (a) Boc_2O, DMAP, DMF, 100%; (b) Br_2, $NaHCo_3$, THF/H_2O, 95%; (c) LDA, RX, THF, −78°C –rt, (RX = EtI **9** 60%; RX = BnBr **12** 83%); (d) BH_3-THF, THF, 0°C, 100%; (e) LDA 2 equiv, PhSeCl 1 equiv, THF, −78°C –rt, 80%; (f) mCPBA, CH_2Cl_2, −78°C –rt, 100%; (g) DIBAH, THF, −20°C, 95%.

tetradehydroaspidospermidine (**23**)[20,21] (Scheme 3). The effects of (−)-14β-ethylrhazinilam **3**, (−)-14β-benzylrhazinilam **4**, (−)- and (+)-14,15-didehydrorhazinilam **5** and **6** on microtubules disassembly and their cytotoxicity are summarized in Table 1. Inhibition of microtubules disassembly was evaluated using tubulin from bovine brain according to the previously described methodology.[8] Cytotoxicity was examined on the KB human cancer line.[22]

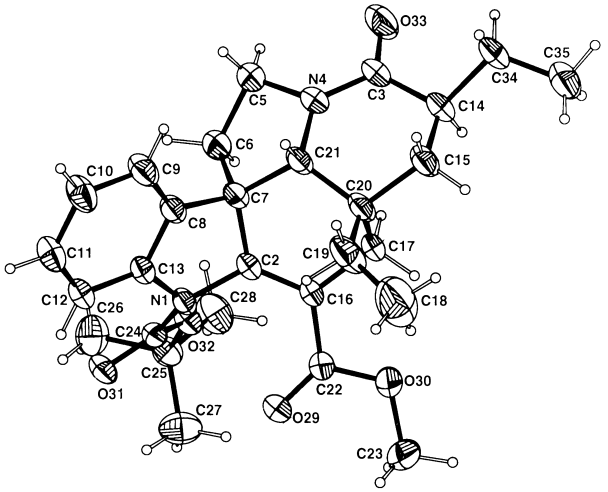


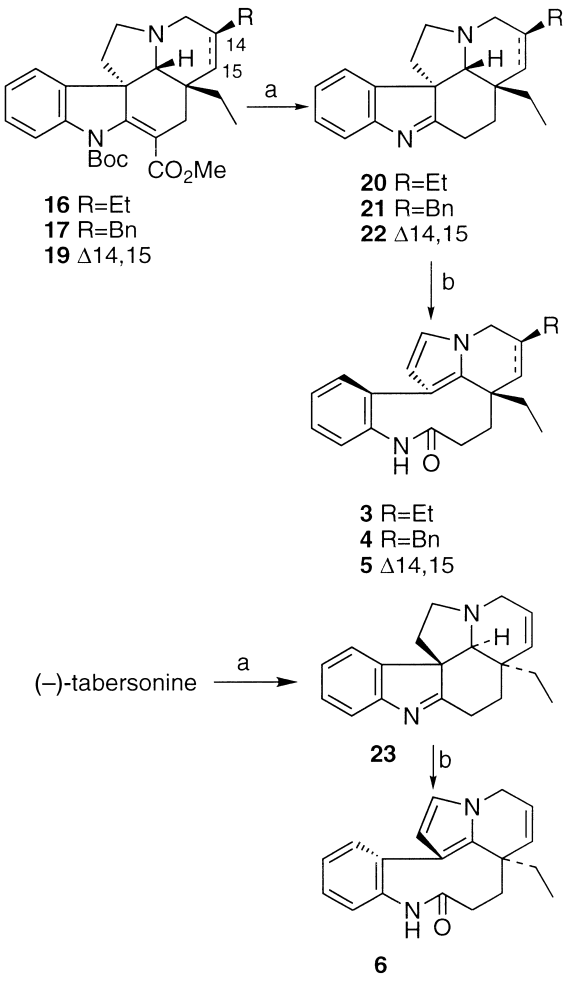
Figure 1. X-ray crytallographic structure of compound **13** (crystallographic numerotation of atoms was used).

The substitution at position 14 with the hydrophobic ethyl and benzyl groups, results in a clear decrease of the activity inhibiting the disassembly of microtubules into tubulin. (−)-14β-Ethylrhazinilam **3** was found 50-fold less active than (−)-rhazinilam **1** and (−)-14β-benzylrhazinilam **4** was found inactive. The unsaturated D-ring derivative, (−)-14,15-didehydrorhazinilam **5** is only 2 times less active than rhazinilam **1**. Unexpectedly, its enantiomer (+)-14,15-didehydrorhazinilam **6** retains some interaction with microtubules. This result is at variance with the fact that the binding interaction with microtubules was shown to be stereoselective in the biphenyl series mimicking the activity of rhazinilam.[13] Regarding the cytotoxicity of the compounds, one

Table 1. Cytotoxicity and antitubulin activity of rhazinilam **1** and analogues **3**, **4**, **5** and **6**

Compound	Cytotoxicity (KB cell line) IC_{50} (μM)[a,b]	Inhibition of microtubules disassembly IC_{50} (μM)[c]
Rhazinilam **1**	2	3
3	100	150
4	44	Inactive
5	4	6
6	300	130

[a] The IC_{50} for cytotoxicity and inhibition of microtubules disassembly was evaluated from measurements run in triplicate.
[b] The cytotoxicity IC_{50} values refer to the concentration of compounds corresponding to 50% growth inhibition after 72 h incubation.
[c] IC_{50} is the concentration of test compound required to inhibit 50% of the rate of microtubules disassembly.



Scheme 3. (a) HCl 12 N, Δ, 5 mn; (b) mCPBA, Ac_2O, NEt_3, CH_2Cl_2, −20°C, 50%.

observes a rather good correlation with the microtubules disassembly assay. However, a discrepancy is observed for compound **4** which is 20 times less cytotoxic than rhazinilam **1** on KB cells but does not show any interaction with microtubules. In this case, the cytotoxicity may be related to a mode of action different from a direct interaction with microtubules.

Conclusion

In summary, this work provides new information on the structure–activity relationships in the rhazinilam series. The presence of hydrophobic groups at C-14 on the D-ring led to a large decrease in the 'antitubulin' activity indicating that this part of the molecule may be in interaction with the binding site. In order to confirm this hypothesis, future studies will concern substitutions at position 14 with polar groups as well as chemical modifications at positions 3 and 15.

Experimental

All reactions were followed by TLC on glass plates coated with Si gel 60 F_{254} silical gel and spots revealed by spraying with a solution of molybdatophosphoric acid in EtOH. Melting points (mp) were measured on a Köfler apparatus. Optical rotations have been measured on a Perkin–Elmer 141 MC polarimeter. Infrared spectra were recorded on a Nicolet FT–IR 205 and UV spectra on a Elmer-Lambda 5 spectrometer. ^1H and ^{13}C NMR spectra were recorded on Bruker AC-200, AC-250 or AC-300 spectrometers using tetramethylsilane as internal standard. Chemical shifts are expressed in part per million (ppm). s, bs, d, bd, t, dd, q and m indicate singlet, broad singlet, doublet, broad doublet, triplet, doublet of doublet, quartet and multiplet, respectively. Mass spectra were measured on a AEI MS-50 spectrometer. All physicochemical measurements and elemental analyses were performed at the ICSN, CNRS, Gif-sur-Yvette, France.

Preparation of 5,21-dihydrorhazinilam *N*-oxide (8). To a solution of **2** (100 mg, 0.36 mmol) in methylene chloride (10 mL) at −20°C was added sodium hydrogen carbonate (36 mg, 0.43 mmol) followed by a solution of *meta*-chloroperoxybenzoic acid (62 mg, 0.36 mmol) in methylene chloride (1 mL). The formation of the *N*-oxide compound was observed by TLC on alumina (eluent CH_2Cl_2:MeOH: 90:10; $R_f \approx 0.8$). An additional solution of *meta*-chloroperoxybenzoic acid (0.25 g, 1.44 mmol) in methylene chloride (4 mL) was then added. The orange solution was then stirred at −20°C until *N*-oxide consumption (4–8 h). The solvent was then evaporated in vacuo and water was added. The mixture was extracted with butan-1-ol (5×). After evaporation of the solvent, the crude product was purified by chromatography on alumina (eluent CH_2Cl_2:MeOH, 90:10) to afford **8** as a yellowish amorphous solid (72 mg, 65%); ^1H NMR (250 MHz, $CDCl_3$) δ 0.65 (3H, t, $J = 7.3$ Hz, H-18); 1.20 (1H, m, H-19); 1.29 (1H, m, H-19); 1.44 (2H, m, H-15 + H-17); 1.74 (4H, m, 2H-14 + H-15 + H-17); 1.97 (1H, m, H-16); 2.16 (1H, m, H-16); 3.51 (1H, m, H-3); 3.84 (1H, m, H-3); 4.10 (1H, m, H-5); 4.37 (1H, s, H-21); 4.59 (1H, m, H-5); 5.85 (1H, s, H-6); 7.16 (1H, m, H-12); 7.25 (2H, m, H-10 + H-11); 7.50 (1H, m, H-9); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 7.4 (C-18); 19.1 (C-14); 27.5 (C-17); 27.7 (C-16); 32.5 (C-19); 34.7 (C-15); 44.9 (C-20); 66.8 (C-3); 73.2 (C-5); 92.2 (C-20); 125.8 (C-6); 126.3 (C-12); 127.2 + 129.5 (C-10 + C-11); 129.0 (C-9); 133.3 (C-7); 136.0 (C-8); 138.0 (C-13); 178.3 (C-2); IR ($CHCl_3$) ν 1666 cm^{-1}; MS (CI+) *m/z* 295 ($[M-H_2O+H]^+$).

Preparation of rhazinilam (1) from 5,21-dihydrorhazinilam *N*-oxide (8). Acetic anhydride (5.23 μL, 1 mmol) was added to a suspension of **8** (20 mg, 0.064 mmol) in methylene chloride at 0°C. The mixture was allowed to warm to room temperature and was stirred for 30 min. Water was then added, the aqueous layer was extracted with methylene chloride (2×). The combined organic layers were dried over sodium sulfate and the solvent was removed in vacuo to yield pure **1** as a white amorphous solid (15.2 mg, 81%); ^1H NMR (300 MHz, $CDCl_3$) δ 0.72 (3H, t, $J = 7.3$ Hz, H-18); 1.25 (1H, dq, $J = 14.6$, 7.3 Hz, H-19); 1.47 (1H, dq, $J = 14.6$, 7.3 Hz, H-19); 1.49 (1H, m, H-17β); 1.96 (1H, m, H-16α); 2.24 (1H, m, H-14α); 2.38 (1H, m, H-16β); 2.46 (1H, m, H-17α);

3.79 (1H, m, H-3β); 4.01 (1H, m, H-3α); 5.76 (1H, m, H-6); 6.51 (1H, m, H-5); 6.69 (1H, bs, NH); 7.21 (1H, m, H-12); 7.30 + 7.35 (2H, m, H-10 + H-11); 7.43 (1H, m, H-9); ^{13}C NMR (75 MHz, $CDCl_3$) δ 8.2 (C-18); 19.5 (C-14); 28.2 (C-16); 30.2 (C-19); 33.1 (C-15); 36.7 (C-17); 38.9 (C-20); 46.1 (C-3); 109.9 (C-6); 117.4 (C-7); 119.1 (C-5); 126.9 (C-12); 127.2 + 128.0 (C-10 + C-11); 130.6 (C-20); 131.5 (C-9); 138.2 (C-8); 140.4 (C-13); 177.4 (C-2); MS (CI +) *m*/*z* 295 ($[M+H]^+$).

'One pot' preparation of (−)-rhazinilam 1 from 2. To a solution of **2** (0.50 mmol) in methylene chloride (10 mL) at −20°C was added sodium hydrogen carbonate (50 mg, 0.60 mmol) followed by a solution of *meta*-chloroperoxybenzoic acid (86 mg, 0.50 mmol) in methylene chloride (1 mL). The formation of the N_b-oxide compound was observed by TLC on alumina (eluent CH_2Cl_2:MeOH: 90:10; $R_f \approx 0.8$). An additionnal solution of *meta*-chloroperoxybenzoic acid (0.35 g, 2.00 mmol) in methylene chloride (4 mL) was then added. The orange solution was then stirred at −20°C until N_b-oxide consumption (4–8 h). The formation of the 5,21-dihydrorhazinilam N_b-oxide was observed by TLC on alumina (eluent CH_2Cl_2:MeOH: 90:10; $R_f \approx 0.5$). Triethylamine (0.7 mL, 5.00 mmol) was then added, followed by acetic anhydride (74 μL, 1.00 mmol). The mixture was allowed to warm to room temperature and was stirred for 30 min. Water was then added, the aqueous layer was extracted with methylene chloride (2×). The combined organic layers were dried over sodium sulfate and the solvent was removed in vacuo. Excess triethylamine was removed by azeotropic distillation with ethanol. The crude product (brown oil) was chromatographed on silica gel to yield **1** (50%).

(N_a-*tert*-Butoxycarbonyl)vincadifformine (10). *tert*-Butoxycarbonic anhydride (4.6 g, 28 mmol), 4-(*N*,*N*-dimethylamino)pyridine (0.33 g, 2.8 mmol) and (+)-vincadifformine **7** (4.6 g, 14 mmol) were dissolved in minimum dimethylformamide. A strong gas evolution occurred. The mixture was stirred at room temperature overnight. *tert*-Butoxycarbonic anhydride (2.3 g, 14 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (0.165 g, 1.4 mmol) were then added again. After stirring 1 h at room temperature, a white precipitate appeared. The solvent was evaporated. The crude product was purified by flash chromatography (eluent heptane:ethyl acetate, 80:20) to provide **10** (5.66 g) in 95% yield as a yellowish solid. Compound **10** was further recrystallized from ethyl acetate as yellowish needles; mp 149–150°C (AcOEt); $[\alpha]_D^{25}$ + 115.4° (*c* 1.20, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.56 (3H, t, *J* = 7.4 Hz, H-18); 0.70 (1H, qd, *J* = 14.8, 7.4 Hz, H-19); 1.13 (1H, qd, *J* = 14.8, 7.4 Hz, H-19); 1.24 (1H, m, H-15β); 1.53 (9H, s, $C(C\underline{H}_3)_3$); 1.59 (1H, m, H-14β); 1.64 (1H, m, H-6β); 1.77 (1H, m, H-15α); 1.86 (1H, m, H-14α); 1.98 (1H, m, H-17β); 2.16 (1H, m, H-6α); 2.26 (1H, m, H-3β); 2.34 (1H, s, H-21); 2.37 (1H, m, H-5β); 2.91 (1H, m, H-5α); 3.01 (1H, m, H-17α); 3.12 (1H, m, H-3α); 3.75 (3H, s, $CO_2C\underline{H}_3$); 7.01 (1H, m, H-10); 7.15 (1H, m, H-9); 7.19 (1H, m, H-11); 7.65 (1H, m, H-12); ^{13}C NMR (75 MHz, $CDCl_3$) δ 7.2 (C-18); 22.4 (C-14); 28.0 (C-17); 28.3 ($C(\underline{C}H_3)_3$); 28.9 (C-19); 32.9 (C-15); 38.0 (C-20); 42.7 (C-6); 51.6 ($CO_2\underline{C}H_3$); 51.8 (C-3 + C-5); 53.2 (C-7); 71.1 (C-21); 82.0 ($\underline{C}(CH_3)_3$); 112.4 (C-16); 116.1 (C-12); 120.5 (C-9); 123.5 (C-10); 127.3 (C-11); 138.5 (C-8); 140.7 (C-13); 149.6 (C-2); 151.3 ($\underline{C}O_2C(CH_3)_3$); 168.3 ($\underline{C}O_2CH_3$); IR ($CHCl_3$) ν 1717 cm^{-1}; MS (EI) *m*/*z* 438 (M^+); 338. Anal. calcd (found) for $C_{26}H_{34}N_2O_4$: C, 71.21 (70.94); H, 7.81 (7.88); N, 6.39 (6.22); O, 14.59 (14.78).

Oxidation of compound 10. A solution of bromine (0.3 mL, 5.86 mmol) in tetrahydrofuran (40 mL) was added dropwise at 20°C to a stirred solution of **10** (1 g, 2.28 mmol) and sodium carbonate (0.5 g, 4.72 mmol) in tetrahydrofuran:water 1:1 (200 mL). The red solution was decolorized by treatment with a saturated solution of sodium thiosulfate. Sodium chloride was added until the layers separated. The aqueous layer was then extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate and the solvent was evaporated under vacuo to yield 1.3 g of crude product as a slight yellow oil. The residue was chromatographed on silica gel (eluent heptane:acetone: 70:30) to afford **11** (0.47 g, 45%) and **12** (0.42 g, 43%) as colorless oils. Compound **11** was further crystallized from ethyl acetate for analysis.

3-oxo-(N_a-*tert*-Butoxycarbonyl)vincadifformine (11). Mp 193–4°C (AcOEt); $[\alpha]_D^{25}$ +27.4° (*c* 1.43, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.67 (3H, t, *J* = 7.4 Hz, H-18); 1.04 (1H, dq, *J* = 14.8, 7.4 Hz, H-19); 1.16 (1H, dq, *J* = 14.8, 7.4 Hz, H-19); 1.44 (1H, m, H-15β); 1.58 (9H, s, $C(C\underline{H}_3)_3$); 1.91 (1H, m, H-6β); 1.96 (1H, m, H-15α); 2.05 (1H, m, H-6α); 2.16 (1H, m, H-17α); 2.30 (1H, m, H-14); 2.39 (1H, m, H-14); 2.46 (1H, m, H-17β); 3.32 (1H, m, H-5β); 3.51 (1H, s, H-21); 3.77 (3H, s, $CO_2C\underline{H}_3$); 4.11 (1H, m, H-5α); 7.08 (1H, m, H-10); 7.15 (1H, m, H-9); 7.28 (1H, m, H-11); 7.67 (1H, m, H-12); ^{13}C NMR (75 MHz, $CDCl_3$) δ 7.5 (C-18); 27.8 (C-19); 28.1 ($C(\underline{C}H_3)_3$); 29.9 (C-15); 30.6 (C-17 + C-14); 39.1 (C-6 + C-20); 42.4 (C-5); 51.6 ($CO_2\underline{C}H_3$); 54.4 (C-7); 66.8 (C-21); 83.0 ($\underline{C}(CH_3)_3$); 110.4 (C-16); 116.2 (C-12); 121.2 (C-9); 124.0 (C-10); 128.3 (C-11); 136.5 (C-8); 140.6 (C-13); 148.7 (C-2); 150.6 ($\underline{C}O_2C(CH_3)_3$); 167.6 ($\underline{C}O_2CH_3$); 170.9 (C-3); IR ($CHCl_3$) ν 1724; 1625 cm^{-1}; MS (CI +) *m*/*z* 453 ($[M+H]^+$); 353. Anal. calcd (found) for $C_{26}H_{32}N_2O_5$: C, 69.00 (68.82); H, 7.13 (7.18); N, 6.19 (6.08); O, 17.68 (17.53).

5-oxo-(N_a-*tert*-Butoxycarbonyl)vincadifformine (12). $[\alpha]_D^{25}$ +54.5° (*c* 0.94, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.61 (3H, t, *J* = 7.4 z, H-18); 0.85 (1H, dq, *J* = 14.8, 7.4 Hz, H-19); 1.24 (1H, dq, *J* = 14.8, 7.4 Hz, H-19); 1.54 (9H, s, $C(C\underline{H}_3)_3$); 1.55 (1H, m, H-15β); 1.63 (2H, m, H-14); 1.91 (1H, m, H-15α); 2.10 (1H, m, H-17β); 2.48 (1H, m, H-6β); 2.58 (1H, m, H-17α); 2.77 (1H, m, H-3β); 2.88 (1H, m, H-6α); 3.77 (3H, s, $CO_2C\underline{H}_3$); 3.78 (1H, s, H-21); 4.34 (1H, m, H-3α); 7.09 (1H, m, H-10); 7.15 (1H, m, H-9); 7.26 (1H, m, H-11); 7.67 (1H, m, H-12); ^{13}C NMR (75 MHz, $CDCl_3$) δ 6.8 (C-18); 19.8 (C-14); 26.7 (C-17); 28.1 ($C(\underline{C}H_3)_3$); 28.9 (C-19); 33.0 (C-15); 37.0 (C-20); 40.0 (C-3); 46.6 (C-6); 51.7 (C-7 + $CO_2\underline{C}H_3$); 66.1 (C-21); 82.8 ($\underline{C}(CH_3)_3$); 110.8 (C-16); 116.2 (C-12); 120.6 (C-9); 124.3 (C-10); 128.2 (C-11); 136.4 (C-8); 140.8 (C-13); 148.5 (C-2); 150.8

($\underline{C}O_2C(CH_3)_3$); 167.4 ($\underline{C}O_2CH_3$); 170.0 (C-5); IR ($CHCl_3$) ν 1723; 1678 cm^{-1}; MS (CI+) *m/z* 453 ($[M+H]^+$); 353; HRMS (CI+) *m/z* calcd (found) for $C_{26}H_{33}N_2O_5$ (MH^+): 453.2390 (453.2397).

14β-Ethyl-3-oxo-(*N*$_a$-*tert*-butoxycarbonyl)vincadifformine (13). To a stirred solution of diisopropylamine (0.62 mL, 4.42 mmol) in anhydrous tetrahydrofuran (5 mL) at −78°C was added *n*-butyl lithium 1.6 M in hexane (2.8 mL, 4.42 mmol). After 15 min at −78°C, a solution of compound **11** (1 g, 2.21 mmol) in anhydrous tetrahydrofuran (10 mL) was added, followed after 30 min at −78°C by ethyl iodide (0.36 mL, 4.42 mmol). Then the mixture was allowed to warm to room temperature over 2 h. The yellow solution was quenched carefully with water. The aqueous layer was extracted with ethyl acetate (2×). The combined organic layers were dried over sodium sulfate and the solvent was evaporated in vacuo. The crude product (yellow oil, 1 g) was chromatographed on silica gel (eluent heptane:acetone: 70:30) to yield 0.64 g of the title compound as a colorless oil (60%). Compound **13** was crystallized from cyclohexane for analysis: mp 192–193°C (cyclohexane); $[\alpha]_D^{25}$ + 82.1° (*c* 1.23, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.65 (3H, t, *J* = 7.4 Hz, H-18); 0.89 (1H, dq, *J* = 14.8, 7.4 Hz, H-19); 0.99 (3H, t, *J* = 7.4 Hz, $CHCH_2C\underline{H}_3$); 1.27 (1H, dq, *J* = 14.8, 7.4 Hz, H-19); 1.55 (9H, s, $C(C\underline{H}_3)_3$); 1.59 (1H, m, H-15β); 1.60 (1H, m, $CHC\underline{H}_2CH_3$); 1.84 (1H, m, H-6β); 1.92 (1H, m, H-6α); 1.97 (1H, m, H-15αa); 1.99 (1H, m, $CHC\underline{H}_2CH_3$); 2.26 (1H, m, H-17β); 2.34 (1H, m, H-17α); 2.38 (1H, m, H-14α); 3.16 (1H, m, H-5β); 3.77 (3H, s, $CO_2C\underline{H}_3$); 3.79 (1H, s, H-21); 4.36 (1H, m, H-5α); 7.08 (1H, m, H-10); 7.20 (1H, m, H-9); 7.27 (1H, m, H-11); 7.68 (1H, m, H-12); ^{13}C NMR (75 MHz, $CDCl_3$) δ 7.4 (C-18); 11.4 ($CHCH^2\underline{C}H_3$); 25.3 ($CH\underline{C}H_2CH_3$); 27.7 (C-17); 28.3 ($C(\underline{C}H_3)_3$); 29.1 (C-19); 34.9 (C-15); 37.4 (C-20); 39.7 (C-14); 41.0 (C-6); 43.1 (C-5); 51.8 ($CO_2\underline{C}H_3$); 55.0 (C-7); 66.4 (C-21); 83.0 ($\underline{C}(CH_3)_3$); 110.8 (C-16); 116.5 (C-12); 120.8 (C-9); 124.0 (C-10); 128.4 (C-11); 136.5 (C-8); 140.8 (C-13); 149.6 (C-2); 150.9 ($\underline{C}O_2C(CH_3)_3$); 167.6 ($\underline{C}O_2CH_3$); 171.2 (C-3); IR ($CHCl_3$) ν 1724; 1625 cm^{-1}; MS (CI+) *m/z* 481 ($[M+H]^+$); 381. Anal. calcd (found) for $C_{28}H_{36}N_2O_5$: C, 69.98 (70.02); H, 7.55 (7.34); N, 5.83 (5.67); O, 16.64 (16.59).

X-ray determination of compound **13**:[23] Mr = 478.57, orthorhombic, space group P212121, a = 7.7849(3), b = 11.932(1), c = 28.486(1) Å, V = 2646.0(3) Å^3, Z = 4, ρ = 1.201 g cm^{-1}. Of the 7229 reflections collected, 4351 reflections ($I > 2\sigma(I)$) were used for the refinement. The final residuals were R1 = 0.0506, *w*R2 = 0.1398, and GOF = 1.031. Structure was solved by direct methods with SHELX86[24] and refined by the full-matrix least squares approximation based on F^2 with SHELXL93[25] programs. Refinement was anisotropic for all non-H atoms. Hydrogen atoms were located from a difference map and refined isotropically.

14β-Benzyl-3-oxo-(*N*$_a$-*tert*-butoxycarbonyl)vincadifformine (14). Compound **14** was obtained from **11** (1 g, 2.21 mmol) by the above procedure using diisopropylamine (0.31 mL, 2.21 mmol), *n*-butyllithium (1.4 mL, 2.24 mmol), and benzyl bromide (0.27 mL, 2.27 mmol). The crude product (yellow oil, 1.2 g) was chromatographed on silica gel (eluent heptane:ethyl acetate, 70:30) to yield 1.0 g of the title compound as a colorless oil (83%); $[\alpha]_D^{25}$ + 114.1° (*c* 1.07, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.52 (3H, t, *J* = 7.4 Hz, H-18); 0.82 (1H, dq, *J* = 14.8, 7.4 Hz, H-19); 1.21 (1H, dq, *J* = 14.8, 7.4 Hz, H-19); 1.54 (1H, m, H-15β); 1.54 (9H, s, $C(C\underline{H}_3)_3$); 1.79 (1H, m, H-15α); 1.84 (1H, m, H-6β); 1.95 (1H, m, H-6α); 2.14 (1H, m, H-17β); 2.34 (1H, m, H-17α); 2.72 (1H, m, H-14α); 2.75 (1H, m, $C\underline{H}_2$-Φ); 3.15 (1H, m, H-5β); 3.47 (1H, m, $C\underline{H}_2$-Φ); 3.75 (3H, s, $CO_2C\underline{H}_3$); 3.75 (1H, s, H-21); 4.43 (1H, m, H-5α); 7.07 (1H, m, H-10); 7.17 (1H, m, H-9); 7.22 (1H, m, H-11); 7.24 (3H, m, H-Φ_o + H-Φ_p); 7.31 (2H, m, H-Φ_m); 7.67 (1H, m, H-12); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 7.2 (C-18); 27.1 (C-17); 28.2 ($C(\underline{C}H_3)_3$); 28.8 (C-19); 35.0 (C-15); 37.0 (C-20); 38.4 ($\underline{C}H_2$-Φ); 39.6 (C-14); 41.4 (C-6); 43.1 (C-5); 51.8 ($CO_2\underline{C}H_3$); 54.9 (C-7); 66.3 (C-21); 82.9 ($\underline{C}(CH_3)_3$); 110.7 (C-16); 116.4 (C-12); 120.6 (C-9); 123.9 (C-10); 126.4 (C-Φ_p); 128.3 (C-11); 128.5 (C-Φ_m); 129.3 (C-Φ_o); 136.4 (C-8); 139.4 (C_q-Φ); 140.6 (C-13); 149.6 (C-2); 150.8 ($\underline{C}O_2C(CH_3)_3$); 167.4 ($\underline{C}O_2CH_3$); 170.0 (C-3); IR ($CHCl_3$) ν 1722; 1625 cm^{-1}; MS (CI+) *m/z* 543 ($[M+H]^+$); 453; 443; HRMS (CI+) *m/z* calcd (found) for $C_{33}H_{39}N_2O_5$ (MH^+): 543.2859 (543.2870).

Compounds 15a and 15b. Compounds **15a** and **15b** were obtained from **11** (3.89 g, 8.61 mmol) by the above procedure using diisopropylamine (2.41 mL, 17.2 mmol), *n*-butyllithium (10.8 mL, 17.3 mmol), and a solution of phenylselenenyl chloride (1.65 g, 8.62 mmol) in anhydrous tetrahydrofuran (10 mL). The crude product (yellow oil, 5.5 g) was chromatographed on silica gel (eluent heptane:ethyl acetate, 50:50) to afford **15b** (1.39 g, 26%) as white needles and **15a** (2.80 g, 54%) as a colorless oil.

14α-Phenylselenenyl-3-oxo-(*N*$_a$-*tert*-butoxycarbonyl)-vincadifformine (15a). $[\alpha]_D^{25}$ −46.5° (*c* 1.14, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.62 (3H, t, *J* = 7.4 Hz, H-18); 1.19 (1H, dq, *J* = 14.8, 7.4 Hz, H-19); 1.21 (1H, dq, *J* = 14.8, 7.4 Hz, H-19); 1.57 (9H, s, $C(C\underline{H}_3)_3$); 1.90 (1H, m, H-6β); 1.97 (1H, m, H-15β); 2.02 (1H, m, H-6α); 2.15 (1H, m, H-17α); 2.26 (1H, m, H-15α); 2.40 (1H, m, H-17β); 3.33 (1H, m, H-5β); 3.76 (3H, s, $CO_2C\underline{H}_3$); 3.90 (1H, s, H-21); 4.04 (1H, m, H-14β); 4.13 (1H, m, H-5α); 7.10 (1H, m, H-10); 7.14 (1H, m, H-9); 7.29 (1H, m, H-11); 7.34 (3H, m, H-Φ_p + H-Φ_m); 7.67 (1H, m, H-12); 7.70 (2H, m, H-Φ_o); ^{13}C NMR (75 MHz, $CDCl_3$) δ 7.3 (C-18); 28.1 ($C(\underline{C}H_3)_3$); 28.8 (C-19); 30.5 (C-17); 36.3 (C-15); 39.1 (C-20); 39.6 (C-6); 40.3 (C-14); 43.3 (C-5); 51.7 ($CO_2\underline{C}H_3$); 54.9 (C-7); 65.8 (C-21); 83.1 ($\underline{C}(CH_3)_3$); 110.3 (C-16); 116.3 (C-12); 121.2 (C-9); 124.1 (C-10); 128.2 + 128.4 (C-Φ_p + C-11); 128.9 (C_q-Φ); 129.3 (C-Φ_m); 134.5 (C-Φ_o); 136.2 (C-8); 140.7 (C-13); 149.1 (C-2); 150.7 ($\underline{C}O_2C(CH_3)_3$); 167.7 ($\underline{C}O_2CH_3$); 168.8 (C-3); IR ($CHCl_3$) ν 1727; 1648 cm^{-1}; MS (CI+) *m/z* 609 ($[M+H]^+$); 509; 453; 353. Anal. calcd (found) for $C_{32}H_{36}N_2O_5Se$: C, 63.26 (63.31); H, 5.97 (6.07); N, 4.61 (4.55); O, 13.17 (13.24).

14β-Phenylselenenyl-3-oxo-(*N*$_a$-*tert*-butoxycarbonyl)-vincadifformine (15b). Mp 185–186°C (heptane–ethyl acetate);

$[\alpha]_D^{25}$ −19.5° (*c* 1.13, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 0.45 (3H, t, *J* = 7.3H z, H-18); 0.93 (1H, dq, *J* = 14.6, 7.3 Hz, H-19); 1.05 (1H, dq, *J* = 14.6, 7.3 Hz, H-19); 1.52 (1H, m, H-15β); 1.57 (9H, s, $C(C\underline{H}_3)_3$); 1.92 (1H, m, H-6β); 2.08 (1H, m, H-17α); 2.09 (1H, m, H-6α); 2.17 (1H, m, H-15α); 2.48 (1H, m, H-17β); 3.43 (1H, m, H-5β); 3.46 (1H, s, H-21); 3.74 (3H, s, $CO_2C\underline{H}_3$); 4.07 (1H, m, H-14α); 4.08 (1H, m, H-5α); 7.08 (1H, m, H-10); 7.13 (1H, m, H-9); 7.28 (4H, m, H-11 + H-Φ_p + H-Φ_m); 7.62 (2H, m, H-Φ_o); 7.65 (1H, m, H-12); ^{13}C NMR (75 MHz, $CDCl_3$) δ 7.5 (C-18); 27.7 (C-19); 28.2 ($C(\underline{C}H_3)_3$); 32.1 (C-17); 38.4 (C-6); 39.0 (C-15); 41.1 (C-20); 43.1 (C-5); 43.5 (C-14); 51.8 ($CO_2\underline{C}H_3$); 54.5 (C-7); 67.0 (C-21); 83.4 ($\underline{C}(CH_3)_3$); 110.3 (C-16); 116.4 (C-12); 121.5 (C-9); 124.2 (C-10); 128.0 + 128.6 (C-Φ_p + C-11); 129.2 (C-Φ_m + C_q-Φ); 135.1 (C-Φ_o); 136.6 (C-8); 141.9 (C-13); 148.5 (C-2); 150.8 ($\underline{C}O_2C(CH_3)_3$); 167.6 ($\underline{C}O_2CH_3$); 169.6 (C-3); IR ($CHCl_3$) ν 1728; 1656 cm^{-1}; MS (CI+) *m*/*z* 609 ($[M+H]^+$); 509; 453; 353. Anal. calcd (found) for $C_{32}H_{36}N_2O_5Se$: C, 63.26 (63.01); H, 5.97 (6.18); N, 4.61 (4.54); O, 13.17 (13.25).

Borane reduction of the amide group on compounds 13 and 14. General procedure. To a solution of **13** or **14** (0.25 mmol) in anhydrous tetrahydrofuran (5 mL) at 0°C was added dropwise a solution of borane–tetrahydrofuran complex 1 M in tetrahydrofuran (1.25 mL, 1.25 mmol). The mixture was stirred at 0°C for 15 min and then quenched carefully with water. After the gas evolution has finished, the solution was treated with 10% hydrochloric acid for 30 min. Sodium hydroxide was then added until pH≈13. Sodium chloride was added until demixion. The aqueous layer was extracted with ethyl acetate (2×). The combined organic layers were dried over sodium sulfate and the solvent was removed in vacuo to afford **16** or **17**, respectively.

14β-Ethyl-(N_a-*tert*-butoxy-carbonyl)vincadifformine (16). Colorless oil (100%); $[\alpha]_D^{25}$ + 83.7° (*c* 1.13, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 0.57 (3H, t, *J* = 7.4 Hz, H-18); 0.69 (1H, qd, *J* = 14.8, 7.4 Hz, H-19); 0.86 (1H, m, H-15β); 0.93 (3H, t, *J* = 7.4 Hz, CH-CH_2-$C\underline{H}_3$); 1.13 (1H, qd, *J* = 14.8, 7.4 Hz, H-19); 1.24 (2H, m, $\underline{C}H$-$C\underline{H}_2$-CH_3); 1.53 (9H, s, $C(C\underline{H}_3)_3$); 1.65 (1H, m, H-6β); 1.82 (1H, m, H-14α); 1.84 (1H, m, H-15α); 1.88 (1H, m, H-3β); 2.00 (1H, m, H-17β); 2.18 (1H, m, H-6α); 2.33 (1H, s, H-21); 2.36 (1H, m, H-5β); 2.91 (1H, m, H-5α); 2.97 (1H, m, H-17α); 3.18 (1H, m, H-3α); 3.75 (3H, s, $CO_2C\underline{H}_3$); 7.01 (1H, m, H-10); 7.15 (1H, m, H-9); 7.19 (1H, m, H-11); 7.65 (1H, m, H-12); ^{13}C NMR (75 MHz, $CDCl_3$) δ 7.2 (C-18); 11.7 (CH-CH_2-$\underline{C}H_3$); 27.4 (CH-$\underline{C}H_2$-CH_3); 28.3 ($C(\underline{C}H_3)_3$); 28.8 (C-17); 29.0 (C-19); 34.6 (C-14); 38.0 (C-20); 40.4 (C-15); 42.9 (C-6); 51.6 (C-5 + $CO_2\underline{C}H_3$); 53.0 (C-7); 57.4 (C-3); 71.1 (C-21); 82.0 ($\underline{C}(CH_3)_3$); 112.5 (C-16); 116.1 (C-12); 120.5 (C-9); 123.5 (C-10); 127.3 (C-11); 138.5 (C-8); 140.7 (C-13); 149.6 (C-2); 151.3 ($\underline{C}O_2C(CH_3)_3$); 168.2 ($\underline{C}O_2CH_3$); IR ($CHCl_3$) ν 1721 cm^{-1}; MS (IC+) *m*/*z* 467 ($[M+H]^+$); 367; HRMS (CI+) *m*/*z* calcd (found) for $C_{28}H_{39}N_2O_4$ (MH^+): 467.2910 (467.2886).

14β-Benzyl-(N_a-*tert*-butoxycarbonyl)vincadifformine (17). Colorless oil (100%); $[\alpha]_D^{25}$ + 99.7° (*c* 1.05, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 0.55 (3H, t, *J* = 7.4 Hz, H-18); 0.71 (1H, qd, *J* = 14.8, 7.4 Hz, H-19); 0.99 (1H, m, H-15β); 1.13 (1H, qd, *J* = 14.8, 7.4 Hz, H-19); 1.51 (9H, s, $C(C\underline{H}_3)_3$); 1.63 (1H, m, H-6β); 1.83 (1H, m, H-15α); 1.96 (1H, m, H-3β); 1.98 (1H, m, H-17β); 2.13 (1H, m, H-6α); 2.20 (1H, m, H-14α); 2.35 (1H, m, H-21 + H-5β); 2.46 (1H, m, $C\underline{H}_2$-Φ); 2.57 (1H, m, $C\underline{H}_2$-Φ); 2.82 (1H, m, H-5α); 2.95 (1H, m, H-17α); 3.04 (1H, m, H-3α); 3.74 (3H, s, $CO_2C\underline{H}_3$); 7.00 (1H, m, H-10); 7.14 (1H, m, H-9); 7.19 (4H, m, H-11 + H-Φ_o + H-Φ_p); 7.30 (2H, m, H-Φ_m); 7.65 (1H, m, H-12); ^{13}C NMR (75 MHz, $CDCl_3$) δ 7.2 (C-18); 28.3 ($C(\underline{C}H_3)_3$); 28.7 (C-17); 28.9 (C-19); 34.9 (C-14); 38.2 (C-20); 40.6 (C-15); 41.3 ($\underline{C}H_2$-Φ); 42.9 (C-6); 51.5 (C-5); 51.6 ($CO_2\underline{C}H_3$); 52.9 (C-7); 57.3 (C-3); 71.0 (C-21); 82.0 ($\underline{C}(CH_3)_3$); 112.3 (C-16); 116.1 (C-12); 120.5 (C-9); 123.5 (C-10); 126.0 (C-Φ_p); 127.3 (C-11); 128.4 (C-Φ_m); 129.0 (C-Φ_o); 138.4 (C-8); 140.3 (C_q-Φ); 140.7 (C-13); 149.7 (C-2); 151.3 ($\underline{C}O_2C(CH_3)_3$); 168.2 ($\underline{C}O_2CH_3$); IR ($CHCl_3$) ν 1717 cm^{-1}; MS (CI+) *m*/*z* 529 ($[M+H]^+$); 429; HRMS (CI+) *m*/*z* calcd (found) for $C_{33}H_{41}N_2O_4$ (MH^+): 529.3067 (529.3076).

3-Oxo-(N_a-*tert*-butoxycarbonyl)tabersonine (18). To a stirred solution of **15a** or **15b** (0.8 g, 1.32 mmol) in methylene chloride (20 mL) at −78°C was added dropwise a solution of *meta*-chloroperoxybenzoic acid (0.25 g, 1.45 mmol) in methylene chloride (3 mL). The solution was then allowed to warm to room temperature. After 1 h at room temperature, the solution turned red indicating the spontanous elimination of the selenoxide. Solid sodium hydroxyde was added to aid elimination in the case of **15b**. Water was then added. The aqueous layer was extracted with methylene chloride (2×). The combined organic layers were dried over sodium sulfate and the solvent was removed in vacuo to afford compound **18** as a white amorphous solid (0.59 g, 100%). It was further crystallized from heptane/acetone for analysis; mp 196–7°C (heptane–acetone); $[\alpha]_D^{25}$ −19.1° (*c* 1.10, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 0.71 (3H, t, *J* = 7.4 Hz, H-18); 1.13 (1H, dq, *J* = 14.8, 7.4 Hz, H-19); 1.25 (1H, dq, *J* = 14.8, 7.4 Hz, H-19); 1.58 (9H, s, $C(C\underline{H}_3)_3$); 1.98 (1H, m, H-6β); 2.09 (1H, m, H-6α); 2.31 (1H, m, H-17α); 2.43 (1H, m, H-17β); 3.33 (1H, m, H-5β); 3.78 (3H, s, $CO_2C\underline{H}_3$); 4.03 (1H, s, H-21); 4.20 (1H, m, H-5α); 5.98 (1H, m, H-15); 6.43 (1H, m, H-14); 7.10 (1H, m, H-10); 7.21 (1H, m, H-9); 7.29 (4H, m, H-11); 7.68 (1H, m, H-12); ^{13}C NMR (75 MHz, $CDCl_3$) δ 7.4 (C-18); 26.6 (C-19); 27.9 ($C(\underline{C}H_3)_3$); 28.6 (C-17); 40.2 (C-20); 42.2 (C-6); 42.3 (C-5); 51.6 ($CO_2\underline{C}H_3$); 54.1 (C-7); 64.5 (C-21); 82.9 ($\underline{C}(CH_3)_3$); 109.4 (C-16); 116.0 (C-12); 120.9 (C-9); 123.2 (C-15); 123.9 (C-10); 128.2 (C-11); 135.9 (C-8); 140.4 (C-13); 145.0 (C-14); 149.9 (C-2); 150.5 ($\underline{C}O_2C(CH_3)_3$); 161.2 (C-3); 167.1 ($\underline{C}O_2CH_3$); IR ($CHCl_3$) ν 1726; 1664 cm^{-1}; MS (CI+) *m*/*z* 451 ($[M+H]^+$); 351. Anal. calcd (found) for $C_{26}H_{30}N_2O_5$: C, 69.31 (69.07); H, 6.71 (6.93); N, 6.22 (5.93); O, 17.76 (18.11).

(N_a-*tert*-Butoxycarbonyl)tabersonine (19). To a solution of **18** (0.4 g, 0.89 mmol) in anhydrous tetrahydrofuran (20 mL) at −20°C was added dropwise a solution of diisobutylaluminum hydride 1 M in hexane (4.5 mL, 4.5 mmol). The mixture was stirred at −20°C for 15 min

and then quenched carefully with water. After the gas evolution has finished, sodium hydroxide was added and the solution was stirred until the aqueous layer was extracted with ethyl acetate (2×). The combined organic layers were dried over sodium sulfate and the solvent was removed in vacuo to give 0.45 g of a yellowish oil. The residue was then chromatographed on silica gel (eluent heptane:acetone, 80:20) to afford **19** (0.37 g, 95%): $[\alpha]_D^{25}$ +34.8° (*c* 1.23, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 0.61 (3H, t, *J* = 7.4 Hz, H-18); 0.97 (1H, qd, *J* = 14.8, 7.4 Hz, H-19); 1.10 (1H, qd, *J* = 14.8, 7.4 Hz, H-19); 1.55 (9H, s, $C(C\underline{H}_3)_3$); 1.78 (1H, m, H-6β); 2.16 (1H, m, H-6α); 2.29 (1H, m, H-17β); 2.51 (1H, m, H-5β); 2.65 (1H, s, H-21); 2.73 (1H, m, H-17α); 3.04 (1H, m, H-5α); 3.09 (1H, m, H-3β); 3.50 (1H, m, H-3α); 3.76 (3H, s, $CO_2C\underline{H}_3$); 5.65 (1H, m, H-15); 5.81 (1H, m, H-14); 7.03 (1H, m, H-10); 7.19 (1H, m, H-9); 7.22 (1H, m, H-11); 7.66 (1H, m, H-12); ^{13}C NMR (75 MHz, $CDCl_3$) δ 7.6 (C-18); 26.5 (C-19); 28.3 ($C(\underline{C}H_3)_3$); 31.4 (C-17); 41.6 (C-20); 43.0 (C-6); 51.0 (C-5); 51.5 (C-3); 51.6 ($CO_2\underline{C}H_3$); 52.6 (C-7); 68.0 (C-21); 82.3 ($\underline{C}(CH_3)_3$); 111.7 (C-16); 116.1 (C-12); 121.0 (C-9); 123.5 (C-10); 125.2 (C-14); 127.5 (C-11); 132.8 (C-15); 138.5 (C-8); 140.7 (C-13); 150.1 (C-2); 151.2 ($\underline{C}O_2C(CH_3)_3$); 168.2 ($\underline{C}O_2CH_3$); IR ($CHCl_3$) ν 1719 cm^{-1}; MS (CI+) *m/z* 437 ($[M+H]^+$); 337; HRMS (CI+) *m/z* calcd (found) for $C_{26}H_{33}N_2O_4$ (MH^+): 437.2440 (437.2440).

Preparation of 1,2-didehydroaspidospermidine analogues 20, 21, 22. General procedure. A suspension of **16**, **17**, **19** (2.96 mmol) in hydrochloric acid 12 N (15 mL) was heated to reflux (110°C) for 5 min. The yellow solution was then cooled to 0°C and a 32% ammonia solution was slowly added until pH≈13. The white suspension was extracted with methylene chloride (3×). Then the combined organic layers were dried over sodium sulfate and the solvent was evaporated in vacuo to yield **20**, **21** or **22**, respectively.

14β-Ethyl-1,2-didehydro-aspidospermidine (20). Yellow oil (100%); $[\alpha]_D^{25}$ +214.6 (*c* 0.49, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 0.50 (3H, t, *J* = 7.4 Hz, H-18); 0.65 (3H, m, 2H-19 + H-15β); 0.92 (3H, t, *J* = 7.4 Hz, CH-CH_2-$C\underline{H}_3$); 1.23 (2H, m, CH-$C\underline{H}_2$-CH_3); 1.56 (1H, m, H-15α); 1.61 (1H, m, H-17); 1.65 (1H, m, H-6β); 1.81 (2H, m, H-3β + H-14α); 2.18 (1H, m, H-6α); 2.39 (1H, s, H-21); 2.44 (1H, m, H-17); 2.60 (1H, m, H-5β); 2.76 (1H, m, H-16); 3.09 (1H, m, H-16); 3.18 (1H, m, H-5α); 3.22 (1H, m, H-3α); 7.15 (1H, m, H-10); 7.27 (1H, m, H-11); 7.32 (1H, m, H-9); 7.51 (1H, m, H-12); ^{13}C NMR (75 MHz, $CDCl_3$) δ 7.3 (C-18); 11.6 (CH-CH_2-$\underline{C}H_3$); 23.7 (C-16); 27.2 (CH-$\underline{C}H_2$-CH_3); 27.9 (C-17); 29.7 (C-19); 34.2 (C-14); 35.4 (C-6); 36.5 (C-20); 40.7 (C-15); 54.3 (C-5); 57.4 (C-3); 60.9 (C-7); 78.9 (C-21); 120.0 (C-12); 120.9 (C-9); 125.1 (C-10); 127.4 (C-11); 147.0 (C-8); 154.4 (C-13); 192.1 (C-2); IR ($CHCl_3$) ν 2964, 1577, 1455, 1216 cm^{-1}; MS (CI+) *m/z* 309 ($[M+H]^+$); HRMS (CI+) *m/z* calcd (found) for $C_{21}H_{29}N_2$ (MH^+): 309.2331 (309.2332).

14β-Benzyl-1,2-didehydroaspidospermidine (21). Yellow oil (100%); $[\alpha]_D^{25}$ +183.7° (*c* 1.75, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 0.49 (3H, t *J* = 7.3 Hz, H-18); 0.65 (2H, m, 2H-19); 0.78 (1H, m, H-15β); 1.56 (1H, m, H-15α); 1.60 (1H, m, H-17); 1.65 (1H, m, H-6β); 1.90 (1H, m, H-3β); 2.15 (1H, m, H-6α); 2.21 (1H, m, H-14α); 2.42 (2H, s, H-21 + $C\underline{H}_2$-Φ); 2.43 (1H, m, H-17); 2.58 (2H, m, H-5 + $C\underline{H}_2$-Φ); 2.74 (1H, m, H-16); 3.04 (1H, m, H-16); 3.10 (2H, m, H-3α + H-5α); 7.18 (4H, m, H-10 + H-Φ_o + H-Φ_p); 7.30 (4H, m, H-9 + H-11 + H-Φ_m); 7.51 (1H, m, H-12); ^{13}C NMR (75 MHz, $CDCl_3$) δ 7.4 (C-18); 23.9 (C-16); 28.2 (C-17); 29.8 (C-19); 34.7 (C-14); 35.5 (C-6); 36.9 (C-20); 41.1 (C-15); 41.2 ($\underline{C}H_2$-Φ); 54.3 (C-5); 57.5 (C-3); 61.1 (C-7); 78.9 (C-21); 120.2 (C-12); 121.1 (C-9); 125.2 (C-10); 126.0 (C-Φ_p); 127.6 (C-11); 128.4 (C-Φ_m); 129.0 (C-Φ_o); 140.3 (C_q-Φ); 147.1 (C-8); 154.7 (C-13); 192.0 (C-2); IR ($CHCl_3$) ν 2967, 1578, 1455, 1243 cm^{-1}; MS (CI+) *m/z* 371 ($[M+H]^+$); HRMS (CI+) *m/z* calcd (found) for $C_{26}H_{31}N_2$ (MH^+): 371.2487 (371.2471).

(+)-1,2,14,15-Tetradehydroaspidospermidine (22). Yellow oil (95%); $[\alpha]_D^{25}$ +135.0 (*c* 1.24, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 0.57 (3H, t, *J* = 7.3 Hz, H-18); 0.90 (2H, q, *J* = 7.3 Hz, 2H-19); 1.68 (1H, m, H-6β); 1.74 (1H, m, H-17); 2.28 (1H, m, H-6α); 2.55 (1H, m, H-17); 2.73 (1H, s, H-21); 2.83 (1H, m, H-5β); 2.86 (1H, m, H-16); 2.99 (1H, m, H-16); 3.10 (1H, m, H-3β); 3.31 (1H, m, H-5α); 3.52 (1H, m, H-3α); 5.52 (1H, m, H-15); 5.69 (1H, m, H-14); 7.16 (1H, m, H-10); 7.29 (1H, m, H-11); 7.35 (1H, m, H-9); 7.52 (1H, m, H-12); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 8.1 (C-18); 24.6 (C-16); 27.4 (C-19); 29.8 (C-17); 35.6 (C-6); 40.3 (C-20); 51.4 (C-3); 53.3 (C-5); 60.8 (C-7); 73.1 (C-21); 120.0 (C-12); 121.1 (C-9); 124.5 (C-14); 125.2 (C-10); 127.6 (C-11); 134.2 (C-15); 147.3 (C-8); 154.2 (C-13); 190.1 (C-2); IR ($CHCl_3$) ν 2968, 1575, 1456, 1251 cm^{-1}; MS (CI+) *m/z* 279 ($[M+H]^+$); HRMS (CI+) *m/z* calcd (found) for $C_{19}H_{23}N_2$ (MH^+): 279.1861 (279.1861).

'One pot' preparation of (−)-rhazinilam analogues 3, 4, 5 and 6. Compounds **3**, **4**, **5** and **6** were obtained from **20**, **21**, **22** and **23**, respectively, following the experimental conditions described for the 'one pot' preparation of rhazinilam (**1**) (see above).

14β-Ethylrhazinilam (3). Purified by chromatography (eluent heptane:acetone, 70:30), **3** was obtained as a white amorphous solid (50%); $[\alpha]_D^{25}$ −316.5° (*c* 1.37, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 0.71 (3H, t, *J* = 7.3 Hz, H-18); 0.99 (3H, t, *J* = 7.3 Hz, CH-CH_2-$C\underline{H}_3$); 1.22 (1H, dq, *J* = 14.6, 7.3 Hz, H-19); 1.27 (2H, q, *J* = 7.3 Hz, CH-$C\underline{H}_2$-CH_3); 1.47 (3H, m, H-19 + 2H-15); 1.50 (1H, m, H-17β); 1.94 (1H, m, H-16α); 2.23 (1H, m, H-14α); 2.37 (1H, m, H-16β); 2.47 (1H, m, H-17α); 3.34 (1H, m, H-3β); 4.05 (1H, m, H-3α); 5.76 (1H, m, H-6); 6.49 (1H, m, H-5); 6.68 (1H, bs, NH); 7.20 (1H, m, H-12); 7.32 (2H, m, H-10 + H-11); 7.43 (1H, m, H-9); ^{13}C NMR (75 MHz, $CDCl_3$) δ 8.3 (C-18); 11.3 (CH-CH_2-$\underline{C}H_3$); 27.2 (CH-$\underline{C}H_2$-CH_3); 28.1 (C-16); 30.1 (C-19); 31.7 (C-14); 37.3 (C-17); 39.5 (C-20); 40.3 (C-15); 51.8 (C-3); 109.9 (C-6); 117.2 (C-7); 119.1 (C-5); 126.9 (C-12); 127.3 + 128.1 (C-10 + C-11); 130.6 (C-20); 131.6 (C-9); 138.2 (C-8); 140.4 (C-13); 177.5 (C-2); IR ($CHCl_3$) ν 1665 cm^{-1}; MS (CI+) *m/z* 323 ($[M+H]^+$); HRMS

(CI+) m/z calcd (found) for $C_{21}H_{27}N_2O$ (MH^+): 323.2124 (323.2116).

14β-Benzylrhazinilam (4). Purified by chromatography (eluent heptane:acetone, 80:20), **4** was obtained as a pale yellow oil (50%); $[\alpha]_D^{25}$ −292.5° (*c* 1.00, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 0.70 (3H, t, J = 7.4 Hz, H-18); 1.21 (1H, qd, J = 14.8, 7.4 Hz, H-19); 1.43 (1H, m, H-17β); 1.46 (1H, qd, J = 14.8, 7.4 z, H-19); 1.50 (2H, m, H-15); 1.92 (1H, m, CH-16α); 2.35 (1H, m, CH-16β); 2.44 (1H, m, H-17α); 2.66 (3H, m, CH-14α + C$\underline{H}_2$-Φ); 3.41 (1H, m, H-3β); 3.90 (1H, m, H-3α); 5.74 (1H, m, H-6); 6.43 (1H, m, H-5); 6.71 (1H, bs, NH); 7.18 (1H, m, H-12); 7.21 (3H, m, H-Φ_o + H-Φ_p); 7.31 (4H, m, CH-10 + CH-11 + H-Φ_m); 7.41 (1H, m, H-9); ^{13}C NMR (75 MHz, $CDCl_3$) δ 8.2 (C-18); 28.0 (C-16); 29.9 (C-19); 31.9 (C-14); 37.4 (C-17); 39.5 (C-20); 40.3 (C-15); 40.9 (C$\underline{H}_2$-Φ); 51.7 (C-3); 110.0 (C-6); 117.2 (C-7); 119.1 (C-5); 126.4 (C-Φ_p); 126.9 (C-12); 127.2 + 128.1 (C-10 + C-11); 128.6 (C-Φ_m); 129.0 (C-Φ_o); 130.3 (C-21); 131.5 (C-9); 138.1 (C-8); 139.1 (C_q-Φ); 140.4 (C-13); 177.5 (C-2); IR ($CHCl_3$) ν 1662 cm^{-1}; MS (CI+) m/z 385 ($[M+H]^+$); HRMS (CI+) m/z calcd (found) for $C_{26}H_{29}N_2O$ (MH^+): 385.2280 (385.2277).

(−)-14,15-Didehydrorhazinilam (5). Purified by chromatography (eluent heptane:acetone, 70:30), **20** was obtained as a white amorphous solid (50%); $[\alpha]_D^{25}$ −293.5° (*c* 1.31, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 0.66 (3H, t, J = 7.4 Hz, H-18); 1.04 (1H, dq, J = 14.8, 7.4 Hz, H-19); 1.61 (1H, dq, J = 14.8, 7.4 Hz, H-19); 1.73 (1H, m, H-17); 2.00 (1H, m, H-16α); 2.06 (1H, m, H-17); 2.35 (1H, m, H-16α); 4.47 (2H, m, H-3); 4.56 (1H, m, H-3); 5.53 (1H, m, H-15); 5.88 (1H, m, H-6); 6.62 (1H, m, H-5); 6.94 (1H, bs, NH); 7.25 (1H, m, H-12); 7.32 + 7.36 (2H, m, H-10 + H-11); 7.40 (1H, m, H-9); ^{13}C NMR (75 MHz, $CDCl_3$) δ 9.1 (C-18); 28.5 (C-16); 29.8 (C-19); 39.6 (C-17); 41.7 (C-20); 44.4 (C-3); 110.1 (C-6); 116.2 (C-7); 118.5 (C-5); 119.6 (C-14); 127.1 (C-12); 127.4 (C-11); 127.8 (C-21); 128.1 (C-10); 131.0 (C-9); 134.5 (C-15); 138.1 (C-8); 139.8 (C-13); 177.0 (C-2); IR ($CHCl_3$) ν 1665 cm^{-1}; MS (CI+) m/z 293 ($[M+H]^+$); HRMS (CI+) m/z calcd (found) for $C_{19}H_{21}N_2O$ (MH^+): 293.1654 (293.1663).

(+)-14,15-Didehydrorhazinilam (6). Purified by chromatography (eluent heptane:acetone, 70:30), **6** was obtained as a white amorphous solid (50%); $[\alpha]_D^{25}$ +292.9° (*c* 1.28, $CHCl_3$); HRMS (CI+) m/z calcd (found) for $C_{19}H_{21}N_2O$ (MH^+): 293.1654 (293.1660).

Acknowledgements

We are very grateful to Professor P. Potier for his continuous interest and encouragement and to Dr T. Sévenet and Dr C. Thal for helpful discussions. We would like to thank RPR and CNRS for a grant (to C.D.) and J. Hannart (Omnium Chimique) for a gift of (+)-vincadifformine. We also express our appreciation to C. Gaspard for the cytotoxicity assays and to P. Pineau for the high scale synthesis of 3-oxo-(N_a-*tert*-butoxycarbonyl)-vincadifformine.

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