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# BISINDOLE ALKALOIDS CONDENSED WITH A CYCLOPROPANE RING, PART 2. CYCLOPROPANO-VINORELBINE AND ITS DERIVATIVES

**Péter Keglevich,[a] László Hazai,[a] Zsófia Dubrovay,[b] Zsuzsanna Sánta,[b] Miklós Dékány,[b] Csaba Szántay, Jr.,[b] György Kalaus,[a] and Csaba Szántay[a]**

[a]Department of Organic Chemistry and Technology, University of Technology and Economics, Budapest, Hungary, H-1111 Budapest, Gellért tér 4. Hungary, [b]Spectroscopic Research Division, Gedeon Richter Plc., H-1475 Budapest 10, P. O. Box 27, Hungary; E-mail: szantay@mail.bme.hu

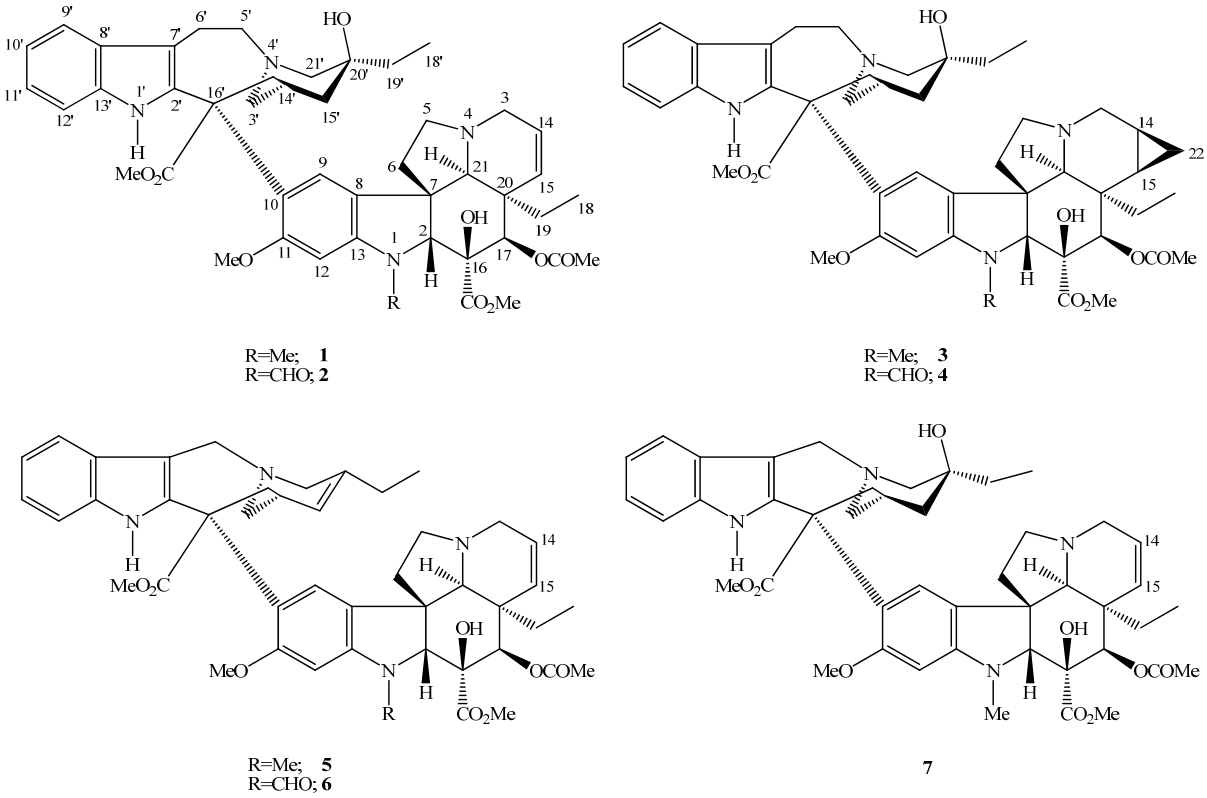
*Dedicated to Professor Isao Kuwajima on the occasion of his 77th birthday*

**Abstract** – Vinorelbine condensed with a cyclopropane ring in the position 14 and 15 of the vindoline monomer part was synthesized and was found to have excellent antitumor activity. Further derivatives of cyclopropano-vinorelbine were prepared, the vincristine-like derivative, the $N^1$-formyl-cyclopropano-vinorelbine, and the hydrated vinorelbine analogue, *i.e.* 5'-desmethylene-cyclopropano-vinblastine.

## INTRODUCTION

Recently[1] we reported the synthesis and cytotoxic activity of cyclopropano-vinblastine (**3**) and cyclopropano-vincristine (**4**), obtained by condensing the therapeutically used anticancer agents vinblastine (**1**) and vincristine (**2**) with a cyclopropane ring (Scheme 1). Compounds **3** and **4** have significant tumor cell inhibiting activity on different tumor types and tumor cell lines. As a continuation of this research work further derivatives of bioactive dimer alkaloids were investigated.

Vinorelbine (Navelbin) (**5**) is a third-generation *Vinca* alkaloid, which was obtained semi-synthetically[2] from dehydrovinblastine[3] isolated from *Catharanthus roseus* and synthesized by the well-known coupling reactions.[4-7] The pharmacological properties and clinical use of vinorelbine are presented in several reviews.[8-11] Vinorelbine has remarkable activity in metastatic breast cancer[12,13] and is successfully approved for the treatment of non-small cell lung cancer (NSCLC).[14-16]




**Scheme 1**

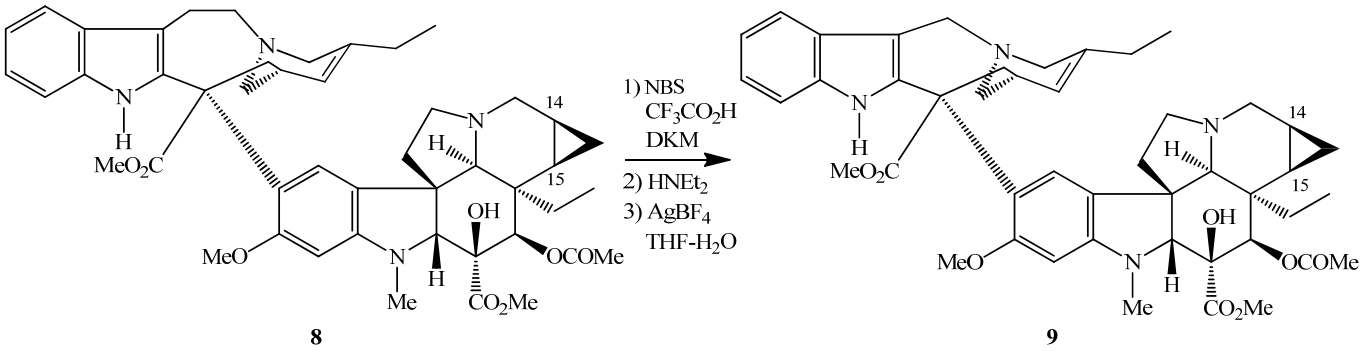
The formyl analog of vinorelbine (**6**) and the hydrated derivative (**7**) are known compounds, however, with only few biological data.[17,18] Compound **6** is actually a vincristine analogue, and the hydrated derivative (**7**) can be considered as nor-5'-vinblastine. Therefore we performed the cyclopropanation of the carbon-carbon double bond of the vindoline part of compounds **5**, **6** and **7**.

## RESULTS AND DISSCUSSION

### Synthesis

Vinorelbine (**5**) cyclopropanated in positions 14 and 15 (**9**) was the first target compound (Scheme 2). 14,15-Cyclopropano-vinorelbine (**9**) was synthesized from the corresponding 14,15-cyclopropano-anhydrovinblastine[1] (**8**) analogously with the method of Song et al.[2] (Scheme 2), when the 9 membered ring of the catharanthin part was contracted to an 8 membered ring by oxidative removal of the 5'-methylene group.

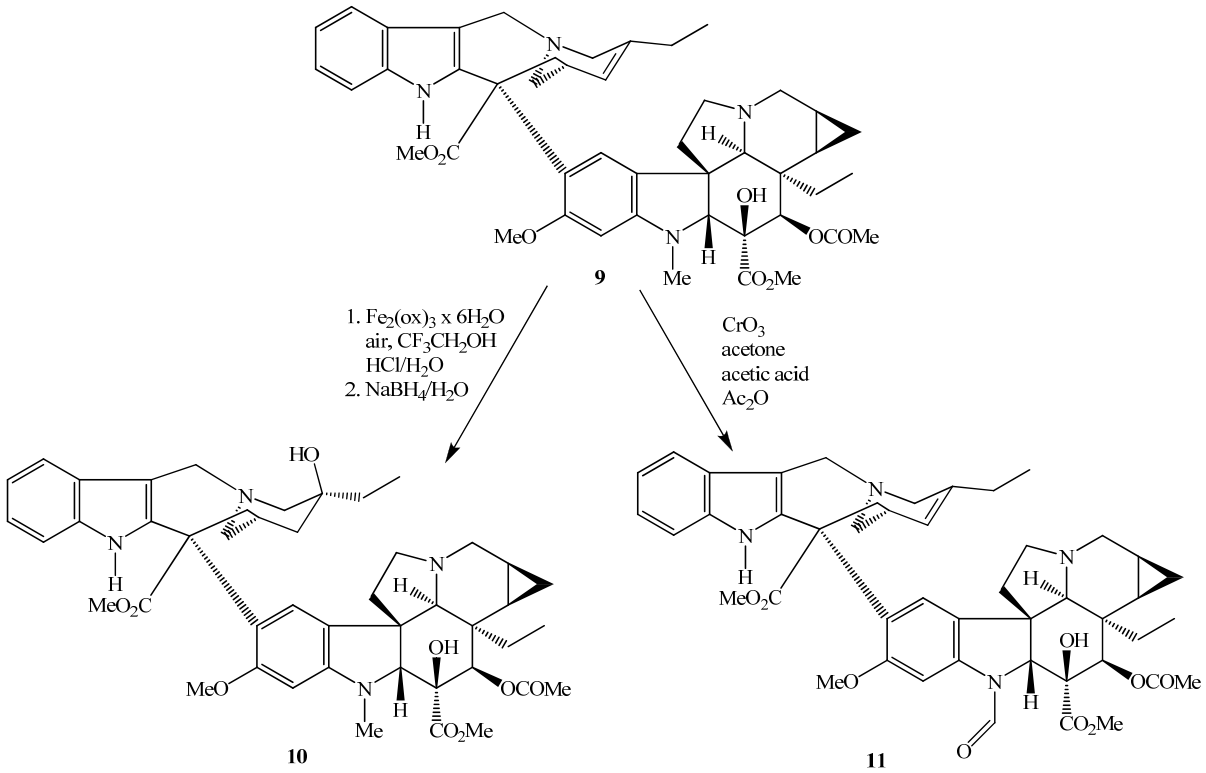
14,15-Cyclopropano-anhydrovinblastine[1] (**8**) in dichloromethane solution was allowed to react with *N*-bromosuccinimide in the presence of trifluoroacetic acid and the reaction mixture was treated with diethylamine at -70 °C and then $AgBF_4$ at 50 °C. After processing the reaction mixture 14,15-cyclopropano-vinorelbine (**9**) was obtained in form of a sulfate salt in 52% yield.




**8** **9**

**Scheme 2**

Nor-5'-vinblastine cyclopropanated in positions 14 and 15 (**10**) was prepared from cyclopropano-vinorelbine (**9**) analogously with the known oxidation method.[7,19] The reaction was carried out with iron(III) oxalate and air in diluted aqueous hydrogen chloride solution in the presence of 2,2,2-trifluoroethanol. After treating the reaction mixture with sodium borohydride in water, hydrated cyclopropano-vinorelbine (**10**) as a sulfate salt was isolated in 16% yield (Scheme 3).




**9** **10** **11**

**Scheme 3**

The next step was the synthesis of compound **11**, the vincristine analogue of vinorelbine with a formyl group on the nitrogen of the vindoline part of the dimer alkaloid. The oxidation reaction used for the preparation of vincristine[20] proved to be the successful method.

Oxidation of 14,15-cyclopropano-vinorelbine (**9**) was achieved with $CrO_3$ at -55 $^oC$ in a mixture of acetone, acetic acid and acetic anhydride. After purification and treating with sulfuric acid, the sulfate salt of *N*-formyl-cyclopropano-vinorelbin (**11**) was obtained in 17% yield.

**Biology**

The synthesized new dimer alkaloids (**9**, **10** and **11**) were investigated on different tumor cell lines at the US National Institute of Health (NIH). These experiments on 56 different tumor cell lines embracing 9 frequently occurring tumor types demonstrated the therapeutic activity of these substances in comparison with vinorelbine. Tumor types and cell lines were the following: leukemia (CCRF-CEM, HL(60)-TB, K-562, MOLT-4, RPMI-8226, SR), non-small cell lung cancer (A549/ATCC, EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, NCI-H460, NCI-H522), colon cancer (COLO 205, HCC-2998, HCT-116, HCT-15, HT-29, KM12, SW-620), CNS cancer (SF-268, SF-295, SF-539, SNB-19, SNB-75, U251), melanoma (LOX IMVI, MALME-3M, M14, MDA-MB-435, SK-MEL-2, SK-MEL-28, SK-MEL-5, UACC-257, UACC-62), ovarian cancer (IGROV1, OVCAR-3, OVCAR-4, OVCAR-8, NCI/ADR-RES, SK-OV-3), renal cancer (786-0, A498, ACHN, CAKI-1, RXF 393, SN12C, TK-10, UO-31), prostate cancer (PC-3, DU-145), breast cancer (MCF7, MDA-MB-231/ATCC, HS 578T, BT-498, T-47D, MDA-MB-468). The NCI screening procedures were described[21] as were the origins and processing of the cell lines.[21-23] In Table 1 percentages of growths are listed for the reference alkaloids and the appropriate cyclopropano derivatives at the concentration of $10^{-5}M$. The bigger negative numbers show more significant decrease of cell number or stronger inhibition effects.

From cell lines of **leukemia** on human leukemia HL-60(TB) cells cyclopropano-vinorelbine (**9**) showed the highest cell number decrease activity similarly to vinorelbine. On the other two cell lines RPMI-8226 and SR, the new compounds (**9**, **10** and **11**) presented rather moderate antitumor effect.

On cell lines of **non-small cell lung cancer**, only on cells of HOP-92 and NCI-H522 were medium activities observed, which were not higher than with vinorelbine.

One of the most important results is presented on the cell lines of **colon cancer**. All three compounds (**9**, **10** and **11**) showed excellent antitumor effect on cell line COLO-205. Most importantly the inhibiting effect of cyclopropano-vinorelbine (**9**) was stronger than with vinorelbine.

In **CNS cancer**, **ovarian cancer**, **renal cancer** and **prostate cancer**, only moderate or small antitumor activity was observed on the investigated tumor cell lines.

Unique cell inhibiting effects were obtained on **melanoma** cell lines. On cells MALME-3M, MDA-MB-435 and SK-MEL-5 cyclopropano-vinorelbine (**9**) showed the highest activities compared to vinorelbine, which possessed antitumor activity only on SK-MEL-5 cells. On MDA-MB-435 cell lines significant inhibition effects were observed in the case of compounds **10** and **11**.

Cyclopropano-vinorelbine (**9**) showed cell number inhibiting activities on two cell lines (MDA-MB-213/ATCC and MDA-MB-468) of **breast cancer**, however, these effects proved to be less important than in the case of colon cancer and melanoma.

**Table 1**. Inhibition effect of vinorelbine derivatives (**9**, **10** and **11**) against different cell lines of different tumors

| | **VNR** | **9** | **10** | **11** |
|---|---|---|---|---|
| **Leukemia** | | | | |
| HL-60(TB) | -55 | -57.3 | -36.5 | -19.4 |
| RPMI-8226 | | -20.3 | | -6.8 |
| SR | -15 | -19.3 | | |
| **Non-small cell lung cancer** | | | | |
| HOP-92 | -20 | | -12.0 | -16.4 |
| NCI-H522 | | -14.5 | -20.3 | |
| **Colon cancer** | | | | |
| COLO-205 | -35 | -86.0 | -49.5 | -78.5 |
| HT29 | | -23.3 | -2.3 | -5.1 |
| **CNS cancer** | | | | |
| SF-295 | -20 | | -13.9 | -32.8 |
| SF-539 | -35 | -17.5 | -0.3 | -18.1 |
| **Melanoma** | | | | |
| MALME-3M | -5 | -63.9 | | |
| M14 | | -22.3 | -23.2 | |
| MDA-MB-435 | | -57.7 | -40.0 | -57.1 |
| SK-MEL-5 | -60 | -79.9 | | -26.9 |
| **Ovarian cancer** | | | | |
| NCI/ADR-RES | | | | -28.8 |
| SK-OV-3 | | -24.0 | | |
| **Renal cancer** | | | | |
| A498 | -20 | -24.5 | -8.1 | -8.2 |
| **Prostate cancer** | | | | |
| DU-145 | -20 | -17.9 | | -9.8 |
| **Breast cancer** | | | | |
| MDA-MB-213/ATCC | | -45.4 | | -24.0 |
| BT-549 | | | -23.1 | |
| MDA-MB-468 | | -31.7 | | |

**VNR**: vinorelbine, **9:** 14,15-cyclopropano-vinorelbine,
**10:** 14,15-cyclopropano-nor-5'-vinblastine, **11**: $N^1$-formyl-14,15-cyclopropano-vinorelbine

## EXPERIMENTAL

### General

Melting points were measured on a VEB Analytik Dresden PHMK-77/1328 apparatus and are uncorrected. IR spectra were recorded on Zeiss IR 75 and 80 instruments. NMR measurements were performed on a Varian 800 MHz NMR spectrometer equipped with a $^{1}H\{^{13}C/^{15}N\}$ Triple Resonance $^{13}C$ Enhanced Salt Tolerant Cold Probe operating at 800 MHz for $^{1}H$ and 201 MHz for $^{13}C$, and a Varian 500 MHz NMR spectrometer equipped with a $^{1}H$ $\{^{13}C/^{15}N\}$ 5 mm PFG Triple Resonance $^{13}C$ Enhanced Cold Probe operating at 500 MHz for $^{1}H$ and 125 MHz for $^{13}C$. Chemical shifts are given on the delta scale as parts per million (ppm) with tetramethylsilane (TMS) ($^{1}H$) or dimethyl sulfoxide-$d_6$ ($^{13}C$) as the internal standard (0.00 ppm and 39.5 ppm, respectively). $^{1}H$-$^{1}H$, direct $^{1}H$-$^{13}C$, and long-range $^{1}H$-$^{13}C$ scalar spin-spin connectivities were established from 2D gDQFCOSY, zTOCSY, gHSQCAD, and gHMBCAD experiments, respectively. All pulse sequences were applied by using the standard spectrometer software package. All experiments were performed at 298 K. HRMS analyses were performed on an LTQ FT Ultra (Thermo Fisher Scientific, Bremen, Germany) system. The ionization method was ESI operated in positive ion mode. For the 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 induce fragmentation. The protonated molecular ion peaks were fragmented by CID at a normalized collision energy of 35–55%. The samples were dissolved in methanol. Data acquisition and analysis were accomplished with Xcalibur software version 2.0 (Thermo Fisher Scientific). TLC was carried out using Kieselgel $60F_{254}$ (Merck) glass plates.

**14,15-Cyclopropano-vinorelbine (9).** 14,15-Cyclopropano-anhydrovinblastine (**8**) (244 mg; 0.302 mmol) was solved in dry $CH_2Cl_2$ (9 mL) and at -70 $^{o}$C in the dark, under Ar *N*-bromosuccinimide (64 mg; 0.362 mmol) was added in a mixture of $CH_2Cl_2$ (7.2 mL) and trifluoroacetic acid (0.13 mL; 1.66 mmol), then diethylamine (0.21 mL; 2.07 mmol) in $CH_2Cl_2$ (1.8 mL) was dropped to the reaction mixture. After stirring at -70 $^{o}$C for 1.5 h 235 mg (1.21 mmol) of $AgBF_4$ dissolved in THF-distilled water mixture (72 mL) was added; the reaction mixture was stirred further in dark under Ar for 3 h at 50 $^{o}$C. The reaction mixture was filtrated, 10% aqueous sodium carbonate was added to the filtrate to pH 9, after which the THF was evaporated. The residue was extracted with $CH_2Cl_2$ (3x20 mL), the combined organic phase was washed with saturated aqueous sodium chloride, dried with magnesium sulfate, and evaporated to dryness. The crude product was purified with preparative TLC (EtOAc-MeOH-$NEt_3$, 97:3:3) and 128 mg (0.162 mmol) base was obtained. The base was dissolved in a mixture of $CH_2Cl_2$ (1.16 mL) and EtOH (0.84 mL), then 0.86 mL of sulfuric acid (0.162 mmol)-EtOH mixture was added and the solution was evaporated to dryness. After treating the residue with $Et_2O$, 142 mg (52%) of 14,15-cyclopropano-

vinorelbine sulfate was obtained as pale yellow crystals. Mp 221-223 °C. Molecular weight (base) 792.96 g/mol ($C_{46}H_{56}N_4O_8$), molecular weight (sulfate) 891.04 g/mol ($C_{46}H_{58}N_4O_{12}S$). TLC (base) ($CH_2Cl_2$-methanol 10:1), $R_f$=0.32. TLC (sulfate) ($CH_2Cl_2$-MeOH 10:1), $R_f$=0.32. IR (sulfate) (KBr) 3432, 2927, 1741, 1613, 1617, 1501, 1433, 1374, 1229, 1038 $cm^{-1}$. $^1$H-NMR (500 MHz, DMSO-$d_6$) δ 0.35 (m, 1H, $H_\alpha$-15); 0.55 (m, 1H, $H_x$-22); 0.90 (m, 1H, $H_y$-22); 0.85 (t, *J*= 7.5 Hz, 3H, $H_3$-18); 1.05 (t, *J*= 7.0 Hz, 3H, $H_3$-18'); 1.08 (m, 1H, $H_\alpha$-14); 1.08 (ABq, *J*= 14.1 Hz, *J*= 7.4 Hz, 1H, $H_x$-19); 1.62 (br, 1H, $H_\alpha$-6); 1.74 (ABq, *J*= 14.1 Hz, *J*= 7.4 Hz, 1H, $H_y$-19); 1.82 (br, 1H, H-14'); 1.86 (br, 1H, $H_\alpha$-5); 1.96 (s, 3H, $H_3$-C(17)OCOCH$_3$); 1.99 (m, 1H, $H_\alpha$-3); 2.00 (br, 1H, $H_\beta$-6); 2.06 (m, 1H, $H_2$-19'); 2.08 (s, 1H, H-21); 2.52 (br, 1H, $H_\alpha$-17'); 2.61 (s, 3H, $H_3$-N(1)CH$_3$); 2.85 (dd, *J*= 13.2 Hz, *J*= 4.0 Hz, 1H, $H_\alpha$-3'); 2.98 (br, 1H, $H_\beta$-17'); 3.03 (br, 1H, $H_\beta$-5); 3.10 (br, 1H, $H_\beta$-3); 3.38 (br, 1H, $H_\beta$-3'); 3.43 (s, 1H, H-2); 3.66 (s, 3H, $H_3$-C(16)COOCH$_3$); 3.67 (s, 3H, $H_3$-C(16')COOCH$_3$); 3.72 (d, *J*= 16.7 Hz, 1H, $H_x$-21'); 3.81 (s, 3H, $H_3$-C(11)OCH$_3$); 3.96 (d, *J*= 16.7 Hz, 1H, $H_y$-21'); 4.52 (d, *J*= 14.2 Hz, 1H, $H_\alpha$-5'); 4.93 (d, *J*= 14.2 Hz, 1H, $H_\beta$-5'); 5.17 (s, 1H, H-17); 5.80 (br, 1H, H-15'); 6.02 (s br, 1H, H-9); 6.43 (s, 1H, H-12); 7.19 (m, 1H, H-10'); 7.22 (m, 1H, H-11'); 7.48 (d, *J*= 8.1 Hz, 1H, H-12'); 7.79 (d, *J*= 8.1 Hz, 1H, H-9'). $^{13}$C-NMR (200 MHz, DMSO-$d_6$) δ 8.1 (C-22); 8.6 (C-18); 11.3 (C-14); 11.6 (C-18'); 15.7 (C-15); 20.9 (C-C(17)OCO<u>C</u>H$_3$); 26.6 (C-19'); 26.8 (C-14'); 34.2 (C-17'); 34.5 (C-19); 37.9 (C-1); 40.3 (C-20); 44.6 (C-6); 43.2 (C-3'); 46.2 (C-5'); 51.6 (C-21'); 51.8 (C-5); 51.9 (C-C(16)COO<u>C</u>H$_3$); 52.8 (C-3); 52.9 (C-C(16')COO<u>C</u>H$_3$); 56.2 (C-C(11)O<u>C</u>H$_3$); 67.7 (C-21); 76.1 (C-17); 78.0 (C-16); 82.5 (C-2); 94.3 (C-12); 104.2 (C-7'); 112.0 (C-12'); 118.3 (C-9'); 118.6 (C-10); 119.8 (C-10'); 122.2 (C-11'); 122.2 (C-15'); 123.4 (C-8'); 123.5 (C-9); 127.8 (C-8); 135.9 (C-2'); 131.1 (C-20'); 134.8 (C-13'); 152.7 (C-13); 158.0 (C-11); 169.9 (C- C(17)O<u>C</u>OCH$_3$); 171.3 (C-C(16)<u>C</u>OOCH$_3$); 173.2 (C-C(16')<u>C</u>OOCH$_3$). HRMS: 793.41693 ($C_{46}H_{57}O_8N_4$ /$M_{base}$+H/; calc. 793.41709). ESI-MS-MS (793.42@cid35) (rel. int. %): 733(100); 683(30); 672(41); 612(29); 580(16); 562(15); 552(17); 510(20); 471(48); 389(19); 357(8); 323(8).

**14,15-Cyclopropano-nor-5'-vinblastine (10)**. 2.525 g (5.22 mmol) of $Fe_2$(oxalate)$_3$x6$H_2O$ was dissolved in distillated water (725 mL) and air was bubbled through the solution at 0 °C for 15 min. Then 138 mg (0.174 mmol) of 14,15-cyclopropano-vinorelbine (**9**) was added in a mixture of 0.1*M* HCl (4.2 mL), distilled water (4.2 mL) and 2,2,2-trifluoroethanol (0.85 mL) and a solution of $NaBH_4$ (132 mg, 3.48 mmol) in water (8.4 mL) was dropped to the reaction mixture. After stirring for 30 min the reaction mixture was treated with 25% $NH_4OH$ solution (19 mL) and was extracted with $CH_2Cl_2$-MeOH 9:1 mixture (3x100 mL). The combined organic phase was washed with water (100 mL), dried with magnesium sulfate and the solvent was evaporated in vacuum. The crude product was purified by preparative TLC (EtOAc-MeOH-$NHEt_2$, 92:4:4) and 26 mg (0.032 mmol) of product was obtained which

was immediately converted to sulfate salt. The base was dissolved in $CH_2Cl_2$ (0.26 mL) and EtOH (0.17 mL) and 0.17 mL of a mixture of sulfuric acid (0.032 mmol) in EtOH was added and evaporated to dryness. The solid residue was treated with $Et_2O$, filtered and 25 mg (16%) of 14,15-cyclopropano-nor-5'-vinblastine (**10**) sulfate salt was obtained as pale yellow crystals. Mp 235-237 °C. IR (KBr) 3425, 2961, 1741, 1617, 1460, 1234, 1039, 747 $cm^{-1}$. $^1$H-NMR[24] (500 MHz, DMSO-$d_6$ + TFA-*d*) δ (ppm) 0.56 (1H, H-15); 0.78 (1H, $H_\alpha$-22); 0.83 (3H, $H_3$-18); 0.90 (t, $J$ = 7.6 Hz, 3H, $H_3$-18'); 0.98 (1H, $H_\beta$-22); 1.37 (1H, H-14); 1.54 (1H, $H_x$-15'); 1.55 (1H, H-14'); 1.57 (2H, $H_2$-19'); 1.84 (2H, $H_2$-19); 2.04 (s, 3H, C(17)OCOCH$_3$); 2.04 (1H, $H_y$-15'); 2.07 (1H, $H_\alpha$-6); 2.34 (1H, $H_\beta$-6); 2.63 (1H, $H_x$-17'); 2.66 (s, 3H, N(1)CH$_3$); 2.93 (1H, $H_y$-17'); 3.29 (1H, $H_x$-21'); 3.29 (1H, H-21); 3.42 (1H, $H_x$-3'); 3.44 (1H, $H_y$-21'); 3.66 (s, 3H, C(16')COOCH$_3$); 3.58 (1H, $H_y$-3'); 3.61 (s, 1H, H-2); 3.65 (2H, $H_2$-5); 3.71 (2H, $H_2$-3); 3.77 (s, 3H, C(16)COOCH$_3$); 3.80 (s, 3H, C(11)OCH$_3$); 4.72 (dd, $J$ = 14.5 Hz, $J$ = 8.5 Hz, 1H, $H_x$-5'); 5.00 (dd, $J$ = 14.5 Hz, $J$ = 4.8 Hz, 1H, $H_y$-5'); 5.19 (s, 1H, H-17); 6.46 (s, 1H, H-12); 7.08 (t, 1H, H-10'); 7.14 (t, 1H, H-11'); 7.42 (d, 1H, H-12'); 7.65 (d, 1H, H-9'); 8.19 (1H, N(4)H); 10.36 (1H, N(4')H). $^{13}$C-NMR (125 MHz, DMSO-$d_6$ + TFA-*d*) δ (ppm) 6.8 (C-22); 6.9 (C-18'); 7.4 (C-18); 10.0 (C-14); 15.1 (C-15); 20.6 (C(17)OCO<u>C</u>H$_3$); 23.9 (C-14'); 34.4 (C-19'); 35.3 (C-19); 36.5 (C-15'); 38.2 (N(1)CH$_3$); 39.7 (C-20); 40.7 (C-3'); 43.0 (C-6); 50 br (C-3); 50.4 (C-5'); 51 br (C-5); 51.1 (C-7); 52.7 (C(16')COO<u>C</u>H$_3$, C(16)COO<u>C</u>H$_3$); 56.2 (C(11)O<u>C</u>H$_3$); 58.8 (C-21'); 67.5 (C-21); 68.2 (C-20'); 74.7 (C-17); 77.8 (C-16); 79.6 (C-2); 94.9 (C-12); 111.9 (C-12'); 117.8 (C-9'); 119.4 (C-10'); 122.1 (C-11'); 127.6 (C-8'); 137 br (C-2'); 135.2 (C-13'); 152.3 (C-13); 158.3 (C-11); 169.8 (C(17)O<u>C</u>OCH$_3$); 171.5 (C(16)<u>C</u>OOCH$_3$); 173.4 (C(16')<u>C</u>OOCH$_3$). HRMS: 811.42724 ($C_{46}H_{59}O_9N_4$; calc. 811.42766). ESI-MS-MS (CID=35 %) (rel. int. %): 751(100); 733(10); 701(10); 691(8); 672(6); 657(9); 612(43); 580(19); 562(15); 552(12); 528(29); 483(51); 471(22); 423(20); 399(11); 389(39); 381(9); 357(13); 341(14).

***N*$^1$-Formyl-14,15-cyclopropano-vinorelbine (11).** 14,15-Cyclopropano-vinorelbine sulfate (**9**) (135 mg; 0.152 mmol) was dissolved in a mixture of acetone (36 mL) and acetic acid (8.3 mL), and at -55 °C $CrO_3$ (82 mg; 0.821 mmol) in acetic anhydride (30.4 mL) was added. After stirring for 20 min, 25% $NH_4OH$ at -50 °C was added to pH 9 keeping the temperature of the reaction mixture below 50 °C, and distilled water (100 mL) was added. The mixture was extracted with $CH_2Cl_2$ (5x40 mL), the combined organic phase was washed with water (2x60 mL), dried with magnesium sulfate and evaporated to dryness. Formic acid (2.4 mL) and acetic anhydride (0.4 mL) were added to the residue and after allowing the solution to stand at room temperature for 15 min, distilled water (50 mL) was added and basified with 25% $NH_4OH$ to pH 9. The solution was extracted with $CH_2Cl_2$ (5x20 mL), the combined organic phase was dried with magnesium sulfate and the solvent was evaporated. The crude product was purified by

preparative TLC (EtOAc-MeOH-$NHEt_2$, 97:3:3) and 52 mg (0.064 mmol) of product (**11**) was obtained, which was transformed immediately to the sulfate salt. The product was dissolved in a mixture of $CH_2Cl_2$ (0.48 mL) and EtOH (0.35 mL), then 0.35 mL of a mixture of sulfuric acid (0.064 mmol)-EtOH was added. The solution was evaporated to dryness and after treating the residue with $Et_2O$ 24 mg (17%) $N^1$-formyl 14,15-cyclopropano-vinorelbine sulfate was obtained as pale yellow crystals. Mp 217-219 °C. IR (KBr) 3472, 2995, 1744, 1678, 1599, 1459, 1233, 1118, 701 $cm^{-1}$. $^1$H-NMR[24] (800 MHz, DMSO-$d_6$ + TFA-*d*) δ (ppm) 0.54 (1H, H-15); 0.70 (1H, $H_\alpha$-22); 0.92 (t, $J$ = 6.5 Hz, 3H, $H_3$-18); 1.07 (t, $J$ = 7.5 Hz, 3H, $H_3$-18'); 1.20 (1H, $H_\beta$-22); 1.22 (1H, $H_x$-19); 1.34 (1H, H-14); 1.85 (1H, H-14'); 1.71 (1H, $H_y$-19); 2.00 (s, 3H, C(17)OCO$CH_3$); 2.02 (1H, $H_\alpha$-6); 2.07 (2H, $H_2$-19'); 2.20 (1H, $H_\beta$-6); 2.60 (1H, $H_x$-17'); 2.85 (1H, $H_y$-17'); 3.04 (1H, $H_x$-5); 3.14 (1H, $H_x$-3); 3.16 (1H, $H_x$-21'); 3.59 (s, 3H, C(16')COO$CH_3$)*; 3.69 (s, 3H, C(16)COO$CH_3$)*; 3.70 (1H, H-21); 3.70 (1H, $H_y$-5); 3.76 (1H, $H_y$-21'); 3.76 (1H, $H_y$-3); 3.78 (1H, $H_x$-3'); 3.92 (s, 3H, C(11)O$CH_3$); 4.06 (1H, $H_y$-3'); 4.54 (s, 1H, H-2); 4.56 (1H, $H_x$-5'); 4.95 (1H, $H_y$-5'); 4.96 (s, 1H, H-17); 5.80 (1H, H-15'); 7.10 (t, 1H, H-10'); 7.16 (t, 1H, H-11'); 7.47 (d, 1H, H-12'); 7.49 (s, 1H, H-12); 7.72 (d, 1H, H-9'); 8.35 (1H, N(4)H); 9.05 (s, 1H, N(1)CHO); 10.71 (1H, N(4')H). $^{13}$C-NMR (200 MHz, DMSO-$d_6$ + TFA-*d*) δ (ppm) 7.1 (C-22); 7.3 (C-18); 11.6 (C-18'); 10.3 (C-14); 15.2 (C-15); 20.5 (C(17)OCO<u>C</u>$H_3$); 26.6 (C-19'); 35.8 (C-19); 39.9 (C-20); 40.4 (C-6); 46.8 (C-5'); 50.4 (C-5); 51.0 (C-3); 51.4 (C-21'); 52.0 (C-3'); 52.7 (C(16')COO<u>C</u>$H_3$, C(16)COO<u>C</u>$H_3$); 56.7 (C(11)O<u>C</u>$H_3$); 65.5 (C-21); 75.2 (C-17); 77.6 (C-16); 69.3 (C-2); 96.1 (C-12); 112.4 (C-12'); 118.3 (C-9'); 119.7 (C-10'); 122.3 (C-15'); 122.5 (C-11'); 128.3 (C-8'); 131.7 (C-20'); 134.3 br (C-2'); 135.6 (C-13'); 141.3 (C-13); 158.3 (C-11); 162.0 (N(1)CHO); 169.7 (C(17)O<u>C</u>O$CH_3$); 169.8 (C(16')<u>C</u>O$OCH_3$)$^+$; 172.9 (C(16)<u>C</u>O$OCH_3$)$^+$.

(*,$^+$ interchangeable ambiguous assignments). HRMS: 807.39603 ($C_{46}H_{55}O_9N_4$; calc. 807.39636). ESI-MS-MS (CID=35 %) (rel. int. %): 747(100); 705(8); 687(27); 654(13); 626(16); 616(7); 594(21); 566(13); 534(4); 527(4); 495(6).

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24. The structure elucidation and spectral assignment of the compounds were based on thorough investigations involving 1D and 2D NMR measurements that proved the correctness of the structures unambiguously. However, due to the fact that the spectra of both compounds (**10** and **11**) consisted of

broad and severely overlapping signals, the multiplicities and integral intensities were mostly not directly extractable from the 1D $^{1}$H NMR spectra. Thus, in the given NMR characterization multiplicities are denoted only where experimentally accessible, and the signal intensities refer to the theoretical ones inferred from the structures.