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SYNTHESIS AND *IN VITRO* ANTITUMOR EFFECT OF NEW VINDOLINE DERIVATIVES COUPLED WITH AMINO ACID ESTERS

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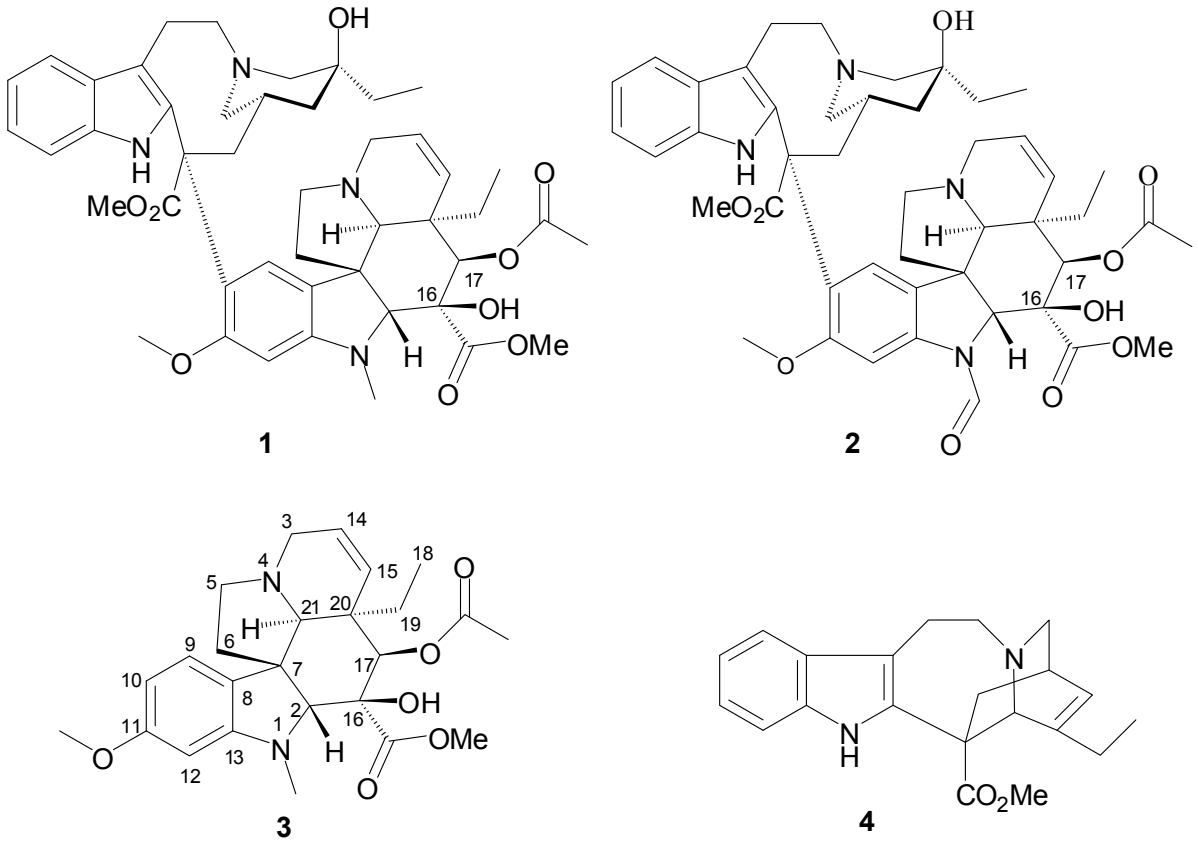
Abstract – 10-Bromovindoline and its 14,15-dihydro- and 14,15-cyclopropano derivatives were coupled in the position 16 with (L)- and (D)-tryptophan methyl esters. The tryptophan derivatives of vindoline were synthesized starting from the 16-carboxylic acid hydrazides *via* the corresponding azides which were allowed to react with the amino acid esters. The new compounds showed antitumor activity against human leukemia (HL-60) cells *in vitr*o.

INTRODUCTION

The *Vinca* alkaloids vinblastine (**1**) and vincristine (**2**) have been widely used in antitumor therapy for about 50 years. These dimeric alkaloids have two monomer alkaloid parts: vindoline (**3**) and catharanthine (**4**) (Scheme 1). The chemistry and pharmacology of vinblastine and vincristine are well known, and a number of their derivatives were synthesized to improve their therapeutic properties.[1] However, vindoline and its derivatives were found to be biologically insignificant, therefore there is much fewer data on their chemistry and biological effect in the literature.[2]



Scheme 1

Our research project was conceived on the basis of two interesting experiences in connection with dimeric alkaloids.

Firstly, some amino acid derivatives of vinblastine and vincristine were synthesized by coupling amino acid esters with the vindoline part in position 16.[3] The obtained conjugates exhibited significant antitumor effect against P388 and L1210 leukemia in mice. At the same time (D)- and (L)-tryptophan derivatives at the 16-position of desacetylvinblastine were conjugated through the carboxyl group with oligoarginine octapeptide as a carrier peptide at the *N*-terminus by Bánóczi *et al*.[4] One of the obtained stereoisomers showed a selective cytotoxic effect against the HL-60 human leukemia cells of higher proliferation rate.

Secondly, for dihydrovinblastine (*i.e.* vinblastine saturated in the 14,15-position of the vindoline part) a decreased antitumor activity and toxicity was observed, probably due to a different mechanism of action from vinblastine.[5]

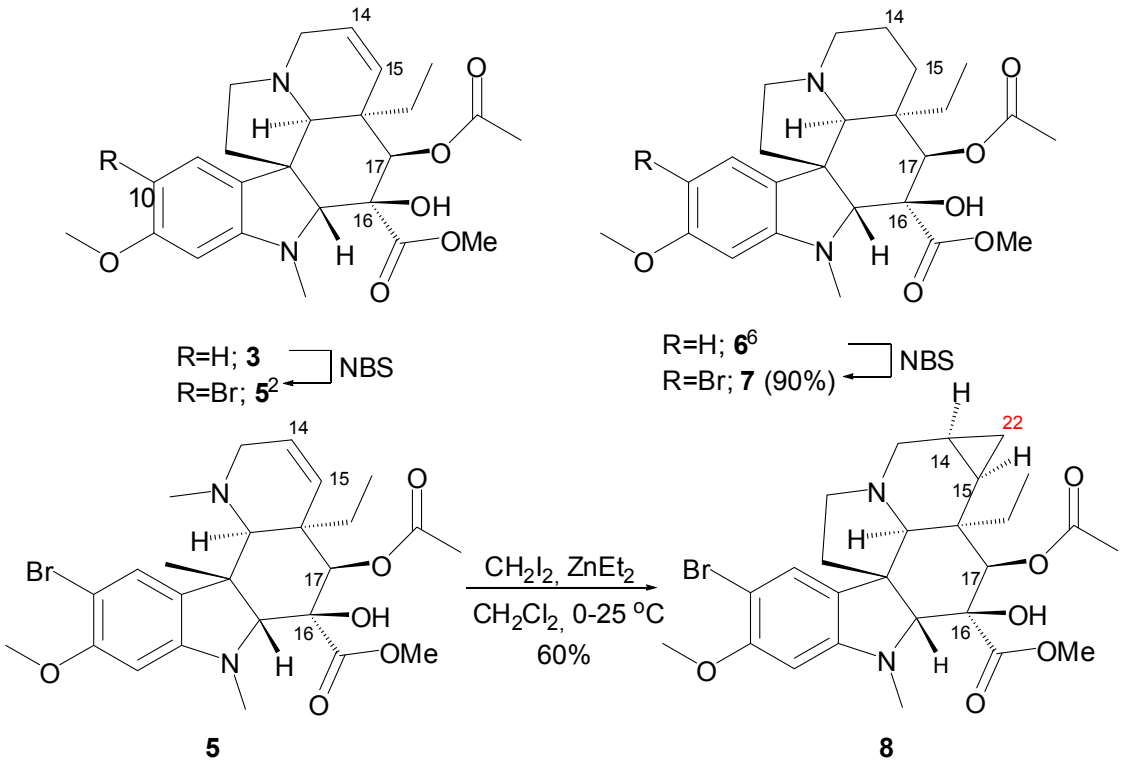
These observations suggest that amino acid conjugation and reduction of the 14,15-position both alter the mechanism of action. Based on these observations our goal was to couple vindoline (**3**), 14,15-dihydrovindoline (**6**), and vindoline condensed with a cyclopropane ring in position 14,15, with

(L)- and (D)-tryptophan methyl ester, as in the case of desacetylvinblastine,[4] at position 16 with a view to screening their biological effect. The azide coupling method known from peptide chemistry was used as the key step in the coupling of vindoline with the amino group of the amino acid ester.

RESULTS AND DISCUSSION

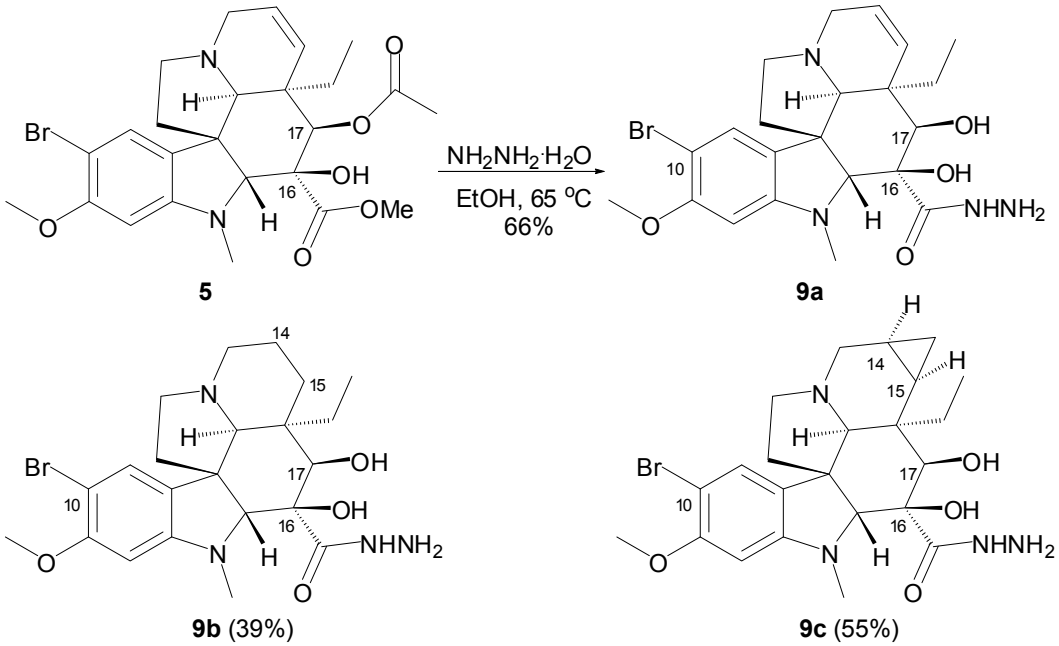
1. Chemistry

The first task was to protect position 10 of vindoline, because in the presence of sodium nitrite used in the preparation of the corresponding azide, a nitrosation reaction took place in position 10, resulting in 10-nitrosovindoline.[2] In this procedure a bromo substituent was used to protect position 10 (Scheme 2).



Scheme 2

10-Bromovindoline (**5**) was prepared by us from vindoline (**3**) in a simple bromination reaction using *N*-bromosuccinimide.[2] 14,15-Dihydrovindoline (**6**) is known from the literature,[6] and was obtained by catalytic hydrogenation of vindoline (**3**). Analogously with the previous reaction, bromination of the saturated derivative **6** resulted in 10-bromo-14,15-dihydrovindoline (**7**). The cyclopropane ring was built into position 14,15 by a typical Simmons-Smith reaction from 10-bromovindoline (**5**) using diethylzinc and diiodomethane, yielding compound **8**.



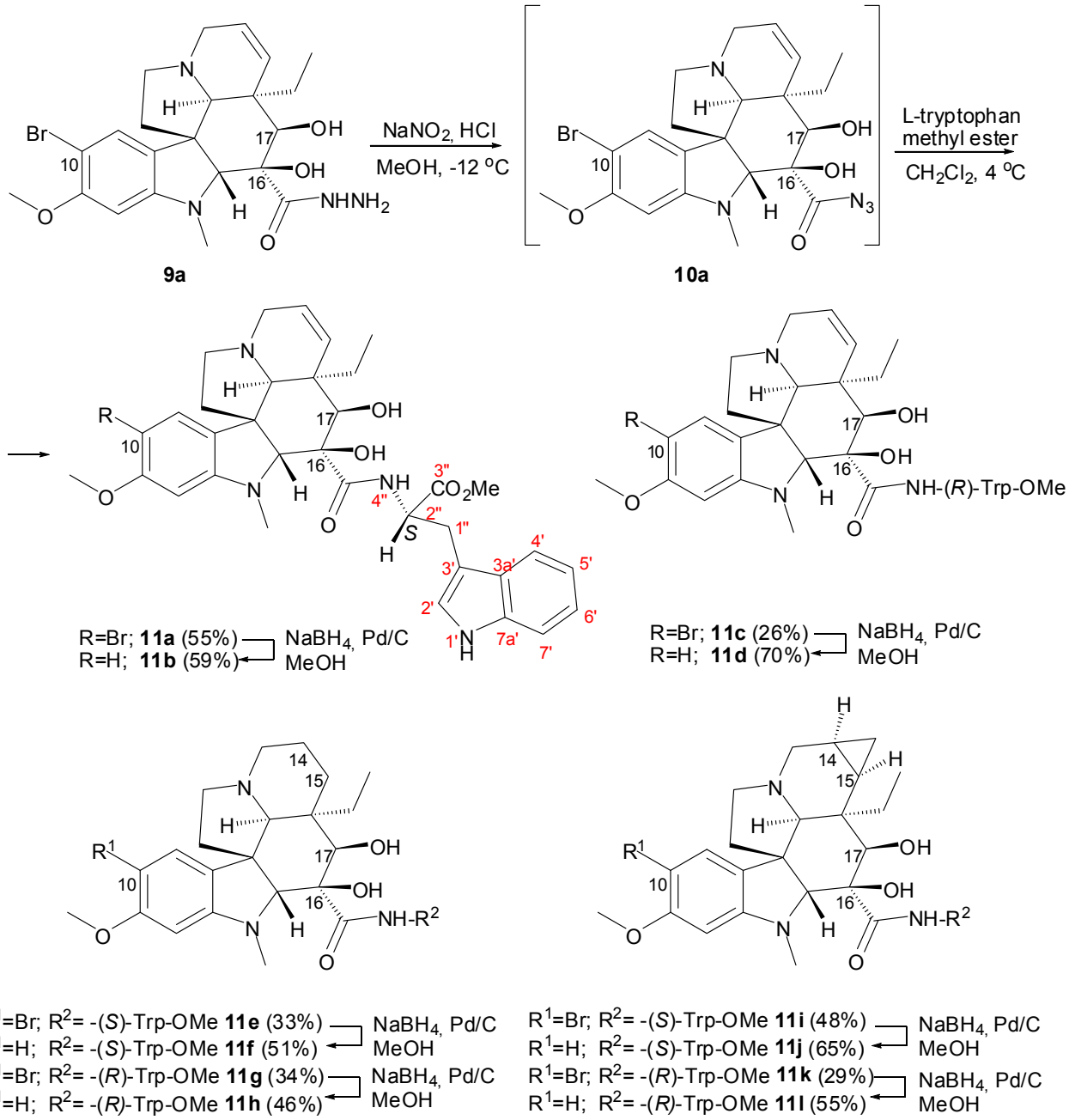
Scheme 3

Hydrazides of 10-bromovindoline (**9a**), 14,15-dihydrovindoline (**9b**), and 14,15-cyclopropanovindoline (**9c**), were synthesized from the corresponding bromo esters 10-bromovindoline (**5**), 10-bromo-14,15-dihydrovindoline (**7**), and 10-bromo-14,15-cyclopropanovindoline (**8**), respectively, with hydrazine hydrate (Scheme 3). Hydrazine, as a strong nucleophile, caused desacetylation in position 17 during the reaction, and so the 17-desacetylated hydrazides were obtained as products.

Coupling the vindoline derivatives with tryptophan methyl esters was achieved by preparation of the azides (Scheme 4). The reaction of hydrazide **9a** with sodium nitrite in methanol in the presence of hydrochloric acid resulted in azide **10a**, which was then allowed to react without isolation with (L)-tryptophan methyl ester to yield the vindoline conjugate **11a**.

Using this procedure 14,15-dihydrovindoline and 14,15-cyclopropanovindoline were also coupled with (L)-tryptophan methyl ester, yielding compounds **11e** and **11i**, respectively. The derivatives containing (D)-tryptophan methyl ester (**11c**, **11g**, **11k**) were synthesized analogously.

In the case of compounds **11a**, **11c**, **11e, 11g, 11i** and **11k**, the bromo substituent was removed from position 10 by hydrogenolysis to give compounds **11b**, **11d**, **11f, 11h, 11j** and **11l**, respectively.



Scheme 4

2. Biology

The influence of the different modifications of the ring system and/or the conjugation with tryptophan on the *in vitro* cytostasis of HL-60 human leukemia cells was analysed by using MTT assay (Table 1). Vindoline, as expected (**2**), exhibited no effect in the concentration range applied (2.6 x 10^{-4}-10^{2} μM). Compound **11f** showed the highest activity IC_{50} = 27.5±1.5). In this compound there is a saturated double bond (14,15-dihydro moiety), but no Br in position 10. The appearance of Br at this position resulted in a significant (2-fold) drop in cytostatic activity (IC_{50} = 60.1±15.0 for **11e** vs. 27.5±1.5 for **11f**). The importance of the saturated bond between C14 and C15 could be emphasized also by the comparison of

the compounds **11b** and **11f**. Compound **11f** with saturation was more effective then compound **11b** with double bond (IC_{50} = 27.5±1.5 for **11f** vs 54.3±6.7 for **11b**). The introduction of Br at position 10 into compound **11b** resulted in essentially no change in the IC_{50} values (IC_{50} = 54.3±6.7 for **11b** vs. 56.8±9.8 for **11a**). However, the introduction of 14(*S*),15(*R*)-cyclopropano part into compound **11b** decreased the activity of both non-brominated (**11j**) and brominated (**11i**) derivatives (IC_{50} = 54.3±6.7 for **11b** vs. 77.1±8.1 for **11j** or 75.3±2.3 for **11i**). The comparison of cytostatic activity of pairs with L- or D-Trp methylester shows no markedly altered IC_{50} values in 10-bromo substituted and unsubtituted compounds (IC_{50} = 56.8±9.8 for **11a** vs 73.8±10.4 for **11c** and IC_{50} = 54.3±6.7 for **11b** vs 73.0±2.3 **11d**), 10-bromo substituted compounds with saturated bond between C14 and C15 (IC_{50} = 60.1±15.0 for **11e** vs 60.8±1.3 for **11g**) or with added 14(*S*),15(*R*)-cyclopropano ring (IC_{50} = 75.3±2.3 for **11i** vs. IC_{50} = 72.6±4.0 for **11k**). Interestingly, the 14(*S*),15(*R*)-cyclopropano derivative of D-TrpOMe (IC_{50} >100 for **11l**) was inactive, while the conjugate with L-TrpOMe (IC_{50} = 77.1±8.1 for **11j**) had measurable *in vitro* cytostatic effect. Also was difference in the activity of the best compound with L-TrpOMe (IC_{50} = 27.5±1.5 for **11f**) and its D-TrpOMe derivative (IC_{50} = 78.4±9.7 for **11h**).

Table 1. Cytostatic activity of vindoline derivatives on HL-60 cells

Compound	Code	IC_{50} (μM)±s.d.
Methyl-{*N*-[10-bromo-17-*O*-desacetyl-16-des(methoxycarbonyl)-vindoline-16-carbonyl]-L-Trp}	**11a**	56.8±9.8
Methyl-{*N*-[10-bromo-17-*O*-desacetyl-16-des(methoxycarbonyl)-vindoline-16-carbonyl]-D-Trp}	**11c**	73.8±10.4
Methyl-{*N*-[17-*O*-desacetyl-16-des(methoxycarbonyl)-vindoline-16-carbonyl]-L-Trp}	**11b**	54.3±6.7
Methyl-{*N*-[17-*O*-desacetyl-16-des(methoxycarbonyl)-vindoline-16-carbonyl]-D-Trp}	**11d**	73.0±2.3
Methyl-{*N*-[10-bromo-14,15-dihydro-17-*O*-desacetyl-16-des(methoxycarbonyl)vindoline-16-carbonyl]-L-Trp}	**11e**	60.1±15.0
Methyl-{*N*-[14,15-dihydro-17-*O*-desacetyl-16-des(methoxycarbonyl)-vindoline-16-carbonyl]-L-Trp}	**11f**	27.5±1.5
Methyl-{*N*-[10-bromo-14,15-dihydro-17-*O*-desacetyl-16-des(methoxycarbonyl)vindoline-16-carbonyl]-D-Trp}	**11g**	60.8±1.3
Methyl-{*N*-[14,15-dihydro-17-*O*-desacetyl-16-des(methoxycarbonyl)vindoline-16-carbonyl]-D-Trp}	**11h**	78.4±9.7
Methyl-{*N*-[10-bromo-14(*S*),15(*R*)-cyclopropano-17-*O*-desacetyl-16-des(methoxycarbonyl)vindoline-16-carbonyl]-L-Trp}	**11i**	75.3±2.3
Methyl-{*N*-[10-bromo-14(*S*),15(*R*)-cyclopropano-17-*O*-desacetyl-16-des(methoxycarbonyl)vindoline-16-carbonyl]-D-Trp}	**11k**	72.6±4.0

Methyl-{*N*-[14(*S*),15(*R*)-cyclopropano-17-*O*-desacetyl-16-des(methoxycarbonyl)vindoline-16-carbonyl]-L-Trp}	**11j**	77.1±8.1
Methyl-{*N*-[14(*S*),15(*R*)-cyclopropano-17-*O*-desacetyl-16-des(methoxycarbonyl)vindoline-16-carbonyl]-D-Trp}	**11l**	>100
Vindoline	**3**	>100

EXPERIMENTAL

General

Melting points 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 dimethylsulfoxide-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 35–50%. 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.

10-Bromo-14,15-dihydrovindoline (7). Dihydrovindoline[6] (**6**) (280 mg, 0.61 mmol) was dissolved in CH_2Cl_2 (10 mL) and 114 mg (0.64 mmol) of *N*-bromosuccinimide was added. The reaction mixture was stirred for 45 min. Then the solution was washed with 5% aq. sodium hydrogen carbonate (10 mL) and with water (10 mL). The organic layer was dried with magnesium sulfate and the solvent was evaporated in vacuum. 295 mg (90%) of **7** was obtained, mp 229 °C. TLC (CH_2Cl_2-MeOH 10:1), R_f = 0.80. $[\alpha]^{32}_{D}$ +6.5 (*c* 1, CH_2Cl_2). IR (KBr): 3439, 1738, 1462, 1244, 1043, 888, 812 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.46 (t, *J* = 7.5 Hz, 3H, H_3-18); 0.93 (ABq, *J* = 14.4 Hz, *J* = 7.5 Hz, 1H, H_x-19); 1.15 (td, *J*

= 13.8 Hz, *J* = 3.7 Hz, 1H, H_{ax}-15); 1.25 (d br, *J* = 13.8 Hz, 1H, H_{eq}-15); 1.30 (ABq, *J* = 14.4 Hz, *J* = 7.5 Hz, 1H, H_y-19); 1.56 (d br, *J* = 12.8 Hz, 1H, H_{eq}-14); 1.73 (qt, *J* = 12.8 Hz, *J* = 3.7 Hz, 1H, H_{ax}-14); 1.98 (s, 3H, H_3-C(17)OCOCH$_3$); 2.08 (br, 1H, H_α-3); 2.15-2.23 (m, 2H, H_2-6); 2.27 (br, 1H, H-21); 2.57 (s, 3H, H_3-N(1)CH$_3$); 2.59 (br, 1H, H_α-5); 3.09 (d br, *J* = 9.1 Hz, 1H, H_β-3); 3.16 (t br, *J* = 9.1 Hz, 1H, H_β-5); 3.57 (s, 1H, H-2); 3.69 (s, 3H, H_3-C(16)COOCH$_3$); 3.79 (s, 3H, H_3-C(11)OCH$_3$); 5.37 (s, 1H, H-17); 6.43 (s, 1H, H-12); 7.36 (s, 1H, H-9); 9.32 (s, 1H, OH). ^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ 7.8 (C-18); 20.7 (C-C(17)OCO<u>C</u>H$_3$); 22.2 (C-14); 29.5 (C-15); 32.8 (C-19); 37.9 (C-1); 42.4 (C-20); 43.3 (C-6); 50.7 (C-3); 51.7 (C-5); 52.0 (C-C(16)COO<u>C</u>H$_3$); 52.0 (C-7); 56.3 (C-C(11)O<u>C</u>H$_3$); 71.5 (C-21); 75.1 (C-17); 77.8 (C-16); 83.0 (C-2); 95.2 (C-12); 99.2 (C-10); 126.7 (C-9); 127.5 (C-8); 153.5 (C-13); 156.0 (C-11); 169.6 (C-C(17)O<u>C</u>OCH$_3$); 172.1 (C-C(16)<u>C</u>OOCH$_3$). HRMS: 537.15972 ($C_{25}H_{34}O_6N_2Br$; calc. 537.15948). ESI-MS-MS (rel. int. %): 477(100); 445(5); 417(7); 266(11).

10-Bromo-14(*S*),15(*R*)-cyclopropanovindoline (8). 10-Bromovindoline[2] (**5**) (1.466 g, 2.74 mmol) was dissolved in CH_2Cl_2 (50 mL) under argon, and at 0 °C diethylzinc (6.99 mL, 6.99 mmol) in 1M hexane solution, then diiodomethane (0.44 mL, 5.46 mmol) were injected into the solution. The reaction mixture was stirred for 30 min at 0 °C and then for 6 h at room temperature. After allowing to stand overnight, further diethylzinc (6.99 mL) and diiodomethane (0.44 mL) were added. After stirring for 4 h at room temperature the reaction mixture was filtered, CH_2Cl_2 (100 mL) was added to the filtrate and was washed with water (100 mL). The aqueous phase was washed with CH_2Cl_2 (2 x 50 mL). The combined organic phase was dried with magnesium sulfate and the solvent was evaporated in vacuum. The crude product was purified by preparative TLC (CH_2Cl_2-MeOH 20:1) and 910 mg (60%) of **8** was isolated as a yellow solid, mp 226-228 °C. TLC (CH_2Cl_2-MeOH 20:1), R_f = 0.50. $[\alpha]^{22}_D$ -50.9 (*c* 1, $CHCl_3$). IR (KBr): 3436, 2970, 1748, 1728, 1253, 1188, 960 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 0.29 (m, 1H, H_α-15); 0.60 (m, 1H, H_x-22); 0.67 (t, *J* = 7.5 Hz, 3H, H_3-18); 0.78 (ABq, *J* = 14.1 Hz, *J* = 7.0 Hz, 1H, H_x-19); 0.81 (m, 1H, H_y-22); 1.14 (m, 1H, H_α-14); 1.71 (ABq, *J* = 14.1, *J* = 7.0 Hz, 1H, H_y-19); 1.97 (s, 3H, H_3-C(17)OCOCH$_3$); 2.40 (dd, *J* = 10.8 Hz, *J* = 3.6 Hz, 1H, H_α-3); 2.10 (m, 1H, H_α-6); 2.19 (ddd, *J* = 13.5 Hz, *J* = 10.0 Hz, *J* = 3.3 Hz, 1H, H_β-6); 2.36 (s, 1H, H-21); 2.40 (dd, *J* = 10.8 Hz, *J* = 3.6 Hz, 1H, H_α-3); 2.46 (td, *J* = 10.0 Hz, *J* = 9.1 Hz, 1H, H_α-5); 2.58 (s, 3H, H_3-N(1)CH$_3$); 3.11 (td , *J* = 9.1 Hz, *J* = 3.3 Hz, 1H, H_β-5); 3.19 (d br, *J* = 10.8 Hz, 1H, H_β-3); 3.46 (s, 1H, H-2); 3.67 (s, 3H, H_3-C(16)COOCH$_3$); 3.81 (s, 3H, H_3- C(11)OCH$_3$); 5.21 (s, 1H, H-17); 6.42 (s, 1H, H-12); 7.36 (s, 1H, H-9); 7.86 (s, 1H, OH). ^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ 7.9 (C-18); 8.6 (C-22); 11.4 (C-14); 15.8 (C-15); 32.8 (C-19); 20.8 (C-C(17)OCO<u>C</u>H$_3$); 38.4 (C-1); 40.4 (C-20); 44.4 (C-6); 51.7 (C-7); 51.8 (C-C(16)COO<u>C</u>H$_3$); 52.3 (C-5); 52.5 (C-3); 56.2 (C-C(11)O<u>C</u>H$_3$); 69.0 (C-21); 76.4 (C-17); 77.9 (C-16); 83.1 (C-2); 94.9 (C-12); 99.0 (C-10); 126.4 (C-9); 127.4 (C-8); 152.9 (C-13); 155.9 (C-11); 169.9 (C-C(17)O<u>C</u>OCH$_3$); 171.6

(C-C(16)COOCH$_3$). HRMS: 549.15945 ($C_{26}H_{34}O_6N_2Br$; calc. 549.15948). ESI-MS-MS (rel. int. %): 489(100); 471(3); 429(7); 401(3); 266(23).

Bromo-17-*O*-desacetyl-16-des(methoxycarbnyl)vindoline-16-carbohydrazide (9a). 10-Bromovindoline[2] (**5**) (1.16 g, 2.17 mmol) was dissolved in a mixture of 10 mL of dry EtOH and 8 mL of $CHCl_3$. 14 mL of 64% hydrazine hydrate was added, then the reaction mixture was stirred at 60 °C for 72 h under argon. The solution was evaporated to dryness *in vacuo* and the residue was dissolved in water (40 mL) and was extracted with 5 x 40 mL of $CHCl_3$. The organic layer was dried with magnesium sulfate and the solvent was evaporated under vacuum. 710 mg (66%) of **9a** was obtained by preparative thin layer chromatographhy of the residue (CH_2Cl_2-MeOH 10:1), mp 143-145 °C. TLC (CH_2Cl_2-MeOH 10:1), R_f = 0.36. $[\alpha]^{24}_D$ +3.86 (*c* 1, CH_2Cl_2). IR (KBr): 3410, 3300, 1670, 1625, 1590, 1490, 1190, 1030, 790 cm^{-1}. ^{1}H NMR (DMSO-d_6): 0.63 (t, 3H, CH_3-18); 0.92 (m, 1H, CH_2-19$_x$); 1.36 (m, 1H, CH_2-19$_y$); 2.16 (t, 2H, CH_2-6$_\alpha$,6$_\beta$); 2.61 (q, 1H, CH_2-5$_\alpha$); 2.70 (s, 1H, CH-21); 2.72 (s, 3H, N^1-CH_3); 2.84 (d, 1H, CH_2-3$_x$); 3.19-3,31 (M, 2H, CH_2-5$_\beta$,3$_y$); 3.48 (s, 1H, CH-2); 3.85 (s, 3H, CH_3O-11); 3.90 (s, 2, CH-17 & OH-17); 4.28 (s, 2H, NH_2); 5.57 (d, 1H, CH-15); 5.78 (dd, 1H, CH-14); 6.30 (s, 1H, CH-12); 7.26 (s, 1H, CH-9); 8.57 (s, 1H, NH); 8.83 (s, 1H, OH-17) ppm. HRMS: 493.14431 ($C_{22}H_{30}O_4N_4Br$; calc. 493.14449). ESI-MS-MS (rel. int. %): 475(63); 457(3); 443(71); 433(100); 373(12); 266(45); 222(4).

10-Bromo-14,15-dihydro-17-*O*-desacetyl-16-des(methoxycarbonyl)vindoline-16-carbohydrazide (9b). 10-Bromo-14,15-dihydrovindoline (**7**) (1.36 g, 2.53 mmol) was treated with 17 mL of 64% hydrazine hydrate in a mixture of 12 mL of dry EtOH and 10 mL of $CHCl_3$ as discussed in the case of hydrazide **9a**. 486 mg (39%) of **9b** was obtained as a foam. TLC (CH_2Cl_2-MeOH 10:1), R_f = 0.53. $[\alpha]^{32}_D$ +57.2 (*c* 1, CH_2Cl_2). IR (KBr): 3426, 2962, 2938, 1666, 1605, 1496, 1467, 1228, 1041, 880 cm^{-1}. 1H NMR (500 MHz, DMSO-d_6) δ 0.60 (t, *J* = 7.4 Hz, 3H, H_3-18); 0.64 (ABq, *J* = 13.0 Hz, *J* = 7.4 Hz, 1H, H_y-19); 0.95 (ABq, *J* = 13.0 Hz, *J* = 7.4 Hz, 1H, H_x-19); 0.73 (td, *J* = 13.0, *J* = 4.4 Hz, 1H, H_{ax}-15); 1.46 (d br, *J* = 13.0 Hz, 1H, H_{eq}-14); 1.80 (qt, *J* = 13.0 Hz, *J* = 2.8 Hz, 1H, H_{ax}-14); 1.96 (m, 1H, H_α-3); 2.00 (s, 1H, H-21); 2.07 (d br, *J* = 13.0 Hz, 1H, H_{eq}-15); 2.10-2.20 (m, 2H, H_2-6); 2.42 (q, *J* = 9.3 Hz, 1H, H_α-5); 2.64 (s, 3H, H_3-N(1)CH_3); 3.00 (d br, *J* = 10.0 Hz, 1H, H_β-3); 3.06 (ddd br, *J* = 9.3 Hz, *J* = 7.7 Hz, *J* = 2.3 Hz, 1H, H_β-5); 3.33 (s, 1H, H-2); 3.78 (s, 3H, H_3-C(11)OCH_3); 4.11 (d, *J* = 7.3 Hz, 1H, OH-17); 4.22 (d, *J* = 7.3 Hz, 1H, H-17); 4.26 (br, 2H, NH_2) 6.35 (s, 1H, H-12); 7.20 (s, 1H, H-9); 8.80 (s, 1H, NH); 9.09 (s, 1H, OH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 7.7 (C-18); 22.9 (C-14); 29.9 (C-15); 34.8 (C-19); 39.1 (C-1); 39.6 (C-20); 45.0 (C-6); 51.4 (C-3); 52.0 (C-5); 52.2 (C-7); 56.2 (C-C(11)O$\underline{C}H_3$); 70.1 (C-17); 75.4 (C-21); 78.8 (C-16); 85.2 (C-2); 94.9 (C-12); 98.5 (C-10); 126.3 (C-9); 128.9 (C-8); 154.2 (C-13);

155.7 (C-11); 172.1 (C-C(16)CONHNH$_2$). HRMS: 495.15995 ($C_{22}H_{32}O_4N_4Br$; calc. 495.16014). ESI-MS-MS (rel. int. %): 477(35); 459(3); 445(14); 435(100); 266(19).

10-Bromo-14(*S*),15(*R*)-cyclopropano-17-*O*-desacetyl-16-des(methoxycarbonyl)vindoline-16-carbohydrazide (9c). 10-Bromo-14,15-cyclopropanovindoline (**8**) (882 mg, 1.605 mol) was treated with 10 mL of 64% hydrazine hydrate in dry EtOH (10 mL) for 24 h as discussed in the case of hydrazide **9a**. 444 mg (55%) of **9c** was obtained which was used in further reactions without any purification. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.52 (m, 2H, H$_\alpha$-15, H$_x$-22); 0.79 (t, J = 7.3 Hz, 3H, H$_3$-18); 0.84 (ABq, J = 13.4 Hz, J = 7.3 Hz, 1H, H$_x$-19); 0.92 (m, 1H, H$_y$-22); 1.12 (m, 1H, H$_\alpha$-14); 1.28 (ABq, J = 13.4 Hz, J = 7.3 Hz, 1H, H$_y$-19); 2.01 (m, 1H, H$_\alpha$-6); 2.08 (ddd, J = 13.9 Hz, J = 10.3 Hz, J = 3.9 Hz, 1H, H$_\beta$-6); 2.28 (s, 1H, H-21); 2.42 (m, 2H, H$_\alpha$-3, H$_\alpha$-5); 2.65 (s, 3H, H$_3$-N(1)CH$_3$); 3.06 (td, J = 9.2 Hz, J = 3.9 Hz, 1H, H$_\beta$-5); 3.18 (m, 1H, H$_\beta$-3); 3.29 (s, 1H, H-2); 3.56 (d, 1H, C(17)OH) 3.79 (s, 3H, H$_3$-C(11)OCH$_3$); 3.95 (d, 1H, H-17); 4.27 (s br, 2H, NH$_2$); 6.30 (s, 1H, H-12); 7.23 (s, 1H, H-9); 7.81 (s, 1H, C(16)OH); 8.91 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 7.9 (C-18); 8.2 (C-22); 11.0 (C-14); 15.0 (C-15); 35.9 (C-19); 38.3 (C-1); 40.1 (C-20); 45.2 (C-6); 51.7 (C-7); 52.0 (C-5); 52.5 (C-3); 56.1 (C-C(11)OCH$_3$); 70.8 (C-21); 73.0 (C-17); 78.7 (C-16); 84.2 (C-2); 94.0 (C-12); 97.7 (C-10); 126.1 (C-9); 127.5 (C-8); 153.0 (C-13); 155.8 (C-11); 171.2 (C-C(16)CONHNH$_2$). HRMS: 507.16017 ($C_{23}H_{32}O_4N_4Br$; calc. 507.16014). ESI-MS-MS (rel. int. %): 489(45); 472(4); 457(19); 447(50); 417(4); 389(100); 266(29); 240(2).

General method for coupling [10-bromo-17-*O*-desacetyl-16-des(methoxycarbonyl)vindoline-16-carbonyl]azides with tryptophan methyl esters

Methyl {*N*-[10-bromo-17-*O*-desacetyl-16-des(methoxycarbonyl)vindoline-16-carbonyl]-L-tryptophanate} (11a). Hydrazide **9a** (250 mg, 0.51 mmol) was dissolved in a mixture of MeOH (8 mL) and 1N hydrochloric acid (29 mL), and at -12 °C sodium nitrite (81 mg, 1.17 mmol) was added. After stirring for 10 min, the pH of the reaction mixture was adjusted to 8-8.5 with saturated aqueous sodium hydrogen carbonate. Then the solution was extracted with CH_2Cl_2 (4 x 20 mL), the organic layer was washed with brine and dried with magnesium sulfate. Subsequently the CH_2Cl_2 solution was concentrated to ¼ part and the azide (**10a**) was used without isolation in the coupling reaction with L-tryptophan methyl ester (0.51 mmol) in CH_2Cl_2 (2 mL). The reaction mixture was kept in a refrigerator at 4 °C for a week. The dark solution was washed with water (8 ml) and dried with magnesium sulfate. The solvent was evaporated in vacuum and the residue was purified by preparative thin-layer chroatography (CH_2Cl_2-MeOH 20:1). 190 mg (55%) of **11a** was isolated, mp 133-134 °C, HCl salt of **11a**, mp 178-181 °C. TLC (CH_2Cl_2-MeOH 20:1), R_f = 0.40. $[\alpha]^{20}_D$ -18.1 (*c* 1, MeOH; **11a**. HCl).

IR (KBr):3400, 2960, 1740, 1660, 1500, 1250, 1030, 740 cm^{-1}. ^1H NMR (DMSO-d_6): 0.55 (t, 3H, CH_3-18); 0.97 (m, 1H, CH_2-19$_x$); 1.40 (m, 1H, CH_2-19$_y$); 1.99 (s, 1H, CH-21); 2.33-2.59 (m, 3H, CH_2-5$_\alpha$,6$_\alpha$,6$_ß$); 2.61 (s, 3H, N^1-CH_3); 3.16-3.78 (m, 3H, CH_2-5$_ß$,6$_\alpha$,6$_ß$); 3.56 (s, 3H, CH_3O-11); 3.66 (s, 1H, CH-2); 3.81 (s, 3H, CH_3-36); 4.15 (s, 1H, CH-17); 4.63 (d, 1H, CH-24); 5.72 (d, 1H, CH-15); 5.85 (dd, 1H, CH-14); 6.37 (s, 1H, CH-12); 6.99 (t, 1H, CH-33); 7.06 (t, 1H, CH-32); 7.31 (s, 1H, CH-27); 7.36 (d, 1H, CH-34); 7.48 (d, 1H, CH-31); 7.54 (s, 1H, CH-9); 8.18 (d, 1H, NH-23); 8.96 (s, 1H, OH-16); 11.02 (s, 1H, indol NH-28) ppm. HRMS: 679.21261 ($C_{34}H_{40}O_6N_4Br$; calc. 679.21257). ESI-MS-MS (rel. int. %): 661(100); 619(5); 443(85); 433(78); 408(6); 373(18); 266(31).

Methyl {*N*-[10-bromo-17-*O*-desacetyl-16-des(methoxycarbonyl)vindoline-16-carbonyl]-D-tryptophanate} (11c). Hydrazide **9a** (250 mg, 0.51 mmol) was converted to the azide (**10a**) which was treated with D-tryptophan methyl ester (0.51 mmol) as discussed in the case of compound **11a**. 90 mg (26%) of **11c** was isolated, mp 137-138 °C. TLC: CH_2Cl_2-MeOH 20:1. R_f = 0.30. $[\alpha]^{23}_D$ -1.1 (*c* 1, CH_2Cl_2). IR (KBr): 3400, 2980, 2940, 1750, 1680, 1620, 1500, 1230, 1050, 880, 820, 750 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ 0.56 (t, *J* = 7.3 Hz, 3H, H_3-18); 0.80 (ABq, *J* = 14.0, *J* = 7.3 Hz, 1H, H_x-19); 1.21 (ABq, *J* = 14.0 Hz, *J* = 7.3 Hz, 1H, H_y-19); 1.99 (m, 1H, H_α-6); 2.06 (m, 1H, H_β-6); 2.09 (s, 3H, H_3-N(1)CH_3); 2.57 (ddd, *J* = 9.7 Hz, *J* = 9.0 Hz, *J*= 7.4 Hz, 1H, H_α-5); 2.61 (s, 1H, H-21); 2.79 (d, *J* = 16.1 Hz, 1H, H_α-3); 3.03 (s, 1H, H-2); 3.19 (m, 2H, H_2-1"); 3.22 (td, *J* = 9.0 Hz, *J* = 4.0 Hz, 1H, H_β-5); 3.35 (m, 1H, H_β-3); 3.60 (s, 3H, H_3-C(3")OOCH_3); 3.74 (s, 3H, H_3-C(11)OCH_3); 3.76 (d, *J* = 7.4 Hz, 1H, H-17); 3.96 (d, *J* = 7.2 Hz, 1H, C(17)OH); 4.57 (ddd, 1H, *J* = 8.5 Hz, *J* = 7.3 Hz, *J* = 6.4 Hz, H-2"); 5.50 (d, *J* = 10.4 Hz, 1H, H-15); 5.74 (ddd, *J* = 10.4, *J* = 5.1, *J* = 1.2 Hz, 1H, H-14); 6.14 (s, 1H, H-12); 7.00 (ddd, *J* = 7.7 Hz, *J* = 7.0 Hz, *J* = 0.9 Hz, 1H, H-5'); 7.05 (ddd, *J* = 7.9 Hz, *J* = 7.0 Hz, *J* = 0.9 Hz, 1H, H-6'); 7.20 (s, 1H, H-9); 7.25 (d, *J* = 2.2 Hz, 1H, H-2'); 7.31 (dt, *J* = 7.9 Hz, *J* = 0.9 Hz, 1H, H-7'); 7.57 (d, *J* = 7.7 Hz, 1H, H-6'); 7.86 (d, *J* = 8.4 Hz, 1H, H-4"); 8.97 (s, 1H, C(16)OH); 10.88 (d, *J* = 2.2 Hz, 1H, H-1'). ^{13}C NMR (125 MHz, DMSO-d_6) δ 7.5 (C-18); 27.5 (C-1"); 32.0 (C-19); 37.5 (C-1); 42.3 (C-20); 44.5 (C-6); 50.3(C-3); 50.5 (C-5); 51.9 (C-C(3")OO<u>C</u>H_3); 52.1 (C-7); 52.9 (C-2"); 56.1 (C- C(11)O<u>C</u>H_3); 67.3 (C-21); 72.6 (C-17); 79.5 (C-16); 83.4 (C-2); 93.7 (C-12); 97.3 (C-10); 109.4 (C-3'); 111.4 (C-7'); 118.2 (C-4'); 118.4 (C-5'); 121.0 (C-6'); 122.6 (C-14); 124.0 (C-2'); 126.1 (C-9); 126.6 (C-8); 127.3 (C-3a'); 131.5 (C-15); 136.1 (C-7a'); 153.0 (C-13); 155.8 (C-11); 172.0 (C-C(16)<u>C</u>ONH); 172.1 (C-3"). HRMS: 679.21253 ($C_{34}H_{40}O_6N_4Br$; calc. 679.21257). ESI-MS-MS (rel. int. %): 661(100); 618(10); 443(58); 433(65); 408(6); 373(22); 266(33).

Methyl {*N*-[10-bromo-14,15-dihydro-17-*O*-desacetyl-16-des(methoxycarbonyl)vindoline-16-carbonyl]-L-tryptophanate} (11e). Hydrazide **9b** (254 mg, 0.51 mmol) was converted to the corresponding azide

which was treated with L-tryptophan methyl ester (0.51 mmol) as discussed in the case of compound **11a**. 115 mg (33%) of **11e** was isolated. TLC: CH_2Cl_2-MeOH 10:1. R_f = 0.54. $[\alpha]^{32}_D$ +30.8 (*c* 0.5, CH_2Cl_2). IR (KBr): 3405, 2945, 1742, 1667, 1605, 1496, 1356, 1340, 1254, 1177, 880, 813, 743 cm^{-1}. ^1H NMR (800 MHz, DMSO-d_6) δ 0.55 (t, *J* = 7.3 Hz, 3H, H_3-18); 0.65 (ABq, *J* = 13.7 Hz, *J* = 7.3 Hz, 1H, H_x-19); 0.70 (td, *J* = 13.6 Hz, *J* = 4.2 Hz, 1H, H_{ax}-15); 0.95 (ABq, *J* = 13.7 Hz, *J* = 7.3 Hz, 1H, H_y-19); 1.49 (dqi, *J* = 13.1 Hz, *J* = 3.3 Hz, 1H, H_{eq}-14); 1.78 (qt, *J* = 13.1 Hz, *J* = 3.9 Hz, 1H, H_{ax}-14); 1.95 (ddd, *J* = 13.1 Hz, *J* = 11.0 Hz, *J* = 2.4 Hz, 1H, H_{ax}-3); 2.03 (s, 1H, H-21); 2.07 (d, *J* = 13.6 Hz, 1H, H_{eq}-15); 2.09-2.18 (m, 2H, H_2-6,); 2.43 (q, *J* = 9.5 Hz, 1H, $H_α$-5); 2.62 (s, 3H, H_3-N(1)CH_3); 3.03 (d, *J* = 11.0 Hz, 1H, H_{eq}-3); 3.07 (td, *J* = 9.5 Hz, *J* = 2.0 Hz, 1H, $H_β$-5); 3.20 (ABd, *J* = 14.7, *J* = 6.1 Hz, 1H, H_x-1"); 3.25 (ABd, *J* = 14.7 Hz, *J* = 6.1 Hz, 1H, H_x-1"); 3.30 (s, 1H, H-2); 3.55(s, 3H, H_3-C(3")$OOCH_3$); 3.78 (s, 3H, H_3-C(11)OCH_3); 4.24 (d, *J* = 7.6 Hz, 1H, H-17); 4.38 (d, *J* = 7.6 Hz, 1H, C(17)OH); 4.64 (dt, 1H, *J* = 7.3 Hz, *J* = 6.1 Hz, H-2"); 6.34 (s, 1H, H-12); 6.99 (ddd, *J* = 7.8 Hz, *J* = 7.0 Hz, *J* = 0.9 Hz, 1H, H-5'); 7.08 (ddd, *J* = 8.1 Hz, *J* = 7.0 Hz, *J* = 1.1 Hz, 1H, H-6'); 7.20 (s, 1H, H-9); 7.26 (d, *J* = 2.3 Hz, 1H, H-2'); 7.35 (dt, *J* = 8.1 Hz, *J* = 0.8 Hz, 1H, H-7'); 7.57 (d, *J* = 7.8 Hz, 1H, H-6'); 7.88 (d, *J* = 7.3 Hz, 1H, H-4"); 9.49 (s, 1H, C(16)OH); 10.91 (d, *J* = 2.4 Hz, 1H, H-1'). ^{13}C NMR (200 MHz, DMSO-d_6) δ 7.7 (C-18); 23.0 (C-14); 27.2 (C-1"); 29.9 (C-15); 34.8 (C-19); 39.6 (C-20); 39.7 (C-1); 45.1 (C-6); 51.4 (C-3); 51.9 (C-C(3")OO<u>C</u>H_3); 52.0 (C-5); 52.5 (C-2"); 52.8 (C-7); 56.3 (C-C(11)O<u>C</u>H_3); 70.4 (C-17); 75.4 (C-21); 79.2 (C-16); 85.1 (C-2); 95.0 (C-12); 98.6 (C-10); 108.7 (C-3'); 111.5 (C-7'); 118.3 (C-4'); 118.6 (C-5'); 121.1 (C-6'); 124.3 (C-2'); 126.4 (C-9); 127.3 (C-3a'); 128.8 (C-8); 136.2 (C-7a'); 154.4 (C-13); 155.8 (C-11); 172.2 (C-C(16)<u>C</u>ONH); 173.6 (C-3"). HRMS: 681.22855 ($C_{34}H_{42}O_6N_4Br$; calc. 681.22822). ESI-MS-MS (rel. int. %): 663(83); 621(2); 463(2); 445(21); 435(100); 417(4); 410(2); 266(12).

Methyl {*N*-[10-bromo-14,15-dihydro-17-*O*-desacetyl-16-des(methoxycarbonyl)vindoline-16-carbonyl]-D-tryptophanate} (11g). Compound **11g** (118 mg, 34% yield) was prepared analogously with compound **11e**, mp 152 °C. TLC: CH_2Cl_2-MeOH 10:1. R_f = 0.54. $[\alpha]^{32}_D$ +45.4 (*c* 0.5, CH_2Cl_2). IR (KBr): 3397, 2946, 1740, 1670, 1604, 1496, 1355, 1341, 1254, 1177, 880, 813, 743 cm^{-1}. ^1H NMR (800 MHz, DMSO-d_6) δ 0.55 (t, *J* = 7.3 Hz, 3H, H_3-18); 0.57 (ABq, *J* = 13.7 Hz, *J* = 7.3 Hz, 1H, H_x-19); 0.70 (td, *J* = 13.6 Hz, *J* = 4.2 Hz, 1H, H_{ax}-15); 0.85 (ABq, *J* = 13.7 Hz, *J* = 7.3 Hz, 1H, H_y-19); 1.45 (dqi, *J* = 13.2 Hz, *J* = 3.6 Hz, 1H, H_{eq}-14); 1.78 (qt, *J* = 13.2 Hz, *J* = 3.9 Hz, 1H, H_{ax}-14); 1.95 (m, 1H, H_{ax}-3) 1.96 (s, 1H, H-21); 1.99-2.12 (m, 3H, H_2-6, H_{eq}-15); 2.12 (s, 3H, H_3-N(1)CH_3); 2.41 (q, *J* = 9.5 Hz, 1H, $H_α$-5); 2.96 (s, 1H, H-2); 3.00 (d, *J* = 10.6 Hz, 1H, H_{eq}-3); 3.06 (td, *J* = 9.5 Hz, *J* = 1.5 Hz, 1H, $H_β$-5); 3.20 (m, 2H, H_2-1"); 3.63 (s, 3H, H_3-C(3")$OOCH_3$); 3.74 (s, 3H, H_3-C(11)OCH_3); 4.12 (d, *J* = 7.5 Hz, 1H, H-17); 4.23 (d, *J* = 7.5 Hz, 1H, C(17)OH); 4.62 (ddd, 1H, *J* = 8.5 Hz, *J* = 7.1 Hz, *J* = 6.4 Hz, H-2"); 6.23 (s, 1H, H-12); 7.00 (ddd, *J* = 7.8 Hz, *J* = 7.0 Hz, *J* = 0.8 Hz, 1H, H-5'); 7.06 (ddd, *J* = 8.0 Hz, *J* = 7.0 Hz, *J* = 1.1 Hz, 1H,

H-6'); 7.17 (s, 1H, H-9); 7.23 (d, *J* = 2.4 Hz, 1H, H-2'); 7.33 (dt, *J* = 8.0 Hz, *J* = 0.8 Hz, 1H, H-7'); 7.57 (d, *J* = 7.8 Hz, 1H, H-6'); 7.87 (d, *J* = 8.5 Hz, 1H, H-4"); 9.44 (s, 1H, C(16)OH); 10.88 (d, *J* = 2.4 Hz, 1H, H-1'). ^{13}C NMR (200 MHz, DMSO-d_6) δ 7.7 (C-18); 22.9 (C-14); 27.7 (C-1"); 29.9 (C-15); 34.8 (C-19); 38.8 (C-1); 39.7 (C-20); 45.1 (C-6); 51.4 (C-3); 52.0 (C-5); 52.1 (C-C(3")OO<u>C</u>H$_3$); 52.4 (C-7); 53.2 (C-2"); 56.3 (C-C(11)O<u>C</u>H$_3$); 70.0 (C-17); 75.5 (C-21); 79.1 (C-16); 84.9 (C-2); 95.0 (C-12); 98.6 (C-10); 109.5 (C-3'); 111.6 (C-7'); 118.3 (C-4'); 118.6 (C-5'); 121.2 (C-6'); 124.2 (C-2'); 126.4 (C-9); 127.4 (C-3a'); 128.9 (C-8); 136.3 (C-7a'); 154.3 (C-13); 155.8 (C-11); 172.4 (C-C(16)<u>C</u>ONH); 173.4 (C-3"). HRMS: 681.22813 ($C_{34}H_{42}O_6N_4Br$; calc. 681.22822). ESI-MS-MS (rel. int. %): 663(1); 621(3); 463(2); 445(24); 435(100); 417(4); 266(15).

Methyl {*N*-[10-bromo-14(*S*),15(*R*)-cyclopropano-17-*O*-desacetyl-16-des(methoxycarbonyl)vindoline-16-carbonyl]-L-tryptophanate} (11i). Hydrazide **9c** (254 mg, 0.50 mmol) was converted to the corresponding azide which was treated with L-tryptophan methyl ester (0.50 mmol) as discussed in the case of compound **11a**. 168 mg (48%) of **11i** was isolated, mp 148-150 °C. TLC: CH_2Cl_2-MeOH 20:1. R_f = 0.41. $[\alpha]^{29}_D$ +20.2 (*c* 1, CH_2Cl_2). IR (KBr): 3406, 1742, 1669, 1603, 1497, 1458, 1230, 1042, 743 cm^{-1}. ^{1}H NMR (800 MHz, DMSO-d_6) δ 0.53 (m, 1H, H$_\alpha$-15); 0.64 (m, 1H, H$_x$-22); 0.79 (t, *J* = 7.3 Hz, 3H, H$_3$-18); 0.87 (ABq, *J* = 13.4 Hz, *J* = 7.3 Hz, 1H, H$_x$-19); 0.98 (q, 1H, *J* = 5.3 Hz, H$_y$-22); 1.16 (m, 1H, H$_\alpha$-14); 1.29 (ABq, *J* = 13.4 Hz, *J* = 7.3 Hz, 1H, H$_y$-19); 1.99 (dt, *J* = 13.4 Hz, *J* = 8.2 Hz, 1H, H$_\alpha$-6); 2.07 (ddd, *J* = 13.4 Hz, *J* = 10.5 Hz, *J* = 4.3 Hz, 1H, H$_\beta$-6); 2.34 (s, 1H, H-21); 2.43 (m, 1H, H$_\alpha$-5); 2.45 (dd, *J* = 10.7 Hz, *J* = 3.6 Hz, 1H, H$_\alpha$-3); 2.64 (s, 3H, H$_3$-N(1)CH$_3$); 3.06 (td, *J* = 9.2 Hz, *J* = 4.3 Hz, 1H, H$_\beta$-5); 3.18 (d, *J* = 10.7 Hz, 1H, H$_\beta$-3); 3.20 and 3.25 (ABd, *J* = 14.8 Hz, *J* = 5.8 Hz, 2H, H$_2$-1"); 3.26 (s, 1H, H-2); 3.56 (s, 3H, H$_3$-C(3")OOCH$_3$); 3.74 (d, *J* = 7.8 Hz, 1H, C(17)OH); 3.79 (s, 3H, H$_3$-C(11)OCH$_3$); 3.97 (d, *J* = 7.8 Hz, 1H, H-17); 4.66 (dt, 1H, *J* = 7.6 Hz, *J* = 5.8 Hz, H-2"); 6.28 (s, 1H, H-12); 6.99 (ddd, *J* = 7.9 Hz, *J* = 7.0 Hz, *J* = 0.9 Hz, 1H, H-5'); 7.07 (ddd, *J* = 8.1 Hz, *J* = 7.0 Hz, *J* = 0.9 Hz, 1H, H-6'); 7.24 (s, 1H, H-9); 7.26 (d, *J* = 2.5 Hz, 1H, H-2'); 7.34 (dt, *J* = 8.1 Hz, *J* = 0.9 Hz, 1H, H-7'); 7.52 (d, *J* = 7.9 Hz, 1H, H-6'); 7.81 (d, *J* = 7.6 Hz, 1H, H-4"); 8.26 (s, 1H, C(16)OH); 10.93 (d, *J* = 2.5 Hz, 1H, H-1'). ^{13}C NMR (200 MHz, DMSO-d_6) δ 7.8 (C-18); 8.2 (C-22); 11.1 (C-14); 15.0 (C-15); 27.1 (C-1"); 35.7 (C-19); 38.8 (C-1); 40.0 (C-20); 45.0 (C-6); 51.7 (C-5); 51.8 (C-C(3")OO<u>C</u>H$_3$); 51.9 (C-7); 52.4 (C-3, C-2"); 56.1 (C-C(11)O<u>C</u>H$_3$); 70.6 (C-21); 73.1 (C-17); 79.0 (C-16); 83.9 (C-2); 94.0 (C-12); 97.7 (C-10); 108.5 (C-3'); 111.3 (C-7'); 118.3 (C-4'); 118.4 (C-5'); 120.9 (C-6'); 124.2 (C-2'); 126.1 (C-9); 127.2 (C-8); 127.3 (C-3a'); 136.0 (C-7a'); 153.2 (C-13); 155.8 (C-11); 171.8 (C-3"); 172.0 (C-C(16)<u>C</u>ONH). HRMS: 693.22832 ($C_{35}H_{42}O_6N_4Br$; calc. 693.22822). ESI-MS-MS (rel. int. %): 675(100); 633(3); 475(4); 457(9); 447(28); 419(4); 389(28); 266(9).

Methyl {*N*-[10-bromo-14(*S*),15(*R*)-cyclopropano-17-*O*-desacetyl-16-des(methoxycarbonyl)vindoline-16-carbonyl]-D-tryptophanate} (11k). Compound **11k** (29% yield) was prepared analogously with compound **11i**, mp 136-138 °C. TLC: CH_2Cl_2-MeOH 20:1. R_f = 0.59. $[\alpha]^{24}_D$ -13.0 (*c* 1, CH_2Cl_2). IR (KBr): 3405, 2930, 1742, 1671, 1603, 1497, 1457, 1230, 1081, 741 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ 0.47 (m, 1H, H_α-15,); 0.5 (m, 1H, H_x-22); 0.74 (t, *J* = 7.3 Hz, 3H, H_3-18); 0.77 (ABq, *J* = 13.0 Hz, *J* = 7.3 Hz, 1H, H_x-19); 0.89 (q, 1H, *J* = 5.1 Hz, H_y-22); 1.11(m, 1H, H_α-14); 1.21 (ABq, *J* = 13.0 Hz, *J* = 7.3 Hz, 1H, H_y-19); 1.88 (dt, *J* = 13.0 Hz, *J* = 8.2 Hz, 1H, H_α-6); 2.04 (s, 3H, H_3-N(1)CH_3); 2.04 (m, 1H, H_β-6); 2.26 (s, 1H, H-21); 2.43 (m, 2H, H_α-3, H_α-5); 3.07 (td , *J* = 9.3 Hz, *J* = 3.7 Hz, 1H, H_β-5); 3.17 (d, *J* = 10.7 Hz, 1H, H_β-3); 3.20 (m, 2H, H_2-1"); 2.85 (s, 1H, H-2); 3.52 (d, *J* = 7.4 Hz, 1H, C(17)OH); 3.64 (s, 3H, H_3-C(3")OOCH_3); 3.75 (s, 3H, H_3-C(11)OCH_3); 3.86 (d, *J* = 7.4 Hz, 1H, H-17); 4.56 (ddd, 1H, *J* = 8.4 Hz, *J* = 8.0 Hz, *J* = 5.7 Hz, H-2"); 6.17 (s, 1H, H-12); 6.99 (ddd, *J* = 7.8 Hz, *J* = 7.0 Hz, *J* = 1.0 Hz, 1H, H-5'); 7.04 (ddd, *J* = 8.1 Hz, *J* = 7.0 Hz, *J* = 1.2 Hz, 1H, H-6'); 7.19 (s, 1H, H-9); 7.25 (d, *J* = 2.2 Hz, 1H, H-2'); 7.31 (dt, *J* = 8.1 Hz, *J* = 1.0 Hz, 1H, H-7'); 7.59 (d, *J* = 7.8 Hz, 1H, H-6'); 7.91 (d, *J* = 8.4 Hz, 1H, H-4"); 8.08 (s, 1H, C(16)OH); 10.87 (d, *J* = 2.2 Hz, 1H, H-1'). ^{13}C NMR (125 MHz, DMSO-d_6) δ 7.6 (C-18); 8.1 (C-22); 10.9 (C-14); 15.0 (C-15); 28.0 (C-1"); 35.7 (C-19); 37.8 (C-1); 39.9 (C-20); 44.7 (C-6); 51.8 (C-7); 51.9 (C-5); 52.1 (C-C(3")OO<u>C</u>H_3); 52.4 (C-3); 53.0 (C-2"); 56.1 (C-C(11)O<u>C</u>H_3); 70.9 (C-21); 72.4 (C-17); 78.9 (C-16); 83.6 (C-2); 93.9 (C-12); 97.6 (C-10); 109.4 (C-3'); 111.4 (C-7'); 118.2 (C-4'); 118.3 (C-5'); 120.9 (C-6'); 124.1 (C-2'); 126.2 (C-9); 127.2 (C-8); 127.3 (C-3a'); 136.0 (C-7a'); 152.9 (C-13); 155.6 (C-11); 171.7 (C-3"); 172.1 (C-C(16)<u>C</u>ONH). HRMS: 693.22822 ($C_{35}H_{42}O_6N_4Br$; calc. 693.22822). ESI-MS-MS (rel. int. %): 675(100); 633(3); 475(3); 457(8); 447(25); 419(3); 389(22); 266(8).

Methyl {*N*-[17-*O*-desacetyl-16-des(methoxycarbonyl)vindoline-16-carbonyl]-L-tryptophanate} (11b). Methyl {*N*-[10-bromo-17-*O*-desacetyl-16-des(methoxycarbonyl)vindoline-16-carbonyl]-L-tryptophanate} (**11a**) (126 mg, 0.19 mmol) was dissolved in MeOH (5 mL). In the presence of palladium on charcoal (150 mg) at 10 °C sodium borohydride (424 mg, 11.2 mmol) was added to the reaction mixture under argon with stirring. After 30 min a few drops of acetic acid were added, the catalyst was filtered and the pH of the solution was adjusted to 9 with aqueous ammonia. The mixture was extracted with CH_2Cl_2 (3 x 20 mL), the combined organic phase was dried with magnesium sulfate and the solvent was evaporated under vacuum. The product (**11b**) was isolated by preparative thin-layer chromatography (CH_2Cl_2-MeOH 20:1) in 66 mg (59%) yield. TLC: CH_2Cl_2-MeOH 20:1. R_f = 0.65. IR (KBr): 2930, 1736, 1656, 1613, 1500, 1434, 1167, 1068, 815, 738 cm^{-1}. ^1H NMR (800 MHz, CD_3CN + D_2O 1:1) δ 0.61 (t, *J* = 7.4 Hz, 3H, H_3-18); 0.93 (ABq, *J* = 13.8 Hz, *J* = 7.4 Hz, 1H, H_x-19); 1.21 (ABq, *J* = 13.8 Hz, *J* = 7.4 Hz, 1H, H_y-19); 2.08-2.16 (m, 1H, H_2-6); 2.54 (td, *J* = 10.0 Hz, *J* = 7.8 Hz, 1H, H_α-5); 2.61 (s, 3H, H_3-N(1)CH_3);

2.71 (s, 1H, H-21); 2.84 (ddd, *J* = 16.2 Hz, *J* = 2.4 Hz, *J* = 1.6 Hz, 1H, H_{α}-3,); 3.25 (ABd, *J* = 14.9 Hz, *J* = 5.8 Hz, 2H, H_x-1"); 3.28 (td , *J* = 10.0 Hz, *J* = 8.3 Hz, *J* = 5.4 Hz, 1H, H_{β}-5); 3.34 (ABd, *J* = 14.9 Hz, *J* = 5.8 Hz, 2H, H_y-1"); 3.40 (s, 1H, H-2); 3.43 (ddd, *J* = 16.2 Hz, *J* = 5.0 Hz, *J* = 1.5 Hz, 1H, H_{β}-3); 3.62 (s, 3H, H_3-C(3")OOCH_3); 3.73 (s, 3H, H_3-C(11)OCH_3); 3.91 (s, 1H, H-17); 4.77 (t, 1H, *J* = 5.8 Hz, H-2"); 5.56 (ddd, *J* = 10.2 Hz, *J* = 2.4 Hz, *J* = 1.5 Hz, 1H, H-15); 5.87 (ddd, *J* = 10.2 Hz, *J* = 5.0 Hz, *J* = 1.6 Hz, 1H, H-14); 6.08 (d, *J* = 2.3 Hz, 1H, H-12); 6.26 (dd, *J* = 8.2, *J* = 2.3 Hz, 1H, H-10); 6.95 (d, *J* = 8.2 Hz, 1H, H-9); 7.05 (ddd, *J* = 7.9 Hz, *J* = 7.0 Hz, *J* = 0.9 Hz, 1H, H-5'); 7.14 (ddd, *J* = 8.0 Hz, *J* = 7.0 Hz, *J* = 0.9 Hz, 1H, H-6'); 7.19 (s, 1H, H-2'); 7.42 (dt, *J* = 8.0 Hz, *J* = 0.9 Hz, 1H, H-7'); 7.54 (dt, *J* = 7.8 Hz, *J* = 0.9 Hz, 1H, H-6'). ^{13}C NMR (200 MHz, CD_3CN+D_2O 1:1) δ 7.7 (C-18); 27.8 (C-1"); 32.8 (C-19); 39.3 (C-1); 43.3 (C-20); 45.0 (C-6); 51.2 (C-3, C-5); 52.8 (C-C(3")OO$\underline{C}H_3$); 53.2 (C-2"); 53.4 (C-7); 55.7 (C-C(11)O$\underline{C}H_3$); 67.9 (C-21); 74.3 (C-17); 80.8 (C-16); 84.3 (C-2); 95.8 (C-12); 104.5 (C-10); 109.3 (C-3'); 112.2 (C-7'); 119.0 (C-4'); 119.6 (C-5'); 122.3 (C-6'); 123.8 (C-9); 124.8 (C-2'); 125.1 (C-14); 126.2 (C-8); 127.8 (C-3a'); 130.8 (C-15); 136.9 (C-7a'); 154.7 (C-13); 161.5 (C-11); 173.5 (C-3"); 173.9 (C-C(16)$\underline{C}$ONH). HRMS: 601.30174 ($C_{34}H_{41}O_6N_4$; calc. 601.30206). ESI-MS-MS (rel. int. %): 583(100); 541(4); 408(6); 365(85); 355(63); 337(4); 295(8); 188(23).

Methyl {*N*-[17-*O*-desacetyl-16-des(methoxycarbonyl)vindoline-16-carbonyl]-D-tryptophanate} (11d). The bromo derivative **11c** (165 mg, 0.243 mmol) was reduced analogously as described in the case of **11b**. Compound **11d** was isolated by preparative thin-layer chromatography (CH_2Cl_2-MeOH 10:1) in 94 mg (70%) yield. TLC: CH_2Cl_2-MeOH 10:1. R_f = 0.70. $[\alpha]^{25}_D$ +7.4 (*c* 1, $CHCl_3$). IR (KBr): 3403, 2925, 1736, 1666, 1617, 1500, 1225, 796 cm^{-1}. ^{1}H-NMR (800 MHz, DMSO-d_6) δ 0.53 (t, *J* = 7.4 Hz, 3H, H_3-18); 0.80 (ABq, *J* = 13.8 Hz, *J* = 7.4 Hz, 1H, H_x-19); 1.22 (ABq, *J* = 13.8 Hz, *J* = 7.4 Hz, 1H, H_y-19); 1.97-2.07 (m, 2H, H_2-6); 2.58 (s, 1H, H-21); 2.51-2.55 (m, 1H, H_{α}-5); 2.78 (dt, *J* = 16.3 Hz, *J* = 2.3 Hz, 1H, H_{α}-3); 2.07 (s, 3H, H_3-N(1)CH_3); 3.24 (td , *J* = 9.2 Hz, *J* = 4.2 Hz, 1H, H_{β}-5); 3.35 (dd, *J* = 16.3 Hz, *J* = 5.1 Hz, 1H, H_{β}-3); 3.15-3.22 (m, 2H, H_2-1"); 3.01 (s, 1H, H-2); 3.61 (s, 3H, H_3-C(3")OOCH_3); 3.78 (d, *J* = 7.1 Hz, 1H, C(17)OH); 3.65 (s, 3H, H_3- C(11)OCH_3); 3.95 (d, *J* = 7.8 Hz, 1H, H-17); 4.58 (td, 1H, *J* = 8.4 Hz, *J* = 5.8 Hz, H-2"); 5.49 (ddd, *J* = 10.1 Hz, *J* = 2.3 Hz, *J* = 1.5 Hz, 1H, H-15); 5.73 (ddd, *J* = 10.1 Hz, *J* = 5.1 Hz, *J* = 1.5 Hz, 1H, H-14); 5.92 (d, *J* = 2.3 Hz, 1H, H-12); 6.15 (dd, *J* = 8.1 Hz, *J* = 2.3 Hz, 1H, H-9); 7.01 (ddd, *J* = 7.8 Hz, *J* = 7.0 Hz, *J* = 0.9 Hz, 1H, H-5'); 7.06 (ddd, *J* = 8.0 Hz, *J* = 7.0 Hz, *J* = 1.1 Hz, 1H, H-6'); 6.91 (d, *J* = 8.1 Hz, 1H, H-9); 7.24 (d, *J* = 2.3 Hz, 1H, H-2'); 7.32 (dt, *J* = 8.0 Hz, *J* = 0.8 Hz, 1H, H-7'); 7.57 (d, *J* = 7.8 Hz, 1H, H-6'); 7.85 (d, *J* = 8.4 Hz, 1H, H-4"); 8.94 (s, 1H, C(16)OH); 10.90 (d, *J* = 2.3 Hz, 1H, H-1'). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 7.6 (C-18); 27.5 (C-1"); 31.8 (C-19); 37.5 (C-1); 42.3 (C-20); 44.6 (C-6); 50.4 (C-3); 50.7 (C-5); 51.9 (C-C(3")OO$\underline{C}H_3$); 52.1 (C-7); 52.8 (C-2"); 55.0 (C- C(11)O$\underline{C}H_3$); 67.4 (C-21); 72.7 (C-17); 79.7 (C-16); 83.5 (C-2); 94.3 (C-12); 103.2 (C-10); 109.3

(C-3'); 111.4 (C-7'); 118.2 (C-4'); 118.4 (C-5'); 121.0 (C-6'); 122.5 (C-14); 124.0 (C-2'); 122.7 (C-9); 125.4 (C-8); 127.3 (C-3a'); 131.6 (C-15); 136.1 (C-7a'); 153.5 (C-13); 160.3 (C-11); 171.1 (C-3"); 171.2 (C- C(16)CONH). HRMS: 601.30225 ($C_{34}H_{41}O_6N_4$; calc. 601.30206). ESI-MS-MS (rel. int. %): 583(100); 541(4); 408(8); 365(65); 355(55); 337(3); 295(8); 188(25).

Methyl {*N*-[14,15-dihydro-17-*O*-desacetyl-16-des(methoxycarbonyl)vindoline-16-carbonyl]-L-trypto-phanate} (11f). The bromo derivative **11e** (226 mg, 0.33 mmol) was reduced analogously as described in the case of **11b**. Compound **11f** was isolated by preparative thin-layer chromatography (CH_2Cl_2-MeOH 10:1) in 102 mg (51%) yield. TLC: CH_2Cl_2-MeOH 10:1. R_f = 0.70. IR (KBr): 3407, 2948, 1742, 1669, 1500, 1458, 1254, 1170, 742 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ 0.56 (t, J = 7.3 Hz, 3H, H_3-18); 0.60 (ABq, J = 13.3 Hz, J = 7.3 Hz, 1H, H_x-19); 0.96 (ABq, J = 13.3 Hz, J = 7.3 Hz, 1H, H_y-19); 0.74 (ddd, J = 13.4 Hz, J = 12.9 Hz, J = 3.8 Hz, 1H, H_{ax}-15); 1.49 (d br, J = 12.9 Hz, 1H, H_{eq}-14); 1.89 (qt, J = 12.9 Hz, J = 3.8 Hz, 1H, H_{ax}-14); 1.97 (m, 1H, $H_α$-5); 1.99 (s, 1H, H-21); 2.07 (dt, J = 13.4, J = 3.8 Hz, 1H, H_{eq}-15); 2.10-2.20 (m, 1H, H_2-6); 2.58 (s, 3H, H_3-N(1)CH_3); 2.37 (m, 1H, $H_α$-3,); 3.04 (d, J = 9.8 Hz, 1H, $H_β$-5); 3.22 (ABd, J = 14.7 Hz, J = 6.0 Hz, 2H, H_x-1"); 3.23 (ABd, J = 14.7 Hz, J = 6.0 Hz, 2H, H_y-1"); 3.27 (s, 1H, H-2); 3.10 (m, 1H, $H_β$-3); 3.55 (s, 3H, H_3-C(3")OOCH_3); 3.68 (s, 3H, H_3-C(11)OCH_3); 4.25 (d, J = 7.3 Hz, 1H, H-17); 4.34 (d, J = 7.3 Hz, 1H, H-C(17)OH); 4.67 (dt, 1H, J = 7.3 Hz, J = 6.0 Hz, H-2"); 6.12 (d, J = 2.3 Hz, 1H, H-12); 6.24 (dd, J = 8.2 Hz, J = 2.3 Hz, 1H, H-10); 6.93 (d, J = 8.2 Hz, 1H, H-9); 6.98 (ddd, J = 7.9 Hz, J = 7.1 Hz, J = 0.9 Hz, 1H, H-5'); 7.07 (ddd, J = 8.1 Hz, J = 7.1, J = 1.0 Hz, 1H, H-6'); 7.26 (d, J = 2.3 Hz, 1H, H-2'); 7.33 (d, J = 8.1 Hz, 1H, H-7'); 7.51 (d, J = 7.9 Hz, 1H, H-6'); 7.86 (d, J = 7.3 Hz, 1H, H-4"); 9.40 (s, 1H, C(16)OH); 10.91 (d, J = 2.3 Hz, 1H, H-1'). ^{13}C NMR (125 MHz, DMSO-d_6) δ 7.6 (C-18); 22.9 (C-14); 27.1 (C-1"); 29.9 (C-15); 34.6 (C-19); 39.4 (C-1); 39.8 (C-20); 45.1 (C-6); 51.4 (C-5); 51.8 (C-C(3")OO$\underline{C}H_3$); 52.1 (C-3); 52.3 (C-7); 52.6 (C-2"); 55.0 (C-C(11)O$\underline{C}H_3$); 70.3 (C-17); 75.5 (C-21); 79.2 (C-16); 85.1 (C-2); 95.6 (C-12); 104.2 (C-10); 108.6 (C-3'); 111.4 (C-7'); 118.2 (C-4'); 118.4 (C-5'); 120.9 (C-6'); 122.9 (C-9); 124.1 (C-2'); 127.1 (C-3a'); 127.6 (C-8); 136.0 (C-7a'); 154.7 (C-13); 160.2 (C-11); 172.1 (C-3"); 173.5 (C-C(16)CONH). HRMS: 603.31743 ($C_{34}H_{43}O_6N_4$; calc. 603.31771). ESI-MS-MS (rel. int. %): 585(100); 543(2); 410(3); 385(2); 367(28); 357(97); 339(4); 297(1); 188(16).

Methyl {*N*-[14,15-dihydro-17-*O*-desacetyl-16-des(methoxycarbonyl)vindoline-16-carbonyl]-D-trypto phanate} (11h). The bromo derivative **11g** (570 mg, 0.836 mmol) was reduced analogously as described in the case of **11b**. Compound **11h** was isolated by preparative thin-layer chromatography (CH_2Cl_2-MeOH 10:1) in 234 mg (46%) yield. TLC: CH_2Cl_2-MeOH 10:1. R_f = 0.8. $[α]^{25}_D$ +53.4 (*c* 1, $CHCl_3$). IR (KBr): 3397, 2950, 1740, 1667, 1616, 1500, 1254, 1061, 969, 743 cm^{-1}. ^1H-NMR (500 MHz, DMSO-d_6) δ 0.52 (t, J = 7.4 Hz, 3H, H_3-18); 0.56 (ABq, J = 13.4 Hz, J = 7.4 Hz, 1H, H_x-19); 0.68 (td, J

= 14.0 Hz, *J* = 4.1 Hz, 1H, H_{ax}-15); 0.88 (ABq, *J* = 13.4 Hz, *J* = 7.4 Hz, 1H, H_y-19); 1.44 (d, *J* = 12.8 Hz, 1H, H_{eq}-14); 1.81 (qt, *J* = 12.8 Hz, *J* = 3.6 Hz, 1H, H_{ax}-14); 1.89-1.98 (m, 1H, H_{ax}-3) 1.92 (s, 1H, H-21); 1.98-2.15 (m, 2H, H_2-6); 2.02 (d, 1H, *J* =14.0 Hz, H_{eq}-15); 2.08 (s, 3H, H_3-N(1)CH_3); 2.36 (q, *J* = 9.1 Hz, 1H, H_α-5); 2.96 (s, 1H, H-2); 3.00 (d, *J* = 10.2 Hz, 1H, H_{eq}-3); 3.09 (td, *J* = 8.7 Hz, *J* = 1.6 Hz, 1H, H_β-5); 3.14-3.24 (m, 2H, H_2-1''); 3.62 (s, 3H, H_3-C(3'')OOCH_3); 3.65 (s, 3H, H_3-C(11)OCH_3); 4.14 (d, *J* = 7.5 Hz, 1H, H-17); 4.19 (d, *J* = 7.5 Hz, 1H, C(17)OH); 4.62 (ddd, 1H, *J* = 8.4 Hz, *J* = 7.4 Hz, *J* = 6.0 Hz, H-2''); 6.01 (d, 1H, *J* = 2.3 Hz, H-12); 6.21 (dd, 1H, *J* = 8.3 Hz, *J* = 2.3 Hz, H-10); 6.89 (d, 1H, *J* = 8.3 Hz, H-9); 6.99 (ddd, *J* = 7.9 Hz, *J* = 7.2 Hz, *J* = 0.9 Hz, 1H, H-5'); 7.05 (ddd, *J* = 8.1 Hz, *J* = 7.2 Hz, *J* = 1.1 Hz, 1H, H-6'); 7.23 (d, *J* = 2.3 Hz, 1H, H-2'); 7.31 (dt, *J* = 8.1 Hz, *J* = 0.9 Hz, 1H, H-7'); 7.56 (d, *J* = 7.9 Hz, 1H, H-6'); 7.87 (d, *J* = 8.4 Hz, 1H, H-4''); 9.32 (s, 1H, C(16)OH); 10.87 (d, *J* = 2.3 Hz, 1H, H-1'). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 7.6 (C-18); 22.8 (C-14); 27.6 (C-1''); 29.9 (C-15); 34.6 (C-19); 38.6 (C-1); 39.6 (C-20); 45.0 (C-6); 51.4 (C-3); 51.9 (C-5); 52.1 (C-C(3'')OO<u>C</u>H_3); 52.2 (C-7); 52.9 (C-2''); 55.0 (C- C(11)O<u>C</u>H_3); 69.8 (C-17); 75.6 (C-21); 79.1 (C-16); 84.9 (C-2); 95.6 (C-12); 104.2 (C-10); 109.2 (C-3'); 111.4 (C-7'); 118.1 (C-4'); 118.4 (C-5'); 121.0 (C-6'); 124.1 (C-2'); 122.9 (C-9); 127.2 (C-3a'); 127.5 (C-8); 136.1 (C-7a'); 154.6 (C-13); 160.1 (C-11); 172.2 (C-3''); 173.4 (C-C(16)<u>C</u>ONH). HRMS: 603.31753 ($C_{34}H_{43}O_6N_4$; calc. 603.31771). ESI-MS-MS (rel. int. %): 585(100); 543(2); 410(4); 385(2); 367(29); 357(99); 339(5); 297(1); 188(19).

Methyl {*N*-[14(*S*),15(*R*)-cyclopropano-17-*O*-desacetyl-16-des(methoxycarbonyl)vindoline-16-carbonyl]-L-tryptophanate} (11j). The bromo derivative **11i** (390 mg, 0.563 mmol) was reduced analogously as described in the case of **11b**. Compound **11j** was isolated by preparative thin-layer chromatography (CH_2Cl_2-MeOH 10:1) in 225 mg (65%) yield, mp 117-119 °C. TLC: CH_2Cl_2-MeOH 10:1. R_f = 0.67. $[\alpha]^{22}_D$ +33.03 (*c* 1, $CHCl_3$). IR (KBr): 3631, 3407, 3011, 2808, 1743, 1666, 1501, 1169, 793, 741, 587 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.53 (1H, m, H-15); 0.64 (1H, m, H-22); 0.77 (3H, t, *J* = 7.3 Hz, H-18); 0.85 (1H, m, H-19); 0.99 (1H, m, H-22); 1.16 (1H, m, H-14); 1.28 (1H, m, H-19); 2.02 (2H, m, H-6); 2.30 (1H, s, H-21); 2.38 (1H, m, H-5); 2.41 (1H, m, H-3); 2.60 (3H, s, 1-CH_3); 3.09 (1H, td, H-5); 3.21 (1H, m, H-3); 3.22 (1H, s, H-2); 3.22 (2H, m, H-1''); 3.56 (3H, s, 3''-OMe); 3.68 (3H, s, 11-OMe); 3.72 (1H, d, *J* = 7.9 Hz, 17-OH); 3.99 (1H, d, *J* = 7.9 Hz, H-17); 4.66 (1H, dt, J_x =7.6 Hz, J_y = 5.8 Hz, H-2''); 6.06 (1H, d, *J* = 2.2 Hz, H-12); 6.20 (1H, dd, J_x = 8.2 Hz, J_y = 2.2 Hz, H-10); 6.93 (1H, d, *J* = 8.2 Hz, H-9); 6.98 (1H, ddd, J_x = 7.9 Hz, J_y = 7.0 Hz, J_z = 0.7 Hz, H-5'); 7.07 (1H, ddd, J_x = 8.1 Hz, J_y = 7.0 Hz, J_z = 1.0 Hz, H-6'); 7.25 (1H, d, *J* = 2.1 Hz, H-2'); 7.34 (1H, dt, J_x = 8.1 Hz, J_y = 0.7 Hz, H-7'); 7.51 (1H, d, *J* = 7.9 Hz, H-4'); 7.81 (1H, d, *J* = 7.6 Hz, 4''-NH); 8.19 (1H, sbr, 16-OH); 10.93 (1H, br, 1'-NH) ppm. ^{13}C NMR (125 MHz, DMSO-d_6) δ 7.9 (C-18); 8.2 (C-22); 11.1 (C-14); 15.1 (C-15); 27.2 (C-1''); 35.6 (C-19); 38.8 (1-Me); 40.0 (C-20); 45.2 (C-6); 51.8 (3''-OMe); 51.9 (C-7); 52.0 (C-5); 52.4 (C-2'');

52.6 (C-3); 55.0 (11-OMe); 70.9 (C-21); 73.1 (C-17); 79.2 (C-16); 84.0 (C-2); 94.7 (C-12); 103.6 (C-10); 108.5 (C-3'); 111.3 (C-7'); 118.3 (C-4'); 118.4 (C-5'); 120.9 (C-6'); 122.7 (C-9); 124.2 (C-2'); 126.2 (C-8); 127.2 (C-3a'); 136.0 (C-7a'); 153.7 (C-13); 160.3 (C-11); 171.9 (16-CO); 172.0 (C-3'') ppm. HRMS: 615.31741 ($C_{35}H_{43}O_6N_4$; calc. 615.31771). ESI-MS-MS (rel. int. %): 597(100); 555(3); 422(6); 397(4); 379(14); 369(44); 341(5); 311(34); 188(2).

Methyl {*N*-[14(*S*),15(*R*)-cyclopropano-17-*O*-desacetyl-16-des(methoxycarbonyl)vindoline-16-carbonyl]-D-tryptophanate} (11l). The bromo derivative **11k** (390 mg, 0.563 mmol) was reduced analogously as described in the case of **11b**. Compounds **11l** was isolated by preparative thin-layer chromatography (CH_2Cl_2-MeOH 10:1) in 190 mg (55%) yield, mp 75 °C. TLC: CH_2Cl_2-MeOH 10:1. R_f = 0.85. $[\alpha]^{22}_D$ -8.81 (*c* 1, $CHCl_3$). IR (KBr): 3565, 3405, 2958, 1743, 1615, 1501, 1228, 1079, 742, 540 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ 0.48 (1H, m, H-15); 0.54 (1H, m, H-22); 0.71 (3H, t, J = 7.1 Hz, H-18); 0.77 (1H, m, H-19); 0.90 (1H, m, H-22); 1.11 (1H, m, H-14); 1.20 (1H, m, H-19); 1.89 (1H, m, H-6); 2.02 (1H, m, H-6); 2.20 (1H, s, H-21); 2.37 (1H, m, H-5); 2.37 (1H, m, H-3); 2.03 (3H, s, 1-CH_3); 3.10 (1H, td, J_x = 9.4 Hz, J_y = 3.8 Hz, H-5); 3.20 (1H, m, H-3); 2.84 (1H, s, H-2); 3.20 (2H, m, H-1''); 3.64 (3H, s, 3''-OMe); 3.65 (3H, s, 11-OMe); 3.49 (1H, d, 17-OH); 3.89 (1H, d, H-17); 4.58 (1H, ddd, J_x = 8.4 Hz, J_y = 8.0 Hz, J_z = 5.8 Hz, H-2''); 5.95 (1H, d, H-12); 6.17 (1H, dd, J_x = 8.2 Hz, J_y = 2.2 Hz, H-10); 6.89 (1H, d, J = 8.2 Hz, H-9); 6.99 (1H, ddd, J_x = 7.9 Hz, J_y = 7.0 Hz, J_z = 0.8 Hz, H-5'); 7.04 (1H, ddd, J_x = 7.9 Hz, J_y = 7.0 Hz, J_z = 1.2 Hz, H-6'); 7.24 (1H, d, J = 2.3 Hz, H-2'); 7.31 (1H, dt, J_x = 7.9 Hz, J_y = 0.8 Hz, H-7'); 7.59 (1H, d, J = 7.9 Hz, H-4'); 7.88 (1H, d, J = 8.4 Hz, 4''-NH); 8.01 (1H, s, 16-OH); 10.88 (1H, dbr, 1'-NH) ppm. ^{13}C NMR (125 MHz, DMSO-d_6) δ 7.9 (C-18); 8.3 (C-22); 11.0 (C-14); 15.1 (C-15); 27.4 (C-1''); 35.6 (C-19); 37.9 (1-Me); 39.9 (C-20); 45.2 (C-6); 51.7 (C-7); 51.9 (3''-OMe); 52.2 (C-5); 52.7 (C-3); 53.0 (C-2''); 55.0 (11-OMe); 71.3 (C-21); 72.6 (C-17); 79.1 (C-16); 83.8 (C-2); 94.7 (C-12); 103.7 (C-10); 109.4 (C-3'); 111.4 (C-7'); 118.2 (C-4'); 118.4 (C-5'); 120.9 (C-6'); 122.7 (C-9); 124.1 (C-2'); 126.3 (C-8); 127.3 (C-3a'); 136.1 (C-7a'); 153.5 (C-13); 160.2 (C-11); 172.0 (16-CO); 172.2 (C-3'') ppm. HRMS: 615.31714 ($C_{35}H_{43}O_6N_4$; calc. 615.31771). ESI-MS-MS (rel. int. %): 597(100); 555(4); 422(11); 397(3); 379(16); 369(52); 341(6); 311(31); 188(16).

Cells

HL-60 human leukemia cells (ATCC: CCL-240) were cultured in RPMI-1640 medium supplemented with 10% FCS (fetal calf serum, Sigma Ltd.), 2 mM L-glutamine and 160 μg/mL gentamycin. Cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2.

Analysis of *in vitro* cytostatic effect

The *in vitro* cytostatic effect of the compounds was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-

diphenyltetrazolium bromide-assay (MTT-assay).[7] For the experiment, 5×10^3 cells per well were plated on 96-well plates. After 24 h incubation at 37 °C, cells were treated for 3 h with the compound dissolved in serum-free medium. All the compounds tested were used in the 2.6×10^{-4}-10^2 μM concentration range. Cells treated with serum-free medium for 3 h were used as negative control. To determine the cytostatic effect, cells were washed twice with serum-free medium and cultured for further 72 h in serum containing medium. On day 4, MTT-assay was carried out MTT solution was added to each well (final concentration: 367 μg/mL). After incubation for 3.5 h, purple crystals were formed by indicating the activity of mitochondrial dehydrogenase enzyme of living cells. Cells were centrifuged for 5 min at 863 g and the supernatant was removed. Crystals were dissolved in DMSO and the optical density (OD) of the samples was measured at λ = 540 and 620 nm using an ELISA Reader (Labsystems MS reader, Finland). OD_{620} was substracted from OD_{540}. The percent of cytostasis was calculated using the following equation:

$$\text{Cytostasis \%} = [1-(OD_{treated}/OD_{control})]\times 100$$

Where $OD_{treated}$ and $OD_{control}$ correspond to the optical densities of treated and control cells, respectively. Cytostasis % was plotted as a function of concentration, fitted to a sigmoidal curve, and the IC_{50} value was determined on the basis of this curve.

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