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Synthesis and structure–activity relationships study of novel anti-tumor carbamate anhydrovinblastine analogues

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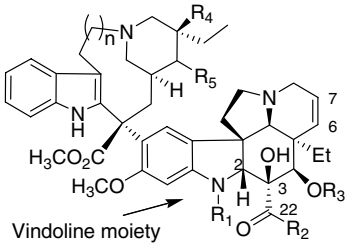
Abstract—A series of 3-demethoxycarbonyl-3-carbamate methyl anhydrovinblastine derivatives (compounds **8b**–**32b**) were designed, synthesized, and evaluated for their inhibition activities against human non-small cell lung cancer cell line (A549) and a human cervix epithelial adenocarcinoma cell line (HeLa). The structure–activity relationships of this new series are described in this paper. Cytotoxicity data revealed that the size of substituents and substitution position had important influence on cytotoxic activity. On two cell lines, compounds (**8b** and **30b**) had more potent cytotoxic activity than the lead compound (**1e**, AVLB). The preliminary antitumor studies of **8b** in vivo showed that it might be promising for the development of new antitumor agents.

1. Introduction

Vinblastine (**1a**) and vincristine (**1b**) have been recognized as clinically important antitumor agents because of their strong antineoplastic activity against a wide spectrum of human tumors for more than 40 years (Fig. 1).[1] These two alkaloids, although structurally almost identical, differ markedly in the type of tumors that they affect and in their toxicological properties.[2] Therefore, it is desirable to identify structural modification that would yield more effective and less toxic analogues. Most of research has mainly focused on the semi-synthesis or total synthesis of vinblastine analogues from the two parts of the dimeric structure, the vindoline and velbenamine portions, by carbon skeleton modification and functional group transformation.[3,4] To date, these efforts have led to two approved drugs, vindesine (**1c**)[5] and vinorelbine (**1d**, NVB)[6], and more than a dozen candidates in clinical evaluation, such as anhydrovinblastine (**1e**, AVLB)[7]. Compared with vincristine and NVB, AVLB has been shown to decrease toxicity to inhibit the growth of lymphomas and certain solid tumors, specifically cervical, lung, breast, and colon cancer.[8] In order to investigate structural requirement of cytotoxic activity of AVLB, we were interested in exploiting the 22-position in the vindoline moiety of anhydrovinblastine as part of an extended SAR study. In this report, a new series of 3-demethoxycarbonyl-3-carbamate methyl anhydrovinblastine derivatives were synthesized and tested for cytotoxic activity in vitro against A549 and HeLa cell lines.



	R_1	R_2	R_3	R_4	R_5	n
1a	Me	OMe	COMe	OH	H	1
1b	CHO	OMe	COMe	OH	H	1
1c	Me	NH_2	OH	OH	H	1
1d	Me	OMe	COMe	=		0
1e	Me	OMe	COMe	=		1

Figure 1. Vinca alkaloids structure.

Keywords: Antitumor; Anhydrovinblastine derivatives; Carbamate; Cytotoxicity.
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2. Chemistry

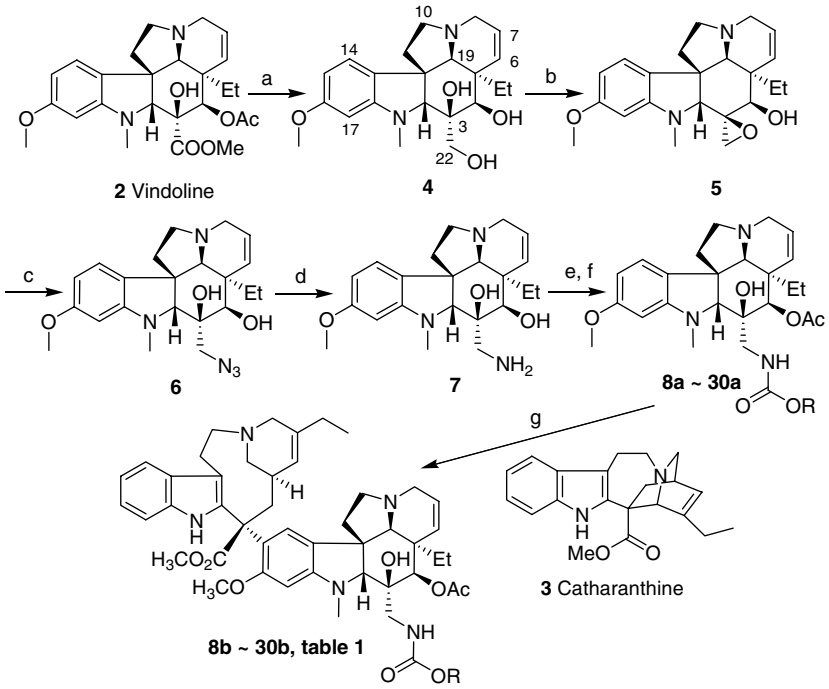
Most of semi-synthesized vinblastine derivatives reported previously were prepared by functional group transformation from vinblastine. Due to its complex structural features and chemical instability, reactions used in functional group transformations usually needed mild conditions. Under these conditions our designed carbamate analogues could not be afforded by anhydrovinblastine as starting material. As we know, coupling of the two biosynthetical precursor monomers, vindoline (**2**) and catharanthine (**3**), can form 3′,4′-anhydrovinblastine by the modified Polonovski-Potier reaction.[9] Vindoline[10], the major alkaloid in whole plant of *Catharanthus roseus*, was easier to be obtained and modified than the dimer vinca alkaloids. Thus, our synthetical strategy was to first prepare the amine (**7**) and its carbamate derivatives (**8a**–**32a**) from vindoline (**2**), and then couple with **3** to prepare the targeted derivatives (**8b**–**32b**).

As shown in Scheme 1, the important intermediate amine (**7**) was prepared in following four steps. Reduction of **2** by $LiAlH_4$ could provide the triol **4** (98%), which was treated with 4-toluene sulfonyl chloride in THF including 50% sodium hydroxide solution at 80 °C to result in the formation of 3β,22-epoxide (**5**) in 80% yield. The ring-opening reaction of the epoxide (**5**) afforded **6** in excellent yield (91%) with azide in the presence of NH_4Cl, which was easy to be purified.[11] Treatment of **6** with $LiAlH_4$ at 0 °C provided **7** in 70% total yield from vindoline. Then **7** was treated with different chloroformats in the presence of diisopropylethylamine (DIPEA) in dichloromethane (DCM) and then acetylated for 4-OH to furnish compounds **8a**–**30a** (80%), which were subsequently coupled with **3** to produce the carbamate anhydrovinblastine derivatives (**8b**–**30b**). Chloroformates could be obtained from commercial suppliers or be synthesized by different alcohols with bis(trichloromethyl)carbonate in the presence of DIPEA in DCM.[12] Synthesis of inner carbamate compound **31b** and reverse carbamate compound **32b** was also achieved for better complement of the SARs according to Scheme 2. Compound **21a** underwent intramolecular attack reaction with sodium hydride to afford **31a**, and then coupled with **3** to produce compound **31b**. For synthesis of compound **32a**, **32** was first prepared by isobutyl amine and carbonyldiimidazole (CDI) in DCM with good yield.[13] Subsequently, the triol **4** was treated with **32** in the presence of sodium hydride in THF and then acetylated to yield compound **32a**, which was coupled with **3** to furnish the compound **32b**.

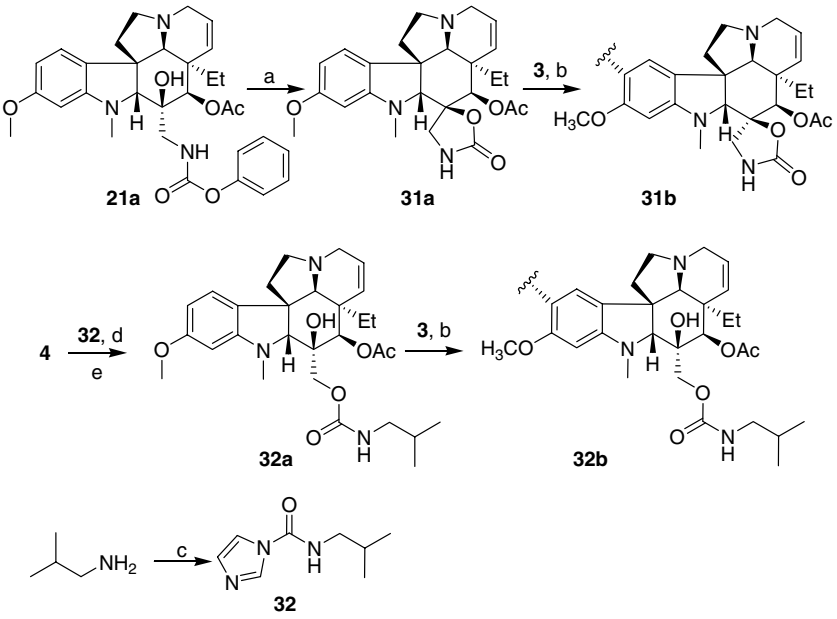
3. Result and discussion

Total 25 dimeric compounds (**8b**–**32b**) were tested for their cytotoxicity against human non-small cell lung cancer cell line (A549) and a human cervix epithelial adenocarcinoma cell line (HeLa) by sulforhodamine B (SRB) assay[14] employing AVLB as positive control. The IC_{50} values for the inhibition of proliferation are shown in Tables 1 and 2. The details for bioassay procedures are described in Section 5.

The methyl carbamate derivative (**8b**) initially synthesized showed more potent cytotoxic activity (IC_{50} values on A549 and HeLa cell lines are 38 and 9 nM, respectively) than positive control AVLB (49 nM and



Scheme 1. Reagents and conditions: (a) $LiAlH_4$, THF, 0 °C–rt, 4 h, 98%; (b) i—THF, 50%NaOH–H_2O, $Bu_4N^+I^-$, 50 °C, 30 min; ii—4-toluene sulfonyl chloride, 80 °C, 3 h, 80%; (c) NaN_3, CH_3OH/H_2O (8:1), 90 °C, 24 h, 91%; (d) $LiAlH_4$, THF, 0 °C–rt, 4 h, 98%; (e) ClCOOR, DIPEA, DCM; (f) Ac_2O, pyridine; (g) i—$FeCl_3$, HCl–H_2O buffer (pH 2), 8 h; ii—$NaBH_4$, NH_4OH, 0 °C, 15 min.



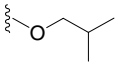
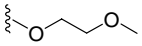
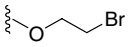
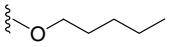
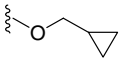
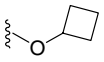
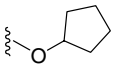
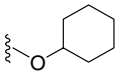
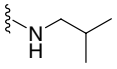
Scheme 2. Reagents and conditions: (a) NaH, THF, 0 °C–rt, 2 h, 40%; (b) i—$FeCl_3$, HCl–H_2O buffer (pH 2), 8 h; ii—$NaBH_4$, NH_4OH, 0 °C, 15 min; (c) CDI, DCM, 0 °C–rt, 24 h; (d) NaH, THF, 0 °C–rt, 4 h; (e) Ac_2O, pyridine.

26 nM). These results indicated that it was suitable for improving the cytotoxicity to introduce the carbamate group at C_{22} of vindoline moiety. So, our present work focused on exploring variants of the substituted alkyl and aromatic groups in **8b** and studying their effects on cytotoxicity. From Table 1, we observed a significant loss of cytotoxicity against HeLa cell with increasing the size of alkyl groups by one additional carbon (**8b**, **9b**, **10b**, **11b**, and **15b**). These results also could be obtained from the aliphatic ring substitution (**17b**, **18b**, and **19b**). Reverse carbamate was found to be much less active (**32b** vs **12b**), which showed the C_{22}–NH was favorable to its cytotoxic activity. 2-Methoxyl ethyl carbamate (**13b**) had comparable activity to the ethyl carbamate (**9b**), while 2-bromoethyl group (**14b**) was much less active (IC_{50} = 14 nM for **13b** vs 16 nM for **9b** vs 30 nM for **14b**). Cyclopropylmethyl carbamate (**16b**) also demonstrated potent inhibitory activity (IC_{50} = 13 nM), which had more activity than isobutyl group (**12b**, IC_{50} = 67 nM). The spiro carbamate (**31b**) showed low activities (IC_{50} = 387 nM), indicating the alkyl substituents are favorable to their cytotoxicities. Similar SAR results of alkyl carbamates also could be found aganist the A549 cell line. Overall, the size of alkyl substituents had more effect on A549 than on HeLa cell line. Small alkyl carbamate analogues, such as **8b**, **9b**, **10b**, **13b**, **16b**, and **17b,** had more than or equal activity to AVLB against HeLa cell line, while only **8b** had more activity against A549.

In our further study, carbamates substituted with aromatic groups were synthesized and tested. Compounds **20b**, **21b**, and **22b** also showed low activities against HeLa cell line for their steric effects. (Table 2) Among the three analogues, 4-methoxy-substituted phenyl group was beneficial to its cytotoxic activity (IC_{50} values for **22b** and **20b** are 49 and 199 nM, respectively). Interestingly, we have found **23b** (54 nM) had a significant increase of cytotoxicity against HeLa cell with benzyl group instead of phenyl group. Subsequently, we further investigated the impact of different substituted benzyl groups on their cytotoxicities. Introduction of electron-donating (OMe) or electron-withdrawing groups (Cl and NO_2) at the 4-position of benzyl group afforded compounds (**25b**, **28b**, and **30b**) with a 3-fold increase in cytotoxic activity against HeLa cell line than AVLB, while 2-methoxyl (**24b**), 3-chloro (**27b**), and 2-nitro benzyl carbamates (**29b**) showed slightly lower activities. Piperonyl (**26b**) was only slightly less cytotoxic than single methoxyl substituent at 4-position (**25b**). However, 2-methoxyl (**24b**) and 3-chloro benzyl carbamate (**27b**) were 4-fold less active against A549 cell line than AVLB, 4-methoxyl (**25b**) and 4-chloro (**28b**) also showed slightly lower activity. Only 4-nitro benzyl carbamate (**30b**, IC_{50} = 38 nM) showed potency increase as methyl carbamate (**8b**) than AVLB. In general, all aromatic carbamate analogues were less sensitive against A549 than HeLa cell line. The same SAR results were to be found that 4-substituted benzyls were beneficial to their cytotoxicities, and benzyl carbamates were more potent than phenyl carbamates.

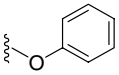
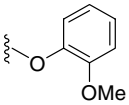
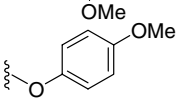
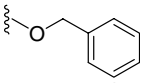
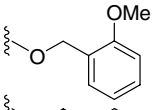
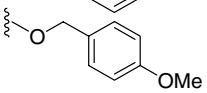
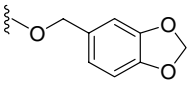
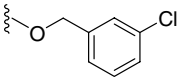
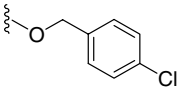
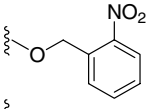
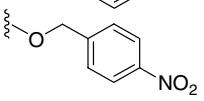
Compound **8b**, which was both potent on two cell lines and easily prepared, was chosen to further evaluate antitumor efficacy in vivo. The antitumor activity of **8b** in vivo was evaluated at doses of 1.5, 3, and 6 mg/kg in nude mice bearing A549 xenografts as the animal model in comparison with compound **1d**. Compound **8b** demonstrated a dose-dependent inhibition of A549 tumor and superior tumor inhibitory activity with 68.5% inhibition at a dose of 6 mg/kg, which showed comparable effect to the clinical drug vinorelbine (**1d**) at a dose of 10 mg/kg. The detailed experimental data are shown in Table 3.

Table 1. Cytotoxic activity of aliphatic substituted analogues

Compound[a]	R	IC_{50} (μM)	
		A549	HeLa
AVLB		0.049 ± 0.005	0.026 ± 0.002
8b		0.038 ± 0.003	0.009 ± 0.001
9b		0.051 ± 0.004	0.016 ± 0.001
10b		0.162 ± 0.011	0.027 ± 0.003
11b		0.604 ± 0.011	0.096 ± 0.009
12b		0.066 ± 0.005	0.067 ± 0.008
13b		0.065 ± 0.008	0.014 ± 0.001
14b		0.131 ± 0.017	0.030 ± 0.003
15b		0.426 ± 0.036	0.184 ± 0.014
16b		0.061 ± 0.004	0.013 ± 0.002
17b		0.121 ± 0.007	0.023 ± 0.002
18b		0.177 ± 0.023	0.132 ± 0.009
19b		0.381 ± 0.037	0.250 ± 0.026
31b		0.631 ± 0.051	0.387 ± 0.035
32b		0.485 ± 0.020	0.186 ± 0.014

[a] Ditartrate of all compounds was used in bioassays.

Table 2. Cytotoxic activity of aromatic substituted analogues

Compound[a]	R	IC_{50} (μM)	
		A549	HeLa
20b		0.110 ± 0.010	0.199 ± 0.018
21b		0.609 ± 0.068	0.148 ± 0.005
22b		0.365 ± 0.032	0.049 ± 0.005
23b		0.057 ± 0.008	0.054 ± 0.002
24b		0.200 ± 0.014	0.069 ± 0.003
25b		0.067 ± 0.009	0.008 ± 0.001
26b		0.075 ± 0.007	0.017 ± 0.002
27b		0.203 ± 0.018	0.039 ± 0.005
28b		0.089 ± 0.011	0.006 ± 0.001
29b		0.061 ± 0.009	0.066 ± 0.007
30b		0.038 ± 0.004	0.010 ± 0.001

[a] Ditartrate of all compounds was used in bioassays.

4. Conclusion

A series of carbamate anhydrovinblastine derivatives (**8b**–**32b**) were designed and synthesized. All the compounds were evaluated for their growth inhibition activities against two tumor cell lines (A549 and HeLa). The SAR information collected so far suggested that the size and position of the carbamate substituents at the 22-position were both important factors for cytotoxic activity in vitro. Compounds **8b** and **30b** maintained potent cytotoxic activities in two tumor cell lines. The preliminary antitumor studies of **8b** in vivo showed that it might be promising for the development of new antitumor agents.

5. Experimental

5.1. Materials and methods

Unless otherwise mentioned, all chemicals and materials were used as received from commercial suppliers without further purification. Tetrahydrofuran (THF) was distilled from sodium/benzophenone under nitrogen. Dichloromethane was distilled from calcium hydride. Thin layer chromatography (TLC) plates (silica gel 60 GF, with glass support) from Yantai jiangyou company were used for monitoring progress of a reaction and visualized with 254 nm UV light and/or spray by a 10% solution of ceric ammonium sulfate (CAS) in phosphoric acid. NMR spectra were recorded on a Varian Mercury-VX300 Fourier transform spectrometer or a Bruker AM-400 spectrometer. The chemical shifts are

Table 3. In vivo anti-tumor efficacy of compound **8b**[a] against non-small cell lung cancer A549 xenografts

Group	Dose (mg/kg)	Mice (*n*)	Body weight (g)	Tumor volume (mm^3) $X \pm SD$		Relative TV ($X \pm SD$)	Inhibition (%)
		Initial/end	Initial/end	Initial	end		
Control		12/12	18.8/21.4	214 ± 48	2194 ± 868	11.06 ± 5.49	
8b	1.5	6/6	19.2/21.3	256 ± 41	2080 ± 542	8.18 ± 2.20	26.0
8b	3	6/6	19.3/20.4	228 ± 68	1545 ± 410	6.96 ± 1.79	37.1*
8b	6	6/6	19.1/18.8	241 ± 30	808 ± 336	3.48 ± 1.83	68.5*
1d	10	6/6	19.0/16.4	218 ± 54	550 ± 206	2.66 ± 1.18	75.9*

[a] The in vivo experiment was carried out in the nude mice bearing A549 xenografts and the compounds were intravenously given. Ditartrate of all compounds was used in bioassays.

* $P < 0.01$ versus control group.

reported in ppm using the δ 7.26 signal of $CDCl_3$ (^1H NMR) and the δ 77.23 signal of $CDCl_3$ (^{13}C NMR) as internal standards. IR spectra were recorded on a Perkin-Elmer 577 spectrometer. EI-MS was obtained on a SHIMADZU GCMS-QP5050A spectrometer. ESI-MS was run on a Bruker Esquire 3000 plus spectrometer in MeOH.

5.1.1. 3,4,22-Triol-3-demethoxycarbonyl-3-hydroxylmethyl-4-deacetyl-vindoline (4). To a suspension of $LiAlH_4$ (114 mg, 3.00 mmol) in dry THF (10 mL) at 0 °C, a solution of vindoline (486 mg, 1.00 mmol) in dry THF (10 mL) was added slowly. The reaction mixture was stirred at room temperature for 4 h and then quenched cautiously by the subsequent addition of water (114 μL), 15% NaOH (aq) (114 μL), and water (342 μL). The resulting suspension was warmed to room temperature and stirred for 10 min. The suspension was filtered through a fritted funnel and the filtrate was concentrated in vacuo to give crude triol. The product was used in the next step without purification. $[\alpha]_D^{20}$ + 45° (*c* 0.20, $CHCl_3$), ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.73 (br s, 1 H), 6.82 (d, *J* = 8.0 Hz, 1H), 6.30 (dd, *J* = 8.0, 2.4 Hz, 1H), 6.12 (d, *J* = 2.4 Hz, 1H), 5.88 (dd, *J* = 10.0, 4.8 Hz, 1H), 5.60 (d, *J* = 10.0 Hz, 1H), 3.93 (d, *J* = 11.2 Hz, 1H), 3.78 (s, 3H), 3.72 (d, *J* = 11.2 Hz, 1H), 3.52 (s, 2H), 3.45 (dd, *J* = 15.6, 4.4 Hz, 1H), 3.35 (td, *J* = 9.2, 4.4 Hz, 1H), 3.00 (s, 3H), 2.83 (dt, *J* = 15.6, 2.0 Hz, 1H), 2.57 (s, 1H), 2.53–2.50 (m, 2H), 2.25–2.16 (m, 3H), 1.36–1.31 (m, 1H), 0.94–0.88 (m, 1H), 0.62 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 160.9, 154.6, 130.8, 126.5, 124.3, 122.9, 104.6, 96.3, 81.0, 77.4, 75.2, 68.3, 65.7, 55.4, 51.8, 51.7, 51.3, 44.8, 43.5, 40.4, 32.5, 7.9. EIMS (*m*/*z*) 386 (M^+), 368, 355, 297, 212, 188, 174, 162, 135, 93.

5.1.2. 3-Demethoxycarbonyl-3-hydroxylmethyl-3β,22-epoxide-4-deacetyl-vindoline (5). To a solution of triol **4** (3.86 g, 10 mmol) in 25 mL THF were added 50% NaOH solution (4 g) and *n*-$Bu_4N^+I^-$ (36.9 mg, 0.1 mmol). After stirring at 50 °C for 30 min, Toluene-4-sulfonyl chloride (2.09 g, 11 mmol) was added slowly. The mixture was stirred at 80 °C for 3 h, and then was diluted with EtOAc (50 mL)and H_2O (30 mL). The organic layer was washed with H_2O (20 mL), brine (20 mL) and dried (Na_2SO_4). Removal of the solvents was followed by column chromatography on silica gel (7:1, hexanes/acetone) to afford the compound **5** as a white solid in 80% (2.95 g) yield. $[\alpha]_D^{20}$ + 4.0° (*c* 0.21, $CHCl_3$), ^1H NMR ($CDCl_3$, 400 MHz) δ: 6.84 (d, *J* = 8.0 Hz, 1H), 6.10 (dd, *J* = 8.0, 2.4 Hz, 1H), 6.03 (dd, *J* = 10.0, 4.8 Hz, 1H), 5.90 (d, *J* = 2.4 Hz, 1H), 5.48 (d, *J* = 10.0 Hz, 1H), 4.07 (s, 1H), 3.78 (s, 3H), 3.54 (dd, *J* = 16.4, 4.8 Hz, 1H), 3.34–3.30 (m, 1H), 3.01 (s, 1H), 2.97 (s, 1H), 2.94 (s, 3H), 2.88 (dt, *J* = 16.4, 2.0 Hz, 1H), 2.57 (d, *J* = 4.0 Hz, 1H), 2.48 (d, *J* = 4.0 Hz, 1H), 2.42–2.34 (m, 2H), 1.67–1.63 (m, 1H), 1.41–1.35 (m, 1H), 1.12–1.07 (m, 1H), 0.79 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 161.1, 151.3, 130.5, 127.1 (2C), 121.2, 100.4, 92.3, 78.6, 68.7, 64.6, 63.7, 55.3, 54.2, 53.3, 51.6, 50.8, 45.4, 40.4, 33.6, 27.2, 8.4. EIMS (*m*/*z*) 368 (M^+), 339, 297, 188, 174, 121, 107, 93.

5.1.3. 3-Demethoxycarbonyl-3-azidomethyl-4-deacetyl-vindoline (6). A solution of compound **5** (8 mmol, 2.95 g) in a 8:1 MeOH/H_2O mixture (72 mL) was treated with NaN_3 (2.6 g, 40 mmol) and NH_4Cl (0.936 g, 17.6 mmol), and the reaction mixture was stirred at 80 °C for 20 h. After cooling EtOAc (100 mL)and H_2O (100 mL) were added. The aqueous layer was additionally extracted with EtOAc (2× 30 mL) and the combined organic layer was washed with brine (100 mL) and dried (Na_2SO_4). Removal of the solvents was followed by column chromatography on silica gel (8:1, hexanes/acetone) to afford the compound **6** as a white solid in 91% (2.99 g) yield. $[\alpha]_D^{20}$ − 69° (*c* 0.18, $CHCl_3$), IR (KBr): 3405, 2964, 2102, 1616, 1500, 1463, 1278, 1222, 1164, 1084 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.68 (br s, 1H), 6.87 (d, *J* = 8.4 Hz, 1H), 6.30 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.09 (d, *J* = 2.4 Hz, 1H), 5.88 (dd, *J* = 10.4, 4.8 Hz, 1H), 5.58 (d, *J* = 10.4 Hz, 1H), 3.85 (d, *J* = 11.2 Hz, 1H), 3.78 (s, 3H), 3.61 (s, 1H), 3.46 (dd, *J* = 16.0, 4.8 Hz, 1H), 3.42–3.37 (m, 2H), 3.20 (d, *J* = 11.2 Hz, 1H), 2.93 (s, 3H), 2.81 (dt, *J* = 16.0, 2.0 Hz, 1H), 2.51 (s, 1H), 2.48–2.43 (m, 1H), 2.29–2.23 (m, 2H), 1.92 (d, *J* = 12.4 Hz, 1H), 1.29–1.23 (m, 1H), 0.89–0.83 (m, 1H), 0.58 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 161.0, 154.8, 130.2, 126.7, 125.3, 122.9, 104.8, 96.5, 80.7, 78.9, 75.4, 68.9, 55.9, 55.5, 52.2, 51.8, 51.4, 44.7, 43.7, 40.6, 32.7, 7.9. ESIMS (*m*/*z*) 412.2 $[M+1]^+$.

5.1.4. 3-Demethoxycarbonyl-3-aminomethyl-4-deacetyl-vindoline (7). To a suspension of $LiAlH_4$ (800 mg, 21.06 mmol) in dry THF (20 mL) at 0 °C, a solution of compound **6** (2.89 g, 7.02 mmol) in dry THF (20 mL) was added slowly. The reaction mixture was stirred at room temperature for 4 h and then quenched cautiously by the subsequent addition of water (0.8 mL), 15% NaOH (aq) (0.8 mL), and water

(2.4 mL). The resulting suspension was warmed to room temperature and stirred for 10 min. The suspension was filtered through a fritted funnel and the filtrate was concentrated in vacuo to give amine **7**. The product was used in the next step without purification. $[\alpha]_D^{20}$ + 38° (*c* 0.09, $CHCl_3$), ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.64 (s, 1 H), 6.87 (d, *J* = 8.0 Hz, 1H), 6.28 (dd, *J* = 8.0, 2.4 Hz, 1H), 6.06 (d, *J* = 2.4 Hz, 1H), 5.87–5.79 (m, 2H), 3.78 (s, 4H), 3.45 (dd, *J* = 15.6, 4.4 Hz, 1H), 3.36–3.32 (m, 1H), 3.24 (s, 1H), 3.20 (d, *J* = 11.6 Hz, 1H), 3.16 (d, *J* = 11.6 Hz, 1H), 2.92 (s, 3H), 2.83 (dt, *J* = 15.6, 2.0 Hz, 1H), 2.59 (s, 1H), 2.52–2.47 (m, 1H), 2.21–2.15 (m, 2H), 1.42–1.37 (m, 1H), 0.94–0.88 (m, 1H), 0.65 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 160.8, 154.4, 131.9, 126.5, 122.9, 122.8, 104.2, 96.1, 84.5, 78.4, 75.8, 68.1, 55.4, 52.5, 51.5, 51.3, 49.7, 45.2, 43.6, 41.5, 32.4, 7.9. EIMS (*m*/*z*) 385 (M^+), 368, 355, 297, 194, 174, 162, 152, 135, 122, 93.

5.2. General procedure for the preparation of 8a–30a

A solution of the alcohol (1.50 mmol) in dry CH_2Cl_2 (10 ml) was treated with bis(trichloromethyl)carbonate (147 mg, 0.50 mmol) followed by the dropwise addition of a solution of diisopropylethylamine (0.26 mL, 1.5 mmol) in CH_2Cl_2 (5 ml) at 0 °C. This was allowed to warm to room temperature and stirred a further 1 h to obtain chloroformate solution in CH_2Cl_2. Then, the prepared chloroformate was added dropwise to a solution of compound **7** (1 mmol) and diisopropylethylamine (0.20 mL, 1.2 mmol) in dry CH_2Cl_2 (10 ml) at room temperature. After stirring for 4 h, this mixture was quenched by adding methanol (2 mL) with vigorous stirring. This was extracted with CH_2Cl_2 (twice) and the combined organic extracts were washed with brine and dried (Na_2SO_4). The residue was added to pyridine (1.0 mL) and Ac_2O (1.0 mL) at room temperature. After stirring the reaction mixture for 8 h, saturated $NaHCO_3$ (5 mL) and EtOAc (20 mL) were added, and the organic phase was washed with H_2O (3× 10 mL) and brine (10 mL), and was dried, concentrated, and purified by flash chromatography (5:1 hexanes/acetone) to provide a white solid (**8a**–**30a**).

5.2.1. 3-Demethoxycarbonyl-3-(methoxycarbonylamino)-methyl-vindoline (8a). Compound **8a** was prepared from methyl chloroformate as material. Yield: 85%; ^1H NMR ($CDCl_3$, 300 MHz): δ: 9.04 (s), 6.84 (d, *J* = 8.1 Hz, 1H), 6.28 (dd, *J* = 8.1, 2.1 Hz, 1H), 6.12 (d, *J* = 2.1 Hz, 1H), 5.85 (dd, *J* = 10.2, 4.5 Hz, 1H), 5.34 (d, *J* = 10.2 Hz, 1H), 5.29 (s, 1H), 4.98 (s, 1H), 3.82 (s, 3H), 3.75 (s, 3H), 3.47 (m, 1H), 3.40 (s, 1H), 3.32 (m, 3H), 3.09 (d, *J* = 12.6 Hz, 1H), 2.87 (s, 3H), 2.82 (d, *J* = 15.6 Hz, 1H), 2.59 (s, 1H), 2.49 (m, 1H), 2.30–2.12 (m, 2H), 2.08 (s, 3H), 1.27 (m, 1H), 0.98 (m, 1H), 0.49 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 170.7, 161.1, 157.3, 154.5, 130.5, 125.8, 124.2, 122.7, 105.2, 96.8, 82.0, 77.2, 76.0, 67.5, 55.4, 52.3, 52.2, 51.7, 50.9, 45.2, 44.8, 42.9, 40.7, 31.5, 21.0, 7.6. ESIMS (*m*/*z*) 486.3 $[M+1]^+$.

5.2.2. 3-Demethoxycarbonyl-3-(ethoxycarbonylamino)-methyl-vindoline (9a). Compound **9a** was prepared from ethyl chloroformate as material. Yield: 81%; ^1H NMR ($CDCl_3$, 300 MHz): δ: 9.05 (s), 6.86 (d, *J* = 8.1 Hz, 1H), 6.30 (dd, *J* = 8.1, 2.4 Hz, 1H), 6.14 (d, *J* = 2.4 Hz, 1H), 5.87 (dd, *J* = 10.2, 4.5 Hz, 1H), 5.34 (d, *J* = 10.2 Hz, 1H), 5.26 (s, 1H), 5.00 (s, 1H), 4.09 (q, *J* = 6.9 Hz, 1H), 3.78 (s, 3H), 3.51 (m, 1H), 3.43 (s, 1H), 3.35 (m, 2H), 3.11 (d, *J* = 12.3 Hz, 1H), 2.90 (s, 3H), 2.81 (d, *J* = 15.9 Hz, 1H), 2.62 (s, 1H), 2.50 (m, 1H), 2.26–2.17 (m, 2H), 2.10 (s, 3H), 1.27 (m, 1H), 1.22 (t, *J* = 6.9 Hz, 3H), 1.00 (m, 1H), 0.51 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 170.2, 160.8, 156.5, 154.1, 130.1, 125.5, 123.8, 122.4, 104.8, 96.4, 81.6, 76.9, 75.6, 67.2, 60.3, 55.0, 51.9, 51.3, 50.6, 44.8, 44.4, 42.6, 40.3, 31.1, 20.6, 14.4, 7.3. ESIMS (*m*/*z*) 500.3 $[M+1]^+$.

5.2.3. 3-Demethoxycarbonyl-3-(isopropyloxycarbonylamino)-methyl-vindoline (10a). Compound **10a** was prepared from isopropanol as material. Yield: 76%; ^1H NMR ($CDCl_3$, 300 MHz): δ: 9.01 (s), 6.78 (d, *J* = 8.1 Hz, 1H), 6.21 (dd, *J* = 8.1, 1.8 Hz, 1H), 6.04 (d, *J* = 1.8 Hz, 1H), 5.78 (dd, *J* = 10.2, 3.9 Hz, 1H), 5.27 (d, *J* = 10.2 Hz, 1H), 5.17 (d, *J* = 6.6 Hz, 1H), 4.92 (s, 1H), 4.79 (m, 1H), 3.67 (s, 3H), 3.40 (m, 2H), 3.35 (s, 1H), 3.24 (m, 1H), 3.02 (d, *J* = 12.6 Hz, 1H), 2.80 (s, 3H), 2.73 (d, *J* = 16.2 Hz, 1H), 2.54 (s, 1H), 2.42 (m, 1H), 2.22–2.11 (m, 2H), 2.00 (s, 3H), 1.20 (m, 1H), 1.11 (d, *J* = 6.0 Hz, 6H), 0.95 (m, 1H), 0.42 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 170.3, 160.8, 156.2, 154.2, 130.2, 125.5, 123.9, 122.4, 104.9, 96.4, 81.7, 77.0, 75.8, 67.5, 67.3, 55.1, 52.0, 51.4, 50.7, 44.9, 44.5, 42.7, 40.3, 31.2, 22.0 (2C), 20.7, 7.4. ESIMS (*m*/*z*) 514.3 $[M+1]^+$.

5.2.4. 3-Demethoxycarbonyl-3-(tertbutyloxycarbonylamino)-methyl-vindoline (11a). Compound **11a** was prepared from di-*tert*-butyl dicarbonate as material. Yield: 86%; ^1H NMR ($CDCl_3$, 300 MHz): δ: 9.01 (s), 6.79 (d, *J* = 8.1 Hz, 1H), 6.21 (dd, *J* = 8.1, 1.8 Hz, 1H), 6.04 (d, *J* = 1.8 Hz, 1H), 5.80 (dd, *J* = 10.2, 4.5 Hz, 1H), 5.28 (d, *J* = 10.2 Hz, 1H), 5.22 (s, 1H), 4.92 (s, 1H), 3.67 (s, 3H), 3.40 (m, 2H), 3.35 (s, 1H), 3.25 (m, 1H), 3.03 (d, *J* = 12.3 Hz, 1H), 2.80 (s, 3H), 2.73 (d, *J* = 15.9 Hz, 1H), 2.54 (s, 1H), 2.43 (m, 1H), 2.22–2.06 (m, 2H), 2.00 (s, 3H), 1.36 (s, 9H), 1.20 (m, 1H), 0.90 (m, 1H), 0.50 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 170.3, 160.9, 156.4, 154.6, 130.8, 126.5, 124.3, 122.7, 104.6, 96.4, 81.6, 79.0, 76.6, 75.7, 68.5, 55.2, 51.9, 51.4, 50.6, 44.9, 44.5, 43.6, 40.5, 31.5, 28.4 (3C), 21.1, 7.7. ESIMS (*m*/*z*) 528.4 $[M+1]^+$.

5.2.5. 3-Demethoxycarbonyl-3-(isobutyloxycarbonylamino)-methyl-vindoline (12a). Compound **12a** was prepared from iso-butyl chloroformate as material. Yield: 83%;^1H NMR ($CDCl_3$, 300 MHz): δ: 9.01 (s), 6.79 (d, *J* = 8.1 Hz, 1H), 6.21 (dd, *J* = 8.1, 1.8 Hz, 1H), 6.04 (d, *J* = 1.8 Hz, 1H), 5.80 (dd, *J* = 10.2, 4.5 Hz, 1H), 5.28 (d, *J* = 10.2 Hz, 1H), 5.22 (s, 1H), 4.92 (s, 1H), 3.73 (d, *J* = 6.3 Hz, 2H), 3.67 (s, 3H), 3.40 (m, 2H), 3.35 (s, 1H), 3.25 (m, 1H), 3.03 (d, *J* = 12.3 Hz, 1H), 2.80 (s, 3H), 2.73 (d, *J* = 15.9 Hz, 1H), 2.54 (s, 1H), 2.43 (m, 1H), 2.22–2.06 (m, 2H), 2.00 (s, 3H), 1.78 (m, 1H), 1.20 (m, 1H), 0.90 (m, 1H), 0.80 (d, *J* = 6.6 Hz, 6H), 0.42 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz):

δ: 170.3, 160.8, 156.7, 154.2, 130.2, 125.5, 123.9, 122.4, 104.8, 96.4, 81.6, 77.0, 75.7, 70.6, 67.3, 55.1, 51.9, 51.4, 50.6, 44.9, 44.5, 42.6, 40.3, 31.2, 27.8, 20.7, 18.8 (2C), 7.3. ESIMS (*m*/*z*) 528.3 $[M+1]^+$.

5.2.6. 3-Demethoxycarbonyl-3-(2-methoxylethyloxycarbonylamino)-methyl-vindoline (13a). Compound **13a** was prepared from 2-methoxylethanol as material. Yield: 65%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 8.98 (s), 6.81 (d, *J* = 8.4 Hz, 1H), 6.24 (d, *J* = 8.4 Hz, 1H), 6.07 (s, 1H), 5.80 (dd, *J* = 10.2, 4.2 Hz, 1H), 5.39 (d, *J* = 7.2 Hz, 1H), 5.30 (d, *J* = 10.2 Hz, 1H), 4.94 (s, 1H), 4.14 (t, *J* = 6.3 Hz, 2H), 3.71 (s, 3H), 3.50 (t, *J* = 6.3 Hz, 2H), 3.50–3.18 (m, 3H), 3.38 (s, 1H), 3.30 (s, 3H), 3.05 (d, *J* = 12.6 Hz, 1H), 2.83 (s, 3H), 2.75 (d, *J* = 15.3 Hz, 1H), 2.56 (s, 1H), 2.45 (q, *J* = 9.0 Hz, 1H), 2.24–2.08 (m, 2H), 2.04 (s, 3H), 1.26–1.18 (m, 1H), 0.99–0.87 (m, 1H), 0.45 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 170.5, 161.0, 156.5, 154.4, 130.3, 125.7, 124.0, 122.6, 105.1, 96.6, 81.7, 77.1, 75.9, 70.9, 67.5, 63.7, 58.8, 55.2, 52.1, 51.5, 50.8, 45.0, 44.6, 42.8, 40.5, 31.4, 20.8, 7.5. ESIMS (*m*/*z*) 530.3 $[M+1]^+$.

5.2.7. 3-Demethoxycarbonyl-3-(bromoethyloxycarbonylamino)-methyl-vindoline (14a). Compound **14a** was prepared from 2-Bromoethanol as material. Yield: 60%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 9.12 (s), 6.87 (d, *J* = 8.4 Hz, 1H), 6.32 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.15 (d, *J* = 2.4 Hz, 1H), 5.89 (dd, *J* = 10.5, 3.6 Hz, 1H), 5.43 (m, 1H), 5.38 (d, *J* = 10.5 Hz, 1H), 5.01 (s, 1H), 4.35 (t, *J* = 6.3 Hz, 2H), 3.79 (s, 3H), 3.49 (t, *J* = 6.3 Hz, 2H), 3.52–3.30 (m, 3H), 3.43 (s, 1H), 3.14 (d, *J* = 13.2 Hz, 1H), 2.91 (s, 3H), 2.83 (d, *J* = 14.7 Hz, 1H), 2.64 (s, 1H), 2.53 (q, *J* = 9.0 Hz, 1H), 2.25–2.10 (m, 2H), 2.12 (s, 3H), 1.26–1.18 (m, 1H), 1.10–0.87 (m, 1H), 0.52 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 170.4, 161.0, 155.8, 154.3, 130.3, 125.6, 124.0, 122.6, 105.1, 96.6, 82.0, 77.0, 75.8, 67.4, 64.1, 55.2, 52.2, 51.5, 50.8, 45.2, 44.6, 42.8, 40.7, 31.3, 29.5, 20.9, 7.4. ESIMS (*m*/*z*) 578.2 $[M+1]^+$.

5.2.8. 3-Demethoxycarbonyl-3-(*n*-pentanyloxycarbonylamino)-methyl-vindoline (15a). Compound **15a** was prepared from *n*-pentanol as material. Yield: 62%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 9.05 (s), 6.82 (d, *J* = 8.1 Hz, 1H), 6.25 (dd, *J* = 8.1, 2.1 Hz, 1H), 6.04 (d, *J* = 2.1 Hz, 1H), 5.82 (dd, *J* = 10.2, 3.3 Hz, 1H), 5.31 (d, *J* = 10.2 Hz, 1H), 5.25 (d, *J* = 10.2 Hz, 1H), 4.96 (s, 1H), 3.97 (t, *J* = 6.3 Hz, 2H), 3.71 (s, 3H), 3.45 (m, 2H), 3.39 (s, 1H), 3.29 (m, 1H), 3.07 (d, *J* = 12.3 Hz, 1H), 2.84 (s, 3H), 2.77 (d, *J* = 15.9 Hz, 1H), 2.58 (s, 1H), 2.46 (m, 1H), 2.26–2.10 (m, 2H), 2.05 (s, 3H), 1.53 (m, 2H), 1.26(m, 5H), 0.93 (m, 1H), 0.74 (m, 5H), 0.46 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 170.4, 160.9, 156.9, 154.4, 130.3, 125.6, 124.0, 122.6, 105.0, 96.5, 81.8, 77.1, 75.9, 67.4, 64.8, 55.2, 52.1, 51.6, 50.8, 45.0, 44.6, 42.8, 40.5, 31.3, 28.7, 27.9, 22.3, 20.9, 14.0, 7.5. ESIMS (*m*/*z*) 542.4 $[M+1]^+$.

5.2.9. 3-Demethoxycarbonyl-3-(cyclopropylmethyloxycarbonylamino)-methyl-vindoline (16a). Compound **16a** was prepared from cyclopropylmethanol as material. Yield: 63%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 9.07 (s), 6.86 (d, *J* = 8.4 Hz, 1H), 6.30 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.14 (d, *J* = 2.4 Hz, 1H), 5.87 (dd, *J* = 10.2, 4.5 Hz, 1H), 5.36 (d, *J* = 10.2 Hz, 2H), 5.00 (s, 1H), 3.86 (d, *J* = 7.2 Hz, 2H), 3.78 (s, 3H), 3.54–3.46 (m, 2H), 3.45 (s, 1H), 3.38–3.31 (m, 1H), 3.12 (d, *J* = 12.6 Hz, 1H), 2.90 (s, 3H), 2.81 (d, *J* = 15.9 Hz, 1H), 2.62 (s, 1H), 2.56–2.46 (m, 1H), 2.28–2.18 (m, 2H), 2.11 (s, 3H), 1.34–1.26 (m, 1H), 1.13–1.03 (m, 1H), 1.00–0.94 (m, 1H), 0.51 (t, *J* = 7.2 Hz, 3H), 0.51 (d, *J* = 4.8 Hz, 2H), 0.26 (d, *J* = 4.8 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 170.4, 160.9, 156.8, 154.4, 130.3, 125.6, 124.0, 122.6, 105.0, 96.5, 81.7, 77.1, 75.9, 69.4, 67.4, 55.2, 52.1, 51.5, 50.8, 45.0, 44.6, 42.8, 40.4, 31.3, 20.8, 10.1, 7.4, 3.0 (2× C). ESIMS (*m*/*z*) 526.3 $[M+1]^+$.

5.2.10. 3-Demethoxycarbonyl-3-(cyclobutyloxycarbonylamino)-methyl-vindoline (17a). Compound **17a** was prepared from cyclobutanol as material. Yield: 65%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 9.06 (s), 6.86 (d, *J* = 8.4 Hz, 1H), 6.30 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.14 (d, *J* = 2.4 Hz, 1H), 5.87 (dd, *J* = 10.2, 4.8 Hz, 1H), 5.36 (d, *J* = 10.2 Hz, 1H), 5.27 (d, *J* = 7.5 Hz, 1H), 4.99 (s, 1H), 4.90 (m, 1H), 3.78 (s, 3H), 3.48 (m, 2H), 3.43 (s, 1H), 3.35 (m, 1H), 3.09 (d, *J* = 12.3 Hz, 1H), 2.89 (s, 3H), 2.80 (d, *J* = 15.9 Hz, 1H), 2.62 (s, 1H), 2.49 (m, 1H), 2.32–2.14 (m, 4H), 2.10 (s, 3H), 2.01 (m, 2H), 1.73 (m, 1H), 1.57 (m, 1H), 1.28 (m, 1H), 0.98 (m, 1H), 0.51 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 170.1, 160.7, 155.7, 154.1, 130.1, 125.4, 123.8, 122.4, 104.8, 96.4, 81.6, 76.8, 75.6, 68.3, 67.1, 54.9, 51.9, 51.2, 50.5, 44.7, 44.4, 42.5, 40.3, 31.1, 30.3, 30.1, 20.6, 12.9, 7.2. ESIMS (*m*/*z*) 526.4 $[M+1]^+$.

5.2.11. 3-Demethoxycarbonyl-3-(cyclopentyloxycarbonylamino)-methyl-vindoline (18a). Compound **18a** was prepared from cyclopentanol as material. Yield: 58%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 8.97 (s), 6.78 (d, *J* = 8.4 Hz, 1H), 6.48 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.04 (d, *J* = 2.4 Hz, 1H), 5.79 (dd, *J* = 10.2, 4.8 Hz, 1H), 5.27 (d, *J* = 10.2 Hz, 1H), 5.14 (d, *J* = 7.5 Hz, 1H), 4.97 (m, 1H), 4.91 (s, 1H), 3.67 (s, 3H), 3.38 (m, 2H), 3.34 (s, 1H), 3.25 (m, 1H), 3.20 (d, *J* = 12.3 Hz, 1H), 2.80 (s, 3H), 2.73 (d, *J* = 15.6 Hz, 1H), 2.54 (s, 1H), 2.40 (m, 1H), 2.21–2.13 (m, 2H), 2.00 (s, 3H), 1.72 (m, 2H), 1.58 (m, 4H), 1.44 (m, 2H), 1.24 (m, 1H), 0.92 (m, 1H), 0.42 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 170.2, 160.8, 156.4, 154.2, 130.2, 125.5, 123.9, 122.4, 104.8, 96.4, 81.7, 77.0 (2C), 75.7, 67.3, 55.0, 51.9, 51.4, 50.7, 44.9, 44.4, 42.6, 40.2, 32.6 (2C), 31.2, 23.4 (2C), 20.7, 7.3. ESIMS (*m*/*z*) 540.4 $[M+1]^+$.

5.2.12. 3-Demethoxycarbonyl-3-(cyclohexanyloxycarbonylamino)-methyl-vindoline (19a). Compound **19a** was prepared from cyclohexanol as material. Yield: 56%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 8.97 (s), 6.79 (d, *J* = 8.4 Hz, 1H), 6.22 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.06 (d, *J* = 2.4 Hz, 1H), 5.79 (dd, *J* = 10.2, 4.8 Hz, 1H), 5.29 (d, *J* = 10.2 Hz, 1H), 5.19 (d, *J* = 5.7 Hz, 1H), 4.93 (s, 1H), 4.53 (m, 1H), 3.69 (s, 3H), 3.40 (m, 2H), 3.37 (s, 1H), 3.27 (m, 1H), 3.04 (d, *J* = 12.3 Hz, 1H), 2.82 (s, 3H), 2.74 (d, *J* = 15.6 Hz, 1H), 2.55 (s, 1H), 2.42 (m, 1H), 2.25–2.15 (m, 2H), 2.02 (s, 3H), 1.75 (m, 2H), 1.62 (m, 2H), 1.42 (m, 1H), 1.26 (m, 6H), 0.88 (m, 1H), 0.44 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 170.3, 160.9, 156.3, 154.3, 130.3, 125.6,

124.0, 122.5, 104.9, 96.4, 81.7, 77.1, 75.8, 72.6, 67.4, 55.1, 52.0, 51.5, 50.7, 45.0, 44.5, 42.7, 40.3, 31.9, 31.3 (2C), 25.5, 23.7 (2C), 20.8, 7.4. ESIMS (*m*/*z*) 554.4 $[M+1]^+$.

5.2.13. 3-Demethoxycarbonyl-3-(phenyloxycarbonylamino)-methyl-vindoline (20a). Compound **20a** was prepared from phenol as material. Yield: 65%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 9.18 (s), 7.30 (t, J = 7.2 Hz, 2H), 7.01 (m, 3H), 6.86 (dd, J = 8.4, 1.8 Hz, 1H), 6.30 (dd, J = 8.4, 1.8 Hz, 1H), 6.14 (s, 1H), 5.87 (dd, J = 10.2, 4.5 Hz, 1H), 5.76 (d, J = 7.5 Hz, 1H), 5.37 (d, J = 9.9 Hz, 1H), 5.01 (s, 1H), 3.74 (s, 3H), 3.60 (m, 1H), 3.47 (s, 1H), 3.42(m, 1H), 3.34 (m, 1H), 3.22 (d, J = 12.6 Hz, 1H), 2.88 (s, 3H), 2.80 (d, J = 15.9 Hz, 1H), 2.65 (s, 1H), 2.49 (m, 1H), 2.26–2.16 (m, 2H), 2.10 (s, 3H), 1.28 (m, 1H), 1.00 (m, 1H), 0.52 (t, J = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 170.5, 160.9, 154.6, 154.2, 151.0, 130.2, 129.1 (2C), 125.5, 125.0, 124.0, 122.6, 121.5 (2C), 105.3, 96.7, 82.3, 76.8, 75.8, 67.2, 55.2, 52.2, 51.3, 50.7, 45.2, 44.5, 42.7, 40.9, 31.2, 20.8, 7.4. ESIMS (*m*/*z*) 548.3 $[M+1]^+$.

5.2.14. 3-Demethoxycarbonyl-3-(2-methoxyl-phenyloxycarbonylamino)-methyl-vindoline (21a). Compound **21a** was prepared from 2-methoxyl-phenol as material. Yield: 60%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 9.16 (s), 7.15 (t, J = 7.2 Hz, 1H), 7.07(d, J = 7.2 Hz, 1H), 7.01 (m, 3H), 6.94–6.87 (m, 3H), 6.31 (dd, J = 8.4, 1.8 Hz, 1H), 6.15 (s, 1H), 5.89 (dd, J = 10.2, 4.5 Hz, 1H), 5.78 (d, J = 8.1 Hz, 1H), 5.38 (d, J = 10.5 Hz, 1H), 5.03 (s, 1H), 3.78 (s, 6H), 3.60 (m, 1H), 3.53 (s, 1H), 3.47(m, 1H), 3.35 (m, 1H), 3.22 (d, J = 12.6 Hz, 1H), 2.88 (s, 3H), 2.82 (d, J = 15.9 Hz, 1H), 2.66 (s, 1H), 2.52 (m, 1H), 2.26–2.16 (m, 2H), 2.12 (s, 3H), 1.28 (m, 1H), 1.00 (m, 1H), 0.53 (t, J = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 170.6, 161.9, 154.5 (2C), 151.8, 140.1, 130.4, 126.5, 125.7, 124.2, 123.4, 122.7, 120.7, 112.3, 105.3, 96.7, 82.0, 77.0, 76.1, 67.4, 55.8, 55.4, 52.3, 51.6, 50.9, 45.3, 44.7, 42.9, 40.7, 31.4, 21.0, 7.6. ESIMS (*m*/*z*) 578.2 $[M+1]^+$.

5.2.15. 3-Demethoxycarbonyl-3-(4-methoxyl-phenyloxycarbonylamino)-methyl-vindoline (22a). Compound **22a** was prepared from 4-methoxyl-phenol as material. Yield: 61%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 9.19 (s), 7.04 (d, J = 7.2 Hz, 2H), 6.87 (m, 3H), 6.33 (dd, J = 8.4, 1.8 Hz, 1H), 6.16 (s, 1H), 5.89 (dd, J = 10.2, 4.5 Hz, 1H), 5.70 (d, J = 7.8 Hz, 1H), 5.38 (d, J = 10.2 Hz, 1H), 5.03 (s, 1H), 3.78 (s, 6H), 3.61 (m, 1H), 3.48 (m, 1H), 3.47(s, 1H), 3.37 (m, 1H), 3.22 (d, J = 12.6 Hz, 1H), 2.95 (s, 3H), 2.84 (d, J = 15.9 Hz, 1H), 2.67 (s, 1H), 2.52 (m, 1H), 2.26–2.16 (m, 2H), 2.12 (s, 3H), 1.28 (m, 1H), 1.00 (m, 1H), 0.53 (t, J = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 170.6, 161.0, 156.8, 155.1, 154.3, 144.6, 130.3, 125.6, 124.2, 122.7, 122.4(2C), 114.2 (2C), 105.3, 96.8, 82.3, 76.8, 75.8, 67.2, 55.5, 55.3, 52.3, 51.4, 50.8, 45.3, 44.7, 42.8, 41.0, 31.3, 21.0, 7.5. ESIMS (*m*/*z*) 578.2 $[M+1]^+$.

5.2.16. 3-Demethoxycarbonyl-3-(benzyloxycarbonylamino)-methyl-vindoline (23a). Compound **23a** was prepared from benzyl chloroformate as material. Yield: 85%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 9.10 (s), 7.30 (m, 5H), 6.86 (d, J = 8.4 Hz, 1H), 6.30 (dd, J = 8.4, 2.4 Hz, 1H), 6.13 (d, J = 2.4 Hz, 1H), 5.87 (dd, J = 10.2, 4.5 Hz, 1H), 5.43 (d, J = 7.5 Hz, 1H), 5.36 (d, J = 10.2 Hz, 1H), 5.09 (s, 2H), 5.01 (s, 1H), 3.77 (s, 3H), 3.50 (m, 2H), 3.42 (s, 1H), 3.34 (m, 1H), 3.14 (d, J = 12.6 Hz, 1H), 2.88 (s, 3H), 2.81 (d, J = 15.9 Hz, 1H), 2.62 (s, 1H), 2.49 (m, 1H), 2.26–2.16 (m, 2H), 2.08 (s, 3H), 1.28 (m, 1H), 1.00 (m, 1H), 0.51 (t, J = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 170.6, 161.1, 156.6, 154.5, 136.8, 130.4, 128.5 (2C), 128.1 (2C), 125.7, 124.2, 122.7, 105.2, 96.7, 81.9, 77.2, 76.0, 67.5, 66.6, 55.4, 52.2, 51.6, 50.9, 45.2, 44.7, 42.9, 40.6, 31.4, 21.0, 7.6. ESIMS (*m*/*z*) 562.3 $[M+1]^+$.

5.2.17. 3-Demethoxycarbonyl-3-(2-methoxylbenzyloxycarbonylamino)-methyl-vindoline (24a). Compound **24a** was prepared from 2-methoxyl benzyl alcohol as material. Yield: 78%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 9.10 (s), 7.23 (d, J = 7.2 Hz, 1H), 7.16 (t, J = 7.2 Hz, 1H), 6.82 (t, J = 7.2 Hz, 1H), 6.77 (d, J = 8.4 Hz, 1H), 6.76 (d, J = 7.2 Hz, 1H), 6.21 (dd, J = 8.4, 2.4 Hz, 1H), 6.04 (s, 1H), 5.77 (dd, J = 10.2, 3.9 Hz, 1H), 5.35 (d, J = 6.3 Hz, 1H), 5.27 (d, J = 10.2 Hz, 1H), 5.07 (s, 2H), 4.93 (s, 1H), 3.70 (s, 3H), 3.66 (s, 3H), 3.47–3.32 (m, 2H), 3.35 (s, 1H), 3.27–3.19 (m, 1H), 3.07 (d, J = 12.9 Hz, 1H), 2.79 (s, 3H), 2.71 (d, J = 15.9 Hz, 1H), 2.52 (s, 1H), 2.42–2.36 (m, 1H), 2.20–2.08 (m, 2H), 1.91 (s, 3H), 1.33–1.22 (m, 1H), 0.95–0.86 (m, 1H), 0.42 (t, J = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 170.4, 160.9, 157.2, 156.6, 154.4, 130.3, 129.3, 129.2, 125.6, 124.9, 124.0, 122.5, 120.3, 110.2, 105.0, 96.5, 81.7, 77.1, 75.9, 67.5, 61.8, 55.2 (2C), 52.0, 51.5, 50.7, 45.2, 44.5, 42.8, 40.4, 31.3, 20.7, 7.4. ESIMS (*m*/*z*) 592.2 $[M+1]^+$.

5.2.18. 3-Demethoxycarbonyl-3-(4-methoxylbenzyloxycarbonylamino)-methyl-vindoline (25a). Compound **25a** was prepared from 4-methoxyl benzyl alcohol as material. Yield: 76%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 8.95 (s), 7.19 (d, J = 8.4 Hz, 2H), 6.78 (d, J = 8.4 Hz, 2H), 6.76 (d, J = 8.1 Hz, 1H), 6.21 (dd, J = 8.1, 1.8 Hz, 1H), 6.04 (s, 1H), 5.77 (dd, J = 10.2, 4.5 Hz, 1H), 5.32 (d, J = 6.3 Hz, 1H), 5.27 (d, J = 10.2 Hz, 1H), 4.93 (s, 3H), 3.67 (s, 6H), 3.43 - 3.38 (m, 2H), 3.33 (s, 1H), 3.26–3.20 (m, 1H), 3.06 (d, J = 12.3 Hz, 1H), 2.78 (s, 3H), 2.71 (d, J = 16.2 Hz, 1H), 2.53 (s, 1H), 2.40 (q, J = 8.7 Hz, 1H), 2.16–2.06 (m, 2H), 1.98 (s, 3H), 1.25–1.17 (m, 1H), 0.94–0.87 (m, 1H), 0.43 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 170.4, 160.9, 159.4, 156.4, 154.3, 130.3, 129.8 (2C), 128.7, 125.6, 124.0, 122.5, 113.7 (2C), 105.0, 96.5, 81.7, 77.0, 75.8, 67.5, 66.2, 55.2, 55.1, 52.1, 51.5, 50.7, 45.1, 44.5, 42.8, 40.4, 31.3, 20.8, 7.4. ESIMS (*m*/*z*) 592.2 $[M+1]^+$.

5.2.19. 3-Demethoxycarbonyl-3-(piperonyloxycarbonylamino)-methyl-vindoline (26a). Compound **26a** was prepared from piperonyl alcohol as material. Yield: 69%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 9.00 (s), 6.83–6.67 (m. 4H), 6.25 (dd, J = 8.1, 1.8 Hz, 1H), 6.08 (s, 1H), 5.84 (s, 2H), 5.80 (dd, J = 10.2, 4.5 Hz, 1H), 5.39 (d, J = 6.0 Hz, 1H), 5.31 (d, J = 10.2 Hz, 1H), 4.96 (s, 1H), 4.92 (s, 2H), 3.70 (s, 6H), 3.50–3.42 (m, 2H), 3.37

(s, 1H), 3.31–3.25 (m, 1H), 3.10 (d, J = 12.3 Hz, 1H), 2.82 (s, 3H), 2.76 (d, J = 16.2 Hz, 1H), 2.57 (s, 1H), 2.45 (q, J = 9.6 Hz, 1H), 2.24–2.09 (m, 2H), 2.02 (s, 3H), 1.28–1.20 (m, 1H), 0.98–0.89 (m, 1H), 0.46 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 170.3, 160.9, 156.3, 154.3, 147.6, 147.3, 130.4, 130.2, 125.6, 123.9, 122.5, 121.8, 108.7, 108.0, 104.9, 100.9, 96.5, 81.7, 77.0, 75.8, 67.4, 66.3, 55.1, 52.0, 51.4, 50.7, 45.1, 44.4, 42.7, 40.4, 31.3, 20.7, 7.4. ESIMS (m/z) 606.2 $[M+1]^+$.

5.2.20. 3-Demethoxycarbonyl-3-(3-chlorobenzyloxycarbonylamino)-methyl-vindoline (27a). Compound **27a** was prepared from 3-chlorobenzyl alcohol as material. Yield: 78%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 9.11 (s), 7.34 (s, 1H), 7.27–7.20 (m. 2H), 6.86 (d, J = 8.4 Hz, 1H), 6.31 (d, J = 8.4 Hz, 1H), 6.14 (s, 1H), 5.88 (dd, J = 10.2, 4.5 Hz, 1H), 5.44 (d, J = 6.0 Hz, 1H), 5.37 (d, J = 10.2 Hz, 1H), 5.06 (s, 2H), 5.01 (s, 1H), 3.79 (s, 3H), 3.56–3.34 (m, 3H), 3.42 (s, 1H), 3.15 (d, J = 12.9 Hz, 1H), 2.88 (s, 3H), 2.82 (d, J = 16.2 Hz, 1H), 2.64 (s, 1H), 2.56–2.47 (m, 1H), 2.33–2.17 (m, 2H), 2.09 (s, 3H), 1.37–1.28 (m, 1H), 1.06–0.94 (m, 1H), 0.52 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 170.4, 161.0, 156.1, 154.3, 138.8, 134.2, 130.3, 129.7, 128.0, 127.7, 125.7, 125.5, 124.0, 122.6, 105.1, 96.6, 81.9, 77.0, 75.9, 67.4, 65.4, 55.2, 52.1, 51.4, 50.8, 45.2, 44.6, 42.8, 40.5, 31.3, 20.8, 7.4. ESIMS (m/z) 596.2 $[M+1]^+$.

5.2.21. 3-Demethoxycarbonyl-3-(4-chlorobenzyloxycarbonylamino)-methyl-vindoline (28a). Compound **28a** was prepared from 4-Chlorobenzyl alcohol as material. Yield: 78%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 9.01 (s), 7.29 (m. 4H), 6.87 (d, J = 8.4 Hz, 1H), 6.31 (d, J = 8.4 Hz, 1H), 6.14 (s, 1H), 5.87 (dd, J = 10.2, 4.5 Hz, 1H), 5.42 (d, J = 6.6 Hz, 1H), 5.36 (d, J = 10.2 Hz, 1H), 5.05 (s, 2H), 5.00 (s, 1H), 3.79 (s, 3H), 3.60–3.25 (m, 3H), 3.41 (s, 1H), 3.14 (d, J = 12.6 Hz, 1H), 2.88 (s, 3H), 2.84 (d, J = 16.2 Hz, 1H), 2.63 (s, 1H), 2.56–2.42 (m, 1H), 2.31–2.14 (m, 2H), 2.09 (s, 3H), 1.40–1.20 (m, 1H), 1.04–0.96 (m, 1H), 0.52 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 170.2, 160.9, 156.1, 154.2, 135.2, 133.5, 130.2, 129.2 (2C), 128.4 (2C), 125.5, 123.9, 122.5, 105.0, 96.5, 81.8, 76.9, 75.7, 67.3, 65.4, 55.0, 52.0, 51.4, 50.6, 45.1, 44.4, 42.7, 40.4, 31.2, 20.6, 7.3. ESIMS (m/z) 596.2 $[M+1]^+$.

5.2.22. 3-Demethoxycarbonyl-3-(2-nitro-benzyloxycarbonylamino)-methyl-vindoline (29a). Compound **29a** was prepared from 2-nitrobenzyl alcohol as material. Yield: 64%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 9.11 (s), 7.97 (d, J = 8.1 Hz, 1H), 7.52 (m, 2H), 7.36 (m. 1H), 6.80 (d, J = 8.4 Hz, 1H), 6.23 (d, J = 8.4 Hz, 1H), 6.06 (s, 1H), 5.81 (dd, J = 10.2, 4.5 Hz, 1H), 5.50 (d, J = 7.5 Hz, 1H), 5.42 (s, 2H), 5.30 (d, J = 10.2 Hz, 1H), 4.94 (s, 1H), 3.68 (s, 3H), 3.49–3.25 (m, 3H), 3.37 (s, 1H), 3.08 (d, J = 12.9 Hz, 1H), 2.81 (s, 3H), 2.76 (d, J = 16.2 Hz, 1H), 2.58 (s, 1H), 2.50–2.41 (m, 1H), 2.24–2.12 (m, 2H), 2.01 (s, 3H), 1.25–1.17 (m, 1H), 0.98–0.84 (m, 1H), 0.44 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 170.5, 161.0, 155.9, 154.3, 147.3, 133.7, 133.4, 130.3, 128.6, 128.4, 125.6, 124.9, 124.1, 122.7, 105.2, 96.7, 82.1, 77.0, 75.9, 67.4, 63.1, 55.3, 52.2, 51.5, 50.8, 45.2, 44.6, 42.8, 40.7, 31.4, 20.9, 7.5. ESIMS (m/z) 607.3 $[M+1]^+$.

5.2.23. 3-Demethoxycarbonyl-3-(4-nitro-benzyloxycarbonylamino)-methyl-vindoline (30a). Compound **30a** was prepared from 4-nitrobenzyl alcohol as material. Yield: 61%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 9.20 (s), 8.21 (d, J = 8.7 Hz, 2H), 8.50 (d, J = 8.7 Hz, 2H), 6.88 (d, J = 8.4 Hz, 1H), 6.32 (dd, J = 8.4, 2.4 Hz, 1H), 6.15 (s, 1H), 5.90 (dd, J = 10.2, 3.6 Hz, 1H), 5.52 (d, J = 7.5 Hz, 1H), 5.38 (d, J = 10.2 Hz, 1H), 5.19 (s, 2H), 5.01 (s, 1H), 3.79 (s, 3H), 3.58–3.33 (m, 3H), 3.42 (s, 1H), 3.16 (d, J = 12.9 Hz, 1H), 2.89 (s, 3H), 2.87 (d, J = 16.2 Hz, 1H), 2.65 (s, 1H), 2.58–2.49 (m, 1H), 2.33–2.23 (m, 2H), 2.09 (s, 3H), 1.34–1.25 (m, 1H), 1.03–0.96 (m, 1H), 0.53 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 170.4, 160.9, 155.8, 154.1, 147.3, 144.2, 130.2, 127.9 (2C), 125.5, 124.0, 123.5 (2C), 122.6, 105.1, 96.6, 82.0, 76.9, 75.7, 67.3, 64.9, 55.1, 52.1, 51.3, 50.7, 45.2, 44.5, 42.7, 40.6, 31.2, 20.7, 7.4. ESIMS (m/z) 607.2 $[M+1]^+$.

5.2.24. 3-Demethoxycarbonyl-3-(2′-oxo-3,5′-spirooxazolidino)-methyl-vindoline (31a). To a solution of compound **20a** (1 mmol, 574 mg) in dry THF (10 mL) was added sodium hydride (60% in oil, 48 mg, 1.2 mmol) at room temperature under a nitrogen atmosphere. After stirred for 2 h, the reaction mixture was washed with saturated ammonium chloride solution and then extracted with EtOAc (twice) and the combined organic extracts were washed with brine, dried (Na_2SO_4), concentrated, and purified by flash chromatography (2:1 hexanes/acetone) to provide a white solid (180 mg, 40%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 6.83 (d, J = 7.8 Hz, 1H), 6.12 (d, J = 7.8 Hz, 1H), 5.96 (s, 1H), 5.87 (dd, J = 9.9, 4.8 Hz, 1H), 5.30 (d, J = 9.9 Hz, 1H), 5.17 (s, 1H), 4.90 (s, 1H), 3.81 (s, 1H), 3.78 (s, 3H), 3.40 (dd, J = 16.5, 4.8 Hz, 1H), 3.18 (s, 2H), 3.21–3.12 (m, 2H), 3.10 (s, 3H), 2.69 (d, J = 16.5 Hz, 1H), 2.65 (s, 1H), 2.39–2.29 (m, 1H), 2.19–2.10 (m, 1H), 2.04 (s, 3H), 1.62–1.52 (m, 1H), 1.30–1.18 (m, 1H), 0.88 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 170.3, 160.9, 158.4, 151.1, 128.2, 127.9, 126.4, 120.4, 100.9, 92.5, 87.1, 75.2, 74.8, 62.5, 55.0, 52.9, 52.3, 51.6, 45.2, 44.4, 43.4, 33.5, 28.5, 20.9, 8.5. ESIMS (m/z) 454.1 $[M+1]^+$.

5.2.25. 3-Demethoxycarbonyl-3-(isobutylcarbamoyloxy)-methyl-vindoline (32a). To a cooled (cold water bath) solution of CDI (2.2 mmol) in CH_2Cl_2 (5 mL) was added the isobutyl amine (2.0 mmol) dropwise. After the solids dissolved, the water bath was removed, and the mixture stirred for a further 24 h. The reaction mixture was diluted with CH_2Cl_2 (5 mL) and quenched with water (20 mL). The aqueous layer was extracted with CH_2Cl_2 (4× 5 mL), the combined organic layers dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo to yield carbamoylimidazoles **30**, which were used in the next reaction without purification. Then, to a solution of compound **4** (1 mmol, 386 mg) and **30** in dry THF (10 mL) was added sodium hydride (60% in

oil, 80 mg, 2 mmol) at room temperature under a nitrogen atmosphere. After stirring for 2 h, the reaction mixture was washed with saturated ammonium chloride solution and then extracted with CH_2Cl_2 (twice) and the combined organic extracts were washed with brine and dried (Na_2SO_4). The residue was added to pyridine (1.0 mL) and Ac_2O (1.0 mL) at room temperature. After stirring the reaction mixture for 8h, saturated $NaHCO_3$ (5 mL) and EtOAc (20 mL) were added, and the organic phase was washed with H_2O (3× 10 mL) and brine (10 mL), and was dried, concentrated, and purified by flash chromatography (4:1 hexanes/acetone) to provide a white solid (275 mg, 52%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 9.10 (s), 6.86 (d, J = 8.4 Hz, 1H), 6.30 (d, J = 8.4 Hz, 1H), 6.12 (s, 1H), 5.89 (dd, J = 10.2, 2.4 Hz, 1H), 5.36 (d, J = 10.2 Hz, 1H), 5.04 (s, 1H), 4.89 (m, 1H), 4.10(s, 2H), 3.79 (s, 3H), 3.63 (s, 1H), 3.52–3.37 (m, 2H), 3.18 (q, J = 6.9 Hz, 1H), 2.88 (s, 3H), 2.82 (d, J = 15.9 Hz, 1H), 2.63 (s, 1H), 2.50 (q, J = 9.3 Hz, 1H), 2.31–2.26 (m, 2H), 2.14 (s, 3H), 1.52–1.41 (m, 2H), 1.38–1.22 (m, 3H), 1.05–0.98 (m, 1H), 1.02 (m, 1H), 0.91 (t, J = 7.2 Hz, 3H), 0.52 (t, J = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 170.4, 160.7, 156.0, 154.1, 129.9, 125.6, 123.9, 122.4, 104.4, 95.9, 81.1, 76.4, 75.9, 67.5, 66.7, 54.9, 51.6, 51.5, 50.5, 44.3, 42.4, 39.4, 31.6, 31.1, 20.6, 19.5, 13.4, 7.3. ESIMS (m/z) 528.2 $[M+1]^+$.

5.3. General procedure for the preparation of 8b–32b

Catharanthine Tartrate (486 mg, 1 mmol) and ferric chloride anhydrous (486 mg, 3 mmol) were combined in a mixture of glycine buffer (containing 320 mg of glycine and 250 mg of sodium chloride in 40 mL of water) and hydrochloric acid (40 mL, 0.1 N) under a nitrogen atmosphere. After 10 min of stirring at room temperature, compound **8a**–**32a** (1 mmol) was added. After 8 h of stirring at room temperature, sodium borohydride (80 mg) in ammonium hydroxide (8 mL) was added dropwise at 0 °C and it was left to react for 15 min. The reaction mixture was extracted with CH_2Cl_2 (4× 20 mL), and the organic phase was filtered with Celite and concentrated at reduced pressure, then the residue was followed by column chromatography on silica gel (100:1, $CHCl_3$/MeOH) to afford the compounds **8b**–**32b** as white solid in over 50% yield.

5.3.1. 3-Demethoxycarbonyl-3-(methoxycarbonylamino)-methyl-anhydrovinblastine (8b). $[\alpha]_D^{20}$ + 60.3° (c 0.74, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 9.26 (s, 1H), 8.04 (s, 1H), 7.51 (d, J = 7.5 Hz, 1H), 7.12 (m, 3H), 6.59 (s, 1H), 6.20 (s, 1H), 5.87 (dd, J = 10.2, 3.9 Hz, 1H), 5.48 (d, J = 5.7 Hz, 1H), 5.42 (d, J = 10.2 Hz, 1H), 5.36 (d, J = 6.6 Hz, 1H), 5.02 (s, 1H), 3.82 (s, 3H), 3.65 (s, 3H), 3.61 (s, 3H), 3.38 (s, 1H), 2.92 (s, 3H), 2.80 (d, J = 16.2 Hz, 1H), 2.60 (s, 1H), 2.14 (s, 3H), 1.93 (q, J = 7.5 Hz, 2H), 1.46 (m, 1H), 1.23 (m, 1H), 0.99 (t, J = 7.5 Hz, 3H), 0.80 (t, J = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 174.9, 171.0, 158.0, 157.4, 153.7, 140.0, 135.1, 131.0, 130.0, 129.6, 124.7, 124.0, 123.7, 123.7, 122.4, 121.8, 119.0, 118.5, 117.5, 110.6, 95.3, 82.2, 76.8, 76.1, 66.1, 56.0, 55.6, 54.7, 52.8, 52.5, 52.2, 50.7, 50.2 (2C), 45.9, 45.2, 44.9, 42.8, 40.8, 34.5, 33.0, 31.6, 28.0, 25.7, 21.1, 12.4, 8.4; ESIMS (m/e) 822.4 $[M+1]^+$. HRESIMS $C_{47}H_{60}N_5O_8$ $[M+H]^+$ calcd for 822.4442, found 822.4439.

5.3.2. 3-Demethoxycarbonyl-3-(ethoxycarbonylamino)-methyl-anhydrovinblastine (9b). $[\alpha]_D^{20}$ + 61.2° (c 0.56, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 9.25 (s, 1H), 8.00 (s, 1H), 7.53 (d, J = 7.5 Hz, 1H), 7.13 (m, 3H), 6.61 (s, 1H), 6.20 (s, 1H), 5.87 (dd, J = 10.2, 3.9 Hz, 1H), 5.48 (d, J = 6.3 Hz, 1H), 5.42 (d, J = 10.2 Hz, 1H), 5.30 (d, J = 3.9 Hz, 1H), 5.03 (s, 1H), 4.09 (q, J = 7.2 Hz, 2H), 3.83 (s, 3H), 3.62 (s, 3H), 3.40 (s, 1H), 2.94 (s, 3H), 2.82 (d, J = 15.9 Hz, 1H), 2.61 (s, 1H), 2.16 (s, 3H), 1.43 (m, 1H), 1.23 (t, J = 7.2 Hz, 3H), 1.21 (m, 1H), 1.00 (t, J = 7.5 Hz, 3H), 0.81 (t, J = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 174.4, 170.8, 157.8, 156.9, 153.8, 137.7, 134.9, 130.8, 129.8, 129.0, 124.7, 123.9, 123.8, 123.3, 122.8, 120.7, 119.4, 118.2, 115.6, 110.6, 95.0, 81.8, 77.0, 75.9, 65.9, 60.7, 55.9, 55.3, 54.4, 52.5, 52.5, 50.9, 50.1, 50.0, 45.4, 45.2, 44.6, 42.7, 40.4, 34.2, 31.5, 31.5, 27.8, 23.2, 21.0, 14.7, 12.4, 8.4; ESIMS (m/e) 836.4 $[M+1]^+$. HRESIMS $C_{48}H_{62}N_5O_8$ $[M+H]^+$ calcd for 836.4598, found 836.4594.

5.3.3. 3-Demethoxycarbonyl-3-(isopropyloxycarbonyl-amino)-methyl-anhydrovinblastine (10b). $[\alpha]_D^{20}$ + 64.2° (c 0.89, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 9.24 (s, 1H), 8.00 (s, 1H), 7.53 (d, J = 7.5 Hz, 1H), 7.18 (m, 3H), 6.60 (s, 1H), 6.20 (s, 1H), 5.87 (dd, J = 10.2, 3.9 Hz, 1H), 5.47 (d, J = 6.0 Hz, 1H), 5.42 (d, J = 10.2 Hz, 1H), 5.25 (d, J = 3.9 Hz, 1H), 5.03 (s, 1H), 4.89 (m, 1H), 3.83 (s, 3H), 3.64 (s, 3H), 3.38 (s, 1H), 2.93 (s, 3H), 2.82 (d, J = 15.9 Hz, 1H), 2.61 (s, 1H), 2.16 (s, 3H), 1.93 (q, J = 7.5 Hz, 2H), 1.43 (m, 1H), 1.25 (m, 1H), 1.23 (d, J = 7.2 Hz, 6H), 1.00 (t, J = 7.5 Hz, 3H), 0.81 (t, J = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 174.9, 171.0, 158.1, 156.7, 153.8, 139.9, 135.1, 131.0, 130.1, 129.6, 124.7, 124.0, 123.9, 123.7, 122.5, 121.7, 119.0, 118.5, 117.3, 110.6, 95.2, 82.2, 77.3, 76.1, 68.0, 66.2, 56.0, 55.6, 54.6, 52.7, 52.5, 52.2, 50.3 (2C), 46.0, 45.3, 44.9, 42.9, 40.7, 34.5, 32.9, 31.7, 28.0, 25.7, 22.3 (2C), 21.1, 12.4, 8.4; ESIMS (m/e) 850.4 $[M+1]^+$. HRESIMS $C_{49}H_{64}N_5O_8$ $[M+H]^+$ calcd for 850.4755, found 850.4758.

5.3.4. 3-Demethoxycarbonyl-3-(tertbutyloxycarbonyl-amino)-methyl-anhydrovinblastine (11b). $[\alpha]_D^{20}$ + 63.1° (c 0.58, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 9.18 (s, 1H), 8.01 (s, 1H), 7.51 (d, J = 7.2 Hz, 1H), 7.12 (m, 3H), 6.60 (s, 1H), 6.20 (s, 1H), 5.87 (dd, J = 10.2, 3.9 Hz, 1H), 5.47 (d, J = 6.0 Hz, 1H), 5.42 (d, J = 10.2 Hz, 1H), 5.17 (d, J = 3.9 Hz, 1H), 5.03 (s, 1H), 3.82 (s, 3H), 3.60 (s, 3H), 3.40 (s, 1H), 2.95 (s, 3H), 2.79 (d, J = 15.9 Hz, 1H), 2.60 (s, 1H), 2.15 (s, 3H), 1.93 (q, J = 7.5 Hz, 2H), 1.43 (s, 9H), 0.99 (t, J = 7.5 Hz, 3H), 0.81 (t, J = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 174.8, 170.8, 158.0, 156.3, 153.7, 140.1, 135.0, 131.1, 130.1, 129.5, 124.6, 123.9, 123.7, 123.6, 122.3, 118.9, 121.6, 118.5, 117.5, 110.5, 95.1, 82.1, 79.1, 77.3, 76.0, 66.1, 55.9, 55.5, 54.6, 52.7, 52.3, 52.3, 50.2 (2C), 46.0, 45.2, 44.6, 42.8, 40.6, 34.4, 33.1, 31.6, 28.5 (3C), 27.9, 25.9, 21.1, 12.4, 8.4; ESIMS

(*m*/*e*) 864.4 $[M+1]^+$. HRESIMS $C_{50}H_{66}N_5O_8$ $[M+H]^+$ calcd for 864.4911, found 864.4914.

5.3.5. 3-Demethoxycarbonyl-3-(isobutyloxycarbonylamino)-methyl-anhydrovinblastine (12b). $[\alpha]_D^{20}$ + 76° (*c* 0.06, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ: 9.30 (s, 1H), 8.00 (s, 1H), 7.53 (d, *J* = 7.5 Hz, 1H), 7.17–7.08 (m, 3H), 6.60 (s, 1H), 6.20 (s, 1H), 5.88 (dd, *J* = 10.2, 3.9 Hz, 1H), 5.47 (d, *J* = 5.4 Hz, 1H), 5.42 (d, *J* = 10.2 Hz, 1H), 5.30 (d, *J* = 3.9 Hz, 1H), 5.04 (s, 1H), 3.84 (d, *J* = 6.0 Hz, 2H), 3.83 (s, 3H), 3.62 (s, 3H), 3.40 (s, 1H), 2.93 (s, 3H), 2.82 (d, *J* = 15.9 Hz, 1H), 2.61 (s, 1H), 2.15 (s, 3H), 1.49–1.44 (m, 1H), 1.28–1.21 (m, 1H), 1.00 (t, *J* = 7.2 Hz, 3H), 0.91 (d, *J* = 6.0 Hz, 6H), 0.81 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 173.6, 170.6, 157.5, 156.8, 153.9, 134.6 (2C), 130.4, 129.4, 128.2, 124.6, 123.9, 123.8, 123.3, 122.6, 119.9, 119.1, 117.7, 112.9, 110.5, 94.7, 81.2, 76.8, 75.7, 70.7, 65.8, 55.7, 54.9, 54.0, 52.5, 52.3, 49.9 (2C), 48.9, 45.1, 44.6, 44.4, 42.4, 39.9, 33.7, 31.2, 29.4, 27.8, 27.5, 20.8, 19.6, 18.8 (2× C), 11.2, 8.1. ESIMS (*m*/*z*) 864.4 $[M+1]^+$. HRESIMS $C_{50}H_{66}N_5O_8$ $[M+H]^+$ calcd for 864.4911, found 864.4915.

5.3.6. 3-Demethoxycarbonyl-3-(2-methoxylethyloxycarbonylamino)-methyl-anhydrovinblastine (13b). $[\alpha]_D^{20}$ + 59.8° (*c* 0.74, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ: 9.26 (s, 1H), 8.04 (s, 1H), 7.48 (d, *J* = 7.2 Hz, 1H), 7.11 (m, 3H), 6.49 (s, 1H), 6.19 (s, 1H), 5.87 (dd, *J* = 10.2, 4.5 Hz, 1H), 5.61 (d, *J* = 5.4 Hz, 1H), 5.50 (d, *J* = 6.6 Hz, 1H), 5.41 (d, *J* = 10.2 Hz, 1H), 4.99 (s, 1H), 4.21 (s, 2H), 3.82 (s, 3H), 3.62 (s, 3H), 3.41 (s, 1H), 3.37 (s, 3H), 2.93 (s, 3H), 2.81 (d, *J* = 16.2 Hz, 1H), 2.59 (s, 1H), 2.14 (s, 3H), 1.99 (q, *J* = 7.5 Hz, 2H), 1.46 (m, 1H), 1.23 (m, 1H), 1.01 (t, *J* = 7.2 Hz, 3H), 0.77 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 174.4, 171.0, 158.0, 156.7, 154.0, 137.2, 135.1, 130.8, 130.0, 129.1, 124.9, 124.2, 123.9, 123.5, 123.1, 120.6, 119.7, 118.4, 115.2, 110.7, 95.1, 81.8, 77.2, 76.1, 71.1, 66.1, 64.0, 59.0, 56.0, 55.5, 54.1, 52.7, 52.7, 50.2 (3C), 45.3, 45.1, 44.8, 42.9, 40.5, 34.3, 31.6, 31.0, 27.9, 22.2, 21.1, 11.9, 8.4; ESIMS (*m*/*e*) 866.4 $[M+1]^+$. HRESIMS $C_{49}H_{64}N_5O_9$ $[M+H]^+$ calcd for 866.4704, found 866.4701.

5.3.7. 3-Demethoxycarbonyl-3-(bromoethyloxycarbonylamino)-methyl-anhydrovinblastine (14b). $[\alpha]_D^{20}$ + 71.4° (*c* 0.61, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ: 9.32 (s, 1H), 8.02 (s, 1H), 7.51 (d, *J* = 7.8 Hz, 1H), 7.12 (m, 3H), 6.60 (s, 1H), 6.20 (s, 1H), 5.87 (dd, *J* = 10.2, 4.5 Hz, 1H), 5.47 (m, 2H), 5.42 (d, *J* = 10.2 Hz, 1H), 5.02 (s, 1H), 4.34 (m, 2H), 3.82 (s, 3H), 3.60 (s, 3H), 3.38 (s, 1H), 2.92 (s, 3H), 2.81 (d, *J* = 16.2 Hz, 1H), 2.61 (s, 1H), 2.15 (s, 3H), 1.92 (q, *J* = 7.5 Hz, 2H), 1.46 (m, 1H), 1.23 (m, 1H), 0.98 (t, *J* = 7.5 Hz, 3H), 0.80 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 174.8, 171.8, 158.0, 156.0, 153.5, 139.9, 135.0, 130.9, 129.9, 129.5, 124.6, 123.8, 123.6, 123.5, 122.3, 121.7, 118.9, 118.4, 117.4, 110.5, 95.2, 82.1, 77.0, 75.9, 65.9, 64.2, 56.0, 55.5, 54.6, 52.7, 52.4, 52.1, 50.1, 50.0, 45.9, 45.1, 44.8, 42.6, 40.8, 34.3, 32.8, 31.5, 29.7, 27.9, 25.6, 21.1, 12.3, 8.3; ESIMS (*m*/*e*) 916.3 $[M+1]^+$. HRESIMS $C_{48}H_{61}BrN_5O_8$ $[M+H]^+$ calcd for 916.3683, found 916.3685.

5.3.8. 3-Demethoxycarbonyl-3-(*n*-pentanyloxycarbonylamino)-methyl-anhydrovinblastine (15b). $[\alpha]_D^{20}$ + 70.4° (*c* 0.45, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ: 9.27 (s, 1H), 8.00 (s, 1H), 7.54 (d, *J* = 7.5 Hz, 1H), 7.13 (m, 3H), 6.60 (s, 1H), 6.20 (s, 1H), 5.87 (dd, *J* = 10.2, 3.9 Hz, 1H), 5.48 (d, *J* = 6.0 Hz, 1H), 5.42 (d, *J* = 10.2 Hz, 1H), 5.30 (d, *J* = 3.9 Hz, 1H), 5.03 (s, 1H), 4.04 (t, *J* = 6.0 Hz, 2H), 3.83 (s, 3H), 3.64 (s, 3H), 3.38 (s, 1H), 2.93 (s, 3H), 2.82 (d, *J* = 15.9 Hz, 1H), 2.61 (s, 1H), 2.16 (s, 3H), 1.60 (m, 2H), 1.46 (m, 1H), 1.26(m, 4H), 1.23 (m, 1H), 1.00 (t, *J* = 7.2 Hz, 3H), 0.89 (d, *J* = 6.6 Hz, 3H), 0.81 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 174.9, 171.0, 158.1, 157.2, 153.7, 140.1, 135.1, 131.0, 130.1, 129.6, 124.7, 124.0, 123.7, 123.7, 122.4, 121.8, 119.0, 118.5, 117.5, 110.6, 95.2, 82.1, 77.2, 76.1, 66.5, 66.1, 56.0, 55.6, 54.7, 52.7, 52.5, 52.3, 50.2 (2C), 46.0, 45.3, 44.9, 42.9, 40.7, 34.5, 33.0, 31.7, 28.3, 28.0, 25.9, 22.4, 22.5, 21.1, 12.4, 10.5, 8.4; ESIMS (*m*/*e*) 878.5 $[M+1]^+$. HRESIMS $C_{51}H_{68}N_5O_8$ $[M+H]^+$ calcd for 878.5068, found 878.5065.

5.3.9. 3-Demethoxycarbonyl-3-(cyclopropylmethyloxycarbonylamino)-methyl-anhydrovinblastine (16b). $[\alpha]_D^{20}$ + 95° (*c* 0.18, $CHCl_3$), ^1H NMR ($CDCl_3$, 300 MHz) δ: 9.27 (s, 1H), 8.00 (s, 1H), 7.53 (d, *J* = 7.5 Hz, 1H), 7.17–7.10 (m, 3H), 6.60 (s, 1H), 6.20 (s, 1H), 5.87 (dd, *J* = 10.2, 4.2 Hz, 1H), 5.47 (d, *J* = 6.0 Hz, 1H), 5.42 (d, *J* = 10.2 Hz, 1H), 5.36 (d, *J* = 4.5 Hz, 1H), 5.03 (s, 1H), 3.89 (d, *J* = 7.2 Hz, 2H), 3.83 (s, 3H), 3.62 (s, 3H), 3.41 (s, 1H), 2.94 (s, 3H), 2.82 (d, *J* = 15.9 Hz, 1H), 2.61 (s, 1H), 2.15 (s, 3H), 1.49–1.44 (m, 1H), 1.28–1.21 (m, 1H), 1.00 (t, *J* = 7.2 Hz, 3H), 0.81 (t, *J* = 7.2 Hz, 3H), 0.51 (d, *J* = 4.8 Hz, 2H), 0.25 (d, *J* = 4.8 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 175.0, 171.0, 158.1, 157.1, 153.8, 140.1, 135.2, 131.2, 130.1, 129.7, 124.7, 124.1, 123.8, 123.8, 122.5, 121.8, 119.0, 118.6, 117.6, 110.7, 95.3, 82.2, 77.3, 76.1, 69.7, 66.3, 56.0, 55.7, 54.7, 52.8, 52.5, 52.4, 50.3 (2C), 46.1, 45.3, 45.0, 43.0, 40.8, 34.5, 33.1, 31.7, 28.0, 25.9, 21.2, 12.5, 10.3, 8.3, 3.2 (2× C); ESIMS (*m*/*z*) 862.4 $[M+1]^+$. HRESIMS $C_{50}H_{64}N_5O_8$ $[M+H]^+$ calcd for 862.4755, found 862.4752.

5.3.10. 3-Demethoxycarbonyl-3-(cyclobutyloxycarbonylamino)-methyl-anhydrovinblastine (17b). $[\alpha]_D^{20}$ + 62.2° (*c* 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ: 9.25 (s, 1H), 8.00 (s, 1H), 7.53 (d, *J* = 7.5 Hz, 1H), 7.13 (m, 3H), 6.60 (s, 1H), 6.20 (s, 1H), 5.87 (dd, *J* = 10.2, 3.9 Hz, 1H), 5.47 (d, *J* = 5.4 Hz, 1H), 5.42 (d, *J* = 10.2 Hz, 1H), 5.30 (d, *J* = 3.9 Hz, 1H), 5.02 (s, 1H), 4.92 (m, 1H), 3.83 (s, 3H), 3.64 (s, 3H), 3.39 (s, 1H), 2.93 (s, 3H), 2.82 (d, *J* = 15.9 Hz, 1H), 2.62 (s, 1H), 2.30 (m, 2H), 2.15 (s, 3H), 2.02 (m, 2H), 1.92 (q, *J* = 7.5 Hz, 2H), 1.73 (m, 1H), 1.57 (m, 1H), 1.25 (m, 1H), 1.00 (t, *J* = 7.5 Hz, 3H), 0.81 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 174.9, 170.9, 158.0, 156.3, 153.7, 140.2, 135.1, 131.1, 130.1, 129.6, 124.7, 124.0, 123.7, 123.7, 122.4, 121.8, 119.0, 118.5, 117.6, 110.6, 95.3, 82.2, 77.1, 76.0, 68.9, 66.0, 56.0, 55.6, 54.7,

52.7, 52.5, 52.3, 50.2 (2C), 46.0, 45.3, 44.7, 42.8, 40.9, 34.4, 33.0, 31.6, 30.7, 30.5, 28.0, 25.9, 21.2, 13.3, 12.4, 8.5; ESIMS (*m/e*) 862.4 $[M+1]^+$. HRESIMS $C_{50}H_{64}N_5O_8$ $[M+H]^+$ calcd for 862.4755, found 862.4754.

5.3.11. 3-Demethoxycarbonyl-3-(cyclopentyloxycarbonylamino)-methyl-anhydrovinblastine (18b). $[\alpha]_D^{20}$ + 68.3° (*c* 0.64, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ: 9.25 (s, 1H), 8.00 (s, 1H), 7.53 (d, *J* = 7.5 Hz, 1H), 7.13 (m, 3H), 6.60 (s, 1H), 6.20 (s, 1H), 5.87 (dd, *J* = 10.2, 3.9 Hz, 1H), 5.47 (d, *J* = 5.1 Hz, 1H), 5.42 (d, *J* = 10.2 Hz, 1H), 5.21 (d, *J* = 3.9 Hz, 1H), 5.08 (m, 1H), 5.03 (s, 1H), 3.83 (s, 3H), 3.64 (s, 3H), 3.40 (s, 1H), 2.93 (s, 3H), 2.82 (d, *J* = 15.9 Hz, 1H), 2.60 (s, 1H), 2.40 (m, 2H), 2.16 (s, 3H), 1.92 (q, *J* = 7.5 Hz, 2H), 1.72 (m, 2H), 1.58 (m, 4H), 1.44 (m, 2H), 1.25 (m, 1H), 1.00 (t, *J* = 7.5 Hz, 3H), 0.81 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 174.9, 170.9, 158.1, 156.9, 153.8, 140.1, 135.2, 131.2, 130.1, 129.6, 124.7, 124.1, 123.8, 123.8, 122.4, 121.8, 119.0, 118.6, 117.5, 110.6, 95.3, 82.2, 77.7, 77.4, 76.1, 66.3, 56.0, 55.7, 54.7, 53.6, 52.7, 52.5, 52.4, 50.3, 46.1, 45.3, 45.0, 42.9, 40.7, 34.5, 33.1, 31.7, 33.0 (2C), 28.0, 26.0, 24.0 (2C), 21.1, 12.5, 8.5; ESIMS (*m/e*) 876.4 $[M+1]^+$. HRESIMS $C_{51}H_{66}N_5O_8$ $[M+H]^+$ calcd for 876.4911, found 876.4915.

5.3.12. 3-Demethoxycarbonyl-3-(cyclohexanyloxycarbonylamino)-methyl-anhydrovinblastine (19b). $[\alpha]_D^{20}$ + 65.0° (*c* 0.58, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ: 9.25 (s, 1H), 8.00 (s, 1H), 7.52 (d, *J* = 7.5 Hz, 1H), 7.13 (m, 3H), 6.60 (s, 1H), 6.20 (s, 1H), 5.87 (dd, *J* = 10.2, 3.9 Hz, 1H), 5.47 (d, *J* = 5.1 Hz, 1H), 5.42 (d, *J* = 10.2 Hz, 1H), 5.26 (d, *J* = 6.0 Hz, 1H), 5.03 (s, 1H), 4.62 (m, 1H), 3.83 (s, 3H), 3.63 (s, 3H), 3.40 (s, 1H), 2.93 (s, 3H), 2.82 (d, *J* = 15.9 Hz, 1H), 2.61 (s, 1H), 2.40 (m, 2H), 2.16 (s, 3H), 1.72 (m, 2H), 1.62 (m, 2H), 1.42 (m, 1H), 1.26 (m, 6H), 1.00 (t, *J* = 7.5 Hz, 3H), 0.81 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 175.0, 171.0, 158.1, 156.7, 153.8, 140.1, 135.2, 131.2, 130.2, 129.7, 124.7, 124.1, 123.9, 123.8, 122.5, 121.8, 119.1, 118.6, 117.6, 110.6, 95.3, 82.2, 77.4, 76.1, 73.0, 66.3, 56.0, 55.7, 54.7, 52.8, 52.5, 52.4, 50.4 (2C), 46.1, 45.3, 45.0, 42.9, 40.7, 34.5, 33.1, 31.7 (2C), 31.7, 28.0, 25.9, 26.0, 24.0 (2C), 21.2, 12.5, 8.5; ESIMS (*m/e*) 890.5 $[M+1]^+$. HRESIMS $C_{52}H_{68}N_5O_8$ $[M+H]^+$ calcd for 890.5068, found 890.5065.

5.3.13. 3-Demethoxycarbonyl-3-(phenyloxycarbonylamino)-methyl-anhydrovinblastine (20b). $[\alpha]_D^{20}$ + 85.7° (*c* 0.35, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ: 9.38 (s, 1H), 8.01 (s, 1H), 7.53 (d, *J* = 7.8 Hz, 1H), 7.35 (t, *J* = 7.8 Hz, 2H), 7.14 (m, 6H), 6.63 (s, 1H), 6.23 (s, 1H), 5.89 (dd, *J* = 10.2, 3.9 Hz, 1H), 5.76 (d, *J* = 7.8 Hz, 1H), 5.46 (m, 2H), 5.07 (s, 1H), 3.83 (s, 3H), 3.63 (s, 3H), 3.45 (s, 1H), 2.99 (s, 3H), 2.85 (d, *J* = 15.9 Hz, 1H), 2.65 (s, 1H), 2.18 (s, 3H), 1.43 (m, 1H), 1.21 (m, 1H), 1.00 (t, *J* = 7.5 Hz, 3H), 0.82 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 174.2, 170.9, 157.8, 154.8, 153.8, 151.1, 134.9 (2C), 130.8, 129.8, 129.3 (2C), 129.3, 128.9, 125.2, 124.8, 123.9, 123.2, 123.0, 121.6 (2C), 120.7, 119.6, 118.9, 118.1, 110.7, 95.3, 82.2, 76.8, 75.8, 65.9, 55.9, 55.3, 54.4, 52.7, 52.6, 52.5, 50.5, 50.1, 50.0, 45.3, 44.9, 42.7, 40.9, 34.1, 33.0, 31.5, 27.8 (2C), 21.1, 11.8, 8.4; ESIMS (*m/e*) 884.4 $[M+1]^+$. HRESIMS $C_{52}H_{62}N_5O_8$ $[M+H]^+$ calcd for 884.4598, found 884.4594.

5.3.14. 3-Demethoxycarbonyl-3-(2-methoxyl-phenyloxycarbonylamino)-methyl-anhydrovinblastine (21b). $[\alpha]_D^{20}$ + 82.0° (*c* 0.35, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ: 9.35 (s, 1H), 8.02 (s, 1H), 7.53 (d, *J* = 7.8 Hz, 1H), 7.14 (m, 5H), 6.94 (d, *J* = 8.4 Hz, 2H), 6.63 (s, 1H), 6.23 (s, 1H), 5.89 (dd, *J* = 10.2, 3.9 Hz, 1H), 5.81 (d, *J* = 8.1 Hz, 1H), 5.47 (m, 2H), 5.06 (s, 1H), 3.83 (s, 3H), 3.81 (s, 3H), 3.64 (s, 3H), 3.49 (s, 1H), 3.00 (s, 3H), 2.85 (d, *J* = 15.9 Hz, 1H), 2.65 (s, 1H), 2.18 (s, 3H), 1.92 (q, *J* = 7.5 Hz, 2H), 1.43 (m, 1H), 1.21 (m, 1H), 1.00 (t, *J* = 7.5 Hz, 3H), 0.83 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 174.9, 171.0, 158.1, 154.7, 153.8, 151.9, 140.2, 140.1, 135.2, 131.2, 130.1, 129.6, 126.6, 124.8, 124.0, 123.8, 123.8, 123.5, 122.5, 121.9, 120.9, 119.1, 118.6, 117.5, 112.5, 110.7, 95.4, 82.2, 77.1, 76.2, 66.2, 56.0, 55.9, 55.7, 54.7, 52.9, 52.6, 52.4, 50.3 (2C), 46.0, 45.3, 45.1, 42.9, 40.9, 34.5, 33.1, 31.7, 28.0, 25.9, 21.2, 12.5, 8.5; ESIMS (*m/e*) 914.4 $[M+1]^+$. HRESIMS $C_{53}H_{64}N_5O_9$ $[M+H]^+$ calcd for 914.4704, found 914.4701.

5.3.15. 3-Demethoxycarbonyl-3-(4-methoxyl-phenyloxycarbonylamino)-methyl-anhydrovinblastine (22b). $[\alpha]_D^{20}$ + 84.1° (*c* 0.35, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ: 9.36 (s, 1H), 8.02 (s, 1H), 7.53 (d, *J* = 7.5 Hz, 1H), 7.14 (m, 5H), 7.02 (d, *J* = 8.4 Hz, 2H), 6.94 (d, *J* = 8.4 Hz, 2H), 6.63 (s, 1H), 6.23 (s, 1H), 5.89 (dd, *J* = 10.2, 3.9 Hz, 1H), 5.81 (d, *J* = 8.1 Hz, 1H), 5.47 (m, 2H), 5.07 (s, 1H), 3.83 (s, 3H), 3.79 (s, 3H), 3.64 (s, 3H), 3.46 (s, 1H), 2.98 (s, 3H), 2.85 (d, *J* = 15.9 Hz, 1H), 2.65 (s, 1H), 2.17 (s, 3H), 1.92 (q, *J* = 7.5 Hz, 2H), 1.43 (m, 1H), 1.21 (m, 1H), 1.00 (t, *J* = 7.5 Hz, 3H), 0.82 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 174.9, 171.0, 158.1, 157.0, 155.4, 153.7, 144.8, 140.2, 135.2, 131.1, 130.1, 129.7, 124.7, 124.0, 123.8, 123.8, 122.6 (2C), 122.5, 122.1, 119.1, 118.6, 117.5, 114.5 (2C), 110.7, 95.5, 82.7, 77.0, 76.0, 66.1, 56.0, 55.7, 54.7, 52.9, 52.7, 52.5, 52.4, 50.3 (2C), 46.0, 45.3 (2C), 42.9, 41.2, 34.5, 33.1, 31.7, 28.0, 26.0, 21.2, 12.4, 8.5; ESIMS (*m/e*) 914.4 $[M+1]^+$. HRESIMS $C_{53}H_{64}N_5O_9$ $[M+H]^+$ calcd for 914.4704, found 914.4703.

5.3.16. 3-Demethoxycarbonyl-3-(benzyloxycarbonylamino)-methyl-anhydrovinblastine (23b). $[\alpha]_D^{20}$ + 67.2° (*c* 0.48, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ: 9.30 (s, 1H), 8.04 (s, 1H), 7.54 (d, *J* = 7.5 Hz, 1H), 7.35 (m, 5H), 7.15 (m, 3H), 6.63 (s, 1H), 6.22 (s, 1H), 5.88 (dd, *J* = 10.2, 4.5 Hz, 1H), 5.48 (m, 2H), 5.42 (d, *J* = 10.2 Hz, 1H), 5.12 (s, 2H), 5.05 (s, 1H), 3.84 (s, 3H), 3.63 (s, 3H), 3.40 (s, 1H), 2.93 (s, 3H), 2.82 (d, *J* = 15.9 Hz, 1H), 2.63 (s, 1H), 2.14 (s, 3H), 1.95 (q, *J* = 7.5 Hz, 2H), 1.46 (m, 1H), 1.28 (m, 1H), 1.01 (t, *J* = 7.5 Hz, 3H), 0.83 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 174.8, 170.8, 157.9, 156.6, 153.5, 140.0, 136.7, 135.0, 131.0, 130.0, 129.5, 128.5 (2C), 128.0, 128.0 (2C), 124.6, 123.9, 123.6, 123.6, 122.3,

121.7, 118.9, 118.4, 117.5, 110.6, 95.2, 82.0, 77.0, 76.0, 66.6, 65.9, 55.9, 55.5, 54.6, 52.6, 52.4, 52.3, 50.0 (2C), 45.9, 45.1, 44.8, 42.7, 40.7, 34.4, 33.0, 31.5, 27.8, 25.9, 21.0, 12.4, 8.4; ESIMS (*m/e*) 898.4 $[M+1]^+$. HRESIMS $C_{53}H_{64}N_5O_8$ $[M+H]^+$ calcd for 898.4755, found 898.4751.

5.3.17. 3-Demethoxycarbonyl-3-(2-methoxylbenzyloxycarbonylamino)-methyl-anhydrovinblastine (24b). $[\alpha]_D^{20}$ + 68.1° (*c* 0.46, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ: 9.26 (s, 1H), 8.03 (s, 1H), 7.53 (d, *J* = 7.5 Hz, 1H), 7.34 (d, *J* = 7.5 Hz, 1H), 7.27 (d, *J* = 7.5 Hz, 1H), 7.13 (m, 3H), 6.93 (d, *J* = 7.5 Hz, 1H), 6.89 (d, *J* = 7.5 Hz, 1H), 6.60 (s, 1H), 6.21 (s, 1H), 5.87 (dd, *J* = 10.2, 3.9 Hz, 1H), 5.44 (m, 3H), 5.17 (s, 2H), 5.04 (s, 1H), 3.83 (s, 6H), 3.62 (s, 3H), 3.38 (s, 1H), 2.93 (s, 3H), 2.81 (d, *J* = 15.3 Hz, 1H), 2.61 (s, 1H), 2.14 (s, 3H), 1.94 (q, *J* = 7.5 Hz, 2H), 1.47 (m, 1H), 1.26 (m, 1H), 1.00 (t, *J* = 7.5 Hz, 3H), 0.82 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 174.9, 170.9, 158.0, 157.7, 156.9, 153.7, 140.1, 135.1, 131.1, 130.1, 129.6, 129.6, 129.4, 125.1, 124.6, 124.0, 123.7, 123.7, 122.4, 121.7, 120.5, 119.0, 118.5, 117.5, 110.6, 110.5, 95.2, 82.0, 77.2, 76.1, 66.1, 62.1, 56.0, 55.6, 55.5, 54.7, 52.7, 52.5, 52.3, 50.2 (2C), 46.0, 45.2, 45.0, 42.8, 40.7, 34.5, 33.0, 31.6, 28.0, 25.9, 21.1, 12.4, 8.4; ESIMS (*m/e*) 928.4 $[M+1]^+$. HRESIMS $C_{54}H_{66}N_5O_9$ $[M+H]^+$ calcd for 928.4860, found 928.4861.

5.3.18. 3-Demethoxycarbonyl-3-(4-methoxylbenzyloxycarbonylamino)-methyl-anhydrovinblastine (25b). $[\alpha]_D^{20}$ + 69.2° (*c* 0.48, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ: 9.24 (s, 1H), 8.03 (s, 1H), 7.53 (d, *J* = 7.5 Hz, 1H), 7.26 (d, *J* = 7.5 Hz, 2H), 7.13 (m, 3H), 6.87 (d, *J* = 7.5 Hz, 2H), 6.61 (s, 1H), 6.20 (s, 1H), 5.87 (dd, *J* = 10.5, 4.5 Hz, 1H), 5.49 (d, *J* = 5.4 Hz, 1H), 5.42 (m, 2H), 5.03 (s, 3H), 3.83 (s, 3H), 3.79 (s, 3H), 3.62 (s, 3H), 3.38 (s, 1H), 2.91 (s, 3H), 2.80 (d, *J* = 16.2 Hz, 1H), 2.61 (s, 1H), 2.13 (s, 3H), 1.94 (q, *J* = 7.5 Hz, 2H), 1.43 (m, 1H), 1.21 (m, 1H), 1.00 (t, *J* = 7.5 Hz, 3H), 0.81 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 174.9, 171.0, 159.6, 158.1, 156.8, 153.7, 140.2, 135.2, 131.2, 130.1, 130.0 (2C), 129.6, 129.0, 124.7, 124.1, 123.8, 123.8, 122.4, 122.8, 119.0, 118.6, 117.6, 114.0 (2C), 110.6, 95.3, 82.1, 77.2, 76.1, 66.5, 66.1, 56.0, 55.7, 55.4, 54.7, 52.8, 52.5, 52.4, 50.2 (2C), 46.0, 45.2, 45.0, 42.9, 40.8, 34.5, 33.1, 31.7, 28.0, 26.0, 21.1, 12.5, 8.5; ESIMS (*m/e*) 928.4 $[M+1]^+$. HRESIMS $C_{54}H_{66}N_5O_9$ $[M+H]^+$ calcd for 928.4860, found 928.4858.

5.3.19. 3-Demethoxycarbonyl-3-(piperonyloxycarbonylamino)-methyl-anhydrovinblastine (26b). $[\alpha]_D^{20}$ + 63.5° (*c* 0.38, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ: 9.26 (s, 1H), 8.03 (s, 1H), 7.52 (d, *J* = 7.5 Hz, 1H), 7.13 (m, 3H), 6.80 (m, 3H), 6.60 (s, 1H), 6.21 (s, 1H), 5.94 (s, 2H), 5.87 (dd, *J* = 10.5, 4.5 Hz, 1H), 5.49 (d, *J* = 6.0 Hz, 1H), 5.42 (d, *J* = 10.5 Hz), 5.03 (s, 3H), 5.00 (s, 2H), 3.83 (s, 3H), 3.62 (s, 3H), 3.35 (s, 1H), 2.91 (s, 3H), 2.81 (d, *J* = 15.9 Hz, 1H), 2.61 (s, 1H), 2.13 (s, 3H), 1.94 (q, *J* = 7.5 Hz, 2H), 1.43 (m, 1H), 1.21 (m, 1H), 1.00 (t, *J* = 7.5 Hz, 3H), 0.81 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 174.9, 171.0, 158.0, 156.7, 153.7, 147.8, 147.6, 140.0, 135.1, 131.1, 130.6, 130.0, 129.6, 124.7, 124.0, 123.7, 123.7, 122.4, 122.1, 121.8, 119.0, 118.5, 117.4, 110.6, 109.0, 108.3, 101.2, 95.3, 82.1, 77.2, 76.1, 66.7, 66.1, 56.0, 55.6, 54.7, 52.7, 52.5, 52.3, 50.2 (2C), 46.0, 45.2, 44.9, 42.8, 40.8, 34.5, 33.0, 31.7, 27.9, 25.8, 21.1, 12.4, 8.4; ESIMS (*m/e*) 942.4 $[M+1]^+$. HRESIMS $C_{54}H_{64}N_5O_{10}$ $[M+H]^+$ calcd for 942.4653, found 942.4651.

5.3.20. 3-Demethoxycarbonyl-3-(3-chlorobenzyloxycarbonylamino)-methyl-anhydrovinblastine (27b). $[\alpha]_D^{20}$ + 65.1° (*c* 0.49, $CHCl_3$); ^1H NMR ($CDCl_3$, 0 MHz): δ: 9.21 (s, 1H), 7.94 (s, 1H), 7.45 (d, *J* = 7.5 Hz, 1H), 7.16 (m, 4H), 7.04 (m, 3H), 6.53 (s, 1H), 6.13 (s, 1H), 5.80 (dd, *J* = 10.2, 3.9 Hz, 1H), 5.38 (m, 3H), 5.05 (s, 2H), 4.96 (s, 1H), 3.76 (s, 3H), 3.57 (s, 3H), 3.31 (s, 1H), 2.84 (s, 3H), 2.75 (d, *J* = 15.9 Hz, 1H), 2.54 (s, 1H), 2.06 (s, 3H), 1.87 (q, *J* = 7.5 Hz, 2H), 1.39 (m, 1H), 1.18 (m, 1H), 0.93 (t, *J* = 7.5 Hz, 3H), 0.74 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 174.9, 171.0, 158.1, 156.5, 153.7, 140.0, 139.0, 135.2, 134.5, 131.1, 130.1, 129.9, 129.6, 128.3, 128.0, 126.0, 124.7, 124.0, 123.7, 123.7, 122.5, 121.9, 119.1, 118.6, 117.4, 110.7, 95.4, 82.0, 77.2, 76.1, 66.1, 65.8, 56.0, 55.7, 54.7, 52.8, 52.6, 52.3, 50.2 (2C), 46.0, 45.3, 45.0, 42.9, 40.9, 34.5, 33.0, 31.7, 28.0, 25.7, 21.1, 12.4, 8.5; ESIMS (*m/e*) 932.4 $[M+1]^+$. HRESIMS $C_{53}H_{63}ClN_5O_8$ $[M+H]^+$ calcd for 932.4365, found 932.4361.

5.3.21. 3-Demethoxycarbonyl-3-(4-chlorobenzyloxycarbonylamino)-methyl-anhydrovinblastine (28b). $[\alpha]_D^{20}$ + 65.7° (*c* 0.47, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ: 9.29 (s, 1H), 8.02 (s, 1H), 7.53 (d, *J* = 7.8 Hz, 1H), 7.30 (m, 4H), 7.13 (m, 3H), 6.61 (s, 1H), 6.20 (s, 1H), 5.87 (dd, *J* = 10.2, 3.9 Hz, 1H), 5.45 (m, 3H), 5.06 (s, 2H), 5.03 (s, 1H), 3.83 (s, 3H), 3.62 (s, 3H), 3.37 (s, 1H), 2.90 (s, 3H), 2.81 (d, *J* = 15.9 Hz, 1H), 2.61 (s, 1H), 2.12 (s, 3H), 1.93 (q, *J* = 7.5 Hz, 2H), 1.47 (m, 1H), 1.24 (m, 1H), 1.00 (t, *J* = 7.5 Hz, 3H), 0.81 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 174.9, 171.0, 158.1, 156.5, 153.7, 140.2, 135.5, 135.2, 134.0, 131.2, 130.1, 129.7, 129.6 (2C), 128.8 (2C), 124.7, 124.0, 123.8, 123.7, 122.5, 122.0, 119.1, 118.6, 117.6, 110.7, 95.4, 82.3, 77.2, 76.1, 66.1, 65.9, 56.1, 55.7, 54.7, 52.8, 52.6, 52.4, 50.3 (2C), 46.1, 45.3, 45.0, 42.9, 40.9, 34.5, 33.1, 31.7, 28.0, 26.0, 21.2, 12.5, 8.5; ESIMS (*m/e*) 932.4 $[M+1]^+$. HRESIMS $C_{53}H_{63}ClN_5O_8$ $[M+H]^+$ calcd for 932.4365, found 932.4362.

5.3.22. 3-Demethoxycarbonyl-3-(2-nitro-benzyloxycarbonylamino)-methyl-anhydrovinblastine (29b). $[\alpha]_D^{20}$ + 66.4° (*c* 0.48, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ: 9.35 (s, 1H), 8.03 (s, 1H), 8.07 (d, *J* = 8.1 Hz, 1H), 7.60 (m, 2H), 7.52 (d, *J* = 7.5 Hz, 1H), 7.46 (m, 1H), 7.13 (m, 3H), 6.62 (s, 1H), 6.22 (s, 1H), 5.88 (dd, *J* = 10.2, 3.9 Hz, 1H), 5.51 (m, 3H), 5.51 (s, 2H), 5.05 (s, 1H), 3.83 (s, 3H), 3.64 (s, 3H), 3.41 (s, 1H), 2.93 (s, 3H), 2.83 (d, *J* = 16.2 Hz, 1H), 2.63 (s, 1H), 2.14 (s, 3H), 1.93 (q, *J* = 7.5 Hz, 2H), 1.47 (m, 1H), 1.24 (m, 1H), 1.00 (t, *J* = 7.5 Hz, 3H), 0.82 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 174.9, 170.9, 158.1, 156.0, 153.6, 147.2, 140.1, 135.1, 133.7, 133.7, 131.1, 130.0, 129.6, 128.8, 128.6, 125.0, 124.7, 124.0, 123.7, 123.7, 122.4, 122.0, 119.0, 118.6, 117.5, 110.6, 95.3, 82.3, 77.1, 76.0, 66.1, 63.3, 56.1, 55.6,

54.7, 52.8, 52.5, 52.4, 50.2 (2C), 46.0, 45.3, 45.0, 42.8, 40.9, 34.5, 33.1, 31.6, 28.0, 26.0, 21.1, 12.4, 8.4; ESIMS (*m/e*) 943.4 $[M+1]^+$. HRESIMS $C_{53}H_{63}N_6O_{10}$ $[M+H]^+$ calcd for 943.4605, found 943.4607.

5.3.23. 3-Demethoxycarbonyl-3-(4-nitro-benzyloxycarbonylamino)-methyl-anhydrovinblastine (30b). $[\alpha]_D^{20}$ + 66.7° (*c* 0.49, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ: 9.36 (s, 1H), 8.19 (d, *J* = 8.7 Hz, 2H), 8.03 (s, 1H), 7.52 (d, *J* = 7.5 Hz, 1H), 7.52 (d, *J* = 8.7 Hz, 2H), 7.13 (m, 3H), 6.61 (s, 1H), 6.21 (s, 1H), 5.88 (dd, *J* = 10.2, 3.9 Hz, 1H), 5.49 (m, 3H), 5.19 (s, 2H), 5.03 (s, 1H), 3.83 (s, 3H), 3.62 (s, 3H), 3.38 (s, 1H), 2.90 (s, 3H), 2.83 (d, *J* = 16.2 Hz, 1H), 2.63 (s, 1H), 2.13 (s, 3H), 1.93 (q, *J* = 7.5 Hz, 2H), 1.47 (m, 1H), 1.24 (m, 1H), 1.00 (t, *J* = 7.5 Hz, 3H), 0.81 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 174.9, 170.9, 158.1, 156.2, 153.6, 147.7, 144.4, 140.1, 135.2, 131.0, 130.0, 129.6, 128.2 (2C), 124.7, 123.9, 123.8 (3C), 123.7, 122.5, 122.1, 119.0, 118.5, 117.6, 110.6, 95.4, 82.4, 77.1, 76.0, 66.1, 65.2, 56.1, 55.7, 54.7, 52.8, 52.5, 52.3, 50.2 (2C), 46.0, 45.3, 45.1, 42.8, 41.0, 34.5, 33.1, 31.7, 28.0, 25.9, 21.1, 12.4, 8.4; ESIMS (*m/e*) 943.4 $[M+1]^+$. HRESIMS $C_{53}H_{63}N_6O_{10}$ $[M+H]^+$ calcd for 943.4605, found 943.4606.

5.3.24. 3-Demethoxycarbonyl-3-(2′-oxo-3,5′-spirooxazolidino)-methyl-anhydrovinblastine (31b). $[\alpha]_D^{20}$ − 30.7° (*c* 0.43, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ: 8.43 (s, 1H), 7.51 (d, *J* = 7.2 Hz, 1H), 7.12 (m, 3H), 6.63 (s, 1H), 6.03 (s, 1H), 5.80 (s, 1H), 5.75 (dd, *J* = 10.2, 3.9 Hz, 1H), 5.46 (d, *J* = 5.7 Hz, 1H), 5.27 (d, *J* = 10.2 Hz, 1H), 5.23 (s, 1H), 3.82 (s, 3H), 3.79 (s, 1H), 3.63 (s, 3H), 3.12 (s, 3H), 2.39 (s, 1H), 2.03 (s, 3H), 0.99 (t, *J* = 7.5 Hz, 3H), 0.90 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 175.4, 171.3, 158.4, 158.0, 150.4, 140.2, 135.3, 131.4, 129.2, 127.9, 126.8, 126.8, 124.3, 122.5, 122.1, 118.9, 118.8, 118.3, 116.9, 110.7, 90.9, 87.2, 74.8 (2C), 62.7, 56.2, 55.9, 54.3, 53.5, 52.5, 52.4, 52.2, 51.5, 46.2, 45.5, 44.9, 44.4, 34.8, 34.0, 33.6, 28.9, 27.9, 25.9, 21.3, 12.4, 9.2; ESIMS (*m/e*) 790.4 $[M+1]^+$. HRESIMS $C_{46}H_{56}N_5O_7$ $[M+H]^+$ calcd for 790.4179, found 790.4180.

5.3.25. 3-Demethoxycarbonyl-3-(isobutylcarbamoyloxy)-methyl-anhydrovinblastine (32b). $[\alpha]_D^{20}$ + 68.0° (*c* 0.38, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ: 9.29 (s, 1H), 8.03 (s, 1H), 7.53 (d, *J* = 7.5 Hz, 1H), 7.14 (m, 3H), 6.60 (s, 1H), 6.18 (s, 1H), 5.88 (dd, *J* = 10.2, 3.9 Hz, 1H), 5.51 (s, 1H), 5.42 (d, *J* = 10.2 Hz, 1H), 5.06 (s, 1H), 4.90(m, 1H), 4.10 (s, 2H), 3.84 (s, 3H), 3.63 (s, 3H), 3.54 (s, 1H), 2.92 (s, 3H), 2.81 (d, *J* = 15.6 Hz, 1H), 2.61 (s, 1H), 2.19 (s, 3H), 1.94 (q, *J* = 7.5 Hz, 2H), 1.46 (m, 1H), 1.23 (m, 1H), 1.03 (t, *J* = 7.5 Hz, 3H), 0.92 (d, *J* = 7.2 Hz, 3H), 0.82 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ: 174.9, 171.0, 158.1, 156.3, 153.6, 140.2, 135.1, 131.2, 129.9, 129.6, 124.8, 124.0, 123.9, 123.8, 122.4, 121.5, 119.0, 118.5, 117.5, 110.6, 94.8, 81.8, 76.9, 76.3, 67.0, 66.4, 56.0, 55.6, 54.6, 52.6, 52.5, 52.4, 50.5, 50.2, 46.1, 45.2, 42.7, 40.9, 40.0, 34.5, 33.0, 32.1, 31.6, 27.9, 25.9, 21.1, 20.0, 13.8, 12.4, 8.4; ESIMS (*m/e*) 864.3 $[M+1]^+$. HRESIMS $C_{50}H_{66}N_5O_8$ $[M+H]^+$ calcd for 864.4911, found 864.4913.

5.4. In vitro cytotoxicity assay

Ditartrate of all compounds was used in bioassays. AVLB and NVB were purchased from Shanghai Kang'ai Biological Product Co., Ltd. A human non-small cell lung cancer cell line (A549) and a human cervix epithelial adenocarcinoma cell line (HeLa), obtained from American Type Culture Collection (Rockville, MD), were used for the cytotoxicity assay in vitro by sulforhodamine B (SRB) assay[14]. Briefly, the cells were seeded at 6000 cells/well in 96-well plates (Falcon, CA, USA) and allowed to attach overnight. The cells were treated in triplicate with grade concentrations of compounds at 37 °C for 72 h. Then, they were fixed with 10% trichloroacetic acid and incubated for 60 min at 4 °C. Then, the plates were washed and dried. SRB solution (0.4% w/v in 1% acetic acid) was added and the culture was incubated for an additional 15 min. After the plates were washed and dried, bound stain was solubilized with Tris buffer, and the optical densities were read on the plate reader (model VERSA Max, Molecular Devices) at 515 nm (A_{515}). The results were expressed as IC_{50} (the compound concentration required for 50% growth inhibition of tumor cells), which was calculated by the Logit method. The mean IC_{50} was determined from the results of three independent tests.

5.5. In vivo antitumor assay of 8b

Female 7-week-old specific pathogen free (SPF) BALB/cA-nude mice (weight, 18–22 g) were obtained from Shanghai Laboratory Animal Center, Chinese Academy of Sciences. A549 cells (2.5×10^6) were subcutaneously implanted into the axilla region of mice. After tumor volume reached 100–300 mm^3, mice were intravenously given **8b** on day 1 at the doses of 1.5 mg/kg, 3 mg/kg, and 6 mg/kg, and **1d** on days 1 and 4 at the dose of 10 mg/kg. Tumor volume and mice weight were measured three times a week. Tumor growth inhibition was calculated as [(Average relative tumor volume of control group − Average relative tumor volume of test group)/Average relative tumor volume of control group] × 100% on day 12 after dosing. Data were presented as means ± SD, and significance was assessed with Student's *t* test. Difference was considered significant at $p < 0.01$.

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