Structure and Synthesis of Vindolicine and Derivatives

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Structure and Synthesis of Vindolicine and Derivatives

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This article describes the reaction of vindoline with formaldehyde and trimethyl orthoformate to prepare vindolicine, tris-vindolicinyl methane and higher molecular weight homologues. The synthesis of 10-formyl vindoline as an intermediate allowed further exploration of its chemistry, in particular the reaction with acetone which yielded a symmetrical dimer, which was further reacted with vindoline to give molecules containing three and four vindoline units. These molecules were characterized by NMR and for some of them (vindolicine, 10-formyl vindoline, 10-(1′-(but-1′-en-3′-one))-vindoline) by X-ray crystallography. Depending on the substitution and on the absence of axes of symmetry, the NMR spectra displayed non-equivalent spin systems for the vindoline moieties. The dimer formed from the double condensation of 10-formyl vindoline with acetone showed cytotoxic activity in the micromolar range.

Introduction

Vindolicine **(1)** is a very special compound among the many alkaloids from the periwinkle, *Catharanthus roseus.* It was first considered as a monomer, related to vindoline **(2)**,[1] then a bis-indole structure was proposed at a symposium in Kyoto,[2] but never published in a regular journal until 1976, where it appeared in an article dealing with ^{13}C NMR of alkaloids.[3] In the meantime, the compound was isolated from *Catharanthus longifolius* and *ovalis.*[4] The ^{13}C NMR spectrum of vindolicine was again published in 1983, with a double set of signals corresponding to each of the moieties.[5] As noticed in a patent, vindolicine has long been the indole alkaloid with the highest molecular weight.[6] It consists of two vindoline units linked by a methylene between the C-10s and thus possesses a symmetry axis. Consequently, the ^{1}H and ^{13}C NMR spectra are simplified and show a single set of signals for the two vindoline parts and a singlet for the methylene. The "double set" assignment seems odd and may be due to spectrometer instability or to the sample not being vindolicine. Unlike other dimeric indole alkaloids from *C. roseus*, vindolicine is not cytotoxic but it was recently demonstrated to possess antidiabetic properties.[7]

Vindoline **(2)**, the monomer constituent of vindolicine is, by far, the most abundant alkaloid of *C. roseus* and is isolated on an industrial scale along with catharanthine, as part of the synthesis of the drug navelbine and of related compounds.[8] Since vindoline is in excess compared to the accompanying catharanthine, it is readily available for further experimentation. The most frequently used sequences of derivatization take advantage of nucleophilicity of vindoline with electrophiles.[9] To the best of our knowledge, the only alternative to nucleophilic addition is the addition of radicals to vindoline developed by Boger et al.[10] Among single electrophiles, we reported additions of vindoline to benzoquinone[8] and as a follow-up of this approach, we wish to describe here the reaction of vindoline with formaldehyde and trimethyl orthoformate and hence, the synthesis of vindolicine and of related compounds.

Results and Discussion

Synthesis of vindolicine

Even though the biosynthetic pathway for vindoline was long ago deciphered in great details,[11] nothing is known on the biosynthesis of vindolicine. The hypothesis of its formation during methylene chloride extraction is ruled out since the molecule is highly stable in this solvent.[12] From a biosynthetic standpoint, formaldehyde, produced through the serine pathway, is the best candidate for the supernumerary carbon atom introduction and it was found to be the reagent of choice to perform the one-carbon atom homologation of vindoline and "dimerization". Solid paraformaldehyde, beforehand depolymerized in acidic water, was preferred over formalin to avoid the presence of methanol. The reaction was conducted in acidic water, at room temperature, and led to the formation of **(1)**. Long reaction times and higher temperatures led to the concomitant formation of high molecular weight oligomers of vindoline and formaldehyde, such as, for example, "trimer" **(3)** and "tetramer" **(4)** (Scheme 1). Vindolicine was purified by medium pressure liquid chromatography and obtained in a crystalline state.

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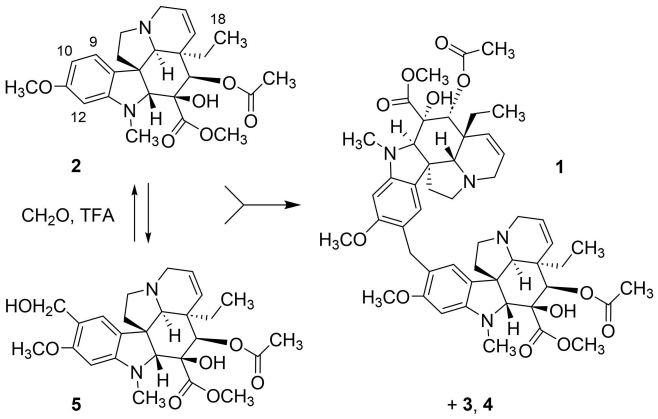


Scheme 1. Synthesis of vindolicine **(1)** and of oligomers.

The NMR spectra of vindolicine showed a single set of signals for the vindolines and a singlet at δ 3.70 for the supplementary methylene (δ ^{13}C 30.2). An X-ray crystal structure was obtained, and it showed the integrity of the two vindoline moieties (Figure 1). In the crystal, the aromatic rings are not coplanar, their dihedral angle is ca 115° and the distance between the two methyl groups of the ethyl side chains is 3 Å. This short distance explains the shielding of these protons in the NMR spectrum (δ 0.33 ppm). The most unexpected information obtained from the NMR experiments is the observation of an NOE between the aromatic H-9 and the methyl triplet of CH_3-18. The distance between these atoms is far too long for an intra-vindoline effect and this corresponds to an NOE between H-9 of one of the vindolines and CH_3-18 of the other.

Structure of oligomers 3 and 4

The second more polar compound from the condensation of formaldehyde with vindoline contained three vindoline units as shown by the ^{1}H-NMR spectrum, which displayed distinct sets of signals for each of the sub-units. The aromatic part of the spectrum consisted of five singlets, three H-9s (δ 6.72, 6.59, 6.48) and two H-12s (δ 6.07, 6.05), according to the HSQC, indicating that one of the vindolines in vindolicine was substituted by another 10-methylene-vindoline. The third substitution by a methylene-vindoline had thus occurred on one of the C-12, thus establishing the structure of **(3)** (Figure 2).

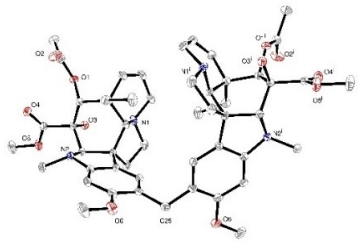


Figure 1. ORTEP representation of vindolicine.

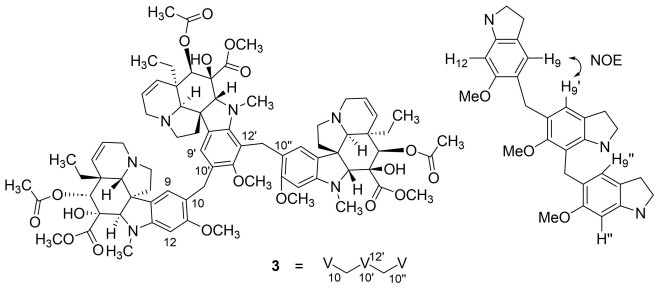


Figure 2. Structure of trimer **(3)**.

For sake of simplicity, compound **(3)** may be designated as $V_{10}CH2_{10}$-V_{12} $CH2_{10}V$ with V standing for vindoline "trimers" are uncommon and the only examples present in the literature are the "natural" trimer described in ref [6] and of unsolved structure, and the trimers produced by Fe-III oxidation of vindoline.[13a] Although possible with ultra-high field spectrometers,[14] complete NMR assignment of **(3)**, where each signal appears as a triad, is beyond the scope of this article and only tentative assignments are proposed. The starting point for the analysis is the assignment of the aromatic protons with the observation of a long-range coupling for the sole pair of singlets at δ 6.48 and 6.07, assigned to either H-9 and −12 or to H-9'' and −12''. The other two H-9s showed a ROE interaction compatible only with the H-9 and H-9′ of structure **(3)** represented below. The COSY and NOESY information allowed to unequivocally assign the aromatic protons as: H-9 (6.72), H-9′ (6.59), H-9'' (6.48), H-12 (6.07) and H-12'' (6.05). From there on, the H-12 allowed to recognize the N-CH_3 and therefrom the H-2s and the rest of the vindolines.

The most polar compound **(4)** was assigned a tetrameric structure based on its mass spectrum. It might be assumed that compound **(4)** corresponds to a double elongation of vindolicine or to addition of formaldehyde and vindoline to compound **(3)**. Two structural possibilities are left corresponding to additions on the remaining reactive centers C-12s. In the above notation, **(4)** is either: V_{-10} $CH2_{10}$-V_{-12} $CH2_{10}$-V_{12} $CH2_{10}$ V or V_{10} $CH2_{12}$ V_{-10} $CH2_{10}$-V_{-12} $CH2_{10}$-V, the latter formula being characterized by a symmetry center. The ^{1}H-NMR spectrum of **(4)** showed signals for two sets of vindolines, corresponding to a symmetrical molecule with some of them, but not all, assignable to "inner" and "outer" vindolines. Two singlets were observed for the four H-9 and those belonging to the outer vindolines H-9s (δ 6.43) were distinguished by the coupling with H-12s (δ 6.06). The ROESY experiment allowed to assign the "outer" aromatic OMe to the most deshielded signals (δ 3.85) while the inner OMe appeared as shielded in ^{1}H-NMR (δ 3.44) and deshielded in ^{13}C NMR (δ 61.2). The first effect is due to the magnetic anisotropy linked to the neighboring aromatic ring, while the second one is due to steric encumbrance. Compound **(4)** is thus $(V_{10}$ $CH2_{12}$ $V_{-10})_2CH2$ (Figure 3). The formation of **(3)** and **(4)** demonstrates that although electrophilic addition to C-10 is preferred, addition to C-12 is possible when the C-10 position is occupied. We never observed additions to C-9, for stereo-electronic reasons and because of

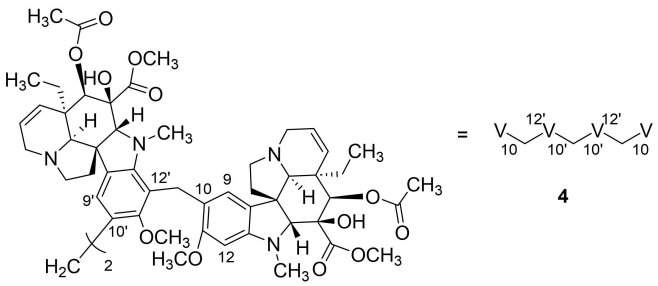


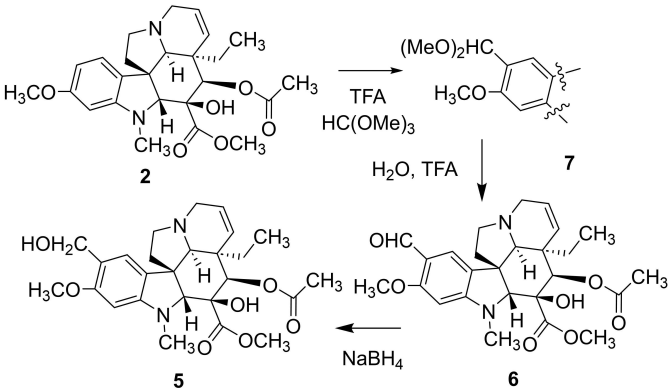
Figure 3. Structure of tetramer (**4**).

the presence of a near-by encumbered quaternary carbon atom.

Reaction mechanism

It is likely that 10-hydroxymethyl vindoline (**5**) is an intermediate in the sequence. Despite numerous attempts, it could not be isolated from the direct reaction of vindoline with formaldehyde and when an excess of this reagent was used, an intractable mixture was obtained. The primary alcohol was however prepared by an indirect route through reduction of aldehyde (**6**). Compound (**6**), 10-formyl vindoline, has been obtained on attempted dichlorocarbene addition on vindoline and by Palladium catalyzed formylation of vindoline.[13] We found it convenient to prepare it by formylation of vindoline with a threefold excess of trimethyl orthoformate under acidic conditions (Scheme 2). As expected, (**6**) shows an extended chromophore with λ_{max} at 348 nm. Compounds (**5**) and (**6**) were obtained in a crystalline state and their structures were confirmed by X-ray crystallography (Figure 4).

The hypothesis of the intermediacy of **5** in the formation of vindolicine was tested by mixing equimolecular amounts of **5** and of vindoline in the presence of TFA. The ^{1}H-NMR spectrum of the crude mixture showed the exclusive formation of vindolicine with some remaining vindoline in an approximate 7 to 3 ratio. Pure vindolicine was isolated from this mixture in 50% yield. The non-quantitative yield of this reaction may be explained by a leak of formaldehyde due to the reversibility of the formylation step (Scheme 3). To support this hypothesis, **5** alone was treated with TFA and vindolicine and vindoline were formed.



Scheme 2. Synthesis of intermediate 10-hydroxymethyl-vindoline (**5**).

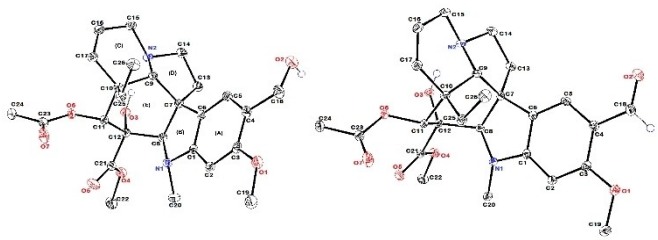
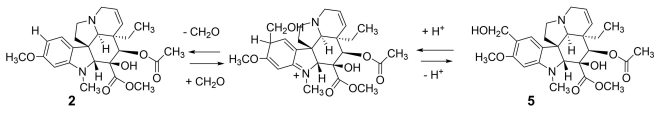


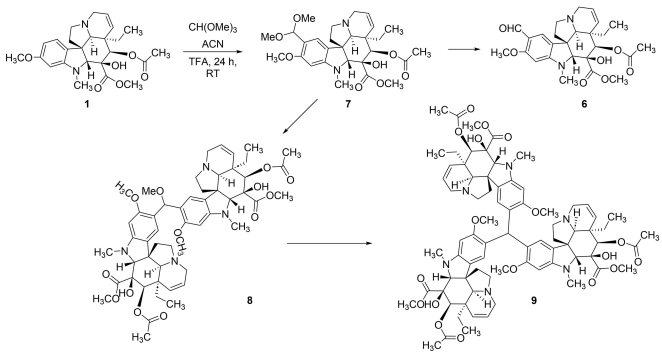
Figure 4. X-ray crystal structures of (**5**) and (**6**).

Synthesis of tris-vindolinyl-methane (9)

The preparation of 10-formyl vindoline (**6**) led us to consider trimethyl orthoformate as a template in the construction of tris-vindolyl-methane. When used in excess over vindoline, this reagent led to ketal (**7**), which yielded aldehyde (**6**) after aqueous work-up. Under these conditions, no products of high molecular weight were formed, but when a 3.5 to 1 ratio of vindoline to trimethyl orthoformate was used, a bis adduct (**8**) and a tri-adduct (**9**) were formed (Scheme 4). Their structures were determined by NMR and mass spectrometry. Compound (**9**) was the expected product of the triple substitution and it showed a molecular ion at m/z 1379 in the positive ESI mode, i.e. three vindolinyl units plus a methine. The molecule possesses a three-fold axis of symmetry and therefore a single set of signals is observed for the three vindolines. The triplet of CH_3-18 is the most shielded resonance at δ⊥⊥0.25 and as in vindolicine, it shows NOEs with the aromatic protons H-9 and H-12 of adjacent sub-units. The methine appears as a sharp singlet at δ_H 6.14, δ_C 37.3, compared to δ_H 3.71, δ_C 30.2 in



Scheme 3. Reversibility of the formation of (**5**) from (**2**).

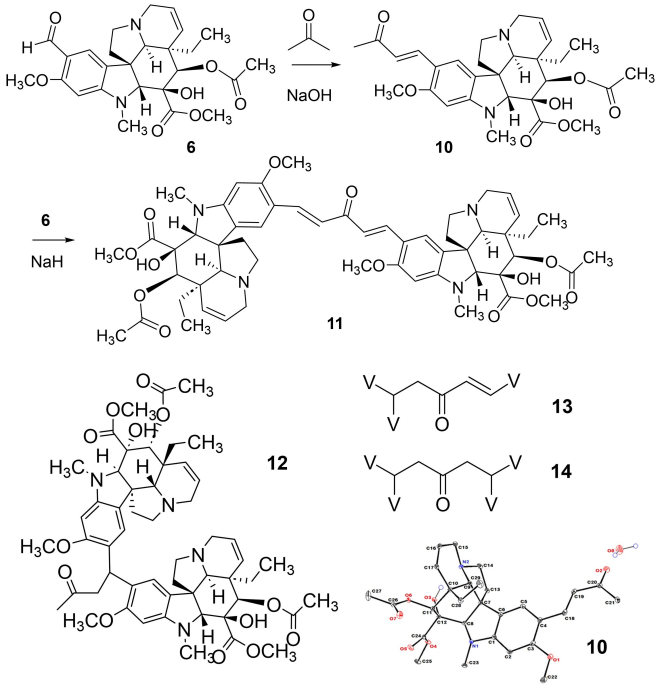


Scheme 4. Synthesis of tris-vindolinyl-methane (**9**).

vindolicine. The second product **(8)** is the result of incomplete substitution and NMR and MS allow its identification as 10′-OMe vindolicine. The introduction of a methoxy group on the methylene bridge destroys the symmetry present in vindolicine, and the resonances of the two moieties become non-equivalent.

Reactions of 10′-formyl vindoline with acetone

10′-Formyl vindoline **(6)** was reacted with acetone in the presence of a base to give butenone derivative **(10)** as the main product (Scheme 5). The new double bond was *trans* as shown by the large coupling constant (J = 16.2 Hz) and its presence induced a downfield shift for H-9 (δ 7.47). No other damage occurred to the molecule, the structure of which was safely established by X-ray crystallography. Deprotonation of **(10)** with NaH and addition of a second molecule of 10′-formyl vindoline yielded the symmetrical bis adduct **(11)**. Compounds **(10)** and **(11)** showed extended UV chromophores with λ_{max} at 473 nm for **(11)** and 393 nm for **(10)**. Owing to electron deficiency on the side chains, they were prone to further addition of vindoline and **(10)** gave a bis-vindolinyl adduct **(12)**, and **(11)** gave a tris vindolinyl adduct **(13)** and tetra vindolinyl adduct **(14)**. The formation of these adducts does not give rise to the creation of new stereogenic center and therefore these compounds are unique.



Scheme 5. Synthesis of acetone adduct **(10–14)** and ORTEP of **(10)**.

Synthesis of vindolicinone

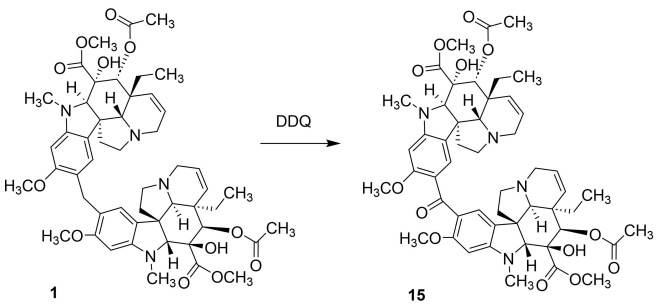
With vindolicine at hand, it was tempting to explore its chemistry and, in particular, the oxidation of the methylene bridge, which would open a route to olefins substituted by vindolines. The methylene bridge was easily oxidized by DDQ in the presence of water under Yonemitsu conditions (Scheme 6). Ketone **15**, named here vindolicinone, was obtained as a pale-yellow solid in ca 60% yield. It showed an extended chromophore with λmax 370 nm.

Symmetry considerations

At the onset of the work on vindolicine, there have been discussions on the unicity or not of the NMR signals for the two vindoline moities. These two "vindolines" are exchangeable through a rotation around an axis bisecting the central methylene and therefore show magnetic equivalence; the same considerations hold for vindolicinone, tris-vindolinyl-methane and bis-acetone adduct **(11)**. In "trimer" **(3)**, the three vindoline units are chemically different and give rise to three distinct but difficult-to-assign sets of signals. In "tetramer" **(4)**, there is an axis of symmetry exchanging the inner vindolines on one hand and the outer ones on the other, producing two sets of signals. When the central methylene is substituted (viz compound **(8)**), the exchange after rotation is no longer possible and the signals of the two vindoline units become nonequivalent. This is the case of compounds **(12)** and **(13)** although in this later example the isolated vindoline shows a third set of signals. The tetrasubstituted acetone adduct **(14)** only shows two sets of signals because the VCHV radicals present on each side of the molecule are exchangeable through rotation around the carbonyl and therefore are equivalent. However, the two vindolines which substitute the same carbon atom are not exchangeable through rotation and display anisochronous signals.

Biological properties of the new molecules

Although vindoline is not a cytotoxic molecule, we felt that the reactivity of 10-hydroxymethyl vindoline **(5)** would cause cell damages through reaction with electron rich biomolecules



Scheme 6. Synthesis of vindolicinone **(15)**.

(nucleic acids, peptides). This was not the case however and the molecule was found inactive against HeLa, PC3 and MB-23 cell lines at micromolar concentrations. Cytotoxicity at the micromolar level was found on the bis acetone adduct **(11)**, but this maybe simply due to the Michael acceptor character of the molecule.[15] On the larvicidal assay, 10-hydroxymethyl vindoline **(5)** showed a 98% inhibition at 100 μM concentration. Compound **(15)** showed potent α-glucosidase inhibitory activity with 92% inhibition at 0.5 mM concentration and was inactive against 3T3 cell lines.

Conclusions

The availability of vindoline makes it an attractive starting point for the construction of libraries of compounds for biological investigation. Its relatively high molecular weight allows the preparation of molecules in the 1000–1500 Dalton range, a domain which is rarely explored.[16] So far, the best and unique market for vindoline remains the preparation of semisynthetic navelbine but there remains a host of simple and elaborated electrophiles to investigate in order to give alternative value to this resource.

Experimental Section

General experimental procedures

Optical rotations were determined in MeOH with an Anton Paar MCP 5100 polarimeter at 25 °C and on JASCO P-2000 spectrometer in 1 mL cells. Fourier transform-infrared (FT-IR) measurements were carried out using a Perkin Elmer Spectrum Two spectrometer in the ATR mode and on Bruker Vector 22 FT-IR spectrometer (France) on KBr disk. HR-ESI-MS experiments were performed using a SYNAPT G2 si, Waters instrument (Manchester, UK). Thermo-scientific model-300 spectrometer was used to record the UV spectra. ^{1}H-(400, 500 or 600 MHz), and ^{13}C-NMR (100, 125 or 150 MHz) spectra were recorded on Bruker Avance-or Neo spectrometers. Single-crystal X-ray diffraction data of compounds **5**, **6** and **10** was collected on Bruker APEXII D8 Venture diffractometer, fitted with PHOTON 100 detector (CMOS technology), and fine-focus sealed tube having X-ray source [Cu Kα radiation $\lambda = 1.54178$ Å]. Reflection intensities were integrated using SAINT software. Absorption correction was done on M-multi-scan. Structures were solved on SHELXTL. X-ray data for compound **1** were collected at low temperature (180 K) on an Agilent Gemini diffractometer using a graphite-monochromated Cu–Kα Enhance radiation (λ = 1.54184 Å) and equipped with an Oxford Instrument Cooler Device. Deposition numbers 2310835 (for **1**), 2310923 (for **5**), 2310924 (for **6**) and 2310925 (for **10**) contain the supplementary crystallographic data for this paper. These data are provided free of charge by the joint Cambridge Crystallographic Data Centre and Fachinformationszentrum Karlsruhe Access Structures service.

Synthesis of vindolicine 1 and of oligomers 3 and 4

Vindoline (961 mg, 2.1 mmoles) is dissolved in 10 mL 2 N HCl and 0.4 mL of a solution prepared from 200 mg *p*-formaldehyde in 2 mL 2 N HCl is added (full dissolution requires sonication). The solution is stirred for 17 hours at room temperature and develops a blue color. It is then neutralized with sodium bicarbonate in the presence of 50 mL DCM. The phases are separated, and the organic layer is dried over Na_2SO_4 and evaporated. One obtains 1.1 g of a blue solid which is purified by flash chromatography on 25 g of Sigel.

Vindolicine **(1)**: off white powder; $[\alpha]_D^{20}$-48° (c 0.014, $CHCl_3$); mp = 255–260 °C; UV (MeOH): λ_{max} 219, 260 and 310 nm; IR (KBr): 3441, 2926, 2855, 1736, 1612, 1498, 1461, 1372, 1249, 1119, 1044, 813 cm^{-1}; ESI-MS (+ve) *m/z*: 925 $(M+H)^+$; ^{1}H-NMR (500 MHz, CD_3OD): δ 6.86 (2H, s, H-9), 6.20 (2H, s,H-12), 5.83 (2H, ddd, J = 10.1, 4.5, 1.2 Hz, H-14), 5.35 (2H, s, H-17), 5.13 (2H, brd, J = 10.1 Hz, H-15), 3.79 (6H, s, C-11 OC$\underline{H}_3$), 3.76 (6H, s, COOC$\underline{H}_3$), 3.71 (2H, s, H-10′), 3.53 (2H, s, H-2), 3.47 (2H, dd, J = 16.5, 4.5 Hz, H-3β), 3.38 (2H, dt, J = 3.6, 18.2 Hz, H-5), 2.81 (2H, bd, J = 16.5 Hz, H-3α), 2.64 (6H, s, NC$\underline{H}_3$), 2.59 (2H, s, H-21), 2.53 (2H, dd, J = 8.5 Hz, H-5), 2.27 (4H, m, H-6), 1.99 (6H, s, OCOC$\underline{H}_3$), 1.50 (2H, dq, J = 14.5, 7.3 Hz, H-19), 1.01 (2H, dq, J = 14.5, 7.3 Hz, H-19), 0.33 (6H, t, J = 7.3 Hz, C$\underline{H}_3$-18); ^{13}C-NMR (125 MHz, CD_3OD): δ 173.6 (C$\underline{O}$OCH$_3$), 172.5 (O$\underline{C}$OCH$_3$), 159.9 (C-11), 153.2 (C-13), 131.4 (C-15), 125.6 (C-14), 125.1 (C-9), 125.0 (C-8), 123.0 (C-10), 94.8 (C-12), 84.8 (C-2), 81.0 (C-16), 77.6 (C-17), 68.2 (C-21), 56.0 (C-11 O$\underline{C}$H$_3$), 54.3 (C-7), 53.0 (C-5), 52.8 (COO$\underline{C}$H$_3$), 52.1 (C-3), 44.8 (C-6), 44.4 (C-20), 39.7 (N-$\underline{C}$H$_3$), 32.0 (C 19), 30.2 (C-10′), 20.8 (OCO$\underline{C}$H$_3$), 8.4 (C-18).

X-ray analysis of vindolicine: data were collected at low temperature (180 K) on a Gemini Rigaku diffractometer using a graphite-monochromated Cu–Kα Enhance radiation (λ = 1.54184) and equipped with an Oxford Instrument Cooler Device. The final unit cell parameters have been obtained by means of a least square refinement. The structure has been solved by Direct methods using SIR92 and refined by means of least squares procedures on a F2 with the program SHELXL9 included in the software package WinGX version 1.63.[17–19] The Atomic Scattering Factors were taken from international tables for X-Ray Crystallography.[20] All hydrogen atoms were geometrically placed and refined by using a riding model, except for atom H3B, which has been localized by Fourier differences and isotropically refined. Although the molecule is an organic compound with light elements, it was possible to determine its absolute configuration under Cu–Kα radiation. All non-hydrogen atoms were anisotropically refined, and in the last cycles of refinement a weighting scheme was used, where weights are calculated from the following formula: $w = 1/[\sigma^2(Fo^2) + (aP)^2 + bP]$ where $P = (Fo^2 + 2Fc^2)/3$. Drawing was performed with the program ORTEP32 with 30% probability displacement ellipsoids for non-hydrogen atoms.

Compound **3**: amorphous solid; $[\alpha]_D^{20}$-108° (c 1.2, MeOH); UV (MeOH): λ_{max} 212, 258 and 308 nm; IR (neat, ATR): 3617, 3455, 2949, 1738, 1615, 1371, 1222, 1039 cm^{-1}; ESI-MS (+ve) *m/z*: 1393.6848 (calc for $C_{77}H_{97}N_6O_{18}$: 1393.6859, $M+H^+$), 697, 465; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 9.75, 9.52 and 9.25 (3 OH), 6.72, 6.59 and 6.48 (3H, 3s, H-9), 6.07 and 6.05 (2H, 2s, H-12), 5.82 (3H, 3 brd, H-14), 5.44, 5.34 and 5.31 (3H, 3s, H-17), 5.24 (3H, 3brd, H-15), 3.85, 3.71 and 3.50 (9H, 3s, C-11 OC$\underline{H}_3$), 3.85 and 3.74 (2H, AB system, J = 16 Hz, 2 H-10′), 3.77 and 3.69 (2H, AB system, 2 H-10″), 3.78, 3.75 and 3.70 (9H, 3s, COOC$\underline{H}_3$), 3.66, 3.64 and 3.54 (3H, 3s, H-2), 2.68, 2.64 and 2.51 (9H,3s, NC$\underline{H}_3$), 2.64, 2.56 and 2.56 (3H, 3s, H-21), 2.04, 2.03 and 2.02 (9H, 3s, OCOC$\underline{H}_3$), 0.42, 0.37 and 0.22 (9H, 3t, J = 7.3 Hz, CH$_3$-18); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 171.9, 171.7, 171.2, 170.8, 170.7, 170.5 (C$\underline{O}$OCH$_3$ and O$\underline{C}$OCH$_3$), 158.8, 158.6, 157.9 (C-11), 152.0, 151.4, 151.3 (C-13), 130.7, 130.6, 130.4 (C-15), 128.9, 127.3 (C-8), 124.3, 124.1, 124.0 (C-14), 124.9, 122.3, 121.6 (C-9), 93.6, 92.9 (C-12), 84.4, 83.3, 83.1 (C-2), 80.1, 79.8, 79.5 (C-16), 76.5, 76.3, 76.0 (C-17), 66.7, 66.3, 65.1 (C-21), 61.0, 55.5, 55.4 (C-11 O$\underline{C}$H$_3$), 52.2, 52.1, 52.0 (COO$\underline{C}$H$_3$), 53.0 (C-7), 52.8 (C-5), 52.1 (C-3), 44.3 (C-6), 44.8 (C-20), 43.9, 39.0, 38.7 (N-$\underline{C}$H$_3$), 30.7, 30.4, 30.1 (C 19), 29.8 (C-10′), 25.3 (C-10″), 21.0 (OCO$\underline{C}$H$_3$), 8.1, 7.65, 7.3 (C-18).

Compound **4**: amorphous solid; $[\alpha]_D^{20}$-470° (c 0.61, MeOH); UV (MeOH): λ_{max} 210, 258 and 306 nm; IR (neat, ATR): 3463, 2949, 1739, 1619, 1222, 1040 cm^{-1}; ESI-MS (+ve) *m/z* 1861.9148 (calc for $C_{103}H_{128}N_8O_{24}$: 1861.9197, $M+H^+$), 931, 621, 466; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 9.63 and 9.23 (4H, 2brs, OH), 6.73 (2H, s, H-9′), 6.43 (2H, s, H-9), 6.06 (2H, s, H-12), 5.86, 5.80 (4H, dd, H-14), 5.33, 5.31 (4H, 2s, H-17), 5.29, 5.23 (4H, brd, H-15), 3.85 (6H, s, Ar-OCH_3), 3.73, 3.77 (12 H, s, $COOCH_3$), 3.44 (6H, s, Ar-OCH_3), 3.84 and 3.71 (2H, AB system, J=16 Hz, H-10′), 3.77 and 3.67 (2H, AB system, H-10″), 3.64, 3.57 (4H, s, H-2), 2.67, 2.48 (12H, s, NCH_3), 2.68, 2.56 (4H, s, H-21), 2.06, 2.05 (12H, s, $OCOCH_3$), 0.51, 0.23 (12H, t, J=7.3 Hz, H_3-18); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 171.7, ($COOCH_3$), 170.7 ($OCOCH_3$), 159.3 (inner C-11), 157.8 (outer C-11), 152.1 (C-13), 151.7 (C-13), 130.6 (C-15), 125.6 (C-8), 124.3 (C-14), 122.4 (inner C-9), 122.2 (outer C-9), 122.9 (C-10), 92.9 (C-12), 84.8, 83.2 (2 C-2) 80.1 (C-16), 76.1 (C-17), 66.6, 65.3 (2 C-21), 61.2, 55.5 (2 C-11 OCH_3), 52.3 ($COOCH_3$), 53.0 (C-7), 50.9 (C-5), 50.6 (C-3), 44.3 (C-6), 42.8 (C-20), 43.6, 39.0 (2 N-CH_3), 31.5, 25.7 (2 C-10 CH_2), 30.6, 30.3 (2 C 19), 21.2 ($OCOCH_3$), 8.2, 7.7 (2 C-18).

Synthesis of compound 6

To a stirred suspension of vindoline (100 mg, 0.21 mmol) in acetonitrile (0.3 mL), trimethyl orthoformate (0.1 mL, 0.60 mmol) was added. The solution was stirred at room temperature for 5 minutes and 0.2 mL TFA was rapidly added. The deep blue solution was stirred for 30 minutes at room temperature and then neutralized with 0.1 N aqueous sodium carbonate, which was extracted with DCM, dried over sodium sulfate and evaporated. A gummy material was obtained, which was dissolved in minimum amount of DCM followed by dropwise addition of hexane. The precipitate was filtered washed with hexane and dried at room temperature to obtain a white powder (95 mg, 95%).

Compound **6**: $[\alpha]_D^{20}$-83° (c 10, $CHCl_3$); mp = 250–253 °C, *Rf* = 0.4 (silica gel, MeOH/DCM 99/1); UV (MeOH); λ_{max} (log ε)220, 261, 303 and 348 (4.79) nm; IR (KBr): 3431, 3027, 2968, 2933, 2877, 2851, 1736, 1658, 1607, 1474, 1433, 1375, 1242, 1149, 1118, 1089, 1041, 953, 845, 815, 744 cm^{-1}; ESI-MS (+ve) *m/z* 485 $(M+H)^+$; ^{1}H-NMR (600 MHz, CD_3OD): δ 10.0 (1H, s, H-10′), 7.54 (1H, s, H-9), 6.20 (1H, s,H-12), 5.89 (1H, dd, J=10.0, 4.3 Hz, H-14), 5.22 (1H, m, H-15), 5.22 (1H, s, H-17), 3.95 (3H, s, C-11 OCH_3), 3.90 (1H, s, H-2), 3.78 (3H, s, $COOCH_3$), 3.47 (1H, dd, J=16.0, 4.9 Hz, H-3β), 3.41 (1H, dt, J=4.5, 9.0 Hz, H-5), 2.94 (1H, bd, J=16.0 Hz, H-3α), 2.92 (1H, s, H-21), 2.84 (3H, s, N-CH_3), 2.72 (1H, dt, J=6.4, 10.2 Hz, H-5), 2.28 (2H, m, H-6), 2.01 (3H, s, $OCOCH_3$),) 1.57 (1H, dq, J=14.7, 7.2 Hz, H-19), 1.10 (1H, dq, J=14.7, 7.2 Hz, H-19), 0.57 (3H, t, J=7.2 Hz, H-18); ^{13}C-NMR (150 MHz, CD_3OD): δ 188.8 (C −10′), 173.2 ($COOCH_3$), 172.3 ($OCOCH_3$), 167.6 (C-11), 160.7 (C-13), 131.0 (C-15), 127.0 (C-8), 126.0 (C-14), 123.6 (C-9), 117.5 (C-10), 91.6 (C-12), 83.8 (C-2), 80.6 (C-16), 77.3 (C- 17), 67.4 (C-21), 56.5 (C-11 OCH_3), 53.4 (C-7), 53.0 ($COOCH_3$), 51.9 (C-5), 51.6 (C-3), 44.4 (C-6), 44.2 (C-20), 36.6 (N-CH_3), 32.1 (C 19), 29.7), 20.7 ($OCOCH_3$), 7.8 (C-18).

A solution of **6** (100 mg, 0.20 mmol) in acetonitrile (0.5 mL) was stirred at room temperature for 5 minutes and then $NaBH_4$ (14.9 mg, 0.40 mmol) was added. After stirring at room temperature for 3 hours, the reaction mixture was quenched with water. The precipitate was filtered on paper and the aqueous layer was extracted with CH_2Cl_2. The extract was dried over sodium sulfate. filtered, concentrated under reduced pressure and purified by column chromatography on silica gel (ethyl acetate: hexane, 7:3) to give compound **5** (80 mg, 80%).

Compound **5**: white powder$[\alpha]_D^{20}$-51° (c 10, MeOH);mp = 222–225 °C, *Rf* = 0.3 (silica gel, MeOH/DCM 98/2); UV (MeOH); $\lambda_{max;}$ 221, 260, 307 nm; IR (KBr): 3436, 2941, 2872, 1738, 1611, 1495, 1462, 1219, 1191, 1362, 1143, 1130, 1106, 1044, 1025, 930, 900, 872, 790, 741, 705 cm^{-1}; ESI-MS (+ve) *m/z* 487.4 $(M+H)^+$; ^{1}H-NMR (500 MHz, CD_3OD): δ 7.08 (1H, s, H-9), 6.25 (1H, s, H-12), 5.86 (1H, ddd, J=10.1, 4.8, 1.5 Hz, H-14), 5.37 (1H, s, H-17), 5.18 (1H, brd, J=10.1 Hz, H-15), 4.53 (d, J=12.5 Hz, H-10′), 4.48 (d, J=12.5 Hz, H-10′), 3.83 (3H, s, C-11 OCH_3), 3.77 (3H, s, $COOCH_3$), 3.59 (1H, s, H-2), 3.48 (1H, ddd, J=16.2, 4.8, 1 Hz, H-3β), 3.40 (1H, dt, J=4.8, 9.3 Hz, H-5), 2.89 (1H, bd, J=16.2 Hz, H-3α), 2.75 (1H, s, H-21), 2.67 (3H, s, N-CH_3), 2.62 (1H, m, H-5), 2.37, (1H, m, H-6), 2.26 (1H, m, H-6), 2.00 (3H, s, $OCOCH_3$), 1.57 (1H, dq, J=14.4, 7.4, H-19), 1.08 (1H, dq, J=14.4, 7.4 Hz, H-19), 0.49 (3H, t, J=7.4 Hz, H-18); ^{13}C-NMR (125 MHz, CD_3OD): δ 173.6 ($COOCH_3$), 172.5 ($OCOCH_3$), 160.2 (C-11), 154.6 (C-13), 131.3 (C-15), 125.7 (C-14), 125.0 (C-8), 124.2 (C-9), 122.0 (C-10), 94.5 (C-12), 84.8 (C-2), 81.0 (C-16), 77.7 (C- 17), 68.0 (C-21), 60.6 (C-10′), 56.0 (C-11 OCH_3), 54.3 (C-7), 52.82 ($COOCH_3$), 52.80 (C-5), 52.0 (C-3), 44.9 (C-6), 44.3 (C-20), 39.3 (NCH_3), 32.0 (C-19), 20.8 ($OCOCH_3$), 8.1 (C-18).

Synthesis of vindolicine from vindoline and 10-hydroxymethyl vindoline

A mixture of **5** (100 mg, 0.20 mmol) and **2** (100 mg, 0.21 mmol) was dissolved in 1 mL of acetonitrile at room temperature. After 5 minutes stirring, TFA (0.5 mL) was added. The mixture was stirred at room temperature for 30 minutes, then neutralized with 0.1 N sodium carbonate, extracted with CH_2Cl_2 (3×20 mL), dried over sodium sulfate, and concentrated under reduced pressure. The crude reaction mixture was purified on alumina (MeOH/DCM 99.8/0.2) to give vindolicine in 85% yield.

Synthesis of trisvindolicine 9

Trimethyl orthoformate (0.01 mL, 0.06 mmol) was added to a stirred solution of vindoline (100 mg, 0.219 mmol) in 0.1 mL acetonitrile, followed by 0.01 mL of TFA. The reaction mixture turned deep blue. Stirring was pursued for 24 h at room temperature. The reaction mixture was neutralized with 0.1 N sodium carbonate and extracted with DCM (3×20 mL), dried over sodium sulfate, and concentrated under reduced pressure. Purification of the crude reaction mixture on alumina gave compounds **8** (20 mg, 20%, eluted with MeOH/DCM 99.8/0.2) and **9** (50 mg, 50%, eluted with MeOH /DCM 99/1). Compound **8**: white powder $[\alpha]_D^{20}$-70° (c 10, MeOH); mp 185–190 °C; *Rf* = 0.5 (silica gel, MeOH/DCM 97/3); UV (MeOH); $\lambda_{max;}$ 229, 261, 310 nm; IR (KBr): 3308, 2930, 2865, 2358, 2344, 2183, 2155, 2100, 2028, 20114, 1998, 17734, 1615, 1463, 1373, 1142, 955, 907, 889, 799, 741 cm^{-1}; ESI-MS (+ve) *m/z* 955 $(M+H)^+$; ^{1}H-NMR (600 MHz, CD_3OD): δ 7.09, 6.80 (2H, 2s, H-9), 6.23, 6.21 (2H, 2s, H-12), 5.87, 5.84 (2H, m, H-14), 5.87 (1H, s, C-10′), 5.36, 5.26 (2H, 2s, H-17), 5.20, 5.14 (2H, brd, J=10.1 Hz, H-15), 3.81, 3.72 (6H, s, C-11 OCH_3), 3.765, 3.757 (6H, 2s, $COOCH_3$), 3.58, 3.56 (2H, 2s, H-2), 2.78, 2.51 (2H, 2s, H-21), 2.69, 2.65, (6H, 2s, N-CH_3), 2.00, 1.98 (6H, 2s, $OCOCH_3$), 0.54, 0.20 (3H, 2t, J=7.2 Hz, H-18); ^{13}C-NMR (150 MHz, CD_3OD): δ 173.5, 173.4 ($COOCH_3$), 172.5, 172.4 ($OCOCH_3$), 160.4, 160.2 (C-11), 154.2, 154.1 (C-13), 131.4. 131.3 (C-15), 125.8, 125.7 (C-14), 124.9, 124.7 (C-8), 123.3, 122.1 (C-9), 122.9 (C-10′), 95.0, 94.6 (C-12), 84.6, 84.4 (C-2) 81.1, 81.0 (C-16), 77.6, 77.5 (C-17), 74.6 (C-10′), 67.7, 67.6 (C-21), 57.0(C-10′ OCH_3),56.4, 56.2 (C-11 OCH_3), 54.4, 54.2 (C-7), 52.8 ($COOCH_3$), 52.6, 52.3 (C-5), 52.0, 51.9 (C-3), 45.3, 44.8 (C-6), 44.3, 44.2 (C-20), 39.4, 39.0 (N-CH_3), 31.9, 31.7 (C 19), 20.8, ($OCOCH_3$), 8.5, 8.3 (C-18). Compound **9**: white powder. $[\alpha]_D^{20}$-194° (*c* 10,MeOH); mp = 200-205 °C; *Rf* = 0.2 (silica gel, MeOH/DCM 96/3); UV (MeOH): $\lambda_{max;}$ 221, 261 and, 311 nm; IR (KBr): 3308, 2930, 2865, 2358, 2344, 2183, 2155, 2100, 2028, 20114, 1998, 17734, 1615, 1463, 1373, 1142, 955, 907, 889, 799, 741 cm-^{1}; ESI-MS (+ve) *m/z* 1379 $(M+H)^+$; ^{1}H-NMR (400 MHz, CD_3OD): δ 6.50 (3H, s, H-9), 6.21 (3H, s,H-12), 6.14 (1H, s, H-10′), 5.82 (3H, br dd, J=10.2, 4.1 Hz, H-14), 5.30

(3H, s, H-17), 5.15 (3H, br d, J=10.2 Hz, H-15), 3.76 (9H, s, COOC$\underline{H}_3$), 3.67 (9H, s, C-11 OC$\underline{H}_3$), 3.53 (3H, s, H-2), 3.43 (3H, br dd, J=16.4, 4.8 Hz, H-3β), 3.36 (3H, m, H-5), 2.70 (3H, br d, J=16.4 Hz, H-3α), 2.65 (9H, s, N-C$\underline{H}_3$), 2.44 (3H, s, H-21), 2.40 (3H, m, H-5), 2.24–2.12 (6H, m, H-6), 1.98 (9H, s, OCOC$\underline{H}_3$), 1.48 (3H, dq, J=14.0, 7.2 Hz, H-19), 1.05 (3H, dq, J=14.0, 7.2 Hz, H-19), 0.25 (3H, t, J=7.2 Hz, H-18); ^{13}C-NMR (100 MHz, CD_3OD): δ 173.4 (C$\underline{O}$OOCH$_3$), 172.4 (O$\underline{C}$OCH$_3$), 160.0 (C-11), 152.9 (C-13), 131.4 (C-15), 126.3 (C-8), 125.7 (C-14), 124.3 (C-9), 123.9 (C-10), 95.5 (C-12), 84.4 (C-2) 81.2 (C-16), 77.4 (C-17), 67.6 (C-21), 56.4 (C-11 O$\underline{C}$H$_3$), 54.4 (COO$\underline{C}$H$_3$), 52.8 (C-7), 52.5 (C-5), 52.0 (C-3), 45.4 (C-6), 44.2 (C-20), 39.5 (N-$\underline{C}$H$_3$), 37.3 (C-10′), 31.8 (C-19), 20.8 (OCO$\underline{C}$H$_3$), 8.8(C-18).

Synthesis of acetone adduct 10

A solution of compound **6** (100 mg, 0.20 mmol) in acetone (2.0 mL) was treated with solid sodium hydroxide (70 mg, 1.75 mmol). The reaction mixture was stirred at room temperature for 1 h and turned yellow. The reaction was neutralized by 1 N HCl, extracted with DCM, which was dried over sodium sulfate before concentration under reduced pressure. Purification by column chromatography afforded **10** (70 mg, 70%, eluted with MeOH/DCM 99.5/0.5) as a yellow crystalline solid. Compound **10**: mp=130-132 °C; $[\alpha]_D^{20}$ = −210 (*c* 0.3, MeOH); UV (λ_{max} MeOH): 223, 315, 393 nm; IR (KBr): 2936, 2360, 2340, 1733, 1660, 1595,1504, 1432, 1371, 1335, 1432, 1371, 1335, 1146, 1089, 1033, 931, 887, 833, 736, 692, 668 cm^{-1}; ^{1}H-NMR (600 MHz, CD_3OD): δ 7.93 (1H, d, J=16.2 Hz, H-10′), 7.47 (1H, s, H-9), 6.66 (1H, d, J=16.2 Hz, H-10″), 6.25 (1H, s, H-12), 5.89 (1H, ddd, J=10.2, 5.0, 1.5 Hz, H-14), 5.28 (1H, s, H-17), 5.21 (1H, br d, J=10.2 Hz, H-15), 3.93 (3H, s, C-11 OC$\underline{H}_3$), 3.79 (1H, s, H-2), 3.78 (s, 3H, CO_2CH_3), 3.48 (1H, br, dd, J=16.2, 5.0 Hz, H-3β), 3.41 (1H, dt, J=4.5, 9.1 Hz, H-5), 2.94 (1H, br d, J=16.2 Hz, H-3α), 2.92 (1H, s, H-21), 2.78 (3H, s, N-C$\underline{H}_3$), 2.72 (1H, m, H-5), 2.31 (s, 3H, C″CO$\underline{C}$H$_3$), 2.30 (2H, m, H-6), 2.00 (3H, s, OCOC$\underline{H}_3$), 1.58 (1H, dq, J=14.5, 7.3 Hz, H-19), 1.09 (1H, dq, J=14.5, 7.3 Hz, H-19), 0.55 (3H, t, J=7.3 Hz, H-18); ^{13}C-NMR (150 MHz, CD_3OD): δ 201.9 (C″H$\underline{C}$O), 173.3 (C$\underline{O}$OOCH$_3$), 172.4 (O$\underline{C}$OCH$_3$) 162.8 (C-11), 157.8 (C-13), 141.8 (C-10′), 131.2 (C-15), 126.8 (C-8), 125.9 (C-14), 123.8 (C-9), 122.9 (C-10″), 115.0 (C-10), 93.0 (C-12), 84.2 (C-2), 80.7 (C-16), 77.4 (C- 17), 67.5 (C-21), 56.4 (C-11 O$\underline{C}$H$_3$), 53.9 (C-7), 53.0 (COO$\underline{C}$H$_3$), 52.2 (C-5), 51.8 (C-3), 44.5 (C-6), 44.3 (C-20), 37.5 (N-$\underline{C}$H$_3$), 32.0 (C 19), 29.9 (C-10′″$\underline{C}$H$_3$), 20.8 (OCO$\underline{C}$H$_3$), 7.9 (C-18).

Synthesis of compound 11

To a stirred suspension of NaH (60% in mineral oil, 120 mg, 3 mmol) in 0.3 mL acetonitrile at room temperature, were successively added compound **10** (30 mg, 0.05 mmol) and after 1 h, compound **6** (30 mg, 0.06 mmol). After 1h of stirring, the orange mixture was quenched with HCl and extracted with DCM. The organic phase was dried over anhydrous sodium sulfate and concentrated. The residue was purified by column chromatography to give **11** (15 mg, 50%, eluted with MeOH/DCM 99/1) as an orange solid.mp=240 °C, R*f*=0.3 (silica gel, MeOH/DCM 97/3); $[\alpha]_D^{20}$ = −110 (*c* 10, MeOH); UV (λ_{max} MeOH): 220, 283, 473 nm; IR (KBr): 3413, 2936, 2357, 1738, 1568, 1432, 1372, 1338, 1170, 1146, 1104, 1033, 1010, 930, 871, 810, 741, 682 cm^{-1}; ESI-MS (+ve) *m/z* 991 (M+H)$^+$; ^{1}H-NMR (600 MHz, CD_3OD): δ 8.03 (1H, d, J=16.2 Hz, H-10′), 7.52 (1H, s, H-9), 7.05 (1H,d, J=16.2 Hz, H-10′), 6.25 (1H, s, H-12), 5.90 (1H, ddd, J=10.2, 4.8, 1.2 Hz, H-14), 5.29 (1H, s, H-17), 5.22 (1H, br d, J=10.2 Hz, H-15), 3.94 (3H, s, C-11 OC$\underline{H}_3$), 3.80 (1H, s, H-2), 3.78 (s, 3H, $CO_2C\underline{H}_3$), 3.49 (1H, dd, J=16.8, 4.8 Hz, H-3β), 3.43 (1H, m, H-5), 2.95 (1H, m, H-3α), 2.93 (1H, s, H-21), 2.79 (3H, s, N-C$\underline{H}_3$), 2.73 (1H, m, H-5), 2.32 (2H, m, H-6), 2.00 (3H, s, OCOC$\underline{H}_3$), 1.58 (1H, dq, J=14.7, 7.3 Hz, H-19), 1.12 (1H, dq, J=14.7, 7.3 Hz, H-19), 0.58 (3H, t, J=7.3 Hz, H-18); ^{13}C-NMR (150 MHz, $CDCl_3$): δ 192.1 (C-10′″), 173.3 (COOCH$_3$), 172.4 (OCOCH$_3$), 163.0 (C-11), 157.6 (C-13), 140.2 (C-10′), 131.2 (C-15), 126.8 (C-8), 125.9 (C-14), 124.0 (C-9), 122.5 (C-10″), 115.9 (C-10), 93.1 (C-12), 84.2 (C-2), 80.8 (C-16), 77.4 (C-17), 67.6 (C-21), 56.4 (C-11 O$\underline{C}$H$_3$), 53.9 (COO$\underline{C}$H$_3$), 53.0 (C-7), 52.2 (C-5), 51.8 (C-3), 44.6 (C-6), 44.3 (C-20), 37.6 (N-$\underline{C}$H$_3$), 32.1 (C 19), 20.8 (OCOCH$_3$), 8.0 (C-18).

Synthesis of compound 12

A mixture of **10** (30 mg, 0.05 mmol) and **2** (90 mg, 0.20 mmol) was stirred for 10 min in a mixture of 2.2 mL acetonitrile and 0.9 mL H_2O. Trifluoroacetic acid (0.6 mL) was added and the reaction was stirred for 24 hours at room temperature. The reaction mixture was neutralized with sodium carbonate and extracted with DCM. The extract dried over with Na2SO4 and concentrated under reduced pressure. Purification of the crude reaction mixture on alumina gave compound **15** (10 mg, 37%, eluted with MeOH/DCM 2/98): white amorphous solid; $[\alpha]_D^{25}$ = −140° (c 0.3, MeOH); mp=185–189 °C; R*f*=0.3 (silica gel, MeOH/DCM 98/2); UV (λ_{max} MeOH): 229, 261, 311; IR (KBr): 3852, 3710, 3680, 2972, 2922, 2865, 2359, 2341, 2051, 2026, 1733, 1616, 1500, 1456, 1371, 1239, 1016, 843, 722, 680, 668, 654, cm^{-1}; ESI-MS (+ve) *m/z* 981.6 (M+H);^{1}H-NMR (600 MHz, CD_3OD): δ 6.89, 6.74 (2H, 2s, H-9), 6.21 (2H, s, H-12), 5.85 (2H, m, H-14), 5.35, 5.27 (2H, 2s, H-17), 5.16 (2H, 2d, H-15), 5.09 (1H, t, J= 7.6 Hz, C$\underline{H}$CH$_2$CO), 3.79, 3.75 (6H, 2s, C-11 OC$\underline{H}_3$), 3.76 (6H, s, COOC$\underline{H}_3$), 3.54, 3.52 (2H, 2s, H-2), 3.45 (2H, *m*, H-3b), 3.37 (2H, *m*, H-5), 2.99, (2H, m, CHC$\underline{H}_2$CO), 2.86, 2.77 (2H, 2br d, $J_{3\alpha,3\beta}$=16.3 H-3a), 2.69, 2.54 (2H, 2s, H-21), 2.66, 2.62 (6H, 2s, N-C$\underline{H}_3$), 2.50 (2H, m, H-5), 2.26 (4H, m, H-6), 2.06 (3H, s, CH$_2$COC$\underline{H}_3$),1.99, 1.98 (6H, 2s, OCOC$\underline{H}_3$), 1.54 (2H, m, H-19), 1.04–0.96 (2H, m, H-19), 0.45, 0.25 (6H, 2t, J= 7.2 Hz, H-18); ^{13}C-NMR (150 MHz, CD_3OD): δ 211.5 (CH$_2$C$\underline{O}$), 173.5, 173.4 (C$\underline{O}$OCH$_3$), 172.5, (O$\underline{C}$OCH$_3$), 159.7 (C-11), 153.3, 153.2, (C-13), 131.4, 131.3 (C-15), 125.7, 125.6 (C-14), 125.2 (C-8), 124.8, 124.7 (C-10), 123.4, 122.7 (C-9), 95.4, 95.0 (C-12), 84.8, 84.5 (C-2) 81.1, 81.0 (C-16), 77.6, 77.5 (C-17), 67.8 (C-21), 56.3, 56.2 (C-11 O$\underline{C}$H$_3$), 54.5, 54.3 (C-7), 52.8 (COO$\underline{C}$H$_3$), 52.7, 52.6 (C-5), 52.1, 52.0 (C-3), 49.7 (C$\underline{H}_2$CO), 45.1, 44.8 (C-6), 44.4, 44.2 (C-20), 39.6, 39.3(N-C$\underline{H}_3$), 34.8 (C$\underline{H}$CH$_2$CO), 32.0, 31.8 (C-19), 29.4 (CO$\underline{C}$H$_3$), 20.8 (OCO$\underline{C}$H$_3$), 8.6, 8.5 (C-18).

Synthesis of compound 13

A mixture of compound **11** (100 mg, 0.1 mmol) and of vindoline **2** (184 mg, 0.40 mmol) in 4 mL DCM and 2 mL H_2O was stirred at room temperature for 10 min. Trifluoroacetic acid (1.3 mL) was added to the reaction mixture which turned green. After stirring for 24 hours at room temperature aqueous sodium carbonate was added until alkaline pH. The aqueous phase was extracted with DCM (3x20 mL) and the extract dried over with Na_2SO_4 and concentrated under reduced pressure. The extract was chromatographed on alumina gave compound **13** (30 mg, 30%, eluted with MeOH/DCM 1/99) as a yellow powder $[\alpha]_D^{26}$ = −196° (c 0.31, MeOH); mp=210-220 °C, *Rf*=0.5 (silica gel, MeOH/DCM 96/4); UV (MeOH); $\lambda_{max;}$ 214, 261, 311 nm; IR (KBr): 3412, 2934, 2367, 2304, 2173, 2059, 2100, 2012, 1984, 1738, 1682, 1592, 1505, 1455, 1433, 1372, 1224, 1086, 954, 929, 834, 681955, 907, 889, 799, 741 cm^{-1}; ESI-MS (+ve) *m/z* 1447.8 (M+H); ^{1}H-NMR (600 MHz, CD_3OD): δ 7.95 (1H, d, J= 16.2 Hz C$\underline{H}$CHCO), 7.44, 6.89, 6.76 (3H, 3s, H9), 6.68 (1H, d, J= 16.2 Hz, CHC$\underline{H}$CO), 6.25, 6.21, 6.19 (3H, 3s, H-12), 5.90, 5.84, 5.78 (3H, 3 br dd, J=10.3, 4.8 Hz, H-14), 5.36, 5.28, 5.26 (3H, 3s, H-17), 5.16 (1H, overlap, CH$_2$C$\underline{H}$CO), 5.22, 5.16, 5.10 (3H, 3d, overlap, J= 10.3 Hz, H-15), 3.89, 3.76, 3.75, (9H, 3s, C-11 OC$\underline{H}_3$), 3.80, 3.61 (3H, 2s, H-53.0, 2), 3.78, 3.76, 3.75 (9H, 3s, COOC$\underline{H}_3$), 3.14 (2H, m, C$\underline{H}_2$CHCO), 2.94, 2.55, 2.50 (3H, 3s, H-21), 2.80, 2.64, 2.61 (9H, 3s, N-C$\underline{H}_3$), 2.00, 1.98, 1.98 (9H, 3s, OCOC$\underline{H}_3$), 0.55, 0.41, 0.32 (9H, 3t, J=

7.2 Hz, H-18); ^{13}C-NMR (125 MHz CD_3OD): δ 202.8 (CHCHCO), 173.6, 173.4, 173.3 ($COOCH_3$), 172.5, 172.4, 172.3 ($OCOCH_3$), 162.8, 159.8, 159.7 (C-11), 157.7, 153.2, 153.1 (C-13), 140.8 (CH=CHCO), 131.3, 131.3, 131.2 (C-15), 122.0, 123.7, 123.6 (C-9), 125.8, 125.1, 126.7 (C-8), 125.7, 125.7, 125.9 (C-14), 124.4, 124.9, 115.0 (C-10), 123.7 (CH=CHCO), 95.5, 95.1, 93.0 (C-12), 84.8, 84.4, 84.1 (C-2), 81.2, 80.9, 80.8 (C-16), 77.6, 77.5, 77.4 (C- 17), 68.2, 67.7, 67.2 (C-21), 56.4, 56.3, 56.3 (C-11 OCH_3,), 54.3, 53.9 (C-7), 53.0, 52.9, 52.8, ($COOCH_3$), 53.0, 52.8, 52.6 (C-5), 52.1, 51.9, 51.7 (C-3), 45.1 ($CHCH_2CO$), 44.8, 44.6 (C-6), 44.2, 44.2, 44.4 (C-20), 35.8 (C-10) $CHCH_2CO$, 37.5, 39.3, 39.5 (C-1), 32.2, 32.1, 31.8 (C-19), 20.8 ($OCOCH_3$), 8.75, 8.73, 8.03 (C-18).

Synthesis of compound 14

Trifluoroacetic acid (4 mL) was added to a stirred solution of **13** (300 mg, 0.30 mmol) and **2** (600 mg, 1.31 mmol) in DCM (12 mL).and H_2O (6 mL). The green mixture was stirred for 48 hours at room temperature. The reaction mixture was neutralized with sodium carbonate and extracted with DCM. The extract dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified on alumina to give compounds **14** (150 mg, 37%): white powder; $[\alpha]_D^{25} = -120$ (c 0.3, MeOH); mp = 290–300 °C; R*f* = 0.1 (silica gel, MeOH/DCM 96/4); UV (λ_{max} MeOH): 219, 261, 311; IR (KBr): 3305, 2935, 2871, 2360, 2341, 2073, 2079, 2002, 1735, 1615, 1498, 1455, 1432, 1371, 1223, 929, 872, 817, 742, 699, 683, 669, cm^{-1}; ESI-MS (+ve) *m/z* 1905.9 (M + H); ^{1}H-NMR (600 MHz, CD_3OD): δ 6.77, 6.75 (4H, 2s, H-9), 6.22, 6.20 (4H, s, H-12), 5.85 (4H, m, H-14), 5.34, 5.29 (4H, 2s, H-17), 5.16 (6 H, m, $CHCH_2CO$ H-15), 3.77, 3.73 (12H, 2s, C-11 OCH_3), 3.76 (12H, s, $COOCH_3$), 3.54, 3.51 (4H, 2s, H-2), 3.47 (4H, m, H-3b), 3.45 (4H, m, H-5), 2.88 (4H, m, $CHCH_2CO$), 2.79 (4H, m, H-3a), 2.67, 2.62 (12H, 2s, N-CH_3), 2.62, 2.56 (4H, 2s, H-21), 2.53 (4H, m, H-5), 2.22 (8H, m, H-6), 1.99, 1.98 (12H, 2s, $OCOCH_3$), 1.52 (4H, m, H-19), 1.04, (4H, m, H-19), 0.41, 0.30 (12H, 2t, $J_{18,19} = 7.2$ Hz, H-18); ^{13}C-NMR (150 MHz, CD_3OD): δ 213 ($CHCH_2CO$), 173.5, 173.4 ($COOCH_3$), 172.4, 172.4 ($OCOCH_3$), 159.8, 159.6 (C-11), 153.3, 153.2 (C-13),131.3, 131.2 (C-15), 125.6, 124.6 (C-8), 125.7, 125.6 (C-14), 123.4, 122.7 (C-9), 124.7, 124.6 (C-10), 95.6, 95.0 (C-12), 84.7, 84.4 (C-2) 80.9 (C-16), 77.6, 77.4 (C-17), 67.8, 67.7 (C-21), 56.2 (C-11 OCH_3), 52.8 ($COOCH_3$), 54.4, 54.3 (C-7), 52.0, 51.9 (C-5), 52.6 (C-3), 48.8 ($CHCH_2CO$), 44.9, 44.8 (C-6), 44.9, 44.2 (C-20), 35.2 ($CHCH_2CO$), 39.6, 39.2 (N-CH_3), 32.0, 31.8 (C 19), 20.7 ($OCOCH_3$), 8.81, 8.68 (C-18).

Synthesis of vindolicinone 15

Vindolicine **1** (100 mg, 0.10 mmol) and 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) (181 mg, 0.79 mmol) were dissolved in 0.8 mL diethyl ether at 0 °C under N_2. Water (100 μL) and acetic acid (100 μL) were added, and the solution was stirred for 6 hours at 0 °C, and 24 h at room temperature. The reaction mixture was neutralized with aqueous sodium carbonate and extracted with DCM Purification of the crude reaction mixture on alumina gave compound **15** (60 mg, 60%, eluted with hexane/acetone 3:7) as a lemon yellow powder; $[\alpha]_D^{20}$-135° (c 0.007, MeOH); mp = 232–235 °C, UV (MeOH): λ_{max}(log ε) 229, 261 and 370 (3.99) nm; IR (KBr): 3440, 2965, 2855, 1735, 1610, 1498, 1461, 1372, 1249, 1119, 1172, 1044, 740 cm^{-1}; ESI-MS (+ve) *m/z*: 939 $(M+H)^+$; ^{1}H-NMR (600 MHz, CD_3OD): δ 7.24 (2H, s, H-9), 6.18 (2H, s,H-12), 5.87 (2H, brdd, $J = 9.5$, 4.5 Hz, H-14), 5.33 (2H, s, H-17), 5.20 (2H, brd, $J = 9.5$ Hz, H-15), 3.79 (8H, s, $COOCH_3$, H-2), 3.62 (6H, s, C-11 OCH_3), 3.46 (2H, dd, $J = 16.2$, 4.5 Hz, H-3), 3.42 (2H, m, H-5), 2.90 (2H, bd, $J = 16.2$ Hz, H-3), 2.83 (2H, s, H-21), 2.78 (6H, s, N-CH_3), 2.66 (2H, m, H-5), 2.33, (4H, m, 2 H-6), 2.02 (6H, s, $OCOCH_3$), 1.62 (2H, dq, $J = 14.7$, 7.2 Hz, H-19), 1.21 (2H, dq, $J = 14.7$, $J = 7.2$ Hz, H-19), 0.55 (6H, t, $J = 7.2$ Hz, H-18); ^{13}C-NMR (150 MHz, CD_3OD): δ 195.6 (C-10′), 173.4 ($COOCH_3$), 172.5 ($OCOCH_3$), 163.1 (C-11), 157.9 (C-13), 131.2 (C-15), 126.2 (C-9), 125.9 (C-14), 125.4 (C-8), 123.3 (C-10), 93.0 (C-12), 84.2 (C-2), 80.9 (C-16), 77.5 (C-17), 67.8 (C-21), 56.2, (C-11 OCH_3), 53.8 (C-7), 53.0 ($COOCH_3$), 52.3 (C-5), 51.8 (C-3), 44.4 (C-6), 44.3 (C-20), 37.7 (N-CH_3), 32.0 (C 19), 20.8 ($OCOCH_3$), 8.1 (C-18).

Author Contributions

This project was devised and conducted by G.M. who also wrote most of the article. Experiments were done by Asia who also wrote a preliminary draft of the article; The X-ray structure elucidations were performed by Y. S. and L.V.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

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