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Graphical Abstract

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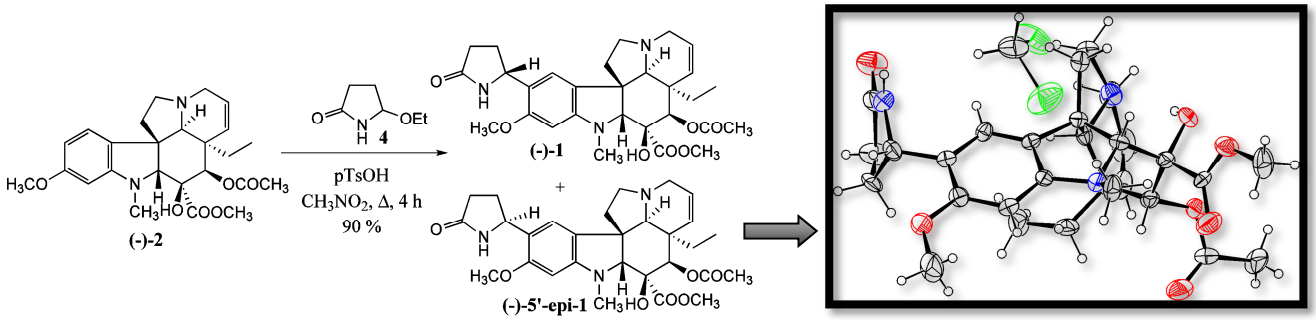

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A simple synthesis of bannucine and 5'-epibannucine from (−)-vindoline

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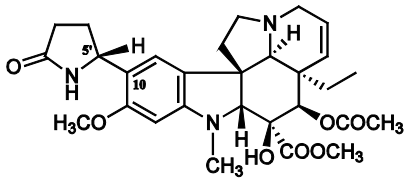
ABSTRACT

Bannucine is an *Aspidosperma alkaloid* isolated from the dried leaves of *Catharanthus roseus*. The molecule is a derivative of vindoline bearing a C10 substituent, a pattern common to the antineoplastic dimeric indole alkaloids of Catharanthus roseus. In bannucine, a 2-pyrrolidone moiety is attached at C5' to the aromatic ring of the vindoline core at C10. In the present work we report the synthesis of bannucine and its 5'-epimer from natural (−)-vindoline using a cyclic *N*-acyliminium ion intermediate whose *N*-acylaminocarbinol precursor is synthesized by the partial reduction of succinimide. We also describe the separation and the structural analysis of the two epimers, using among others, single crystal X-ray diffraction methods, in order to clarify the orientation of the proton attached to the C5' carbon. The *in vitro* antineoplastic activity of the pure epimers was also investigated, but none of the two substances showed significant activity on the examined tumor cell lines.

1. Introduction

Catharanthus roseus (L.) G. Don is a herbaceous evergreen plant endemic to Madagascar. It is grown around the globe as an ornamental plant and also as a medical plant used for the treatment of a wide range of diseases including hypertension, malaria, diabetes and Hodgkin's lymphoma.[1] During the past decades numerous alkaloids have been isolated from *Catharanthus roseus*, among which the most important ones are vinblastine and vincristine. These two antineoplastic dimeric indole alkaloids are used in the clinical treatment of certain types of cancer including leukemias, non-small cell lung cancer and Hodgkin's lymphoma. The isolation and structural analysis of potentially antineoplastic alkaloids derived from *Catharanthus roseus* is still a relevant field of research today. The structure elucidation, rational synthesis and chemical modification of the isolated compounds provide a constant challenge for organic chemists.[2]

The *Aspidosperma alkaloid* bannucine (**(−)-1**, **Figure 1.**) was isolated by Atta-ur-Rahman and co-workers from the dried leaves of Catharanthus roseus in 1986.[3] The molecule is a derivative of vindoline (**(−)-2**) bearing a C10 substituent, a pattern common to the antineoplastic dimeric indole alkaloids. In bannucine, a 2-pyrrolidone moiety is attached at C5' to the aromatic ring of the vindoline core at C10. Apart from spectroscopic structure elucidation the authors also described the new compound by giving its melting point (152-154 °C) and specific rotation ($[\alpha]_D$ = −33 (c = 0.26; chloroform)). Based on their NMR measurements the authors assigned a β-orientation to the proton attached to C5'.



(-)-1

Figure 1. The structure of bannucine **((−)-1)**

During our work we set the aim of synthesizing bannucine from natural (−)-vindoline.

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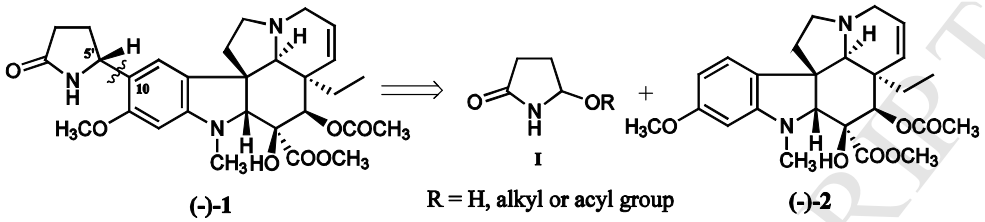
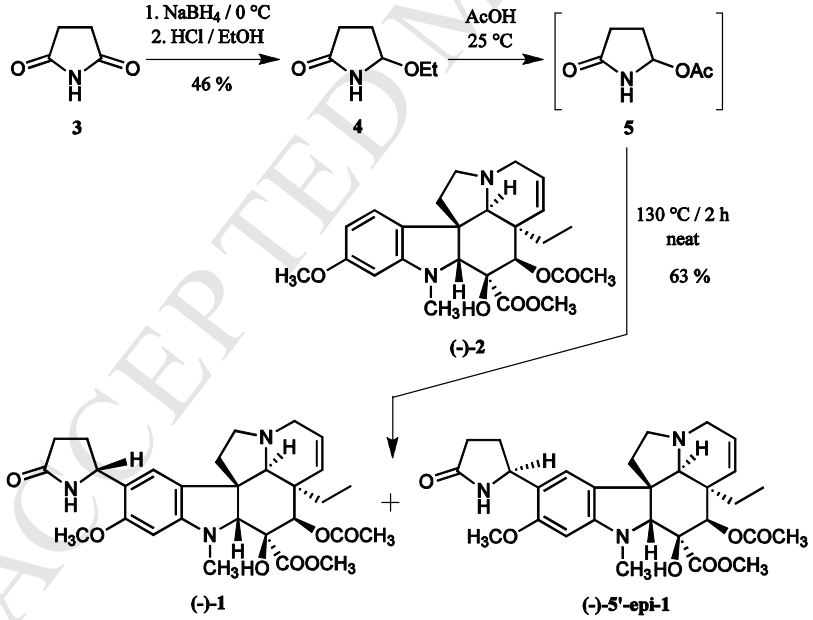
2. Results and discussion

N-acylaminocarbinol derivatives, as well as the *N*-acyliminium ions that arise from them, are often used as electrophilic reagents and preferred intermediates during the synthesis of alkaloids, especially for the construction of heterocyclic units.[4] The reactivity of vindoline (**(−)-2**) and the use of these reagents provide an opportunity for the synthesis of bannucine (**(−)-1**). The electron-rich aromatic ring of vindoline (**(−)-2**) is highly activated toward electrophilic substitution; its most reactive position is C10, owing to the directing effects of the methoxy and the dialkylamino group.[5-7] Considering the statements above we proposed a simple retrosynthetic scheme for the synthesis of bannucine (**(−)-1**) (**Scheme 1.**). Disconnection of the C10-C5' bond leads to the cyclic *N*-acylaminocarbinol reagent of type **I**, and vindoline (**(−)-2**).



Scheme 1. Retrosynthetic analysis of bannucine (**(−)-1**)

We first synthesized 5-acetoxypyrrolidine-2-one (**5**) according to the procedure described in the literature.[8-9] Compound **5** can be prepared from succinimide (**3**) in two steps (**Scheme 2.**). The partial reduction of **3** yields 5-ethoxypyrrolidine-2-one (**4**),[8] which is converted to acetate **5** by stirring in acetic acid at ambient temperature.[9] Considering the sensitivity of *N*-acylaminocarbinol acetate **5**, no attempts were made at its purification, instead it was used immediately as an oil in the next reaction. Vindoline (**(−)-2**) was allowed to react with an excess of acetate **5** at 130 °C under neat conditions for 2 hours, according to the procedure described by Nagasaka et al.[9] The reaction gave a mixture of bannucine and 5'-epibannucine in an acceptable yield (**Scheme 2.**). Purification of the epimeric mixture was carried out by column chromatography. The pure epimeric mixture was crystallized by trituration in ether and characterized by its melting point (151-153 °C) and specific rotation ($[\alpha]_D$ = −52.3 (c = 0.26; $CHCl_3$)).

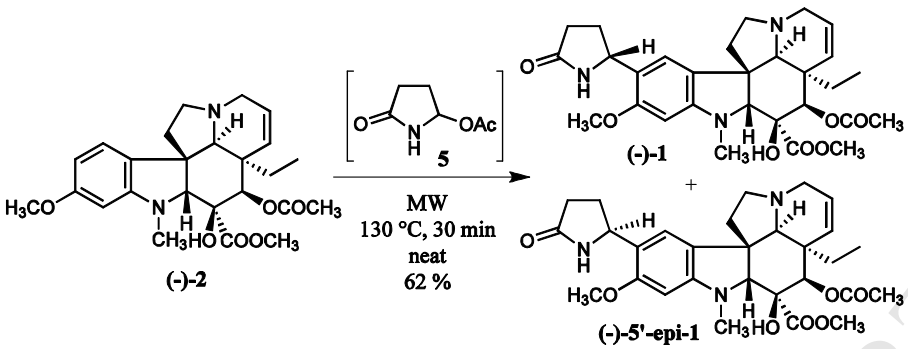


Scheme 2. Synthesis of bannucine epimers under neat conditions

Optimization of the excess of reagent **5** used is included in **Table 1.** (entries 1-5). Since raising the amount of the *N*-acylaminocarbinol reagent to more than 5 equivalents relative to vindoline did not result in a higher yield, experiments hereafter were conducted using 5 equivalents of acetate **5** relative to **(−)-2**.

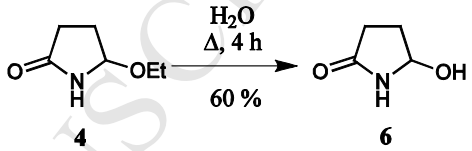
Next, we investigated the possibility of preparing the epimeric mixture using different methods. Applying microwave heating to neat mixtures of a five-fold excess of the *N*-acylaminocarbinol acetate **5** and vindoline (**(−)-2**) at 130 °C for different durations gave oily reaction mixtures (**Scheme 3.**) (**Table 1.**, entries 6-8). A shorter reaction time resulted in a clear reaction mixture, while longer reaction times gave lower yields presumably due to the thermal degradation of the product, which resulted in the formation of tarry impurities in the reaction mixture. The highest yield (62%) was reached after a 30 minute run at 130 °C (**Table 1.**, entry 8), which was virtually equal to that of the conventional heating method (**Table 1.**, entry 3). Compared to conventional heating, the advantage of using microwave heating was a significantly shorter reaction time and a reduced amount of tarry

side products, owing to the direct and uniform heating of the reaction mixture.

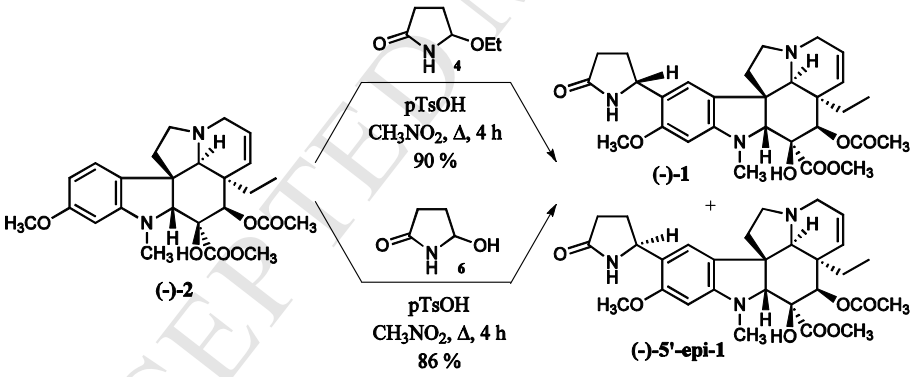


Scheme 3. Synthesis of bannucine epimers using microwave irradiation

Next, we investigated the acid catalysed solution-phase synthesis of the bannucine epimers. Small-scale model experiments were conducted to establish the most suitable solvent for the reaction (**Table 1.**, entries 9-15). Reactions were run in boiling solvents for 4 hours, with catalytic amounts of *p*-toluenesulfonic acid. The best solvent was found to be nitromethane, with the isolated yield 77%. The suitability of nitromethane can probably be attributed to its ability to solvate the ionic intermediates of the reaction due to its high polarity. A larger scale (100 mg) experiment in nitromethane gave the title compounds in an improved 90% overall yield (**Table 1.**, entry 16), likely due to decreased loss of material during isolation (**Scheme 5.**). Under the same conditions, amidocarbinol **6**, which was prepared by hot water hydrolysis of **4** (**Scheme 4.**),[10] gave virtually the same yield (**Table 1.**, entry 17), which comes as no surprise, considering that the same *N*-acyliminium intermediate (**7**) is involved in the reaction (**Scheme 6.**).



Scheme 4. Synthesis of 5-hydroxypyrrolidine-2-one (**6**)



Scheme 5. Acid catalysed solution-phase synthesis of bannucine epimers

Table 1. Yield of the bannucine epimers under different conditions

Entry	Reagent	Excess of reagent (mol. equiv.)	Solvent	Catalyst	Temp. (°C)	Time (h)	Heating	Yield*, † (%)
1	**5**	2	-	-	130	2	oil bath	31
2	**5**	3	-	-	130	2	oil bath	39
3	**5**	5	-	-	130	2	oil bath	63
4	**5**	10	-	-	130	2	oil bath	66
5	**5**	20	-	-	130	2	oil bath	64
6‡	**5**	5	-	-	130	2	MW	44
7‡	**5**	5	-	-	130	1	MW	50
8‡	**5**	5	-	-	130	0.5	MW	62

9	**4**	5	EtOAc	pTsOH	77 (Δ)	4	oil bath	22
10	**4**	5	THF	pTsOH	66 (Δ)	4	oil bath	56
11	**4**	5	DMF	pTsOH	120	4	oil bath	25
12	**4**	5	Dioxane	pTsOH	101 (Δ)	4	oil bath	66
13	**4**	5	Toluene	pTsOH	111 (Δ)	4	oil bath	36
14	**4**	5	CH_3NO_2	pTsOH	101 (Δ)	4	oil bath	77
15	**4**	5	CH_3CN	pTsOH	81 (Δ)	4	oil bath	58
16[#]	**4**	5	CH_3NO_2	pTsOH	101 (Δ)	4	oil bath	90
17[#]	**6**	5	CH_3NO_2	pTsOH	101 (Δ)	4	oil bath	86

*Experiments were run with 71 mg vindoline (**(−)-2**), except where otherwise noted.
[†] Isolated yield for the epimeric mixture of bannucine (**(−)-1**) and 5'-epibannucine (**(−)-5'-epi-1**). The ratio of bannucine and 5'-epibannucine in the product was identical (2:3) in all of the experiments.
[‡] Microwave reactions were run with 212 mg vindoline (**(−)-2**) (for details see the Experimental section).
[#] Larger-scale experiments were run with 100 mg vindoline (**(−)-2**), accounting for a higher isolated yield.

According to the NMR measurements of the product the mixture contained the two epimers in an approximate ratio of 2:3. Separation of the epimers was accomplished by multiple consecutive chromatographic purifications on preparative TLC plates. The structures of the pure epimers were elucidated by spectroscopic methods including MS and NMR measurements. In comparing our NMR results with those of Atta-ur-Rahman et al.,[3] we found that the synthetic epimers **(–)-1** and **(–)-5'-epi-1** and the bannucine isolated by them had very similar ^{13}C NMR spectra. However, some of our ^{13}C NMR assignments differ from those given by Atta-ur-Rahman et al.; in particular, we concluded that the chemical shift values for the OCO<u>C</u>H$_3$ and C4' carbons had been misassigned in the original work, presumably because of the lower spectral resolution available at that time. For this reason we herein provide our own assignments in the experimental section, as deduced from higher resolution ^{13}C NMR and ^{13}C–^{1}H correlation spectra (HSQC and HMBC).

The configuration of the stereogenic carbon at C5' could not be assigned unambiguously solely on the basis of the NMR spectra. In order to be able to assign the configuration of the carbon at C5' in each epimer, we grew a single crystal of the epimer with the lower R_f value, the component which is more abundant in the epimeric mixture, and could also be isolated with a higher yield. The unknown configuration was elucidated by X-ray crystallography (XRD) (**c.f. Figure 2.**). The absolute configuration of C5' turned out to be *S*, which translates to the α-orientation of the hydrogen at C5'. Therefore the examined isomer is 5'-epibannucin (**(−)-5'-epi-1**), and the ratio of epimers in the original mixture was **(−)-1** : **(−)-5'-epi-1** = 2:3. Further reactions yielded the epimers in approximately the same ratio.

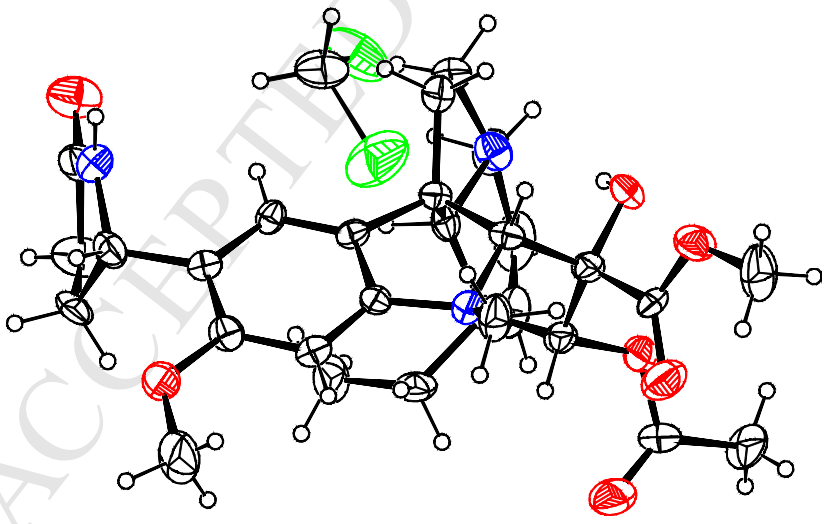


Figure 2. The XRD structure of 5'-epibannucine shown in 30% atomic displacement ellipsoids

The absolute configuration of the crystal could be ascertained by both the deduction of the configuration based on the known stereocenters and by the use of the anomalous dispersion effect mainly due to the dichloromethane solvent molecule. This latter proved to be successful even in this case when only a slightly populated solvent was retained in the crystal. The site occupancies were modelled as 50% population of the dichloromethane atomic positions. Both the Flack[11] and the Hooft parameters[12] show that the X-ray structure is the correct hand of the crystal. Analysis of the absolute structure using Bayesian likelihood methods was done using PLATON.[13] The results indicated that the absolute structure had been correctly assigned. The calculated probability that the structure is inverted is given as 0 (**Figure 3.**). As only a part of the original solvent quantity was retained in the crystal it might still be useful like a "heavy atom" derivative in defining the absolute stereochemistry.

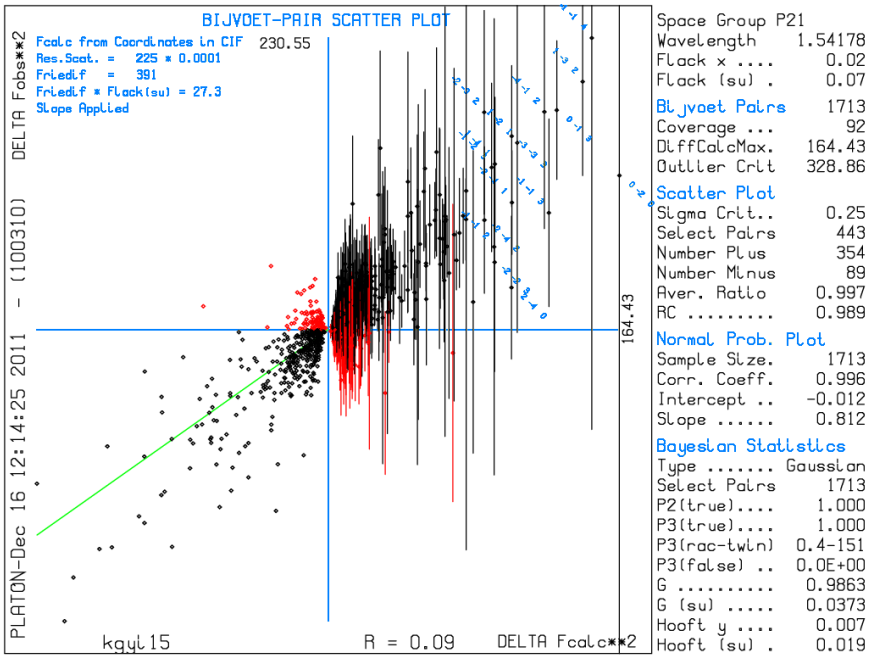
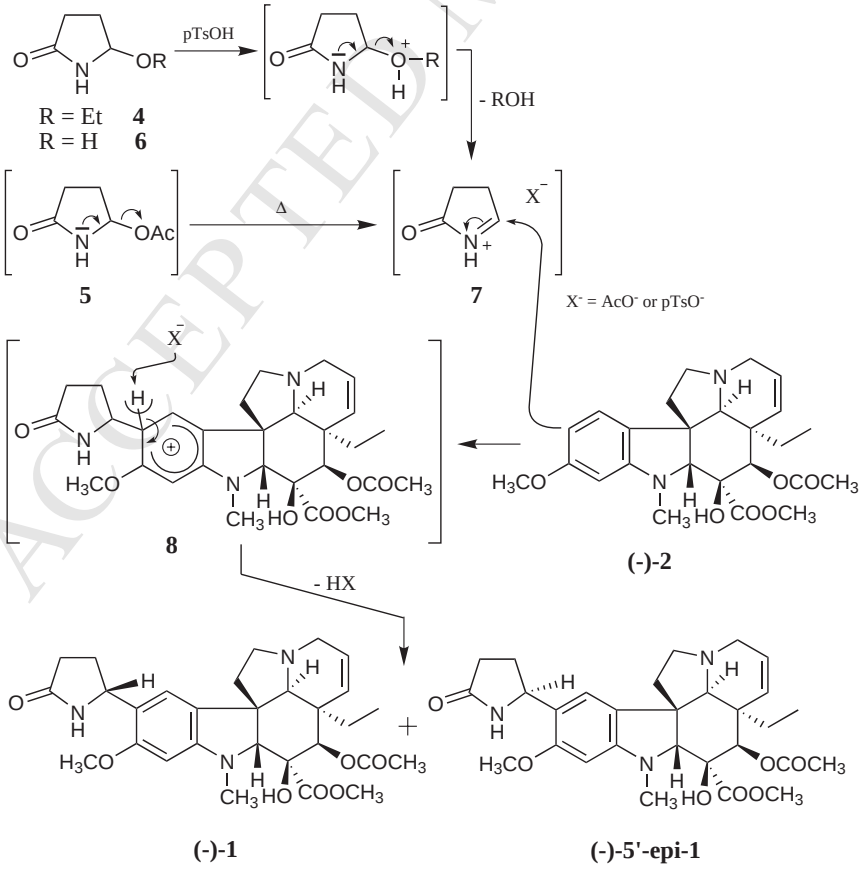


Figure 3. Bijvoet pair analysis[12] of the 5'-epibannucin (**(−)-5'-epi-1**) : dichloromethane 1 : 0.5 crystal

Based on the literature, we proposed a plausible mechanism for the formation of the bannucine epimers. Depending on the *N*-acylaminocarbinol reagent used, either the protonation of **4** or **6**, or the thermal heterolysis of **5** leads to the formation of the *N*-acyliminium salt **7**, which is an electrophile reactive enough to attack the aromatic ring of vindoline (**(−)-2**) at C10. The reaction follows the general mechanism of electrophilic aromatic substitutions: the σ-complex (**8**) formed in the reaction is deprotonated by the acetate or tosylate anion yielding a mixture of bannucine (**(−)-1**) and 5'-epibannucine (**(−)-5'-epi-1**) (**Scheme 6.**).



Scheme 6. Proposed reaction mechanism for the formation of the bannucine epimers

The pure epimers of bannucine were characterized by their melting points and specific rotations. Bannucine (**(−)-1**) melted at 284-286 °C with a specific rotation $[\alpha]_D$ = −12.3 (c = 0.26; $CHCl_3$), while 5'-epibannucine (**(−)-5'-epi-1**) melted at 191-193 °C and had specific rotation $[\alpha]_D$ = −72.3 (c = 0.26; $CHCl_3$). Comparison of the melting points and specific rotations we measured for the pure epimers to those from the literature[3] gave no leads in the identification of the epimers (**Figure 4.**). Based on the fact that the specific rotation given by Atta-ur-Rahman et al. for the substance they regarded as pure bannucine ($[\alpha]_D$ = −33 (c = 0.26; $CHCl_3$))[3] significantly differs from the value measured by us, and that it falls between the specific rotations we measured for bannucine (**(−)-1**) ($[\alpha]_D$ = −12.3 (c = 0.26; $CHCl_3$)) and 5'-epibannucine (**(−)-5'-epi-1**) ($[\alpha]_D$ = −72.3 (c = 0.26; $CHCl_3$)), it is likely that the substance Atta-ur-Rahman et al. isolated from *Catharanthus roseus* was not pure bannucine, but in fact a mixture of the two epimers. This assumption is corroborated by the fact that the melting point given by Atta-ur-Rahman et al. for their substance (152-154 °C),[3] is very close to the one we measured for the solid epimeric mixture that we synthesized (151-153 °C), and it is much lower than the melting point we have measured for each pure epimer (bannucine (**(−)-1**) mp.: 284-286 °C; 5'-epibannucine (**(−)-5'-epi-1**) mp.: 191-193 °C).

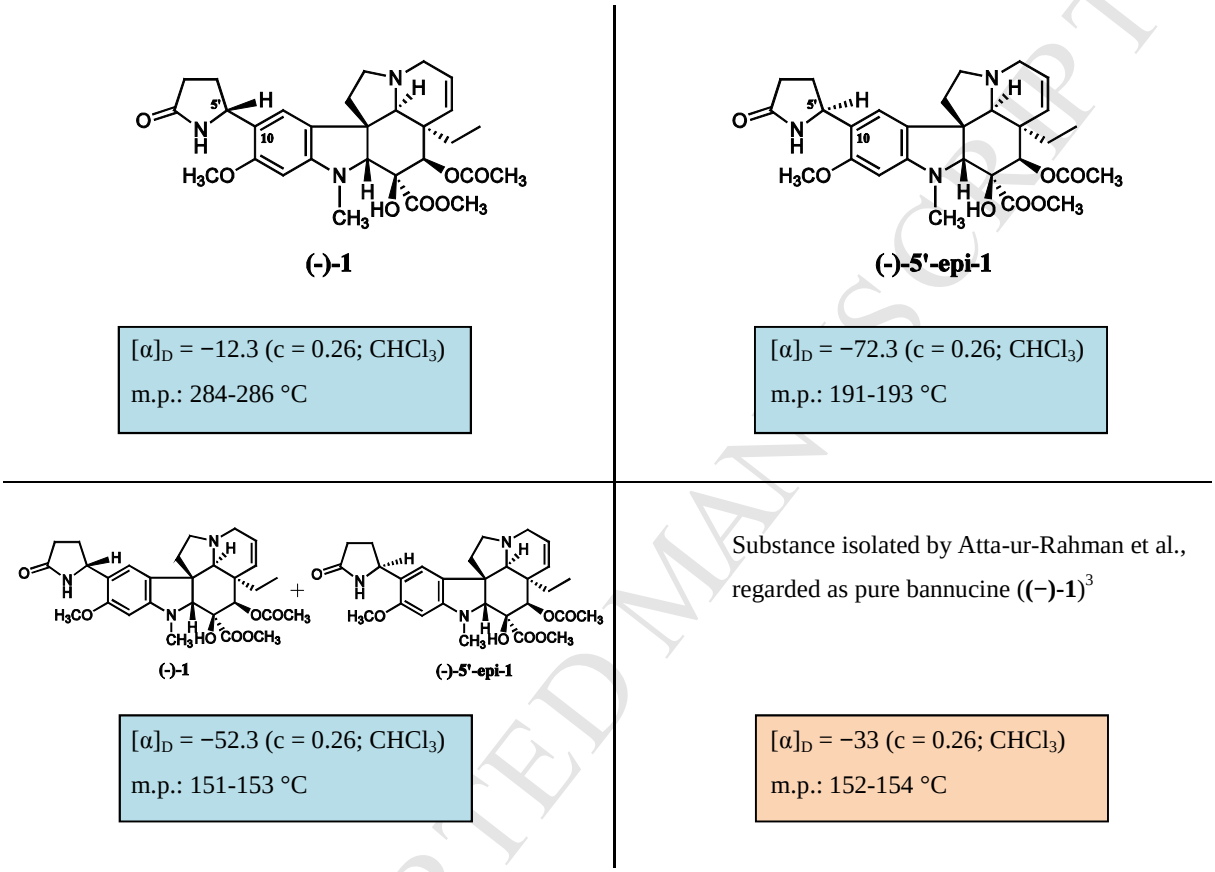


Figure 4. Comparison of own and literature data: melting points and specific rotations of the epimeric mixture and of the pure epimers

3. Biology

The *in vitro* antineoplastic activity of the pure epimers (**(−)-1**) and (**(−)-5'-epi-1**) was investigated at the National Institutes of Health (NIH), where the proliferation inhibitory effect of the two samples was measured against different tumor cell lines. The experiments were performed on 57 different tumor cell lines embracing 9 frequently occurring tumor types. The tumor types and cell lines were the following: leukemia (CCRF-CEM, HL-60(TB), K-562, MOLT-4, SR), non-small cell lung cancer (A549/ATCC, EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, NCI-H460, NCI-H522), colon cancer (COLO 205, HCC-2998, HCT-116, HCT-15, HT29, KM12, SW-620), CNS cancer (SF-268, SF-295, SF-539, SNB-19, SNB-75, U251), melanoma (LOX IMVI, MALME-3M, M14, MDA-MB-435, SK-MEL-2, SK-MEL-28, SK-MEL-5, UACC-257, UACC-62), ovarian cancer (OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, NCI/ADR-RES, SK-OV-3), renal cancer (786-0, A498, ACHN, CAKI-1, RXF 393, SN12C, TK-10, UO-31), prostate cancer (PC-3, DU-145), breast cancer (MCF7, HS 578T, BT-549, T-47D, MDA-MB-468).

The NCI screening procedures were conducted as described in the literature,[14] and the origins and processing of the cell lines were also described.[14-16] The inhibitory effect of **(−)-1** and **(−)-5'-epi-1** samples were measured against the listed tumor cell lines at the concentration of 2.5×10^{-6} M and 10^{-5} M respectively, but both epimers were ineffective on the examined tumor cell lines (no negative growth percentage value was greater than 20%).

4. Conclusion

In the present work we successfully elaborated the synthesis of the *Aspidosperma alkaloid* bannucine (**(–)-1**) and its 5'-epimer (**(–)-5'-epi-1**) from natural (–)-vindoline (**(–)-2**). In our synthesis we applied a cyclic *N*-acyliminium intermediate, whose *N*-

acylaminocarbinol precursor was synthesized by the partial reduction of succinimide. The epimers were separated by preparative TLC and fully characterised, including X-ray crystallography, which allowed the unambiguous assignment of the configuration of the C5' carbon in each epimer, allowing clarification of the data previously reported for bannucine (**(–)-1**). The *in vitro* antineoplastic activity of the pure epimers was tested against 57 different tumor cell lines, but none of the two substances showed significant antitumor activity.

5. Experimental section

5.1. General

Melting points were measured on a SANYO Gallenkamp apparatus and are uncorrected. IR spectra were recorded on a Bruker FT-IR instrument. Optical rotations were measured on a PerkinElmer 241 polarimeter at ambient temperature, at the D-line of sodium (λ = 589.3 nm). ^{1}H NMR and ^{13}C NMR measurements were performed on Varian 400 MHz, Varian 500 MHz and Varian 800 MHz spectrometers. Chemical shifts are given on the delta scale as parts per million (ppm) with tetramethylsilane (TMS) (^{1}H) or dimethylsulfoxide-d_6 (^{13}C) as the internal standard (0.00 ppm and 39.5 ppm, respectively). MS spectra were recorded on VG-Trio-2 and Finnigan MAT 95SQ instrument using EI or ESI techniques. HRMS analyses were performed on an LTQ FT Ultra (Thermo Fischer Scientific, Bremen, Germany) system. Preparative TLC was carried out using Kieselgel 60 F_{254} (Merck) coated glass plates. Column chromatography was performed using Geduran Si 60 (Merck) silica. Investigations using microwave heating were conducted in a single-mode Discover System (CEM Corporation) using sealed reaction vessels (borosilicate, 10 mL volume). Experiments were performed in temperature control mode (using the built-in calibrated IR sensor) with 200 W initial power. The reaction mixtures were stirred („low" setting), the specified time is equal to the time at set point. (–)-Vindoline (isolated from *Catharanthus roseus*) was provided by Gedeon Richter Plc.

5.2. X-ray diffraction

This experiment was carried out at ambient temperature on an apparently intact crystal selected from a batch vial. All data collections were run on a RIGAKU R-AXIS RAPID II image plate diffractometer using Cu-K_α radiation. Crystal data: $C_{29.5}H_{38}ClN_3O_7$, Fwt.: 582.08, white, prism, size: 0.03 × 0.02 × 0.01 mm, space group $P2_1$, a = 11.4810(10) Å, b = 12.1940(10) Å, c = 12.7760(10) Å, β = 115.174(4)°, V = 1618.7(2) Å^3, T = 293(2) K, Z = 2, F(000) = 618, D_x = 1.194 Mg/m^3, μ = 1.429 mm^{-1}. A crystal of 5'-epibannucin was mounted on a loop. Cell parameters were determined by least-squares of the setting angles of 5730 (6.84 ≤ θ ≤ 54.23) reflections. Intensity data were collected on a R-AXIS RAPID diffractometer (graphite monochromator; Cu-K_α radiation, λ = 1.54178 Å) at 293(2) K in the range 6.83 ≤ θ ≤ 54.22 using θ/2θ scans. A total of 8724 reflections were collected of which 3778 were unique [$R_{(int)}$ = 0.0396, $R(\sigma)$ = 0.0861]; intensities of 2824 reflections were greater than 2(σI). Completeness to 2θ = 0.984. A numerical absorption correction was applied to the data (the minimum and maximum transmission factors were 0.952 and 0.994). The structure was solved by direct methods[17] (and subsequent difference syntheses). Anisotropic full-matrix least-squares refinement[17] on F^2 for all non-hydrogen atoms yielded R_1 = 0.0893 and wR^2 = 0.2111 for 2824 [I > 2(σI)] and R_1 = 0.1172 and wR^2 = 0.2290 for all (3778) intensity data, (number of parameters = 394, goodness-of-fit = 1.083, the Flack absolute structure parameter x = 0.02(7), Hooft y = 0.01(2), the maximum and mean shift/esd is 0.004 and 0.001). The maximum and minimum residual electron density in the final difference map was 0.52 and -0.28 e·Å^{-3}. Hydrogen atomic positions were calculated from assumed geometries. Hydrogen atoms were included in structure factor calculations but they were not refined. The isotropic displacement parameters of the hydrogen atoms were approximated from the U(eq) value of the atom they were bonded to. Crystal structure data are deposited with the Cambridge Crystallographic Data Centre under CCDC 1063928 (**(−)-5'-epi-1**) and can be obtained free of charge upon application.

5-Ethoxypyrrolidine-2-one (4)
To a solution of succinimide (**3**) (7.156 g, 72.22 mmol) in ethanol (300 mL) at 0 °C was added sodium borohydride (4.00 g, 105.74 mmol) in one portion. The reaction mixture was stirred at 0 °C for 4 h, during which time every 15 min 5 drops of 2 M ethanolic hydrogen chloride solution were added. Then the reaction mixture was acidified to pH=3 with 2 M ethanolic hydrogen chloride solution over 30 min, after which it was stirred at 5 °C for 45 min. Then the reaction mixture was neutralised (pH=7) with 5% ethanolic potassium hydroxide solution and evaporated *in vacuo* to give a syrupy solid, which was suspended in chloroform (80 mL), filtered, and the precipitate washed with chloroform (3×20 mL). The filtrate was evaporated *in vacuo* to give a colourless oil, which was dissolved in dichloromethane (80 mL) and washed with water (3×10 mL). The aqueous phase was extracted with dichloromethane (6×20 mL), then the organic phases were unified, dried ($MgSO_4$) and the solvent evaporated *in vacuo* to give the *title compound* **4** (4.327 g, 46%) as a colourless oil, which crystallized on standing, giving white crystals, m.p. 51-53 °C (lit.: 48-53 °C);[8] R_f (acetone) 0.72; ν_{max}(KBr) 3200, 2978, 1707, 1689, 1668, 1457, 1282, 1250, 1067, 986 cm^{-1}; δ_H (400 MHz, DMSO-d_6) 8.62 (1 H, s, NH), 4.89-4.83 (1 H, m, H-5), 3.54-3.44 (1 H, m, OCH_{2y}), 3.35-3.27 (1 H, m, OCH_{2x}), 2.30-2.11 (1 H, m, H_y-4), 2.30-2.11 (1 H, m, H_y-3), 2.05-1.95 (1 H, m, H_x-3), 1.89-1.78 (1 H, m, H_x-4), 1.10 (3 H, t, J 7.0 Hz, CH_3); δ_C (100.6 MHz, DMSO-d_6) 177.4 (CON), 85.0 (C-5), 61.6 (OCH_2), 28.1 (C-3), 27.7 (C-4), 15.1 (CH_3); MS(ESI): 130 ($C_6H_{12}NO_2$); ESI-MS-MS (cid=35) (rel. int. %): 84(100).

5-Hydroxypyrrolidine-2-one (6)
A solution of 5-ethoxypyrrolidin-2-one (**4**) (2.0 g, 15.5 mmol) in water (25 mL) was refluxed for 3 h. The solvent was evaporated *in vacuo* to give a colourless oil, which was triturated with ethyl acetate to give a white solid. The solid was filtered and recrystallized from acetone to give the *title compound* **6** (0.89 g, 60%) as a white solid, m.p. 94-96 °C (lit.: 90 °C);[13] R_f (33% hexane/acetone) 0.19; ν_{max}(KBr) 3254, 2996, 2962, 1668, 1475, 1415, 1323, 1271, 1166, 1101, 1070, 1016cm^{-1}; δ_H (400 MHz, DMSO-d_6) 8.18 (1 H, s, NH), 5.70 (1 H, d, J 6.9 Hz, OH), 5.06 (1 H, m, H-5), 2.33-2.23 (1 H, m, H_y-3), 2.24-2.14 (1 H, m, H_y-4), 2.03-1.94 (1 H, m, H_x-3), 1.75-1.66 (1 H, m, H_x-4); δ_C (100.6 MHz, DMSO-d_6) 176.7 (CON),78.5 (C-5), 30.3 (C-4), 28.4 (C-3); HRMS: 102.05491 ($C_4H_8NO_2$; calc. 102.05496); ESI-MS-MS (cid=55) (rel. int. %): 85(100).

Bannucine ((−)-1) and 5'-epibannucine ((−)-5'-epi-1)

method a): neat mixture, conventional heating

A solution of 5-ethoxypyrrolidine-2-one (**4**) (300 mg, 2.32 mmol) in acetic acid (10 mL) was stirred at room temperature for 24 h. The solvent was evaporated *in vacuo* in a water bath of 35-40 °C, then vindoline (**(−)-2**) (212 mg, 0.46 mmol) was added and the neat mixture was stirred at 130 °C for 2 h. The arising dark brown oil, which solidified on standing at room temperature, was

dissolved in dichloromethane (20 mL), the solution was washed with saturated sodium bicarbonate solution (10 mL), dried ($MgSO_4$) and the solvent evaporated *in vacuo*. Purification of the crude product by column chromatography (acetone) gave the epimeric mixture of the *title compounds* **(−)-1** and **(−)-5'-epi-1** (158 mg, 63%) as an off-white amorphous solid, which was crystallized by trituration in ether to give white crystals, m.p. 151-153 °C (lit. (bannucine): 152-154 °C);[3] R_f (9% MeOH/CH_2Cl_2) 0.68 and 0.63; $[\alpha]_D^{25}$ −52.3 (*c* 0.26, $CHCl_3$) (lit. (bannucine): $[\alpha]_D^{25}$ −33 (*c* 0.26, $CHCl_3$));[3] ν_{max}(KBr) 3573, 3311, 3028, 2965, 2946, 2877, 1740, 1694, 1612, 1599, 1500, 1465, 1431, 1374, 1242, 1047 cm^{-1}. Comparison of the ^1H and ^{13}C NMR spectra of the epimeric mixture to literature data[3] showed that the substance we synthesized was indeed a mixture of bannucine and its C5'-epimer. Epimeric ratios can be calculated from the integrated intensities of the following protons: H-C(16)OH, H-1', H-17, $H_α$-3, H-21, H_x-4' (see the spectra of the pure epimers in the Supporting Information). MS(ESI): 540 ($C_{29}H_{38}N_3O_7$); ESI-MS-MS (cid=35) (rel. int. %): 522(10); 480(100); 448(5); 271(28).

method b): neat mixture, microwave heating

A solution of 5-ethoxypyrrolidine-2-one (**4**) (300 mg, 2.32 mmol) in acetic acid (10 mL) was stirred at room temperature for 24 h. The solution was transferred to a standard 10 mL MW vessel and the solvent was evaporated *in vacuo* in a water bath of 35-40 °C. Vindoline (**(−)-2**) (212 mg, 0.46 mmol) was added, the vessel was sealed and the neat mixture was heated at 130 °C in a microwave reactor for 30 min. The arising colourless oil was dissolved in dichloromethane (20 mL), the solution was washed with saturated sodium bicarbonate solution (10 mL), dried ($MgSO_4$) and the solvent evaporated *in vacuo*. Purification of the crude product by column chromatography (acetone) gave the epimeric mixture of the *title compounds* **(−)-1** and **(−)-5'-epi-1** (156 mg, 62%) as an off-white amorphous solid, which was crystallized by trituration in ether to give white crystals. The physical and spectral characteristics of the product are the same as in *method a)*.

method c): acid catalysed solution-phase reaction

To a mixture of vindoline (**(−)-2**) (100 mg, 0.22 mmol) and 5-ethoxypyrrolidine-2-one (**4**) (141 mg, 1.09 mmol) in nitromethane (5 mL) a catalytic amount of *p*-toluenesulfonic acid was added and the mixture refluxed for 4 h. The solvent was evaporated *in vacuo*, the residue was dissolved in dichloromethane (20 mL), the solution was washed with saturated sodium bicarbonate solution (10 mL), dried ($MgSO_4$) and the solvent evaporated *in vacuo*. Purification of the crude product by column chromatography (acetone) gave the epimeric mixture of the *title compounds* **(−)-1** and **(−)-5'-epi-1** (106 mg, 90%) as an off-white amorphous solid, which was crystallized by trituration in ether to give white crystals. The physical and spectral characteristics of the product are the same as in *method a)*.

Separation of the epimers of bannucine

Purification of an epimeric mixture of bannucine (**(−)-1**) and 5'-epibannucine (**(−)-5'-epi-1**) (158 mg) by preparative TLC (9% MeOH/CH_2Cl_2) gave the *title compounds* as white amorphous solids, which were crystallized by trituration in ether. Bannucine (**(−)-1**) (40 mg, 25%) was obtained as a white solid, m.p. 284-286 °C (lit. (bannucine): 152-154 °C);[3] R_f (9% MeOH/CH_2Cl_2) 0.68; $[\alpha]_D^{25}$ −12.3 (*c* 0.26, $CHCl_3$) (lit. (bannucine): $[\alpha]_D^{25}$ −33 (*c* 0.26, $CHCl_3$));[3] ν_{max}(KBr) 3315, 2945, 2876, 2834, 1735, 1710, 1612, 1599, 1497, 1466, 1448, 1430, 1377, 1342, 1259, 1243, 1224, 1047, 813, 759 cm^{-1}; δ_H (799.7 MHz, DMSO-d_6) 8.78 (1 H, s,C(16)OH), 7.70(1 H, s, H-1'), 6.97 (1 H, s, H-9), 6.33 (1 H, s, H-12), 5.82 (1 H, ddd, *J* 10.1, 4.9, 1.7 Hz, H-14), 5.19 (1 H, s, H-17), 5.09 (1 H, dt, *J* 10.1, 2.0 Hz, H-15), 4.80 (1 H, dd, *J* 7.7, 5.8 Hz, H-5'), 3.78 (3 H, s,C(11)OCH_3), 3.66 (3 H, s,C(16)COOCH_3), 3.53 (1 H, s, H-2), 3.43 (1 H, dd, *J* 16.4, 4.9 Hz, $H_β$-3), 3.29 (1 H, td, *J* 9.0, 4.1 Hz, $H_β$-5), 2.82 (1 H, brd, *J* 16.4 Hz, $H_α$-3), 2.66 (1 H, s, H-21), 2.60 (3 H, s, H_3-1), 2.58-2.54 (1 H, m, $H_α$-5), 2.38-2.33 (1 H, m, H_y-4'), 2.24-2.18 (2 H, buried m, H_2-6), 2.21-2.12 (2 H, buried m, H_2-3'), 1.94 (3 H, s, C(17)OCOCH_3), 1.69-1.65 (1 H, m,H_x-4'), 1.47-1.43 (1 H, m, H_y-19), 0.95-0.91 (1 H, m, H_x-19), 0.43 (3 H, t, *J* 7.5 Hz, H_3-18); δ_C (201.1 MHz DMSO-d_6) 177.1 (C-2'), 171.6 (C(16)COOCH$_3$), 170.0 (C(17)OCOCH$_3$), 157.3 (C-11), 152.5 (C-13), 130.0 (C-15), 124.31 (C-14), 124.28 (C-8), 122.1 (C-10), 119.7 (C-9), 93.7 (C-12), 82.9 (C-2), 78.7 (C-16), 75.9 (C-17), 66.1 (C-21), 55.6 (C(11)OCH$_3$), 52.3 (C-7), 51.7 (C(16)COOCH$_3$), 51.5 (C-5'), 51.2 (C-5), 50.5 (C-3), 43.7 (C-6), 42.5 (C-20), 38.6 (C-1), 30.6 (C-19), 30.0 (C-3'), 29.1 (C-4'), 20.7 (C(17)OCOCH$_3$), 7.6 (C-18); MS(ESI): 540 ($C_{29}H_{38}N_3O_7$); ESI-MS-MS (cid=35) (rel. int. %): 522(10); 480(100); 448(5); 271(26).

5'-Epibannucine (**(−)-5'-epi-1**) (75 mg, 47%) was obtained as a white solid, m.p. 191-193 °C (lit. (bannucine): 152-154 °C);[3] R_f (9% MeOH/CH_2Cl_2) 0.63; $[\alpha]_D^{25}$ −72.3 (*c* 0.26, $CHCl_3$) (lit. (bannucine): $[\alpha]_D^{25}$ −33 (*c* 0.26, $CHCl_3$));[3] ν_{max}(KBr) 3573, 3321, 2960, 2877, 1741, 1694, 1615, 1600, 1500, 1466, 1433, 1373, 1241, 1047, 740 cm^{-1}; δ_H (799.7 MHz, DMSO-d_6) 8.89 (1 H, s,C(16)OH),7.78 (1 H, s, H-1'), 6.98 (1 H, s, H-9), 6.33 (1 H, s, H-12), 5.82 (1 H, ddd, *J* 10.1, 4.9, 1.4 Hz, H-14), 5.16 (1 H, s, H-17), 5.09 (1 H, dt, *J* 10.1, 1.4 Hz, H-15), 4.82 (1 H, dd, *J* 7.5, 6.0 Hz, H-5'), 3.78 (3 H, s, C(11)OCH_3), 3.65 (3 H, s, C(16)COOCH_3), 3.54 (1 H, s, H-2), 3.40 (1 H, ddd, *J* 16.4, 4.9, 1.4 Hz, $H_β$-3), 3.29 (1 H, td, *J* 9.1, 4.3 Hz, $H_β$-5), 2.88 (1 H, dt, *J* 16.4, 1.4 Hz, $H_α$-3), 2.70 (1 H, s, H-21), 2.62-2.59 (1 H, buried m, $H_α$-5), 2.60 (3 H, s, H_3-1), 2.38-2.36 (1 H, m, H_y-4'), 2.24-2.19 (2 H, m, H_2-6), 2.18-2.14 (1 H, m, H_y-3'), 2.12-2.08 (1 H, m, H_x-3'), 1.93 (3 H, s,C(17)OCOCH_3),1.59-1.56 (1 H, m, H_x-4'), 1.48-1.45 (1 H, m, H_y-19), 0.89-0.86 (1 H, m,H_x-19), 0.41 (3 H, t, *J* 7.4 Hz,H_3-18); δ_C (201.1 MHz DMSO-d_6) 177.0 (C-2'), 171.6 (C(16)COOCH$_3$), 170.1 (C(17)OCOCH$_3$), 157.2 (C-11), 152.4 (C-13), 129.8 (C-15), 124.6 (C-14), 124.1 (C-8), 122.0 (C-10), 119.5 (C-9), 93.7 (C-12), 82.9 (C-2), 78.7 (C-16), 75.9 (C-17), 65.8 (C-21), 55.6 (C-C(11)OCH$_3$), 52.4 (C-7), 51.7 (C(16)COOCH$_3$), 51.1 (C-5'), 50.9 (C-5), 50.3 (C-3), 43.4 (C-6), 42.4 (C-20), 38.6 (C-1), 30.4 (C-19), 30.0 (C-3'), 29.9 (C-4'), 20.7 (C(17)OCOCH$_3$), 7.4 (C-18); MS(ESI): 540 ($C_{29}H_{38}N_3O_7$); ESI-MS-MS (cid=35) (rel. int. %): 522(10); 480(100); 448(5); 271(26).

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Supplementary data

Supplementary data (^1H, ^{13}C, HSQC and HMBC NMR spectra of bannucine and 5'-epibannucine) associated with this article can be found in the online version, at http://xxxxxxxxxxxxxxxxxxx.

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