Vinblastine-Type Antitumor Alkaloids: A Method for Creating New C17 Modified Analogues

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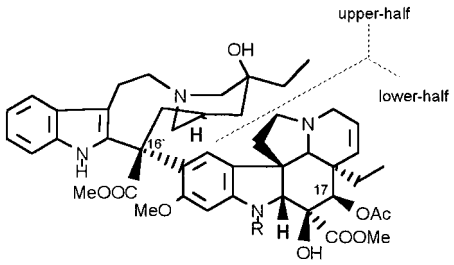
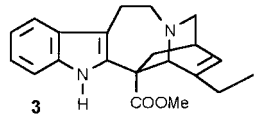
Vinblastine (**1**) and vincristine (**2**) (Figure 1), currently widely used in chemotherapy,[1] have attracted a great deal of attention from the synthetic point of view by virtue of their intriguing structural features, potent pharmacology activity, and limited natural availability from vegetable sources.[2] As a consequence, a number of approaches to **1** and **2** are known, all based on the coupling of catharanthine (**3**) or a synthetically generated upper-half to the naturally occurring lower-half vindoline (**4**) (Figure 2).[3] A large number of analogues, modified at −COOMe and −OAc in the lower-half, have been prepared. However, a full understanding of the structure−activity relationship is far from clear, and therefore, any synthetic route that leads to a modified vincristine- or vinblastine-type alkaloid must be given due consideration.[4]

In this report we describe the synthesis of the binary alkaloid **13**, its subsequent stereoselective hydroxylation to **14**, and its reaction with tri-*O*-acetylguanosine to give the novel adduct **15** (Figure 3), the first example of a C17-modified vinblastine-like alkaloid.



1 R = CH_3
2 R = CHO

Figure 1.



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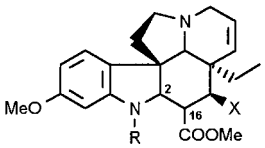

4	2βH	R = Me	16βOH	X = OAc
7	$\Delta_{2,16}$	R = H		X = H
8	2βH	R = H	16βH	X = H

Figure 2.

In a previous paper we suggested[5] a project for the regio- and stereoselective hydroxylation, at the C17 position, of the anhydrocarbomethoxyvelbanamine/11-methoxytabersonine binary compound **5** by benzeneseleninic anhydride (BSA);[6] this was a convenient and innovative approach toward the preparation of vinblastine and vincristine analogues bearing different substituents at C17. We realized a model study describing the successful hydroxylation at C17 of the binary alkaloid eburnamine/11-methoxytabersonine **6a** (Figure 4). A subsequent elaboration to the eburnamine/vindoline binary compound and the preparation of 17*β*-substituted derivatives having vinblastine-type structures resulted in the adduct **6b** that has anticancer activity, although low. We have now extended these results to the more precious binary compounds with 20-dehydroxycarbomethoxyvelbanamine in the "top-half".

Our first goal was to prepare the anhydrocarbomethoxyvelbanamine/11-methoxytabersonine binary derivative **5** (Figure 3); however, the coupling between catharanthine (**3**) and 11-methoxytabersonine (**7**) (Figure 2) by the Polonovski−Potier[3c] reaction gave only a 4% yield of the desired compound. The application of the methodology described by Szantay[3f] did not improve the

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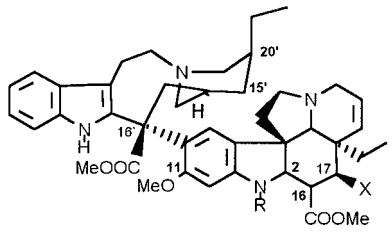

5	$\Delta_{20',15'}$	R = H	$\Delta_{2,16}$	X = H
9	$\Delta_{20',15'}$	R = H		X = H
10	$\Delta_{20',15'}$	R = Me		X = H
11		R = H		X = H
12		R = Me		X = H
13		R = H	$\Delta_{2,16}$	X = H
14		R = H	$\Delta_{2,16}$	X = OH

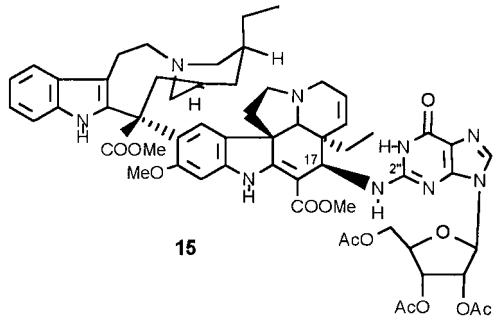

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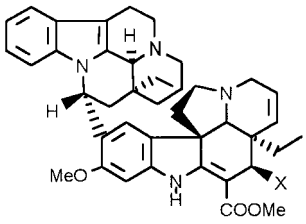
Figure 3.



6a X= H
6b X= O-triacetylguanosine

Figure 4.

result; therefore, we then considered modifying the starting compounds. Our reasoning was that the presence of the β-anilino acrylic system of 11-methoxytabersonine (**7**) reduces the nucleophilic character of C10 that, in the case of vindoline (**4**), is very high. In fact this obstacle was removed by reducing **7** with $NaBH_3CN$,[7] resulting in 2,16-dihydro-11-methoxytabersonine (**8**) (Figure 2). The coupling of **8** with **3** was realized in an aqueous medium in the presence of ferric chloride, in accordance with the Szantay modification of the previous Kutney methodology.[3d] This simple and economical reaction furnished the binary compound **9** in 65% yield. The formation of the C2−C16 double bond and the introduction of a hydroxy function at the C17 of **9** with benzeneseleninic anhydride (BSA) in benzene at 35 °C was unsuccessful and gave only an unseparable mixture of products. The presence of the C15′−C20′ double bond confers instability to the binary compound **9**, as was demonstrated in the case of anhydrovinblastine.[8a]

To avoid the formation of products deriving from the oxidative process, the C15′−C20′ double bond of **9** was hydrogenated (Pd/C)[8b] and the obtained product (**11**, 85% yield) was oxidized with BSA. Compounds **13** and **14** were isolated from the reaction mixture in 9 and 62% yields, respectively.

Compounds **9** and **11** gave us the occasion to prepare, by reductive amination (HCHO, $NaBH_3CN$), compounds **10** (75%) and **12** (64%), derivatives that exhibit a structural and chemical similarity to anhydrovinblastine and vinblastine. The signs of the Cotton effect curves in **9** (208 (−37), 225 (+16)), **11** (208 (−37), 222 (+17)) and **12** (208 (−20), 223 (+65)) were as expected for the C16′ natural stereochemistry.[3a,9]

Having developed a valuable route to the C17 hydroxylated dimeric compound **14**, we were then confronted with the task of stereoselectively introducing different groups by the Lewis acid mediated addition of nucleophilic compounds. The hydroxylated binary derivative **14** was treated with tri-*O*-acetylguanosine in the presence of TBDMSCl and *N*,*N*-diisopropylethylamine (DIPEA).[6c] The reaction proceeded stereoselectively to give, as the unique product, the adduct **15** in 59% yield. In the ^{1}H NMR spectrum the H-17 proton is present at δ 5.21 as a broad signal confirming the formation of the C−N bond. It is expected that by changing the nucleophile other potential antineoplastic binary alkaloids can be prepared, and this highlights the flexibility of this methodology to generate different libraries for further biological investigation. The possibility of obtaining 11-methoxytabersonine from *Vinca herbacea*[10] on a gram scale, before it is metabolized to vindoline,[11] highlights the relevance of this challenging project. It is clear that vindoline is a "closed product", as it does not offer any possibility of modification.

Pharmacological evaluations will be reported in due course.

Experimental Section[6c]

2,16-Dihydro-11-methoxytabersonine (8): R_f 0.23 (75:25:0.5 hexane−EtOAc−EtOH); $[\alpha]_D$ −15.5 (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.58 (3 H, t, J = 7.5 Hz), 0.85 (1 H, q, J = 7.5 Hz), 1.01 (1 H, q, J = 7.5 Hz), 1.64 (1 H, t, J = 13.5, 7.5 Hz), 1.71 (1 H, dd, J = 13.5, 3.5 Hz), 2.00 (1 H, dt, J = 14, 9 Hz), 2.18 (1 H, ddd, J = 14, 9, 3 Hz), 2.20 (1 H, brs), 2.30 (1 H, q, J = 9 Hz), 2.58 (1 H, dt, J = 16, 1.5 Hz), 3.18 (1 H, dt, J = 9, 4.5 Hz), 3.29 (1 H, dd, J = 16, 6 Hz), 3.68 (3 H, s), 3.72 (1 H, dt, J = 13.5, 3.5 Hz), 3.74 (3 H, s), 4.05 (1 H, d, J = 3.5 Hz), 4.10−4.40 (1 H, br signal), 5.36 (1 H, brd, J = 10 Hz), 5.73 (1 H, ddd, J = 10, 5, 1.5 Hz), 6.10 (1 H, d, J = 2 Hz), 6.19 (1 H, dd, J = 8, 2 Hz), 6.88 (1 H, d, J = 2 Hz); ^{13}C NMR (acetone-d_6) δ 9.2, 32.5, 35.2, 39.1, 41.1, 44.5, 52.3, 52.8, 53.4, 54.4, 56.2, 69.0, 70.5, 96.2, 104.6, 124.9, 125.3, 127.8, 136.6, 154.1, 162.5, 176.2; HRMS calcd for $C_{22}H_{28}O_3N_2$ 368.2100, found 368.2112.

1-Demethyl-16-dehydroxy-17-deacetoxyanhydrovinblastine (9). Catharanthine (**3**) hydrochloride (440 mg, 1.2 mmol) and ferric chloride hexahydrate (875 mg, 3.24 mmol) were combined in a mixture of glycine buffer (24 mL, containing 174 mg of glycine and 174 mg of sodium chloride in 30 mL of water) and hydrochloride acid (12 mL, 0.1 N) under a nitrogen

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atmosphere. After 10 min of stirring at room temperature, **8** (441 mg, 1.2 mmol) was added. After 2 h of stirring at room temperature, sodium borohydride (50 mg) in ammonium hydroxide (5 mL) was added dropwise. The reaction mixture was extracted with CH_2Cl_2. Flash chromatography (39:1 EtOAc−MeOH) afforded **9** (550 mg, 65%): R_f 0.3 (39:1 EtOAc−MeOH) (CAS yellow − red); $[\alpha]_D$ +63.4 (*c* 0.57, $CHCl_3$); CD (MeOH) λ ($\Delta\epsilon$) 208 (−37), 225 (+16); mp 178−190 °C; ^{1}H NMR (acetone-d_6) δ 0.86 (3 H, t, J = 7.5 Hz), 1.18 (3 H, t, J = 7.5 Hz), 0.98−1.17 (1 H, m), 1.20−1.31 (1 H, m), 1.54−2.05 (6 H, m), 2.08−2.21 (1 H, m), 2.26 (1 H, brs), 2.32−2.63 (3 H, m), 2.75−2.89 (1 H, m), 2.99−3.52 (10 H, m), 3.53 (3 H, s), 3.69 (3 H, s), 3.76 (4 H, m), 4.10 (1 H, m), 5.39 (1 H, d, J = 9 Hz), 5.43 (1 H, d, J = 7 Hz), 5.78 (1 H, dd, J = 9, 4.5 Hz), 6.32 (1 H, s), 6.69 (1 H, s), 7.01 (1 H, t, J = 7 Hz), 7.09 (1 H, t, J = 7 Hz), 7.30 (1 H, d, J = 7 Hz), 7.48 (1 H, d, J = 7 Hz), 8.60−8.81 (2 H, brs); ^{13}C NMR (acetone-d_6) δ 9.1, 12.8, 27.8, 28.3, 32.5, 34.5, 34.9, 35.9, 40.8, 43.6, 44.4, 47.3, 51.3 (2C), 51.6, 52.2, 52.5, 53.8, 54.0, 55.1, 56.1, 67.9, 68.4, 94.0, 111.5, 117.3, 118.9, 119.4, 120.4, 122.6, 125.2 (2C), 125.6, 127.1, 130.1, 133.1, 134.9, 136.0, 141.1, 152.6, 158.4, 175.4 (2C); HRFABMS calcd for $C_{43}H_{53}O_5N_4$ (MH^+) 705.4016, found (MH^+) 705.4033.

15′,20′-Dihydro-1-demethyl-16-dehydroxy-17-deacetoxyanhydrovinblastine (11). Compound **9** (400 mg, 0.568 mmol) was dissolved in MeOH (10 mL), and Pd/C (5%, 20 mg) was added. The mixture was hydrogenated at room temperature and atmospheric pressure for 3 h. The solution was filtered through Celite and evaporated. Flash chromatography (19:1 EtOAc−MeOH) afforded **11** (384 mg, 96%): R_f 0.15 (19:1 EtOAc−MeOH) (CAS yellow); $[\alpha]_D$ +51.5 (*c* 2, $CHCl_3$); CD (MeOH) λ ($\Delta\epsilon$) 208 (−37), 222 (+17); ^{1}H NMR (acetone-d_6) δ 0.74 (3 H, t, J = 7 Hz), 0.90 (3 H, t, J = 7 Hz), 1.10−3.45 (26 H, m), 2.29 (1 H, brs), 3.55 (3 H, s), 3.68 (3H, s), 3.76 (4H, m), 4.11 (1H, brs), 5.39 (1H, brd, J = 9 Hz) 5.78 (1H, dd, J = 9, 4.5 Hz), 6.31 (1H, s), 6.63 (1H, s), 6.99−7.16 (2H, m), 7.31 (1H, d, J = 7 Hz), 7.48 (1H, d, J = 7 Hz), 8.60 (1H, brs), 8.81 (1H, brs); ^{13}C NMR (MeOH-d_4) δ 9.2, 11.4, 20.6, 28.7, 29.8, 32.7, 35.1, 35.5 (2C), 38.8, 40.3, 41.3, 41.5, 44.9, 45.7, 51.9, 52.2, 52.6, 52.9, 53.5, 54.4, 55.4, 56.3, 68.4, 69.8, 94.5, 111.7, 115.7, 118.8, 119.5, 120.3, 123.7, 125.3, 125.7, 127.9, 129.7, 131.9, 135.5, 136.6, 153.6, 158.7, 176.7, 176.9; HRFABMS calcd for $C_{43}H_{55}N_4O_5$ (MH^+) 707.4172, found (MH^+) 707.4156.

Oxidation of 15′,20′-Dihydro-1-demethyl-16-dehydroxy-17-deacetoxyanhydrovinblastine (11). A solution of **11** (200 mg, 0.28 mmol) in benzene (25 mL) was treated with benzeneseleninic anhydride (217 mg, 0.62 mmol), and the mixture was heated at 35 °C for 12 h. The solvent was evaporated. Flash chromatography (20:1 EtOAc−5% NH_4OH in MeOH) afforded **13** (18 mg, 9%) and **14** (125 mg, 62%). For **13**: R_f 0.8 (20:1 EtOAc−5% NH_4 OH in MeOH) (CAS blue); ^{1}H NMR (acetone-d_6) δ 0.64 (3 H, t, J = 7 Hz), 0.92 (3 H, t, J = 7 Hz), 1.30−3.51 (27 H, m), 3.65 (3 H, s), 3.71 (3 H, s), 3.80 (3 H, s), 5.68 (1 H, brd, J = 10 Hz), 5.75 (1 H, dd, J = 10, 4.5 Hz), 6.82 (1 H, s), 7.14 (1 H, s), 7.21 (1 H, t, J = 7 Hz), 7.27 (1 H, t, J = 7 Hz), 7.38 (1 H, d, J = 7 Hz), 7.45 (1 H, d, J = 7 Hz), 9.15−9.35 (2 H, br signal). For **14**: R_f 0.45 (20:1 EtOAc−5% NH_4 OH in MeOH) (CAS yellow); $[\alpha]_D$ −38 (*c* 0.25, CH_2Cl_2); ^{1}H NMR (acetone-d_6) δ 0.67 (3 H, t, J = 7 Hz), 0.85−1.20 (5 H, m), 1.30−3.60 (23 H, m), 3.68 (3 H, s), 3.75 (3 H, s), 3.81 (3 H, s), 4.60 (1 H, d, J = 2 Hz), 5.76 (1 H, d, J = 10 Hz), 5.96 (1 H, dd, J = 10, 4 Hz), 6.87 (1 H, s), 7.12 (1 H, s), 7.21 (1 H, t, J = 7 Hz), 7.27 (1 H, t, J = 7 Hz), 7.37 (1 H, d, J = 7 Hz), 7.45 (1 H, d, J = 7 Hz); ^{13}C NMR (acetone-d_6) δ 7.8, 12.8, 23.9, 28.7, 29.8, 32.7, 36.3, 36.9, 39.1, 41.3, 43.2, 44.9, 46.2, 51.1, 51.5, 52.1, 52.4, 53.4, 54.0, 54.9, 56.5, 69.5, 71.2, 96.8, 99.0, 107.0, 115.7, 118.8, 120.6, 120.8, 122.1, 126.9, 128.1, 129.7, 130.0, 132.0, 134.9, 137.1, 149.9, 157.9, 168.2, 172.3, 174.8; FABMS 721; HRFABMS calcd for $C_{43}H_{53}N_4O_6$ (MH^+) 721.3965, found (MH^+) 721.3947.

Preparation of 16-Dehydroxy-17-deacetoxyanhydrovinblastine (10) and 15′,20′-Dihydro-16-dehydroxy-17-deacetoxyanhydrovinblastine (12). Substrate **9** or **11** (0.070 mmol) was dissolved in CH_3CN (10 mL). To the stirred solution (acetate buffer, pH 4.2) were added aqueous CH_2O (37%, 25 mL, 0.31 mmol) and $NaCNBH_3$ (15 mg, 0.24 mmol) at room temperature. After 1 h, HCl (0.1 N) was added, and the reaction mixture was poured into water, basified (NH_4OH), and extracted with CH_2Cl_2. For **10**: Flash chromatography (7:3 EtOAc − CH_2Cl_2); R_f 0.25 (7:3 EtOAc−CH_2Cl_2) (CAS blue−green); $[\alpha]_D$ +38 (*c* 0.4, MeOH); CD (MeOH) λ ($\Delta\epsilon$) 208 (−20), 223 (+65); ^{1}H NMR (acetone-d_6) δ 0.85 (3 H, t, J = 7 Hz), 1.01 (3 H, t, J = 7 Hz), 1.10−3.50 (23 H, m), 2.20 (1 H, brs), 2.81 (3 H, s), 3.55 (3 H, s) 3.75 (4 H, m), 3.82 (3 H, s), 4.01 (1 H, brs), 5.39 (1 H, brd, J = 9 Hz), 5.45 (1 H, d, J = 6.7 Hz), 5.76 (1 H, dd, J = 9, 4.5 Hz), 6.37 (1 H, s), 6.66 (1 H, s), 7.01 (1 H, t, J = 7 Hz), 7.08 (1 H, t, J = 7 Hz), 7.29 (1 H, d, J = 7 Hz), 7.67 (1 H, d, J = 7 Hz), 8.75 (1 H, brs); ^{13}C NMR (acetone-d_6) δ 8.6, 12.3, 27.3, 27.7, 31.9, 33.9, 34.4, 35.4, 38.7, 40.1, 43.8, 44.5, 50.9, 51.2, 51.6, 52.6, 53.2, 53.5, 53.8, 54.4, 54.9, 56.1, 68.5, 76.7, 93.0, 110.9, 118.0, 118.3, 118.9, 121.5, 122.1, 124.2, 124.6, 124.7, 126.5, 129.7, 131.9, 134.7, 136.6, 140.1, 153.6, 159.9, 176.8, 177.1; HRMS calcd for $C_{44}H_{54}N_4O_5$ 718.4094, found 718.4085; HRFABMS calcd for $C_{44}H_{54}N_4O_5$ (MH^+) 719.4172, found (MH^+) 719.4161. For **12**: flash chromatography (EtOAc); R_f 0.25 (EtOAc) (CAS blue); ^{1}H NMR (acetone-d_6) δ 0.81 (3 H, t, J = 7 Hz), 0.96 (3 H, t, J = 7 Hz), 1.10−3.60 (26 H, m), 2.18 (1 H, s), 2.71 (3 H, s), 3.60 (3 H, s), 3.70 (3 H, s), 3.81 (4 H, m), 4.41 (1 H, d, J = 6 Hz), 5.33 (1 H, d, J = 9 Hz), 5.76 (1 H, dd, J = 9, 4.5 Hz), 6.29 (1 H, s), 6.42 (1 H, s), 7.09 (1 H, t, J = 7 Hz), 7.18 (1 H, t, J = 7 Hz), 7.35 (1 H, t, J = 7 Hz), 7.61 (1 H, d, J = 7 Hz), 8.90 (1 H, brs); HRFABMS calcd for $C_{44}H_{57}N_4O_5$ (MH^+) 721.4329, found (MH^+) 721.4344.

Preparation of 15′,20′-Dihydro-1-demethyl-2,16-didehydro-16-dehydroxy-17-triacetyl guanosil-17-deacetoxyanhydrovinblastine (15). To a solution of **14** (100 mg, 0.141 mmol) in CH_2Cl_2 (10 mL) was added tri-*O*-acetylguanosine (67 mg, 0.169 mmol). The mixture was cooled (0 °C), and DIPEA (0.3 mmol) followed by TBDMSCl (0.3 mmol) were added dropwise. Stirring was continued for 15 h at room temperature. Flash chromatography (12:1 $CHCl_3$−MeOH) gave **15** (83 mg, 59%): R_f 0.35 (12:1 $CHCl_3$−MeOH) (CAS yellow); $[\alpha]_D$ −85 (*c* 0.14, CH_2Cl_2); CD (MeOH) λ ($\Delta\epsilon$) 212 (−48), 243 (+8); ^{1}H NMR (acetone-d_6) δ 0.77 (3H, t, J = 7 Hz), 0.92 (3H, t, J = 7 Hz), 0.98−4.01 (25H, m), 2.01 (3H, s), 2.05 (3H, s), 2.10 (3H, s), 3.68 (3H, s), 3.80 (3H, s), 3.90 (3H, s) 4.35 (3H, m), 5.21 (1H, br signal), 5.65−5.81 (2H, m), 5.91−6.02 (2H, m), 6.07−6.15 (1H, m), 6.89 (1H, s), 7.16 (1H, s), 7.22 (1H, t, J = 7 Hz), 7.30 (1H, d, J = 7 Hz), 7.39 (1H, d, J = 7 Hz), 7.47 (1H, d, J = 7 Hz), 7.70 (1H, s), 8.70 (1H, brs), 9.55 (1H, brs), 10.35 (1H, brs) {when the spectrum was recorded at 100 °C, two signals appeared at δ 5.21 (1H, d, J = 7 Hz) and 7.91 (1H, d, J = 7 Hz) due to H-17 and (C2″)-NH, respectively}; HRFABMS calcd for $C_{59}H_{70}N_9O_{13}$ (MH^+) 1112.5093, found (MH^+) 1112.5076.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of **8**−**11**, and **14** and ^{1}H spectra of **12**, **13**, and **15** (13 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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