Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 16, pp. 1999-2004, 1994
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0960-894X/94 $7.00+0.00

0960-894X(94)00276-2

A NEW APPROACH TO SYNTHESIS OF VINBLASTINE ANALOGS. ADDITION OF ORGANOZINC REAGENTS TO THE DIHYDROPYRIDINIUM ION GENERATED BY FRAGMENTATIVE COUPLING OF CATHARANTHINE AND VINDOLINE.

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Abstract

The dihydropyridinium ion generated by Potier-Polonovski coupling of catharanthine and vindoline reacts with organozinc reagents to give either 15' or 21'-substituted adducts. The former are unstable enamines but 20'-deoxyvinblastine analogs can be isolated after $NaBH_4$ reduction. 21'-Phenylanhydrovinblastine and the 15'-ethyl, 15'-*tert*-butyl and 15'-phenyl analogs of 20'-deoxyvinblastine show activity in the micromolar range as tubulin assembly inhibitors.

Despite the clinical importance of the *bis*-indole alkaloids vincristine (VCR) and vinblastine (VLB) in treatment of various neoplastic diseases, the range of analogs which have been available has been quite limited and as a result many aspects of structure-activity relationships remain uninvestigated.[1] The majority of analogs have involved addition of substituents at the C16 carbonyl group.[2] The C5'-nor analog, vinorelbine, is obtained by fragmentation of anhydrovinblastine-N-oxide or the corresponding 7'-bromoindolenine.[3] Some examples of functionality and stereochemical modifications in the C15', C20' region have been obtained by chemical transformation of vinblastine.[2] The synthetic work of Kuehne has provided unnatural alkyl substituents at C20'.[4]

In a study of the mechanism of oxidative fragmentation of catharanthine we demonstrated that the dihydropyridinium ion intermediates could be isolated in a state of purity satisfactory for further chemical manipulation.[5] The dihydropyridinium ion **A** which is generated by Potier-Polonovsky fragmentation of catharanthine in the presence of vindoline, has been observed before but previous chemical transformations have focused on *in situ* reduction to $\Delta^{20,21}$ or $\Delta^{15,20}$ anhydrovinblastine.[6] The ion is sufficiently stable that if the CH_2Cl_2 is evaporated at ~1.0 mm, ~0°, it can be subjected to further chemical reactions. In this study the objective was to obtain C15 or C21 substituted vinblastine analogs by addition of organozinc reagents to ion **A**.

We have reported a study of the reactions of organozinc reagents with the 1-benzyl-3-ethyl-5,6-dihydropyridinium ion.[7] That study indicated that dialkyl and diarylzinc reagents could add either in a 1,2 or 1,4-manner. Alkylzinc reagents prepared from Grignard reagents under conditions where $MgBr_2$ remained soluble gave mainly 1,2 addition, while reagents prepared in such a way that $MgBr_2$ was absent gave mainly 1,4-addition. On the other hand, alkynyl, alkenyl and allyl zinc

reagents appeared to prefer 1,2 addition regardless of the reaction conditions. Both dimethylcuprate and diphenylcuprate gave exclusively 1,4 addition to 1-benzyl-3-ethyl-5,6-dihydropyridinium ion. In this study we used diethylzinc, diphenylzinc, *bis*-(*t*-butyl)zinc and *bis*-(phenylethynyl)zinc. All were prepared in such a way that $MgBr_2$ was absent.[8]

The formation of ion **A** is known to be stereoselective under Potier-Polonovsky fragmentative-coupling conditions at temperatures below-50°.[5,9] The configuration at C16′ is such that the CO_2CH_3 group is **β** and a conformation similar to that found for vinblastine and its analogs can be assumed. In this conformation both C21′and C15′ appear to be more accessible from the **α**-face than from the **β**-face, suggesting added substituents will have the **α**-configuration. Since the products of 1,4 addition at C15′ are enamines they were expected to be unstable. The crude products of addition of organozinc reagents which were believed to result from addition at C15′ were immediately reduced with $NaBH_4$, thereby adding a hydrogen at C20′. Thus the stereochemistry at C15′ and C20′ is established by 1,4-addition followed by reduction whereas only stereochemistry at the C21′ carbon is involved for 1,2-addition. Table 1 gives the yields and observed HRMS MW for the isolated addition products.

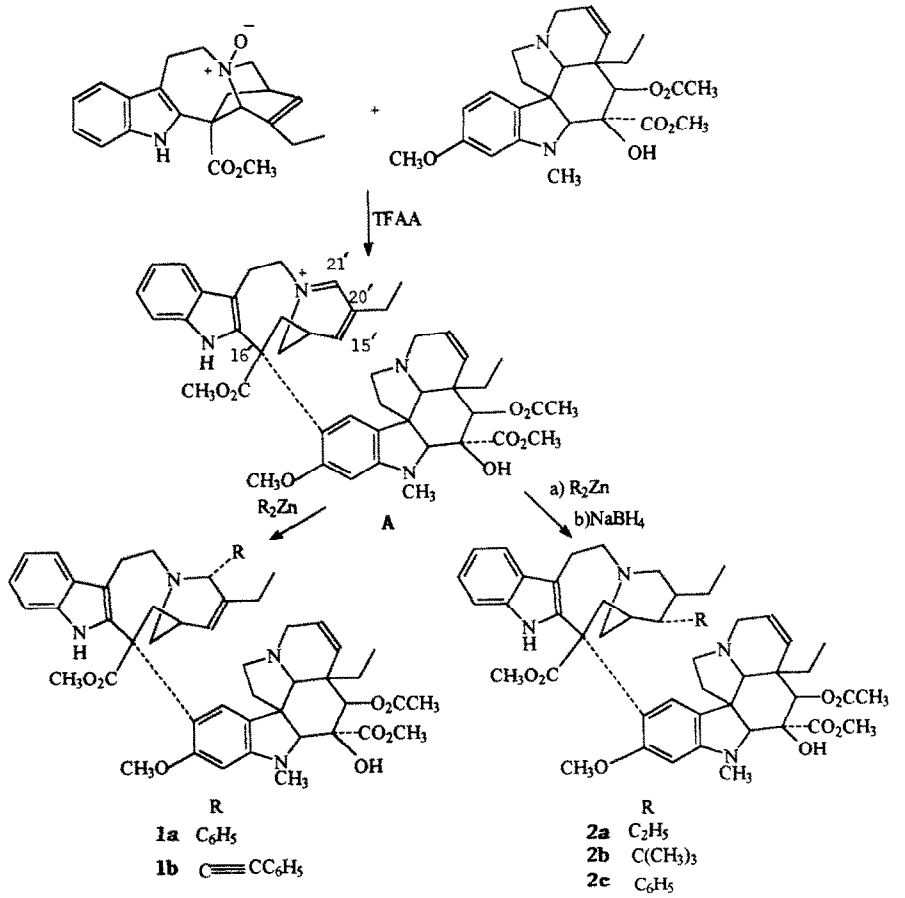

Table I. Characteristics of Reaction Products

Reactant	Product	Yield%	HRMS Calcd	HRMS Found	Tubulin Assembly Inhibition, IC_{50} μM[a]
Ph_2Zn	**1a**	36	868.4411(M^+)	868.4384	1.4, 1.6
$(PhC{\equiv}C)_2Zn$	**1b**	11	893.4489 (MH^+)	893.4526	21, 28
$(C_2H_5)_2Zn$	**2a**	33	822.4568(M^+)	822.4586	1.3, 1.4
$(t\text{-}C_4H_9)_2Zn$	**2b**	30	851.4959(MH^+)	851.5061	0.4, 0.4
Ph_2CuLi	**2c**	28	871.4646(M^+)	871.4622	0.5, 0.6
	VLB				0.5, 0.3

[a]Polymerization was assayed in a PMEDG buffer system containing 1% DMSO using tubulin at a concentration of 3-3.5 mg/ml.

With the published assignments of the ^1H and ^{13}C spectra of vinblastine as a guide,[10] interpretation of the spectra of **2b** was undertaken by use of 2D ^1H-^{13}C correlations, ^{13}C-DEPT assignment of CH_n type and HMBC long range coupling correlations. The assignments made are shown in Figure 1. Confirmatory aspects of these assignments are the peaks assigned to the new *t*-butyl substituent, identification of eight methylene groups consistent with the fragmented *iboga* structure and confirmation of the introduction of a C10 substituent on the vindoline ring.

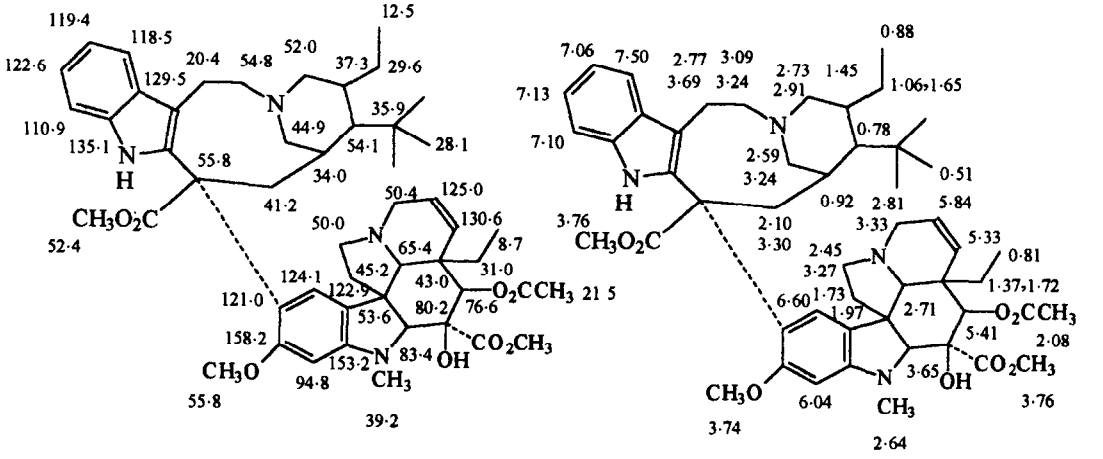

Figure 1. Carbon and Proton NMR Assignments for **2b**.

The NMR spectra of the other products have not been completely assigned but characteristic features of the spectra support the structural assignments. The proton NMR spectra are tabulated in Table 2. In the 21′-phenyl adduct **1a**, in addition to the vindoline singlets at 6.18 and 6.63, there is a doublet at 5.68 due to the C15′ vinyl hydrogen. Other prominent features of the spectrum are the two ethyl triplets at 0.82 and 0.93 ppm. The ^{13}C spectrum shows 24 sp^3 carbon peaks above 90 ppm, 22 sp^2 peaks between 90 and 160 ppm and 3 carbonyl peaks. The 21′-phenylethynyl derivative **1b** shows the vindoline aromatic singlets at 6.13 and 6.67 ppm and vinyl multiplets at 5.30 and 5.05 ppm. A doublet at 5.42 is assigned to the C15′ vinyl hydrogen. The ethyl triplets appear at 0.82 and 1.06 ppm.

The product **2a** from addition of diethylzinc has two aromatic singlets at 6.10 and 6.62, which confirm C-10 substitution of vindoline. The C-14 and C-15 vinyl peaks of vindoline are also evident at 5.87 (d of d) and 5.38 (d) respectively. Singlets appear at 2.03, 2.69, 3.60 and 3.78 ppm with the latter evidently resulting from overlap of both the

Table 2. Proton NMR Peaks[a]

1a[b]	1b	2a	2b	2c
0.82 t (J=7)	0.82 t (J=7) 3H	0.45 t (b)	0.51 s (9H)	0.78 t (J=7)
0.93 t (J=7)	1.06 t (J=7) 3H	0.62-0.78 m	0.8 m	0.84 t (J=7)
1.3-1.4 m	1.2-1.3 m	0.84 t (J=7)	0.81 t	0.9-1.2 m
1.72 q (J=7) 2H	1.3-1.45 m	0.90 t (J=7)	0.88 t (J=7)	1.25-1.35 m
1.75-1.9 m	1.75-1.95 m	1.05-1.30 m	1.06 m 1H	1.55-1.80 m
2.13 s 3H	2.12 s 3H	1.3-1.42 m	1.36 m (b)	1.95-2.05 m
2.15-2.25 m	2.1-2.25 m	1.45-1.58 m	1.45 m 1H	2.09 s
2.48-2.56 m	2.25-2.35 m	1.7-1.8 m	1.65 m 1H	2.35-2.45 m
2.64 s 1H	2.4-2.5 m	2.03 s 1H	1.73 m 2H	2.61 s 1H
2.75 s 3H	2.61 s 1H	2.10 s 3H	1.95-2.10 m	2.66 s 3H
2.90 d (J=15) 1H	2.71 s 3H	2.45-2.55 m	2.08 s 3H	2.75-2.85 m
3.06-3.20 m	2.75-2.85 m	2.69 s 3H	2.45 m 1H	3.03-3.10 dd (b)
3.25-3.45 m	3.1-3.45 m	2.8-2.9 m	2.59 d	3.15-3.40 m
3.65 s 3H	3.62 s 3H	3.08-3.2 m	2.64 s 3H	3.64 s 3H
3.82 s 3H	3.73 s 1H	3.25-3.3 m	2.65-2.85 m	3.68 s 1H
3.86 s 3H	3.80 s 3H	3.33-3.40 dd 1H	2.91 t 1H	3.74 s 3H
4.23 s 1H	3.83 s 3H	3.60 s 3H	3.09 dd 1H	3.78 s 3H
5.31 d (J=10) 1H	4.22 s 1H	3.68 s 1H	3.20-3.35 m	5.27 d (J=11)
5.51 s 1H	5.30 d (J=10) 1H	3.78 s 6H	3.55 m	5.83 dd (J=11,4)
5.68 d (J=6) 1H	5.42 d (J=6) 1H	5.38 d (J=10) 1H	3.6-3.7 m	6.02 s 1H
5.86 dd (J=10,4) 1H	5.50 s 1H	5.44 s 1H	3.65 s 1H	6.53 s 1H
6.18 s 1H	5.85 dd (J=10,4)	5.87 dd (J=10,4)	3.75 s 6H	7.05-7.25 m
6.63 s 1H	6.13 s 1H	6.10 s 1H	5.33 m	7.53 d (J=8)
7.05-7.15 m	6.67 s 1H	6.62 s 1H	5.41 s 1H	8.03 s
7.20-7.30 m	7.05-7.2 m	7.10-7.20 m	5.84 dd 1H	
7.47 d (J=8)	7.2-7.25 m	7.55 d (J=7) 1H	6.04 s 1H	
8.05 s 1H	7.3-7.43 m	7.95 s (b)	6.60 s 1H	
	7.53 d (J=8) 1H	9.78 s 1H	7.05-7.15 m	
	8.04 s		7.50 d (J=8) 1H	
			7.87 s	
			9.68 s	

[a] In $CDCl_3$ at 300 MHz

[b] This spectrum shows an impurity of ~ 15% revealed by singlets at 5.48, 6.14 and 6.55

C16′ and C16 ester methoxy groups. The high field region of the ^{1}H-NMR spectrum shows sharp ethyl triplets at 0.84 and 0.90 ppm. There is a broadened peak of similar intensity at 0.45 ppm. The ^{13}C-NMR spectrum shows 29 peaks above 90 ppm of which 3 were of reduced intensity and one was broadened. The anticipated structure has 29 saturated carbons, four of which are quaternary. There are 16 peaks due to sp^2-C between 110 and 160 ppm, of which 8 appear to be unprotonated on the basis of intensity (structure **2a** has 8 protonated and 7 unprotonated sp^2 carbons). There are three carbonyl peaks. The 15′-phenyl adduct **2c** shows overlapping triplets near 0.8 ppm and the characteristic vindoline singlet at 6.02 and 6.53 ppm. There are 35 observable peaks in the sp^3 region (<90 ppm) of the ^{13}C-NMR spectrum and 17 in the sp^2 region, 11 of which are protonated judging by the DEPT spectrum. Eleven protonated sp^2 carbons are anticipated for the addition of a second phenyl substituent.

For compounds **2a**, **2b** and **2c** new stereocenters are created at C15′ and C20′. While the spectra cannot rule out small amounts of stereoisomers it appears each of the products is primarily a single stereoisomer. On the basis of the projected conformation of the intermediate ion **A**, we speculate that the C20′ substituent is added from the α face.

The *bis*-indole alkaloids are known to function as anti-mitotic agents[11]. This activity is believed to be based on interaction with tubulin and inhibition of tubulin assembly provides an *in vitro* indication of activity. We used the assembly inhibition assay derived from that of Gaskin, Cantor and Shelansky using bovine brain tubulin.[12] The data are given in Table 1. Except for the 21′-phenylethynyl adduct **1b**, the compounds are assembly inhibitors with IC_{50} values in the range of 1μM, which is comparable to VLB under the same conditions.

The 15′-*tert*-butyl derivative **2b** was subjected to the National Cancer Institute *in vitro* screening review as NCS-663567. Most cell lines were inhibited at micromolar concentrations $GI_{50}=10^{-6}$-10^{-7}μM but only a few showed net reduction in viable cells $LC_{50} > 10^{-5}$μM. By way of comparison, VLB shows $GI_{50} < 10^{-10}$ M. VCR shows GI_{50} values in the 10^{-7}M range and complete ihibition of growth with $TGI<10^{-6}$M for the most sensitive cell lines.

General Procedure for Generation of Dihydropyridinium A and Reaction with Organozinc Reagents. Catharanthine-N-oxide (352 mg, 1.0 mmol) was dissolved in CH_2Cl_2 and cooled to -60°. Vindoline (456 mg, 1.0 mmol) was added. To this solution there was added TFAA (0.50 ml, 3.5 eq) and the solution was stirred at -50 to -65° for 2 h. The solution was evaporated under vacuum (~0.5 mm) at 0° for 20-30 min. The residue was dissolved in anhydrous THF at 0°. To this solution there was added a solution of the appropriate organozinc reagent (0.5M, 4 eq.). The reaction mixture was stirred and slowly allowed to warm to room temperature over 6 h. The reaction mixture was then shaken with a buffer prepared from equal volumes of saturated 1:1:1 NaCl, $NaHCO_3$ and NH_4Cl. The mixture was extracted with CH_2Cl_2 and the combined extracts were washed with water. The dried (Na_2SO_4) extract was then evaporated and purified by flash chromatography. Commercial diethylzinc was used. Diphenylzinc was prepared from phenylmagnesium bromide and zinc bromide in the presence of dioxane which precipitates $MgBr_2$.[8] *Bis*-(phenylethynyl)zinc was prepared from phenylacetylene and diethylzinc.[13] *Bis*-*t*-butylzinc was prepared from *t*-butyllithium and $ZnBr_2$. Diphenylcuprate was prepared from phenyllithium and CuI.

Acknowledgement

This research was supported by NIH Grant GM-41105.

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(*Received in USA* 11 *April* 1994; *accepted* 11 *July* 1994)