material). Reminimization was then done in AMBER.

The octanucleotide duplex d(GATGCATC)$_2$ was constructed and minimized in AMBER using Arnott's B DNA geometry.[24] Figure 3 shows a schematic for this sequence. Minimized saframycin A species were docked onto it near G4 using MIDAS. In many cases, it was obvious that certain orientations would give unsatisfactory models. For protonated saframycin A hydroquinone the covalent models were made in both the 3′ and 5′ direction. Subsequent models were made only in the 3′ direction. Coordinates of the docked models were captured and the structures were refined in AMBER to a root mean square value of <0.1 kcal/mol Å. They were then subjected to 48 ps of molecular dynamics at 300 ± 10 K in AMBER, with a temperature increase from 10 to 300 K in the first 16 ps. The equilibrium conditions were non-classical dynamics with velocity scaling (constant temperature). There was no periodicity and shake was on. A control file for molecular dynamics is given in the supplementary material. The resulting structures were then reminimized using molecular mechanics under the conditions described above.

Helix distortion enthalpies (Table I) were calculated by subtracting the helix enthalpies in the adduct from the enthalpies of the unbound helix, and drug distortion enthalpies were calculated in the same way. Net binding enthalpies were obtained by adding the total intermolecular binding and the distortion enthalpies. Enthalpies for the binding of the drug to individual DNA residues were generated by the analysis module (ANAL) of AMBER 3.0. Hydrogen-bond data were generated in the same way.

Solvation and counterions were added to the completed model of protonated saframycin A with 3′*R* geometry. This was done by placing the complex in a box of water extending ±7 Å from its farthest coordinates. Solvated sodium cations were placed at the bisector of each phosphate group at a distance of 3.0 Å from the phosphorus atom[25] and a solvated chloride ion was placed near the protonated amino group. The system was then minimized in AMBER until the root mean square gradient was <0.2 kcal/mol Å. The model was then submitted for molecular dynamics in AMBER for 15 ps at 300 ± 10 K (starting at 10 K) and then reminimized using molecular mechanics. A control file for molecular mechanics on the solvated adduct is given in the supplementary material.

Acknowledgment. We thank Dr. Peter A. Kollman for a copy of AMBER 3.0, and Dr. Timothy P. Wunz for assistance in the use of this program. Dr. Tadashi Arai generously sent us a sample of saframycin A.

Registry No. Saframycin A, 66082-27-7; saframycin A dihydroquinone, 133966-18-4; d(GATGCATC), 133983-37-6.

Supplementary Material Available: Tables of bond, angle, and torsional parameters for the cyano group of saframycin A, atomic charges for saframycin A species, and input and control files for AMBER (7 pages). Ordering information is given on any current masthead page.

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New α-Amino Phosphonic Acid Derivatives of Vinblastine: Chemistry and Antitumor Activity

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A series of new amino phosphonic acid derivatives of vinblastine (**1**, VLB) has been synthesized and tested in vitro and in vivo for antitumor activity. The compounds were obtained from O^4-deacetyl-VLB azide (**5**). All of the new products studied were capable of inhibiting tubulin polymerization in vitro. The most potent antitumor compounds bore an alkyl substituent on the phosphonate. In these compounds, the antitumor activity strongly depended on the stereochemistry of the phosphonate. The phosphonate (1*S*)-[1-[[[O^4-deacetyl-3-de(methoxycarbonyl)vincaleukoblastin-3-yl]carbonyl]amino]-2-methylpropyl]phosphonic acid diethyl ester (**15**) exhibited a remarkable activity against cancer cell lines both in vitro and in vivo.

Bisindole alkaloids (*Vinca* alkaloids) extracted from the Madagascan periwinckle (*Catharantus roseus*) are complex, dimeric structures that occupy a particular place among natural substances. The antitumor activity of this class of compounds was discovered during the 1960s by serendipity, and this instigated many studies concerning the chemical, pharmacological, and clinical aspects of these substances.[1-3]

Vinblastine (**1**, VLB) and vincristine (**2**, VCR) have been used in human anticancer chemotherapy for several years (Chart I). It is striking that a minor structural modification (compared with the size and complexity of these compounds) induces very different clinical responses, as VLB and VCR differ only by the transformation, by oxidation, of the indolic methyl of vindoline into a formyl group.

The mechanism of action of *Vinca* alkaloids at the molecular level is not well known, but it is generally considered that they inhibit microtubule formation and subsequently arrest cells in mitosis.[4] Microtubules are involved in many other essential biological processes and inhibiting their formation with drugs can produce important secondary effects. In addition, the antitumor activity of the *Vinca* alkaloids seems to depend critically on their uptake by, and release from, the tumor cells.[5]

Numerous structural analogues have been prepared in order to reduce clinical side effects (particularly neuro-

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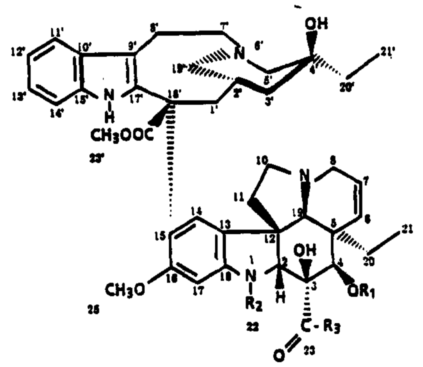
Chart I



		R_1	R_2	R_3
VLB	1	$COCH_3$	CH_3	OCH_3
VCR	2	$COCH_3$	CHO	OCH_3
Vinglycinate	3	$COCH_2N(CH_3)_2$	CH_3	OCH_3
Vintrypol	4	H	CH_3	L-Trp-OC_2H_5

toxicity) and to broaden the spectra of activity of these compounds.[2,3,6]

Chemists have grafted amino acid vectors onto the vindoline part of VLB to facilitate transport of these large alkaloid molecules. Vinglycinate (3) (an *N,N*-dimethyl derivative of glycine in the O^4 position)[7] and vintryptol (4), a deacetylated VLB that has been grafted onto an L-tryptophan used in the ethyl ester form,[8] illustrate such an approach. The latter compound was particularly interesting since it showed no evidence of neurotoxicity in Phase I clinical trials.[9]

As α-amino phosphonic acids are considered as bioisosters of natural amino acids and are widely used to modify biological processes,[10,11] we wondered whether these vectors could be used to generate valuable analogues of VBL and VCR.

The present work deals with the chemical properties of these new compounds and their antitumor activity, in comparison with reference *Vinca* alkaloids: VLB (1), VCR (2), and vintryptol (4). In particular, (i) the nature of the substituent borne by the α-amino phosphonate and (ii) the stereochemistry of these amino acids were examined. This last point will be more thoroughly discussed on account of the surprising results obtained in the present work.

Chemistry

The compounds studied here have been synthesized starting from compound 1 via the O^4-deacetyl-VLB azide (5) (Scheme I).[2]

The condensations of 5 with the α-amino phosphonates 6 were performed in cold methylene chloride. The products were purified by preparative HPLC and their structures established (IR, UV, ^{13}C and ^1H NMR, and MS). The overall yield of pure derivatives was in the range of 30 to 50%.

The condensation of a racemic phosphonate onto VLB (1), which is a chiral molecule, gives rise to the formation of two diastereoisomers, which are epimers. For practical reasons, in order to ascertain the influence of the substituent R borne by the phosphonate, the first screening tests were performed by using mixtures of epimers (Table I, compounds **10**, **13**, **16**, **19**, **22**, **23**, and **24**).

Next, given the results of Table I, some of the most promising individual epimers were prepared in stereochemically pure form according to one of the following methods.

Scheme I

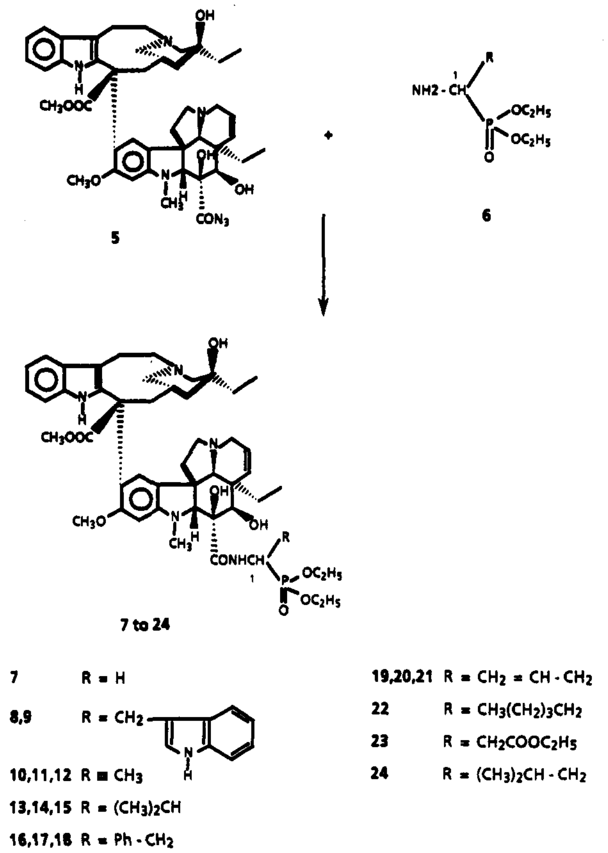
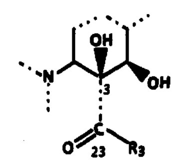


Table I. α-Amino Phosphonic Derivatives of Vinblastine



no.	R_3[a]	no.	R_3[a]
7	Gly(P)-$(OC_2H_5)_2$	16	*dl*-Phe(P)-$(OC_2H_5)_2$
8	*l*-Tryp(P)-$(OC_2H_5)_2$	17	Phe(P)-$(OC_2H_5)_2$[b]
9	*d*-Tryp(P)-$(OC_2H_5)_2$	18	Phe(P)-$(OC_2H_5)_2$[b]
10	*dl*-Ala(P)-$(OC_2H_5)_2$	19	*dl*-α-allyl-Gly(P)-$(OC_2H_5)_2$[c]
11	*l*-Ala(P)-$(OC_2H_5)_2$	20	α-allyl-Gly(P)-$(OC_2H_5)_2$[b]
12	*d*-Ala(P)-$(OC_2H_5)_2$	21	α-allyl-Gly(P)-$(OC_2H_5)_2$[b]
13	*dl*-Val(P)-$(OC_2H_5)_2$	22	*dl*-α-*n*-pentyl-Gly(P)-$(OC_2H_5)_2$[d]
14	*l*-Val(P)-$(OC_2H_5)_2$	23	*dl*-Glu(P)-$(OC_2H_5)_3$[e]
15	*d*-Val(P)-$(OC_2H_5)_2$	24	*dl*-Leu(P)-$(OC_2H_5)_2$

[a] α-amino phosphonic acid diethyl esters. [b] Isolated after HPLC, starting from epimeric mixtures of 16 and 19. No direct information on the configuration at C^1. [c] *dl*-(1-aminobuten-3-yl)-phosphonic acid diethyl ester. [d] *dl*-(1-Aminohexyl)phosphonic acid diethyl ester. [e] *dl*-1-Amino-1-(diethylphosphono)propionic acid ethyl ester.

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Table II. Configuration of the α-Amino Phosphonic Acid Diethyl Esters

α-amino phosphonic acid diethyl esters			corresponding α-amino phosphonic acids			
no.		$[\alpha]_D^{20}$,[a] deg	no.	$[\alpha]_D^{20}$,[b] deg	$[\alpha]_D^{20}$ (lit.), deg	abs config
6a	*l*-Tryp(P)-$(OC_2H_5)_2$	−18.0				
6b	*d*-Tryp(P)-$(OC_2H_5)_2$	+18.6				
6c	*l*-Ala(P)-$(OC_2H_5)_2$	−5.2	**27c**	−11.3	−16.9[c]	*R*
6d	*d*-Ala(P)-$(OC_2H_5)_2$	+5.7	**27d**	+13.7	+16.8,[c] +12.14[d]	*S*[e]
6e	*l*-Val(P)-$(OC_2H_5)_2$	−9.5[f]	**27e**	+0.9	+10,[g] +0.6[h]	*R*
6f	*d*-Val(P)-$(OC_2H_5)_2$	+9.5[f]	**27f**	−0.3	−10,[g] −0.6[h]	*S*[i]

[a] *c* 1, $CHCl_3$. [b] *c* 0.5, NaOHN. [c] *c* 2, NaOHN, reported in ref 20. [d] *c* 0.5, NaOHN, reported in ref 13. [e] Reference 20, ref 15. [f] From a pure oil. [g] *c* 2, NaOHN, reported in ref 14; but as reported by the authors of the ref 21. [This specific rotation value for the valine analogue is an apparent error and should be printed as 1.0.] [h] *c* 5, NaOHN, reported in ref 21. [i] Reference 14.

Table III. Comparison of Physicochemical Data for Epimers

carbon no.	11	12	14	15	17	18	20	21
				^{1}H NMR[a]				
6	5.79	5.72	5.74	5.68	5.74	5.68	5.73	5.67
7	5.84	5.82	5.84	5.81	5.81	5.80	5.83	5.80
19	2.59	2.57	2.56	2.53	2.58	2.53	2.57	2.51
22	2.84	2.78	2.83	2.79	2.82	2.75	2.78	
21′	0.94	0.92	0.93	0.89	0.93	0.89	0.90	0.89
				^{13}C NMR[a]				
2	84.00	84.33	84.00	84.30	83.80	84.40	83.80	84.03
4		73.80	73.70	74.30	73.70	74.10	73.80	74.06
8	50.45	50.64	50.30	50.70	50.60	50.60	50.30	50.60
10	49.94	50.18	49.60	50.70	50.10	50.50	50.00	50.40
17	93.40	93.60	93.40	93.70	93.40	93.70	93.30	93.40
21′	8.67	8.64	8.63	8.57	8.70	8.63	8.64	8.59
				Polarity of Compounds[b]				
t_R	4.46	5.00	4.58	5.69	6.94	8.08	5.06	5.77

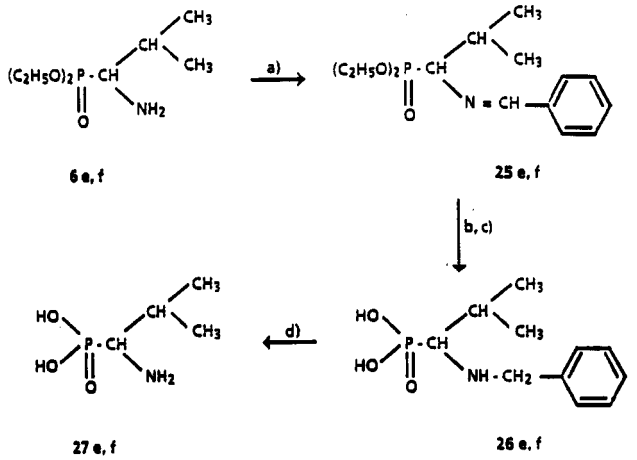
[a] 400 MHz, $CDCl_3$, δ given in ppm/TMS. [b] HPLC: Lichrosorb RP18 5 μm; MeOH–$NaHPO_4$ 0.01M (4:1); flow, 2 mL/min. Retention time (t_R) given in min.

Method A: Preparative HPLC. Being diastereoisomers, the pairs of epimers could be separated after reverse phase HPLC. Only small quantities of pure epimers could be obtained by this technique, however, and, furthermore, no direct information about the stereochemistry at C^1 (α to phosphorus) could be acquired. Compounds **17**, **18**, **20**, and **21** were obtained by this method.

Method B: Phosphonate Resolution. Three racemic phosphonates **6**, Ala(P)-$(OC_2H_5)_2$, Val(P)-$(OC_2H_5)_2$, and Trypt(P)-$(OC_2H_5)_2$, were resolved in the diethyl ester form, using (+)- and (−)-dibenzoyltartaric acids. The optical purity attained for each enantiomer was up to 99.5% and was assessed by derivatization with camphanyl chloride and CPV studies. The rotation values obtained are listed in Table II. These optically pure diesters were then separately condensed with the azide **5**. Compounds **8**, **9**, **11**, **12**, **14**, and **15** were prepared by this method in an essentially pure form, and their physicochemical properties are reported in Table III.

Configuration at C^1 (α to Phosphorus). The configuration at the C^1 position was determined by starting from the precursor phosphonate. Despite the fact that almost all the phosphonic analogues of the corresponding natural α amino acids have been synthesized,[12] only few asymmetrical syntheses[13-15] and few systematic studies on the chemical[16] or physicochemical[17] correlations giving access to their absolute configurations have been published. At this time, only Tyr(P), the phosphonated analogue of tyrosine has been found as a natural compound.[18,19] For compounds **11**, **12** and **14**, **15** the assignment of the C^1 position of the phosphonate was based upon X-ray crystallographic data from the literature for two of the phosphonic acid (Ala(P) and Val(P)). As the data are missing for the other phosphonates, a tentative assignment for the configuration at C^1 for the compounds **17**, **18** and **20**, **21** was inferred by analogy using physicochemical data obtained for the epimers.

Scheme II[a]



[a] (a) PhCHO, toluene, reflux; (b) H_2–PtO_2, EtOH; (c) HBr, CH_3COOH; (d) H_2–Pd/C, EtOH.

Assignment for Compounds 11, 12 and 14, 15. (Ala(P)-$(OC_2H_5)_2$ (6c and 6d). The hydrolysis in an acidic medium (HBr/acetic acid) gave the corresponding diacids **27c** and **27d**. Their rotations are in agreement with those in the literature (Table II). Since the absolute configuration of *d*-Ala(P) (**27d**) had been established by X-ray,[20] the corresponding diesters **6d** and the compound **12** have the same *S* configuration at C^1.

Val(P)-$(OC_2H_5)_2$ (6e and 6f). The hydrolysis conditions used for **27c** and **27d** gave intractable residues where R is isopropyl. We had to use a more laborious method (Scheme II): protection of the primary amine as its *N*-benzyl derivative, hydrolysis of the diester, and then debenzylation to yield **27e** and **27f**. The optical rotations found are in good agreement with those reported by Kafarski,[21] and as the absolute configuration of *d*-Val(P) **27f**

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has previously been established by X-ray,[14] **6f** and **15** have the same *S* configuration at C^1.

Tentative Assignment of Configurations at C^1 (α to Phosphorus) for Compounds 17, 18 and 20, 21. The ^{13}C and ^1H NMR spectra of compounds **11**, **12** and **14**, **15** are not very different from the spectra obtained under similar conditions for VLB (**1**): the presence of the phosphonic residue does not significantly modify the alkaloid backbone. Nevertheless, when the spectra of two epimers (**11**, **12** and **14**, **15**) are compared (Table III), slight differences in the position of the signals in the vicinity of the phosphonic residue can be observed. For instance, a systematic shift in ^1H NMR spectra appears for positions at carbon 6, 7, 19, 22, and 21′ when **11** is compared to **12** and **14** to **15**. A similar phenomenon occurs when **17** is compared to **18** and **20** to **21**. Hence, these changes seem to be related to the C^1 configuration.

Furthermore, the polarity on HPLC seems to vary in the same way when pairs of diastereoisomeric epimers are compared (Table III, footnote *b*), both of the *S* compounds **12** and **15** are less polar than their *R* epimers **11** and **14**.

Thus, considering the compounds where *R* is alkyl, we can postulate tentatively that the less polar compounds have the *S* configuration at C^1: **12**, **15**, **18**, and **21**. These results are consistent with the biological data we have obtained, since only the compounds assigned the *S* configuration show potent antitumor activity.

Pharmacology

The study of the biological properties of these new VLB (**1**) derivatives was carried out in vitro on leukemia L1210 cells in culture and in vivo on P388 leukemia-inoculated mice. In order to examine their mechanism of action, we measured their capacity to inhibit tubulin polymerization.

The most potent compounds in vitro generally bear an alkyl group at C^1 (**1**, **13**, **19**, **27**, **23**, **24**). The compounds substituted with an aromatic group at this position are less active (**8**, **9**, **16**). Moreover, the antitumor activity presents a dramatic stereoselectivity: one of the epimers, the less polar compound, shows almost all of the activity (**12**, **15**, **21**) while the other epimer is almost inactive. The active epimers are 5 to 10 times more potent than the reference drugs **1** and **2** in inhibiting the growth of L1210 cells in culture.

In vivo, the antitumor activity of the compounds shows a remarkable correlation with the results obtained in vitro. The most active compounds **12** and **15** are efficient at doses 10 to 20 times lower than the reference compounds **1** and **2** and allow much higher *T*/*C* ratios to be reached. After treatment with **15**, one-third of the P388-bearing animals were long-term survivors, a result that is uncommon with *Vinca* alkaloid derivatives. The most active compound, **15**, has been more extensively studied in comparison with its epimer **14**.[22]

All of these compounds are inhibitors of tubulin polymerization. Their inhibitory potency ranges between 0.07 μM (for VLB) to 0.2 μM (for VCR) (Table IV). There is no obvious direct correlation between the potency on this test and the nature of the phosphonate grafted onto VLB (**1**).

Discussion

The present work deals with the synthesis and evaluation, in vitro and in vivo, of new VLB (**1**) derivatives bearing an α-amino phosphonate group **6** at C.[23] Some of our derivatives, namely, those bearing an alkyl R residue, were more cytotoxic than either the parent VLB and VCR or their carboxylic counterparts. These high activities are closely related to the stereochemistry of the phosphonate, the most active compounds **12** and **15** having the *S* configuration. These two derivatives are 7-fold more cytotoxic and more active in vivo (both in terms of increase of life span and long-term survivors) than VLB. Moreover, their optimal dosages are at least 10-fold lower than that of VLB.

The high potencies of **12** and **15** are not easily explained, since our results show that (i) the presence of the α-amino phosphonate does not modify the conformation of the alkaloid skeleton and the ability of the compounds to interact with tubulin and (ii) the epimers of **12** and **15**, namely, **11** and **14**, inhibit tubulin polymerization to the same extent but are clearly less active antitumor agents.

Hence, although the overall mechanism of action of these compounds probably lies in their ability to inhibit tubulin polymerization, the higher potency of their epimers seems not to be due to a better interaction with this target. This lack of correlation between cytotoxic activity and inhibition of tubulin polymerization has previously been pointed out for the currently used *Vinca* alkaloid derivatives.[23,24] In this case, the different growth inhibition induced by VCR and VLB was found to correlate with their intracellular retention by tumor cells.[5]

Studies conducted on the tritiated **14** and **15** have shown that (i) **15** was more accumulated by the tumor cells in culture than its inactive epimer **14** and (ii) **14** was more rapidly released from cells than the active epimer **15**, which is strongly retained.[25]

Table IV. Biological Activity of Compounds

no.	cytotoxicity[a] IC_{50} (nM)	antitumor activity against P388 leukemia in mice[b] opt dosage[c] (mg/kg)	*T*/*C* (%) range[d]	LTS[e] (60 days)	inhibitn of tubulin polymrzn IC_{50} (μM)
1	3.15	3.00	157–203	02/45	0.07
2	4.85	3.00	165–181	00/15	0.50
4		100.00	150–285	02/54	0.20
7	17.75	3.00	230	01/06	0.30
8	36.67	40.00	197	00/06	0.10
9	64.67	40.00	188	00/06	0.10
10	2.50	0.50	151–480	07/96	0.10
11	37.00	1.00	145	00/06	0.08
12	0.40	0.25	342	02/06	0.20
13	0.64	0.50	160–447	11/54	0.30
14	10.92	3.00	135	00/06	0.80
15	0.43	0.15	168–600	08/25	0.40
16	46.00	5.00	149–224	00/12	0.07
17	14.26	20.00	261	01/05	
18	9.64	8.00	229	00/05	
19	3.70	1.00	234–302	02/12	0.30
20	26.40	12.00	195	00/05	
21	1.29	1.00	294	00/05	
22	3.97	3.00	203	00/06	0.02
23	2.77	3.00	122	00/06	0.30
24	3.13	1.50	261	01/06	

[a] Inhibition of L1210 cell proliferation measured by [^3H] incorporation assays (mean of 2 to 5 values obtained in independent experiments). [b] Mice were inoculated ip on day 0 with 10^6 P388 cells. Treatment is given ip on day 1. [c] Dosage that gives the best *T*/*C*. [d] Range of *T*/*C* obtained at the optimal dosage in independent experiments.

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These results suggest that the potency of **15** is due to both cellular accumulation and retention.

These properties observed in human solid tumor cell lines[25] present numerous advantages over the existing *Vinca* derivatives and make these new derivatives very promising. Compound **15** is currently in Phase I clinical trials.

Experimental Section

Vinblastine sulfate was obtained from ORIL, Bolbec, France. Melting points were determined on a Köfler apparatus and are uncorrected. Optical rotations were measured (g/100 mL) on a Perkin-Elmer 241 polarimeter. Spectral data were obtained with a Perkin-Elmer Model 682 IR spectrophotometer (γ cm^{-1}, $CHCl_3$ or KBr) and a Perkin-Elmer Model λ 7 UV spectrophotometer (CH_3OH, max, nm). Positive (MH^+) ion FAB spectra were obtained on a Nermag R10-10C apparatus. The samples were dissolved in a glycerol–thioglycerol matrix (1:1) and ionization was effected by a beam of krypton ions accelerated through 6 to 8 KeV. ^1H and ^{13}C NMR spectra were recorded on Brucker W360 and Brucker WP60 NMR spectrometers ($CDCl_3$, Me_4Si as internal standard). HPLC analyses were carried out on a Waters system consisting of two Models 590 HPLC pumps, a UV Waters Model M455 detector (detection at 205 nm), and a data module Perkin-Elmer LCI 100.

Cell Culture Study. L1210 cells were cultivated in an RPMI 1640 medium supplemented with 10% FCS (Fetal calf serum, Gibco) 2 mM L-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, and 10 mM HEPES buffer (pH = 7.4). Cytotoxicity was measured by the inhibition of [^{3}H]thymidine ([^{3}H]-T 43 Ci/mmol, CEA, France) incorporation. The cells in the exponential phase of growth (10^5 cells/mL) were incubated with the tested drugs (5 concentrations in triplicate) for 24 h and then were labeled with 0.5 μCi/mL [^{3}H]-T for 3 h in 96-well microplates. Cells were then harvested onto filters, lyzed, and washed with water, and the filters were counted in a Beckman Model LS 3801 counter. Results are expressed as IC_{50}, the drug concentration that inhibits by 50% [^{3}H]-T incorporation with respect to untreated cells.

In Vivo Activity. The antitumor activities were evaluated on the experimental P388 leukemia. Murine P388 leukemic cells were given by Mario Negri Institute (Milano, Italy) and were maintained by ip inoculation into DBA_2 mice (Iffa Credo, France). For the chemotherapeutic assays, the cells were inoculated ip (10^6 cells) into DBA_2 mice on day 0. The drugs as sulfate salts were dissolved in saline and injected ip on day 1. The results are expressed in term of T/C in % (median survival time of treated animals divided by median survival of controls) and LTS (survivors up to 60 days).

Tubulin Polymerization. Tubulin was partially purified by successive polymerization/depolymerization cycles according to the method of Shelanski.[26] The final preparation contained approximately 50 mg per starting dog brain (90 g). The inhibition assays were conducted by turbidimetry at 350 nm in a total reaction volume of 500 μL containing 0.5 mg/mL of tubulin preparation and 10 μL of DMSO in which were solubilized the compounds. Controls were systematically run in parallel with DMSO alone. The compounds were tested between the concentrations of 10^{-6} and 10^{-9} M. The values of the various rates of polymerization (two separated experiments) were plotted versus the concentration of *Vinca* derivatives and the IC_{50} appreciated graphically.

General Procedure for Preparation of Compounds 7 to 24. Compounds were prepared by coupling azide **5** to appropriate α-amino phosphonate **6**. The procedure for the preparation of the azide from VLB was based on that of Barnett[2] and Rao.[8] The phosphonates **6** were obtained by following published procedures or as described below. For the coupling reactions, an equimolar solution of **5** and **6** in methylene chloride was kept in the dark for 48 h, with stirring at room temperature. Depending on the structure of the product, different procedures of purification were utilized as described below.

Epimeric Mixtures (Compounds **10, 13, 16, 19, 22, 23, 24**) and **7**. Methylene chloride was evaporated in vacuo and the crude product purified by chromatography (silica gel, 230–400 mesh; Merck-Clevenot, France; toluene–ethanol (4:1)). The separations were monitored by TLC (silica gel 60 F 254; Merck-Clevenot, France; 5 × 10 cm; toluene–ethanol (4:1)) and the product was analyzed by HPLC: CH_3CN–Na_2HPO_4 0.01 M, (7:3); Lichrosorb RP18 5 μm (Merck-Clevenot); solvent flow, 2 mL/mn.

Pure Epimers. Method A (Compounds **17, 18, 20, 21**). After evaporation of the methylene chloride, the crude products were solubilized in the minimum of ethanol and the separation of the epimers was carried out by HPLC: MeOH–Na_2HPO_4 0.01 M (7:3); Lichroprep RP18 15–25 μm. The fractions containing the more polar epimer were combined, methanol was evaporated in vacuo, and the resulting aqueous solution was extracted with methylene chloride (2 × 50 mL). The organic solution was washed with water (1 × 50 mL), dried ($MgSO_4$), and evaporated in vacuo. The second less polar epimer was obtained by following the same treatment.

Method B (Compounds **8, 9, 11, 12, 14, 15**). The crude products were purified by HPLC: Lichroprep RP18 15–25 μm; MeOH–Na_2HPO_4 0.01 M (4:1).

Sulfates were obtained by addition of a theoretical quantity of sulfuric ethanol (2%) prepared starting from ethanol and 1 N sulfuric acid. For these pure epimers and compound **7**, ^1H and ^{13}C NMR data are available as supplementary material.

Compound 7 ([[[[*O*^{4}-deacetyl-3-de(methoxycarbonyl)-vincaleukoblastin-3-yl]carbonyl]amino]methyl]phosphonic acid diethyl ester) from Gly(P)-$(OC_2H_5)_2$[27] and 5: yield 47%, TLC R_f = 0.31; HPLC, 1 peak, t_R = 6.5; MS (FAB), m/z 904 (MH^+).

Compound 10 from *dl*-Ala(P)-$(OC_2H_5)_2$[28] and 5: yield 30%, TLC R_f = 0.31; HPLC, 2 peaks 1/1, t_R = 5.66, 6.80; MS (FAB), m/z 932 (MH^+ + 14), 918 (MH^+), 900, 886, 872, 858.

Compound 13 from *dl*-Val(P)-$(OC_2H_5)_2$[28] and 5: yield 33%, TLC R_f = 0.35; HPLC, 2 peaks 1/1, t_R = 5.56, 6.02; MS (FAB), m/z 946 (MH^+), 709, 651.

Compound 16 from *dl*-Phe(P)-$(OC_2H_5)_2$[28] and 5: yield 40%, TLC R_f = 0.38; HPLC, 2 peaks 1/1, t_R = 10.02, 10.99; MS (FAB), m/z 994 (MH^+), 992, 962, 934, 856.

Compound 19 from *dl*-α-allyl-Gly(P)-$(OC_2H_5)_2$[29] and 5: yield 25%, TLC R_f = 0.37; HPLC, 2 peaks 55/45, t_R = 6.57, 7.43; MS (FAB), m/z 944 (MH^+).

Compound 22 from *dl*-α-*n*-pentyl-Gly(P)-$(OC_2H_5)_2$[29] and 5: yield 35%, TLC R_f = 0.36; HPLC, 2 peaks 1/1, t_R = 7.08, 8.16; MS (FAB), m/z 974 (MH^+), 972, 651, 649, 571, 542, 355.

Compound 23 from *dl*-Glu(P)-$(OC_2H_5)_2$[29] and 5: yield 47%, TLC R_f = 0.25; HPLC, 2 peaks 1/1, t_R = 7.63, 8.03; MS (FAB), m/z 990 (MH^+), 988, 752, 651, 649, 571, 542, 355.

Compound 24 from *dl*-Leu(P)-$(OC_2H_5)_2$[28] and 5: yield 31%, TLC R_f = 0.36; HPLC, (MeOH–Na_2HPO_4 0.01 M (4:1), 2 peaks 6/4, t_R = 13.64, 17.89; MS (FAB), m/z 974 (MH^+ + 14), 960 (MH^+), 944, 928, 900, 709, 651, 649, 604, 571, 542, 355, 86.

Compounds 17 and 18 from 16: **16** (0.3 g) gave **17** (0.1 g) (yield 66%; HPLC, 2 peaks (98.2/1.8)) and **18** (0.13 g) (yield 86%; HPLC, 2 peaks (1.5/98.5)).

Compounds 20 and 21 from 19: **19** (0.350 g) gave **20** (0.15 g) (yield 85%; HPLC, 1 peak) and **21** (0.13 g) (yield 74%; HPLC, 2 peaks (2.3/97.7)).

Compound 8 from *l*-Tryp(P)-$(OC_2H_5)_2$ (6a) and 5: yield 54%; MS (FAB), m/z 1047 (MH^+ + 14), 1033 (MH^+), 709, 691, 679, 651, 571, 542, 355, 295, 272, 130, 124.

Compound 9 from *d*-Tryp(P)-$(OC_2H_5)_2$ (6b) and 5: yield 55%; MS (FAB), m/z 1033 (MH^+), 709, 679, 651, 571, 542, 517, 355, 297.

Compound 11 ((1*R*)-[1-[[[*O*^{4}-deacetyl-3-de(methoxycarbonyl)vincaleukoblastin-3-yl]carbonyl]amino]ethyl]-phosphonic acid diethyl ester) from *l*-Ala(P)-$(OC_2H_5)_2$ (6c)

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and 5: yield 29%; $[\alpha]^{20}_D$ + 18.7° (*c* 1, MeOH); UV 214 (50 207), 266 (15 544); IR 1730 (ester), 1665 (amide), 1300–1150 (P=O); MS (FAB), *m/z* 932 (MH^+ + 14), 918 (MH^+), 916, 900, 886, 858, 709, 542, 355, 337, 323, 295, 154, 144, 122, 108.

Compound 12 from *d*-Ala(P)-$(OC_2H_5)_2$ (6d) and 5: yield 30%; $[\alpha]^{20}_D$ + 20.6° (*c* 1, MeOH); UV 214 (50 116), 266 (15 291); IR 1730 (ester), 1670 (amide), 1300–1150 (P=O); MS (FAB), *m/z* 932 (MH^+ + 14), 930 (MH^+ + 12), 918 (MH^+), 900, 886, 858, 651, 649, 571, 355, 337, 323, 295, 154, 144, 122, 108.

Compound 14 from *l*-Val(P)-$(OC_2H_5)_2$ (6e) and 5: yield 32%; UV 214 (43 472), 266 (13 975); IR 1730 (ester), 1675 (amide), 1300–1150 (P=O); MS (FAB), *m/z* 946 (MH^+), 944, 928, 926, 914, 886, 709, 649, 604, 571, 124, 122.

Compound 15 ((1*S*)-[1-[[[*O*⁴-deacetyl-3-de(methoxycarbonyl)vincaleukoblastin-3-yl]carbonyl]amino]-2-methylpropyl]phosphonic acid diethyl ester) from *d*-Val-(P)-$(OC_2H_5)_2$ (6f) and 5: yield 29%; UV 214 (45 043), 264 (13 707); IR 1730 (ester), 1675 (amide), 1300–1150 (P=O); MS (FAB), *m/z* 946 (MH^+), 944, 928, 926, 914, 886, 709, 649, 604, 571, 124, 122.

Synthesis of Phosphonates. *l*-Tryp(P)-$(OC_2H_5)_2$ (6a). The synthesis of *d,l*-Tryp(P)-$(OC_2H_5)_2$ was performed by following exactly the procedure described for the preparation of the corresponding *d,l*-Tryp(P)-$(OCH_3)_2$,[30] but starting with the triethyl ester of phosphonoacetic acid. Then a solution of *d,l*-Tryp-(P)-$(OC_2H_5)_2$ previously obtained (7.1 g, 24 mmol) in ethanol (25 mL) was poured into a solution of dibenzoyl-D-tartaric acid monohydrate (9 g, 24 mmol) in ethanol (50 mL). The mixture was stirred for 1 h at room temperature and filtered and the precipitate was washed with ethanol (1 × 30 mL) and dried. Two recrystallizations from ethanol (60 mL) afforded 6.9 g of a compound melting at 225–226 °C. The phosphonate **6a** was liberated with 1 N aqueous NaOH and extracted with methylene chloride (2 × 30 mL). The combined organic solutions were washed with water, dried ($MgSO_4$), and evaporated in vacuo to yield a colorless oil: yield 75%; $[\alpha]^{20}_D$ −18° (*c* 1, $CHCl_3$); IR 3500–3100 (NH, NH_2), 1280–1200 (P=O), 1080–1020 (P—O—C); 745 (P—O—C); ^1H NMR δ 8.8 (1 H, br s, NH), 7.8–7.1 (5 H, m). Anal. ($C_{14}H_{21}N_2O_3P$) C, H, N.

***d*-Trypt(P)-$(OC_2H_5)_2$ (6b).** A solution of *d,l*-Trypt(P)-$(OC_2H_5)_2$ (7.1 g, 24 mmol) in ethanol was treated as described above with 9 g (24 mmol) of dibenzoyl-L-tartaric acid monohydrate to yield a salt melting at 217–218 °C. **6b** was liberated as above: colorless oil, 2.7 g, 75%; $[\alpha]^{20}_D$ +18°6 (*c* 1, $CHCl_3$); IR; ^1H NMR. Anal. ($C_{14}H_{21}N_2O_3P$) C, H, N.

***l*-Ala(P)-$(OC_2H_5)_2$ (6c).** A solution of *d,l*-Ala(P)-$(OC_2H_5)_2$ (27 g, 0.149 mol) in ethanol (200 mL) was poured into a solution of dibenzoyl-D-tartaric acid monohydrate (56 g, 0.149 mol) in ethanol (350 mL). The mixture was treated as described above. Two recrystallizations from ethanol (65 mL) afforded 18 g of a compound melting at 210 °C. The phosphonate **6c** was liberated with concentrated NaOH and obtained as a colorless oil (3.9 g): yield 32%; $[\alpha]^{20}_D$ −5.3° (*c* 1, $CHCl_3$); IR 3360–3280 (NH, NH_2), 1235 (P=O). Anal. ($C_6H_{16}NO_3P$) C, H, N.

***d*-Ala(P)-$(OC_2H_5)_2$ (6d).** The same procedure was applied to a solution of *d,l*-Ala(P)-$(OC_2H_5)_2$ (48.5 g, 0.267 mol) in ethanol (250 mL) and dibenzoyl-L-tartaric acid monohydrate (95.9 g, 0.267 mol) in ethanol (750 mL). Salt mp: 212 °C; oil (6.3 g); yield 26%; $[\alpha]^{20}_D$ +5.7° (*c* 1, $CHCl_3$); IR. Anal. ($C_6H_{16}NO_3P$) C, H, N.

***l*-Val(P)-$(OC_2H_5)_2$ (6e).** A solution of *d,l*-Val(P)-$(OC_2H_5)_2$ (30 g, 0.143 mol) in ethanol (150 mL) was poured into a solution of dibenzoyl-D-tartaric acid monohydrate (54 g, 0.143 mol) in ethanol (1350 mL). After being stirred at room temperature, the precipitate was filtered and recrystallized five times from ethanol (5 × 600 mL). Salt, mp: 232 °C, 30 g. The phosphonate **6e** was liberated with concentrated NaOH as described before and obtained as a colorless oil (9.6 g): yield 64%; $[\alpha]^{2-}_D$ −9.47° (from the pure oil).

***d*-Val(P)-$(OC_2H_5)_2$ (6f),** as described for 6e. A solution of *d,l*-Val(P)-$(OC_2H_5)_2$ (30 g, 0.143 mol) in ethanol (150 mL) was poured into a solution of dibenzoyl-L-tartaric acid monohydrate (54 g, 0.143 mol) in ethanol (1350 mL). Salt mp: 225 °C, 15 g; colorless oil (4.5 g); yield 30%; $[\alpha]^{20}_C$ +9.47° (from the pure oil); ^1H NMR δ 4.15 (4 H, m), 2.85 (1 H, dd), 2.15 (1 H, m), 1.95 (2 H, m, D_2O exchangeable), 1.35 (6 H, t), 1.05 (6 H, m). Anal. ($C_8H_{20}NO_3P$) C, H, N.

***l*-(1-Amino-2-methylpropyl)phosphonic Acid (27f). (a) *l*-[1-(Benzylimino)-2-methylpropyl]phosphonic Acid Diethyl Ester (25f).** A solution of **6f** (4 g, 0.019 mol) and benzaldehyde (2 g, 0.019 mol) in toluene (50 mL) was refluxed, the water formed being removed by azeotropic distillation. The cold solution was evaporated to yield 5.6 g (99%) of an oil, which was used in the next step: $[\alpha]^{20}_D$ −40.4° (*c* 1, $CHCl_3$); IR 1640 (C=N), 1160–1260 (P=O); ^1H NMR δ 8.2 (d, 1 H, N=CH), 7.75 (m, 2 H, Ar), 7.4 (m, 3 H, Ar), 3.9–4.3 (m, 4 H, OCH_2), 3.45 (dd, 1 H, CHP), 2.2–2.5 (m, 1 H, $CH(CH_3)_2$), 1.2–1.5 (m, 6 H, CH_3CH_2P), 1.05 (m, 6 H, $(CH_3)_2CH$). Anal. ($C_{15}H_{24}NO_3P$) C, H, N.

(b) 1-[1-(Benzylamino)-2-methylpropyl]phosphonic Acid (26f). A solution of **25f** (5.3 g, 0.018 mol) in ethanol (20 mL) was hydrogenated over PtO_2 (0.2 g) at room temperature and atmospheric pressure. After absorption of 1 equiv of H_2, the solution was filtered and the solvent was evaporated to yield 5.3 g (100%) of an oil: IR, disappearance of C=N bond; ^1H NMR; disappearance of d at δ = 8.2. The crude oil was then treated during 3 h at reflux with 100 mL of a solution of acetic acid containing 10 mL of aqueous hydrobromic acid (48%). The solvent was removed under reduced pressure and the residue dried overnight on P_2O_5 and then dissolved in ethanol (50 mL). The acid was precipitated by slow addition of 10 mL of propylene oxide. After filtration: 2.6 g (61%); mp 260 °C; $[\alpha]^{20}_D$ −37.4° (*c* 1, EtOH); ^1H NMR (D_2O) δ 7.5 (m, 5 H, Ar), 4.45 (d, 2 H, CH_2Ar), 3.1 (dd, 1 H, HC(P)N), 2.3 (m, 1 H, $CH(CH_3)_2$), 1.1 (m, 6 H, $CH(CH_3)_2$).

(c) 27f. A solution of **26f** (2.4 g, 0.01 mol) in ethanol (100 mL) was stirred during 8 h at 40 °C, over 10% Pd/C (0.3 g), under an atmosphere of hydrogen (normal pressure). After filtration, the precipitate was washed with water (3 × 25 mL), and the combined aqueous solutions were evaporated under reduced pressure. The residue was then triturated in ethanol to give after filtration 1.2 g (80%) of crystals: mp >260; $[\alpha]^{20}_D$ −0.3° (*c* 2, NaOHN); ^1H NMR (D_2O) δ 3.1 (1 H, HC(P)N), 2.2 (m, 1 H, $CH(CH_3)_2$), 1.1 (m, 6 H, $(CH_3)_2CH$). Anal. ($C_4H_{12}NO_3P$) H, N; C. Calcd: 31.38. Found: 32.07.

***d*-(1-Amino-2-methylpropyl)phosphonic Acid (27e). (a) *d*-[1-(Benzylimino)-2-methylpropyl]phosphonic Acid Diethyl Ester (25e).** **25e** was prepared as previously described starting from **6e** (α^{20}_D −9.5) (1 g, 0.005 mol) and benzaldehyde (0.5 g, 0.005 mol) in toluene (10 mL) to yield an oil: 1.4 g (94%); $[\alpha]^{20}_D$ +40.8° (*c* 1, $CHCl_3$); IR; ^1H NMR.

(b) *d*-[1-(Benzylamino)-2-methylpropyl]phosphonic acid (26e); crystals, 0.65 g (74%); mp >260 °C; $[\alpha]^{20}_D$ +37.1° (*c* 1, EtOH); IR; ^1H NMR.

(c) 27e: crystals; 0.3 g (79%); mp >260 °C; $[\alpha]^{20}_D$ +0.9° (*c* 2, NaOHN); ^1H NMR. Anal. ($C_4H_{12}NO_3P$) C, H, N.

Supplementary Material Available: ^1H NMR and ^{13}C NMR spectral data of compounds **7–9, 11, 12, 14, 15, 17, 18, 20,** and **21** (11 pages). Ordering information is given on any current masthead page.

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