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Syntheses and biological evaluation of vinblastine congeners †

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Sixty-two congeners of vinblastine (VLB), primarily with modifications of the piperidine ring in the carbomethoxycleavamine moiety of the binary alkaloid, were synthesized and evaluated for cytotoxicity against murine L1210 leukemia and RCC-2 rat colon cancer cells, and for their ability to inhibit polymerization of microtubular protein at $<10^{-6}$ M, and for induction of spiralization of microtubular protein, and for microtubular disassembly at 10^{-4} M concentrations. An ID_{50} range of $>10^{7}$ M concentrations was found for L1210 inhibition by these compounds, with the most active 1000× as potent as vinblastine.

Introduction

The development of practical, enantioselective total syntheses of the clinically effective anticancer alkaloids vinblastine (VLB, **1**) and vincristine (VCR, **2**, Scheme 1)[1,2] provided the first opportunity to systematically synthesize molecular modifications for a study of structure–activity relationships. Structural changes of the bridged piperidine ring of the carbomethoxycleavamine (top) segment of these binary alkaloids led to a range of cytotoxicities with $ID_{50} > 10^{-6}$ M to $ID_{50} = 10^{-13}$ M, thus establishing the importance of this segment of the molecule for VLB-like biological activity. Key features of our synthetic strategy are the facile, stereoselective assembly of the structural moiety required for coupling to the vindoline (bottom) segment and the high stereoselectivity of that process for the specific C-14′–16′ relative stereochemistry,[3,4] which is required for VLB-like biological activity (see Scheme 1). The compounds of this paper were all prepared by this methodology. When designated with an R in the Tables, they were synthesized at Hoffmann–La Roche.

Biological results

Our initial synthesis of the simple vinblastine analogue lacking the 20′-ethyl and hydroxyl functions (**3**),[3,4] showed that this compound, like VLB, exhibits cytotoxicity against L1210 leukemia cells (albeit about 300× less potently, Table 1), and cytotoxicity against RCC rat colon cancer cells with 1/100 the potency of VLB. At the biochemical level it inhibits tubulin polymerization at a stoichiometric concentration (10^{-6} M) and VLB-like disassembly of microtubules at higher concentration (10^{-4} M). Microtubular protein single spirals were formed with compound **3** at 10^{-4} M concentration, while VLB produces spiral aggregates at that concentration.[5,6]

The epimeric 20′-deoxyvinblastine (**4**) and 20′-deoxyleurosidine (**5**) showed 1/10 and 1/100, respectively, of the cytotoxic potency of VLB against L1210 and RCC-2 cells,[5–7] and 20′-deoxyvinblastine (**4**) inhibits tubulin polymerization at a substoichiometric concentration (10^{-7} M), like VLB, while its C-20′ epimer **5** is only as potent as the 20′-deethyl analogue **3** in that test. Like vinblastine, both compounds **4** and **5** gave spiral aggregates with microtubular protein and disassembled microtubules at 10^{-4} M concentration.

Since the introduction of an equatorial 20′-ethyl substituent resulted in a substantial increase in cytotoxic potency and achieved a VLB-like substoichiometric level of inhibition of tubulin polymerization, it was of interest to explore the structure–activity sensitivity to substitution at C-20′ with other alkyl substituents. Increased homologation of the C-20′ ethyl group gave a drop, rather than a further increase in L1210 and RCC-2 cytotoxicity for the 20′-equatorial *n*-propyl congener **6R**, to about one tenth of the potency of the 20′-deoxy VLB congener **4**. Similar results were found for its C-20′ epimer **7R** relative to 20′-deoxyleurosidine (**5**). While the C-20′ propyl congeners still produced an inhibition of tubulin polymerization, no spiralization was found at higher concentrations with microtubular protein.

Replacement of the equatorial C-20′ substituents by a benzyl group (**8R**) resulted in loss of all VLB-like activity, while an axial C-20′ benzyl group (**9R**) still provided modest L1210 cytotoxicity and inhibition of tubulin polymerization.

Decreasing the size of the C-20′ substituent, on the other hand, led to increased potency. Thus, both C-20′ methyl congeners (**10** and **11**) are almost as active as vinblastine (**1**) in cytotoxicity with L1210 cells and they are slightly more potent in inhibition of tubulin polymerization, in disassembly of microtubules and in formation of spiral aggregates with microtubular protein. Indeed, single spirals were produced with low concentrations ($1–2 \times 10^{-7}$ M) of each compound with microtubular protein. Introduction of combined α and β-methyl substitution at C-20′ (**12**), however, resulted in activities closer to those of the 20′-deethyl-20′-deoxy congener **3**, and loss of the activity of microtubule disassembly. The introduction of two C-20′ ethyl substituents (**13R**) led to a further decrease in activity.

These results suggest that substitution at C-20′ modulates VLB-like activity at the biochemical and the cellular levels, not primarily by affecting the conformational shape of the bridged piperidine ring (deformation towards half-chair or boat) but, rather by the need for a lipophylic substituent with limited

† Electronic supplementary information (ESI) available: Full experimental procedures and UV, IR, ^{1}H NMR, ^{13}C NMR and MS data for all compounds. See http://www.rsc.org/suppdata/ob/b2/b209990j/

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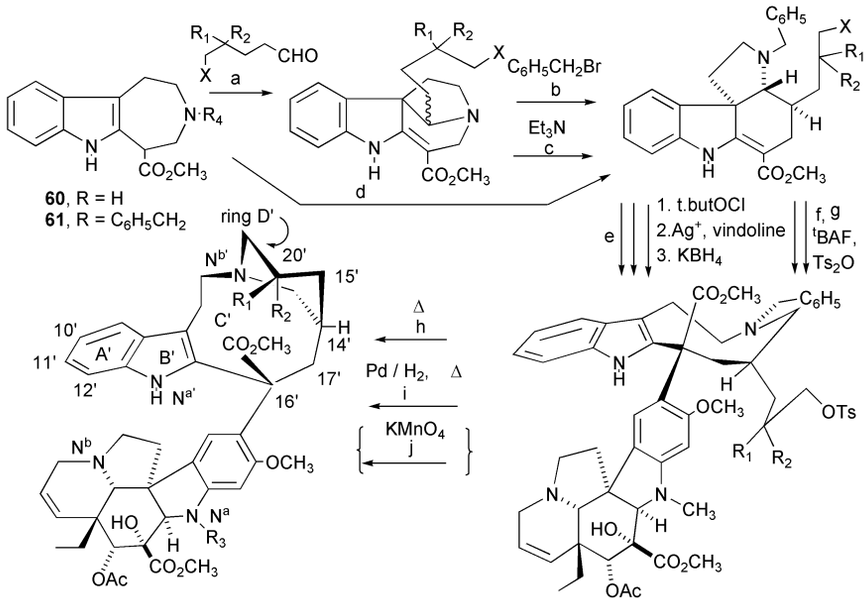


R_1=Et, R_2=OH, R_3=Me, vinblastine (**1**)
R_1=Et, R_2=OH, R_3=CHO, vincristine (**2**)
R_1=OH, R_2=Et, R_3=Me, leurosidine (**29**)
R_1=R_2=H, R_3=Me (**3**)
R_1=R_2=H, R_3=CHO (**37**)
R_1=Et, R_2=H, R_3=Me (**4**)
R_1=Et, R_2=H, R_3=CHO (**38**)
R_1=H, R_2=Et, R_3=Me (**5**)
R_1=H, R_2=Et, R_3=CHO (**39**)
R_1=C_3H_7, R_2=H, R_3=Me (**6R**)
R_1=C_3H_7, R_2=H, R_3=CHO (**40R**)
R_1=H, R_2=C_3H_7, R_3=Me (**7R**)
R_1=H, R_2=C_3H_7, R_3=CHO (**41R**)
R_1=Bz, R_2=H, R_3=Me (**8R**)
R_1=H, R_2=Bz, R_3=Me (**9R**)
R_1=Me, R_2=H, R_3=Me (**10**)
R_1=H, R_2=Me, R_3=Me (**11**)
R_1=R_2=R_3=Me (**12**)
R_1=R_2=Et, R_3=Me (**13R**)

Scheme 1 *Typical reaction conditions for formation of C-20′ varied substitution of vinblastine congeners:* (a) **60** (R_4 = H), respective aldehyde (X = TMS or tBDMS), THF, rt, 24 h; (b) $C_6H_5CH_2Br$, THF, refl. 24 h; (c) MeOH, Et_3N or DIBEA, refl. 6 h or 6 d; (d) 61 (R_4 = $C_6H_5CH_2$), respective aldehyde, toluene, refl. 12 h; (e) 1) tBOCl, Et_3N, CH_2Cl_2, 0 °C; 2) vindoline·HCl, acetone, 0 °C, $AgBF_4$, HBF_4·Et_2O, 25 min; 3) KBH_4, HOAc, rt, 30 min; (f) tBAF, THF, rt, 2 h; (g) Ts_2O, Et_3N, CH_2Cl_2, 0 °C to rt, 2.5 h; (h) toluene, refl. 6 h to 5 d; (i) 1) 10% Pd/C, H_2, MeOH, 6 h, rt; 2) toluene refl. 6 h; (j) CH_2Cl_2, HOAc, $KMnO_4$, hexaoxocyclooctadecane, −72 °C, 1 h.

volume of radial motion. To test this hypothesis, we synthesized C-15′–20′ ring *cis*-fused deoxy-VLB congeners, where the C-20′ substituent is now tied back to the piperidine ring. Indeed, it was found that VLB-like activity was substantially increased. The C-20′ *S* six-membered ring compound **14** (see Table 1 and Scheme 7) is 500× more potent and the corresponding C-20′ *R* fused ring **15** is 1000× more potent than VLB in L1210 cytotoxicity, while the activity with RCC-2 cells is equivalent to that of 20′-deoxyvinblastine (**4**). The corresponding five-membered ring analogues **16** and **17** (see Table 1 and Scheme 6) gave activity profiles similar to those of the C-20′ ethyl compounds **4** and **5**.

Even the C-15′–20′ benzo analogue **18** (see Table 1 and Scheme 8), with four coplanar carbon atoms of the piperidine ring D′, still shows cytotoxicities equal to those of 20′-deoxyvinblastine (**4**). All of these ring-fused congeners also exhibit full VLB-type activity at the biochemical level with tubulin and with microtubules.

The extraordinary high L1210 cytotoxicity of the C-15′–20′ fused cyclohexane congeners **14** and **15** prompted us to synthesize the corresponding tetrahydropyran analogues **19** and **20** (see Scheme 9). These compounds showed a 4–5 × 10^5 drop in cytotoxic potency relative to the cyclohexane fused congeners **14**,**15**, to the range of 20′-deoxy VLB activity.

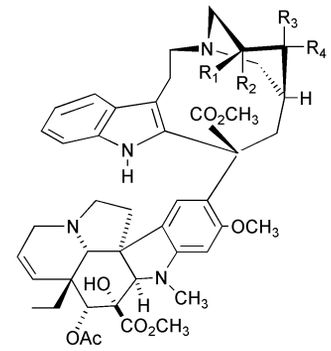
In an alternative approach, the C-20′ spiro five and six-membered ring substituted congeners **21** and **22** (see Scheme 3) were synthesized. While these compounds were not expected to display the high activity of the C-15′–20′ ring-fused compounds (restricted rotation C-20′ monoalkyl class), it could be hoped that they would have greater VLB activity than the C-20′ diethyl compound **13R**. Accordingly, these compounds showed full VLB activity at the biochemical level (tubulin polymerization inhibition at substoichiometric concentration with spiral aggregation at higher concentration) and 20′-deethyl-20′-deoxy VLB-like cytotoxicity with RCC-2 cells, but they were not cytotoxic to L1210 cells at concentrations considered for activity ($<10^{-6}$ M).

This same activity profile was also found with 20′-deethyl-20′-deoxy VLB congeners in which the D′-piperidine ring had been enlarged to a seven (**23**) or eight (**24**) membered ring (see Table 2, Scheme 4). Molecular modeling suggests that in common with the 20′-spiro substituted compounds **21** and **22**, compounds **23** and **24** have a conformation with a shielded ring C′ nine-membered ring cavity.

The lack of L1210 cytotoxicity, despite good inhibition of tubulin polymerization, suggests a possible failure of the congeners **21**–**24** to penetrate the cell membrane. Accordingly, on electroporation they were found to have very high cytotoxic activity.[8]

Based on the hypothesis that shielding of $N^{b'}$ to protonation, solvation, and/or binding might be responsible for inhibition of transport of compounds **21**–**24** into the cell, and a consequent lack of cytotoxicity, a more accessible basic nitrogen was introduced by formation of the hydrazide **25** (see Scheme 4 and

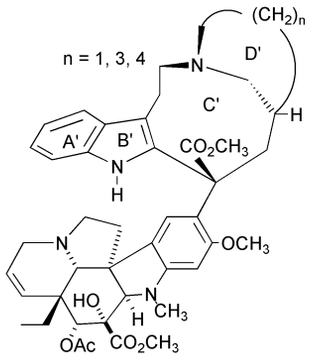
Table 1



					Population growth IC_{50}/M		Microtubule		
Compound	R_1	R_2	R_3	R_4	L1210 leukemia	RCC-2 Colon adenocarcinoma	Microtuble assembly IC_{50}/M	Disassembly into spiral aggregates at 10^{-4} M	Spiralization of MTP at 10^{-4} M
1[a]	Et	OH	H	H	4×10^{-10}	3×10^{-9}	3×10^{-7}	+	+
3[a]	H	H	H	H	1×10^{-7}	5×10^{-7}	2×10^{-6}	+	+, Single spirals
4[a]	Et	H	H	H	3×10^{-9}	3×10^{-8}	2×10^{-7}	+	+
5[a]	H	Et	H	H	2×10^{-8}	5×10^{-7}	1×10^{-6}	+ (5×10^{-5} M)	+ (5×10^{-5}M)
6R[a]	Propyl	H	H	H	5×10^{-8}	6×10^{-7}	3×10^{-6}	N.D.	±, Amorphous aggregates
7R[a]	H	Propyl	H	H	8×10^{-8}	6×10^{-7}	2×10^{-6}	−	±, Amorphous aggregates
8R[a]	$CH_2C_6H_5$	H	H	H	$>1 \times 10^{-6}$	N.D.	No effect, at 1×10^{-5}		− (5×10^{-5}M)
9R[a]	H	$CH_2C_6H_5$	H	H	5×10^{-7}	N.D.	3×10^{-6}	−	−
10[a]	Me	H	H	H	1×10^{-9}	3×10^{-8}	2×10^{-7} single spirals	+	+
11[a]	H	Me	H	H	1×10^{-9}	5×10^{-8}	1×10^{-7} single spirals	+	+
12[a]	Me	Me	H	H	3×10^{-8}	8×10^{-7}	3×10^{-6}	−	+
13R[a]	Et	Et	H	H	5×10^{-6}	$>1 \times 10^{-6}$	No effect at 2×10^{-5}	−	N.D.
14	$R_2 + R_4 = -(CH_2)_4-$, $R_1 = R_3 = H$				8×10^{-13}	2×10^{-8}	1×10^{-7}	+	+
15	$R_1 + R_3 = -(CH_2)_4-$, $R_2 = R_4 = H$				3×10^{-13}	2×10^{-8}	1×10^{-7}	+	+
16	$R_2 + R_4 = -(CH_2)_3-$, $R_1 = R_3 = H$				2×10^{-8}	N.D.	3×10^{-6}	+	+
17	$R_1 + R_3 = -(CH_2)_3-$, $R_2 = R_4 = H$				1×10^{-9}	5×10^{-7}	2×10^{-7}	+	+
18	Benzo		Benzo		3×10^{-9}	5×10^{-8}	1×10^{-7}	+(5×10^{-5}M)	+(5×10^{-5}M)
19	$R_1 + R_3 = (CH_2)O(CH_2)_2$, $R_2 = R_4 = H$				5×10^{-8}	N.D.	1×10^{-6}	N.D.	N.D.
20	$R_2 + R_4 = (CH_2)O(CH_2)_2$, $R_1 = R_3 = H$				3×10^{-8}	N.D.	2×10^{-6}	N.D.	N.D.
21	$-(CH_2)_4-$		H	H	$>10^{-6}$	5×10^{-7}	5×10^{-7}	+	+
22	$-(CH_2)_5-$		H	H	$>10^{-6}$	5×10^{-7}	5×10^{-7}	+	+
27	CH_3	OH	H	H	1×10^{-9}	1×10^{-8}	1×10^{-6}	+	+
28	OH	CH_3	H	H	4×10^{-7}	6×10^{-7}	No effect at 2×10^{-5}	−	−
29	OH	Et	H	H	1×10^{-6}	$>10^{-6}$	1×10^{-5}	−	−, Short MT

[a] Denotes methanesulfonate salt. The corresponding compounds without designation R were also tested as free bases and found to have the same activities as these salts.

Table 2



Compound		Population growth microtubule IC_{50}/M		Microtuble		
		L1210 leukemia	RCC-2 colon adenocarcinoma	Microtuble assembly IC_{50}/M	Disassembly into spiral aggregates at 10^{-4} M	Spiralization of MTP at 10^{-4} M
23	D′7 ring	$>10^{-6}$	5×10^{-7}	2×10^{-7}	+	+
23[a]		5×10^{-8}	N.D.	2×10^{-6}	N.D.	N.D.
24	D′8 ring	$>10^{-6}$	5×10^{-7}	2×10^{-7}	+	+
26R	D′5 ring	$>10^{-6}$	$>10^{-6}$	1.5×10^{-5}	+	±, amorphous aggregates

[a] Denotes methanesulfonate salt.

Table 4) of the seven-membered ring D′ congener **23**. Indeed, this compound, like VLB hydrazide (**175**), had almost the L1210 cytotoxicity of VLB (**1**). Finally, administration of the dimethanesulfonate salt of the base **23** also resulted in modest L1210 cytotoxicity, comparable to that of the unsubstituted piperidine ring D′ compound **3**.[9]

The corresponding lower homologue with a five-membered ring D′ (**26R**) in place of the piperidine ring, was found to lack all VLB-like activity.

Considering that the methyl homologue **10** of 20′-deoxyvinblastine (**4**) has a relatively greater L1210 cytotoxicity than the ethyl compound **4**, and approaches that of vinblastine, it seemed likely that introduction of an axial C-20′ hydroxyl substituent into this compound could provide another VLB congener with superior activity. However, evaluation of 20′-methyl-20′-deethyl vinblastine (**27**) showed that here the axial C-20′ hydroxyl substituent did not enhance cytotoxic potency. The corresponding C-20′ epimer, 20′-methyl-20′-deethylleurosidine (**28**) showed no VLB-type tubulin activity, as expected from the lack of such activity with leurosidine (**29**).

The syntheses and evaluation of a group of vinblastine congeners with decreased biological activities also provided a scale for consideration of structure–activity changes based on introduction of substituents on the aromatic ring of the carbomethoxycleavamine (top) moiety of the binary alkaloids (Table 3). Thus syntheses of 11′-methoxy-20′-deethyl-20′-deoxyvinblastine (**30**) gave a congener which is more potent in inhibiting tubulin polymerization than the parent demethoxy compound **3**, but which lacks the tubulin spiralization effect at high concentration and, which is also less cytotoxic. A decrease or loss of activity was seen with 11′-methoxy-20′-deoxyleurosidine (**31R**) and 11′-methoxy-20′-deoxyvinblastine (**32R**) was even found to lack all VLB-like activity.

In 10′-methoxy-20′-deoxyvinblastine (**33R**) moderate L1210 and RCC-2 cytotoxicity and inhibition of tubulin polymerization are retained and with its C-20′ epimer (**34R**) we also observed disassembly of microtubules and tubulin spiralization, as with the parent compound **5**. Introduction of an 11′-bromo substituent (**35**) led to loss of the tubulin spiralization activity of 20′-deethyl-20′-deoxyvinblastine (**3**), while a 10′-chloro derivative **36** showed an increase in L1210 cytotoxicity and in potency of tubulin polymerization inhibition.

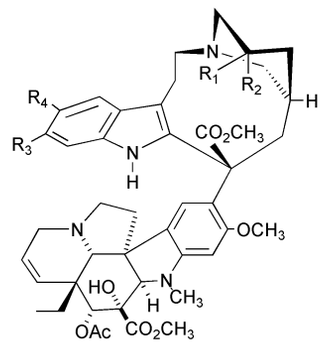
Oxidation of some of the preceding vinblastine congeners to the corresponding vincristine series (Table 4) resulted only in minor perturbation of the cytotoxicity and tubulin polymerization inhibition values for 20′-deethyl-20′-deoxyvincristine (**37**), 20′-deoxyvincristine (**38**) and its C-20′ epimer (**39**). Of interest, however, is the 100× increased inhibition of tubulin polymerization seen with the C-20′ propyl homologues (**40R** and **41R**). Indeed, these compounds were the most potent of all examined in this test.

Since the tryptophan derived amide derivative of vinblastine (**42**, Fig. 1) had been reported to have a better therapeutic index than vinblastine (however, with decreased potency),[10] we also examined that compound and the corresponding 20′-deoxy and 20′-*epi*-20′-deoxy VLB tryptophan derivatives (**43** and **44**, Fig. 1). All three compounds were found to be equivalent in L1210 cytotoxicity and in inhibition of tubulin polymerization, but the deoxy compounds **43** and **44** were less potent in RCC-2 cytotoxicity (Table 4). On the other hand, the carboxylic acid **45**, derived from 20′-deoxyvinblastine (**4**), was as potent in RCC-2 cytotoxicity as the methyl ester **4**.

The isolation of peptides that home specifically to tumor blood vessels has led to their attachment to doxorubicin for its targeted drug delivery with diminished general cytotoxicity.[11] We therefore attached such a peptide to vinblastine and to our seven-membered ring D′ congener deacetyl-**23**. While congener **23** was not transported into L1210 cells as the free base (see above) the resulting bridged CRGNC peptide derivative **176** now gave some inhibition of L1210 cells as the free base. This compound and a corresponding VLB derivative **177** (Fig. 1) showed the anticipated attenuation of L1210 cell inhibition (Table 4) that would suggest their *in vivo* investigation for targeted delivery.

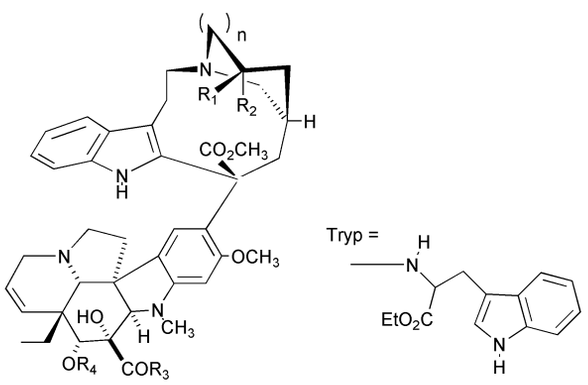
A unique feature of our synthetic route to vinblastine congeners is the ability to generate these compounds as atropisomers (isolable conformational isomers, Fig. 2),[2,4,7] which can be thermally converted to products with the natural conformation. Examination of the atropisomers of the potent agents VLB (**1**), 20′-deoxy VLB (**4**) and its C-20′ epimer **5**, of the corresponding methyl analogues **10** and **11**, and of the C-15′–16′ cyclohexyl fused compound **14** showed that, as expected from the drastically altered shape and the increased binding ability of the piperidine nitrogen in the atropisomers,

Table 3

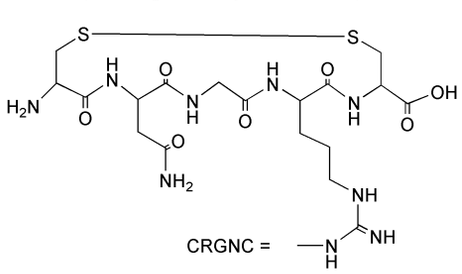


Compound	R_1	R_2	R_3	R_4	Population growth IC_{50}/M: L1210 leukemia	Population growth IC_{50}/M: RCC-2 Colon adenocarcinoma	Microtubule: Microtuble assembly IC_{50}/M	Microtubule: Disassembly into spiral aggregates at 10^{-4} M	Microtubule: Spiralization of MTP at 10^{-4} M
30	H	H	OMe	H	1×10^{-6}	$>5 \times 10^{-6}$	2×10^{-7}	−	−, short MT
31R[a]	H	Et	OMe	H	1×10^{-6}	N.D.	2×10^{-6}	−	±, amorphous aggregates
32R[a]	Et	H	OMe	H	$>1 \times 10^{-6}$	$>10^{-6}$	no effect at 1.3×10^{-5}	−	−
33R[a]	Et	H	H	OMe	1×10^{-8}	5×10^{-7}	2×10^{-6}	−	±, amorphous aggregates
34R[a]	H	Et	H	OMe	5×10^{-8}	5×10^{-8}	2×10^{-6}	+	+
35	H	H	Br	H	1×10^{-7}	1×10^{-6}	1×10^{-6}	−	−
36[a]	H	H	H	Cl	2×10^{-8}	N.D.	5×10^{-7}	N.D.	N.D.

[a] Denotes methanesulfonate salt. The corresponding compound without designation R was also tested as free base and found to have the same activities as the salt.



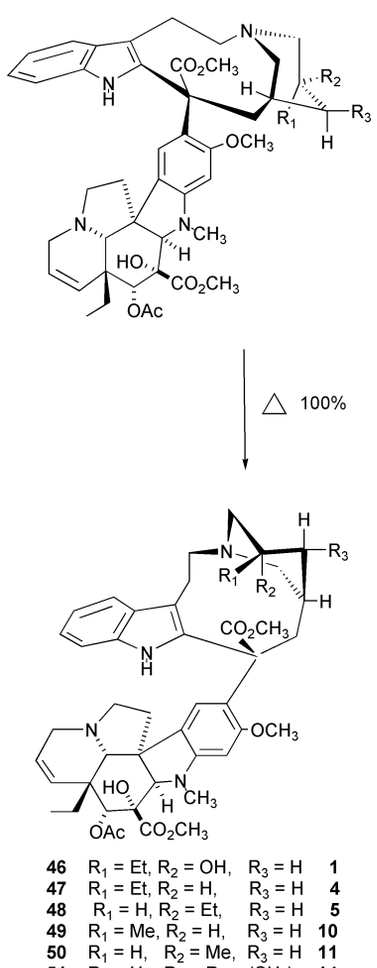
42 R_1 = Et, R_2 = OH, R_3 = Tryp, R_4 = Ac, n = 1
43 R_1 = Et, R_2 = H, R_3 = Tryp, R_4 = H, n = 1
44 R_1 = H, R_2 = Et, R_3 = Tryp, R_4 = H, n = 1
45 R_1 = Et, R_2 = H, R_3 = OH, R_4 = Ac, n = 1
25 R_1 = R_2 = H, R_3 = $NHNH_2$, R_4 = H, n = 2
175 R_1 = Et, R_2 = OH, R_3 = $NHNH_2$, R_4 = H, n = 1
176 R_1 = R_2 = H, R_3 = CRGNC, R_4 = H, n = 2
177 R_1 = Et, R_2 = OH, R_3 = CRGNC, R_4 = H, n = 1



Vinblastine congeners with modifications of vindolinyl methoxycarbonyl function

Fig. 1

these compounds **46**–**51** did not inhibit tubulin polymerization nor generate spiral aggregates from microtubular protein (Table 5. The marginal cytotoxicities shown in the table may be due to slight contamination by the corresponding very potent active atropisomers)

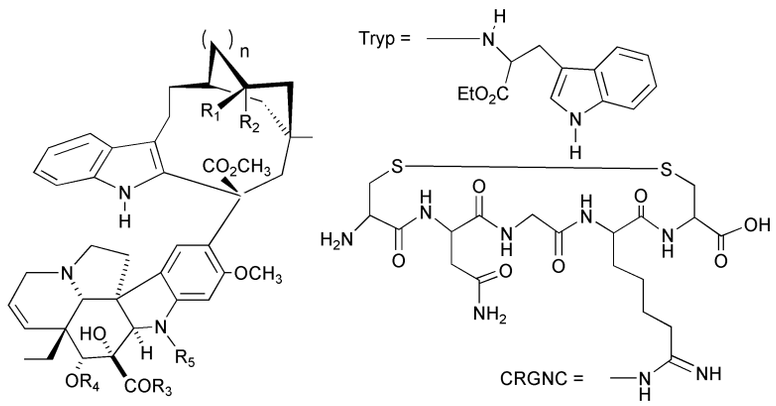


46 R_1 = Et, R_2 = OH, R_3 = H **1**
47 R_1 = Et, R_2 = H, R_3 = H **4**
48 R_1 = H, R_2 = Et, R_3 = H **5**
49 R_1 = Me, R_2 = H, R_3 = H **10**
50 R_1 = H, R_2 = Me, R_3 = H **11**
51 R_1 = H, R_2 = R_3 = $(CH_2)_4$ **14**

Higher energy atropisomers of vinblastine congeners and their thermal conformational inversion to the natural vinblastine-type conformation

Fig. 2

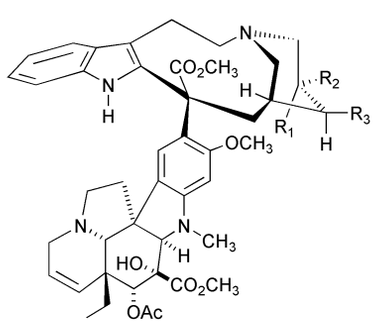
Table 4



Compound	R_1	R_2	R_3	R_4	R_5	*n*	Population growth IC_{50}/M: L1210 leukemia	Population growth IC_{50}/M: RCC-2 Colon adenocarcinoma	Microtubule: Microtuble tubulin IC_{50}/M	Microtubule: Disassembly into spiral aggregates at 10^{-4}/M	Microtubule: Spiralization of MTP at 10^{-4} M
37[a]	H	H	OMe	Ac	CHO	1	4×10^{-7}	N.D.	2×10^{-6}	N.D.	N.D.
38[a]	Et	H	OMe	Ac	CHO	1	7×10^{-10}	7×10^{-9}	2×10^{-7}	+	+
39[a]	H	Et	OMe	Ac	CHO	1	2×10^{-8}	N.D.	5×10^{-7}	+	+
40R[a]	propyl	H	OMe	Ac	CHO	1	1×10^{-7}	5×10^{-7}	1×10^{-8}	+	±, amorphous material
41R[a]	H	propyl	OMe	Ac	CHO	1	5×10^{-8}	7×10^{-7}	1×10^{-8}	−	±, amorphous material
42[a]	Et	OH	Tryp	Ac	Me	1	4×10^{-8}	3×10^{-8}	3×10^{-7}	N.D.	N.D.
43	Et	H	Tryp	H	Me	1	5×10^{-8}	2×10^{-7}	3×10^{-7}	(P)	(P)
44[a]	H	Et	Tryp	H	Me	1	4×10^{-8}	1×10^{-7}	3×10^{-7}	N.D.	N.D.
45	Et	H	OH	H	Me	1	6×10^{-9}	4×10^{-8}	6×10^{-7}	N.D.	N.D.
25[a]	H	H	$NHNH_2$	H	Me	2	5×10^{-9}	N.D.	1×10^{-6}	N.D.	N.D.
175	Et	OH	$NHNH_2$	H	Me	1	3×10^{-9}	N.D.	5×10^{-6}	N.D.	N.D.
176	H	H	CRGNC	H	Me	2	4×10^{-7}	N.D.	5×10^{-6}	N.D.	N.D.
177	Et	OH	CRGNC	H	Me	1	4×10^{-8}	N.D.	9×10^{-6}	N.D.	N.D.

[a] Denotes methanesulfonate salt. The corresponding compounds without designation R were also tested as free bases and found to have the same activities as these salts.

Table 5

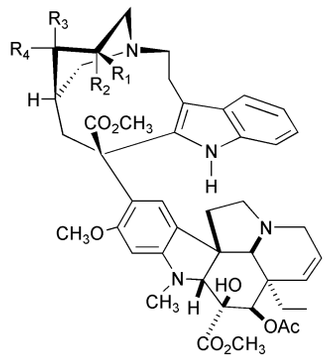


Compound	R_1	R_2	R_3	Population growth IC_{50}(M): L1210 leukemia	Population growth IC_{50}(M): RCC-2 Colon adenocarcinoma	Microtubule: Microtuble assembly IC_{50}/M	Microtubule: Disassembly into spiral aggregates at 10^{-4} M	Microtubule: Spiralization of MTP at 10^{-4} M
46	Et	OH	H	$>10^{-6}$	$>10^{-6}$	No effect, at 1×10^{-5}	−	−
47	Et	H	H	5×10^{-7}	3×10^{-7}	6×10^{-6}	±,MT and spirals	+, Single spirals
48	H	Et	H	5×10^{-7}	$>10^{-6}$	No effect, at 1×10^{-5}	−	−
49	Me	H	H	1.8×10^{-7}	4×10^{-6}	No effect, at 5×10^{-5}	$-(5 \times 10^{-5})$	$-(5 \times 10^{-5})$
50	H	Me	H	1.5×10^{-5}	3×10^{-5}	No effect, at 5×10^{-5}	$-(5 \times 10^{-5})$	$-(5 \times 10^{-5})$
51	H	$-(CH_2)_4-$		5×10^{-8}	N.D.	No effect, at 1×10^{-5}	$-(5 \times 10^{-5})$	±, Amorphous aggregates (5×10^{-5}M)

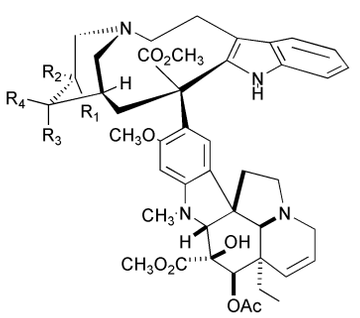
Possible contamination of **51** with **16**.

This inactivity was also found with the C-14′,16′-*epi*-compound vincovaline (**52**, Fig. 3, Table 6),[12] its atropisomer **53** (Table 7), 20′-deoxyvincovaline (**54**), its atropisomer **55**, the corresponding C-20′ epimer **56**, the C14′,16′,20′-*epi*-isomers **57** and **58** of the α and β-cyclopentyl fused compounds **18** and **19** and the atropisomer **59** of the diastereomer of the cyclohexyl

Table 6

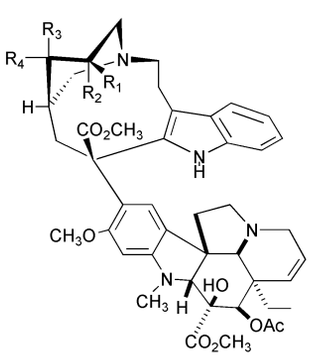


Compound	R_1	R_2	R_3	R_4	Population growth IC_{50}/M: L1210 leukemia	Population growth IC_{50}/M: RCC-2 Colon adenocarcinoma	Microtubule: Microtuble assembly IC_{50}/M	Microtubule: Disassembly into spiral aggregates at 10^{-4} M	Microtubule: Spiralization of MTP at 10^{-4} M
52	Et	OH	H	H	$>1 \times 10^{-4}$	N.D.	$>10^{-4}$	−	−, Short MT
54	Et	H	H	H	1.5×10^{-5}	5×10^{-6}	No effect at 5×10^{-5}	−	−
57	$R_2 + R_4 = -(CH_2)_3-$, $R_1 = R_3 = H$ Possible contamination of **57** with **16**				8×10^{-8}	$>10^{-6}$	1×10^{-4}	−	−, Short MT
58	$R_1 + R_3 = -(CH_2)_3-$, $R_2 = R_4 = H$ Possible contamination of **58** with **17**				5×10^{-7}	$>10^{-6}$	no effect at 1×10^{-5}	−	−, Short MT



53 R_1 = Et, R_2 = OH, $R_3 = R_4$ = H
55 R_1 = Et, R_2 = H, $R_3 = R_4$ = H
[**59** R_1 = H, $R_2 = R_4 = (CH_2)_4$, R_3 = H]

100%



52 R_1 = Et, R_2 = OH, $R_3 = R_4$ = H
54 R_1 = Et, R_2 = H, $R_3 = R_4$ = H
[**56** R_1 = H, R_2 = Et, $R_3 = R_4$ = H
57 R_1 = H, $R_2 = R_4 = (CH_2)_3$, R_3 = H
58 $R_1 = R_3 = (CH_2)_3$, $R_2 = R_4$ = H]

C-14′, 16′-epi-vinblastine (vincovaline) congeners with conformational thermal inversion of their higher energy atropisomers

Fig. 3

fused compound **14**. While these diastereomers of active VLB congeners lack the fundamental ability to affect tubulin polymerization and therefore do not qualify as additional VLB congeners, they were found to provide a new series of compounds that can modulate the activity of active cytotoxic agents and, that can overcome multidrug resistance. Those results are described in a separate publication.[13]

Synthetic methodology

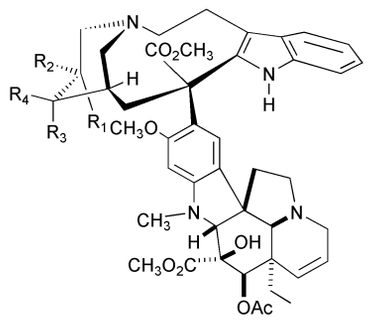
The carbomethoxy cleavamine (top) segment of the described VLB congeners, with variations of C-20′ substitution, were all generated using procedures analogous to those developed for the 20′-ethyl compounds.[7] While condensations of aldehydes containing variations of the eventual C-20′ substituents were initially carried out with the N^b-unsubstituted indoloazepine **60**,[14] with a following N^b-benzylation of the resulting epimeric bridged azepines and rearrangement, later syntheses of the key tetracyclic intermediates used condensations of the N^b-benzyl-indoloazepine **61**, cracked to an acrylate amine at 110 °C (Scheme 1).[15]

The aldehyde precursors for 20′-deoxyvinblastine (**4**), 20′-deoxyleurosidine (**5**) and their C-20′ methyl analogues **10** and **11** were generated by alkylation of valerolactone, with eventual selective reduction of an ester function after introduction into tetracyclic vinylogous urethane intermediates **62** for generation of the tetracyclic vindoline coupling precursors **63** (Scheme 2).

In syntheses of the C-20′ propyl analogues **6R** and **7R** it was found that the tetracyclic intermediates **64** could be synthesized more effectively by making the intermediate aldehyde **65** by reaction of a pyrrolidine enamine derivative of pentanal with acrylonitrile, followed by borohydride reduction of the resulting aldehyde, O-silylation and reduction of the nitrile function by diisobutylaluminum hydride (DIBAL-H).

In the C-20′methyl series, cyclization of the D′-*seco*-tosylate intermediates **66**–**69** produced a mixture of "natural" (lower energy) and "unnatural" (higher energy) atropisomers of the final products, with the ratio depending on the diastereomeric relationship of the *seco*-intermediates. From the corresponding cyclization the C-20′ ethyl compounds 20′-deoxyvinblastine (**4**), its C-14′,16′,20′ epimer 20′-deoxyvincovaline (**54**), 20′-deoxyleurosidine (**5**) and its C-14′,16′,20′ epimer had been obtained as high–low energy atropisomers in respective ratios of 3 : 97, 43 : 57, 28 : 72, and 60 : 40, while the ratios for the C-20′ methyl analogues **10**,**72**,**11**,**73**, were 5 : 95, 46 : 54, 29 : 71 and 58 : 42, in good agreement, based on isolated product

Table 7



Compound	R_1	R_2	R_3	R_4	Population growth IC_{50}/M L1210 leukemia	Population growth IC_{50}/M RCC-2 Colon adenocarcinoma	Microtubule Microtuble assembly IC_{50}/M	Microtubule Disassembly into spiral aggregates at 10^{-4} M	Microtubule Spiralization of MTP at 10^{-4} M
53	Et	OH	H	H	$>10^{-6}$	N.D.	No effect, at 1×10^{-4}	–	–
55	Et	H	H	H	5×10^{-7}	$>10^{-6}$	1×10^{-4}	–	–, short MT
56	H	Et	H	H	$>10^{-6}$	$>10^{-6}$	No effect, at 1×10^{-4}	–	–
59	$R_2 + R_4 = -(CH_2)_4-$, $R_1 = R_3 = H$				5×10^{-7}	N.D.	No effect, at 1.5×10^{-5}	–	±, Amorphous aggregates

yields. Complete inversion of the high to low energy atropisomer in refluxing toluene was seen in each case. In the C-20′ propyl series, the higher energy atropisomers were not isolated but they were converted directly to the "natural" type atropisomers by heating of the crude reaction products in toluene.

For synthesis of the C-20′ dimethyl congener **12** of vinblastine, the required 4,4-dimethyl-5-functionalized-valeraldehyde **74a** (Scheme 3) was prepared by sodium hydroxide catalyzed alkylation of isobutyraldehyde with acrylonitrile (49% of **75a**), followed by sodium borohydride reduction of of the aldehyde function (95%) and protection of the resulting alcohol **76a** as its *tert*-butyldimethylsilyl ether (**77a**, 69%) prior to reduction of the nitrile function (79%) with diisobutyl aluminum hydride (DIBAL-H). Condensation of the aldehyde **74a** with the indoloazepine **60**,[14] subsequent benzylation, rearrangement, coupling to vindoline and reduction proceeded in the usual fashion (*i.e.* Scheme 1).[7] Cyclization of a C-21′ tosylate **81a** (R = Me) required five days at reflux in toluene to furnish, after debenzylation, the product **12** in the "natural" atropisomeric form.[16]

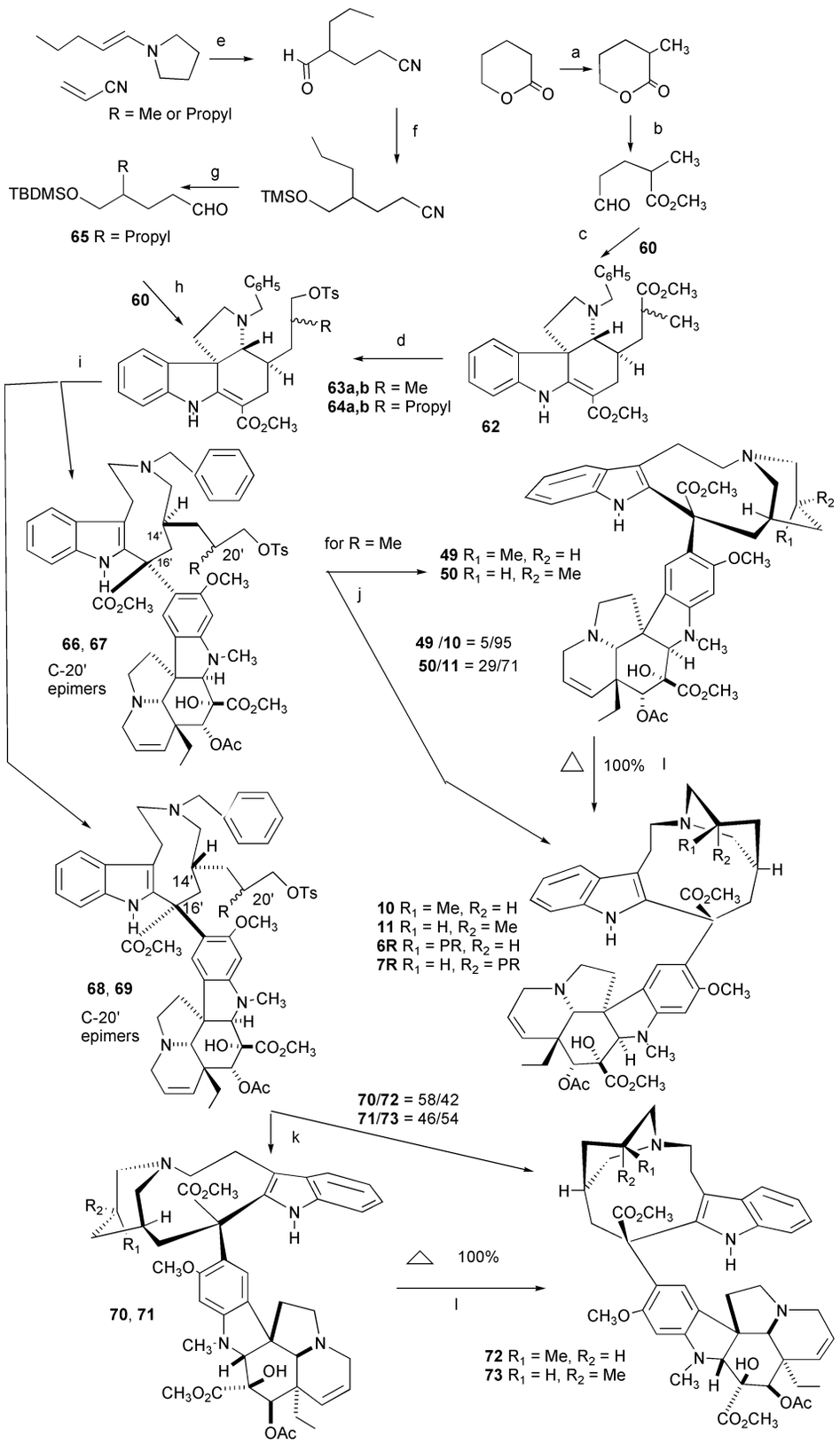
The C-20′ diethyl compound **13R** and the spiro five and six-membered ring congeners **21** and **22** were prepared analogously. Here, a higher energy atropisomer (**82**), was isolated and could be thermally converted to the lower energy atropisomer (**13R**,**21**,**22**) in refluxing toluene.

Syntheses of the seven and eight-membered ring D′ homologues **23** and **24** of 20′-deethyl-20′-deoxyvinblastine (**3**) were obtained, respectively, by condensation of 6-*tert*-butyldimethylsilyloxyhexanal and 7-*tert*-butyldimethylsilyloxy heptanal (**84**,**85**, Scheme 4) with the indoloazepines **60** or **61**, followed by the rearrangement, vindoline coupling and cyclization steps described for the lower homologue **3**.[4] Diastereomeric coupling products **86**,**87**, and **88**,**89**, were obtained in 1 : 1 ratios in the two homologous series. Again, atropisomeric products **90**,**92** and **91**,**93** were formed in the ring D′ cyclization and inversion of the higher energy atropisomers **90**–**93** to the lower energy atropisomers **23**,**94**, and **24**,**95** was found on heating. Conformational inversion of these D′ enlarged atropisomers to the lower energy atropisomeric form still required refluxing toluene, thus suggesting that the major barrier to this inversion is passage of the C-3′ methylene group through the cavity of the nine-membered ring C′, rather than a conformational change of ring D′.

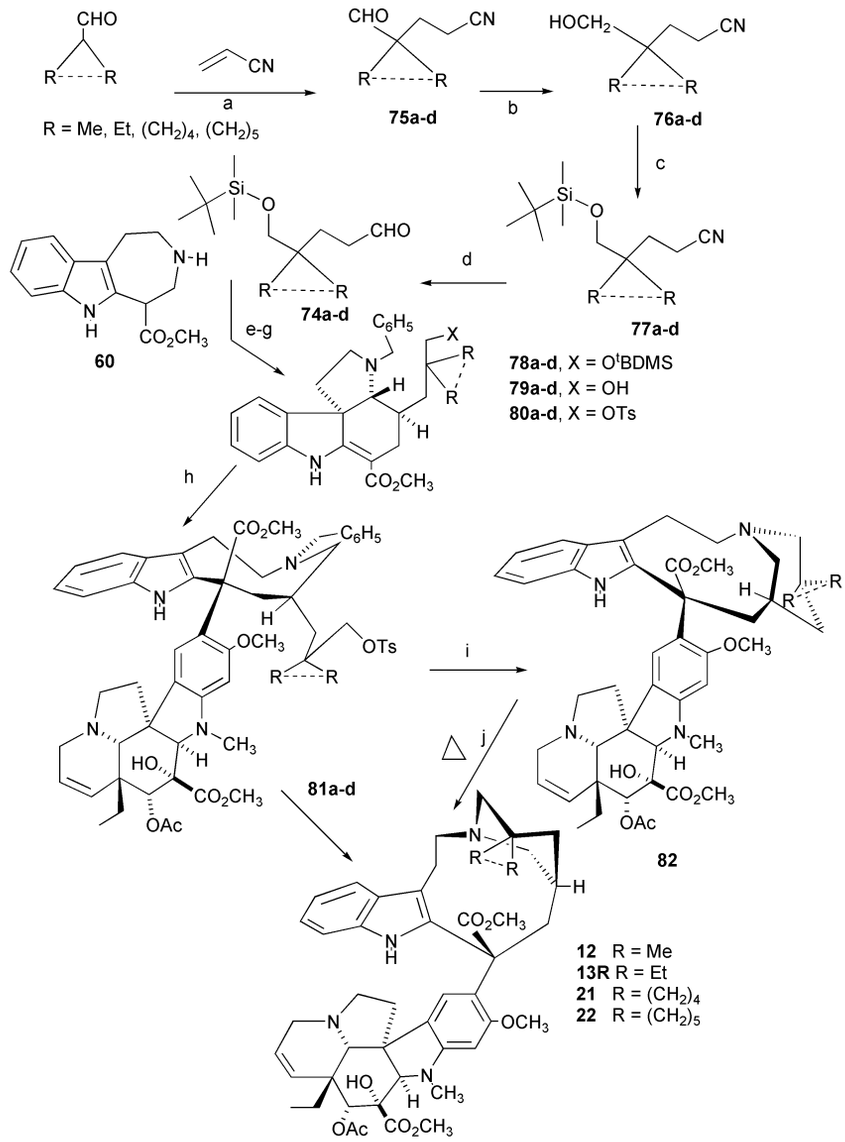
For syntheses of the C-15′, 20′ cyclopentyl *cis*-fused VLB congeners **16** and **17** (Scheme 6), the *cis*-2-[(acetoxy)methyl]-cyclopentaneacetaldehyde **96** was prepared from *cis*-bicyclo-[3.3.0]oct-6-ene-3-one (**97**,[17] Scheme 5). Hydrogenation of the double bond (100%) and Baeyer–Villiger oxidation (79%) afforded the lactone **98**. Its reduction with DIBAL-H (87%) provided the lactol product **99**. Attempts to condense this aldehyde equivalent with the indoloazepine **60** failed. Consequently, the lactol ring was opened by formation of the dithiolan alcohol **100** (79%). Formation of its acetate (95%) and liberation of the aldehyde function with mercuric acetate provided the aldehyde **96** (78%).

Condensation of the aldehyde **96** with the indoloazepine **60** gave the racemic acetates **101**,**102**. They were methanolized to the corresponding alcohols **103**,**104**, which were chromatographically separated and tosylated (**105**,**106**), and followed by our usual sequence for elaboration of D′-*seco*-binary alkaloids. Two sets of C-14′–15′ diastereomeric tosylates **107**,**108** and **109**,**110** were obtained (Scheme 6). Their difference in rate of cyclization allowed definition of their relative stereochemistry. The C-14′–15′ PREF,[18] 14′α–H and the C-14′–15′ PREF,14′β–H fused products **109**,**110** were assigned to the slower cyclization (4 h at reflux in THF) and their corresponding C-14′–15′ PARF diastereomers **107**,**108** to the faster cyclization (at room temperature and complete in <2 h in THF at reflux), based on generation of 1,3-diaxial repulsions of ring D′ substituents at C-14′, C-20′ and on $N^{b'}$ in generation of the quaternary salts corresponding to the former pair **109**,**110**. The absolute configuration at C-16′,14′,15′,20′ within each set of diastereomers follows from the NMR chemical shift of the vindolinyl ethyl group at δ 0.82 for the C-16′*S*, 14′*R* eventual debenzylation products **16** and **17** as contrasted with their diasteromers (*i.e.* **16** *vs.* **111**),[4] as well as from the potent VLB-type biological activity of products **16** and **17**, that is absent in the C-16′*R*, 14′*S* compounds. Atropisomers of these compounds (which are typically found to have characteristic very broad NMR signals in all such isolated VLB congeners,[1,3] see below), could not be detected in these syntheses.

The starting material for syntheses of the C-15′, 20′ *cis*-fused cyclohexyl VLB congeners **14** and **15**, the atropisomer **51** of the former and its diastereomer **59**, was 2-indanol (**112**, Scheme 5). Its hydrogenation over 5% Rh/C provided a diastereomeric mixture of alcohols (59%), which was oxidized to the ketone **113** (96%). A Baeyer–Villiger oxidation to the lactone **114** (95%) and its reduction with DIBAL-H provided a lactol product **115** (80%). Again, direct condensation of this product with the indoloazepine **60** could not be achieved. On derivatization with 1,2-ethanedithiol, the dithiolan alcohol **116** was formed



Scheme 2 *Reagents, conditions and yields:* (R = Me), (a) LDA, MeI, HMPA, −78 °C to −40 °C, 3 h (66–77%); (b) 1) MeOH, conc. H_2SO_4, 45 min; 2) PCC, NaOAc, CH_2Cl_2, 30 min (40%); (c) 1) **60**, MeOH, rt, 20 h; 2) $C_6H_5CH_2Br$, THF, refl. 2 d; 3) Et_3N, THF, refl. 10 h (85–90%); (d) 1) LAH, THF, 0 °C (55%); 2) Ts_2O, Et_3N, CH_2Cl_2, 0 °C to rt (62–71%); (i) 1) tBOCl, Et_3N, CH_2Cl_2, 0 °C; 2) vindoline·HCl, acetone, 0 °C, $AgBF_4$, HBF_4·Et_2O, 25 min; 3) KBH_4, HOAc, rt, 30 min (89%/72% R = Me); (j) 1) a: toluene, 110 °C, 17 h; b: toluene, 110 °C, 5 d; 2) 10% Pd/C, H_2, MeOH a: 81% **10**, 4% **49**; b: 61% **11**, 25% **50**; (k) 1) a: toluene, 110 °C, 20 h; b: toluene, 110 °C, 7 d; 2) 10% Pd/C, H_2, MeOH a: 37% **72**, 51% **70**; b: 41% **71**, 47% **73**); (l) toluene, refl. (100%) (R = *n*-propyl), (e) 1) dioxane, refl. 16 h; 2) H_2O, refl. 8 h, 3) aq. HCl 15 min (33%); (f) 1) $NaBH_4$, MeOH, 5 °C; 2) aq. HCl (96%); 3) tBDMSCl, CH_2Cl_2, 4 °C, DIPEA, 4) DMAP, rt, 66 h (83%); (g) DIBAL-H, toluene, −70 °C, 3 h (81%); (h) 1) **60**, MeOH, rt, 19 h; 2) $C_6H_5CH_2Br$, THF, rt, 19 h; 3) MeOH, DIPEA. refl. 3 h (82%); 4) KF (H_2O), CH_3CN, $BnEt_3NCl$, refl. 48 h, (82%); 5) TsCl, DMAP, PYD, rt, 26 h (77%); (i) 1) tBOCl, Et_3N, CH_2Cl_2, 0 °C; 2) vindoline·HCl, acetone, rt, $AgBF_4$, HBF_4·Et_2O, 80 min; 3) $NaBH_4$, HOAc, rt, 30 min (38% **66**, 42% **67**); (j + l) 1) xylene, 140 °C, 22 h; 2) 10% Pd/C, H_2, MeOH, 11 or 6.5 h; 3) tol. refl. 4 h (37% **6R**, 41% **7R**).

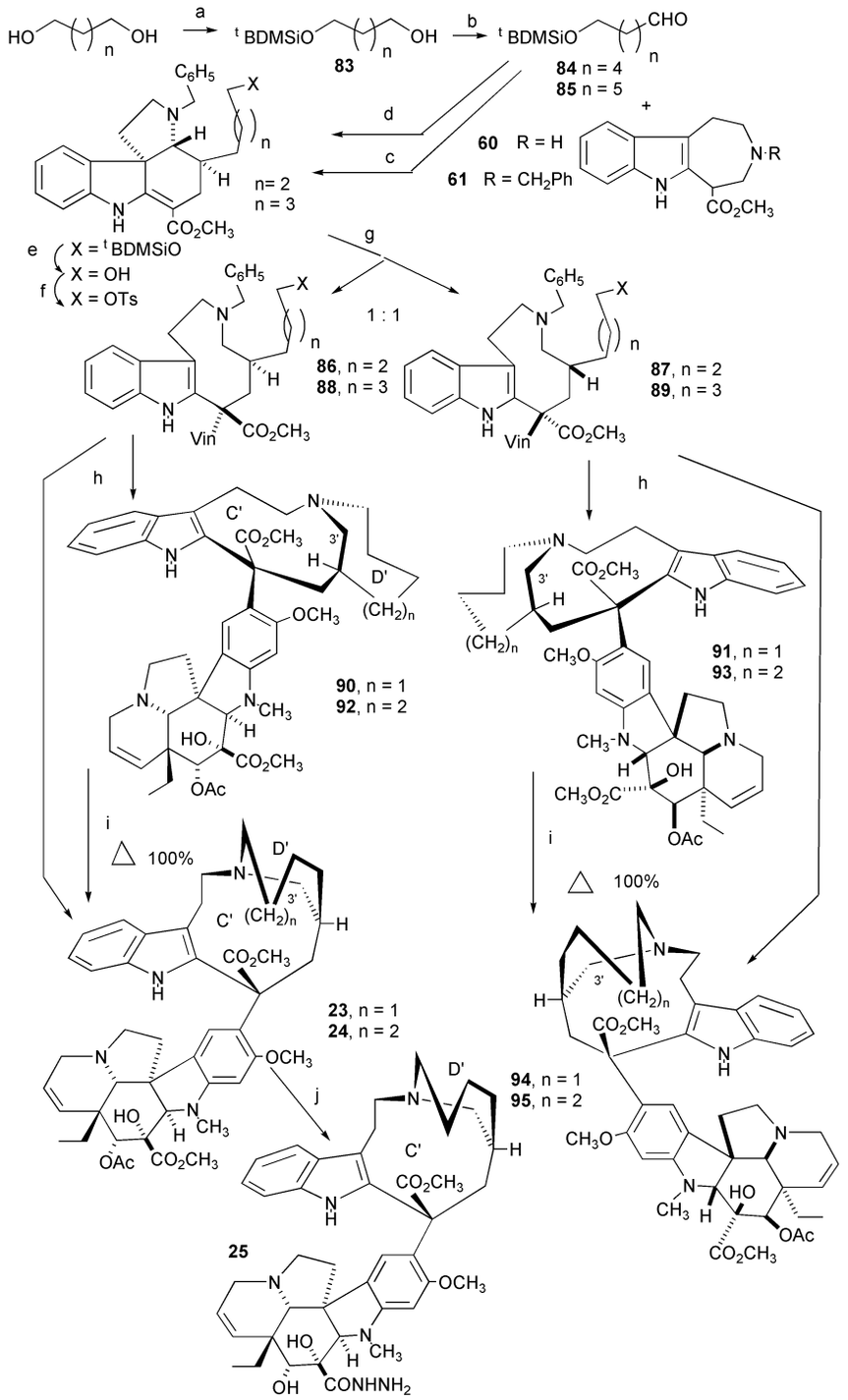


Scheme 3 *Reagents, conditions and yields:* (a) for **75**, ref. 24, R = Me, isobutyraldehyde, hydroquinone, acrylonitrile, dioxane, aq. NaOH, 65 °C, 150 min (49% yield); (b) for **76**, $NaBH_4$, MeOH, 1 h, 0 °C (R = Me, 95%; R = $(CH_2)_4$, 85%; R = $(CH_2)_5$, 98%); (c) for **77**, TBDMSCl, Et_3N or DIEA, CH_2Cl_2, DMAP, 0 °C to 20 °C, 16 h (R = Me, 69%; R = $(CH_2)_4$, 92%; R = $(CH_2)_5$, 75%); (d) 1) DBAL-H, CH_2Cl_2, −78 °C, 6 h; 2) 1 M HCl, 0 °C (**74**, R = Me, 79%; R = $(CH_2)_4$, 72%; R = $(CH_2)_5$, 70%); (e) 1) **60**, THF or MeOH or CH_2Cl_2, rt, 24 h; 2) $C_6H_5CH_2Br$, THF, refl. 24 h; 3) MeOH, Et_3N, refl. 6 d (**78**, R = Me, 70%; R = Et, 88%; R = $(CH_2)_4$, 98%; R = $(CH_2)_5$, 99%); (f) TsOH or tBAF, THF, rt, 2 h (**79**, R = Me, 98%; R = Et, 86% (made with 48% aq. HF); R = $(CH_2)_4$, 91%; R = $(CH_2)_5$, 92%); (g) Ts_2O or TsCl, Et_3N, DMAP, CH_2Cl_2, 0 °C to rt, 2.5 h (**80**, R = Me, 72%; R = Et, 96%; R = $(CH_2)_4$, 94%; R = $(CH_2)_5$, 95%; (h) 1) tBOCl, Et_3N, CH_2Cl_2, 0 °C 15 min; 2) vindoline·HCl, acetone, 0 °C, $AgBF_4$, $HBF_4{\cdot}Et_2O$, 30 min; 3) KBH_4, HOAc, rt, 20 min (**81**, R = Me, two diastereomers, 75%; R = Et, 47%, R = $(CH_2)_5$, 34%, its 14′*R*,16′*R* diastereomer 53%; (i) 1) toluene, refl. R = Me, 5 days, R = $(CH_2)_4$, 4 h for 14′*S*,16′*S* series; refl 13 h for R = Me, 14′*R*,16′*R* series; 2) 10% Pd/C, H_2, MeOH; 3) toluene, reflux 6 h (12, 29%; its 14′*R*,16′*R* epimer, 29%; **13R**, 45%; **22**, 82%; its 14′*R*, 16′*R* diastereomer, 85%).

(64%). Acetylation of the hydroxyl group (98%) and liberation of the aldehyde function with HBF_4in aqueous THF provided the aldehyde **117** (95%). After generation of two racemic cyclohexane C-15–20 *cis*-fused D-*seco*-ψ-vincadifformine congeners **118**,**119** (Scheme 7), chromatographic separation of diastereomers and formation of C-21 tosylates, coupling to vindoline and reduction with potassium borohydride, two sets of diastereomeric intermediates **124**,**125** and **126**,**127** were obtained. Their respective cyclizations required 22 h *vs.* 3 h in refluxing toluene. After debenzylation and chromatography, pure samples of the VLB congeners **14** and **15**, the atropisomer **51** of **14** and its C-16′,14′,15′,20′-*epi*-diastereomer (**59**) were obtained.

Synthesis of the benzo analogue **18** of VLB was derived from Baeyer–Villiger oxidation of 2-indanone (**128**, Scheme 8). The resulting lactone **129** (90%), on DIBAL-H reduction, provided a lactol product **130**, which could be condensed directly with the indoloazepine **60**, in contrast to failure of such condensations with the lactols **99** and **115**. (Lactol ring stabilization by ring fusion in lactols **99** and **115**, in contrast to the unsubstituted δ-lactol,[3] is cancelled by introduction of two trigonal centers in the benzolactol **130**). After *N*-benzylation and rearrangement (50% overall, the alcohol function in the D-*seco*-ψ-vincadifformine congener **131** was protected with a *tert*-butyldimethylsilyl group (**132**, 93%) before coupling to vindoline. The eventual diastereomeric products **133** (41%) and **134** (47%) were desilylated with *p*-toluenesulfonic acid in aqueous THF (88%) and the resulting alcohols **135**,**136** cyclized by reaction with *p*-toluenesulfonic anhydride to give, after debenzylation (52% overall), the diastereomeric benzo VLB congeners **18** and **137**.

For syntheses of the tetrahydropyran fused congeners **19** and **20**, tetrahydropyran-4-one was subjected to a Horner–Emmons condensation, followed by ester hydrolysis. The resulting mixture of conjugated and unconjugated acids **138** and **139** (17 : 83) was condensed with formaldehyde in acetic acid and

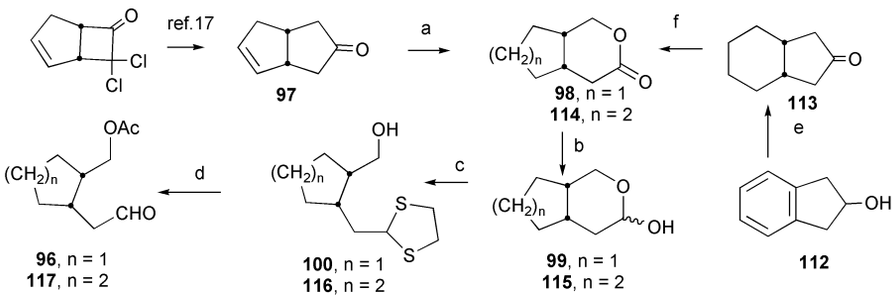


Scheme 4 *Reagents, conditions and yields are for 7-membered ring products* **23** and **94**; products **24** and **95** are made analogously: (a) tBDMSiCl, Et_3N, DMAP, 54%; (b) Swern oxidation, 94%; (c) **61**, neat, 130 °C; (d) 1) **60**, MeOH; 2) $PhCH_2Br$; 3) Et_3N, MeOH, refl. (e) HF, aq. CH_3CN, c + e 97%; (f) Ts_2O, Et_3N, DMAP, 98%; (g) 1) tBuOCl, Et_3N; 2) vindoline, $AgBF_4$; 3) KBH_4, AcOH, 80%, 1 : 1 **86** and **87**; (h) 1) sealed tube, CH_3CN, 110 °C; 2) Pd/C, H_2, 67% **90**, 12% **23**; (i) toluene, 110 °C, >98%; (h,i) 1) toluene, reflux; 2) Pd/C, H_2; 3) toluene reflux, 84%; (j) hydrazine, MeOH, reflux, 94%.

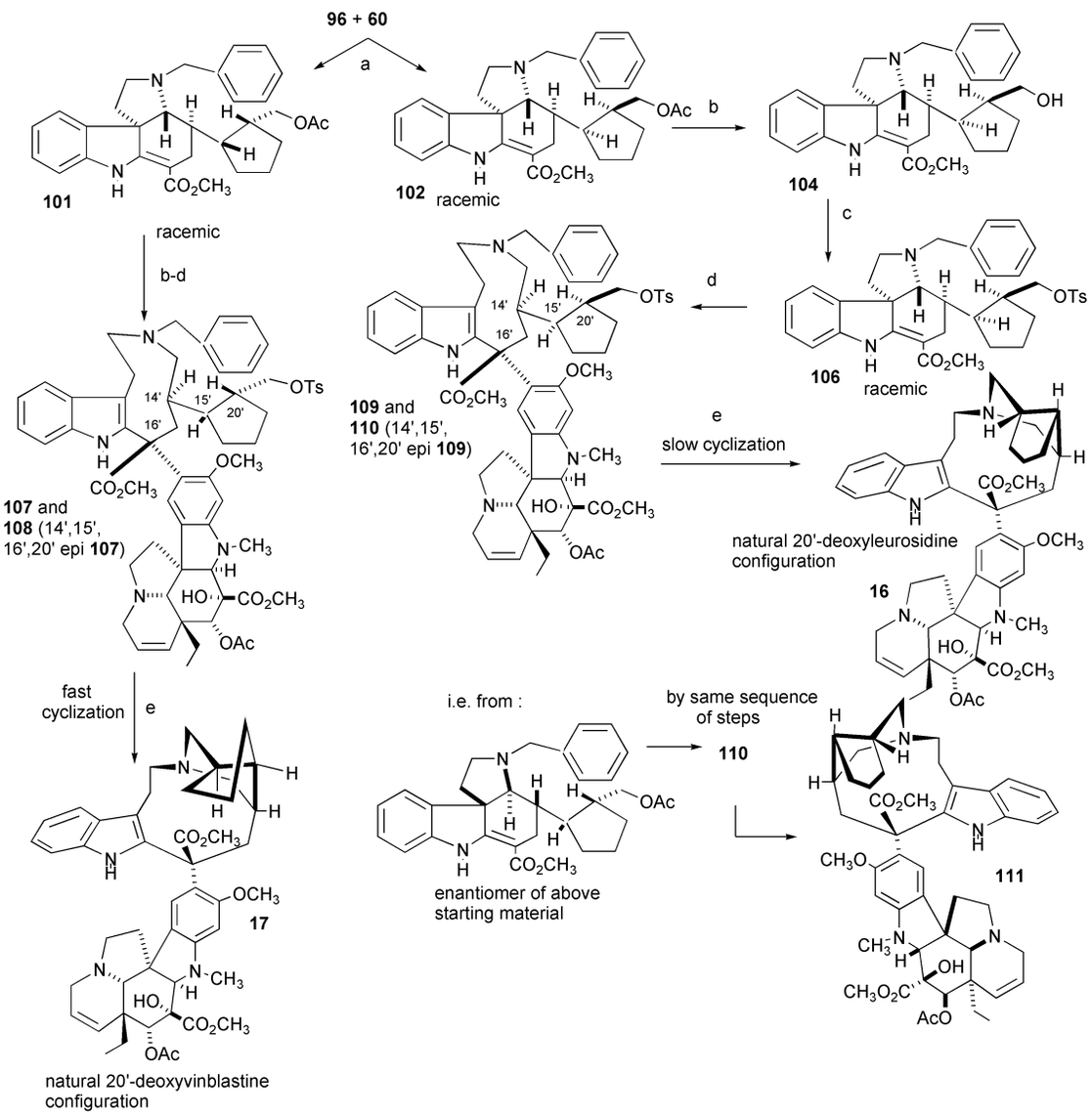
1.5% H_2SO_4 to give a 48% yield of the α,β–unsaturated lactone **140**. Its hydrogenation over Pt led to a 7 : 1 ratio of *cis–trans* fused lactones **141a**,**b** in 88% yield. A more favorable 14 : 1 ratio, but only an 18% yield, was obtained from hydrogenation of the β,γ–unsaturated lactone **142**, that could be generated by isomerization of the double bond with KOH and recyclization of the acid (57%). While its hydrogenation with Pt in ethyl acetate now produced an anticipated more favorable 14 : 1 ratio of *cis–trans* lactones **141a**,**b**, the major products of the reaction were derived from hydrogenolysis to form *cis*- and *trans*-3-methyltetrahydropyran-4-yl-acetic acids in a 9 :1 ratio. Complete hydrogenolysis was obtained, as expected, by hydrogenation with 10% Pd/C in ethanol (see Table 8).

A reduction of the 7 : 1 lactone mixture **141a**,**b** with DIBAL-H provided the corresponding lactols **143a**,**b**. In order to liberate a free aldehyde function, desired for optimum condensation with an *N*-alkylindoloazepine, the lactols **143a**,**b** were opened with ethanedithiol, and the resulting thioacetal alcohols **144a**,**b** were then converted to their acetate derivatives **145a**,**b**. Thioacetal cleavage with mercuric oxide then provided the aldehydes **146a**,**b** (7 : 1 *cis–trans*).

Condensation of the aldehydes **146a**,**b** with the indoloazepine **147**[19] bearing a chiral ferrocenyl N^b-substituent, diastereoselectively generated the tetracyclic amines **148**,**149** (54%), which were separated chromatographically from their minor diastereomers **150**,**151** (9%) The major diastereomers



Scheme 5 *Reagents, conditions and yields:* (a) 1) 10% Pd/C, H_2, EtOAc, 25 psi, 90 min (100%); 2) MCPBA, $NaHCO_3$, CH_2Cl_2, 20 °C, 24 h; 3) $Na_2S_2O_3$, 20 min (79% **98**); (b) DIBAL-H, CH_2Cl_2, −78 °C to 0 °C, 1 h (87% **99**, 80% **115**); (c) $(CH_2)_2(SH)_2$, 12 M HCl, 0 °C to 20 °C (79% **100**, 64% **116**); (d) 1) AcCl, Pyd. CH_2Cl_2, 0 °C to 20 °C, 0.5 h; (95%, 98%); 2) HgO, THF, HBF_4, 5 min (78% **96**, 94% **117**); (e) 1) 5% Rh/C, H_2, MeOH, 20 psi, 20 °C (59%); 2) PCC, CH_2Cl_2, 2 h, 20 °C (96%); (f) 1) MCPBA, CH_2Cl_2, $NaHCO_3$, 20 °C, 17 h; 2) $Na_2S_2O_3$,15 min (95% **114**).

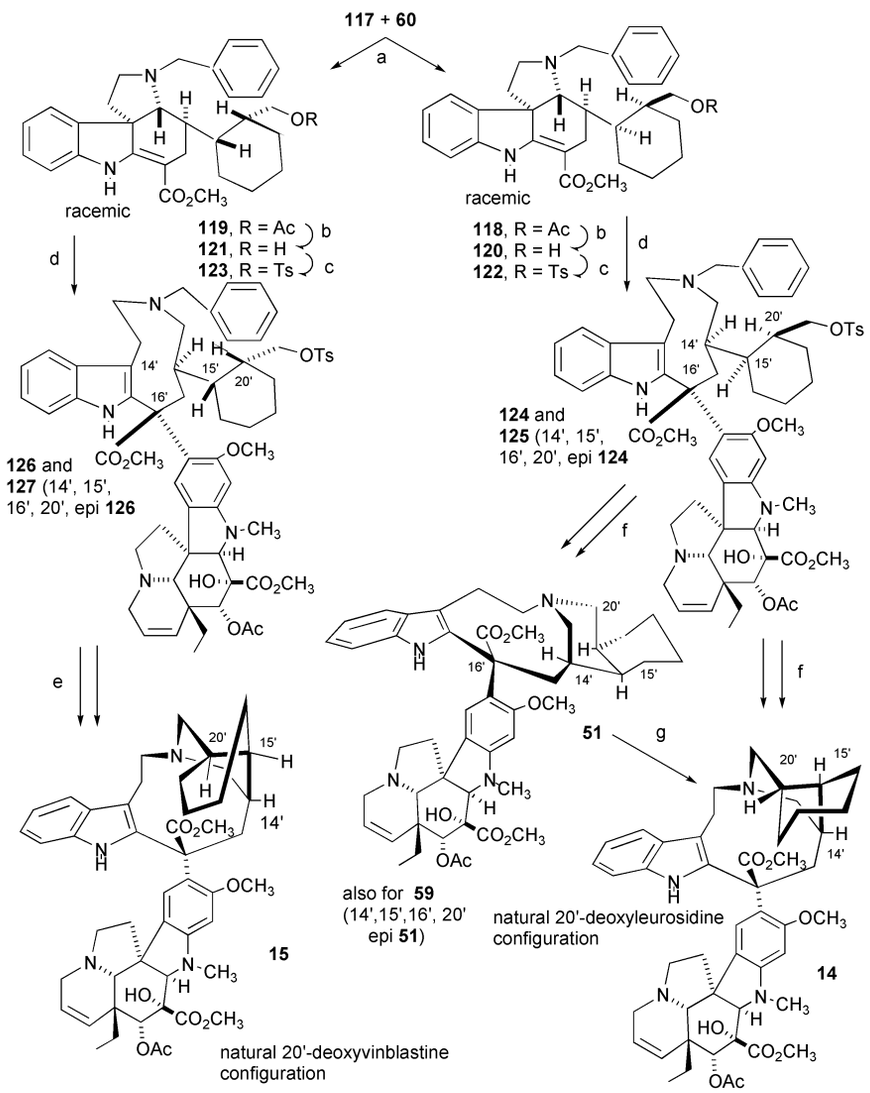


Scheme 6 a) 1) CH_2Cl_2, 20 °C, 18 h; 2) $CHCl_3$, $C_6H_5CH_2Br$, refl. 4 h; 3) MeOH, Et_3N, refl. 18 h (84% **101**, **102**, 1 : 1); (b) K_2CO_3, MeOH, H_2O, refl. 0.5 h, chromatographic separation (52% **103**, 46% **104**); (c) Ts_2O, CH_2Cl_2, Et_3N, 0 °C to 20 °C, 18 h (91% **105**, 74% **106**); (d) 1) tBOCl, Et_3N, 2) vindoline, $AgBF_4$, $HBF_4{\cdot}Et_2O$; 3) KBH_4, HOAc (48% **107** + **108**, including their quaternary salt cyclization products); 67% **109** + **110**); (e) 1) THF, refl. 2 h; 2) 10% Pd/C, H_2, MeOH, 2 h (39% **17**, 37% 14′,15′,16′,20′ ***epi*-17**, 35% **16**, 36% **111**).

were then subjected to replacement of the ferrocenyl N^b-substituent by a benzyl group (**152**,**153**, 36%, 2 steps), and to acetate methanolysis. Chromatographic separation of the diastereomeric alcohols **154**,**155** and their tosylation (**156**,**157**, 82%, 2 steps) was followed by our usual coupling sequence with vindoline. The indoloazonines **158** and **159** were then cyclized at 110 °C. Hydrogenolysis of the quaternary N^b-benzyl substituent provided the tetrahydropyran derivatives **19** and **20** (22% and 24% respectively, 5 steps). Here the final cyclization required more strenuous conditions than those used with the corresponding cyclohexyl substituted indoloazonines **124**–**127**. Consequently, debenzylation of the resulting pentacyclic quaternary salts gave only 5% of the higher energy atropisomer **160a** in addition to the lower energy conformer **160b** of product **20**.

Table 8 Hydrogenation of unsaturated lactones **140** and **142**. Product a: 3-methyltetrahydropyran-4-yl-acetic acid. Product b: ethyl 3-hydroxy-methyltetrahydropyran-4-yl-acetate. All reactions at 1 atm H_2

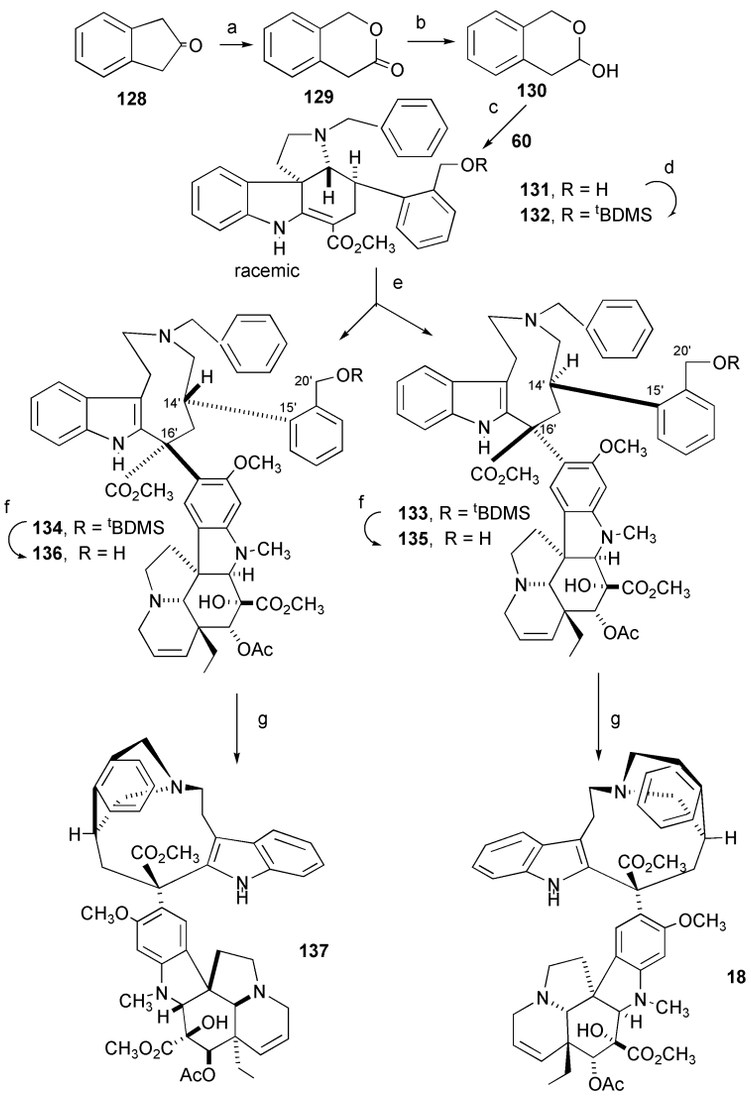
			Product yield (the ratio of *cis* to *trans*)		
Lactone	Catalyst	Solvent	**141a/141b**	a	b
140	10% Pd/C	EtOH	12%, 9:1	3.8%, 4:1	62.3%, 3.6:1
140	10% Pd/C	AcOEt	84.6%, 4.5:1		
140	10% Pd/C	AcOH	74.0%, 3.5:1		
140	PtO_2	AcOEt	88.3%, 7.2:1		
140	PtO_2	THF	87.1%, 6.9:1		
140	PtO_2	t-BuOH	68.3%, 7.2:1		
142	10% Pd/C	EtOH		77.9%, 3.6:1	
142	PtO_2	AcOEt	18.2%, 14.3:1	48.3%, 8.5:1	
140 or 142	Pd/C or PtO_2	CCl_4	No reaction		



Scheme 7 (a) 1) CH_2Cl_2, 20 °C, 16 h (95%); 2) CH_2Cl_2, $C_6H_5CH_2Br$, refl. 3 h; 3) MeOH, Et_3N, refl. 4 h (90% **118**, **119**, 1 : 1); (b) NaOMe, MeOH, rt, 3 h, chromatographic separation (**120**, 41% **121**, 47%); (c) Ts_2O, CH_2Cl_2, Et_3N, DMAP, 0 °C, 48 h (86% **122**, 82% **123**); (d) 1) tBOCl, Et_3N, CH_2Cl_2, 0 °C; 2) vindoline·HCl, acetone, 0 °C, $AgBF_4$, HBF_4·Et_2O, 30 min; 3) KBH_4, HOAc, rt, 35 min; (e) 1) toluene, reflux 3 h; 2) 10%Pd/C, H_2, MeOH, 3) toluene, reflux, 8 h, (d + e 20% **15**, 20% 14′,15′,16′, 20′-***epi*-15**); (f) 1) toluene, refl. 22 h; 2) 10%Pd/C, H_2, MeOH (d + f 27% **14**, 23% 14′,15′,16′, 20′-***epi*-14**); (g) toluene, reflux, 8 h.

No conformational high energy isomer could be isolated from cyclization of the diastereomeric tosylate **158**, where only the lower energy atropisomer **19** was obtained in analogy to the cyclohexane fused congener **15**.

The methyl analogues **27** and **28** of vinblastine (**1**) and leurosidine (**29**), their C-14′,16′ epimeric diastereomers (*i.e.* **161**) and corresponding atropisomers of these compounds (*i.e.* **162a**,**b**,) were obtained by the procedures used for syntheses of the parent C-20′ ethyl compounds.[1,2] Enantioselectivity at C-20′ was achieved by use of the chiral aldehydes **167**,**168** on condensation with the *N*-benzyl indoloazepine **61** (Scheme 10). The use of trimethylsilyl protected alcohols was found to be preferable for synthesis of the vinblastine homologue **27** and the homologue **161** of 20′-*epi*-vincovaline because of a cleaner final cleavage of the silyl ether function in the final reaction step. However, the *tert*-butyldimethylsilyl derivatives



Scheme 8 (a) 1) MCPBA, $NaHCO_3$, CH_2Cl_2, 20 °C; 2) $Na_2S_2O_3$ (90%); (b) DIBAL-H, CH_2Cl_2, −78 °C, 2 h, 0 °C, 15 min (91%); (c) 1) CH_2Cl_2, $B(OH)_3$, rt, 3 d; 2) $C_6H_5CH_2Br$, $CHCl_3$, 8 h refl. 3) Et_3N, MeOH, 10 h refl. (50%); (d) tBDMSCl, Et_3N, CH_2Cl_2, rt, 5 min (93%); (e) 1) tBOCl, Et_3N, CH_2Cl_2, 0 °C, 5 min; 2) vindoline·HCl, acetone, 0 °C, $AgBF_4$, $HBF_4{\cdot}Et_2O$, 30 min; 3) KBH_4, HOAc, rt, 35 min (41% **133**, 47% **134**); (f) TsOH, THF, H_2O, 20 °C, 45 min (88% **135**, 89% **136**); (g) 1) Ts_2O, Et_3N, CH_2Cl_2, 20 °C, 35 min; 2) 10% Pd/C, H_2, MeOH, 20 °C, 30 min (52% **18**).

could be used in the C-20′ epimeric series for synthesis of the homologue **28** of leurosidine (**29**). Coupling of the tetracyclic intermediates **169a**,**b** and **170a**,**b** with vindoline, and reduction, provided the binary tosylates **171a**,**b** and **172a**,**b**.[1,2] On treatment with tetrabutylammonium fluoride these furnished the epoxides **173a**,**b**. Their cyclization and debenzylation gave a preponderance of the higher energy atropisomeric products **162a**,**b** over the lower energy conformers **27** and **161**. Heating in toluene at reflux resulted in complete transformation to the latter products.

Alternatively, the C-21′ tosylates **171a**,**b** and **172a**,**b** cyclized on heating in methanol to give the quaternary salts **163a**,**b** and **164a**,**b**. Their debenzylation provided the silyl ethers **165a**,**b** and **166a**,**b**. A final cleavage of the trimethylsilyl ether **165a** provided the 20′-methyl homologue **27** of vinblastine in its lower energy conformation. The TBDMS ether **166a** required more forcing conditions for cleavage, thus resulting in partial decomposition.

The ring A′ methoxy and halogen subtituted compounds **30**–**35** were obtained from the corresponding indoloazepines.[20] Noteworthy was the formation of a substantial amount of C-16′–14′ PREF products in the vindoline coupling reaction of the C-11 methoxylated D-*seco*-ψ-vincadifformines. This decreased C-16′–14′ PARF selectivity is in agreement with the reaction mechanism postulated for these coupling reactions,[4,7] which is based on a carbocation intermediate. On stabilization by a C-11 methoxy substituent this cation has an increased lifetime that allows instability of its initial conformation.

Compounds in which the vindoline moiety is oxidized to the vincristine series, **37**–**41**, or are converted to tryptophan derivatives, **42**–**44**, and the acid **45**, were obtained by the methodologies known for such transformations.[21–23]

Acknowledgements

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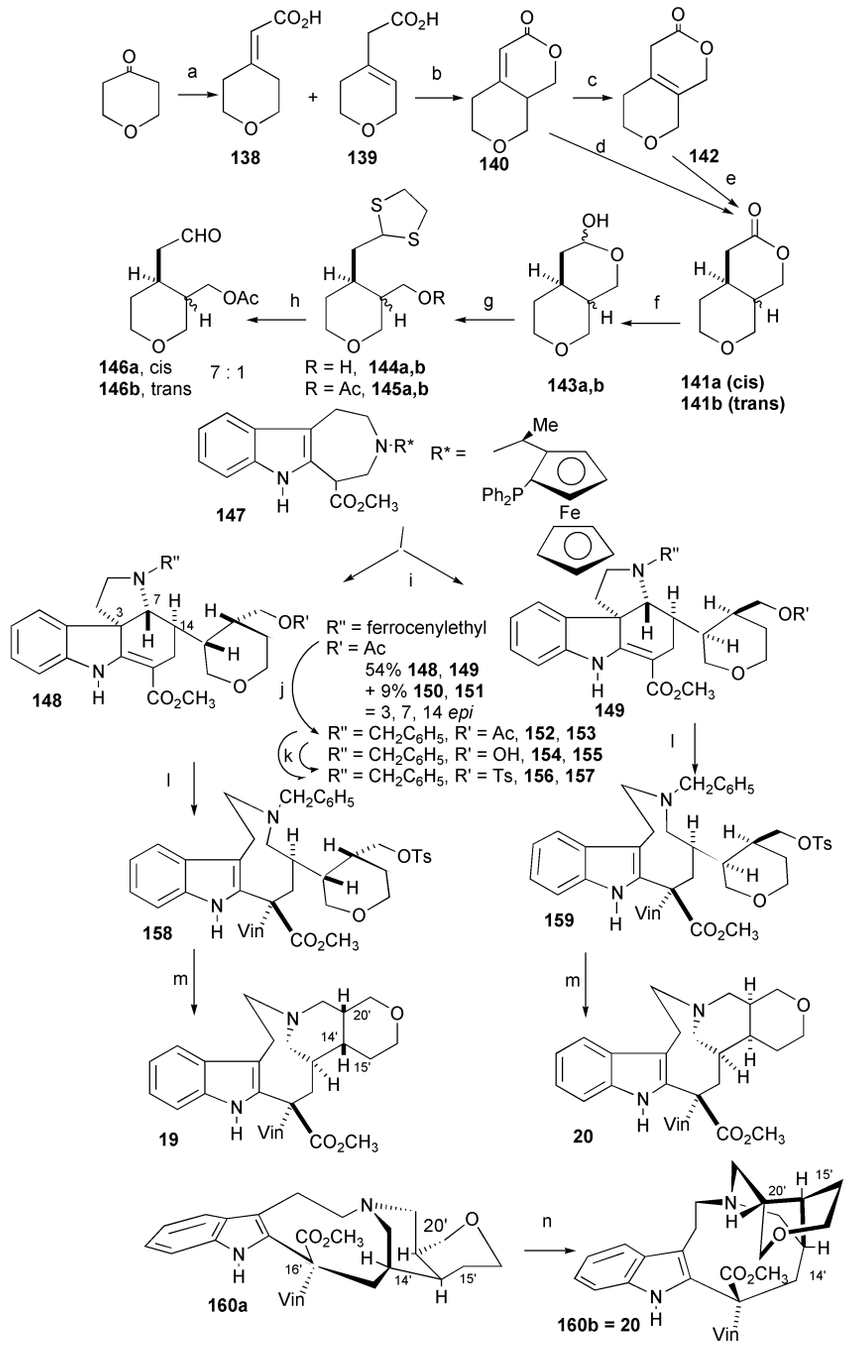
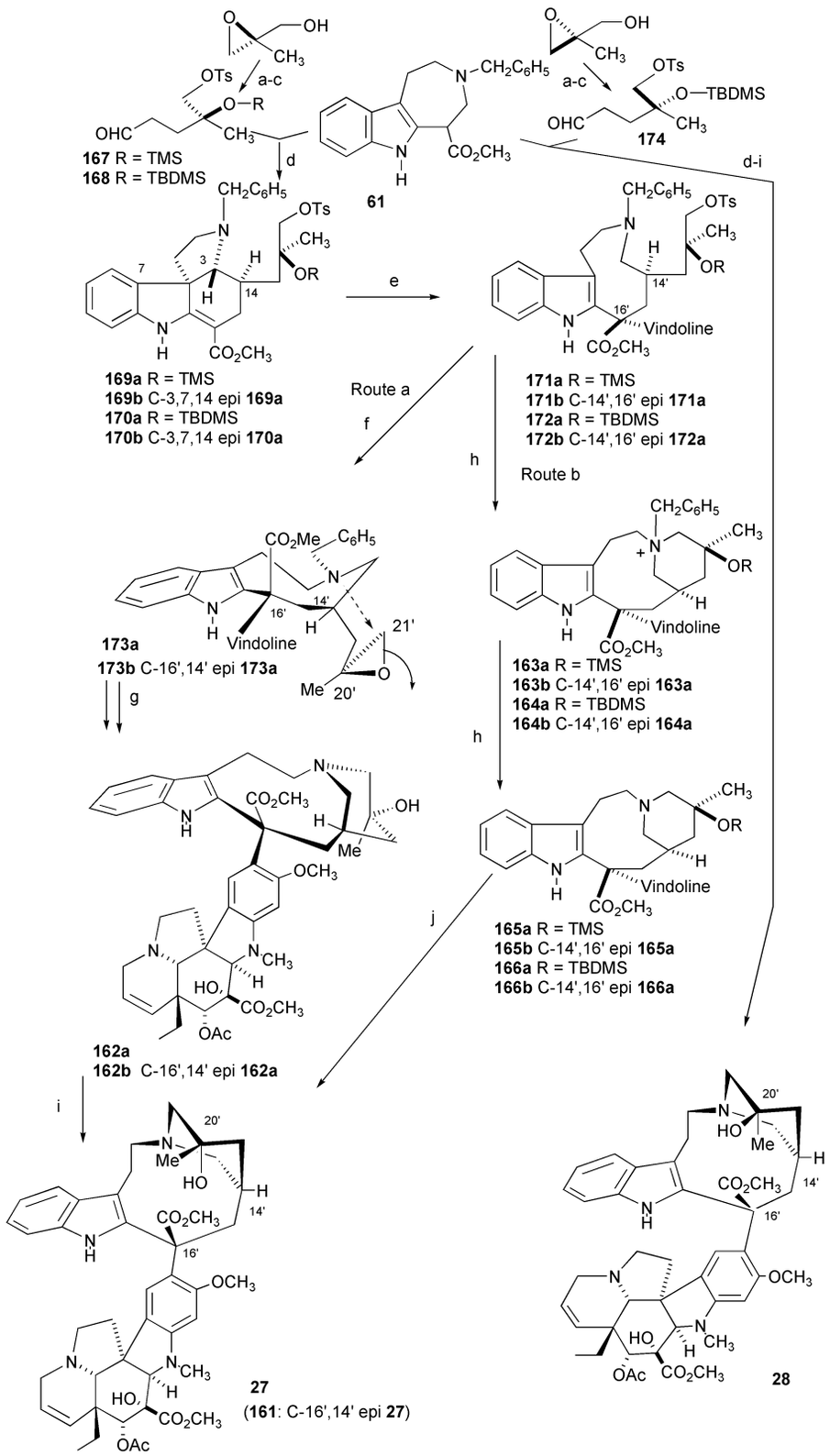


Scheme 9 *Reagents and conditions*: (a) 1) $Ph_3P{=}CHCO_2Me$, toluene, 20% $PhCO_2H$, refl. 8 h, 96%; 2) 50% KOH, EtOH, refl. 20 h; 3) dil. HCl, 94% (**138** : **139** = 17 : 83); (b) (CH_2O), AcOH, 1.5% conc. H_2SO_4, refl. 10 h, 48%; (c) 1) 5% KOH–EtOH, refl. 25 h; 2) dil. HCl, 53%; (d, e) see Table 8; (f) DIBAL-H, CH_2Cl_2, −78 to 0 °C, 77%; (g) 1) 1,2-ethanedithiol, conc. HCl, 60%; 2) AcCl, pyridine, CH_2Cl_2, 99%; (h) 48% HBF_4, THF–H_2O, 84%. (i) R'' = ferrocenylethyl, C_6H_6, 36 h refl. 63%; R'' = Bn, toluene, 110 °C 70%; (j) 1) AcOH, 70 °C; 2) $PhCH_2Br$, Et_3N, K_2CO_3, 36%; (k) 1) NaOMe, MeOH refl. chromatographic separation of diastereomers **154**, **155**; 2) Ts_2O, DMAP, 84% **156**, 82% **157**; (l) 1) tBuOCl, Et_3N; 2) vindoline, HBF_4, $AgBF_4$; 3) KBH_4; (m) 1) 110 °C, CH_3CN, sealed tube; 2) Pd/C, H_2, 22% **19** from **154**, 24% **20** from **155**; (n) toluene, 110 °C.



Scheme 10 (a) CuI, CH_2=$CHCH_2MgCl$, THF, −60 °C to −20 °C (66%); (b) Et_3N, DMAP, TsCl, CH_2Cl_2, 20 h, rt (88%); (c) 1) TMSCl, DIEA, THF, 30 min (86%); TBDMSTf (95%); 2) O_3, CH_2Cl_2, −78 °C, 10 min; 3) $(C_6H_5)_3P$, −78 °C to rt, 18 h (81%, 74%); (d) toluene, refl. 15 h (57%, 65%); (e) 1) tBOCl, Et_3N, CH_2Cl_2, 0 °C; 2) vindoline, acetone, 0 °C, 15 min, $AgBF_4$, $HBF_4.Et_2O$, 15 min; 3) KBH_4, HOAc, rt, 30 min (58%); (f) TBAF, THF, rt, 15 min (85%, 73% **173a**, 91%, 77% **173b**); (g) 1) MeOH, 1.5 eq. HOAc, refl. 12 h; 2) 10% Pd/C, H_2, MeOH, 3 h (52% **162a**, 30% **27**); 46% **162b**, 17% **161**; (h) 1) **171a**, MeOH, refl. 36 h, 2) 10% Pd/C, H_2, MeOH, 2.5 h (67% **165a**); 1) **172a**/**172b**, MeOH, 120 °C, 19/29 h; 2) Pd/C, H_2, MeOH, 72% **166a**, 63% **166b**; 3) TBAF, THF, 45 min (76% **27**); (i) toluene, refl. 6 h (100%); (j) TBAF, THF, 1.5 h refl. Analogous conditions for transformations from aldehyde **174** to product **28**.

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