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SYNTHESIS AND ANTIANGIOGENIC ACTIVITY OF NEW CARBOHYDRATE DERIVATIVES

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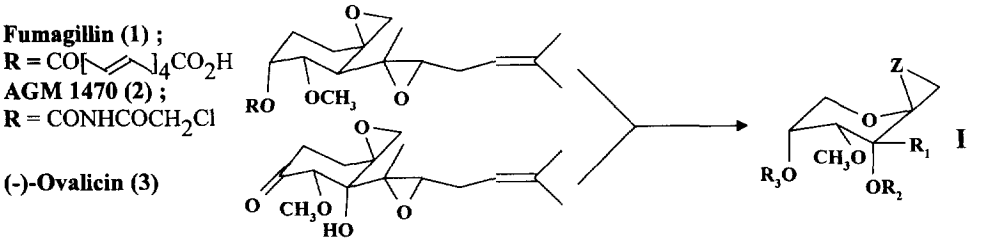
Abstract: Carbohydrate based analogues of the natural product (-)-Ovalicin have been synthesized, and shown to be potent inhibitors of angiogenesis. Copyright © 1996 Elsevier Science Ltd

Introduction.

Angiogenesis, the process of new capillary blood vessels formation is a characteristic of a number of important physiological (embryogenesis[2], ovulation[3], wound repair[4]) as well as pathological (diabetic retinopathy[5], rheumatoid arthritis[5], cancerous growth[6]) events. Consequently, inhibition of angiogenesis has been proposed as a potential approach toward the discovery of novel anti-tumor agents[7]. In 1990 fumagillin **1** was reported to have potent anti-angiogenic activity,[8] and recently its semi-synthetic analogue, TNP-470 (AGM-1470) **2** has entered a phase I clinical study in patients with advanced cervix cancer[9]. (-)-Ovalicin **3**, a natural product[10] very similar in structure to Fumagillin **1**, has been shown to possess interesting antibiotic, anti-tumor and immunosupressive activities[11]. Apart from the total synthesis of **3** in racemic[12] and enantioselective[13] form and the total synthesis of racemic **1**[14], only a few derivatives of these molecules have been so far described[15]. In order to investigate the structural requirements for their antiangiogenic properties we have envisaged the synthesis of pyranyl derivatives which are hybrids of TNP-470 **2** and Ovalicin **3** (Figure 1) giving, as such, structures related to **I**.

Figure 1



Our retrosynthetic analysis (Scheme 1) suggested that D-Fructose as starting material will meet the stereochemical requirements of target structures such as **I**. As shown, D-Fructose possesses four contiguous

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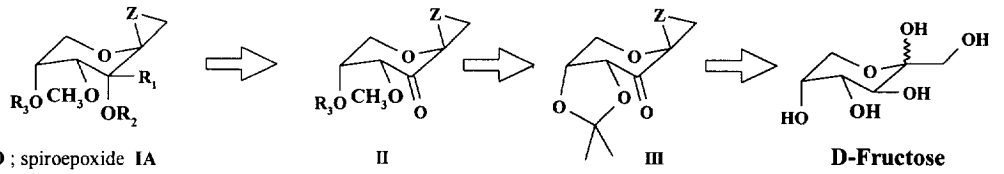
chiral centers ideally located to built a tetrahydropyran structure with the absolute configuration of TNP-470 **2** and/or (-)-Ovalicin **3**. Moreover, this strategy offers the possibility to introduce a spiroepoxide ring on the anomeric center (i.e., Z = O) mimicking the parent Ovalicin structure but also different spirocycles (i.e., Z = $OC(Me)_2O$ or OCO_2) capable of keeping a rigid conformation to the target molecules (i.e., OR_3 axial as seen in TNP-470). Following the above strategy we have been able to synthesize three distinct families (e.g., **IA**, **IB** and **IC**) of new psicopyranoside derivatives bearing different types of C-3 lipophilic side chain.

Scheme 1



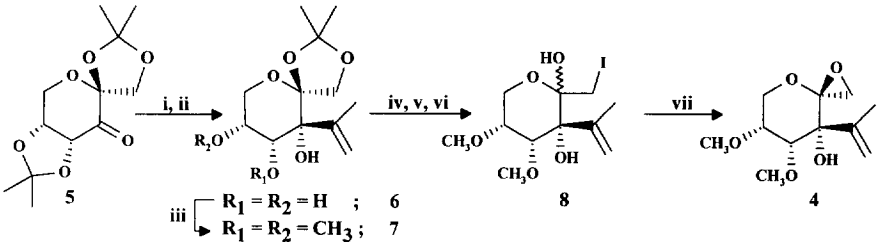
Z = O ; spiroepoxide **IA**
Z = $OC(Me)_2O$; spiroacetonide **IB**
Z = OCO_2 ; spirocarbonate **IC**

Chemistry.

The Spiroepoxide series IA,

Although anomeric spiroepoxides derived from fructopyranose have been described by Campbell[16], the stability of such a reactive function needed first to be assessed through the synthesis of simple analogues. In this aim, spiro anomeric epoxide **4** bearing a stable 5-OCH_3 substituant and lacking the C-3 epoxy lipophilic side chain was prepared as shown (Scheme 2).

Scheme 2



i. 2-Bromopropene, tert-BuLi, Et_2O, Toluene, -78°C, 50% ; ii. AcOH, H_2O, rt, 98% ; iii. NaH, CH_3I, DMF, rt, 59% ; iv. acidic resin, H_2O, 60°C, 70% ; v. TsCl, pyridine, rt, 74%; vi. NaI, acetone, rt, 70% ; vii. Ag_2O, dioxane, rt, 85%.

Thus treatment of the known ketone **5**[17] with 2-lithiopropene at -78°C followed by selective acidic hydrolysis of the 4,5-acetonide function gave stereoselectively diastereomer **6**. The resulting triol **6** was selectively methylated on the two secondary hydroxyl groups, to afford **7**. Hydrolysis of the 1,2-spiroacetonide function of **7** yielded the desired triol (mixture of α/β anomers) which upon treatment with tosyl chloride and sodium iodide in acetone gave iodohydrin **8**, precursor of anomeric spiroepoxide **4**.

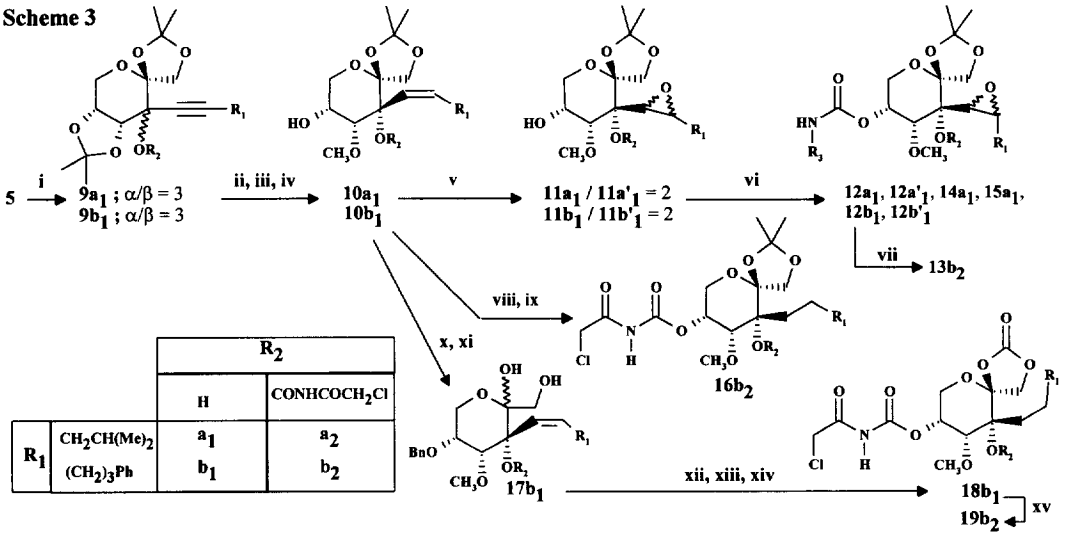
In order to complete the last step we used the very mild conditions previously described by Campbell et al.[18] employing silver(I) oxide at room temperature.

Although spiroepoxide **4** was shown by ^{1}H-NMR to possess the desired anomeric β stereochemistry, the rather unstable nature of the anomeric spiroepoxide function, encouraged us not to continue in the preparation of Ovalicin-like dioxa-spiro[2,5]octanes. Consequently, we made the choice to focus our efforts on the second and third series (type **IB** and **IC**; Scheme 1) in which the anomeric center has been locked into an acetonide or a carbonate ring giving rise to a rigid trioxa-spiro[4.5]decane structure.

The Spiroacetonide IB and Spirocarbonate IC series,

With the anomeric center secured onto an isopropylidene or a carbonate ring our work became more predictable and several structures related to **IB** and **IC** were prepared following the synthetic routes depicted below (Scheme 3 and 4).

Scheme 3



i. H—≡—R_1 , nBuLi, THF, -78°C, **$9a_1$** (89%) and **$9b_1$** (81%) ; **ii.** H_2, Lindlar, C_6H_6, rt, **a_1** and **b_1** (85%) ; **iii.** AcOH, H_2O, rt, **a_1** and **b_1** (90%) ; **iv.** Bu_2SnO, MeOH, rfx. then CH_3I, dioxane, rfx., **$10a_1$** (65%) and **$10b_1$** (73%) ; **v.** mCPBA, $NaHCO_3$, CH_2Cl_2, rt, **$11a_1$** + **$11a'_1$** (75%) and **$11b_1$** + **$11b'_1$** (72%) ; **vi.** R_3NCO, CH_2Cl_2, 0°C, $R_3 = COCH_2Cl$, **$12a_1$** (87%) and **$12a'_1$** (90%) ; **$12b_1$**, (83%) and **$12b'_1$** (64%) ; $R_3 = CO_2Et$, **$14a_1$** (95%) ; $R_3 = CH_2CO_2Et$, **$15a_1$** (83%) ; **vii.** $ClCH_2CONCO$, THF, rfx., **$13b_2$** (maj., 30%). **viii.** H_2, Pd/C, AcOEt, rt, **b_1** (97%) ; **ix.** $ClCH_2CONCO$, DMAP, CH_2Cl_2, 0°C, **$16b_2$** (82%) ; **x.** BnBr, NaH, NBu_4I, THF, rt, **b_1** (92%) ; **xi.** acidic resin, THF, H_2O, 70°C, **$17b_1$** (74%) ; **xii.** CDI, THF, CH_2Cl_2, rfx., **b_1** (68%) ; **xiii.** = **viii.**, **b_1** (92%) ; **xiv.** $ClCH_2CONCO$, CH_2Cl_2, 0°C, **$18b_1$** (50%) ; **xv.** $ClCH_2CONCO$, CH_2Cl_2, rt, **$19b_2$** (83%).

Stereoselective introduction of the C-3 lipophilic side chain proceeded efficiently with metallated alkynes, giving a 3:1 mixture of the readily separable α, β diastereomers **$9a_1$** and **$9b_1$**. The α-diastereomer was subjected

to catalytic hydrogenation over the Lindlar catalyst, to afford the corresponding *Z*-olefin. ^{1}H-NMR experiments confirmed both the *Z*-configuration of these olefins, and the relative stereochemistry of the desired α-isomer. After hydrolysis of the 4,5-acetonide ring, the resulting triols were selectively methylated. at their equatorial hydroxyl group using Anderson methodology[19] to give alkene **10a$_1$** and **10b$_1$** in good yield. Epoxidation of the resulting double bond gave a separable 2:1 mixture of oxiranes **11a$_1$ / 11a'$_1$** (75% yield) or **11b$_1$ / 11b'$_1$** in 72% yield. However, we were unable to determine the absolute configuration of the newly formed epoxy groups by NMR spectroscopy in these compounds.

Isomers **11a$_1$ / 11a'$_1$** and **11b$_1$ / 11b'$_1$** were carbamoylated on their secondary hydroxyl group using common isocyanates. For example, **11a$_1$** yielding **12a$_1$**, **14a$_1$** and **15a$_1$** when subjected respectively to chloroacetyl isocyanate, ethoxycarbonyl isocyanate and isocyanato ethyl acetate. Bis-chloroacetyl carbamates (e.g., **16b$_2$** and **19b$_2$**) have also been synthesized in this series as shown Scheme 3.

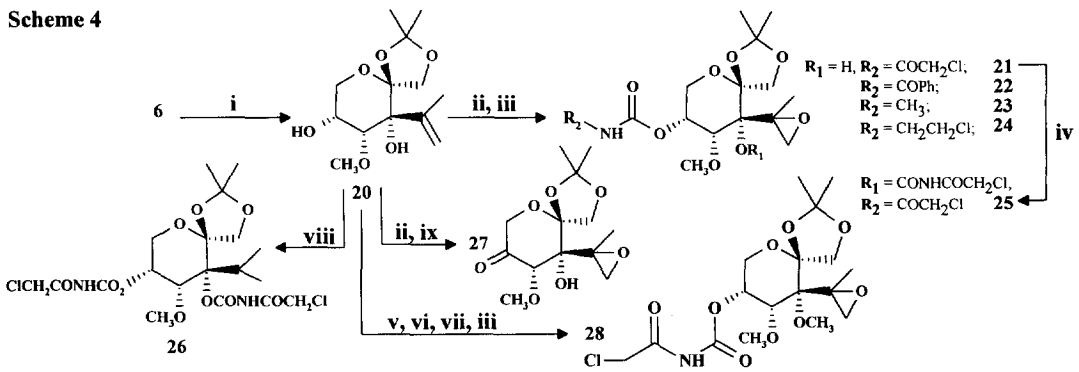
As previously mentioned (*supra*) a third series (Spirocarbonate family **IC**) of rigidified bicyclic pyranosides have been prepared using standard organic reactions (Scheme 3).

Among the molecules synthesized in this series mono-carbamoyl **18b$_1$** and bis-carbamoyl derivative **19b$_2$** showed promising antiangiogenic activities as will be discussed in the last section of this paper.

At this stage of our study we decided to investigate the influence of the C-3 chain length on the biological activity.

In this aim a family of isoprenylepoxides have been prepared as shown below. Diol **20**, a versatile synthon toward the synthesis of this series, was derived from triol **6** (see Scheme 1) using dibutyltin oxide and iodomethane as for preparation of **10a$_1$** and **10b$_1$** (*supra*). Ovalicin-like derivative **27** was synthesized from **20** *via* an epoxidation / chromium-oxidation sequence.

Scheme 4



i. Bu_2SnO, MeOH, rfx then CH_3I, dioxane, rfx., 92% ; **ii.** mCPBA, CH_2Cl_2, rt, 86% ; **iii.** R_2NCO, CH_2Cl_2, 0°C, 79% (**21**), 70% (**22**), 95% (**23**), 97% (**24**), 46% (**28**) ; **iv.** $ClCH_2CONCO$, THF, rfx., 17% (**25**) ; **v.** TBSCl, Imidazole, DMF, rt, 96% ; **vi.** NaH, THF, rfx then CH_3I, rfx., 70% ; **vii.** TBAF, THF, 0°C to rt, 98% ; **viii.** H_2, Pd/C, AcOEt, rt then $ClCH_2CONCO$, CH_2Cl_2, rt, 72% (**26**, 2 steps) ; **ix.** PDC, mol. sieves, CH_2Cl_2, rt, 46%.

Biological results and discussion.

The search for molecules displaying a high antiangiogenic profile with a cellular antiproliferative level resembling that of TNP-470, prompted us to evaluate the ability of our new compounds to specifically[20] inhibit angiogenesis using the chick chorioallantoic membrane (CAM)[21] assay. Their capability to inhibit the proliferation of tumor cells in culture (A431 human epidermoid carcinoma) as well as primary culture of endothelial cells from pork aorta has also been evaluated[22] and compared to those of Ovalicin **1**, Fumagillin **2** and TNP-470 **3** (Table).

Table : Anti-angiogenic activity of representative compounds in the CAM assay and *in vitro* antiproliferative effect against on A431 human epidermoid carcinoma cells and primary endothelial cells from pork aorta (ECPA).

Cpd. N°	*Spiro Family*	*CAM assay % of eggs with avascular zone 125 nmol of cpd.*	*A431 Antiprolif. level IC_{50} (µM)*	*ECPA Antiprolif. level IC_{50} (µM)*	*Cpd. N°*	*Spiro Family*	*CAM assay % of eggs with avascular zone 125 nmol of cpd*	*A431 Antiprolif. level IC_{50} (µM)*	*ECPA Antiprolif. level IC_{50} (µM)*
1 (Ovalicin)		[a] N.D.	7.5	[b](0.1 nM), 10 µM	**$16b_2$**	IB	88	1.2	0.8
2 (Fumagillin)		80	48	[b](0.1 nM), 100 µM	**$18b_1$**	IC	95	11	14
3 (TNP-470)		85	5.3	[b](0.001 nM), 12 µM	**$19b_2$**	IC	80	2	2.4
4	IA	32	>500	>500	**21**	IB	84	84	57
$12a_1$	IB	68	44	178	**22**	IB	55	>500	363
$12a'_1$	IB	75	44	124	**23**	IB	51	>500	195
$12b_1$	IB	76	29	90	**24**	IB	10	>500	278
$12b'_1$	IB	79	19	68	**25**	IB	62	38	122
$13b_2$	IB	63	3.7	16	**26**	IB	95	5.6	23
$14a_1$	IB	11	471	398	**27**	IB	48	>500	119
$15a_1$	IB	21	442	343	**28**	IB	16	39	175

[a] Not Determined.

[b] It is of interest to note that Ovalicin **1**, Fumagillin **2** and TNP-470 **3** possess a dual activity on endothelial cells, showing a first inhibition at low doses (cytostaticity) and a second one at higher concentrations (cytotoxicity)[23]. Only one IC_{50} is given for other compounds since they do not induce a detectable first phase of inhibition in the nM range.

Among the numerous derivatives synthesized, the 5-O-chloroacetylcarbamates displayed the highest antiangiogenic profile in the CAM assay (e.g., **$12a_1$** vs **$14a_1$** and **$15a_1$** or **21** vs **22-24**).

The lipophilicity of the C-3 epoxy sidechain (e.g., **$12a_1$**, **$12a'_1$** vs **$12b_1$**, **$12b'_1$** or vs **21**) as well as the stereochemistry of the epoxy group (**$12a_1$** vs **$12a'_1$** and **$12b_1$** vs **$12b'_1$**) do not seem to play a crucial role in the antiangiogenic activity (e.g., **$16b_1$** vs **$16b'_1$**). While introduction of a second chloroacetylcarbamate moiety on the C-3 tertiary hydroxyl group lowered significantly the CAM activity (e.g., **$13b_2$** vs **$12b_1$**, **25** vs **21** or **$19b_2$** vs **$18b_1$**), alkylation with a methyl residu led to marginally active compounds (e.g., **28** vs **21**).

Elimination of the oxirane ring of bis-chloroacetylcarbamates increased their antiangiogenic profile (**$16b_2$** vs **$13b_2$** and **26** vs **25**) while replacing the spiroacetonide ring of **$16b_2$** by a spirocarbonate one (i.e., **$19b_2$**) do not affect the CAM activity. Moreover, as already mentioned in the acetonide series monocarbamates are better inhibitors of neovascularisation than bis-carbamoyl derivatives (e.g., **$18b_1$** vs **$19b_2$**). Among the molecules showing a CAM activity higher than 60% and a cytotoxicity against A431 and endothelial cell lines,

comparable with that of TNP-470, five of them (i.e., **$13b_2$**, **$16b_2$**, **$18b_1$**, **$19b_2$** and **26**), have been selected for *in vivo* biological evaluation as new anti-cancer agents.

In conclusion, this letter describes the synthesis of new fructo and psicopyranosides[24], analogues of Ovalicin and/or TNP-470 which are potent inhibitors of neovascularisation in the CAM assay.

Such optically active derivatives with a broader spectrum of activity than the parent compounds have been prepared from cheap commercially available D-Fructose in 7 to 10 chemical steps and their *in vivo* evaluation will give us valuable informations for further clinical development.

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References and Notes:

1. *Present Address :* Dr. P. Léon, 6 Place des Alpes, 78280 Guyancourt, France.
Prof. D.C. Billington, Department of Pharmaceutical and Biological Sciences, Aston University, Aston Triangle, Birmingham, B4 7ET, UK.
2. a) Gonzalez-Crussi, F. *Am. J. Anat.*, **1971**, *130*, 441. b) Coffin, J.D.; Poole, T.J. *Development*, **1988**, *102*, 735.
3. For review see Findlay, J.K. *J. Endocrinol.*, **1986**, *111*, 357.
4. Madri, J.A.; Pratt, B.M. *Clark R.A.F. and Henson P.M. (eds.). Plenum. New York.* **1988**, 337
5. Davis, M.D.; *Diabetes Metabol. Rev.*, **1988**, *4*, 291.
6. a) Folkman, J. *Sci. Am..*, **1976**, *234*, 58. b) Folkman, J.; Cotran, R. *Int. Rev. Exp. Pathol.*, **1976**, *16*, 207. c) Folkman, J. *Adv. Cancer Res.*, **1985**, *43*, 175.
7. a) Folkman, J. *Ann. Surg.*, **1972**, *175*, 409. b) Folkman, J. *De Vita, V.T. Jr; Hellman, S.A.; Rosenberg, S.A. (eds.) Lippincott. Philadelphia*, **1985**, 42. c) Folkman, J.; *Engl. J. Med.*, **1989**, *320*, 1211.
8. Ingber, D.; Fujita, T.; Kishimoto, S.; Sudo, K.; Kanamaru, T.; Brem, H.; Folkman, J. *Nature*, **1990**, *348*, 555.
9. Levy, T. ; Kudelka, A. ; Verschraegen, C.F. ; Edwards, C.L. ; Freedman, R.S. ; Kaplan, A. ; Kieback, D. ; Steger, M. ; Mante, R. ; Gutterman, J. ; Piamsomboon, S. ; Termrungruanglert, W. ; Kavanagh, J.J. *87th Annual Meeting of the American Association for Cancer Research, Washington DC.*, April 20-24th, **1996**, abst. N°1140.
10. a) Sigg, H.P.; Weber, H.P. *Helv. Chim. Acta.*, **1968**, *51*, 1395; b) Bollinger, P.; Sigg, H.P.; Weber, H.P. *Helv. Chim. Acta.*, **1973**, *56*, 819.
11. a) Zimmerman, W.A.; Hartmann, G.R. *Eur. J. Biochem.*, **1981**, *118*, 143; b) Borel, J.F.; Lazary, S.; Staehelin, H. *Agents and Actions*, **1974**, *4*, 357.
12. Corey, E.J. ; Dittami, J.P. *J. Am. Chem. Soc.*, **1985**, *107*, 256.
13. a) Corey, E.J.; Guzman-Perez, A.; Noe, M.C. *J. Am. Chem. Soc.*, **1994**, *116*, 12109. b) Bath, S.; Billington, D.C.; Géro, S.; Quiclet-Sire, B.; Samadi, M. *J. Chem. Soc., Chem. Commun.*, **1994**, 1495.
14. Corey, E.J.; Snider, B. B. *J. Am. Chem. Soc.*, **1972**, *94*, 2549.
15. a) Marui, S. ; Itoh, F. ; Kozai, Y. ; Sudo, K. ; Kishimoto, S. *Chem. Pharm. Bull.*, **1992**, *40*, 96 ; b) Marui, S. ; Kishimoto, S. *Chem. Pharm. Bull.*, **1992**, *40*, 575 ; c) Marui, S. ; Yamamoto, T. ; Sudo, K. ; Akimoto, H. ; Kishimoto, S. *Chem. Pharm. Bull.*, **1995**, *43*, 588.
16. Campbell, M.M.; Heffernan, G.D. *Tetrahedron Lett.*, **1991**, *32*, 1237.
17. Brady, JR. R.F. *Carbohydr. Res.*, **1970**, *15*, 35.
18. Campbell, M.M.; Heffernan, G.D.; Lewis, T. *Carbohydr. Res.*, **1994**, *251*, 243.
19. Nashed, M.A.; Anderson, L. *Tetrahedron Lett.*, **1976**, 3503.
20. Evaluation of the percentage of eggs with an avascular zone, allows the identification of specific antiangiogenic derivatives devoid of toxicity. Compounds showing local hemorrhage, marked distorsion of large preexisting vessels and/or death of the embryo were not selected as potential antiangiogenic drugs.
21. Crum, R.; Szabo, S.; Folkman, J. *Science*, **1985**, *230*, 1375.
22. For related methodology see Pierré, A. ; Kraus-Berthier, L. ; Atassi, G. ; Cros, S. ; Poupon, M-F. ; Lavielle, G. ; Berlion, M. ; Bizzari, J-P. *Cancer Res.*, **1991**, *51*, 2312.
23 Kusaka, M. ; Sudo, K. ; Matsutani, E. ; Kozai, Y. ; Marui, S. ; Fujita, T. ; Ingber, D. ; Folkman, J. *Br. J. Cancer*, **1994**, *69*, 212
24. All synthetic intermediates and final compounds gave satisfactory ^1H NMR, ^{13}C NMR, mass spectral, and elemental analysis data, in full agreement with their assigned structures and stereochemistries. Experimental procedures and analytical data have been published elsewhere ; Adir and Co ; European Patent, 0699679 (EP 0699679), p. 96-130711/14, **1996**.

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