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(Z)-1,4-Diamino-2-butene as a Vector of Boron, Fluorine, or Iodine for Cancer Therapy and Imaging: Synthesis and Biological Evaluation

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Abstract—Polyamine vectors are attractive for tumor targeting. We envisaged *(Z)*-1,4-diamino-2-butene (***Z*-DAB**), an unsaturated analogue of putrescine as vector of ^{10}B, ^{18}F and ^{131}I for boron neutron capture therapy (BNCT), and tumor imaging by positron emission tomography or scintigraphy respectively. In the present work, the synthesis and characterization of new derivatives of ***Z*-DAB** were reported. ***Z*-DAB** was actively transported in cells via the polyamine transport system and converted into the spermidine analogue. *(E)*-2-iodo-1,4-diamino-2-butene (***E*-I-DAB**) was not taken up by the polyamine transport system and may not be suitable for tumor imaging. In contrast, *(Z)*-2-{4-(5,5-dimethyl-[1,3,2]dioxaborinan-2-yl)phenyl}methyl-1,4-diamino-2-butene (***Z*-4-Bbz-DAB**) was a substrate of the transport system and allowed significant boron accumulation in 3LL cells. Its potential in BNCT will be evaluated.

Introduction

Putrescine, spermidine and spermine are ubiquitous low molecular weight biogenic amines, essential for cell growth and differentiation.[1] In eukaryotic cells, complex, highly regulated biosynthetic and catabolic pathways determine the intracellular pools of polyamines. Cells are also equipped with active transport systems, allowing them to take up polyamines from the environment.[2] The activity of the uptake system is under the control of the intracellular free polyamine pool. Structural features, the enhanced requirement of tumor cells for the polyamines, and the characteristics of the transport system make the polyamines attractive vectors for tumor targeting. The following aspects are important in this regard:

i The specificity of the polyamine transport system is not stringent. A wide variety of polyamine derivatives and analogues are able to use this system.[2]

ii The activity of the polyamine transport system is higher in transformed cells than in their normal counterparts.[3,4] In vivo, tissues with a high transport rate, such as liver, and tissues with a high demand for polyamines, such as tumors[5] and prostate[6] take up radiolabeled polyamines in greater amounts than other tissues.

iii Depletion of intracellular polyamines, for example by 2-(difluoromethyl)ornithine (DFMO), an inactivator of ornithine decarboxylase, greatly enhances polyamine uptake by various cell lines.[7–10] In vivo, the polyamine content of tumor cells is more efficiently depleted by DFMO than that of non- or slow-growing cells. As a consequence, treatment with DFMO increases the uptake rate of tumors, which readily accumulate high amounts of polyamines.[11–13]

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All these properties have led to the design of polyamine derivatives for tumor targeting.[2] In particular, the polyamine transport system has been targeted for the selective accumulation of various isotopes in cancer cells for therapy or tumor imaging. BNCT is a method for the treatment of brain tumors and melanomas. It requires the accumulation of the boron isotope ^{10}B within the tumor cells and relies on the cytotoxic action of energy-rich α particles formed by irradiation of the ^{10}B isotope with low energy neutrons (for reviews, see refs 14 and 15). *o*-Carborane cages attached to the primary or secondary amino groups of spermidine and spermine have been synthesized for BNCT, but they were too toxic for clinical use.[16,17] Recently, we reported the synthesis of boron-containing benzyl polyamines.[18] These derivatives, which exhibit low cytotoxicity, affinity for DNA, allow efficient boron accumulation in tumor cells in vitro. They are potential candidates for BNCT. Fluorinated putrescine or spermidine derivatives were also designed and evaluated for Positron Emission Tomography (PET), a non-invasive tumor-imaging modality.[18–22] Finally, iodine-substituted diamines were studied for application in ^{125}I scintigraphy.[23,24]

(Z)-1,4-Diamino-2-butene (***Z*-DAB**, Fig. 1) is an unsaturated derivative of putrescine. Previous reports have demonstrated that 1,4-diamino-2-butene (**DAB**, isomer not specified) is a substrate of spermidine synthase, which converts **DAB** into unsaturated spermidine.[25,26] Only the (*cis*) isomer of unsaturated spermidine can be converted into the spermine homologue by spermine synthase.[25,27] However, in contrast to spermidine or spermine, none of the higher homologues of ***Z*-DAB** is degraded along the retroconversion pathway, which involves acetylation by spermidine/spermine N^1-acetyl transferase and oxidative splitting by polyamine oxidase.[27] **DAB** and its higher homologues reverse the cytostatic effect of DFMO.[25,27] ***Z*-DAB** competitively inhibits the uptake of putrescine,[28] which suggests that it may use the polyamine transport system. Hence, in highly proliferating cells treated with DFMO, ***Z*-DAB** may be efficiently trapped via the polyamine transport system.

Because of its metabolic and transport properties, ***Z*-DAB** is a potential tumor-targeting vector. New boron-, fluorine-, and iodine-containing derivatives of ***Z*-DAB** (Fig. 1) were designed to specifically target tumor cells for BNCT, PET, and scintigraphy, respectively. Biological properties of these derivatives are reported with special focus on interactions with the polyamine transport system and metabolic pathways of the polyamines. In particular, the cytotoxicity and cell accumulation of the derivatives were studied in CHO cells and in a polyamine transport deficient CHO mutant cell line (CHO-MG).[29] Studies were also performed on 3LL cells (Lewis lung carcinoma) because of our past experience with close related compounds on this cell line[18] and the availability of the in vivo model in our laboratory.[30]

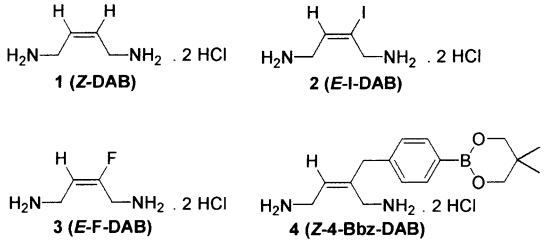


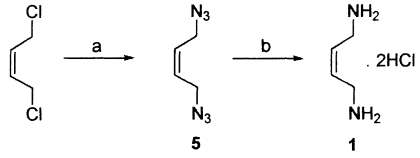
Figure 1. Structure of ***Z*-DAB** derivatives.

Results

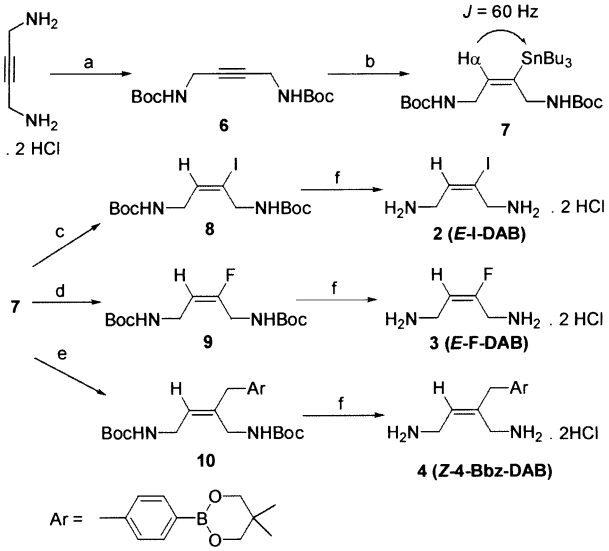
Chemical syntheses

Synthesis of the target compound *(Z)*-1,4-diamino-2-butene dihydrochloride (**1**, ***Z*-DAB**) is shown in Scheme 1. The *(Z)*-1,4-dichloro-2-butene was employed as starting material. This reagent reacted with sodium azide at room temperature in DMSO to give the diazide **5** in quantitative yield. Due to the suspected instability of this intermediate, it was used directly without further purification in the next step. Reduction of the azido groups by an in situ Staudinger reaction[31] gave the desired product **1** in 76% overall yield.

(*E*)-2-Iodo-1,4-diamino-2-butene dihydrochloride (**2, *E*-I-DAB**),*(E)*-2-fluoro-1,4-diamino-2-butene dihydrochloride (**3**, ***E*-F-DAB**), and *(Z)*-2-{4-(5,5-dimethyl-[1,3,2]dioxaborinan - 2 - yl)phenyl}methyl - 1,4 - diamino - 2 - butene dihydrochloride (**4**, ***Z*-4-Bbz-DAB**) were synthesized by



Scheme 1. Results and conditions: (a) NaN_3, DMSO, 25 °C; (b) PPh_3, HCl, 25 °C, 76%.



Scheme 2. Results and conditons: (a) $(Boc)_2O$, Et_3N, THF, rt, 83%; (b) $HSnBu_3$, $PdCl_2(PPh_3)_2$, THF, rt, 98%; (c) I_2, CH_2Cl_2, rt, 72%; (d) 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate), CH_3CN, rt, 40%; (e) $BrCH_2Ar$, $Pd(PPh_3)_4$, toluene, reflux, 51%; (f) HCl (12 N), EtOH, rt, **2**, 88%, **3**, 80%, **4**, 87%.

a versatile route illustrated in Scheme 2 from 1,4-diamino-2-butyne dihydrochloride. Protection of the amino groups was performed with $(Boc)_2O$ in THF containing 2 equiv of Et_3N. Hydrostannation of **6** in the presence of a catalytic amount of $Pd(PPh_3)_4$ at room temperature, the key step of our synthesis, afforded the corresponding vinylstannane **7** in a 95% yield.[32] The reaction is highly stereoselective, *syn* addition being deduced from the small ^3J coupling constant between Sn and Hα (60 Hz). On reaction with I_2 at room temperature, **7** gave *(E)*-2-iodo-1,4-di-(*tert*-butyloxycarbonyl)-amino-2-butene **8** in 72% yield with total conservation of stereochemistry. Electrophilic fluorination of vinylstannane **7** was performed using commercially available 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate).[33] *(E)*-2-Fluoro-1,4-di-(*tert*-butyloxycarbonyl) amino-2-butene **9** was obtained in 40% yield, accompanied by 20% of protonolysis of the vinylstannane. Cross-coupling reaction of **7** with the 2,2-dimethylpropane-1,3-diol ester of the [4-bromomethyl)phenyl]boronic acid[34] in the presence of palladium catalyst provided the corresponding 2-substituted-1,4-di-(*tert*-butyloxycarbonyl)amino-2-butene **10** in 51% yield. Deprotection of the geometrically pure dehydroputrescines derivatives **8**, **9**, **10** was achieved with aqueous concentrated hydrochloric acid in ethanol.

Biological studies

Effect on the growth of CHO and polyamine transport deficient CHO-MG cells. Up to 1 mM, ***Z*-DAB** had no effect on the growth of CHO and CHO-MG cells. ***Z*-4-Bbz-DAB** and ***E*-I-DAB** showed moderate cytotoxic effects on CHO cells with IC_{50} values of 340 and 90 μM, respectively (Table 1). CHO-MG cells were significantly less sensitive to ***Z*-4-Bbz-DAB** (IC_{50} value around 1 mM) than CHO cells. In contrast, ***E*-I-DAB** had similar growth inhibitory effect on both CHO and CHO-MG cells.

Accumulation in CHO and CHO-MG cells. Accumulation of ***Z*-DAB** derivatives was studied after a 1- or 4-h exposure to 250 μM (Table 2). Intracellular levels of ***Z*-DAB** and ***Z*-4-Bbz-DAB** were significantly higher in CHO than in CHO-MG cells (at least 2-fold higher). In contrast, CHO and CHO-MG cells accumulated equivalent amounts of ***E*-I-DAB**.

Effect on putrescine uptake by 3LL cells. In 3LL cells, ***Z*-DAB** and ***Z*-4-Bbz-DAB** were weak inhibitors of putrescine uptake with IC_{50} values of 250 and 535 μM, respectively (Table 1). In contrast, up to 1 mM, ***E*-I-DAB** had no effect on putrescine transport.

Effect on the growth of 3LL cells. The effects of ***Z*-DAB** and its derivatives on the growth of 3LL cells were similar to those observed on CHO cells. While ***Z*-DAB** had very little effect on cell growth (about 25% inhibition at 1 mM), ***Z*-4-Bbz-DAB** and ***E*-I-DAB** inhibited cell growth with IC_{50} values around 300 μM (Table 1). At concentrations exceeding the IC_{50} values of ***Z*-4-Bbz-DAB** and ***E*-I-DAB**, cells were swelling and granular vacuoles developed in the cytoplasm. The same morphological changes were observed in CHO cells.

The influence of DFMO on the cytotoxicity of ***Z*-DAB** derivatives was studied by monitoring 3LL cell growth after a 24 h pre-incubation with 5 mM DFMO, followed by a 48 h exposure to the drugs. As shown in Fig. 2, all derivatives, as free spermidine, antagonized DFMO-induced cell growth arrest. They supported growth of putrescine- and spermidine-depleted cells. Total reversion of the DFMO effect required at least 50 times higher concentrations of ***Z*-DAB**, than of spermidine. ***Z*-4-Bbz-DAB** and ***E*-I-DAB** also antagonized the effect of DFMO but less efficiently than ***Z*-DAB**.

Accumulation and metabolism of *Z*-DAB derivatives in 3LL cells. 3LL cells exposed for 24 h to ***Z*-DAB** accumulated 2-fold higher amounts of the drug than after a 4 h incubation (Table 3). DFMO-pretreated cells exposed to ***Z*-DAB** for 4 h accumulated 2-fold higher amounts of the drug than control cells ($p < 0.05$). After a 24 h exposure, drug levels of ***Z*-DAB** were similar in DFMO-pretreated and control cells. Co-treatment with an inhibitor of spermidine synthase [*trans*-4-methylcyclohexylamine (4MCHA)][35,36] resulted in increased intracellular levels of ***Z*-DAB** in both control and DFMO-pretreated cells ($p < 0.05$).

HPLC chromatograms revealed the presence of two ***Z*-DAB** metabolites (M1 and M2 with retention times of 20.7 and 25.3 min, respectively), which eluted close to

Table 1. Inhibitory effects of (*Z*)-1,4-diaminobutene derivatives on cell growth, polyamine uptake, and ODC activity[a]

	Cell growth[b]			ODC[c]	[^{14}C] putrescine uptake[d]
	3LL	CHO	CHO-MG		
	IC_{50} (μM)			IC_{50} (μM)	IC_{50} (μM)
***Z*-DAB**	> 1000	> 1000	> 1000	30	260
***Z*-4-Bbz-DAB**	298	338	1003*	> 200[e]	535
***E*-I-DAB**	380	87	108	80	> 500[f]

[a]All data are expressed as mean (SD < 20%).
[b]Effect on cell growth was measured after a 72-h incubation period.
[c]Inhibition of ODC activity was determined in freshly prepared extracts of kidneys of testosterone-stimulated DBA/2 mice.
[d]Effect of ***Z*-DAB** derivatives on putrescine uptake was determined in 3LL cells, after a 20-min incubation with ***Z*-DAB** derivatives and [^{14}C] putrescine (0.5 μCi; 10 μM).
[e]A 200-μM dose induced a 35% inhibition of ODC activity.
[f]A 500-μM dose provoked a 18% inhibition of putrescine uptake.
*Significantly different from values in CHO cells ($p < 0.05$).

Table 2. Accumulation of (*Z*)-1,4-diaminobutene derivatives in CHO and in polyamine transport deficient CHO-MG cells

	CHO		CHO-MG	
	nmol/mg protein[a]			
Incubation time[b]	1 h	4 h	1 h	4 h
***Z*-DAB**	8.6 ± 1.4	9.6 ± 1.1	3.9 ± 0.4*	4.5 ± 1.1*
***Z*-4-Bbz-DAB**	1.1 ± 0.2	3.0 ± 0.2	b.d.l.*	1.5 ± 0.4*
***E*-I-DAB**	12.8 ± 0.6	10.1 ± 1.8	9.8 ± 1.0	8.4 ± 1.7

[a]Compounds were determined by HPLC. All data are expressed as mean ± SD. b.d.l., below detection limit (< 0.2 nmol/mg of protein).
[b]Cells were treated with 250 μM derivative for 1 or 4 h.
*Significantly different from values in CHO cells ($p < 0.05$).

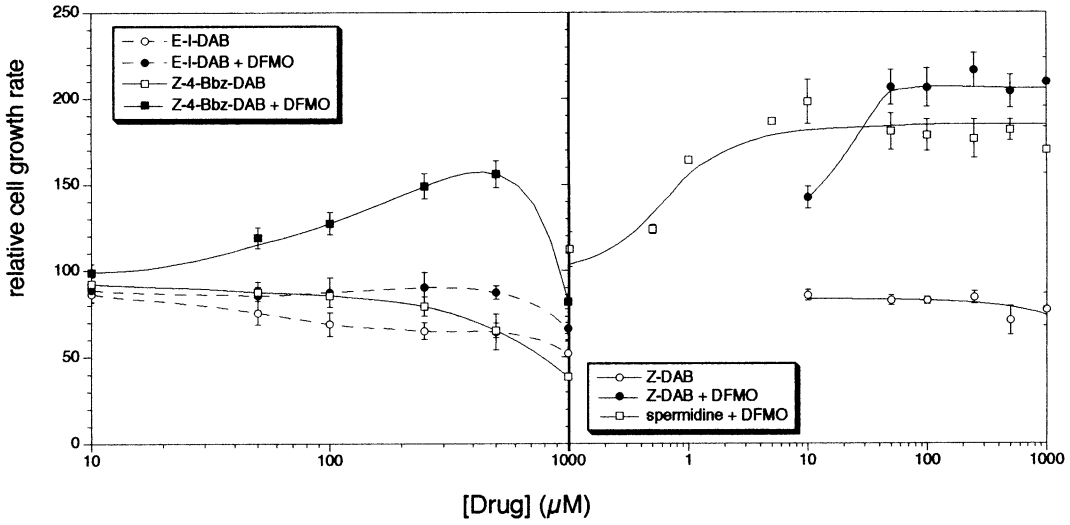


Figure 2. Effect of ***Z*-DAB** derivatives on the growth of 3LL cells in absence or after pretreatment with DFMO. 24 h after seeding, 5 mM DFMO was added to the 3LL cell culture medium for 24 h, followed by 48-h exposure to spermidine or to a derivative of ***Z*-DAB**. The relative cell growth rate was calculated from the value of cell growth of the corresponding control cells cultured in absence ($OD_{540nm} = 2.01$) or in presence ($OD_{540nm} = 0.57$) of DFMO.

Table 3. Accumulation of (*Z*)-1,4-diaminobutene in 3LL cells and their effect on polyamine metabolism

Treatment	Time of exposure (h)	Putrescine[a]	Spermidine[a]	Spermine[a]	***Z*-DAB**[a]	***Z*-DAB** derivative[a,b]	Peak 1[c]	Peak 2[c]
None	—	0.94	9.10	5.91	—	—	—	—
DFMO[d]	—	b.d.l.[e]	b.d.l.[e]	4.25	—	—	—	—
4MCHA[f]	—	5.79	1.69	6.98	—	—	—	—
DFMO[d]/4MCHA[f]	—	b.d.l.[e]	b.d.l.[e]	4.65	—	—	—	—
***Z*-DAB**[g]	4	0.20	9.07	6.83	3.56	—	b.d.l.[e]	b.d.l.[e]
***Z*-DAB**[g]/DFMO[d]	4	0.19	1.49	7.43	7.07*	—	67.09	b.d.l.[e]
***Z*-DAB**[g]	24	0.16	3.91	6.53	7.80	—	117.40	14.44
***Z*-DAB**[g]/DFMO[d]	24	0.11	3.07	2.32	7.20	—	171.14*	141.91*
***Z*-DAB**[g]/4MCHA[f]	24	2.61	0.81	6.04	11.84	—	b.d.l.[e]	b.d.l.[e]
***Z*-DAB**[g]/DFMO[d]/4MCHA[f]	24	0.14	0.27	4.17	23.80*,†	—	b.d.l.[e]	b.d.l.[e]
***Z*-4-Bbz-DAB**[h]	24	0.19	5.44	7.88	Traces	1.61 (1.82)[i]	—	—
***Z*-4-Bbz-DAB**[h]/DFMO[d]	24	b.d.l.[e]	b.d.l.[e]	4.80	Traces	1.49 (1.49)[i]	—	—
***Z*-4-Bbz-DAB**[h]/4MCHA[f]	24	7.16	1.84	4.80	n.m.[j]	1.53	—	—
***Z*-4-Bbz-DAB**[h]/DFMO[d]/4MCHA[f]	24	b.d.l.[e]	b.d.l.[e]	4.21	Traces	1.70	—	—
***E*-I-DAB**[g]	4	0.10	6.42	4.00	b.d.l.[e]	8.15	—	—
***E*-I-DAB**[g]/DFMO[d]	4	0.05	0.27	4.93	b.d.l.[e]	8.72	—	—
***E*-I-DAB**[g]	24	0.17	4.88	3.89	b.d.l.[e]	6.93	—	—
***E*-I-DAB**[g]/DFMO[d]	24	0.02	0.03	3.03	b.d.l.[e]	8.05	—	—

[a]Data are mean of values expressed as nmol/mg protein measured by HPLC (SD < 20%).
[b]***Z*-DAB** derivative measured is ***Z*-4-Bbz-DAB** or ***E*-I-DAB**.
[c]Data are expressed as area 10^3/mg protein of additional peaks detected by HPLC at 20.7 and 25.3 min, respectively, for peaks 1 and 2 (mean).
[d]Cells were incubated with 5 mM DFMO for 24 h before derivative addition.
[e]Below detection limit: < 0.05 nmol/mg protein for putrescine and spermidine; < 0.2 nmol/mg protein for ***Z*-DAB** and its derivatives; < 5.10^3/mg protein for peak 1 and 2.
[f]Cells were incubated with 250 μM 4MCHA for 24 h before derivative addition.
[g]Cells were treated with 250 μM derivative for 24 h.
[h]Cells were treated with 138 μM derivative for 24 h.
[i]Values of boron measurement by ICP-AES method.
[j]Not measurable because of the broad putrescine peak.
*Significantly different from values in control cells ($p < 0.05$); † significantly different from values in 4MCHA-treated cells ($p < 0.05$).

spermidine and spermine (retention times of 20.6 and 25.1 min), respectively. M1 was detected as early as 4 h after ***Z*-DAB** addition in DFMO-pretreated cells, but not in control cells. After 24 h, both M1 and M2 were detected in 3LL cells. Higher amounts of these metabolites were measured in cells pre-treated with DFMO ($p < 0.05$). 4MCHA prevented the formation of both metabolites.

During a 4-h exposure, 3LL cells accumulated 2-fold higher amounts of ***E*-I-DAB** compared with ***Z*-DAB** ($p < 0.05$). However, longer exposure did not result in enhanced intracellular level of ***E*-I-DAB**. Cells pre-treated with DFMO accumulated similar amounts than control cells. HPLC chromatograms did not show any evidence of metabolization of ***E*-I-DAB**.

***Z*-4-Bbz-DAB** was poorly taken up by 3LL cells. After a 24-h exposure, 3LL cells accumulated less ***Z*-4-Bbz-DAB** than ***Z*-DAB**. Neither DFMO nor 4MCHA affected the accumulation of the derivative. Boron concentrations determined by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) were similar to the amount of drug measured by HPLC. Traces of ***Z*-DAB** were detected in all cells treated with ***Z*-4-Bbz-DAB**.

Effect on polyamine pools. In 3LL cells, all three derivatives induced a decrease of putrescine pools within 24 h by approximately 80% of the control values, and a reduction of spermidine levels between 40 and 60% of control values (Table 3). Spermine levels were only affected by treatment with ***E*-I-DAB**, which caused a 35% decrease.

DFMO-treated cells exposed for 24 h to ***Z*-DAB** exhibited a dramatic decrease of their spermine pools, along with a significant accumulation of spermidine. Addition of ***E*-I-DAB** or ***Z*-4-Bbz-DAB** had no effect on the polyamine pools of DFMO-treated cells.

Inhibition of spermidine synthase by 4MCHA in 3LL cells led to a 6-fold increase of putrescine pools and a 5-fold decrease of spermidine levels. Addition of ***Z*-DAB** to these 4MCHA-treated cells reduced amplification of putrescine levels, while spermidine pools were more profoundly diminished. ***Z*-4-Bbz-DAB** did not modify the effect of 4MCHA in 3LL cells.

Both ***Z*-DAB** and ***E*-I-DAB** inhibited ornithine decarboxylase, with IC_{50} values of 30 and 80 μM, respectively (Table 1). Although less efficient, ***Z*-4-Bbz-DAB** also decreased ODC activity (35% inhibition at 200 μM).

Discussion

Three derivatives of ***Z*-DAB** carrying an atom of boron, fluorine, or iodine, were synthesized for potential application in cancer therapy or imaging. Biological studies with the fluorinated derivative could not be performed because of the instability of the drug. The metabolic and transport properties of the other derivatives are discussed in comparison with the features of their parent compound.

1. ***Z*-DAB** was significantly metabolized in 3LL cells. Accumulation of ***Z*-DAB** was paralleled by the appearance of two new compounds. It is known that both ***Z*-DAB** and its spermidine homologue are substrates of spermidine and spermine synthase, respectively, although the rates of reaction are reduced and the K_M are increased, as compared with the natural substrates.[25–27] In 3LL cells, inhibition of spermidine synthase with 4MCHA prevented the formation of both metabolites and increased the intracellular levels of ***Z*-DAB**. Based on these observations and on their chromatographic behavior, the metabolites were tentatively identified as unsaturated spermidine and spermine analogues. In DFMO-pretreated cells, the formation of the spermine analogue was greatly enhanced because the depletion of spermidine reduced the competition for spermidine synthase and favored the conversion of the spermidine derivative. In contrast, ***E*-I-DAB** and ***Z*-4-Bbz-DAB** did not cause formation of detectable amounts of a new compound. Previous work demonstrated the stringency of spermidine synthase for the 1,4-butanediamine moiety.[26] However, Sarhan and coll.[25] showed that substituents on the four-carbon chain were tolerated within certain limits, with a better tolerance for substituents in the 2-position. The bulky iodine atom and the boron-containing benzyl moiety prevent interaction of the derivatives with the synthase due to steric hindrance. In the case of ***Z*-4-Bbz-DAB**, trace amounts of ***Z*-DAB** were detected in 3LL cells, suggesting that the compound may lose to some extent its boron-containing substituent. In a recent study, a similar loss was observed from a derivative of spermidine with the same boron-containing moiety attached to the central amino group of spermidine.[18] Previous studies have demonstrated that ***Z*-DAB** was a substrate of diamine oxidase.[37] We also observed that this enzyme (data not shown) oxidized the iodine-, but not the boron-containing derivative of ***Z*-DAB**. However, aminoguanidine, an inhibitor of diamine oxidase, did not modify the cytotoxicity or the accumulation of ***Z*-DAB** and ***E*-I-DAB** in 3LL cells (data not shown).

2. Polyamine patterns were modified in 3LL cells treated with ***Z*-DAB** (Table 3). In agreement with previous studies,[38] ***Z*-DAB** was found to be a potent inhibitor of ODC (Table 1) although less potent than the (*E*)-isomer.[39,40] Depletion of putrescine and spermidine pools may result from ODC inhibition. However, a similar effect was observed in cells treated with ***Z*-4-Bbz-DAB**, a much weaker ODC inhibitor. This suggests that other mechanisms may be involved in polyamine depletion. Because of the polyamine-like structure of ***Z*-DAB** and its derivatives down-regulation of the expression of ODC by feedback mechanisms have to be considered.

In DFMO-pretreated cells, the profound depletion of spermine pools induced by ***Z*-DAB** can be explained by the effective competition of ***Z*-DAB** and its spermidine analogues with the natural polyamines for the synthases. Such competitions also account for the presence of elevated amounts of putrescine and spermidine in DFMO-pretreated cells.

3. ***Z*-DAB** antagonized the growth inhibitory effect of DFMO in 3LL cells, as has previously been described in chick embryos.[25] The partial repletion of putrescine and spermidine pools by ***Z*-DAB** and its spermidine-like aminopropylation derivative may contribute to the reversion of the DFMO effect. However, it is known that certain homologues of putrescine and spermidine are able to reverse the growth arrest produced by exposure of tumor cells to DFMO, probably by their ability to occupy polyamine binding sites and prevent vital processes of cell division.[41,42] This property is likely to be shared by ***Z*-DAB** and its spermidine analogue[25,27] as well as by ***Z*-4-Bbz-DAB**, which, in the absence of putrescine and spermidine, also reversed efficiently the cytostatic effect of DFMO. Presumably, the bulky

iodine atom diminishes the affinity of ***E*-I–DAB** for the polyamine binding sites by steric hindrance as is evidenced by its poor ability to reverse the DFMO effect.

4. Previous studies have shown that ***Z*-DAB** is a weak competitor of putrescine uptake by rat lung.[28] Its low affinity was imputed to the unsaturation, which reduces the flexibility and the distance between the amino groups of the diamine. ***Z*-DAB** was also found to be a weak competitor for putrescine uptake in 3LL cells. Substituents in the 2-position further reduced the affinity of ***Z*-DAB** for the polyamine transport system. However, despite their low affinity, ***Z*-DAB** and ***Z*-4-Bbz-DAB** use the polyamine transport system as was evidenced by the greater accumulation in CHO cells than in CHO-MG mutant cells, which lack the polyamine transport system. The up-regulation of the polyamine transport system in polyamine-depleted 3LL cells induced a 2-fold higher accumulation of ***Z*-DAB** after a 4 h-exposure. Because of the conversion of ***Z*-DAB** into its spermidine and spermine analogues, the enhanced accumulation was not obvious after a 24 h-exposure. DFMO-induced upregulation of the transport activity did not result in enhanced accumulation of ***Z*-4-Bbz-DAB**. We have discussed earlier the possibility that this compound may induce feedback regulatory mechanisms leading to ODC inhibition. It is known that one regulatory pathway of polyamine transport involves the induction of antizyme, which inhibits ODC but also the activity of the polyamine uptake.[43] Such effect could antagonize the DFMO-induced upregulation of the transport system and prevent a higher accumulation of the derivative. In contrast to ***Z*-DAB** and its boron-containing derivative, ***E*-I-DAB** was not transported by the polyamine transport system probably because of the steric hindrance of the iodine atom.

High tumor/normal tissue ratios are a requirement for drugs used in BNCT as well as for probes used in imaging. The rationale behind the synthesis of new (*Z*)-1,4-diamino-2-butene derivatives was to develop compounds sharing the ability of the natural polyamines to be taken up by cells via the polyamine transport system. If derivatives are substrates of this system, the DFMO-induced upregulation of the activity of the polyamine transport system should allow their preferential accumulation in tumor cells. ***Z*-DAB** is a potential vector because it shares transport properties with the natural polyamines. However, the substitution on the 2-position may induce profound alterations of its properties. An iodine atom in 2-position abolished its ability to serve as a substrate for the polyamine transport system. Therefore, ***E*-I-DAB** is not useful for selective tumor imaging. In contrast, substitution with the boron-containing benzyl moiety did not prevent the ability of the derivative to use the polyamine transport system. Boron delivery by ***Z*-4-Bbz-DAB** into 3LL cells (around 1.4 μg of B/g of 3LL cells) was of the same order of magnitude as that achieved by boronated *N*-benzyl polyamines, or by borocaptate sodium (BSH) or (L)-*p*-boronophenylalanine (L-BPA), the two reference compounds used in BNCT.[18] Nevertheless, ***Z*-4-Bbz-DAB** accumulation could not be enhanced by depletion of putrescine and spermidine pools by DFMO. This may restrain its potential for BNCT. In vivo studies will be performed to fully evaluate the clinical interest of ***Z*-4-Bbz-DAB** for BNCT.

Experimental

Chemicals

Usual laboratory chemicals were purchased from Merck (Darmstadt, Germany) or Sigma Chemical Co. (St Louis, MO, USA) and used directly without further purification unless otherwise specified. [^{14}C] putrescine (118 mCi/mmol) and [^{14}C] ornithine (61 mCi/mmol) were from Amersham France (Les Ulis, France) and Pico-Fluor 15 from Packard Instr. Chemical Operations (Groningen, The Netherlands). DFMO was obtained from Ilex Oncology (San Antonio, TX, USA). Data are given as mean values ± SD of three or more experiments. Comparisons between means were made using the Student's *t*-test assuming significance at $p < 0.05$.

Chemical syntheses

General procedures. Tetrahydrofuran (THF) was distilled from deep blue solutions of sodium/benzophenone ketyl prior to use. Methylene chloride was shaken with concentrated H_2SO_4, dried over K_2CO_3, and distilled. All melting points were determined on a Kofler apparatus and are uncorrected. NMR spectra were recorded on a 200- or 300-MHz spectrometer operating in the Fourrier transform mode. ^{13}C NMR spectra were obtained with broadband proton decoupling. Chemical shifts were expressed as δ value with TMS (tetramethylsilane) or CCl_3F (for ^{19}F NMR) as internal standard. For ^{11}B NMR, the chemical shifts are in ppm relative to $BF_3{\cdot}OEt_2$. HRMS were obtained with a Varian MAT 311 mass spectrometer (Centre Régional de Mesures Physiques de l'Ouest). Microanalyses were performed at the Central Laboratory for Analysis, CNRS, Lyon (France). Silica gel 60F254 was used for column chromatography.

(*Z*)-1,4-Diazido-2-butene (5). To a stirred solution of *(Z)*-1,4-dichloro-2-butene (0.5 mL, 4.75 mmol) in DMSO (12 mL), NaN_3 (2.77 g, 42.7 mmol) was added and stirring was continued for 10 min at room temperature. Water (10 mL) was added to the solution, which was then extracted with AcOEt (3× 15 mL). The extract was dried ($MgSO_4$), concentrated to afford crude product **5** as a colorless oil (400 mg) which was used without further purification. ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.83 (m, 4H), 5.83 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 46.9, 127.8.

(*Z*)-1,4-Diamino-2-butene dihydrochloride (*Z*-DAB, 1). To a solution of the diazide **5** (230 mg) in 3 mL tetrahydrofuran was added a solution of triphenylphosphine (0.88 g, 3.35 mmol) in tetrahydrofuran (2 mL) with stirring. After 1 h at room temperature, the reaction mixture was heated at 50 °C for 3 h. An aqueous solution of hydrochloric acid (1 mL, 3 M) was added and the tem-

perature was maintained at 50 °C for another 1 h. The solvents were removed in vacuo and the residue was partitioned between methylene chloride (10 mL) and water (10 mL). The aqueous phase was extracted with methylene chloride (3 × 10 mL). Removal of the water under reduce pressure (40 °C) gave the diamine dihydrochloride **1** as a colorless solid (200 mg, 76%), which was recrystallized from a mixture of methanol–ether (1/1). Mp of *N*,*N*′-dibenzoyl derivative: 177–178 °C [lit,[44] mp 177.5–178.5 °C]. ^{1}H NMR (300 MHz, D_2O) δ 3.55 (d, J = 5.5 Hz, 4H), 5.69 (t, J = 5.5 Hz, 2H). ^{13}C NMR (75.5 MHz, D_2O) δ 38.7, 129.5. Anal. calcd for $C_4H_{12}N_2Cl_2$: C, 30.21; H, 7.60; N, 17.61. Found: C, 30.25; H, 7.58; N, 17.63.

1,4-di-(*tert*-Butoxycarbonyl)amino-2-butyne (6). To a stirred solution of 1,4-diamino-2-butyne dihydrochloride (2 g, 12.7 mmol) and Et_3N (3.9 mL, 28 mmol) in THF (13 mL) cooled to 0 °C was added dropwise a solution of $(Boc)_2O$ (6.11 g, 28 mmol) in THF (6 mL). The stirring was continued for 12 h at room temperature and the solvent was evaporated under vacuum. The residue was taken up in Et_2O (100 mL) and the solution was washed with a 0.01 N HCl solution (25 mL), then with water (25 mL), and finally with a 5% $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$, filtered, and evaporated under vacuum to give **6** (3 g, 83%) as a colorless solid which was used without further purification. Mp 120–121 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 32;1.41 (s, 18H), 3.91 (d, J = 5.4 Hz, 4H), 4.73 (bs, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.3, 30.5, 79.2, 79.9, 155.4.

***(E)*-2-(Tributylstannyl)-1,4-di(*tert*-butoxycarbonyl)-amino-2-butene (7).** To a THF solution (10 mL) of **6** (1 g, 3.52 mmol) containing $PdCl_2(PPh_3)_2$ (70 mg, 0.02 equiv) was added tributyltin hydride (1.42 mL, 5.28 mmol) dropwise with a syringe over a period of ca. 5–10 min. The originally light yellow solution abruptly turned orange-brown. After 30 min, an additional 0.2 equiv of tributyltin hydride was added, and stirring was continued for about 10 min. THF was then evaporated. The oily residue, which was now contaminated with black palladium impurities, was purified by column chromatography on silica gel (210–400 mesh), initially with 100% heptane to remove tin byproducts, followed by ethyl acetate/heptane (20/80). The desired vinylstannane **7** was obtained as a colorless oil (1.98 g, 98%); R_f = 0.25 (ethyl acetate/heptane = 1:9). ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.89 (m, 18H), 1.27 (m, 9H), 1.44 (s, 9H), 1.45 (s, 9H), 3.80 (d, J = 6.4 Hz, 2H), 3.94 (m, 2H), 4.75 (br s, 1H), 4.88 (br s, 1H), 5.63 [t, J = 6.4 Hz, 1H, $J(^{117}SnH)$ = 62 Hz, $J(^{119}SnH)$ = 64 Hz]. ^{13}C NMR (50 MHz, $CDCl_3$) δ 9.7, 13.7, 27.4, 28.4, 29.1, 38.1, 42.0, 77.1, 77.7, 138.4, 145.0, 155.6, 155.9. Anal. calcd for $C_{26}H_{52}N_2O_4Sn$: C, 54.27; H, 9.11; N, 4.87. Found: C, 54.30; H, 9.13; N, 4.90.

***(E)*-2-Iodo-1,4-di-(*tert*-butyloxycarbonyl)amino-2-butene (8).** A 0.17 M methylene chloride solution of iodine (1 mL) was added, over a period of 15 min, into a stirred solution of 100 mg (0.17 mmol) of **7** in methylene chloride (1 mL) under argon atmosphere. After 1 h at room temperature, the reaction mixture was washed twice with aqueous sodium bisulfite (10%) and then once with 5 mL of a 10% aqueous solution of potassium fluoride to convert tributyltin iodide into tributyltin fluoride.[45] The insoluble tributyltin fluoride was eliminated by filtration, and the organic phase was decanted and dried over magnesium sulfate. After evaporation of methylene chloride, the residue was purified by flash chromatography to afford the product **8** as a colorless oil (52 mg, 72%). R_f = 0.25 (ethyl acetate/heptane = 2:8). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.43 (s, 9H), 1.46 (s, 9H), 3.80 (d, J = 7.0 Hz, 2H), 4.18 (m, 2H), 5.15 (br s, 1H), 5.40 (br s, 1H), 6.38 (t, J = 7.0 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.4, 39.3, 46.4, 79.4, 80.1, 125.9, 139.7, 155.6, 155.8. Anal. calcd for $C_{24}H_{25}N_2O_4I$: C, 54.15; H, 4.73; N, 5.26. Found: C, 54.18; H, 4.74; N, 5.27.

***(E)*-2-Fluoro-1,4-di-(*tert*-butyloxycarbonyl)amino-2-butene (9).** 1-Chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]-octane bis(tetrafluoroborate) (370 mg, 1.04 mol) was added to a solution of vinyl stannane **7** (300 mg, 0.52 mmol) in dry CH_3CN (6 mL) and the stirring was continued at room temperature under argon atmosphere. After 6 days, the yellowish mixture was poured onto a plug of silica gel in a fritted glass funnel and eluted with hexane. The fractions containing product were combined and evaporated by rotary evaporator. Flash chromatography on silica gel afforded 64 mg of the product **9** as colorless oil (40%). R_f = 0.20 (ethyl acetate/heptane = 2:8). ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.42 (s, 9H), 1.44 (s, 9H), 3.73 (d, J = 8.6 Hz, 2H), 3.88 (d, J = 21.9 Hz, 2H), 5.11 (m, 2H), 5.30 (dt, J = 8.6, 19.0 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.4, 37.2, 37.9, 79.4, 80.1, 106.0 (J = 21.5 Hz), 128.8 (J = 245.7 Hz), 155.9. ^{19}F NMR (282 MHz, $CDCl_3$) δ −107.73. Anal. calcd for $C_{14}H_{25}N_2O_4F$: C, 55.25; H, 8.28; N, 9.20. Found: C, 55.30; H, 8.32; N, 9.22.

***(Z)*-2-{4-(5,5-Dimethyl-[1,3,2]dioxaborinan-2-yl)phenyl}-methyl-1,4-di-(*tert*-butyloxycarbonyl)amino-2-butene (10).** To a schlenk was successively added 40 mg of tetrakis(triphenylphosphine)palladium (0.035 mmol), 16 mL of dry toluene, 615 mg of 2-[4-(bromomethyl)phenyl]-5,5-dimethyl-1,3,2-dioxaborane (1.73 mmol) and 1 g of vinylstannane **7**. The resulting solution was stirred at reflux for 18 h, then cooled, partitioned between 30 mL of ether and 30 mL of one-third saturated potassium fluoride, and vigorously stirred for 30 min. The resulting precipitate of tri-*n*-butylstannyl fluoride was removed by gravity filtration, and the organic layer was separated, washed with brine, and dried ($MgSO_4$). The product **10** was isolated by flash chromatography as colorless oil (430 mg, 51%). R_f = 0.3 (ethyl acetate/heptane = 3:7). ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.02 (s, 6H), 1.44 (s, 18H), 3.37 (s, 2H), 3.72 (d, J = 6.2 Hz, 2H), 3.74–3.78 (m, 6H), 4.80 (br s, 1H), 5.15 (br s, 1H), 5.40 (m, 1H), 7.16 (d, J = 7.6 Hz, 2H), 7.74 (d, J = 7.6 Hz, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.9, 28.4, 31.9, 37.6, 39.0, 42.2, 72.3, 79.0, 127.7, 128.3, 134.1, 139.0, 142.0, 156.0. Anal. calcd for $C_{26}H_{41}N_2O_6B$: C, 63.94; H, 8.46; N, 5.74. Found: C, 63.93; H, 8.47; N, 5.77.

General procedure of amino-group deprotection

A solution of *N-tert*-butoxycarbonyl derivative (3 mmol) in ethanol (26 mL) was treated by a 12 M aqueous solution of hydrochloric acid (6.4 mL) at room temperature overnight. After removal of the solvent, the mixture was diluted in water (10 mL) and washed with ether. The aqueous solution was concentrated and dried under high vacuum.

***(E)*-2-Iodo-1,4-diamino-2-butene dihydrochloride (*E*-I-DAB, 2).** Hygroscopic colorless solid; 88%. ^{1}H NMR (200 MHz, D_2O) δ 3.61 (d, $J=7.4$ Hz, 2H), 3.91 (s, 2H), 6.59 (t, $J=7.4$ Hz, 1H). ^{13}C NMR (50 MHz, D_2O) δ 38.5, 44.8, 96.2, 139.0. Anal. calcd for $C_4H_{11}N_2Cl_2I$: C, 16.86; H, 3.89; N, 9.83. Found: C, 16.92; H, 3.93; N, 9.87.

***(E)*-2-Fluoro-1,4-diamino-2-butene dihydrochloride (*E*-F-DAB, 3).** Amorphous solid; 80%. ^{1}H NMR (200 MHz, D_2O) δ 3.52 (d, $J=8.3$ Hz, 2H), 3.79 (d, $J=19.4$ Hz, 2H), 5.52 (dt, $J=8.3$, 18.9 Hz, 1H). ^{13}C NMR (50 MHz, D_2O) δ 37.6, 38.0, 107.5 ($J=246.2$ Hz), 128.0. ^{19}F NMR (282 MHz, D_2O) δ −102.69. HRMS (EI) calcd for $C_4H_9N_2F$ (M^+) 104.0750, found 104.0751.

***(Z)*-2-{4-(5,5-Dimethyl-[1,3,2]dioxaborinan-2-yl)phenyl}-methyl-1,4-diamino-2-butene dihydrochloride (*Z*-4-Bbz-DAB, 4).** Amorphous solid; 87%. ^{1}H NMR (200 MHz, D_2O) δ 0.84 (s, 6H), 3.36 (s, 4H), 3.60 (s, 2H), 3.67 (s, 2H), 3.78 (d, $J=7.3$ Hz, 2H), 5.69 (t, $J=7.3$ Hz, 1H). ^{13}C NMR (50 MHz, D_2O) δ 20.7, 37.0, 37.7, 40.7, 68.4, 126.0, 129.2, 134.6, 138.1, 140.6. ^{11}B NMR (96 MHz, D_2O) δ 26.5. HRMS (EI) calcd for $C_{16}H_{25}N_2O_2B$ (M^+) 288.2009, found 288.2010.

Biological studies

Cells. 3LL murine Lewis lung carcinoma, CHO, and CHO-MG^R (provided by Dr. W. Flintoff, University of Western Ontario) cells were cultured in RPMI 1640 medium (Eurobio, Les Ulis, France) supplemented with 10% fetal calf serum, 2 mM glutamine, streptomycin (100 μg/mL) and penicillin (100 U/mL) (Biomerieux, Marcy l'Etoile, France). Two μg/mL L-proline was added in the culture medium for CHO-MG^R cells. Cells were grown at 37 °C under a humidified 5% CO_2 atmosphere. As other diamines, ***Z*-DAB** and its derivatives were not substrates of bovine serum amine oxidase (BSAO). This allows the study of the drugs to be carried out in culture medium with serum and without the addition of aminoguanidine, an inhibitor of BSAO.

Inhibition of putrescine uptake. 3LL cells were seeded in 12-well plates (Becton Dickinson, Oxnard, CA, USA) 24 h before the experiment (initial density = 0.5 10^6 cells/mL). Cells were then incubated for 20 min with 10 μM [^{14}C] putrescine (0.5 μCi) at 37 °C (or 4 °C for control of passive transport) in the absence or presence of putrescine derivatives. After three washes with phosphate-buffered saline, cells were dissolved in 0.5 mL of NaOH 0.1 N and radioactivity was determined on a 0.35 mL aliquot of the cell suspension after mixing with 2 mL of a scintillation cocktail (Pico-Fluor 15). Protein content was assayed on 0.1 mL of the cellular suspension by reaction with the Folin Phenol reagent.[46]

In vitro evaluation of drugs cytotoxicity/cytostasy. Cell growth effect was assayed in 96-wells plates (Becton Dickinson, Oxnard, CA, USA). Initial density was 1000 cells/well for 3LL, CHO and CHO-MG cells. Cell growth rates were determined by measuring formazan formation from 3-[4,5-dimethylthiazol-2-yl)]2,5-diphenyltetrazolium bromide (MTT) using a Titertek Multiskan MCC/340 microreader (Labsystems, Life Sciences Interactions, Cergy-Pontoise, France) for absorbance measurements.[47]

Accumulation of DAB derivatives in cells. Cells were cultured in tissue culture flasks at an initial density of 5×10^4/mL. Unless stated otherwise, drugs were added 24 h after seeding. Cells were washed three times with phosphate-buffered saline and harvested using trypsine/EDTA.

Determination of polyamines and of (*Z*)-DAB derivatives by HPLC. Cells were disrupted by sonication in 0.5 mL perchloric acid (0.2 N). After centrifugation (2500g), the supernatant was used for HPLC analysis. Protein content was determined on cell pellets according to Lowry et al.[46]

Determination of polyamines, *Z*-DAB, and *E*-I-DAB. Natural polyamines, ***Z*-DAB**, and ***E*-I-DAB** were separated by HPLC, after reaction with dansyl chloride, as previously described.[48] The following gradient was used for separation of the dansyl derivatives: 0 min (10% B); 15 min (50% B); 25 min (100% B); 29 min (100% B); 30 min (10% B); 33 min (10% B). Retention times of ***Z*–DAB**, putrescine, ***E*-I-DAB**, spermidine, and spermine were 13.3, 14.0, 14.8, 20.6, and 25.1 min, respectively.

Determination of polyamines and *Z*-4-Bbz-DAB. Polyamines and ***Z*-4-Bbz-DAB** were determined by separation of the ion pairs formed with *n*-octanesulfonic acid on a reversed-phase column, reaction of the column effluent with *o*-phtalaldehyde and *N*-acetylcysteine, and monitoring of fluorescence intensity, as previously described.[49] Retention times of putrescine, ***Z*-DAB**, ***Z*–4-Bbz-DAB**, spermidine and spermine were 14.9, 18.8, 22.0, and 26.2 min, respectively.

Boron measurements. 3LL cells were processed as previously described for boron analysis by ICP-AES.[18] ICP-AES determinations of boron were done using the 249.773 nm line on a ICP atomic emission spectrometer (JY 38 S, Jobin-Yvon ISA, Longjumeau, France).

Determination of ODC activity. A previously established [^{14}C]CO_2 trapping method was used with minor modifications.[48] Kidneys of testosterone-stimulated DBA2 mice were homogenized in 20 volumes of ice-cold Tris–HCl buffer (25 mM, pH 7.5) containing 1 mM dithiothreitol, 0.1 mM EDTA and 0.02% Brij 35. After a 15 min centrifugation at 25,000*g*, 190 μL aliquots of

the supernatants were used per assay (total assay volume 250 μL). Ornithine concentration per assay was 0.1 μmol (0.5 μCi). Blanks contained 5 mM DFMO in order to inactivate ODC.

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References and Notes

1. Cohen, S. S. *A Guide to the Polyamines*; Oxford University Press: Oxford/New York, 1998.
2. Seiler, N.; Delcros, J.-G.; Moulinoux, J.-P. *Int. J. Biochem. Cell Biol.* **1996**, *28*, 843.
3. Chen, K. Y.; Rinehart, C. A. *Biochem. Biophys. Res. Commun.* **1981**, *101*, 243.
4. Bachrach, U.; Seiler, N. *Cancer Res.* **1981**, *41*, 1205.
5. Volkow, N.; Goldman, S. S.; Flamm, E. S.; Cravioto, H.; Wolf, A. P.; Brodie, J. D. *Science* **1983**, *221*, 673.
6. Clarck, R. B.; Fair, W. R. *J. Nucl. Med.* **1975**, *16*, 337.
7. Alhonen-Hongisto, L.; Seppänen, P.; Jänne, J. *Biochem. J.* **1980**, *192*, 941.
8. Minchin, R. F.; Raso, A.; Martin, R. L.; Ilett, K. F. *Eur. J. Biochem.* **1991**, *200*, 457.
9. Porter, C. W.; Ganis, B.; Vinson, T.; Marton, L. J.; Kramer, D. L.; Bergeron, R. J. *Cancer Res.* **1986**, *46*, 6279.
10. Rinehart, C. A.; Chen, K. Y. *J. Biol. Chem.* **1984**, *259*, 4750.
11. Chaney, s. E.; Kobayaschi, K.; Goto, R.; Digenis, G. A. *Life Sci.* **1983**, *32*, 1237.
12. Heston, W. D. W.; Kadmon, D.; Covey, D. F.; Fair, W. R. *Cancer Res.* **1984**, *44*, 1034.
13. Redgate, E. S.; Grudziak, A. G.; Deutsch, M.; Boggs, S. S. *Int. J. Radiat. Oncol., Biol., Phys.* **1997**, *38*, 169.
14. Hawthorne, M. F. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 950.
15. Soloway, A. H.; Tjarks, W.; Barnum, B. A.; Rong, F.-G.; Barth, R. F.; Codogni, I. M.; Wilson, J. G. *Chem. Rev.* **1998**, *98*, 1515.
16. Cai, J. P.; Soloway, A. H.; Barth, R. F.; Adams, D. M.; Hariharan, J. R.; Wyzlic, I. M.; Radcliffe, K. *J. Med. Chem.* **1997**, *40*, 3887.
17. Zhuo, J.-C.; Cai, J.; Soloway, A. H.; Barth, R. F.; Adams, D. M.; Ji, W.; Tjarks, W. *J. Med. Chem.* **1999**, *42*, 1282.
18. Martin, B.; Possémé, F.; LeBarbier, C.; Carreaux, F.; Carboni, B.; Seiler, N.; Moulinoux, J.-P.; Delcros, J.-G. *J. Med. Chem.* **2001**, *44*, 3653.
19. Hull, W. E.; Kunz, W.; Port, R. E.; Seiler, N. *NMR Biomed.* **1988**, *1*, 11.
20. Hwang, D. R.; Jerabek, P. A.; Kadmon, D.; Kilbourn, M. R.; Patrick, T. B.; Welch, M. J. *Int. J. Radiat. Appl. Instrum., Part [A]* **1986**, *37*, 607.
21. Hwang, D. R.; Lang, L. X.; Mathias, C. J.; Kadmon, D.; Welch, M. J. *J. Nucl. Med.* **1989**, *30*, 1205.
22. Hwang, D. R.; Mathias, C. J.; Welch, M. J.; McGuire, A. H.; Kadmon, D. *Int. J. Radiat. Appl. Instrum., Part [B]* **1990**, *17*, 525.
23. Michelot, J. M.; Moreau, M.-F. C.; Labarre, P. G.; Madelmont, J.-C.; Veyre, A. J.; Papon, J. M.; Parry, D. F.; Bonafous, J. F.; Boire, J.-Y. P.; Desplanches, G. G.; Bertrand, S. J.; Meyniel, G. *J. Nucl. Med.* **1991**, *32*, 1573.
24. Michelot, J. M.; Moreau, M.-F. C.; Veyre, A. J.; Bonafous, J. F.; Bacin, F. J.; Madelmont, J.-C.; Bussiere, F.; Souteyrand, P. A.; Mauclaire, L. P.; Chossat, F. M.; Papon, J. M.; Labarre, P. G.; Kauffmann, P.; Plagne, R. J. *J. Nucl. Med.* **1993**, *34*, 1260.
25. Sarhan, S.; Dezeure, F.; Seiler, N. *Int. J. Biochem.* **1987**, *19*, 1037.
26. Samejima, K.; Nakazawa, Y. *Arch. Biochem. Biophys.* **1980**, *201*, 214.
27. Pegg, A. E.; Nagarajan, S.; Nafiey, S.; Ganem, B. *Biochem. J.* **1991**, *274*, 167.
28. O'Sullivan, M. C.; Golding, B. T.; Smith, L. L.; Wyatt, I. *Biochem. Pharmacol.* **1991**, *41*, 1839.
29. Mandel, J. L.; Flintoff, W. F. *J. Cell. Physiol.* **1978**, *97*, 335.
30. Seiler, N.; Delcros, J. G.; Vaultier, M.; LeRoch, N.; Havouis, R.; Douaud, F.; Moulinoux, J. P. *Cancer Res.* **1996**, *56*, 5624.
31. Gololobov, Y. G.; Zhmurova, I. N.; Kasukhin, L. F. *Tetrahedron* **1981**, *37*, 437.
32. Lebarbier, C.; Carreaux, F.; Carboni, B. *Tetrahedron Lett.* **1999**, *40*, 6233.
33. Matthews, D. P.; Miller, S. C.; Jarvi, E. S.; Sabol, J. S.; McCarthy, J. R. *Tetrahedron Lett.* **1993**, *34*, 3057.
34. Takeuchi, M.; Mizuno, T.; Shinmori, H.; Nakashima, M.; Shinkai, S. *Tetrahedron* **1996**, *52*, 1195.
35. Shirahata, A.; Takahashi, N.; Beppu, T.; Hosoda, H.; Samejima, K. *Biochem. Pharmacol.* **1993**, *45*, 1897.
36. Beppu, T.; Shirahata, A.; Takahashi, N.; Hosoda, H.; Samejima, K. *J. Biochem.* **1995**, *117*, 339.
37. He, Z.; Nadkarni, D. V.; Sayre, L. M.; Greenaway, F. T. *Biochim. Biophys. Acta* **1995**, *1253*, 117.
38. Havis, N. D.; Walters, D. R.; Foster, S. A.; Martin, W. P.; Cook, F. M.; Robins, D. J. *Pestic. Sci.* **1994**, *41*, 61.
39. Relyea, N.; Rando, R. R. *Biochem. Biophys. Res. Com.* **1975**, *67*, 392.
40. Kameji, T.; Murakami, Y.; Hayashi, S.-I. *J. Biochem.* **1979**, *86*, 191.
41. Porter, C. W.; Bergeron, R. J. *Science* **1983**, *219*, 1083.
42. Porter, C. W.; Cavanaugh, P. F.; Stolowich, N.; Ganis, B.; Kelly, E.; Bergeron, R. J. *Cancer Res.* **1985**, *45*, 2050.
43. Mitchell, J. L. A.; Judd, G. G.; Bareyal-Leyser, A.; Ling, S. Y. *Biochem. J.* **1994**, *299*, 19.
44. Fabiano, E.; Golfind, B. T.; Sadeghi, M. M. *Synthesis* **1987**, 190.
45. Harpp, D. N.; Gongras, M. J. *J. Am. Chem. Soc.* **1988**, *110*, 7737.
46. Lowry, O. H.; Rosenbrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265.
47. Mosmann, T. J. *Immunol. Methods* **1983**, *65*, 55.
48. Delcros, J. G.; Vaultier, M.; Roch, N. L.; Havouis, R.; Moulinoux, J. P.; Seiler, N. *Anti-Cancer Drug Des.* **1997**, *12*, 35.
49. Seiler, N.; Knödgen, B. *J. Chromatogr.* **1980**, *221*, 227.