N-Benzylpolyamines as Vectors of Boron and Fluorine for Cancer Therapy and Imaging: Synthesis and Biological Evaluation

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Received April 9, 2001

Cancer cells have high-affinity polyamine uptake systems with a low stringency for structural features. Putrescine, spermidine, and spermine have, therefore, been considered as potential vectors for the selective accumulation in tumors of therapeutically or diagnostically useful structures and elements. We envisaged *N*-benzyl derivatives of the polyamines as vectors of ^{10}B and ^{18}F for boron neutron capture therapy (BNCT) and tumor imaging by positron emission tomography (PET), respectively. In the present work, the synthesis, transport characteristics, DNA-binding properties, and cytotoxicity of several *N*-benzyl derivatives of putrescine and spermidine are described. The fluorinated spermidine derivative *N*-{3-[(4-aminobutyl)amino]-propyl}[(4-fluorophenyl)methyl]amine (**N^1-4-Fbz-spd**) may be useful for PET because of its high accumulation in cancer cells via the polyamine transport system. Among the boron-containing benzyl polyamines, *N*-(4-aminobutyl){[4-(dihydroxyboryl)phenyl]methyl}amine (**4-Bbz-put**) and *N*-{3-[(4-aminobutyl)amino]propyl}{[4-(dihydroxyboryl)phenyl]methyl}amine (**N^1-4-Bbz-spd**) should be suitable for BNCT, because their accumulation in B16 melanoma cells was more efficient than that of borocaptate and borophenylalanine, two reference compounds used in BNCT.

Introduction

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

(i) The specificity of the polyamine transport system is not stringent. A wide variety of synthetic polyamine derivatives and analogues are capable of using 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 polyamine concentrations, e.g., 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 nongrowing or slow-growing cells. As a consequence, polyamine depletion increases the uptake rate of tumor cells, which readily accumulate high amounts of polyamines.[11−13]

(iv) Another property of interest of spermidine and spermine for tumor targeting is their high-affinity binding to DNA.[14] If a polyamine derivative is taken up via the polyamine transport system, it will in part be located in the cell nucleus.

All these properties have led many researchers to design polyamine derivatives for tumor targeting. Known chemotherapeutic drugs have been conjugated to polyamines to enhance cytotoxicity to tumor cells and to diminish secondary effects on normal cells. For example, DNA damage by a chlorambucil−spermidine conjugate was enhanced,[15] and among others, nitroimidazole[16] and aziridine[17] polyamine conjugates have been synthesized, with improved therapeutic indexes.

The polyamine transport system has also been used to accumulate various isotopes in several cancer cells, for either cancer curing or tumor imaging. Boron neutron capture therapy (BNCT) is an emerging modality for the treatment of brain tumors and melanomas. It relies on the formation of energy-rich α particles with a short action radius by irradiation of the ^{10}B isotope with low-energy neutrons ($^{10}B(n,\alpha)^7Li$); for reviews, see refs 18 and 19. *o*-Carborane cages attached to the primary or secondary amino groups of spermidine and spermine have been made.[20,21] In vitro, these compounds bind to DNA and accumulate in tumor cells similarly

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10.1021/jm010897q CCC: $20.00
Published on Web 10/18/2001

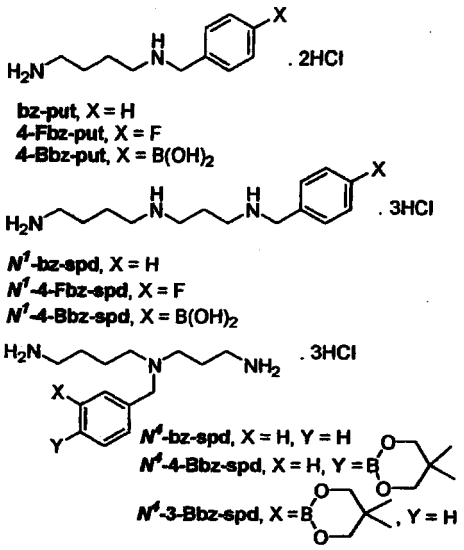


Figure 1. *N*-Benzylpolyamines.

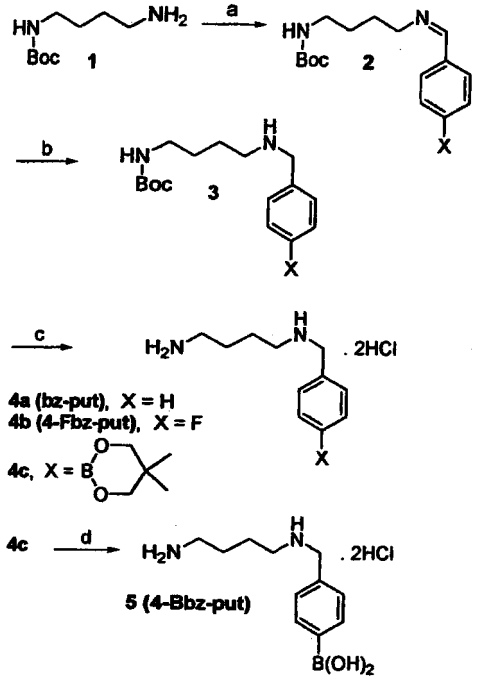
as borocaptate (BSH) and *p*-borophenylalanine (BPA), which are in clinical trial. However, the cytotoxicity of the carboranes prohibits their use. Fluorine-containing polyamines have been suggested for noninvasive imaging of tumors by ^{18}F positron emission tomography (PET; for reviews, see refs 22 and 23) or by ^{19}F NMR. Various fluorinated putrescine derivatives have been synthesized for this purpose.[24−27] Finally, iodine-substituted diamines were studied for application in ^{125}I scintigraphy.[28,29]

Because conjugates of spermine exhibit high toxicity,[20,30] we focused our effort on putrescine and spermidine derivatives. To be taken up via the polyamine transport system, spermidine derivatives should preferentially be substituted on the N^1-amino group, so that the aminobutyl moiety is free to interact with the transporter,[31,32] but N^4-substituted spermidines are also substrates of the polyamine transport system.[33] On the basis of these results, we envisaged to prepare spermidine derivatives carrying a heterosubstituted benzyl substituent, either on the terminal nitrogen (**N^1-bz-spd**, **N^1-4-Fbz-spd**, **N^1-4-Bbz-spd**) or on the central nitrogen (**N^4-bz-spd**, **N^4-4-Bbz-spd**, **N^4-3-Bbz-spd**) (Figure 1) as potential agents for BNCT or PET imaging. Boron- or fluorine-substituted *N*-benzylputrescines (**bz-put**, **4-Fbz-put**, and **4-Bbz-put**) were also synthesized. Boron and fluorine were preferentially introduced in the *para*-position of the aromatic moiety, because of the greater stability of the resulting products. In vitro biological properties of these compounds were evaluated in comparison with the unsubstituted parent *N*-benzylpolyamines. This included DNA-binding, cytotoxicity, and the ability to use the polyamine transport system. The accumulation of the compounds in target cells is also reported.

Chemical Syntheses

Syntheses of the target compounds *N*-(4-aminobutyl)-(phenylmethyl)amine dihydrochloride (**4a**, **bz-put**), *N*-(4-aminobutyl)[(4-fluorophenyl)methyl]amine dihydrochloride (**4b**, **4-Fbz-put**), and *N*-(4-aminobutyl){[4-(dihydroxyboryl)phenyl]methyl}amine dihydrochloride (**5, 4-Bbz-put**) are shown in Scheme 1. The mono-Boc-protected 1,4-diaminobutane **1**, obtained by reaction of $(Boc)_2O$ with a large excess of 1,4-diaminobutane,[34] was employed as starting material. Reductive amination of **1** to **3** was achieved in two steps: imine **2** was obtained from **1** and benzaldehyde derivatives, and was then further reduced to **3** with $NaBH_4$. 2-(4-Formylphenyl)-5,5-dimethyl-1,3,2-dioxaborane was prepared from 4-bromobenzaldehyde by a described procedure.[35] The *tert*-butoxycarbonyl protecting group was removed quantitatively by acidic treatment of **3**. Impurities from **3** were completely removed by organic extractions, to afford products **4** of high purity. Boronic acid **5** from 2,2-dimethylpropane-1,3-diol boronate ester **4c** was prepared by reacting phenylboronic acid in a two-phase ether/water system.[36] This procedure permits **5** to be obtained in high purity owing to the differences in solubility. The starting 2,2-dimethylpropane-1,3-diol ester **4c** and the free boronic acid are soluble in the aqueous phase while the 2,2-dimethylpropane-1,3-diol phenylboronate ester accumulates in the organic phase.

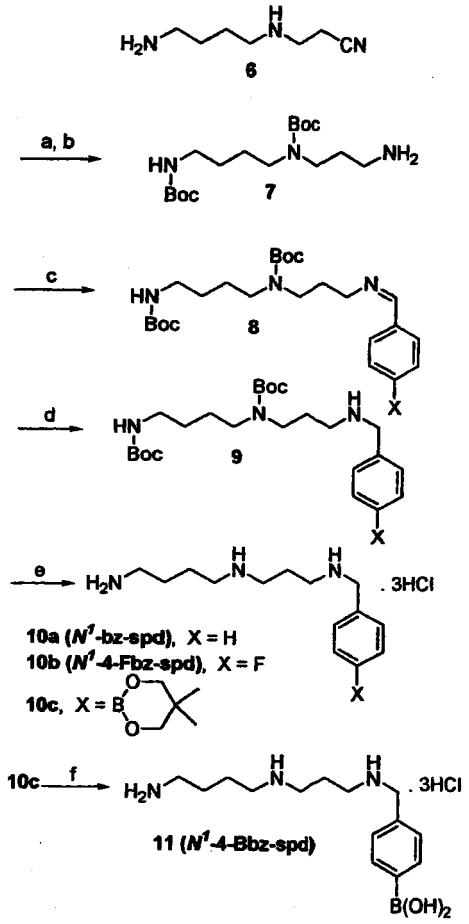
Scheme 1[a]



[a] Reagents: (a) XPhCHO, 3 Å molecular sieves, Et_2O; (b) $NaBH_4$, EtOH; (c) HCl (12 M), EtOH; (d) $PhB(OH)_2$, Et_2O/H_2O.

N-{3-[(4-Aminobutyl)amino]propyl}(phenylmethyl)-amine trihydrochloride (**10a**, **N^1-bz-spd**), *N*-{3-[(4-aminobutyl)amino]propyl}[(4-fluorophenyl)methyl]amine trihydrochloride (**10b**, **N^1-4-Fbz-spd**), and *N*-{3-[(4-aminobutyl)amino]propyl}{[4-(dihydroxyboryl)phenyl]-methyl}amine trihydrochloride (**11**, **N^1-4-Bbz-spd**) were synthesized by the route illustrated in Scheme 2 from 3-[(4-aminobutyl)amino]propanenitrile[37] (**6**). *tert*-Butyl *N*-(3-aminopropyl)-*N*-{4-{[(*tert*-butyloxy)carbonyl]amino}-butyl}carbamate (**7**) was obtained from **6** according to the literature[38] by protection of the primary and secondary amino groups with a Boc, and reduction of the nitrile group. Benzylation of **7** with substituted benzaldehydes was effected by reductive amination in high yields. Removal of the Boc groups by acidic cleavage afforded the target compounds **10a** and **10b. N^1-4-Bbz-spd (11)** was obtained from **10c** using the same process as that used for the preparation of **5**.

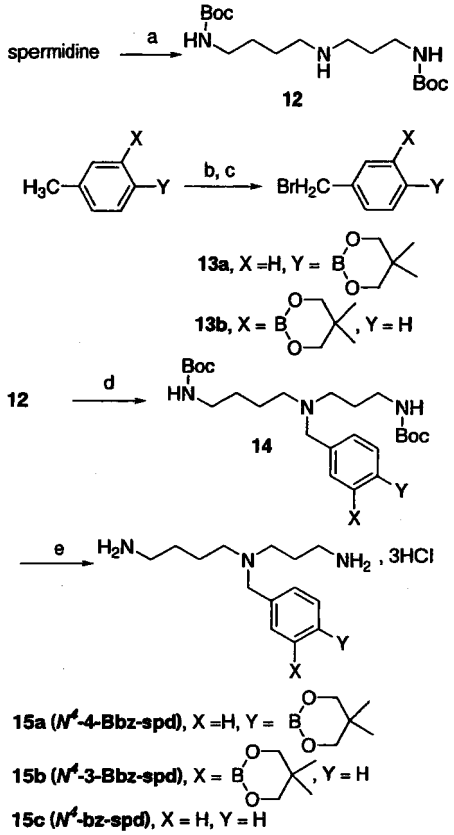
Scheme 2[a]



[a] Reagents: (a) $(Boc)_2O$, THF; (b) H_2, 5 bar, Raney-Ni, EtOH, NaOH; (c) XPhCHO, 3 Å molecular sieves, Et_2O; (d) $NaBH_4$, EtOH; (e) HCl (12 M), EtOH; (f) $PhB(OH)_2$, Et_2O/H_2O.

The desired target compounds *N*-(3-aminobutyl)-*N*(3-aminopropyl){{4-(5,5-dimethyl[1,3,2]dioxaborinan-2-yl)-phenyl}methyl}amine trihydrochloride (**15a**, **N^4-4-Bbz-spd**), and *N*-(3-aminobutyl)-*N*-(3-aminopropyl){{3-(5,5-dimethyl[1,3,2]dioxaborinan-2-yl)phenyl}methyl}-amine trihydrochloride (**15b**, **N^4-3-Bbz-spd**) were obtained according to a convergent synthetic route illustrated in Scheme 3 from spermidine and phenyl boronic acid derivatives. The *tert*-butyl *N*-{4-{*N*-{3-[(*tert*-butyloxycarbonyl)amino]propyl}amino}butyl}carbamate (**12**) was first synthesized from the free triamine using 2-[(*tert*-butoxycarbonyl)oxyimine]-2-phenylacetonitrile (Boc-ON), to selectively protect the primary amino groups.[32] The 2,2-dimethylpropane-1,3-diol ester of the [4-(bromomethyl)phenyl]- and [3-(bromomethyl)-phenyl]boronic acids **13a** and **13b** were prepared by halogenation of the corresponding (4-methylphenyl)- and (3-methylphenyl)boronate with *N*-bromosuccinimide.[39] Alkylation of **12** was performed in the presence of $NaHCO_3$ and NaI. Deprotection of the resulting spermidine derivatives **14** was achieved with aqueous concentrated hydrochloric acid in ethanol. *N*-(3-Aminobutyl)-*N*-(3-aminopropyl)(phenylmethyl)amine[40] trihydrochloride (**15c**, **N^4-bz-spd**) was prepared in a similar way from **12** and benzyl bromide. It should be noted that the *N*-benzylated spermidines **15** are quite hygroscopic.

Scheme 3[a]



[a] Reagents: (a) Boc-ON, THF; (b) 2,2-dimethylpropane-1,3-diol, Et_2O; (c) NBS, AIBN, CCl_4; (d) **13a** or **13b** or $PhCH_2Br$, $NaHCO_3$, NaI; (e) HCl (12 M), EtOH.

Table 1. DNA Binding Affinity of *N*-Benzylpolyamine Derivatives[a]

compound	IC_{50} (μM)[b]	compound	IC_{50} (μM)[b]
putrescine	>11000	**N^1-bz-spd**	107 ± 9
bz-put	3109 ± 765	**N^1-4-Fbz-spd**	341 ± 42
4-Fbz-put	>3000	**N^1-4-Bbz-spd**	37 ± 2
4-Bbz-put	1382 ± 243	**N^4-bz-spd**	310 ± 39
spermidine	98 ± 20	**N^4-4-Bbz-spd**	102 ± 0.6

[a] As measured by displacement of ethidium bromide from calf thymus DNA. [b] Concentration of *N*-benzylpolyamine derivative required to decrease fluorescence intensity of the DNA−ethidium bromide complex by 50%. (Data are expressed as mean ± SD).

Biological Studies

1. DNA Binding Assay. IC_{50} values, defined as the concentration of a polyamine derivative required to decrease the fluorescence of the ethidium bromide−DNA complex by 50%, are reported in Table 1. As observed with their natural counterparts, spermidine derivatives were better ligands for DNA than the corresponding putrescine derivatives. Notably, **N^1-bz-spd** exhibited an affinity for DNA equivalent to that of spermidine, whereas **N^4-bz-spd** was 3 times less efficient. Addition of a boron group to the parent *N*-benzylpolyamines increased the ability to displace ethidium bromide. **N^1-4-Bbz-spd** exhibited about a 2.5 times higher affinity for DNA than the natural spermidine. In contrast, fluorinated compounds displayed a weaker affinity for DNA than the parent compounds.

2. Ability of Polyamine Derivatives To Use the Polyamine Transport System. The ability of the derivatives to be taken up by active transport was

Table 2. Accumulation of *N*-Benzylpolyamine Derivatives in CHO and Polyamine Transport Deficient CHO-MG Cells[a]

	dose (μM)	CHO (nmol/mg of protein)	CHO-MG (nmol/mg of protein)	ratio CHO/CHO-MG
bz-put	50	0.40 ± 0.03	0.10 ± 0.02[b]	4.0
4-Fbz-put	50	0.90 ± 0.05	0.45 ± 0.04[b]	2.0
4-Bbz-put	50	5.76 ± 0.25	0.51 ± 0.06[b]	11.3
N^1-bz-spd	50	17.58 ± 2.8	bdl[b]	>35
N^1-4-Fbz-spd	10	9.40 ± 0.89	bdl[b]	>19
N^1-4-Bbz-spd	50	4.78 ± 0.46	bdl[b]	>9.6
N^4-bz-spd	250	13.64 ± 2.60	5.55 ± 0.72[b]	2.5
N^4-4-Bbz-spd	250	5.05 ± 0.68	3.84 ± 0.87[b]	1.3
N^4-3-Bbz-spd	250	5.65 ± 0.02	3.71 ± 0.09[b]	1.5

[a] CHO and CHO-MG cells were incubated for 24 h with the compounds. Levels of *N*-benzylpolyamine derivatives were determined by HPLC (data are expressed as mean ± SD). bdl = below detection limit (<0.5 nmol/mg of protein). [b] $p < 0.01$: significantly different from values for CHO cells.

Table 3. Effect of *N*-benzylpolyamine Derivatives, BSH, and BPA on 3LL Cell Growth

	IC_{50} (μM)[a,e]		effect of DFMO pretreatment[b,e]
	72 h incubation	48 h incubation	48 h incubation
BSH	147 ± 50	287 ± 20	ND[c]
BPA[d]	>100	>100	ND[c]
bz-put	604 ± 32	993 ± 62	partial reversion (>50 μM)
4-Fbz-put	468 ± 21	568 ± 64	partial reversion (>10 μM)
4-Bbz-put	>1000	>1000	partial reversion (>50 μM)
N^1-bz-spd	9.3 ± 0.9	31.0 ± 1.0	synergy (IC_{50} = 11.9 ± 3.6 μM)
N^1-4-Fbz-spd	2.3 ± 0.8	4.7 ± 1.1	synergy (IC_{50} = 0.5 ± 0.3 μM)
N^1-4-Bbz-spd	400 ± 35	824 ± 176	partial reversion (>1 μM)
N^4-bz-spd	>1000	>1000	complete reversion (>100 μM)
N^4-4-Bbz-spd	>1000	>1000	complete reversion (>10 μM)
N^4-3-Bbz-spd	>1000	>1000	ND[c]

[a] Compounds were added to the culture medium 24 h after seeding. [b] Twenty-four hours after seeding, 5 mM DFMO was added for a 24 h preincubation period before addition of other drugs. [c] ND = not determined. [d] Because of its poor solubility, the cytotoxicity of BPA was not tested at concentrations superior to 100 μM. [e] All data are expressed as mean ± SEM.

determined by comparing their accumulation in CHO cells and in mutant polyamine transport deficient CHO-MG cells. As shown in Table 2, at concentrations that did not affect cell viability, all derivatives were more actively taken up by CHO than by CHO-MG cells. The highest CHO/CHO-MG accumulation ratios were observed with the N^1-benzylspermidine derivatives. These were not detected in CHO-MG cells. In contrast, the accumulation ratios for the N^4-spermidine derivatives were low, despite their considerable accumulation in CHO cells, which was of the same order of magnitude as that of the N^1-derivatives. Among the putrescine derivatives, **4-Bbz-put** showed the greatest accumulation in CHO cells and an accumulation ratio superior to 11.

3. Studies on Lewis Lung Carcinoma Cells (3LL Cells). Effect of Polyamine Derivatives on Cell Growth and Polyamine Metabolism of 3LL Cells. The effect of the drugs on the growth of 3LL cells was evaluated using an MTT assay. The concentrations of the drugs causing 50% cell growth inhibition (IC_{50}) are reported in Table 3.

Among the N^1-benzylspermidines, the fluorine-containing derivative was the most cytotoxic (IC_{50} around 2 μM at 72 h), followed by the compound without a substituent on the benzyl moiety, while the boronated derivative showed considerably less growth inhibition (IC_{50} around 400 μM at 72 h). The N^4-benzylspermidines did not show any cytotoxicity on 3LL cells up to 1 mM. *N*-Benzylputrescine exhibited a very moderate effect on 3LL cell growth ($IC_{50} \approx 600$ μM). Its fluorinated analogue **4-Fbz-put** was slightly more cytotoxic, while the boron-containing derivative did not affect cell growth up to 1 mM. The boronated compounds appear even less cytotoxic than BSH.

Table 4. Polyamine Levels in 3LL Cells after Treatment with *N*-Benzylpolyamine Derivatives[a]

	drug	dose (μM)	putrescine (nmol/mg of protein)	spermidine (nmol/mg of protein)	spermine (nmol/mg of protein)
control[b]	none		0.74 ± 0.21	9.28 ± 1.17	7.24 ± 0.55
	N^4-4-Bbz-spd	50	0.12 ± 0.03	15.54 ± 0.73	11.28 ± 0.39
DFMO[c]	none		Bdl	bdl	6.68 ± 0.67
	bz-put	50	Bdl	0.89 ± 0.07	5.79 ± 0.11
	4-Fbz-put	50	Bdl	0.92 ± 0.27	10.99 ± 2.30
	4-Bbz-put	50	Bdl	1.07 ± 0.08	7.52 ± 0.002
		250	1.31 ± 0.30	2.28 ± 0.00	7.66 ± 0.20
	N^1-bz-spd	10	Bdl	bdl	7.23 ± 0.29
	N^1-4-Fbz-spd	1	Bdl	bdl	8.25 ± 1.11
	N^1-4-Bbz-spd	50	Bdl	0.14 ± 0.07	7.25 ± 0.31
	N^4-bz-spd	250	Bdl	4.15 ± 0.46	8.64 ± 0.60
	N^4-4-Bbz-spd	50	Bdl	8.92 ± 0.88	7.40 ± 0.07

[a] The polyamine content was evaluated by HPLC (data are expressed as mean ± SD). bdl = below detection limit. [b] 3LL cells were incubated for 24 h in the presence or absence of **N^4-4-Bbz-spd** before processing. [c] 3LL cells were incubated for 24 h with polyamine derivatives after a 24 h preincubation period in the presence of 5 mM DFMO.

Most of the benzyl derivatives had no or a limited effect on intracellular polyamine pools. At cytotoxic concentrations, N^1-benzylspermidine and its fluorine analogue induced a significant decrease of intracellular putrescine levels but did not affect spermidine and spermine concentrations (data not shown). Both **N^4-4-Bbz-spd** and **N^4-3-Bbz-spd** derivatives induced a decrease in putrescine and an approximate increase of both spermidine and spermine concentrations by 60% (Table 4), whereas **N^4-bz-spd** did not affect polyamine levels at the same concentration.

The influence of DFMO on the cytotoxicity of the polyamine derivatives was studied by monitoring 3LL cell growth after a 24 h preincubation with 5 mM DFMO, followed by a 48 h exposure to the drugs (Table 3). Combined exposure with DFMO greatly enhanced the cytotoxicity of N^1-benzylspermidine and of its fluorinated analogue. In the presence of DFMO, the IC_{50} values of **N^1-bz-spd** and **N^1-4-Fbz-spd** were diminished by 90%. In contrast, all the other *N*-benzyl derivatives antagonized DFMO-induced cell growth arrest; i.e., they supported growth of putrescine- and spermidine-depleted cells, as shown in Figure 2 for spermidine and N^4-benzylspermidine derivatives. However, total reversion of the DFMO effect required 10 and 100 times higher concentrations of **N^4-4-Bbz-spd** and **N^4-bz-spd,** respectively, than of spermidine. The putrescine derivatives as well as **N^1-4-Bbz-spd** reversed only partially the effect of DFMO (see Figure 2).

In 3LL cells pretreated with DFMO, intracellular putrescine and spermidine were below the detection level (Table 4). Polyamine pools remained unchanged in 3LL cells treated with **N^1-bz-spd** and **N^1-4-Fbz-spd**, the derivatives showing a synergistic effect with DFMO. In contrast, significant amounts of spermidine were detected in cells treated with the other derivatives. In cells treated with 50 μM **N^4-4-Bbz-spd**, spermidine

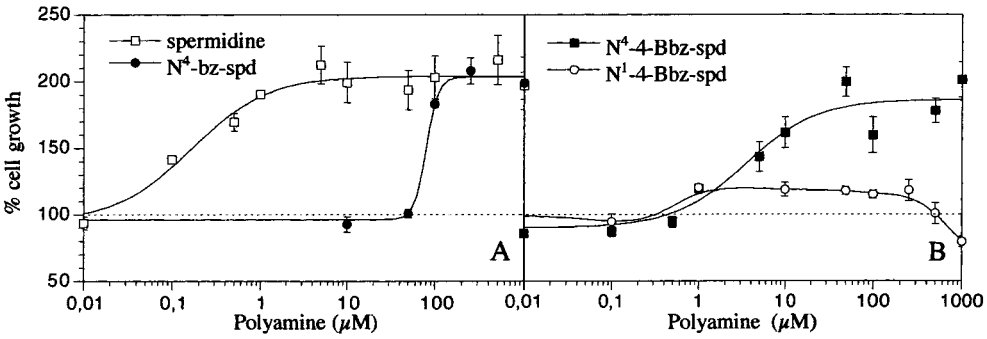


Figure 2. Reversion of the cytostatic effect of DFMO by spermidine and *N*-benzylspermidines in 3LL cells: (A) effect of spermidine and **N^4-bz-spd**; (B) effect of **N^4-4-Bbz-spd** and **N^1-4-Bbz-spd**. Twenty-four hours after seeding, 5 mM DFMO was added to the 3LL cell culture medium for 24 h, followed by a 48 h incubation period with spermidine or a spermidine derivative. The percent cell growth was calculated from the value of the cell growth of control cells treated only with DFMO.

Table 5. Effect of a DFMO Pretreatment on the Accumulation of N-benzyl Polyamine Derivatives in 3LL Cells

		HPLC measurements[c] drug (nmol/mg of protein)			ICP-AES measurements[c] boron (nmol/mg of protein)	
drug	dose (μM)	without DFMO[a]	with DFMO[b]	ratio with/without DFMO	without DFMO[a]	with DFMO[b]
BSH	50	ND	ND	ND	0.82 ± 0.30	ND
BPA	50	ND	ND	ND	0.80 ± 0.09	ND
bz-put	50	0.34 ± 0.19	1.03 ± 0.22[d]	3.0		
4-Fbz-put	50	0.55 ± 0.06	1.31 ± 0.04[d]	2.4		
4-Bbz-put	10	0.52 ± 0.01	3.37 ± 0.06[d]	6.5		
	50	1.32 ± 0.27	8.44 ± 1.27[d]	6.4	1.29 ± 0.10	6.73 ± 1.69
N^1-bz-spd	10	9.00 ± 0.46	23.67 ± 2.46[d]	2.7		
N^1-4-Fbz-spd	1	5.51 ± 0.29	12.19 ± 2.37[d]	2.2		
	10	12.87 ± 1.18	ND	ND		
N^1-4-Bbz-spd	50	2.01 ± 0.73	4.18 ± 1.27[d]	2.1	2.70 ± 1.22	5.68 ± 0.43
N^4-bz-spd	10	2.59 ± 0.04	5.52 ± 0.36[d]	2.1		
	50	4.92 ± 0.00	6.92 ± 0.66[d]	1.4		
	250	6.11 ± 0.41	5.46 ± 0.35	0.9		
N^4-4-Bbz-spd	50	2.60 ± 0.50	0.98 ± 0.17	0.4	3.46 ± 1.13	1.83 ± 0.20[e]
	250	3.35 ± 0.51	2.55 ± 0.05	0.8		
N^4-3-Bbz-spd	250	4.52 ± 0.67	3.93 ± 0.26	0.9		

[a] Cells were incubated for 24 h with the derivatives or BSH. [b] After a 24 h incubation period with 5 mM DFMO, cells were further incubated for 24 h with the *N*-benzylpolyamine derivatives. [c] ND = not done. Data are expressed as mean ± SD. [d] $p < 0.01$: significantly different from control values without DFMO. [e] $p < 0.01$: significantly different from values measured by HPLC.

levels were restored to normal values, but putrescine remained undetectable. In contrast, in cells treated with 250 μM **4-Bbz-put**, putrescine pools were replenished.

Accumulation of Polyamine Derivatives in 3LL Cells. Intracellular levels of the polyamine derivatives were determined in 3LL cells after a 24 h exposure at concentrations that did not affect cell growth, by HPLC of the respective compound. The accumulation of the derivatives in cells pretreated with DFMO for 24 h is also reported (Table 5).

At identical extracellular concentrations, 3LL cells accumulated higher amounts of spermidine than of the putrescine derivatives. The fluorine-containing polyamine derivatives exhibited accumulation in 3LL cells comparable to that of their parent compounds. In contrast, the boron-containing moiety enhanced the accumulation of the putrescine derivative but diminished that of the spermidine derivatives.

Pretreatment of 3LL cells with DFMO enhanced the accumulation of all derivatives except that of **N^4-4-Bbz-spd**. The highest difference between DFMO-treated and control cells was observed with **4-Bbz-put** (ratio around 6, while it was only around 2 for the other derivatives). With **N^4-bz-spd**, the accumulation ratio decreased from 2.1 to 0.9 when concentrations in the medium increased from 10 to 250 μM. For **N^4-4-Bbz-spd**, the ratio remained below 1 in the range 50−250 μM.

Measurement of the cellular boron content was performed in 3LL cells treated with the boron-containing analogues (Table 5). For all boron-containing polyamines, the boron content was similar to that of the amount of the drug determined by HPLC. The same observation was made in DFMO-treated cells, except in cells exposed to **N^4-4-Bbz-spd**, where the boron content was significantly higher. All boron-containing polyamine derivatives allowed boron accumulations similar to or higher than those of BSH and BPA. When 3LL cells were pretreated with DFMO, boron concentrations with polyamine derivatives were 2−8-fold higher than with BSH or BPA.

4. Accumulation of Boronated Polyamines in Various Cell Lines. As melanoma and brain tumors are the main targets for BNCT, accumulation of boron-containing derivatives was studied in murine C6 glioblastoma and B16 melanoma cells, as well as in human U251 glioblastoma cells (Table 6). **4-Bbz-put** and **N^1-4-Bbz-spd** accumulated more efficiently in B16 cells than in C6 and U251 cells. In contrast, accumulation of **N^4-4-Bbz-spd** was superior in glioblastoma cells. Pretreatment of the cells with DFMO enhanced the

Table 6. Accumulation of Boron-Containing *N*-Benzylpolyamine Derivatives in Melanoma[a] and Glioblastoma[b] Cells

drug (50 μM, 24 h exposure)		C6 (nmol/mg of protein)[c]	U251 (nmol/mg of protein)[c]	B16 (nmol/mg of protein)[c]
4-Bbz-put	control[d]	0.09 ± 0.02	0.21 ± 0.07	5.77 ± 0.47
	DFMO[e]	0.41 ± 0.03[f]	0.22 ± 0.01	20.41 ± 0.05[f]
	ratio ± DFMO	4.6	1.1	3.5
N^1-4-Bbz-spd	control[d]	1.63 ± 0.03	1.65 ± 0.04	4.75 ± 0.11
	DFMO[e]	3.51 ± 0.06[f]	2.52 ± 0.08[f]	9.26 ± 0.95[f]
	ratio ± DFMO	2.2	1.5	2.0
N^4-4-Bbz-spd	control[d]	2.92 ± 0.22	2.12 ± 0.03	0.92 ± 0.24
	DFMO[e]	2.50 ± 0.25	1.79 ± 0.02[f]	0.45 ± 0.03
	ratio ± DFMO	0.9	0.8	0.5

[a] Murine B16 melanoma cells. [b] Rat C6 glioblastoma cells and human U251 glioblastoma cells. [c] Levels of *N*-benzylpolyamine derivatives were measured by HPLC (data are expressed as mean ± SD). [d] Cells were incubated with 50 μM boron-containing polyamine for 24 h. [e] After a 24 h incubation period with 5 mM DFMO, cells were further incubated for 24 h with 50 μM polyamine derivatives. [f] $p < 0.01$: significantly different from control values without DFMO.

accumulation of **4-Bbz-put** in C6 and B16 cells about 4 times, but had no effect in U251 cells. DFMO pretreatment increased the accumulation of **N^1-4-Bbz-spd** by a factor of 2 in all three cell lines. In contrast, **N^4-4-Bbz-spd** amounts were in all cell lines similar or lower in DFMO-treated vs normal cells.

Discussion

The rationale behind the synthesis of new *N*-benzylpolyamines was to develop compounds sharing some properties of the natural polyamines, particularly their low cytotoxicity and their ability to be taken up by cells via a specific polyamine transport system. The up-regulation of the activity of this transport system in cancer cells after treatment with DFMO allows the preferential accumulation of drugs in the tumor cells if they are transported by this system. High tumor/normal tissue ratios are a requirement for drugs used in BNCT as well as for probes used in imaging.

All three parent *N*-benzylpolyamines use the polyamine transport system as became obvious from the greater accumulation in CHO cells than in CHO-MG mutant cells, which lack the polyamine transport system. However, uptake was highly dependent on the nature of the polyamine and the position of the benzyl moiety on the polyamine chain. Earlier studies demonstrated a relationship between the number of protonated nitrogens of the polyamine derivative and its affinity for the polyamine transport system.[41−45] The higher number of positive charges is the reason for the more efficient accumulation of the triamines **N^1-bz-spd** and **N^4-bz-spd** in comparison with that of the diamine **bz-put**. Despite comparable accumulation of **N^1-bz-spd** and **N^4-bz-spd** in CHO cells, their accumulation in the mutant CHO-MG cells was strikingly different. **N^1-bz-spd** levels were below the detection limit**.** Its entry into cells appears to be entirely dependent on the presence of an active polyamine transport system. In contrast, CHO-MG cells accumulated significant amounts of **N^4-bz-spd**; the relative accumulation ratio between CHO and CHO-MG cells did not exceed 4 (while it was superior to 35 with **N^1-bz-spd**). A greater importance of the polyamine transport system in the uptake of the N^1-derivative is in agreement with previous studies, which showed a higher affinity for the transporter of N^1-spermidine derivatives when compared to their N^4-homologues.[31,32,43] However, the ability of **N^4-bz-spd** to accumulate independently of the presence of an active polyamine transporter indicates either the presence of another transport system, or more likely that plasma membranes are more permeable to this compound.

Fluorine substitution on the benzyl moiety of both **bz-put** and **N^1-bz-spd** did not alter greatly cellular uptake. In contrast, the boronic acid substitution, a bulkier substituent, had a significant impact on uptake. It reduced 2−3 times the accumulation of the spermidine derivatives in CHO and 3LL cells. The boronate group mainly affects the ability of the drug to use the polyamine transporter since polyamine transport deficient cells accumulated similar amounts of **N^4-4-Bbz-spd** and **N^4-bz-spd**. The position of the substituent on the benzyl moiety has little influence on transport as is evident from the similar behavior of **N^4-4-Bbz-spd** and **N^4-3-Bbz-spd**. In contrast, the boronic substitution of **bz-put** considerably increased its cellular uptake.

Another argument in favor of the use of the polyamine transport system by the polyamine derivatives is their increased accumulation in polyamine-depleted cells. Polyamine depletion results in the up-regulation of the polyamine transport system. Depletion of putrescine and spermidine by treatment with DFMO is known to increase the accumulation rate of polyamine-related drugs.[33,46−48] The uptake of all polyamine derivatives reported here was enhanced in DFMO-treated 3LL cells, except for N^4-spermidine derivatives. This discrepancy will be discussed later.

Among the three parent compounds, only **N^1-bz-spd** exhibited a significant cytotoxicity on 3LL cells, with an IC_{50} around 10 μM. This is in agreement with previous studies showing that N^1-substituted spermidines and spermine were more toxic than their N^4-substituted homologues.[20] As has been previously mentioned, introduction of fluorine into the benzene moiety increased and a boronic acid substituent decreased the cytotoxicity of the derivative. This effect was most striking in the N^1-spermidine series. The reduced toxicity is presumably related to the lower lipophilicity of the derivative as evidenced by a significant reduction in the chromatographic retention time on a reversed-phase column as ion pairs with *n*-octanesulfonic acid (**N^1-4-Bbz-spd,** 22.9 min; **N^1-bz-spd,** 28.1 min; **N^1-4-Fbz-spd**, 28.4 min; **4-Bbz-put,** 17.0 min; **bz-put**, 23.2 min; **4-Fbz-put**, 24.88 min). Reduced lipophilicity may impair interactions with intracellular sites involved in growth support.

Combined exposure of cells to DFMO and to one of the two cytotoxic drugs **N^1-bz-spd** and **N^1-4-Fbz-spd** produced a synergistic cytotoxic effect, most probably

due to the higher amounts of drug accumulating in DFMO-treated cells. In contrast, all other drugs reversed partially or completely the cytostatic effect of DFMO. In those cells, putrescine and/or spermidine could be detected, while they were below the detection limit in DFMO-treated cells not exposed to the drugs. Incubation of the drugs in culture medium at 37 °C for several days did not lead to the formation of free polyamines. This indicates that the putrescine and spermidine detected in DFMO-treated cells were formed inside the cells. The newly formed putrescine and spermidine counteract growth inhibition by DFMO. It is known that *N*-benzyl derivatives of putrescine[49] and *N*,*N*′-bis(benzyl)polyamine analogues[50] are substrates of polyamine oxidase, which splits the conjugates into the parent amine and benzaldehyde. It is likely that both fluorinated and boronated putrescine and N^1-spermidine derivatives are also substrates of this oxidase. In support of this assertion, we observed that, in CHO cells, intracellular levels of **bz-put** or **4-Bbz-put** were 2−3-fold higher in the presence of MDL 72527, an inhibitor of polyamine oxidase[51] (data not shown).

In contrast to a previous report,[33] **N^4-bz-spd** was found to reverse the effect of DFMO. However, the reversion was observed only in cells treated with at least 100 μM drug, a dose higher than the one used in the previous study. A similar effect was observed with its boron-containing derivative at 10 times lower concentration. In cells treated with DFMO and with the two derivatives, repletion of the spermidine pool was observed. In DFMO-treated cells, 50 μM **N^4-4-Bbz-spd** produced spermidine levels equivalent to those measured in normal cells. In addition, the boron content of the cells was superior to the amount of drug, as determined by HPLC. These observations strongly suggest that free spermidine is formed as the result of intracellular catabolism of **N^4-4-Bbz-spd**. Its degradation also occurs in normal cells, as is evident from the increased spermidine and spermine levels after exposure of cells to **N^4-4-Bbz-spd**. The enhanced formation of spermidine in cells treated with **N^4-4-Bbz-spd** indicates that the presence of the boron moiety on the benzyl group of **N^4-bz-spd** favors its degradation. In cells treated with the N^1-benzylspermidine derivatives, intracellular spermidine formation was observed only in the case of the boron-containing derivatives. The catabolic pathway responsible for this degradation remains to be clarified. The repletion of spermidine pools observed in cells treated with the N^4-spermidine derivatives is likely to be responsible for the prevention of the up-regulation of the polyamine transport activity by DFMO. This, along with the intracellular degradation of the drugs, explains why DFMO did not improve the accumulation of the N^4-benzylspermidine derivatives.

Potentials of the Fluorine-Containing Benzylpolyamines in PET. As ^{18}F is a short-lived isotope (half-life 110 min), preparation of ^{18}F-labeled compounds useful for PET should not exceed 2 h. Syntheses of **4-Fbz-put** and **N^1-4-Fbz-spd** are based on reductive amination of selectively protected polyamines with 4-fluorobenzaldehyde. This efficient, regioselective, and rapid sequence might be applicable to the corresponding labeled compound, since the [^{18}F]benzaldehyde can easily be prepared.[52] Among the two fluorinated compounds, the **N^1-4-Fbz-spd** derivative is probably a good candidate for PET because of its high accumulation in cancer cells via the polyamine transport system. Its cytotoxicity may not prevent its use, since in PET the concentrations to be administrated are low.

Potentials of the Boron-Containing Benzylpolyamines in BNCT. To become a candidate for BNCT, a boron-containing drug should be nontoxic, allow production of an intracellular boron content >10 μg/g in cancer tissue, and exhibit high tumor selectivity.

As reported above, none of our boron-containing drugs show significant toxicity to 3LL cells. They were considerably less cytotoxic than the clinically used BSH (IC_{50} = 150 μM, Table 2), and they appear to be less toxic than the previously reported carborane-containing polyamines (IC_{50} to F98 cells below 100 μM after 24 h of treatment[20,21]). The three boron-containing benzylpolyamines allowed us to accumulate boron to around 2 μg/g of 3LL cells. Despite the fact that each molecule carries only 1 boron atom, the amount of boron accumulated was higher than that achieved with BSH (only 0.7 μg of B/g of 3LL cells), a molecule carrying 12 boron atoms, and with BPA (0.9 μg of B/g of 3LL cells). Because the uptake of **4-Bbz-put** and **N^1-4-Bbz-spd** is highly dependent on the polyamine transport activity, pretreatment of 3LL cells with DFMO improved boron accumulation (up to 6 μg of B/g of cells). The high affinity of the polyamines to DNA[14] is another property of interest in BNCT. **N^1-4-Bbz-spd** exhibited higher affinity for DNA than spermidine. This property allows vectorization of boron into the nuclei and thus should favor its efficacy in BNCT, as cell killing is thought to be more effective with boron close to DNA.[53]

Targets for BNCT include brain tumors and melanoma. Murine as well as human glioblastoma cells accumulated only low levels of **4-Bbz-put**, **N^1-4-Bbz-spd,** and **N^4-4-Bbz-spd** even after pretreatment with DFMO. Only up to 3 μg of B/g of cells accumulated within 24 h when treated with 0.5 μg of B/mL of medium. Previously, the in vitro and in vivo accumulation of the acid form of **N^4-4-Bbz-spd** in glioma cells was reported.[21] F98 cells accumulated around 100 μg of B/g of cells after 48 h of incubation with 5.1 μg of B/mL of medium. However, in mice bearing intracerebral GL261 gliomas, this compound (delivered by continuous subcutaneous infusion by an Alzet pump) does not allow sufficient boron accumulation in the tumor tissue. Other N^4-spermidine derivatives carrying a carborane moiety (10 boron atoms) did not produce higher boron concentrations in F98 glioma cells in vitro. In vivo, the boron content in the GL261 tumor was only 7 μg of B/g of tumor after infusion with the carborane analogue of **N^4-4-Bbz-spd**.[21] Up to now, in vivo assays with boron-containing N^1-spermidine or *N*-putrescine derivatives have not been reported. However, our results suggest that boron-containing benzylpolyamines may not be suitable for BNCT of brain tumors.

In B16 melanoma cells, the polyamine derivatives **4-Bbz-put** and **N^1-4-Bbz-spd** allowed the accumulation of boron up to 5 μg of B/g of cells with only 0.55 μg of B/mL in the medium. As expected from their ability to use the polyamine transport system, pretreatment with DFMO increased boron accumulation in B16 cells (up to 18 μg of B/g of B16 cells). In a previous report, B16

cells exposed for 18 h to BPA or BSH at 5 or 10 μg of boron/mL of medium were shown to accumulate 12.5 μg of B/g of cells (results estimated on the basis of 2 mg/10^6 B16 cells).[54] Others have reported boron concentrations of 20 μg/g of B16 cells after treatment with BPA at 11 μg of B/mL of medium.[55] These results suggest **4-Bbz-put** and **N^1-4-Bbz-spd** for BNCT of melanomas.

An essential requirement for drugs used in BNCT is a high tumor selectivity in vivo. The ability of the drugs to use the polyamine transport system is a basis for tumor selectivity, in particular, in combination with DFMO, which up-regulates the transport rate selectively in the tumor tissue.[11−13] As compared with N^1-spermidine derivatives, results reported in this study have shown that the intracellular uptake of N^4-spermidine derivatives was poorly dependent on the polyamine transport system. Previous biodistribution studies with a N^4-carborane-containing spermidine derivative revealed that this compound allowed the accumulation of boron concentrations in B16 melanoma in vivo to up to 18 μg of B/g of tumor.[21] Tumor/blood and tumor/skin ratios were superior to 3, but were not judged sufficient for a clinical use by the authors. The most promising derivatives described in this work are likely to be **4-Bbz-put** and **N^1-4-Bbz-spd** since their accumulation is highly dependent on the presence of active transport, especially since tumor selectivity can be improved by pretreatment with DFMO. In addition, their solubility in water and their low toxicity are advantages over the currently used drugs.

The potentials of *N*-benzylpolyamines as vectors of boron for tumor targeting are evidenced by the present in vitro study. In vivo experiments are in progress on murine melanoma tumors, to further evaluate *N*-benzylpolyamines as potential drugs for BNCT. In addition, the synthesis of new benzylpolyamine derivatives with more than one boron atom is being designed.

Experimental Section

Reagents were purchased from chemical companies and used directly without further purification unless otherwise specified. Diethyl ether (Et_2O) and tetrahydrofuran (THF) were distilled from deep blue solutions of sodium/benzophenone ketyl prior to use. All melting points were determined on a Kofler apparatus and are uncorrected. NMR spectra were recorded on Bruker AC 200 (200 MHz for ^{1}H and 50.3 MHz for ^{13}C) and Bruker AC 300 (300 MHz for ^{1}H, 75.5 MHz for ^{13}C and 96 MHz for ^{11}B) spectrometers operating in the Fourier transform mode. ^{13}C NMR spectra were obtained with broadband proton decoupling. Chemical shifts are expressed as δ values with tetramethylsilane or CCl_3F (for ^{19}F NMR) as internal standard. For ^{11}B NMR, the chemical schifts are given in parts per million relative to $BF_3{\cdot}OEt_2$. High-resolution mass spectra were obtained with a Varian MAT 311 mass spectrometer (Centre Régional de Mesures Physiques de l'Ouest, Rennes, France). Microanalyses were performed at the Central Laboratory for Analysis (CNRS, Lyon, France). Silica gel 60F254 was used for column chromatography.

1. Chemical Syntheses. General Procedure of Imine Formation. To a stirred solution of amine (11 mmol) and 3 Å molecular sieves (2 g) in anhydrous Et_2O (50 mL) was added a solution of aldehyde (10 mmol) in anhydrous Et_2O (30 mL). The mixture was stirred at room temperature for 16 h, until the imine formation was complete (monitored by TLC). After filtration, the solvent was evaporated under vacuum to give the desired product, which was used without further purification.

Data for *tert*-Butyl *N*-{4-[(Phenylmethylidene)amino]-butyl}carbamate (2a): prepared from **1** and benzaldehyde; colorless oil; 98%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.28 (s, 1H), 7.80−7.65 (m, 2H), 7.45−7.30 (m, 3H), 4.85 (br, 1H), 3.61 (t, J = 6.7 Hz, 2H), 3.17 (t, J = 6.2 Hz, 2H), 1.80−1.50 (m, 4H), 1.44 (s, 9H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 161.1, 156.0, 136.1, 130.6, 128.6, 128.0, 78.9, 61.1, 40.3, 28.4, 28.1, 27.8.

Data for *tert*-Butyl *N*-{4-[(4-Fluorophenyl)methylidene]-amino}butyl}carbamate (2b): prepared from **1** and 4-fluorobenzaldehyde; colorless oil; 95%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.17 (s, 1H), 7.80−7.58 (m, 2H), 7.08−6.92 (m, 2H), 4.65 (br, 1H), 3.60−3.50 (m, 2H), 3.18−3.00 (m, 2H), 1.75−1.30 (m, 13H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 160.1, 155.0, 130.4, 130.2, 116.3, 115.8, 61.4, 39.3, 27.4, 27.1, 26.8; ^{19}F NMR (282 MHz, $CDCl_3$) δ −110.160.

Data for *tert*-Butyl *N*-{4-{{[4-(5,5-Dimethyl[1,3,2]dioxaborinan-2-yl)phenyl]methylidene}amino}butyl}carbamate (2c): prepared from **1** and 2-(4-formylphenyl)-5,5-dimethyl-1,3,2-dioxaborane; colorless solid; 96%; mp = 118−120 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.28 (s, 1H), 7.85 (d, J = 8.1 Hz, 2H), 7.70 (d, J = 8.1 Hz, 2H), 4.70 (br, 1H), 3.78 (s, 4H), 3.70−3.58 (m, 2H), 3.25−3.10 (m, 2H), 1.82−1.40 (m, 13H), 1.05 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 161.5, 156.0, 137.9, 134.1, 133.9, 127.2, 72.3, 61.3, 40.2, 28.4, 28.1, 27.9, 21.9; HRMS (EI) calcd for $C_{21}H_{33}BN_2O_4$ (M^+) 388.2533, found 388.2548.

Data for *tert*-Butyl *N*-{3-[(Phenyl)methylidene]amino}-propyl}-*N*-{4-[(*tert*-butyloxy)carbonyl]amino}butyl}-carbamate (8a): prepared from **7** and benzaldehyde; colorless oil; 95%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.20 (s, 1H); 7.72−7.58 (m, 2H), 7.45−7.26 (m, 3H); 4.65 (br, 1H), 3.65−3.47 (m, 2H), 3.30−3.00 (m, 6H), 2.00−1.25 (m, 24H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 161.7, 156.4, 156.0, 136.6, 131.0, 128.9, 128.4, 79.6, 79.4, 59.5, 47.1, 45.6, 40.6, 28.8, 27.8, 26.1.

Data for *tert*-Butyl *N*-{3-[(4-Fluorophenyl)methylidene]-amino}propyl}-*N*-{4-[(*tert*-butyloxy)carbonyl]amino}-butyl}carbamate (8b): prepared from **7** and 4-fluorobenzaldehyde; colorless oil; 96%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.17 (s, 1H), 7.70−7.56 (m, 2H), 7.10−6.92 (m, 2H), 4.62 (br, 1H), 3.62−3.45 (m, 2H), 3.33−2.95 (m, 6H), 1.95−1.72 (m, 2H), 1.60−1.25 (m, 22H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 160.2, 156.4, 156.0, 136.9, 130.4, 130.2, 116.2, 115.8, 79.6, 79.4, 59.4, 47.1, 45.6, 40.6, 28.8, 28.7, 27.8, 26.1; ^{19}F NMR (282 MHz, $CDCl_3$) δ −110.250.

Data for *tert*-Butyl *N*-{3-{{[4-(5,5-Dimethyl[1,3,2]dioxaborinan-2-yl)phenyl]methylidene}amino}propyl}-*N*-{4-{[(*tert*-butyloxy)carbonyl]amino}butyl}carbamate (8c): prepared from **7** and 2-(4-formylphenyl)-5,5-dimethyl-1,3,2-dioxaborane; colorless oil; 98%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.21 (s, 1H), 7.78 (d, J = 8.0 Hz, 2H), 7.60 (d, J = 8.0 Hz, 2H), 4.65 (br, 1H), 3.69 (s, 4H), 3.60−3.45 (m, 2H), 3.28−2.92 (m, 6H), 1.95−1.76 (m, 2H), 1.52−1.25 (m, 22H), 0.94 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 160.6, 155.0, 154.6, 136.9, 133.3, 126.1, 78.2, 71.2, 58.2, 45.7, 44.3, 39.2, 30.8, 27.4, 26.4, 20.9; ^{11}B NMR (96 MHz, $CDCl_3$) δ 27.4.

General Procedure of Reduction. $NaBH_4$ (20 mmol) was added in small portions to a solution of imine (10 mmol) in dry ethanol (60 mL) at 0 °C. After being stirred for 4 h, the mixture was concentrated under vacuum. The residue was taken up in water (60 mL) and extracted with CH_2Cl_2 (3 × 60 mL). The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by flash chromatography on silica gel (210−400 mesh).

Data for *tert*-Butyl *N*-{4-[(4-Fluorophenyl)methyl]-amino}butyl}carbamate (3b): prepared from **2b**; colorless oil; 85% (R_f = 0.4, ethyl acetate/methanol, 1:1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.28−7.13 (m, 2H), 7.00−6.82 (m, 2H), 5.23 (br, 1H), 3.67 (s, 2H), 3.25−3.15 (m, 2H), 2.65−2.50 (m, 2H), 1.59−1.28 (m, 14H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 148.5, 130.1, 129.9, 115.7, 115.3, 77.9, 53.7, 49.3, 41.0, 28.8, 28.3, 27.8; ^{19}F NMR (282 MHz, $CDCl_3$) δ −116.509.

Data for *tert*-Butyl *N*-{4-{{[4-(5,5-Dimethyl[1,3,2]dioxaborinan-2-yl)phenyl]methyl}amino}butyl}carbamate (3c): prepared from **2c**; colorless solid; 65% (R_f = 0.2, methanol); mp = 112−113 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ

7.78 (d, J = 8.0 Hz, 2H), 7.32 (d, J = 8.0 Hz, 2H), 4.88 (br, 1H), 3.82 (s, 2H), 3.80 (s, 4H), 3.22−3.10 (m, 2H), 2.75−2.60 (m, 2H), 1.62−1.40 (m, 14H), 1.05 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 155.0, 141.7, 133.0, 128.4, 126.3, 114.6, 77.9, 71.2, 52.9, 47.8, 39.4, 30.8, 27.4, 26.8, 26.3, 20.9; ^{11}B NMR (96 MHz, $CDCl_3$) δ 26.7; HRMS (EI) calcd for $C_{21}H_{35}BN_2O_4$ (M^+) 390.2690, found 390.2691.

Data for *tert*-Butyl *N*-{3-[(Phenylmethyl)amino]propyl}-*N*-{4-{[(*tert*-butyloxy)carbonyl]amino}butyl}-carbamate (9a): prepared from **8a**; colorless oil; 79% (R_f = 0.3, ethyl acetate/methanol, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37−7.15 (m, 5H), 4.65 (br, 1H), 3.75 (s, 2H), 3.32−3.00 (m, 6H), 2.58 (t, J = 6.9 Hz, 2H), 1.80−1.32 (m, 25H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 155.0, 154.5, 139.2, 127.4, 127.2, 125.9, 78.3, 53.0, 45.6, 39.2, 27.5, 27.4, 26.4.

Data for *tert*-Butyl *N*-{3-{[(4-Fluorophenyl)methyl]-amino}propyl}-*N*-{4-{[(*tert*-butyloxy)carbonyl]amino}-butyl}carbamate (9b): prepared from **8b**; colorless oil; 85% (R_f = 0.4, ethyl acetate/methanol, 1:1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.28−7.15 (m, 2H), 6.97−6.83 (m, 2H), 4.62 (br, 1H), 3.66 (s, 2H), 3.25−2.98 (m, 6H), 2.58−2.45 (m, 2H), 1.75−1.28 (m, 25H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 155.0, 154.6, 135.0, 128.7, 128.6, 114.3, 113.9, 78.3, 52.3, 45.5, 39.2, 27.5, 27.4, 26.4; ^{19}F NMR (282 MHz, $CDCl_3$) δ −116.523.

Data for *tert*-Butyl *N*-{3-{{[4-(5,5-Dimethyl[1,3,2]dioxaborinan-2-yl)phenyl]methyl}amino}propyl}-*N*-{4-{[(*tert*-butyloxy)carbonyl]amino}butyl}carbamate (9c): prepared from **8c**; colorless oil; 65% (R_f = 0.2, methanol); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.68 (d, J = 8.0 Hz, 2H), 7.20 (d, J = 8.0 Hz, 2H), 4.70 (br, 1H), 3.69 (s, 2H), 3.67 (s, 4H), 3.23−2.92 (m, 6H), 2.51 (t, J = 6.7 Hz, 2H), 1.70−1.22 (m, 25 H), 0.94 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 155.0, 154.6, 141.8, 133.0, 126.3, 78.2, 71.2, 53.1, 45.6, 39.2, 30.8, 27.5, 27.4, 26.4, 20.9; ^{11}B NMR (96 MHz, $CDCl_3$) δ 26.5. Anal. ($C_{29}H_{50}BN_3O_6$) C, H.

General Procedure of Alkylation. To a solution of **12** (43.4 mmol) in dry tetrahydrofuran/dimethyl sulfoxide (120 mL/30 mL) were added the bromide derivative (34.7 mmol), $NaHCO_3$ (86.8 mmol), and NaI (13 mmol). The mixture was heated at reflux for 6 h. After removal of the tetrahydrofuran, the mixture was diluted with ethyl acetate and washed with H_2O. $MgSO_4$ was added, and filtration was followed by the evaporation of the solvent. The residue was purified by flash chromatography on silica gel (210−400 mesh).

Data for *tert*-Butyl *N*-{4-{*N*-{3-[(*tert*-Butyloxycarbonyl)amino]propyl}{[4-(5,5-dimethyl[1,3,2]dioxaborinan-2-yl)phenyl]methyl}amino}butyl}carbamate (14a): prepared from **12** and 2-[4-(bromomethyl)phenyl]-5,5-dimethyl-1,3,2-dioxaborane (**13a**); yellow oil; 51% (R_f = 0.5 heptane/ethyl acetate, 8:2); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.74 (d, J = 7.9 Hz, 2H), 7.28 (d, J = 7.9 Hz, 2H), 5.40 (br, 1H), 4.70 (br, 1H), 3.76 (s, 4H), 3.51 (s, 2H), 3.15−2.98 (m, 4H), 2.52−2.37 (m, 4H), 1.62−1.47 (m, 6H), 1.44 (s, 18H), 1.02 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 156.0, 141.9, 133.9, 128.2, 78.0, 77.9, 72.2, 58.9, 53.8, 52.7, 40.8, 40.1, 31.8, 28.5, 28.4, 27.8, 26.6, 24.2, 21.9; HRMS (EI) calcd for $C_{29}H_{50}BN_3O_6$ (M^+) 547.3792, found 547.3794.

Data for *tert*-Butyl *N*-{4-{*N*-{3-[(*tert*-Butyloxycarbonyl)amino]propyl}{[3-(5,5-dimethyl[1,3,2]dioxaborinan-2-yl)phenyl]methyl}amino}butyl}carbamate (14b): prepared from **12** and 2-[3-(bromomethyl)phenyl]-5,5-dimethyl-1,3,2-dioxaborane (**13b**); yellow oil; 58% (R_f = 0.5 heptane/ethyl acetate, 8:2); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.70−7.20 (m, 4H), 5.33 (br, 1H), 4.75 (br, 1H), 3.76 (s, 4H), 3.52 (s, 2H), 3.18−2.92 (m, 4H), 2.51−2.35 (m, 4H), 1.72−1.28 (m, 24H), 1.00 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 156.1, 134.5, 132.8, 127.7, 78.0, 77.9, 72.3, 58.7, 53.2, 52.7, 40.8, 40.1, 31.9, 28.5, 28.4, 27.8, 26.6, 24.2, 21.9; HRMS (EI) calcd for $C_{29}H_{50}BN_3O_6$ (M^+) 547.3792, found 547.3794.

Data for *tert*-Butyl *N*-{4-{*N*-{3-[(*tert*-Butyloxycarbonyl)-amino]propyl}(phenylmethyl)amino}butyl}carbamate[56] (14c): prepared from **12** and benzyl bromide; yellow oil; 70% (R_f = 0.5, heptane:ethyl acetate, 9:1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.35−7.20 (m, 5H), 5.48 (br, 1H), 4.80 (br, 1H), 3.50 (s, 2H), 3.25−3.00 (m, 4H), 2.52−2.33 (m, 4H), 1.70−1.41 (m, 24H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 156.0, 139.4, 128.9, 128.2, 127.0, 78.9, 78.7, 58.8, 53.3, 52.3, 40.4, 39.8, 28.5, 28.4, 27.8, 26.6, 24.2. Anal. ($C_{24}H_{41}N_3O_4$) C, H, N.

General Procedure of Amino-Group Deprotection. A solution of *N*-*tert*-butoxycarbonyl derivative (3 mmol) in ethanol (26 mL) was treated with 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.

Data for *N*-(4-Aminobutyl)(phenylmethyl)amine Dihydrochloride (bz-put, 4a): prepared from **3a**; colorless solid; 95%; mp 251−253 °C; ^{1}H NMR (200 MHz, D_2O) δ 7.28−7.17 (m, 5H), 3.97 (s, 2H), 2.93−2.68 (m, 4H), 1.60−1.42 (m, 4H); ^{13}C NMR (50 MHz, D_2O) δ 131.0, 130.2, 130.0, 129.6, 51.4, 46.7, 39.2, 24.3, 23.1; HRMS (EI) calcd for $C_{11}H_{18}N_2$ (M^+) 178.1469, found 178.1471.

Data for *N*-(4-Aminobutyl)[(4-fluorophenyl)methyl]-amine Dihydrochloride (4-Fbz-put, 4b): prepared from **3b**; colorless solid; 92%; mp 266−268 °C; ^{1}H NMR (200 MHz, D_2O) δ 7.47−7.34 (m, 2H), 7.20−7.05 (m, 2H), 4.14 (s, 2H), 3.09 (t, J = 6.9 Hz, 2H), 3.00 (t, J = 7.4 Hz, 2H), 1.75−1.59 (m, 4H); ^{13}C NMR (50 MHz, D_2O) δ 132.5, 132.3, 116.7, 116.2, 50.7, 46.6, 39.1, 24.3, 23.0; ^{19}F NMR (282 MHz, D_2O) δ − 111, 106; HRMS (EI) calcd for $C_{11}H_{17}N_2F$ (M^+) 196.1376, found 196.1372. Anal. ($C_{11}H_{19}N_2Cl_2F$) C, H, N.

Data for *N*-(4-Aminobutyl){{4-(5,5-dimethyl[1,3,2]dioxaborinan-2-yl)phenyl}methyl}amine Dihydrochloride (4c): prepared from **3c**; colorless solid; 90%; mp > 300 °C; ^{1}H NMR (200 MHz, D_2O) δ 7.95 (d, J = 8.0 Hz, 2H), 7.60 (d, J = 8.0 Hz, 2H), 4.38 (s, 2H), 3.48 (s, 4H), 3.30−3.08 (m, 4H), 1.95−1.78 (m, 4H), 0.97 (s, 6H); ^{13}C NMR (50 MHz, D_2O) δ 134.8, 133.5, 129.6, 68.4, 51.3, 46.8, 39.2, 36.7, 24.3, 23.1, 20.8.

Data for *N*-{3-[(4-Aminobutyl)amino]propyl}(phenylmethyl)amine Trihydrochloride (N^1-bz-spd, 10a): prepared from **9a**; colorless solid; 95%; mp > 300 °C; ^{1}H NMR (200 MHz, D_2O) δ 7.48−7.26 (m, 5H), 4.13 (s, 2H), 3.15−2.80 (m, 8H), 2.10−1.88 (m, 2H), 1.76−1.55 (m, 4H); ^{13}C NMR (50 MHz, D_2O) δ 130.8, 130.2, 130.1, 129.7, 51.6, 47.4, 44.8, 44.2, 39.2, 24.3, 23.1, 23.0; HRMS (EI) calcd for $C_{14}H_{26}N_3$ (M^+ + H) 236.2127, found 236.2137. Anal. ($C_{14}H_{28}N_3Cl_3$) C, H.

Data for *N*-{3-[(4-Aminobutyl)amino]propyl}[(4-fluorophenyl)methyl]amine Trihydrochloride (N^1-4-Fbz-spd, 10b): prepared from **9b**; colorless solid; 93%; mp > 300 °C; ^{1}H NMR (300 MHz, D_2O) δ 7.52−7.43 (m, 2H), 7.26−7.12 (m, 2H), 4.21 (s, 2H), 3.20−2.95 (m, 8H), 2.15−2.00 (m, 2H), 1.82−1.65 (m, 4H); ^{13}C NMR (50 MHz, D_2O) δ 132.5, 132.4, 116.7, 116.2, 50.9, 47.4, 44.8, 44.2, 39.1, 24.2, 23.1, 23.0; ^{19}F NMR (282 MHz, D_2O) δ −110.988; HRMS (EI) calcd for $C_{14}H_{25}N_3F$ (M^+ + H) 254.2033, found 254.2032. Anal. ($C_{14}H_{27}N_3FCl_3$) C, H.

Data for *N*-{3-[(4-Aminobutyl)amino]propyl}{{4-(5,5-dimethyl[1,3,2]dioxaborinan-2 -yl)phenyl}methyl}amine Trihydrochloride (10c): prepared from **9c**; colorless solid; 90%; mp > 300 °C; ^{1}H NMR (300 MHz, D_2O) δ 7.30 (d, J = 8.5 Hz, 2H), 6.88 (d, J = 8.5 Hz, 2H), 4.13 (s, 2H), 3.32 (s, 4H), 3.15−2.91 (m, 8H), 2.16−2.00 (m, 2H), 1.80−1.65 (m, 4H), 0.82 (s, 6H); HRMS (EI) calcd for $C_{19}H_{34}BN_3O_2$ (M^+) 347.2744, found 347.2748.

Data for *N*-(3-Aminobutyl)-*N*-(3-aminopropyl){{4-(5,5-dimethyl[1,3,2]dioxaborinan-2-yl)phenyl}methyl}-amine Trihydrochloride (N^4-4-Bbz-spd, 15a): prepared from **14a**; amorphous solid; 92%; ^{1}H NMR (300 MHz, D_2O) δ 7.83 (d, J = 8.1 Hz, 2H), 7.52 (d, J = 8.1 Hz, 2H), 4.42 (s, 2H), 3.34 (s, 4H), 3.28−3.18 (m, 4H), 3.10−2.95 (m, 4H), 2.26−2.05 (m, 2H), 1.81−1.64 (m, 4H), 0.82 (s, 6H); ^{13}C NMR (50 MHz, D_2O) δ 134.9, 131.4, 130.7, 68.7, 57.5, 52.3, 49.9, 39.1, 36.9, 36.3, 24.2, 22.0, 20.8, 20.6; ^{11}B NMR (96 MHz, D_2O) δ 29.5; HRMS (EI) calcd for $C_{19}H_{34}BN_3O_2$ (M^+) 347.2744, found 347.2748.

Data for *N*-(3-Aminobutyl)-*N*-(3-aminopropyl){{3-(5,5-dimethyl[1,3,2]dioxaborinan-2-yl)phenyl}methyl}-amine Trihydrochloride (N^4-3-Bbz-spd, 15b): prepared from **14b**; amorphous solid; 90%; ^{1}H NMR (300 MHz, D_2O) δ

7.92−7.50 (m, 4H), 4.44 (s, 2H), 3.35 (s, 4H), 3.36−3.20 (m, 4H), 3.10−2.95 (m, 4H), 2.30−2.15 (m, 2H), 1.95−1.60 (m, 4H), 0.83 (s, 6H); HRMS (EI) calcd for $C_{19}H_{34}BN_3O_2$ (M^+) 347.2744, found 347.2748.

Data for *N*-(3-Aminobutyl)-*N*-(3-aminopropyl)(phenylmethyl)amine Trihydrochloride (N^4-4-bz-spd, 15c): prepared from **14c**; amorphous solid; 95%; ^{1}H NMR (200 MHz, D_2O) δ 7.42−7.28 (m, 5H), 4.26 (s, 2H), 3.20−3.00 (m, 4H), 2.96−2.80 (m, 4H), 2.15−1.42 (m, 6H); ^{13}C NMR (50 MHz, D_2O) δ 131.4, 130.8, 129.3, 129.1, 57.8, 52.4, 49.9, 39.2, 37.0, 24.3, 22.1, 21.0; HRMS (EI) calcd for $C_{14}H_{25}N_3$ (M^+) 235.2048, found 235.2050.

General Procedure for the Cleavage of Boronic Ester. To a stirred solution of the boronic ester (1 mmol) in water (15 mL) was added phenylboric acid (1 mmol) and ether (15 mL). After 3 h at room temperature the ether layer was removed and replaced by fresh ether (15 mL). After a further 3 h, the ether layer was again removed and replaced. The mixture was then stirred for an additional 3 h. The ether layer was removed, and the aqueous phase was washed with ether. The aqueous solution was concentrated and dried under high vacuum.

Data for *N*-(4-Aminobutyl){[4-(dihydroxyboryl)phenyl]methyl}amine Dihydrochloride (4-Bbz-put, 5): prepared from **4c**; colorless solid; 95%; mp 262−264 °C; ^{1}H NMR (200 MHz, D_2O) δ 7.88 (d, J = 8.0 Hz, 2H), 7.55 (d, J = 8.0 Hz, 2H), 4.30 (s, 2H), 3.12 (t, J = 6.8 Hz, 2H), 3.05 (t, J = 6.7 Hz, 2H), 1.90−1.70 (m, 4H); ^{13}C NMR (50 MHz, D_2O) δ 134.7, 133.4, 129.6, 51.3, 47.4, 39.2, 24.3, 23.1; ^{11}B NMR (96 MHz, $CDCl_3$) δ 29.3; HRMS (EI) calcd for $C_{25}H_{30}BN_4O_6$ with matrix 3-nitrobenzyl alcohol (M^+ + H) 493.2258, found 493.2262. Anal. ($C_{11}H_{21}BN_2O_2Cl_2$) C, H, N.

Data for *N*-{3-[(4-Aminobutyl)amino]propyl}{[4-(dihydroxyboryl)phenyl]methyl}amine Trihydrochloride (N^1-4-Bbz-spd, 11): prepared from **10c**; colorless solid; 93%; mp > 300 °C; ^{1}H NMR (300 MHz, D_2O) δ 7.80 (d, J = 7.9 Hz, 2H), 7.45 (d, J = 7.9 Hz, 2H), 4.25 (s, 2H), 3.22−2.95 (m, 8H), 2.18−2.03 (m, 2H), 1.80−1.66 (m, 4H); ^{13}C NMR (50 MHz, D_2O) δ 134.8, 133.2, 129.6, 51.5, 47.4, 44.8, 44.4, 39.2, 24.3, 23.1, 23.0; ^{11}B NMR (96 MHz, D_2O) δ 28.5; HRMS (EI) calcd for $C_{28}H_{37}BN_5O_6$ with matrix 3-nitrobenzyl alcohol (M^+ + H) 550.2837, found 550.2840.

2. Biological Studies. Unless otherwise stated, the usual laboratory chemicals were purchased from Merck (Darmstadt, Germany) or Sigma Chemical Co. (St. Louis, MO). DFMO was obtained from Ilex Oncology (San Antonio, TX). BPA and BSH were purchased from Boron Biologicals Inc. (Raleigh, NC). Data are given as mean values and SD (or SEM) of three or more experiments. Comparisons between means were made using the Student's t-test assuming significance at $p < 0.01$.

a. Ethidium Bromide Displacement Assay. The release of ethidium bromide from DNA was monitored fluorimetrically as previously described[57] using a Fluorolog 2 spectrofluorimeter (Jobin-Yvon ISA, Longjumeau, France) at excitation and emission wavelengths of 546 and 595 nm, respectively. Calf thymus DNA (0.2 A_{260} unit) was added to 3 mL of buffer (5 mM NaCl/1 mM sodium cacodylate, pH 7) containing 2.0 μM ethidium bromide. The complex was titrated by adding μL volumes of concentrated solutions of polyamines or polyamine derivatives at room temperature. The fluorescence intensity was corrected for the dilution.

b. Cell Culture. Murine 3LL Lewis lung carcinoma, murine B16 melanoma, rat C6 glioblastoma, human U251 glioblastoma (obtained from the brain tumor tissue bank at the Department of Neurosurgery, University of California, San Francisco), CHO, and CHO-MG^R (provided by Dr. W. Flintoff, University of Western Ontario) cells were grown in RPMI 1640 medium (Eurobio, Les Ulis, France) supplemented with 10% fetal calf serum, 2 mM glutamine, streptomycin (50 μg/mL), and penicillin (100 U/mL) (Biomerieux, Marcy l'Etoile, France). A 2 μg/mL sample of L-proline was added to the culture medium for CHO-MG^R cells. Cells were grown at 37 °C under a humidified 5% CO_2 atmosphere. Because none of the drugs tested were substrates of the bovine serum amine oxidase (data not shown), their culture in the presence of calf serum did not require the addition of aminoguanidine, an inhibitor of this enzyme.

c. In Vitro Evaluation of Drug Cytotoxicity/Cytostasy. The effect of the polyamine derivatives on cell growth was assayed in 96-well plates (Becton Dickinson, Oxnard, CA). Twenty-four hours after seeding (initial density 1000 3LL cells/well), drugs were added in culture medium. 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.[58]

d. Cellular Uptake. Cells were cultured in tissue culture flasks with initial densities of 5×10^4 cells/mL. Unless stated otherwise, drugs were added 24 h after seeding. Cells were harvested using trypsine/EDTA. All cells were washed three times with phosphate-buffered saline before processing.

e. Determination of Polyamines and of Polyamine Derivatives. Cells were disrupted by sonication in 0.2 N perchloric acid and proteins precipitated by centrifugation at 1500*g*. Each supernatant was frozen at −20 °C until analysis by HPLC. The protein content was determined using the Folin phenol reagent.[59] Polyamines and their derivatives were determined by separation of the ion pairs formed with *n*-octanesulfonic acid (for polyamines and all derivatives except for **4-Fbz-put**) or *n*-hexanesulfonic acid (for **4-Fbz-put**) on a reversed-phase column, reaction of the column effluent with *o*-phthalaldehyde and *N*-acetylcysteine, and monitoring of fluorescence intensity as previously described.[60]

f. Boron Measurements. Capped Teflon digestion bombs containing the cells in 2 mL of 60% nitric acid were heated for a 90 min cycle using a microwave oven (MDS-8ID, CEM, Mattews; maximal power 630 ± 70 W). After cooling, 2 mL of 30% hydrogen peroxide was added. After another heating cycle, the samples were transferred to polypropylene tubes and completed to 5 mL with Millipore water. Boron standards were prepared from a boron atomic absorption standard solution (Sigma) and diluted in a mixture of nitric acid, hydrogen peroxide, and water (2:2:1). ICP-AES determinations of boron were done using the 249.773 nm line on an ICP atomic emission spectrometer (JY 38 S, Jobin-Yvon ISA, Longjumeau, France).

Acknowledgment. We are indebted to Pr. W. Flintoff (University of Western Ontario, London, Canada) for providing the CHO-MG cells. We acknowledge the Ministère de la Recherche et de la Technologie (MRT), the Centre National de la Recherche Scientifique (CNRS), the Association pour la Recherche sur le Cancer (ARC), the Ligue Nationale Contre le Cancer (LNCC—Comités d'Ille-et-Vilaine, du Morbihan et des Côtes d'Armor), and the Conseil Régional de Bretagne (CRB) for financial support and for fellowships to F.P. (CRB), B.M. (LNCC and ARC), and C.L. (MRT). Our sincere thanks are also due to D. Desplanques and A. Papillon (Laboratoire Interrégional de la DGCCRF, Rennes, France) for giving us access to the ICP-atomic emission spectrometer as well as for their appreciable advice and technical assistance. We also gratefully acknowledge R. Havouis for the HPLC determinations.

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JM010897Q