Journal of Medicinal Chemistry Article

J. Med. Chem. 2010, 53, 7647–7663 7647
DOI: 10.1021/jm1007648

Targeting the Polyamine Transport System with Benzazepine- and Azepine-Polyamine Conjugates[†]

Sophie Tomasi,[‡] Jacques Renault,[‡] Bénédicte Martin,[§] Stephane Duhieu,[§] Virginie Cerec,[§] Myriam Le Roch,[‡] Philippe Uriac,[‡] and Jean-Guy Delcros*[,§]

[‡]*Produits Naturels–Synthèses–Chimie Médicinale, Sciences Chimiques de Rennes, CNRS UMR 6226, Faculté de Pharmacie, Université Rennes 1, Université Européenne de Bretagne, Rennes Cedex, France, and* [§]*Groupe de Recherche en Thérapeutique Anticancéreuse, Faculté de Médecine, Université Rennes 1, Université Européenne de Bretagne, Rennes Cedex, France*

Received June 22, 2010

The polyamine transport system (PTS) whose activity is up-regulated in cancer cells is an attractive target for drug design. Two heterocyclic (azepine and benzazepine) systems were conjugated to various polyamine moieties through an amidine bound to afford 18 compounds which were evaluated for their affinity for the PTS and their ability to use the PTS for cell delivery. Structure–activity relationship studies and lead optimization afforded two attractive PTS targeting compounds. The azepine–spermidine conjugate **14** is a very selective substrate of the PTS that may serve as a vector for radioelements used for diagnoses or therapeutics in nuclear medicine. The nitrobenzazepine–spermine conjugate **28** is a very powerful PTS inhibitor with very low intrinsic cytotoxicity, able to prevent the growth of polyamine depleted cells in presence of exogenous polyamines.

Introduction

Polyamines are ubiquitous organic polycations present in all living organisms (Figure 1). Although their precise functions remain unknown, polyamines are essential in the regulation of cell proliferation and differentiation. The remarkable complexity of the mechanisms controlling their homeostasis stresses their exceptional importance. All cells are equipped with a multifaceted and highly regulated enzymatic machinery allowing polyamine synthesis, retroconversion, and degradation. Cells also possess active transport systems allowing import and export of polyamines. On a molecular level, polyamine transport systems (PTS[a]) controlling the import of exogenous polyamines have been characterized in bacteria, in yeast,[1,2] and in the protozoan parasites *Leishmania* and *Trypanosoma*.[3,4] In contrast, polyamine transport in mammalian cells remains a measurable import process[5–8] still waiting to be molecularly characterized.

PTS have been recognized as potential targets for therapeutic intervention in cancers.[8–10] Many cancer cells exhibit elevated polyamine import activity,[5,11,12] probably due to their enhanced need for these growth supporting factors. The elevated activity of the PTS along with its broad structural tolerance which allows the import of non-native polyamine conjugates, provide an opportunity to selectively target cancer cells.[5,10] The literature reports many examples of polyamine conjugates with cytotoxic drugs,[13–21] but only a few of them display enhanced cytotoxicity to cancer cells over their normal counterparts in vitro.[22,23] The first successful design was reported recently by Barret and his colleagues, who developed a spermine–epipodophyllotoxin conjugate with selective uptake via the PTS and a wide therapeutic index, able to induce complete regression in human breast tumor xenograft model after i.p. or oral administrations.[24] In addition, they also describe the development of fluorophor-labeled polyamine probes to identify tumors expressing a highly active PTS.[11,25,26] Attempts have been also made to use polyamines as selective vectors of radioelements for tumor therapy or imaging.[27–33]

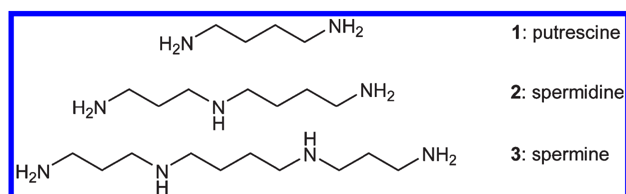


Figure 1. Structures of naturally occurring putrescine (**1**), spermidine (**2**), and spermine (**3**).

Because of the high affinity of some polyamine conjugates for the PTS, such structures have also been designed toward the identification of polyamine transport inhibitors.[34–37] Indeed the ability of polyamine biosynthesis inhibitors (e.g., α-difluoromethylornithine, an ornithine decarboxylase inhibitor (DFMO)) to completely deplete internal polyamines and therefore inhibit cancer cell growth, is overcome by the importation of polyamines from external sources, hence the need of potent PTS inhibitors. In particular, spermine conjugates with amino acids such as lysine seems to be very promising.[35,38,39]

The lack of knowledge on the molecular nature of the PTS still renders difficult a rational design of PTS-targeting drugs. Therefore, to identify molecular recognition elements and to delineate the structural tolerance accommodated by

[†]This work is dedicated to the memory of Professor Nikolaus Seiler (1931–2006), an outstanding scientist and mentor and pioneer in the polyamine field, and of Dr. Pierre Guénot (1948–2007).

*To whom correspondence should be addressed. Phone: 33.(0)4.78.78.59.71. Fax: 33.(0)4.78.78.28.87. E-mail: delcros@lyon.fnclcc.fr. Address: Apoptosis Cancer & Development Laboratory, CNRS UMR5238, Centre Léon Bérard, 28 Rue Laennec, 69008 Lyon, France.

[a]Abbreviations: PTS, polyamine transport system; CHO, Chinese hamster ovary; DFMO, α-difluoromethylornithine; CHO-MG, Chinese hamster ovary cells polyamine transport deficient mutant; L1210, mouse leukemia cells; PBS, phosphate buffered saline.

© 2010 American Chemical Society
Published on Web 10/06/2010

R^1		Free polyamines	Azepine-polyamine conjugates	Benzazepine-polyamine conjugates
R^1		H_2N-R^1	Azepine ring; Amidine bond; Polyamine moiety	Benzazepine ring; Amidine bond; Polyamine moiety; R^2
NH_2 (x) 2HCl	x=4	**1**	**11** (Az 4)	**19**: R^2=H (BzAz 4)
	x=8	**4**	**12** (Az 8)	**20**: R_2=H (BzAz 8)
NH_2 (x, y) 3HCl	x=3, y=4	] **2**	**14** (Az 3,4)	**23**: R^2=H (BzAz 3,4)
	x=4, y=3		**15** (Az 4,3)	
	x=3, y=3	**5**	**13** (Az 3,3)	
	x=4, y=4	**6**	**16** (Az 4,4)	
	x=3, y=5	**7**	**17** (Az 3,5)	
NH_2 (x, y, z) 4HCl	x=3, y=4, z=3	**3**	**18** (Az 3,4,3)	**22**: R^2=H (BzAz 3,4,3)
				26: R^2=Br
				27: R^2=OCH_3
				28: R^2= NO_2
				29: R^2= NH_2
	x=3, y=3, z=3	**8**		**21**: R^2=H (BzAz 3,3,3)
	x=4, y=4, z=4	**9**		**25**: R^2=H (BzAz 4,4,4)
OH, OH, NH_2 4HCl		**10**		**24**: R_2=H

Figure 2. Structure and nomenclature of free (**1**−**10**) and conjugated (**11**−**29**) polyamines.

the PTS, systematic structure−activity relationship studies were carried out on a series of polyamine conjugates with complex systems such as arenes, azamacrocycles, or aromatic heterocycles[16,23,32,40−44] All these conjugates were screened in terms of their affinity for the PTS as well as for their ability to be delivered in various cell lines possessing or not an active and/or activated PTS. To further extend our knowledge of the structure−activity relationship of polyamine conjugates and based on our previous study confirming the size of substituents as a limiting factor for the conjugate selectivity on PTS,[43] a similar approach was carried out on two new series of benzazepine and azepine heterocyclic systems coupled to various polyamine scaffolds by an amidine bond (Figure 2). This amidine function leads to the rapid access of various chain moieties and allows keeping of the positive charge in physiological conditions in comparison to the amide one.

Results and Discussion

Synthesis. The final compounds **11**−**29** were polyamine conjugates and were all evaluated as hydrochloride salts. Their heterocyclic moiety, either an azepine or a benzazepine ring, was linked to natural or synthetic polyamine chain, diamines, triamines or tetramines, through an amidine bond (Figure 2). Despite the various conjugation methods used, all starting materials were identical and consisted of free or suitably protected polyamines and lactams.

Preparation of Polyamines. Most polyamines used were either commercial (putrescine **1**, spermine **3**, norspermidine **5**) or known compounds (Figure 3): mono-Boc putrescine

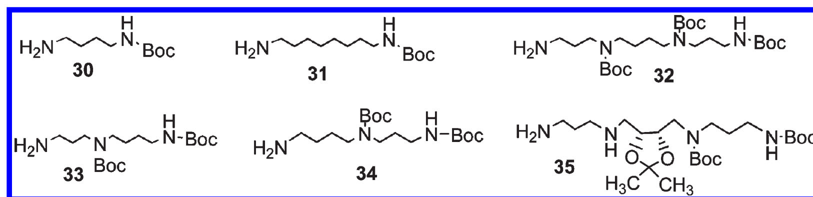
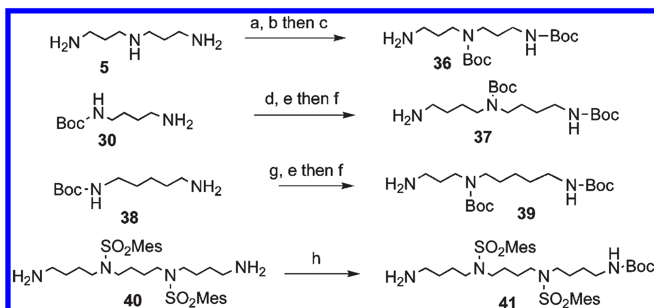


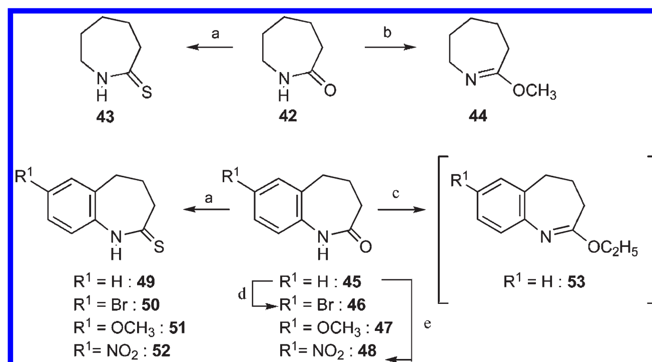
Figure 3. Structures of protected polyamines (**30**−**35**).

Scheme 1[a]



[a] Reagents: (a) ethyl trifluoroacetate (1 equiv); (b) $(Boc)_2O$ (3 equiv); (c) K_2CO_3 (5.2 equiv); (d) 4-bromobutyronitrile (1 equiv), KF/Celite; (e) $(Boc)_2O$ (1.5 equiv); (f) H_2, Raney Ni, NH_3/EtOH; (g) acrylonitrile (1 equiv); (h) $(Boc)_2O$ (1 equiv).

Scheme 2[a]



[a] Reagents: (a) Lawesson's reagent, dioxane or toluol; (b) $(CH_3)_2SO_4$, toluol; (c) $(C_2H_5)_3OBF_4$, CH_2Cl_2; (d) Br_2, CH_3COOH; (e) H_2SO_4, HNO_3.

30,[45] mono-Boc octanediamine **31**,[46] di-Boc spermidines **33**,[47] and **34**,[48] tri-Boc spermine **32**,[49] or protected dihydroxyspermine **35**.[50]

Adapted procedures were carried out to afford protected triamines **36**,[49] **37**,[49] and **39**[49] (Scheme 1) in a 39%, 44%, and 75% yields, respectively. The mono Boc protected compound **41** resulted from a classical monoprotection of the dimesitylhomospermine **40** (Scheme 1).[51]

Preparation of Lactams. The ε-caprolactam **42** is commercially available. The benzazepinones **45** and **47** were prepared from α-tetralone and 6-methoxy-1-tetralone, whose oxime groups underwent Beckmann rearrangement.[52,53] In the case of **47**, the Beckmann rearrangement resulted in the formation of two regioisomers that could be separated. The substituted analogues **46** and **48** were obtained from **45**: its nitration was achieved according to classical techniques[54,55] to provide **48** in good yield. The access to the bromo analogue **46** had been described through the reduction of the nitro group to an amine followed by a Sandmeyer reaction,[53,54] but for this work, we simply transposed chlorination conditions[56] to the bromination of **45** and directly obtained **46**, the bromination site being confirmed by ^{13}C NMR spectroscopy.

Preparation of Thiolactams and Iminoethers. Thiolactams **43** and **49**−**52** were prepared in good yields (80%) by reaction of lactams **42** and **45**−**48** with Lawesson's reagent (Scheme 2).[57]

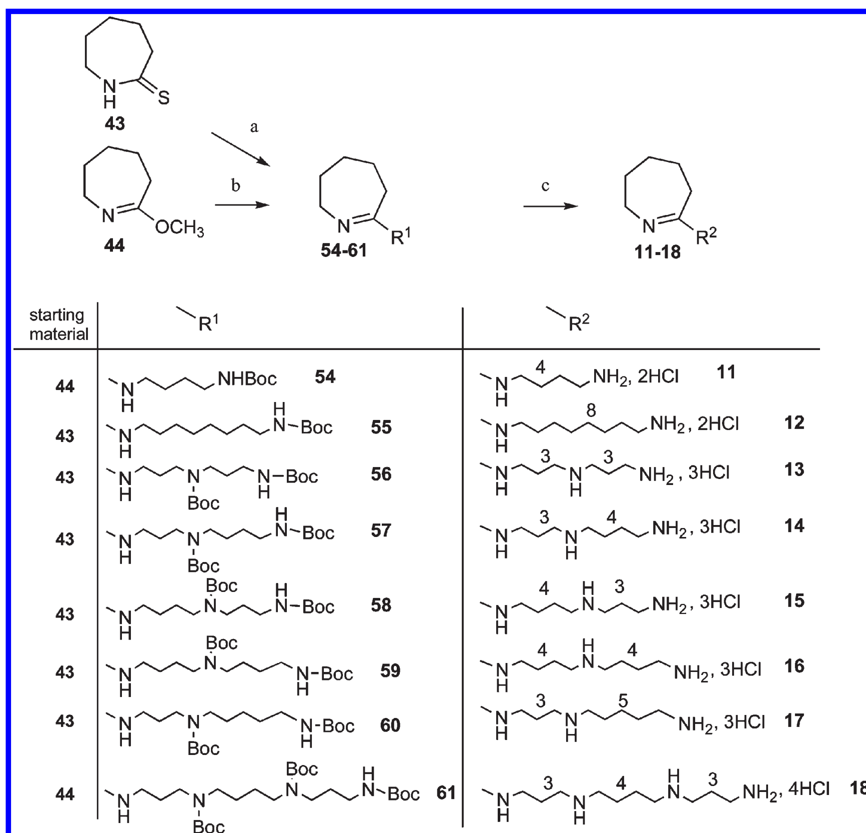
The lactams **42** and **45** could also be converted to iminoethers (Scheme 2). Two methods were carried out. The first method used dimethylsulfate as a methyl donor[58] and was applied to the lactam **42** to give **44** with a moderate yield (35%). For the second method, triethyloxonium tetrafluoroborate and the benzazepinone **45** were solved together in anhydrous dichloromethane to give the imidate **53**, which was not isolated.[59]

Conjugation. The coupling of the heterocycles to the polyamine chain was then carried out: the amidines **54**−**71** were obtained either from thiolactams **43** or **49**−**52** or from iminoethers **44** or **53**. Classically, thiolactams are reacted with the polyamine moiety in the presence of a base (triethylamine or excessive polyamine) and mercury(II) chloride ($HgCl_2$).[60] This reaction produces HgS and HCl that can be trapped by a base. Practical reasons prompted us to carry out different procedures depending on the polyamine moiety (symmetrical or unsymmetrical) as well as on the thiolactam lipophilicity. Our initial attempts consisted of direct conjugation of a free polyamine (putrescine or spermine) with the ε-caprothiolactam **43**. Despite formation of the expected amidine, the final isolation (extraction followed by chromatographic purification) could not be achieved because of the high hydrophilicity of the final compound. Thus, conjugation of the ε-caprothiolactam (Scheme 3) always involved the use of equimolar protected polyamines whose lipophilic protective groups not only prevented the formation of regioisomers (for unsymmetrical polyamines) but also facilitated the isolation of the compounds **54**−**61**. The use of protective groups was also necessary to the regioselective conjugation of unsymmetrical polyamines **33** and **35** to the thiolactam **49** (Scheme 4, syntheses of **66**−**67**). The free secondary amine of **35** did not notably react in our conditions. Finally, the substituted lactams **50**−**52** were coupled to the readily accessible tri-Boc spermine **32** and furnished **69**−**71** in moderate to good yield.

In contrast, the direct coupling (Scheme 4) of lipophilic thiolactam **49** to unprotected symmetrical chains such as putrescine **1**, 1,8-diaminooctane **4**, norspermine **5**, or spermine **3** was successful for the preparation of **62**−**65**. An excess of the polyamine could be advantageously used instead of triethylamine (TEA): it prevented the formation of bis-conjugates and was easily removed by extraction in aqueous medium. As we observed for the partially protected chain **35**, secondary amines did not react and the expected amidines **62**−**65** could be isolated and purified by the usual chromatographic methods.

The second route to amidines used iminoethers to avoid the presence of highly toxic mercury salts. Thus, in the first attempt (Scheme 3), the iminoether **44** was isolated and

Scheme 3[a]



[a] Reagents: (a) protected PA R^1-H (1 equiv), TEA (4 equiv), $HgCl_2$, THF, Δ; (b) protected excessive PA R^1-H, Δ; (c) 3 M HCl, C_2H_5OH.

reacted with the protected putrescine **30** to give **54** that was not isolated. Similarly (Scheme 4), the protected amine **41** was added to **53**, and the desired compound **68** was obtained in low yield (18% from the lactam **45**).

The amidines **55**−**71** were purified by column chromatography and characterized by usual techniques such as IRFT and ^{1}H NMR spectroscopy.

The nitro group of **71** was reduced by H_2 in the presence of Pd/C to provide **72** quantitatively.

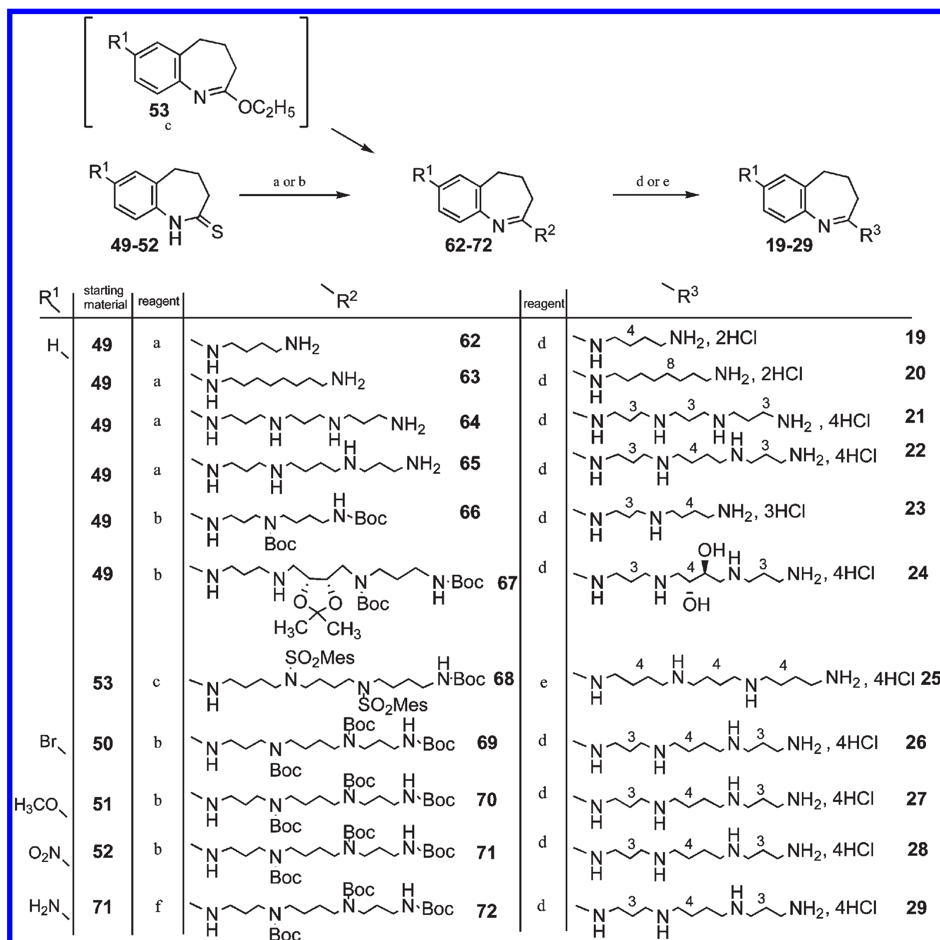
Last, the compounds **54**−**72** (with the exception of **68**) were deprotected using 2 M HCl in ethanol to afford the hydrochlorides **11**−**29**. HBr was used for simultaneous deprotection of the sulphonamide and Boc groups of **68**,[51] which gave the hydrochloride **25** after alkalinization in aqueous NaOH, extraction, and final treatment with HCl.

All final compounds **11**−**29** were pure according to TLC, HPLC, and elemental analysis criteria. They were fully analyzed by usual techniques of IRFT, ^{1}H and ^{13}C NMR and HRMS.

Affinity for the PTS. The intrinsic affinities of the conjugated and unsubstituted polyamine systems for the PTS (K_i values) were determined in a competitive assay with radiolabeled spermidine as previously reported.[16,41] The following trend toward increasing transporter affinity (lower K_i value) was observed for both free or substituted polyamines: diamines < triamines < tetramines. Although similar trends were observed in a series of conjugated polyamines, the conjugates with diamine or triamine chains generally displayed lower affinity (higher K_i values) than the unsubstituted homologues (e.g., **14** or **23** vs **2**; **13** vs **5**). In contrast, the opposite trend was observed with the tetramine conjugates (lower K_i values) (e.g., **18** or **22** vs **3**; **25** vs **9**). These observations are in total agreement with previous data collected with other polyamine conjugate systems.[16,40,43] This suggests that the heterocyclic moiety itself participates in the binding of the conjugate to the PTS. We earlier postulated the existence of an hydrophobic pocket of set dimensions adjacent to the PTS which serves as a docking site for the hydrophobic cargo tethered to the polyamine chain.[42] The fit binding of the azepine or benzazepine moiety into this pocket could participate in the high affinity of tetramine conjugates. In addition, the polyamine conjugates with the bulkier benzazepine cargo had higher affinity for the PTS than their homologues conjugated to azepine. The higher affinity of the benzazepine conjugates may be related to their higher hydrophobicity, leading to a tighter anchorage to the hydrophobic pocket.[42] A similar trend was already observed in a series of arenes tethered to polyamines, with the following trend toward increasing transporter affinity: benzyl < naphtyl < anthracenyl.[23]

As observed for unsubstituted polyamines, the number of nitrogen centers (e.g., **14** vs **12**) as well as the tether (number of CH_2 spacer units) between the nitrogen centers (e.g., **13** vs **14** vs **16**) had a dramatic effect on the K_i value of conjugated polyamines. In the azepine-triamine series, a clear preference for the aminobutyl spacer was evident (e.g., **16** vs **14** and **17**). The preference was also for a terminal aminobutyl moiety (**14** vs **15**). The presence of hydrophilic hydroxyl groups on the central aminobutyl chain of spermine was deleterious for the affinity of both the free and conjugated polyamines. Hydroxyl groups may cause steric hindrance or may hamper hydrophobic interactions between the central methylene chain and hydrophobic residues of the PTS. Such interactions have been shown to be important to the recognition of bacterial polyamine uptake systems.[1]

Scheme 4[a]



[a] Reagents: (a) excessive R^2-H, $HgCl_2$, THF, Δ; (b) R^2-H (1 equiv), TEA (4 equiv), $HgCl_2$, THF, Δ; (c) **41** (0.9 equiv), TEA (0.9 equiv), CH_2Cl_2, 0 °C then 40 °C; (d) 3 M HCl, C_2H_5OH; (e) 30% HBr in HOAc/PhOH/CH_2Cl_2 then HCl; (f) H_2, $Pd(OH)_2$/C.

Transport and Selectivity. Two cellular models were chosen to explore the selectivity of the conjugates toward the PTS: (i) L1210 cells in absence or presence of DFMO, inhibition of ornithine decarboxylase by DFMO leads to a significant increase in polyamine uptake as a reaction to the depletion of intracellular polyamine pools,[61] (ii) chinese hamster ovary (CHO) cells and the polyamine transport deficient mutant CHO-MG cells.[62] These cells were challenged with the conjugates and intracellular levels were monitored by HPLC. Polyamine conjugates that selectively target the PTS should display an enhanced accumulation in cells with greater PTS activity (e.g., CHO vs CHO-MG; DFMO-treated L1210 vs L1210).

First, the accumulation of the conjugates containing naturally occurring polyamines was determined in L1210 and CHO cells after a 24 h exposure at various concentrations (ranging from 0.1 to 100 μM). Both cell types accumulated, in a concentration dependent manner, measurable amounts of the conjugates (Figure 4A1−B1).

The accumulation of these derivatives was strongly dependent on the PTS activity because their accumulation into CHO-MG cells was significantly reduced when compared to CHO (Figure 5). Co-treatment of L1210 cells with DFMO which induces an upregulation of the PTS activity, enhanced the accumulation of all conjugates but **19** and **23** (Figure 6). However, the latter displayed a high CHO/CHO-MG accumulation ratio (around 11), suggesting a high selectivity for the PTS in CHO cells. The absence of observable enhancement of its accumulation in DFMO-treated cells may have several explanations. There may be differences in the fine specificity of the PTS in the different cellular models. In this context, it has to be noted that while the amounts accumulated for most analogues were similar in both cells, the accumulation of **15** was six times higher in CHO than in L1210 cells (Figure 4). It has also been reported that free polyamines, formed as the result of intracellular catabolism of some polyamine derivatives, may prevent the up-regulation of the PTS activity by DFMO. Such mechanism was proposed to explain the absence of DFMO-enhanced accumulation of N^4-benzylspermidine derivatives.[32]

The nature of the polyamine vector has a strong impact on the accumulation of the conjugates. CHO and L1210 cells accumulated higher amounts of spermidine than the spermine or the putrescine conjugates (Figure 4). Although spermine confers a much higher affinity of the conjugates for the PTS (Table 1), it was not an efficient vector for their accumulation into cells, a trend already reported for other spermine conjugates.[40,43] In addition, it also has to be noted that for a given polyamine vector and despite their higher affinity for the PTS cells accumulated lesser amounts of the benzazepine than azepine conjugates (e.g., **14** vs **23**). So the size and/or the hydrophobicity of the cargo influence the interaction with the PTS in two opposite ways. A bulkier and/or more hydrophobic cargo confers more affinity for the PTS but reduces the amount transported.

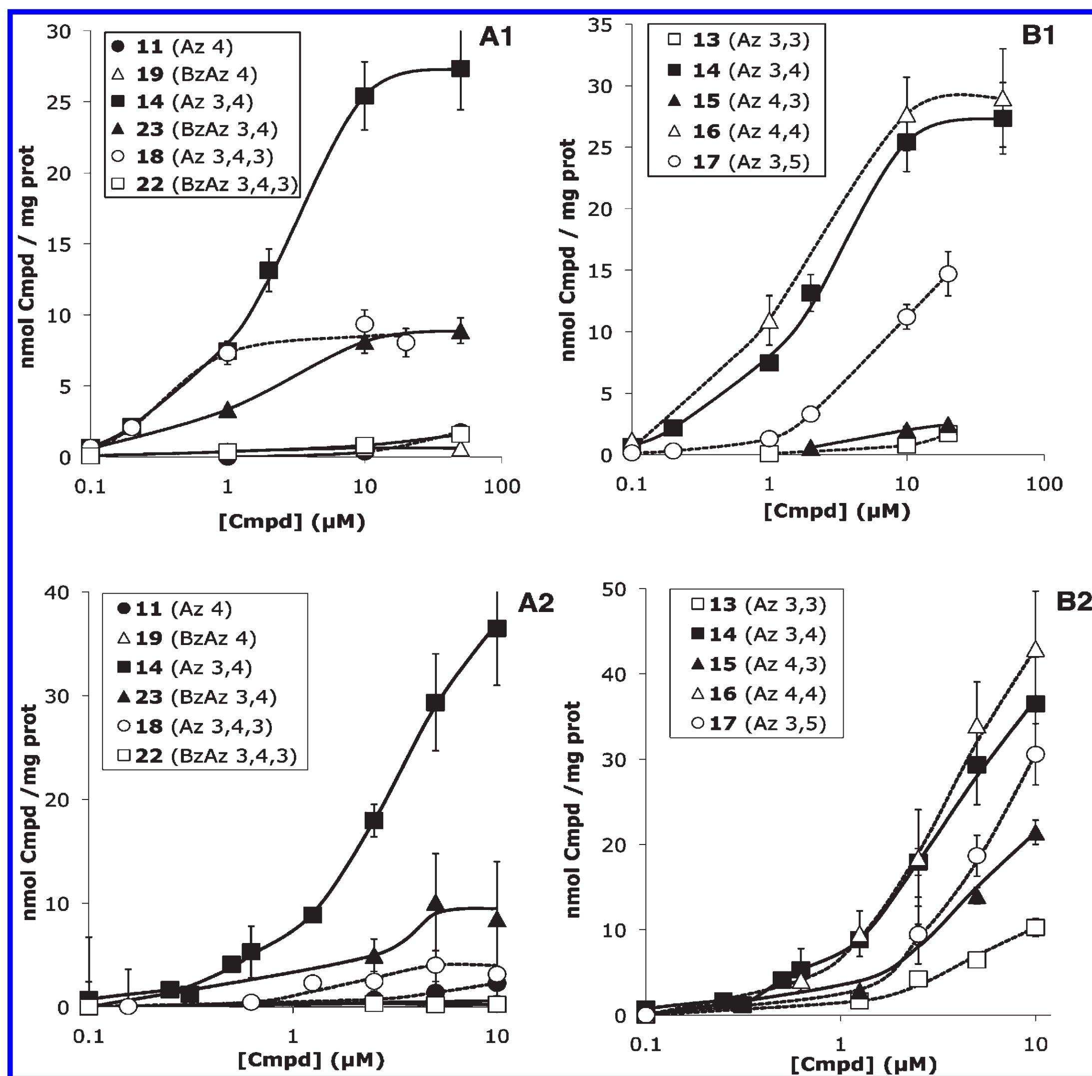


Figure 4. Cellular uptake of naturally occurring polyamine conjugates (A) and triamine−azepine conjugates (B) in L1210 (1) and CHO (2) cells cultured 24 h in presence of the compounds. Intracellular concentrations were determined by HPLC as described in the Experimental Section. Results are the mean of triplicates. Bars, SD.

Altogether these observations confirm previous studies indicating that spermidine is a better vector than putrescine or spermine for cell delivery using the PTS.[32,40,43] To determine the influence of the spermidine scaffold, we studied the accumulation of azepine conjugates with various triamines: homospermidine, norspermidine, N^1- or N^8-tethered spermidine, and N^3-aminopropyl-cadaverine. All triamine conjugates were actively taken up by a PTS dependent mechanism in CHO and L1210 cells as suggested by their very high CHO/CHO-MG accumulation ratio and their enhanced accumulation in DFMO-treated L1210 cells. The terminal aminobutyl motif appeared to be advantageous for accumulation into L1210 and CHO cells. Indeed, both cells accumulated higher amount of N^1-spermidine (**14**) and homospermidine (**16**) conjugates than N^8-spermidine (**15**) and norspermidine (**13**) conjugates (Figure 4). The aminobutyl motif seems to be the optimum size because the derivative with a terminal aminopentyl (**17**) accumulated less than the homologue with a terminal aminobutyl (**14**). In this triamine series, there is a good correlation between the affinity of the compounds for the PTS and their quantitative and selective cell delivery. The more efficient delivery was observed with compounds **14** and **16**. Despite their slight difference in affinity for the PTS (Table 1), they were both accumulated to similar amounts in L1210 as well as in CHO cells. Both compounds had an effect on cell growth as assessed using the MTT assay (Figure 7). This effect was rather cytostatic than cytotoxic because over 95% of the cells remains viable after a 48 h treatment with 100 μM of the compounds as determined using a Trypan blue assay (data not shown). **16** had a stronger impact on L1210 cells: for instance, 10 μM of **16** induced a 65% reduction in cell growth as assessed using the MTT assay after 48 h culture (compared with 30% reduction with **14**). A similar assay performed in presence of DFMO demonstrated another difference in the behavior of the two compounds: **16** partially antagonized the cytostatic effect of DFMO. Because of its lower impact on cell growth and its lack of antagonism toward DFMO, **14** appears to be a more suitable vector for cell delivery than **16**. A time-course study shows that in presence of DFMO the accumulation of **14** is rapid, with a plateau reached 8 h after the beginning of exposure, time where DFMO treated cells have accumulated around 4 times more compound **14** than the control cells (Figure 8).

Its is of interest to identify PTS-selective structures with high accumulation in cancer cells and devoid of cytotoxicity. Various structures including unconjugated polyamines,[28−31,63−65]

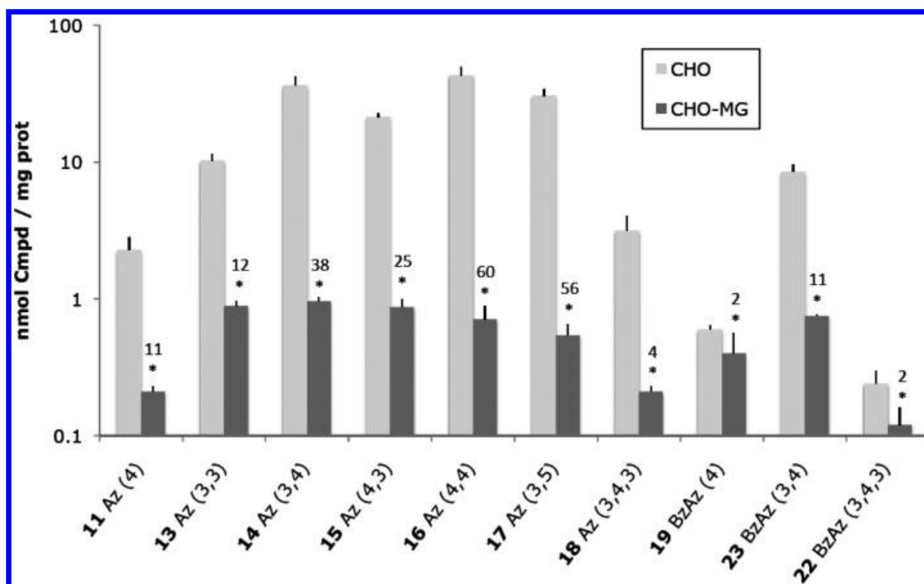


Figure 5. Cellular uptake of the conjugates in CHO and CHO-MG cells. Conjugates were added 24 h after seeding and collected 24 h later. Intracellular levels of conjugates were determined by HPLC on perchloric extracts; mean values (SD) from three determinations. * $p < 0.05$, significantly different from values determined in CHO cells. CHO/CHO-MG accumulation ratios are indicated as bold numbers over the CHO-MG bars.

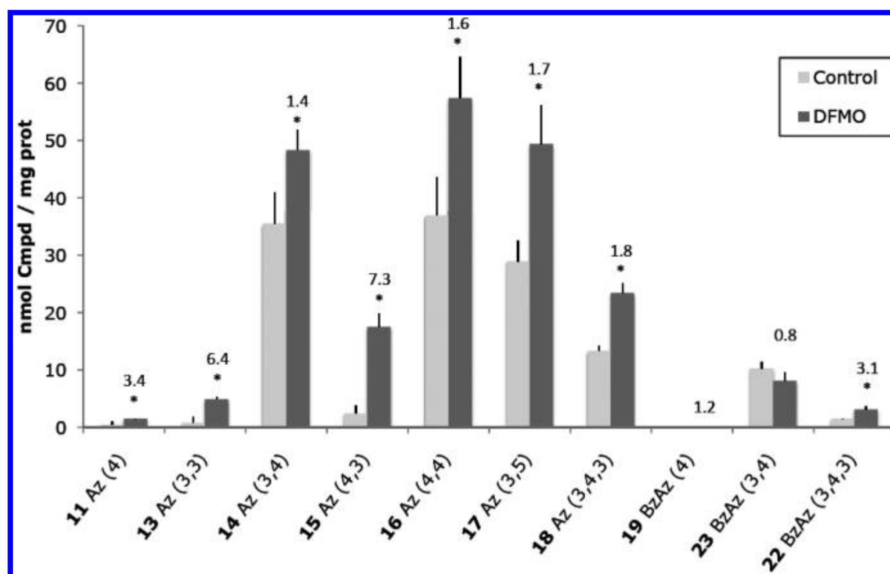


Figure 6. Effect of DFMO on intracellular accumulation of polyamine conjugates in L1210 cells. All cells were challenged with 10 μM conjugates at the time of seeding in presence or absence of DFMO (5 mM). Cells were collected 48 h later, and intracellular levels of conjugates were determined by HPLC as described in the Experimental Section. Values represent mean values (SD) from three determinations. * $p < 0.05$, significantly different from values determined in control cells. DFMO/control accumulation ratios are indicated as bold numbers over the DFMO bars.

conjugated polyamines such as *N*-benzylpolyamines,[32] or polyamine analogues such as (*Z*)-1,4-diamino-2-butene[33] have been envisaged as vector of various isotopes such as boron, fluorine or iodine for cancer therapy and imaging. But because of their cytotoxicity, low selectivity, and/or low accumulation rate in cells, their development was not pursued. The azepine–spermidine conjugate **14** shares all properties required for an effective PTS-targeting vector. In comparison of the behavior of **14** with that of N^1-benzylspermidine, a closely structurally related compound,[32] demonstrates its higher potential: its accumulation in cells (e.g., CHO) is two times higher; **14** is a weak cytostatic agent that can be used in combination of DFMO (while N^1-benzylspermidine is cytotoxic in the 10 μM range, and DFMO synergizes its cytotoxicity). Although we cannot predict how isotopes carried by the azepine moiety will affect the properties of **14**, boron, iodine, and fluorine-substituted **14** are currently being designed.

Polyamine Transport Inhibition. The importation of exogenous natural polyamines annihilates the growth-inhibitory efficiency of DFMO-induced polyamine depletion in vivo.[66] To circumvent this problem, the systematic reduction of exogenous polyamine sources using a combination of polyamine-deficient diet with a decontamination of the gastrointestinal tract have proved to be a viable strategy to recover the effectiveness of DFMO but clearly lack tumor specificity.[67,68] A most straightforward approach is the use of PTS inhibitors capable to prevent the uptake of natural polyamines. A highly specific and selective PTS inhibitor should be characterized by: (i) a high affinity for

Table 1. K_i Values for the Inhibition of Spermidine Transport by Free or Conjugated Polyamines in L1210 Cells

polyamine side chain	(cmpd) K_i^a (μM) {K_m/K_i ratio}[b] free polyamine	azepine conjugates	benzazepine conjugates
putrescine	(**1**) 208 ± 16.3 [c] *{0.011}*	(**11**) 763 ± 103 *{0.003}*	(**19**) 35.1 ± 1.0 *{0.067}*
diaminooctane	(**4**) 24.1 ± 0.8 *{0.098}*	(**12**) 183 ± 12 *{0.013}*	(**20**) 126 ± 10 *{0.019}*
norspermidine	(**5**) 5.12 ± 0.21 *{0.46}*	(**13**) 161 ± 5.4 *{0.015}*	NA[d]
spermidine		(**14**) 14.6 ± 0.9 *{0.16}*	(**23**) 4.40 ± 0.27 *{0.54}*
		(**15**) 44.7 ± 1.2 *{0.053}*	NA
homospermidine	(**6**) 2.30 ± 0.08 *{1.03}*	(**16**) 5.47 ± 0.19 *{0.43}*	NA
aminopropyl-diaminopentane	(**7**) 8.03 ± 0.50 *{0.29}*	(**17**) 45.9 ± 2.8 *{0.051}*	NA
norspermine	(**8**) 1.51 ± 0.12 *{1.57}*	NA	(**21**) 0.88 ± 0.04 *{2.69}*
spermine	(**3**) 1.34 ± 0.31 *{1.77}*	(**18**) 0.35 ± 0.01 *{6.77}*	(**22**) 0.15 ± 0.03 *{15.8}*
			(**26**) 0.14 ± 0.01 *{16.9}*
			(**27**) 0.23 ± 0.01 *{10.3}*
			(**28**) 0.12 ± 0.02 *{19.8}*
			(**29**) 0.19 ± 0.01 *{12.5}*
homospermine	(**9**) 0.73 ± 0.06 *{3.25}*	NA	(**25**) 0.20 ± 0.01 *{11.9}*
dihydroxy-spermine	(**10**) 5.27 ± 0.41 *{0.45}*	NA	(**24**) 2.25 ± 0.30 *{1.05}*

[a] K_i values were calculated from the half-maximal inhibitory concentration (IC_{50}) estimated by iterative curve fitting for sigmoidal equations describing polyamine uptake velocity in the presence of growing concentrations of antagonist. [b] The K_m value for spermidine uptake (2.37 ± 0.45 μM) was determined by Lineweaver–Burke analysis of transport velocity at increasing radiolabeled substrate concentrations. [c] Data are expressed as mean (±SD) from three separate determinations. [d] NA: not available

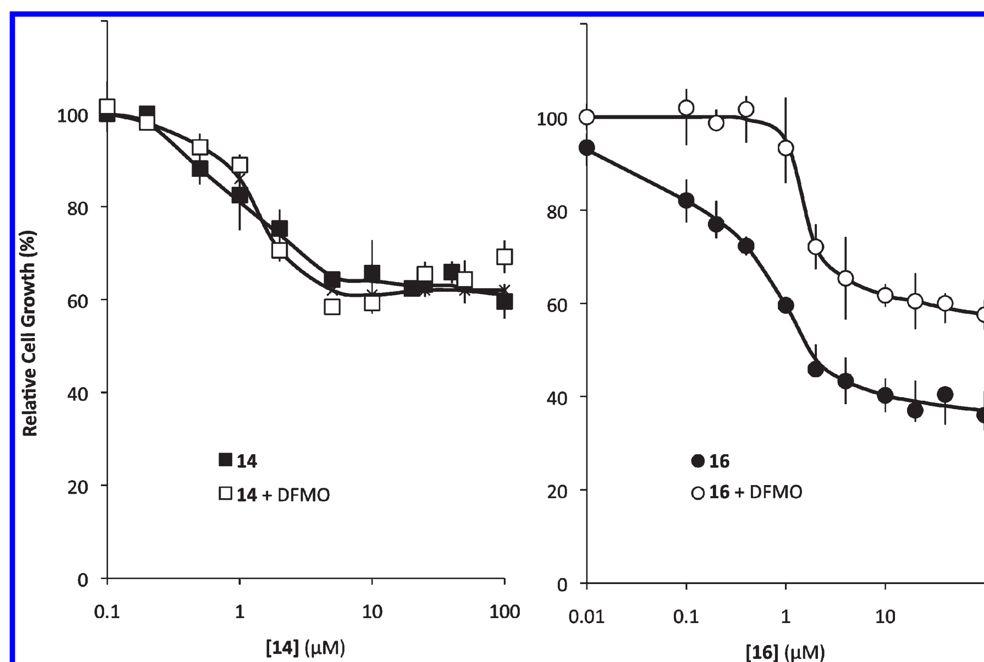


Figure 7. Effect of the conjugates **14** and **16** on L1210 cell growth in the presence or absence of DFMO. Cells were cultured for 48 h with the conjugates in the presence or absence of 5 mM DFMO. Cell growth rates were determined using the MTT assay. The relative cell growth rates were calculated from the value of cell growth of the corresponding control cells cultured in the absence or in the presence of DFMO. Data are mean of triplicates. Bars, SD.

the transporter, (ii) a very low cytotoxicity, the sole inhibition of the PTS should not affect cell growth or viability in normal conditions, (iii) an absence of DFMO antagonizing effect because the major therapeutic application of PTS inhibitors is to be given in association with this ODC inhibitor.

With its high affinity for the PTS (K_i = 0.15 μM), low uptake (Figure 4), and limited cytotoxicity (Figure 9), the benzazepine–spermine conjugate **22** is an attractive lead. However, **22** antagonizes partially the cytostatic effect of DFMO (Figure 9). Two strategies were followed for **22** optimization: (a) variations on the spermine chain, (b) substitution on the benzazepine moiety.

The substitution of the spermine chain by the analogues norspermine (**8**) or homospermine (**9**) reduced to some extent the affinity for the PTS (Table 1) and, in addition, both **21** and **25** were more cytotoxic on L1210 cells than **22** (data not shown).

Four substitutions (−Br, $-OCH_3$, $-NO_2$, $-NH_2$) were performed on the benzene ring of the benzazepine moiety to generate new spermine derivatives. These substitutions did not impair the affinity for the PTS (Table 1), but they greatly affected the cytotoxicity of the compound and its behavior in presence of DFMO (Figure 9). The bromo (**26**) or the methoxy (**27**) derivatives were more cytotoxic than **22**, and

they displayed a synergistic effect in presence of DFMO. The amino derivative **29** had also more inhibitory effect on cell growth and reversed very partially the effect of DFMO. In contrast, the nitro derivative **28** up to 20 μM did not display any effect on cell growth and did not show any synergistic or antagonist effect in presence of DFMO. Therefore the nitro derivative **28** is likely a potent and selective polyamine transport inhibitor. We then investigated its ability to prevent the reversion of DFMO-induced L1210 cell growth inhibition by exogenous naturally occurring polyamines (Figure 10).

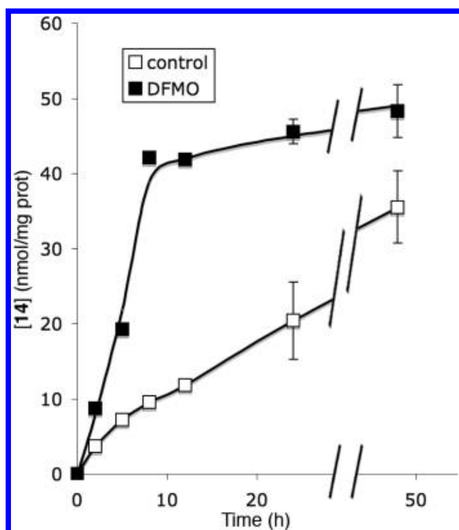


Figure 8. Time course of the accumulation of **14** in L1210 cells cultured in the presence or the absence of DFMO. Conjugate **14** (10 μM) and DFMO (5 mM) were added at the time of seeding. At the indicated time, cells were collected and intracellular levels of **14** were determined by HPLC as described in the Experimental Section. Results are the mean of triplicates. Bars, SD.

Putrescine (**1**), spermidine (**2**), and spermine (**3**) are all three able to antagonize the DFMO-induced cytostatic effect in L1210 cells. The efficiency of **2** and **3** is much higher because almost total reversion of the DFMO effect is observed at 1 μM, while around 20 μM of **1** is required. The coculture of L1210 cells with the derivative **28** at 20 μM (a concentration that did not affect the growth of the cells) prevented almost completely the reversion up to 100 μM of **1**, 20 μM of **2**, and 1 μM of **3**, concentrations a lot higher than those usually found in body fluids.[69−74]

The nitrobenzazepine−spermine conjugate **28** appears to be a potent polyamine transport inhibitor. It displays a very low intrinsic cytotoxicity. DFMO-induced polyamine depletion, which also translates into an activation of the PTS activity, does not enhance the cytotoxicity of **28**, as observed with many polyamine analogues and derivatives.[9,10] In addition, **28** does not antagonize the cytostatic effect of DFMO, demonstrating that **28** does not supply the cells with their polyamine requirements (Figure 10). Such antagonist effect has been reported for derivatives acting as polyamine mimetics and for conjugates capable of releasing polyamines after enzymatic cleavage.[32] All these properties may also be the consequence of a very low accumulation of the compounds inside the cells. However, we could not check

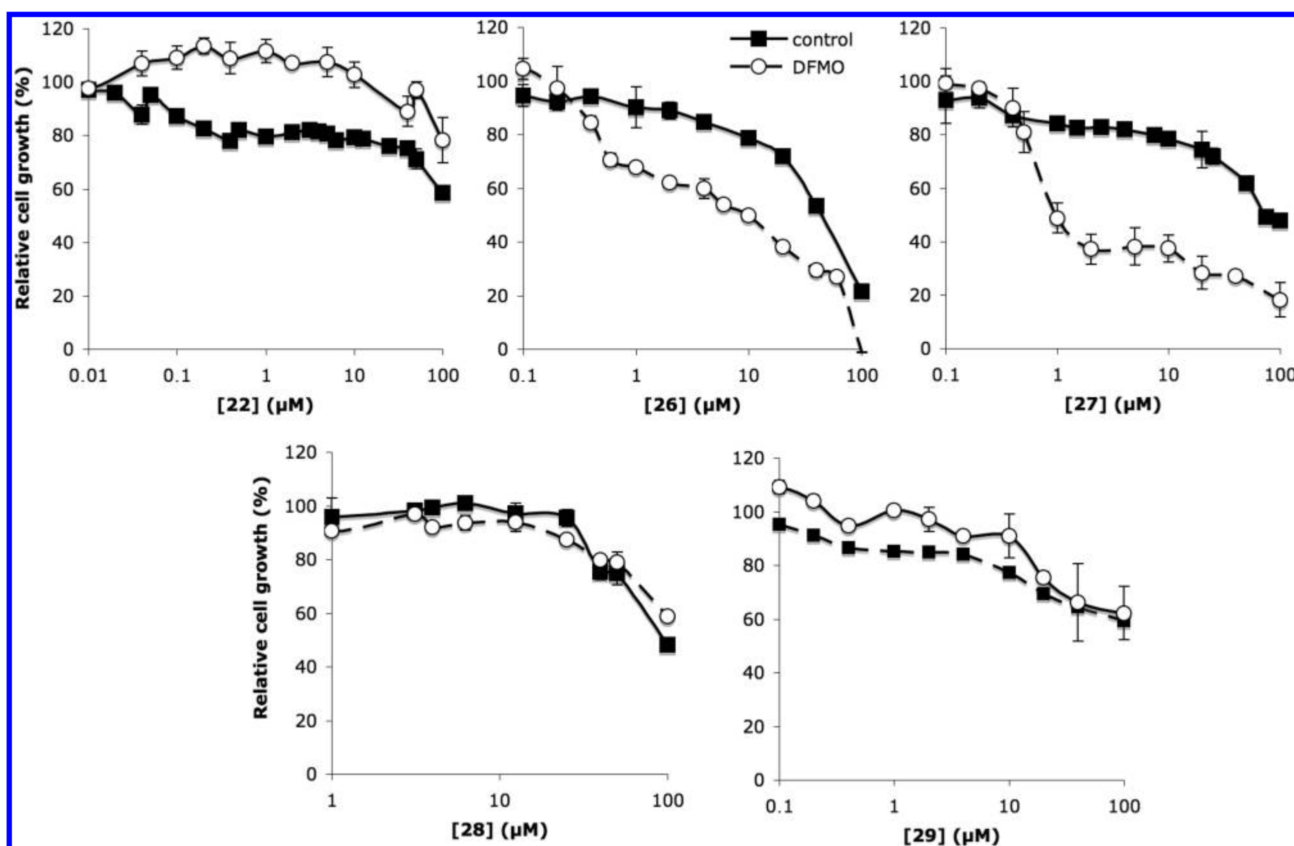


Figure 9. Effect of the conjugates **22** and **26-29** on L1210 cell growth in the presence or absence of DFMO. Cells were cultured for 48 h with the conjugates in the presence or absence of 5 mM DFMO. Cell growth rates were determined using the MTT assay. The relative cell growth rates were calculated from the value of cell growth of the corresponding control cells cultured in the absence or in the presence of DFMO. Data are mean of triplicates. Bars, SD.

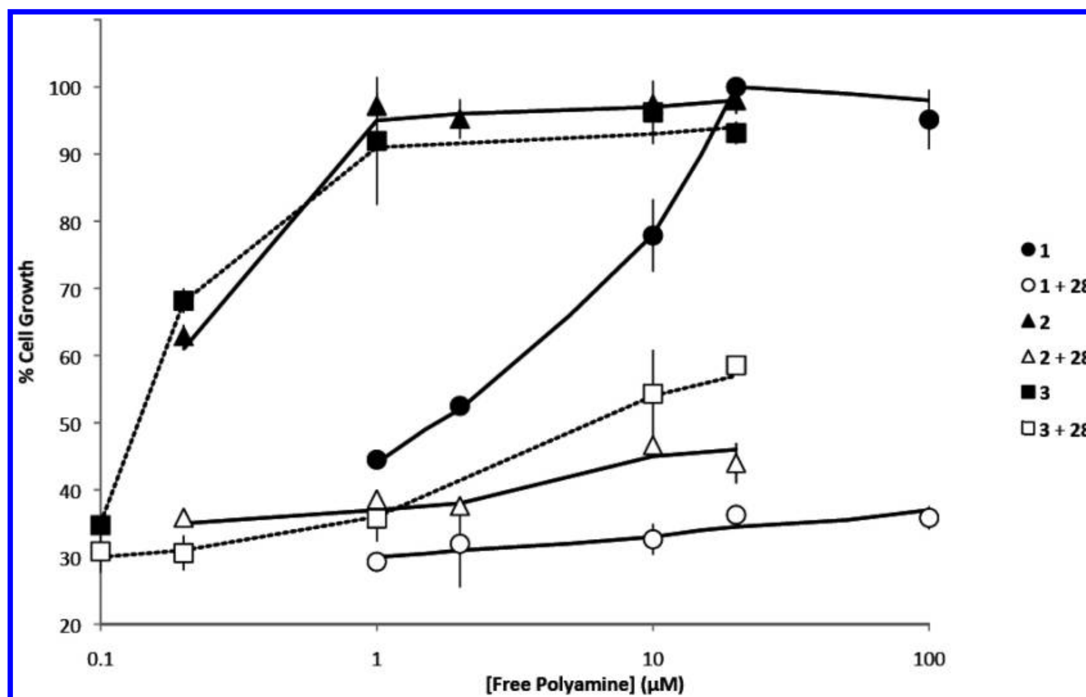


Figure 10. Effect of **28** on the reversion of the DFMO-induced cytostatic effect on L1210 cells by free polyamines. L1210 cells were seeded in presence of 5 mM DFMO. Twenty-four hours later, cells were challenged with free polyamines (**1**, **2**, or **3**) in presence or absence of **28** (20 μM). Cell growth was determined using a MTT assay 48 h later. Data are mean of triplicates. Bars, SD.

this aspect because **28** did not give rise, after derivatization with *o*-phthalaldehyde, to a fluorescent derivative detectable in our HPLC system.

A recent report has demonstrated that the combination of DFMO with new lipophilic−spermine conjugates that are highly potent polyamine transport inhibitors, is a valid approach for cancer therapy in vivo.[38] The intrinsic properties of **28** make it a novel lead for further polyamine-targeted anticancer development.

Conclusion

The structure−activity relationship studies of two families of polyamine conjugates identified in each family a PTS-targeting compound of interest. The azepine−spermidine conjugate **14** is a very specific substrate of the PTS which accumulates to high levels in cells equipped with an active PTS. This compound could serve as a vector to accumulate various isotopes for either cancer curing or tumor imaging. The benzazepine series afforded a high affinity PTS inhibitor. The nitrobenzazepine−spermine **28** prevents the reversion of the DFMO-induced cytostatic effect by exogenous polyamines at physiological concentrations. This compound may serve as an adjuvant in DFMO anticancer therapy which is seriously impaired by the exogenous polyamines that are imported into the cells via the PTS.

Experimental Section

Chemistry. Reagent-grade solvents were purchased from chemical companies and used directly without further purification unless otherwise specified. THF was dried under nitrogen by distillation over sodium and benzophenone and diethylether by distillation over $LiALH_4$. Dry ethanol was stored over 4 Å molecular sieves.

Merck Silica Gel 60 (70−230 mesh) was used as solid phase for column chromatography. Thin-layer chromatographies were performed on Merck Silica Gel 60 F254 (layer thickness: 0.22 mm). Solvent systems (expressed in volume percents) and R_f are indicated in the text. The compounds were visualized using UV light, ninhydrin, iodine, or alkaline solution of $KMnO_4$. FTIR spectra were recorded on a Perkin-Elmer 16 PC instrument (KBr pellets; ν: cm^{-1}). NMR spectra were recorded on a Bruker DMX spectrometer at 500 MHz (^{1}H) or 125 MHz (^{13}C). TMS was used as the internal standard for NMR spectra performed in $CDCl_3$. 3-(Trimethylsilyl)-1-propanesulfonic acid (DSS) was used as the external standard for NMR spectra recorded in D_2O. Attributions in ^{1}H NMR of chemical shifts were performed using selective decoupling experiments and COSY spectra (for **17** and **23**) recorded on a Bruker DMX spectrometer at 500 MHz with a spectral window of 3004 Hz. Broad band and gated decoupling ^{13}C NMR spectra were recorded, and the assignments were made using chemical shifts and coupling constants (^1J and long-range coupling) and HMQC, HMBC spectra (for **17** and **23**). Values with an asterisk (*) can be interchanged. Optical rotations were recorded with a Perkin-Elmer 341 automatic polarimeter at 21.5 °C.

Electronic impact high resolution mass spectra (HRMS EI) were recorded on a Varian MAT 311 double-focusing instrument at the CRMPO (Centre Régional de Mesures Physiques de l'Ouest, Rennes) with a source temperature of 140 °C, an ion accelerating potential of 3 kV and ionizing electrons of 70 eV and 300 μA. High resolution mass spectra determined by liquid secondary ion mass spectrometry (HRMS LSIMS) were performed on a ZabSpec Tof Micromass at the CRPMO with a source temperature of 40 °C, an ion (Cs^+) accelerating potential of 8 kV, and mNBA (*meta*-nitrobenzylic alcohol) as matrix.

HPLC analyses were conducted on an Agilent 1100 series HPLC system equipped with a 1200 series fluorimeter according to versions of a previously described method.[75] Polyamine derivatives were determined by separation of the ion pairs formed with *n*-octanesulfonic acid on a reversed-phase column (C18 column; Nucleosil 5, C18 AB, 100 mm^{-5} μm from Macherey-Nagel, Düren, Germany), reaction of the column effluent with *o*-phthalaldehyde and *N*-acetylcysteine, and monitoring of fluorescence intensity (excitation at 345 nm; emission at 455 nm) as detailed in the Supporting Information.

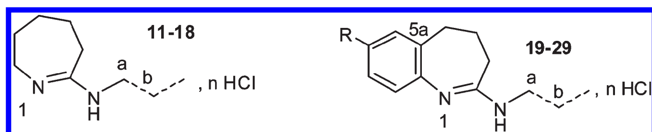


Figure 11. Numbering assignment for heterocycle ring and polyamine chain.

Purities of compounds were >95% as determined by elemental analyses performed by the Laboratoire de Microanalyses (Faculté de Pharmacie, Université Paris XI, Chatenay-Malabry). Purity of the derivatives was also determined using analytical high voltage paper electrophoresis as >95%.[76]

The compounds were numbered for the heterocyclic moiety using IUPAC rules and for the polyamine moiety using letters a−l (Figure 11).

Synthesis. Previously reported procedures were used for the synthesis of compounds **30**−**35**, **39**, **40**.

General Procedure A: Thionation. A solution of lactam and Lawesson's reagent (0.5−1 equiv) in dry dioxane or dry toluol was refluxed for 6 h. After evaporation of the solvent under reduced pressure, the residue was purified by column chromatography.

General Procedure B: Coupling of Thiolactams to Protected Polyamines. To a refluxing solution of thiolactam, suitably protected polyamine (1 equiv) and triethylamine (4 equiv) in dry THF (10−50 mL) was added mercury(II) chloride ($HgCl_2$, 1 equiv). A black precipitate of mercury sulfide HgS was observed, and refluxing was continued for 1 h under stirring. The THF was evaporated under reduced pressure, and the residue was suspended in methanol. The mercury sulfide was removed by filtration and washed with methanol. After evaporation of the solvent under reduced pressure, the residue was dissolved in CH_2Cl_2 and then the organic layer was washed with a 0.2 M aqueous solution of sodium thiosulfate $Na_2S_2O_3$. After drying over K_2CO_3, the organic layer was evaporated under reduced pressure. The residue was purified by column chromatography.

General Procedure C: Coupling Thiolactams to Free Polyamines. To a refluxing solution of thiolactam and free polyamine (10−25 equiv) in 2 mL of dry THF was added mercury(II) chloride $HgCl_2$ (1 equiv). The mixture was refluxed for 1 h, during which a black precipitate of HgS was observed. The THF was removed under reduced pressure, and the residue was suspended in CH_2Cl_2. The mercury sulfide (HgS) was removed by filtration and then the organic layer was washed with a 0.2 M aqueous $Na_2S_2O_3$. After drying over K_2CO_3, the organic layer was evaporated under reduced pressure. The residue was purified by column chromatography.

General Procedure D: Removal of Boc Groups and Preparation of Hydrochlorides. The amidine conjugate was stirred in a 2 M solution of HCl gas in ethanol (1.2 equiv per amino group). After evaporation of the ethanol, the residue was triturated in anhydrous ether to give a white hygroscopic solid.

Data for (6*R*, 7*S*)-*N*¹,*N*⁴-Di-*tert*-butoxycarbonyl-(6,7-*O*-isopropylidene)-6,7-dihydroxyspermine 35.[50] Oil, 21%; R_f 0.44 (CH_3OH/NH_4OH 95/5).

***N*¹,*N*⁴-Di-*tert*-butoxycarbonylnorspermidine 36.** A methanolic solution of norspermidine **5** (5 g, 38.1 mmol, 1 equiv) was cooled to −78 °C, and ethyltrifluoroacetate (1 equiv) was added. The temperature was maintained at −78 °C for 1 h and then allowed to reach 0 °C for 1 h. A methanolic solution of di-*tert*-butyldicarbonate (3 equiv) was then added at room temperature. The mixture was stirred for 1 h, made alkaline with K_2CO_3 (5.2 equiv), and left overnight. After filtration of insoluble salts, the filtrate was evaporated and chromatographed using CH_3OH/NH_4OH 95/5. Colorless oil, 39%; R_f 0.43 (CH_3OH/NH_4OH 95/5).

***N*¹,*N*⁵-Di-*tert*-butoxycarbonylhomospermidine 37.** *N*¹-*tert*-Butoxycarbonylputrescine **30** (0.500 g, 2.7 mmol, 1 equiv), 4-bromobutyronitrile (1 equiv), and KF on Celite (6 equiv) were mixed and stirred in CH_3CN (18 mL) for 24 h at 45 °C. The mixture was filtered, and the insoluble salts were washed with CH_3CN. The filtrate was evaporated under reduced pressure. The residue was dissolved in NaOH 1N (10 mL) and washed with CH_2Cl_2. The organic layer was washed with brine, dried over potassium carbonate, evaporated under reduced pressure, and then chromatographed using $CH_2Cl_2/CH_3OH/NH_4OH$: 80/20/0.5. The pure aminonitrile (0.370 g, 1.45 mmol, 1 equiv) was dissolved in THF and reacted with di-*tert*-butyldicarbonate (1.5 equiv) at room temperature and stirred overnight. The diprotected aminonitrile was purified by column chromatography with Et_2O. This latter (0.460 g, 1.29 mmol) was dissolved in an ethanolic solution of NH_3, and 1 g of Raney Nickel was added. The mixture was stirred under hydrogen (5 bar) at 25 °C for 72 h. The Raney nickel was removed by filtration, and the filtrate was evaporated under reduced pressure. The residue was chromatographed using CH_3OH/NH_4OH 95/5 and TLC was visualized using $KMnO_4$. Oil, 44%; R_f 0.49 (CH_3OH/NH_4OH 95/5).

Data for *N*¹-*tert*-Butoxycarbonylpentane-1,5-diamine 38:[45] Oil, 75%; R_f 0.35 (CH_3OH/NH_4OH 95/5).

***N*⁴,*N*⁹-Di-*tert*-butoxycarbonyl-4-azanonane-1,9-diamine 39.** A methanolic solution of acrylonitrile (0.46 g, 2.27 mmol, 1 equiv) was added dropwise for 1 h at 0 °C to a methanolic solution of **39** (1 equiv). The mixture was stirred overnight. After removal of MeOH, the residue was purified by column chromatography using $CH_2Cl_2/CH_3OH/NH_4OH$: 90/10/0.5. The pure aminonitrile was dissolved in CH_2Cl_2 and reacted with di-*tert*-butyldicarbonate (1.5 equiv) at 0 °C over 3 h and then stirred at room temperature overnight. The residue was chromatographed using Et_2O. The compound was dissolved in an ethanolic solution of NH_3, and a spoon of Raney Nickel was added. The mixture was put for 72 h under hydrogen (6 bar) at 25 °C. The residue was filtered and the precipitate was washed with EtOH and the filtrate was evaporated under reduced pressure. The residue was chromatographed using CH_3OH/NH_4OH 95/5 and TLC was visualized using $KMnO_4$. Oil, 75%; R_f 0.50 (CH_3OH/NH_4OH 95/5).

Data for *N*¹-*tert*-Butoxycarbonyl-*N*⁵,*N*¹⁰-bis(mesitylenesulfonyl)homospermine 41. To a solution of *N*⁵,*N*¹⁰-bis(mesitylenesulfonyl)homospermine **40**[51] (5.68 g, 9.56 mmol, 3 equiv) in CH_2Cl_2 was added dropwise at 0 °C a solution of di-*tert*-butyl dicarbonate (1 equiv) in CH_2Cl_2. After stirring at 0 °C over 3 h then at room temperature overnight, the solvent was evaporated under reduced pressure. Column chromatography using $CH_2Cl_2/MeOH/NH_4OH$: 70/10/1. Oil, 57%; R_f 0.51 ($CH_2Cl_2/MeOH/NH_4OH$ 70/10/1).

Data for 1,3,4,5,6,7-Hexahydro-1-azepin-2-thione 43. General procedure A from **42** (2 g, 17.6 mmol) and Lawesson's reagent (0.5 equiv) in dry toluene (50 mL). Column chromatography using CH_2Cl_2/Et_2O 95/5. White solid, 76%; R_f 0.49 (CH_2Cl_2/Et_2O 95/5).

2-Methoxy-3,4,5,6-tetrahydro-2*H*-azepin-2-one 44. To a warmed solution of ε-caprolactam **42** (11.32 g, 0.1 mol, 1 equiv) in toluol (35 mL) was added dropwise the dimethylsulfate (1 equiv) for 45 min. The reaction mixture was stirred for 16 h and then cooled at room temperature. A 50% aqueous solution of K_2CO_3 was added. When the CO_2 release was finished, stirring was continued for 90 min. The potassium methylsulfate precipitate was eliminated by filtration and washed by diethylether (3 × 10 mL). After decantation, the organic phase was dried over K_2CO_3 and concentrated under reduced pressure. The product was purified by distillation. Liquid, 35%; R_f 0.63 (CH_2Cl_2); Bp_{10} = 80 °C.

7-Bromo-1,3,4,5-tetrahydro-2*H*-1-benzazepin-2-one 46. To a solution of **45** (0.2 g, 1.24 mmol) in a mixture of AcOH (3 mL) and water (1 mL) was added dropwise bromine (0.2 g, 1.30 mmol) dissolved in AcOH (1 mL) for 0.5 h. The solution was stirred for 0.5 h and then cooled to 4 °C and water (20 mL) was added to have a precipitate that was filtered off. Cream solid, 75%; R_f 0.38 (CH_2Cl_2/EtOAc 80/20).

Data for 7-Methoxy-1,3,4,5-tetrahydro-2*H*-1-benzazepin-2-one 47.[53] White solid, 15%; R_f 0.70 (acetone).

7-Nitro-1,3,4,5-tetrahydro-2*H*-1-benzazepin-2-one 48. To a mixture of concentrated sulfuric acid (6.75 mL) and fuming nitric acid (5.4 mL) was added **45** (0.9 g, 5.58 mmol). The suspension was heated to 90 °C for 5 min. Then the mixture was cooled to 0 °C and the precipitate was filtered off and crystallized in a mixture of diethyl ether−hexane (80/20). Yellow solid, 77%; R_f 0.36 (CH_2Cl_2/Et_2O 80/20).

Data for 1,3,4,5-Tetrahydro-2*H*-1-benzazepin-2-thione 49. General procedure A from **45** (2 g, 12.4 mmol) and Lawesson's reagent (1 equiv) in dry toluene (50 mL). Column chromatography using CH_2Cl_2. Yellow oil, 80%; R_f 0.60 (CH_2Cl_2).

7-Bromo-1,3,4,5-tetrahydro-2*H*-1-benzazepin-2-thione 50. General procedure A from **46** (0.3 g, 1.25 mmol) and Lawesson's reagent (0.7 equiv) in dry toluene (20 mL). Column chromatography using CH_2Cl_2. White solid, 60%; R_f 0.19 (CH_2Cl_2/Et_2O 80/20).

7-Methoxy-1,3,4,5-tetrahydro-2*H*-1-benzazepin-2-thione 51. General procedure A from **47** (0.35 g, 1.84 mmol) and Lawesson's reagent (0.5 equiv) in dry toluene (20 mL) without further purification. White solid, 50%; R_f = 0.70 (CH_2Cl_2).

7-Nitro-1,3,4,5-tetrahydro-2*H*-1-benzazepin-2-thione 52. General procedure A from **48** (0.47 g, 2.28 mmol) and Lawesson's reagent (0.5 equiv) in dry toluene (25 mL). Column chromatography using Et_2O/pentane 80/20. White solid, 70%; R_f 0.71 (Et_2O/pentane 80/20).

***N*¹-(4,5-Dihydro-3*H*-azepin-2-yl)-*N*⁴-*tert*-butoxycarbonylputrescine 54.** To a solution of *N*-mono-*tert*-butoxycarbonylputrescine **30** (0.36 g, 1.91 mmol, 1 equiv) in CH_2Cl_2 was added an excess of imidate **44** (5 equiv). The mixture was allowed to warm at 60−70 °C at 15−20 mmHg for 5 h. To remove the excess of imidate, the mixture was warmed at 60 °C under reduced pressure (10^{-2} mmHg). Oil, 91%, R_f 0.65 (isopropylamine/$CH_3OH/CHCl_3$ 2/4/4).

***N*¹-(4,5-Dihydro-3*H*-azepin-2-yl)-*N*⁸-*tert*-butoxycarbonyloctane-1,8-diamine 55.** General procedure B from **43** (0.275 g, 2.13 mmol, 1 equiv) and **31** (1 equiv). Column chromatography using isopropylamine/$CH_3OH/CHCl_3$: 1/4/4. Oil, 72%; R_f 0.35 (isopropylamine/$CH_3OH/CHCl_3$ 1/4/4), 0.18 (CH_3OH/NH_4OH 90/10).

***N*¹-(4,5-Dihydro-3*H*-azepin-2-yl)-*N*⁴,*N*⁷-di-*tert*-butoxycarbonylnorspermidine 56.** General procedure B from **43** (0.201 g, 1.55 mmol, 1 equiv) and **36** (1 equiv). Column chromatography using isopropylamine/$CH_3OH/CHCl_3$: 1/4/4. Oil, 74%; R_f 0.37 (isopropylamine/$CH_3OH/CHCl_3$ 1/4/4), 0.11 (CH_3OH/NH_4OH 90/10).

***N*¹-(4,5-Dihydro-3*H*-azepin-2-yl)-*N*⁴,*N*⁸-di-*tert*-butoxycarbonylspermidine 57.** General procedure B from **43** (0.200 g, 1.55 mmol, 1 equiv) and **33** (1 equiv). Column chromatography using isopropylamine/$CH_3OH/CHCl_3$: 0.5/4/4. Oil, 81%; R_f 0.35 (CH_3OH/NH_4OH 90/10).

***N*¹-(4,5-Dihydro-3*H*-azepin-2-yl)-*N*⁵,*N*⁸-di-*tert*-butoxycarbonylspermidine 58.** General procedure B from **43** (0.135 g, 1.04 mmol, 1 equiv) and **34** (1 equiv). Column chromatography using isopropylamine/$CH_3OH/CHCl_3$: 1/4/4. Oil, 72%; R_f 0.38 (isopropylamine/$CH_3OH/CHCl_3$ 1/4/4).

***N*¹-(4,5-Dihydro-3*H*-azepin-2-yl)-*N*⁵,*N*⁹-di-*tert*-butoxycarbonylhomospermidine 59.** General procedure B from **43** (0.169 g, 1.31 mmol, 1 equiv) and **37** (1 equiv). Column chromatography using isopropylamine/$CH_3OH/CHCl_3$: 1/4/4. Oil, 65%; R_f 0.44 (isopropylamine/$CH_3OH/CHCl_3$ 1/4/4), 0.13 (CH_3OH/NH_4OH 90/10).

***N*¹-(4,5-Dihydro-3*H*-azepin-2-yl)-*N*⁴,*N*⁹-di-*tert*-butoxycarbonyl-4-azanonane-1,9-diamine 60.** General procedure B from **43** (0.176 g, 1.36 mmol, 1 equiv) and **39** (1 equiv). Column chromatography using isopropylamine/$CH_3OH/CHCl_3$: 1/4/4. Oil, 77%; R_f 0.46 (isopropylamine/$CH_3OH/CHCl_3$ 1/4/4), 0.13 (isopropylamine/$CH_3OH/CHCl_3$ 0.5/4/4).

***N*¹-(4,5-Dihydro-3*H*-azepin-2-yl)-*N*⁴,*N*⁹*N*¹²-tri-*tert*-butoxycarbonylspermine 61.** To a solution of *N*¹,*N*⁴,*N*⁹-tri-*tert*-butoxycarbonylspermine **32** (0.500 g, 0.99 mmol, 1 equiv) in DMF was added an excess of imidate **44** (8 equiv). The mixture was allowed to warm at 60−70 °C at 15−20 mmHg for 5 h. To remove the excess of imidate, the mixture was warmed at 60 °C under reduced pressure (10^{-2} mmHg). Column chromatography using CH_3OH/NH_4OH 90/10 (*n*). Oil, 85%; 0.35 (CH_3OH/NH_4OH 90/10).

Data for *N*¹-(4,5-Dihydro-3*H*-1-benzazepin-2-yl)putrescine 62. General procedure C from **49** (0.500 g, 2.82 mmol, 1 equiv) and putrescine **1**. Column chromatography using CH_3OH/NH_4OH: 90/10. Oil, 40%; R_f 0.20 (CH_3OH/NH_4OH 90/10).

Data for *N*¹-(4,5-Dihydro-3*H*-1-benzazepin-2-yl)octane-1,8-diamine 63. General procedure C from **49** (0.300 g, 1.69 mmol, 1 equiv) and 1,8-diaminooctane **4**. Column chromatography using CH_3OH/NH_4OH: 90/10. Oil, 74%; R_f 0.38 (CH_3OH/NH_4OH 90/10).

Data for *N*¹-(4,5-Dihydro-3*H*-1-benzazepin-2-yl)norspermine 64. General procedure B from **49** (0.47 g, 2.65 mmol, 1 equiv) and norspermine **8**. Column chromatography using $MeOH/NH_4OH$: 50/50. Oil, 32%; R_f 0.41 ($MeOH/NH_4OH$ 50/50).

Data for *N*¹-(4,5-Dihydro-3*H*-1-benzazepin-2-yl)spermine 65. General procedure B from **49** (0.24 g, 1.36 mmol, 1 equiv) and spermine **3**. Column chromatography using isopropylamine/$CH_3OH/CHCl_3$: 2/4/4. Oil, 60%; R_f 0.11 (isopropylamine/$CH_3OH/CHCl_3$ 2/4/4).

Data for *N*¹-(4,5-Dihydro-3*H*-1-benzazepin-2-yl)-*N*⁴,*N*⁸-di-*tert*-butoxycarbonyl spermidine 66. General procedure B from **49** (0.150 g, 0.85 mmol, 1 equiv) and *N*⁴,*N*⁸-di-*tert*-butoxycarbonylspermidine **33**. Column chromatography using $CH_2Cl_2/CH_3OH/NH_4OH$: 90/10/0.5. Oil, 80%; R_f 0.36 ($CH_2Cl_2/CH_3OH/NH_4OH$ 90/10/0.5).

(6*R*,7*S*)-*N*¹²-(4,5-Dihydro-3*H*-1-benzazepin-2-yl)-*N*¹,*N*⁴-di-*tert*-butoxycarbonyl-(6,7-*O*-isopropylidene)-6,7-dihydroxyspermine 67. General procedure B from **49** (0.090 g, 0.51 mmol, 1 equiv) and (6*R*,7*S*)-*N*¹,*N*⁴-di-*tert*-butoxycarbonyl-(6,7-*O*-isopropylidene)-6,7-dihydroxyspermine **35**. Column chromatography using $CH_2Cl_2/CH_3OH/NH_4OH$: 90/10/0.7. Oil, 71%; R_f 0.48 ($CH_2Cl_2/CH_3OH/NH_4OH$ 90/10/0.7).

Data for *N*¹-(4,5-Dihydro-3*H*-1-benzazepin-2-yl)-*N*¹⁵-*tert*-butoxycarbonyl-*N*⁵,*N*¹⁰-bis(mesitylenesulfonyl)homospermine 68. To a 1 M solution of triethyloxonium tetrafluoroborate[60] (1.42 mL, 1.5 equiv) in anhydrous CH_2Cl_2 was added dropwise at 0 °C under nitrogen atmosphere a solution of **45** (0.163 g, 0.943 mmol, 1 equiv) in anhydrous CH_2Cl_2. After stirring overnight at room temperature, a solution in anhydrous CH_2Cl_2 of **41** (0.555 g, 0.9 equiv) and of Et_3N (0.085 g, 0.118 mL, 0.9 equiv) was added dropwise at 0 °C. The mixture was stirred at room temperature during 4 h and warmed at 40 °C during 15 mn. After addition of 5 mL of water, an extraction with CH_2Cl_2 (2 × 5 mL) was performed. The organic layers were mixed, washed with KOH 20% (5 mL), and then with H_2O/NaCl, dried over K_2CO_3, filtered, and concentrated under reduced pressure. Column chromatography using CH_3OH/NH_4OH: 99/1. Oil, 18%; R_f 0.39 (CH_3OH/NH_4OH 99/1).

***N*¹-(7-Bromo-4,5-dihydro-3*H*-1-benzazepin-2-yl)-*N*⁴,*N*⁹,*N*¹²-tri-*tert*-butoxycarbonyl-spermine 69.** General procedure B from **50** (0.256 g, 0.59 mmol, 1 equiv) and *N*⁴,*N*⁹,*N*¹²-tri-*tert*-butoxycarbonyl-spermine **32**. Column chromatography using CH_2Cl_2/CH_3OH: 95/5.

Oil, 68%; R_f 0.25 (CH_2Cl_2/CH_3OH 95/5).

***N*¹-(7-Methoxy-4,5-dihydro-3*H*-1-benzazepin-2-yl)-*N*⁴,*N*⁹,*N*¹²-tri-*tert*-butoxycarbonyl-spermine 70.** General procedure B from **51** (0.100 g, 0.48 mmol, 1 equiv) and *N*⁴,*N*⁹,*N*¹²-tri-*tert*-butoxycarbonyl-spermine **32**. Column chromatography using CH_3OH/NH_4OH: 99/1. Oil, 49%; R_f 0.31 (CH_3OH/NH_4OH 99/1).

***N*¹-(7-Nitro-4,5-dihydro-3*H*-1-benzazepin-2-yl)-*N*⁴,*N*⁹,*N*¹²-tri-*tert*-butoxycarbonyl-spermine 71.** General procedure B from **52** (0.200 g, 0.90 mmol, 1 equiv) and *N*⁴,*N*⁹,*N*¹²-tri-*tert*-butoxycarbonyl-spermine **32**. Column chromatography using CH_2Cl_2/CH_3OH: 95/5. Oil, 49%; R_f 0.32 (CH_2Cl_2/CH_3OH 95/5).

***N*¹-(7-Amino-4,5-dihydro-3*H*-1-benzazepin-2-yl)- *N*⁴,*N*⁹,*N*¹²-tri-*tert*-butoxycarbonyl-permine 72.** Pearlman's catalyst (0.8 equiv) was added to **71** (0.200 g, 0.34 mmol, 1 equiv) dissolved in EtOH.

The mixture was stirred for 60 h under an atmosphere of H_2 at room temperature. The mixture was then filtered, washed with EtOH, and concentrated under reduced pressure. Oil, 100%; R_f 0.50 (CH_3OH/NH_4OH 95/5).

***N^1*-(4,5-Dihydro-3*H*-azepin-2-yl)putrescine Hydrochloride. 11** was prepared from **54** using 0.9 M HClg in AcOEt. A recrystallization was performed with an isopropyl alcohol/EtOH 50/50 mixture. White solid, 37%. FTIR 2400 to 3600 + 2057 (NH^+), 1657 (C=N). ^{1}H NMR (500 MHz, D_2O) δ 1.66 (m, 2H, H-6), 1.74−1.81 (m, 8H, H-b, H-c, H-4, H-5), 2.67 (m, 2H, H-3), 3.04 (t, 2H, J = 6.7 Hz, H-d), 3.26 (t, 2H, J = 6.2 Hz, H-a), 3.47 (t, 2H, J = 5 Hz, H-7). ^{13}C NMR (125 MHz, D_2O) δ 23.57 (C-4), 24.26* (C-b), 24.56* (C-c), 27.90 (C-6), 29.40 (C-5), 32.43 (C-3), 39.40 (C-d), 41.59 (C-a), 44.58 (C-7), 169.93 (C-2). HRMS (LSIMS) (m/z) calcd for $C_{10}H_{22}N_3$ (M + H)$^+$ 183.1735; found 183.1743. Anal. ($C_{10}H_{21}N_3$.2HCl.0.25H_2O) C, H, N.

***N^1*-(4,5-Dihydro-3*H*-azepin-2-yl)octane-1,8-diamine Hydrochloride 12.** General procedure D from **55**, white solid, 79%. R_f 0.14 (CH_3OH/NH_4OH 50/50). FTIR 2350 to 3650 + 2020 (NH^+), 1654 (C=N). ^{1}H NMR (500 MHz, D_2O) δ 1.37 (m, 8H, H-c, H-d, H-e, H-f), 1.63−1.70 (m, 6H, H-b, H-g, H-6), 1.73 (m, 2H, H-4), 1.80 (m, 2H, H-5), 2.68 (m, 2H, H-3), 3.02 (t, 2H, J = 7.5 Hz, H-h), 3.22 (t, 2H, J = 7 Hz, H-a), 3.49 (m, 2H, H-7). ^{13}C NMR (125 MHz, D_2O) δ 23.65 (C-4), 25.88, 26.33, 27.00, 27.06, 28.42 (C-b, C-c, C-d, C-e, C-f, C-g), 28.02 (C-6), 29.40 (C-5), 32.42 (C-3), 39.92 (C-h), 42.17 (C-a), 44.27 (C-7), 169.70 (C-2). HRMS (LSIMS) (m/z) calcd for $C_{14}H_{30}N_3$ (M + H)$^+$ 240.2440; found 240.2440. Anal. ($C_{14}H_{29}N_3 \cdot 2HCl \cdot 0.4H_2O$) C, H, N.

***N^1*-(4,5-Dihydro-3*H*-azepin-2-yl)norspermidine Hydrochloride 13.** General procedure D from **56**, white solid, 93%. FTIR 2310 to 3400 + 2001 (NH^+), 1654 (C=N). ^{1}H NMR (500 MHz, D_2O) δ 1.67 (m, 2H, H-6), 1.74 (m, 2H, H-4), 1.80 (m, 2H, H-5), 2.05−2.16 (m, 4H, H-b, H-e), 2.70 (m, 2H, H-3), 3.14 (t, 2H, J = 7.8 Hz, H-f), 3.17−3.22 (m, 4H, H-c, H-d), 3.35 (t, 2H, J = 7 Hz, H-a), 3.51 (m, 2H, H-7). ^{13}C NMR (125 MHz, D_2O) δ 23.45 (C-4), 24.15 (C-b, C-e), 27.76 (C-6), 29.34 (C-5), 32.51 (C-3), 36.92 (C-f), 39.21 (C-a), 44.47 (C-7), 45.06* (C-c), 45.47* (C-d), 170.23 (C-2). HRMS (EI) (m/z) calcd for $C_{12}H_{26}N_4$ M^+ 226.2158; found 226.2148. Anal. ($C_{12}H_{26}N_4 \cdot 3HCl \cdot 0.1H_2O$) C, H, N.

***N^1*-(4,5-Dihydro-3*H*-azepin-2-yl)spermidine Hydrochloride 14.** General procedure D from **57**, white solid, 81%. FTIR 2300 to 3600 + 2077 (NH^+), 1654 (C=N). ^{1}H NMR (500 MHz, D_2O) δ 1.68 (m, 2H, H-6), 1.74 (m, 2H, H-4), 1.80−1.82 (m, 6H, H-e, H-f, H-5), 2.08 (m, 2H, H-b), 2.70 (m, 2H, H-3), 3.07 (t, 2H, J = 7 Hz, H-g), 3.13−3.18 (m, 4H, H-c, H-d), 3.35 (t, 2H, J = 7 Hz, H-a), 3.51 (m, 2H, H-7). ^{13}C NMR (125 MHz, D_2O) δ 23.16 (C-e), 23.46 (C-4), 24.16* (C-b), 24.30* (C-f), 27.76 (C-6), 29.36 (C-5), 32.51 (C-3), 39.19* (C-a), 39.25* (C-g), 44.48 (C-7), 45.32 (C-c), 47.44 (C-d) 170.23 (C-2). HRMS (LSIMS) (m/z) calcd for $C_{13}H_{29}N_4$ (M + H)$^+$ 241.2392; found 241.2395. Anal. ($C_{13}H_{28}N_4 \cdot 3HCl \cdot 0.45H_2O$) C, H, N.

***N^8*-(4,5-Dihydro-3*H*-azepin-2-yl)spermidine Hydrochloride 15.** General procedure D from **58**, white solid, 83%. FTIR 2312 to 3600 + 2043 (NH^+), 1654 (C=N). ^{1}H NMR (500 MHz, D_2O) δ 1.67 (m, 2H, H-6), 1.73−1.81 (m, 8H, H-b, H-c, H-4, H-5), 2.12 (m, 2H, H-f), 2.68 (m, 2H, H-3), 3.13 (t, 4H, J = 7.8 Hz, H-d, H-e), 3.18 (t, 2H, J = 8 Hz, H-g), 3.27 (t, 2H, J = 6.7 Hz, H-a), 3.50 (m, 2H, H-7). ^{13}C NMR (125 MHz, D_2O) δ 23.40 (C-4), 23.53, 24.13, 24.26 (C-b, C-c, C-f), 27.86 (C-6), 29.36 (C-5), 32.46 (C-3), 36.94 (C-g), 41.50 (C-a), 44.36 (C-7), 44.90 (C-e), 47.61 (C-d) 169.94 (C-2). HRMS (LSIMS) (m/z) calcd for $C_{13}H_{29}N_4$ (M + H)$^+$ 241.2392; found 241.2395. Anal. ($C_{13}H_{28}N_4 \cdot 3HCl \cdot 0.6H_2O$) C, H, N.

***N^1*-(4,5-Dihydro-3*H*-azepin-2-yl)homospermidine Hydrochloride 16.** General procedure D from **59**, white solid, 98%. FTIR 2336 to 3610 + 2045 (NH^+), 1662 (C=N). ^{1}H NMR (500 MHz, D_2O) δ 1.66 (m, 2H, H-6), 1.71−1.79 (m, 12H, H-b, H-c, H-f, H-g, H-4, H-5), 2.67 (m, 2H, H-3), 3.05 (t, 2H, J = 7.2 Hz, H-h), 3.08−3.12 (m, 4H, H-d, H-e), 3.25 (t, 2H, J = 6.5 Hz, H-a), 3.49 (m, 2H, H-7). ^{13}C NMR (125 MHz, D_2O) δ 22.99, 23.26 (C-c, C-f), 23.39 (C-4), 24.15, 24.17 (C-b, C-g), 27.72 (C-6), 29.22 (C-5), 32.32 (C-3), 39.05 (C-h), 41.38 (C-a), 44.22 (C-7), 47.12, 47.30 (C-d, C-e) 169.79 (C-2). HRMS (LSIMS) (m/z) calcd for $C_{14}H_{31}N_4$ (M + H)$^+$ 255.2549; found 255.2547. Anal. ($C_{14}H_{30}N_4 \cdot 3HCl \cdot 2H_2O$) C, H, N.

***N^1*-(4,5-Dihydro-3*H*-azepin-2-yl)-azanonane-1,9-diamine Hydrochloride 17.** General procedure D from **60**, white solid, 91%. FTIR 2200 to 3650 + 2034 (NH^+), 1646 (C=N). ^{1}H NMR (500 MHz, D_2O) δ 1.49 (m, 2H, H-f), 1.68−1.81 (m, 10H, H-e, H-g, H-4, H-5, H-6), 2.08 (m, 2H, H-b), 2.70 (m, 2H, H-3), 3.04 (t, 2H, J = 7.5 Hz, H-h), 3.11 (t, 2H, J = 8 Hz, H-d), 3.16 (t, 2H, J = 8 Hz, H-c), 3.36 (t, 2H, J = 7 Hz, H-a), 3.52 (m, 2H, H-7). ^{13}C NMR (125 MHz, D_2O) δ 23.16 (C-f), 23.48 (C-4), 24.19 (C-b), 25.49 (C-e), 26.64 (C-g), 27.77 (C-6), 29.37 (C-5), 32.53 (C-3), 39.30 (C-a), 39.55 (C-h), 44.48 (C-7), 45.26 (C-c), 47.85 (C-d) 170.22 (C-2). HRMS (LSIMS) (m/z) calcd for $C_{14}H_{31}N_4$ (M + H)$^+$ 255.2549; found 255.2551. Anal. ($C_{14}H_{30}N_4 \cdot 3HCl \cdot 0.1H_2O$) C, H, N.

***N^1*-(4,5-Dihydro-3*H*-azepin-2-yl)spermine Hydrochloride 18. 18** was prepared from **61** using 0.9 M HClg in AcOEt. White solid, 26%. FTIR 2500 to 3600 + 2058 (NH^+), 1658 (C=N). ^{1}H NMR (500 MHz, D_2O) δ 1.67 (m, 2H, H-6), 1.73 (m, 2H, H-4), 1.80−1.82 (m, 6H, H-e, H-f, H-5), 2.05−2.13 (m, 4H, H-b, H-i), 2.69 (m, 2H, H-3), 3.11−3.20 (m, 10H, H-c, H-d, H-g, H-h, H-j), 3.34 (t, 2H, J = 7 Hz, H-a), 3.51 (t, 2H, H-7, J = 5 Hz, H-7). ^{13}C NMR (125 MHz, D_2O) δ 23.16 (C-e, C-f), 23.46 (C-4), 24.13* (C-b), 24.17* (C-i), 27.76 (C-6), 29.36 (C-5), 32.52 (C-3), 36.95 (C-j), 39.26 (C-a), 44.48 (C-7), 44.94* (C-c), 45.34* (C-h), 47.38* (C-d), 47.40* (C-g), 170.23 (C-2). HRMS (LSIMS) (m/z) calcd for $C_{16}H_{36}N_5$ (M + H)$^+$ 297.2892; found 297.2826. Anal. ($C_{16}H_{35}N_5 \cdot 4HCl \cdot 2.5H_2O$) N, C; H calcd 9.08, found 8.47.

***N^1*-(4,5-Dihydro-3*H*-1-benzazepin-2-yl)putrescine Hydrochloride 19.** General procedure D from **62**, white solid, 89%; R_f 0.20 (CH_3OH/NH_4OH 90/10). FTIR 2400 to 3600 + 2049 (NH^+), 1668 (C=N). ^{1}H NMR (500 MHz, D_2O) δ 1.76−1.93 (m, 4H, H-b, H-c), 2.31 (m, 2H, H-4), 2.51 (t, 2H, J = 7.2 Hz, H-3), 2.79 (t, 2H, J = 7.3 Hz, H-5), 3.04 (t, 2H, J = 7.0 Hz, H-d), 3.54 (t, 2H, J = 6.5 Hz, H-a), 7.23 (d, 1H, J = 7.6 Hz, H-9), 7.35−7.43 (m, 3H, H-6, H-7, H-8). HRMS (LSIMS) (m/z) calcd for $C_{14}H_{22}N_3$ (M + H)$^+$ 232.1814; found 232.1810. Anal. ($C_{14}H_{21}N_3 \cdot 2HCl \cdot 0.5H_2O$) C, H, N.

***N^1*-(4,5-Dihydro-3*H*-1-benzazepin-2-yl)octane-1,8-diamine Hydrochloride 20.** General procedure **D** from **63**, white solid, 87%; R_f 0.38 (CH_3OH/NH_4OH 90/10). FTIR 2400 to 3400 + 2054 (NH^+), 1663 (C=N). ^{1}H NMR (500 MHz, D_2O) δ 1.40−1.48 (m, 8H, H-c, H-d, H-e, H-f), 1.68* (m, 2H, H-b), 1.77* (m, 2H, H-g), 2.30 (m, 2H, H-4), 2.48 (t, 2H, J = 7 Hz, H-3), 2.77 (t, 2H, J = 7.2 Hz, H-5), 3.01 (t, 2H, J = 7.5 Hz, H-h), 3.49 (t, 2H, J = 7 Hz, H-a), 7.24 (d, 1H, J = 7.6 Hz, H-9), 7.35−7.43 (m, 3H, H-6, H-7, H-8). ^{13}C NMR (125 MHz, D_2O) δ 25.90, 26.28, 27.07, 27.12, 28.45 (C-b, C-c, C-d, C-e, C-f, C-g), 28.87 (C-5), 29.14 (C-3), 29.73 (C-4), 39.90 (C-h), 42.89 (C-a), 123.87 (C-9), 128.26 (C-8), 128.31 (C-7), 130.26 (C-6), 135.19* (C-5a), 135.49* (C-9a), 166.40 (C-2). HRMS (LSIMS) (m/z) calcd for $C_{18}H_{30}N_3$ (M + H)$^+$ 288.2440; found 288.2443. Anal. ($C_{18}H_{29}N_3 \cdot 2HCl \cdot 0.1H_2O$) C, H, N.

***N^1*-(4,5-Dihydro-3*H*-1-benzazepin-2-yl)norspermine Hydrochloride 21.** General procedure C from **64**, white solid, 75%; R_f 0.13 (isopropylamine/CH_3OH/$CHCl_3$ 2/4/4), 0.46 (CH_3OH/NH_4OH: 50/50). FTIR 2450 to 3300 + 2066 (NH^+), 1657 (C=N). ^{1}H NMR (500 MHz, D_2O) δ 2.10−2.23 (m, 6H, H-b, H-e, H-h), 2.33 (m, 2H, H-4), 2.53 (t, 2H, J = 7 Hz, H-3), 2.80 (t, 2H, J = 7.3 Hz, H-5), 3.12−3.28 (m, 10H, H-c, H-d, H-f, H-g, H-i), 3.63 (t, 2H, J = 7 Hz, H-a), 7.26 (d, 1H, J = 8 Hz, H-9), 7.37−7.45 (m, 3H, H-6, H-7, H-8). ^{13}C NMR (125 MHz, D_2O) δ 23.11 (C-e), 24.13* (C-b), 24.32* (C-h), 28.80 (C-5), 29.27 (C-3), 29.80 (C-4), 36.93 (C-i), 39.98 (C-a), 45.01, 45.11, and 45.52 (C-c, C-d, C-f, C-g), 123.96 (C-9), 128.27 (C-8), 128.54 (C-7), 130.29 (C-6), 135.28 (C-5a, C-9a), 167.06 (C-2). HRMS (LSIMS) (m/z) calcd for $C_{19}H_{34}N_5$ (M + H)$^+$ 332.2814; found 332.2812. Anal. ($C_{19}H_{33}N_5 \cdot 4HCl \cdot 2H_2O$) C, H, N.

N^1-(4,5-Dihydro-3*H*-1-benzazepin-2-yl)spermine Hydrochloride 22. General procedure D from **65**, white solid, 73%; R_f 0.16 (isopropylamine/CH_3OH/$CHCl_3$ 2/4/4), 0.43 (CH_3OH/NH_4OH 50/50). FTIR 2500 to 3350 + 2054 (NH^+), 1657 (C=N). ^1H NMR (500 MHz, D_2O) δ 1.80−1.83 (m, 4H, H-e, H-f), 2.10 (m, 2H, H-i), 2.19 (m, 2H, H-b), 2.32 (m, 2H, H-4), 2.52 (t, 2H, J = 7 Hz, H-3), 2.79 (t, 2H, J = 7.3 Hz, H-5), 3.10−3.19 (m, 8H, H-d, H-g, H-h, H-j), 3.24 (t, 2H, J = 8.1 Hz, H-c), 3.61 (t, 2H, J = 7 Hz, H-a), 7.24 (d, 1H, J = 7.5 Hz, H-9), 7.36−7.44 (m, 3H, H-6, H-7, H-8). ^{13}C NMR (125 MHz, D_2O) δ 23.24* (C-e), 23.27* (C-f), 24.19* (C-b), 24.40* (C-i), 28.87 (C-5), 29.35 (C-3), 29.87 (C-4), 37.05 (C-j), 40.11 (C-a), 45.01* (C-c), 45.41* (C-h), 47.49 (C-d, g), 124.04, 128.35, 128.59, 130.36 (C-6, 7, 8, 9), 135.34 (C-5a, C-9a), 167.11 (C-2). HRMS (LSIMS) (*m/z*) calcd for $C_{20}H_{36}N_5$ $(M + H)^+$ 346.2971; found 346.2980. Anal. ($C_{20}H_{35}N_5 \cdot 4HCl \cdot 1.5H_2O$) C, H, N.

N^1-(4,5-Dihydro-3*H*-1-benzazepin-2-yl)spermidine Hydrochloride 23. General procedure D from **66**, white solid, 88%; R_f 0.45 (isopropylamine/CH_3OH/$CHCl_3$ 2/4/4), 0.62 (CH_3OH/NH_4OH 50/50). FTIR 2480 to 3280 + 2052 (NH^+), 1663 (C=N). ^1H NMR (500 MHz, D_2O) δ 1.75−1.84 (m, 4H, H-e, H-f), 2.19 (m, 2H, H-b), 2.28 (m, 2H, H-4), 2.47 (t, 2H, J = 7.1 Hz, H-3), 2.75 (t, 2H, J = 7.3 Hz, H-5), 3.05 (t, 2H, J = 7.2 Hz, H-g), 3.15 (t, 2H, J = 7.4 Hz, H-d), 3.25 (t, 2H, J = 8 Hz, H-c), 3.60 (t, 2H, J = 7.1 Hz, H-a), 7.23 (d, 1H, J = 7 Hz, H-9), 7.32−7.41 (m, 3H, H-6, H-7, H-8). ^{13}C NMR (125 MHz, D_2O) δ 22.81 (C-e), 23.95 (C-b, C-f), 28.42 (C-5), 28.88 (C-3), 29.42 (C-4), 38.84 (C-g), 39.66 (C-a), 44.96 (C-c), 47.12 (C-d), 123.58 (C-9), 127.89 (C-8), 128.07 (C-7), 129.85 (C-6), 134.88 (C-5a, C-9a), 166.65 (C-2). HRMS (LSIMS) (*m/z*) calcd for $C_{17}H_{29}N_4$ $(M + H)^+$ 289.2392; found 289.2390. Anal. ($C_{17}H_{28}N_4 \cdot 3HCl \cdot 0.5H_2O$) C, H, N.

(6*R*,7*S*)-N^1-(4,5-Dihydro-3*H*-1-benzazepin-2-yl)-6,7-dihydroxyspermine Hydrochloride 24. General procedure D from **67**, white solid, 90%; $[\alpha]_D^{21.5} = +0.57°$ (*c* 11.7 mmol, H_2O); R_f 0.10 (isopropylamine/CH_3OH/$CHCl_3$ 2/4/4), 0.60 (CH_3OH/NH_4OH 50/50). FTIR 2300 to 3600 + 2031 (NH^+), 1657 (C=N). ^1H NMR (500 MHz, D_2O) δ 2.14 (m, 2H, H-i), 2.26 (m, 2H, H-b), 2.32 (m, 2H, H-4), 2.53 (t, 2H, J = 7.2 Hz, H-3), 2.81 (t, 2H, J = 7.3 Hz, H-5), 3.16 (t, 2H, J = 7.8 Hz, H-j), 3.21* (m, 2H, H-d), 3.27 (t, 2H, J = 8 Hz, H-h), 3.33 (t, 2H, J = 8.1 Hz, H-c), 3.46* (m, 2H, H-g), 3.65 (t, 2H, J = 7 Hz, H-a), 3.95−4.00 (m, 2H, H-e, H-f), 7.27 (d, 1H, J = 7.6 Hz, H-9), 7.35−7.45 (m, 3H, H-6, H-7, H-8). ^{13}C NMR (125 MHz, D_2O) δ 24.02* (C-i), 24.21* (C-b), 28.81 (C-5), 29.29 (C-3), 29.81 (C-4), 36.96 (C-j), 40.04 (C-a), 45.23* (C-c), 45.65* (C-h), 49.94 (C-d, C-g), 68.65 (C-e, C-f), 123.96 (C-9), 128.29 (C-8), 128.55 (C-7), 130.32 (C-6, 7, 8, 9), 135.29 (C-5a, 9a), 167.09 (C-2). HRMS (LSIMS) (*m/z*) calcd for $C_{20}H_{36}N_5O_2$ $(M + H)^+$ 378.2869; found 378.2864. Anal. ($C_{20}H_{35}N_5O_2$.4HCl.0.5H_2O) C, H, N.

N^1-(4,5-Dihydro-3*H*-1-benzazepin-2-yl)homospermine Hydrochloride 25. The deprotection step of **68** was previously reported.[51] White solid, 60%; R_f 0.17 (isopropylamine/CH_3OH/NH_4OH), 0.40 (CH_3OH/NH_4OH). FTIR 2300 to 3650 + 2078 (NH^+), 1658 (C=N). ^1H NMR (500 MHz, D_2O) δ 1.79−1.87 (m, 12H, H-b, H-c, H-f, H-g, H-j, H-k), 2.33 (m, 2H, H-4), 2.52 (t, 2H, J = 7 Hz, H-3), 2.80 (t, 2H, J = 7.3 Hz, H-5), 3.05−3.16 (m, 10H, H-d, H-e, H-h, H-i, H-l), 3.58 (m, 2H, H-a), 7.25 (d, 1H, J = 7.6 Hz, H-9), 7.37−7.45 (m, 3H, H-6, H-7, H-8). ^{13}C NMR (125 MHz, D_2O) δ 23.14, 23.20, and 23.47 (C-c, C-f, C-g, C-i), 24.31* (C-b), 24.44* (C-k), 28.82 (C-5), 29.20 (C-3), 29.74 (C-4), 39.18 (C-l), 42.25 (C-a) 47.62, 47.30, and 47.47 (C-d, C-e, C-h, C-i), 123.91 (C-9), 128.26 (C-8), 128.43 (C-7), 130.27 (C-6), 135.26 (C-5a, C-9a), 166.75 (C-2). HRMS (LSIMS) (*m/z*) calcd for $C_{22}H_{40}N_5$ $(M + H)^+$ 373.3205; found 373.3293. Anal. ($C_{22}H_{39}N_5 \cdot 4HCl \cdot 3.5H_2O$) N; H calcd 8.65, found 7.98; C calcd 45.37, found 45.94.

N^1-(7-Bromo-4,5-dihydro-3*H*-1-benzazepin-2-yl)-spermine Hydrochloride 26. General procedure D from **69**, white solid, 82%; R_f 0.16 (isopropylamine/CH_3OH/$CHCl_3$ 2/4/4), 0.45 (CH_3OH/NH_4OH 50/50). FTIR 2300 to 3650 + 2054 (NH^+), 1657 (C=N). ^1H NMR (500 MHz, D_2O) δ 1.83 (m, 4H, H-e, H-f), 2.13 (m, 2H, H-i), 2.21 (m, 2H, H-b), 2.31 (m, 2H, H-4), 2.52 (t, 2H, J = 7.2 Hz, H-3), 2.77 (t, 2H, J = 7.2 Hz, H-5), 3.12−3.22 (m, 8H, H-d, H-g, H-h, H-j), 3.24 (t, 2H, J = 8 Hz, H-c), 3.62 (t, 2H, J = 7.2 Hz, H-a), 7.17 (d, 1H, J = 8.3 Hz, H-9), 7.56−7.59 (m, 2H, H-6, H-8). ^{13}C NMR (125 MHz, D_2O) δ 25.71* (C-e), 25.75* (C-f), 26.68* (C-b), 26.85* (C-i), 31.22 (C-4), 31.71 (C-3), 32.00 (C-5), 39.49 (C-j), 42.63 (C-a), 47.48* (C-c), 47.85* (C-h), 49.94* (C-d), 49.95* (C-g), 123.54 (C-7), 128.16 (C-9), 133.63 (C-8), 135.51 (C-6), 137.14* (C-5a), 139.94* (C-9a), 169.54 (C-2). HRMS (LSIMS) (*m/z*) calcd for $C_{20}H_{35}N_5Br$ $(M + H)^+$ 424.2076; found 424.2068. Anal. ($C_{20}H_{34}N_5Br \cdot 4HCl \cdot 2H_2O$) C, H, N.

N^1-(7-Methoxy-4,5-dihydro-3*H*-1-benzazepin-2-yl)spermine Hydrochloride 27. General procedure D from **70**, white solid, 75%; R_f 0.22 (isopropylamine/CH_3OH/$CHCl_3$ 2/4/4), 0.44 (CH_3OH/NH_4OH 50/50). FTIR 2300 to 3650 + 2067 (NH^+), 1654 (C=N). ^1H NMR (500 MHz, D_2O) δ 1.83 (m, 4H, H-e, H-f), 2.12 (m, 2H, H-i), 2.19 (m, 2H, H-b), 2.31 (m, 2H, H-4), 2.51 (t, 2H, J = 7 Hz, H-3), 2.77 (t, 2H, J = 7 Hz, H-5), 3.12−3.21 (m, 8H, H-d, H-g, H-h, H-j), 3.23 (t, 2H, J = 8 Hz, H-c), 3.60 (t, 2H, J = 7 Hz, H-a), 3.87 (s, 3H, OCH_3), 7.00−7.02 (m, 2H, H-6, H-8), 7.20 (d, 1H, J = 8.6 Hz, H-9). ^{13}C NMR (125 MHz, D_2O) δ 23.23* (C-e), 23.32* (C-f), 24.16* (C-b), 24.37* (C-i), 29.03 (C-5), 29.48 (C-3), 29.55 (C-4), 37.00 (C-j), 39.97 (C-a), 44.97* (C-c), 45.38* (C-h), 47.44 (C-d, C-g), 113.37 (C-8), 115.37 (C-6), 125.31 (C-9), 128.44 (C-9a), 136.92 (C-5a), 158.54 (C-7), 166.82 (C-2). HRMS (LSIMS) (*m/z*) calcd for $C_{21}H_{38}N_5O$ $(M + H)^+$ 376.3076; found 376.3077. Anal. ($C_{21}H_{37}N_5O \cdot 4HCl \cdot 2H_2O$) C, H, N.

N^1-(7-Nitro-4,5-dihydro-3*H*-1-benzazepin-2-yl)spermine Hydrochloride 28. General procedure D from **71**, yellow solid, 84%; R_f 0.15 (isopropylamine/CH_3OH/$CHCl_3$ 2/4/4), 0.45 (CH_3OH/NH_4OH 50/50). FTIR 2300 to 3600 + 2059 (NH^+), 1661 (C=N), 1521, 1355 (NO_2). ^1H NMR (500 MHz, D_2O) δ 1.84 (m, 4H, H-e, H-f), 2.13 (m, 2H, H-i), 2.23 (m, 2H, H-b), 2.39 (m, 2H, H-4), 2.60 (t, 2H, J = 7.2 Hz, H-3), 2.92 (t, 2H, J = 7.3 Hz, H-5), 3.12−3.22 (m, 8H, H-d, H-g, H-h, H-j), 3.26 (t, 2H, J = 8 Hz, H-c), 3.68 (t, 2H, J = 7.2 Hz, H-a), 7.45 (d, 1H, J = 8.6 Hz, H-9), 8.17 (dd, 1H, J = 8.8 Hz, J = 2.6 Hz, H-8), 8.30 (d, 1H, J = 2.6 Hz, H-6). ^{13}C NMR (125 MHz, D_2O) δ 23.18* (C-e), 23.22* (C-f), 24.14* (C-b), 24.28* (C-i), 29.04 (C-5), 29.34 (C-3), 29.39 (C-4), 36.94 (C-j), 40.42 (C-a), 44.94* (C-c), 45.30* (C-h), 47.44 (C-d, C-g), 123.70 (C-8), 124.86 (C-6), 125.75 (C-9), 136.76 (C-5a), 141.55 (C-9a), 146.61 (C-7), 167.42 (C-2). HRMS (LSIMS) (*m/z*) calcd for $C_{20}H_{35}N_6O_2$ $(M + H)^+$ 391.2821; found 391.2817. Anal. ($C_{20}H_{34}N_6O_2 \cdot 4HCl \cdot 0.9H_2O$) C, H, N.

N^1-(7-Amino-4,5-dihydro-3*H*-1-benzazepin-2-yl)spermine hydrochloride 29. General procedure D from **72**, cream solid, 59%; R_f 0.13 (isopropylamine/CH_3OH/$CHCl_3$ 2/4/4), 0.37 (CH_3OH/NH_4OH 50/50). FTIR 2300 to 3650 + 2071 (NH^+), 1662 (C=N). ^1H NMR (500 MHz, D_2O) δ 1.82 (m, 4H, H-e, H-f), 2.11 (m, 2H, H-i), 2.21 (m, 2H, H-b), 2.34 (m, 2H, H-4), 2.55 (t, 2H, J = 7.1 Hz, H-3), 2.83 (t, 2H, J = 7.1 Hz, H-5), 3.11−3.21 (m, 8H, H-d, H-g, H-h, H-j), 3.24 (t, 2H, J = 8 Hz, H-c), 3.62 (t, 2H, J = 7.2 Hz, H-a), 7.33−7.37 (m, 3H, H-6, H-7, H-8). ^{13}C NMR (125 MHz, D_2O) δ 23.18* (C-e), 23.18* (C-f), 24.14* (C-b), 24.29* (C-i), 28.85 (C-5), 29.18 (C-3), 29.40 (C-4), 36.96 (C-j), 40.20 (C-a), 44.94* (C-c), 45.33* (C-h), 47.41 (C-d, C-g), 135.84* (C-5a), 137.53* (C-9a), 167.18 (C-2). HRMS (LSIMS) (*m/z*) calcd for $C_{20}H_{37}N_6$ $(M + H)^+$ 361.3080; found 361.3078. Anal. ($C_{20}H_{36}N_6 \cdot 5HCl \cdot 1.3H_2O$) H, N; C calcd 42.42, found 43.79.

Biological Studies. Unless otherwise stated, usual laboratory chemicals were purchased from Merck (Darmstadt, Germany) or Sigma (St Louis, MO, USA). DFMO was obtained from Ilex Oncology (San Antonio, TX, USA) and [^{14}C]spermidine trihydrochloride from Amersham (Les Ulis, France). All data are given as mean values of three or more experiments. Comparisons between means were made using the Student's *t* test assuming significance at $p < 0.05$.

Cell Culture. Murine leukemia L1210, CHO, and CHO-MG cells were grown in RPMI 1640 medium supplemented with 10% fetal calf serum, glutamine (2 mM), penicillin (100 U/mL),

streptomycin (50 μg/mL) (Eurobio, Les Ulis, France) at 37 °C under a humidified 5% CO_2 atmosphere as previously described.[43] To prevent artifactual results due to the oxidation of the conjugates by the serum amine oxidase present in fetal calf serum (Supporting Information Figure 1),[77] cell culture medium was supplemented with aminoguanidine (2 mM).

In Vitro Evaluation of Drugs Cytotoxicity/Cytostasy. The effect of the amidine derivatives on L1210 cell growth was assayed in sterile 96-wells microtiter plates (Becton Dickinson, Oxnard, CA, USA). L1210 cells were seeded at 5×10^4 cells/mL of medium (100 μL per well). Single CHO and CHO-MG cells, harvested by trypsinization, were plated at 2×10^3 cells/mL. Drug solutions (5 μL per well) of appropriate concentration were added at the time of seeding for L1210 cells and after an overnight incubation for CHO and CHO-MG cells. When required, 5 mM DFMO was added in the culture medium at the time of drug addition. After exposure to the drug for 48 h, cell growth was determined by measuring formazan formation from 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium using a Titertek Multiskan MCC/340 microplate reader (Labsystems, Cergy-Pontoise, France) for absorbance (540 nm) measurements.[78]

Spermidine Uptake Inhibition in L1210 Cells. The ability of the amidine derivatives to compete with radiolabeled spermidine for uptake was determined in L1210 cells by a 10 min uptake assay at 37 °C in the presence of increasing concentrations of competitor, using 1 μM [^{14}C]spermidine as substrate. The assay mixture contained 2×10^6 L1210 cells and was performed in a final volume of 0.6 mL Hanks' balanced salt solution supplemented with 20 mM HEPES. Initially, the K_m values of spermidine transport was determined as previously described.[79] K_i values were determined using the Cheng–Prusoff equation[80] from the IC_{50} value derived by iterative curve fitting of the sigmoidal equation describing the velocity of polyamine uptake in the presence of the respective competitor.[34,81]

Cellular Uptake. For determination of the cellular uptake of the derivatives, cells were seeded in culture flasks at 4×10^4 cells/mL for L1210 cells and at 2×10^5 cells/mL for CHO and CHO-MG cells. Drugs were added at the time of seeding for L1210 and 24 h after seeding for CHO and CHO-MG cells. Cells were harvested 24 h (CHO and CHO-MG) or 48 h (L1210) after drug addition. Harvested cells were washed three times in cold 0.14 M NaCl. Cell pellets were disrupted by sonication in 1 mL of 0.2 N $HClO_4$. After a night at 4 °C, homogenates were centrifuged at 15000 rpm for 30 min. Supernatants were used for HPLC determination of polyamine and derivatives as described above. Pellets were dissolved in 0.1 N NaOH and used for protein determination.

Acknowledgment. We are indebted to Professor W. Flintoff (University of Western Ontario, London, Canada) for providing the CHO-MG cells. We are grateful to D. Moncoq and R. Havouis for their technical assistance. We acknowledge the Centre National de la Recherche Scientifique (CNRS), the Association pour la Recherche sur le Cancer (ARC), and the Ligue Nationale Contre le Cancer (LNCC–Comités d'Ille-et-Vilaine, du Morbihan et des Côtes d'Armor) for financial support and for fellowships to S.T. and B.M. (LNCC). EU COST 922 is acknowledged for support. Our sincere thanks are also due to A. Bondon (CNRS UMR 6509, Rennes, France) for performing HMBC and HMQC spectra and to the late Dr. P. Guénot (Centre Régional de Mesures Physiques de l'Ouest, Rennes, France) for mass measurements.

Supporting Information Available: Spectroscopic and purity data for target compounds, HPLC analysis, and additional information. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Igarashi, K.; Ito, K.; Kashiwagi, K. Polyamine uptake systems in *Escherichia coli*. *Res. Microbiol.* **2001**, *152*, 271–278.
(2) Igarashi, K.; Kashiwagi, K. Polyamine transport in bacteria and yeast. *Biochem. J.* **1999**, *344*, 633–642.
(3) Hasne, M. P.; Ullman, B. Identification and characterization of a polyamine permease from the protozoan parasite *Leishmania major*. *J. Biol. Chem.* **2005**, *280*, 15188–15194.
(4) Hasne, M. P.; Coppens, I.; Soysa, R.; Ullman, B. A high-affinity putrescine-cadaverine transporter from *Trypanosoma cruzi*. *Mol. Microbiol.* **2010**, *76*, 78–91.
(5) Seiler, N.; Delcros, J. G.; Moulinoux, J. P. Polyamine transport in mammalian cells. An update. *Int. J. Biochem. Cell Biol.* **1996**, *28*, 843–861.
(6) Belting, M.; Persson, S.; Fransson, L. A. Proteoglycan involvement in polyamine uptake. *Biochem. J.* **1999**, *338*, 317–323.
(7) Soulet, D.; Covassin, L.; Kaouass, M.; Charest-Gaudreault, R.; Audette, M.; Poulin, R. Role of endocytosis in the internalization of spermidine-C(2)-BODIPY, a highly fluorescent probe of polyamine transport. *Biochem. J.* **2002**, *367*, 347–357.
(8) Palmer, A. J.; Wallace, H. M. The polyamine transport system as a target for anticancer drug development. *Amino Acids* **2010**, *38*, 415–422.
(9) Seiler, N. Thirty years of polyamine-related approaches to cancer therapy. Retrospect and prospect. Part 1. Selective enzyme inhibitors. *Curr. Drug Targets* **2003**, *4*, 537–564.
(10) Seiler, N. Thirty years of polyamine-related approaches to cancer therapy. Retrospect and prospect. Part 2. Structural analogues and derivatives. *Curr. Drug Targets* **2003**, *4*, 565–585.
(11) Kruczynski, A.; Vandenberghe, I.; Pillon, A.; Pesnel, S.; Goetsch, L.; Barret, J. M.; Guminski, Y.; Le Pape, A.; Imbert, T.; Bailly, C.; Guilbaud, N. Preclinical activity of F14512, designed to target tumors expressing an active polyamine transport system. *Invest. New Drugs* **2009**, DOI: 10.1007/s10637-009-9328-3.
(12) Seiler, N.; Dezeure, F. Polyamine transport in mammalian cells. *Int. J. Biochem.* **1990**, *22*, 211–218.
(13) Holley, J.; Mather, A.; Cullis, P.; Symons, M. R.; Wardman, P.; Watt, R. A.; Cohen, G. M. Uptake and cytotoxicity of novel nitroimidazole-polyamine conjugates in Ehrlich ascites tumour cells. *Biochem. Pharmacol.* **1992**, *43*, 763–769.
(14) Holley, J. L.; Mather, A.; Wheelhouse, R. T.; Cullis, P. M.; Hartley, J. A.; Bingham, J. P.; Cohen, G. M. Targeting of tumor cells and DNA by a chlorambucil–spermidine conjugate. *Cancer Res.* **1992**, *52*, 4190–4195.
(15) Eiseman, J. L.; Rogers, F. A.; Guo, Y.; Kauffman, J.; Sentz, D. L.; Klinger, M. F.; Callery, P. S.; Kyprianou, N. Tumor-targeted apoptosis by a novel spermine analogue, 1,12-diaziridinyl-4,9-diazadodecane, results in therapeutic efficacy and enhanced radiosensitivity of human prostate cancer. *Cancer Res.* **1998**, *58*, 4864–4870.
(16) Delcros, J. G.; Tomasi, S.; Carrington, S.; Martin, B.; Renault, J.; Blagbrough, I. S.; Uriac, P. Effect of spermine conjugation on the cytotoxicity and cellular transport of acridine. *J. Med. Chem.* **2002**, *45*, 5098–5111.
(17) Suzuki, I.; Shigenaga, A.; Nemoto, H.; Shibuya, M. Synthesis and DNA damaging ability of enediyne–polyamine conjugates. *Tetrahedron Lett.* **2004**, *45*, 1955–1959.
(18) Cunha, A. S.; Lima, E. I. S.; Pinto, A. C.; Esteves-Souza, A.; Echevarria, A.; Camara, C. A.; Vargas, M. D.; Torres, J. C. Synthesis of Novel Naphthoquinone–Spermidine Conjugates and their Effects on DNA-Topoisomerases I and II-α. *J. Braz. Chem. Soc.* **2006**, *17*, 439–442.
(19) Dallavalle, S.; Giannini, G.; Alloatti, D.; Casati, A.; Marastoni, E.; Musso, L.; Merlini, L.; Morini, G.; Penco, S.; Pisano, C.; Tinelli, S.; De Cesare, M.; Beretta, G. L.; Zunino, F. Synthesis and cytotoxic activity of polyamine analogues of camptothecin. *J. Med. Chem.* **2006**, *49*, 5177–5186.
(20) Pang, J. Y.; Long, Y. H.; Chen, W. H.; Jiang, Z. H. Amplification of DNA-binding affinities of protoberberine alkaloids by appended polyamines. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1018–1021.
(21) Verschoyle, R. D.; Carthew, P.; Holley, J. L.; Cullis, P.; Cohen, G. M. The comparative toxicity of chlorambucil and chlorambucil–spermidine conjugate to BALB/c mice. *Cancer Lett.* **1994**, *85*, 217–222.
(22) Cullis, P. M.; Green, R. E.; Merson-Davies, L.; Travis, N. Probing the mechanism of transport and compartmentalisation of polyamines in mammalian cells. *Chem. Biol.* **1999**, *6*, 717–729.
(23) Wang, C.; Delcros, J. G.; Cannon, L.; Konate, F.; Carias, H.; Biggerstaff, J.; Gardner, R. A.; Phanstiel, O., 4th Defining the molecular requirements for the selective delivery of polyamine conjugates into cells containing active polyamine transporters. *J. Med. Chem.* **2003**, *46*, 5129–5138.

(24) Barret, J. M.; Kruczynski, A.; Vispe, S.; Annereau, J. P.; Brel, V.; Guminski, Y.; Delcros, J. G.; Lansiaux, A.; Guilbaud, N.; Imbert, T.; Bailly, C. F14512, a potent antitumor agent targeting topoisomerase II vectored into cancer cells via the polyamine transport system. *Cancer Res.* **2008**, *68*, 9845–9853.

(25) Guminski, Y.; Grousseaud, M.; Cugnasse, S.; Brel, V.; Annereau, J. P.; Vispe, S.; Guilbaud, N.; Barret, J. M.; Bailly, C.; Imbert, T. Synthesis of conjugated spermine derivatives with 7-nitrobenzoxadiazole (NBD), rhodamine and bodipy as new fluorescent probes for the polyamine transport system. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2474–2477.

(26) Annereau, J. P.; Brel, V.; Dumontet, C.; Guminski, Y.; Imbert, T.; Broussas, M.; Vispe, S.; Breand, S.; Guilbaud, N.; Barret, J. M.; Bailly, C. A fluorescent biomarker of the polyamine transport system to select patients with AML for F14512 treatment. *Leuk. Res.* **2010**, *34*, 1383–1389.

(27) Sarhan, S.; Knödgen, B.; Gerhart, F.; Seiler, N. Chain-fluorinated polyamines as tumor markers—I. In vivo transformation of 2,2-difluoroputrescine into 6,6-difluorospermidine and 6,6-difluorospermine. *Int. J. Biochem.* **1987**, *19*, 843–852.

(28) Hull, W. E.; Kunz, W.; Port, R. E.; Seiler, N. Chain-fluorinated polyamines as tumour markers. III. Determination of geminal difluoropolyamines and their precursor 2,2-difluoroputrescine in normal tissues and experimental tumours by in vitro and in vivo 19F NMR spectroscopy. *NMR Biomed.* **1988**, *1*, 11–19.

(29) Hwang, D. R.; Lang, L. X.; Mathias, C. J.; Kadmon, D.; Welch, M. J. *N*-3-[18F]fluoropropylputrescine as potential PET imaging agent for prostate and prostate derived tumors. *J. Nucl. Med.* **1989**, *30*, 1205–1210.

(30) Cai, J. P.; Soloway, A. H.; Barth, R. F.; Adams, D. M.; Hariharan, J. R.; Wyzlic, I. M.; Radcliffe, K. Boron-containing polyamines as DNA targeting agents for neutron capture therapy of brain tumors: synthesis and biological evaluation. *J. Med. Chem.* **1997**, *40*, 3887–3896.

(31) Zhuo, J. C.; Cai, J. P.; Soloway, A. H.; Barth, R. F.; Adams, D. M.; Ji, W. H.; Tjarks, W. Synthesis and biological evaluation of boron-containing polyamines as potential agents for neutron capture therapy of brain tumors. *J. Med. Chem.* **1999**, *42*, 1282–1292.

(32) Martin, B.; Posseme, F.; Le Barbier, C.; Carreaux, F.; Carboni, B.; Seiler, N.; Moulinoux, J. P.; Delcros, J. G. *N*-Benzylpolyamines as vectors of boron and fluorine for cancer therapy and imaging: synthesis and biological evaluation. *J. Med. Chem.* **2001**, *44*, 3653–3664.

(33) Martin, B.; Posseme, F.; Le Barbier, C.; Carreaux, F.; Carboni, B.; Seiler, N.; Moulinoux, J. P.; Delcros, J. G. (*Z*)-1,4-Diamino-2-butene as a vector of boron, fluorine, or iodine for cancer therapy and imaging: synthesis and biological evaluation. *Bioorg. Med. Chem.* **2002**, *10*, 2863–2871.

(34) Covassin, L.; Desjardins, M.; Charest-Gaudreault, R.; Audette, M.; Bonneau, M. J.; Poulin, R. Synthesis of spermidine and norspermidine dimers as high affinity polyamine transport inhibitors. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1709–1714.

(35) Burns, M. R.; Carlson, C. L.; Vanderwerf, S. M.; Ziemer, J. R.; Weeks, R. S.; Cai, F.; Webb, H. K.; Graminski, G. F. Amino acid/spermine conjugates: polyamine amides as potent spermidine uptake inhibitors. *J. Med. Chem.* **2001**, *44*, 3632–3644.

(36) Graminski, G. F.; Carlson, C. L.; Ziemer, J. R.; Cai, F.; Vermeulen, N. M.; Vanderwerf, S. M.; Burns, M. R. Synthesis of bis-spermine dimers that are potent polyamine transport inhibitors. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 35–40.

(37) Covassin, L.; Desjardins, M.; Soulet, D.; Charest-Gaudreault, R.; Audette, M.; Poulin, R. Xylylated dimers of putrescine and polyamines: influence of the polyamine backbone on spermidine transport inhibition. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3267–3271.

(38) Burns, M. R.; Graminski, G. F.; Weeks, R. S.; Chen, Y.; O'Brien, T. G. Lipophilic lysine−spermine conjugates are potent polyamine transport inhibitors for use in combination with a polyamine biosynthesis inhibitor. *J. Med. Chem.* **2009**, *52*, 1983–1993.

(39) Chen, Y.; Weeks, R. S.; Burns, M. R.; Boorman, D. W.; Klein-Szanto, A.; O'Brien, T. G. Combination therapy with 2-difluoromethylornithine and a polyamine transport inhibitor against murine squamous cell carcinoma. *Int. J. Cancer* **2006**, *118*, 2344–2349.

(40) Wang, C.; Delcros, J. G.; Biggerstaff, J.; Phanstiel, O., 4th Molecular requirements for targeting the polyamine transport system. Synthesis and biological evaluation of polyamine-anthracene conjugates. *J. Med. Chem.* **2003**, *46*, 2672–2682.

(41) Wang, C.; Delcros, J. G.; Biggerstaff, J.; Phanstiel, O., 4th Synthesis and biological evaluation of *N*1-(anthracen-9-ylmethyl)triamines as molecular recognition elements for the polyamine transporter. *J. Med. Chem.* **2003**, *46*, 2663–2671.

(42) Gardner, R. A.; Delcros, J. G.; Konate, F.; Breitbeil, F., 3rd; Martin, B.; Sigman, M.; Huang, M.; Phanstiel, O. t. *N*1-substituent effects in the selective delivery of polyamine conjugates into cells containing active polyamine transporters. *J. Med. Chem.* **2004**, *47*, 6055–6069.

(43) Delcros, J. G.; Tomasi, S.; Duhieu, S.; Foucault, M.; Martin, B.; Le Roch, M.; Eifler-Lima, V.; Renault, J.; Uriac, P. Effect of polyamine homologation on the transport and biological properties of heterocyclic amidines. *J. Med. Chem.* **2006**, *49*, 232–245.

(44) Phanstiel, O. t.; Kaur, N.; Delcros, J. G. Structure−activity investigations of polyamine−anthracene conjugates and their uptake via the polyamine transporter. *Amino Acids* **2007**, *33*, 305–313.

(45) Callahan, J. F.; Ashton-Shue, D.; Bryan, H. G.; Bryan, W. M.; Heckman, G. D.; Kinter, L. B.; McDonald, J. E.; Moore, M. L.; Schmidt, D. B.; Silvestri, J. S.; Stassen, F. L.; Nelson, L. S.; Yim, C. F.; Huffman, W. F. Structure−activity relationships of novel vasopressin antagonists containing C-terminal diaminoalkanes and (aminoalkyl)guanidines. *J. Med. Chem.* **1989**, *32*, 391–396.

(46) Hansen, J. B.; Nielsen, M. C.; Ehrbahr, U.; Buchardt, O. Partially protected polyamines. *Synthesis* **1982**, 404–405.

(47) Goodnow, R.; Konno, J. K.; Niwa, M.; Kallimopoulos, T.; Bukownik, R.; Lenares, D.; Nakanishi, K. Synthesis of glutamate receptor antagonist philanthotoxin-433 (PhTX-433) and its analogs. *Tetrahedron* **1990**, *46*, 3267–3286.

(48) Levchine, I.; Rajan, P.; Borloo, M.; Bollaert, W.; Haemers, A. An efficient synthesis of selectively functionalized spermidine. *Synthesis* **1994**, 37–39.

(49) Geall, A. J.; Blagbrough, I. S. Homologation of polyamines in the synthesis of lipo-spermine conjugates and related lipoplexes. *Tetrahedron Lett.* **1998**, *39*, 443–446.

(50) Le Roch, M.; Renault, J.; Penlaë, K.; Uriac, P. Synthesis of dihydroxylated polyamines from an erythronolactone. *Tetrahedron Lett.* **2003**, *44*, 3451–3453.

(51) Bergeron, R. J.; Weimar, W. R.; Wu, Q.; Feng, Y.; McManis, J. S. Polyamine analogue regulation of NMDA MK-801 binding: a structure−activity study. *J. Med. Chem.* **1996**, *39*, 5257–5266.

(52) Horning, V. L.; Stromberg, E. C.; Loyd, H. A. Beckmann rearrangements. An investigation of special cases. *J. Am. Chem. Soc.* **1952**, *74*, 5153–5155.

(53) Büge, A.; Locke, C.; Köhler, T.; Nuhn, P. Synthese von tetrahydrobenzazepinon-phenylhydrazonen. *Arch. Pharm.* (*Weinheim*) **1994**, *327*, 99–103.

(54) Itoh, K.; Miyake, A.; Tada, N.; Hirata, M.; Oka, Y. Synthesis and beta-adrenergic blocking activity of 2-(*N*-substituted amino)-1,2,3,4-tetrahydronaphtalen-1-ol derivatives. *Chem. Pharm. Bull.* (*Tokyo*) **1984**, *32*, 130–151.

(55) Osadchii, S. A.; Kochubei, N. V. Synthesis of 7-mercapto-2,3,4,5-tetrahydro-1*H*-1-benzazepin-2-one. *Russ. J. Appl. Chem.* **1994**, *67*, 1674–1676.

(56) Chen, W. Y.; Gilman, N. W. Synthesis of 7-phenylpyrimido[5,4-*d*][1]benzazepin-2-ones. *J. Heterocycl. Chem.* **1983**, *20*, 663–666.

(57) Tomasi, S.; Eifler-Lima, V. L.; Le Roch, M.; Corbel, J. C.; Renault, J.; Uriav, P.; Mens, T.; Catros-Quemener, V.; Moulinoux, J. P. Depletion of polyamines potentiates the antitumour effect of tetracyclic amidines bearing a putrescine moiety. *Pharm. Sci.* **1997**, *3*, 241–247.

(58) Benson, R. E.; Cairns, T. L. Chemical reactions of caprolactam. *J. Am. Chem. Soc.* **1948**, *70*, 2115–2118.

(59) Weintraub, L.; Oles, S. R.; Kalish, N. A. A convenient general synthesis of amidines. *J. Org. Chem.* **1968**, *33*, 1679–1681.

(60) Foloppe, M. P.; Rault, S.; Robba, M. Pyrrolo[2,1-*c*][1,4]benzodiazepines: a mild conversion of thiolactam into amidine. *Tetrahedron Lett.* **1992**, *33*, 2803–2804.

(61) Alhonen-Hongisto, L.; Seppanen, P.; Janne, J. Intracellular putrescine and spermidine deprivation induces increased uptake of the natural polyamines and methylglyoxal bis(guanylhydrazone). *Biochem. J.* **1980**, *192*, 941–945.

(62) Mandel, J. L.; Flintoff, W. F. Isolation of mutant mammalian cells altered in polyamine transport. *J. Cell Physiol.* **1978**, *97*, 335–344.

(63) Hwang, D. R.; Jerabek, P. A.; Kadmon, D.; Kilbourn, M. R.; Patrick, T. B.; Welch, M. J. 2-[18F]Fluoroputrescine: preparation, biodistribution, and mechanism of defluorination. *Int. J. Rad. Appl. Instrum., A* **1986**, *37*, 607–612.

(64) Dezeure, F.; Sarhan, S.; seiler, N. Chain-fluorinated polyamines as tumor markers—IV. Comparison of 2-fluoroputrescine and 2,2-difluoroputrescine as substrates of spermidine synthase in vitro and in vivo. *Int. J. Biochem.* **1988**, *20*, 1299–1312.

(65) Hwang, D. R.; Mathias, C. J.; Welch, M. J.; McGuire, A. H.; Kadmon, D. Imaging prostate derived tumors with PET and *N*-(3-[18F]fluoropropyl)putrescine. *Int. J. Rad. Appl. Intrum., B* **1990**, *17*, 525–532.

(66) Ask, A.; Persson, L.; Heby, O. Increased survival of L1210 leukemic mice by prevention of the utilization of extracellular

polyamines. Studies using a polyamine-uptake mutant, antibiotics and a polyamine-deficient diet. *Cancer Lett.* **1992**, *66*, 29–34.
(67) Sarhan, S.; Knödgen, B.; Seiler, N. The gastrointestinal tract as polyamine source for tumor growth. *Anticancer Res.* **1989**, *9*, 215–224.
(68) Seiler, N.; Sarhan, S.; Grauffel, C.; Jones, R.; Knödgen, B.; Moulinoux, J.-P. Endogenous and exogenous polyamines in support of tumor growth. *Cancer Res.* **1990**, *50*, 5077–5083.
(69) Nishioka, K.; Romsdahl, M. M. Elevation of putrescine and spermidine in sera of patients with solid tumors. *Clin. Chim. Acta* **1974**, *57*, 155–161.
(70) Marton, L. J.; Heby, O.; Wilson, C. B. Increased polyamine concentrations in the cerebrospinal fluid of patients with brain tumors. *Int. J. Cancer* **1974**, *14*, 731–735.
(71) Loser, C.; Folsch, U. R.; Paprotny, C.; Creutzfeldt, W. Polyamine concentrations in pancreatic tissue, serum, and urine of patients with pancreatic cancer. *Pancreas* **1990**, *5*, 119–127.
(72) Loser, C.; Folsch, U. R.; Paprotny, C.; Creutzfeldt, W. Polyamines in colorectal cancer. Evaluation of polyamine concentrations in the colon tissue, serum, and urine of 50 patients with colorectal cancer. *Cancer* **1990**, *65*, 958–966.
(73) Zhou, G.; Yu, Q.; Ma, Y.; Xue, J.; Zhang, Y.; Lin, B. Determination of polyamines in serum by high-performance capillary zone electrophoresis with indirect ultraviolet detection. *J. Chromatogr., A* **1995**, *717*, 345–349.
(74) Byun, J. A.; Lee, S. H.; Jung, B. H.; Choi, M. H.; Moon, M. H.; Chung, B. C. Analysis of polyamines as carbamoyl derivatives in urine and serum by liquid chromatography-tandem mass spectrometry. *Biomed. Chromatogr.* **2008**, *22*, 73–80.
(75) Seiler, N.; Knödgen, B. High-performance liquid chromatographic procedure for the simultaneous determination of the natural polyamines and their monoacetyl derivatives. *J. Chromatogr.* **1980**, *221*, 227–235.
(76) Bernard, T.; Pocard, J. A.; Perroud, B.; Le Rudulier, D. Variations in the response of salt stressed rhizobium strains to betaines. *Arch. Microbiol.* **1986**, *143*, 359–364.
(77) Gahl, W. A.; Pitot, H. C. Polyamine degradation in foetal and adult bovine serum. *Biochem. J.* **1982**, *202*, 603–611.
(78) Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of radiosensitivity. *Cancer Res.* **1987**, *47*, 943–946.
(79) Clement, S.; Delcros, J. G.; Feuerstein, B. G. Spermine uptake is necessary to induce haemoglobin synthesis in murine erythroleukaemia cells. *Biochem. J.* **1995**, *312*, 933–938.
(80) Cheng, Y.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.
(81) Torossian, K.; Audette, M.; Poulin, R. Substrate protection against inactivation of the mammalian polyamine-transport system by 1-ethyl-3-(3-dimethylaminopropyl)carbodi-imide. *Biochem. J.* **1996**, *319*, 21–26.