Synthesis and Biological Evaluation of Aminopolyamines

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Abstract: Exploitation of the polyamine backbone as a vector for intracellular transport of various pharmacophores has focused largely on fixing the cargo molecule to one of the nitrogens in the linear chain. This communication describes the assembly of a model aminopolyamine analogue, 6-amino-N^1,N^{12}-diethylspermine, and its biological properties. This amino polyamine presents an additional site of attachment for cargo molecules, reduces cell growth, and achieves cellular concentrations that are higher than those of N^1,N^{12}-diethylspermine.

It is now clear that N-alkylated polyamines exhibit antineoplastic activity against a number of murine and human tumor lines in vitro and in vivo.[1,2] Although it is well-established that these polyamine analogues utilize the polyamine transport apparatus for incorporation into cells,[3,4] deplete polyamine pools,[5] drastically reduce the level of ornithine decarboxylase (ODC)[6,7] and *S*-adenosylmethionine decarboxylase (AdoMetDC) activities,[8] and in some cases up-regulate spermidine/spermine N^1-acetyltransferase (SSAT),[9−12] the precise mechanism by which they induce death in tumor cells still remains a mystery.[13] A systematic structure−activity study has shown that very small structural alterations in these polyamine analogues can cause pronounced changes in their biological activity at the cellular and whole animal levels.[5] This is exemplified by the differences in how various analogues compete for transport and how they influence key polyamine biosynthetic enzymes.

We have mapped out the structural boundary conditions set by the polyamine transport apparatus on polyamine analogues.[1,2,5−8,11,14−19] Although steric issues are important,[1,15−17] charge is critical to recognition and transport of these substrates.[1,15,16,19−21] For example, N^1,N^{12}-diethylspermine (DESPM), a tetracation at physiological pH, competes well with spermidine (SPD) for the polyamine transport apparatus and is concentrated to millimolar levels intracellularly. N^1,N^{12}-Bis(2,2,2-trifluoroethyl)spermine (FDESPM) is dicationic at pH 7.4 and competes poorly with SPD for the polyamine transport apparatus. In the picture that emerges, the polyamine transport apparatus provides biological counteranions for the cationic nitrogens. Other investigators have exploited these findings by attaching a variety of cargo molecules to the spermine backbone.[21−24] On the basis of simple electrostatics, when a cargo molecule is neutral or positively charged, binding to the transporter should be more favorable than when the cargo molecule is negatively charged.[1,15−17,25]

Recently, we have shown that it is possible to vector iron chelators intracellularly via the polyamine transport apparatus.[26] A simple bidentate, neutral ligand, 1,2-dimethyl-3-hydroxypyridin-4-one (L1), was conjugated to spermine (SPM), generating 1-(12-amino-4,9-diazadodecyl)-2-methyl-3-hydroxy-4(1*H*)-pyridinone. The denticity of the iron complex was not affected by the attachment of the SPM moiety. L1 does not achieve high concentrations (∼1 μM) in cultured cells. In murine leukemia L1210 cells, all of the indicators of intracellular access, including (1) the effect on cell proliferation (IC_{50}), (2) the ability to compete with radiolabeled spermidine for the polyamine transport apparatus (K_i), (3) the impact on polyamine pools, and (4) effects on the polyamine enzymes ODC, AdoMetDC, and SSAT, were consistent with the polyamine fragment of the polyamine−chelator conjugate serving as a vector. The adduct was transported quite efficiently into the cells; the cellular concentration of 0.39 mM is more than 1900-fold higher than the extracellular treatment concentration and is also nearly 400 times higher than the cellular concentration attained by the parent ligand L1. This vector concept has now been successfully extended to the iron chelator (*S*)-4,5-dihydro-2-(2,4-dihydroxyphenyl)-4-methyl-4-thiazolecarboxylic acid. The norspermidine conjugate (*S*)-4,5-dihydro-2-[2-hydroxy-4-(12-amino-5,9-diazadodecyloxy)phenyl]-4-methyl-4-thiazolecarboxylic acid ethyl ester achieves very high intracellular concentrations relative to its parent ligand (Bergeron et al., manuscript in press).

Exploitation of the polyamine backbone as a vector has focused largely on attaching the cargo molecule to one of the nitrogens in the linear chain, either a terminal primary amine or a secondary internal nitrogen. The nature of the linkage between the nitrogens and the cargo molecule is critical. The linkage can have a profound impact on the molecule's charge disposition and thus its uptake properties. Clearly, an acyl or sulfonyl linkage to the nitrogens prevents protonation at physiological pH.

Consider the linear tetraamine SPM: acylation of a single terminal nitrogen still leaves the requisite three cationic SPD-like sites for binding and transport. However, acylation of a secondary nitrogen interrupts the linear charge array. Although a triamine remains, the systems looks more like a diamine insulated from a monoamine. This problem could be overcome by introducing a primary amine into the polyamine backbone. This primary amine could be linked via acyl or sulfonyl fragments to a variety of cargo molecules without affecting the charge substantially. The current work describes (1) the assembly of such an aminopolyamine pharmacophore, (2) an evaluation of the compound's effects on L1210 cell proliferation, (3) an evaluation of its cellular uptake, and (4) its impact on polyamine pools and enzymes by procedures that are standard in this laboratory. The target chosen for this initial study was a derivative of the well-characterized polyamine analogue DESPM, 6-amino-N^1,N^{12}-diethylspermine (6ADESPM,

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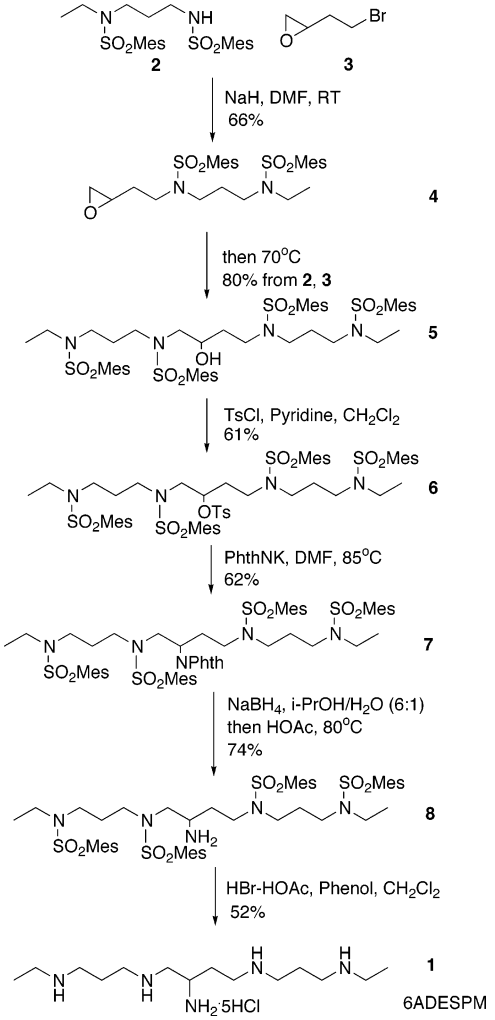
Scheme 1. Synthesis of the Aminopolyamine 6ADESPM



1). Ensuing studies will focus on utilization of this vector for intracellular transport of a variety of ligands.

The synthesis began with the alkylation of *N*,*N*′-bis-(mesitylenesulfonyl)-*N*-ethyl-1,3-diaminopropane (**2**)[19,28] with 4-bromo-1,2-epoxybutane (**3**)[29,30] (Scheme 1). When the anion of sulfonamide **2** (NaH in DMF) was treated with an equimolar amount of **3** at room temperature, selective displacement of bromide occurred,[29] giving epoxide **4** in 66% yield. When the anion of **2** (2 equiv) was heated at 70 °C with ω-bromoepoxide **3**, tetrasulfonamide alcohol **5** was generated in 80% yield. Reaction of **5** with *p*-toluenesulfonyl chloride in pyridine and CH_2Cl_2 afforded secondary tosylate **6** in 61% yield. Displacement of the hindered leaving group of **6** was effected with potassium phthalimide in DMF at 85 °C, providing fully protected pentaamine **7** in 62% yield. Phthalimide **7** was unreactive to prolonged heating with hydrazine in CH_3CH_2OH. However, reduction of **7** with sodium borohydride in aqueous 2-propanol and then heating the mixture with acetic acid[31] gave 6-amino-N^1,N^{12}-diethyl-N^1,N^4,N^9,N^{12}-tetrakis(mesitylenesulfonyl)spermine (**8**) in 74% yield. Removal of the mesitylenesulfonyl protecting groups of **8** using HBr−acetic acid in phenol and CH_2Cl_2 and conversion to the pentahydrochloride salt furnished 6ADESPM (**1**) in 52% yield. It is important to point out that this product is racemic. The synthetic methodology of Scheme 1 in conjuction with the commercial availability of both stereoisomers of epoxide **3** lends itself nicely to assembling each enantiomer of 6ADESPM (**1**). This synthetic route is versatile in that *N*-monoalkylsulfonamides besides **2** could be added to epoxide **4**. Furthermore, the primary amino group of **8** could be modified with alkyl, acyl, or other functionality stable to mesitylenesulfonyl group removal, providing access to analogues of 6ADESPM (**1**).

Table 1. L1210 Cell Growth Inhibition and Transport for Alkylated Spermine Analogues

compound	structure/abbreviation	IC_{50} (μM)[a] 48 h	96 h	K_i (μM)[b]
	DESPM[c]	30	0.2	1.6
	FDESPM[d]	–	> 100	285
	6ADESPM (**1**)	8	0.4	33.7

[a] The IC_{50} was estimated from growth curves for L1210 cells grown in the presence of nine different extracellular concentrations of drug spanning four logarithmic units: 0, 0.03, 0.1, 0.3, 1.0, 3, 10, 30, and 100 μM. IC_{50} data are presented as the mean of at least two experiments with variation from the mean typically 10−25% for the 96 h IC_{50} values. [b] K_i determinations were made by following analogue inhibition of spermidine transport. All polyamine analogues exhibited simple substrate-competitive inhibition of [^{3}H]SPD transport by L1210 cells. Values reported in the table represent the mean of at least two or three experiments with a variation typically less than 10%. [c] From ref 15. [d] From ref 16.

At present, it is unclear how the cell might see 6ADESPM in terms of charge, i.e., as a tetracation or as a fully protonated pentaamine. The pK_a values for the nitrogens remain to be determined. However, on the basis of our previous pK_a studies with the tetraamines N^1,N^{11}-diethylnorspermine (DENSPM), DESPM (Table 1), and N^1,N^{14}-diethylhomospermine (DEHSPM),[20] 6ADESPM is likely to be a tetracation at physiological pH. Although DEHSPM is largely tetracationic at physiological pH, shortening the methylene backbone between the charged centers (e.g., DEHSPM vs DENSPM) profoundly reduces the fraction of tetracation. The shorter insulators between the cationic nitrogens and the resulting electrostatic repulsion impede complete protonation.[32]

The 48 and 96 h IC_{50} values (Table 1) suggest that 6ADESPM (8 μM at 48 h; 0.4 μM at 96 h) behaves more like its tetracationic parent DESPM (30 and 0.2 μM at 48 and 96 h, respectively) than its dicationic analogue FDESPM (>100 μM at 96 h).

The ability of 6ADESPM to compete with radiolabeled SPD for uptake was compared to those of DESPM[15] and FDESPM[16] (Table 1). The dication FDESPM was a poor competitor (K_i = 285 μM), behaving much like putrescine (PUT). DESPM was an excellent competitor (K_i = 1.6 μM). Interestingly, the pentaamine 6ADESPM (K_i = 33.7 μM) does not bind to the transport apparatus nearly as well as does DESPM, leaving questions about the nature of the charge disposition.

L1210 cells were treated with DESPM or 6ADESPM at about 5 times their respective 48 h IC_{50} values[15] (Table 2) to ensure that we are working in a range where growth inhibition is occurring. At 150 μM in the culture media, DESPM depleted PUT and SPD to 0%

Table 2. Effect of Alkylated Spermine Analogues on Polyamine Pools in L1210 Cells

compd	treatment concn (μM)	PUT[a]	SPD[a]	SPM[a]	cellular concn[b]
DESPM[c]	150	0	0	22	1.78
FDESPM[c]	500	87	94	116	<0.02
6ADESPM	50	26	7	32	3.10

[a] Putrescine (PUT), spermidine (SPD), and spermine (SPM) levels after 48 h of treatment are given as percent of the polyamine found in untreated controls. The control values in pmol/10^6 L1210 cells are PUT = 183 ± 15, SPD = 2694 ± 232, SPM = 774 ± 81. [b] The cellular concentration of the analogue is expressed as nmol/10^6 cells. Untreated L1210 cells (10^6) correspond to about 1 μL volume; therefore, the concentration can be estimated as mM. [c] From ref 16.

Table 3. Impact of Alkylated Spermine Analogues on Ornithine Decarboxylase (ODC), *S*-Adenosylmethionine Decarboxylase (AdoMetDC), and Spermidine/Spermine N^1-Acetyltransferase (SSAT) in L1210 Cells[a]

compd	ODC	AdoMetDC	SSAT
DESPM[b]	3	28	460
FDESPM[c]	100	100	97
6ADESPM	16 ± 2	58 ± 8	667 ± 16

[a] Enzyme activity is expressed as percent of untreated control for ODC (1 μM at 4 h), AdoMetDC (1 μM at 6 h), and SSAT (10 μM at 48 h). Each experiment included a positive control that had a known, reproducible impact on enzyme activities (mean ± SD): 1 μM DEHSPM lowered ODC to 6.7 ± 2.6% of the untreated control; 1 μM DEHSPM decreased AdoMetDC to 40.7 ± 6.2% of the untreated control; 2 μM DENSPM increased SSAT to 1029 ± 87% of the untreated control. Data shown in the table represent the mean of at least three experiments and have variances consistent with those suggested by the positive control data presented above. [b] From ref 15. [c] From ref 16.

of control values, reduced SPM to 22% of control, and accumulated to a cellular concentration of 1.78 mM.[16] The polyamine pools of cells treated with 50 μM 6ADESPM were affected far less; PUT, SPD, and SPM were diminished to 26, 7, and 32% of control values, respectively. However, 6ADESPM achieved a level nearly 2 times that of DESPM, 3.10 mM. Although the extracellular treatment concentration of FDESPM (500 μM)[16] was nearly twice its K_i value, the cellular concentration was <2% that of DESPM and <0.6% that of 6ADESPM; polyamine pools remained virtually unchanged.

On a comparative basis, the results (Table 3) are not unexpected and are consistent with the effects of the drug on polyamine pools. The compound with the highest IC_{50}, the highest K_i, and the least effect on polyamine pools, FDESPM, also has no impact on the polyamine enzymes ODC, AdoMetDC, and SSAT.[16] Conversely, DESPM, the compound with the lowest 96 h IC_{50}, the lowest K_i, and the most profound influence on polyamine pools also had the greatest effect on ODC (3% of control) and AdoMetDC (28% of control) activities.[15] The most interesting of these compounds is 6ADESPM. When compared to DESPM, the aminopolyamine has a higher 96 h IC_{50}, a higher K_i, and less of an effect on polyamine pools. 6ADESPM had less of an influence than DESPM on ODC and AdoMetDC activities (16% and 58% of control, respectively); however, SSAT activity was stimulated to a greater degree, 667% of control. Recall that the intracellular concentration of 6ADESPM is nearly 3 times higher than that of DESPM.

A highly flexible synthetic method is now available for the assembly of aminopolyamines. Because of the observed biological profile of 6ADESPM (**1**), a racemic mixture, each enantiomer will be assembled and evaluated. The key synthon for further studies, the tetramesitylenesulfonamide with its single free amine (8), will allow coupling of various cargo molecules without significantly compromising charge distribution. The primary amine can be acylated, and protecting mesitylenesulfonyl groups can then be removed, leaving a vector with four cationic centers.

The resulting 6ADESPM behaves very much like its parent drug, slowing cell growth, competing effectively with SPD for the polyamine transport apparatus, reducing polyamine pools and the activities of ODC and AdoMetDC, and stimulating SSAT. Thus, it would seem that introduction of an amino group into the polyamine backbone of DESPM is compatible with cellular uptake. In fact, this aminopolyamine achieves cellular concentrations that are almost 2 times higher than those of DESPM itself. The question as to whether there is anything patently unusual about the biological properties of this model vector itself has now been answered.

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Supporting Information Available: Experimental procedures and analytical and spectral data for 6ADESPM. This material is available free of charge via the Internet at http://pubs.acs.org.

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