polyamine binders were removed from the negatively charged DNA duplex under the applied electric field used for electrophoresis.

[13] Restriction enzyme *Ban* II cuts pBR322 at a GA(G)GCT(C)C sequence (locations 471 and 485).

[14] A referee suggested that the observed retardation of hydrolysis could have been caused by a small amount of free amine **2** liberated from **1** (used in a 100 μM concentration), such an equilibrium would liberate **2** in a 2.7 μM or less concentration (ref. [10]). A series of experiments with various concentrations of **2** indicated that no inhibition of the nuclease activity occurs with such a small concentration of **2**, indicating in turn that the free amine is not responsible for the observed retardation with **1**.

## Complexation of Antimony(III) by Trypanothione**

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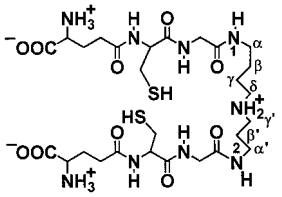
In contrast to almost all other organisms, trypanosomatids can conjugate the sulfur-containing tripeptide glutathione and the polyamine spermidine to form trypanothione ($N^1$,$N^8$-bis(glutathionyl)spermidine).[1–3] Together with trypanothione reductase (TR), the dithiol form of trypanothione ($T(SH)_2$), which is an analogue of glutathione found in the mammalian host, provides a uniquely intracellular reducing environment in parasites. Besides its protective and regulatory roles, $T(SH)_2$ and its related enzymes are essential for growth and survival of these parasites.[4–6] The trypanosomatid protozoan



Trypanothione, $T(SH)_2$

parasite *Leishamnia* is the causative of kala azar and severe forms of leishmaniasis that afflict more than ten million people worldwide. Several antimony compounds are currently the agents of choice for the treatment of leishmaniases and have been used for decades. Analogous to arsenical drugs against African trypanosomes,[7] one of the major targets for antimony drugs is probably $T(SH)_2$. Overproduction of $T(SH)_2$ in cells is also related to resistance to antimonials in *Leishmania*.[8] We show here that $Sb^{III}$ forms a novel complex with $T(SH)_2$ by binding to two sulfur atoms and to the oxygen atom of a water molecule. This appears to be the first chemical characterization of a metal complex of $T(SH)_2$, which may play a role in the mechanism of action of the antimony antileishmanial drugs.

Trypanothione $T(SH)_2$ can be readily obtained by the reduction of trypanothione disulfide ($TS_2$) by tris(2-carboxyethyl)phosphane.[8] The electrospray (ESI) mass spectrum of $T(SH)_2$ at around pH 7 exhibits a cluster of ions at $m/z$ 724.9 corresponding to $C_{27}H_{50}N_9O_{10}S_2$ (calcd 724.3). The reaction product of an antimony compound and $T(SH)_2$ at pH 7.4 displayed a prominent cluster of ions related to the isotopic distribution of Sb and C, with an $[M^+]$ peak at $m/z$ 842.6 corresponding to $SbS_2C_{27}N_9O_{10}H_{48}$ (calcd for $SbT(S)_2$ complex: 843.2; see Figure S1 in the Supporting Information). This represents a 1:1 antimony(III) trypanothione complex, in contrast to the antimony(III) glutathione complex $Sb(GS)_3$.[9]

The $^1H$ NMR signals of $T(SH)_2$ in aqueous solution were assigned with the aid of various 2D TOCSY, NOESY, and ROESY spectra. Signals from pairs of like residues overlap, with only minor differences attributable to the actual asymmetry of the spermidine moiety. This indicates an approximate symmetry of the two halves of $T(SH)_2$ that is similar to that of its oxidatized form.[10] Only four relatively sharp NH peaks and one very broad peak were observed at pH 4.6, and the very broad one disappeared above pH 5. The NH peaks of both Cys and Gly are significantly broadened at pH 7.4 (Figure 1). Addition of 0.5 of a molar equivalent of antimony(III) tartrate at pH 4.6 resulted in a peak at $\delta = 3.36$, while the signal of the $\beta$ protons of the Cys residues of free

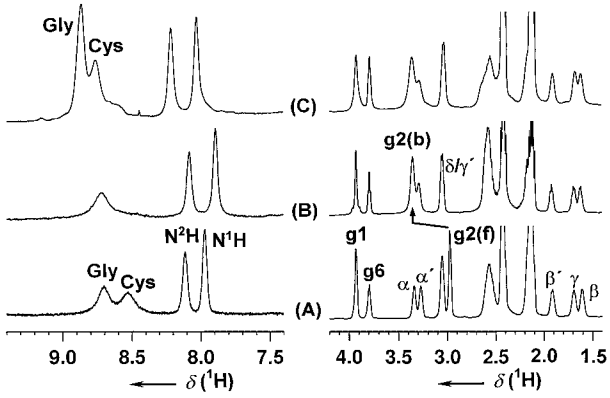



Figure 1. 500 MHz $^1H$ NMR spectra. A) Trypanothione $T(SH)_2$. B) $T(SH)_2$ after addition of antimony(III) tartrate (molar ratio 1:1) at 298 K and pH 7.4; C) as B) but at 278 K. Note the large low-field shifts of the peaks for Cys $\beta$-$CH_2$ (g2) on complexation to $Sb^{III}$. Labels: g1, $\alpha$-$CH_2$ of Gly; g6, $\alpha$-$CH_2$ of Glu.

$T(SH)_2$ at $\delta = 2.97$ decreased in intensity. Similarly, the NH signal of the Cys residue of $T(SH)_2$ at $\delta = 8.54$ decreased in intensity, while a new peak appeared at slightly lower field ($\delta = 8.59$). A 2D TOCSY spectrum confirmed that the peaks at $\delta = 4.52$, 2.97, and 8.54 are coupled to each other, as are those at $\delta = 4.68$, 3.36, and 8.59. The former set can therefore

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be assigned to the $\alpha$-, $\beta$-, and NH protons of the Cys residues of $T(SH)_2$, and the latter to protons of the Cys residues of $Sb^{III}$-bound trypanothione. The 2D exchange spectrum (EXSY, mixing time 400 ms) showed cross-peaks between free and bound forms of both the $\alpha$-CH and $\beta$-$CH_2$ groups of the Cys residues of $T(SH)_2$ at $\delta = 4.68/4.52$ and 3.36/2.97, respectively (data not shown). This suggested that the free and bound forms were in the slow exchange on the $^1H$ NMR time scale at pH 4.6. A cross-peak at $\delta = 4.70/4.34$ was also observed due to the exchange between free and bound forms of tartrate.

After the addition of a total of 1.0 molar equivalent of $Sb^{III}$, the signals of the Cys residues of the bound form further increased in intensity, and less than 20% of the free form remained. The latter totally disappeared when the pH of the solution was higher than 5.6, and the solution was kept at pH 7.4 thereafter. No further shifts in the proton signals of trypanothione were observed when more $Sb^{III}$ was added, and this suggests formation of a stable 1:1 $SbT(S)_2$ complex, in good agreement with the ESI-MS data. The large shifts of the $\beta$ and $\alpha$ protons of Cys residues ($\Delta\delta = 0.40$ and 0.13, respectively; Figure 1 and Table S1 in the Supporting Information) indicated that sulfur atoms are the predominant binding sites for $Sb^{III}$. Surprisingly, the $^1H$ chemical shifts of $N^1H$ and $N^2H$ are shifted to low frequency by $\Delta\delta \approx 0.08$ and 0.03, respectively. Shifts for the remaining protons are minor. The signals of the amino protons of Cys were too broad to be observed at 298 K, but reappeared when the temperature was lowered to 278 K (Figure 1).

The 2D ROESY experiments (mixing time 200 ms) were performed at 298 and 278 K to elucidate the structure of the complex in solution. The important results are summarized below. No amide – amide NOEs were observed, but the cross-peaks for sequence assignment were observed clearly. Unexpectedly a cross-peak between $N^1H$ and $\alpha'$-$CH_2$ was observed (Figure 2), which suggested the distance between these two protons is less than 5 Å. Similarly a cross-peak between $\gamma$-$CH_2$ and $\gamma'$-$CH_2$ was also observed. Surprisingly, a variety of protons have cross-peaks with water, particularly those of the hydrophobic part of spermidine, the $\alpha$- and $\beta$-protonsof Cys, and the $\alpha$-protons of Gly (Figure 2). This clearly demonstrated that the oxygen atom of a water molecule is bound to $Sb^{III}$, and strong hydrogen bonding of the water molecule to the carboxyl oxygen atom of Gly may account for the changes in the chemical shift of $N^1H$ of spermidine after formation of the complex. The distances between the water protons and neighboring protons are probably around 5 Å.

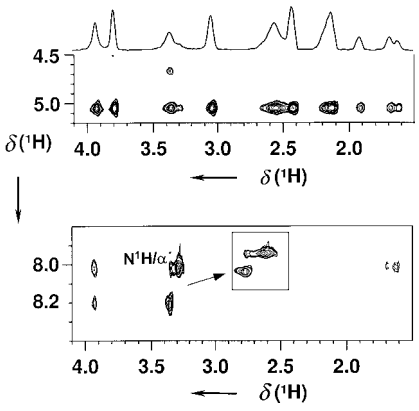


Figure 2. Expansion of the 200 ms 2D ROESY NMR spectra of $SbT(S)_2$ at 278 K (pH 7.4). A variety of cross-peaks were observed between water protons and the hydrophobic part of spermidine, the $\alpha$- and $\beta$-protons of Cys, and the $\alpha$-protons of Gly (top); a section through the 2D ROESY spectrum at the chemical shift of water is plotted above the spectrum. A weak long-range rOe between $N^1H$ and $\alpha'$-$CH_2$ was clearly observed (bottom).

Figure 3 shows a model of $SbT(S)_2$ which is consistent with our experimental results. For comparison, the structure of trypanothione disulfide in a $TS_2$ complex with trypanothione

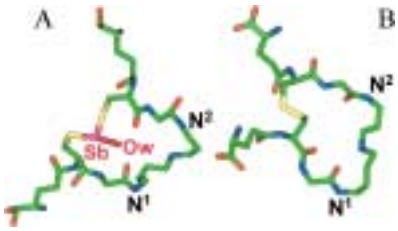


Figure 3. A) Structure for $SbT(S)_2$ which is consistent with the observed NMR rOe; B) crystal structure of the disulfide form of trypanothione from a complex of trypanothione reductase and trypanothione.[11] All hydrogen atoms are omitted for clarity. C, green; N, blue; O, red; S, yellow; Sb, purple.

reductase (TR)[11] is also shown (Figure 3B). The $Sb^{III}$ ion is strongly coordinated to the two sulfur atoms of $T(SH)_2$ (Figure 3A), and the oxygen atom of a water molecule completes the coordination sphere. The presence of an $Sb^{III}$-bound water molecule is necessary, since the distance between $N^1H$ and $\alpha'$-$CH_2$ is otherwise greater than 8 Å, as is that between $\gamma$-$CH_2$ and $\gamma'$-$CH_2$. It is known that the coordination number of $Sb^{III}$ is normally at least three.[12]

The strong binding of $Sb^{III}$ to $T(SH)_2$ suggested that $T(SH)_2$ could protect trypanothione reductase from antimony drugs within whole cells under the conditions used in this work, and this is in contrast to a previous report.[13] The inhibition of TR by $Sb^{III}$ is probably also due to the formation of a covalent bond with the active-site cysteine residues of TR after its reduction by NADPH. Although it is thermodynamically stable, the complex $SbT(S)_2$ is kinetically labile under the conditions used. The exchange of $Sb^{III}$ between free and bound forms of $T(SH)_2$ implies that $Sb^{III}$ is transported by a thiolate "pool", and Sb – TR complex could readily be formed even in the presence of high concentration of $T(SH)_2$. Biological investigations of $SbT(S)_2$ and its inhibiting action on TR are therefore warranted.

*Experimental Section*

Oxidized trypanothione was purchased from Bachem, and tris(2-carboxyethyl)phosphane and antimony tartrate from Aldrich.

$^1H$ NMR spectra were acquired on Bruker DRX500 spectrometer operating at 500.013 MHz. All of the samples (5 mM) for NMR were in $H_2O/D_2O$ (90/10), and several mixing times were chosen in the range of 20 – 75 ms (2D TOCSY), 200 – 400 ms (NOESY), and 200 ms (ROESY). The resonance signal for water was suppressed by the WATERGATE pulsed-field-gradient sequence.[14] Data acquisition and processing conditions have been described previously.[9, 15]

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pH measurements were made with a Corning 440 pH meter equipped with an Aldrich micro-combination electrode calibrated with standard buffer solutions of pH 4, 7, and 10.

Electrospray mass spectrometry (ESI-MS) was performed on a LCQ spectrometer (Finnigan Corporation). The sample was infused at 3 μL min$^{-1}$, and the ions were produced in an atmospheric-pressure ionization (API)/ESI ion source. The source temperature was 453–473 K, and the flow rate of the drying gas was 0.9 L min$^{-1}$. A potential of 3.5 kV was applied to the probe tip, and a cone voltage of 5–10 V over 200–2000 Da was used. The quadrupole was scanned at 100 amu s$^{-1}$. The mass accuracy of all measurements was within 0.5 $m/z$ units. Data acquisition and processing were performed with the Microsoft Windows NT operating system. The mass spectrum was simulated on a PC with IsoPro 3.0.

Molecular modeling was performed by using INSIGHT II (95.0, Biosym-MSI software) with the ESFF force field under the optimized pulldown in the builder module.

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## Supramolecular Modification of the Periphery of Dendrimers Resulting in Rigidity and Functionality**

Maurice W. P. L. Baars, Annika J. Karlsson, Victor Sorokin, Bas F. W. de Waal, and E. W. Meijer*

The number, size, and function of peripheral groups of dendritic macromolecules determine many of the typical dendrimer properties, such as dense-shell packing, overall shape, and multivalency.[1] Properties related to solubility or physical state—semi-crystalline, glass, liquid crystalline, or liquid—are also strongly dependent on the nature of the dendritic end group.[2] Finally, specific interactions of guest molecules with the dendritic hosts rely on both the core and the shell of the dendrimer.[3–11] Most of the end-group modifications are based on covalent bonding, while the use of supramolecular interactions to obtain new dendritic peripheries is limited. Chechik and Crooks showed that ionic bonding between an amine-terminated poly(aminoamine) (PAMAM) and a fatty acid resulted in similar host–guest properties as those of the corresponding covalent amide analogues, while Tomalia and co-workers recently used ionic interactions to assemble dendrimers into higher aggregates.[12] We anticipated that the combination of a dense packing of the shell with the possibility of tuning the functionality of the periphery is of great importance in making dendrimers that can be used as shape-persistent building blocks in nanotechnology. Herein we disclose a general methodology to modify the periphery of poly(propyleneimine) dendrimers using such a supramolecular approach. The covalently attached adamantylurea end groups of the dendrimer are used as a scaffold to reversibly bind glycinylurea building blocks through strong and directional multiple interactions (Scheme 1).

The design of the modification is given in Scheme 1 and the scaffold is based upon DAB-dendr-$(NHCONH\text{-}Ad)_n$ (**1**, with $n = 4$, 8, 16, 32, and 64 for **1a**–**e**, respectively). These dendrimers were selected after studying DAB-dendr-$(NHCO\text{-}Ad)_n$ (**2a**–**e**), DAB-dendr-$(NHCONH\text{-}C_{12}H_{25})_n$, and DAB-dendr-$(NHCO\text{-}C_{12}H_{25})_n$ as well. All dendrimers were synthesized in quantitative yield from DAB-dendr-$(NH_2)_n$ and the corresponding isocyanate or acid chloride and were fully characterized.[13a] The concept of the supramolec-

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