Synthesis and Biological Evaluation of *s*-Triazine Substituted Polyamines as Potential New Anti-Trypanosomal Drugs

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The P2 transporter is a nucleoside transporter which is unique to the protozoan parasite *Trypanosoma brucei*, the causative organism of Human African Trypanosomasis. The transporter has been shown to bind some structural motifs not recognized by other transporters. In this paper we describe the use of the melamine motif, a substrate of the P2 transporter, as a potential tool to selectively deliver polyamine analogues to the parasites. The synthesis of a number of polyamine analogues attached to a variety of melamine analogues is described. Many of the compounds were shown to competitively inhibit uptake of adenosine, indicating that they are recognized by the transporter. Some of the compounds showed good in vitro activity against the parasites.

Introduction

Parasitic diseases cause huge suffering in many parts of the world. However, comparatively little research is done in the field, and available funds for further investigations reflect by no means the number and seriousness of cases.[1] African sleeping sickness (Human African Trypanosomiasis, HAT) has become resurgent at a time when current chemotherapy is failing.[2]

According to the World Health Organization (WHO),[3] more than 60 million people are at risk of HAT with an estimated number of 0.3−0.5 million infected people and about 40 000 deaths reported in 1998. The disease is lethal when untreated. There are no vaccines available, and the treatment, once the parasite has crossed the blood-brain barrier, relies on the use of Melarsoprol and eflornithine when available at this time.[2,4] Melarsoprol is an arsenic based drug with low chemotherapeutic index,[5] which needs to be administered intravenously under medical observation. Severe side effects (myocardial damage, hypertension, exfoliative dermatitis, and reactive encephalopathy)[6] as well as resistance[7] has been reported. Therefore, there is clearly an urgent need for a new generation of drugs.

HAT is caused by the two subspecies *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*. They live in body fluids including the blood and lymph and, in later stages, the cerebrospinal fluid, where they grow and divide by binary fission.[8] The rational approach toward the development of new potential chemotherapeutics focuses on differences in biochemistry and metabolism between the human host and the causative agent.[9] However, the biochemistry and metabolism in trypanosomes are complex and only fragmentarily investigated.[10] In this paper we discuss how selective toxicity may be brought about by selective drug uptake through nutrient transporters in trypanosomes.[11]

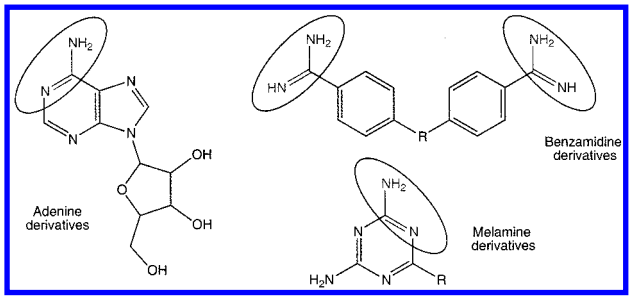

Figure 1. P2 Recognition Motif

Carter and Fairlamb characterized a unique nucleoside transporter in trypanosomes by screening about 190 purine and pyrimidine analogues as substrates for the transporter.[12] The recognition motif for the transporter has been elucidated (Figure 1) and is found on numerous reagents including aromatic amidines and amino substituted 1,3,5-triazines as well as adenine and adenosine.[13,14] 1,3,5-Triazines are generally not recognized by mammalian nucleoside transporters and should therefore offer excellent selectivity between host cells and parasites.[7c,14] Furthermore, a 2,4-diamino-1,3,5-triazine derivative was recently reported as a new lead against trypanosomiasis.[15] Since the 1,3,5-triazine derivatives are selectively taken up by *T. brucei*, it should be possible to selectively transport or target cytotoxic agents to the parasite. In the work described here, polyamines were selected as the cytotoxic agent.

Polyamines have a variety of essential roles within the cell where they are involved in growth and differentiation.[16,17] Polyamine analogues show promise as anticancer agents, antiparasitic agents, antidiarrhoeals, anti-HIV agents, metal chelators, and as gene delivery agents. Polyamine metabolism is known to be a drug target in African trypanosomes as inhibition of the initial polyamine biosynthesis enzyme, ornithine decarboxylase, by DL-α-difluoromethylornithine (DFMO) is

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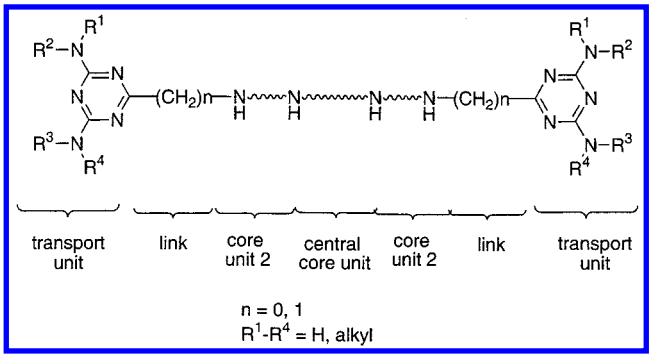


Figure 2. Target Compounds: Lead Structure A

toxic to these cells.[10,18] DFMO toxicity may be partly related to the fact that these parasites lack transporters for polyamines and thus cannot salvage sufficient quantities of polyamines from the plasma to bypass inhibition of polyamine synthesis brought about by ODC inhibition. Furthermore, since the unique thiol trypanothione, a spermidine bridged bis-glutathione, is crucial in the parasite's defense against oxidative stress and redox maintenance, polyamines have an additional vital role in trypanosomes.[19] Polyamine transporters would represent ideal portals of entry for other polyamine analogues with potential cytotoxic activity. However, their absence from bloodstream form *T. brucei*[10] means that alternative routes to target polyamine analogues to these cells are required. The P2 amino-purine transporter offers the potential to target polyamine analogues to trypanosomes.

Therefore we aimed to design, synthesize, and evaluate substituted polyamines, carrying 1,3,5-triazine units, as potential anti-trypanosomal drugs. Preliminary results[13] indicated that this route might be successful, and lead structure A (Figure 2) was used as a starting point for two series of analogues. In a first series, the influence of structural changes of the central core unit was investigated. In a second series, the effect of additional methyl substituents on the 1,3,5-triazine was studied. Here we report the synthesis of the envisaged molecules and their structure−activity relationship with respect to their interaction with the P2 transporter and their activity against *Trypanosoma brucei* sub-species. Activity was also determined against the related parasitic organism *Trypanosoma cruzi* which does not possess a P2 transporter.

Chemistry

The synthesis of polyamines can be accomplished using very different strategies.[16,17] We used a three-step procedure (Scheme 1) to form the polyamine backbones, which were then reacted with suitable 1,3,5-triazines (Schemes 1, 2, and 3). In a first step, commercially available alkyl diamines (**1a**−**f**) were treated with 2 equiv of acrylonitrile in ethanol at room temperature to undergo a double Michael addition reaction. The desired bis-adducts **2a**−**f** were isolated in 62−92% yield after recrystallization, distillation, or column chromatography. No formation of mono Michael adducts was observed. Compounds **2a**−**f** were then BOC-protected under standard conditions using BOC-anhydride in MeOH at room temperature with TEA as additional base. In the presence of only 2 equiv of BOC-anhydride, the sterically more demanding substrates **2d**−**f** formed mixtures of mono (17%, 28%, 33%) and bis-protected products (**3d**−**f**; 78%, 68%, 64%), whereas compounds **2a**−**c** were cleanly converted into the bis-protected products **3a**, **3b**, and **3c** in 97%, 85%, and 76% yield. In a third step, the reduction of the BOC-protected nitriles **3a**−**f** completed the synthesis of the polyamine backbones (**4a**−**f**).

The catalytic hydrogenation of **3c**−**f** turned out to be much more complicated than expected.[20] However, the reduction was finally achieved by catalytic hydrogenation over palladium activated Raney-nickel in dioxane/water with LiOH as base, and the polyamines **4a**−**f** were isolated in 84−94% yield.

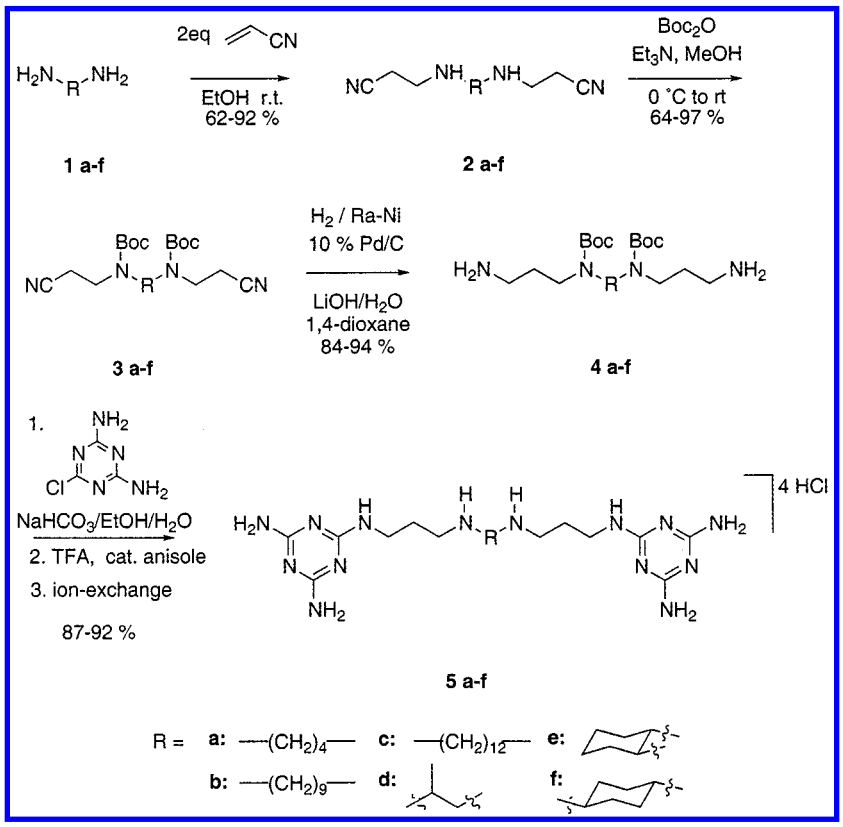
To obtain the first set of compounds (**5a**−**f**), carrying only free amino groups on the triazine units, polyamines **4a**−**f** were arylated with 2-chloro-4,6-diamino-1,3,5-triazine in ethanol/water (1:1) at 80 °C overnight. In the absence of water, virtually no reaction took place; also, using polar aprotic solvents (DMSO, DMF, MeCN) the reaction was found to be impractically slow. Increasing the reaction temperature above 80 °C resulted in partial BOC-deprotection, which left the uncertainty of regioselectivity during further arylation. Monitoring the reaction by mass spectroscopy indicated complete conversion of the starting material (**4a**−**f**) when 2.2 equiv of arylating agent were used; 2-hydroxy-4,6-diamino-1,3,5-triazine was detected as a byproduct (MS). The crude arylated, BOC-protected intermediates were directly deprotected using TFA at room temperature with anisole as *tert*-butyl cation scavenger. Purification was carried out by ion-exchange chromatography on the strongly acidic resin Dowex 50WX2-200, using a rough gradient of aqueous HCl-solution as eluent. The target compounds **5a**−**f** were isolated as tetrahydrochloric salts in 87−92% yield after recrystallization from ethanol/ether.

Finding compound **5c** to be the most active within the first set (compare Table 1), we further investigated the influence of differently substituted 1,3,5-triazine units in the central core C12-series. For comparison, analogues of the central core C9-series were also prepared (Scheme 2).

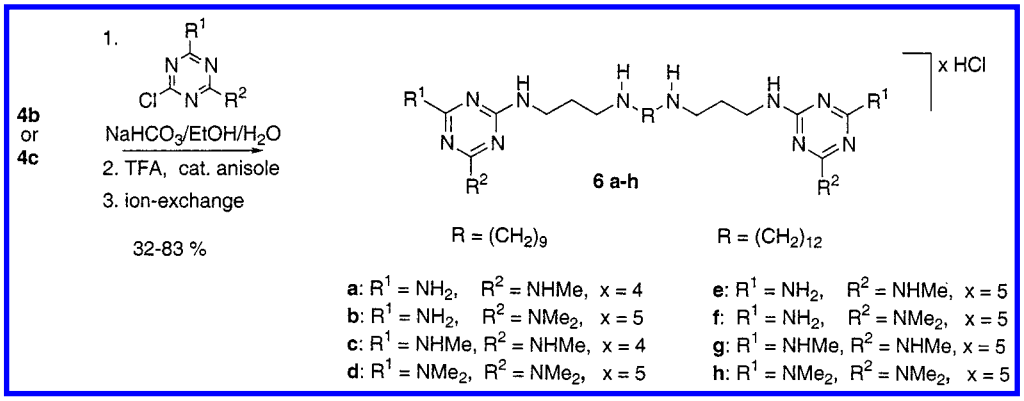
Using the same strategy and similar reaction conditions as for the preparation of **5a**−**f**, we arylated polyamines **4b** and **4c** with four different methylamino substituted 2-chloro-1,3,5-triazines. The arylating agents were prepared in one or two steps from cyanuric chloride by slightly modified literature procedures.[21] They turned out to be significantly less reactive than the unsubstituted 2-chloro-4,6-diamino-1,3,5-triazine, and use of up to 4 equiv of arylating agent was necessary to complete the reaction. After BOC-deprotection, ion-exchange chromatography, and recrystallization (as above), the methylamino substituted target compounds **6a**−**h** were isolated as tetra- or pentahydrochloric salts in 32−83% yield.

Utilizing the comparably low reactivity of the arylating agents used for the preparation of the bistriazine substituted compounds **6a**−**h**, we further investigated the synthesis of monotriazine substituted analogues (**7a**−**e**) without the use of additional protecting group chemistry (Scheme 3). When polyamines **4b** and **4c** were reacted with 2 equiv of a dialkylamino substituted 2-chloro-1,3,5-triazine, MS analysis indicated the highly

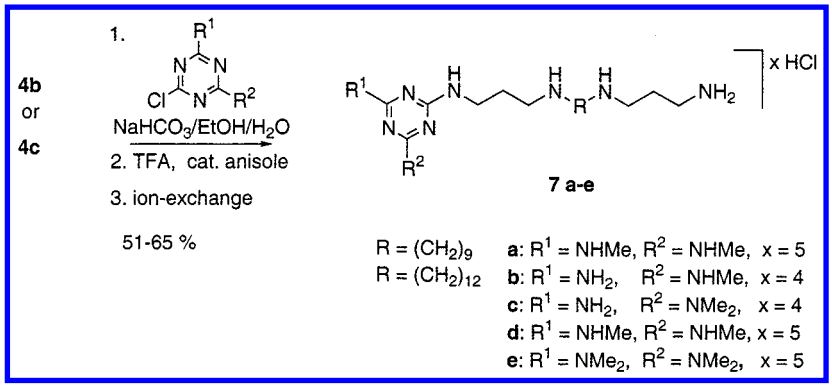
Scheme 1. Variation of Core Units



Scheme 2. Variation of Triazine Units within the C9 and C12 Series, Bis Substitution

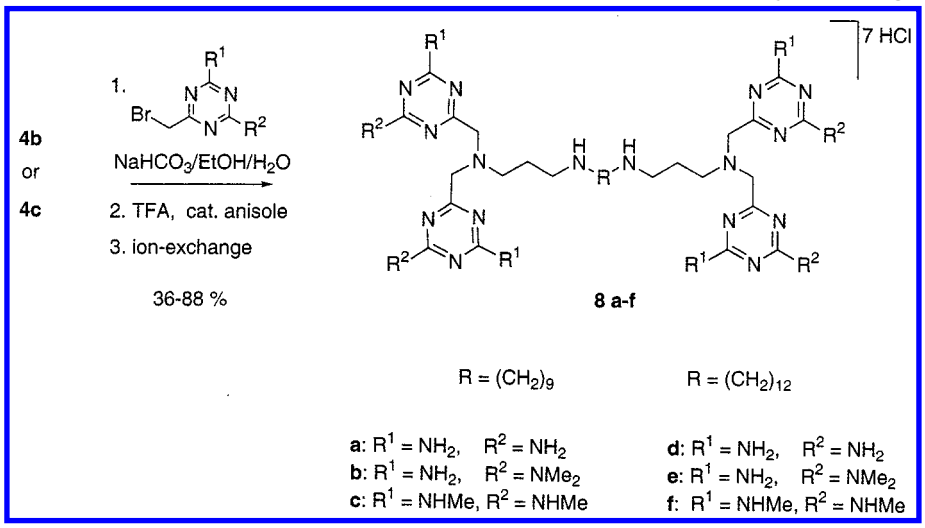


Scheme 3. Variation of Triazine Units within the C9 and C12 Series, Mono Substitution



selective formation of monoadducts within 15−20 h. After BOC-deprotection, ion-exchange chromatography, and recrystallization (as above), the monosubstituted target compounds **7a**, **7d**, and **7e** were isolated as pentahydrochloric salts in 51−65% yield. Analogue reactions with 2-chloro-1,3,5-triazines carrying at least one free NH_2 group were much more difficult to control, and the formation of bis substituted byproducts could not be avoided. After BOC-deprotection, ion-exchange chromatography, and recrystallization (as above) the

Scheme 4. Variation of Triazine Units within the C9 and C12 Series, Additional Methylene Bridge



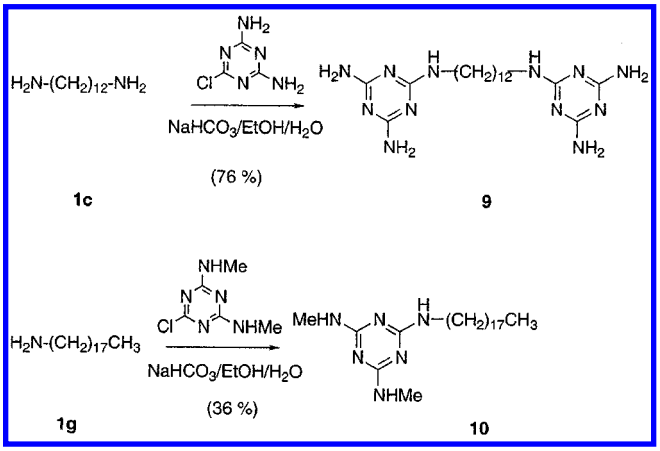
monosubstituted compounds **7b** and **7c** were isolated as tetrahydrochloric salts in a 85:15 and 90:10 ratio (^{1}H NMR) together with their bis substituted analogues **6e** and **6f**.

While in compounds **5a**−**f**, **6a**−**h**, and **7a**−**e** the 1,3,5-triazine units are directly linked to the polyamine backbone, we were also interested in attaching the triazine units via an additional methylene spacer. We reacted polyamines **4b** and **4c** with aminosubstituted 2-bromomethyl-1,3,5-triazines (Scheme 4), which were prepared from sodium dicyanamide or dicyandiamide via substituted biguanides adapting known literature procedures.[22] The stoichiometric reaction between the amino substituted 2-bromomethyl-1,3,5-triazines and polyamines **4b** and **4c** led to mixtures of mono, bis, tris, and tetra alkylated products with bis and tetra adducts dominating. By addition of 4−5 equiv of alkylating agent, the reaction proceeded to the BOC-protected tetra addition adducts. Bis and tris adducts were found only as minor byproducts according to MS analysis. Use of a larger excess of alkylating agent led to the formation of penta- and hexasubstituted ammonium salts. After BOC-deprotection, ion-exchange chromatography, and recrystallization (as above), the aminosubstituted methylene-bridged target compounds **8a**−**f** were isolated as heptahydrochloric salts in 36−88% yield.

Two nonpolyamine substituted triazines (**9** and **10**) were prepared as additional controls to ascertain the necessity of the polyamine functionality (Scheme 5). Starting from 1,12-diaminododecane (**1c**) and 1-aminooctadecane (**1g**), reaction with 2 equiv of an aminosubstiuted 2-chloro-1,3,5-triazine afforded compounds **9** and **10** in 76% and 36% yield after crystallization from ethanol/water (**9**) or column chromatography (**10**).

All synthesized intermediates (**2a**−**f**, **3a**−**f**, **4a**−**f**) and final products (**5a**−**f**, **6a**−**h**, **7a**−**e**, **8a**−**f**, **9**, **10**) were characterized by their ^{1}H NMR, ^{13}C NMR, and mass spectra. Electrospray MS analysis of the target compounds (hydrochloric salts) displayed the M+H$^+$ peak of the free base, and HRMS was performed as a proof of identity. Water and solvent residues were strongly retained by the compounds, which was reflected in the combustion analysis. The microanalytical data was used to determine the number of HCl units per molecule via the N:Cl ratio. Dependent on their pK_a values and the pH of the surrounding solvent, the polyamine targets may exist in different protonated forms.

Scheme 5. Non-Polyamine Analogues



Biological Data

All compounds were assayed for their ability to inhibit adenosine uptake by the P2 transporter which gives an indication of the affinity between the transporter and these molecules (Table 1). In vitro toxicity against intact bloodstream form trypomastigotes (the clinically relevant stage) of *Trypanosoma brucei brucei* and *T. b. rhodesiense* was also measured. All compounds were also assayed for their activity against the related parasite *T. cruzi* (amastigotes), which is the causative agent for Chagas' disease. Since these parasites are intracellular, reagents cannot reach their plasma membranes without first traversing the membrane of the host cells in which they reside, thus precluding the possibility of selective activity through selective uptake.

Interaction with the P2 Transporter in *T. brucei*. All compounds showed dose dependent inhibition of adenosine uptake via the P2 transporter. Most target compounds displayed a moderate affinity toward the P2 transporter with apparent K_i values in the range of 3−70 μM. Compounds **5d** and **5f** were an exception, showing only weak P2 transporter affinity with apparent K_i = 163 μM and 125 μM. Compounds **5c**, **6a**, **6e**,

Table 1. Activities of Compounds

compd	P2 affinity K_i (μM)	*T. b. rhodesiense* IC_{50} (μM)	*T. b. brucei* IC_{50} (μM)	*T. cruzi* IC_{50} (μM)	L-6 rat myoblast cells IC_{50} (μM)
5a (4)[a]	72	160	>100	>160	353
5b (4)	19	128	>100	>140	nd
5c (4)	0.50	16	>100	>13	295
5d (4)	163	>163	>100	>163	362
5e (4)	55	>152	>100	>152	338
5f (4)	125	>152	>100	>152	338
6a (4)	0.75	16	53	>135	285
6b (5)	10	9.6	40	>123	274
6c (4)	14	17	4.0	>130	289
6d (5)	29	1.2	32	16	37.7
6e (5)	0.135	9.8	2.0	116	nd
6f (5)	0.24	3.5	>100	9.7	50.9
6g (5)	3.2	2.3	17	18	143
6h (5)	2.5	0.81	1.7	0.80	6.8
7a (5)	17	0.94	84	23	135.2
7b (4)	0.41	1.1	>200	>154	127
7c (4)	1.0	0.94	20	32	39.9
7d (5)	25	0.58	20	31	57.7
7e (5)	0.6	1.3	14.7	0.71	14.2
8a (7)	82	4.02	32	>88	220
8b (7)	17	0.265	3.0	54.8	177
8c (7)	30	0.265	0.1	76.8	177
8d (7)	6.7	0.57	21	>85	188
8e (7)	4.6	0.44	2.0	>77	105
8f (7)	2.4	0.18	2.9	>77	76.6
9[b] (0)		13		6.0	239
10[b] (0)	-	14		12	48.2

[a] Number of HCl units used in the calculation. [b] Poor solubility. Controls: *T. b. rhodesiense*, melarsoprol IC_{50} = 6.1 nM; *T. cruzi*, benznidazole, IC_{50} = 3.3 μM; *T. b. rhodesiense*, strain STIB 900; *T. b. brucei*, strain 427; *T. cruzi*, strain Tulahuen C4.

6f, **7b**, and **7e** exhibited an exceptionally high affinity with apparent K_i values significantly below 1 μM. Changes in the central core unit and on the triazine rings were reflected by changing K_i values. In unmethylated compounds (**5a**−**f**), an increase in central chain length correlated with increased apparent affinity for the transporter. The introduction of methylamino and dimethylamino substituents on the triazine units was well tolerated, although increasing the extent of methylation on the terminal triazine units leads to a diminuition of apparent affinity.

Thus for the nonyl series (**5b** and **6a**−**d**), monomethylation (**6a**) actually caused a 25-fold increase in affinity compared to **5b**. However, subsequent methylations reduced affinity. A similar trend was observed with the dodecyl series (**5c** and **6e**−**h**). The tetra substituted compounds with the extra methylene bridge (**8**) all showed lower affinity than the corresponding disubstituted compounds (**8a/5b**; **8b/6b**; **8c/6c**; **8d/5c**; **8e/6f**) except **8f/6g** which showed roughly similar affinity. Monosubstitution as compared to disubstitution (**7a**−**e**) led to no effect or loss of affinity for the P2 transporter (compare **7a/6c**; **7b/6e**; **7c/6f**; **7d/6g**) in all cases except one, the fully methylated (**7e/6h**).

Trypanocidal Activity. All compounds, except those of the first series (**5a**−**f**), displayed dose dependent in vitro activities against *T. b. rhodesiense* strain STIB 900 with IC_{50} values ranging from 0.18 μM to 17 μM (Table 1). In the first series (**5a**−**f**), where the central core unit has been varied, **5c**, containing the *n*-dodecyl chain as core unit, showed weak activity (IC_{50} = 16 μM) against *T. b. rhodesiense*. The next most promising compound was the *n*-nonyl compound **5b**. Starting from **5b** (*n*-nonyl chain), analogues were designed by replacing NH_2 groups on the triazine ring with NHMe and NMe_2 groups. Introduction of one (**6a**) or two (**6b** and **6c**) methyl groups per triazine unit resulted in an 10-fold increase in anti-trypanosomal activity. When four methyl groups per triazine unit were introduced (**6d**), an 80-fold increase in activity compared with **5b** was observed. Similarly, NH_2 group replacement starting from **5c** (*n*-dodecyl chain) led to 2−20-fold higher anti-trypanosomal activity for the methylated derivatives **6e**−**h** compared with **5c**.

Monosubstituted compounds showed a slight increase in activity against *T. b. rhodesiense* compared to the disubstituted compounds (**7a/6c**; **7b/6e**; **7c/6f**; **7d/6g**) except for the fully methylated derivative (**7e/6h**) which showed little effect.

Assays, carried out to access in vitro activity against *T. b. brucei* strain 427 displayed similar correlations, although in general *T. b. rhodesiense* strain STIB 900 was more sensitive to the active compounds. Strain and subspecies variability in response to most drugs is a usual feature of trypanosomes. This could be due to any of a number of biochemical differences, including differences in transporter activity responsible for accumulating drug, differences in levels or biophysical properties of drug targets, or other changes in the metabolic milieu which may alter the impact of compounds on viability. We are not currently in a position to identify which features distinguish the parasites with respect to their sensitivity to compounds in the studies reported here.

The most active trypanocidal compounds were obtained when methylamino substituted triazines were attached to the C9- or C12-polyamine precursor via an additional CH_2 linker (**8a**−**f**). Compound **8f** showed especially good activity against *T. b. rhodesiense* with an IC_{50} value of 0.18 μM and good parasite/host selec-

tivity. Other analogues in the same series (**8b**−**e**) showed similarly high activity and good parasite/host selectivity. One analogue carrying methylene-bridged triazine units without any methylamino substituents (**8a**) was significantly less active than **8f**. Comparable results were found for the activities against *T. b. brucei*. The introduction of additional CH_2 linkers between the polyamine backbone and the triazines increased the anti-trypanosomal activity up to 1000-fold. Target compound **8c** was identified as the most active against *T. b. brucei* with an IC_{50} value of 0.1 μM, making it a very suitable candidate for in vivo testing.

Compounds **9** and **10** were prepared to establish the necessity of the polyamine chain. However, these compounds showed poor solubility in aqueous solution, meaning the data for these compounds presented in Table 1 is very approximate. Therefore, while it is unknown if the central amino groups are essential for activity against the molecular target, they are essential for the solubility properties of the compounds.

In Vivo Activity. Compounds **6e**, **8b**, **8c**, and **8e** were tested for toxicity and anti-trypanosomal activity in mice. However, 1 mg kg^{-1} intraperitoneal injection into mice was not curative, and concentrations greater than 10 mg kg^{-1} induced severe acute toxicity.

T. cruzi. *T. cruzi* is phylogenetically related to *T. brucei* and causes Chagas' disease. Both pathogens share a number of biochemical features including the presence of trypanothione. However, there are also key differences between these two organisms at the level of host-pathogen interaction. Critically, *T. brucei* is a parasite which lives free in the bloodstream and cerebrospinal fluid of the host, while *T. cruzi* amastigotes replicate in the cytosol of a number of mammalian cell types. To act against intracellular parasites, drugs must cross host cell membranes too, thus diminishing the prospects of selective toxicity through selective uptake. In general, the compounds were less toxic for *T. cruzi* than *T. brucei*, and the most active ones (**6h** and **7e**) were also those which were among the most cytotoxic for mammalian cells, indicating that these products may enter cells via different routes and induce toxicity within multiple cell types.

Discussion

The P2 amino-purine transporter has been shown to carry a number of compounds into trypanosomes. The structure−activity relationship between this transporter and its substrates has been determined,[7,13,14] and it is possible to generate novel trypanocidal compounds bearing this recognition motif. Addition of the motif to cytotoxic moieties could endow them with the ability to be selectively accumulated and thus selectively toxic to trypanosomes. Polyamine metabolism has been shown to be a good chemotherapeutic target in *T. brucei*. However, these cells lack transporters for polyamines which means that this logical route of targeting polyamine analogues to these cells is not available. Therefore we have developed polyamine analogues bearing groups (1,3,5-triazines) enabling recognition by the P2 amino-purine transporter to see if novel, trypanocidal polyamine analogues could be produced.

The triazine substituted polyamines **5a**−**f**, **6a**−**h**, **7a**−**e**, and **8a**−**f** have been shown to interact with the P2 tranpsorter, based on their ability to inhibit the uptake of radiolabeled adenosine by this route. It is not possible, using this assay, to determine whether these molecules are actually internalized via this route, only that they interact with the transporter. Radiolabeled or other traceable derivatives of the compounds would be required to verify whether they are internalized via this route. Notwithstanding, several compounds proved highly active against both *T. brucei rhodesiense* and *T. brucei brucei* in vitro, although host toxicity precluded full evaluation of these compounds in vivo.

The compounds described here were designed to determine preliminary structure activity relationships. Activities against *T. b. brucei* and *T. b. rhodesiense* (see IC_{50} values in Table 1) varied little when the central core unit was modified (**5a**−**f**) with the exception of **5c**. An increase in activity was found, however, when methylamino or dimethylamino substituted triazine units were used (**6a**−**h**). Analogues of **6a**−**h** carrying only one instead of two triazine units (**7a**−**e**) showed slightly higher activity. A further increase in activity was observed when the triazine units were attached via an additional methylene spacer (**8a**−**f**). These results demonstrate that the triazine moieties play a crucial role in the mode of action of this family of compounds. Currently we cannot explain why the addition of methylene spacers enhances trypanocidal activity. Polyamines with a straight long alkyl chain as central core unit seem to be beneficial. Attempts to assay the substituted 2-chloro- and 2-bromomethyl-1,3,5-triazines themselves for their antitrypanosomal activity failed because of their insolubility in aqueous solution.

There is no obvious correlation between the P2 transporter affinity and the anti-trypanosomal activity of the compounds, leading to the conclusion that uptake through the P2 transporter is not the rate-limiting step in toxicity. We cannot exclude the possibility that these products actually enter cells via routes other than the P2 transporter. In the absence of information about the specific targets of the compounds, nothing can be stated about the altered toxic activity of the products. Other factors apart from the uptake, such as metabolic breakdown and interactions with intracellular targets, are likely to be critical in determining toxicity.

The actual mode of action for the reported triazine substituted polyamines also remains unclear. Kukoamine A,[23] spermine and spermidine derivatives,[24] glutathionyl spermidine phosphinates (GSP),[25] and other polyamine analogues[26] have all been shown to be inhibitors of trypanothione biosynthesis, and it might be speculated that our compounds act in a similar manner against *T. brucei*. However, other modes of action, also including the displacement of polyamines from their normal physiological sites of action or interactions involving metabolites of the triazine substituted polyamines, may also play an important role. To understand and improve the activities of our compounds, further research has to verify intracellular drug targets and possible metabolic pathways.

Conclusion

We have reported the design and synthesis of triazine substituted polyamines as drugs against *T. brucei spp.*,

the causative agent for African sleeping sickness. Four different types of test compounds (**5a**−**f**, **6a**−**h**, **7a**−**e,** and **8a**−**f**) were prepared using a five-step synthesis consisting of Michael addition reaction, BOC-protection, hydrogenation, arylation, and BOC-deprotection. Furthermore, two non-polyamine analogues (**9** and **10**) were prepared by arylation of commercially available amines. The compounds were designed with the intention of selectively targeting to the interior of *T. brucei* via the P2 amino-purine transporter. All molecules were assayed for their affinity for this transporter, and their activity against three different parasitic protozoa (*T. b. rhodesiense*, *T. b. brucei*, and *T. cruzi*). Good activities were found in several cases. Especially the tetra triazine substituted polyamine **8c** showed an excellent toxicity profile with high activity against *T. b. rhodesiense* (IC_{50} = 0.27 μM) and *T. b. brucei* (IC_{50} = 0.10 μM) and a low cytotoxicity against rat L-6 cells (MIC = 177 μM).

Experimental Section

P2 Transporter Assay.[27] A total of 100 μL of *T. b. brucei* 427 cells at 2 × 10^8/mL was added to a buffer containing radiolabeled adenosine and varying concentrations of potential inhibitor. The buffer also contained 1 mM inosine, to block adenosine uptake by the P1 transporter. After a 10 s incubation at room temperature, the cells were spun through a layer of oil (1-Bromododecane, 97%) to form a pellet, and the entire reaction tube was flash frozen in liquid nitrogen. The oil layer containing the pellet was cut off into a scintillation vial, and the radioactivity present was measured by scintillation counter. The apparent K_i value was calculated using the competitive inhibition equation from the Grafit 4.0 software. Each experiment was done in duplicate.

In Vitro Biological Activity. *T. b. brucei*. Serial dilutions of the test compounds from 200 μM were set out in a 96-well plate in duplicate. A total of 100 μL of cells (*T. b. brucei* 427) at 2 × 10^5/ml was added to each well, leaving an initial dilution of 100 μM and 2000 cells per well. After 48 h incubation at 37 °C, Alamar Blue (20 μL)[28] was added to each well, and the plates incubated for a further 24 h. Live cells reduce the Alamar Blue to a pink color by unspecified dehydrogenases, and a concomitant change in absorption at 600 nm can be measured spectrophotometrically. The IC_{50} value was calculated from the raw data using the Grafit 4.0 software. Values were checked by visually examining cell viability and motility in wells.

***T. b. rhodesiense*.** Minimum Essential Medium (50 μL) supplemented with 2-mercaptoethanol and 15% heat-inactivated horse serum[29] was added to each well of a 96-well microtiter plate. Serial drug dilutions were added to the wells. Then 50 μL of trypanosome suspension (*T. b. rhodesiense* STIB 900) was added to each well (final trypanosome concentration 2 × 10^4/mL), and the plate incubated at 37 °C under a 5% CO_2 atmosphere for 72 h. Alamar Blue (10 μL)[28] was then added to each well, and incubation continued for a further 2−4 h. The plate was then read with a Millipore Cytofluor 2300 using an excitation wavelength of 530 nm and emission wavelength of 590 nm. Fluorescence development was expressed as percentage of the control, and IC_{50} values determined.

***T. cruzi*.** Rat skeletal myoblasts (L-6 cells) were seeded in 96-well microtiter plates at 2000 cells/well/100μL in RPMI 1640 medium with 10% FBS and 2 mM L-glutamine. After 24 h, 5000 trypomastigotes of *T. cruzi* (Tulahuen strain C2C4 containing the galactosidase (Lac Z gene)) were added in 100 μL per well with 2× a serial drug dilution. The plates were incubated at 37 °C in 5% CO_2 for 4 days. After 96 h, the minimum inhibitory concentration (MIC) was determined microscopically. For measurement of the IC_{50}, the substrate CPRG/Nonidet was added to the wells. The color reaction which developed during the following 2−4 h was read photometrically at 540 nm. From the sigmoidal inhibition curve, IC_{50} values were calculated. Cytotoxicity was assessed in the same assay using noninfected L-6 cells, and the same serial drug dilution. The MIC was determined microscopically after 4 days.

In Vivo Biological Activity. On day one of the trials groups of five mice were infected with 2 × 10^5 *T. b. brucei* strain 427 by intraperitoneal injection. On days three and four after injection, the groups of mice were treated by intraperitoneal injection with either the test compounds or diminazene aceturate (as a positive control to clear infection). A negative control group received no treatment. The parasitaemia of all mice was checked daily by tail prick examination, and sick animals were euthanized.

Chemistry. General. Purification by column chromatography was performed on Sorbosil C60A silica gel 40−60 μm from Merck. Qualitative thin-layer chromatography (TLC) was done on precoated aluminum sheets silica gel 60 F_{254} from Merck. Compounds were detected either with iodine or 254 nm UV light. All given R_f values refer to the purified material and were obtained under chamber saturation with the applied solvent mixture. Purification by ion exchange chromatography was performed on a Dowex 50WX2-200 resign using an HCl gradient (50 mL of 0 N, 400 mL of 2 N, 300 mL of 5 N in 1:1 H_2O/*i*-PrOH). The progress of elution was monitored by UV absorbance at 240 nm. UV spectra were recorded at a Helios α UV/VIS spectral photometer from Unicam. Melting points were determined with a Gallenkamp melting point apparatus and are not corrected. ^{1}H and ^{13}C NMR data were obtained on a Bruker 300 MHz NMR spectrometer using the applied solvent simultaneously as internal standard. Mass spectra were recorded at a Platform II mass spectrometer (Micromass) from Fisons. Ionization was achieved in the positive electrospray mode using a 1:1 solvent mixture of acetonitrile and water as mobile phase. High-resolution mass spectra were recorded by the National Mass Spectrometry Service Centre in Swansea at a MAT 900 XLT high resolution double focusing mass spectrometer from Finnigan using the same ionization procedure. Accurate mass measurement was performed by peak matching. Combustion analyses were performed by the analytical and chemical consultancy services MEDAC Ltd. Solvents and reagents were used as purchased unless otherwise stated. All yields quoted in this paper were isolated yields. Compounds **2a**,[30,31] **2b**,[32] **2c**,[31] and **3a**[33] have been reported in the literature earlier. The synthesis of **4a**−**f** has been reported separately.[20]

Michael Addition Reaction, General Procedure. A total of 1 equiv of diamine (1 M solution in absolute EtOH) was cooled in an ice bath, and 2 equiv of acrylonitrile (1 M solution in absolute EtOH) was added dropwise at 0 °C. The nearly colorless solution was allowed to warm to room temperature and stirred at room temperature for additional 20 h. After the solvent was removed at reduced pressure, the obtained residue was purified by column chromatography on SiO_2 using 15% MeOH in $CHCl_3$ as eluent, by high vacuum distillation, or by crystallization.

3-(4-[(2-Cyanoethyl)amino]butylamino)propanenitrile (2a). Starting from 1,4-diaminobutane (**1a**, 500 mg, 5.67 mmol) and acrylonitrile (600 mg, 11.3 mmol), **2a** was obtained as pale yellow oil (864 mg, 78%): R_f (SiO_2, 15% MeOH in $CHCl_3$) 0.36; ^{1}H NMR ($CDCl_3$) δ/ppm = 2.93 (t, ^3J = 6.7 Hz), 2.66 (m, 4H), 2.54 (t, ^3J = 6.7 Hz, 4H), 1.68 (m, 2H, NH), 1.54 (m, 4H); ^{13}C NMR ($CDCl_3$) δ/ppm = 119.2 (s), 49.3 (t), 45.4 (t), 28.0 (t), 19.1 (t); MS (ES^+) m/z = 217 ($M+Na^+$, 10%), 195 ($M+H^+$, 100%).

3-(9-[(2-Cyanoethyl)amino]nonylamino)propanenitrile (2b). Starting from 1,9-diaminononane (**1b**, 500 mg, 3.16 mmol) and acrylonitrile (335 mg, 6.31 mmol), **2b** was obtained as pale yellow oil (770 mg, 92%): R_f (SiO_2, 15% MeOH in $CHCl_3$) 0.47; ^{1}H NMR ($CDCl_3$) δ/ppm = 2.96 (t, ^3J = 6.6 Hz, 4H), 2.65 (t, ^3J = 7.1 Hz, 4H), 2.56 (t, ^3J = 6.6 Hz, 4H), 1.51 (m, 4H), 1.39 (bs, 2H, NH), 1.37−1.31 (m, 10H); ^{13}C NMR ($CDCl_3$) δ/ppm = 119.2 (s), 49.6 (t), 45.5 (t), 30.4 (t), 29.9 (t),

29.8 (t), 27.6 (t), 19.1 (t); MS (ES^+) m/z = 287 ($M+Na^+$, 8%), 265 ($M+H^+$, 100%).

3-(12-[(2-Cyanoethyl)amino]dodecylamino)propanenitrile (2c). Starting from 1,12-diaminododecane (**1c**, 500 mg, 2.5 mmol) and acrylonitrile (264 mg, 4.98 mmol), **2c** was obtained as pale yellow oil, which solidified on standing (650 mg, 85%): R_f (SiO_2, 15% MeOH in $CHCl_3$) 0.44; mp 55−56 °C; ^1H NMR ($CDCl_3$) δ/ppm = 2.96 (t, J = 6.6 Hz, 4H), 2.66 (t, J = 7.2 Hz, 4H), 2.56 (t, J = 6.6 Hz, 4H), 1.52 (m, 4H), 1.34 (m, 2H, NH), 1.41−1.26 (m, 16H); ^{13}C NMR ($CDCl_3$) δ/ppm = 119.2 (s), 49.7 (t), 45.5 (t), 30.4 (t), 30.0 (t), 29.9 (t), 27.6 (t), 19.1 (t); MS (ES^+) m/z = 307 ($M+H^+$, 100%)

3-(2-[(2-Cyanoethyl)amino]-1-methylethylamino)propanenitrile (2d). Starting from (±)-1,2-diaminopropane (**1d**, 20 g, 270 mmol) and acrylonitrile (28.6 g, 35.5 mL, 540 mmol), **2d** was obtained as light yellow oil after high vacuum distillation (40.0 g, 82%): R_f (SiO_2, 15% MeOH in $CHCl_3$) 0.32; bp 162−168 °C/0.005 Torr; ^1H NMR ($CDCl_3$) δ/ppm = 3.05−2.82 (m, 4H), 2.97−2.65 (m, 2H), 2.55−2.41 (m, 5H), 1.61 (bs, 2H, NH), 1.06 (d, ^3J = 6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ/ppm = 119.4 (s), 119.3 (s), 55.1 (t), 52.6 (d), 45.5 (t), 43.0 (t), 19.6 (t), 19.3 (t), 19.0 (q); MS (ES^+) m/z = 203 ($M+Na^+$, 15%), 181 ($M+H^+$, 100%)

3-(2-[(2-Cyanoethyl)amino]cyclohexylamino)propanenitrile (2e). Starting from *trans*-1,2-diaminocyclohexane (**1e**, 10.0 g, 87.5 mmol) and acrylonitrile (9.3 g, 11.5 mL, 175 mmol), **2e** was obtained as light yellow viscous oil after high vacuum distillation (12.0 g, 62%): R_f (SiO_2, 15% MeOH in $CHCl_3$) 0.42; bp 180 °C/0.09 Torr; mp 74−75 °C; ^1H NMR ($CDCl_3$) δ/ppm = 3.04/3.02/2.81/2.77 (4t, ^3J = 6.78, 6.78, 6.34, 6.44 Hz, 4H), 2.49 (t, ^3J = 6.78 Hz, 4H), 2.15 (m, 2H), 2.04 (m, 2H), 1.81 (s, 2H, NH), 1.72 (m, 2H), 1.22 (m, 2H), 1.02 (m, 2H); ^{13}C NMR ($CDCl_3$) δ/ppm = 119.4 (s), 61.4 (d), 42.8 (t), 32.1 (t), 25.2 (t), 19.7 (t); MS (ES^+) m/z = 221 ($M+H^+$, 100%).

3-(4-[(2-Cyanoethyl)amino]cyclohexylamino)propanenitrile (2f). Starting from *trans*-1,4-diaminocyclohexane (**1f**, 20 g, 175 mmol) and acrylonitrile (18.6 g, 23 mL, 350 mmol), **2f** was obtained as off-white microcrystalline powder after recrystallization from EtOH (31.8 g, 82%): R_f (SiO_2, 15% MeOH in $CHCl_3$) 0.16; mp 115−116 °C; ^1H NMR ($CDCl_3$) δ/ppm = 2.89 (td, ^3J = 6.59, 4.52 Hz, 4H), 2.49−2.37 (m, 2H), 2.47 (t, ^3J = 6.59 Hz, 4H), 1.90 (m, 4H), 1.19 (m, 2H, NH), 1.16−1.05 (m, 4H); ^{13}C NMR ($CDCl_3$) δ/ppm = 116.8 (s), 53.9 (d), 40.4 (t), 29.9 (t), 17.3 (t); MS (ES^+) m/z = 221 ($M+H^+$, 50%), 151 (100%)

Boc-Protection, General Procedure. A solution of 1 equiv of Michael adduct (0.25 M in MeOH) was cooled to 0 °C, and 2 equiv of triethylamine followed by 2 equiv of di-*tert*-butyl dicarbonate (0.5 M in MeOH) were added. The mixture was stirred at room temperature for 24 h, the solvent was removed under reduced pressure, and the residue was purified by column chromatography on SiO_2 using 5% MeOH in CH_2Cl_2 as eluent.

***N*⁴,*N*⁹-Di(*tert*-butyloxycarbonyl)-3-(4-[(2-cyanoethyl)amino]butylamino)propanenitrile (3a).** Starting from **2a** (864 mg, 4.45 mmol), triethylamine (990 mg, 9.78 mmol), and di-*tert*-butyl dicarbonate (2.14 g, 9.81 mmol), **3a** was obtained as colorless oil, which crystallized on standing at room temperature (1.70 g, 97%): R_f (SiO_2, 5% MeOH in CH_2Cl_2) 0.57; mp 102−104 °C; ^1H NMR ($CDCl_3$) δ/ppm = 3.47 (t, 4H, ^3J = 6.68 Hz), 3.30 (m, 4H), 2.70−2.53 (m, 4H), 1.53 (m, 4H), 1.48 (s, 18H); ^{13}C NMR ($CDCl_3$) δ/ppm = 155.6 (s), 155.2 (s), 118.8 (s), 118.3 (s), 80.9 (s), 48.7 (t), 47.6 (t), 44.4 (t), 43.9 (t), 28.8 (q), 26.4 (t), 25.9 (t), 18.0 (t), 17.4 (t); MS (ES^+) m/z = 433 ($M+K^+$, 20%), 417 ($M+Na^+$, 100%), 412 ($M+NH_4^+$, 30%).

***N*⁴,*N*¹⁴-Di(*tert*-butyloxycarbonyl)-3-(9-[(2-cyanoethyl)amino]nonylamino)propanenitrile (3b).** Starting from **2b** (770 mg, 2.91 mmol), triethylamine (645 mg, 6.37 mmol), and di-*tert*-butyl dicarbonate (1.40 g, 6.41 mmol), **3b** was obtained as colorless oil (1.15 g, 85%): R_f (SiO_2, 5% MeOH in CH_2Cl_2) 0.63; ^1H NMR ($CDCl_3$) δ/ppm = 3.46 (t, 4H, ^3J = 6.77 Hz), 3.25 (t, 4H, ^3J = 7.59 Hz), 2.68−2.52 (m, 4H), 1.58−1.47 (m, 4H), 1.47 (s, 18H), 1.29 (m, 10H); ^{13}C NMR ($CDCl_3$) δ/ppm = 155.8 (s), 155.1 (s), 118.4 (s), 118.9 (s), 80.8 (s), 80.6 (s), 49.0 (t), 48.1 (t), 44.2 (t), 43.8 (t), 29.9 (t), 29.6 (t), 29.1 (t), 28.8 (q), 27.1 (t), 17.9 (t), 17.4 (t); MS (ES^+) m/z = 487 ($M+Na^+$, 80%), 482 ($M+NH_4^+$, 100%), 465 ($M+H^+$, 10%).

***N*⁴,*N*¹⁷-Di(*tert*-butyloxycarbonyl)-3-(12-[(2-cyanoethyl)amino]dodecylamino)propanenitrile (3c).** Starting from **2c** (650 mg, 2.12 mmol), triethylamine (470 mg, 4.64 mmol), and di-*tert*-butyl dicarbonate (1.02 g, 4.67 mmol), **3c** was obtained as colorless oil (820 mg, 76%): R_f (SiO_2, 5% MeOH in CH_2Cl_2) 0.61; ^1H NMR ($CDCl_3$) δ/ppm = 3.46 (t, 4H, ^3J = 6.68), 3.24 (t, 4H, ^3J = 7.59 Hz), 2.68−2.51 (m, 4H), 1.57−1.43 (m, 4H), 1.46 (s, 18H), 1.26 (m, 16H); ^{13}C NMR ($CDCl_3$) δ/ppm = 155.8 (s), 155.1 (s), 118.9 (s), 118.4 (s), 80.8 (s), 80.6 (s), 49.0 (t), 48.2 (t), 44.2 (t), 43.8 (t), 30.0 (t), 29.9 (t), 29.7 (t), 28.8 (q), 27.1 (t), 17.9 (t), 17.4 (t); MS (ES^+) m/z = 545 ($M+K^+$, 15%), 529 ($M+Na^+$, 65%), 524 ($M+NH_4^+$, 100%), 507 ($M+H^+$, 10%).

***N*⁴,*N*⁷-Di(*tert*-butyloxycarbonyl)-3-(2-[(2-cyanoethyl)amino]-1-methylethylamino)propanenitrile (3d).** Starting from **2d** (5.00 g, 27.7 mmol), triethylamine (6.16 g, 60.9 mmol), and di-*tert*-butyl dicarbonate (13.3 g, 60.9 mmol), **3d** was obtained as colorless micro crystalline powder (8.2 g, 78%): R_f (SiO_2, 5% MeOH in CH_2Cl_2) 0.66; mp 173−175 °C; ^1H NMR ($CDCl_3$) δ/ppm = 4.24/4.03 (2m, br, 1H), 3.72−3.33 (m, 4H), 3.33−3.04 (m, 2H), 2.65 (m, 2H), 2.57 (m, 2H), 1.49 (s, 18H), 1.23 (d, 3H, ^3J = 6.59 Hz); ^{13}C NMR ($CDCl_3$) δ/ppm = 155.4 (s), 155.2 (s), 118.8 (s), 118.3 (s), 81.6 (s), 81.4 (s), 50.2 (d), 43.9 (t), 43.9 (t),41.1 (t), 28.8 (q), 17.6 (t), 17.4 (t), 16.5 (q); MS (ES^+) m/z = 403 ($M+Na^+$, 100%), 390 ($M+NH_4^+$, 15%), 381 ($M+H^+$, 20%). A byproduct was isolated as pale yellow oil and identified as the mono protected material (1.33 g, 17%): R_f (SiO_2, 5% MeOH in CH_2Cl_2) 0.59 (tailing); ^1H NMR ($CDCl_3$) δ/ppm = 3.56−3.54 (m, 2H), 3.20 (d, 2H, ^3J = 5.67 Hz), 3.06−2.78 (m, 3H), 2.66 (m, br, 2H), 2.48 (t, 2H, ^3J = 6.31), 1.47 (s, 9H), 1.41 (s, br, 1H, NH), 1.05 (d, 3H, ^3J = 6.40 Hz); ^{13}C NMR ($CDCl_3$) δ/ppm = 155.7 (s), 119.2 (s), 118.8 (s), 81.2 (s), 54.9 (t), 54.4 (t), 53.1 (d), 52.5 (d), 45.5 (t), 43.1 (t), 28.7 (q), 19.7 (t), 19.0 (q), 17.9 (t), 17.1 (t); MS (ES^+) m/z = 561 ($2M+H^+$, 10%), 303 ($M+Na^+$, 50%), 281 ($M+H^+$, 100%), 225 ($M+2H^+$-^{t}Bu$^+$, 60%).

***N*⁴,*N*⁷-Di(*tert*-butyloxycarbonyl)-3-(2-[(2-cyanoethyl)amino]cyclohexylamino)propanenitrile (3e).** Starting from **2e** (5.00 g, 22.7 mmol), triethylamine (5.00 g, 49.4 mmol), and di-*tert*-butyl dicarbonate (10.8 g, 49.5 mmol), **3e** was obtained as colorless micro crystalline powder (6.53 g, 68%): R_f (SiO_2, 5% MeOH in CH_2Cl_2) 0.68; mp 202−204 °C; ^1H NMR ($CDCl_3$) δ/ppm = 4.12−3.98 (m, 1H), 3.86−3.67 (m, 1H), 3.65−3.35 (m, 2H), 3.29−3.05 (m, 2H), 2.74−2.38 (m, 4H), 2.03−1.89 (m, 2H), 1.89−1.75 (m, 2H), 1.57−1.23 (m, 4H), 1.50 (s, 18H); ^{13}C NMR ($CDCl_3$) δ/ppm = 155.5 (s), 118.3 (s), 81.4 (s), 55.3 (d), 39.6 (t), 31.4 (t), 28.8 (q), 25.6 (t), 19.0 (t); MS (ES^+) m/z = 443 ($M+Na^+$, 100), 438 ($M+NH_4^+$, 48%), 421 ($M+H^+$, 30%). A byproduct was isolated as pale yellow oil and identified as the mono protected material (2.06 g, 28%): R_f (SiO_2, 5% MeOH in CH_2Cl_2) 0.63; ^1H NMR ($CDCl_3$) δ/ppm = 4.15−3.96/3.75−3.65 (2m, 1H), 3.65−3.41/3.41−3.24 (2m, 2H), 3.08−2.94 (m, 1H), 2.85−2.59 (m, 3H), 2.53−2.33 (m, 2H), 2.12 (m, 1H), 1.87−1.69 (m, 3H), 1.61−0.98 (m, 6H), 1.49 (s, 9H); ^{13}C NMR ($CDCl_3$) δ/ppm = 156.0 (s), 155.5 (s), 119.3 (s), 118.9 (s), 118.3 (s), 81.4 (s), 81.2 (s), 58.4 (d), 55.3 (d), 42.3 (t), 39.6 (t), 33.1 (t), 31.4 (t), 31.0 (t), 28.8 (q), 26.0 (t), 25.6 (t), 25.2 (t), 20.0 (t), 19.0 (t); MS (ES^+) m/z = 343 ($M+Na^+$, 30%), 321 ($M+H^+$, 100%), 265 ($M+2H^+$-^{t}Bu$^+$, 12%)

***N*⁴,*N*⁹-Di(*tert*-butyloxycarbonyl)-3-(4-[(2-cyanoethyl)amino]cyclohexylamino)propanenitrile (3f).** Starting from **2f** (5.00 g, 22.7 mmol), triethylamine (5.00 g, 49.4 mmol), and di-*tert*-butyl dicarbonate (10.8 g, 49.5 mmol), **3f** was obtained as colorless oil, which crystallized on standing at room temperature (6.09 g, 64%): R_f (SiO_2, 5% MeOH in CH_2Cl_2) 0.69; mp 135−138 °C; ^1H NMR ($CDCl_3$) δ/ppm = 3.90/3.61 (2m, br, 2H), 3.38 (m, 4H), 2.60 (m, 4H), 1.94−1.78 (m, 4H), 1.56 (m, 4H), 1.48 (s, 18H); ^{13}C NMR ($CDCl_3$) δ/ppm = 155.1 (s), 118.3 (s), 81.2 (s), 56.2 (d), 54.4 (d), 40.7 (t), 39.5 (t), 30.3 (t), 28.8 (q), 19.3 (t), 18.4 (t); MS (ES^+) m/z = 443 ($M+Na^+$, 30%), 438 ($M+NH_4^+$, 100%). A byproduct was isolated as pale yellow oil

and identified as the mono protected material (2.37 g, 33%): R_f (SiO_2, 5% MeOH/CH_2Cl_2) 0.24−0.37; ^{1}H NMR ($CDCl_3$) δ/ppm = 3.92−3.67 (m, 1H), 3.38 (t, 2H, ^3J = 7.04 Hz), 2.94 (t, 2H, ^3J = 6.59 Hz), 2.59 (m, 2H), 2.51 (t, 2H, ^3J = 6.59 Hz), 2.46−2.41 (m, 1H), 2.01 (m, 2H), 1.80 (m, 2H), 1.60−1.46 (m, 2H), 1.48 (s, 9H), 1.42 (s, 1H, NH), 1.28−1.14 (m, 2H); ^{13}C NMR ($CDCl_3$) δ/ppm = 155.1 (s), 119.1 (s), 118.3 (s), 81.1 (s), 55.8 (d), 55.1 (d), 42.7 (t), 39.7 (t), 32.9 (t), 29.7 (t), 28.8 (q), 19.6 (t), 19.3 (t); MS (ES^+) m/z = 641 ($2M+H^+$, 70%), 343 ($M+Na^+$, 30%), 321 ($M+H^+$, 100%)

Reduction, General Procedure. A total of 1 equiv of protected material (0.05 M in dioxan/water 4:1) was treated with 1 equiv of Raney-nickel (50% suspension in water), 0.1 equiv of palladium (10% on charcoal), and 3 equiv of lithium hydroxide monohydrate. The reaction mixture was stirred under hydrogen atmosphere at 50 °C for 20 h. The catalyst was filtered off, the solvents were removed in vacuo, and a mixture of 50 mL of water and 25 mL of dichloromethane was added. After phase separation, the aqueous phase was extracted twice with dichloromethane (15 mL), the combined organic layers were dried over sodium sulfate, and the solvent was removed in vacuo. The data for this step (compounds **4a**−**f**) have been reported.[20]

Arylation and Deprotection, General Procedure. A total of 100 mg of reduced material was dissolved in absolute EtOH (10 mL) and water (10 mL). A total of 1−2 equiv of the substituted triazine and 1−2 equiv of $NaHCO_3$ were added, and the mixture was stirred under reflux for 15−20 h. If ES-MS indicated incomplete reaction, more substituted triazine and $NaHCO_3$ were added and reflux continued for another 15−20 h. The solvents were removed in vacuo, and the residue was washed twice with water (5 mL). Anisole (0.2 mL, 1.8 mmol) and trifluoroacetic acid (2.0 mL, 25.9 mmol) were added at 0 °C, and the reaction mixture was stirred at room temperature for 3 h. The trifluoroacetic acid was removed in vacuo, the residue was washed twice with ether (5 mL) and dried in vacuo. The crude product was dissolved or suspended in water (20 mL) and submitted to an ion-exchange column (10 mL Dowex 50WX2−200, H^+-form). The column was washed with water (50 mL) and HCl (2 M in water, 400 mL) and was finally eluted with HCl (5 M in 2-propanol/water 1:1, 300 mL). The solvents were removed in vacuo, and the isolated sticky solids were precipitated from ethanol/ether to yield the hydrochloric salts as powders.

(a) Reactions with 2-Chloro-4,6-diamino-1,3,5-triazine. *N*^{2}-(3-[4-(3-[(4,6-Diamino-1,3,5-triazin-2-yl)amino]propylamino)butyl]aminopropyl)-1,3,5-triazine-2,4,6-triamine Tetrahydrochloride (5a). Starting from **4a** (100 mg, 0.248 mmol), 2-chloro-4,6-diamino-1,3,5-triazine (80 mg, 0.550 mmol), and $NaHCO_3$ (46 mg, 0.548 mmol), **5a** was obtained as a light yellow powder (130 mg, 92%): R_f (SiO_2, 35% aq NH_3/MeOH 1:10): 0.05; mp(sealed tube) dec in two steps: 1. >260 °C, 2. >350 °C; ^{1}H NMR (CD_3OD/D_2O 10:1) δ/ppm = 3.52 (t, 4H, ^3J = 6.50 Hz), 3.09 (m, 8H), 2.03 (tt, 4H ^3J = 7.38 Hz, ^3J = 6.68 Hz), 1.84 (m, 4H); NH exchanged; ^{13}C NMR (CD_3OD/D_2O 10:1) δ/ppm = 162.6 (s), 158.0 (s), 48.2 (t), 46.5 (t), 38.7 (t), 27.2 (t), 24.2 (t); MS (ES^+) m/z = 421 ($M+H^+$, 100%), 211 (($M+2H^+$)/2, 50%); HR-MS (ES^+) calcd for $(C_{16}H_{33}N_{14})^+$: 421.3012; found 421.3012. Anal. ($C_{16}H_{32}N_{14}$·4HCl·1H_2O) H, N; C: calcd, 32.9; found, 33.4; Cl: calcd, 24.3; found, 24.8.

***N*^{2}-(3-[9-(3-[(4,6-Diamino-1,3,5-triazin-2-yl)amino]propylamino)nonyl]aminopropyl)-1,3,5-triazine-2,4,6-triamine Tetrahydrochloride (5b).** Starting from **4b** (100 mg, 0.212 mmol), 2-chloro-4,6-diamino-1,3,5-triazine (68 mg, 0.467 mmol), and $NaHCO_3$ (40 mg, 0.476 mmol), **5b** was obtained as off-white powder (120 mg, 89%): R_f (SiO_2, 35% aq NH_3/MeOH 1:10) 0.16; mp(sealed tube) dec in two steps: 1. >220 °C, 2. >360 °C; ^{1}H NMR (CD_3OD) δ/ppm = 3.52 (m, 4H), 3.06 (m, br, 8H), 2.02 (m, 4H), 1.73 (m, 4H), 1.39 (m, 10H); NH exchanged; ^{13}C NMR (CD_3OD) δ/ppm = 162.8 (s), 158.0 (s), 48.1 (t), 46.7 (t), 38.8 (t), 30.1 (t), 30.0 (t), 27.5 (t), 27.3 (t), 27.2 (t); MS (ES^+) m/z = 491 ($M+H^+$, 100%), 246 (($M+2H^+$)/2, 60%); HR-MS (ES^+) calcd for $(C_{21}H_{43}N_{14})^+$: 491.3795; found 491.3792. Anal.($C_{21}H_{42}N_{14}$·4HCl·1H_2O) C, H, Cl; N: calcd, 30.0; found, 29.2.

***N*^{2}-(3-[12-(3-[(4,6-Diamino-1,3,5-triazin-2-yl)amino]propylamino)dodecyl]amino-propyl)-1,3,5-triazine-2,4,6-triamine Tetrahydrochloride (5c).** Starting from **4c** (100 mg, 0.194 mmol), 2-chloro-4,6-diamino-1,3,5-triazine (62 mg, 0.426 mmol), and $NaHCO_3$ (35 mg, 0.417 mmol), **5c** was obtained as off-white powder (115 mg, 87%): R_f (SiO_2, 35% aq NH_3/MeOH 1:10) 0.26; mp(sealed tube) dec > 210 °C; ^{1}H NMR (CD_3OD) δ/ppm = 3.51 (t, 4H, ^3J = 6.59 Hz), 3.07 (t, 4H, ^3J = 7.59 Hz), 3.01 (t, 4H, ^3J = 8.05 Hz), 2.01 (tt, 4H, ^3J = 7.53, 6.77 Hz), 1.71 (m, 4H), 1.35/1.30 (2m, 16H); NH exchanged; ^{13}C NMR (CD_3OD) δ/ppm = 162.4 (s), 161.2 (s), 158.0 (s), 49.1 (t), 46.3 (t), 38.7 (t), 30.4 (t), 30.3 (t), 30.1 (t), 27.5 (t), 27.1 (t); MS (ES^+) m/z = 533 ($M+H^+$, 100%), 267 (($M+2H^+$)/2, 10%); HR-MS (ES^+) calcd for $(C_{24}H_{49}N_{14})^+$: 533.4264; found 533.4263. Anal. ($C_{24}H_{48}N_{14}$·4HCl·2H_2O) C, H, Cl; N: calcd, 27.4; found, 26.4.

***N*^{2}-(3-[2-(3-[(4,6-Diamino-1,3,5-triazin-2-yl)amino]propylamino)propyl]aminopropyl)-1,3,5-triazine-2,4,6-triamine Tetrahydrochloride (5d).** Starting from **4d** (100 mg, 0.257 mmol), 2-chloro-4,6-diamino-1,3,5-triazine (85 mg, 0.584 mmol), and $NaHCO_3$ (49 mg, 0.583 mmol), **5d** was obtained as a colorless powder (130 mg, 91%): R_f (SiO_2, 35% aq NH_3/MeOH 1:10) 0.30; mp(sealed tube) dec > 240 °C; ^{1}H NMR (CD_3OD) δ/ppm = 3.89−3.74 (m, 1H), 3.55 (m, 5H), 3.43−3.33 (dd, ^3J = 13.36 Hz, 4.40 Hz, 1H), 3.20 (m, 4H), 2.10 (m, 4H), 1.52 (d, 3H, ^3J = 6.59 Hz); EtOH signals at 3.59 (q), 1.18 (t); NH exchanged; ^{13}C NMR (CD_3OD) δ/ppm = 162.9 (s), 158.1 (s), 53.0 (d), 50.6 (t), 47.6 (t), 44.5 (t), 38.8 (t), 38.7 (t), 27.4 (t), 27.2 (t), 15.3 (q); EtOH signals at 53.6 (t), 18.4 (q); MS (ES^+) m/z = 407 ($M+H^+$, 100%), 204 (($M+2H^+$)/2, 50%); HR-MS (ES^+) calcd for $(C_{15}H_{31}N_{14})^+$: 407.2856; found 407.2855. Anal. ($C_{15}H_{30}N_{14}$·4HCl·1EtOH·1H_2O) C, N; H: calcd, 6.9; found, 6.4; Cl: calcd, 23.0; found, 23.7.

***N*^{2}-(3-[2-(3-[(4,6-Diamino-1,3,5-triazin-2-yl)amino]propylamino)cyclohexyl]aminopropyl)-1,3,5-triazine-2,4,6-triamine Tetrahydrochloride (5e).** Starting from **4e** (100 mg, 0.233 mmol), 2-chloro-4,6-diamino-1,3,5-triazine (75 mg, 0.515 mmol), and $NaHCO_3$ (55 mg, 0.655 mmol), **5e** was obtained as a colorless powder (120 mg, 87%): R_f (SiO_2, 35% aq NH_3/MeOH 1:10) 0.45; mp(sealed tube) dec > 240 °C; ^{1}H NMR (CD_3OD) δ/ppm = 3.55 (m, 6H), 3.33 (m, 2H), 3.15 (m, 2H), 2.35 (m, 2H), 2.20 (m, 2H), 2.14 (m, 2H), 1.85 (m, 2H), 1.64 (m, 2H), 1.41 (m, 2H); EtOH signals at 3.59 (q), 1.18 (t); NH exchanged; ^{13}C NMR (CD_3OD) δ/ppm = 162.7 (s), 158.1 (s), 59.1 (d), 44.9 (t), 38.9 (t), 27.7 (t), 27.2 (t), 23.7 (t); EtOH signals at 53.6 (t), 18.4 (q); MS (ES^+) m/z = 447 ($M+H^+$, 100%), 224 (($M+2H^+$)/2, 35%); HR-MS (ES^+) calcd for $(C_{18}H_{35}N_{14})^+$: 447.3169; found 447.3170. Anal. ($C_{18}H_{34}N_{14}$·4HCl·1EtOH·1H_2O) H, N, Cl; C: calcd, 36.6; found, 37.2.

***N*^{2}-(3-[4-(3-[(4,6-Diamino-1,3,5-triazin-2-yl)amino]propylamino)cyclohexyl]aminopropyl)-1,3,5-triazine-2,4,6-triamine Tetrahydrochloride (5f).** Starting from **4f** (100 mg, 0.233 mmol), 2-chloro-4,6-diamino-1,3,5-triazine (75 mg, 0.515 mmol), and $NaHCO_3$ (45 mg, 0.536 mmol), **5f** was obtained as a colorless powder (125 mg, 90%): R_f (SiO_2, 35% aq NH_3/MeOH 1:10) 0.14; mp(sealed tube) dec > 300 °C; ^{1}H NMR (CD_3OD) δ/ppm = 3.52 (m, 4H), 3.11 (m, 6H), 2.30 (m, 4H), 2.01 (m, 4H), 1.58 (m, 4H); NH exchanged; ^{13}C NMR (CD_3OD) δ/ppm = 163.0 (s), 158.4 (s), 57.0 (d), 44.1 (t), 39.1 (t), 28.1 (t), 27.7 (t); MS (ES^+) m/z = 447 ($M+H^+$, 100%), 224 (($M+2H^+$)/2, 60%); HR-MS (ES^+) calcd for $(C_{18}H_{35}N_{14})^+$: 447.3169; found 447.3167. Anal. ($C_{18}H_{34}N_{14}$·4HCl·2H_2O) H, N, Cl; C: calcd, 34.4; found, 33.9.

(b) Bis Additions with Amino Substituted 2-Chloro-1,3,5-triazines. *N*^{2}-(3-((9-((3-((4-Amino-6-(methylamino)-1,3,5-triazin-2-yl)amino)propyl)amino)nonyl)amino)-propyl)-*N*^{4}-methyl-1,3,5-triazine-4,2,6-triamine Tetrahydrochloride (6a). Starting from **4b** (100 mg, 0.212 mmol), 2-chloro-4-amino-6-methylamino-1,3,5-triazine (2 × 75 mg, 2 × 0.470 mmol), and $NaHCO_3$ (2 × 40 mg, 2 × 0.476 mmol), **6a** was isolated as light yellow powder (100 mg, 71%): R_f (SiO_2, 35% aq NH_3/MeOH 1:10) 0.31; mp(sealed tube) > 120 °C slow

dec (foam), > 300 dec; ^{1}H NMR (CD_3OD) δ/ppm = 3.51 (m, 4H), 3.14−2.88 (m, 14H), 2.03 (m, 4H), 1.72 (m, 4H), 1.38 (m, 10H); EtOH signals at 3.61 (q), 1.18 (t); NH exchanged; ^{13}C NMR (CD_3OD) δ/ppm = 161.7 (s), 157.5 (s), 49.2 (t), 46.7 (t), 46.5 (t), 39.0 (t), 38.6 (t), 30.1 (t), 30.0 (t), 28.0 (q), 27.7 (q), 27.5 (t), 27.2 (t); EtOH signals at 53.6 (t), 18.4 (q); MS (ES^+) m/z = 519 ($M+H^+$, 60%), 260 ((M+2H$^+$)/2, 100%); HR-MS (ES^+) calcd for $(C_{23}H_{47}N_{14})^+$: 519.4108; found 519.4102. Anal. ($C_{23}H_{46}N_{14}$·4HCl·1.5EtOH·1H_2O) C, H, N; Cl: calcd, 18.8, found, 19.4.

***N*^{2}-(3-((9-((3-((4-Amino-6-(dimethylamino)-1,3,5-triazin-2-yl)amino)propyl)amino)nonyl)amino)propyl)-*N*^{4},*N*^{4}-dimethyl-1,3,5-triazine-4,2,6-triamine Pentahydrochloride (6b).** Starting from **4b** (100 mg, 0.212 mmol), 2-chloro-4-amino-6-(dimethylamino)-1,3,5-triazine (2 × 80 mg, 2 × 0.461 mmol), and $NaHCO_3$ (2 × 40 mg, 2 × 0.476 mmol), **6b** was isolated as light yellow powder (100 mg, 65%): R_f (SiO_2, 35% aq NH_3/MeOH 1:10) 0.43; mp(sealed tube) > 150 slow dec (foam), 270−290 °C dec; ^{1}H NMR (CD_3OD) δ/ppm = 3.56 (m, br, 4H), 3.23/3.20 (2s, 12H), 3.10 (m, 4H), 3.02 (m, 4H), 2.06 (m, 4H), 1.73 (m, 4H), 1.38 (m, 10H); EtOH signals at 3.61 (q), 1.18 (t); NH exchanged; ^{13}C NMR (CD_3OD) δ/ppm = 163.0 (s), 157.5 (s), 157.0 (s), 49.2 (t), 46.7 (t), 38.9 (t), 37.4 (q), 37.2 (q), 30.1 (t), 30.0 (t), 27.5 (t), 27.2 (t), 27.0 (t); EtOH signals at 53.6 (t), 18.4 (q); MS (ES^+) m/z = 547 ($M+H^+$, 95%), 274 ((M+2H$^+$)/2, 100%); HR-MS (ES^+) calcd for $(C_{25}H_{51}N_{14})^+$: 547.4421; found 547.4423. Anal. ($C_{25}H_{50}N_{14}$·4.5HCl·2EtOH·1H_2O) C, H, N; Cl: calcd, 19.4, found, 20.2.

***N*^{2}-(3-((9-((3-((4,6-Di(methylamino)-1,3,5-triazin-2-yl)amino)propyl)amino)nonyl)amino)propyl)-*N*^{4},*N*^{6}-dimethyl-1,3,5-triazine-6,4,2-triamine Tetrahydrochloride (6c).** Starting from **4b** (100 mg, 0.212 mmol), 2-chloro-4,6-bis-(methylamino)-1,3,5-triazine (2 × 81 mg, 2 × 0.467 mmol), and $NaHCO_3$ (2 × 40 mg, 2 × 0.476 mmol), **6c** was isolated as light yellow powder (90 mg, 61%): R_f (SiO_2, 35% aq NH_3/MeOH 1:10) 0.41; mp(sealed tube) > 120 °C slow dec (foam), 280−290 °C dec; ^{1}H NMR (CD_3OD) δ/ppm = 3.57−3.47 (m, 4H), 3.14−2.99 (m, 8H), 2.98/2.92 (2s, 12H), 2.05 (m, 4H), 1.72 (m, 4H), 1.38 (m, 10H); NH exchanged; ^{13}C NMR (CD_3OD) δ/ppm = 156.4 (s), 156.0 (s), 49.1 (t), 46.7 (t), 46.4 (t), 39.1 (t), 38.9 (t), 38.6 (t), 30.1 (t), 30.0 (t), 27.9 (q), 27.7 (q), 27.5 (t), 27.2 (t), 27.1 (t); MS (ES^+) m/z = 547 ($M+H^+$, 5%), 274 ((M+2H$^+$)/2, 100%); HR-MS (ES^+) calcd for $(C_{25}H_{51}N_{14})^+$: 547.4421; found 547.4418. Anal. ($C_{25}H_{50}N_{14}$·4HCl·1EtOH·2H_2O) C, H, N; Cl: calcd, 18.3; found, 18.8.

***N*^{2}-(3-((9-((3-((4,6-Di(methylamino)-1,3,5-triazin-2-yl)amino)propyl)amino)nonyl)amino)propyl)-*N*^{4},*N*^{4},*N*^{6},*N*^{6}-tetramethyl-1,3,5-triazine-6,4,2-triamine Pentahydrochloride (6d).** Starting from **4b** (100 mg, 0.212 mmol), 2-chloro-4,6-bis(dimethylamino)-1,3,5-triazine (2 × 94 mg, 2 × 0.466 mmol), and $NaHCO_3$ (2 × 40 mg, 2 × 0.476 mmol), **6d** was isolated as light yellow powder (50 mg, 30%): R_f (SiO_2, 35% aq NH_3/MeOH 1:10) 0.61; mp(sealed tube) > 120 °C slow dec (foam), 270−280 °C dec; ^{1}H NMR (CD_3OD) δ/ppm = 3.60 (m, 4H), 3.22 (s, br, 24H), 3.12 (m, 4H), 3.03 (m, 4H), 2.08 (m, 4H), 1.73 (m, 4H), 1.39 (m, 10H); NH exchanged; ^{13}C NMR (CD_3OD) δ/ppm = 163.1 (s), 156.7 (s), 155.2 (s), 49.3 (t), 46.8 (t), 39.0 (t), 37.4 (q), 30.0 (t), 27.5 (t), 27.2 (t), 26.9 (t); MS (ES^+) m/z = 603 ($M+H^+$, 80%), 302 ((M+2H$^+$/2, 100%); HR-MS (ES^+) calcd for $(C_{29}H_{59}N_{14})^+$: 603.5047; found 603.5039. Anal. ($C_{29}H_{58}N_{14}$·5HCl·3H_2O) C, H, N; Cl, calcd, 21.1; found, 21.9.

***N*^{2}-[3-(12-[(3-[4-Amino-6-(methylamino)-1,3,5-triazin-2-yl]aminopropyl)amino]dodecylamino)propyl]-*N*^{4}-methyl-1,3,5-triazine-2,4,6-triamine Pentahydrochloride (6e).** Starting from **4c** (100 mg, 0.194 mmol), 2-chloro-4-amino-6-methylamino-1,3,5-triazine (2 × 68 mg, 2 × 0.426 mmol), and $NaHCO_3$ (2 × 36 mg, 2 × 0.429 mmol) **6e** was isolated as white powder (120 mg, 83%): R_f (SiO_2, 35% aq NH_3/MeOH 1:10) 0.31; mp(sealed tube) > 190 °C slow dec (foam), > 300 °C dec; ^{1}H NMR (CD_3OD) δ/ppm = 3.52 (m, 4H), 3.14−2.91 (m, 8H), 2.98/2.94 (2s, 6H), 2.03 (m, 4H), 1.72 (m, 4H), 1.36−1.32 (m, 16H); EtOH signals at 3.61 (q), 1.18 (t); NH exchanged; ^{13}C NMR (CD_3OD) δ/ppm = 164.5)s), 163.9 (s), 160.7 (s), 158.9 (s), 157.4 (s), 157.0 (s), 49.1 (t), 46.7 (t), 46.4 (t), 39.0 (t), 38.9 (t), 38.6 (t), 30.6 (t), 30.4 (t), 30.2 (t), 28.0 (q), 27.7 (q), 27.6 (t), 27.2 (t), 27.1 (t); EtOH signals at 53.6 (t), 18.4 (q); MS (ES^+) m/z = 597 ($M+HCl+H^+$, 25%), 561 ($M+H^+$, 70%), 281 ((M+2H$^+$)/2, 100%); HR-MS (ES^+) calcd for $(C_{26}H_{53}N_{14})^+$: 561.4577; found 561.4586. Anal. ($C_{26}H_{52}N_{14}$·4.5HCl·1EtOH 2H_2O) C, H, N, Cl.

***N*^{2}-[3-(12-[(3-[4-Amino-6-(dimethylamino)-1,3,5-triazin-2-yl]aminopropyl)amino]dodecylamino)propyl]-*N*^{4},*N*^{4}-dimethyl-1,3,5-triazine-2,4,6-triamine Pentahydrochloride (6f).** Starting from **4c** (100 mg, 0.194 mmol), 2-chloro-4-amino-6-(dimethylamino)-1,3,5-triazine (2 × 74 mg, 2 × 0.426 mmol), and $NaHCO_3$ (2 × 36 mg, 2 × 0.429 mmol), **6f** was isolated as white powder (110 mg, 73%): R_f (SiO_2, 35% aq NH_3/MeOH 1:10) 0.38; mp(sealed tube) > 200 °C slow dec (foam), > 300 °C dec; ^{1}H NMR (CD_3OD) δ/ppm = 3.55 (m, 4H), 3.22/3.18 (2s, 12H), 3.09 (m, 4H), 3.01 (m, 4H), 2.05 (m, 4H), 1.70 (m, 4H), 1.34−1.29 (m, 16H); EtOH signals at 3.61 (q), 1.18 (t); NH exchanged; ^{13}C NMR (CD_3OD) δ/ppm = 162.2 (s), 157.4 (s), 157.2 (s), 49.0 (t), 46.4 (t), 38.8 (t), 37.6 (q), 37.3 (q), 30.3 (t), 30.2 (t), 29.9 (t), 27.3 (t), 27.0 (t), 26.7 (t); EtOH signals at 53.6 (t), 18.4 (q); MS (ES^+) m/z = 625 ($M+HCl+H^+$, 12%), 589 ($M+H^+$, 8%), 295 ((M+2H$^+$)/2, 100%); HR-MS (ES^+) calcd for $(C_{28}H_{57}N_{14})^+$: 589.4890; found 589.4895. Anal. ($C_{28}H_{56}N_{14}$·4.5HCl·1EtOH·1H_2O) C, H, N, Cl.

***N*^{2}-[3-(12-[(3-[4,6-Di(methylamino)-1,3,5-triazin-2-yl]-aminopropyl)amino]dodecylamino)propyl]-*N*^{4},*N*^{6}-dimethyl-1,3,5-triazine-2,4,6-triamine pentahydrochloride (6g).** Starting from **4c** (100 mg, 0.194 mmol), 2-chloro-4,6-bis-(methylamino)-1,3,5-triazine (2 × 75 mg, 2 × 0.432 mmol), and $NaHCO_3$ (2 × 36 mg, 2 × 0.429 mmol), **6g** was isolated as white powder (65 mg, 43%): R_f (SiO_2, 35% aq NH_3/MeOH 1:10) 0.40; mp(sealed tube) > 210 °C dec; ^{1}H NMR (CD_3OD) δ/ppm = 3.57 (m, 4H), 3.08 (m, 4H), 3.03 (m, 4H), 2.98/2.92 (2s, 12H), 2.04 (m, 4H), 1.71 (m, 4H), 1.36−1.31 (m, 16H); EtOH signals at 3.61 (q), 1.18 (t); NH exchanged; ^{13}C NMR (CD_3OD) δ/ppm = 164.4 (s), 156.3 (s), 155.9 (s), 49.2 (t), 49.1 (t), 46.6 (t), 46.3 (t), 45.8 (t), 39.1 (t), 38.9 (t), 38.5 (t), 37.9 (t), 30.5 (t), 30.4 (t), 30.1 (t), 27.7 (q, br), 27.5 (t), 27.2 (t), 27.0 (t), 24.8 (t,); EtOH signals at 53.6 (t), 18.4 (q); MS (ES^+) m/z = 625 ($M+H^{+}$+HCl, 10%), 589 ($M+H^+$, 30%), 295 ((M+2H$^+$)/2, 100%), 227 (15%), 195 (15%); HR-MS (ES^+) calcd for $(C_{28}H_{57}N_{14})^+$: 589.4890; found 589.4899. Anal. ($C_{28}H_{56}N_{14}$·5HCl·2.5EtOH) H, N, Cl; C: calcd, 44.7; found, 45.3.

***N*^{2}-[3-(12-[(3-[4,6-Di(dimethylamino)-1,3,5-triazin-2-yl]-aminopropyl)amino]dodecylamino)propyl]-*N*^{4},*N*^{4},*N*^{6},*N*^{6}-tetramethyl-1,3,5-triazine-2,4,6-triamine Pentahydrochloride (6h).** Starting from **4c** (100 mg, 0.194 mmol), 2-chloro-4,6-bis(dimethylamino)-1,3,5-triazine (2 × 86 mg, 2 × 0.426 mmol), and $NaHCO_3$ (2 × 36 mg, 2 × 0.429 mmol), **6h** was isolated as white powder (95 mg, 59%): R_f (SiO_2, 35% aq NH_3/MeOH 1:10) 0.52; mp(sealed tube) 192−195 °C; ^{1}H NMR (CD_3OD) δ/ppm = 3.59 (m, 4H), 3.21 (s, br, 24H), 3.10 (m, 4H), 3.00 (m, 4H), 2.07 (m, 4H), 1.71 (m, 4H), 1.36−1.31 (2m, 16H); EtOH signals at 3.61 (q), 1.18 (t); NH exchanged; ^{13}C NMR (CD_3OD) δ/ppm = 163.1 (s), 156.6 (s), 155.2 (s), 49.1 (t), 46.5 (t), 38.9 (t), 37.3 (q, br), 30.5 (t), 30.4 (t), 30.1 (t), 27.5 (t), 27.2 (t), 26.8 (t); EtOH signals at 53.6 (t), 18.4 (q); MS (ES^+) m/z = 681 ($M+H^{+}$+HCl, 30%), 645 ($M+H^+$, 35%), 323 ((M+2H$^+$)/2, 100%); HR-MS (ES^+) calcd for $(C_{32}H_{65}N_{14})^+$: 645.5516; found 645.5515. Anal. ($C_{32}H_{64}N_{14}$·4.5HCl·1.5EtOH) C, H, N, Cl.

(c) Mono Additions with Amino Substituted 2-Chloro-1,3,5-triazines. *N*^{2}-(3-((9-((3-((4,6-Di(methylamino)-1,3,5-triazin-2-yl)amino)propyl)amino)nonyl)amino)propyl)-*N*^{4},*N*^{6}-dimethyl-1,3,5-triazine-6,4,2-triamine Pentahydrochloride (7a). Starting from **4b** (100 mg, 0.212 mmol), 2-chloro-4,6-bis(methylamino)-1,3,5-triazine (81 mg, 0.467 mmol), and $NaHCO_3$ (40 mg, 0.476 mmol), **7a** was isolated as off-white powder (60 mg, 48%): R_f (SiO_2, 35% aq NH_3/MeOH 1:10) 0.09; mp(sealed tube) > 150 °C slow dec (foam), 220−225 °C dec; ^{1}H NMR (CD_3OD) δ/ppm = 3.63−3.46 (m, 2H), 3.19−2.88 (m, 16H), 2.20−1.96 (m, 4H), 1.80−1.64 (m, 4H), 1.49−1.27 (m, 10H); EtOH signals at 3.61 (q), 1.18 (t); NH exchanged; ^{13}C NMR (CD_3OD) δ/ppm = 161.7 (s), 161.3 (s), 49.2 (t), 45.9 (t), 37.9 (t), 30.1 (t), 30.0 (t), 27.7 (q), 27.4 (t),

27.2 (t), 25.4 (t); EtOH signals at 53.6 (t), 18.4 (q); MS (ES^+) m/z = 410 ($M+H^+$, 20%), 206 (($M+2H^+$)/2, 100%); HR-MS (ES^+) calcd for $(C_{20}H_{44}N_9)^+$: 410.3720; found 410.3724. Anal. ($C_{20}H_{43}N_9 \cdot 5HCl \cdot 1.5EtOH \cdot 1H_2O$) C, H, N, Cl.

***N*²-[3-(12-[(3-Aminopropyl)amino]dodecylamino)-propyl]-N⁴-methyl-1,3,5-triazine-2,4,6-triamine Tetrahydrochloride (7b).** Starting from **4c** (100 mg, 0.194 mmol), 2-chloro-4-amino-6-methylamino-1,3,5-triazine (63 mg, 0.395 mmol), and $NaHCO_3$ (33 mg, 0.393 mmol), an 85:15 mixture of mono-adduct **7b** and bis-adduct **6e** was isolated as an off-white powder (100 mg): R_f (SiO_2, 35% aq NH_3/MeOH 1:10) 0.07 (**7b**) and 0.32 (**6e**); mp(sealed tube) 280−290 °C dec; ^{1}H NMR (CD_3OD): **7b**; δ/ppm = 3.61−3.46 (m, 2H), 3.19−2.89 (m, 13H), 2.17−1.94 (m, 4H), 1.76−1.61 (m, 4H), 1.43−1.23 (m, 16H); **6e**; δ/ppm = 3.61−3.46 (m, 4H), 3.19−2.89 (m, 14H), 2.17−1.94 (m, 4H), 1.76−1.61 (m, 4H), 1.43−1.23 (m, 16H); NH exchanged; ^{13}C NMR (CD_3OD): **7b**; δ/ppm = 161.3 (s) 160.0 (s) 157.1 (s), 48.8 (t), 45.5 (t), 37.7 (t), 30.0 (t), 29.9 (t), 29.6 (t), 28.1 (q), 27.0 (t), 26.8 (t), 24.9 (t); **6e**; δ/ppm = 161.3 (s), 160.0 (s), 157.1 (s), 49.0 (t), 46.2 (t), 45.9 (t), 38.8 (t), 38.6 (t), 38.4 (t), 30.0 (t), 29.9 (t), 29.6 (t), 28.1 (q), 27.9 (q), 27.0 (t), 26.8 (t), 26.7 (t); MS (ES^+) m/z = 561 ($M_{\mathbf{6e}}+H^+$, 50%), 438 ($M_{\mathbf{7b}}+H^+$, 40%)

***N*²-[3-(12-[(3-Aminopropyl)amino]dodecylamino)-propyl]-*N*⁴,*N*⁴-dimethyl-1,3,5-triazine-2,4,6-triamine Tetrahydrochloride (7c).** Starting from **4c** (100 mg, 0.194 mmol), 2-chloro-4-amino-6-(dimethylamino)-1,3,5-triazine (70 mg, 0.403 mmol), and $NaHCO_3$ (34 mg, 0.405 mmol), a 90:10 mixture of monoadduct **7c** and bis-adduct **6f** was isolated as an off-white powder (80 mg): R_f (SiO_2, 35% aq NH_3/MeOH 1:10) 0.07 (**7c**) and 0.39 (**6f**); mp(sealed tube) 280−290 °C dec; ^{1}H NMR (CD_3OD): **7c**; δ/ppm = 3.69−3.47 (m, 2H), 3.28−2.95 (m, 16H), 2.24−1.99 (m, 4H), 1.83−1.63 (m, 4H), 1.50−1.24 (m, 16H); **6f**; δ/ppm = 3.69−3.47 (m, 4H), 3.28−2.95 (m, 20H), 2.24−1.99 (m, 4H), 1.83−1.63 (m, 4H), 1.50−1.24 (m, 16H); NH exchanged; ^{13}C NMR (CD_3OD): **7c**; δ/ppm = 162.5 (s) 157.4 (s) 157.2 (s), 49.0 (t), 45.8 (t), 37.8 (t), 37.5 (q), 30.4 (t), 30.3 (t), 30.0 (t), 27.4 (t), 27.1 (t), 25.1 (t); **6f**; δ/ppm = 162.5 (s), 157.4 (s), 157.2 (s), 49.1 (t), 46.5 (t), 45.8 (t), 38.8 (t), 37.8 (t), 37.5 (q), 37.3 (q), 30.4 (t), 30.3 (t), 30.0 (t), 27.4 (t), 27.1 (t), 26.8 (t); MS (ES^+) m/z = 589 ($M_{\mathbf{6f}}+H^+$, 40%), 452 ($M_{\mathbf{7c}}+H^+$, 25%)

***N*²-[3-(12-[(3-Aminopropyl)amino]dodecylamino)-propyl]-*N*⁴,*N*⁶-dimethyl-1,3,5-triazine-2,4,6-triamine Pentahydrochloride (7d).** Starting from **4c** (100 mg, 0.194 mmol), 2-chloro-4,6-bis(methylamino)-1,3,5-triazine (70 mg, 0.403 mmol), and $NaHCO_3$ (34 mg, 0.405 mmol), **7d** was isolated as an off-white powder (80 mg, 65%): R_f (SiO_2, 35% aq NH_3/MeOH 1:10) 0.11; mp(sealed tube) 300−305 °C dec; ^{1}H NMR (CD_3OD) δ/ppm = 3.54 (m, 2H), 3.20−2.89 (m, 16H), 2.13 (m, 4H), 1.72 (m, 4H), 1.35/1.30 (2m, 16H); EtOH signals at 3.52 (q), 1.18 (t); NH exchanged; ^{13}C NMR (CD_3OD) δ/ppm = 156.2 (s), 155.8 (s), 49.1 (t,), 45.7 (t), 37.8 (t), 30.3 (t), 30.2 (t), 30.0 (t), 27.7 (q), 27.3 (t), 27.0 (t), 25.1 (t); EtOH signals at 53.6 (t), 18.4 (q); MS (ES^+) m/z = 488 ($M+HCl+H^+$, 30%), 452 ($M+H^+$, 70%), 227 (($M+2H^+$)/2, 60%); HR-MS (ES^+) calcd for $(C_{23}H_{50}N_9)^+$: 452.4189; found 452.4191. Anal. ($C_{23}H_{49}N_9 \cdot 5HCl \cdot 2EtOH$) C, H; N: calcd, 17.4; found, 16.8; Cl: calcd, 24.4; found, 24.9.

***N*²-[3-(12-[(3-Aminopropyl)amino]dodecylamino)-propyl]-*N*⁴,*N*⁴,*N*⁶,*N*⁶-tetramethyl-1,3,5-triazine-2,4,6-triamine Pentahydrochloride (7e).** Starting from **4c** (100 mg, 0.194 mmol), 2-chloro-4,6-bis(dimethylamino)-1,3,5-triazine (80 mg, 0.397 mmol), and $NaHCO_3$ (33 mg, 0.393 mmol), **7e** was isolated as a light yellow powder (80 mg, 62%): R_f (SiO_2, 35% aq NH_3/MeOH 1:10) 0.11; mp(sealed tube) 310−315 °C dec; ^{1}H NMR (CD_3OD) δ/ppm = 3.58 (m, 2H), 3.27−2.95 (m, 22H), 2.12 (m, 4H), 1.71 (m, 4H), 1.35/1.30 (2m, 16H); EtOH signals at 3.52 (q), 1.18 (t); EtOH signals at 3.52 (q), 1.18 (t); NH exchanged; ^{13}C NMR (CD_3OD) δ/ppm = 156.5 (s), 155.1 (s), 49.1 (t), 45.7 (t), 37.8 (t), 37.3 (q), 30.3 (t), 30.2 (t), 29.9 (t), 27.3 (t), 27.0 (t), 25.1 (t); EtOH signals at 53.6 (t), 18.4 (q); MS (ES^+) m/z = 516 ($M+HCl+H^+$, 10%), 480 ($M+H^+$, 30%), 241 (($M+2H^+$)/2, 30%); HR-MS (ES^+) calcd for $(C_{25}H_{54}N_9)^+$: 480.4502; found 480.4501. Anal. ($C_{25}H_{53}N_9 \cdot 5.5HCl \cdot 3EtOH$) C, H, N, Cl.

(d) Tetra-Adducts with Amino Substituted 2-Bromomethyl-1,3,5-triazines. *N*¹,*N*⁹-Di(3-(di((4,6-diamino-1,3,5-triazin-2-yl)methyl)amino)propyl)-1,9-nonanediamine Heptahydrochloride (8a). Starting from **4b** (120 mg, 0.254 mmol), 2-bromomethyl-4,6-diamino-1,3,5-triazine (2 × 115 mg, 2 × 0.564 mmol), and $NaHCO_3$ (2 × 50 mg, 2 × 0.595 mmol), **8a** was isolated as light yellow powder (210 mg, 81%): R_f (SiO_2, 35% aq NH_3/MeOH 1:10) baseline; mp(sealed tube) > 120 °C slow dec (foam), 280−300 °C dec; ^{1}H NMR (CD_3OD) δ/ppm = 3.94/3.92 (2s, 4H, Ar−CH_2−N), 3.16 (m, 4H), 3.03 (m, 4H), 2.83 (m, 4H), 2.06 (m, 4H), 1.72 (m, 4H), 1.38 (m, 10H); EtOH signals at 3.61 (q), 1.18 (t); NH exchanged, Ar−CH_2−N partially exchanged; ^{13}C NMR (CD_3OD) δ/ppm = 167.6 (s), 162.3 (s), 58.3 (t), 58.0 (t), 49.0 (t), 46.8 (t), 30.0 (t), 29.9 (t), 27.4 (t), 27.2 (t), 24.6 (t); EtOH signals at 53.6 (t), 18.4 (q); MS (ES^+) m/z = 765 ($M+H^+$, 5%), 383 (($M+2H^+$/2, 100%), 256 (($M+3H^+$/3, 25%); HR-MS (ES^+) calcd for $(C_{31}H_{57}N_{24})^+$: 765.5198; found 765.5199. Anal. ($C_{31}H_{56}N_{24} \cdot 7HCl \cdot 2.5EtOH \cdot 2H_2O$) C, H, N, Cl.

***N*¹,*N*⁹-Di(3-(((4-amino-6-(dimethylamino)-1,3,5-triazin-2-yl)methyl)amino)propyl)-1,9-nonanediamine Heptahydrochloride (8b).** Starting from **4b** (100 mg, 0.212 mmol), 2-bromomethyl-4-amino-6-(dimethylamino)-1,3,5-triazine (100+50 mg, 0.431+0.215 mmol), and $NaHCO_3$ (36+18 mg, 0.429+0.214 mmol), **8b** was isolated as light yellow powder (150 mg, 63%): R_f (SiO_2, 35% aq NH_3/MeOH 1:10) baseline; mp(sealed tube) > 150 °C slow dec (foam), 285−295 °C dec; ^{1}H NMR (CD_3OD) δ/ppm = 4.00 (s, 4H, Ar−CH_2−N), 3.29/3.24 (2s, 24H), 3.10 (m, 4H), 3.01 (m, 4H), 2.91 (m, 4H), 2.02 (m, 4H), 1.70 (m, 4H), 1.36 (m, 10H); EtOH signals at 3.61 (q), 1.18 (t); NH exchanged, Ar−CH_2−N partially exchanged; ^{13}C NMR (CD_3OD) δ/ppm = 166.6 (s), 162.6 (s), 157.3 (s), 58.3 (t), 57.4 (t), 49.1 (t), 46.7 (t), 37.9 (q), 37.8 (q), 29.9 (t), 29.8 (t), 27.3 (t), 27.1 (t), 24.8 (t); EtOH signals at 53.6 (t), 18.4 (q); MS (ES^+) m/z = 916 ($M+HCl+H^+$, 95%), 878 ($M+H^+$, 10%), 440 (($M+2H^+$)/2, 100%); HR-MS (ES^+) calcd for $(C_{39}H_{73}N_{24})^+$: 877.6450; found 877.6460. Anal. ($C_{39}H_{72}N_{24} \cdot 7.5HCl \cdot 3EtOH \cdot 2H_2O$) C, H, N, Cl.

***N*¹,*N*⁹-Di(3-(((4,6-di(methylamino)-1,3,5-triazin-2-yl)-methyl)amino)propyl)-1,9-nonanediamine Heptahydrochloride (8c).** Starting from **4b** (100 mg, 0.212 mmol), 2-bromomethyl-4,6-bis(methylamino)-1,3,5-triazine (2 × 100+50 mg, 2 × 0.431+0.215 mmol), and $NaHCO_3$ (2 × 36+18 mg, 2 × 0.429+0.214 mmol), **8c** was isolated as light yellow powder (100 mg, 42%): R_f (SiO_2, 35% aq NH_3/MeOH 1:10) baseline; mp(sealed tube) > 150 °C slow dec (foam), > 245 °C dec; ^{1}H NMR (CD_3OD) δ/ppm = 3.87 (s, 4H, Ar−CH_2−N), 3.12 (m, 4H), 3.07−2.95 (m, 4H), 3.05/2.99 (2s, 24H), 2.82 (m, 4H), 2.01 (m, 4H), 1.71 (m, 4H), 1.37 (m, 10H); EtOH signals at 3.61 (q), 1.18 (t); NH exchanged, Ar−CH_2−N partially exchanged; ^{13}C NMR (CD_3OD) δ/ppm = 166.2 (s), 160.3 (s), 58.3 (t), 57.4 (t), 49.0 (t), 46.8 (t), 30.0 (t), 29.9 (t), 28.1 (q), 27.4 (t), 27.1 (t), 24.8 (t), 24.7 (t); EtOH signals at 53.6 (t), 18.4 (q); MS (ES^+) m/z = 916 ($M+HCl+H^+$, 50%), 900 ($M+Na^+$, 20%), 878 ($M+H^+$, 15%), 440 (($M+2H^+$)/2, 100%); HR-MS (ES^+) calcd for $(C_{39}H_{73}N_{24})^+$: 877.6450; found 877.6449; Anal. ($C_{39}H_{72}N_{24} \cdot 7HCl \cdot 2.5EtOH$) C, H, N; Cl: calcd, 19.9; found, 19.4.

***N*¹,*N*¹²-Di(3-[(4,6-diamino-1,3,5-triazin-2-yl)methyl]-aminopropyl)-1,12-dodecanediamine Heptahydrochloride (8d).** Starting from **4c** (100 mg, 0.194 mmol), 2-bromomethyl-4,6-diamino-1,3,5-triazine (3 × 80 mg, 3 × 0.392 mmol), and $NaHCO_3$ (3 × 33 mg, 3 × 0.393 mmol), **8d** was isolated as light yellow powder (146 mg, 71%): R_f (SiO_2, 35% aq NH_3/MeOH 1:10) baseline; mp(sealed tube) > 260 °C dec; ^{1}H NMR (CD_3OD) δ/ppm = 3.92 (s, 6H, Ar−CH_2−N), 3.15 (t, 4H, ^3J = 7.87 Hz), 3.02 (t, 4H, ^3J = 7.87 Hz), 2.81 (t, 4H, ^3J = 7.23 Hz), 2.05 (m, 4H), 1.72 (m, 4H), 1.37−1.32 (m, 16H); EtOH signals at 3.61 (q), 1.18 (t); NH exchanged, Ar−CH_2−N partially exchanged; ^{13}C NMR (CD_3OD) δ/ppm = 167.7 (s), 162.4 (s, br), 58.1 (t), 53.6 (t), 49.1 (t), 46.8 (t), 30.5 (t), 30.4 (t), 30.2 (t), 27.6 (t), 27.3 (t), 24.7 (t); EtOH signals at 53.6 (t), 18.4 (q); MS (ES^+) m/z = 843 ($M+HCl+H^+$, 10%), 807 ($M+H^+$, 5%), 404

((M+2H$^+$)/2, 100%); HR-MS (ES$^+$) calcd for $(C_{34}H_{63}N_{24})^+$: 807.5667; found 807.5671. Anal. ($C_{34}H_{62}N_{24}$·6.5HCl·1EtOH·2H_2O) C, H, N, Cl.

***N*^{1},*N*^{12}-Di[3-([4-amino-6-(dimethylamino)-1,3,5-triazin-2-yl]methylamino)propyl]-1,12-dodecanediamine Heptahydrochloride (8e).** Starting from **4c** (100 mg, 0.194 mmol), 2-bromomethyl-4-amino-6-(dimethylamino)-1,3,5-triazine (2 × 93 mg, 2 × 0.401 mmol), and $NaHCO_3$ (2 × 35 mg, 3 × 0.417 mmol), **8e** was isolated as light yellow powder (200 mg, 88%): R_f (SiO_2, 35% aq NH_3/MeOH 1:10) baseline; mp(sealed tube) 155−165 °C dec; ^{1}H NMR (CD_3OD) δ/ppm = 3.96 (s, 5H, Ar−CH_2−N), 3.22/3.16 (2s, 24H), 3.16 (t, 4H, ^3J = 7.96 Hz), 3.03 (t, 4H, ^3J = 7.78 Hz), 2.83 (t, 4H, ^3J = 7.04 Hz), 2.07 (m, 4H), 1.72 (m, 4H), 1.37−1.33 (m, 16H); EtOH signals at 3.61 (q), 1.18 (t); NH exchanged, Ar−CH_2−N partially exchanged; ^{13}C NMR (CD_3OD) δ/ppm = 166.3 (s), 162.9 (s), 157.6 (s), 58.2 (t), 53.6 (t), 49.8 (t), 46.7 (t), 37.7 (q), 37.6 (q), 30.6 (t), 30.5 (t), 30.2 (t), 27.6 (t), 27.3 (t), 24.7 (t); EtOH signals at 58.3 (t), 18.4 (q); MS (ES$^+$) *m*/*z* = 955 (M+HCl+H$^+$, 20%), 919 (M+H$^+$, 30%), 460 ((M+2H$^+$)/2, 100%); HR-MS (ES$^+$) calcd for $(C_{42}H_{79}N_{24})^+$: 919.6919; found 919.6920. Anal. ($C_{42}H_{78}N_{24}$·6.5HCl·2EtOH·1H_2O) C, H, N, Cl.

***N*^{1},*N*^{12}-Di[3-([4,6-di(methylamino)-1,3,5-triazin-2-yl]-methylamino)propyl]-1,12-dodecanediamine Heptahydrochloride (8f).** Starting from **4c** (100 mg, 0.194 mmol), 2-bromomethyl-4,6-bis(methylamino)1,3,5-triazine (2 × 93 mg, 2 × 0.401 mmol), and $NaHCO_3$ (2 × 35 mg, 3 × 0.417 mmol), **8f** was isolated as light yellow powder (80 mg, 35%): R_f (SiO_2, 35% aq NH_3/MeOH 1:10) baseline; mp(sealed tube) > 130 °C slow dec (foam), > 200 °C dec; ^{1}H NMR (CD_3OD) δ/ppm = 3.82 (s, 4H, Ar−CH_2−N), 3.15 (m, 4H), 3.05−2.89 (m, 28H), 2.77 (m, 4H), 2.03 (m, 4H), 1.72 (m, 4H), 1.36−1.33 (m, 16H); EtOH signals at 3.61 (q), 1.18 (t); NH exchanged, Ar−CH_2−N partially exchanged; ^{13}C NMR (CD_3OD) δ/ppm = 167.7 (s), 166.0(s), 57.8 (t), 53.5 (t), 49.0 (t), 46.8 (t), 30.6 (t), 30.4 (t), 30.2 (t), 27.6 (t), 28.5 (q), 27.9 (q), 27.3 (t), 24.7 (t); EtOH signals at 58.3 (t), 18.4 (q); MS (ES$^+$) *m*/*z* = 955 (M+HCl+H$^+$, 20%), 919 (M+H$^+$, 100%), 460 ((M+2H$^+$)/2, 50%); HR-MS (ES$^+$) calcd for $(C_{42}H_{79}N_{24})^+$: 919.6919; found 919.6933. Anal. ($C_{42}H_{78}N_{24}$·6.5HCl·2EtOH·1H_2O) C, H, N, Cl.

Other Analogues. *N*^{2}-12-[(4,6-Diamino-1,3,5-triazin-2-yl)amino]dodecyl-1,3,5-triazine-2,4,6-triamine (9). 1,12-Diaminododecane (**1c**, 200 mg, 0.998 mmol), 2-chloro-4,6-diamino-1,3,5-triazine (320 mg, 2.20 mmol), and $NaHCO_3$ (185 mg, 2.20 mmol) in a solvent mixture of absolute EtOH (20 mL) and water (20 mL) were stirred at 80 °C for 19 h. The solvents were removed in vacuo, and the residue was washed twice with water (5 mL) and recrystallized from a mixture of hot absolute EtOH (25 mL) and water (20 mL). Compound **9** was isolated as white microcrystalline powder (320 mg, 77%): R_f (SiO_2, 35% aq NH_3/MeOH 1:10) 0.73; mp(sealed tube) > 150 °C slow dec (foam), 168−175 °C; ^{1}H NMR (CD_3OD/[D_6]-DMSO 1:1) δ/ppm = 3.12 (m, 4H), 1.38 (m, 4H), 1.15 (m, 16H); NH exchanged; ^{13}C NMR (CD_3OD/[D_6]-DMSO 1:1) δ/ppm = 167.2 (s), 166.7 (s), 166.3 (s), 40.5 (t), 40.4 (t), 29.9 (t), 29.7 (t), 29.6 (t), 29.5 (t), 27.0 (t); MS (ES$^+$) *m*/*z* = 419 (M+H$^+$, 100%); HR-MS (ES$^+$) calcd for $(C_{18}H_{35}N_{12})^+$: 419.3107; found 419.3103. Anal. ($C_{18}H_{34}N_{12}$·0.9H_2O) C, H, N.

***N*^{2},*N*^{4}-Dimethyl-N^6-octadecyl-1,3,5-triazine-2,4,6-triamine (10).** 1-Aminooctadecane (**1g**, 100 mg, 0.371 mmol), 2-chloro-4,6-bis(methylamino)-1,3,5-triazine (130 mg, 0.749 mmol), and $NaHCO_3$ (65 mg, 0.774 mmol) in a solvent mixture of absolute ethanol (10 mL) and water (10 mL) were stirred at 80 °C for 15 h. The solvents were removed in vacuo, and the residue was separated between CH_2Cl_2 (15 mL) and water (15 mL). The aqueous phase was extracted three times with CH_2Cl_2 (10 mL), and the combined organic phases were dried over sodium sulfate. The solvent was removed in vacuo, and the residue (110 mg, off-white solid) was purified by column chromatography on SiO_2 using 5% MeOH in CH_2Cl_2 as eluent. Compound **10** was isolated as white powder (55 mg, 36%): R_f (SiO_2, 5% MeOH in CH_2Cl_2): 0.20: R_f (SiO_2, 35% aq NH_3/MeOH 1:10) 0.50; mp(sealed tube) 58−61 °C; ^{1}H NMR (CD_3OD/$CDCl_3$ 10:1) δ/ppm = 3.31 (m, 2H), 2.86 (m, 6H), 1.54 (m, 2H), 1.25 (m, 30H), 0.87 (m, 3H); NH exchanged; ^{13}C NMR (CD_3OD/$CDCl_3$ 10:1) δ/ppm = 166.4 (s, br), 41.5 (t), 33.1 (t), 30.8 (t), 30.5 (t), 28.0 (t), 27.7 (q), 23.8 (t), 14.5 (q); MS (ES$^+$) *m*/*z* = 407 (M+H$^+$, 100%); HR-MS (ES$^+$) calcd for $(C_{23}H_{47}N_6)^+$: 407.3862; found 407.3866. Anal. ($C_{23}H_{46}N_6$·0.5H_2O) C, H, N.

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References

(1) (a) Trouiller, P.; Olliaro, P. L. Drug development output: what proportion for tropical diseases? *Lancet* **1999**, *354*, 164. (b) Hirst, S. I.; Stapley, L. A. Parasitology: The dawn of a new millennium. *Parasitol. Today* **2000**, *16*, 1−3.

(2) Barrett, M. P. Problems for the chemotherapy of human African trypanosomiasis. *Curr. Opin. Infect. Dis.* **2000**, *13*, 647−651.

(3) (a) WHO Expert Committee *Control and Surveillance of African Trypanosomiasis*. In *WHO Technical Report Series*; World Health Organisation: Geneva, 1998; Vol. 881. (b) http://www.who.int. (c) http://www.who.int/tdr.

(4) Nichols, A. C.; Yielding, L.; Agbe, S. A. O. A Chlorodiazirine Analog of Pentamidine with Anti-Trypanosomal Activity. *J. Parasitol.* **2000**, *86*, 177−180.

(5) (a) Keiser, J.; Burri, C. Physico-chemical properties of the trypanocidal drug melarsoprol. *Acta Tropica* **2000**, *74*, 101−104. (b) Keiser, J.; Ericsson, O.; Burri, C. Investigations of the Metabolites of the trypanocidal drug melarsoprol. *Clin. Pharmacol. Ther.* **2000**, *67*, 478−488.

(6) Pepin, J.; Milord, F. The treatment of human African trypanosomiasis. *Adv. Parasitol.* **1994**, *33*, 1−47.

(7) (a) Legros, D.; Fournier, C.; Etchegorry, M. G.; Maiso, F.; Szumilin, E. Thera peutic failure of melarsoprol among patients treated for late stage of *T. b. gambiense* human African trypanosomiasis in Uganda. *Bull. Soc. Pathol. Exot.* **1999**, *92*, 171−172. (b) Legros, D.; Evans, S.; Maiso, F.; Enyaru, J. C. K.; Mbulamberi, D. Risk factors for treatment failure after melarsoprol for *Trypanosoma brucei gambiense* trypanosomiasis in Uganda. *Trans. R. Soc. Trop. Med. Hyg.* **1999**, *93*, 439−442. (c) Barrett, M. P.; Fairlamb, A. H. The Biochemical Basis of Arsenical-Diamidine Crossresistance in African Trypanosomes. *Parasitol. Today* **1999**, *15*, 136−140.

(8) (a) Gutteridge, W. E.; Coombs, G. H. *Biochemistry of parasitic protozoa*; Macmillan Press LTD: London, 1977. (b) Molyneux, D. H.; Ashford, R. W. *The biology of trypanosoma and leishmania, parasites of man and domestic animals*; Taylor & Francis Ltd.: London, 1983. (c) Ristic, M. *Infectious blood diseases of man and animals, diseases caused by protista*; Weinman, Ed.; Academic Press: New York, 1968; Vol. 2.

(9) van Calenbergh, S.; Herdewijn, P. Rational Development of New Sleeping Sickness Drugs. *Curr. Med. Chem.* **1997**, *4*, 359−384.

(10) Hide, G.; Mottram, J. C.; Coombs, G. H.; Holmes, P. H. *Trypanosomiasis and Leishmaniasis: Biology and Control*; CAB International: Oxon, 1997.

(11) (a) Zilberstein, D. Transport of nutrients and ions across membranes of trypanosomatid parasites. *Adv. Parasitol.* **1993**, 261−291. (b) Hasne, M. P.; Barrett, M. P. Drug uptake via nutrient transporters in *Trypanosoma brucei*. *J. Appl. Microbiol.* **2000**, *89*, 697−701.

(12) (a) Carter, N. S.; Fairlamb, A. H. Arsenical-resistant trypanosomes lack an usual adenosine transporter. *Nature* **1993**, *361*, 173−175. (b) A. H. Fairlamb, personal communication of unpublished data.

(13) Tye, C.-K.; Kasinathan, G.; Barrett, M. P.; Brun, R.; Doyle, V. E.; Fairlamb, A. H.; Weaver, R.; Gilbert, I. H. An Approach To Use An Unusual Adenosine Transporter To Selectively Deliver Polyamine Analogues To Trypanosomes. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 811−816.

(14) De Koning, H. P.; Jarvis, S. M. Adenosine Transporters in Bloodstream Forms of *Trypanosoma brucei brucei*: Substrate Recognition Motifs and Affinity for Trypanocidal Drugs. *Mol. Pharmacol.* **1999**, *56*, 1162−1170.

(15) (a) Kaminsky, R.; Brun, R. in vitro and in vivo activities of trybizine hydrochloride against various pathogenic trypanosome species. *Antimicrob. Agents Chemother.* **1998**, *42*, 2858−2862.

(b) Bacchi, C. J.; Vargas, M.; Rattendi, D.; Goldberg, B.; Zhou, W. Antitrypanosomal Activity of a New Triazine Derivative, SIPI 1029, In Vitro and *In Model* Infections. *Antimicrob. Agents Chemother.* **1998**, *42*, 2718−2721.

(16) Karigiannis, G.; Papaioannou, D. Structure, Biological Activity and Synthesis of Polyamine Analogues and Conjugates. *Eur. J. Org. Chem.* **2000**, 1841−1863.

(17) (a) Kuksa, V.; Buchan, R.; Lin, P. K. T. Synthesis of Polyamines, Their Derivatives, Analogues and Conjugates. *Synthesis* **2000**, 1189−1207. (b) Tabor, C. W.; Tabor, H. Polyamines. *Annu. Rev. Biochem.* **1984**, *53*, 749−790. (c) Marton, L. J.; Pegg, A. E. Polyamines as Targets for Therapeutic Intervention. *Annu. Rev. Pharmacol. Toxicol.* **1995**, *35*, 55−91.

(18) Phillips, M. A.; Coffino, P.; Wang, C. C. *Trypanosoma Brucei* Ornithine Decarboxylase − Enzyme Purification, Characterization, and Expression in Escherichia-Coli. *J. Biol. Chem.* **1988**, *263*, 17933−17941.

(19) Flohe, L.; Hecht, H. J.; Steinert, P. Glutathione and Trypanothione in Parasitic Hydroperoxide Metabolism. *Free Rad. Biol. Med.* **1999**, *27*, 966−984.

(20) Klenke, B.; Gilbert, I. H. Nitrile reduction in the presence of BOC-protected amino groups by catalytic hydrogenation over palladium activated Raney-Nickel. *J. Org. Chem.* **2001**, *66*, 2480−2483.

(21) (a) Pearlman, W. M.; Banks, C. K. Substituted Chlorodiamino-s-triazines. *J. Am. Chem. Soc.* **1948**, *70*, 3726−3728. (b) Irikura, T.; Abe, Y.; Okamura, K.; Higo, K.; Maeda, A.; Morinaga, F.; Shirai, G.; Hatae, S. New s-triazine derivatives as depressants for reticuloendothelial hyperfunction induced by bacterial endotoxin. *J. Med. Chem.* **1970**, *13*, 1081−1089. (c) Katritzky, A. R.; Oniciu, D. C.; Ghiviriga, I.; Barcock, R. A. 4,6-bis-(N,N-Dialkylamino)-s-triazines and 2,4,6-tris-(N,N-Dialkylamino)-s-triazines − Synthesis, NMR Spectra and Restricted Rotations. *J. Chem. Soc., Perkin Trans. 2*, **1995**, 785−792.

(22) (a) Eisa, H. M.; Tantawy, A. S.; El-Kerdawy, M. M. Synthesis of cetain 2-aminoadamantane derivatives as potential antimicrobial agents. *Pharmazie* **1991**, *46*, 182−185. (b) Kelarev, V. I.; Remizov, A. S.; Karakhanov, R. A.; Polivin, Y. N.; Oietaio, D. Synthesis and properties of sym-triazines. 10. Synthesis of 2,4-diamino-sym-triazines containing a sterically hindered phenol substituent. *Chem. Heterocycl. Compd. Engl. Transl.* **1992**, *28*, 1189−1193. (c) Baker, B. R.; Ho, B.-T. Analogues of tetrahydrofolic acid. XXX. Inhibition of dihydrofolic reductase by some 6-substituted 2,4-diamino-s-triazines. *J. Heterocycl. Chem.* **1965**, *2*, 340−343. (d) Overberger, C. G.; Michelotti, F. W.; Carabateas, P. M. Preparation of triazines by the reaction of biguanide and esters. *J. Am. Chem. Soc.* **1957**, *79*, 941−943.

(23) Ponasik, J. A.; Strickland, C.; Faerman, C.; Savvides, S.; Karplus, P. A.; Ganem, B. Kukoamine A and other hydrophobic acylpolyamines: potent and selective inhibitors of *Crithidia fasciculata* trypanothione reductase. *Biochem. J.* **1995**, *311*, 371−375.

(24) Bonnet, B.; Soullez, D.; Davioud-Charvet, E.; Landry, V.; Horvath, D.; Sergheraert, C. New Spermine and Spermidine Derivatives as Potent Inhibitors of *Trypanosoma cruzi* Trypanothione Reductase. *Bioorg. Med. Chem.* **1997**, *5*, 1249−1256.

(25) Chen, S.; Lin, C.-H.; Walsh, C. T.; Coward, J. K. Novel Inhibitors of Thrypanothione Biosynthesis: Synthesis and Evaluation of a Phosphinate Analogue of Glutathionyl Spermidine (GSP), a Potent, Slow Binding Inhibitor of GSP Synthetase. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 505−510.

(26) O'Sullivan, M. C.; Zhou, Q. Novel Polyamine Derivatives as Potent Competitive Inhibitors of *Trypanosoma Cruzi* Trypanothione Reductase. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1957−1960.

(27) Carter, N. S.; Berger, B. J.; Fairlamb, A. H. Uptake of Diamidine Drugs by the P2 Nucleoside Transporter in Melarsen-sensitive and -resistant *Trypanosoma brucei* brucei. *J. Biol. Chem.* **1995**, *270*, 28153−28157.

(28) Räz, B.; Iten, M.; Grether-Bühler, Y.; Kaminsky, R.; Brun, R. The Alamar Blue assay to determine drug sensitivity of African Trypanosomes (*T. b. rhodesiense and T. b. gambiense*) in vitro. *Acta Tropica* **1997**, *68*, 139−147.

(29) Balz, T.; Baltz, D.; Giroud, C.; Crockett, J. Cultivation in a semi-defined medium of animal infective forms of *Trypanosoma brucei, T. equiperdum, T. evansi, T. rhodesiense* and *T. gambiense. EMBO J.* **1985**, *4*, 1273−1277.

(30) Koermendi, O.; Horvath, D. Vereinfacthte Synthesen Des Spermins. *Acta Chim. Acad. Sci. Hung.* **1954**, *4*, 5−8.

(31) Israel, M.; Rosenfield, J. S., Modest, E. J. Analogs of Spermine and Spermidine. I. Synthesis of Polymethylenepolyamines by reduction of cyanoethylated α,ω-alkylenediamines. *J. Med. Chem.* **1964**, *7*, 710−716.

(32) Weinstock, L. T.; Rost, W. J.; Cheng, C. C. Synthesis of new Polyamine Derivatives for Cancer Chemoterapeutic Studies. *J. Pharm. Sci.* **1981**, *70*, 956−959.

(33) Blessing, T.; Remy, J.-S.; Behr, J.-P. Template oligomerization of DNA-Bound cations produces calibrated nanometric particles. *J. Am. Chem. Soc.* **1998**, *120*, 8519−8520.

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