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Original article

Synthesis of a series of N^6-substituted adenosines with activity against trypanosomatid parasites

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Dedicated to Prof. Dr. Detlef Geffken on the occasion of his 65th birthday.

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ABSTRACT

The involvement of purine salvage in the accumulation of current trypanocidal drugs is important for the treatment of African sleeping sickness. The substrate specificity of essential nucleoside transporters is therefore of physiological and pharmacological interest. With the intention to contribute to the knowledge in the field, a series of 16 adenosine derivatives with substituents in N^6-position were prepared in order to evaluate their potential to inhibit *Trypanosoma brucei* spp. in vitro. An unmodified ribose moiety was selected to conserve key molecular recognition motifs of the arsenal of integral membrane proteins expressed in large numbers on the protozoan plasma membrane. Two of the new compounds prepared using a polymer-assisted acylation protocol showed antitrypanosomal activities in the single digit micromolar concentration range.

1. Introduction

One prominent peculiarity widespread among protozoan parasite species is that these pathogens have lost the capability to synthesize purines de novo. This energy saving biochemical handicap is the cause for fundamental differences in the nucleoside utilization machinery of trypanosomes and mammalian organisms. Each parasite has developed a distinct and unique complement of purine transporters and salvage enzymes that are necessary to use purines from the surrounding environment [1]. As a consequence of this efficient parasitic scavenging system, *Trypanosoma brucei*, the parasite that causes sleeping sickness, is solely dependent on its host to supply purines and therefore susceptible to pharmaceutical intervention with nucleoside antimetabolites and nucleoside derived ligands of nucleoside binding pockets such as cordycepin (3′-deoxyadenosine) [2].

Recently, a thorough characterization of the plasma membrane subproteome of bloodstream forms of *T. brucei* revealed that it contains a large number of integral membrane proteins. Besides the abundance of numerous adenylate cyclases, the investigation disclosed 11 nucleoside/nucleobase transporters, and 15 ion pumps and channels [3]. Thus, the activity and selective toxicity of adenosine derived antitrypanosomal agents will be strongly influenced by these individual cell surface transporters that mediate access to the cell. This is because substrate recognition by nucleobase and nucleoside transporters is strikingly different in humans and protozoa, and purine salvage by the parasites is far more efficient at low substrate concentrations [1].

Some current antiprotozoal agents accumulate in the parasites thus obtaining a reasonable therapeutic selectivity by a concentration effect rather than by interaction with specific intracellular, or even so-called "clean" targets [4,5]. Following this rationale, the use of purine derivatives for the treatment of various protozoan infections, including malaria [6–8] and leishmaniasis [9–13], has been investigated by us as well as others. This report is a continuation of this project.

2. Chemistry

Recently, the endogenous occurrence of various N^6-benzyladenosine derivatives such as the derivative **1**, prepared by our group (Fig. 1) [6], and their cytokinin activity has been unraveled by Dolezal et al. [14]. Thus, the nucleophilic displacement of the chlorine atom in **2** with various amines is a straightforward and

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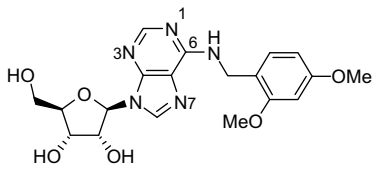

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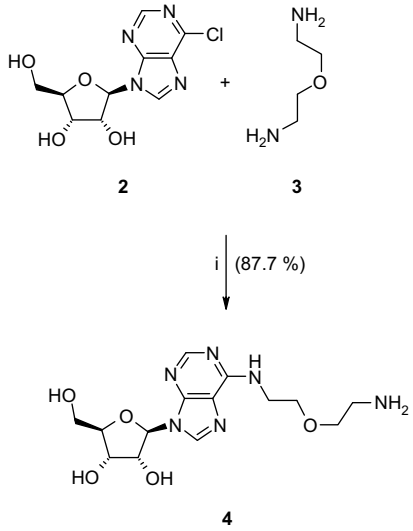
Fig. 1. N^6-(2,4-Dimethoxybenzyl)-adenosine (**1**) – a natural product (prepared by us unknowingly).

highly promising approach for the generation of bioactive compounds. In this project, we aimed at the introduction of amines with a flexible spacer long enough to reach putative lipophilic binding pockets surrounding the targeted adenosine binding motifs. More specific aspects of the rationale for the synthesis of the N^6-substituted adenosines presented here are conferred in the Discussion section. In order to gain access to the target compounds, it would have been one option to synthesize various amines with such a spacer and a lipophilic binding motif attached to it. These amines could have been reacted with **2** to yield novel adenosine derivatives. However, nucleosides formed by the nucleophilic displacement reaction of **2** with amines are often obtained in less than 80% yield and have to be purified by demanding column chromatography.

Therefore, the sequential introduction of the amine-based linker **3** and parallel decoration of the resulting nucleoside **4** with various substituents were envisioned. Thus, one common nucleoside derivative **4** with a spacer of suitable length and polarity was selected and synthesized by the route depicted in Scheme 1 [15]. Only this common scaffold **4** had to be purified by challenging column chromatography. The subsequent introduction of diversity elements through polymer-assisted acylation led to the target compounds that could be isolated by mere filtration over short chromatography columns to remove particulates and invisible impurities.

Initially, a 2-(2-amino-ethoxy)-ethyl substituent was introduced into commercially available 6-deamino-6-chloro adenosine **2** [16] by using an excess of 2-(2-amino-ethoxy)-ethylamine (**3**). The required 2-(2-amino-ethoxy)-ethylamine (**3**), although commercially available, was prepared starting from inexpensive but toxic 1-chloro-2-(2-chloroethoxy)ethane and potassium



Scheme 1. Preparation of N^6-spacer-modified amino scaffold **4**. (i) 1-Propanol, Hünig's base, 12 h 60 °C, 24 h 5 °C, chromatographic purification.

phthalimide followed by treatment with methylamine (not shown). The resulting amine (**3**) is best purified and stored as its dihydrochloride salt. Prior to use, **3** dihydrochloride was suspended in methanol and eluted through a column of strongly basic ion exchange resin with methanol. Aminolysis of **2** with an excess of **3** as free base furnished **4** in good yield.

Meanwhile, polymer-bound acylation reagents were prepared. For the connection of carboxylic acids with primary amines, diverse types of functional anchor groups have been described such as pyrimidinone-linkers [17], *N*-hydroxysuccinimide-linkers [18], *N*-acylindol-linkers [19,20], and 2,4,6-trichloro[1,3,5]triazene-linkers [21], to name only a few. Here, 2,3,5,6-tetrafluoro-4-hydroxybenzoic acid (**5**) was chosen as a commercially available couple & release linker [22]. The labile carboxylic acid-linker bond results from strong electron withdrawing properties of fluoro substituents of the benzene ring. However, the relative high reactivity of this bond is the reason for the limited applicability in solid-phase synthesis protocols. Nevertheless this construct is an attractive choice for the transfer of simple carboxylic acid residues onto nucleophiles such as **4**. During the attachment of 2,3,5,6-tetrafluoro-4-hydroxybenzoic acid (**5**) to an aminomethylated polystyrene through in situ activation by using *N*,*N*′-diisopropylcarbodiimide (DIC) and benzotriazol-1-ol (*N*-hydroxy benzotriazole, HOBt) the unintended formation of phenolic esters occurs. This side reaction can easily be detected by monitoring the diagnostic infrared absorption at 1765 cm^{-1}. Thus, treatment of the resin with a weak base like piperidine and subsequent washing with hydrochloric acid in *N*,*N*-dimethyl formamide (DMF) are advisable. With this simple procedure side-products are destroyed quantitatively and resulting unbound **5** is removed. The appropriate 16 carboxylic acids were then coupled to polymer-bound linker **6** separately. In situ activation by means of DIC treatment in the presence of catalytic amounts of 4-dimethylamino-pyridine (DMAP) yielded 16 polymer reagents of the general structure **7** (Scheme 2).

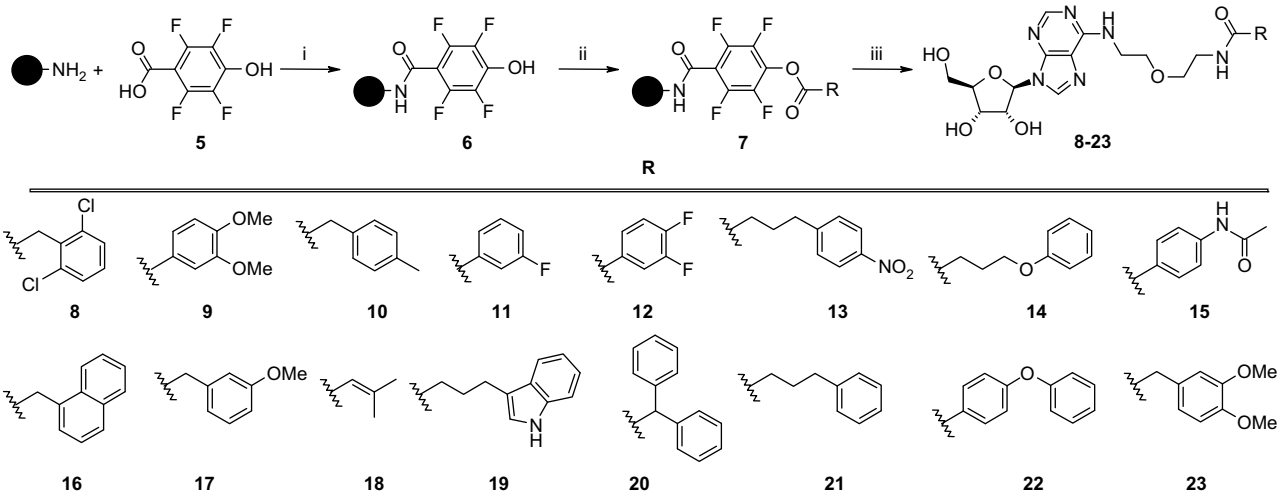
The quantitative acylation of template **4** with an excess of the individual polymer-bound reagents was performed in parallel by reaction in CH_2Cl_2 or THF. After completion of the reactions, the resulting amides **8**–**23** formed were filtered over glass filter funnels and the solvent was removed under reduced pressure. In order to remove notorious invisible impurities such as inorganic salts or tiny polymeric fragments of polystyrene beads all compounds were purified over flash-chromatography columns by using a medium pressure LC system without individual optimization of chromatographic parameters [23].

3. Results

After an operationally simple, parallel chromatographic purification step, 16 adenosine derivatives **8**–**23** were obtained in high yields and excellent purity. The products were subjected to both spectroscopic characterization and biological evaluation. The results of these investigations are reported in Table 1.

4. Discussion

In general, the introduction of structural diversity into the sugar and/or the base moiety of nucleosides represents a validated approach for the generation of active compounds in bioorganic and medicinal chemistry. Specifically, nucleoside transporters of kinetoplastids such as *T. brucei* display high affinity for their substrates and thus can be exploited for the selective accumulation of toxic nucleoside derivatives in pathogenic protozoa [5]. Nonetheless, a critical analysis of the various adenosine transporters that are functionally expressed during the various life-cycle



Scheme 2. Polymer-assisted synthesis of target compounds **8–23**. (i) Coupling conditions (as described [22]): 2,3,5,6-tetrafluoro-4-hydroxybenzoic acid (**5**, 1.7 equiv), DIC (1.5 equiv), DMF, HOBt (1.5 equiv), 25 °C, 16 h, piperidine in DMF, HCl in DMF; (ii) RCOOH (2 equiv) (R s. above), DMAP (0.2 equiv), DIC (2 equiv), DMF, 20 °C, 16 h. (iii) 16 reactions separately: **4**, CH_2Cl_2 or THF, 20 °C, 12 h.

stages of *T. brucei*, has been hampered by the fact that in trypanosomes, which express most genes constitutively in large polycistrons, the presence of mRNA does not guarantee the expression of the corresponding protein product [24].

These membrane proteins have been divided into P1- and P2-type transporters [25]. Both P1 and P2 transporters efficiently transport numerous purine nucleoside analogs, whereas P2 is additionally involved in the accumulation of the front-line trypanocidal drugs pentamidine and melarsoprol by the parasite.

Based on the existing knowledge of P2 substrate recognition motifs, melamine-based nitroheterocycles specifically targeted to the trypanosome were designed and synthesized as novel trypanocides by Baliani et al. [26]. However, it was shown that resistance to the current front-line drugs against African trypanosomiasis might correlate with loss of P2 transporter activity [5]. In this context, studying the P1 adenosine transporters is an important field of research given that the *T. brucei* genome contains multiple equilibrative nucleoside transporter (ENT) family genes encoding P1-type transporters [27].

Al-Salabi et al. reported that the high affinity for purine nucleosides of the NT10/AT-B P1-type transporter is much reduced when the H-bond-accepting ring nitrogen atoms, N3 and N7, are replaced by carbon atoms [28]. Whereas the ribose sugar of adenosine is known not to be involved in the binding of the P2 transporter, the ribose is important for binding to another P1-like transporter, NT9/AT-Dl [28]. For this reason we chose to synthesize adenosine derivatives with an intact sugar moiety in this project. Concerning the aminopurine moiety, the relatively large difference between the adenosine and inosine binding energies can be explained by the existence of a strong H-bond between the 6-amino group and the NT9/AT-D binding pocket, and a possible weak H-bond with N1.

The aminopurine nucleoside cordycepin was shown to be a potent trypanocide and its uptake is mediated by the purine transporters P1 and P2. While cordycepin is highly active in vitro, it shows poor antitrypanocidal activity in vivo unless coadministered with an adenosine deaminase inhibitor. Furthermore, it interacts with multiple mammalian proteins. For instance, it could be shown that cordycepin inhibits the growth of B16-BL6 mouse melanoma cells through the stimulation of the adenosine A3 receptor followed by glycogen synthase kinase-3β (GSK) activation and cyclin D1 suppression. This is of interest for antineoplastic chemotherapy because both inhibitors of cyclin dependant kinases (CDKs) and GSK are promising therapeutic targets [29,30]. Obviously, removal of the 3′OH group is compatible with high trypanocidal activity (cordycepin lacks the 3′-OH function), even if the potentially important influence on the favored sugar pucker in solution at 37 °C remains elusive. However, published models for binding of adenosine to *T. b. brucei* nucleoside transporters suggest that the optimal affinity of adenosine analogs for the *T. b. brucei* P1 nucleoside transporter in some stages of the parasite life-cycle may require intact 3′ and 5′ OH groups, in addition to the N-3 and N-7 H-bond acceptors [28,4,31]. Affinity for the P2 adenine/adenosine transporter is reported to involve the presence of an N^6 H-bond donor in conjunction with an N-1 H-bond acceptor, an aromatic ring contributing to π–π interactions, and a group at the position corresponding to N-9, which takes on electrostatic interactions [5]. On the basis of these findings, various di- or trisubstituted adenosine derivatives are also of interest as potential antitrypanosomal agents. However, di- or trisubstituted adenosine derivatives tend to have a higher log $P_{O/W}$ and are thus prone to passive diffusion through membranes, are not ideal substrates for transport proteins and have poor pharmacokinetic properties. In order to identify active compounds that might effectively use P1 and P2 binding motifs we decided to design N^6-monoalkylated adenosines with

Table 1
Purity and biological activity of target compounds **8–23**.

Entry	Purity[a] (%)	IC_{50}[b] [μM]
8	100	49.7
9	99.8	>170
10	100	n.d.[c]
11	99.6	13.6
12	100	167.3
13	100	88.7
14	100	3.4
15	93.5	131.7
16	97.4	>170
17	99.3	131.9
18	100	125.3
19	99.8	37.6
20	100	85.9
21	99.5	4.0
22	99.2	10.0
23	100	9.2

[a] HPLC, 100% method, detection at 254 nm.
[b] *T. b. rhodesiense*. IC_{50} for standard drug (melarsoprol): 10 nM
[c] Compound **10** was not included in the biological investigation (n.d. = not determined).

a balanced polar surface area, lipophilicity and molmass as well as stability to adenosine deaminases.

The molecular target of the compounds synthesized is not known. Our assumption is that the compounds are accumulated inside the trypanosome and bind to a large number of enzymes, some of which comprise adenosine binding motifs with varying affinities. In this respect, promiscuity in ligand–target interaction with many different enzymes of the adenosine salvage pathway or enzymes utilizing adenosine containing substrates such as ATP or NAD^+ might be regarded as an advantage in terms of the parasites ability to acquire resistance. Additionally, the fact that purine analogs enter the parasites through multiple distinct transport proteins as well as passive diffusion might be suited to prevent the onset of resistance to this class of compounds [32].

The highly analogous compounds **14** and **21** show the same desired activity in the selected model. While single digit micromolar activity is only moderate it seems warranted to make additional modifications to the adenosine molecule in order to generate enough data for a complete SAR-study. The synthesis of new adenosine derived amino-functionalized templates can be achieved either by established procedures or by using novel glycosylation reactions [33,34]. The parallel derivatization, however, can be achieved fast and efficiently with the polymer-bound acylating species reported here.

5. Conclusion

Through introduction of a spacer fragment decorated with a nucleophilic aliphatic primary amine, adenosine was transformed into a scaffold for the introduction of diversity at a predefined position. By using polymer-bound acylating species, clean and high yielding transformation of the unprotected, enantiomerically pure amino alcohol was easily achieved. The complex scaffold did not have to be attached to a linker or polymer but nevertheless the resulting mixtures could easily be separated by filtration. After consumption of the limiting nucleoside derivative, the suspensions obtained contain only the target molecules in solution along with polymer-bound reagents. The already highly pure test compounds could be further purified rapidly and efficiently by flash or medium pressure chromatography, yielding substances of sufficient purity for biological evaluation. This approach enables the rapid generation of complex molecules that are not found in presently known libraries. Such compounds will be useful in providing new insights into the complexities of nucleoside transport in trypanosomes.

6. Experimental section

^{1}H NMR spectra were recorded on a Jeol Eclipse+ 500 spectrometer, using tetramethylsilane as internal standard. The purity of the target compounds was deduced from ^{1}H NMR data as well as evaluated by HPLC, using a Dionex Summit HPLC-system with a diode array detector and CC 125/4 Nucleodur 100-5 C18 ec columns, supplied by Macherey-Nagel and water/methanol gradients. Purity was calculated using the UV data at 254 nm using Chromeleon 6.50 software. A second, lower frequency (220 nm) was monitored in order to be able to identify impurities with low absorption coefficients at the standard wavelength 254 nm, which are notoriously present in small molecule libraries. Due to the fact, that all samples were purified over reversed-phase columns prior to analysis, these impurities – if present – were effectively removed prior to analysis and thus absent in detectable amounts. TLC reaction control was performed on Macherey-Nagel Polygram Sil G/UV_{254} precoated microplates, spots were visualized under UV-illumination at 254 nm. IR-spectra for the detection of erroneously formed 4-hydroxy-2,3,5,6-tetrafluorobenzoic acid esters on the solid support were recorded as KBr tablets on a Nicolet 510P FT-IR spectrometer. High resolution MS data were obtained on a Micromass Autospec instrument (ESI, methanol (1/1, v/v) infusion at 10 μl/min with polyethylene glycol as reference).

6.1. 2-(2-Amino-ethoxy)-ethylamine (*3*)

To a solution of 30 mmol (3.51 ml) 1-chloro-2-(2-chlorethoxy)ethane in 300 ml DMF was added 120 mmol (22.2 g) of potassium phthalimide. The resulting suspension was kept at reflux and stirred for 24 h. The suspension was poured onto crushed ice and the resulting precipitate was filtrated and washed with 70 ml aqueous sodium hydroxide solution (0.1 mol/l), subsequently with 70 ml water. The residue was dried under reduced pressure.

Intermediate product: 1-phthaloyl-2-(2-phthaloyl-ethoxy)-ethane ^{1}H NMR (500 MHz, [D_6]-DMSO and 1 drop D_2O) = δ (ppm) 7.80–7.77 (m, 4H, benzene), 7.69–7.66 (m, 4H, benzene), 3.71–3.66 (m, 4H, ethylene), 3.65–3.60 (m, 4H, ethylene). An amount of 13.7 mmol (5 g) of this material was dissolved in 200 ml of a solution of 33% methylamine in methanol. The mixture was stirred for 5 h. Afterwards the solvent was removed under reduced pressure and the remaining residue was suspended in 8 ml of water. Column chromatography using strongly basic ion exchange resin (Dowex 1 × 2, 200–400 mesh, OH^--form) yielded an aqueous solution of the desired amine, which was further concentrated in vacuo. Yield 49% (699 mg). ^{1}H NMR (500 MHz, [D_6]-DMSO + 1 drop D_2O) = δ (ppm) 3.34 (t, 4H, J = 5.8 Hz, ethylene), 2.65 (t, 4H, J = 5.6 Hz, ethylene).

Storage as hydrochloric acid salt: amine **3** is dissolved in ice cooled diethylether. Subsequent addition of hydrochloric acid leads to the formation of the corresponding crystalline dihydrochloride. Yield 93% ^{1}H NMR (500 MHz, [D_6]-DMSO + 1 drop D_2O) = δ (ppm) 3.95 (t, 4H, J = 4.6 Hz, ethylene), 3.29 (t, 4H, J = 4.6 Hz, ethylene).

6.2. N^6-[2-(2-Amino-ethoxy)-ethyl]adenosine (*4*)

To a solution of 500 mg (1.74 mmol) 1-(6-Chloro-purin-9-yl)-β-D-1-deoxyribofuranose (**2**) in 22.5 ml 1-propanol were added 1.40 g (13.44 mmol) of 2-(2-Amino-ethoxy)-ethylamine (**3**) and 300 μl Hünig's base. The mixture was held at 60 °C for 12 h. Reaction control via TLC indicated quantitative conversion. The solvent was removed under reduced pressure and the remaining oily residue was dissolved in 20 ml methanol/water (1:4). Chromatographic purification was achieved using a short column filled with strongly basic ion exchange resin (Dowex 1 × 2, 200–400 mesh, OH^--form). The product containing fractions was collected and the solvent was removed under in vacuo. Yield 88% (543 mg). ^{1}H NMR (500 MHz, [D_6]-DMSO + 1 drop D_2O) = δ (ppm) 8.35 (s, 1H, C8–H), 8.22 (bs, 1H, C2–H), 7.82 (bs, 1H very broad, N^6–H), 5.89 (d, 1H, J = 6,1 Hz, 1′H), 4.61 (m, 1H, 2′H), 4.15 (m, 1H, 3′H), 3.97 (m, 1H, 4′H), 3.69–3.54 (m, 6H, 5′H overlapping ethylene), 3.39 (t, 2H, J = 5.7 Hz, ethylene), 2.65 (m, 2H, ethylene). ^{13}C NMR: (126 MHz, [D_6]-DMSO + 1 drop D_2O) = δ (ppm) 154.6, 152.2, 148.4, 139.7, 119.7, 87.9, 85.8, 73.4, 72.5, 70.5, 68.5, 61.5, 41.2 (one signal is missing due to peak overlapping) HR-ESI-MS $[M+H]^+$ calcd 355.1730 found 355.1741; mp 147 °C.

6.3. General procedure for the preparation of simple polymer supported acid, using 4-hydroxy-2,3,5,6-tetrafluorobenzoic acid (*5*)

To a flask containing 1 g of 4-hydroxy-2,3,5,6-tetrafluorobenzoic acid loaded onto aminomethylated polystyrene (**6**) (prepared from very high load aminomethylated polystyrene, purchased from Novabiochem, Switzerland, batch number A20540) with an initial loading level of 1.1 mmol/g was added 20 ml DMF and the resin was

allowed to swell for 10 min [22]. Subsequently, 2 equiv (2.2 mmol) of the appropriate carboxylic acid and 0.22 mmol (27 mg) of DMAP were added to the suspended resin. After the addition of 2.2 mmol (195 μl) of DIC, the mixture was agitated at room temperature for 16 h. The resin beads were filtered off and washed exhaustively with DMF (three times 15 ml), dichloromethane (three times 15 ml) and THF (three times 15 ml) and afterwards dried under in vacuo. After careful drying the increase in weight was determined.

6.4. General procedure for the preparation of compounds **8–23** using chemset **7**

200 mg of resin loaded with the appropriate carboxylic acid (loading level approximately 1 mmol carboxylic acid per g) was swollen in 2 ml dichloromethane (or alternatively THF). A solution of **4** in a few drops THF (as little solvent as possible) was added to the suspension. After 12 h agitation at ambient temperature (20 °C) in most cases complete conversion of the amine **4** to the target carboxylic acid amide **8–23** could be observed via TLC. Subsequently the resin was washed with dichloromethane and THF and the collected fractions were evaporated under reduced pressure. To ensure product purity, all samples were further purified via MPLC.

6.5. N^6-(2-{2-[2-(2,6-Dichlorophenyl)acetamido]-ethoxy}ethyl)adenosine (**8**)

Conversion rate 98%. Purity (HPLC after MPLC) = 100% (254 nm). ^{1}H NMR (500 MHz, [D$_6$]-DMSO) = δ (ppm) 8.35 (s, 1H, C8–H), 8.22 (bs, 1H, C2–H), 8.10 (m, 1H, amido), 7.72 (bs, 1H, N^6–H), 7.42 (m, 2H, benzene), 7.29 (m, 1H, benzene), 5.88 (d, 1H, J = 5.6 Hz, 1′H), 5.38 (m, 1H, 3′OH), 5.33 (m, 1H, 5′OH), 5.12 (m, 1H, 2′OH), 4.60 (m, 1H, 2′H), 4.15 (m, 1H, 3′H), 3.96 (m, 1H, 4′H), 3.80 (s, 2H, benzyl–CH$_2$), 3.70–3.58 (m, 6H, 5′H overlapping ethylene), 3.47 (m, 2H, ethylene), 3.36 (m, 2H, ethylene overlapping H$_2$O). HR-ESI-MS [M + H]$^+$ calcd 541.1369 found 541.1387.

6.6. N^6-{2-[2-(3,4-Dimethoxybenzamido)ethoxy]ethyl}adenosine (**9**)

Conversion rate 83%. Purity (HPLC after MPLC) = 99.8% (254 nm). ^{1}H NMR (500 MHz, [D$_6$]-DMSO) = δ (ppm) 8.34 (m, 2H, C8–H overlapping amido), 8.20 (bs, 1H, C2–H), 7.71 (bs, 1H, N^6H), 7.44 (m, 2H, benzene), 6.98 (d, 1H, J = 8.5 Hz, benzene), 5.89 (d, 1H, J = 6.1 Hz, 1′H), 5.37 (d, 1H, J = 6.2 Hz, 3′OH), 5.32 (m, 1H, 5′OH), 5.11 (d, 1H, J = 4.7 Hz, 2′OH), 4.60 (m, 1H, 2′H), 4.14 (m, 1H, 3′H), 3.96 (m, 1H, 4′H), 3.79 (s, 3H, methoxy), 3.78 (s, 3H, methoxy), 3.70–3.60 (m, 4H, 5′H overlapping ethylene), 3.55 (m, 2H, ethylene), 3.40 (m, 2H, ethylene), 3.36 (m, 2H, ethylene overlapping H$_2$O). HR-ESI-MS [M + H]$^+$ calcd 519.2203 found 519.2226.

6.7. N^6-(2-{2-[2-(4-Methylphenyl)acetamido]ethoxy}ethyl)adenosine (**10**)

Conversion rate 100%. Purity (HPLC after MPLC) = 100% (254 nm). ^{1}H NMR (500 MHz, [D$_6$]-DMSO) = δ (ppm) 8.33 (s, 1H, C8–H), 8.20 (bs, 1H, C2–H), 7.98 (bs, 1H, N^6–H), 7.69 (m, 1H, amido), 7.10 (m, 2H, Benzene), 7.05 (m, 2H, Benzene), 5.88 (m, 1H, 1′H), 5.37 (m, 1H, 3′OH), 5.32 (m, 1H, 5′OH), 5.11 (m, 1H, 2′OH), 4.59 (m, 1H, 2′H), 4.14 (m, 1H, 3′H), 3.95 (m, 1H, 4′H), 3.71–3.51 (m, 6H, 5′H overlapping ethylene and benzyl–CH$_2$), 3.43 (m, 2H, ethylene), 3.33 (m, 2H, ethylene), 3.18 (m, 2H, ethylene), 2.23 (s, 3H, methyl). HR-ESI-MS [M + H]$^+$ calcd 487.2305 found 487.2295.

6.8. N^6-{2-[2-(3-Fluorobenzamido)ethoxy]ethyl}adenosine (**11**)

Conversion rate 96%. Purity (HPLC after MPLC) = 99.6% (254 nm). ^{1}H NMR (500 MHz, [D$_6$]-DMSO) = δ (ppm) 8.57 (t, 1H, J = 5.6 Hz, amido), 8.34 (s, 1H, C8–H), 8.20 (bs, 1H, C2–H), 7.74–7.67 (m, 2H, N^6–H overlapping benzene), 7.63 (m, 1H, benzene), 7.50 (m, 1H, benzene), 7.36 (m, 1H, benzene), 5.88 (d, 1H, J = 6,1 Hz, 1′H), 5.39 (m, 1H, 3′OH), 5.33 (m, 1H, 5′OH), 5.13 (m, 1H, 2′OH), 4.60 (m, 1H, 2′H), 4.15 (m, 1H, 3′H), 3.96 (m, 1H, 4′H), 3.71–3.53 (m, 8H, 5′H overlapping ethylene and H$_2$O), 3.43 (m, 2H, ethylene). HR-ESI-MS [M + Na]$^+$ calcd 499.1717 found 499.1743.

6.9. N^6-{2-[2-(3,4-Difluorobenzamido)ethoxy]ethyl}adenosine (**12**)

Conversion rate 100%. Purity (HPLC after MPLC) = 100% (254 nm). ^{1}H NMR (500 MHz, [D$_6$]-DMSO) = δ (ppm) 8.59 (t, 1H, J = 5.4 Hz, amido), 8.34 (s, 1H, C8–H), 8.20 (bs, 1H, C2–H), 8.10 (bs, 1H, N^6–H), 7.88 (m, 1H, benzene), 7.72 (m, 1H, benzene), 7.53 (m, 1H, benzene), 5.89 (d, 1H, J = 6.1 Hz, 1′H), 5.38 (m, 1H, 3′OH), 5.33 (m, 1H, 5′OH), 5.13 (m, 1H, 2′OH), 4.60 (m, 1H, 2′H), 4.15 (m, 1H, 3′H), 3.96 (m, 1H, 4′H), 3.69–3.61 (m, 4H, 5′H overlapping ethylene), 3.55 (m, 2H, ethylene), 3.42 (m, 2H, ethylene), two protons are missing due to signal overlapping with water. HR-ESI-MS [M + H]$^+$ calcd 495.1804 found 495.1778.

6.10. N^6-(2-{2-[4-(4-Nitrophenyl)butanamido]ethoxy} ethyl)adenosine (**13**)

Conversion rate 95%. Purity (HPLC after MPLC) = 100% (254 nm). ^{1}H NMR (500 MHz, [D$_6$]-DMSO) = δ (ppm) 8.34 (s, 1H, C8–H), 8.20 (bs, 1H, C2–H), 8.14 (d, 2H, J = 8.7 Hz, benzene), 7.81 (t, 1H, J = 5.4 Hz, amido), 7.70 (bs, 1H, N^6–H), 7.46 (d, 2H, J = 8.7 Hz, benzene), 5.88 (d, 1H, J = 6.0 Hz, 1′H), 5.38 (d, 1H, J = 6.1 Hz, 3′OH), 5.33 (m, 1H, 5′OH), 5.13 (d, 1H, J = 4.7 Hz, 2′OH), 4.60 (m, 1H, 2′H), 4.15 (m, 1H, 3′H), 3.97 (m, 1H, 4′H), 3.70–3.53 (m, 6H, 5′H ethylene), 3.44 (t, 2H, J = 5.8 Hz, ethylene), 3.20 (m, 2H, ethylene overlapping H$_2$O), 2.69 (t, 2H, J = 7.6 Hz, butanamido), 2.09 (t, 2H, J = 7.4 Hz, butanamido), 1.82 (m, 2H, butanamido). HR-ESI-MS [M + H]$^+$ calcd 546.2312 [M + H]$^+$ found 546.2338.

6.11. N^6-(2-{2-[4-(Phenoxy)butanamido]ethoxy}ethyl)adenosine (**14**)

Conversion rate 98%. Purity (HPLC after MPLC) = 100% (254 nm). ^{1}H NMR (500 MHz, [D$_6$]-DMSO) = δ (ppm) 8.34 (s, 1H, C8–H), 8.21 (bs, 1H, C2–H), 7.86 (t, 1H, J = 5.4 Hz, amido), 7.70 (bs, 1H, N^6–H), 7.26 (m, 2H, phenoxy), 6.90 (m, 3H, phenoxy), 5.88 (d, 1H, J = 6.1 Hz, 1′H), 5.39 (d, 1H, J = 6.2 Hz, 3′OH), 5.33 (m, 1H, 5′OH), 5.13 (d, 1H, J = 4.7 Hz, 2′OH), 4.59 (m, 1H, 2′H), 4.15 (m, 1H, 3′H), 3.94 (m, 3H, 4′H overlapping butanamido), 3.70–3.52 (m, 6H, 5′H overlapping ethylene), 3.43 (t, 2H, J = 5.9 Hz, ethylene), 3.21 (m, 2H, ethylene overlapping H$_2$O), 2.24 (t, 2H, J = 7.4 Hz, butanamido), 1.91 (m, 2H, butanamido). HR-ESI-MS [M + H]$^+$ calcd 517.2411 found 517.2442.

6.12. N^6-{2-[2-(4-Acetamido-benzamido)ethoxy]ethyl} adenosine (**15**)

Conversion rate 93%. Purity (HPLC after MPLC) = 93.5% (254 nm). ^{1}H NMR (500 MHz, [D$_6$]-DMSO) = δ (ppm) 10.09 (s, 1H, acetamido), 8.34 (s, 1H, C8–H), 8.32 (t, 1H, J = 4.6 Hz, amido), 8.21 (bs, 1H, C2–H), 7.78 (d, 2H, J = 8.7 Hz, benzene), 7.73 (bs, 1H, N^6–H), 7.62 (d, 2H, J = 8.6 Hz, benzene), 5.88 (d, 1H, J = 6.1 Hz, 1′H), 5.39 (m, 1H, 3′OH), 5.33 (m, 1H, 5′OH), 5.13 (m, 1H, 2′OH), 4.60 (m, 1H, 2′H), 4.15 (m, 1H, 3′H), 3.96 (m, 1H, 4′H), 3.83–3.52 (m, 8H, 5′H

overlapping ethylene and water), 3.41 (m, 2H, ethylene), 2.06 (s, 3H, acetyl–CH_3). HR-ESI-MS $[M+H]^+$ calcd 538.2026 found 538.2052.

6.13. N^6-(2-{2-[2-(1-Naphthyl)acetamido]ethoxy}ethyl) adenosine (**16**)

Conversion rate 98%. Purity (HPLC after MPLC) = 97.4% (254 nm). ^{1}H NMR (500 MHz, [D_6]-DMSO) = δ (ppm) 8.35 (s, 1H, C8–H), 8.22 (bs, 1H, C2–H), 8.17 (m, 1H, amido), 8.07 (d, 1H, J = 7.4 Hz, naphthyl), 7.89 (m, 1H, naphthyl), 7.79 (m, 1H, naphthyl), 7.71 (bs, 1H, N^6–H), 7.49 (m, 2H, naphthyl), 7.41 (m, 2H, naphthyl), 5.89 (d, 1H, J = 6.0 Hz, 1′H), 5.40 (m, 1H, 3′OH), 5.33 (m, 1H, 5′OH), 5.14 (m, 1H, 2′OH), 4.60 (m, 1H, 2′H), 4.15 (m, 1H, 3′H), 3.96 (m, 1H, 4′H), 3.90 (s, 2H, methylene), 3.71–3.51 (m, 6H, 5′H overlapping ethylene and water), 3.46 (m, 2H, ethylene), 3.24 (m, 2H, ethylene overlapping H_2O). HR-ESI-MS $[M+H]^+$ calcd 523.2305 found 523.2288.

6.14. N^6-(2-{2-[2-(4-Methoxyphenyl)acetamido]ethoxy} ethyl)adenosine (**17**)

Conversion rate 89%. Purity (HPLC after MPLC) = 99.3% (254 nm). ^{1}H NMR (500 MHz, [D_6]-DMSO) = δ (ppm) 8.35 (s, 1H, C8–H), 8.21 (bs, 1H, C2–H), 8.03 (t, 1H, J = 5.4 Hz, amido), 7.70 (bs, 1H, N^6–H), 7.17 (t, 1H, J = 7.8 Hz, benzene), 6.83–6.75 (m, 3H, benzene), 5.89 (d, 1H, J = 6.1 Hz, 1′H), 5.37 (d, 1H, J = 6.2 Hz, 3′OH), 5.33 (m, 1H, 5′OH), 5.13 (d, 1H, J = 4.6 Hz, 2′OH), 4.60 (m, 1H, 2′H), 4.15 (m, 1H, 3′H), 3.96 (m, 1H, 4′H), 3.72 (s, 3H, methoxy), 3.69–3.52 (m, 6H, 5′H overlapping ethylene), 3.45 (t, 2H, J = 5.8 Hz, ethylene), 3.37 (s, 2H, methylene), 3.12 (m, 2H, ethylene). HR-ESI-MS $[M+H]^+$ calcd 503.2254 found 503.2273.

6.15. N^6-{2-[2-(3,3-Dimethylacrylamido)ethoxy]ethyl} adenosine (**18**)

Conversion rate 92%. Purity (HPLC after MPLC) = 100% (254 nm). ^{1}H NMR (500 MHz, [D_6]-DMSO) = δ (ppm) 8.35 (s, 1H, C8–H), 8.22 (bs, 1H, C2–H), 7.72 (m, 2H, amido overlapping N^6–H), 5.89 (d, 1H, J = 6.0 Hz, 1′H), 5.65 (s, 1H; acryl–CH), 5.38 (d, 1H, J = 6.2 Hz, 3′OH), 5.34 (m, 1H, 5′OH), 5.12 (d, 1H, J = 4.7 Hz, 2′OH), 4.60 (m, 1H, 2′H), 4.15 (m, 1H, 3′H), 3.97 (m, 1H, 4′H), 3.71–3.52 (m, 6H, 5′H overlapping ethylene), 3.45 (t, 2H, J = 5.8 Hz, ethylene), 3.22 (m, 2H, ethylene), 2.06 (s, 3H, CH_3), 1.77 (m, 3H, CH_3). HR-ESI-MS $[M+H]^+$ calcd 437.2149 found 437.2164.

6.16. N^6-(2-{2-[4-(3-Indolyl)-butanamido]ethoxy}ethyl) adenosine (**19**)

Conversion rate 92%. Purity (HPLC after MPLC) = 99.8% (254 nm). ^{1}H NMR (500 MHz, [D_6]-DMSO) = δ (ppm) 10.70 (s, 1H, indole–NH), 8.35 (s, 1H, C8–H), 8.21 (bs, 1H, C2–H), 7.79 (m, 1H, amido), 7.70 (bs, 1H, N^6–H), 7.49 (d, 1H, J = 8.2 Hz, indole), 7.32 (d, 1H, J = 8.1 Hz, indole), 7.08 (m, 1H, indole), 7.04 (m, 1H, indole), 6.95 (m, 1H, indole), 5.89 (d, 1H, J = 6.1 Hz, 1′H), 5.38 (d, 1H, J = 6.1 Hz, 3′OH), 5.34 (m, 1H, 5′OH), 5.13 (m, 1H, 2′OH), 4.61 (m, 1H, 2′H), 4.15 (m, 1H, 3′H), 3.97 (m, 1H, 4′H), 3.70–3.52 (m, 6H, 5′H overlapping ethylene and H_2O), 3.44 (t, 2H, J = 5.9 Hz, ethylene), 3.20 (m, 2H, ethylene), 2.66 (t, 2H, J = 7.3 Hz, butyryl), 2.14 (m, 2H, butyryl), 1.85 (m, 2H, butyryl). HR-ESI-MS $[M+H]^+$ calcd 540.2571 found 540.2595.

6.17. N^6-(2-{2-[2-(1,1′-Diphenyl)acetamido]ethoxy}- ethyl)adenosine (**20**)

Conversion rate 90%. Purity (HPLC after MPLC) = 100% (254 nm). ^{1}H NMR (500 MHz, [D_6]-DMSO) = δ (ppm) 8.42 (bs, 1H, N^6H), 8.38 (m, 2H, C8H overlapping amido), 8.25 (s, 1H, C2H), 7.36 (m, 4H, 4-phenoxybenzyl), 7.20 (m, 3H, phenyl), 7.18 (m, 1H, phenyl), 7.10 (t, 1H, 4-phenoxybenzyl, J = 7.4 Hz), 6.96 (m, 4H, 4-phenoxybenzyl), 5.87 (d, 1H, 1′H, J = 5.9 Hz), 4.68 (m, 3H, 2′H overlapping benzyl), 4.07 (m, 1H, 3′H), 3.94 (m, 1H, 4′H), 3.48 (s, 2H, CH_2), 3.36–3.46 (m, 2H, 5′H overlapped by water). HR-ESI-MS $[M+H]^+$ calcd 549.2462 found 549.2486.

6.18. N^6-{2-[2-(4-Phenylbutanamido)ethoxy]ethyl}adenosine (**21**)

Conversion rate 89%. Purity (HPLC after MPLC) = 99.5% (254 nm). ^{1}H NMR (500 MHz, [D_6]-DMSO) = δ (ppm) 8.34 (s, 1H, C8–H), 8.20 (bs, 1H, C2–H), 7.79 (t, 1H, J = 5.4 Hz, amido), 7.70 (bs, 1H, N^6–H), 7.26 (m, 2H, benzene), 7.16 (m, 3H, benzene), 5.89 (d, 1H, J = 6.1 Hz, 1′H), 5.38 (m, 2H, 3′OH overlapping 2′OH), 5.33 (m, 1H, 5′OH), 4.61 (m, 1H, 2′H), 4.15 (m, 1H, 3′H), 3.97 (m, 1H, 4′H), 3.83–3.75 (m, 2H, butyryl), 3.70–3.53 (m, 6H, 5′H overlapping ethylene), 3.45 (t, 2H, J = 6.0 Hz, ethylene), 3.19 (m, 2H, ethylene), 2.54 (t, 2H, J = 7.7 Hz, butyryl), 2.08 (t, 2H, J = 7.4 Hz, butyryl). HR-ESI-MS $[M+H]^+$ calcd 501.2462 found 501.2437.

6.19. N^6-(2-[2-(4-Phenoxybenzamido)ethoxy]ethyl)adenosine (**22**)

Conversion rate 89%. Purity (HPLC after MPLC) = 99.2% (254 nm). ^{1}H NMR (500 MHz, [D_6]-DMSO) = δ (ppm) 8.41 (t, 1H, J = 5.6 Hz, amido), 8.33 (s, 1H, C8–H), 8.21 (bs, 1H, C2–H), 7.86 (d, 2H, J = 8.8 Hz, benzene), 7.73 (bs, 1H, N^6–H), 7.43 (m, 2H, benzene), 7.20 (t, 1H, J = 7.0 Hz, benzene), 7.08 (d, 2H, J = 7.6 Hz, benzene), 7.02 (d, 2H, J = 8.8 Hz, benzene), 5.89 (d, 1H, J = 6.1 Hz, 1′H), 5.38 (d, 2H, J = 6.1 Hz, 3′OH), 5.33 (m, 1H, 5′OH), 5.13 (d, 1H, J = 4.7 Hz, 2′OH), 4.61 (m, 1H, 2′H), 4.15 (m, 1H, 3′H), 3.96 (m, 1H, 4′H), 3.83–3.53 (m, 8H, 5′H overlapping ethylene and H_2O), 3.42 (t, 2H, J = 6.0 Hz, ethylene). HR-ESI-MS $[M+H]^+$ calcd 551.2254 found 551.2246.

6.20. N^6-(2-[2-(3,4-Dimethoxyphenylacetamido)ethoxy] ethyl)adenosine (**23**)

Conversion rate 100%. Purity (HPLC after MPLC) = 100% (254 nm). ^{1}H NMR (500 MHz, [D_6]-DMSO) = δ (ppm) 8.37 (s, 1H, C8–H), 8.20 (bs, 1H, C2–H), 8.05 (t, 1H, J = 5.5 Hz, amido), 7.71 (bs, 1H, N^6–H), 6.83 (m, 2H, benzene), 6.78 (m, 1H, benzene), 5.93 (d, 1H, J = 6.1 Hz, 1′H), 5.37 (d, 1H, J = 6.2 Hz, 3′OH), 5.33 (m, 1H, 5′OH), 5.12 (d, 1H, J = 4.6 Hz, 2′OH), 4.60 (m, 1H, 2′H), 4.15 (m, 1H, 3′H), 3.96 (m, 1H, 4′H), 3.74 (s, 6H, methoxy), 3.69–3.51 (m, 6H, 5′H overlapping ethylene), 3.45 (t, 2H, J = 5.8 Hz, ethylene), 3.36 (s, 2H, benzyl–CH_2), 3.12 (m, 2H, ethylene). HR-ESI-MS $[M+H]^+$ calcd 533.2360 found 533.2377.

6.21. In vitro microplate assay against T. brucei rhodesiense

Minimum essential medium (50 μl) supplemented according to a known procedure with 2-mercaptoethanol and 15% heat-inactivated horse serum was added to each well of a 96-well microtiter plate [35]. Serial drug dilutions were prepared covering a range from 90 to 0.123 μg/ml. Then 10^4 bloodstream forms of *T. b. rhodesiense* STIB 900 in 50 μl were added to each well and the plate incubated at 37 °C under a 5% CO_2 atmosphere for 72 h. Alamar Blue (10 μl containing 12.5 mg resazurin dissolved in 1000 ml distilled water) was then added to each well and incubation continued for

a further 2–4 h. The Alamar blue dye is an indicator of cellular growth and/or viability. The blue, non-fluorescent, oxidized form becomes pink and fluorescent upon reduction by living cells. The plate was then read in a Spectramax Gemini XS microplate fluorometer (Molecular Devices) using an excitation wavelength of 536 nm and emission wavelength of 588 nm [36]. Fluorescence development was measured and expressed as percentage of the control. Data were transferred into the graphic programme Softmax Pro (Molecular Devices) which calculated IC_{50} values. Melarsoprol served as standard.

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