Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 159–165

BIOORGANIC & MEDICINAL CHEMISTRY

Improving an Antitrypanosomal Lead Applying Nucleophilic Substitution on a Safety Catch Linker

Abolfasl Golisade,[a] Claudia Herforth,[a] Ludo Quirijnen,[b] Louis Maes[b] and Andreas Link[a,*]

[a]*Institut für Pharmazie, Abteilung für Pharmazeutische Chemie, Universität Hamburg, Bundesstrasse 45, D-20146 Hamburg, Germany*

[b]*Tibotec Group N.V., Intercity Business Park, Mechelen Noord, Zone L, Generaal de Wittelaan 11B 3, B-2800 Mechelen, Belgium*

Dedicated to Prof. Dr. J. Thiem on the occasion of his 60th birthday

Received 26 March 2001; accepted 10 July 2001

Abstract—In a joint effort with various laboratories we have been aiming at the structure-based design of glycolysis inhibitors as anti-trypanosomal drugs. 2′-Deoxy-2′-(3-methoxybenzamido)-N^6-(1-naphtylmethyl)adenosine (**1a**) was thus revealed as a promising lead structure for the development of selective agents against protozoan parasites. Here we describe the polymer-assisted synthesis of novel amido derivatives of the scaffold 2′-amino-2′-deoxy-N^6-(1-naphtylmethyl)adenosine (**5a**) we reported recently. This building block synthesized in solution was treated with an excess of polymer-supported carboxylic acids leading to chemoselective, practically quantitative conversion of the amine to the desired analogous amides. The best compound (**1h**) from this series was obtained after on-bead nucleophilic substitution of the carboxylic acid equivalent attached to the Kenner safety catch linker and exhibited an improved inhibitory effect on *T. b. brucei* blood stream forms with an IC_{50} of 0.85 μM in vitro

Introduction

The prevalence of African trypanosomiasis, that seemed to decrease steadily in the 1960s, is now greater than ever in sub-Saharan regions of the African continent. Despite many promising reports on novel drugs against trypanosomes under development, research in this area is still highly demanded.[1–4]

Ideally, the quest for selective drugs for the therapy of infectious diseases starts from the identification of target structures that are absent in the host while exhibiting essential functions for the infective agent.[5] Alternatively, targeting the binding sites of coenzyms rather than the often well-conserved active sites of enzymes offers a rational starting point for the generation of selective inhibitors of protozoan parasites.[6,7] In this respect, a broad array of targets that utilize adenosyl-containing substrates like ATP or NAD^+ bear the opportunity for the rational design of ligands starting from the adenosine scaffold. Introducing diversity into either the carbohydrate and/or base subunits of adenosine represents promising strategies to identify inhibitors of enzymes that are dependent on binding nucleoside derivatives to attain activity.

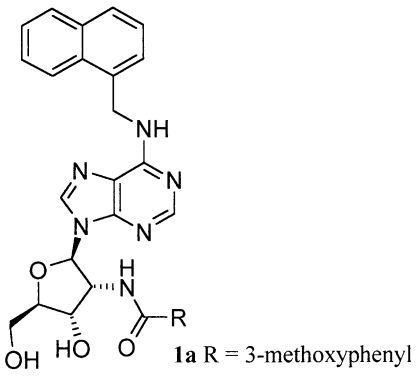
Recent reports have demonstrated that several potent and selective inhibitors of glycolytic enzymes in *Kinetoplastidae* can be discovered by this approach and that these compounds decrease survival of trypanosomes in vitro.[8] One of these seven glycolytic enzymes involved in the conversion of glucose to 3-phosphoglycerate is glyceraldehyde-3-phosphate dehydrogenase GAPDH.[9]

Chemistry

Using information derived from theoretical calculations and experimental data from X-ray crystallography on the binding orientation of an N^6-benzylated NAD^+-derivative to the coenzyme-pocket of protozoal GAPDH, 2′-deoxy-2′-(3-methoxybenzamido)-N^6-(1-naphtylmethyl)adenosine (**1a**) was synthesized and shown to exhibit a marked inhibitory potency and a

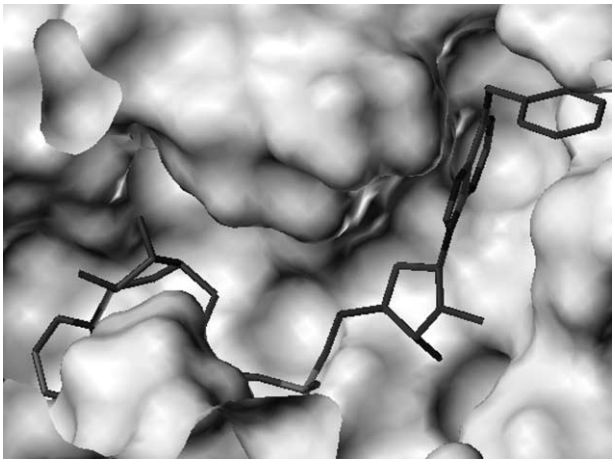
*Corresponding author. Tel.: +49-40-42838-3467; fax: +49-40-42838-6573; e-mail: link@uni-hamburg.de

0968-0896/02/$ - see front matter
PII: S0968-0896(01)00253-X



1a R = 3-methoxyphenyl

Figure 1. Lead structure **1a**.



Picture 1. N^6-benzyl NAD^+-derivative inside *L. mexicana* GAPDH.

considerable selectivity for the protozoal enzymes as opposed to the human counterpart.[10] The development of this compound resulted from the rational attempt to combine structural elements that demonstrated beneficial effects for binding and inhibition, separately (Fig. 1, Picture 1).[7]

The benzyl substituent (Picture 1, upper right corner) of a N^6-benzyl NAD^+-derivative fills a narrow hydrophobic pocket in the non-functional adenosine binding site that may lead to development of ligands with high binding affinity (coordinates from the homologous *Leishmania mexicana* GAPDH; PDB-identifier: 1GYQ, authors: S. Suresh, W. Hol[10]). At the same time, another lipophilic cleft at the 2′-region (lower right corner), that is occluded in the human counterpart of GAPDH, opens the way for the design of selective inhibitors of trypanosomes.[7] Generally, amido substitution of deoxyadenosines leads to compounds with retained hydrogen bond donor capabilities on the substitution site in contrast to *O*-acylated adenosine derivatives.[11]

As to be anticipated, the measured effects of substitution in the 2′-region and on the N^6-nitrogen atom cannot easily be correlated.[11] Therefore, for optimization of the lead structure **1a,** a combinatorial approach allowing for the synthesis of arrays of selected modifications is an interesting objective to be addressed. Because synthetic strategies applying polymer supports as solubility control auxiliaries in order to facilitate the generation of molecular libraries have proven to be a powerful tool in drug discovery, we decided to synthesize a set of lead structure analogues by polymer-supported or polymer-assisted protocols.[12–18] Thus, we applied 2′-amino-2′-deoxy-N^6-(1-naphthylmethyl)adenosine (**5a**) as a core building block to access a series of 2′-amido-2′-deoxy-N^6-(1-naphthylmethyl)adenosine derivatives (**1b–l**).

The synthesis of the structurally demanding common scaffold **5a** was performed from the antiviral drug vidarabine (**2**) following reported procedures.[19,20]

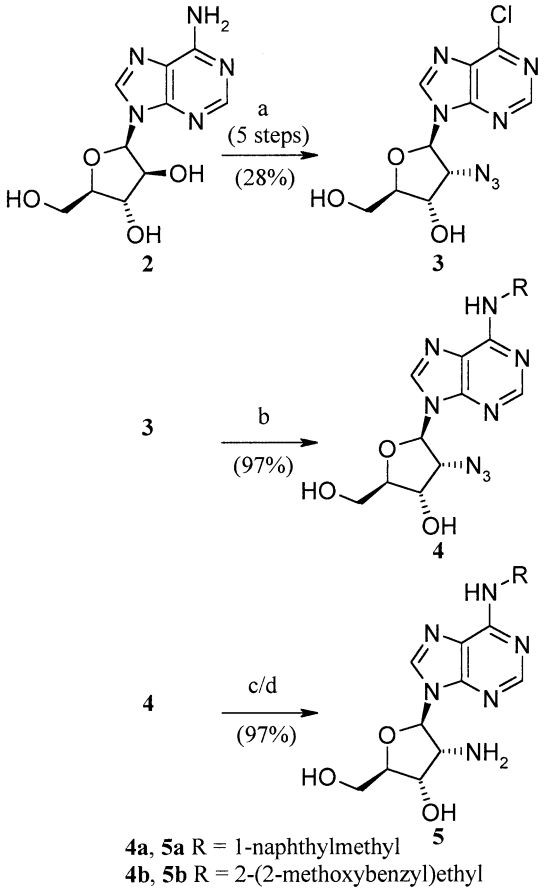
We investigated the versatility of this classical synthesis via 6-chloropurine for the synthesis of alternatively N^6-substituted 2′-amino-2′-deoxyadenosine intermediates because of the general interest in the chemistry of N^6-substituted adenosine derivatives that apparently increased lately.[21] Pd-catalyzed hydrogenation had been reported to proceed slowly and incomplete, for example for 2′-azido-2′-deoxy-N^6-dimethyladenosine by Ikehara and Takatsuka.[22] Consequently we felt the necessity to evaluate the effectiveness of this synthetic step for the preparation of analogous amino nucleoside scaffolds. The synthesis of model compound **5b** was carried out as outlined in Scheme 1 and proceeded well.

For fast and simple introduction of different acid residues in parallel, a polymer-assisted acylation protocol was applied. Therefore commercially available carboxylic acids were coupled to the Kenner safety catch linker improved by Backes et al., by established amide bond forming procedures.[23–30]

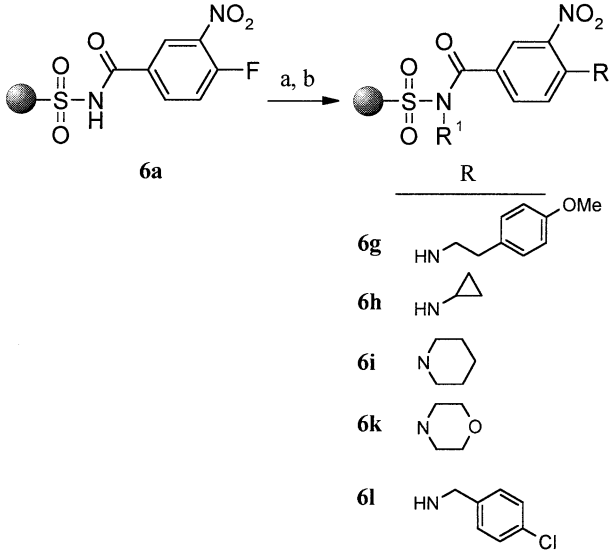
Prior to the polymer-assisted reaction with scaffold **5a**, the Kenner linker has to be activated by *N*-alkylation to form a good leaving group as described by Backes et al. or by use of trimethylsilyldiazomethane.[26,29] The polymer bound acids subsequently form the desired amides upon agitation with a solution of an appropriate amine at slightly elevated temperatures.

With the intention of widening the range of structural modifications achievable on the 2′-amino position beyond the scope of commercially available carboxylic acids, intermediate nucleophilic substitution of polymer-bound residues as a source for diversity was incorporated into the sequence. Because the range of available amines is exceptionally broad, this drug discovery approach might be regarded as especially attractive.[31] 4-Fluoro-3-nitro-benzoic acid was thus attached to the sulfamoyl linker yielding intermediate **6a** and subsequently converted to the corresponding aniline derivatives **6g–l** by treatment with excess amines (Scheme 2).

Alkylation with bromoacetonitrile as activation step for the Kenner linker is reported to give poor results for aromatic residues adjacent to the sulfamoyl attachment site.[26] For that reason we used prolonged reaction times for this activation protocol and employed commercially



Scheme 1. Synthesis of compounds **5a** and **5b**. (a) (i) TIPDSCl, pyridine; (ii) TfCl, DMAP, CH_2Cl_2; (iii) NaN_3, DMF; (iv) CCl_4, isoamyl nitrite, 60 °C; (v) TBAF, THF, 50 °C, 5 h; (b) **4a**: 1-naphthylmethylamine, 1-propanol, 50 °C, 16 h, **4b**: 2-(2-methoxyphenyl)ethylamine, 1-propanol, 50 °C, 16 h; (c) **5a**: InN/H_4Cl, EtOH ΔT; (d) **5b**: Pd/C 10%, H_2 (2 bar), dioxane, 6 h.



Scheme 2. Formation of aniline derivatives **6g–l** from fluoro-analogue **6a**. (a) Appropriate amine (excess), DMF. $R^1 = H$; (b) Activation by treatment with trimethylsilyldiazomethan in hexanes for 24 h ($R^1 = CH_3$) or $BrCH_2CN$, DIPEA in NMP 48 h ($R^1 = CH_2CN$).

available trimethylsilyl diazomethane as an alternative activation reagent to ensure optimal results and for means of comparison. However, in both cases, the yields of the compounds obtained were generally good. The predicted less effective protocol using bromoacetonitrile turned out to be slightly superior within this series of compounds. Apparently, the advantage of a putative higher ratio of activation with trimethylsilyldiazomethan is overcompensated by the resulting less effective activation as compared to the electron-withdrawing variant (Table 1).

A novel interesting alternative activation procedure via Mitsunobu reaction of polymer bound acyl sulfonamides using pentafluorobenzyl alcohol, triphenylphosphine, and diisopropyl azodicarboxylate was added to this list of techniques only recently.[32] Alkylation of the aniline-nitrogen in **6g–l** was observed in neither case.

Biological evaluation

The series of 2′-amido-2′-deoxy-N^6-(1-naphthylmethyl)-adenosines **1b–l** as well as the related compounds **4a**, **4b** and **5a**, **5b** were tested for in vitro activity upon trypomastigotes (*T. b. brucei*) as described by Hirumi et al.[33] For means of comparison, the most active compound of this class **1a** reported so far was included in the evaluation as well as N^6-(1-naphthylmethyl)adenosine (**7**) itself (structure not shown). The results obtained are summarized below (Table 2).

Discussion

Because the Kenner linker modified by Ellman and his group tolerates strongly basic or acidic conditions prior to activation, on-bead modifications of carboxylic acids attached to the linker permit the construction of polymer bound carboxylic acid equivalent libraries. We demonstrated its value as a tool for the simple on-bead modification by nucleophilic substitution as source of diversity. Subsequent parallel modification of aminodeoxynucleosides was shown to be a straightforward concept to obtain desired 2′-amido-2′-deoxy-N^6-(1-naphthylmethyl)adenosines. The first series of compounds obtained by this approach showed marked inhibitory activity in vitro. Compounds **1b** and **1h–l** displayed inhibitory concentrations comparable to the analogue with the lowest IC_{50} versus *T. b. brucei* GAPDH described before (**1a**), all in the same order of

Table 1. Results of alternative activation protocols

Entry 1	From **6h–l**, R1 = CH_3		From **6h–l**, R1 = CH_2CN	
	Purity[a] (%)	Yield[b] (%)	Purity[a] (%)	Yield[b] (%)
h	92	88	94	96
i	94	85	96	92
k	92	96	97	95
l	95	94	95	96

[a]Purity of crude reaction filtrate determined via semi-preparative MPLC, 100% method, detection at 254 nm.
[b]Isolated material obtained by evaporation of purified product containing fractions.

Table 2. In vitro activity upon trypomastigotes (*T. b. brucei*)

Compd	Yield (%)[a]	Residue R in structures **1**	IC_{50}[b] (μM)
1a	95	3-Methoxyphenyl	5
1b	97	2-(3-Indolyl)ethyl	4
1c	96	3-(3-Indolyl)propyl	19
1d	93	3-(3,5-Dichlorophenoxy)propyl	>32
1e	96	(3-Thienyl)methyl	>32
1f	95	3,5-Difluorophenyl	17
1g	85	4-[2-(4-Methoxyphenyl)ethyl]amino-3-nitrophenyl	16
1h	88–96	4-Cyclopropylamino-3-nitrophenyl	0.85
1i	85–92	3-Nitro-[4-(piperid-1-yl)]phenyl	3.5
1k	95–96	3-Nitro-[4-(morpholin-1-yl)]phenyl	4
1l	94–96	(4-Chlorobenzyl)amino-3-nitrophenyl	8
4a			>32
4b			>32
5a			>32
5b			>32
7			4
Suramin			0.059

[a]Isolated material obtained by evaporation of purified product containing fractions.
[b]IC_{50} values were determined by measuring growth inhibition at 3–5 concentrations. IC_{50} values for **1h** and **1i** were confirmed by repeated experiments (identical conditions, 5 concentrations) and are reported as arithmetic mean of repeated experiments.

magnitude. The IC_{50} of the apparently most active compound **1h** of 0.85 μM was confirmed in repeated experiments, ranging from 0.7 μM to 1.0 μM.

Surprisingly even the 2′-unsubstituted adenosine derivative N^6-(1-naphthylmethyl)adenosine (**7**) included for means of comparison, showed a marked inhibition of *T. b. brucei* trypomastigotes of $IC_{50} = 4$ μM in vitro. This result is not in accordance with results from enzyme based assays. The IC_{50} for the highly analogous GAPDH of *L. mexicana* has been determined to be much higher (150 μM).[8] Therefore the question arises, whether the activity of **7** versus trypomastigotes is not attributed to inhibition of GAPDH alone. This finding will be addressed by future investigations. Therefore compounds **1h** and **7** will be immobilized on agarose beads and surface plasmon resonance detection devices and used to screen for hitherto not identified binding partners from cell fractions and an array of commercially available enzymes. This well established technique was shown to be a valuable tool in a project for the evaluation of target structures for the novel class of cyclin-dependent kinase inhibitors called paullones we reported recently.[34] The increased inhibitory activity of compound **1h** in comparison to the lead **1a** will most likely become helpful in this approach because weak affinity binders usually do not lead to straightforward target identification.

In our initial attempts unchanged acid equivalents were transferred to building blocks originating from solution-phase chemistry.[30] Because the coupling to the resin and subsequent transfer to building blocks in solution after activation is just an advanced use of coupling reagents, this approach cannot be regarded as a solid-phase synthesis (SPOS) technique. Due to this reason, we consider this application of the Kenner linker for the construction of chemoselective acylating reagents a special kind of polymer-assisted solution phase (PASP) synthesis.[35,36] In the series **1g–l** reported here, acid residues are altered by on-bead modification prior to activation of the linker and subsequent transfer. In this case one should not consider this approach a PASP sequence but more or less a SPOS or PASP/SPOS hybride technique. Because acid residues can be transferred that otherwise would have had to be synthesized and purified separately, the additional operations of attachment and activation become much more economical and this approach represents a considerable advantage in the process of 'high-speed-analogueing'.

Conclusion

We were successful in the identification of a lead structure analogue with improved biological activity using on-bead nucleophilic substitution on a safety catch linker as source for diversity. Additional modifications of the improved lead structure **1h** in the region of the 5′-OH seem to be beneficial in cases where the binding region of the nicotinamide moiety of NAD^+ can be targeted as well.[37] Because structural modifications in different molecular regions of this class of compounds are not easy to correlate due to conformational flexibility, a library of polymer-supported acids obtained as described above might become a valuable tool for the synthesis of amide libraries based on multi-substituted nucleoside scaffolds in the future.

Experimental

Identity of all compounds was assigned by NMR spectroscopy. Sample purity was deduced from ^{1}H NMR data as well as evaluated by MPLC. Purity is reported as purity of crude products prior to purification (percentage of target compound contained in residue from evaporated reaction mixture). Yields are reported as isolated material obtained by evaporation of purified product containing fractions. ^{1}H NMR spectra were recorded on a Bruker AMX 400 spectrometer, using tetramethylsilane as internal standard. MPLC simultaneous purity analyses/purifications were performed using a

Büchi 681 pump (flow rate 10 mL, MeOH/H_2O 70:30), and UV-detector (254 nm) with Merck 310-25 Lobar-LiChroprep®-RP-18 columns. TLC was performed on Macherey-Nagel Polygram® Sil G/UV_{254} precoated microplates, spots were visualized under UV-illumination. Infrared spectra were recorded using KBr pellets on a Perkin Elmer 1660 FT-IR spectrometer. MS data (FAB) was obtained on a Finnigan MAT 311A instrument with *m*-nitrobenzylic alcohol as matrix. Solvents were purified according to standard procedures and freshly distilled prior to use. Standard glass ware was oven dried at 150 °C and kept in a desiccator.

General procedure A for the synthesis of the polymer supported acids 6h–l. To a flask containing 52.0 g of dry 4-sulfamylbenzamidomethyl polystyrene with an initial loading level of 1.25 mmol/g as determined by elemental analysis (prepared from very high load aminomethylated polystyrene batch number A20540, purchased from Novabiochem, Switzerland) was added 500 mL of THF. The resin was allowed to swell at room temperature for 2 h. In another flask, 44.4 g (240 mmol) of 4-fluoro-3-nitro benzoic acid was dissolved in 500 mL dry THF and preactivated via in situ anhydride formation by adding 18.6 mL (120 mmol) *N*,*N*-diisopropylcarbodiimide overnight. **CAUTION**: *N*,*N*-diisopropylcarbodiimide may lead to severe allergic reactions, strictly avoid skin contact. After addition of 15 mL Hünig's base (88 mmol) and 500 mg (4.1 mmol) 4-(dimethylamino)pyridine as catalyst, to the swollen resin, the coupling mixture was added. The resulting reaction mixture was agitated at room temperature for 48 h. The resin beads were filtered off and washed exhaustively with THF (two times), methanol (two times), and THF (two times) resulting in 53.48 g resin after careful drying in vacuo. This weight increase of 11.48 g corresponds to a practically quantitative conversion of 4-sulfamylbenzamidomethyl polystyrene to [(4-fluoro-3-nitrobenzoyl)4-sulfamyl]benzamidomethyl polystyrene **6a** resulting in a loading level of 1.00 mmol/g. For the derivatization of **6a** to resins **6h–l**, 2.0 g **6a** (2.0 mmol) each was treated with 20 mmol of the appropriate amine in 25 mL DMF overnight and subsequently washed with DMF (three times), methanol (two times), and THF (two times).

Activation method A1. 400 mg of resin **6h–l** were subsequently swollen in THF and treated with 4.0 mL (trimethylsilyl)diazomethane 1 M solution in hexanes (4.0 mmol) purchased from Aldrich for 24 h and washed with THF (10 mL), methanol (three times 10 mL), and THF (10 mL).

Activation method A2. The sulfonamide linker of 400 mg **6h–l** was activated for cleavage by alkylation with 640 μL (9 mmol) bromoacetonitrile **CAUTION**: alkylating agent, strictly avoid skin contact, and 340 μL (2 mmol) Hünig's base in 3 mL 1-methylpyrrolidone for 48 h. The dark brown slurry was washed with dry dimethylsulfoxide (5×5 mL) and THF (5×10 mL) yielding off-white to yellowish resin particles.

General procedure B for the synthesis of 2′-amido-2′-deoxy-N^6-(1-naphthylmethyl)adenosines 1h–l. The polymer supported activated acids **6h–l** were transferred to the amino group of 4.06 mg (10 μmol) of 2′-amino-2′-deoxy-N^6-(1-naphthylmethyl)adenosine (**5a**) by shaking at 55 °C in 5 mL THF. The reaction was monitored by TLC and terminated when the starting material was quenched (6–24 h). Polymer beads and particulates were removed by filtration, the beads where washed exhaustively with dry THF and the combined THF fractions were evaporated. ^{1}H NMR experiments of the crude material obtained as well as MPLC analyses revealed high purity of the compounds and the absence of impurities in detectable quantities other than traces of starting material or acid hydrolyzed from the resin.

2′-[(4-Cyclopropylamino-3-nitro)benzamido]-2′-deoxy-N^6-(1-naphthylmethyl)adenosine (1h). Compound **1h** was prepared from resin **6h** synthesized by general procedure A using cyclopropyl amine. Following general procedure B, activation method A1 88%, activation method A2 96% yellow product was obtained. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) = 8.77 (bs, 1H,), 8.66 (s, 1H), 8.58 (d, 1H, J = 8.14 Hz), 8.37 (s, 1H), 8.29–8.14 (m, 3H), 8.05–8.02 (m, 1H), 7.96–7.94 (m, 1H), 7.83–7.81 (m, 1H), 7.59–7.52 (m, 2H), 7.46–7.34 (m, 3H), 6.28 (d, 1H, J = 8.14 Hz), 5.40–5.31 (m, 1H), 5.18 (bs, 2H), 4.35 (d, 1H, J = 5.09 Hz), 4.14–4.08 (m, 1H), 3.77–3.69 (m, 1H), 3.65–3.58 (m, 1H), 2.72–2.63 (m, 1H), 0.91–0.87 (m,2H), 0.68–0.59 (m, 2H), HRFAB-MS $[M+H]^+$ calcd = 611.2367 found = 611.2351, MPLC purity: 92% for A1, 94% for A2.

2′-Deoxy-N^6-(1-naphthylmethyl)-2′-[3-nitro-4-(piperid-1-yl)benzamido]adenosine (1i). Compound **1i** was prepared from resin **6i** synthesized by general procedure A using piperidine. Following general procedure B, activation method A1 85%, activation method A2 92% orange product was obtained. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 8.46 (bs, 1H,), 8.38 (d, 1H, J = 2.03 Hz), 8.10–8.07 (m, 1H), 7.99–7.97 (dd, 1H, J = 2.03, 6.61 Hz), 7.90–7.88 (m, 1H), 7.85–7.83 (d, 1H, J = 8.14 Hz), 7.78 (bs, 1H), 7.56–7.51 (m, 3H), 7.46–7.43 (t, 1H, J = 8.14, 7.12 Hz), 7.10–7.08 (d, 1H, J = 8.65 Hz), 6.21 (bs, 1H), 6.07 (bs, 1H), 5.83 (d, 1H, J = 4.07 Hz), 5.70 (dd, 1H, J = 3.06, 5.59), 5.30 (bs and s overlapped, 3H), 5.21 (dd, 1H, J = 4.07, 4.58), 4.59–4.52 (m, 1H), 4.14–4.11 (m, 1H), 3.99–3.92 (m, 1H), 3.17–3.14 (m, 4H), 1.78–1.61 (m, 6H), HRFAB-MS $[M+H]^+$ calcd = 638.2601 found = 638.2588, MPLC purity: 94% for A1, 96% for A2.

2′-Deoxy-2′-[4-(morphol-1-yl)-3-nitro]benzamido-N^6-(1-naphthylmethyl)adenosine (1k). Compound **1k** was prepared from resin **6k** synthesized by general procedure A using morpholine. Following general procedure B, activation method A1 96%, activation method A2 95% yellow product was obtained. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 8.46 (bs, 1H), 8.42 (d, 1H, J = 2.03 Hz), 8.10–8.03 (m, 2H), 7.90–7.87 (m, 1H), 7.85 (d, 1H, J = 8.65 Hz), 7.78 (bs, 1H), 7.56–7.49 (m, 1H), 7.44 (t, 1H, J = 8.14, 7.12 Hz), 7.13 (d, 1H, J = 9.16 Hz), 6.20 (bs, 1H), 6.04 (bs, 1H), 5.84 (d, 1H, J = 4.07 Hz), 5.71 (dd, 1H, J = 2.54, 6.11 Hz), 5.30 (bs and s overlapped, 3H), 5.22 (dd, 1H, J = 4.07, 4.58 Hz), 4.60–4.54 (m, 1H),

4.14 (dd, 1H, $J=1.02$, 11.70), 3.98–3.96 (m, 1H), 3.86 (t, 4H, $J=4.58$ Hz), 3.17 (t, 4H, $J=4.58$), HRFAB-MS $[M+H]^+$ calcd = 640.2394, found = 640.2395, MPLC purity: 92% for A1, 97% for A2.

2′-[4-(4-Chlorobenzyl)amino-3-nitro]benzamido-2′-deoxy-N^6-(1-naphthylmethyl)adenosine (1l). Compound **1l** was prepared from resin **6l** synthesized by general procedure A using 4-chlorobenzyl amine. Following general procedure B, activation method A1 94%, activation method A2 96% yellow product was obtained. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 8.83 (d, 1H, $J=1.52$ Hz), 8.70 (t, 1H, $J=5.09$ Hz), 8.46 (bs, 1H), 8.10–8.07 (m, 1H), 8.00–7.97 (m, 1H), 7.90–7.88 (m, 1H), 7.85 (d, 1H, $J=8.14$ Hz), 7.79 (bs, 1H), 7.56–7.51 (m, 4H), 7.44 (t, 1H, $J=4.14$, 7.12 Hz), 7.37 (d, 2H, $J=8.65$ Hz), 7.29 (d, 2H, $J=8.65$ Hz), 6.84 (d, 1H, $J=9.16$ Hz), 6.19 (bs, 1H), 6.05 (bs, 1H), 5.83 (d, 1H, $J=4.58$ Hz), 5.69 (dd, 1H, $J=3.05$, 4.59 Hz), 5.30 (bs and s overlapped, 3H), 5.19 (dd, 1H, $J=4.07$, 4.58 Hz), 4.60 (d, 2H, $J=5.59$ Hz), 4.57–4.55 (m, 1H), 4.14 (d, 1H, $J=11.19$ Hz), 3.98–3.96 (m, 1H), HRFAB-MS $[M+H]^+$ calcd = 694.2055, found = 694.2074, MPLC purity: 95% for A1 and A2.

2′-Azido-2′-deoxy-N^6-[2-(2-methoxyphenyl)ethyl]adenosine (4b). An amount of 330 mg (1.06 mmol) **3** was dissolved in 20 mL 1-propanol. To this solution were added 200 mg (1.35 mmol) 2-(2-methoxyphenyl)ethylamine and 250 μL (1.50 mmol) Hünig's base. The reaction mixture was stirred at 50 °C for 16 h. Evaporation of the solvent and purification via column chromatography (4.5×15 cm, *n*-hexane/EtOAc 1:1) gave 430 mg (97%) of a pale yellow foam. ir (KBr): 2110 cm^{-1} (N_3). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) = 8.38 (s, 1H), 8.24 (bs, 1H), 7.89 (s, 1H), 7.21–7.15 (m, 2H), 6.99 (d, 2H, $J=7.63$ Hz), 6.85 (t, 1H, $J=7.63$, 7.12 Hz), 6.07–6.04 (m, 2H, 1′H and 3′OH overlapped), 5.30 (t, 1H, $J=5.60$ Hz), 4.64 (t, 1H, $J=6.10$, 5.60 Hz), 4.57–4.51 (m, 1H), 4.01 (m, 1H), 3.78 (s, 3H); 3.72–3.64 (m, 3H), 3.60–3.53 (m, 1H), 2.91 (t, 2H, $J=7.12$ Hz) HRFAB-MS $[M+H]^+$ calcd: 433.1737 found: 433.1742.

2′-Amino-2′-deoxy-N^6-[2-(2-methoxyphenyl)ethyl]adenosine (5b). A solution of 300 mg (0.74 mmol) **4b**, in dioxane (150 mL) was purged with nitrogen and charged with 10% Pd-C (100 mg). The reaction mixture was shaken under hydrogen (pressure 2 bar) for 6 h. The catalyst was removed by filtration through silica gel. Evaporation of the solvent and purification over silica gel (1.5×15 cm, EtOAc) and DOWEX™ 2×OH (4.5×15 cm, MeOH/H_2O 70:30) sequentially gave 290 mg (97%) of the title compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) = 8.29 (bs, 1H) 8.21 (bs, 1H), 7.84 (bs, 1H), 7.21–7.15 (m, 1H), 6.97 (d, 2H, $J=8.14$ Hz), 6.85 (t, 1H, 1′H, $J=7.63$, 7.12 Hz), 5.68 (d, 1H, $J=8.14$ Hz), 5.56–5.46 (m, 2H), 4.04–3.94 (m, 3H), 3.78 (s, 3H), 3.71–3.63 (m,3H), 3.57–3.48 (m, 1H); 2.91 (t, 2H, $J=7.63$, 7.12 Hz), HRFAB-MS $[M+H]^+$ calcd: 401.1937 found: 401.1913.

In vitro activity upon trypomastigotes (*T. b. brucei*). Blood stream forms of *T. b. brucei* were cultivated in HMI-9 medium as described by Hirumi et al.[33] In a 96-well microplate, 10,000 haemoflagellates were incubated at different drug concentrations for 4 days. Parasite multiplication was evaluated microscopically. IC_{50} values were determined by measuring growth inhibition at 3–5 concentrations. Suramin was included as standard agent.

Acknowledgements

This work was supported by the Fonds der Chemischen Industrie FCI, the Deutsche Pharmazeutische Gesellschaft DPhG, and the Deutsche Forschungsgemeinschaft DFG (Graduiertenkolleg 464). Helpful discussion and encouragement by Prof. Dr. D. Geffken is gratefully acknowledged.

References and Notes

1. *Control and Surveillance of African Trypanosomiasis Report of a WHO Expert Committee*; WHO: Geneva, 1998; Vol. 881.
2. Pécoul, B.; Gastellu, M. *Lancet* **1999**, *354*, 955.
3. Kaminsky, R.; Brun, R. *Antimicrob. Agents Chemother.* **1998**, *42*, 2858.
4. Zhou, W.; Xin, Z.; Zhang, X.; Shen, J.; Qiu, Q. *Yao Hsueh Hsueh Pao* **1996**, *31*, 823.
5. Jomaa, H.; Wiesner, J.; Sanderbrand, S.; Altincicek, B.; Weidemeyer, C.; Hintz, M.; Turbachova, I.; Eberl, M.; Zeidler, J.; Lichtenthaler, H.; Soldati, D.; Beck, E. *Science* **1999**, *285*, 1573.
6. Opperdoes, F. R. *Annu. Rev. Microbiol.* **1987**, *41*, 127.
7. Verlinde, C. M. L. J.; Callens, M.; Van Calenbergh, S.; Van Aerschot, A.; Herdewijn, P.; Hannaert, V.; Michels, P. A. M.; Opperdoes, F. R.; Hol, W. G. J. *J. Med. Chem.* **1994**, *37*, 3605.
8. Aronov, A. M.; Verlinde, C. L. M. J.; Hol, W. G. J.; Gelb, M. H. *J. Med. Chem.* **1998**, *41*, 4790.
9. Verlinde, C. M. L. J.; Hannaert, V.; Blonski, C.; Willson, M.; Périé, J. J.; Fothergill-Gillmore, L. A.; Opperdoes, F. R.; Hol, W. G. J.; Michels, P. A. M. *Drug Res. Updates* **2001**, *4*, 1.
10. Aronov, A. M.; Suresh, S.; Buckner, F. S.; Voorhis, W. C. V.; Verlinde, C. L. M. J.; Opperdoes, F. R.; Hol, W. G. J.; Gelb, M. H. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4273.
11. Van Calenbergh, S.; Verlinde, C. M. L. J.; Soenens, J.; De Bruyn, A.; Callens, M.; Blaton, N. M.; Peeters, O. M.; Rozenski, J.; Hol, W. G.; Herdewijn, P. *J. Med. Chem.* **1995**, *38*, 3838.
12. Früchtel, J. S.; Jung, G. *Angew. Chem. Int. Ed.* **1996**, *35*, 17.
13. Hall, S. *Mol. Diversity* **1999**, *4*, 131.
14. James, I. W. *Mol. Diversity* **1998**, *3*, 181.
15. Nefzi, A.; Dooley, C. T.; Ostresh, J. M.; Houghten, R. A. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2273.
16. Smith, J. R. *Biomed. Health Res.* **1999**, *22*, 171.
17. Flynn, D. L. *Med. Res. Rev.* **1999**, *19*, 409.
18. Link, A. *Angew. Chem. Int. Ed.* **2000**, *39*, 4039.
19. Robins, M.; Wnuk, S.; Hernández-Thirring, A.; Samano, M. *J. Amer. Chem. Soc.* **1996**, *118*, 11341.
20. Golisade, A.; Van Calenbergh, S.; Link, A. *Tetrahedron* **2000**, *56*, 3167.
21. Lin, X.; Robins, M. *Org. Lett.* **2000**, *2*, 3497.
22. Ikehara, M.; Takatsuka, Y. *Chem. Pharm. Bull.* **1978**, *26*, 985.

23. Kenner, G. W.; McDermott, J. R.; Sheppard, R. C. *J. Chem. Soc., Chem. Commun*. **1971**, 636.
24. Backes, B. J.; Ellman, J. A. *J. Am. Chem. Soc*. **1994**, *116*, 11171.
25. Backes, B. J.; Harris, J. L.; Leonetti, F.; Craik, C. S.; Ellman, J. A. *Nat. Biotechnol*. **2000**, *18*, 187.
26. Backes, B. J.; Virgilio, A. A.; Ellman, J. A. *J. Am. Chem. Soc*. **1996**, *118*, 3055.
27. Backes, B. J.; Ellman, J. A. *J. Org. Chem*. **1999**, *64*, 2322.
28. Yang, L.; Morriello, G. *Tetrahedron Lett*. **1999**, *40*, 8197.
29. Ingenito, R.; Bianchi, E.; Fattori, D.; Pessi, A. *J. Am. Chem. Soc*. **1999**, *121*, 11369.
30. Link, A.; Van Calenbergh, S.; Herdewijn, P. *Tetrahedron Lett*. **1998**, *39*, 5175.
31. Zaragoza, F.; Stephensen, H. *Angew. Chem. Int. Ed*. **2000**, *39*, 554.
32. Shen, D.-M.; Shu, M.; Chapman, K. *Org. Lett*. **2000**, *2*, 2789.
33. Hirumi, H.; Hirumi, K. *J. Parasitology* **1989**, *75*, 985.
34. Leost, M.; Schultz, C.; Link, A.; Wu, Y.; Biernat, J.; Mandelkow, E.; Bibb, J.; Snyder, G.; Greengard, P.; Zaharevitz, D.; Gussio, R.; Senderowicz, A.; Sausville, E.; Kunick, C.; Meijer, L. *Eur. J. Biochem*. **2000**, *267*, 5983.
35. Flynn, D. L.; Devraj, R.; Naing, W.; Parlow, J.; Weidner, J.; Yang, S. *Med. Chem. Res*. **1998**, *8*, 219.
36. Curran, D. P. *Angew. Chem. Int. Ed*. **1998**, *37*, 1174.
37. Kennedy, K. J.; Bressi, J. C.; Gelb, M. H. *Bioorg. Med. Chem. Lett*. **2001**, *11*, 95.