A new entry to adenosine analogues via purine nitration - Combinatorial synthesis of antiprotozoal agents and adenosine receptor ligands
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This chapter contains an account of the research that was carried out in collaboration with Dr. Margot Beukers, Jacobien von Freitag Drabbe Künzel and Prof. Dr. Ad IJzerman from Leiden University.

**ABSTRACT**

Two types of conformationally restricted adenosine analogues were synthesised by methods involving nucleophilic nitro substitution. Type I contains a tether between N^6^ and C2, allowing for the spatial confinement of pharmacophores. Type II contains a chain connecting C5’ and C2, thereby covalently restricting the nucleoside in the syn conformation. Binding studies at adenosine receptors revealed A3 selectivity of nucleosides of type I, while the complete absence of receptor affinity of the syn restricted adenosine analogues II confirmed that binding to the receptor requires the anti conformation.
4.1 INTRODUCTION

In the previous chapter the synthesis of 2,N6-disubstituted adenosine analogues was described. The 2-nitro group, introduced by our TBAN-TFAA purine nitration method, was used both as an enhancer of C-6 electrophilicity and as a leaving group in nucleophilic substitution reactions. In our ongoing efforts to exploit the benefit of the 2-nitro group, we considered it an appealing idea to use it for intramolecular substitution reactions, thus allowing the formation of cyclophanes. A few 2,N6-polymethylene bridged adenosine derivatives have been reported, but these compounds have not been evaluated biologically (see Figure 4.1). The preparation of these cyclophanes involved elaborate construction of the purine skeleton. The natural nucleotide cyclic ADP-ribose (cADPR), a general mediator involved in Ca2+ signalling, contains a diphosphate-riboisyl bridge between N-1 and C-5'. cADPR is readily hydrolysed at the unstable N-1-glycosidic linkage and many stable analogues have been synthesised since the discovery of this natural cyclic nucleotide in 1987.

![Polymethylene bridged adenosine analogue](image1)

![cyclic ADP-ribose](image2)

**Figure 4.1. Literature examples of cyclonucleosides**

The rotational freedom of pharmacophores on the purine ring can be restricted by attaching them to the cyclophanes. In this way better insight in the binding of ligands to their biological targets can be obtained. In adenosine receptor research structure-activity relationships dictate that the 2'- and 3'-hydroxyl groups be unaffected and that an N6-hydrogen atom is mandatory for adenosine receptor agonist activity. With the objective of synthesising agonists for the adenosine receptor, two macrocyclic motifs then immediately come to mind (see Figure 4.2). The first comprises a connection between N6 and C2, accomplished by ring-closure of a chain connected to N6 (ring A) via nucleophilic nitro substitution. The second theme consists of a connection between the ribose 5' and the purine C2 position, achieved by cyclisation of a chain connected to the ribose 5'-position (ring B). Depending on the rings-size a marked influence on the purine-ribose syn/anti ratio can be expected (vide infra).
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N\textsuperscript{6}(R)-(Phenylisopropyl)adenosine, R-PIA, is a potent and selective A\textsubscript{1} receptor ligand that contains a phenyl pharmacophore.\textsuperscript{5} The phenyl group is relatively mobile in this molecule and by conformationally restricting this aromatic moiety more insight might be obtained in the structure and mutual orientation of the binding regions at the receptor. An approach constraining the phenyl group onto the adenine framework has been reported by Quinn and coworkers.\textsuperscript{6} They prepared several R-PIA congeners and investigated efficacy and affinity towards the rat adenosine A\textsubscript{1} (rA\textsubscript{1}) and A\textsubscript{2A} (rA\textsubscript{2A}) receptors and found agonist activity and selectivity for the rA\textsubscript{1} receptor. The affinity of their most active compound, with a K\textsubscript{i} value of 0.61 \textmu M at the rA\textsubscript{1} receptor, was about 500 fold lower than for R-PIA with a K\textsubscript{i} value of 1.2 nM. The authors inferred the presence of a N\textsuperscript{6}-proton (purine numbering), a known structural necessity for agonist activity,\textsuperscript{4} by tautomerism. Moreover N-1 is substituted, although this might not necessarily have a deleterious effect, since various N-1 substituted\textsuperscript{7} and 1-deaza\textsuperscript{8} adenosine analogues are known as potent adenosine receptor agonists.

Figure 4.2. General idea for synthesising macrocyclic adenosine analogues.

More phenyl containing adenosine analogues are known as active adenosine receptor agonists. As shown in Figure 4.4, N\textsuperscript{6}-Phenyl adenosine is an A\textsubscript{1} selective agonist,\textsuperscript{9} N\textsuperscript{6}-benzyl
adenosine also exhibits $A_1$ selectivity, albeit less pronounced.\textsuperscript{10} If the location of this phenyl group would be constricted by tethering it to the purine part and subsequently allowing the phenylene moiety to ‘walk’ through this tether as depicted in Figure 4.5, one would increase the knowledge of the spatial position of the phenyl group required for selective binding to the different adenosine receptors.

The orientation of the ribose moiety with respect to the nucleobase, is an important factor that determines the binding of nucleosides to adenosine receptors.\textsuperscript{11} This orientation is characterised by the glycosidic torsion angle $\chi$, which is defined by the dihedral angle between $C8\text{-}N9\text{-}C1'\text{-}O4'$. Nucleosides exist in two predominant rotamers called syn and anti. For the syn conformer $\chi$ has a typical value of $230 \pm 30^\circ$, while for the anti conformation it is $45 \pm 40^\circ$.

5'-N-Ethylcarboxamido adenosine, NECA, is a high affinity, non-selective adenosine receptor agonist (Figure 4.6).\textsuperscript{12} The x-ray structure of this compound revealed a syn conformation around the glycosyl linkage in which a hydrogen bond is observed between the N-3 position of the adenine ring and the NH of the carboxamide group.\textsuperscript{13} In solution, NECA prefers a syn conformation as determined from its nuclear Overhauser effect (NOE) of the $^1H$ NMR spectra.\textsuperscript{14} Although initially this conformation was thought to be one of the important factors responsible for receptor binding activity,\textsuperscript{13} later publications seriously doubted this idea. Moreover, an anti configuration between purine base and ribose moiety has frequently been
proclaimed to be imperative for receptor binding of adenosine derivatives, i.e. with a ‘normal’ 5’-hydroxyl group. In a reported evaluation of C2-alkynyl-NECA derivatives, which also displayed a preferred syn conformation in solution, the syn conformational requirement was questioned by the absence of positive correlations of the glycosyl conformation and sugar-puckering to the receptor affinity. While NECA in the crystal structure is in the syn conformation due to the intramolecular hydrogen bonding between N-3 and NH of the carboxamide, molecular modelling has shown that the energy barrier between the syn and the anti conformers is only 2.1 kcal/mol in favour of the syn conformation. These findings supported the idea that NECA can readily adopt both the syn and the anti conformation in solution, therefore not excluding the proposed anti mode of binding to the receptor.

To obtain more clarity in this matter, nucleosides ought to be synthesised and evaluated at the adenosine receptors that are constrained in certain conformations, like cyclonucleosides which are fixed by a bridge between the sugar and nucleobase moieties. In our opinion, cyclonucleosides with a connection between the purine 2 and the ribose 5’ position will be appropriate syn restricted conformers, cf. ring B in Figure 4.2, with all essential requirements for receptor binding still present: an intact purine system, a N⁶-proton and the 2’ and 3’ hydroxyl groups.

In this chapter the synthesis and biological evaluation of several 2,N⁶- and 2,5’-cyclophane adenosine derivatives will be described.

### 4.2 Synthetic approach towards 2,N⁶ tethered adenosine analogues

With the solid phase route towards 2,N⁶-disubstituted adenosine analogues described in Chapter 2 in mind, a solid supported synthesis of 2,6-tethered adenosine analogues was envisaged, aiming for profitable use of the pseudo-dilution effect. Since the molecules are anchored to the solid phase, they may be more or less prevented from interaction with each other, thus lowering the chance of intermolecular side reactions. In this respect cyclisation
reactions can be achieved on solid support, while cross-couplings will be thwarted in the absence of interaction between the sites. Successful macrocyclisations are known on solid support.\textsuperscript{19} For preliminary studies 1,6-diaminohexane was selected as a simple \(\omega\)-diamine for coupling to solid supported 2-nitro-6-chloropurine riboside 4 (see Chapter 2) as depicted in Scheme 4.1. Several attempts at ring-closing 5 by displacement of the 2-nitro group failed.

Analysis of the reaction by cleaving the nucleoside from the resin learned that no cyclisation had occurred. Instead an unidentified mixture of polymers was obtained. Cross-coupling and amidation of the amino-tether to the carboxyl linker were suspected, although this was not further investigated.

During the past three decades there has been much debate on the matter of site isolation versus site interactions.\textsuperscript{20} The current view is that a dynamic equilibrium exists between site separation and site isolation and that this equilibrium is influenced by factors like resin capacity and cross-linking.\textsuperscript{21} In addition, the reactivity of the reactants determines whether they are suitable for ring-closing reactions on solid support. Examples of attempted macrocycle preparation on solid phase are known where besides the desired ring-closing metathesis primarily cross-coupling reactions were observed.\textsuperscript{22}

The solid phase approach made us clear that the macrocyclisation was not a straightforward process. Therefore we switched to a solution phase strategy for obtaining these cyclophanes, allowing for closer monitoring of this reaction.

### 4.3 Macrocycles derived from symmetrical diamines

When dealing with symmetrical diamines the approach depicted in Scheme 4.2 was envisaged. The diamine is selectively coupled to suitably protected 2-nitro-6-chloropurine riboside by
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**Scheme 4.2.** Solution phase approach with symmetrical diamines.

Adding the chloropurine to an excess of diamine. Subsequent ring closure by nitro substitution will be effected by heating the coupled free amine under high dilution conditions to avoid dimerisation. Removal of the protective groups then leads to the 2,N*6*-tethered macrocyclic adenosine analogues.

Several diamines were chosen for this strategy, ranging from simple ω-alkyl diamines, that are commercially available to more complicated amines that contain pharmacophores in the chain. In order to create a series containing a phenyl ring in the tether ortho- and para-bis(cyanomethyl)benzenes were hydrogenated using platinum(IV)oxide in an acidic solution (Scheme 4.3). After trituration diamines 7 (ortho) and 8 (para) were isolated as their di-HCl salts.

**Scheme 4.3.** Catalytic hydrogenation of ω- and ρ-bis(cyanomethyl)benzenes.

Substitution of the chloro atom in TBS-protected 2-nitro-6-chloropurine riboside 9\(^{23a}\) by several diamines proceeded smoothly and in good yields furnishing the cyclisation precursors 10a-g (see Scheme 4.4 and Table 4.1 on page 74). Cyclisation in acetonitrile with a nucleoside

**Scheme 4.4.** (a) diamine 5-10 equiv, Et\(_3\)N, CH\(_2\)Cl\(_2\), 0°C; (b) DIPEA, CH\(_3\)CN (1 mM), Δ, 3-7 d; (c) NH\(_4\)F, MeOH, Δ.
Table 4.1. Reactions with symmetrical diamines.

<table>
<thead>
<tr>
<th>Diamine</th>
<th>Coupling</th>
<th>Cyclisation</th>
<th>Deprotection</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₂₂</td>
<td>10a 88%</td>
<td>11a 36%</td>
<td>12a 56%</td>
</tr>
<tr>
<td>NH₂₂</td>
<td>10b 88%</td>
<td>11b 63%</td>
<td>12b 63%</td>
</tr>
<tr>
<td>NH₂₂</td>
<td>10c 76%</td>
<td>11c 52%</td>
<td>12c 44%</td>
</tr>
<tr>
<td>(H₂C)₆</td>
<td>10d 87%</td>
<td>11d 49%</td>
<td>12d 45%</td>
</tr>
<tr>
<td>NH₂₂</td>
<td>10e 74%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(H₂C)₉</td>
<td>10f 75%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NH₂₂</td>
<td>10g 90%</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Structure number followed by isolated yields; Pure product after trituration.

A concentration of 1 mM required refluxing for several days. Compounds 11a-d were isolated in moderate to good yields. All attempts at cyclising amines 10e-g failed. Apparently, a considerable degree of rotational freedom is required for successful nitro substitution. Subsequent removal of the silyl protecting groups from 11a-d with ammonium fluoride furnished 2,N₆-bridged adenosine analogues 12a-d in moderate isolated yields after trituration with diethyl ether.

### 4.4 MACROCYCLES DERIVED FROM ASYMMETRICAL DIAMINES

With the idea of confining a phenyl moiety in the tether-ring to various positions we made the phenyl ring ‘walk’ through the tether in order to obtain information on the optimal position of the aromatic moiety required for binding to the receptor (Figure 4.7). From the cyclisation experiments of 10d and 10e with chains containing a phenylene group we learned that only a meta orientation of the aminoalkyl anchors as in 10d resulted in ring closure. With the symmetrical derivative 12d already in hand, synthesis of the asymmetrical analogues required another approach. Because the 2-nitro group is not substituted by aniline nitrogen nucleophiles as discussed in Chapter 2, synthesis of the 2-anilino derivative was not undertaken.

![Figure 4.7. Phenyl moiety ‘walking’ through the tether.](image-url)
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**Scheme 4.5.** Approach towards asymmetric tethers.

The scenario for generating the asymmetrical tethered adenosine analogues required a slight modification of the method described for the symmetrical macrocycles. This is depicted in Scheme 4.5. Coupling of a mono N-Fmoc-protected diamine to the C-6 position of 2-nitro-6-chloropurine 9 is followed by removal of the Fmoc group with a non-nucleophilic base, like DBU, to avoid premature nucleophilic substitution of the 2-nitro group. Cyclisation and desilylation as described in the previous paragraph will then lead to the desired phenylene tethered cyclophanes. First the Fmoc-protected phenylene diamine precursors were synthesised.

**Scheme 4.6.**

- (a) 3-azidopropylphosphonium bromide, KO\(_2\)Bu, THF, 0 °C, 86%; (b) \(\text{H}_2\), Pd/C, MeOH, 74%; (c) FmocCl, DIPEA, CH\(_2\)Cl\(_2\), 0 °C, 79%.

The synthesis of 1-amino-3-(4-N-Fmoc-aminobutyl)benzene 16 involved Wittig coupling of 3-nitrobenzaldehyde 13 and 3-azidopropylphosphorous ylid, which was generated in situ from 3-azidopropylphosphonium bromide via a modified literature procedure (Scheme 4.6).\(^{24}\) The olefination resulted exclusively in the formation of Z-alkene 14. Subsequent catalytic hydrogenation with palladium on carbon as the catalyst efficiently reduced both the nitro and azido groups and the double bond yielding diamine 15. Fmoc protection of the aliphatic amino functionality of 15 gave 3-(4-N-Fmoc-aminobutyl)aniline 16.

**Scheme 4.7.**

- (a) FmocCl, DIPEA, CH\(_2\)Cl\(_2\), 0 °C, 94%; (b) Boc\(_2\)O, CH\(_2\)Cl\(_2\), 82%.

R = Fmoc
R = Boc
For the synthesis of the N-Fmoc-3-(3-aminopropyl)benzylamine 22 and 3-(3-N-Fmoc-aminopropyl)benzylamine 26, a complementary strategy was pursued. First propargylamine was either Fmoc or Boc protected via modified literature methods to obtain 17 and 18 in good yields (Scheme 4.7). Under Sonogashira conditions acetylenes 17 and 18 were coupled to Fmoc or Boc protected 3-iodobenzylamine, 19 and 23 respectively, providing the orthogonally protected diamino precursors 20 and 24 (Scheme 4.8). Catalytic hydrogenation with palladium on carbon furnished arylalkanes 21 and 25. Subsequent removal of the Boc groups under acidic conditions gave N-Fmoc-3-(3-aminopropyl)benzylamine 22 and 3-(3-N-Fmoc-aminopropyl)-benzylamine 26.

Scheme 4.8. (a) FmocCl, DIPEA, CH₂Cl₂, 85%; (b) Boc₂O, CH₂Cl₂, 84%; (c) [PPh₃]₄Pd, Cul, Et₃N, DMF, 20: 70%, 24: 69%; (d) H₂, Pd/C, EtOAc, quantitative; (e) TFA, CH₂Cl₂, quantitative.

Scheme 4.9. (a) mono-Fmoc-diamine, DIPEA, CH₂Cl₂; (b) DBU, CH₂Cl₂, 10h: 80%, 10i: 61%, 10j: 63%; (c) DIPEA, CH₃CN 1 mM, 6 d 11h: 45%; (d) Et₃N·3HF, THF, 12h: 61%.
Coupling of Fmoc-amines 16, 26 and 22 to 2-nitro-6-chloropurine 9 and subsequent Fmoc removal with DBU proceeded smoothly providing 2-nitro adenosine analogues 10h,j, respectively (Scheme 4.9). Ring-closure was only observed for the m=0,n=4 derivative 10h allowing for cycloadenosine 11h in 45% yield. Removal of the silyl protecting groups eventually gave the m=0,n=4 phenylene tethered adenosine analogue 12h. Despite ample efforts the cyclisation of the m=1,n=3 derivative 10i and the m=3,n=1 derivative 10j was not accomplished; instead decomposition was observed upon prolonged heating.

4.5 ‘Open’ 2,6 disubstituted analogues

In order to compare the binding affinity of the cyclic analogues with their ‘open’ counterparts the latter were synthesised as depicted in Scheme 4.10. For the preparation of the open congener of the C10 macrocycle 12c, triacetyl protected 2-nitro-6-chloropurine riboside 27 was stirred in neat 1-pentylamine. In this one pot reaction both substitution of the chloro and nitro groups and aminolysis of the acetate groups were effected furnishing the pure dipentyl analogue 28 in 50% yield. The open counterpart of phenylene cyclophane 12d was generated by allowing 27 to react selectively at the 6 position with phenethylamine at 0°C to give the 6-substituted analogue 29 quantitatively. Stirring this compound in 70% aqueous ethylamine resulted in 2-nitro substitution and acetate aminolysis. Pure 2-ethylamino-N6-phenethyl adenosine 30 was isolated in 37% yield.

Scheme 4.10. (a) 1-pentylamine, neat, 50%; (b) phenethylamine, DIPEA, CH2Cl2, 0°C; (c) 70% aqueous EtNH2, 37% (2 steps).
4.6 2,5'-Tethered Adenosine Analogues

In order to study whether NECA, a high affinity, non-selective adenosine receptor agonist (see Figure 4.6), is able to bind to the receptor in the syn conformation, we chose to force this nucleoside in the syn conformation by linking the purine 2- and the sugar 5'-position by short chains. The constricted NECA congener 38 and propylcarboxamide analogue 39 were synthesised as depicted in Scheme 4.11. 2-Nitro adenosine 31\textsuperscript{21,23} was 2',3'-isopropylidene protected under standard conditions to give acetonide 32. The TEMPO-iodobenzene diacetate oxidising system reported for the 5'-oxidation of common 2',3'-protected nucleosides was applied to 2',3'-isopropylidene protected 2-nitro adenosine 32 generating 5'-carboxylic acid 33 in 85% yield.\textsuperscript{26} Coupling of monotritylated 1,2-diaminoethane and 1,3-diaminopropane to carboxylic acid 33 by using EDC-HOBt as standard coupling reagents generated amides 34 and 35. Intramolecular nitro substitution was realised by acidolysis of the trityl groups and subsequent heating in the presence of 20 equivalents of DIPEA in a diluted acetonitrile solution. Probably, the template effect due to hydrogen bonding between N-3 and the carboxamide NH greatly facilitated ring closure and lactams 36 and 37 were obtained in high

\[
\text{Scheme 4.11. (a) } \text{HC(OCH}_3)\text{, } \rho \text{TsOH-H}_2\text{O, acetone, 61%; (b) TEMPO, iodobenzene diacetate, CH}_3\text{CN, H}_2\text{O, 85%; (c) tritylaminealkylamine, EDC, HOBt, DMF, THF, 34: 80%, 35: 78%; (d) TFA, CH}_2\text{Cl}_2, \text{then MeOH; (e) DIPEA, CH}_3\text{CN, 80 °C, 36: 79%, 37: 99% (over 2 steps); (f) TFA-H}_2\text{O, 38: 79%, 39: 80%.}
\]
yield. Removal of the isopropylidene group under aqueous acidic conditions furnished the desired carboxamide cycloadenosine derivatives 38 and 39.

Another approach leaving the 5'-methylene group intact, thereby creating syn restricted adenosine analogues, was investigated as shown in Scheme 4.12. The amino acids glycine and β-alanine, protected as their sodium salts, were coupled to 2',3'-isopropylidene protected 2-nitro adenosine 32 by displacement of the nitro group. After acidification with acetic acid carboxylic acids 40 and 41 directly crystallised from the reaction mixture. Lactonisation of 41 with EDC-DMAP furnished lactone 42 in 49% yield. Hydrolysis of the isopropylidene protecting group furnished the desired cycloadenosine derivative 43. All attempts at cyclising 40, for example with EDC-DMAP, Mukayama's reagent or the pentafluorophenyl ester, resulted in formation of a mixture of polymers and no further efforts were made to obtain this lactone.

Scheme 4.12. (a) sodium glycinate or sodium β-alaninate, DMF-H$_2$O, 80 °C, then HOAc, 40: 75%, 41: 64%; (b) EDC, DMAP, DMF, 49%; (c) TFA-H$_2$O, 74%.

**Conformational Analysis**

It has been shown that in purine nucleosides the H2' chemical shift can be used as an indicator of the sugar-base orientation. Typical chemical shift values for H-2' measured in DMSO are around 5.0 ppm for adenosine analogues with a strong preference for the syn conformation, like 8-(α-hydroxyisopropyl)adenosine with 4.98 ppm, while analogues that are restricted to the anti conformation show values around 4.2 ppm, like 8,5'-cyclo-8-oxoadenosine with 4.24 ppm. The change in chemical shift of H-2' has been attributed to the influence exerted on this proton by the ring current of the purine system and the anisotropy of N-3, when the orientation of the nucleobase changes from anti to syn. Since the syn-anti equilibrium is very rapid with respect to the NMR time scale, the chemical shift will have an intermediate value determined by the probability of finding the molecule in one of the two
conformations. Thus for adenosine, with an H-2' chemical shift value of 4.62 ppm, a slight preference for the syn conformation was inferred.

In Table 4.2 the H-2'-chemical shift values are displayed of the syn restricted (carboxamido) adenosine analogues 38, 39 and 43. The values for adenosine and NECA have been added for comparison. The chemical shift value of 5.06 ppm of H-2' of the syn restricted adenosine analogue, lactone 43, shows that the maximum value is indeed around 5.0 ppm. For the carboxamido derivatives 38 and 39 a similar trend is observed. The more the nucleoside is confined to the syn orientation, the higher the chemical shift value of H-2'; cf. NECA with 4.62 ppm, trimethylene bridged carboxamide 39 with 4.83 ppm and dimethylene bridged carboxamide 39 with 4.99 ppm. The di- and trisubstituted 5'-carboxamido-adenosine analogues discussed in Chapter 3 display typical H-2'-chemical shift values between 4.60 and 4.73 ppm.

Table 4.2. 1H NMR H-2' chemical shift values for (carboxamido) adenosine analogues.a

<table>
<thead>
<tr>
<th>Compound</th>
<th>adenosine</th>
<th>lactone 43 (CH2)2 tether</th>
<th>NECA</th>
<th>carboxamide 38 (CH2)2 tether</th>
<th>carboxamide 39 (CH2)3 tether</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ (H-2')</td>
<td>4.62</td>
<td>5.06</td>
<td>4.62</td>
<td>4.99</td>
<td>4.83</td>
</tr>
</tbody>
</table>

a measured in d6-DMSO at 27 °C.

Besides the use of the chemical shift value of H-2' as a tool for estimation of the syn-anti rotamer distribution, also NOE experiments can be employed to determine the predominant conformation. If saturation of H-8 of the purine ring produces a strong NOE at H-1' and small ones at H-2' and H-3', then the syn orientation should be preferred. In a reverse experiment saturation of H-1' should render a significant NOE at H-8. If, on the other hand, saturation of H-8 results in a strong NOE at H-2' and a smaller one at H-1', the anti orientation should dominate. An elaborate study was reported by Seela's group concerning the application of NOE spectroscopy for a semiquantitative estimation of the syn-anti conformer population of nucleosides. Their method involved the set-up of a calibration graph by using the NOE values of H-1', H-2' and H-3' after irradiation of H-8 of a syn and anti fixed nucleoside respectively. Thus, they estimated a 60% syn population for adenosine.

In NOE measurements three different cases can be discerned depending on the tumbling rate of the measured molecules. They are referred to as the fast, the intermediate and the slow motion regimes. For molecules that tumble rapidly compared to the spectrometer observation frequency, the NOE has a positive value. At the other extreme, relatively slowly tumbling molecules show negative NOE values. Between these two extremes lies the intermediate region in which the NOE changes sign and even can become zero. Within this region the magnitude and sign of the NOE is highly sensitive to the rate of the molecular motions. The point at which this region is entered will be dependent on a number of factors; the size and shape of the molecule, solution conditions, like viscosity, temperature and possibly pH, and
We investigated the effect of solvent viscosity and spectrometer field strength on NOE intensities of adenosine derivatives, applied to determine the syn-anti rotamer distribution. Table 4.3 shows the enhancements of H-1’ and H-2’ obtained after saturation of H-8 of adenosine, NECA and the syn restricted (carboxamido) adenosine analogues 38, 39 and 43. Saturation of H-8 gives more reliable results than saturation of H-1’, since the latter can confer its energy to neighbouring protons (see experimental part). Clearly, the results obtained with a 400 MHz machine at 27 °C are quite unreliable, indicating that the intermediate molecular tumbling region applies to these conditions. Raising the temperature to 60 °C, thereby reducing the viscosity of the solvent, significantly improves the NOE data, but a slightly different distribution is found as compared with the measurements on the 200 MHz spectrometer. This minor difference is most probably the consequence of the temperature effect on the syn-anti rotamer equilibrium. Although not explicitly mentioned in their publication, the NOE’s reported by Seela’s group were probably measured on a 400 MHz spectrometer at room temperature, judging from the similar values found by us at that observation frequency.28 Our findings put the reliability and usefulness of their extended NOE-publication to question.

For NECA only irradiation of H-1’ was reported in the literature and 22 % enhancement of H-8 was obtained with a 100 MHz spectrometer.14 In our experiments H-8 enhancements of 15.1 % (60 °C, 400 MHz) and 17.5 % (200 MHz) were obtained, which is in agreement with their measurements at 100 MHz.
4.7 BINDING STUDIES AT THE ADENOSINE RECEPTORS

The conformationally restricted adenosine analogues were tested in radioligand binding assays to determine their affinity for the human adenosine receptors hA$_1$, hA$_{2A}$, hA$_{2B}$ and hA$_3$. Binding affinities at the adenosine receptors are given in micromolar or percentage displacement of the radioligand at 10 or 1 micromolar (see Table 4.4). For comparison, the affinities of the nonselective adenosine receptor agonist NECA have been added. They were determined with the same test system.

With the exception of 12a which showed minor displacement of the radioligand, the tested compounds did not display binding to the low affinity hA$_{2B}$ receptor. This is not surprising, since even the most active agonists known for this receptor, like NECA, have affinities in the low micromolar range. Another observed motif is the lack of affinity for the hA$_{2A}$ receptor of all tested compounds. Generally, A$_{2A}$ selectivity is favoured by large C2 substituents on adenosine, whereas N$^6$-substituted analogues favour binding to the A$_1$ receptor. Remarkably, for the tested 2,N$^6$-disubstituted adenosine analogues hA$_{2A}$ affinity is virtually absent, while various 2,N$^6$-modified adenosine analogues were reported as highly active and selective agonists for this receptor.

Quite remarkable is the hA$_3$ selectivity of the 2,N$^6$-cyclophanes. A clear relation can be deduced between tether size and hA$_3$ selectivity and activity. The affinity for the hA$_3$ receptor increases in the order C$_6$<C$_9$<C$_{10}$<C$_{phénylène}$, going from 4.33 ± 0.59 μM for 12a to 1.47 ±0.10 μM for 12d. One can imagine that the more rotational freedom the tether has, i.e. with increasing tether size, the better it is capable of folding into a hydrophobic pocket. For the hA$_1$ receptor the opposite is observed: affinities fall off in approximately the same order, leading to an increasing hA$_3$ vs hA$_1$ selectivity. For comparison, the conformationally restricted PIA-analogues reported by Quinn’s group$^6$ (see Figure 4.3) displayed low micromolar affinities for the rA$_1$ receptor, but no affinities for the A$_3$ receptor were reported.

The hA$_3$ selectivity of the 2,N$^6$-bridged adenosine derivatives indicates that at the hA$_3$ receptor the C-2 and N$^6$ regions seem to have partial overlap, while for the hA$_1$ receptor different binding sites for C2 and N$^6$ substituents are recognised, which was suggested before. Accordingly, hA$_1$ affinity is fairly restored when the 2,N$^6$ chain is broken, for example in compound 28, allowing the two substituents to occupy different pockets. The two alkyl substituents in 28 are not well tolerated by the hA$_3$ receptor, judging from the reduced affinity. For hA$_3$ binding activity an aromatic group linked to N$^6$ is favourable, as can be seen from the most active cyclophane 12d and ‘open’ structure 30 which both contain a 2-phenethyl group on N$^6$. The latter compound even has the highest hA$_3$ affinity of all tested compounds, with a K$_i$ value of 0.32 μM. The beneficial effect on A$_3$ affinity of an aromatic residue on N$^6$ is best
### Table 4.4. Affinities of the conformationally restricted adenosine analogues in radioligand binding assays at the human A₁, A₂A, A₂B and A₃ receptors.

<table>
<thead>
<tr>
<th>Conformationally Restricted Analogue</th>
<th>Kᵢ ± SEM in μM (n=3) or % displacement at 10 μM or 1 μM* (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>hA₁&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2,N&lt;sup&gt;6&lt;/sup&gt;-cyclic analogues</td>
<td></td>
</tr>
<tr>
<td>12a</td>
<td>40.4 %</td>
</tr>
<tr>
<td>12b</td>
<td>41.4 %</td>
</tr>
<tr>
<td>12c</td>
<td>29.2 %</td>
</tr>
<tr>
<td>12d</td>
<td>15.3 %</td>
</tr>
<tr>
<td>12h&lt;sup&gt;e&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>open analogues</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>3.52 ± 0.38</td>
</tr>
<tr>
<td>30</td>
<td>39.3 %</td>
</tr>
<tr>
<td>2,5'-cyclic analogues</td>
<td></td>
</tr>
<tr>
<td>38&lt;sup&gt;*&lt;/sup&gt;</td>
<td>2.85 %</td>
</tr>
<tr>
<td>39&lt;sup&gt;*&lt;/sup&gt;</td>
<td>20.4 %</td>
</tr>
<tr>
<td>43&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0 %</td>
</tr>
<tr>
<td>NECA&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.012 (0.096-0.015)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Displacement of [³H]DPCPX from human A₁ receptors expressed on CHO cells; <sup>b</sup> displacement of [³H]-ZM241,385 from human A₂A receptors expressed on CHO cells; <sup>c</sup> displacement of [³H]DPCPX from human A₂B receptors expressed on CHO cells; <sup>d</sup> displacement of [¹²⁵I]-ABMECA from human A₃ receptors expressed on HEK293 cells; <sup>e</sup> insoluble; <sup>f</sup> not determined; <sup>g</sup> values taken from reference 30.

Illustrated by N<sup>6</sup>-(3-iodo-4-amino-benzyl)-5'-methylcarboxamido adenosine, IAB-MECA, one of the highest affinity agonists known for the human A₃ receptor with a Kᵢ value of 0.64 nM.\(^{33}\)

While phenylene derivative 12d displayed the highest A₃ affinity from the tested cyclophanes, unfortunately the influence of a migrating phenylene moiety in the tether could not be studied, because the m=0,n=4 analogue 12h appeared insoluble in the test medium and cyclisation of the m=1,n=3 and m=3,n=1 derivatives could not be realised, as described in Section 4.4.

The syn restricted analogues 38, 39 and 43 displayed a near complete lack of affinity for all adenosine receptors. These findings indeed corroborate the idea that an anti-orientation between purine base and sugar is imperative for binding to the adenosine receptors.\(^{11,15}\) On the other hand it should be noted that not just the syn-anti conformation but also the spatial orientation of the amide substituents in 38 and 39, cf. the N-ethyl group in NECA, and the sugar puckering might have an influence on receptor binding.
4.8 CONCLUDING REMARKS

Various macrocyclic adenosine derivatives were prepared by methods involving nucleophilic nitro displacement. In adenosine receptor studies the 2,N\textsuperscript{6}-bridged adenosine analogues show selectivity for the adenosine A\textsubscript{3} receptor, thereby revealing that in the A\textsubscript{3} receptor partial overlap may exist between the C2 and N\textsuperscript{6} binding domains. The complete absence of receptor affinity of the syn restricted 2,5'-tethered (5'-carboxamido)adenosine analogues emphasises that binding to the receptor requires the nucleoside anti conformation.

4.9 ACKNOWLEDGEMENTS

Jacobien von Freitag Drabbe Künzel, kindly acknowledged for performing the receptor binding studies, Dr. Margot Beukers and Prof. Dr. Ad IJzerman are much appreciated for the pleasant cooperation. Martin Wanner is gratefully acknowledged for the synthetic contribution to this chapter and for measuring the NOE spectra with the valued assistance of Lidy van der Burg.

4.10 EXPERIMENTAL

**General information.** For experimental details see section 2.8. Radioligand binding studies were performed as described in reference 30. NOE's were measured on a Bruker ARX 400 (400 MHz) spectrometer or on a Bruker AC 200 (200 MHz) machine applying a 4.5 seconds presaturation time (see Table 4.5 at the end of this section).

1,3-Bis-(2-aminoethyl)benzene (7). A suspension of 1,3 bis-(cyanomethyl)benzene (1.0 g; 6.4 mmol), platinum(IV)oxide (100 mg; 0.44 mmol), conc. HCl (1.3 mL) in EtOH (20 mL) was stirred vigorously under a hydrogen atmosphere (balloon) for 3 days. After filtration over highflow the solvent was evaporated. Trituration with a mixture of MeOH/EtOAc afforded the diamine-2 HCl salt as a grey solid (0.73 g; 3.1 mmol; 48%). \textsuperscript{1}H NMR (d\textsubscript{6}-DMSO) δ 8.16 (bs, 6H, NH\textsubscript{3}+), 7.30 (t, J 7.4, 1H, H\textsubscript{Ar}), 7.18-7.15 (m, 3H, H\textsubscript{Ar}), 3.05-3.02 (m, 4H, 2xCH\textsubscript{2}), 2.92-2.88 (m, 4H, 2xCH\textsubscript{2}). The free amine was obtained by taking the di-HCl salt (0.6 g; 2.5 mmol) up in EtOH (2.5 mL) and adding sat. aqueous K\textsubscript{2}CO\textsubscript{3} (10 mL). The suspension was stirred for 45 min. and was extracted with CH\textsubscript{2}Cl\textsubscript{2} (2x25 mL). The collected organic layers were washed with brine (1x25 mL) and dried with Na\textsubscript{2}SO\textsubscript{4} to yield 7 as a viscous liquid (0.37 g; 2.3 mmol; 90%).

1,4-Bis-(2-aminoethyl)benzene (8). This compound was synthesised by the method described for 1,3-bis-(2-aminoethyl)benzene 7. The diamine-2 HCl salt of 8 was isolated as an off-white solid (0.88 g; 3.7 mmol; 58%). \textsuperscript{1}H NMR (d\textsubscript{6}-DMSO) δ 8.10 (bs, 6H, NH\textsubscript{3}+), 7.24 (s,4H, H\textsubscript{Ar}), 3.05-2.99 (m, 4H, 2xCH\textsubscript{2}), 2.89-2.85 (m, 4H, 2xCH\textsubscript{2}).

2',3',5'-Tri-O-tert-butyldimethylsilyl-2-nitro-N\textsuperscript{6}-(6-aminoheptyl)-adenosine (10a). 2-Nitro-6-chloro-(2,3,5-tri-O-tert-butyldimethylsilyl-β-D-ribofuranosyl)-9H-purine 9 (0.165 g; 0.25 mmol) was added to a stirred solution of 1,6-diaminohexane (0.29 g; 2.5 mmol) in dry CH\textsubscript{2}Cl\textsubscript{2} (20 mL) at -18 °C. After stir-
rings for 30 min at 0 °C the mixture was immediately applied to a silica column. Flash chromatography (CH₃Cl₂/EtOH 99:1→CH₂Cl₂/EtOH/Et₂N 88:10.2) afforded amine 10a as a colourless glass (0.16 g; 0.21 mmol; 88%). The product was immediately used for the next step.

2',3',5'-Tri-O-tert-butylidemethylsilyl-2-nitro-N⁴-(9-aminononyl)-adenosine (10b). The same method was used as described for amine 10a. Flash chromatography (CH₂Cl₂/MeOH 99:1→CH₂Cl₂/MeOH/Et₂N 88:10:2) afforded amine 10b as a yellow foam (0.172 g; 0.22 mmol; 88%). ¹H NMR (δ 8.28 (s, 1H, H-8), 6.25 (bs, 1H, NH), 5.96 (d, J 4.6, 1H, H-1'), 4.72 (m, 1H, H-2'), 4.32 (t, J 3.9, 1H, H-3'), 4.16-4.06 (m, 2H, H-4' and H-5'), 3.79 (dd, J 11.0 and 2.7, 1H, H-5')₃, 3.68-3.66 (m, 2H, CH₂), 3.52-3.48 (m, 2H, CH₂), 2.80 (t, J 7.1, 2H, NH₂), 1.70-1.54 (m, 4H, 2xCH₂), 1.38-1.26 (m, 10H, 5xCH₂), 0.95, 0.93 and 0.81 (3xs, 3x9H, 3xBu) 0.15, 0.13, 0.11, 0.09, -0.02 and -0.17 (6xs, 6x3H, 6xCH₃).

2',3',5'-Tri-O-tert-butylidemethylsilyl-2-nitro-N⁴-(10-aminodecyl)-adenosine (10c). The same method was used as described for amine 10a. Cycloadenosine 10c was isolated as a yellow foam (0.150 g; 0.199 mmol; 76%). ¹H NMR (δ 8.28 (s, 1H, H-8), 6.67 (s, 1H, H-8), 5.94 (d, J 5.6, 1H, H-1'), 4.96 (m, 1H, H-1'), 4.38 (m, 1H, H-3'), 4.06-4.04 (m, 2H, H-4' and H-5'), 3.77-3.75 (m, 1H, H-5'), 3.44-3.39 (m, 2H, CH₂), 3.35-3.33 (m, 2H, CH₂), 2.78 (m, 2H, NH₂), 1.68-1.42 (m, 4H, 2xCH₂), 1.28-1.22 (m, 12H, 6xCH₂), 0.96, 0.89 and 0.72 (3xs, 3x9H, 3xBu) 0.15, 0.14, 0.12, 0.07, -0.09 and -0.31 (6xs, 6x3H, 6xCH₃).

2',3',5'-Tri-O-tert-butylidemethylsilyl-2-nitro-N⁴-[3-(2-aminoethyl)-phenyl]-ethyl]-adenosine (10d). A solution of 2-nitro-6-chloro-(2,3,5-tri-O-tert-butyldimethylsilyl-β-D-ribofuranosyl)-9H-purin-9e (0.165 g; 0.25 mmol) in dry CH₂Cl₂ (10 mL) was added dropwise to a stirred solution of 1,3-bis-(2-aminoethyl)benzene 7 (0.37 g; 2.3 mmol) and Et₃N (0.35 mL; 2.5 mmol) in dry CH₂Cl₂ (40 mL) at 0 °C. After stirring for 2 h silica gel (3 g) was added and the solvent was evaporated. Flash chromatography (CH₂Cl₂/MeOH/Et₂N 92:5:3→87:10:3) afforded the product as a colourless glass (0.34 g; 0.43 mmol; 87%). ¹H NMR (δ 8.28 (s, 1H, H-8), 7.24 (t, J 7.8, 1H, Hₐ), 7.10-7.06 (m, 3H, Hₐ), 6.44 (bs, 1H, NH), 5.95 (d, J 4.6, 1H, H-1'), 4.74-4.71 (m, 1H, H-2'), 4.33-4.31 (m, 1H, H-3'), 4.15-4.07 (m, 2H, H-4' and H-5'), 3.96-3.94 (m, 2H, CH₂), 3.79 (dd, J 11.0 and 2.7, 1H, H-5'), 3.00-2.97 (m, 4H, 2xCH₂), 2.74 (t, J 6.6, 2H, CH₂), 0.95, 0.93 and 0.81 (3xs, 3x9H, 3xBu) 0.15, 0.13, 0.11, 0.09, -0.02 and -0.17 (6xs, 6x3H, 6xCH₃). m/z 802.4523 (M⁺+H, C₃₈H₅₈N₆O₆S₂₃ requires 802.4539).

2',3',5'-Tri-O-tert-butylidemethylsilyl-2-nitro-N⁴-[2-[4-(2-aminoethyl)-phenyl]-ethyl]-adenosine (10e). The same procedure was used as described for amine 10d. Amine 10e was isolated as a yellow foam (174 mg; 0.22 mmol; 74%). ¹H δ 8.27 (s, 1H, H-8), 7.24-7.12 (m, 4H, Hₐ), 6.54 (bs, 1H, NH), 5.96 (d, J 4.6, 1H, H-1'), 4.80-4.76 (m, 1H, H-2'), 4.33-4.31 (m, 1H, H-3'), 4.17-4.06 (m, 2H, H-4' and H-5'), 3.96-3.90 (m, 2H, CH₂), 3.81-3.78 (m, 1H, H-5'), 3.28-3.26 (m, 2H, CH₂), 3.05-2.92 (m, 4H, 2xCH₂), 0.95, 0.93 and 0.81 (3xs, 3x9H, 3xBu) 0.14, 0.11, 0.10, 0.09, -0.01 and -0.20 (6xs, 6x3H, 6xCH₃). Attempts at cyclising this compound failed.

2',3',5'-Tri-O-tert-butylidemethylsilyl-2-nitro-N⁴-(3-cis-aminomethyl-cyclohexylmethyl)-adenosine (10f). A mixture of cis,1,3-bis-(aminomethyl)cyclohexane-2 HCl (0.50 g; 2.5 mmol), obtained by addition of HCl to a cis,trans mixture (Aldrich) and selective crystallisation, and Cs₂CO₃ (2.44 g; 7.5 mmol) in dry THF (40 mL) was stirred vigorously stirred at 0 °C. After 5 min a solution of 2-nitro-6-chloro-(2,3,5-tri-O-tert-butylidemethylsilyl-β-D-ribofuranosyl)-9H-purin-9e (0.328 g; 0.5 mmol) in dry THF (10 mL) was added dropwise. After stirring for 30 min silica gel (3 g) was added and the solvent was evaporated.
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Flash chromatography (CH₂Cl₂/MeOH/Et₃N 92:5:3→82:15:3) afforded the product as a colourless glass (0.29 g; 0.37 mmol; 75 %).¹¹H NMR (δ(DMSO) 8.82 (bs, 1H, NH), 8.62 (s, 1H, H-8), 5.92 (d, J 5.4, 1H, H-1'), 4.94 (m, 1H, H-2'), 4.38 (m, 1H, H-3'), 4.03 (m, 1H, H-4'), 3.78-3.75 (m, 1H, H-5_3'), 3.45-3.15 (m, 5H, H-5', 2xCH₂), 2.36 (m, 1H, HCH), 1.80-1.58 (m, 5H, HCH and 2xCH₂), 1.21-1.19 (m, 1H, HCH), 0.840-0.79 (m, 2H, CH₂), 0.60-0.54 (m, 1H, HCH), 0.92, 0.87 and 0.73 (3x, 3xH, 3xCH₃) 0.13, 0.11, 0.06, 0.05, -0.09 and -0.28 (6x, 6x3H, 6xCH₃). Attempts at cyclising this compound failed.

2',3',5'-Tri-O-tert-butyl(dimethyl)silyl-2-nitro-N⁶-[2-(4-aminoethyl)-piperazin-1-yl-ethyl]-adenosine (10g).

2',3',5'-Tri-O-tert-butyl(dimethyl)silyl-2-nitro-N⁶-[3-(4-aminobutyl)phenyl]-adenosine (10h).

The crude Fmoc protected product was taken up in CH₂Cl₂ (4 mL), cooled to 0 °C and DBU (0.104 mL; 0.70 mmol) was added. After stirring for 1 h the solution was diluted with light petroleum (4 mL) and immediately applied to a silica column. Flash chromatography (CH₂Cl₂/MeOH 98:2→95:5→CH₂Cl₂/MeOH/Et₃N 88:10:2) furnished free amine 10h as a yellow foam (0.318 g; 0.397 mmol; 80%).¹¹H NMR δ 8.45 (s, 1H, H-8), 7.14 (s, 1H, NH), 7.86 (s, 1H, HAr), 7.74 (d, J 7.5, 2H, Fmoc), 7.64-7.61 (m, 1H, HAr), 7.58 (d, J 7.4, 2H, Fmoc), 7.39-7.25 (m, 5H, Hfmoct and HAr), 7.00 (d, J 7.2, 1H, HAr), 6.02 (d, J 4.4, 1H, H-1'), 4.90 and 4.59 (2xbs, 1H, NH rotamers), 4.69 (t, J 4.3, 1H, H-2'), 4.38 (d, J 6.9, 2H, Fmoc), 4.33 (t, J 4.1, 1H, H-3'), 4.22-4.11 (m, 3H, Hfmoct, H-4' and H-5_3'), 3.82 (dd, J 11.2 and 2.5, 1H, H-3'), 3.27-3.22 and 3.13-3.10 (2xns, 2H, CH₂ rotamers), 2.71 (t, J 7.0, 2H, CH₂), 1.76-1.55 (m, 4H, 2xCH₂), 0.97, 0.93 and 0.82 (3x, 3xH, 3xCH₃) 0.17, 0.16, 0.11, 0.10, 0.00 and -0.13 (6x, 6x3H, 6xCH₃).

The crude Fmoc protected product was taken up in CH₂Cl₂ (4 mL), cooled to 0 °C and DBU (0.104 mL; 0.70 mmol) was added. After stirring for 1 h the solution was diluted with light petroleum (4 mL) and immediately applied to a silica column. Flash chromatography (CH₂Cl₂/MeOH 98:2→95:5→CH₂Cl₂/MeOH/Et₃N 88:10:2) furnished free amine 10h as a yellow foam (0.318 g; 0.397 mmol; 80%).¹¹H NMR δ 8.45 (s, 1H, H-8), 7.91 (s, 1H, NH), 7.80 (s, 1H, HAr), 7.66 (dd, J 8.0, J 1.5, 1H, HAr), 7.34 (t, J 7.8, 1H, HAr), 7.01 (d, J 7.8, 1H, HAr), 6.04 (d, J 4.6, 1H, H-1'), 4.71 (t, J 4.4, 1H, H-2'), 4.34 (t, J 4.1, 1H, H-3'), 4.18-4.10 (m, 2H, H-4' and H-5_3'), 3.82 (dd, J 11.2 and 2.7, 1H, H-5_3'), 2.76-2.67 (m, 4H, CH₂ and NH₂), 1.76-1.70 (m, 2H, CH₂), 1.57-1.51 (m, 2H, CH₂), 1.16-1.12 (m, 2H, CH₂), 0.97, 0.94 and 0.83 (3x, 3x9H, 3xCH₃) 0.18, 0.16, 0.11, 0.10, 0.00 and -0.14 (6x, 6x3H, 6xCH₃).
Conformationally restricted adenosine analogues

2',3',5'-Tri-O-tert-butyldimethylsilyl-2-nitro-N6-(3-[3-(aminomethyl)phenyl]propyl)-adenosine (10i).

Following the method described for adenosine analogue 10i, the Fmoc protected product was acquired (0.44 g; 0.40 mmol; 64%). 1H NMR δ 8.25 (s, 1H, H-8), 7.75 (d, J 7.5, 2H, H_{Fmoc}), 7.59 (d, J 7.2, 2H, H_{Fmoc}), 7.39 (t, J 7.4, 2H, H_{Fmoc}), 7.31-7.23 (m, 5H, H_{Fmoc} and H_{Ar}), 7.14-7.10 (m, 1H, H_{Ar}), 6.09 (bs, 1H, NH), 5.94 (d, J 4.6, 1H, H-1'), 5.29 and 5.11 (2xs, 1H, NH rotamers), 4.70 (m, 2H, H-2'), 4.45 (d, J 6.9, 2H, H_{Fmoc}), 4.37 (d, J 5.6, 2H, ArCH2NH), 4.32 (t, J 4.0, 1H, H-3'), 4.23 (t, J 6.9, 1H, H_{Fmoc}), 4.15-4.08 (m, 2H, H-4' and H-5'), 3.79 (dd, J 11.1 and 2.7, 1H, H-5'\textsubscript{b}), 3.74-3.72 (m, 2H, CH2), 2.76 (t, J 7.5, 2H, CH2), 2.07-2.03 (m, 2H, CH2), 0.95, 0.93 and 0.81 (3xs, 3x9H, 3xtBu) 0.15, 0.14, 0.11, 0.09, -0.02 and -0.17 (6xs, 6x3H, 6xCH3).

Free amine 10j was obtained as a yellow foam (0.314 g; 0.391 mmol; 98%) after flash chromatography. The product was immediately used for cyclisation experiments. Cyclisation was not accomplished.

2',3',5'-Tri-O-tert-butyldimethylsilyl-N6-hexamethylene-2-aminoadenosine (11a). A solution of amine 10a (0.16 g; 0.21 mmol) and DIPEA (0.29 mL; 1.6 mmol) in dry acetonitrile (200 mL) was refluxed under a nitrogen atmosphere for 3 days. Evaporation of the solvent and flash chromatography (light petroleum/EtOAc 4:1) afforded cycloadenosine 11a as a colourless glass (53 mg; 0.075 mmol; 36%). 1H NMR δ 7.75 (s, 1H, H-8), 5.99 (t, J 5.6, 1H, NH), 5.82 (d, J 4.7, 1H, H-1'), 4.96 (t, J 5.9, 1H, NH), 4.57 (t, J 4.5, 1H, H-2'), 4.29 (t, J 4.2, 1H, H-3'), 4.06 (dd, J 7.1 and 4.2, 1H, H-4'), 3.98 (dd, J 11.3 and 4.2, 1H, H-5'), 3.76 (dd, J 11.3 and 2.9, 1H, H-5'\textsubscript{b}), 3.57-3.52 (m, 2H, CH2), 3.43-3.37 (m, 2H, CH2), 1.66-1.60 (m, 4H, 2xCH2), 1.44-1.40 (m, 10H, 5xCH2), 0.94, 0.92 and 0.83 (3xs, 3x9H, 3xtBu) 0.11, 0.10, 0.09, 0.08, -0.03 and -0.11 (6xs, 6x3H, 6xCH3).

2',3',5'-Tri-O-tert-butyldimethylsilyl-N6-nonamethylene-2-aminoadenosine (11b). The same method was used as described for cycloadenosine 11a. Cycloadenosine 11b was isolated as a colourless glass (86 mg; 0.11 mmol; 63%). 1H NMR δ 7.72 (s, 1H, H-8), 5.82 (d, J 4.8, 1H, H-1'), 5.76 (t, J 6.5, 1H, NH), 4.80 (t, J 6.6, 1H, NH), 4.53 (t, J 4.5, 1H, H-2'), 4.29 (t, J 4.1, 1H, H-3'), 4.06 (dd, J 7.0 and 4.0, 1H, H-4'), 3.99 (dd, J 11.2 and 4.3, 1H, H-5'), 3.76 (dd, J 11.2 and 2.9, 1H, H-5'\textsubscript{b}), 3.57-3.51 (m, 2H, CH2), 3.41-3.35 (m, 2H, CH2), 1.66-1.60 (m, 4H, 2xCH2), 1.44-1.40 (m, 10H, 5xCH2), 0.94, 0.92 and 0.83 (3xs, 3x9H, 3xtBu) 0.11, 0.10, 0.09, 0.08, -0.03 and -0.11 (6xs, 6x3H, 6xCH3).

2',3',5'-Tri-O-tert-butyldimethylsilyl-N6-decamethylene-2-aminoadenosine (11c). The same method was used as described for cycloadenosine 11a. Cycloadenosine 11c was obtained as a white foam (75 mg; 0.098 mmol; 52%). 1H NMR δ 7.70 (s, 1H, H-8), 5.82 (d, J 5.1, 1H, H-1'), 5.49 (t, J 6.5, 1H, NH), 4.65 (t, J 4.7, 1H, H-2'), 4.56 (t, J 6.4, 1H, NH), 4.29 (t, J 3.9, 1H, H-3'), 4.07-4.05 (m, 1H, H-4'), 3.99 (dd, J 11.2 and 4.6, 1H, H-5'), 3.76 (dd, J 11.2 and 3.0, 1H, H-5'\textsubscript{b}), 3.67-3.64 (m, 2H, CH2), 3.53-3.50
(m, 2H, CH₂), 1.64-1.54 (m, 4H, 2xCH₂), 1.43-1.27 (m, 12H, 6xCH₃), 0.94, 0.92 and 0.82 (3x, 3xH, 3xtBu) 0.11, 0.10, 0.09, 0.08, -0.04 and -0.15 (6x, 6xH, 6xCH₃).

2',3',5'-Tri-O-tert-butyldimethylsilyl-protected cycloadenosine (11d). A solution of amine 10d (100 mg; 0.13 mmol) and DIPEA (0.2 mL; 1.3 mmol) in dry acetonitrile (90 mL) was refluxed under a nitrogen atmosphere for 7 days. Evaporation of the solvent and flash chromatography (light petroleum/EtOAc 4:1) afforded cycloadenosine 11d as a colourless glass (47 mg; 0.064 mmol; 49%). ¹H NMR δ 7.85 (s, 1H, H-8), 7.60 (s, 1H, HA), 6.73-6.64 (m, 3H, H₂A), 5.74 (bs, 1H, NH), 5.46 (t, J 6.9, 1H, NH), 4.69-4.53 (m, 4H, H-l', H-2', CH₂), 4.19 (dd, J 4.2 and 2.9, 1H, H-3'), 4.01 (dd, J 6.7 and 2.9, 1H, H-4'), 3.90 (m, 1H, H-5'), 3.72 (dd, J 11.2 and 2.6, 1H, H-5'), 3.36-3.26 (m, 2H, CH₂), 3.11-3.04 (m, 2H, CH₂), 2.49-2.43 (m, 2H, CH₂), 0.94, 0.91 and 0.78 (3x, 3xH, 3xtBu) 0.11, 0.10, 0.08, 0.07, -0.11 and -0.29 (6x, 6xH, 6xCH₃).

2',3',5'-Tri-O-tert-butyldimethylsilyl-protected cycloadenosine (11h). A solution of free amine 10h (0.318 g; 0.40 mmol) and DIPEA (0.5 mL; 3.1 mmol) in dry acetonitrile (200 mL) was refluxed under a nitrogen atmosphere for 6 days. Evaporation of the solvent and flash chromatography (light petroleum/EtOAc/Et₂N 3:1:0.04) afforded the cycloadenosine 11h as a light yellow foam (0.134 g; 0.178 mmol; 45%). ¹H NMR δ 8.45 (bs, 1H, NH), 7.84 (s, 1H, H-8), 7.56 (s, 1H, H₂A), 7.19 (t, J 7.8, 1H, H₆A), 6.86 (d, J 7.7, 2H, H₆A), 5.88 (d, J 5.0, 1H, H-1'), 4.74 (m, 1H, NH), 4.66 (t, J 4.6, 1H, H-2'), 4.31 (t, J 3.9, 1H, H-3'), 4.10-4.08 (m, 1H, H-4'), 4.01 (dd, J 11.2 and 4.5, 1H, H-5'), 3.80 (dd, J 11.2 and 2.8, 1H, H-5'), 3.55 (dd, J 12.4 and 6.3, 2H, CH₂), 2.71 (t, J 7.3, 2H, CH₂), 1.87-1.82 (m, 2H, CH₂), 1.77-1.72 (m, 2H, CH₂), 0.97, 0.95 and 0.84 (3x, 3xH, 3xtBu) 0.14, 0.13, 0.12, 0.11, -0.02 and -0.14 (6x, 6xH, 6xCH₃).

N₂,N₆-hexamethylene-2-aminoadenosine (12a). A solution of cycloadenosine 11a (53 mg; 0.075 mmol) and ammonium fluoride (0.185 mg; 5 mmol) in MeOH (4 mL) was refluxed for 20 h. After allowing the sample to cool to rt silica gel was added (1 g) and the solvent was evaporated. Flash chromatography (EtOAc/MeOH 99:1→EtOAc/MeOH 88:12) followed by crystallisation from water yielded cycloadenosine 12a as a white solid (15.3 mg; 0.042 mmol; 56%); mp 213-216 °C. ¹H NMR (d₆-DMSO) δ 7.87 (s, 1H, H-8), 7.68 (t, J 5.2, 1H, NH), 6.42 (t, J 5.6, 1H, NH), 5.71 (d, J 6.4, 1H, H-1'), 5.54 (bs, 1H, OH), 5.40 (bs, 1H, OH), 5.15 (bs, 1H, OH), 4.55 (t, J 5.5, 1H, H-2'), 4.10 (m, 1H, H-3'), 3.91 (m, 1H, H-4'), 3.67-3.62 (m, 1H, H-5'), 3.58-3.52 (m, 1H, H-5'), 3.42-3.39 (m, 2H, CH₂), 3.29-3.26 (m, 2H, CH₂), 1.63-1.59 (m, 4H, 2xCH₂), 1.51-1.43 (m, 4H, 2xCH₂). m/z 365.1946 (M⁺+H, C₁₅H₂₅N₆O₄ requires 365.1967).

N₂,N₆-nonamethylene-2-aminoadenosine (12b). The same procedure was used as described for cycloadenosine 12a. The crude product was obtained as a white solid (35 mg; 0.086 mmol; 78%) after flash chromatography. Trituration with Et₂O afforded pure cycloadenosine 12b as a white solid (28 mg; 0.069 mmol; 63%); mp 193-195 °C. ¹H NMR (d₆-DMSO) δ 7.87 (s, 1H, H-8), 7.53 (t, J 6.1, 1H, NH), 6.24 (t, J 6.1, 1H, NH), 5.70 (d, J 6.3, 1H, H-4'), 5.51 (bs, 1H, OH), 5.37 (bs, 1H, OH), 5.13 (bs, 1H, OH), 4.53 (m, 1H, H-2'), 4.10 (m, 1H, H-3'), 3.91 (m, 1H, H-4'), 3.66-3.63 (m, 1H, H-5'), 3.55-3.52 (m, 1H, H-5'), 3.37-3.31 (m, 2H, CH₂), 3.27-3.23 (m, 2H, CH₂), 1.61-1.51 (m, 4H, 2xCH₂), 1.42-1.35 (m, 10H, 5xCH₂). m/z 407.2406 (M⁺+H, C₁₅H₂₃N₆O₄ requires 407.2407).

N₂,N₆-decamethylene-2-aminoadenosine (12c). The same procedure was used as described for cycloadenosine 12a. The crude product was obtained as a white solid (30 mg; 0.071 mmol; 73% ) after flash chromatography. Trituration with Et₂O afforded the pure product as a white solid (18 mg;
volatile ss gave the crude product which was taken up in warm CH₂Cl₂. 3.43 (t, J 6.3, 1H, H'1), 5.43 (bs, 1H, OH), 5.36 (d, J 6.2, 1H, OH), 5.11 (d, J 4.0, 1H, OH), 4.54 (m, 1H, H-2'), 4.11 (m, 1H, H-3'), 3.91 (m, 1H, H-4'), 3.67-3.63 (m, 1H, H-5'), 3.57-3.40 (m, 3H, H-5' ' and CH₂), 3.35-3.32 (m, 2H, CH₂), 1.61-1.51 (m, 4H, 2xCH₂), 1.40-1.25 (m, 12H, 6xCH₃). m/z 421.2554 (M⁺+H, C₂₀H₃₁N₆O₄ requires 421.2563).

Deprotection of cycloadenosine 11d. A solution of tri-TBDMS protected cycloadenosine 11d (32 mg; 0.043 mmol) and ammonium fluoride (80 mg; 2.15 mmol) in MeOH (2 mL) was refluxed for 2 h. After allowing the sample to cool to rt silica gel was added (30 mg) and the solvent was evaporated. Flash chromatography (CH₂Cl₂/MeOH 99:1→85:15) afforded crude 12d. A pure sample was obtained by tritylation with Et₂O yielding cycloadenosine 12d as a white solid (8 mg; 0.019 mmol; 45%). ^1H NMR (d₆-DMSO) δ 7.93 (s, 1H, H-8), 7.72 (s, 1H, H₆), 7.09 (bs, 1H, NH), 6.69-6.64 (m, 3H, H₆), 5.95 (bs, 1H, NH), 5.58 (d, J 4.6, 1H, H-1'), 5.43 (bs, 1H, OH), 5.33 (bs, 1H, OH), 5.09 (bs, 1H, OH), 4.55-4.39 (m, 3H, H-2', CH₂), 4.04 (m, 1H, H-3'), 3.85 (m, 1H, H-4'), 3.61-3.46 (m, 2H, H-5), 3.27-3.18 (m, 2H, CH₂), 3.01-2.92 (m, 2H, CH₂), 2.45-2.34 (m, 2H, CH₂). m/z 413.1935 (M⁺+H, C₂₀H₂₅N₆O₄ requires 413.1937).

Deprotection of cycloadenosine 11h. A mixture of tri-TBDMS protected cycloadenosine 11h (48 mg; 0.077 mmol) and Et₃N·3 HF (0.2 mL) in THF (1 mL) was stirred for 3 days. The white precipitate was filtered off and washed with THF (2 mL). Trituration with MeOH afforded pure cycloadenosine 12h as a white solid (19 mg; 0.047 mmol; 61%); mp >250 °C, decomp. ^1H NMR (d₆-DMSO) δ 9.39 (bs, 1H, NH), 8.47 (s, 1H, H₆), 8.07 (s, 1H, H-8), 7.22 (d, J 8.0, 1H, NH), 7.14 (t, J 7.7, 1H, H₆), 6.80 (d, J 7.2, 1H, H₆), 6.52 (bs, 1H, NH), 5.79 (d, J 5.9, 1H, H-1'), 5.40 (d, J 6.0, 1H, OH), 5.20 (bs, 1H, OH), 5.13 (d, J 4.4, 1H, OH), 4.55 (m, 1H, H-2'), 4.14 (m, 1H, H-3'), 3.92 (m, 1H, H-4'), 3.69-3.65 (m, 1H, H-5'), 3.58-3.55 (m, 1H, H-5'), 3.43-3.38 (m, 2H, CH₂), 2.62-2.58 (m, 2H, CH₂), 1.70-1.61 (m, 4H, 2xCH₂). m/z 413.1937 (M⁺+H, C₂₀H₂₅N₆O₄ requires 413.1937).

1,3-Azidopropylphosphonium bromide. 1,3-Azidopropylphosphonium bromide was synthesised according to a modified literature procedure. ^24 A mixture of triphenylphosphine (26.2 g; 50 mmol) and 1,3-dibromopropane (40 mL; 200 mmol) in toluene (125 mL) was stirred at 80 °C for 18 h. Filtration furnished 3-bromopropyltriphenylphosphonium bromide as a white solid (23.6 g; 50 mmol), which was used without further purification. A mixture of 3-bromopropyltriphenylphosphonium bromide (9.29 g; 20 mmol) and sodium azide (1.95 g; 30 mmol) in H₂O-EtOH (1:1) was refluxed for 10 h. After concentration the mixture was extracted with CH₂Cl₂. Drying with Na₂SO₄ and evaporation of the volatiles gave the crude product which was taken up in warm CH₂Cl₂ and precipitated with EtOAc and then Et₂O. The product was filtered and dried in vacuo at 50 °C to yield azidopropylphosphonium bromide as a white solid (8.0 g; 19 mmol; 94%). ^1H NMR (d₆-DMSO) δ 7.95-7.90 (m, 3H, H₆), 7.87-7.77 (m, 12H, H₆), 3.70-3.63 (m, 2H, CH₂), 3.55 (t, J 6.5, 2H, CH₂), 1.82-1.76 (m, 2H, CH₂).

ω-Azido alkene (14). Potassium tert-butoxide (1.79 g; 16 mmol) was added in 3 portions to a solution of 3-nitrobenzaldehyde (2.71 g; 18 mmol) and azidopropylphosphonium bromide (6.82 g; 16 mmol) in THF (70 mL) at 0 °C. After stirring for 30 min. the reaction mixture was quenched with NH₄Cl. After 5 min water was added and the mixture was extracted with EtOAc. Flash chromatography (light petroleum/EtOAc 2:1) afforded Z-azido alkene 14 (3.0 g; 13.8 mmol; 86%). ^1H NMR δ 8.13 (m, 2H, H₆), 7.59 (d, J 7.7, 1H, H₆), 7.52 (t, J 7.7, 1H, H₆), 6.62 (d, J 11.6, 1H, ArCHCH₃), 5.86-5.80 (m, 1H, ArCHCH₃), 3.43 (t, J 6.7, 2H, -CH₂N₃), 2.63-2.57 (m, 2H, -CH₂CH₂N₃).
3-(4-Aminobutylaniline (15). A mixture of azide 14 (2.5 g; 11.5 mmol) and 10 wt-% palladium on carbon (0.5 g) in MeOH (50 mL) was stirred under a hydrogen atmosphere (balloon) for 1 night. Extra palladium on carbon was added (0.2 g) and the reaction was allowed to continue for 16 h. The mixture was filtrated over highflow and the filtrate was concentrated to dryness. Flash chromatography (EtOAc/MeOH/conc. NH₄OH 78:20:2) afforded 3-(4-aminobutylaniline 15 (1.4 g; 8.5 mmol; 74%).

3-(4-Fmoc-aminobutylaniline (16). A mixture of 3-(4-aminobutylaniline 15 (0.198 g; 1.21 mmol), DIPEA (0.24 mL; 1.4 mmol) and Fmoc chloride (0.337 g; 1.3 mmol) in CH₂Cl₂ (4 mL) was stirred at 0 °C for 3 h. The mixture was diluted with light petroleum and immediately applied to a silica column. Flash chromatography (EtOAc/light petroleum 1:1) afforded 3-(4-Fmoc-aminobutylaniline 16 as a white solid (0.368 g; 95 mmol; 79%). ¹H NMR δ 7.76 (d, J 7.5, 2H, Fmoc), 7.59 (d, J 7.2, 2H, H₃Fmoc), 7.40 (t, J 7.4, 2H, Fmoc), 7.32 (dt, J 7.4 and 1.0, 2H, H₃Fmoc), 7.07 (t, J 7.1, 1H, H₃Fmoc), 6.58 (d, J 7.4, 1H, H₃Fmoc), 4.70 and 4.43 (2xbs, 1H, NH rotamers), 4.40 (d, J 6.8, 2H, H₃Fmoc), 4.21 (t, J 6.8, 1H, Fmoc), 3.60 (bs, 2H, NH₂), 3.22-3.20 and 3.10-3.08 (2xm, 2H, CH₂ rotamers), 2.54 (t, J 7.2, 2H, CH₂), 1.64-1.55 (m, 4H, 2xCH₂).

N-Fmoc-propargylamine (17). This compound was synthesised according to a modified literature procedure. A mixture of propargylamine (0.688 mL; 10 mmol), DIPEA (2.09 mL; 12 mmol) and Fmoc chloride (2.59 g; 10 mmol) in CH₂Cl₂ (20 mL) was stirred at 0 °C for 3 h. The mixture was stirred for an additional hour at rt. The organic layer was washed with water (2x20 mL) and dried with Na₂SO₄. After evaporation of the solvent the crude product was triturated with Et₂O/light petroleum to afforded Fmoc-propargylamine 17 as a white solid (2.6 g; 94 mmol; 94%). ¹H NMR δ 7.77 (d, J 7.5, 2H, Fmoc), 7.59 (d, J 7.3, 2H, H₃Fmoc), 7.41 (t, J 7.4, 2H, H₃Fmoc), 7.31 (dd, J 7.4 and 0.9, 2H, H₃Fmoc), 4.95 (bs, 1H, NH), 4.43 (d, J 7.0, 2H, H₃Fmoc), 4.25-4.22 (m, 1H, H₃Fmoc), 4.00 (m, 2H, CH₂), 2.26 (t, J 2.4, 1H, CCH).

N-Boc-propargylamine (18). This compound was synthesised according to a modified literature procedure. A mixture of propargylamine (0.688 mL; 10 mmol) and Boc₂O (2.4 g; 11 mmol) in CH₂Cl₂ (20 mL) was stirred for 4 h. Evaporation of the solvent and crystallisation from light petroleum at −20 °C afforded N-Boc-propargylamine 18 as a white solid (1.27 g; 8.2 mmol; 82%). ¹H NMR δ 4.68 (bs, 1H, NH), 3.92 (m, 2H, CH₂), 2.26 (t, J 2.5, 1H, CCH), 1.45 (s, 9H, t-Bu).

N-Fmoc-3-iodobenzylamine (19). A mixture of 3-iodobenzylamine (0.47 g; 2.0 mmol), DIPEA (0.39 mL; 2.2 mmol) and Fmoc chloride (0.52 g; 2 mmol) in CH₂Cl₂ (10 mL) was stirred at 0 °C for 3 h. The mixture was washed with water (2x10mL), the organic layer was dried with Na₂SO₄ and the solvent was evaporated. Trituration with Et₂O afforded N-Fmoc-3-iodobenzylamine 19 as a white solid (0.78 g; 1.71 mmol; 85%). ¹H NMR δ 7.76 (d, J 7.6, 2H, H₃Fmoc), 7.63-7.58 (m, 3H, H₃Fmoc and H₃A), 7.41 (t, J 7.4, 2H, H₃Fmoc), 7.32 (t, J 7.3, 2H, H₃Fmoc), 7.24-7.21 (m, 1H, H₃A), 7.07 (t, J 7.6, 1H, H₃A), 5.06 (bs, 1H, NH), 4.48 (d, J 6.7, 2H, H₃Fmoc), 4.33 (d, J 5.9, 2H, CH₂), 4.23 (t, J 6.7, 1H, H₃Fmoc).

Sonogashira coupling of acetylene 18 and aryl iodide 19. By the method described for aryl acetylene 24 aryl acetylene 20 was obtained as a solid after trituration with Et₂O/light petroleum (0.335 g; 0.695 mmol; 70%). ¹H NMR δ 7.77 (d, J 7.5, 2H, H₃Fmoc), 7.60 (d, J 7.4, 2H, H₃Fmoc), 7.41 (t, J 7.4, 2H,
**Conformationally restricted adenosine analogues**

H$_{\text{Fmoc}}$, 7.38-7.20 (m, 6H, H$_{\text{Fmoc}}$ and H$_{\text{Ar}}$), 5.06 (bs, 1H, NH), 4.73 (bs, 1H, NH), 4.47 (d, J 6.8, 2H, H$_{\text{Fmoc}}$), 4.36 (d, J 5.9, 2H, ArCH$_2$), 4.25 (t, J 6.8, 1H, H$_{\text{Fmoc}}$), 4.15 (m, 2H, CH$_2$), 1.47 (s, 9H, t-Bu).

**Catalytic hydrogenation of acetylene 20.** A suspension of acetylene 20 (0.30 g; 0.62 mmol) and 10 wt-% palladium on carbon (100 mg) in MeOH (10 mL) was stirred at 50 °C for 20 h under a hydrogen atmosphere (balloon). The mixture was filtered over highflow and the filtrate was concentrated to dryness affording arylalkane 21 (0.30 g; 0.62 mmol; 99%). $^1$H NMR δ 7.77 (d, J 7.5, 2H, H$_{\text{Fmoc}}$), 7.60 (d, J 7.3, 2H, H$_{\text{Fmoc}}$), 7.40 (t, J 7.4, 2H, H$_{\text{Fmoc}}$), 7.32-7.23 (m, 3H, H$_{\text{Fmoc}}$ and H$_{\text{Ar}}$), 7.11-7.09 (m, 3H, H$_{\text{Ar}}$), 5.12 and 4.93 (2xbs, 1H, NH rotamers), 4.56 (bs, 1H, NH), 4.45 (d, J 6.9, 2H, H$_{\text{Fmoc}}$), 4.36 (d, J 5.8, 2H, ArCH$_2$NH), 4.23 (t, J 6.9, 1H, H$_{\text{Fmoc}}$), 3.15-3.12 (m, 2H, CH$_2$), 2.63 (t, J 7.7, 2H, CH$_2$), 1.80 (quintet, J 7.7, 2H, CH$_2$), 1.46 (s, 9H, t-Bu).

**N-Fmoc-3-(3-aminopropyl)benzylamine (22).** By the method described for amine 26 the crude arylpropylamine-TFA salt 22 was obtained as a solid (0.31 mg; 0.62 mmol; 99%). This compound was used without further purification.

**N-Boc-3-iodobenzylamine (23).** A mixture of 3-iodobenzylamine (0.5 g; 2.15 mmol) and Boc$_2$O (0.65 g; 3.0 mmol) in dry CH$_2$Cl$_2$ (5 mL) was stirred for 4 h at rt. The mixture was diluted with light petroleum and immediately applied to a silica column. Flash chromatography (EtOAc/light petroleum 1:4) afforded N-Boc-3-iodobenzylamine 23 as an oil (0.60 g; 1.80 mmol; 84%). $^1$H NMR δ 7.63 (s, 1H, H$_{\text{Ar}}$), 7.59 (d, J 7.9, 1H, H$_{\text{Ar}}$), 7.24 (d, J 7.8, 1H, H$_{\text{Ar}}$), 7.06 (t, J 7.8, 1H, H$_{\text{Ar}}$), 4.81 (bs, 1H, NH), 4.26-4.27 (m, 2H, CH$_2$), 1.46 (s, 9H, t-Bu).

**Sonogashira coupling of acetylene 17 and aryl iodide 23.** To an argon flushed solution of acetylene 17 (0.415 g; 1.5 mmol) and aryl iodide 23 (0.275 g; 0.87 mmol) in dry DMF (3 mL) was added copper(l)iodide (0.038 g; 0.2 mmol), palladium(0)tetrakis(triphenylphosphine) (0.115 g; 0.1 mmol) and triethylamine (0.152 mL; 1.1 mmol). The mixture was stirred for 4 h under an argon atmosphere. Water (10 mL) was added and the mixture was extracted with Et$_2$O (2×5 mL). The combined organic layers were dried with Na$_2$SO$_4$ and the solvent was evaporated. Flash chromatography (EtOAc/light petroleum 1:2) afforded aryl acetylene 24 as a solid (0.278 g; 0.595 mmol; 69%). $^1$H NMR δ 7.77 (d, J 7.6, 2H, H$_{\text{Fmoc}}$), 7.61 (d, J 7.1, 2H, H$_{\text{Fmoc}}$), 7.40 (t, J 7.4, 2H, H$_{\text{Fmoc}}$), 7.35-7.24 (m, 6H, H$_{\text{Fmoc}}$ and H$_{\text{Ar}}$), 5.00 (bs, 1H, NH), 4.79 (bs, 1H, NH), 4.44 (d, J 7.0, 2H, H$_{\text{Fmoc}}$), 4.30-4.20 (m, 5H, CH$_2$, CH$_2$ and H$_{\text{Fmoc}}$), 1.46 (s, 9H, t-Bu).

**Catalytic hydrogenation of aryl acetylene 24.** A suspension of acetylene 24 (0.1 g; 0.21 mmol) and 10 wt-% palladium on carbon (5 mg) in EtOAc (4 mL) was stirred under a hydrogen atmosphere (balloon) for 3 h. The mixture was filtered over highflow and the filtrate was concentrated to dryness offering arylalkane 25 (0.1 g; 0.21 mmol; 99%). $^1$H NMR δ 7.76 (d, J 7.5, 2H, H$_{\text{Fmoc}}$), 7.59 (d, J 7.4, 2H, H$_{\text{Fmoc}}$), 7.40 (t, J 7.4, 2H, H$_{\text{Fmoc}}$), 7.31 (t, J 7.4, 2H, H$_{\text{Fmoc}}$), 7.26-7.23 (m, 1H, H$_{\text{Ar}}$), 7.12-7.07 (m, 3H, H$_{\text{Ar}}$), 4.82-4.73 and 4.45 (2xms, 2H, 2xNH rotamers), 4.41 (d, J 6.8, 2H, H$_{\text{Fmoc}}$), 4.30-4.20 (m, 3H, CH$_2$ and H$_{\text{Fmoc}}$), 3.25-3.20 and 3.11-3.08 (2xms, 2H, CH$_2$ rotamers), 2.65-2.57 (m, 2H, CH$_2$ rotamers), 1.85-1.82 and 1.73-1.69 (2xms, 2H, CH$_2$ rotamers), 1.46 (s, 9H, t-Bu).

**3-(3-N-Fmoc-aminopropyl)benzylamine (26).** TFA (1 mL) was added to a solution of arylalkane 25 (0.1 g; 0.21 mmol) in dry CH$_2$Cl$_2$ (2 mL). The solution was stirred for 1 h. The mixture was diluted with toluene (2 mL) and the volatiles were evaporated. Co-evaporation with toluene (2 mL) furnished
the crude benzylamine-TFA salt 26 (104 mg; 0.21 mmol; 99%). This compound was used without further purification.

2-Pentylamino-N4-pentyladenosine (28). A solution of 2-nitro-6-chloro-9(2,3,5-triacetyl-β-D-ribofuranosyl)-9H-purine 27,23 (250 mg, 0.55 mmol) in 1-pentyamine (5 mL) was stirred under a nitrogen atmosphere for 16 h. The solution was evaporated to dryness and the residue was subjected to flash chromatography (methyl-t-butylether with 5→15% MeOH). Drying in vacuo at 55 °C for 2 days and trituration with Et2O furnished adenosine analogue 28 (116 mg, 0.27 mmol; 50%) as a white solid, mp 144–145 °C. 1H-NMR (d6-DMSO) δ 7.88 (s, 1H, H-8), 7.28 (br s, 1H, N6-H), 5.72 (d, J 6.1, 1H, H-1'), 5.35 (d, J 6.1, 1H, OH), 5.19 (br s, 1H, OH), 5.11 (1H, d, J 4.6, OH), 4.60 (1H, m, H-2'), 4.12 (dd, J 8.0 and 3.5, 2H, H-3'), 3.90 (m, 1H, H-4'), 3.67-3.62 (m, 1H, H-5'), 3.41-3.29 (m, 2H, NCH2), 1.62-1.51 (m, 4H, pentyl), 1.32-1.28 (m, 8H, pentyl), 0.90-0.987 (m, 6H, pentyl); m/z 413.2740 (M+H, C20H33N6O4 requires 423.2720).

2',3',5'-Tri-O-acetyl-2-nitro-adenosine (32). A solution of 2-nitro-6-chloro-9-(2,3,5-triacetyl-β-D-ribofuranosyl)-9H-purine (0.30 g; 0.66 mmol) and DIPEA (0.22 mL; 1.32 mmol) in CH2Cl2 (8 mL) at 0 °C and the solution was stirred for 8 h under a nitrogen atmosphere. EtOAc (20 mL) was added and the mixture was washed with 1% aqueous oxalic acid (3x15 mL). Drying with Na2SO4 and evaporation of volatiles afforded crude 29 as a yellow foam, which was used without further purification. 1H-NMR δ 8.08 (s, 1H, H-8), 7.34-7.22 (m, 5H, H6), 6.32 (bs, 1H, NH), 6.22 (d, J 5.3, 1H, H-1'), 5.74 (t, J 5.4, 1H, H-2'), 5.65-5.60 (m, 1H, H-3'), 4.48-4.42 (m, 3H, H-5' and H-6'), 4.00-3.95 (m, 2H, NCH2), 3.02 (t, J 7.0, 2H, PhCH2), 2.17, 2.12 and 2.09 (3xs, 3x3H, 3xAc).

2-Ethylamino-N6-phenethyladenosine (30). A solution of 2-nitro-N6-phenethyladenosine 29 (194 mg, 0.36 mmol) in 70% ethylamine in water (25 mL) was stirred for 4 h. The product was adsorbed to silica and volatiles were evaporated. Flash chromatography (methyl-t-butylether with 5→15% MeOH), and drying in vacuo at 55 °C for 1 day was followed by trituration with Et2O to yield adenosine analogue 30 as a white solid (55 mg; 0.13 mmol; 37%), mp 180–181 °C. 1H-NMR (d6-DMSO) δ 7.90 (s, 1H, H-8), 7.37 (br s, 1H, N6-H), 7.34-7.20 (m, 5H, H6), 6.27 (br s, 1H, N5-H), 5.74 (d, J 6.1, 1H, H-1'), 5.36 (d, J 6.1, 1H, OH), 5.17 (br s, 1H, OH), 5.12 (1H, d, J 4.6, OH), 4.63-4.60 (1H, m, H-2'), 4.12 (dd, J 8.1 and 4.6, 2H, H-3'), 3.91-3.89 (m, 1H, H4'), 3.68-3.62 (m, 3H, CH2 and H5'), 3.57-3.51 (m, 1H, H-5'b), 3.30-3.25 (m, 2H, NCH2), 3.08-3.02 (m, 2H, NCH2), 2.92 (t, J 8.0, 2H, CH2), 1.15 (t, J 7.1, 3H, CH3). m/z 415.2097 (M+H, C20H27N6O4 requires 415.2094).

2',3'-O-Isopropylidene-2-nitro-adenosine (32). A mixture of 2-nitro adenosine8,23 31 (0.936 g; 3 mmol), pTsOH-H2O (0.95 g; 5 mmol) and trimethyl orthoformate (1.64 mL; 15 mmol) in dry acetone (60 mL) was stirred for 20 min. The mixture was neutralised with triethylamine (0.696 mL; 5 mmol) and the solvent was evaporated. Flash chromatography (EtOAc with 6% MeOH) furnished 2',3'-protected 2-nitro-adenosine 32 as a yellow solid (0.641 g; 1.82 mmol; 61%). mp 154-157 °C. 1H-NMR (CDCl3/d6-DMSO) δ 8.30 (s, 1H, H-8), 7.35 (bs, 2H, NH2), 5.93 (d, J 3.8, 1H, H-1'), 4.99 (dd, J 6.0 and 3.8, 1H, H-2'), 4.90 (dd, J 6.0 and 1.9, 1H, H-3'), 4.29 (s, 1H, H-4'), 3.71 (dd, J 12.5 and 2.4, 1H, H-5'a), 3.59 (dd, J 12.4 and 3.0, 1H, H-5'b), 1.45 (s, 3H, CH3), 1.19 (s, 3H, CH3).

Mono-N-trityl-1,2-diaminoethane. A solution of trityl chloride (3.5 g; 12.5 mmol) in CH2Cl2 (50 mL) was added dropwise to a stirred solution of 1,3 diaminoethane (5.0 mL; 75 mmol) in CH2Cl2 (50 mL).
When the reaction was complete as judged by TLC analysis, the mixture was washed with 1 M NaOH (25 mL) and water (2 x 25 mL) and dried with Na2SO4. Flash chromatography (EtOAc/MeOH/NH4OH 83:15:2 afforded the product as an oil (3.0 g; 9.9 mmol; 79%). 1H-NMR (d6-DMSO) δ 7.42 (d, J 7.6, 6H, trityl), 7.30 (t, J 7.6, 6H, triyl), 7.19 (t, J 7.6, 3H, triyl), 2.68-2.64 (m, 2H, CH2), 2.03-2.00 (m, 2H, CH2).

**Mono-N-trityl-1,3-diaminopropane.** The same procedure was used as described for mono-N-trityl-1,2-diaminoethane affording mono-N-trityl-1,3-diaminopropane as an oil (2.8 g; 8.8 mmol; 70%). 1H-NMR (d6-DMSO) δ 7.41 (d, J 7.5, 6H, trityl), 7.30 (t, J 7.5, 6H, triyl), 7.19 (t, J 7.5, 3H, triyl), 2.57 (t, J 6.8, 2H, CH2), 2.02 (t, J 6.8, 2H, CH2), 1.54 (quintet, J 6.8, 2H, CH2).

2',3'-O-Isopropylidene-2-nitro-adenosine-5'-carboxylic acid (33). This compound was synthesised according to a modified literature procedure.26 A mixture of iodobenzene diacetate (0.354 g; 1.10 mmol), TEMPO (16 mg; 0.10 mmol) and 2',3'-O-isopropylidene-2-nitro-adenosine 32 (0.176 mg; 0.50 mmol) in water/acetonitrile/light petroleum/toluene 1:1:1:1 (2 mL) was stirred for 4 h. The resulting precipitate was filtered and triturated with Et2O to yield 5'-carboxylic acid 33 as a solid (156 mg; 0.426 mmol; 85%); mp 165 °C decomp. 1H-NMR (d6-DMSO) δ 12.74 (bs, 1H, COOH), 8.56 (s, 1H, H-8), 8.29 (s, 2H, NH2), 6.43 (s, 1H, H-1'), 5.67 (m, 1H, H-3'), (d, J 5.6, 1H, H-2'), 4.74 (s, 1H, H-4'), 1.54 (s, 3H, CH3), 1.38 (s, 3H, CH3).

2',3'-O-Isopropylidene-2-nitro-5'-N-(2-[N-trityl-amino]ethyl)carboxamidoadenosine (34). A mixture of 2',3'-O-isopropylidene-2-nitro-adenosine-5'-carboxylic acid 33 (73 mg; 0.20 mmol), N-Trityl-1,2-diaminoethane (76 mg; 0.25 mmol), EDC (76 mg; 0.40 mmol) and HOBr (7 mg; 0.05 mmol) in DMF-THF (1:2; 3 mL) was stirred for 1.5 h. The mixture was diluted with Et2O (6 mL) and washed with water (3x5 mL). The combined water layers were extracted with Et2O (6 mL) and the organic layers were combined and dried with Na2SO4. Evaporation of the volatiles afforded the crude carboxamide 34 as a yellow solid (103 mg; 0.16 mmol; 80%), which was used without further purification. 1H-NMR δ 8.09 (s, 1H, H-8), 7.32-7.13 (m, 16H, CONH and triyl), 6.36 (bs, 2H, NH2), 6.10 (d, J 3.6, 1H, H-1'), 5.30-5.24 (m, 2H, H-2' and H-3'), 4.75 (m, 1H, H-4'), 3.66-3.60 (m, 1H, HCH), 3.10-3.08 (m, 1H, HCH), 2.23-2.20 (m, 1H, HCH), 2.11-2.08 (m, 1H, HCH), 1.65 (s, 3H, CH3), 1.37 (s, 3H, CH3).

2',3'-O-Isopropylidene-2-nitro-5'-N-(3-[N-trityl-amino]propyl)carboxamidoadenosine (35). This compound was prepared by the method described for carboxamide adenosine 34. Flash chromatography (EtOAc) furnished carboxamide 35 as a yellow solid (104 mg; 0.157 mmol; 78%). 1H-NMR δ 7.93 (s, 1H, H-8), 7.43-7.18 (m, 15H, CONH and triyl), 6.88 (bs, 1H, CONH), 6.62 (bs, 2H, NH2), 6.15 (s, 1H, H-1'), 5.38 (s, 1H, H-2'), 5.28 (s, 1H, H-3'), 4.72 (s, 1H, H-4'), 3.40-3.36 (m, 1H, HCH), 3.31-3.28 (m, 1H, HCH), 2.03-1.99 (m, 1H, HCH), 1.79-1.70 (m, 3H, HCH and CH2), 1.67 (s, 3H, CH3), 1.33 (s, 3H, CH3).

2',3'-O-Isopropylidene-N2,N5'-dimethylene-5'-carboxamido-2-aminoadenosine (36). Crude tritylamine 34 (90 mg; 0.14 mmol) was dissolved in CH2Cl2 (2 mL) and TFA was added (2 mL) upon which the solution turned bright yellow. After stirring for 30 seconds MeOH (2 mL) was added and the solution was stirred for 1 min until the yellow colour disappeared. Toluene was added (20 mL) and volatiles were evaporated (20 mbar, bath temperature ≤ 40 °C!). A second co-evaporation with toluene (5 mL) afforded the free amine·TFA salt which was taken up in acetonitrile (16 mL). DIPEA (0.5 mL; 2.8 mmol) was added and the solution was refluxed for 4 h. The product was adsorbed to silica and volatiles were evaporated. Flash chromatography (EtOAc with 0->20% MeOH) afforded lactam 36 (40 mg; 0.11 mmol; 79%). 1H-NMR (d6-DMSO) δ 9.76 (bs, 1H, CONH), 7.93 (s, 1H, H-8), 7.22 (bs,
2',3'-O-Isopropylidene-N^2,N^2'-trimethylene-5'-carboxamido-2-aminoadenosine (37). This compound was prepared by the method described for amine 36. Flash chromatography (EtOAc with 5–8% MeOH) gave lactam 37 (58.5 mg; 0.156 mmol; 99%). \(^1\)H-NMR (d_6-DMSO) \(\delta\) 9.27 (bs, 1H, CONH), 7.94 (s, 1H, H-8), 6.95 (bs, 2H, NH2), 6.86 (dd, J = 8.1 and 5.6, 1H, NH), 6.04 (d, J = 5.4, 1H, H-1'), 5.21 (t, J = 5.4, 1H, H-2'), 4.97 (dd, J = 5.9 and 1.2, 1H, H-3'), 4.51 (d, J = 1.2, 1H, H-4'), 3.36-3.32 (m, 1H, HCH), 3.16-3.10 (m, 2H, HCH and HCH), 2.99-2.95 (m, 1H, HCH), 2.11-2.09 (m, 1H, HCH), 1.61 (s, 3H, CH3), 1.33 (s, 3H, CH3), 1.31-1.26 (m, 1H, HCH).

N^2,N^2'-dimethylene-5'-carboxamido-2-aminoadenosine (38). Lactam 36 (39 mg; 0.11 mmol) was dissolved in TFA-water (9:1; 2 mL). After stirring for 15 min the mixture was evaporated to dryness (20 mbar, bath temperature \(< 40\degree C\)). Flash chromatography (EtOAc/Methanol 8:2) and trituration with Et\(_2\)O afforded lactam 38 as a solid (29 mg; 0.087 mmol; 79%). \(^1\)H-NMR (d_6-DMSO) \(\delta\) 9.78 (bs, 1H, CONH), 7.89 (s, 1H, H-8), 7.00 (bs, 2H, NH2), 6.89 (m, 1H, NH), 5.83 (d, J = 8.3, 1H, H-1'), 5.69 (d, J = 3.6, 1H, OH), 5.59 (d, J = 6.7, 1H, OH), 5.01-4.98 (m, 1H, H-2'), 4.34-4.31 (m, 2H, H-3' and H-4'), 3.87-3.82 (m, 2H, HCH and HCH), 3.49-3.42 (m, 1H, CH), 3.10-3.06 (m, 1H, CH), 2.79-2.73 (m, 1H, CH). m/z 322.1271 (M^+H, C\(_{12}\)H\(_{16}\)N\(_2\)O requires 322.1264).

N^2,N^2'-trimethylene-5'-carboxamido-2-aminoadenosine (39). This compound was prepared by the method described for lactam 38. Lactam 39 was isolated as a white solid (36.5 mg; 0.087 mmol; 79%), mp 177-179 \degree C. \(^1\)H-NMR (d_6-DMSO) \(\delta\) 9.57 (bs, 1H, CONH), 7.88 (s, 1H, H-8), 6.97-6.93 (m, 3H, NH2 and NH), 5.83-5.80 (m, 2H, H-1' and OH), 5.48 (d, J = 6.9, 1H, OH), 4.86-4.81 (m, 1H, H-2'), 4.29 (s, 1H, H-4'), 4.09 (t, J = 4.1, 1H, H-3'), 3.24-3.11 (m, 4H, H-5' and NH), 2.15-2.12 (m, 1H, HCH), 1.31-1.25 (m, 1H, HCH). m/z 336.1424 (M^+H, C\(_{13}\)H\(_{18}\)N\(_2\)O requires 336.1420).

2',3'-Isopropylidene-2-(N-glycyl)adenosine (40). A mixture of 2',3'-isopropylidene-2-nitro-adenosine 32 (100 mg; 0.31 mmol) and sodium glycinate-5 H\(_2\)O (0.29 g; 1.55 mmol) in DMF-H\(_2\)O (1:1; 3 mL) was stirred at 80 \degree C for 2 h. After allowing the mixture to cool down to rt the pH was adjusted to \(\approx 5-6\) with acetic acid (420 \(\mu\)L) upon which the product precipitated from the solution. Filtration and washing with water (0.5 mL) afforded 2-N-glycyl-adenosine 40 as a white solid (88 mg; 0.23 mmol; 75%). \(^1\)H-NMR (d_6-DMSO) \(\delta\) 7.92 (s, 1H, H-8), 6.85 (bs, 2H, NH2), 6.50 (bs, 1H, NH), 6.00 (d, J = 2.4, 1H, H-1'), 5.41 (m, 1H, H-2'), 4.99 (dd, J = 6.2 and 2.7, H-3'), 4.12 (m, 1H, OH), 3.87-3.81 (m, 3H, H-4' and CH\(_2\)), 3.57-3.50 (m, 2H, H-5'), 1.54 (s, 3H, CH3), 1.35 (s, 3H, CH3).

2',3'-Isopropylidene-2-N-\(\beta\)-alanyl adenosine (41). This compound was prepared by the method described for 2-N-glycyl-adenosine 40 using sodium \(\beta\)-alaninate-3 H\(_2\)O. 2-N-\(\beta\)-alanyl-adenosine 41 was obtained as a white solid (76 mg; 0.193 mmol; 64%), mp 205-207 \degree C. \(^1\)H-NMR (d_6-DMSO) \(\delta\) 12.12 (bs, 1H, COOH), 7.91 (s, 1H, H-8), 6.81 (bs, 2H, NH2), 6.31 (t, J = 5.8, 1H, NH), 6.01 (d, J = 2.2, 1H, H-1'), 5.41 (m, 1H, H-2'), 5.06 (bs, 1H, OH), 4.99 (dd, J = 6.2 and 2.8, H-3'), 4.14-4.10 (m, 1H, H-4'), 3.57-3.41 (m, 4H, H-5' and NHCH\(_2\)), 2.54 (d, J = 1.9, 2H, CH\(_2\)CO), 1.54 (s, 3H, CH3), 1.34 (s, 3H, CH3).

Lactonisation of 41. A mixture of 2-N-\(\beta\)-alanyl-adenosine 41 (39.4 mg; 0.10 mmol), EDC (105 mg; 0.55 mmol) and DMAP (18.3 mg; 0.15 mmol) in THF-DMF (8:3; 5.5 mL) was stirred for 16 h. Aque-
Conformationally restricted adenosine analogues

ous work-up and flash chromatography (EtOAc with 5→10% MeOH) furnished lactone 42 as a solid (18.5 mg; 0.049 mmol; 49%). 

H-NMR (d$_6$-DMSO) 7.87 (s, 1H, H-8), 6.74 (bs, 2H, NH$_2$), 6.61 (dd, J 7.7 and 5.4, 1H, NH), 5.90 (d, J 3.5, 1H, H-1'), 5.45 (dd, J 6.5 and 3.5, 1H, H-2'), 5.29 (dd, J 6.5 and 3.7, H-3'), 4.89 (dd, J 12.9 and 1.4, 1H, H-5''a), 4.29 (m, 1H, H-4'), 4.12-4.04 (m, 1H, NH), 3.19-3.12 (m, 1H, H-$\text{NHCH}$), 2.65 (dd, J 13.7 and 6.5, 1H, CO$_\text{HCH}$), 2.05 (dd, J 13.7 and 8.9, 1H, CO$_\text{HCH}$), 1.58 (s, 3H, CH$_3$), 1.35 (s, 3H, CH$_3$).

Deprotection of lactone 42. Isopropylidene protected lactone 42 was dissolved in TFA-H$_2$O 9:1 (2 mL). After stirring for 15 min the solution was evaporated to dryness (20 mbar, bath temperature ≤ 40 °C). Flash chromatography (EtOAc with 15% MeOH) furnished a glass which was triturated with a mixture of MeOH and Et$_2$O to yield lactone 43 as a white solid (12.1 mg; 0.036 mmol; 74%); mp 190 °C. 

H-NMR (d$_6$-DMSO) 7.93 (s, 1H, H-8), 7.21 (bs, 2H, NH$_2$), 6.89 (bs, 1H, NH), 5.62 (d, J 5.4, 1H, H-1'), 5.34 (bs, 1H, OH), 5.20 (bs, 1H, OH), 5.06 (dd, J 5.4 and 5.1, 1H, H-2'), 4.96 (d, J 12.2, 1H, H-5''a), 4.55 (dd, J 5.1 and 4.1, H-3'), 4.03 (m, 1H, H-4'), 3.99-3.93 (m, 1H, H-$\text{NHCH}$), 3.87 (d, J 12.2, 1H, H-5''b), 3.26-3.22 (m, 1H, H-$\text{NHCH}$), 2.65 (dd, J 13.7 and 6.3, 1H, CO$_\text{HCH}$), 2.12 (t, J 12.9, 1H, CO$_\text{HCH}$). m/z 337.1260 (M$^+$+H, C$_{13}$H$_{17}$N$_6$O$_5$ requires 337.1260).

Table 4.5. Nuclear Overhauser Enhancements (%).

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*Samples of 8-10 mg in 0.6 mL d$_6$-DMSO; bFrom 2 experiments; cValues taken from ref. 28.

4.11 REFERENCES