A new entry to adenosine analogues via purine nitration - Combinatorial synthesis of antiprotozoal agents and adenosine receptor ligands
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Solid phase synthesis of 2,N^6-disubstituted adenosine analogues

**ABSTRACT**
A small combinatorial library of 2,N^6-disubstituted adenosine analogues III was prepared on solid support. Nitration of polystyrene supported 6-chloropurine riboside furnished 2-nitro-6-chloropurine nucleoside II, a highly reactive difunctionalised species. Amines were selectively introduced by 6-chloro displacement at room temperature without affecting the 2-nitro group. Subsequent substitution of the 2-nitro group by amines was achieved at 80-90 °C. Removal of the riboside protective groups under mildly acidic conditions, followed by cleavage of the nucleosides from the resin, yielded 2,N^6-disubstituted adenosine analogues III.
2.1 INTRODUCTION

The solid phase combinatorial synthesis of di- and trisubstituted purines, to obtain for instance cyclin-dependent kinase inhibitors, is well documented. However, reports of solid supported syntheses of purine nucleosides are restricted to the synthesis of DNA- and RNA-oligomers and the more stable carbocyclic analogues. The general interest in the synthesis and application of adenosine analogues prompted us to fill up this synthetical void and to develop a solid phase route towards the combinatorial substitution of purine riboside systems. We chose to introduce diversity elements on the purine ring at the 2 and the 6 position, since several 2,N\textsuperscript{6}-disubstituted analogues of adenosine are described as potent adenosine receptor agonists and inhibitors of \textit{Trypanosoma brucei} phosphoglycerate kinase. Moreover, adenosine analogues are potential therapeutics against malaria caused by drug-resistant \textit{Plasmodium falciparum}.

2.2 FUNCTIONALISING THE PURINE SKELETON

The introduction of amino substituents on the 2- and 6-positions is generally achieved via nucleophilic displacement of 2,6-dihalogenated purine systems. The 2-halogen functionality is typically introduced by conversion of an amino group, as present in guanine or guanosine derivatives, to halogen substituents via a diazotation-halogenation sequence (Scheme 2.1). A recently developed method published by Kato and coworkers involves 2-halogenation of 2-tributylstannyl-6-chloropurine riboside, which is obtained by lithiation-stannylation of 6-chloropurine riboside.

![Scheme 2.1. Known literature methods for functionalisation of the purine 2-position.](image-url)
The purine 6-position is the most reactive towards nucleophilic substitution and, although activated by the electron-withdrawing effect of a halogen atom on C-2, moderately elevated temperatures (25-80 °C) are usually applied for the introduction of aliphatic or aromatic amines at C-6. Displacement of the halogen on the 2-position requires more forcing conditions (120-130 °C) and is restricted to aliphatic amines. These harsh conditions are not favoured in (automated) solid phase synthesis.

Recently, the functionalisation of the 2-position in triacetyl-protected 6-chloropurine riboside 1 by nitration with a mixture of tetrabutylammonium nitrate and trifluoroacetic anhydride (TBAN-TFAA) was developed in our group (Scheme 2.2 and see also chapter 6). The electrophilicity of the purine C-6 in 2 was greatly enhanced by the 2-nitro group, and introduction of amino substituents could be achieved at temperatures below 0 °C, as was shown in the synthesis of several 2-nitro-N^6-substituted adenosine analogues 3, identified as high affinity adenosine receptor agonists. Under these conditions the nitro and acetate groups were not affected. During ammonolysis of the acetate protecting groups of 3 undesired substitution of the nitro group was observed. Intrigued by this nitro substitution, we dissolved 2-nitro-6-chloropurine riboside triacetate 1 in n-butylamine at room temperature, which gave 2,6-dibutylaminopurine riboside 4 in 88 % yield (Scheme 2.3). This preliminary result prompted us to explore the nitro displacement for beneficial use.

![Scheme 2.2. TBAN-TFAA nitration followed 6-chlorosubstitution by nitrogen nucleophiles.](image)

![Scheme 2.3. 6-Chloro and 2-nitro substitution with n-butylamine.](image)
Chapter 2

2.3 The nitro group as a leaving group

The nitro group is rarely encountered as a leaving group in \( S_NAr \) reactions; instead, it mainly serves as a precursor to an amino substituent via a reductive pathway. This is primarily due to the poor synthetic availability of nitrobenzenes containing ortho or para electron-withdrawing substituents, which considerably facilitate aromatic nucleophilic substitution. Classical, electrophilic nitrination of aromatic rings containing one electron-withdrawing group predominantly results in the formation of meta-substituted products. In heterocyclic systems, such as pyridines and pyrimidines, α- and γ-nitrogen atoms are strongly activating towards \( S_NAr \) reactions. But again these electron poor positions cannot be nitrated by classical methods. In this view, the introduction of a nitro group on the electrophilic 2-position in the purine ring is exceptional and most likely proceeds via a radical process, thus creating a highly activated nitro substituent. A conclusive mechanistic study of this nitrination reaction is presented in chapter 6.

The nitro group acting as a leaving group has been reported to be particularly successful in activated aromatic systems, comparable to fluorine. In \( S_NAr \) reactions an approximate order of leaving group ability is: \( F > NO_2 > OTs > SOPh > Cl,Br,I \). Of course, this greatly depends on the nature of the nucleophile and aromatic substrate. With translation of the substitution to the solid phase in mind, the 2-nitro displacement at room temperature is a considerable improvement compared with the 2-halogen substitutions, that require elevated temperatures even in solution.

Having established that the 6-chloro and the 2-nitro groups are convenient handles for introducing structural diversity on the purine skeleton, we turned our attention to combinatorial solid phase synthesis. In this chapter the development of a solid phase route towards \( 2,N^6 \)-disubstituted adenosine analogues is described, which is validated through the synthesis of a small combinatorial library.

2.4 Solid supported syntheses

A solid phase modification of nucleosides was envisaged via coupling of the riboside 5'-hydroxyl to a polystyrene resin, leaving the purine system free for substitution (Scheme 2.4). Next, functionalisation of the solid supported 6-chloropurine riboside 5 by TBAN-TFAA nitrination would lead to the highly reactive 2-nitro-6-chloro-purine system 6. This on-resin nitrination offers considerable advantages over the coupling of an already 2,6-difunctionalised purine riboside: the attack of nucleophilic species on the activated electrophilic C-6 in the nitrated system under coupling conditions is prevented, and furthermore, 6-chloropurine riboside is commercially available. Introduction of diversity elements on the purine scaffold would provide, after deprotection and cleavage, the disubstituted purine ribosides 7.
Disubstituted adenosine analogues

Scheme 2.4. Solid phase strategy towards 2,6-disubstituted purine ribosides.

Carboxypolystyrene 8, the solid phase version of the benzoyl protecting group for alcohols, was selected as a solid support, since it is stable towards acidic and moderately basic/nucleophilic conditions. Alcohols are easily coupled to this resin by using standard esterification reagents, e.g. diisopropylcarbodiimide, DIC, in combination with DMAP. For reasons of solubility and selectivity, 6-chloropurine riboside was 2',3'-diol-protected prior to attachment to the solid support. The 2',3'-isopropylidene protected 6-chloropurine riboside 9 was esterified to carboxypolystyrene 8 in the presence of DIC and catalytic DMAP leading to immobilised 6-chloropurine 10 (Scheme 2.5). A malachite green test after 16 hours proved that no remaining COOH-groups were present on the resin.

Scheme 2.5. Coupling of the nucleoside to carboxypolystyrene resin.

In solution the TBAN-TFAA nitration of 6-chloropurine riboside triacetate 2 is performed at 0 °C.16 For simplicity, however, room temperature conditions are preferred in automated solid phase syntheses. The nitration of solid supported 10 at room temperature was optimised to 90 % using a 0.15 M solution of TBAN-TFAA (= 3 equivalents) in dichloromethane. After cleaving the nucleosides from the resin using a cocktail of sodium methoxide in methanol-THF, the conversion of 10 to 11 was determined by NMR and HPLC analysis of the crude mixture of products. The quantitative substitution of the chloro and nitro groups by methoxy groups provides a mixture of 6-methoxy- and 2,6-dimethoxypurine ribosides 12 and 13.
corresponding to starting material and product, respectively. It is remarkable that no electrophilic nitration of the polystyrene matrix is observed, thus supporting the radical mechanism of the TBAN-TFAA nitration (see chapter 6).

Chloro displacement of 11 by aliphatic or aromatic amines, in the presence of diisopropylethylamine (DIPEA) occurred in dichloromethane at room temperature without affecting the nitro group (Scheme 2.7). These solid phase substitution reactions proceeded considerably slower as compared to those in solution, where nitro displacement was already observed at room temperature. In the next step, amines were introduced by nitro substitution of solid supported 14, which required elevated temperatures. N-Methyl-2-pyrrolidone (NMP) appeared to be a valuable solvent, because its resin swelling properties are excellent and a large temperature range can be applied. Subjecting resin 14 to an amine and DIPEA in NMP at 80-90 °C for 24 hours led to efficient formation of 15. With aromatic amines as nucleophiles substitution of the 2-nitro group was not accomplished. Undesired aminolysis of the ester linkage, resulting in cleavage of the nucleoside from the resin, was not observed under the different reaction conditions applied.

Scheme 2.6. Nitration on solid support and monitoring by cleavage from the resin.

Scheme 2.7. Selective aminations. (a) DIPEA, CH₂Cl₂, rt, 4 h; (b) DIPEA, NMP, 80-90 °C, 24 h.
**PROTECTIVE GROUP ISSUES**

At this point, the removal of the 2',3'-isopropylidene group from 15 confronted us with significant problems. Usually, this protecting group is removed in aqueous solutions containing AcOH. These conditions, however, are not compatible with polystyrene resins, since they do not swell in protic solvents. Trifluoroacetic or hydrochloric acid in dichloromethane or THF were used instead. When applied to resin-bound 15, concomitant cleavage of the glycosidic bond was observed, while under milder acidic conditions removal of the isopropylidene group was sluggish and incomplete.

![Scheme 2.8. Selective 5'-desilylation.](image)

Therefore we focussed on the TBDMS-ether, which can be removed without the use of acid. 2',3'-DiTBDMS protected 6-chloropurine riboside 17 was obtained in 96 % yield by efficient and selective acid catalysed removal of the 5'-TBDMS-ether from 2',3',5'-tri-O-TBDMS protected nucleoside 16 (Scheme 2.8).

Coupling of 2',3'-diTBDMS protected 6-chloropurine riboside 17 to carboxypolystyrene 8 proceeded smoothly, but resin-bound 6-chloropurine 18 did not give a clean nitration. Furthermore, on-resin deprotection with various fluoride salts, for example NH₄F, TBAF and 3 HF-Et₃N, was obstructed by incomplete removal of the silyl groups.

![Scheme 2.9. Solid phase approach using 2',3'-diTBDMS protected 6-chloropurine riboside.](image)
Eventually, we switched to the 2',3'-methoxymethylidene protecting group, a more acid labile variant of the isopropylidene moiety (Scheme 2.10). 2',3'-Methoxymethylidene protected 6-chloropurine riboside 20 was prepared by condensation of 6-chloropurine riboside 19 with trimethyl-orthoformate according to a literature procedure. As expected, coupling to resin 8 proceeded without difficulty furnishing immobilised 21. The nitration of 21 provided a clean conversion to 22 and after the selective amination steps leading to 23 and then 24, complete on-resin deprotection of 24 was achieved under mild conditions (0.1 M pTsOH in

Scheme 2.11. (a) 0.15 M TBAN-TFAA, CH₂Cl₂; (b) R¹-NH₂, DIPEA, CH₂Cl₂; (c) R²-NH₂, DIPEA, NMP, 80-90 °C; (d) pTsOH-H₂O, CH₂Cl₂-MeOH 97:3; (e) NaOCH₃, MeOH, THF.
dichloromethane-methanol 97:3) to render 25 without affecting the glycosidic bond. Cleavage of the resin-bound disubstituted nucleosides was brought about with a mixture of sodium methoxide in methanol-THF to yield the desired nucleoside analogues 26.

2.5 Library synthesis

A small combinatorial library was synthesised in order to validate the developed solid phase sequence from methoxymethylidene protected 6-chloropurine riboside 20 to adenosine analogues 26 (see Table 2.1 on page 38). Most of the amines selected for substitution at the 2- and the 6-position are active pharmacophores known from adenosine receptor studies and trypanosomal research, for example cyclopentylamine, 3-iodo-benzylamine, diphenyl-ethylamine, aniline and histamine. Compounds 26a-p were obtained in 64-97% purity after cleavage, as determined by HPLC. Minor amounts of side-products could be traced back to incomplete nitration or nitro substitution. In order to biologically evaluate the library higher purities were mandatory. Therefore, products 26a-p were purified using semi-preparative HPLC, furnishing the 2,N<sup>6</sup>-disubstituted adenosine analogues in acceptable overall yields and high purity.

2.6 Concluding remarks

In conclusion, it was shown that resin bound 6-chloropurine riboside could be efficiently nitrated by the TBAN-TFAA mixture without affecting the polystyrene matrix. The resulting 2-nitro group in the purine ring not only activates the C-6 position towards nucleophilic attack, but can also be easily substituted by nucleophiles. The solid phase sequence we developed opens the way to generate larger combinatorial libraries of disubstituted adenosine analogues. Biological evaluation of the synthesised adenosine analogues is described in chapter 5. Further synthetic efforts addressing substitution reactions at the 2-nitro purine system are discussed in the two following chapters.

2.7 Acknowledgements

Bert van Groen and Ron Groenestein are much appreciated for realising my ideas about modifying standard glass reaction tubes so that they are suitable for solid phase synthesis in Radley's Carousel Reaction Station™ and easy work-up afterwards on the IST VacMaster-20 Sample Processing Station™.
Table 2.1. Library of disubstituted adenosine analogues.

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<th>Purity after prep-HPLC (%)</th>
<th>Yield after prep-HPLC (%)</th>
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2.8 Experimental

General Information

All reactions involving moisture sensitive compounds were carried out under a dry nitrogen atmosphere. Dichloromethane (phosphorous pentaoxide and calciumhydride), tetrahydrofuran (sodium/benzophenone ketyl) and light petroleum (60-80) were distilled freshly prior to use. All other commercially available chemicals were used without further purification. Peptide grade solvents were used for solid phase chemistry. Carboxypolystyrene (0.88 mmol g⁻¹) was purchased from Rapp Polymere, Tübingen, Germany. Solution phase reactions were monitored by using thin-layer chromatography (TLC) on silica coated plastic sheets (Merck silica gel 60 F254) with the indicated eluent. The compounds were visualised by UV light (254 nm), I₂ or spraying with a solution of ninhydrin in EtOH (0.4%) followed by charring at 140 °C. Flash chromatography²⁹ refers to purification using the indicated eluent and Acros silica gel (0.030-0.075 mm). Infrared spectra of resins were measured in KBr using a DRIFT module (Bruker), vibrations are reported in cm⁻¹. Nuclear magnetic resonance spectra (¹H NMR and ¹³C NMR, APT) were determined in the indicated solvent using a Bruker ARX 400 (¹H: 400 MHz, ¹³C: 100 MHz) at 300 K, unless indicated otherwise. Peakshapes in the NMR spectra are indicated with the symbols 'q' (quartet), 'dq' (double quartet), 't' (triplet), 'dt' (double triplet), 'd' (doublet), 'dd' (double doublet), 's' (singlet), 'bs' (broad singlet) and 'm' (multiplet). Perdeuterated solvents were obtained from Cambridge Isotope Laboratories Ltd. Chemical shifts (δ) are given in ppm downfield from tetramethylsilane (¹H, ¹³C) and coupling constants J in Hz. NH and OH signals were identified after mixing the sample with a drop of D₂O. Melting points were measured with a Leitz melting point microscope and are uncorrected. Mass spectra and accurate mass measurements were performed using a JEOL JMS-SX/SX 102 A Tandem Mass Spectrometer using Fast Atom Bombardment (FAB). A resolving power of 10,000 (10% valley definition) for high resolution FAB mass spectrometry was used. Analytical HPLC was performed on a C18 column (Inertsil ODS-3, particle size 3 mm; 4.6mm×50mm) using the following elution gradient: linear gradient of 5% to 95% aqueous CH₃CN containing 0.04% HCO₂H over 5 min, then 95% aqueous CH₃CN containing 0.04% HCO₂H for 2 min at 2.0 mL min⁻¹. Semi-preparative HPLC was performed on a C18 column (Polygosil 60 C-18, particle size 10 mm; 20mm×250mm) using one of the following elution gradients: Method A, linear gradient of 5% to 95% aqueous CH₃CN containing 0.04% HCO₂H over 15 min, then 95% aqueous CH₃CN containing 0.04% HCO₂H for 6 min at 7.0 mL min⁻¹ Method B, linear gradient of 5% to 95% aqueous CH₃CN over 15 min, then 95% aqueous CH₃CN for 6 min at 7.0 mL min⁻¹. Products were detected at λ = 254 nm.

General Solid Phase Procedures: Large-scale solid phase reactions (> 200 mg of resin) were performed in dried glass scintillation vessels, bubbling nitrogen gas through the resin suspension. Small-scale solid phase reactions (100-200 mg of resin) were run under a nitrogen atmosphere in Radleys Carousel Reaction Station™ using oven-dried modified glass reaction tubes. The tubes were fitted with a glass frit and luer tip to facilitate work-up on the IST VacMaster-20 Sample Processing Station™. Small-scale reactions were gently stirred with a magnetic stirring bar. The modified tubes were heated in a sand-bath fitted in the Carousel Reaction Station™. Resins were suspended in 1 mL solvent/100 mg resin. The resins were washed according to the indicated sequence.

2-n-Butylamino-N⁸-n-butyladenosine (4). A solution of 2-nitro-6-chloro-(2,3,5-tri-acetyl-β-D-ribofuranosyl)9H-purine 2 (75 mg, 0.16 mmol) in n-butylamine (2 mL) was stirred under a nitrogen atmosphere for 16 h. The solution was evaporated to dryness and the residue was subjected to flash chromatography (EtOAc with 5-15% MeOH). Drying in vacuo at 55 °C for 16 h and trituration with Et₂O furnished 4 (55 mg, 88%) as a white solid, mp 141-142 °C. ¹H-NMR (CDCl₃) δ 7.88 (1H, s, H-8), 7.29
(1H, br s, NβH), 6.19 (1H, br s, 2-NH), 5.73 (1H, d, J 6.1, H-1'), 5.35 (1H, d, J 6.1, OH), 5.18 (1H, br s, OH), 5.10 (1H, d, J 4.6, OH), 4.60 (1H, m, H-2'), 4.13 (2H, dd, J 8.1 and 4.6, H-3'), 3.90 (1H, dd, J 7.2 and 3.7, H-4'), 3.63 (1H, m, H-5'), 3.52 (1H, m, H-5'), 3.42 (2H, m, NCH₂), 3.24 (2H, m, NCH₂), 1.55 (4H, m, butyl), 1.34 (4H, m, butyl), 0.91 (6H, t, J 7.3, butyl); m/z 395.2415 (M⁺H, C₁₈H₃₁N₆O₄ requires 395.2407).

6-Chloro-(2,3-di-O-tert-butyldimethylsilyl-β-D-ribofuranosyl)-9H-purine (17): This compound was synthesised following a modified literature procedure.²⁷ To a stirred solution of 6-chloro-(2,3,5-tri-O-tert-butyldimethylsilyl-f}-D-ribofuranosyl)-9H-purine 16 (3.75 g; 5.96 mmol) in THF (75 mL) was added aqueous TFA (38 mL, TFA-H₂O 1:1) at 0 °C. After stirring for 4.5 h at 0 °C, the reaction mixture was neutralised with saturated aqueous NaHCO₃ and diluted with ethyl acetate (200 mL). After separation, the organic phase was washed with H₂O and brine, dried over anhydrous Na₂SO₄ and evaporated at reduced pressure. The residue was subjected to flash chromatography (light petroleum-EtOAc 1:1) to provide 17 as a white solid (2.98 g; 5.78 mmol; 96 %), m.p 156-157 ºC. The product was recrystallised from light petroleum-EtOAc prior to coupling to the resin. Η-NMR (CDCl₃) δ 8.78 (1H, s, H-2'), 8.19 (1H, s, H-8), 5.88 (1H, d, J 7.8, H-1'), 5.52 (1H, d, J 9.7, OH), 5.52 (1H, dd, J 7.8 and 4.6, H-2'), 4.34 (1H, d, J 4.6, H-3'), 4.19 (1H, m, H-4'), 3.94 (1H, d, J 13.0, H-5'), 3.73 (1H, dd, J 13.0 and 9.7, H-5'), 0.95 and 0.74 (18H, 2xs, 2xt-Bu), 0.13, -0.13, -0.65 (all 3H, s, SiCH₃).

General procedure for the coupling of 2',3'-diole protected 6-chloropurine ribosides to carboxypolystyrene. To a suspension of carboxypolystyrene (1.0 g; 0.88 mmol) in 10 mL of CH₂Cl₂ was added the 2',3'-diole protected 6-chloropurine riboside (1.78 mmol), DIPCIDI (0.28 mL; 1.78 mmol) and DMAP (43 mg; 0.35 mmol). The reaction was monitored with a malachite green test.²³ After 16 h the reaction was complete and the resulting resin was washed with CH₂Cl₂ (6×), MeOH, CH₂Cl₂, MeOH, Et₂O, CH₂Cl₂, Et₂O, CH₂Cl₂ and dried in vacuo at 50 ºC.

General procedure for the nitration of resin-bound 2',3'-diole protected 6-chloropurine ribosides. A 0.15 M nitrating mixture was prepared at 0 °C by adding TFAA (0.54 mL; 2.55 mmol) to a solution of tetrabutylammonium nitrate (TBAN, 0.77 g; 3 mmol) in dry CH₂Cl₂ (17 mL) during 2 min. After stirring for 10 min this solution was added via syringe to the resin-bound 2',3'-diole protected 6-chloropurine riboside (0.88 mmol). After 2.5 h the resin was washed with CH₂Cl₂ (6×), MeOH, CH₂Cl₂, MeOH, Et₂O, CH₂Cl₂, Et₂O, CH₂Cl₂ and dried in vacuo at 50 ºC.

General procedure for the amination by chloro substitution of resin-bound 2-nitro-6-chloropurines. To a suspension of the resin-bound 2-nitro-6-chloropurine (0.54 mmol) in CH₂Cl₂ (8 mL) was added DIPEA (0.73 mL; 4.32 mmol) and the amine (3.24 mmol). After 4 h the resin was washed with CH₂Cl₂ (6×), MeOH, CH₂Cl₂, MeOH, Et₂O, CH₂Cl₂, Et₂O and CH₂Cl₂ and dried in vacuo at 50 ºC. For aromatic amines a longer reaction time (16 h) was employed to ensure complete chloro substitution.

General procedure for the amination by nitro substitution of resin-bound 2-nitro-6-aminopurines. To a suspension of the resin-bound 2-nitro-6-aminopurine (0.10 mmol) in NMP (1.5 mL) was added DIPEA (0.14 mL; 0.8 mmol) and cyclopentylamine (60 µL; 0.6 mmol). After heating at 80-90 °C for 24 h the resin was washed with NMP(3x), CH₂Cl₂ (3x), MeOH, CH₂Cl₂, MeOH, Et₂O, CH₂Cl₂, Et₂O and CH₂Cl₂.
General procedure for the removal of the 2',3'-methoxymethylidene group from resin bound 24 (25). Resin 24 (0.10 mmol) was washed twice with a solution of pTsOH-H₂O (18 mg/mL) in CH₂Cl₂-MeOH (97:3). After subjecting to this solution for 16 h resin 25 was washed with CH₂Cl₂-MeOH (97:3; 3x), CH₂Cl₂-DIPEA (90:10; 3x), CH₂Cl₂ (3x), MeOH, CH₂Cl₂, MeOH, Et₂O, CH₂Cl₂, Et₂O and CH₂Cl₂.

General procedure for cleavage of the 2,N⁴-disubstituted adenosine analogues from resin 25 (26). To a suspension of resin 25 (0.10 mmol) in THF (1.5 mL) was added a solution of 5.24 M NaOMe in MeOH (76 µL; 0.40 mmol). After 1 h the resin was washed with THF (2x), MeOH, THF and MeOH. The washings were passed over an SPE column (Supelco, packed with 1 g silica gel) and analysed with HPLC. The products 26d, 26h, 26l and 26p were purified via semi-preparative HPLC using method A. All other products were purified by using method B. The products were isolated by lyophilisation furnishing the 2,N⁴-disubstituted adenosine analogues 26a-p as white solids.

2-Cyclopentylamino-N⁴-cyclopentyladenosine (26a). ¹H-NMR (d₆-DMSO) δ 7.89 (1H, s, H-8), 7.10 (1H, br s, N⁶-H), 6.12 (1H, br s, 2-NH), 5.73 (1H, d, J 6.0, H-1'), 5.36 (1H, d, J 4.7, OH), 5.12 (2H, m, 2xOH), 4.59 (1H, br, H-2'), 4.49 (1H, br, NCH), 4.15 (2H, m, NCH and H-3'), 3.89 (1H, dd, J 4.5, H-4'), 3.63 (1H, m, H-5'a), 3.53 (1H, m, H-5'b), 1.92 (4H, m, cyclopentyl), 1.66 (4H, m, cyclopentyl), 1.49 (8H, m, cyclopentyl); m/z 419.2416 (M⁺+H, C₂₀H₂₉N₆O₄ requires 419.2407).

2-(N₂-Tryptamino)-N⁴-cyclopentyladenosine (26b). ¹H-NMR (d₆-DMSO) δ 10.80 (1H, s, indole NH), 7.92 (1H, s, H-8), 7.62 (1H, d, J 7.8, indole 4-H), 7.35 (1H, d, J 7.9, indole 7-H), 7.18 (2H, br, N⁶-H and indole 2-H), 7.08 (1H, t, J 7.9, indole 6-H), 7.00 (1H, t, J 7.8, indole 5-H), 6.28 (1H, br s, 2-NH), 5.79 (1H, d, J 6.0, H-1'), 5.38 (1H, d, J 5.9, OH), 5.23 (1H, br s, OH), 5.12 (1H, d, J 4.4, OH), 4.61 (1H, m, H-2'), 4.53 (1H, br, NCH), 4.15 (1H, m, H-3'), 3.93 (1H, dd, J 6.9 and 3.5, H-4'), 3.64 (1H, m, H-5'a), 3.42 (3H, m, H-5'b and NCH₂CH₂), 2.97 (2H, t, J 7.5, NCH₂CH₂), 1.94 (2H, m, cyclopentyl), 1.69 (2H, m, cyclopentyl), 1.56 (4H, m, cyclopentyl); m/z 494.2489 (M⁺+H, C₂₅H₃₂N₇O₄ requires 494.2516).

2-(2-Benzoyloxyethylamino)-N⁴-cyclopentyladenosine (26c). ¹H-NMR (d₆-DMSO) δ 7.91 (1H, s, H-8), 7.35 (4H, m, Ar-H), 7.29 (1H, m, Ar-H), 7.21 (1H, br s, N⁶-H), 6.17 (1H, br s, 2-NH), 5.74 (1H, d, J 6.1, H-1'), 5.36 (1H, d, J 5.9, OH), 5.23 (1H, br s, OH), 5.13 (1H, d, J 4.3, OH), 4.59 (1H, m, H-2'), 4.52 (2H, s, PhCH₂), 4.45 (1H, br, NCH), 4.13 (1H, m, H-3'), 3.91 (1H, m, H-4'), 3.57 (6H, m, 5'-H, NCH₂CH₂, NCH₂CH₂), 1.94 (2H, m, cyclopentyl), 1.64 (2H, m, cyclopentyl), 1.53 (4H, m, cyclopentyl); m/z 485.2507 (M⁺+H, C₂₅H₃₃N₇O₄ requires 485.2512).

2-(N₂-Histidyl)-N⁴-cyclopentyladenosine (26d). ¹H-NMR (d₆-DMSO) δ 8.21 (1H, br s, Im-2-H), 7.90 (1H, s, H-8), 7.59 (1H, br s, Im-NH), 7.15 (1H, br s, Im-H), 6.86 (1H, br s, Im 4-H), 6.31 (1H, br s, 2-NH), 5.74 (1H, d, J 5.6, H-1'), 4.63 (1H, m, H-2'), 4.48 (1H, br, NCH), 4.25 (1H, m, H-3'), 3.91 (1H, dd, J 7.8 and 3.9, H-4'), 3.65 (1H, m, H-5'a), 3.51 (3H, m, H-5'b and NCH₂CH₂), 2.73 (2H, m, NCH₂CH₂), 1.92 (2H, m, cyclopentyl), 1.66 (2H, m, cyclopentyl), 1.52 (4H, m, cyclopentyl); m/z 445.2299 (M⁺+H, C₂₀H₂₉N₅O₄ requires 445.2312).

2-Cyclopentylamino-N⁴-(2,2-diphenylethyl)adenosine (26e). ¹H-NMR (d₆-DMSO) δ 7.84 (1H, s, H-8), 7.31 (8H, m, Ar-H), 7.19 (3H, m, Ar-N and N⁶-H), 6.28 (1H, br s, 2-NH), 5.71 (1H, d, J 5.2, H-1'), 5.36 (1H, br s, OH), 5.11 (2H, br s, 2xOH), 4.62 (1H, m, H-2'), 4.55 (1H, m, Ph₂CH), 4.22 (1H, br, NCH), 4.12 (1H, m, H-3'), 4.03 (2H, m, Ph₂CHCH₂), 3.88 (1H, m, H-4'), 3.64 (1H, m, H-5'a), 3.51 (1H, m, 41
2-(N$_6$-Tryptamino)-N$_4$-(2,2-diphenylethyl)adenosine (26f). $^1$H-NMR (d$_6$-DMSO) $\delta$ 10.82 (1H, s, indole NH), 7.89 (1H, s, H-8), 7.63 (1H, d, J 7.5, indole 4-H), 7.36 (1H, d, J 8.0, indole 7-H), 7.23 (12H, m, Ar-H, N$_6$-H and indole 2-H), 7.08 (1H, m, indole 6-H), 6.98 (1H, m, indole 5-H), 6.49 (1H, br s, 2-NH), 5.77 (1H, m, H-1'), 4.59 (2H, m, H-2' and Ph$_2$CH), 4.13 (1H, m, H-3'), 4.03 (3H, m, H-4' and Ph$_2$CHCH$_2$), 3.49 (4H, m, 5'-H and NCH$_2$CH$_2$), 2.98 (2H, m, NCH$_2$CH$_3$); m/z 606.2822 (M$^+$H, C$_{34}$H$_{36}$N$_7$O$_4$ requires 606.2829).

2-(2-Benzoxo-ethylamino)-N$_4$-(2,2-diphenylethyl)adenosine (26g). $^1$H-NMR (d$_6$-DMSO) $\delta$ 7.86 (1H, s, H-8), 7.36–7.26 (14H, m, Ar-H and N$_6$-H), 7.19 (2H, m, Ar-H), 6.35 (1H, br s, 2-NH), 5.72 (1H, d, J 5.6, H-1'), 5.35 (1H, d, J 6.0, OH), 5.22 (1H, br s, OH), 5.12 (1H, d, J 4.4, OH), 4.61 (1H, m, H-2'), 4.56 (1H, m, Ph$_2$CH), 4.53 (1H, s, PhCH$_2$), 4.12 (1H, m, H-3'), 4.03 (2H, m, Ph$_2$CHCH$_2$), 3.88 (1H, m, H-4'), 3.58 (6H, m, 5'-H, NCH$_2$CH$_2$); m/z 597.2846 (M$^+$H, C$_{33}$H$_{37}$N$_6$O$_4$ requires 597.2825).

2-(N$_6$-Histamino)-N$_4$-(2,2-diphenylethyl)adenosine (26h). $^1$H-NMR (d$_6$-DMSO) $\delta$ 8.18 (1H, br s, Im-2-H), 7.85 (1H, s, H-8), 7.62 (1H, br s, Im-NH), 7.29 (9H, m, Ar-H and N$_6$-H), 7.19 (2H, m, Ar-H), 6.85 (1H, br s, Im-4-H), 6.48 (1H, br s, 2-NH), 5.72 (1H, d, J 5.0, H-1'), 4.61 (2H, m, H-2' and Ph$_2$CH), 4.23 (1H, m, H-3'), 4.03 (2H, m, Ph$_2$CHCH$_2$), 3.90 (1H, m, H-4'), 3.58 (4H, m, 5'-H and NCH$_2$CH$_2$), 2.85 (2H, m, NCH$_2$CH$_3$); m/z 557.2641 (M$^+$H, C$_{29}$H$_{33}$N$_6$O$_4$ requires 557.2625).

2-Cyclopentylamino-N$_4$-(3-iodobenzyl)adenosine (26i). $^1$H-NMR (d$_6$-DMSO) $\delta$ 7.97 (1H, br s, N$_6$-H), 7.94 (1H, s, H-8), 7.74 (1H, s, Ph 2-H), 7.59 (1H, d, J 7.7, Ph 4-H), 7.37 (1H, d, J 7.7, Ph 6-H), 7.12 (1H, t, J 7.7, Ph 5-H), 6.21 (1H, br s, 2-NH), 5.74 (1H, d, J 6.0, H-1'), 5.36 (1H, d, J 6.0, OH), 5.11 (2H, m, 2xOH), 4.57 (3H, m, H-2' and N$_6$-CH$_2$), 4.12 (2H, m, H-3' and NCH), 3.89 (1H, dd, J 7.5 and 3.9, H-4'), 3.65 (1H, m, H-5'), 3.54 (1H, m, H-5'), 1.84 (2H, m, cyclopentyl), 1.64 (2H, m, cyclopentyl), 1.45 (4H, m, cyclopentyl); m/z 567.1216 (M$^+$H, C$_{27}$H$_{28}$N$_6$O$_4$ requires 567.1217).

2-(N$_6$-Tryptamino)-N$_4$-(3-iodobenzyl)adenosine (26j). $^1$H-NMR (d$_6$-DMSO) $\delta$ 10.79 (1H, s, indole NH), 8.01 (1H, br s, N$_6$-H), 7.96 (1H, s, H-8), 7.75 (1H, s, Ph 2-H), 7.57 (2H, m, indole 4-H and Ph 4-H), 7.36 (2H, m, indole 7-H and Ph 6-H), 7.09 (3H, m, indole 2-H, indole 6-H and Ph 5-H), 6.98 (1H, m, indole 5-H), 6.36 (1H, br s, 2-NH), 5.79 (1H, d, J 5.6, H-1'), 5.39 (1H, d, J 6.1, OH), 5.19 (1H, br s, OH), 5.12 (1H, d, J 4.7, OH), 4.62 (3H, m, H-2' and N$_6$-CH$_2$), 4.15 (1H, m, H-3'), 3.92 (1H, dd, J 7.3 and 3.8, H-4'), 3.65 (1H, m, H-5'), 3.52 (3H, m, H-5', and NCH$_2$CH$_2$), 2.93 (2H, t, J 7.4, NCH$_2$CH$_3$); m/z 642.1337 (M$^+$H, C$_{27}$H$_{29}$N$_6$O$_4$I requires 642.1326).

2-(2-Benzoxoethylamino)-N$_4$-(3-iodobenzyl)adenosine (26k). $^1$H-NMR (d$_6$-DMSO) $\delta$ 8.01 (1H, br s, N$_6$-H), 7.95 (1H, s, H-8), 7.73 (1H, s, Ph 2-H), 7.58 (1H, d, J 7.9, Ph 4-H), 7.32 (6H, m, C$_6$H$_5$CH$_2$O and Ph 6-H), 7.10 (1H, t, J 7.9, Ph 5-H), 6.26 (1H, br s, 2-NH), 5.74 (1H, d, J 5.9, H-1'), 5.37 (1H, d, J 5.8, OH), 5.22 (1H, br s, OH), 5.13 (1H, d, J 4.4, OH), 4.54 (3H, m, H-2' and N$_6$-CH$_2$), 4.46 (2H, m, PhCH$_2$), 4.13 (1H, m, H-3'), 3.91 (1H, m, H-4'), 3.65 (1H, m, H-5'), 3.52 (5H, m, H-5', NCH$_2$CH$_2$); m/z 633.1298 (M$^+$H, C$_{26}$H$_{30}$N$_6$O$_5$I requires 633.1322).

2-(N$_6$-Histamino)-N$_4$-(3-iodobenzyl)adenosine (26l). $^1$H-NMR (d$_6$-DMSO) $\delta$ 8.17 (1H, br s, Im-2-H), 7.99 (1H, br s, N$_6$-H), 7.94 (1H, s, H-8), 7.75 (1H, s, Ph 2-H), 7.58 (2H, m, Ph 4-H and Im-NH), 7.38 (1H, d, J 7.7, Ph 6-H), 7.10 (1H, t, J 7.7, Ph 5-H), 6.81 (1H, br s, Im-4-H), 6.40 (1H, br s, 2-NH), 5.75
Disubstituted adenosine analogues

(1H, d, J 5.6, H-1'), 5.02 (3H, band, 3xOH), 4.62 (3H, m, H-2' and N6CH2), 4.24 (1H, m, H-3'), 3.90 (1H, m, H-4'), 3.67 (1H, m, H-5'), 3.51 (3H, m, H-5'b, NCH2CH2), 2.73 (2H, m, NCH2CH2); m/z 593.1116 (M++H, C21H26N6O4 requires 593.1122).

2-Cyclopentylamino-N6-phenyladenosine (26m). 'H-NMR (d6-DMSO) δ 9.51 (1H, br s, N6-H), 8.07 (1H, s, H-8), 8.05 (2H, d, J 7.7, Ph-2-H), 7.29 (2H, t, J 7.7, Ph-2-H), 6.99 (1H, t, J 7.7, Ph-4-H), 6.61 (1H, br s, 2-NH), 5.80 (1H, d, J 6.0, H-1'), 5.41 (1H, d, J 4.1, OH), 5.15 (1H, d, J 4.7, OH), 5.05 (1H, br s, OH), 4.64 (1H, m, H-2'), 4.18 (2H, m, H-3' and NCH), 3.91 (1H, dd, J 7.8 and 4.2, H-4'), 3.67 (1H, m, H-5'), 3.56 (1H, m, H-5'), 1.94 (2H, m, cyclopentyl), 1.70 (2H, m, cyclopentyl), 1.53 (4H, m, cyclopentyl); m/z 427.2117 (M++H, C21H27N6O4 requires 427.2094).

2-(N6-Tryptamino)-N6-phenyladenosine (26n). 'H-NMR (d6-DMSO) δ 10.82 (1H, s, indole NH), 9.46 (1H, br s, N6-H), 8.10 (1H, s, H-8), 8.03 (2H, d, J 7.7, Ph-2-H), 7.63-7.59 (1H, m, indole 4H), 7.35 (1H, d, J 8.1, indole 7-H), 7.25 (2H, t, J 7.7, Ph-3-H), 7.20 (1H, s, indole 2-H), 7.08 (1H, m, indole 6-H), 6.99 (2H, m, indole 5-H and Ph 4-H), 6.74 (1H, br s, 2-NH), 5.85 (1H, d, J 4.9, H-1'), 5.43 (1H, d, J 6.0, OH), 5.15 (1H, d, J 4.7, OH), 5.11 (1H, m, OH), 4.64 (1H, m, H-2'), 4.18 (1H, m, H-3'), 3.95 (1H, dd, J 7.5 and 4.0, H-4'), 3.60 (4H, m, 5'-H and NCH2CH2), 2.93 (2H, t, J 7.6, NCH2CH2); m/z 502.2236 (M++H, C25H28N6O4 requires 502.2203).

2-(2-Benzoyl-ethylamino)-N6-phenyladenosine (26o). 'H-NMR (d6-DMSO) δ 9.47 (1H, br s, N6-H), 8.09 (1H, s, H-8), 8.01 (2H, d, J 8.0, Ph-2-H), 7.30 (7H, m, C6H5CH2O and Ph 3-H), 6.99 (1H, t, J 7.3, Ph 4-H), 6.65 (1H, br s, 2-NH), 5.81 (1H, d, J 6.0, H-1'), 5.41 (1H, d, J 6.0, OH), 5.16 (1H, d, J 4.8, OH), 5.12 (1H, br s, OH), 4.63 (1H, m, H-2'), 4.53 (2H, s, PhCH2), 4.16 (1H, m, H-3'), 3.93 (1H, m, H-4'), 3.59 (6H, m, 5'-H, NCH2CH2); m/z 493.2209 (M++H, C25H29N6O5 requires 493.2199).

2-(N6-Histamino)-N6-phenyladenosine (26p). 'H-NMR (d6-DMSO) δ 9.45 (1H, br s, N6-H), 8.20 (1H, br s, Im-NH), 8.08 (1H, s, H-8), 8.03 (2H, d, J 7.6, Ph-2-H), 7.59 (1H, s, Im-2-H), 7.28 (2H, t, J 7.6, Ph 3-H), 6.99 (1H, t, J 7.6, Ph 4-H), 6.86 (1H, s, Im-4-H), 6.77 (1H, br s, 2-NH), 5.81 (1H, d, J 5.7, H-1'), 5.22 (3H, band, 3xOH), 4.64 (1H, m, H-2'), 4.27 (1H, m, H-3'), 3.93 (1H, m, H-4'), 3.67 (1H, m, H-5'), 3.51 (3H, m, H-5'b, NCH2CH2), 2.81 (2H, m, NCH2CH2); m/z 453.2011 (M++H, C21H25N6O4 requires 453.1999).

2.9 References

21. 2-Nitro-6-chloropurine riboside triacetate quickly reacts at the 6-position with e.g. triethylamine and 4-DMAP.
24. Toluene is only slowly nitratated under the applied TBAN-TFAA conditions.