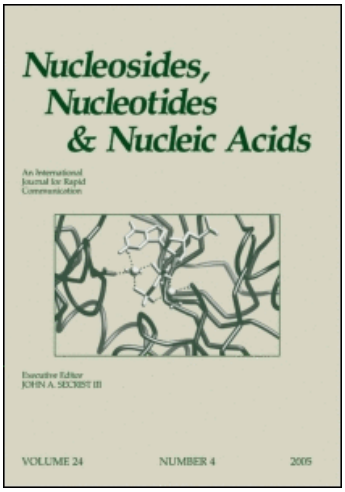

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Synthesis of an Uncharged cAMP-Analogue

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SYNTHESIS OF AN UNCHARGED cAMP-ANALOGUE

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Abstract. 3'-O,5'-N-(N-phenylsulfonyliminocarbonyl)-5'-amino-5'-deoxy adenosine, an uncharged cAMP-analogue was synthesized. This was accomplished by treatment of 5'-amino-5'-deoxy-2',3'-O-isopropylidene adenosine with dimethyl N-phenylsulfonyldithio-carbamate. After removal of the isopropylidene protecting group and treatment of the intermediate with benzoyl chloride, cyclisation was carried out in DMF containing 10 equivalents of potassium tert-butoxide. Final deprotection of the adenine moiety was carried out with hydrazine hydrate.

Introduction

The responses to the second messengers cyclic AMP (cyclic adenosine monophosphate) and cyclic GMP (cyclic guanosine monophosphate) can be regulated both at the level of degradation as well as synthesis. Cyclic nucleotide phosphodiesterases are very important enzymes which modulate the intracellular response to cyclic AMP and cyclic GMP, by catalyzing the hydrolysis of the 3',5'-cyclic nucleotides with formation of the nucleoside 5'-O-monophosphates.

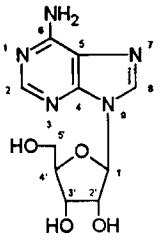
In the early seventies, it was recognized that multiple molecular forms of phosphodiesterases exist which differ in molecular mass, substrate specificity, sensitivity to inhibitors, intracellular location, immunologic reactivity, kinetic characteristics and regulation by allosteric effectors (Phosphodiesterase I, II and III with several subclasses for type I and III). They play an important role in several physiological processes such as cardiac muscle contraction, platelet aggregation, nerve transmission and lipolysis.

Selective inhibitors of the different forms of phosphodiesterases can mimic the response to a variety of endogenous substances which act via stimulation of cyclic AMP or cyclic GMP synthesis including epinephrine, prostaglandines, adenosine, glucagon, atrial natriuretic factor and histamine.

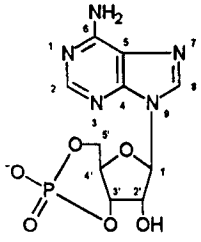
The differences in the distribution of phosphodiesterases and the differences in intracellular location imply that such inhibitors may produce a tissue specific response.

Recently, griseolic acid, a potent non-selective cyclic nucleotide-phosphodiesterase inhibitor with a nucleoside-based structure, has been isolated[1] and derivatives of this compound have been synthesized[2].

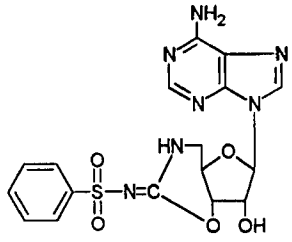
Here we present the synthesis of a first representative of a potential class of cyclic AMP analogues. The cyclic phosphate group has been replaced by an isourea function. As the isourea function is further substituted with an electron withdrawing group, this functionality is essentially uncharged at physiological pH. This could benefit the cellular uptake of the analogue. The choice of the phenyl substituent is based upon the observation that antagonists usually have supplementary hydrophobic groups, when their structure is compared with the structure of the agonist.



Adenosine



cAMP
Cyclic Adenosine-3'-O,5'-O-monophosphate



3'-O,5'-N-(N-phenylsulfonyliminocarbonyl)
5'-amino-5'-deoxy Adenosine

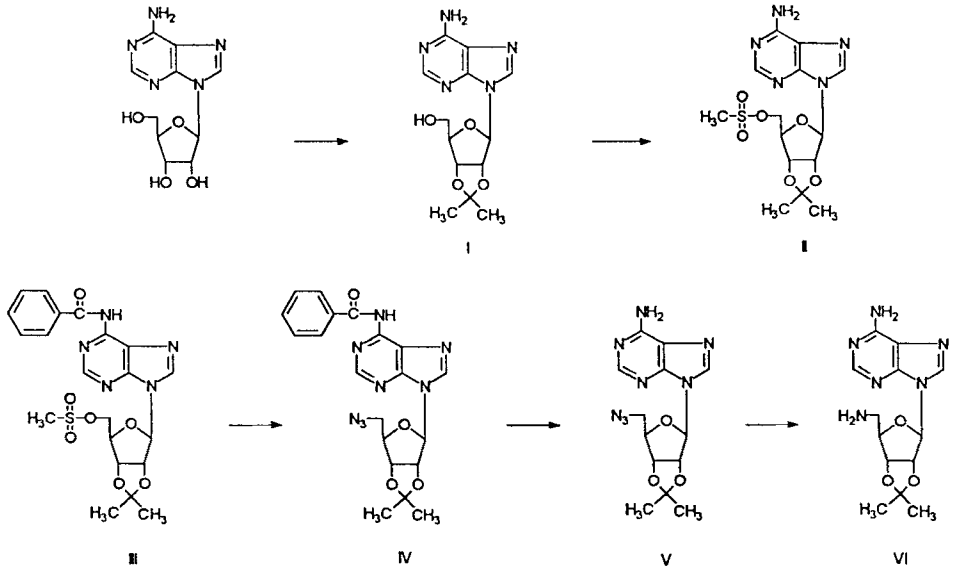
Scheme I

Chemistry

For the synthesis of 5',3'-cyclic derivatives of adenosine one could envisage a ring closure reaction starting from the 5'-derivatised compound or starting from the 3'-derivatised compound. A primary hydroxyl group is generally more available for functionalisation than a secondary hydroxyl group. Besides, starting from a 3'-O-functionalized adenosine molecule, prior protection of the 2'-hydroxyl group to prevent 2',3'-cyclisation would be necessary. Our strategy, therefore, involves a derivatisation of the 5'-function of adenosine followed by a cyclisation reaction with the 3'-hydroxyl group.

The primary 5'-hydroxyl group of adenosine is sterically less hindered than the 2'- and 3'-secondary hydroxyl groups. This difference could be exploited for a selective functionalisation at the 5'-position. However, due to the vicinal position of both

secondary hydroxyl groups, the 2'-hydroxyl group is more acidic than the 5'-hydroxyl group. Therefore, mesylation of unprotected adenosine gives a complex reaction mixture[3]. We decided to use an isopropylidene group to protect the 2'- and 3'- hydroxyl groups during the mesylation reaction.



Scheme II

Reaction of adenosine with acetone results in the formation of the 2',3'-acetonide. Conditions were as described by Hampton[4]. Since the reaction seemed to progress rather slowly, 2,2-dimethoxypropane was added to remove the water formed and thus drive the reaction to completion[5]. This resulted in a yield of 88 % of 2',3'-O-isopropylidene adenosine (I) after 7 hours of reaction. Selective mesylation of the 5'-hydroxyl of I is possible without protecting the aromatic amine[6]. The reaction was carried out as described for the 5'-O-tosylation reaction[7]. Pyridine served both as a solvent for the reaction and as a base, and it also generates a low concentration of the much more reactive alkoxide ion of the sugar[8]. The reaction proceeded smoothly at 0°C and was completed after one hour.

During nucleophilic substitution of the 5'-tosyl function of 5'-O-tosyl-2',3'-O-isopropylidene adenosine with external nucleophiles, a side reaction could be expected, due to the proximity of the N^3-function of the adenosine base[9]. This intramolecular

attack results in the formation of a 5'-O,N^3 cyclic derivative. This side reaction can be prevented by lowering the nucleophilicity of the N^3-atom[10]. Therefore the 6-amino group of 5'-O-mesyl-2',3'-O-isopropylidene adenosine (II) was protected by benzoylation[11].

Introduction of an azido function was then carried out without complications using the method of Jahn[10]. The reaction proceeded at 80°C and was completed after 45 minutes.

Reduction of the azido function in position 5' generates a primary aliphatic amine. This aliphatic amine is more basic than the aromatic amine. A benzoyl migration to the more electron rich centre could be expected. To avoid the displacement reaction, the N^6-benzoyl group is removed with ammonia in methanol[12] before catalytic reduction of the azido group is performed. The reduction was carried out in ethanol with Pd-C as catalyst[13].

The second part of the synthetic scheme (scheme III) is the synthesis of the 3'-O,5'-N-cyclic compound using dimethyl N-phenylsulfonyldithiocarbamate as reagent, which was synthesized according to Gompper and Hägele[14].

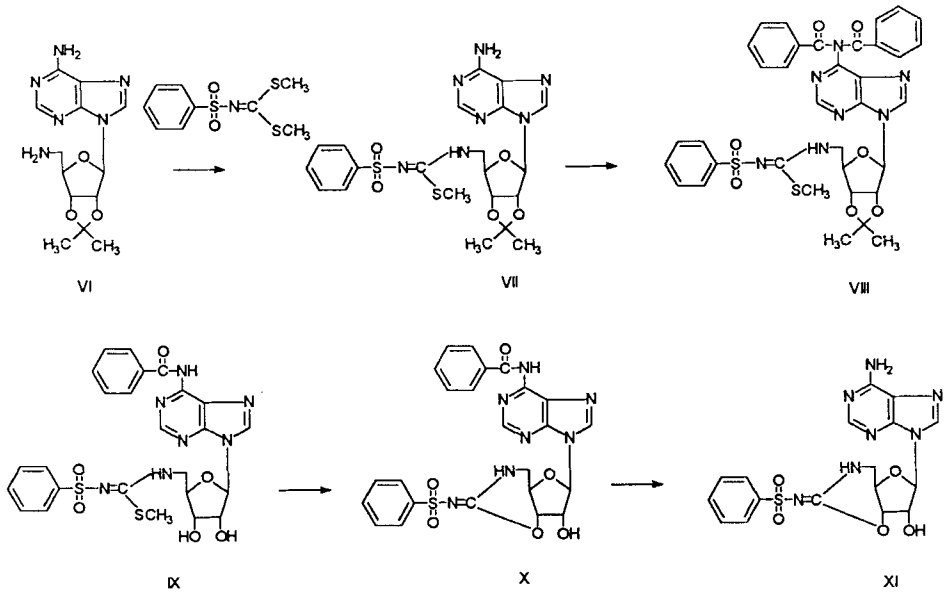
Reaction of 5'-amino-5'-deoxy-2',3'-O-isopropylidene adenosine (VI) with dimethyl N-phenylsulfonyldithiocarbamate gives 1-(5'-deoxy-2',3'-O-isopropylidene-adenosin-5'-yl)-2-methyl-3-phenylsulfonyl-isothiourea (VII) in 93 % yield. After removal of the isopropylidene protecting group, the cyclisation reaction was attempted using several reaction circumstances. However, this reaction invariably results in complex reaction mixtures from which no desired compound could be isolated. This is due to the presence of the unprotected nucleophilic purine base. Therefore the exocyclic amino function was again protected by benzoylation before deprotection of the 2'- and 3'-hydroxyl with a 90 % solution of trifluoroacetic acid was carried out. Aqueous acetic acid was less effective[15].

Several basic substances such as sodium hydride, DBU and butyl lithium were tried to close the six membered ring but the reaction was only successful using potassium tert-butoxide in dimethylformamide.

Finally, the base protecting group had to be removed. Deprotection circumstances using ammonia were not effective due to opening of the six membered ring. However, treatment with hydrazine hydrate for six hours yielded the desired compound.

Conclusions

This article describes the succesful synthesis of a neutral cyclic AMP analogue. The reaction scheme included several protection - deprotection strategies in order to avoid side reactions. 2',3'-Protection is necessary to functionalize selectively the 5'-position.



Scheme III

Transient base protection is needed to prevent attack of the N^3-nucleophilic centre on the 5'-position. The crucial cyclisation step could be accomplished using potassium tert-butoxide in DMF. This reaction also requires a base protecting step. Final deprotection was accomplished using hydrazine hydrate in pyridine/acetic acid.

Data on the biological activity of the resulting compound will be presented elsewhere.

Experimental section

Ultraviolet spectra were recorded with a Philips PU 8700 UV/VIS spectrophotometer. The ^1H NMR and ^{13}C NMR spectra were determined with a JEOL FX90Q spectrometer with tetramethylsilane as internal standard for the ^1H NMR spectra and DMSO-d6 (39,6 ppm), $CDCl_3$ (76,9 ppm) or CD_3OD (49,9 ppm) for the ^{13}C NMR spectra (s = singlet, d = doublet, t = triplet, br s = broad signal, m = multiplet). Mass spectra were obtained using a Kratos Concept 1H mass spectrometer (LSIMS : liquid secondary ion mass spectrum, CI : chemical ionisation). Melting points were determined with a Büchi-Tottoli apparatus and are uncorrected. Precoated Merck silicagel F254 plates were used for TLC, and the spots were examined with UV light and sulfuric acid - anisaldehyde spray. Column chromatography was performed on Merck silicagel (0,063 -

0,200 mm). Anhydrous solvents were obtained as follows : pyridine was dried by distillation after it had been refluxed for 24h over potassium hydroxide, water was removed from N,N-dimethylformamide by distillation with benzene followed by distillation in vacuo and acetone was refluxed over calcium chloride and distilled.

N^6-benzoyl-2',3'-O-isopropylidene-5'-O-mesyl adenosine (III)

8,72 g (0,028 mol) of 2',3'-O-isopropylidene adenosine[4] (I) was evaporated three times with pyridine. Then it was dissolved in dry pyridine and cooled in an icebath. To the clear solution were added 1,1 equivalents of mesyl chloride (2,42 ml - 0,0312 mol), followed by another 0,5 equivalents (1,1 ml - 0,014 mol) after one hour. The reaction was completed after two hours at 0°C. Benzoylation was performed overnight at room temperature with 4 equivalents of benzoyl chloride (13,23 ml - 0,1136 mol). After addition of 5 ml of methanol, the reaction mixture was evaporated and purified by extraction (ethyl acetate/saturated sodium bicarbonate) followed by column chromatography (CH_2Cl_2, 100; CH_2Cl_2/MeOH, 99-1) to give 10,54 g N^6,N^6-dibenzoyl-5'-O-mesyl-2',3'-O-isopropylidene adenosine (0,018 mol - 63 %).

^{1}H NMR (DMSO-d6) : δ 1,35 (s, 3H, CH_3), 1,57 (s, 3H, CH_3), 3,09 (s, 3H, CH_3SO_2-), 4,42 (br s, 3H, 4'-H + 5'-H), 5,09 (dd, 1H, 3'-H), 5,56 (br s, 1H, 2'-H), 6,38 (br s, 1H, 1'-H), 7,53 (m, 6H, benzoyl o, p), 7,80 (m, 4H, benzoyl m), 8,72 (s, 1H, 8-H), 8,76 (s, 1H, 2-H) ppm.

^{13}C NMR (DMSO-d6) : δ 172,7 (C=O), 152,8 (C-6), 152,5 (C-2), 151,6 (C-4), 146,1 (C-8), 133,9 (benzoyl), 129,6 (benzoyl m,p), 127,9 (benzoyl o), 125,3 (C-5), 114,6 (-C-(OR)$_2$), 90,4 (C-1'), 84,5 (C-4'), 83,7 (C-3'), 81,2 (C-2'), 69,6 (C-5'), 37,3 (CH_3SO_2-), 27,4 (CH_3), 25,6 (CH_3) ppm.

9,12 g (0,0154 mol) of this product was treated with 2M NH_3/Ethanol. The reaction mixture was evaporated after 1,5 hours and purified by column chromatography (CH_2Cl_2/MeOH, 99-1) to yield 6,61 g of III (0,0135 mol - 88 %).

UV (MeOH): λ_{max} = 279 nm (ε = 15300)

^{1}H NMR (DMSO-d6) : δ 1,37 (s, 3H, CH_3), 1,58 (s, 3H, CH_3), 3,14 (s, 3H, CH_3SO_2-), 4,46 (br s, 3H, 4'-H + 5'-H), 5,14 (br s, 1H, 3'-H), 5,56 (br s, 1H, 2'-H), 6,40 (br s, 1H, 1'-H), 7,60 (m, 3H, benzoyl), 8,07 (m, 3H, benzoyl), 8,60 (s, 1H, 8-H), 8,78 (s, 1H, 2-H), 11,21 (br s, 1H, NH) ppm.

^{13}C NMR (DMSO-d6) : δ 165,7 (C=O), 151,7 (C-6), 150,6 (C-2), 143,2 (C-4), 133,4 (C-8), 132,4 (benzoyl), 128,4 (benzoyl m,p), 127,4 (benzoyl o), 125,7 (C-5), 113,7 (-C-(OR)$_2$), 89,4 (C-1'), 80,8, 83,3, 83,8 (C-2', C-3' and C-4'), 69,0 (C-5'), 36,8 (CH_3SO_2-), 26,9 (CH_3), 25,2 (CH_3) ppm.

LSIMS (glycerol) : 490 $(M + H)^+$, 384 (M-benzoyl)$^+$, 135 (adenine + H)$^+$, 105 (benzoyl)$^+$, 77 (phenyl)$^+$;
HRMS calculated for $C_{21}H_{24}N_5O_7S$: 490,1396 $(M + H)^+$, found 490,1404.

5'-azido-5'-deoxy-N^6-benzoyl-2',3'-O-isopropylidene adenosine (IV)

To a solution of 2,59 g (5,29 mmol) of III in 50 ml of DMF was added, 4 equivalents of sodium azide (1,38 g - 0,0212 mol) and the reaction was heated at 80°C for 45 minutes. Excess sodium azide was removed by filtration. The resulting solution was diluted with ethyl acetate, washed with water and evaporated to give a solid residue. Purification of the solid was performed by column chromatography (CH_2Cl_2/MeOH, 98-2) giving 1,94 g of IV (4,45 mmol - 84%).
UV (MeOH) : λ_{max} = 279,6 nm (ε = 16900)
IR : 2100 cm^{-1} (N_3).
^1H NMR (DMSO-d_6) : δ 1,36 (s, 3H, CH_3), 1,58 (s, 3H, CH_3), 3,63 (d, 2H, 5'-H), 4,40 (m, 1H, 4'-H), 5,06 (dd, 1H, 3'-H), 5,58 (m, 1H, 2'-H), 6,35 (d, 1H, 1'-H), 7,60 (m, 3H, benzoyl o,p), 8,10 (m, 2H, benzoyl m), 8,69 (s, 1H, 8-H), 8,78 (s, 1H, 2-H), 11,21 (br s, 1H, NH) ppm.
^{13}C NMR (DMSO-d_6) : δ 170,0 (C=O), 152,1 (C-6), 150,6 (C-2), 143,6 (C-4), 133,6 (C-8), 132,8 (benzoyl), 128,7 (benzoyl m,p), 128,1 (benzoyl o), 125,7 (C-5'), 114,2 (-C-(OR)2), 89,6 (C-1'), 81,7, 83,3 and 84,9 (C-2', C-3' and C-4'), 51,9 (C-5'), 27,8 (CH_3), 25,4 (CH_3) ppm.
CI (i-C_4H_{10}) : 437 $(M + H)^+$, 136 (adenine + 2H)$^+$, 105 (benzoyl)$^+$, 77 (phenyl)$^+$;
HRMS calculated for $C_{20}H_{21}N_8O_4$: 437,1685 $(M + H)^+$, found 437,1714.

5'-azido-5'-deoxy-2',3'-O-isopropylidene adenosine (V)

In 25 ml of methanol saturated with ammonia was taken up 480 mg (1,10 mmol) of IV. The solution was kept at room temperature for 40 hours and was evaporated to a yellow oil. Purification by column chromatography (CH_2Cl_2/MeOH, 98-2) yielded 240 mg of V (0,72 mmol - 66 %).
^1H NMR (DMSO-d_6) : δ 1,34 (s, 3H,CH_3), 1,55 (s, 3H, CH_3), 3,58 (d, 2H, 5'-H), 4,32 (m, 1H, 4'-H), 5,00 (dd, 1H, 3'-H), 5,52 (dd, 1H, 2'-H), 6,21 (d, 1H, 1'-H), 7,39 (s, 2H, NH_2), 8,12 (s, 1H, 8-H), 8,34 (s, 1H, 2-H) ppm.
^{13}C NMR (DMSO-d_6) : δ 25,5 (CH_3), 27,3 (CH_3), 52,0 (C-5'), 81,8, 83,3 and 84,9 (C-2', C-3' and C-4'), 89,6 (C-1'), 114,2 (-C-(OR)2), 119,0 (C-5), 140,9 (C-8), 149,2 (C-4), 153,2 (C-2), 156,2 (C-6) ppm.
LSIMS (glycerol) : 332 M^+, 136 (adenine + 2H)$^+$;
HRMS calculated for $C_{13}H_{17}N_8O_3$: 333,1423 $(M + H)^+$, found 333,1434.

5'-amino-5'-deoxy-2',3'-O-isopropylidene adenosine (VI)

A solution of 200 mg (0,60 mmol) of V in 10 ml of ethanol was hydrogenated for five hours at 45 psi in the presence of 20 mg 10 % Pd-C. The catalyst was removed by filtration. After evaporation, the title compound was purified by column chromatography (CH_2Cl_2/MeOH, 80-20; CH_2Cl_2/MeOH/Et_3N, 80-20-1) and isolated in 70 % yield (0,42 mmol - 130 mg).

VI could also be synthesized (in a one pot procedure) starting from 11,75 g (0,0382 mol) of I without any intermediate purification. A total yield of 4,80 g of the purified (column chromatography CH_2Cl_2/MeOH, 80-20) product was obtained (0,0157 mol - 41 %).

UV(MeOH) : λ_{max} = 259 nm (ε = 15100)

^{1}H NMR (DMSO-d6) : δ 1,33 (s, 3H, CH_3), 1,54 (s, 3H, CH_3), 2,76 (br s, 2H, 5'-H), 4,10 (m, 1H, 4'-H), 4,98 (dd, 1H, 3'-H), 5,45 (dd, 1H, 2'-H), 6,09 (d, 1H, 1'-H), 7,31 (s, 2H, NH_2 Ar.), 8,14 (s, 1H, 8-H), 8,36 (s, 1H, 2-H) ppm.

^{13}C NMR (DMSO-d6) : δ 156,1 (C-6), 153,0 (C-2), 149,2 (C-4), 140,3 (C-8), 119,3 (C-5), 113,6 (-C-(OR)2), 89,4 (C-1'), 87,0 (C-4'), 81,8 and 83,1 (C-2' and C-3'), 43,5 (C-5'), 27,3 (CH_3), 25,5 (CH_3) ppm.

CI (NH_3) : 307 $(M + H)^+$, 172 $(M\text{-adenine})^+$, 136 $(adenine + 2H)^+$;

HRMS (i-C_4H_{10}) calculated for $C_{13}H_{19}N_6O_4$: 307,1519 $(M + H)^+$, found 307,1489.

1-(5'-deoxy - 2',3'-O-isopropylidene adenosin-5'-yl) - 2-methyl - 3-phenylsulfonyl-isothiourea (VII)

To a solution of 610 mg (1,99 mmol) of VI in 20 ml pyridine, 1 equivalent dimethyl N-phenylsulfonyldithiocarbamate (521 mg - 1,99 mmol) was added. Reaction proceeded at 80°C for 4 hours. The pyridine was evaporated. Purification by column chromatography (CH_2Cl_2/MeOH, 99-1) yielded 960 mg of VII (1,85 mmol - 93 %).

UV (MeOH): λ_{max} = 250,5 nm (ε = 32200)

^{1}H NMR (DMSO-d6) : δ 1,31 (s, 3H, CH_3), 1,52 (s, 3H, CH_3), 2,38 (s, 3H, SCH_3), 3,62 (br s, 2H, 5'-H), 4,34 (br s, 1H, 4'-H), 5,05 (dd, 1H, 3'-H), 5,46 (br s, 1H, 2'-H), 6,18 (br s, 1H, 1'-H), 7,34 (s, 2H, NH_2), 7,55 (m, 3H, phenylsulfon o,p), 7,73 (m, 2H, phenylsulfon m), 8,18 (s, 1H, 8-H), 8,31 (s, 1H, 2-H), 8,56 (br s, 1H, NH) ppm.

^{13}C NMR (DMSO-d6) : δ 156,1 (C-6), 152,7 (C-2), 150,0 (phenylsulfon), 148,7 (C-4), 140,1 (C-8), 131,7 (phenylsulfon p), 128,6 (phenylsulfon m), 125,9 (phenylsulfon o), 119,3 (C-5), 113,5 (-C-(OR)2), 89,0 (C-1'), 83,7 (C-4'), 81,8 and 83,1 (C-2' and C-3'), 45,4 (C-5'), 27,0 (CH_3), 25,3 (CH_3), 14,1 (SCH_3) ppm.

CI (NH_3) : 520 $(M + H)^+$, 472 $(M - SCH_3)^+$, 307 $(M\text{-}\varnothing\text{-}SO_2\text{-}N{=}C\text{-}SCH_3)^+$, 136 $(adenine + 2H)^+$;

LSIMS (thioglycerol); HRMS calculated for $C_{21}H_{26}N_7O_5S_2$: 520,1436 $(M + H)^+$, found 520,1420.

1-(5'-deoxy - N^6,N^6-dibenzoyl - 2',3'-isopropylidene adenosin-5'-yl) - 2-methyl - 3-phenylsulfonyl-isothiourea (VIII)

A mixture of 900 mg (1,73 mmol) of VII and 4 equivalents of benzoylchloride (0,8 ml - 6,9 mmol) in 15 ml of dry pyridine was stirred for two hours at room temperature, 5 ml water was added and the reaction mixture was evaporated. The residue was dissolved in ethyl acetate, washed with saturated sodium bicarbonate solution, dried (Na_2SO_4) and evaporated. Column chromatographic purification (CH_2Cl_2/MeOH, 98-2) yielded 1,22 g of VIII (1,68 mmol - 97 %).

UV (MeOH): λ_{max} = 244,5 nm (ε = 40800), 272 nm (ε = 21300)

^1H NMR (DMSO-d6) : δ 1,31 (s, 3H, CH_3), 1,52 (s, 3H, CH_3), 2,33 (s, 3H, SCH_3), 3,61 (br s, 2H, 5'-H), 4,33 (br s, 1H, 4'-H), 5,05 (br s, 1H, 3'-H), 5,48 (br s, 1H, 2'-H), 6,32 (d, 1H, 1'-H), 7,51 (m, 9H, Ar.), 7,74 (m, 6H, Ar.), 8,09 (br s, 1H, NH), 8,69 (s, 1H, 8-H), 8,76 (s, 1H, 2-H) ppm.

^{13}C NMR (DMSO-d6) : δ 171,8 (C=O), 152,0 (C-6), 151,8 (C-2), 149,5 ($ArSO_2$), 145,9 (C-4), 133,5 (C-8), 133,2 (Ar.), 128,8 (Ar.p), 128,6 (Ar.m), 128,1 (Ar.o), 125,9 (C-5), 113,6 (-C-(OR)2), 89,2 (C-1'), 84,0 (C-4'), 81,4 and 83,0 (C-2' and C-3'), 45,0 (C-5'), 26,9 (CH_3), 25,2 (CH_3), 14,0 (SCH_3) ppm.

LSIMS (glycerol) : 728 M^+, 622 (M-benzoyl)$^+$, 344 (N^6-dibzadenine + 2H)$^+$;

HRMS calculated for $C_{35}H_{34}N_7O_7S_2$: 728,1961 (M + H)$^+$, found 728,1975.

1-(N^6-benzoyl-5'-deoxy-adenosin-5'-yl)-2-methyl-3-phenylsulfonyl--isothiourea (IX)

The isopropylidene protecting group was removed from 1,17 g (1,61 mmol) of VIII by dissolving the compound into 20 ml of a 90 % solution of trifluoroacetic acid in water. The solution was kept for 40 minutes at room temperature, then the mixture was neutralized by pouring it into a saturated bicarbonate solution and evaporated. The reaction product was adsorbed on Celite and purified by column chromatography (CH_2Cl_2/MeOH, 95-5) to give 673 mg of IX (1,15 mmol - 71 %).

UV (MeOH): λ_{max} = 233 nm (ε = 36900); λ_{max} = 279 nm (ε = 23000); λ_{min} = 264 nm (ε = 18200)

^1H NMR ($CDCl_3$) : δ 2,34 (s, 3H, SCH_3), 3,73 (br s, 2H, 5'-H), 4,22 (m, 2H, 4'-H + 3'-H), 4,84 (t, 1H, 2'-H), 5,51 (br s, 2H, 2'-HO + 3'-HO), 6,03 (d, 1H, 1'-H), 7,53 (m, 6H, Ar.o,p), 7,73 (m, 2H, Ar.m), 8,00 (m, 2H, Ar.m), 8,68 (s, 1H, 8-H), 8,73 (s, 1H, 2-H), 9,00(br s, 1H, NH) ppm.

^{13}C NMR ($CDCl_3$) : δ 165,7 (C=O), 152,0 (C-6), 151,5 (C-2), 150,5 ($ArSO_2$), 143,6 (C-4), 133,5 (C-8), 132,3 (Ar.), 131,7 (Ar.o), 128,6 (Ar.p), 128,4 (Ar.m), 125,9 (C-5), 88,2 (C-1'), 81,9 (C-4'), 71,3 and 72,6 (C-2' and C-3'), 45,6 (C-5'), 14,1 (SCH_3) ppm.

LSIMS (thioglycerol) : 584 M^+, 240 (N^6-bzadenine + 2H)$^+$;

HRMS calculated for $C_{25}H_{26}N_7O_6S_2$: 584,1385 (M + H)$^+$, found 584,1395.

5'-amino-5'-deoxy-N^6-benzoyl-3'-O,5'-N-(N-phenylsulfonyliminocarbonyl) adenosine (X)

To a solution of 580 mg (0,99 mmol) of IX in 30 ml of dry DMF was added 10 equivalents of tBuOK (1,12 g - 9,9 mmol). After stirring for two hours at 80°C, the reaction was virtually complete. The mixture was neutralized with aqueous hydrogen chloride to pH 5 and evaporated. Purification by column chromatography (CH_2Cl_2/MeOH, 98-2; 90-10) was only partially successful. Plate chromatography gave a pure NMR-sample. Crystallization from MeOH/CH_3CN gave analytically pure compound. mp : 243°C (Decomp)

UV (MeOH) : λ_{max} = 280,0 nm (ε = 20000)

^1H NMR (DMSO-d6): δ 6,23 (s, 1H, 1'-H), 7,58 (m, 6H, Ar, O, p), 7,85 (m, 2H, Ar.m), 8,06 (m, 2H, Ar.m), 8,62 (s, 1H, 8-H), 8,75 (s, 1H, 2-H) ppm and disappearance of the SCH_3 signal at 2,3 ppm.

^{13}C NMR (DMSO-d6) : δ 166,0 (C=O), 156,8 (C=N), 152,0 (C-6), 151,6 (C-2), 150,8 (Ar.SO_2), 143,6 (C-4), 133,7 (C-8), 132,6 (Ar.), 131,8 (Ar.o), 128,8 (Ar.p), 128,6 (Ar.m), 126,4 (C-5), 92,5 (C-1'), 76,9 (C-4'), 70,9 (C-3'), 68,4 (C-2'), 44,6 (C-5') ppm.

LSIMS (3-nitrobenzylalcohol) : 536 M^+, 240 (N^6-bzadenine + 2H)$^+$, 136 (adenine + 2H)$^+$; HRMS calculated for $C_{24}H_{22}N_7O_6S$: 536,1352 (M + H)$^+$, found 536,1379.

5'-amino-5'-deoxy-3'-O,5'-N-(N-phenylsulfonyliminocarbonyl) adenosine (XI)

To a solution of 120 mg (0,22 mmol) of X in pyridine/acetic acid (4:1) were added 4 equivalents of hydrazine hydrate (34 mg - 0,897 mmol). The reaction mixture was stirred for six hours at room temperature and poured in a mixture of ethyl acetate/water. The water layer was extracted three times with ethyl acetate. The combined organic layers were evaporated to dryness. The resulting product was dissolved in pyridine/methanol (2:1) and purified by preparative TLC (silica gel : crystallized ether, CH_2Cl_2/MeOH 90:10) to give 72 mg of X and 22 mg of XI from MeOH/CH_3CN (23 %) . mp : 196°C (Decomp)

UV λ_{max} = 259 nm (ε = 14000)

^1H NMR (CD_3OD) : δ 3,68 (m, 2H, 5'-H), 4,30 (m, 1H, 4'-H), 4,84 (d, 1H, 2'-H), 5,13 (dd, 2H, 3'-H), 6,09 (S, 1H, 1'-H), 7,54 (m, 3H, Ar.), 7,91 (d, 2H, Ar.), 8,19 and 8,18 (2 x S, 2H, 8-H and 2-H) ppm.

^{13}C NMR (CD_3OD) : δ 159,5 ($^{N=C-N}_{\ \ O}$), 157,6 (C-6), 154,5 (C-2), 150,5 (C-4), 144,1 (C-i), 142,0 (C-8), 133,7 (Ar., p), 130,2 (Ar., m), 127,9 (Ar., O), 121,0 (C-5), 94.9 (C-1'), 78,8 (C-4'), 72,9 (C-2'), 70,0 (C-3'), 46,5 (C-5') ppm.

LSIMS (thio-2,2'-bis(ethanol) with addition of trifluoroacetic acid) : 432 (M + H)$^+$, 167 ($\varnothing SO_2NC$); HRMS calculated for $C_{17}H_{18}N_7O_5S$: 432,1090 (M + H)$^+$, found 432,1064.

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