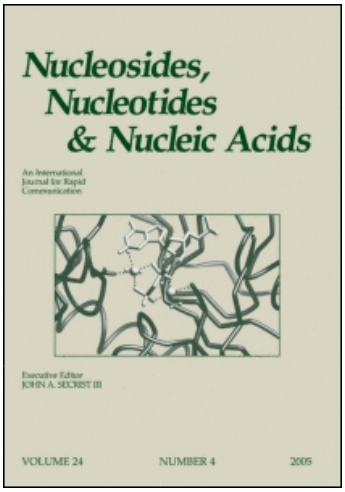

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Synthesis of 3'-Fluoro-3'-deoxy-N^6-cyclopentyladenosine

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SYNTHESIS OF 3'-FLUORO-3'-DEOXY-N^6-CYCLOPENTYLADENOSINE

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Abstract : Starting from 9-(β-D-xylofuranosyl)-6-chloropurine, the title compound was prepared in four steps. Reaction with cyclopentylamine followed by treatment of the 2'-O,5'-O-ditritylated material with diethylaminosulfur trifluoride (DAST), yielded after deprotection the desired compound.

Introduction

Adenosine (figure 1.A) plays an important role as local modulator in the mammalian body. It has been implied in the normal functioning of cardiovascular, renal, immunological and hormonal systems[1,2].

Its effects are mediated via G protein coupled adenosine receptors, of which at least three subtypes exist, *viz.* A_1, A_2, and A_3. The distribution of these receptors is quite ubiquitous, explaining the plethora of effects mentioned above[3,4].

Pathological circumstances, however, usually demand selective intervention. As an example, A_2-selective agonists may be useful as antipsychotics[5,6]. However, compounds developed so far also show profound effects in the cardiovascular and other systems, which can be regarded as side effects in that case[4].

Therefore, selectivity, either at the receptor level or in tissue distribution and disposition, is crucial. It has been shown that substitution at the adenosine molecule can lead to very potent, and

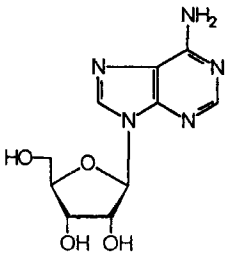

figure 1.A

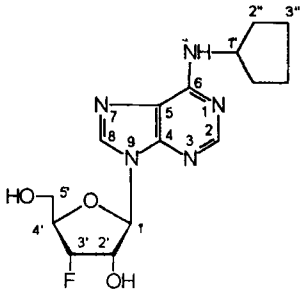

figure 1.B

selective adenosine receptor agonists[7]. In particular the N^6-and C2-region have been investigated in this respect in a triad of organic chemistry, molecular modelling and pharmacological testing[3,8,9]. Little is known, however, about the effect of modifications at the ribofuranosyl moiety.

To fill this gap in current knowledge, we decided to synthesize a sugar modified adenosine analogue. 3'-Fluoro-3'-deoxy-N^6-cyclopentyladenosine (figure 1.B) was designed to act as potential A_1-receptor (ant)agonist. The cyclic, hydrophobic N^6-monosubstituent was expected to impose this selectivity[8]. The sugar part was modified by exchange of the 3'-hydroxyl function with a 3'-fluorine. Fluorine is often used in biological chemistry to replace a hydroxyl group[10]. The carbon-fluorine bond distance is comparable to the carbon-oxygen bond length. Furthermore, the electronegativity of fluorine is comparable to that of oxygen. Finally, fluorine can act as a hydrogen bond acceptor, but unlike the hydroxyl group can not function as hydrogen donor.

Chemistry

The synthesis of N^6-monosubstituted adenosine[11,12] as well as that of 3'-fluoro-3'-deoxyadenosine[13,14,15,16] has been described before. Combination of both strategies was however not tempting, due to expected low yields and long-winded chemistry.

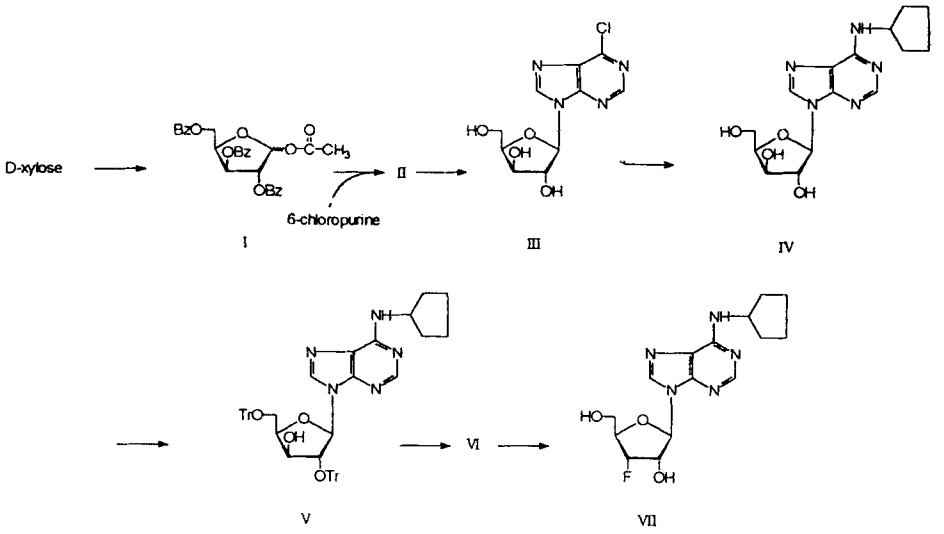

figure 2

We chose a more straightforward reaction scheme (figure 2). Since the introduction of fluorine with DAST in general occurs with inversion of configuration[16], the synthesis was started from D-xylose. The sugar was converted into 1-O-acetyl-2,3,5-tri-O-benzoyl-D-xylofuranose (figure 2.I) as described by Nakayama et al.[17]. Condensation with 6-chloropurine and deprotection with MeOH/NH_3 yielded the key intermediate 9-(β-D-xylofuranosyl)-6-chloropurine (figure 2.III). For the condensation reaction, the mixture of protected sugar and base was treated with a mixture of trimethylsilyl trifluoromethane sulfonate, trimethylsilyl chloride and hexamethyldisilazane. This procedure is a slightly modified version of the method used by Vorbrüggen and Bennua[18]. The condensation yielded exclusively the β-nucleoside[19].

6-Chloropurine was chosen as the heterocyclic base to gain easy access to any kind of N^6-monosubstituted adenosine analogue, i.e. by simple nucleophilic substitution with the appropriate amine. Reaction of 9-(β-D-xylofuranosyl)-6-chloropurine with cyclopentylamine in refluxing ethanol in the presence of triethylamine, gave N^6-cyclopentyl-9-(β-D-xylofuranosyl)-adenine (figure 2.IV). This compound was converted into its 2'-O,5'-O-dityrityl analogue (figure 2.V). The

trityl protecting group was chosen to avoid elimination as side reaction during treatment with the nucleophile. Reaction with DAST in dry dichloromethane and deprotection with 80% acetic acid at 100°C yielded 3'-fluoro-3'-deoxy-N^6-cyclopentyladenosine (figure 2.VII). The position and orientation[13,14,16,20] of the fluorine atom was confirmed by NMR analysis.

Conclusions

A straigtforward strategy with limited protection and deprotection steps to synthesize N^6-substituted 3'-deoxy-3'-fluoroadenosine analogues is described. This has allowed us to complete the synthesis of 3'-fluoro-3'deoxy-N^6-cyclopentyladenosine in seven steps starting from D-xylose. This compound, tested as an adenosine receptor agonist, displayed modest activity [$IC_{50}(A_1$ - GTP) = 2.3 ± 0.9 μM; $IC_{50}(A_1$ + GTP) = 11.9 ± 0.2 μM; $IC_{50}(A_2)$ = 91 ± 2.5 μM]. The parent compound N^6-cyclopentyladenosine, when tested under identical conditions, displayed a 100 - 1000-fold higher affinity for both receptor systems, indicating that the 3'-OH group in its interaction with the receptor is essential as hydrogen bond donor rather than acceptor.

Experimental

Chemistry

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-d_6 (39.6 ppm), CD_3OD (49.9 ppm) or $CDCl_3$ (76.9 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. Precoated Machery-Nagel Alugram^R Sil G/UV_{254} plates were used for TLC, and the spots were examined with UV-light and sulfuric acid - anisaldehyde spray. Column chromatography was performed on silica gel (0.060 - 0.200 mm pore diameter ca 6 nm). Anhydrous solvents were obtained as follows : pyridine and triethylamine were dried by distillation after they had

been refluxed for 24 h on potassium hydroxide; acetonitrile and dichloromethane were refuxed on calcium hydride before distillation; methanol and ethanol were refluxed on magnesium/iodine overnight prior to distillation.

1-O-acetyl-2,3,5-tri-O-benzoyl-D-xylofuranose (I)

This compound was prepared in a yield of 60 % following a method described by Nakayama and Saneyoshi[17].

9-(2,3,5-tri-O-benzoyl-β-D-xylofuranosyl)-6-chloropurine (II) and 9-(β-D-xylofuranosyl)-6-chloropurine (III)

6-Chloropurine (2.80 g, 0.018 mol) and I[17] (0.016 mol, 8.38 g) were weighed together in a reaction flask and 100 ml of anhydrous acetonitrile was added. To the mixture were added : hexamethyldisilazane (0.016 mol, 3.48 ml), trimethylsilyl chloride (0.025 mol, 3.15 ml) and trimethylsilyl triflate (0.025 mol, 4.77 ml). The mixture was refluxed for 2.5 h, dichloromethane was added and the solution was extracted with a sat. $NaHCO_3$ solution. The organic phase was dried over $MgSO_4$ and evaporated. An analytical sample of 9-(2,3,5-tri-o-benzoyl-β-D-xylofuranosyl)-6-chloropurine was prepared by column chromatography (hexane-EtOAc 80:20) and crystallized from EtOH. The rest of the compound was purified after deprotection.

UV (MeOH) : λ_{max} : 265 and 233 nm.

^1H NMR ($CDCl_3$) : δ 4.83 (2H, d, J = 5.8 Hz, 5'-H), 5.07 (1H, dt, J = 4.0 Hz and 5.8 Hz, 4'-H), 6.03 (1H, dd, J = 4.0 and 1.6 Hz, 3'-H), 6.34 (1H, dd, J = 2.0 and 1.6 Hz, 2'-H), 6.49 (1H, d, J = 2.1 Hz, 1'-H), 7.25-8.20 (15H, m, benzoyl), 8.57 and 8.64 (2H, 2xs, 2-H and 8-H) ppm.

^{13}C NMR ($CDCl_3$) : δ 165.9, 164.8, 164.8 (3 x CO), 152.2 (C-2 and C-6), 143.2 (C-8),131.7 (C-5), 134.0, 133.3, 130.0, 129.6, 128.7, 128.6, 128.4 and 128.1 (benzoyl), 88.6 (C-1'), 79.8, 79.7 (C-4' and C-3'), 75.3 (C-2') and 61.6 (C-5') ppm.

Chemical impact mass spectrum (CIMS) - iC_4H_{10} : 599 $(M+H)^+$, 155 $(B+2H)^+$; HR calculated for $C_{31}H_{24}N_4O_7Cl$: 599.1333, found 599.1391.

The crude II was deprotected with methanolic ammonia. After the mixture had been stirred for 6h at room temperature, the solvent was removed in vacuo. The product was purified by column chromatography

(CH_2Cl_2/MeOH 98:2 to 95:5) to give 1.79 g of III (38% over two steps).
^1H NMR (CD_3OD) : δ 8.78 and 8.72 (2H, 2xs, 2-H and 8-H), 6.07 (1H, d, J = 1.2 Hz, 1'-H), 4.38 (1H, s, 2'-H), 4.30 (1H, m, 4'-H), 4.12 (1H, m, 3'-H) and 3.81 (1H, m, 5'-H) ppm.
^{13}C NMR (CD_3OD) : δ 154.0 (C-2), 153.7 (C-6), 151.6 (C-4), 148.0 (C-8), 133.6 (C-5), 92.5 (C-1'), 86.8 (C-4'), 82.9 (C-2'), 77.1 (C-3') and 61.8 (C-5') ppm.
Electron impact mass spectrum (EIMS) : 286 $(M)^+$, 155 $(B+2H)^+$; HR calculated for $C_{10}H_{11}N_4O_4Cl$: 286.0469, found 286.0452.

9-(β-D-xylofuranosyl)-N^6-cyclopentyladenine (IV)

To a solution of 140 mg (0.488 mmol) of III in 10 ml of dry ethanol was added triethylamine (4.88 mmol, 0.68 ml) and cyclopentylamine (1.465 mmol, 0.145 ml) and the reaction mixture was refluxed with stirring for 2 h. The solvent was removed in vacuo and the residue was purified by preparative TLC on silica gel (CH_2Cl_2/MeOH 90:10) to yield 98 mg of IV (60%).
UV (MeOH) : λ_{max} = 270 nm (ε = 15700)
^1H NMR (DMSO-d_6) : δ 1.10-2.10 (m, cyclopentyl), 3.73 (2H, m, 5'-H_2), 4.11 (2H, m, 3'-H and 4'-H), 4.35 (1H, m, 2'-H), 4.79 (1H, t, 5'-OH), 5.91 (2H, 1'-H and OH), 7.72 (1H, d, J = 8.0 Hz, N-H), 8.22 (2-H), 8.28 (8-H) ppm.
^{13}C NMR (DMSO-d_6) : δ 154.4 (C-6), 152.3 (C-2), 148.5 (C-4), 139.5 (C-8), 119.0 (C-5), 89.6 (C-1'), 83.5 (C-4'), 81.0 (C-3'), 75.4 (C-2'), 59.6 (C-5'), 52.7 (C-1"), 32.3 (C-2")and 23.6 (C-3") ppm.
EIMS 335 $(M)^+$, 204 $(B+2H)^+$; HR calculated for $C_{15}H_{21}N_5O_4$: 335.1593, found 335.1585.

9-(2,5-di-O-trityl-β-D-xylofuranosyl)-N^6-cyclopentyladenine (V)

After being coevaporated two times with dry pyridine, 68 mg (0.20 mmol) of IV was dissolved in dry pyridine. Trityl chloride (0.81 mmol, 0.226 g) was added, the mixture was stirred for 18 h at 90°C, evaporated to an oil and extracted (CH_2Cl_2/sat.$NaHCO_3$). The organic layer was dried over $MgSO_4$, evaporated and purified by preparative TLC on silica gel (CH_2Cl_2/MeOH 98:2) to give 35 mg of V (20%).
UV (MeOH) : λ_{max} = 270 nm (ε = 19400).

^1H NMR ($CDCl_3$) : δ 1.3-2.2 (cyclopentyl + OH), 3.51 (2H, m, J = 10.8, 7.0 and 4.7 Hz, 5'-H), 4.04 (1H, dd, J = 11.0 and 3.2 Hz, 3'-H), 4.30 (1H, m, J = 7.0, 4.7 and 3.2 Hz, 4'-H), 4.62 (1H, d, J = 1.02 Hz, 2'-H), 5.41 (1H, d, J = 1.18 Hz, 1'-H), 5.72 (1H, d, J = 7.70 Hz, NH), 6.88 (1H, s, 2-H), 7.1-7.5 (phenyl) and 8.21 (1H, s, 8-H) ppm.
^{13}C NMR ($CDCl_3$) : δ 156.4 (C-6), 154.4 (C-2), 151.8 (C-4), 143.8 and 143.6 (trityl), 140.2 (C-8), 128.6 - 126.7 (trityl), 102.7 (C-5), 88.5 (C-1'), 86.9 (C-4'), 84.3 (C-3'), 82.5 (C-2'), 62.5 (C-5'), 52.8 (C-1"), 33.3 (C-2") and 23.6 (C-3") ppm.
Liquid secondary ion mass spectrum (LSIMS)-thioglycerol : 820 $(M+H)^+$, 204 $(B+2H)^+$; HR calculated for $C_{53}H_{50}N_5O_4$: 820.3863, found 820.3874.

2',5'-Di-O-trityl-3'-fluoro-3'-deoxy-N^6-cyclopentyladenosine (VI) and 3'-fluoro-3'-deoxy-N^6-cyclopentyladenosine (VII)

DAST (1.46 mmol, 190 μl) was added to a solution of 120 mg (0.146 mmol) of V in dry dichloromethane. The reaction mixture was stirred for 4 h at room temperature and then washed with sat. $NaHCO_3$ solution. The organic phase was dried, evaporated and purified by preparative TLC on silica gel yielding 70 mg of VI (60%).
UV (MeOH) : λ_{max} = 270.4 nm
^1H NMR ($CDCl_3$) : δ 2.2 - 1.7 (br, cyclopentyl), 3.34 (1H, dd, J = 4.3 and 10.5 Hz, H-5'A), 3.01 (1H, dd, J = 3.7 and 10.5 Hz, H-5'B), 3.58 (1H, dd, J = 52.9 and 3.7 Hz, H-3'), 4.22 (1H, dt, J = 27.0 and 4.0 Hz, H-4'), 5.12 (1H, ddd, J = 21.3, 7.5 and 4.1 Hz, H-2'), 5.75 (1H, d, J = 8Hz, NH), 6.26 (1H, d, J = 7.4 Hz, H-1'), 7.43 - 7.00 (30H, m, Ar), 8.15 (1H, s, 8-H) and 7.79 (1H, s, 2-H) ppm.
^{13}C NMR ($CDCl_3$) : δ 154.4 (C-6), 153.0 (C-2), 149.2 (C-4), 143.2 (trityl), 138.8 (C-8), 128.5 - 127.1 (trityl), 120.1 (C-5), 90,4 (d, $^1J_{3',F}$ = 188 Hz, C-3'), 87.4, 87.0 and 86.2 (C-1', trityl), 82.5 (d, $^2J_{2',F}$ = 23.2 Hz, C-2'), 75.1 (d, $^2J_{4',F}$ = 15.9 Hz, C-4'), 62.8 (d, $^3J_{5',F}$ = 11.0 Hz, C-5'), 52.4 (C-1"), 33.4 (C-2") and 23.6 (C-3") ppm.
LSIMS (thioglycerol) : 822 $(M+H)^+$, 204 $(B+2H)^+$; HR calculated for $C_{53}H_{49}N_5O_3F$: 822.3819, found 822.3792.

Deprotection of VI (60 mg, 0.073 mmol) was complete after heating for 30 min on a steambath in 80% acetic acid. The solvent was

evaporated and coevaporated with toluene. The remaining oil was dissolved in water and washed with diisopropyl ether. The water layer was evaporated and purified by preparative TLC followed by HPLC on silica gel (CH_2Cl_2/MeOH 95:5) giving 17 mg of VII (70%). The HPLC-system consisted of a Gilson model 303 Pump and Model 802 C Manometric Module, a silicagel column (KG = 60 ± 15 μ), a Pye Unicam LC-UV detector and a recorder. A sample was dissolved in mobile phase CH_2Cl_2-MeOH, 5% MeOH; injected and eluated from the analytical column at a flow rate of 1,25 cm^3/min. Detection was by monitoring of the absorbance at 269 nm. Retention time of the product was 8 minutes.
UV (MeOH) : λ_{max} = 268.4 nm (ε = 15400).
^1H NMR (CD_3OD) : δ 2.45 - 1.44 (br s, cyclopentyl), 3.83 (2H, d, J = 2.2 Hz, H-5'), 4.42 (1H, dt, J = 27.7 and 2.2 Hz, H-4'), 4.84 (1H, ddd, $J_{2',F}$ = 24.1, 7.9 Hz and 4.1 Hz, H-2'), 5.12 (1H, dd, J = 4.2 and 56.0 Hz, H-3'), 5.99 (1H, d, J = 7.9 Hz, H-1'), 8.20 (2xs, H-2 and H-8) ppm.
^{13}C NMR (CD_3OD) : δ 155.8 (C-6), 153.6 (C-2), 147.6 (C-4), 141.5 (C-8), 121.0 (C-5), 94.4 (d, $^1J_{3',F}$ = 181.9 Hz, C-3'), 90.3 (C-1'), 86.3 (d, $^2J_{2',F}$ = 22.8 Hz, C-2'), 74.4 (d, $^2J_{4',F}$ = 15.1 Hz, C-4'), 63.0 (d, $^3J_{5',F}$ = 11.0 Hz, C-5'), 53.8 (C-1"), 33.9 (C-2") and 24.7 (C-3") ppm.
EIMS : 337 (M), 204 (B+2H) ; HR calculated for $C_{15}H_{20}N_5O_3F$: 337.1550, found 337.1549

Biology

Adenosine A_1 receptor affinities were determined in radioligand binding studies on rat corical membranes with [^{3}H]DPCPX as the radioligand according to a protocol published previously[19]. Measurements with [^{3}H]DPCPX were performed in the presence and absence of 1 mM GTP. Adenosine A_2 receptor affinities were determined on rat striatial membranes with [^{3}H]CGS 21680 as the radioligand[20,21]. Data were analysed with InPlot 4.0 (GraphPad Software, Inc., San Diego, CA, USA).

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