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SYNTHESIS OF 1,5-ANHYDRO-2-(N^6-CYCLOPENTYLADENIN-9-YL)-2-DEOXY-D-*ALTRO*HEXITOL

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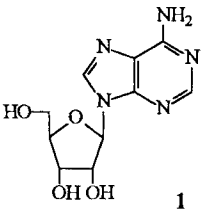
Abstract: The N^6-cyclopentyladenosine (CPA) analogue (**4**) was synthesized in 10 steps starting from glucose. The results of the radioligand binding assays are consistent with the thus far published findings that compounds containing a six-membered moiety at N^9 exhibit extremely weak affinity for adenosine receptors. Replacement of the ribofuranosyl moiety of CPA (**2**) by a 2-deoxy-D-*altro*hexitol moiety is sufficient to completely abolish its agonist activity.

The endogenous purine adenosine (**1**) influences numerous processes troughout the body[1], including the cardiovascular, renal, nervous and immunological systems. The observed physiological effects of adenosine are the result of its interaction with specific membrane-bound receptors. Direct binding studies with radiolabeled ligands with high affinity for adenosine receptors have facilitated the identification of receptors in various tissues. At least two extracellular adenosine receptors are coupled to adenylate cyclase. One, termed A_1, is a high affinity receptor that inhibits adenylate cyclase, which is present in adipocytes and heart and brain cells. The other, termed A_2, is more ubiquitous and is a low affinity receptor that activates adenylate cyclase[2,3]. Recently, a third subtype (A_3) of the purinoreceptors has been identified.

The A_1 and A_2 receptors have a distinct pharmacology and tissue distribution that offer the potential for the development of selective modulators of adenosine related effects that may have use as therapeutic entities. With the use of the A_2 binding assay it has been possible to measure the A_1/A_2 affinity ratios of different adenosine agonists and antagonists leading to the identification of compounds with selectivity for one or the other receptor subtype. A series of N^6-alkyladenosines and N^6-cycloalkyladenosines[4] have been reported to be A_1 receptor-selective agonists. Among these, N^6-cyclopentyladenosine (CPA, (**2**)) showed high affinity (0.59 nM) and 780 fold selectivity for the A_1 receptor[4].

Our effort in this area is directed towards the development of a novel selective adenosine agonist as a potential therapeutic agent. Data for modifications at the ribofuranosyl moiety do not abound. To further

fill this gap in current knowledge, we decided to synthesize a sugar modified adenosine analogue. 1,5-Anhydro-2-(N^6-cyclopentyladenin-9-yl)-2-deoxy-D-*altro*hexitol (**4**) was designed to act as potential A_1 receptor agonist. The cyclic hydrophobic N^6-monosubstituent was expected to impose this selectivity[5]. The sugar part was modified by enlargement of the pentose to a tetrahydropyran ring. We opted for this six membered ring as the 'ribo' analogue of the 1,5-anhydro-2,3-dideoxy-D-arabinohexitol nucleosides[6]. 1,5-Anhydro-2-(adenin-9-yl)-2-deoxy-D-*altro*hexitol (**3**) was also synthesized in order to be able to compare the receptor affinity of adenosine (**1**) and CPA (**2**) with the analogues containing a 6-membered ring.



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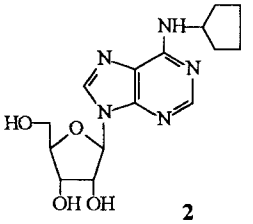

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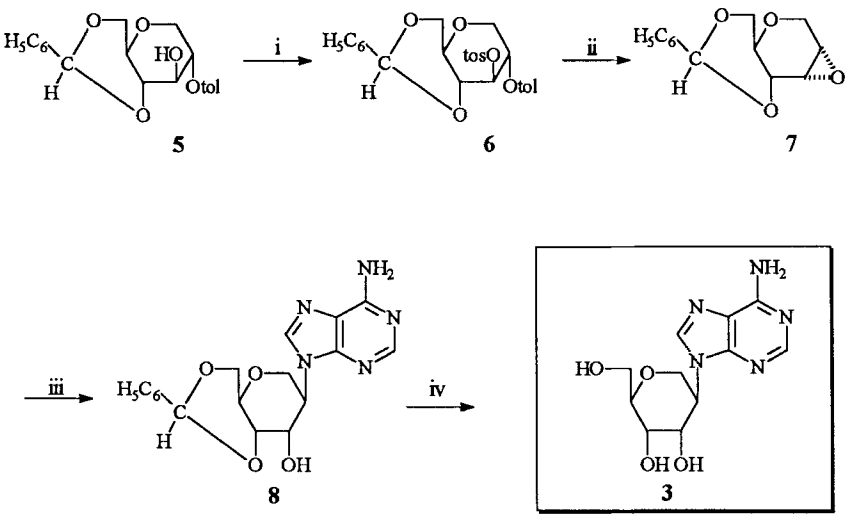
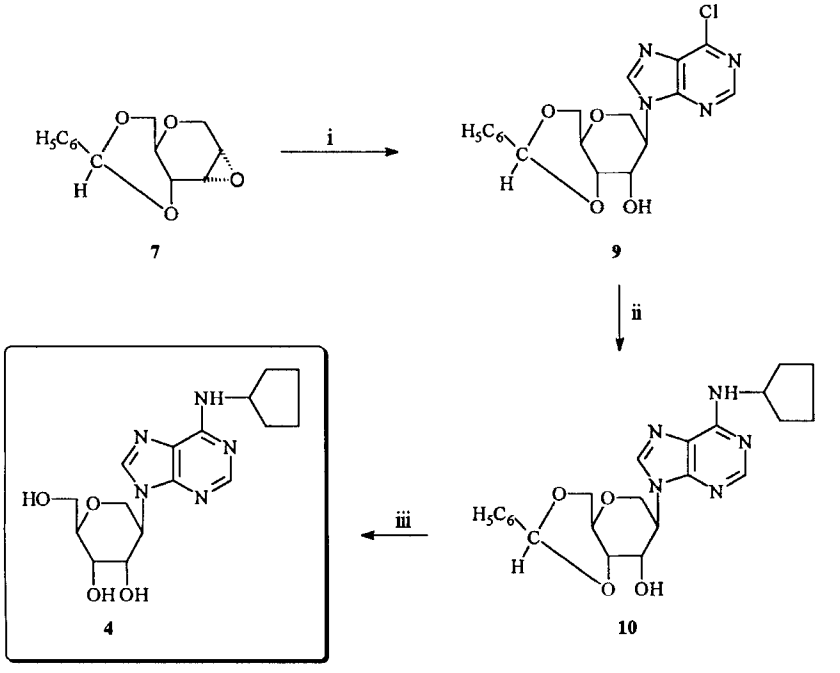
1,5-Anhydro-4,6-*O*-benzylidene-2-*O*-p-toluoyl-D-glucitol (**5**)[6] was used as starting material for the synthesis of the desired compounds (**3**) and (**4**). After tosylation of the C-3 hydroxylfunction, treatment of (**6**) with sodium methoxide led to concomitent hydrolysis and epoxide formation. 1,5:3,4-Anhydro-4,6-*O*-benzylidene-D-allitol (**7**) was obtained in 39 % overall yield starting from glucose. For the synthesis of (**3**), the adenine base was introduced by nucleophilic opening of the epoxide. Reaction of the sodium salt of adenine with (**7**) in DMF at 100 °C afforded (**8**) in 54 % yield. The reaction is regioselective and only yielded the C-2 substituted derivative which has a D-*altro*-configuration. This can be rationalized by the trans-diaxial opening of the oxirane ring in the conformationally rigid molecule (**7**). Removal of the benzylidene protecting group of (**8**) with 80 % of acetic acid gave the title compound (**3**) in 78 % yield[7]. For the synthesis of (**4**), the epoxide (**7**) was reacted with 6-chloropurine in the presence of K_2CO_3 in DMF at 80°C. Only 16 % of (**9**) could be obtained after 48 h reaction time. The difficulty in opening the epoxide with 6-chloropurine was further demonstrated by recovery of 38 % of the starting material (**7**). Nucleophilic substitution of the 6-chloropurine derivative (**9**) was accomplished with cyclopentylamine in boiling ethanol, using an excess of the amine (or TEA) as proton scavenger. The reaction was followed by deprotection with 80 % acetic acid to give (**4**) in 61 % yield[8].

The adenosine (**3**) and CPA (**4**) analogues were tested as adenosine receptor agonist by the *[^{3}H]DPCPX* binding assay for the A_1 adenosine receptor and the [^{3}H]CGS 21680 binding assay for the A_2 adenosine receptor. Radioligand binding showed that (**4**) gave only A_1 binding and did not interact with the A_2 receptor. However, the adenosine A_1 receptor affinity was very low [IC_{50} (A_1-GTP) > 100 μM; IC_{50} (A^1 + GTP) > 100 μM]. N^6-Cyclopentyladenosine (**2**), when tested under identical conditions displayed a 10^4-10^5 fold higher affinity, indicating that structural requirements for the ribofuranosyl



5 6 7 8 3

(i) $CH_3C_6H_4SO_2Cl$, Pyridine; (ii) NaOMe, toluene, 50°C; (iii) NaH, adenine, DMF, 100°C; (iv) HOAc 80%, 80°C.



7 9 10 4

(i) 6-chloropurine, K_2CO_3, DMF, 80°C; (ii) $C_5H_9NH_2$, EtOH, reflux; (iii) HOAc 80%, 50°C.

moiety are very strict. The adenosine analogue (**3**) showed no receptor binding at concentrations up to 10^{-4} M. This result confirms the findings of Taylor *et al.*[9] that ring opening or enlargement of the pentose to a hexose ring are detrimental to receptor affinity. As shown here, replacement with a 2-deoxy-D-*altro*hexitol likewise completely abolished all agonist activity.

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7. Analysis of compound **3**:

   Mp: 219-222°C; UV(MeOH): λ_{max} 262 nm (ε = 12000); CIMS (iC4H10): m/e 282 (MH^+), 136 (BH_2^+).

   ^{1}H NMR (DMSO-d_6): δ 3.42-3.78 (m, 4H), 4.09 (m, 3H), 4.57 (m, 1H), 4.68 (t, 1H, 6'-OH), 4.76 (d, 1H), 5.46 (d, 1H, J = 4 Hz), 7.26 (s, 2H, NH_2), 8.17 (s, 1H), 8.35 (s, 1H) (H-2, H-8) ppm.

   ^{13}C NMR (DMSO-d_6): δ 55.4 (C-2'), 60.5 (C-6'), 63.6, 64.3 (C-3', C-4'), 67.7 (C-1'), 77.4 (C-5'), 118.4 (C-5), 140.3 (C-8), 149.9 (C-4), 152.8 (C-2), 156.1 (C-6) ppm.

   Anal. ($C_{11}H_{15}N_5O_4xH_20$)C, H, N.
8. Analysis of compound **4**:

   UV(MeOH): λ_{max} 271 nm (ε = 12000); FDMS: m/e 350 (MH^+), 204 (BH_2^+); exact mass calculated for $C_{16}H_{23}N_5O_4$: 349.1750, found: 349.1812;

   ^{1}H NMR ($CDCl_3$): δ 1.05-2.35 (m, 9H, cyclopentyl), 3.42-3.80 (m, 4H), 4.10 (m, 3H), 4.57 (d, 1H), 4.76 (t, 1H, 6'-OH), 4.79 (d, 1H), 5.47 (d, J = 4 Hz, 1H), 7.32 (d, J = 8 Hz, 1H, NH), 8.22 (s, 1H), 8.28 (s, 1H) (H-2, H-8) ppm; ^{13}C NMR ($CDCl_3$): δ 23.6 (CH_2), 33.3 (CH_2), 52.3 (CH), 55.6 (C-2'), 60.8 (C-6'), 63.8, 64.4 (C-3', C-4'), 68.0 (C-1'), 77.4 (C-5'), 119.1 (C-5), 138.4 (C-8), 149.2(C-4), 153.5 (C-2), 154.5 (C-6) ppm.

   Anal. ($C_{16}H_{23}N_5O_4x0.75H_2O$)
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