A new entry to adenosine analogues via purine nitration - Combinatorial synthesis of antiprotozoal agents and adenosine receptor ligands
Rodenko, B.

Citation for published version (APA):
A new entry to adenosine analogues via purine nitration

Combinatorial synthesis of antiprotozoal agents and adenosine receptor ligands

In the body adenosine plays an important role as a regulator of many aspects of cellular metabolism. This endogenous nucleoside is related to bioactive adenine nucleotides such as adenosine mono-, di- and triphosphate and cyclic adenosine monophosphate and it is present as a structural element in RNA inter alia. Adenosine mediates many of its physiological effects via the G-protein coupled adenosine receptors, of which four subtypes have been characterised: the A₁, A₂A, A₂B and A₃ receptors. In the introductory Chapter 1 the therapeutic implications of drugs acting at these adenosine receptors are indicated. Selective activation of these receptors can be achieved by modifying the endogenous agonist adenosine. Modified adenosine analogues also have potential as antiprotozoal therapeutics. Whereas mammalian cells can synthesise purine nucleosides de novo, protozoan parasites are entirely dependent on purine salvage from their host. These parasites have evolved intricate uptake mechanisms with a broad tolerance for purines and purine nucleosides, including unnatural, potentially cytostatic nucleoside analogues. Parasitic diseases, like malaria and the African sleeping
sickness, threatens nearly half the world’s population. Drugs currently used for the treatment of these diseases often have serious side effects and are becoming increasingly useless due to the rapid evolution of drug resistance. Obviously, the development of new antiparasitic drugs is of cardinal importance. Progress in molecular biology allows for the high-throughput screening of drug candidates. Acceleration of the costly drug development process demands the production of a large amount of compounds in a short period. Combinatorial parallel synthesis either in solution or on solid phase is generally considered as the answer, which makes the development of new, fast sorting synthetic methodology a highly relevant matter.

In Chapter 2 the development of the first reported library of nucleoside monomers entirely prepared on a solid support is described. In this case the nucleoside is attached to the solid support by an ester linkage between the nucleoside 5'-hydroxyl group and a carboxyl functionalised polystyrene resin. Nitration of the purine ring in 1 furnished 2-nitro-6-chloropurine nucleoside 2, 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,N6-disubstituted adenosine analogues 3.

To expand the solid phase methodology to the modification of the ribosyl moiety, a sequence was developed involving the safety-catch principle, described in Chapter 3. A safety-catch linker remains inert during the solid supported diversification steps and can be ‘switched
on' at will, to allow for cleavage of the substrate from the resin. A 5′-carboxylate nucleoside scaffold is attached to the solid support via an aryl hydrazide linkage. Following diversification reactions at the purine system and removal of the 2′,3′-protective groups, the hydrazide linkage in 4 is oxidised. The resulting acyl diazene species 5 reacts in situ with amines present, thus releasing 5′-carboxamidoadenosine analogues 6. Two small libraries were synthesised composed of 5′,N6-disubstituted and 2′,5′,N6-trisubstituted carboxamidoadenosine analogues.

Chapter 4 deals with the construction of conformationally restricted adenosine analogues, making use of macrocyclisations involving the nitro substitution reactions, that were so fruitfully applied in the solid supported syntheses described in the preceding chapters. Two types of conformationally restricted adenosine analogues were synthesised. Type I contains a tether between N6 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.

The in vitro antiprotozoal evaluation of the adenosine analogues that were synthesised as discussed in the preceding chapters is described in Chapter 5. Several compounds were identified that displayed significant growth inhibitory activity against Trypanosoma brucei rhodesiense, the parasite that causes African sleeping sickness, and against Plasmodium falciparum, the parasite that is responsible for the most lethal form of malaria.

The versatile purine nitrination reaction constitutes the key step in the synthetic strategies described in this thesis. The mechanism of the purine nitrination with a mixture of tetrabutylammonium nitrate and trifluoroacetic anhydride was elucidated by using NMR spectroscopy, as reported in Chapter 6. Extensive monitoring of the nitrination of 9 excluded direct nitrination of the highly electrophilic C2 position and demonstrated that this reaction occurred in a three step process. Electrophilic attack by trifluoroacetyl nitrate (TFAN) on the purine N7 position results in a nitrammonium species that is trapped by a trifluoroacetate anion furnishing nitramine intermediate 10. A subsequent nitramine rearrangement generates C2-nitro species 11 that immediately eliminates TFA to give 2-nitro-6-chloro purine 12.
involvement of radicals during the nitramine rearrangement was unequivocally established by $^{15}$N-CIDNP NMR. A radical trapping experiment disclosed that 65-70% of the nitramine rearrangement takes place intermolecularly.