GHENT UNIVERSITY

FACULTY OF PHARMACEUTICAL SCIENCES

Department of Pharmaceutics

Laboratory for Medicinal Chemistry

Master thesis performed at:

UNIVERSITA DEGLI STUDI DI CAMERINO

SCHOOL OF PHARMACY

Medicinal Chemistry Unit

Academic year 2013-2014

Synthesis of Ligands for GPR17

Gilles DEGEZELLE

First Master of Drug Development

Promoter

Prof. Dr. apr. S. Van Calenbergh

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ABSTRACT

GPR17 can be called an interesting receptor, not only for its promising role as a target to treat demyelinating diseases, obesity, or to suppress pulmonary inflammation, but as well for its fiercely debated ligand profile. Although deorphanization has been claimed in 2006, still GPR17 can be seen as an orphan receptor because of conflicting results obtained by different research groups.

In 2006, uracil nucleotides and cysteinyl leukotrienes were identified as natural ligands of the GPR17 receptor. Since GPR17 is phylogenetically located right between the P2Y (nucleotides as natural ligand) and cysteinyl leukotriene receptor, this dual receptor was considered plausible. Later, the role of the cysteinyl leukotrienes was doubted and in other reports even both ligands were questioned. Until this day, no reasonable explanation was found for the incoherent results, but the planned collaboration between the different research groups should provide clarity.

Meanwhile, no restraint in research to possible GPR17 ligands can be permitted because of GPR17's promising pathological role, despite the uncertainty about the ligand profile. The most recent studies show that antagonists in particular would be of greatest importance. Among the few GPR17 antagonists, the bisphosphate nucleotides, MRS2179 and PF4, show high affinity potential. In order to study the importance of the sugar moiety of these two nucleotides in the binding with the receptor, new phosphorylated adenine derivatives bearing an alkyl chain in 9-position which can mimic the 2'-deoxyribose of the GPR17 antagonists MRS2179 and PF 4, have been designed.

The aim of this thesis was to find an appropriate synthetic approach for these compounds. A successful pathway has been created, but further investigation on the phosphorylation reactions is needed, especially on the purification. Nevertheless, a good and efficient synthesis of the intermediary just before the phosphorylation reactions was obtained and allows this further mandatory research.

SAMENVATTING

GPR17 kan op zijn minst een interessante receptor genoemd worden. Niet alleen vanwege zijn veelbelovende rol in de behandeling van demyeliniserende aandoeningen, obesitas of in de onderdrukking van pulmonaire inflammatie, maar ook om zijn fel bediscussieerd ligand profiel. Hoewel in 2006 de ontdekking van de natuurlijke liganden van GPR17 geclaimd werd, kan GPR17 nog steeds als een weesreceptor gezien worden vanwege tegenstrijdige resultaten verkregen door verschillende onderzoeksgroepen.

In 2006 werden de uracil nucleotiden en cysteïnylleukotriënen geïdentificeerd als de natuurlijke liganden van de GPR17 receptor. Aangezien GPR17 fylogenetisch vlak tussen de P2Y- (met nucleotiden als de natuurlijke ligand) en cysteinylleukotrieenreceptor gelegen is, werd deze dubbele receptor aannemelijk geacht. Later werd de rol van de cysteïnylleukotriënen in twijfel getrokken en in andere verslagen werd zelfs de rol van beide liganden ondermijnd. Tot op de dag van vandaag, werd er geen redelijke verklaring gevonden voor de onsamenhangende resultaten, maar de geplande samenwerking tussen de verschillende onderzoeksgroepen moet duidelijkheid scheppen.

Ondertussen kan er zich geen terughoudendheid in het onderzoek naar mogelijke GPR17 liganden worden gepermitteerd vanwege de veelbelovende pathologische rol van GPR17, dit ondanks de onzekerheid over het ligand profiel. De meest recente studies tonen aan dat vooral de antagonisten van groot belang zijn. Tussen het beperkt aantal gekende GPR17 antagonisten vertonen de bisfosfaat nucleotiden, MRS2179 en PF4, een hoge affiniteit. Om de invloed van de suikergroep van deze twee nucleotiden op de affiniteit voor de receptor te bestuderen werden nieuwe gefosforyleerde adeninederivaten met een alkylketen op de 9-positie ontworpen die de 2'-desoxyribose van MRS2179 en PF 4 kunnen nabootsen.

Het doel van deze thesis was om een geschikte synthetische benadering voor deze verbindingen te vinden. Een succesvolle route is gevonden, maar verder onderzoek naar de fosforyleringsreacties zijn nog nodig, vooral op naar de opzuivering ervan. Desalniettemin is een goede en efficiënte synthese verkregen van het intermediair vlak voor de fosforylatiereacties, wat het verdere nodige onderzoek mogelijk maakt.

ACKNOWLEDGMENT

Although I'm not a hero in expressing my gratitude, I'm taking the challenge. Especially since there are some people who certainly deserve this honour.

First of all, I would like to thank Prof. Dr. apr. Serge van Calenbergh, for making this wonderful Erasmus possible and for his pioneering role in the Erasmus program at our faculty.

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Next, my gratitude goes out to dr. Catia Lambertucci for the follow-up on my research and finishing my thesis. She also made me realize that I am "very lucky to work with phosphates" and … I wouldn't dare do deny that.

Definitely not to forget is my admiration for Ajiroghene Thomas, who has been my teacher and supervisor, but just as much a friend. His everlasting patience with me surely spared him some of his life years. I wish him a lot of success in his future career and hope to see him back in Belgium soon.

For the fatiguing yet entertaining moments in the lab I have to thank my lab partners. Angela and Alice, to whom I wish much prosperity in their future work and life. I enjoyed the pleasant music of Andrea and wish him every success in his PhD. My horribly bad sense of humour I could share with Justine, my fellow Erasmus student, with whom I have made fantastic trips through Italy.

Finally, I address a word of thanks to my family and girlfriend who supported me in this Erasmus period and also made the whole adventure possible.

TABLE OF CONTENTS

LIST OF USED ABBREVIATIONS

δ	Chemical shift
μ	Magnetic moment
AgRP	Agouti-related peptide
ATP	Adenosine triphosphate
BH_2OCH_3	Methoxyborane
BH_3	Borane
cAMP	Cyclic adenosine monophosphate
CysLT	Cysteinyl leukotriene
DCM	Dichloromethane
DMF	*N*,*N*-Dimethylformamide
DMSO-d_6	Deuterated dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EC_{50}	Half maximal effective concentration
EL2	2^{nd} extracellular loop
ESI	Electron spray ionization
GPCR	G protein-coupled receptor
H_3PO_3	Phosphorous acid
HCl	Hydrochloric acid
hGPR17-L	Long human GPR17-isoform
hGPR17-S	Short human GPR17-isoform
HPLC	High-performance liquid chromatography
Hz	Hertz
I	Spin quantum number
J	Coupling constant
K_2CO_3	Potassium carbonate
MS	Mass spectroscopy
m/z	Mass-to-charge ratio
N_2	Nitrogen gas
Na_2SO_4	Sodium sulfate
$NaBH_4$	Sodium borohydride

$NaHCO_3$	Sodium bicarbonate
NH_4HCO_3	Ammonium bicarbonate
NMR	Nuclear magnetic resonance
NOE	Nuclear Overhauser effect
OLG	Oligodendrocyte
OPC	Oligodendrocyte progenitor cell
PBr_3	Phosphorous tribromide
$POCl_3$	Phosphoryl chloride
ppm	Parts per million
RT	Room temperature
^{35}SGTPγS	Guanosine 5'-O-[gamma-thio]triphosphate
SIM	Single ion monitoring
TEA	Triethylamine
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TM3	3rd transmembrane helix
TM6	6th transmembrane helix
TM7	7th transmembrane helix
TMS	Tetramethylsilane
TMSBr	Bromotrimethylsilane
TPPO	Triphenylphosphine oxide
UDP	Uridine diphosphate
UV	Ultraviolet

1. INTRODUCTION

1.1. DISCOVERY OF GPR17

G protein-coupled receptors (GPCRs) represent, with approximately 800 members, the largest class of receptors found in the human genome (1). The theory of their common transmembrane 7-helix structure was confirmed with the crystallisation of rhodopsin. This structure implies an alternation of hydrophilic and lipophilic amino acid sequences with some typical anchor points that have been preserved in all GPCRs. The completion of the human genome made it possible to search for these patterns in DNA, which evoked the discovery of many new GPCRs (2). For those so-called orphan receptors, no ligands or any biological function was known. Given the variety of possible therapeutic effects, deorphanizing these receptors became a whole new interesting research field.

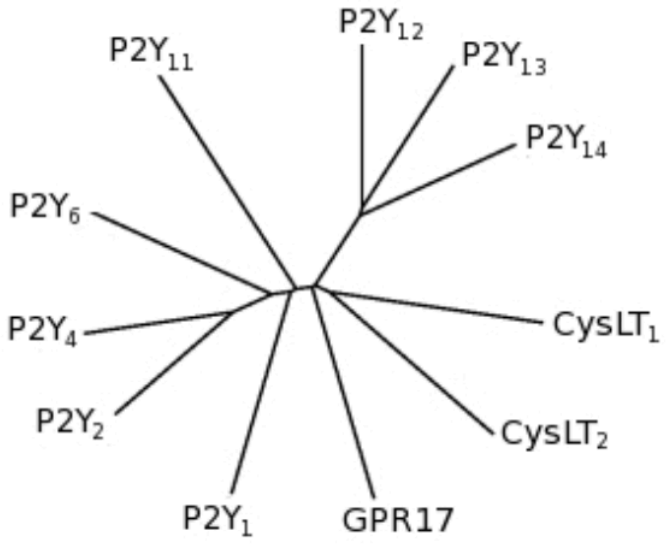


Figure 1.1: Phylogenetic tree showing the relationship between GPR17, P2Y, and CysLT receptors (3)**.**

In this particular way GPR17 (previously R12) was discovered in 1998 (4) and a deorphanizing report was published in 2006 (5). Phylogenetically, GPR17 can be positioned right between the P2Y- and cysteinyl leukotriene (CysLT) receptor (Figure 1.1). In order to fully understand GPR17, a closer look can be taken at those two receptors ligands and their biological function.

1.2. THE P2Y-RECEPTOR

The P2Y-receptor family is a subdivision of the purinergic receptors that are G-protein coupled and are activated by nucleotides. They mediate immensely diverse biological functions and are widely spread in almost all human tissues. Eight human subtypes are identified and cloned, $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$. The missing numbers represent non-mammalian subtypes, or receptors for which there is no sufficient evidence of responsiveness to nucleotides (6).

Roughly four pharmacological groups can be distinguished among the human P2Y receptors (6):

1) receptors that preferably respond to adenine nucleotides such as $P2Y_{1,11,12,13}$;

2) uracyl nucleotides-preferring receptors $P2Y_4$ and $P2Y_6$;

3) the $P2Y_2$ receptors that show a mixed selectivity;

4) the $P2Y_{14}$ receptors that respond exclusively to the sugar nucleotides like UDP-glucose or UDP-galactose.

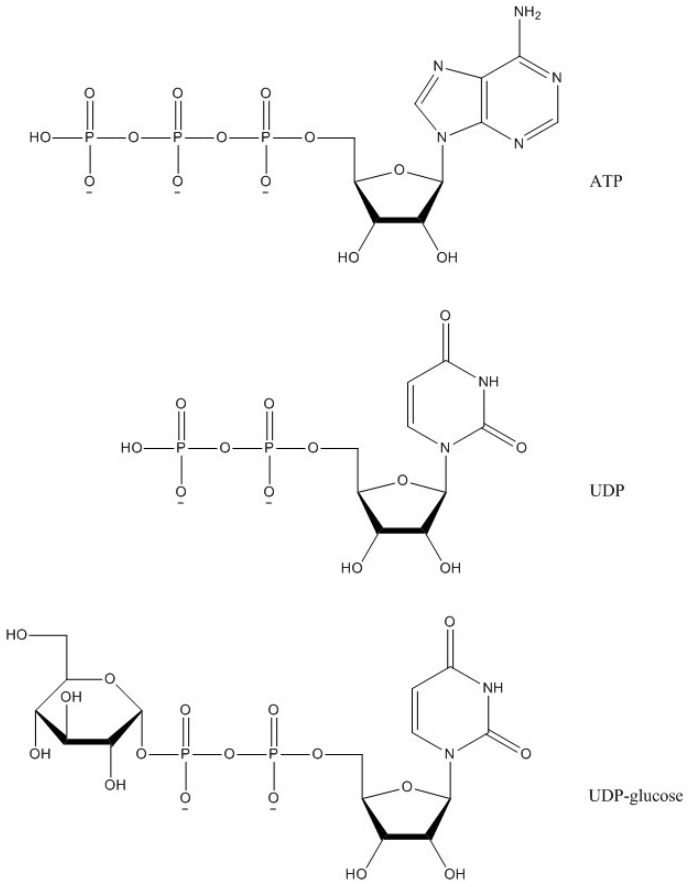


Figure 1.2: Structure of ATP, UDP, and UDP-glucose.

Phylogenetically and structurally, the P2Y receptors can be divided into 2 subgroups (Figure 1.1). The first subgroup includes $P2Y_{1,2,4,6,11}$ and the second subgroup with the highest sequence identity includes $P2Y_{12,13,14}$. All human P2Y receptors hold a typical H-X-X-R/K motif in TM6, which is believed to be important for agonist activity. The first subgroup also shares a TM7 Q/K-X-X-R motif for agonist affinity whereas this is replaced in the second subgroup with K-E-X-X-L. Site-directed mutagenesis of the $P2Y_1$ and $P2Y_2$ receptors shows that these positively charged residues (H, R, and K) are crucial to interact with the negative phosphate groups of nucleotides. The two subgroups are also distinguished in their G-protein coupling. All the receptors of the first group mediate their effects trough $G_{q/11}$, which stimulates phospholipase C, whilst the other group uses $G_{i/o}$, inhibiting cAMP production (6,7).

1.3. THE CYSTEINYL LEUKOTRIENE RECEPTOR (CYSLTR)

The cysteinyl leukotrienes (LTC4, LTD4, LTE4) (Figure 1.3) are large lipophilic mediators derived from arachidonic acid that act in various immune processes. Therefore their receptors, $CysLT_1R$ and $CysLT_2R$, are good contenders as a target to cure or treat immune related diseases. Their role is best known in asthma and allergic rhinitis but they also have functions in cancer and cardiovascular, gastrointestinal, skin, and immune disorders (8).

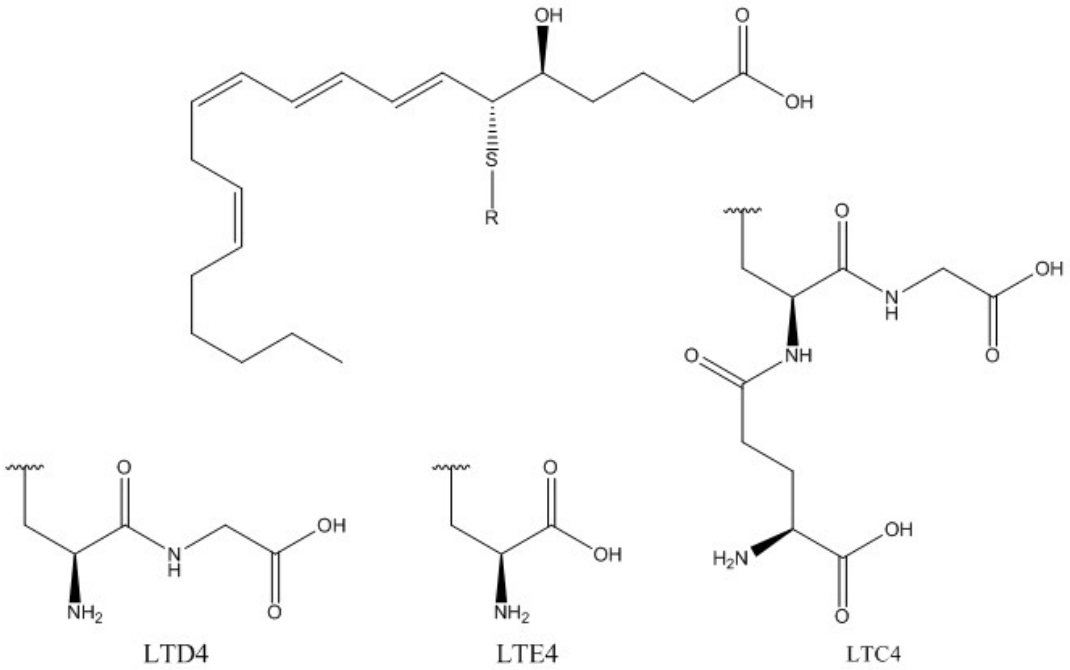


Figure 1.3: the cysteinyl leukotrienes LTC4, LTD4, and LTE4.

The two receptors share a 38% amino acid homology and are categorized as rhodopsin-like GPCRs who belong to the purine receptor cluster (2). This is because they surprisingly have a higher commonality with the P2Y-receptors than with the other leukotriene receptors, despite their great difference in ligands (9). The presumption of two different cysteinyl leukotriene receptors was originally made by whether they are sensitive to the "classical" antagonists such as zafirlukast and montelukast or not. Only the $CysLT_1$ receptor has an affinity to these antagonists (10).

1.4. BIOLOGICAL ROLES OF GPR17

1.4.1. Brain repair

Prior to the GPR17 deorphanizing report of Ciana et al. (2006), a functional crosstalk was discovered between the nucleotide and CysLT systems in brain inflammation and neurological diseases. Microglia are seen as the pathological sensors in the brain and they co-release both mediators in these circumstances (11). This theory received much support with the identification of GPR17 as a new dual receptor with two distinct binding sites: one sensitive to uracil nucleotides and the other to cysLTs. All the more because GPR17 receptors are highly expressed in brain, heart, and kidney, typically organs that undergo damage due to ischemia. Also an increased neuronal GPR17 expression was observed after ischemia and the knockdown of GPR17 in an ischemic rat model prevented evolution of ischemic brain damage suggesting a possible anti-neurodegenerative role for GPR17-antagonists (5,12).

Conflicting conclusions were made when GPR17 was identified as one of the three genes that are exclusively expressed in adult hippocampal neural progenitor cells (namely the low-affinity nerve growth factor receptor, GPR17, and C-C chemokine receptor type 7). These are multipotent stem cells that can differentiate into neurons, oligodendrocytes (OLG) and astrocytes, all three cell types of the central nervous system. They are considered as the most likely targets to heal neurodegenerative disorders (13). In this respect, not antagonists but GPR17-agonists would be of great interest to stimulate those stem cells.

It is clear that the expression pattern of GPR17 during ischemia evolution must be a changing given and may play a role in both neuronal death and brain repair. Additional

research with an ischemic mouse model showed the following chronology. Within 24 h after middle cerebral artery occlusion (to create an ischemic model), GPR17 was strongly upregulated inside the injured area compared to unlesioned regions. This upregulation is associated with the cell death of those damaged neurons resulting in declining expression of GPR17. As from 48 h to 72 h, a second wave of GPR17 induction is noticeable. Microglia/macrophages at the border of the injured area are recruited towards the ischemic damage and express GPR17. At the same time, oligodendrocyte progenitor cells (OPCs) (which are GPR17 positive) around the lesion start to proliferate to mature cells, initiating remyelination (14). Confirmation of the same function for GPR17 in the human brain was obtained from neurosurgical traumatic brain injury samples where GPR17 antibodies labelled dying neurons, reactive astrocytes, and activated microglia/macrophages that were infiltrating the lesion (15).

Focussing on the remyelination, the maturation of OPCs is of utmost importance. GPR17 expression on OPCs peaks in immature pre-oligodendrocytes whereas it is absent in mature OLGs (16). Stimulation of GPR17 on OPCs causes differentiation break in the immature nonmyelinating stage making GPR17 function as a brake in negatively regulating OLG maturation (17). Hence, it can be concluded that the inhibition of GPR17 may have a high potential in demyelinating diseases such as multiple sclerosis.

1.4.2. Food intake regulation

Recently, GPR17 was found to play a central role in the regulation of appetite in mice (18). Hypothalamic neurons expressing Agouti-related peptide (AgRP) are mainly responsible for food intake. Stimulation of these neurons in mice resulted within minutes in voracious food intake (19). Conversely, ablation of AgRP neurons in adult mice resulted in starvation (20). The hormones insulin and leptin act here as appetite suppressants by inhibiting the AgRP neurons, but surprisingly the ablation of the insulin or leptin receptor in AgRP neurons had only minor effects (18). In the search for a target to inhibit the AgRP neuron activity a protein called FoxO1 was encountered that integrates both leptin and insulin signalling. Mice lacking this transcription factor in the AgRP neurons are lean, have less appetite, showed improved glucose homeostasis, and increased insulin and leptin sensitivity. It was remarked that in these knockdown mice, GPR17 expression had significantly decreased.

Intracerebroventricular injections of the GPR17 antagonist Cangrelor were able to induce the same food intake diminution making GPR17 a high potential target for treating eating disorders such as obesity (18).

1.4.3. Pulmonary inflammation

As suggested by Maekawa et al., GPR17 is a negative regulator of the $CysLT_1$ receptor (see below), suppressing $CysLT_1R$-mediated responses in, for example, pulmonary inflammation (21). In an experiment with GPR17 knockdown mice, a broad role for $CysLT_1R$ in antigen presentation and downstream phases of allergic pulmonary inflammation was revealed. These $CysLT_1R$ mediated responses are thoroughly downregulated by GPR17 expression (22).

1.5. GPR17 CHARACTERISTICS

1.5.1. GPR17 isoforms and their expression pattern

Two splice variants are known for the receptor differing only in the length of the N-terminus. This causes different ligand-binding profiles, as it is an extracellular part of the receptor. The long human isoform, here referred to as hGPR17-L, is 28 amino acids longer than the short variant, hPGR17-S (Figure 1.4).

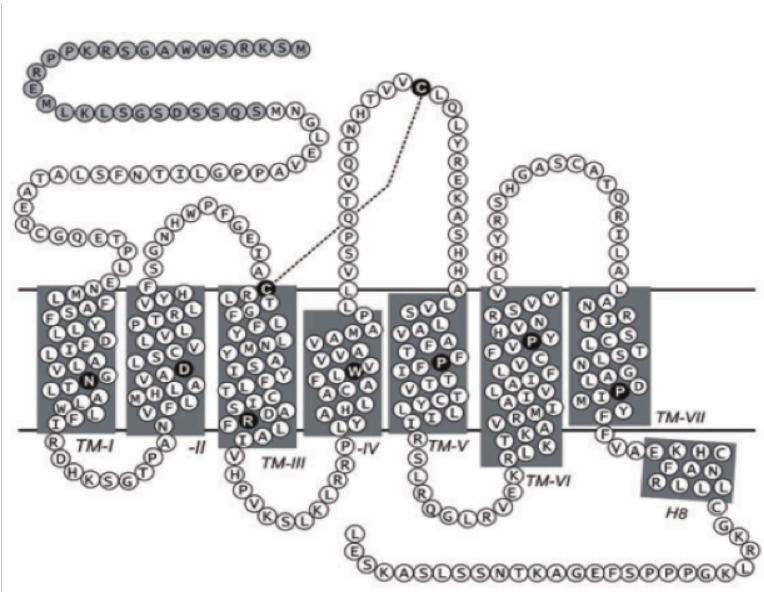


Figure 1.4: Serpentine model of hGPR17. The grey residues represent the 28 additional amino acids of the long splice variant compared to the small isoform. The highly conserved residues among rhodopsin-like 7TM receptors are indicated as black circles with the disulphide-bridge between EL2 and TM3 as a stapled line (23).

As mentioned before, GPR17 is expressed in kidney and heart but especially in the brain. Isoform-specific determination of the expression pattern showed overall higher expression of hGR17-S in the brain which represents a 10-fold of hGPR17-L gene expression. Completely contrary to this, hGPR17-L is the dominant form in the heart and even the only variant in the kidneys suggesting tissue-specific roles in the respective organs (23).

1.5.2. Ligands of GPR17

Since the GPR17 deorphanization report in 2006, much conflicting results are reported regarding ligand specificity in which the duality of the receptor is strongly disputed. Originally GPR17 was identified by Ciana et al. (2006) to be responsive to both uracil nucleotides (with UDP-galactose = UDP > UDP-glucose) and cysLTs (with LTC_4 >> LTD_4), corresponding to the normal affinities for respectively P2Y and CysLT receptors (5). These results were obtained through ^{35}SGTPγS binding assays, cAMP inhibition assays and single-cell calcium imaging from 1321N1, COS7, CHO, and HEK293 cell expressing both human and rat GPR17.

Newly synthesised agonists were reported by Buccioni et al. (2011) with PF9 (Figure 1.5) as the most active compound (about 30 times higher affinity than UDP-galactose) (24). PF9 is now commercially available from Tocris Bioscience.

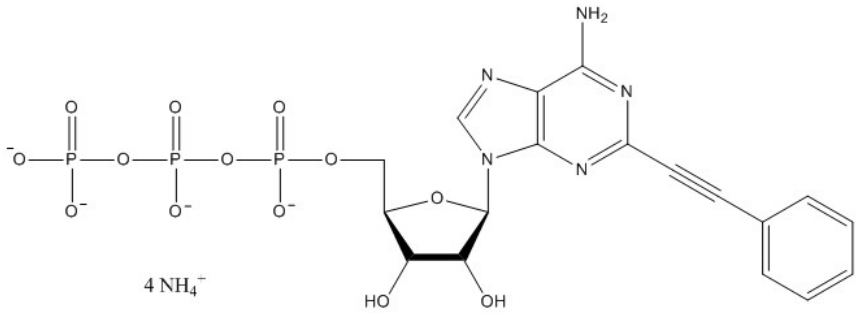


Figure 1.5: Nucleotide-pocket agonist PF9.

As well, antagonists were found to inhibit the function of GPR17: Cangrelor, a $P2Y_{12}$/$P2Y_{13}$ antagonist, and the $P2Y_1$ antagonist MRS2179 could inhibit the effects of nucleotides. CysLTs were antagonized by Montelukast and Pranlukast, both $CysLT_1$ antagonists (Figure 1.6) (5).

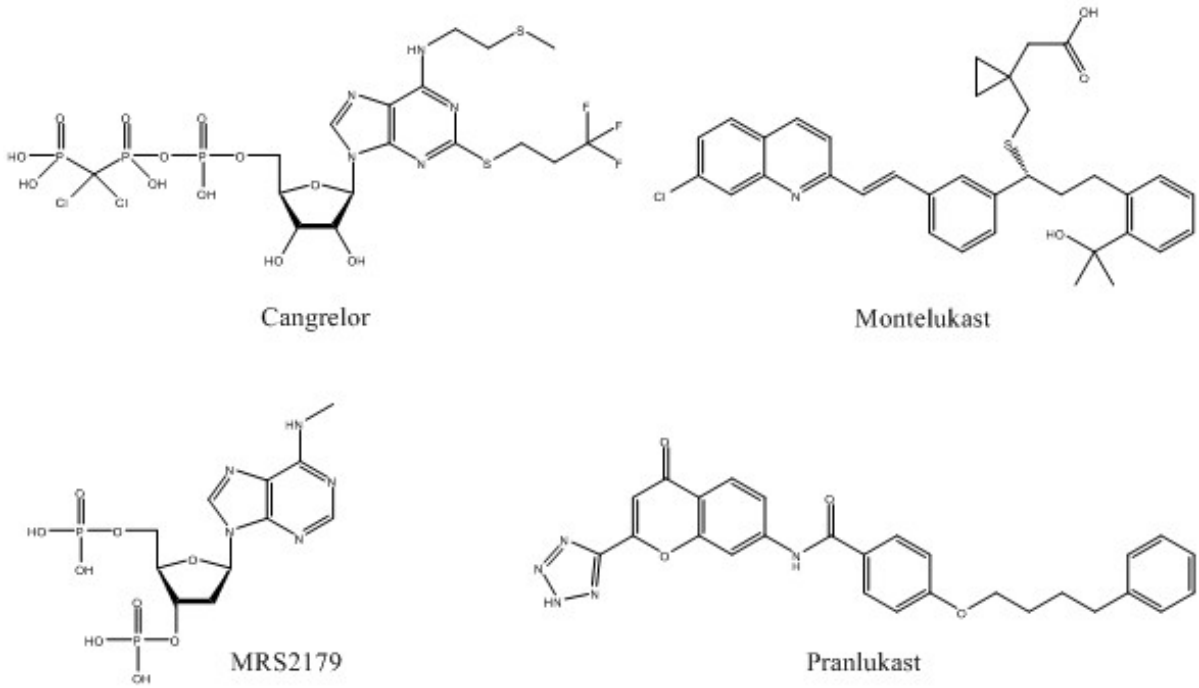


Figure 1.6: GPR17 antagonists Cangrelor and MRS2179 (nucleotide-pocket) and Montelukast and Pranlukast (cysLT-pocket).

Because of the interestingly results and possible biological functions, research was continued by this group based on this report (3,16,24–28). From this, more information was obtained about the functional cross-talk between the two allosteric binding places in which the purinergic ligand is able to mediate a heterologous regulation of the cysLT binding site. Because the cysLTs are not able to perform this effect on the purinergic binding site, the nucleotides exhibit a kind of dominance over the cysLTs. Homologous desentization/resensitization occurred with the typical kinetics of GPCRs (27).

Research on GPR17 by different groups, however, led to contradictory conclusions. Benned-Jensen and Rosenkilde contested the cysLT affinity by GPR17 and reported only receptor activation by UDP and the UDP-sugars (23). Even more noticeable, Maekawa et al. concluded that GPR17 was not activated by any of these presumed agonists but suggested a regulatory role for GPR17, inhibiting the response of CysLTR1 on its mediators (21). A more detailed research was performed by Qi Ai-Dong et al. expressing GPR17 on more possible cell lines and coupling the receptor with all known G-proteins. However, none of the suggested ligands showed affinity for GPR17 but instead confirming the theory by Maekawa et al. of GPR17 as a CysLTR1 regulator. As well, no possible explanations were found for the discrepancies between the research groups (29).

There is an urgent need for clarity about GPR17s pharmacological profile. It may be possible that GPR17 can act, depending on specific pathophysiological conditions, through a ligand-dependent or a ligand-independent pathway (27). In this regard, an exchange of assays and cooperation between the different research groups can possibly answer many questions. Finally, a possible incorrect identification was suggested which involves the heterooligomerization of an orphan receptor with endogenous P2Y receptors that are normally not expressed, but are recruited because of the orphan receptor expression (29).

Very recently, a completely new kind of GPR17 agonist was discovered by Hennen et al. (2013), namely MDL29,951 (Figure 1.7). Even in a complex environment of endogenous purinergic receptors, the agonist very selective for the GPR17 receptor. Also remarkable was that MDL29,951-activated GPR17 could mediate signalling trough various second messenger pathways to initiate its biological function: G proteins of the $G\alpha_i$, $G\alpha_s$, and $G\alpha_q$ subfamily, as well as β-arrestins. Furthermore also GV150526A was identified as a GPR17 agonist (Figure 1.7) (17).

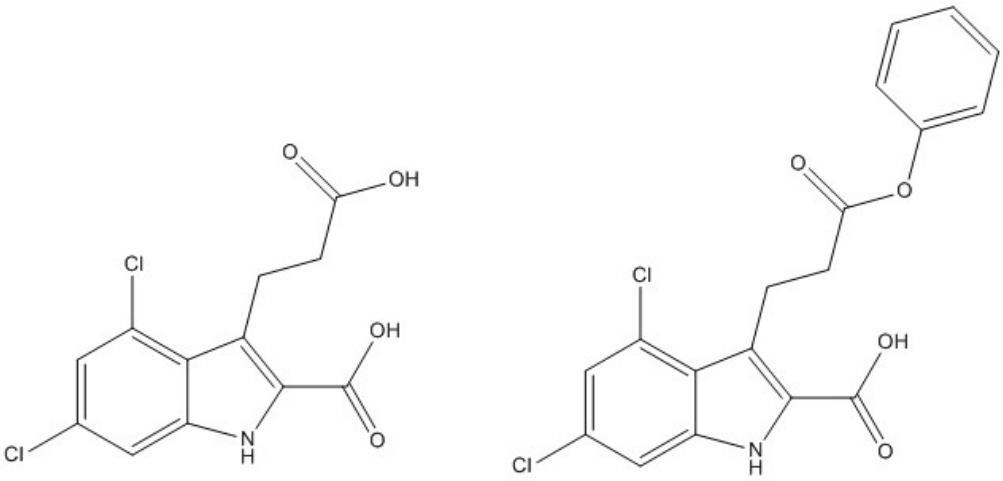


Figure 1.7: Chemical structures of GPR17 agonists MDL29,951 and GV150526A.

2. OBJECTIVES

As described in the introduction chapter, recent studies suggest that in pathological conditions, such as multiple sclerosis, GPR17 activation acts as a brake on remyelination. These observations lead to expectations that GPR17 antagonists could raise that brake. Also, in cerebral ischemia, antagonizing GPR17 could block lesion growth.

Among the few GPR17 antagonists known, there are some adenine bisphosphate nucleotides, such as MRS2179 and PF4, which show an EC_{50} of 508 and 582 nM, respectively. These two compounds share the common feature to be 2'-deoxyribose derivatives. Hence, with the aim to study the importance of the sugar moiety of these two nucleotides in the binding with the receptor, new phosphorylated adenine derivatives bearing an alkyl chain in 9-position which can mimic the 2'-deoxyribose of the GPR17 antagonists MRS2179 and PF 4, have been designed and must be synthesized (Figure 3.1).

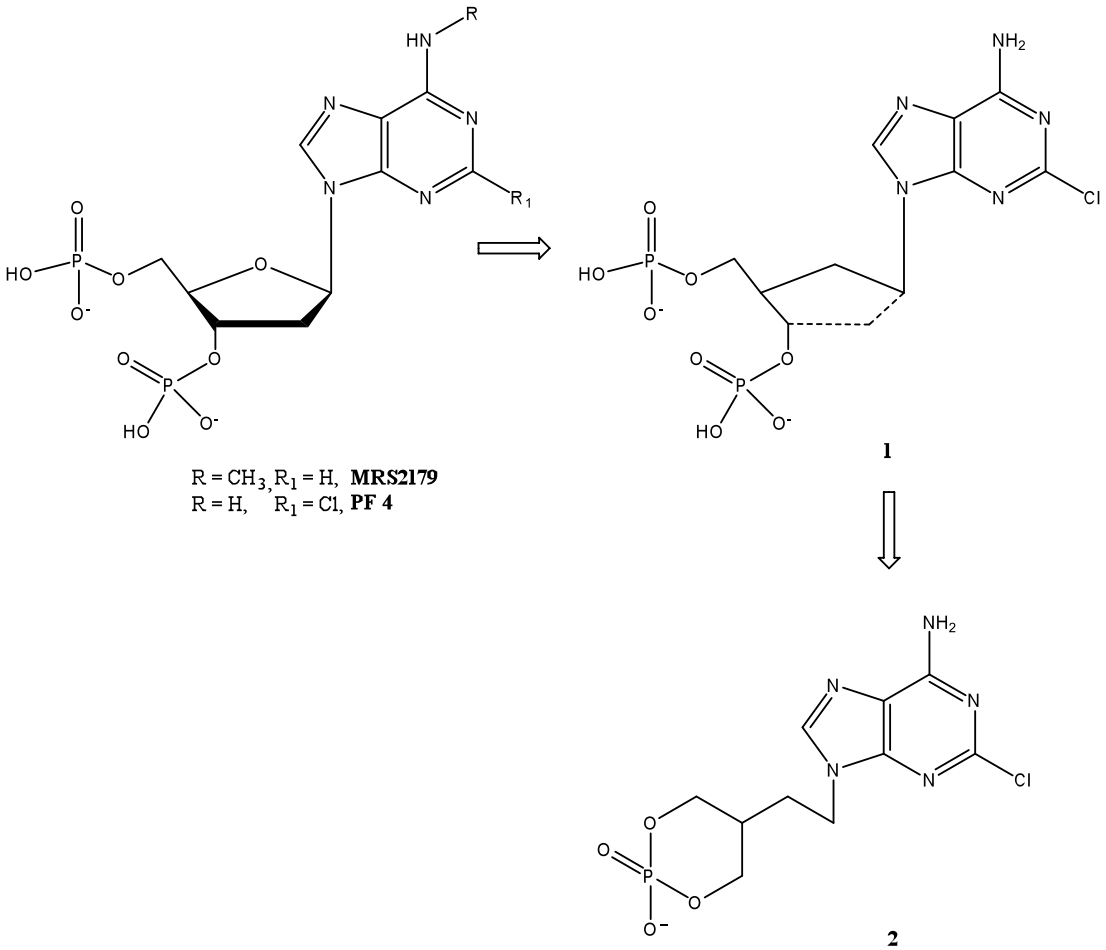


Figure 3.1: Rational design of new GPR17 ligands.

3. MATERIALS AND METHODS

3.1. MATERIALS

TLC Silica gel 60 $_{F254}$ glass plates (5x10 cm) from Merck (Darmstadt, Germany) as well as TLC Silica gel 60 $_{F254}$ aluminium sheets (20x20 cm) from the same company were used for Thin Layer Chromatography (TLC). Preparative TLC was performed on Alugram® Sil G/UV$_{254}$ plates (20x20 cm) from Macharey-Nagel (Düren, Germany). To visualize the spots on TLC, UV-detection (254 nm), an iodine chamber or basic permanganate solution was used.

Silica gel column chromatography was always performed with silica purchased from Fluka Analytical (Steinheim, Germany): Silica gel, high purity grade, pore size 60Å, 230-400 mesh particle size, 40-63 µm particle size, for flash column chromatography.

Solvents and reagents used were always purchased from Sigma-Aldrich (Steinheim, Germany), Fluka Chemika (Steinheim, Germany) or Alfa Aesar (Heysham, United Kingdom).

3.2. METHODS

3.2.1. Nuclear Magnetic Resonance (NMR) (30,31)

NMR is an important spectroscopy technique routinely used by chemists to study or confirm a chemical structure. NMR is a phenomenon, which occurs when nuclei with a property called spin and in a static magnetic field are exposed to a second oscillating magnetic field.

Many atom nuclei (consisting of protons and neutrons) behave as if they are spinning on an axis and posses spin. Consequence is that they generate in this way a magnetic moment (μ). Nuclei have a spin when the number of protons, or the number of neutrons, or both, are odd. This because the spins from two protons compensate each other and the same applies to two neutrons, but the spins of a neutron and a proton can never cancel each other out. The most commonly used nuclei to perform NMR are ^{1}H, ^{13}C, ^{19}F, and ^{31}P, because their spin *I* is equal to ½ and the analysis of this spin is the most straightforward one. For the ^{1}H-nucleus, two spin states exist, - ½ and + ½, which creates an opposite pointing magnetic moment, but they do not differ in energy. However, when an external magnetic field is applied, energy is required to go from the spin state with an aligned magnetic moment (low

energy), to the spin state with its magnetic moment opposed to the external magnetic field (high energy).

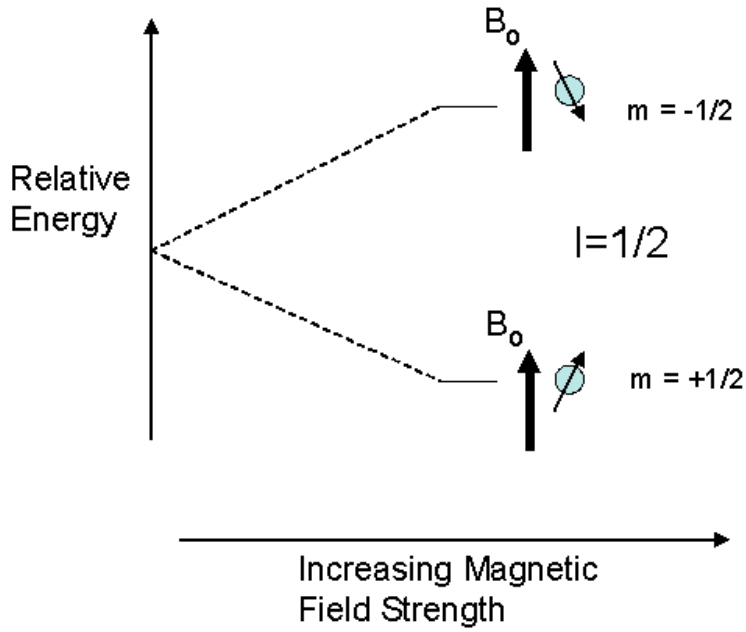


Figure 3.1: The two allowed spin states for a ^{1}H-nucleus (31)**.**

Magnetic resonance takes place when aligned nuclei change their spin orientation towards the applied field by absorbing energy. This energy transfer only occurs when the electric field that is generated by the spinning nuclei, and the electromagnetic irradiation that is applied to create resonance, have the same frequency.

However, electrons can influence the resonance of a proton because they act as a shield protecting the proton from the external magnetic field. Because of the proportional relationship between the influence of the external magnetic field and the frequency of the electric field of the proton, the resonance of the proton is dependent of the electron density surrounding the proton. The more a proton is surrounded by electrons, the weaker is the prevalence of the external magnetic field and the lower is the required frequency to resonance the proton.

Resonance frequencies are reported as how far they are shifted from a reference compound. The twelve protons of tetramethylsilane (TMS) are highly shielded and give a sufficient strong signal to make TMS the ideal reference. In order to neutralize the influence of various magnets, the chemical shift (δ) is defined as the shift in Hz divided by the frequency of the external magnetic field in MHz, giving values in the ppm-range.

A proton can be affected by the spin states of the protons on its neighbour carbon atom. This leads to spin-spin splitting in which the signal splits into *n*+1 peaks with *n* as the number of protons bound to the adjacent carbon atom. The intensity ratios of such a multiplet follow the numbers of Pascal's Triangle. For example: the ratios of the signals of a quartet will be 1:3:3:1. The distance between those peaks is called the coupling constant (*J*). It is a measurement of how strong these interactions between neighbour protons is and gives information about bond distance and angles.

The NMR spectrum is represented with the resonance frequency on one axis, and on the other axis, the intensity of the peak at that frequency. The ^{1}H-NMR spectrum can decipher a great wealth of information about the structure of a molecule. First, the number of resonance peaks tells how many different proton groups are present in a molecule. Secondly the position of those peaks in the resonance spectrum provides information about the electron density around the proton group. Furthermore is the area under the peak important for the number of protons in its group. And finally, the splitting pattern tells us how many neighbour protons are present around the group of protons.

3.3.2. Mass Spectroscopy (MS) (32,33)

A mass analysis is a possible method to analyze and identify compounds in really small amounts. In this thesis MS analysis is performed on a quadrupole ESI-mass apparatus (HP 1100 MSD). Generally, methanol is used to solubilize the product, with the exception of the phosphates that were dissolved in water. After the sample is introduced it undergoes first ionization (here with an electron spray ionizator (ESI)), which creates fragment ions as well as molecular ions. After ionization, the ions are separated in compliance with their m/z value or mass to charge ratio by a mass analyzer (here with a quadrupole).

The ESI technique involves the spraying of the solution of the sample through a highly charged needle capillary (Figure 3.2). Charged droplets are produced in which the ions are solvated. By nebulizing with an inert gas such as nitrogen, the droplet size is decreased. Columbic repulsion between the charges in the decreasing droplet increases until the Rayleigh limit is reached. Subsequently, Coulombic explosions create individual gas phase analyte ions that are guided trough ion optics into the mass analyzer.

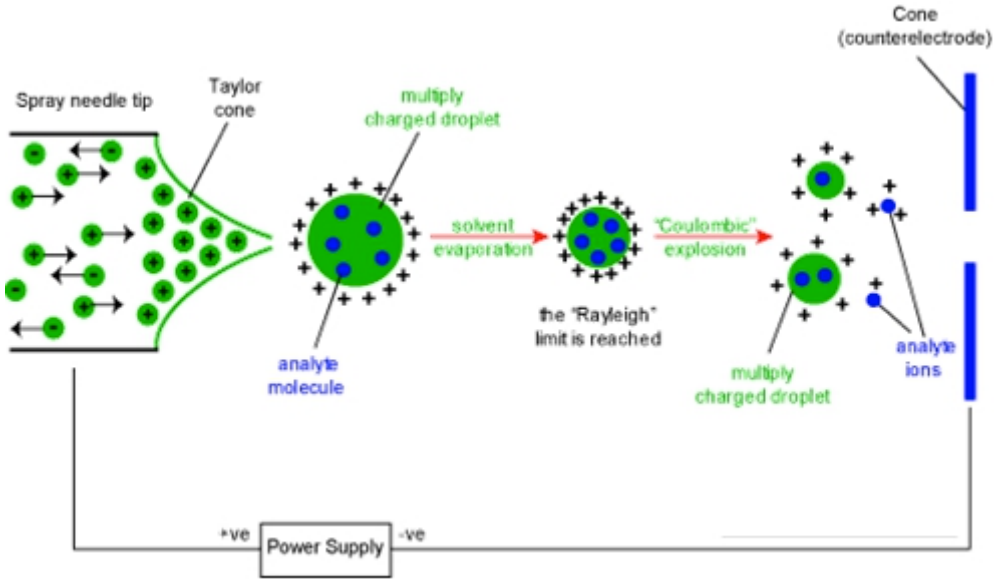


Figure 3.2: Schematic diagram of a typical ESI MS source (33).

The analyzer, the quadrupole, consists of four bars, two pairs of metallic rods (Figure 3.3). One pair has a positive electrical potential and the other pair has the negative potential. In this way, alternating the potentials of the bars can create an oscillating electric field. Only the ions with a particular m/z ration will resonate and have a stable and good trajectory to pass the quadrupole. The quadrupole can be used in two modes: SCAN or SIM (single ion monitoring). The SCAN mode is used for qualitative and quantitative analyses and rapidly iterates through all the possible m/z values multiple times to register all ions formed. In the SIM mode, the quadrupole only scans for one particular m/z value. In this way the SIM mode has a lower detection limit and is more sensitive, so it is used for quantitative analyses.

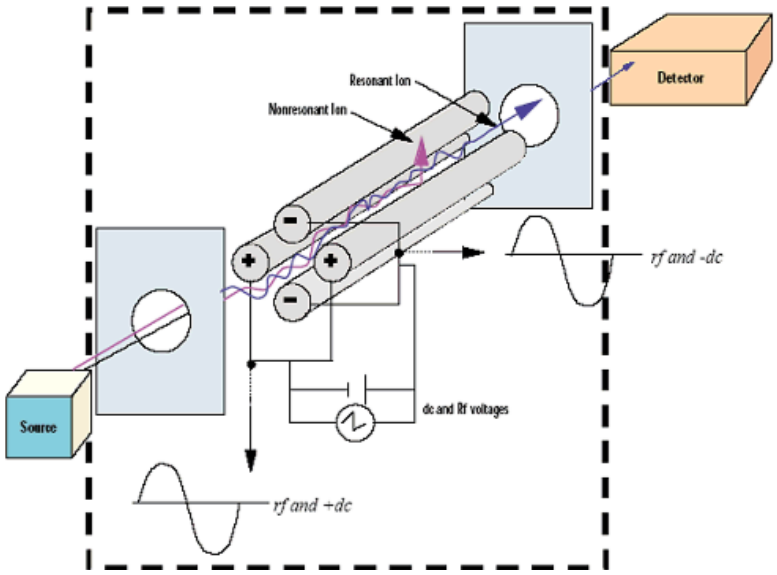


Fig 3.3. A quadrupole (32)

The last step in the mass spectrometry is the detection. An electron multiplier is used to convert negative and positive ions into electrons, which are then amplified in a cascade where each electron can induce emission of roughly 3 electrons. The result is a shower of electrons, all triggered by just one electron, that is collected by a metal anode (32).

4. CHEMISTRY

In order to obtain the desired compounds, **1** and **2** (see Figure 4.1), the strategy was to first synthesise the sugar-like chain, then attach the purine in the correct form and finally phosphorylate the compound (Figure 4.1). This consecution is also the only possible one for multiple reasons. An earlier attachment of phosphates at the sugar-like chain would result in a molecule incapable of further reaction because of its instability. Secondly, formation of the right chain directly on the purine wouldn't be convenient due to the high cost of the purine itself, and modification of the purine with the carbon chain already attached could give undesirable chain adjustments.

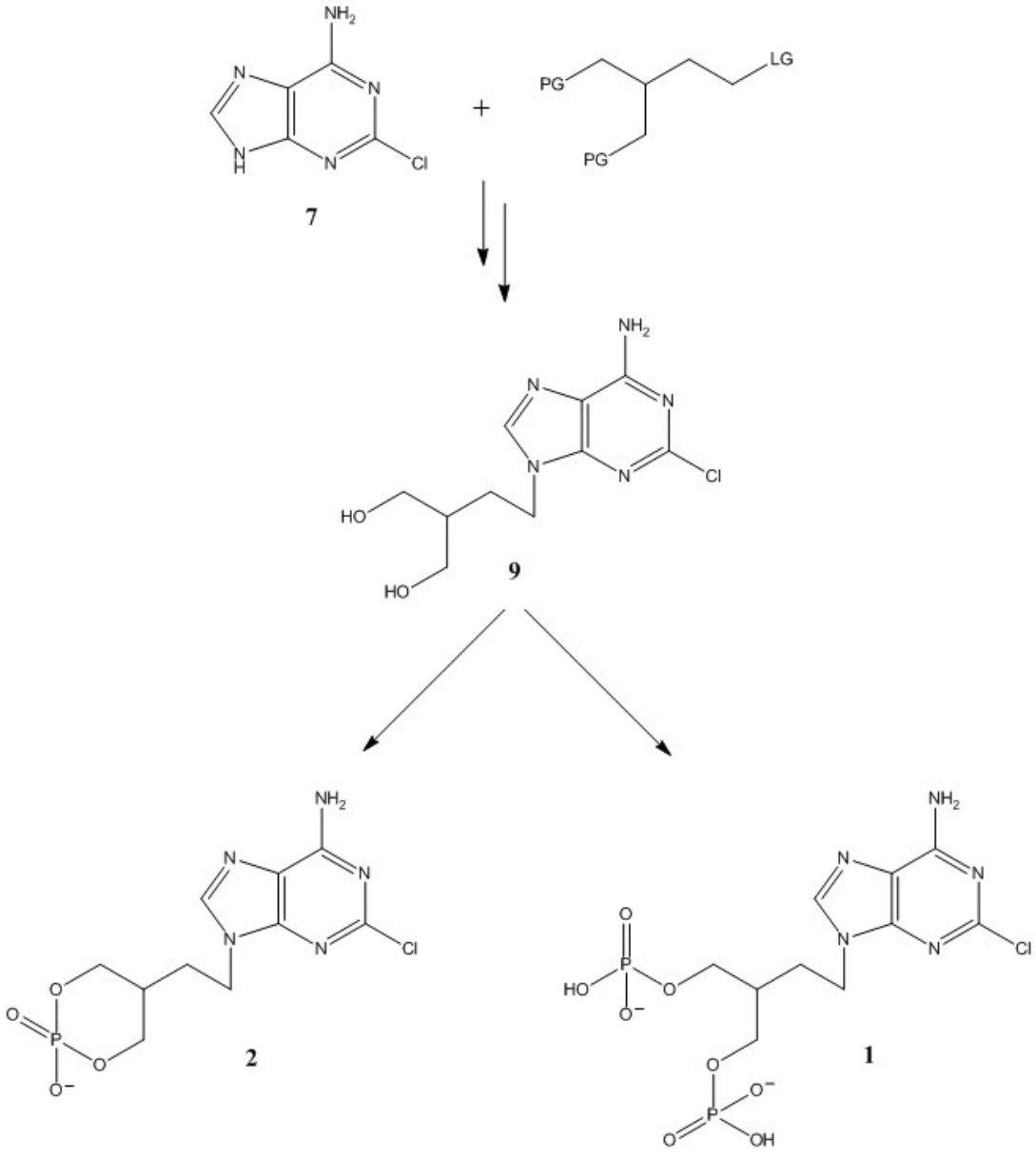


Figure 4.1: Synthetic approach.

4.1. SYNTHESIS OF THE SUGAR-LIKE CHAIN

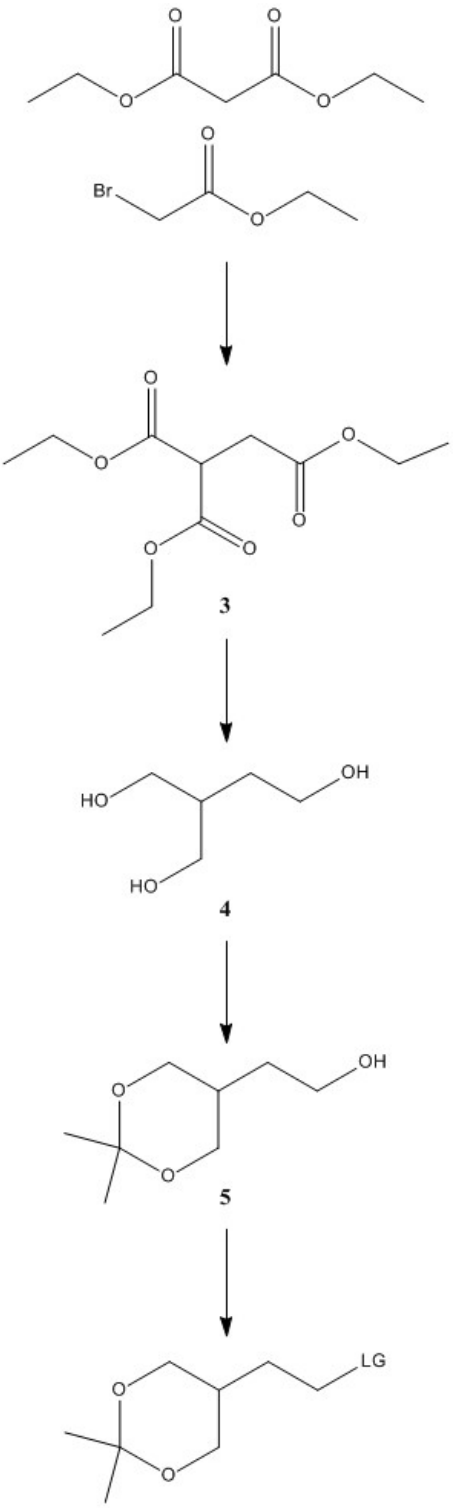


Figure 4.2: Synthetic approach of the sugar-like chain.

The chain that has to mimic the 2'-deoxyribose of nucleotides is compound **4**, which is obtained through reduction of the tricarboxylate **3**. This, latter compound has been prepared from diethylmalonate and ethyl bromoacetate (Figure 4.2).

In order to make a correct and efficient coupling with the suitable purine derivative, a good leaving group is needed on the chain, but to put it correctly, the other two hydroxyl groups must be protected first. With the aid of dimethoxypropane, two hydroxyl groups are incorporated in a ring structure like the dioxane ring (compound **5**), but a seven-membered ring can be formed as well, although in lower yield. Later on, this protection can be removed in simple acidic conditions. However, this implicates that until then, slightly basic conditions must be maintained.

Now only one hydroxyl group is left and can be modified in a good leaving group. At first, the substitution of the hydroxyl group to a bromine atom was chosen because of its stability and good leaving capability. After bromination trials failed, another group was tried. Mesyl is a very good leaving group but has instead a low stability so that after mesylation, the linking must happen quickly. A third possibility is tosylation, which displays higher stability and consequently lower leaving properties, but has the advantage of being visible under UV-light (convenient

in TLC). However, with a coupling yield of 37.86%, the mesylation reaction to give derivative **6** was successful enough to continue.

4.1.1 Synthesis of triethyl-1,1,2-ethanetricarboxylate (3)

The tricarboxylate **3** was formed by nucleophilic substitution of diethylmalonate by ethyl bromoacetate. First a proton could easily be subtracted from diethylmalonate with sodiumhydride to obtain a strong nucleophile. Then, the activated diethylmalonate could attack ethyl bromoacetate through a S_N2 reaction mechanism (Figure 4.3).

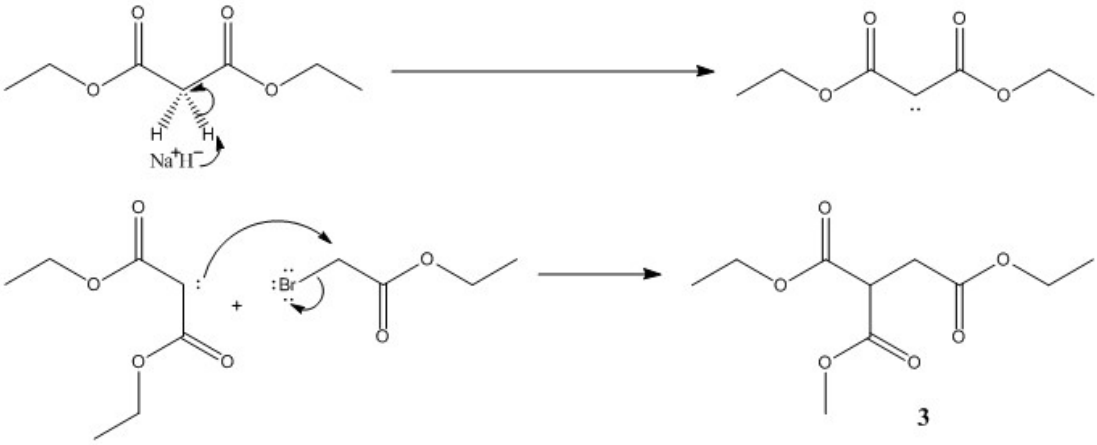


Figure 4.3: Reaction mechanism for the synthesis of compound 3.

4.1.2. Synthesis of 2-hydroxymethyl-1,4-butanediol (4)

A procedure with sodium borohydride ($NaBH_4$) was chosen for the reduction of the esters (Figure 4.4 and Figure 4.5). Normally $NaBH_4$ is not strong enough to reduce esters but in mixed solvents of tert-butyl alcohol and methanol a reduction was observed with a high yield (34).

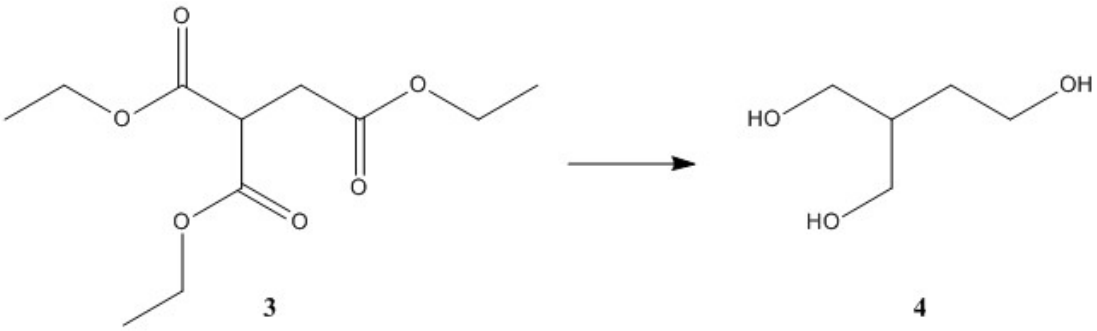


Figure 4.4: Reaction scheme for the synthesis of compound 4.

In a first step $NaBH_4$ is activated by methanol (Figure 4.5). This ester group can push more electrons towards boron, which facilitates the hydride ion forming and makes the molecule strong enough to reduce an ester. The reduction results in an aldehyde, which can be further reduced to the desired alcohol by $NaBH_4$ as well as by its methoxy variant. This reaction takes place three times because of the number of ester groups, but the amount of $NaBH_4$ was kept low since as well BH_3 or BH_2OCH_3 have still reductive capacities for respectively aldehydes and esters. Given that the yield, in comparison to the yield in the literature of 100%, was low (39.97%), a higher amount of $NaBH_4$ may offer a solution. Also, the slower addition of methanol could increase the efficiency.

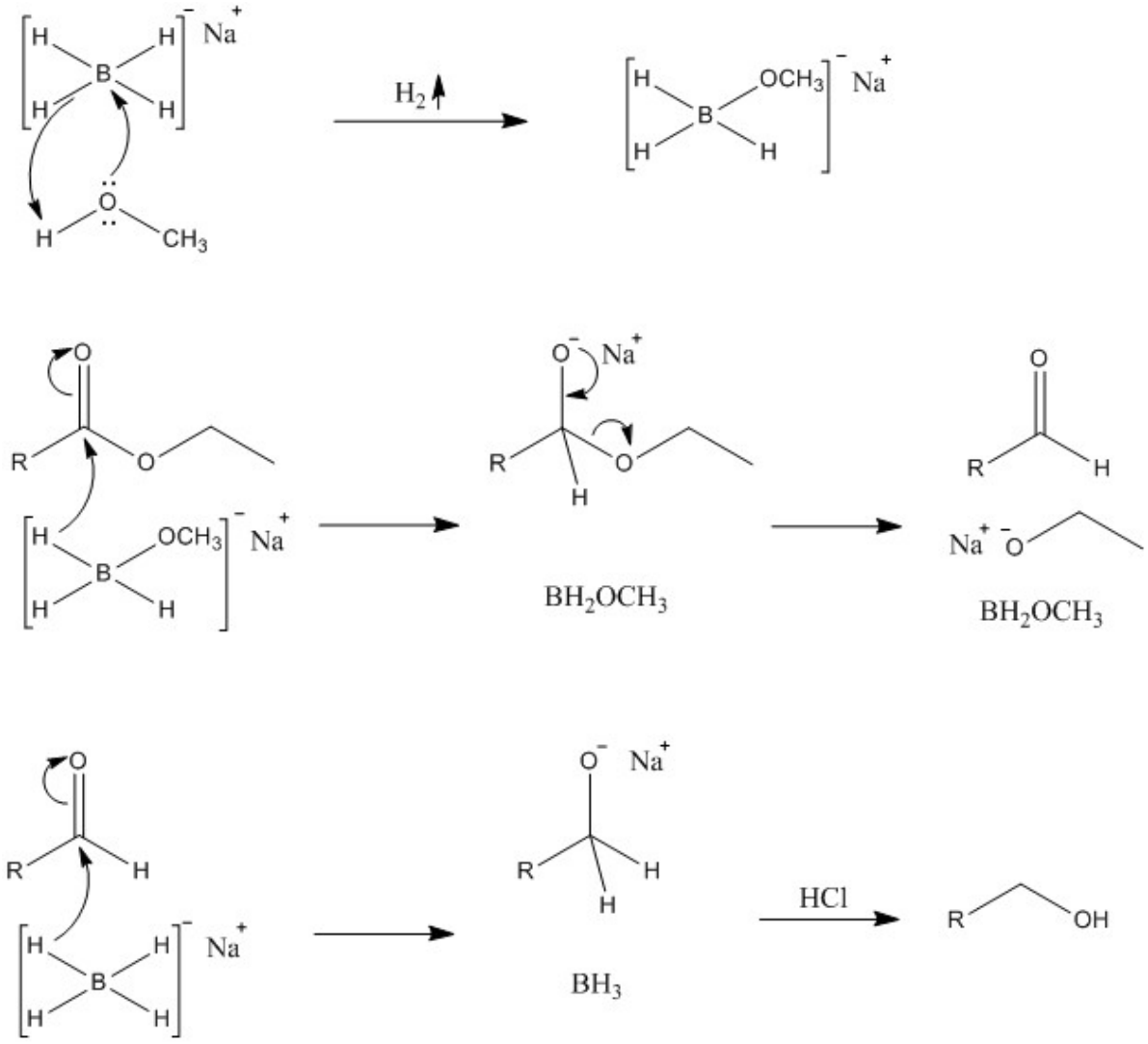


Figure 4.5: Reaction mechanism of the reduction, which takes place 3 times on compound 3 to form 4.

4.1.3. Synthesis of 2,2-dimethyl-5-(2'-hydroxyethyl)-1,3-dioxane (5)

For the protection of two alcohol groups, again, a protocol could be found (35). Based on the experience of the lab, some adjustments were made. Temperature was increased to 65°C instead of room temperature (RT). Also dimethylformamide (DMF) was used instead of tetrahydrofuran (THF) because THF boils at 66°C. Despite these changes very little product was seen on TLC. Therefore, 50% more reagent (dimethoxypropane) and twice as much catalyst (p-toluene sulfonic acid monohydrate) was used in a new test reaction. On TLC, an improvement was noticeable although not sufficient. A new strategy was the use of a Dean-Stark apparatus to trap the formed methanol and push the reaction in this way more towards the product. However, in a new reaction after a whole night no methanol was collected and the yield totalled only 6.89%. Finally, by increasing the temperature again (to 80°C), adding three times as much reagent, and a double amount of catalyst, a reasonable yield of 30.60% was obtained. It must be noticed that with this protection, the alcohol group that was meant to stay unprotected can also participate in the reaction forming a seven-membered ring variant. This side product was successfully purged away from the desired product by flash column chromatography.

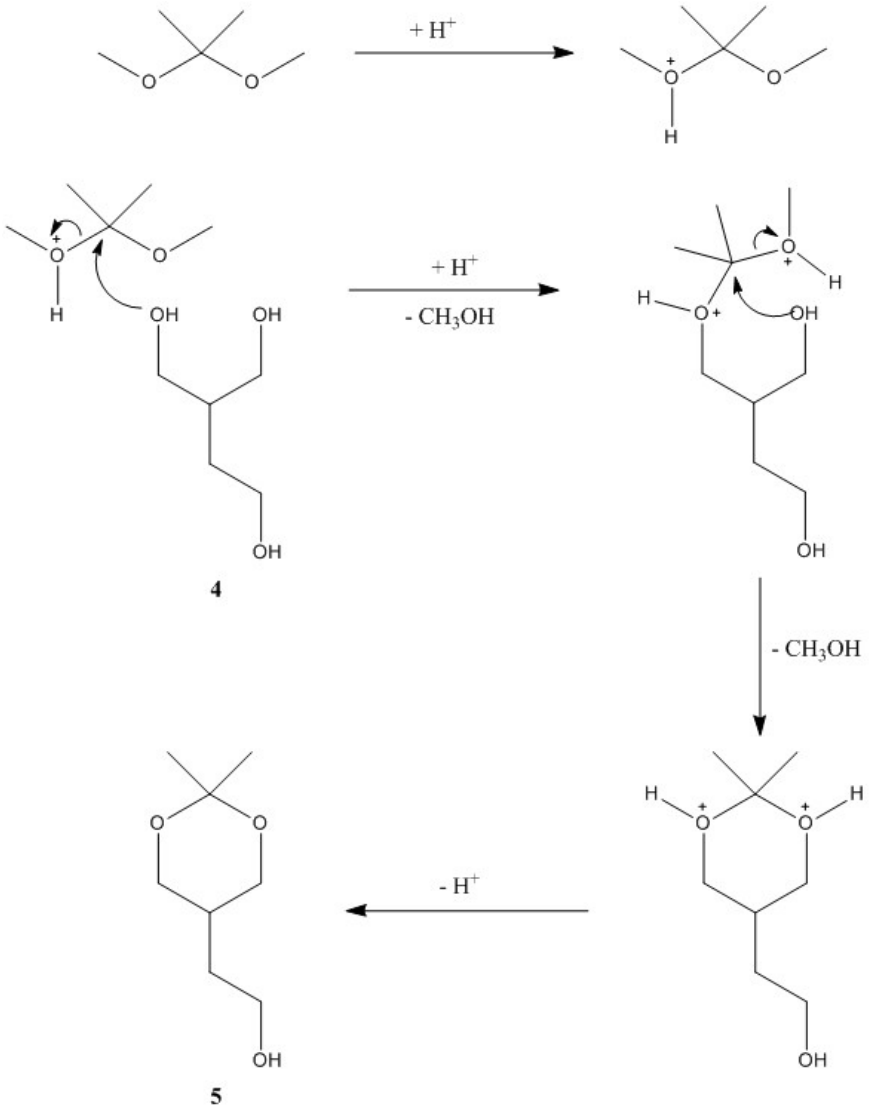


Figure 4.6: Reaction mechanism of the protection to form compound 5.

The reaction can proceed because of the presence of a catalyst that can provide protons to make the methoxygroups of dimethoxypropane good leaving groups in such way that the alcohol groups of **4** can attack and close the dioxane ring. At the end of the reaction p-toluene sulfonic acid can recover its protons (Figure 4.6).

4.1.4. Attempted synthesis of 5-(2'-bromoethyl)-2,2-dimethyl-1,3-dioxane

To attach bromide as a good leaving group, the Appel-reaction was used (Figure 4.7) (36). The reaction starts with the transmission from a bromide from carbon tetrabromide to triphenylphosphine obtaining the phosphonium salt and a very reactive tertiary carbanion. This carbanion will subtract a proton from the alcohol group resulting in the corresponding alkoxide and bromoform. An intermediate is formed by nucleophilic substitution of the

bromide ion by the alkoxide. Next the bromide attacks in a S_N2 reaction resulting in the desired compound and triphenylphosphine oxide (TPPO).

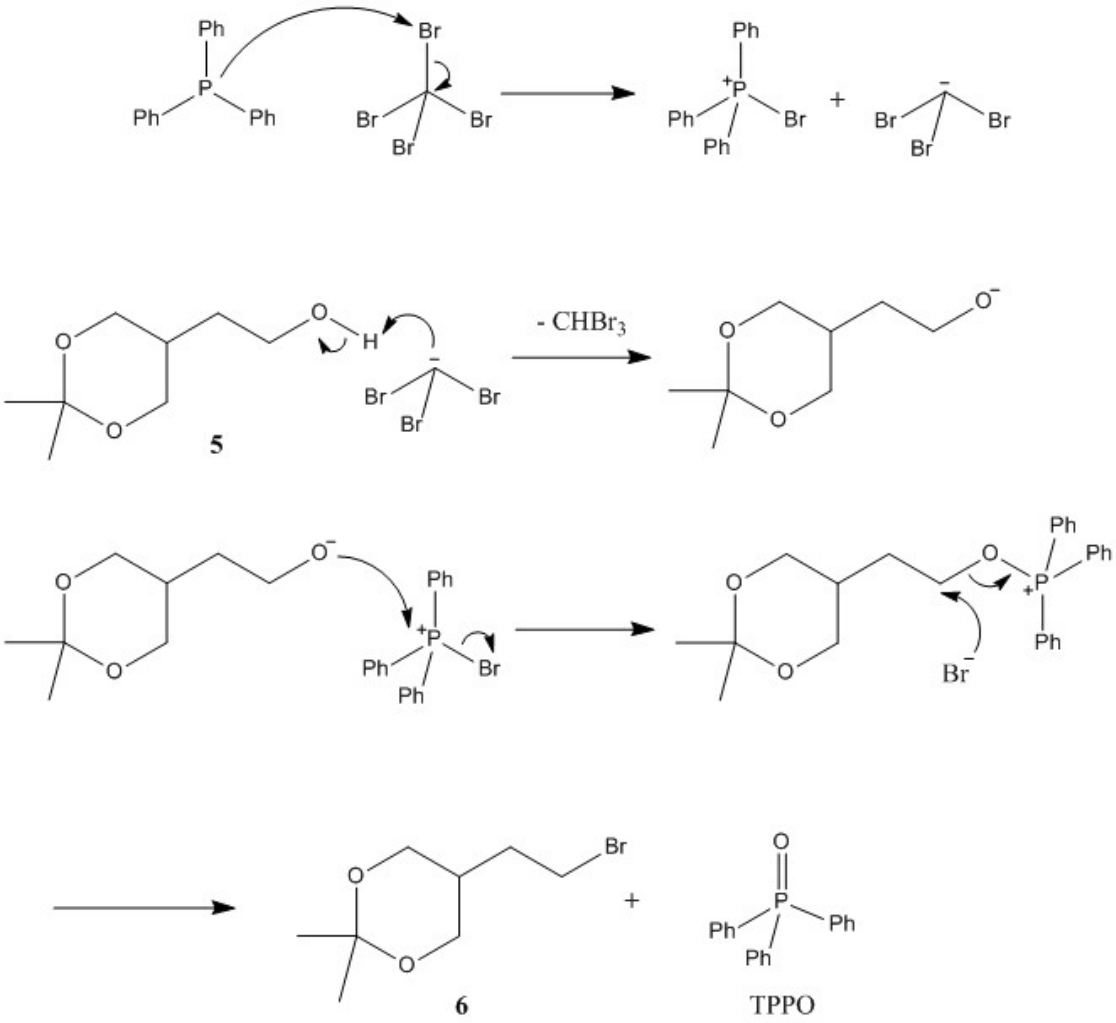


Figure 4.7: Appel reaction mechanism.

The found procedure proposed an extraction with hexane but didn't work, resulting only in a partial isolation of TPPO and no product could be seen. However, the reaction was monitored by TLC and no starting material could be seen. A second extraction of the water phase, this time with ethyl acetate, resulted in an amount that was to big to be only the desired product. In a further attempt to remove TPPO a filtration on silica gel was performed using an increasing polarity of the eluent but without result. Since this purification prior to the attachment of the purine is of utmost importance, a new reaction was necessary.

Another bromination strategy was found with phosphorous tribromide (PBr_3) (Figure 4.8). Here the alcohol group attacks and substitutes a bromine. This bromide formed attacks on his turn the intermediate in a S_N2 reaction with formation of the desired molecule and PBr_2OH, which can still function as a brominating agent until it is fully reacted to

phosphorous acid (H_3PO_3) (Figure 4.8). On HPLC-MS no product could be seen after leaving the reaction for a whole weekend.

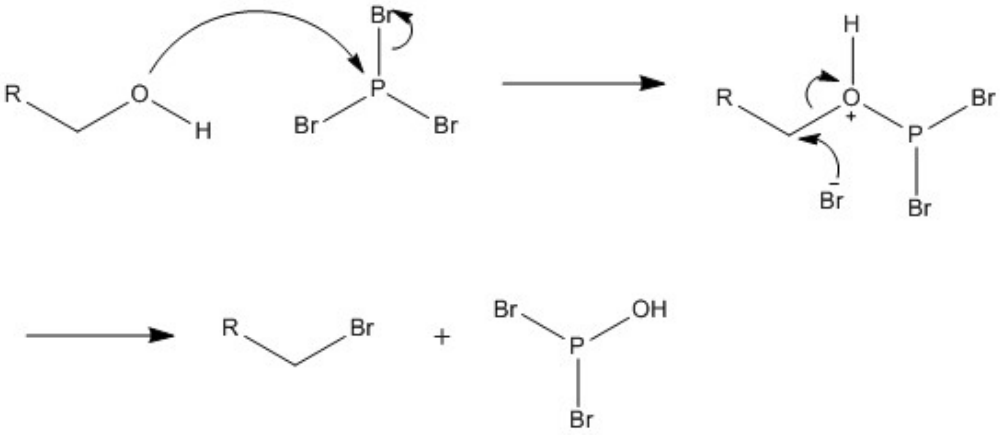


Figure 4.8: Bromination mechanism with phosphorous tribromide.

4.1.5. Synthesis of 2-(2,2-dimethyl-1,3-dioxan-5-yl)ethyl methanesulfonate (6)

Since no suitable bromination procedure was found, mesylation was tried (Figure 4.9). A patent procedure was followed but resulted in an incomplete reaction (37). The amount of methanesulfonyl chloride was tripled after insufficient reaction progress was detected by TLC. Therefore, an increase of base was performed to avoid a decrease in pH since acidic conditions deteriorate the protection. Consequently, the wash with concentrated hydrochloric acid (HCl) was replaced by water.

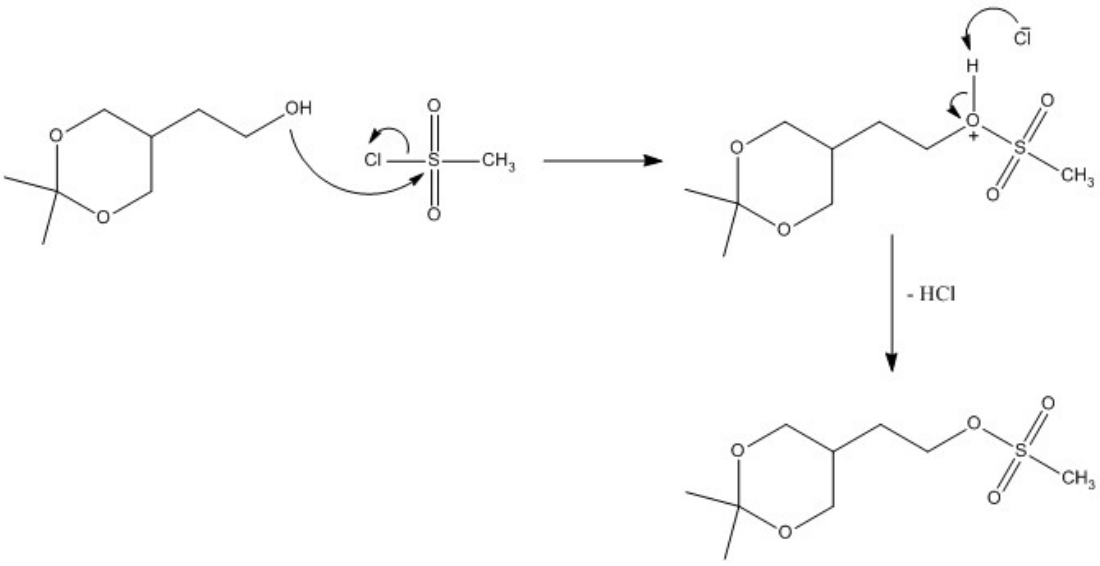


Figure 4.9: Mesylation mechanism.

4.2. SYNTHESIS OF THE PURINE DERIVATIVE (**7**)

The suitable purine, 6-amino-2-chloro-adenine was prepared using an in-house procedure starting from 2,6-dichloropurine treated with ammonia under high pressure and temperature. At fixed temperatures, this nucleophilic aromatic substitution is selective for the C6 position on purines (Figure 4.10).

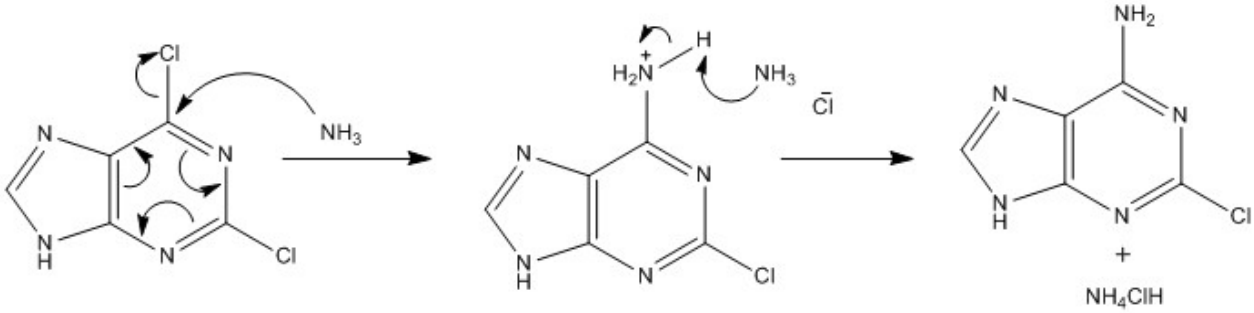


Figure 4.10: nucleophillic aromatic substitution at the purine.

4.3. LINKING AND PHOSPHORYLATIONS

Conjoining the chain and the purine block can give two isomer products, N7 and N9, of which the latter one is the desired form (**8**) and is always obtained in a higher yield. Nonetheless should the formation of N7-isomer be restricted to an absolute minimum by working for example at a lower temperature. Because of the use of an instable but very reactive leaving group this is more likely to be successful. Purification of both isomers is postponed until after deprotection (in acidic conditions) whereby more polar groups are available to separate them on a silica gel column chromatography. This gives better separation with the N7-form the most polar isomer. In the procedures followed, the N7-isomer has not been obtained in detectable amount.

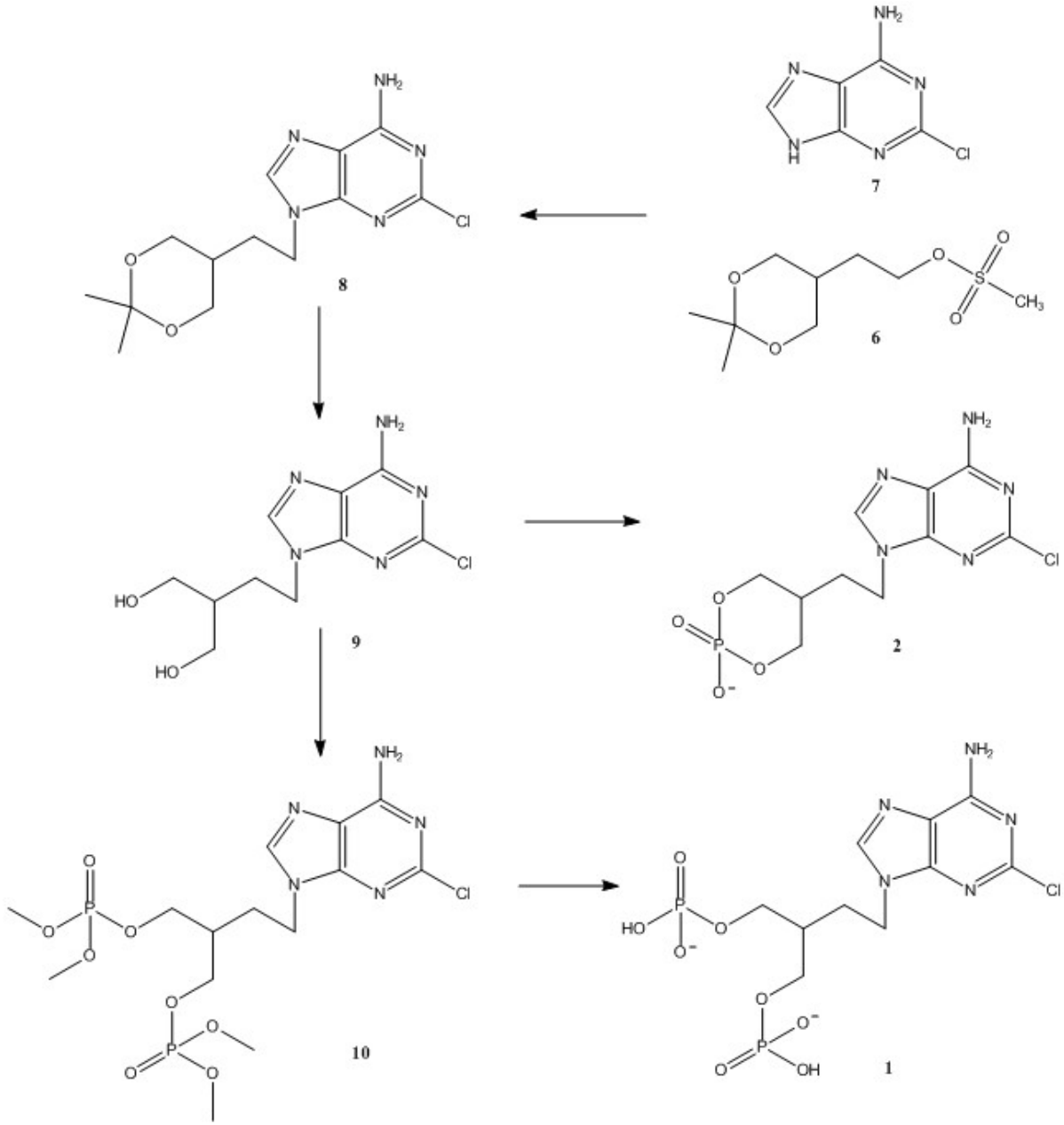


Figure 4.11: Linking of the purine to the chain, deprotection, and phosphorylation approach.

When phosphoryl chloride ($POCl_3$) is used for phosphorylation on compound **9**, a cyclic phosphate (**2**) is obtained (Figure 4.11). In order to achieve a bisphosphorylation (**1**), a protected intermediate that can not bind to two alcohol groups is necessary. With the aid of phosphoramidite and subsequent oxidation by hydrogen peroxide, the bisoxydimethylphosphate (**10**) is the intermediate that makes the formation of a cyclic phosphate impossible.

4.3.1. Synthesis of 2-chloro-9-(2-(2,2-dimethyl-1,3-dioxan-5-yl)eth-1-yl)-9H-adenine (8)

The coupling was based on the same patent as followed in the mesylation step (37). This procedure permitted a rapid succession of the two reactions, which is necessary because of the instability of the mesylate. First a base, here potassium carbonate (K_2CO_3), activates the purine, making it nucleophillic enough to substitute the mesylate in the next step in a S_N2 reaction (Figure 4.12).

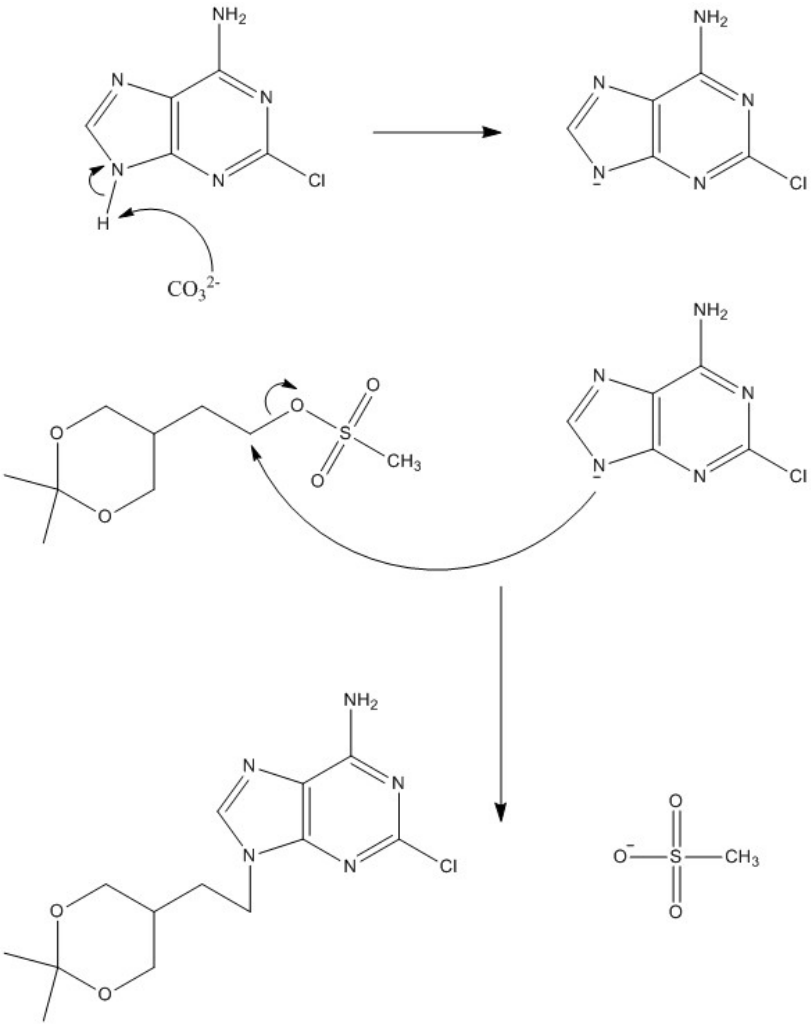


Figure 4.12: Linking of the purine to the chain.

Very importantly is that the substitution appears on the N9-position of the purine. Therefore the reaction temperature was lowered to room temperature to favour the N9-variant. Successfully, after 15 h no N7 was seen on TLC but only very little N9-product was formed. Hence, the temperature was raised to 65°C as prescribed in the protocol. In this, another compound is formed together with the desired one even if in lower yield. After a first column the compounds were not separated but the N9-substituted compound could be

isolated with preparative TLC and characterized by NMR. The amount of the other compound was too small to be analysed. No quantitative separation was necessary yet because this was planned after the deprotection. In this way more polar groups are present and a good separation is more likely. Remarkably, on a big scale, no other side products appeared to be present in appreciable amount.

4.3.2. Synthesis of 2-Chloro-9-[4-hydroxy-3-(hydroxymethyl)but-1-yl]-9H-adenine (9)

To deprotect the two alcohol groups a simple treatment with acid was sufficient and almost quantitative (85.68%). First, the strong acid protonates the ether oxygen, making it a better leaving group. Then, when the ring is opened, the second oxygen is protonated as well, and the protection removed (Figure 4.13).

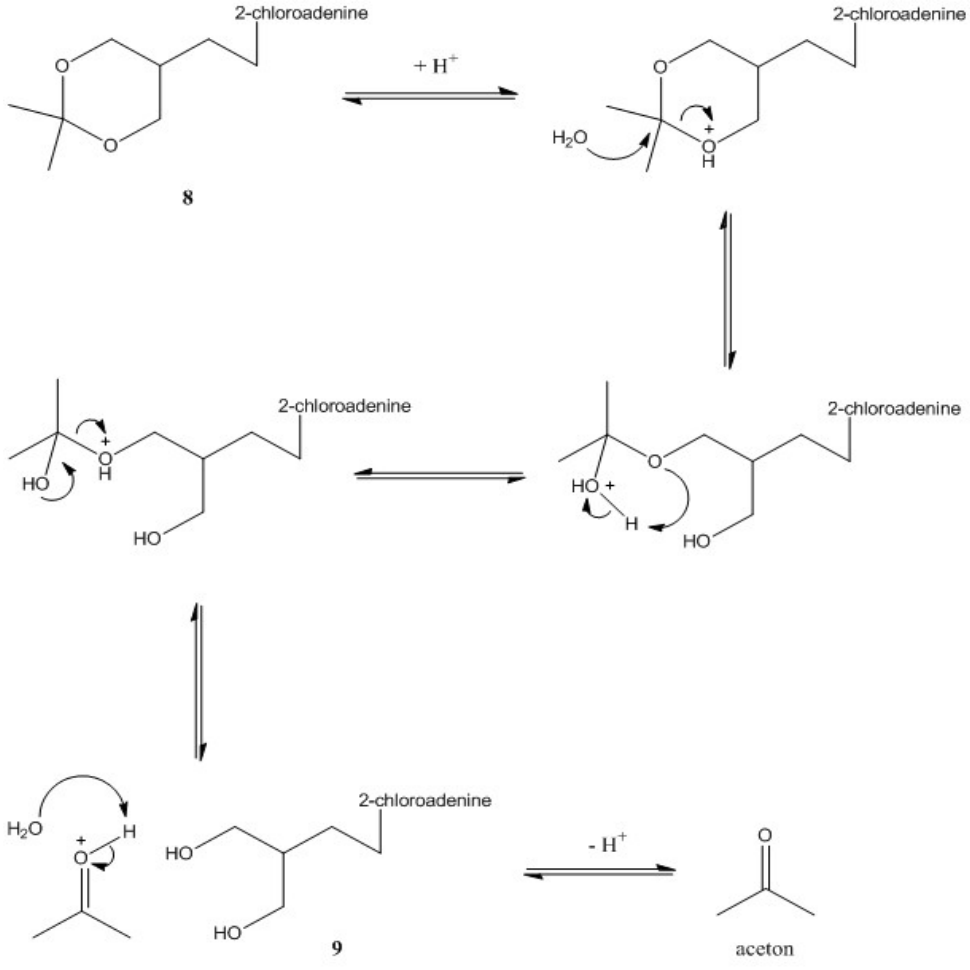


Figure 4.13: Mechanism of the deprotection in acidic conditions.

Since this is the last step before the phosphorylation, a compound with good purity is desired. After a column, the N9-isomer was confirmed with a NOE-experiment (Nuclear Overhauser Effect). By attacking the protons of the chain closest to the N-coupling with the

purine (see arrows Figure 4.14), an effect should be seen on the protons closest to them. In a N7-isomer the 6-amino group is close enough and gets affected, while in a N9-isomer no influence is noticed as was noticed on our compound.

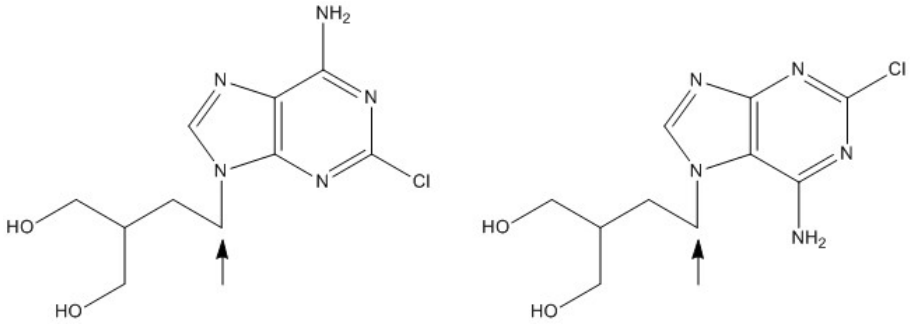


Figure 4.14: Attacked protons (arrows) in the NOE-experiment on the N7 and N9 isomer.

4.3.4. Attempted synthesis of 2-Chloro-9-[4-hydroxy-3-(hydroxymethyl)but-1-yl]-9H-adenine cyclic phosphate (2)

For the cyclic phosphorylation (Figure 4.15), use was made of an in-house procedure with $POCl_3$. The reaction was performed under anhydrous conditions in trimethyl phosphate and the released acid was captured with proton sponge. Purification required two Sephadex columns with a gradient elution from 0M to 0.5M NH_4HCO_3. However, about the spot that we have purified can not be concluded with certainty that it is the cyclic phosphate.

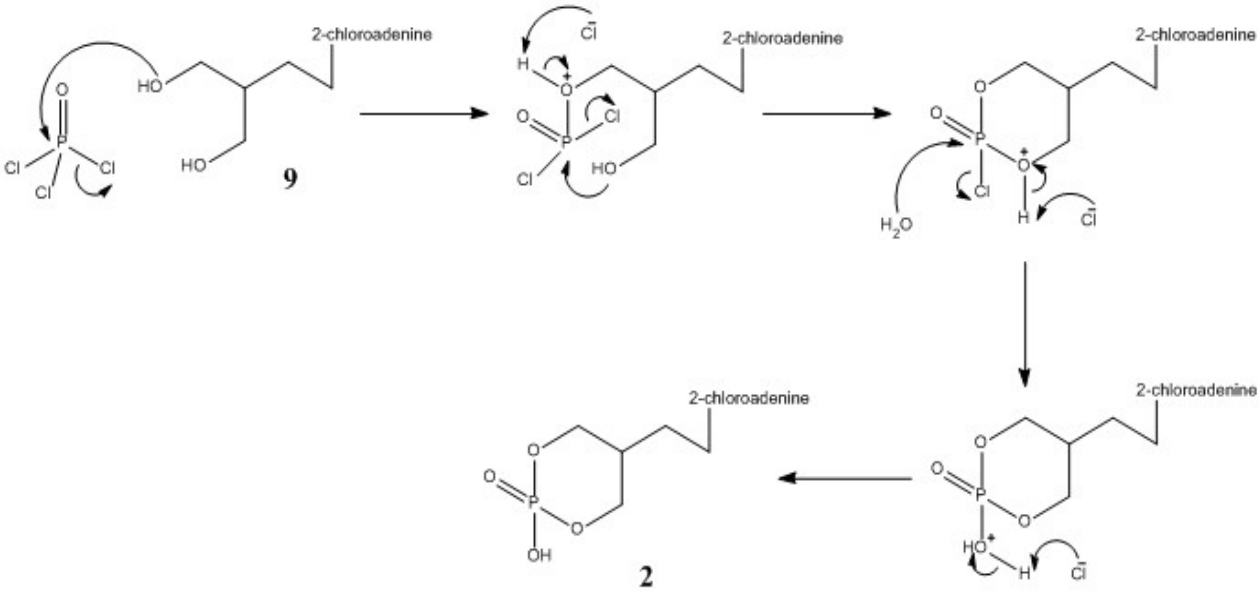


Figure 4.15: Mechanism of the synthesis of the cyclic phosphate

4.3.5. Attempted synthesis of 2-[2-(6-amino-2-chloro-9H-purin-9-yl)ethyl]propane-1,3-bisoxy(dimethylphosphate) (10)

The bisoxy(dimethyl)phosphate was achieved with O,O-dimethoxy-N,N-diisopropylphosphoramidite together with tetrazol as an activating ligand (Figure 4.16). First the tetrazol takes in the place of the diisopropylamine and attaches to the protected phosphate. Then it is easier for the alcohol groups to attack the phosphate because tetrazol can act as a good leaving group. The obtained phosphite triester is oxidized with hydrogen peroxide to give the bisoxy(dimethyl)phosphate. Since the phosphate is protected, no Sephadex column was necessary to purify this compound but instead a normal silica gel column chromatography was performed. However, NMR spectrum could not give exclusive evidence that compound **10** was obtained, partially because of the low quantity. In order to form conclusions, a higher amount of compound should be synthesized and examined.

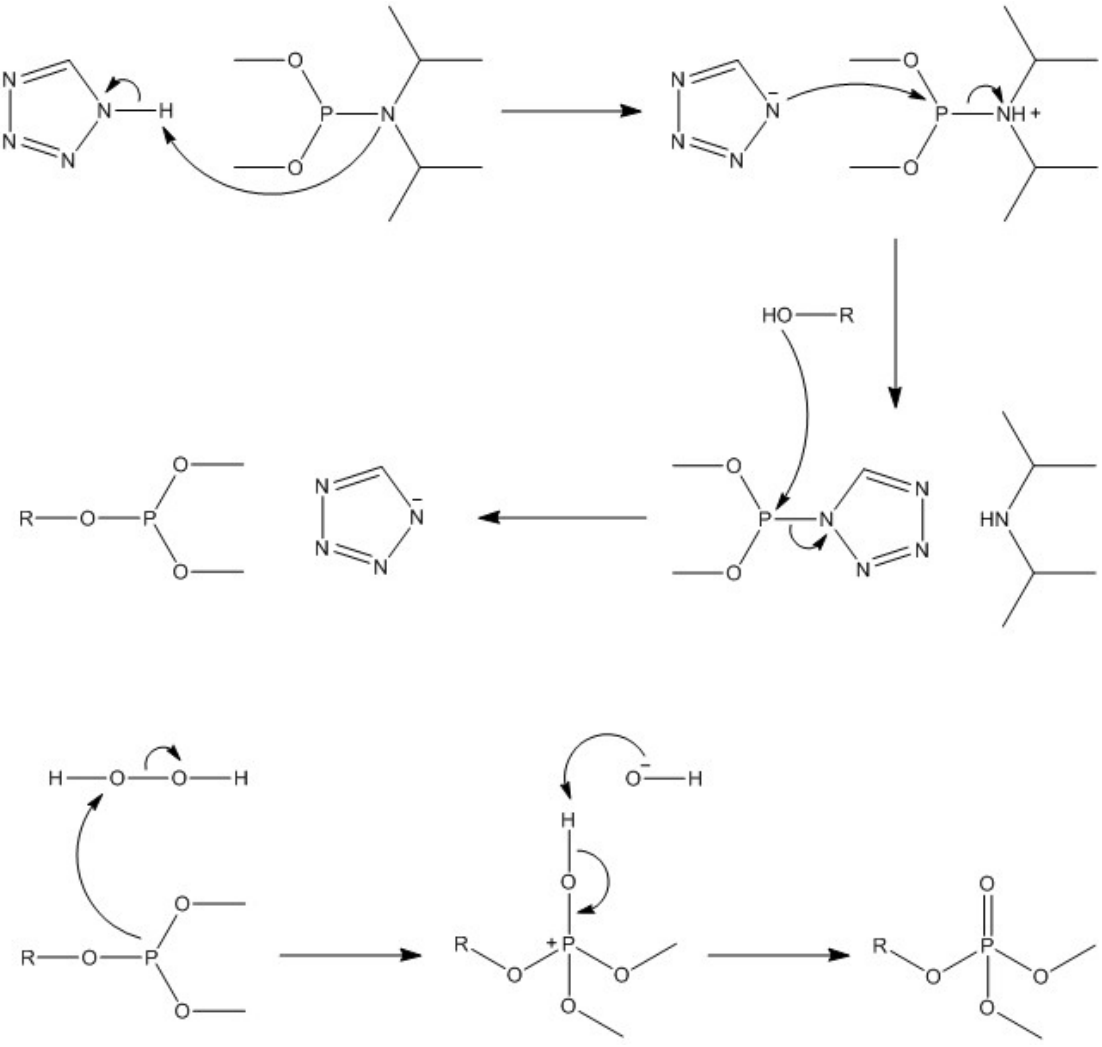


Figure 4.16: Mechanism of the dimethylphosphorylation of one alcohol group. On the desired compound, this reaction takes place twice.

4.3.6. Planned synthesis of 2-[2-(6-amino-2-chloro-9H-purin-9-yl)ethyl]propane-1,3-bisoxy(diammoniumphosphate) (1)

Although no bis(dimethyl)phophate (**10**) was obtained, the strategy for deprotection to finally obtain the bisphosphate (**1**) was the following. A protocol was found that suggested this step with the aid of bromotrimethylsilyl (TMSBr) (38).

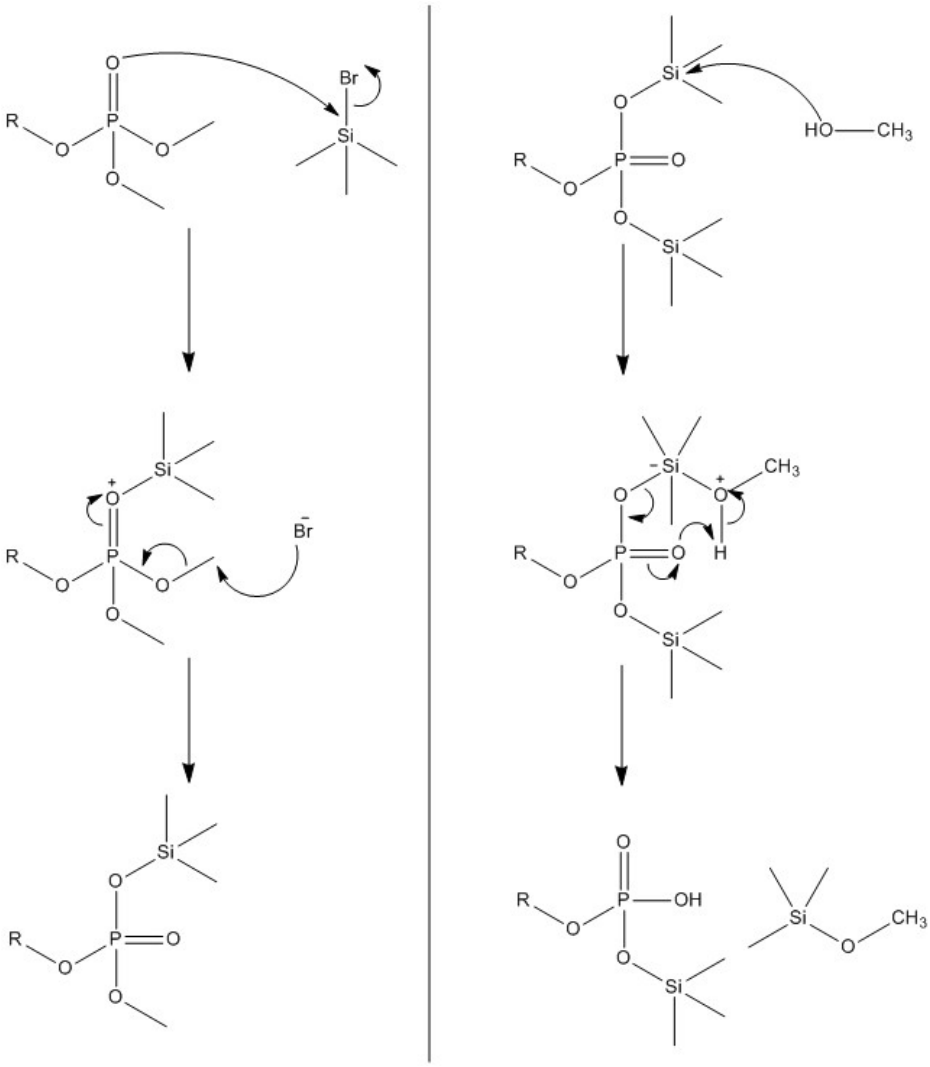


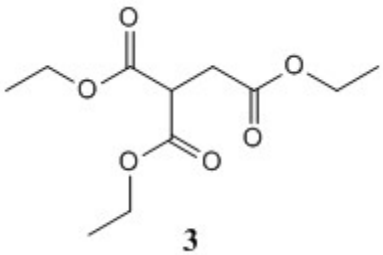
Figure 4.17: Reaction mechanism of one dimethylphosphate deprotection. The left reaction proceeds twice with the starting product of the right-wing reaction as its final result. The right reaction is performed twice as well with each time the removal of one silicium group.

To deprotect one dimethylphosphate the following reaction mechanism was found. In a first step, trimethylsilyl groups replace the methyl groups. Since there are two methyl groups, the reaction on the right side of Figure 4.17 takes place twice. Subsequently, the addition of methanol to the reaction mixture is able to break of the silyl groups and form the desired final compound (left side of Figure 4.17 and takes place twice as well).

5. EXPERIMENTAL SECTION

5.1. SYNTHESIS OF TRIETHYL-1,1,2-ETHANETRICARBOXYLATE (**3**)

Diethyl malonate (40.44 g, 252.50 mmol) was dropwise added over a period of 1h to an ice-cooled suspension of sodium hydride (60% in mineral oil, 7.25 g, 302.10 mmol) in dry THF (300 mL) under N_2-atmosphere. The reaction mixture was allowed to stir at RT for 1.5 h. Afterwards the mixture was cooled again to 0°C to slowly add ethyl bromoacetate (25 mL, 225.45 mmol) over a period of 0.5 h. The ice bath was removed and the reaction was finally stirred for another 2.5h at RT. A mixture of 1.5N HCl (15 mL) and ice (400 mL) was added to the reaction and extracted with dichloromethane (DCM) (3x300 mL). After drying with Brine and concentration under vacuum, the crude was purified by column chromatography over silica gel (4% ethyl acetate in petroleum ether) to give the title compound as a yellow oil (quantitative).

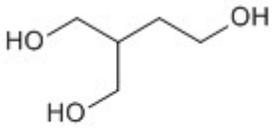


3

^{1}H NMR (DMSO-d_6): δ 1.15 (m, 9H, 3 x CH_3), 2.80 (d, J = 7.2 Hz, 2H, CH_2), 3.77 (t, J = 6.4 Hz, 1H, CH), 4.09 ppm (m, 6H, 3 x CH_3).

5.2. SYNTHESIS OF 2-HYDROXYMETHYL-1,4-BUTANEDIOL (**4**)

To a refluxing solution of triethyl-1,1,2-ethanetricarboxylate (1.940 g, 7.88 mmol) in tert-butyl alcohol (15.76 mL) at 86°C, sodium borohydride (789 mg, 20.80 mmol) was added followed by the slow addition of methanol (0.985 mL) in 3 aliquots over 0.5 h. The reaction mixture was further heated for 0.5h and then allowed to cool down. The reaction was carefully neutralized with HCl 6M and extracted with ethanol followed by drying of the organic phase with brine and filtration in vacuo. After concentration under vacuum, the crude was purified by column chromatography over silica gel (10% methanol in DCM) to give the title compound as a yellow oil (378 mg, 3.15 mmol, 39.97%).

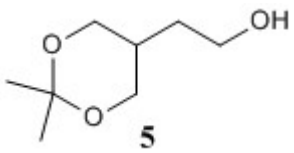


4

^{1}H NMR (DMSO-d_6): δ 1.37 (q, J = 6.8 Hz, 2H, CH_2), 1.54 (sept, J = 6.0 Hz, 1H, CH), 3.35 (m, 4H, CH_2), 3.41 (q, J = 6.8 Hz, 2H, CH_2), 4.34 (t, J = 5.2 Hz, 2H, 2 x OH), 4.40 ppm (t, J = 5.2 Hz, 1H, OH).

5.3. SYNTHESIS OF 2,2-DIMETHYL-5-(2'-HYDROXYETHYL)-1,3-DIOXANE (**5**)

To a solution of 2-(hydroxymethyl)butane-1,4-diol (11.757 g, 97.85mmol) in DMF (55mL) under N_2-atmosphere, dimethoxy propane (34.242 g, 328.77 mmol) and p-toluene sulfonic acid monohydrate (1.860 g, 9.78 mmol) were added. The resulting solution was refluxed overnight at 80°C. The reaction mixture was then neutralized by addition of triethylamine (TEA), the solvent was removed under vacuum, and the residue was purified by silica gel column chromatography (3% to 5% methanol in chloroform) to give the title compound as a yellow oil (4.796 g, 29.94 mmol, 30.60%).

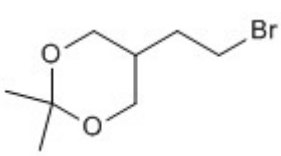


5

^{1}H NMR (DMSO-d_6): δ 1.37 (q, J = 6.8 Hz, 2H, CH_2), 1.54 (sept, J = 6.0 Hz, 1H, CH), 3.35 (m, 4H, CH_2), 3.41 (q, J = 6.8 Hz, 2H, CH_2), 4.34 (t, J = 5.2 Hz, 2H, 2 x OH), 4.40 ppm (t, J = 5.2 Hz, 1H, OH).

5.4. ATTEMPTED SYNTHESIS OF 5-(2'-BROMOETHYL)-2,2-DIMETHYL-1,3-DIOXANE

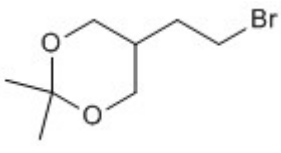
To an ice-cooled solution under N_2-atmosphere of 5-(2-hydroxyethyl)-2,2-dimethyl-1,3-dioxane (514 mg, 3.21 mmol) and carbon tetrabromide (1.598 g, 4.82 mmol) in DMF (9.3 mL), triphenylphosphine (1.264 g, 4.82 mmol) was added and the reaction mixture was stirred for 0.5 h at 0°C. Saturated aqueous $NaHCO_3$ (4.65 mL) and water (4.65 mL) was added followed by extraction with ethyl acetate, drying with Na_2SO_4, and filtration in vacuo.



Still, TPPO was present and in a second attempt a filtration on silica (n-hexane, diethylether, DCM, and ethyl acetate/DCM) was performed, but the TPPO was irremovable.

5.5. ATTEMPTED SYNTHESIS OF 5-(2'-BROMOETHYL)-2,2-DIMETHYL-1,3-DIOXANE

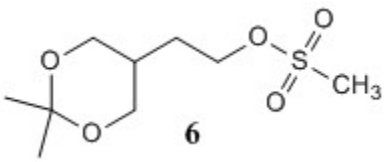
Under a N_2-atmosphere, pyridine (0.300 mL) and phosphorous tribromide (0.324 mL) were added to an ice-cooled solution of 5-(2-hydroxyethyl)-2,2-dimethyl-1,3-dioxane (201 mg, 1.25 mmol) in anhydrous toluene (2.50 mL). The reaction mixture was allowed to warm to RT and stirred for 2 days. Afterwards, the mixture was poured in saturated aqueous



$NaHCO_3$ (3 mL), extracted with ethyl acetate (3 x 3mL), dried with Na_2SO_4 and filtered in vacuo. An HPLC-MS analysis was performed but could not confirm the title compound.

5.6. SYNTHESIS OF 2-(2,2-DIMETHYL-1,3-DIOXAN-5-YL)ETHYL METHANESULFONATE (**6**)

To an ice-cooled solution of 5-(2-hydroxyethyl)-2,2-dimethyl-1,3-dioxane (208 mg, 1.30 mmol) in anhydrous toluene (6.50 mL) under N_2-atmosphere was added TEA (0.572 mL). Then the mixture was further cooled to -10°C to slowly add methanesulphonyl chloride (0.375 mL) drop by drop. The reaction was allowed to heat up to RT and stirred for 3h. The work-up was performed by adding water, extraction with DCM, drying with Na_2SO_4 and filtration in vacuo. No column was performed because of the instability of the compound and the linking was carried out immediately after this reaction. However, NMR confirmation of the title compound (quantitative) was made on the test reaction, which also detected the presence of methanesulphonic acid.

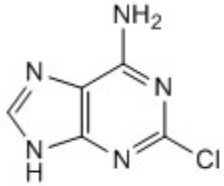


6

^{1}H NMR (DMSO-d_6): δ 1.25 (s, 3H, CH_3), 1.28 (q, J = 6.8 Hz, 2H, CH_2), 1.32 (s, 3H, CH_3), 1.78 (m, 1H, CH), 3.37 (q, J = 6.4 Hz, 2H, CH_2), 3.48 (m, 2H, CH_2), 3.73 (dd, J = 4.0 Hz, J = 4.4 Hz, 2H, CH_2), 4.45 ppm (t, J = 5.0 Hz, 1H, CH_2).

5.7. SYNTHESIS OF 6-AMINO-2-CHLOROADENINE (**7**)

A metallic vessel was cooled in liquid N_2 and then fluxed with N_2 for 15 minutes. Next, liquid ammonia was condensed into the bomb and 2,6-dichloropurine (2.599 g, 13.75 mmol) was added. The vessel was tightly closed and heated in the oven at 100°C for 12h. Afterwards, the vessel was submerged in liquid N_2 again, then opened and the ammonia was left to escape. The resulting solid was washed with water on a filter to remove the salts to obtain the pure titled product (2.116 g, 12.48 mmol, 90.76%).

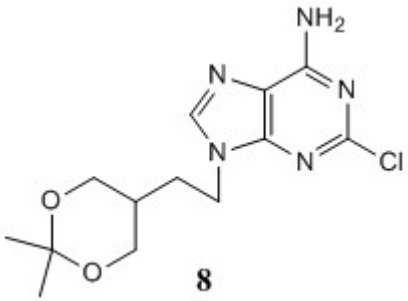


7

^{1}H NMR (DMSO-d_6): δ 7.64 (br s, 2H, NH_2), 8.10 (s, 1H, H-8), 13.02 ppm (br s, 1H, NH-9).

5.8. SYNTHESIS OF 2-CHLORO-9-(2-(2,2-DIMETHYL-1,3-DIOXAN-5-YL)ETH-1-YL)-9H-ADENINE (**8**)

DMF (46,40 mol) is added to 5-(2-mesylethyl)-2,2-dimethyl-1,3-dioxane (5.760 g, 24.17 mol), followed by anhydrous potassium carbonate (16.702 g, 120.85 mol) and 2-amino-6-chloroadenine (3.484 g, 20.54 mol). The reaction mixture, under N_2-atmosphere, was stirred for 12h at 65°C. After the reaction mixture cooled down to RT, water (60 mol) was added to form a precipitate. Stirring continued for 1 h at RT with subsequent filtration and wash of the solid with water. The crude was purified by silica gel column chromatography (3% to 5% methanol in DCM) to give the title compound as an off white powder (2.853 g, 9.15 mol, 37.86%).

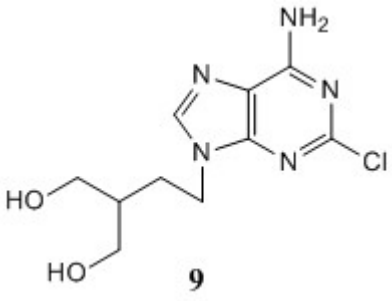


8

^{1}H NMR (DMSO-d_6): δ 1.24 (s, 3H, CH_3), 1.28 (s, 3H, CH_3), 1.56 (m, 1H, CH), 3.54 (m, 2H, CH_2), 3.75 (dd, J = 5.6 Hz, 2H, CH_2), 4.08 (t, J = 6.0 Hz, 2H, CH_2), 7.77 (br s, 2H, NH_2), 8.18 ppm (s, 1H, H-8).

5.9. SYNTHESIS OF 2-CHLORO-9-[4-HYDROXY-3-(HYDROXYMETHYL)BUT-1-YL]-9H-ADENINE (**9**)

To a solution of protected purine derivative **8** (2.853g, 9.15 mmol) in acetonitrile (50 mL) was added HCl 1M (50 mL). After 0.5 h, the reaction mixture was neutralized with saturated $NaHCO_3$ and dried under vacuum. The crude was purified by column chromatography over silica gel (10% methanol in DCM) to give the title compound as a white powder (2.130 g, 7.84 mmol, 85.68%).

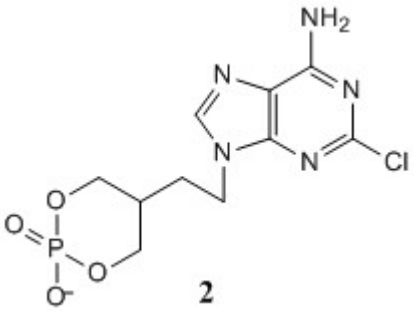


9

^{1}H NMR (DMSO-d_6): δ 0.96 (sept, J = 6.4 Hz, 1H, CH), 1.32 (q, J = 7.2 Hz, 2H, CH_2), 2.89 (m, 2H, CH_2), 2.60 (m, 2H, CH_2), 3.74 (t, J = 7.2 Hz, 2H, CH_2), 4.04 (m, 2H, 2xOH), 7.87 (br s, 2H, NH_2), 8.24 ppm (s, 1H, H-8).

5.10. ATTEMPTED SYNTHESIS OF 2-CHLORO-9-[4-HYDROXY-3-(HYDROXYMETHYL)BUT-1-YL]-9H-ADENINE CYCLIC PHOSPHATE (**2**)

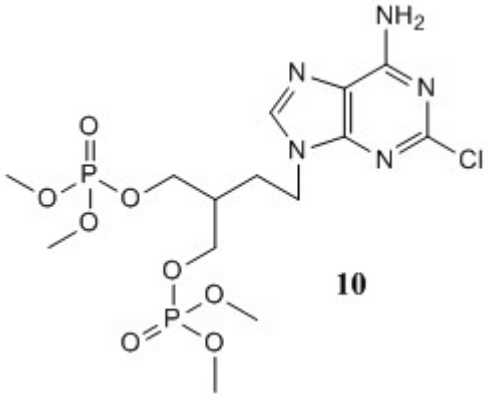
A solution of 9 (104 mg, 0.38 mmol) in trimethyl phosphate (1.90 mL) under N_2-atmosphere is cooled down to 0°C in an ice bath. Proton sponge (122 mg, 0.57 mmol) and subsequently $POCl_3$ (132 µL, 160 mg, 1.14 mmol) was added. The reaction mixture was allowed to heat up to RT and left stirring for another 3 h. Two Sephadex columns were performed (gradient from 0M to 0.5M NH_4HCO_3), but the putative cyclic phosphate spot could not be confirmed with NMR. The reaction will have to be performed again under different conditions in order to obtain a mixture that can be purified better.



2

5.11. ATTEMPTED SYNTHESIS OF 2-[2-(6-AMINO-2-CHLORO-9H-PURIN-9-YL)ETHYL]PROPANE-1,3-BISOXY(DIMETHYLPHOSPHATE) (**10**)

A solution of **9** (101 mg, 0.37 mmol) and 0,0-dimethoxy-N,N-diisopropylphosphoramidite (303 µL, 359 mg, 1.86 mmol) in anhydrous acetonitrile (1.86 mL) and under N_2-atmosphere was cooled down to -30°C. Subsequently tetrazol (4.13mL 0.45M solution in acetonitrile) was added slowly and the reaction was allowed to heat up to RT. After 0.5 h, the reaction was cooled down again, this time to -20°C, to add hydrogen peroxide (1.86 mL). Finally the mixture was left to react further for 1 h at RT. Silica gel column chromatography (30% cyclohexane, 68% DCM, 1% methanol, and 1% TEA) was performed to purify the crude. The NMR spectrum shows most likely the titled compound, but there is no conclusive evidence for this. A greater amount of compound and better separation will be necessary in order to be able to have indisputable proof of presence.



10

6. CONCLUSION AND FURTHER PROSPECTS

The identification of the role of GPR17 in pathologies such as cerebral ischemia and multiple sclerosis suggests this receptor as a new target for the treatment of these pathologies. In particular, compounds able to antagonize the activation of GPR17 could be useful to initiate remyelation in damaged brain tissue or block the progression of a lesion after ischemic injury.

Some adenosine bisphosphate nucleotides have been identified as antagonists of GPR17. Analogues of these compounds in which the adenine moiety is bound with a branched alkyl chain and phosphorylated in two different positions can be of particular interest to study the impact of the sugar moiety on the affinity with the receptor. These nucleotide bisphosphate analogues have been designed and a synthetic route was mapped and assessed.

During the course of this work, it was possible to obtain a useful intermediate (compound **9**) in the synthesis of the desired compounds. Because the phosphorylating reactions showed to many impurities that were difficult to abate, no conclusions can be made. In order to obtain a better and more efficient separation, use could be made of HPLC with either a reversed phase or an ion-exchange column.

Furthermore, the used synthetic route was successful enough to be used in further research to explore the effect of N6-substitutions or alkylations on the C2-position. Of course, these modifications on the purine must still be carried out before it is linked to the sugar-like chain.

7. LITERATURE

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