GHENT UNIVERSITY

FACULTY OF PHARMACEUTICAL SCIENCES

Department of Pharmaceutics

Laboratory for Medicinal Chemistry

Master thesis performed at:

UNIVERSITA DEGLI STUDI DI CAMERINO

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Medicinal Chemistry Unit

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SYNTHETIC APPROACH FOR THE PREPARATION OF 2-ALKOXY-5'-*N*-ETHYLCARBOXAMIDOADENOSINE DERIVATIVES

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First Master of Drug Development

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ABSTRACT

Adenosine is the natural agonist of four adenosine receptors: A_1, A_{2A}, A_{2B} and A_3, which all are G protein coupled-receptors. The resulting effect after stimulation varies with the cell type, number of expressed receptors and amount of agonist present. Since the adenosine receptor subtypes are ubiquitously expressed throughout the body (and sometimes co-expressed), the search for selective agonists is still pending, even after thirty years of research.

The A_{2A} receptor is particularly involved in inflammation processes, wound healing and sleep regulation. Therefore, development of potent and selective agonists could be useful in the treatment of diverse inflammatory diseases (rheumatoid arthritis, asthma etc.). Furthermore, topical application for the purpose of wound healing would comprise a significant improvement on current treatment. However this promising therapeutic potential, there are only two clinically approved drugs upon the present day, namely adenosine (Adenocard®) and regadenoson (Lexiscan®). They are used to terminate supraventricular tachycardia and as a myocardial stress agent respectively, leaving the above mentioned possibilities still open.

A_{2A} receptor agonists are, with only a few exceptions, structurally related to the natural ligand. It has been shown that modifications of the ribose 4' CH_2OH and C-2, N^6-position of the purine are possible to remain agonist activity. In this thesis we chose to combine a 5'-*N*-ethylcarboxamido and a C-2 phenethoxy modification to possibly improve selectivity for the A_{2A} receptor. Additionally, this compound would serve as a general template in view of synthesizing a series of 2-(aryl)alkyloxy 5'-*N*-ethylcarboxamido derivatives.

Initially, a convergent synthetic approach was proposed. However, substantial difficulties in combining ribose protection / deprotection and introducing the 4' CH_2OH substituent were encountered, resulting in a switch towards a divergent approach.
After two attempts to reduce the amount of synthetic steps that are described in literature procedures, we finally obtained a key intermediate. This should now be further functionalized to yield the desired compound.

SAMENVATTING

Adenosine is de natuurlijke ligand voor vier adenosine receptoren: A_1, A_{2A}, A_{2B} and A_3, die allemaal G protein coupled-receptors zijn. Stimulatie door de natuurlijke ligand varieert naargelang het celtype, expressieniveau van de receptor en de hoeveelheid agonist aanwezig in het milieu. Aangezien ieder subtype alomtegenwoordig tot expressie komt in het gehele lichaam (en er mogelijks zelfs co-expressie optreedt), is onderzoek naar selectieve liganden nog steeds gaande.

De A_{2A} receptor heeft vooral een functie in inflammatoire processen, wondheling en de regulatie van slaap. Selectieve agonisten zouden kunnen gebruikt worden in het bestrijden van inflammatoire aandoeningen zoals asthma, rheumatoïde arthritits etc. Daarenboven zou topische applicatie een verbetering kunnen betekenen ten aanzien van de huidige therapie inzake wondheling. Desondanks het veelbelovende therapeutisch potentieel van deze liganden, zijn er tot op heden slechts twee agonisten op de markt. Dit is adenosine (Adenocard®) zelf en het in 2008 goedgekeurde regadenoson (Lexiscan®), echter niet voor bovengenoemde indicaties. Ze worden namelijk gebruikt voor het beëindigen van supraventriculaire tachycardie en “stress agent” in myocard imaging studies, respectievelijk.

Alle gekende A_{2A} agonisten zijn, behalve enkele uitzonderingen, structureel afgeleid van de adenosine scaffold. Talloze modificaties hebben uitgewezen dat enkel de 4’ CH_2OH, C-2- en N^6-positie kunnen gewijzigd worden. In deze thesis hebben we geopteerd om een 5’-*N*-ethylcarboxamide en C-2 fenylethoxy modificatie te combineren met de bedoeling om de selectiviteit voor de A_{2A} receptor te verhogen. Deze molecule dient tevens ook als eerste test van de syntheseweg, die later zou kunnen gebruikt worden om een analoge serie van derivaten te bekomen.

De initiële convergente benadering bleek niet te voldoen om de ribose bescherming / ontscherming te combineren met de introductie van de 4’ CH_2OH substituent. Hierdoor werd overgeschakeld op een divergente aanpak. Na twee pogingen om het aantal synthesestappen, zoals beschreven in de literatuur, te verkorten, werd uiteindelijk een sleutelintermediair bekomen. Dit zou in een volgend stadium verder moeten worden gemodificeerd om het beoogde product te verkrijgen.

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TABLE OF CONTENTS

1. INTRODUCTION 1
1.1. ADENOSINE 1
1.2. PURINERGIC RECEPTORS 3
1.2.1. General introduction 3
1.2.2. Adenosine receptors 4
1.2.2.1. G protein coupled-receptors (GPCRs) 4
1.2.2.2. Adenosine receptor signalling 5
1.2.3. The A_{2A} receptor 8
1.2.3.1. Structure-Activity Relationship (SAR) of A_{2A} receptor agonists 8
1.2.3.2. Therapeutic potential of A_{2A} receptor agonists 12
2. OBJECTIVES 17
3. MATERIALS AND METHODS 18
3.1. METHODS 18
3.1.1. Nuclear Magnetic Resonance (NMR) [48] 18
3.1.1.1. The principle of NMR 18
3.1.1.2. The NMR spectrum 20
3.1.1.3. Instrumentation 20
3.1.2. Mass Spectroscopy (MS) [49, 50] 20
3.1.2.1. The principle of MS 20
3.1.2.2. The mass spectrum 22
3.1.2.3. Instrumentation 22
3.2. MATERIALS 22
4. CHEMISTRY 24
4.1. RETROSYNTHETIC ANALYSIS 24
4.1.1. Convergent approach 26
4.1.1.1. Synthesis of 2-chloroadenine (**3**) 26
4.1.1.2. Synthesis of 2-phenethoxyadenine (**2**) 27
4.1.1.3. Attempted synthesis of 1-*O*-acetyl-2,3-*O*-dibenzoyl-β-D-ribofuranose (**8**) 27
4.1.1.4. Synthesis of 5-*O*-triphenylmethylene-β-D-ribofuranose (**18**) 28
4.1.2. Divergent approach 29
4.1.2.1. Synthesis of 2',3'-*O*-isopropylideneguanosine (**19**) 30
4.1.2.2. Synthesis of sodium 2',3'-*O*-isopropylideneguanosine-5'-carboxylate (**20**) 30
4.1.2.3. Reaction mechanism of TEMPO / BAIB (bisacetoxy-iodobenzene) oxidation 30
4.1.2.4. Attempted synthesis of 2-amino-6-chloro-9-(2',3'-*O*-isopropylidene-5'-*N*-ethylcarboxamido-β-D-ribofuranosyl)purine (**21**) 32
4.1.2.5. Attempted synthesis of 2-amino-6-chloro-9-(2',3'-*O*-isopropylidene-5'-*N*-ethylcarboxamido-β-D-ribofuranosyl)purine (**21**) 32
4.1.2.6. Synthesis of 2-amino-6-chloro-9-(2',3'-*O*-isopropylidene-5'-*N*-ethylcarboxamido-β-D-ribofuranosyl)purine (**21**) 33
4.1.2.7. Synthesis of 2-amino-6-chloro-9-(2',3'-*O*-isopropylidene-5'-*N*-ethylcarboxamido-β-D-ribofuranosyl)purine (**21**) 34
4.1.2.8. Reaction mechanism of the simultaneous chlorination and amination 34
4.1.2.9. Adapted divergent synthetic scheme 35

4.1.2.10. Synthesis of 2-amino-2’-3’-*O*-isopropylidene-5’-*N*-ethylcarboxamidoguanosine (**25**) ... 36
4.1.2.11. Synthesis of *O*6-(benzotriazol-1-yl)-2-amino-9-(2’-3’-*O*-isopropylidene-5’-*N*-ethylcarboxamido-β-D-ribofuranosyl)purine (**27**) ... 37
4.1.2.12. Proposed reaction mechanism of phosphonium salt formation and subsequent displacement ... 38
4.1.2.13. Attempted synthesis of 6-*O*-(benzotriazol-1-yl)-2-phenethoxy-9-(2’,3’-*O*-isopropylidene-5’-*N*-ethylcarboxamido-β-D-ribofuranosyl)purine (**28**) 39
4.1.2.14. Proposed reaction mechanism for non-aqueous diazotization / dediazoniation. ... 40
5. EXPERIMENTAL SECTION ... 42
5.1. CONVERGENT APPROACH ... 42
5.1.1. Synthesis of the purine building block ... 42
5.1.1.1. Synthesis of 2-chloroadenine (**3**) ... 42
5.1.1.2. Synthesis of 2-phenethoxyadenine (**2**) ... 42
5.1.2. Synthesis of the ribose building block ... 43
5.1.2.2. Attempted synthesis of 1-*O*-acetyl-2,3-*O*-dibenzoyl-β-D-ribofuranose (**8**) ... 43
5.1.2.3. Synthesis of 5-*O*-triphenylmethylene-β-D-ribofuranose (**18**) ... 43
5.2. DIVERGENT APPROACH ... 44
5.2.1. Synthesis of 2’,3’-*O*-isopropylideneguanosine (19) ... 44
5.2.2. Synthesis of sodium 2’,3’-*O*-isopropylideneguanosine-5’-carboxylate (20) ... 45
5.2.3. Attempted synthesis of 2-amino-6-chloro-9-(2’,3’-*O*-isopropylidene-5’-*N*-ethylcarboxamido-β-D-ribofuranosyl)purine (21) ... 45
5.2.4. Attempted synthesis of 2-amino-6-chloro-9-(2’,3’-*O*-isopropylidene-5’-*N*-ethylcarboxamido-β-D-ribofuranosyl)purine (21) ... 46
5.2.5. Synthesis of 2-amino-6-chloro-9-(2’,3’-*O*-isopropylidene-5’-*N*-ethylcarboxamido-β-D-ribofuranosyl)purine (21) ... 46
5.2.6. Synthesis of 2-amino-6-chloro-9-(2’,3’-*O*-isopropylidene-5’-*N*-ethylcarboxamido-β-D-ribofuranosyl)purine (21) ... 47
5.2.7. Synthesis of 2’-3’-*O*-isopropylideneguanosine-5’-*N*-ethylcarboxamide (25) ... 48
5.2.8. Synthesis of *O*6-(benzotriazol-1-yl)-2’-3’-*O*-isopropylideneguanosine-5’-*N*-ethylcarboxamide (27) ... 48
5.2.9. Attempted synthesis of 6-*O*-(benzotriazol-1-yl)-2-phenethoxy-9-(2’,3’-*O*-isopropylidene-5’-*N*-ethylcarboxamido-β-D-ribofuranosyl)purine (28) ... 49
6. CONCLUSION AND FUTURE PROSPECTS ... 51
7. LITERATURE ... 52

LIST OF USED ABBREVIATIONS

δ	Chemical shift
μ	Magnetic moment
ac	Alternating current
AC	Adenylate (adenylyl) cyclase
ADA	Adenosine deaminase
ADP	Adenosine-5'-diphosphate
Ado	Adenosine
AMP	Adenosine-5'-monophosphate
AR	Adenosine receptor
ATP	Adenosine-5'-triphosphate
BAIB	Bis-acetoxyiodobenzene
BOP	Benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate
cAMP	Cyclic adenosine-5'-monophosphate
CBP	CREB-binding protein
CoA	Coenzyme A
CRE	cAMP responsive element
CREB	cAMP responsive element binding protein
DAG	Diacylglycerol
DBU	1,8-diazabicylcoundec-7-ene
dc	Direct current
DEA	*N*,*N*-diethylaniline
DMSO-d_6	Dimethyl sulfoxide (deuterated)
ESI	Electrospray ionization
FAD	Flavine adenine dinucleotide
GDP	Guanosine-5'-diphosphate
GPCR	G-protein-coupled-receptor
GTP	Guanosine-5'-triphosphate
Hz	Hertz
I	Spin quantum number
IP_3	Inositol-1,4,5-triphosphate
J	Coupling constant

K_i	Inhibition constant
MAPK	Mitogen-activated protein kinase
MeCN	Acetonitrile
MeOH	Methanol
MPI	Myocardial perfusion imaging
MS	Mass spectroscopy
m/z	Mass-to-charge ratio
NAD	Nicotinamide adenine dinucleotide
NECA	5'-*N*-ethylcarboxamidoadenosine
NF-κB	Nuclear factor-κB
NMR	Nuclear magnetic resonance
PDE	Phosphodiesterase
PIP_2	Phosphatidylinositol-4,5-bisphosphate
PKA	Protein kinase A
PKC	Protein kinase C
PLC	Phospholipase C
ppm	Parts per million
SAM	S-adenosyl methionine
SAR	Structure-activity-relationship
TEA	Tetra-ethylammoniumchloride
TEMPO	(2,2,6,6-tetramethyl-piperidin-1-yl)-oxyl
THF	Tetrahydrofuran
TNF	Tumor necrosis factor
tPA	Tissue plasminogen activator
UDP	Uridine-5'-diphosphate
UTP	Uridine-5'-triphosphate
VEGF	Vascular endothelial growth factor

1. INTRODUCTION

1.1. ADENOSINE

Adenosine (Ado) (FIGURE 1.1) is a nucleoside that for one part consists of the heterocyclyc base adenine, which is linked by a β-glycosidic bond to a D-ribofuranose moiety. It is mainly present as the nucleotide adenosine triphosphate (ATP), a RNA building block. ATP is not only a constitutional element, it also serves as an energy transfer molecule, since it is transformed to adenosine diphosphate (ADP) or adenosine monophosphate (AMP). ATP also serves as substrate for kinases, a class of enzymes that catalyzes phosphorylation of other molecules. Intracellularly it is converted by adenylate cyclase (AC) to cyclic adenosine monophosphate (cAMP), a secondary messenger in signal transduction [1]. Also related to adenosine are the cofactors nicotinamide adenine dinucleotide (NAD), flavine adenine dinucleotide (FAD), coenzyme A (CoA) and the methylating agent, S-adenosyl methionine (SAM) [2].

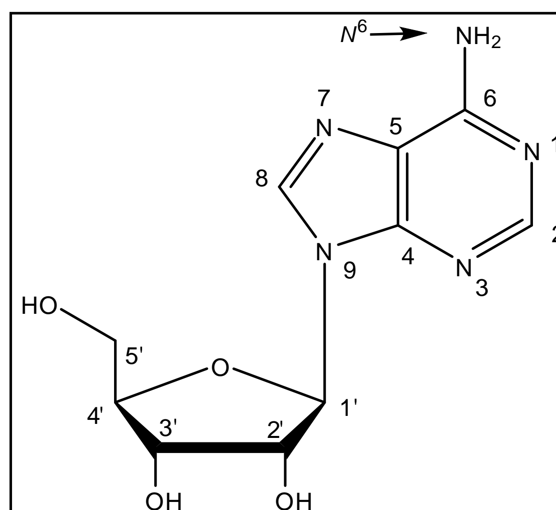


FIGURE 1.1: STRUCTURE OF ADENOSINE

Under normal physiological conditions, adenosine is constantly formed and degraded intra- as well as extracellularly. Adenosine is produced intracellularly by two mechanisms. First it can be formed by hydrolysis of S-adenosyl-homocysteine. Secondly, AMP-dephosphorylation by means of 5’-nucleotidase also gives rise to adenosine. The origin of this AMP is either adenosine itself (which underwent phosphorylation by adenosine kinase) or ATP breakdown. The formed adenosine can be transformed into inosine by adenosine deaminase (ADA) or transported extracellularly [3]. Transmembrane transport is accomplished by facilitated diffusion. This process is equilibrative, which means that an excess of adenosine either intra-

or extracellular is equalized through transport in the other direction. These bidirectional transporters are called ENT_1 and ENT_2 [4]. It has been reported however, that passive diffusion of adenosine can happen, but only in cases of extreme production (e.g. hypoxia) [3].

The origin of extracellular adenosine is versatile. First it can be transported as such from the cells (by mechanisms mentioned above). Next, adenosine is released from certain cell types upon stimulation by e.g. glutamatergic agonists (such as NMDA) or nitric oxide. It was also found that activation of D_1-receptors enhances adenosine release, but does not have a role in maintaining basal levels [3]. Finally, it can be produced from enzymatic breakdown of extracellular nucleotides (ATP, ADP, AMP) (FIGURE 1.2). The last and rate-determining step in this cascade is the dephosphorylation of AMP by ecto-5'-nucleotidase [5]. Transformation of extracellular cAMP by phosphodiesterase (PDE) is another possibility of producing adenosine [3, 5].

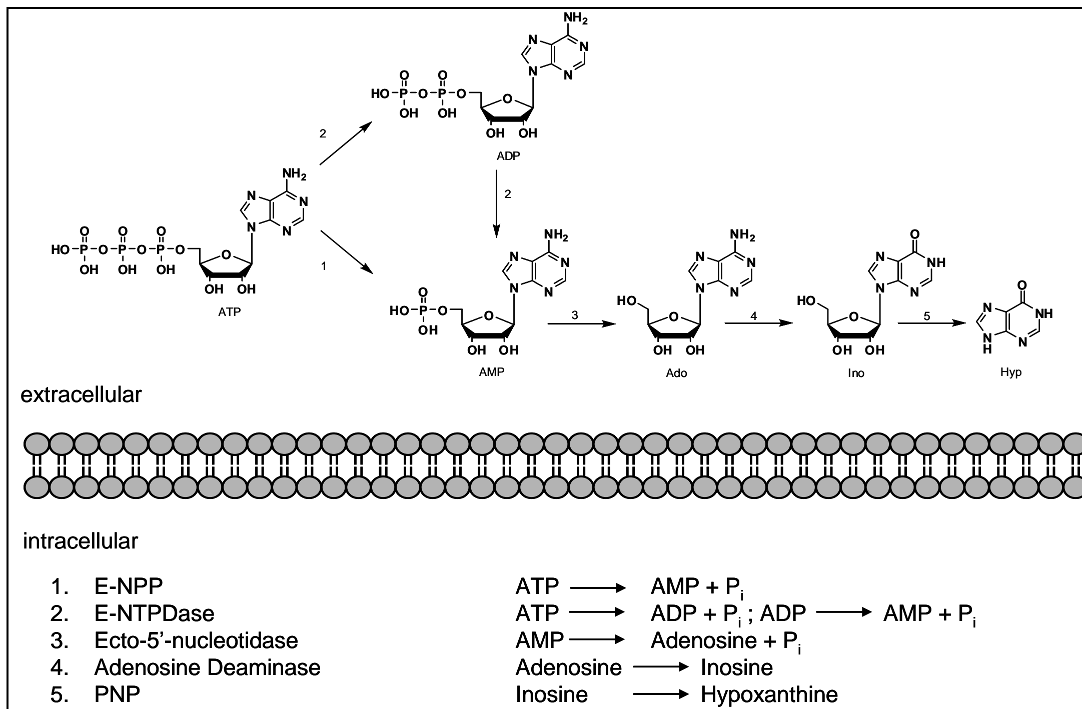


FIGURE 1.2: EXTRACELLULAR BREAKDOWN OF ADENOSINE DERIVED NUCLEOTIDES. (adapted from [6]) The converting enzymes are most abundant on the cell surface (namely membrane-bound); however they can also be found in soluble form. They are then referred to as exonucleotidases [5]. **Ado**= adenosine, **Ino**= inosine, **Hyp**= hypoxanthine, **E-NPP**= ecto-nucleotide pyrophosphatase/phosphodiesterase, **E-NTPDase**= ectonucleoside triphosphate diphosphohydrolase, **PNP**: purine nucleoside phosphorylase.

The extracellularly formed adenosine can undergo re-uptake or enzymatic breakdown to inosine by ADA [6]. A great difference in relation to classical neurotransmitters is the fact that re-uptake of adenosine (into neurons) goes rather slow [7].

The increase of extracellular adenosine was previously thought to originate from intracellular production and subsequent transportation. However, recent research has focussed mainly on extracellular nucleotides [8]. A few mechanisms need to be considered:

- Cell death or apoptosis causes intracellular nucleotides to be set free in the extracellular space [7].
- Storage vesicles in excitory / secretory cells (for example nerve terminals, circulating platelets…) which release ATP together with other neurotransmitters via Ca^{2+}-mediated exocytosis [6].
- Transient release under various conditions such as mechanical stress, hypotonic swelling, serotonin etc [6].
- Co-release of ATP together with other neurotransmitters [9].

1.2. PURINERGIC RECEPTORS

1.2.1. General introduction

The action of extracellular adenosine was first described in 1929 by Dury and Szent-Györgyi. They found that this nucleoside had potent vasodilating and bradycardic properties [10]. In 1971, Burnstock proposed the concept of purinergic receptors and published evidence thereof one year later [11]. This is a logic consequence of the fact that ATP and adenosine were also thought to behave as neurotransmitters. However, it was not until the mid-1990’s that this concept was generally accepted [9]. In 1978, Burnstock proposed a subdivision of the purinoreceptors: the P1 (with Ado as selective agonist) and the P2 (with ATP/ADP as selective agonists) [12]. Nowadays, however, it is recognized that on some P2Y receptors, UTP (uridine triphosphate) is as equipotent as ATP. Also, the $P2Y_6$ receptor is UDP (uridine diphosphate) – selective, while $P2Y_{14}$ receptors are selective for UDP-glucose and UDP-galactose [13].

Upon the present day, four adenosine receptors (ARs) exist, named: A_1, A_{2A}, A_{2B} and A_3. All four ARs are members of the rhodopsin-like GPCR-(G protein coupled-receptor) superfamily [14]. Furthermore, in the class of the P2 receptors a subdivision is made into P2X and P2Y receptors. The former consists of seven human receptors (which are ligand-gated ion channels) [15], the latter of eight receptors, which are also GPCRs [16]. (FIGURE 1.3)

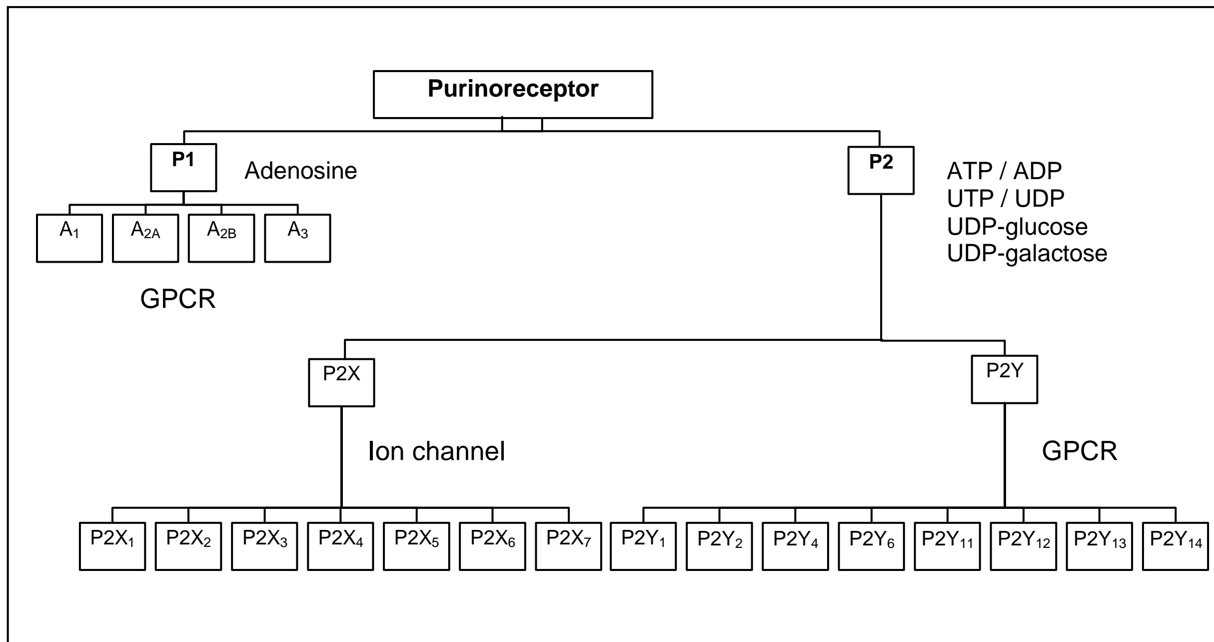


FIGURE 1.3: THE PURINORECEPTOR SUBDIVISION

1.2.2. Adenosine receptors

1.2.2.1. G protein coupled-receptors (GPCRs)

As mentioned before, all four ARs are GPCRs, which contain seven (I-VII) transmembrane α-helical domains (TM1-TM7) [7]. The ARs belong to the rhodoposin family (family A) [17]. Most related are the A_{2A} and A_{2B} (with a 59% sequence similarity), while the A_1 and A_3 receptor show a 49% sequence similarity [18]. An overall 56% sequence identicalness in the transmembrane helices is preserved in all four receptors. Moreover, an average identity of 71% in the upper part of the TM-domains (ligand recognition domain) has been observed. Despite this structural resemblance, they all have different length, N-terminal region, intra/extracellular loops and function of C-terminal region. An interesting feature of the A_{2A} receptor is the 120 amino acid extension at the C-terminus, which is not present in the other three [19]. All ARs have been cloned (since the 1990s) from human as well as other mammalian species, with the A_3-receptor being the last [3].

Until a few years ago, all information about these receptors was either conceived from pharmacological assays or by means of homology modelling using the rhodopsin receptor in its inactive state. However, in 2008, Jaakola et al. and Magnani et al. succeeded to crystallize the A_{2A} receptor [20]. Even more recent, two research groups were able to crystallize the same receptor, but bound to its natural ligand adenosine [21] and two agonists: NECA [21] and UK432097 [22] (**6**, **12** FIGURE 1.8, respectively).

1.2.2.2. Adenosine receptor signalling

ARs, as other GPCRs, mediate their response after agonist association by a signalling cascade. Upon binding of an agonist, the receptor undergoes a conformation change resulting in the association of a guanine nucleotide-protein (G protein) to the receptor-agonist complex. This G protein is composed of three proteins named α, β and γ. An activation-inactivation cycle of this protein is responsible for translating the initial conformational change of the receptor into cellular response [23].

The β and γ proteins are part of an undissociable complex (βγ) and form a functional complex. The α-subunit is able to bind and hydrolyze the nucleotide guanosinetriphosphate (GTP) into guanosinediphosphate (GDP), and controls the cycle. The α-subunit bound to GDP is associated with the βγ-subunit in the basal state. This form is recognized by an activated receptor, which underwent a conformation change, and association results in a ternary complex (agonist-receptor-G-protein). As a result, GDP dissociates from the α-subunit and is replaced by GTP, which on its turn induces another conformational change. As a consequence, the α- and βγ-subunits dissociate. These free proteins couple to other systems, eventually leading to cellular response(s). Ending of the cycle is realized by an intrinsic hydrolysis-activity of the α-subunit forming GDP, which results in reassociation with the βγ-subunit [23]. (FIGURE 1.4)

Generally, signalling of ARs happens through classical pathways, namely by (in)activation of adenylyl (adenylate) cyclase (AC). However, it has become clear that also other mechanisms are implicated, e.g. phospholipase C (PLC), Ca^{2+}- and mitogen-activated protein kinases (MAPK's) [18].

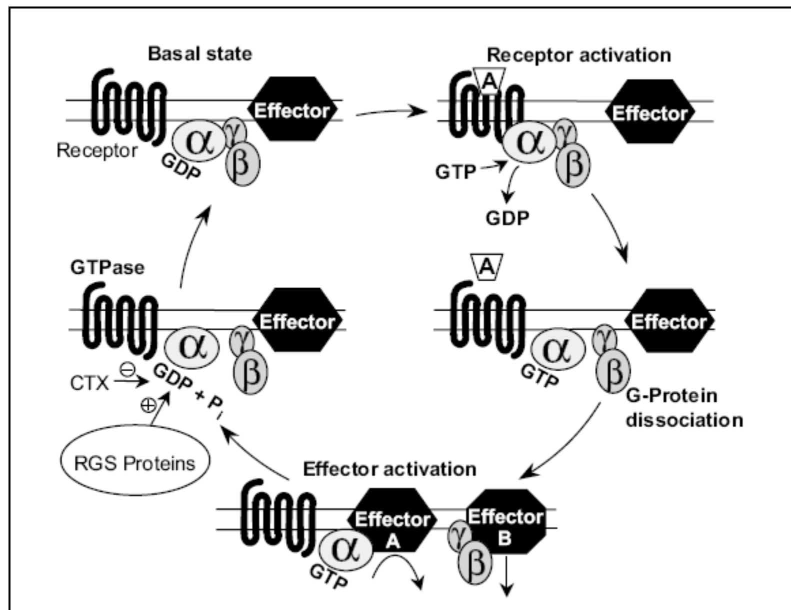


FIGURE 1.4: ACTIVATION AND INACTIVATION CYCLE OF THE G PROTEIN [23]. **A**= Agonist, **CTX**= Cholera Toxin; a bacterial toxin that effects constitutional α-subunit activation by inhibiting its GTP-hydroxylase (GTPase) activity, **RGS**= Regulators of G-Protein Signalling; a class of proteins able to increase the GTPase rate of the α-subunit.

The A_1 and A_3 receptors are negatively coupled to AC, by means of G_i protein activation. This results in reduced formation of cAMP and subsequent diminished activity of protein kinase A (PKA). The βγ-subunit couples positively to PLC. Activation of this enzyme leads to hydrolysis of phospholipide phosphatidylinositol-4,5-bisphosphate (PIP_2), forming two other second messengers diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP_3). IP_3 on its turn activates an intracellular Ca^{2+}-channel, increasing the intracellular [Ca^{2+}]. This leads to a higher activity of protein kinase C (PKC) and other Ca^{2+}-binding proteins, e.g. calmodulin. Additional binding of DAG to PKC is necessary for activation of this kinase. The activated PKC is able to phosphorylate other intracellular proteins and thereby regulating their activity. The A_1-receptor is coupled via a G_O-protein, which inhibits voltage-dependent Ca^{2+}-channels and activates pertussis toxin-sensitive K^+- and K_{ATP}-channels especially in cardiac and neuronal tissue [18, 23, 24]. (FIGURE 1.5)

The A_{2A} and A_{2B} receptors – in contrast to the A_1 and A_3 receptors – couple positively to AC by G_S. In the striatum, however, the A_{2A} receptor couples through G_{olf}, a similar protein to G_S. Activation of AC leads to an increase in cAMP and subsequent higher activity of PKA, which phosphorylates other proteins such as ion

channels, PDEs, and other phosphoproteins. The A_{2B} receptor has been reported to couple positively to PLC via a G_q-mechanism, also resulting in activation [18, 24].

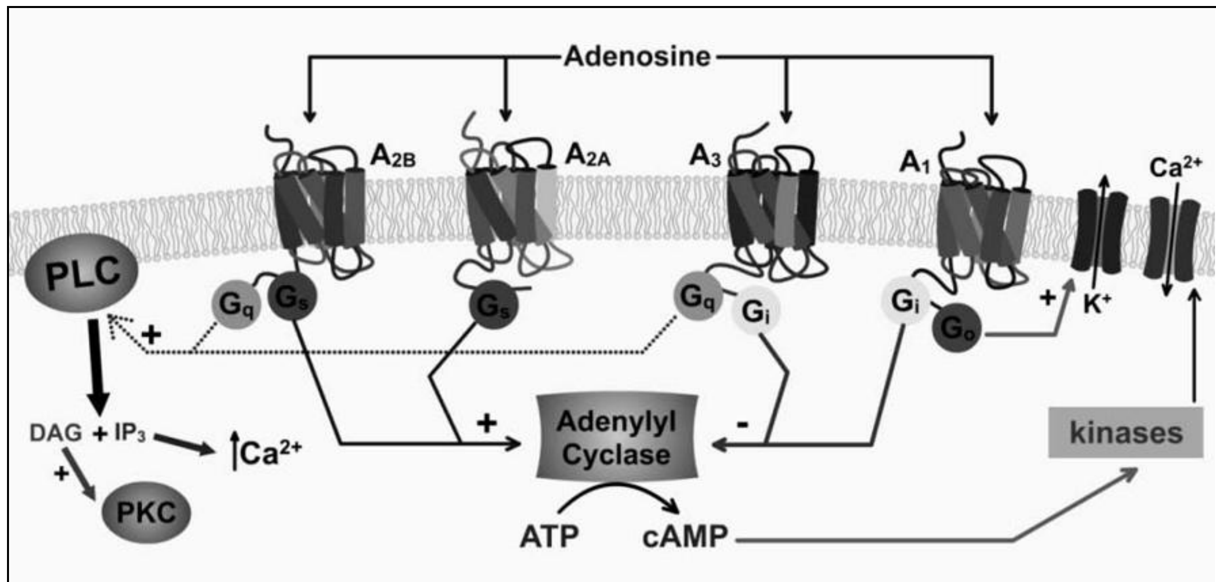


FIGURE 1.5: SUMMARY OF CLASSICAL ADENOSINE RECEPTOR SIGNALLING [19].

The A_{2A} receptor has a significantly longer carboxy-terminus and all the extra amino acids (120) are remarkably conserved among species [19, 20]. As a result, other signalling pathways, which are G protein independent, were found. This domain is also responsible for the formation of heterodimeric complexes with other receptor subtypes, e.g. dopaminergic D_2 receptors [24].

Responses of ARs after agonist activation have been found to desensitize as is the case for all GPCRs. The A_3 receptor is desensitized most rapidly. For the A_{2A} receptor both homo- as well as heterologous desensitization can occur. The former occurs probably by a guanine regulating kinase (GRK) and arrestin-dependent process. The latter has been reported to take place as a result of changes in G-protein subunit expression, AC enzyme function, and PDE-activity [25]. Recently, a new paradigm in relation to A_{2A} receptor signalling based on the bifunctionality of β-arrestins has been proposed. Herein, the β-arrestins are not only responsible for the desensitization and internalization of the receptor, but have also the ability to initiate their own signalling [26].

1.2.3. The A_{2A} receptor

The existence of different types of ARs was first suggested in 1979, following observations that adenosine and its derivatives, were able to increase or decrease cAMP. On this basis, receptors were divided into A_1 (inhibiting) and A_2 (activating) receptors. In 1983, Daly et al. found two subsets of A_2 ARs, both activating adenylate cyclase, but significantly different in their affinity to adenosine. The A_{2A} receptor exhibited high affinity (0.1 – 1.0 µM), while the A_{2B} receptor showed low affinity (≥ 10 µM) towards adenosine. The coding genes for the A_{2A} receptor in humans are found on chromosome 22, and exhibit polymorphism. Furthermore, a high base pair homology (82.9%) between mouse and human receptors is noticed [27].

1.2.3.1. Structure-Activity Relationship (SAR) of A_{2A} receptor agonists

Since the recognition of the clinical utility of Adenosine (**1**, Figure 1.7) in the 1980s [28], the search for potent, selective and metabolically stable A_{2A} agonists continues even today. The problem with administration of adenosine is the short duration of action. The reason for this is obvious: extensive extracellular metabolism and re-uptake [3-6].

Upon the present day, almost all known AR agonists are derivatives of the natural ligand [29]. Nonetheless, substituted pyridines have been reported to exhibit A_{2B} agonist properties [30]. In addition, it is has recently been found that the natural cyclic monoterpene, limonene, exhibits A_{2A} agonist activity [31]. After testing numerous modifications, it was concluded that the adenosine scaffold should be preserved for agonist activity. The only possible positions that can be modified are the 4' CH_2OH of the ribose and the C-2 and N^6-position of the purine. Any of these modifications renders the derivatives metabolically stable [29]. A summary of the SAR for A_{2A} agonists is represented in FIGURE 1.6.

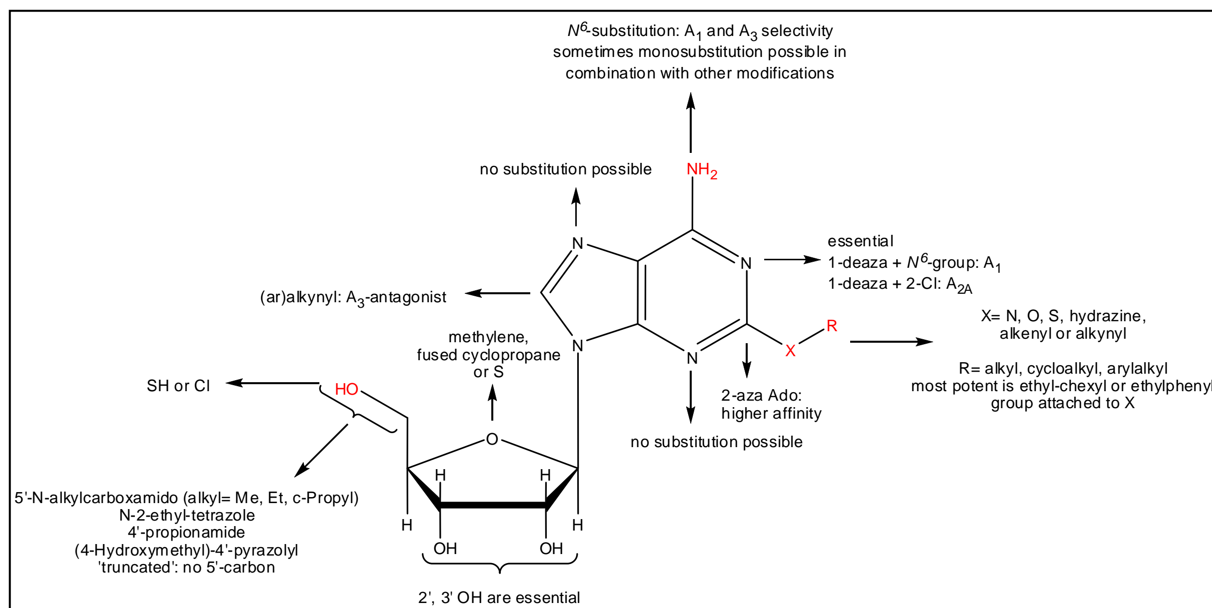


FIGURE 1.6: SUMMARY OF STRUCTURE-ACTIVITY-RELATIONSHIP (SAR) OF A_{2A} AGONISTS.

Ribose modifications

An intact ribose moiety containing the 2' and 3' OH-groups is essential for full agonist activity, although the 3' OH is more tolerant towards substitution [29]. The endocyclic oxygen in the furanose-ring can be replaced by an isosterical sulphur, which leads to an improvement in affinity for A_{2A} [19]. Furthermore the oxygen might also be substituted by a sole methylene group or a methylene part of a fused cyclopropane ring. This modification led to compounds having very low A_{2A} affinity and high selectivity for A_1 and A_3 ARs [29]. Recently, derivatives containing a cyclopentane ring have been reported as potent A_{2A} agonists (K_i ± 5 nM for the most potent ones), showing mild selectivity in respect to A_3 receptors [32]. The 4' CH_2OH is one of the three main positions for modification. Removal of the 5' OH leads to a decrease in potency, but various combinations of substitution restore potent agonist activity. Replacement by a chlorine or a thiol results in greater affinity. Next, an *N*-alkylcarboxamido group is well tolerated by all ARs (as it was first observed by the non-selective agonist *N*-ethylcarboxamido, NECA (**6**, FIGURE 1.8)). The best alkyl chains connected to this amide are methyl, ethyl or cyclopropyl [29].

Change of the 4' CH_2OH by a 2-ethyltetrazole (in combination with a C-2 substituent) led also to potent and quite selective agonists (**2**, Figure 1.7) [33]. A recent paper reports on derivatives bearing either a 4' inverse amide or a 4'

heterocycle. The most potent derivatives have a propionamide or a 4-hydroxymethyl-1-pyrazolyl moiety in combination with a cyclopentane ring and extensive purine modifications (**3**, **4**, Figure 1.7) [32]. The latest paper describes agonists having a 'truncated' (absence of the 5' carbon) five-membered ring containing either an oxygen or sulphur and substitution at C-2 position (**5**, Figure 1.7). It was found that these compounds show dual acting features, namely A_{2A} agonistic together with A_3 antagonistic function [34].

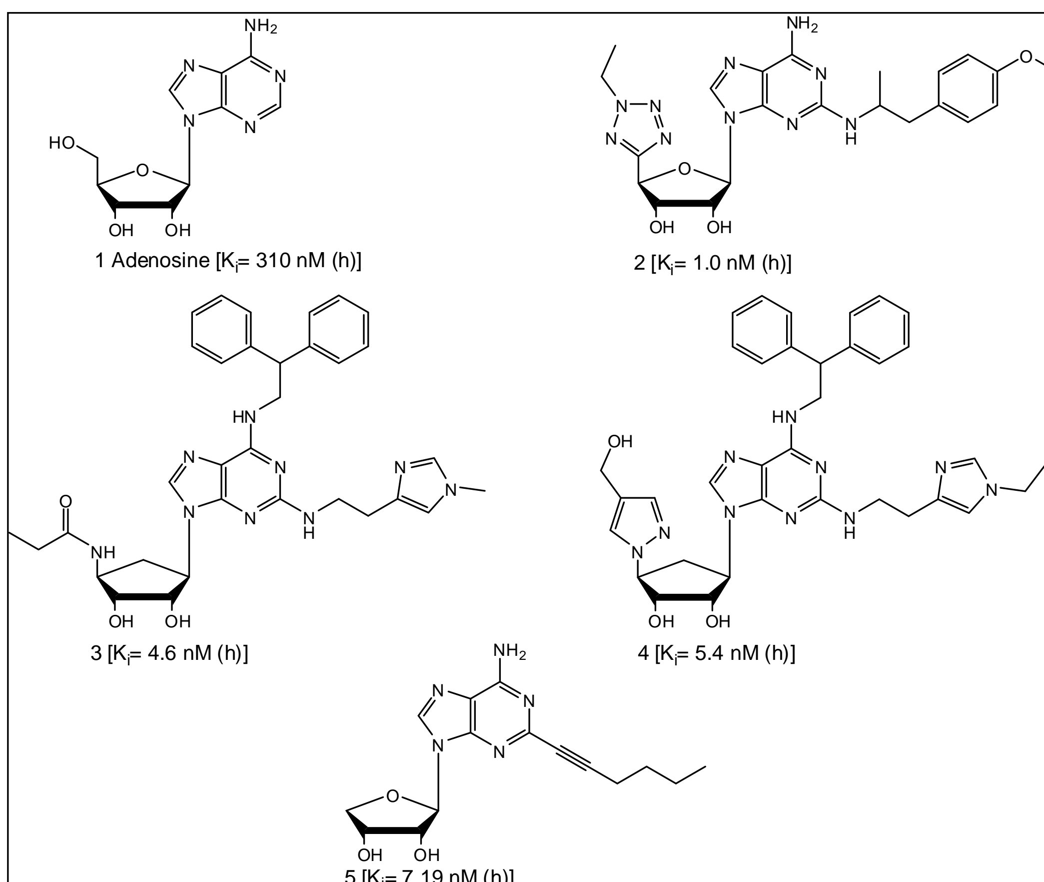


FIGURE 1.7: STRUCTURES OF SOME A_{2A} RECEPTOR AGONISTS. **(h)**= K_i-values determined at human A_{2A} receptors. References for listed K_i values: **1** [35], **2** [33], **3-4** [32], **5** [34].

Purine modification

As is the case for ribose modifications, only few changes in the purine moiety are allowed to maintain or increase affinity for the A_{2A} receptor. It was found that the nitrogen atoms in 3 and 7 position are essential for agonist activity at all AR subtypes. Furthermore, 1-deaza derivatives have diminished affinities and show A_1 selectivity when combined with an N^6-modification [29]. It has also been found that a 2-aza modification of adenosine exhibits a greater binding affinity. Alterations made at the C-8 position of the purine lead to different effects at all ARs, so it is difficult to

draw any general conclusion. Nonetheless, substitution at the C-8 position with (ar)alkynyl chains results in A_3 antagonists [19, 34].

Introducing substituents at the amino group in 6 position (even more when bulky disubstitution at C-2 and N^6) is disadvantageous for A_{2A} affinity. In fact, most of the N^6-substituents lead to A_1 selectivity [19, 29]. Despite these general trends, some changes at N^6-position have been found to increase affinity at the A_{2A}-AR. Examples are the introduction of a 2,2-diphenylethyl group [32, 36, 37] or a 1-ethyl-propyl group [32]. It needs to be noted, however, that also substitution at C-2 and 4' CH_2OH position were present. Furthermore, not all of these compounds have been tested at all four receptors, so conclusions about selectivity are not readily possible.

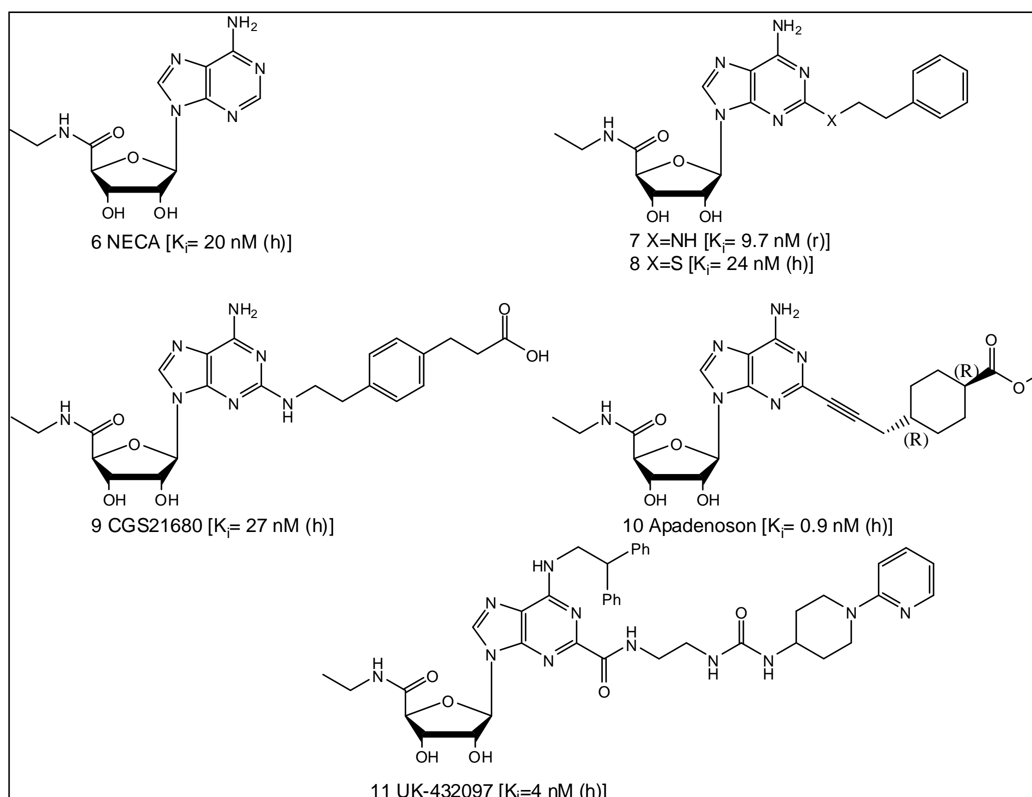


FIGURE 1.8: STRUCTURES OF SOME NECA DERIVATIVES SHOWED TO BE A_{2A} RECEPTOR AGONISTS. **(h)**= K_i-values determined at human A_{2A} receptor. **(r)**= K_i-values determined at rat A_{2A} receptor. References for listed K_i-values: **6-8** [29], **9-11** [35].

Modifications at the C-2 position of the purine with (aryl)alkylamino (**7**, **9**), (aryl)alkylthio (**8**), (aryl)alkoxy, (ar)alkynyl (**10**) (and subsequent alkenyl reduction products), hydrazine, and even N-carboxamido (**11**, FIGURE 1.8) groups have been

tried [29, 36, 37]. It was found that a phenylethyl or cyclohexylethyl group directly coupled to the heteroatom or triple bond showed the greatest affinities [19, 29].

In summary, appropriate 4’ CH_2OH modifications combined with a bulky C-2 substitution may afford promising A_{2A} receptor agonists. These large C-2 substituents, however, need to exist of a quite rigid spacer and bulkiness can not be extreme [29].

1.2.3.2. Therapeutic potential of A_{2A} receptor agonists

The effects of adenosine *in vivo* are widespread and pleiotropic. The cellular response upon agonism or antagonism of the ARs greatly depends on the expression of the four ARs in the targeted tissue. Since they can be co-expressed, selectivity is of major importance to reduce possible side-effects [24]. The most important (possible) applications of A_{2A} receptor ligands are either related to cells of the striatopallidal area in the brain, the immune, and cardiovascular system. The reason for this is obvious: these regions have the highest expression of this AR-subtype [3].

Myocardial stress agents

Coronary artery disease consists of plaque build-up on the artery wall (atherosclerosis), causing reduced blood flow to the heart muscle itself. Progressive closure of the coronary vessels may lead to angina pectoris and even heart attack. This disease accounts for merely twenty percent of deaths in the U.S. Coronary artery disease is detected by myocardial (stress-rest) perfusion imaging (MPI), which primary aim at visualizing the stenosis. The accuracy of this test is greatly influenced by the distribution of a radio-active labelled tracer. Sufficient distribution is normally achieved by patient exercise. However, some patients are unable to exercise sufficiently or the risk of intense exercise is too high, necessitating pharmacological intervention. One approach, being used for decades, is the continuous intravenous administration of adenosine, mimicking the exercise stress. Adenosine exerts a vasodilating effect on coronary and peripheral arterial beds by activating A_{2A} receptors (via a G_s-induced cAMP increase). Activation of ARs results in increased coronary blood flow (distributing the tracer) and unfortunately, tachycardia. Furthermore, since adenosine is a non-selective agonist, it also causes a negative

dromotropic and inotropic effect. These side-effects are mediated by A_1 receptor activation and cause major patient discomfort. Bolus injection of a selective A_{2A} agonist, regadenoson (FIGURE 1.9), has the advantage not to activate the A_1 receptor and subsequently improving patient comfort [38-40].

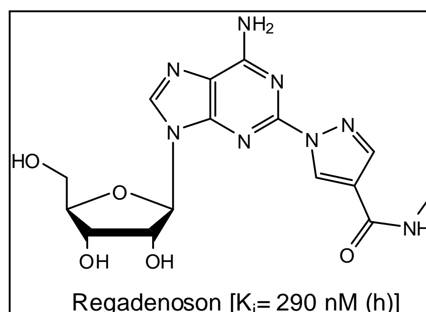


FIGURE 1.9: STRUCTURE OF REGADENOSON. **(h)**= K_i-values determined at human A_{2A} receptor [35].

Inflammation

Inflammation is an important mechanism for the protection against pathogens. After recognition, activated immune cells secrete pro-inflammatory cytokines and chemokines, leading to infiltration of macrophages, lymphocytes and neutrophils. Other inflammatory effects include vasodilatation, increased vascular permeability (to increase infiltration). Secretion of tumor necrosis factor (TNF) and lymphotoxins results in the destruction of pathogens and infected host-cells. However, these responses can, depending on the strength of reaction, cause substantial collateral damage. Furthermore, alterations in this inflammatory process are at the basis of pathophysiological acute and chronic inflammation. In the search for physiological mechanisms to down regulate inflammation, a role for ARs was discovered [41].

(Anti-) inflammatory effects of ARs have been deduced from the observation that extracellular adenosine concentrations increase tremendously in situations of cellular distress and excessive tissue damage. Extracellular adenosine accumulation is mostly induced by tissue hypoxia. Hypoxia inhibits the enzyme adenosine kinase, which converts over 80% of the adenosine to AMP under normoxic conditions. Furthermore, also direct release of adenosine (and adenosine related-nucleotides) after cell lysis contributes to the steep increase. It was found that A_{2A}-receptor signalling plays a crucial role in the physiological downregulation of inflammatory responses [41, 42]. (FIGURE 1.7)

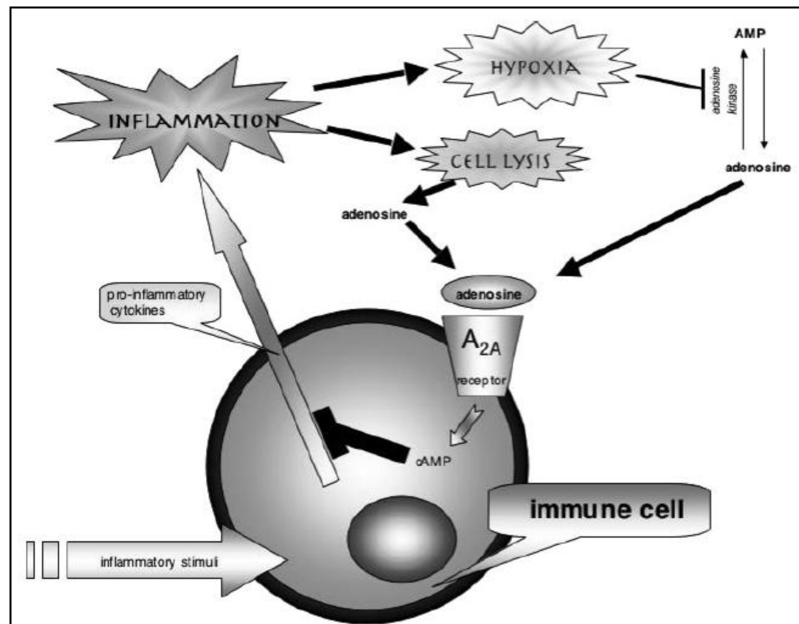


FIGURE 1.7: SIMPLIFIED REPRESENTATION OF THE 'METABOLIC SWITCH' MODEL LEADING TO DOWN-REGULATION OF INFLAMMATION [41].

Activation of A_{2A} receptors leads to an intracellular increase of cAMP after G_S-mediated AC activation. This rise in cAMP-levels effects many other signalling pathways. Amongst them, PKA, a kinase that activates the nuclear substrate cAMP responsive element-binding protein (CREB) by phosphorylation, is the most important. CREB is a protein able to activate genes with cAMP responsive elements (CRE) in their promoters. This activation subsequently leads to altered (higher) transcription. Next, phosphorylated CREB competes with nuclear factor-κB (NF-κB) p65 for a co-factor named CREB-Binding Protein, CBP. CBP is also used by NF-κB, to exert its transcriptional activity, which results in increased cytokine expression. These cytokines, such as TNF-α, are the main cause of inflammation. In this way activated A_{2A} receptors are able to – via CREB – regulate TNF-α expression. Besides this pathway, the rise in cAMP and PKA activation are also linked to other signalling molecules leading to suppressed cytokine release [42, 43]. (FIGURE 1.8)

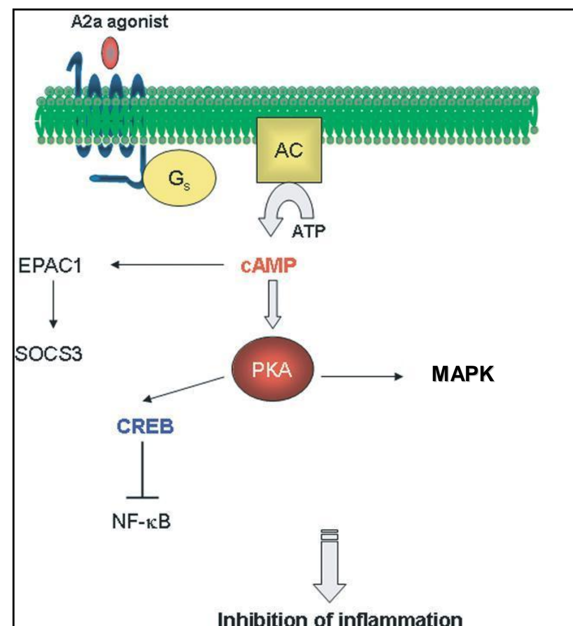


FIGURE 1.8: A_{2A}-RECEPTOR SIGNALLING IN RELATION TO INFLAMMATION INHIBITION. (adapted from [42]). **EPAC1**= Exchange protein directly activated by cAMP-1, **SOCS3**= Suppressor of cytokine signalling-3, **MAPK**= Mitogen activated protein kinase.

This anti-inflammatory effect might be used in the treatment of inflammatory respiratory diseases, rheumatoid arthritis, ischemia reperfusion injury and also complications of diabetes [41].

Wound healing

Foot ulcers are a major complication of patients suffering from diabetes. This condition greatly affects patient quality of life. Current treatment is either based on less effective non-pharmacological measures or topical administration of growth factors, which can cause malignancies. Therefore, a lot of effort has been put to reveal pathophysiological processes of (diabetic foot) ulcers. A first phase in the complex process of wound healing is haemostasis and inflammation. The formation of a blood clot enables recirculation and provides an attachment point for tissue regeneration. Inflammation causes leukocyte infiltration and release of cytokines and chemokines, which stimulate fibroblasts and epithelial cells. However, prolonged or aggravated inflammation is detrimental for adequate healing; with a prominent role for TNF-α. As mentioned above, adenosine plays a substantial role in release of cytokines and chemokines, mostly through A_{2A} receptors. Better control over the inflammation process, would ensure a more proper healing process [44].

A second phase consists of collagen synthesis by fibroblasts. This process has been shown to depend on an A_{2A} receptor activation and subsequent MAPK-signalling pathway. Since most diabetic patients suffer from endothelial dysfunction and consequently reduced neovascularisation, wound healing is impaired. Adenosine can increase the expression of vascular endothelial growth factor (VEGF), and thereby stimulate both angiogenesis and vasculogenesis. However, also other adenosine stimulated pathways are responsible for angiogenesis. Finally, proteases degrade the provisional extracellular matrix, which is then replaced by a definite one. A_{2A} activation leads to increased intracellular cAMP-levels, that are responsible for acute tPA (tissue plasminogen activator) release. This serine protease is the main initiator for further activation of other proteases, which degrade the primary formed extracellular matrix [43, 45].

Sleep disorders

Since adenosine acts as a local agent enabling energy homeostasis it should come as no surprise that it also affects sleep and wakefulness. A period of sleep deprivation causes an increase in adenosine levels, which can induce sleep. Furthermore an overexpression of cytosolic ADA (responsible for extracellular adenosine breakdown) has shown to increase wakefulness. It was long emphasized that this effect was mediated by A_1 receptors, but A_1 knock-out mice showed quite normal sleep patterns. It has also known for a long time that caffeine (found to be a non-selective A_{2A} antagonist [46]), increases attention and arousal. Direct injection of A_2 agonists into the brain, on the other hand, was able to induce sleep, by mediating the effects of prostaglandin D_2 (a sleep-inducing substance). A_{2A} receptors co-expressed with D_2 receptors in the basal ganglia of the brain are also believed to be responsible for sleep control [8].

Clinical approved drugs

Upon the present day, there are only two A_{2A}-agonists approved and commercially available. The natural agonist itself (Adenocard®) is used for terminating paroxysmal supraventricular tachycardia, Wolff-Parkinson-White syndrome (both via A_1 receptors) and as a diagnostic tool. Nowadays however, regadenoson (Lexiscan®; approved in 2008 by the FDA) has mainly replaced adenosine for the latter indication [24].

2. OBJECTIVES

Since adenosine receptor research started about thirty years ago, a vast amount of possible agonists has been synthesized and tested. Therefore, a primary aim of this thesis was to compile a database consisting of AR agonists, with the focus on the A_{2A} subtype. This literature search resulted in a significant number of analogues structurally derived from Ado structure. However, 2-alkoxy / 2-araloxy-5'-*N*-ethylcarboxamidoadenosine were scarce.

In 2007, a NECA derivative, bearing a 2-(indol-3-yl)-ethoxy group was synthesized by Adachi et al., as A_{2B} agonist. This compound showed only moderate affinity for all ARs and was not selective [47]. It was believed that the combination of an appropriate C-2 substitution combined with the replacement of the 4' CH_2OH with a *N*-ethylcarboxamido group would result in potent, but in particular selective A_{2A} agonists. C-2 substitutions bearing a cyclohexylethyl or phenylethyl group bound to a hetero-atom (in adenosine as well as in NECA derivatives), were already found to result in potent agonists [29].

Thus, the objective of this thesis was the synthesis of a NECA derivative bearing a phenethoxy group at C-2 position (**1**, FIGURE 2.1). Furthermore, special attention was to be paid to the versatile character of the synthetic procedure in order to enable facile synthesis of a series of related derivatives.

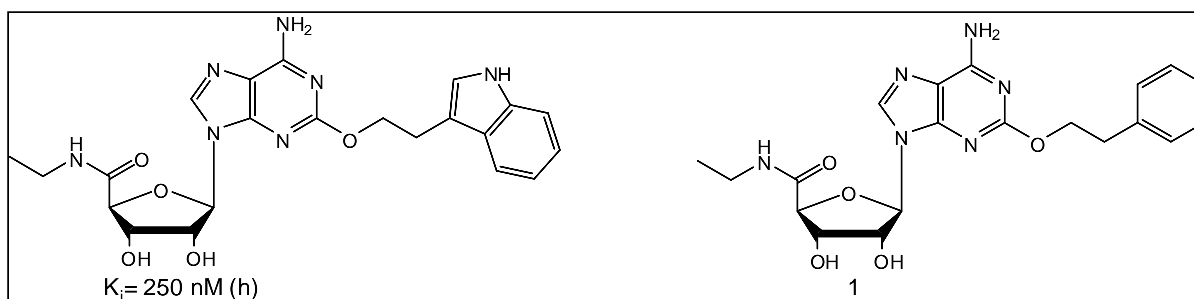


FIGURE 2.1: STRUCTURES OF 6-AMINO-2-(INDOL-3-YL)-ETHOXY-9-(5'-*N*-ETHYLCARBOXAMIDO-β-D-RIBOSFURANOSYL)PURINE AND 6-AMINO-2-PHENETHOXY-9-(5'-*N*-ETHYLCARBOXAMIDO-β-D-RIBOSFURANOSYL)PURINE (**1**). K_i-value determined at human A_{2A} receptor [47].

3. MATERIALS AND METHODS

3.1. METHODS

3.1.1. Nuclear Magnetic Resonance (NMR) [48]

3.1.1.1. The principle of NMR

NMR is an important spectroscopic technique, mostly used for structure elucidation and confirmation, since it provides information about the carbon-hydrogen network of a molecule. Together with other spectroscopic techniques such as infra-red (IR), which gives information about functional groups, and mass spectroscopy, it is possible to elucidate even very complex compound structures.

Many nuclei possess a property called “spin”: their nuclei (consisting of protons and neutrons) behave as if they were spinning. Among these, the most commonly used are ^{1}H, ^{13}C and ^{31}P. However, any nucleus that has either an odd mass, an odd atomic number or both, possesses the property of “spin”. Every nucleus with a spin can only adopt a quantized number of spin states and is determined by its nuclear spin quantum number *I*. The number *I* is a physical constant and for each nucleus there are $2I + 1$ allowed spin states, with integral differences ranging from $-I$ to $+I$. For the ^{1}H-nucleus, *I* is ½ and there exist two spin states, namely –½ and +½. These different spin states are of equal energy in the absence of an external magnetic field (B_0). However, when an external magnetic field is applied, these two states differ in energy. The reason for this being the fact that moving charged particles (here the positively charged nucleus) generate their own magnetic field. For example: the hydrogen nucleus may have a clockwise (+½) or a counter clockwise (–½) spin and the magnetic moments (μ) are pointed in opposite directions. (FIGURE 3.1) When an external magnetic field is applied, the magnetic moment of the nuclei is either aligned (low energy) or opposed (high energy). This difference in energy depends on the strength of the field applied and also on the type of nucleus considered.

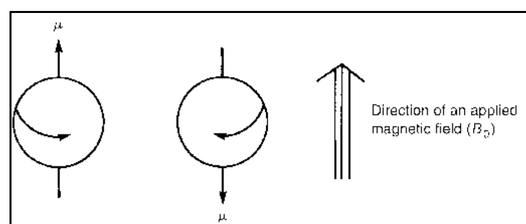


FIGURE 3.1: THE TWO ALLOWED SPIN STATES FOR A ^{1}H-NUCLEUS [48].

Magnetic resonance takes place when aligned nuclei are induced to absorb energy, and thereby change their spin orientation towards the applied field. Protons, for example, absorb energy since the magnetic field causes them to precess around its own spin axis. Precession occurs with a distinct angular frequency (Larmor frequency) and is directly proportional to the external field strength. As a result, an electric field with the same frequency is generated. When electromagnetic irradiation with equal frequency is applied, an energy transfer can occur, finally resulting in spin change. This process is called resonance and the nucleus is said to have resonance with the incoming wave.

However, not all protons in a molecule exhibit the same resonance. This variability is due to a different electron density surrounding each proton. As a result, each proton is shielded from the external magnetic field by different amount. The greater the electron density surrounding a proton, the lower the field a proton will experience. Because of a lower field, a slower precession (smaller angular frequency) and absorption of electromagnetic irradiation takes place. The resulting differences in frequency can be very small and exactly measuring them at sufficient precision is difficult. Therefore, a reference compound, tetramethylsilane (TMS) is added to the sample. This compound has twelve protons which are more shielded than those of most other molecules. Consequently, resonance frequencies are reported as how far (in Hz) they shifted from the reference signal. As mentioned before, frequencies and therefore also the frequency difference, depends on the external magnetic field strength. To avoid confusion by using different magnets, all shifts are reported normalized to the field applied. The chemical shift (δ) is defined as:

$$\delta = \frac{\text{shift in Hz}}{\text{operating frequency in MHz}} \qquad \text{EQUATION 3.1}$$

Chemical shifts express the amount a given proton deviates from the reference signal (by definition given the value zero) in parts per million (ppm).

Another phenomenon in ^{1}H-NMR spectroscopy is that a proton can “feel” the number of equivalent protons bound to the adjacent carbon atom. This leads to spin-spin splitting, and can be explained by the (*n*+1)-rule. The signal of a proton will split

into (n+1)-signals, whereby n is the number of adjacent equivalent protons. The intensity ratios of a multiplet follow the numbers in Pascal's Triangle. For example: the signal of a triplet will have intensity ratios of 1 : 2 : 1. The distance between the peaks is called the coupling constant or J. It is, in fact, a measure of how strongly a certain nucleus is affected by the spin states of its neighbour. J is calculated as the distance between two peaks and expressed in Hertz (Hz). Furthermore, coupling constants of protons groups that split each other must be identical (experimental error taken into account).

3.1.1.2. The NMR spectrum

An NMR spectrum represents the intensity as a function of resonance frequency (represented relative to a reference as chemical shift). An enormous amount of information can be deduced from a spectrum. First, the number of resonance peaks is an indication for the amount of different protons present in the molecule. Secondly, the position (chemical shift) in the spectrum provides knowledge about the chemical environment. Next, the area under a peak is a measure for the number of protons. Finally, the splitting pattern (the count of peaks as well as their ratio) indicates the identity of the neighbouring protons.

3.1.1.3. Instrumentation

For the recording of all ^{1}H-NMR spectra, a Varian Mercury 400 MHz spectrometer was used. All samples were dissolved in DMSO-d_6.

3.1.2. Mass Spectroscopy (MS) [49, 50]

3.1.2.1. The principle of MS

Mass spectroscopy (MS) is an analytical technique also used for identification of organic compounds. Firstly, the introduced sample undergoes ionization, yielding molecular as well as fragment ions. Depending on the type of ionization source both positive as negative ions can be formed. After ionization, ions are directed to a mass analyzer, which separates ions by their m/z (mass-to-charge) ratio.

A wide range of both ionization sources and mass analyzers are commercially available. All mass spectra in this thesis are recorded on an electrospray ionization –

single quadrupole mass spectrometer with a direct liquid injection as sample introduction.

Electrospray Ionization (ESI) is a kind of desorption ionization technique, operated under atmospheric pressure and temperature. A liquid sample is introduced into a small capillary needle at a constant flow rate. This needle is maintained at a potential of several kilovolts. As a result, charged droplets (i.e. a spray) are formed at the tip of the capillary. Some molecules however, possess a charge (depending on the pH of the solvent) from themselves, while other compounds form adducts with present ions such as H^+, Na^+, K^+, Cl^-… The remaining solvent is removed by guiding the spray through another capillary while orthogonally applying a drying gas. By decreasing the droplet size through evaporation, the charge density increases until the surface tension is not longer able to support the charge. At that point, 'Coulombic explosion' occurs, which tears down the droplet in several smaller ones. Repetition of this process ultimately leads to formation of (multiply) charged analyte molecules.

The formed ions need to be separated based on their m/z ratio by a mass analyzer before detection. A quadrupole consists of four parallel cylindrical rods, whereby two opposing rods are connected electrically. One pair is attached to the positive pole of a varying dc source and the other pair connected to the negative end. Furthermore, 180° out of phase radio-frequency ac voltages are applied to each pair of rods. Ions are first accelerated by a potential difference. Simultaneously, both the ac and dc voltages are increased while keeping their ratio constant. This changes the ion trajectories depending on their m/z ratio. At any given moment, only ions with a certain m/z ratio will have a stable trajectory in the oscillating electrical fields and therefore reach the detector (most commonly an electron-multiplier). Ions that do not exhibit a stable trajectory will collide with one of the rods and be neutralized by it.

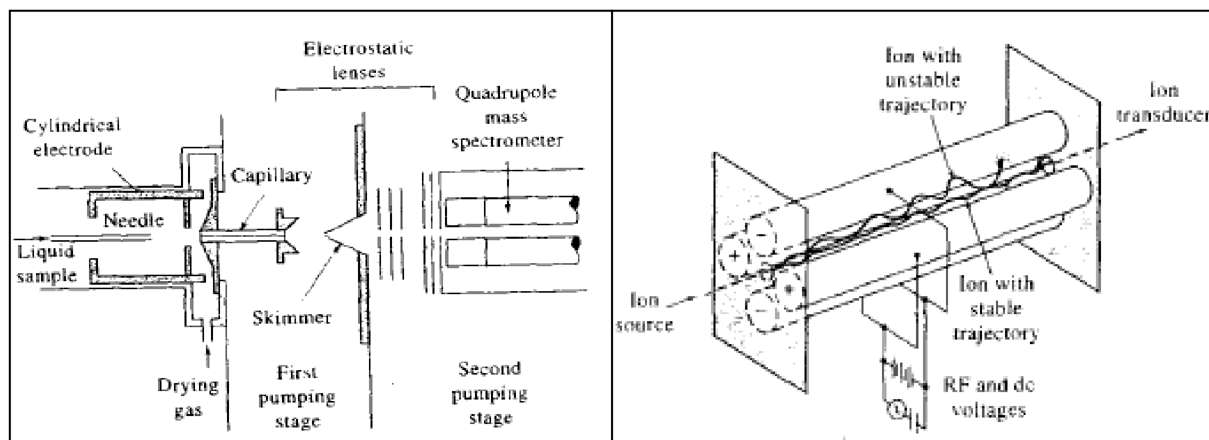


FIGURE 3.2: (left) GENERAL INSTRUMENTATION OF AN ESI-QUADRUPOLE MASS SPECTROMETER, (right) DETAIL OF A QUADRUPOLE MASS ANALYSER (SKOOG, D. A., et al. 2007). [50]

3.1.2.2. The mass spectrum

After ionization, separation of ions based on m/z ratio and detection, a mass spectrum is generated. A mass spectrum depicts the relative abundance (the most abundant ion is assigned value 100%) as a function of the mass-to-charge ratio. The amount of certain ions is characteristic for a specific molecule (its structure as well as composition). The presence of the molecular ions gives information about the entire molecule, while fragments can be used for structure elucidation. Given the fact that ESI is a soft ionization technique, little fragmentation is observed. Therefore, mass-spectrometry will primarily be used for further structure conformation (and detection of the presence of impurities) after ^{1}H-NMR analysis.

3.1.2.3. Instrumentation

In this thesis, all mass spectra were recorded on an ESI – Single Quadrupole Mass Spectrometer from Agilent Technologies Inc. (Santa Clara (CA), USA).

3.2. MATERIALS

Thin Layer Chromatography (TLC) was performed using SIL G-25 UV_{254} glass plates (5 x 10cm) from Macherey-Nagel (Düren, Germany) and cut to appropriate size before use. Visualization occurred by UV-detection (254 nm), iodine chamber or basic permanganate solution. Reversed phase TLC was performed using RP-C_{18} F_{254S} glass plates (5 x 10 cm) from Merck (Darmstadt, Germany) and cut to appropriate size before use.

Preparative TLC was performed using Silica Gel GF 500 μm (20 x 20 cm) glass plates purchased from Analtech Inc. (Newark (DE), USA).

Silica gel column chromatography was always performed using Silica Gel 60 purchased from Fluka Analytical (Steinheim, Germany).

Reagents used were purchased from Fluka Chemika (Steinheim, Germany), Panreac (Barcelona, Spain), Simga-Aldrich (Steinheim, Germany). Amano Lipase PS (crude enzyme) was purchased from Sigma Aldrich (Steinheim, Germany). Liquid ammonia was purchased from Ucar Speciality Gases (Westerlo, Belgium).

Solvents used were purchased from Carlo Erba reagents (Milano, Italy) or Sigma-Aldrich (Steinheim, Germany).

4. CHEMISTRY

4.1. RETROSYNTHETIC ANALYSIS

In order to obtain compound **1**, both a convergent and divergent approach were investigated. A convergent approach would allow simultaneous synthesis of the purine and ribose building block, with coupling being the penultimate step. This would ensure a minimum of different characterizations, which comprises a great advantage in synthesizing a series. 2-Chloroadenine (**3**, FIGURE 4.1) was therefore found to be a good purine starting material, but because of its relative high cost, 2,6-dichloropurine (**4**, FIGURE 4.1) was eventually selected.

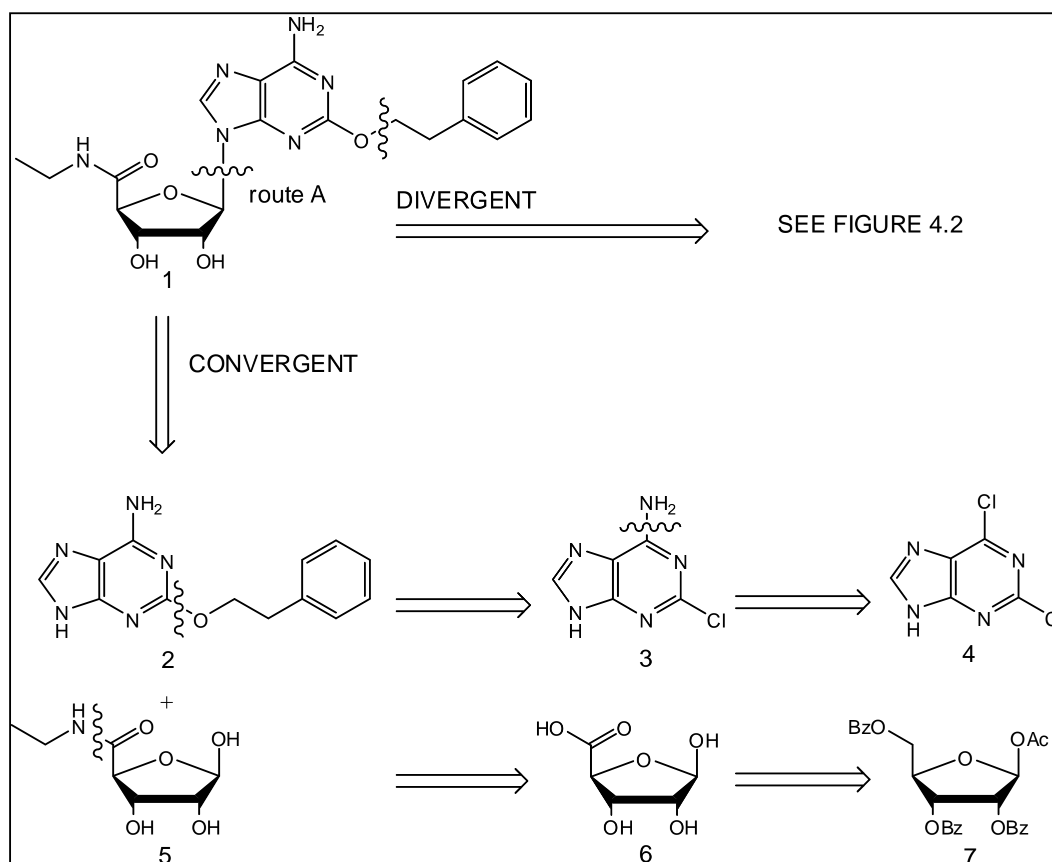


FIGURE 4.1: CONVERGENT RETROSYNTHETIC ANALYSIS.

Deciding on an appropriate ribose proved more challenging because of the apparent difficulties (reported low yield, multiple steps) in introducing suitable protecting groups and to carry out the 5' carbon modification. Compound **7** (FIGURE 4.1) was chosen, since recently Martinez-Montero et al. described a biocatalytic, selective deprotection of a structurally related derivative in high yield. [51]

Investigation of a divergent approach gave also rise to a versatile possible synthetic route with the most crucial step being the transformation of the C-2 amino into a hydroxyl group (path A, FIGURE 4.2) or direct coupling with the desired alcohol to obtain the aralkoxy derivative (path B, FIGURE 4.2). Therefore, the N^6-position needs to be functionalized with a suitable leaving group, which can undergo aminolysis after C-2 modification. An obvious and straightforward choice would be 2-amino-6-chloropurine-9-β-D-ribofuranose (**13**) following the literature procedure for the synthesis of 6-amino-2-(indol-3-yl)ethoxy-9-(5'-*N*-ethylcarboxamido-β-D-ribofuranosyl)purine [47]. However, in view of product price, this was not an option. Therefore, a suitable N^6 modified derivate **10**, starting from the relative inexpensive building block guanosine (**12**), is to be prepared. (FIGURE 4.2)

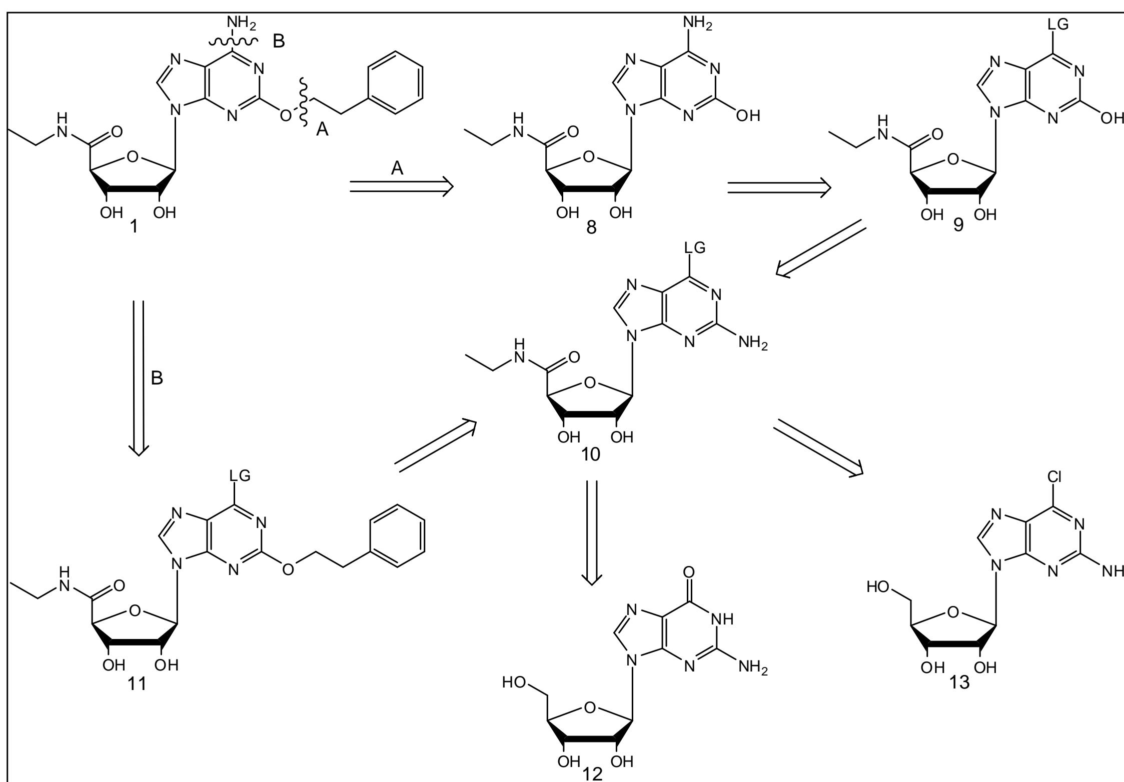


FIGURE 4.2: DIVERGENT RETROSYNTHETIC ANALYSIS.

In view of appealing reported yields and the simultaneous preparation of both constituents a convergent approach was initially pursued.

4.1.1. Convergent approach

The proposed convergent synthetic route to obtain compound **1** is depicted in FIGURE 4.3. Reaction conditions are either derived from literature [51-53], or existing in-house procedures.

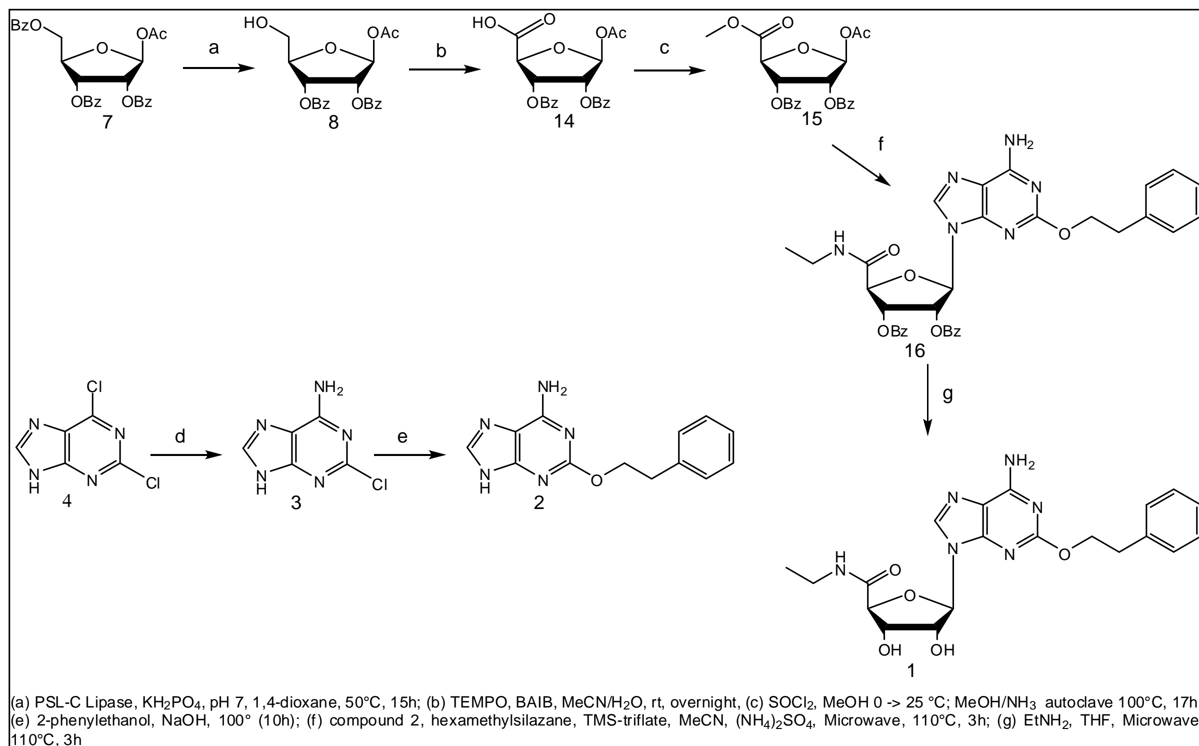


FIGURE 4.3: PROPOSED CONVERGENT SYNTHESIS OF **1**.

4.1.1.1. Synthesis of 2-chloroadenine (**3**)

The convergent approach started with the nucleophilic aromatic substitution on 2,6-dichloropurine (**4**) using an in-house procedure employing liquid ammonia. Rigourous temperature control resulted in selective N^6-position substitution. (FIGURE 4.4)

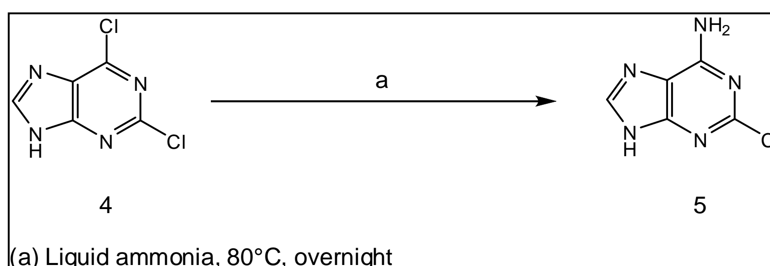


FIGURE 4.4: SYNTHESIS OF 2-CHLOROADENINE (**5**).

4.1.1.2. Synthesis of 2-phenethoxyadenine (**2**)

2-phenethoxyadenine (**2**) was prepared by nucleophilic aromatic substitution as described for 2-butoxyadenine in literature by Kurimoto, A. et al. [52]. Preparation of the phenylethoxy nucleophile was achieved by reacting solid sodium in the alcohol. Difficulties upon purification were encountered since the mixture formed a gel-like solid after cooling below 110 °C. Furthermore, the possible presence of unreacted sodium was not favourable towards the work-up. For this reason, use of the same work-up procedure as in [54] was not applied. Destruction of sodium occurred through adding of ethanol. Evaporation of the remaining solvent and liquid extraction with hydrochloric acid did not give the desired result, so column chromatography for purification was used. In view of this, another attempt was made to synthesize **2** using a modified in-house procedure. Replacing the sodium by NaOH resulted in a slower reaction and above all lower yielding method. (FIGURE 4.5)

A possible improvement could be the use of a strong, non-nucleophilic base such as sodium hydride, 1,8-diazabicyclo-undec-7-ene (DBU) or lithiumdiisopropylamide (LDA). Nonetheless, derivatization of **4** in N-9 position with a 3,4-dihydropyran, subsequent amination followed by nucleophilic aromatic substitution and deprotection has already shown to be a viable alternative [55].

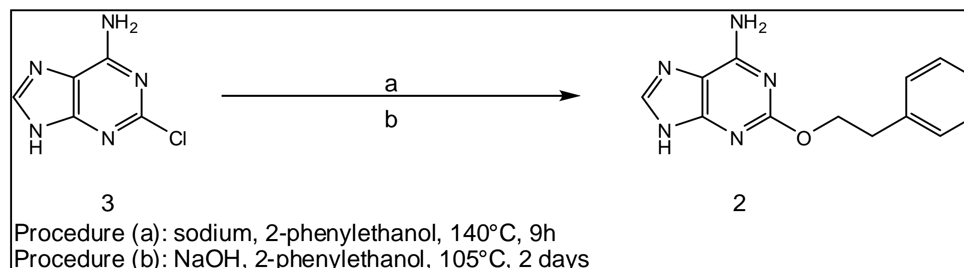


FIGURE 4.5: SYNTHESIS OF 2-PHENETHOXYADENINE (**2**).

4.1.1.3. Attempted synthesis of 1-*O*-acetyl-2,3-*O*-dibenzoyl-β-D-ribofuranose (**8**)

Martinez-Montero, S. et al. reported the selective enzymatic hydrolysis of di-*O*-toluoyl-1,2-dideoxyribose [51], which we attempted on compound **8**. A first attempt showed no reaction upon continuous monitoring by TLC. Since a suspension of **7** was formed, low solubility and subsequent accessibility might have been the problem. Therefore, in a second attempt 1,4-dioxane was added until complete dissolution changing the ratio of 2:1 phosphate buffer/1,4-dioxane to 1:2. Also this attempt proved to be unsuccessful after continuous TLC monitoring for 2 days. A

possible explanation might be the lowered solubility of *Pseudomonas cepacia* crude lipase. Furthermore, the presence of both the acetyl and benzoyl group in 1 and 2 position respectively cause additional sterical hindrance, leading to unavailability of the active centre. This explanation thereby defies the initial assumption of structural resemblance.

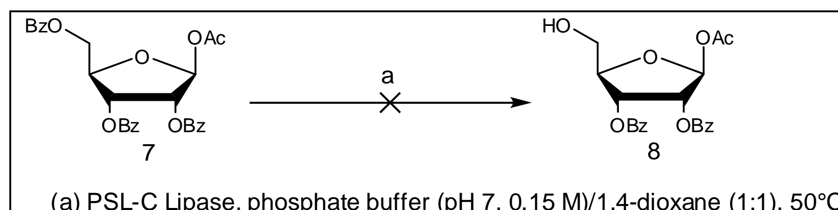


FIGURE 4.6: ATTEMPTED SYNTHESIS OF 1-*O*-ACETYL-2,3-*O*-DIBENZOYL-β-D-RIBOFURANOSE (**8**).

Selective enzymatic deprotection of 7 failed, which obliged revision of the proposed synthetic route.

It was decided to selectively protect the 5'-OH as a trityl-ether, which could later be cleaved under mild acidic conditions, while leaving a suitable ester protecting group (such as acetate, benzoate) intact.

4.1.1.4. Synthesis of 5-*O*-triphenylmethylene-β-D-ribofuranose (**18**)

Protection of β-D-ribofuranose (**17**) occurred through an S_N1 reaction mechanism following a literature procedure [56]. This procedure was chosen because it excludes the use of pyridine. It was found that the reaction produced the title compound in low yield (9%).

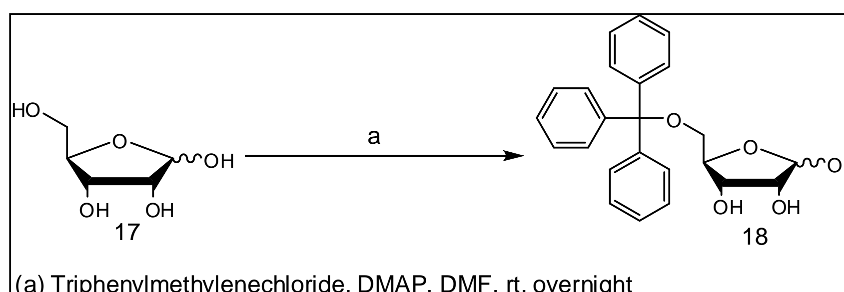


FIGURE 4.8: SYNTHESIS OF 5-*O*-TRIPHENYLMETHYLENE-β-D-RIBOFURANOSE (**18**).

An alternative could be producing the 5-O-tritylether combined with 1,2,3-tri-*O*-benzoyl groups in one step [57].

Considering the low yield of the 5’ OH protection and difficulties encountered with the purine building block it was decided to switch to a divergent synthetic approach.

4.1.2. Divergent approach

The initially selected divergent approach to obtain compound **1** is depicted in FIGURE 4.9. The proposed synthetic route is based on existing literature procedures: [47, 53, 58].

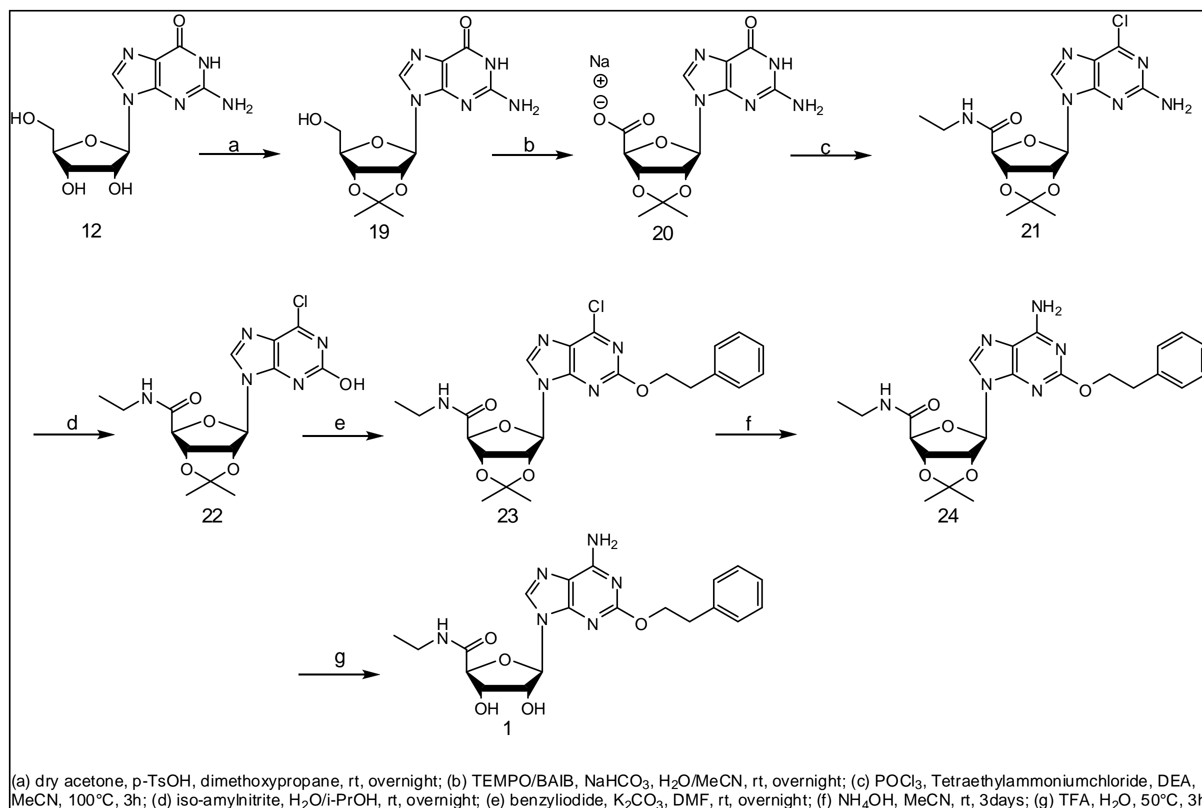
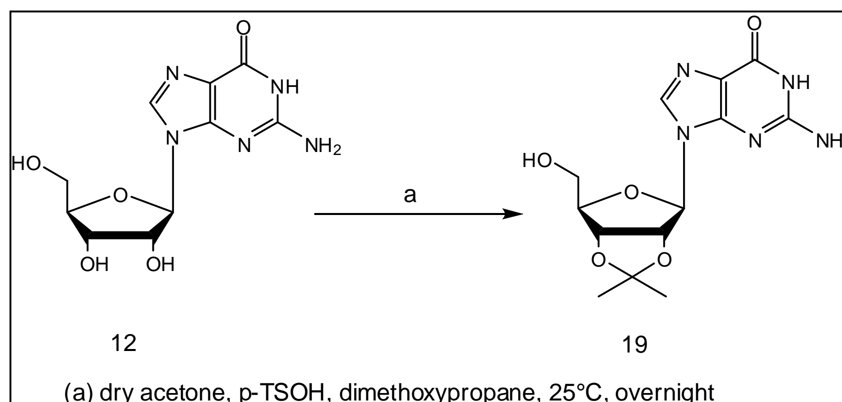


FIGURE 4.9: PROPOSED DIVERGENT SYNTHETIC ROUTE

In literature there are three suitable leaving groups for *N*[6] modification described, namely: a benzotriazole [53, 59], a chlorine [53] or an arylsulphonate [60]. Although chlorination is reported to be capricious, it was initially tried in view of preparing intermediate **21** in one step instead of two. Secondly, the existence of an in-house procedure for the chlorination of 2’,3’,5’-*O*-triacetylguanosine in high yield (80 %) contributed to this choice.

4.1.2.1. Synthesis of 2’,3’-*O*-isopropylideneguanosine (**19**)

Selective protection of the 2’,3’-*syn* diol was achieved by acetal formation with dimethoxypropane in acetone and p-toluenesulfonic acid (p-TSOH) [53]. Since the reaction is reversed when large quantities of water are present, dry acetone was used.

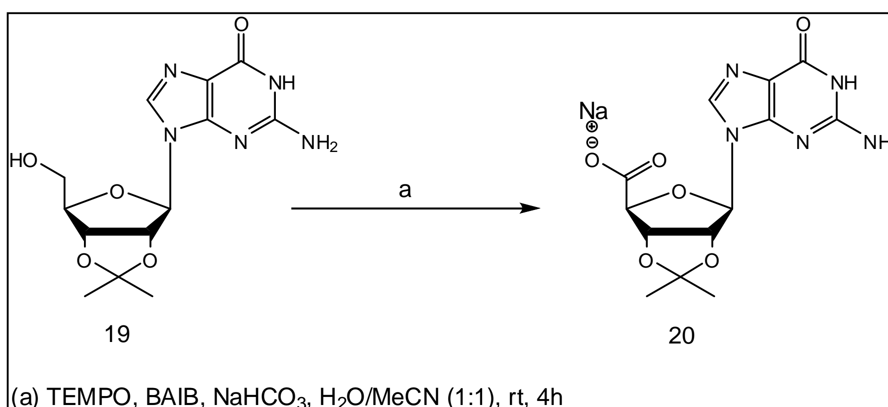


(a) dry acetone, p-TSOH, dimethoxypropane, 25°C, overnight

FIGURE 4.10: SYNTHESIS OF 2’,3’-*O*-ISOPROPYLIDENEGUANOSINE (**19**).

4.1.2.2. Synthesis of sodium 2’,3’-*O*-isopropylideneguanosine-5’-carboxylate (**20**)

A vast amount of oxidizing agents could be used for transforming hydroxyl groups into carboxylic acids. (2,2,6,6-Tetramethylpiperidin-1-yl)-oxyl (TEMPO) mediated oxidations are mild and in particular environmental friendly. A literature procedure [58] was slightly modified, resulting in higher yield.



(a) TEMPO, BAIB, $NaHCO_3$, H_2O/MeCN (1:1), rt, 4h

FIGURE 4.11: SYNTHESIS OF SODIUM 2’-3’-*O*-ISOPROPYLIDENEGUANOSINE-5’-CARBOXYLATE (**20**).

4.1.2.3. Reaction mechanism of TEMPO / BAIB (bisacetoxy-iodobenzene) oxidation

TEMPO is a stable radical species due to the absence of an α-hydrogen atom. It can either be oxidized or reduced to the corresponding oxoammonium salt and

hydroxylamine respectively. The oxidizing species is the oxoammonium salt, which would imply stoichiometric use of the radical or salt. However, it is also possible to regenerate the radical in situ by adding a secondary oxidant, in this case BAIB, resulting in a catalytic cycle. The proposed reaction mechanism is depicted in FIGURE 4.12.

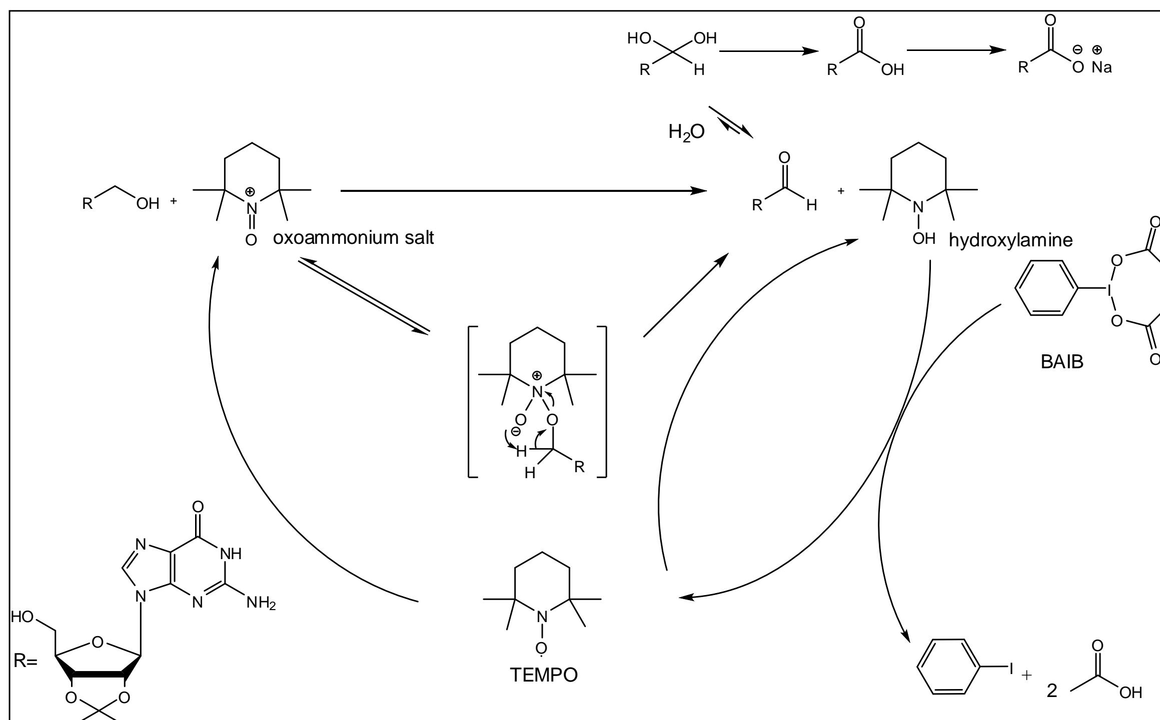


FIGURE 4.12: PROPOSED REACTION MECHANISM FOR THE TEMPO/BAIB OXIDATION OF A PRIMARY ALCOHOL INTO CARBOXYLIC ACID.

Under alkaline conditions, a compact five-membered transition state is formed between the alcohol and the oxoammonium salt. This undergoes an elimination-like reaction to form the aldehyde and the hydroxylamine. The aldehyde can be hydrated and one of the two resulting hydroxyl groups be oxidized to give the carboxylic acid. In the presence of $NaHCO_3$ however, the salted form is found after an acid-base reaction. TEMPO is regenerated from the hydroxylamine, which is oxidized by the secondary oxidant, BAIB. In contrast to other TEMPO-catalytic cycles, the oxoammonium salt seems not to be formed by oxidation of TEMPO, but rather due to an acetic acid catalyzed dismutation of the radical. The initial acetic acid to start the catalytic cycle is generated by ligand exchange of the alcohol on BAIB [61].

4.1.2.4. Attempted synthesis of 2-amino-6-chloro-9-(2’,3’-*O*-isopropylidene-5’-*N*-ethylcarboxamido-β-D-ribofuranosyl)purine (**21**)

Preparation of 5’-*N*-ethylcarboxamidoAdo has been performed in different ways. It was believed that simultaneous activation of the carboxylate and N^6-chlorination could be achieved, finishing by carrying out amination in order to obtain **21**. This strategy has already been applied on 2’,3’-O-isopropylideneinosine-5’-carboxylic acid using thionyl chloride [62]. However, since phosphorus oxychloride ($POCl_3$) is commonly used for N^6-chlorination [53, 60, 62], it was decided to proceed in this way first. Since TLC showed no complete disappearance of the starting material after 5 minutes at 100 °C, cycles of 5 minutes were repeated until complete consumption (additional 15 minutes). After adding of ethylamine and purification ^{1}H-NMR did not show the formation of the N^6-chloro derivative **21** but only the ethylcarboxamido resulting in 2-amino-2’-3’-*O*-isopropylidene-5’-*N*-ethylcarboxamidoguanosine (**25**). (FIGURE 4.13)

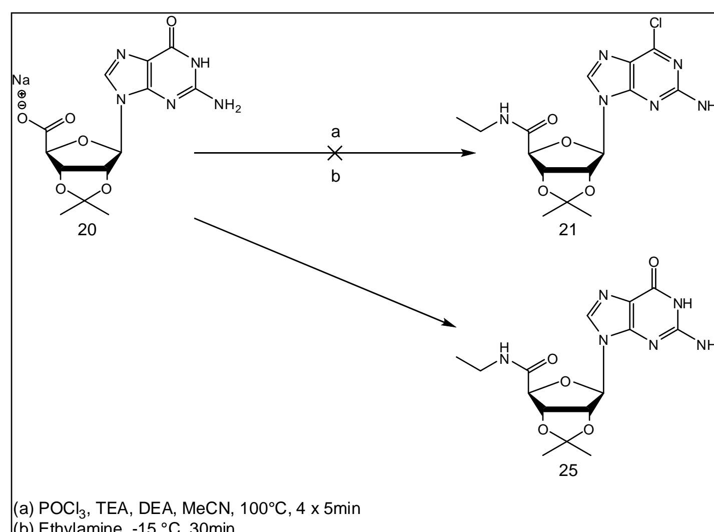


FIGURE 4.13: ATTEMPTED SYNTHESIS OF 2-AMINO-6-CHLORO-9-(2’,3’-*O*-ISOPROPYLIDENE-5’-*N*-ETHYLCARBOXAMIDE-β-D-RIBOFURANOSYL)PURINE (**21**).

4.1.2.5. Attempted synthesis of 2-amino-6-chloro-9-(2’,3’-*O*-isopropylidene-5’-*N*-ethylcarboxamido-β-D-ribofuranosyl)purine (**21**)

In a second attempt to synthesize compound **21** it was decided to employ the procedure as described for the inosine derivative [62]. Complete consumption of the starting material was observed after 5 hours, but TLC showed the formation of **25**, however in a higher yield. A possible explanation for not obtaining of compound **21**,

might be the reduced acidity of the amide in respect to inosine due to the presence of the electron withdrawing C-2 amino group.

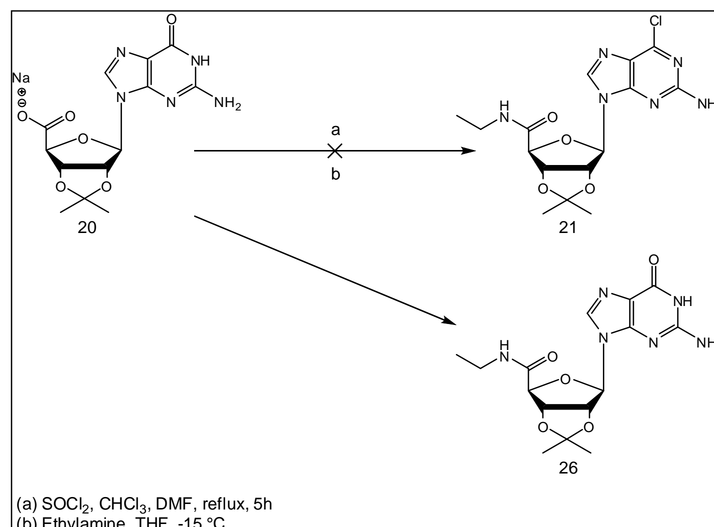


FIGURE 4.14: ATTEMPTED SYNTHESIS OF 2-AMINO-6-CHLORO-9-(2',3'-*O*-ISOPROPYLIDENE-5'-*N*-ETHYLCARBOXAMIDO-β-D-RIBOFURANOSYL)PURINE (**21**).

4.1.2.6. Synthesis of 2-amino-6-chloro-9-(2',3'-*O*-isopropylidene-5'-*N*-ethylcarboxamido-β-D-ribofuranosyl)purine (**21**)

A third attempt to synthesize compound **21** started from the earlier obtained 2-amino-2'-3'-*O*-isopropylidene-5'-*N*-ethylcarboxamide-guanosine (**25**). An advantage of this trial was the fact that the reaction could now be followed by TLC. An existing literature procedure was applied [53]. Continuous monitoring by TLC showed disappearance of the starting material in 90 minutes. After work-up including preparative TLC, compound **21** was isolated.

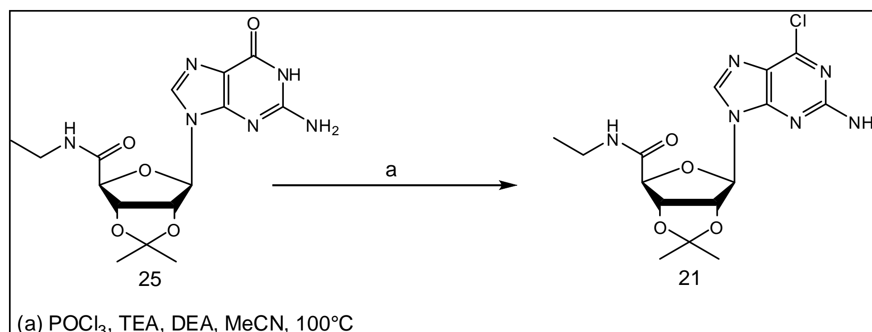


FIGURE 4.15: SYNTHESIS OF 2-AMINO-6-CHLORO-9-(2',3'-*O*-ISOPROPYLIDENE-5'-*N*-ETHYLCARBOXAMIDO-β-D-RIBOFURANOSYL)PURINE (**21**).

4.1.2.7. Synthesis of 2-amino-6-chloro-9-(2',3'-*O*-isopropylidene-5'-*N*-ethylcarboxamido-β-D-ribofuranosyl)purine (**21**)

Because we now had a reference sample at our disposal, synthesis of **21** was again executed, with increased reaction time (2.5 hours). After adding ethylamine and work-up, impure product (TLC iodine staining) was isolated. A major side-product of the reaction was analyzed and ^{1}H-NMR showed additional presence of 3 sets of protons. Moreover, the signal for the C-2 NH_2 in **21** was found about 1 ppm more downfield than the one observed in **26**. This pointed towards concomitant formation of 2-amino-6-ethylamino-9-(2',3'-*O*-isopropylidene-5'-*N*-ethylcarboxamide-β-D-ribofuranosyl)purine as the structure for **26**. Attempts (washing, recrystallization and even a second silica gel purification) to further purify **21** were not successful. (FIGURE 4.16)

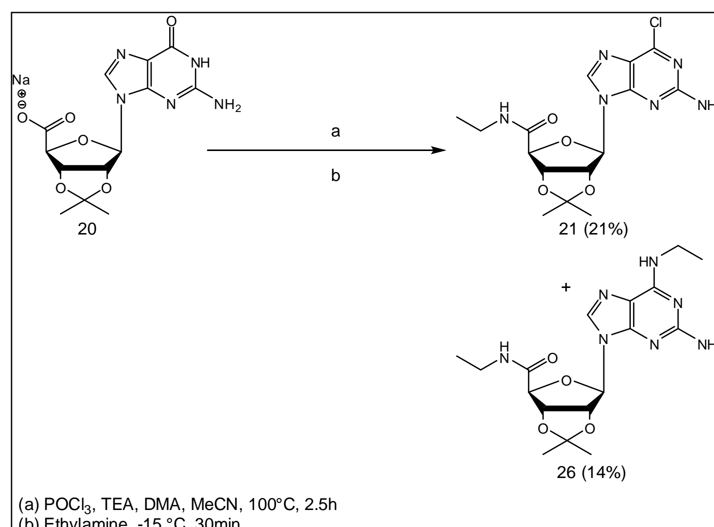


FIGURE 4.16: SYNTHESIS OF 2-AMINO-6-CHLORO-9-(2',3'-*O*-ISOPROPYLIDENE-5'-*N*-ETHYLCARBOXAMIDO-β-D-RIBOFURANOSYL)PURINE (**21**).

4.1.2.8. Reaction mechanism of the simultaneous chlorination and amination

Activation of the carboxylate by chlorination with $POCl_3$ takes place through an S_N2 substitution reaction. Replacement of introduced dichlorophosphoryloxy leaving group by a chlorine as the consequence of a nucleophilic acyl substitution leads to the acyl chloride intermediate (which can not be isolated due to its reactivity). Due activated acyl species, reaction with a weak nucleophile such as ethylamine is fast.

At higher temperature, keto-enol tautomerization happens fast, after which the enol can attack the tetrahydral centre of phosphorus oxychloride. Hereby, a good

leaving group is introduced in N^6 position. The formed HCl is captured by the weak base present in the reaction medium, namely *N*,*N*-diethylaniline (DEA). Nucleophilic aromatic displacement of the dichlorophosphoryloxy leaving group by a chlorine atom occurs slower than its aliphatic counterpart; therefore needing a higher temperature and longer reaction time. Addition of a quaternary ammonium salt, such as tetra-ethylammoniumchloride (TEA) increases the presence of chlorine atoms in the medium. However, since derivative **26** was isolated in quite high amount in respect to the desired product, it might be concluded that displacement in N^6-position was not complete upon adding of ethylamine.

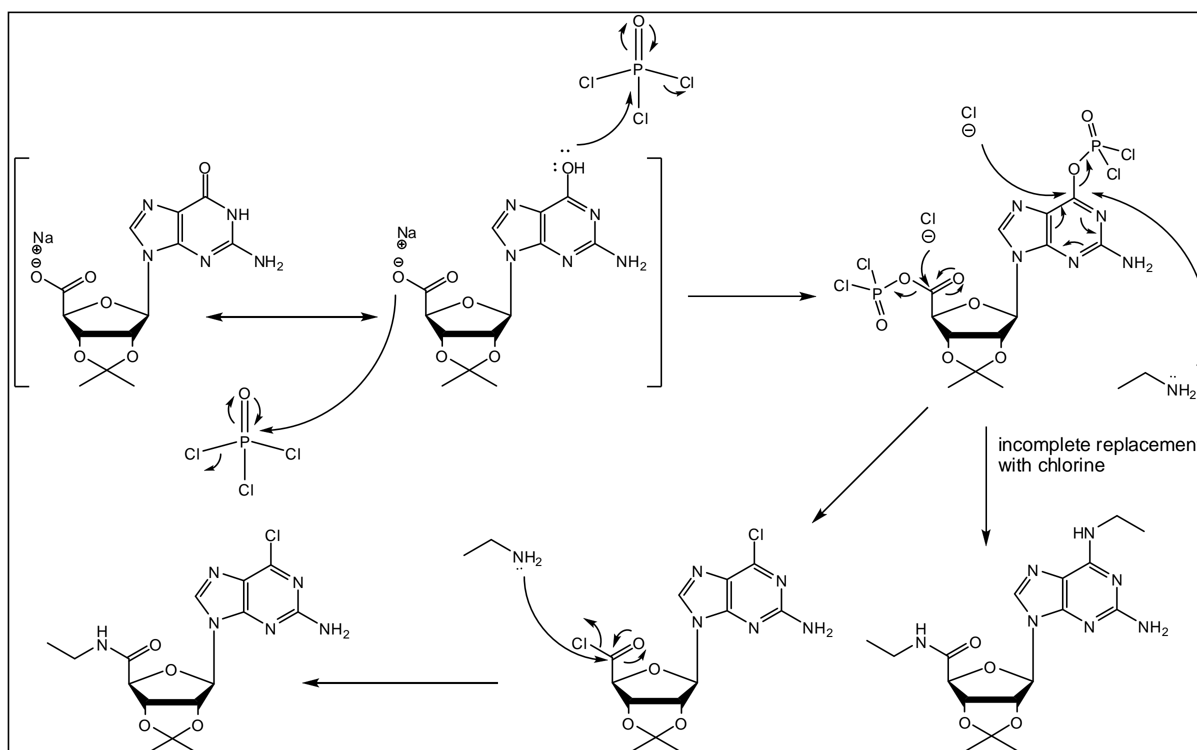


FIGURE 4.17: REACTION MECHANISM FOR SIMUTANEOUS CHLORINATION.

The simultaneous preparation of both the ethylcarboxamide and N^6-derivative proved less promising (low yield, difficult separation) than initially expected. In view of this, an alteration was made by first preparing compound 25 and subsequently introducing a benzotriazole moiety as N^6-substituent instead of a chlorine leaving group.

4.1.2.9. Adapted divergent synthetic scheme

Introducing another N^6-leaving group should improve yield, but results in an additional synthetic step. However, Sakakibara, N. et al. described a procedure for

the synthesis of 2-alkoxyadenosine derivatives using a one step non-aqueous diazotization-dediazoniation reaction [63]. Implementing this procedure would mean an improvement to the procedure generally followed in literature, which employs aqueous diazotization resulting in the C-2 hydroxy intermediate. Subsequent treatment with a weak base and S_N2 displacement on a primary substrate introduces the (ar)alkyl chain, leading to the C-2 ether [47].

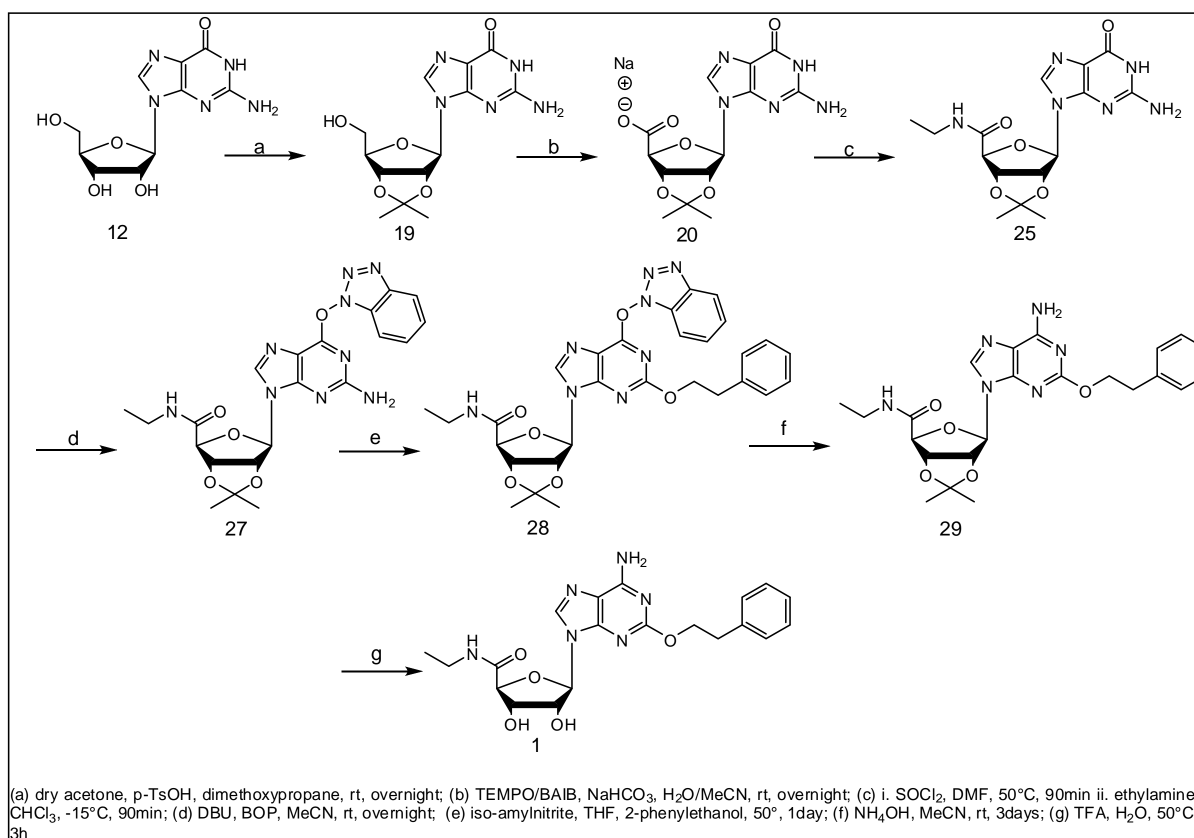


FIGURE 4.18: ADAPTED PROPOSED DIVERGENT SYNTHETIC SCHEME.

4.1.2.10. Synthesis of 2-amino-2'-3'-*O*-isopropylidene-5'-*N*-ethylcarboxamidoguanosine (**25**)

In order to obtain compound **25**, an in-house procedure employing thionyl chloride both as reactant and solvent was used. The reaction proceeds via a nucleophilic acyl substitution mechanism which eventually leads to the activated acylchloride intermediate. Treatment of the acylchloride with ethylamine, results in amide formation through another nucleophilic acyl substitution. The reaction yielded **25** in inferior yield than obtained in 4.2.1.5. Employing that procedure could definitely be an improvement. Nevertheless, even a 54% yield is quite low, so amide formation by using another acid activating agent, such as benzotriazol-1-

yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBop) might improve the yield significantly [47].

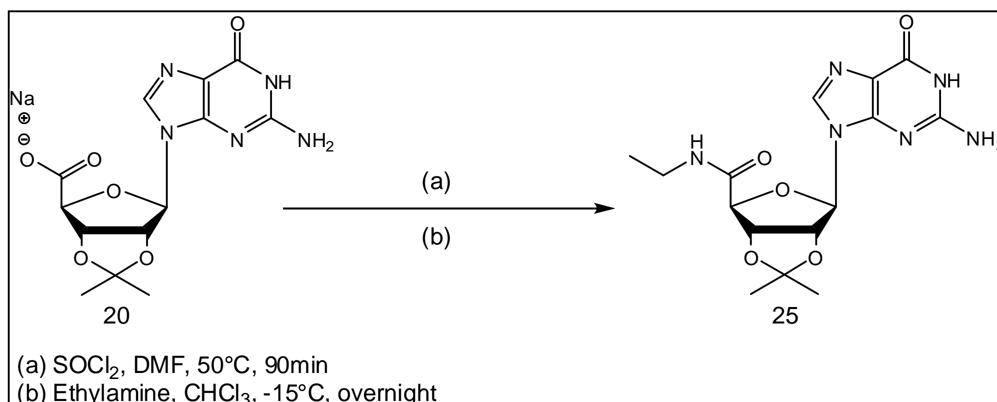


FIGURE 4.19: SYNTHESIS OF 2-AMINO-2',3'-*O*-ISOPROPYLIDENE-5'-*N*-ETHYLCARBOXAMIDOGUANOSINE (25).

4.1.2.11. Synthesis of O^6-(benzotriazol-1-yl)-2-amino-9-(2'-3'-*O*-isopropylidene-5'-*N*-ethylcarboxamido-β-D-ribofuranosyl)purine (**27**)

A literature procedure for synthesis of intermediate **27** was used [53]. As was expected, this method comprised a significant improvement in terms of convenience and yield in comparison with the previously tried chlorination. However, after washing the organic layer 5x with water and 1x brine, as described in literature, TLC showed still slight presence of probably DBU and / or unreacted BOP. Since DBU has a high boiling point it was decided to further wash with water (2x) and brine (1x). Afterwards recrystallization from isopropanol was necessary to obtain a pure product. The additional manipulations could account for obtaining a lower yield than reported [53]. An improvement to this procedure could be the use of the less toxic PyBop-reagent as substituent for BOP (benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate). This excludes the formation of the carcinogenic hexamethylphosphoramide. (FIGURE 4.20) The reaction mechanism is discussed below and involves the formation of a phosphonium salt, which undergoes nucleophilic aromatic displacement (S_NAr) to give the O^6-benzotriazol derivative.

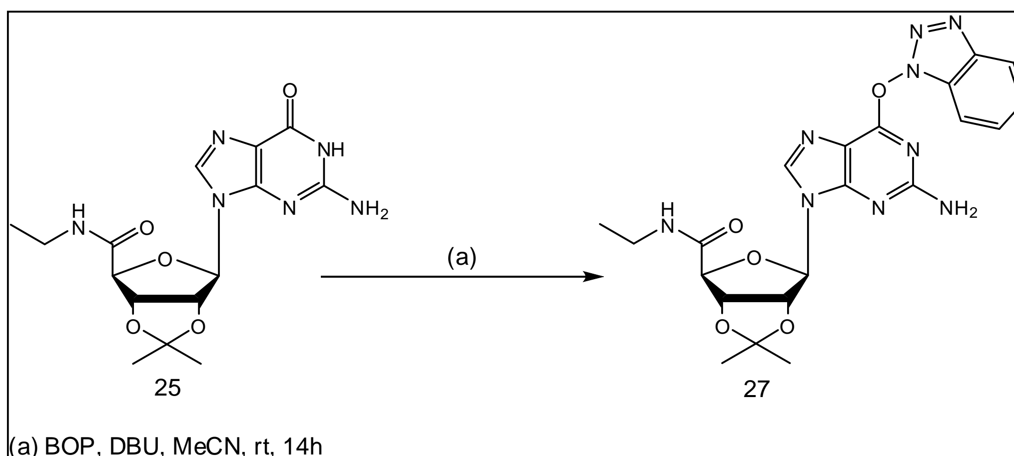


FIGURE 4.20: SYNTHESIS OF 6-*O*-(BENZOTRIAZOL-1-YL)-2-AMINO-9-(2'-3'-*O*-ISOPROPYLIDENE-5'-*N*-ETHYLCARBOXAMIDO-β-D-RIBOFURANOSYL)PURINE (27).

4.1.2.12. Proposed reaction mechanism of phosphonium salt formation and subsequent displacement

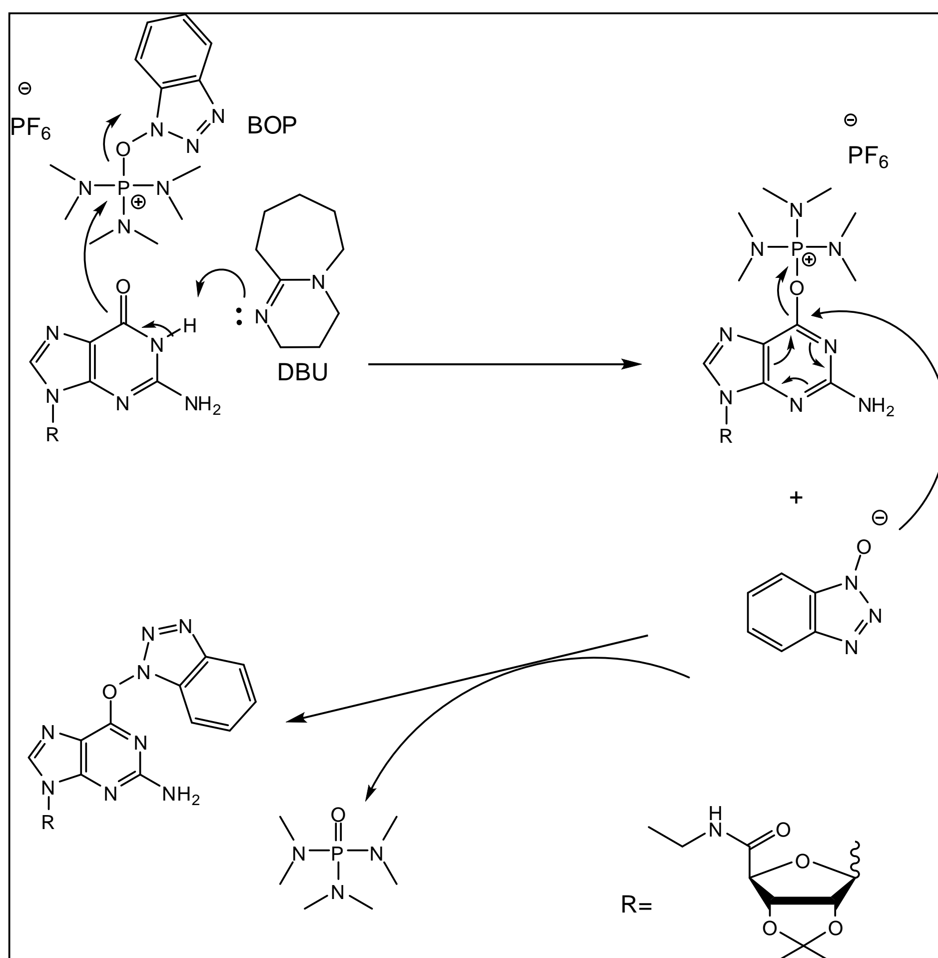


FIGURE 4.21: PROPOSED REACTION MECHANISM OF THE PHOSPHONIUM SALT FORMATION AND SUBSEQUENT NUCLEOPHILIC DISPLACEMENT.

The strong, non-nucleophilic base 1,8-diazabicylcoundec-7-ene (DBU) will abstract the amide proton of the purine, which leads to an electrostatic attraction between of the negatively charged purine and positively charged phosphonium species. As a consequence, the benzotriazole moiety will function as a leaving group, resulting in a stable phosphonium salt intermediate. In the absence of other nucleophiles, the hydroxybenzotriazole will act as one in a nucleophilic aromatic substitution reaction (S_NAr) [59]. (FIGURE 4.21)

4.1.2.13. Attempted synthesis of 6-*O*-(benzotriazol-1-yl)-2-phenethoxy-9-(2',3'-*O*-isopropylidene-5'-*N*-ethylcarboxamido-β-D-ribofuranosyl)purine (**28**)

Recently, Sakakibara N. et al. reported on the direct formation of C-2 ethers, by employing non-aqueous diazotization/dediazoniation reaction. However, they reported other N^6-modified nucleosides and used low-boiling point alcohols; justifying there use as both nucleophile and solvent. Since it was intended to apply this procedure on substrate **28** with 2-phenylethanol (which has a boiling point of 220 °C) a suitable solvent needed to be selected. Since diazotization reactions might take place by radical intermediates, the use of halogenated solvents was thereby excluded in order to avoid C-2 halogenated side-products [64]. Additionally, the solvent should preferably dissolve both starting material as well as 2-phenylethanol. Taking all of this into consideration, tetrahydrofuran (THF) was selected. It was recognized however, that since THF is fully miscible with the formed water in the reaction, competition might occur. The C-2 phenylethoxy derivative could not be found after purification of the reaction mixture by preparative TLC. However, the C-2 hydroxy side-product was recovered in 18 % yield, which is remarkably high since only one equivalent of water is formed during the reaction. It might also be concluded that the used amount of 2-phenylethanol was not high enough. Furthermore, analysis of the presumed product fraction showed the formation of compound **30**, which was not predicted.

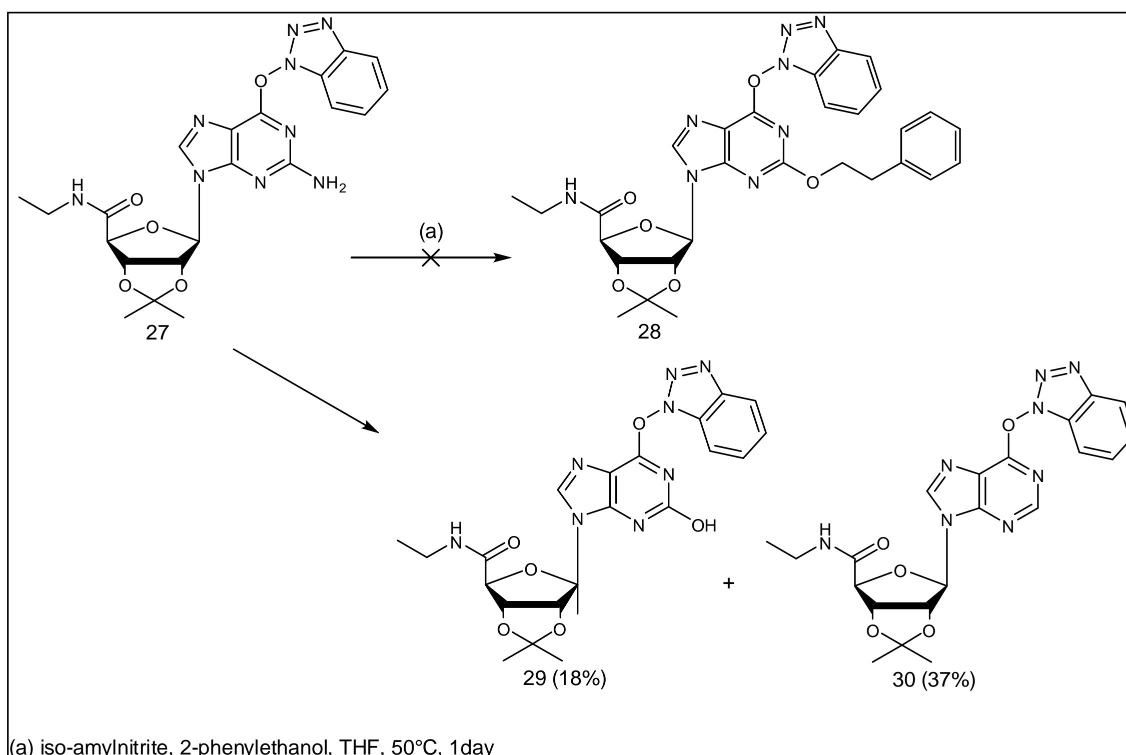


FIGURE 4.22: ATTEMPTED SYNTHESIS 6-*O*-(BENZOTRIAZOL-1-YL)-2-PHENETOXY-9-(2',3'-*O*-ISOPROPYLIDENE-5'-*N*-ETHYLCARBOXAMIDO-β-D-RIBOFURANOSYL)PURINE (**28**).

Another approach to obtain **28** could be the investigation of a solid-phase extraction technique for removal of excess 2-phenylethanol in order to use the alcohol as a solvent. Furthermore, direct aminolysis on the crude mixture would lead to a more polar and therefore easier-separable compound.

4.1.2.14. Proposed reaction mechanism for non-aqueous diazotization / dediazoniation.

Iso-amylnitrite is a nitrite ester that can easily decompose to lead to a diazonium salt as the reaction intermediate. This intermediate is resonance stabilized, however to a lesser extent because of the electron withdrawing character of the benzotriazole. The formed C-2 diazogroup can undergo nucleophilic attack, preferably with the strongest nucleophile present in the reaction medium. Ideally, this should be the alcohol either applied as solvent or in excess. However, since water molecules are more nucleophilic and less sterically hindered than e.g. 2-phenylethanol, the formation of side-product **29** was expected. Nonetheless, the reaction mechanism proposed by [63] is not sufficient to explain the formation of compound **30** in quite high yield.

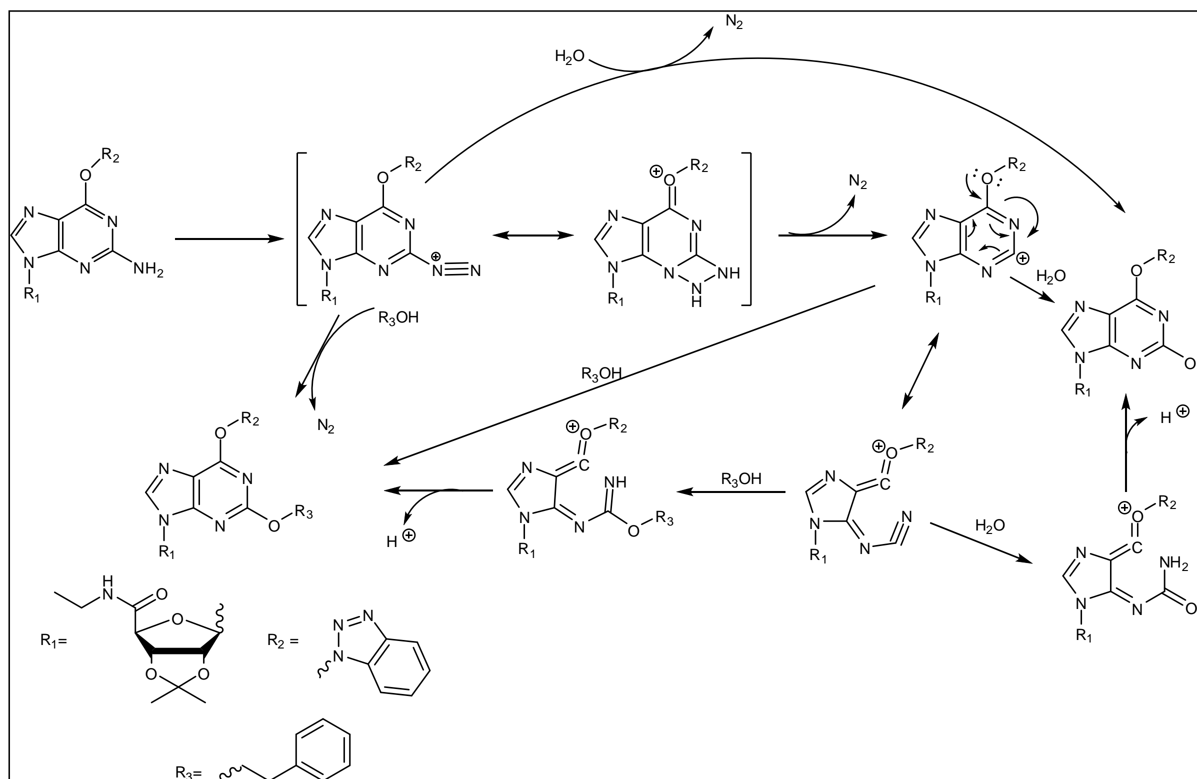


FIGURE 4.23: PROPOSED REACTION MECHANISM FOR NON-AQUEOUS DIAZOTIZATION / DEDIAZONIATION.

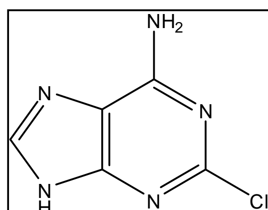
5. EXPERIMENTAL SECTION

5.1. CONVERGENT APPROACH

5.1.1. Synthesis of the purine building block

5.1.1.1. Synthesis of 2-chloroadenine (**3**)

A stainless steel reaction bomb was cooled by submerging in liquid N_2 and subsequently fluxed with N_2 for 15 minutes. Next, liquid ammonia was condensed into the bomb followed by the addition of **4** (1.022 g, 5.04 mmol). The reaction vessel was sealed and left to react at 80°C overnight. The mixture was submerged in liquid N_2, the vessel opened and the ammonia left to escape. The resulting solid was washed with a mixture of water and MeOH (1:1), filtered, yielding a tan solid as the product **3** (685 mg, 4.04 mmol, 75%).



^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.62 (2H, brs, NH_2, exchangeable); 8.11 (1H, s, H-8); 12.69 (1H, brs, NH-9, exchangeable).

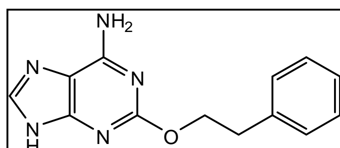
5.1.1.2. Synthesis of 2-phenethoxyadenine (**2**)

Procedure A:

2-phenylethanol (4.5 mL) was stirred under N_2-atmosphere and sodium (200 mg, 8.70 mmol) added. After ceasing of gas formation, 2-chloroadenine (**3**) (150 mg, 0.885 mmol) was added to the mixture and heated to 100 °C which was increased to 140 °C after 30 minutes and left to react for 9 hours. The mixture was allowed to cool to room temperature and ethanol was added (20 mL), and after 1 hour removed by rotary-evaporation. The remaining solid was partioned between water (50 mL) and ethyl acetate (50 mL), further extracted with ethyl acetate (2x 50 mL). The aqueous layer was acidified with HCl 2N and extracted again with ethyl acetate (3x 50 mL). TLC showed the product present in the first combined organic layer; which was dried over sodium sulphate, filtered and volatiles removed by evaporation under reduced pressure. The remaining oil was pre-adsorbed on silica gel and purified by silica gel chromatography (100 % $CHCl_3$ to 99/1 $CHCl_3$:MeOH). This yielded 2-phenethoxyadenine as a yellowish solid (33 mg, 0.129 mmol, 15%).

Procedure B:

2-phenethylethanol (3.5 mL) was stirred under N_2-atmosphere and pulverized sodium hydroxide (76 mg, 1.90 mmol) added. After 15 minutes, 2-chloroadenine (**3**) (200 mg, 1.18 mmol) was added to the mixture and stirred at 105 °C for 8 hours, after which it was transferred into a stainless steel bomb and reacted for 2 days in an oven at 110 °C. The remaining brown liquid was adsorbed on silica gel prior to purification by silica gel chromatography (100% DCM to 95/5 DCM:MeOH) yielding a yellow solid (24 mg, 0.094 mmol, 8%) as the product.



^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.97 (2H, t, *J*= 6.8 Hz, CH_2-Ar); 4.36 (2H, t, *J*= 6.8 Hz, O-CH_2); 7.08 (2H, s, NH_2, exchangeable); 7.28 (5H, m, Ar-H); 7.85 (1H, s, H-8); 12.55 (1H, br s, NH-9, exchangeable).

5.1.2. Synthesis of the ribose building block

5.1.2.1. Attempted synthesis of 1-*O*-acetyl-2,3-*O*-dibenzoyl-β-D-ribofuranose (**8**)

1-*O*-acetyl-2,3,5-*O*-tribenzoyl-β-D-ribofuranose (**7**) (100 mg, 0.198 mmol) was dissolved in 1,4-dioxane (1 mL) and potassium phosphate buffer (2 mL, pH 7, 0.15 M) added under stirring at 50 °C resulting in a white suspension. Next, crude Amano Lipase PS was added and the mixture stirred for 15 hours. Since no product was visualized, the mixture was let to react for another 2 days, after which no formation of any product was observed by TLC.

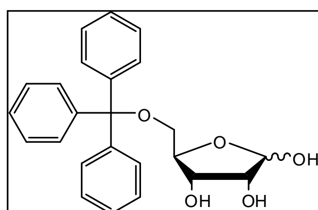
5.1.2.2. Attempted synthesis of 1-*O*-acetyl-2,3-*O*-dibenzoyl-β-D-ribofuranose (**8**)

1-*O*-acetyl-2,3,5-*O*-tribenzoyl-β-D-ribofuranose (**7**) (107 mg, 0.212 mmol) was suspended in potassium phosphate buffer (2 mL, pH 7, 0.15 M) at 50 °C and 1,4-dioxane added until complete dissolution (4 mL). Next, crude Amano Lipase PS was added; the resulting suspension stirred for 2 days. Continuous monitoring by TLC showed only the spot of the starting material.

5.1.2.3. Synthesis of 5-*O*-triphenylmethylene-β-D-ribofuranose (**18**)

β-D-ribofuranose (**17**) (204 mg, 1.40 mmol) was dissolved in anhydrous DMF (2 mL) under N_2-atmosphere after which triphenylmethylenechloride (413 mg, 1.48 mmol), 4-dimethylaminopyridine (DMAP) (14 mg, 0.115 mmol) and triethylamine (340 µL, 2.44 mmol) were added. The resulting mixture was stirred at room temperature

overnight. The resulting yellow solution was poured into ice-water and extracted with DCM (3 x 30 mL). The organic layers were combined and washed with saturated ammonium chloride solution (1 x 30 mL), water (2 x 30 mL), dried over sodium sulphate and evaporated till dryness. The remaining solid was purified by silica gel chromatography (DCM:MeOH:triethylamine, 95/5/0.05), subsequently recrystallized from EtOH to give **18** as a white solid (50 mg, 0.127 mmol, 9 %).

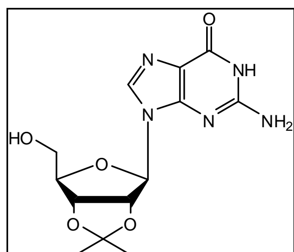


^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.92 (1H, m, CH_2); 3.08 (1H, m, CH_2); 3.74 (1H, m, 2'-H); 3.79 (1H, m, 3'-H); 3.95 (1H, m, 4'-H); 4.72 (1H, d, *J*= 13.6 Hz, OH, exchangeable); 4.73 (1H, d, *J*= 13.6 Hz, OH, exchangeable); 5.18 (1H, dd, *J*= 4.4 Hz, 7.6 Hz, 1'-H); 5.86 (1H, d, *J*= 8.0 Hz, 8.0 Hz, OH, exchangeable); 7.31 (15H, m, Ar-H). **Exact mass**: (ESI-MS): calculated for $C_{24}H_{24}ClO_5^-$: [M+Cl$^-$]= 427.1, found 427.0.

5.2. DIVERGENT APPROACH

5.2.1. Synthesis of 2',3'-*O*-isopropylideneguanosine (19)

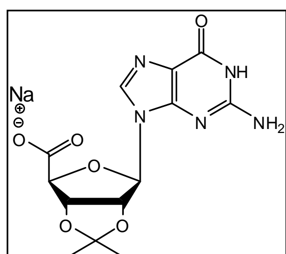
Previously dried guanosine (**12**) (2.007 g, 7.086 mmol) was stirred in anhydrous acetone (80 mL). To the reaction mixture was added p-toluenesulfonic acid (1.332 g, 7 mmol) and 2,2-dimethoxypropane (20 mL) and the resulting yellowish suspension stirred at room temperature overnight. The reaction mixture was then evaporated to dryness and taken up into water (15 mL). Next, solid $NaHCO_3$ (598 mg, 7.12 mmol) was portion wise added and the foamy suspension stirred for 2 hours. Saturated $NaHCO_3$ solution (13.5 mL) was added and stirred for another 2 hours. The resulting suspension was filtered in vacuo and the resulting solid washed with cold water, yielding a white solid as the product (**19**) (1.979 g, 6.12 mmol, 86%).



^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.29 (3H, s, CH_3-isopropylidene); 1.49 (3H, s, CH_3-isopropylidene); 3.47-3.54 (2H, m, 5'-CH_2); 4.08-4.11 (1H, m, 3.0 Hz, 4'-H); 4.94 (1H, dd, *J*=2.8 Hz, 6.4 Hz, 3'-H); 5.00 (1H, br s, 5'-OH, exchangeable); 5.17 (1H, dd, *J*= 2.8 Hz, 6.4 Hz, 2'-H); 5.90 (1H, d, *J*= 2.4 Hz, 1'-H); 6.49 (2H, br s, NH_2, exchangeable); 7.91 (1H, s, H-8); 10.66 (1H, br s, NH-1, exchangeable).

5.2.2. Synthesis of sodium 2’,3’-*O*-isopropylideneguanosine-5’-carboxylate (20)

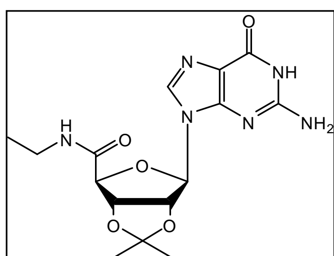
2’-3’-*O*-isopropylideneguanosine (**19**) (800 mg, 2.47 mmol) was taken together with BAIB (1.748 g, 5.43 mmol), TEMPO (87 mg, 0.56 mmol) and solid $NaHCO_3$ (421 mg, 5.01 mmol). A mixture of acetonitrile (4.3 mL) and water (4.3 mL) were added to give an orange suspension which was stirred at room temperature for 4 hours. The resulting precipitate was filtered and sequentially (5 times) washed with diethylether and acetone to yield a white solid as the product **20** (827 mg, 2.30 mmol, 93 %).



^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.26 (3H, s, CH_3-isopropylidene); 1.47 (3H, s, CH_3-isopropylidene); 4.23 (1H, s, 4’-H); 4.84 (1H, dd, 2’-H); 4.94 (1H, d, *J*= 7.2 Hz, 3’-H); 5.89 (1H, d, *J*= 2.8 Hz, 1’-H); 6.60 (2H, br s, NH_2, exchangeable); 8.50 (1H, br s, H-8); 10.86 (1H, br s, NH-1, exchangeable).

5.2.3. Attempted synthesis of 2-amino-6-chloro-9-(2’,3’-*O*-isopropylidene-5’-*N*-ethylcarboxamido-β-D-ribofuranosyl)purine (21)

Sodium 2’,3’-*O*-isopropylideneguanosine-5’-carboxylate (**20**) (205 mg, 0.571 mmol) and TEA (193 mg, 1.16 mmol) were previously dried under vacuum to constant weight. Under rigorously anhydrous conditions were added N,N-diethylaniline (100 µL, 0.629 mmol) and anhydrous acetonitrile (20 mL). The resulting suspension was cooled to 0 °C in an ice-salt bath and stirred for 15 minutes, after which phosphorus oxychloride (300 µL, 3.34 mmol) was added. The mixture was placed in an oil bath at 100 °C for 5 minutes. Since TLC showed no complete consumption of the starting material, so heating continued until disappearance. The mixture was allowed to cool to room temperature and volatiles were removed by rota-evaporation. The resulting brown oil was cooled to -15 °C and an excess of ethylamine added, resulting in a brown solution. After 30 minutes ethylamine was left to escape overnight while allowing warming to room temperature. Purification by silica gel chromatography (gradient elution 95/5 to 90/10; $CHCl_3$:MeOH) yielded slightly impure 2’-3’-*O*-isopropylideneguanosine-5’-*N*-ethylcarboxamide (**25**) (60 mg, 0.165 mmol, 29 %).



^{1}H-NMR (400 MHz, DMSO-d_6): δ 0.72 (3H, t, *J*= 6.8 Hz, CH_3); 1.31 (3H, s, CH_3-isopropylidene); 1.48 (3H, s, CH_3-isopropylidene); 2.78-2.96 (2 x 1H, m, CH_2); 4.45 (1H, d, *J*=

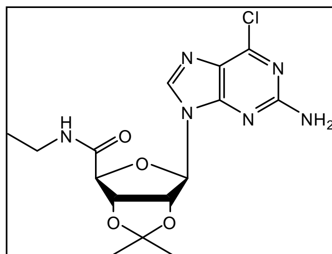
2.0 Hz, 4'-H); 5.25 (1H, d, *J*= 6.0 Hz, 3'-H); 5.43 (1H, dd, *J*= 2.0 Hz, 6.0 Hz, 2'-H); 6.11 (1H, s, 1'-H); 6.40 (2H, br s, NH_2, exchangeable); 7.36 (1H, t, *J*= 5.6 Hz, NH); 7.82 (1H, br s, H-8); 10.63 (1H, br s, NH-1, exchangeable).

5.2.4. Attempted synthesis of 2-amino-6-chloro-9-(2',3'-*O*-isopropylidene-5'-*N*-ethylcarboxamido-β-D-ribofuranosyl)purine (21)

Sodium 2',3'-*O*-isopropylideneguanosine-5'-carboxylate (**20**) (107 mg, 0.298 mmol) was suspended in freshly distilled chloroform (1.4 mL) under anhydrous conditions, to which was added anhydrous *N*,*N*-dimethylformamide (DMF) (65 µL, 0.840 mmol) and thionyl chloride (115 µL, 1.58mmol). The suspension was placed in a pre-heated oil bath to gentle reflux for 5 hours. A yellow solution was formed, which was evaporated till dryness; the resulting yellow oil dissolved in anhydrous THF (2 mL). Next, an excess of ethylamine was added at -15 °C and allowed to gradually warm to room temperature. The remaining solution was evaporated to dryness and pre-adsorbed on silica gel prior to purification by silica gel chromatography (50/40/10; $CHCl_3$:c-hexane:MeOH) to yield 2'-3'-*O*-isopropylideneguanosine-5'-*N*-ethylcarboxamide (**25**) (59 mg, 0.162 mmol, 54%) as an off-white solid.

5.2.5. Synthesis of 2-amino-6-chloro-9-(2',3'-*O*-isopropylidene-5'-*N*-ethylcarboxamido-β-D-ribofuranosyl)purine (21)

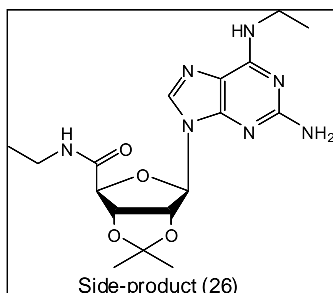
2'-3'-*O*-isopropylideneguanosine-5'-*N*-ethylcarboxamide (**25**) (59 mg, 0.162 mmol) and TEA (60 mg, 0.362 mmol) were previously dried under vacuum to constant weight. Under anhydrous conditions were added anhydrous acetonitrile (4.2 mL) and freshly distilled *N*,*N*-diethylaniline (34 µL, 0.214 mmol). The yellow solution was cooled to 0 °C in an ice-salt bath and allowed to stir for 15 minutes, after which phosphorus oxychloride (88 µL, 0.964 mmol) was added. The solution was transferred to a pre-heated oil bath at 100 °C and reacted until consumption of the starting material by TLC was observed. The remaining orange solution was evaporated to dryness and partioned between ice-water (30 mL) and chloroform (30 mL). 30 mL of a saturated $NaHCO_3$-solution was cautiously added, after which the water layer was extracted with chloroform (2x 30 mL). The organic layers were combined, dried over sodium sulphate, filtered, evaporated to dryness after which the resulting yellow oil was subjected to preparative TLC ($CHCl_3$:MeOH; 92/8) yielding **21** as a yellow solid (20 mg, 0.052 mmol, 32%).



^{1}H-NMR (400 MHz, DMSO-d_6): δ 0.58 (3H, t, *J*= 7.2 Hz); 1.33 (3H, s, CH_3-isopropylidene); 1.50 (3H, s, CH_3-isopropylidene); 2.68-2.72 (1H, m, CH_2); 2.82-2.81 (1H, m, CH_2); 4.50 (1H, d, *J*= 2.0 Hz, 4'-H); 5.37 (1H, d, *J*= 6.0 Hz, H-3'); 5.52 (1H, dd, *J*= 2.0 Hz, 6.0 Hz, H-2'); 6.24 (1H, s, H-1'); 6.88 (2H, br s, NH_2, exchangeable); 7.42 (1H, t, *J*= 5.6 Hz; NH); 8.20 (1H, s, H-8). **Exact mass**: (ESI+MS): calculated for $C_{15}H_{20}ClN_6O_4^+$: $[M+H]^+$= 383.12; found $[M+H]^+$= 383.13.

5.2.6. Synthesis of 2-amino-6-chloro-9-(2',3'-*O*-isopropylidene-5'-*N*-ethylcarboxamido-β-D-ribofuranosyl)purine (21)

Sodium 2',3'-*O*-isopropylideneguanosine-5'-carboxylate (**20**) (392 mg, 1.09 mmol) and TEA (732 mg, 4.42 mmol) were previously dried under vacuum to constant weight. Under anhydrous conditions were added anhydrous acetonitrile (40 mL) and freshly distilled *N*,*N*-diethylaniline (456 µL, 3.26 mmol). The resulting suspension was cooled to 0 °C in an ice-salt bath and stirred for 15 minutes, after which phosphorus oxychloride (1.20 mL, 13.1 mmol) was added. The mixture was placed in an oil bath at 100 °C for 2.5 hours. The mixture was allowed to cool to room temperature and volatiles were removed by rota-evaporation and further drying on the oil pump. The resulting brown oil was dissolved in freshly distilled chloroform (7.5 mL), cooled to -15 °C and an excess of ethylamine added, resulting in a brown solution. After 30 minutes ethylamine was left to escape, while allowing warming to room temperature. The remaining solution was evaporated to dryness. To the remaining oil was added chloroform (150 mL), ice water (150 mL) and 150 mL saturated $NaHCO_3$ solution. The water layer was extracted with chloroform (2 x 150 mL), dried over sodium sulphate, filtered and evaporated to dryness. The remaining oil was pre-adsorbed on silica gel and purified by chromatography (CH_2Cl_2:MeOH, 100 to 95/5) yielding impure **21** (90 mg, 0.235 mmol, 21%). Side-product **26** was isolated (60 mg, 0. 146 mmol, 14 %).



^{1}H-NMR (400 MHz, DMSO-d_6): δ 0.71 (3H, t, *J*= 7.2 Hz, CH_3-carboxamide); 1.10 (3H, t, *J*= 7.6 Hz, CH_3); 1.32 (3H, s, CH_3-isopropylidene); 1.50 (3H, s, CH_3-isopropylidene); 2.84-3.31 (2H, m, CH_2-carboxamide); 3.40 (2H, m, CH_2-N^6); 4.43 (1H, d, *J*= 2.0 Hz, 4'-H); 5.28 (1H, dd, *J*= 1.6 Hz, 6.0 Hz, 2'-H); 5.37 (1H, dd, *J*= 2.0 Hz, 6.0 Hz, 3'-H); 5.79 (2H, br s, NH_2, exchangeable); 6.12 (1H, d, 2.0 Hz, 1'-H); 7.32 (1H, br s, NH-ethylamine exchangeable); 7.37 (1H, t, *J*= 5.6 Hz, NH-carboxamide); 7.80 (1H, s, H-8).

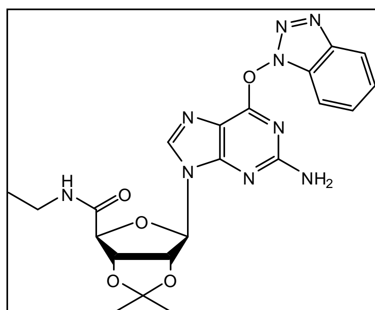
5.2.7. Synthesis of 2'-3'-*O*-isopropylideneguanosine-5'-*N*-ethylcarboxamide (25)

Sodium 2',3'-*O*-isopropylideneguanosine-5'-carboxylate (**20**) (855 mg, 2.38 mmol) was dried to constant weight under vacuum. Anhydrous DMF (200 µL, 2.59 mmol) was added, the suspension cooled to 0 °C in an ice-salt bath and stirred for 15 minutes. Thionyl chloride (8.6 mL, 118.5 mmol) was added allowed to react for 90 minutes in an oil bath at 50 °C. The resulting orange solution was evaporated to dryness, subsequently co-evaporated with toluene (2 x 10 mL) and dried on the oil pump. The resulting oil was cooled to -15 °C and an excess of ethylamine was added. The remaining solution was allowed to gradually warm to room temperature and stirred overnight. The ethylamine was let to escape and further evaporated to dryness. The remaining oil was pre-adsorbed on silica gel and purified by chromatography (chexane/$CHCl_3$/MeOH, 60/34/6). The orange solid was washed with MeCN to obtain **25** as a white solid (315 mg, 0.865 mmol, 36%).

5.2.8. Synthesis of *O*^{6}-(benzotriazol-1-yl)-2'-3'-*O*-isopropylideneguanosine-5'-*N*-ethylcarboxamide (27)

Previously dried (6 hours) 2'-3'-*O*-isopropylideneguanosine-5'-*N*-ethylcarboxamide (**25**) (160 mg, 0.439 mmol) was suspended in anhydrous MeCN (10 mL). BOP (290 mg, 0.659 mmol) and DBU (100 µL, 0.668 mmol) were added obtaining a yellowish solution within 5 minutes. The reaction mixture was kept under N_2-atmosphere at room temperature for 14 hours. The mixture was diluted with ethyl acetate (40 mL), washed with water (5 x 20 mL) and brine (1 x 10 mL); additional washing with water (2 x 20 mL) and brine (1 x 10 mL) was necessary. The organic layer was dried over sodium sulphate, evaporated to dryness yielding a slight yellow

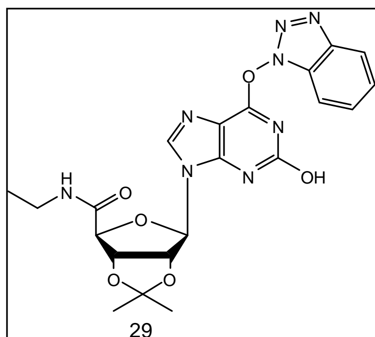
solid, which was recrystallized from isopropanol to give the product (**27**) as a fine white powder (130 mg, 0.270, 62 %).



^{1}H-NMR (400 MHz, DMSO-d_6): δ 0.64 (3H, t, CH_3); 1.33 (3H, s, CH_3-isopropylidene); 1.50 (3H, s, CH_3-isopropylidene); 2.74-2.92 (2x 1H, m, CH_2); 4.51 (1H, d, *J*= 2.0 Hz, 4'-H); 5.38 (1H, d, *J*= 5.6 Hz, 3'-H); 5.50 (1H, dd, *J*=1.6 Hz, 6.4 Hz, 2'-H); 6.27 (1H, s, 1'-H); 6.67 (2H, br s, NH_2, exchangeable); 7.46 (1H, m, NH); 7.51-7.54 (1H, m, Ar-H); 7.64 (2H, m, Ar-H); 7.16 (1H, d, *J*= 8.4 Hz, Ar-H); 8.19 (1H, s, H-8). **Exact mass**: (ESI+MS): calculated for [$C_{21}H_{23}N_9NaO_5^+$]: $[M+Na]^+$= 504.2; found $[M+Na]^+$= 504.1.

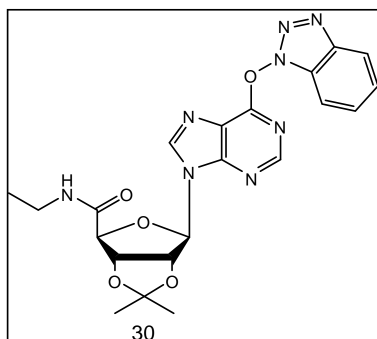
5.2.9. Attempted synthesis of 6-*O*-(benzotriazol-1-yl)-2-phenethoxy-9-(2',3'-*O*-isopropylidene-5'-*N*-ethylcarboxamido-β-D-ribofuranosyl)purine (28)

Previously dried (**27**) (83 mg, 0.172 mmol) was dissolved in anhydrous THF (7 mL), 2-phenylethanol (100 µL, 0.909 mmol) added and let to stir at 0 °C in an ice-salt bath for 15 minutes. Then, iso-amylnitrite (120 µL, 0.893 mmol) was added and the mixture heated to 50 °C in an oil bath until TLC showed complete disappearance of the starting material (**27**). The volatiles were removed under reduced pressure and the remaining yellow oil-like solution purified by preparative TLC ($CHCl_3$/c-hexane, 50:50; afterwards 2x $CHCl_3$:MeOH 98:2). Side products 2-hydroxy-6-*O*-(benzotriazol-1-yl)-9-(2',3'-*O*-isopropylidene-5'-*N*-ethylcarboxamido-β-D-ribofuranosyl)purine (**29**) was isolated (15 mg, 0.031 mmol, 18%) and 6-*O*-(benzotriazol-1-yl)-9-(2',3'-*O*-isopropylidene-5'-*N*-ethylcarboxamido-β-D-ribofuranosyl)purine (**30**) (30 mg, 0.064 mmol, 37%).



^{1}H-NMR (400 MHz, DMSO-d_6): δ 0.61 (3H, m, CH_3); 1.32 (3H, s, CH_3-isopropylidene); 1.51 (3H, s, CH_3-isopropylidene); 2.72 (1H, m, CH); 2.88 (1H, m, CH); 4.54 (1H, s, 4'-H); 5.42 (1H, br s, 3'-H); 5.51 (1H, br s, 2'-H); 6.34 (1H, br s, 1'-H); 7.35 (1H, m, NH); 7.57 (1H, m, Ar-H); 7.66 (2H, d, *J*= 3.6 Hz, Ar-H); 8.19 (1H, d, *J*= 8.4 Hz, Ar-H); 8.42 (1H, s, H-8); 12.16 (1H, br s, OH

exchangeable). **Exact mass**: (ESI-MS): calculated for [$C_{21}H_{21}N_8O_6^-$]: [M-H]$^-$= 481.2; found: [M-H]$^-$= 481.0.



^{1}**H-NMR** (400 MHz, DMSO-d_6): δ 0.51 (3H, t, *J*= 7.6 Hz, CH_3); 1.33 (3H, s, CH_3-isopropylidene); 1.51 (3H, s, CH_3-isopropylidene); 2.69-2.76 (1H, m, CH_2); 2.76-2.78 (1H, m, CH_2); 4.62 (1H, d, *J*= 2.0 Hz, 4'-H); 5.43 (1H, dd, *J*= 2.0 Hz, 6.0 Hz, 3'-H); 5.51 (1H, d, *J*= 6.0 Hz, 2'-H); 6.54 (1H, s, 1'-H); 7.56-7.57 (1H, m, NH); 7.58-7.59 (1H, m, Ar-H); 7.65 (2H, d, *J*= 3.6 Hz); 8.21 (1H, m, Ar-H); 8.45 (1H, s C-2 H); 8.81 (1H, s, H-8). **Exact mass**: (ESI-MS): calculated for [$C_{21}H_{22}ClN_8O_5^-$]: [M+Cl]$^-$= 501.1; found [M+Cl]$^-$= 501.0.

6. CONCLUSION AND FUTURE PROSPECTS

The objective of this thesis was to find and execute a synthetic route for derivative **1**. By selecting appropriate literature procedures, and taking benefit from experience present in the Medicinal Chemistry Unit, both a covergent as well as divergent route has been proposed.

However, during the execution of the selected procedure multiple difficulties were encountered, which led to changing the synthetic strategy several times. First of all, a convergent approach did not meet the expectations due to cumbersome protection / deprotection steps of the ribose moiety. In the case of biocatalytic deprotection, it can now be stated that the assumed structural similarity was probably not correct. Solubility issues most likely further contributed to non-functioning. Secondly, tritylation proved to be too low-yielding for further use; however direct combination with protection of the other hydroxyl groups as an ester might be an improvement. Additional low yielding of purine modification led us to pursue a divergent approach.

Two attempts were made to reduce the amount of steps after investigation of literature procedures. Firstly, a direct acid activation combined with purine chlorination succeeded after four attempts. However, the yield was inferior to the known two steps procedure, and the pure product could not be isolated. Employing a benzotriazole N^6-substitution on the 5’ *N*-ethylcarboxamido derivative **25** proceeded in higher yield and involved less laborious purification. A subsequent attempt to directly functionalize the C-2 position on the purine proved not successful. Nonetheless, the 2-hydroxy derivative **29** was found in a quite high yield as by-product. Future efforts should be directed at obtaining this C-2 OH intermediate in acceptable yield and using it for the further conversion to the desired 2-phenylethyl ether.

7. LITERATURE

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