THYMIDINE KINASE AS A MOLECULAR TARGET FOR THE
DEVELOPMENT OF NOVEL ANTICANCER AND ANTIBIOTIC AGENTS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

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ABSTRACT

The purpose of this dissertation is to develop novel boron-delivery agents for the treatment of glioblastoma multiforme (GBM) and to discover novel antibiotics for the treatment of anthrax infections.

GBM is one of the most aggressive of all cancers. Despite significant advances in the treatment of almost all other types of cancer, the survival of patients diagnosed with GBM has remained almost unchanged for decades. The GBM accounts for approximately 50% of newly diagnosed primary brain tumors and its yearly incidence in the USA is 4.3 cases per 100,000 people. *Bacillus anthracis* is a Gram-positive, spore-forming, anaerobic bacterium that causes anthrax. Due to the infectiousness of *B. anthracis* spores and the high mortality of inhalational anthrax, the major concern with anthrax in the 21st century is its use as a biological weapon. Therefore, both GBM and anthrax infections are major public health concerns in USA. Thymidine kinase (TK), a key enzyme of the salvage pathway for DNA biosynthesis, is an attractive molecular target for both diseases because of its high activity in tumor cells and its frequent occurrence in pathogenic bacteria. The goal of our research is to develop TK-targeting anticancer agents for GBM and TK-targeting antibiotics for *B. anthracis*.

In GBM-related research, boron neutron capture therapy (BNCT) was chosen as the therapeutic modality. BNCT kills tumor cells without damaging normal brain tissue
by utilizing a nuclear reaction of the boron-10 isotope \(^{10}\text{B}\) with thermal neutrons. Previous structure-activity relationship (SAR) studies of 3-carboranyl thymidine analogues (3CTAs) identified one promising boron-delivery agent for BNCT, designated N5-2OH. Two feasible synthetic routes for the preparation of \(^{10}\text{B}\)-enriched N5-2OH were developed and it was evaluated as a boron-delivery agent for BNCT in pilot neutron irradiation experiments. Treatment of rats bearing intracerebral F98 glioma with \(^{10}\text{B}\)-enriched N5-2OH and neutron irradiation resulted in prolonged survival compared with control groups, indicating that \(^{10}\text{B}\)-enriched N5-2OH has a significant potential as a boron-delivery agent for BNCT of GBM. Stereochemical- and geometrical N5-2OH isomers were synthesized and their substrate specificities for human thymidine kinase 1 (hTK1) were evaluated to complete SAR studies of the N5-2OH series. A computational model for the binding of the N5-2OH series to the active site of hTK1 was also developed.

In order to improve physicochemical properties of 3CTAs, zwitterionic \textit{nido} 3CTAs were synthesized and evaluated as boron-delivery agents for BNCT. All compounds of this 3CTA subclass were good substrates for hTK1. In \textit{in vivo} studies, one zwitterionic \textit{nido} 3CTA accumulated selectively in mice implanted with TK1-expressing L929 (wt) tumors \textit{versus} those implanted with L929 TK1 (-) tumors. In addition, it showed improved water solubility compared with N5-2OH. The zwitterionic \textit{nido} \textit{m}-carborane system constituted a novel boron-carrying moiety for BNCT drug development, and thus, its unique properties were further investigated. The zwitterionic \textit{nido} \textit{m}-carborane cluster exhibited unexpected chemical features by reacting with the carbonyl group of ketones or aldehydes without addition of acid catalyst to afford zwitterionic iminium-substituted carborane.
In anthrax-related research, a compound library composed of known antiviral and anticancer drugs was screened in two assay systems to identify a lead compound for the development of novel anthrax-specific antibiotics. Phosphoryl transfer assays showed that most of the tested thymidine analogues of this library were good substrates of both TK from *B. anthracis* (*Ba*TK) and hTK1. However, only FLT and Floxuridine showed activity in *in vitro* toxicity studies with *B. anthracis* Sterne.

Due to the limited success in identifying a lead compound out of a library of known antiviral and anticancer drugs, twenty thymidine analogues were designed and synthesized as potential inhibitors of *Ba*TK and thymidine monophosphate kinase of *B. anthracis* (*Ba*TMPK), which is another key enzyme in DNA biosynthesis. The inhibitors were designed to mimic thymidine triphosphate (dTTP), the endogenous feedback inhibitor of *Ba*TK. The phosphate moiety of dTTP was replaced with sulfonamide, amide, urea, thiourea, or triazole groups in these inhibitors. Some of the urea-, thiourea-, and triazole-linked inhibitors exhibited relatively low IC$_{50}$ values in preliminary growth inhibition studies with *B. anthracis* Sterne. Overall, the concept of novel antibiotics targeting *Ba*TK and/or *Ba*TMPK was validated and two inhibitors were selected as lead compounds for further structural optimization.
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<td>HOMO</td>
<td>Highest occupied molecular orbital</td>
</tr>
<tr>
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<td>High performance liquid chromatography</td>
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<td>HRMS</td>
<td>High resolution mass spectroscopy</td>
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<td>hTK1</td>
<td>Human thymidine kinase 1</td>
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<td>hTMPK</td>
<td>human thymidine monophosphate kinase</td>
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<td>Hertz</td>
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<td>IC_{50}</td>
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<td>IR</td>
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xx
<table>
<thead>
<tr>
<th>Abbreviation</th>
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<td>LDL</td>
<td>Low-density lipoprotein</td>
</tr>
<tr>
<td>LUMO</td>
<td>Lowest occupied molecular orbital</td>
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<td>m</td>
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<td>Molar</td>
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<td>Monophosphate</td>
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<td>Normality</td>
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<td>n-Butyllithium</td>
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<td>4-Methyl morpholine N-oxide</td>
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<td>Ortho</td>
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<td>Osmium tetraoxide</td>
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<td>Para</td>
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<td>TBAF</td>
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</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
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</tr>
<tr>
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</tr>
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<td><em>Ureaplasma urealyticum</em> thymidine kinase</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
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CHAPTER 1

THYMIDINE KINASE AS A MOLECULAR TARGET FOR
ANTICANCER, ANTIVIRAL, AND ANTIBIOTIC THERAPY

1.1. Introduction

DNA (2’-deoxyribonucleic acid) is a polynucleotide chain molecule that encodes genetic information. DNA biosynthesis requires four types of 2’-deoxyribonucleoside triphosphates (dNTPs) as essential precursors, which are 2’-deoxyadenosine triphosphate (dATP), 2’-deoxyguanosine triphosphate (dGTP), 2’-deoxycytidine triphosphate (dCTP), and thymidine triphosphate (dTTP). Each dNTP is composed of three subunits: a nitrogen-containing base, a five-carbon sugar, and three phosphate groups, as shown in Figure 1.1. The nitrogen-containing bases are classified into two pyrimidine bases, thymine (T) and cytosine (C), and two purine bases, adenine (A) and guanine (G). The carbohydrate unit in DNA is 2’-deoxy-D-ribose while D-ribose is found in RNA.

1.2. De novo pathway versus salvage pathway of dNTPs

dNTPs are biosynthesized by two pathways: De novo pathway and salvage pathway,\(^\text{1,2}\) which are depicted in Figure 1.2. The de novo synthesis of dNTPs begins with basic cellular building blocks such as glutamine, glycine, aspartate, 5-
phosphoribosyl-1-pyrophosphate, CO₂ and folic acid. In case of purine-based dNTPs, inosine monophosphate (IMP) is the key intermediate while uridine monophosphate (UMP) is the key component in pyrimidine-based dNTP biosynthesis. Ribonucleotide reductase (RR) is another key enzyme of the de novo pathway, which reduces ribose in all four ribonucleotides to 2′-deoxyribose.

![Diagram of DNA structure](image)

**Figure 1.1.** Structures of 2′-deoxyribonucleoside triphosphates (dNTPs).

dTTP: Thymidine triphosphate, dCTP: 2′-deoxycytidine triphosphate, dATP: 2′-deoxyadenosine triphosphate, dGTP: 2′-deoxyguanosine triphosphate.

The salvage pathway utilizes 2′-deoxyribonucleosides (dNs) from intra- or extracellular pools generated from DNA catabolism. Deoxyribonucleoside kinases (dNKs) catalyzes the initial transfer of the γ-phosphate from ATP to the 5′-hydroxyl group of dNs to form 2′-deoxyribonucleoside-5′-monophosphates (dNMPs). This step is generally considered to be the rate-limiting step in the dNK salvage pathway. The dNMPs are further phosphorylated to dNDPs (2′-deoxyribonucleoside-5′-diphosphates) by nucleoside monophosphate kinases (NMPKs). Finally, nucleoside diphosphate kinases
(NDPKs) catalyze the formation of dNTPs. The initial phosphorylation by dNKs is irreversible, while the following two steps catalyzed by NMPKs and NDPKs are reversible. Intracellularly, dephosphorylation of dNMPs occurs primarily via 5’-nucleotidases (5’-NTs).^{5}

**Figure 1.2.** *De novo* and salvage pathways of dNTP synthesis


**1.3. dNKs (Deoxyribonucleoside kinases)**

Mammals have four distinct dNKs; thymidine kinase 1 (TK1), deoxycytidine kinase (dCK), thymidine kinase 2 (TK2), and deoxyguanosine kinase (dGK).^{1} TK1 and dCK are found in the cytosol while TK2 and dGK are located in the mitochondria. TK1
converts thymidine (dThd) and 2’-deoxyuridine (dUrd) to the corresponding monophosphates (dTMP and dUMP) in the presence of ATP and Mg\(^{2+}\). Deoxycytidine kinase (dCK) phosphorylates endogenous 2’-deoxycytidine (dCyd), 2’-deoxyguanosine (dGuo), and 2’-deoxyadenosine (dAdo) to the corresponding monophosphates (dCMP, dGMP, and dAMP, respectively). Mitochondrial TK2 phosphorylates pyrimidine-based dNs (dThd, dUrd, and dCyd) while dGK phosphorylates purine-based dNs (dGuo and dAdo). Human TK1 (hTK1) shares high amino acid sequence homology with those of other vertebrates such as mouse, hamster, and chicken.\(^6\) The mouse dCK protein sequence is 88% homologous to human dCK.\(^7\)

*Drosophila melanogaster* (Fruit fly) has a single multisubstrate deoxyribonucleoside kinase (*Dm*-dNK), which phosphorylates all four natural substrates (dThd, dCyd, dGuo, and dAdo) with higher efficiency.\(^8,9\) Silk moth and mosquito also contain a multisubstrate kinase, indicating that insects may only have a single type of dNK.\(^10,11\)

TK1-like sequences are found in several bacteria such as the Gram-positive *Bacillus anthracis* and *Staphylococcus aureus*, the Gram-negative *Escherichia coli*, and the mycoplasma bacterium *Ureaplasma urealyticum*.\(^12-14\) Lactobacilli and Bacilli possess additional kinases to phosphorylate the other three natural substrates.\(^15\) However, *E. coli* has only a TK activity but lacks dCK and dGK activities.\(^14\) On the other hand, *Mycobacterium tuberculosis* is devoid of any TK activity.\(^15\)

Several herpes viruses have TK activity including herpes simplex virus 1 and 2 (HSV1-TK and HSV2-TK), Varicella Zoster virus (VZV-TK), equine herpes virus 4 (EHV4-TK), Epstein-Barr virus (EBV-TK) and Kaposi's sarcoma-associated herpesvirus
(KSHV)/Human Herpesvirus 8 (HHV-8). Two major differences exist between hTK1 and viral TKs. First, all viral TKs except HSV2-TK have additional thymidylate kinase activity, which converts dTMP into dTDP. Secondly, viral TKs have a broad substrate specificity, and thus, phosphorylate purine and pyrimidine nucleosides while hTK1 accepts only endogenous dThd and dUrd. In particular, HSV-TK can convert therapeutic nucleosides with acyclic an sugar moiety, such as acyclovir, ganciclovir, and penciclovir, into the corresponding nucleoside monophosphates. Poxviruses are an exception among the viruses because it is believed that they contain a TK1-like kinase.16,17

<table>
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<tr>
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<th>hTK2 (23304350)</th>
<th>dCK (33357874)</th>
<th>dGK (17943105)</th>
<th>dNK (31615910)</th>
<th>HSV1-TK</th>
<th>VZV-TK</th>
<th>EBV-TK</th>
<th>UuTK</th>
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<td>47%</td>
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</tbody>
</table>

Table 1.1. Protein sequence identities of dNKs generated with BLAST 2.2.6

aIdentification number of the proteins from the National Center for Biological Information (NCBI)
b n.s: No significant similarity
Based on the amino acid sequences and substrate specificities, the known dNKs are classified into three subgroups: 1) TK1-like kinases, 2) TK2, dCK, dGK, and Dm-dNK, and 3) viral TKs. As shown in Table 1.1, hTK1 has 36% and 39% sequence identities with *Ureaplasma urealyticum* thymidine kinase (*UuTK*) and *Clostridium Acetobutylicum* thymidine kinase (*CaTK*), respectively. In the second group, there is at least 30% sequence identity among TKs while the sequence identities among the viral TKs range from 25% to 39%. It should be noted that some scientists only postulate two subgroups of dNKs, the TK1-like kinases and the other dNKs.18,19

1.4. Crystal structures of dNKs

Several dNK X-ray crystal structures have been published. Table 1.2 summarizes structural information (e.g., PDB ID, resolution, and ligands of dNKs deposited in Protein Data Bank (PDB). Figure 1.3 shows three-dimensional (3D) X-ray crystal structures of dNKs. X-ray crystal structures of human dNKs except TK2 were determined during the last five years.20-24 The first X-ray crystal structure of a human dNK, the mitochondrial dGK, was reported by Eklund and coworkers in 2001.21 Surprisingly, ATP was found to bind into the substrate-binding site, not in the phosphate-donor site of the dimeric 3D structure of dGK. Lavie and coworkers reported the crystal structure of dCK in complex with its endogenous substrate dCyd and with the anticancer prodrugs AraC and gemcitabine. dCK was also crystallized as a homodimeric protein.22 All dCK crystal structures contained adenosine diphosphate (ADP) in the phosphate-donor site. Very recently, dCK complexed with ADP and clofarabine was reported.24 Clofarabine is a purine-nucleoside chemotherapeutic agent for the treatment of leukemias and other
hematological malignancies. The structural analysis of dCK-clofarabine-ADP complex revealed that the purine-derived nucleoside (clofarabine) displays the same binding pattern as the pyrimidine-based nucleosides (AraC and gemcitabine). Two different groups reported crystal structures of hTK1 in complex with dTTP, which is the endogeneous feedback inhibitor of this kinase. Both structures were crystallized as truncated tetramers, in which each monomer is missing 41 amino acids at the C-terminal region. It was reported, however, that removal of this C-terminal region did not affect hTK1 activity.

*Dm*-dNK structures were determined as dimers in complex with either of the two substrates dThd or dCyd, or the feedback inhibitor dTTP. The substrate binding site of *Dm*-dNK was similar to those of dGK and dCK. However, there was a significant density for Mg$^{2+}$ in the vicinity of β- and γ-phosphates binding sites in the *Dm*-dNK structure. The Mg$^{2+}$ appeared to play a crucial role in inducing conformational changes of *Dm*-dNK upon dTTP binding. Thymidine kinase from *Ureaplasma urealyticum* (*Uu*TK), a human pathogen in the urogenital tract that can cause pregnancy complications, was solved as a tetramer in complex with dTTP or dThd. dThd alone binds to the active site of *Uu*TK in a similar way as the dThd portion in dTTP. Thymidine kinase from *Clostridium acetobutylicum* (*Ca*TK), which is a Gram-positive, sporulating, anaerobe capable of naturally producing acetone, butanol, and ethanol, was co-crystallized with ADP. Due to the absence of substrate or feedback inhibitor, the substrate binding site appears to be more open compared to the hTK1 and *Uu*TK.
<table>
<thead>
<tr>
<th>dNKs</th>
<th>PDB ID</th>
<th>Resolution (Å)</th>
<th>Ligands</th>
<th>Reference</th>
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<td>1XBT</td>
<td>2.40</td>
<td>dTTP</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>1W4R</td>
<td>1.83</td>
<td>dTTP</td>
<td>20</td>
</tr>
</tbody>
</table>

| dCK  | 1P60   | 1.96           | dCyd and ADP | 22    |
|      | 1P62   | 1.90           | Gemcitabine and ADP | 22    |
|      | 1P5Z   | 1.60           | AraC and ADP  | 22    |
|      | 2A7Q   | 2.55           | Clofarabine and ADP | 24    |
|      | 2A2Z   | 3.02           | dCyd and UDP  | 27    |

| dGK  | 1JAG   | 2.80           | ATP      | 21    |
|      | 2B8T   | 2.00           | dThd    | 12    |
|      | 1XMR   | 2.50           | dTTP    | 23    |

| uTK  | 1J90   | 2.56           | dCyd    | 21    |
|      | 1OT3   | 2.50           | dThd    | 26    |
|      | 1OE0   | 2.40           | dTTP    | 26    |

| HSV1-TK | 1VTK | 2.75 | ADP and dTMP | 28 |
|         | 2VTK | 2.80 | ADP and dThd | 28 |
|         | 3VTK | 3.00 | ADP and 5-IdUMP | 28 |
|         | 1KI2 | 2.20 | Ganciclovir | 29 |
|         | 1KI3 | 2.37 | Penciclovir | 29 |
|         | 1KI7 | 2.20 | 5-iododeoxyuridine | 29 |
|         | 1KI8 | 2.20 | BVDU | 29 |
|         | 1E2I | 1.90 | Acyclovir | 30 |

| VZV-TK | IOSN | 3.20 | BVDU-MP and ADP | 31 |
|        | 1P6X | 2.00 | dThd  | 32 |
| EHV4-TK | 1P75 | 3.02 | TP5A  | 32 |
|         | 1P73 | 2.70 | TP4A  | 32 |
|         | 1P72 | 2.10 | dThd and ADP | 32 |

Table 1.2. Structural information of dNKs deposited in Protein Data Bank

There have been several reports on the crystal structures of herpes simplex virus 1 thymidine kinase (HSV1-TK).\(^{28-30,33-35}\) X-ray crystal structures of HSV1-TK were determined in complex with various substrates including dThd, dTMP, 5-iodo-2′-deoxyuridine (5-IdUrd), 5-iodo-2′-deoxyuridine monophosphate (5-IdUMP), and the
antiviral drugs (E)-5-(2-bromovinyl)-2’-deoxyuridine (BVDU), acyclovir, ganciclovir, and penciclovir.\textsuperscript{28-30,33-35} Thymidine kinase of varicella zoster virus (VZV-TK), which causes both a chicken pox in children and shingles in adults, was determined as a monomer in complex with BVDU-MP and ADP.\textsuperscript{31} Thymidine kinase of equine herpes virus 4 (EHV4-TK), which causes respiratory disease and, less commonly, abortion in horses, was crystallized in complex with dThd and two so-called bisubstrate inhibitors with either four or five phosphate units covalently inserted between the 5’-positions of dThd and adenosine.\textsuperscript{32}

1.5. Clinical implications of dNKs

The therapeutic activity of anticancer and antiviral agents derived from nucleosides depends on the action of dNKs, which usually catalyzes the initial step in the conversion of nontoxic nucleoside prodrugs into the corresponding toxic nucleoside triphosphates. The anti-HIV prodrugs zidovudine (AZT) and stavudine (d4T) are activated by hTK1,\textsuperscript{36,37} while anticancer prodrugs gemcitabine, cytarabine, clofarabine, cladribine, and fludarabine undergo monophosphorylation by the action of dCK.\textsuperscript{38-42} With the exception of AZT, the initial 5’-monophosphorylation by hTK1 and dCK is the rate-limiting step in this activation process.\textsuperscript{36-43} The antiviral agents acyclovir and ganciclovir are converted into the monophosphates by HSV1-TK. Further phosphorylation is then carried out by both viral and cellular enzymes in the host. The specific toxic effects towards lytic viruses or proliferative cancer cells are predominantly exerted by the active triphosphate forms of these prodrugs, which block DNA synthesis by inhibiting DNA polymerases or HIV reverse transcriptase and by incorporating into 3’-terminal ends of DNA chains.\textsuperscript{36-43}
Figure 1.3. Crystal structures of dNKs

TK1-like family: (a) hTK1 with dTTP, (b) UuTK with dTTP, (c) CaTK with ADP. dCK, dGK, and Dm-dNK family: (d) dCK with dCyd and ADP, (e) dGK with ATP, (f) Dm-dNK with dCyd. Viral TK family: (g) HSV1-TK with dThd and ADP, (h) VZV-TK with BVDU-MP and ADP, (i) EHV4-TK with dThd and ADP
Furthermore, dNKs have become attractive candidates in suicide gene therapy and cancer diagnostics. In suicide gene therapy, a dNK-encoded gene is inserted into the genome of target cells. The expressed dNK protein activates a specific nucleoside prodrug, which is usually not activated by cellular dNKs in the host. The current most widely used combination is HSV-tk gene and ganciclovir. Tumors cells transfected with the HSV-tk gene express the corresponding HSV-TK, which phosphorylates ganciclovir selectively in the transfected cells. The monophosphate is further di- and triphosphorylated by NMPK (and partially also HSV-TK) and NDPK, respectively. Incorporation of ganciclovir triphosphates into the DNA chain leads to the termination of DNA replication, and thus, resulting in tumor cell death.

More recently, dNKs have also been explored as molecular targets to detect tumor progression and viral infections. Nucleoside prodrugs used for this purpose include 3’-fluoro-3’-deoxythymidine (FLT), 1-(2-fluoro-5-methyl-β-arabinofuranosyl)uracil (FMAU), and 1-(2-fluoro-5-iodo-β-arabinofuranosyl)uracil (FIAU), which have the potential to be used as Positron Emission Tomography (PET) agents in radiolabeled form.

1.6. Human thymidine kinase 1 (hTK1)

1.6.1. hTK1 and TK2

Although TK2 is an isoenzyme of hTK1, it has significantly different biochemical properties. The human TK1 gene is located on chromosome 17 and that of TK2 is on chromosome 16. Both enzymes do not share significant homology in their amino acid sequences as shown in Table 1.1. The expression of hTK1 is cell cycle-dependent, whereas the TK2 activity is constant throughout the cell cycle. The monomeric molecular
weights of hTK1 and TK2 are 25.5 kDa and 29 kDa, respectively.\textsuperscript{57,58} Both hTK1 and TK2 phosphorylate dThd and dUrd efficiently, but TK2 can also accept dCyd as a substrate. The limited dimensions of the dThd-binding site in hTK1 crystal structure reflect its narrow substrate specificity. Nevertheless, hTK1 can phosphorylate dThd analogues with modifications at the N-3, C-5 position, and the 3’-position of the 2’-deoxyribose.\textsuperscript{4,59-62} In particular, bulky substitutions at the N-3 position of dThd are tolerated by hTK1.\textsuperscript{59,61,62} On the other hand, TK2 primarily tolerates modification at the 2’-ara position of dThd or dUrd and it is also more susceptible to bulkier modification patterns at C-5 than hTK1.\textsuperscript{60,63}

No hTK1 gene polymorphisms or phenotypic alterations have been reported in humans until now. However, inactivation of the cytosolic TK1 gene in mice, which eliminates dTMP synthesis \textit{via} the salvage pathway, had a profound effect on their health, even though the \textit{de novo} pathway remained intact under these conditions.\textsuperscript{64} On the other hand, mutations in TK2 resulted in devastating myopathy and depletion of muscular mitochondrial DNA in infancy.\textsuperscript{65,66}

\textbf{1.6.2. Biochemical properties of hTK1}

During the cell cycle, the activity of hTK1 remains very low in early G1 phase, begins to increase at late G1/S-phase, and reaches its maximum during S-phase, in which DNA is synthesized. hTK1 is degraded during mitosis, and thus, undetectable during G1 phase. hTK1 activity is regulated during the cell cycle through phosphorylation. Hyperphosphorylated hTK1 decreases affinity for dThd.\textsuperscript{67,68} Serine 13 of hTK1 is the residue involved in the mitotic phosphorylation. Ser 13 is a highly conserved amino acid
residue in vertebrate TK1s. Mutation of serine 13 into alanine 13 resulted in abolishment of mitotic phosphorylation of hTK1.\textsuperscript{67,68}

ATP is a co-substrate of hTK1 and the phosphate donor for dThd and dUrd. It also acts as a positive effector, controlling a slow transition from low dThd-affinity hTK1 dimer ($K_M = 15$ $\mu$M) to a high affinity tetramer ($K_M = 0.7$ $\mu$M). hTK1 has a 20-fold higher affinity for dThd in the presence of ATP than in the absence of ATP.\textsuperscript{69} The hTK1 dimer appeared to be dominant in G0 and G1 phases while the tetramer was predominantly found in S phase.\textsuperscript{68}

1.7. TK1 and drug design

TK2, dCK, and dGK are synthesized at relatively constant rates throughout the cell cycle. hTK1 activity, however, is only found in proliferating cells, and thus, this enzyme is widely distributed and expressed in malignant tumors,\textsuperscript{1,43} including those of the brain,\textsuperscript{70,71} pancreas,\textsuperscript{52} and lung.\textsuperscript{72} This makes the enzyme a very attractive molecular target for the design of novel anticancer agents and tumor-prognostic markers. The recently determined hTK1 crystal structures\textsuperscript{20,23} also allow structure-based drug design \textit{in silico}. The work described in this dissertation utilized hTK1 as a molecular target for the discovery of novel agents for the treatment of brain tumors by boron neutron capture therapy (BNCT), which will be discussed in Chapters 2-4. Knowledge and experience accumulated in the anticancer project were then applied to discovery of novel antibiotics targeting \textit{BaTK}, which is the family of TK1-like kinases, for the treatment of anthrax infections (Chapter 5).
2.1. Glioblastoma multiforme (GBM)

Glioblastoma multiforme (GBM) is one of the most malignant and aggressive of all tumors. Despite significant advances in the treatment of many other cancer types using surgery, chemotherapy, and radiation therapy, the survival of patients diagnosed with GBM has remained almost unchanged for decades. The GBM accounts for approximately 50% of newly diagnosed primary brain tumors in USA and Europe, and the incidence is 4.3 cases per 100,000 people per year from 1999 to 2002 in USA (http://www.cdc.gov). Current standard therapy for GBM consists of surgical resection followed by radiotherapy. However, the 5-year survival rate for GBM patients over 45 is 2% or less and the median survival is generally less than one year from the time of diagnosis. This failure to cure GBM patients with surgery is due to the inability of completely eradicating microinvasive tumor cells within the brain, mainly within a 2 cm-margin of the surgical resection cavity. The success of conventional chemotherapy in the treatment of GBM is also limited, probably because many anticancer agents cannot...
penetrate the blood-brain barrier (BBB) effectively. The most commonly used chemotherapeutic drugs for GBM are temozolomide and BCNU (carmustine). Radiation therapy also has limited success in the treatment of GBM due to the damage to normal brain tissue and spinal cord.

In a recent randomized trial with 573 GBM patients from 85 centers, 84% percent of whom had undergone prior debulking surgery, radiotherapy plus temozolomide, a conventional DNA methylating chemotherapeutic agent, resulted in a median survival of 14.6 months compared with 12.1 months for radiotherapy alone. This 2.5 months-increase is probably one of the most significant documented improvements in the treatment of GBM during the last three decades but it also bears testimony to the grim reality that this form of cancer remains to be almost completely incurable in contrast with other forms of cancer.

Therefore, there is a pressing obligation to develop a broad range of novel innovative treatment concepts and strategies for GBM. Several of these innovative modalities have shown promising results in preclinical evaluation and clinical trials. These include X-radiation sensitization, photodynamic therapy, signal transduction inhibition, angiogenesis inhibition, radiation therapies including BNCT, immunotherapy including vaccines, gene therapy, oncolytic viruses, and stem cell therapy. BNCT may have the potential to improve the outcome of GBM treatment because it can direct radiation damage selectively to tumor
cells by employing molecular-targeting strategies.

2.2. Boron neutron capture therapy (BNCT)

2.2.1. Principles

BNCT is a binary chemotherapeutic method for the treatment of cancers, which is based on the nuclear reaction between boron-10 isotope ($^{10}$B) and low energy thermal neutrons. Both components alone are harmless in humans. However, when $^{10}$B atom is irradiated with thermal or epithermal neutrons, an unstable $^{11}$B isotope is produced, which subsequently undergoes fission to yield two high linear energy transfer (LET) particles: a $^4$He nuclei and a $^7$Li nuclei (Figure 2.1). These particles have very short path lengths (9 and 5 µm, respectively), which approximate the diameter of a cell (10-15 µm). Provided that $^{10}$B can be accumulated selectively in cancer cells, subsequent neutron irradiation can selectively kill these cells without damaging the surrounding normal cells due to the locally-restricted lethal effect.

![Figure 2.1](Image)

Figure 2.1. Nuclear capture and fission reaction by $^{10}$B and thermal neutron

2.2.2. Keys to success of BNCT
For successful BNCT, $^{10}$B-delivery agents should fulfill four criteria. First, a minimum accumulation of 20 $\mu$g of non-radioactive $^{10}$B per gram of tumor tissue, which corresponds to $\sim 10^9$ boron atoms per tumor cell, is required. Secondly, $^{10}$B should be delivered selectively to tumor cells and attained while at the same time the concentration of $^{10}$B in surrounding normal tissue should be kept low to minimize damage to normal tissue. It is crucial that the ratio of tumor to brain (T/Br) and tumor to blood (T/Bl) boron concentration is higher than 4. Thirdly, $^{10}$B should be retained in the tumor tissue for a sufficient period of time to carry out BNCT. Finally, $^{10}$B-delivery agents should have minimal systemic toxicity. Currently, no single boron-delivery agent satisfies all four criteria.

### 2.2.3. Boron-delivery agents in clinical trials

Only two boron-containing compounds have been evaluated extensively in clinical trials. One is (L)-4-dihydroxyborylphenylalanine (BPA), an amino acid derivative, the other is sodium mercaptoundecahydro closo-dodecaborate (BSH), a polyhedral borane anion (Figure 2.2). BPA has been used in the form of a fructose complex because of its low water solubility. The BPA-fructose complex showed a moderately selective accumulation in tumors cells. The advantages of BSH as boron-delivery agents are high boron percentage and low systemic toxicity. However, BSH was found to be very sensitive to oxidation when exposed to air. In addition, the T/Br ratio of BSH ranged from 1.0 to 1.3. Although the safety of both drugs has been well established, they have not demonstrated tumor-selective targeting. Therefore, there is an urgent need to discover novel tumor-selective boron-delivery agents for BNCT.
2.3. Boronated thymidine analogues as BNCT agents

2.3.1. Boron-containing cellular building blocks

Many BNCT agents have been designed and synthesized as boronated analogues of endogenous cellular building blocks because tumors cells grow rapidly, and thus, have an increased requirement for cellular building blocks compared with normal cells. Prerequisite for the success of this strategy is, of course, that such conjugates of biomolecules with the boron moiety are recognized by tumor cells in the same way as the corresponding unaltered biomolecules.

Tumor cells require a substantial amount of nucleic acid precursors for DNA biosynthesis. As discussed in Chapter 1, tumors cells synthesize dNTPs via salvage and de novo pathways. Boron-containing nucleosides, in particular derivatives of dThd and dUrd, have been intensively studied as BNCT agents because of their potential metabolic incorporation into tumors cells. The biochemical pathway of these agents will be discussed in detail in Chapter 2.3.2.
Amino acids and related peptides are also required as cellular building blocks in proliferating cells to a greater extent. BPA is known to accumulate selectively in tumor cells based on its ability to serve as a substrate of the (L)-amino acid membrane transporter,\textsuperscript{140-142} which is overexpressed in several forms of cancer due to the higher demand for amino acids.\textsuperscript{143} Increased expression of the glucose transporter was also found in tumor cells,\textsuperscript{144} and thus, boronated glucose could be taken up in tumors by utilizing this elevated glucose transport system.

Polyamines (e.g. putrescine, spermidine, and spermine) are important biochemical components for tumor cell proliferation and differentiation. Polyamine transport systems are up-regulated in rapidly growing tumor cells, and thus boron-containing polyamines could be transported more effectively into tumor cells.\textsuperscript{145} Boron-containing porphyrins also showed selective uptake and retention in tumor cells. It appears that these structures are “hitchhiking” with low-density lipoproteins (LDLs), which are selectively taken up by many tumor cells because they have an increased demand for cholesterol, and thus, overexpress LDL receptors on their cell membrane.\textsuperscript{134-139}

### 2.3.2. Biochemical metabolic pathway of boron-containing thymidine analogues

A possible salvage pathway for activation of boron-containing dThd or dUrd analogues is shown in Figure 2.3. Boronated dThd analogues (dThds) may enter cells either via passive diffusion or facilitated and/or concentrative nucleoside transport. Provided boronated dThds are good substrates for phosphorylating enzymes such as hTK1, TMPK, or NDPK, metabolites of boronated dThds are accumulated in the cells due to the acquired phosphate groups. Nucleoside transporters do not accept nucleotides
as substrates and the negatively charged phosphate moieties prevent their own efflux by passive diffusion. However, monophosphates (MPs) of boronated dThds could be exported by nucleotide efflux pumps such as MRP-4 and MRP-5. The key regulatory enzyme in the metabolism of boronated dThds presumably is hTK1. As discussed in Chapter 1, the expression of this kinase is tightly cell cycle-regulated and active enzyme is found only in S-phase. Therefore, conversion of boronated dThds to the corresponding dTMPs by hTK1 may result in their selective transient intracellular entrapment.

**Figure 2.3.** Biochemical salvage pathway of boron-containing thymidines.
It has been suggested that boron-containing nucleosides and nucleotides require access to cell nuclei in order to be active as BNCT agents and the hypothetical incorporation of boronated dNTPs into DNA has spurred early efforts to synthesize boron-containing nucleoside prodrugs as BNCT agents. However, in antimetabolite chemotherapy, active triphosphates are also responsible for most of the severe toxic side-effects, mainly in bone-marrow and intestinal epithelium. Triphosphates of boron-containing nucleoside prodrugs could exert a similar toxicity profile without neutron radiation when administered systemically at high doses. In particular, triphosphates of dThds substituted with a boron moiety at the N-3 position potentially terminate DNA synthesis because substitution at the N-3 position of thymine interferes with the base-pairing with the complementary adenine. Therefore, the monophosphorylation and/or diphosphorylation of boronated dThds by hTK1 and TMPK, and the subsequent transient intracellular entrapment may be sufficient for BNCT.

2.3.3. hTK1 and brain tumors

TK1 and dCK activities of the rat F98 glioma, murine L929 TK1 (+), and L929 TK1 (-) cell lines were reported by Barth and coworkers. The rat F98 glioma cell line exhibits similar growth and invasive characteristics to human GBM, and therefore, it is considered as a suitable rat model for human GBM studies. As shown in Table 2.1, TK1 activities were 356 pmol/mg/minutes for F98 glioma and 228 pmol/mg/minutes for L929 TK1 (wt) cell lines, which express TK1. TK1 activities in F98 glioma and L929 TK1 (wt) cell lines were significantly higher than L929 TK1 (-) cells and normal brain. dCK activity was 3-5 fold higher in tumor cell lines than in normal brain. These data indicate
that hTK1 is an attractive target enzyme in the discovery of novel boron-delivery agents for BNCT. It should be noted that murine TK1 and hTK1 have very similar substrate specificities.\textsuperscript{151}

<table>
<thead>
<tr>
<th>Cell line</th>
<th>TK1 Activity (pmol/mg/min)</th>
<th>dCK Activity (pmol/mg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F98 glioma</td>
<td>356 ± 18</td>
<td>43 ± 5</td>
</tr>
<tr>
<td>L929 TK1 (wt)</td>
<td>228 ± 16</td>
<td>30 ± 2</td>
</tr>
<tr>
<td>L929 TK1 (-)</td>
<td>9 ± 3</td>
<td>29 ± 3</td>
</tr>
<tr>
<td>Normal brain (cortex)</td>
<td>≤ 5</td>
<td>≤ 10</td>
</tr>
</tbody>
</table>

Table 2.1. TK1 and dCK activities in brain tumor cell lines and normal brain.\textsuperscript{70}

\textsuperscript{a} Activities of TK1 and dCK were determined by measuring the phosphorylation of [methyl-\textsuperscript{3}H] dThd and [methyl-\textsuperscript{3}H] dCyd using total protein extracts from the cell lines indicated.

\textbf{2.3.4. Evaluation of hTK1 substrate characteristics of boron-containing dThds in phosphorylation transfer assay (PTA).}

Figure 2.4 shows the phosphorylation of dThd by hTK1, which catalyzes the transfer of γ-phosphate group of ATP to the 5′-hydroxyl group of dThd or boron-containing dThds. The reaction by hTK1 produces dTMP and ADP. In a phosphoryl transfer assay (PTA), the [γ-\textsuperscript{33}P]ATP is used as a phosphate donor. In the case of boronated dThds which are good substrate of hTK1, radioactive \textsuperscript{33}P is transferred from [γ-\textsuperscript{33}P]ATP to the 5′-hydroxyl group of boronated dThds to produce boronated \textsuperscript{33}PdTMPs. Phosphorimaging is utilized to quantitate the production of radioactive boronated dTMPs.
2.4. Carboranes as boron moieties of BNCT agents

The early interest in the polyhedral boron and carborane cluster was their development and use as high-energy fuels for aircrafts and rockets in 1950-60s.\textsuperscript{152,153} Another interest that emerged soon thereafter was their medical application in BNCT. In particular, carboranes (C$_2$B$_{10}$H$_{12}$) are advantageous for drug design in BNCT because of their high boron content and stability.\textsuperscript{106} Synthetic and structural versatility as well as lack of toxicity are other beneficial factors.\textsuperscript{154}

2.4.1 Carborane structures

Polyhedral carboranes (C$_2$B$_{10}$H$_{12}$) are three-dimensional $\sigma$-aromatic structures. They are composed of two carbon atoms, ten boron atoms, and twelve hydrogen atoms. One of the striking structural features of carboranes is their icosahedral geometry in

\textbf{Figure 2.4.} 5'-Monophosphorylation of dThd or boronated dThds by hTK1
which the carbon and boron atoms are hexavalent. Closo-carboranes are available in the form of three geometrical isomers (ortho, meta, and para) based on the position of the two carbon atoms in the cage scaffold (Figure 2.5).

\[\text{closo-o-Carborane} \quad [c-o-C_2B_{10}H_{12}] \quad \text{closo-m-Carborane} \quad [c-m-C_2B_{10}H_{12}] \quad \text{closo-p-Carborane} \quad [c-p-C_2B_{10}H_{12}]\]

\[\text{nido-o-Carborane} \quad [n-o-C_2B_9H_{12}] \quad \text{nido-m-Carborane} \quad [n-m-C_2B_9H_{12}] \quad \text{nido-p-Carborane} \quad [n-p-C_2B_9H_{12}]\]

**Figure 2.5.** Closo- and nido structures of o-, m-, and p-carborane

### 2.4.2. Preparation of carboranes

Closo o-carborane can be prepared in two steps by refluxing decaborane (B$_{10}$H$_{14}$) in acetonitrile, followed by refluxing the resulting decaborane-acetonitrile complex with acetylene in toluene or benzene.$^{154,155}$ Recently, reaction of decaborane with alkynes in the presence of ionic liquids produced closo o-carboranes in high yields.$^{156}$ Closo m-carborane is produced by thermal rearrangement of closo o-carborane at 400-500 °C. Closo p-carborane is obtained via the same process by exposing closo m-carborane to 600-700 °C. Since the preparation of o-carboranes is the simplest of the three isomers, it is the least expensive, and therefore, has been studied extensively both in naturally-occurring abundance and $^{10}$B-enriched form.
Cage degradation of neutral closo o-carborane into negatively charged water-soluble nido o-carborane occurs by treatment with Brønsted bases such as amines, alkoxides and hydroxides.\textsuperscript{154} Although m-carborane is more resistant to the degradation by Brønsted bases than o-carborane, it is easily converted into water-soluble nido m-carborane by treatment with the fluoride ion.\textsuperscript{157-160} Transformation of closo p-carborane into nido p-carborane requires extremely harsh reaction conditions.\textsuperscript{161}

2.4.3. The chemistry of carboranes

Carboranes are in general extremely stable to oxidative, reductive, and acidic conditions. However, substitution reactions at both C-H and B-H vertices of these clusters occur under specific conditions. The hydrogen atoms attached to the carbon atoms can be removed by the treatment of n-butyllithium (n-BuLi) in benzene, tetrahydrofuran (THF), or diethylether. Reported pKa values of o-, m-, and p-carborane in ethanol are 24, 33 and 40, respectively.\textsuperscript{162} As a result, C-H vertices of o-carborane are activated more easily than those of m- and p-carborane. The weakly acidic C-H protons in carboranes have the potential for hydrogen bond formation\textsuperscript{162,163} and their overall hydrophilicity decreases in the order o- > m- > p-carborane.\textsuperscript{164} C-lithium salts of all three carborane isomers undergo nucleophilic substitution reactions with alkyl halides or tosylates.

Since carboranes are aromatic, they also undergo electrophilic substitution in a fashion similar to benzene. The B-H vertices antipodal to the carbon atoms of closo o-carborane (9B and 12B of closo o-carborane in Figure 2.5) are more susceptible to electrophilic substitution than the other B-H vertices due to the electronegativity
difference between carbon and boron.\textsuperscript{154} Halogen atoms can be introduced preferentially at both antipodal boron atoms using molecular halogens in the presence of Lewis acids such as aluminum chloride.\textsuperscript{165,166} They can be replaced with alkyl, allyl, aryl, or other halides using palladium-catalyzed cross-coupling reactions.\textsuperscript{167-169}

2.4.4. Theoretical calculations of carboranes

Structural determinations of carboranes have been achieved by gas-phase electron diffraction.\textsuperscript{170,171} However, there are no reported X-ray crystal structures of carboranes due to the cage disorder by rotation of the pseudo-spherical cluster and difficulties in distinguishing between the boron and carbon atoms. By utilizing the C-H acidity of carboranes, crystal structures of carboranes have been determined in complex with molecules containing heteroatoms (e.g., N, O, S, P, and halogens), which are able to participate in hydrogen-bonding with the acidic C-H of carboranes. The structural data obtained experimentally have been utilized to construct more accurate carborane models theoretically. Semi-empirical, \textit{ab initio} Hartree-Fock (HF), and density functional theory (DFT) calculations of all carboranes have been carried out by a number of research groups.\textsuperscript{171-174} Geometries and parameters (e.g. bond length, bond angle, and charge) of carboranes calculated at the HF and DFT levels were in good agreement with those determined experimentally.\textsuperscript{171-174} However, structural information of carboranes obtained from theoretical calculations have not been utilized for docking studies of carborane-containing compounds with macromolecules because current molecular docking programs (e.g. Sybyl, Autodock) do not provide parameters for the hexavalent boron atom of carboranes.
2.5. Carborane-containing dThds.

2.5.1. dThds substituted with a carborane cage at the carbohydrate portion

The first reported carborane-containing nucleoside was a uridine derivative substituted with carborane at the 2´-position.\textsuperscript{175} A number of additional uracil/thymine-based nucleosides with carborane cages linked to the 2´-, 3´-, or 5´-positions of ribose or 2´-deoxyribose were synthesized.\textsuperscript{119,120,176} PTAs with recombinant hTK1 showed that introduction of the carborane cluster at the 3´-position of dThd is moderately tolerated by hTK1.\textsuperscript{120}

2.5.2. dThds substituted with a carborane cage at the C-5 position

A basic motivation of the strategy to introduce the carborane cluster at the 5-position of dUrd or dThd certainly stemmed from the fact that substitution of CH\textsubscript{3} at the C-5 position of dThd with iodine and bromine resulted in dUrd prodrugs such as 5-iodo- and 5-bromo-2´-deoxyuridine, which are excellent substrates for phosphorylating enzymes. Indeed, metabolites of both prodrugs are able to substitute for dTTP during DNA synthesis to a high extent.\textsuperscript{177} Among dThds and dUrds substituted with the carborane cage at the 5-position, CDU [5-(1-o-carboranyl)-2´-deoxyuridine, Figure 2.6] was selected as a potential candidate for BNCT and further evaluated \textit{in vitro} and \textit{in vivo}.\textsuperscript{118,178}
2.5.3. *dThds substituted with a carborane cage at the N-3 position (3CTAs)*

In 1996, Lunato reported that *dThds* substituted with a carborane cluster at the N-3 position of *dThd* were phosphorylated by recombinant *hTK1*. These novel *dThd*-based prodrugs with a carborane cage at the N-3 position were later termed 3-carboranyl thymidine analogues (3CTAs). Since the first report of 3CTAs appeared, 3CTAs have been extensively studied as boron-delivery agents for BNCT by Tjarks, Eriksson, Barth, and their coworkers. A series of 3CTAs includes *dThds* substituted with hydrophilicity-enhancing dihydroxypropyl group at the carborane cage or flexible ethyleneoxide moieties between the *dThd* scaffold and carborane cage. One of these 3CTAs, designated N5-2OH (3-[5-{2-(2,3-dihydroxyprop-1-yl)-o-carboran-1-yl}pentan-1-yl]thymidine, Figure 2.6), was identified as a lead compound and extensively evaluated biochemically and biologically. N5-2OH had a good *hTK1* phosphorylation rate (41% relative to that of *dThd*) and the highest $k_{cat}/K_M$ value of all 3CTAs (35.8% relative to that of *dThd*). Furthermore, in the absence of endogenous *dThd*, N5-2OH was a better substrate of *hTK1* than FLT ($k_{cat}/K_M = 7.6\%$ that of *dThd*) and comparable to AZT ($k_{cat}/K_M = 43.7\%$ that of *dThd*). In the presence of *dThd*, N5-2OH was a moderate inhibitor of *dThd*.

![Figure 2.6. Potential dThd-based BNCT agents in preclinical trials](image)
phosphorylation with an IC₅₀ of 9.3 µmol/L. However, N5-2OH was not phosphorylated by other dNKs including TK2, Dm-dNK, and dCK.
CHAPTER 3

$^{10}$B-ENRICHED N5-2OH:
A PROMISING BORON-DELIVERY AGENT FOR BNCT

3.1. Background and rationale

N5-2OH was selected as the first lead compound of our 3CTA library because it exhibited higher phosphorylation rates (PRs) by hTK1 and superior \textit{in vitro} biological properties compared with other 3CTAs.$^{59,70,181}$ It also showed physiochemical properties suitable for effective penetration of the BBB ($\text{logP} = 2.09)$.\textsuperscript{181} Some biological data of N5-2OH are summarized in Table 3.1.

Although N5-2OH was found to be a promising boron-delivery agent for BNCT of GBM, some drawbacks were identified as well. First, preparation of a sufficient amount of N5-2OH in $^{10}$B-enriched form was a prerequisite to investigate its activity in neutron irradiation experiments with tumor/rodent models. The isotopes of the element boron occur with a natural abundance of 19.8\% for $^{10}$B and 80.2\% for $^{11}$B. However, only $^{10}$B has the capacity to capture thermal neutrons effectively, and thus, can be utilized for BNCT.$^{106}$ In order to reduce their quantities by a factor of 4, and consequently, decrease systemic toxicity, clinical BNCT agents are usually administered in $^{10}$B-enriched form.$^{106}$
Both clinical BNCT agents, BPA and BSH discussed in Chapter 2, have been synthesized and evaluated in $^{10}$B-enriched form.$^{100,148}$

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Model</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorylation$^a$</td>
<td>hTK1 phosphorylation</td>
<td>$41 \pm 5^d$</td>
</tr>
<tr>
<td></td>
<td>TK2 phosphorylation</td>
<td>$0.002^b$</td>
</tr>
<tr>
<td>In vitro</td>
<td>Rat F98 glioma</td>
<td>70 µM</td>
</tr>
<tr>
<td>cytotoxicity$^c$</td>
<td>Murine L929 fibroblast (wt)</td>
<td>23 µM</td>
</tr>
<tr>
<td></td>
<td>Murine L929 TK1 (-)</td>
<td>58 µM</td>
</tr>
<tr>
<td>In vitro</td>
<td>Rat F98 cell line</td>
<td>25.7 ± 6.0 µg B/10⁹ cells</td>
</tr>
<tr>
<td>uptake$^d$</td>
<td>Murine L929 (wt)</td>
<td>92.0 ± 20.0 µg B/10⁹ cells</td>
</tr>
<tr>
<td></td>
<td>Murine L929 TK1 (-)</td>
<td>9.3 ± 2.7 µg B/10⁹ cells</td>
</tr>
</tbody>
</table>

Table 3.1. Summary of biological data of N5-2OH$^{70}$

$^a$Value was determined using 10 µM of N5-2OH as described in Chapter 2.3.4 and expressed relative to dThd. The value of dThd was set to 100.

$^b$Value was determined as described in Chapter 2.3.4 using 100 µM of N5-2OH

$^c$The values represent the concentration of N5-2OH, which produced a 50% reduction in cell viability.

$^d$Cells were incubated with 17.5 µM of N5-2OH for 24 h at 37 °C, followed by harvest and boron determination by means of DCP-AES.$^{183}$

Secondly, the evaluation of optically pure epimers and geometrical isomers of N5-2OH was crucial to complete structure-activity relationship (SAR) studies. Previous biological studies were carried out with the mixture of two epimers of the o-carboranyl form of N5-2OH, which have $(R)$- and $(S)$-configuration at C-16 marked with ‘*’ in Scheme 3.1. Therefore, both stereochemically pure forms of o-carboranyl N5-2OH as well as geometrical isomers derived from m- and p-carborane were prepared.
Finally, the elucidation of the binding mode of N5-2OH in the active site of hTK1 was not possible until the end of 2004, when the first hTK1 crystal structure was published. hTK1 has a very narrow substrate specificity compared with the other dNKs, accepts only two natural substrates (dThd and dUrd) and only minor modifications at both nucleosides are tolerated. For the design of improved 3CTAs, it was of pivotal importance to understand why N5-2OH and other 3CTAs, having a bulky carborane cluster and a long alkyl or ethylene oxide spacer group at the N-3 position, were accepted as substrates of hTK1. Therefore, detailed computational docking studies with various geometric N5-2OH isomers using both hTK1 crystal structures and a homology model of hTK1 were carried out.

3.2. Preparation and analysis of 10B-enriched N5-2OH

3.2.1. Synthesis

The original 10-step synthesis of o-carboranyl N5-2OH with naturally-occurring boron isotope distribution (termed as 11B-abundant) and as a mixture of two epimers was reported by Al-Madhoun and coworkers. Although this lengthy procedure was suitable for the preparation of sufficient quantities of N5-2OH and several of its homologues for initial SAR studies, it was found to be inefficient and costly for the synthesis of N5-2OH in 10B-enriched form.

To obtain 10B-enriched N5-2OH, two possible synthetic routes were designed utilizing commercially available 10B-enriched o-carborane or decaborane. The first 4-step route, starting from 10B-enriched o-carborane (98% 10B), is shown in Scheme 3.2. Scheme 3.3 shows an alternative synthetic sequence starting from 10B-enriched
decaborane (99.5% $^{10}$B). Prior to the use of expensive $^{10}$B-enriched starting materials, $^{11}$B-abundant N5-2OH was synthesized to identify optimal reaction conditions.

Scheme 3.1. Synthesis of $^{11}$B-abundant N5-2OH by Al-Madhoun et al.$^{59}$

Reagents: (a) 3-aminopropylamide, 1,3-diaminopropane; (b) AcCl, pyridine, Et$_2$O; (c) B$_{10}$H$_{14}$, CH$_3$CN, toluene; (d) Tris(dibenzylideneacetone)dipalladium, bis(diphenylphosphino)ethane, allylethyl carbonate, THF; (e) OsO$_4$, NMO, THF; (f) $p$-TsOH, CH$_3$C(OCH$_3$)$_2$CH$_3$; (g) K$_2$CO$_3$, acetone, H$_2$O; (h) $p$-TsCl, Et$_3$N, DMAP, CH$_2$Cl$_2$; (i) dThd, K$_2$CO$_3$, DMF, acetone; (j) 17% HCl, CH$_3$OH.
**Scheme 3.2.** Preparation of $^{10}$B-enriched N5-2OH from o-carborane

Reagents and reaction conditions: (a) n-BuLi, allyl bromide, THF, rt, 12 h; (b) n-BuLi, 1,5-pentanediol di-p-tosylate, benzene, 5-10 °C, 1 h; (c) dThd, K$_2$CO$_3$, DMF/acetone (1:1), 35 °C, 96 h; (d) OsO$_4$, NMO, 1,4-dioxane.

Reaction of an o-carborane lithium salt, generated from o-carborane by treatment with n-BuLi in THF at -78 °C, with allyl bromide provided compound **3.2.A**. Homobifunctional 1,5-pentanediol di-p-tosylate was chosen to connect the carborane cage with the N-3 position of dThd through a pentylene spacer. Treatment of 1,5-pentanediol with p-toluenesulfonyl chloride and triethylamine in dichloromethane, followed by recrystallization in ethyl acetate, afforded 1,5-pentanediol di-p-tosylate as a white solid in 75% yield. To prevent formation of by-products **3.3.C** and **3.3.D** (Figure 3.1) during the second step of Scheme 3.1, the lithium salt of **3.2.A** was dissolved in benzene and the resulting solution was added very slowly to a solution of 1,5-pentanediol di-p-tosylate in benzene at 5-10 °C. Stirring of the reaction mixture at 5-10 °C for 1 h and subsequent quenching with water afforded compound **3.3.A** in 70% yield.
Previously, it was reported that compound 3.2.A isomerizes in the presence of potassium tert-butoxide in tert-butanol or benzene at 40-45 °C to produce 1-(trans-1-propenyl)-o-carborane 3.3.D. The formation of 3.3.D was observed at room temperature. However, lowering the reaction temperature to 5-10 °C significantly reduced the formation of 3.3.D. Isomerization of the allyl group was also observed in step c of Scheme 3.2. When compound 3.3.A was reacted with dThd in DMF/acetone (1:1) at 35 °C for 96 h, 3.4.A was obtained in a ratio of > 30:1 with the olefin isomer 3.4.C, which was not easily separated from 3.4.A by silica gel chromatography. Higher temperature (50 °C) reaction condition produced 3.4.A and its olefin isomer 3.4.C in a 1:1 ratio. When the mixture of 3.4.A and 3.4.C was treated with the osmium tetroxide (OsO₄) and 4-methylmorpholine N-oxide (NMO), only 3.4.A was dihydroxylated to
afford target compound 3.5.A. Compound 3.4.C appeared to be more resistant to the dihydroxylation reaction presumably due to steric hindrance by the bulky o-carborane cluster. Using reaction conditions optimized for the preparation of 3.5.A, $^{10}$B-enriched N5-2OH (3.5.B) was obtained in 4 steps from $^{10}$B-enriched o-carborane in 15% overall yield by following the same synthetic route.

Scheme 3.3. Synthesis of $^{10}$B-enriched N5-2OH from decaborane

Reagents and reaction conditions: (a) (bmin)Cl, toluene, reflux, 10 min; (b) n-BuLi, allyl bromide, THF, rt, 12 h; (c) TBAF, THF; (d) n-BuLi, 1,5-pentanediol di-p-tosylate, benzene, 5-10 °C, 1 h; (e) dThd, K$_2$CO$_3$, DMF/acetone (1:1), 35 °C, 96 h; (f) OsO$_4$, NMO, 1,4-dioxane.

The alternative synthetic route for $^{10}$B-enriched N5-2OH (3.5.B), starting from $^{10}$B-enriched decaborane, is shown in Scheme 3.3. By employing biphasic reaction conditions reported by Sneddon and coworkers,$^{156}$ the reaction of decaborane with trimethylsilyl acetylene in a mixture of 1-butyl-3-methylimidazolium (bmin) chloride and toluene afforded compound 3.6 in 50% yield. In contrast, the conventional reaction of a decaborane-acetonitrile adduct with trimethylsilyl acetylene,$^{154}$ gave compound 3.6 in only 23% yield. Lithium salt formation of the compound 3.6 with n-BuLi, followed by addition of allyl bromide, provided compound 3.7 in 60% yield. Removal of the trimethylsilyl group of 3.7 using tetrabutylammonium fluoride (TBAF)$^{186}$ gave
compound 3.2.B (80% yield), which was further processed as described in Scheme 3.2, to obtain target compound 3.5.B.

### 3.2.2 Analysis

Infrared (IR) and high-resolution mass spectroscopy (HRMS) analysis of \(^{11}\text{B}\)-abundant N5-2OH (3.5.A) and \(^{10}\text{B}\)-enriched N5-2OH (3.5.B) revealed interesting differences as a result of the isotopic composition of both compounds. The IR spectra exhibited minor differences in the B-H stretching band of 2580 cm\(^{-1}\) for 3.5.A versus 2587 cm\(^{-1}\) for 3.5.B. Electrospray ionization (ESI) mass spectra of 3.5.A and 3.5.B showed significant differences in their isotope patterns. The base peak of 3.5.A (M + Na)\(^+\) centered around 551 Da while that of 3.5.B (M + Na)\(^+\) appeared at 543 Da (Figure 3.2). A gaussian-shaped curve distribution, which is typical for \(^{11}\text{B}\)-abundant carborane-containing molecules, was only observed in HRMS spectrum of 3.5.A.

![Figure 3.2. ESI-mass spectra of compounds 3.5.A and 3.5.B](image-url)
Recently, it was reported that the reaction of dThd with 8-dioxane-3-cobalt bis(dicarbolide) using sodium hydride as a base produced a mixture of O-4 and N-3 alkylated product. Since there was also a possibility of producing O-4 alkylated product when dThd was condensed with the carboranyl alkyltosylate under the mild basic conditions (potassium carbonate in acetone/DMF at 35 °C) used in our studies, selective N-3 alkylation was confirmed using 2D-NMR studies.

![2D-HMBC spectrum of compound 3.5.A](image)

**Figure 3.3.** 2D-HMBC spectrum of compound 3.5.A
A previous analysis by 1D-NMR confirmed N-3 alkylation in N5-2OH solely by typical differences of $^{13}$C-chemical shift between 4-OCH$_2$- and 3-NCH$_2$- groups.\textsuperscript{179} The 2D-HMBC (Heteronuclear Multiple Bond Correlation) NMR was selected because it shows the coupling between proton nuclei and carbon nuclei, which are separated by one or two bonds. Thus, if O-4 alkylated product had been generated under the mild basic condition, the hydrogens at C-8 would have coupled with C-4 and C-5. However, they showed coupling with C-2 and C-4, establishing that it is N-3 alkylated product. Prior to the 2D-HMBC experiment, each proton and carbon peak of compound 3.5.A was assigned by $^1$H-NMR, $^1$H-$^1$H COSY, $^{13}$C-NMR, $^{13}$C-DEPT, and $^1$H-$^{13}$C HMQC (all of the spectra are shown in the Appendix A). As shown in Figure 3.3, two hydrogens at C-8 coupled with both carbonyl carbons (C-2 and C-4). Thus, the 2D-HMBC experiment confirmed exclusive N-3 alkylation in compound 3.5.A.

3.3. Preparation and analysis of pure N5-2OH epimers and geometrical isomers

3.3.1. Synthesis

The synthetic procedure (Scheme 3.1) reported by Al-Madhoun \textit{et al.}\textsuperscript{59} and the synthetic routes described in Scheme 3.2 and 3.3 provided N5-2OH as a mixture of two epimers due to dihydroxylation of the allylic function with OsO$_4$. The epimers have different configuration at the C-16 position of N5-2OH. Since the pure stereoisomers are more potent and less toxic than a mixture in some cases of drug development, pure epimers of N5-2OH were prepared from chiral starting materials as shown in Scheme 3.4. Compound 3.8.A was prepared in 63\% yield by reacting o-carborane with (S)-(+) 2,2-dimethyl-1,3-dioxolane-4-ylmethyl p-tosylate. Addition of a solution of the lithium salt
Condensation of compound 3.9.A with dThd, followed by deprotection of the isopropylidene group under the acidic condition, afforded compound 3.11.A with (R)-configuration at C-16. The second epimer (3.11.B) was prepared from (R)-2,2-dimethyl-1,3-dioxolane-4-ylmethyl p-tosylate in accordance with the procedure described for 3.11.A. It should be noted that this synthetic route is potentially suitable for the preparation of N5-2OH in $^{10}$B-enriched form. In addition, this synthetic route eliminates
the use of toxic OsO₄, which was applied for the dihydroxylation of the allylic group in
the reaction sequences shown in Schemes 3.1, 3.2, and 3.3.

Scheme 3.5. Synthetic route for geometrical N5-2OH isomers

Reagents and reaction conditions: (a) n-BuLi, 2,2-dimethyl-1,3-dioxolane-4-yl methyl p-tosylate, benzene, 
rt, 14 hr; (b) n-BuLi, 1,5-pentanediol di-p-tosylate, benzene, 5-10 °C, 1 hr; (c) dThd, K₂CO₃, DMF/acetone 
(1:1), 50 °C, 48 h; (f) 17% HCl, MeOH.

The synthetic approach described in Scheme 3.4 was also used for the preparation of 
the m-, and p-carboranyl isomers of N5-2OH. (R/S)-mixtures of compounds 3.11.C,
3.11.D, and 3.11.E were synthesized in 4 steps from o-, m- and p-carborane in overall 15%, 19%, and 16% yield, respectively (Scheme 3.5).

3.3.2. Analysis

A chiral solvating agent was used to determine the ratio of (R)- and (S)-epimers of 3.5.A. When one equivalent of (S)-(+)-2,2,2-trifluoro-1-(9-anthryl)-ethanol (Pirkle alcohol) was treated with 3.5.A in CDCl₃, the ¹H-NMR spectrum showed a partial overlap of two triplets for H-1´ with different intensities (Figure 3.4). In contrast, when the pure epimers 3.11.A and 3.11.B were treated with the Pirkle alcohol under the same conditions, single triplets for H-1´ appeared at 6.13 and 6.10 ppm, respectively. Two signals were also observed for the hydroxyl proton at C-16 of 3.5.A and 3.11.C while only one single peak was observed for that of the pure epimers (3.11.A and 3.11.B). Based on this analysis, compound 3.5.A consisted of approximately 3.11.A and 3.11.B in a 2:3 ratio. Steric hindrance by o-carborane and close proximity of thymidine to the allylic group appeared to cause the unequal compositions of (R)- and (S)-epimers of 3.5.A. When acetone-d⁶ was used instead of CDCl₃, no splitting pattern or chemical shift change was observed, probably because of the carbonyl group of acetone-d⁶, which prevented the OH group of Pirkle alcohol from forming hydrogen bonds with OH group (C-16) of 3.5.A.
Figure 3.4. Partial $^1$H-NMR spectra of 3.11.A, 3.11.B, 3.11.C, and 3.5.A with (S)-(+) 2,2,2-trifluoro-1-(9-anthryl)-ethanol in CDCl$_3$ at 300 K

The optical rotation values for 3.5.A (-5.1), 3.11.A (+17.5), and 3.11.B (-14.8) indicated a similar epimeric composition for 3.5.A. Efforts to determine the epimeric ratio of 3.5.A by HPLC using either a reversed phase (C18) column or a β-cyclodextrin-based chiral column (CYCLOBOND I, Advanced Separation Technologies Inc.) were not successful. We did also not observe any differences in CD spectra of epimers 3.11.A and 3.11.B presumably due to the fact that C-16 of N5-2OH is separated from thymine, the major chromophore, by multiple bonds.

Purity verification of all target compounds by analytical reversed-phase (C18) HPLC using methanol/water and acetonitrile/water gradient systems revealed interesting retention times, in particular for the geometrical isomers of N5-2OH (Table 3.2).
Water/acetonitrile gradient (100:0 to 70:30 over 5 min, from 70:30 to 40:60 over 25 min, from 40:60 to 0:100 over 20 min) with a flow rate of 1 mL/min was applied to determine retention times of \(3.11.C\), \(3.11.D\), and \(3.11.E\). As already discussed in Chapter 2.4.3, the hydrophobicity of three carborane isomers decreases in the order of \(p- > m- > o-\)-carborane presumably due to the decreasing potential of C-H for hydrogen bond formation.\(^{162,164}\) Using the water/acetonitrile gradient system, however, the retention times of the \(o-\) (\(3.11.C\)), \(m-\) (\(3.11.D\)), and \(p-\)(\(3.11.E\)) carboranyl N5-2OH isomers, and thus their hydrophobicities decreased notably in the order of \(3.11.C > 3.11.D > 3.11.E\) (Table 3.2), which is also demonstrated by the HPLC chromatogram of a mixture of the three isomers shown in Figure 3.5.

![Figure 3.5](image)

**Figure 3.5.** HPLC spectrum of a mixture of geometrical N5-2OH isomers (\(3.11.C\), \(3.11.D\), and \(3.11.E\)) using water/acetonitrile gradient
This trend was also observed when methanol/water gradient was used. Accordingly, the ratios of apolar surface areas (APSA) to polar surface areas (PSA) for 3.11.A, the (R)-epimer of 3.11.D, and the (R)-epimer of 3.11.E, calculated with VEGA ZZ 2.0.4 software (Milano, Italy) decreased in the order 3.11.A > (R)-3.11.D > (R)-3.11.E (Table 3.2).

<table>
<thead>
<tr>
<th>Compound</th>
<th>hTK1 PR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Retention time (RP-18)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>(APSA/PSA) ratio&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>N5-2OH&lt;sup&gt;d&lt;/sup&gt;</td>
<td>39 ± 8</td>
<td>26.9 min</td>
<td></td>
</tr>
<tr>
<td>3.5.A</td>
<td>39 ± 6</td>
<td>26.9 min</td>
<td></td>
</tr>
<tr>
<td>3.5.B (&lt;sup&gt;10&lt;/sup&gt;B enriched)</td>
<td>36 ± 7</td>
<td>26.5 min</td>
<td></td>
</tr>
<tr>
<td>3.11.A (R)</td>
<td>34 ± 7</td>
<td>26.7 min</td>
<td>3.31 (461.4 Å&lt;sup&gt;2&lt;/sup&gt; / 139.3 Å&lt;sup&gt;2&lt;/sup&gt;)</td>
</tr>
<tr>
<td>3.11.B (S)</td>
<td>33 ± 6</td>
<td>26.6 min</td>
<td></td>
</tr>
<tr>
<td>3.11.C (R/S, o-)</td>
<td>32 ± 7</td>
<td>26.9 min</td>
<td></td>
</tr>
<tr>
<td>3.11.D (R/S, m-)</td>
<td>42 ± 4</td>
<td>25.7 min</td>
<td>3.16 (492.7 Å&lt;sup&gt;2&lt;/sup&gt; / 156.1 Å&lt;sup&gt;2&lt;/sup&gt;)</td>
</tr>
<tr>
<td>3.11.E (R/S, p-)</td>
<td>45 ± 6</td>
<td>23.7 min</td>
<td>3.02 (491.8 Å&lt;sup&gt;2&lt;/sup&gt; / 162.9 Å&lt;sup&gt;2&lt;/sup&gt;)</td>
</tr>
</tbody>
</table>

**Table 3.2.** hTK1 PRs, retention time, and ratios of apolar to polar surface area (APSA/PSA) of the N5-2OH isomers

<sup>a</sup> Compound concentrations were 40 µM and the DMSO concentration was set to 1%. Enzyme activities were determined using a spectrophotometric assay.

<sup>b</sup> Water/acetonitrile gradient (100:0 to 70:30 over 5 min, from 70:30 to 40:60 over 25 min, from 40:60 to 0:100 over 20 min) with a flow rate of 1 mL/min was applied.

<sup>c</sup> PSAs and APSAs of compounds 3.5.A and (R)-epimers of 3.11.D and 3.11.E were calculated using the VEGA ZZ 2.0.4 program.

<sup>d</sup> N5-2OH was synthesized using the method reported by Al-Madhoun et al.®
3.4. In silico Studies

Recently, crystal structures of hTK1 (PDB ID: 1W4R and 1XBT),20,23 UuTK (PDB ID: 1XMR and 2B8T),12,23 and CaTK (PDB ID: 1XX6) were published and are now available for structure-based drug design. The crystal structures of hTK1 and UuTK were determined as tetramers containing dThd or dTTP.20,23 However, CaTK was crystallized as a dimer in complex with ADP. A visual inspection indicated that the hTK1s and UuTKs may represent “closed” TK forms while CaTK may constitute an “open” or “semi-open” TK form. In the case of many other nucleoside kinases and nucleoside monophosphate kinase, binding of the substrates and ATP appears to be associated with a conformational change from an open unoccupied form, over a partially closed form involving substrate- or ATP binding, to a closed form binding both substrates and ATP.189-193

The structures of 3.11.A and the (R)-epimers of 3.11.D and 3.11.E were optimized at the level of B3LYP/6-31G* using the Gaussian 03 Program running on an Itanium 2 Cluster at the Ohio Supercomputer Center (Figure 3.6). Conformational and geometrical parameters of the dThd scaffold of all three compounds were compared with the analyzed dThd structure reported by Ghomi and coworkers.195-197 The lowest energy conformer of dThd has C-2′-endo/anti conformation.195-197 The glycosyl torsion angle (O-4′, C-1′, N-1, and C-2) in dThd was found to be between 90° and 270° for the anti orientation and between -90° and +90° for the syn orientation.195-197 Quantum mechanical calculations of 3.11.A and the (R)-epimers of 3.11.D and 3.11.E at the B3LYP/6-31G* level showed a stable C-2′-endo/anti conformation of dThd scaffold in all three compounds. The glycosyl torsion angles of optimized 3.11.A and the (R)-epimers of
3.11.D and 3.11.E were +232°, +229°, and +229°, respectively. Also, all of them were C-2’-endo conformers. Interestingly, the distance between O-3’ and the hydrogen of the OH group attached to the C-17 position of 3.11.A was 2.005 Å, and thus, intramolecular hydrogen bonding is conceivable. This specific interaction may render 3.11.A more amphiphilic than the (R)-epimers of 3.11.D and 3.11.E (Figure 3.6), which may also explain the observed differences in retention times in HPLC experiments and APSA/PSA ratios (Table 3.2).

![Figure 3.6](image)

**Figure 3.6.** The optimized structures of 3.11.A and (R)-epimers of 3.11.D and 3.11.E at the B3LYP/6-31G* level using Gaussian 03 program.

Structures were visualized with GaussView3.0 (Gaussian Inc., Pittsburg, PA). Each solid sphere stands for atoms as follows: Red (oxygen), blue (nitrogen), dark gray (carbon), pink (boron), light gray (hydrogen).

For docking studies, the coordinates of 3.11.A and the (R)-epimers of 3.11.D and 3.11.E were transferred into Sybyl 7.1 (Tripos Inc., St. Louis, Missouri) and the atom type of the boron atoms of the carborane cages were changed to the C.3 atom type because Sybyl 7.1 does not provide default parameters for calculations involving molecules with hexavalent boron atoms of the carborane cluster. This manipulation of
boron atom types was described previously by us and was found to be suitable for docking studies with other carborane-containing molecules.\textsuperscript{198,199} The three compounds were then docked into the active site of both available hTK1 crystal structures\textsuperscript{20,23} using the FlexX module of Sybyl 7.1. However, none of the N5-2OH isomers was found to bind to the dTTP binding site effectively.

Since both hTK1 crystal structures presumably are closed forms of the enzyme, we further analyzed the binding of 3.11.A and the (R)-epimers of 3.11.D and 3.11.E to an “open” or “semi-open” form of hTK1, as described in the following. The amino acid sequence identity of CaTK, presumably representing an “open” or “semi-open” form, with hTK1 is 39%. Thus, a homology model of hTK1 was generated using CaTK as a template. The initial coordinates of the hTK1 homology model were constructed with SWISS-MODEL (Version 36.0003).\textsuperscript{200} The obtained coordinates were then transferred to Sybyl 7.1 and hydrogen atoms were added. The hydrogen atom positions were minimized until an RMS of 0.005 kcal·mol\textsuperscript{-1}Å\textsuperscript{-1} was reached using the Powell method. This homology model was solvated with water molecules using the Solvent/Solvate option.\textsuperscript{201} The solvated homology model was minimized until the gradient reached 0.05 kcal·mol\textsuperscript{-1}Å\textsuperscript{-1} using the Tripos Force Field. For docking studies, the active site was generated by selecting the same amino acid residues that are located within a radius of 12.0 Å from dTTP in the hTK1 crystal structure (PDB ID:1W4R).\textsuperscript{20}
Figure 3.7. (A) hTK1 crystal structure (PDB ID: 1W4R) with dTTP
(B) hTK1 homology model docked with 3.11.A
(C) hTK1 homology model docked with (R)-epimer of 3.11.D
(D) hTK1 homology model docked with (R)-epimer of 3.11.E

a: lasso domain, b: loop connecting the β2 and β3 strands, c: extended cleft between the lasso domain and the loop connecting the β2 and β3 strands
As shown in Figure 3.7.A and 3.7.B, the difference in the distance between the loop connecting the β2 and β3 strands (b) and the “lasso” domain (a) in the crystal structure of hTK1 and the homology model of hTK1 is substantial. We acknowledge the limitations of docking studies involving a homology model. Eventually, only an in-depth X-ray crystallographic analysis can reveal realistic dimensions of open and closed forms of hTK1. Nevertheless, in contrast with the hTK1 crystal structures, docking of compounds 3.11.A and (R)-epimers of 3.11.D and 3.11.E into the homology model of hTK1 revealed an interesting binding pattern. The bulky carboranyl side chain at N-3 position of 3.11.A is oriented towards the extended gap (c) between the loop connecting the β2 and β3 strands (b) and the “lasso” domain (a).

We hypothesize that this extended gap allows effective binding of 3.11.A to the active site of the open form of hTK1 and that partial closure of the lasso due to binding of 3.11.A will eventually result in an orientation of the 2´-deoxyribose portion that allows for an effective transfer of the γ-phosphate from ATP to the 5´- or 3´-hydroxyl group of 3.11.A while the carborane cluster of 3.11.A is relocated to the enzyme surface through unfolding and extension of the pentylene spacer.

This model accounts for the efficient phosphorylation of 3.11.A by hTK1 (Table 3.2). It also provides an explanation for the apparent lack of steric factors on the phosphorylation of various N5-2OH isomers by hTK1 (Table 3.2), since the carborane cage, which is the center of all steric alterations, is located towards the surface of the enzyme (Figure 3.7.A through 3.7.D). The (R)-epimer of m-carboranyl N5-2OH (3.11.D) docked in a similar way to the hTK1 homology model as 3.11.A. However, in the case of the (R)-epimer of 3.11.E, the dihydroxypropyl group attached to p-carborane projects
away from the active site of hTK1 (Figure 3.7.D) while the same group attached to o-carborane in 3.11.A apparently folds back to the enzyme surface. The specific orientation of the dihydroxypropyl group in the (R)-epimer of 3.11.E may cause less steric hindrance during the closure of the “lasso” portion of hTK1 compared with 3.11.A, which could explain the slightly higher relative hTK1 PR (Table 3.2).

3.5. Biological evaluation

3.5.1 Phosphoryl transfer assays (PTAs)

The substrate specificity of hTK1 for all synthesized compounds was evaluated using recombinant human hTK1 in PTAs. PRs of the synthesized compounds ranged from 33% to 45% relative to that of dThd (Table 3.2). 10B-enriched N5-2OH (3.5.B) showed a PR very similar to that of 11B-abundant N5-2OH (3.5.A). There was no significant difference between the PRs of the pure epimers 3.11.A and 3.11.B. Therefore, the stereochemistry at the C-16 position apparently did not affect the phosphorylation by hTK1. The PRs of the m- and p-carboranyl isomers of N5-2OH (3.11.D and 3.11.E) were also similar to that of the o-carboranyl N5-2OH (3.11.C). These results demonstrated that the dihydroxypropyl group as well as the carborane cage is most likely located outside of the active site of hTK1, which is also supported by the modeling of N5-2OH binding to hTK1, as discussed in Chapter 3.4. It appears that the dihydroxypropyl group attached to the carborane cage of the N5-2OH series contributes only to their hydrophilicity but does not affect their binding to hTK1. It should be warranted that pure epimers of 3.11.D and 3.11.E are synthesized to prove our hypothesis. The hTK1 PRs of the N5-2OH series were generated in collaboration with Dr. Staffan Eriksson and coworkers.
3.5.2. In vivo studies

Since no significant difference in hTK1 PRs among the synthesized N5-2OH series was observed, $^{11}$B-abundant N5-2OH (3.5.A) was chosen for in vivo biodistribution studies to validate its activity. For these studies, the F98 glioma cell line was selected to induce brain tumors in rats because the biological behavior of intracerebral tumors produced in this way simulates that of human GBM, as discussed in Chapter 2. Also, substrate specificities of rat TK1 are very similar to those of hTK1.$^{151}$

F98 glioma cells were stereotactically implanted into the right cerebral hemisphere of Fisher rats. Fourteen days later, two compounds (3.5.A and BPA) were administered by convection-enhanced delivery (CED). CED can delivery drugs to large regions of the brain by applying a pressure gradient to establish bulk flow during interstitial infusion without significant functional or structural damage to the brain. N5-2OH (3.5.A) (78 µg of boron) was solubilized in 10 µL of aqueous 50% DMSO solution and BPA-fructose complex (78 µg of boron) in PBS. The rats were euthanized at 1 and 2.5 h after CED. Boron concentration of the collected tissue and blood was determined by direct current plasma-atomic emission spectroscopy (DCP-AES).$^{183}$ Table 3.3 summarizes the boron biodistribution of 3.5.A and BPA in F98 glioma-bearing rats. N5-2OH (3.5.A) exhibited high T/Br ratios (> 8) at 1 h and 2.5 h after CED administration. In contrast, the retention ratio of BPA in tumor to normal brain or blood was much lower, in particular after 2.5 h (< 2).$^{70}$

This work was carried out in collaboration with Drs. Rolf F. Barth and Weilian Yang.
### Table 3.3. Boron biodistribution in F98-glioma-bearing rats at 1 and 2.5 h after administration of BPA and N5-2OH by CED.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Time (h)</th>
<th>Tumor (µg/g)</th>
<th>Ipsilateral brain (µg/g)</th>
<th>Contralateral brain (µg/g)</th>
<th>Blood (µg/g)</th>
<th>T/Br ratio$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5.A</td>
<td>1.0</td>
<td>41 ± 11</td>
<td>5 ± 4</td>
<td>&lt; 0.5</td>
<td>&lt; 0.5</td>
<td>8.2</td>
</tr>
<tr>
<td>BPA</td>
<td>1.0</td>
<td>68 ± 18</td>
<td>19 ± 14</td>
<td>4 ± 2</td>
<td>4 ± 4</td>
<td>3.6</td>
</tr>
<tr>
<td>3.5.A</td>
<td>2.5</td>
<td>16 ± 2</td>
<td>2 ± 2</td>
<td>&lt; 0.5</td>
<td>&lt; 0.5</td>
<td>8.0</td>
</tr>
<tr>
<td>BPA</td>
<td>2.5</td>
<td>24 ± 8</td>
<td>15 ± 8</td>
<td>8 ± 4</td>
<td>4 ± 2</td>
<td>1.6</td>
</tr>
</tbody>
</table>

$^a$ The tumor to brain ratio was calculated from boron concentration of tumor and ipsilateral brain.

### 3.5.3. Neutron irradiation studies

Since N5-2OH (3.5.A) showed promising biodistribution in rats bearing intracerebral F98 glioma, $^{10}$B-enriched N5-2OH (3.5.B) was used for neutron irradiation studies in the same rodent/tumor model at the Massachusetts Institute of Technology (MIT) Nuclear Research Laboratory (Cambridge, MA). F98 glioma-bearing rats were divided into three groups of 7-9 animals each, as follows: Group I, 500 µg of 3.5.B, administered intracerebrally (i.c.) by CED, followed by neutron irradiation; Group II received 50% DMSO alone by CED, followed by neutron irradiation; and Group III received 500 µg of 3.5.B by CED without neutron irradiation. Biodistribution of 3.5.B was determined in a group of 4 rats that received 3.5.B, as per Groups I and III, but these animals were euthanized 24 hours later and samples of tumor, blood and brain were analyzed for boron content by DCP-AES and the corresponding boron concentrations were 17.3 ± 4.3 µg/g for tumor and < 0.5 µg/g for both normal brain and blood.
Survival data are summarized in Table 3.4. Animals that received 3.5.B, followed by BNCT, had a mean survival time of 37 ± 7 days compared to 31 ± 3 days for irradiated controls and 25 ± 2 days for untreated controls, and the statistical difference between these groups was significant (p < 0.02). These preliminary survival data are encouraging since they were carried out without optimizing formulation, dosing and delivery of 3.5.B. Furthermore, it should be noted that the plasma concentrations of dThd, which competes with 3.5.B at the active site of hTK1, are 10 times lower in humans than in rodents.\textsuperscript{204} therefore, BNCT with 3.5.B could be significantly more effective in humans than in rodents.

Neutron irradiation experiments were carried out in collaboration with Drs. Rolf F. Barth and Weilian Yang (OSU) and Drs. Peter J. Binns, Kent J. Riley and Jeffrey A. Coderre (MIT).
3.6. Summary and conclusions

Two feasible synthetic routes for the preparation of $^{10}$B-enriched N5-2OH were developed and $^{10}$B-enriched N5-2OH was evaluated as a boron-delivery agent for BNCT in pilot neutron irradiation experiments. Treatment of rats, bearing intracerebral F98 glioma, with $^{10}$B-enriched N5-2OH (3.5.B) and neutron irradiation resulted in prolonged survival compared to control groups of animals, indicating that $^{10}$B-enriched N5-2OH has a significant potential as a boron-delivery agent for BNCT of GBM. Stereochemical- and geometrical N5-2OH isomers were synthesized and their specificities as substrate for hTK1 were evaluated to complete the SAR studies of the N5-2OH series. A computational model for the binding of N5-2OH to the active site of hTK1 was developed. hTK1 PRs and docking studies indicated that the dihydroxypropyl group attached to the carborane cluster of the N5-2OH isomers may only function as a hydrophilicity-enhancing structural element, but does not increase the binding affinity to the active site of hTK1. Overall, the studies described in Chapter 3 identified $^{10}$B-enriched N5-2OH as one of the most promising novel BNCT agents.

The work described in Chapter 3 was partially published in *Cancer Research* (Barth *et al.*, 2004, 64, 6287-6295).

3.7. Outlook: Challenges ahead

Although the pilot neutron irradiation studies with $^{10}$B-enriched N5-2OH (3.5.B) produced very promising results, further structural improvement of 3CTAs is warranted. A major problem of N5-2OH is its low solubility in water necessitating the use 50% aquesous DMSO for *in vivo* studies (see Chapters 3.4 and 3.5). Approaches to overcome
the low water-solubility of N5-2OH will be addressed in Chapter 4 in more detail. The second concern is the real-time pharmacokinetics of $^{10}$B-enriched N5-2OH in clinical studies since the measurement of tissue boron concentration via DCP-AES is only suitable to a limited extent for this purpose. One solution would be the introduction of a radioactive isotope such as $^{123}$I into 3CTAs, which would allow real-time imaging via Single Photon Emission Computed Tomography (SPECT). Attachment of other iodine isotopes, such as $^{125}$I or $^{131}$I, could even lead to the use of 3CTAs in the conventional radiation therapy.\textsuperscript{205}

![Scheme 3.6. Iodination of N5-2OH](image)

Reagents and conditions: (a) I$_2$, AlCl$_3$, CH$_2$Cl$_2$, reflux, 48 h.

A major problem associated with the biomedical applications of drugs labeled with radioactive iodines is susceptibility to dehalogenation. Release of radioiodine from drugs leads to the accumulation of significant quantities of radioactive iodine in the
thyroid and stomach. Iodine linked to boron atoms of the carborane cage in 3CTAs may overcome this problem of cleavage of carbon-iodine bonds because the B-I bond in the carborane cage in 3CTAs is expected to be less susceptible enzymatic and hydrolytic cleavage than the C-I bond.

Therefore, we have conducted the pilot experiment shown in Scheme 3.6. Direct iodination of N5-2OH was carried out with I₂ in dichloromethane in the presence of AlCl₃ at 40 °C for 48 h (Scheme 3.6). Products (3.12.A, 3.12.B, and 3.12.C) were isolated by analytical HPLC. The structures of 3.12.A, 3.12.B, and 3.12.C were confirmed by ¹H-NMR, HRMS, and ¹¹B-NMR. Interestingly, another set of three iodinated compounds separated by silica gel chromatography were identified as the thymine derivatives of 3.12.A, 3.12.B, and 3.12.C. This demonstrated that the glycosidic bond is to some extent susceptible to cleavage under the reaction conditions. To our knowledge, this is the first report on the direct iodination of the closo-carborane cage in a bioactive molecule, although the exact positions of the iodine substituents at the carborane cages in 3.12.A, 3.12.B, and 3.12.C have not yet been assigned.

Eventually, the success of N5-2OH iodination may facilitate pharmacokinetic studies of ¹⁰B-enriched N5-2OH. By applying the route described in Scheme 3.6, radioactive iodine can be attached directly to the carborane cage by using radioactive I₂. Alternative radioiodination of 3.12.A, 3.12.B, and 3.12.C may be achieved by palladium-catalyzed nucleophilic substitution using radioactive NaI (e.g Na¹²³I) and Herrmann’s catalyst.
CHAPTER 4

INVESTIGATION OF BIOCHEMICAL, BIOLOGICAL, AND CHEMICAL PROPERTIES OF ZWITTERIONIC NIDO CARBORANE

4.1. Background and rationale

Research activities on discovering novel BNCT agents in our laboratories have focused on the synthesis and biological evaluation of N5-2OH, as discussed in Chapter 3. Although the evaluation of N5-2OH in preclinical BNCT studies produced very promising results, a major drawback of this 3CTA is the lack of water-solubility, precipitated by the high hydrophobicity of its closo o-carborane cluster. Several strategies have been explored to improve the water-solubility of 3CTAs including the introduction of multiple hydroxyl groups, PEGylation, and formation of anionic or zwitterionic nido-carboranyl 3CTAs, the latter being the topic of this Chapter.

Biomolecules substituted with negatively charged nido-carborane are usually water-soluble because of the anionic character of the nido-carborane cage. In addition, the transformation of closo carboranyl biomolecules into their nido counterparts has only a minimal effect on their overall structure, and thus, provides a means of manipulating physicochemical properties while maintaining biological activities. However, it seems
unlikely that such anionic structures can cross cell membranes effectively via passive diffusion due to their highly hydrophilic character. Therefore, several 3CTAs containing a zwitterionic NH$_3^+$-nido $m$-carborane were synthesized and evaluated biologically (Chapter 4.2).

The basis for the unique physicochemical properties of zwitterionic NH$_3^+$-nido $m$-carboranyl 3CTAs is the intramolecular charge compensation between the positively charged ammonium group and the negatively charged nido $m$-carborane cage, presumably leading to structures with balanced hydrophilicity/hydrophobicity properties. A few nido o-carboranes that were substituted with charge-compensating sulfonium, pyridinium, or phosphonium group at a boron atom of the cluster have been reported. Therefore, we investigated an alternative zwitterionic NH$_3^+$-nido $m$-carborane system both experimentally and theoretically (Chapter 4.3).

4.2. Synthesis and biological evaluation of zwitterionic nido 3CTAs for BNCT

4.2.1. Synthesis

Zwitterionic NH$_3^+$-nido $m$-carborane containing 3CTAs were synthesized as shown in Scheme 4.1. Starting material 4.1 was prepared on a gram scale applying a procedure previously reported by Kahl and coworkers. The tosylates used for the alkylation of 4.1 were either purchased [ethyleneglycol di-$p$-tosylate, di(ethyleneglycol) di-$p$-tosylate] or prepared from 1,3-propanediol, 1,4-butanediol, 1,5-pentanediol, and 1,6-hexanediol as described previously. Alkylation of 4.1 with the alkanediol di-$p$-tosylate and di(ethyleneglycol) di-$p$-tosylate using 2.2 equivalents of n-BuLi in benzene afforded the $m$-carboranyl derivatives 4.2-4.7 in 14-45% yield. The relatively low yield
of 4.7 (14%) is probably due to both the electron-withdrawing effect of the oxygen atom in the diethylene ether spacer and the low solubility of di(ethyleneglycol) di-p-tosylate in benzene.

Scheme 4.1. Synthesis of zwitterionic *nido* 3CTAs and the neutral *closo* counterparts

Reagents and conditions: (a) Alkanediol di-p-tosylates, n-BuLi, benzene, 10 °C, 0.5-1 h; (b) dThd, K₂CO₃, DMF/acetone (1:1), 50 °C, 48 h; (c) CF₃COOH, CH₂Cl₂, rt, 24 h; (d) TBAF, THF, 70 °C, 1-2 h.

Condensation of compounds 4.2-4.7 with dThd was carried out in the presence of K₂CO₃ in DMF/acetone (1:1) at 50 °C for 48 h to provide compounds 4.8-4.13 in 50-85% yield. The tert-butoxycarbonyl (Boc) group was subsequently removed with
trifluoroacetic acid (TFA) in dichloromethane at room temperature for 24 h to give the neutral closo m-carboranyl 3CTAs (4.14.A-4.19.A) in 69-87% yield. Treatment of 4.14.A-4.19.A with tetrabutylammonium fluoride hydrate (TBAF•H2O) in THF at 70 °C for 1-2 h and subsequent acidic workup provided the corresponding zwitterionic nido 3CTAs (4.14.B-4.19.B) in 50-70% yield. Formation of the nido carboranes was monitored by IR, which showed the disappearance of the typical B-H band of closo carboranes around 2590 cm⁻¹ and emergence of the B-H band of nido-carboranes around 2530 cm⁻¹.

4.2.2. Analysis

Analytical C18- and C8 reversed phase HPLC (RP-18 and RP-8) was carried out with compounds 4.14.A/B-4.19.A/B for the following reasons: (i) to verify their purity, (ii) to obtain amounts of 1-3 mg in highly purified form for PTAs (Figure 4.2 and Table 4.1), and (iii) to attain retention times as measures of their lipophilicities (Table 4.1). Water/methanol gradient (100:0 to 70:30 over 10 min, from 70:30 to 90:10 over 20 min, and from 90:10 to 0:100 over 10 min) with a flow rate of 1 mL/min was applied for all target compounds using RP-8 and RP-18. The obtained retention times of 4.14.A/B-4.19.A/B ranged from 14.3 to 24.8 min while those of AZT and nido o-carboranyl 4.20 (see Figure 4.2 for the structure) were 12.2 and 11.8 min, respectively. Compound 4.20 and AZT were selected as control compounds as representatives for pure anionic nido 3CTAs and established therapeutic nucleoside prodrugs, respectively. It should be noted that all zwitterionic nido 3CTAs had longer retention times than AZT, which is known to penetrate cell membranes mainly via passive diffusion.
<table>
<thead>
<tr>
<th>Compound</th>
<th>TK1 (10 µM)</th>
<th>TK2 (100 µM)</th>
<th>RP-18 retention times</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.14.A</td>
<td>71.9 ± 5.1</td>
<td>&lt; 0.1</td>
<td>19.01</td>
</tr>
<tr>
<td>4.14.B</td>
<td>89.4 ± 1.2</td>
<td>&lt; 0.1</td>
<td>14.32&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4.15.A</td>
<td>58.2 ± 2.5</td>
<td>&lt; 0.1</td>
<td>19.08</td>
</tr>
<tr>
<td>4.15.B</td>
<td>64.6 ± 4.0</td>
<td>&lt; 0.1</td>
<td>14.96</td>
</tr>
<tr>
<td>4.16.A</td>
<td>58.0 ± 5.3</td>
<td>0.4</td>
<td>20.81</td>
</tr>
<tr>
<td>4.16.B</td>
<td>63.8 ± 3.1</td>
<td>0.1</td>
<td>15.38</td>
</tr>
<tr>
<td>4.17.A</td>
<td>45.1 ± 2.6</td>
<td>0.4</td>
<td>22.65</td>
</tr>
<tr>
<td>4.17.B</td>
<td>50.7 ± 1.6</td>
<td>1.2</td>
<td>16.36</td>
</tr>
<tr>
<td>4.18.A</td>
<td>14.2 ± 2.1</td>
<td>0.7</td>
<td>24.76</td>
</tr>
<tr>
<td>4.18.B</td>
<td>45.4 ± 2.0</td>
<td>1.3</td>
<td>17.77</td>
</tr>
<tr>
<td>4.19.A</td>
<td>48.4 ± 1.6</td>
<td>0.9</td>
<td>18.08</td>
</tr>
<tr>
<td>4.19.B</td>
<td>75.1 ± 0.6</td>
<td>0.5</td>
<td>14.48</td>
</tr>
<tr>
<td>4.20</td>
<td>44.8 ± 1.7</td>
<td>N/A</td>
<td>11.78</td>
</tr>
<tr>
<td>N5-2OH&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.0 ± 5.0</td>
<td>&lt; 0.1</td>
<td>22.54</td>
</tr>
<tr>
<td>AZT&lt;sup&gt;c&lt;/sup&gt;</td>
<td>52.0</td>
<td>4.0</td>
<td>12.22</td>
</tr>
<tr>
<td>dThd&lt;sup&gt;d&lt;/sup&gt;</td>
<td>100.0</td>
<td>100.0</td>
<td>8.72</td>
</tr>
</tbody>
</table>

Table 4.1. hTK1/TK2 PRs and RP-18 retention times of 3CTAs, AZT, and dThd

<sup>a</sup> Two peaks were observed. The average value of both peaks (14.21 min and 14.43 min) was inserted. <sup>b,c</sup> hTK1 and TK2 PRs were reported previously. 43,59<sup>d</sup> PR for dThd were set to 100. Mean ± SD values are based on three experiments for recombinant hTK1 and one experiment for TK2. N/A: Not determined
Interestingly, during RP-18 HPLC analysis of compound 4.14.B, two close peaks were observed at 14.21 and 14.43 min. RP-8 HPLC analysis produced a similar pattern with peaks at 14.49 and 14.65 min. This was not the case for 4.14.A-4.19.A and 4.15.B-4.19.B. The compounds corresponding to these peaks were isolated and analyzed by NMR and HRMS. While HRMS data for both compounds were indicative for the structure of 4.14.B, several differences were observed in the $^1$H-NMR and $^{13}$C-NMR spectra, in particular for the signals of the C-1’ proton (6.27 ppm and 6.36 ppm) and the N-CH$_2$ protons, as shown in Figure 4.1. Similar chemical shifts patterns were not observed for 4.14.A-4.19.A and 4.15.B-4.19.B.

![Figure 4.1. Partial $^1$H-NMR spectra of two pure epimers of 4.14.B](image-url)
Degradation of \textit{closo} \textit{m}-carborane into its \textit{nido} \textit{m}-counterpart introduces a chiral element,\textsuperscript{158-160,174} which in the case of the zwitterionic 3CTAs \textbf{4.14.B-4.19.B} results in a mixture of two epimers. The close proximity of the bulky \textit{nido} carborane cluster to the dThd scaffold in \textbf{4.14.B} could cause additional steric constraint emphasizing structural differences between both epimeric structures (Figure \textbf{4.1}). However, this splitting pattern might also derive from an atropisomeric effect due to hindered rotation of the single bonds between \textit{nido} carborane cluster and dThd scaffold. If the obtained fractions from HPLC were two pure atropisomers, increasing the temperatures should promote their interconversion. However, we did not observe any changes in \textsuperscript{1}H-NMR spectra of both forms that were obtained before and after 1 h-reflux in methanol, indicating the formation of pure epimers (Figure \textbf{4.1}). Further analysis, ideally through X-ray crystallography, would be required to substantiate this hypothesis.

\textbf{4.2.3 Phosphoryl transfer assays (PTAs)}

The $\beta$-autoradiogram depicted in Figure \textbf{4.2} shows the monophosphate (MP) products of the neutral \textit{closo} carboranyl \textbf{4.15.A, 4.17.A}, and N5-2OH, anionic \textit{nido} carboranyl \textbf{4.20}, zwitterionic \textit{nido} carboranyl (\textbf{4.15.B} and \textbf{4.17.B}), and dThd (‘b’). In previous studies with similar 3CTAs, combined $\alpha/\beta$-autoradiography provided evidence that the observed intense spots with high $R_f$ values (‘a’) are typical for MPs of 3CTAs.\textsuperscript{59,61,62} The migration of the MPs also provides information on hydrophilicity differences between the anionic \textit{nido}-, zwitterionic \textit{nido}-, and neutral \textit{closo} 3CTAs. It is obvious that the $R_f$ values of the MPs of the zwitterionic \textit{nido} 3CTAs (\textbf{4.15.B} and \textbf{4.17.B}) are between the MPs of neutral \textit{closo}- and anionic \textit{nido} 3CTAs.

The reaction products were separated by PEI-cellulose TLC and visualized by $^{32}$P-autoradiography. (a) MP products of the compounds 4.20, 4.15.B, 4.15.A, 4.17.B, 4.17.A, and N5-2OH. (b) dTMP.

The exact PRs of compounds 4.14.A/B-4.19.A/B relative to that of dThd with hTK1 and TK2 are summarized in Table 4.1. The hTK1 PRs of compounds 4.14.A-4.19.A (neutral closo 3CTAs) ranged from 71.9 ± 5.1% to 14.2 ± 2.1% and those of compounds 4.14.B-4.19.B (zwitterionic nido 3CTAs) ranged from 89.4 ± 1.2% to 45.4 ± 2.0% (Table 4.1). The hTK1 PR of 4.14.B (89.4 ± 1.2%) is the highest among the reported 3CTAs. Preliminary enzyme kinetic analysis of 4.14.B resulted in a $k_{cat}/K_M$ value
of ~ 49% relative to that of dThd. The hTK1 PRs of the zwitterionic nido \textit{m}-carboranyl series \textbf{4.14.B-4.19.B} were 5-30% higher than those of the corresponding neutral \textit{closo m}-carboranyl series \textbf{4.14.A-4.19.A}. The hTK1 PRs decreased in both series with increasing spacer length almost in a linear fashion except compounds \textbf{4.19.A/B}, which have diethylene ether spacers.

As shown in Table \textbf{4.1}, the hTK1 PRs of \textbf{4.14.A/B-4.19.A/B} are inversely proportional to their RP-18 retention times, and thus, to their lipophilicity. In the cases of zwitterionic compound \textbf{4.19.B}, having a diethylene ether spacer, the RP-18 retention time (14.48 min) and the hTK1 PR (75%) are more comparable with those of compounds \textbf{4.14.B} (14.31 min and 89 \%) and \textbf{4.15.B} (14.96 min and 65 \%), having ethylene- and propylene spacers, rather than those of \textbf{4.17.B} (16.36 min and 51 \%) and \textbf{4.18.B} (17.77 min and 45 \%) with pentylene- and hexylene spacers comparable in lengths to the diethylene ether spacer in \textbf{4.19.B}. Therefore, it appears that the determining factor for their hTK1 PRs of zwitterionic \textit{nido} 3CTAs is their lipophilicity rather than their spacer lengths. However, we cannot exclude the possibility that the oxygen atom in the spacer of \textbf{4.19.B} affects the hTK1 PR through specific hydrogen-bonding interactions in the substrate binding site of hTK1.

Only at high substrate concentrations of 100 μM, compounds \textbf{4.16.A/B-4.19.A/B}, having butylene-, pentylene-, hexylene-, and diethylene ether spacers, appeared to be phosphorylated to a limited extent by TK2 with PRs ranging from 0.1-1.3\%, while \textbf{4.14.A/B-4.15.A/B} with shorter ethylene- and propylene spacers apparently were not substrates of TK2.
Overall, zwitterionic nido 3CTAs showed a unique hydrophilcity/hydrophobicity balance between neutral closo 3CTAs, which are not sufficiently soluble in water for preclinical studies, and anionic nido 3CTAs, which are presumably too hydrophilic to cross cell membranes via passive diffusion. Therefore, zwitterionic nido 3CTAs may be able to penetrate cell membranes by passive diffusion while having increased water solubility compared with N5-2OH.

All PRs were obtained in collaboration with Dr. Staffan Eriksson and coworkers.

4.2.4. In vivo studies

Although compound 4.14.B has better enzymatic properties than compound 4.15.B, the latter was selected for preliminary in vivo biodistribution studies in mice bearing subcutaneous (s.c.) L929 (wt) or L929 TK1 (-) tumors because it was available in sufficient quantities. BPA and N5-2OH were used as reference compounds. All compounds were injected intratumorally (i.t.) twice with a 2-h interval. Each injection included compound quantities equivalent to 50 µg boron. This procedure mimics to some degree the condition of CED to brain tumors, which is the proposed route of administration for 3CTAs (Chapter 3.5.2). Boron concentrations in L929 (wt) tumors were 21.7 ± 9.1, 29.8 ± 7.4, and 4.0 ± 2.1 µg/g tumor for 4.15.B, N5-2OH, and BPA, respectively, at 2 h following the 2nd injection (Figure 4.4). The corresponding values for the L929 TK1 (-) tumors, were 7.0 ± 5.9, 12.1 ± 7.7, and 5.8 ± 3.1 µg/g tumor. Boron concentrations in blood, skin, and liver were < 0.5 µg/g tissue in both tumor/rodent models. The values for 4.15.B and N5-2OH in L929 (wt) tumors were comparable and in the concentration range necessary for BNCT, while those for BPA were significantly
lower. The values for nucleoside analogues 4.15.B and N5-2OH in L929 TK1 (-) tumors were 3.1 and 2.5 times lower than those in L929 (wt) tumors. However, the amino acid BPA was 1.5 times higher in L929 TK1 (-) tumors than in L929 (wt) tumors.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Boron concentration (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L929 (wt)</td>
</tr>
<tr>
<td>4.15.B</td>
<td>21.7 ± 9.1</td>
</tr>
<tr>
<td>N5-2OH</td>
<td>29.8 ± 7.4</td>
</tr>
<tr>
<td>BPA</td>
<td>4.0 ± 2.1</td>
</tr>
</tbody>
</table>

Table 4.2. Tumor uptake of 4.15.B, N5-2OH, and BPA in mice bearing s.c. implants of L929 (wt) and L929 TK1 (-) tumor.

Compound quantities equivalent to a total of 100 µg boron were given in two intratumoral injections in a 2-h interval. Boron concentrations in tissues were determined at 2 h following the second injection by DCP-AES. Each data point represents the arithmetic mean ± SD of 4 mice.

These results indicated that the uptake of 4.15.B and N5-2OH is related to some form of nucleoside metabolism, presumably intracellular entrapment through 5’-monophosphorylation by hTK1. However, we cannot exclude the possibility that cellular efflux mechanisms specific to L929 TK1 (wt) and L929 TK1 (-) may contribute to the observed biodistribution patterns.

As expected, the zwitterionic compound 4.15.B showed improved water solubility compared to N5-2OH. In order to dissolve compound quantities equivalent to 50 µg boron, 15 µL of DMSO/H2O (70:30) were required for N5-2OH while 4.15.B was
dissolved completely in 10 µL of DMSO/H₂O (24:76), indicating significantly increased water solubility properties of **4.15.B**.

The *in vivo* studies described in Chapter 4.2.4 were carried out in collaboration with Dr. Rolf F. Barth and coworkers.

### 4.2.5. Summary and Conclusions

Six zwitterionic NH₃⁺-*nido* m-carborane containing 3CTAs and their corresponding neutral NH₂-*closo* m-carborane substituted counterparts were synthesized. Their PRs were evaluated with recombinant hTK1 and TK2. The obtained hTK1 PRs ranged from 89% to 14% relative to dThd and appeared to be dependent mainly on the lipophilicity of the compounds although structural contributions, such as the spacer length between carborane cluster and dThd scaffold, may also be of importance. The hTK1 PR of zwitterionic compound **4.14.B** (89%) was the highest determined for any 3CTA. HPLC RP-18 retention times indicated that the lipophilicity of zwitterionic compounds such as **4.14.B** and **4.15.B** may be sufficient for passive diffusion through cell membranes. One zwitterionic compound **4.15.B** was preferentially taken up in L929 (wt) tumors implanted in nude mice and showed significantly improved water solubility compared with N5-2OH. Overall, zwitterionic *nido* 3CTAs appears to have similar or even improved biological properties than N5-2OH in combination with improved physicochemical properties (e.g. water solubility). It should be noted that **4.14.B** presumably has biodistribution and water-solubility properties that are superior to those of **4.15.B**.
The work described in Chapter 4.2 was published in the *Journal of Medicinal Chemistry* (Byun *et al.* 2005, 48, 1188-1198) and in *Applied Radiation and Isotopes* (Byun *et al.* 2004, 61, 1125-1130).

### 4.3. Experimental and theoretical studies of zwitterionic *nido m*-carborane system

The zwitterionic *nido m*-carboranyl 3CTAs described in Chapter 4.2 showed balanced hydrophilicity/hydrophobicity properties unlike those of lipophilic *closo* and hydrophilic *nido* 3CTAs, demonstrating the usefulness of NH$_3^+$-*nido m*-carborane as a novel boron moiety in BNCT drug design. Since there has been no previous report on this specific carborane cage structure, we further investigated some of its synthetic, spectroscopic, and theoretical features.

#### 4.3.1. Synthesis

![Scheme 4.2. Synthesis of zwitterionic *nido m*-carborane (4.22)](image)

Reagents and conditions: (a) 1) n-BuLi/THF/CO$_2$; 2) PCl$_5$/toluene; 3) (CH$_3$)$_3$SiN$_3$/toluene, t-BuOH, reflux; (b) CF$_3$COOH, dichloromethane, rt, 12 h; (c) TBAF, THF, 70 °C, 1.5 h.

Compound 4.1 was prepared from *m*-carborane (1,7-C$_2$B$_{10}$H$_{12}$) in 3 steps as described previously.$^{121}$ Deprotection of the Boc group was achieved by treatment with
TFA at room temperature for 12 h to afford 1-amino-1,7-carborane (4.21) in 85% yield. Reaction of compound 4.21 with TBAF in THF for 1.5 h at 70 °C afforded zwitterionic nido m-carborane (4.22) in 61% yield.

4.3.2. Structural analysis

![Figure 4.3. Structures of 4.21 and 4.22 optimized at the B3LYP/6-31G* level](image)

The bridging hydrogen of compound 4.22, designated as 25H in Figure 4.3, was observed at -1.99 ppm in $^1$H-NMR due to nuclear shielding by the electron-rich nido m-carborane cluster. This phenomenon has been discussed previously by Onak et al. The reduced shielding in the electron deficient closo-carborane cluster and increased shielding in the electron-rich zwitterionic nido-carborane affect the chemical shifts of the hydrogen atoms (17H) attached to the carbon at the 9-position of both compounds. Hydrogen peak of 17H in 4.22 appeared at 1.43 ppm while that of compound 4.21 appeared at 3.23 ppm.
$^{13}$C-NMR spectra showed similar patterns. Chemical shifts for the two carbons (9C and 21C) of 4.22 were 32.83 ppm and 53.77 ppm, respectively. In contrast, two peaks of 4.21 were observed in a low-field region (9C: 54.65 ppm, 21C: 90.45 ppm).

Figure 4.4. $^{11}$B (proton-decoupled)-$^{11}$B (proton-decoupled) COSY spectrum of 4.22
\(^{11}\)B-\(^{11}\)B correlation spectroscopy (\(^{11}\)B-\(^{11}\)B COSY) shown in Figure 4.4, in combination with common prediction rules for borane or heteroborane clusters,\(^{219}\) provided the complete assignment for the nine boron atoms of compound 4.22. According to these prediction rules, signals for boron atoms with higher coordination number appear in a high-field region and substitution of boron with carbon in the cage scaffold causes a low-field shift of the boron atom opposite to carbon.\(^{219}\) Based on these rules, 2B and 3B (Figure 4.3) were expected to appear in a high-field region because both of them coordinate five boron atoms (2B: 1B, 3B, 4B, 6B and 7B, 3B: 2B, 4B, 7B, 8B and 10B). Three boron atoms (5B, 4B and 7B) were expected in a low-field region because 5B coordinates only two boron atoms (8B and 10B), and 4B and 7B are at the position antipodal to 9C and 21C, respectively. Nine boron atoms of compound 4.22 were assigned via the \(^{11}\)B-\(^{11}\)B COSY spectrum shown in Figure 4.4.

IR spectra of compounds 4.21 and 4.22 showed two distinctive stretching bands for B-H and C-N. B-H stretching bands for 4.21 and 4.22 were at 2595 cm\(^{-1}\) and 2535 cm\(^{-1}\) respectively. This frequency change is a major spectroscopic feature to distinguish closo carboranes from their nido counterparts. The C-N stretching band of compound 4.21 was 1611 cm\(^{-1}\), which is very close to the typical C=N stretching band while that of compound 4.22 was 1467 cm\(^{-1}\). Recently, Boyd and coworkers reported exo \(\pi\)-bond formation between a carbon atom of \(o\)-carborane and several \(\pi\)-donor substituents (OH, NH\(_2\), NH, and CH\(_2\)) attached to it.\(^{212}\) The observed exo C-N distance in 1-amino-2-phenyl-\(ortho\)-carborane was 1.392 Å, which is shorter than that of a typical C-N single bond (~ 1.47 Å). Therefore, we hypothesized that the C-N bond of compound 4.21 also has exo C-N \(\pi\)-bonding character.

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4.3.3. Theoretical calculations

Methods: Theoretical *ab initio* HF and DFT calculations for compounds 4.21 and 4.22 were performed using the Gaussian 03 program running on an Itanium 2 Cluster at Ohio Super Computer Center. The structures were optimized at the HF/6-31G*, HF/6-31G**, B3LYP/6-31G*, and B3LYP/6-31G** levels. NMR chemical shift calculations were performed with the gauge-including atomic orbitals (GIAO) method using the optimized geometries in acetonitrile (CH₃CN) solution. Theoretical $^{11}$B-NMR chemical shifts were computed versus the chemical shift of diborane (16.6 ppm) and then converted to the BF₃•Et₂O scale. $^1$H-NMR and $^{13}$C-NMR chemical shifts were referenced to tetramethylsilane (TMS) standard.

Table 4.3 lists the total energies of the optimized structures of 4.21 and 4.22 at the HF and DFT levels and Table 4.4 summarizes the charge distributions of the heavy atoms and the bridging hydrogen of 4.21 and 4.22.

<table>
<thead>
<tr>
<th>Compound</th>
<th>HF/6-31G*</th>
<th>HF/6-31G**</th>
<th>B3LYP/6-31G*</th>
<th>B3LYP/6-31G**</th>
</tr>
</thead>
</table>

Table 4.3. Total energies of optimized structures 4.21 and 4.22
<table>
<thead>
<tr>
<th>Atom</th>
<th>4.21 Mulliken charge</th>
<th>4.21 APT charge</th>
<th>4.22 Mulliken charge</th>
<th>4.22 APT charge</th>
</tr>
</thead>
<tbody>
<tr>
<td>1B</td>
<td>0.098</td>
<td>0.117</td>
<td>-0.015</td>
<td>0.185</td>
</tr>
<tr>
<td>2B</td>
<td>0.032</td>
<td>0.061</td>
<td>-0.001</td>
<td>-0.035</td>
</tr>
<tr>
<td>3B</td>
<td>0.035</td>
<td>0.062</td>
<td>0.122</td>
<td>-0.001</td>
</tr>
<tr>
<td>4B</td>
<td>0.023</td>
<td>0.162</td>
<td>0.023</td>
<td>0.248</td>
</tr>
<tr>
<td>5B</td>
<td>0.081</td>
<td>0.171</td>
<td>0.088</td>
<td>0.260</td>
</tr>
<tr>
<td>6B</td>
<td>0.044</td>
<td>0.150</td>
<td>0.012</td>
<td>0.225</td>
</tr>
<tr>
<td>7B</td>
<td>0.005</td>
<td>0.169</td>
<td>0.031</td>
<td>0.272</td>
</tr>
<tr>
<td>8B</td>
<td>0.042</td>
<td>0.140</td>
<td>0.019</td>
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</tr>
<tr>
<td>9C</td>
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<td>-0.199</td>
<td>-0.311</td>
<td>-0.319</td>
</tr>
<tr>
<td>10B</td>
<td>0.050</td>
<td>0.114</td>
<td>0.002</td>
<td>0.122</td>
</tr>
<tr>
<td>21C</td>
<td>-0.038</td>
<td>0.167</td>
<td>-0.097</td>
<td>-0.125</td>
</tr>
<tr>
<td>22B</td>
<td>0.066</td>
<td>0.193</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>24N</td>
<td>-0.605</td>
<td>-0.487</td>
<td>-0.560</td>
<td>-0.198</td>
</tr>
<tr>
<td>26H</td>
<td>-0.002</td>
<td>-0.120</td>
<td>0.046</td>
<td>-0.047</td>
</tr>
</tbody>
</table>

**Table 4.4.** Mulliken- and APT charges of heavy atoms and the bridging hydrogen atom for **4.21** and **4.22** at the B3LYP/6-31G** level.

Gaussian 03 program provides two types of atomic charge populations as default settings: Mulliken and atomic polar tensor (APT). As shown in Table 4.4, there is a significant difference of atomic charge distributions between Mulliken charge and APT charge for compounds **4.21** and **4.22**. The boron atoms 5B and 22B, which are attached to the carbon atoms of **4.21**, are expected to have the highest positive charge because they are susceptible to removal by fluoride treatment. APT charge calculation showed that these two boron atoms (5B and 22B) of **4.21** were indeed those with the highest charges.
(0.171 and 0.193) while Mulliken charge calculations identified 1B (0.099) as the most positively charged boron atom. Distances between 21C and 24N of optimized structures 4.21 and 4.22 at the B3LYP/6-31G** level were 1.4309 Å and 1.5078 Å, respectively, indicating exo C-N π-bond character of compound 4.21.

Theoretical chemical shifts of nine boron atoms for 4.22 were obtained using GIAO/11B-NMR calculations at the HF/6-31G*, HF/6-31G**, B3LYP/6-31G*, and B3LYP/6-31G** levels. Table 4.5 summarizes the experimental and theoretical 11B-NMR chemical shifts of compound 4.22. NMR chemical shifts obtained experimentally showed strong correlations ($r^2$: 0.9957) with those calculated using DFT (B3LYP/6-31G* and B3LYP/6-31G**) methods.

<table>
<thead>
<tr>
<th>Atom</th>
<th>Experimental$^a$</th>
<th>HF/6-31G*</th>
<th>HF/6-31G**</th>
<th>B3LYP/6-31G*</th>
<th>B3LYP/6-31G**</th>
</tr>
</thead>
<tbody>
<tr>
<td>4B</td>
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<td>2.58</td>
<td>3.45</td>
<td>-4.08</td>
<td>-2.81</td>
</tr>
<tr>
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<td>0.20</td>
<td>1.02</td>
<td>-5.82</td>
<td>-4.55</td>
</tr>
<tr>
<td>7B</td>
<td>-3.6</td>
<td>-7.21</td>
<td>-6.29</td>
<td>-6.30</td>
<td>-5.01</td>
</tr>
<tr>
<td>10B</td>
<td>-15.75</td>
<td>-16.23</td>
<td>-15.12</td>
<td>-17.44</td>
<td>-16.14</td>
</tr>
<tr>
<td>8B</td>
<td>-19.53</td>
<td>-17.71</td>
<td>-17.11</td>
<td>-21.94</td>
<td>-20.66</td>
</tr>
<tr>
<td>6B</td>
<td>-20.07</td>
<td>-18.69</td>
<td>-17.77</td>
<td>-22.55</td>
<td>-21.30</td>
</tr>
<tr>
<td>1B</td>
<td>-20.07</td>
<td>-22.50</td>
<td>-21.65</td>
<td>-23.26</td>
<td>-21.97</td>
</tr>
<tr>
<td>3B</td>
<td>-31.95</td>
<td>-32.50</td>
<td>-31.55</td>
<td>-33.34</td>
<td>-32.07</td>
</tr>
<tr>
<td>2B</td>
<td>-33.59</td>
<td>-35.08</td>
<td>-33.95</td>
<td>-35.39</td>
<td>-34.10</td>
</tr>
</tbody>
</table>

$r^2$ | 0.9646 | 0.9659 | 0.9957 | 0.9957 |

| Standard deviation | 2.4824 | 2.4382 | 0.8663 | 0.8659 |

Table 4.5. Experimental and theoretical (GIAO) 11B-NMR chemical shifts of 4.22

$^a$Experimental NMR chemical shifts were recorded in deuteriated CH$_3$CN solution at 25 °C.
4.3.4. Observations

![Proton-decoupled $^{11}$B-NMR spectra of compound 4.22 in deuteriated acetone solution recorded as a function of time](image)

**Figure 4.5.** Proton-decoupled $^{11}$B-NMR spectra of compound 4.22 in deuteriated acetone solution recorded as a function of time

The 0 min spectrum was recorded right after dissolving 4.22 in deuteriated acetone.

During $^{11}$B-NMR studies with compound 4.22 in deuteriated acetone (CD$_3$COCD$_3$), we observed time-dependant disappearance and emergence of signals, as shown in Figure 4.5. Interestingly, we noticed that the solution of 4.22 in CD$_3$COCD$_3$ at 180 min contained a UV active compound that could be detected at 254 nm via TLC analysis. To explore potential solvent effects on NMR chemical shifts and the formation of an UV active component, the capacity of various solvents (acetone, methylethylketone,
acetonitrile, benzene, chloroform, diethyl ether, dimethylsulfoxide, ethyl acetate, hexane, methanol, tetrahydrofuran, and water) to form an UV-active complex with 4.22 was studied by UV/TLC analysis. Only acetone and methylethylketone (CH₃CH₂COCH₃) appeared to interact 4.22 to form UV-active species. Indeed, reaction of 4.22 with acetone at room temperature for 6 h, and subsequent removal of acetone under reduced pressure yielded the iminium-containing compound 4.23 in a quantitative yield (Scheme 4.3). The chemical structure of 4.23 was confirmed by ¹H-, ¹³C-, ¹¹B-NMR and HRMS. Compound 4.23 was fairly stable in solvents such as methanol, benzene, and dichloromethane although a small amount (~ 10%) of 4.22 was detected after 24 h at room temperature.

Scheme 4.3. Preparation of iminium-containing compounds (4.23-4.25) from 4.22
The UV absorption spectra (Figure 4.6) of 10^{-5} M solutions of compounds 4.22 and 4.23 in methanol showed bands at the 220 nm and 253 nm regions. Only the 220 nm band is a typical characteristic for the 3D σ-aromatic system of carboranes.\textsuperscript{154} The band at 253 nm had significant intensity only in the case of 4.23 whereas it was reduced to merely a shoulder in 4.22. A similar UV absorption shift was reported previously for the σ-aromatic closo-p-carborane cluster when conjugated with π-aromatic benzene.\textsuperscript{220} To the best of our knowledge, however, compound 4.23 is the first example of stable conjugation of a 3D σ-aromatic system of a nido carborane cluster with a non-aromatic iminium group, resulting in a significant alteration in the UV absorption. We hypothesize that an electron delocalization from the negatively charged nido m-carborane cluster to the iminium double bond is responsible for this bathochromic shift.

**Figure 4.6.** UV absorption spectra of compound 4.22 and 4.23 (10^{-5} M solutions in MeOH)
Molecular Orbital (HOMO/LUMO) calculations (Figure 4.7) for 4.22 and 4.23 at the B3LYP/6-31G** level supported the bathochromic shift of 4.22 and 4.23 observed in UV experiment. As shown in Figure 4.7, in the case of the conjugated molecule 4.23, alternating-phase orbitals with similar sizes stemming from iminium nitrogen group and the nido carborane cluster overlapped completely. In particular, the HOMO of 4.23 showed a pattern of overlapping orbitals similar to that of benzene. In contrast, there is no significant overlap of alternating-phase orbitals between carborane cluster and nitrogen in 4.22. The energy gap between HOMO and LUMO of compound 4.23 was 4.98 eV, which is notably smaller than that of compound 4.22 (5.52 eV). It is well known that conjugation increases with a decreasing the energy gap between HOMO and LUMO decreases.221

Figure 4.7. Diagrams of HOMO and LUMO for 4.22, 4.23, and benzene
4.3.5. Applications of novel zwitterionic nido m-carborane cluster

As mentioned in Chapter 4.3.4, treatment of compound 4.22 with methylethylketone (CH$_3$CH$_2$COCH$_3$) also generated a UV active (254 nm) component, indicating that the carbonyl (C=O) functional group in ketones and probably aldehydes is susceptible to attack by the ammonium group of compound 4.22. Therefore, we tested the reactivity of 4.22 with several ketones (e.g, acetophenone, cyclohexanone, benzophenone, methylethylketone) and aldehydes (e.g, acrolein, acetaldehyde, anisaldehyde and 2,4-dimethoxybenzaldehyde) in benzene without addition of any catalyst. With the exception of benzophenone, all of these ketones and aldehydes presumably generated iminium-type compounds as demonstrated by UV/TLC inspection at 254 nm. However, carboxylic acids (e.g, cinnamic acid) treated with 4.22 did not generate iminium-type compounds. Two iminium compounds (4.24 and 4.25 in Scheme 4.3), derived from cyclohexanone and 2,4-dimethoxybenzaldehyde, precipitated from benzene and their structures were confirmed by $^1$H-, $^{13}$C-NMR and HRMS.

The unique chemical reactivity of compound 4.22 may be of great interest to organic chemists. A plausible mechanism for the reaction of compound 4.22 with ketones or aldehydes, involving acidic catalysis by an “internal” hydrogen atom, is shown in Scheme 4.4 using acetone as an example. A free electron pair of the carbonyl oxygen of acetone captures a proton of the ammonium group of 4.22. The protonated carbonyl group is now sufficiently electrophilic to be attacked by the emerged nucleophilic amine group to generate a tetrahedral intermediate. Eventually, the formation of a stable conjugated system between carborane cluster and iminium (C=\(\text{N}^+\)) group in 4.23 is the driving force for the elimination of water in the last step.
In general, amines react with carbonyl groups of aldehydes or ketones in the presence of acid catalyst under reflux and formation of imine- or iminium-containing compounds is accomplished by removing water through azeotropic distillation. In contrast, acetone and compound \textbf{4.22} do not require any acid catalyst, reflux, and azeotropic distillation to generate compound \textbf{4.23}. In addition, hydrolysis of \textbf{4.23} occurred under very mild condition (5\% NaHCO\textsubscript{3}/EtOH, rt, 2 h) to regenerate \textbf{4.22}. Therefore, we believe the reactivity of the ammonium function attached to the zwitterionic \textit{nido} \textit{m}-carboarne cages of the 3CTAs (\textbf{4.14.B-4.19.B}) will be of no consequence for their biological activities under physiological conditions. However, it is conceivable that the unique chemical properties of compound \textbf{4.22} could be utilized to facilitate organic reactions (e.g. Michael addition) or to detect carbonyl impurities in analytical chemistry.

\textbf{4.3.6. Summary and conclusions}

The zwitterionic ammonium-substituted \textit{nido} \textit{m}-carborane cluster (\textbf{4.22}) was synthesized and its experimental data were compared with those calculated at the \textit{ab initio} HF and DFT levels using Gaussian 03\textsuperscript{194} GIAO/\textsuperscript{11}B-NMR chemical shifts for
compound 4.22 obtained at the B3LYP/6-31G* and B3LYP/6-31G** levels showed strong correlations with the experimental values. The zwitterionic NH₃⁺-nido m-carborane 4.22 exhibited unique chemical properties by reacting with the carbonyl group of ketones or aldehydes without addition of acid catalyst at room temperature. The zwitterionic iminium-substituted compound 4.23 showed a bathochromic shift in UV experiment due to the stable conjugation of the σ-aromatic carborane cage with the iminium group. The unique structural, physicochemical, and chemical properties of the novel zwitterionic NH₃⁺-nido m-carborane structure may lead to a wide range of novel applications in organic chemistry and boron chemistry.

4.4. Outlook

Based on the evaluation of its enzymatic and physicochemical properties, zwitterionic compound 4.14.B has been identified as one of two “2nd Generation 3CTA” which have the potential to replace N5-2OH, the current lead compound of our 3CTA library. The other “2nd Generation 3CTA” is the compound 4.26 shown in Figure 4.8. Compound 4.26 was synthesized and evaluated as a boron-delivery agent by Narayanasamy et al.211

![Figure 4.8. Chemical structures of 2nd generation 3CTAs](image-url)
The nature of chirality in 1,2-disubstituted nido \textit{o}-carboranes or 1,7-disubstituted nido \textit{m}-carboranes has been poorly studied.\cite{174,222,223} Therefore, the possibility of simple chromatographic separation of pure epimers of \textbf{4.14.B} may be a useful tool to initiate more detailed studies on the chirality of nido \textit{m}-carborane. The dThd moiety in both epimers of \textbf{4.14.B} could be removed with hydrazine (NH$_2$NH$_2$).\cite{224} Alternatively, one could attach dThd or a comparable structure to NH$_3^+$-nido \textit{m}-carborane via a linker that is easily cleaved. To substantiate our hypothesis that pure epimers rather than atropisomers of \textbf{4.14.B} have been separated by HPLC, analysis of two pure epimers by X-ray crystallography is warranted. X-ray crystal structure of iminium compounds \textbf{4.23}, \textbf{4.24} or \textbf{4.25} would also be determined.
CHAPTER 5

DISCOVERY OF NOVEL ANTIBIOTICS TARGETING THYMIDINE KINASE
AND THYMIDINE MONOPHOSPHATE KINASE
FROM BACILLUS ANTHRACIS

5.1. Introduction

Bacillus anthracis (B. anthracis), the agent that causes anthrax infections, is a
Gram-positive, facultative, anaerobic bacterium. Anthrax infection is classified into three
types by the route of exposure: cutaneous, inhalational, and gastrointestinal anthrax.225
Humans can be infected with anthrax by handling infected animals, inhaling anthrax
spores, or eating uncooked meat of infected animals.225 Anthrax spores can survive for
many years in the soil and can be easily transmitted to humans. Regardless of the
exposure route to humans, anthrax spores are phagocytosed by macrophages where they
germinate.226 Cutaneous anthrax is the most common while the inhalational anthrax is the
most deadly form.

Interest in anthrax pathogenesis emerged as a consequence of the intentional
attack via contaminated letters with anthrax spores in 2001 in the USA, and thus, the
primary concern with anthrax in the 21st century is its use as a biological weapon.227
FDA-approved antibiotics for the treatment of anthrax infections include ciprofloxacin
(fluoroquinolones), doxycycline (tetracyclines), and penicillin G (β-lactams). An anthrax vaccine (Biothrax®, BioPort Corporation, Lansing, MI) is available for prevention of anthrax disease. However, resistance could be developed towards the current antibiotics and vaccination of the large population is difficult. Therefore, there is an urgent need to discover novel antibiotics for the treatment of unvaccinated individuals who may become exposed to anthrax or infected with antibiotic-resistant strains of *B. anthracis*.

### 5.2. Rationale

The physiological function of typical salvage pathway enzymes in prokaryotes is not known. But it is very likely that they complement the *de novo* pathway of the DNA precursor synthesis. It is also possible that these enzymes are essential for colonization and pathogenicity in certain situations of bacterial growth. A large fraction of multidrug-resistant *Staphylococci* has proven to be dependent on an exogenous supply of dThd for growth. *B. anthracis* strains lacking thymidylate synthase (TS) activity relied on the salvage pathway for dTTP production for survival. Figure 5.1 depicts biochemical pathways for the synthesis of dTTP in *B. anthracis*. *B. anthracis* thymidine kinase (*BaTK*) catalyzes the production of dTMP from dUrd and dThd in the salvage pathway while TS plays a pivotal role in transferring a methyl group from 5,10-methylene tetrahydrofolate to dUMP for dTMP production in the *de novo* pathway. dUMP is synthesized from dUTP by a dUTPase and from dCMP by a dCMP deaminase in the *de novo* pathway. The role of thymidine monophosphate kinase (TMPK) for dTTP
production is even more crucial than those of TK and TS because it is supplied with dTMP both by salvage and de novo pathways.

Figure 5.1. Salvage and de novo biochemical pathways for dTTP synthesis


BaTK may be an attractive molecular target for novel antibiotics for the treatment of anthrax infections. It could be utilized to activate a nucleoside-based prodrug, which is converted to a toxic dNTP by dNKs in a “traditional” way\textsuperscript{36,41,42} or it could be targeted
with a \(Ba\)TK-specific inhibitor. In the latter case, a \(Ba\)TK-specific inhibitor may be used in combination with an inhibitor of TS or other crucial enzymes in the \textit{de novo} pathway. To our knowledge, substrates or inhibitors targeting bacterial TK have not yet been developed for therapeutic purposes. Recently, however, the known TK substrate 1-(2’-deoxy-2’-fluoro-\(\beta\)-D-arabinofuranosyl)-5-[\(^{125}\)I]iodouracil (F\(^{125}\)IAU) proved to be a useful tool for the diagnosis of various localized bacterial infections by \textit{Staphylococcus aureus} and \textit{Enterococcus faecalis}.\(^5\)\

\textit{B. anthracis} thymidine monophosphate kinase (\(Ba\)TMPK) is a more attractive target than \(Ba\)TK because dTMPs supplied by salvage and \textit{de novo} pathways are diphosphorylated only by \(Ba\)TMPK. There is no report on transport of dTDP into bacteria and, therefore, it is likely that dTDP production in \textit{B. anthracis} relies solely on the action of \(Ba\)TMPK. Unless \textit{B. anthracis} possesses an unknown alternative mechanism to generate or transport dTDP, \(Ba\)TMPK provides an excellent molecular target for the discovery of novel antibiotics for the treatment of anthrax infections.

5.3. Evaluation of agents with established molecular targets in mammalian and viral systems as potential antibiotics against \textit{B. anthracis}

The structures of agents used in enzyme and toxicity studies described below are shown in Figure 5.2. Their established biological activities in mammalian and viral systems are summarized briefly as follows. The dUrd analogues substituted with halogen atoms or the ethyl group at the 5-position (5-FdUrd, 5-CldUrd, 5-BrdUrd, 5-IdUrd, and 5-EtdUrd) are known to be good substrates for hTK1 and their triphosphates are substrates for human DNA polymerase. Floxuridine (5-FdUrd) and 5-FU are strong TS
inhibitors. dThd analogues substituted with bulky groups at the N-3 position (3-isopropyl-dThd, 3-propargyl-dThd, and N5-2OH) are effectively phosphorylated by hTK1 and UuTK, however, not much is known about their subsequent metabolism.\textsuperscript{12,43} dThd analogues modified at the 3’-position are tolerated moderately by hTK1 as substrates.\textsuperscript{147,233} They are also further phosphorylated to their triphosphate forms, which are relatively poor substrates of human DNA polymerase. However, triphosphates of AZT and d4T are good HIV (human immunodeficiency virus) reverse transcriptase inhibitors.\textsuperscript{234}

The anticancer agents gemcitabine, ara-C, and cladribine are all effectively monophosphorylated by dCK. Their corresponding triphosphates are powerful competitive inhibitors of human DNA polymerase and they terminate DNA synthesis through their incorporation into DNA. Gemcitabine is also a highly effective inhibitor of RR, another key enzyme in the \textit{de novo} pathway of DNA synthesis. Acyclovir, ganciclovir, and BVDU are converted to their monophosphates by HSV-TK but not by hTK1. Their triphosphates are inhibitors of HSV-DNA polymerase. Ganciclovir is also monophosphorylated by cytomegalovirus (CMV) pUL97 protein kinase and its triphosphate is an effective inhibitor of DNA polymerase, which is utilized in HSV-tk/ganciclovir suicide gene therapy. Hydroxyurea is a RR inhibitor and 6-mercaptopurine (6-MP) inhibits various enzymes specific to purine nucleotide \textit{de novo} biosynthesis.
Figure 5.2. Chemical structures of the existing compound library
<table>
<thead>
<tr>
<th>Compound</th>
<th>PR&lt;sup&gt;a&lt;/sup&gt; (%) BaTK</th>
<th>PR&lt;sup&gt;a&lt;/sup&gt; (%) hTK1</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; at 6 h (µM)</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; at 20 h (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-FdUrd (Flouxuridine)</td>
<td>91 ± 5</td>
<td>95&lt;sup&gt;12&lt;/sup&gt;</td>
<td>7.0</td>
<td>1.8</td>
</tr>
<tr>
<td>5-FU</td>
<td>-</td>
<td>-</td>
<td>2.9</td>
<td>2.9</td>
</tr>
<tr>
<td>5-EtdUrd</td>
<td>83 ± 6</td>
<td>80&lt;sup&gt;12&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5-CldUrd</td>
<td>80 ± 4</td>
<td>196&lt;sup&gt;12&lt;/sup&gt;</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
</tr>
<tr>
<td>5-BrdUrd</td>
<td>67 ± 4</td>
<td>80&lt;sup&gt;235&lt;/sup&gt;</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
</tr>
<tr>
<td>5-IdUrd</td>
<td>88 ± 5</td>
<td>170&lt;sup&gt;12&lt;/sup&gt;</td>
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<td>-</td>
</tr>
<tr>
<td>BVDU</td>
<td>&lt; 0.1</td>
<td>&lt; 1&lt;sup&gt;235&lt;/sup&gt;</td>
<td>&gt; 250</td>
<td>&gt; 250</td>
</tr>
<tr>
<td>3-Methyl-dThd</td>
<td>35 ± 6</td>
<td>43&lt;sup&gt;12&lt;/sup&gt;</td>
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<td>-</td>
</tr>
<tr>
<td>3-Propargyl-dThd</td>
<td>31 ± 3</td>
<td>21&lt;sup&gt;12&lt;/sup&gt;</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
</tr>
<tr>
<td>3-Isopropyl-dThd</td>
<td>23 ± 6</td>
<td>17&lt;sup&gt;12&lt;/sup&gt;</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
</tr>
<tr>
<td>N5-2OH</td>
<td>30 ± 5</td>
<td>41&lt;sup&gt;235&lt;/sup&gt;</td>
<td>10.0</td>
<td>22.1</td>
</tr>
<tr>
<td>AZT</td>
<td>47 ± 8</td>
<td>52&lt;sup&gt;12&lt;/sup&gt;</td>
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<td>&gt; 1000</td>
</tr>
<tr>
<td>d4T</td>
<td>8 ± 1</td>
<td>7&lt;sup&gt;43&lt;/sup&gt;</td>
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<td>&gt; 500</td>
</tr>
<tr>
<td>FLT</td>
<td>68 ± 3</td>
<td>30&lt;sup&gt;12&lt;/sup&gt;</td>
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<td>127</td>
</tr>
<tr>
<td>D-FIAU</td>
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<td>76&lt;sup&gt;12&lt;/sup&gt;</td>
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<tr>
<td>L-FMAU&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>-</td>
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<tr>
<td>DOT&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>-</td>
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<tr>
<td>3´-Azidomethyl-dThd</td>
<td>31 ± 7</td>
<td>15&lt;sup&gt;12&lt;/sup&gt;</td>
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</tr>
</tbody>
</table>

Table 5.1. PRs and in vitro growth inhibition of the existing compound library

Table 5.1 continued
Table 5.1 continued

<table>
<thead>
<tr>
<th>Compound</th>
<th>PR</th>
<th>IC50 (µM)</th>
<th>IC12 (µM)</th>
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<td>15^{12}</td>
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<tr>
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<td>-</td>
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<td>-</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Cladribine</td>
<td>-</td>
<td>-</td>
<td>&gt;250</td>
</tr>
<tr>
<td>Acyclovir</td>
<td>-</td>
<td>-</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Ganciclovir</td>
<td>-</td>
<td>-</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>-</td>
<td>-</td>
<td>&gt;500</td>
</tr>
<tr>
<td>6-Mercaptopurine</td>
<td>-</td>
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<td>&gt;200</td>
</tr>
</tbody>
</table>

Table 5.1. PRs and in vitro growth inhibition of the existing compound library.

a PR for dThd was set to 100 with both TKs. The concentrations of compounds and [γ-\(^{32}\)P]ATP were 100 µM. b Compounds were a gift from Dr. Serge Van Calenbergh from the Ghent University, Ghent, Belgium. c Compounds were a gift from Dr David Chu from the University of Georgia, Athens, GA.

5.3.1. \textit{BaTK and hTK1 PRs}

The \textit{BaTK} PRs of compounds substituted with halogens or the ethyl group at the 5-position (5-F, 5-Cl, 5-Br, 5-I, and 5-EtdUrd) ranged from 67 to 91% relative to that of dThd (Table 5.1). The activity decreased drastically (< 0.1%) with larger substitution, such as the bromovinyl group in BVDU. All dThd analogues substituted at the 3’-position were good substrates of \textit{BaTK} (24-68% PRs) except for d4T. Moderate activities (23-35% PRs) were also observed for dThd analogues modified at the N-3 position. D-FIAU had a high relative PR of 79% in \textit{BaTK}, which supports its potential use as a diagnostic agent for localized bacterial infections.\textsuperscript{54} Overall, the PRs observed for \textit{BaTK} were comparable with those observed for hTK1 (Table 5.1). Except AZT, however, the \textit{BaTK} PRs for most of the tested 3’-substituted compounds were higher than those for hTK1, indicating that selectivity for \textit{BaTK versus hTK1} may be achieved through 3’-modification. The PTAs described in this chapter were carried out in collaboration with Dr. Staffan Eriksson and coworkers.

5.3.2. \textit{Molecular modeling}

Extensive studies with \textit{M. tuberculosis} TMPK indicated sensitivity to inhibition by dThd and dTMP analogues with 3’-modifications such as the 3’-azido-, 3’-fluoro, 3’-azidomethyl and 3’-fluoromethyl group.\textsuperscript{236-238} As shown in Table 5.1, the PRs of 3’-azidomethyl-Thd, 3’-fluoromethyl-Thd, and FLT were about two times higher with \textit{BaTK} compared with hTK1. In order to elucidate whether 3’-modifications at dThd have the potential to generate selective inhibitor/substrate for \textit{BaTK versus hTK1}, we investigated the dimension of the binding pocket around the 3’-edge of the dThd scaffold.
in a homology model of *BaTK* and the crystal structure of hTK1 using Site ID module implemented in Sybyl 7.1 (Figure 5.3).

**Figure 5.3.** Dimensions of the dTTP binding pocket in hTK1 crystal structure (A) and the *BaTK* homology model (B).

Although there is higher sequence identity (62%) between *BaTK* and *CaTK* than between *BaTK* and hTK1 (40%) or *UuTK* (43%), crystal structures of the latter two were used as templates to build the homology model of *BaTK*. These two TKs presumably represent ‘closed’ form while *CaTK* may represent an ‘open’ form as discussed in Chapters 1 and 3. Standard docking programs are able to enrich known ligands for a specific protein target out of large libraries consisting of ligands and non-ligands.\(^{202,239}\) However, the nature of the protein structure plays a crucial role in the level of enrichment. In general, holo (closed) conformations appear to be more effective than apo (open) conformations.\(^{202}\) We hypothesize that, in particular, for the identification of inhibitor
binding, holo conformations are essential. As already discussed in Chapter 3, the computational analysis of substrate binding may also necessitate the use of apo conformations.

Comparative Site ID analysis of the active site of BaTK homology model and hTK1 crystal structure showed that BaTK has a larger cleft around the C-3’/C-4’ edges of 2’-deoxyribose than hTK1 (Figure 5.3), indicating that modification at this position may result in selective binding to BaTK versus hTK1. Indeed, based on both the docked poses and their binding energies, several of C-3’-branched dThd triphosphates analogues (e.g., -CH2C≡CH, -CH2CH=CH2, -CH2N3, -CH2SH, -NHCH3, -OCH3 and -SCH3) built in silico showed better binding to the BaTK homology model than to the hTK1 crystal structure (PDB ID: 1W4R) when docked with the FlexX module of Sybyl 6.9. However, in vitro toxicity studies of AZT, FLT, 3’-aminomethyl-dThd, and 3’-hydroxymethyl-dThd in B. anthracis Sterne indicated that 3’-modification at dThd alone may not have the potential to exert significant toxicity in B. anthracis (Table 5.1).

5.3.3. In vitro toxicity studie with B. anthracis Sterne

B. anthracis Sterne strain (34F2), an attenuated strain of B. anthracis, was used in in vitro toxicity studies. It has been used previously as a surrogate for the B. anthracis Ames, a virulent strain.240 We carried out in vitro toxicity studies of the existing library with B. anthracis Sterne. Among all screened dThd and dUrd analogues, only Floxuridine (1.8 μM), N5-2OH (22.1 μM) and FLT (127 μM) had IC50 values < 200 μM at 20 h incubation. Gemcitabine and 5-FU showed low IC50 values at 0.11 μM and 2.9 μM, respectively. This result implied that the functions of RR or TS are crucial for
growth of \textit{B. anthracis} Sterne. Floxuridine is a very good substrate both of \textit{BaTK} (PR: 91\%) and hTK1 (PR: 95\%). This agent is an effective inhibitor of TS as a monophosphate, and a good terminator of DNA biosynthesis by incorporating into both DNA and RNA in triphosphate form. The moderate toxicity observed for N5-2OH appeared to be due to its high lipophilicity (see Chapter 3) rather than its capacity to function as a \textit{BaTK} substrate and/or inhibitor. Its high lipophilicity may allow N5-2OH to interact with membrane components of \textit{B. anthracis} Sterne, thereby causing toxicity.\textsuperscript{70,181} Lipophilicity-dependent toxicity of carborane-containing compounds has been previously demonstrated in mammalian systems.\textsuperscript{130,133} FLT is phosphorylated more than twice as effectively by \textit{BaTK} than by hTK1 and it also showed moderate toxicity in \textit{B. anthracis} Sterne after 20 h incubation. FLT toxicity was not observed after 6 h incubation, indicating that toxic FLT metabolites are accumulated in \textit{B. anthracis} Sterne over a longer time period. Except for BVDU, all other dUrd analogues were efficiently phosphorylated by \textit{BaTK} in PTAs but they were apparently not toxic to \textit{B. anthracis} Sterne in \textit{in vitro} toxicity studies.

This is in a sharp contrast with mammalian or viral systems which are highly sensitive to these compounds. Possible explanations for the lack of toxicity could be the fact that these agents are not effectively transported into the interior of \textit{B. anthracis} Sterne or the conversion of the 5´-monophosphates to triphosphate forms by TMPK and NDPK of \textit{B. anthracis} Sterne is hampered. It is also conceivable that the triphosphates are not substrates of \textit{B. anthracis} Sterne DNA polymerase or they are ineffective as feedback inhibitors of \textit{BaTK}.
However, high and moderate toxicities observed for Floxuridine and FLT indicate that transport of dThd analogues with 3’- and 5-modifications into B. anthracis Sterne is possible and that their metabolites can exert toxicity. Some reports indicate low utilization levels for TKs in Gram-positive bacteria and thus low dependence on salvage pathways for their DNA synthesis.\textsuperscript{241,242} This may, in particular, be the case for the M9 minimal growth medium used in our toxicity studies, which only contains phosphate, chloride, glucose, and amino acids but no nucleoside or nucleobase supplements. This may cause downregulation of nucleoside transport mechanism and salvage pathway enzymes in B. anthracis Sterne. This explanation would also account for the high toxicities observed for floxuridine and 5-FU, which are TS inhibitors. Remarkable is the observed toxicity of gemcitabine in B. anthracis Sterne, which is comparable to that of enrofloxacin, the fluoroquinolone-class antibiotic we used as a positive control for \textit{in vitro} toxicity studies. Gemcitabine utilizes both salvage (dCK) and \textit{de novo} pathway enzymes (RR) for its activity. This substantiates the initial assumption (Chapter 5.2) that both pathways must be targeted in B. anthracis for nucleoside-based antibiotic therapy of anthrax. It remains to be determined whether gemcitabine can be effective as an antibiotic at plasma concentrations that are significantly lower than those used in cancer chemotherapy.

The \textit{in vitro} toxicity studies described in Chapter 5.3.2 were carried out in collaboration with Dr. Andrew J. Phipps in the OSU Department of Veterinary Biosciences.

\textbf{5.4. \textit{Ba}TK- and/or \textit{Ba}TMPK-specific inhibitors}
5.4.1. Drug design

Studies described in Chapter 5.3 indicated that the concept of dThd or dUrd prodrug-based antibiotics for anthrax may have limited potential. Therefore, we focused our efforts on the discovery of agents that could function as selective inhibitors of BaTK and/or BaTMPK that do not require metabolic activation. Such inhibitors could compete with endogenous substrates (dThd, dUrd, dTMP) of BaTK/BaTMPK and thereby decrease dTTP production. The resulting imbalanced dNTP pool could affect DNA synthesis in B. anthracis, and thus, reduce its growth and spread.

BaTK belongs to the TK1-like kinase and has 40% and 43% sequence identity with hTK1 and UuTK, respectively. Crystal structures of hTK1 and UuTK were determined very recently, most of which are in complex with dTTP, the endogenous feedback inhibitor of hTK1 and UuTK, in the active site. Therefore, dTTP was utilized as a template to design novel BaTK/BaTMPK inhibitors.

Compounds containing a triphosphate group, however, are inherently unstable under physiological conditions and they can not cross cell membranes because of their highly ionic character. These features render a triphosphate-containing compound completely unsuitable as a drug. Therefore, research on stable phosphate mimics with increased lipophilicity has been extensive. These mimics include acyclic structures and five- or six-membered homo- or heterocyclic (N, O, S) ring systems substituted with e.g. hydroxyl, amino, methoxy, sulfonate, sulfonamide, carboxylic, keto, thioketo, chlorine, fluorine, urea, thiourea, phosphoamide, or carbamate groups as well as triphosphates in which backbone oxygen atoms were replaced with e.g. amino, methylene, fluoromethylene, hydroxymethylene, chloromethylene, borane, and cyanoborane groups.
Some of these phosphate mimics had already found successful application in the (bio)synthesis of natural and synthetic antibiotics. These include the natural product Nucleocidin (Figure 5.4), an inhibitor of protein synthesis with broad antibacterial spectrum, and Salicyl-AMS (Figure 5.4), an inhibitor of the siderophore biosynthesis with a salicylsulfamoyl group at the 5′-position. Both of these agents demonstrated antimicrobial activity, and therefore, it is reasonable to assume that 

\[ Ba\text{TK}/Ba\text{TMPK} \] inhibitor containing stable lipophilic phosphate mimics at the 5′-position should be able to enter the interior of \( B.\text{anthracis} \).

Despite significant structural differences (Figures 5.6 and 5.7), TKs and TMPKs catalyze very similar biochemical processes. This may be the reason why TKs of most HSVs have both TK and TMPK activity. Both enzymes possess a binding site for dThd or dTMP, embedded in the interior of the enzymes, an adenosine binding site, located on the surface of the enzymes, and a bridging phosphate binding site, which accommodates either three (TK) or four (TMPK) phosphate moieties. Therefore, design

\[ \text{Figure 5.4. Known antibiotics substituted with phosphate mimics at the 5′-position} \]
strategies for BaTK inhibitors based on phosphate mimics are also applicable to BaTMPK inhibitors.

The design of selective inhibitors of BaTMPK may be more attractive than those of BaTK because the sequence identity between human TMPK (hTMPK) and BaTMPK is only 21% while that of hTK1 and BaTK is 40%. Various crystal structures of hTMPK as well as those of Escherichia coli (E. coli), Mycobacterium tuberculosis (M. tuberculosis), Yeast, and Staphylococcus aureus (S. aureus) have been determined.278-285 In particular, E. coli and S. aureus TMPK (SaTMPK) have 34% and 49% sequence identity with BaTMPK, which allowed us to build a homology model of BaTMPK.

Figure 5.5. Design of phosphate-mimicking dThd analogues as BaTK/BaTMPK inhibitors
Figure 5.5 shows strategies for the design of BaTK and BaTMPK inhibitors containing phosphate mimics. To the best of our knowledge, none of these phosphate mimics has been used previously for the design and synthesis of dThd analogues. The dThd scaffold was kept intact because it provides the element of selective affinity for both enzymes. Sulfonamide-, urea-, thiourea-, amide-, and triazole linkers were chosen to replace the α-phosphate moiety of dTTP. In particular, triazole has an interesting linker property because it has a much stronger dipole moment than an amide bond, and thus, the nitrogen atoms of the triazole group act as hydrogen bond acceptors. To increase lipophilicity, the benzene moiety was attached as a replacement of the β-phosphate of dTTP. Additional polar groups (e.g. SO₂CH₃, CO₂CH₃, and SO₂NH₂) were attached to the benzene moiety to substitute for the terminal γ-phosphate moiety of dTTP. All three groups were expected to interact with the phosphate-loop (P-loop) region of both enzymes via hydrogen-bonding and electrostatic interaction.

5.4.2. Synthesis

Scheme 5.1 Synthesis of 5′-amino-5′-deoxythymidine as a key intermediate

Reagents and conditions: (a) p-toluenesufonyl chloride (1.1 eq), pyridine, 0 °C → rt; (b) NaN₃, DMF, 70 °C, 12 h; (c) H₂, 5% Pd/C, EtOH, RT, 24 h.
The preparation of the designed compounds shown in Figure 5.5 was achieved by utilizing compounds 5.2 and 5.3 as key intermediates. Compound 5.3 was obtained according to a reported procedure\textsuperscript{287,288} by selective tosylation of the 5′-position of dThd, followed by the introduction of azide, and palladium-catalyzed hydrogenation.

**Scheme 5.2.** Synthesis of sulfonamide-linked dThd inhibitors

Reagents and condition: (a) the appropriate sulfonyl chloride, TEA, DMF, rt, 6 h.

Sulfonamide-linked dThd inhibitors (5.4-5.7) were prepared in 65-80% yield by reacting 5.3 with the appropriate sulfonyl chlorides [4-(methylsulfonyl)benzenesulfonyl chloride for 5.4, 3-(methylsulfonyl)benzenesulfonyl chloride for 5.5, 2-(methylsulfonyl)benzenesulfonyl chloride for 5.6, 1-naphthalenesulfonyl chloride for 5.7] in the presence of triethylamine (Scheme 5.2).
Scheme 5.3. Synthesis of thiourea- and urea-linked dThd inhibitors

Reagents and condition: (a) the appropriate phenylisothiocyanate or phenylisocyanate, DMAP, pyridine, rt, 12 h.

Thiourea-linked compound 5.8 was prepared by reacting compound 5.3 with 2-(methylthio) phenylisothiocyanate and catalytic amount of DMAP in pyridine. The *meta*- and *para*-isomers (5.9-5.10) were prepared in 73% and 83% yield by using 3-(methylthio)- and 4-(methylthio) phenylisothiocyanate, respectively. Identical reaction conditions were used for the synthesis of the urea-linked dThd inhibitors 5.11-5.13. Treatment of 3-(methylthio)-, 4-(methylthio)phenylisocyanate, and methyl 3-isocyanatobenzoate with 5.3 afforded 5.11 (68% yield), 5.12 (73% yield), and 5.13 (52% yield), respectively.
Scheme 5.4. Synthesis of amide-linked dThd inhibitors

Reagents and condition: (a) the appropriate benzoic acid, DCC, HOBt, DMF, rt, 12 h.

Various benzene carboxylic acids (see experimental section) were coupled with 5.3 using 1-hydroxybenzotriazole (HOBt) and 1,3-dicyclohexylcarbodiimide (DCC) to afford the amide-linked inhibitors 5.14-5.18 in yields ranging from 38% to 58%. When methyl hydrogen phthalate was reacted with 5.3, compound 5.19 was synthesized in 28% yield via the intermediate 5.19.I, as shown in Scheme 5.4. This reaction was presumably facilitated by the close proximity of CO$_2$Me and NH groups resulting in the formation of the phthalimide via intramolecular cyclization.
The triazole-linked inhibitors 5.20-5.23 were prepared by reacting the azide 5.2 with the appropriate alkynes (see experimental section for details). Formation of 1,4-substituted-1,2,3-triazoles from azides and alkynes had been described as ‘click chemistry’. No protecting groups are required in this reaction. Copper (I) is the most powerful catalyst of this 1,3-dipolar cycloaddition of azides and alkynes. We used the ‘copper (II)/ascorbate system’, which generates the Cu (I) catalyst in situ by reducing CuSO₄ with sodium ascorbate. To our knowledge, this is the first report on the triazole-linked dThds. However, the obtained yields were very low (8-15%).

5.4.3 Biological evaluation

In vitro toxicity studies of compounds 5.1-5.23 were carried out using B. anthracis Sterne. As shown in Table 5.2, all of the thiourea- and urea-linked compounds except compound 5.13 produced inhibition at 6 h incubation. In particular, the IC₅₀ values
of compounds substituted with SCH$_3$ group at the meta position of the phenyl ring (5.9 and 5.11) were very low with 5.3 µM and 0.48 µM, respectively. However, their activities deteriorated dramatically at 20 h incubation. The amide-linked compounds with SCH$_3$ (5.16) and CO$_2$Me (5.17) at phenyl ring also displayed significant inhibitory activity at 6 h but not at 20 h. Compounds 5.9-5.12 and 5.16-5.17 have common structural features, which could have been metabolized by enzymes of *B. anthracis* Sterne. SCH$_3$ is easily oxidized to sulfoxide or sulfone and CO$_2$Me could be hydrolyzed by esterases to give CO$_2$H. Urea and thiourea bonds could also be cleaved to produce the inactive precursor 5.3. This hypothesis is supported by the IC$_{50}$ of compound 5.15, a potential metabolite of compound 5.16, which was inactive at 6 h and 20 h. Also, we observed decomposition of urea-, thiourea-, and amide-linked dThds in DMSO even if they were kept at -20 °C.

The structurally different triazole-linked dThd analogues were active at 6 h in the range of 84 to 430 µM concentration. Interestingly, compound 5.21 retained its activity to some degree until 20 h (IC$_{50}$: 303 µM). It is conceivable that the triazole-linked compounds are in general more resistant to metabolism or hydrolysis compared with the urea-, thiourea-, and amide-linked compounds and thus, displayed activities up to 20 h. While transformation to inactive metabolites may be an explanation for the deteriorating activities observed for several compounds in *B. anthracis* Sterne, it could be another explanation that they have a bacteriostatic rather than a bactericidal effect and delayed the growth of *B. anthracis* Sterne, which eventually reached a plateau of growth after 20 h.
Table 5.2. *In vitro* IC$_{50}$ of dThd analogues against *B. anthracis* Sterne

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<th>IC$_{50}$ (µM) at 20 h</th>
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</tr>
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</table>

N/A: not determined
5.4.4. Molecular modeling

5.4.4.1. B. anthracis thymidine kinase (BaTK)

![Image of molecular structures](image)

**Figure 5.6.** The hTK1 crystal structure with dTTP (A) and the BaTK homology model docked with compound 5.11 (B) and 5.21 (C)

Docking of compounds 5.1-5.23 into the crystal structure of hTK1 and the homology model of BaTK (Chapter 5.3.2) demonstrated that all thiourea- and urea-linked dThds (5.8-5.13) bind to the active site of both enzymes in a fashion that is similar to that of dTTP (Figure 5.6). The structures of urea-linked 5.11 and triazole-linked 5.21 docked into the active site of the BaTK homology model are shown in Figure 5.5. Both the urea group in 5.11 and the triazole moiety in 5.21 overlapped with the α-phosphate in dTTP, as predicted. Most of the sulfonamide-, amide-, and triazole-linked inhibitors (5.4-5.7, 5.14-5.18, and 5.20-5.23) also showed similar binding patterns compared with that of
dTTP in both evaluated 3D-structures. Only the phthalimide-linked compound 5.19 did not dock to either of these structures.

**5.4.4.2. *B. anthracis* thymidine monophosphate kinase (BaTMPK)**

TMPK from *S. aureus* (*SaTMPK*) was used as a template to build a homology model of *BaTMPK* because *S. aureus* and *B. anthracis* are Gram-positive bacteria and their TMPKs have high sequence identity (49%) as mentioned in Chapter 5.4.1. Recently, three crystal structures of *SaTMPK* (PDB ID: 2CCG, 2CCJ, and 2CCK) were determined.\(^{291}\) Surprisingly, two poses of dTMP were found in the one of the *SaTMPK* crystal structures (PDB ID: 2CCJ) as shown in Figure 5.7.B. In one dTMP structure, the monophosphate group interacting with P-loop region was projected towards the putative ADP binding site (Figure 5.7.B). However, the monophosphate group of the other dTMP projected towards a pocket generated by the c- and d-loops (Figure 5.7.B).

The guanidine side chain of Arg 106 in the d-loop of *SaTMPK* interacts with the α-phosphate of the second dTMP structure and may contribute the dTMP binding. This arginine residue in the d-loop region is found in *SaTMPK*, *BaTMPK* and *E. coli* TMPK, but not human, yeast, and *M. tuberculosis* TMPK. Docking studies of compounds 5.11 and 5.21 into the *BaTMPK* homology model exhibited that the phosphate-mimicking portions of their docked poses are located very close to d-loop region, indicating that these two compounds in *BaTMPK* may have a binding mode similar to the second dTMP in *SaTMPK* (Figure 5.7.C and 5.7.D).
Figure 5.7. (A) Crystal structure of hTMPK with dTMP and ADP, (B) Crystal structure of SaTMPK with dTMP, (C) BaTMPK homology model with 5.11, (D) BaTMPK homology model with 5.21.

a: P-loop, b: Lid-region, c: Loop missing in 2CCK, d: Loop with Arg 105
The carbonyl oxygen of urea in **5.11** is 1.89 Å away from the guanidine group in Arg 109 of **BaTMPK** which corresponds to Arg 106 in **SaTMPK**. The binding pattern of compound **5.9** in **BaTMPK** was similar to the first dTMP binding pose in **SaTMPK** (Figure 5.7.E). In contrast, docking of **5.9, 5.11, and 5.21** into the crystal structure of **hTMPK** crystal structure showed the different binding pattern. The thymine regions of all three compounds overlapped the β-phosphate of ADP and the phenyl ring in these structures overlapped the 2’-deoxyribose moiety of dTMP substrate (Figure 5.7.F). Such an inversed binding pattern also has the potential to inhibit substrate binding. Indeed, a similar inversed binding pattern has been found for AZTMP both in the crystal structures of **hTMPK** and of **TMPK** from *M. tuberculosis*. It has been suggested that this specific interaction of AZT-MP with these enzymes may contribute to its toxicity in both systems.
5.5. Summary and conclusions

A compound library composed of known antiviral and anticancer drugs was screened to find a lead compound for the development of potential nucleoside-based antibiotics for the treatment of anthrax infections. PTAs showed that most of dThd and dUrd analogues of this compound library were good substrates for BaTK as well as hTK1. Compounds substituted with a bulky group at 3’-position were better substrates of BaTK than of hTK1, implying this position can be utilized to achieve selectivity for BaTK versus hTK1. This finding was substantiated by computational Site ID studies. In vitro toxicity studies of this library in B. anthracis Sterne showed that all of dThd and dUrd analogues were inactive except FLT and Floxuridine. However, the IC$_{50}$ of FLT is too high (127 µM) for selecting it as a lead compound and the activity of Floxuridine appeared to be derived from inhibiting TS rather than from blocking salvage pathway enzymes.

Twenty dThd analogues were designed and synthesized as potential inhibitors of BaTK and/or BaTMPK. These structures were designed to mimic dTTP, the endogenous feedback inhibitor of BaTK. The phosphate moiety of dTTP was replaced with sulfonamide-, amide-, urea-, thiourea-, or triazole groups in these inhibitors. Some of the urea-, thiourea-, and triazole-linked inhibitors exhibited relatively low IC$_{50}$ values in toxicity studies with B. anthracis Sterne, indicating that they may have potential as lead compounds. Molecular modeling studies of the active compounds revealed that their binding patterns in BaTK and BaTMPK are similar to those of dTTP in hTK1 and dTMP in SaTMPK. Overall, a concept of novel antibiotics targeting BaTK and BaTMPK was
validated and two compounds (5.11 and 5.21) were selected as lead compounds for further structural optimization of this series.

5.6. Outlook

The biological evaluation of the twenty potential BaTK and/or BaTMPK inhibitors should be substantiated with enzyme inhibition studies including hTK1, BaTK, hTMPK and BaTMPK. Also, crystal structures of BaTK and BaTMPK have to be generated to replace the homology models of both enzymes used in our studies for the development of more accurate binding models of active compounds. Finally, further toxicity studies with active inhibitors, including additional time points between 6 and 20 h, have to be carried out to elucidate the observed phenomenon of deteriorating toxicities at 20 h. A final analysis of this study will be only possible when these additional experiments are accomplished.
CHAPTER 6

SUMMARY AND CONCLUSIONS

TK is a key nucleoside metabolizing enzyme in the DNA biosynthesis in many organisms. It is a promising molecular target for the development of anticancer drugs because it has a very high activity in all types of tumor cells but not in most normal resting cells. Also, the frequent occurrence of TK activity in \textit{B. anthracis} makes the enzyme a promising target for novel alternative antibiotics for anthrax infections. This dissertation aimed at two research goals: One was to develop hTK1-targeting BNCT agents to treat human GBM and the other was to discover novel \textit{BaTK/BaTMPK}-targeting antibiotics for anthrax infections.

GBM is one of the most malignant and aggressive of all tumors. Despite significant advances in the treatment of many other cancer types using surgery, chemotherapy, and radiation therapy, the survival of patients diagnosed with GBM has remained almost unchanged for decades. \textit{B. anthracis}, the agent that causes anthrax, is a Gram-positive, spore-forming, anaerobic bacterium. Due to the infectiousness of \textit{B. anthracis} spores by the respiratory route and the high mortality of inhalational anthrax, it can be utilized as a bioweapon. Therefore, both GBM and anthrax infections are major public health concerns in USA.
In our research related to the development of novel anticancer agents for the treatment of GBM, BNCT was chosen as the therapeutic modality. BNCT selectively kills tumor cells without damaging normal brain tissue by utilizing a nuclear reaction of boron-10 isotope \(^{10}\text{B}\) with thermal neutrons. Previous SAR studies of 3CTAs identified one promising boron-delivery agent designated N5-2OH. Two feasible synthetic routes for the preparation of \(^{10}\text{B}\)-enriched N5-2OH were developed from commercial sources, \(\text{o-carborane}\) and \(\text{decaborane}\). \(^{10}\text{B}\)-enriched N5-2OH was evaluated as a boron-delivery agent for BNCT in pilot neutron irradiation experiments at MIT. Treatment of rats, bearing intracerebral F98 glioma, with \(^{10}\text{B}\)-enriched N5-2OH and neutron irradiation resulted in prolonged survival compared with control groups, indicating that \(^{10}\text{B}\)-enriched N5-2OH has a significant potential as a BNCT agent for the treatment of human GBM.

Stereochemical- and geometrical N5-2OH isomers were synthesized and their substrate characteristics for hTK1 were evaluated to complete the SAR studies of the N5-2OH series. Stereochemical- and geometrical N5-2OH isomers had hTK1 PRs in the range of 32\% to 45\% and did not show any significant difference among the isomers. Binding modes of several geometrical N5-2OH isomers to the active site of hTK1 were elucidated by carrying out the docking studies of N5-2OH series with the homology model of hTK1 and the hTK1 crystal structure. The results showed that the bulky carborane cluster at the N-3 position of dThd projected towards the surface area rather than into the active site of an open form of hTK1 homology model, and thus, allowed effective phosphorylation.

To improve physicochemical properties (e.g. water solubility) of 3CTAs, novel zwitterionic \(\text{nido m-carboranyl} \) 3CTAs were synthesized and evaluated as boron-delivery
agents for BNCT. All of them were good substrates of hTK1 and showed balanced hydrophilicity/lipophilicity properties. One zwitterionic nido 3CTA was preferentially taken up in L929 (wt) tumors implanted in nude mice comparable to N5-2OH and showed significantly improved water solubility compared with N5-2OH. One zwitterionic nido m-carboranyl 3CTA was classified as a “2nd Generation 3CTA”, which has the potential to replace N5-2OH, the current lead compound of our 3CTA library.

The unique properties of the zwitterionic nido m-carborane system were investigated experimentally and theoretically. Chemical shifts for the nine boron atoms of the zwitterionic nido m-carborane cluster obtained at the B3LYP/6-31G* and B3LYP/6-31G** level showed strong correlations with the corresponding experimental values. Interestingly, it was found that the zwitterionic nido m-carborane system exhibited a unique reactivity with the carbonyl group of ketones or aldehydes, forming zwitterionic iminium-substituted carboranes without addition of acid catalyst. The iminium-substituted carborane exhibited a bathochromic shift in UV experiment due to the conjugation of σ-aromatic system of anionic nido m-carborane and the double bond of the positively charged iminium group. The observation was supported by the overlap of alternating-phase orbitals in HOMO and LUMO and by a small energy gap between HOMO and LUMO.

In the project related to the development of novel antibiotics, the compound library composed of known nucleoside-based antiviral and anticancer drugs was screened to identify a lead compound for the development of potential antibiotics for treatment of anthrax infections. PTAs showed that most of the tested dThd or dUrd analogues of this library were good substrates of BaTK as well as hTK1. However, in vitro toxicity studies
of this library with *B. anthracis* Sterne showed that most of these compounds are non-toxic except Fluorouridine, which inhibits TS in the *de novo* pathway of DNA biosynthesis, and FLT, which was active only at 20 h incubation.

Twenty dThd-based *Ba*TK/*Ba*TMPK inhibitors were designed and synthesized as potential *Ba*TK and/or *Ba*TMPK inhibitors. The inhibitor structures mimicked dTTP, the endogenous feedback inhibitor of *Ba*TK. The α-phosphate moiety of dTTP was replaced with sulfonamide, amide, urea, thiourea, or triazole group. Some of the urea-, thiourea-, and triazole-substituted dThd inhibitors exhibited relatively low IC$_{50}$ values at 6 h in toxicity studies with *B. anthracis* Sterne. However, most of them were inactive at 20 h incubation, indicating that they may be converted rapidly into inactive forms under *in vitro* toxicity assay conditions.

Overall, the development of novel hTK1-targeting boron-delivery agents for BNCT was successful and $^{10}$B-enriched N5-2OH showed highly promising results in preclinical neutron irradiation studies. Our design concept for the discovery of novel antibiotics targeting *Ba*TK and/or *Ba*TMPK was validated and two dTTP-mimicking inhibitors were selected as lead compounds for further structural optimization.
CHAPTER 7

EXPERIMENTAL SECTION

7.1. General Methods

$^1$H-NMR, $^{13}$C-NMR, and $^{11}$B-NMR spectra were obtained on Bruker 250, 300 or 400 MHz ($^1$H resonance frequency) FT-NMR instruments. Chemical shifts are reported in parts per million (ppm). The coupling constants are reported in Hertz (Hz). High resolution electrospray ionization (HR-ESI) mass spectra were recorded on a Micromass QTOF-Electrospray mass spectrometer and a 3-Tesla Finnigan FTMS-2000 Fourier Transform mass spectrometer at The Ohio State University Campus Chemical Instrumentation Center. Electron-impact (EI) mass spectra were obtained with a Kratos MS-25 mass spectrometer using 70 eV ionization conditions at The Ohio State University Department of Chemistry. IR spectra were recorded on a Protégé 460 IR Spectrometer using sodium chloride monocrystal discs. UV spectra were obtained on a Perkin-Elmer UV/VIS/NIS spectrometer. Optical rotations were measured on a Perkin-Elmer 241 automatic polarimeter. Compound visualization on Silica Gel 60 F$_{254}$ precoated TLC plates (0.25 mm layer thickness) (Merck, Darmstadt, Germany) was attained by UV light and KMnO$_4$ spray. Carborane-containing compounds were selectively visualized by spraying a solution of 0.06% PdCl$_2$ /1% aqueous HCl on TLC plates and subsequent
heating to ~ 120 °C. Reagent grade solvents were used for column chromatography using Silica gel 60, particle size 0.040-0.063 mm (Merck, New Jersey).

Analytical HPLC data of the target compounds were obtained with reversed phase C8 (RP-8) and C18 (RP-18) 250×4 LiChrosphere 100 Å [5 µm] columns (Merck,) using a Rainin HPLC instrument equipped with a Dynamax DA controller, HPXL pumps, and a Dynamax UV-1 detector (Rainin Instrument Company Inc., Woburn, MA, USA). HPLC grade water, methanol, and acetonitrile were used as solvents. Four gradient systems and two analytical columns were used as follows.

Method A: Water/methanol gradient (100:0 to 30:70 over 10 min, from 30:70 to 10:90 over 20 min, and from 10:90 to 0:100 over 10 min) with a flow rate of 1 mL/min was applied on RP-18 column.

Method B: Water/acetonitrile gradient (100:0 to 70:30 over 5 min, from 70:30 to 40:60 over 25 min, from 40:60 to 0:100 over 20 min) with a flow rate of 1 mL/min was applied on RP-18 column.

Method C: Water/methanol gradient (100:0 to 70:30 over 10 min, from 70:30 to 90:10 over 20 min, and from 90:10 to 0:100 over 10 min) with a flow rate of 1 mL/min was applied on RP-18 column.

Method D: Water/methanol gradient (100:0 to 70:30 over 10 min, from 70:30 to 90:10 over 20 min, and from 90:10 to 0:100 over 10 min) with a flow rate of 1 mL/min was applied on RP-8 column.

Reagent grade chemicals were obtained from commercial vendors and used as such. Decaborane (99.5% 10B-enriched) and o-carborane (98.0 % 10B-enriched) were purchased from Katchem (Prague, Czech Republic) and osmium tetroxide from Strem
Chemicals (Newburyport, MA). Anhydrous benzene and THF were obtained using distillation from sodium bezophenone ketyl prior to use. All reactions were carried out under argon atmosphere. *Caution: Decaborane is a highly toxic, impact sensitive compound, which forms explosive mixtures especially with halogenated materials. Osmium tetroxide is a highly toxic and flammable liquid. A careful study of the MSDS is advisable before usage of both chemicals.*

7.2. Chapter 3

![Chemical Structure](image)

(2,3-Propen-1-yl)-o-carborane (3.2.A)

To a solution of o-carborane (4.26 g, 30 mmol) in THF (250 mL) was added a solution of n-BuLi (12.6 mL, 31.5 mmol, 2.5 M solution in hexanes) at -78 °C. The reaction mixture was gradually warmed to room temperature and stirred for 1 h. Subsequently, the reaction mixture was cooled down to -78 °C and allyl bromide (2.85 mL, 33 mmol) was added slowly. The mixture was warmed to room temperature and stirred for additional 12 h. Distilled water (50 mL) was added and excess THF was removed under reduced pressure. The residue was extracted with ethylacetate (150 mL × 2), the combined organic layers were washed with d-HCl solution (100 mL) and brine (100 mL), and dried over magnesium sulfate. After filtration and evaporation, the residue was purified by silica gel column chromatography using hexanes as the eluent to give
compound 3.2.A (3.23 g, 59%). $R_f$ 0.50; $^1$H-NMR (CDCl$_3$) $\delta$ 2.94 (d, 2H, allyl-CH$_2$, $J = 7.5$ Hz), 3.56 (br s, 1H, H-C$_{carborane}$), 5.06-5.16 (dq, 1H, C=CH$_2$, $J = 16.8, 1.3$ Hz), 5.18-5.24 (dq, 1H, C=CH$_2$, $J = 10.0, 1.3$ Hz) 5.59-5.77 (m, 1H, CH=C); $^{13}$C-NMR (CDCl$_3$) $\delta$ 41.65 (CH$_2$), 59.44 (C$_{carborane}$-H), 73.50 (C$_{carborane}$-C), 121.02 (C=C), 131.19 (C=C); MS (HR-ESI) C$_5$H$_{16}$B$_{10}$Na (M+Na)$^+$ calcd 209.2080, found 209.2067.

(2,3-Propen-1-yl)-o-carborane (3.2.B)

Compound 3.2.B was prepared from $^{10}$B-enriched o-carborane in 75% yield adapting the procedure described for compound 3.2.A. $R_f$ 0.50 (only hexanes); $^1$H-NMR (CDCl$_3$) $\delta$ 2.93 (d, 2H, allyl-CH$_2$, $J = 7.5$ Hz), 3.55 (br s, 1H, H-C$_{carborane}$), 5.11 (dd, 1H, C=CH$_2$, $J = 16.8, 1.2$ Hz), 5.20 (d, 1H, C=CH$_2$, $J = 9.9$ Hz), 5.62-5.77 (m, 1H, CH=C); $^{13}$C-NMR (CDCl$_3$) $\delta$ 41.66 (CH$_2$), 59.43 (C$_{carborane}$-H), 73.50 (C$_{carborane}$-C), 121.02 (C=C), 131.21 (C=C); MS (HR-El) C$_5$H$_{16}$B$_{10}$ (M$^+$) calcd 176.2569, found 176.2567.

5-[2-(2,3-Propen-1-yl)-o-carboran-1-yl]pentyl tosylate (3.3.A)
To a solution of compound 3.2.A (1.20 g, 6.57 mmol) in benzene (30 mL) was added a solution of n-BuLi (2.89 mL, 7.23 mmol, 2.5 M in hexanes) at 10 °C over a period of 20 min. The solution was stirred at room temperature for 1 h and added dropwise to a solution of 1,5-pentanediol di-p-tosylate (3.25 g, 7.87 mmol) in benzene (20 mL) at 10 °C. The reaction mixture was stirred for 1 h at the same temperature, quenched with distilled water (20 mL) and extracted with ethylacetate (40 mL × 3). The combined organic layers were washed with brine (20 mL) and dried over magnesium sulfate. After filtration and evaporation, the residue was purified by silica gel column chromatography using hexanes/ethyl acetate (4:1) as the eluent to give compound 3.3.A (2.08 g, 70%). \( R_f \) 0.25; \(^1\)H-NMR (CDCl\(_3\)) \( \delta \) 1.29-1.49 (m, 4H, CH\(_2\)), 1.52-1.69 (m, 2H, CH\(_2\)), 2.09-2.12 (m, 2H, CH\(_2\)-C\(_{\text{carborane}}\)), 2.43 (s, 3H, CH\(_3\)), 2.89 (dt, 2H, allyl-CH\(_2\), \( J = 7.2, 1.2 \) Hz), 3.98 (t, 2H, CH\(_2\)OTs, \( J = 6.2 \) Hz), 5.07 (dq, 1H, C=CH\(_2\), \( J = 16.8, 1.3 \) Hz), 5.16 (dq, 1H, C=CH\(_2\), \( J = 10.0, 1.3 \) Hz), 5.64-5.81 (m, 1H, CH=C), 7.33 (d, 2H, ArH, \( J = 13.7 \) Hz), 7.76 (d, 2H, ArH, \( J = 13.7 \) Hz); \(^{13}\)C-NMR (CDCl\(_3\)) \( \delta \) 21.60 (CH\(_2\)), 25.04 (CH\(_2\)), 28.33 (CH\(_2\)), 28.83 (CH\(_2\)), 34.60 (CH\(_2\)), 39.28 (CH\(_2\)), 69.85 (O-CH\(_2\)), 77.97 (C\(_{\text{carborane}}\)-C), 78.89 (C\(_{\text{carborane}}\)-C), 119.45 (C=C), 127.78 (ArC), 129.86 (ArC), 132.50 (C=C), 132.93 (ArC), 144.87 (ArC); MS (HR-ESI) C\(_{17}\)H\(_{32}\)B\(_{10}\)O\(_3\)S\(_1\)Na (M+Na)\(^+\) calcd 447.2973, found 447.2983.

\[
\text{TsO} \quad \text{(3.3.A)}
\]

5-[2-(2,3-Propen-1-yl)-\( \sigma \)-carboran-1-yl]pentyl tosylate (3.3.B)
Compound 3.3.B was prepared from 3.2.B in 65% yield adapting the procedure described for compound 3.3.A. \( R_f \) 0.24 (hexanes/ethylacetate, 4:1); \(^1\)H-NMR (CDCl\(_3\)) \( \delta \) 1.23-1.36 (m, 2H, CH\(_2\)), 1.42-1.50 (m, 2H, CH\(_2\)), 1.60-1.67 (m, 2H, CH\(_2\)), 2.07-2.15 (m, 2H, CH\(_2\)-C\(_{\text{carborane}}\)), 2.43 (s, 3H, CH\(_3\)), 2.89 (d, 2H, allyl-C\(_2\) \( J = 7.1 \) Hz), 3.99 (t, 2H, CH\(_2\)OTs, \( J = 6.1 \) Hz), 5.07 (d, 1H, C=CH\(_2\), \( J = 16.9 \) Hz), 5.16 (d, 1H, C=CH\(_2\), \( J = 10.0 \) Hz), 5.68-5.77 (m, 1H, CH=C), 7.33 (d, 2H, ArH, \( J = 8.0 \) Hz), 7.76 (d, 2H, ArH, \( J = 8.0 \) Hz); \(^{13}\)C-NMR (CDCl\(_3\)) \( \delta \) 21.66 (CH\(_2\)), 25.11 (CH\(_2\)), 28.39 (CH\(_2\)), 28.88 (CH\(_2\)), 34.67 (CH\(_2\)), 39.36 (CH\(_2\)), 69.84 (O-CH\(_2\)), 77.97 (C\(_{\text{carborane}}\)-C), 78.85 (C\(_{\text{carborane}}\)-C), 119.47 (C=C), 127.85 (ArC), 129.89 (ArC), 132.58 (C=C), 132.96 (ArC), 144.90 (ArC); MS (HR-ESI) C\(_{17}H\(_{32}\)B\(_{10}\)O\(_3\)S\(_1\)Na (M+Na)+ calcd 439.3264, found 439.3271.

3-{5-[2-(2,3-Propen-1-yl)-o-carboran-1-yl]pentan-1-yl}thymidine (3.4.A)

To a solution of compound 3.3.A (1.98 g, 4.66 mmol) in DMF/acetone (1:1, 40 mL) were added dThd (2.82 g, 11.64 mmol) and potassium carbonate (2.57 g, 18.59 mmol). The mixture solution was stirred for 96 h at 35 °C, filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography using dichloromethane/acetone (4:1) as the eluent to give compound 3.4.A (1.21 g, 52 %). \( R_f \) 0.21; \(^1\)H-NMR (CD\(_3\)OD) \( \delta \) 1.31-1.40 (m, 2H, CH\(_2\)), 1.52-1.67 (m, 4H, CH\(_2\)), 1.89 (d,
3H, CH₃, J = 1.2 Hz), 2.11-2.31 (m, 4H, H-2´ and CH₂-C_carborane), 3.05 (dt, 2H, allyl-CH₂, J = 7.2 Hz, 1.2 Hz), 3.71 (dd, 1H, H-5´, J = 12.0, 3.1 Hz), 3.79 (dd, 1H, H-5´, J = 12.00, 3.7 Hz), 3.87-3.93 (m, 3H, H-4´ and CH₂) 4.36-4.41 (m, 1H, H-3´), 5.12-5.16 (m, 1H, C=CH₂), 5.19-5.20 (m, 1H, C=CH₂) 5.72-5.86 (m, 1H, CH=CH) 6.29 (t, 1H, H-1´, J = 6.9 Hz), 7.82 (d, 1H, H-6, J = 1.2 Hz); ¹³C-NMR (CD₃OD) δ 13.23 (CH₃), 27.21 (CH₂), 27.88 (CH₂), 30.32 (CH₂), 35.62 (CH₂), 40.08 (CH₂), 41.34 (CH₂), 41.85 (CH₂), 62.75 (C-5´), 72.09 (C-3´), 80.03 (C_carborane-C), 81.28 (C_carborane-C), 87.07 (C-1´), 88.85 (C-4´), 110.68 (C-5) 119.92 (C=C), 134.31(C=C), 136.43 (C-6), 152.28 (C-2), 165.34 (C-4); MS (HR-ESI) C₂₀H₃₈B₁₀N₂O₅Na (M+Na)⁺ calcd 517.3676, found 517.3651.

3-{5-[2-(2,3-Propen-1-yl)-o-carboran-1-yl]pentan-1-yl}thymidine (3.4.B)

Compound 3.4.B was prepared from 3.3.B in 79% yield adapting the procedure described for compound 3.4.A. ¹H-NMR (CD₃OD) δ 1.31-1.38 (m, 2H, CH₂), 1.54-1.65 (m, 4H, CH₂), 1.89 (d, 3H, CH₃, J = 1.1 Hz), 2.12-2.30 (m, 4H, H-2´ and CH₂-C_carborane), 3.05 (d, 2H, allyl-CH₂, J = 7.3 Hz), 3.71 (dd, 1H, H-5´, J = 12.0, 3.1 Hz), 3.79 (dd, 1H, H-5´, J = 12.00, 3.6 Hz), 3.88-3.92 (m, 3H, H-4´ and CH₂), 4.37-4.40 (m, 1H, H-3´), 5.14-5.19 (m, 2H, C=CH₂) 5.76-5.86 (m, 1H, CH=C), 6.29 (t, 1H, H-1´, J = 6.9 Hz), 7.83 (d, 1H, H-6, J = 1.1 Hz); ¹³C NMR (CD₃OD)
δ 13.22 (CH₃), 27.21 (CH₂), 27.88 (CH₂), 30.33 (CH₂), 35.63 (CH₂), 40.09 (CH₂), 41.34 (CH₂), 41.84 (CH₂), 62.76 (C-5´), 72.11 (C-3´), 80.08 (C_carborane-C), 81.32 (C_carborane-C), 87.08 (C-1´), 88.87 (C-4´), 110.70 (C-5) 119.90 (C=C), 134.35 (C=C), 136.45 (C-6), 152.31 (C-2), 165.38 (C-4); MS (HR-ESI) C₂₀H₃₈B₁₀N₂O₅Na (M+Na)+ calcd 509.3972, found 509.3969.

3-{5-[2-(2,3-Dihydroxyprop-1-yl)-o-carboran-1-yl]pentan-1-yl}thymidine (3.5.A)

To a solution of compound 3.4.A (980 mg, 1.98 mmol) and NMO (500 mg, 4.26 mmol) in 1,4-dioxane (20 mL) was added an aqueous solution of OsO₄ (0.1 g in 10 mL H₂O). The reaction mixture was protected from light by covering the reaction flask with aluminum foil, stirred at room temperature for 6 h, quenched with a concentrated aqueous solution of Na₂S₂O₃ (500 mg, 3.16 mmol), and excess solvents were removed under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane/acetone (1:1) as the eluent to give compound 3.5.A (400 mg, 40 %). R_f 0.30; [α]D²⁵ -5.1 (c = 0.10; MeOH); ¹H-NMR (CD₃OD) δ 1.28-1.39 (m, 2H, CH₂), 1.52-1.67 (m, 4H, CH₂), 1.89 (d, 3H, CH₃, J = 1.2 Hz), 2.12-2.36 (m, 5H, H-2´ and CH₂-C_carborane, and CH(OH)-CH₂-C_carborane), 2.56 (dd, 1H, CH(OH)-CH₂-C_carborane, J = 15.8, 1.7 Hz), 3.33 (dd, 1H, CH₂OH, J = 11.0, 6.5 Hz) 3.47 (dd, 1H, CH₂OH, J = 11.0, 5.3 Hz), 3.71 (dd, 1H, H-5´, J = 12.0, 3.7 Hz), 3.74-3.80 (m, 1H, CH(OH)-CH₂OH), 3.80 (dd, 1H,
H-5′, \( J = 12.0, 3.1 \) Hz), 3.87-3.93 (m, 3H, H-4′ and CH\(_2\)N), 4.36-4.41 (m, 1H, H-3′), 6.29 (t, 1H, H-1′, \( J = 6.9 \) Hz), 7.83 (d, 1H, H-6, \( J = 1.2 \) Hz); \(^{13}\)C-NMR (CD\(_3\)OD) \( \delta \) 13.23 (CH\(_3\)), 27.29 (CH\(_2\)), 27.90 (CH\(_2\)), 30.38 (CH\(_2\)), 35.80 (C-C\(_{\text{carborane}}\)), 39.89 (C-C\(_{\text{carborane}}\)), 41.34 (C-2′), 41.92 (CH\(_2\)N), 62.76 (C-5′), 66.91 (O-CH\(_2\)), 72.11 (O-CH), 72.21 (C-3′), 80.44 (C\(_{\text{carborane}}\)-C), 81.89 (C\(_{\text{carborane}}\)-C), 87.10 (C-1′), 88.87 (C-4′), 110.71 (C-5), 136.47 (C-6), 152.33 (C-2), 165.43 (C-4); \(^{11}\)B-NMR (CD\(_3\)OD) \( \delta \) -7.4 (8B), -1.9 (2B); IR 1054, 1099, 1269, 1472, 1627, 1666, 2580 (B-H), 2935, 3405; MS (HR-ESI) C\(_{20}\)H\(_{40}\)B\(_{10}\)N\(_2\)O\(_7\)Na (M+Na\(^+\)) calcd 551.3747, found 551.3728; HPLC retention time: 26.9 min (Method A), 21.1 min (Method B).

\[
\text{HO} \quad \text{N} \quad \text{N} \\
\text{O} \quad \text{O} \quad \text{O} \\
\text{OH} \quad \text{OH} \quad \text{OH} \\
\sigma ^{10}\text{B-H}
\]

3-{5-[2-(2,3-Dihydroxyprop-1-yl)-o-carboran-1-yl]pentan-1-yl}thymidine (3.5.B)

Compound 3.5.B was prepared from 3.4.B in 40% yield adapting the procedure described for compound 3.5.A. \( R_f \) 0.30 (dichloromethane/acetone, 1:1); \(^1\)H-NMR (CD\(_3\)OD) \( \delta \) 1.28-1.39 (m, 2H, CH\(_2\)), 1.52-1.67 (m, 4H, CH\(_2\)), 1.89 (d, 3H, CH\(_3\), \( J = 1.1 \) Hz), 2.17-2.39 (m, 5H, H-2′ and CH\(_2\)-C\(_{\text{carborane}}\), and CH(OH)-CH\(_2\)-C\(_{\text{carborane}}\)), 2.56 (dd, 1H, CH(OH)-CH\(_2\)-C\(_{\text{carborane}}\), \( J = 15.8, 1.6 \) Hz), 3.33 (dd, 1H, CH\(_2\)OH, \( J = 11.0, 6.5 \) Hz), 3.46 (dd, 1H, CH\(_2\)OH, \( J = 11.0, 5.3 \) Hz), 3.72 (dd, 1H, H-5′, \( J = 12.0, 3.7 \) Hz), 3.76-3.80 (m, 1H, CH(OH)-CH\(_2\)OH), 3.79 (dd, 1H, H-5′, \( J = 12.0, 3.1 \) Hz), 3.81-3.92 (m, 3H, H-4′ and}
CH$_2$N), 4.37-4.40 (m, 1H, H-3’), 6.30 (t, 1H, H-1’, $J$ = 6.7 Hz), 7.83 (d, 1H, H-6, $J$ = 1.1 Hz); $^{13}$C-NMR (CD$_3$OD) $\delta$ 13.21 (CH$_3$), 27.29 (CH$_2$), 27.91 (CH$_2$), 30.39 (CH$_2$), 35.83 (CH$_2$), 39.92 (CH$_2$), 41.34 (CH$_2$), 41.92 (CH$_2$), 62.77 (C-5’), 66.92 (O-CH$_2$), 72.13 (O-CH$_2$), 72.23 (C-3’), 80.49 (C$_{carborane}$-C), 81.94 (C$_{carborane}$-C), 87.11 (C-1’), 88.89 (C-4’), 110.72 (C-5), 136.49 (C-6), 152.34 (C-2), 165.45 (C-4); IR 1054, 1097, 1267, 1470, 1625, 1664, 1690, 2587 (B-H), 2926, 3385; MS (HR-ESI) C$_{20}$H$_{40}$B$_{10}$N$_2$O$_7$Na (M+Na)$^+$ calcd 543.4027, found 543.4003; HPLC retention time: 26.5 min (Method A), 21.0 min (Method B).

**tert-Butyldimethylsilyl-o-carborane (3.6)**

Method I: Decaborane (635 mg, 5.21 mmol) was dissolved in acetonitrile (10 mL) and refluxed at 80 °C for 30 min. The solution turned yellow due to the formation of the decaborane-acetonitrile adducts. To this solution was added tert-butyldimethylsilylacetylene (0.75 mL, 3.85 mmol) in toluene (10 mL) and the resulting reaction mixture was refluxed for 24 h. The dark brown solution was cooled to room temperature and methanol (1 mL) was added. After evaporation, the residue was purified by silica gel column chromatography using hexanes as the eluent to give the compound 3.6 (299 mg, 23%).

Method II: To a solution of 1-butyl-3-methylimidazolium chloride (990 mg) in toluene (10 mL) was added decaborane (122 mg, 1 mmol) and tert-butyldimethylsilylacetylene
(0.56 mL, 3 mmol) at 120 °C under argon atmosphere. The mixture was stirred vigorously at the same temperature for 10 min. After removal of toluene, the residue was purified by silica gel column chromatography using hexane as the eluent to give the compound 3.6 (125 mg, 50%). \( R_f \) 0.50 (100% pentane); \(^1\)H-NMR (CDCl\textsubscript{3}) \( \delta \) 0.91 (s, 6H, -Si(CH\textsubscript{3})\textsubscript{2}), 1.22 (s, 9H, C(CH\textsubscript{3})\textsubscript{3}), 3.56 (br s, 1H, H-C\textsubscript{carborane}); \(^{13}\)C-NMR (CDCl\textsubscript{3}) \( \delta \) -5.34 (CH\textsubscript{3}), 13.21 (CH\textsubscript{3}), 28.87 (CH\textsubscript{2}), 59.44 (C\textsubscript{carborane}-H), 73.50 (C\textsubscript{carborane}-C); MS(HR-EI) C\textsubscript{8}H\textsubscript{26}B\textsubscript{10}Si (M\textsuperscript{+}) calcd 250.8765, found 250.8654.

![Chemical structure](image)

\( \text{Si} \quad \circ: ^{10}\text{B-H} \)

### 1-(\textit{tert-Butyldimethylsilyl})-2-(2,3-propen-1-yl)-\( \alpha \)-carborane (3.7)

To a solution of 3.6 (290 mg, 1.16 mmol) in THF (50 mL) was added n-BuLi (0.464 mL, 1.16 mmol, 2.5 M solution in hexanes) at -78 °C over a period of 10 min. The solution was gradually warmed to room temperature and was stirred for 1 h. Allyl bromide (0.10 mL, 1.2 mmol) was added to the solution at -78 °C and the reaction mixture was stirred at room temperature for 12 h. Distilled water (50 mL) was added and THF was removed under reduced pressure. The residue was extracted with ethylacetate (30 mL × 4). The combined organic layers were washed with d-HCl solution (100 mL) and brine (100 mL), and dried over MgSO\textsubscript{4}. After filtration and evaporation, the residue was purified by silica gel column chromatography using hexanes as the eluent to give compound 3.7 (201 mg, 60 %). \( R_f \) 0.60 (100% hexanes); \(^1\)H-NMR (CDCl\textsubscript{3}) \( \delta \) 0.92 (s, 6H, 128
-Si(CH₃)₂), 1.23 (s, 9H, C(CH₃)₃), 2.93 (d, 2H, allyl-CH₂, J = 7.5 Hz), 5.06-5.16 (m, 1H, C=CH₂), 5.18-5.24 (m, 1H, C=CH₂), 5.59-5.77 (m, 1H, CH=C); ¹³C-NMR (CDCl₃) δ -5.28 (CH₃), 13.21 (CH₃), 21.90 (CH₃), 28.87 (CH₂), 41.65 (CH₂), 72.76 (C₉-carborane-C), 73.50 (C₉-carborane-C), 121.02 (C=C), 131.19 (C=C); MS (HR-EI) C₁₁H₃₀B₁₀Si (M⁺) calcd 290.2080, found 290.2067.

![Structure of (2,3-Propen-1-yl)-o-carborane (3.2.B)](image)

(2,3-Propen-1-yl)-o-carborane (3.2.B)

To a solution of compound 3.7 (170 mg, 0.96 mmol) in THF (10 mL) was added TBAF (1.5 mL, 1.0 M in THF) at -78 °C. The mixture was stirred at room temperature for 2 h, and then poured into ice-water (30 mL), extracted with diethyl ether (30 mL × 3). The combined organic layers were washed with brine and dried over MgSO₄. After filtration and evaporation, crude compound 3.2.B (135 mg, 80%) was obtained. Analytical data were identical to those for the 3.2.B preparation described above.

![Structure of (R)-4-(o-Carboran-1-yl)methyl-2,2-dimethyl-1,3-dioxolane (3.8.A)](image)

(R)-4-(o-Carboran-1-yl)methyl-2,2-dimethyl-1,3-dioxolane (3.8.A)

To a solution of o-carborane (213 mg, 1.5 mmol) in benzene (5 mL) was added a solution of n-BuLi (0.66 mL, 1.65 mmol, 2.5 M solution in hexanes) at 0 °C. The
solution was first stirred for 30 min at 0 °C and subsequently for 30 min at room temperature. The reaction mixture was again cooled to 5 °C and (S)-(+) -2,2-dimethyl-1,3-dioxolane-4-ylmethyl p-tosylate (429 mg, 1.5 mmol) in benzene (10 mL) was added dropwise. The reaction mixture was stirred at room temperature for 14 h. Subsequently, distilled water (10 mL) was added and excess benzene was removed under reduced pressure. The residue was extracted with ethylacetate (50 mL), the organic layer was washed with d-HCl solution (20 mL) and brine (20 mL) and dried over MgSO4. After filtration and evaporation, the residue was purified by silica gel columns chromatography using hexanes/ethylacetate (25:1) as the eluent to give compound 3.8.A (242 mg, 63%).

Rf 0.12; 1H-NMR (CDCl3) δ 1.31 (s, 3H, CH3), 1.37 (s, 3H, CH3), 2.39 (dd, 1H, CH2, J = 15.1, 3.4 Hz), 2.47 (dd, 1H, CH2, J = 15.1, 9.1 Hz), 3.47 (dd, 1H, CH2, J = 8.4, 5.9 Hz), 4.02 (br s, 1H, H-Ccarborane), 4.05 (dd, 1H, CH2, J = 8.4, 6.2 Hz), 4.17-4.23 (m, 1H, CH);

13C-NMR (CDCl3) δ 25.4 (CH3), 26.9 (CH3), 42.0 (CH2-Ccarborane), 59.8 (H-C-carborane), 68.7 (CH2), 72.4 (CH2-C-carborane), 73.8 (CH), 110.2 [C(CH3)2]

(S)-4-((o-Carboran-1-yl)methyl-2,2-dimethyl-1,3-dioxolane (3.8.B)

Compound 3.8.B was prepared from (R)-(-)-2,2-dimethyl-1,3-dioxolane-4-ylmethyl p-tosylate in 60% yield adapting the procedure described for compound 3.8.A.

Rf 0.12 (hexanes/ethylacetate, 25:1); 1H-NMR (CDCl3) δ 1.31 (s, 3H, CH3), 1.37 (s, 3H, CH3), 2.39 (dd, 1H, CH2, J = 15.1, 3.5 Hz), 2.47 (dd, 1H, CH2, J = 15.1, 9.1 Hz), 3.47 (dd,
1H, CH2 J = 8.4, 5.9 Hz), 4.02 (br s, 1H, H-C_{carborane}), 4.05 (dd, 1H, CH2, J = 8.4, 6.2 Hz), 4.18-4.23 (m, 1H, CH); $^{13}$C-NMR (CDCl$_3$) δ 25.4 (CH$_3$), 26.9 (CH$_3$), 42.0 (CH$_2$-C_{carborane}), 59.8 (H-C_{carborane}), 68.7 (CH$_2$), 72.4 (CH$_2$-C_{carborane}), 73.8 (CH), 110.2 [C(CH$_3$)$_2$]

4-($o$-Carboran-1-yl)methyl-2,2-dimethyl-1,3-dioxolane (3.8.C)

Compound 3.8.C was prepared from 2,2-dimethyl-1,3-dioxolane-4-ylmethyl p-tosylate in 65% yield adapting the procedure described for compound 3.8.A. $R_f$ 0.12 (hexanes/ethylacetate, 25:1); $^1$H-NMR (CDCl$_3$) δ 1.31 (s, 3H, CH$_3$), 1.36 (s, 3H, CH$_3$), 2.38 (dd, 1H, CH$_2$, J = 15.2, 3.9 Hz), 2.47 (dd, 1H, CH$_2$, J = 15.2, 8.8 Hz), 3.47 (dd, 1H, CH$_2$, J = 8.4, 5.9 Hz), 4.02 (br s, 1H, H-C_{carborane}), 4.05 (dd, 1H, CH$_2$, J = 8.4, 6.1 Hz), 4.18-4.23 (m, 1H, CH); $^{13}$C-NMR (CDCl$_3$) δ 25.8 (CH$_3$), 27.3 (CH$_3$), 42.4 (CH$_2$-C_{carborane}), 60.3 (H-C_{carborane}), 69.1 (CH$_2$), 72.8 (CH$_2$-C_{carborane}), 74.2 (CH), 110.6 [C(CH$_3$)$_2$]

4-($m$-Carboran-1-yl)methyl-2,2-dimethyl-1,3-dioxolane (3.8.D)

Compound 3.8.D was prepared from $m$-carborane and 2,2-dimethyl-1,3-dioxolane-4-ylmethyl p-tosylate in 59% yield adapting the procedure described for compound 3.8.A. $R_f$ 0.16 (hexanes/ethylacetate, 15:1); $^1$H-NMR (CDCl$_3$) δ 1.30 (s, 3H, CH$_3$), 2.42 (dd, 1H, CH$_2$, J = 15.2, 4.3 Hz), 2.57 (dd, 1H, CH$_2$, J = 15.2, 8.8 Hz), 3.47 (dd, 1H, CH$_2$, J = 8.4, 5.9 Hz), 4.02 (br s, 1H, H-C_{carborane}), 4.05 (dd, 1H, CH$_2$, J = 8.4, 6.2 Hz), 4.18-4.23 (m, 1H, CH); $^{13}$C-NMR (CDCl$_3$) δ 25.4 (CH$_3$), 26.9 (CH$_3$), 42.0 (CH$_2$-C_{carborane}), 59.8 (H-C_{carborane}), 68.7 (CH$_2$), 72.4 (CH$_2$-C_{carborane}), 73.8 (CH), 110.2 [C(CH$_3$)$_2$]
CH₃), 1.34 (s, 3H, CH₃), 2.07 (dd, 1H, CH₂-Ccarborane, J = 14.7, 5.3 Hz), 2.29 (dd, 1H, CH₂-Ccarborane, J = 14.7, 5.6 Hz), 2.92 (br s, 1H, H-Ccarborane), 3.42-3.48 (m, 1H, CH₂), 4.01-4.07 (m, 2H); ¹³C-NMR (CDCl₃) δ 25.35 (CH₃), 26.86 (CH₃), 41.18 (CH₂-Ccarborane), 55.26 (H-Ccarborane), 69.00 (CH₂), 72.59 (CH₂-Ccarborane), 74.66 (CH), 109.13 [C(CH₃)₂]

![Structure](image)

4-(p-Carboran-1-yl)methyl-2,2-dimethyl-1,3-dioxolane (3.8.E)

Compound 3.8.E was prepared from p-carborane and 2,2-dimethyl-1,3-dioxolane-4-ylmethyl p-tosylate in 50% yield adapting the procedure described for compound 3.8.A. 

Rₛ 0.18 (hexanes/ethylacetate, 15:1); ¹H-NMR (CDCl₃) δ 1.26 (s, 3H, CH₃), 1.29 (s, 3H, CH₃), 1.76 (dd, 1H, CH₂-Ccarborane, J = 14.9, 6.8 Hz), 1.98 (dd, 1H, CH₂-Ccarborane, J = 14.9, 5.4 Hz), 2.63 (br s, 1H, H-Ccarborane), 3.34 (dd, 1H, CH₂, J = 8.1, 5.9 Hz), 3.81-3.86 (m, 1H, CH), 3.95 (dd, 1H, CH₂, J = 8.1, 5.9 Hz); ¹³C-NMR (CDCl₃) δ 25.71 (CH₃), 27.21 (CH₃), 43.42 (CH₂-Ccarborane), 59.24 (H-Ccarborane), 69.42 (CH₂), 74.81 (CH), 81.33 (CH₂-Ccarborane), 109.18 [C(CH₃)₂]
(R)-5-[2-(2,3-Isopropylidenedioxyprop-1-yl)-o-carboran-1-yl]pentyl tosylate (3.9.A)

To a solution of compound 3.8.A (129 mg, 0.5 mmol) in benzene (5 mL) was added n-BuLi (0.22 mL, 0.55 mmol, 2.5 M solution in hexanes) at 5 °C over a period of 20 min. The solution was first stirred at the same temperature for 30 min and then at room temperature for 30 min. The solution was then slowly added to a solution of 1,5-pentanediol di-p-tosylate (330 mg, 0.80 mmol) in benzene (10 mL) at 5 °C (ice bath). The reaction mixture was stirred for 1 h at the same temperature. Distilled water (5 mL) was added and the reaction mixture was extracted with ethylacetate (15 mL × 3). The combined organic layers were washed with brine (20 mL) and dried over magnesium sulfate. After filtration and evaporation, the residue was purified by silica gel column chromatography using hexanes/ethylacetate (4:1) as the eluent to give compound 3.9.A (144 mg, 58 %). Rf 0.21; ¹H-NMR (CDCl₃) δ 1.31 (s, 3H, CH₃), 1.35 (s, 3H, CH₃), 1.41-1.51 (m, 2H, CH₂), 1.60-1.67 (m, 2H, CH₂), 2.06-2.23 (m, 4H, CH₂ and CH₂-C(carborane), 2.34 (dd, 1H, CH₂-C(carborane, J = 15.5, 5.1 Hz), 2.41 (dd, J = 15.5, 6.5 Hz), 2.43 (s, 3H, CH₃), 3.54 (dd, 1H, CH₂, J = 8.3, 6.5 Hz), 3.99 (t, 2H, OCH₂, J = 6.2 Hz), 4.11 (dd, 1H, CH₂, J = 8.3, 6.0 Hz), 4.19-4.25 (m, 1H, CH), 7.32 (d, 2H, ArH, J = 8.1 Hz), 7.75 (d, 2H, ArH, J = 8.1 Hz); ¹³C-NMR (CDCl₃) δ 21.62 (CH₃), 25.05 (CH₃), 25.25 (CH₃), 26.79 (CH₂), 28.35 (CH₂), 28.87 (CH₂), 34.81 (CH₂-C(carborane), 39.40 (CH₂-C(carborane), 69.04 (CH₂), 69.81 (CH₂OTs), 74.39 (CH), 76.86 (C-C(carborane), 79.62 (C-C(carborane), 109.54 [C(CH₃)₂), 127.80 (ArC), 129.87 (ArC), 132.90 (ArC), 144.86 (ArC); MS (HR-ESI) C₂0H₃₈B₁₀O₅S₁Na (M+Na)⁺ calcd 521.3352, found 521.3347.
(S)-5-[2-(2,3-Isopropylidenedioxyprop-1-yl)-6-carboran-1-yl]pentyl tosylate (3.9.B)

Compound 3.9.B was prepared from 3.8.B in 62% yield adapting the procedure described for compound 3.9.A. $R_f$ 0.21 (hexanes/ethylacetate, 4:1); $^1$H-NMR (CDCl$_3$) $\delta$ 1.32 (s, 3H, CH$_3$), 1.36 (s, 3H, CH$_3$), 1.42-1.51 (m, 2H, CH$_2$), 1.61-1.68 (m, 2H, CH$_2$), 2.02-2.23 (m, 4H, CH$_2$ and CH$_2$-C$_{carborane}$), 2.34 (dd, 1H, CH$_2$-C$_{carborane}$, $J$ = 15.4, 5.2 Hz), 2.42 (dd, 1H, CH$_2$-C$_{carborane}$, $J$ = 15.4, 6.5 Hz), 2.45 (s, 3H, CH$_3$), 3.54 (dd, 1H, CH$_2$, $J$ = 8.3, 6.4 Hz), 3.99 (t, 2H, OCH$_2$, $J$ = 6.2 Hz), 4.12 (dd, 1H, CH$_2$, $J$ = 8.3, 6.0 Hz), 4.19-4.25 (m, 1H, CH), 7.32 (d, 2H, ArH, $J$ = 8.1 Hz), 7.75 (d, 2H, ArH, $J$ = 8.1 Hz); $^{13}$C-NMR (CDCl$_3$) $\delta$ 21.64 (CH$_3$), 25.07 (CH$_3$), 25.26 (CH$_3$), 26.81 (CH$_2$), 28.37 (CH$_2$), 28.89 (CH$_2$), 34.83 (CH$_2$-C$_{carborane}$), 39.42 (CH$_2$-C$_{carborane}$), 69.07 (CH$_2$), 69.81 (CH$_2$OTs), 74.41 (CH), 76.84 (C-C$_{carborane}$), 79.62 (C-C$_{carborane}$), 109.55 [C(CH$_3$)$_2$], 127.82 (ArC), 129.87 (ArC), 132.92 (ArC), 144.87 (ArC); MS (HR-ESI) C$_{20}$H$_{38}$B$_{10}$O$_5$S$_1$Na (M+Na)$^+$ calcd 521.3352, found 521.3345.

5-[2-(2,3-Isopropylidenedioxyprop-1-yl)-6-carboran-1-yl]pentyl tosylate (3.9.C)

Compound 3.9.C was prepared from 3.8.C in 60% yield adapting the procedure described for compound 3.9.A. $R_f$ 0.21 (hexanes/ethylacetate, 4:1); $^1$H-NMR (CDCl$_3$) $\delta$ 134
1.32 (s, 3H, CH₃), 1.36 (s, 3H, CH₃), 1.42-1.51 (m, 2H, CH₂), 1.61-1.68 (m, 2H, CH₂),
2.02-2.23 (m, 4H, CH₂ and CH₂-C(carborane)), 2.34 (dd, 1H, CH₂-C(carborane), \( J = 15.4 \), 5.2 Hz),
2.42 (dd, 1H, CH₂-C(carborane), \( J = 15.4 \), 6.4 Hz), 2.44 (s, 3H, CH₃), 3.55 (dd, 1H, CH₂, \( J = 8.2 \), 6.5 Hz), 3.99 (t, 2H, OCH₂, \( J = 6.1 \) Hz), 4.12 (dd, 1H, CH₂, \( J = 8.2 \), 6.1 Hz), 4.20-4.26 (m, 1H, CH), 7.33 (d, 2H, ArH, \( J = 8.1 \) Hz), 7.76 (d, 2H, ArH, \( J = 8.1 \) Hz); ¹³C-
NMR (CDCl₃) \( \delta \) 21.7 (CH₃), 25.0 (CH₃), 25.3 (CH₃), 26.8 (CH₂), 28.7 (CH₂), 30.9 (CH₂),
34.8 (CH₂-C(carborane), 39.5 (CH₂-C(carborane), 69.1 (CH₂), 70.8 (CH₂OTs), 74.5 (CH), 109.6
[CH(CH₃)₂], 127.8 (ArC), 129.9 (ArC), 133.0 (ArC), 144.9 (ArC); MS (HR-ESI)
C₂₀H₃₈B₁₀O₅S₁Na (M+Na)+ calcd 521.3352, found 521.3365.

5-[2-(2,3-Isopropylidenedioxyprop-1-yl)-m-carboran-1-yl]pentyl tosylate (3.9.D)

Compound 3.9.D was prepared from 3.8.D in 64% yield adapting the procedure
described for compound 3.9.A. \( R_f \) 0.21 (hexanes/ethylacetate, 4:1); ¹H-NMR (CDCl₃) \( \delta \)
1.20-1.28 (m, 4H, CH₂), 1.30 (s, 3H, CH₃), 1.34 (s, 3H, CH₃), 1.55-1.60 (m, 2H, CH₂),
1.81-1.85 (m, 2H, CH₂-C(carborane), 2.04 (dd, 1H, CH₂-C(carborane), \( J = 14.7 \), 5.4 Hz), 2.27 (dd,
1H, CH₂-C(carborane), \( J = 14.7 \), 5.6 Hz), 2.44 (s, 3H, CH₃), 3.42-3.48 (m, 1H, CH₂), 3.97 (t,
2H, OCH₂, \( J = 6.3 \) Hz), 4.00-4.05 (m, 2H), 7.32 (d, 2H, ArH, \( J = 8.2 \) Hz), 7.75 (d, 2H,
ArH, \( J = 8.2 \) Hz); ¹³C-NMR (CDCl₃) \( \delta \) 21.64 (CH₃), 24.67 (CH₂), 25.36 (CH₃), 26.88
(CH₃), 28.38 (CH₂), 29.16 (CH₂), 36.62 (CH₂-C(carborane), 41.18 (CH₂-C(carborane), 69.02
(CH₃), 70.2 (CH₂OTs), 72.06 (C-C₃carborane), 74.65 (CH), 76.06 (C-C₃carborane), 109.08 (C(CH₃)₂), 127.84 (Ar-C), 129.84 (Ar-C), 133.01 (Ar-C), 144.78 (Ar-C); MS (HR-ESI) C₂₀H₃₈B₁₀O₅S₁Na (M+Na)⁺ calcd 521.3352, found 521.3336.

5-[2-(2,3-Isopropylidenedioxyprop-1-yl)-p-carboran-1-yl]pentyloxytosylate (3.9.E)

Compound 3.9.E was prepared from 3.8.E in 65% yield adapting the procedure described for compound 3.9.A. R₇ 0.21 (hexanes/ethylacetate, 4:1); ¹H-NMR (CDCl₃) δ 0.99-1.13 (m, 4H, CH₂), 1.26 (s, 3H, CH₃), 1.29 (s, 3H, CH₃), 1.48-1.55 (m, 4H, CH₂ and CH₂-C₃carborane), 1.75 (dd, 1H, CH₂-C₃carborane, J = 14.9, 7.1 Hz), 1.98 (dd, 1H, CH₂-C₃carborane, J = 14.9, 5.4 Hz), 2.43 (s, 3H, CH₃), 3.34 (dd, 1H, CH₂-C₃carborane, J = 8.2, 6.1 Hz), 3.78-3.86 (m, 1H, CH), 3.92 (t, 2H, OCH₂, J = 6.4 Hz), 3.99-4.03 (m, 1H, CH₂), 7.32 (d, 2H, ArH, J = 8.2 Hz), 7.75 (d, 2H, ArH, J = 8.2 Hz); ¹³C-NMR (CDCl₃) δ 21.63 (CH₃), 24.82 (CH₂), 25.30 (CH₃), 26.81 (CH₃), 28.66 (CH₂), 30.90 (CH₂), 37.45 (CH₂-C₃carborane), 41.32 (CH₂-C₃carborane), 69.04 (CH₂), 70.03 (CH₂OTs), 74.55 (CH), 75.12 (C-C₃carborane), 78.15 (C-C₃carborane), 108.78 (C(CH₃)₂), 127.84 (Ar-C), 129.83 (Ar-C), 133.13 (Ar-C), 144.73 (Ar-C); MS (HR-ESI) C₂₀H₃₈B₁₀O₅S₁Na (M+Na)⁺ calcd 521.3352, found 521.3339.
(R)-3-{5-[2-(2,3-Isopropylidenedioxyprop-1-yl)-o-carboran-1-yl]pentan-1-yl}thymidine (3.10.A)

To a solution of compound 3.9.A (125 mg, 0.25 mmol) in a mixture of DMF and acetone (10 mL, 1:1) was added dThd (150 mg, 0.62 mmol) and potassium carbonate (150 mg, 1.13 mmol). The reaction mixture was stirred at 50 °C for 48 h. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel columns chromatography using ethylacetate/methanol (20:1) as the eluent to give compound 3.10.A (88 mg, 62%). $R_f\ 0.29; ^1H-NMR (MeOH-d$_4$) δ 1.30-1.35 (m, 2H, CH$_2$) 1.32 (s, 3H, CH$_3$), 1.34 (s, 3H, CH$_3$), 1.54-1.65 (m, 4H, CH$_2$), 1.89 (d, 3H, CH$_3$, $J = 1.1$ Hz), 2.15-2.28 (m, 2H, H-2'), 2.28-2.34 (m, 2H, CH$_2$-C$_{carborane}$), 2.51 (dd, 1H, CH$_2$-C$_{carborane}$, $J = 15.9, 7.9$ Hz), 2.58 (dd, 1H, CH$_2$-C$_{carborane}$, $J = 15.9, 3.5$ Hz), 3.53 (dd, 1H, CH$_2$, $J = 8.2, 7.2$ Hz), 3.72 (dd, 1H, H-5', $J = 12.0, 3.7$ Hz), 3.79 (dd, 1H, H-5', $J = 12.0, 3.7$ Hz), 3.88-3.92 (m, 3H, H-4' and CH$_2$N), 4.10 (dd, 1H, CH$_2$, $J = 8.3, 6.1$ Hz), 4.23-4.29 (m, 1H, CH), 4.37-4.40 (m, 1H, H-3'), 6.30 (t, 1H, H-1', $J = 6.8$ Hz), 7.83 (d, 1H, H-6, $J = 1.1$ Hz); $^{13}$C-NMR (MeOH-d$_4$) δ 13.24 (CH$_3$), 25.85 (CH$_3$), 27.26 (CH$_3$), 27.92 (CH$_2$), 30.34 (CH$_2$), 35.73 (CH$_2$-C$_{carborane}$), 40.25 (CH$_2$-C$_{carborane}$), 41.35 (C-2'), 41.87 (CH$_2$-N), 62.76 (C-5'), 69.99 (CH$_2$), 72.11 (C-3'), 75.95 (CH), 79.53 (CH$_2$-C$_{carborane}$), 81.77 (CH$_2$-C$_{carborane}$), 87.05 (C-1'), 88.86 (C-4'),
110.70 (C-5), 110.89 [C(\text{CH}_3)_2], 136.44 (C-6), 152.30 (C-2), 165.36 (C-4); MS (HR-ESI) 
C_{23}H_{44}B_{10}N_{2}O_{7}Na (M+Na)^+ calcd 591.4061, found 591.4063.

(S)-3-\{5-\{2-(2,3-Isopropylidenedioxyprop-1-yl)-\text{o-carboran-1-yl}\text{pentan-1-yl}\}\text{thymidine (3.10.B)}

Compound 3.10.B was prepared from 3.9.B in 65% yield adapting the procedure described for compound 3.10.A. $R_f$ 0.29 (ethylacetate/methanol, 20:1); $^1$H-NMR (MeOH-d$^4$) $\delta$ 1.30-1.35 (m, 2H, CH$_2$) 1.32 (s, 3H, CH$_3$), 1.34 (s, 3H, CH$_3$), 1.54-1.65 (m, 4H, CH$_2$), 1.89 (d, 3H, CH$_3$, $J = 1.1$ Hz), 2.14-2.28 (m, 2H, H-2$^\prime$), 2.28-2.34 (m, 2H, CH$_2$C$_{\text{carborane}}$), 2.51 (dd, 1H, CH$_2$C$_{\text{carborane}}$, $J = 15.9$, 7.9 Hz), 2.58 (dd, 1H, CH$_2$C$_{\text{carborane}}$, $J = 15.9$, 3.5 Hz), 3.53 (dd, 1H, CH$_2$, $J = 8.2$, 7.2 Hz), 3.71 (dd, 1H, H-5$^\prime$, $J = 12.0$, 3.7 Hz), 3.79 (dd, 1H, H-5$^\prime$, $J = 12.0$, 3.7 Hz), 3.88-3.92 (m, 3H, H-4$^\prime$ and CH$_2$N), 4.10 (dd, 1H, CH$_2$, $J = 8.3$, 5.9 Hz), 4.23-4.29 (m, 1H, CH), 4.37-4.40 (m, 1H, H-3$^\prime$), 6.30 (t, 1H, H-1$^\prime$, $J = 6.8$ Hz), 7.83 (d, 1H, H-6, $J = 1.1$ Hz); $^{13}$C-NMR (MeOH-d$^4$) $\delta$ 13.23 (CH$_3$), 25.85 (CH$_3$), 27.20 (CH$_3$), 27.26 (CH$_2$), 27.93 (CH$_2$), 30.35 (CH$_2$), 35.74 (CH$_2$C$_{\text{carborane}}$), 40.25 (CH$_2$C$_{\text{carborane}}$), 41.35 (C-2$^\prime$), 41.87 (CH$_2$N), 62.76 (C-5$^\prime$), 69.99 (CH$_2$), 72.11 (C-3$^\prime$), 75.96 (CH), 79.54 (CH$_2$C$_{\text{carborane}}$), 81.78 (CH$_2$C$_{\text{carborane}}$), 87.06 (C-1$^\prime$), 88.87 (C-4$^\prime$),
110.70 (C-5), 110.90 \([C(CH_3)_2]\), 136.45 (C-6), 152.31 (C-2), 165.37 (C-4); MS (HR-ESI) C\(_{23}\)H\(_{44}\)B\(_{10}\)N\(_{2}\)O\(_{7}\)Na (M+Na\(^+\)) calcd 591.4061, found 591.4067.

![Chemical structure of compound 3.10.C](image)

3-\{5-[2-(2,3-Isopropylidenedioxyprop-1-yl)-\(\alpha\)-carboran-1-yl]pentan-1-yl\}thymidine (3.10.C)

Compound 3.10.C was prepared from 3.9.C in 60% yield adapting the procedure described for compound 3.10.A. \(R_f\) 0.29 (ethylacetate/methanol, 20:1); \(^1\)H-NMR (MeOH-\(d_4\)) \(\delta\) 1.30-1.35 (m, 2H, CH\(_2\)), 1.32 (s, 3H, CH\(_3\)), 1.34 (s, 3H, CH\(_3\)), 1.54-1.65 (m, 4H, CH\(_2\)), 1.89 (d, 3H, CH\(_3\), \(J = 1.2\) Hz), 2.14-2.28 (m, 2H, H-2\(^\prime\)), 2.28-2.34 (m, 2H, CH\(_2\)-C\(_{\text{carborane}}\)), 2.51 (dd, 1H, CH\(_2\)-C\(_{\text{carborane}}\), \(J = 15.9, 7.9\) Hz), 2.58 (dd, 1H, CH\(_2\)-C\(_{\text{carborane}}\), \(J = 15.9, 3.4\) Hz), 3.54 (dd, 1H, CH\(_2\), \(J = 8.2, 6.5\) Hz), 3.71 (dd, 1H, H-5\(^\prime\), \(J = 12.0, 3.7\) Hz), 3.79 (dd, 1H, H-5\(^\prime\), \(J = 12.0, 3.7\) Hz), 3.88-3.91 (m, 3H, H-4\(^\prime\) and CH\(_2\)N), 4.09 (dd, 1H, CH\(_2\), \(J = 8.1, 6.1\) Hz), 4.23-4.29 (m, 1H, CH), 4.37-4.40 (m, 1H, H-3\(^\prime\)), 6.30 (t, 1H, H-1\(^\prime\), \(J = 6.8\) Hz), 7.83 (d, 1H, H-6, \(J = 1.2\) Hz); \(^{13}\)C-NMR (MeOH-\(d_4\)) \(\delta\) 13.22 (CH\(_3\)), 25.84 (CH\(_3\)), 27.19 (CH\(_3\)), 27.26 (CH\(_2\)), 27.92 (CH\(_2\)), 30.35 (CH\(_2\)), 35.74 (CH\(_2\)-C\(_{\text{carborane}}\)), 40.25 (CH\(_2\)-C\(_{\text{carborane}}\)), 41.35 (C-2\(^\prime\)), 41.87 (CH\(_2\)-N), 62.76 (C-5\(^\prime\)), 70.00 (CH\(_2\)), 72.12 (C-3\(^\prime\)), 75.97 (CH), 79.54 (CH\(_2\)-C\(_{\text{carborane}}\)), 81.78 (CH\(_2\)-C\(_{\text{carborane}}\)), 87.06 (C-1\(^\prime\)), 88.88 (C-4\(^\prime\)), 139
110.70 (C-5), 110.90 (C(CH₃)₂), 136.49 (C-6), 152.32 (C-2), 165.38 (C-4); MS (HR-ESI) C₂₃H₄₄B₁₀N₂O₇Na (M+Na)⁺ calcd 591.4061, found 591.4061.

![Chemical Structure](image)

**3-\{5-[2-(2,3-Isopropylidenedioxyprop-1-yl)-m-carboran-1-yl]pentan-1-yl\}thymidine (3.10.D)**

Compound **3.10.D** was prepared from **3.9.D** in 57% yield adapting the procedure described for compound **3.10.A**. \(R_f\) 0.25 (ethylacetate/methanol, 25:1); \(^1\)H-NMR (Acetone-\(d^6\)) \(\delta\) 1.20-1.24 (m, 2H, CH\(_2\)) 1.27 (s, 3H, CH\(_3\)), 1.30 (s, 3H, CH\(_3\)), 1.37-1.45 (m, 2H, CH\(_2\)), 1.50-1.58 (m, 2H, CH\(_2\)), 1.82 (s, 3H, CH\(_3\)), 1.95-2.00 (m, 2H, H-2´), 2.21-2.24 (m, 4H, CH\(_2\)-C\(_{carborane}\)), 3.44 (dd, 1H, CH\(_2\), \(J = 7.5, 5.8\) Hz), 3.76-3.85 (m, 4H, CH\(_2\)N and H-5´), 3.92 (m, 1H, H-4´), 4.02-4.10 (m, 2H, CH\(_2\)), 4.23 (t, 1H, OH-5´, \(J = 4.8\) Hz), 4.39 (d, 1H, OH-3´, \(J = 3.9\) Hz) 4.45-4.49 (m, 1H, H-3´), 6.32 (t, 1H, H-1´, \(J = 6.7\) Hz), 7.81 (s, 1H, H-6); \(^{13}\)C-NMR (Acetone-\(d^6\)) \(\delta\) 13.28 (CH\(_3\)), 25.76 (CH\(_3\)), 26.93 (CH\(_3\)), 27.10 (CH\(_2\)), 27.75 (CH\(_2\)), 30.32 (CH\(_2\)), 37.38 (CH\(_2\)-C\(_{carborane}\)), 41.13 (CH\(_2\)-C\(_{carborane}\)), 41.89 (C-2´), 42.92 (CH\(_2\)-N), 62.49 (C-5´), 69.38 (CH\(_3\)), 71.78 (C-3´), 75.36 (CH), 86.19 (C-1´), 88.44 (C-4´), 109.59 (C-5), 109.62 [CH\(_3\)], 135.26 (C-6), 151.48 (C-2), 163.52 (C-4); MS (HR-ESI) C₂₃H₄₄B₁₀N₂O₇Na (M+Na)⁺ calcd 591.4061, found 591.4046.
3-\{5-\{2-(2,3-Isopropylidenedioxyp-1-yl)-p-carboran-1-yl|pentan-1-yl\}thymidine

(3.10.E)

Compound 3.10.E was prepared from 3.9.E in 62% yield adapting the procedure
described for compound 3.10.A. Rf 0.25 (ethylacetate/methanol, 25:1); 1H-NMR
(MeOH-d$_4$) $\delta$ 1.10-1.20 (m, 4H, CH$_2$) 1.25 (s, 3H, CH$_3$), 1.27 (s, 3H, CH$_3$), 1.47-1.53 (m,
2H, CH$_2$), 1.60-1.64 (m, 2H, CH$_2$-Ccarborane), 1.78-1.93 (m, 2H, CH$_2$-Ccarborane), 1.89 (d,
3H, CH$_3$, J = 1.2 Hz), 2.14-2.29 (m, 2H, H-2'), 3.30-3.32 (m, 1H, H-5'), 6.28 (t, 1H, H-1', J = 6.7 Hz),
7.82 (d, 1H, H-6, J = 1.2 Hz); 13C-NMR (MeOH-d$_4$) $\delta$ 13.20 (CH$_3$), 25.66 (CH$_3$), 27.16
(CH$_3$), 27.32 (CH$_2$), 28.01 (CH$_2$), 30.29 (CH$_2$), 38.73 (CH$_2$-Ccarborane), 41.34 (CH$_2$-
Ccarborane), 41.99 (C-2'), 42.96 (CH$_2$-N), 62.76 (C-5'), 69.93 (CH$_2$), 72.10 (C-3'), 75.89
(CH), 77.21 (C-Ccarborane), 81.43 (C-Ccarborane), 87.09 (C-1'), 88.87 (C-4'), 110.23 (C-5),
110.68 [C(CH$_3$)$_3$], 136.46 (C-6), 152.28 (C-2), 165.39 (C-4); MS (HR-ESI)
C$_{23}$H$_{44}$B$_{10}$N$_2$O$_7$Na (M+Na)$^+$ calcd 591.4061, found 591.4062.
(R)-3-{5-[2-(2,3-Dihydroxyprop-1-yl)-o-carboran-1-yl]pentan-1-yl}thymidine (3.11.A)

To a solution of compound 3.10.A (40 mg, 0.07 mmol) in MeOH (5 mL) was added a mixture of 3N-HCl and EtOH (1 mL, 1:1). The reaction mixture was stirred at room temperature for 20 h. Potassium carbonate (17 mg) was added to the reaction mixture, which was then stirred for 30 min at room temperature. The reaction mixture was filtered using a Buchner funnel to remove the solids and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel columns chromatography using ethylacetate/methanol (15:1) as the eluent to give compound 3.11.A (30 mg, 81%).

$R_f$ 0.25; $[a]_D^{25} +17.5$ ($c = 0.15$, MeOH); $^1$H-NMR (MeOH-d$_4$) $\delta$ 1.30-1.38 (m, 2H, CH$_2$), 1.55-1.65 (m, 4H, CH$_2$), 1.89 (d, 3H, CH$_3$, $J = 1.1$ Hz), 2.15-2.39 (m, 5H, H-2’, CH$_2$-C$_{carborane}$, and CH(OH)-CH$_2$-C$_{carborane}$), 2.56 (dd, 1H, CH$_2$-C$_{carborane}$, $J = 15.8$, 1.5 Hz), 3.34 (dd, 1H, CH$_2$OH, $J = 11.0$, 6.5 Hz), 3.47 (dd, 1H, CH$_2$OH, $J = 11.0$, 5.3 Hz), 3.72 (dd, 1H, H-5’, $J = 12.1$, 3.7 Hz), 3.75-3.79 (m, 1H, CH(OH)-CH$_2$OH), 3.79 (dd, 1H, H-5’, $J = 12.1$, 3.2 Hz), 3.88-3.91 (m, 3H, H-4’ and CH$_2$N), 4.37-4.40 (m, 1H, H-3’), 6.29 (t, 1H, H-1’, $J = 6.8$ Hz), 7.83 (d, 1H, H-6, $J = 1.1$ Hz); $^{13}$C-NMR (MeOH-d$_4$) $\delta$ 13.26 (CH$_3$), 27.27 (CH$_2$), 27.88 (CH$_2$), 30.36 (CH$_2$), 35.77 (CH$_2$), 39.84 (CH$_2$), 41.32 (CH$_2$), 41.92 (CH$_2$), 62.73 (O-CH$_2$), 66.89 (O-CH$_2$), 72.07 (O-CH$_2$), 72.17 (O-CH$_2$), 80.39 (C$_{carborane}$-C), 81.85 (C$_{carborane}$-C), 87.06 (O-CH), 88.81 (O-CH), 110.67 (C-5), 136.44 (C-6), 152.26 (C-2), 165.37 (C-4); MS (HR-ESI) C$_{20}$H$_{46}$B$_{10}$N$_2$O$_7$Na (M+Na)$^+$ calcld 551.3747, found 551.3755; HPLC retention time: 26.7 min (Method A), 21.1 min (Method B).
(S)-3-{5-[2-(2,3-Dihydroxyprop-1-yl)-o-carboran-1-yl]pentan-1-yl}thymidine

(3.11.B)

Compound 3.11.B was prepared from 3.10.B in 85% yield adapting the procedure described for compound 3.11.A. $R_f$ 0.25 (ethylacetate/methanol, 15:1); $[\alpha]_D^{25}$ -14.8 ($c = 0.1$, MeOH); $^1$H-NMR (MeOH-d$_4$) $\delta$ 1.30-1.38 (m, 2H, CH$_2$), 1.54-1.65 (m, 4H, CH$_2$), 1.89 (d, 3H, CH$_3$, $J = 1.1$ Hz), 2.15-2.39 (m, 5H, H-2’, CH$_2$-C$_{carborane}$, and CH(OH)-CH$_2$-C$_{carborane}$), 2.56 (dd, 1H, CH$_2$-C$_{carborane}$, $J = 15.8$, 1.6 Hz), 3.33 (dd, 1H, CH$_2$OH, $J = 11.0$, 6.5 Hz), 3.47 (dd, 1H, CH$_2$OH, $J = 11.0$, 5.3 Hz), 3.72 (dd, 1H, H-5’, $J = 12.1$, 3.7 Hz), 3.75-3.79 (m, 1H, CH(OH)-CH$_2$OH), 3.79 (dd, 1H, H-5’, $J = 12.1$, 3.2 Hz), 3.88-3.92 (m, 3H, H-4’ and CH$_2$N), 4.37-4.40 (m, 1H, H-3’), 6.29 (t, 1H, H-1’, $J = 6.8$ Hz), 7.83 (d, 1H, H-6, $J = 1.1$ Hz); $^{13}$C-NMR (MeOH-d$_4$) $\delta$ 13.24 (CH$_3$), 27.28 (CH$_2$), 27.90 (CH$_2$), 30.37 (CH$_2$), 35.79 (CH$_2$), 39.87 (CH$_2$), 41.33 (CH$_2$), 41.92 (CH$_2$), 62.75 (O-CH$_2$), 66.90 (O-CH$_2$), 72.09 (O-CH$_2$), 72.20 (O-CH$_2$), 80.42 (C$_{carborane}$-C), 81.87 (C$_{carborane}$-C), 87.09 (O-CH), 88.85 (O-CH), 110.69 (C-5), 136.46 (C-6), 152.30 (C-2), 165.41 (C-4); MS (HR-ESI) C$_{20}$H$_{40}$B$_{10}$N$_2$O$_7$Na (M+Na)$^+$ calcd 551.3747, found 551.3749; HPLC retention time: 26.6 min (Method A), 21.1 min (Method B).
3-{5-[2-(2,3-Dihydroxyprop-1-yl)-o-carboran-1-yl]pentan-1-yl}thymidine (3.11.C)

Compound 3.11.C was prepared from 3.10.C in 80% yield following the procedure described for compound 3.11.A. 

$R_f$ 0.25 (ethylacetate/methanol, 15:1); $^1$H-NMR (MeOH-d$_4$) $\delta$ 1.30-1.37 (m, 2H, CH$_2$), 1.55-1.65 (m, 4H, CH$_2$), 1.89 (d, 3H, CH$_3$, $J$ = 1.1 Hz), 2.18-2.42 (m, 5H, H-2', CH$_2$-C$_{\text{carborane}}$ and CH(OH)-CH$_2$-C$_{\text{carborane}}$), 2.55 (dd, 1H, CH$_2$-C$_{\text{carborane}}$, $J$ = 15.8, 1.5 Hz), 3.33 (dd, 1H, CH$_2$OH, $J$ = 11.0, 6.5 Hz), 3.46 (dd, 1H, CH$_2$OH, $J$ = 11.0, 5.3 Hz), 3.71 (dd, 1H, H-5', $J$ = 12.1, 3.7 Hz), 3.74-3.79 (m, 1H, CH(OH)-CH$_2$OH), 3.79 (dd, 1H, H-5', $J$ = 12.1, 3.2 Hz), 3.88-3.92 (m, 3H, H-4' and CH$_2$N), 4.37-4.40 (m, 1H, H-3'), 6.29 (t, 1H, H-1', $J$ = 6.8 Hz), 7.83 (d, 1H, H-6, $J$ = 1.1 Hz); $^{13}$C-NMR (MeOH-d$_4$) $\delta$ 13.22 (CH$_3$), 27.29 (CH$_2$), 27.90 (CH$_2$), 30.38 (CH$_2$), 35.81 (CH$_2$), 39.90 (CH$_2$), 41.33 (CH$_2$), 41.92 (CH$_2$), 62.76 (O-CH$_2$), 66.91 (O-CH$_2$), 72.12 (O-CH$_2$), 72.21 (O-CH$_2$), 80.45 (C$_{\text{carborane}}$-C), 81.90 (C$_{\text{carborane}}$-C), 87.10 (O-CH), 88.88 (O-CH), 110.71 (C-5), 136.48 (C-6), 152.33 (C-2), 165.44 (C-4); MS (HR-ESI) C$_{20}$H$_{40}$B$_{10}$N$_2$O$_7$Na (M+Na)$^+$ calcd 551.3747, found 551.3753; HPLC retention time: 26.9 min (Method A), 21.0 min (Method B).
3-{5-[7-(2,3-Dihydroxyprop-1-yl)-m-carboran-1-yl]pentan-1-yl}thymidine (3.11.D)

Compound 3.11.D was prepared from 3.10.D in 88% yield following the procedure described for compound 3.11.A. \( R_f \) 0.25 (ethylacetate/methanol, 15:1); \(^1\)H-NMR (MeOH-d\textsuperscript{4}) \( \delta \) 1.21-1.28 (m, 2H, CH\textsubscript{2}), 1.38-1.44 (m, 2H, CH\textsubscript{2}), 1.53-1.60 (m, 2H, CH\textsubscript{2}), 1.89 (d, 3H, CH\textsubscript{3}, \( J \) = 1.1 Hz), 1.92-1.98 (m, 2H, CH\textsubscript{2}-C\textsubscript{carborane}), 2.16-2.30 (m, 4H, H-2' and CH\textsubscript{2}-C\textsubscript{carborane}), 3.26 (dd, 1H, CH\textsubscript{2}OH, \( J \) = 11.1, 6.1 Hz), 3.36 (dd, 1H, CH\textsubscript{2}OH, \( J \) = 11.1, 5.5 Hz), 3.55-3.60 (m, 1H, CH(OH)-CH\textsubscript{2}OH), 3.72 (dd, 1H, H-5', \( J \) = 12.1, 3.7 Hz), 3.79 (dd, 1H, H-5', \( J \) = 12.1, 3.2 Hz), 3.86-3.92 (m, 3H, H-4' and CH\textsubscript{2}N), 4.37-4.40 (m, 1H, H-3’), 6.29 (t, 1H, H-1’, \( J \) = 6.8 Hz), 7.83 (d, 1H, H-6, \( J \) = 1.1 Hz); \(^{13}\)C-NMR (MeOH-d\textsuperscript{4}) \( \delta \) 13.22 (CH\textsubscript{3}), 27.40 (CH\textsubscript{2}), 28.05 (CH\textsubscript{2}), 30.74 (CH\textsubscript{2}), 37.98 (CH\textsubscript{2}), 41.34 (CH\textsubscript{2}), 41.80 (CH\textsubscript{2}), 42.01 (CH\textsubscript{2}), 62.75 (O-CH\textsubscript{2}), 66.94 (O-CH\textsubscript{2}), 72.10 (O-CH\textsubscript{2}), 72.35 (O-CH\textsubscript{2}), 75.21 (C\textsubscript{carborane-C}), 77.68 (C\textsubscript{carborane-C}), 87.11 (O-CH), 88.86 (O-CH), 110.69 (C-5), 136.47 (C-6), 152.29 (C-2), 165.40 (C-4); MS (HR-ESI) C\textsubscript{20}H\textsubscript{46}B\textsubscript{10}N\textsubscript{2}O\textsubscript{7}Na (M+Na)\textsuperscript{+} calcd 551.3747, found 551.3740; HPLC retention time: 25.7 min (Method A), 21.0 min (Method B).
3-{5-[12-(2,3-Dihydroxyprop-1-yl)-p-carboran-1-yl]pentan-1-yl}thymidine (3.11.E)

Compound 3.11.E was prepared from 3.10.E in 88% yield following the procedure described for compound 3.11.A. $R_f$ 0.25 (ethylacetate/methanol, 15:1); $^1$H-NMR (MeOH-$d^4$) δ 1.10-1.20 (m, 4H, CH$_2$), 1.47-1.53 (m, 2H, CH$_2$), 1.60-1.66 (m, 3H, CH$_2$-C$_{carborane}$), 1.89 (d, 3H, CH$_3$, $J$ = 1.1 Hz), 1.89-1.92 (m, 1H, CH$_2$-C$_{carborane}$), 2.14-2.29 (m, 2H, H-2`), 3.16 (dd, 1H, CH$_2$OH, $J$ = 11.0, 6.0 Hz), 3.24 (dd, 1H, CH$_2$OH, $J$ = 11.0, 5.6 Hz), 3.32-3.38 (m, 1H, CH), 3.71 (dd, 1H, H-5`, $J$ = 12.1, 3.7 Hz), 3.79 (dd, 1H, CH$_2$OH, $J$ = 12.1, 3.1 Hz), 3.83 (t, 2H, CH$_2$N, $J$ = 7.6 Hz), 3.88-3.91 (m, 1H, H-4`), 4.36-4.40 (m, 1H, H-3`), 6.28 (t, 1H, H-1`, $J$ = 6.8 Hz), 7.82 (d, 1H, H-6, $J$ = 1.1 Hz); $^{13}$C-NMR (MeOH-$d^4$) δ 13.17 (CH$_3$), 27.33 (CH$_2$), 28.01 (CH$_2$), 30.27 (CH$_2$), 38.71 (CH$_2$), 41.34 (CH$_2$), 42.00 (CH$_2$), 42.52 (CH$_2$), 62.76 (O-CH$_2$), 66.87 (O-CH$_2$), 72.07 (O-CH$_2$), 72.16 (O-CH$_2$), 78.74 (C$_{carborane}$-C), 81.23 (C$_{carborane}$-C), 87.12 (O-CH), 88.86 (O-CH), 110.69 (C-5), 136.43 (C-6), 152.28 (C-2), 165.38 (C-4); MS (HR-ESI) C$_{20}$H$_{40}$B$_{10}$N$_2$O$_7$Na (M+Na)$^+$ calcd 551.3747, found 551.3746; HPLC retention time: 23.7 min (Method A), 19.9 min (Method B).

Iodination of N5-2OH (3.12.A-C)
To a solution of compound 3.5.A (90 mg, 0.17 mmol) in dichloromethane (15 mL) was added I₂ (70 mg, 0.26 mmol) and AlCl₃ (4.5 mg, 0.034 mmol). The reaction mixture was stirred at 40 °C for 48 h. Aqueous solution of Na₂S₂O₃ (70 mg) was added to the reaction mixture and stirred at room temperature for additional 30 min. Ethylacetate (15 mL) was added and the organic layer was separated, washed with brine, and dried over magnesium sulfate. After filtration and evaporation, the residue was separated by silica gel column chromatography using ethylacetate and methanol (15:1) as the eluent, followed by separation of three products on the RP-18 analytical column using the method A.

3-{5-[2-(2,3-Dihydroxyprop-1-yl)-9-iodo-o-carboran-1-yl]pentan-1-yl}thymidine (3.12.A)

Yield: 7 %; HPLC retention time: 26.5 min (Method A); ¹H-NMR (CD₃OD) δ 1.34-1.40 (m, 2H, CH₂), 1.58-1.63 (m, 4H, CH₂), 1.88 (d, 3H, CH₃, J = 1.2 Hz), 2.12-2.37 (m, 5H, H-2’ and CH₂-C-carborane, and CH(OH)-CH₂-C-carborane), 2.46 (dd, 1H, CH(OH)-CH₂-C-carborane, J = 15.8, 1.7 Hz), 3.31 (dd, 1H, CH₂OH, J = 11.0, 6.5 Hz) 3.46 (dd, 1H, CH₂OH, J = 11.0, 5.3 Hz), 3.67-3.69 (m, 1H, CH(OH)-CH₂OH), 3.71 (dd, 1H, H-5’, J = 12.0, 3.7 Hz), 3.79 (dd, 1H, H-5’, J = 12.0, 3.2 Hz), 3.89-3.93 (m, 3H, H-4’ and CH₂N), 4.37-4.40 (m, 1H, H-3’), 6.30 (t, 1H, H-1’, J = 6.9 Hz), 7.83 (d, 1H, H-6, J = 1.2 Hz);
\[^{11}\text{B-NMR (CD}_3\text{OD)} \delta -14.8, -9.9, -6.3; \ MS (HR-ESI) \text{C}_{20}\text{H}_{39}\text{B}_{10}\text{IN}_{2}\text{O}_{7}\text{Na (M+Na)}^+ \text{ calcd 677.2714, found 677.2759.}\]

\[3\{-5-[2-(2,3\text{-Dihydroxyprop-1-yl})-9,12\text{-diodo-o-carboran-1-yl}][\text{pentan-1-yl}]\text{thymidine (3.12.B)}\]

Yield: 16 %; HPLC retention time 28.9 min (Method A); \[^{1}\text{H-NMR (CD}_3\text{OD)} \delta 1.32\text{-1.40 (m, 2H, CH}_2\text{), 1.56\text{-1.66 (m, 4H, CH}_2\text{), 1.89 (d, 3H, CH}_3\text{, }J = 1.2 \text{ Hz), 2.14\text{-2.37 (m, 5H, H-2’ and CH}_2\text{-C}_{\text{carborane}}, \text{and CH(OH)}\text{-CH}_2\text{-C}_{\text{carborane}}, 2.52 (dd, 1H, CH(OH)}\text{-CH}_2\text{-C}_{\text{carborane}, J = 15.8, 1.7 Hz), 3.31 (dd, 1H, CH}_2\text{OH, }J = 11.0, 6.5 \text{ Hz) 3.48 (dd, 1H, CH}_2\text{OH, }J = 11.0, 5.3 \text{ Hz), 3.71\text{-3.74 (m, 1H, CH(OH)}\text{-CH}_2\text{OH), 3.72 (dd, 1H, H-5’, }J = 12.0, 3.7 \text{ Hz), 3.79 (dd, 1H, H-5’, }J = 12.0, 3.2 \text{ Hz), 3.89\text{-3.92 (m, 3H, H-4’ and CH}_2\text{N), 4.37\text{-4.40 (m, 1H, H-3’), 6.30 (t, 1H, H-1’, }J = 6.9 \text{ Hz), 7.83 (d, 1H, H-6, }J = 1.2 \text{ Hz); }^{13}\text{C-NMR (CD}_3\text{OD)} \delta 13.21 (\text{CH}_3), 27.12 (\text{CH}_2), 27.78 (\text{CH}_2), 30.31 (\text{CH}_2), 35.32 (\text{C}_{\text{carborane}), 39.49 (\text{C}_{\text{carborane}}, 41.34 (\text{C}-2’), 41.83 (\text{CH}_2\text{N}), 62.78 (\text{C}-5’), 66.83 (\text{O-CH}_2), 72.00 (\text{O-CH}), 72.13 (\text{C}-3’), 78.37 (\text{C}_{\text{carborane}-\text{C}), 79.66 (\text{C}_{\text{carborane}-\text{C}), 87.13 (\text{C}-1’), 88.89 (\text{C}-4’), 110.73 (\text{C}-5), 136.50 (\text{C}-6), 152.35 (\text{C}-2), 165.44 (\text{C}-4); }^{11}\text{B-NMR (CD}_3\text{OD)} \delta -12.6, -6.6, -4.0; \ MS (HR-ESI) \text{C}_{20}\text{H}_{38}\text{B}_{10}\text{I}_2\text{N}_{2}\text{O}_{7}\text{Na (M+Na)}^+ \text{ calcd 803.1680, found 803.1683.}\]

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3-{5-[2-(2,3-Dihydroxyprop-1-yl)-9,11,12-triiodo-o-carboran-1-yl]pentan-1-yl}thymidine (3.12.C)

Yield: 10%; HPLC retention time 30.62 min (Method A); \(^{1}\)H-NMR (CD\(_3\)OD) \(\delta\) 1.35-1.39 (m, 2H, CH\(_2\)), 1.57-1.65 (m, 4H, CH\(_2\)), 1.89 (d, 3H, CH\(_3\), \(J = 1.2\) Hz), 2.17-2.33 (m, 5H, H-2´ and CH\(_2\)-C\(_{carborane}\), and CH(OH)-CH\(_2\)-C\(_{carborane}\)), 2.54 (dd, 1H, CH(OH)-CH\(_2\)-C\(_{carborane}\), \(J = 15.8, 1.7\) Hz), 3.33 (dd, 1H, CH\(_2\)OH, \(J = 10.9, 6.4\) Hz) 3.49 (dd, 1H, CH\(_2\)OH, \(J = 10.9, 5.2\) Hz), 3.72 (dd, 1H, H-5´, \(J = 12.0, 3.7\) Hz), 3.72-3.74 (m, 1H, CH(OH)-CH\(_2\)OH), 3.79 (dd, 1H, H-5´, \(J = 12.0, 3.2\) Hz), 3.89-3.93 (m, 3H, H-4´ and CH\(_2\)N), 4.37-4.40 (m, 1H, H-3´), 6.30 (t, 1H, H-1´, \(J = 6.9\) Hz), 7.83 (d, 1H, H-6, \(J = 1.2\) Hz); \(^{11}\)B-NMR (CD\(_3\)OD) \(\delta\) -15.0, -10.0, -6.2; MS (HR-ESI) C\(_{20}\)H\(_{37}\)B\(_{10}\)I\(_3\)N\(_2\)O\(_7\)Na (M+Na\(^+\)) calcd 929.0646, found 929.0649.

7.3. Chapter 4
1-{(tert-Butyloxy)carbonylamino}-1,7-closo-\(m\)-carborane (4.1)

Compound 4.1 was synthesized according to a procedure reported by Karl and coworkers.\(^{121}\) \(^1\)H-NMR (CDCl\(_3\)) \(\delta\) 1.46 (s, 9H, t-butyl), 2.93 (br s, 1H, H-C\(_{\text{carborane}}\)), 5.17 (br s, 1H, NH); MS (HR-ESI) \(C_7H_{21}B_{10}NO_2Na\) (M+Na)\(^+\) calcd: 282.2468, found: 282.2478.

\[\text{Structure of Compound 4.1}\]

2-{[7-(tert-Butyloxy)carbonylamino]-closo-\(m\)-carboran-1-yl}ethyl tosylate (4.2)

n-BuLi in hexanes (2.5 M, 1.53 mL, 3.83 mmol) was slowly added to a solution of compound 4.1 (450 mg, 1.74 mmol) in benzene (15 mL) at 5-10 °C and stirred for 20 min at the same temperature. The mixture was warmed to room temperature and stirred for additional 30 min. The solution was added very slowly to a solution of ethylene glycol di-\(p\)-tosylate (1.6 g, 4.32 mmol) in benzene (5 mL) at 5-10 °C. The mixture was stirred for 30 min at the same temperature, distilled water (20 mL) was added, and benzene was removed under reduced pressure. The residue was extracted with ethyl acetate (15 mL \(\times\) 3) and the combined organic layers were washed with brine, and dried over magnesium sulfate. After filtration and evaporation, the residue was purified by silica gel column chromatography (hexanes/ethylacetate, 12:1) to give compound 4.2 as colorless oil in 35% (280 mg) yield. \(R_f\) 0.15; \(^1\)H-NMR (CDCl\(_3\)) \(\delta\) 1.40 (s, 9H, C(CH\(_3\))\(_3\)), 2.29 (t, 2H, CH\(_2\)-C\(_{\text{carborane}}\), \(J = 7.1\) Hz), 2.44 (s, 3H, CH\(_3\)), 3.94 (t, 2H, CH\(_2\)O, \(J = 7.1\) Hz),
5.07 (s, 1H, NH), 7.34 (d, 2H, Ar-H, \(J = 8.3\) Hz), 7.76 (m, 2H, Ar-H, \(J = 8.3\) Hz); \(^{13}\)C NMR (CDCl\(_3\)) \(\delta\) 21.69 (CH\(_3\)), 28.13 (CH\(_3\)), 33.47 (CH\(_2\)-C\(_{\text{carborane}}\)), 67.35 [OC(CH\(_3\))\(_3\)], 69.37 (CH\(_2\)O), 80.72 (C\(_{\text{carborane}}\)-C), 82.89 (C\(_{\text{carborane}}\)-C), 127.93 (Ar-C), 129.98 (Ar-C), 132.97 (Ar-C), 145.05 (Ar-C), 152.04 (C=O); MS (HR-ESI) C\(_{16}\)H\(_{31}\)B\(_{10}\)NO\(_5\)SNa (M+Na\(^{+}\)) calcd: 480.2833, found: 480.2802.

![Chemical structure](image)

**3-{{[7-(tert-Butyloxy)carbonylamino]-closo-m-carboran-1-yl}propyl tosylate (4.3)}**

Compound 4.3 was prepared according to the procedure described for compound 4.2 using n-BuLi in hexanes (2.5 M, 1.04 mL, 2.60 mmol), compound 4.1 (307 mg, 1.18 mmol), and 1,3-propanediol di-p-tosylate (1.00 g, 2.6 mmol). Purification by silica gel column chromatography (hexanes/ethylacetate, 12:1) gave 4.3 as colorless oil in 36% (200 mg) yield. \(R_f\) 0.16; \(^1\)H-NMR (CDCl\(_3\)) \(\delta\) 1.40 (s, 9H, C(CH\(_3\))\(_3\)), 1.63-1.67 (m, 2H, CH\(_2\)), 1.90-1.97 (m, 2H, CH\(_2\)-C\(_{\text{carborane}}\)), 2.43 (s, 3H, CH\(_3\)), 3.91 (t, 2H, CH\(_2\)O, \(J = 7.0\) Hz), 5.13 (s, 1H, NH), 7.33 (d, 2H, Ar-H, \(J = 8.1\) Hz), 7.73 (d, 2H, Ar-H, \(J = 8.1\) Hz); \(^{13}\)C NMR (CDCl\(_3\)) \(\delta\) 21.65 (CH\(_3\)), 28.14 (CH\(_3\)), 28.99 (CH\(_2\)), 33.03 (CH\(_2\)-C\(_{\text{carborane}}\)), 69.01 [OC(CH\(_3\))\(_3\)], 72.37 (CH\(_2\)O), 80.72 (C\(_{\text{carborane}}\)-C), 81.70 (C\(_{\text{carborane}}\)-C), 127.89 (Ar-C), 129.96 (Ar-C), 132.78 (Ar-C), 145.04 (Ar-C), 151.85 (C=O); MS (HR-ESI) C\(_{17}\)H\(_{33}\)B\(_{10}\)NO\(_5\)SNa (M+Na\(^{+}\)) calcd: 494.2975, found: 494.2997.
4-[[7-(tert-Butyloxy)carbonylamino]-closo-m-carboran-1-yl]butyl tosylate (4.4)

Compound 4.4 was prepared according to the procedure described for compound 4.2 using n-BuLi in hexanes (2.5 M, 1.00 mL, 2.50 mmol), compound 4.1 (258 mg, 1.00 mmol), and 1,4-butanediol di-p-tosylate (600 mg, 1.51 mmol). Purification by silica gel column chromatography (hexanes/ethylacetate, 14:1) gave 8 as colorless oil in 43% (210 mg) yield. Rf 0.14; 1H-NMR (CDCl3) δ 1.25-1.35 (m, 4H, CH2), 1.41 (s, 9H, C(CH3)3), 1.49-1.52 (m, 2H, CH2), 1.81-1.88 (m, 2H, CH2-Carborane), 2.44 (s, 3H, CH3), 3.95 (t, 2H, CH2O, J = 6.2 Hz), 5.09 (s, 1H, NH), 7.34 (d, 2H, Ar-H, J = 8.0 Hz), 7.77 (d, 2H, Ar-H, J = 8.0 Hz); 13C NMR (CDCl3) δ 21.64 (CH3), 25.81 (CH2), 28.12 (CH3), 28.25 (CH2), 36.31 (CH2-Carborane), 69.65 [O(CH3)3], 73.21 (CH2O), 80.51 (C-carborane-C), 81.63 (C-carborane-C), 127.86 (Ar-C), 129.93 (Ar-C), 132.87 (Ar-C), 144.89 (Ar-C), 151.86 (C=O); MS (HR-ESI) C18H35B10NO5Sn (M+Na)+ calcd: 508.3147, found: 508.3169.

5-[[7-(tert-Butyloxy)carbonylamino]-closo-m-carboran-1-yl]pentyl tosylate (4.5)

Compound 4.5 was prepared according to the procedure described for compound 4.2 using n-BuLi in hexanes (2.5 M, 1.82 mL, 4.56 mmol), compound 4.1 (560 mg, 2.17
mmol), and 1,5-pentanediol di-p-tosylate (983 mg, 2.39 mmol). Purification by silica gel column chromatography (hexanes/ethylacetate, 14:1) gave 4.5 as colorless oil in 39% (420 mg) yield. $R_f$ 0.16; $^1$H-NMR (CDCl$_3$) $\delta$ 1.20-1.28 (m, 4H, CH$_2$), 1.42 (s, 9H, C(CH$_3$)$_3$), 1.57-1.62 (m, 2H, CH$_2$), 1.83-1.89 (m, 2H, CH$_2$-C$_{\text{carborane}}$), 2.45 (s, 3H, CH$_3$), 3.98 (t, 2H, CH$_2$O, $J = 6.2$ Hz), 5.12 (s, 1H, NH), 7.34 (d, 2H, Ar-H, $J = 7.9$ Hz), 7.78 (d, 2H, Ar-H, $J = 7.9$ Hz); $^{13}$C-NMR (CDCl$_3$) $\delta$ 21.63 (CH$_3$), 24.88 (CH$_2$), 28.12 (CH$_3$), 28.35 (CH$_2$), 29.10 (CH$_2$), 36.76 (CH$_2$-C$_{\text{carborane}}$), 70.03 [O$(\text{CH}_3)_3$], 73.60 (CH$_2$O), 80.52 (C$_{\text{carborane}}$-C), 81.59 (C$_{\text{carborane}}$-C), 127.84 (Ar-C), 129.85 (Ar-C), 133.02 (Ar-C), 144.79 (Ar-C), 151.89 (C=O); MS (HR-ESI) C$_{19}$H$_{37}$B$_{10}$NO$_5$SNa (M+Na)$^+$ calcd: 522.3288, found: 522.3263.

![Chemical Structure](image)

6-[(7-(tert-Butyloxy)carbonylamino]-closo-m-carboran-1-yl]hexyl tosylate (4.6)

Compound 4.6 was prepared according to the procedure described for compound 4.2 using n-BuLi in hexanes (2.5 M, 1.00 mL, 2.50 mmol), compound 4.1 (258 mg, 1.00 mmol), and 1,6-hexanediol di-p-tosylate (650 mg, 1.52 mmol). Purification by silica gel column chromatography (hexanes/ethylacetate, 15:1) gave 4.6 as colorless oil in 45% (230 mg) yield. $R_f$ 0.16; $^1$H-NMR (CDCl$_3$) $\delta$ 1.10-1.12 (m, 2H, CH$_2$), 1.21-1.27 (m, 4H, CH$_2$), 1.40 (s, 9H, C(CH$_3$)$_3$), 1.55-1.59 (m, 2H, CH$_2$), 1.82-1.86 (m, 2H, CH$_2$-C$_{\text{carborane}}$), 2.43 (s, 3H, CH$_3$), 3.97 (t, 2H, CH$_2$O, $J = 6.4$ Hz), 5.11 (s, 1H, NH), 7.32 (d, 2H, Ar-H, $J$
\[ \text{H}\begin{array}{c}
\text{N} \\
\text{O} \\
\text{O} \\
\text{S} \\
\text{O}
\end{array}\text{O} \]

2-{2-\{7-(tert-Butyloxy)carbonylamino]-closo-\textit{m}-carboran-1-yl\}ethylxyethyl tosylate (4.7)

Compound 4.7 was prepared according to the procedure described for compound 4.2 using n-BuLi in hexanes (2.5 M, 2.04 mL, 5.10 mmol), compound 4.1 (600 mg, 2.32 mmol), and di(ethylene glycol) di-\textit{p}-tosylate (1.05 g, 2.55 mmol). Purification by silica gel column chromatography (hexanes/ethylacetate, 5:1) gave 4.7 as colorless oil in 14\% (160 mg) yield. \( R_f \) 0.19, \textbf{1}H-NMR (CDCl\textsubscript{3}): \( \delta \) 1.40 (s, 9H, C(CH\textsubscript{3})\textsubscript{3}), 2.09 (t, 2H, CH\textsubscript{2}-C\textsubscript{carborane}, \( J = 6.8 \text{ Hz} \)), 2.44 (s, 3H, CH\textsubscript{3}), 3.30 (t, 2H, CH\textsubscript{2}O, \( J = 6.8 \text{ Hz} \)), 3.53 (t, 2H, CH\textsubscript{2}O, \( J = 4.5 \text{ Hz} \)), 4.11 (t, 2H, CH\textsubscript{2}O, \( J = 4.5 \text{ Hz} \)), 5.13 (br s, 1H, NH), 7.32 (d, 2H, Ar-H, \( J = 7.9 \text{ Hz} \)), 7.77 (d, 2H, Ar-H, \( J = 7.9 \text{ Hz} \)); MS (HR-ESI) C\textsubscript{18}H\textsubscript{35}B\textsubscript{10}NO\textsubscript{6}SNa (M+Na\textsuperscript{+}) calcd: 524.3086, found: 524.3257.
3-\{2-[(7-(\text{tert}-\text{Butyloxy})\text{carbonylamino})-\text{closo}-m-\text{carboran}-1-\text{yl}]\text{ethan}-1-\text{yl}\}\text{thymididine}

(4.8)

A solution of compound 4.2 (270 mg, 0.59 mmol), dThd (440 mg, 1.82 mmol), potassium carbonate (450 mg, 3.26 mmol) in DMF/acetone (20 mL, 1:1) was stirred at 50 °C for 48 h. The solution was filtered and evaporated. The residue was dissolved in the water (20 mL) and extracted with ethyl acetate (20 mL × 4). The combined organic layers were washed with brine and dried over magnesium sulfate. After filtration and evaporation, the residue was purified by silica gel column chromatography (ethyl acetate/methanol, 15:1) to give compound 4.8 as a white solid in 64% (200 mg) yield. \( R_f \) 0.15; \(^1\)H-NMR (CDCl\(_3\)) \( \delta \) 1.39 (s, 9H, C(CH\(_3\))\(_3\)), 1.86 (s, 3H, CH\(_3\)), 2.15-2.20 (m, 2H, H-2\(´\)), 2.27-2.32 (t, 2H, CH\(_2\)-C\(_{\text{carborane}}\), \( J = 6.3 \) Hz), 3.82-3.99 (m, 5H, H-4\(´\), H-5\(´\) and CH\(_2\)N), 4.51-4.55 (m, 1H, H-3\(´\)), 5.34 (s, 1H, NH), 6.17 (t, 1H, H-1\(´\), \( J = 6.7 \) Hz), 7.43 (s, 1H, H-6); \(^{13}\)C-NMR (MeOH-d\(_4\)) \( \delta \) 13.09 (CH\(_3\)), 28.51 (CH\(_3\)), 34.21 (CH\(_2\)-C\(_{\text{carborane}}\)), 41.31 (CH\(_2\)N), 41.39 (C-2\(´\)), 62.69 (C-5\(´\)), 71.45 (C-3\(´\)), 72.01 [OC(CH\(_3\))\(_3\)], 81.66 (C\(_{\text{carborane}}\)-C), 83.21 (C\(_{\text{carborane}}\)-C), 87.12 (C-1\(´\)), 88.85 (C-4\(´\)), 110.60 (C-5), 136.65 (C-6), 151.86 (C=O), 154.58 (C=O), 164.92 (C=O); MS (HR-ESI) C\(_{19}\)H\(_{37}\)B\(_{10}\)N\(_3\)O\(_7\)Na (M+Na\(^+\)) calcd: 550.3542, found: 550.3578.
3-{{7-(tert-Butyloxy)carbonylamino}-closo-m-carboran-1-yl}propan-1-yl}thymidine (4.9).

Compound 4.9 was prepared according to the procedure described for compound 4.8 using compound 4.3 (170 mg, 0.36 mmol), dThd (104 mg, 0.43 mmol), potassium carbonate (150 mg, 1.08 mmol). Purification by silica gel column chromatography (ethyl acetate/methanol, 15:1) gave 4.9 as a white solid in 85% (166 mg) yield. \( R_f \) 0.17; \(^1^H-NMR (CDCl_3) \delta 1.40 (s, 9H, C(CH_3)_3), 1.56-1.67 (m, 2H, CH_2), 1.90 (s, 3H, CH_3), 1.90-2.02 (m, 2H, H-2'), 2.30-2.38 (m, 2H, CH_2-C_{carborane}), 3.80-3.98 (m, 5H, H-4', H-5' and CH_2N), 4.56-4.59 (m, 1H, H-3'), 5.15 (s, 1H, NH), 6.17 (t, 1H, H-1'), 7.33 (s, 1H, H-6); \(^1^C-NMR (MeOH-d^4) \delta 13.22 (CH_3), 28.53 (CH_2), 29.10 (CH_3), 36.53 (CH_2-C_{carborane}), 41.32 (CH_2N), 41.55 (C-2'), 62.72 (C-5'), 72.04 [O(C(CH_3)_3)], 74.09 (C-3'), 81.62 (C_{carborane}-C), 82.99 (C_{carborane}-C), 87.10 (C-1'), 88.84 (C-4'), 110.68 (C-5), 136.61 (C-6), 152.22 (C=O), 154.59 (C=O), 163.31 (C=O); MS (HR-ESI) \( C_{20}H_{39}B_{10}N_3O_7Na \) (M+Na)\(^+\) calcd: 564.3616, found: 564.3638.
3-{4-[(7-(tert-Butyloxy)carbonylamino)-closo-m-carboran-1-yl]butan-1-yl}thymidine (4.10).

Compound 4.10 was prepared according to the procedure described for compound 4.8 using compound 4.4 (206 mg, 0.42 mmol), dThd (252 mg, 1.04 mmol), potassium carbonate (275 mg, 1.99 mmol). Purification by silica gel column chromatography (dichloromethane/acetone, 2:1) gave 14 as a white solid in 69% (160 mg) yield. $R_f$ 0.19; $^1$H-NMR ($CDCl_3$) $\delta$ 1.35-1.38 (m, 2H, CH$_2$), 1.40 (s, 9H, C(CH$_3$)$_3$), 1.48-1.50 (m, 2H, CH$_2$), 1.90 (d, 3H, CH$_3$, $J$ = 1.1 Hz), 1.92-1.97 (m, 2H, CH$_2$-C$_{carborane}$), 2.30-2.46 (m, 2H, H-2´), 3.80-3.94 (m, 4H, H-5´ and CH$_2$N), 3.98-4.00 (m, 1H, H-4´), 4.59-4.61 (m, 1H, H-3´), 6.11 (t, 1H, H-1´, $J$ = 6.8 Hz), 7.29 (d, 1H, H-6, $J$ = 1.1 Hz); $^{13}$C-NMR ($CDCl_3$) $\delta$ 13.27 (CH$_3$), 26.87 (CH$_2$), 27.09 (CH$_2$), 28.14 (CH$_3$), 36.47 (CH$_2$-C$_{carborane}$), 40.11 (CH$_2$N), 40.55 (C-2´), 62.45 (C-5´), 71.57 [OC(CH$_3$)$_3$], 71.84 (C-3´), 80.40 (C$_{carborane}$-C), 81.53 (C$_{carborane}$-C), 86.81 (C-1´), 88.00 (C-4´), 110.35 (C-5), 135.05 (C-6), 150.84 (C=O), 151.97 (C=O), 163.17 (C=O); MS (HR-ESI) C$_{21}$H$_{41}$B$_{10}$N$_3$O$_7$Na (M+Na)$^+$ calcd: 578.3773, found: 578.3887.
3-{5-[7-(tert-Butyloxy)carbonylamino]-closo-m-carboran-1-yl}pentan-1-yl|thymidine (4.11).

Compound 4.11 was prepared according to the procedure described for compound 4.8 using compound 4.5 (380 mg, 0.76 mmol), dThd (460 mg, 1.9 mmol), potassium carbonate (420 mg, 3.04 mmol). Purification by silica gel column chromatography (dichloromethane/acetone, 2:1) gave 4.11 as a white solid in 79% (340 mg) yield. $R_f$ 0.21; $^1$H-NMR (acetone-$d^6$) $\delta$ 1.23-1.32 (m, 2H, CH$_2$), 1.36 (s, 9H, C(CH$_3$)$_3$), 1.39-1.45 (m, 2H, CH$_2$), 1.52-1.61 (m, 2H, CH$_2$), 1.81-1.82 (d, 3H, CH$_3$, $J$ = 1.2 Hz), 2.02-2.07 (m, 2H, CH$_2$-C$_{\text{carborane}}$), 2.20-2.25 (m, 2H, H-2´), 3.75-3.79 (m, 2H, H-5´), 3.81-3.87 (m, 2H, CH$_2$N), 3.90-3.94 (m, 1H, H-4´), 4.21-4.26 (t, 1H, OH-5´, $J$ = 5.0 Hz), 4.38-4.40 (d, 1H, OH-3´, $J$ = 4.2 Hz), 4.44-4.51 (m, 1H, H-3´), 6.33 (t, 1H, H-1´, $J$ = 6.6 Hz), 7.49 (br s, 1H, NH), 7.81 (q, 1H, H-6, $J$ = 1.2 Hz); $^{13}$C-NMR (CDCl$_3$) $\delta$ 13.03 (CH$_3$), 26.24 (CH$_2$), 26.94 (CH$_2$), 27.94 (CH$_3$), 31.24 (CH$_2$), 36.34 (CH$_2$-C$_{\text{carborane}}$), 40.21 (CH$_2$N), 40.85 (C-2´), 61.98 (C-5´), 70.97 [OC(CH$_3$)$_3$], 73.65 (C-3´), 80.56 (C$_{\text{carborane}}$-C), 81.11 (C$_{\text{carborane}}$-C), 86.37 (C-1´), 86.94 (C-4´), 109.81 (C-5), 134.55 (C-6), 150.70 (C=O), 151.98 (C=O), 163.27 (C=O); MS (HR-ESI) C$_{22}$H$_{43}$B$_{10}$N$_3$O$_7$Na (M+Na)$^+$ calcd: 592.3999, found: 592.3986.
3-{6-[(7-(tert-Butyloxy)carbonylamino)-closo-m-carboran-1-yl]hexan-1-yl}thymidine (4.12).

Compound 4.12 was prepared according to the procedure described for compound 4.8 using compound 4.6 (208 mg, 0.40 mmol), dThd (262 mg, 1.08 mmol), potassium carbonate (273 mg, 1.98 mmol). Purification by silica gel column chromatography (dichloromethane/acetone, 2:1) gave 4.12 as a white solid in 55% (128 mg) yield. $R_f$ 0.23; $^1$H-NMR (CDCl$_3$) $\delta$ 1.18-1.29 (m, 6H, CH$_2$), 1.39 (s, 9H, C(CH$_3$)$_3$), 1.52-1.54 (m, 2H, CH$_2$), 1.86-1.89 (m, 2H, CH$_2$-C$_{carborane}$), 1.90 (d, 3H, CH$_3$, $J$ = 1.0 Hz), 2.30-2.40 (m, 2H, H-2’), 3.79-3.92 (m, 4H, H-5’ and CH$_2$N), 3.97-4.00 (m, 1H, H-4’), 4.55-4.59 (m, 1H, H-3’), 6.15 (t, 1H, H-1’, $J$ = 6.8 Hz), 7.32 (d, 1H, H-6, $J$ = 1.1 Hz); $^{13}$C-NMR (CDCl$_3$) $\delta$ 13.28 (CH$_3$), 26.37 (CH$_2$), 27.26 (CH$_2$), 28.13 (CH$_3$), 28.67 (CH$_2$), 29.71 (CH$_2$), 36.96 (CH$_2$-C$_{carborane}$), 40.08 (CH$_2$N), 41.15 (C-2’), 62.40 (C-5’), 71.52 [OC(CH$_3$)$_3$], 74.03 (C-3’), 80.45 (C$_{carborane}$-C), 81.59 (C$_{carborane}$-C), 86.86 (C-1’), 87.65 (C-4’), 110.33 (C-5), 134.86 (C-6), 150.95 (C=O), 152.02 (C=O), 163.30 (C=O); MS (HR-ESI) C$_{23}$H$_{45}$B$_{10}$N$_3$O$_7$Na (M+Na)$^+$ calcd: 606.4085, found: 606.4249.

\[
\begin{align*}
&\text{HO} \\
&\text{N} \\
&\text{O} \\
&\text{O} \\
&\text{N} \\
&\text{O} \\
&\text{O} \\
&\text{OH}
\end{align*}
\]

3-{2-[(7-(tert-Butyloxy)carbonylamino)-closo-m-carboran-1-yl]ethyloxyethyl} thymidine (4.13).
Compound 4.13 was prepared according to procedure described for compound 4.8 using compound 4.7 (160 mg, 0.32 mmol), dThd (200 mg, 0.80 mmol), potassium carbonate (175 mg, 1.28 mmol). Purification by silica gel column chromatography (ethyl acetate/methanol, 15:1) gave 4.13 as a white solid in 50% (90 mg) yield. \( R_f \) 0.15; \(^1\)H-NMR (acetone-\(d^6\)) \( \delta \) 1.38 (s, 9H, C(CH\(_3\))\(_3\)), 1.83 (d, 3H, CH\(_3\), \( J = 1.2 \) Hz), 2.15-2.20 (m, 2H, CH\(_2\)-C\(_{\text{carborane}}\)), 2.22-2.26 (m, 2H, H-2´), 3.40 (t, 2H, OCH\(_2\), \( J = 6.3 \) Hz), 3.57 (t, 2H, OCH\(_2\), \( J = 6.0 \) Hz), 3.75-3.81 (m, 2H, H-5´), 3.94-3.96 (m, 1H, H-4´), 4.08 (t, 2H, CH\(_2\)N, \( J = 6.0 \) Hz), 4.26 (t, 1H, OH-5´, \( J = 5.1 \) Hz), 4.41 (d, 1H, OH-3´, \( J = 4.2 \) Hz), 4.47-4.50 (m, 1H, H-3´), 6.32-6.37 (t, 1H, H-1´, \( J = 6.9 \) Hz), 7.47 (br s, 1H, NH), 7.82-7.83 (d, 1H, H-6, \( J = 1.2 \) Hz); \(^{13}\)C-NMR (MeOH-\(d^4\)) \( \delta \) 13.30 (CH\(_3\)), 28.27 (CH\(_3\)), 37.41 (CH\(_2\)-C\(_{\text{carborane}}\)), 40.40 (CH\(_2\)N), 41.15 (C-2´), 62.74 (C-5´), 67.45 (CH\(_2\)), 69.37 (CH\(_2\)), 71.80 [OC(CH\(_3\))\(_3\)], 72.02 (C-3´), 79.45 (C-3´, C\(_{\text{carborane}}-\text{C} \)), 80.79 (C-3´, C\(_{\text{carborane}}-\text{C} \)), 86.40 (C-1´), 88.54 (C-4´), 109.79 (C-5), 135.62 (C-6), 151.67 (C=O), 153.26 (C=O) 163.75 (C=O); MS (HR-ESI) C\(_{21}\)H\(_{41}\)B\(_{10}\)N\(_3\)O\(_8\)Na (M+Na\(^+\)) calcd: 594.3788, found: 594.3777.


Trifluoroacetic acid (0.5 mL, 0.74 mmol) was added to a solution of compound 4.8 (195 mg, 0.37 mmol) dissolved in dichloromethane (10 mL) 0 °C. The mixture was
stirred at room temperature for 24 h. After evaporation, triethylamine (1 mL) and distilled water (10 mL) were added. (Caution: It is necessary to remove CF$_3$COOH completely by evaporation to avoid the generation of toxic fumes due to acid-base reaction). The residue was extracted with ethyl acetate (20 mL × 5). The combined organic layers were washed with brine, and dried over magnesium sulfate. After filtration and evaporation, the residue was purified by silica gel column chromatography (ethyl acetate/MeOH, 15:1) to give compound 4.14.A as a white solid in 85% (135 mg) yield. $R_f$ 0.13; $^1$H-NMR (MeOH-d$_4$) $\delta$ 1.87 (d, 3H, CH$_3$, $J = 1.2$ Hz), 2.11-2.29 (m, 4H, CH$_2$-C$_{carborane}$ and H-2´), 3.72 (dd, 1H, H-5´, $J = 12.0$, 3.1 Hz), 3.80 (dd, 1H, H-5´, $J = 12.0$, 3.1 Hz), 3.86-3.93 (m, 3H, H-4´ and CH$_2$N), 4.34-4.38 (m, 1H, H-3´), 6.25 (t, 1H, H-1´, $J = 6.5$ Hz), 7.81 (d, 1H, H-6, $J = 1.2$ Hz); $^{13}$C-NMR (MeOH-d$_4$) $\delta$ 13.08 (CH$_3$), 34.03 (CH$_2$-C$_{carborane}$), 41.31 (CH$_2$N), 41.45 (C-2´), 62.71 (C-5´), 72.04 (C-3´), 74.68 (C$_{carborane}$-C), 87.13 (C-1´), 88.88 (C-4´), 89.93 (C$_{carborane}$-C), 110.61 (C-5), 136.66 (C-6), 151.90 (C-2), 164.95 (C-4); MS (HR-ESI) C$_{14}$H$_{29}$B$_{10}$N$_3$O$_5$Na (M+Na)$^+$ calcd: 450.3016, found: 450.3022; HPLC retention time: 19.01 min (Method C), 18.47 min (Method D)

![Chemical Structure](attachment:chemical_structure.png)

3-[3-(7-Amino-closo-m-carboran-1-yl)propan-1-yl]thymidine (4.15.A).
Compound **4.15.A** was prepared according to the procedure described for compound **4.14.A** using compound **4.9** (1.10 g, 2.03 mmol) and trifluoroacetic acid (2.74 mL, 4.06 mmol). Purification by silica gel column chromatography (ethyl acetate/methanol, 15:1) gave **4.15.A** as a white solid in 87% (780 mg) yield. \( R_f \) 0.16; \(^1\)H-NMR (MeOH-d\(^4\)) \( \delta \) 1.60-1.73 (m, 2H, CH\(_3\)), 1.90 (s, 3H, CH\(_3\), \( J = 1.1 \) Hz), 1.95-2.02 (m, 2H, CH\(_2\)-C\(_{\text{carborane}}\)), 2.13-2.32 (m, 2H, H-2’), 3.69-3.84 (m, 4H, H-5’ and CH\(_2\)N), 3.88-3.93 (m, 1H, H-4’), 4.36-4.41 (m, 1H, H-3’), 6.29 (t, 1H, H-1’, \( J = 6.6 \) Hz), 7.82 (d, 1H, H-6, \( J = 1.1 \) Hz); \(^{13}\)C-NMR (MeOH-d\(^4\)) \( \delta \) 13.16 (CH\(_3\)), 29.16 (CH\(_2\)), 35.30 (CH\(_2\)-C\(_{\text{carborane}}\)), 41.31 (CH\(_2\)N), 41.44 (C-2’), 62.74 (C-5’), 72.06 (C-3’), 74.71 (C\(_{\text{carborane}}\)-C), 87.12 (C-1’), 88.87 (C-4’), 91.18 (C\(_{\text{carborane}}\)-C), 110.68 (C-5), 136.62 (C-6), 152.24 (C-2), 165.31 (C-4); MS (HR-ESI) C\(_{15}\)H\(_{31}\)B\(_{10}\)N\(_3\)O\(_5\)Na (M+Na)\(^+\) calcd 464.3178, found 464.3184; HPLC retention time: 19.08 min (Method C), 18.54 min (Method D).

![Image of compound 4.15.A](image)

3-[(4-(7-Amino-closo-\( m \)-carboran-1-yl)butan-1-yl)thymidine (4.16.A).

Compound **4.16.A** was prepared according to the procedure described for compound **4.14.A** using compound **4.10** (95 mg, 0.16 mmol) and trifluoroacetic acid (0.22 mL, 0.32 mmol). Purification by silica gel column chromatography (ethyl acetate/methanol, 15:1) gave **4.16.A** as a white solid in 69% (48 mg) yield. \( R_f \) 0.18; \(^1\)H-
NMR (MeOH-d$_4$) δ 1.34-1.39 (m, 2H, CH$_2$), 1.49-1.55 (m, 2H, CH$_2$), 1.89-1.90 (d, 3H, CH$_3$, $J$ = 1.1 Hz), 1.96-2.01 (m, 2H, CH$_2$-C$_{carborane}$), 2.15-2.29 (m, 2H, H-2´), 3.72 (dd, 1H, H-5´, $J$ = 12.1, 3.4 Hz), 3.79 (dd, 1H, H-5´, $J$ = 12.1, 3.4 Hz), 3.85-3.91 (m, 3H, CH$_2$N and H-4´), 4.37-4.39 (m, 1H, H-3´), 6.29 (t, 1H, H-1´, $J$ = 6.4 Hz), 7.84 (d, 1H, H-6, $J$ = 1.1 Hz); $^{13}$C-NMR (MeOH-d$_4$) δ 13.21 (CH$_3$), 27.99 (CH$_2$), 28.30 (CH$_2$), 37.46 (CH$_2$-C$_{carborane}$), 41.38 (CH$_2$N), 41.56 (C-2´), 62.76 (C-5´), 72.11 (C-3´), 75.36 (C$_{carborane}$-C), 87.16 (C-1´), 88.90 (C-4´), 89.45(C$_{carborane}$-C), 110.70 (C-5), 136.51 (C-6), 152.29 (C-2), 165.37 (C-4); MS (HR-ESI) C$_{16}$H$_{33}$B$_{10}$N$_3$O$_5$Na (M+Na)$^+$ calcd: 478.3316, found: 478.3309; HPLC retention time: 20.81 min (Method C), 19.88 min (Method D).

![Chemical structure](image)

3-[5-(7-Amino-closo-m-carboran-1-yl)pentan-1-yl]thymidine (4.17.A).

Compound 4.17.A was prepared according to the procedure described for compound 4.14.A using compound 4.11 (330 mg, 0.58 mmol) and trifluoroacetic acid (0.78 mL, 1.16 mmol). Purification by silica gel column chromatography (ethyl acetate/methanol, 15:1) gave 4.17.A as a white solid in 81% (220 mg) yield. $R_f$ 0.20; $^1$H-NMR (MeOH-d$_4$) δ 1.22-1.30 (m, 2H, CH$_2$), 1.34-1.45 (m, 2H, CH$_2$), 1.51-1.60 (m, 2H, CH$_2$), 1.89 (d, 3H, CH$_3$, $J$ = 1.2 Hz), 1.92-1.99 (m, 2H, CH$_2$-C$_{carborane}$), 2.19-2.25 (m, 2H, H-2´), 3.71 (dd, 1H, H-5´, $J$ = 12.0, 3.7 Hz), 3.79 (dd, 1H, H-5´, $J$ = 12.0, 3.7 Hz), 3.85-
3.93 (m, 3H, H-4’ and CH2N), 4.36-4.41 (m, 1H, H-3’), 6.29 (t, 1H, H-1’, J = 6.5 Hz), 7.82 (d, 1H, H-6, J = 1.2 Hz); 13C-NMR (acetone-d6) δ 13.20 (CH3), 27.37 (CH2), 28.04 (CH2), 30.73 (CH2), 37.79 (CH2-Ccarborane), 41.31 (CH2N), 42.01 (C-2’), 62.75 (C-5’), 72.09 (C-3’), 75.50 (Ccarborane-C), 87.11 (C-1’), 88.86 (C-4’), 89.41(Ccarborane-C), 110.71 (C-5), 136.47 (C-6), 152.30 (C-2), 165.41 (C-4); MS (HR-ESI) C17H35B10N3O5Na (M+Na)+ calcd: 492.3472, found: 492.3459; HPLC retention time: 22.65 min (Method C), 21.25 min (Method D).

3-[5-(7-Amino-closo-m-carboran-1-yl)hexan-1-yl]thymidine (4.18.A).

Compound 4.18.A was prepared according to the procedure described for compound 4.14.A using compound 4.12 (84 mg, 0.14 mmol) and trifluoroacetic acid (0.19 mL, 0.28 mmol). Purification by silica gel column chromatography (ethyl acetate/methanol, 15:1) gave 4.18.A as a white solid in 78% (53 mg) yield. Rf 0.21; 1H-NMR (MeOH-d4) δ 1.21-1.28 (m, 4H, CH2), 1.33-1.39 (m, 2H, CH2), 1.53-1.58 (m, 2H, CH2), 1.89 (d, 3H, CH3, J = 1.1 Hz), 1.91-1.96 (m, 2H, CH2-Ccarborane), 2.15-2.29 (m, 2H, H-2’), 3.71 (dd, 1H, H-5’, J = 12.1 & 3.4 Hz), 3.79 (dd, 1H, H-5’, J = 12.1, 3.4 Hz), 3.86-3.91 (m, 3H, CH2N and H-4’), 4.37-4.39 (m, 1H, H-3’), 6.30 (t, 1H, H-1’, J = 6.9 Hz), 7.83 (d, 1H, H-6, J = 1.1 Hz); 13C-NMR (MeOH-d4) δ 13.20 (CH3), 27.46 (CH2), 28.32
(CH₂), 29.77 (CH₂), 30.67 (CH₂), 31.02 (CH₂), 37.90 (CH₂-Ccarborane), 41.33 (CH₂N), 42.15 (C-2’), 62.76 (C-5’), 72.10 (C-3’), 75.58 (Ccarborane-C), 87.11 (C-1’), 88.87 (C-4’), 89.40 (Ccarborane-C), 110.71 (C-5), 136.46 (C-6), 152.31 (C-2), 165.41 (C-4); MS (HR-ESI) C₁₈H₃₇B₁₀N₃O₅Na (M+Na)⁺ calcd: 506.3629, found: 506.3811; HPLC retention time: 24.76 min (Method C), 23.35 min (Method D).


Compound 4.19.A was prepared according to the procedure described for compound 4.14.A using compound 4.13 (60 mg, 0.10 mmol) and trifluoroacetic acid (0.14 mL, 0.20 mmol). Purification by silica gel column chromatography (ethyl acetate/methanol, 15:1) gave 4.19.A as a white solid in 85% (40 mg) yield. Rf 0.13; ¹H-NMR (MeOH-d⁴) δ 1.91 (d, 3H, CH₃, J = 1.2 Hz), 2.07-2.17 (m, 2H, CH₂-Ccarborane), 2.21-2.29 (m, 2H, H-2’), 3.37 (m, 2H, OCH₂, J = 5.7 Hz), 3.59 (t, 2H, OCH₂, J = 5.6 Hz), 3.71 (dd, 1H, H-5’, J = 12.0, 3.7 Hz), 3.79 (dd, 1H, H-5’, J = 12.0, 3.7 Hz), 3.88-3.92 (m, 2H, H-4’), 4.14 (m, 2H, CH₂N, J = 5.7 Hz), 4.36-4.41 (m, 1H, H-3’), 6.32 (t, 1H, H-1’, J = 6.9 Hz), 7.85 (d, 1H, H-6, J = 1.2 Hz); ¹³C-NMR (MeOH-d⁴) δ 13.30 (CH₃), 37.73 (CH₂-Ccarborane), 41.37 (CH₂N), 42.43 (C-2’), 62.78 (C-5’), 67.90 (CH₂), 70.08 (CH₂), 72.15 (C-3’), 73.26 (Ccarborane-C), 87.16 (C-1’), 88.89 (C-4’), 89.74 (Ccarborane-C), 110.77 (C-5),
136.70 (C-6), 152.44 (C-2), 165.53 (C-4); MS (HR-ESI) C$_{16}$H$_{33}$B$_{10}$N$_3$O$_6$Na (M+Na)$^+$
calcd: 494.3264, found: 494.3250; HPLC retention time: 18.08 min (Method C), 17.61
min (Method D).


Tetrabutylbutylammonium fluoride hydrate (200 mg, 0.63 mmol) was added to a
solution of compound 4.14.A (50 mg, 0.12 mmol) in THF (2 mL) and stirred at 70 °C for
1 h. The progress of the reaction was monitored by the IR. Distilled water (5 mL) was
added at 0 °C, followed by the addition of 3N-hydrochloric acid to adjust the pH to 2-3.
The solution was extracted with ethyl acetate (20 mL × 5) and the combined organic
layers were washed with brine, and dried over magnesium sulfate. After filtration and
evaporation, the residue was purified by silica gel column chromatography (ethyl acetate/
methanol, 15:1, 1% acetic acid) to give compound 4.14.B in 70% (35 mg) yield. $R_f$ 0.10;
$^1$H-NMR (MeOH-d$_4$) δ -1.94 (br s, 1H, H$_µ$), 1.90 (d, 3H, CH$_3$, $J = 1.1$ Hz), 1.92-1.97 (m, 2H, CH$_2$-C$_{carborane}$), 2.16-2.32 (m, 2H, H-2´'), 3.72 (m, 2H, H-5´), 3.88-3.93 (m, 1H, H-4´),
4.02-4.22 (m, 2H, CH$_2$N), 4.36-4.41 (m, 1H, H-3´), 6.27 & 6.36 (t, 1H, H-1´, $J = 6.7$ Hz),
7.81 & 7.82 (d, 1H, H-6, $J = 1.1$ Hz); $^{13}$C-NMR (MeOH-d$_4$) δ 13.28 (CH$_3$), 36.56 &
36.64 (CH$_2$-C$_{carborane}$), 41.21 & 41.42 (CH$_2$N), 44.70 & 44.82 (C-2´), 62.74 & 92.91 (C-
5’), 72.00 & 72.12 (C-3’), 86.94 & 87.28 (C-1’), 88.84 (C-4’), 111.72 & 110.20 (C-5), 136.23 & 136.41 (C-6), 152.32 (C-2), 165.63 & 165.70 (C-4); 11B NMR (MeOH-d4): δ -33.41, -32.35, -20.19, -15.71, -2.24, -0.58; MS (HR-ESI) C14H30B9N3O5Na (M+Na)+ calcd: 441.2968, found: 441.2964; HPLC retention time: 14.21 & 14.43 min (Method C), 14.49 & 14.65 min (Method D).

3-[3-(7-Ammonium-nido-m-carboran-1-yl)propan-1-yl]thymidine (4.15.B).

Compound 4.15.B was prepared according to the procedure described for compound 4.14.B using compound 4.14.A (25 mg, 0.057 mmol) and tetrabutylbutylammonium fluoride hydrate (77 mg, 0.33 mmol). Purification by silica gel column chromatography (ethyl acetate/methanol, 15:1, 1% acetic acid) gave 4.15.B in 61% (15 mg) yield. Rf 0.12; 1H-NMR (MeOH-d4) δ -1.86 (br s, 1H, Hµ) 1.54-1.82 (m, 4H, CH2-Ccarborane and CH2), 1.90 (d, 3H, CH3, J =1.1 Hz), 2.14-2.30 (m, 2H, H-2´), 3.72 (dd, 1H, H-5´, J =12.0, 3.1 Hz), 3.80 (dd, 1H, H-5´, J =12.0, 3.1 Hz), 3.89-3.94 (m, 3H, H-4´ and CH2N), 4.36-4.42 (m, 1H, H-6), 6.30 (t, 1H, H-1´, J = 6.8 Hz), 7.82 (d, 1H, H-6, J =1.1 Hz); 13C-NMR (MeOH-d4) δ 13.23 (CH3), 31.74 (CH2), 37.01 (CH2-Ccarborane), 41.35 (CH2N), 42.81 (C-2´), 62.76 (C-5´), 72.07 (C-3´), 87.11 (C-1´), 88.85 (C-4´), 111.75 (C-5), 136.38 (C-6), 152.32 (C-2), 165.45 (C-4); 11B-NMR (MeOH-d4): δ -36.85,
3-[4-(7-Ammonium-\textit{nido-m}-carboran-1-yl)butan-1-yl]thymidine (4.16.B).

Compound 4.16.B was prepared according to the procedure described for compound 4.14.B using compound 4.16.A (45 mg, 0.099 mmol) and tetrabutylbutylammonium fluoride hydrate (105 mg, 0.33 mmol). Purification by silica gel column chromatography (ethyl acetate/methanol, 15:1, 1% acetic acid) gave 4.16.B in 66% (29 mg) yield. $R_f$ 0.13; $^1$H-NMR (MeOH-d$_4$) $\delta$ -1.92 (br s, 1H, H$_\mu$) 1.64-1.75 (m, 6H, CH$_2$-C$_{\text{carborane}}$ and CH$_2$), 1.90 (s, 3H, CH$_3$), 2.05-2.33 (m, 2H, H-2$'$), 3.72 (dd, 1H, H-5$'$, $J = 12.0$, 3.7 Hz), 3.79 (dd, 1H, H-5$'$, $J = 12.0$, 3.7 Hz), 3.88-3.91 (m, 3H, H-4$'$ and CH$_2$N), 4.36-4.41 (m, 1H, H-3$'$), 6.29 (t, 1H, H-1$'$, $J = 6.6$ Hz), 7.82 (s, 1H, H-6); $^{13}$C-NMR (MeOH-d$_4$) $\delta$ 13.24 (CH$_3$), 28.81 (CH$_2$), 31.18 (CH$_2$), 39.57 (CH$_2$-C$_{\text{carborane}}$), 41.38 (CH$_2$N), 42.58 (C-2$'$), 62.76 (C-5$'$), 72.08 (C-3$'$), 87.15 (C-1$'$), 88.87 (C-4$'$), 110.76 (C-5), 136.41 (C-6), 152.34 (C-2), 165.48 (C-4); $^{11}$B-NMR (MeOH-d$_4$) $\delta$ -33.94, -32.18, -20.29, -16.10, -1.88, -0.52; MS (HR-ESI) C$_{\text{16}}$H$_{\text{34}}$B$_{\text{9}}$N$_{\text{3}}$O$_{\text{5}}$Na (M+Na)$^+$ calcd: 469.3282, found: 469.3274; HPLC retention time: 15.38 min (Method C), 15.43 min (Method D).
3-[5-(7-Ammonium-nido-m-carboran-1-yl)pentan-1-yl]thymidine (4.17.B).

Compound **4.17.B** was prepared according to the procedure described for compound **4.14.B** using compound **4.17.A** (50 mg, 0.11 mmol) and tetrabutylbutylammonium fluoride hydrate (125 mg, 0.40 mmol). Purification by silica gel column chromatography (ethyl acetate/methanol, 15:1, 1% acetic acid) gave **4.17.B** in 65% (33 mg) yield. Rf 0.15; ¹H-NMR (MeOH-d⁴) δ -1.88 (br s, 1H, Hµ), 1.32-1.38 (m, 2H, CH₂), 1.56-1.78 (m, 6H, CH₂-Ccarborane and CH₂), 1.90 (d, 3H, CH₃, J = 1.2 Hz), 2.13-2.33 (m, 2H, H-2´), 3.72 (dd, 1H, H-5´, J = 12.0, 3.8 Hz), 3.79 (dd, 1H, H-5´, J = 12.0, 3.8 Hz) 3.84-3.86 (m, 3H, CH₂N and H-4´), 4.36-4.41 (m, 1H, H-3´), 6.23 (t, 1H, H-1´, J = 6.9 Hz), 7.82 (d, 1H, H-6, J = 1.2 Hz); ¹³C NMR (MeOH-d⁴) δ 13.24 (CH₃), 28.28 (CH₂), 28.69 (CH₂), 33.69 (CH₂), 39.73 (CH₂-Ccarborane), 41.34 (CH₂N), 42.55 (C-2´), 62.77 (C-5´), 72.09 (C-3´), 87.15 (C-1´), 88.87 (C-4´), 110.77 (C-5), 136.44 (C-6), 152.34 (C-2), 165.49 (C-4); MS (HR-ESI) C₁₇H₃₆B₉N₃O₅Na (M+Na)⁺ calcd: 483.3420, found: 483.3420, C₁₇H₃₆B₉N₃O₅Na₂ (M-H+Na₂)⁺ calcd: 505.3240, found: 505.3240; HPLC retention time: 16.36 min (Method C), 16.12 min (Method D).
3-[6-(7-Ammonium-nido-\(m\)-carboran-1-yl)hexan-1-yl]thymidine (4.18.B).

Compound 4.18.B was prepared according to the procedure described for compound 4.14.B using compound 4.18.A (40 mg, 0.083 mmol) and tetrabutylbutylammonium fluoride hydrate (100 mg, 0.32 mmol). Purification by silica gel column chromatography (ethyl acetate/methanol, 15:1, 1% acetic acid) gave 4.18.B in 53% (21 mg) yield. R\(_f\) 0.17; \(^1\)H-NMR (MeOH-d\(^4\)) \(\delta\) -1.89 (br s, 1H, H\(\mu\)) 1.30-1.38 (m, 4H, CH\(_2\)), 1.64-1.79 (m, 6H, CH\(_2\)-C\(_{\text{carborane}}\) and CH\(_2\)), 1.90 (d, 3H, CH\(_3\), \(J = 1.1\) Hz), 2.13-2.29 (m, 2H, H-2\(´\)), 3.71 (dd, 1H, H-5\(´\), \(J = 12.0\), 3.0 Hz), 3.80 (dd, 1H, H-5\(´\), \(J = 12.0\), 3.0 Hz), 3.90-3.92 (m, 3H, H-4\(´\) and CH\(_2\)N), 4.36-4.41 (m, 1H, H-3\(´\)), 6.29 (t, 1H, H-1\(´\), \(J = 6.5\) Hz), 7.82 (d, 1H, H-6, \(J = 1.1\) Hz); \(^{13}\)C-NMR (MeOH-d\(^4\)) \(\delta\) 13.23 (CH\(_3\)), 28.06 (CH\(_2\)), 28.63 (CH\(_2\)), 30.69 (CH\(_2\)), 33.94 (CH\(_2\)), 39.78 (CH\(_2\)-C\(_{\text{carborane}}\)), 41.34 (CH\(_2\)N), 42.45 (C-2\(´\)), 62.77 (C-5\(´\)), 72.11 (C-3\(´\)), 87.12 (C-1\(´\)), 88.88 (C-4\(´\)), 110.74 (C-5), 136.43 (C-6), 152.33 (C-2), 165.46 (C-4); \(^{11}\)B-NMR (MeOH-d\(^4\)) \(\delta\) -33.87, -32.15, -20.15, -15.94, -1.91, -0.32; MS (HR-ESI) C\(_{18}\)H\(_{38}\)B\(_9\)N\(_3\)O\(_5\)Na (M+Na\(^+\)) calcd: 497.3597, found: 497.3596; HPLC retention time: 17.77 min (Method C), 17.23 min (Method D).

Compound 4.19.B was prepared according to the procedure described for compound 4.14.B using compound 4.19.A (60 mg, 0.13 mmol) and tetrabutylbutylammonium fluoride hydrate (150 mg, 0.48 mmol). Purification by silica gel column chromatography (ethyl acetate/methanol, 13:1, 1% acetic acid) gave 4.19.B in 50% (30 mg) yield. \( R_f \) 0.11; \(^1\)H-NMR (MeOH-d\(^4\)) \( \delta \) -1.94 (br s, 1H, H\( \mu \)) 1.55-1.69 (m, 2H, CH\(_2\)-C\(_{\text{carborane}}\)), 1.90 (d, 3H, CH\(_3\), \( J = 1.1 \) Hz), 2.15-2.33 (m, 2H, H-2'), 3.54-3.63 (m, 4H, OCH\(_2\)) 3.70 (dd, 1H, H-5', \( J = 12.1, 3.2 \) Hz), 3.79 (dd, 1H, H-5', \( J = 12.1, 3.2 \) Hz), 3.88-3.92 (m, 1H, H-4') 4.13 (t, 2H, CH\(_2\)N, \( J = 6.2 \) Hz), 4.36-4.41 (m, 1H, H-3'), 6.29 (t, 1H, H-1', \( J = 6.8 \) Hz), 7.81 (d, 1H, H-6, \( J = 1.1 \) Hz); \(^1\)C-NMR (MeOH-d\(^4\)) \( \delta \) 13.25 (CH\(_3\)), 38.82 (CH\(_2\)-C\(_{\text{carborane}}\)), 41.38 (CH\(_2\)N), 41.40 (C-2'), 62.76 (C-5'), 67.77 (CH\(_2\)), 72.06 (C-3'), 74.57 (CH\(_2\)), 87.18 (C-1'), 88.86 (C-4'), 110.68 (C-5), 136.57 (C-6), 152.41 (C-2), 165.52 (C-4); \(^{11}\)B-NMR (MeOH-d\(^4\)): \( \delta \) -33.86, -32.26, -20.23, -16.08, -2.00, -0.57; MS (HR-ESI) C\(_{16}\)H\(_{34}\)B\(_9\)N\(_3\)O\(_6\)Na (M+Na\(^+\)) calcd: 485.3232, found: 485.3232; HPLC retention time: 14.48 min (Method C), 14.63 min (Method D).
7-Amino-7, 9-nido-m-carborane (4.21)

Compound 4.21 was prepared from m-carborane by applying the procedure reported by Kahl et al.\textsuperscript{121} $R_f$ 0.32 (hexanes/ethylacetate, 1:1); $^1$H-NMR (CD$_3$CN) $\delta$ 3.23 (br s, 1H, H-C$_{carborane}$), 3.78 (br s, 2H, NH$_2$); $^{13}$C-NMR (CD$_3$CN) $\delta$ 54.65 (H-C$_{carborane}$), 90.45 (N-C$_{carborane}$); $^{11}$B NMR (CD$_3$CN) $\delta$ -12.14, -11.56, -9.57, -6.99, -0.62; IR (cm$^{-1}$) 3392 (m), 3332, 2595 (s), 1611 (m), 1218, 1054; MS (HR-ESI) C$_2$H$_{14}$B$_9$N$_1$Na$_1$ (M+H)$^+$ calcd: 160.2125, found: 160.2134.

7-Ammomium-7, 9-nido-m-carborane (4.22)

To a solution of tetrabutylammonium fluoride hydrate (2.50 g, 7.94 mmol) in THF (25 mL) was slowly added a solution of compound 4.21 (420 mg, 2.64 mmol) in THF (5 mL) at 70 °C. The solution was stirred at the same temperature for 1.5 h. After cooling to room temperature, distilled water (10 mL) was added, followed by evaporation of excess THF. Aqueous 3N hydrochloric acid (3 mL) was added to adjust the pH to 2-3. The solution was extracted with ethyl acetate (30 mL $\times$ 2) and the combined ethylacetate layers were washed with brine and dried over magnesium sulfate. After filtration and
evaporation, the residue was purified by silica gel column chromatography (hexanes/ethylacetate, 1:1) to give compound **4.22** in 61% (240 mg, 1.61 m mol) yield. $R_f$ 0.24; $^1$H-NMR (CD$_3$CN) $\delta$ -1.99 (br s, 1H, bridging H), 1.43 (br s, 1H, H-C$_{carborane}$), 6.04 (br s, 3H, NH$_3$); $^{13}$C-NMR (CD$_3$CN) $\delta$ 32.83 (H-C$_{carborane}$), 53.77 (N-C$_{carborane}$); $^{11}$B-NMR (CD$_3$CN) $\delta$ -33.59, -31.95, -20.07, -19.53, -15.75, -3.60, -2.82, 0.82; IR (cm$^{-1}$) 3215 (s), 3189, 2535 (s), 1467 (s); MS (HR-ESI) C$_2$H$_{14}$B$_9$N$_1$Na$_1$ (M+Na)$^+$ calcd: 173.1900, found: 173.1897.

![Chemical Structure](image)

**7-Isopropylideneammonium-7, 9-nido-m-carborane (4.23)**

Compound **4.22** (50 mg, 0.34 mmol) was dissolved in anhydrous acetone (5 mL) and stirred at room temperature for 6 hours. After evaporation of excess acetone, the residue was purified by silica gel flash column chromatography (hexanes/ethylacetate, 1:1) to give compound **4.23** in 95% yield. $R_f$ 0.27; $^1$H-NMR (CD$_3$CN) $\delta$ -1.92 (br s, 1H, bridging H), 1.54 (br s, 1H, H-C$_{carborane}$), 2.35 (s, 3H, CH$_3$), 2.51 (s, 3H, CH$_3$), 10.37 (br s, 3H, C=NH); $^{13}$C-NMR (CD$_3$CN) $\delta$ 23.09 (CH$_3$), 25.56 (CH$_3$), 32.64 (H-C$_{carborane}$), 61.59 (N-C$_{carborane}$), 193.08 (C=N); $^{11}$B-NMR (CD$_3$CN) $\delta$ -32.93, -31.59, -19.75, -19.33, -18.47, -15.59, -2.48, -2.04, 0.21; IR (cm$^{-1}$) 3268 (s), 2529 (s), 1662 (s), 1424, 1003; MS (HR-ESI) C$_5$H$_{18}$B$_9$N$_1$Na$_1$ (M+Na)$^+$ calcd: 213.2215, found: 213.2216.
**7-Cyclohexylideneammonium-7, 9-nido-m-carborane (4.24)**

Compound 4.22 (100 mg, 0.68 mmol) and cyclohexanone (0.13 mL, 1.36 mmol) were dissolved in anhydrous benzene (3 mL) and stirred at room temperature for 6 h. The precipitated solid was collected by filtration and washed with hexanes to give compound 4.24 in 68% yield. \( R_f \) 0.24 (hexanes/ethylacetate, 2:1); \(^1\)H-NMR (CD_3OD) \( \delta \) -1.92 (br s, 1H, bridging H), 1.43 (br s, 1H, H-Carborane), 3.93 (s, 3H, CH_3), 4.04 (s, 3H, CH_3), 6.67 (d, 1H, ArH, \( J = 2.2 \) Hz), 6.75 (dd, 1H, ArH, \( J = 8.8, 2.2 \) Hz), 7.67 (d, 1H, ArH, \( J = 8.8 \) Hz), 8.45 (s, 1H, CH=N), 10.68 (br s, 1H, NH); \(^{13}\)C-NMR (CD_3OD) \( \delta \) 25.30 (CH_2), 28.08 (CH_2), 28.72 (CH_2), 33.15 (H-Carborane), 33.75 (CH_2), 35.82 (CH_2), 62.57 (N-Carborane), 196.08 (C=N); MS (HR-ESI) \( C_8H_{22}B_9N \) (M+Na)^+ calculated: 252.2561, found: 252.2570.

**7-(2,4-Dimethoxy)benzylideneammonium-7, 9-nido-m-carborane (4.25)**

Compound 4.22 (100 mg, 0.68 mmol) and 2,4-dimethoxybenzaldehyde (0.2 mL, 1.20 mmol) were dissolved in anhydrous benzene (3 mL) and stirred at room temperature
for 6 hours. The precipitated solid was collected by filtration and washed with hexanes to
give compound 4.25 in 61% yield. $R_f$ 0.29 (hexanes/ethylacetate, 2:1); $^1$H-NMR
(CD$_3$CN) $\delta$ -1.92 (br s, 1H, bridging H), 1.57 (br s, 1H, H-C$_{carborane}$), 3.93 (s, 3H, CH$_3$),
4.04 (s, 3H, CH$_3$), 6.67 (d, 1H, ArH, $J = 2.2$ Hz), 6.75 (dd, 1H, ArH, $J = 8.8$, 2.2 Hz),
7.67 (d, 1H, ArH, $J = 8.8$ Hz), 8.45 (s, 1H, CH=N), 10.68 (br s, 1H, NH); $^{13}$C-NMR
(CD$_3$CN) $\delta$ 33.02 (H-C$_{carborane}$), 57.32 (OCH$_3$), 57.90 (OCH$_3$), 65.10 (N-C$_{carborane}$), 99.74
(ArC), 109.30 (ArC), 109.99 (ArC), 139.86 (ArC), 163.86 (C=N), 164.88 (ArC), 167.37
(ArC); MS (HR-ESI) C$_{11}$H$_{22}$B$_9$NO$_2$ (M+Na)$^+$ calculated: 320.2458, found: 320.2447.

7.4. Chapter 5

Compounds 5.1-5.3 were prepared from dThd by adapting procedures reported
previously.$^{287,288}$ The reported $^1$H- and $^{13}$C-NMR data of 5.1-5.3 were identical with those
obtained by us.

5´-O-Tosylthymidine (5.1)

$^1$H-NMR (DMSO-d$_6$) $\delta$ 1.77 (s, 3H, CH$_3$), 2.06-2.16 (m, 2H, H-2´), 2.41 (s, 3H,
CH$_3$), 3.85-3.88 (m, 1H, H-4´), 4.13-4.17 (m, 1H, H-3´), 4.16 (dd, 1H, H-5´, $J = 10.7$, 5.5
Hz), 4.25 (dd, 1H, H-5´, $J =$10.7, 3.2 Hz), 5.42 (d, 1H, OH, $J = 4.3$ Hz), 6.14 (t, 1H, H-1´,
$J = 7.0$ Hz, 7.38 (s, 1H, H-6), 7.47 (d, 2H, ArH, $J = 8.3$ Hz), 7.79 (d, 2H, ArH, $J = 8.3$ Hz), 11.3 (s, 1H, NH); $^{13}$C-NMR (DMSO-d$_6$) $\delta$ 12.04 (CH$_3$), 21.07 (CH$_3$), 38.32 (C-2'), 69.90 (C-5'), 70.12 (C-3'), 83.17 (C-1'), 83.96 (C-4'), 109.76 (C-5), 127.57 (ArC), 130.15 (ArC), 132.10 (ArC), 135.84 (C-6), 145.10 (ArC), 150.32 (C-2), 163.60 (C-4).

5'-Azido-5'-deoxythymidine (5.2)

$^{1}$H-NMR (DMSO-d$_6$) $\delta$ 1.79 (s, 3H, CH$_3$), 2.04-2.28 (m, 2H, H-2'), 3.50 (d, 2H, H-5', $J = 5.0$ Hz), 3.81-3.87 (m, 1H, H-4'), 4.17-4.21 (m, 1H, H-3'), 5.39 (d, 1H, OH, $J = 4.2$ Hz), 6.20 (t, 1H, H-1', $J = 6.9$ Hz), 7.49 (s, 1H, H-6), 11.3 (s, 1H, NH); $^{13}$C-NMR (CD$_3$OD) $\delta$ 12.05 (CH$_3$), 38.05 (C-2'), 51.67 (C-5'), 70.70 (C-3'), 83.84 (C-1'), 84.51 (C-4'), 109.77 (C-5), 136.03 (C-6), 150.44 (C-2), 163.62 (C-4).
5´-Amino-5´-deoxythymidine (5.3)

$^1$H-NMR (DMSO-d$_6$) $\delta$ 1.79 (s, 3H, CH$_3$), 2.02-2.13 (m, 2H, H-2´), 2.72 (d, 2H, NH$_2$), 3.50-3.58 (m, 2H, H-5), 3.62-3.65 (m, 1H, H-4´), 4.16-4.21 (m, 1H, H-3´), 5.14 (s, 1H, OH), 6.14 (t, 1H, H-1´, $J = 6.9$ Hz), 7.65 (s, 1H, H-6); $^{13}$C NMR (DMSO-d$_6$) $\delta$ 12.05 (CH$_3$), 38.05 (C-2´), 51.66 (C-5´), 70.70 (C-3´), 83.83 (C-1´), 84.51 (C-4´), 109.77 (C-5), 136.03 (C-6), 150.43 (C-2), 163.61 (C-4)

General procedure for the synthesis of sulfonamide-linked BaTK/BaTMPK inhibitors (5.4-5.7)

To a solution of 5´-amino-5´-deoxythymidine 5.3 (60 mg, 0.25 mmol) and the indicated sulfonylchloride (0.28 mmol) [see below] in DMF (5 mL) was added triethylamine (0.5 mL). The resulting solution was stirred for 6 h at room temperature. The solution was cooled to 4 °C, triethylamine hydrochloride was removed by filtration, and the filtrate was concentreated. The residue was triturated with methanol to give a crystalline solid, which was collected by filtration. Washing the solid with hexanes/ethylacetate (1:1) afforded the following products.

5´-Deoxy-5´-[4-(methylsulfonyl)benzenesulfonamido]thymidine (5.4)
Reaction of 4-(methylsulfonyl)benzenesulfonyl chloride with 5.3 provided compound 5.4 (92 mg, 80%). \(R_f\) 0.10 (dichloromethane/methanol, 10:1); \(^1\)H-NMR (DMSO-d<sub>6</sub>) \(\delta\) 1.78 (s, 3H, CH<sub>3</sub>), 2.00-2.15 (m, 2H, H-2\(^{-}\)), 2.98 (dd, 1H, H-5\(^{-}\), \(J = 13.6, 6.7\) Hz), 3.11 (dd, 1H, H-5\(^{-}\), \(J = 13.6, 4.1\) Hz), 3.29 (s, 3H, SO<sub>2</sub>CH<sub>3</sub>), 3.68-3.72 (m, 1H, H-4\(^{-}\)), 4.12-4.15 (m, 1H, H-3\(^{-}\)), 5.33 (d, 1H, OH, \(J = 4.3\) Hz), 6.11 (t, 1H, H-1\(^{-}\), \(J = 6.8\) Hz), 7.49 (s, 1H, H-6), 8.04 (d, 2H, ArH, \(J = 8.4\) Hz), 8.13 (d, 2H, ArH, \(J = 8.4\) Hz), 8.21 (s, 1H, NH), 11.25 (s, 1H, NH); \(^1\)C-NMR (DMSO-d<sub>6</sub>) \(\delta\) 12.10 (CH<sub>3</sub>), 38.19 (C-2\(^{-}\)), 43.14 (C-5\(^{-}\)), 44.71 (SO<sub>2</sub>CH<sub>3</sub>), 70.84 (C-3\(^{-}\)), 83.86 (C-1\(^{-}\)), 84.69 (C-4\(^{-}\)), 109.70 (C-5), 127.48 (ArC), 128.12 (ArC), 136.28 (C-6), 144.00 (ArC), 145.15 (ArC), 150.44 (C-2), 163.72 (C-4); MS (HR-ESI) C<sub>17</sub>H<sub>21</sub>N<sub>3</sub>O<sub>8</sub>S<sub>2</sub> (M+Na)<sup>+</sup> calcd 482.0668, found 482.0672.

\[ \text{5'}-\text{Deoxy-5'}-[3-(methylsulfonyl)benzenesulfonamido]thymidine (5.5) \]

Reaction of 3-(methylsulfonyl)benzenesulfonyl chloride with 5.3 provided compound 5.5 (89 mg, 78%). \(R_f\) 0.16 (dichloromethane/methanol, 10:1); \(^1\)H-NMR (MeOH-d<sub>4</sub>) \(\delta\) 1.89 (d, 3H, CH<sub>3</sub>, \(J =1.1\) Hz), 2.00-2.23 (m, 2H, H-2\(^{-}\)), 3.10-3.25 (m, 2H, H-5\(^{-}\)), 3.18 (s, 1H, SO<sub>2</sub>CH<sub>3</sub>), 3.78-3.84 (m, 1H, H-4\(^{-}\)), 4.27-4.33 (m, 1H, H-3\(^{-}\)), 6.14 (t, 1H, H-1\(^{-}\), \(J = 6.8\) Hz), 7.52 (d, 1H, H-6, \(J = 1.1\) Hz), 7.82 (t, 1H, ArH, \(J = 7.9\) Hz), 8.17 (d, 1H, ArH, \(J = 7.9\) Hz), 8.18 (d, 1H, ArH, \(J = 7.9\) Hz), 8.40 (t, 1H, ArH, \(J = 1.7\) Hz);
$^{13}$C-NMR (MeOH-d$_4$) δ 12.38 (CH$_3$), 40.18 (C-2'), 44.17 (C-5'), 45.77 (SO$_2$CH$_3$), 72.47 (C-3'), 86.23 (C-1'), 86.61 (C-4'), 111.88 (C-5), 126.86 (ArC), 131.79 (ArC), 132.22 (ArC), 132.83 (ArC), 138.30 (C-6), 143.46 (ArC), 143.88 (ArC), 152.28 (C-2), 166.39 (C-4); MS (HR-ESI) C$_{17}$H$_{21}$N$_3$O$_8$S$_2$ (M+Na)$^+$ calcd 482.0668, found 482.0666.

5'-Deoxy-5'-[2-(methylsulfonyl)benzenesulfonamido]thymidine (5.6)

Reaction of 2-(methylsulfonyl)benzenesulfonyl chloride with 5.3 provided compound 5.6 (83 mg, 72%). $R_f$ 0.16 (dichloromethane/methanol, 10:1); $^1$H-NMR (MeOH-d$_4$) δ 1.88 (d, 3H, CH$_3$, $J = 1.1$ Hz), 2.15-2.25 (m, 2H, H-2'), 3.17 (dd, 1H, H-5', $J = 13.4$, 6.1 Hz), 3.24 (dd, 1H, H-5', $J = 13.4$, 3.9 Hz), 3.40 (s, 1H, SO$_2$CH$_3$), 3.82-3.86 (m, 1H, H-4'), 4.26-4.30 (m, 1H, H-3'), 6.11 (t, 1H, H-1', $J = 6.9$ Hz), 7.42 (d, 1H, H-6, $J = 1.1$ Hz), 7.86-7.90 (m, 2H, ArH), 8.21-8.24 (m, 1H, ArH), 8.27-8.31 (m, 1H, ArH); $^{13}$C-NMR (MeOH-d$_4$) δ 12.46 (CH$_3$), 40.10 (C-2'), 44.54 (C-5'), 45.90 (SO$_2$CH$_3$), 72.45 (C-3'), 85.85 (C-1'), 86.41 (C-4'), 111.94 (C-5), 132.73 (ArC), 133.82 (ArC), 134.92 (ArC), 135.57 (ArC), 138.03 (C-6), 139.68 (ArC), 140.20 (ArC), 152.17 (C-2), 166.39 (C-4); MS (HR-ESI) C$_{17}$H$_{21}$N$_3$O$_8$S$_2$ (M+Na)$^+$ calcd 482.0668, found 482.0673.
5′-Deoxy-5′-(naphthalene-1-sulfonamido)thymidine (5.7)

Reaction of 1-naphthlenesulfonyl chloride with 5.3 provided compound 5.7 (70 mg, 65%). \( R_f \) 0.15 (dichloromethane/methanol, 10:1); \(^1\)H-NMR (MeOH-d\(^4\)) \( \delta \) 1.85 (d, 3H, \( \text{CH}_3 \), \( J = 1.1 \) Hz), 2.12-2.19 (m, 2H, H-2′), 3.08 (dd, 1H, H-5′, \( J = 14.0, 6.0 \) Hz), 3.24 (dd, 1H, H-5′, \( J = 14.0, 4.1 \) Hz), 3.75-3.81 (m, 1H, H-4′), 4.23-4.27 (m, 1H, H-3′), 6.05 (t, 1H, H-1′, \( J = 6.9 \) Hz), 7.46 (d, 1H, H-6, \( J = 1.1 \) Hz), 7.53-7.70 (m, 3H, ArH), 8.00 (dd, 1H, ArH, \( J = 7.8, 1.5 \) Hz), 8.12 (d, 1H, ArH, \( J = 7.8 \) Hz), 8.20 (dd, 1H, ArH, \( J = 7.8, 1.5 \) Hz), 8.69 (dd, 1H, ArH, \( J = 7.8, 1.5 \) Hz); \(^1\)C-NMR (MeOH-d\(^4\)) \( \delta \) 12.33 (\( \text{CH}_3 \)), 40.08 (C-2′), 45.73 (C-5′), 72.56 (C-3′), 86.30 (C-1′), 86.84 (C-4′), 111.70 (C-5), 125.25 (ArC), 125.87 (ArC), 127.95 (ArC), 129.01 (ArC), 129.47 (ArC), 130.07 (ArC), 130.14 (ArC), 135.19 (ArC), 135.81 (ArC), 136.71 (ArC), 138.45 (C-6), 152.21 (C-2), 166.39 (C-4); MS (HR-ESI) C\(_{20}\)H\(_{21}\)N\(_3\)O\(_6\)S (M+Na\(^+\)) calcd 454.1049, found 454.1031.

General procedure for the synthesis of urea- or thiourea-linked \( \text{BaTK/ BaTMPK} \) inhibitors (5.8-5.13)

To a solution of 5′-amino-5′-deoxythymidine 5.3 (60 mg, 0.25 mmol) and the indicated phenylisocyanate or phenylisothiocyanate (0.28 mmol) [see below] in pyridine (10 mL) was added 4-(dimethylamino)pyridine (6 mg, 0.05 mmol). The resulting solution
was stirred for 12 h at room temperature. The solvent was evaporated and the residue was purified by silica gel column chromatography using ethylacetate/methanol (25:1) as the eluent to give the following products.

5´-Deoxy-5´-[(2-(methylthio)anilinothiocarbonyl)amino]thymidine (5.8)

Reaction of 2-(methylthio)phenyl isothiocyanate with 5.3 provided compound 5.8 (80 mg, 76%). \( R_f \) 0.30 (ethylacetate/methanol, 25:1); \( ^1\)H-NMR (MeOH-d\(^4\)) \( \delta \) 1.88 (s, 3H, CH\(_3\)), 2.22-2.25 (m, 2H, H-2´), 2.40 (s, 3H, SCH\(_3\)), 3.83-3.98 (m, 2H, H-5´), 4.02-4.06 (m, 1H, H-4´), 4.34-4.38 (m, 1H, H-3´), 6.19 (t, 1H, H-1´, \( J = 6.9 \) Hz), 7.18 (dd, 1H, ArH, \( J = 7.8, 7.4 \) Hz ), 7.23 (dd, 1H, ArH, \( J = 7.8, 7.4 \) Hz), 7.34 (d, 2H, ArH, \( J = 7.8 \) Hz ), 7.52 (s, 1H, H-6); \( ^{13}\)C-NMR (MeOH-d\(^4\)) \( \delta \) 12.47 (CH\(_3\)), 15.42 (CH\(_3\)), 39.96 (C-2´), 49.65 (C-5´), 73.09 (C-3´), 86.34 (C-1´), 87.00 (C-4´), 111.81 (C-5), 126.74 (ArC), 128.07 (ArC), 129.09 (ArC), 129.45 (ArC), 126.25 (ArC), 138.12 (C-6), 141.03 (ArC), 152.28 (C-2), 166.43 (C-4), 182.27 (C=S); MS (HR-ESI) C\(_{18}\)H\(_{22}\)N\(_4\)O\(_4\)S\(_2\)Na (M+Na\(^+\)) calcd 445.0980, found 445.0995.
5′-Deoxy-5′-\{[3-(methylthio)anilinothiocarbonyl]amino\}thymidine (5.9)

Reaction of 3-(methylthio)phenyl isothiocyanate with 5.3 provided compound 5.9 (77 mg, 73%). $R_f$ 0.31 (ethylacetate/methanol, 25:1); $^1$H-NMR (MeOH-d$_4$) $\delta$ 1.86 (d, 3H, $CH_3$, $J = 1.0$ Hz), 2.22-2.30 (m, 2H, H-2′), 2.45 (s, 3H, $SCH_3$), 3.77-3.98 (m, 2H, H-5′), 4.04-4.10 (m, 1H, H-4′), 4.32-4.38 (m, 1H, H-3′), 6.21 (t, 1H, H-1′, $J = 6.9$ Hz), 7.02-7.04 (m, 1H, ArH), 7.06 (t, 1H, ArH, $J = 6.9$ Hz) 7.23 (d, 1H, ArH, $J = 7.9$ Hz), 7.27-7.30 (m, 1H, ArH), 7.50 (d, 1H, H-6, $J = 1.0$ Hz); $^{13}$C-NMR (MeOH-d$_4$) $\delta$ 12.40 (CH$_3$), 15.43 (CH$_3$), 38.85 (C-2′), 50.31 (C-5′), 73.12 (C-3′), 86.10 (C-1′), 87.11 (C-4′), 111.97 (C-5), 121.61 (ArC), 122.71 (ArC), 124.50 (ArC), 130.51 (ArC), 138.16 (C-6), 140.15 (ArC), 141.43 (ArC), 152.30 (C-2), 166.36 (C-4), 182.66 (C=S); MS (HR-ESI) C$_{18}$H$_{22}$N$_4$O$_4$S$_2$Na (M+Na)$^+$ calcd 445.0980, found 445.0995.

5′-Deoxy-5′-\{[4-(methylthio)anilinothiocarbonyl]amino\}thymidine (5.10)
Reaction of 4-(methylthio)phenyl isothiocyanate with 5.3 provided compound 5.10 (88 mg, 83%). \( R_f \) 0.26 (ethylacetate/methanol, 25:1); \( ^1\text{H-NMR (MeOH-d}^4\text{)} \) \( \delta \) 1.87 (s, 3H, CH\(_3\)), 2.22-2.28 (m, 2H, H-2’), 2.45 (s, 3H, SCH\(_3\)), 3.82-3.96 (m, 2H, H-5’), 4.04-4.07 (m, 1H, H-4’), 4.34-4.38 (m, 1H, H-3’), 6.20 (t, 1H, H-1’, \( J = 6.9 \text{ Hz} \)), 7.22-7.27 (m, 4H, ArH), 7.52 (s, 1H, H-6); \( ^{13}\text{C-NMR (MeOH-d}^4\text{)} \) \( \delta \) 12.41 (CH\(_3\)), 16.00 (CH\(_3\)), 39.84 (C-2’), 49.71 (C-5’), 73.08 (C-3’), 86.17 (C-1’), 87.08 (C-4’), 111.87 (C-5), 126.34 (ArC), 128.43 (ArC), 138.17 (C-6), 152.29 (C-2), 166.38 (C-4), 182.80 (C=S); MS (HR-ESI) \( \text{C}_{18}\text{H}_{22}\text{N}_{4}\text{O}_{4}\text{S}_2\text{Na (M+Na)}^+ \text{ calcd} \) 445.0980, found 445.1007.

\[ \text{5'-Deoxy-5'-(3-(methylthio)anilinocarbonyl} \text{amino]thymidine (5.11)} \]

Reaction of 3-(methylthio)phenyl isocyanate with 5.3 provided compound 5.11 (69 mg, 68%). \( R_f \) 0.36 (ethylacetate/methanol, 15:1); \( ^1\text{H-NMR (DMSO-d}^6\text{)} \) \( \delta \) 1.77 (s, 3H, CH\(_3\)), 2.04-2.19 (m, 2H, H-2’), 2.42 (s, 3H, SCH\(_3\)), 3.19-3.48 (m, 2H, H-5’), 3.76-3.79 (m, 1H, H-4’), 4.15-4.16 (m, 1H, H-3’), 5.33 (d, 1H, OH, \( J = 4.2 \text{ Hz} \)), 6.18 (t, 1H, H-1’, \( J = 7.4 \text{ Hz} \)), 6.32 (t, 1H, NH, \( J = 5.8 \text{ Hz} \)), 6.78 (d, 1H, ArH, \( J = 7.8 \text{ Hz} \)), 7.05 (d, 1H, ArH, \( J = 7.8 \text{ Hz} \)), 7.15 (t, 1H, ArH, \( J = 7.8 \text{ Hz} \)), 7.43 (s, 1H, ArH), 7.52 (s, 1H, H-6), 8.58 (s, 1H, NH), 11.32 (s, 1H, NH); \( ^{13}\text{C-NMR (DMSO-d}^6\text{)} \) \( \delta \) 12.06 (CH\(_3\)), 14.57 (CH\(_3\)), 38.39
(C-2´), 41.43 (C-5´), 71.13 (C-3´), 83.74 (C-1´), 85.30 (C-4´), 109.77 (C-5), 114.18 (ArC), 114.60 (ArC), 118.46 (ArC), 129.14 (ArC), 136.07 (C-6), 138.38 (ArC), 140.91 (ArC), 150.48 (C-2), 155.12 (C=O), 163.70 (C-4); MS (HR-ESI) C_{18}H_{22}N_{4}O_{5}S (M+Na)^+ calcd 429.1209, found 429.1214

\[
\begin{align*}
\text{5´-Deoxy-5´-\{[4-(methylthio)anilinocarbonyl]amino\}thymidine (5.12)}
\end{align*}
\]

Reaction of 4-(methylthio)phenyl isocyanate with 5.3 provided compound 5.12 (74 mg, 73%). \( R_f \) 0.34 (ethylacetate/methanol, 15:1); \(^1\)H-NMR (DMSO-d\(^6\)) \( \delta \) 1.77 (s, 3H, CH\(_3\)), 2.04-2.17 (m, 2H, H-2´), 2.40 (s, 3H, SCH\(_3\)), 3.17-3.43 (m, 2H, H-5´), 3.75-3.78 (m, 1H, H-4´), 4.17-4.21 (m, 1H, H-3´), 5.37 (d, 1H, OH, \( J = 4.2 \) Hz), 6.15 (t, 1H, H-1´, \( J = 7.4 \) Hz), 6.88 (t, 1H, NH, \( J = 5.8 \) Hz), 7.14 (d, 2H, ArH, \( J = 8.6 \) Hz), 7.37 (d, 2H, ArH, \( J = 8.6 \) Hz), 7.66 (s, 1H, H-6), 9.38 (s, 1H, NH), 11.28 (s, 1H, NH); \(^{13}\)C-NMR (DMSO-d\(^6\)) \( \delta \) 11.90 (CH\(_3\)), 16.26 (CH\(_3\)), 38.50 (C-2´), 40.50 (C-5´), 70.82 (C-3´), 83.39 (C-1´), 85.21 (C-4´), 109.80 (C-5), 118.06 (ArC), 127.96 (ArC), 128.47 (ArC), 136.13 (C-6), 138.60 (ArC), 150.45 (C-2), 155.49 (C=O), 163.71 (C-4); MS (HR-ESI) C\(_{18}\)H\(_{22}\)N\(_4\)O\(_5\)S (M+Na)^+ calcd 429.1209, found 429.1212.
5´-Deoxy-5´-[[3-(methoxycarbonyl)anilinocarbonyl]amino]thymidine (5.13)

Reaction of methyl 3-isocyanatobenzoate with 5.3 provided compound 5.13 (54 mg, 52%). Rf 0.34 (ethylacetate/methanol, 15:1); 1H-NMR (DMSO-d6) δ 1.77 (s, 3H, CH3), 2.07-2.16 (m, 2H, H-2´), 3.23-3.46 (m, 2H, H-5´), 3.75-3.80 (m, 1H, H-4´), 3.83 (s, 3H, OCH3), 4.16-4.18 (m, 1H, H-3´), 5.32 (d, 1H, OH, J = 4.2 Hz), 6.18 (t, 1H, H-1´, J = 7.4 Hz), 6.36 (t, 1H, NH, J = 5.8 Hz), 7.36 (t, 1H, ArH, J = 7.9 Hz), 7.49 (dt, 1H, ArH, J = 7.9, 1.8 Hz), 7.56 (dt, 1H, ArH, J = 7.9, 1.8 Hz), 7.52 (s, 1H, H-6), 8.12 (t, 1H, ArH, J = 1.8 Hz), 8.81 (s, 1H, NH), 11.29 (s, 1H, NH); 13C-NMR (DMSO-d6) δ 11.98 (CH3), 38.17 (C-2´), 40.79 (C-5´), 52.04 (OCH3), 71.11 (C-3´), 83.76 (C-1´), 85.25 (C-4´), 109.74 (C-5), 116.65 (ArC), 118.00 (ArC), 122.07 (ArC), 129.02 (ArC), 130.04 (ArC), 136.02 (C-6), 140.79 (ArC), 150.45 (C-2), 155.09 (C=O), 163.69 (C-4), 166.25 (C=O); MS (HR-ESI) C19H22N4O7 (M+Na)+ calcd 441.1386, found 441.1391.

General procedure for the synthesis of amide-linked BaTK/BaTMPK inhibitors (5.14-5.19)

To a solution of 5´-amino-5´-deoxythymidine 5.3 (60 mg, 0.25 mmol) and the indicated benzoic acid (0.30 mmol) [See below] in DMF (8 mL) was added 1-hydroxybenzotriazole (HOBt, 41 mg, 0.30 mmol). A solution of 1,3-
dicyclohexylcarbodiimide (DCC, 62 mg, 0.30 mmol) in DMF (2 mL) was added slowly and the resulting reaction mixture was stirred for 12 h at room temperature. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using ethylacetate/methanol (20:1) as the eluent to give the following products.

![Chemical structure of 5'-Deoxy-5'-[4-(sulfamoyl)benzeneamido]thymidine (5.14)](image)

**5'-Deoxy-5'-[4-(sulfamoyl)benzeneamido]thymidine (5.14)**

Reaction of 4-(sulfamoyl)benzoic acid with 5.3 provided compound 5.14 (51 mg, 48%).

$R_f$ 0.22 (ethylacetate/methanol, 15:1); $^1$H-NMR (DMSO-d$_6$) $\delta$ 1.77 (s, 3H, CH$_3$), 2.07-2.18 (m, 2H, H-2’), 3.52-3.55 (m, 2H, H-5’), 3.88-3.90 (m, 1H, H-4’), 4.24-4.26 (m, 1H, H-3’), 5.33 (d, 1H, OH, $J$ = 4.4 Hz), 6.15 (t, 1H, H-1’, $J$ = 6.7 Hz), 7.48 (s, 2H, NH$_2$), 7.52 (s, 1H, H-6), 7.90 (d, 2H, ArH, $J$ = 8.3 Hz), 8.01 (d, 2H, ArH, $J$ = 8.3 Hz), 8.82 (t, 1H, NH, $J$ = 5.6 Hz), 11.30 (s, 1H, NH); $^{13}$C-NMR (DMSO-d$_6$) $\delta$ 11.96 (CH$_3$), 38.50 (C-2’), 41.67 (C-5’), 71.26 (C-3’), 84.03 (C-1’), 84.71 (C-4’), 109.62 (C-5), 125.60 (ArC), 127.88 (ArC), 136.17 (ArC), 137.11 (C-6), 146.30 (ArC), 150.41 (C-2), 163.68 (C-4), 165.47 (C=O); MS (HR-ESI) C$_{17}$H$_{20}$N$_4$O$_7$S (M+Na)$^+$ calcd 447.0950, found 447.0953.
5´-Deoxy-5´-[4-(methylsulfonyl)benzeneamido]thymidine (5.15)

Reaction of 4-(methylsulfonyl)benzoic acid with 5.3 provided compound 5.15 (56 mg, 53%). Rf 0.27 (ethylacetate/methanol, 15:1); ¹H-NMR (DMSO-d⁶) δ 1.76 (s, 3H, CH₃), 2.07-2.17 (m, 2H, H-2´), 3.28 (s, 3H, SO₂CH₃), 3.54-3.58 (m, 2H, H-5´), 3.90-3.94 (m, 1H, H-4´), 4.25-4.29 (m, 1H, H-3´), 5.40 (d, 1H, OH, J = 4.3 Hz), 6.15 (t, 1H, H-1´, J = 6.8 Hz), 7.53 (s, 1H, H-6), 8.02-8.18 (m, 4H, ArH), 9.02 (s, 1H, NH), 11.38 (s, 1H, NH); ¹³C-NMR (DMSO-d⁶) δ 12.10 (CH₃), 38.11 (C-2´), 42.14 (C-5´), 44.19 (SO₂CH₃), 70.89 (C-3´), 83.81 (C-1´), 84.56 (C-4´), 110.11 (C-5), 127.40 (ArC), 128.64 (ArC), 139.50 (C-6), 143.33 (ArC), 150.64 (C-2), 164.02 (C-4), 165.62 (ArC); MS (HR-ESI) C₁₈H₂₁N₃O₇S (M+Na)⁺ calcd 446.0998, found 446.1002.

5´-Deoxy-5´-[4-(methylthio)benzeneamido]thymidine (5.16)
Reaction of 4-(methylthio)benzoic acid with 5.3 provided compound 5.16 (57 mg, 58%). $R_f$ 0.37 (ethylacetate/methanol, 15:1); $^1$H-NMR (DMSO-d$_6$) $\delta$ 1.76 (s, 3H, CH$_3$), 2.07-2.12 (m, 2H, H-2’), 2.51 (s, 3H, SCH$_3$), 3.49-3.52 (m, 2H, H-5’), 3.88-3.90 (m, 1H, H-4’), 4.22-4.26 (m, 1H, H-3’), 5.32 (d, 1H, OH, $J$ = 4.3 Hz), 6.14 (t, 1H, H-1’, $J$ = 6.8 Hz), 7.32 (d, 2H, ArH, $J$ = 8.4 Hz), 7.51 (s, 1H, H-6), 7.81 (d, 2H, ArH, $J$ = 8.4 Hz), 8.58 (t, 1H, NH, $J$ = 5.6 Hz), 11.30 (s, 1H, NH); $^{13}$C-NMR (DMSO-d$_6$) $\delta$ 12.45 (CH$_3$), 14.59 (CH$_3$), 41.90 (C-2’), 49.08 (C-5’), 71.76 (C-3’), 84.84 (C-1’), 85.21 (C-4’), 110.47 (C-5), 125.48 (ArC), 128.32 (ArC), 130.47 (ArC), 136.88 (C-6), 143.51 (ArC), 150.99 (C-2), 164.59 (C-4), 167.14 (C=O); MS (HR-ESI) C$_{18}$H$_{21}$N$_3$O$_5$S (M+Na)$^+$ calcd 414.1100, found 414.1104.

5’-Deoxy-5’-[4-(methoxycarbonyl)benzeneamido]thymidine (5.17)

Reaction of mono-methyl terephthalate with 5.3 provided compound 5.17 (45 mg, 43%). $R_f$ 0.37 (ethylacetate/methanol, 15:1); $^1$H-NMR (DMSO-d$_6$) $\delta$ 1.76 (s, 3H, CH$_3$), 2.08-2.22 (m, 2H, H-2’), 3.51-3.56 (m, 2H, H-5’), 3.88 (s, 3H, OCH$_3$), 3.88-3.92 (m, 1H, H-4’), 4.24-4.26 (m, 1H, H-3’), 5.31 (d, 1H, OH, $J$ = 4.4 Hz), 6.14 (t, 1H, H-1’, $J$ = 6.7 Hz), 7.51 (s, 1H, H-6), 7.97 (d, 2H, ArH, $J$ = 7.3 Hz), 8.04 (d, 2H, ArH, $J$ = 7.3 Hz), 8.80 (t, 1H, NH, $J$ = 5.0 Hz), 11.28 (s, 1H, NH); $^{13}$C-NMR (MeOH-d$_4$) $\delta$ 11.93 (CH$_3$), 38.36...
(C-2′), 41.67 (C-5′), 52.33 (OCH₃), 71.28 (C-3′), 84.06 (C-1′), 87.73 (C-4′), 109.60 (C-5), 127.62 (ArC), 129.11 (ArC), 131.79 (ArC), 136.15 (ArC), 138.34 (C-6), 150.41 (C-2), 163.68 (C-4), 165.65 (C=O), 165.68 (C=O), 169.36 (C=O); MS (HR-ESI) C₁₉H₂₁N₃O₇ (M+Na)+ calcd 426.1277, found 426.1285.

![5'-Deoxy-5'-[3-(methoxycarbonyl)benzeneamido]thymidine (5.18)](image)

5'-Deoxy-5'-[3-(methoxycarbonyl)benzeneamido]thymidine (5.18)

Reaction of methyl hydrogen isophthalate with 5.3 provided compound 5.18 (38 mg, 38%). Rf 0.36 (ethylacetate/methanol, 15:1); ¹H-NMR (MeOH-d₄) δ 1.82 (s, 3H, CH₃), 2.25-2.30 (m, 2H, H-2′), 3.63-3.78 (m, 2H, H-5′), 3.93 (s, 3H, OCH₃), 4.02-4.06 (m, 1H, H-4′), 4.34-4.38 (m, 1H, H-3′), 6.22 (t, 1H, H-1′, J = 7.4 Hz), 7.50 (s, 1H, H-6), 7.59 (t, 1H, ArH, J = 7.8 Hz), 8.09 (dt, 1H, ArH, J = 7.8, 1.2 Hz), 8.17 (dd, 1H, ArH, J = 7.8, 1.2 Hz), 8.50 (t, 1H, ArH, J = 1.2 Hz); ¹³C-NMR (MeOH-d₄) δ 12.29 (CH₃), 40.11 (C-2′), 42.88 (C-5′), 52.86 (OCH₃), 73.06 (C-3′), 86.43 (C-1′), 87.03 (C-4′), 111.75 (C-5), 129.42 (ArC), 130.02 (ArC), 131.88 (ArC), 132.87 (ArC), 133.45 (ArC), 136.04 (ArC), 138.20 (C-6), 152.32 (C-2), 163.36 (C-4), 167.73 (C=O), 169.36 (C=O); MS (HR-ESI) C₁₉H₂₁N₃O₇ (M+Na)+ calcd 426.1277, found 426.1289.
5′-Deoxy-5′-(phthalimido)thymidine (5.19)

Reaction of methyl hydrogen phthalate with 5.3 provided compound 5.19 (26 mg, 28%). Rf 0.57 (ethylacetate/methanol, 15:1); 1H-NMR (DMSO-d6) δ 1.82 (s, 3H, CH3), 2.07-2.26 (m, 2H, H-2´), 3.78 (dd, 1H, H-5´, J = 14.2, 7.7 Hz), 3.85 (dd, 1H, H-5´, J = 14.2, 5.6 Hz), 3.99-4.04 (m, 1H, H-4´), 4.22-4.26 (m, 1H, H-3´), 5.37 (d, 1H, OH, J = 4.1 Hz), 6.14 (t, 1H, H-1´, J = 6.8 Hz), 7.56 (s, 1H, H-6), 7.83-7.92 (m, 4H, ArH), 11.25 (s, 1H, NH); 13C-NMR (DMSO-d6) δ 12.86 (CH3), 38.84 (C-2´), 47.37 (C-5´), 72.44 (C-3´), 83.19 (C-1´), 84.18 (C-4´), 109.55 (C-5), 123.14 (ArC), 131.43 (ArC), 134.54 (ArC), 136.12(C-6), 150.36 (C-2), 163.65 (C-4), 167.86 (C=O); MS (HR-ESI) C18H17N3O6 (M+Na)+ calcd 394.1015, found 394.1024.

General procedure for the synthesis of triazole-linked BaTK/BaTMPK inhibitors (5.20-5.23)

Compound 5.2 (65 mg, 0.25 mmol) and the indicated alkyne (0.25 mmol) [see below] were dissolved in a mixture of water and tert-BuOH (6 mL, 1:1). Freshly prepared sodium ascorbate solution (0.05 mL, 1 M solution in water) was added, followed by the addition of freshly prepared copper (II) sulfate pentahydrate (0.01 mL, 0.5 M solution in water). During vigorously stirring of the reaction mixture at room temperature for 48 h, a
precipitate formed. The reaction mixture was diluted with 20 mL of water and cooled to 0 °C. A yellow precipitate was collected by filtration and dried in vacuum to give the following products.

![Diagram of 5'-Deoxy-5'-[4-phenyl-(1,2,3)triazol-1-yl]thymidine (5.20)](image)

5'-Deoxy-5'-[4-phenyl-(1,2,3)triazol-1-yl]thymidine (5.20)

Reaction of ethynylbenzene with 5.2 provided compound 5.20 (14 mg, 15%). \( R_f \) 0.18 (dichloromethane/methanol, 10:1); \(^1\)H-NMR (DMSO-d\(^6\)) \( \delta \) 1.68 (s, 3H, CH\(_3\)), 2.07-2.21 (m, 2H, H-2’), 3.78 (s, 3H, OCH\(_3\)), 4.11-4.14 (m, 1H, H-4’), 4.29-4.33 (m, 1H, H-3’), 4.67 (dd, 1H, H-5’, \( J = 14.3, 6.8 \) Hz), 4.76 (dd, 1H, H-5’, \( J = 14.3, 4.5 \) Hz), 5.53 (d, 1H, OH, \( J = 4.4 \) Hz), 6.19 (t, 1H, H-1’, \( J = 6.8 \) Hz), 7.24 (s, 1H, H-6), 7.31-7.44 (m, 3H, ArH), 7.84 (d, 2H, ArH, \( J = 7.5 \) Hz), 8.57 (s, 1H, ArH), 11.31 (s, 1H, NH); \(^{13}\)C-NMR (DMSO-d\(^6\)) \( \delta \) 11.96 (CH\(_3\)), 37.85 (C-2’), 51.09 (C-5’), 71.38 (C-3’), 83.72 (C-1’), 84.65 (C-4’), 115.16 (C-5), 122.19 (ArC-triazole), 125.10 (ArC), 127.87 (ArC), 128.87 (ArC), 130.59 (ArC), 138.20 (C-6), 146.35 (ArC-triazole), 152.32 (C-2), 163.36 (C-4); MS (HR-ESI) \( C_{18}H_{19}N_5O_4 \) (M+Na\(^+\)) calcd 392.1335, found 392.1332.
5′-Deoxy-5′-[4-(4-fluorophenyl)-(1,2,3)triazol-1-yl]thymidine (5.21)

Reaction of 1-ethynyl-4-fluorobenzene with 5.2 provided compound 5.21 (13 mg, 13%). $R_f$ 0.14 (dichloromethane/methanol, 10:1); $^1$H-NMR (DMSO-d$_6$) $\delta$ 1.69 (s, 3H, CH$_3$), 2.11-2.21 (m, 2H, H-2′), 4.09-4.14 (m, 1H, H-4′), 4.28-4.32 (m, 1H, H-3′), 4.67 (dd, 1H, H-5′, $J$ = 14.5, 6.3 Hz), 4.76 (dd, 1H, H-5′, $J$ = 14.5, 4.7 Hz), 5.52 (d, 1H, OH, $J$ = 4.4 Hz), 6.19 (t, 1H, H-1′, $J$ = 6.8 Hz), 7.23 (s, 1H, H-6), 7.28 (t, 2H, ArH, $J$ = 8.7 Hz), 7.88 (dd, 2H, ArH, $J$ = 8.3, 5.7 Hz), 8.55 (s, 1H, ArH), 11.28 (s, 1H, NH); $^{13}$C-NMR (DMSO-d$_6$) $\delta$ 12.00 (CH$_3$), 37.88 (C-2′), 51.12 (C-5′), 70.52 (C-3′), 83.72 (C-1′), 83.98 (C-4′), 109.82 (C-5), 115.71 (ArC), 115.93 (ArC), 122.12 (ArC-triazole), 127.11 (ArC), 127.19 (ArC), 135.95 (C-6), 145.51 (ArC-triazole), 150.44 (C-2), 160.55 (ArC), 162.98 (ArC), 163.57 (C-4); MS (HR-ESI) C$_{18}$H$_{18}$FN$_3$O$_4$ (M+Na)$^+$ calcd 410.1241, found 410.1241.
5′-Deoxy-5′-[4-(4-methoxyphenyl)-(1,2,3)triazol-1-yl]thymidine (5.22)

Reaction of 4-ethynylanisole with 5.2 provided compound 5.22 (11 mg, 10%). \( R_f \)
0.18 (dichloromethane/methanol, 10:1); \(^1^H\)-NMR (DMSO-d6) \( \delta \) 1.69 (s, 3H, CH3), 2.07-
2.21 (m, 2H, H-2′), 3.78 (s, 3H, OCH3), 4.08-4.12 (m, 1H, H-4′), 4.28-4.32 (m, 1H, H-
3′), 4.65 (dd, 1H, H-5′, \( J = 14.2, 6.7 \) Hz), 4.73 (dd, 1H, H-5′, \( J = 14.2, 4.5 \) Hz), 5.53 (d,
1H, OH, \( J = 4.3 \) Hz), 6.19 (t, 1H, H-1′, \( J = 6.8 \) Hz), 7.00 (d, 2H, ArH, \( J = 8.7 \) Hz), 7.24 (s,
1H, H-6), 7.76 (d, 2H, ArH, \( J = 8.7 \) Hz), 8.45 (s, 1H, ArH), 11.31 (s, 1H, NH); \(^1^C\)-NMR
(DMSO-d6) \( \delta \) 12.04 (CH3), 38.75 (C-2′), 51.94 (C-5′), 56.00 (OCH3), 71.44 (C-3′), 83.34
(C-1′), 84.65 (C-4′), 109.78 (C-5), 115.16 (ArC), 124.11 (ArC-triazole), 127.36 (ArC),
134.93 (ArC), 135.91 (C-6), 147.18 (ArC-triazole), 152.32 (C-2), 159.88 (ArC), 163.53
(C-4); MS (HR-ESI) C19H21N5O5 (M+Na)+ calcd 422.1440, found 422.1452.

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5′-Deoxy-5′-[4-(pyridine-3-yl)-(1,2,3)triazol-1-yl]thymidine (5.23)

Reaction of 3-ethynlpyridine with 5.2 provided compound 5.23 (7 mg, 8%). \( R_f \)
0.06 (dichloromethane/methanol, 8:1); \(^1^H\)-NMR (DMSO-d6) \( \delta \) 1.68 (s, 3H, CH3), 2.08-
2.20 (m, 2H, H-2′), 4.09-4.15 (m, 1H, H-4′), 4.27-4.33 (m, 1H, H-3′), 4.67 (dd, 1H, H-5′,
\( J = 14.3, 6.5 \) Hz), 4.79 (dd, 1H, H-5′, \( J = 14.3, 4.9 \) Hz), 5.46 (d, 1H, OH, \( J = 4.3 \) Hz),
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6.19 (t, 1H, H-1’, J = 6.8 Hz), 7.24 (s, 1H, H-6), 7.39-7.66 (m, 2H, ArH), 8.23 (d, 1H, ArH, J = 7.4 Hz), 8.69 (s, 1H, ArH), 8.86-8.89 (m, 1H, ArH), 11.29 (s, 1H, NH); MS (HR-ESI) C17H18N6O4 (M+Na)+ calcd 393.1287, found 393.1310.

7.5. Phosphoryl transfer assays (PTAs)

Chapter 3 and 4: PTAs with purified recombinant hTK1 and TK2, were carried out as described previously with minor modifications.59,61 dThd and 3CTAs were dissolved in DMSO to produce stock solutions of 10 and 100 µM concentrations. The reaction mixtures contained 10 µM of compounds for hTK1 assays and 100 µM of 3CTA for TK2 assays, 100 µM ATP with 0.03 µM [γ-32P]-ATP (Amersham Pharmacia Biotech, IL, USA), 50 mM Tris-HCl (pH 7.6), 5 mM MgCl2, 125 mM KCl, 10 mM DTT, and 0.5 mg/mL bovine serum albumin (BSA). The final concentrations of DMSO were set to 1%. The reaction mixtures were incubated at 37 °C for 20 min in presence of 50 ng of enzyme. Subsequently, the enzyme was heat-inactivated for 2 min at 95 °C. The reaction mixtures were centrifuged and 1 µL sample portions were spotted on PEI-cellulose TLC plates (Merck, USA). The TLC plates were placed overnight in a solvent system (isobutyric acid/ ammonium hydroxide/ water, 66:1:33). The radiolabeled spots were visualized by phospho-imaging (Fuji Film, Science Lab., Image Gauge V3.3) and phosphorylation values of 3CTAs were expressed relative to that of dThd.

Alternatively, PRs in hTK1 were determined by measuring the change of ADP production in absorbance at 340 nm, caused by NADH oxidation in a coupled enzyme system with pyruvate kinase and lactate dehydrogenase. The standard reaction mixture contained 40 µM 3CTA (N5-2OH isomers, Chapter 3), 20 mM Tris-HCl (pH 7.6), 50
mM KCl, 2 mM MgCl₂, 0.5 mM ATP, 5 mM DTT, 1 mM phosphoenolpyruvate, 0.5 units/mL pyruvate kinase, 0.5 units/mL lactate dehydrogenase, 0.1 mM NADH and 0.6 µg enzyme in a total volume of 0.25 mL. The reaction was performed at 24 °C with a Cary 3 spectrophotometer (Varian Techtron, Mulgrave, Australia) and started by the addition of dThd or 3CTAs. The enzyme activity values were calculated from the slope of the absorbance graph. The activity of hTK1 with 20 µM dThd was 640 nmol dTMP formed per min and mg hTK1 protein.

All measurements were carried out at least three times and the mean and the standard deviation (SD) were calculated.

**Chapter 5: B. anthracis** Sterne strain (34F2) tk gene was cloned by Dr. Andrew Phipps and coworkers at the OSU Department of Veterinary Biosciences. Recombinant BaTK was expressed and purified by Dr. Staffan Eriksson and coworkers at the Swedish University of Agricultural Sciences in Uppsala, Sweden. The standard PTAs contained 100 µM nucleoside, 50 mM Tris-HCl (pH 7.6), 2 mM MgCl₂, 2 mM ATP, 100 µM ATP with 0.03 µM \[\gamma^{32P}\]-ATP (Amersham Pharmacia Biotech, IL, USA), 0.5 mg/mL BSA, 5 mM DTT, 15 mM NaF and 10 ng of BaTK in a total volume of 50 µL. The monophosphate products were separated by thin layer chromatography and quantified by phospho-imaging (Fuji Image Gauge V3.3)

Alternatively, BaTK activity with the nucleosides was followed by the ADP production in a coupled enzyme system with pyruvate kinase and lactate dehydrogenase. The standard reaction mixture contained 50 mM Tris-HCl (pH 7.6), 2 mM MgCl₂, 1 mM ATP, 5 mM DTT, 1 mM phosphoenolpyruvate, 5 units/mL pyruvate kinase, 5 units/mL lactate dehydrogenase, 100 µM NADH and 5 µg BaTK in a total volume of 0.5-1 mL.
The reaction was performed at 37 °C with a Cary 3 spectrophotometer (Varian Techtron, Mulgrave, Australia).

All measurements were carried out at least three times and the mean and the standard deviation (SD) were calculated.

7.6. In vitro toxicity studies

*B. anthracis* Sterne was cultured in M9 minimal media with amino acids for 12 to 18 h at 37 °C with constant shaking. An aliquot of the overnight culture was diluted with fresh M9 minimal media with amino acids to an optical density in the range of 0.050 to 0.100 at 600 nm. Each compound was dissolved in DMSO to produce the corresponding stock solutions of 200 or 500 µM concentrations, respectively. Stock solutions of low concentrations (0.1 µM - 100 µM) were prepared by diluting the initial stock solutions with the M9 minimal media. Each stock solution was freshly prepared before the culture experiment. The stock solution (50 µL) and the culture media (5 mL) were mixed and incubated at 37 °C with constant shaking. An aliquot of the culture was removed at 4, 6 and 20 h and the optical density at 600 nm was determined. The percent of growth inhibition was determined using the formula \(\%I = \frac{A_{600nm}^{test}}{A_{600nm}^{control}} \times 100\). The minimal inhibitory concentration (IC\textsubscript{50}) was determined by linear regression analysis using Pharmacokinetic compartment model 107 (inhibitory effect sigmoid E\textsubscript{max} model) implemented in WinNonlin Professional (version 5.0.1, Cary, NC). Enrofloxacin (Baytril\textsuperscript{®}, Bayer Health Care, Shawnee Mission, Kansas) was included at 100 ng/mL as a positive control.
7.7. *In vivo* uptake experiments (Chapter 4)

L929 (wt) and L929 TK1 (-) tumors were established in nude mice by implanting 10^6 cells subcutaneously (s.c.) into the flank of the animals. Two weeks later, the tumors had attained a weight of 0.3 to 1.1 g. Mice were injected intratumorally (i.t.) over a period of 2 min. with quantities N5-2OH, 4.15.B, and BPA equivalent to 50 µg boron solubilized in 15 µL of 70% aqueous DMSO, 10 µL of 24% aqueous DMSO, and 15 µL of PBS, respectively. This was followed by a second injection 2 hours later. In both injections, 0.17 mol equivalents of 5-FdUrd was co-injected to suppress the *de novo* synthesis of dThd nucleotides. Boron concentrations in tissues were determined DCP-AES.\(^{183}\) Each point represents the arithmetic mean ± SD of 4 mice.

7.8. Preclinical BNCT experiments (Chapter 3)

BNCT was performed 14 days following intracerebral stereotactic implantation of F98 glioma cells. Rats were transported to the Nuclear Reactor Laboratory at the Massachusetts Institute of Technology (MIT) and then randomized on the basis of weight into experimental groups of 7-9 animals each as follows: Group I, 500 µg of \(^{10}\)B-enriched N5-2OH (3.5.B) in 200 µl of 50% DMSO, administered intracerebrally (i.c.) over a period of 24 hours by CED using ALZET minipumps (model #2001D) at a flow rate of 8 µl/h, followed by irradiation with a collimated beam of thermal neutrons at a reactor power of 4.8 MW-min; Group II received 50% DMSO alone by CED, as per Group I, followed by neutron irradiation; and Group III received 500 µg of 3.5.B in 50% DMSO \via CED without neutron irradiation. Group IV received 500 µg of \(^{10}\)B-enriched N5-2OH and processed for boron determinations by DCP-AES as described previously.\(^{183}\) All
irradiated rats were anesthetized with a mixture of ketamine and xylazine. BNCT was carried out at the MITR-II reactor in the MIT irradiation facility, which produces a beam of high intensity thermal neutrons without any contaminating fast neutrons. After completion of BNCT, the animals were held at MIT for ~ 3 days to allow induced radioactivity to decay before they were returned to The Ohio State University (Columbus, OH) for clinical monitoring.

7.9. Molecular modeling

7.9.1. Homology modeling

The initial coordinates of each homology model (hTK1, BaTK, and BaTMPK) were constructed with SWISS-MODEL (Version 36.0003) using the indicated template proteins (1XX6 for hTK1, 1W4R for BaTK and 2CCJ for BaTMPK). The obtained coordinates were then transferred to Sybyl 7.1 and hydrogen atoms were added. The hydrogen atom positions were minimized until an RMS of 0.005 kcal·mol\(^{-1}\)Å\(^{-1}\) was reached using the Powell method. The homology models were solvated with water molecules using the Solvent/Solvate option.\(^{201}\) The solvated homology models were minimized until the gradient reached 0.05 kcal·mol\(^{-1}\)Å\(^{-1}\) was reached using Tripos Force Field. For docking studies, the active sites were generated by selecting the same amino acid residues that are located within a radius of 12.0 Å from a ligand in the template crystal structure.

7.9.2. Theoretical calculations using Gaussian 03 program
Theoretical calculations for 3.11.A, (R)-epimers of 3.11.D-3.11.E, and 4.21-4.23 using the Gaussian 03 program (Gaussian Inc., Wallingford, CT) were carried out by running on an Itanium 2 Cluster at Ohio Super Computer Center. The structures were fully optimized at HF/6-31G*, HF/6-31G**, B3LYP/6-31G*, B3LYP/6-31G**. All optimized geometries showed real frequencies. NMR chemical shift calculations of 4.22 were performed with the gauge-including atomic orbitals (GIAO) method using the optimized geometries in acetonitrile (CH$_3$CN) solution. Theoretical $^{11}$B-NMR chemical shifts were computed versus the chemical shift of diborane (16.6 ppm) and then converted to the BF$_3$-Et$_2$O scale. $^1$H-NMR and $^{13}$C-NMR chemical shifts were referenced to the tetramethylsilane (TMS) standard.

7.9.3. Calculation of polar surface areas (PSAs) and apolar surface areas (APSAs)

Compounds 3.11.A, (R)-epimers of 3.11.D, and (R)-epimer of 3.11.E were optimized as described above with Gaussian 03 and parameters were transferred to HyperChem™ 7.51 for windows (Hypercube, Inc., Gainesville, FL). The boron atom type of the carborane cages in the optimized structures was changed into the C.3 carbon atom type. For (A)PSA calculations, structure parameters were transferred to the VEGA ZZ 2.0.4 program (Milano, Italy). A probe radius of 0.5 was used for the calculation of (A)PSAs.

7.9.4. Docking studies using the FlexX module in Sybyl 7.1

Compounds 3.11.A, (R)-epimers of 3.11.D, and (R)-epimer of 3.11.E optimized using Gaussian 03 as described above were saved in mol2 file format and transferred to
Sybyl 7.1 (Tripos Inc, St. Lewis, Missouri). Atom and bond types of the compounds were manually adjusted for docking studies in Sybyl 7.1. Specifically, the atom type of hexavalent boron atoms in the carborane cage was modified to the C.3 atom type because parameters for boron atoms of carborane cages are not available in Sybyl 7.1. For docking studies, the active site was generated by selecting the amino acids that are located within a radius of 12.0 Å from ligands in the crystal structures (1W4R for hTK1 and 1E2F for hTMPK). In case of the homology model, the active site was generated by selecting the same amino acid residues that are located within a radius of 12.0 Å from ligands in the corresponding crystal structures or template protein structures (1W4R for hTK1, 1W4R for BaTK and 2CCJ for BaTMPK). Docking of the compound library into the active site was performed with the FlexX module in Sybyl 7.1.
BIBLIOGRAPHY


Ph-1,2-C₂B₁₀H₁₀ (X = F, OH or NH₂) and related anions. Dalton Transactions, 2004(17): p. 2786-2799.


APPENDIX A

\(^1\)H-NMR AND \(^{13}\)C-NMR SPECTRA OF 3.5.A
$^1$H-NMR and 2D-COSY spectra of 3.5.A
$^{13}$C-NMR and $^{13}$C-DEPT135 spectra of 3.5.A
2D-HMQC spectrum of 3.5.A