Design and Synthesis of Inhibitors for the Human Geranylgeranyl Pyrophosphate Synthase

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Abstract

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Synthesis of potent, selective and drug-like inhibitors of the human geranylgeranyl pyrophosphate synthase (hGGPPS) has been a challenge in the last decades. Many reports highlight the finding of potent or selective inhibitors but all of them are substrate-mimic based inhibitors. Consequently, our efforts have been focused toward the synthesis of compounds also featuring drug-like structures. Herein, the synthesis of various potent and selective inhibitors of hGGPPS are reported. Those inhibitors are bisphosphonate-containing compounds harbouring well established drug-like heterocyclic cores with various side chain substituents. Potent molecules such as those reported in this thesis could serve as molecular probes for exploring the role of hGGPPS in human diseases such as multiple myeloma (MM), bone diseases and Alzheimer's disease.

Résumé

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La synthèse de molécules puissantes, selective et ayant une structure ressemblant à un médicament qui inhibe la géranylgéranyl pyrophasphate synthase humaine (hGGPPS) a été considéré comme un défi depuis la dernière décennie. Plusieurs articles soulignent la synthèse d'inhibiteurs sélectifs ou puissant, mais aucun de ceux-ci ne présente de structure ressemblant à un médicament. Nos efforts ont été placés sur la synthèse de molécules qui possédait aussi une structure de médicament. Ici, la synthèse de puissantes différentes molécules qui possèdent aussi des structures similaires à des médicaments est présentée. Ces inhibiteurs contiennent un groupement bisphosphonate, des hétérocycles reconnus comme ayant une structure de médicament et des chaînes latérales qui elles contiennent plusieurs groupements fonctionnels. Les puissantes molécules décrient dans cette thèse pourraient servir à sonder différentes maladies tel que le myélome multiple, des maladies osseuses et la maladie de l'Alzheimer.

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Frequently Used Symbols and Abbreviations

AD	Alzheimer's disease
CuTC	Copper(I) thiophene-2-carboxylate
°C	Degree Celsius
Cdc4	Cell division cycle 4
$CDCl_3$	Deuterated chloroform
D_2O	Deuterium dioxide
dba	dibenzylideneacetone
DCM	Dichloromethane
DIPEA	N,N-Diisopropylethylamine
DMAPP	Dimethylallyl pyrophosphate
DMA	Dimethylacetamide
DMB	2,4-Dimethoxybenzylamine
DMF	N,N-dimethylformamide
DMSO	Dimethylsulfoxide
Et	Ethyl
EtOAc	Ethyl acetate
EtOH	Ethanol
FPP	Farnesyl pyrophosphate
FPPS	Farnesyl pyrophosphate synthase
FTase	Farnesyltransferase
GGPP	Geranylgeranyl pyrophosphate
GGPPS	Geranylgeranyl pyrophosphate synthase

GGTase-I	Geranylgeranyltransferase I
GGTase-II	Geranylgeranyltransferase II
GTPase	Small guanine triphosphate binding proteins
GSK-3β	Glycogen synthase kinase 3 beta
h	Hour
HBTU	N,N,N′,N′-Tetramethyl-O-(1H-benzotriazol-1-yl)uranium hexafluorophosphate
HCl	Hydrochloric acid
hFPPS	Human farnesyl pyrophosphate synthase
hGGPPS	Human geranylgeranyl pyrophosphate synthase
HMG-CoA reductase	3-hydroxy-3-methyl-glutaryl Coenzyme A reductase
HPLC	High-performance liquid chromatography
IC_{50}	Inhibitor concentration causing 50% inhibition
IPP	Isopentenyl pyrophosphate
KF	Potassium fluoride
LC	Liquid Chromatography
MeOH	Methanol
MHz	Megahertz
MM	Multiple Myeloma
m-RNA	messenger Ribonucleic acid
MS	Mass Spectrometry
m/z	Mass-to-charge ratio
NMR	Nuclear Magnetic Resonance

N-BP	Nitrogen-containing Bisphosphonates
NH_4OH	Ammonium hydroxide
NEt_3	Triethylamine
NMDA	N-methyl-D-aspartate
OAc	Acetate
$POCl_3$	Phosphorus oxychloride
PPh_3	Triphenylphosphine
PPi	Inorganic pyrophosphate
P-Tau	Phosphorylated tau
Quant.	Quantitative
r.t.	Room temperature
REP	Rab escort protein
SAR	Structure-activity relationship
t-BuOK	Potassium tert-butoxide
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
TMS-Br	Trimethylsilyl bromide

Chapter 1: Introduction

1.1 The Mevalonate Pathway

The mevalonate pathway is responsible for the biosynthesis of all isoprenoids, which are themselves precursors to various metabolites such as cholesterol, heme A, ubiquinone, dolichol, vitamin D and lipoproteins, bile acids.[1-3] The two key isoprenoids, farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) serve as substrates for the post-translational prenylation of small guanosine triphosphate binding proteins (GTPases) (Fig. 1.3) Prenylation acts as a molecular switch that promotes protein-protein interactions or protein-membrane interactions.[4, 5] Prenylation also plays a vital role in cell signalization and proliferation. [2, 5] Furthermore, the implications of prenylation in human pathology has made it a target for drug discovery, especially in oncology treatment, bone diseases and Alzheimer's disease.

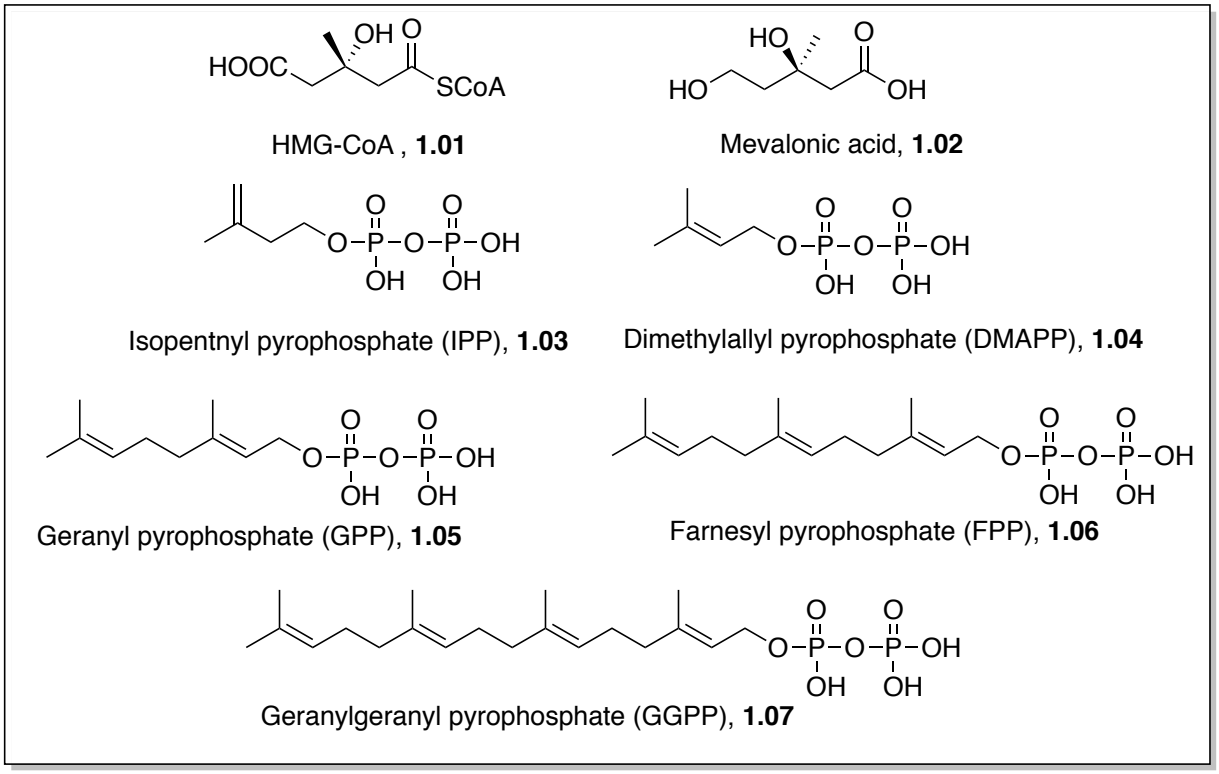


Figure 1.1. Structure of isoprenoids and precursors present in the mevalonate pathway.

The first and rate-limiting step of the mevalonate pathway is the conversion of HMG-CoA to mevalonic acid (mevalonate) by HMG-CoA reductase.[6, 7] This enzyme is targeted by a widely used class of prescription drugs, the statins. They are clinically validated cholesterol-lowering agents that inhibit competitively the HMG-CoA reductase by blocking its active site and thus preventing the substrate of the enzyme from bind.[8] Lovastatin (Mevacor® **1.08**), Pravastatin (Pravachol® **1.09**) and Atorvastatin (Lipitor® **1.10**) are perhaps the most well-known examples.[8, 9] (Fig. 1.2). Direct downstream metabolites of mevalonic acid, isopentenyl pyrophosphate (IPP) and its isomer, dimethyl allyl pyrophosphate (DMAPP) are key precursors to all isoprenoids. The human farnesyl pyrophosphate (hFPPS) catalyze the condensation of those 5-carbon units. DMAPP and IPP are condensed to afford the geranyl pyrophosphosphate (GPP) and then consecutively condensation of another IPP unit with GPP to afford FPP which constitute a 15-carbon chain.[10] FPP is a precursor to numerous isoprenoids, such as dolichol, haem A and ubiquinone. (Fig. 1.3)The hFPPS enzyme is targeted by a class of prescription drugs known as the nitrogen-containing bisphosphonates (N-BPs), such as zoledronic acid (Zometa® **1.11**) and risedronic acid (Actonel® **1.12**).[11] (Fig. 1.2). Those drugs are commonly used to treat Paget's disease, osteoporosis and metastatic bone diseases.[12]

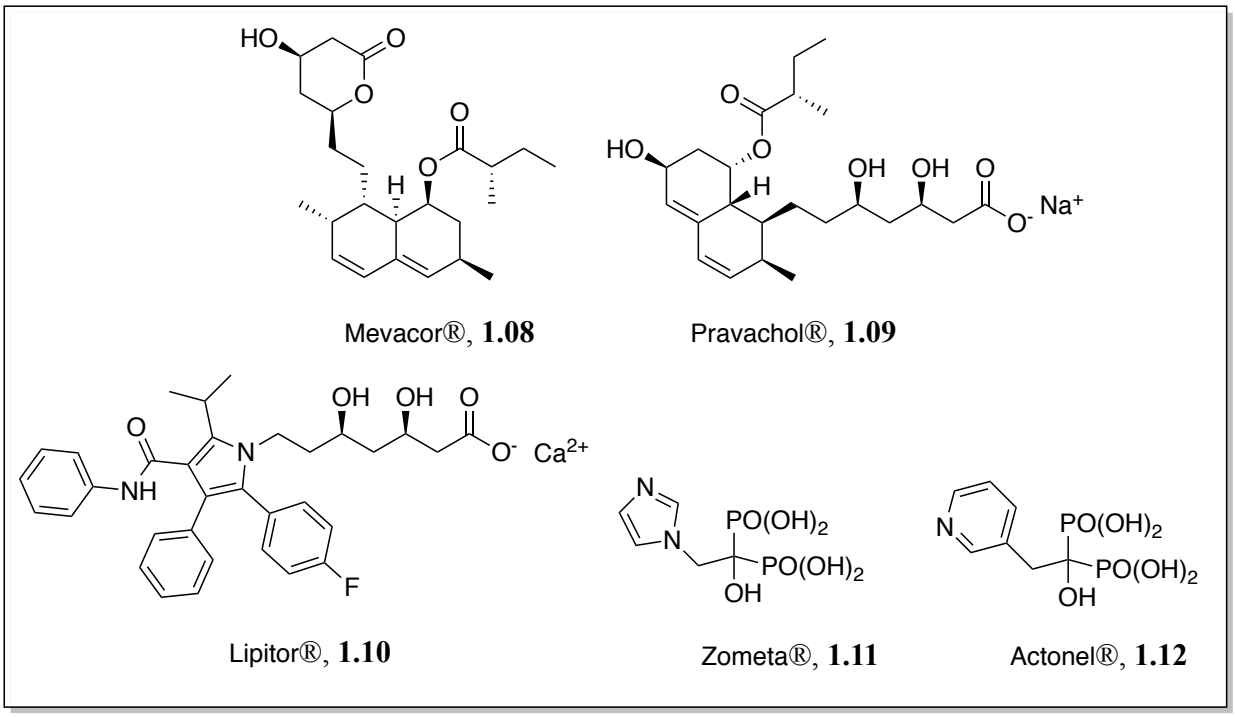


Figure 1.2. Clinically validated drug of the mevalonate pathway.

The next step in the biosynthesis of isoprenoids involves the condensation of FPP with IPP by the human geranylgeranyl pyrophosphate synthase (hGGPPS) to afford the 20-carbon chain metabolite GGPP. GGPP is also involved in the prenylation of proteins. Although, most of the prenylated proteins have a specific substrate, either FPP or GGPP, there are biochemical redundancy mecanisms where cross prenylation has been observed.[13]

Prenylation is an important post-translational modification for small GTPases where either a farnesyl unit or a geranylgeranyl unit is covalently attached to a protein. The most commonly known GTPases come from the Ras super family. It makes for more than 150 humain G-proteins which can undergo catalytic post-translational modification via farnesyalation or geranylgeranylation.[14,15] Prenylation can be carried out by three different prenyltransferase, farnesyl transferase (FTase), geranylgeranyl transferase I (GTPase), and geranylgeranyl

transferase II (GTPase II).[14] Prenylation selectivity of GGTase I and FTase is controlled by the terminal CAAX motif of small GTPases. Depending on X, farnesylation or geranylgeranylation will occur.[16] Where X is methionine, serine, cysteine or glutamine farnesylation will be predominant and where X is leucine or isoleucine geranylgeranylation will prevail.[17] An important feature of prenylation is that the two GGTase do not prenylate the same G-proteins; this is not a redundancy mechanism. GGTase I is responsible for the geranylgeranylation of Ras and Rho, while GGTase II is responsible for the prenylation of Rab protein together with Rab escort protein (REP).[18] Prenylation by GGTase II does not require the same CAAX motif that FTase and GGTase I use and for this reason, cross-prenylation of FTase and GGTase II substrate has never been reported.[19] However, cross-prenylation between FTase and GGTase I has been observed in many cases and it was proposed as the reason for the failure of FTase inhibitors in clinical trials.[20, 21] For instance, when exposed to farnesyl transferase inhibitors, N-Ras and K-Ras can maintain their functions by been geranylgeranylated by GGTase I instead of farnesylated.[22]

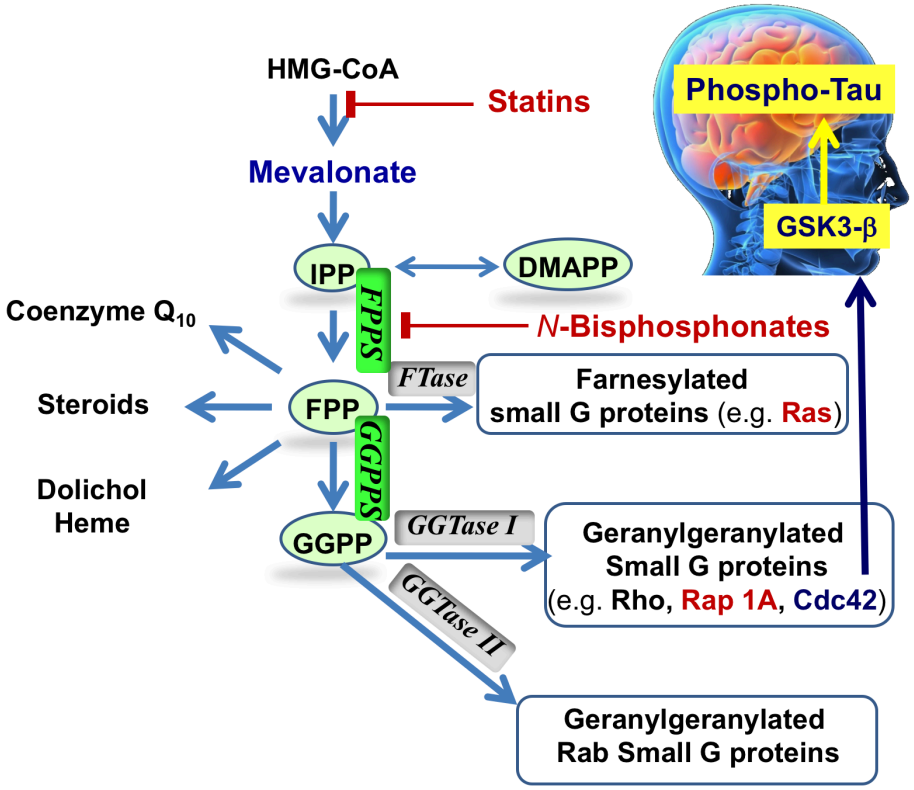


Figure 1.3. Representation of key steps in the Mevalonate pathway. The classes of clinically validated inhibitors (shown in red) for their respective enzymes (boxed in green). The prenyl transferases are boxed in grey.

1.2 Human geranylgeranyl pyrophosphate (hGGPPS)

The human geranylgeranyl pyrophosphate synthase (hGGPPS) catalyzes the elongation of FPP to GGPP via condensation of one FPP unit with an IPP unit. (Fig. 1.4) The principal role attributed to GGPP is the post-translational modification of G-Protein.[23] Prenylation has several functions, first and foremost, it increases lipophilicity of the proteins and therefore its cell membrane affinity. Since GGPP is bigger than FPP, geranylgeranylation increases membrane affinity by a larger extent.[24] Furthermore, prenylation is vital for cell survival due to the plethora of biological functions associated with small GTPases. For instance, Ras proteins play a key role in cell proliferation, survival and differentiation.[25] Rho proteins which include the well-known Rac

and Cdc42 regulate the shape of the cell, its movement and interactions with other cells.[26] Rab proteins which are mostly prenylated by GGTase II regulate endocytosis and membrane trafficking. Those proteins are geranylgeranylated by GGTase II since they harbor a -CC, -CXC, -CCXX, or –CXXX terminal motif instead of a CAAX motif required for prenylation via FTase and GTase I.[18] Inhibiting GGTase II would therefore presumably lead to no cross-prenylation. Additionally, RalA and RalB which are part of the Rap sub classes have been proven to be exclusively geranylgeranylated.[27, 28] The Rap GTPases are essential for cell-cell interaction. [29]

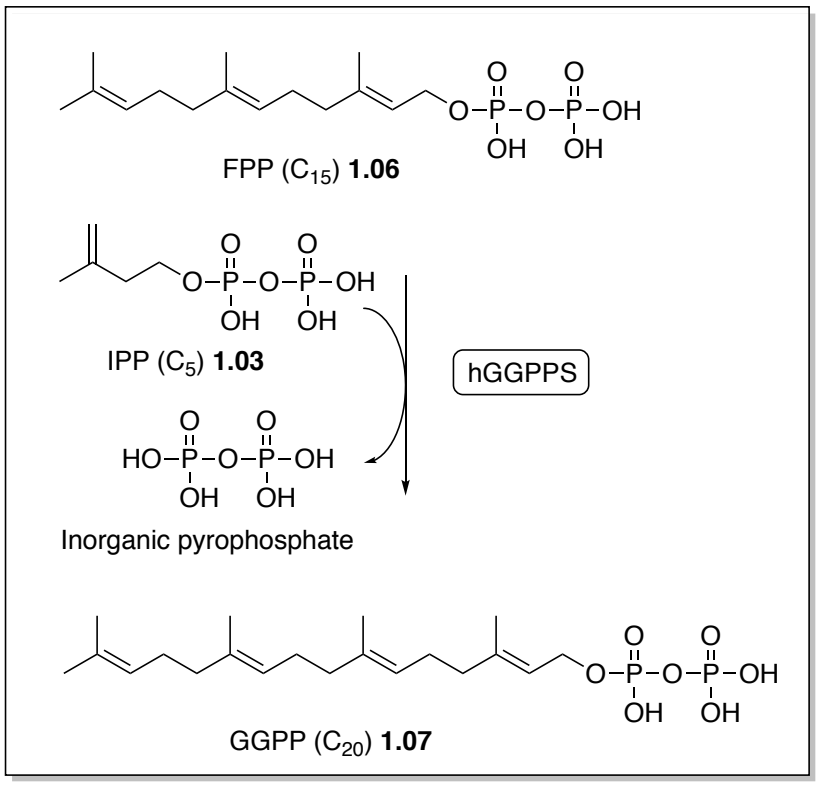


Figure 1.4. Biosynthesis of GGPP catalyzed by hGGPPS.

To date, only one crystal structure of the human GGPPS has been reported by Oppermann's group.[3] The enzyme has a hexameric nature, composed of trimers of dimers (Fig. 1.5), a form shared by mammalian and insect GGPPSs. In contrast, archeal, bacterial, fungal and plant GGPPS express a dimeric form and can be crystalized much more easily. For example, Oldfield's group

co-crystalized numerous GGPPS inhibitors with *Saccharomyces cerevisiae* GGPPS.[30] However, the *Saccharomyces cerevisiae* GGPPS only shares 43% sequence identity and 60% similarity with hGGPPS making it difficult to draw comparison in between the two enzymes inhibition patterns.[31] Kinetic studies and crystallographic evidences suggest that hGGPPS has a feedback mechanism where GGPP inhibit hGGPPS.[3] In fact, GGPP was shown to compete for binding competitively with FPP. However, GGPP was unintentionally co-crystalized with hGGPPS and found to bind in an adjacent pocket to the active site and not the substate FPP pocket, which would suggest that this pocket may play a role in an inhibitory conformation.[3] To date, no human GGPPS crystal structure with an inhibitor bound in its active site has been reported. Potent and selective inhibitors that could rigidify the enzyme for crystallography are required to further investigate and map the different enzymatic pockets of hGGPPS.

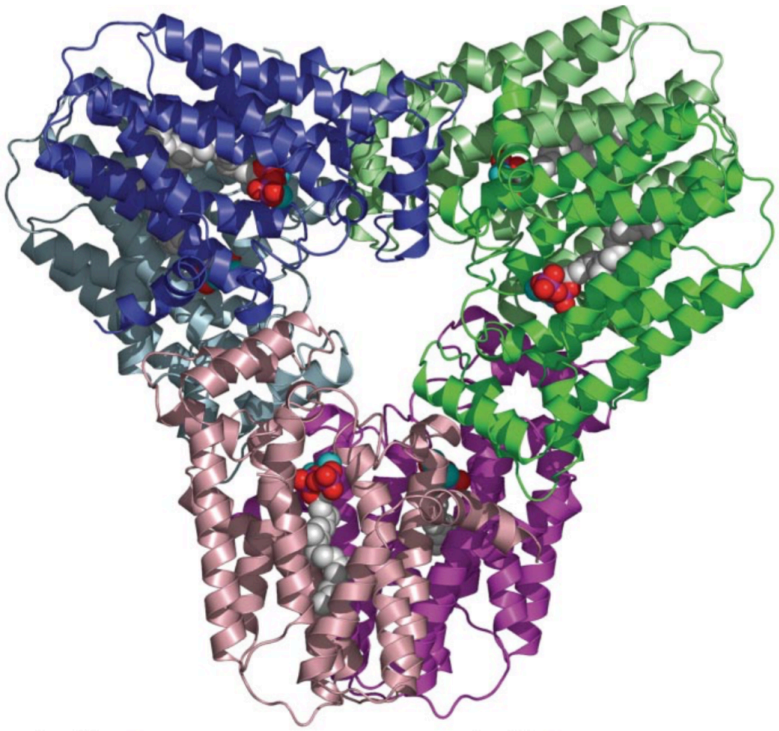


Figure 1.5. Crystal structure of hGGPPS (PDB: 2Q80). Each dimer represented by two similar colors. Each monomer has a GGPP bound into it (red and grey spheres) with Magnesium ions (cyan spheres)

1.3 The Human GGPPS as a therapeutic target

Given the numerous functions of hGGPPS, it has been proposed as a potential therapeutic target against several human pathologies. However, since none of the known inhibitors of this enzyme have advanced to clinical development, hGGPPS remain a non-validated therapeutic target. hFPPS inhibitors which already have clinically validated drugs currently on the market as previously mentioned. The N-BPs are primarily used against bone disease such as Paget's disease, osteoporosis and metastatic bone tumors.[2, 32] Unfortunately, they do have limitations due to their systemic distribution. Since they are highly charged species that binds to the bone tissues, they present poor systemic distribution which prohibit them from having effectiveness against human pathology outside of bone tissues.[33] Side effects of the drug include nephron toxicity sometimes resulting in kidney failure, osteonecrosis of the jaw and gastrointestinal irritation.[34] Therefore, hGGPPS inhibitors could be a great alternative to treat cancer malignancies. Nevertheless, N-BPs are growing in popularity and remain a good treatment for bone-related malignancies.

Another potential therapeutic target for hGGPPS is Alzeihmer's disease.[35] Alzheimer disease is the main cause of dementia in Canada and according to *Alzheimer Society Canada,* it costs more than 10.4 billion dollars every year to treat dementia and that represent a huge financial burden.[36] Moreover, medications on the market can only treat symptoms of Alzheimer. Only two class of drugs have yet been approved, cholinesterase inhibitors and NMDA receptor antagonist which are both use to lessen memory loss and confusion.[37] Small GTPases play a crucial role in cell survival and neuronal plasticity. The prenylation cascade from GGPP → RhoA-cdc42 →GSK3-β → phosphorylated-Tau has been proposed as largely responsible for hyper-phosphorylation of the tau protein (P-Tau) in the brain and tangle formation in the neurons.[38] P-Tau is the hallmark of

neurofibrillary tangle formation in the brain and progression of the Alzheimer's disease. Providing a new drug-like compound that inhibits hGGPPS would lead to better understand of hGGPPS mechanism of action and thus better help understand a possible cause of AD. Previous studies have demonstrated a link between the level of GGPP in the brain of mice and their age.[35] Furthermore, it has been reported that the level of hGGPPS mRNA and GGPP metabolite in AD brains are higher than in the brain of control subjects of the same age and gender.[39] These results are consistent with the finding of our collaborator Prof. Juders Poirier (Professor of Medicine and Psychiatry, McGill University, Director, Research Program on Aging, Cognition and Alzheimer's Disease and Associate Director, Centre for the Studies on the Prevention of Alzheimer's disease, Douglas Mental Health University Institute). However, selective inhibitors of hGGPPS, with good drug-like properties, have not being identified. Consequently, validation of hGGPPS as a potential therapeutic target for the prevention of AD remains speculative.

1.4 Inhibitors of the hGGPPS

A limited number of hGGPPS inhibitors have been reported to date. While some of the reported inhibitors show good selectivity and potency, none of them were co-crystallized with hGGPPS. Moreover, most of them do not show promising "drug-like" structures, since they are for the most part substrate mimic with long sp^3 and sp^2 carbon chains that are likely susceptible to oxidation and fast clearance. Furthermore, all of these substrate mimic inhibitors harbor the well-known bisphosphonate moiety that is also present in the N-BP inhibitors for hFPPS. This fact is not surprising since hGGPPS and hFPPS are thought to share similar enzymatic mechanism.[3] However, hGGPPS has a bigger enzymatic cavity than hFPPS and consequently, it has been shown that inhibitors with a long and rigid side chain are more selective in inhibiting hGGPPS.

One of the first selective inhibitors of hGGPPS (over hFPPS) was reported by Oldfield's group (Fig. 1.6; compound **1.13**).[44] This compound is composed of a long alkyl chain with several aromatic rings attached to an α-hydroxy bisphosphonate.[40] This inhibitor resembles N-BPs with longer side chain. The extended lipophilic chain was design to prevent the inhibitor from binding to the small enzymatic pocket of hFPPS and hence providing it with selectivity towards hGGPPS. While its potency remains low, Oldfield's goal was achieved in showing that long rigid side chain provides selectivity to hGGPPS. Another strategy was to simply add a long and flexible alkyl chain to a risedronate-like compound, such as **1.15**. Those types of molecules were design as dual inhibitor for hGGPPS and hFPPS since the long lipophilic tail could fit in both enzymatic pockets.[40] Those dual inhibitors further prove what Oldfield suggested, rigid side chains are required to provide selectivity towards hGGPPS. It is postulated that the side chain of **1.15** folds itself to fit into the hFPPS pocket. The most potent and selective inhibitors known to date were synthesized by Wiemer's group (Fig. 1.6). Compound **1.14** is composed of a bisphosphonate moiety with two geranyl chains forming a V-shape that acts as a substrate mimic and is thought to bind in the FPP pocket and the product GGPP inhibitory pocket of hGGPPS.[30] This fact is what is postulated to provide increased potency of such compounds. However, the most potent and selective compound reported to date are composed of only one geranyl chain.[41] Compound **1.16** also harbour the well-known bisphosphonate moiety. As for now, non-bisphosphonate compounds with a "drug-like" structure have not been reported.

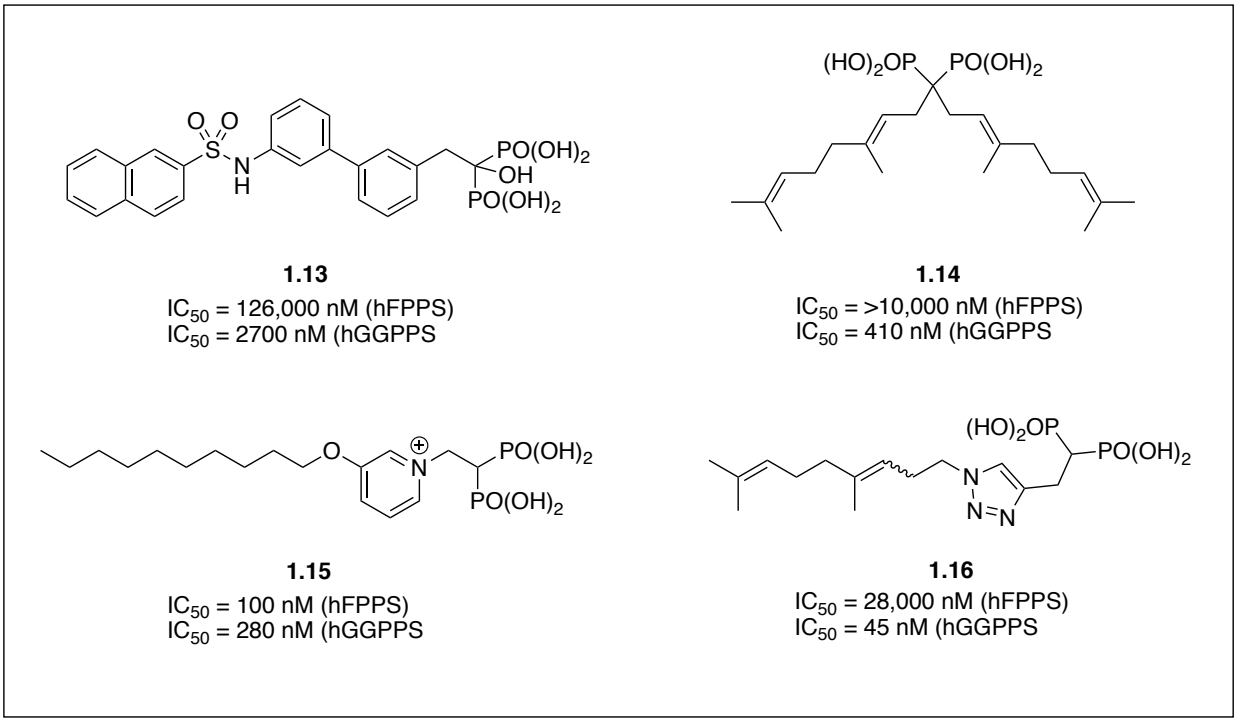


Figure 1.6. Structure, potency and selectivity of hGGPPS inhibitors.

1.5 "Hit identification" of the hGGPPS inhibitors

Numerous reports have been published highlighting the use of the thienopyrimidine core in the design of biologically active compounds and approved drugs used for the treatment for fungal[42] , microbial[43] and viral[44] infections. Our group previously reported the synthesis of various inhibitors harbouring a thieno[2,3-*d*]pyrimidine and phosphonate chemotypes as hFPPS inhibitors.[45, 46] (Fig 1.7)

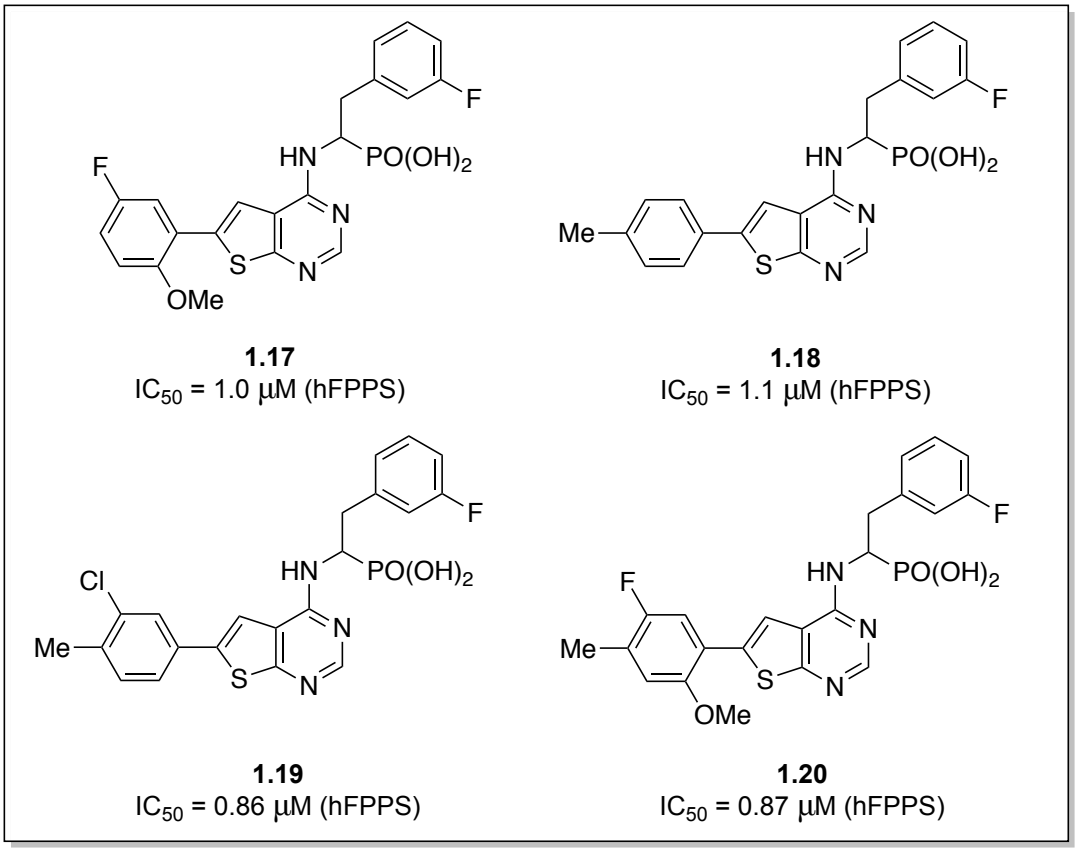


Figure 1.7. Potent inhibitors of hFPPS.

Additionally, our group screened a library of thienopyrimidine-based bisphosphonate compounds for probing their selectivity between hFPPS and hGGPPS using *in vitro* inhibition assays.[45] (Fig. 1.8) Based on this screen, analogs were identified that exhibited a slightly higher selectivity for inhibiting hGGPPS as compared to hFPPS. For instance, compound **1.22** which is a bisphosphonate analog of compounds **1.16** to **1.20** shows a 2 folds selectivity for hGGPPS over hFPPS. Moreover, when the aromatic group is moved from the C_6 position to C_2, selectivity for hGGPPS increases by another 2 folds. In addition to that, when further elongating the side chain at the C_2 position, selectivity increases by another 4 folds making compound **1.23** 31 times more selective for hGGPPS than for hFPPS. To access whether the selectivity comes only from longer side chain, compound **1.24** was synthesized and tested. This compound is substituted at the C_6

position with a long side chain and shows no potency for both enzymes up to a concentration of 10 uM. This proves that the binding mode of the bisphosphonate is crucial and that substitution at C_2 is needed.

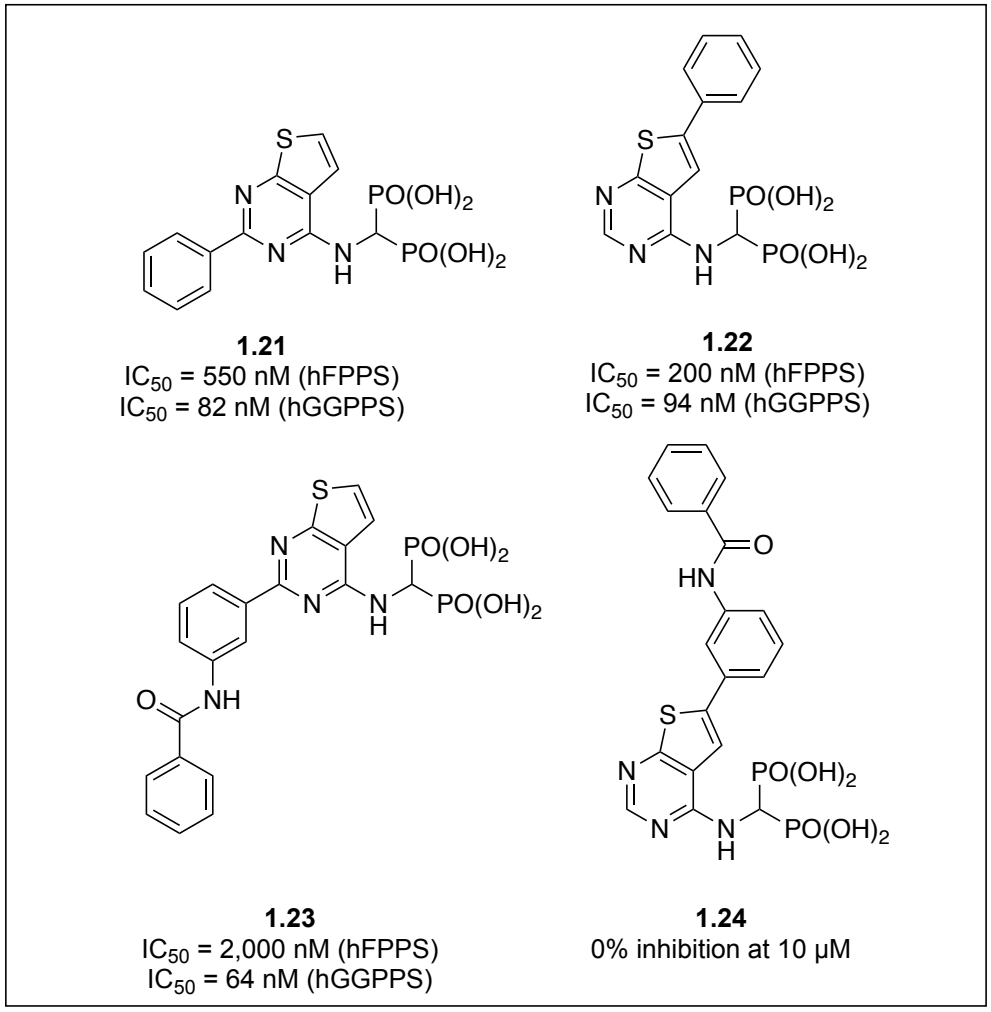


Figure 1.8. Pharmacophore mapping of hGGPPS.

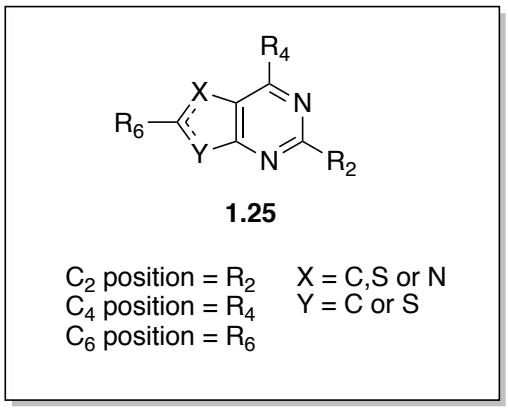


Figure 1.9. Scaffold substitutions.

Probing of the hGGPPS active site cavity was then pursued by synthesizing various compounds structurally related to **1.25**. (Fig. 1.9) Long aromatic side chain at C_2 position were found to increase selectivity and potency towards hGGPPS. Harbouring a bisphosphonate moiety at the C_4 position was also found to be crucial for maintaining good activity in our *in vitro* assays. Based on those finding, our group started building a structure-activity relationship model, which will be discussed in chapter 3.

1.6 Scaffold elaboration

Preliminary screen and optimization of various compounds led us to further investigate analogs with a general structure of **1.25**. The potency and their structure-activity relationship (SAR) of these analogs will be discussed in chapter 3. In drug discovery, it is well known that scaffold "hopping" can often lead to significant improvements in the overall biopharmaceutical properties of class of compounds, including cell-based potency, metabolic stability, oral bioavailability and systemic exposure, without affecting the intrinsic potency (*i.e.* affinity for binding to the biological target) of the compounds. Additionally, analogs based on a single scaffold would be a major liability in terms of patent protection. Finally, it is possible that changing the

scaffold of our compounds could improve the overall potency of these compounds by slightly changing the interactions made in the hGGPPS binding cavity. This would change the SAR of the new series of compounds and consequently, another library of those type of scaffolds would be needed. The primary focus of this thesis is the synthesis of two new scaffolds as well as the building of a library of compounds for those new scaffolds. Therefore, various ways of making similar scaffolds to thieno[2,3-d]pyrimidine has been explored.

Retrosynthetic analysis of fused heterocyclic compounds like thienopyrimidine reveals two simple disconnections to obtain two different ring systems. (Fig. 1.10) From this analysis, it is clear that at least two routes are viable to form those type of heterocyclic compounds, either through first the elaboration of a tetra-substituted pyrimidine or the elaboration of a di-substituted 5-membered heteroaromatic ring.

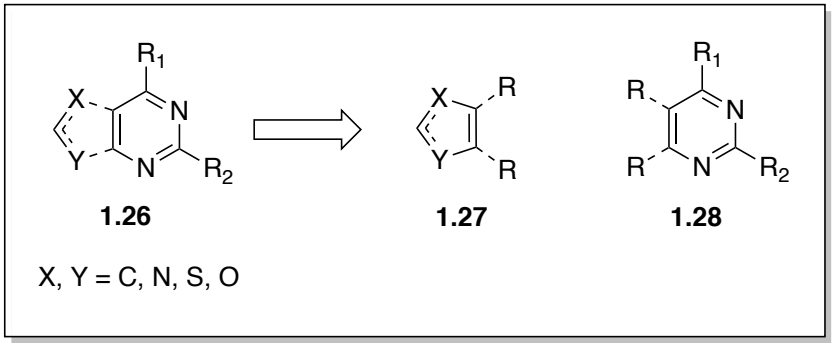
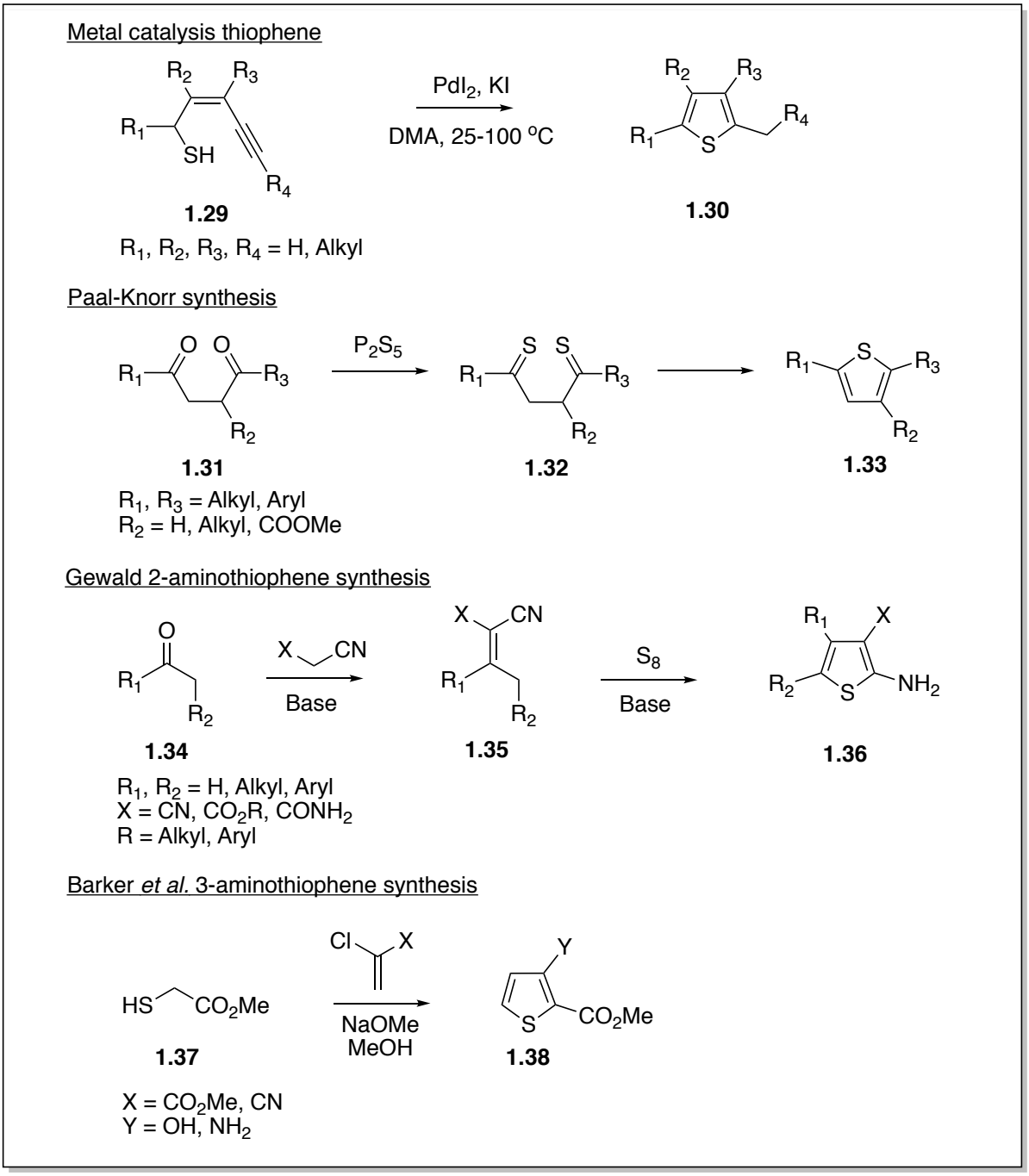


Figure 1.10. Retrosynthetic analysis of fused heterocyclic pyrimidine compounds.

While the synthesis of a tetra substituted pyrimidine can be quite challenging and limited, di substituted thiophene or thiazole are either readily available or can be synthesized through known procedures.[47] (Scheme 1.1) Many procedure have been reported for the synthesis of substituted thiophenes ranging from metal catalyzed reactions to the well-known Gewald or Paal-Knorr thiophene synthesis. Metal catalyzed reactions are often used to add substituents directly on

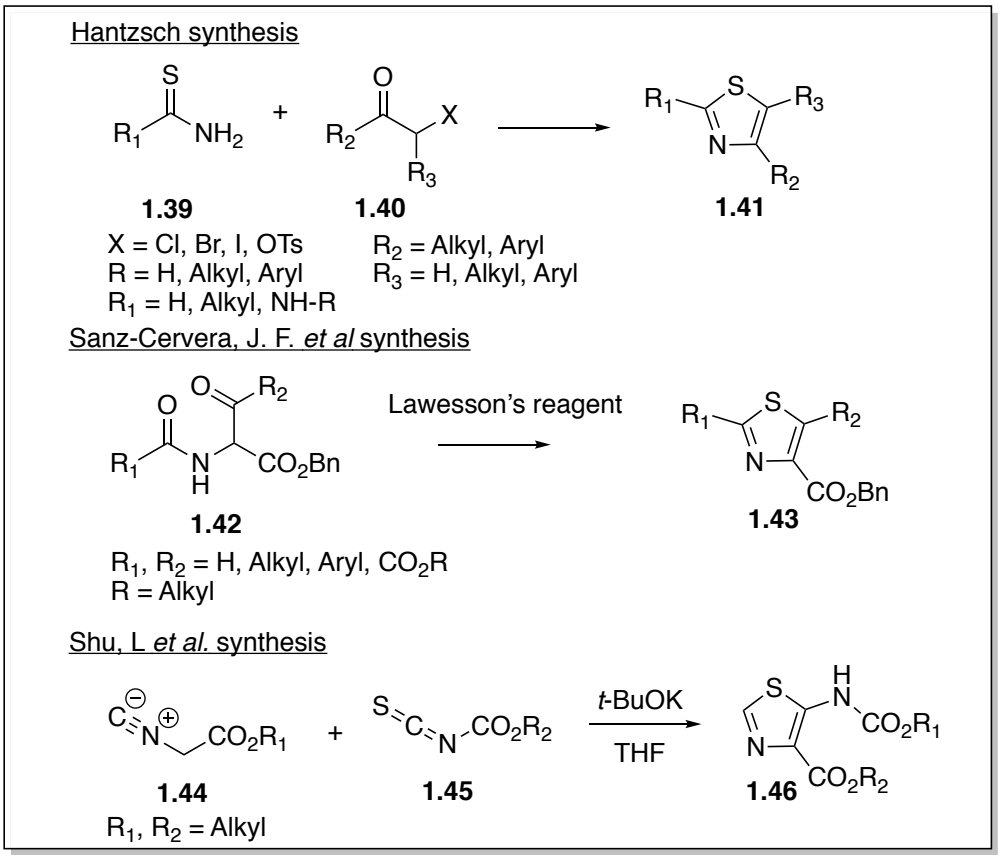
a thiophene, but rarely used for the formation of a thiophene itself. This is probably owing to their limited substrate scope due to their low tolerance of functional groups, especially to sulphur.[48, 49] On the other hand, Paal-Knorr synthesis is more versatile in that it tolerates more functional groups but is limited in the substitution patterns it has.[50, 51] (Scheme 1.1) The well-known Gewald reaction has generally moderate yield, but offers access to 2-aminothiophene and a wide variety of functional groups which can be used to elaborate a pyrimidine ring.[52] Modifications of this reaction have also been made, utilizing its ylidene intermediate. The Gewald reaction undergoes Knoevenagel condensation of a carbonyl (**1.34**) with a malononitrile derivative to form a stable ylidene (**1.38**). It then cyclizes in presence of elemental sulfur to afford an analog of **1.36**. Since the reaction can be done step wise, more elaborated ylidene can be first synthesized and then used in the cyclization reaction.[53] Other modifications have been described. For instance, the use of 1,4-dithiane-2,5-diol, a dimer of 2-mercapto acetaldehyde has been used as a source of carbonyl for condensation and of sulfur for cyclization. This allows cyclization without the addition of elemental sulfur. A brilliant use of this modification was made by Barker *et al*.[53] (Scheme 1.1) His group took advantage of this and used a similar protocol to gain access to 3-aminothiophene which was not possible with the original Gewald procedure. Access to 3-amino or 2-aminotriophene is critical for elaboration of a pyrimidine ring and so modifications of the Gewald synthesis. These procedures were chosen as our synthetic protocol. (Scheme 2.1, 2.2)



Scheme 1.1. Substituted thiophene synthesis.

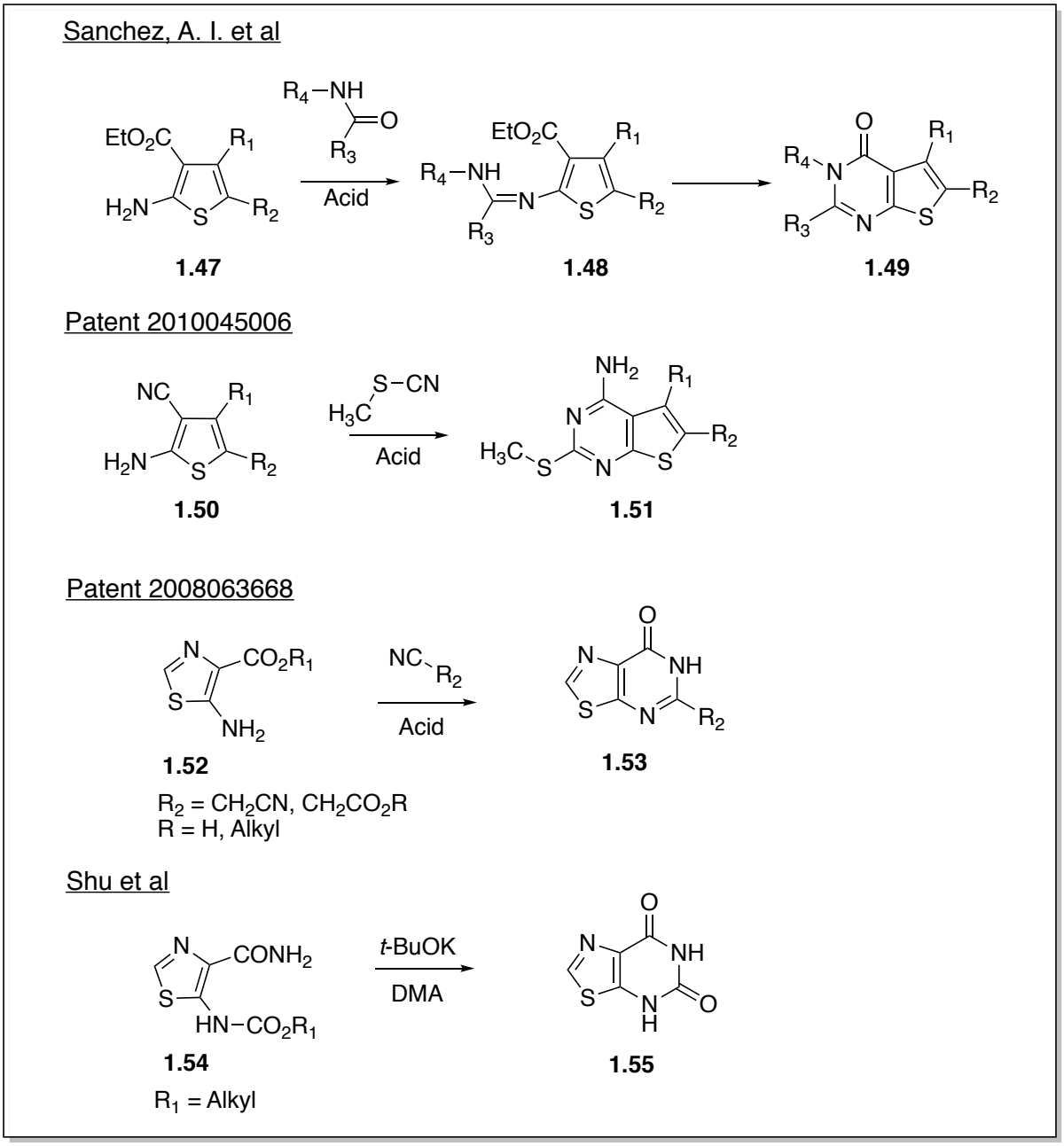
The same retrosynthetic analysis can be applied for the preparation of thiazolopyrimidine. For the same reason, synthesis of substituted thiazole to achieve formation annulation of a pyrimidine ring is also desired. Thiazole rings can be obtained by the well-known Hantzsch

synthesis. Modification to the Hantzsch protocole include the Holzapfel-Meyers-Nicolaou modification which allows elaboration of a thiazole ring from a chiral thioamide (1.39 where R_1 is a chiral center) without racemization.[54] Intramolecular reactions can also be achieved by introduction of a thionating agent such as Lawesson's reagent to promote a cyclization reaction when two carbonyl group are present.[55] (Scheme 1.2) However, an even simpler method was developed by Shu *et al* which enable access to the desired di substituted thiazole from readily available starting materials. The thiazoles formed by this method are also well suited for the formation of thiazolopyrimidine ring. It is worth noting that while many other protocols are available for formation of thiazole containing a substituent with a chiral group, those were not required in our synthetic scheme.



Scheme 1.2. Substituted thiazole synthesis.

Elaborating the synthesis of a pyrimidine ring from a di-substituted thiophene is a well-known transformation, several protocols are available. Most of the procedures which allow for the formation of a C_2 substituted pyrimidine include a nucleophilic nitrogen at the 2 position and an electrophilic carbonyl carbon at the 3 position (**1.47**).[56, 57] (Scheme 1.3) In fact, formation of a Schiff base followed by a nucleophilic attack an electrophilic carbon to form a pyrimidinone is one of the most used strategy. Similar strategies have been reported using thiocyanates.[58] (Scheme 1.3) However, they require strong acidic condition to activate the nitrile group directly attached to the thiophene. The same conditions involved in synthesis of **1.51** can be used for the synthesis of thiazolopyrimidine **1.53**.[59] This methodology can be further extended to the synthesis of thieno[3,2-*d*]pyrimidine. Intramolecular reaction to elaborate a thiazolopyrimidinone is also possible.[60] A strong base such as t-BuOK is used to deprotonate the nucleophilic nitrogen of **1.54** which then attack the electrophilic carbon of the carbamate moiety to form the pyrimidinone ring. This methodology gives access to thiazolopyrimidinone which can be further modified to be used in our synthetic route.



Scheme 1.3. Synthesis of thienopyridime, thiazolopyrimidone and thiazolopyrimidinone from di-substituted thiophenes and di-substituted thiazoles.

1.7 Thesis objectives

The main goals of my studies were to design and synthesis various hGGPPS inhibitors. More specifically, the synthesis of new heterocyclic cores for our inhibitors that were only

thieno[2,3-d]pyrimidine cores harbouring a bisphosphonate moiety. The synthesis of a monophosphonate was also a main goal of the research. This was done in order to evaluate if this functional group had any potential to increase the drug-like character of our inhibitors by reducing the net charge of the compounds in biologicaly relevent media. Lastly, the synthesis of a small library of compounds with the new heterocyclic cores was also a key objectives in order to to increase our structure-activity relationship.

The design of bisphosphonate containing compounds was based off of previous work from my group highlighted in **Chapter 1**, section **1.5**. The synthesis of the monophosphonate and bisphophonate compounds are summerized in **Chapter 2**, section **2.2**. The structure-activity relationship is brielfy described in **Chapter 3**, section **3.2**. Most of the compounds were not tested at the time of the submission of the thesis, therefore conclusions on the SAR remain only preliminary.

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Chapter 2: Synthesis of hGGPPS Inhibitors

2.1 Introduction:

Numerous reports have been published highlighting the use of thienopyrimidine core in the design of biologically active compounds with properties as antifungal, antibacterial and other therapeutic agents. Various methodologies have also been reported for the preparations of highly substituted pyridines and thienopyrimidines, as briefly summarized in section 1.6. Some of these methods were utilized in our synthetic protocol to achieve the synthesis of substituted thieno[2,3-*d*]pyrimidine, thieno[3,2-*d*]pyrimidine and thiazolo[5,4-*d*]pyrimidine.

2.2 Contribution of author:

The synthesis all the compounds shown in Figure 2.1 was achieved as part of this MSc thesis.

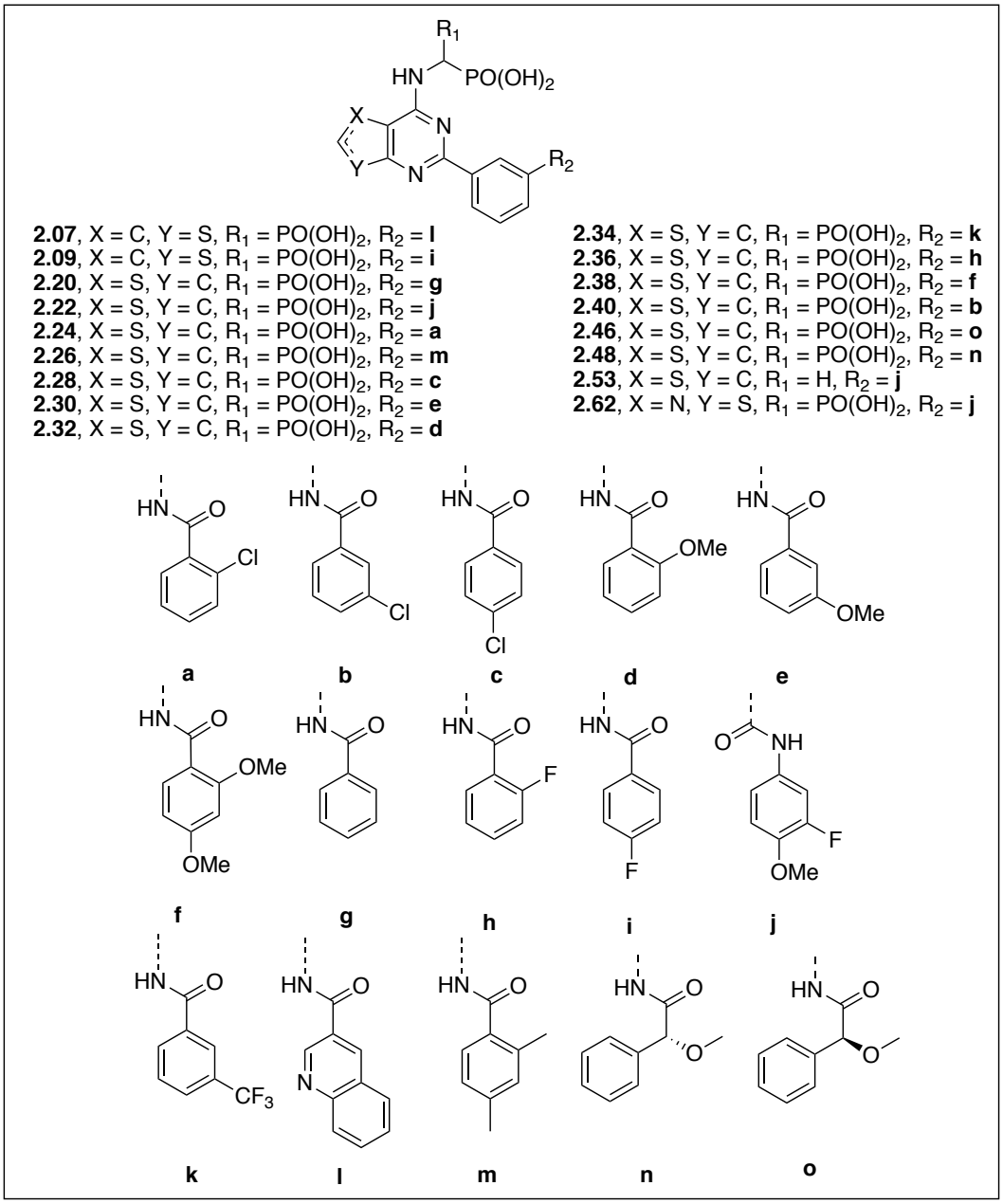
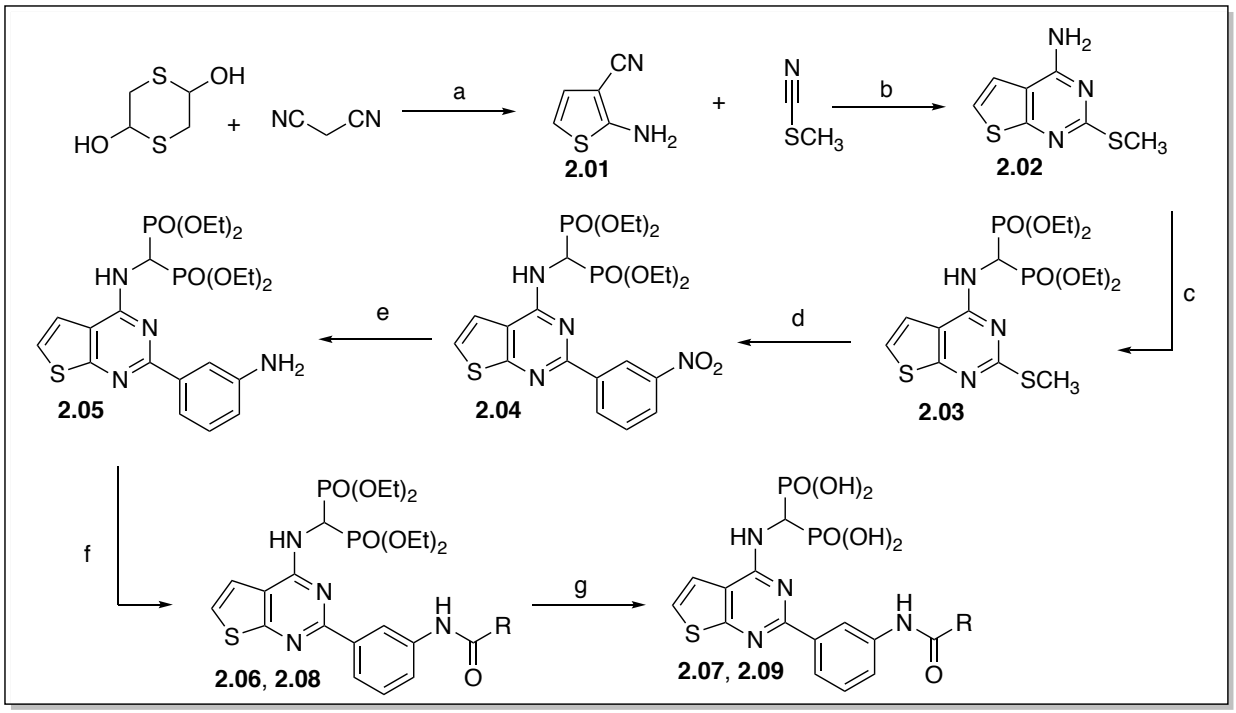


Figure 2.1. Analogs synthesized contributing to the structure-activity relationship studies.

2.3 Chemistry

The strategy used for the synthesis of thieno[2,3-*d*]pyrimidine-based inhibitors is outlined in Scheme **2.1**. Gewald synthesis of thiophene was used to generate the desired substituted intermediate **2.01**.[1] Cyclization of the thiophene in presence of methyl-thiocyanate gave the desired substituted thieno[2,3-*d*]pyrimidine scaffold **2.02**, which can be further modified at either the C_2 and/or the C_4 position. Installation of the tetraethyl bisphosphonate ester at C_4 was first conducted by using triethyl orthoformate and diethyl phosphite at relatively high temperature (130°C).[2] Elongation at the C_2 position was realized by the Liebeskind-Srogl coupling, which is thought to involve the mechanism depicted in Figure **2.2**.[3] This modified Suzuki-like reaction was used to couple arylthiomethyl ethers to aryl boronic acid harboring various functional group. The reaction is catalyzed by copper (I) thiocarboxylate (CuTC) and various palladium (0) catalysts. CuTC was proven to be essential and could not be replaced by any copper halides suggesting an important role for the carboxylate moiety. The carboxylate is thought to activate the boronic acid by formation of a dative bond. This idea stems from the fact that the reaction occurs without a base, proposing that the boron specie must be activated by another specie which could be the carboxylate.[3] (Figure **2.2**). Additionally, in some cases, the addition of zinc acetate led to higher yields, likely due to the formarion of a organometalic species which prevent nitrogen-containing groups from coordinating to Pd (0) or to the boronic acid. Further modifications to the coupled aryl moiety were subsequently accomplished by reduction of the nitro group of intermediate **2.04** to the amino group using tin(II) chloride.[4] Amide coupling with different carboxylic acids was achieve using HBTU as the coupling reagent to convert intermediate **2.05** to the phosphonate tetraester **2.06** and **2.08**.[5]



Scheme 2.1. Procedure for synthesis of thieno[2,3-d]pyrimidine based inhibitors.

Reagents and reaction conditions: (a) NEt_3, MeOH/DMF, 50 °C, 1h (42%); (b) 4M HCl in dioxane, 70 °C, 24h, (80%); (c) Triethyl orthoformate, Diethyl phosphite, 130 °C, 40h, (81%); (d) Aryl boronic acid, CuTC, $Pd_2(dba)_3$, dioxane, 80 °C, 4h, (87%); (e) $SnCl_2 \cdot H_2O$, EtOH, 80 °C, 2h, (84%); (f) HBTU, DIPEA, DMF, r.t., 12h, (37 - 65%); (g) TMS-Br, MeOH, r.t., 3 - 5 days, (63 - 74%).

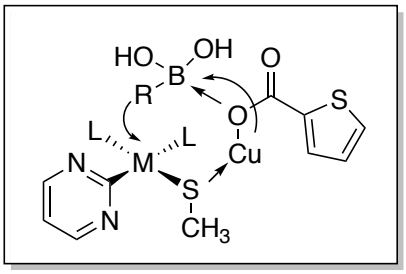
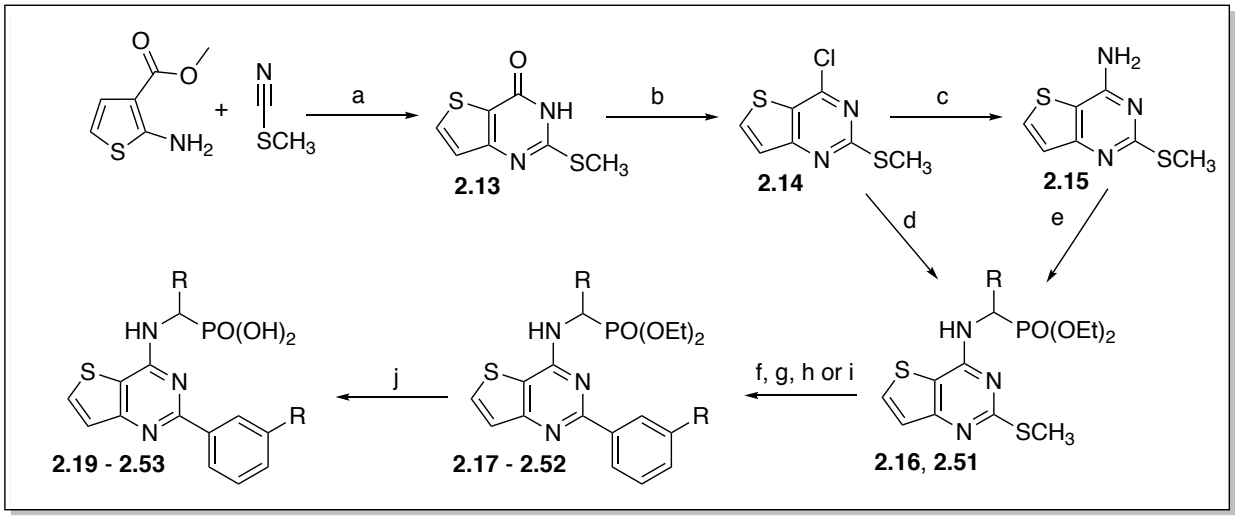


Figure 2.2. Proposed mechanism of the Liebeskind-Srogl coupling reaction.

As previously mentioned in chapter1, the synthetic route to thieno[3,2-*d*]pyrimidinone is similar to its isomer, thieno[2,3-*d*]pyrimidinone. Here, readily available methyl carboxylate

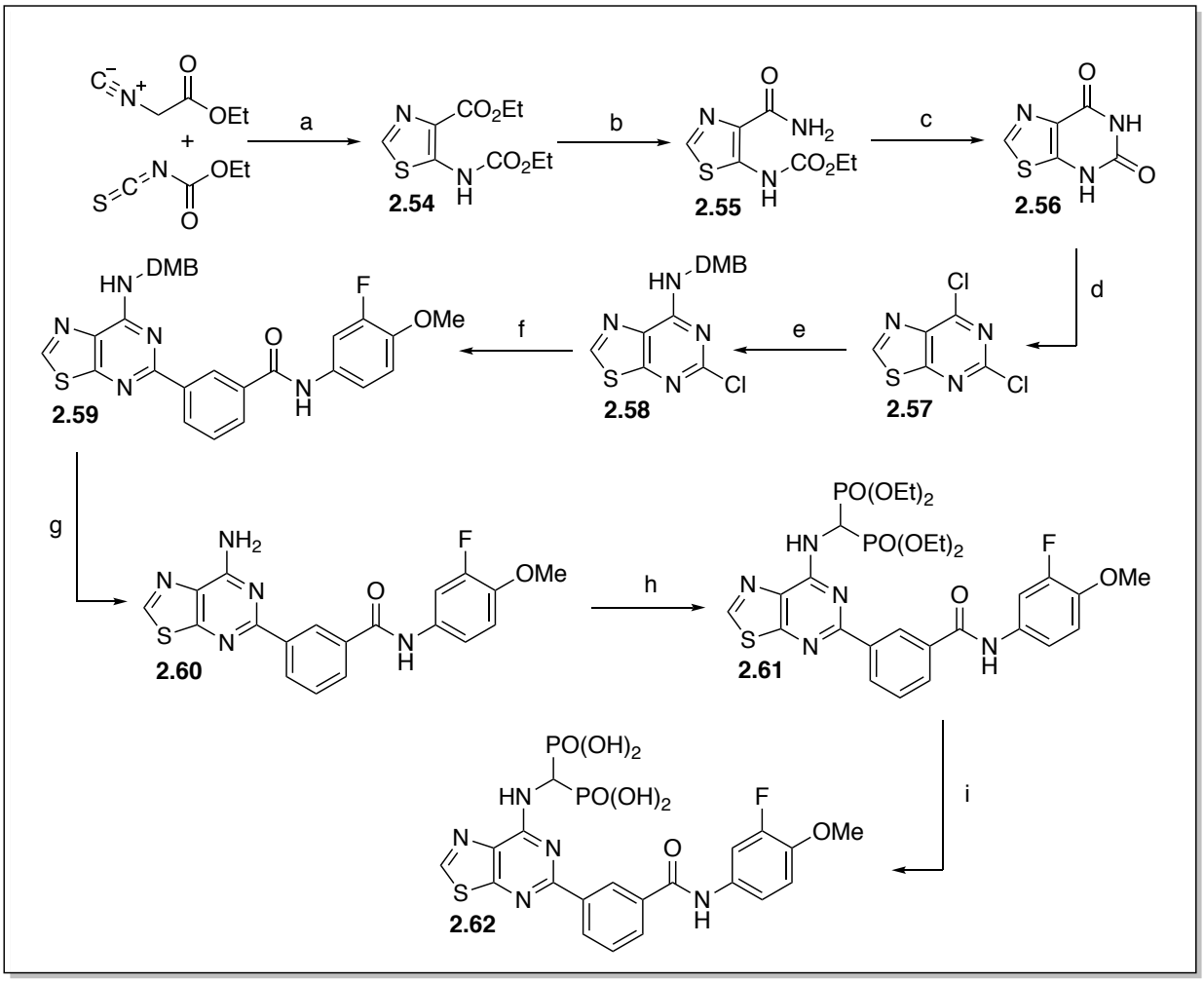
aminothiophene was directly used to elaborate the pyrimidone ring **2.13**. The compound was then subjected to phosphorus oxychloride conditions to get chloropyrimidine **2.14**. Further modification to conversion to the key amino-thienopyrimidine **2.15** or **2.16** was achieved by nucleophilic substitution either with ammonium hydroxide under pressured conditions or by treatment of amino-phosphonate under basic conditions. Having those two key amines in hands, two pathways were envisioned. One where a Suzuki-like coupling would permit further elaboration of the compound through amide coupling and the other one, where a fully elaborated side chain would be installed through the same coupling reaction.



Scheme 2.2. Procedure for synthesis of thieno[3,2-d]pyrimidine based inhibitors.

Reagents and reaction conditions: (a) 4M HCl in dioxane, 90 °C, 48h, (80% - quant.); (b) $POCl_3$, 106 °C, 12h, (65 - 93%); (c) Conc. Aqueous Ammonia, 90 °C, 12h, (70 - 92%); (d) diethyl (aminomethyl) phosphonate, NEt_3, dioxane, 100 °C, 72h, (58%); (e) Triethyl orthoformate, Diethyl phosphite, 130 °C, 40h, (40 - 62%); (f) Aryl boronic acid, CuTC, $Pd_2(dba)_3$, $Zn(OAc)_2$ dioxane, 80 °C, 12h, (60 - 87%) (g) H_2, (1atm), Pd/C (10 wt. %), EtOAc, 18h, (64 - 82%); (h) HBTU, DIPEA, DMF, r.t., 12h, (56 - 91%); (i) Aryl boronic acid, CuTC, $Pd_2(dba)_3$, $Zn(OAc)_2$ dioxane, 80 °C, 12h, (56%); (j) TMS-Br, MeOH, r.t., 3-6 days (31 - 78%).

Another strategy was elaborated to form thiazolo[5,4-*d*]pyrimidine inhibitors. Isocyanoacetate and isothiocyanate were reacted in presence of base to afford the substituted thiazole **2.54**. From that point reactions were performed to afford dichlorothiazolo[5,4-*d*]pyrimidine following Shu's and coworkers procedure described in chapter 1, summarized in scheme **1.2** and **1.3**.[6] Protection of the C_7 position was then performed by nucleophilic substitution of 2-4 dimethoxy benzyl amine (DMB). After Suzuki coupling, the protecting group, DMB, was removed by treatment with TFA in DCM to afford **2.60**. Installation of the bisphosphonate ester and deprotection were achieved using the same procedure as previously described in Scheme **2.2**.



Scheme 2.3. Procedure for synthesis of thiazolo[5,4-d]pyrimidine based inhibitors.

Reagents and reaction conditions: (a) t-BuOK, THF, - 40 to 0 °C, 2h, (76%); (b) NH_4OH, EtOH, 40 °C, 16h, (71%); (c) DMA, t-BuOK, 100 °C, 2h, (79%); (d) $POCl_3$, 106 °C, 3.5h, (57%); (e) 2,4-Dimethoxy benzyl amine, DIPEA, DMSO, r.t., 3h, (59%); (f) Aryl boronic acid, $Pd(PPh_3)_4$, KF, MeOH/dioxane, 90 °C, 18h, (88%); (g) TFA, DCM, 40 °C, 72h, (72%); (h) Triethyl orthoformate, Diethyl phosphite, 130 °C, 48h (40 - 60%); (i) TMS-Br, MeOH, r.t., 5 days, (77%).

2.4 References

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2.5 Experimental

General

All NMR spectra were obtained on a Bruker AV400 400MHz spectrometers or a Bruker AV500 500MHz spectrometer. LC trace and low resolution mass (MS) were obtained using Waters ALLIANCE® instrument (e2695 with 2489 UV detector, 3100 mass spectrometer, C18 5µm column). High resolution mass spectra were obtained using electrospray ionization (ESI+/-).

General Methods:

General method for the synthesis of tetraethyl bisphosphonate esters (A):

4-amino-thienopyrimidine intermediate (1.0 eq), diethyl phosphite (7.0 eq) and triethyl orthoformate (1.7 eq) were dissolved in toluene (2.2 ml). The mixture was stirred and heated at 130 °C for 48 h. The reaction was monitored by TLC and LC-MS. Upon completion, the reaction was cooled to r.t. and concentrated *in vacuo*. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument (solvent gradient from 0 to 100% EtOAc in Hexane, then from 0 to 20% MeOH in EtOAc).

General method for the Liebeskind-Srogl cross coupling (B):

Thiomethyl ether intermediate (1.0 eq), boronic acid (2.5 eq), CuTC (2.5 eq), zinc acetate (1.4 eq) and 1,4-dioxane (0.6 ml) were added into an oven-dried microwave tube. The mixture was stirred and bubbled with argon for 10 minutes, then $Pd_2(dba)_3$ (0.05 eq) was added and the vial was sealed. The reaction was then stirred and heated to 80 °C for 36h (under argon). The reaction was followed by TLC and/or by LC-MS. Upon completion, the reaction was diluted with ethyl acetate. The solution was filtered with filter paper and the residue was washed with ethyl acetate. The filtrate

transferred into a separating funnel and washed with 10% aqueous ammonium hydroxide (three times), followed by brine. The organic extract was dried over sodium sulfate and concentrated *in vacuo*. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument (solvent gradient from 0 to 100% EtOAc in Hexane, then from 0 to 20% MeOH in EtOAc).

General method for amide coupling (C):

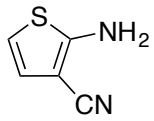
The amine intermediate (1 eq), carboxylic acid (1.1 eq) and HBTU (1.1 eq) were dissolved in dry DMF (1.9 ml) under argon atmosphere. To this solution, DIPEA (0.03 ml, 2 eq) was added and stirred at r.t. The reaction was followed by TLC. Upon completion of the reaction, brine (10 ml) was added and the mixture was extracted with ethyl acetate (20 ml; twice). The organic phase was washed with saturated ammonium chloride (10 ml), brine, dried over sodium sulfate and concentrated *in vacuo*. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument (solvent gradient from 0 to 100% EtOAc in Hexane, then from 0 to 15% MeOH in EtOAc).

General method for the deprotection of the bisphosphonate to the phosphonic acid (D):

The bisphosphonate ester (1 eq) was dissolved in dry DCM (5.0 ml) and the solution was cooled to 0 °C. TMS-Br (15 eq) was then added and the mixture was stirred at r.t. for 3 to 7 days. The reaction was followed by ^{31}P-NMR. After completion, it was concentrated *in vacuo*. The residue was added with methanol (1.0 ml), swirled and sonicated briefly. The solvent was then removed under vacuum (methanol and sodium bicarbonate in the receiving flask), and additional methanol (1.0 ml) was added to the resulting residue. This procedure was repeated several times (3 to 5

times). The residue was dissolved with a few drops of methanol, and then water (few drops) was added to precipitate out the product which was filtered, washed with double deionized water, HPLC acetonitrile (1.0 ml), HPLC chloroform or ethyl ether (1.0 ml), HPLC hexane and then dried *in vacuo*.

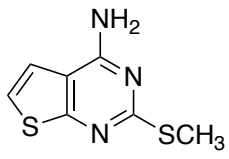
2-aminothiophene-3-carbonitrile (2.01)



1,4-dithiane-2,5-diol (5.00 g, 32.8 mmol) and malononitrile (4.34 g, 65.7 mmol) were dissolved in DMF (0.3 ml, cat.) and anhydrous methanol (20 ml). To the stirring solution was added EtN_3 (4.58 ml, 32.8 mmol) (dropwise). The reaction mixture was then stirred and heated at 50 °C for 1 hour. The reaction was followed by TLC. Upon completion, the reaction was cooled down to r.t. Then hexane (10ml) was added to the reaction mixture. The reaction was further cooled down at -4 °C (in the freezer) for 1 hour. The mixture was then filtered and the residue was washed with hexane. The residue was then dried *in vacuo* to afford a crude light brown solid (1.72 g, 42%), which was used in the next step without further purification.

^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 6.73 (d, J = 5.7 Hz, 1H), 6.35 (d, J = 5.7 Hz, 1H), 4.75 (br s, 2H).

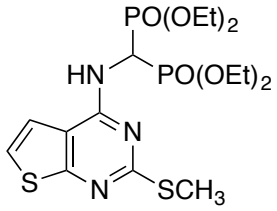
2-(methylthio)thieno[2,3-*d*]pyrimidin-4-amine (2.02)



4M HCl in 1,4-dioxane (21 ml, 82.9 mmol) was added to solid **2.01** (1.72 g, 13.8 mmol), followed by methyl thiocyanate (1.00 g, 13.8 mmol). The resulting suspension was heated to 70 °C in a sealed pressured tube for 24 h. The reaction was monitored by TLC. The mixture was allowed to cool to r.t. and the resulting greenish precipitate was collected by vacuum filtration. The green solid **2.02** was dissolved in ethyl acetate and washed with saturated aqueous sodium bicarbonate, layers separated and the aqueous phase was extracted further with ethyl acetate (thrice). The organic extracts were combined, washed with hexane, dried over sodium sulfate and concentrated *in vacuo*. Product was obtained as light brown solid (2.18 g, 80%), which was used in the next step without further purification.

^{1}H NMR (500 MHz, DMSO-d$_6$): δ (ppm) 7.55 (br s, 2H), 7.47 (d, *J* = 5.9 Hz, 1H), 7.36 (d, *J* = 5.9 Hz, 1H), 2.46 (s, 3H). **^{13}C NMR (126 MHz, DMSO-d$_6$):** δ 167.5, 166.5, 158.3, 120.7, 120.2, 113.4, 13.8. MS [ESI$^+$] m/z: 198.0 $[M + H]^+$

Tetraethyl (((2-(methylthio)thieno[2,3-*d*]pyrimidin-4-yl)amino)methylene) bis(phosphonate) (2.03)

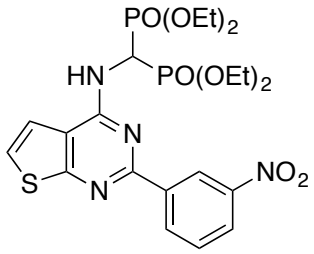


Compound **2.03** was synthesized using the general procedure **A**. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument (1.10 g, 21%).

^{1}H NMR (500 MHz, DMSO-d$_6$): δ 8.70 (d, *J* = 9.7 Hz, -NH), 7.97 (d, *J* = 6.0 Hz, 1H), 7.45 (d, *J* = 6.0 Hz, 1H), 5.70 (td, *J* = 23.6, 9.7 Hz, 1H), 4.14 – 4.02 (m, 8H), 2.50 (s, 3H), 1.21 (t, *J* = 7.1 Hz, 6H), 1.14 (t, *J* = 7.0 Hz, 6H). **^{13}C NMR (126 MHz, DMSO-d$_6$):** δ 167.3, 165.4, 155.1 (t, *J* =

4.1 Hz), 12.1, 120.1, 113.6, 62.9 – 62.7 (m), 44.4 (t, *J* = 147.3 Hz), 16.2 – 16.1 (m), 13.5. **^{31}P NMR (203 MHz, DMSO-d$_6$):** δ 16.77 (s). MS [ESI$^+$] m/z: 484.4 $[M + H]^+$

Tetraethyl (((2-(3-nitrophenyl)thieno[2,3-*d*]pyrimidin-4-yl)amino)methylene) bis(phosphonate) (2.04)

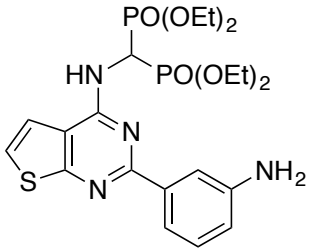


Compound **2.03** (131 mg, 0.27 mmol), 3-nitrophenylboronic acid (113 mg, 0.68 mmol), CuTC (154 mg, 0.81 mmol) and Pd(dppf)Cl_2 (22.1 mg, 0.03 mmol) were added into an oven-dried round bottom flask. The flask was evacuated and purged with argon, then 1,4-dioxane (1.5 ml) was added. The flask was sealed and heated to 50 °C for 4h under argon atmosphere. The reaction was monitored by TLC. Upon completion, the reaction was diluted with ethyl acetate and filtered, the residue was washed with ethyl acetate. The filtrate was washed with 10% aqueous ammonium hydroxide (thrice), followed by brine. The organic extract was dried over sodium sulfate and concentrated *in vacuo*. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument (solvent gradient from 0 to 100% EtOAc in Hexane, then from 0 to 15% MeOH in EtOAc). Product was obtained as light yellow solid (100 mg, 67%)

^{1}H NMR (500 MHz, DMSO-d$_6$): δ 9.12 (t, *J* = 1.9 Hz, 1H), 8.85 (d, *J* = 9.6 Hz, -NH), 8.80 (d, *J* = 7.9 Hz, 1H), 8.35 (m, 1H), 8.14 (d, *J* = 6.0 Hz, 1H), 4.23 – 4.02 (m, 8H), 1.20 (t, *J* = 7.0 Hz, 6H), 1.12 (t, *J* = 7.0 Hz, 6H). **^{13}C NMR (126 MHz, DMSO-d$_6$):** δ 167.8, 156.5 (t, *J* = 4.0 Hz), 156.4, 148.7, 139.6, 134.1, 130.8, 125.3, 124.9, 122.4, 120.8, 116.4, 63.4 – 63.2 (m), 45.0 (t, *J* =

147.2 Hz), 16.7 – 16.6 (m). **^{31}P NMR (203 MHz, DMSO-d$_6$):** δ 16.86 (s). MS [ESI$^+$] m/z: 559.3 $[M + H]^+$

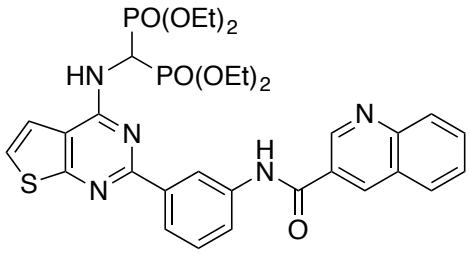
Tetraethyl(((2-(3-aminophenyl)thieno[2,3-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonate) (2.05)



Solid **2.04** (101 mg, 0.18 mmol) was dissolved in ethanol (1.8 ml) in a pressured vessel. $SnCl_2 \cdot H_2O$ (203 mg, 0.90 mmol) was then added and the vessel was sealed. The mixture was stirred and heated at 80 °C for 2h. The reaction was monitored by TLC and LC-MS. Upon completion, the solution was cooled to r.t. and saturated sodium bicarbonate solution was slowly added to the solution. The resulting mixture was extracted with ethyl acetate (thrice), brine and dried over sodium sulfate. It was then concentrated *in vacuo*. Product was isolated as yellow solid (80 mg, 84%), which was used in the next step without further purification.

^{1}H NMR (500 MHz, DMSO-d$_6$): δ 8.52 (d, *J* = 9.7 Hz, 1H), 8.06 (d, *J* = 6.0 Hz, 1H), 7.64 (t, *J* = 1.9 Hz, 1H), 7.58 (d, *J* = 6.0 Hz, 1H), 7.53 (d, *J* = 7.7 Hz, 1H), 7.14 (t, *J* = 7.8 Hz, 1H), 6.67 (dd, *J* = 7.9, 1.5Hz, 1H), 5.22 (br s, 2H), 4.17 – 4.05 (m, 8H), 1.16 (t, *J* = 7.0 Hz, 6H), 1.11 (t, *J* = 7.0 Hz, 6H). **^{31}P NMR (203 MHz, DMSO-d$_6$):** δ 17.18 (s). MS [ESI$^-$] m/z: 527.2 $[M - H]^-$

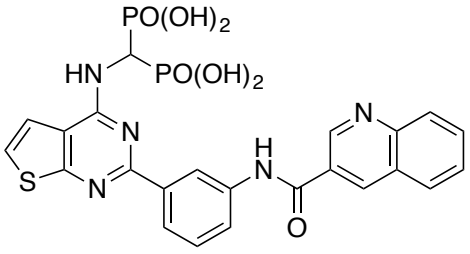
Tetraethyl(((2-(3-(quinoline-3-carboxamido)phenyl)thieno[2,3-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonate) (2.06)



Compound **6** was synthesized following the general procedure **C**. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument. The product was obtained as a yellow solid (23 mg, 37%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.46 (d, *J* = 2.2 Hz, 1H), 8.75 (d, *J* = 2.0 Hz, 1H), 8.53 (s, 1H), 8.43 (s, 1H), 8.30 (d, *J* = 7.9 Hz, 1H), 8.19 (t, *J* = 8.7 Hz, 2H), 7.99 (d, *J* = 7.9 Hz, 1H), 7.88 – 7.82 (m, 1H), 7.66 (t, *J* = 7.1 Hz, 1H), 7.55 (t, *J* = 7.9 Hz, 1H), 7.36 (d, *J* = 6.0 Hz, 1H), 7.32 (d, *J* = 6.0 Hz, 1H), 5.99 (td, *J* = 21.7, 10.0 Hz, 1H), 5.88 (d, *J* = 9.5 Hz, 1H), 4.32 – 4.12 (m, 8H), 1.24 (t, *J* = 7.0 Hz, 12H). **^{31}P NMR (203 MHz, $CDCl_3$)** δ 16.87. MS (ESI^+): (*m/z*) $[M + H]^+$ 684.34

(((2-(3-(quinoline-3-carboxamido)phenyl)thieno[2,3-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonic acid) (2.07)

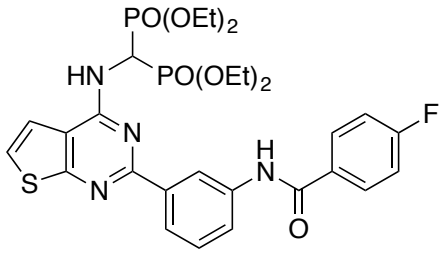


Compound **7** was synthesized following the general procedure **B**. The product was obtained as a yellowish powder (12.4 mg, 74%). **^{1}H NMR (500 MHz, D_2O)** δ 9.16 (s, 1H), 8.83 (s, 1H), 8.27

(s, 1H), 8.12 – 8.03 (m, 2H), 7.98 (d, *J* = 8.5 Hz, 1H), 7.88 (d, *J* = 8.0 Hz, 1H), 7.82 (t, *J* = 7.6 Hz, 1H), 7.66 (t, *J* = 7.4 Hz, 1H), 7.56 (t, *J* = 7.9 Hz, 1H), 7.43 (d, *J* = 5.9 Hz, 1H), 7.35 (d, *J* = 5.9 Hz, 1H), 5.26 (t, *J* = 19.3 Hz, 1H). **^{31}P NMR (203 MHz, D_2O)** δ 13.77. **^{13}C NMR (126 MHz, D_2O)** δ 165.9, 165.1, 159.1, 156.7, 147.9, 147.3, 138.1, 137.3, 137.2, 132.2, 129.4, 129.3, 127.8, 127.0, 126.3, 125.9, 125.0, 123.3, 123.0, 120.7, 118.5, 115.7. C-α to the bisphosphonate was observed by HSQC. HSQC (^{1}H-^{13}C): 1H at δ 5.26 correlates to 13C-α at δ 50.2

HRMS (ESI-) calculated for $C_{23}H_{17}N_5O_7P_2S$ *m/z* $[M - 2H]^-$, 284.5167; found, *m/z* 284.5163

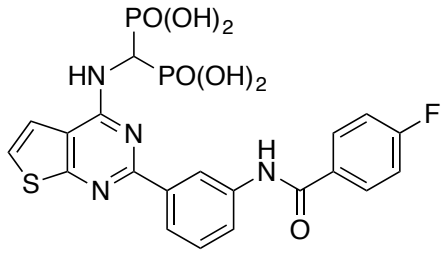
Tetraethyl(((2-(3-(4-fluorobenzamido)phenyl)thieno[2,3-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonate) (2.08)



Compound **8** was synthesized following the general procedure **C**. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument. Product was obtained as a yellow solid (48 mg, 65%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.39 (s, 1H), 8.28 (d, *J* = 7.9 Hz, 1H), 8.14 (d, *J* = 7.6 Hz, 1H), 8.03 – 7.90 (m, 3H), 7.52 (t, *J* = 8.0 Hz, 1H), 7.37 (d, *J* = 6.0 Hz, 1H), 7.21 (t, *J* = 8.5 Hz, 2H), 5.97 (d, *J* = 9.9 Hz, 1H), 5.78 (s, 1H), 4.35 – 4.08 (m, 8H), 1.29 – 1.19 (m, 12H). **^{31}P NMR (203 MHz, $CDCl_3$)** δ 16.84. MS (ESI^+): (*m/z*) $[M + H]^+$ 651.31

(((2-(3-(4-fluorobenzamido)phenyl)thieno[2,3-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonic acid) (2.09)

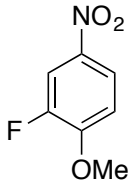


Compound **9** was synthesized following the general procedure **D**. The product was obtained as a beige powder (26.1 mg, 63%).

^{1}H NMR (400 MHz, D_2O) δ 8.37 (s, 1H), 8.20 (d, *J* = 7.9 Hz, 1H), 8.05 – 7.98 (m, 2H), 7.93 (d, *J* = 8.1 Hz, 1H), 7.70 – 7.60 (m, 2H), 7.51 (d, *J* = 6.0 Hz, 1H), 7.39 – 7.29 (m, 2H), 5.17 (t, *J* = 19.0 Hz, 1H). **^{31}P NMR (162 MHz, D_2O)** δ 13.93 (s). **^{13}C NMR (126 MHz, D_2O)** δ 168.5, 165.8, 165.4, 163.8, 159.7, 156.9, 138.5, 137.4, 130.1, 130.0, 129.4, 125.1, 124.1, 122.9, 121.7, 118.9, 115.8, 115.7, 115.5, 49.5 (t, *J* = 122.7 Hz).

HRMS (ESI-) calculated for $C_{20}H_{15}FN_4NaO_7P_2S$ *m/z* $[M-H+Na]^-$, 559.0024; found, *m/z* 559.0010

2-fluoro-1-methoxy-4-nitrobenzene (2.10)

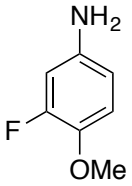


2-fluoroanisole (500 mg, 3.96 mmol), water (25 ml), 96% v/v sulfuric acid (67 ml) and nitric acid (0.3 ml, 6.1 mmol) were added and stirred at 0 °C. To this cold mixture was added dropwise solution of sodium nitrite (271 mg, 3.93 mmol) in water (5 ml). After addition of all the components, the reaction mixture was warmed to r.t. and stirred for 3h, then diluted with water

(300ml) and placed in the refrigerator over-night. The precipitate that formed was collected by filtration and purified by column chromatography. The desired compound was obtained as a yellowish powder (398.0 mg, 59%), which was used with no further purification in the next reaction step.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07 (ddd, *J* = 9.1, 2.7, 1.5 Hz, 1H), 7.99 (dd, *J* = 10.7, 2.7 Hz, 1H), 7.07 – 7.01 (m, 1H), 4.00 (s, 3H). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 153.5 (d, *J* = 10.08 Hz), 152.3, 150.3, 121.1 (d, *J* = 3.78 Hz), 112.3 (t, *J* = 20.16 Hz), 112.2, 56.8.

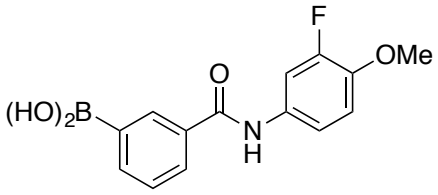
3-fluoro-4-methoxyaniline (2.11)



Compound **2.10** (100 mg, 0.58 mmol) and 10% wt. palladium on carbon (12.4 mg, 0.12 mmol) were added in a round bottom flask. The flask was purged with a hydrogen balloon and then, methanol (5 ml) was added. The reaction flask atmosphere was vacuumed every 2-3h and flushed with hydrogen to remove any air in the flask. The reaction mixture was stirred for 24h under hydrogen atmosphere (1atm balloon). The reaction was monitored by LCMS. Upon completion, the catalyst was removed by vacuum filtration over a celite pad. The filtrate was concentrated *in vacuo*. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument (solvent gradient from 0 to 80% EtOAc in Hexane). The reaction yields a brownish solid (71.2 mg, 86%).

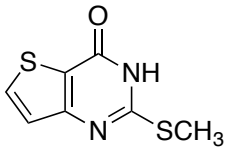
^{1}H NMR (500 MHz, $CDCl_3$) δ 6.79 (t, *J* = 9.0 Hz, 1H), 6.47 (dd, *J* = 12.7, 2.7 Hz, 1H), 6.38 (d, *J* = 8.6 Hz, 1H), 3.81 (s, 3H), 3.49 (s, 2H).

(3-((3-fluoro-4-methoxyphenyl)carbamoyl)phenyl)boronic acid (2.12)



Compound **2.12** was synthesized following the general procedure **C**. The product was obtained as a white solid (101 mg, 58%), which was used with no further purification in the next reaction step. **^{1}H NMR (500 MHz, CD_3OD)** δ 8.20 (s, 1H), 7.93 (s, 2H), 7.62 (dd, *J* = 13.3, 2.5 Hz, 1H), 7.50 (s, 1H), 7.39 (d, *J* = 8.8 Hz, 1H), 7.09 (t, *J* = 9.1 Hz, 1H), 3.88 (s, 3H). MS [ESI^+] m/z: 290.0 $[M + H]^+$

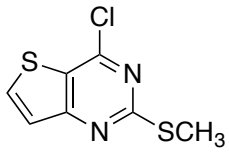
2-(methylthio)thieno[3,2-*d*]pyrimidin-4(3*H*)-one (2.13)



Methyl-3-amino-2-thiophenecarboxylate (1.00g, 6.36 mmol), methyl thiocyanate (465 mg, 6.36 mmol) and 4M hydrochloric acid in dioxane (9.5 ml) were added into a pressured vessel. The resulting suspension was stirred and heated to 90 °C for 24h. The reaction was followed by TLC. Upon completion, the mixture was cooled to r.t. and the resulting white precipitate was collected by vacuum filtration. The solid residue was washed with ethanol followed by hexane. Product was obtained as a white solid (1.19 g, 94%), which was used with no further purification in the next reaction step.

^{1}H NMR (500 MHz, DMSO) δ 8.14 (d, *J* = 5.2 Hz, 1H), 7.31 (d, *J* = 5.2 Hz, 1H), 2.54 (s, 3H). **^{13}C NMR (126 MHz, DMSO)** δ 158.2, 157.7, 157.3, 135.1, 124.5, 119.1, 12.9. MS [ESI^+] m/z: 199.4 $[M + H]^+$

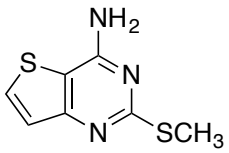
4-chloro-2-(methylthio)thieno[3,2-*d*]pyrimidine (2.14)



Phosphorus oxychloride (17.9 ml, 192 mmol) was added to compound **2.13** (3.80 g, 19.2 mmol) in a reaction flask and heated at 106 °C (reflux) for 12h. The reaction was followed by TLC. Upon completion, the phosphorus oxychloride was distilled out of the reaction flask and the remaining reaction mixture was cooled down to 0 °C. The mixture was then neutralized (pH = 7) by the addition of sodium bicarbonate solution (slow addition was key to avoid foaming). The resulting mixture was extracted with DCM (thrice). The combined organic layer was washed with brine and then concentrated *in vacuo*. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument (solvent gradient from 0 to 100% EtOAc in Hexane). The product was obtained as a white powder (3.04 g, 73%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (d, *J* = 5.5 Hz, 1H), 7.45 (d, *J* = 5.5 Hz, 1H), 2.65 (s, 3H). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 168.9, 162.5, 154.6, 137.5, 126.1, 124.2, 14.7. MS [ESI^+] m/z: 217.0 and 219.0 $[M + H]^+$

2-(methylthio)thieno[3,2-*d*]pyrimidin-4-amine (2.15)

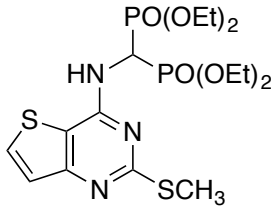


Ammonium hydroxide (28% NH_3 in water) (18.7 ml, 592 mmol) was added to compound **2.14** (1.28 g, 5.93 mmol) in a pressured vessel. The reaction was stirred and heated to 90 °C for 12h (overnight). The reaction was followed by TLC. Upon completion, the mixture was cooled to r.t.

and then filtered. The resulting residue was washed with water and dried *in vacuo*. The product was obtained as a light green powder (1.06 g, 91%), which was used with no further purification in the next reaction step.

^{1}H NMR (500 MHz, DMSO) δ 8.06 (d, *J* = 5.4 Hz, 1H), 7.48 (s, 2H), 7.26 (d, *J* = 5.3 Hz, 1H), 2.46 (s, 3H). **^{13}C NMR (126 MHz, DMSO)** δ 166.7, 160.5, 157.7, 133.7, 123.6, 110.8, 13.3. MS [ESI$^+$] m/z: 198.1 $[M + H]^+$

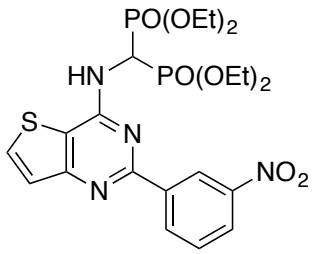
Tetraethyl (((2-(methylthio)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene) bis(phosphonate) (2.16)



Compound **2.16** was synthesized following the general procedure **A**. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument. Product was obtained as light brown solid (1.00 g, 55%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73 (d, *J* = 5.3 Hz, 1H), 7.33 (d, *J* = 5.3 Hz, 1H), 5.70 (td, *J* = 21.9, 9.8 Hz, 1H), 5.49 (d, *J* = 9.2 Hz, 1H), 4.29 – 4.12 (m, 8H), 2.58 (s, 3H), 1.33 – 1.22 (m, 12H). **^{31}P NMR (203 MHz, $CDCl_3$)** δ 16.37. **^{13}C NMR (126 MHz, $CDCl_3$)** δ 167.9, 161.4, 155.1, 132.4, 124.6, 112.1, 63.9 (m), 44.7 (t, *J* = 146.8 Hz), 16.5 (m), 14.4. MS (ESI$^+$): (*m/z*) $[M + H]^+$ 484.2

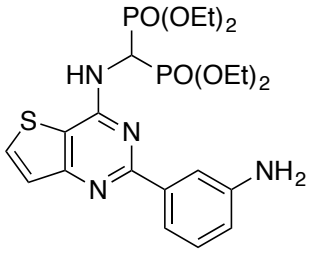
Tetraethyl(((2-(3-nitrophenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonate) (2.17)



Compound **2.17** was synthesized following the general procedure **B**. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument. The product was obtained as a white solid (201.1 mg, 87%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.31 (s, 1H), 8.80 (d, *J* = 7.8 Hz, 1H), 8.31 (d, *J* = 9.4 Hz, 1H), 7.85 (d, *J* = 5.3 Hz, 1H), 7.66 (t, *J* = 8.0 Hz, 1H), 7.55 (d, *J* = 5.3 Hz, 1H), 5.88 (td, *J* = 21.8, 9.8 Hz, 1H), 5.62 (d, *J* = 9.6 Hz, 1H), 4.33 – 4.13 (m, 8H), 1.27 (dt, *J* = 24.1, 7.1 Hz, 12H). **^{31}P NMR (203 MHz, $CDCl_3$)** δ 16.46. **^{13}C NMR (126 MHz, $CDCl_3$)** δ 161.6, 158.6, 156.1, 148.8, 140.2, 133.9, 132.8, 129.5, 125.6, 124.8, 123.3, 115.0, 63.9 (m), 45.1 (t, *J* = 147.4 Hz), 16.5 (m). MS (ESI^+): (*m/z*) $[M + H]^+$ 559.3

Tetraethyl(((2-(3-aminophenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonate) (2.18)

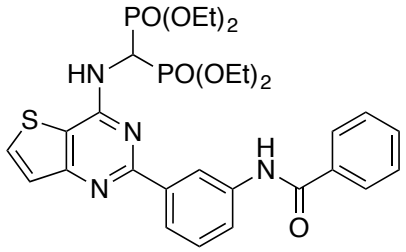


Compound **2.18** (100 mg, 0.18 mmol) amd 10% wt. of palladium on carbon (4 mg, 0.04 mmol) were added in a round bottom flask. The flask was purged with a hydrogen balloon and then,

methanol (5 ml) was added. The reaction flask atmosphere was vacuumed every 2-3h and flushed with hydrogen to remove any air in the flask. The reaction mixture was stirred for 24h under hydrogen atmosphere (1atm balloon). The reaction was monitored by LCMS. Upon completion, the catalyst was removed by vacuum filtration over a celite pad. The filtrate was concentrated *in vacuo*. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument (solvent gradient from 0 to 100% EtOAc in Hexane, then from 0 to 20% MeOH in EtOAc). The reaction yields a yellowish solid (77.3 mg, 82%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 (d, *J* = 7.7 Hz, 1H), 7.79 (s, 1H), 7.72 (d, *J* = 5.3 Hz, 1H), 7.45 (d, *J* = 5.3 Hz, 1H), 7.21 (t, *J* = 7.8 Hz, 1H), 6.77 (d, *J* = 7.7 Hz, 1H), 5.97 (t, *J* = 21.8 Hz, 1H), 5.74 (s, 1H), 4.18 (d, *J* = 27.2 Hz, 9H), 3.90 (s, 2H), 1.19 (dd, *J* = 12.7, 6.7 Hz, 13H). **^{31}P NMR (203 MHz, $CDCl_3$)** δ 16.74. **^{13}C NMR (126 MHz, $CDCl_3$)** δ 161.5, 160.7, 155.5, 146.6, 139.0, 132.1, 129.2, 125.4, 118.6, 117.2, 114.6, 113.9, 63.7 (m), 44.5 (t, *J* = 146.7 Hz), 16.3 (m). MS (ESI^+): (*m/z*) $[M + H]^+$ 530.3

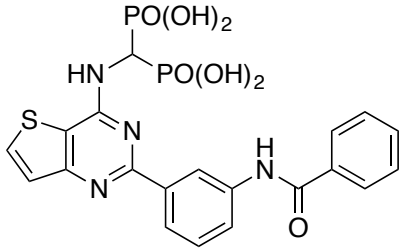
Tetraethyl(((2-(3-benzamidophenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonate) (2.19)



Compound **2.19** was synthesized following the general procedure **C**. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument. The product was obtained as a yellowish solid (30.9 mg, 57%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53 (s, 1H), 8.43 (s, 1H), 8.25 (d, *J* = 7.9 Hz, 1H), 8.22 (d, *J* = 7.8 Hz, 1H), 7.94 (d, *J* = 7.4 Hz, 2H), 7.84 (d, *J* = 5.4 Hz, 1H), 7.64 (d, *J* = 5.5 Hz, 1H), 7.52 (t, *J* = 7.9 Hz, 2H), 7.47 (t, *J* = 7.4 Hz, 2H), 5.97 (s, 2H), 4.20 (s, 8H), 1.23 (dd, *J* = 14.9, 7.2 Hz, 12H). **^{31}P NMR (162 MHz, $CDCl_3$)** δ 16.38. **^{13}C NMR (126 MHz, $CDCl_3$)** δ 166.1, 159.8, 155.9, 138.8, 137.7, 135.0, 133.3, 131.9, 129.6, 128.9, 128.5, 127.4, 127.0, 124.9, 124.5, 123.1, 119.8, 114.5, 110.4 (m), 64.2 (m), 44.8, 16.5 (m).

(((2-(3-benzamidophenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonic acid) (2.20)

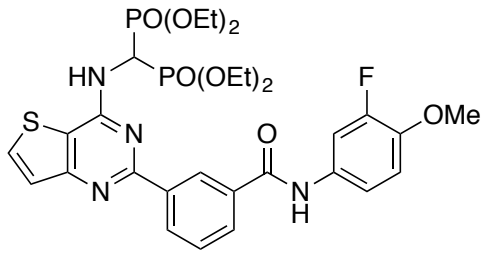


Compound **2.20** was synthesized following the general procedure **D**. The product was obtained as a white solid (10.9 mg, 66%).

^{1}H NMR (500 MHz, D_2O) δ 8.37 (s, 1H), 8.20 (d, *J* = 7.8 Hz, 1H), 8.01 (d, *J* = 5.4 Hz, 1H), 7.96 (d, *J* = 7.5 Hz, 2H), 7.92 (d, *J* = 8.0 Hz, 1H), 7.71 – 7.56 (m, 4H), 7.45 (d, *J* = 5.4 Hz, 1H), 5.08 (t, *J* = 19.1 Hz, 1H). **^{31}P NMR (203 MHz, D_2O)** δ 13.97. **^{13}C NMR (126 MHz, D_2O)** δ 169.8, 161.0, 158.7, 157.1, 139.0, 137.4, 134.0, 133.3, 132.4, 129.5, 128.8 (2 C), 127.5 (2 C), 125.33, 124.3, 123.4, 122.1, 115.0, 50.3.

HRMS (ESI-) calculated for $C_{20}H_{17}O_7N_4P_2S$ *m/z* $[M - H]^-$, 519.02987; found, *m/z* 519.02918

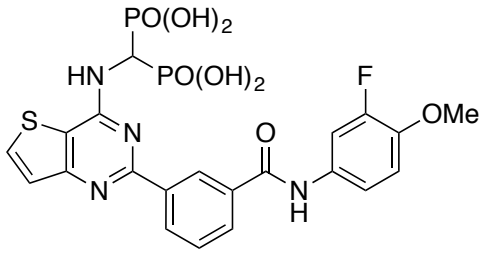
Tetraethyl (((2-(3-((3-fluoro-4-methoxyphenyl)carbamoyl)phenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonate) (2.21)



Compound **2.21** was synthesized following the general procedure **C**. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument. The product was obtained as a yellowish solid (39.3 mg, 56%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.37 (s, 1H), 9.26 (s, 1H), 8.56 (d, *J* = 7.8 Hz, 1H), 8.09 (d, *J* = 7.7 Hz, 1H), 7.71 (d, *J* = 5.3 Hz, 1H), 7.67 (dd, *J* = 13.1, 2.1 Hz, 1H), 7.61 (d, *J* = 8.5 Hz, 1H), 7.51 (t, *J* = 7.7 Hz, 1H), 7.43 (d, *J* = 5.3 Hz, 1H), 6.86 (t, *J* = 9.1 Hz, 1H), 6.54 (s, 1H), 5.62 (t, *J* = 18.3 Hz, 1H), 4.15 (dd, *J* = 42.1, 11.0 Hz, 8H), 3.83 (s, 3H), 1.18 (dd, *J* = 13.0, 7.0 Hz, 12H). **^{31}P NMR (203 MHz, $CDCl_3$)** δ 17.06. **^{13}C NMR (126 MHz, $CDCl_3$)** δ 165.4, 161.4, 159.7, 155.9 (t, *J* = 3.78 Hz), 152.9, 150.9, 144.0 (d, *J* = 11.34 Hz), 138.1, 134.6, 132.5(t, *J* = 10.08 Hz), 130.9, 129.8, 128.8, 127.0, 125.3, 116.2 (d, *J* = 2.52 Hz), 114.2, 113.5 (d, *J* = 2.52 Hz), 109.5 (d, *J* = 22.68 Hz), 63.7 (m), 56.6, 46.8 (t, = 148.7 Hz), 16.3 (m). MS (ESI^+): (*m/z*) $[M + H]^+$ 681.4

(((2-(3-((3-fluoro-4-methoxyphenyl)carbamoyl)phenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonic acid) (2.22)

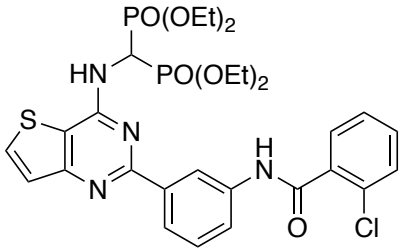


Compound **2.22** was synthesized following the general procedure **D**. The product was obtained as a white solid (15.8 mg, 57%).

^{1}H NMR (400 MHz, DMSO) δ 10.46 (s, 1H), 8.96 (s, 1H), 8.63 (d, *J* = 7.8 Hz, 1H), 8.24 (d, *J* = 5.2 Hz, 1H), 8.05 (d, *J* = 7.7 Hz, 1H), 7.79 (dd, *J* = 13.8, 2.4 Hz, 1H), 7.69 (t, *J* = 7.8 Hz, 1H), 7.55 (d, *J* = 5.3 Hz, 2H), 7.18 (t, *J* = 9.4 Hz, 1H), 5.60 – 5.44 (m, 2H), 3.84 (s, 3H). **^{31}P NMR (162 MHz, DMSO)** δ 13.46. **^{13}C NMR (126 MHz, D_2O)** δ 168.9, 160.6, 158.8, 157.2, 152.3, 150.4, 144.4, 144.3, 138.4, 133.9, 133.4, 132.1, 130.4 (d, *J* = 9.4 Hz), 129.2, 129.2, 126.9, 123.5, 115.0, 113.9, 111.1, 111.0, 56.3, 49.7

HRMS (ESI-) calculated for $C_{23}H_{10}FN_{12}P_2S$ *m/z* $[M - H]^-$, 567.0337; found, *m/z* 567.0328

tetraethyl(((2-(3-(2-chlorobenzamido)phenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonate) (2.23)

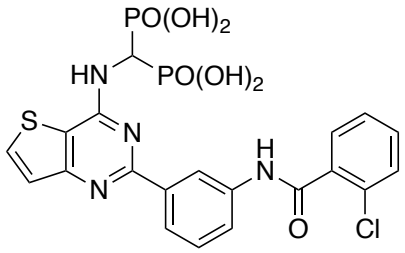


Compound **2.23** was synthesized following the general procedure **C**. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument. The product was obtained as a yellowish solid (85.9 mg, 91%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.50 (d, *J* = 4.9 Hz, 2H), 8.22 (d, *J* = 7.8 Hz, 1H), 8.08 (d, *J* = 8.8 Hz, 1H), 7.73 (d, *J* = 5.3 Hz, 1H), 7.63 (dd, *J* = 7.5, 1.4 Hz, 1H), 7.50 – 7.42 (m, 2H), 7.39 – 7.25 (m, 3H), 5.96 (td, *J* = 21.9, 9.7 Hz, 1H), 5.52 (d, *J* = 9.4 Hz, 1H), 4.28 – 4.07 (m, 8H), 1.20 (t, *J* = 7.1 Hz, 12H). **^{31}P NMR (203 MHz, $CDCl_3$)** δ 16.59. **^{13}C NMR (126 MHz, $CDCl_3$)** δ 164.8, 161.5,

160.1, 155.7, 138.9, 138.2, 135.5, 132.2, 131.4, 130.7, 130.2, 129.9, 129.2, 127.1, 125.4, 124.5, 122.2, 119.5, 114.2, 63.7 (dt, *J* = 16.6, 3.0 Hz), 44.6 (t, *J* = 147.0 Hz), 16.3 (dt, *J* = 8.1, 2.8 Hz).

(((2-(3-(2-chlorobenzamido)phenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonic acid) (2.24)

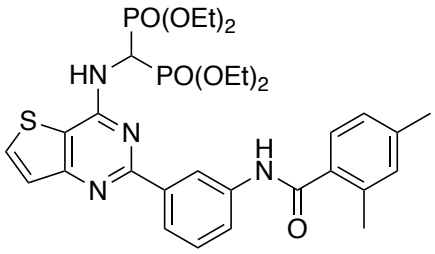


Compound **2.24** was synthesized following the general procedure **D**. The product was obtained as a white solid (41.0 mg, 58%).

^{1}H NMR (500 MHz, D_2O) δ 8.36 (s, 1H), 8.23 (d, *J* = 8.1 Hz, 1H), 8.02 (d, *J* = 5.4 Hz, 1H), 7.99 (d, *J* = 9.0 Hz, 1H), 7.72 – 7.65 (m, 2H), 7.62 (dd, *J* = 8.1, 1.1 Hz, 1H), 7.57 (td, *J* = 7.8, 1.7 Hz, 1H), 7.52 (td, *J* = 7.5, 1.3 Hz, 1H), 7.46 (d, *J* = 5.4 Hz, 1H), 5.08 (t, *J* = 19.1 Hz, 1H). **^{31}P NMR (203 MHz, D_2O)** δ 13.75. **^{13}C NMR (126 MHz, D_2O)** δ 168.9, 160.9, 158.6, 157.1, 139.1, 137.0, 134.8, 133.2, 131.9, 130.3, 130.0, 129.6, 128.6, 127.3, 125.6, 123.6, 123.4, 121.5, 115.1. C-α to the bisphosphonate was observed by HSQC. HSQC (^{1}H-^{13}C): 1H at δ 5.08 correlates to 13C-α at δ 50.7

HRMS (ESI-) calculated for $C_{20}H_{16}O_7N_4ClP_2S$ *m/z* $[M-H]^-$, 552.9908; found, *m/z* 552.9903

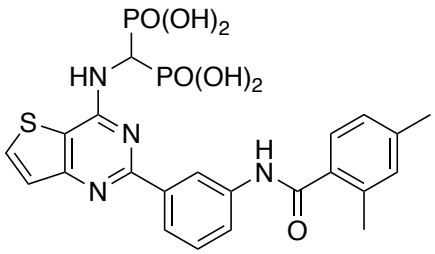
tetraethyl(((2-(3-(2,4-dimethylbenzamido)phenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonate) (2.25)



Compound **2.25** was synthesized following the general procedure **C**. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument. The product was obtained as a yellowish solid (89.0 mg, 84%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.44 (s, 1H), 8.21 (d, *J* = 7.8 Hz, 1H), 8.08 – 8.01 (m, 2H), 7.74 (d, *J* = 5.3 Hz, 1H), 7.43 (dd, *J* = 33.4, 6.5 Hz, 3H), 7.05 – 6.98 (m, 2H), 5.97 (td, *J* = 21.9, 9.8 Hz, 1H), 5.51 (d, *J* = 9.4 Hz, 1H), 4.22 – 4.13 (m, 8H), 2.46 (s, 3H), 2.31 (s, 3H), 1.20 (t, *J* = 7.1 Hz, 12H). **^{31}P NMR (203 MHz, $CDCl_3$)** δ 16.60. **^{13}C NMR (126 MHz, $CDCl_3$)** δ 168.3, 161.6, 160.3, 155.7, 140.3, 138.9, 138.7, 136.7, 133.5, 132.2, 132.0, 129.2, 126.9, 126.4, 125.5, 124.1, 122.0, 119.3, 114.2, δ 63.7 (dt, *J* = 15.1, 3.0 Hz), 44.6 (t, *J* = 148.3 Hz), 21.3, 19.9, 16.4 (dt, *J* = 8.5, 2.8 Hz).

(((2-(3-(2,4-dimethylbenzamido)phenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonic acid) (2.26)

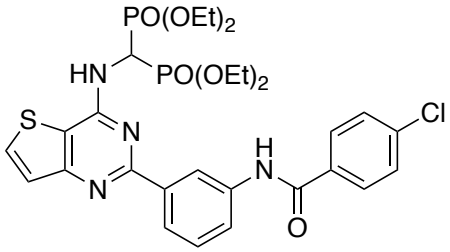


Compound **2.26** was synthesized following the general procedure **D**. The product was obtained as a white solid (47.0 mg, 64%).

^{1}H NMR (500 MHz, D_2O) δ 8.32 (s, 1H), 8.20 (d, *J* = 7.8 Hz, 1H), 8.03 (d, *J* = 5.4 Hz, 1H), 7.96 (d, *J* = 9.0 Hz, 1H), 7.66 (t, *J* = 7.9 Hz, 1H), 7.51 (d, *J* = 7.8 Hz, 1H), 7.46 (d, *J* = 5.4 Hz, 1H), 7.26 (s, 1H), 7.22 (d, *J* = 7.8 Hz, 1H), 5.11 (t, *J* = 19.2 Hz, 1H), 2.45 (s, 3H), 2.39 (s, 3H). **^{31}P NMR (203 MHz, D_2O)** δ 13.66. **^{13}C NMR (126 MHz, D_2O)** δ 172.0, 161.0, 158.6, 157.1, 141.3, 139.1, 137.4, 136.0, 133.2, 132.7, 131.5, 129.6, 127.2, 126.5, 125.3, 123.8, 123.5, 121.6, 115.1, 50.7, 20.4, 18.6.

HRMS (ESI-) calculated for $C_{22}H_{21}O_7N_4P_2S$ *m/z* $[M-H]^-$, 547.0612; found, *m/z* 547.0611

tetraethyl(((2-(3-(4-chlorobenzamido)phenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonate) (2.27)

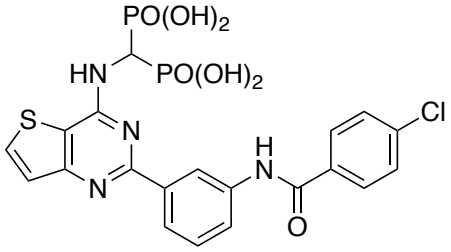


Compound **2.27** was synthesized following the general procedure **C**. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument. The product was obtained as a yellowish solid (77.0 mg, 81%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.71 (s, 1H), 8.54 (s, 1H), 8.19 (d, *J* = 7.8 Hz, 1H), 8.01 (s, 1H), 7.86 (d, *J* = 8.2 Hz, 2H), 7.72 (d, *J* = 5.3 Hz, 1H), 7.48 – 7.38 (m, 2H), 7.37 (d, *J* = 8.4 Hz, 2H), 6.02 (s, 1H), 5.52 (d, *J* = 8.4 Hz, 1H), 4.22 (s, 3H), 4.16 (d, *J* = 10.9 Hz, 8H), 1.20 (q, *J* = 6.8 Hz, 12H). **^{31}P NMR (203 MHz, $CDCl_3$)** δ 16.67. **^{13}C NMR (126 MHz, $CDCl_3$)** δ 164.9, 161.7, 160.3,

155.7, 138.9, 138.4, 137.9, 133.4, 132.3, 129.1, 128.9, 128.8, 125.5, 124.4, 122.6, 120.0, 114.2, 63.8 (m), 44.6 (m), 16.37 (dt, *J* = 10.1, 2.7 Hz).

(((2-(3-(4-chlorobenzamido)phenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonic acid) (2.28)

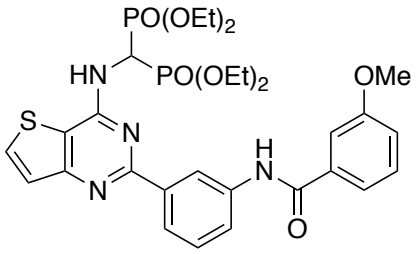


Compound **2.28** was synthesized following the general procedure **D**. The product was obtained as a white solid (35.4 mg, 55%).

^{1}H NMR (500 MHz, D_2O) δ 8.37 (s, 1H), 8.19 (d, *J* = 7.7 Hz, 1H), 8.03 (d, *J* = 5.4 Hz, 1H), 7.94 (d, *J* = 8.5 Hz, 3H), 7.66 (d, *J* = 7.9 Hz, 1H), 7.62 (d, *J* = 8.6 Hz, 2H), 7.46 (d, *J* = 5.4 Hz, 1H), 5.19 – 5.07 (m, 1H). **^{31}P NMR (203 MHz, D_2O)** δ 13.76. **^{13}C NMR (126 MHz, D_2O)** δ 168.8, 161.0, 158.6, 157.1, 139.0, 137.9, 137.4, 133.2, 132.6, 129.6, 129.1, 128.9, 125.4, 124.2, 123.5, 122.1, 115.1, 50.8.

HRMS (ESI-) calculated for $C_{20}H_{16}O_7N_4ClP_2S$ *m/z* $[M-H]^-$, 552.9909; found, *m/z* 552.9902

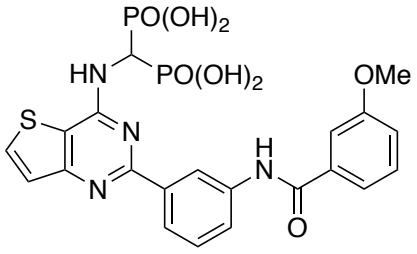
tetraethyl(((2-(3-(3-methoxybenzamido)phenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonate) (2.29)



Compound **2.29** was synthesized following the general procedure **C**. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument. The product was obtained as a yellowish solid (47.0 mg, 75%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.48 (s, 1H), 8.35 (s, 1H), 8.23 (d, *J* = 7.9 Hz, 1H), 8.10 (d, *J* = 8.1 Hz, 1H), 7.77 (d, *J* = 5.3 Hz, 1H), 7.52 – 7.43 (m, 4H), 7.36 (t, *J* = 7.9 Hz, 1H), 7.05 (dd, *J* = 7.9, 2.2 Hz, 1H), 6.00 (td, *J* = 21.8, 9.7 Hz, 1H), 5.53 (d, *J* = 9.7 Hz, 1H), 4.25 – 4.18 (m, 8H), 3.85 (s, 3H), 1.22 (td, *J* = 7.1, 2.3 Hz, 12H). **^{31}P NMR (203 MHz, $CDCl_3$)** δ 16.67. **^{13}C NMR (126 MHz, $CDCl_3$)** δ 165.76, 161.68, 160.29, 160.01, 155.76, 138.96, 138.49, 136.55, 132.30, 129.78, 129.31, 125.57, 124.37, 122.43, 119.64, 119.03, 118.10, 114.29, 112.66, 63.84 (dt, *J* = 16.3, 3.0 Hz), 55.55, 44.66 (t, *J* = 145.8 Hz), 16.42 (dt, *J* = 9.5, 2.8 Hz). MS (ESI^+): (*m/z*) $[M + H]^+$ 663.3

(((2-(3-(3-methoxybenzamido)phenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonic acid (2.30)

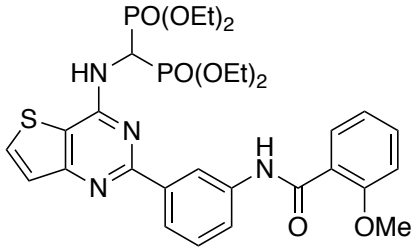


Compound **2.30** was synthesized following the general procedure **D**. The product was obtained as a white solid (21.1 mg, 54%).

^{1}H NMR (500 MHz, D_2O) δ 8.38 (s, 1H), 8.20 (d, *J* = 7.7 Hz, 1H), 8.03 (d, *J* = 5.4 Hz, 1H), 7.92 (d, *J* = 7.7 Hz, 1H), 7.66 (t, *J* = 7.9 Hz, 1H), 7.60 – 7.53 (m, 3H), 7.46 (d, *J* = 5.4 Hz, 1H), 7.28 (d, *J* = 6.4 Hz, 1H), 5.10 (t, *J* = 19.1 Hz, 1H), 3.94 (s, 3H). **^{31}P NMR (203 MHz, D_2O)** δ 13.72. **^{13}C NMR (126 MHz, D_2O)** δ 169.3, 161.0, 159.1, 158.6, 157.1, 139.0, 137.4, 135.6, 133.2, 130.2, 129.6, 125.4, 124.4, 123.5, 122.2, 120.2, 118.3, 115.1, 112.7, 55.6, 50.8 (m).

HRMS (ESI-) calculated for $C_{21}H_{19}O_8N_4P_2S$ *m/z* $[M-H]^-$, 549.0404; found, *m/z* 549.0402

tetraethyl(((2-(3-(2-methoxybenzamido)phenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonate) (2.31)

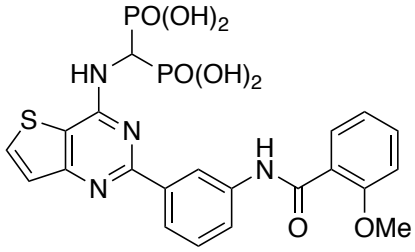


Compound **2.31** was synthesized following the general procedure **C**. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument. The product was obtained as a yellowish solid (41.0 mg, 65%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.95 (s, 1H), 8.50 (t, *J* = 1.8 Hz, 1H), 8.32 (dd, *J* = 7.8, 1.8 Hz, 1H), 8.24 (dt, *J* = 7.8, 1.2 Hz, 1H), 8.12 (d, *J* = 9.2 Hz, 1H), 7.82 (d, *J* = 5.3 Hz, 1H), 7.56 – 7.48 (m, 3H), 7.15 (t, *J* = 7.9 Hz, 1H), 7.07 (d, *J* = 8.2 Hz, 1H), 6.01 (td, *J* = 21.9, 9.8 Hz, 1H), 5.55 (d, *J* = 9.0 Hz, 1H), 4.35 – 4.17 (m, 8H), 4.12 (s, 3H), 1.26 (td, *J* = 7.1, 2.9 Hz, 12H). **^{31}P NMR (203 MHz, $CDCl_3$)** δ 16.63. **^{13}C NMR (126 MHz, $CDCl_3$)** δ 163.4, 161.7, 160.5, 157.4, 155.73, 138.9,

138.8, 133.3, 132.6, 132.3, 129.3, 125.6, 124.1, 122.8, 121.9, 121.7, 120.0, 114.2, 111.6, 63.8 (dt, J = 17.6, 3.1 Hz), 44.7 (t, J = 147.6 Hz), 16.4 (dt, J = 7.8, 2.9 Hz). MS (ESI^+): (m/z) $[M + H]^+$ 663.3

(((2-(3-(2-methoxybenzamido)phenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonic acid) (2.32)

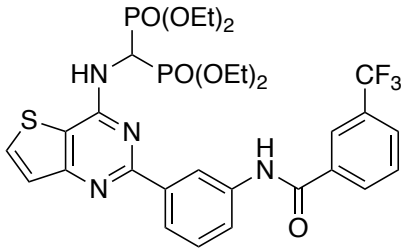


Compound **2.32** was synthesized following the general procedure **D**. The product was obtained as a white solid (29.2 mg, 75%).

^{1}H NMR (500 MHz, D_2O) δ 8.35 (s, 1H), 8.21 (d, J = 7.7 Hz, 1H), 8.03 (d, J = 5.4 Hz, 1H), 7.87 (dd, J = 24.0, 7.8 Hz, 2H), 7.69 – 7.60 (m, 2H), 7.46 (d, J = 5.4 Hz, 1H), 7.27 (d, J = 8.4 Hz, 1H), 7.19 (t, J = 7.5 Hz, 1H), 5.12 (t, J = 19.0 Hz, 1H), 4.03 (s, 3H). **^{31}P NMR (203 MHz, D_2O)** δ 13.78. **^{13}C NMR (126 MHz, D_2O)** δ 168.0, 161.1, 158.6, 157.1, 139.1, 137.3, 133.6, 133.2, 130.0, 129.6, 125.6, 124.3, 123.5, 122.5, 122.0, 121.1, 115.1, 112.4, 56.0, 50.8.

HRMS (ESI-) calculated for $C_{21}H_{19}O_8N_4P_2S$ m/z $[M-H]^-$, 549.0404; found, m/z 549.0377

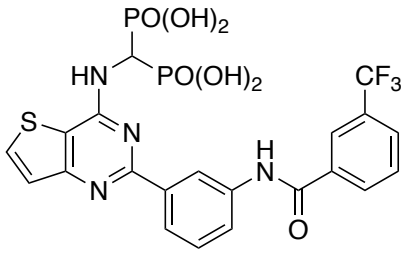
tetraethyl(((2-(3-(3-(trifluoromethyl)benzamido)phenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonate) (2.33)



Compound **2.33** was synthesized following the general procedure **C**. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument. The product was obtained as a yellowish solid (54.0 mg, 82%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.65 (s, 1H), 8.53 (s, 1H), 8.26 – 8.18 (m, 2H), 8.12 (d, *J* = 7.8 Hz, 1H), 8.05 (d, *J* = 7.8 Hz, 1H), 7.78 – 7.73 (m, 2H), 7.58 (t, *J* = 7.8 Hz, 1H), 7.50 – 7.46 (m, 1H), 7.45 (d, *J* = 7.9 Hz, 1H), 6.01 (td, *J* = 21.9, 9.8 Hz, 1H), 5.49 (d, *J* = 9.3 Hz, 1H), 4.28 – 4.13 (m, 8H), 1.22 (q, *J* = 7.1 Hz, 12H). **^{31}P NMR (203 MHz, $CDCl_3$)** δ 16.66. **^{13}C NMR (126 MHz, $CDCl_3$)** δ 164.5, 161.7, 160.3, 155.8, 139.0, 138.2, 136.0, 132.3, 131.4, 131.1, 130.7, 129.3, 129.3, 128.3 (d, *J* = 3.7 Hz), 125.6, 124.7, 124.6 (d, *J* = 3.8 Hz), 122.6, 120.0, 114.3, 63.9 (m), 44.7 (t, *J* = 147.8 Hz), 16.4 (dt, *J* = 10.9, 2.9 Hz). MS (ESI^+): (*m/z*) $[M + H]^+$ 701.3

(((2-(3-(3-(trifluoromethyl)benzamido)phenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonic acid) (2.34)

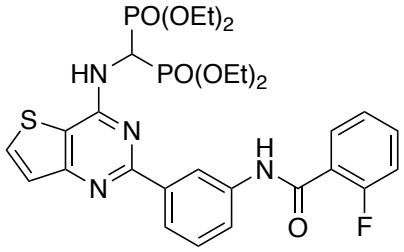


Compound **2.34** was synthesized following the general procedure **D**. The product was obtained as a white solid (21.7 mg, 40%).

^{1}H NMR (500 MHz, D_2O) δ 8.39 (s, 1H), 8.29 (s, 1H), 8.21 (t, *J* = 7.8 Hz, 2H), 8.08 – 7.89 (m, 3H), 7.78 (t, *J* = 7.9 Hz, 1H), 7.66 (t, *J* = 8.0 Hz, 1H), 7.47 (d, *J* = 5.4 Hz, 1H), 5.14 (t, *J* = 19.1 Hz, 1H). **^{31}P NMR (203 MHz, D_2O)** δ 13.73. **^{13}C NMR (126 MHz, D_2O)** δ 168.2, 160.9, 158.6, 157.1, 139.0, 137.3, 134.8, 133.2, 131.0, 129.6, 129.5, 128.8, 125.4, 124.9, 124.5, 124.8, 123.5, 122.7, 121.9, 115.1, 50.7.

HRMS (ESI-) calculated for $C_{21}H_{16}O_7N_4F_3P_2S$ *m/z* $[M-H]^-$, 587.0150; found, *m/z* 587.0173

tetraethyl(((2-(3-(2-fluorobenzamido)phenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonate) (2.35)

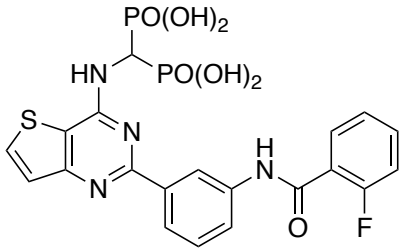


Compound **2.35** was synthesized following the general procedure **C**. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument. The product was obtained as a yellowish solid (79.0 mg, 86%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.63 (d, *J* = 14.6 Hz, 1H), 8.50 (s, 1H), 8.25 (d, *J* = 7.9 Hz, 1H), 8.15 (td, *J* = 7.9, 1.7 Hz, 1H), 8.07 – 8.04 (m, 1H), 7.79 (d, *J* = 5.3 Hz, 1H), 7.53 – 7.47 (m, 3H), 7.32 – 7.27 (m, 1H), 7.18 (dd, *J* = 11.9, 8.2 Hz, 1H), 5.97 (td, *J* = 21.9, 9.8 Hz, 1H), 5.51 (d, *J* = 9.3 Hz, 1H), 4.30 – 4.13 (m, 8H), 1.26 – 1.20 (m, 12H). **^{31}P NMR (203 MHz, $CDCl_3$)** δ 16.60. **^{13}C NMR (126 MHz, $CDCl_3$)** δ 161.7, 161.5 (d, *J* = 3.3 Hz), 161.4, 160.2, 159.5, 155.8, 155.8, 155.7, 139.1, 138.1, 133.8 (d, *J* = 9.3 Hz), 132.3, 129.3, 125.6, 125.1 (d, *J* = 3.2 Hz), 124.7, 122.7,

121.6 (d, *J* = 11.4 Hz), 120.0, 116.3, 116.1, 114.3, 63.8 (dt, *J* = 16.7, 3.1 Hz), 44.6 (t, *J* = 147.4 Hz), 16.4 (dt, *J* = 8.6, 2.9 Hz).

(((2-(3-(2-fluorobenzamido)phenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonic acid) (2.36)

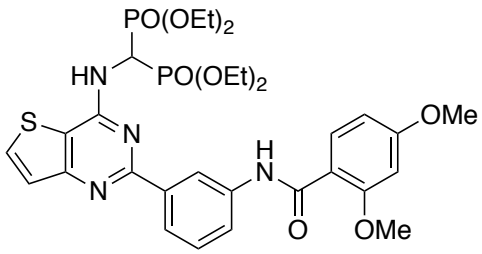


Compound **2.36** was synthesized following the general procedure **D**. The product was obtained as a white solid (32.5 mg, 49%).

^{1}H NMR (500 MHz, D_2O) δ 8.39 (s, 1H), 8.22 (d, *J* = 7.9 Hz, 1H), 8.03 (d, *J* = 5.4 Hz, 1H), 7.96 (d, *J* = 8.1 Hz, 1H), 7.88 – 7.83 (m, 1H), 7.67 (t, *J* = 7.9 Hz, 2H), 7.47 (d, *J* = 5.4 Hz, 1H), 7.41 (t, *J* = 7.6 Hz, 1H), 7.36 (dd, *J* = 10.6, 8.7 Hz, 1H), 5.06 (t, *J* = 19.1 Hz, 1H). **^{31}P NMR (203 MHz, D_2O)** δ 13.67. **^{13}C NMR (126 MHz, D_2O)** δ 166.3, 161.0, 160.6, 158.6, 157.1, 139.1, 137.1, 133.8 (d, *J* = 8.9 Hz), 133.2, 129.9, 129.6, 125.6, 124.8 (d, *J* = 3.4 Hz), 124.1, 123.5, 121.9, 116.6, 116.4, 115.1, 50.8 (m).

HRMS (ESI-) calculated for $C_{20}H_{16}O_7N_4FP_2S$ *m/z* $[M-H]^-$, 537.0189; found, *m/z* 537.0204

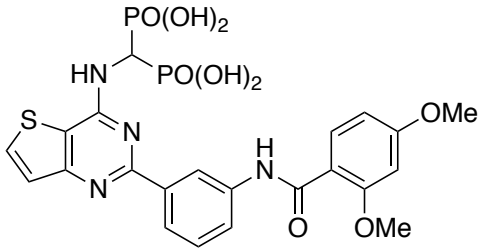
tetraethyl(((2-(3-(2,4-dimethoxybenzamido)phenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonate) (2.37)



Compound **2.37** was synthesized following the general procedure **C**. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument. The product was obtained as a yellowish solid (74.5 mg, 76%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.82 (s, 1H), 8.44 (s, 1H), 8.27 (d, *J* = 8.8 Hz, 1H), 8.20 (d, *J* = 7.8 Hz, 1H), 8.10 (d, *J* = 9.2 Hz, 1H), 7.80 (d, *J* = 5.3 Hz, 1H), 7.52 (d, *J* = 5.3 Hz, 1H), 7.47 (t, *J* = 7.9 Hz, 1H), 6.64 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.55 (d, *J* = 2.3 Hz, 1H), 5.98 (td, *J* = 21.9, 9.8 Hz, 1H), 5.48 (d, *J* = 9.5 Hz, 1H), 4.31 – 4.13 (m, 9H), 4.07 (s, 3H), 3.86 (s, 3H), 1.23 (td, *J* = 7.1, 1.8 Hz, 13H). **^{31}P NMR (203 MHz, $CDCl_3$)** δ 16.63. **^{13}C NMR (126 MHz, $CDCl_3$)** δ 163.9, 163.3, 161.8, 160.5, 158.7, 155.7, 139.0, 138.8, 134.4, 132.2, 129.2, 125.6, 123.9, 122.8, 119.9, 114.9, 114.2, 105.8, 98.9, 63.8 (dt, *J* = 18.5, 3.1 Hz), 56.4, 55.7, 44.6 (t, *J* = 146.8 Hz), 16.4 (dt, *J* = 8.1, 2.9 Hz).

(((2-(3-(2,4-dimethoxybenzamido)phenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonic acid) (2.38)

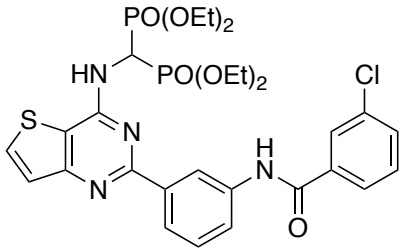


Compound **2.38** was synthesized following the general procedure **D**. The product was obtained as a white solid (31.4 mg, 50%).

^{1}H NMR (500 MHz, D_2O) δ 8.26 (s, 1H), 8.12 (d, *J* = 7.9 Hz, 1H), 7.94 (d, *J* = 5.4 Hz, 1H), 7.83 (d, *J* = 9.3 Hz, 1H), 7.75 (d, *J* = 7.5 Hz, 1H), 7.56 (t, *J* = 7.9 Hz, 1H), 7.38 (d, *J* = 5.4 Hz, 1H), 6.70 (d, *J* = 7.2 Hz, 2H), 3.97 (s, 3H), 3.85 (s, 3H). **^{31}P NMR (203 MHz, D_2O)** δ 13.88. **^{13}C NMR (126 MHz, D_2O)** δ 166.9, 163.7, 161.1, 159.2, 158.7, 157.2, 139.0, 137.4, 133.2, 132.4, 129.5, 125.4, 124.4, 123.5, 122.0, 115.1, 114.2, 106.2, 98.7, 56.2, 55.7, 50.8.

HRMS (ESI-) calculated for $C_{22}H_{21}O_9N_4P_2S$ *m/z* $[M-H]^-$, 579.0489; found, *m/z* 579.0510

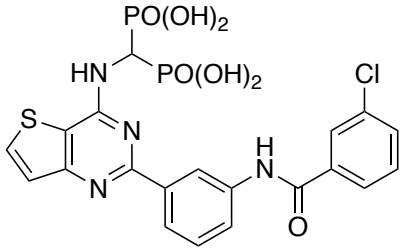
tetraethyl(((2-(3-(3-chlorobenzamido)phenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonate) (2.39)



Compound **2.39** was synthesized following the general procedure **C**. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument. The product was obtained as a yellowish solid (63.2 mg, 67%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.66 (s, 1H), 8.53 (s, 1H), 8.20 (d, *J* = 7.9 Hz, 1H), 8.05 – 7.98 (m, 1H), 7.91 (t, *J* = 1.8 Hz, 1H), 7.79 (d, *J* = 7.8 Hz, 1H), 7.73 (d, *J* = 5.3 Hz, 1H), 7.50 – 7.39 (m, 3H), 7.35 (t, *J* = 7.9 Hz, 1H), 6.02 (td, *J* = 21.9, 9.7 Hz, 1H), 5.54 (d, *J* = 9.4 Hz, 1H), 4.28 – 4.07 (m, 8H), 1.21 (td, *J* = 7.1, 3.2 Hz, 12H). **^{31}P NMR (203 MHz, $CDCl_3$)** δ 16.68. **^{13}C NMR (126 MHz, $CDCl_3$)** δ 164.5, 161.6, 160.2, 155.6, 138.8, 138.2, 136.8, 134.7, 132.2, 131.6, 129.9, 129.1, 127.7, 125.5, 125.4, 124.4, 122.5, 119.9, 114.2, 63.8 (dt, *J* = 12.9, 3.0 Hz), 44.6 (t, *J* = 146.7 Hz), 16.3 (dt, *J* = 9.3, 2.8 Hz).

(((2-(3-(3-chlorobenzamido)phenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonic acid) (2.40)

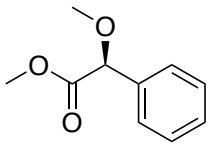


Compound **2.40** was synthesized following the general procedure **D**. The product was obtained as a white solid (24.6 mg, 39%).

^{1}H NMR (500 MHz, D_2O) δ 8.41 (s, 1H), 8.23 (d, *J* = 7.9 Hz, 1H), 8.05 (d, *J* = 5.4 Hz, 1H), 8.00 (s, 1H), 7.91 (t, *J* = 9.4 Hz, 2H), 7.68 (q, *J* = 8.0, 7.2 Hz, 2H), 7.60 (t, *J* = 7.9 Hz, 1H), 7.48 (d, *J* = 5.4 Hz, 1H), 5.10 (d, *J* = 19.1 Hz, 1H). **^{31}P NMR (203 MHz, D_2O)** δ 13.69. **^{13}C NMR (126 MHz, D_2O)** δ 168.3, 160.9, 158.7, 157.1, 139.0, 137.3, 135.9, 134.2, 133.2, 132.2, 130.4, 129.6, 127.6, 125.8, 125.4, 124.2, 123.5, 122.0, 115.1, 50.8.

HRMS (ESI-) calculated for $C_{20}H_{16}O_7N_4ClP_2S$ *m/z* $[M-H]^-$, 552.9920; found, *m/z* 552.9909

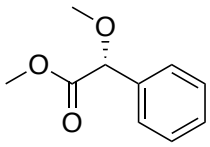
methyl (*S*)-2-methoxy-2-phenylacetate (2.41)



A solution of n-BuLi (2.5M in hexane) (2.7 ml, 6.90 mmol) was added slowly to a stirring solution of anhydrous DMSO (5ml). S-Mandelic acid (500mg, 3.29 mmol) dissolved in DMSO (1.3 ml) was added to the solution of n-BuLi and the resulting mixture was stirred under nitrogen atmosphere for 2h at r.t. Methyl iodide (0.47ml, 7.56 mmol) was then added and the reaction mixture and the reaction was stirred overnight. Upon completion of the reaction, the mixture was poured into water and extracted with ether. The combined ethereal layers were washed with brine, dried over sodium sulfate and concentrated *in vacuo* to afford the desired product as an uncolored oil (522mg, 77%), which was used with no further purification in the next step.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (dd, *J* = 7.9, 1.4 Hz, 2H), 7.39 – 7.33 (m, 3H), 4.78 (s, 1H), 3.72 (s, 3H), 3.41 (s, 3H). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 171.3, 136.3, 128.9, 128.8, 127.4, 82.7, 57.5, 52.4.

methyl (*R*)-2-methoxy-2-phenylacetate (2.42)

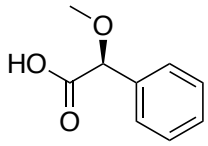


A solution of n-BuLi (2.5M in hexane) (2.7 ml, 6.90 mmol) was added slowly to a stirring solution of anhydrous DMSO (5ml). S-Mandelic acid (500mg, 3.29 mmol) dissolved in DMSO (1.3 ml) was added to the solution of n-BuLi and the resulting mixture was stirred under nitrogen atmosphere for 2h at r.t. Methyl iodide (0.47ml, 7.56 mmol) was then added and the reaction

mixture and the reaction was stirred overnight. Upon completion of the reaction, the mixture was poured into water and extracted with ether. The combined ethereal layers were washed with brine, dried over sodium sulfate and concentrated *in vacuo* to afford the desired product as an uncolored oil (497mg, 74%), which was used with no further purification in the next step.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46 (dd, *J* = 8.0, 1.5 Hz, 2H), 7.41 – 7.35 (m, 3H), 4.80 (s, 1H), 3.74 (s, 3H), 3.43 (s, 3H). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 171.2, 136.3, 128.9, 128.8, 127.3, 82.7, 57.5, 52.4.

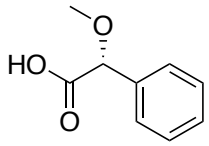
(*S*)-2-methoxy-2-phenylacetic acid (2.43)



Lithium hydroxide monohydrate (105 mg, 2.50 mmol) was added to a stirring solution of compound **2.41** was dissolved in a mixture of water (2.50 ml), methanol (2.50 ml) and THF (1.25 ml). The solution was stirred for 24h at room temperature. Upon completion, the reaction was quenched with aqueous HCl (1M) and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and concentrated *in vacuo*. The product was obtained as an off white-yellow solid (411mg, 99%), which was used with no further purification in the next step.

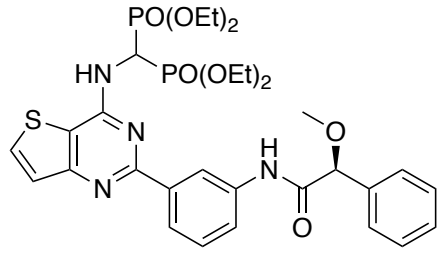
^{1}H NMR (500 MHz, DMSO-d_6) δ 7.40 – 7.31 (m, 5H), 4.76 (s, 1H), 3.30 (s, 3H). **^{13}C NMR (126 MHz, DMSO)** δ 171.8, 137.3, 128.3, 128.2, 127.1, 81.7, 56.7. MS (ESI^-): (*m/z*) $[M + H]^-$ 165.0

(*R*)-2-methoxy-2-phenylacetic acid (2.44)



Lithium hydroxide monohydrate (105 mg, 2.50 mmol) was added to a stirring solution of compound **2.42** was dissolved in a mixture of water (2.50 ml), methanol (2.50 ml) and THF (1.25 ml). The solution was stirred for 24h at room temperature. Upon completion, the reaction was quenched with aqueous HCl (1M) and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and concentrated *in vacuo*. The product was obtained as an off white-yellow solid (405mg, 97%), which was used with no further purification in the next step. **^{1}H NMR (500 MHz, DMSO-d_6)** δ 7.40 – 7.32 (m, 5H), 4.75 (s, 1H), 3.30 (s, 3H). **^{13}C NMR (126 MHz, DMSO)** δ 171.8, 137.4, 128.3, 128.2, 127.1, 81.7, 56.7. MS (ESI$^-$): (*m/z*) $[M + H]^-$ 165.0

tetraethyl(((2-(3-(2-methoxy-2-phenylacetamido)phenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene)(*S*)-bis(phosphonate) (2.45)

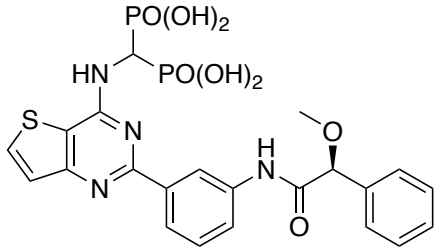


Compound **2.45** was synthesized following the general procedure **C**. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument. The product was obtained as an off-white solid (112 mg, 87%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.79 (s, 1H), 8.44 (t, *J* = 1.9 Hz, 1H), 8.20 (dt, *J* = 7.9, 1.3 Hz, 1H), 7.98 (ddd, *J* = 8.1, 2.3, 1.0 Hz, 1H), 7.76 (d, *J* = 5.3 Hz, 1H), 7.51 – 7.43 (m, 3H), 7.41 (t, *J*

= 7.9 Hz, 1H), 7.38 – 7.25 (m, 3H), 5.99 (td, *J* = 21.8, 9.8 Hz, 1H), 5.88 (d, *J* = 10.0 Hz, 1H), 4.75 (s, 1H), 4.28 – 4.11 (m, 8H), 3.44 (s, 3H), 1.30 – 1.15 (m, 12H). **^{31}P NMR (203 MHz, $CDCl_3$)** δ 16.64. **^{13}C NMR (126 MHz, $CDCl_3$)** δ 168.6, 161.4, 160.0, 155.6, 138.8, 137.6, 136.6, 132.3, 129.1, 128.5, 128.5, 127.0, 125.3, 124.1, 121.5, 118.9, 114.2, 83.9, 63.7 (dt, *J* = 13.6, 2.9 Hz), 57.3, 44.4 (t, *J* = 147.6 Hz), 16.2 (dd, *J* = 5.5, 2.7 Hz). MS (ESI^+): (*m/z*) $[M + H]^+$ 663.4 Chiral HPLC: 60% ee

(*S*)-(((2-(3-(2-methoxy-2-phenylacetamido)phenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonic acid) (2.46)

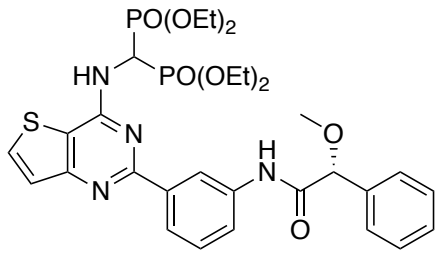


Compound **2.47** was synthesized following the general procedure **D**. The product was obtained as a white solid (50.6 mg, 61%).

^{1}H NMR (500 MHz, D_2O) ^{1}H NMR (500 MHz, Deuterium Oxide) δ 8.30 (t, *J* = 2.0 Hz, 1H), 8.18 (dt, *J* = 8.0, 1.4 Hz, 1H), 8.01 (d, *J* = 5.4 Hz, 1H), 7.76 (ddd, *J* = 8.0, 2.2, 1.1 Hz, 1H), 7.62 – 7.45 (m, 6H), 7.44 (d, *J* = 5.4 Hz, 1H), 5.11 – 5.00 (m, 2H), 3.53 (s, 3H). **^{31}P NMR (203 MHz, D_2O)** δ 13.53. **^{13}C NMR (126 MHz, D_2O)** δ 172.1, 160.9, 158.7, 157.1, 139.0, 136.4, 136.4, 133.3, 129.5, 129.3, 129.1, 127.6, 125.7, 124.1, 123.5, 122.0, 115.0, 83.5, 57.2, 50.3 (m).

HRMS (ESI-) calculated for $C_{22}H_{21}O_8N_4P_2S$ *m/z* $[M-H]^-$, 563.0561; found, *m/z* 563.0554

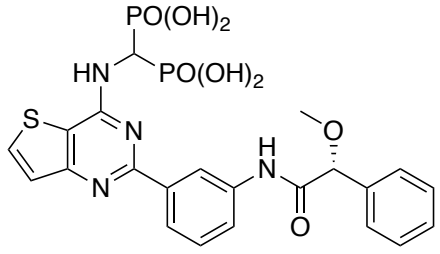
tetraethyl(((2-(3-(2-methoxy-2-phenylacetamido)phenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene)(*R*)-bis(phosphonate) (2.47)



Compound **2.45** was synthesized following the general procedure **C**. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument. The product was obtained as an off-white solid (114 mg, 89%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.78 (s, 1H), 8.44 (t, *J* = 1.9 Hz, 1H), 8.21 (dt, *J* = 7.8, 1.3 Hz, 1H), 8.00 (ddd, *J* = 8.1, 2.3, 1.0 Hz, 1H), 7.78 (d, *J* = 5.4 Hz, 1H), 7.52 – 7.44 (m, 3H), 7.42 (d, *J* = 7.9 Hz, 1H), 7.40 – 7.32 (m, 2H), 7.32 – 7.28 (m, 1H), 5.99 (td, *J* = 21.9, 9.8 Hz, 1H), 5.81 (d, *J* = 9.8 Hz, 1H), 4.77 (s, 1H), 4.31 – 4.11 (m, 8H), 3.46 (s, 3H), 1.27 – 1.17 (m, 12H). **^{31}P NMR (203 MHz, $CDCl_3$)** δ 16.66. **^{13}C NMR (126 MHz, $CDCl_3$)** δ 168.6, 161.5, 160.1, 155.7, 138.9, 137.7, 136.7, 132.4, 129.2, 128.6, 128.6, 127.1, 125.4, 124.2, 121.6, 118.9, 114.2, 83.9, 63.7 (dt, *J* = 13.9, 3.0 Hz), 57.3, 44.5 (t, *J* = 146.8 Hz), 16.3 (dd, *J* = 6.0, 2.9 Hz). MS (ESI^+): (*m/z*) $[M + H]^+$ 663.4 Chiral HPLC: 60% ee

(*R*)-(((2-(3-(2-methoxy-2-phenylacetamido)phenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methylene)bis(phosphonic acid) (2.48)

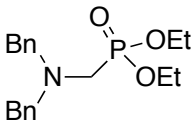


Compound **2.48** was synthesized following the general procedure **D**. The product was obtained as a white solid (48.4 mg, 58%).

^{1}H NMR (500 MHz, D_2O) δ 8.22 (t, *J* = 2.0 Hz, 1H), 8.10 (dt, *J* = 8.1, 1.4 Hz, 1H), 7.93 (d, *J* = 5.4 Hz, 1H), 7.68 (ddd, *J* = 8.1, 2.2, 1.1 Hz, 1H), 7.55 – 7.38 (m, 6H), 7.36 (d, *J* = 5.3 Hz, 1H), 5.04 – 4.92 (m, 2H), 3.45 (s, 3H). **^{31}P NMR (203 MHz, D_2O)** δ 13.54. **^{13}C NMR (126 MHz, D_2O) δ** 172.1, 160.9, 158.7, 157.1, 139.0, 136.4, 136.4, 133.3, 129.5, 129.3, 129.1, 127.6, 125.7, 124.1, 123.5, 122.0, 115.0, 83.5, 57.2, 50.3 (m).

HRMS (ESI-) calculated for $C_{22}H_{21}O_8N_4P_2S$ *m/z* $[M-H]^-$, 563.0561; found, *m/z* 563.0551

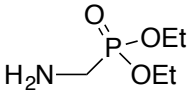
Diethyl ((dibenzylamino)methyl)phosphonate (2.49)



36% v/v aqueous formaldehyde (0.93 ml) was added to a stirring solution of dibenzylamine (1.95 ml, 10.14 mmol) and diethylphosphite (1.57 ml, 12.17 mmol) dissolved in THF (3 ml). The reaction was stirred and heated to 50 °C for 24h. The reaction was followed by TLC. Upon completion of the reaction, the mixture was concentrated *in vacuo*. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument (solvent gradient from 0 to 100% EtOAc in Hexane). The product was obtained as a transparent oil (2.70 g, 77%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 (d, *J* = 7.3 Hz, 4H), 7.34 (t, *J* = 7.5 Hz, 4H), 7.27 (t, *J* = 7.3 Hz, 2H), 4.09 (p, *J* = 7.1 Hz, 4H), 3.81 (s, 4H), 2.92 (d, *J* = 10.4 Hz, 2H), 1.32 (t, *J* = 7.1 Hz, 6H). **^{31}P NMR (203 MHz, $CDCl_3$)** δ 25.68.

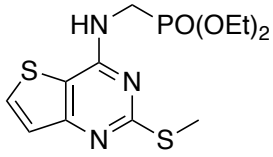
Diethyl (aminomethyl)phosphonate (2.50)



Concentrated hydrochloric acid (12N) (0.14 ml, 1.73 mmol) was added to a stirring solution of compound **2.49** (600 mg, 1.73 mmol) and 10% wt palladium on carbon (91.7 mg, 0.17 mmol) dissolved in ethanol (3.6 ml). The flask was purged with hydrogen and stirred for 4h at r.t. The reaction was followed by TLC. Upon completion, the mixture was neutralized with sodium hydroxide. The mixture was then filtered on a celite pad. The filtrate was then concentrated *in vacuo*. Compound **2.50** was obtained as a transparent liquid (287.0 mg, 99%), which was used with no further purification in the next step.

^{1}H NMR (500 MHz, $CDCl_3$) δ 4.09 – 3.79 (m, 4H), 2.84 (d, *J* = 10.3 Hz, 2H), 1.30 (s, 2H), 1.17 (t, *J* = 7.1 Hz, 6H). **^{31}P NMR (203 MHz, $CDCl_3$)** δ 27.48. MS (ESI^+): (*m/z*) $[M + H]^+$ 168.1

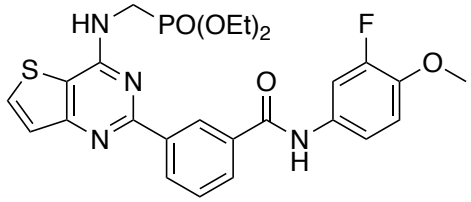
Diethyl (((2-(methylthio)thieno[3,2-*d*]pyrimidin-4-yl)amino)methyl)phosphonate (2.51)



Trimethylamine (0.2 ml, 1.384 mmol) was added to a stirring solution of compound **2.14** (231 mg, 1.384 mmol) and compound **2.50** (150 mg, 0.692 mmol) dissolved in dioxane (1.0 ml). The solution was stirred and heated for 72h at 100 °C. The reaction was followed by TLC. Upon completion, the mixture was concentrated *in vacuo*. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument (solvent gradient from 0 to 100% EtOAc in Hexane, then 0 to 20% MeOH in EtOAc). Compound **2.51** was obtained as a yellowish solid (139.0 mg, 58%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64 (d, *J* = 5.3 Hz, 1H), 7.27 (d, *J* = 5.3 Hz, 1H), 5.61 (s, 1H), 4.17 (m, 6H), 2.59 (s, 3H), 1.31 (t, *J* = 7.1 Hz, 6H). **^{31}P NMR (203 MHz, $CDCl_3$)** δ 22.86 **^{13}C NMR (126 MHz, $CDCl_3$)** δ 167.9, 161.0, 156.1, 131.7, 124.6, 112.1, 62.8, 36.3 (d, *J* = 156 Hz), 16.6, 14.4. MS (ESI^-): (*m/z*) $[M - H]^-$ 346.08

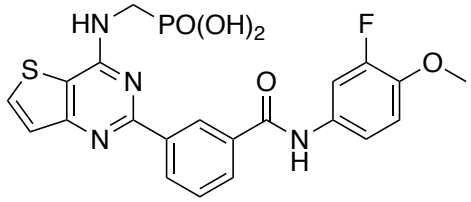
Diethyl(((2-(3-((3-fluoro-4-methoxyphenyl)carbamoyl)phenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methyl)phosphonate (2.52)



Compound **2.52** was synthesized following the general procedure **B**. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument. The product was obtained as a yellowish solid (45.0 mg, 23%).

^{1}H NMR (500 MHz, DMSO-*d*6) δ 10.44 (s, 1H), 9.06 (s, 1H), 8.66 (d, *J* = 7.8 Hz, 1H), 8.45 (t, *J* = 5.9 Hz, 1H), 8.19 (d, *J* = 5.3 Hz, 1H), 8.03 (d, *J* = 7.8 Hz, 1H), 7.78 (dd, *J* = 13.7, 2.5 Hz, 1H), 7.66 (t, *J* = 7.7 Hz, 1H), 7.57 (d, *J* = 8.9 Hz, 1H), 7.53 (d, *J* = 5.3 Hz, 1H), 7.18 (t, *J* = 9.4 Hz, 1H), 4.22 (dd, *J* = 10.4, 6.0 Hz, 2H), 4.04 (s, 4H), 3.84 (s, 3H), 1.11 (t, *J* = 7.0 Hz, 6H). **^{31}P NMR (203 MHz, DMSO-*d*6)** δ 23.47.

(((2-(3-((3-fluoro-4-methoxyphenyl)carbamoyl)phenyl)thieno[3,2-*d*]pyrimidin-4-yl)amino)methyl)phosphonic acid (2.53)

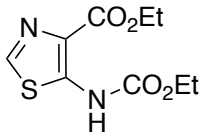


Compound **2.53** was synthesized following the general procedure **D**. The product was obtained as a yellowish solid (12.4 mg, 31%).

^{1}H NMR (500 MHz, D_2O) δ 8.51 (s, 1H), 8.35 (d, *J* = 7.5 Hz, 1H), 8.00 (d, *J* = 5.3 Hz, 1H), 7.86 (d, *J* = 7.8 Hz, 1H), 7.60 (t, *J* = 7.8 Hz, 1H), 7.44 – 7.38 (m, 2H), 7.21 (d, *J* = 8.7 Hz, 1H), 7.08 (t, *J* = 9.2 Hz, 1H), 3.89 (s, 3H), 3.78 (d, *J* = 13.0 Hz, 2H). **^{31}P NMR (203 MHz, D_2O)** δ 13.96. **^{13}C NMR (126 MHz, D_2O)** δ 168.1, 160.1, 152.0, 150.1, 143.9, 143.8, 138.0, 133.5, 131.8, 130.5, 130.4, 129.0, 129.0, 126.5, 123.3, 117.8, 113.5, 110.2, 110.0, 56.1, 40.3.

HRMS (ESI-) calculated for $C_{21}H_{17}FN_4O_5PS$ *m/z* $[M - H]^-$, 487.0647; found, *m/z* 487.0635

Ethyl 5-((ethoxycarbonyl)amino)thiazole-4-carboxylate (2.54)

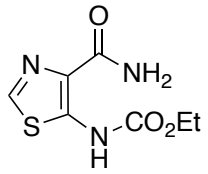


Ethyl isocyanoacetate (500 mg, 4.42 mmol) was added dropwise to a stirring solution of t-BuOK (546 mg, 4.86 mmol) dissolved in anhydrous THF (10.0 ml) at - 40 °C. Ethoxycarbonyl isothiocyanate (609 mg, 4.64 mmol) was then added dropwise to the solution. The temperature of the mixture was rose to 0 °C over 1.5h. The solution was then quenched by the addition of glacial acetic acid (2.5 ml). The resulting mixture was diluted with water. The mixture was extracted with

ethyl acetate (thrice), washed with brine and dried over sodium sulfate. The solution was then concentrated *in vacuo*. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument (solvent gradient from 0 to 100% EtOAc in Hexane). The product was obtained as an orange solid (821 mg, 76%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.01 (s, 1H), 8.59 (s, 1H), 4.33 (q, J = 7.1 Hz, 2H), 4.26 (q, J = 7.1 Hz, 2H), 1.31 (t, J = 7.1 Hz, 3H), 1.28 (t, J = 7.1 Hz, 3H). **^{13}C NMR (126 MHz, DMSO-d_6)** δ 163.7, 152.6, 146.5, 145.1, 126.8, 62.7, 60.8, 14.2, 14.1. MS (ESI$^+$): (*m/z*) $[M + H]^+$ 245.15

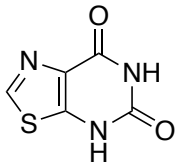
Ethyl (4-carbamoylthiazol-5-yl)carbamate (2.55)



Ethanol (0.8 ml) was added to compound **2.54** (820mg, 3.36 mmol) and the mixture was stirred and heated at 40 °C until all the solid was dissolved. Water (1.6 ml) and ammonium hydroxide (5.8 ml, 84 mmol) were then to the solution and the temperature was increased to 80 °C for 30 minutes. The reaction was then cooled down to r.t. and the resulting mixture was filtered. The residue was rinsed with several portions of water, and dried *in vacuo*. The product was obtained as a white solid (510 mg, 71%), which was used with no further purification in the next step.

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.89 (s, 1H), 8.58 (s, 1H), 7.83 (d, J = 49.4 Hz, 2H), 4.23 (q, J = 7.1 Hz, 2H), 1.27 (t, J = 7.1 Hz, 3H). MS (ESI$^+$): (*m/z*) $[M + H]^+$ 216.08

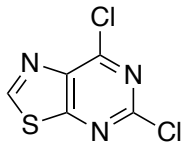
3a,7a-dihydrothiazolo[5,4-*d*]pyrimidine-5,7(4*H*,6*H*)-dione (2.56)



t-BuOK (625.6 mg, 5.58 mmol) was added to a stirring solution of compound **2.55** (400 mg, 1.86 mmol) dissolved in DMA (10ml) under argon atmosphere. The mixture was then stirred and heated at 100 °C for 1 hour. The reaction was then cooled down to r.t. and filtered. The residue was rinsed with water and then dried *in vacuo*. The product was isolated as an off-white solid (267 mg, 85 %), which was used with no further purification in the next step.

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.98 (s, 1H), 11.30 (s, 1H), 8.71 (s, 1H). **^{13}C NMR (126 MHz, DMSO)** δ 157.7, 150.3, 149.7, 146.2, 130.2.

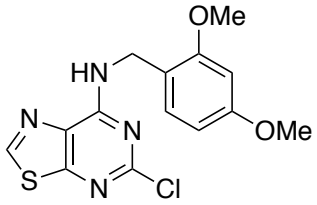
5,7-dichlorothiazolo[5,4-*d*]pyrimidine (2.57)



DIPEA (0.7 ml, 3.84 mmol) was slowly added to a stirring solution of compound **2.56** (500 mg, 2.96 mmol) dissolved in neat phosphorus oxychloride (4.1ml, 44.34 mmol) under argon atmosphere. The solution was stirred at room temperature for 1 hour. After the fact, the temperature was increased 95 °C for 2.5 hours. Upon completion of the reaction, phosphorus oxychloride was distilled out and the oily residue was then dissolve in ethyl acetate. The ethyl acetate layer was washed with sodium bicarbonate (twice). The organic layer was collected, dried with sodium sulfate and concentrated *in vacuo*. The product was obtained as an off-white solid (353 mg, 58%), which was used with no further purification in the next step.

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.70 (s, 1H). MS (ESI$^+$): (*m/z*) $[M + H]^+$ 206.0 and 208.0

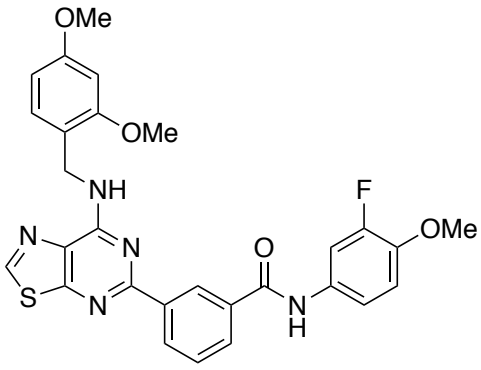
5-chloro-*N*-(2,4-dimethoxybenzyl)thiazolo[5,4-*d*]pyrimidin-7-amine (2.58)



DIPEA (1.27 ml, 7.28 mmol) was added to a stirring solution of compound **2.57** (1.00g, 4.85 mmol) and dimethoxybenzylamine (1.06g, 6.31 mmol) dissolved in DMSO (13 ml). The solution was stirred at room temperature for 3 hours. The reaction was followed by TLC. Upon completion, the mixture was poured into water (55 ml) and cooled to °C for 20 minutes. The cold mixture was filtered. The solid obtained was washed with water and then dried *in vacuo*. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument (solvent gradient from 0 to 50% EtOAc in Hexane). Product was obtained as a yellowish solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.66 (s, 1H), 7.30 (d, *J* = 8.1 Hz, 1H), 6.81 (s, 1H), 6.44 (d, *J* = 10.7 Hz, 2H), 4.73 (d, *J* = 5.8 Hz, 2H), 3.84 (s, 3H), 3.80 (s, 3H). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 161.9, 160.9, 158.8, 156.9, 156.3, 150.6, 131.0, 130.1, 117.7, 104.0, 98.7, 55.5, 55.5, 40.5. MS (ESI$^+$): (*m/z*) $[M + H]^+$ 337.2

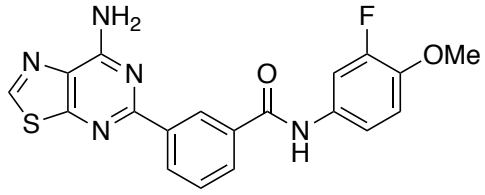
3-(7-((2,4-dimethoxybenzyl)amino)thiazolo[5,4-*d*]pyrimidin-5-yl)-*N*-(3-fluoro-4-methoxyphenyl)benzamide (2.59)



KF (58 mg, 1.00 mmol) was added to a stirring solution of compound **2.58** (135 mg, 0.401 mmol) and compound **2.12** (151 mg, 0.521 mmol) dissolved in methanol (5.4 ml) and dioxane (2.7 ml) under argon atmosphere. $Pd(PPh_3)_4$ (46 mg, 0.040 mmol) was added to the solution while argon was bubbled into the reaction mixture. The vial was sealed and the reaction mixture was stirred and heated to 90 °C for 16 hours. The reaction was followed by TLC. Upon completion, the reaction mixture was cooled down to r.t. and extracted with DCM. The organic layer was washed with brine and then concentrated *in vacuo*. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument (solvent gradient from 0 to 100% EtOAc in Hexane). Product was obtained as an off-white solid (217 mg, 98%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.45 (s, 1H), 9.27 (s, 1H), 8.89 (s, 1H), 8.58 (t, J = 5.7 Hz, 1H), 8.55 (d, J = 7.7 Hz, 1H), 8.02 (d, J = 7.7 Hz, 1H), 7.79 – 7.73 (m, 1H), 7.62 (s, 1H), 7.55 (s, 1H), 7.26 (d, J = 8.3 Hz, 1H), 7.18 (t, J = 9.3 Hz, 1H), 6.56 (s, 1H), 6.43 (d, J = 10.1 Hz, 1H), 4.79 (d, J = 5.6 Hz, 2H), 3.85 (s, 3H), 3.84 (s, 3H), 3.70 (s, 3H).

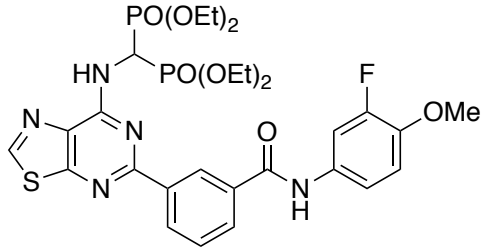
3-(7-aminothiazolo[5,4-*d*]pyrimidin-5-yl)-*N*-(3-fluoro-4-methoxyphenyl)benzamide (2.60)



Neat TFA (4.0 ml, 52 mmol) was added to a stirring solution of compound **2.59** (212 mg, 0.388 mmol) dissolved in DCM (10.0 ml). The solution was stirred at r.t. for 72 hours. The reaction was followed by TLC. Upon completion, the solution was diluted with more DCM (50 ml). The reaction mixture was quenched by slow addition of sodium bicarbonate. The organic layer was washed with brine, dried with sodium sulfate and concentrated *in vacuo*. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument (solvent gradient from 0 to 100% EtOAc in Hexane). The product was obtained a slightly orange solid (116 mg, 75%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.45 (s, 1H), 9.24 (s, 1H), 8.91 (s, 1H), 8.56 (d, J = 7.7 Hz, 1H), 8.03 (d, J = 7.5 Hz, 1H), 7.88 (s, 2H), 7.77 (d, J = 13.1 Hz, 1H), 7.62 (s, 1H), 7.55 (s, 1H), 7.18 (t, J = 9.3 Hz, 1H), 3.84 (s, 3H). MS (ESI$^+$): (*m/z*) $[M + H]^+$ 396.31

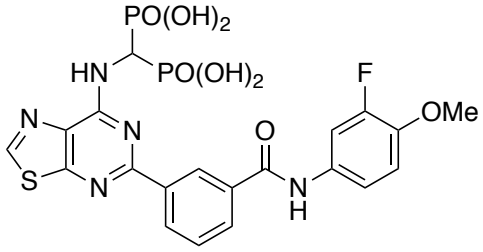
Tetraethyl (((5-(3-((3-fluoro-4-methoxyphenyl)carbamoyl)phenyl)thiazolo[5,4-*d*]pyrimidin-7-yl)amino)methylene)bis(phosphonate) (2.61)



Compound **2.61** was synthesized following the general procedure **A**. The product was purified by normal phase column chromatography on silica gel using Combiflash instrument (solvent gradient from 0 to 100% EtOAc in Hexane). Product was obtained as an off-white solid (25 mg, 30%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.47 (s, 1H), 9.39 (s, 1H), 9.08 (brs, 1H), 8.65 (dt, *J* = 1.4, 7.9 Hz, 1H), 8.09 (dt, *J* = 1.4, 8.0 Hz, 1H), 7.98 (s, 1H), 7.78 (dd, *J* = 2.5, 13.7 Hz, 1H), 7.70 (t, *J* = 7.8 Hz, 1H), 7.58 (d, *J* = 8.7 Hz, 1H), 7.18 (t, *J* = 9.4 Hz, 1H), 5.68 (m, 1H), 4.19 – 4.04 (m, 8H), 3.84 (s, 3H), 1.14 (dt, *J* = 7.0, 24.2 Hz, 12H). **^{31}P NMR (203 MHz, DSMO-d_6)** δ 16.66. MS (ESI$^+$): (*m/z*) $[M + H]^+$ 683.49

(((5-(3-((3-fluoro-4-methoxyphenyl)carbamoyl)phenyl)thiazolo[5,4-*d*]pyrimidin-7-yl)amino)methylene)bis(phosphonic acid) (2.62)



Compound **37** was synthesized following the general procedure **B**. The product was obtained as beige solid (52 mg, 78%).

^{1}H NMR (500 MHz, D_2O) δ 8.99 (s, 1H), 8.71 (s, 1H), 8.48 (d, *J* = 7.8 Hz, 1H), 7.96 (d, *J* = 7.7 Hz, 1H), 7.66 (t, *J* = 7.8 Hz, 1H), 7.43 (dd, *J* = 12.7, 2.3 Hz, 1H), 7.25 (d, *J* = 8.9 Hz, 1H), 7.15 (t, *J* = 9.1 Hz, 1H), 5.03 – 4.91 (m, 1H), 3.87 (s, 3H). **^{31}P NMR (203 MHz, D_2O)** δ 13.32. **^{13}C NMR (126 MHz, D_2O)** δ 168.5, 160.5 (d, *J* = 16.0 Hz), 155.4, 153.0, 152.2, 150.3, 144.2 (d, *J* = 10.8 Hz), 137.7, 133.9, 132.1, 130.5 (d, *J* = 9.5 Hz), 130.0, 129.6, 129.2, 127.0, 118.6 (d, *J* = 3.1 Hz), 113.9 (d, *J* = 2.0 Hz), 110.9 (d, *J* = 22.1 Hz), 56.3, 50.3.

HRMS (ESI-) calculated for $C_{20}H_{16}FN_5O_8P_2S$ *m/z* $[M - 2H]^-$, 283.5095; found, *m/z* 283.5106

Chapter 3: Structure-activity relationship of inhibitors

3.1 Rationale

Our SAR is based on the premise that longer side chains at the C_2 position of the thieno[2,3-*d*]pyrimidine core dictate the potency and selectivity of our bisphosphonate compounds in inhibiting hGGPPS over hFPPS. This hypothesis is based on the results previously discussed in Chapter 1 (Fig. 1.8). Fundamentally, our goal is to design selective and potent inhibitors of hGGPPS that would allow evaluation of the biological properties of hGGPPS. Additionally, these compounds can be used to obtain structural information (e.g. X-ray structures of enzyme/inhibitor complexes) that can further guide drug discovery. Since the bisphosphonate moiety seems to be crucial to maintain activity, almost all of our compounds are characterized by this pharmacophore. So far, our attempt at improving selectivity have been directed towards the terminal group of our lipophilic chain that is most of the time a substituted aromatic ring. Attention has also been put on the linker of the two rings generally present in our inhibitors and some fine-tuning in the scaffold for selectivity improvement and possibly metabolic stability improvements. (Fig. 3.1)

3.2 Results and discussion

Most of the compounds prepared as part of this thesis are currently undergoing biological evaluation. Consequently, only a few key examples have been evaluated and can be reported at this time. Our compounds are first screened for activity in our in-house enzymatic inhibition assay, as previously reported.[1] It allows for quick determination of in vitro potency raking between structurally related compounds in inhibiting the wild type hGGPPS. First, the percentage of inhibition of our compounds at 0.1μM and 1μM is evaluated, then IC_{50} is measured when the previous results are promising (e.g. >50% inhibition at 1 μM). Some of our compounds were also

tested in in an antiproliferative assay using multiple myeloma (MM) cancer cells RPMI 8226 RPMI.[2]

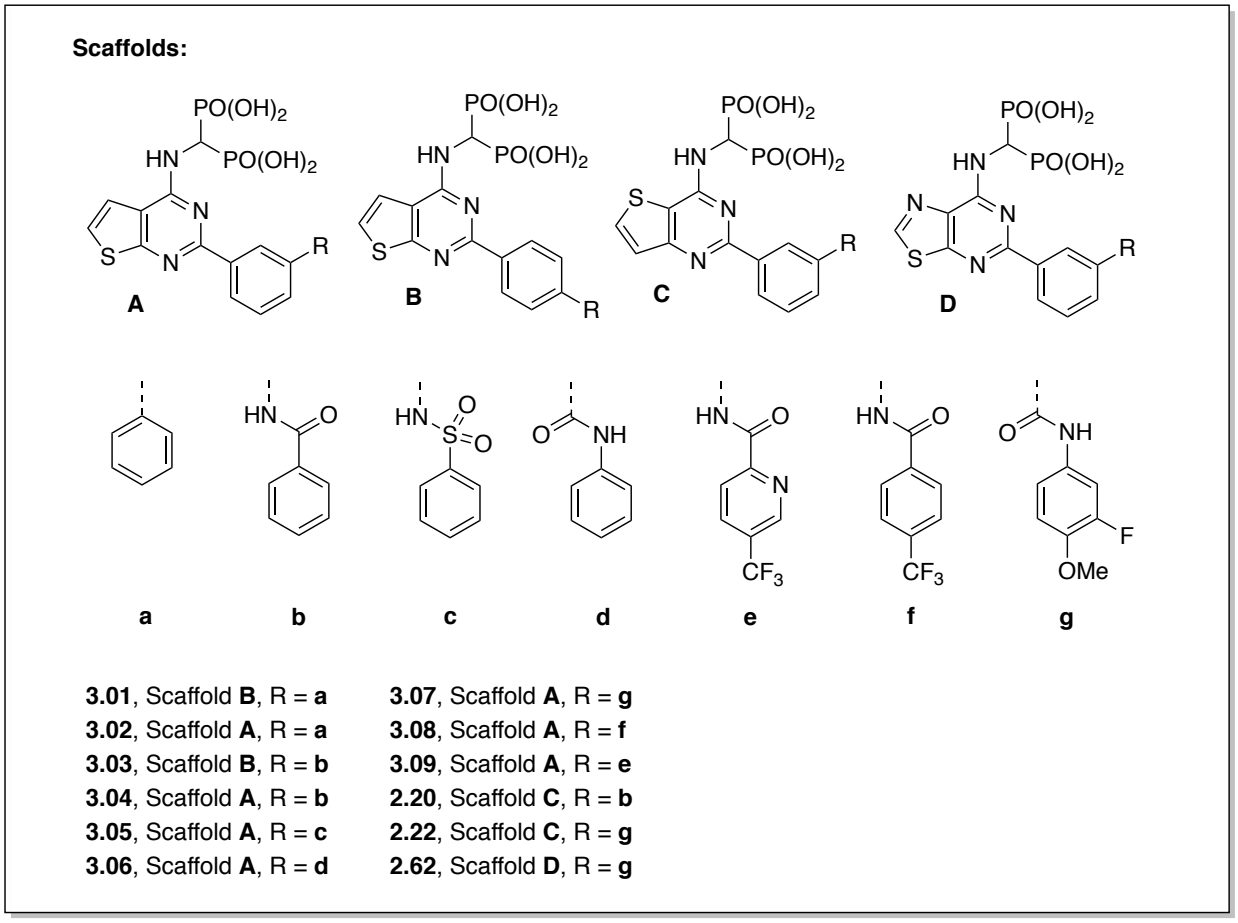


Figure 3.1. Summary of key compounds for our SAR of hGGPPS.

It is important to note that development of SAR is in progress, consequently, only some preliminary trends can be reported. For clarity purposes, all comparisons will be made using percentage inhibition at 1 μM unless otherwise stated. One of the first observations made is that compound having a linker at the *meta* position are more potent than those having a linker at the *para* position. (e.g. **3.03** versus **3.04** in table 3.1). Sulfonamides are often described as bioisostere of amides, in our case, they do not appear to improve the level of activity *in vitro*. On the other hand, having the nitrogen of the amide moiety connected directly to the terminal aromatic ring

(**3.06**) has a slightly positive effect. (e.g. **3.04** versus **3.05** and **3.06** in table 3.1) However, more analogs need to be synthesized in order to confirm those preliminary observations. When looking at scaffolds, compounds having a "sulfur up" (thieno[3,2-*d*]pyrimidine) seems to maintain the same level of potency then our previously best in class scaffold "sulfur down" (thieno[3,2-*d*]pyrimidine) both in enzymatic assay and in MM cells. (e.g. **2.20**, **2.22** versus **3.04**, **3.07**) For instance, **2.22** and **3.07** which are scaffold isomers of one another have similar EC_{50}, 192 nM and 171 nM respectively which is in the same considered the variability of the assay. (e.g. 2 folds' variability) Additionally, thiazolopyrimidine scaffold (e.g. scaffold D, Fig. 3.1), shows similar results to its analogs **2.22** and **3.07** in MM assays. However, no conclusion can be drawn from those comparisons since they are based on single comparison. More data will be needed to confirm that all scaffolds tested have the same activity. Compounds in Fig 3.1 are relatively similar and show similar activity in our enzymatic assay, mostly lower than 2 folds' variability. However, clear differences can be observed in the 8226 RPMI cell assay. A 30 folds difference in activity can be observed between compound **3.09** and **3.08** which are both composed of scaffold A but have a p-trifluoromethyl pyridine side chain and a p-trifluoromethyl phenyl side chain respectively.

Compound	% Inhibition at 0.1 μM	% Inhibition at 1 μM	IC_{50} (nM)	EC_{50} (nM)
3.01	50	75	n.d.	n.d.
3.02	51	72	n.d.	n.d.
3.03	33	53	113	n.d.
3.04	51	76	64	n.d.
3.05	29	55	n.d.	n.d.
3.06	63	80	45	462[a]
3.07	59	86	86	171[b]
3.08	75	85	n.d.	712[a]
3.09	62	85	n.d.	21310[a]
2.20	65	90	n.d.	326[a]
2.22	58	79	n.d.	192[a]
2.62	n.d.	n.d.	n.d.	285[c]

Table 3.1. Activity of key compounds in 8226 RPMI cell assay and enzyme inhibition data (IC_{50}, percentage inhibition at 0.1 and 1 μM of inhibitor).

[a] EC_{50} values shown are taken from a single assay of n =1
[b] EC_{50} values shown are average of n =2
[c] EC_{50} values shown are average of n =3
n.d.: not determined.

3.3 Future directions

A synthetic route towards thiazolopyrimidine scaffold has been established but more analogs need to be made to confirm our structure-activity relationship with this new core. In the research presented, a focus has been put on the "terminal" aromatic group moiety but other moieties remain unexplored. Our best compounds already show submicromolar inhibition in vitro

and in 8226 RPMI cell assays. Exploration of the SAR and designing selective and potent inhibitors of hGGPPS that would allow evaluation of the biological properties of hGGPPS remains our main goals.

3.4 References

1. Leung, C.-Y.; Langille, A. M.; Mancuso, J.; Tsantrizos, Y. S. Discovery of thienopyrimidine-based inhibitors of the human farnesyl pyrophosphate synthase—Parallel synthesis of analogs via a trimethylsilyl ylidene intermediate. *Bioorganic Med. Chem.* **2013,** 21, 2229.

2. Lin, Y.-S.; Park, J.; De Schutter, J. W.; Huang, X. F.; Berghuis, A. M.; Sebag, M.; Tsantrizos, Y. S. Design and Synthesis of Active Site Inhibitors of the Human Farnesyl Pyrophosphate Synthase: Apoptosis and Inhibition of ERK Phosphorylation in Multiple Myeloma Cells. *J. Med. Chem.* **2012,** 55, 3201.

Contributions to Knowledge

Developed a synthetic protocol for C_2 and C_4 substituted thieno[3,2-*d*]pyrimidine where C_2 is a monophosphonate or a bisphosphonate and C_4 are various chains composed mainly of aromatic moieties. These inhibitors are selective in inhibiting hGGPPS over hFPPS and currently the best known "drug-like" inhibitors of the targeted enzyme (hGGPPS) with an EC_{50} in the submicromolar range in inhibiting cancer cell proliferation. These inhibitors can be synthesized in a library mode via the developed of our synthetic protocol. Also developed a synthetic protocol for C_5 and C_7 substituted thiazolo[5,4-*d*]pyrimidine where C_7 is a bisphosphonate and C_5 are various chains composed mainly of aromatic moieties. These inhibitors are also selective in

inhibiting hGGPPS (over hFPPS) and exhibit potency (EC_{50}) in blocking cancer cell proliferation in the low nanomolar range.

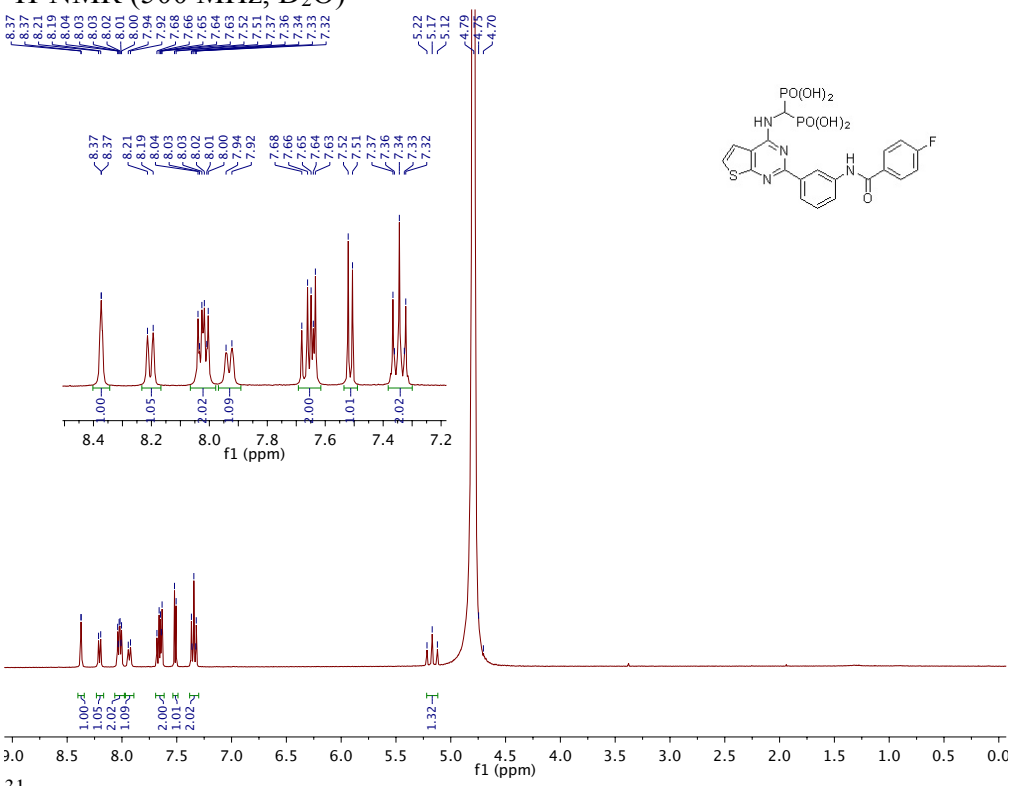
Figure 2.3 in **chapter 2** present all the analogs made during this thesis. The synthesis for each intermediate in also described in chapter 2. ^{1}H, ^{13}C and ^{31}P NMR are available in Appendix I, LC trace are available in Appendix II and HRMS are available in chapter 2.

Appendix I

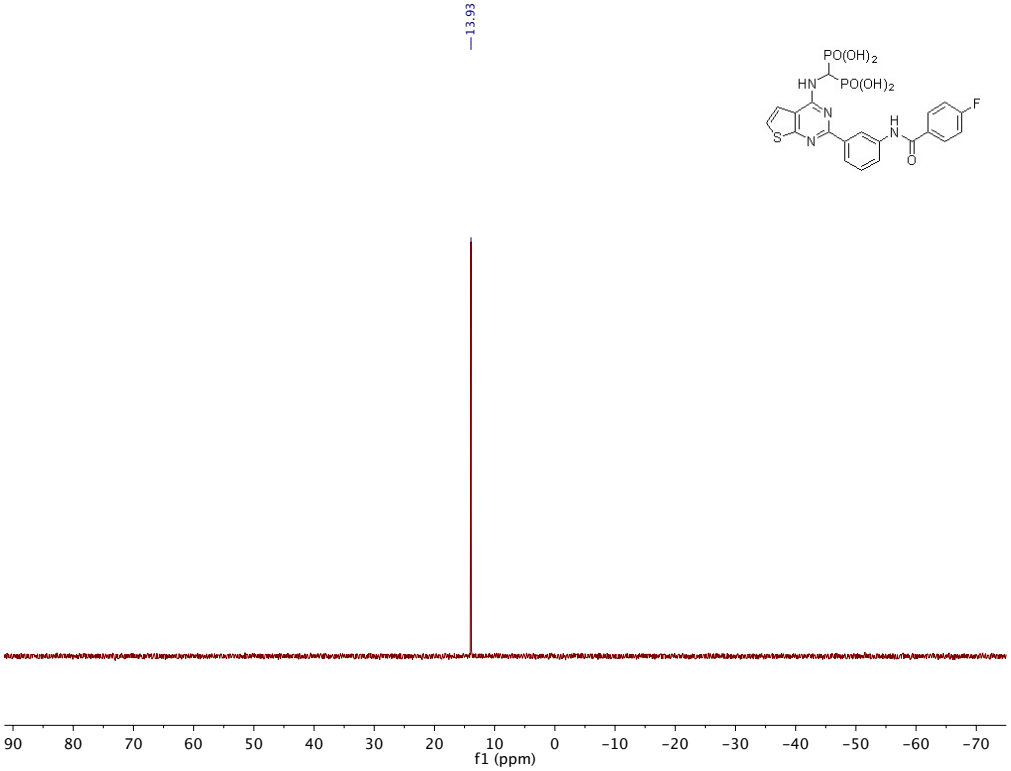
NMR spectra of final compounds

Inhibitor **2.07**

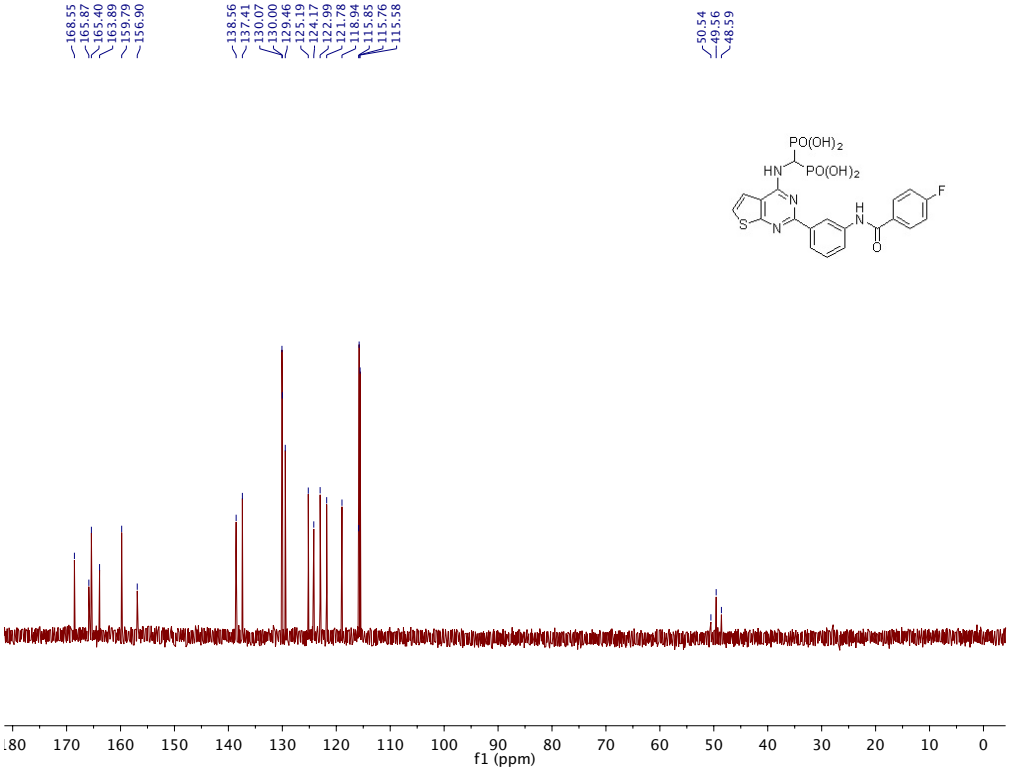
^{1}H-NMR (500 MHz, D_2O)



^{31}P NMR (162 MHz, D_2O)

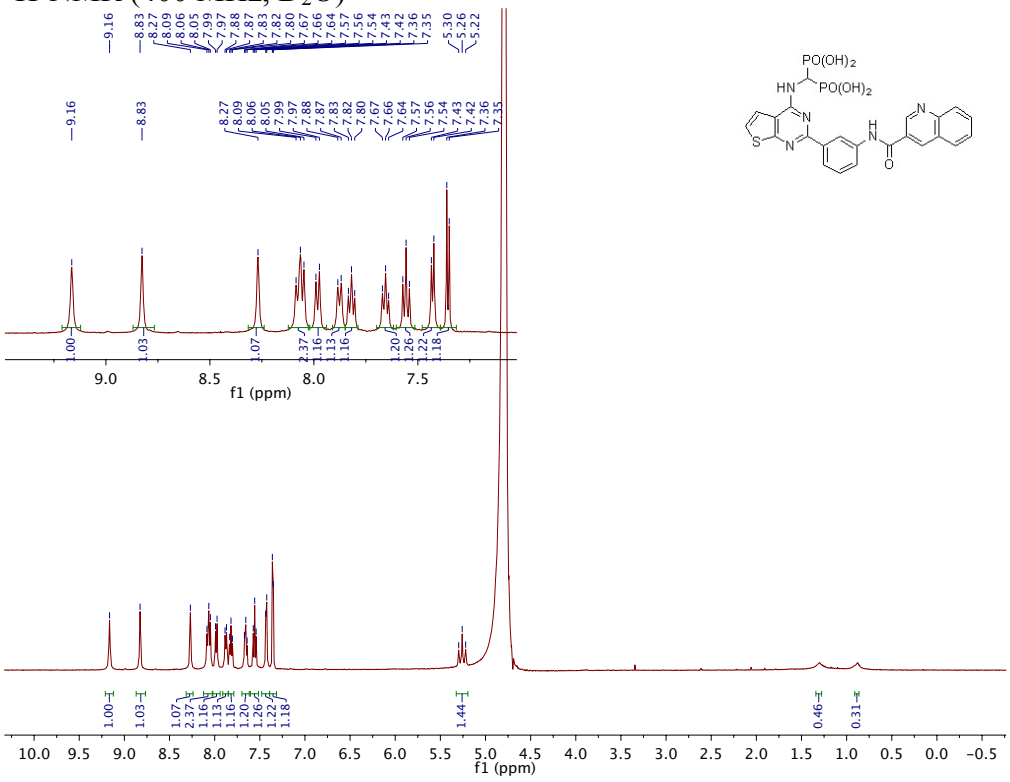


^{13}C NMR (126 MHz, D_2O)

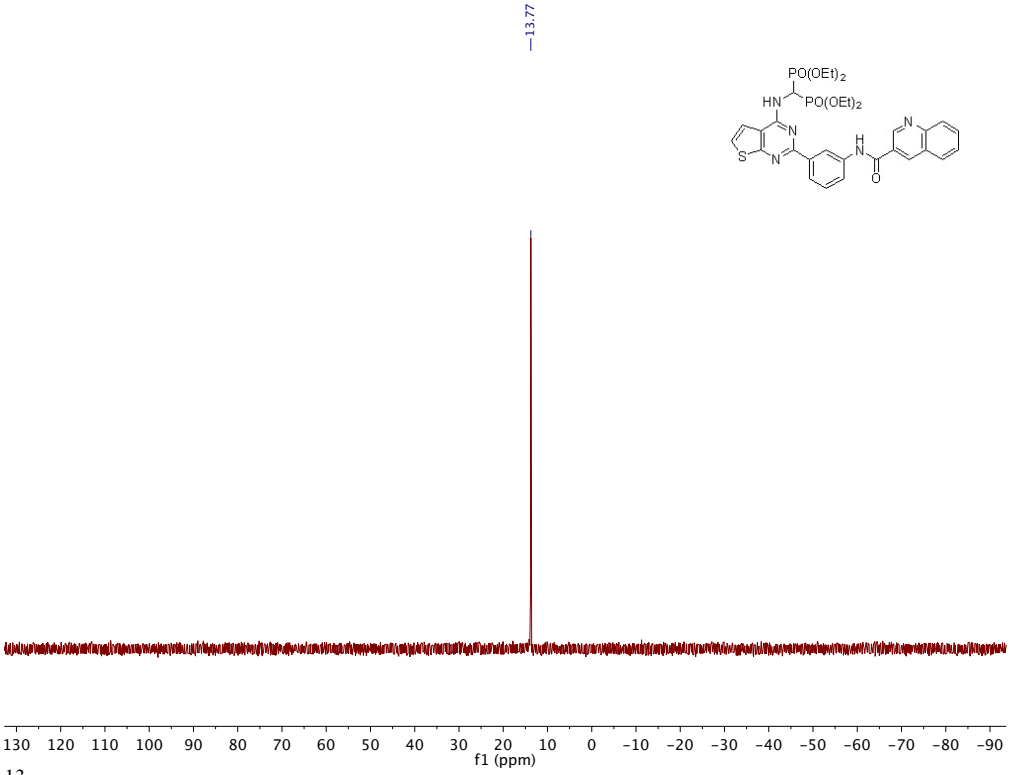


Inhibitor **2.09**

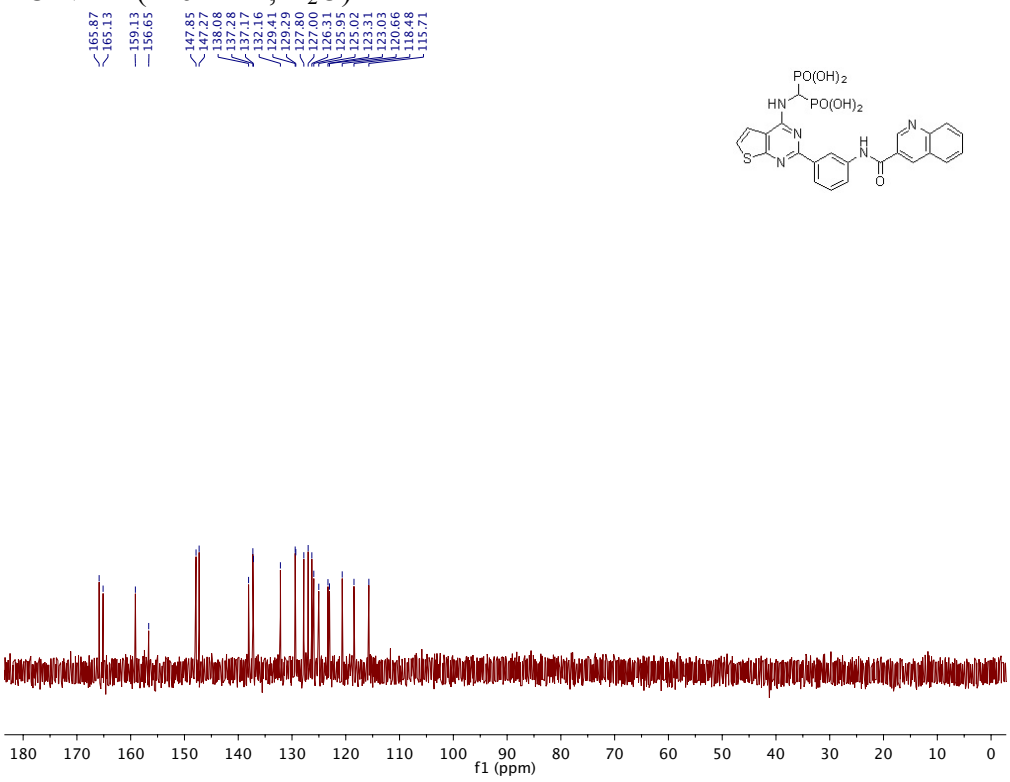
^{1}H-NMR (400 MHz, D_2O)



^{31}P NMR (203 MHz, D_2O)

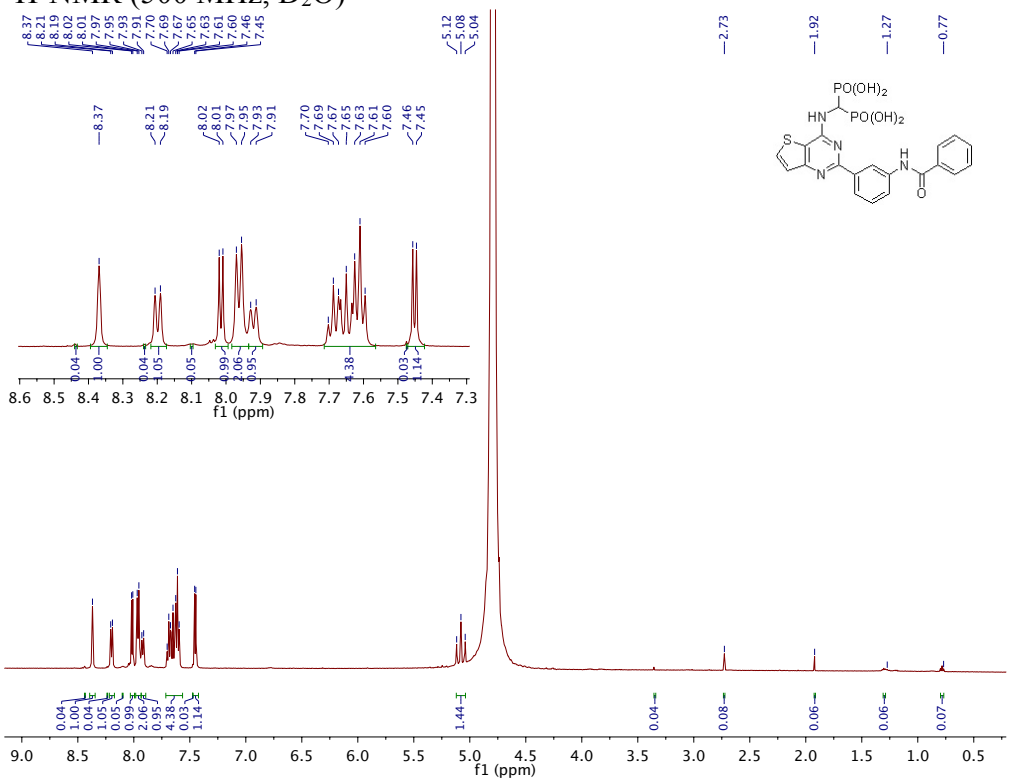


^{13}C NMR (126 MHz, D_2O)

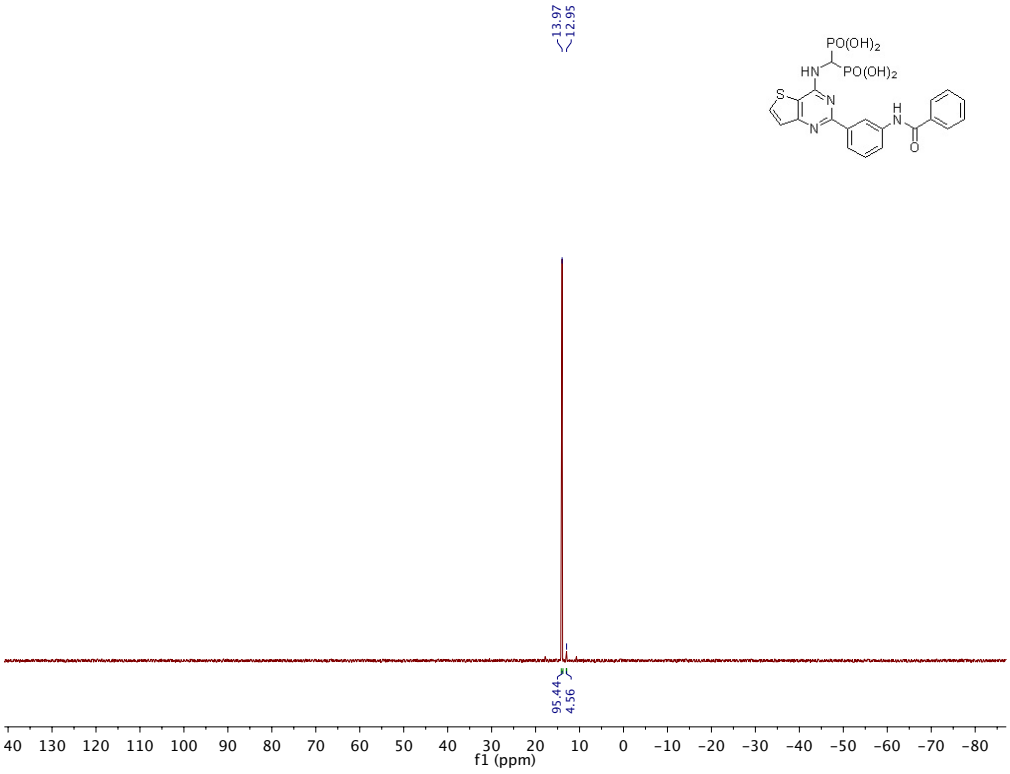


Inhibitor **2.20**

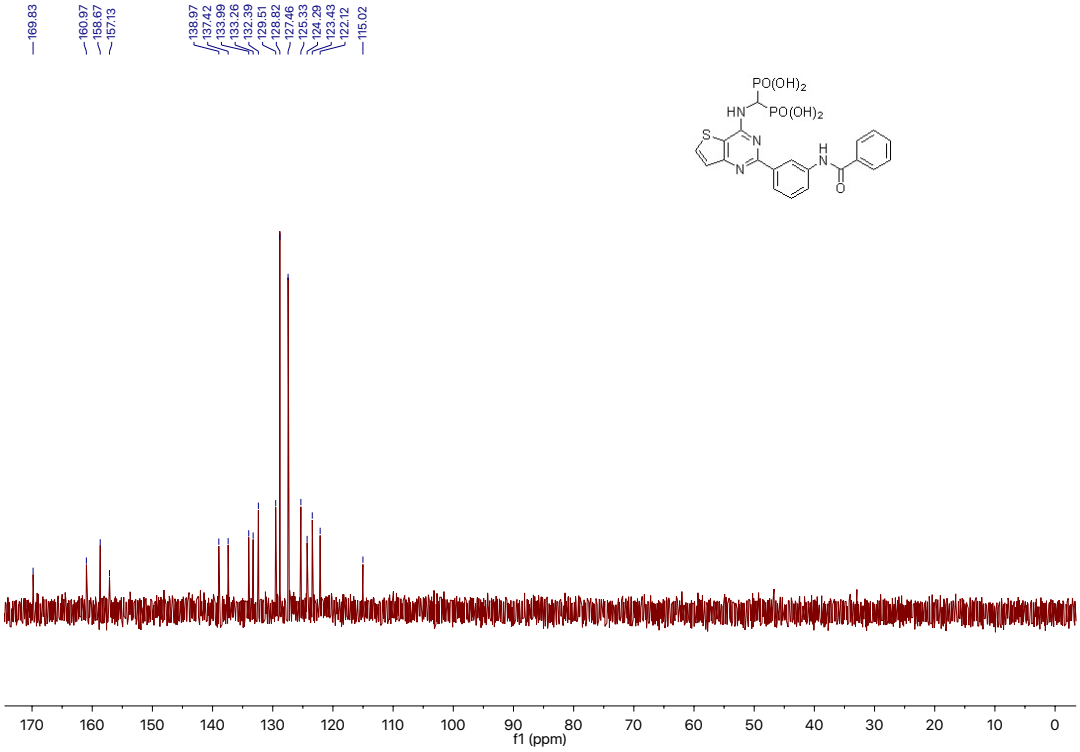
^{1}H-NMR (500 MHz, D_2O)



^{31}P NMR (203 MHz, D_2O)

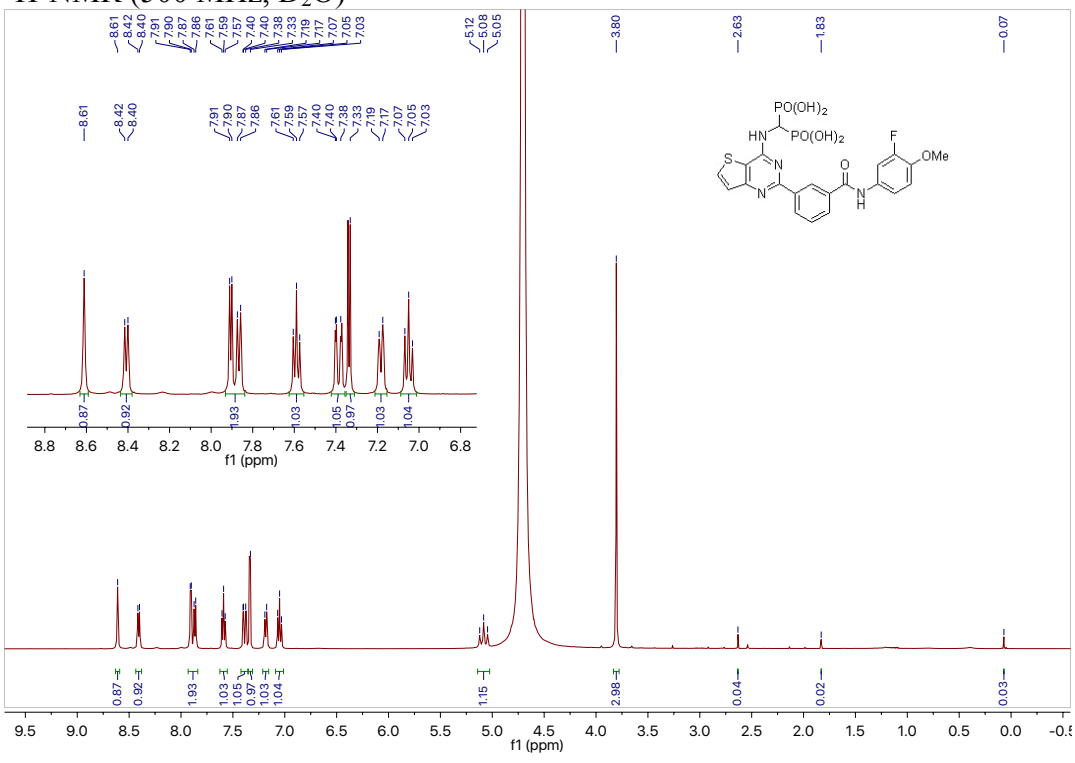


^{13}C NMR (126 MHz, D_2O)

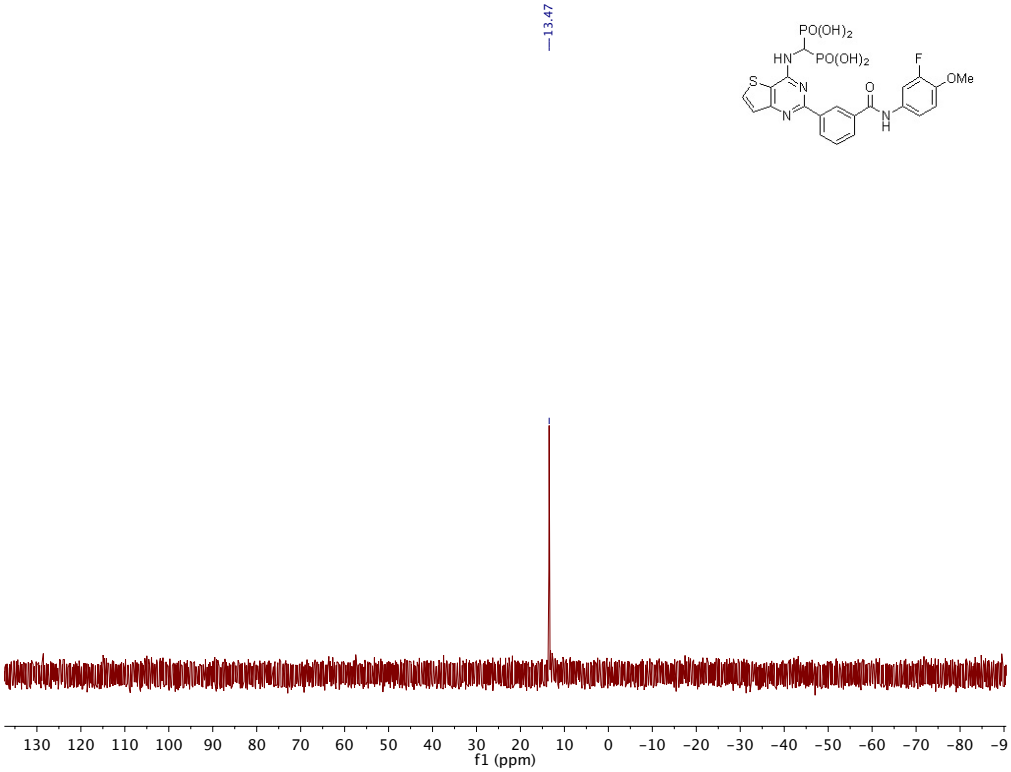


Inhibitor **2.22**

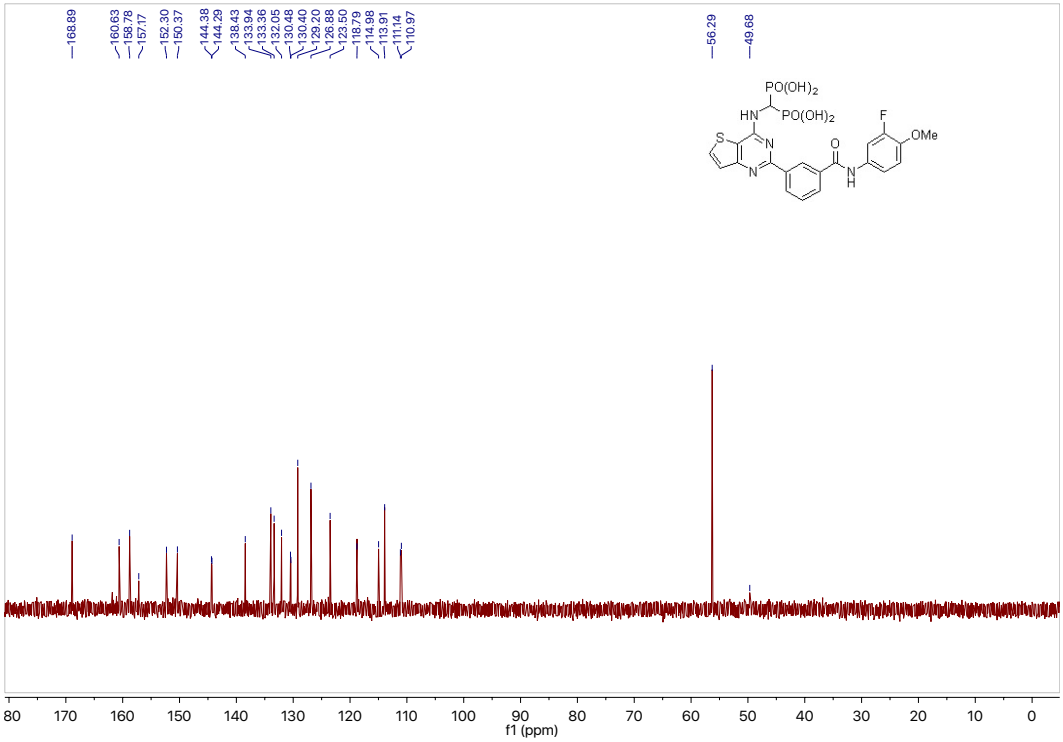
^{1}H-NMR (500 MHz, D_2O)



^{31}P NMR (203 MHz, D_2O)

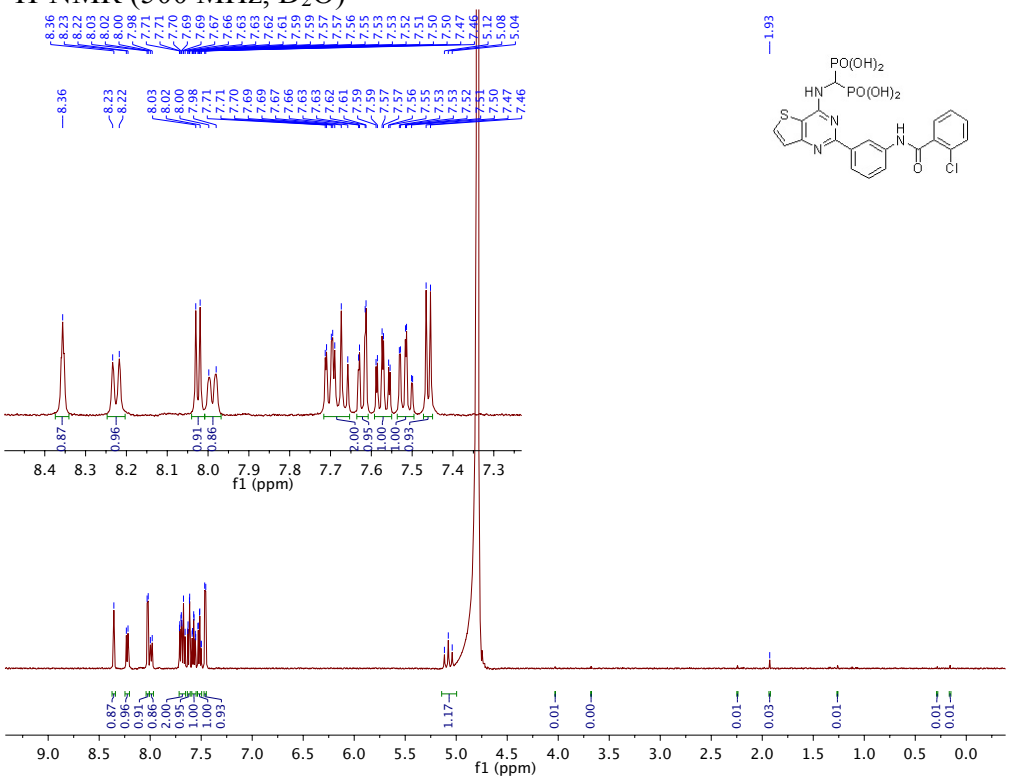


^{13}C NMR (126 MHz, D_2O)

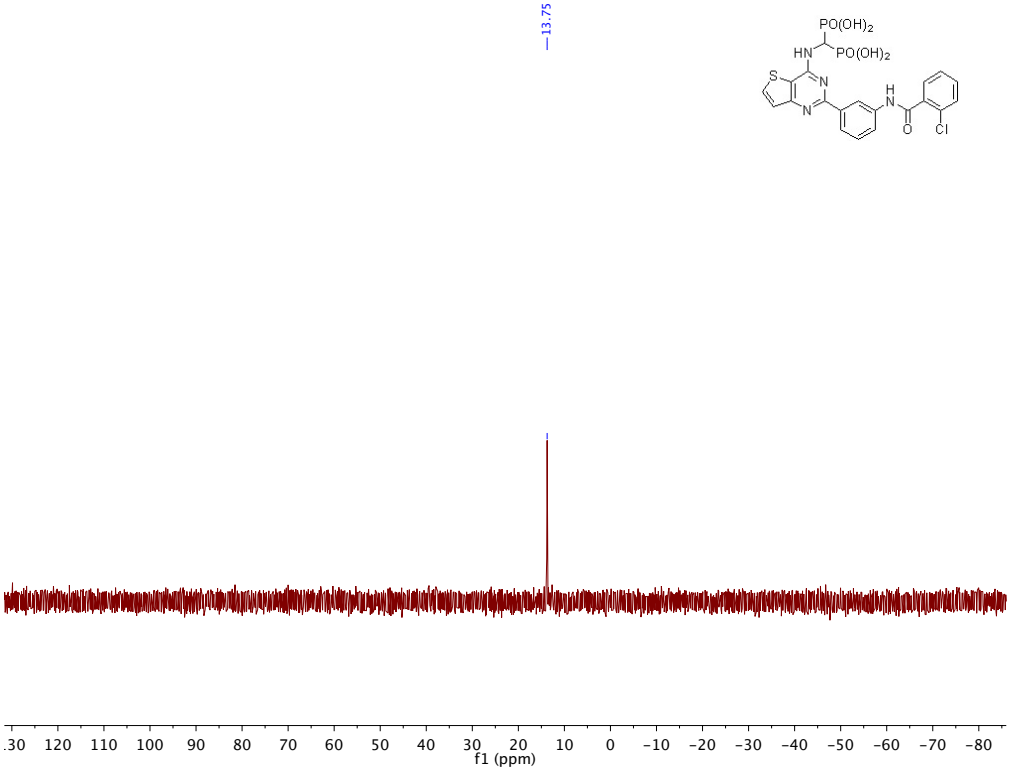


Inhibitor **2.24**

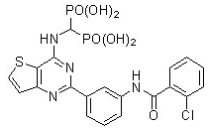
^{1}H-NMR (500 MHz, D_2O)

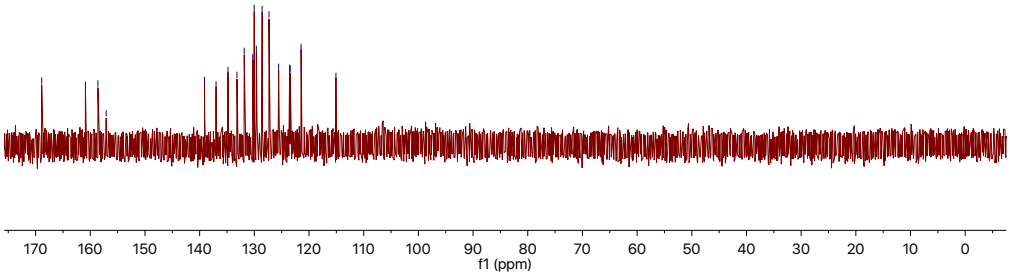


^{31}P NMR (203 MHz, D_2O)



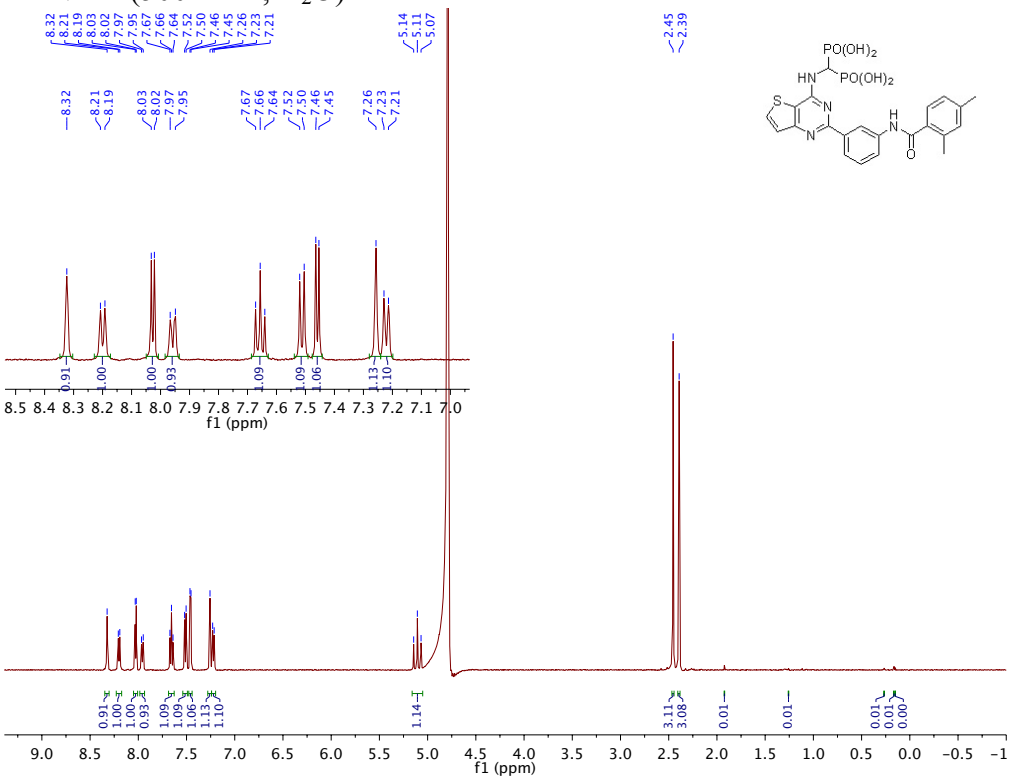
^{13}C NMR (126 MHz, D_2O)



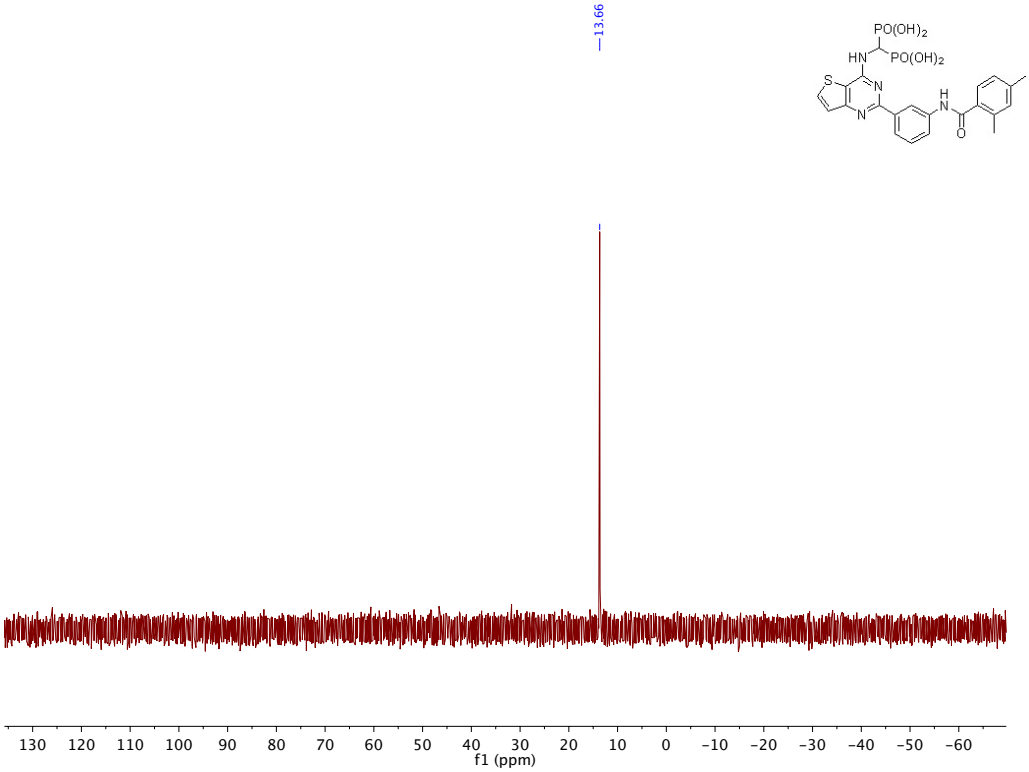


Inhibitor **2.26**

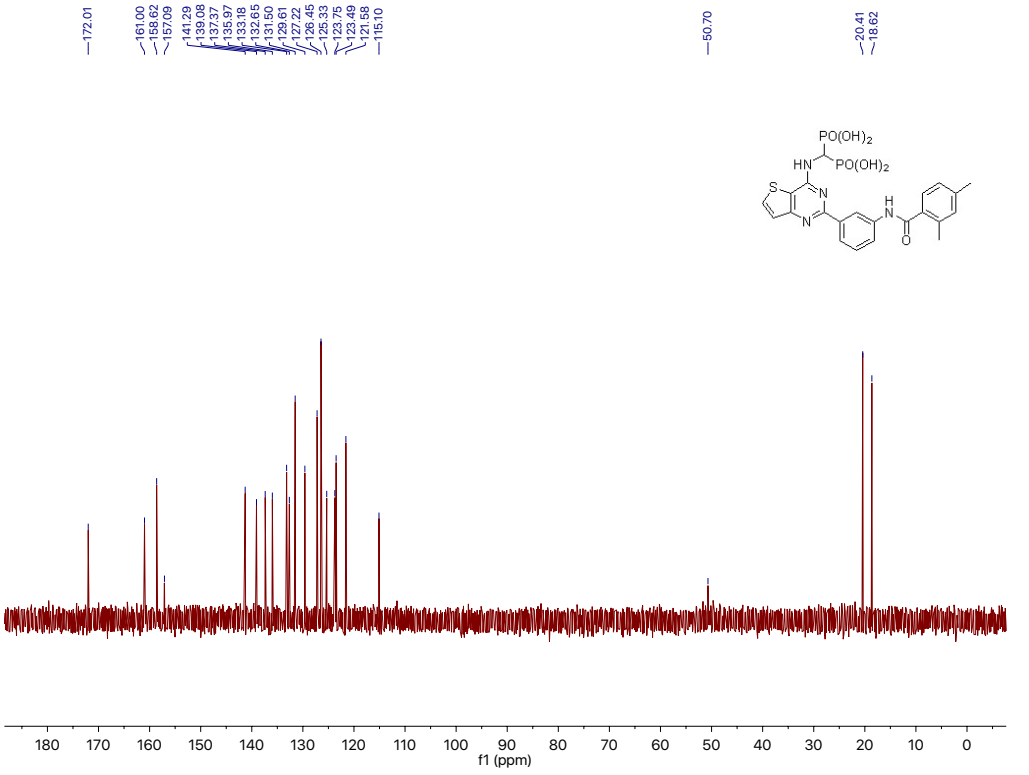
^{1}H-NMR (500 MHz, D_2O)



^{31}P NMR (203 MHz, D_2O)

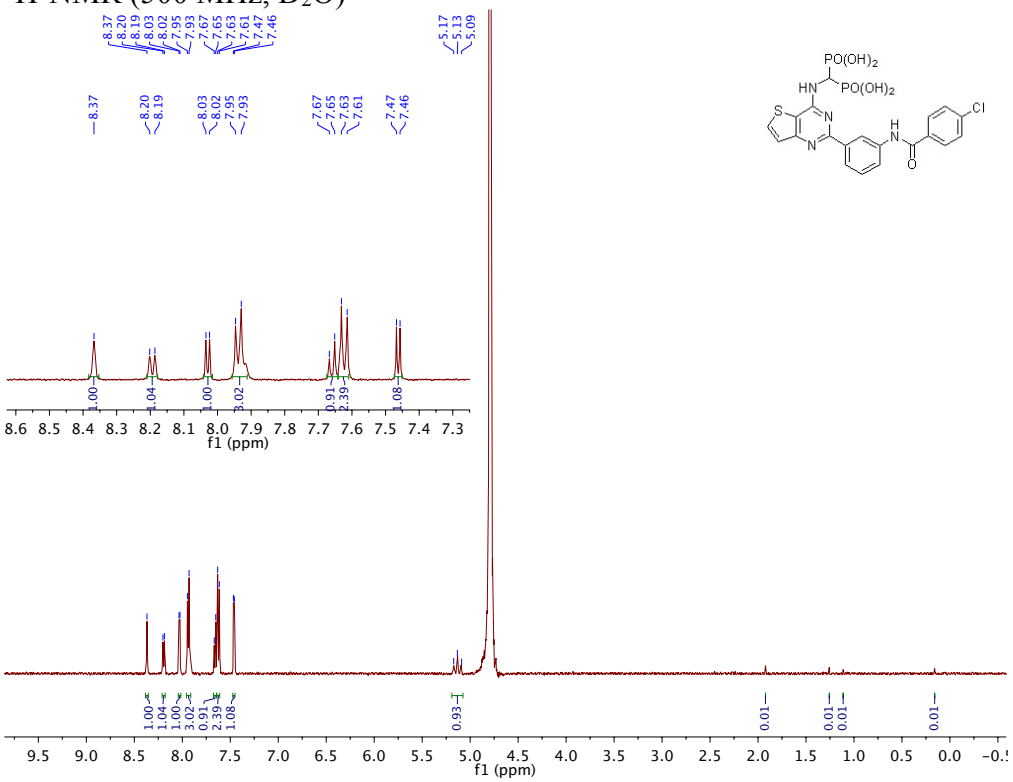


^{13}C NMR (126 MHz, D_2O)

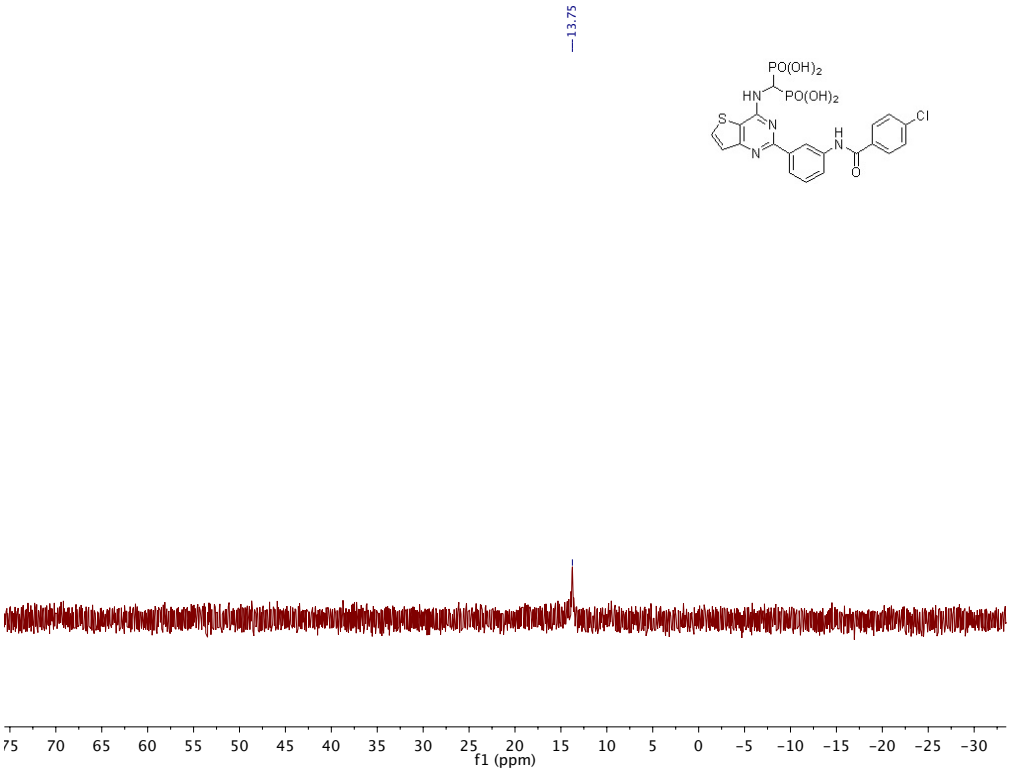


Inhibitor **2.28**

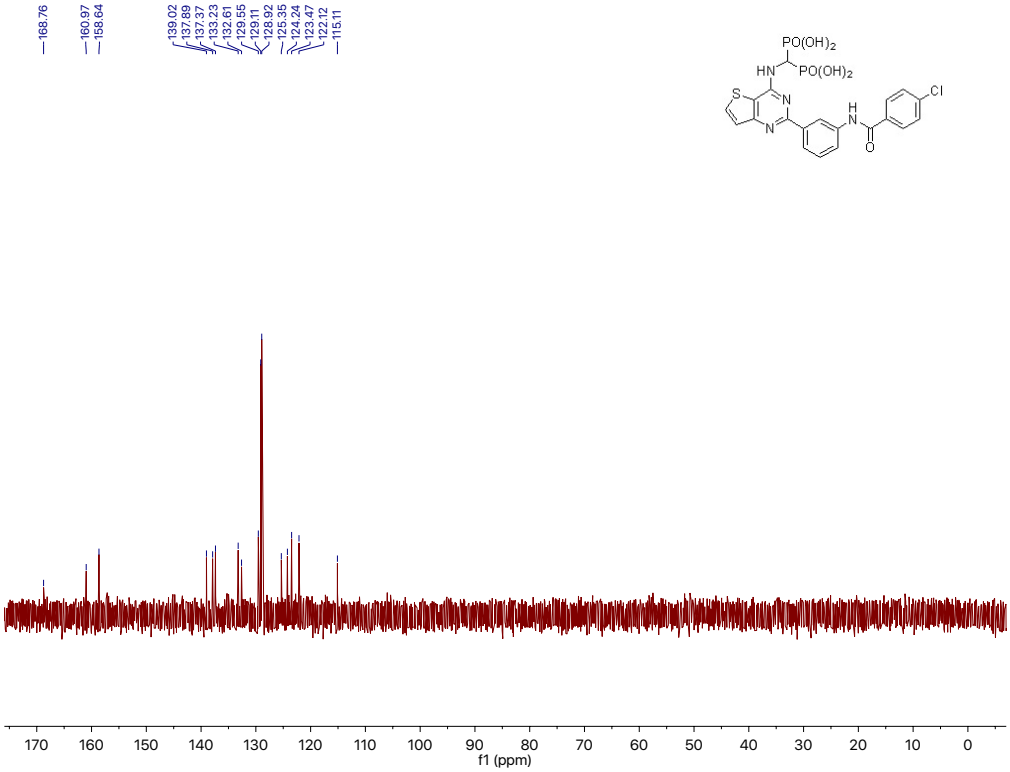
^{1}H-NMR (500 MHz, D_2O)



^{31}P NMR (203 MHz, D_2O)

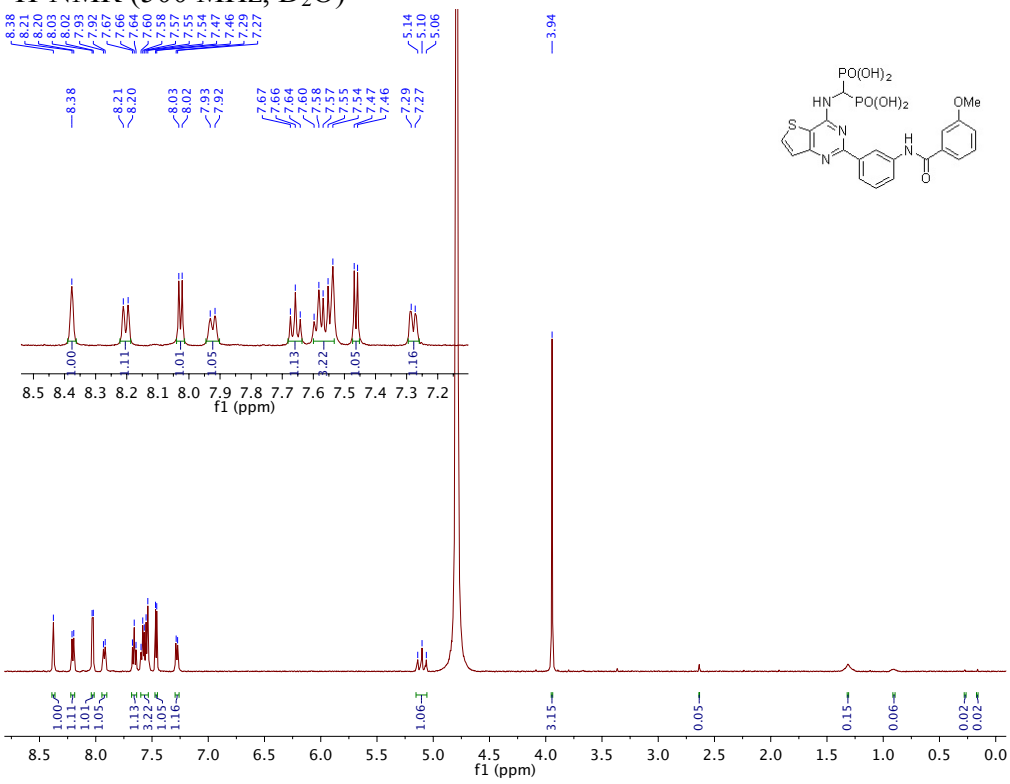


^{13}C NMR (126 MHz, D_2O)

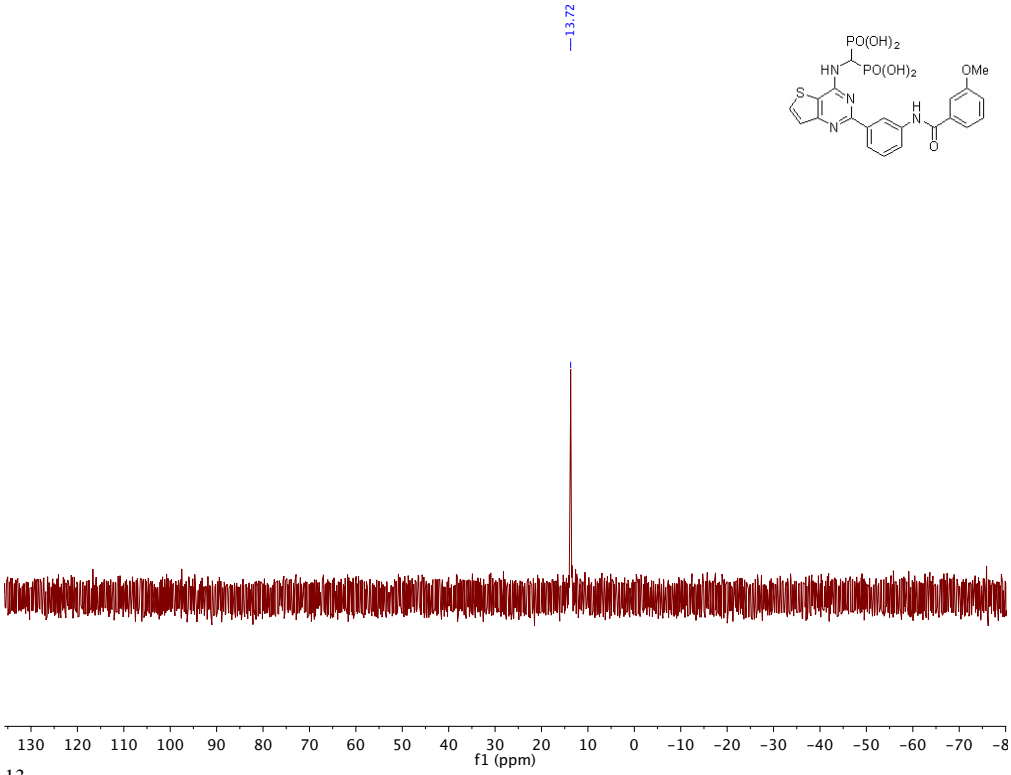


Inhibitor **2.30**

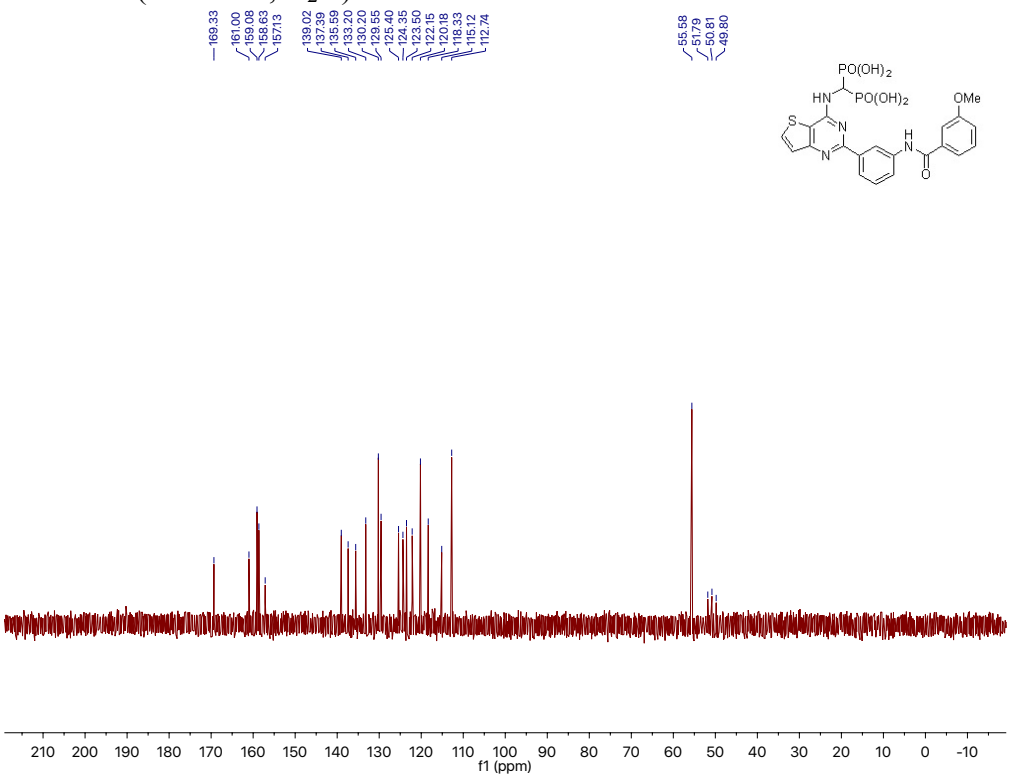
^{1}H-NMR (500 MHz, D_2O)



^{31}P NMR (203 MHz, D_2O)

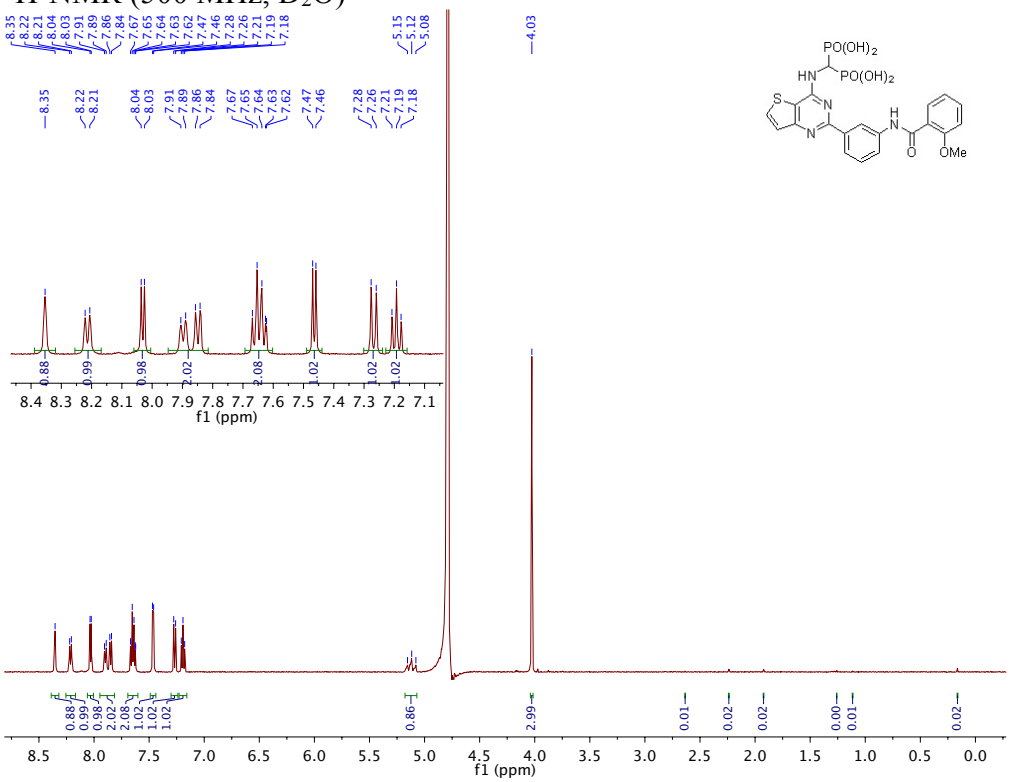


^{13}C NMR (126 MHz, D_2O)

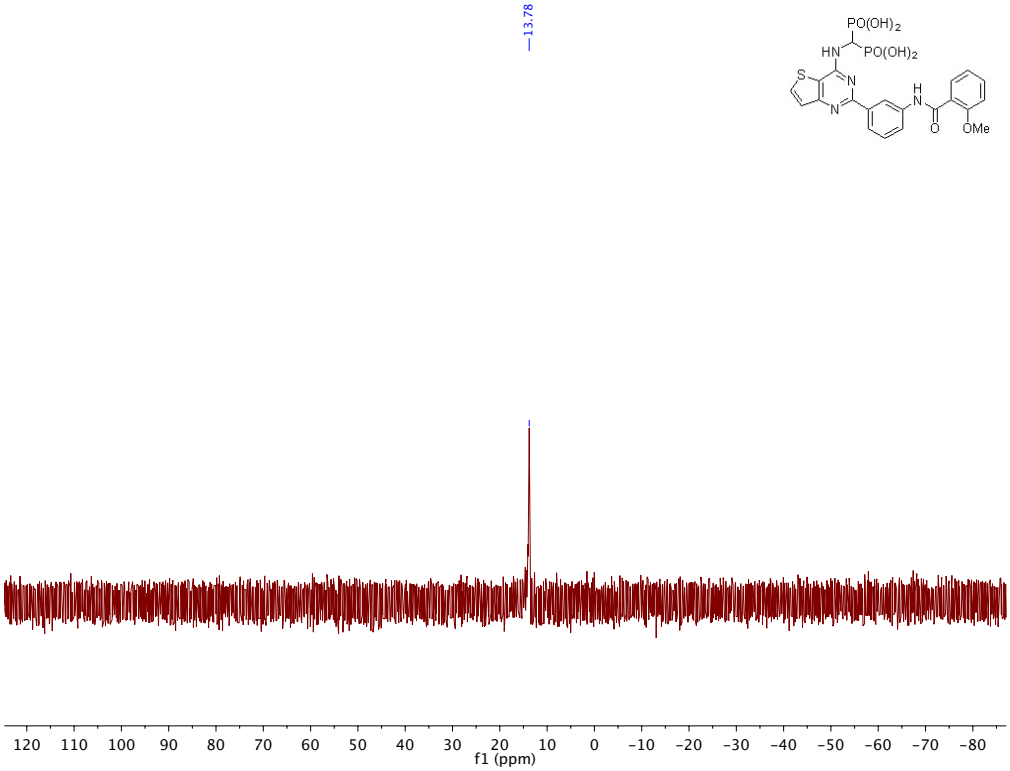


Inhibitor **2.32**

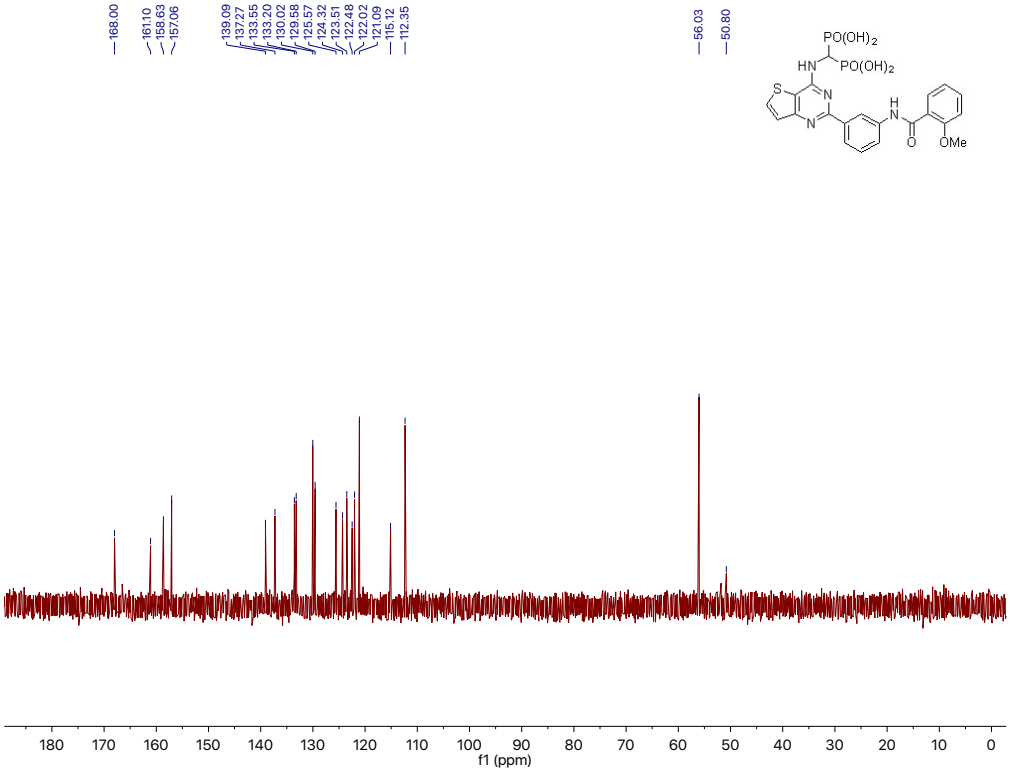
^{1}H-NMR (500 MHz, D_2O)



^{31}P NMR (203 MHz, D_2O)

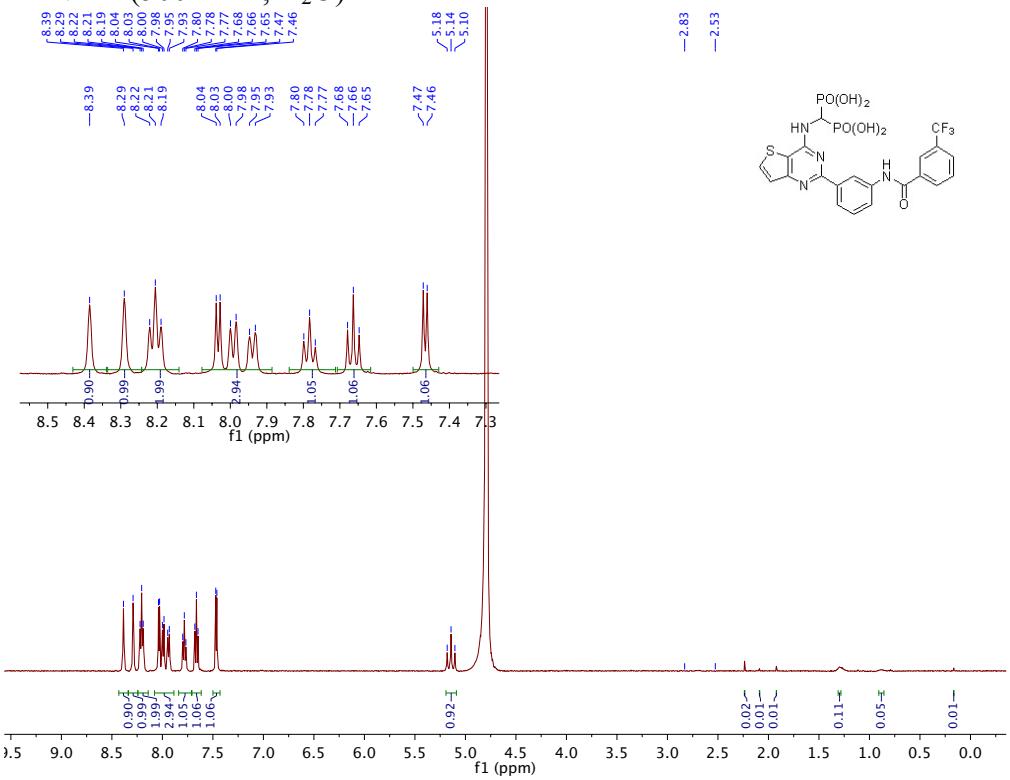


^{13}C NMR (126 MHz, D_2O)

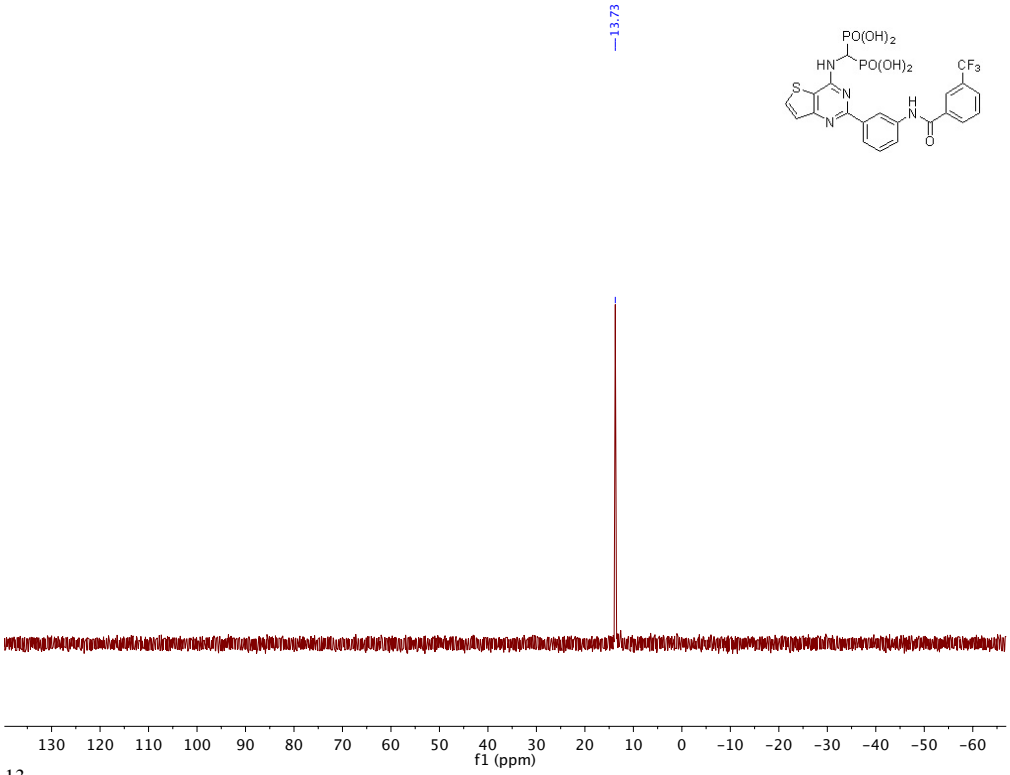


Inhibitor **2.34**

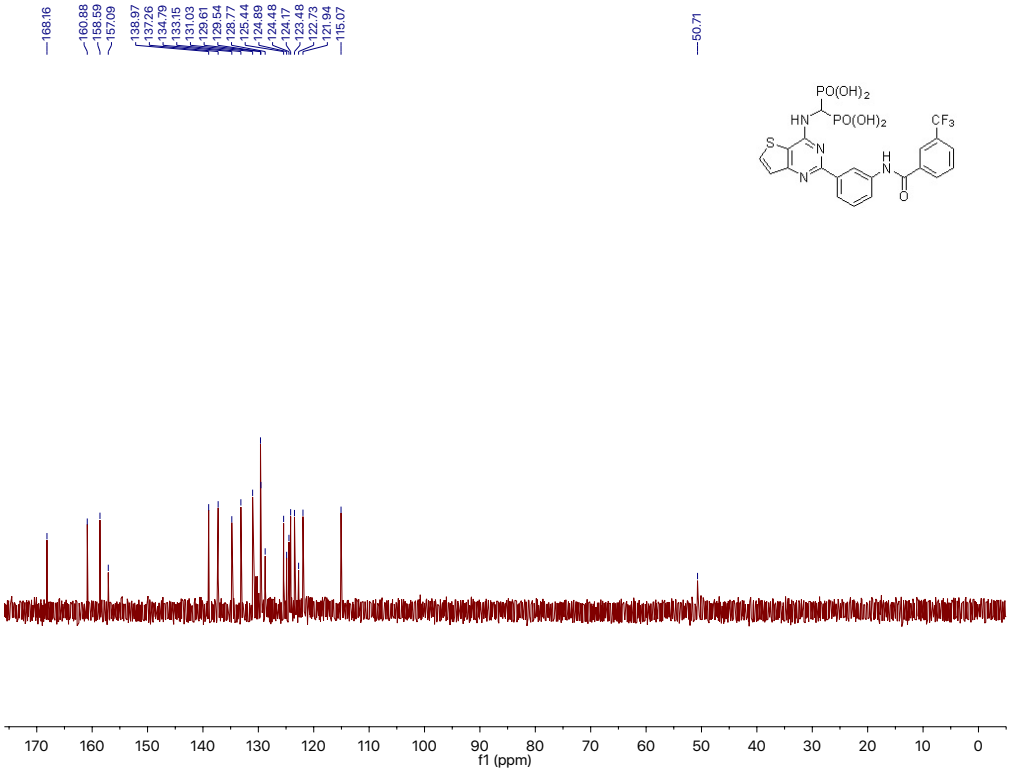
^{1}H-NMR (500 MHz, D_2O)



^{31}P NMR (203 MHz, D_2O)

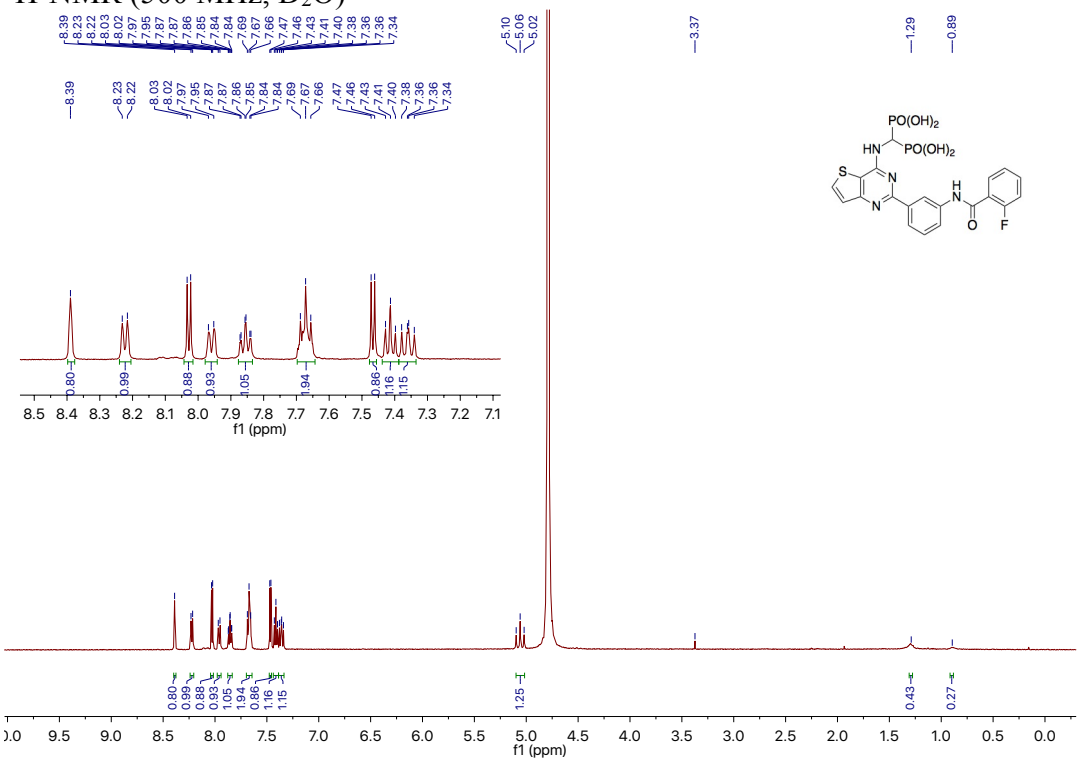


^{13}C NMR (126 MHz, D_2O)

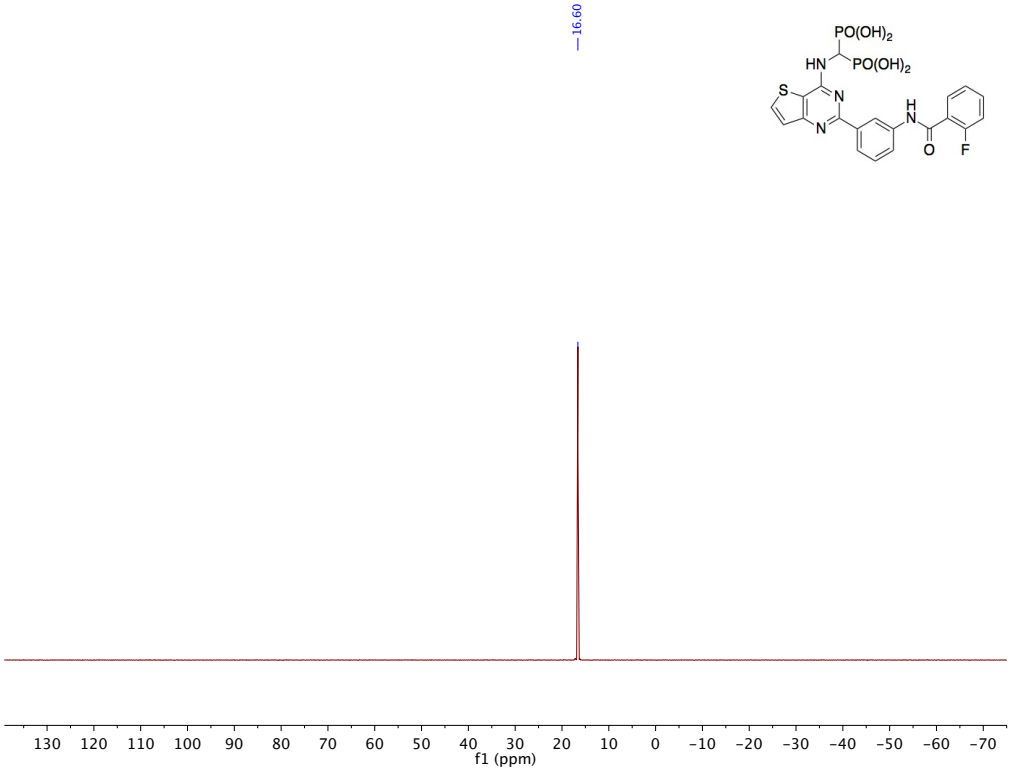


Inhibitor **2.36**

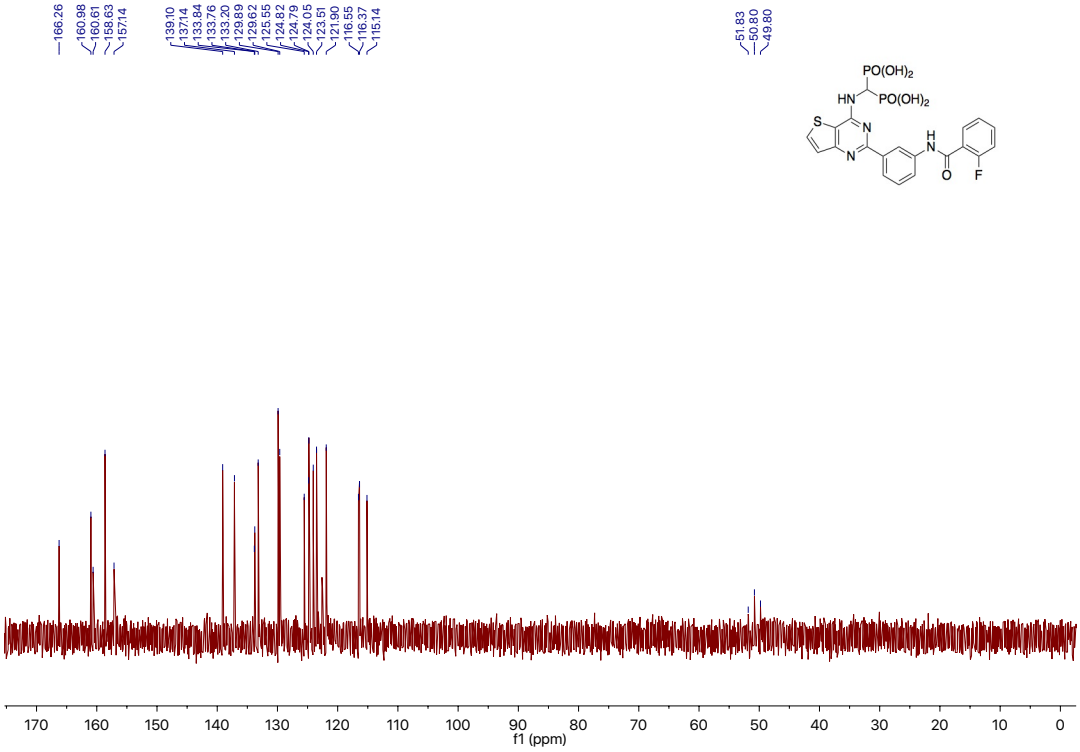
^{1}H-NMR (500 MHz, D_2O)



^{31}P NMR (203 MHz, D_2O)

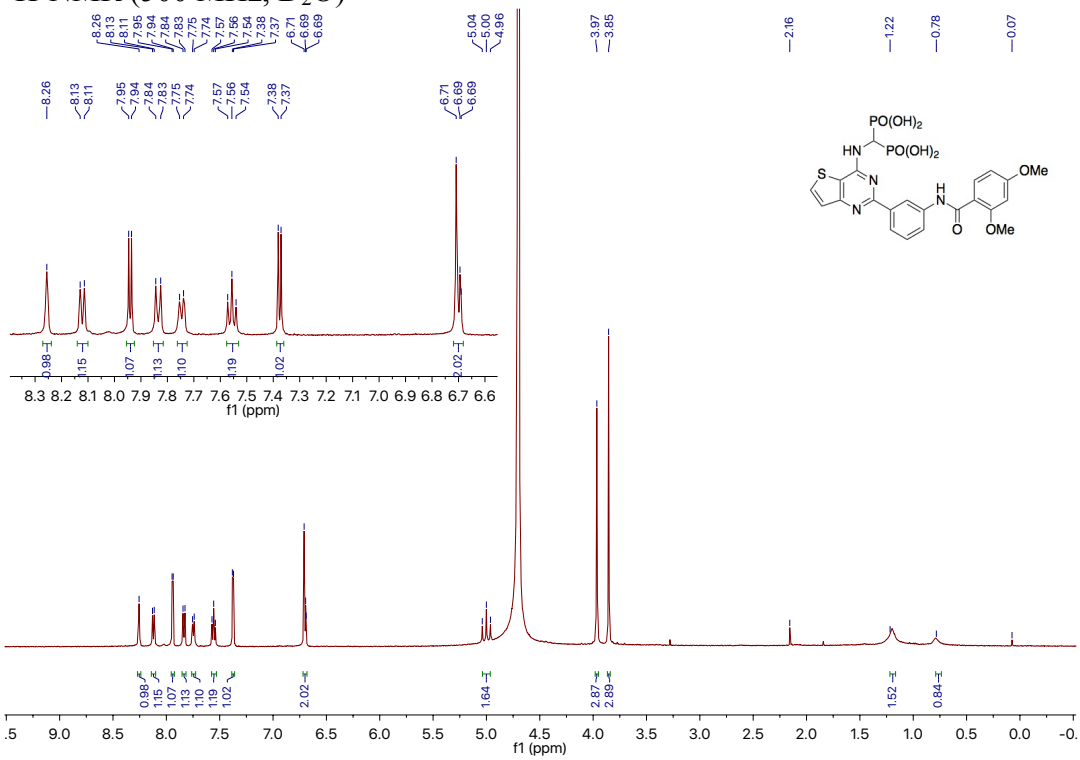


^{13}C NMR (126 MHz, D_2O)

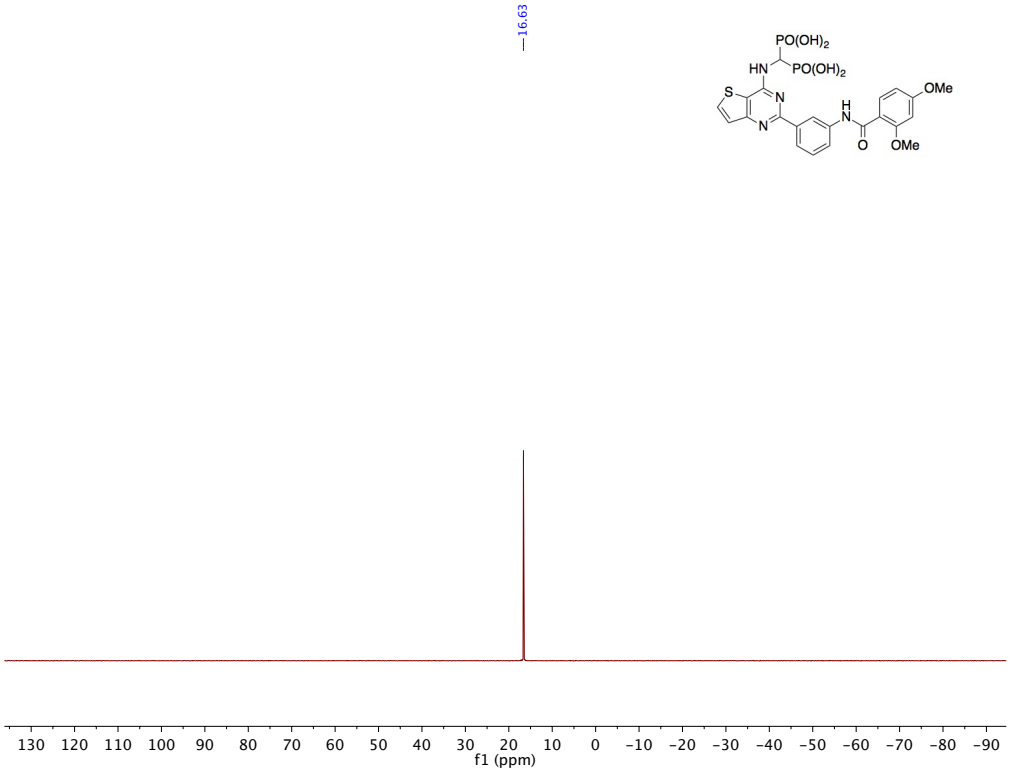


Inhibitor **2.38**

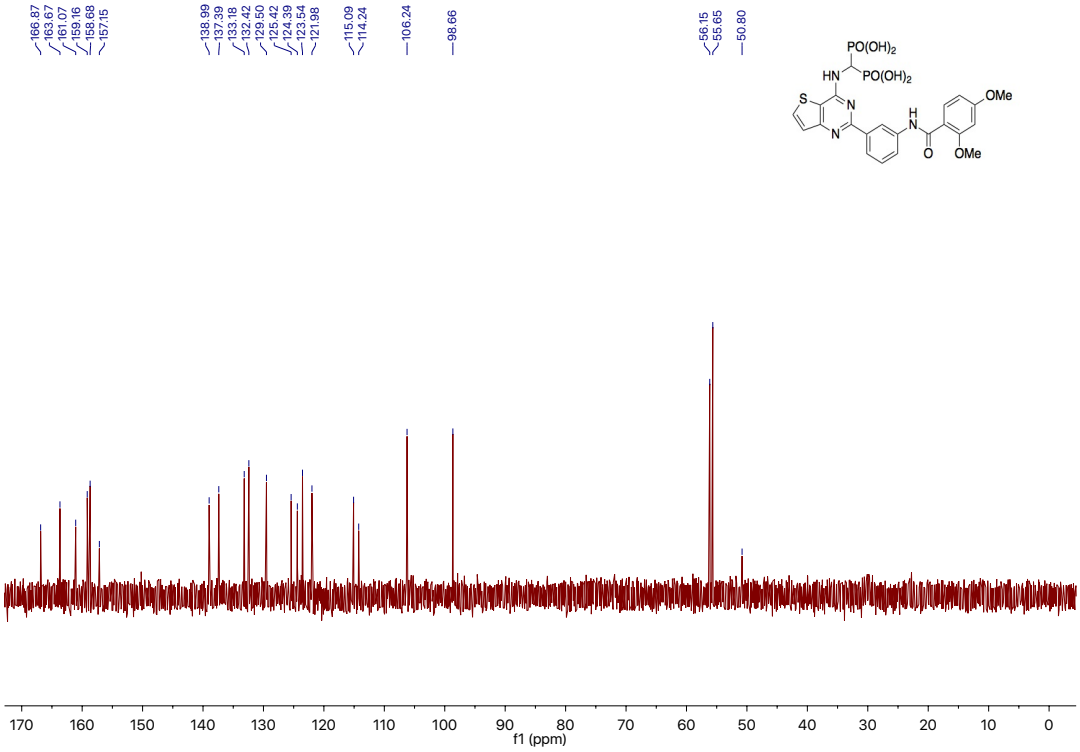
^{1}H-NMR (500 MHz, D_2O)



^{31}P NMR (203 MHz, D_2O)

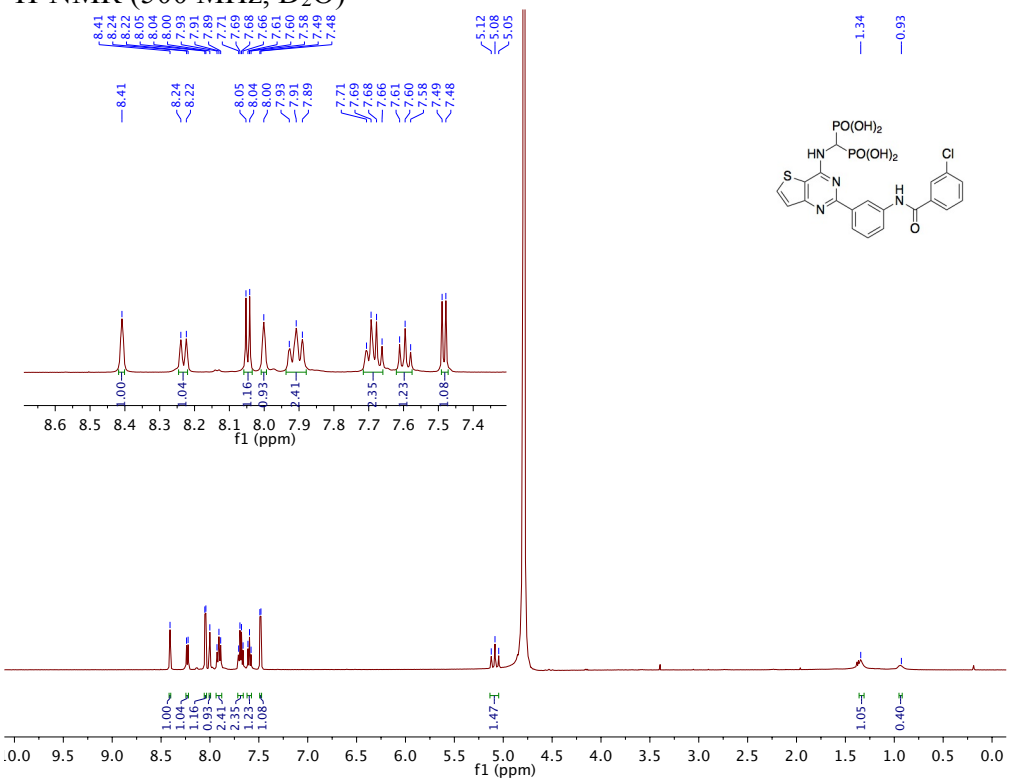


^{13}C NMR (126 MHz, D_2O)

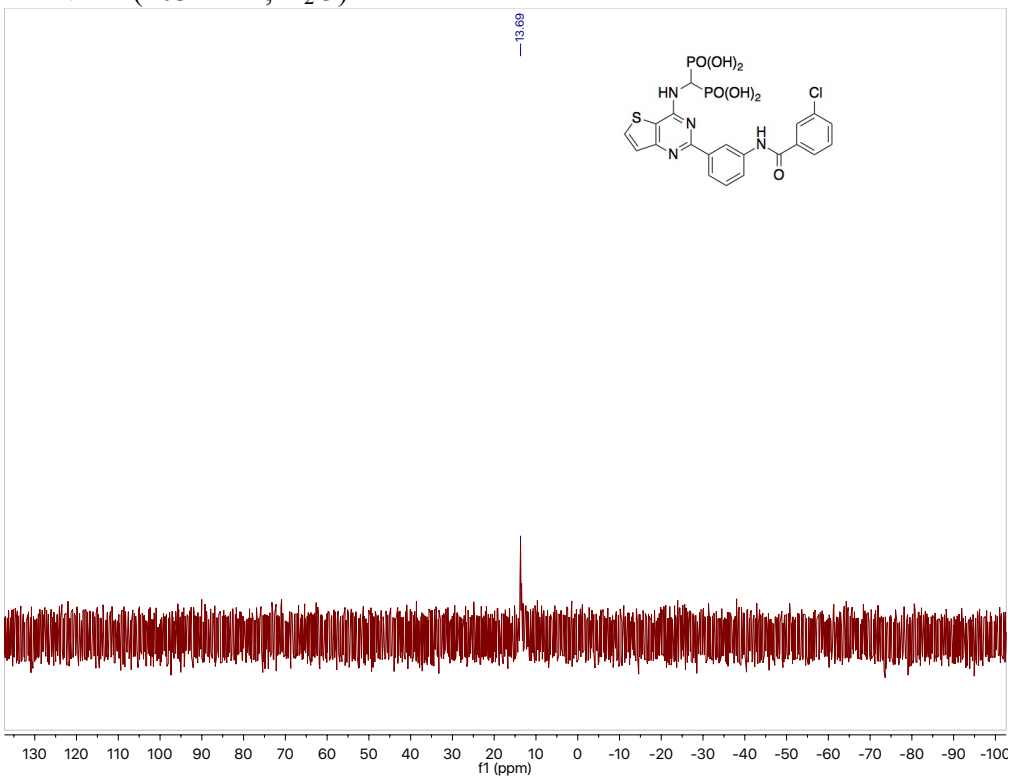


Inhibitor **2.40**

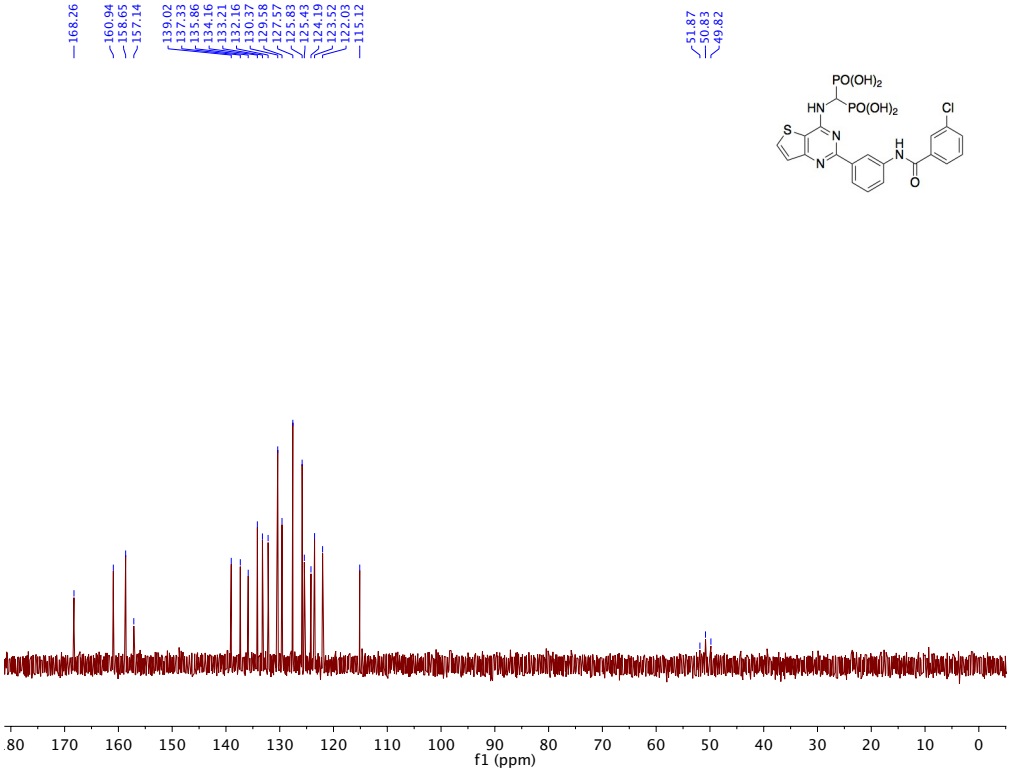
^{1}H-NMR (500 MHz, D_2O)



^{31}P NMR (203 MHz, D_2O)

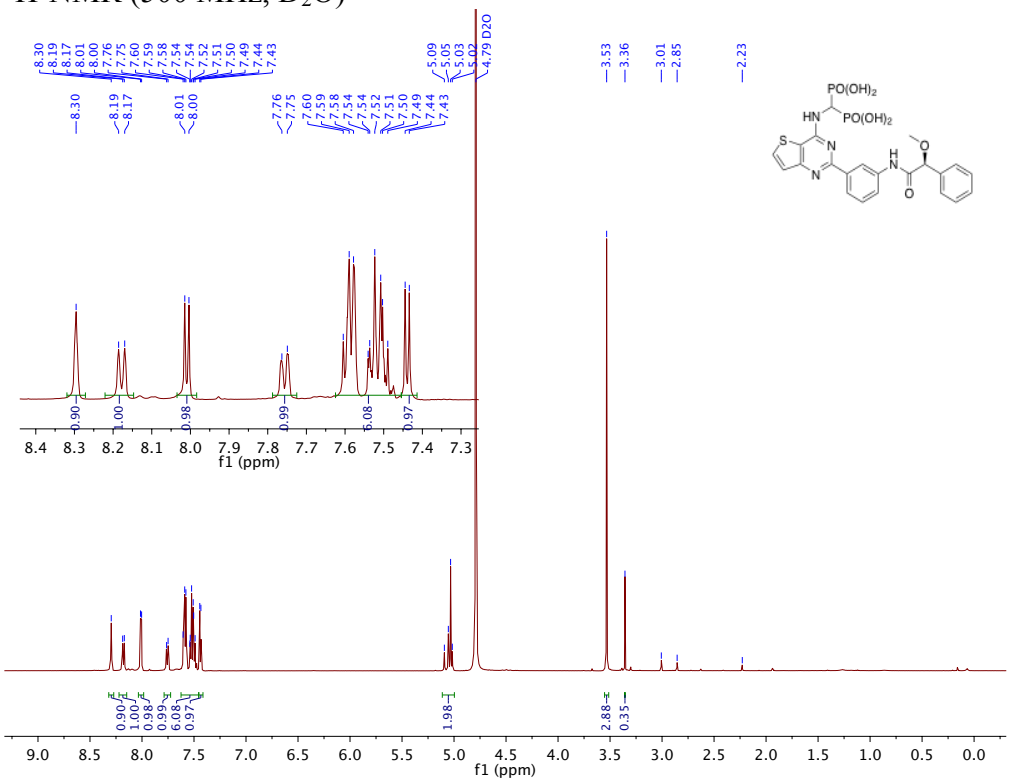


^{13}C NMR (126 MHz, D_2O)

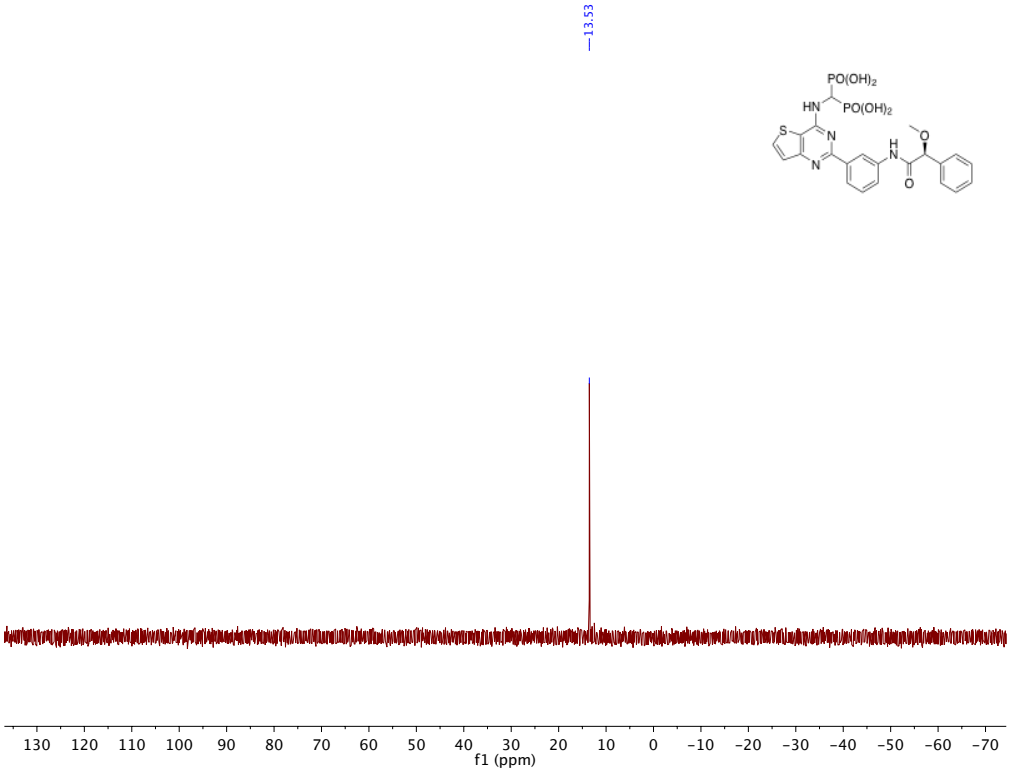


Inhibitor **2.46**

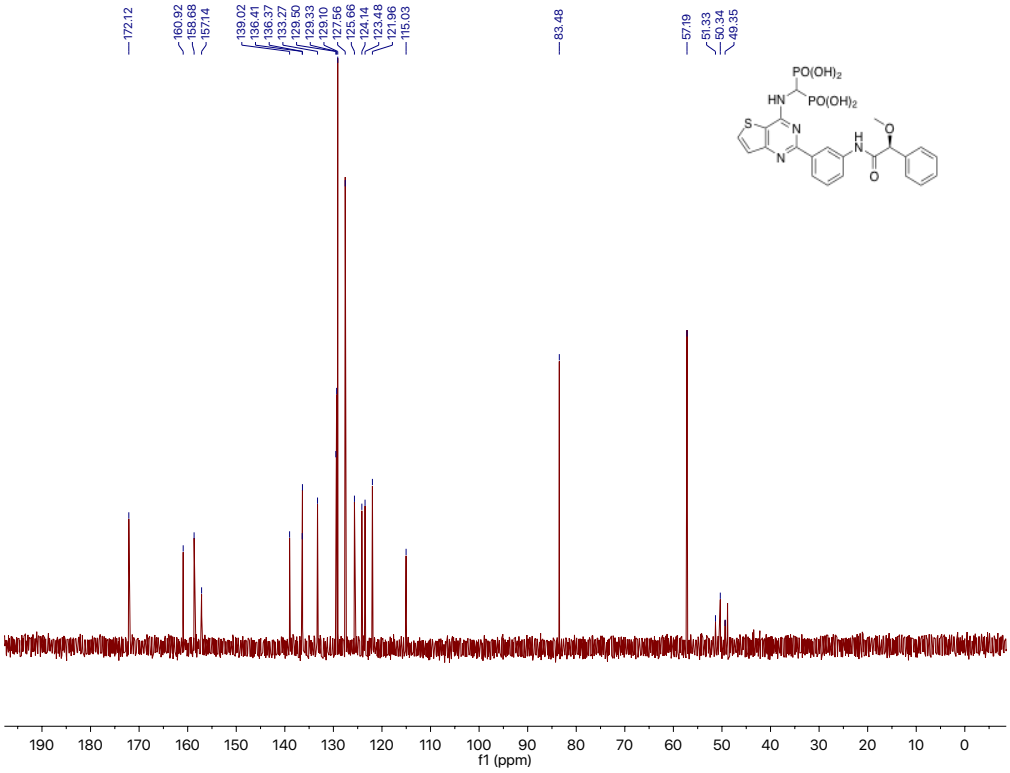
^{1}H-NMR (500 MHz, D_2O)



^{31}P NMR (203 MHz, D_2O)

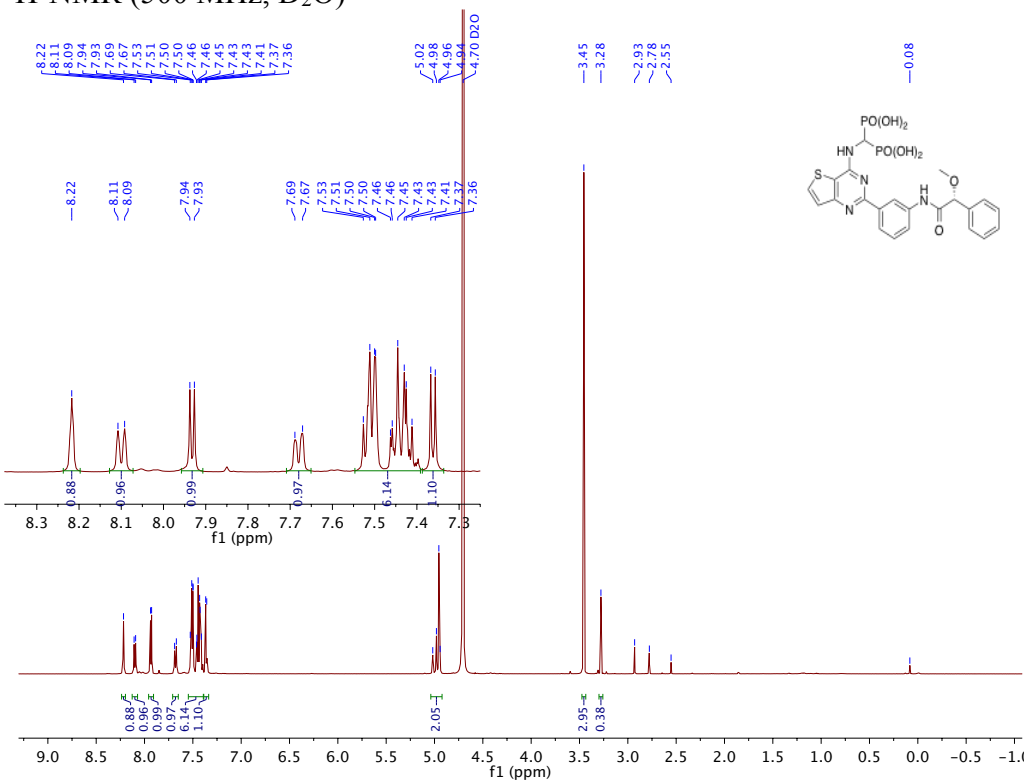


^{13}C NMR (126 MHz, D_2O)

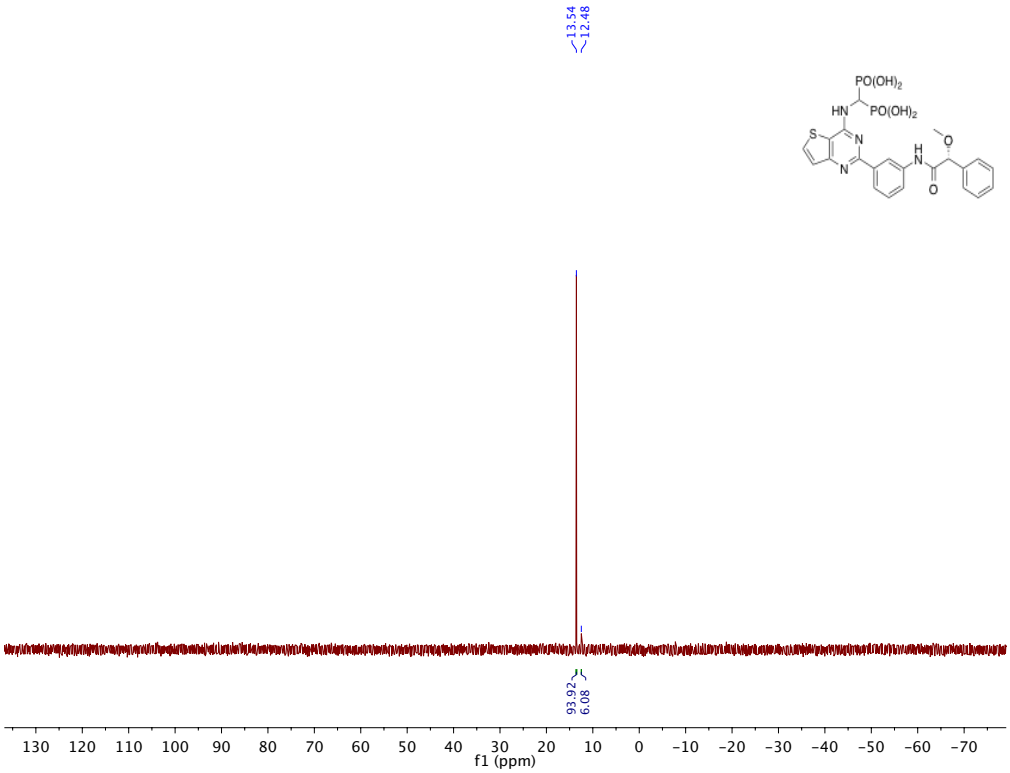


Inhibitor **2.48**

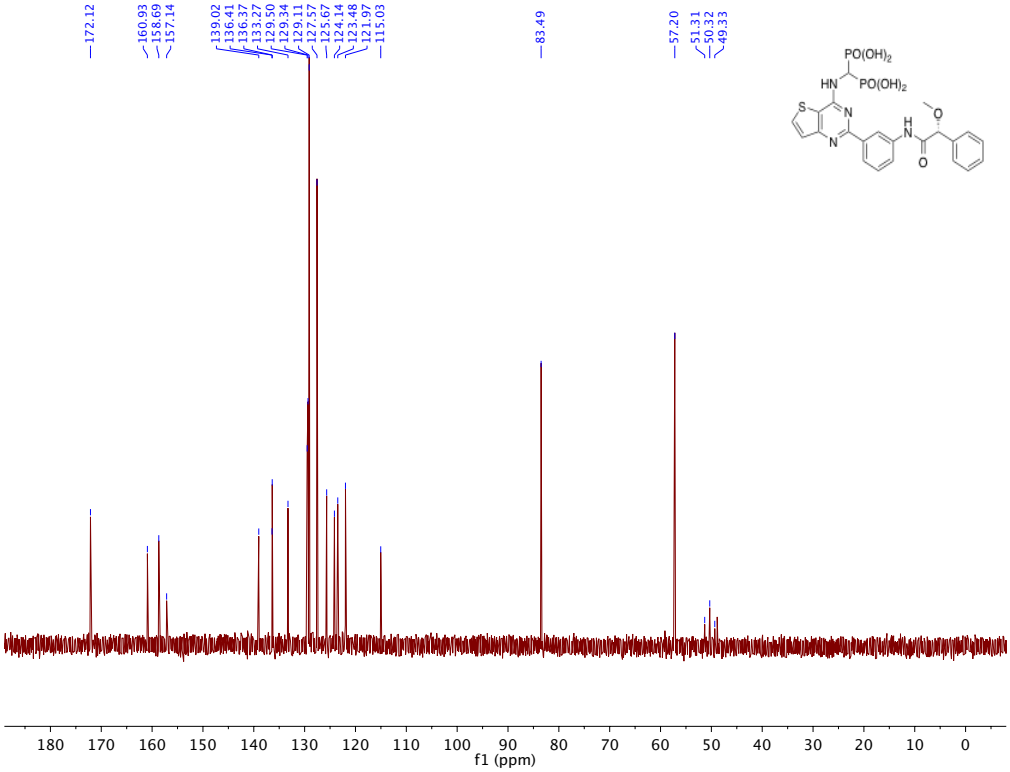
^{1}H-NMR (500 MHz, D_2O)



^{31}P NMR (203 MHz, D_2O)

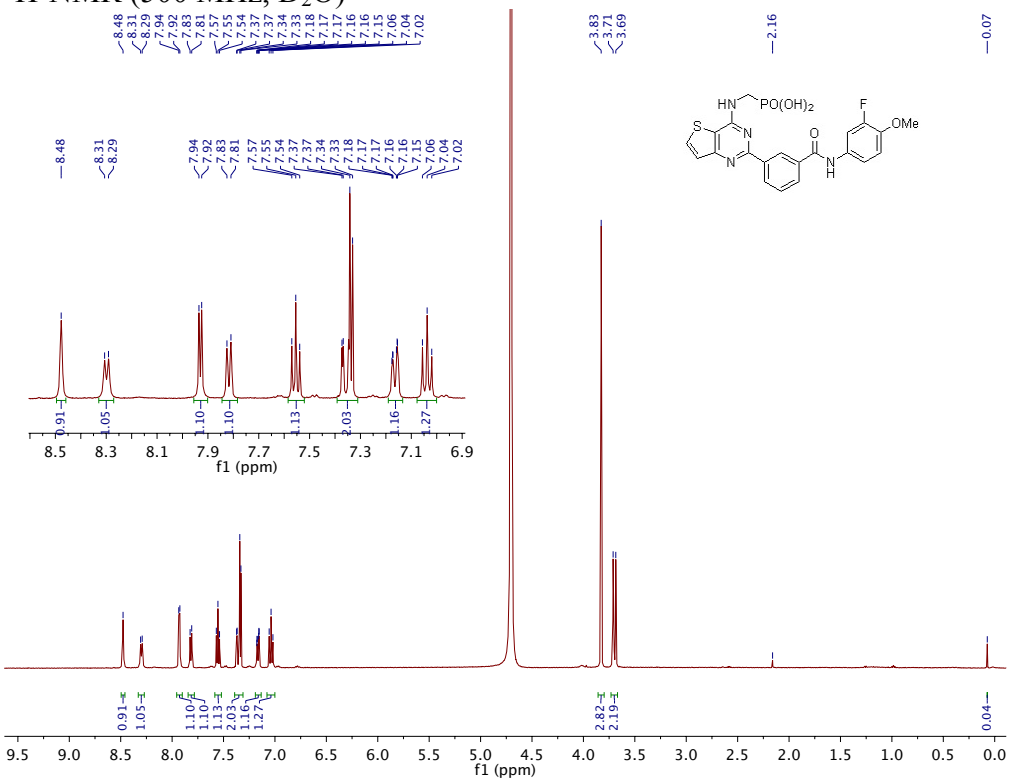


^{13}C NMR (126 MHz, D_2O)

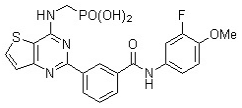


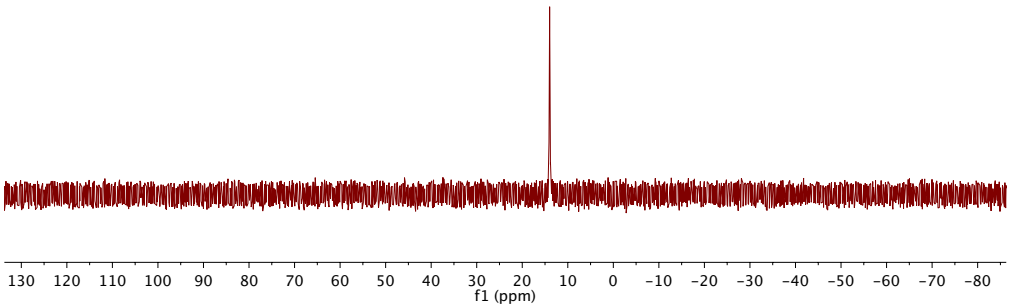
Inhibitor **2.53**

^{1}H-NMR (500 MHz, D_2O)

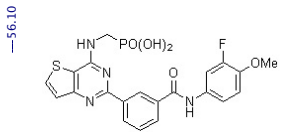


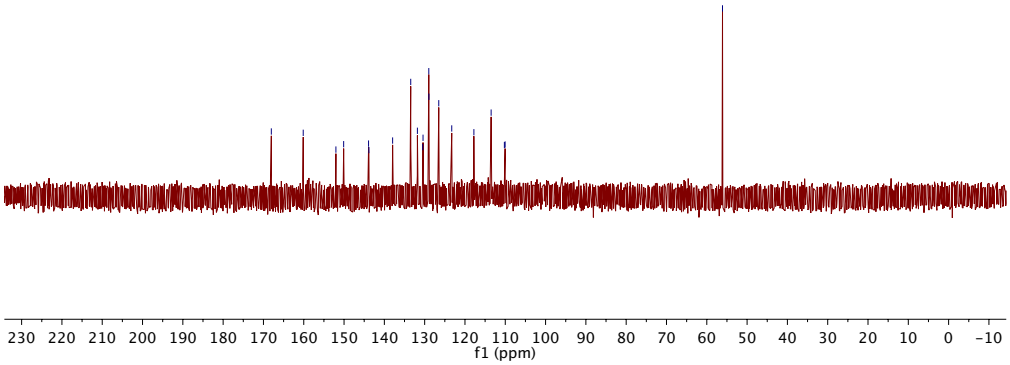
^{31}P NMR (203 MHz, D_2O)





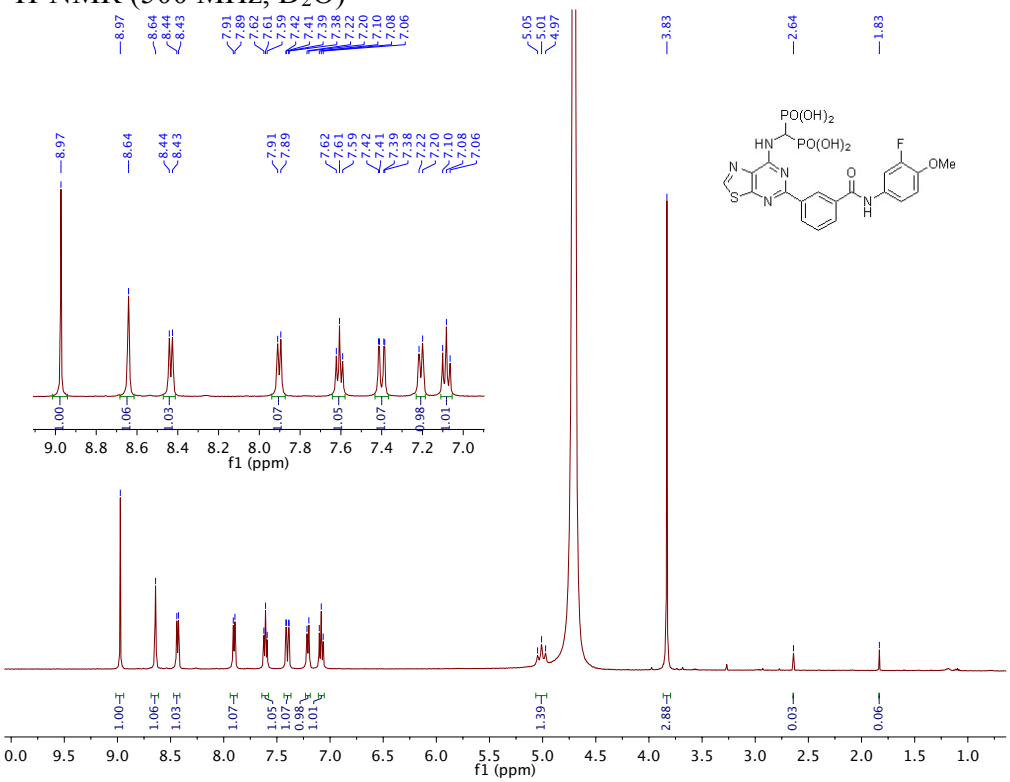
^{13}C NMR (126 MHz, D_2O)



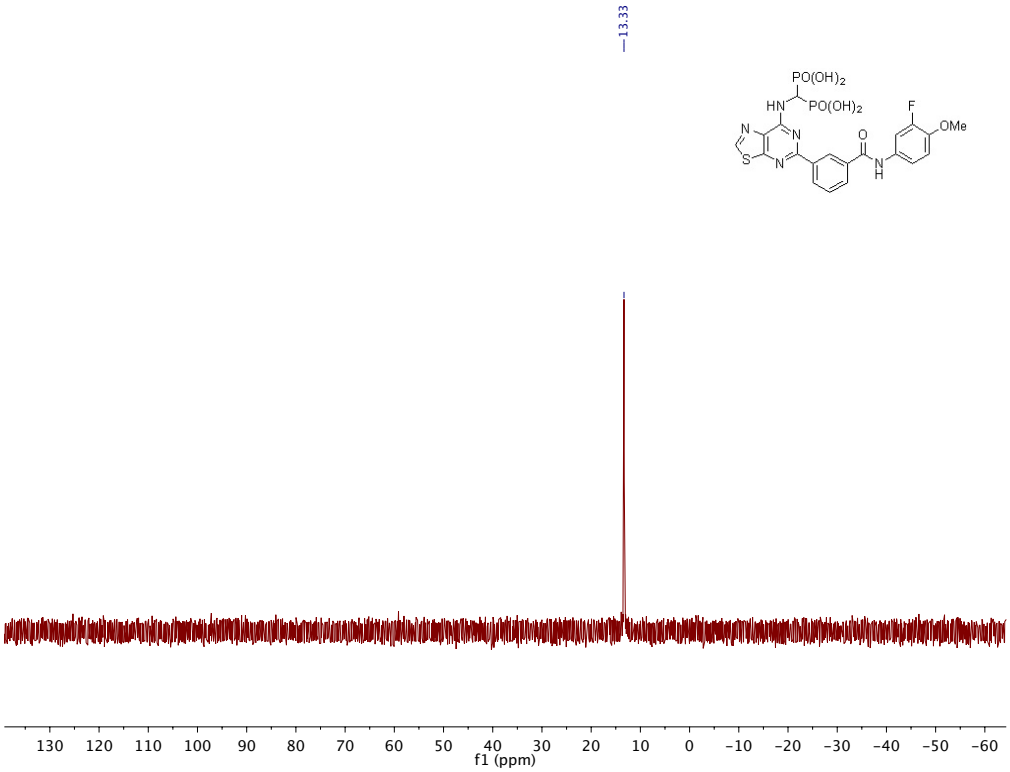


Inhibitor **2.62**

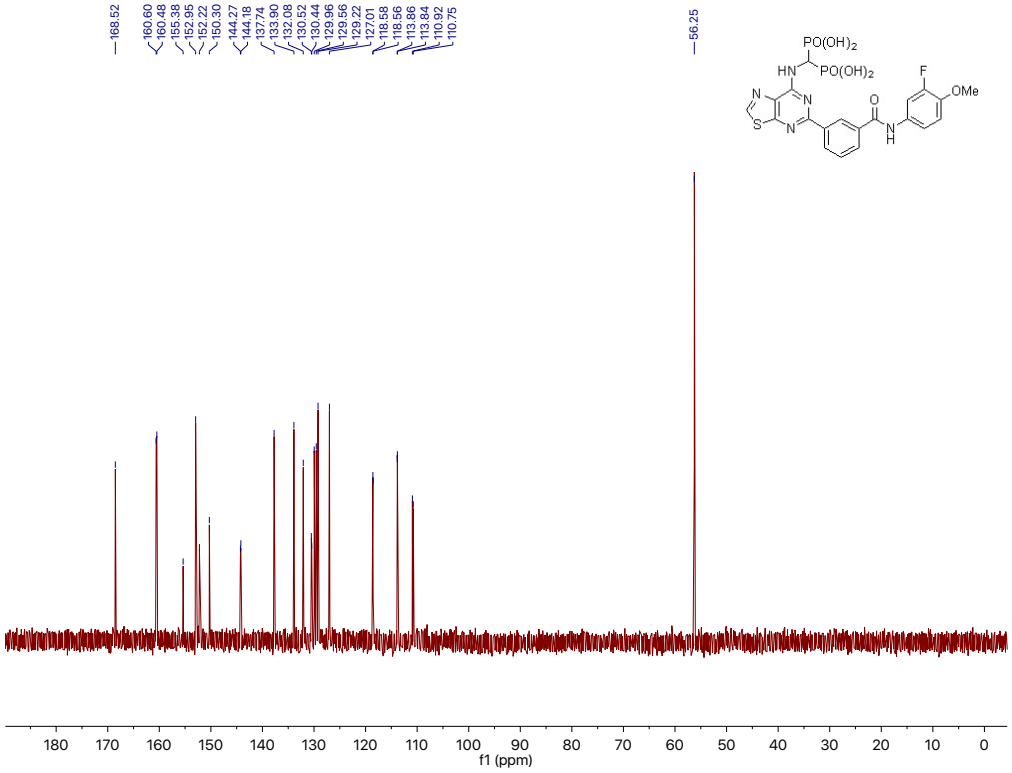
^{1}H-NMR (500 MHz, D_2O)



^{31}P NMR (203 MHz, D_2O)



^{13}C NMR (126 MHz, D_2O)

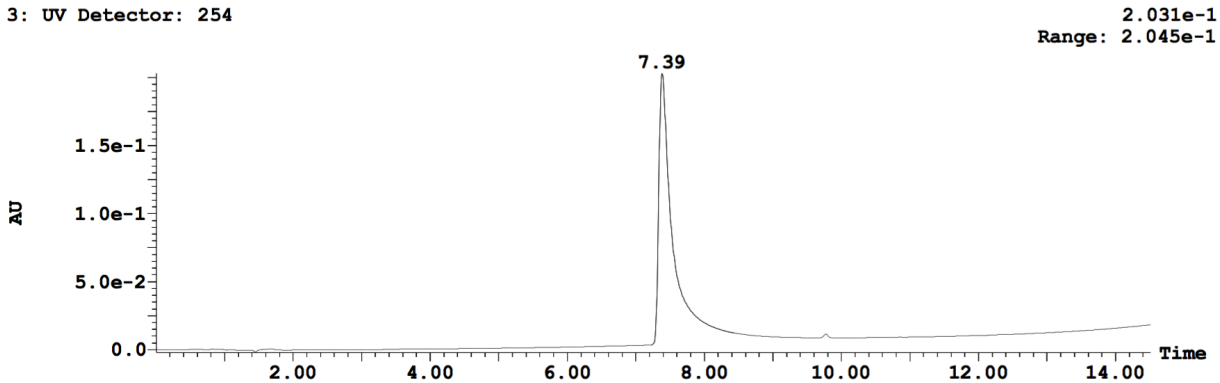


Appendix II

HPLC UV trace of final compounds

Inhibitor **2.07**

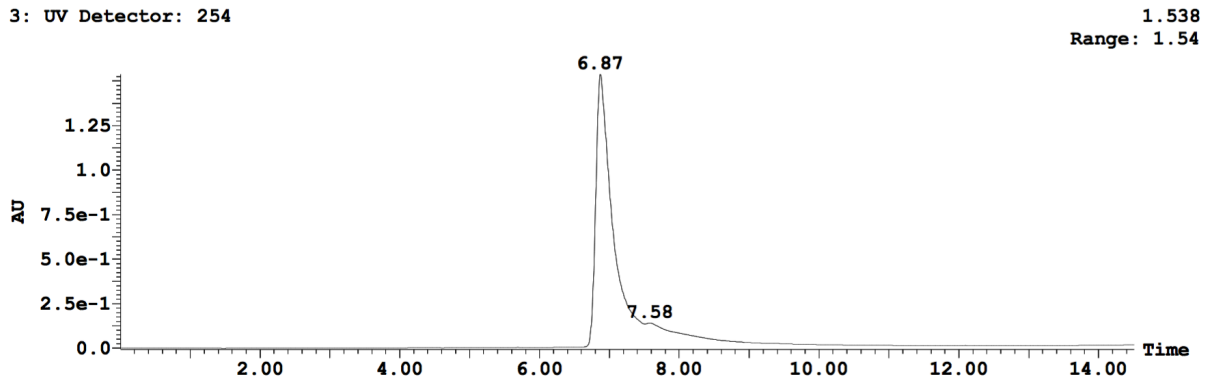
Tsantrizos Lab Page 1
LC-MS Sample Report for Felix
Sample: 1 Vial:1:A,5 ID:FV-01-09
File:FV-01-09 Date:06-Feb-2018 Time:15:57:33
Description: Method:C:\MassLynx\Atl_C18_15min_H2O_ACN_FA_NO_MS.olp



Peak Number	Time	AreaAbs	Area %Total	Height	Mass Found
1	7.39	46574	100.00	198415	

Inhibitor **2.09**

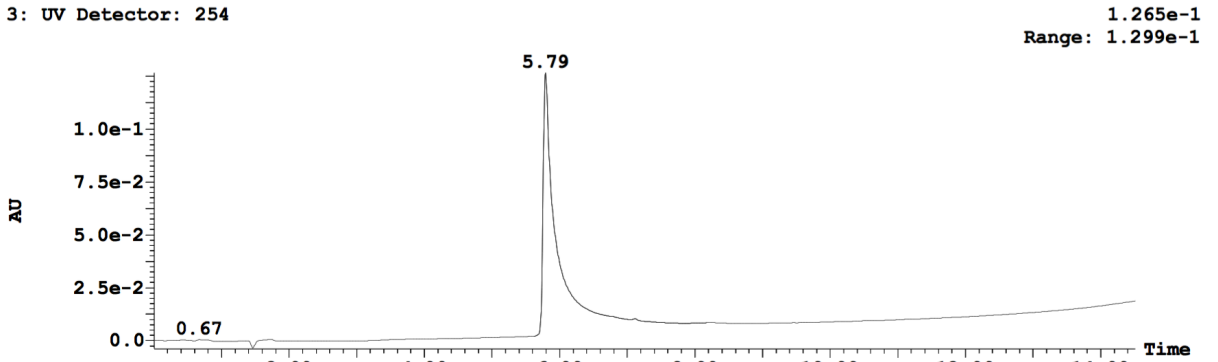
Tsantrizos Lab Page 1
LC-MS Sample Report for Felix
Sample: 1 Vial:1:B,9 ID:FV-01-10
File:FV-01-10 Date:07-Feb-2018 Time:19:42:56
Description: Method:C:\MassLynx\Atl_C18_15min_H2O_ACN_FA_NO_MS.olp



Peak Number	Time	AreaAbs	Area %Total	Height	Mass Found
1	6.87	432472	86.15	1528075	
2	7.58	69530	13.85	123447	

Inhibitor **2.20**

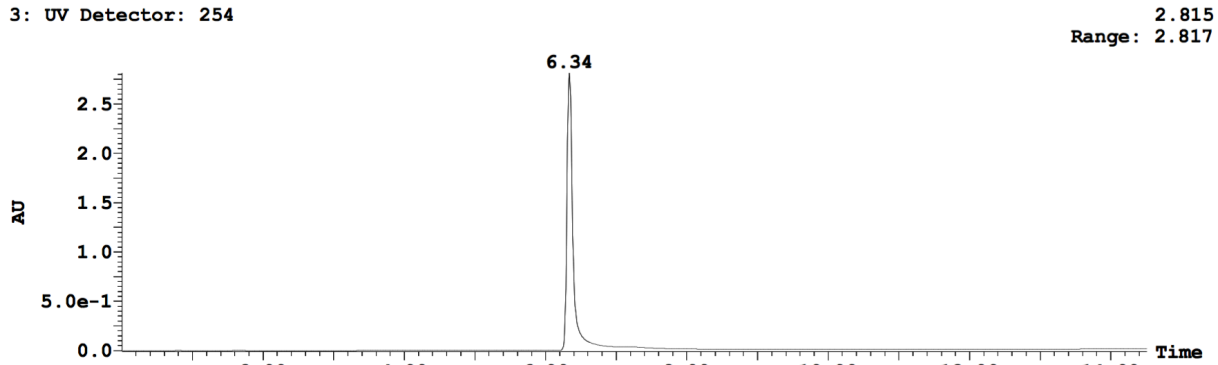
Tsantrizos Lab Page 1
LC-MS Sample Report for Felix
Sample: 1 Vial:1:A,1 ID:FV-01-63
File:FV-01-63 Date:23-Oct-2017 Time:19:06:35
Description: Method:C:\MassLynx\Atl_C18_15min_H2O_ACN_FA_NO_MS.olp



Peak Number	Time	AreaAbs	Area %Total	Height	Mass Found
2	5.79	30738	94.57	123864	
3	7.11	1764	5.43	4557	

Inhibitor **2.22**

Tsantrizos Lab Page 1
LC-MS Sample Report for Felix
Sample: 1 Vial:1:A,4 ID:FV-01-84
File:FV-01-84 Date:05-Jul-2017 Time:10:20:25
Description: Method:C:\MassLynx\Atl_C18_Pos_Neg_15min_H2O_ACN_FA.olp

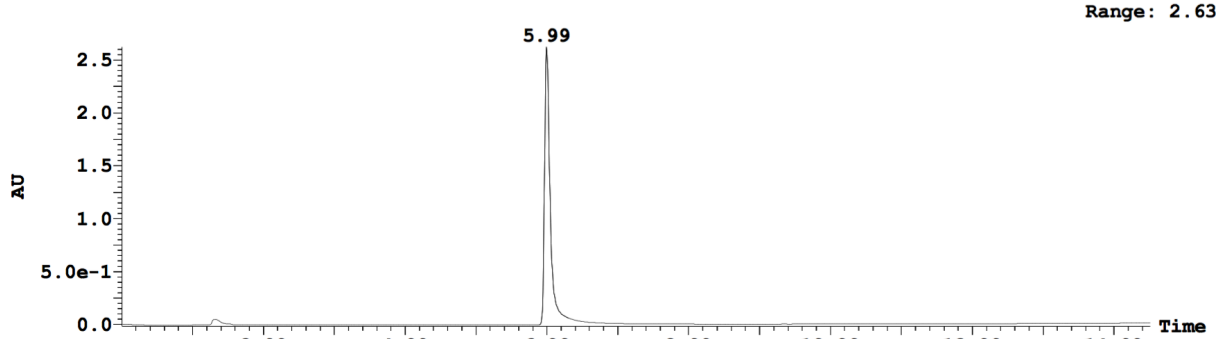


Peak Number	Time	AreaAbs	Area %Total	Height	Mass Found
1	6.34	257691	100.00	2789467	

Inhibitor **2.24**

Tsantrizos Lab Page 1
LC-MS Sample Report for Felix
Sample: 1 Vial:1:A,7 ID:fv-02-116
File:fv-02-116 Date:26-Apr-2018 Time:16:43:18
Description: Method:C:\MassLynx\Atl_C18_Pos_Neg_15min_H2O_ACN_FA.olp

3: UV Detector: 254 2.622
Range: 2.63

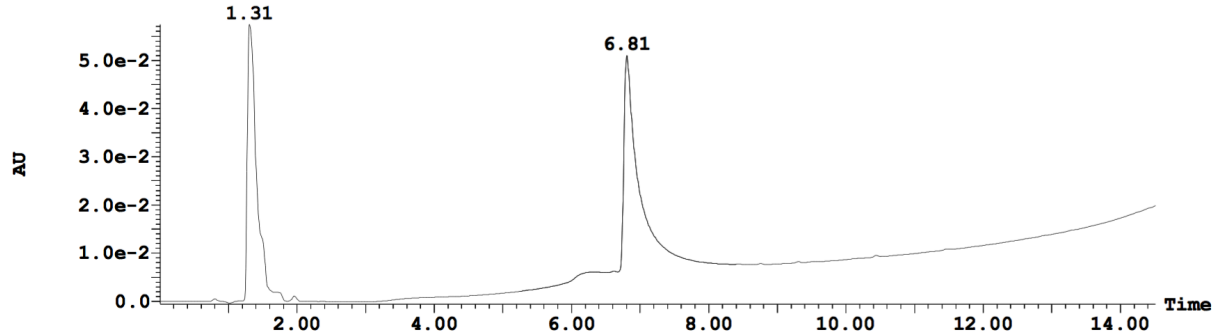


Peak Number	Time	AreaAbs	Area %Total	Height	Mass Found
3	5.99	235199	100.00	2609635	

Inhibitor **2.26**

Tsantrizos Lab Page 1
LC-MS Sample Report for Felix
Sample: 2 Vial:1:A,8 ID:fv-02-117
File:fv-02-117 Date:26-Apr-2018 Time:17:03:25
Description: Method:C:\MassLynx\Atl_C18_Pos_Neg_15min_H2O_ACN_FA.olp

3: UV Detector: 254 5.744e-2
Range: 5.784e-2

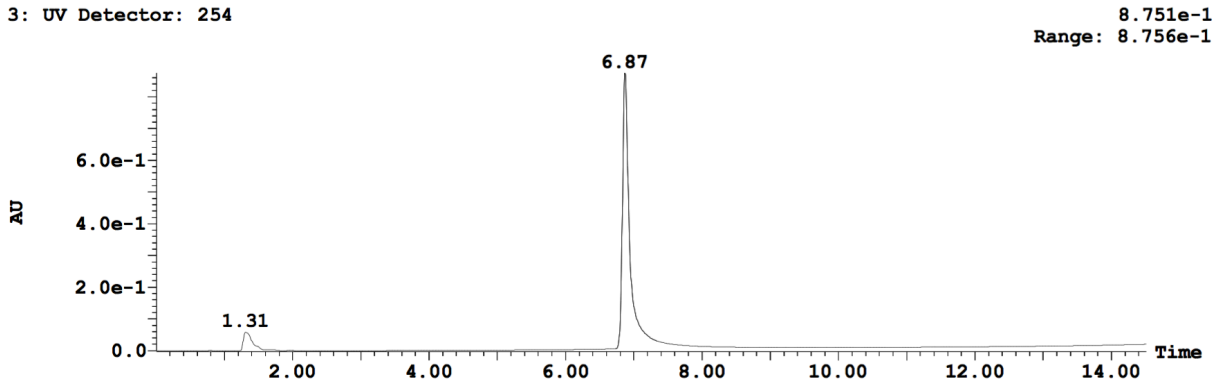


Peak Number	Time	AreaAbs	Area %Total	Height	Mass Found
2	6.32	1174	8.03	2062	
3	6.62	192	1.31	1777	
4	6.81	13255	90.65	46126	

Inhibitor **2.28**

Tsantrizos Lab Page 1
LC-MS Sample Report for Felix

Sample: 3	Vial:1:A,9	ID:fv-02-118
File:fv-02-118	Date:26-Apr-2018	Time:17:22:59
Description:	Method:C:\MassLynx\Atl_C18_Pos_Neg_15min_H2O_ACN_FA.olp	

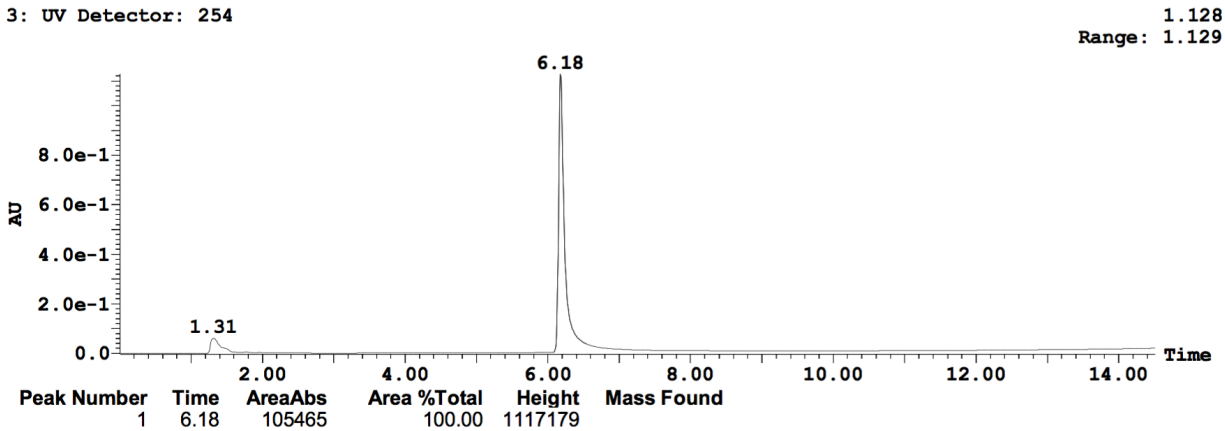


Peak Number	Time	AreaAbs	Area %Total	Height	Mass Found
1	6.87	102816	100.00	863992	

Inhibitor **2.30**

Tsantrizos Lab Page 1
LC-MS Sample Report for Felix

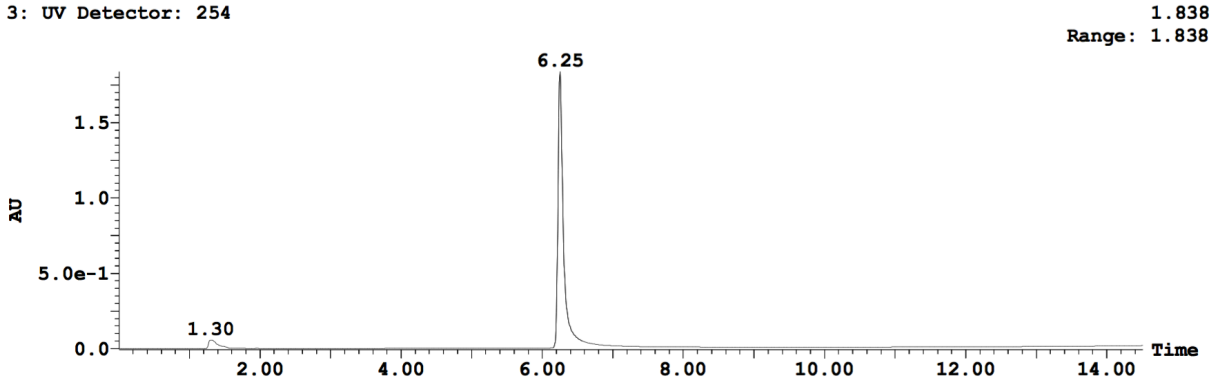
Sample: 2	Vial:1:B,8	ID:fv-02-120
File:fv-02-120	Date:27-Apr-2018	Time:16:47:20
Description:	Method:C:\MassLynx\Atl_C18_Pos_Neg_15min_H2O_ACN_FA.olp	



Peak Number	Time	AreaAbs	Area %Total	Height	Mass Found
1	6.18	105465	100.00	1117179	

Inhibitor **2.32**

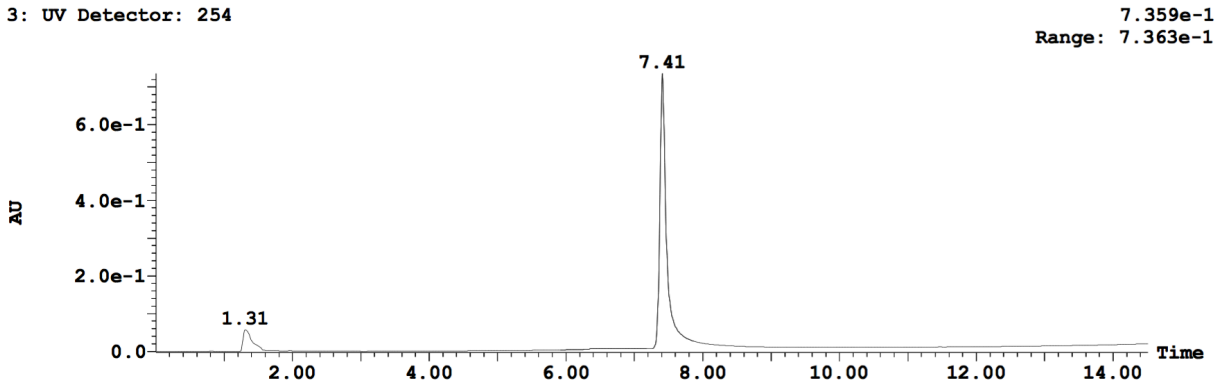
Tsantrizos Lab Page 1
LC-MS Sample Report for Felix
Sample: 3 Vial:1:B,9 ID:fv-02-121
File:fv-02-121 Date:27-Apr-2018 Time:17:06:55
Description: Method:C:\MassLynx\Atl_C18_Pos_Neg_15min_H2O_ACN_FA.olp



Peak Number	Time	AreaAbs	Area %Total	Height	Mass Found
2	6.25	156091	100.00	1821114	

Inhibitor **2.34**

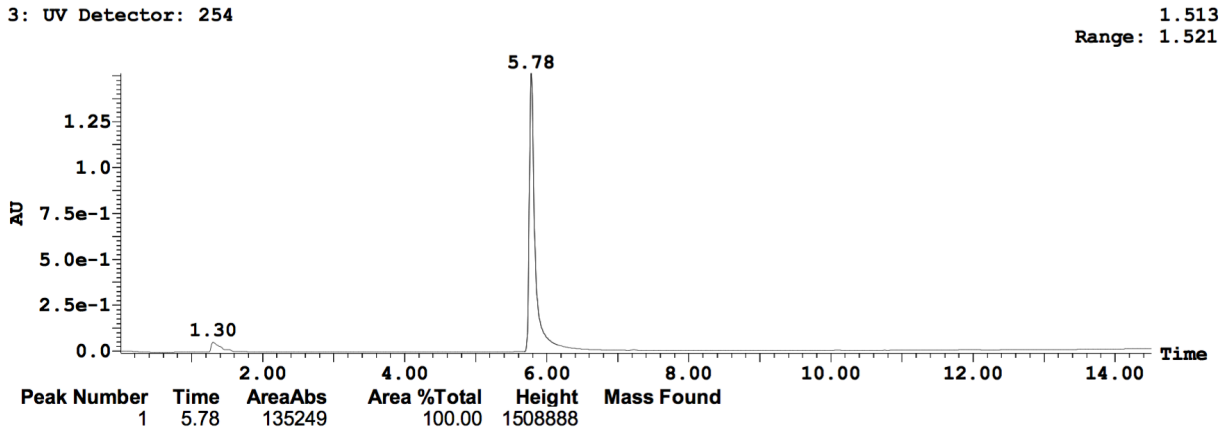
Tsantrizos Lab Page 1
LC-MS Sample Report for Felix
Sample: 4 Vial:1:B,10 ID:fv-02-122
File:fv-02-122 Date:27-Apr-2018 Time:17:26:30
Description: Method:C:\MassLynx\Atl_C18_Pos_Neg_15min_H2O_ACN_FA.olp



Peak Number	Time	AreaAbs	Area %Total	Height	Mass Found
7	6.60	1045	1.30	1719	
13	7.41	79574	98.70	723573	

Inhibitor **2.36**

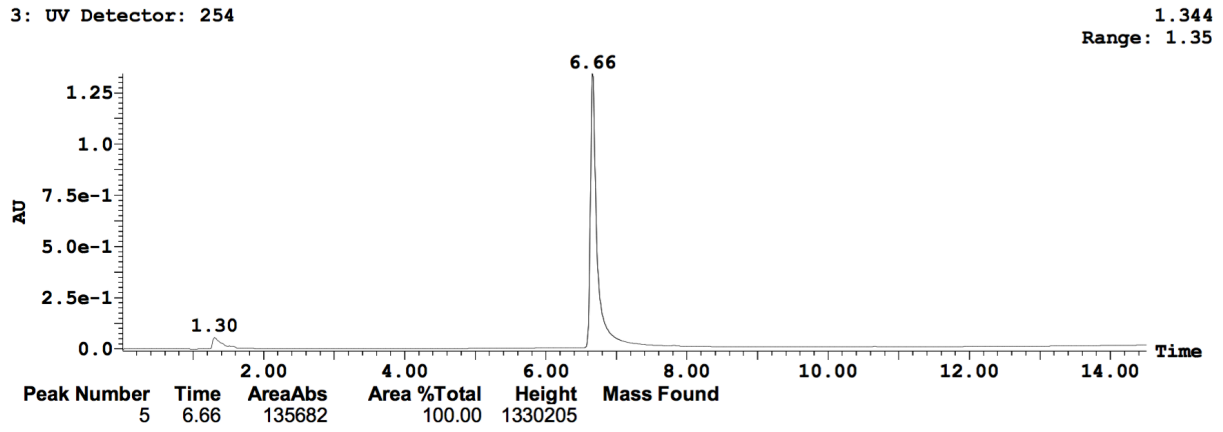
Tsantrizos Lab Page 1
LC-MS Sample Report for Felix
Sample: 1 Vial:1:C,12 ID:fv-02-131
File:fv-02-131 Date:28-May-2018 Time:15:00:25
Description: Method:C:\MassLynx\Atl_C18_15min_H2O_ACN_FA_NO_MS.olp



Peak Number	Time	AreaAbs	Area %Total	Height	Mass Found
1	5.78	135249	100.00	1508888	

Inhibitor **2.38**

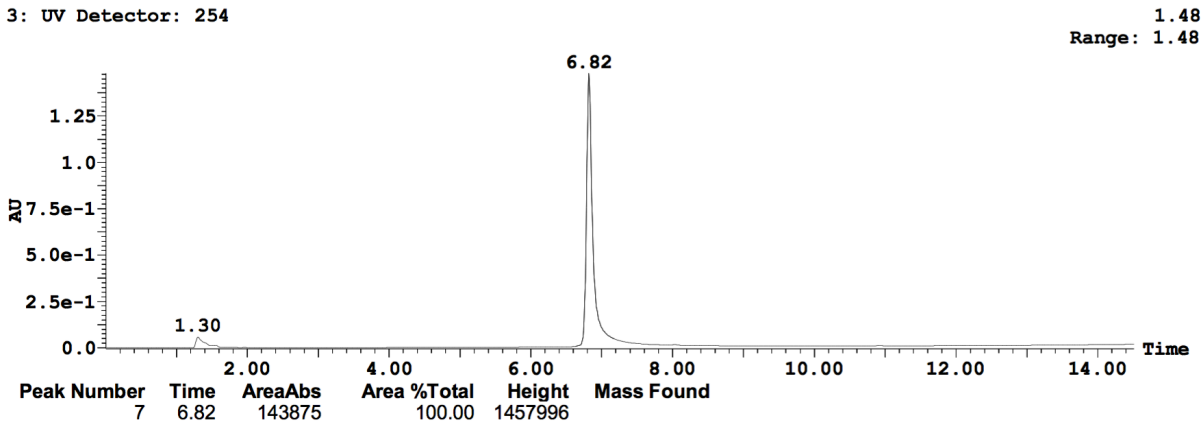
Tsantrizos Lab Page 1
LC-MS Sample Report for Felix
Sample: 2 Vial:1:D,1 ID:fv-02-132
File:fv-02-132 Date:28-May-2018 Time:15:23:32
Description: Method:C:\MassLynx\Atl_C18_15min_H2O_ACN_FA_NO_MS.olp



Peak Number	Time	AreaAbs	Area %Total	Height	Mass Found
5	6.66	135682	100.00	1330205	

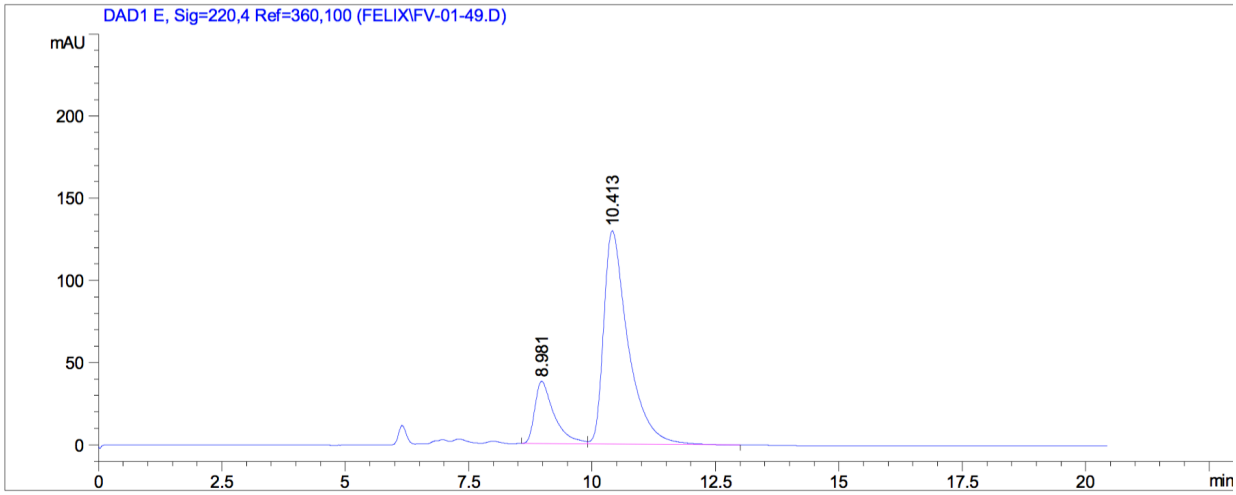
Inhibitor **2.40**

Tsantrizos Lab Page 1
LC-MS Sample Report for Felix
Sample: 3 Vial:1:D,2 ID:fv-02-133
File:fv-02-133 Date:28-May-2018 Time:15:43:08
Description: Method:C:\MassLynx\Atl_C18_15min_H2O_ACN_FA_NO_MS.olp



Peak Number	Time	AreaAbs	Area %Total	Height	Mass Found
7	6.82	143875	100.00	1457996	

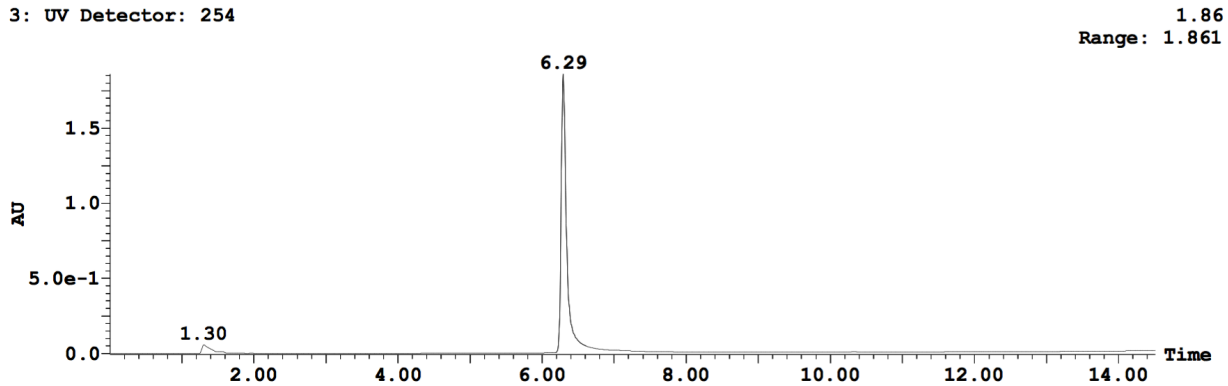
Precursor to **2.46** (**2.45** bisphosphonate ester) Chiral HPLC



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.981	BV	0.3969	1027.06641	37.81532	18.7118
2	10.413	VB	0.5035	4461.80713	129.63609	81.2882

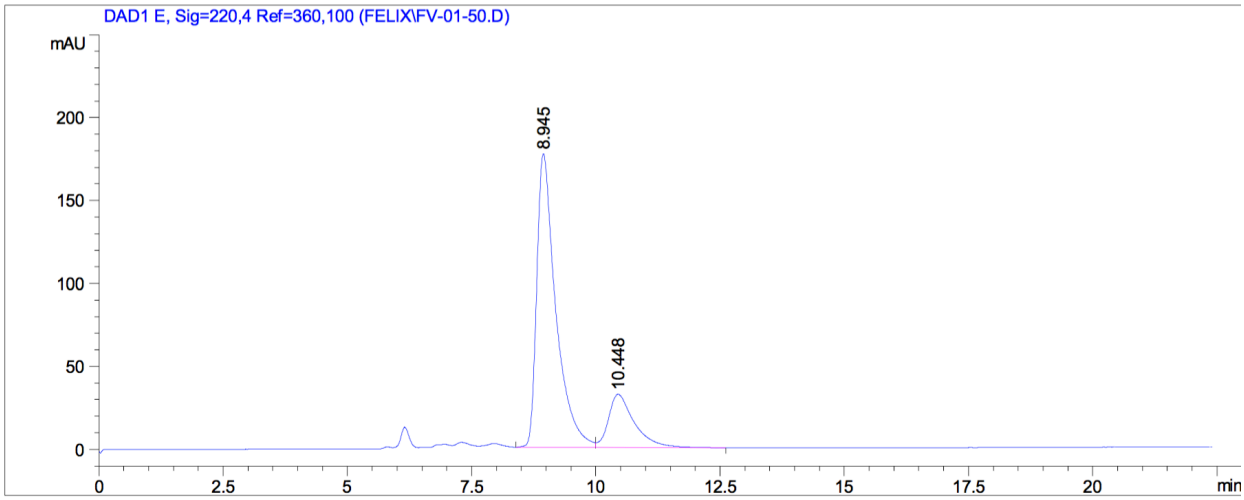
Inhibitor **2.46**

Tsantrizos Lab Page 1
LC-MS Sample Report for Felix
Sample: 4 Vial:1:D,3 ID:fv-01-64
File:fv-01-64 Date:28-May-2018 Time:16:02:43
Description: Method:C:\MassLynx\Atl_C18_15min_H2O_ACN_FA_NO_MS.olp



Peak Number	Time	AreaAbs	Area %Total	Height	Mass Found
3	6.29	156183	100.00	1842999	

Precursor to **2.48** (**2.47** bisphosphonate ester) Chiral HPLC

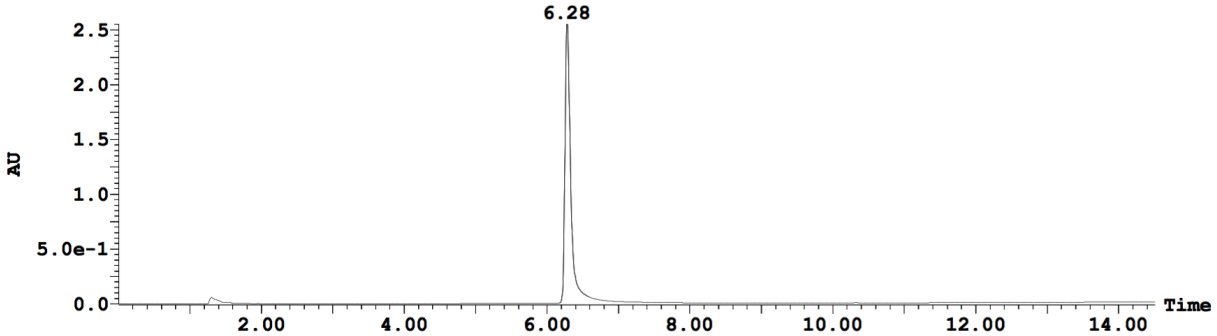


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.945	BV	0.3882	4704.65674	176.95755	80.1734
2	10.448	VB	0.5269	1163.44714	32.09912	19.8266

Inhibitor **2.48**

Tsantrizos Lab Page 1
LC-MS Sample Report for Felix
Sample: 5 Vial:1:D,4 ID:fv-01-65
File:fv-01-65 Date:28-May-2018 Time:16:22:18
Description: Method:C:\MassLynx\Atl_C18_15min_H2O_ACN_FA_NO_MS.olp

3: UV Detector: 254 2.552
Range: 2.552

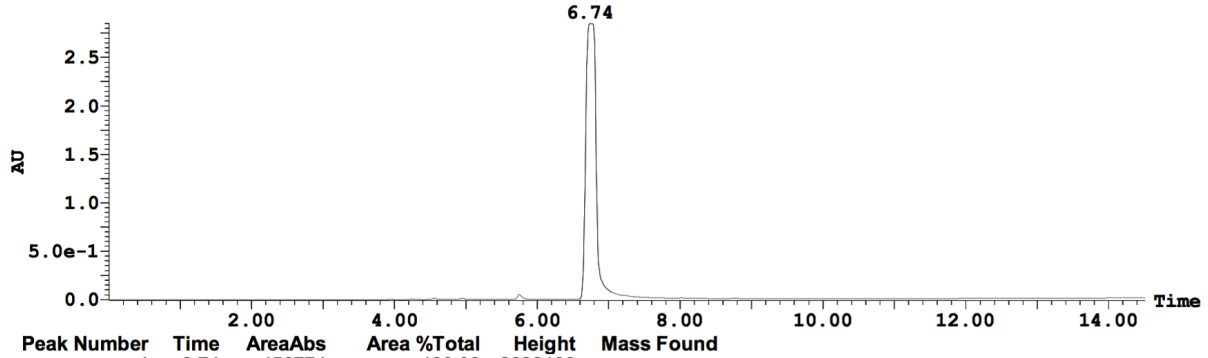


Peak Number	Time	AreaAbs	Area %Total	Height	Mass Found
2	6.28	226788	100.00	2529304	

Inhibitor **2.53**

Tsantrizos Lab Page 1
LC-MS Sample Report for Felix
Sample: 3 Vial:1:A,7 ID:FV-01-108
File:FV-01-108 Date:06-Feb-2018 Time:16:36:52
Description: Method:C:\MassLynx\Atl_C18_15min_H2O_ACN_FA_NO_MS.olp

3: UV Detector: 254 2.85
Range: 2.851



Peak Number	Time	AreaAbs	Area %Total	Height	Mass Found
4	6.74	456774	100.00	2822189	

Inhibitor **2.62**

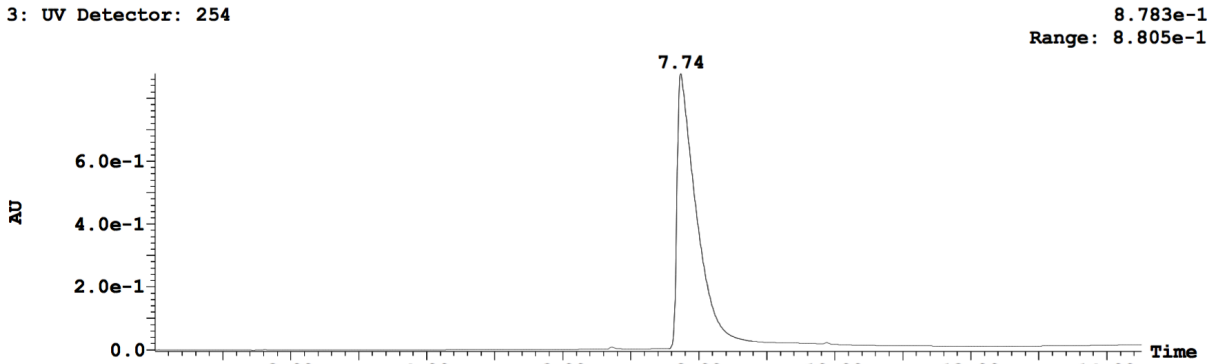
Tsantrizos Lab Page 1

LC-MS Sample Report for Felix

Sample: 1 Vial:1:H,7 ID:FV-01-117

File:FV-01-117 Date:20-Jan-2018 Time:11:41:15

Description: Method:C:\MassLynx\Atl_C18_15min_H2O_ACN_FA_NO_MS.olp



Peak Number	Time	AreaAbs	Area %Total	Height	Mass Found
2	7.74	282141	100.00	871244	