University of Sussex

A CONVENIENT SYNTHESIS OF BIOACTIVE CYCLOHEXENEPHOSPHONATES

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Abbreviations

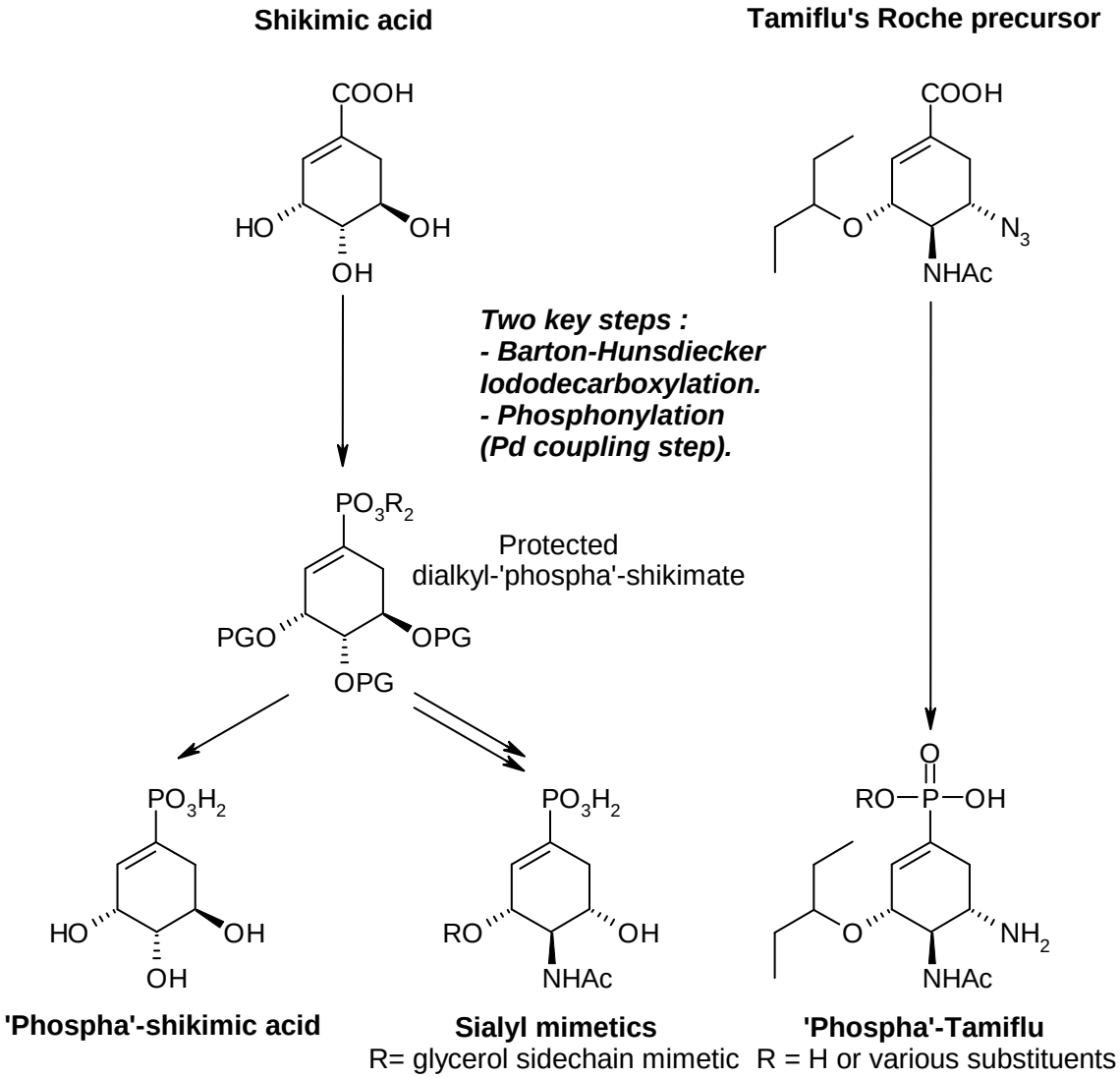
Ac	Acetyl
AcOH	Acetic acid
AIBN	Azobisisobutyronitrile
Ala	Alanine
Arg	Arginine
Asp	Aspartic acid
Bn	Benzyl
BnBr	Benzyl bromide
Boc	*tert*-Butyloxycarbonyl
Boc-ON	2-(*tert*-Butoxycarbonyloxyimino)-2-phenylacetonitrile
n-Bu_3SnH	*tri*-*n*-Butyltin hydride
Cat.	Catalytic
DABCO	1,4-Diazabicyclo[2.2.2]octane
DANA	2-Deoxy-2,3-dehydro-*N*-acetylneuraminic acid
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
DIAD	Diisopropyl azodicarboxylate
DMAP	4-Dimethylaminopyridine
DMF	Dimethyl formamide
DMP	2,2-Dimethoxypropane
Dpephos	Bis(2-diphenylphosphinophenyl)ether
DPPA	Diphenylphosphoryl azide
dppf	1,1’-Bis(diphenylphosphino)ferrocene
EA	Ethyl acetate
Glu	Glutamic acid
gpc	gel permeation chromatography
HA	Hemagglutinin
His	Histidine
HR-ESI-MS	High resolution electro-spray ionisation mass spectroscopy
IBX	*o*-Iodoxybenzoic acid
Ile	Isoleucine

IMP	Inosine monophosphate
J	Coupling constant (Hz)
KSAc	Potassium thioacetate
LHMDS	Lithium bis(trimethylsilyl)amide
Me	Methyl
MeOH	Methanol
Me_3P	Trimethyl phosphine
MES	4-Morpholinoethanesulfonic acid
MOM	Methoxymethyl
MOMCl	Methoxymethyl chloride
Ms	Mesylate
MUNANA	2'-(4-Methylumbelliferyl)-α-D-*N*-acetylneuraminic acid
NA	Neuraminidase
NaCl	Sodium chloride
NADP	Nicotinamide adenine dinucleotide phosphate
$NaHCO_3$	Sodium hydrogenocarbonate
NaOH	Sodium hydroxide
NBA	*N*-bromoacetamide
NEt_3	Triethylamine
NH_4Cl	Ammonium chloride
NMR	Nuclear magnetic resonance
PhSH	Thiophenol
$P(OMe)_3$	Trimethylphosphite
p-TsOH	*para*-toluene sulfonic acid
RNA	Ribonucleic acid
RT	Room temperature
SA	Sialic acid
SDH	Shikimate dehydrogenase
Ser	Serine
S_N2	Bimolecular nucleophilic substitution
TBAI	*tetra*-*n*-Butyl ammonium iodide
TBAF	*tetra*-*n*-Butyl ammonium fluoride
TBDMS	*tert*-Butyldimethylsilyl
TBDPS	*tert*-Butyldiphenylsilyl

THF	Tetrahydrofuran
TIPS	Triisopropylsilyl
TFA	Trifluoroacetic acid
TLC	Thin layer chromatography
TMSBr	Trimethylsilyl bromide
Tol	Toluene
Tr	Trityl
TS	Trans sialidase
TsCl	*para*-Toluene sulfonylchloride
Tyr	Tyrosine
UV	Ultraviolet

Abstract

Influenza virus infection and the shikimic acid pathway are two of many examples of microbe-host interactions and microbial biosynthetic pathways that are interesting for investigation by means of small molecules. A particularly interesting structural motif common to both is the cyclohexenecarboxylic acid. In the former, this structural motif has been employed as a mimetic of the sialyl cation intermediate and forms the scaffold of the anti-influenza drug and neuraminidase inhibitor Oseltamivir (or Tamiflu^{TM}). In the latter pathway, crucial modifications towards aromatic amino acids are carried out via shikimic acid, a cyclohexenecarboxylic acid, as a substrate.



Scheme 1. Strategic overview for the synthesis of important ‘phospha’-isosteres.

A straightforward method to replace the carboxylate moiety in such structures with a phosphonate would provide access to a wide variety of mimetics, for instance monoesters, that still retain a negative charge under physiological conditions usually required for bioactivity (Scheme **1**).

The aim of this research project, as presented in Scheme 1, was to develop an efficient synthesis of the cyclohexenephosphonate scaffold from chiral pool precursors via two key steps, a Hunsdiecker-Barton iododecarboxylation followed by a palladium-mediated coupling step to introduce the phosphonate moiety, thus giving a convenient access to interesting bioactive molecules. This approach has successfully been applied to the shikimic acid to afford ‘phospha’-shikimic acids and 3-dehydro-‘phospha’-shikimic acids, and further development of this strategy has led to the synthesis of ‘phospha’-Tamiflu and its derivatives from an Oseltamivir precursor.

Towards a convenient synthesis of bioactive cyclohexenephosphonates

I.1. Theoretical part

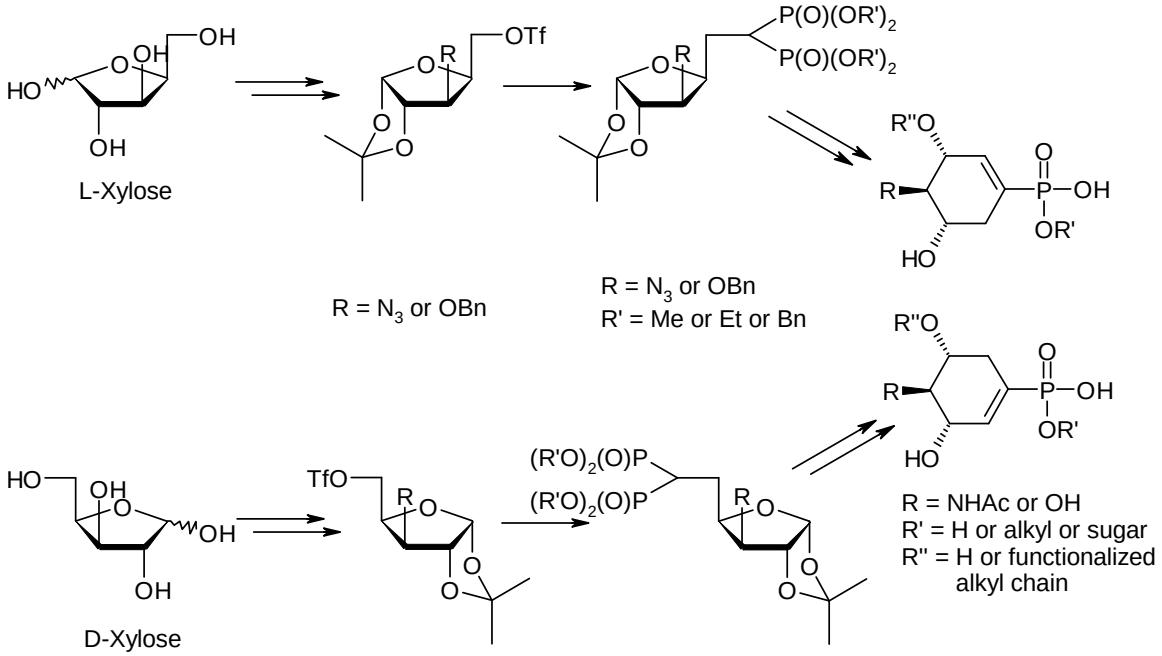
I.1.1. Previous work

Work by Streicher *et al.*[1-6] has been focused on the synthesis of carbocyclic sialylmimetics and more specifically the use of cyclohexenephosphonates as scaffolds for sialidase inhibitor librairies, mainly oriented towards the inhibition of parasitic and bacterial sialidases. These cyclohexenephosphonates, which retain the half-chair conformation of the sialidase reaction transition state and retain a negative charge required for recognition by the enzyme, allow attachment of a spacer molecule or additional sugar moieties, which is of importance due to the more complex functionality of bacterial or protozoal sialidases in contrast to the influenza sialidase for which very potent inhibitors have already been developed.

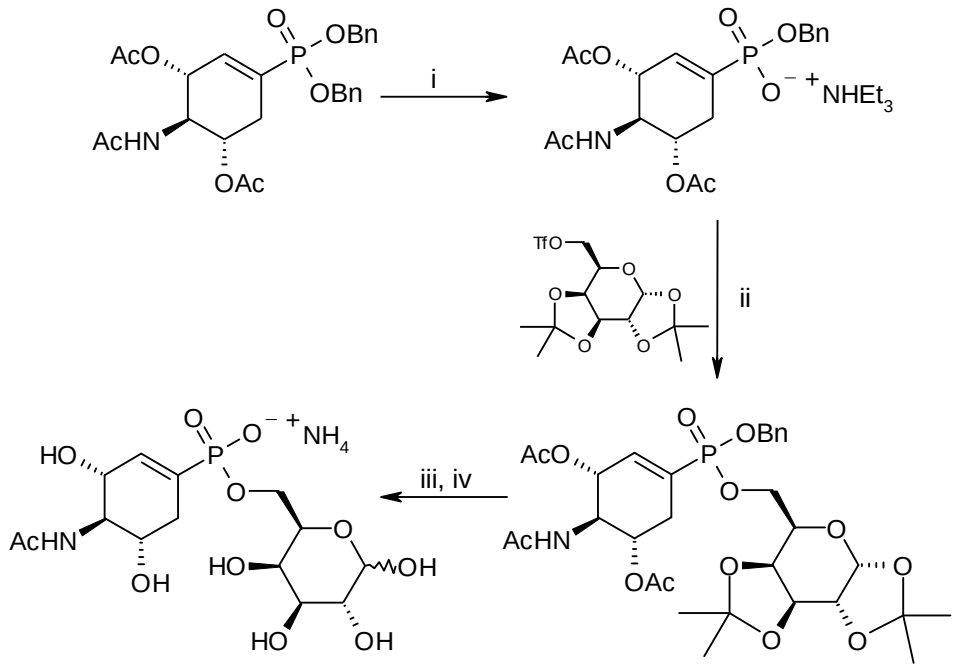
A strategy was developed to synthesize D- and L-*xylo* configured cyclohexenephosphonates from D- and L-xylose respectively, by chain elongation and cyclization utilizing an intramolecular Horner-Wadsworth-Emmons-type condensation as outlined in Scheme **2**.[6] This methodology was inspired by the synthesis of shikimic acid and analogs introduced by both Fleet *et al.*[7, 8] (synthesis of (-)-shikimic acid from D-mannose) and Vasella and coworkers [9, 10] (synthesis of (-)-shikimic acid from D-lyxose).

This synthetic approach towards cyclohexenephosphonates allowed further exploration of the structural space beyond the sialidase active site and was used for the design and synthesis of novel sialidase inhibitor libraries. It led to the synthesis of diethyl phosphonates,[1-3] which was then extended to the synthesis of dibenzyl and dimethyl phosphonates.[4, 5] These allowed, after partial saponification with thiophenol and triethylamine, the synthesis of mixed diesters containing aliphatic moieties or aglycon mimetics through a Mitsunobu condensation or alkylation with suitable triflates. Thus, introduction of a hydrophobic group or a sugar moiety to the phosphonate were

successfully achieved.[4, 5] The synthesis of pseudo-sialosides via di-benzyl phosphonates[4] is shown in Scheme **3** below.



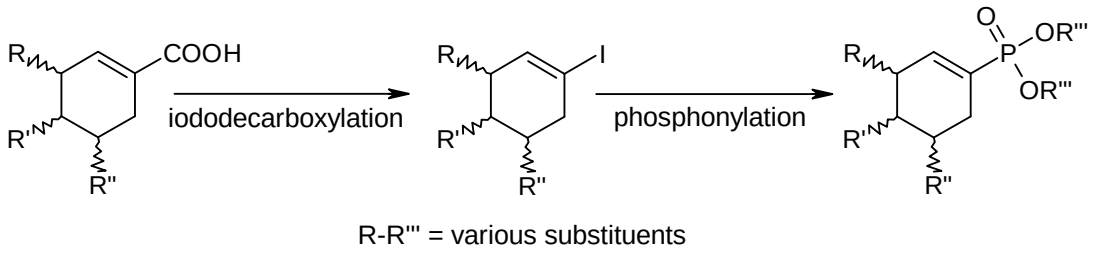
Scheme 2. Previous group work:
Synthesis of D- and L-*xylo* configured cyclohexephosphonates from D- and L-Xylose.



Scheme 3. Strategy for the synthesis of mixed diester phosphonates:
Reagents & conditions: (i) $PhSH/NEt_3$; (ii) sugar triflate, DMF; (iii) $H_2/Pd/C$ then $NH_3/MeOH$; (iv) CF_3COOH.

One shortcoming of the routes above (Scheme 3) is the relatively high total number of synthetic steps from the chiral-pool starting material, leading to moderate overall yields. A new synthetic approach towards phospha-isosteres of cyclohexenylcarboxylic acids, as described there-in, is the direct replacement of the carboxylic acid moiety by the phosphonic acid moiety. The most effective way to achieve this utilizes a halodecarboxylation of the carboxylic acid followed by a conversion of the resulting vinyl halide into a vinyl phosphonate diester. An overview of the halodecarboxylation and the phosphonylation steps is presented below.

I.1.2. Barton-Hunsdiecker's iododecarboxylation and Hirao's coupling reaction



Scheme 4. Introduction to the iododecarboxylation and phosphonylation steps.

I.1.2.1. *The halodecarboxylation reaction*

The generation of carboxyl radicals and trapping of the ensuing alkyl radicals by various radical-trapping agents allows the transformation of carboxylic acids into a range of diverse functional groups. These reactions are known as reductive decarboxylation, oxidative decarboxylation, decarboxylative halogenation, chalcogenation, phosphorylation, oxygenation and amination and decarboxylation with subsequent C-C bond formation. Different methods exist for the generation of carboxyl radicals obtained by homolytic cleavage from suitable precursors containing a weak carboxyl-X bond.[11] These carboxyl radical precursors are:

- Acyl hypohalites used in the classical Hunsdiecker reaction.[12, 13] Acyl hypohalites are prepared *in situ* by reaction of a silver salt of the carboxylic acid with a halogen. This method is used for carbon-halogen bond formation.

- Lead(IV) carboxylates used in the Kochi variant[14] of the Hunsdiecker reaction. Lead tetraacetate is employed to generate a weak bond between the acid and the lead by exchange of an acetate of lead tetraacetate for the acid. This method is used for the synthesis of alkyl halides from carboxylic acids.
- *O*-Acyl oximes prepared by reaction of benzophenone oxime with acyl chlorides and used as synthetic precursors for photochemical arylation of aromatic compounds.[15]
- *O*-Acyl thiohydroxamates developed by Barton *et al.*,[16] prepared by reaction of acyl chlorides with *N*-hydroxy-pyridine-2-thione. This method to generate the carboxyl radical tolerates a much wider range of functional groups as it operates under much milder conditions. After decarboxylation, the alkyl radical can be trapped with a variety of radical-trapping reagents. The reactions are initiated thermally in appropriate solvents or by white light photolysis.

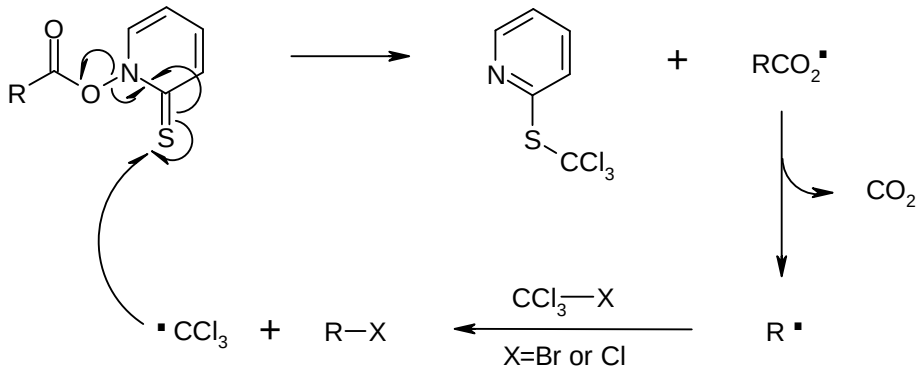
In the interest of the following research work, only the decarboxylative halogenation reaction will be discussed here.

The discovery of the halodecarboxylation was made by Borodin in 1861 involving the preparation of methyl bromide from silver acetate.[17] Hunsdiecker investigated the reaction for the synthesis of aliphatic halides in the early 1940's.[12, 13] Since then, the common name given to the reaction has been the Hunsdiecker reaction. The Hunsdiecker reaction consists of the degradation of a silver salt of the carboxylic acid in anhydrous medium by means of halogen to obtain the corresponding halide of one less atom than the original acid. The reaction can be done with other salts than silver salts such as mercury(II),[18] thallium(I)[19] and lead(IV)[14] salts. The reaction can be expressed by the following equation (M is metal and X is halogen):

$$RCOOM + X_2 \longrightarrow RX + CO_2 + MX$$

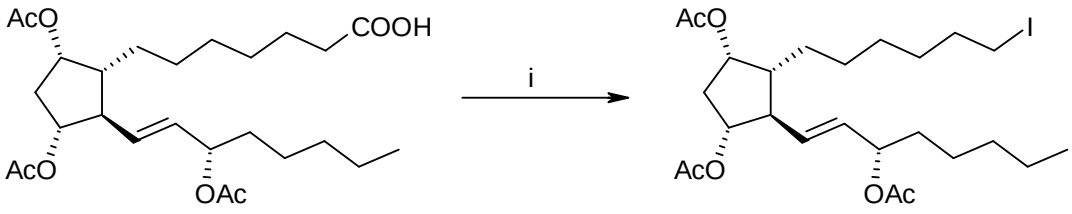
The classical Hunsdiecker reaction is carried out in tetrachloromethane under reflux and optimal yields are obtained with bromine, followed by chlorine and then iodine. An investigation by Barton of the halodecarboxylation showed that photochemical decarboxylation of acyl hypoiodites provided a method for the preparation of alkyl iodides by using lead tetraacetate and iodine as reagents[20]. The difficulties, expense and toxicity associated with the use of metal salts have led to the development of more wide-ranging and generally applicable conditions to carry out the decarboxylative

halogenations such as the photolytic or thermal decomposition of *O*-acyl thiohydroxamates in halogen donor solvents developed by Barton in the 1980's.[16, 21, 22] This decarboxylative halogenation proceeds by a radical chain mechanism (as shown in Scheme **5**) under very mild conditions. Decarboxylative chlorination or bromination of thiohydroxamate esters is achieved using carbon tetrachloride or bromotrichloromethane respectively as the solvent and trapping species and the reaction is initiated by heating under reflux or by UV irradiation induced photolysis.



Scheme 5. Halodecarboxylation chain mechanism from the thiohydroxamate ester.

Decarboxylative iodination by this method uses iodoform as an iodine donor in benzene or cyclohexene. So far, the halodecarboxylation had been investigated mainly on aliphatic carboxylic acids. Barton then extended the scope of his procedure to aromatic carboxylic acids.[23] Following Barton's procedures, the alkyl iodides are the halides formed in the lowest yield and although well investigated for aliphatic and aromatic carboxylic acids, very little has been reported on vinyl carboxylic acids.[11, 22] More recently, Eaton and co-workers[24] proposed 2,2,2-trifluoroiodoethane as a more convenient and efficient iodinating agent which they used successfully in their synthesis of iodocubanes.



Scheme 6. Iododecarboxylation step in the synthesis of prostaglandin phosphonic acids.
Reagents & conditions: (i) oxalyl chloride, DMF_{cat}, anhydrous CH_2Cl_2 then sodium salt of N-hydroxypyridine-2-thione, $DMAP_{cat}$, CF_3CH_2I, anhydrous CH_2Cl_2, hν, reflux, 56%.

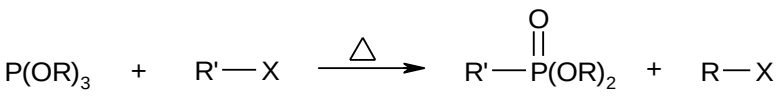
Likewise, this source of iodide radical has been used in the iododecarboxylation reaction involved in the synthesis of prostaglandin phosphonic acids by Kende and co-workers (Scheme **6**).[25]

Over the last decade, new protocols for the Hunsdiecker reaction have been developed, mainly for the synthesis of aromatic and aryl-substituted vinyl halides. Roy and co-workers[26, 27] developed a catalytic Hunsdiecker-like protocol, using *N*-bromosuccinimide as halogenating reagent and lithium acetate as catalyst. Tokuda *et al.*[28, 29] developed microwave induced halodecarboxylation of aromatic and α,β-unsaturated carboxylic acids, leading to aryl halides and (*E*)-β-arylvinyl halides respectively, carried out in the presence of a catalytic amount of LiOAc and with *N*-halosuccinimide as halogenating reagent.[28, 29] Alternatively Jain *et al.*[30] carried out the microwave irradiation using *N*-chlorobenzotriazole. Another procedure developed by Tokuda *et al.*[31] led to the stereoselective synthesis of (*Z*)-1-bromo-1-alkenes from the corresponding 2,3-dibromoalkanoic acids using a triethylamine/DMF system and microwave irradiation. The development of more environmentally friendly 'green' Hunsdiecker reactions have as well been achieved, using inorganic bromide (KBr) and hydrogen peroxide in the presence of molybdenium (VI) as catalyst[32] or using *N*-halosuccinimide in micellar media.[33]

The modified Barton-Hunsdiecker halodecarboxylation procedure, introduced by Eaton[24] and Kende[25], was chosen for the detailed investigation of the reaction conditions.

I.1.2.2. *Hirao's palladium-promoted coupling reaction*

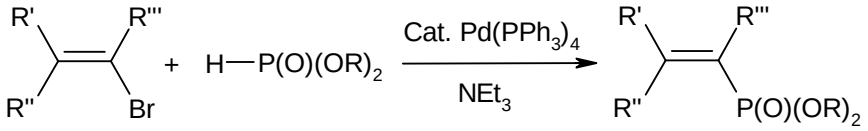
One of the most versatile pathways for the sp^3-carbon-posphorus bond formation is the Michaelis-Arbuzov reaction.[34] The reaction involves the S_N2 reaction of esters of trivalent phosphorus with alkyl halides to yield dialkyl alkylphosphonates under heating conditions, it thus converts a trivalent phosphorus into a pentavalent phosphorus.



Scheme 7. Michaelis-Arbuzov reaction.

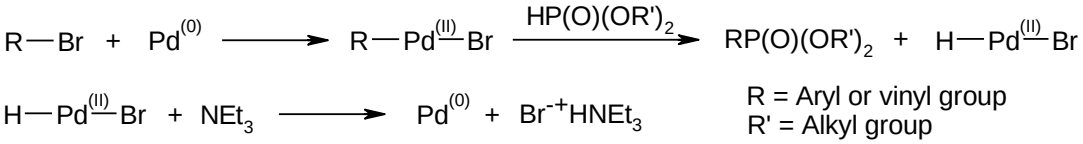
Due to the SN_2–type mechanism of the reaction, aryl and vinyl halides are unreactive towards trialkyl phosphites. Nevertheless, Arbuzov products of aryl halides were obtained by free-radical chemistry, for example by photolysis and heating in presence of trialkyl phosphites[35, 36] or by photostimulated reaction of halogenobenzene derivatives with dialkyl phosphite anions as nucleophiles in liquid ammonia[37, 38] or in DMF.[39]
More recently, vinyl and arylphosphonates were synthesized by Bentrude and co-workers[40] in good yields, following generation of vinyl and aryl radicals from the corresponding bromides, under typical AIBN/*n*-Bu_3SnH conditions in refluxing benzene in the presence of an excess of $P(OMe)_3$. The reaction was not stereospecific as the *E* or *Z* stereochemistry of the starting vinyl bromides was lost during the reaction and a mixture of the *E*/*Z* phosphonate compounds was obtained.

The difficulty of the sp^2-carbon-phosphorus bond formation has been mainly overcome by palladium-catalyzed coupling reactions. Hirao[41] developed in the early 1980's a methodology to convert vinyl bromides into dialkyl vinyl phosphonates stereoselectively,[42] and aryl bromides into dialkyl arylphosphonates in good yields.[43]



Scheme 8. Hirao's reaction.

The reactions of vinyl and aryl bromides with *O*,*O*-dialkyl phosphonates (also called dialkyl phosphites) under Hirao's experimental conditions are carried out in the presence of triethylamine and a catalytic amount of tetrakis(triphenylphosphine) palladium in toluene at 90°C under a nitrogen atmosphere. The palladium(0) species undergoes oxidative addition with vinyl or aryl bromides to give the aryl-palladium complex. The attack of dialkyl phosphite to the vinyl/aryl palladium complex leads to the formation of the dialkyl vinyl/aryl phosphonate. The base, triethylamine, regenerates the palladium(0) species, which is then available for another reaction cycle, by formation of the salt of NEt_3 with HBr. This formation path of dialkyl vinyl/aryl phosphonates is outlined in Scheme **9**.

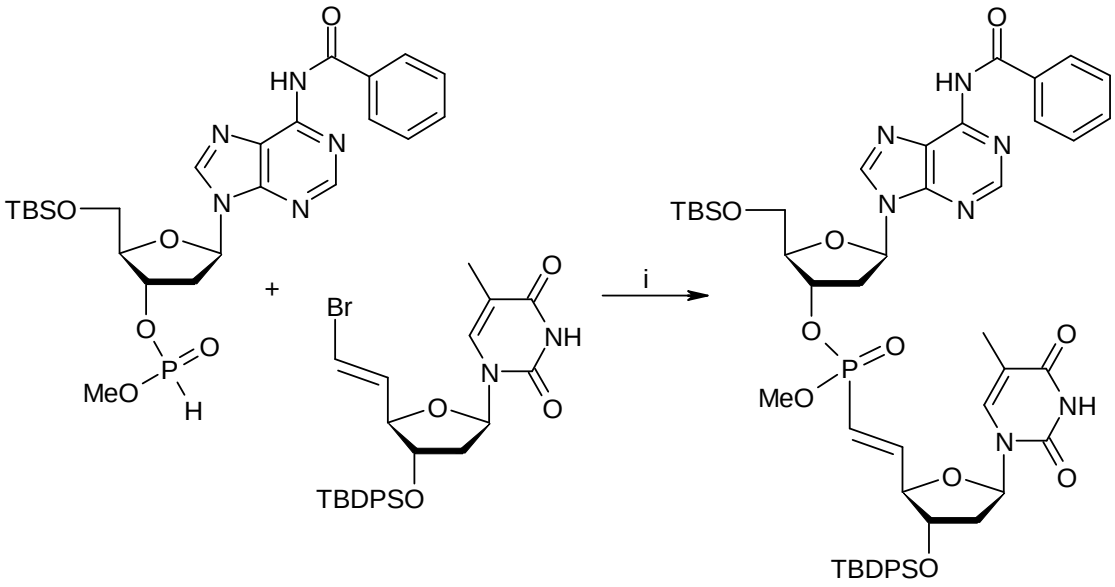


Scheme 9. Reaction cycle for the formation of dialkyl vinyl/aryl phosphonates.

Hirao's procedure has since been widely used for the synthesis of dialkyl alkenylphosphonates from alkenyl bromides and iodides.[44-48] Notably, reactions using Hirao's conditions have also been applied to (un)substituted cyclohexene ring systems carrying a vinylic bromide or iodide.[42, 49] Some of these reactions have been carried out with variations to the original protocol by the use of different bases, for example DABCO[50] or caesium carbonate and *N*,*N*-dimethylethylenediamine.[51]

Holt and co-workers[52] applied Hirao's conditions to couple alkenyl triflates with dialkyl phosphites in good yields. Hirao's conditions were also used by Xu *et al.* for the synthesis of unsymmetrical alkyl arylphenylphosphinates,[53] functionalized alkyl alkylarylphosphinates,[54] alkylarylphenylphosphine oxides[55] from aryl halides and for the synthesis of alkyl alkenylmethyl and alkenylphenylphosphinates from arylvinyl and vinyl halides.[56]

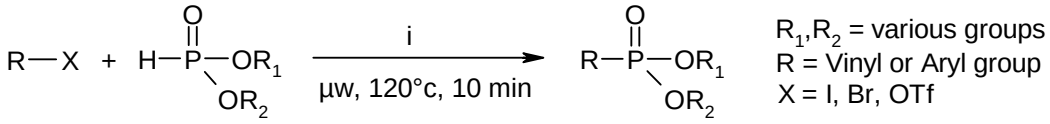
Moreover, an improved procedure for the synthesis of vinylphosphonate-linked nucleic acids has been developed by Hayes and co-workers (Scheme **10**).[57]



Scheme 10. *Reagents & conditions:* (i) $Pd(OAc)_2$, dppf, THF, reflux, propylene oxide, 92% yield.

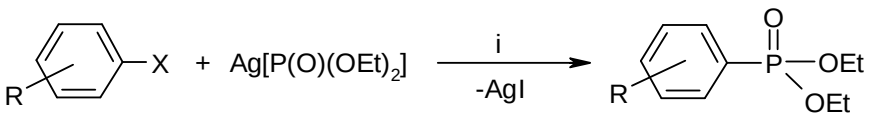
The couplings of hindered H-phosphonate diesters with vinylic bromides were achieved by using propylene oxide as an alternative HBr scavenger (replacing the triethylamine) and using a different catalyst system by generating the catalyst in situ from palladium acetate and 1,1'-bis(diphenylphosphino)ferrocene (dppf) in refluxing THF.

Recently, Stawinski and co-workers developed a general and efficient method for the formation of the sp^2-carbon-phosphorus bond by a microwave-assisted palladium-catalyzed cross-coupling of aryl and vinyl halides with various H-phosphonate diesters in good yields,[58] as outlined in Scheme **11**.



Scheme 11. *Reagents & conditions:* (i) 5 mol % $Pd(PPh_3)_4$, Cs_2CO_3 or NEt_3, THF.

As a final example of a metal-catalyzed process to generate dialkyl arylphosphonates, Stockland *et al.* developed a room temperature Hirao reaction.[59] The reaction still utilizes Pd (0) as catalyst, which is formed in situ from palladium acetate and bis(2-diphenylphosphinophenyl)ether (dpephos) as supporting ligand, and silver phosphonates as transmetallating agents to afford diethyl arylphosphonates from aryl iodides in moderate to good yields, as described in Scheme **12**.



Scheme 12. Hirao's reaction at room temperature:
Reagents & conditions: (i) $Pd(OAc)_2$ 5 mol %, dpephos 10 mol %, THF, 25°C.

Bentrude's conditions and subsequently Hirao's conditions were chosen to be investigated as the second key step involved in the synthesis of the cyclohexenephosphonate scaffold and more specifically, to achieve the phosphonylation of our vinyl iodide.

I.1.3. Shikimic acid and the shikimate pathway

I.1.3.1. *Shikimic acid*

(-)-Shikimic acid was first isolated by Ekmann in 1885 from the fruit of *Illicium religiosum*. It is from this oriental plant, named shikimi-no-ki in Japanese that the name shikimic acid was derived.

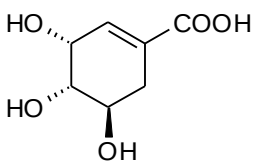


Figure 1. (-)-shikimic acid

(-)-Shikimic acid plays an important role in a biosynthetic pathway, known as the shikimate pathway, which leads ultimately to the biosynthesis of three aromatic amino acids, L-phenylalanine, L-tyrosine and L-tryptophan.

(-)-Shikimic acid has been selected as the starting material of choice (compared to quinic acid) for the industrial synthesis of Oseltamivir Phosphate (Tamiflu^{TM}) by chemists at F. Hoffmann-La Roche Ltd. Indeed, (-)-Shikimic acid is now available in ton quantities either by extraction from the fruit *Illicium verum* (Chinese star anise) or by fermentation using a genetically engineered E. coli strain developed by Frost and co-workers[60, 61] and technically established at Roche. It was recently reported that *Liquidambar styraciflua*, more commonly known as the sweetgum tree, could be a renewable source of shikimic acid.[62] In fact, it was shown that the sweetgum tree can yield shikimic acid in amounts comparable to that of *Illicium verum*, through the seeds of its annual fruit.

Due to its biological importance, there has been a wide interest in finding alternative synthetic routes to (-)-shikimic acid. Its first total syntheses in a racemic form were performed by Raphael[63] and Smissman[64, 65] employing an identical synthetic route, using the Diels-Alder reaction of acrylic acid (or methyl acrylate in Smissman's synthesis) with (1E,3E)-1,4-diacetoxy-1,3-butadiene as starting materials. Since then, many different syntheses of (-)-shikimic acid and its racemic form have been reported. Synthetic approaches based on the Diels-Alder reaction, syntheses from benzene and its derivatives, chiral pool-based syntheses from (-)-quinic acid and from carbohydrates were used to achieve the synthesis of (-)-shikimic acid. Two detailed reviews about this work, the first by Searle and co-workers [66] (1993) and the second by Singh and Jiang[67] (1998), give a good overview of these synthetic approaches. Over the last

decade, interest in the shikimic acid synthesis[68-70] has declined as interest in novel synthetic routes to synthesize shikimic acid-like molecules have increased.[71-76]

I.1.3.2. *Shikimate pathway*

As mentioned previously, though in less detail, the shikimate pathway, a biosynthetic pathway which is absent in mammals, allows the biosynthesis of aromatic amino acids and aromatic compounds such as ubiquinones, naphthoquinones and folates. The shikimate pathway is found only in micro organisms (bacteria, parasites and microbial eukaryotes) and plants. As a consequence, the shikimic acid pathway provides a valuable roadmap for engineering new herbicides and herbicide-resistant crops, as well as new antibiotic and antiparasitic drugs by targeting inhibition of its enzymes. In this context, significant research is being undertaken to fully understand this pathway and to elucidate structural data of enzymes of importance implicated in the shikimate pathway in order to facilitate the direction of new small molecule inhibitor design.

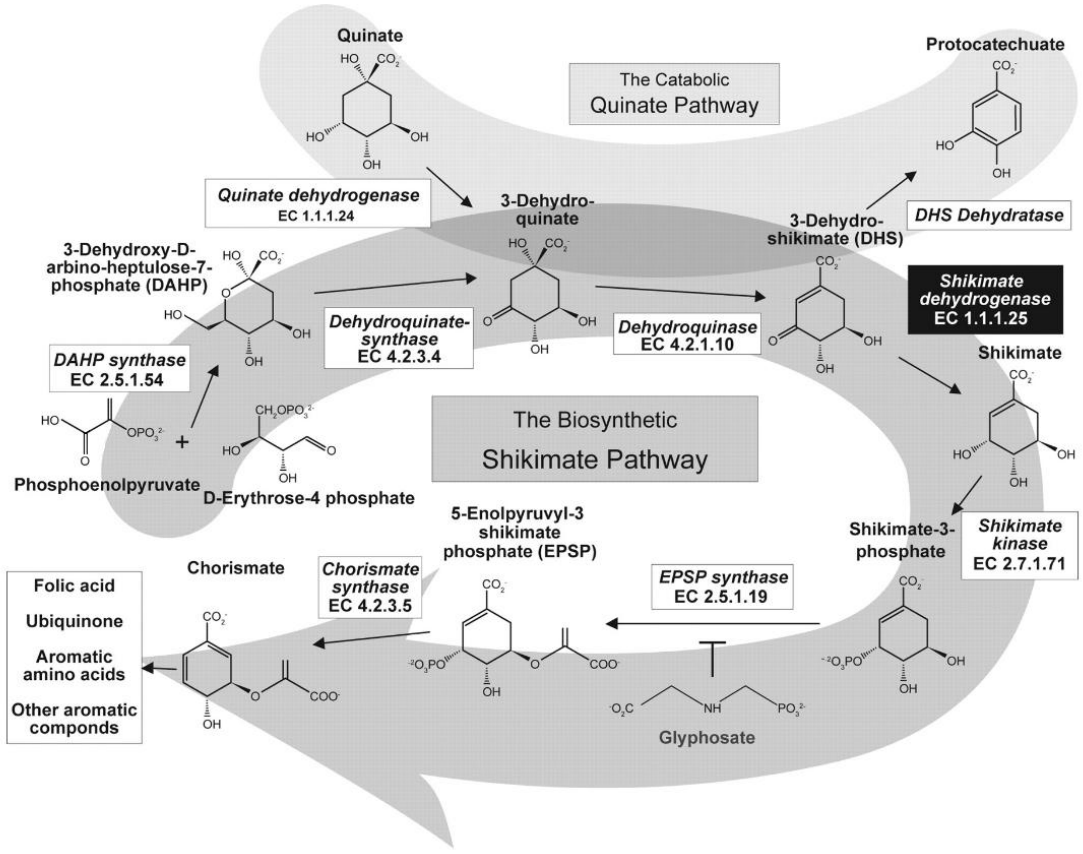


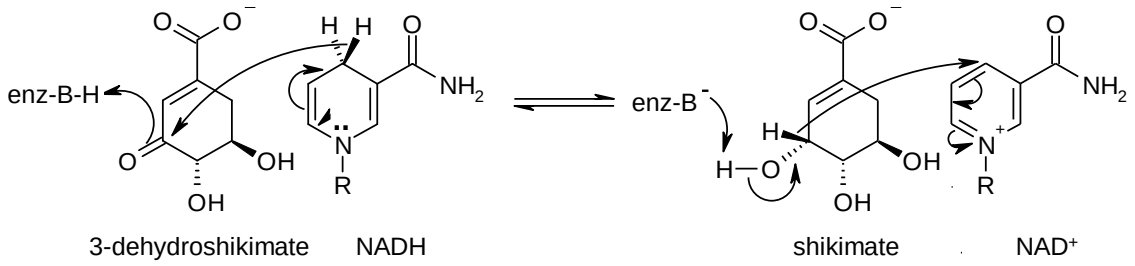
Figure 2. The shikimate pathway (Corrected figure taken with permission from the *Journal of Biological Chemistry* **2005**, *280*, 7162).

In a sequence of seven metabolic steps the shikimate pathway transforms D-Erythrose-4-phosphate and phosphoenolpyruvate into chorismate, the latter being the precursor to the synthesis of aromatic amino acids (Figure **2**[77]). The pathway enzymes are: 2-keto-3-deoxy-D-arabinoheptulosonate-7-phosphate synthase, dehydroquinate synthase, 5-dehydroquinate dehydratase, shikimate dehydrogenase, shikimate kinase, 3-enoylpyruvylshikimate-5-phosphate synthase and chorismate synthase, following the transformation of D-Erythrose-4-phosphate into chorismate.

In this biological pathway, shikimic acid is the intermediate synthesized by the shikimate dehydrogenase which allows the reversible reduction of 3-dehydroshikimate into shikimate. This is the justification why designing and synthesizing new shikimic acid mimetics could lead to the discovery of new antimicrobials.

I.1.3.3. *Shikimate dehydrogenase*

Shikimate dehydrogenase (SDH) catalyses the fourth step of the shikimate pathway and is responsible for the reversible reduction of 3-dehydroshikimate to shikimate in the presence of the cofactor NAD(P)H (Scheme **13**[78]).



Scheme 13. Overall reaction mechanism for the reduction of 3-dehydroshikimate to shikimate using NADH.

In fungi and yeast, SDH exists as a component of the pentafunctional AROM polypeptide which catalyses five of the seven steps of the shikimate pathway. In plants, SDH is associated with 3-dehydroquinate dehydratase as a bifunctional enzyme.[79, 80] In bacteria, three types of SDH have been characterized to date, AroE, YdiB and SDH-L.[81] They are members of the quinate/shikimate 5-dehydrogenase family. Crystal structures of both the AroE and YdiB proteins reveal the presence of a NAD(P)-binding and catalytic site.

AroE is a monofunctional enzyme specific to the shikimate pathway (identified in *Escherichia coli*, *Salmonella typhimurium* and many other microbial species) and catalyses the reduction of 3-dehydroshikimate to shikimate in the presence of NADPH. In contrast, YdiB, which is represented in a lower percentage of organisms, is a bifunctional enzyme used in both shikimate and quinate pathway. YdiB reduces 3-dehydroshikimate to shikimate and 3-dehydroquinate to quinate in the presence of either NADH or NADPH. Nevertheless, the biological function of YdiB is not fully clear as it is not known if 3-dehydroshikimate or quinate is its natural substrate.[77, 79] The SDH-L (shikimate dehydrogenase-like) protein has been identified only in a small group of organisms and it has been shown that SDH-L requires NADPH for the reduction of 3-dehydroshikimate to shikimate.[81] Structural studies of dehydrogenases AroE and YdiB show a three-dimensional conserved protein structure and indicate that their substrate and nucleotide-binding sites are also highly conserved.[81]

More especially, these enzymes contain two α/β domains connected by two α-helices and separated by a wide cleft which is the active site of these enzymes. This deep cavity is contiguous with the hydride acceptor $NAD(P)^+$ co-factor which is binding to the C-terminal domain being a Rossmann domain[82] in the structure (Figure **3**[80]).

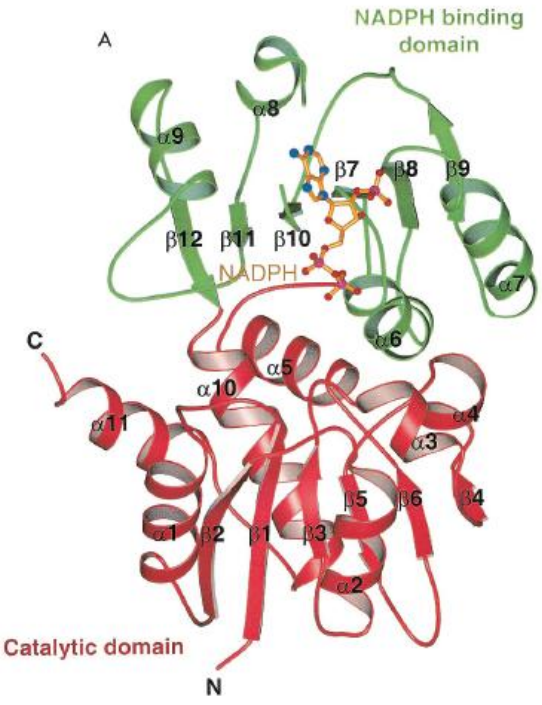


Figure 3. Ribbon diagram of the AroE : NADPH complex structure. The NADPH binding domain is shown in green at the top, the catalytic domain is shown in red at the bottom, and NADPH is shown in a ball-and-stick representation (Figure taken with permission from the *Journal of Bacteriology* **2003**, *185*, 4144).

The key residues interacting with the substrate and with the co-factor are well conserved in all the SDH orthologues. Figure **4**[79] shows a view of AroE active site with 3-dehydroshikimate and the cofactor $NADP^+$ and Figure **5**[78] shows a view of YdiB active site with the shikimate.

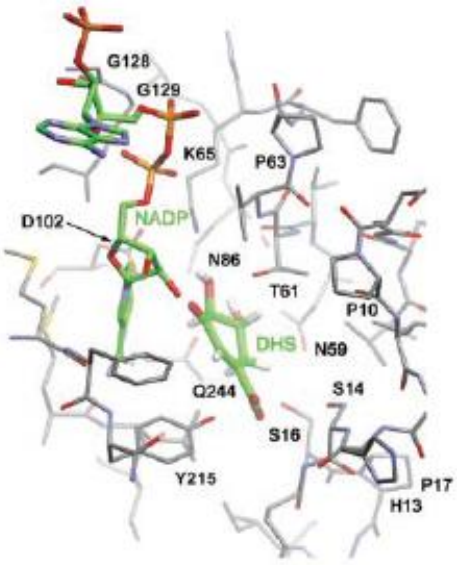


Figure 4. Molecular model of the binding of dehydroshikimate to the active site of AroE (Figure taken with permission from the *Journal of Biological Chemistry* **2003**, *278*, 19463)

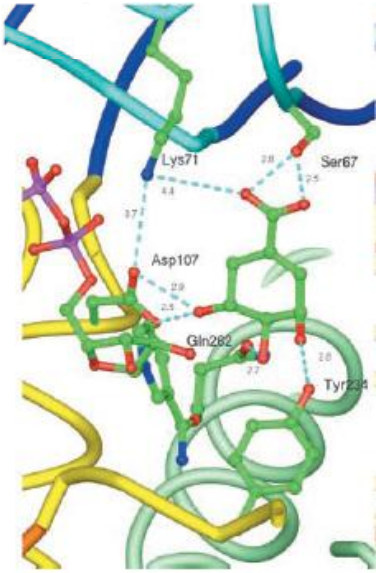


Figure 5. Proposed binding mode for a molecule of shikimate in the active site of YdiB. Distances are given in Å. All of the side chains shown here are 100% conserved in the shikimate dehydrogenase cluster of orthologous genes with the exception of Ser-67 (which is only substituted with threonine) and Tyr-234 (which is present in 37 of 43 sequences) (Figure taken with permission from the *Journal of Biological Chemistry* **2003**, *278*, 19176).

This therefore indicates that all the different types of SDH use a similar catalytic mechanism and that rational drug design of potent inhibitors may be possible. Even though the determination of several SDH crystal structures and the efforts being made to understand the reaction mechanism involved in SDH may provide enough structural information for rational drug discovery, no SDH efficient inhibitors have yet been found. However, some inhibitors of SDH from *Helicobacter pylori* have recently been discovered using a high throughput screening.[83] These compounds are curcumin **A**, 3-(2-naphthyloxy)-4-oxo-2-trifluoromethyl)-4H-chromen-7-yl 3-chlorobenzoate **B**, butyl 2-{[3-(2-naphthyloxy)-4-oxo-2-(trifluoromethyl)-4H-chromen-7-yl]oxy}propanoate **C**, 2-({2-[(2-{[2-(2,3-dimethylanilino)-2-oxoethyl]sulfanyl}-1,3-benzothiazol-6-yl)amino]-

2-oxoethyl}sulfanyl)-*N*-(2-naphthyl) acetamide **D**, and maesaquinone diacetate **E**, with IC_{50} values of 15.4, 3.9, 13.4, 2.9, and 3.5μM, respectively.

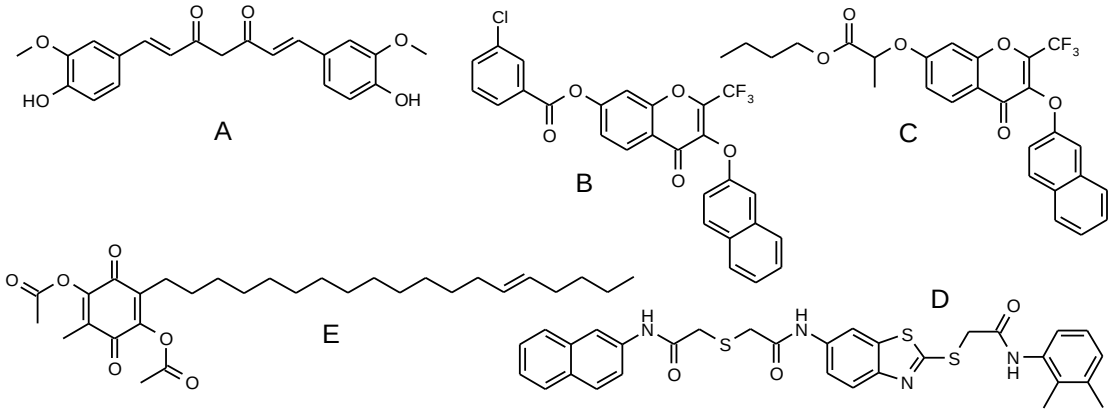


Figure 6. Chemical structure of compounds A-E.

These inhibitors, which are not the result of rational drug discovery, possess new chemical scaffolds which could lead to the development of novel SDH inhibitors.

I.1.3.4. *Towards Shikimic acid mimetics*

The fundamental aim of this research work was to find a novel and effective synthetic approach towards cyclohexenephosphonates from chiral cyclohexene carboxylate precursors.

It was thus decided to apply this approach to shikimic acid to afford 'phospha'-shikimic acid and derivatives. Shikimic acid is a particularly interesting starting material to work with as it is both an important metabolite in its own right and a starting material in anti-influenza drug synthesis.[84, 85] Moreover, the replacement of the carboxylate functionality by a phosphonate moiety enables the retention of the necessary pharmacophores in the target molecules. This has some precedent, since the phosphonate moiety is frequently used as a bioisostere of carboxylate groups in drug design and synthesis,[2-4, 86, 87] retaining the negative charge, present under physiological conditions, which is essential for activity and opening space to incorporate lateral functionality on phosphonate monoesters.

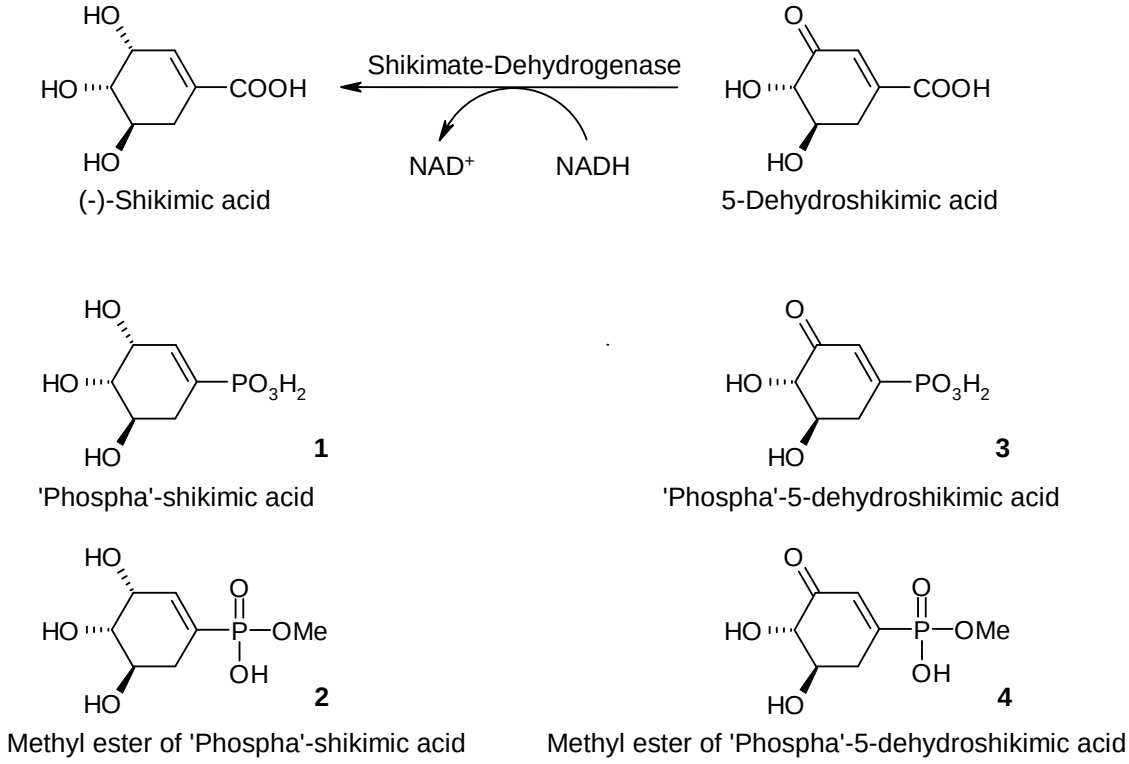
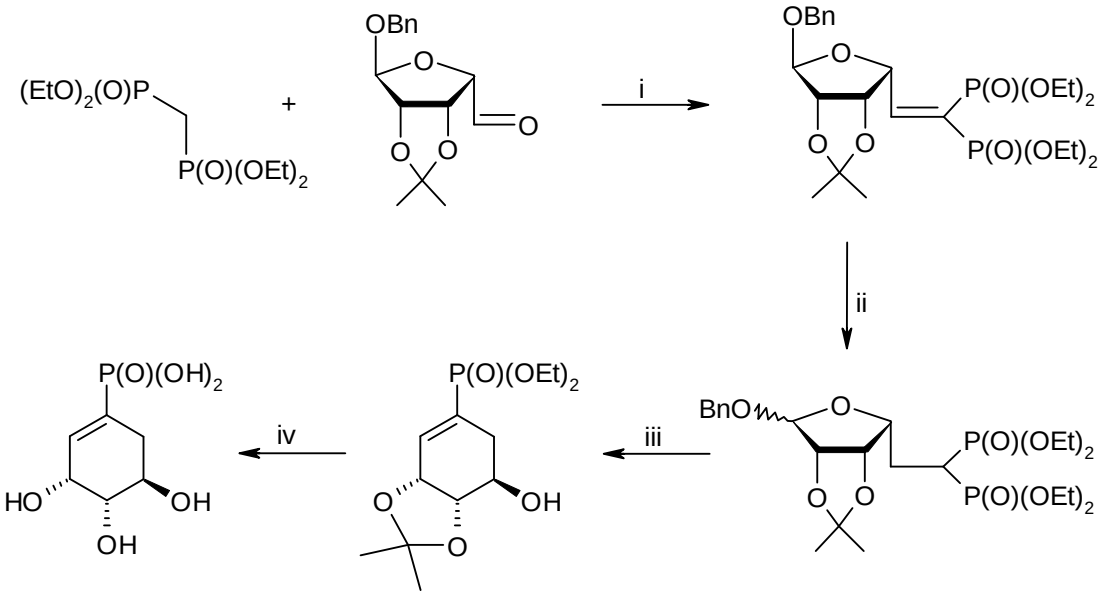


Figure 7. Targeted phospha-isosteres of shikimic acid.

As shown in Figure **7** the synthetic targets are the ammonium salts of [(3R,4S,5R)-trihydroxy-1-cyclohexene-1-phosphonic acid] **1**, [methyl (3R,4S,5R)-trihydroxy-1-cyclohexene-1-phosphonate] **2**, [(4S,5R)-dihydroxy-3-oxo-1-cyclohexene-1-phosphonic acid] **3** and [methyl (4S,5R)-dihydroxy-3-oxo-1-cyclohexene-1-phosphonate] **4.**

It is important to note that 'phospha'-shikimic acid **1** has previously been synthesized by Mirza *et al.*[9, 10] They designed a synthetic route leading to the synthesis of shikimic acid and its phosphonate analogue. As illustrated in Scheme **14**, condensation of a suitably protected D-lyxose 5-aldehyde with tetraethyl methylenediphosphonate (or diethyl ethoxycarbonylmethylphosphonate) afforded the Knoevenagel product which after hydrogenation and intramolecular Horner-Wadsworth-Emmons olefination afforded the protected cyclohexene precursor to 'phospha'-shikimic acid (or shikimic acid). Subsequent hydrolysis of the phosphonate ester (or carboxylic ester) and of the ketal afforded 'phospha'-shikimic acid (or shikimic acid).

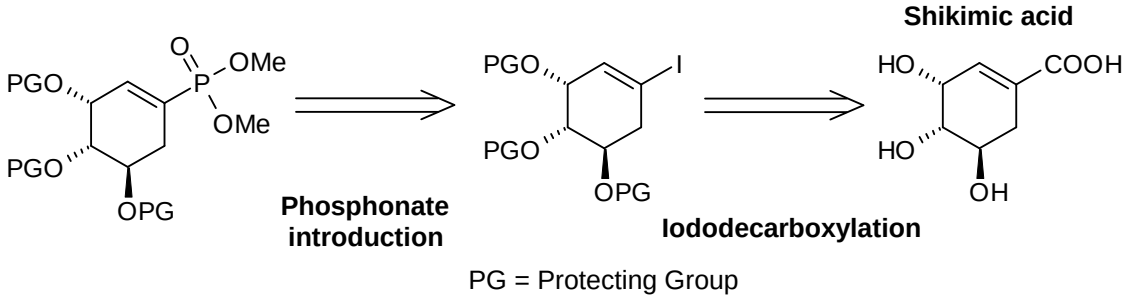


Scheme 14. Mirza's 'phospha'-shikimic acid synthesis:
Reagents & conditions: (i) N-methylmorpholine, $TiCl_4$, CCl_4, THF, 64%; (ii) H_2, 10% Pd/C, EtOH; (iii) NaOEt, EtOH, 42%; (iv) Me_3SiBr, $CHCl_3$ then H_2O, 93%.

I.2. Results and discussion - Synthesis of phospha-isosteres of shikimic acid

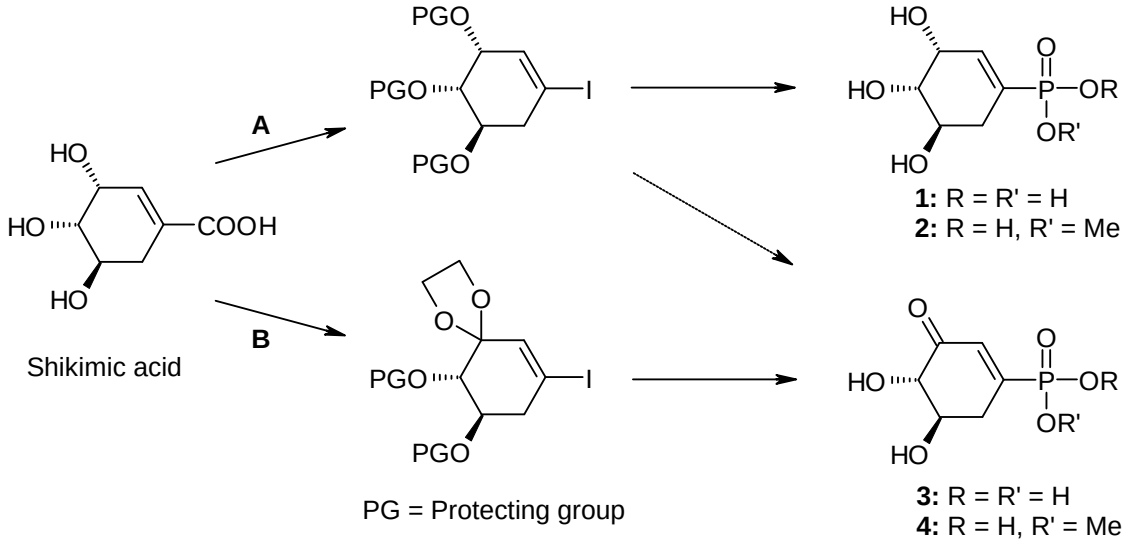
I.2.1. Retrosynthetic analysis and synthetic strategies towards phospha-shikimic acids and derivatives

The most effective route to achieve the synthesis of 'phospha'-shikimic acid would proceed via the protection of the various shikimic acid hydroxyl groups followed by a halodecarboxylation of the carboxylic acid and a subsequent conversion of the resulting vinyl halide into the respective phosphonate diester, as shown in Scheme **15** below.



Scheme 15. Retrosynthetic analysis.

Two different routes have been explored. Route A was based on halodecarboxylation of a protected shikimic acid followed by a phosphonylation leading to phospha-shikimic acid **1** and its methyl ester **2**. Oxidation of the 3-OH at a later stage (after phosphonylation) led to only the methyl ester of 3-dehydro phospha-shikimic acid **4**. Route B, which has the oxidation step placed prior to the halodecarboxylation, allowed the synthesis of 3-dehydro 'phospha'-shikimic acid **3** and its methyl ester **4**. Route B was investigated to assess the effects of protecting groups in the synthetic process and to improve final deprotection of the dehydro derivative.

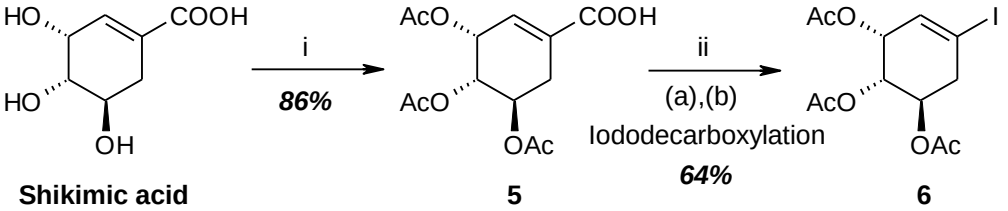


Scheme 16. Strategies towards phospha-isosteres of shikimic acid.

I.2.2. Route A.

I.2.2.1. *Synthesis of 1-iodo-(3R,4S,5R)-tri-acetoxy-1-cyclohexene **6** from shikimic acid.*

In order to carry out the halodecarboxylation step, the various hydroxyl groups first needed to be protected. Shikimic acid was thus acetylated. Acetylation was achieved with acetic anhydride and a catalytic amount of sulphuric acid, but the protected shikimic acid was only obtained in a moderate yield of 34%. The protection was improved using standard conditions, acetic anhydride in pyridine,[88] to give acetylated compound **5** in good yield (Scheme **17**).



Scheme 17. *Reagents & conditions:* (i) pyridine/acetic anhydride; (ii) (a) oxalyl chloride, DMF_{cat}, anhydrous CH_2Cl_2; (b) sodium salt of N-hydroxypyridine-2-thione, $DMAP_{cat}$, CF_3CH_2I, anhydrous CH_2Cl_2, irradiation by a 250W floodlamp.

During the early stages of the synthetic work, decarboxylation attempts with silver salts of the carboxylic acid with I_2 (Hunsdiecker conditions) or with Pb(IV) and halide salts such as LiCl and LiBr (Kochi conditions) were carried out. None of them gave satisfying results. Instead, decomposition of the starting material was observed.

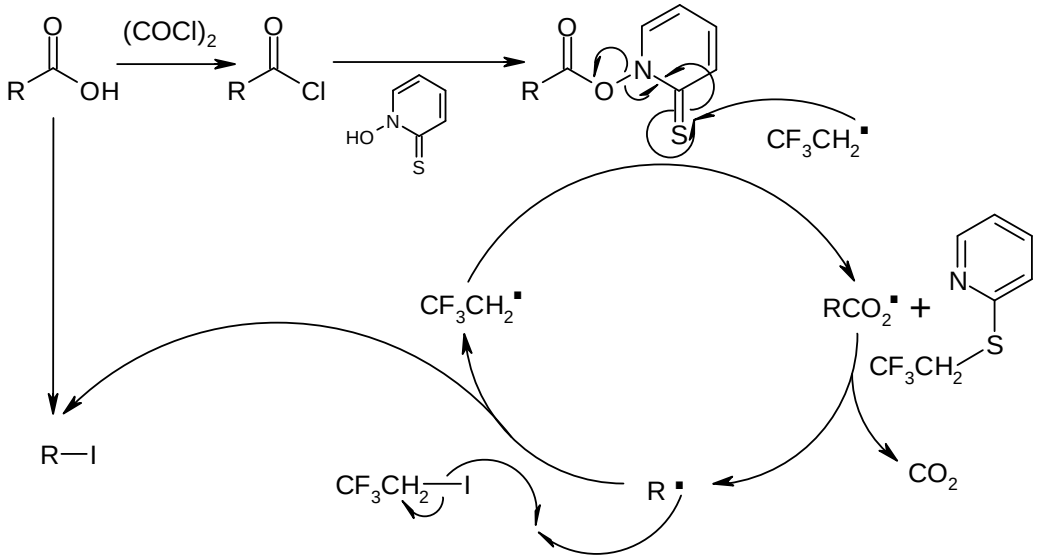
The classic Hunsdiecker reaction[12, 13] and its Kochi variant,[14] particularly in its metal-free version introduced by Barton,[16, 22] is well established for the conversion of carboxylic acids into halides via a radical mechanism. The protected shikimic acid was thus converted to 1-iodo-(3*R*,4*S*,5*R*)-tri-acetoxy-1-cyclohexene **6** using a modified Barton-Hunsdiecker halodecarboxylation reaction,[25] specifically here an iodinative decarboxylation.[23, 24]

The decarboxylative halogenation has been well described by Barton and co-workers for both aliphatic and aromatic acids via a radical decomposition of the thiohydroxamic ester (derived from the reaction between the acid chloride and *N*-hydroxypyridine-2-thione) in the presence of a halogen source.[16, 21, 23] Interestingly, a thorough search of the literature revealed that very little has been reported on its application to vinyl carboxylic acids,[11, 29, 89] particularly when considering that vinyl halides and mainly iodides are very useful substrates in a variety of organic reactions, namely C-C coupling reactions.

The Barton procedure is a one pot conversion of carboxylic acid functionalized compounds to their halogenated analogues, which requires a two step methodology:
(a) the conversion of the carboxylic acid to the acid chloride with oxalyl chloride in the presence of a catalytic amount of dimethyl formamide in anhydrous dichloromethane;

(b) the decarboxylation step via the formation of the thiohydroxamic ester. (Scheme **18**). The acid chloride is added to a mixture of the sodium salt of *N*-hydroxypyridine-2-thione, the halogen donor 2-iodo-1,1,1-trifluoroethane (CF_3CH_2I), with a catalytic amount of 4-dimethylaminopyridine in anhydrous DCM, the solution then being irradiated with a 250W UV electric flood lamp.

The reaction proceeds by a radical chain mechanism[16] involving a carbon radical intermediate which reacts with the halogen donor to form the desired halogen derivative (Scheme **18)**.



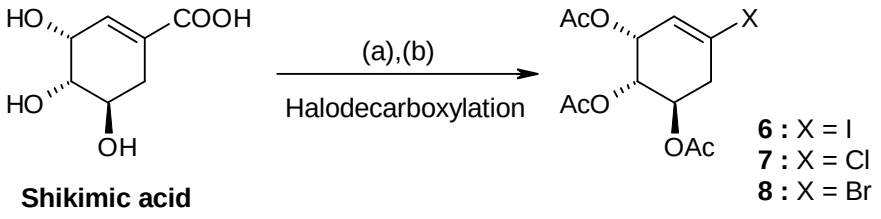
Scheme 18. Iododecarboxylation mechanism.

A study on suitable conditions for the halodecarboxylation of acetylated shikimic acid **5** was carried out. Different conditions were applied to the system in order to optimize the reaction. It was assumed that the acid chloride was always formed quantitatively, therefore the second step of the procedure became the main focus and was investigated by varying certain reaction parameters. The reactions containing different equivalents of halogen donors were irradiated with different UV sources for various reaction times.
The Table **1** below summarizes the conditions used.

Entry	Halogen donor	Equivalent	Light	Time (min)	Yield (%)	Compound
1	CF_3CH_2I	11	250W electric flood lamp	60	57	**6**
2	CF_3CH_2I	11	250W electric flood lamp	240	28	**6**
3	CF_3CH_2I/ ICH_2CH_2I	2/3.5	250W electric flood lamp	90	20	**6**
4	ICH_2CH_2I	11	250W electric flood lamp	120	5	**6**
5	CF_3CH_2I	5	250W electric flood lamp	90	64	**6**
6	CF_3CH_2I	6	250W electric flood lamp	75	57	**6**
7	CF_3CH_2I	11	125W UV (365-366 nm)	60	27	**6**
8	CF_3CH_2I	5	400W UV (365-366 nm)	60	17	**6**
9	CCl_4	11	250W electric flood lamp	60	23	**7**
10	CCl_4	11	125W UV (365-366 nm)	60	8	**7**
11	CBr_4	11	250W electric flood lamp	75	36	**8**

Table 1. Halodecarboxylation reaction optimization table. The starting material is the acetylated shikimic acid.

Clearly from the table above, it can be concluded that 5 or 6 equivalents of CF_3CH_2I are enough for the iododecarboxylation reaction to proceed, along with irradiation of the mixture by a 250W electric flood lamp for at least one hour. These became the standard conditions for the iododecarboxylation reaction of protected shikimic acid. Use of the common iodine source diiodoethane or irradiation with specific UV-sources did not result in improved yields. Chloro- and bromo-decarboxylation proved to be less efficient as trial experiments afforded the 1-chloro-(3R,4S,5R)-tri-acetoxy-1-cyclohexene **21** and 1-bromo-(3*R*,4*S*,5*R*)-tri-acetoxy-1-cyclohexene **22** in low yields of 23% and 36% respectively. (Scheme **19**).

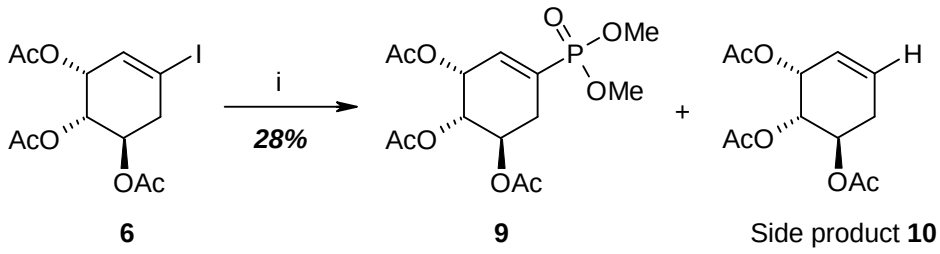


Shikimic acid

Scheme 19. *Reagents & conditions:* (a) oxalyl chloride, DMF_{cat}, anhydrous CH_2Cl_2; (b) sodium salt of N-hydroxypyridine-2-thione, $DMAP_{cat}$, halogen donor (see **Table 1**), anhydrous CH_2Cl_2, irradiation by UV light.

I.2.2.2. *Introduction of the phosphonate group: Synthesis of Dimethyl (3R,4S,5R)-tri-acetoxy-1-cyclohexene-1-phosphonate **9** and deprotection.*

Initially it was thought that phosphonylation would occur under radical conditions, following Bentrude's conditons for the synthesis of vinyl and arylphosphonates,[40] using AIBN and *n*-Bu_3SnH at 80°C in the presence of an excess of trimethylphosphite. This reaction was not successful. Purification and subsequent NMR analysis of what was thought to be the product indicated that acetyl groups had been removed and that no vinylphosphonate proton, usually characterized as a doublet around 6.5 ppm, could be detected.



Scheme 20. *Reagents & conditions:* (i) $HP(O)(OMe)_2$, $Pd(PPh_3)_4$, anhydrous NEt_3, anhydrous Toluene, 80°C.

It was therefore decided to synthesize the vinylphosphonate using a palladium(0) catalyzed step.[47] Utilizing the experimental conditions employed by Hirao,[41, 42] this coupling step involves the reaction of vinyl-iodide **6** with dimethylphosphite and triethylamine in the presence of a catalytic amount of tetrakis(triphenylphosphine) palladium. Phosphonylation occurred but only with poor yields. The low yield of this reaction was contributed to the base labile acetyl groups which display instability to the conditions utilizing triethylamine with heating.[90]

Another peculiarity of this reaction is the formation of a side product **10** which is obtained in a non-negligible amount (18% yield) compared to compound **9** (28% yield). At present, the mechanism of this deiodination remains to be established. Hydrodehalogenation as a side reaction has already been observed using the same experimental conditions of Hirao's reaction.[45] Such products have also been mentioned in the literature.[39]

The crystal structure of dimethyl (3R,4S,5R)-tri-acetoxy-1-cyclohexene-1-phosphonate **9** was determined and is shown in Figure **8** below. As expected for the cyclohexene ring, the crystal structure displays a half-chair conformation with C-5 and C-4 respectively above and below the plane formed by the four atoms H, C-3, C-6 and P which are bonded to the two sp^2 carbons C-2 and C-1. Acetoxy groups on C-4 and C-5 are pseudo axial and the acetoxy group on C-3 is pseudo equatorial.

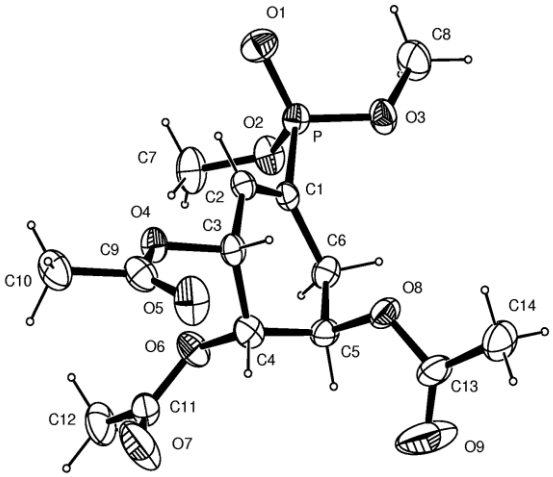
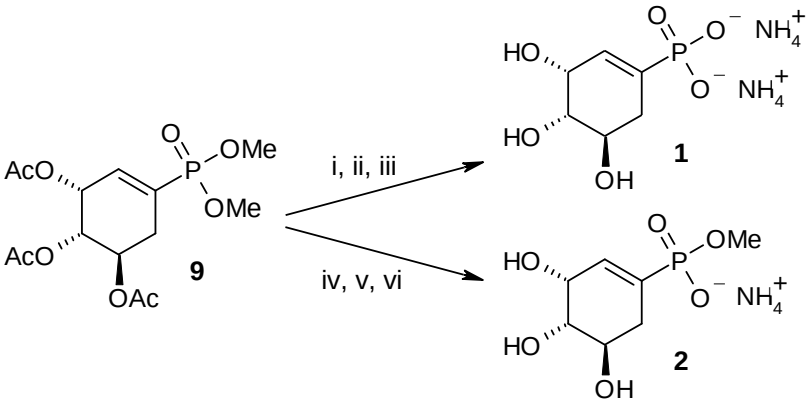


Figure 8. ORTEP-generated structure of acetylated 'phospha'-shikimic acid dimethyl ester **9**.

Following the phosphonate introduction, the first target compounds **1** and **2** were obtained by saponification of the acetyl esters and total (using TMSBr) or partial cleavage (using NaI) of the methyl phosphonates (Scheme **21**).



Scheme 21. *Reagents & conditions:* (i) bromotrimethylsilane, lutidine, anhydrous CH_2Cl_2; (ii) aqueous ammonia (10%); (iii) gel permeation chromatography (gpc); (iv) NaI, anhydrous acetone, reflux; (v) aqueous ammonia (10%); (vi) gpc.

Protected ‘phospha’-shikimic acid **9** was subjected to cleavage of the methyl phosphonates using TMSBr and lutidine in anhydrous CH_2Cl_2 for 4 hours, giving the free phosphonic acid which was carried to the next step without further purification. Analysis by NMR of the crude product revealed that the phosphonate was deprotected. Deacetylation of the latter compound was carried out in aqueous ammonia leading to ‘Phospha’-shikimic acid **1** which was directly purified by gel permeation chromatography on a Biogel P4 column.
Mono-demethylation of compound **9** was achieved using sodium iodide in dry acetone under reflux conditions to afford the sodium salt.[91] Deprotection of the hydroxyl groups was achieved with aqueous ammonia affording the mono-methyl ‘Phospha’-shikimic acid **2** which was purified by gel permeation chromatography on a Biogel P4 column.

In only three steps starting from the shikimic acid, the overall yield of this synthesis of ‘phospha’-shikimic acid dimethyl ester **9** is 14%. In order to improve the yield of the phosphonylation step and to access the 3-dehydro ‘phospha’-shikimic acid, a novel and more efficient synthesis of protected ‘phospha’-shikimic acid was thus required involving a new protecting group strategy.

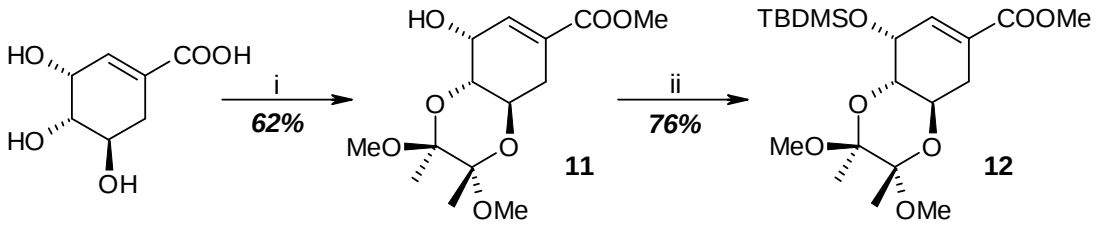
I.2.3. A alternate protecting group strategy towards shikimic acid derivatives.

I.2.3.1. *A new protecting group strategy.*

We noted previously that the difficulties encountered during formation of phosphonate **9** could be due to its protecting group pattern that is the acetyl groups which were labile under the conditions used (triethylamine with heating). As a consequence, protecting groups unaffected under nucleophilic and basic conditions, such as ketals or silyl ethers, were used in an attempt to introduce the dialkyl phosphonate in better yields.
Moreover, protection of the hydroxyl groups of the shikimic acid had to be done in a manner to access specifically the hydroxyl group belonging to C-3 and alter it in a chemoselective manner to afford the dialkyl (4S,5R)-dihydroxy-3-oxo-1-cyclohexene-1-phosphonate.

To begin with, a regioselective protection was needed and specifically a trans-vicinal protection of the hydroxyl groups belonging to C-4 and C-5 of the shikimic acid[92, 93] in order to protect independently the hydroxyl group belonging to the C-3.

We therefore started the synthesis with esterification and protection of shikimic acid as the methyl ester-4,5-trans diketal **11** in one step according to a published procedure[92] (Scheme **22**).

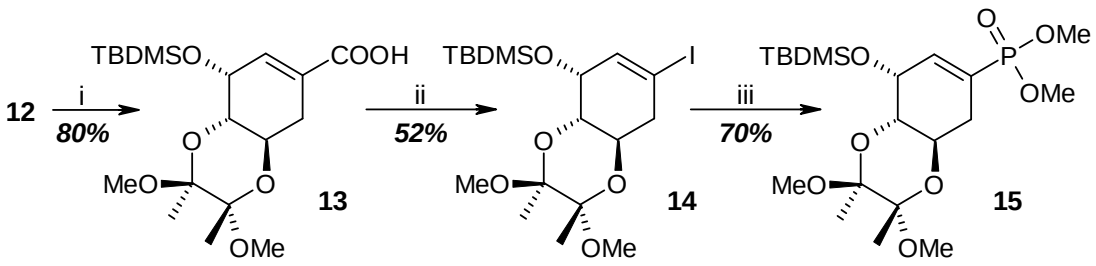


Scheme 22. *Reagents & conditions:* (i) $CH(OMe)_3$, 2,3-butanedione, CSA_{cat}, MeOH; (ii) TBDMSCl, imidazole , CH_2Cl_2.

The regioselective protection of the trans-vicinal diol was achieved in presence of butane-2,3-dione, a catalytic amount of (+)-camphorsulfonic acid (CSA), and trimethyl orthoformate in methanol under reflux (90°C) for 72h to give compound **11** in 62% yield after purification. The protected cis-vicinal diol is also obtained but not characterized and used to recycle compound **11**. Unfortunately, even using the same conditions, the yields were not fully comparable to the literature yields of 80 and 87%.[92, 93]

Silylation of the 3-OH was carried out reacting compound **11** with *tert*-butyldimethylsilyl chloride in anhydrous CH_2Cl_2, in presence of imidazole and a catalytic amount of DMAP for 24h at room temperature to give silylated compound **12** in 76% yield.[93]

I.2.3.2. *Iododecarboxylation and phosphonate introduction: key steps towards 3-dehydro-'phospha'-shikimic acid derivatives.*



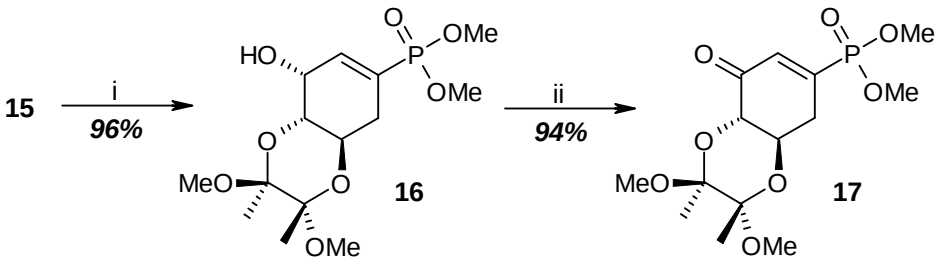
Scheme 23. *Reagents & conditions:* (i) NaOH 0.5M, MeOH; (ii) (a) oxalyl chloride, DMF_{cat}, anhydrous CH_2Cl_2; (b) sodium salt of N-hydroxypyridine-2-thione, $DMAP_{cat}$, CF_3CH_2I, anhydrous CH_2Cl_2, irradiation by a 250W floodlamp; (iii) dimethylphosphite, $Pd(PPh_3)_4$, anhydrous NEt_3, anhydrous toluene.

Saponification of protected shikimic acid **12**, followed by the two key steps iododecarboxylation and phosphonate introduction afforded protected 'phospha'-shikimic acid **15** (Scheme **23**).

Demethylation of compound **12** was carried out in a 1:1 mixture of aqueous NaOH (0.5M) and MeOH to give free acid **13** in 80% yield after purification. The latter compound was iododecarboxylated as described earlier to furnish the iodo derivative **14** which was obtained in 39% yield. However, 25% of the starting material **13** was recovered. This could be due to the quality of the oxalyl chloride used and/or the length of time the sample was irradiated. Based on the recovered starting material, the yield of this iododecarboxylation step is 52%.

Introduction of the phosphonate group was achieved under the same conditions as described earlier to obtain phosphonate derivative **15**. As expected, the new desired phosphonate derivative **15** was obtained in an increased 70% yield thus making this protecting group pattern a superior choice for the palladium coupling step.

At this stage of the synthesis, five steps from the shikimic acid, the overall yield of the synthesis of intermediate **15** was 14% which unfortunately would mean no improvement overall in the generation of 'phospha'-shikimic acids **1** and **2**. However, this new protecting group strategy allowed for a significant improvement in the yield of the phosphonate introduction step.



Scheme 24. *Reagents & conditions:* (i) TBAF, THF; (ii) IBX, anhydrous acetone, reflux.

Cleavage of the silyl ether was achieved under anhydrous conditions with tetrabutylammonium fluoride in THF to afford the deprotected alcohol **16** in 96% yield (Scheme **24**). The crystal structure of dimethyl (3R,4S,5R)- 3-hydroxy-4,5-(2,3-dimethoxy-butan-2,3-dioxy)-cyclohex-1-ene-1-phosphonate **16** was determined and is shown in Figure **9**.

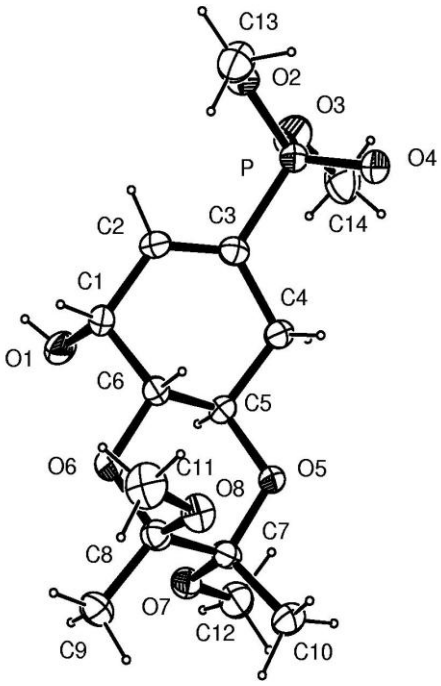
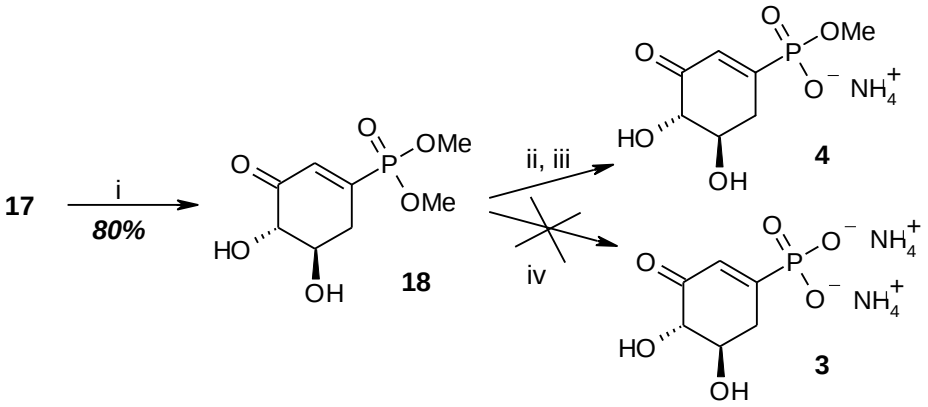


Figure 9. ORTEP-generated structure of alcohol **16**.

Oxidation of **16** was carried out by a simple and efficient procedure[94], using IBX a mild oxidant, and proceeded in high yields affording the protected 3-dehydro-'phospha'-shikimic acid derivative **17**. Following this procedure, alcohol **16** was dissolved in acetone, IBX was added to the solution which was heated to 65°C for 2 to 3 hours to give the ketone **17** in 94% yield.



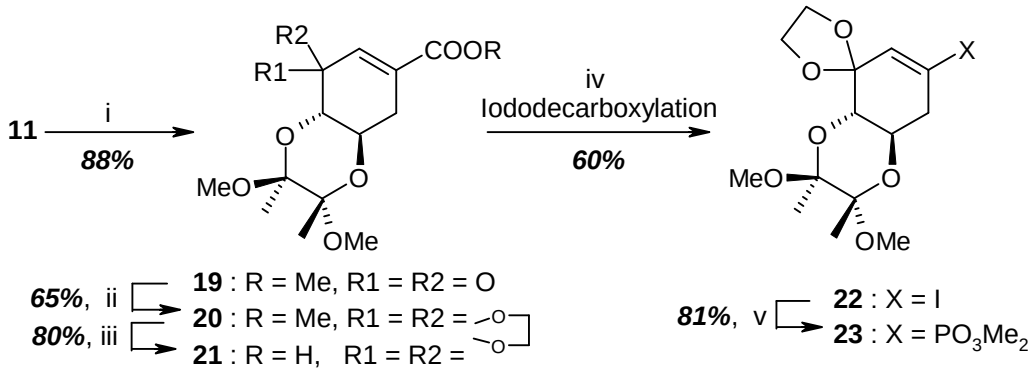
Scheme 25. *Reagents & conditions:* (i) TFA/H_2O : 9/1; (ii) NaI, anhydrous acetone, reflux; (iii) gpc; (iv) TMSBr, lutidine, anhydrous CH_2Cl_2.

The first deprotection step (Scheme **25**) was the acidic hydrolysis of the 4,5-trans diketal derivative **17** with aqueous trifluoroacetic acid which afforded after flash chromatography the trans-diol **18** in 80% yield. The following deprotection step to achieve was the partial cleavage of the dimethylphosphonate **18**. Three attempts were carried out without giving any results (aqueous ammonia (10% w/w), sodium hydroxide (0.05 M) and thiophenol (7 eq)/NEt_3 (14 eq). Finally, monomethyl ester **4** was successfully generated by iodide-mediated *O*-alkyl cleavage[91] and purification by gel permeation chromatography.

The total cleavage of the dimethylphosphonate was regrettably not achieved from compound **18**. Indeed the usual conditions, bromotrimethyl silane and lutidine in anhydrous DCM, were thought not to be adequate conditions for the deprotection of this compound bearing an enolisable carbonyl group. Protection of the carbonyl group was thus a necessity.

I.2.4. Route B.

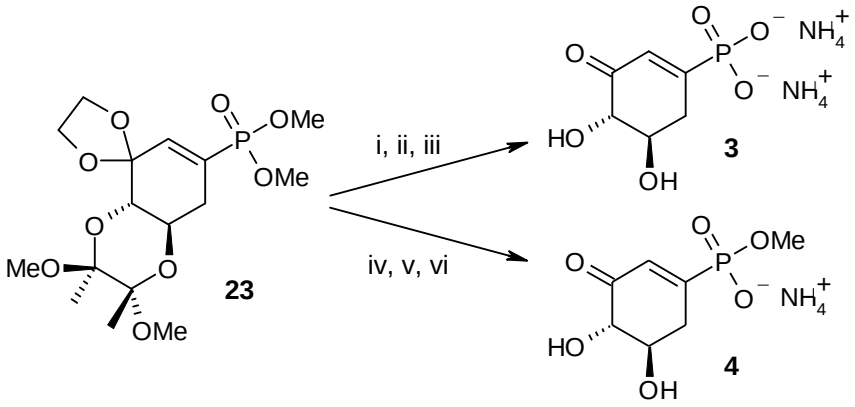
Route B involved oxidation and protection of the ketone before the iododecarboxylation and phosphonylation steps (Scheme **26**). Route B gave access to target compounds **3** and **4** as it allowed the total cleavage of the methyl phosphonate esters (Scheme **27**).



Scheme 26. *Reagents & conditions:* (i) IBX, anhydrous acetone, reflux; (ii) 2-bromoethanol, *t*-BuOK, DMF; (iii) NaOH 0.5M, MeOH; (iv) (a) Oxalyl chloride, DMF_{cat}, anhydrous CH_2Cl_2; (b) sodium salt of *N*-hydroxypyridine-2-thione, $DMAP_{cat}$, CF_3CH_2I, anhydrous CH_2Cl_2, irradiation by a 250W floodlamp; (v) dimethylphosphite, $Pd(PPh_3)_4$, anhydrous NEt_3, anhydrous toluene.

IBX-oxidation of the protected shikimic acid **11** possessing the free hydroxyl group at C-3 afforded the keto-derivative **19** in 88% yield, which was then protected by acetalization of the carbonyl group with 2-bromoethanol under basic conditions[95] to afford the dioxolane **20** in 65% yield. The carboxylic methyl ester **20** was saponified under basic conditions to yield the free acid **21** in 80% yield. The Hunsdiecker-Barton iododecarboxylation of compound **21** into its iododerivative **22** was carried out and afforded a slightly better yield than those obtained previously (60% yield, based on consumed starting material). The phosphonate coupling of compound **22** with dimethylphosphite under usual conditions afforded compound **23** in an increased yield of 82%.

After six steps, using the shikimic acid as a starting material, the overall yield for the formation of the protected 3-dehydro-'phospha'-shikimic acid **23** was 14%. Again, the overall yield of this synthesis was not improved. This is because the necessary protecting group steps proved not to be quantitative and time restrictions prevented their optimization in this project. It may be conceivable that optimized conditions may improve the overall yield significantly. However, this protecting group pattern offered a superior route to carry out the iododecarboxylation and the phosphonylation reactions in better yields.



Scheme 27. *Reagents & conditions:* (i) TMSBr, lutidine, anhydrous CH_2Cl_2; (ii) TFA/H_2O : 9/1; (iii) gpc; (iv) TFA/H_2O : 9/1; (v) NaI, anhydrous acetone, reflux; (vi) gpc.

Compound **23** was first reacted with TMSBr and lutidine in anhydrous CH_2Cl_2 to give the intermediate phosphonic acid which was subjected to acidic deprotection (aqueous TFA) of the ketals and was then purified by gel permeation chromatography to afford target compound **3**.

To obtain target compound **4**, deprotection of the carbonyl and hydroxyl groups was first achieved with aqueous TFA. Then, mono-demethylation of the crude intermediate **18** was carried out using sodium iodide in dry acetone under reflux conditions to afford the sodium salt of compound **4**. Purification by gel permeation chromatography afforded the ammonium salt of compound **4**.

I.2.5. Dehydroquinase assay

Dehydroquinase, which catalyses the reversible dehydration of 3-dehydro-quinate into 3-dehydroshikimate, is involved in the biosynthetic shikimate pathway and in the catabolic quinate pathway (as shown in Figure **2**).

Inhibitory activities of target compounds **1**, **2**, **3** and **4** in a dehydroquinase assay[96] could not be assessed due to time constraints but will be shortly investigated in the laboratory. Type I and type II dehydroquinase enzymes will be assayed by monitoring the increase in absorbance at 234 nm in the UV spectrum due to the absorbance of the enone-carboxylate chromophore of 3-dehydroshikimate.

I.3. Conclusion.

We achieved a novel extension of the Hunsdiecker-Barton iododecarboxylation to vinyl-, in particular trihydroxy- and dihydroxyketo-cyclohexenylcarboxylic acids with different protecting groups. Our application of the iododecarboxylation in combination with the phosphonylation represents a short and effective route to ‘phospha’-shikimic acid and its derivatives. As a consequence, our approach could be used for the synthesis of phospha-isosteres of various bioactive molecules. We therefore decided to apply our strategy to the synthesis of phospha-isosteres of the influenza neuraminidase inhibitor Oseltamivir as described in the following chapter.

Efficient synthesis of novel, highly active phospha-isosteres of the Influenza neuraminidase inhibitor Oseltamivir.

I.4. Theoretical part.

I.4.1. Influenza.

I.4.1.1. *Introduction to influenza viruses*

Influenza is an infectious disease caused by enveloped RNA viruses of the Orthomyxoviridae family, the Influenza viruses (A, B and C). Influenza A viruses are the cause of all flu pandemics and are known to infect birds, mammals and humans. The natural host for these viruses are wild birds. Influenza B viruses are known to infect humans and seals and influenza C viruses are known to infect humans and pigs. In humans, the site of infection by influenza A virus is the respiratory epithelium lining the trachea. Thus influenza is a respiratory tract infection and the common symptoms in humans are fever, headache, sore throat, coughing, muscle pain and fatigue. In certain cases, influenza can cause pneumonia which can be fatal. Influenza viruses that infect birds are generally described as avian influenza viruses.

Influenza A viruses are divided into subtypes based on the two main viral surface glycoproteins Hemagglutinin (H or HA) and Neuraminidase (N or NA) on the basis of which they are classified. There are 16 known hemagglutinin subtypes (H1-H16) and 9 known neuraminidase subtypes (N1-N9) and many different combinations of these proteins are possible.[97] For example, some subtypes of influenza A, like H1N1 (1918), H2N2 (1957) and H3N2 (1968) viruses have caused illness in people worldwide during the twentieth century.[98, 99]

Three prominent subtypes of the avian flu are known to infect both birds and people. These subtypes are influenza A H5, influenza A H7 and influenza A H9. All known subtypes of influenza A viruses are circulating among wild birds which do not usually fall ill when infected with avian influenza A viruses, while domestic poultry fall ill and usually die from avian influenza.
Influenza A and B viruses are further classified into strains. Avian influenza A virus strains are classified as low pathogenic or highly pathogenic on the basis of genetic features of the virus and the severity of the illness they can cause. Low pathogenic avian influenza A viruses are usually associated with mild disease in poultry whereas highly pathogenic viruses can cause severe illness and high mortality in poultry.[97]

I.4.1.2. *Influenza A virus H5N1*

More specifically, avian influenza A (H5N1) virus is considered to be highly pathogenic. This virus can cause rapid and fatal illness in many bird populations. Its spread in poultry and wild birds in many countries has raised concerns about the increased risk of transmission of H5N1 to humans, although it seems that avian influenza A viruses do not easily infect humans. They may be transmitted from animals to humans directly from intimate contact with birds, from avian virus-contaminated environments or through an intermediate host. The possibility of human to human transmission is being intensively investigated.[100, 101]

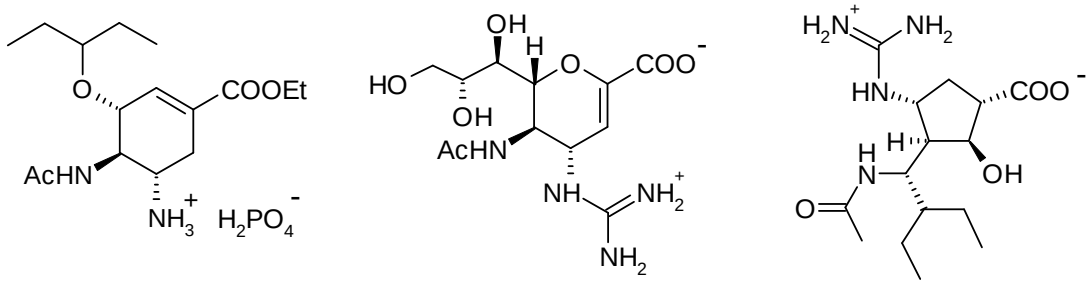
Since the pathogenic virus has been isolated from a farmed goose in 1996 in China and fatal human infection cases have been reported in 1997 in Hong Kong, the avian influenza A H5N1 virus is considered by health authorities as a potential threat to

human beings and has therefore been studied intensively worldwide. Indeed, the influenza A H5N1 virus meets two of the three criteria for a new pandemic influenza virus, it is able to replicate in human beings and a major part of the human population does not have antibodies to the virus. The third criterium is the potential spread of the virus from man to man which has so far not been observed.[102] Since then, outbreaks in many types of poultry, domestic animals and humans have been reported westwards across Asia, Europe, the Middle East and North Africa.

The WHO (World Health Organization), on the 2nd June 2009, confirmed 433 cases of H5N1 in humans in Azerbaijan, Bangladesh, Cambodia, China, Djibouti, Egypt, Indonesia, Iraq, Myanmar, Lao People's Democratic Republic, Nigeria, Pakistan, Thailand, Turkey and Vietnam, leading to 262 deaths.[103]

As mitigation measures, vaccines and antivirals are options. Definitive vaccines have not yet been made but prototypes against the H5N1 strain are being studied and produced. Vaccines against influenza always need to be updated due to the antigenic drift of these viruses and, in case of a pandemic, vaccines would not be manufactured in time, so stockpiles of antiviral drugs are necessary to combat the avian flu.[99, 104] There are 5 different classes of compounds active against influenza A virus, with some being currently available and some being developed:[105]

- Neuraminidase inhibitors such as Oseltamivir, Zanamivir and Peramivir (which is still being studied),



Tamiflu™, Oseltamivir Relanza™, Zanamivir Peramivir

Figure 10. Neuraminidase inhibitors.

- M2 ion channel blockers such like amandatine and rimandatine, which were the first antiviral treatments for influenza (Figure **11**),

- IMP dehydrogenase inhibitors such as ribarivin and viramidine (Figure **11**),

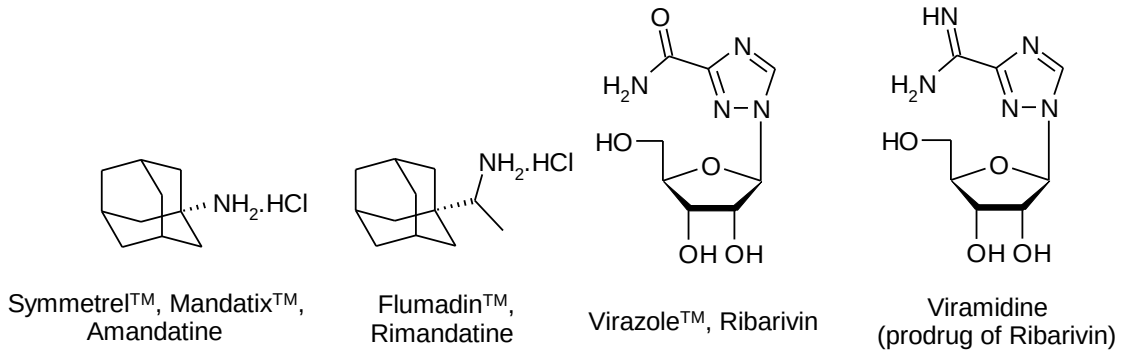


Figure 11. M2 ion channel blockers and IMP dehydrogenase inhibitors.

- Interferones (which are natural proteins produced by the cells of the immune system) and siRNAs,
- RNA polymerase inhibitors such as T705 and flutimide (Figure **12**).

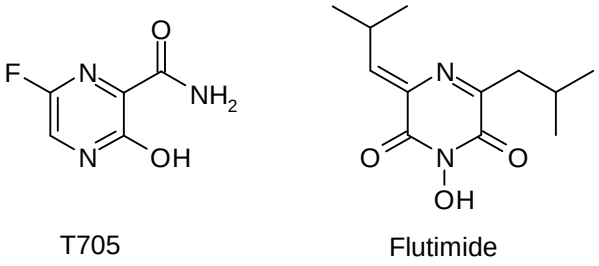


Figure 12. RNA polymerase inhibitors.

I.4.1.3. *The role of neuraminidase and hemagglutinin in influenza virus replication*

The influenza virus membrane contains two surface glycoproteins (hemagglutinin and neuraminidase) which give the virus the ability to cause the disease, as illustrated below in Figure **13**. The hemagglutinin binds to the terminal sialic acid found on the host cell's surface glyco-proteins allowing the influenza virus to attach and penetrate the host cell. Following cell entry, the M2 protein, located in the viral envelope, allows acid to enter the virus, thus enabling the release of the virus genetic material (the RNAs and polymerase enzymes) into the host cell. Then the ribonucleic acid strands are replicated in the nucleus of the infected host cell and packaged into new virions which migrate to the cell membrane as they are produced. The neuraminidase proteins intervene at this

stage. They cleave the terminal sialic acids from the cell surface proteins in order to release the new virus which will thus propagate the infection by invading other host cells.

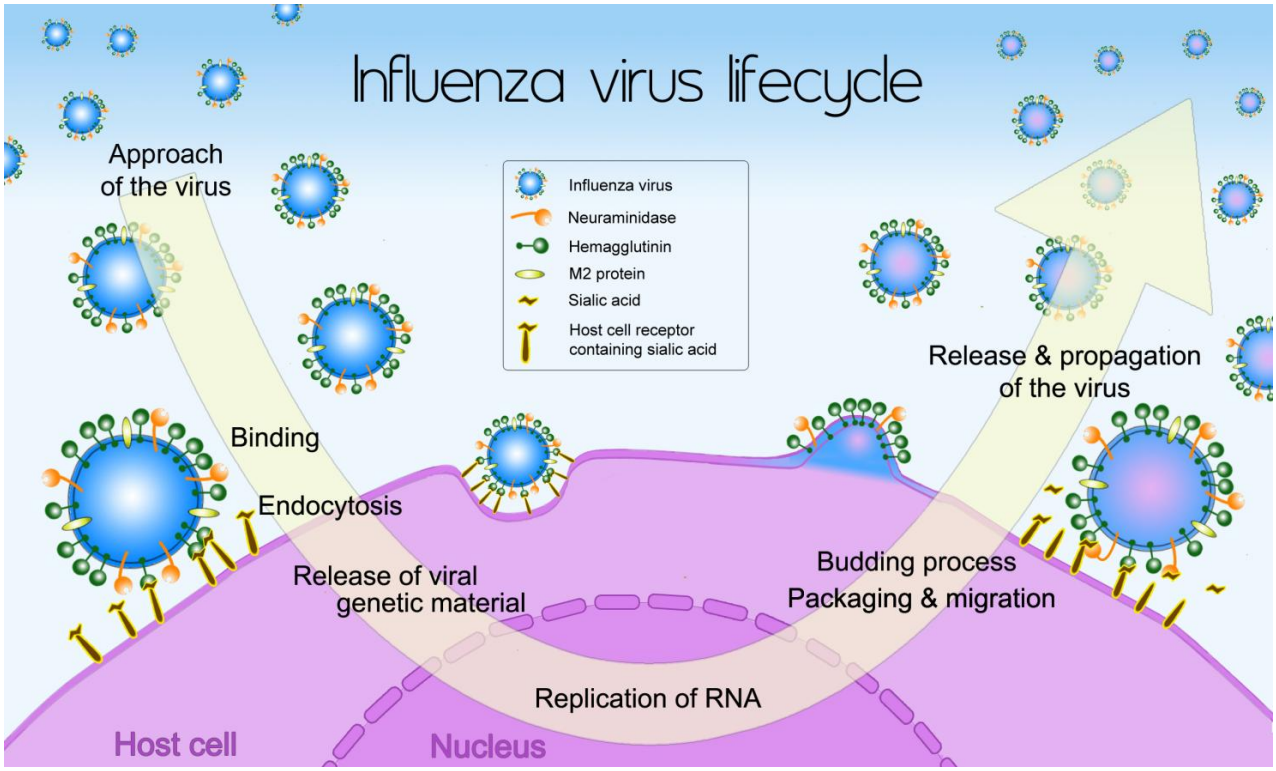


Figure 13. Influenza virus life cycle.

The M2 protein inhibitors, the amandatines which interfere with viral uncoating inside the cell, are associated with some toxic effects and with rapid emergence of drug-resistance. In consequence, much work has been focused on the design of neuraminidase inhibitors for which the mechanism of action is illustrated in Figure **14**[104]. Designed to be analogues of sialic acid, the natural neuraminidase substrate, or structurally mimicking the oxocarbenium cation intermediate during sialic acid hydrolysis by influenza virus neuraminidase, NA inhibitors are, in contrast to the amandatines, less likely to promote the development of drug-resistant influenza and less toxic.[104-106]

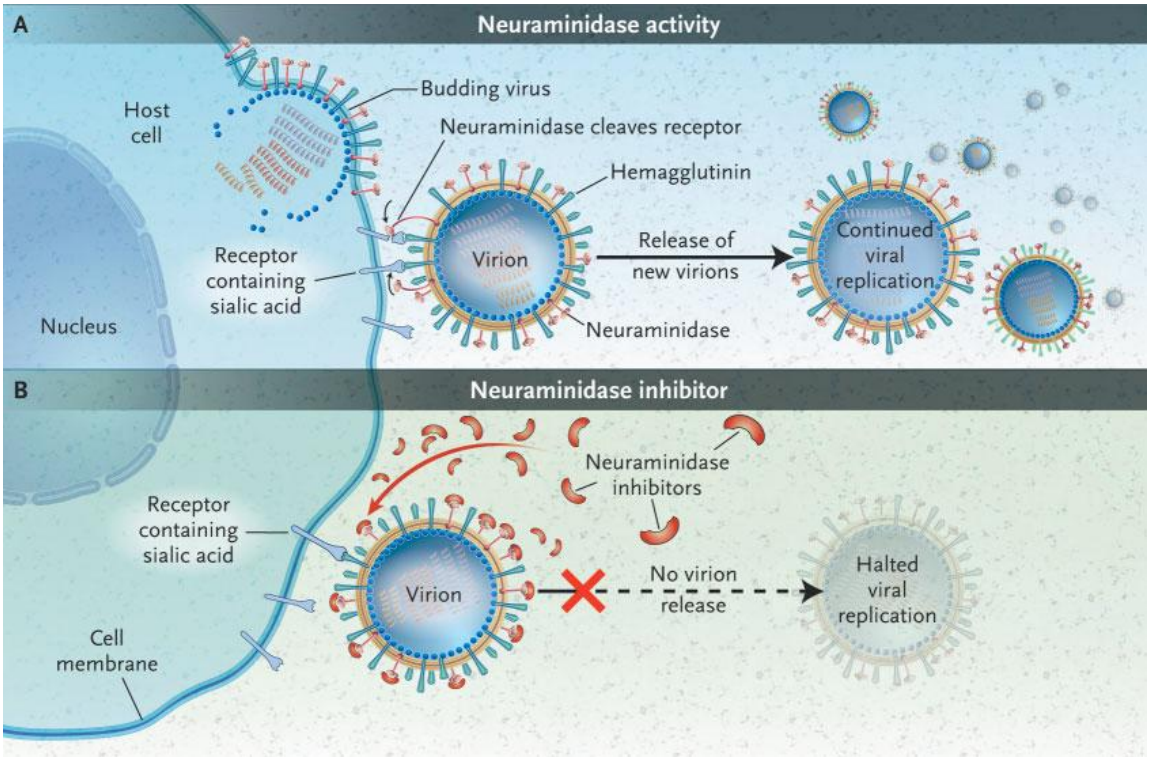


Figure 14. Mechanism of action of Neuraminidase Inhibitors: Panel A shows the action of neuraminidase in the continued replication of virions in influenza infection. The replication is blocked by neuraminidase inhibitors (Panel B), which prevent virions from being released from the surface of infected cells (Figure taken with permission from the *New England Journal of Medicine* **2005**, *353*, 1363).

I.4.1.4. *The influenza virus haemagglutinin membrane glycoprotein*

As described previously, the haemagglutinin glycoprotein (HA) of the influenza virus membrane is responsible for binding of the virus to cell-surface receptors to initiate virus infection. The structure of the influenza virus HA complexed with its receptor (sialic acid) was reported in the late 1980's by Wiley and co-workers.[107]

The haemagglutinin glycoprotein of influenza virus is a trimer, shaped as an elongated cylinder of 135 Å in length with a triangular cross-section varying in radius from 15 to 40 Å. As an illustration, the structures of H5 (A/Dk/Sing/97) avian and H9 (A/Sw/9/98) swine HA are shown below.[109]

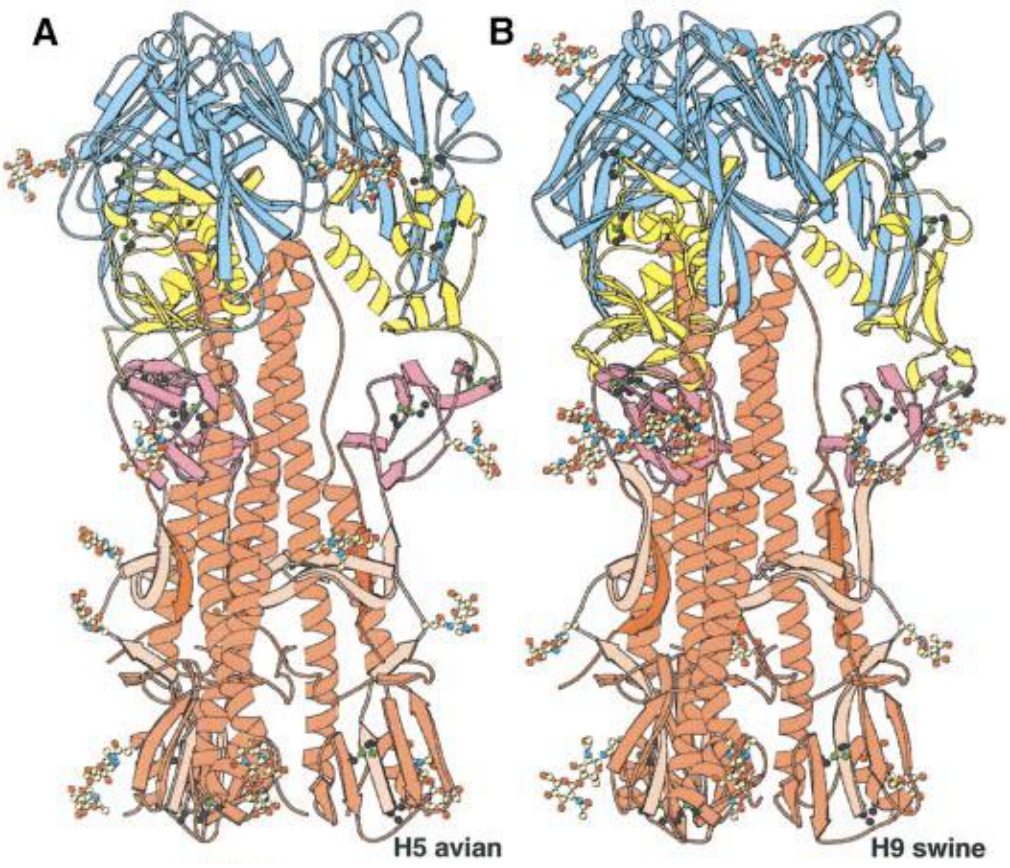


Figure 15. Ribbon diagram of the trimer of H5 (A/Dk/Sing/97) avian HA colored by subdomains: receptor subdomain R (blue), vestigial enzyme subdomain E' (yellow), HA_2 stem F subdomain (red), F' subdomain HA_1 1-52 (pink), F' subdomain HA_1 275-307 (purple). Oligosaccharides are coloured by atom type. (B) Trimer of H9 swine (A/Sw/9/98) HA. (Figure taken with permission from the *Embo Journal* **2002**, *21*, 865).

Each of the identical subunits consists of two disulfide-linked glycopolypeptides, categorized as HA_1 and HA_2. The HA structure is divided into two distinct regions:[108]

- a long fibrous region, containing residues from both HA_1 and HA_2, consisting of a triple-stranded coiled-coil of α-helices anchored in the membrane by HA_2.
- a globular region of antiparallel β-sheets which sits on the top of the fibrous region and contains residues only from HA_1. It contains the host-receptor binding site and the variable antigenic determinants. Due to the presence of these antibody binding sites, HA is a key target for the human host immune system. During antigenic drift, mutations in HA structure preventing antibodies to bind, allow the virus to cause news epidemics. As a consequence these antibody-binding sites are important targets for vaccine development.

There are two different linkages between sialic acid and the penultimate galactose residues of carbohydrate side chains found in nature, the Neu5Ac α(2,3)-Gal and Neu5Ac α(2,6)-Gal glycosidic linkages.

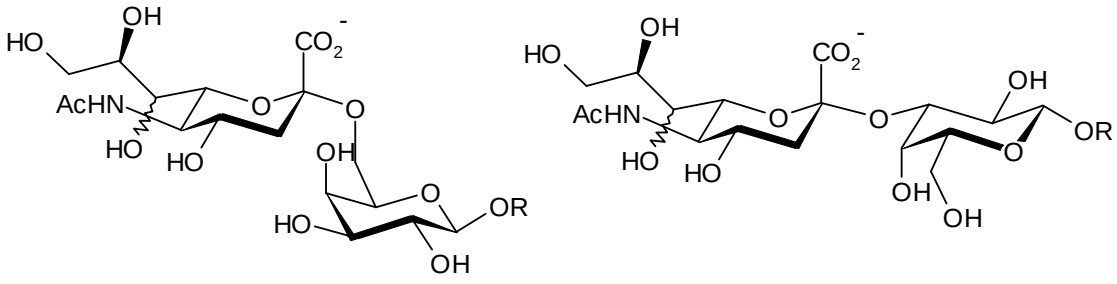


Figure 16. Naturally occurring α(2,3)- and α(2,6)-sialoglycoconjugates.

Extensive surveys of influenza viruses isolated from a variety of species have shown that linkage recognition specificity correlates with species specificity for infection. HAs show differences in the specificity of receptor binding based on the recognition of sialic acids, either α(2,3)- or α(2,6)-linked to galactose. HAs of avian viruses prefer sialic acid (2,3)-linked, which is predominant in avian enteric tracts where these viruses replicate. By contrast, the major linkage found in the human respiratory tract is a α(2,6)-linkage and perhaps consequently HAs of human viruses preferentially recognize sialic acid in α(2,6)-linkage. Swine viruses recognize sialic acid in either linkage.[110-116] Significant differences have been observed between the conformations of α(2,3)- and α(2,6)-linked sialosides when binding to HAs.[117, 118]

This receptor-binding specificity suggests that HAs of avian influenza viruses need to adapt to infect and replicate in different species. Consequently, the conversion of avian HA to one that preferentially recognize sialic acid in an α(2,6)-linkage to terminal galactose residues of glycoconjugates is an important step in the generation of pandemic strains. The HA mutations responsible for the binding of H5N1 influenza A viruses to human-type sialoglycoconjugate receptors have been addressed by various methods, which showed that few amino acid changes would allow the avian virus HA to switch from recognizing preferentially a α(2,3)-linkage to a α(2,6)-linkage.[119-121]

I.4.1.5. *The influenza virus neuraminidase membrane glycoprotein*

As described previously, neuraminidase removes sialic acid from cell-surface receptors to facilitate virus release and propagation to uninfected cells. There are nine subtypes for neuraminidase (N1-N9) which belong to two phylogenetically distinct groups.[122] Group-1 contains N1, N4, N5 and N8 subtypes and group-2 contains N2, N3, N6, N7 and N9.

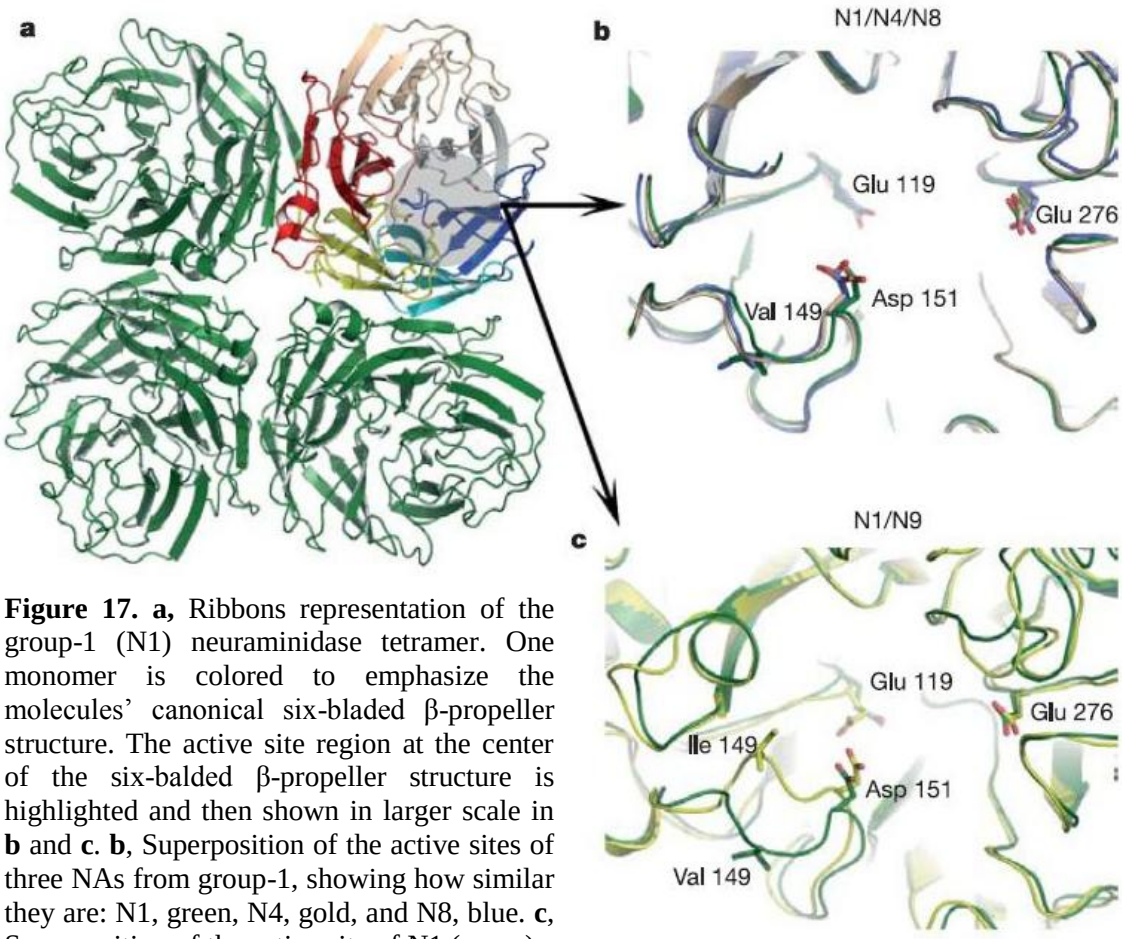


Figure 17. a, Ribbons representation of the group-1 (N1) neuraminidase tetramer. One monomer is colored to emphasize the molecules' canonical six-bladed β-propeller structure. The active site region at the center of the six-balded β-propeller structure is highlighted and then shown in larger scale in **b** and **c**. **b**, Superposition of the active sites of three NAs from group-1, showing how similar they are: N1, green, N4, gold, and N8, blue. **c**, Superposition of the active site of N1 (green) and N9 (yellow) NAs, demonstrating that N9 is different to N1 in the 150-loop region. Conserved residues such as Glu 119, Asp 151 and Glu 276 and the hydrophobic residue at position 149 are shown in stick representation (Figure taken with permission from *Nature* **2006**, *443*, 45).

The structure of the influenza virus glycoprotein neuraminidase was resolved by X-ray crystallography for the group-2 neuraminidases N2 and N9 subtypes in the 1980's,[123, 124] and more recently (within the last decade), the crystal structures of three group-1 neuraminidases from the N1, N4 and N8 subtypes have been determined.[122, 125]

The neuraminidase structure is a tetramer with a box-shaped head (100x100x60 Å). Each monomer is composed of six topological antiparallel β-sheets arranged in a propeller formation. The N1 neuraminidase tetramer is shown in Figure **17a**.[122]

The recent structural comparison of group-1 and group-2 neuraminidases, in complex with and without their inhibitors (oseltamivir, zanamivir, DANA and peramivir), illustrates that their active sites are almost identical (Figure **17c**[122]).

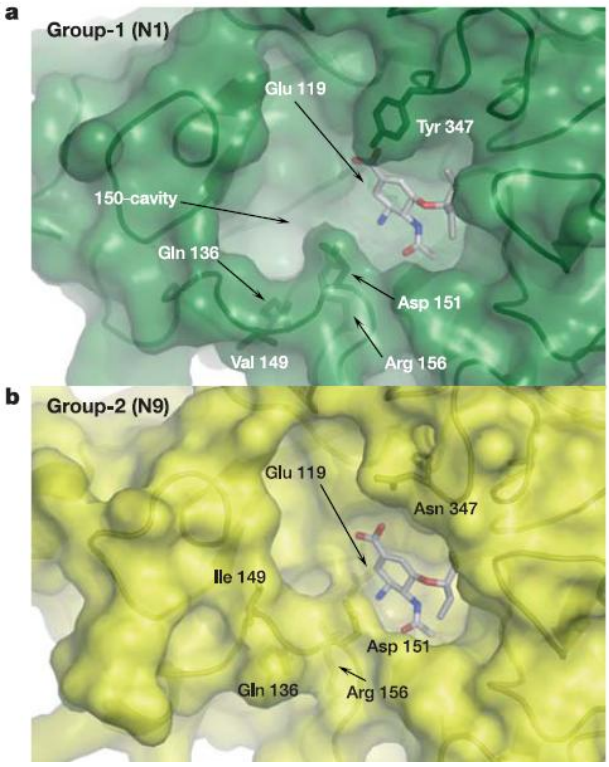


Figure 18. Molecular surfaces of group-1 and group-2 NAs with bound oseltamivir showing the 150-cavity in the group-1 structure that arises because of the distinct configuration of the 150-loop. **a, b,** N1 (a, green) and N9 (b, yellow) shown in surface representation with the protein main shown in 'worm' representation (Figure taken with permission from *Nature* **2006**, *443*, 45).

However, some specific conformational differences can be observed between the two groups centred around the '150-loop' (residues 147-152) which opens a cavity adjacent to the active site in the crystal structures of group-1 NAs (Figure **18**).[122] This open form of the '150-loop' implicates the potential of new opportunities for antiviral drug design.[122, 125, 126]

In contrast to the receptor-binding specificity observed for HAs towards α(2,3)- and α(2,6)-linked sialylgalactosides depending on infected species, it is less clear what NAs' preferences are towards α(2,3)- or α(2,6)-linked sialylgalactosides during hydrolysis of

the ketosidic linkage. It has been shown that avian influenza virus NAs have a pronounced preference for α(2,3)-linked sialylgalactosides. Regarding human influenza virus NAs, they act on both linkages but still with a preference for α(2,3)-linked sialylgalactosides.[127-129] These data are generally obtained by analyzing the reaction kinetics for the hydrolysis of the two substrates by a given neuraminidase or whole virus.

By synthesizing sialoglycoconjugate mimetics (compounds **46** and **54**), we suggest inhibitor-based tools for the characterization of influenza virus NAs. Structural comparaison of group-1 and group-2 neuraminidases in complex with our sialoglycoconjugate mimetics **46** and **54** could give precious information on receptor binding specificity of the neuraminidase active site.

I.4.2. Oseltamivir.

I.4.2.1. *A neuraminidase inhibitor.*

Oseltamivir was developed by Gilead Sciences[84, 130, 131] and is currently marketed by Hoffmann-La Roche under the trade name Tamiflu™.[85, 132] It is used in the treatment and prophylaxis of both Influenza A and B viruses which includes the H5N1 virus. Oseltamivir is the first commercially developed orally active neuraminidase inhibitor. It is an ethyl ester prodrug, which is enzymatically hydrolysed to the free carboxylate which is the active metabolite.

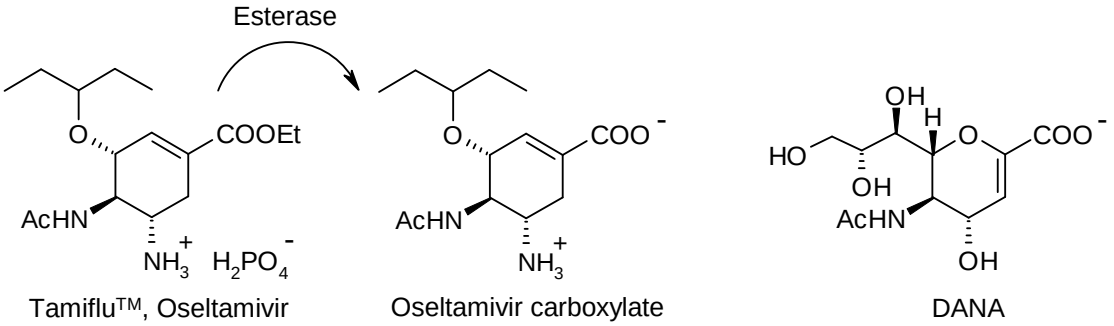
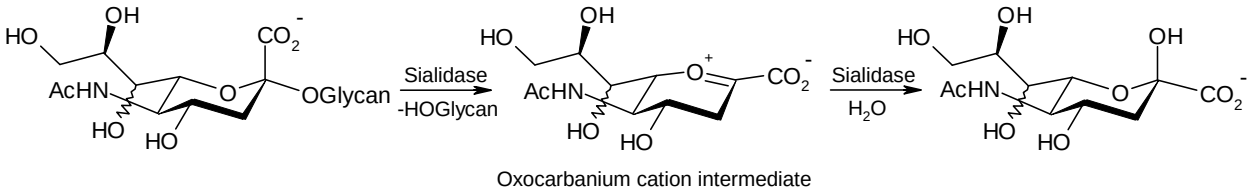


Figure 19. Oseltamivir, DANA.

Oseltamivir is the successful result of structure-based inhibitor design using the crystallographic data of two of the group-2 neuraminidases N2 and N9.[123, 124] Oseltamivir (like DANA (Figure **19**) and zanamivir (Figure **10**)) mimics the

oxocarbenium-ion transition state occurring during sialoside hydrolysis by influenza virus neuraminidase.



Scheme 28. Sialoside hydrolysis by influenza virus neuraminidase.

Studies of the mechanism of sialoside hydrolysis by influenza virus neuraminidase demonstrated that to allow hydrolysis, the $^{2}C_{5}$ chair conformation of the sialoside pyran ring is distorted towards a boat conformation through complexation of the carboxylate in equatorial position with three arginine residues in the enzyme's active site (the arginine triad conserved in sialidases). Following protonation of the glycosidic oxygen in axial position, the glycosidic bond breaks and the system is proposed to run through an oxocarbenium-ion type intermediate with half chair conformation before being trapped by a water molecule to release the sialic acid, product of the hydrolysis.[133, 134] The oseltamivir carboxylate and its interaction with neuraminidase residues are shown in Figure **20**.

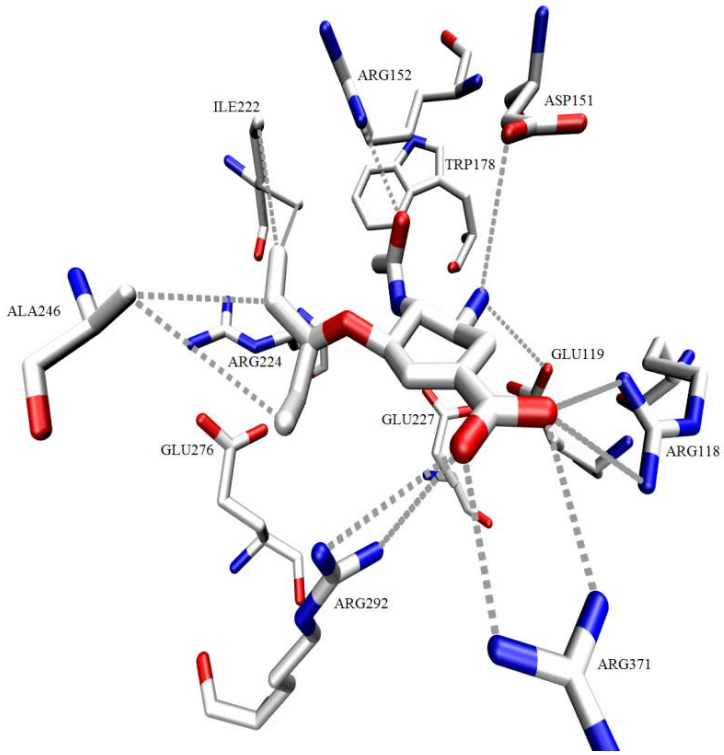


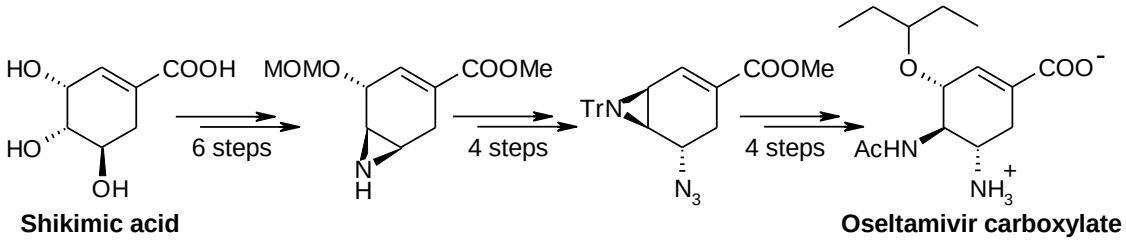
Figure **20**. Neuraminidase active site designations and important interactions of Oseltamivir carboxylate (Figure drawn using Visual Molecular Dynamics (VMD) with the pdb 2ht7, crystal structure of N8 neuraminidase in open complex with oseltamivir).

Different types of interactions are occurring between the ligand and the active site. Two are charge-charge interactions. The first one is the carboxylate group acting as a negatively ionisable group (at the C1 position) in interaction with three positive charges belonging to Arg 118, Arg 292 and Arg 371 (the arginine triad). The second interaction results from the positive ionisable amino group (at the C5 position) facing three negative charges belonging to Glu 119, Glu 227 and Asp 151. The pentyl chain of the ether group at the C3 position undergoes hydrophobic interactions in the active site, the residues Ile 222, Arg 224, Ala 246 and Glu 276 surround it. The other interactions are hydrogen-bond interactions. Two are hydrogen-bond donors, the cation NH_3^+ (at the C5 position) and the N-H of the acetamide group (at the C4 position) and one is a hydrogen-bond acceptor, the carbonyl of the acetamide group.
The success of structure-based neuraminidase inhibitor design has been attributed to proposals that the active sites of the neuraminidases are highly conserved across all influenza A and B virus strains. Mutations are thus unlikely to happen, in fact they would compromise the neuraminidase activity. However, a few mutations are known to occur in resistant subtypes of influenza virus, taking place in the sites of Arg 292, His 274 and Glu 119.[98]

I.4.2.2. *Oseltamivir syntheses.*

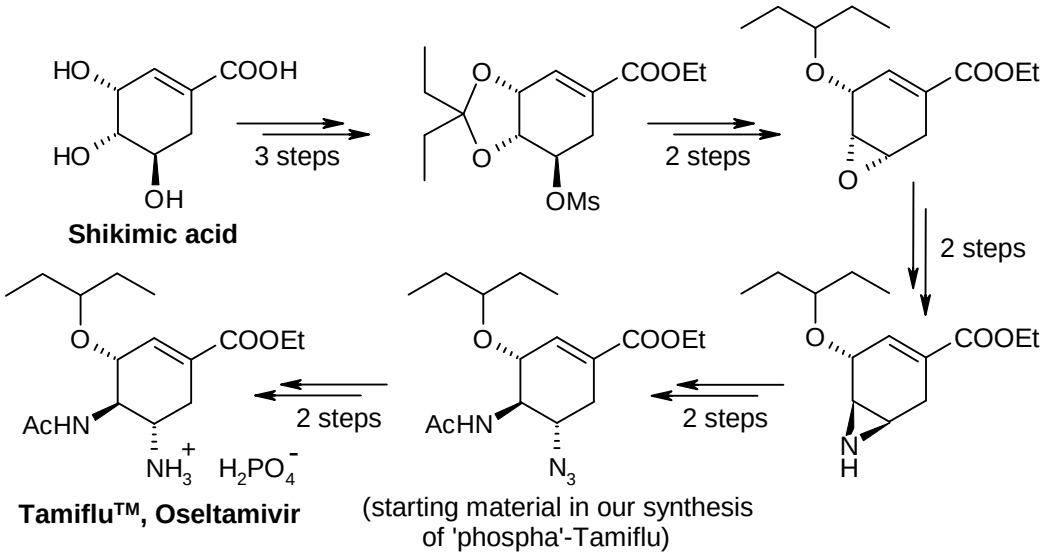
Tamiflu (Oseltamivir phosphate) has acquired importance due to its activity against both influenza A and B viruses. Indeed, Tamiflu has been stockpiled by governments worldwide in order to protect humans against a potential future pandemic. Considering the amount of Tamiflu required worldwide in the event of a pandemic, a lot of effort has been put into finding an efficient synthetic route to produce it in large quantities.

The first discovery and synthesis of Oseltamivir were made by Gilead Sciences.[84] Using (-)-shikimic acid as starting material, Oseltamivir was synthesized using a set of S_N2 reactions to obtain the required L-*xylo* ring configuration and the late introduction of the pentyloxy sidechain by opening of a tritylaziridine, allowing for introduction of sidechain group diversity which is critical in the drug discovery process (Scheme **29**).[130]



Scheme 29. Overview of Gilead's first synthesis of Oseltamivir.

The first investigated routes to synthesize Oseltamivir used (-)-shikimic acid and (-)-quinic acid as starting materials. However, (-)-shikimic acid, with its 1,2-double bond already in place and a synthesis of oseltamivir which had a higher overall yield, led it to become the starting material of choice.[131] Extensive development by Gilead Sciences and by Roche led to the current industrial synthesis of Oseltamivir phosphate from (-)-shikimic acid in a sequence of 10 steps with a 35% overall yield. The synthesis proceeds through a 3,4-pentylidene 5-mesylshikimate intermediate which is regioselectively reduced to an epoxide intermediate. The azide based stereoselective opening of the latter leads to the formation of an aziridine intermediate which is then converted to the acetamidoazide (starting material used in our synthesis of 'phospha'-Tamiflu and derivatives). Oseltamivir phosphate is then obtained by reduction of the azide and formation of the phosphate salt (Scheme **30**).[85, 131, 132, 135]



Scheme 30. Overview of the Hoffmann-La Roche Ltd industrial synthesis of Oseltamivir.

To address initial uncertainties regarding supply of (-)-shikimic acid and the hazards involved with large scale use of azides, the Roche group developed alternate approaches. An azide-free approach closely related to the industrial synthesis, a Diels-Alder approach (reaction of furan with ethyl acrylate) and an enzymatic desymmetrization approach using 2,6-dimethoxyphenol as starting material were investigated.[85, 132, 136, 137]

Nonetheless, the increasing significance of Tamiflu has prompted several renowned academic laboratories to compete in the search for an effective and elegant synthesis. As a result, a considerable number of novel syntheses of Tamiflu has been reported in recent years.[138, 139] The following section gives a short overview of these attempts to find new synthetic routes to Tamiflu.

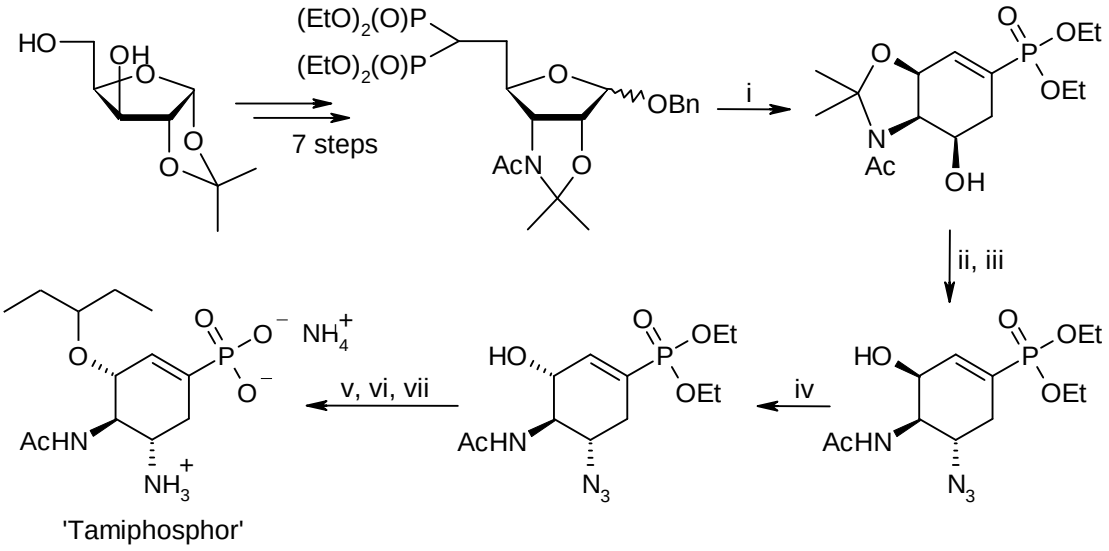
- Fang *et al.* have developed synthetic routes leading to Tamiflu and its phosphonate congener 'Tamiphosphor'.[140, 141] Following Streicher and co-workers' synthetic approach to L-xylo cyclohexenephosphonates,[1-5] their first synthesis used D-xylose as chiral precursor and an intramolecular Horner-Wadsworth-Emmons reaction as key step to form the cyclohexene core of the Tamiflu. Their second synthesis (11 steps, 21% overall yield) used bromoarene *cis*-1,2-dihydrodiol as starting material and an organometallic coupling reaction as key step.
- Corey and co-workers reported a synthesis using a catalytic and enantioselective Diels-Alder reaction developed in his group, employing butadiene and trifluoroethyl acrylate as starting materials. Oseltamivir phosphate was synthesized in 11 steps and about 30% overall yield.[142, 143]
- Shibasaki and co-workers reported enantioselective approaches using a catalytic desymmetrization of meso-aziridines synthesized from 1,4-cyclobutadiene.[144, 145] These initial synthetic routes are relatively long with very low overall yields, prompting them to develop a completely different synthetic route using a Diels-Alder reaction between siloxy diene and fumaroyl chloride and a Curtius rearrangement as key steps.[146] This route required less steps than their first and second generation syntheses. Recently, they reported a synthesis of Tamiflu (in 12 steps, 16% overall yield) using a barium-catalyzed asymmetric Diels-Alder type reaction.[147]

- Fukuyama and co-workers used an asymmetric Diels-Alder reaction between dihydropyridine and acroleine as the first step, followed by a bromolactonization and a Hofmann rearrangement as key transformations in the synthesis of Tamiflu in 11 steps and about 6% overall yield.[148, 149]
- Cong and Yao reported a ring-closing metathesis-based synthetic route to synthesize Tamiflu from L-serine as starting chiral material, in 18 steps and 16% overall yield.[150]
- Trost and Zhang reported a concise synthesis of Oseltamivir by desymmetrization of a racemic bicyclic lactone using their own Pd-ligand system, in 8 steps and an overall yield of 30%.[151]
- Kann and co-workers' route to Oseltamivir relied on the synthesis of an enantiomerically pure iron carbonyl complex. The synthesis contains 12 steps with an overall yield of 4%.[152]
- Banwell and co-workers reported a longer synthesis of Tamiflu from enantiomerically pure cis-1,2-catechol (16 steps) compared to Fang's second synthesis (11 steps).[153]
- Hayashi and co-workers reported a high yielding synthesis by three 'one-pot' operations, affording Oseltamivir in an overall yield of 57% in only 9 reactions.[154]
- Mandai and Oshitari reported an asymmetric synthesis of Oseltamivir from D-mannitol without any chromatographic purification (18 steps, 7% overall yield).[155] They reported another enantioselective synthesis from L-methionine in 18 steps and 8%.[156]
- Nie and Shi reported two different syntheses from (-)-shikimic acid very similar to Roche's industrial synthesis.[157, 158]

I.4.3. 'Phospha'-Tamiflu syntheses.

During our work on the synthesis of 'phospha'-Tamiflu, Fang *et al*. achieved the syntheses of Oseltamivir, its phosphonate congener ('phospha'-Tamiflu or 'Tamiphosphor') and the guanidine analogues.[140] These syntheses were inspired by the synthetic strategy of Streicher *et al*. towards cyclohexenephosphonates using D- and L-xylose as chiral precursors.[1-4, 6] In brief, after appropriate protection and modification of xylose, an intramolecular Horner-Wadsworth-Emmons reaction was carried out to

furnish the cyclohexene scaffold of the target compounds. Substitution of the hydroxyl group belonging to carbon 5 by an azido group with inversion of configuration, followed by inversion of configuration of the hydroxyl group at carbon 3, introduction of the ether side chain, azide reduction and saponification afforded ‘Tamiphosphor’ (Scheme **31**).



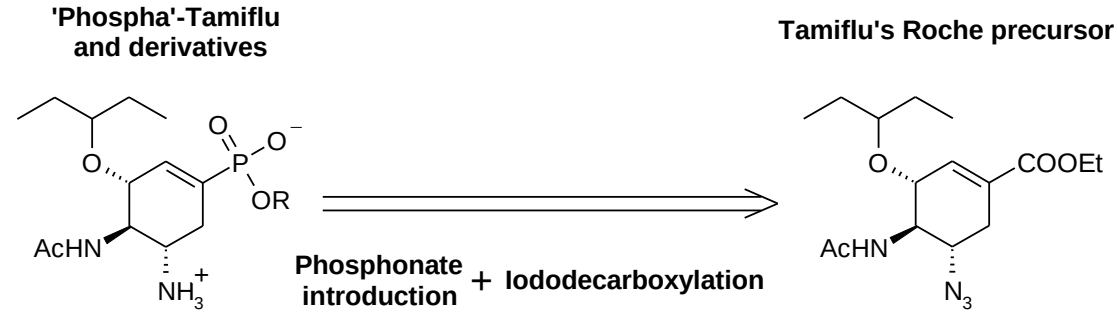
Scheme 31. Fang *et al*. synthesis of ‘phospha’-Tamiflu using D-xylose as starting material. *Reagents & conditions:* (i) H_2, Pd/C, EtOH, 25°C, 24h; NaOEt, EtOH, 25°C, 5h, 80%; (ii) $(PhO)_2PON_3$, (*i*-Pr)N=C=N(*i*-Pr), PPh_3, THF, 25°C, 48h; (iii) HCl, EtOH, reflux, 1h, 74%; (iv) Tf_2O, pyridine, CH_2Cl_2, -15 to -10°C, 2h ; KNO_2, 18-crown-6, DMF, 40°C, 24h, 71%; (v) $Cl_3CC(=NH)OCHEt_2$, CF_3SO_3H, CH_2Cl_2, 25°C, 16h, 82%; (vi) H_2, Lindlar catalyst, EtOH, 25°C, 16h, 85%; (vii) TMSBr, $CHCl_3$, 25°C, 24h, aqueous NH_4HCO_3, lyophilization, 85%.

In order to shorten this synthesis and improve the overall yield, Fang *et al*. designed another synthesis of Oseltamivir and ‘Tamiphosphor’.[141] An azide-free synthesis was developed utilizing the readily available starting material bromoarene *cis*-1,2-dihydrodiol which allows a late-stage functionalization by transformation of the bromine atom into a carboxylate or a phosphonate by the use of organometallic coupling reactions. It must be noted that like us, they used a modified Hirao’s procedure[50] to introduce the phosphonate moiety. The palladium-promoted coupling reaction of vinyl halides with dialkyl phosphites to furnish vinyl phosphonates is the obvious reaction of choice due to its convenience and effectiveness.[41, 42]

I.5. Results and discussion.

I.5.1. Strategy towards ‘phospha’-Tamiflu and its derivatives.

The first objective was to apply the strategy developed for the synthesis of ‘phospha’-shikimic acid to the synthesis of the ‘phospha’-Tamiflu. Taking advantage of the availability of the industrial precursor of Tamiflu, the acetamido-azide precursor could be used as a starting material. Our Hunsdiecker-Barton iododecarboxylation methodology and the subsequent palladium-mediated phosphonylation step should afford the ‘phospha’-Tamiflu in yields high enough to allow for derivatisation as well.

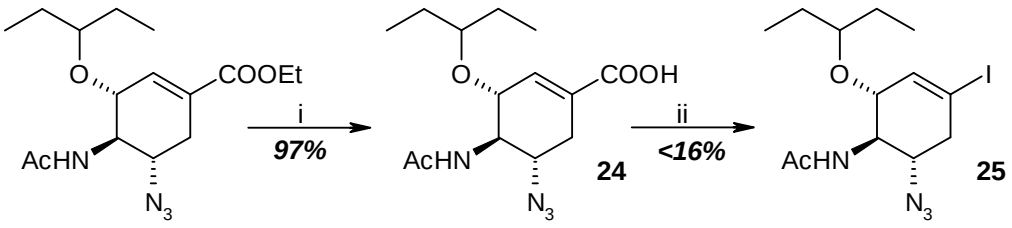


Scheme 32. Strategy towards ‘phospha’-Tamiflu and its derivatives.

I.5.2. Key steps towards the key intermediate **34**.

I.5.2.1. *A first approach.*

In order to allow the halodecarboxylation step, Tamiflu’s precursor was saponified to give the free acid **24** in 97% yield. Our tried and tested conditions for the iododecarboxylation were applied to the latter compound. Unfortunately, this step always resulted in low yields. The iodo-derivative compound **25** was difficult to purify due to the formation of many side-products with similar TLC behaviour (the side products have not been characterized). Moreover, no starting material was recovered from the reaction.



Scheme 33. *Reagents & conditions:* (i) NaOH 0.5M, dioxane; (ii) (a) oxalyl chloride, DMF_{cat}, anhydrous CH_2Cl_2; (b) N-hydroxypyridine-2-thione, $DMAP_{cat}$, CF_3CH_2I, anhydrous CH_2Cl_2, irradiation by a 250W floodlamp.

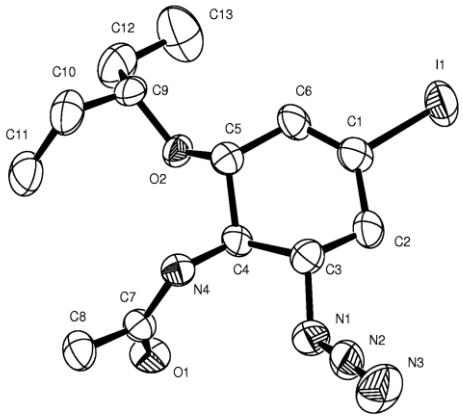
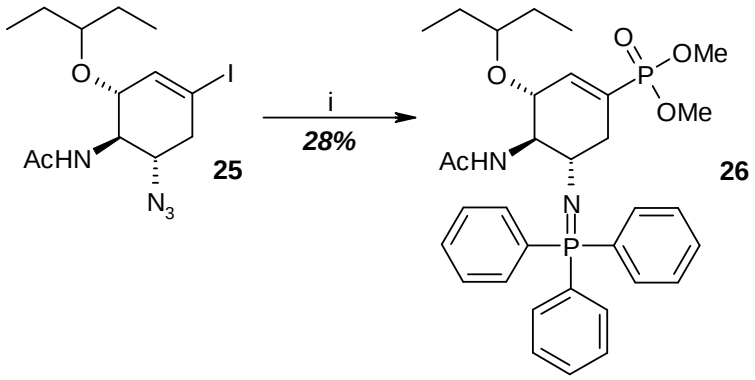


Figure 21. ORTEP-generated structure of iodo compound **25**.

With compound **25** in hand, a few phosphonylation attempts were then carried out leading to the unexpected and undesired compound **26** in moderate yields (see Scheme **34**). The use of tetrakis(triphenylphosphine)palladium(0) for the coupling of the iodo-azide **25** with dimethyl phosphonate was not the best choice for the reaction as it allowed the triphenylphosphine to convert the azide into the iminophosphorane derivative.



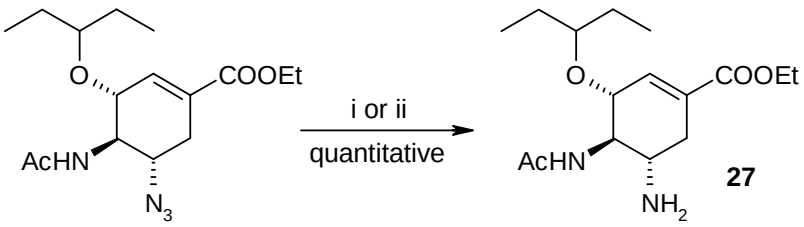
Scheme 34. *Reagents & conditions:* (i) Dimethylphosphite, $Pd(PPh_3)_4$, anhydrous NEt_3, anhydrous THF.

In order to avoid this unwelcome conversion of the azide into the iminophosphorane, phosphonylation was attempted using an alternative palladium catalyst. The use of bis(dibenzylideneacetone)palladium(0) ($Pd_2(dba)_3$) did not bring any improvement to the reaction. The iodo compound was not activated by the catalyst and showed no sign of reaction even after five hours refluxing in anhydrous THF.

Consequently, reduction of the azide to the amine followed by protection of the amine was a necessary step prior to the palladium-mediated coupling with dimethyl phosphonate. However the Hunsdiecker-Barton iododecarboxylation step needed to be optimized as well in order to gain access to a suitably amino-protected phosphonate key compound. From that, the synthesis of 'phospha'-Tamiflu and its derivatives would only require standard deprotection and alkylation steps.

I.5.2.2. *Protected amino derivatives of Tamiflu's precursor.*

The initial idea was to synthesize protected amino compounds to study their effect on the decarboxylation step. Unfortunately, due to time restriction and the project moving on, this study was not fully completed. The next paragraphs show the synthesis of three different carbamates (Boc, Fmoc and benzyl) and one acetamide (trifluoroacetamide).
The azide group of the industrial Tamiflu precursor was first reduced to the amine which was then protected as its carbamate. Azides may be converted to amines using the Staudinger reaction[159-161] (with the use of a trialkyl phosphine) or by hydrogenation (using in our case the Lindlar catalyst[162] due to the required presence of the alkene on the cyclohexene ring). Tamiflu's precursor was thus subjected independently to both reactions giving the free amine in quantitative yields, the hydrogenation being a much more convenient and easier procedure compared to the Staudinger reaction.

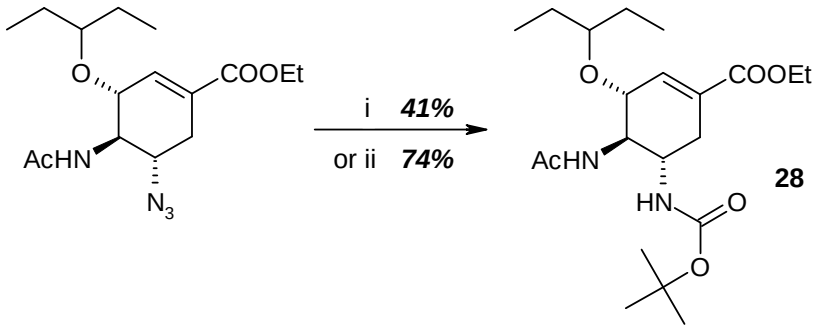


Scheme 35. *Reagents & conditions:* (i) Pd, Lindlar's catalyst, H_2, EtOH; (ii) (a) PMe_3, anhydrous THF, (b) H_2O.

Three different carbamates have been used as protecting groups[90], the *t*-butyl (Boc) carbamate cleaved by acidic hydrolysis, the benzyl (Cbz or Z) carbamate cleaved by catalytic hydrogenolysis and the 9-fluorenylmethyl (Fmoc) carbamate cleaved by β-elimination with base.

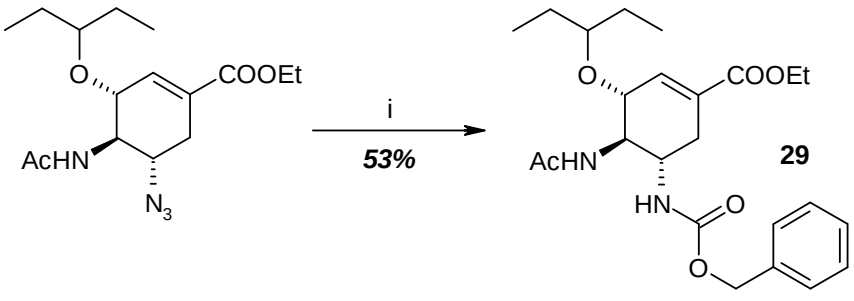
Since Sato *et al*.[163] showed that reduction of azides by hydrogenation followed by addition of di-*t*-butyl dicarbonate (Boc_2O) provided the Boc-protected amines, reduction and protection steps are frequently performed in a one-pot two stage procedure to transform azides into protected amines.

This way, the Boc-protected compound **28** was first synthesized following Afonso's procedure,[164] using the Staudinger reaction with PMe_3 and reacting the iminophosphorane formed with Boc_2O, in a moderate yield of 41%.



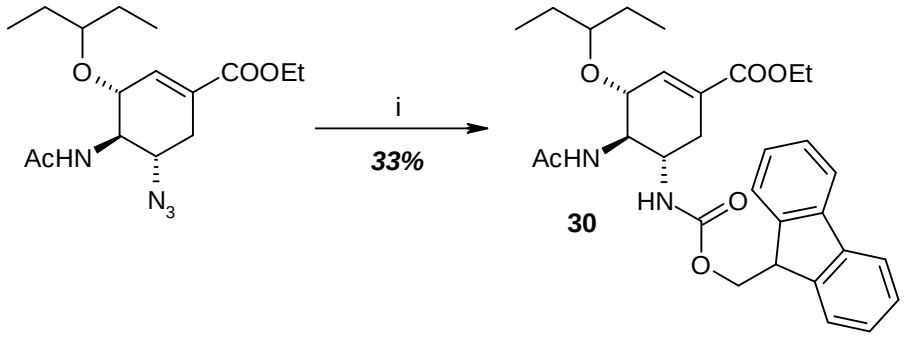
Scheme 36. *Reagents & conditions:* (i) (a) PMe_3, anhydrous THF, (b) Boc_2O, (c) H_2O; (ii) (a) PMe_3, anhydrous THF, (b) Boc-ON, (c) H_2O.

An improved procedure[165] using 2-(*t*-butoxycarbonyloxyimino)-2-phenylacetonitrile (Boc-ON) as an activated Boc-transferring agent instead of Boc-anhydride led to the Boc-protected compound **28** in an improved yield of 74 %.



Scheme 37. *Reagents & conditions:* (i) (a) PMe_3, anhydrous THF, (b) benzyl chloroformate, (c) $NaHCO_3$.

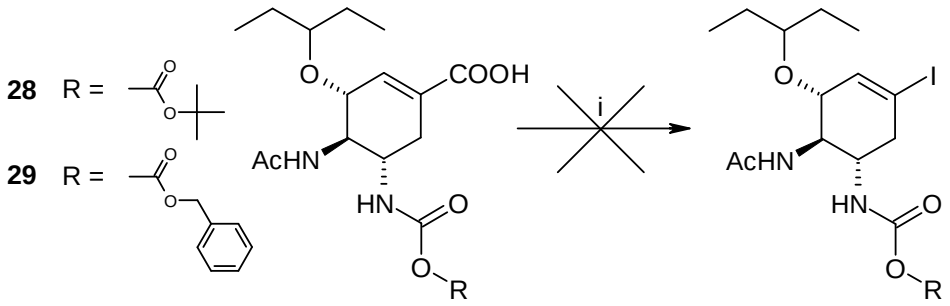
Benzyl carbamate **29** was obtained in 53% yield using a similar one-pot transformation involving reduction of the azide by PMe_3 followed by addition of benzyl chloroformate.



Scheme 38. *Reagents & conditions:* (i) (a) PMe_3, anhydrous THF, (b) FmocCl, (c) NH_4Cl.

Following the same procedure, reduction of the azide by PMe_3 and addition of 9-fluorenylmethyl chloroformate afforded 9-fluorenyl carbamate **30** in 33% yield.

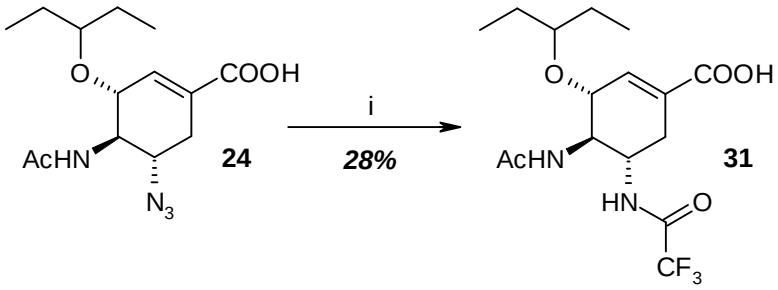
Compounds **28** and **29** were saponified under basic conditions (NaOH 0.5M) and purified by flash chromatography to give the free acid derivatives which were not characterized as they were used to ascertain the effect of the carbamate protecting groups on the iododecarboxylation. Unfortunately this reaction failed in both cases as shown below in Scheme **39**.



Scheme 39. *Reagents & conditions:* (i) (a) oxalyl chloride, DMF_{cat}, anhydrous CH_2Cl_2; (b) N-hydroxypyridine-2-thione, $DMAP_{cat}$, CF_3CH_2I, anhydrous CH_2Cl_2, irradiation by a 250W floodlamp.

Attempts were made to synthesize the amino protected compound as the trifluoro acetamide. The trifluoro acetamide derivative was synthesized from the free carboxylic acid **24** as it would be impossible to saponify the ester in presence of the trifluoro acetamide due to its lability under basic conditions. The azide was reduced with PMe_3

and the iminophosphorane thus formed was reacted with trifluoroacetic anhydride to give the trifluoro acetamide **31** in 22 % yield. Unfortunately, due to time restriction **31** was not subjected to the iododecarboxylation.

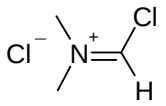


Scheme 40. *Reagents & conditions:* (i) (a) PMe_3, anhydrous THF, (b) $(CF_3CO)_2O$, (c) NH_4Cl.

I.5.2.3. *Optimised route leading to key monoalkyl phosphonate intermediate **34**.*

As shown previously, the iododecarboxylation of compound **24** always resulted in low yields. It was thought that the formation of the acyl chloride was certainly the major issue causing the halodecarboxylation step to be unsuccessful. A new experimental route was thus needed to convert the carboxylic acid into the acyl chloride. The use of oxalyl chloride with a catalytic amount of DMF leads to the formation of the highly electrophilic and cationic Vilsmeier reagent, which reacts rapidly with the carboxylic acid to form the acyl chloride and regenerates DMF.

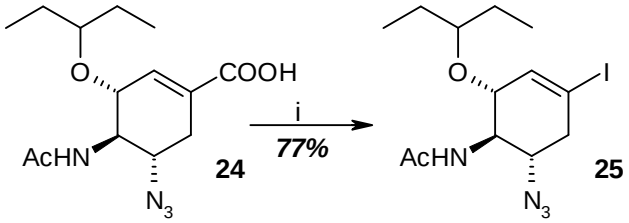
Therefore, the alternative route utilizes a stoichiometric amount of Vilsmeier reagent to react with the carboxylic acid, instead of different chlorinating agents (such as $SOCl_2$ and PCl_5), to generate the acyl chloride in situ. The Vilsmeier reagent can be formed by reaction between DMF and activated chlorinated compounds such as thionyl chloride ($SOCl_2$),[166] $POCl_3$,[167] $COCl_2$ or oxalyl chloride.[168]



Vilsmeier reagent

Formation of the Vilsmeier reagent was achieved using thionyl chloride and DMF in stoichiometric amounts. Thionyl chloride is the reagent of choice because of its lower cost, compared to oxalyl chloride, and its reaction with DMF only provides the Vilsmeier reagent and a gaseous by-product SO_2. The Vilsmeier reagent is obtained as a white powder after evaporation of SO_2 under high vacuum.

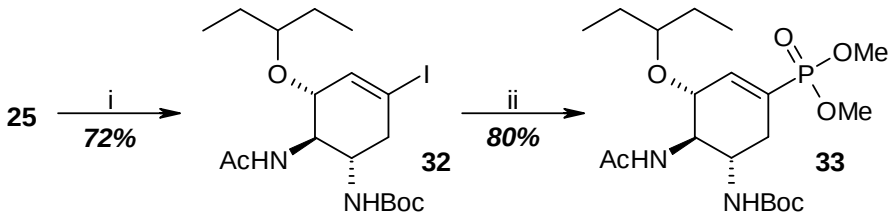
The Hunsdiecker-Barton iododecarboxylation of compound **24** was carried out following the same procedure as used previously except that in the first step oxalyl chloride in presence of a catalytic amount of DMF was replaced by a stoichiometric amount of the Vilsmeier reagent with the carboxylic acid **24**.



Scheme 41. *Reagents & conditions:* (i) (a) Vilsmeier reagent, anhydrous CH_2Cl_2; (b) N-hydroxypyridine-2-thione, $DMAP_{cat}$, CF_3CH_2I, anhydrous CH_2Cl_2, irradiation by a 250W floodlamp.

This modified procedure proved to be a success, the vinyl iodide **25** was obtained in a very good yield of 77% based on consumed starting material. It is important to note that for the second step of the halodecarboxylation procedure, the addition of the acyl chloride to the mixture of *N*-hydroxypyridine-2-thione, 2-iodo-1,1,1-trifluoroethane and a catalytic amount of DMAP in anhydrous CH_2Cl_2 must be done without further evaporation and dilution of the mixture containing the acyl chloride (as it was done when using oxalyl chloride).

In order to avoid the formation of the iminophosphorane during the palladium-mediated phosphonylation step, the azide **25** needed first to be reduced and protected. The *tert*-butyl carbamate protecting group introduced in an efficient way as described in a procedure by Ariza *et al.* [165] with the use of Boc-ON was the protecting group of choice due to its stability under basic conditions and its effective introduction.



Scheme 42. *Reagents & conditions:* (i) (a) PMe_3, anhydrous THF, (b) Boc-ON, anhydrous THF, (c) H_2O; (ii) dimethylphosphite, $Pd(PPh_3)_4$, anhydrous NEt_3, anhydrous toluene.

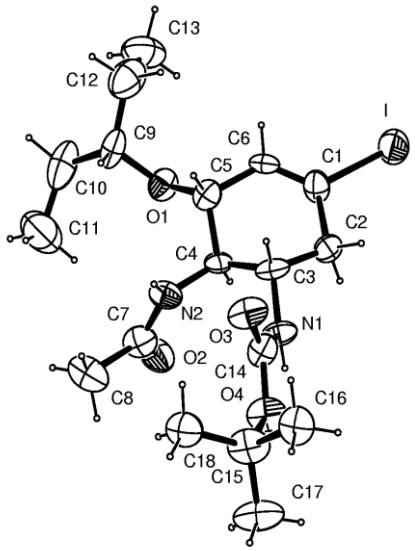
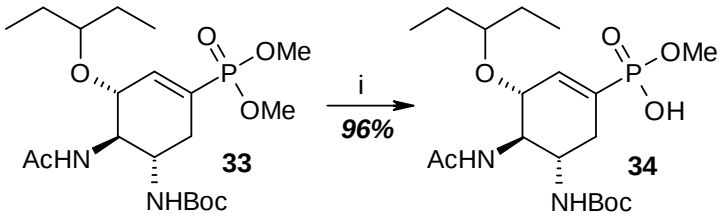


Figure 22. ORTEP-generated structure of iodo compound **32**.

The Boc protected amine **32** was obtained in 72% yield. The palladium coupling step of compound **32** with dimethylphosphite furnished cyclohexenephosphonate **33** in 80% yield with no traceable by-products. The mono-saponification was achieved under basic conditions (NaOH 0.25M) to afford monoester **34** in 96% yield.



Scheme 43. *Reagents & conditions:* (i) NaOH 0.25M, dioxane, then Amberlite IR-120 (H^+).

The key cyclohexenephosphonate monomethyl ester intermediate **34** was synthesized in a moderate overall yield of 41% in five steps from the industrial Tamiflu precursor. Monoester **34** is the key precursor in the following syntheses of all phosphonate monoesters, employing a previously established mixed diester strategy:[4, 5, 169, 170] Esterification via condensation under Mitsunobu conditions or via alkylation with triflates, followed by selective cleavage of the methyl ester group.

I.5.3. Synthesis of 'phospha'-Tamiflu derivatives

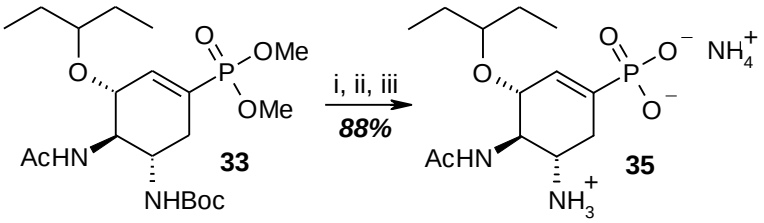
To demonstrate the versatility of the approach and to obtain a structurally diverse set of exemplary inhibitors, we chose to synthesize:

- the phospha-isostere **35** of Tamiflu and its methyl ester **36** as proof of principle,[171]
- the hexyl ester **40** having a hydrophobic aglycone mimetic,[171]
- the two galactosyl esters **46** and **54** with the natural sialic acid aglycone galactose mimicking the α2-6- and α2-3-sialoglycoconjuguates respectively,[172]
- the hexyl thioacetate ester **60**, allowing access to multimeric inhibitors and immobilization.

Compounds **35**, **36**, **40**, **46** and **54** should be then tested for inhibition of the neuraminidase activity of an H1N1 virus (A/Norway/1758/07) in collaboration with the National Institute for Medical Research (NIMR, London, UK).

I.5.3.1. *Synthesis of 'phospha'-Tamiflu **35** and its methyl ester **36***

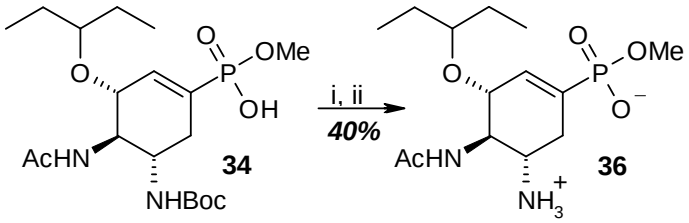
'Phospha'-Tamiflu was synthesized from dimethylphosphonate **33** (Scheme **44)**.



Scheme 44. *Reagents & conditions:* (i) TMSBr, lutidine, anhydrous CH_2Cl_2; (ii) TFA/H_2O : 1/1; (iii) gel permeation chromatography (gpc) (0.1M NH_4HCO_3).

Complete demethylation of **33** by stirring at room temperature with TMSBr and lutidine for 9 hours and removal of the Boc protecting group under standard acidic conditions (TFA/H_2O : 1/1) afforded Phospha-Tamiflu **35** in 88% yield after gpc. Cleavage of the pentyl ether was observed when compound **33** was left for considerably longer time with TMSBr and lutidine.

The monomethyl ester **36** was obtained by a simple deprotection step from compound **34** in 40% yield after gel permeation chromatography.

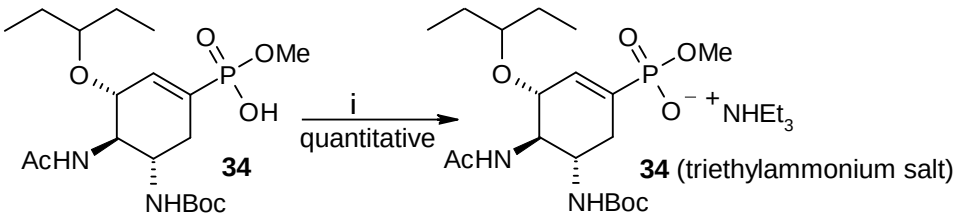


Scheme 45. *Reagents & conditions:* (i) TFA/H_2O : 1/1 ; (ii) gpc.

I.5.3.2. *Introduction of an hydrophobic moiety by alkylation: synthesis of the hexyl ester **40** of 'phospha'-Tamiflu*

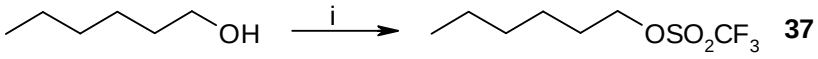
A convenient approach to synthesize mixed diesters of cyclohexenephosphonates has already been reported by our group, based on esterification by alkylation of cyclohexenephosphonate monoester with suitable alkyl triflates or by condensation using Mitsunobu conditions[4, 5] We could also show that, alkylation of cyclohexenephosphonate monobenzyl esters with octyl triflate can be achieved in good yield.[4] The same methodology was thus applied to the synthesis of the hexyl methyl 'phospha'-Tamiflu derivative from cyclohexenephosphonate monomethyl ester **34** and hexyl triflate **37**.

In order to achieve the alkylation step, formation of the triethyl ammonium salt of compound **34** and synthesis of hexyl triflate **37** were first required.



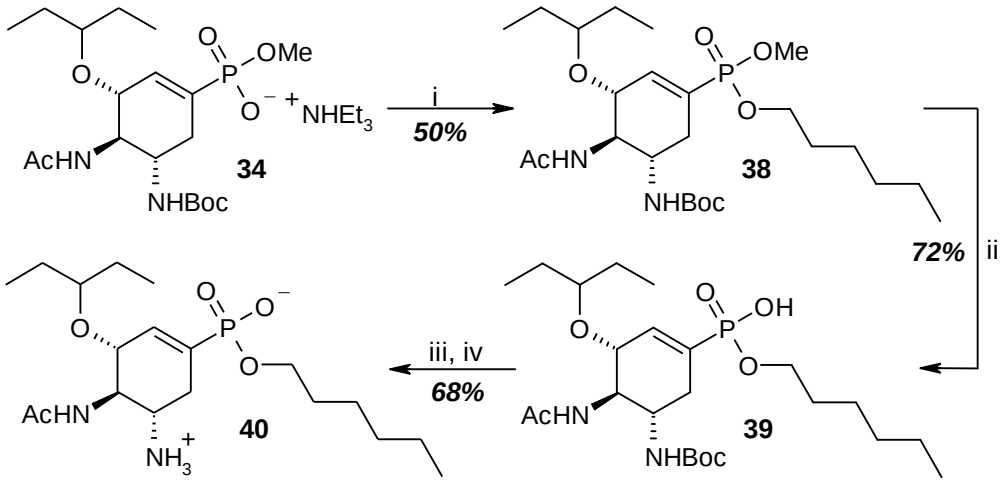
Scheme 46. *Reagents & conditions:* (i) NEt_3, dioxane.

The triethyl ammonium phosphonate salt of **34** was obtained by stirring the free acid with a few drops of triethyl amine in dioxane. After lyophilisation triethyl ammonium salt **34** was obtained as a white powder.



Scheme 47. *Reagents & conditions:* (i) Triflic anhydride, NEt_3, anhydrous toluene.

Hexyl triflate **37** was synthesized from commercially available hexanol by reacting the latter with triflic anhydride in presence of NEt_3 in anhydrous toluene. A simple extraction was sufficient to obtain hexyl triflate **37** as a colourless oil which was only characterized by ^1H NMR spectroscopy and used without further purification.



Scheme 48. *Reagents & conditions:* (i) hexyl-triflate **37**, anhydrous DMF; (ii) thiophenol, anhydrous NEt_3, anhydrous THF; (iii) TFA/H_2O : 1/1; (iv) gpc.

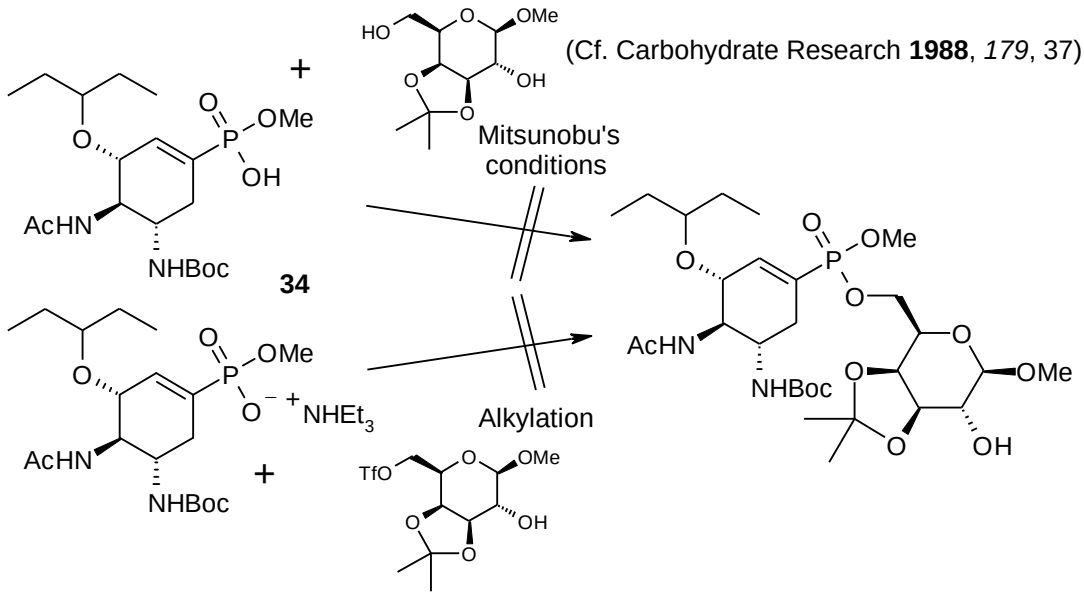
Alkylation of the triethyl ammonium salt of compound **34** with hexyl triflate **37** gave an inseparable mixture of diastereomeric hexyl methyl phosphonates **38** in a moderate 50% yield, unreacted starting material could be recovered. Selective demethylation of diastereoisomers **38** using thiophenol and NEt_3 afforded hexyl phosphonate **39** in 72% yield. Boc-cleavage under acidic conditions (TFA/H_2O : 1/1) followed by purification by gpc furnished target 'phospha'-Tamiflu monohexyl ester **40** in 68% yield.

I.5.3.3. *Introduction of a sugar moiety by alkylation: Synthesis of α(2-6)-sialoglycoconjugate mimetic **46***

It has been shown by us that esterification of substituted cyclohexenephosphonate monoalkyl esters with carbohydrates could be achieved in moderate yields via alkylation with a triflated sugar or via condensation using Mitsunobu conditions.[4] The same methodologies were thus applied to the synthesis of α(2,6)-sialoglycoconjugate mimetic **46**.

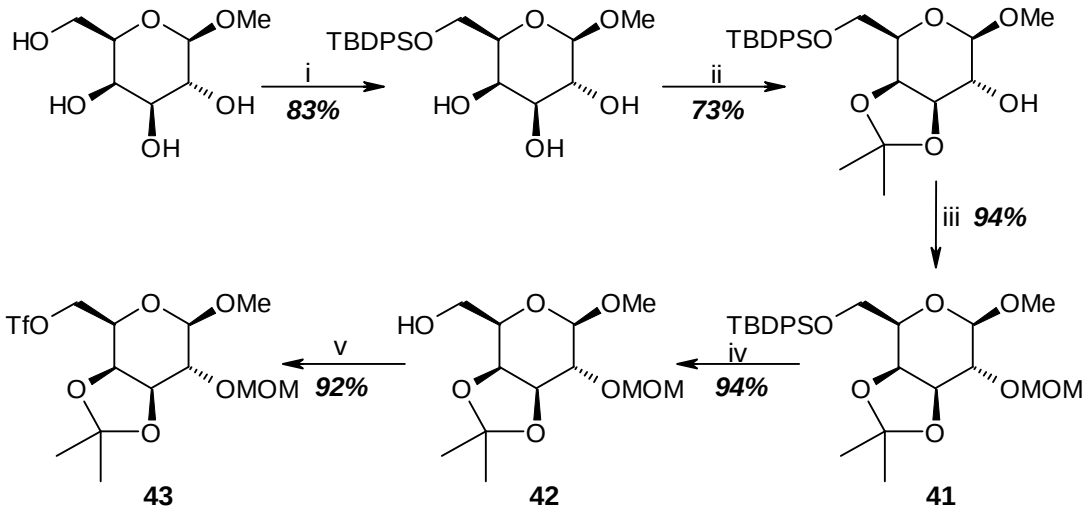
The first attempt to esterify the methyl phosphonic acid **34** under Mitsunobu conditions[169, 173, 174] with a suitable but not fully protected galactopyranoside derivative[175] is shown in Scheme **49**. It was thought that the unprotected secondary alcohol would not be an issue and interfere in the reaction as the primary alcohol is more reactive. Unfortunately the reaction did not proceed even after days of stirring and heating. The starting material was however recovered.

We thus decided to try modified Mitsunobu conditions[176] including the use of the more electron-deficient tri-(4-chlorophenyl)phosphine to increase the electrophilicity of the phosphorus and a base such as triethyl amine which would act as a catalyst. As this failed as well, we then synthesized the 6-*O*-galactosyl triflate to undergo alkylation of the triethyl ammonium phosphonate salt **34**, but unfortunately alkylation was not observed.



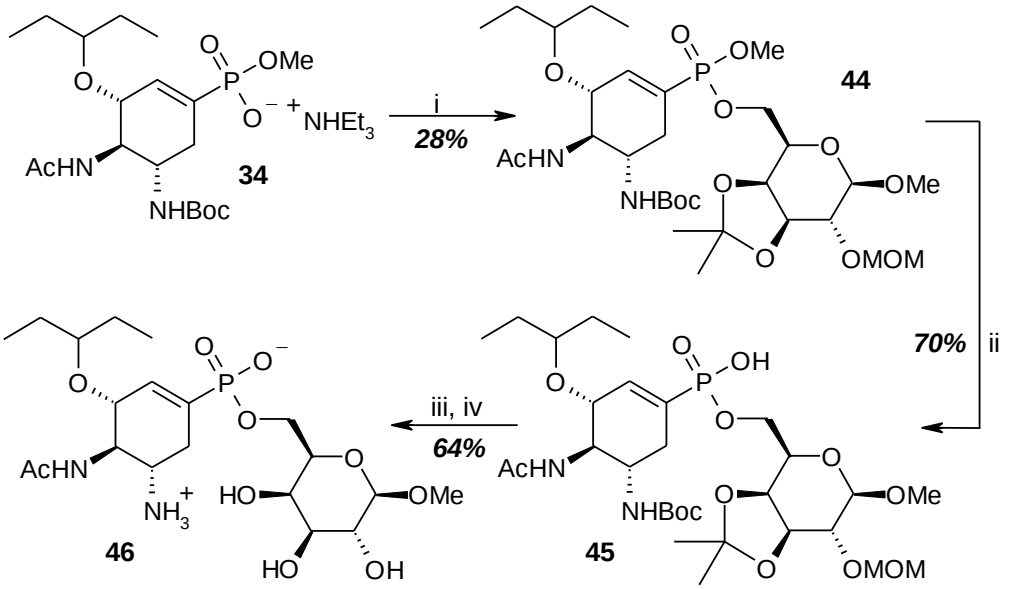
Scheme 49. Esterification trials under Mitsunobu's conditions and by alkylation.

We thus concluded that complete protection of the sugar was thus required for the alkylation to proceed. The synthesis of fully protected sugar triflate **43** is shown below (Scheme **50**).



Scheme 50. *Reagents & conditions:* (i) TBDPSCl, imidazole, anhydrous DMF; (ii) DMP, *p*-$TsOH_{cat}$, acetone; (iii) NaH, MOMCl, anhydrous THF; (iv) TBAF, anhydrous THF; (v) lutidine, triflic anhydride, -30°C.

The first two protection steps of *β*-D-galactopyranoside were achieved following literature procedures.[177] Silylation of the primary alcohol with TBDPSCl in anhydrous DMF in the presence of imidazole followed by isopropylidenation with 2,2-dimethoxypropane in dry acetone in the presence of a catalytic amount of para-toluenesulfonic acid, which are standard protection steps in carbohydrate chemistry, afforded Methyl 6-*O*-(*tert*-butyldiphenylsilyl)-3,4-*O*-isoprolylidene-*β*-D-galactopyranoside in good yields. Methoxymethylation[178] of the latter compound was carried out using NaH and MOMCl in anhydrous THF to afford the fully protected sugar **41** in 94% yield., which was then desilylated using TBAF in anhydrous THF to obtain the desired hydroxyl derivative **42** in 94% yield. Triflation of primary alcohol **42** with triflic anhydride and lutidine, gave sugar triflate **43** in 92% yield.



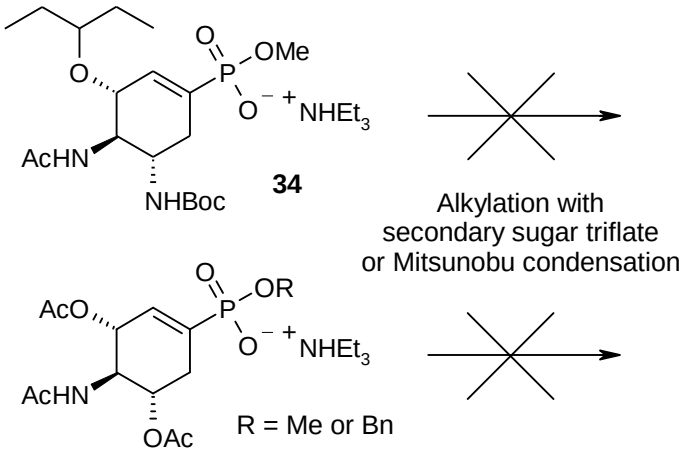
Scheme 51. *Reagents & conditions:* (i) Galactose-triflate **43**, anhydrous DMF; (ii) thiophenol, anhydrous NEt_3, anhydrous THF; (iii) TFA/H_2O : 1/1; (iv) gpc.

Triflated sugar **43** was used to alkylate the triethylammonium salt of monomethyl ester **34** to give mixed diester **44** in 28% yield (scheme **51**). The unreacted starting material **34** was recovered during purification. Cleavage of the methyl ester of diastereoisomers **44** with thiophenol and triethylamine afforded monoester **45** in 70% yield. Final deprotection was achieved in aqueous trifluoroacetic acid to furnish α(2,6)-sialoside mimetic **46** in 64% yield after purification by gel-permeation chromatography.

I.5.4. An alternative approach to mixed diesters of ‘phospha’-Tamiflu

I.5.4.1. *Introduction of a sugar moiety by phosphonate coupling: Synthesis of α(2,3)-sialoglycoconjugate mimetic **54***

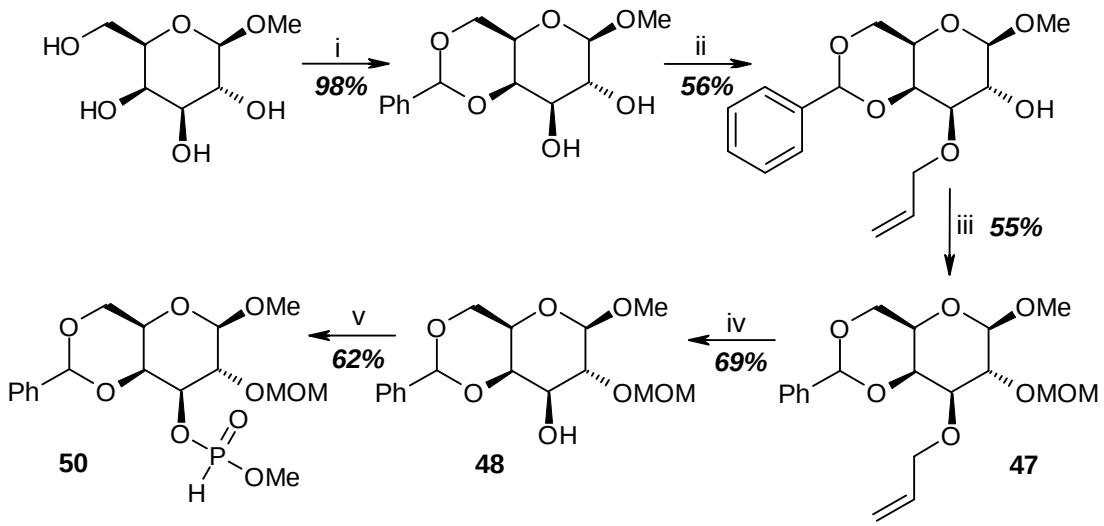
As it has been reported previously,[5] attempts to esterify cyclohexenephosphonate-monobenzyl esters and cyclohexephosphonate-monomethyl esters via alkylation with secondary sugar triflates or condensation under Mitsunobu conditions with secondary sugar alcohols failed. As expected, these attempts also failed with the ‘phospha’-Tamiflu derivative **34**.



Scheme 52. Attempted esterification with secondary sugar alcohol.

To overcome this issue which was probably due to steric hindrance, a direct Pd(0)-mediated coupling with a respective sugar-modified phosphonic acid was envisaged. The synthesis of this protected methyl galactosyl phosphonic acid **50** is summarized in Scheme **53**.

Methyl 2-*O*-methoxymethyl-4,6-*O*-benzylidene-β-D-galactopyranoside **48** was synthesized by selective protection and deprotection steps starting with methyl-*β*-D-galactopyranoside. The first two protecting steps were carried out as described in literature. Benzylidenation[179] with benzaldehyde dimethyl acetal and *p*-TsOH in acetonitrile gave methyl 4,6-*O*-benzylidene-β-D-galactopyranoside in 98% yield. Selective allylation[180-183] occurred in two steps, activation of the 2,3-diol with dibutyltin oxide[184] in dry toluene under reflux to give the cyclic dibutylstannylidene derivative which was reacted with allyl bromide and TBAI under reflux to give a 3.2 : 1 mixture of the 3-*O*- and 2-*O*- allyl ethers, which were easily separated by flash chromatography, in 56% and 17% yield respectively. Methyl 3-*O*-allyl-4,6-*O*-benzylidene-β-D-galactopyranoside was then momylated[178] to provide the fully protected sugar **47** in 55% yield. Direct palladium mediated allylic cleavage [183, 185] of **47** was achieved with $PdCl_2$ in aqueous acetic acid in the presence of sodium acetate to furnish 3-*O*- deprotected sugar **48** in 69% yield.



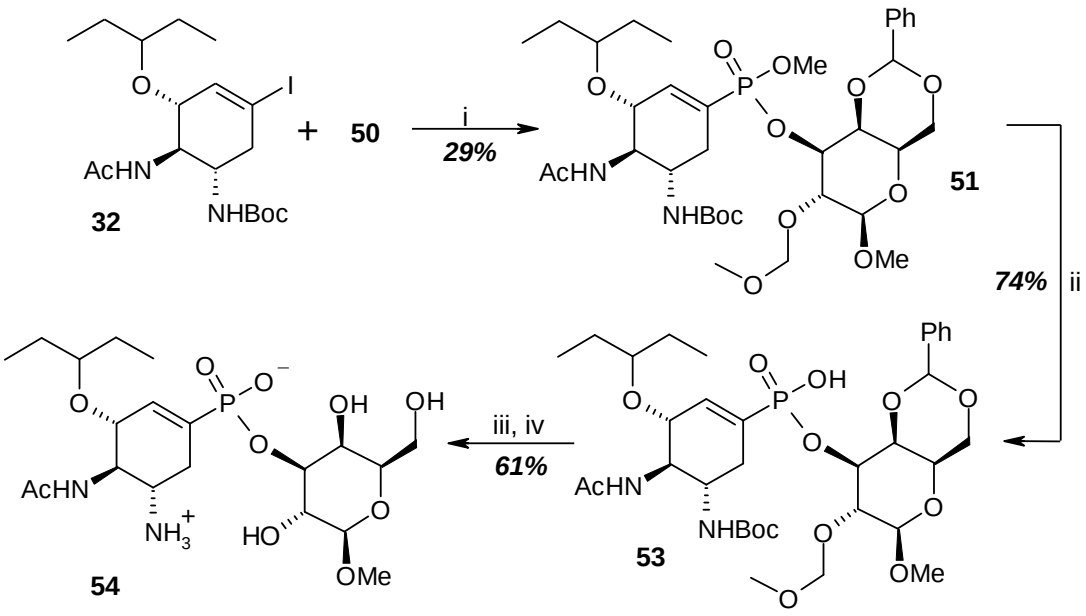
Scheme 53. *Reagents & conditions:* (i) benzaldehyde dimethyl acetal, *p*-$TsOH_{cat}$, acetonitrile; (ii) (a) dibutyltin oxide, anhydrous toluene, reflux, (b) TBAI, allylbromide, anhydrous toluene, reflux; (iii) NaH, MOMCl, dry THF; (iv) NaOAc, $PdCl_2$, $AcOH/H_2O$: 10/1; (v) chloro(dimethyl)phosphine **49**, *N*,*N*-diisopropylethyl amine, anhydrous CH_2Cl_2.

Dimethyl chlorophosphonate **49** was obtained by reaction of a 2 to 1 mixture of trimethylphosphite and phosphorus trichloride respectively, and further purification by distillation under reduced pressure (34°C, 42 Torr).[186]

$$PCl_3 + 2\ P(OMe)_3 \longrightarrow 3\ P(OMe)_2Cl \quad \mathbf{49}$$

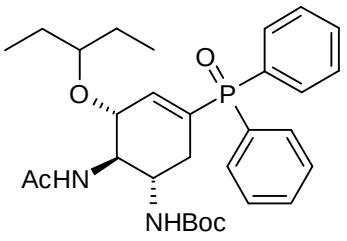
Scheme 54. Dimethyl chlorophosphonate formation.

The desired mixed diester **50** was synthesized in 62% yield by phosphonylation of the secondary alcohol **48** with dimethyl chlorophosphonate **49** to give the triester which immediately hydrolyzed on silica during purification by flash chromatography to give **50** (Scheme **53**).



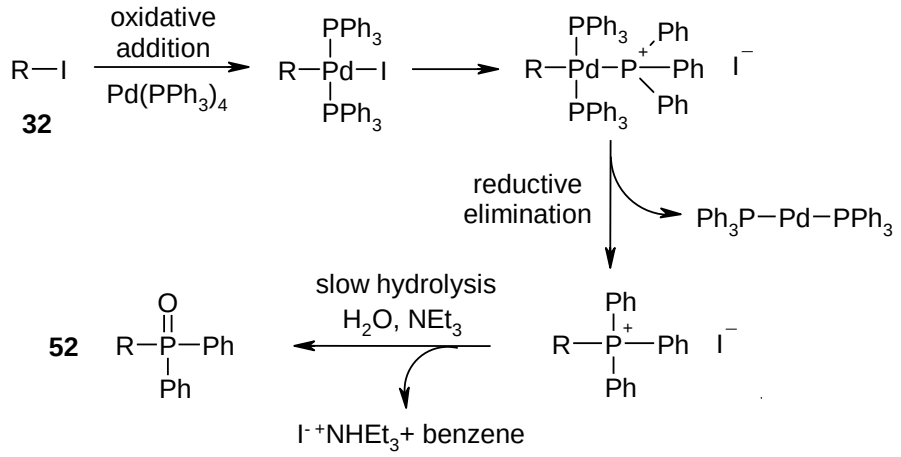
Scheme 55. *Reagents & conditions:* (i) anhydrous NEt_3, $Pd(PPh_3)_4$; (ii) thiophenol, anhydrous NEt_3, anhydrous THF; (iii) TFA/H_2O : 1/1; (iv) gpc.

Coupling of H-phosphonate **50** with vinyl iodide **32** using $Pd(PPh_3)_4$ furnished protected diastereomeric mixed diesters **51** in a moderate 29% yield. However, no dehalogenation product (as observed previously) had formed but instead the phosphine oxide **52** was obtained as a side-product in 9% yield. A coupling reaction between PPh_3 and vinyl iodide **32** seems to occur but no formal mechanism has been elucidated. It is thought that substituted cyclohexene triphenylphosphonium iodide is formed and is slowly hydrolysed[187] during work-up and purification by flash chromatography to form phosphine oxide **52** (Scheme **56**).



52

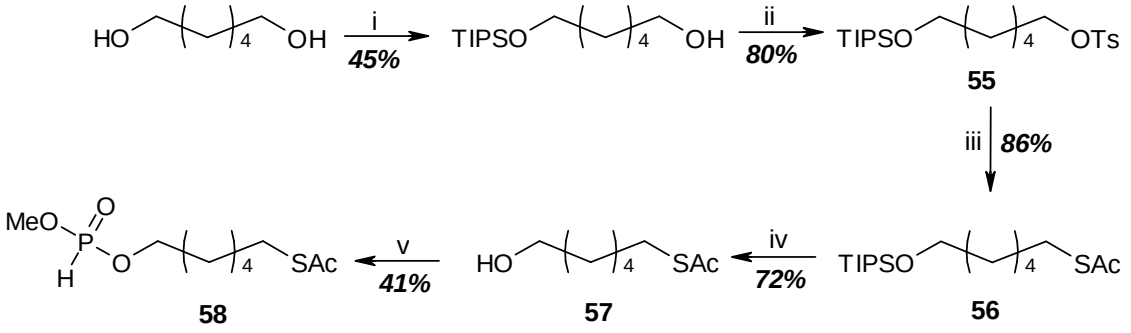
The modified procedure, introduced by Hayes and co-workers[57] (Scheme **10)** using propylene oxide as an alternative HI scavenger and using palladium acetate and 1,1'-bis(diphenylphosphino)ferrocene (dppf) instead of $Pd(PPh_3)_4$, would be an interesting alternative to investigate for this coupling step.



Scheme 56. Supposed mechanism of side-product phosphine oxide **52**.

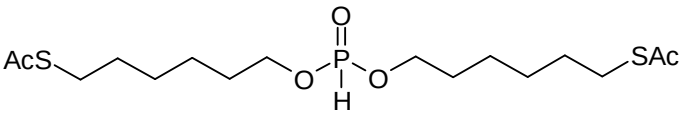
The mixed diester **51** was then subjected to final deprotection steps. Selective phosphonate demethylation converted the mixture of diastereoisomers **51** into the monoester **53** in 74% yield which was deprotected in aqueous acetic acid to furnish α(2,3)-sialoside mimetic **54** in 61% yield after purification by gel-permeation chromatography. A selective observation of phosphonate ester protons by ^{1}H spin-echo difference (SED) spectroscopy[188] of compound **54** confirmed the 'phospha'-Tamiflu moiety linkage to the 3-position of the galactose (see Appendix 2 : NMR spectrum of compound **54**).

I.5.4.2. *Introduction of a variable ω-thioacetyl linker for immobilisation and oligomerisation of the 'phospha'-Tamiflu motif*



Scheme 57. *Reagents & conditions:* (i) imidazole, TIPSCl; (ii) TsCl, DMAP, anhydrous pyridine, 0°C; (iii) KSAc, pyridine; (iv) TBAF, AcOH, THF; (v) chloro(dimethyl)phosphine **49**, *N*,*N*-diisopropylethyl amine, anhydrous CH_2Cl_2.

Hexane-1,6-diol was mono-protected as the triisopropylsilyl ether[189] in a moderate 45% yield due to the inevitable formation of the disilyl ether. The remaining free alcohol was then tosylated[190] to furnish **55** in 80% yield. The tosylate was substituted by KSAc in pyridine to give **56** in a good 86% yield, which was desilylated with TBAF in presence of acetic acid in THF to afford ω-thioacetyl hexanol **57** in 72% yield. Phosphonylation of **57** with dimethyl chlorophosphonate **49** gave the phosphite which was directly hydrolyzed on silica during purification by flash chromatography to furnish the desired mixed diester hydrogen phosphite **58** in 41% yield. By-product **59** was isolated in 11% yield and fully characterized.



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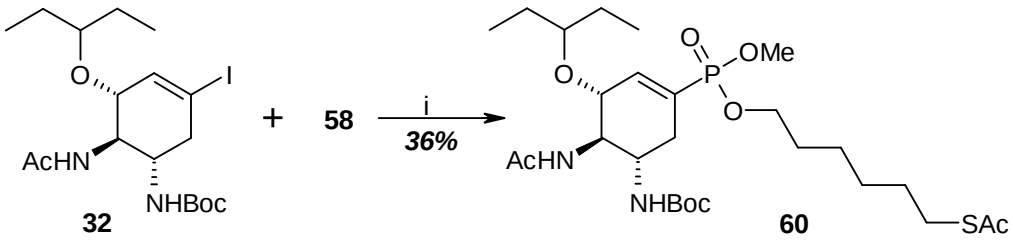
Finally, *O*-methyl *O*-(ω-thioacetyl hexyl) phosphonic acid **58** was coupled to vinyl iodide **32** to give the diastereoisomers of methyl (ω-thioacetoxy hexyl)-'phospha'-tamiflu **60** in 36% yield.



Scheme 58. *Reagents & conditions:* (i) anhydrous NEt_3, $Pd(PPh_3)_4$.

I.5.5. Inhibition of neuraminidase activity

In collaboration with the National Institute for Medical Research (NIMR, London, UK), phosphonates **35**, **36**, **40**, **46** and **54** were investigated for inhibitory activity in a whole-virus assay using allantoic fluid from infected eggs (Table **2**).[171, 172] In this standard assay, which is widely used in the influenza field, the inhibition constant K_i is obtained by measuring the effect of the inhibitor on the rate of MUNANA hydrolysis.[191-196]

35	**36**	**40**	**46**	**54**	Oseltamivir
0.17 ± 0.08	0.30 ± 0.04	0.22 ± 0.03	0.74 ± 0.08	0.29 ± 0.02	0.14 ± 0.02

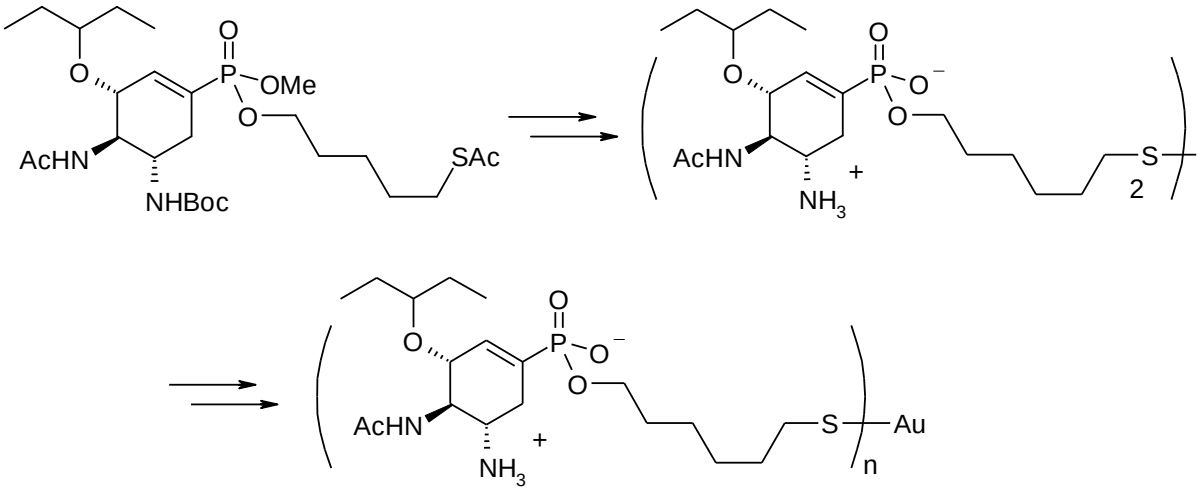
Table 2. Inhibition constants (K_i [nM]) for target phosphonates.
(Inhibition of Munana hydrolysis catalysed by neuraminidase from influenza virus A/Norway/1758/07; Inhibition constants (K_i) were determined as described in III.2.3 Inhibition of neuraminidase activity of influenza viruses from allantoic fluid from infected eggs; K_M for MUNANA = 6.4 ± 0.6 µM)

'Phospha'-Tamiflu and its monoesters display an inhibitory activity against influenza virus neuraminidase very much in the same range as Oseltamivir itself. This was further confirmed by inhibition of neuraminidase from Oseltamivir-resistant virus (A/Norway/1735/07, K_M for MUNANA = 11.1 ± 0.6 µM) by 'phospha'-Tamiflu **35** with a K_i of 48 ± 8 nM compared with 36 nM for Oseltamivir.[171] These findings are important as they allow the conclusion that the inhibitory properties of Oseltamivir are fully retained when the carboxylate is substituted by a monoalkyl phosphonate which retains a negative charge under physiological conditions.

Regarding sialylgalactoside mimetics **46** (α(2,6)-sialogalactoside mimetic) and **54** (α(2,3)-sialogalactoside mimetic), a significant difference in their respective inhibitory activity against influenza virus neuraminidase was observed.[172] α(2,3)-sialylgalactoside mimetic **54** inhibits somewhat more strongly than the isomeric α(2,6)-sialylgalactoside mimetic **46**. In the absence of detailed structural data, this finding cannot be directly correlated with the substrate specificity of the virus but it does demonstrate that the set of the two compounds is useful to establish a fingerprint for the neuraminidase which is, at least partly, governed by its substrate specificity. In other words, the ratio of the inhibition constants of **46** and **54** should show some proportionality to the ratio of the binding constants of the respective α(2,3)- and α(2,6)-sialylgalactoside substrates.

I.5.6. Future scope

Compound **60** will be used as a precursor to the synthesis of the 'Phospha'-Tamiflu dimmer which will then be tested for inhibitory activity against influenza virus neuraminidase. The synthesis of a multivalent inhibitor will as well be investigated. Immobilization of Tamiflu on nanoparticles (gold nanoparticles or CdSe-nanodots) or on resin is in the pipeline of the group's work as illustrated in Scheme **59**.



Scheme 59. From compound 60 to 'Phospha'-Tamiflu dimer to 'Phospha'-Tamiflu nano-gold.particules.

Immobilization of Tamiflu has already been reported by Shibasaki,[197] replacing the pentyl ether pharmacophore of Oseltamivir by a linker. Our 'phospha'-Tamiflu approach would allow immobilization without impairing the Oseltamivir pharmacophore.

To further investigate the binding specificity of neuraminidase towards α(2,6)- or α(2,3)-linked sialylgalactosides and to probe the cavity adjacent to the active site, the '150-loop' (representing residues 147 to 152) observed in the crystal structures of group-1 neuraminidases, it could be of interest to synthetise α(2,6)- and α(2,3)-sialyloligosaccharide mimetics possessing a longer carbohydrate chain. Inhibition data and structural data of such mimetics could lead to a better understanding of neuraminidase activity.

I.6. Conclusion

We have achieved the novel extension of the Hunsdiecker-Barton decarboxylation to 1-cyclohexenecarboxylic acids and successfully applied it to the synthesis of ‘phospha’-shikimic acid and derivatives as well as to the synthesis of ‘phospha’-Tamiflu and derivatives. We thus provide access not only to the potent inhibitor ‘phospha’-Tamiflu **35** but also to its monoesters. This approach allows for adding functionality to the Oseltamivir pharmacophore, without compromising the inhibitory activity and allowing for the presentation of the Tamiflu motif on an unlimited variety of carrier structures.

Experimental section

I.7. General procedures

All reactions were carried out in oven dried glassware with magnetic stirrers. All reagents were purchased from commercial suppliers and used without further purification. Methanol and DMF were purchased anhydrous and used as received. Methylene chloride was distilled twice before use and solvents for chromatography (toluene and ethyl acetate) were distilled before use. All other solvents used in the reactions were distilled from appropriate drying agents prior to use. Fine chemicals were purchased from Aldrich-, Sigma- or Acros-Chemicals and were of the highest purity available. Reactions were monitored by TLC (Thin Layer Chromatography) using pre-coated silica gel 60 F_{254} plates (pre-coated plastic sheets with a 0.20 mm layer of silica gel with fluorescent indicator UV_{254}) (POLYGRAM SIL G/UV_{254}, Macherey-Nagel). Compounds were detected by UV absorption and/or by staining with a molybdenum phosphate reagent (20g ammonium molybdate and 0.4g cerium(IV) sulfate in 400mL of 10% aqueous sulfuric acid) or with a basic $KMnO_4$ solution and subsequent heating at 120°C for a few minutes. Chromatography purification was performed using silica gel 60Å 'Davisil' (particle size 35-70µm) from Fisher Scientific, UK. Silica-based MPLC chromatography was carried out on the Büchi Sepacore system equipped with glass columns packed with LiChroprep Si 60 (15-25 µm) from Merck, Darmstadt, Germany. Gel permeation chromatography was carried out in the 1-10 mg scale on a XK 16/70 column (bed volume 130 mL), from Amersham packed with Sefadex G-10 (particle size 40-120 µm) and 0.1M NH_4HCO_3 as buffer. Detection was achieved with a differential refractometer from Knauer, Berlin, Germany. ^{1}H NMR, ^{13}C NMR, ^{31}P NMR and all multidimensional spectra were recorded on Varian VNMRS spectrometers (600 MHz, 500 MHz or 400 MHz, see compound characterisation for individual experiments). Chemical shifts in ^{1}H and ^{13}C NMR spectra were referenced to the residual proton resonance of the respective deuterated solvents, $CDCl_3$ (7.24 ppm), D_2O (4.80 ppm) and D_2O in CD_3OD (4.88 ppm). For ^{31}P NMR spectra H_3PO_4 was used as external standard (0 ppm). In some cases, ^{13}C chemical shifts were deduced from heteronuclear multiple spin correlation (HSQC) spectra. The H_{6ax} and H_{6eq} assignments refer to the pseudoaxial and pseudoequatorial protons in the cyclohexene systems, respectively, obtained by ROESY spectroscopy. In pseudo-disaccharidic systems the

cyclohexene ring is indicated by the suffix ‘a’, the sugar by the suffix ‘b’. Diastereomic mixtures of mixed diesters were indicated by the suffix ‘h’ (higher moving) and ‘l’ (lower moving) but in most cases no attemps of separation were made.

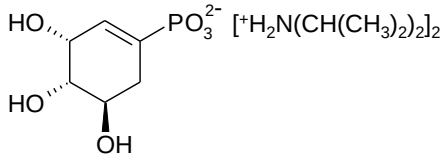
HR-ESI-MS (High Resolution Electro-Spray Ionisation Mass Spectrometry) was ascertained for all compounds on a Brucker Daltonics Apex III in positive mode with MeOH and/or H_2O as solvents.

Fluorescence was measured in a JASCO FP-6300 fluorimeter.

I.8. Synthetic procedures and compound characterization

I.8.1. ‘Phospha’-shikimic derivatives

Di(diisopropyl)ammonium [(3R,4S,5R)-trihydroxy-1-cyclohexene-1-phosphonate] (1)



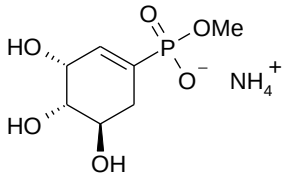
Compound **9** (10 mg, 0.027 mmol) was dissolved in dichloromethane (1 mL) and trimethylsilyl bromide (36 µL, 0.27 mmol) and lutidine (38 µL, 0.324 mmol) were added to the solution. After 4 hours stirring, the solvent was evaporated and aqueous ammonia 10% (1.5 mL) was added. After 2 hours stirring, the mixture was frozen and lyophilized. The residue was purified on a Biogel P4 column to afford, after lyophilization, compound **1** as the ammonium salt. **1** was dissolved in H_2O (2mL), a drop of diisopropyl amine was added, the mixture was stirred for 5 min, then frozen and lyophilized to afford the diisopropyl ammonium salt of compound **1**.

^{1}H NMR (600 MHz, D_2O) δ_H 6.18 (d, 1H, $J_{P\text{-}2}$ = 17.9 Hz, H-2), 4.34-4.31 (m, 1H, H-3), 3.96-3.90 (m, 1H, H-5), 3.66 (dd, 1H, J = 9.7, 4.4 Hz, H-4), 3.52 (sept, J = 6.6 Hz, 4H, NCH), 2.77 (ddd, J = 17.4, 6.3, 6.3 Hz, 1H, H-6’), 2.19 (dd, J = 17.6, 8.3 Hz, 1H, H-6), 1.31 (d, J = 6.6 Hz, 24H).

^{13}C NMR (600 MHz, D_2O) δ_C 139.42 (d, J = 165 Hz, C-1), 128.59 (m, C-2), 72.78 (C-4), 67.11, 66.99, 66.92 (1s, 1d, C-3, C-5), 47.15 (NCH), 33.59 (d, J = 8.9 Hz, C-6), 18.20.

^{31}P NMR (161.9 MHz, D_2O) δ_P 10.80.

Ammonium [methyl (3R,4S, 5R)-trihydroxy-1-cyclohexene-1-phosphonate] (2)



Compound **9** (11 mg, 0.030 mmol) was dissolved in dry acetone (2 mL) and NaI (20 mg, 0.133 mmol) was added to the solution under nitrogen atmosphere. The mixture was heated to reflux for 8 hours and then the solvent was evaporated. Aqueous ammonia 10% was added to the residue, and the mixture was stirred for 3 hours, was then frozen and lyophilized. The residue was purified on a Biogel P4 column to afford compound **2**.

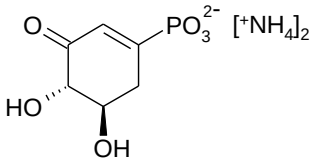
^{1}H NMR (500 MHz, D_2O) δ_H 6.42 (d, $J_{P\text{-}2}$ = 19.3 Hz, 1H, H-2), 4.45 (s, 1H, H-3), 4.05-3.99 (m, 1H, H-5), 3.81-3.74 (m, 1H, H-4), 3.57 (d, J = 10.7 Hz, 3H, POCH_3), 2.77-2.57 (m, 1H, H-6'), 2.25-2.15 (m, 1H, H-6).

^{13}C NMR (500 MHz, D_2O) δ_C 135.48 (d, J = 7.3 Hz, C-2), 132.29 (d, J = 170.6 Hz, C-1), 71.98 (C-4), 66.49/66.33 (C-3/C-5), 51.61 (m, POMe), 32.37 (d, J = 9.1 Hz, C-6).

^{31}P NMR (121.4 MHz, D_2O) δ_P 16.49.

HR-ESI-MS calculated for $C_7H_{12}O_6P$ $(M+H+Na)^+$ 247.0342200, found 247.0341957.

Diammonium [(4S, 5R)-dihydroxy-3-oxo-1-cyclohexene-1-phosphonate] (3)



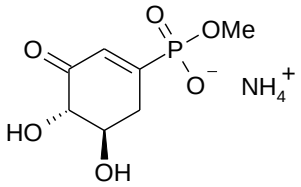
Compound **23** (10 mg, 0.025 mmol) was dissolved in dichloromethane (1 mL) and trimethylsilyl bromide (36 µL, 0.25 mmol) and lutidine (38 µL, 0.3 mmol) were added to the solution. After 12 hours stirring, TMSBr (36 µL, 0.25 mmol) and lutidine (38 µL, 0.3 mmol) were added to the mixture. After few hours, the solvent was evaporated and the residue was dissolved in 2 mL of aqueous trifluoroacetic acid (TFA:H_2O ; 9:1). After overnight stirring, the mixture was frozen and lyophilized. Gel permeation chromatography on a Biogel P4 column afforded compound **3**.

^{1}H NMR (500 MHz, D_2O) δ_H 6.48 (d, $J_{2,P}$ = 16.5 Hz, 1H, H-2), 4.31 (d, J = 11.3 Hz, 1H, H-4), 4.07-3.98 (m, 1H, H-5), 3.18-3.08 (m, 1H, H-6'), 2.73-2.63 (m, 1H, H-6).

^{13}C NMR (400 MHz, D_2O) δ_C 128.72 (d, J = 7.3 Hz, C-2), 127.24 (d, J = 181.6 Hz, C-1), 78.34 (C-4), 71.53 (d, J =12.0 Hz, C-5), 34.93 (d, J = 7.4 Hz, C-6).

^{31}P NMR (121.4 MHz, D_2O) δ_P 8.16.

Ammonium [methyl (4S, 5R)-dihydroxy-3-oxo-1-cyclohexene-1-phosphonate] (4)



From **18**: Compound **18** (8 mg, 0.034 mmol) was dissolved in dried acetone (1 mL) and NaI (15 mg, 0.10 mmol) was added to the solution under nitrogen atmosphere. The mixture was heated to reflux for 8 hours and then the solvent was evaporated. The residue was purified on a Biogel P4 column to afford compound **4**.

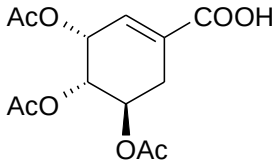
From **23**: Aqueous trifluoroacetic acid (TFA:H_2O ; 9:1) (2 mL) was added to compound **23** (30 mg, 0.076 mmol) and the mixture was stirred overnight. More aqueous TFA (2 mL) was added and the mixture stirred for 24 hours. After evaporation under vacuum, the deprotected "dimethylphosphonate" compound was purified by flash chromatography on a Pasteur pipette (EA:MeOH ; 10:1). The pure intermediate (13.5 mg, 0.057 mmol) was dissolved in dried acetone. NaI (25 mg, 0.17 mmol) was added under nitrogen atmosphere. The mixture was heated to reflux, stirred for 6 hours, then frozen and lyophilized. Gel permeation chromatography on a Biogel P4 column afforded compound **4**.

^{1}H NMR (400 MHz, D_2O) δ_H 6.55 (dd, $J_{2,P}$ = 17.9, 3.0 Hz, 1H, H-2), 4.36 (d, J = 11.2 Hz, 1H, H-4), 4.07 (ddd, J = 10.5, 10.5, 5.2 Hz, 1H, H-5), 3.64 (d, J = 10.9 Hz, 3H, $POCH_3$), 3.05 (ddd, J = 17.9, 9.7, 5.2, 1H, H-6'), 2.68 (dddd, J = 17.9, 10.1, 3.0, 3.0 Hz, 1H, H-6).

^{13}C NMR (400 MHz, D_2O) δ_C 131.89 (d, J = 7.3 Hz, C-2), 78.38 (C-4), 71.10 (d, J = 13.2 Hz, C-5), 52.18 (d, J = 5.5 Hz, POMe), 34.25 (d, J = 7.6 Hz, C-6).

^{31}P NMR (121.4 MHz, D_2O) δ_P 12.84.

(3R,4S,5R)-tri-acetoxy-1-cyclohexene-1-carboxylic acid (5)



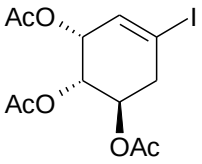
Shikimic acid (0.4 g, 2.3 mmol) was dissolved in pyridine/acetic anhydride (2/1 ; 9 mL).After 5 hours stirring, the solution was concentrated under vacuum. Toluene was added to the mixture to help remove pyridine by co-evaporation. The residue was dissolved in ethyl acetate and washed with HCl 1M (10 mL x 3) and then with brine (5 mL x 3). The organic phase was dried with $MgSO_4$, evaporated and the residue was purified by flash chromatography (Tol:EA:Acetic acid; 3:1:0.5%) to furnish compound **5** (0.635 g, 2.1 mmol) in 88% yield. R_f = 0.27 (Tol:EA ; 2:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ_H 10.72 (bs, 1H, COOH), 6.82 (s, 1H, H-2), 5.71 (s, 1H, H-3), 5.24 (m, 2H, H-4, H-5), 2.85 (d, *J* = 17.5 Hz, 1H, H-6'), 2.39 (d, *J* = 18.1 Hz, 1H, H-6), 2.05, 2.04, 2.02 (3s, 9H, 3 $OC(O)CH_3$).

^{13}C NMR (300 MHz, $CDCl_3$) δ_C 170.32, 170.16, 170.08 (*C*OOH, 3 O*C*OCH_3), 135.04 (C-2), 130.73 (C-1), 67.55, 66.83 (C-4, C-5), 66.10 (C-3), 28.10 (C-6), 21.05, 20.81 (3 $OCOCH_3$).

HR-ESI-MS calculated for $C_{13}H_{16}O_8$ $(M+Na)^+$ 323.0735470, found 323.0737386.

1-iodo-(3R,4S,5R)-tri-acetoxy-1-cyclohexene (6)



Triacetoxyshikimic acid **5** (50 mg, 0.166 mmol) was dissolved in dry CH_2Cl_2 (0.5 mL) in which DMF (0.12 µL) had been added. To the solution was added, under dinitrogen, oxalyl chloride (0.11 mL, 1.24 mmol). The mixture was stirred at room temperature under dinitrogen for 45 min and then concentrated under vacuum. The residue was taken up in CH_2Cl_2 (0.5 mL).

Meanwhile a mixture of the sodium salt of *N*-hydroxypyridine-2-thione (27 mg, 0.18 mmol), DMAP (1.8 mg, 0.015 mmol), 2-iodo-1,1,1-trifluoroethane 5eq (CF_3CH_2I) (0.08 mL, 0.83 mmol) was mixed by stirring in dry CH_2Cl_2 (1 mL) under nitrogen in a quartz tube.

5 min before adding the residue of the first step, the quartz tube, still under nitrogen, was brought to reflux by irradiation with a 250W floodlamp. The solution of acid chloride was then added to this second mixture which was irradiated for 45 min.

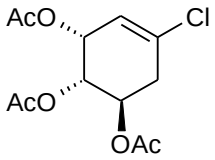
After removal of the solvent, the mixture was purified directly by flash chromatography (Tol:EA ; 5:1) to give compound **6** in 58%. R_f = 0.42 (Tol:EA ; 5:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ_H 6.31-6.24 (m, 1H, H-2), 5.43-5.36 (m, 1H, H-3), 5.24-5.11 (m, 2H, H-4, H-5), 3.13 (dd, J = 17.9, 4.8 Hz, 1H, H-6'), 2.58 (dd, J = 17.5, 5.3 Hz, 1H, H-6), 2.02, 2.01, 1.99 (3s, 9H, 3 $OC(O)CH_3$).

^{13}C NMR (300 MHz, $CDCl_3$) δ_C 170.09, 170.01, 169.97 (3 $OCOCH_3$), 133.22 (C-2), 98.19 (C-1), 68.07, 67.80, 67.68 (C-3, C-4, C-5), 43.78 (C-6), 21.01, 20.92, 20.85 (3 $OCOCH_3$).

HR-ESI-MS calculated for $C_{12}H_{15}IO_6$ $(M+Na)^+$ 404.9806440, found 404.9805523.

1-chloro-(3R,4S,5R)-tri-acetoxy-1-cyclohexene (7)

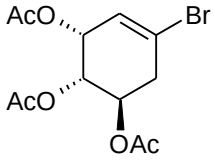


Synthesis of **7**: see synthesis of compound **6**, CCl_4 (11 eq) was used as the halogen donor. Yield: 24%.

^{1}H NMR (300 MHz, $CDCl_3$) δ_H 5.83 (d, 1H, H-2), 5.57 (dd, $J_{3,4}$ = 4.4, $J_{3,2}$ = 4.4 Hz, 1H, H-3), 5.32-5.24 (m, 1H, H-5), 5.17 (dd, $J_{4,5 \text{ or } 3}$ = 9.4, $J_{4,\ 3 \text{ or } 5}$ = 4.0 Hz, 1H, H-4), 2.98 (dd, $J_{6',6}$ = 18.1, $J_{6',5}$ = 6.2 Hz, 1H, H-6'), 2.45 (dd, $J_{6,6'}$ = 18.2, $J_{6,5}$ = 7.1 Hz, 1H, H-6), 2.06, 2.05, 2.03 (3s, 9H, 3 OAc);

HR-ESI-MS calculated for $C_{12}H_{15}ClO_6Na$ $(M+Na)^+$ 313.0446680, found 313.0449370.

1-bromo-(3R,4S,5R)-tri-acetoxy-1-cyclohexene (8)



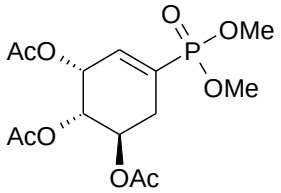
Synthesis of **8**: see synthesis of compound **6**, CBr_4 (11 eq) was used as the halogen donor. Yield: 36%.

^{1}H NMR (300 MHz, $CDCl_3$) δ_H 6.05 (d, $J_{2,3}$ = 5.09 Hz, 1H, H-2), 5.51 (dd, $J_{3,4}$ = 4.5, $J_{3,2}$ = 4.5 Hz, 1H, H-3), 5.31-5.22 (m, 1H, H-5), 5.18 (dd, $J_{4,5\text{ or }3}$ = 9.4, $J_{4,3\text{ or }5}$ = 4.0 Hz, 1H, H-4), 3.10 (dd, $J_{6',6}$ = 18.3, $J_{6',5}$ = 5.9 Hz, 1H, H-6'), 2.56 (dd, $J_{6,6'}$ = 18.3, $J_{6,5}$ = 6.3 Hz, 1H, H-6), 2.06, 2.05, 2.03 (3s, 9H, 3 OAc);

^{13}C NMR (300 MHz, $CDCl_3$) δ_C 125.48 (C-2), 70.15 (C-4), 67.47 (C-5), 67,28 (C-3), 39.93 (C-6), 21.15, 21.24, 21.32 (3 $COCH_3$);

HR-ESI-MS calculated for $C_{12}H_{15}BrO_6Na$ $(M+Na)^+$ 356.9935390 found 356.9944219.

Dimethyl (3R,4S,5R)-tri-acetoxy-1-cyclohexene-1-phosphonate (9)



Anhydrous toluene was degassed in dinitrogen. Compound **6** (48 mg, 0.13 mmol) in dry toluene (0.5 mL) was added to a solution of tetrakis triphenylphosphine palladium (0.2 equiv, 30 mg) in dry toluene (0.5 mL), under a dinitrogen atmosphere. To that mixture was added a solution of triethylamine (4 equiv, 96 µL) and dimethylphosphite (4 equiv, 52 µL) in dry toluene (0.5 mL), under dinitrogen. The mixture was heated to 80°C and maintained at this temperature for 3 hours. After cooling down at room temperature, the reaction was quenched by addition of saturated aqueous NH_4Cl (1 mL).The mixture was extracted with toluene, washed with brine and the solvent evaporated. Purification by flash chromatography (Tol:EA ; 5:1 -> 2:1) gave the desired compound **9** in 28% yield. R_f = 0.02 (Tol:EA ; 5:1).

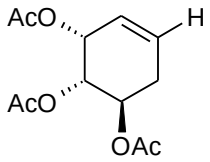
^{1}H NMR (300 MHz, $CDCl_3$) δ_H 6.52 (d, $J_{2,P}$ = 21.1 Hz, 1H, H-2), 5.67 (s, 1H, H-3), 5.29-5.18 (m, 2H, H-4, H-5), 3.74, 3.70 (2d, J = 3.8 Hz, 6H, 2 OCH_3), 2.78 (d, J = 18.5 Hz, 1H, H-6'), 2.28 (d, J = 17.9 Hz, 1H, H-6), 2.05, 2.04, 2.02 (3s, 9H, 3 $OC(O)CH_3$).

^{13}C NMR (300 MHz, $CDCl_3$) δ_C 170.11, 170.08, 170.02 ($OCOCH_3$), 136.91 (d, J = 8.9 Hz, C-2), 129.48 (d, J =184.0 Hz, C-1), 67.95, 66.73 (d, J = 13.7 Hz, C-4, C-5), 66.02 (d, J = 21.6 Hz, C-3), 52.97, 52.93 (d, J = 5.9 Hz, 2 POMe), 29.10 (d, J = 9.3 Hz, C-6), 21.16, 20.93, 20.91 (3 OCO*C*H_3).

^{31}P NMR (121.4 MHz, $CDCl_3$) δ_P 19.15.

HR-ESI-MS calculated for $C_{14}H_{21}O_9P$ $(M+Na)^+$ 387.0812160, found 387.0815399.

(3R,4S,5R)-tri-acetoxy-cyclohex-1-ene (10)

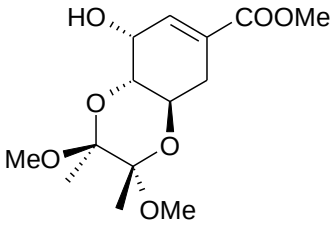


Side product from the reaction above formed in 18% yield. R_f = 0.41 (Tol:EA ; 5:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.90-5.81 (m, 1H, H-2), 5.72-5.64 (m, 1H, H-1), 5.57 (dd, $J_{3,4}$ = 4.3, $J_{3,2}$ = 4.3 Hz, 1H, H-3), 5.32-5.22 (m, 1H, H-5), 5.15 (dd, $J_{4,5 \text{ or } 3}$ = 10.2, $J_{4,3 \text{ or } 5}$ = 4.0 Hz, 1H, H-4), 2.74 (ddd, $J_{6',6}$ = 18.5, $J_{6',5}$ = 4.9, $J_{6',1}$ = 4.9 Hz, 1H, H-6'), 2.22-2.09 (m, 1H, H-6), 2.06, 2.04, 2.01 (3s, 9H, 3 OAc);

HR-ESI-MS calculated for $C_{12}H_{16}O_6Na$ $(M+Na)^+$ 279.0832730 found 279.0839093.

Methyl (3R,4S,5R)-3-hydroxy-4,5-(2,3-dimethoxy-butan-2,3-dioxy)-cyclohex-1-ene-1-carboxylate (11)



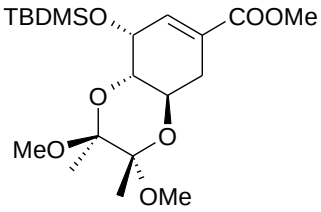
To a mixture of shikimic acid (0.5 g, 2.87 mmol), butane-2,3-dione (0.5 mL, 2 equiv), a catalytic amount of (+)-camforsulfonic acid (CSA_{cat}) in methanol (10 mL) was added trimethylorthoformate (1.57 mL, 5 equiv). The mixture was refluxed under nitrogen for 72h. After being cooled to room temperature, saturated aqueous $NaHCO_3$ (5 mL) was added to the mixture and stirred for 5 to 10 min. Removal of the solvent and purification by flash chromatography (Tol:EA ; 5:1 -> 1:1) yields compound **11** in 62%. R_f = 0.67 (EA:MeOH ; 100:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ_H 6.88 (dd, J =4.9, 2,6 Hz, 1H, H-2), 4.36 (dd, J = 4.7 Hz, 4.7 Hz, 1H, H-3), 4.07 (ddd, J =10.7 Hz, 10.7, 5.8 Hz, 1H, H-5), 3,72 (s, 3H, $COOCH_3$), 3.60 (dd, J = 10.7 Hz, 4.3 Hz, 1H, H-4), 3.25, 3.23 (2s, 6H, 2 OCH_3), 2.81 (dd, J =17.5, 5.6 Hz, 1H, H-6'), 2.22 (ddd, J =17.5, 10.3, 2.4 Hz, 1H, H-6), 1.31, 1.28 (2s, 6H, 2 CH_3).

^{13}C NMR (300 MHz, $CDCl_3$) δ_C 166.71 ($COOCH_3$), 135.24 (C-2), 131.76 (C-1), 100.11, 99.32, 70.64 (C-4), 65.13 (C-3), 62.50 (C-5), 52.16 ($COCH_3$), 48.17, 48.05 (2 OCH_3), 30.22 (C-6), 17.97, 17.78 (2 CH_3).

HR-ESI-MS calculated for $C_{14}H_{22}O_7$ $(M+Na)^+$ 325.1252420, found 325.1257742.

Methyl (3R,4S,5R)-3-[(*tert*-butyldimethylsilyl)oxy]-4,5-(2,3-dimethoxy-butan-2,3-dioxy)-cyclohex-1-ene-1-carboxylate (12)



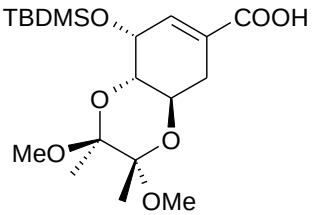
To a solution of compound **11** (0.309 mg, 1.02 mmol), imidazole (167 mg, 2.45 mmol), and a catalytic amount of dimethylaminopyridine ($DMAP_{Cat}$; 0.05 eq) in anhydrous DCM (5 mL) was added *tert*-butyldimethylsilyl chloride (185 mg, 1.23 mmol). The mixture was stirred at room temperature for 24h. Saturated aqueous $NaHCO_3$ (5 mL) was added to the reaction mixture which was then extracted with DCM (15 mL x 3), washed with brine (10 mL x 2) and dried over $MgSO_4$. The solvent was removed and the product was purified by flash chromatography (Tol:EA ; 10:1 then 5:1) to yield compound **12** in 76%. R_f = 0.59 (Tol:EA ; 5:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ_H 6.75 (dd, *J* =5.3, 2,6 Hz, 1H, H-2), 4.29 (dd, *J* = 4.5, *J* = 4.5 Hz, 1H, H-3), 4.09 (ddd, *J* =10.6, 10.60, 6.0 Hz, 1H, H-5), 3.72 (s, 3H, $COOCH_3$), 3.45 (dd, *J* = 10.7, 4.4 Hz, 1H, H-4), 3.22,3.20 (2s, 6H, 2 OCH_3), 2.78 (dd, *J* = 17.5, 6.0 Hz, 1H, H-6'), 2.19 (ddd, *J* = 17.5, 10.6, 2.6 Hz, 1H, H-6), 1.27, 1.26 (2s, 6H, 2 CH_3), 0.86 (s, 9H, *t*-Bu), 0.10, 0.08 (2s, 6H, 2 $SiCH_3$).

^{13}C NMR (300 MHz, $CDCl_3$) δ_C 167.26 (COOMe), 136.92 (C-2), 129.91 (C-1), 99.68, 98.90, 70.97 (C-4), 66.14 (C-3), 62.55 (C-5), 52.18 ($COCH_3$), 47.96, 47.80 (2 OCH_3), 30.54 (C-6), 25.91 ($C(CH_3)_3$), 18.55 ($SiC(CH_3)_3$), 18.05, 17.87 (2 CH_3), –4.49, -4.62 (2 $SiCH_3$).

HR-ESI-MS calculated for $C_{20}H_{36}O_7Si$ $(M+Na)^+$ 439.2124900, found 439.2122511.

(3R,4S,5R)-3-[(*tert*-butyldimethylsilyl)oxy]-4,5-(2,3-dimethoxy-butan-2,3-dioxy)-cyclohex-1-ene-1-carboxylic acid (13)



To compound **12** (300 mg, 0.72 mmol) diluted in MeOH (1 mL), was added a solution of aqueous NaOH 0.5M (2 mL). The mixture was stirred for 16h. Amberlite IR-120 H^+ was added until the pH=7 to neutralize the solution which was then filtered, evaporated,

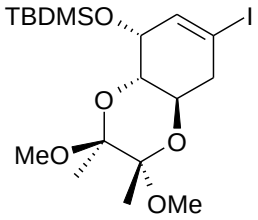
diluted in DCM, washed with brine, and evaporated. The product was purified by flash chromatography (Tol:EA:Acetic acid ; 5:1:0.2% and then 3:1:0.2%) to afford compound **13** in 80% yield. R_f = 0.12 (Tol:EA ; 5:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ_H 6.87 (dd, *J* = 5.4, 2,3 Hz, 1H, H-2), 4.31 (dd, *J* = 4.6, 4.6 Hz, 1H, H-3), 4.11 (ddd, *J* =10.6, 10.6, 6.0 Hz, 1H, H-5), 3.47 (dd, *J* = 10.8, 3.9 Hz, 1H, H-4), 3.23, 3.21 (2s, 6H, 2 OCH_3), 2.77 (dd, *J* = 17.5, 6.0 Hz, 1H, H-6'), 2.20 (ddd, *J* = 17.3, 10.3, 2.3 Hz, 1H, H-6), 1.27, 1.26 (2s, 6H, 2 CH_3), 0.86 (s, 9H, *t*-Bu), 0.10, 0.08 (2s, 6H, 2 $SiCH_3$).

^{13}C NMR (300 MHz, $CDCl_3$) δ_C 171.83 (COOH), 139.19 (C-2), 129.41 (C-1), 99.74, 98.99, 70.95 (C-4), 66.10 (C-3), 62.52 (C-5), 48.04, 47.90 (2 OCH_3), 30.27 (C-6), 25.97 ($C(CH_3)_3$), 18.57 ($SiC(CH_3)_3$), 18.07, 17.90 (2 CH_3), –4.49, -4.61 (2 $SiCH_3$).

HR-ESI-MS calculated for $C_{19}H_{34}O_7Si$ $(M+Na)^+$ 425.1972650, found 425.1966011.

1-iodo-(3R,4S,5R)-3-[(*tert*-butyldimethylsilyl)oxy]-4,5-(2,3-dimethoxy-butan-2,3-dioxy)-cyclohex-1-ene (14)



Compound **13** (124 mg, 0.308 mmol) was dissolved in dry CH_2Cl_2 (1 mL) in which DMF (0.3 μL) had been added. To the solution was added, under dinitrogen, oxalyl chloride (0.13 mL, 1.54 mmol). The mixture was allowed to stay at room temperature stirring under dinitrogen for 45 min (until there is no more bubbling) and then concentrated under vacuum. The residue was taken up in dry CH_2Cl_2 (1 mL).

Meanwhile, a mixture of *N*-hydroxypyridine-2-thione (47 mg, 0.37 mmol), DMAP (4 mg, 0.1 equiv), 2-iodo-1,1,1-trifluoroethane (0.12 mL, 1.23 mmol) was mixed by stirring in dry DCM (1 mL) under nitrogen in a quartz tube.

5 min before adding the residue of the first step, the quartz tube, still under nitrogen, is brought to reflux by irradiation with a 250W floodlamp. The solution of acid chloride was then added to this second mixture, and irradiation was continued for a further 30 min.

After removal of the solvent, the mixture was purified directly by flash chromatography (Tol:EA, 10:1 and then 5:1) to give compound **14** in 39% of yield. 25% of starting

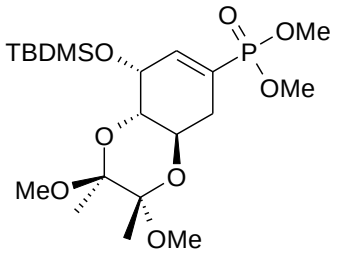
material **13** was recovered. The yield based on recovered starting material is 52%. R_f = 0.80 (Tol:EA ; 5:1).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 6.28 (dd, *J* = 5.7, 2.3 Hz, 1H, H-2), 4.18 (ddd, *J* = 10.5, 10.5, 6.3 Hz, 1H, H-5), 4.07-4.0 (m, 1H, H-3), 3.46 (dd, *J* = 10.7, 3.6 Hz, 1H, H-4), 3.21, 3.19 (2s, 6H, 2 OCH_3), 2.85 (dd, *J* = 17.6, 6.3 Hz, 1H, H-6'), 2.58 (ddd, *J* = 17.6, 10.0, 2.5 Hz, 1H, H-6), 1.25, 1.23 (2s, 6H, 2 CH_3), 0.86 (s, 9H, *t*-Bu), 0.07, 0.05 (2s, 6H, 2 $SiCH_3$).

^{13}C NMR (500 MHz, $CDCl_3$) δ_C 138.3 (C-2), 99.72, 99.0, 97.17 (C-1), 70.23 (C-4), 68.86 (C-3), 63.53 (C-5), 48.02,47.91 (2 OCH_3), 45.51 (C-6), 26.02 (*t*-Bu), 18.60 ($SiC(CH_3)_3$), 18.03, 17.90 (2 CH_3), –4.43,-4.59 (2 $SiCH_3$)

HR-ESI-MS calculated for $C_{18}H_{33}IO_5Si$ $(M+Na)^+$ 507.1030910, found 507.1034148.

Dimethyl (3R,4S,5R)-3-[(*tert*-butyldimethylsilyl)oxy]-4,5-(2,3-dimethoxy-butan-2,3-dioxy)-cyclohex-1-ene-1-phosphonate (15)



Anhydrous toluene was degassed in dinitrogen. Compound **14** (169 mg, 0.35 mmol) dissolved in dry toluene (1 mL) was added to a solution of tetrakis triphenylphosphine palladium (40 mg, 0.1 equiv) in dry toluene (1 mL) saturated under a dinitrogen atmosphere. To that mixture was added a solution of triethylamine (147 µL, 1.05 mmol) and dimethylphosphite (65 µL, 0.7 mmol) in dry toluene (1 mL), under nitrogen. The mixture was heated to 80°C and maintained at this temperature for 4 hours. After cooling down at room temperature, the reaction was quenched by addition of saturated aqueous NH_4Cl (3 mL). The mixture was then extracted with toluene, washed with brine and the solvent evaporated. Purification by flash chromatography (Tol:EA ; 5:1 -> 1:1) gave the desired compound **15** in 70% yield. R_f = 0.10 (Tol:EA ; 3:1).

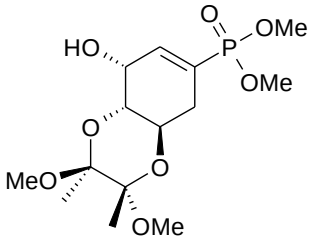
^{1}H NMR (300 MHz, $CDCl_3$) δ_H 6.59 (ddd, $J_{2,P}$ = 20.8, 4.6, 2,3 Hz, 1H, H-2), 4.25 (dd, *J* = 4.5, 4.5 Hz, 1H, H-3), 4.12 (ddd, *J* = 10.4, 10.4, 6.1 Hz, 1H, H-5), 3.47 (dd, *J* = 10.8, 3.9 Hz, 1H, H-4), 3.22, 3.20 (2s, 6H, 2 OCH_3), 2.54 (ddd, *J* = 16.8, 8.2, 6.4 Hz, 1H, H-6'), 2.23-2.09 (m, 1H, H-6), 1.27, 1.25 (2s, 6H, 2 CH_3), 0.85 (s, 9H, *t*-Bu), 0.09, 0.06 (2s, 6H, 2 $SiCH_3$).

^{13}C NMR (500 MHz, $CDCl_3$) δ_C 141.79 (d, *J* = 7.6 Hz, C-2), 126.96 (d, *J* = 181.9 Hz, C-1), 99.81, 99.12, 70.99 (d, *J* = 2.4 Hz, C-4), 66.41 (d, *J* = 21.7 Hz, C-3), 62.48 (d, *J* = 14.4 Hz, C-5), 52.75, 52.73 (2d, *J* = 5.7 Hz, $P(OCH_3)_2$), 48.07, 47.94 (2 OCH_3), 31.03 (d, *J* = 9.4 Hz, C-6), 25.94 (*t*-Bu), 18.08/17.89 (2 CH_3), –4.44, -4.63 (2 $SiCH_3$).

^{31}P NMR (161.9 MHz, $CDCl_3$) δ_P 20.70.

HR-ESI-MS calculated for $C_{20}H_{39}O_8PSi$ $(M+Na)^+$ 489.2011710, found 489.2044024.

Dimethyl (3R,4S,5R)-3-hydroxy-4,5-(2,3-dimethoxy-butan-2,3-dioxy)-cyclohex-1-ene-1-phosphonate (16)



Compound **15** (0.122 g, 0.26 mmol) was dissolved in anhydrous THF (2 mL) and TBAF (0.1 g, 0.31 mmol) was added to the solution. The mixture was stirred at room temperature for 3h, then quenched by addition of saturated aqueous NH_4Cl and extracted with diethyl ether. Purification by flash chromatography (Tol:EA ; 3:1 to 1:1) gave compound **16** in 96% of yield. R_f 0.49 (EA:MeOH ; 5:1).

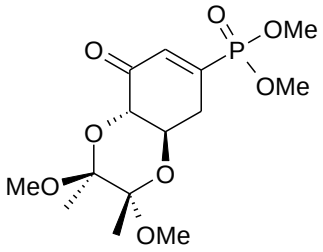
^{1}H NMR (300 MHz, $CDCl_3$) δ_H 6.68 (ddd, $J_{2,P}$ = 21.0, *J* = 4.6, 2.4 Hz, 1H, H-2), 4.33 (dd, *J* = 4.5, 4.5 Hz, 1H, H-3), 4.09 (ddd, *J* = 10.6, 10.6, 5.7 Hz, 1H, H-5), 3.72, 3.68 (2d, *J* = 3.9 Hz, 6H, $P(OCH_3)_2$), 3.60 (dd, *J* = 10.8, 4.2 Hz, 1H, H-4), 3.24, 3.23 (2s, 6H, 2 OCH_3), 2.75 (bs, 1H, OH), 2.58 (ddd, *J* = 16.9, 8.7, 5.7 Hz, 1H, H-6'), 2.29-2.14 (m, 1H, H-6), 1.31, 1.27 (2s, 6H, 2 CH_3).

^{13}C NMR (300 MHz, $CDCl_3$) δ_C 139.55 (d, *J* = 8.5 Hz, C-2), 129.50 (d, *J* = 181.3 Hz, C-1), 100.27, 99.54, 70.62 (d, *J* = 2.3 Hz, C-4), 65.51 (d, *J* = 21.7 Hz, C-3), 62.42 (d, *J* = 14.4 Hz, C-5), 52.96, 52.96 (2d, *J* = 5.8 Hz, $P(OCH_3)_2$), 48.32, 48.25 (2 OCH_3), 30.72 (d, *J* = 9.6 Hz, C-6), 18.06, 17.88 (2 CH_3).

^{31}P NMR (161.9 MHz, $CDCl_3$) δ_P 19.69.

HR-ESI-MS calculated for $C_{14}H_{25}O_8P$ $(M+Na)^+$ 375.1176890, found 375.1179254.

Dimethyl (4S,5R)-4,5-(2,3-dimethoxy-butan-2,3-dioxy)-3-oxo-cyclohex-1-ene-1-phosphonate (17)



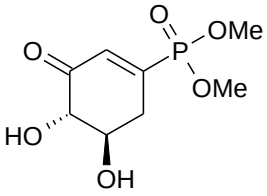
Compound **16** (42 mg, 0.125 mmol) was dissolved in acetone (2 ml). IBX (105 mg, 3 eq) was added to the solution. The mixture was stirred for 2h 30min at 65°C. The mixture was then filtered through celite and the filtrate was evaporated under vacuum. Purification by flash chromatography (EA) gave the ketone **17** in 94% yield. R_f = 0.39 (EA).

^{1}H NMR (300 MHz, $CDCl_3$) δ_H 6.58 (dd, $J_{2,P}$ = 20.6, J = 2.9 Hz, 1H, H-2), 4.31 (d, J = 11.7 Hz, 1H, H-4), 4.08 (ddd, J = 11.2, 11.2, 5.2 Hz, 1H, H-5), 3.80, 3.76 (2d, J = 2.6 Hz, 6H, $P(OCH_3)_2$), 3.27, 3.23 (2s, 6H, 2 OCH_3), 2.86 (ddd, J = 17.9, 10.3, 5.3 Hz, 1H, H-6'), 2.61 (dddd, J = 17.8, 10.6, 3.2, 3.2 Hz, 1H, H-6), 1.39, 1.29 (2s, 6H, 2 CH_3).

^{13}C NMR (300 MHz, $CDCl_3$) δ_C 136.5 (C-2), 75.6 (C-4), 48.9, 48.6 (2 OCH_3), 31.26 (C-6), 18.0, 17.9 (2 CH_3).

^{31}P NMR (121.4 MHz, $CDCl_3$) δ 17.69.

Dimethyl (4S,5R)-dihydroxy-3-oxo-1-cyclohexene-1-phosphonate (18)



Compound **17** (0.030 g, 0.0856 mmol) was dissolved in aqueous trifluoroacetic acid (TFA:H_2O ; 9:1 ; 3 mL). The solution was stirred overnight then frozen and lyophilized. Purification by flash chromatography (EA:MeOH:Et_3N ; 5:1:0.2% -> 1:1:0.2%) gave compound **18** in 79% yield. R_f = 0.20 (EA:MeOH ; 5:1).

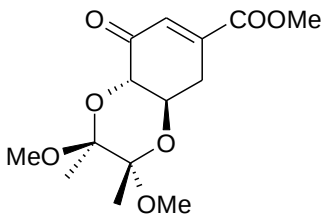
^{1}H NMR (600 MHz, $CDCl_3$) δ_H 6.70 (dd, $J_{2,P}$ = 20.2, J = 3.1 Hz, 1H, H-2), 4.11 (d, J = 10.9 Hz, 1H, H-4), 3.93 (ddd, J = 10.6, 10.6, 5.3 Hz, 1H, H-5), 3.81, 3.79 (2d, J = 6.5 Hz, 6H, $P(OCH_3)_2$), 3.00 (ddd, J = 18.3, 10.4, 5.3 Hz, 1H, H-6'), 2.56 (dddd, J = 18.3, 10.1, 3.2, 3.2 Hz, 1H, H-6).

^{13}C NMR (600 MHz, $CDCl_3$) δ_C 197.69 (d, J = 25.4 Hz, C-3), 147.34 (d, J = 178.4 Hz, C-1), 134.55 (d, J = 8.0 Hz, C-2), 79.46 (C-4), 72.37 (d, J = 14.85 Hz, C-5), 53.62, 53.58 ($POCH_3$), 32.95 (d, J = 7.5 Hz, C-6).

^{31}P NMR (121.4 MHz, $CDCl_3$) δ_P 17.14.

HR-ESI-MS calculated for $C_8H_{13}O_6P$ $(M+Na)^+$ 259.0342940, found 259.0341957.

Methyl (4S,5R)-4,5-(2,3-dimethoxy-butan-2,3-dioxy)-3-oxo-cyclohex-1-ene-1-carboxylate (19)



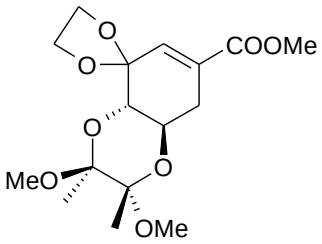
Compound **11** (120 mg, 0.40 mmol) was dissolved in acetone (4 ml). IBX (331 mg, 1.20 mmol) was added to the solution. The mixture was stirred for 3 hours at reflux under nitrogen. The mixture was then filtered through celite and the filtrate was evaporated under vacuum. Purification by flash chromatography (Tol:EA ; 3:1) gave the ketone **19** in 88% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ_H 6.78 (d, J = 3.1 Hz, 1H, H-2), 4.29 (d, J = 11.5, 1H, H-4), 4.07 (ddd, J = 10.7, 10.7, 5.3 Hz, 1H, H-5), 3.81 (s, 3H, $COOCH_3$), 3.27, 3.22 (2s, 6H, 2 OCH_3), 3.05 (dd, J = 18.5, 5.3 Hz, 1H, H-6'), 2.61 (ddd, J = 18.3, 10.6, 3.1 Hz, 1H, H-6), 1.39, 1.30 (2s, 6H, 2 CH_3).

^{13}C NMR (300 MHz, $CDCl_3$) δ_C 194.66 (C-3), 166.00 ($COOCH_3$), 144.81 (C-1), 132.86 (C-2), 100.50, 99.48, 75.26 (C-4), 67.22 (C-5), 53.14 ($COOCH_3$), 48.64, 48.28 (2 OCH_3), 30.65 (C-6), 17.81, 17.73 (2 CH_3).

HR-ESI-MS calculated for $C_{14}H_{20}O_7$ $(M+Na)^+$ 323.1100290, found 323.1101241.

Methyl (4S,5R)-3,3-(ethan-1,2-dioxy)-4,5-(2,3-dimethoxy-butan-2,3-dioxy)-cyclohex-1-ene-1-carboxylate (20).



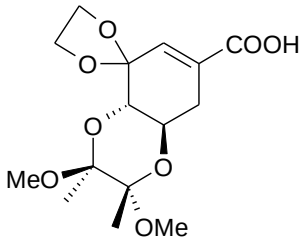
To a stirred solution of compound **19** (177 mg ; 0.59 mmol) and 2-bromoethanol (250 µL ; 6 eq) in anhydrous DMF (2 mL) at –60°C under nitrogen was added dropwise a solution of *t*-BuOK (397 mg ; 3.54 mmol) in anhydrous DMF (2 mL) for 10 min. Then the mixture was stirred until ambient temperature was reached. Then aqueous NH_4Cl was added and the mixture was extracted with EA (4 x 10 mL). Combined organic phases were washed with brine (2 x 5 mL) and dried with $MgSO_4$. After filtration, the solvent was removed under vacuum and the residue was purified by flash chromatography (Tol:EA ; 5:1) to afford compound **20** in 65%.

^{1}H NMR (300 MHz, $CDCl_3$) δ_H 6.50 (d, J = 2.5 Hz, 1H, H-2), 4.22, 4.10 (m, 2H, -OCH_2CH_2O-), 4.07-3.93 (m, 3H, -OCH_2CH_2O-, H-5), 3.78 (d, J = 11.1 Hz, 1H, H-4), 3.72 (s, 3H, $COOCH_3$), 3.25 (s, 6H, 2 OCH_3), 2.77 (dd, J = 17.5, 6.0 Hz, 1H, H-6'), 2.25 (ddd, J = 17.5, 10.4, 2.8 Hz, 1H, H-6), 1.30, 1.28 (2s, 6H, 2 CH_3).

^{13}C NMR (300 MHz, $CDCl_3$) δ_C 166.57 ($COOCH_3$), 136.37 (C-2), 130.53 (C-1), 105.19 (C-3), 99.60, 99.13, 73.58 (C-4), 67.33, 66.43 (-OCH_2CH_2O-), 65.37 (C-5), 52.36 ($COOCH_3$), 48.18, 48.00 (2 OCH_3), 29.59 (C-6), 18.01, 17.99 (2 CH_3).

HR-ESI-MS calculated for $C_{16}H_{24}O_8$ $(M+Na)^+$ 367.1363780, found 367.1363388.

(4S,5R)-3,3-(ethan-1,2-dioxy)-4,5-(2,3-dimethoxy-butan-2,3-dioxy)-cyclohex-1-ene-1-carboxylic acid (21)



To compound **20** (220 mg, 0.64 mmol) dissolved in MeOH (1 mL), was added $NaOH_{(aq)}$ 0.5M (3 mL). The mixture was stirred overnight. Amberlite IR-120 H^+ was added until the pH=7 to neutralize the solution which was then filtered (rinsing the Amberlite with

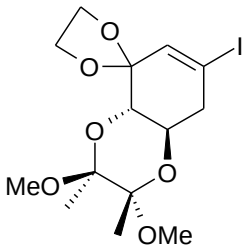
MeOH), evaporated, diluted in DCM, washed with brine, and evaporated. The product was purified by flash chromatography (Tol:EA ; 3:1) to afford acid **21** in 82% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ_H 6.60 (d, J = 2.3 Hz, 1H, H-2), 4.21-4.11 (m, 2H, -OCH_2CH_2O-), 4.07-3.94 (m, 3H, -OCH_2CH_2O-, H-5), 3.79 (d, J = 11.3 Hz, 1H, H-4), 3.25 (s, 6H, 2 OCH_3), 2.75 (dd, J = 17.5, 5.8 Hz, 1H, H-6'), 2.25 (ddd, J = 17.6, 10.4, 2.6 Hz, 1H, H-6), 1.30, 1.28 (2s, 6H, 2 CH_3).

^{13}C NMR (300 MHz, $CDCl_3$) δ_C 170.99 (COOH), 138.42 (C-2), 129.98 (C-1), 105.02 (C-3), 99.64, 99.17, 73.42 (C-4), 67.36, 66.47 (-OCH_2CH_2O-), 65.30 (C-5), 48,21, 48,02 (2 OCH_3), 29.27 (C-6), 18.01, 17.98 (2 CH_3).

HR-ESI-MS calculated for $C_{15}H_{22}O_8$ $(M+Na)^+$ 353.1207980, found 353.1206888.

1-iodo-(4S,5R)-3,3-(ethan-1,2-dioxy)-4,5-(2,3-dimethoxy-butan-2,3-dioxy)-cyclohex-1-ene (22)



Compound **21** (80 mg, 0.24 mmol) was dissolved in dry CH_2Cl_2 (1 ml) in which DMF (0.3 µL) had been added. To the solution was added, under dinitrogen, oxalyl chloride (0.11 mL, 1.29 mmol). The mixture was allowed to stay at room temperature stirring under dinitrogen for 45 min and then concentrated under vacuum. The residue was taken up in dry CH_2Cl_2 (1 ml).

Meanwhile, a mixture of *N*-hydroxypyridine-2-thione (46 mg, 0.31 mmol), DMAP (3 mg, 0.1 equiv), 2-iodo-1,1,1-trifluoroethane (0.10 mL, 1.03 mmol) was mixed by stirring in dry DCM (2 mL) under nitrogen in a quartz tube.

5 min before adding the residue of the first step, the quartz tube, still under nitrogen, is brought to reflux by irradiation with a 250W floodlamp. The solution of acid chloride was then added to this second mixture which was irradiated for a further 30 min.

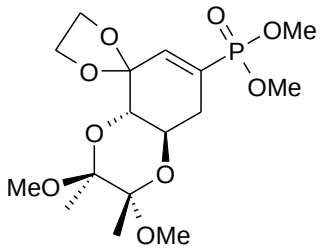
After removal of the solvent, the mixture was purified directly by flash chromatography (Tol:EA, 10:1 and then 7:1) to give compound **22** in 60% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ_H 6.09 (d, J = 2.1 Hz, 1H, H-2), 4.24-3.85 (m, 5H, -OCH_2CH_2O-, H-5), 3.75 (d, J = 11.1 Hz, 1H, H-4), 3.24, 3.23 (2s, 6H, 2 OCH_3), 2.84 (dd, J = 17.4, 6.3 Hz, 1H, H-6'), 2.63 (ddd, J = 17.4, 10.0, 2.5 Hz, 1H, H-6), 1.27, 1.24 (2s, 6H, 2 CH_3).

^{13}C NMR (300 MHz, $CDCl_3$) δ_C 138.35 (C-2), 106.33 (C-3), 99.54, 99.22, 97.40 (C-1), 73.29 (C-4), 67.09, 66.24 (-OCH_2CH_2O-), 65.81 (C-5), 48.22, 47.99 (2 OCH_3), 44.32 (C-6), 18.00, 17.93 (2 CH_3).

HR-ESI-MS calculated for $C_{14}H_{21}IO_6$ $(M+Na)^+$ 435.0279560, found 435.0275025.

Dimethyl (4S,5R)-3,3-(ethan-1,2-dioxy)-4,5-(2,3-dimethoxy-butan-2,3-dioxy)-cyclohex-1-ene-1-phosphonate (23)



Anhydrous toluene was degassed in dinitrogen. Compound **22** (160 mg, 0.39 mmol) in dry toluene (2 mL) was added to a solution of tetrakis triphenylphosphine palladium (67 mg, 0.15 equiv) in dry toluene (1 mL) saturated under a dinitrogen atmosphere. To that mixture was added a solution of triethylamine (165 µL, 1.16 mmol) and dimethylphosphite (106 µL, 1.16 mmol) in dry toluene (1 mL), under nitrogen. The mixture was heated to 80°C and maintained at this temperature for 3 hours. After cooling down at room temperature, the reaction was quenched by addition of saturated aqueous NH_4Cl (3 mL). The mixture was then extracted with toluene, washed with brine and the solvent evaporated. Purification by flash chromatography (Tol:EA ; 1:1 -> 0:1) gave dimethyl phosphonate **23** in 81% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ_H 6.32 (dd, $J_{2,P}$ = 21.5, J = 2.2 Hz, 1H, H-2), 4.17-4.07 (m, 2H, -OCH_2CH_2O-), 4.06-3.90 (m, 3H, -OCH_2CH_2O-, H-5), 3.76 (d, J = 10.9 Hz, 1H, H-4), 3.70, 3.67 (2d, J = 5.4 Hz, 6H, $P(OCH_3)_2$), 3.23 (s, 6H, 2 OCH_3), 2.61-2.47 (m, 1H, H-6'), 2.25-2.11 (m, 1H, H-6), 1.28, 1.25 (2s, 6H, 2 CH_3).

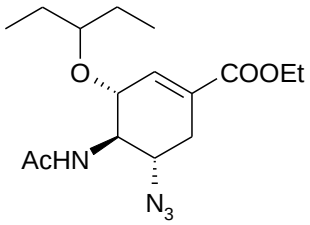
^{13}C NMR (300 MHz, $CDCl_3$) δ_C 141.25 (d, J = 7.5 Hz, C-2), 127.93 (d, J = 180.8 Hz, C-1), 105.08 (d, J = 25.0 Hz, C-3), 99.82, 99.37, 73.54 (d, J = 2.2 Hz, C-4), 67.46, 66.57 (-OCH_2CH_2O-), 65.49 (d, J = 15.1 Hz, C-5), 53.10, 53.10 (2d, J = 5.7 Hz, $P(OCH_3)_2$), 48.42, 48.22 (2 OCH_3), 30.09 (d, J = 8.8 Hz, C-6), 18.16, 18.14 (2s, 2 CH_3).

^{31}P NMR (121.4 MHz, $CDCl_3$) δ_P 20.16.

HR-ESI-MS calculated for $C_{16}H_{27}O_9P$ $(M+Na)^+$ 417.1281860, found 417.1284901.

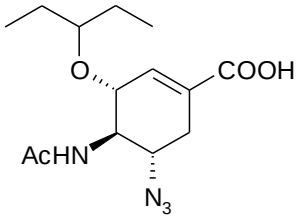
I.8.2. 'Phospha'-Tamiflu derivatives

Ethyl (3*R*,4*R*,5*S*)-4-acetamido-5-azido-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate.



Tamiflu's precursor was obtained from F. Hoffman-La Roche Ltd. Analytical purity was confirmed by NMR.

(3*R*,4*R*,5*S*)-4-acetylamino-5-azido-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid (24).

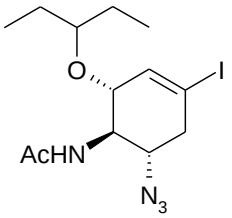


Oseltamivir precursor (1 g, 2.95 mmol) was dissolved in dioxane (6 mL), a NaOH-solution (0.5 M, 6 mL) was added and the mixture was stirred overnight. The mixture was neutralized with Amberlite IR-120 (H^+), filtered and lyophilized. The residue was purified by flash chromatography (Tol:EA:AcOH ; 1:1:0.5%) to afford the free acid **24** (889 mg, 97% yield) as a colorless solid. R_f = 0.27 (Tol:EA, 1:1).

^{1}H NMR (500 MHz, CD_3OD) δ_H 6.85 (s, 1H, H-2), 4.27 (s, 1H, H-3), 3.90-3.80 (m, 2H, H-4, H-5), 3.48-3.42 (m, 1H, pentyl-CH), 2.89 (d, *J* = 18.6 Hz, 1H, H-6), 2.31-2.22 (m, 1H, H-6'), 2.07 (s, 3H, $COCH_3$), 1.63-1.53 (m, 4H, 2 pentyl-CH_2), 1.01-0.93 (m, 6H, 2 pentyl-CH_3).

HR-ESI-MS calculated for $C_{14}H_{22}N_4O_4$ $(M+Na)^+$ 333.1534270, found 333.1533263.

(3*R*,4*R*,5*S*)-4-acetylamino-5-azido-3-(1-ethylpropoxy)-1-iodocyclohexene (25)



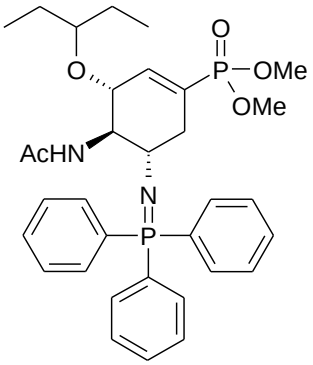
Under an atmosphere of dry nitrogen, the free acid **24** (250 mg, 0.81 mmol) and (chloromethylene)dimethyliminium chloride (Vilsmeier reagent, 124 mg, 0.97 mmol) were dissolved in dry CH_2Cl_2 (2 mL) and the mixture was stirred for 40 min at room temperature. Simultaneously, using a quartz tube, *N*-hydroxypyridine-2-thione (144 mg, 0.97 mmol), DMAP (50 mg, 0.4 mmol) and 2-iodo-1,1,1-trifluoroethane (0.317 mL, 3.22 mmol) were dissolved in dry CH_2Cl_2 (2 mL) under an atmosphere of dry nitrogen by help of an ultrasonic bath. The mixture was irradiated and heated to reflux with a 250W floodlamp for 5 minutes, followed by addition of the acyl chloride mixture. Irradiation and refluxing was then continued for a further 30 min. After evaporation of the solvent, the mixture was purified by flash chromatography (Tol:EA; 5:1 -> 2:1) to give the iodo derivative **25** (129 mg, 0.33 mmol) as a pale yellow crystalline solid and starting material **24** (117 mg, 0.38 mmol). Yield: 41% (77% based on consumed starting material). R_f = 0.53 (Tol:EA; 1:1).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 6.26 (s, 1H, H-2), 5.74 (bd, J = 6.4 Hz, 1H, NH), 4.38-4.31 (m, 2H, H-3, H-5), 3.29-3.19 (m, 2H, H-4, pentyl-CH), 2.89 (dd, J = 17.5 Hz, 1H, H-6), 2.57-2.48 (m, 1H, H-6'), 1.99 (s, 3H, $COCH_3$), 1.50-1.39 (m, 4H, 2 pentyl-CH_2), 0.88-0.83 (m, 6H, 2 pentyl-CH_3).

^{13}C NMR (125.8 MHz, $CDCl_3$) δ_C 171.2, 139.1 (C-2), 92.3 (C-1), 82.2 (pentyl-CH), 75.6 (C-3), 57.94 (C-4 or C-5), 57.92 (C-5 or C-4), 44.9 (C-6), 26.5, 25.9 (2 pentyl-CH_2), 23.8, 9.8, 9.5 (2 pentyl-CH_3).

HR-ESI-MS calculated for $C_{13}H_{21}IN_4O_2$ $(M+Na)^+$ 415.0606840, found 415.0601400.

Dimethyl (3*R*,4*R*,5*S*)-4-acetylamino-5-(triphenylphosphoranylidene)amino-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate (26).

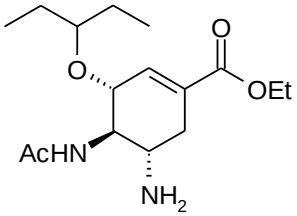


Under an atmosphere of dry nitrogen, tetrakis triphenylphosphine palladium (142 mg, 0.12 mmol) and azo-vinyl iodide **32** (161 mg, 0.41 mmol) were dissolved in anhydrous toluene (3 mL). Triethylamine (229 µL, 1.64 mmol) and dimethylphosphite (150 µL, 1.64 mmol) were added to the solution and the mixture was stirred at 65 °C for 2 hours. PPh_3 (108 mg, 0.41 mmol) was added to the mixture which was stirred for one more hour at the same temperature. After cooling to room temperature, the reaction was quenched by addition of saturated aqueous NH_4Cl (3 mL). CH_2Cl_2 (30 mL) was added and the organic phase was extracted with NH_4Cl (10 mL), washed with brine (2x10 mL), dried over $MgSO_4$ and evaporated. Purification by flash chromatography (EA:MeOH; 10:1 -> 2:1) gave the vinyl phosphonate **26** (72 mg, 0.12 mmol, 29%). R_f = 0.28 (EA:MeOH; 3:1).

^{1}H NMR (300 MHz, CD_3OD) δ_H 7.93-7.83 (m, 3H), 7.80-7.68 (m, 12H), 7.47 (d, *J* = 21.9 Hz, 1H, H-2), 4.17 (bd, *J* = 7.8 Hz, 1H), 3.89-3.79 (m, 1H), 3.66 (d, *J* = 11.4 Hz, 3H, OCH_3), 3.59 (d, *J* = 11.4 Hz, 3H, OCH_3), 3.52-3.44 (m, 1H), 3.37-3.30 (m, 1H), 2.60-2.38 (m, 1H, H-6), 2.34-2.20 (m, 1H, H-6'), 1.72 (s, 3H, $COCH_3$), 1.51-1.37 (m, 4H, 2 pentyl-CH_2), 1.51-1.37 (m, 6H, 2 pentyl-CH_3).

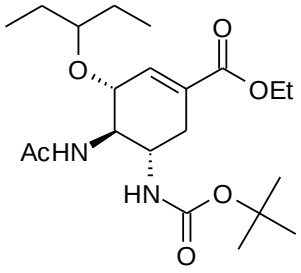
HR-ESI-MS calculated for $C_{33}H_{42}N_2O_5P$ $(M+H)^+$ 609.2669270, found 609.2673651.

Ethyl (3*R*,4*R*,5*S*)-4-acetylamino-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate (27).



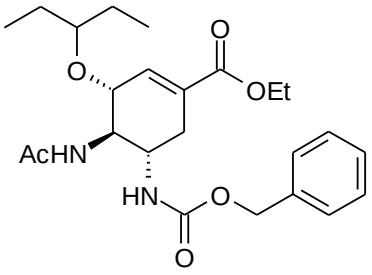
Under an atmosphere of dry nitrogen, the Lindlar catalyst (151,4 mg) was added to Tamiflu's precursor (400 mg, 1.19 mmol) and the mixture was dissolved in EtOH (6 mL). The mixture was stirred overnight and then filtered through celite. Removal of the solvent under vacuum afforded compound **27** (366 mg, 1.17 mmol) in 98% yield.
HR-ESI-MS calculated for $C_{24}H_{32}N_2O_7$ $(M+Na)^+$ 335.1943750, found 335.1941285.

Ethyl (3*R*,4*R*,5*S*)-4-acetylamino-5-*N*-(*tert*-Butoxycarbonyl)-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate (28).



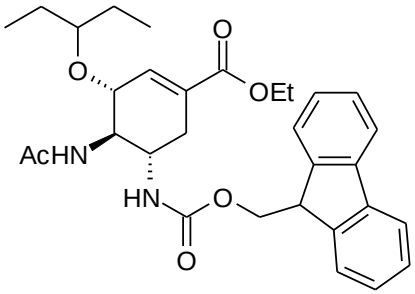
Under an atmosphere of dry nitrogen, Tamiflu's precursor (200 mg, 0.59 mmol) was dissolved in dry THF, PMe_3 was added (0.65 mL of 1M solution in THF, 0.65 mmol) and the mixture was stirred at room temperature for 2 hours. When TLC indicated the absence of starting material, the mixture was cooled to -16°C and a solution of 2-(*tert*-Butoxycarbonyloxyimino)-2-phenylacetonitrile (Boc-ON, 160 mg, 0.65 mmol) in THF (1 mL) was added via cannula. The mixture was then stirred for 3.5 hours, during which it was allowed to come to room temperature. Water (6 mL) was added and the solution was stirred for additional few minutes. The solution was extracted with EA (30 mL) and then washed with saturated aqueous NaCl (2x20 mL), the organic phase was dried over $MgSO_4$, concentrated and the residue was purified by flash chromatography (Tol:EA; 5:1 -> 1:1) to afford compound **28** (175 mg, 0.42 mmol, 72%). R_f = 0.32 (Tol:EA; 1:1).
HR-ESI-MS calculated for $C_{21}H_{36}N_2O_6$ $(M+Na)^+$ 435.2471280, found 435.2465580.

Ethyl (3*R*,4*R*,5*S*)-4-acetylamino-5-*N*-(benzyloxycarbonyl)-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate (29).



Under an atmosphere of dry nitrogen, Tamiflu's precursor (50 mg, 0.15 mmol) was dissolved in dry THF, PMe_3 was added (0.16 mL of 1M solution in THF, 0.16 mmol) and the mixture was stirred at room temperature for 2 hours. When TLC indicated the absence of starting material, the mixture was cooled to -16°C and a solution of benzyl chloroformate (420 µL, 2.95 mmol) in THF (1 mL) was added via cannula. The mixture was then stirred for 3.5 hours, during which it was allowed to come to room temperature. A few milliliters (5 mL) of semi-saturated $NaHCO_3$ were added and the solution was stirred for additional few minutes. The solution was extracted with CH_2Cl_2 (20 mL) and then washed with saturated aqueous NaCl (2x10 mL). The organic phase was dried over $MgSO_4$, concentrated and the residue was purified by flash chromatography (Tol:EA; 5:1 -> 1:1) to afford compound **29** (35 mg, 0.08 mmol, 35%).
R_f = 0.29 (Tol:EA; 1:1).
HR-ESI-MS calculated for $C_{24}H_{34}N_2O_6$ $(M+Na)^+$ 469.2315650, found 469.2309079.

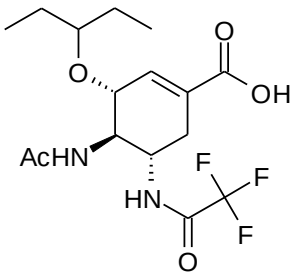
Ethyl (3*R*,4*R*,5*S*)-4-acetylamino-5-*N*-(9-fluorenylmethoxycarbonyl)-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate (30).



Under an atmosphere of dry nitrogen, Tamiflu's precursor (50 mg, 0.15 mmol) was dissolved in dry THF, PMe_3 was added (0.22 mL of 1M solution in THF, 0.22 mmol) and the mixture was stirred at room temperature for 2 hours. When TLC indicated the absence of starting material, the mixture was cooled to -16°C and a solution of

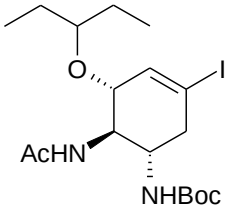
FMOCCl (191 mg, 0.74 mmol) in THF (1 mL) was added via cannula. The mixture was then stirred overnight, during which it was allowed to come to room temperature. A few milliliters (5 mL) of semi-saturated $NaHCO_3$ were added and the solution was stirred for additional few minutes. The solution was extracted with EA (15 mL) and subsequently washed with saturated aqueous NaCl (2x10 mL). The organic phase was dried over $MgSO_4$, concentrated and the residue was purified by flash chromatography (Tol:EA; 5:1 -> 1:1) to afford compound **30** (21 mg, 0.05 mmol, 27%).
HR-ESI-MS calculated for $C_{31}H_{38}N_2O_6$ $(M+Na)^+$ 557.2622300, found 557.2622081.

(3*R*,4*R*,5*S*)-4-acetylamino-5-trifluoroacetylamino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid (31).



Under an atmosphere of dry nitrogen, (3*R*,4*R*,5*S*)-4-acetylamino-5-azido-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid (50 mg, 0.15 mmol) was dissolved in dry THF, PMe_3 was added (0.18 mL of 1M solution in THF, 0.177 mmol) and the mixture was stirred at room temperature for 2 hours. When TLC indicated the absence of starting material, the mixture was cooled to -16°C and a solution of trifluoroacetic anhydride (103 µL, 0.74 mmol) in THF (1 mL) was added via cannula. The mixture was then stirred for 3.5 hours, during which it was allowed to come to room temperature. A few milliliters (5 mL) of semi-saturated NH_4Cl were added and the solution was stirred for additional few minutes. EA (15 mL) was added and the solution was extracted with saturated aqueous NaCl (2x10 mL), the organic phase was dried over $MgSO_4$, concentrated and the residue was purified by flash chromatography (Tol:EA; 5:1 -> 1:1) to afford compound **30** (26 mg, 0.05 mmol, 33%). R_f = 0.09 (Tol:EA; 3:1).
HR-ESI-MS calculated for $C_{16}H_{23}F_3N_2O_5$ $(M+Na)^+$ 403.1454850, found 403.1451275.

(3*R*,4*R*,5*S*)-4-acetylamino-5-*N*-*tert*-Butoxycarbonyl-amino-3-(1-ethylpropoxy)-1-iodocyclohexene (32)



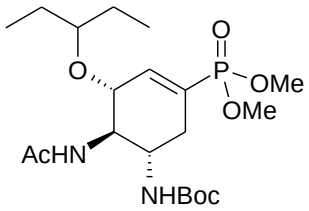
Under an atmosphere of dry nitrogen, the azido compound **25** (236 mg, 0.6 mmol) was dissolved in dry THF, PMe_3 was added (0.661 mL of 1M solution in THF, 0.66 mmol) and the mixture was stirred at room temperature for 1 hour. When TLC indicated the absence of starting material, the mixture was cooled to -16°C and a solution of 2-(*tert*-Butoxycarbonyloxyimino)-2-phenylacetonitrile (Boc-ON, 222 mg, 0.9 mmol) in THF (1 mL) was added via cannula. The mixture was then stirred for 3.5 hours, during which it was allowed to come to room temperature. Water (2 mL) was added and the solution was stirred for additional few minutes. The solution was extracted with CH_2Cl_2 (30 mL) and then washed with saturated aqueous NaCl (2x20 mL). The organic phase was dried over $MgSO_4$, concentrated and the residue was purified by flash chromatography (Tol:EA; 5:1 -> 1:1) to afford compound **32** (202 mg, 0.43 mmol, 72%) as a pale yellow crystalline solid. R_f = 0.39 (Tol:EA; 1:1).

^{1}H NMR (600 MHz, $CDCl_3$) δ_H 6.31 (s, 1H, H-2), 5.51 (bd, *J* = 8.1 Hz, 1H, NHAc), 5.25 (bd, *J* = 8.1 Hz, 1H, NHBoc), 4.10-4.05 (m, 1H, H-4), 3.85-3.79 (m, 1H, H-5), 3.76 (bs, 1H, H-3), 3.28 (bs, 1H, pentyl-CH), 2.85 (bd, *J* = 17.9, 1H, H-6), 2.60 (dd, *J* = 17.4, 7.5 Hz, 1H, H-6'), 1.96 (s, 3H), 1.51-1.43 (m, 4H, 2 pentyl-CH_2), 1.40 (s, 9H), 0.86 (m, 6H, 2 pentyl-CH_3).

^{13}C NMR (150.9 MHz, CDCl3) δ_C 170.9, 156.2, 138.2 (C-2), 95.1 (C-1), 82.2 (pentyl-CH), 79.9, 77.8, (C-3), 53.3 (C-4), 50.6 (C-5), 45.2 (C-6), 28.6, 26.3, 26.0 (2 pentyl-CH_2), 23.5, 9.7, 9.5 (2 pentyl-CH_3).

HR-ESI-MS calculated for $C_{18}H_{31}IN_2O_4$ $(M+Na)^+$ 489.1238250, found 489.1220716.

Dimethyl (3*R*,4*R*,5*S*)-4-acetylamino-5-*N*-*tert*-Butoxycarbonyl-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate (33)



Under an atmosphere of dry nitrogen, tetrakistriphenylphosphine palladium (230 mg, 0.2 mmol) and vinyl iodide **32** (620 mg, 1.33 mmol) were dissolved in anhydrous toluene (10 mL). Triethylamine (0.74 mL, 5.32 mmol) and dimethylphosphite (0.49 mL, 5.32 mmol) were added to the solution and the mixture was stirred at 75 °C for 90 minutes. After cooling to room temperature, the reaction was quenched by addition of saturated aqueous NH_4Cl (10 mL). CH_2Cl_2 (50 mL) was added and the organic phase was extracted with NH_4Cl (10 mL), washed with brine (2x10 mL), dried over $MgSO_4$ and evaporated. Purification by flash chromatography (EA:MeOH; 1:0 -> 6:1) gave the desired vinyl phosphonate **33** in 80% yield as a white glassy solid. R_f = 0.42 (EA:MeOH; 6:1).

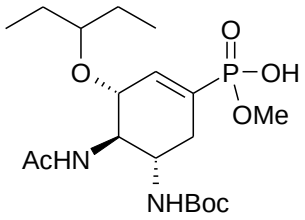
^{1}H NMR (600 MHz, CDCl3) δ_H 6.58 (d, *J*P-2 = 22.1 Hz, 1H, H-2), 5.88 (d, *J* = 9.4 Hz, 1H, NHAc), 5.12 (d, *J* = 9.2 Hz, 1H, NHBoc), 4.07-4.00 (m, 1H, H-4), 3.90 (bs, 1H, H-3), 3.80-3.72 (m, 1H, H-5), 3.69 (d, *J* = 10.8 Hz, 3H, OCH_3), 3.68 (d, 3H, *J* = 10.8 Hz, OCH_3), 3.32 (m, 1H, pentyl-CH), 2.57 (m, 1H, H-6), 2.18 (m, 1H, H-6'), 1.95 (s, 3H, $COCH_3$), 1.51-1.42 (m, 4H, 2 pentyl-CH_2), 1.38 (s, 9H, $C(CH_3)_3$), 0.85-0.84 (m, 6H, 2 pentyl-CH_3).

^{13}C NMR (150.9 MHz, CDCl3) δ_C 171.0, 156.4, 142.6 (d, J_{P-2} = 8.1 Hz, C-2), 126.6 (d, J_{P-1} = 182.9 Hz, C-1), 82.4 (pentyl-CH), 79.9, 76.3 (d, J_{P-3} = 22.2 Hz, C-3), 54.5 (C-4), 52.8, (d, *J* = 6.4 Hz, OCH_3), 52.7 (d, *J* = 6.1 Hz, OCH_3), 49.4 (d, J_{P-5} = 14.2 Hz, C-5), 31.4 (d, J_{P-6} = 9.5 Hz, C-6), 28.5, 26.3, 25.8 (2 pentyl-CH_2), 23.5, 9.7, 9.3 (2 pentyl-CH_3).

^{31}P NMR (161.9 MHz, $CDCl_3$) δ_P 19.58.

HR-ESI-MS calculated for $C_{20}H_{37}N_2O_7P$ $(M+Na)^+$ 471.2260180, found 471.2230592.

Methyl [(3*R*,4*R*,5*S*)-4-acetylamino-5-*N*-*tert*-Butoxycarbonyl-amino-3-(1-ethylpropoxy)-1-cyclohexene]-1-phosphonic acid (34)



Phosphonate **33** (100 mg, 0.22 mmol) was dissolved in dioxane (2 mL), NaOH-solution (0.25M, 2 mL) was added and the mixture was stirred overnight at room temperature, neutralised with Amberlite IR-120 (H+) and lyophilised. Purification by flash chromatography (EA:MeOH; 6:1 -> 1:2) yielded phosphonic acid **34** (93 mg, 0.21 mmol) in 96% yield as a white solid. R_f = 0.21 (CH_2Cl_2:MeOH, 2:1). The product can be converted to the ammonium salt by lyophilisation from 0.1 M (NH_4HCO_3-solution).

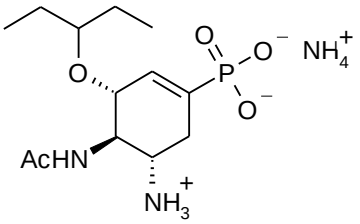
^{1}H NMR (500 MHz, D_2O) δ_H 6.38 (d, $J_{P\text{-}2}$ = 19.8 Hz, 1H, H-2), 4.30 (bd, J = 6.8 Hz, 1H, H-3), 3.89 (dd, J = 10.1 Hz, 1H, H-4), 3.80 (m, 1H, H-5), 3.64-3.54 (m, 1H, pentyl-CH), 3.58 (d, J = 10.6 Hz, 3H, OCH_3), 2.61 (m, 1H, H-6), 2.30 (m, 1H, H-6'), 2.09 (s, 3H, $COCH_3$), 1.69-1.46 (m, 4H, 2 pentyl-CH_2), 1.49 (s, 9H, $C(CH_3)_3$), 0.97 (t, J = 7.3 Hz, 3H, pentyl-CH_3), 0.91 (t, J = 7.3 Hz, 3H, pentyl-CH_3).

^{13}C NMR (125.8 MHz, D_2O) δ_C 174.2, 157.6, 136.9 (m, C-2), 131.1 (d, $J_{P\text{-}1}$ = 171.7 Hz, C-1), 84.1 (pentyl-CH), 81.0, 76.8 (C-3), 55.6 (C-4), 51.7 (d, J = 5.1 Hz, OCH_3), 49.3 (d, $J_{P\text{-}5}$ = 14.3 Hz, C-5), 31.2 (d, $J_{P\text{-}6}$ = 9.8 Hz, C-6), 27.6, 25.7, 25.3 (2 pentyl-CH_2), 22.2, 8.7, 8.6 (2 pentyl-CH_3).

^{31}P NMR (161.9 MHz, D_2O) δ_P 15.3.

HR-ESI-MS calculated for $C_{19}H_{35}N_2O_7P$ $(M+Na)^+$ 457.2104680, found 457.2074091.

Diammonium [(3*R*,4*R*,5*S*)-4-acetylamino-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (35)



Under an atmosphere of dry nitrogen, compound **33** (11 mg, 0.025 mmol) was dissolved in dry CH_2Cl_2 (1 mL). Lutidine (34 µL, 0.294 mmol) and trimethylsilyl bromide (33 µL, 0.245 mmol) were added and the mixture was stirred at room temperature for 9h. The

solvent was evaporated and TFA (50% in water, 2 mL) was added. After 1 hour of stirring at room temperature, the mixture was frozen and lyophilised. The residue was purified by gel permeation chromatography to afford compound **35** (7 mg, 88 %) as a white solid.

^{1}H NMR (600 MHz, D_2O) δ_H 6.34 (d, $J_{P\text{-}2}$ = 19.5 Hz, 1H, H-2), 4.27 (bd, J = 8.9 Hz, 1H, H-3), 4.09 (dd, J = 11.7, 8.8 Hz, 1H, H-4), 3.61-3.54 (m, 2H), 2.87-2.82 (ddd, J = 17.0, 7.9, 5.4 Hz ,1H, H-6_{ax}), 2.55-2.49 (ddddd, J = 17.0, 10.7, 5.9, 2.9 Hz, 1H, H-6_{eq}), 2.11 (s, 3H), 1.63-1.55 (m, 3H), 1.52-1.45 (m, 1H), 0.92 (t, J = 7.5 Hz, 3H), 0.87 (t, J = 7.5 Hz, 3H).

^{13}C NMR (150.9 MHz, D_2O) δ_C 175.1, 134.1 (d, $J_{P\text{-}2}$ = 6.9 Hz, C-2), 132.1 (d, $J_{P\text{-}1}$ = 173.5 Hz, C-1), 84.3 (pentyl-CH), 75.9 (d, $J_{P\text{-}3}$ = 18.4 Hz, C-3), 52.9 (C-4), 49.7 (d, $J_{P\text{-}5}$ = 13.2 Hz, C-5), 29.2 (d, $J_{P\text{-}6}$ = 10.8 Hz, C-6), 25.4, 25.1 (2 pentyl-CH_2), 22.3, 8.5, 8.4 (2 pentyl-CH_3).

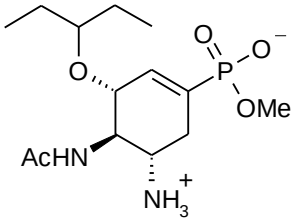
^{31}P NMR (161.9 MHz, D_2O) δ_P 10.24.

HR-ESI-MS calculated for $C_{13}H_{25}N_2O_5P$ $(M+H)^+$ 321.1573660, found 321.1573849.

HR-ESI-MS calculated for $C_{13}H_{25}N_2O_5P$ (M+Na)+ 343.1398270, found 343.1393295.

HR-ESI-MS calculated for $C_{13}H_{25}N_2O_5P$ (2M+Na)+ 663.2897800, found 663.2894380.

Ammonium [methyl (3*R*,4*R*,5*S*)-4-acetylamino-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (36)



Compound **34** (44 mg, 0.101 mmol) was dissolved in aqueous TFA (50%, 4 mL) and the mixture was stirred at room temperature for 2 hours. Following lyophilisation, the residue was purified by gel permeation chromatography and again lyophilised to afford compound **36** (13 mg, 0.04 mmol) as a white solid in 40% yield.

^{1}H NMR (500 MHz, D_2O) δ_H 6.44 (d, $J_{P\text{-}2}$ = 19.3 Hz, 1H, H-2), 4.34 (bd, J = 9.7 Hz, 1H, H-3), 4.11 (dd, J = 10.0 Hz, 1H, H-4), 3.65-3.56 (m, 2H, H-5, pentyl-CH), 3.59 (d, J = 10.8 Hz, 3H, OCH_3), 2.83 (m, 1H, H-6), 2.50 (m, 1H, H-6'), 2.16 (s, 3H, $COCH_3$), 1.69-1.49 (m, 4H, 2 pentyl-CH_2), 0.97 (t, 3H, pentyl-CH_3), 0.93 (t, 3H, pentyl-CH_3).

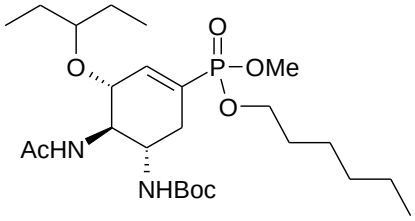
^{13}C NMR (500 MHz, D_2O) δ_C 175.1, 137.0-136.8 (m, C-2), 129.4 (d, $J_{P\text{-}1}$ = 173.2 Hz, C-1), 84.2 (pentyl-CH), 76.0 (d, $J_{P\text{-}3}$ = 18.9 Hz, C-3), 53.2 (C-4), 51.8-51.7 (m, OMe),

49.6 (d, $J_{P\text{-}5}$ = 13.8 Hz, C-5), 29.5 (d, $J_{P\text{-}6}$ = 12.1 Hz, C-6), 25.5, 25.2 (2 pentyl-CH_2), 22.3, 8.6, 8.5 (2 pentyl-CH_3).

^{31}P NMR (161.9 MHz, D_2O) δ_P 14.17.

HR-ESI-MS calculated for $C_{14}H_{27}N_2O_5P$ $(M+Na)^+$ 357.1562790, found 357.1549796.

Methyl 1-hexyl (3*R*,4*R*,5*S*)-4-acetylamino-5-*N*-*tert*-Butoxycarbonyl-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate (38)



Synthesis of hexyl triflate **37**: Under an atmosphere of dry nitrogen, hexanol (191 µL, 1.52 mmol) was dissolved in anhydrous toluene (5 mL), NEt_3 (274 µL, 1.97 mmol) was added with stirring and the mixture was cooled to -20°C. Triflic acid anhydride (305 µL, 1.82 mmol), dissolved in anhydrous toluene (5 mL) was added in portions to the mixture which was stirred for 1 hour and then allowed to warm up to room temperature. The colourless upper layer formed was evaporated to give an oil (hexyl triflate) which was used without further purification. The purity of **37** was confirmed by NMR.

Alkylation:

Compound **34** (26 mg, 0.06 mmol) was converted to its triethyl ammonium salt by dilution in distilled water (1 mL), addition of a few drops of NEt_3, stirring for 10 min, and lyophilisation to give a white solid. The triethyl ammonium salt of compound **34** was dissolved in anhydrous DMF (1 mL) and hexyl-triflate **37** (50 µL) was added to the solution via a syringe. The mixture was stirred for 24 hours at room temperature. Following evaporation of the solvent, the residue was purified by flash chromatography (Tol:EA; 1:1 -> 0:1) to give compound **38** (15.5 mg, 0.03 mmol) as mixture of diastereomers in 50% yield. R_f = 0.13 (EA).

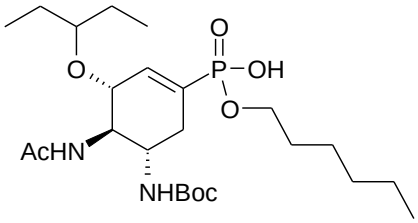
^{1}H NMR (600 MHz, $CDCl_3$) δ_H 6.58 (d, $J_{P\text{-}2}$ = 21.7 Hz, 1H, H-2), 5.65 (bd, J = 8.3 Hz, 1H, NHAc), 5.00 (m, 1H, NHBoc), 4.13-3.92 (m, 3H, H-4, $POCH_2$-), 3.82-3.75 (m, 1H, H-5), 3.69, 3.67 (2d, J = 7.3 Hz, 3H, OCH_3), 3.31 (m, 1H, pentyl-CH), 2.58 (m, 1H, H-6), 2.17 (m, 1H, H-6'), 1.96 (s, 3H, $COCH_3$), 1.67-1.60 (m, 2H), 1.52-1.44 (m, 4H), 1.40 (s, 9H, $C(CH_3)_3$), 1.37-1.21 (m, 6H), 0.89-0.83 (m, 9H).

^{13}C NMR (150.9 MHz, $CDCl_3$, 2 sets of signals) δ_C 171.0 (2s), 156.5 (2s), 142.1 (2d, J_{P-2} = 7.0 Hz, C-2), ~127.1 (2d, J_{P-1} = 183.7 Hz, C-1), 82.3 (pentyl-CH), 80.0, 76.5 (2d, J_{P-3} = 21.7 Hz, C-3), 66.5 (2d, J = 5.9 Hz, -OCH_2-), 60.6, 54.7 (2s, C-4), 52.7 (2d, J = 6.0 Hz, POMe), 49.4 (2d, J_{P-5} = 6.8 Hz, C-5), 31.5 (2s, C-6), 30.65 (2d, J = 6.1 Hz), 29.9, 28.5, 26.3, 25.8 (2 pentyl-CH_2), 25.4, 23.6, 22.7, 14.2, 9.8, 9.3 (2 pentyl-CH_3).

^{31}P NMR (242.9 MHz, $CDCl_3$) δ_P 18.07, 18.05.

HR-ESI-MS calculated for $C_{25}H_{47}N_2O_7P$ $(M+Na)^+$ 541.3032520, found 541.3013095.

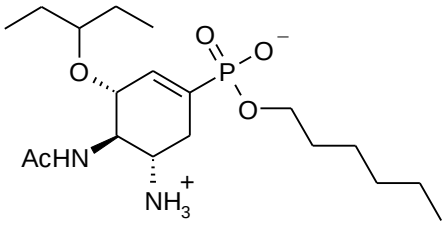
1-hexyl [(3*R*,4*R*,5*S*)-4-acetylamino-5-*N*-*tert*-Butoxycarbonyl-amino-3-(1-ethylpropoxy)-1-cyclohexene]-1-phosphonic acid (39)



Under atmosphere of dry nitrogen, compound **38** (12 mg, 0.023 mmol) was dissolved in anhydrous THF (1 mL) and anhydrous NEt_3 (46 µL, 0.32 mmol) and thiophenol (17 µL, 0.16 mmol) were added to the solution. After 48 hours of stirring at room temperature, the same amounts of anhydrous NEt_3 (46 µL, 0.32 mmol) and thiophenol (17 µL, 0.16 mmol) were added and the mixture was stirred for additional 24 h. Following evaporation of the solvent, purification by flash chromatography (EA:MeOH, 3:1 -> 1:2) afforded the monoester **39** (8.5 mg, 0.017 mmol) in 72% yield. Analysis by NMR indicated the absence of the methyl ester signal.

HR-ESI-MS calculated for $C_{24}H_{45}N_2O_7P$ $(M+Na)^+$ 527.2860840, found 527.2856594.

Ammonium [1-hexyl (3*R*,4*R*,5*S*)-4-acetylamino-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (40)



The monoester **39** (7 mg, 0.0139 mmol) was dissolved in aqueous TFA (50%, 1 mL) and the mixture was stirred at room temperature for 2 hours, followed by lyophilisation.

The residue was purified by gel permeation chromatography to afford compound **40** in 68% yield.

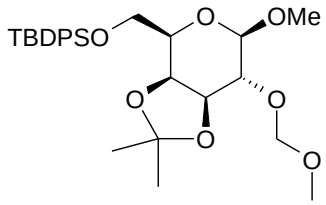
^{1}H NMR (500 MHz, D_2O) δ_H 6.42 (d, $J_{P\text{-}2}$ = 19.4 Hz, 1H, H-2), 4.33 (bd, J = 9.0 Hz, 1H, H-3), 4.10 (dd, J = 11.6 Hz, 8.9 Hz, 1H, H-4), 3.92-3.85 (m, 2H, OCH_2), 3.64-3.57 (m, 2H, H-5, pentyl-CH), 2.85 (m, 1H, H-6), 2.52 (m, 1H, H-6'), 2.16 (s, 3H, $COCH_3$), 1.74-1.48 (m, 6H), 1.48-1.33 (m, 6H), 1.01-0.89 (m, 9H).

^{13}C NMR (125.8 MHz, D2O) δ_C 175.1, 136.1 (d, J = 7.0 Hz, C-2), 130.2 (d, J = 174.0 Hz, C-1), 84.0 (pentyl-C), 75.9 (d, J = 19.6 Hz, C-3), 65.3 (d, J = 5.5 Hz, OCH_2), 53.1 (C-4), 49.7 (d, J = 14.2 Hz, C-5), 30.7, 29.9 (d, J = 6.6 Hz), 29.5 (d, J = 10.9 Hz, C-6), 25.5, 25.1, 24.7, 22.3, 21.8, 13.3, 8.6, 8.5 (2 pentyl-CH_3).

^{31}P NMR (161.9 MHz, D_2O) δ_P 12.55.

HR-ESI-MS calculated for $C_{19}H_{37}N_2O_5P$ $(M+Na)^+$ 427.2327960, found 427.2332299.

Methyl 6-*O*-(*tert*-butyldiphenylsilyl)-3,4-*O*-isopropylidene-2-*O*-methoxymethyl-β-D-galactopyranoside (41).

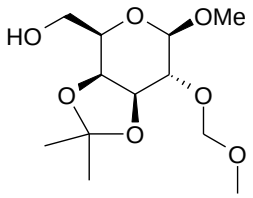


Methyl 6-*O*-(*tert*-butyldiphenylsilyl)-3,4-*O*-isopropylidene-ß-D-galactopyranoside[177] (516 mg, 1.09 mmol) was dissolved in dry THF (4 mL) and the solution was cooled to -20°C. Subsequently, NaH (32 mg, 1.31 mmol), suspended in dry THF (3 mL) and MOMCl (100 µL, 1.31 mmol) were added dropwise, the mixture was stirred overnight and allowed to come up to room temperature. The reaction was quenched by addition of saturated aqueous NH_4HCO_3 (10 mL). CH_2Cl_2 (40 mL) was added to the solution which was then extracted with NH_4HCO_3 (10 mL), washed with brine (2x10 mL), the organic phase was dried over $MgSO_4$ and the solvent evaporated. Purification by flash chromatography (Tol:EA, 1:0 -> 10:1) gave compound **41** (531 mg, 1.03 mmol) in 94% yield as a colorless oil. R_f = 0.34 (Tol:EA, 10:1).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.71-7.61 (m, 4H, TBDPS), 7.44-7.33 (m, 6H, TBDPS), 4.84, 4.74 (2d, J = 6.2 Hz, 2H, methoxymethyl-CH_2), 4.23 (bd, J = 5.1 Hz, 1H, H-4), 4.13 (d, J = 8.3 Hz, 1H, H-1), 4.09 (dd, 1H, H-3), 3.99-3.89 (m, 2H, H-6, H-6'), 3.82 (dd, 1H, H-5), 3.58 (dd, 1H, H-2), 3.47 (s, 3H, methoxymethyl-CH_3), 3.41 (s, 3H, OCH_3), 1.50, 1.33 (2s, $C(CH_3)_2$), 1.04 (s, 9H, $C(CH_3)_3$).

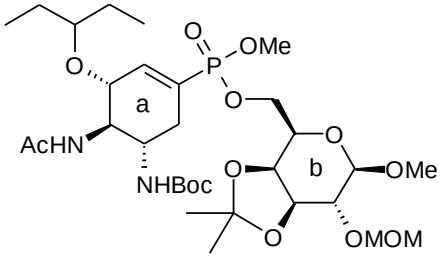
^{13}C NMR (125.8 MHz, $CDCl_3$) δ_C 135.8 (2s), 133.5, 133.4, 129.9, 127.9, 127.8, 109.8, 103.4 (C-1), 96.2 (methoxymethyl-CH_2), 78.8 (C-3), 76.0 (C-2), 73.4 (C-4), 73.3 (C-5), 62.8 (C-6), 56.5 (OCH_3), 55.4 (methoxymethyl-CH_3), 27.8, 26.7, 26.4, 19.2.
HR-ESI-MS calculated for $C_{28}H_{40}O_7Si$ $(M+Na)^+$ 539.2428640, found 539.2435513.

Methyl 3,4-*O*-isopropylidene-2-*O*-methoxymethyl-β-D-galactopyranoside (42)



Compound **41** (444 mg, 0.883 mmol) and TBAF (334 mg, 1.06 mmol) were dissolved in dry THF (10 mL) and the mixture was stirred overnight at room temperature. Evaporation of the solvent and purification by flash chromatography (Tol:EA, 5:1 -> 0:1) gave compound **42** (220 mg, 0.814 mmol) in 92% yield. R_f = 0.45 (EA).
^{1}H NMR (500 MHz, $CDCl_3$) δ_H 4.85, 4.75 (2d, J = 6.4 Hz, 2H, methoxymethyl-CH_2), 4.16 (d, J = 8.0 Hz, 1H, H-1), 4.14-4.09 (m, 2H, H-3, H-4), 3.96 (m, 1H, H-6), 3.81 (m, 2H, H-5, H-6'), 3.58 (dd, J = 7.8 Hz, 6.4 Hz, 1H, H-2), 3.50 (s, 3H, methoxymethyl-CH_3), 3.39 (s, 3H, OCH_3), 2.17 (bdd, J = 8.5, 3.5 Hz, 1H, OH), 1.49, 1.31 (2s, $C(CH_3)_2$).
^{13}C NMR (125.8 MHz, $CDCl_3$) δ_C 110.5, 103.7 (C-1), 96.4 (methoxymethyl-CH_2), 79.1 (C-3 or C-4), 76.1 (C-2),74.3 (C-3 or C-4), 73.3 (C-5), 62.7 (C-6), 57.0, 55.7, 28.0, 26.7.
HR-ESI-MS calculated for $C_{12}H_{22}O_7$ $(M+Na)^+$ 301.1255650, found 301.1257742.

Methyl (methyl 3,4-*O*-isopropylidene-2-*O*-methoxymethyl-β-D-galactopyranos-6-yl) [(3*R*,4*R*,5*S*)-4-acetamido-3-amino-3-(1,1-dimethylethyloxycarbonylamino)-5-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (44).



Synthesis of Methyl 3,4-*O*-isopropylidene-2-*O*-methoxymethyl-6-*O*-trifluoromethanesulfonyl-β-D-galactopyranoside (galactose triflate) **43**:

Under an atmosphere of dry nitrogen, alcohol **42** (146 mg, 0.54 mmol) was dissolved in dry CH_2Cl_2 (2 mL), lutidine (125 µL, 1.08 mmol) was added and the mixture was stirred for a few minutes before being cooled to -30°C. Triflic anhydride, dissolved in dry CH_2Cl_2 (1 mL) was added to the mixture via syringe and the mixture was stirred for 2 hours at this temperature. CH_2Cl_2 (5 mL) was added and the mixture was subsequently extracted with NH_4HCO_3-solution (2x3 mL) and KH_2PO_4-solution (2x3 mL). The organic phase was dried over $MgSO_4$ and the solvent was evaporated. The residue was purified on a short pad of silica flushed with CH_2Cl_2 to give triflate **43** (228 mg, 0.565 mmol) in 98% yield. The purity of **43** was confirmed by NMR.

Alkylation:

Compound **34** (50 mg, 0.115 mmol) was converted to its triethyl ammonium salt by dilution in distilled water (1 mL), addition of a few drops of NEt_3, stirring for 10 min and lyophilization to give a white solid. The salt was dissolved in anhydrous DMF (0.5 mL) and galactose-triflate **43** (71 mg, 0.173 mmol), dissolved in anhydrous DMF (0.5 mL) was added. The mixture was stirred for 48 hours at room temperature. Following evaporation of the solvent, the residue was purified by flash chromatography (EA:MeOH, 1:0 -> 10:1) to give compound **44** (22 mg, 0.032 mmol) as a white solid in 28% yield. R_f = 0.41 (EA:MeOH, 10:1). Approximately 50 % of unreacted starting material (phosphonate **34**) could be recovered.

^{1}H NMR (500 MHz, CD_3OD) δ_H 6.70, 6.68 (2d, $J_{P\text{-}2}$ = 22.4 Hz, 1H, H-2a (h,l)), 6.51 (d, 1H, NH), 4.90, 4.76 (2d, J = 6.3 Hz, 2H, methoxymethyl-CH_2), 4.35-4.25, 4.25-4.12 (2m, 7H, H-3a, H-1b, H-3b, H-4b, H-5b, H-6b, H-6b'), 3.94 (m, 1H, H-4a), 3.85 (d, J = 11.1 Hz, 3H, $POCH_3$), 3.81 (m, 1H, H-5a), 3.60 (m, 1H, H-2b), 3.57, 3.56 (2s, 3H,

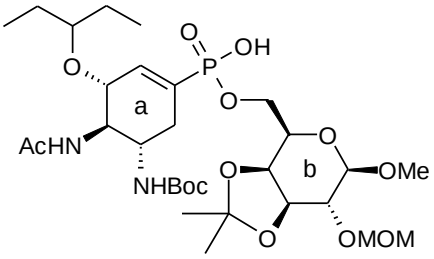
methoxymethyl-CH_3(h,l)), 3.49 (m, pentyl-CH), 3.46 (s, 3H, OCH_3), 2.64 (m, 1H, H-6a), 2.32 (m, 1H, H-6a'), 2.03 (s, 3H, $COCH_3$), 1.65-1.54 (m, 4H, 2 pentyl-CH_2), 1.56, 1.40 (2s, 6H, $C(CH_3)_2$), 1.50 (s, 9H, $C(CH_3)_3$), 0.99 (t, 3H, pentyl-CH_3), 0.95 (t, 3H, pentyl-CH_3).

^{13}C NMR (125.8 MHz, CD_3OD) δ_C 173.9, 158.1, 144.82 (2d, J_{P-1} = 6.9 Hz, C-2a), 111.5, 104.8 (C-1b), 97.2 (d, methoxymethyl-CH_2), 83.9 (d, pentyl-CH), 80.5, 80.2, 77.5 (d, J = 21.0 Hz, C-3a), 77.3 (C-2b), 74.9, 72.9 (2d), 66.4 (2d), 57.3 (d, $POCH_3$), 56.3 (d, C-4a), 55.9 (-OCH_3), 53.7 (methoxymethyl-CH_3), 50.6 (C-5a), 32.2 (C-6a), 28.9, 28.3, 27.4, 26.9, 26.8 (2 pentyl-CH_3), 23.1, 10.05, 9.7 (2 pentyl-CH_3).

^{31}P NMR (161.9, CD_3OD) δ_P 19.11.

HR-ESI-MS calculated for $C_{31}H_{55}N_2O_{13}P$ $(M+Na)^+$ 717.3359900, found 717.3333975.

(methyl 3,4-*O*-isopropylidene-2-*O*-methoxymethyl-β-D-galactopyranos-6-yl) [(3*R*,4*R*,5*S*)-4-acetylamino-5-*N*-*tert*-Butoxycarbonyl-amino-3-(1-ethylpropoxy)-1-cyclohexene]-1-phosphonic acid (45)

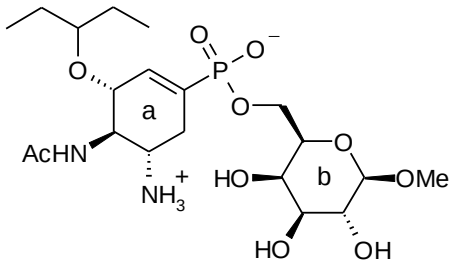


Under atmosphere of dry nitrogen, compound **44** (22 mg, 0.032 mmol) was dissolved in anhydrous THF (1 mL) and anhydrous NEt_3 (14 equiv, 62 μL) and thiophenol (7 equiv, 23 μL) were added to the solution. After 48 hours of stirring at room temperature, the same amounts of anhydrous NEt_3 (14 equiv) and thiophenol (7 equiv) were added and the mixture was stirred for additional 24 h. After evaporation of the solvent, purification by flash chromatography (EA:MeOH, gradient 5:1 -> 1:1) afforded the monoester **45** in 70% yield. R_f = 0.14 (EA:MeOH, 10:1). Analysis by NMR indicated the absence of the methyl ester signal.

^{31}P NMR (161.9, CD_3OD) δ_P 11.45.

HR-ESI-MS calculated for $C_{30}H_{53}N_2O_{13}P$ $(M+Na)^+$ 703.3170980, found 703.3177474.

(Methyl -β-D-galactopyranos-6-yl) [(3*R*,4*R*,5*S*)-4-acetamido-3-amino-5-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (46)



The monoester **45** (12 mg, 0.0176 mmol) was dissolved in aqueous TFA (50%, 2 mL) and the mixture was stirred at room temperature for 2 hours, followed by lyophilisation. The residue was purified by gel permeation chromatography to afford compound **46** (8 mg) in 64 % yield.

^{1}H NMR (600 MHz, D_2O) δ_H 6.42 (d, $J_{P\text{-}2}$ = 19.5 Hz, 1H, H-2a), 4.36 (d, J = 7.9 Hz, 1H, H-1b), 4.29 (bd, J = 8.8 Hz, 1H, H-3a), 4.08 (dd, J = 11.6 Hz, 9.0 Hz, 1H, H-4a), 4.00 (d, J = 3.4 Hz, 1H, H-4b), 3.95 (dd, J = 6.5 Hz, 6.5 Hz, 1H, H-6b), 3.97-3.93 (1H, H-5b), 3.86 (dd, 1H, H-6b'), 3.69 (dd, J = 9.9 Hz, 3.5 Hz, 1H, H-3b), 3.60 (s, 3H, OCH_3), 3.57-3.52 (m, 3H, pentyl-CH, H-5a, H-2b), 2.84 (m, 1H, H-6a), 2.52 (m, 1H, H-6a'), 2.11 (s, 3H, $COCH_3$), 1.63-1.45 (m, 4H, 2 pentyl-CH_2), 0.92 (t, 3H, pentyl-CH_3), 0.87 (t, 3H, pentyl-CH_3).

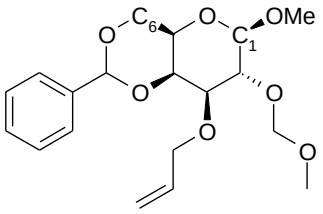
^{13}C NMR (150.9 MHz, D_2O) δ_C 175.1, 137.1 (d, J = 7.0 Hz, C-2a), 129.5 (d, J = 174.2 Hz, C-1a), 103.8 (C-1b), 84.2 (pentyl-CH), 75.9 (d, J = 19.7 Hz, C-3a), 73.5 (d, J = 7.8 Hz, C-6b), 72.6 (C-3b), 70.6 (C-2b), 68.1 (C-4b), 62.6 (d, J = 4.5 Hz, C-5b), 57.2 (OCH_3), 53.1 (C-4a), 49.6 (d, J = 13.9 Hz, C-5a), 29.3 (d, J = 11.5 Hz, C-6a), 25.4, 25.1 (pentyl-CH_2), 22.3 ($COCH_3$), 8.5, 8.4 (pentyl-CH_3).

^{31}P NMR (161.9 MHz, D_2O) δ_P 13.0.

HR-ESI-MS calculated for $C_{20}H_{37}N_2O_{10}P$ $(M+H)^+$ 497.2269930, found 497.2258584.

HR-ESI-MS calculated for $C_{20}H_{37}N_2O_{10}P$ $(M+Na)^+$ 519.2067700, found 519.2078030.

Methyl 2-*O*-methoxymethyl-3-*O*-allyl-4,6-*O*-benzylidene-β-D-galactopyranoside (47)



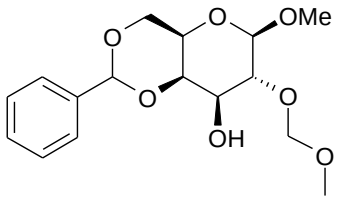
To a solution of Methyl 3-*O*-allyl-4,6-*O*-benzylidene-β-D-galactopyranoside (0.514 g, 1.59 mmol) in dry THF (4 mL), cooled to 0°C in an atmosphere of nitrogen, was added NaH (57.4 mg, 2.39 mmol) as a suspension in dry THF (3mL). The mixture was stirred for 15 min and then cooled to -20°C. MOMCl (157 µL, 2.07 mmol) was added dropwise, the mixture was allowed to warm up to room temperature and stirred overnight. The reaction was quenched by addition of saturated aqueous NH_4HCO_3 (5 mL). CH_2Cl_2 (40 mL) was added and the mixture was extracted with NH_4HCO_3 (2x10 mL), washed with brine (2x10 mL), the organic phase was dried over $MgSO_4$ and evaporated. Purification of the residue by flash chromatography (Tol:EA 5:1->1:5) gave compound **47** (0.323 g, 0.88 mmol, 55%) as a white solid. R_f = 0.53 (Tol:EA 1:1).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.50 (2H, m), 7.36-7.31 (3H, m), 5.91 (ddd, J = 17.3, 10.4, 5.7 Hz, 1H, allyl-$CHCH_2$), 5.50 (s, 1H, CHPh), 5.29 (dd, J = 17.2, 1.6 Hz, 1H, allyl-$CHCH_2$), 5.16 (dd, J = 10.4, 1.5 Hz, 1H, allyl-$CHCH_2$), 4.82, 4.80 (2d, J = 6.2 Hz, 2H, methoxymethyl-CH_2), 4.29 (dd, $J_{6,6'}$ = 12.3, $J_{6,5}$ = 1.4 Hz, 1H, H-6), 4.25 (d, $J_{1,2}$ = 7.8 Hz, 1H, H-1), 4.21-4.11 (m, 4-H, 3H, allyl-OCH_2), 4.03 (dd, $J_{6',6}$ = 12.3, $J_{6',5}$ = 1.6 Hz, 1H, H-6'), 3.84 (dd, $J_{2,3}$ = 9.7, $J_{2,1}$ = 7.9 Hz, 1H, H-2), 3.53 (s, 3H, OCH_3), 3.44 (dd, $J_{3,2}$ = 9.8, $J_{3,4}$ = 3.6 Hz, 1H, H-3), 3.42 (s, 3H, methoxymethyl-CH_3), 3.34 (s, 1H, H-5);

^{13}C NMR (125.8 MHz, $CDCl_3$) δ_C 138.01, 135.17, 129.08, 128.24, 126.72, 117.34, 104.22 (C-1), 101.52 (Ph-CH), 97.65 (methoxymethyl-CH_2), 79.21 (C-3), 74.46 (C-2), 73.72 (C-4), 71.02, 69.40 (C-6), 66.61 (C-5), 56.79 (OCH_3), 56.07 (methoxymethyl-CH_3);

HR-ESI-MS calculated for $C_{19}H_{26}O_7$ $(M+Na)^+$ 389.1575350, found 389.1570743.

Methyl 2-*O*-methoxymethyl-4,6-*O*-benzylidene-β-D-galactopyranoside (48)



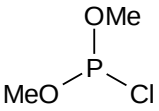
A mixture of compound **47** (0.316 g, 0.86 mmol), NaOAc (0.212 g, 2.59 mmol) and $PdCl_2$ (0.168 g, 0.95 mmol) in AcOH (4 mL) and H_2O (0.4 mL) was stirred for 5 hours at room temperature. The reaction mixture was evaporated in *vacuo*. The residue was dissolved in CH_2Cl_2 (20 mL) and washed successively with saturated aqueous $NaHCO_3$ (10 mL) and H_2O (10 mL), the organic phase was dried over $MgSO_4$ and evaporated to dryness. Purification of the residue by flash chromatography (Tol:EA ; 3:1 -> 1:3) gave compound **48** (0.194 g, 0.595 mmol, 69%). R_f = 0.17 (Tol:EA 1:1).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.52-7.46 (m, 2H), 7.37-7.30 (m, 3H), 5.53 (s, 1H, CHPh), 4.84, 4.76 (2d, J = 6.5 Hz, 2H, methoxymethyl-CH_2), 4.32 (d, $J_{6,6'}$ = 12.5 Hz, 1H, H-6), 4.25 (d, $J_{1,2}$ = 6.5 Hz, 1H, H-1), 4.20 (s, 1H, H-4), 4.05 (d, $J_{6',6}$ = 12.5 Hz, 1H, H-6'), 3.70-3.62 (m, 2H, H-3, H-2), 3.55-3.52 (m, 3H, OCH_3), 3.44-3.41 (m, 4H, H-5, methoxymethyl-CH_3), 3.01 (bd, J = 5.4 Hz, 1H, OH);

^{13}C NMR (125.8 MHz, $CDCl_3$) δ_C 137.81, 129.36, 128.38, 126.74, 103.77 (C-1), 101.76 (pentyl-CH), 97.74, 77.92 (C-3), 75.78, 72.48 (C-2, C-4), 69.30 (C-6), 66.71 (C-5), 57.15 (OCH_3), 56.03;

HR-ESI-MS calculated for $C_{16}H_{22}O_7$ $(M+Na)^+$ 349.1252960, found 349.1257742.

Chloro(dimethyl)phosphine (49)

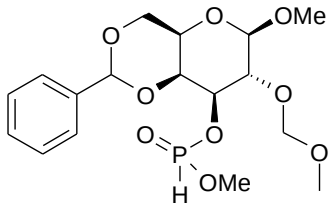


Under an atmosphere of nitrogen, $P(OMe)_3$ (16.2 mL, 137 mmol) was added dropwise to PCl_3 (6 mL, 68.6 mmol) at 0°C. The colorless solution turned slightly orange as soon as the mixture was heated up gradually to 90°C. The mixture was stirred at 90°C for 1 hour and 30 min. The residue was then distilled under reduced pressure (34°C, 42 Torr).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 3.68 (d, J = 11.1 Hz).

^{31}P NMR (161.9 MHz, $CDCl_3$) δ_P 169.28.

_O_-Methyl _O_-(methyl 2-_O_-methoxymethyl-4,6-_O_-benzylidene-β-D-galactopyranos-3-yl) phosphonic acid (50)



Under an atmosphere of nitrogen, compound **48** (31 mg, 0.095 mmol) was dissolved in dry CH_2Cl_2 (1 mL). *N*,*N*-diisopropyl ethyl amine (49 µL, 0.285 mmol) was added to the solution which was then cooled to 0°C. Chloro(dimethyl)phosphine **49** (40 µL) was added dropwise, the reaction mixture was allowed to come to room temperature and stirred overnight. Solid $NaHCO_3$ (50 mg) was added to the reaction mixture, followed by MeOH (0.5 mL) and silica (50 mg). The mixture was evaporated *in vacuo* to dryness. Purification by flash chromatography (Tol:EA:MeOH 1:1:0 -> 0:10:1) gave compound **50** (24 mg, 0.059 mmol, 62%). Upper spot on tlc: R_f = 0.18, lower spot on tlc: R_f= 0.16 (EA:MeOH 20:1).

NMR data of the isomer corresponding to the upper spot:

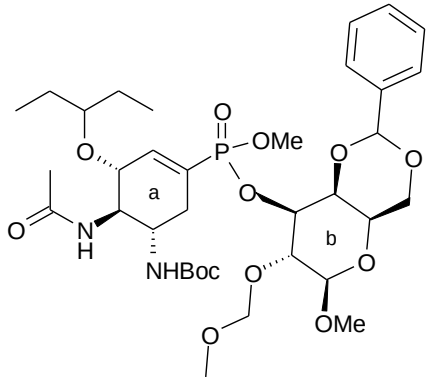
^{1}H NMR (600 MHz, $CDCl_3$) δ_H 7.51-7.48 (m, 2H), 7.32-7.36 (m, 3H), 6.93 (d, $J_{H,P}$ = 717.2 Hz, 1H, H-P), 5.55 (s, 1H, CHPh), 4.87, 4.72 (2d, J = 6.3 Hz, 2H, methoxymethyl-CH_2), 4.45-4.50 (m, 1H, H-3), 4.38 (d, J = 3.7 Hz, 1H, H-4), 4.32 (dd, $J_{6,6'}$ = 12.4, $J_{6,5}$ = 1.2 Hz, 1H, H-6), 4.28 (d, $J_{1,2}$ = 7.7 Hz, 1H, H-1), 4.05 (dd, $J_{6',6}$ = 12.5, $J_{6',5}$ = 1.6 Hz, 1H, H-6'), 3.90 (dd, $J_{2,3}$ = 9.8, $J_{2,1}$ = 7.7 Hz, 1H, H-2), 3.70 (d, J = 12.1 Hz, 3H, $POCH_3$), 3.54 (s, 3H, OCH_3), 3.44 (s, 1H, H-5), 3.38 (s, 3H, methoxymethyl-CH_3);

^{13}C NMR (150.9 MHz, $CDCl_3$) δ_C 137.73, 129.33, 128.40, 126.57, 104.17 (C-1), 101.43 (benzylidene-CH), 97.64 (methoxymethyl-CH_2), 76.25 (d, $J_{3,P}$ = 5.9 Hz, C-3), 75.85 (C-4), 73.98 (d, $J_{2,P}$ = 5.7 Hz, C-2), 69.14 (C-6), 66.13 (C-5), 57.23 (OCH_3), 56.42 (methoxymethyl-CH_3), 51.90 (d, J = 5.8 Hz, $POCH_3$);

^{31}P NMR (161.9 MHz, $CDCl_3$) δ_P (8.19), 9.92;

HR-ESI-MS calculated for $C_{17}H_{25}O_9P$ $(M+Na)^+$ 427.1143900, found 427.1128400.

Methyl (methyl 2-*O*-methoxymethyl-4,6-*O*-benzylidene-β-D-galactopyranos-3-yl) [(3R,4R,5S)-4-acetamido-3-(1,1-dimethylethyloxycarbonylamino)-5-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (51)



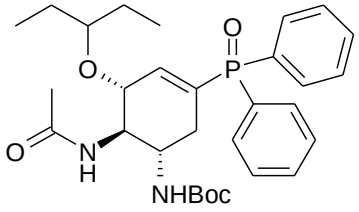
The phosphinic acid **50**, tetrakis triphenylphosphine palladium (7 mg, 6.3 µmol) and vinyl iodide **32** (20 mg, 0.042 mmol) were mixed under an atmosphere of dry nitrogen. The mixture was dissolved in anhydrous toluene (3 mL) and triethylamine (8.8 µL, 0.063 mmol) was added. The mixture was stirred at 85°C for 2.5 hours. After cooling to room temperature, the reaction was quenched by addition of saturated aqueous NH_4Cl (10 mL). CH_2Cl_2 (15 mL) was added to the solution which was then extracted with NH_4Cl (5 mL), washed with brine (2x5 mL), the organic phase was dried over $MgSO_4$ and the solvent was evaporated. Purification by flash chromatography (Tol:EA:MeOH 1:2:0 -> 0:10:1) gave a mixture of phosphonate diastereoisomers **51** (9 mg, 0.012 mmol, 30%). R_f = 0.34 (EA:MeOH 10:1). The product contains a cyclohexenenyl diphenylphosphine oxide impurity which could be separated and characterised only after the following synthetic step (compound **52**).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 6.66/6.53 (2d, $J_{2a,P}$ = 22 Hz, 1H, H-2a of the 2 diastereoisomers);

^{31}P NMR (161.9 MHz, $CDCl_3$) δ_P 18.93/18.07 (2 diastereoisomers);

HR-ESI-MS calculated for $C_{35}H_{55}N_2O_{13}P$ $(M+Na)^+$ 765.3321640, found 765.3333975.

Diphenyl [(3R,4R,5S)-4-acetamido-3-(1,1-dimethylethyloxycarbonylamino)-5-(1-ethylpropoxy)-1-cyclohexene] phosphine oxide (52)

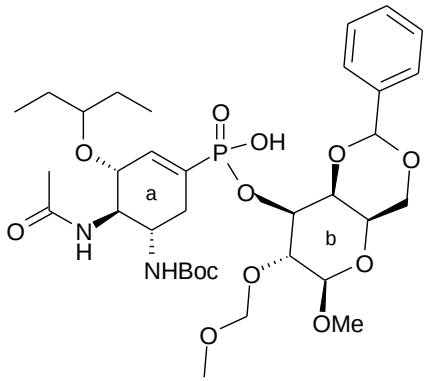


^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.72-7.62 (m, 4H), 7.58-7.42 (m, 6H), 6.22 (d, *J* = 19 Hz, 1H, H-2), 5.71 (d, *J* = 8.95 Hz, 1H, NHAc), 4.93 (d, *J* = 8.95 Hz, 1H, NHBoc), 4.13-4.03 (m, 1H, H-4), 3.88 (d, *J* = 7.3 Hz, 1H, H-3), 3.85-3.78 (m, 1H, H-5), 3.21-3.15 (m, 1H, H-7), 2.68-2.59 (m, 1H, H-6), 2.21-2.12 (m, 1H, H-6'), 1.94 (s, 3H, NHAc), 1.46-1.39 (m, 4H), 1.37 (s, 9H), 0.82 (t, *J* = 7.2 Hz, 3H), 0.69 (t, *J* = 7.2 Hz, 3H).

^{31}P NMR (161.9 MHz, $CDCl_3$) δ_P 28.12.

HR-ESI-MS calculated for $C_{30}H_{41}N_2O_5P$ $(M+Na)^+$ 563.2635950, found 563.2645300.

(Methyl 2-*O*-methoxymethyl-4,6-*O*-benzylidene-β-D-galactopyranos-3-yl) [(3R,4R,5S)-4-acetamido-3-(1,1-dimethylethyloxycarbonylamino)-5-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (53)



Under an atmosphere of dry nitrogen, compound **51** (8 mg; 0.011 mmol) was dissolved in anhydrous THF (1 mL). Anhydrous NEt_3 (22 µL, 0.156 mmol) and thiophenol (8 µL, 0.078 mmol) were added to the solution. After 48 h of stirring at room temperature, more anhydrous NEt_3 (22 µL, 0.156 mmol) and thiophenol (8 µL, 0.078 mmol) were added to the mixture which was stirred for another 24 h at room temperature. After evaporation of the solvent, purification by flash chromatography (EA:MeOH 10:1->1:5) afforded compound **53** (6 mg, 8.2 µmol, 74%).

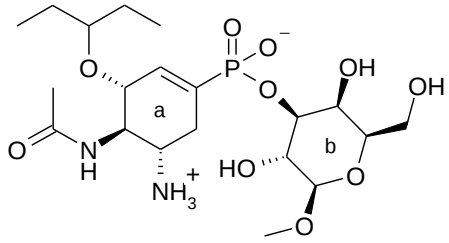
^{1}H NMR (600 MHz, CD_3OD) δ_H 7.56-7.52 (bs, 2H), 7.38-7.33 (bs , 3H), 6.48 (d, $J_{2a,P}$ = 19.8 Hz, 1H, H-2a), 5.65 (s, 1H, CHPh), 4.89-4.82 (2d, J = 5.9 Hz, 1H, methoxymethyl-CH_2), 4.41-4.36 (m, 2H, H-1b, H-4b), 4.23 (d, $J_{6b,6b'}$ = 12.4 Hz, 1H, H-6b), 4.21-4.14 (m, 2H, H-6b', H-2b/H-3b), 4.08 (d, J = 6.7 Hz, 1H, H-3a), 3.89 (dd, J = 10.0 Hz, 1H, H-4a), 3.81 (dd, J = 8.7 Hz, 1H, H-2b/H-3b), 3.77-3.71 (m, 1H, H-5a), 3.57 (s, 4H, H-5b, OCH_3), 3.46 (s, 3H, methoxymethyl-CH_3), 3.45-3.42 (m, 1H, pentyl-CH), 2.77-2.70 (m, 1H, H-6a), 2.43-2.35 (m, 1H, H-6a'), 1.97 (s, 3H), 1.60-1.43 (m, 4H), 1.41 (s, 9H), 0.95-0.88 (m, 6H);

^{13}C NMR (150.9 MHz, CD_3OD) δ_C 173.71 ($NHCOCH_3$), 158.09 ($NHCOOC(CH_3)_3$), 139.95, 137.66 (m, C-2a), 129.85, 129.04, 127.76, 105.40 (C-1b), 102.27 (benzylidene-CH), 98.20 (methoxymethyl-CH_2), 83.39 (pentyl-CH), 80.12, 78.10 (d, $J_{3a,P}$ = 19.85 Hz, C-3a), 77.04 (C-4b), 76.05 (d, $J_{3b\text{-}C/2b\text{-}C,P}$ = 5.1 Hz, C-3b/C-2b), 74.88 (d, $J_{3b\text{-}C/2b\text{-}C,P}$ = 5.95 Hz, C-3b/C-2b), 70.19 (C-6b), 67.86 (C-5b), 57.31 (OCH_3), 56.94 (d, $J_{4a,P}$ = 2.6 Hz, C-4a), 56.64 (methoxymethyl-CH_3), 51.20 (d, $J_{5a,P}$ = 13.6 Hz, C-5a), 32.96 (d, $J_{6a,P}$ = 9.6 Hz, C-6a), 28.91, 27.55, 26.91, 23.19, 10.44, 9.84;

^{31}P NMR (161.9 MHz, CD_3OD) δ_P 10.87;

HR-ESI-MS calculated for $C_{34}H_{53}N_2O_{13}P$ $(M+Na)^+$ 775.3140430, found 775.3153421.

(Methyl-β-D-galactopyranos-3-yl) [(3R,4R,5S)-4-acetamido-3-amino-5-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (54)



Compound **53** (6 mg, 8.2 µmol) was dissolved in a solution of 1:1 TFA/H_2O (2 mL) and the mixture was stirred at room temperature for 3 hours. The mixture was lyophilized and the residue was purified by gel permeation chromatography to afford compound **54** (2.5 mg, 5µmol, 61%) as a white powder after lyophilization.

^{1}H NMR (600 MHz, D_2O) δ_H 6.42 (d, $J_{2a,P}$ = 20.4 Hz, 1H, H-2a), 4.39 (d, $J_{1b,2b}$ = 8.3 Hz, 1H, H-1b), 4.22 (d, $J_{3a,4a}$ = 8.5 Hz, 1H, H-3a), 4.08 (d, $J_{4b,3b}$ = 3.3 Hz, 1H, H-4b), 4.02 (ddd, $J_{3b,2b}$ = 8.8, $J_{3b,4b}$ = 3.4 Hz, 1H, H-3b), 3.89 (dd, $J_{4a,5a}$ = 11.0, $J_{4a,3a}$ = 9.4 Hz, 1H, H-4a), 3.84-3.71 (m, 3H, H-6b, H-6b', H-5b), 3.64 (dd, J = 9.6, 8.2 Hz, 1H, H-2b), 3.60 (s, 3H, OCH_3), 3.54 (tt, J = 5.6 Hz, 1H, pentyl-CH), 3.27 (s, 1H, H-5a), 2.81-2.73

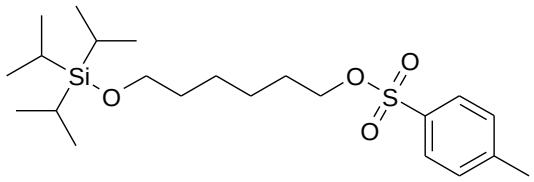
(m, 1H, H-6a), 2.45-2.37 (m, 1H, H-6a'), 2.10 (s, 3H, $COCH_3$), 1.63-1.54 (m, 3H), 1.52-1.44 (m, 1H), 0.92 (t, J = 7.4 Hz, 3H, pentyl-CH_3), 0.87 (t, $J_{2,P}$ = 7.4 Hz, 3H, pentyl-CH_3);

^{13}C NMR (150.9 MHz, D_2O) δ_C 174.92 ($NHCOCH_3$), 136.77 (d, $J_{2a,P}$ = 7.3 Hz, C-2a), 103.52 (C-1b), 84.08 (pentyl-CH), 81.14, 76.61 (d, $J_{3b,P}$ = 5.8 Hz, C-3b), 76.32 (d, $J_{3a,P}$ = 20.4 Hz, C-3a), 74.68 (C-5b), 69.66 (d, $J_{2b,P}$ = 5.6 Hz, C-2b), 68.35 (C-4b), 60.80 (C-6b), 57.12 (OCH_3), 49.49 (C-5a), 31.15 (C-6a), 25.45, 25.10, 22.26 ($NHCOCH_3$), 8.51, 8.48;

^{31}P NMR (242.9 MHz, D_2O) δ_P 13.06;

HR-ESI-MS calculated for $C_{19}H_{35}N_2O_{10}P$ $(M+Na)^+$ 519.2078, found 519.2062.

Toluene-4-sulfonic acid 6-(triisopropylsilyloxy) hexyl ester (55)



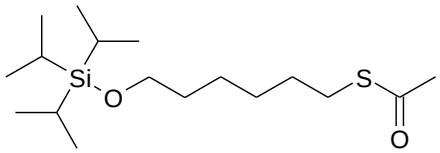
Under an atmosphere of dry nitrogen, 6-(Triisopropylsilyloxy) hexan-1-ol (3.86 g, 14.06 mmol) was dissolved in dry pyridine (12 mL). DMAP (172 mg, 1.406 mmol) and *p*-toluene sulfonyl chloride (4.02 g, 21.09 mmol) were added to the solution and the mixture stirred a 0°C for 4 hours. The reaction was quenched by addition of saturated aqueous NH_4Cl (5 mL). After evaporation to dryness of the mixture, the residue was dissolved in DCM (15 mL) which was then extracted with NH_4Cl (5 mL), washed with brine (2x5 mL), the organic phase was dried over $MgSO_4$ and the solvent evaporated. Purification by flash chromatography (Tol:EA ; 1:0 -> 10:1) gave compound **55** (4.792 g, 0.011 mol, 80%) as an incolored oil. R_f = 0.19 (Tol).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J*= 8.1, 3.3 Hz, 2H), 7.32 (d, *J*= 7.7, 2.7 Hz, 2H), 4.04-3.96 (m, 2H), 3.65-3.57 (m, 2H), 2.43 (d, *J*= 3.2 Hz, 3H), 1.63 (bs, 2H), 1.46 (bs, 2H), 1.34-1.24 (m, 4H), 1.07-0.98 (m, 21H).

^{13}C NMR (150.9 MHz, $CDCl_3$) δ 144.79, 133.58, 129.99, 128.08, 70.83, 63.39, 32.93, 29.09, 25.47, 25.44, 21.86, 18.23, 12.23.

HR-ESI-MS calculated for $C_{22}H_{40}O_4SSi$ $(M+Na)^+$ 451.2295600, found 451.2308781.

6-(triisopropylsilyloxy) hexyl thioacetate (56)



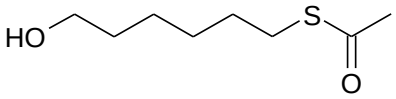
Compound **55** (4.792 g, 0.011 mol) was dissolved in pyridine (65 mL), then KSAc (3.83 g, 0.033 mol) was added to the solution. The mixture was stirred for 5 hours at room temperature. Pyridine was removed under high vacuum and the residue was dissolved in DCM (50 mL). The solution was washed with saturated aqueous NH_4Cl (2x15 mL), NaCl (20 mL), the organic phase was dried over $MgSO_4$ and the solvent evaporated. Purification by flash chromatography (Tol) afforded compound **56** (3.208 g, 0.0096 mol, 86%) as an incolored oil. R_f = 0.46 (Tol).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 3.65 (t, *J*= 6.5 Hz, 2H), 2.85 (t, 7.3 Hz, 2H), 2.30 (s, 3H), 1.60-1.48 (m, 4H), 1.39-1.31 (m, 4H), 1.08-1.00 (m, 21H).

^{13}C NMR (150.9 MHz, $CDCl_3$) δ_C 196.17, 63.53, 33.06, 30.84, 29.75, 29.36, 28.89, 25.62, 18.26, 12.27.

HR-ESI-MS calculated for $C_{17}H_{36}O_2SSi$ $(M+Na)^+$ 355.2095450, found 355.2097487.

Thioacetate hexanol (57)



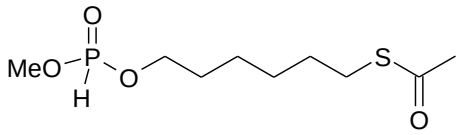
Compound **56** (0.874 g, 2.63 mmol) was dissolved in THF (20 mL), then AcOH (1 mL) and TBAF (3.32 g, 10.51 mmol) were successively added. The mixture was stirred overnight. The solution was washed with saturated aqueous $NaHCO_3$ (2x15 mL), NaCl (20 mL), the organic phase was dried over $MgSO_4$ and the solvent evaporated. Purification by flash chromatography (Tol) afforded compound **57** (0.333 g, 1.889 mol, 72%) as an incolored oil. R_f = 0.17 (Tol:EA ; 5:1).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 3.53 (t, *J*= 6.5 Hz, 2H), 2.79 (t, 7.4 Hz, 2H), 2.24 (s, 3H), 2.10 (s, 1H, OH), 1.54-1.44 (m, 4H), 1.35-1.26 (m, 4H).

^{13}C NMR (150.9 MHz, $CDCl_3$) δ_C 196.23, 62.69, 32.59, 30.68, 29.53, 29.09, 28.59, 25.32.

HR-ESI-MS calculated for $C_8H_{16}O_2S$ $(M+H_20+H)^+$ 195.1048370, found 195.1049416.

_O_-methyl _O_-(hexyl thioacetate) phosphonic acid (58)



Under an atmosphere of nitrogen, compound **57** (333 mg, 1.89 mmol) was dissolved in dry DCM (5 mL) at 0°C. *N*,*N*-diisopropyl ethyl amine (0.99 mL, 5.67 mmol) was added to the solution. Chloro(dimethyl)phosphine **49** (0.6 mL) was added drop by drop, the reaction mixture was allowed to come up to room temperature and stirred overnight. Solid $NaHCO_3$ (a spatula) was added to the reaction mixture, followed by MeOH (2 mL) and silica (a few spatula). The mixture was evaporated to dryness. Purification by flash chromatography (Tol:EA ; 5:1 -> 1:3) gave compound **58** (208 mg, 0.818 mmol, 41%) as a yellowish liquid-oil. R_f = 0.15 (Tol:EA ; 1:1).

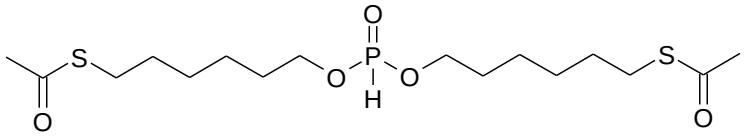
^{1}H NMR (500 MHz, $CDCl_3$) δ_H 6.75 (d, *J*= 694.5 Hz, 1H, PH), 4.09-3.99 (m, 2H, -O-CH_2-), 3.74 (d, *J*= 12.4 Hz, 3H, POMe), 2.84 (t, *J*= 7.3 Hz, 2H, -CH_2-S-), 2.29 (s, 3H), 1.71-1.62 (m, 2H), 1.60-1.52 (m, 2H), 1.38 (s, 4H).

^{13}C NMR (150.9 MHz, $CDCl_3$) δ_C 196.06, 65.92 (d, *J*= 6.1 Hz), 52.14 (d, *J*= 5.6 Hz, POMe), 30.83 ($COCH_3$), 30.45 (d, *J*= 6.2 Hz), 29.59, 29.14, 28.41, 25.23.

^{31}P NMR (161.9 MHz, $CDCl_3$) δ_P 9.15.

HR-ESI-MS calculated for $C_9H_{19}O_4PS$ $(M+Na)^+$ 277.0629110, found 277.0633874.

_O_-_O_-di(hexyl thioacetate) phosphonic acid (59)



By-product **59** (83 mg, 0.208 mmol, 11%) from the formation of compound **58**.

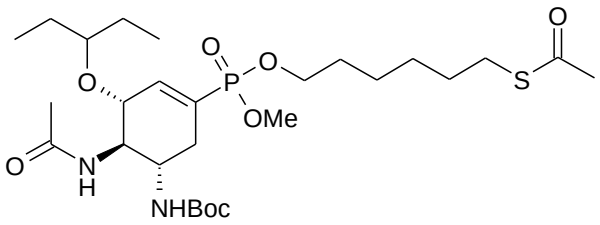
^{1}H NMR (500 MHz, $CDCl_3$) δ_H 6.75 (d, *J*= 692.8 Hz, 1H, PH), 4.08-3.96 (m, 4H, -O-CH_2-), 2.86 (t, *J*= 7.3 Hz, 4H, -CH_2-S-), 2.28 (s, 6H), 1.69-1.62 (m, 4H), 1.58-1.50 (m,4H), 1.36 (s, 8H).

^{13}C NMR (150.9 MHz, $CDCl_3$) δ_C 195.97, 65.76 (d, *J*= 6.0 Hz), 30.79 ($COCH_3$), 30.42 (d, *J*= 6.3 Hz), 29.56, 29.10, 28.39, 25.22.

^{31}P NMR (161.9 MHz, $CDCl_3$) δ_P 7.69.

HR-ESI-MS calculated for $C_{16}H_{31}O_5PS_2$ $(M+Na)^+$ 421.1243, found 421.1240.

Methyl (hexyl thioacetate) [(3R,4R,5S)-4-acetamido-3-(1,1-dimethylethyloxycarbonylamino)-5-(1-ethylpropoxy)-1-cyclohexene-1-phosphonate] (60)



Under an atmosphere of dry nitrogen, were added to the phosphinic acid **58**, tetrakistriphenylphosphine palladium (14 mg, 0.012 mmol) and vinyl iodide **32** (37 mg, 0.078 mmol). The mixture was dissolved in anhydrous toluene (3 mL), then triethylamine (8.8 µL, 0.063 mmol) was added. The mixture was heated to 80°C, stirred and maintained at this temperature for 3 hours. After cooling down at room temperature, the reaction was quenched by addition of saturated aqueous NH_4Cl (3 mL). DCM (15 mL) was added to the solution which was then extracted with NH_4Cl (3 mL), washed with brine (2x3 mL), the organic phase was dried over $MgSO_4$ and the solvent evaporated. Purification by flash chromatography (EA:MeOH ; 1:0 -> 5:1) gave the desired vinyl phosphonate **60** (17 mg, 0.0287 mmol, 36%). R_f = 0.38 (EA:MeOH ; 20:1).

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 6.58 (d, *J*= 21.8 Hz, 1H, H2), 5.76, 5.73 (2d, *J*= 10.0 Hz, 1H, NHAc), 5.12, 5.06 (2d, *J*= 8.9 Hz, 1H, NHBoc), 4.08-3.92 (m, 3H, H4, -O-CH_2-), 3.89 (s, 1H, H3), 3.81-3.72 (m, 1H, H5), 3.68, 3.67 (2dd, *J*= 11.0, 1.9 Hz, 3H, POMe), 3.31 (bs, 1H, H7), 2.84 (t, *J*= 7.3 Hz, 2H, -S-CH_2-), 2.62-2.54 (m, 1H, H6), 2.29 (s, 3H, thioacetate), 2.23-2.13 (m, 1H, H6'), 1.95 (s, 3H, acetamide), 1.63 (bs, 2H), 1.59-1.52 (m, 2H), 1.51-1.43 (m, 4H), 1.39 (s, 9H), 1.36 (bs, 4H), 0.89-0.81 (m, 6H).

^{13}C NMR (150.9 MHz, $CDCl_3$) δ_C 196.25, 171.01 (acetamide), 156.50 (carbamate), 142.29 (m, C2), 82.37 (C7), 79.92, 76.52 (m, C3), 66.19 (m, -OCH_2-), 54.73 (d, *J*= 11.8 Hz, C4), 52.69 (2d, *J*= 5.9 Hz, POMe), 49.42 (2d, *J*= 14.2 Hz, C5), 31.49 (m, C6), 30.84 ($SCOCH_3$), 30.48 (m), 29.59, 29.13 (d, *J*= 4.2 Hz), 28.55 (Boc), 28.43 (d, *J*= 4.2 Hz), 26.33, 25.79, 25.25, 23.57 ($NHCOCH_3$), 9.77, 9.33. C1 missing.

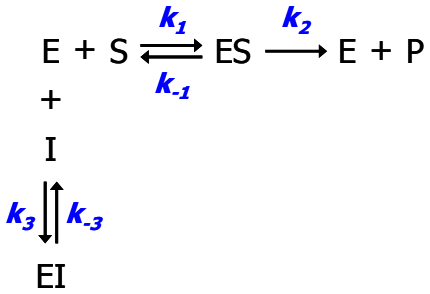
^{31}P NMR (161.9 MHz, $CDCl_3$) δ_P 18.29, 18.27.

HR-ESI-MS calculated for $C_{27}H_{49}N_2O_8PS$ $(M+Na)^+$ 615.2829770, found 615.2839449.

I.8.3. Inhibition of neuraminidase activity of influenza viruses from allantoic fluid from infected eggs

Neuraminidase (NA) enzymatic activity was studied using the fluorescent substrate 2'-4-methylumbelliferyl-α-D-*N*-acetylneuraminic acid (MUNANA). Measurements were made at 37°C in 32.5 mM MES (pH 6.5) + 4 mM $CaCl_2$ using a JASCO FP-6300 fluorimeter with excitation at 365 nm and emission at 450 nm. Michaelis-Menten constants for enzyme, $K_m = (k_2 + k_1)/k_1$, were determined using standard initial rate measurements with estimated neuraminidase concentrations in the range 0.1 to 0.5 nM and MUNANA concentrations in the range 2 to 200 µM.

Inhibition constants (K_i) were determined by measuring the extent to which different concentrations of inhibitor reduced the steady-state rate of MUNANA hydrolysis. The data were interpreted using the following simple model for competitive inhibition in which E, S, and I represent neuraminidase, MUNANA, and inhibitor, respectively:



The reduced rate of hydrolysis of MUNANA observed in the presence of inhibitor is predicted by the following equation (Rameix-Welti *et al.*)

$$V_I = \frac{V_0([S]+K_m)}{[S]+K_m\left(1+\frac{[I]}{K_I}\right)}$$

Where V_1 is the steady-state rate for MUNANA hydrolysis in the presence of inhibitor at concentration [I], V_0 is the steady-state rate for MUNANA hydrolysis in the absence of inhibitor, [S] is the MUNANA concentration, K_m is the Michaelis-Menten constant for hydrolysis of MUNANA and K_i is the dissociation constant for the enzyme-inhibitor complex. Because these inhibitors show a slow approach to the new-steady state rate (see Collins *et al.*) it is necessary to confirm that the new-steady state rate had been

reached; this was done by demonstrating that the first derivative of the fluorescence change (proportional to the NA activity) became constant at long times.

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List of publications and posters.

Publications:

- 'Efficient Synthesis of Highly Active Phospha-Isosteres of the Influenza Neuraminidase Inhibitor Oseltamivir'; *ChemMedChem*, **2009**, 4(3), 335-7.
- 'Galactose-Conjugates of the Oseltamivir Pharmacore – New Tools for the Characterization of Influenza Virus Neuraminidases'; B. Carbain, S. R. Martin, P. J. Collins, H. Streicher, *Org. Biomol. Chem*, **2009**, 7, 2570–2575.
- In preparation: 'New aspects of the Hunsdiecker-Barton decarboxylation – syntheses of phospha-shikimic acid and derivatives'.

Posters:

- **XXth International Symposium on Medicinal Chemistry, 2008, Vienna, Austria**: 'A convenient synthesis of bioactive cyclohexenephosphonates'.
- **Glycomics, 2008, London, UK**: 'Efficient Synthesis of Novel, Highly Active Phospha-Isosteres of Shikimic Acid and the Influenza Neuraminidase Inhibitor Oseltamivir'.

Appendix 1 : Crystal structures

ORTEP-generated structure of (3*R*,4*S*,5*R*)-tri-acetoxy-1 cyclohexene-1-phosphonate **9**

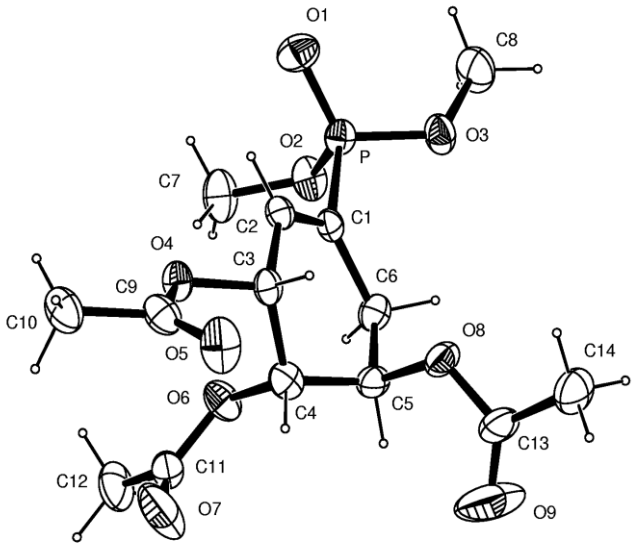


Table 1. Crystal data and structure refinement of compound 9.

Empirical formula	C14 H21 O9 P	
Formula weight	364.28	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	$P2_1$ (No.4)	
Unit cell dimensions	a = 7.6921(3) Å	α = 90°.
	b = 8.6347(2) Å	β = 91.420(1)°.
	c = 13.5463(4) Å	γ = 90°.
Volume	899.46(5) $Å^3$	
Z	2	
Density (calculated)	1.35 Mg/m^3	
Absorption coefficient	0.20 mm^{-1}	
F(000)	384	
Crystal size	0.15 x 0.15 x 0.05 mm^3	

Theta range for data collection	3.55 to 26.71°.
Index ranges	-9<=h<=9, -10<=k<=10, -16<=l<=16
Reflections collected	11218
Independent reflections	3572 [R(int) = 0.069]
Reflections with I>2sigma(I)	2817
Completeness to theta = 26.71°	95.7 %
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3572 / 1 / 222
Goodness-of-fit on F^2	1.013
Final R indices [I>2sigma(I)]	R1 = 0.051, wR2 = 0.098
R indices (all data)	R1 = 0.074, wR2 = 0.107
Absolute structure parameter	0.01(12)
Largest diff. peak and hole	0.26 and -0.28 e.Å^{-3}

Data collection KappaCCD, Program package WinGX, Abs correction not applied, Refinement using SHELXL-97, Drawing using ORTEP-3 for Windows.

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^{2}x 10^3).

U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
P	3928(1)	2471(1)	3821(1)	25(1)
O(1)	4327(3)	2441(3)	4883(2)	38(1)
O(2)	4198(3)	862(2)	3276(2)	34(1)
O(3)	1993(3)	2858(2)	3493(2)	33(1)
O(4)	9170(3)	5809(2)	3457(2)	26(1)
O(5)	9512(3)	8298(3)	3006(2)	44(1)
O(6)	8326(3)	4079(3)	1763(2)	33(1)
O(7)	10543(4)	5279(4)	1060(3)	71(1)
O(8)	4626(3)	6682(2)	1701(2)	32(1)
O(9)	4629(6)	7251(5)	110(2)	113(2)

C(1)	5114(4)	3844(3)	3126(2)	22(1)
C(2)	6368(4)	4674(3)	3583(2)	23(1)
C(3)	7419(4)	5839(4)	3036(2)	24(1)
C(4)	7445(4)	5536(4)	1942(2)	29(1)
C(5)	5614(4)	5281(4)	1525(2)	27(1)
C(6)	4718(4)	3942(4)	2031(2)	29(1)
C(7)	5893(5)	142(4)	3361(3)	45(1)
C(8)	573(5)	2027(5)	3918(3)	50(1)
C(9)	10095(4)	7149(4)	3375(3)	32(1)
C(10)	11915(4)	6947(5)	3783(3)	39(1)
C(11)	9809(5)	4099(5)	1270(3)	37(1)
C(12)	10417(5)	2504(5)	1051(3)	49(1)
C(13)	4153(5)	7539(5)	920(2)	41(1)
C(14)	2984(5)	8831(5)	1182(3)	45(1)

Table 3. Bond lengths [Å] and angles [°].

P-O(1)	1.463(2)
P-O(3)	1.578(2)
P-O(2)	1.590(2)
P-C(1)	1.780(3)
O(2)-C(7)	1.446(4)
O(3)-C(8)	1.439(4)
O(4)-C(9)	1.364(4)
O(4)-C(3)	1.450(4)
O(5)-C(9)	1.193(4)
O(6)-C(11)	1.337(4)
O(6)-C(4)	1.452(4)
O(7)-C(11)	1.203(5)
O(8)-C(13)	1.334(4)
O(8)-C(5)	1.452(4)
O(9)-C(13)	1.192(4)
C(1)-C(2)	1.341(4)
C(1)-C(6)	1.508(4)

C(2)-C(3)	1.498(4)
C(3)-C(4)	1.506(5)
C(4)-C(5)	1.520(5)
C(5)-C(6)	1.518(5)
C(9)-C(10)	1.503(5)
C(11)-C(12)	1.486(6)
C(13)-C(14)	1.481(5)

O(1)-P-O(3)	117.04(13)
O(1)-P-O(2)	114.47(15)
O(3)-P-O(2)	100.76(12)
O(1)-P-C(1)	115.75(14)
O(3)-P-C(1)	101.64(13)
O(2)-P-C(1)	105.17(14)
C(7)-O(2)-P	117.8(2)
C(8)-O(3)-P	120.1(2)
C(9)-O(4)-C(3)	115.7(2)
C(11)-O(6)-C(4)	118.7(3)
C(13)-O(8)-C(5)	117.8(3)
C(2)-C(1)-C(6)	123.3(3)
C(2)-C(1)-P	118.9(2)
C(6)-C(1)-P	117.6(2)
C(1)-C(2)-C(3)	121.4(3)
O(4)-C(3)-C(2)	107.5(2)
O(4)-C(3)-C(4)	110.4(2)
C(2)-C(3)-C(4)	112.9(3)
O(6)-C(4)-C(3)	109.4(3)
O(6)-C(4)-C(5)	104.2(2)
C(3)-C(4)-C(5)	110.8(3)
O(8)-C(5)-C(6)	108.4(3)
O(8)-C(5)-C(4)	107.6(2)
C(6)-C(5)-C(4)	111.7(3)
C(1)-C(6)-C(5)	113.8(3)
O(5)-C(9)-O(4)	123.2(3)

O(5)-C(9)-C(10)	126.0(3)
O(4)-C(9)-C(10)	110.8(3)
O(7)-C(11)-O(6)	122.7(3)
O(7)-C(11)-C(12)	125.8(3)
O(6)-C(11)-C(12)	111.4(3)
O(9)-C(13)-O(8)	122.0(4)
O(9)-C(13)-C(14)	125.5(3)
O(8)-C(13)-C(14)	112.5(3)

ORTEP-generated structure of (3*R*,4*S*,5*R*)-3-[(*tert*-butyldimethylsilyl)oxy]-4,5-(2,3-dimethoxy-butan-2,3-dioxy)-cyclohex-1-ene-1-carboxylic acid **13**

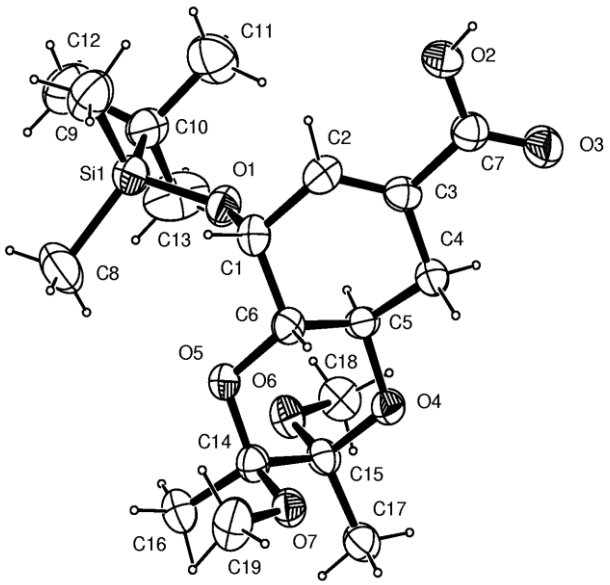


Table 1. Crystal data and structure refinement of compound 13.

Empirical formula	C19 H34 O7 Si
Formula weight	402.55
Temperature	173(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	P1 (No.1)

Unit cell dimensions	a = 6.9400(5) Å	α = 97.908(4)°.
	b = 11.6981(9) Å	β = 94.516(4)°.
	c = 14.0445(9) Å	γ = 90.602(3)°.
Volume	1125.59(14) $Å^3$	
Z	2	
Density (calculated)	1.19 Mg/m^3	
Absorption coefficient	0.14 mm^{-1}	
F(000)	436	
Crystal size	0.15 x 0.10 x 0.01 mm^3	
Theta range for data collection	3.40 to 22.95°	
Index ranges	-7<=h<=7, -12<=k<=12, -15<=l<=15	
Reflections collected	11214	
Independent reflections	5933 [R(int) = 0.058]	
Reflections with I>2sigma(I)	4705	
Completeness to theta = 22.95°	99.0 %	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	5933 / 3 / 513	
Goodness-of-fit on F^2	1.020	
Final R indices [I>2sigma(I)]	R1 = 0.053, wR2 = 0.111	
R indices (all data)	R1 = 0.079, wR2 = 0.125	
Absolute structure parameter	0.11(17)	
Largest diff. peak and hole	0.20 and -0.29 e.$Å^{-3}$	

The very thin crystal gave weak and limited diffraction.

Two essentially identical independent molecules labeled equivalently.

Data collection KappaCCD, Program package WinGX, Abs correction not applied, Refinement using SHELXL-97, Drawing using ORTEP-3 for Windows

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3).

U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
Si(1)	3340(2)	8794(1)	1870(1)	45(1)
O(1)	5074(5)	7982(3)	2251(2)	40(1)
O(2)	9602(6)	8596(3)	5115(3)	50(1)
O(3)	11642(5)	7252(3)	4546(2)	45(1)
O(4)	7685(4)	4815(3)	1751(2)	34(1)
O(5)	3902(4)	5640(3)	1921(2)	32(1)
O(6)	6039(4)	5291(3)	341(2)	38(1)
O(7)	4338(4)	3697(3)	2093(2)	35(1)
C(1)	5104(7)	7296(4)	3019(3)	35(1)
C(2)	6756(7)	7738(4)	3738(3)	36(1)
C(3)	8464(7)	7222(4)	3774(3)	33(1)
C(4)	8908(7)	6149(4)	3118(3)	35(1)
C(5)	7418(7)	5938(4)	2272(3)	32(1)
C(6)	5426(7)	6042(4)	2643(3)	31(1)
C(7)	10063(8)	7685(4)	4515(3)	37(1)
C(8)	1321(9)	7879(6)	1217(5)	77(2)
C(9)	2423(9)	9741(5)	2908(4)	63(2)
C(10)	4570(9)	9626(4)	1027(4)	49(1)
C(11)	6397(9)	10260(5)	1569(5)	68(2)
C(12)	3201(11)	10528(5)	655(5)	75(2)
C(13)	5214(13)	8785(6)	177(5)	82(2)
C(14)	4212(7)	4537(4)	1438(3)	33(1)
C(15)	6196(7)	4484(4)	1004(3)	33(1)
C(16)	2521(7)	4274(5)	680(4)	43(1)
C(17)	6696(7)	3282(4)	538(4)	41(1)
C(18)	7735(8)	5468(5)	-139(4)	47(1)
C(19)	2622(8)	3563(5)	2595(4)	53(2)
Si(1B)	3939(2)	4623(1)	6485(1)	39(1)
O(1B)	2164(4)	3659(3)	6143(2)	37(1)
O(2B)	-2382(6)	3278(3)	3312(3)	48(1)

O(3B)	-4449(5)	2090(3)	3875(2)	46(1)
O(4B)	-477(4)	661(3)	6695(2)	33(1)
O(5B)	3313(4)	1453(3)	6494(2)	34(1)
O(6B)	1233(5)	1606(3)	8094(2)	39(1)
O(7B)	2876(5)	-557(3)	6326(2)	37(1)
C(1B)	2108(7)	2721(4)	5389(3)	36(1)
C(2B)	437(7)	2902(4)	4675(3)	38(1)
C(3B)	-1273(7)	2361(4)	4648(3)	33(1)
C(4B)	-1719(7)	1519(4)	5320(3)	36(1)
C(5B)	-192(7)	1616(4)	6166(3)	31(1)
C(6B)	1768(7)	1591(4)	5784(3)	32(1)
C(7B)	-2857(8)	2543(4)	3918(3)	37(1)
C(8B)	5986(8)	3945(5)	7121(5)	64(2)
C(9B)	4810(8)	5197(5)	5418(4)	56(2)
C(10B)	2776(8)	5748(4)	7311(4)	47(1)
C(11B)	916(9)	6163(5)	6800(4)	60(2)
C(12B)	4144(10)	6805(5)	7615(5)	71(2)
C(13B)	2252(10)	5241(6)	8212(4)	68(2)
C(14B)	1033(7)	585(4)	7434(3)	35(1)
C(15B)	3004(7)	504(4)	6989(3)	33(1)
C(16B)	508(8)	-473(4)	7893(4)	43(1)
C(17B)	4690(7)	510(5)	7728(4)	42(1)
C(18B)	-419(8)	1943(5)	8598(4)	50(1)
C(19B)	4574(8)	-822(5)	5817(4)	54(2)

Table 3. Bond lengths [Å] and angles [°].

Si(1)-O(1)	1.643(3)
Si(1)-C(8)	1.857(6)
Si(1)-C(9)	1.864(6)
Si(1)-C(10)	1.880(5)
O(1)-C(1)	1.429(5)
O(2)-C(7)	1.323(6)
O(3)-C(7)	1.213(6)

O(4)-C(15)	1.424(5)
O(4)-C(5)	1.435(5)
O(5)-C(14)	1.400(6)
O(5)-C(6)	1.434(5)
O(6)-C(15)	1.414(6)
O(6)-C(18)	1.428(6)
O(7)-C(14)	1.435(6)
O(7)-C(19)	1.448(6)
C(1)-C(2)	1.504(7)
C(1)-C(6)	1.514(6)
C(2)-C(3)	1.336(7)
C(3)-C(4)	1.501(6)
C(3)-C(7)	1.503(7)
C(4)-C(5)	1.504(6)
C(5)-C(6)	1.516(7)
C(10)-C(13)	1.537(8)
C(10)-C(12)	1.544(8)
C(10)-C(11)	1.552(8)
C(14)-C(16)	1.520(7)
C(14)-C(15)	1.547(6)
C(15)-C(17)	1.522(7)
Si(1B)-O(1B)	1.658(3)
Si(1B)-C(8B)	1.861(6)
Si(1B)-C(9B)	1.863(5)
Si(1B)-C(10B)	1.866(5)
O(1B)-C(1B)	1.414(6)
O(2B)-C(7B)	1.346(6)
O(3B)-C(7B)	1.214(6)
O(4B)-C(14B)	1.429(5)
O(4B)-C(5B)	1.443(5)
O(5B)-C(15B)	1.411(5)
O(5B)-C(6B)	1.432(5)
O(6B)-C(14B)	1.406(6)
O(6B)-C(18B)	1.425(6)

O(7B)-C(19B)	1.440(6)
O(7B)-C(15B)	1.443(6)
C(1B)-C(2B)	1.509(7)
C(1B)-C(6B)	1.525(6)
C(2B)-C(3B)	1.335(7)
C(3B)-C(7B)	1.481(7)
C(3B)-C(4B)	1.501(6)
C(4B)-C(5B)	1.520(6)
C(5B)-C(6B)	1.500(6)
C(10B)-C(13B)	1.536(7)
C(10B)-C(12B)	1.542(8)
C(10B)-C(11B)	1.543(8)
C(14B)-C(16B)	1.525(7)
C(14B)-C(15B)	1.547(7)
C(15B)-C(17B)	1.501(7)

O(1)-Si(1)-C(8)	110.2(3)
O(1)-Si(1)-C(9)	110.2(2)
C(8)-Si(1)-C(9)	109.4(3)
O(1)-Si(1)-C(10)	102.8(2)
C(8)-Si(1)-C(10)	111.0(3)
C(9)-Si(1)-C(10)	113.0(3)
C(1)-O(1)-Si(1)	128.9(3)
C(15)-O(4)-C(5)	113.0(3)
C(14)-O(5)-C(6)	113.2(3)
C(15)-O(6)-C(18)	115.8(4)
C(14)-O(7)-C(19)	115.1(4)
O(1)-C(1)-C(2)	107.4(4)
O(1)-C(1)-C(6)	110.4(4)
C(2)-C(1)-C(6)	109.2(4)
C(3)-C(2)-C(1)	122.5(4)
C(2)-C(3)-C(4)	123.6(4)
C(2)-C(3)-C(7)	120.6(4)
C(4)-C(3)-C(7)	115.8(4)

C(3)-C(4)-C(5)	110.5(4)
O(4)-C(5)-C(4)	109.1(4)
O(4)-C(5)-C(6)	111.5(4)
C(4)-C(5)-C(6)	108.6(4)
O(5)-C(6)-C(1)	109.1(4)
O(5)-C(6)-C(5)	113.2(3)
C(1)-C(6)-C(5)	108.8(4)
O(3)-C(7)-O(2)	123.6(5)
O(3)-C(7)-C(3)	121.9(5)
O(2)-C(7)-C(3)	114.5(5)
C(13)-C(10)-C(12)	110.2(5)
C(13)-C(10)-C(11)	108.2(5)
C(12)-C(10)-C(11)	108.9(5)
C(13)-C(10)-Si(1)	109.5(4)
C(12)-C(10)-Si(1)	110.6(4)
C(11)-C(10)-Si(1)	109.4(4)
O(5)-C(14)-O(7)	111.2(4)
O(5)-C(14)-C(16)	106.1(4)
O(7)-C(14)-C(16)	111.6(4)
O(5)-C(14)-C(15)	111.1(4)
O(7)-C(14)-C(15)	103.9(4)
C(16)-C(14)-C(15)	113.0(4)
O(6)-C(15)-O(4)	111.5(4)
O(6)-C(15)-C(17)	112.9(4)
O(4)-C(15)-C(17)	105.9(4)
O(6)-C(15)-C(14)	103.5(4)
O(4)-C(15)-C(14)	109.4(4)
C(17)-C(15)-C(14)	113.8(4)
O(1B)-Si(1B)-C(8B)	109.9(2)
O(1B)-Si(1B)-C(9B)	110.4(2)
C(8B)-Si(1B)-C(9B)	109.2(3)
O(1B)-Si(1B)-C(10B)	102.9(2)
C(8B)-Si(1B)-C(10B)	111.8(3)
C(9B)-Si(1B)-C(10B)	112.5(2)

C(1B)-O(1B)-Si(1B)	127.9(3)
C(14B)-O(4B)-C(5B)	112.8(3)
C(15B)-O(5B)-C(6B)	112.7(3)
C(14B)-O(6B)-C(18B)	116.4(4)
C(19B)-O(7B)-C(15B)	115.0(4)
O(1B)-C(1B)-C(2B)	107.5(4)
O(1B)-C(1B)-C(6B)	110.1(4)
C(2B)-C(1B)-C(6B)	108.9(4)
C(3B)-C(2B)-C(1B)	122.8(4)
C(2B)-C(3B)-C(7B)	121.0(4)
C(2B)-C(3B)-C(4B)	123.5(4)
C(7B)-C(3B)-C(4B)	115.5(4)
C(3B)-C(4B)-C(5B)	110.3(4)
O(4B)-C(5B)-C(6B)	111.3(4)
O(4B)-C(5B)-C(4B)	108.2(3)
C(6B)-C(5B)-C(4B)	108.6(4)
O(5B)-C(6B)-C(5B)	113.6(4)
O(5B)-C(6B)-C(1B)	108.5(4)
C(5B)-C(6B)-C(1B)	108.5(4)
O(3B)-C(7B)-O(2B)	122.8(5)
O(3B)-C(7B)-C(3B)	123.5(4)
O(2B)-C(7B)-C(3B)	113.7(5)
C(13B)-C(10B)-C(12B)	109.5(5)
C(13B)-C(10B)-C(11B)	108.9(5)
C(12B)-C(10B)-C(11B)	107.9(5)
C(13B)-C(10B)-Si(1B)	109.5(4)
C(12B)-C(10B)-Si(1B)	110.7(4)
C(11B)-C(10B)-Si(1B)	110.2(4)
O(6B)-C(14B)-O(4B)	111.6(4)
O(6B)-C(14B)-C(16B)	113.5(4)
O(4B)-C(14B)-C(16B)	105.3(4)
O(6B)-C(14B)-C(15B)	103.2(3)
O(4B)-C(14B)-C(15B)	109.5(3)
C(16B)-C(14B)-C(15B)	113.8(4)

O(5B)-C(15B)-O(7B)	110.5(3)
O(5B)-C(15B)-C(17B)	106.1(4)
O(7B)-C(15B)-C(17B)	111.5(4)
O(5B)-C(15B)-C(14B)	110.9(4)
O(7B)-C(15B)-C(14B)	104.9(3)
C(17B)-C(15B)-C(14B)	113.1(4)

Hydrogen bonds with H..A < r(A) + 2.000 Angstroms and <DHA > 110 deg.

D-H	d(D-H)	d(H..A)	<DHA	d(D..A)	A
O2-H2X	0.78	2.09	153	2.814	O7B_a [x+1, y+1, z]
O2B-H2Y_a	0.73	2.11	171	2.827	O7 [x-1, y, z]

ORTEP-generated structure of Dimethyl (3*R*,4*S*,5*R*)- 3-hydroxy-4,5-(2,3-dimethoxy-butan-2,3-dioxy)-cyclohex-1-ene-1-phosphonate **16**

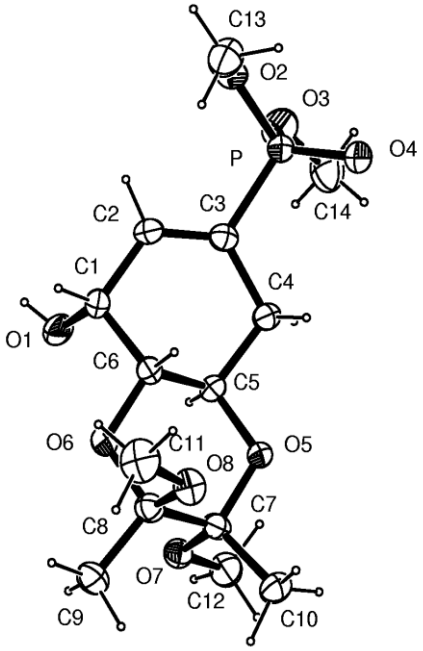


Table 1. Crystal data and structure refinement of compound 16.

Empirical formula	C14 H25 O8 P	
Formula weight	352.31	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	$P2_1$ (No.4)	
Unit cell dimensions	a = 7.5515(2) Å	α = 90°.
	b = 9.4704(4) Å	β = 100.943(2)°.
	c = 11.8545(4) Å	γ = 90°.
Volume	832.37(5) $Å^3$	
Z	2	
Density (calculated)	1.41 Mg/m^3	
Absorption coefficient	0.20 mm^{-1}	
F(000)	376	
Crystal size	0.20 x 0.15 x 0.10 mm^3	
Theta range for data collection	3.50 to 26.04°	
Index ranges	-8<=h<=9, -11<=k<=11, -14<=l<=14	
Reflections collected	12562	
Independent reflections	3257 [R(int) = 0.046]	
Reflections with I>2sigma(I)	2997	
Completeness to theta = 26.04°	99.2 %	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3257 / 1 / 218	
Goodness-of-fit on F^2	1.054	
Final R indices [I>2sigma(I)]	R1 = 0.034, wR2 = 0.080	
R indices (all data)	R1 = 0.040, wR2 = 0.083	
Absolute structure parameter	0.04(9)	
Largest diff. peak and hole	0.26 and -0.29 e.$Å^{-3}$	

The hydroxyl H atom was refined.

Data collection KappaCCD, Program package WinGX, Abs correction not applied, Refinement using SHELXL-97, Drawing using ORTEP-3 for Windows

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3).

U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
P	3514(1)	7841(1)	3229(1)	25(1)
O(1)	-1550(2)	7421(2)	5265(1)	37(1)
O(2)	2500(2)	8886(2)	2303(1)	29(1)
O(3)	3055(2)	6441(2)	2519(1)	40(1)
O(4)	5445(2)	8120(2)	3628(1)	37(1)
O(5)	3463(2)	7222(2)	7552(1)	22(1)
O(6)	167(2)	8639(2)	7339(1)	24(1)
O(7)	1425(2)	6178(2)	8555(1)	26(1)
O(8)	2607(2)	9793(2)	8478(1)	30(1)
C(1)	-316(3)	8554(3)	5273(2)	26(1)
C(2)	707(3)	8412(3)	4292(2)	27(1)
C(3)	2333(3)	7828(3)	4408(2)	23(1)
C(4)	3353(3)	7241(2)	5533(2)	25(1)
C(5)	2219(3)	7244(2)	6465(2)	22(1)
C(6)	1067(3)	8559(2)	6384(2)	23(1)
C(7)	2612(3)	7326(2)	8521(2)	23(1)
C(8)	1385(3)	8649(3)	8413(2)	25(1)
C(9)	216(3)	8733(3)	9320(2)	32(1)
C(10)	4140(3)	7367(3)	9562(2)	32(1)
C(11)	1818(4)	11164(3)	8329(2)	41(1)
C(12)	2210(3)	4812(2)	8587(2)	33(1)
C(13)	2760(4)	10404(3)	2472(2)	40(1)
C(14)	3924(4)	5138(3)	2916(3)	47(1)

Table 3. Bond lengths [Å] and angles [°].

P-O(4)	1.4687(16)
P-O(2)	1.5655(16)
P-O(3)	1.5732(18)
P-C(3)	1.7952(19)
O(1)-C(1)	1.420(3)
O(2)-C(13)	1.459(3)
O(3)-C(14)	1.434(3)
O(5)-C(7)	1.423(2)
O(5)-C(5)	1.443(2)
O(6)-C(8)	1.422(2)
O(6)-C(6)	1.429(2)
O(7)-C(7)	1.414(3)
O(7)-C(12)	1.420(3)
O(8)-C(8)	1.416(3)
O(8)-C(11)	1.425(3)
C(1)-C(6)	1.518(3)
C(1)-C(2)	1.519(3)
C(2)-C(3)	1.330(3)
C(3)-C(4)	1.513(3)
C(4)-C(5)	1.522(3)
C(5)-C(6)	1.511(3)
C(7)-C(10)	1.522(3)
C(7)-C(8)	1.549(3)
C(8)-C(9)	1.518(3)

O(4)-P-O(2)	115.83(9)
O(4)-P-O(3)	115.32(10)
O(2)-P-O(3)	97.44(9)
O(4)-P-C(3)	111.09(9)
O(2)-P-C(3)	107.55(9)
O(3)-P-C(3)	108.58(11)
C(13)-O(2)-P	119.42(15)

C(14)-O(3)-P	120.65(16)
C(7)-O(5)-C(5)	113.74(14)
C(8)-O(6)-C(6)	112.66(15)
C(7)-O(7)-C(12)	115.92(17)
C(8)-O(8)-C(11)	115.93(19)
O(1)-C(1)-C(6)	110.52(18)
O(1)-C(1)-C(2)	110.89(19)
C(6)-C(1)-C(2)	107.34(16)
C(3)-C(2)-C(1)	123.50(18)
C(2)-C(3)-C(4)	123.30(17)
C(2)-C(3)-P	120.14(15)
C(4)-C(3)-P	116.36(14)
C(3)-C(4)-C(5)	112.15(16)
O(5)-C(5)-C(6)	109.77(17)
O(5)-C(5)-C(4)	106.72(16)
C(6)-C(5)-C(4)	110.60(17)
O(6)-C(6)-C(5)	110.33(17)
O(6)-C(6)-C(1)	109.60(16)
C(5)-C(6)-C(1)	110.22(18)
O(7)-C(7)-O(5)	110.85(16)
O(7)-C(7)-C(10)	112.59(18)
O(5)-C(7)-C(10)	105.50(16)
O(7)-C(7)-C(8)	104.59(16)
O(5)-C(7)-C(8)	110.08(16)
C(10)-C(7)-C(8)	113.33(18)
O(8)-C(8)-O(6)	111.03(18)
O(8)-C(8)-C(9)	112.43(19)
O(6)-C(8)-C(9)	105.66(16)
O(8)-C(8)-C(7)	103.97(16)
O(6)-C(8)-C(7)	110.12(18)
C(9)-C(8)-C(7)	113.74(18)

Hydrogen bonds with H..A < r(A) + 2.000 Angstroms and <DHA > 110 deg.

D-H	d(D-H)	d(H..A)	<DHA	d(D..A)	A
O1-H1X	0.76	2.02	175	2.772	O4 [x-1, y, z]

ORTEP-generated structure of (3*R*,4*R*,5*S*)-4-acetylamino-5-azido-3-(1-ethylpropoxy)-1-iodocyclohexene **25**

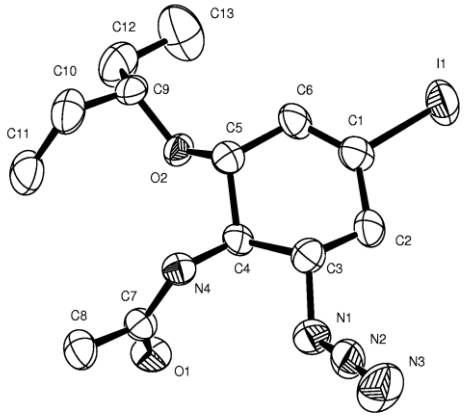


Table 1. Crystal data and structure refinement of compound 25.

Empirical formula	C13 H20 I N4 O2	
Formula weight	391.23	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	$P2_12_12_1$ (No.19)	
Unit cell dimensions	a = 4.8452(1) Å	α = 90°.
	b = 19.0439(5) Å	β = 90°.
	c = 36.6182(9) Å	γ = 90°.
Volume	3378.82(14) $Å^3$	
Z	8	
Density (calculated)	1.54 Mg/m^3	
Absorption coefficient	1.90 mm^{-1}	
F(000)	1560	
Crystal size	0.50 x 0.25 x 0.02 mm^3	
Theta range for data collection	3.51 to 26.00°	

Index ranges	-3<=h<=5, -23<=k<=23, -45<=l<=45
Reflections collected	25623
Independent reflections	6531 [R(int) = 0.069]
Reflections with I>2sigma(I)	4539
Completeness to theta = 26.00°	99.2 %
Tmax. and Tmin.	0.9634 and 0.8497
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	6531 / 0 / 360
Goodness-of-fit on F^2	1.075
Final R indices [I>2sigma(I)]	R1 = 0.050, wR2 = 0.122
R indices (all data)	R1 = 0.088, wR2 = 0.144
Absolute structure parameter	0.03(3)
Largest diff. peak and hole	1.17 and -0.85 e.$Å^{-3}$

There are two independent molecules, one of which has disorder in the ethyl groups.. The disordered C atoms in the ethyl groups of molecule B were left isotropic.

Data collection KappaCCD, Program package WinGX, Abs correction MULTISCAN
Refinement using SHELXL-97, Drawing using ORTEP-3 for Windows

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3).

βU(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
I(1)	8324(1)	7250(1)	4302(1)	71(1)
O(1)	12634(8)	4203(3)	5393(1)	46(1)
O(2)	8652(9)	4551(2)	4503(1)	39(1)
N(1)	9812(15)	5848(3)	5606(2)	59(2)
N(2)	8786(16)	6200(3)	5851(2)	57(2)
N(3)	8100(19)	6509(4)	6102(2)	83(2)
N(4)	8193(10)	4549(3)	5311(1)	37(1)
C(1)	8086(14)	6356(4)	4637(2)	46(2)

C(2)	9013(16)	6460(3)	5020(2)	53(2)
C(3)	8209(16)	5841(3)	5260(2)	44(2)
C(4)	8854(12)	5139(3)	5074(2)	35(1)
C(5)	7269(12)	5065(3)	4713(2)	37(1)
C(6)	7228(14)	5750(3)	4501(2)	47(2)
C(7)	10165(14)	4134(3)	5449(2)	39(2)
C(8)	9123(13)	3550(3)	5695(2)	49(2)
C(9)	6898(14)	4099(3)	4288(2)	47(2)
C(10)	6025(15)	3474(4)	4506(2)	60(2)
C(11)	8452(17)	3010(4)	4631(2)	64(2)
C(12)	8520(20)	3941(4)	3938(2)	71(2)
C(13)	8900(30)	4586(5)	3702(2)	94(3)
I(1B)	3850(2)	6438(1)	3456(1)	97(1)
O(1B)	-2344(9)	5300(4)	1677(2)	78(2)
O(2B)	2419(8)	4429(2)	2470(1)	46(1)
N(1B)	253(15)	6791(4)	2065(2)	71(2)
N(2B)	905(17)	6887(3)	1743(2)	74(2)
N(3B)	1280(20)	7036(4)	1447(2)	97(3)
N(4B)	2150(9)	5389(3)	1834(1)	39(1)
C(1B)	3134(18)	6046(4)	2928(2)	57(2)
C(2B)	1603(17)	6541(4)	2682(2)	54(2)
C(3B)	2201(14)	6346(3)	2281(2)	46(2)
C(4B)	1712(13)	5565(3)	2215(2)	39(1)
C(5B)	3558(14)	5113(3)	2454(2)	40(1)
C(6B)	3947(16)	5421(4)	2828(2)	59(2)
C(7B)	109(15)	5256(4)	1600(2)	50(2)
C(8B)	1003(16)	5055(5)	1223(2)	62(2)
C(9B)	4350(15)	3851(4)	2405(2)	56(2)
C(10B)	4490(30)	3647(9)	2012(4)	63(5)
C(11B)	1840(20)	3413(5)	1851(3)	87(3)
C(12B)	3640(40)	3224(9)	2671(4)	72(5)
C(13B)	4000(30)	3374(5)	3044(3)	102(4)
C(9C)	4350(15)	3851(4)	2405(2)	56(2)
C(10C)	2630(30)	3268(8)	2231(4)	52(4)

C(11C)	1840(20)	3413(5)	1851(3)	87(3)
C(12C)	5840(40)	3661(11)	2721(5)	87(6)
C(13C)	4000(30)	3374(5)	3044(3)	102(4)

Table 3. Bond lengths [Å] and angles [°].

I(1)-C(1)	2.101(7)
O(1)-C(7)	1.221(7)
O(2)-C(5)	1.414(7)
O(2)-C(9)	1.443(7)
N(1)-N(2)	1.228(8)
N(1)-C(3)	1.485(9)
N(2)-N(3)	1.139(9)
N(4)-C(7)	1.338(8)
N(4)-C(4)	1.456(8)
C(1)-C(6)	1.324(9)
C(1)-C(2)	1.486(9)
C(2)-C(3)	1.521(9)
C(3)-C(4)	1.531(8)
C(4)-C(5)	1.536(8)
C(5)-C(6)	1.518(9)
C(7)-C(8)	1.518(9)
C(9)-C(10)	1.496(10)
C(9)-C(12)	1.534(11)
C(10)-C(11)	1.539(11)
C(12)-C(13)	1.513(12)
I(1B)-C(1B)	2.104(7)
O(1B)-C(7B)	1.224(9)
O(2B)-C(5B)	1.418(7)
O(2B)-C(9B)	1.463(9)
N(1B)-N(2B)	1.232(9)
N(1B)-C(3B)	1.495(9)
N(2B)-N(3B)	1.134(9)
N(4B)-C(7B)	1.333(8)

N(4B)-C(4B)	1.450(7)
C(1B)-C(6B)	1.306(10)
C(1B)-C(2B)	1.499(10)
C(2B)-C(3B)	1.544(9)
C(3B)-C(4B)	1.527(9)
C(4B)-C(5B)	1.517(9)
C(5B)-C(6B)	1.504(9)
C(7B)-C(8B)	1.497(10)
C(9B)-C(10B)	1.492(17)
C(9B)-C(12B)	1.577(18)
C(10B)-C(11B)	1.481(18)
C(12B)-C(13B)	1.407(18)

C(5)-O(2)-C(9)	115.5(5)
N(2)-N(1)-C(3)	114.7(6)
N(3)-N(2)-N(1)	172.1(9)
C(7)-N(4)-C(4)	121.5(5)
C(6)-C(1)-C(2)	124.5(6)
C(6)-C(1)-I(1)	120.2(5)
C(2)-C(1)-I(1)	115.3(5)
C(1)-C(2)-C(3)	111.4(6)
N(1)-C(3)-C(2)	110.6(6)
N(1)-C(3)-C(4)	106.2(5)
C(2)-C(3)-C(4)	111.6(5)
N(4)-C(4)-C(3)	111.3(5)
N(4)-C(4)-C(5)	109.5(5)
C(3)-C(4)-C(5)	111.1(5)
O(2)-C(5)-C(6)	108.8(5)
O(2)-C(5)-C(4)	107.2(5)
C(6)-C(5)-C(4)	111.6(5)
C(1)-C(6)-C(5)	123.4(6)
O(1)-C(7)-N(4)	124.9(6)
O(1)-C(7)-C(8)	120.3(6)
N(4)-C(7)-C(8)	114.7(6)

O(2)-C(9)-C(10)	110.4(6)
O(2)-C(9)-C(12)	105.7(6)
C(10)-C(9)-C(12)	115.8(6)
C(9)-C(10)-C(11)	113.5(6)
C(13)-C(12)-C(9)	112.3(7)
C(5B)-O(2B)-C(9B)	115.8(5)
N(2B)-N(1B)-C(3B)	115.4(7)
N(3B)-N(2B)-N(1B)	172.0(9)
C(7B)-N(4B)-C(4B)	123.6(5)
C(6B)-C(1B)-C(2B)	123.8(7)
C(6B)-C(1B)-I(1B)	122.0(6)
C(2B)-C(1B)-I(1B)	114.2(5)
C(1B)-C(2B)-C(3B)	109.0(6)
N(1B)-C(3B)-C(4B)	111.8(6)
N(1B)-C(3B)-C(2B)	104.5(6)
C(4B)-C(3B)-C(2B)	110.8(5)
N(4B)-C(4B)-C(5B)	109.7(5)
N(4B)-C(4B)-C(3B)	110.8(5)
C(5B)-C(4B)-C(3B)	111.7(5)
O(2B)-C(5B)-C(6B)	111.5(5)
O(2B)-C(5B)-C(4B)	108.5(5)
C(6B)-C(5B)-C(4B)	112.3(6)
C(1B)-C(6B)-C(5B)	124.8(7)
O(1B)-C(7B)-N(4B)	124.1(7)
O(1B)-C(7B)-C(8B)	120.6(7)
N(4B)-C(7B)-C(8B)	115.3(6)
O(2B)-C(9B)-C(10B)	112.4(8)
O(2B)-C(9B)-C(12B)	109.3(8)
C(10B)-C(9B)-C(12B)	114.0(10)
C(11B)-C(10B)-C(9B)	115.0(11)
C(13B)-C(12B)-C(9B)	114.7(12)

ORTEP-generated structure of (3*R*,4*R*,5*S*)-4-acetylamino-5-*N*-*tert*-Butoxycarbonyl-amino-3-(1-ethylpropoxy)-1-iodocyclohexene **32**

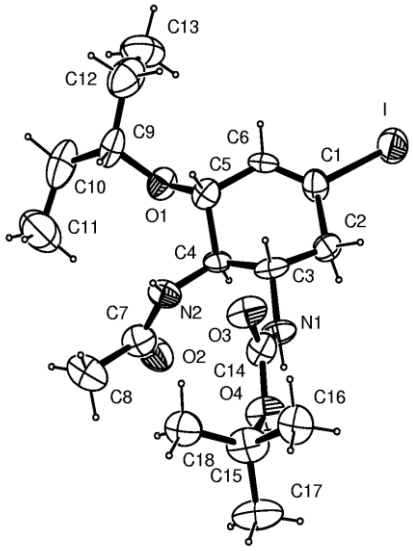


Table 1. Crystal data and structure refinement of compound 32.

Empirical formula	C18 H31 I N2 O4	
Formula weight	466.35	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	$P2_1$ (No.4)	
Unit cell dimensions	a = 12.2334(15) Å	α = 90°.
	b = 4.9607(6) Å	β = 105.910(7)°.
	c = 18.6244(19) Å	γ = 90°.
Volume	1086.9(2) $Å^3$	
Z	2	
Density (calculated)	1.43 Mg/m^3	
Absorption coefficient	1.50 mm^{-1}	
F(000)	476	
Crystal size	0.30 x 0.02 x 0.01 mm^3	
Theta range for data collection	3.41 to 25.95°	

Index ranges	-14<=h<=15, -6<=k<=5, -22<=l<=22
Reflections collected	7090
Independent reflections	3663 [R(int) = 0.092]
Reflections with I>2sigma(I)	2313
Completeness to theta = 25.95°	95.8 %
Tmax. and Tmin.	0.9809 and 0.7795
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3663 / 1 / 238
Goodness-of-fit on F^2	1.044
Final R indices [I>2sigma(I)]	R1 = 0.074, wR2 = 0.148
R indices (all data)	R1 = 0.133, wR2 = 0.177
Absolute structure parameter	-0.01(6)
Largest diff. peak and hole	1.28 and -0.76 e.$Å^{-3}$ (near I)

The H atoms on N were refined.

Data collection KappaCCD, Program package WinGX, Abs correction MULTISCAN
Refinement using SHELXL-97, Drawing using ORTEP-3 for Windows

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3).

U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
I	2724(1)	5924(3)	4507(1)	75(1)
O(1)	2482(7)	3805(15)	1689(4)	46(2)
O(2)	5185(11)	6756(17)	1530(6)	59(3)
O(3)	6576(8)	-1655(16)	3359(6)	49(3)
O(4)	8009(7)	1432(15)	3443(4)	51(2)
N(1)	6280(10)	2800(20)	3424(7)	44(3)
N(2)	4896(9)	2590(20)	1934(5)	43(2)
C(1)	3354(10)	4330(20)	3659(6)	42(3)
C(2)	4672(9)	4130(20)	3877(6)	37(3)

C(3)	5051(9)	2540(20)	3301(6)	40(3)
C(4)	4401(9)	3530(20)	2512(6)	36(3)
C(5)	3183(10)	2700(20)	2369(6)	44(3)
C(6)	2731(9)	3640(20)	3011(6)	40(3)
C(7)	5312(13)	4280(30)	1504(8)	49(4)
C(8)	5876(13)	2980(20)	972(8)	63(4)
C(9)	1873(12)	1830(20)	1161(6)	56(4)
C(10)	1591(15)	3260(30)	392(7)	82(5)
C(11)	2634(15)	3940(40)	118(10)	105(6)
C(12)	829(11)	700(40)	1374(7)	75(4)
C(13)	-52(12)	2830(40)	1380(9)	82(5)
C(14)	6926(8)	610(30)	3399(5)	42(3)
C(15)	8895(11)	-630(20)	3408(8)	57(4)
C(16)	9101(11)	-2310(20)	4126(7)	54(3)
C(17)	9921(10)	1150(30)	3454(8)	70(4)
C(18)	8508(12)	-2220(30)	2705(7)	58(3)

Table 3. Bond lengths [Å] and angles [°].

I-C(1)	2.093(11)
O(1)-C(5)	1.430(12)
O(1)-C(9)	1.441(13)
O(2)-C(7)	1.242(14)
O(3)-C(14)	1.196(16)
O(4)-C(14)	1.368(14)
O(4)-C(15)	1.504(14)
N(1)-C(14)	1.355(16)
N(1)-C(3)	1.464(15)
N(2)-C(7)	1.351(17)
N(2)-C(4)	1.449(14)
C(1)-C(6)	1.284(14)
C(1)-C(2)	1.553(15)
C(2)-C(3)	1.504(15)
C(3)-C(4)	1.546(15)

C(4)-C(5)	1.499(15)
C(5)-C(6)	1.522(15)
C(7)-C(8)	1.498(19)
C(9)-C(12)	1.543(19)
C(9)-C(10)	1.548(17)
C(10)-C(11)	1.54(2)
C(12)-C(13)	1.51(2)
C(15)-C(18)	1.489(18)
C(15)-C(17)	1.517(19)
C(15)-C(16)	1.539(18)

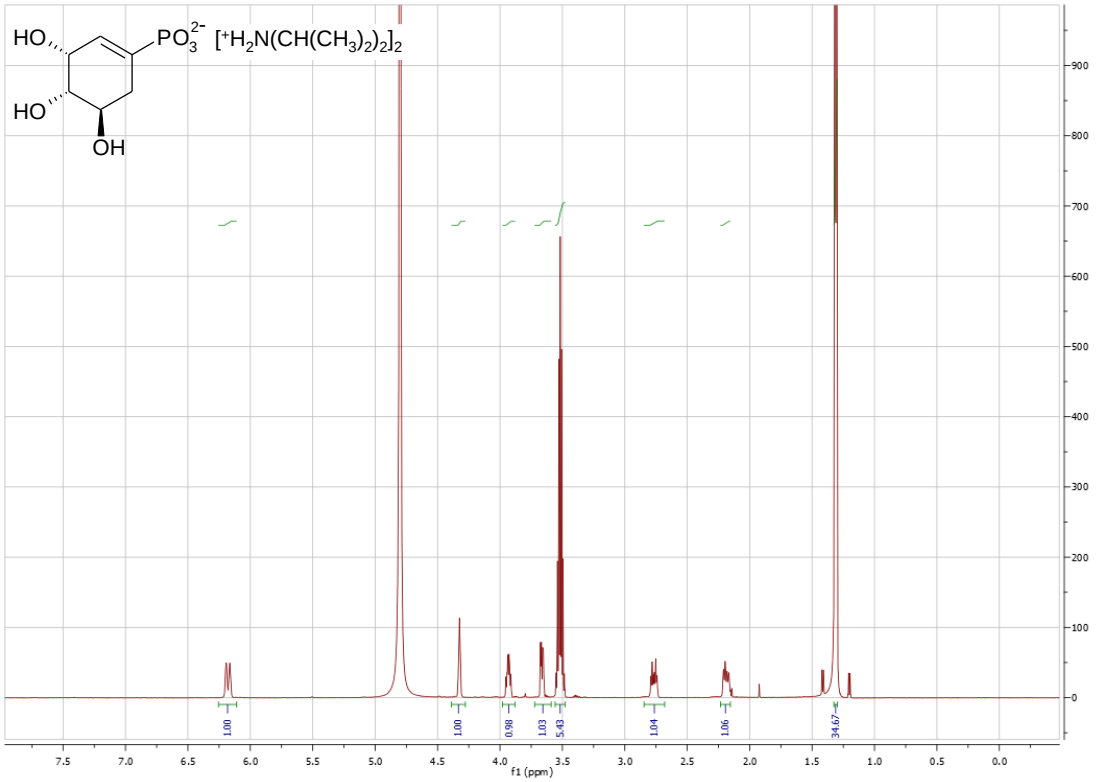
C(5)-O(1)-C(9)	114.6(8)
C(14)-O(4)-C(15)	119.5(10)
C(14)-N(1)-C(3)	120.4(10)
C(7)-N(2)-C(4)	122.9(10)
C(6)-C(1)-C(2)	122.4(10)
C(6)-C(1)-I	124.2(9)
C(2)-C(1)-I	113.4(7)
C(3)-C(2)-C(1)	110.4(8)
N(1)-C(3)-C(2)	110.2(9)
N(1)-C(3)-C(4)	111.0(9)
C(2)-C(3)-C(4)	109.4(9)
N(2)-C(4)-C(5)	112.2(9)
N(2)-C(4)-C(3)	113.2(9)
C(5)-C(4)-C(3)	108.0(8)
O(1)-C(5)-C(4)	112.1(9)
O(1)-C(5)-C(6)	108.6(9)
C(4)-C(5)-C(6)	109.5(8)
C(1)-C(6)-C(5)	124.7(10)
O(2)-C(7)-N(2)	121.3(15)
O(2)-C(7)-C(8)	122.4(16)
N(2)-C(7)-C(8)	116.2(11)
O(1)-C(9)-C(12)	112.3(11)
O(1)-C(9)-C(10)	105.4(10)

C(12)-C(9)-C(10)	114.4(11)
C(11)-C(10)-C(9)	114.4(13)
C(13)-C(12)-C(9)	113.0(14)
O(3)-C(14)-N(1)	123.8(11)
O(3)-C(14)-O(4)	127.5(11)
N(1)-C(14)-O(4)	108.7(12)
C(18)-C(15)-O(4)	109.8(10)
C(18)-C(15)-C(17)	114.6(12)
O(4)-C(15)-C(17)	101.5(10)
C(18)-C(15)-C(16)	114.4(11)
O(4)-C(15)-C(16)	106.2(10)
C(17)-C(15)-C(16)	109.3(11)

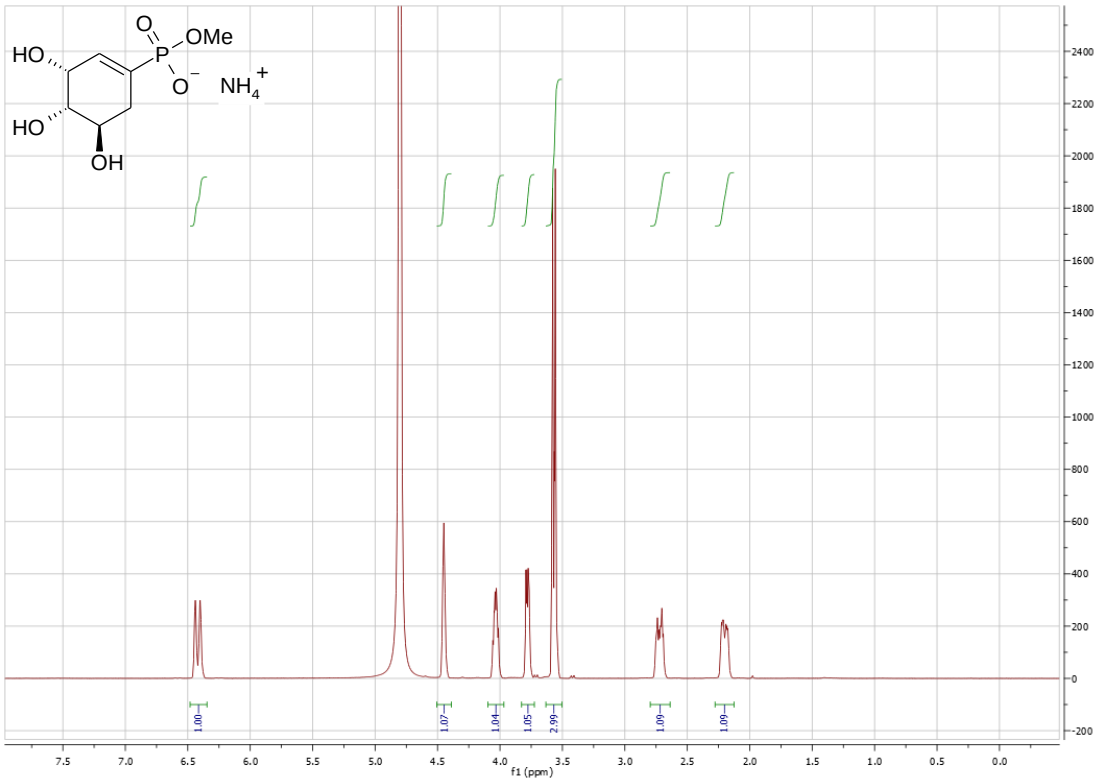
Hydrogen bonds with H..A < r(A) + 2.000 Angstroms and <DHA > 110 deg.

D-H	d(D-H)	d(H..A)	<DHA	d(D..A)	A
N1-H1	0.91	1.99	144	2.779	O3 [x, y+1, z]
N2-H2	0.96	2.12	159	3.035	O2 [x, y-1, z]

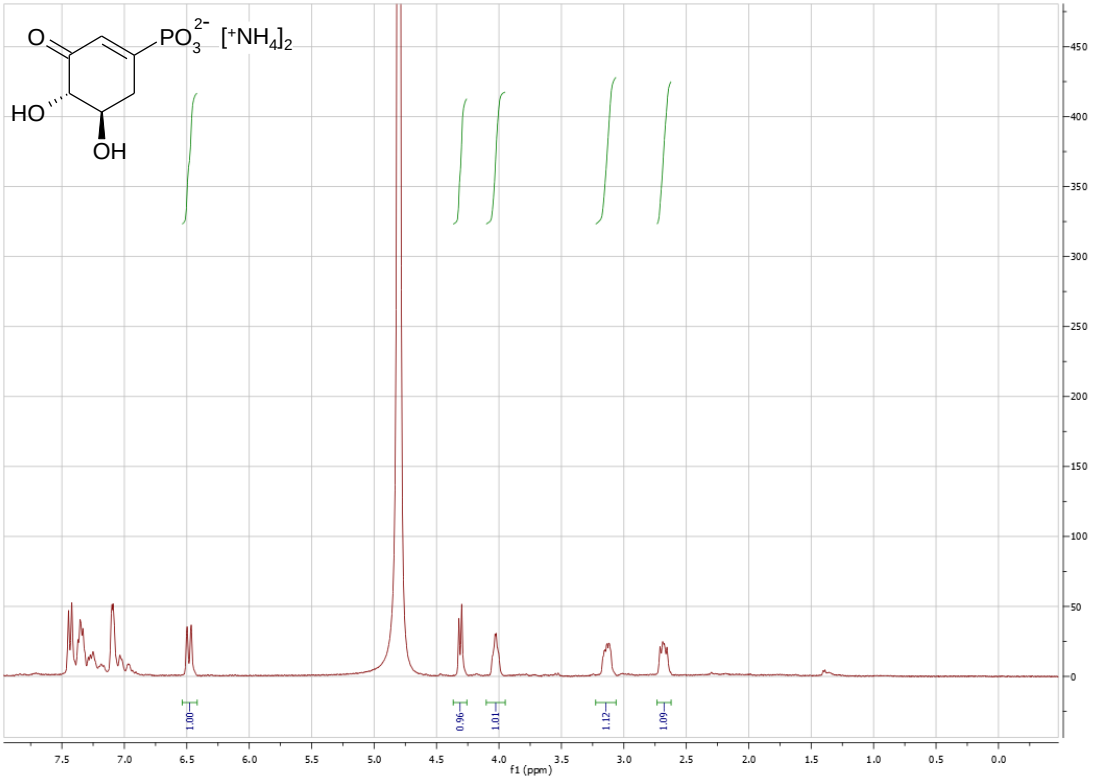
Appendix 2 : ^{1}H NMR Spectra



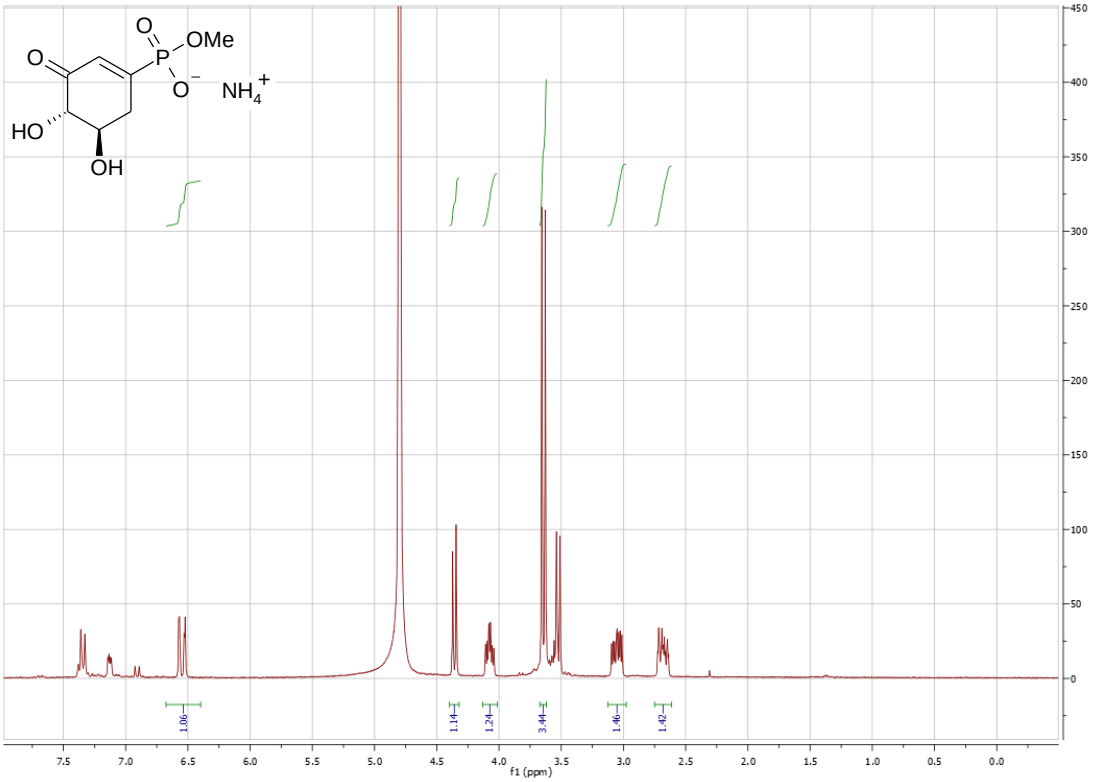
^{1}H-NMR (600 MHz, D_2O) of compound **1**



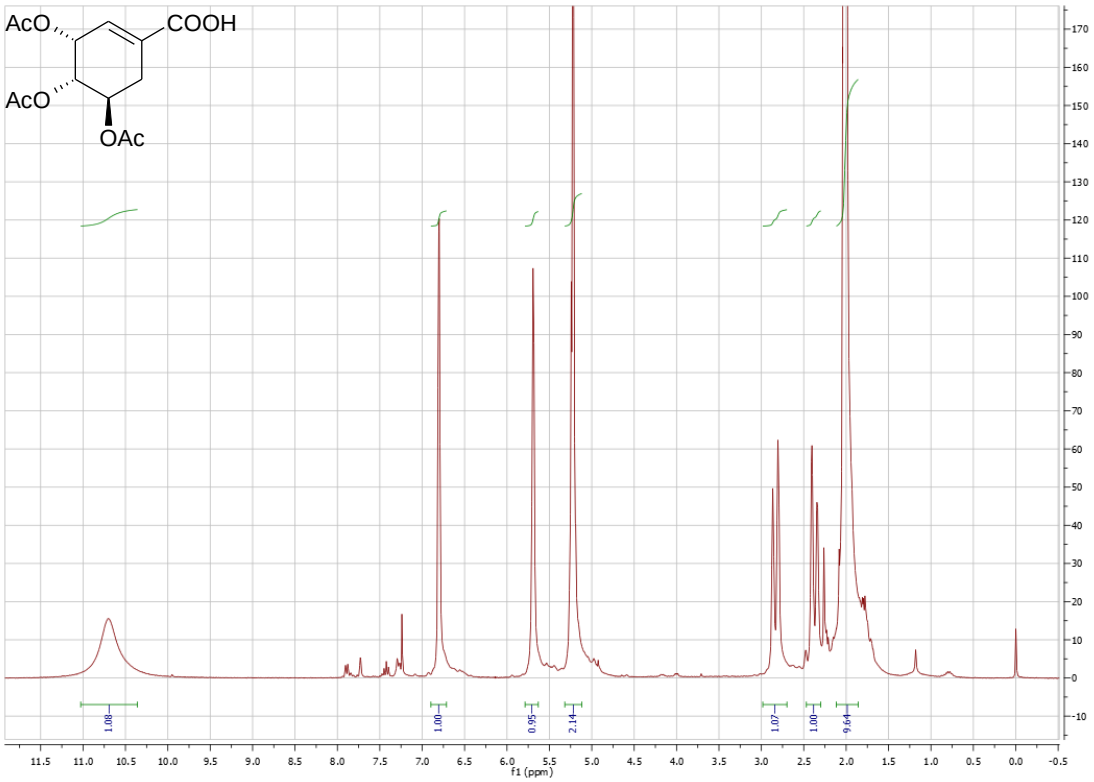
^{1}H-NMR (500 MHz, D_2O) of compound **2**



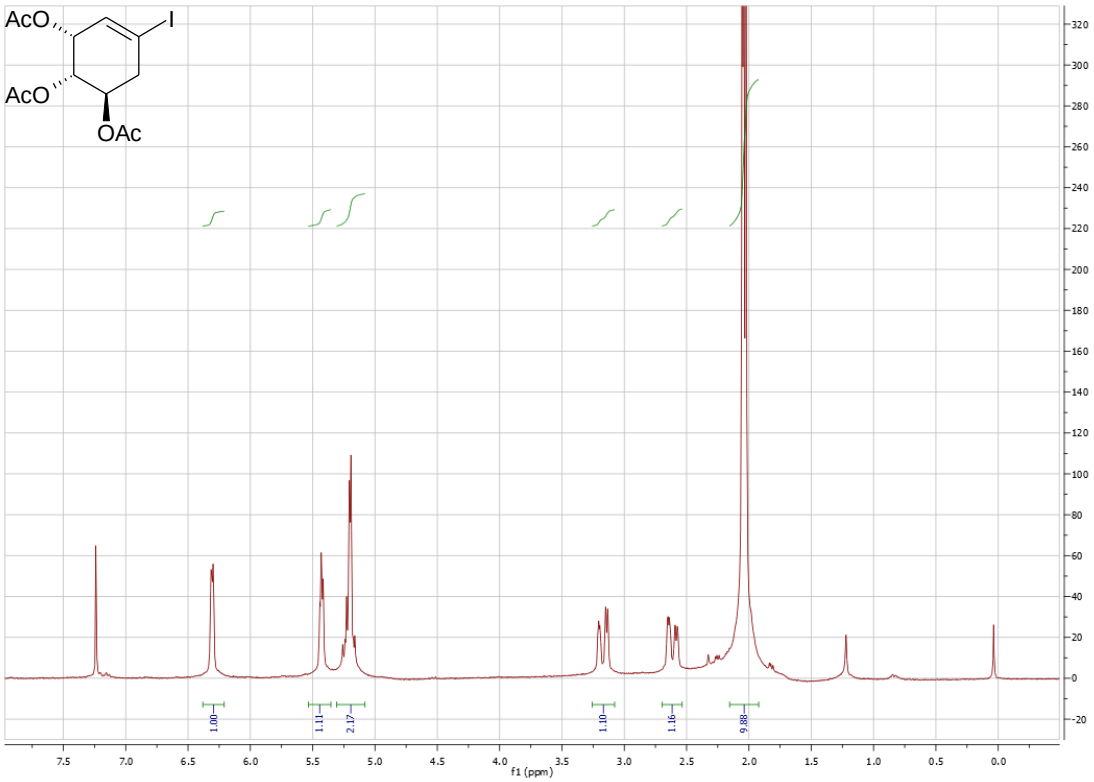
^{1}H-NMR (500 MHz, D_2O) of compound **3**



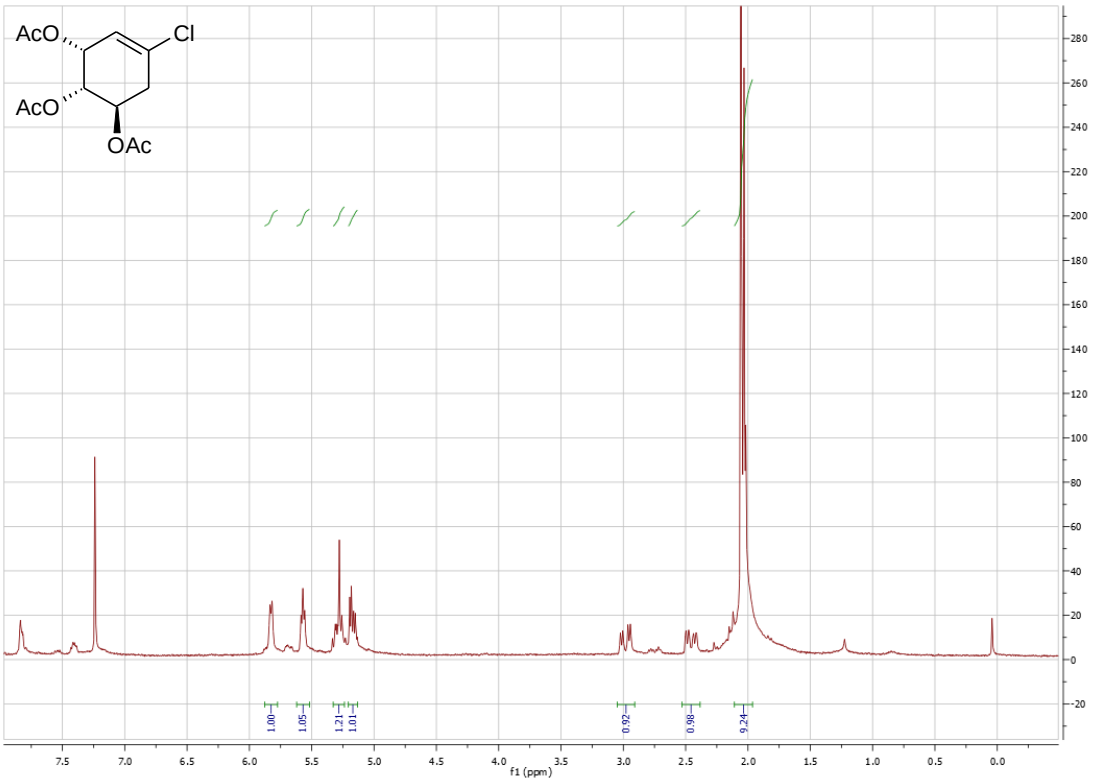
^{1}H-NMR (400 MHz, D_2O) of compound **4**



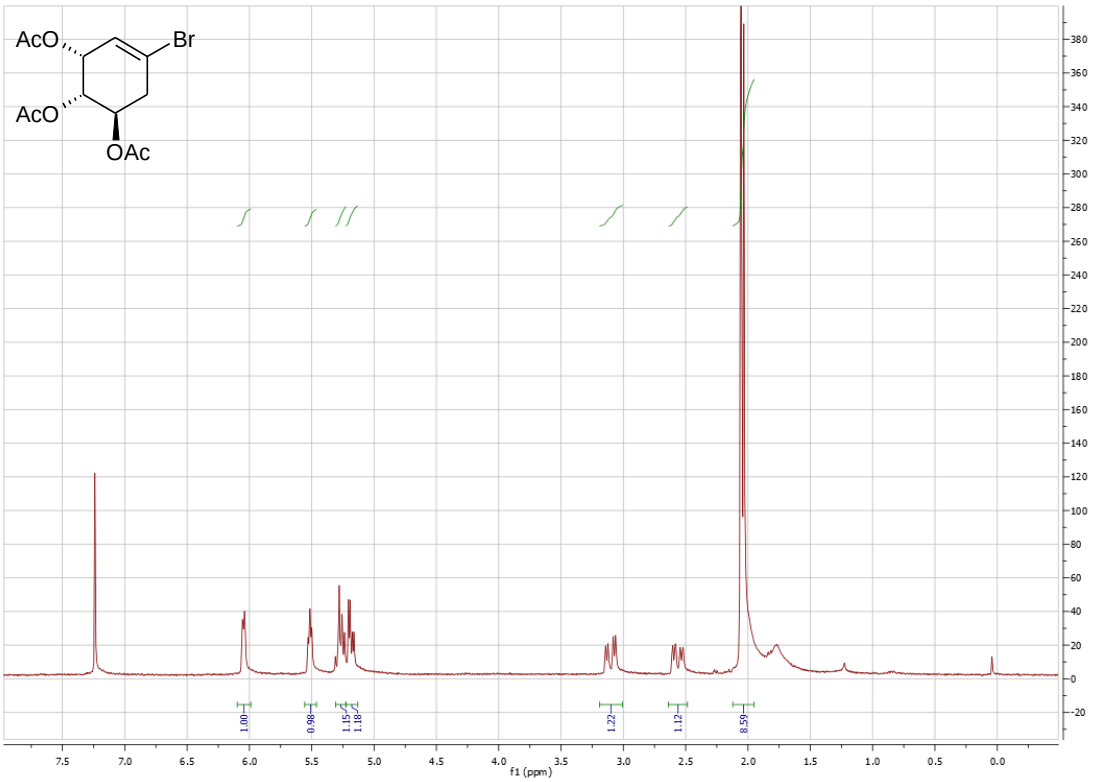
^{1}H-NMR (300 MHz, $CDCl_3$) of compound **5**



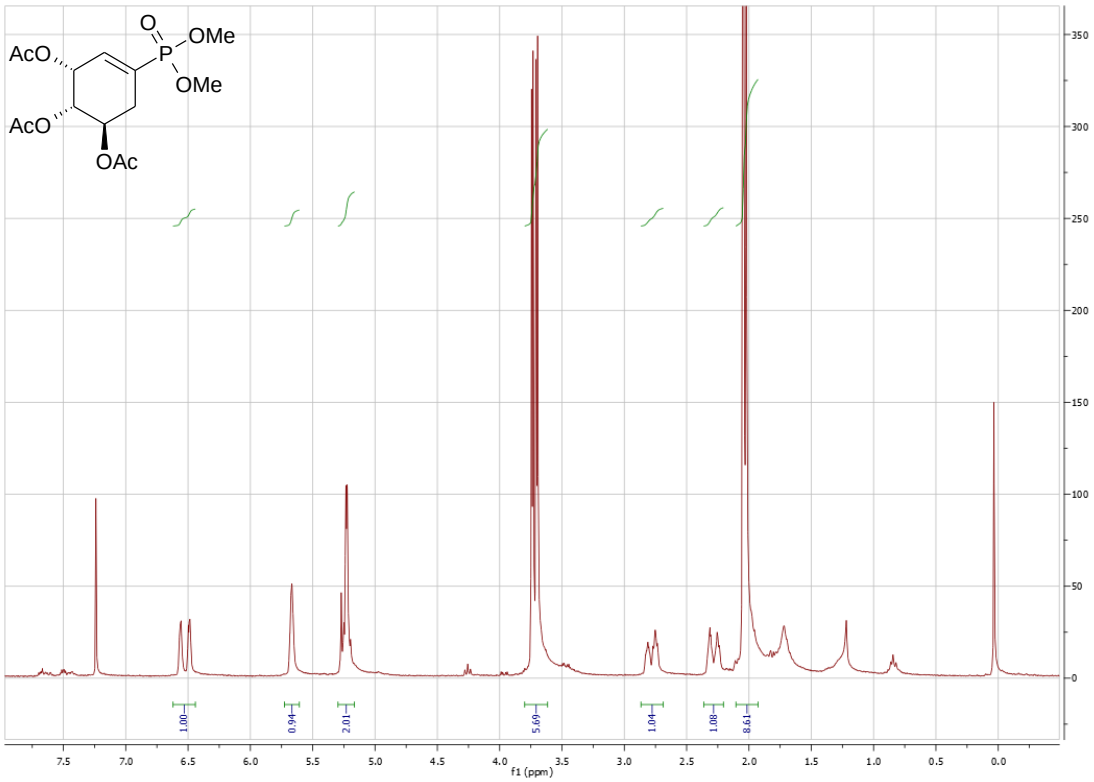
^{1}H-NMR (300 MHz, $CDCl_3$) of compound **6**



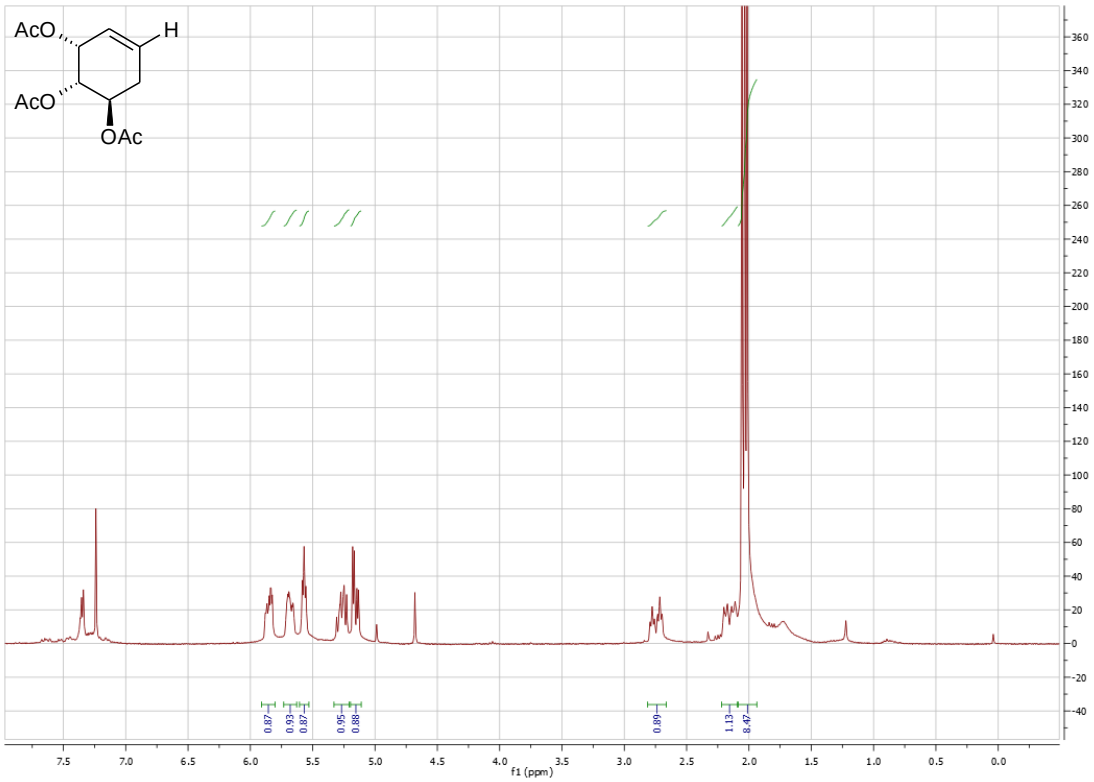
^{1}H-NMR (300 MHz, $CDCl_3$) of compound **7**



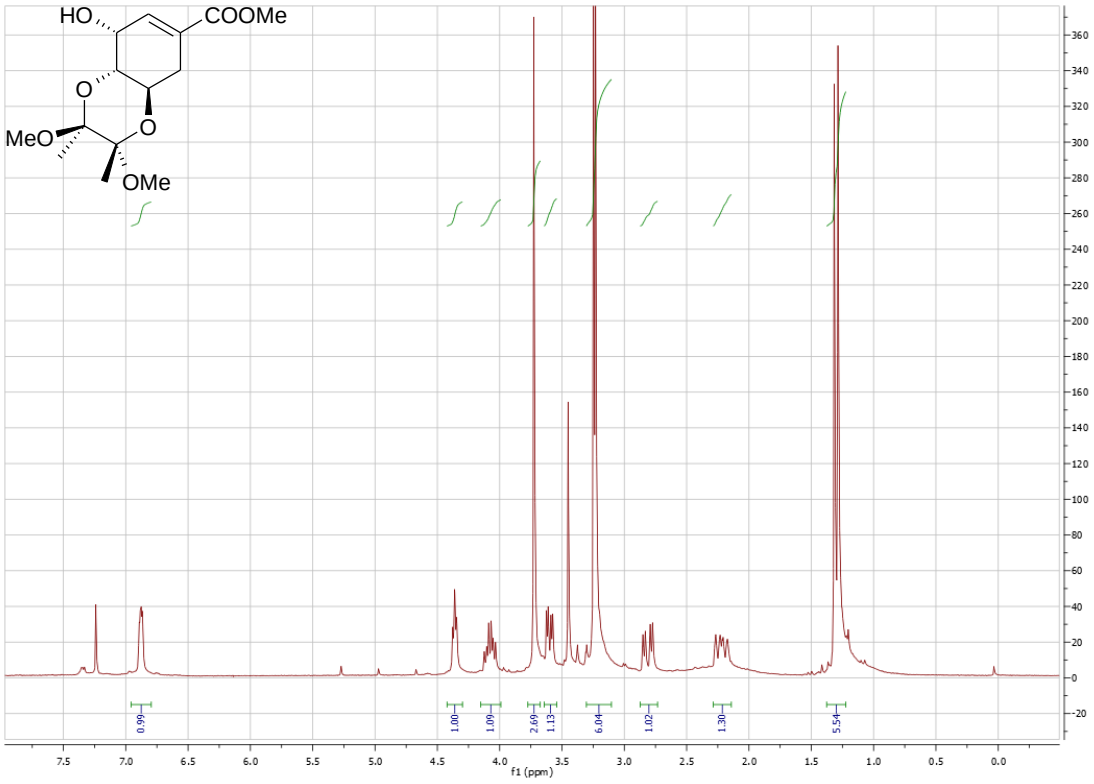
^{1}H-NMR (300 MHz, $CDCl_3$) of compound **8**



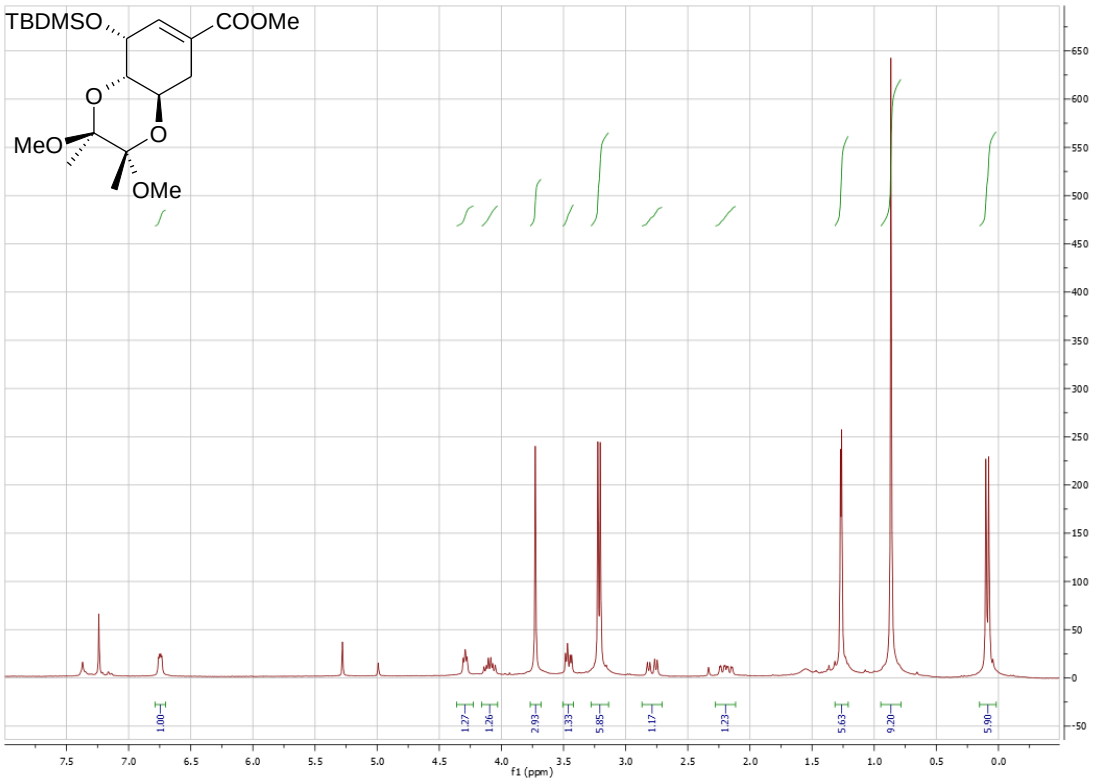
^{1}H-NMR (300 MHz, $CDCl_3$) of compound **9**



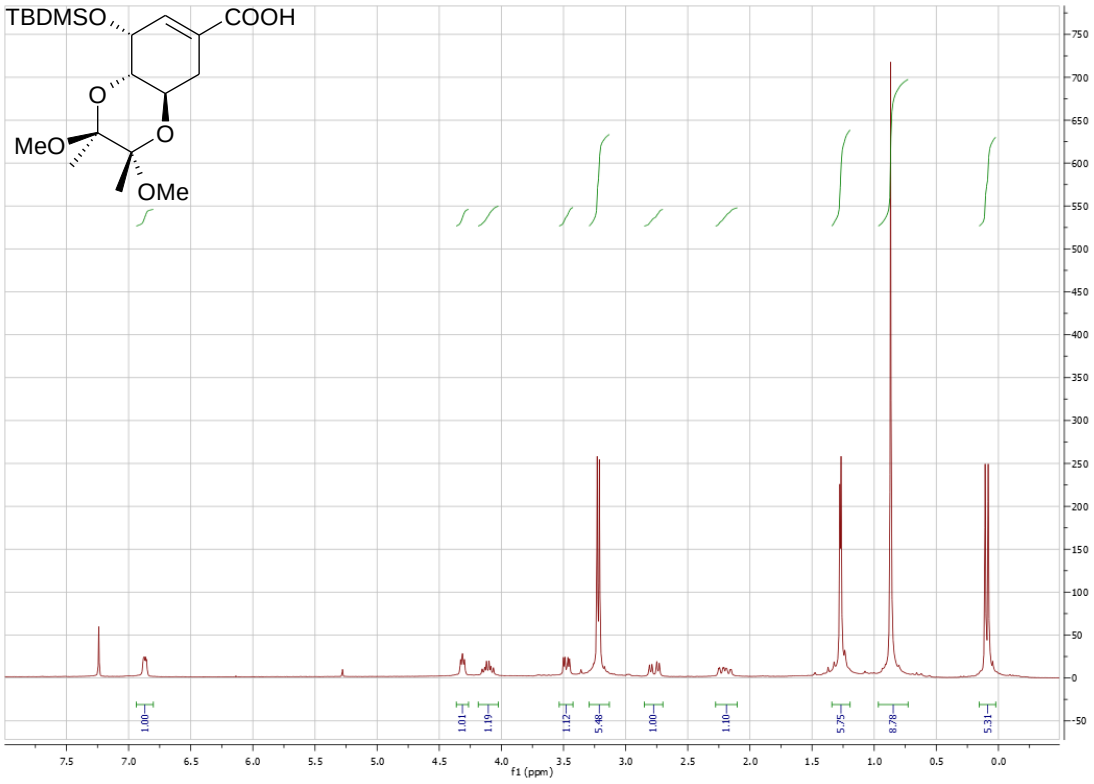
^{1}H-NMR (300 MHz, $CDCl_3$) of compound **10**



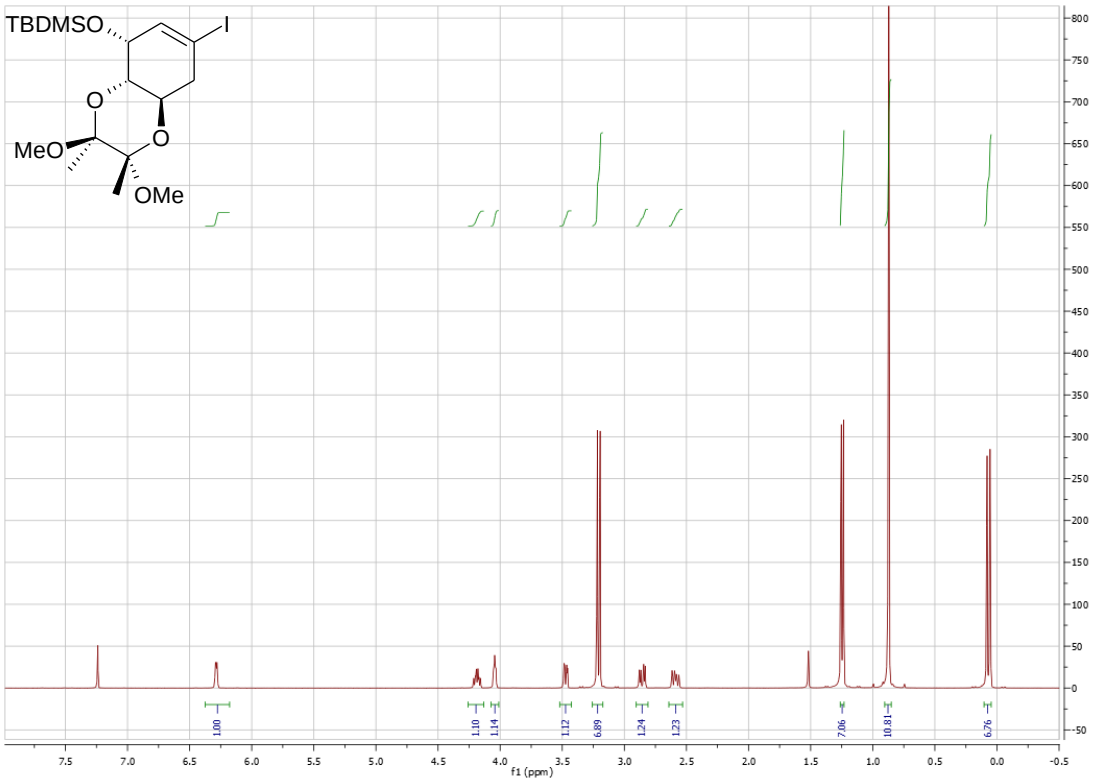
^{1}H-NMR (300 MHz, $CDCl_3$) of compound **11**



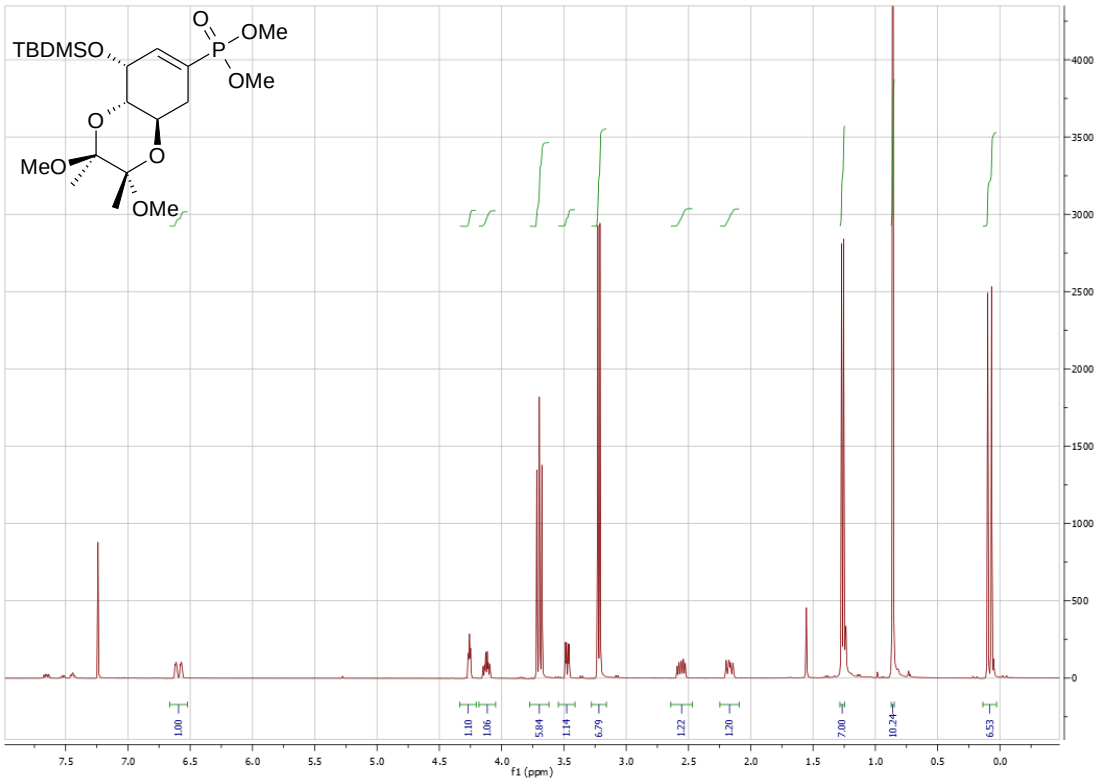
^{1}H-NMR (300 MHz, $CDCl_3$) of compound **12**



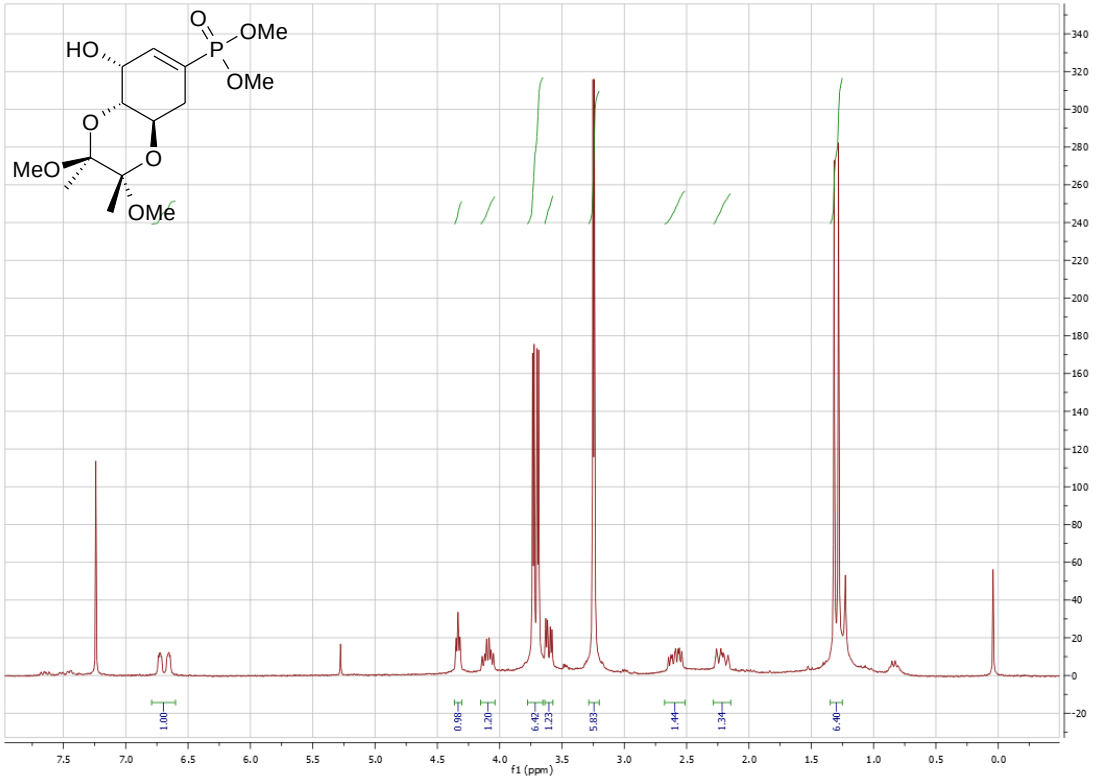
^{1}H-NMR (300 MHz, $CDCl_3$) of compound **13**



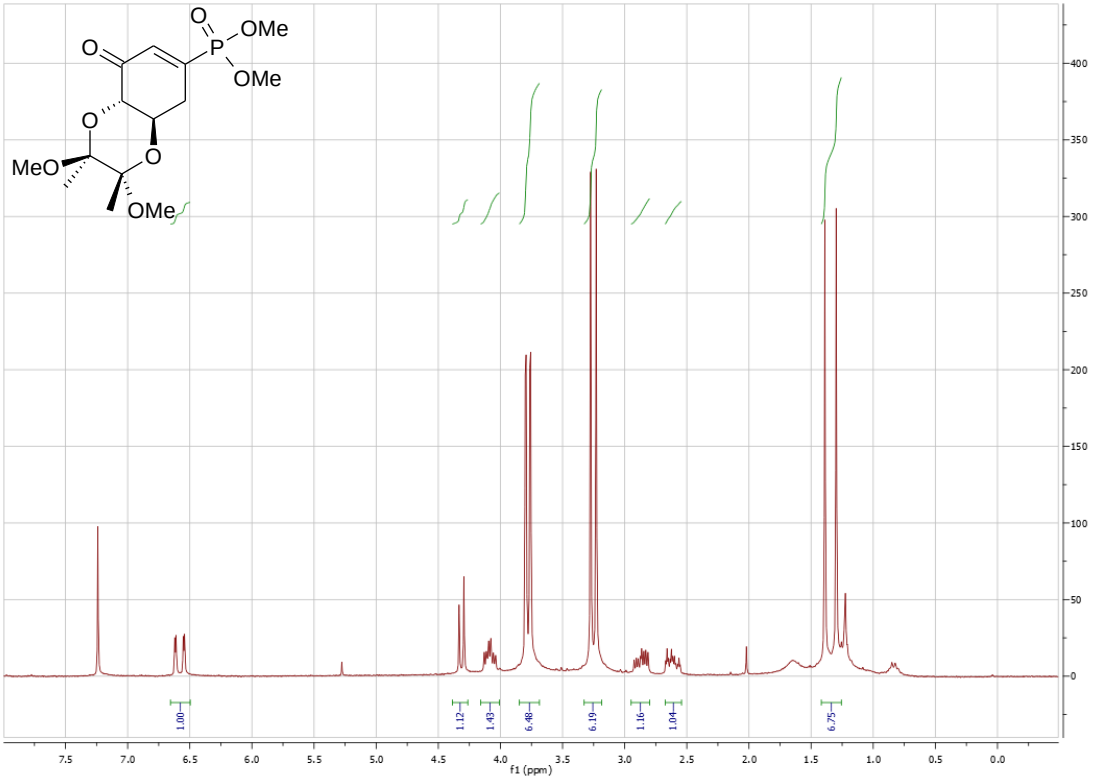
^{1}H-NMR (500 MHz, $CDCl_3$) of compound **14**



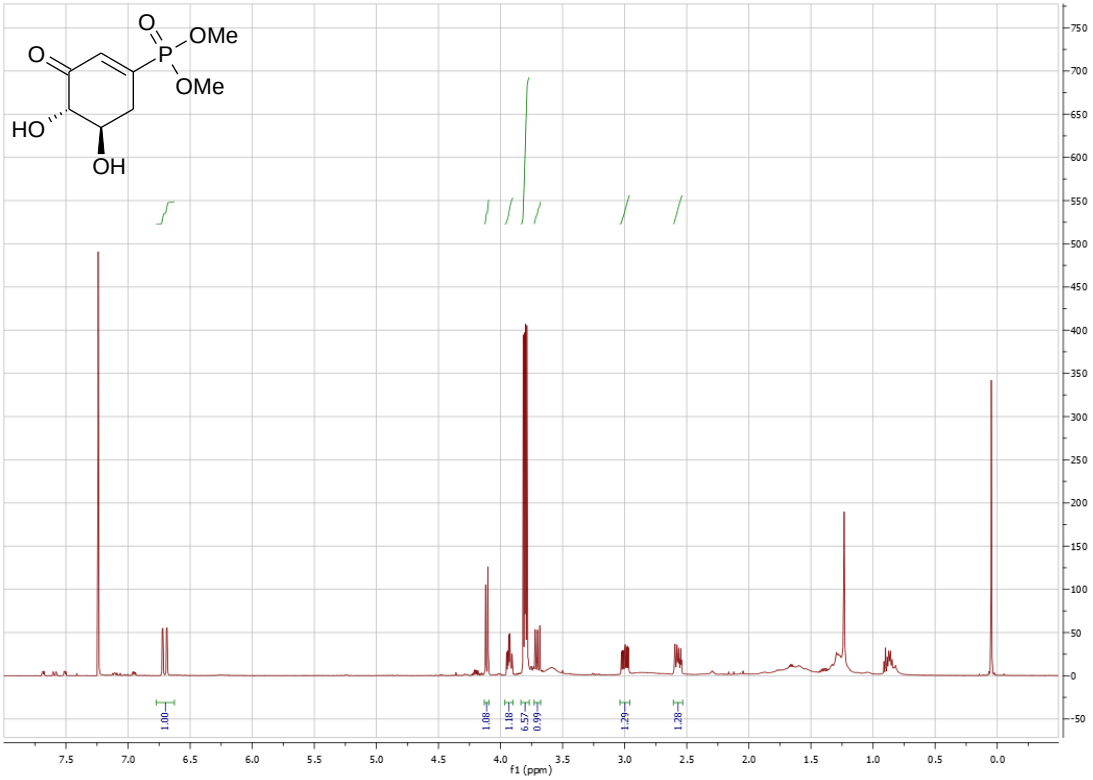
^{1}H-NMR (500 MHz, $CDCl_3$) of compound **15**



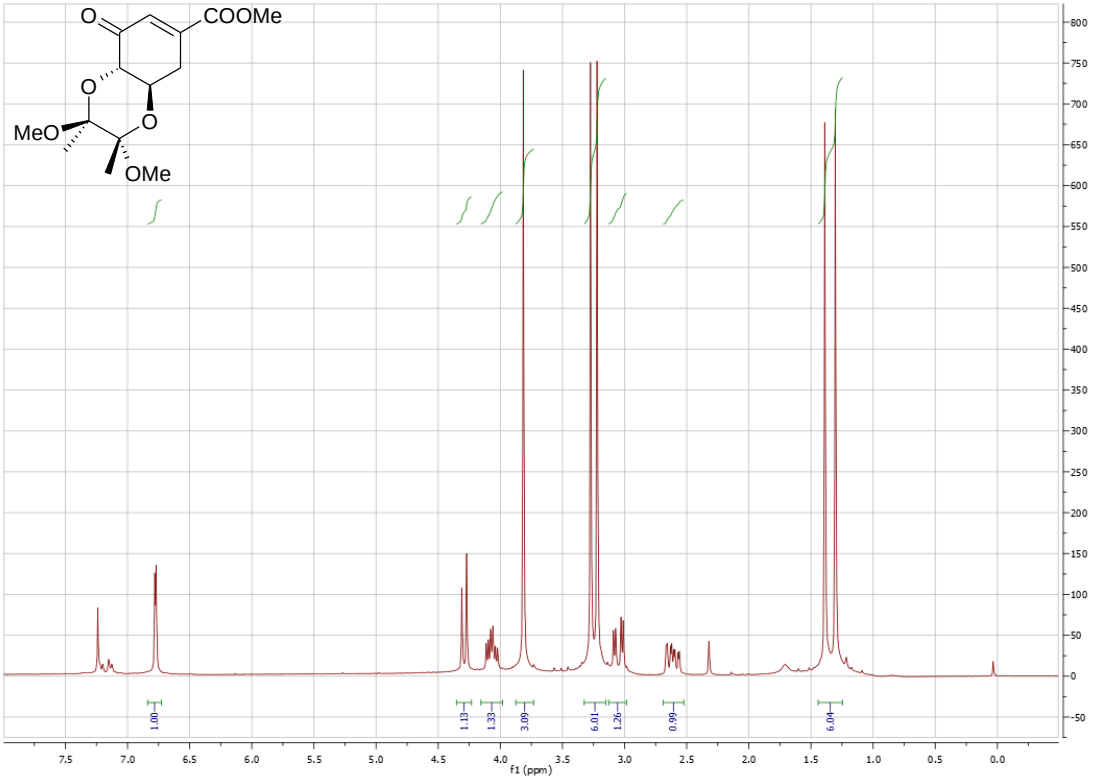
^{1}H-NMR (300 MHz, $CDCl_3$) of compound **16**



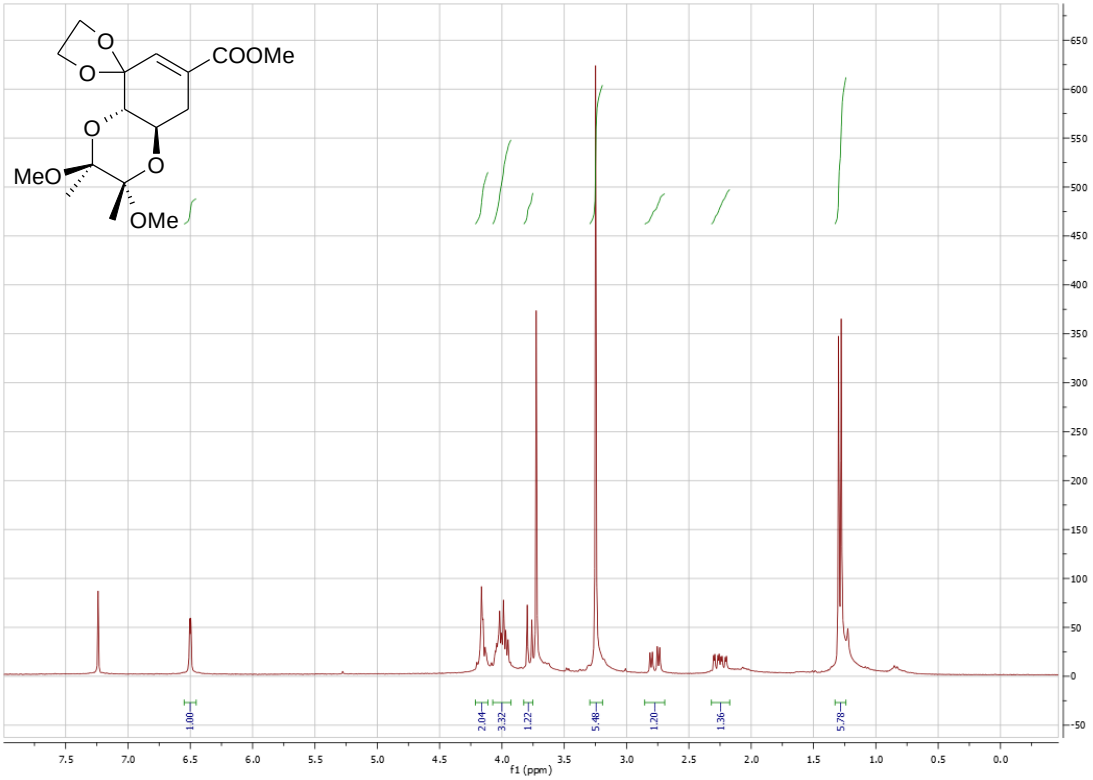
^{1}H-NMR (300 MHz, $CDCl_3$) of compound **17**



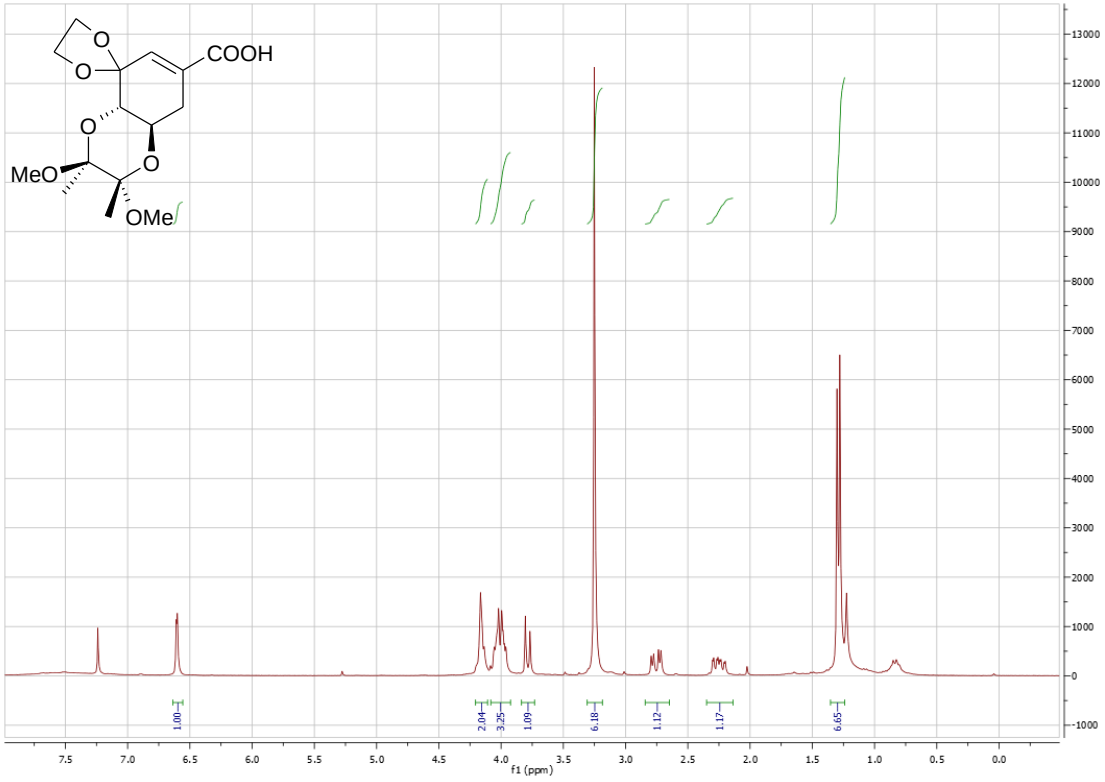
^{1}H-NMR (600 MHz, $CDCl_3$) of compound **18**



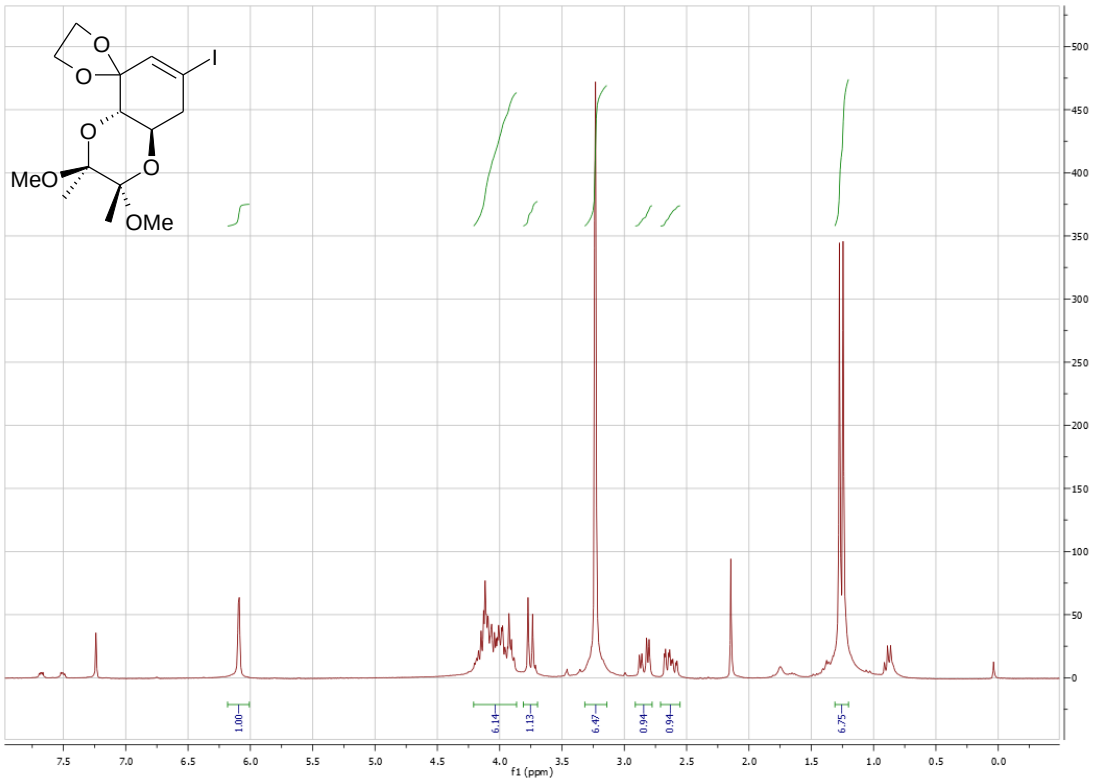
^{1}H-NMR (300 MHz, $CDCl_3$) of compound **19**



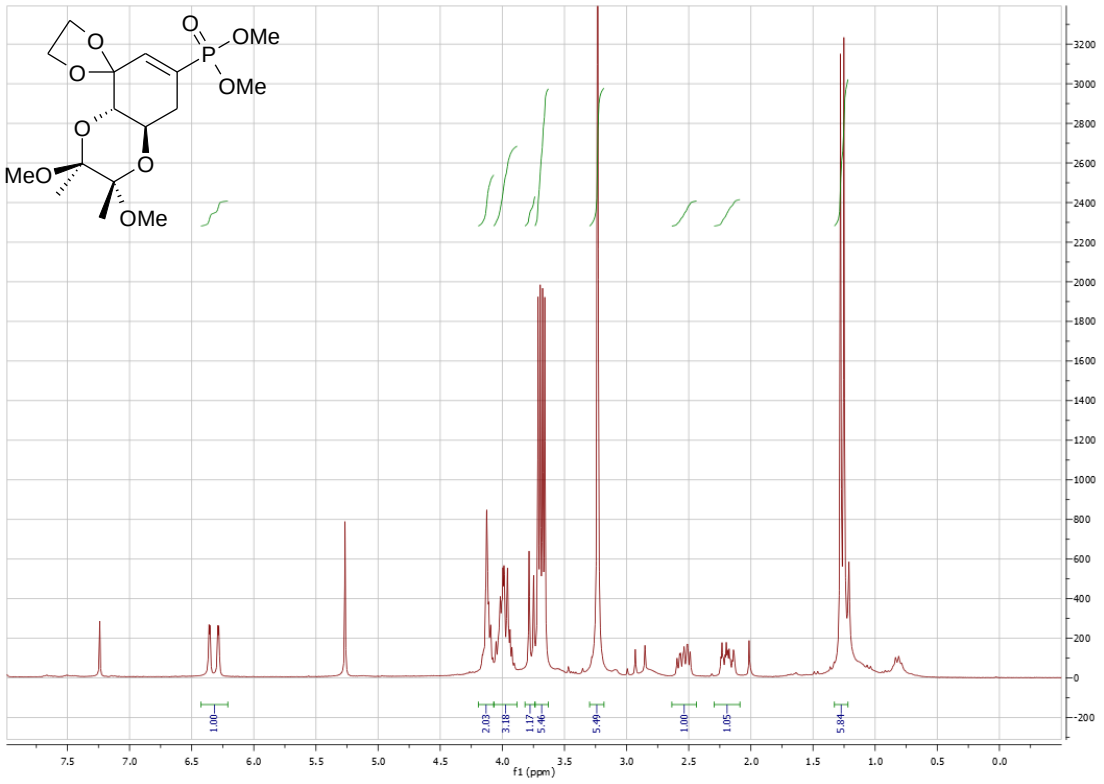
^{1}H-NMR (300 MHz, $CDCl_3$) of compound **20**



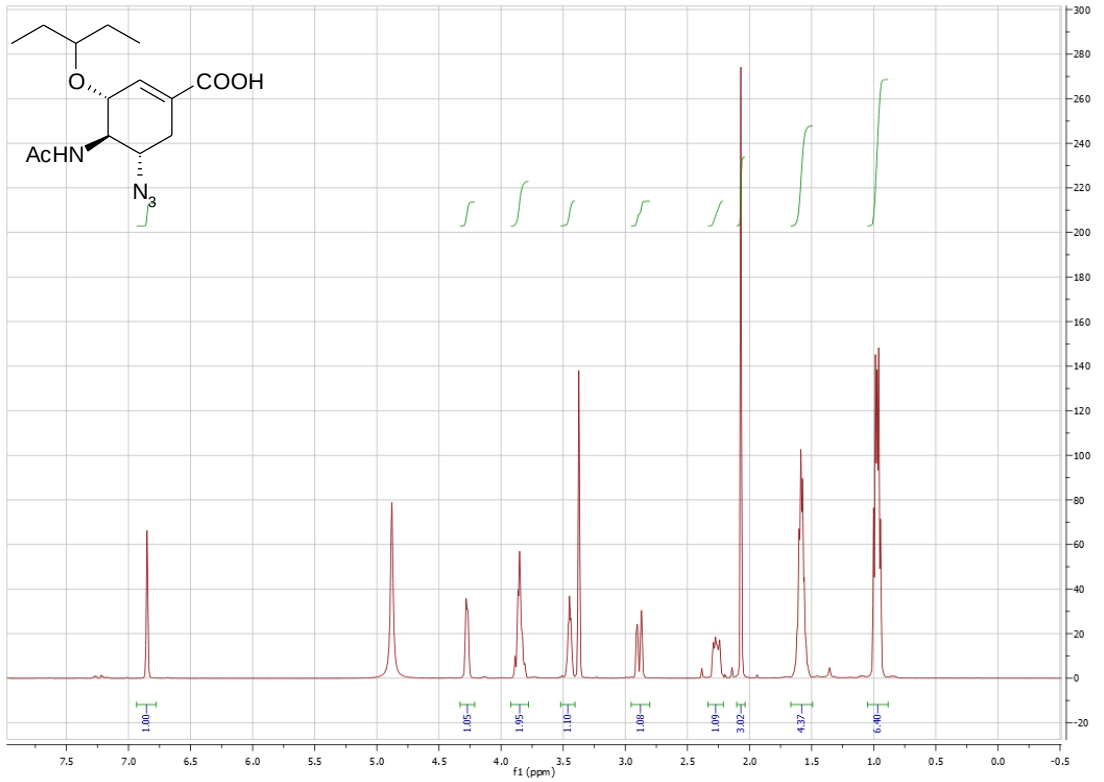
^{1}H-NMR (300 MHz, $CDCl_3$) of compound **21**



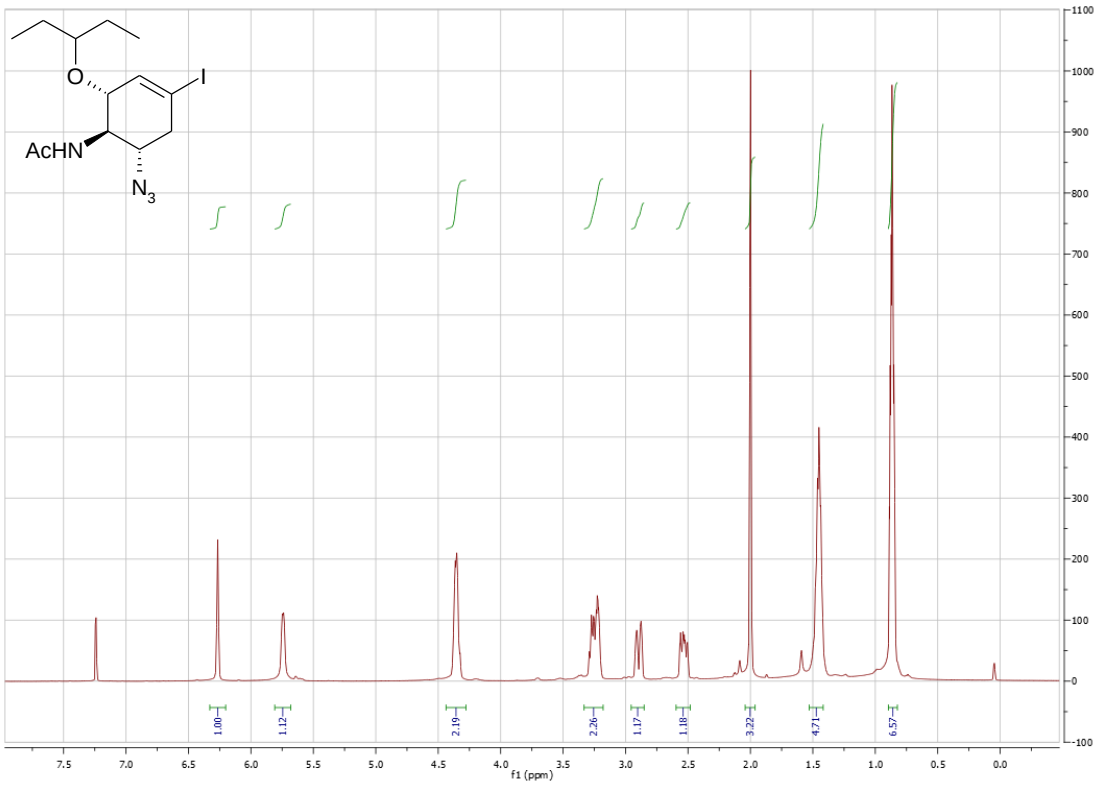
^{1}H-NMR (300 MHz, $CDCl_3$) of compound **22**



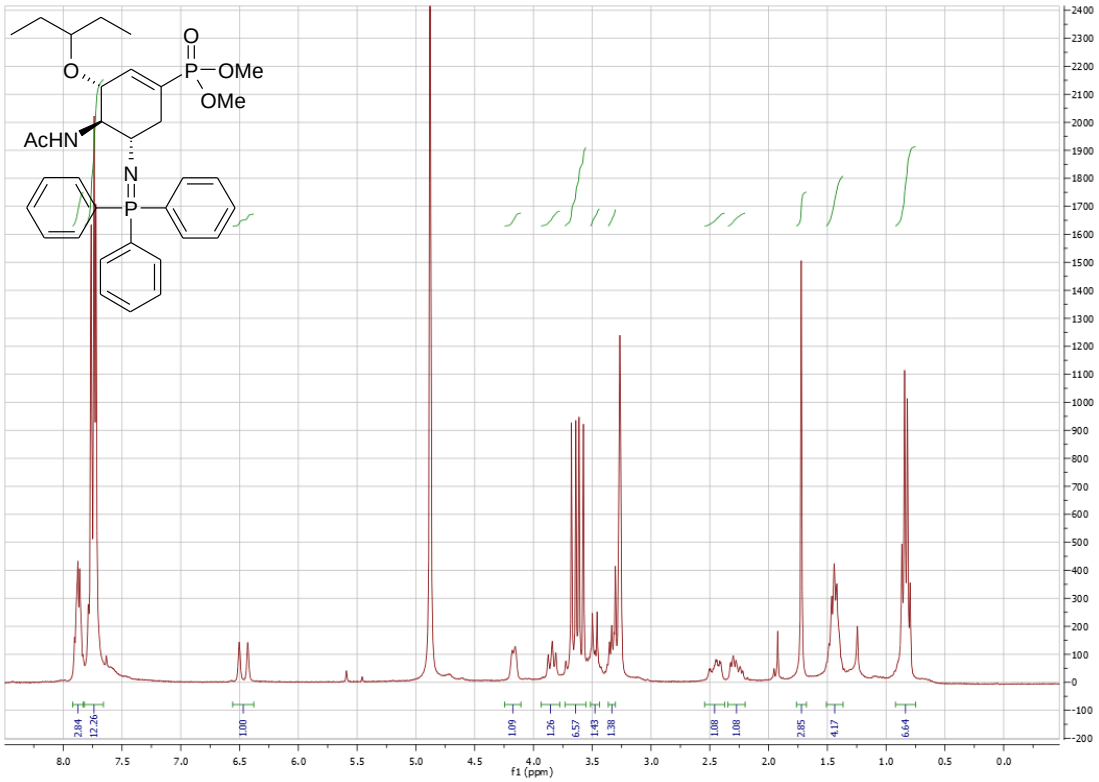
^{1}H-NMR (300 MHz, $CDCl_3$) of compound **23**



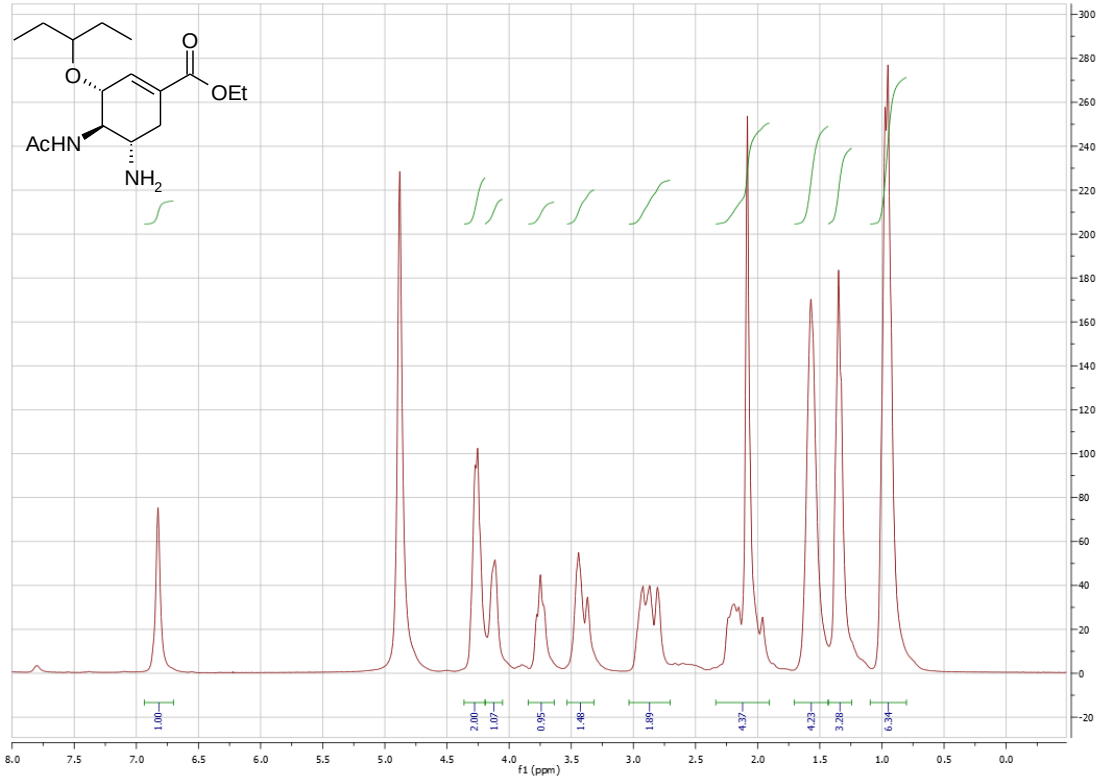
^{1}H-NMR (500 MHz, CD_3OD) of compound **24**



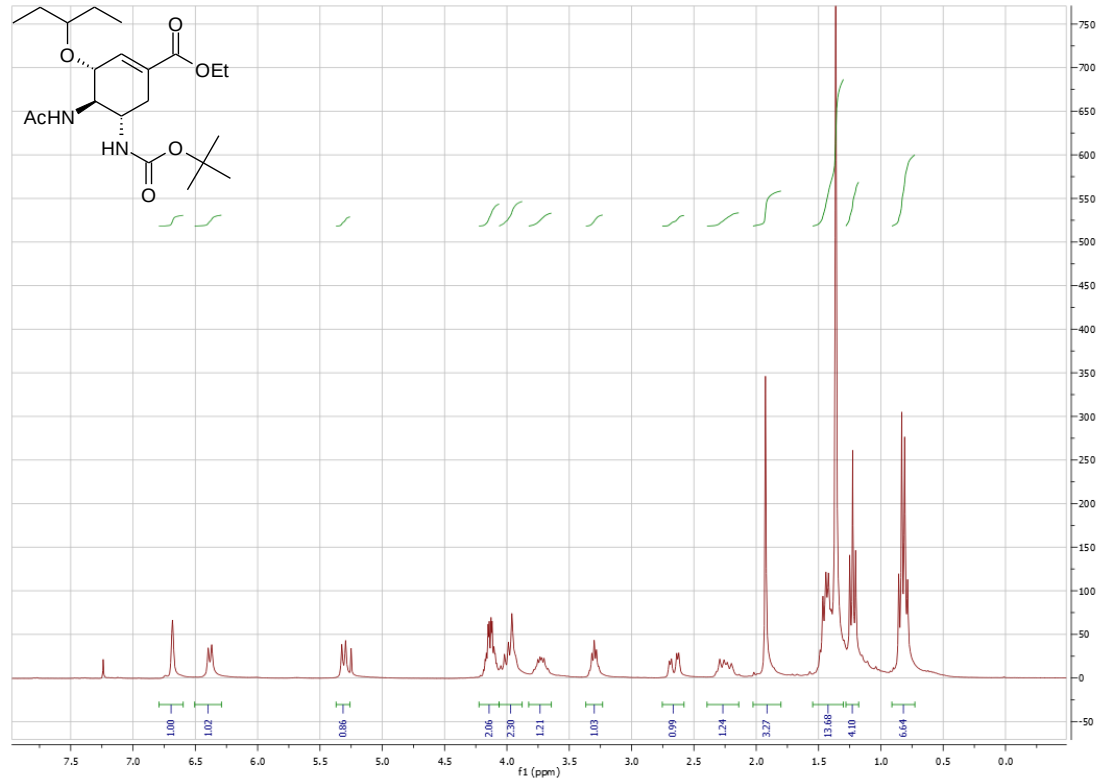
^{1}H-NMR (500 MHz, $CDCl_3$) of compound **25**



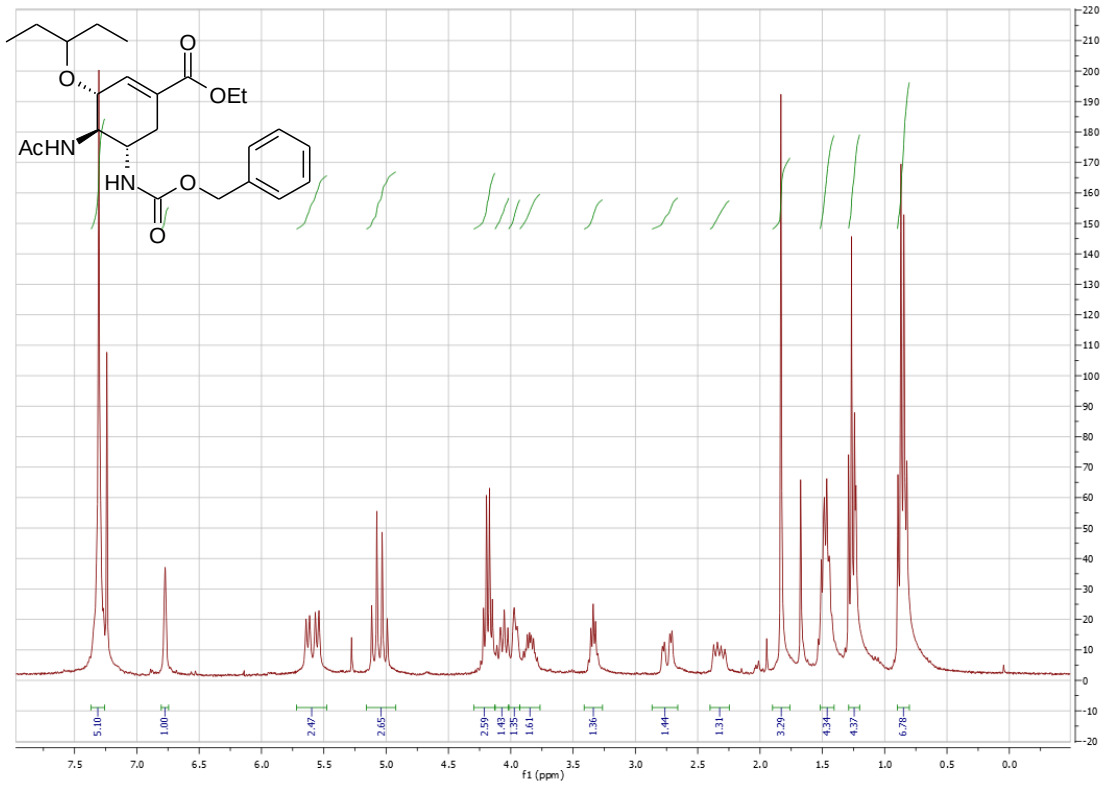
^{1}H-NMR (300 MHz, CD_3OD) of compound **26**



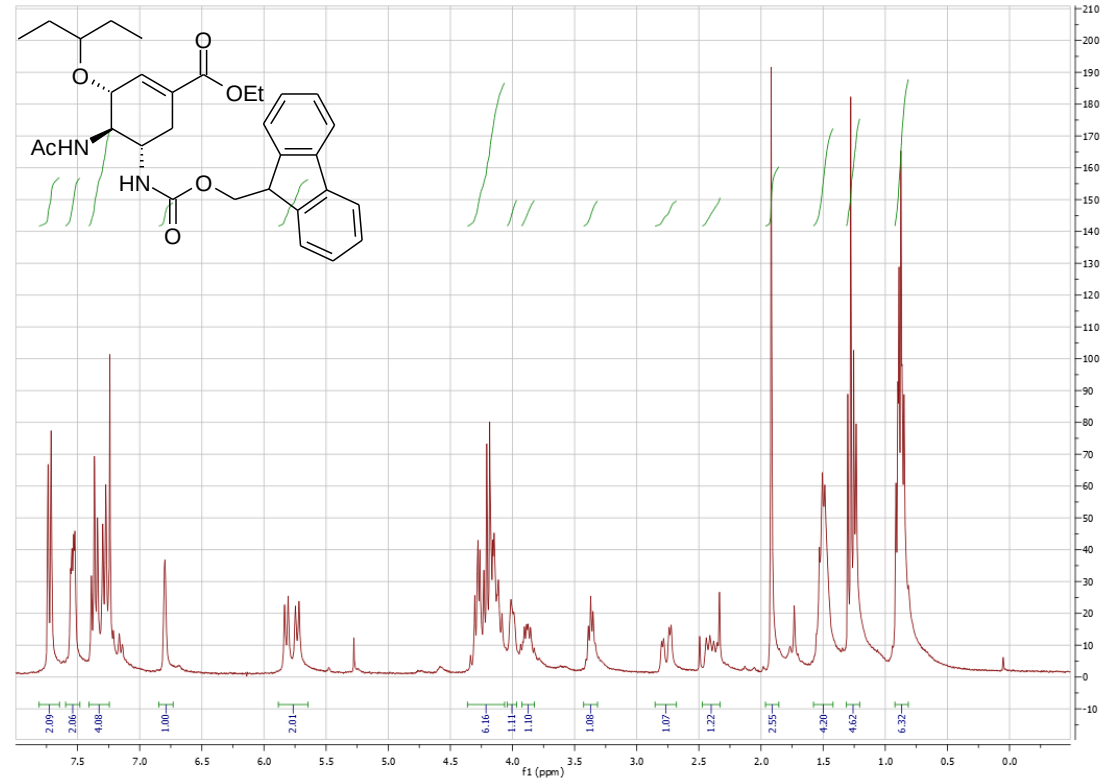
^{1}H-NMR (300 MHz, CD_3OD) of compound **27**



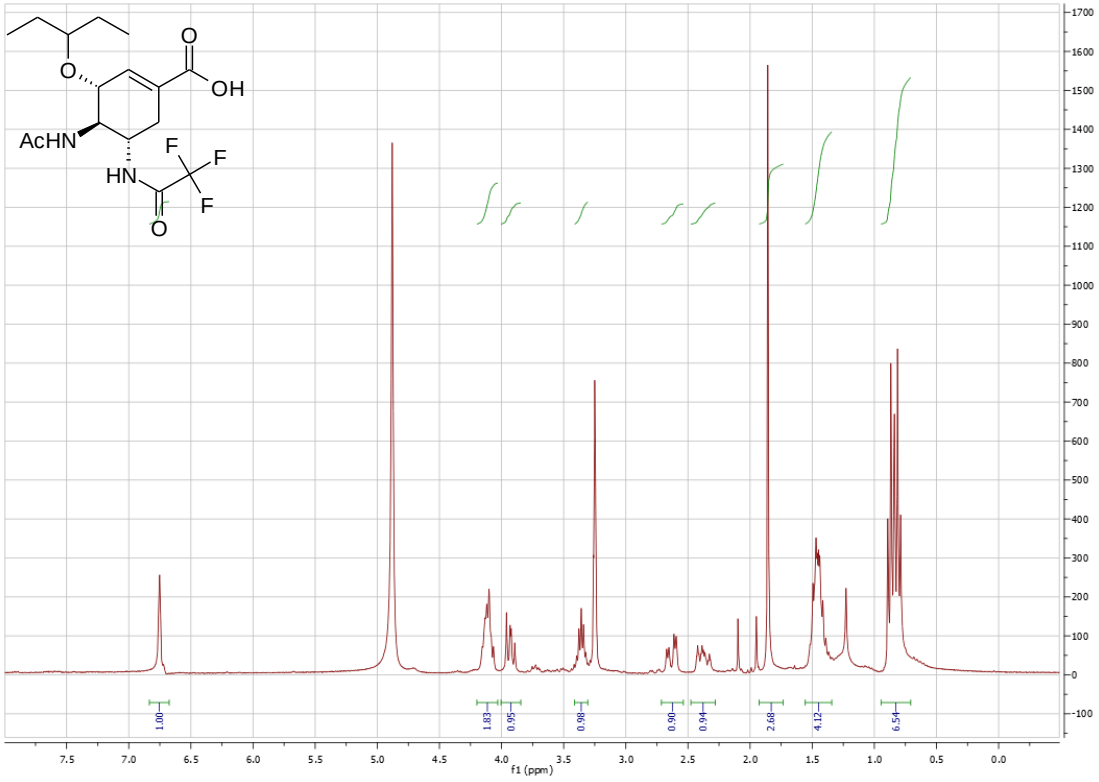
^{1}H-NMR (300 MHz, $CDCl_3$) of compound **28**



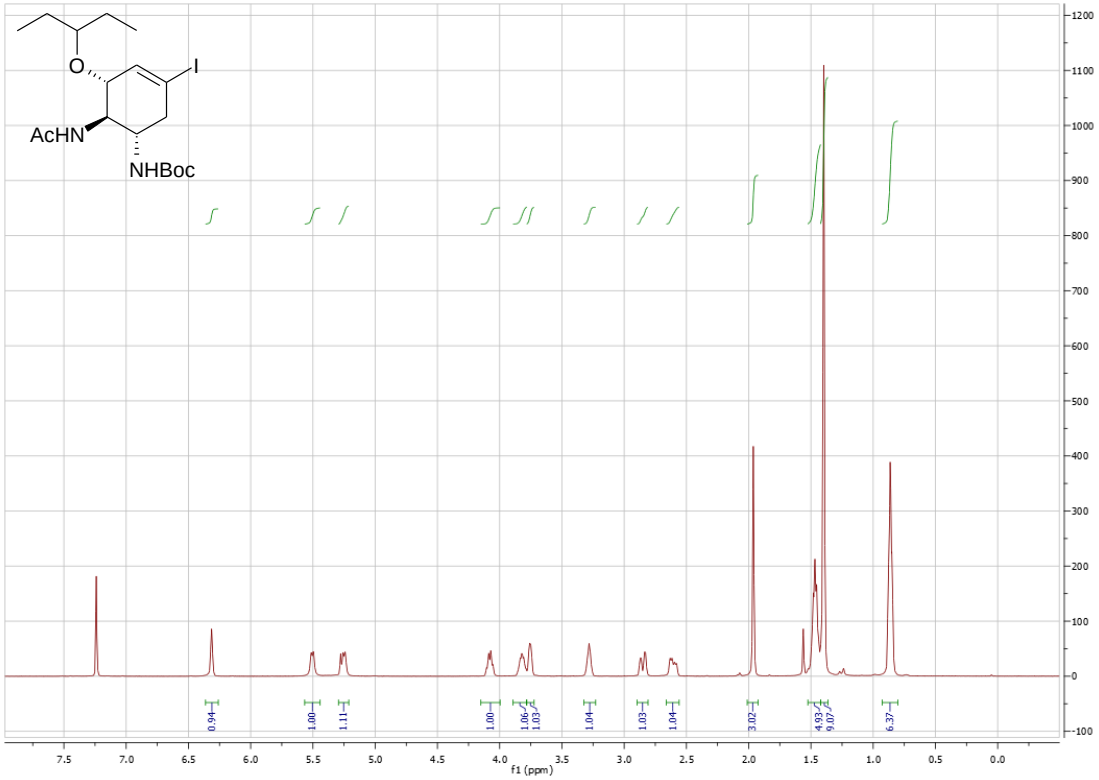
^{1}H-NMR (300 MHz, $CDCl_3$) of compound **29**



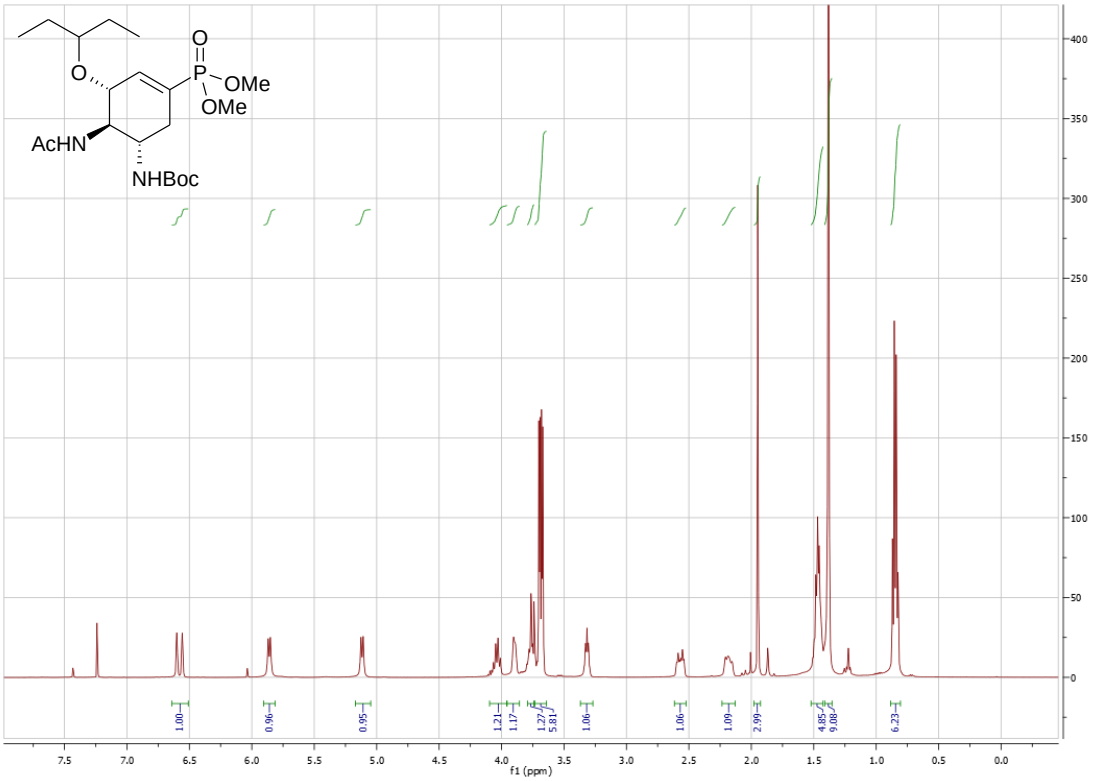
^{1}H-NMR (300 MHz, $CDCl_3$) of compound **30**



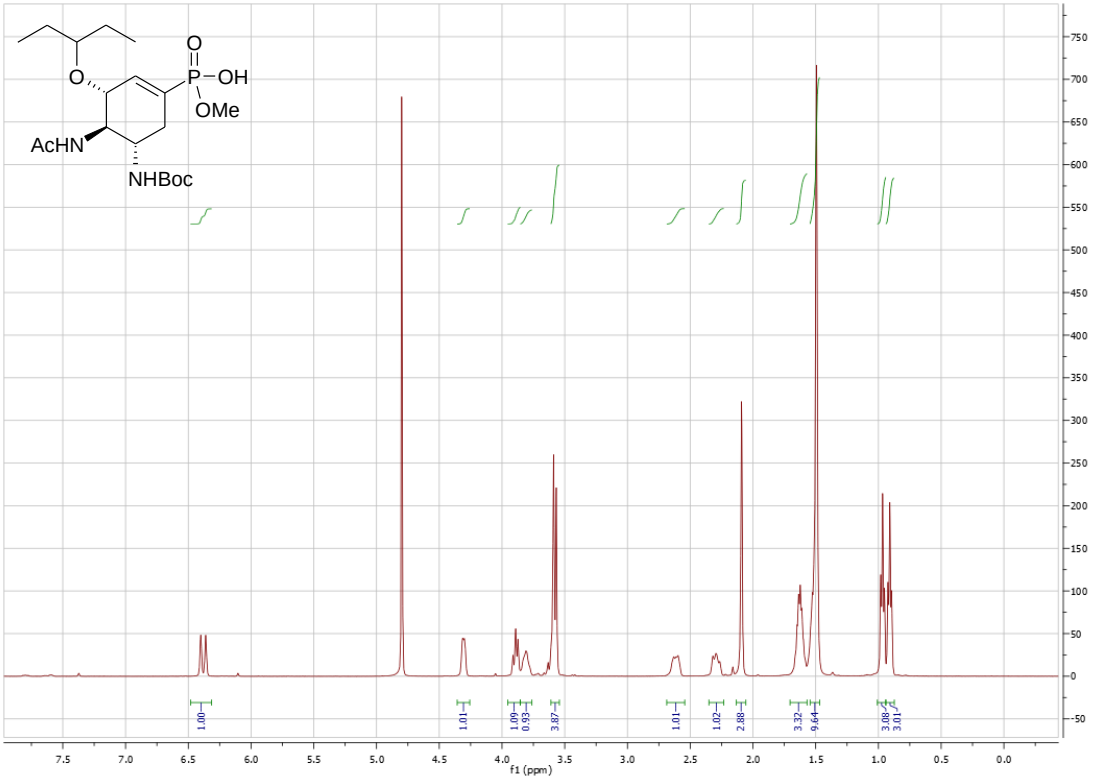
^{1}H-NMR (300 MHz, CD_3OD) of compound **31**



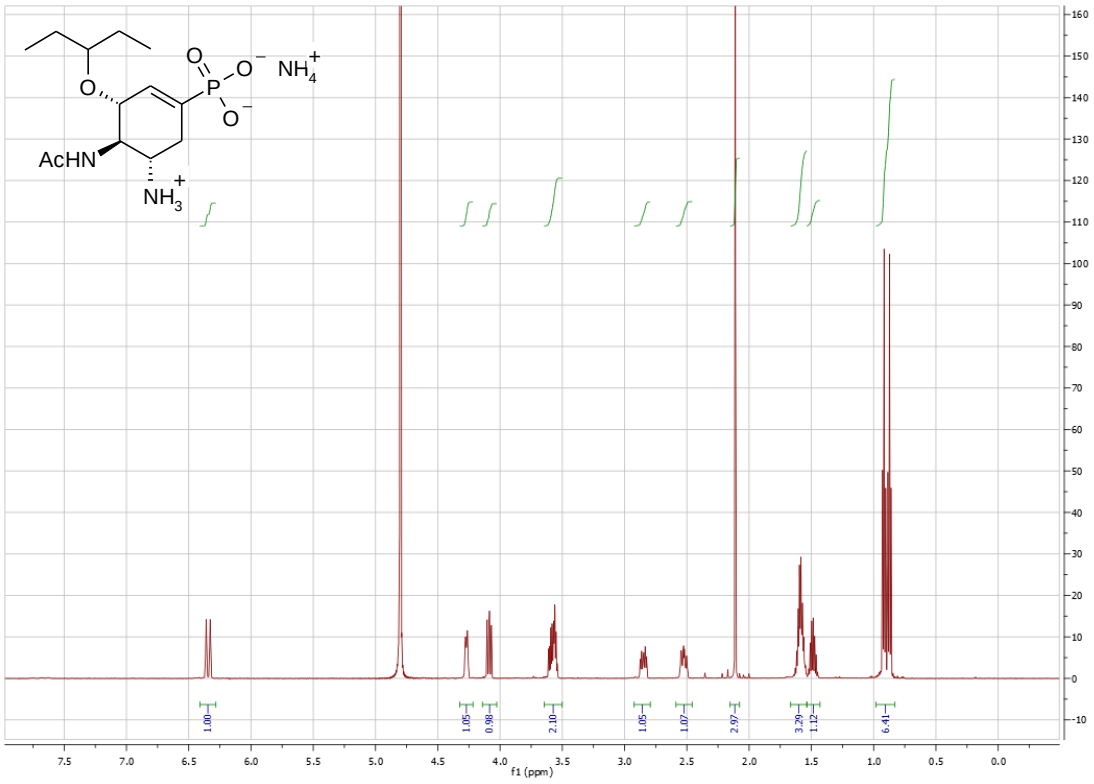
^{1}H-NMR (500 MHz, $CDCl_3$) of compound **32**



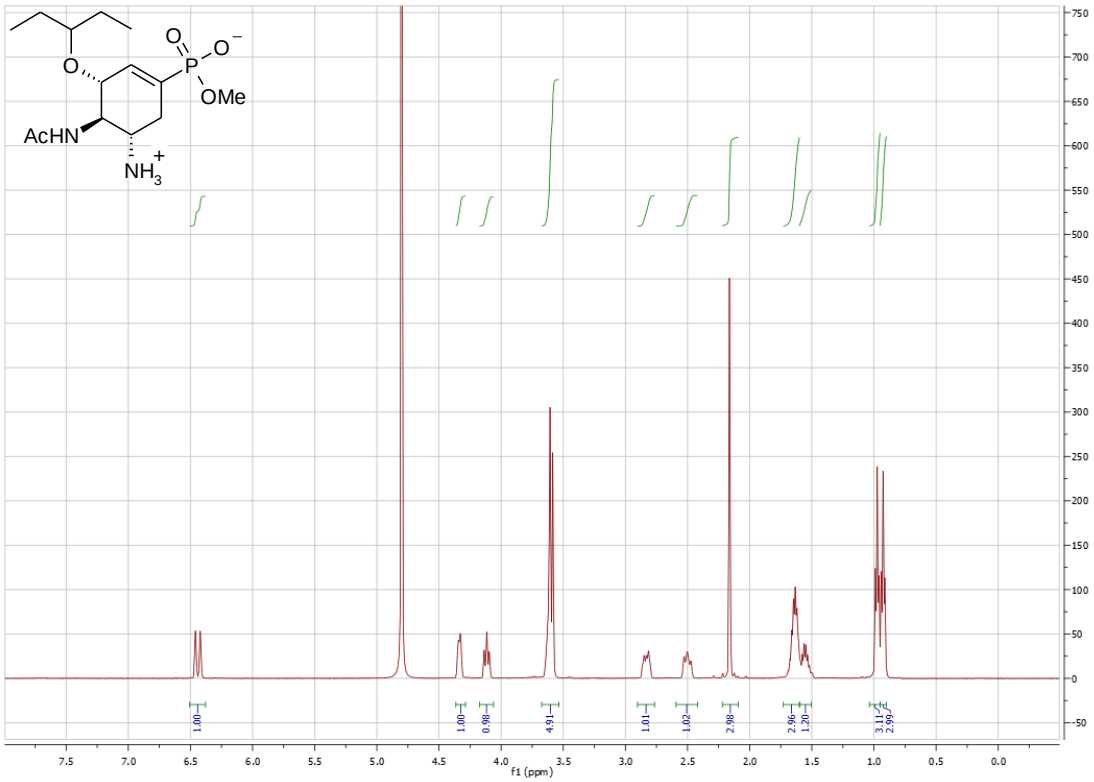
^{1}H-NMR (500 MHz, $CDCl_3$) of compound **33**



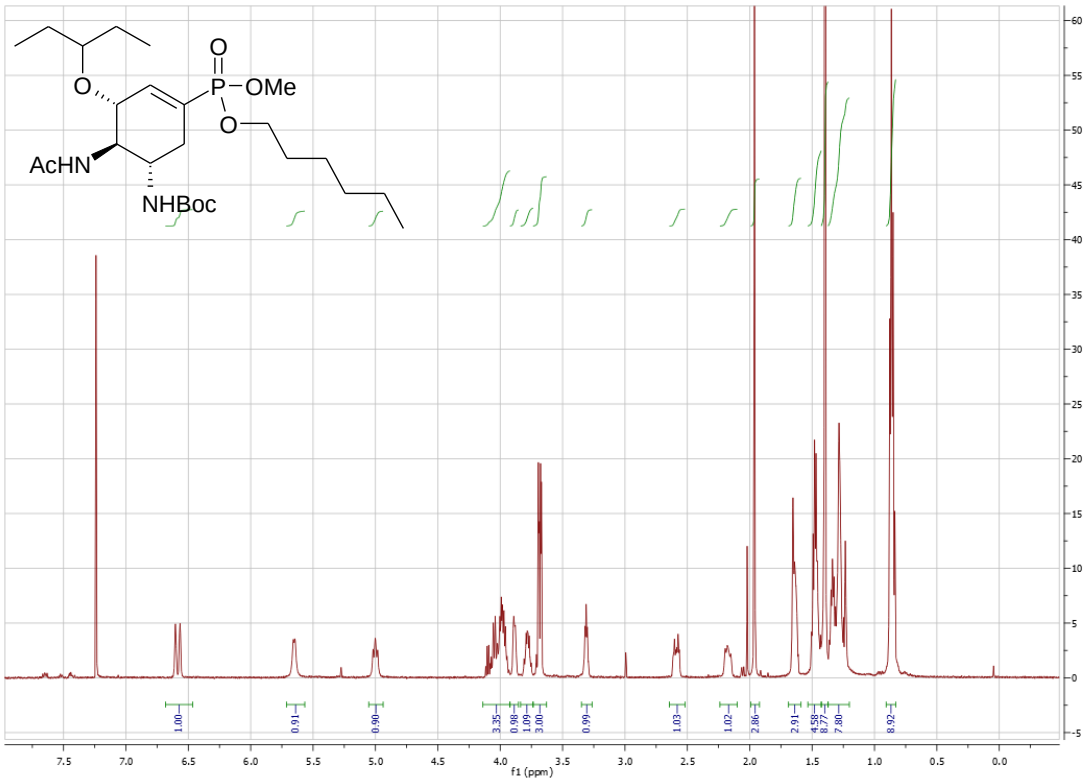
^{1}H-NMR (500 MHz, D_2O) of compound **34**



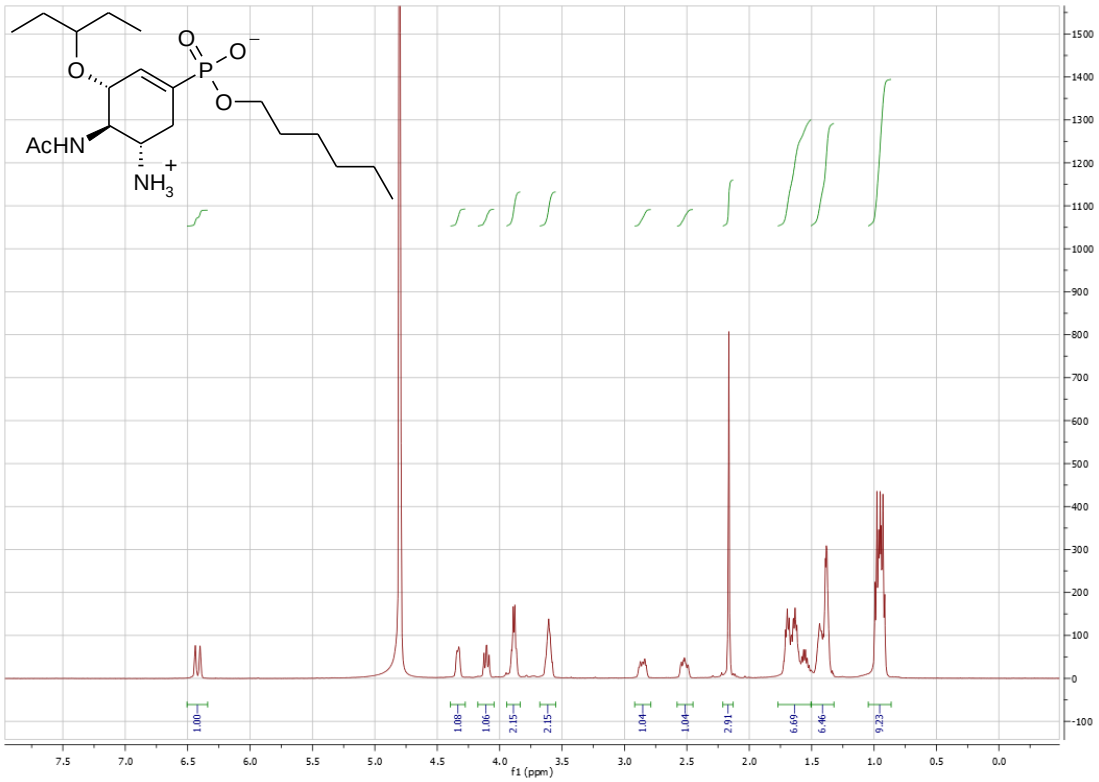
^{1}H-NMR (600 MHz, D_2O) of compound **35**



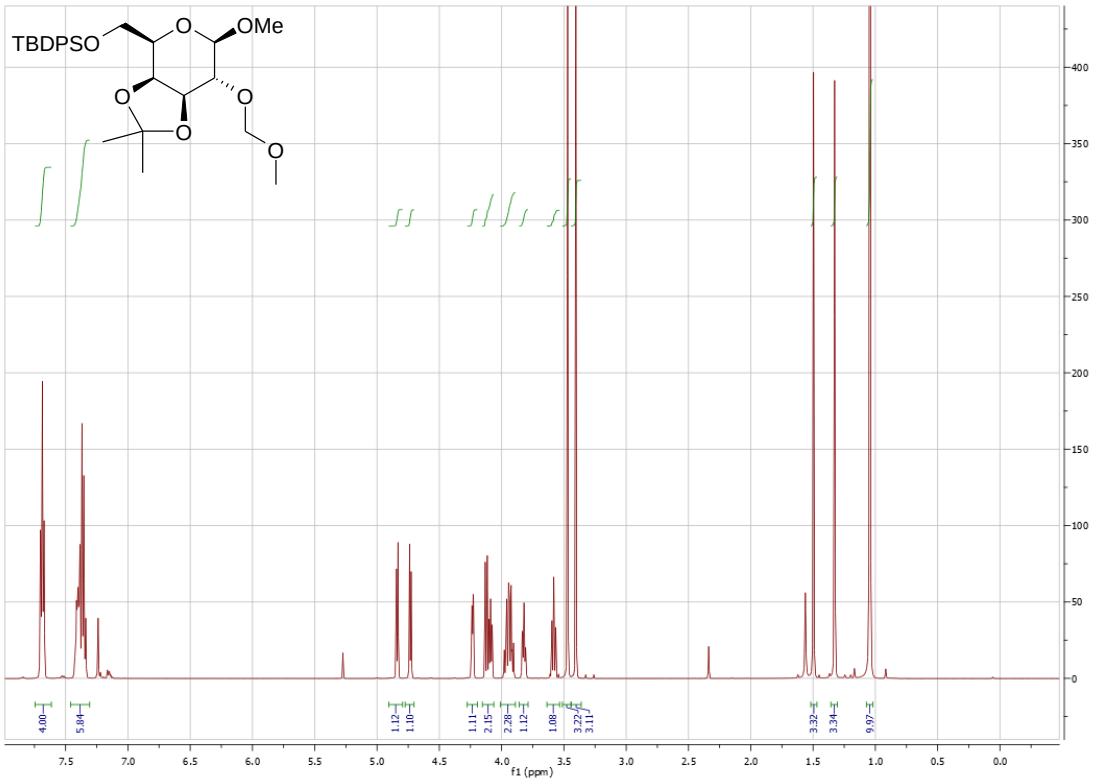
^{1}H-NMR (500 MHz, D_2O) of compound **36**



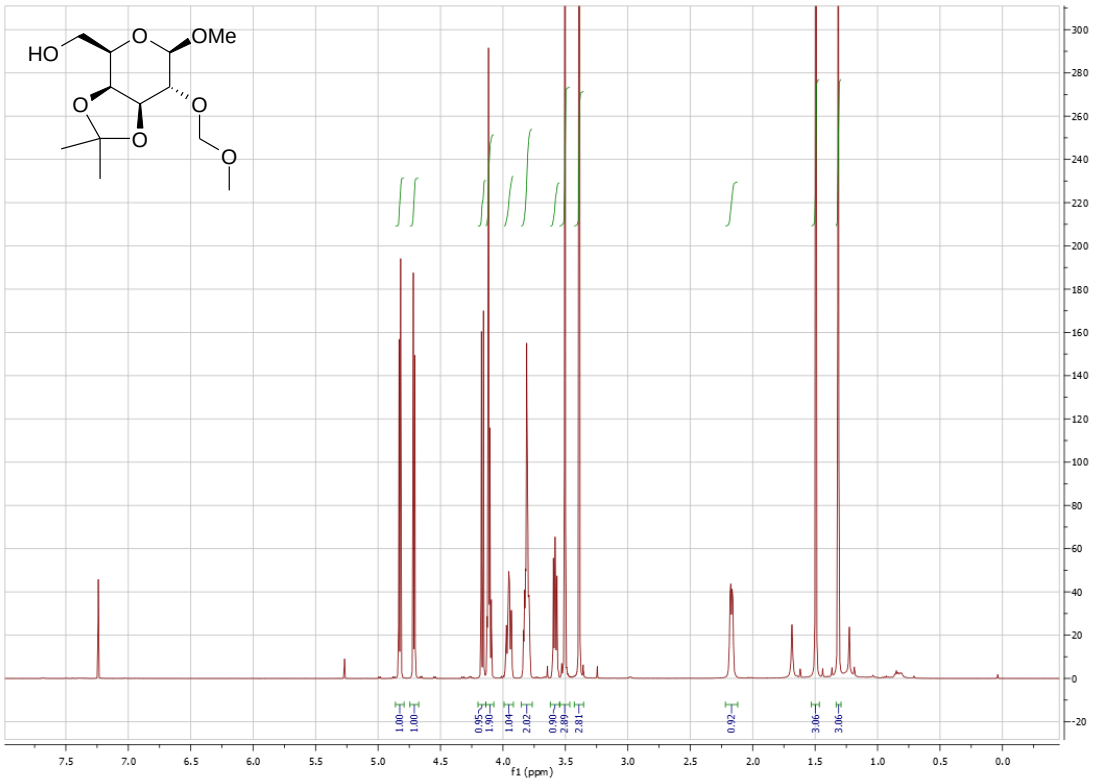
^{1}H-NMR (600 MHz, $CDCl_3$) of compound **38**



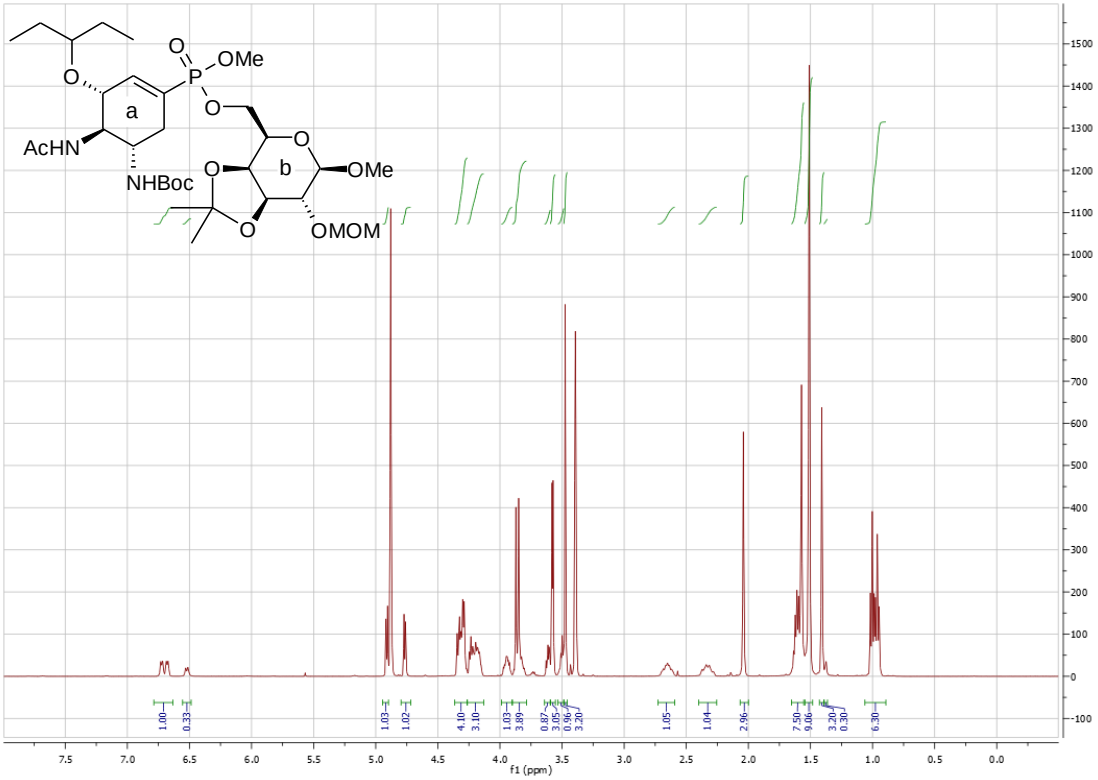
^{1}H-NMR (500 MHz, D_2O) of compound **40**



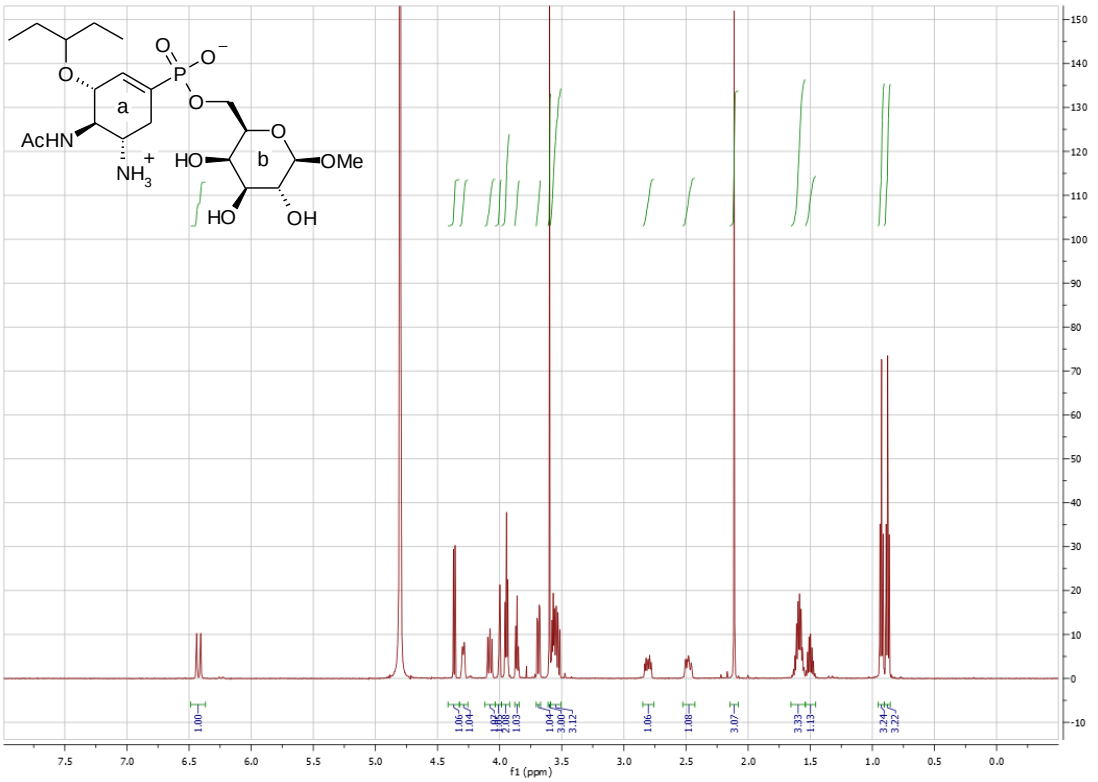
^{1}H-NMR (500 MHz, $CDCl_3$) of compound **41**



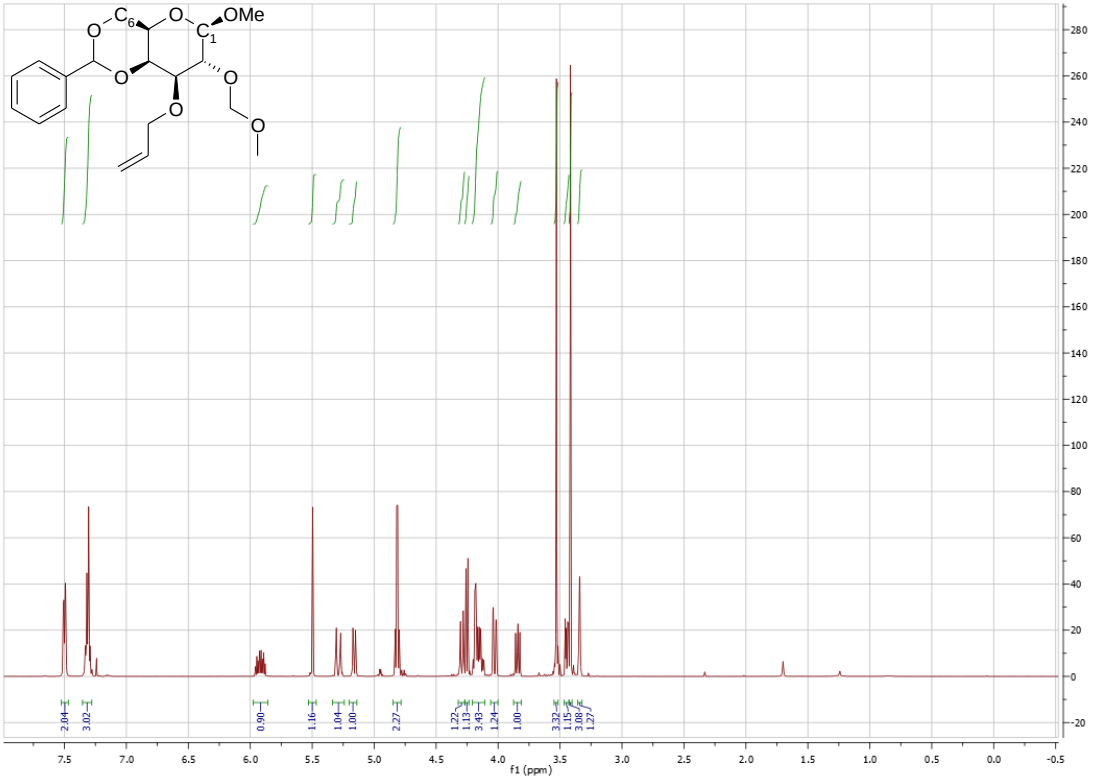
^{1}H-NMR (500 MHz, $CDCl_3$) of compound **42**



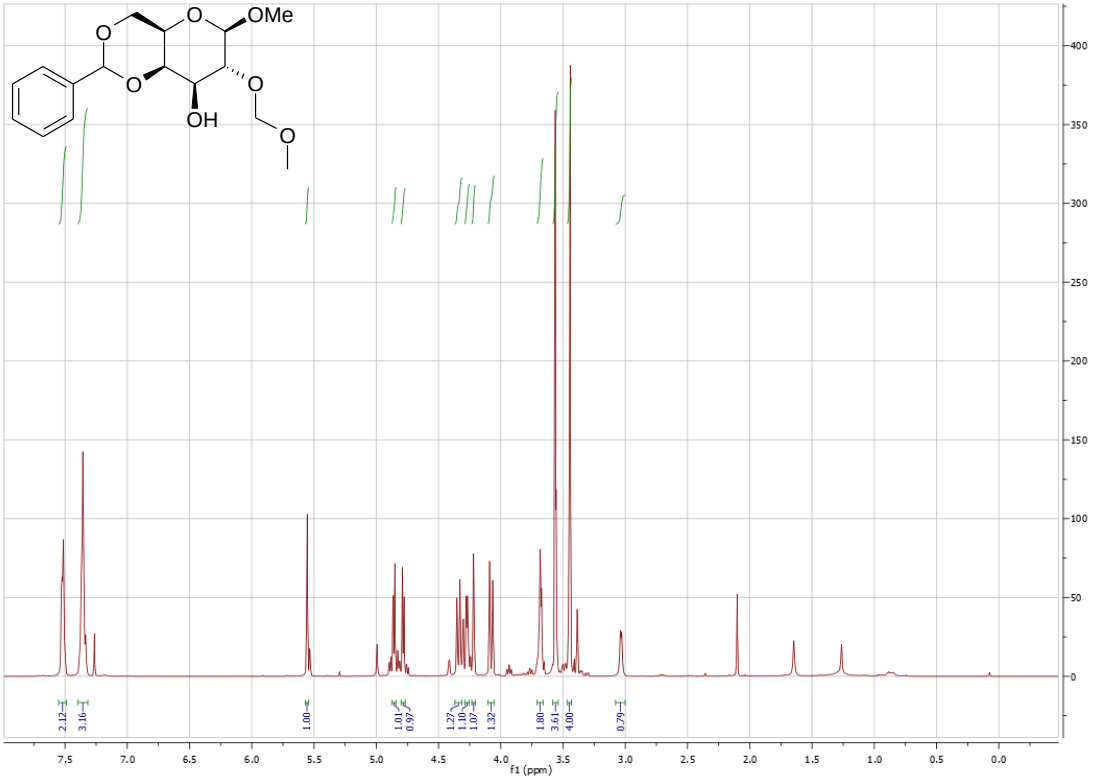
^{1}H-NMR (500 MHz, CD_3OD) of compound **44**



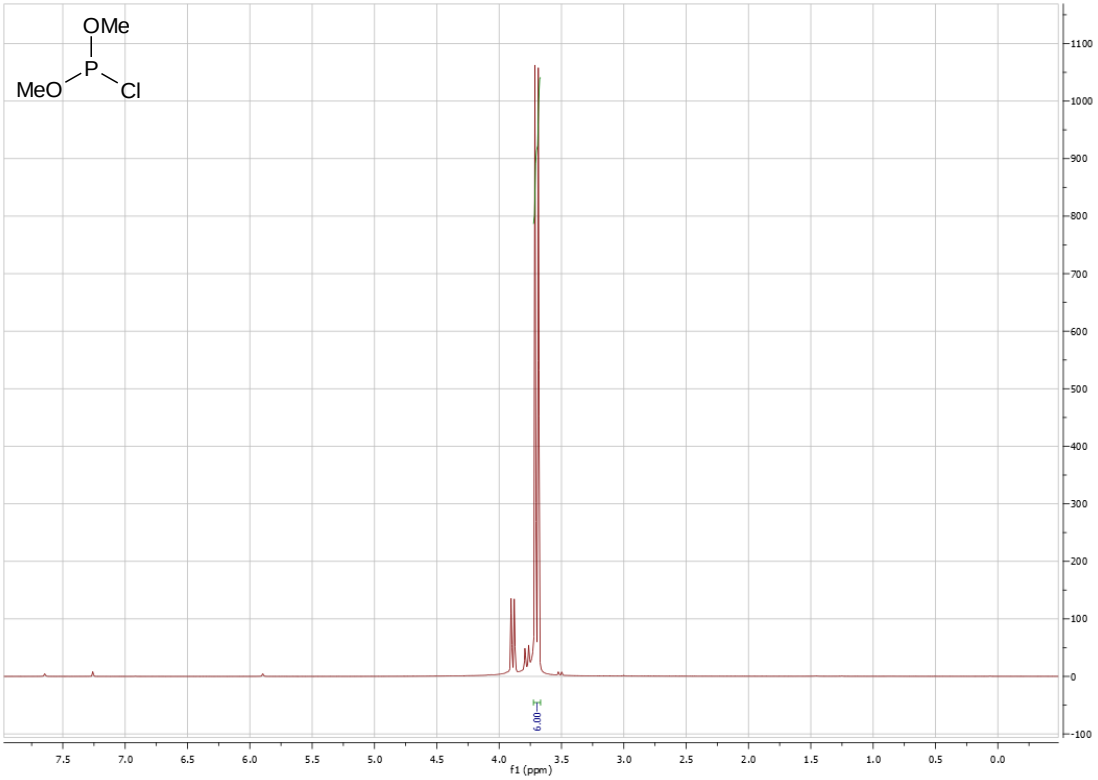
^{1}H-NMR (600 MHz, D_2O) of compound **46**



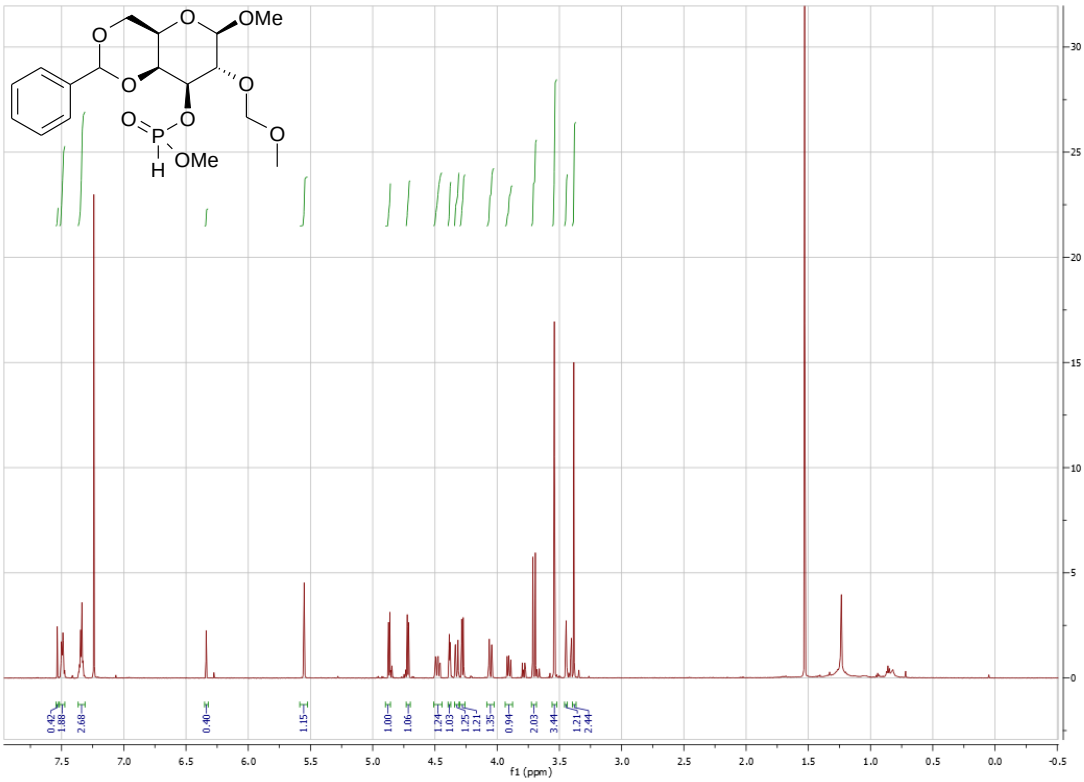
^{1}H-NMR (500 MHz, $CDCl_3$) of compound **47**



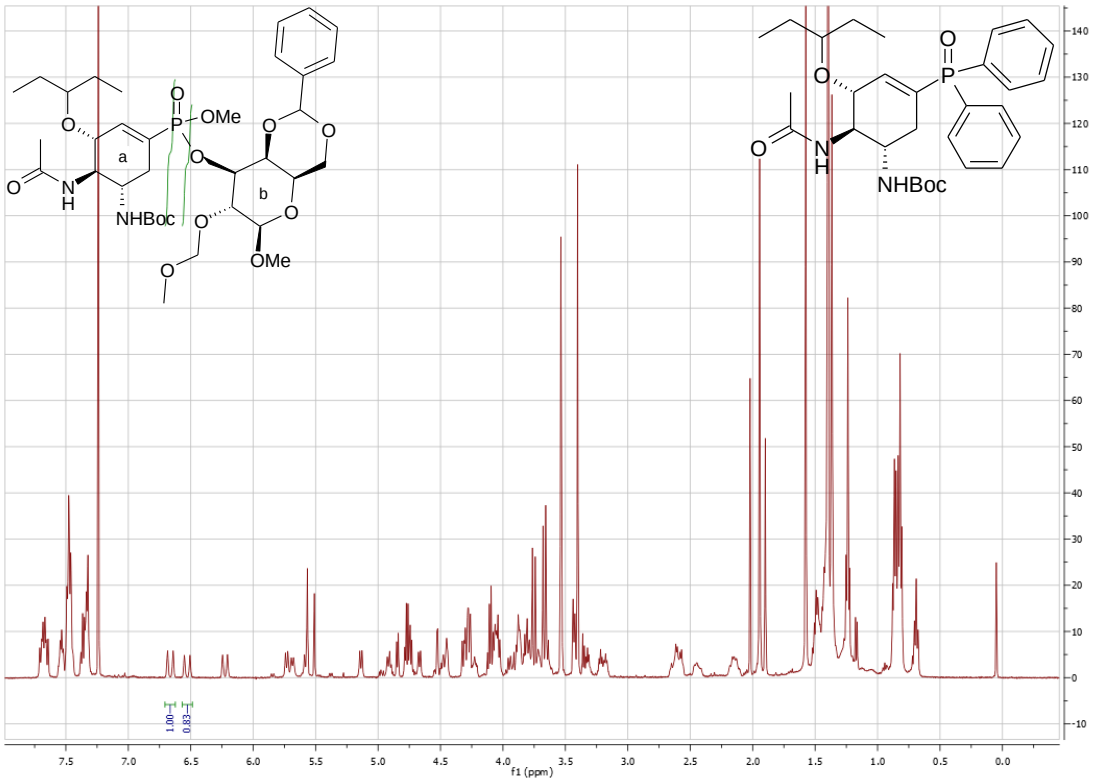
^{1}H-NMR (500 MHz, $CDCl_3$) of compound **48**



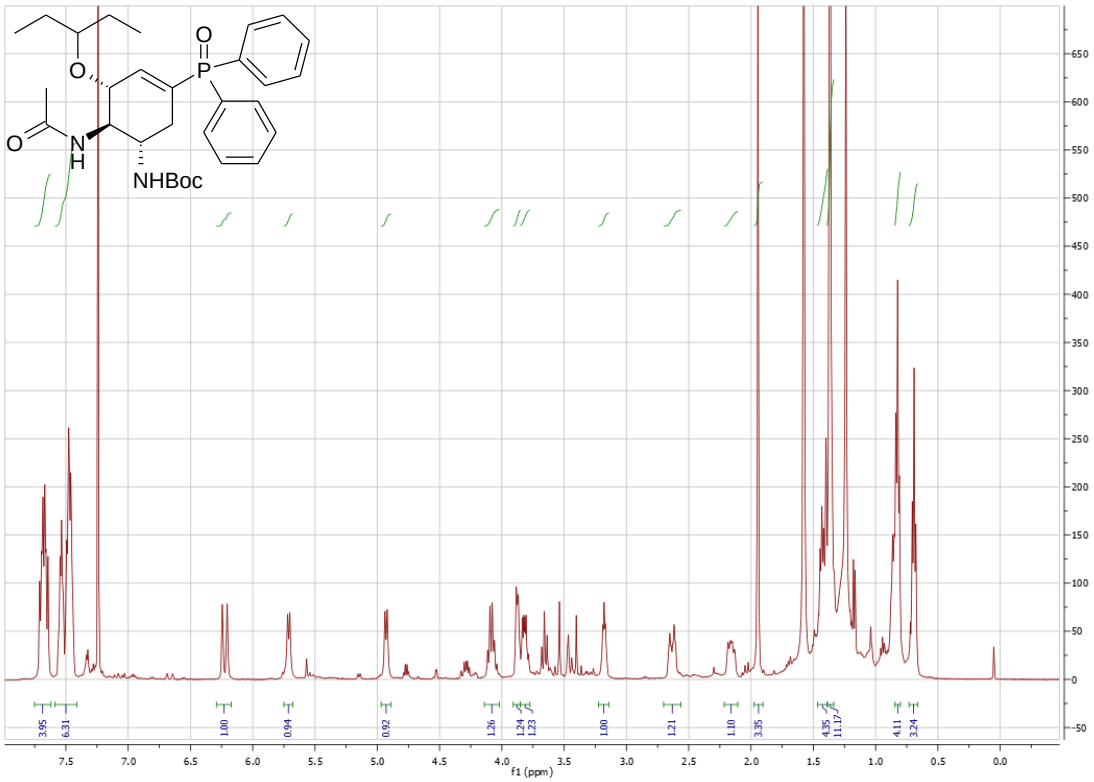
^{1}H-NMR (400 MHz, $CDCl_3$) of compound **49**



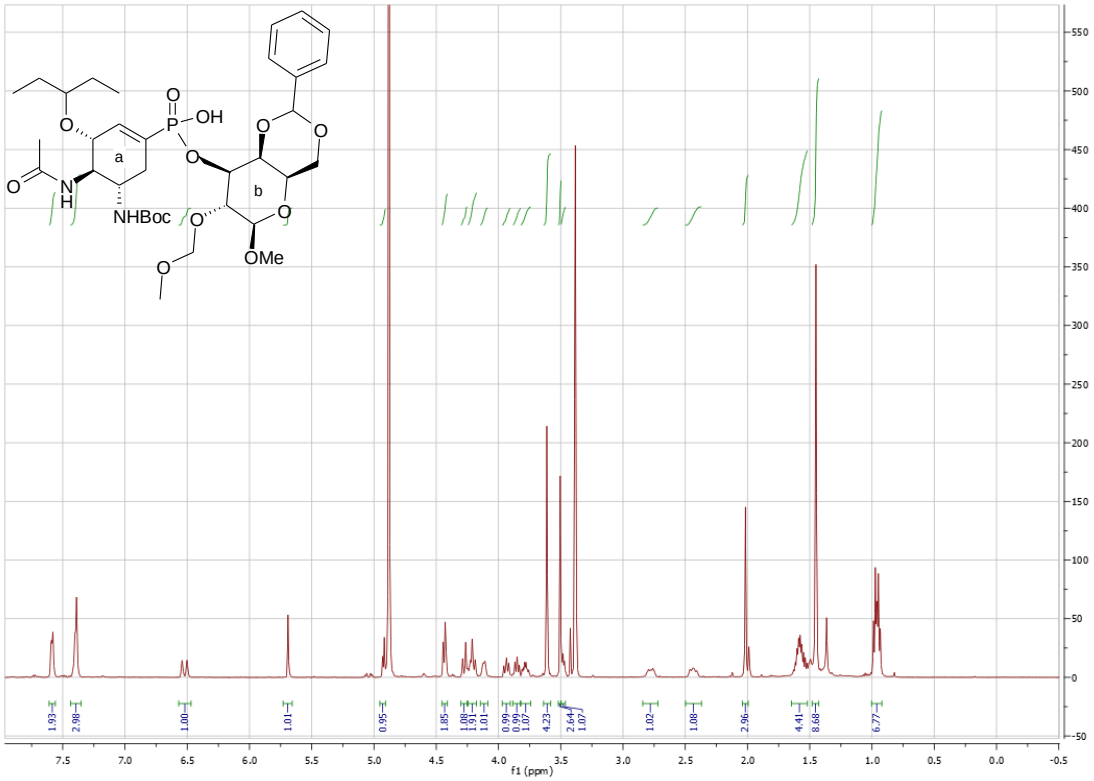
^{1}H-NMR (600 MHz, $CDCl_3$) of compound **50**



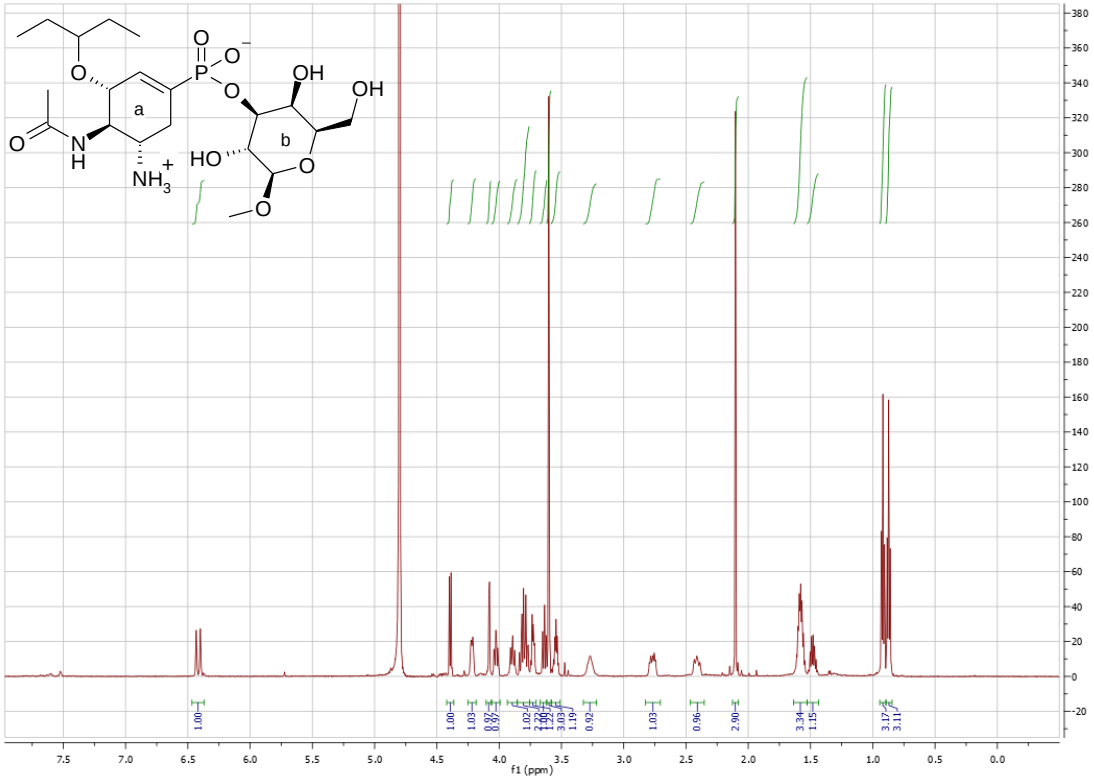
^{1}H-NMR (500 MHz, $CDCl_3$) of compound **51** + side product **52**



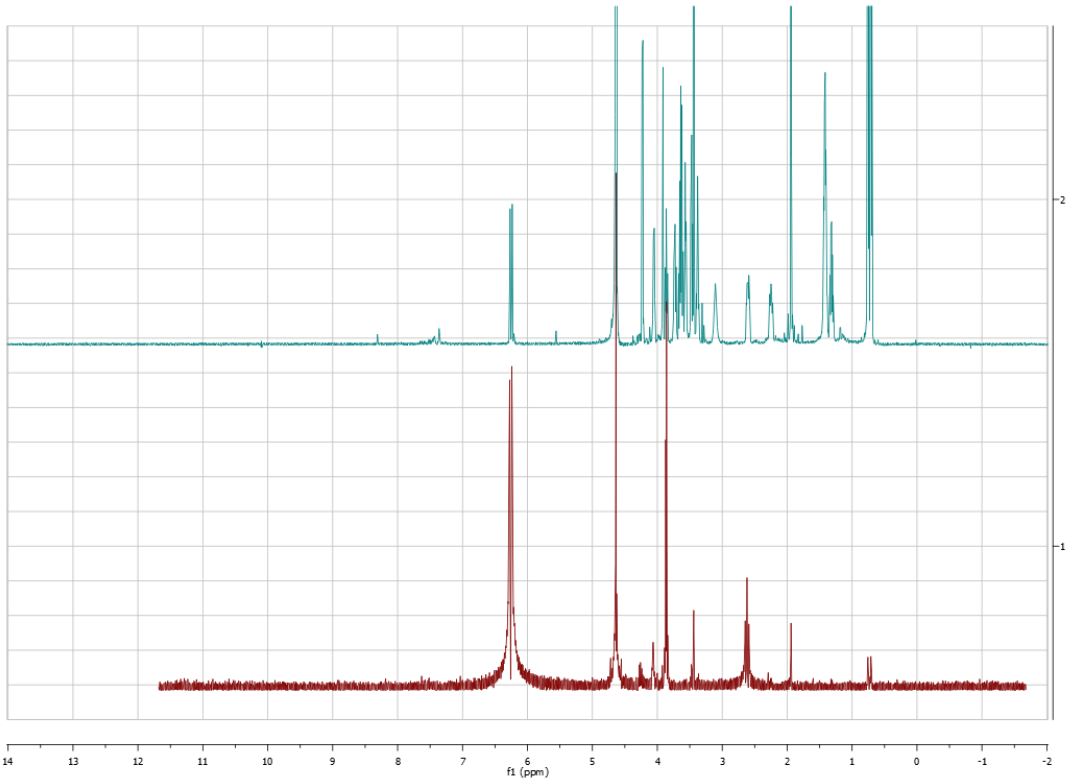
^{1}H-NMR (500 MHz, $CDCl_3$) of side product **52**



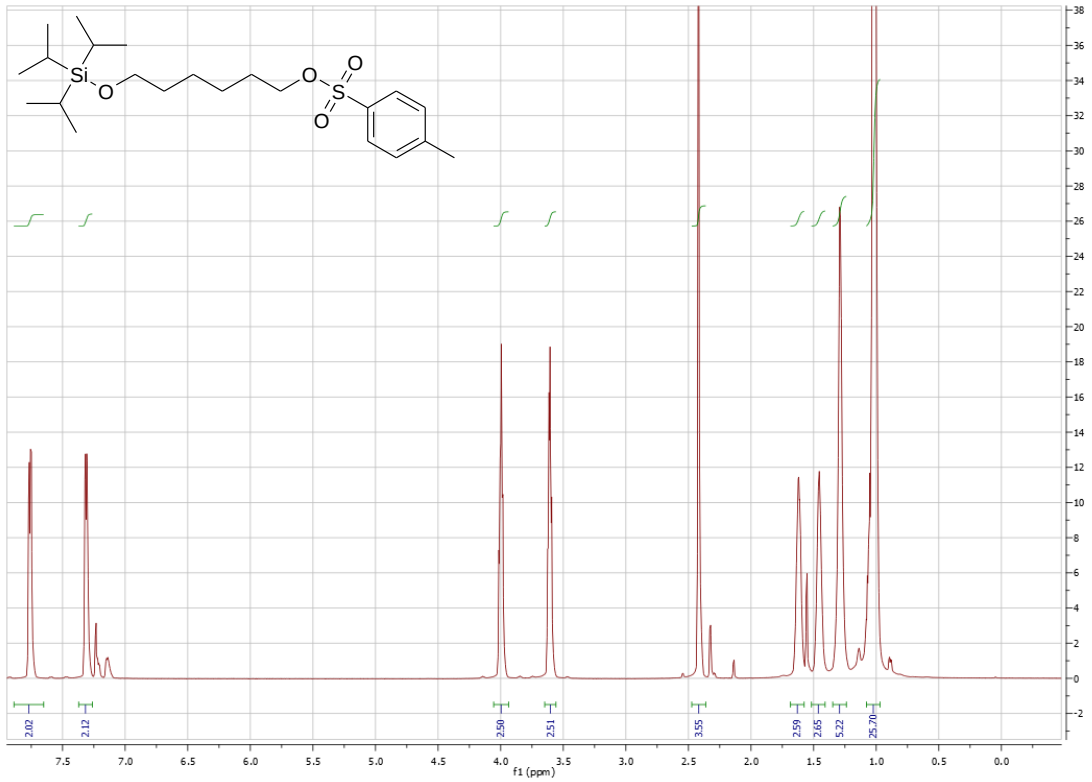
^{1}H-NMR (600 MHz, CD_3OD) of compound **53**



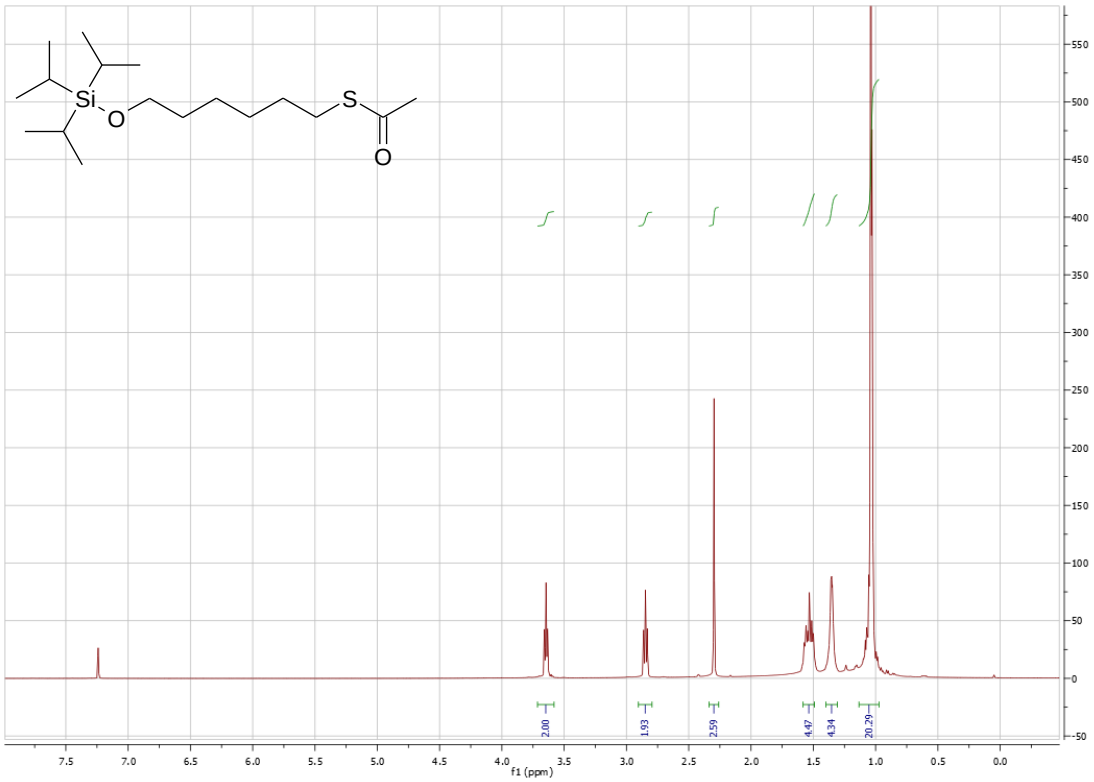
^{1}H-NMR (600 MHz, D_2O) of compound **54**



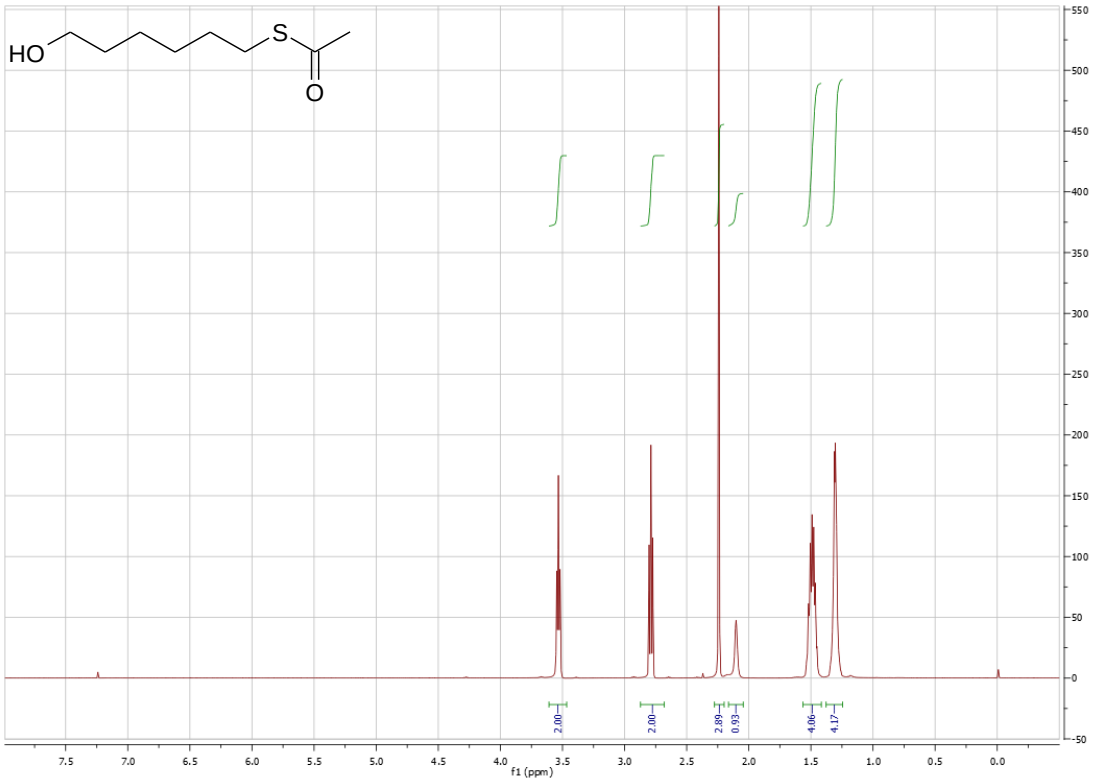
SED-NMR (600 MHz, D_2O) of compound **54**



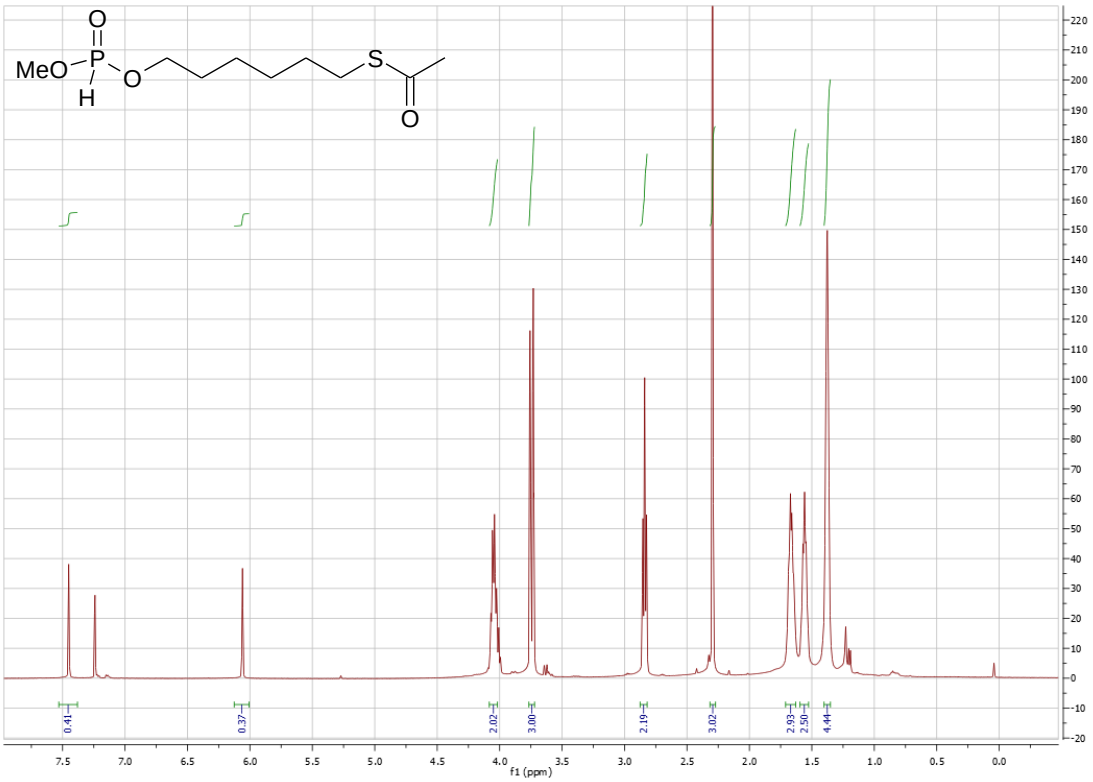
^{1}H-NMR (500 MHz, $CDCl_3$) of compound **55**



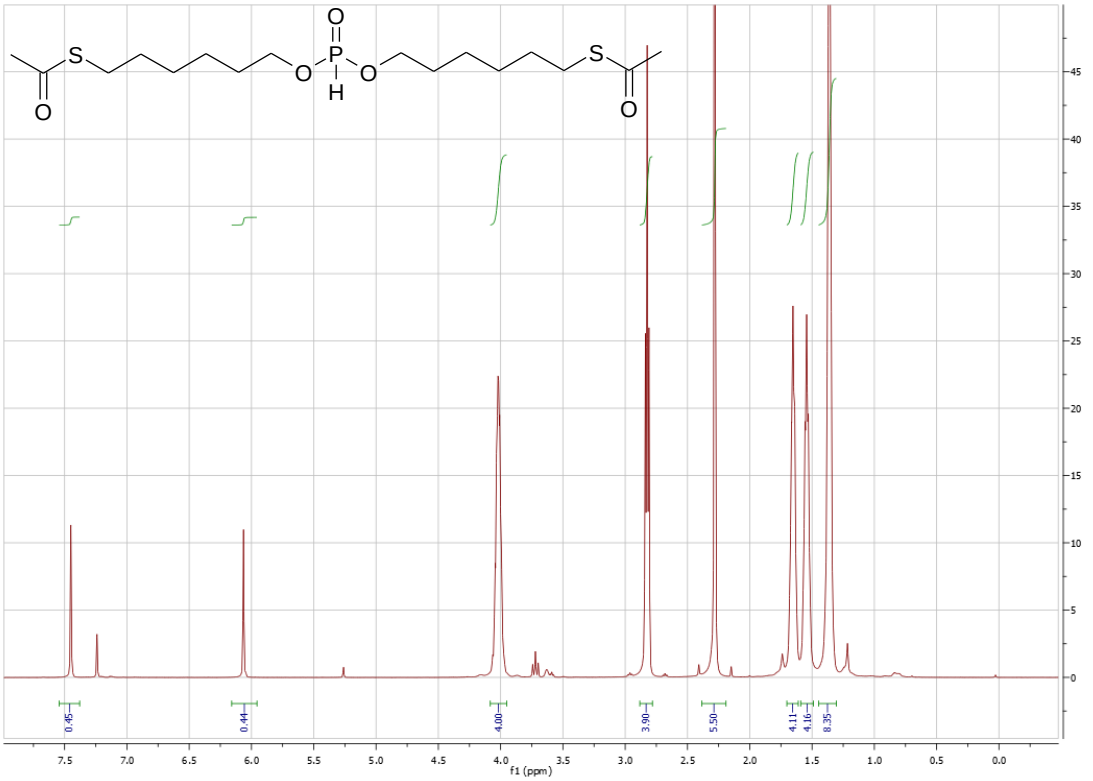
^{1}H-NMR (500 MHz, $CDCl_3$) of compound **56**



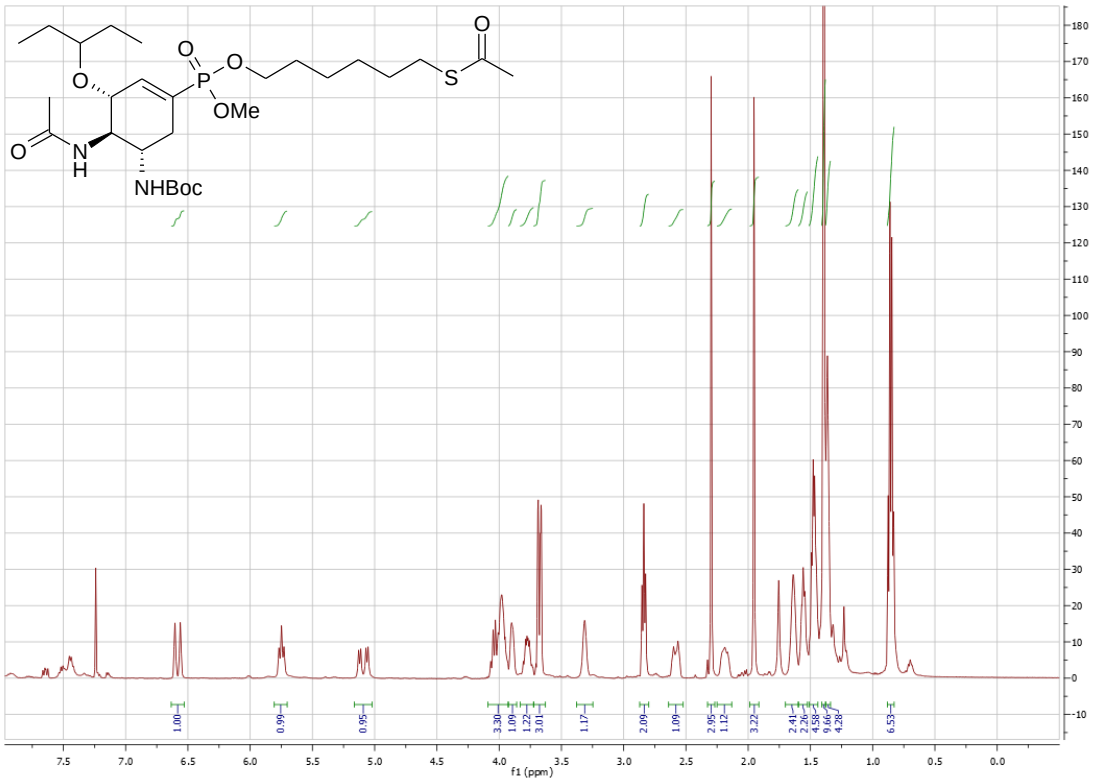
^{1}H-NMR (500 MHz, $CDCl_3$) of compound **57**



^{1}H-NMR (500 MHz, $CDCl_3$) of compound **58**

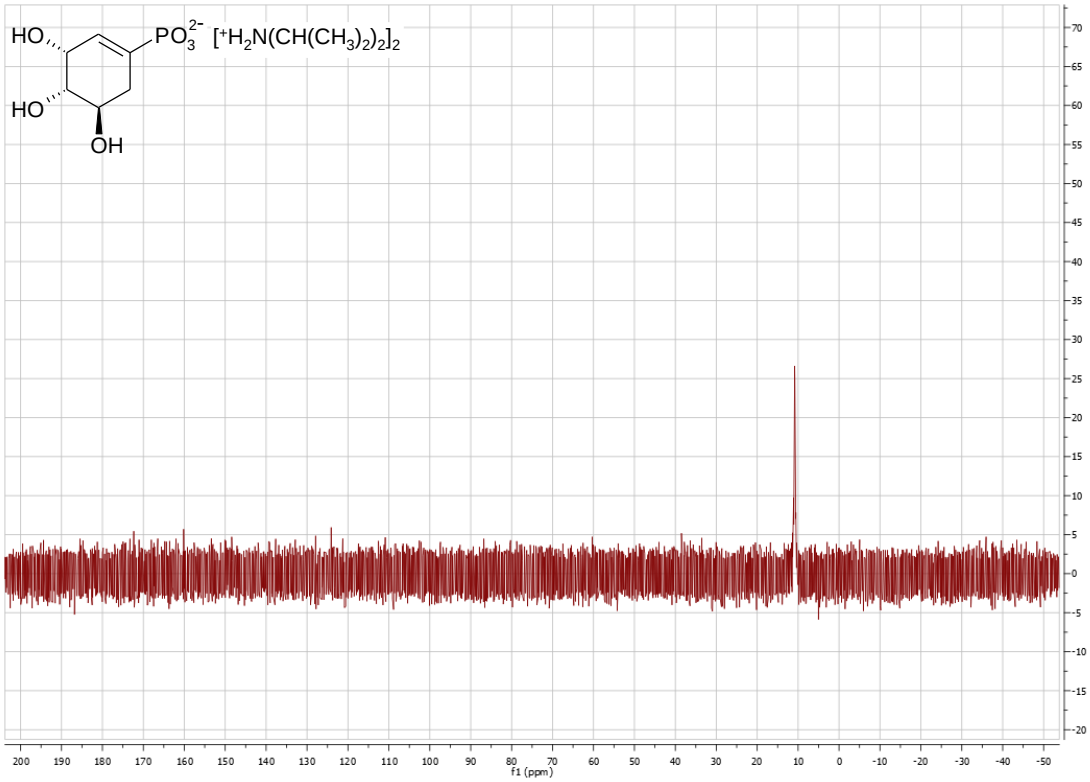


^{1}H-NMR (500 MHz, $CDCl_3$) of compound **59**

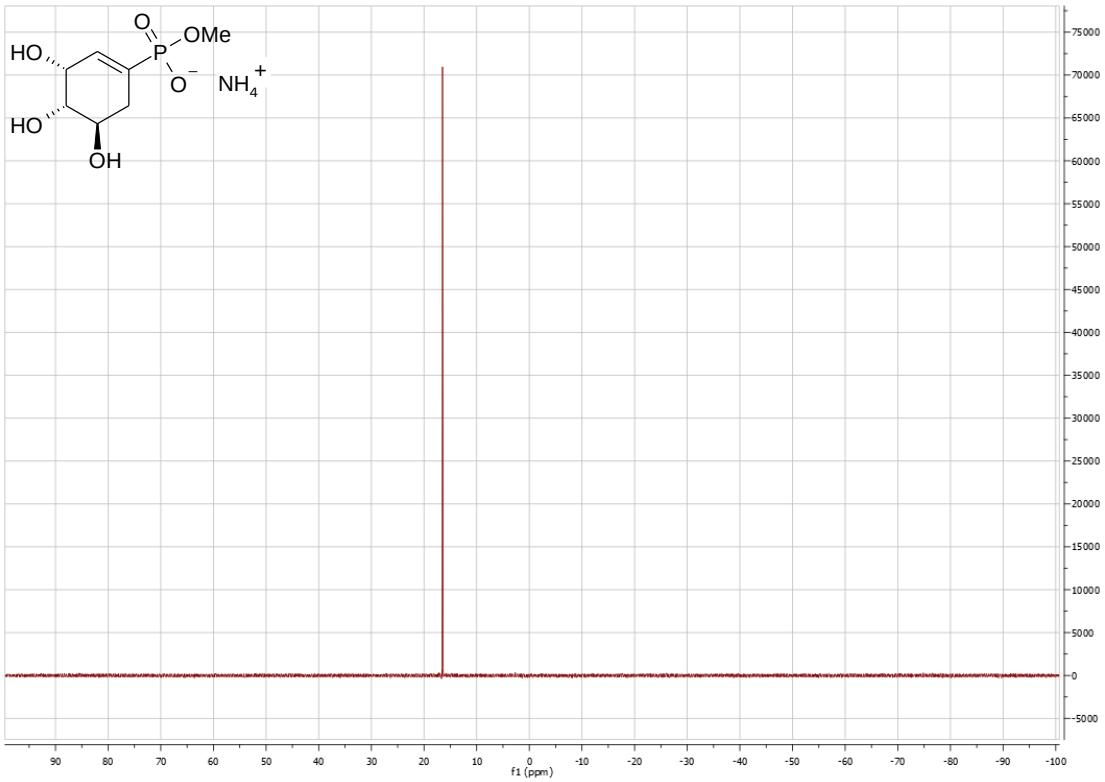


^{1}H-NMR (500 MHz, $CDCl_3$) of compound 60

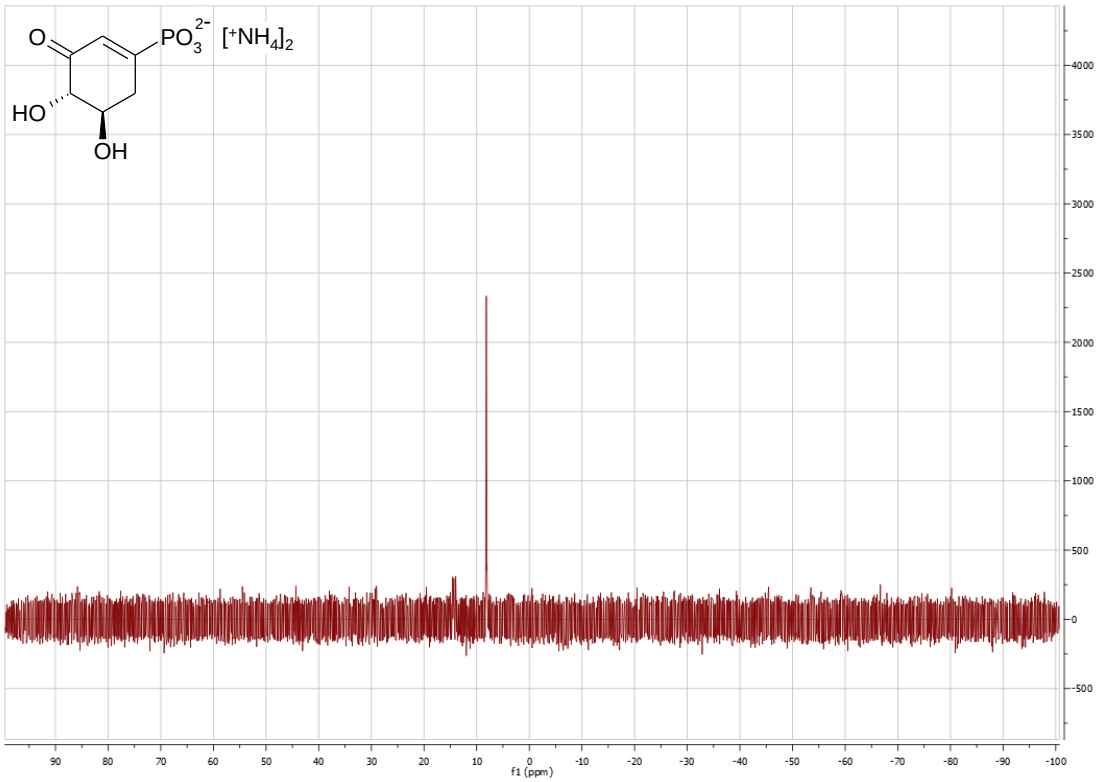
Appendix 3 : ^{31}P NMR Spectra



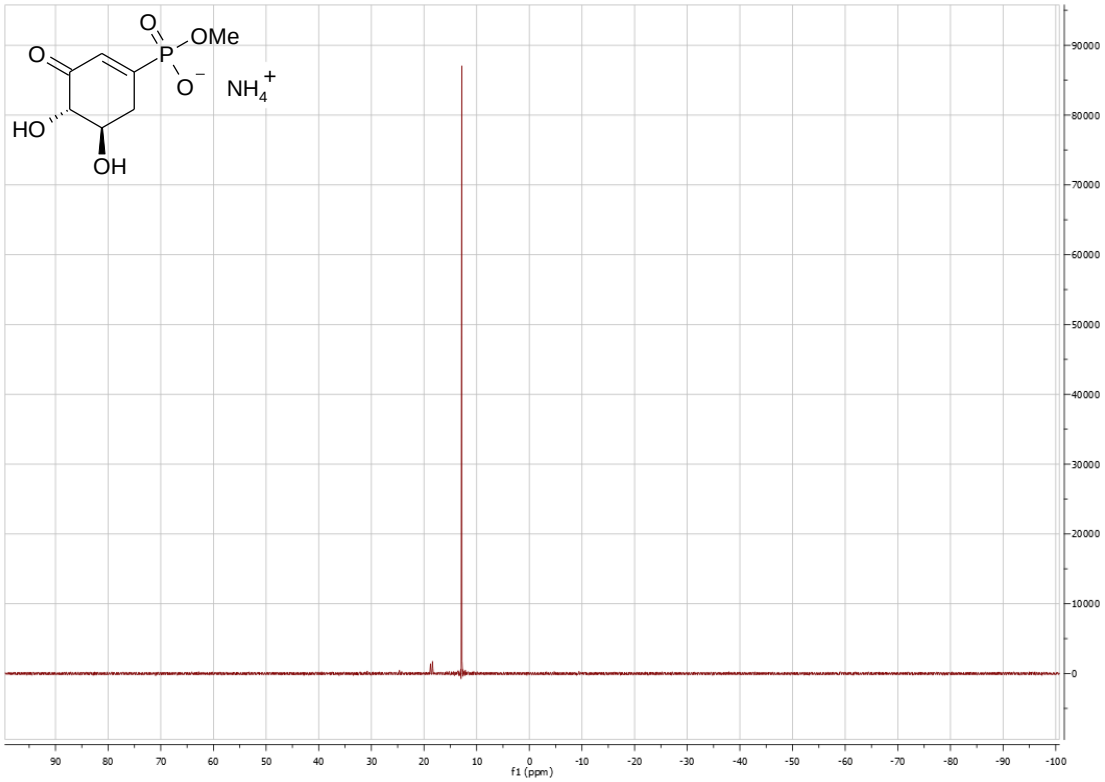
^{31}P-NMR (161.9 MHz, D_2O) of compound **1**



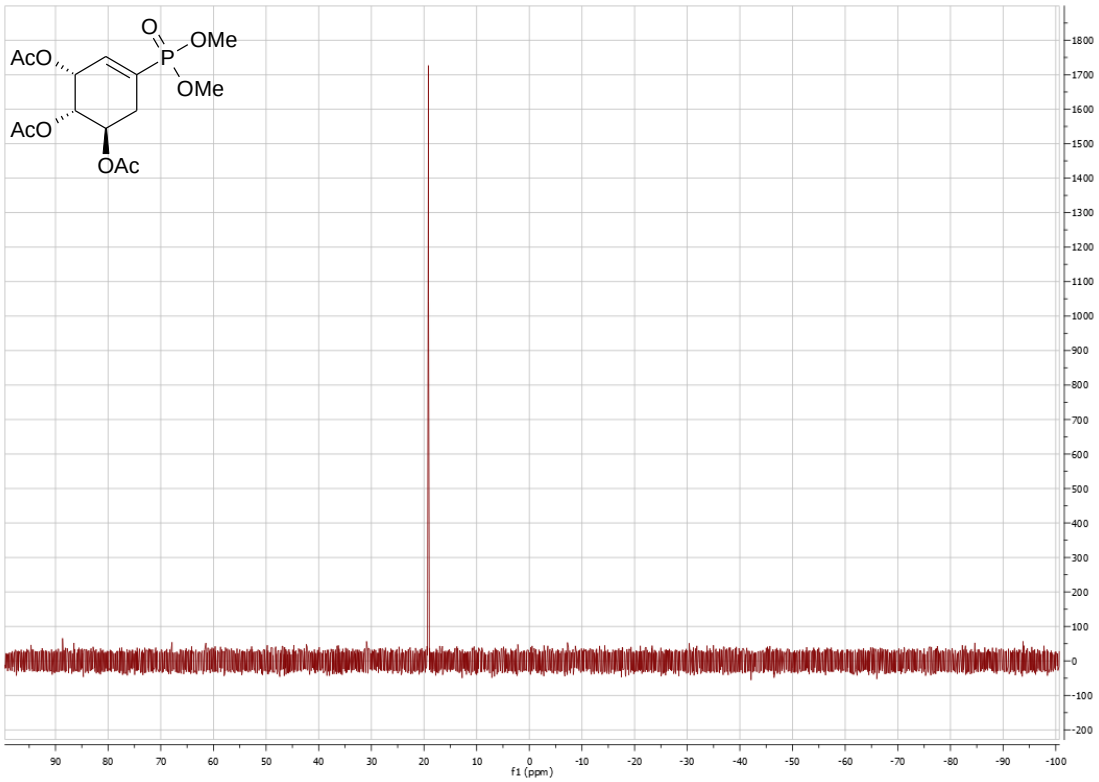
^{31}P-NMR (121.4 MHz, D_2O) of compound **2**



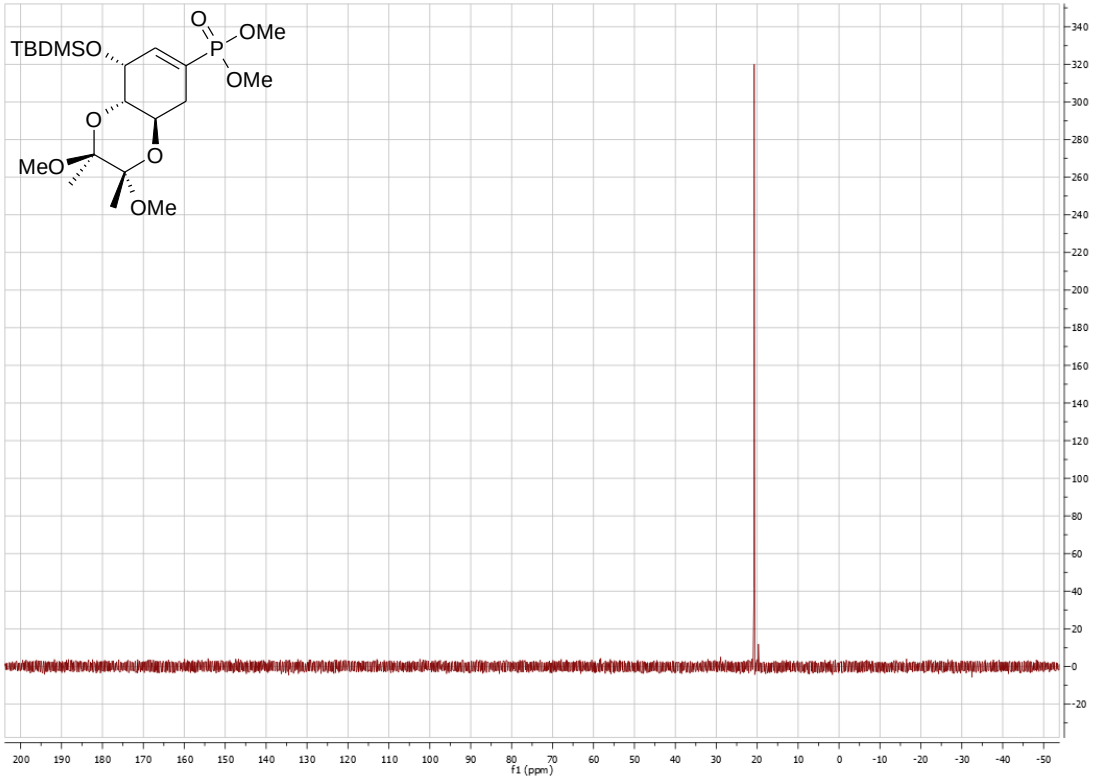
^{31}P-NMR (121.4 MHz, D_2O) of compound **3**



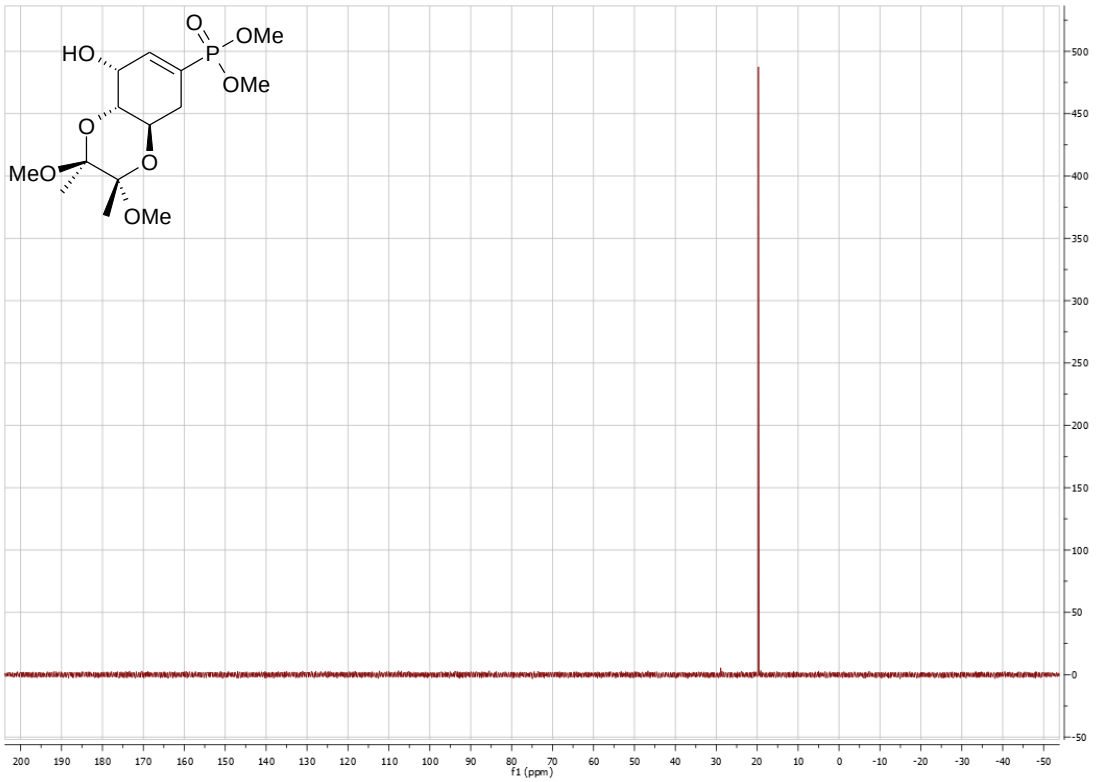
^{31}P-NMR (121.4 MHz, D_2O) of compound **4**



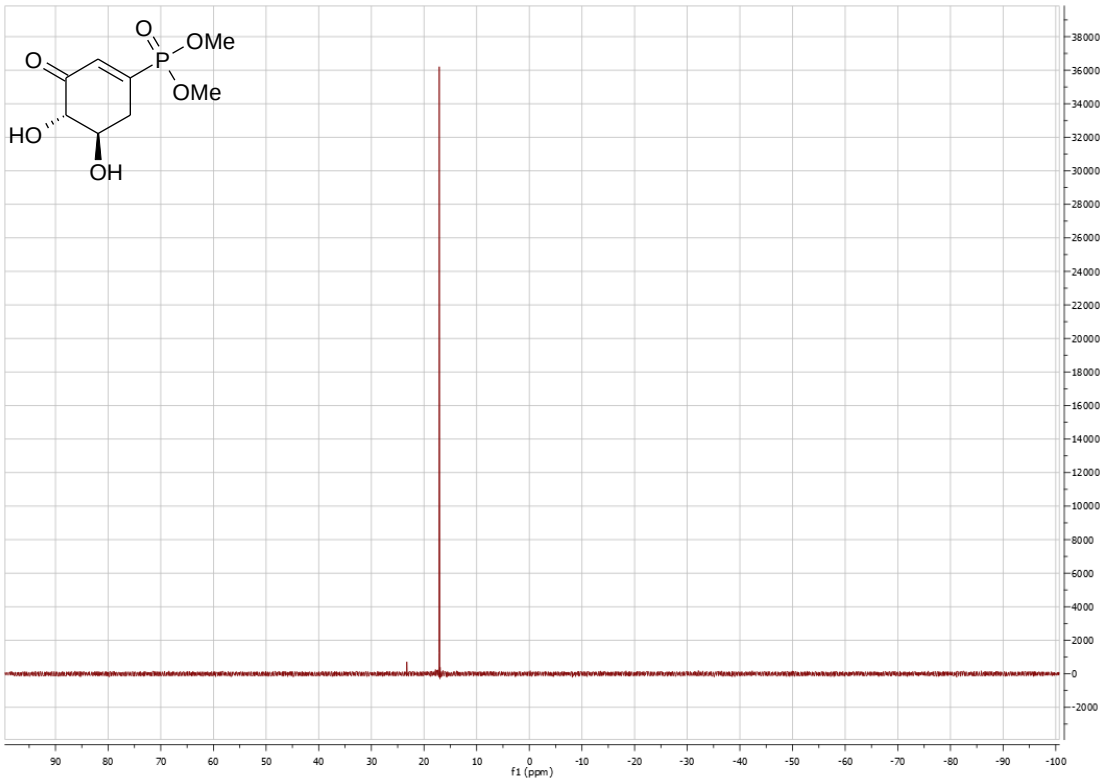
^{31}P-NMR (121.4 MHz, $CDCl_3$) of compound **9**



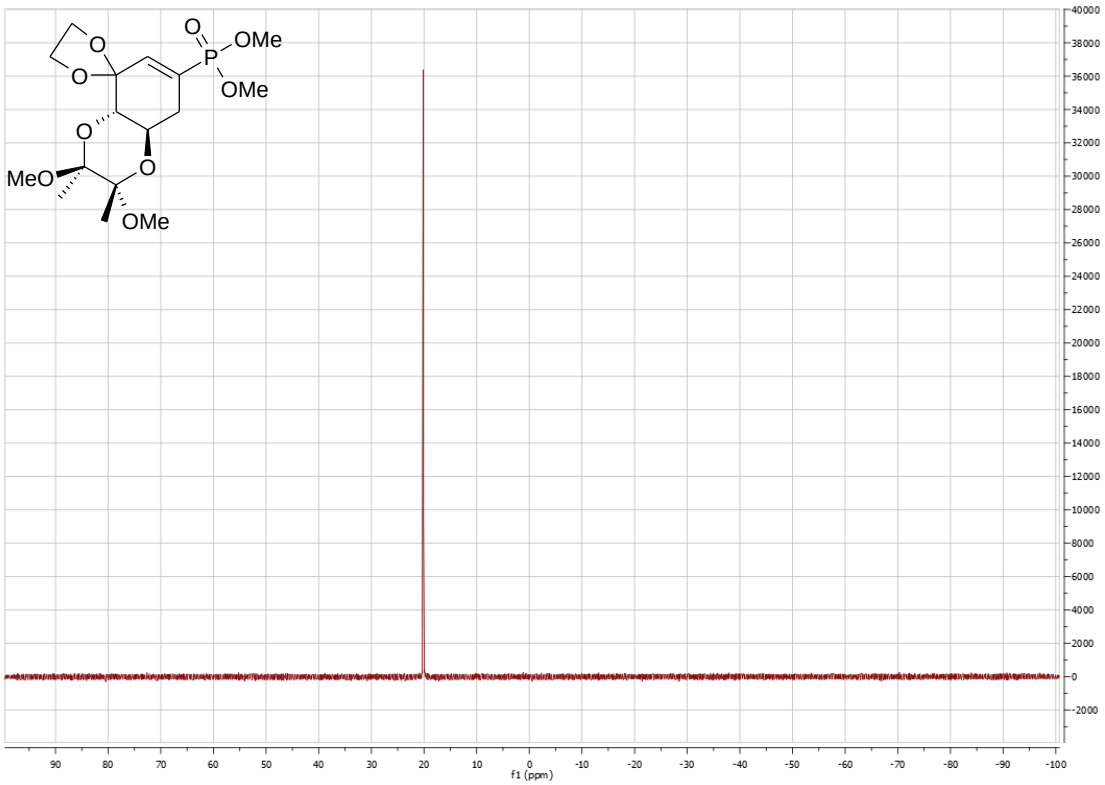
^{31}P-NMR (161.9 MHz, $CDCl_3$) of compound **15**



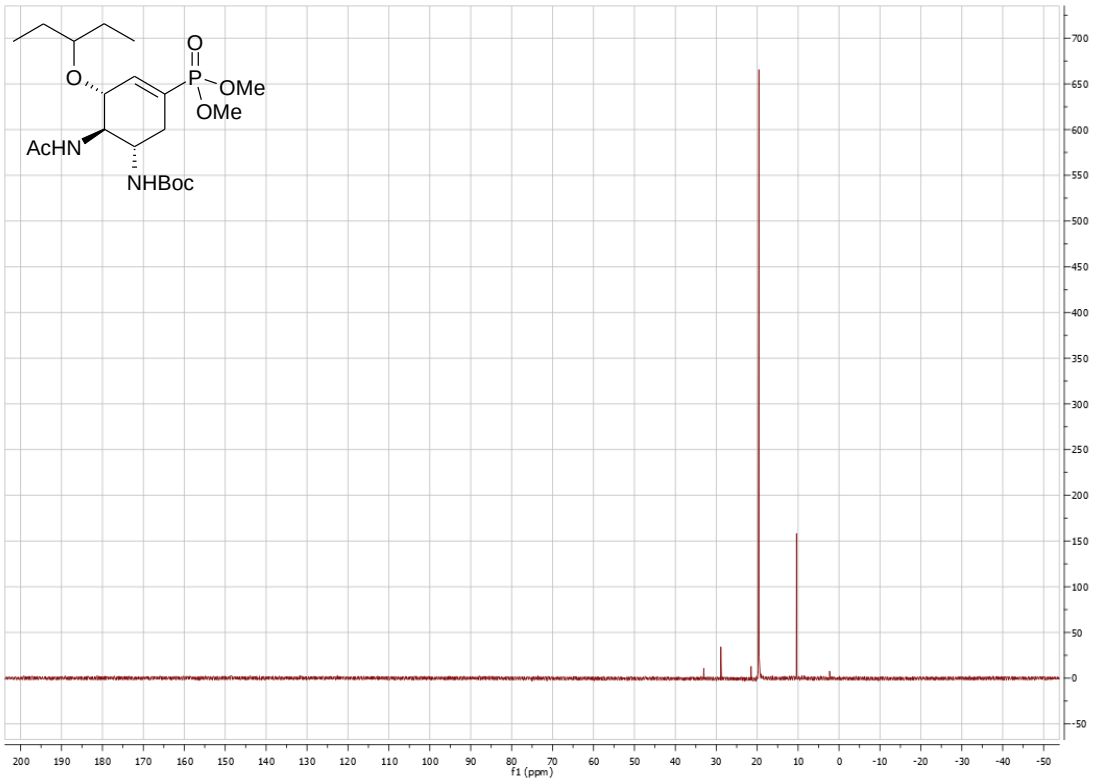
^{31}P-NMR (161.9 MHz, $CDCl_3$) of compound **16**



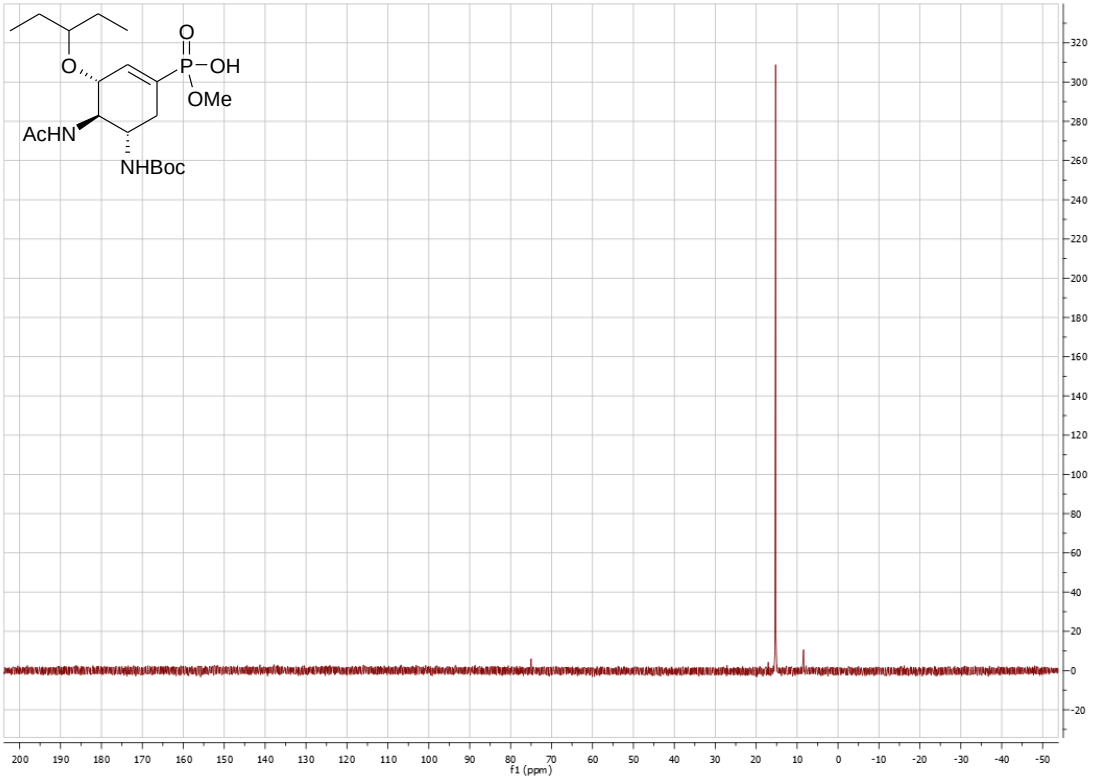
^{31}P-NMR (121.4 MHz, $CDCl_3$) of compound **18**



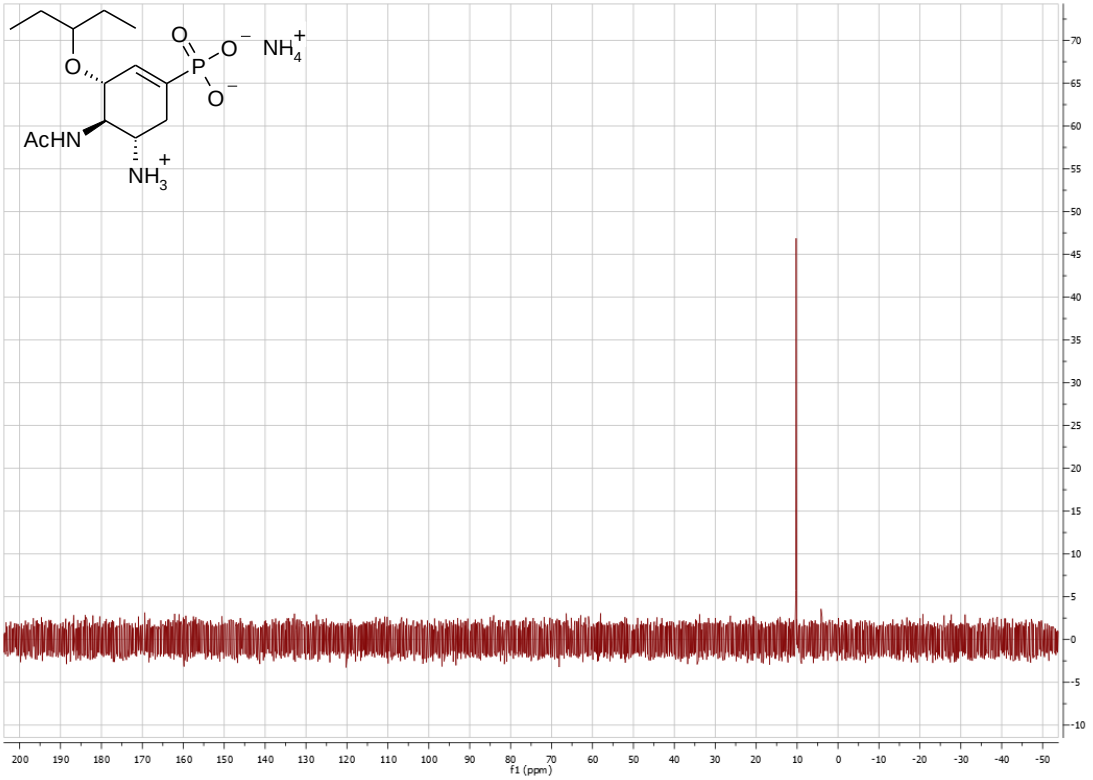
^{31}P-NMR (121.4 MHz, $CDCl_3$) of compound **23**



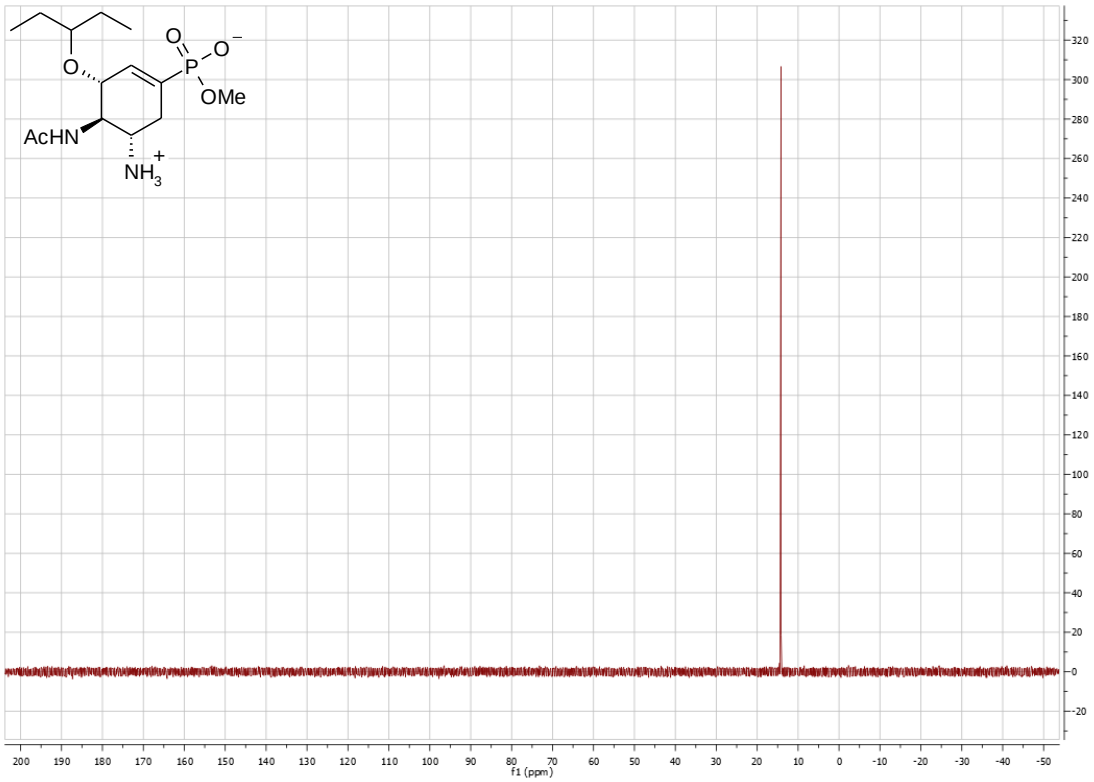
^{31}P-NMR (161.9 MHz, $CDCl_3$) of compound **33**



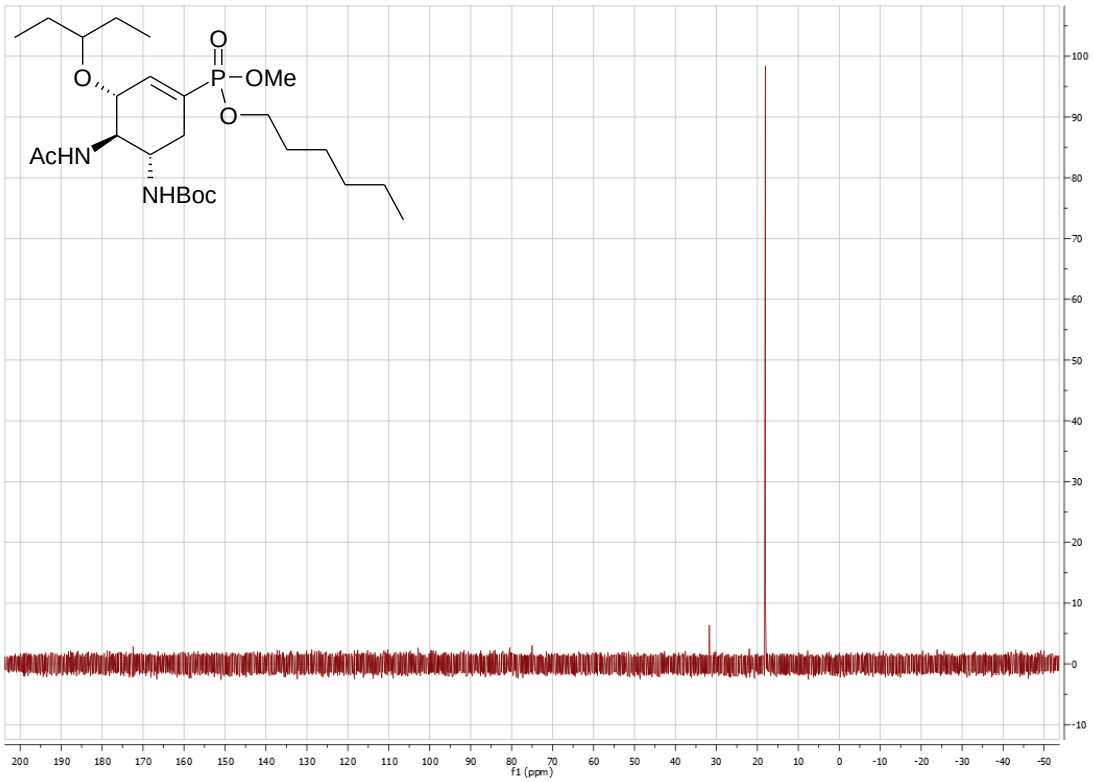
^{31}P-NMR (161.9 MHz, D_2O) of compound **34**



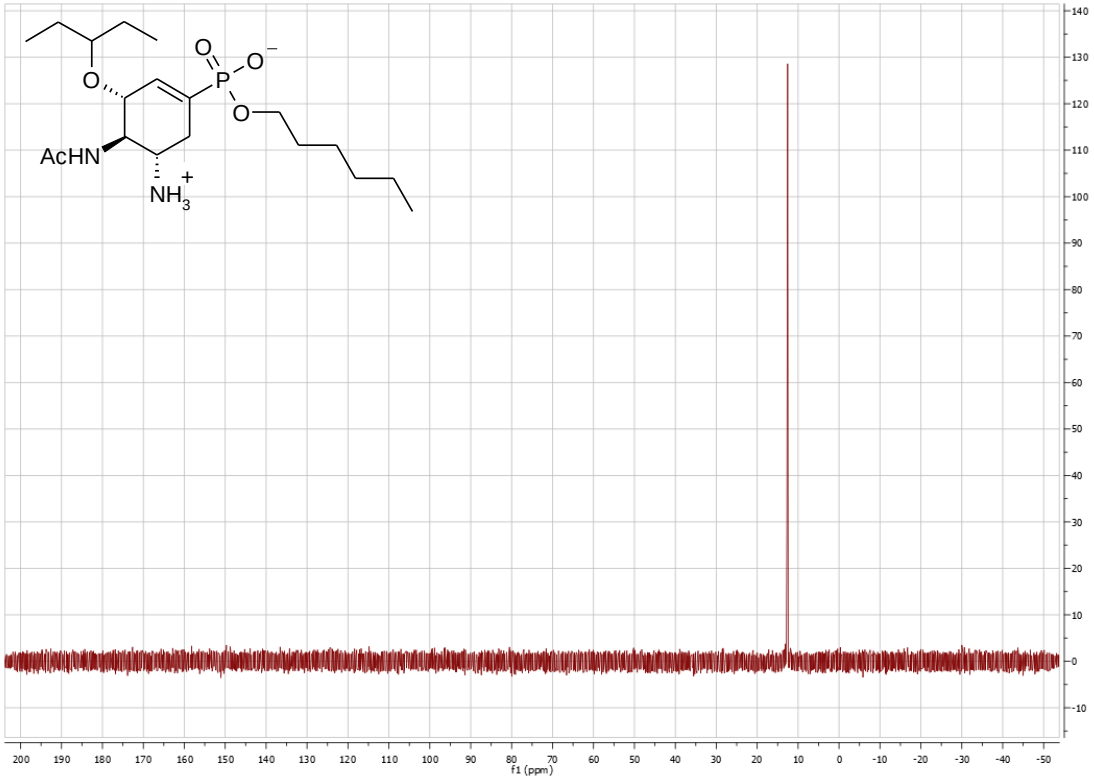
^{31}P-NMR (161.9 MHz, D_2O) of compound **35**



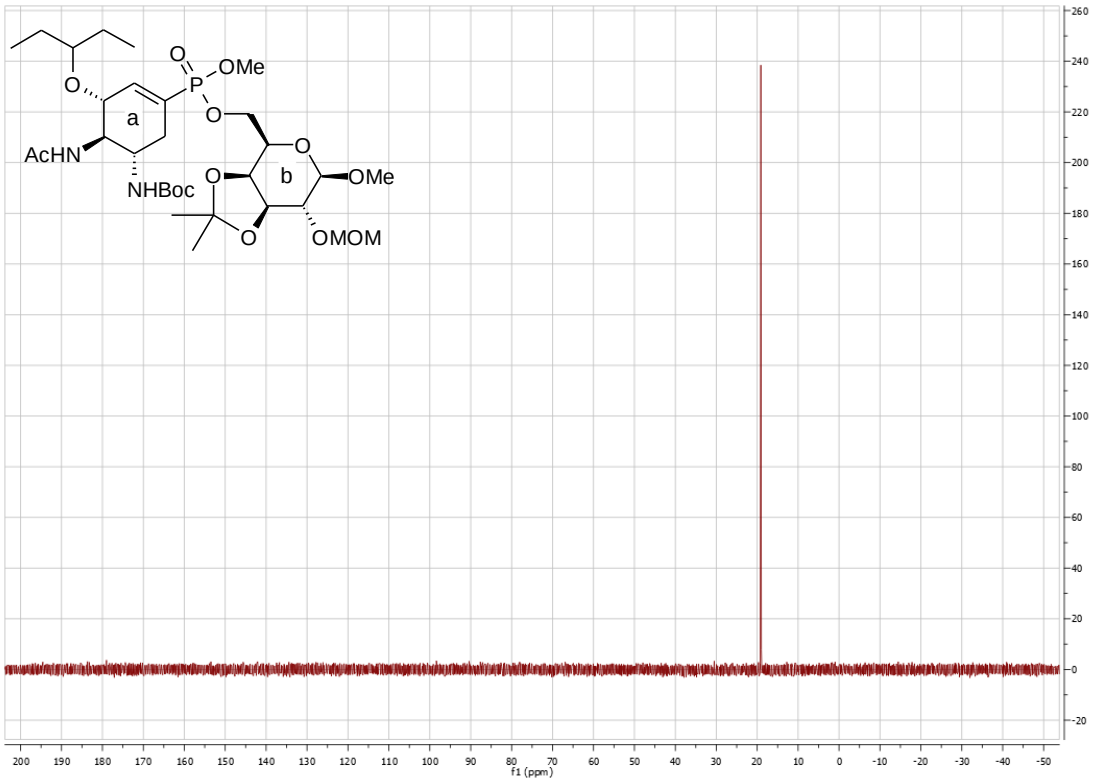
^{31}P-NMR (161.9 MHz, D_2O) of compound **36**



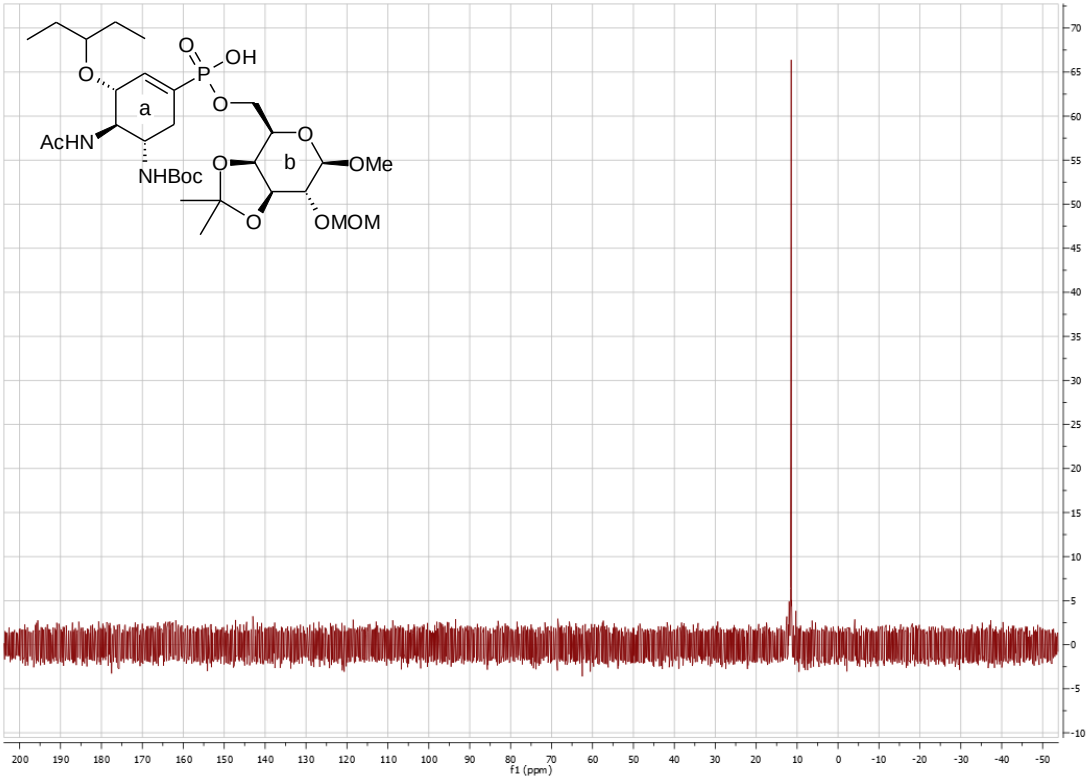
^{31}P-NMR (242.9 MHz, $CDCl_3$) of compound **38**



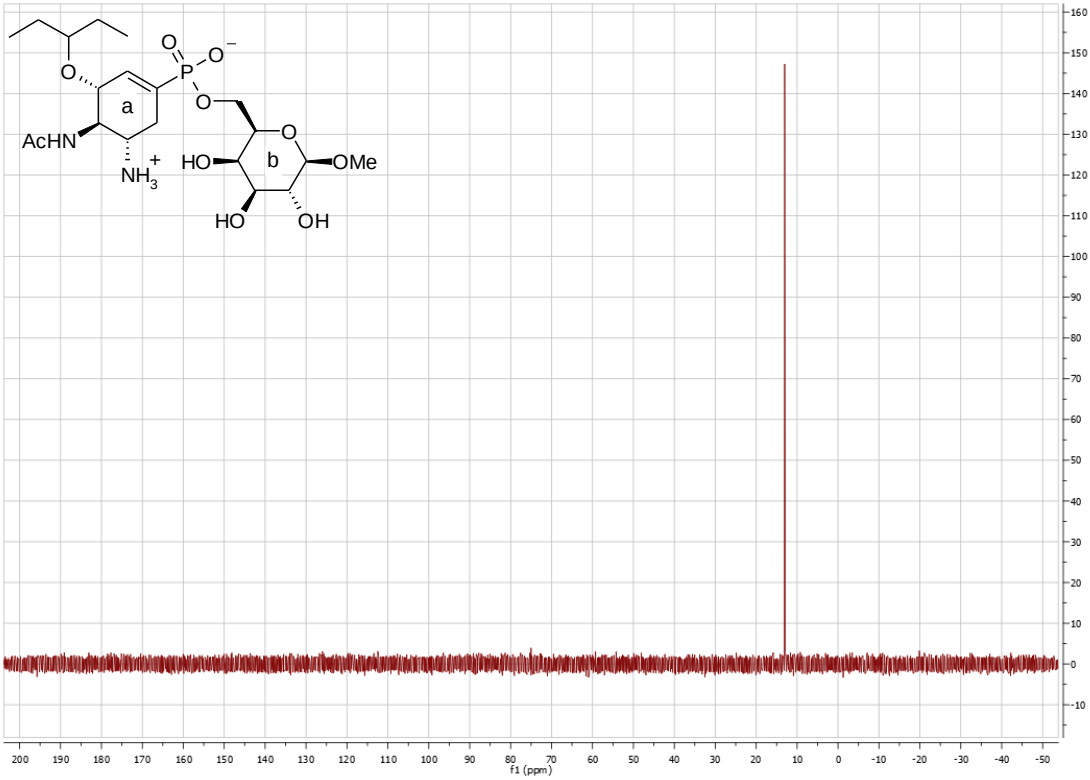
^{31}P-NMR (161.9 MHz, D_2O) of compound **40**



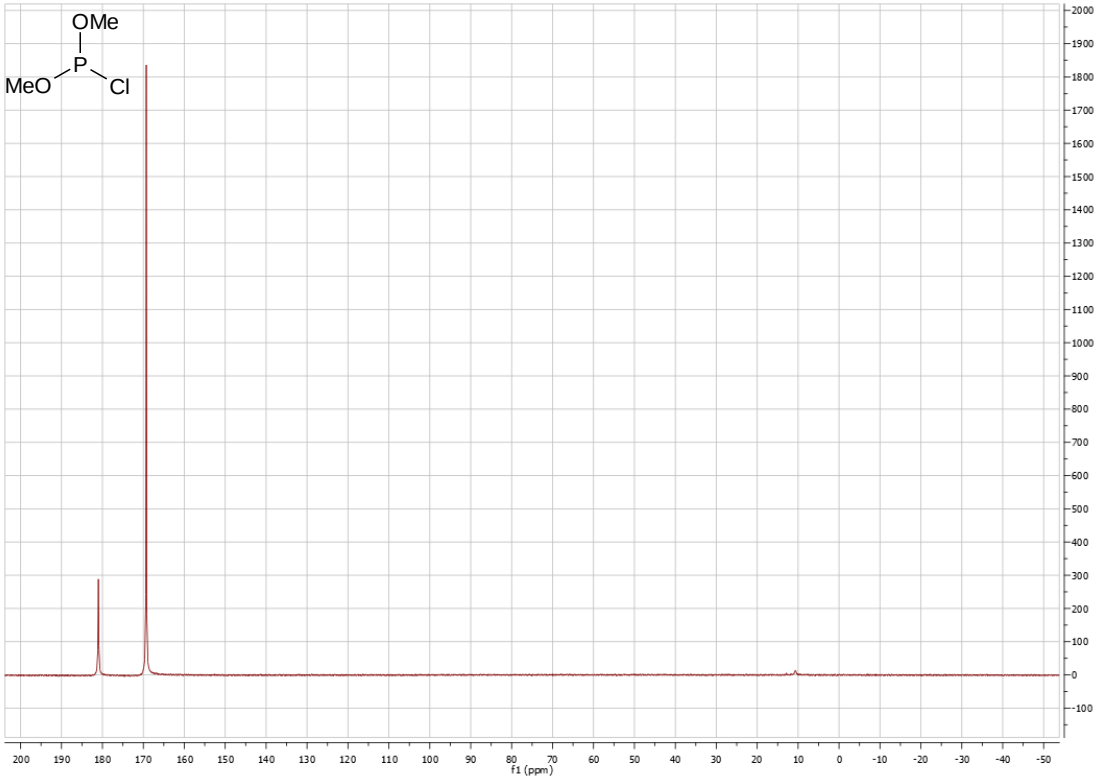
^{31}P-NMR (161.9 MHz, CD_3OD) of compound **44**



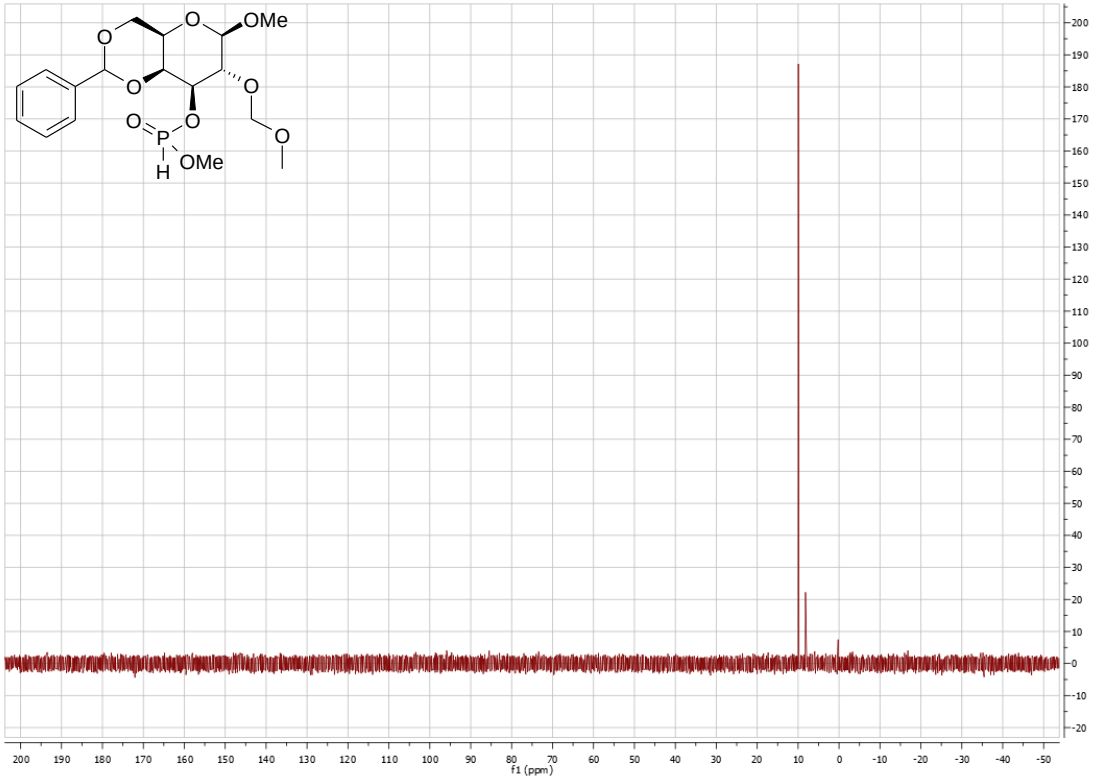
^{31}P-NMR (161.9 MHz, CD_3OD) of compound **45**



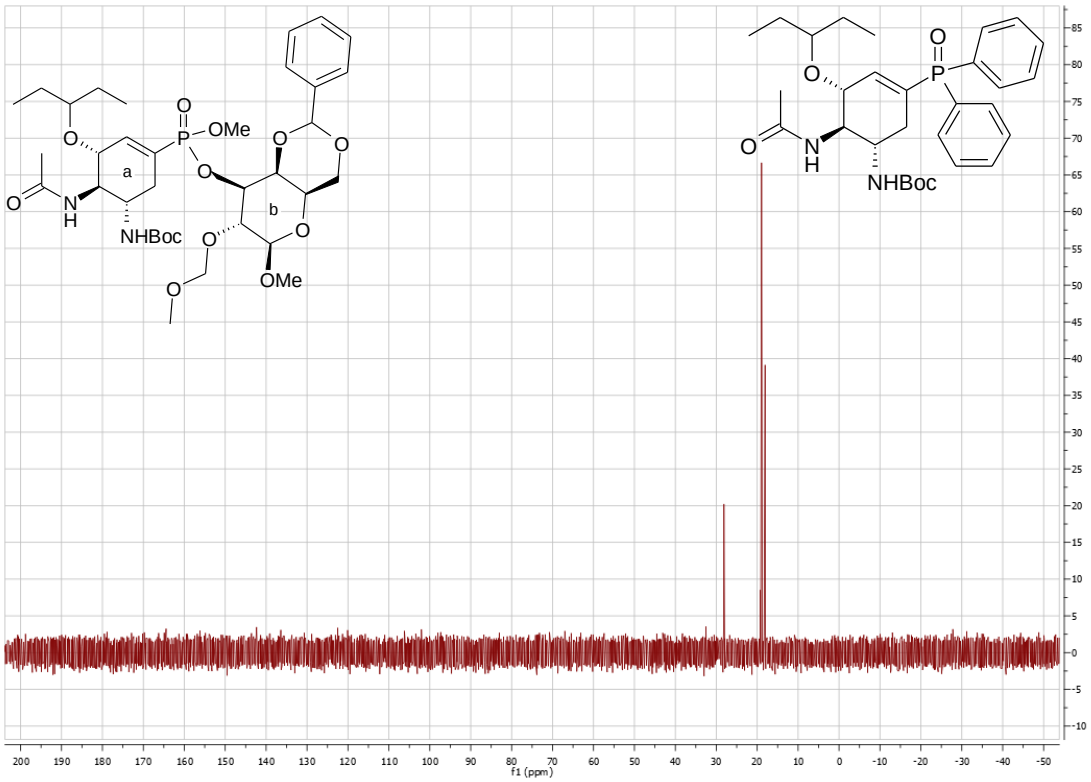
^{31}P-NMR (161.9 MHz, D_2O) of compound **46**



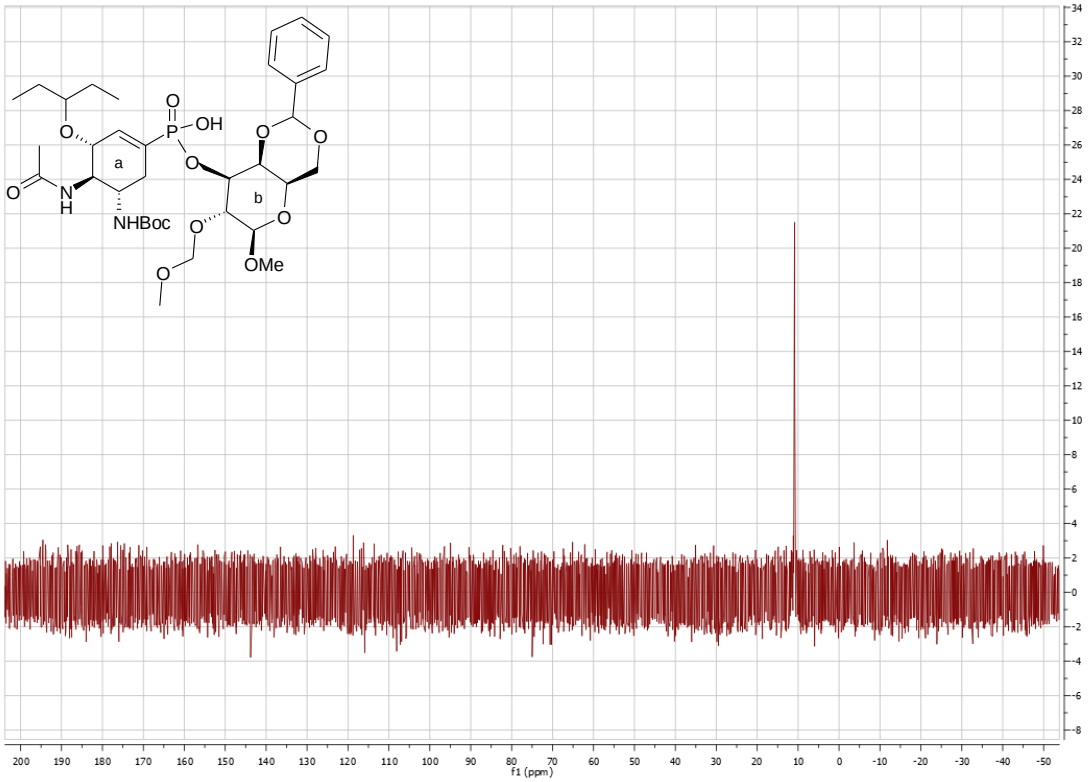
^{31}P-NMR (161.9 MHz, $CDCl_3$) of compound **49**



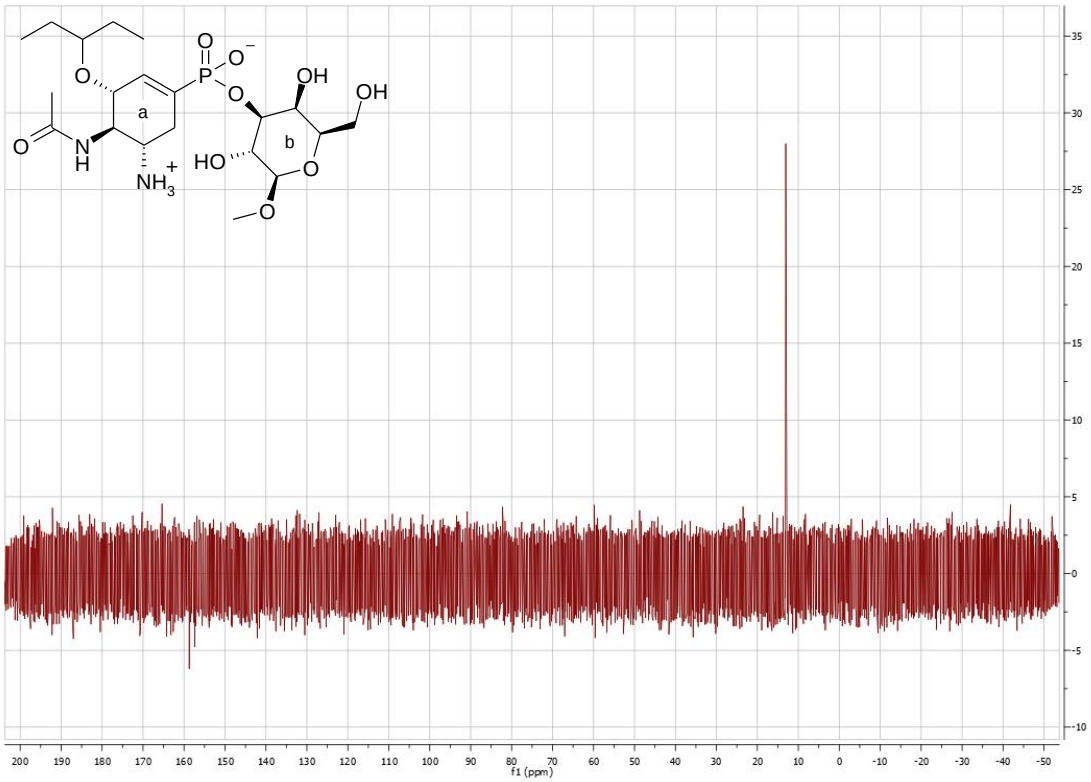
^{31}P-NMR (161.9 MHz, $CDCl_3$) of compound **50**



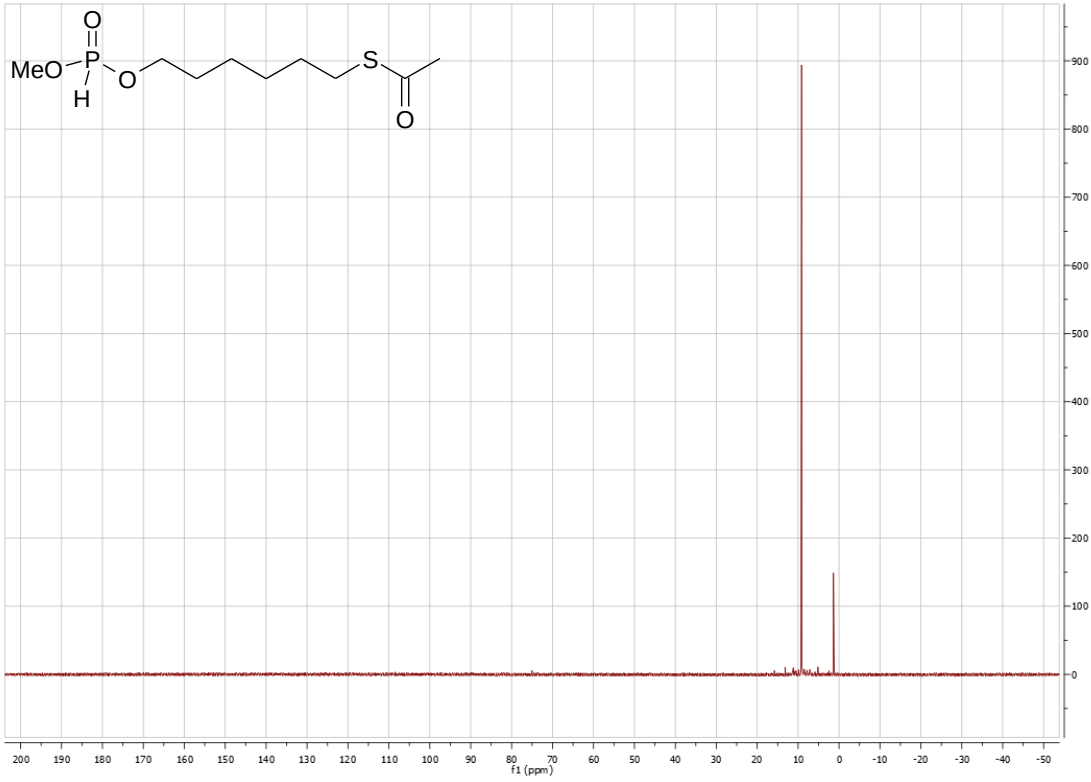
^{31}P-NMR (161.9 MHz, $CDCl_3$) of compound **51** + side product **52**



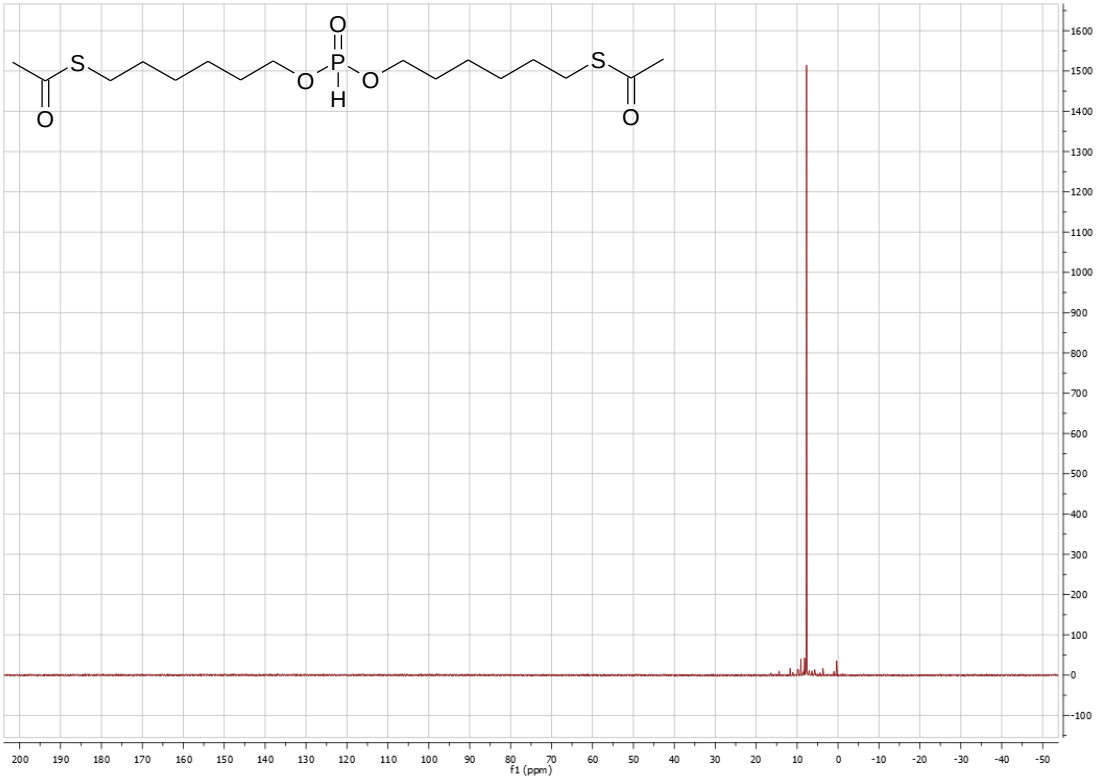
^{31}P-NMR (161.9 MHz, CD_3OD) of compound **53**



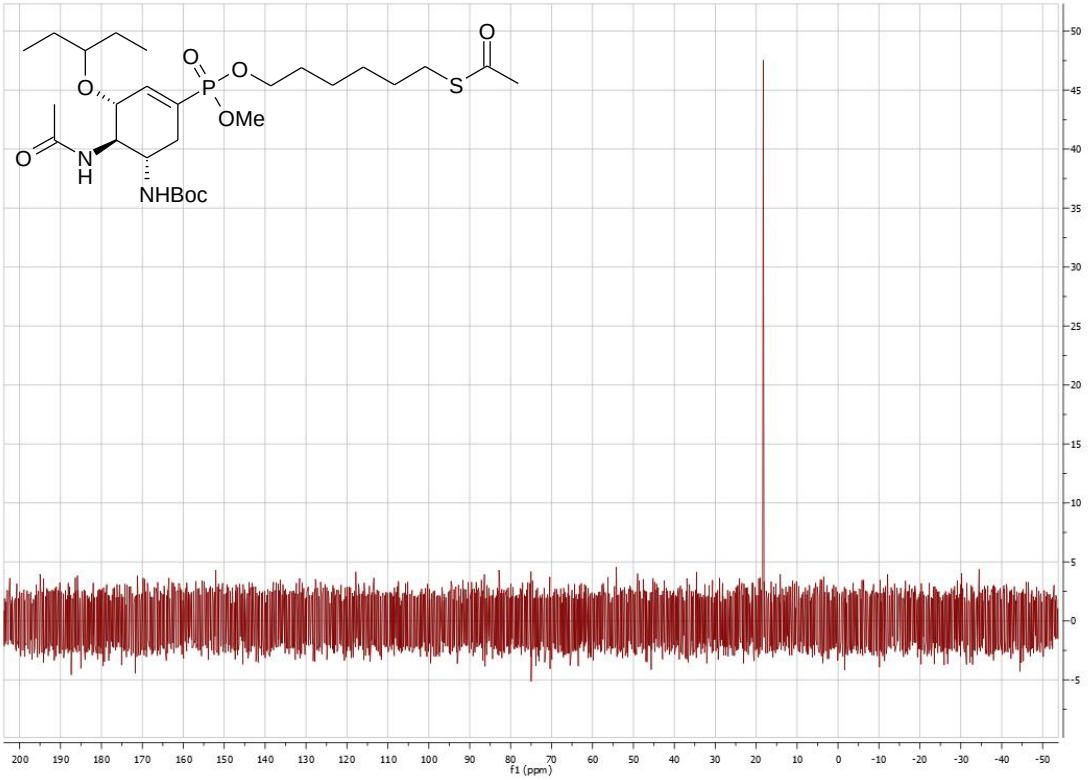
^{31}P-NMR (242.9 MHz, D_2O) of compound **54**



^{31}P-NMR (161.9 MHz, $CDCl_3$) of compound **58**



^{31}P-NMR (161.9 MHz, $CDCl_3$) of compound **59**



^{31}P-NMR (161.9 MHz, $CDCl_3$) of compound **60**