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Synthesis of Phosphonic Acid Probes for Studies on
Phosphonate Metabolizing Enzymes

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Deutsche Zusammenfassung

Die Erforschung von Phosphonat (Pn) abbauenden Enzymen ist nach wie vor eine wissenschaftliche Nische, welche jedoch in den letzten Jahrzehnten vermehrt für Aufsehen gesorgt hat. Grund hierfür ist die Erkenntnis, dass es einen direkten Zusammenhang mit den biogeochemischen Zyklen der Natur (P, C und N-Zyklus) gibt. Da ein tieferes Verständnis des Pn-Abbaus zudem höchstwahrscheinlich zu medizinischen Innovationen führen wird, ist es notwendig Verbindungen herzustellen, welche als Grundlage für mechanistische Untersuchungen des Pn Metabolismus benötigt werden.

Die gegenständliche Masterarbeit beschäftigt sich daher mit der Synthese diverse Substanzen für die Studien des Enzym Komplexes C-P Lyase sowie der Hilfsenzyme des hydrolytischen Pn-Abbauweges. Die Arbeit diskutiert die erfolgreiche Synthese von (2-(4-Nitrophenoxy)ethyl)phosphonsäure (**NPEP**), einen Substrat Analogon der C-P Lyase, welches als Indikator für C-P Lyase-aktivität in Umweltproben dienen soll. Weiters werden verschiedene Syntheseroute für (N-Dansyl-3-aminopropyl)2-tryptamine-ethylphosphinat (**DTAP**), welches in gleicher Art wie das zuvor erwähnte **NPEP** als C-P Lyase Indikator dienen soll, diskutiert. Zuletzt beschäftigt sich die Arbeit mit mehreren Versuchen beide Enantiomere von 2-Amino-1-fluoroethylphosphonsäure (**FAEP**) zu synthetisieren. **FAEP** ist ein potenzieller Inhibitor des Enzyms Phosphonate breakdown factor A (PbfA) welches jedoch über einen bislang unbekannten Mechanismus in Anwesenheit von Phosphonate breakdown factor F (PbfF) hydrolytisch gespalten werden kann.

Abstract

Research on phosphonate (Pn) degrading enzymes is today still a scientific niche but received considerable attention within the past decades as its importance and relations to the major biogeochemical cycles (P, C, and N cycle) came to light. Studying the underlying mechanism of Pn breakdown furthermore has the potential to lead to significant medical innovations. Thus, synthetic efforts are needed to facilitate enzyme mechanistic studies on Pn degrading enzymes.

The current thesis aimed at the synthesis of varied compounds needed to study open questions regarding the radical C–P lyase enzyme complex, as well as on auxiliary enzymes needed for hydrolytic Pn degradation. It presents the successful synthesis of (2-(4-nitrophenoxy)ethyl)phosphonic acid (**NPEP**), a C–P lyase substrate analogue which is planned to be used as indicator of C–P lyase activity in complex environmental samples. The publication further discusses varied synthetic approaches towards (N-dansyl-3-aminopropyl)2-tryptamine ethyl phosphin (**DTAP**), which aims to detect C–P lyase activity in a similar way. Both compounds will facilitate the discovery of unstudied C–P lyase homologs and therefore help in investigating this breakdown mechanism. Additionally, attempts to access both enantiomers of 2-amino-1-fluoroethylphosphonate (**FAEP**) are described. This compound is a potential inhibitor of phosphonate breakdown factor A (PbfA), which can be degraded hydrolytically by an unknown mechanism in the presence of phosphonate breakdown factor F (PbfF). The latter is hypothesized to possess additional pharmaceutical value as a drug against phosphonate producing organisms.

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1 Introduction

1.1 Organophosphonic Acids

Organophosphonic acids (Pns) are a class of (bio)molecules, mainly defined by their characteristic C–P bond, which makes them highly stable against enzymatic degradation. The stable P–C bond is often associated with selective bioactivity towards microbes and other higher-order organisms and distinguishes them from the isosteric and isoelectronic phosphate monoesters (Figure 1).¹

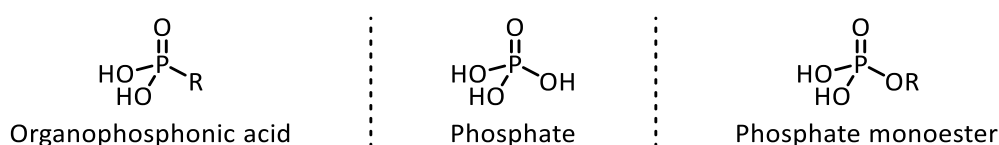


Figure 1 - Chemical structures of Pns, phosphate and phosphate monoesters.

The ionized form of a Pn is able to mimic both a phosphate monoester and a carboxylate. Despite their geometrical similarity, Pns exhibit one rather substantial difference compared to phosphate (mono)esters: the enhanced stability of the carbon-phosphorus bond. Even though the bond dissociation energy of a P–O bond shows an increase of 70–100 kJ mol^{–1} compared to C–P bonds, it is crucial to understand that these values are based on homolytic cleavage. The real strength of the C–P bond arises from its significant stability against heterolytic cleavage. The transition state necessary for C–P bond cleavage of Pns is unfavored from a geometrical point of view. Moreover, this cleavage would result in a carbanionic species which is unstable compared to alkoxides formed by phosphate hydrolysis. The lack of electron lone pairs in carbanions further rules out any Lewis-acid anion stabilization.²

Apart from the use of Pns as bulk products in industrial processes as, for example, cleaning detergents, washing agents and anti-scalants, Pns especially show potential in the biomedical and pharmaceutical sectors.³ They are studied mainly – but not exclusively – in the field of antiviral drugs, often targeting DNA viruses and retroviruses. Prime examples for such drugs are *cidofovir*, which proved to be effective in the treatment of many viral infections, or *adefovir*, licensed to be used as oral prodrug form to treat hepatitis B.^{4,5} The key factor for the immense use of Pns as drugs is their chameleon-like ability to mimic phosphates, as well as the tetrahedral transition states formed during ester and amide bond formation as well as

hydrolysis, which allows them to function as highly efficient substrate or transition state analogues.^{4,6}

As indicated above, Pns can be of synthetic and biogenic origin. The intrinsic high stability of the C–P bond once enabled the discovery of the first naturally occurring phosphonic acid in the late 1950s. 2-Aminoethylphosphonic acid (**2-AEP**) was isolated from sheep rumen protozoa and proved stable against acidic hydrolysis as well against combustion for elemental analysis.²

Phosphorus represents a life-limiting nutrient for all life forms on Earth and is the restraining growth factor in many – especially aqueous - ecosystems. Thus, some microorganisms evolved mechanisms to degrade and digest Pns, and use them as an (alternative) phosphorus source.¹ Scientific research about the potential usefulness and environmental role of natural Pns started around 1950-1960. However, most of today's knowledge was acquired within the last 20 to 30 years, especially assisted by genome mining techniques.⁷

1.2 C–P Bond Cleavage

The fascinating stability of the C–P bond of phosphonates led to many questions concerning the natural degradation of such compounds. Comprehensive research within the past 60 years so far uncovered three different C–P breakdown routes, expressed in a variety of different microorganisms. These differ in the nature of the actual C–P cleavage reaction and are thus called the oxidative, hydrolytic and radical pathway.

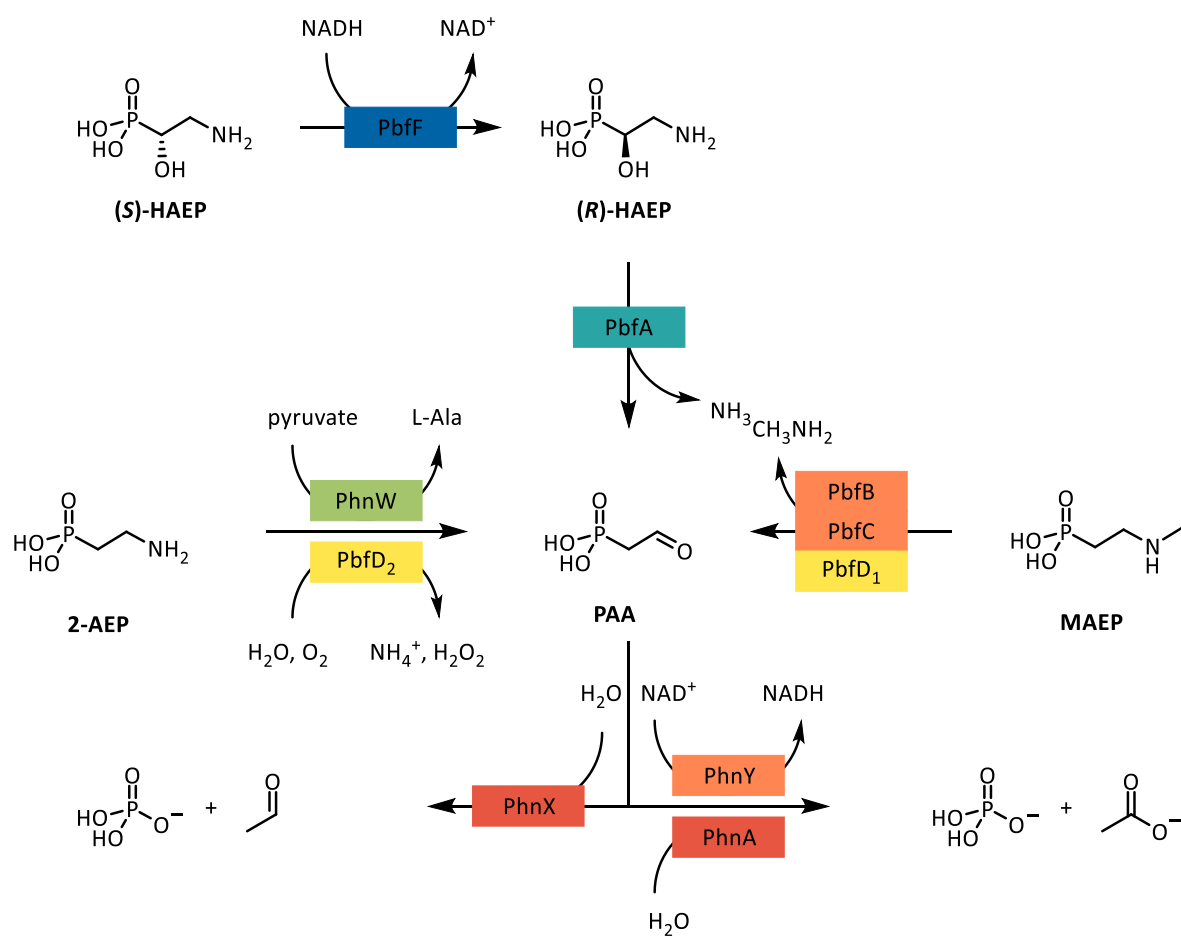
As far as we know today, the substrate scope for the oxidative pathway is limited to **2-AEP**, 2-(*N,N,N*-trimethylammonio)-ethylphosphonic acid (**TMAEP**), methylphosphonic acid (**MePn**) and 3-aminopropylphosphonic acid (**3-APP**).⁸ The breakdown typically starts with a stereospecific α -hydroxylation next to the phosphorus of the given substrate, followed by C–P cleavage by an enzyme referred to as PhnZ. Said hydroxylation is catalyzed by non-heme iron 2-oxoglutarate (Fe/2OG) dependent oxygenases (PhnY* and its homologs) and consumes equimolar amounts of molecular oxygen. As this oxidative pathway is the most recently discovered phosphonate breakdown mechanism, most of the published data is still incomplete, including a few controversial mechanistic propositions.⁹

The focus of this master's thesis however lies on the synthesis of compounds needed to study the two other C–P cleavage routes: the hydrolytic- as well as the radical pathway. Both will therefore be discussed in more detail in the following subchapters.

1.3 The Hydrolytic Pathway

The first discovered phosphonate degradation pathway had a very limited substrate scope and only 2-aminoethyl phosphonate (**2-AEP**) and phosphonoalanine (**PnAla**) were accepted as substrates. Both are first transaminated by PhnW and PalA, respectively, which leads to the formation of phosphonoacetaldehyde (**PAA**) from **2-AEP** and phosphonopyruvate from **PnAla**, (see Scheme 1). The formed carbonyl compounds are subsequently hydrolyzed (PhnX) to acetaldehyde or pyruvate and phosphate. Alternatively, **PAA** can be oxidized to phosphonoacetate by PhnY which enables the successive cleavage to acetate and phosphate, facilitated by PhnA (Scheme 1).^{10–12}

The narrow substrate specificity of this pathway raised questions about the evolutionary benefits compared to the way more promiscuous radical C–P cleavage pathway. This bottleneck was recently discovered to be partly bridged by the action of auxiliary enzymes, referred to as phosphonate breakdown factors (Pbfs). Current research showed a variety of Pbfs to catalyze reactions finally giving β -oxo-phosphonates that can be degraded by the original PhnWAY pathway. The β -carbonyl moiety thereby acts as electron sink to enable the hydrolytic cleavage. For instance, PbfA is encoded in over 13% of bacterial gene clusters dedicated to hydrolytic Pn cleavage and converts (*R*)-2-amino-1-hydroxyethylphosphonate [(*R*)-**HAEP**] to **PAA** via a 1-2-elimination mechanism (Scheme 1). PbfB, PbfC and PbfD₁ widen the substrate diversity by oxidizing *N*-methyl-2-aminoethylphosphonate (**MAEP**) to **PAA**. Additionally, PbfD₂ was discovered to catalyze the oxidative deamination of **2-AEP** to **PAA**.¹³ Another supplemental enzyme, termed PbfF, was recently studied and shown to transform (*S*)-**HAEP** to (*R*)-**HAEP**, which is again a substrate for PbfA.¹⁴ PbfF was initially hypothesized to be a dehydrogenase, but further studies found evidence for it to be a NAD(P)-dependent racemase. PbfF is structurally quite distinct when compared to analogous NAD-dependent enzymes which catalyze similar isomerizations and was so far only discovered in two strains of *Alphaproteobacteria*.



Scheme 1 – Summary of the known hydrolytic Pn breakdown pathway for 2-AEP and analogs. Enzyme types are indicated by the following color-code: red: hydrolases; orange: dehydrogenases; yellow: oxidases; green: transaminases; green: lyases; Blue: isomerases.

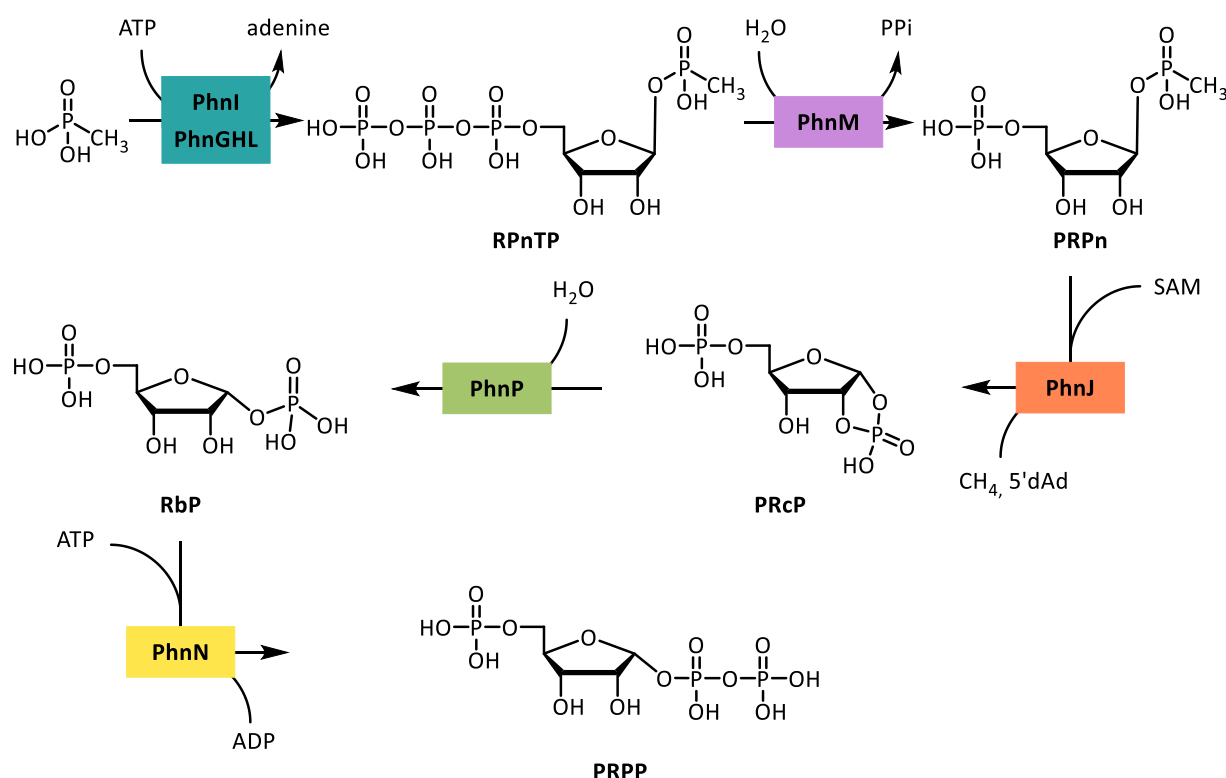
While these additional enzymes broaden the substrate scope of hydrolytic Pn breakdown tremendously, hydrolytic cleavage still remains rather substrate specific. Therefore, the hydrolytic Pn degradation pathway does not tolerate a broad range of substrates similar to the oxidative pathway.

1.4 The C–P Lyase Pathway

In the 1960s, both ethyl- and methylphosphonate were found to be processed by *Escherichia coli* (*E. coli*) to yield inorganic phosphate as well as ethane or methane. However, no specific enzyme responsible for this transformation could be characterized at the time. Given the fact that hydrolytic P–C cleavage through *phnX* only tolerates aminophosphonates as substrates, it seemed likely that the degradation observed in *E. coli* must follow a different mechanism. Further investigations indicated that *E. coli* exclusively breaks down Pns in phosphorus-scarce areas. C–P lyase was later found to consist of multiple individual enzymes, many of which are so far not completely understood. Extensive studies led to the discovery of the *pho* operon (= *phnCDEFGHIJKLMNOP*) which encodes the observed degradation of phosphonates by the so-called C–P lyase complex. Homology comparisons showed the *phnF* genes to repress C–P lyase expression until binding of an unknown molecule which seems to be indicative of phosphorus scarce areas. *PhnCDE* genes facilitate the transport of the substrates into the cell. The *phnIJM* genes were identified to form the essential catalytic core system necessary for C–P bond cleavage.^{15–17}

The phosphonate disassembly starts with *PhnI*, a nucleosidase protein, cleaving adenine from adenosine triphosphate (ATP) in order to manufacture D-ribose-5-triphosphate (**RTP**) which is subsequently bound to the substrate-phosphonate by *PhnGHL*, producing an α -D-ribose-1-alkylphosphonate-5-triphosphate (**RPnTP**). Followingly, the amidohydrolase *PhnM* liberates pyrophosphate from **RPnTP**, resulting in the formation of α -D-ribose-1-alkylphosphinate-5-phosphate (**PRPn**). The key step, the cleavage of the C–P bond, is catalyzed by *PhnJ*, which needs S-adenosyl methionine (SAM) as cofactor. The *PhnJ* reaction is initiated by an iron-sulfur cluster, which cleaves the carbon-sulfur bond of the SAM cofactor, creating a 5'-desoxyadenosyl radical and methionine. Multiple hydrogen transfer reactions ultimately result in the formation of a thiyl radical from cysteine. Said radical attacks the phosphorus atom of the phosphonate resulting in the creation of an unstable pentavalent radical intermediate. Subsequent homolytic cleavage of the P–C bond yields an α -D-ribose-1,2-cyclic phosphate-5-phosphate (**PRcP**) as well as the respective hydrocarbon. The cyclic phosphorus-containing molecule is then partially hydrolyzed to α -D-ribosyl-1-5-bisphosphate (**RbP**) by *PhnP*. An additional phosphate is bound to the molecule by *PhnN*, forming 5-phospho-ribosyl-1-diphosphate (**PRPP**). **PRPP** itself can either be further converted into pyrophosphate and

adenosine monophosphate by a phosphoribosyl transferase or simply utilized in the biosynthesis of NAD (see Scheme 2).^{18–21}



Scheme 2 - Schematic overview of the key steps of Pn breakdown by C-P lyase on the example of methylphosphonic acid; ATP: adenosine triphosphate; RPNTP: α -D-ribose-1-alkylphosphonat-5-triphosphate; PPI: diphosphate; PRPn: α -D-ribose-1-alkylphosphinate-5-phosphate; SAM: S-adenosyl methionine; 5'dAd: 5'-deoxyadenosine; PRcP: D-ribose-1,2-cyclic phosphate-5-phosphate; RbP: α -D-ribosyl-1-5-bisphosphate; PRPP: 5-phospho-ribosyl-1-diphosphate.

Genes *phnOP* proved to be non-essential for the studied phosphonate-breakdown but catalyzed further modifications after the C-P bond cleavage.

In contrast to hydrolytic and oxidative Pn cleavage, C-P lyase catalyzed Pn catabolism was found to tolerate a very broad range of substrate including both alkyl substituted Pns as well as aryl Pns. Even anthropogenic phosphonates such as glyphosate can be converted into inorganic phosphate and the corresponding hydrocarbon.²² Current research suggests that the stability of the formed radical intermediate does not influence the success of the breakdown, which would explain this immense substrate scope.²³

1.5 Research Questions

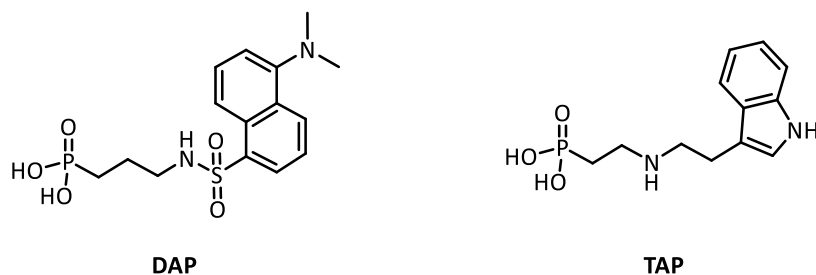
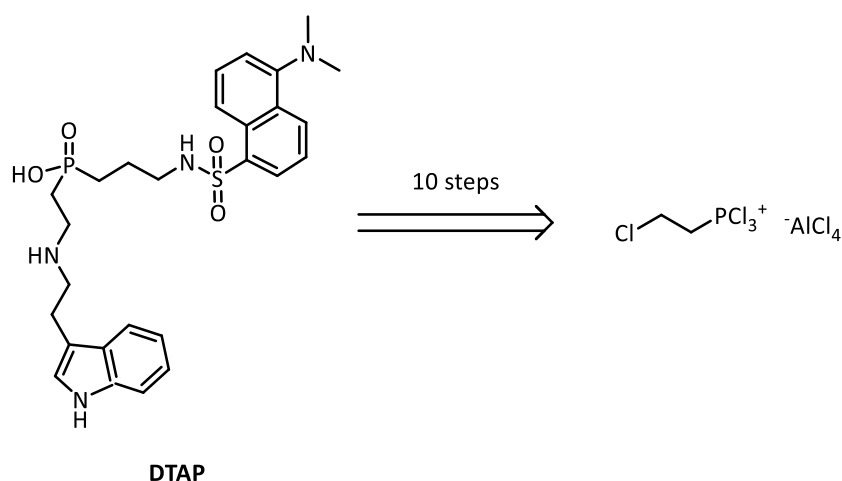
C–P Lyase Detection

While the overall breakdown of phosphonates *via* the C–P lyase pathway is understood, many mechanistic details remain unexplained. For instance, PhnI requires the presence of PhnG, PhnH and PhnL for cleaving adenine from ATP, however it is unclear why this companionship is necessary. Furthermore, another active site between PhnI and PhnJ has been discovered recently which appears to be essential for the breakdown mechanism, but the exact role and mechanism of this active site is not understood.^{24,25}

Over time, a steadily increasing number of C–P lyase expressing bacterial strains were found in nature and it is known that there are differences in substrate specificity between organisms. However, most studies on C–P lyase focus on the *E. coli* variant, which is for example not able to degrade glyphosate, while other C–P lyase homologs are. Being able to isolate a broader range of C–P lyase expressing organisms would therefore significantly help to understand this complex enzymatic system. Current methods for C–P lyase activity detection often rely on messenger RNA transcripts and have proven to be very time consuming and tedious. As the enzyme complex appears to be able to break down the majority of Pns tested so far, it seemed reasonable to use fluorescing groups to detect the starting materials and products of this transformation. And indeed, Zechel *et al.* could show that *N*-dansyl-3-aminopropyl phosphonic acid (**DAP**) is accepted as a substrate. However, the use of an enzyme substrate, bearing a functional group that only becomes UV-active/fluorescent upon cleavage of the P–C bond, would be a way more convenient screening method. This would even allow to detect C–P lyase activity in complex environmental samples without intermediate work-up. The availability of a C–P lyase specific detection method would reduce the effort to identify new homologs of this enzyme drastically.

Based on the work of a former group member, a FRET-substrate (Förster resonance energy transfer) for C–P lyase was developed. The main concept behind this compound was the parallel presence of a fluorophore as well as a photo quencher. This way, fluorescence would become detectable upon cleavage of one of the two groups as the quenching mechanism is limited to distances between 1-10 nm between both moieties.²⁶ (*N*-dansyl-3-aminopropyl)-2-tryptamine ethyl phosphinate (**DTAP**) was hypothesized to be a good substrate for C–P lyase

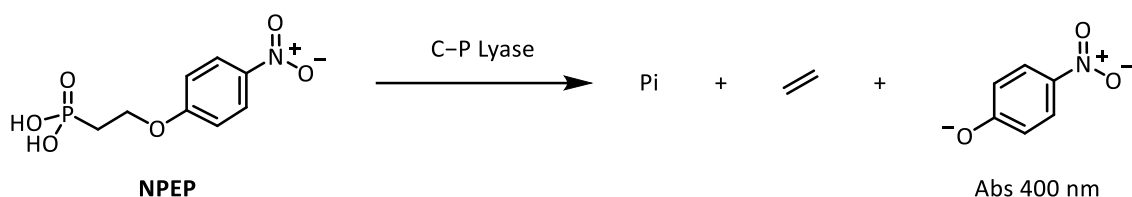
based on the work of Zechel *et al.*²⁷ However, the purification of the final compound turned out to be troublesome, and the first synthetic steps were tedious, low-yielding and required the use of harmful highly halogenated compounds (Scheme 3, top). Thus, a different synthetic approach to access this compound is needed, which was the first aim of this thesis. **DTAP** additionally requires the synthesis of the corresponding metabolites resulting upon P–C bond cleavage for comparative reasons (see Scheme 3, bottom). The synthesis of these reference compounds was further achieved relying on known routes.²⁶



*Scheme 3 – Top: Previous approach for the synthesis **DTAP**; Bottom: Hypothesized metabolites resulting from **DTAP** by the action of C-P lyase (N-dansyl-3-aminopropyl phosphonic acid (**DAP**) and 2-tryptamine ethyl phosphonic acid (**TAP**).*

Another compound that can be envisaged for this purpose is (2-(4-nitrophenoxy)ethyl)phosphonic acid (**NPEP**), which bears a nitrophenolate group. Current knowledge about the C–P lyase degradation pathway suggests that such a compound would be transformed into inorganic phosphate (Pi), ethene and 4-nitrophenonolate upon action of the enzyme complex (Scheme 4). *p*-Nitrophenolate has a quite distinct absorption band with a maximum at 400 nm and would therefore also enable a simple photometrical detection of

C-P lyase activity even in low concentrations where direct ethene detection is impracticable.^{26,28}

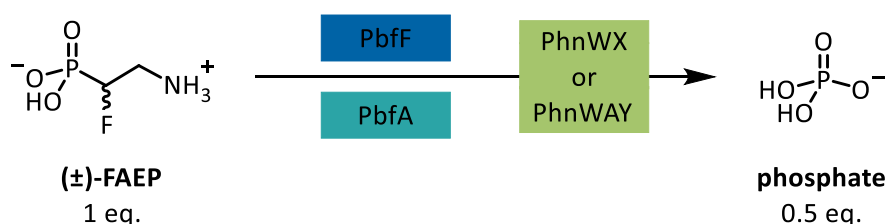


*Scheme 4 - Hypothesized breakdown of **NPEP** to nitrophenolate, ethene and inorganic phosphate (Pi) catalyzed by C-P lyase.*

The syntheses of this fluorophore therefore represent the second research goal of this thesis.

Phosphonate Breakdown Factor F

PbF (see chapter 1.3) was shown to accomplish the isomerization of (*S*)-**HAEP** to (*R*)-**HAEP**. As such it broadens the substrate scope of hydrolytic P-C bond cleavage pathway. However, enzyme assays using a combination of PbF, PbfA and either PhnWX or PhnWAY showed (±)-2-amino-1-fluoroethylphosphonate [(±)-**FAEP**] also to be partly degraded to inorganic phosphate (Scheme 5). (±)-**FAEP** was originally synthesized as potential inhibitor for PbfA. This finding is particularly intriguing as (±)-**FAEP** catabolism approximately corresponds to a conversion of 50% of the used substrate. This strongly indicates that only one of the two possible enantiomers of **FAEP** might be tolerated. Therefore, one enantiomer might either not be degraded or have the desired inhibitory effect on PbfA.¹



Scheme 5 – PbfF-catalyzed partial breakdown of (±)-FAEP to phosphate.

In order to proof this hypothesis and to study the underlying mechanistic details, enantiopure samples (*R*)-**FAEP** and (*S*)-**FAEP** are needed. Their synthesis was therefore the third research goal of this thesis.

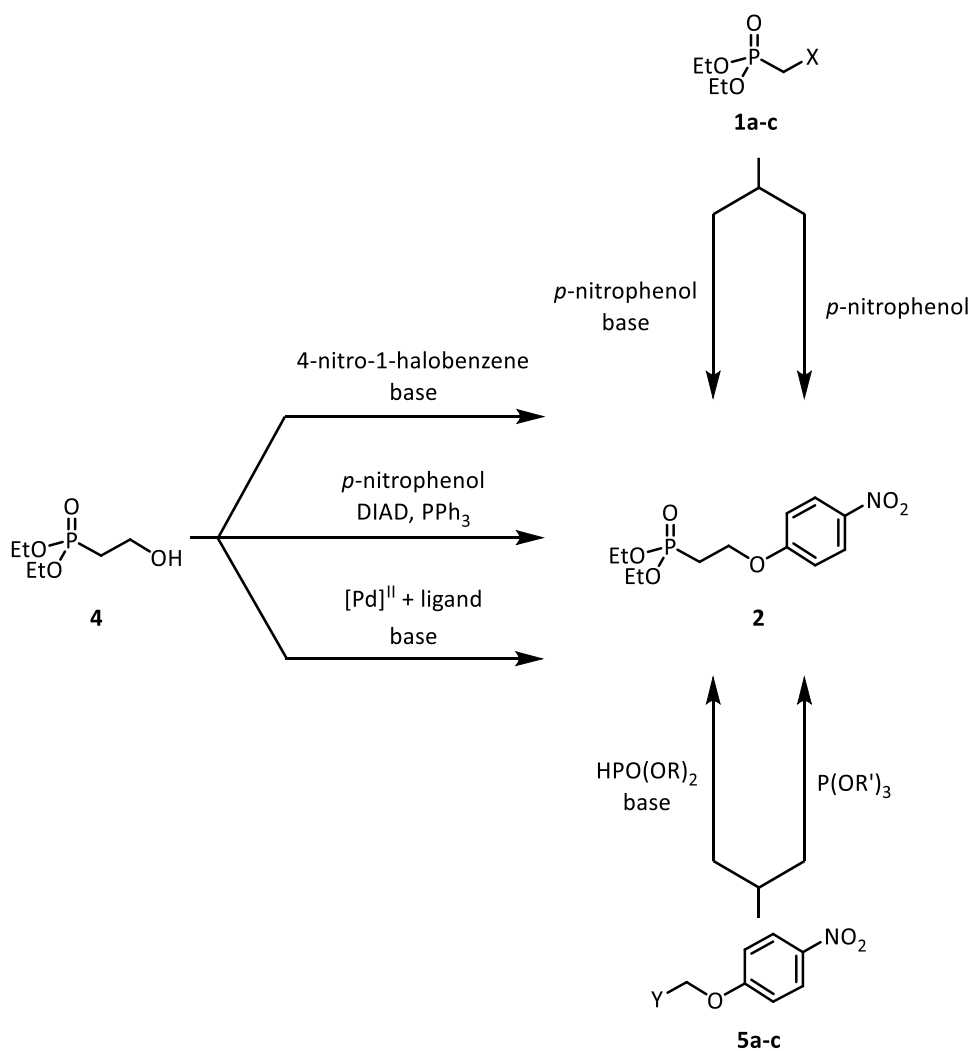
¹ Unpublished data (27/12/25)

2 Results and Discussion:

2.1 2-(4-Nitrophenoxy)ethyl phosphonic acid

Even though the target compound, 2-(4-nitrophenoxy)ethyl phosphonic acid (**NPEP**), is a rather small molecule, many different approaches for its synthesis can be conceived. Possible cuts could be made between the phenol-moiety and the adjacent C₂-unit or between the latter and the phosphorus moiety. Classical nucleophilic substitution reactions seemed to be the most straight forward approach to join the three parts of the molecule; but in which order had to be figured out first.

Possible approaches included a conventional Williamson-ether-type synthesis, using *p*-nitrophenol under basic conditions as nucleophile and a haloalkyl-phosphonate as electrophile (Scheme 6). The second method exploits the high reactivity of an acyl halide in combination with *p*-nitrophenol for the formation of the desired bond. This approach would furthermore prevent elimination, which is considered a classical side reaction in the formerly discussed approach. Alternatively, the synthesis of **NPEP** can also be envisaged starting from 2-hydroxyethylphosphonate (**4**) in combination with another nucleophilic species. In case of difficulties under basic conditions, a primary hydroxy-phosphonate is further the ideal substrate to react with *p*-nitrophenol *via* a Mitsunobu reaction. The fifth envisaged option, which is also the only catalytic approach, planned to make use of a palladium catalyst to facilitate the reaction between *p*-nitrophenyl bromide and a primary alcohol. Last but not least, the P–C bond can also be envisaged to be formed last. This could either be done by reacting an H-phosphonate, after deprotonation, with 2-(*p*-nitrophenoxy)ethyl bromide (**5a**) or by using an Arbuzov reaction between a suitable alkyl- or acyl halide and a trialkyl phosphite. Acyl halides show a rapid increase in reactivity towards Arbuzov reactions when compared to alkyl halides. However, they also necessitate further reduction as well as dehydration of the formed α -oxo phosphonate.



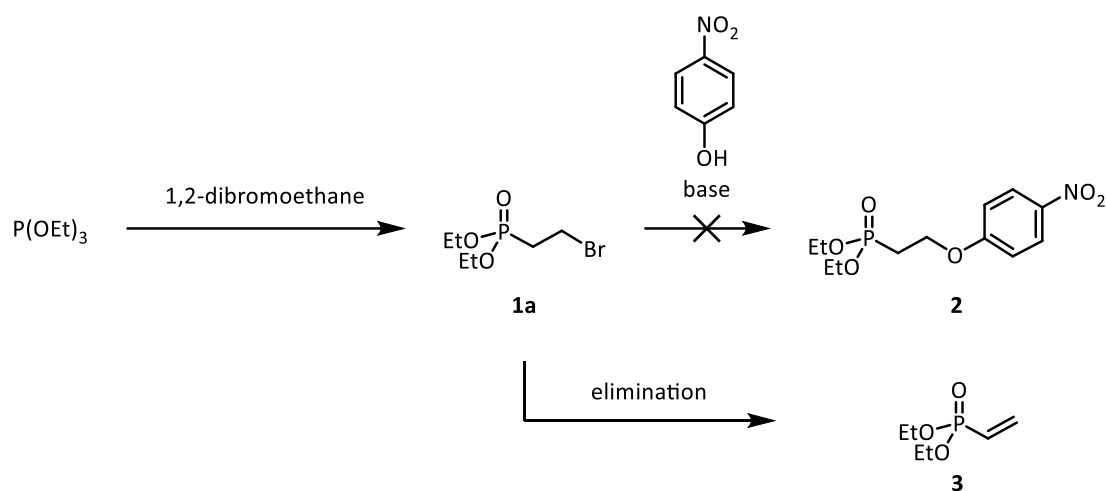
Scheme 6 - Proposed approaches towards ethyl-protected **NPEP** (**2**); $\text{X} = \text{CH}_2\text{Br}$ (**1a**), $\text{C}(\text{O})\text{Cl}$ (**1b**), CH_2Cl (**1c**); $\text{Y} = \text{CH}_2\text{Br}$ (**5a**), $\text{C}(\text{O})\text{Cl}$ (**5b**), CH_2Cl (**5c**); $\text{R} = -\text{OEt}$, $-\text{OiPr}$, $-\text{OPh}$, $-\text{OBn}$; $\text{R}' = -\text{OMe}$, $-\text{OEt}$.

(2-(4-Nitrophenoxy)ethyl)phosphonic acid – Substitution

As described in the previous chapter (2.1), the synthesis of **NPEP** was originally planned to involve the nucleophilic substitution of diethyl 2-bromoethylphosphonate (**1a**) with *p*-nitrophenol. The Arbuzov reaction was originally heated to reflux for 16 to 20 h under inert conditions. However, reducing the reaction time to 2.5 h increased the yield of **1a** and reduced byproduct formation.

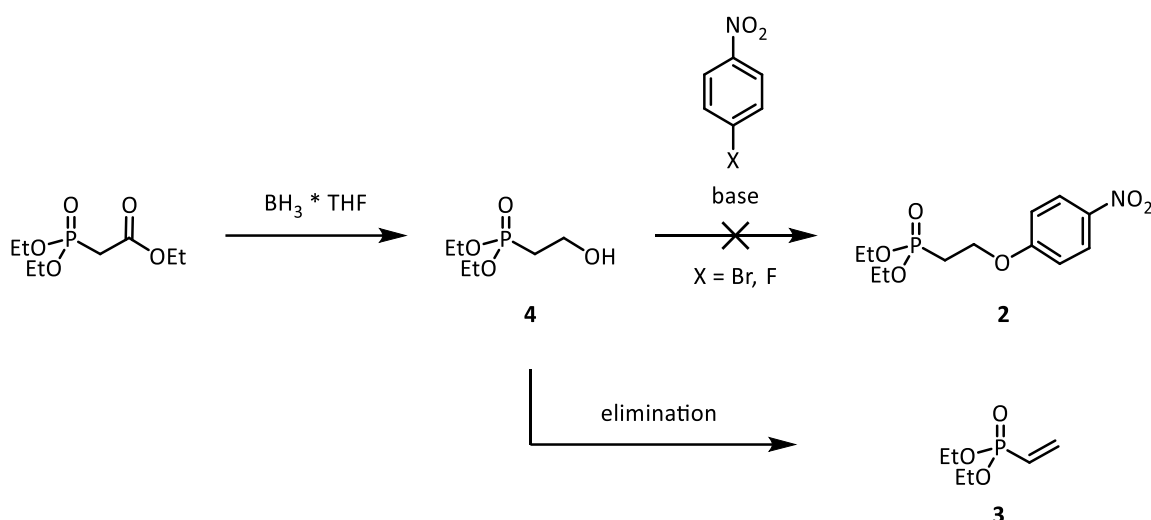
While on paper the deprotonated nitrophenol should be nucleophilic enough to attack the shown bromide, the desired compound could not be obtained through this approach. The use of sodium hydride as base and TBAI as phase transfer catalyst, exclusively led to the elimination product **3** [^1H NMR: 6.40-6.00 (m, 3 H, $\text{P}-\text{CH}=\text{CH}_2$); ^{31}P NMR: δ_{P} 17.58 ppm], regardless of the reaction temperature (0°C to 70°C tested) (Scheme 7). A similar setup, using

phenol instead of *p*-nitrophenol was carried out, producing only the elimination product. Preformation of the needed nitrophenolate and removal of excess base before addition of 2-bromoethylphosphonate (**1a**) to the reaction vessel led to a mixture of unreacted starting material and elimination product as could be judged by ^1H NMR spectroscopy. The last substitution reaction that was studied was set up using Cs_2CO_3 as base. This idea was inspired by the synthesis of **5a** from *p*-nitrophenol and 1,2-dibromoethane under basic conditions. As this reaction only seemed to work when using Cs_2CO_3 instead of K_2CO_3 , it was hypothesised that the same principle might apply to the reaction of **1a** with *p*-nitrophenol. Unfortunately, the reaction again only produced the elimination product.



Scheme 7 – Attempted synthesis of *NPEP* via substitution and isolated product **3**.

As the utilization of *p*-nitrophenol as nucleophile failed, an alternative nucleophilic aromatic substitution approach was envisaged as a work-around, starting from diethyl 2-hydroxyethyl phosphonate (**4**), which can be easily obtained from the reduction of triethyl phosphonoacetate using a borane-tetrahydrofuran (THF) complex (Scheme 8).

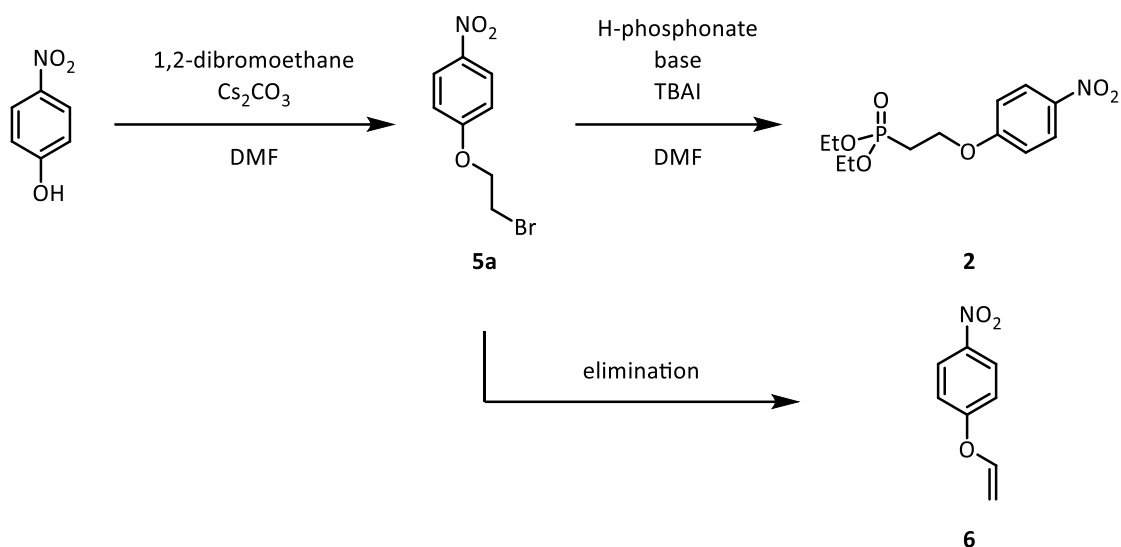


Scheme 8 – Attempted synthesis of NPEP via nucleophilic aromatic substitution starting from triethylphosphonoacetate.

The hypothesis, that deprotonated alcohol **4** would attack 4-fluoro-1-nitrobenzene or 4-bromo-1-nitrobenzene and consecutively result in the formation of the corresponding Meisenheimer complex was tested using different solvents (THF and DMF) and bases (Cs_2CO_3 , K_2CO_3 and NaH). Unfortunately, again only mixtures of **4** and the elimination product **3** were obtained as judged by NMR spectroscopy.

(2-(4-Nitrophenoxy)ethyl) phosphonic acid – H-Phosphonate

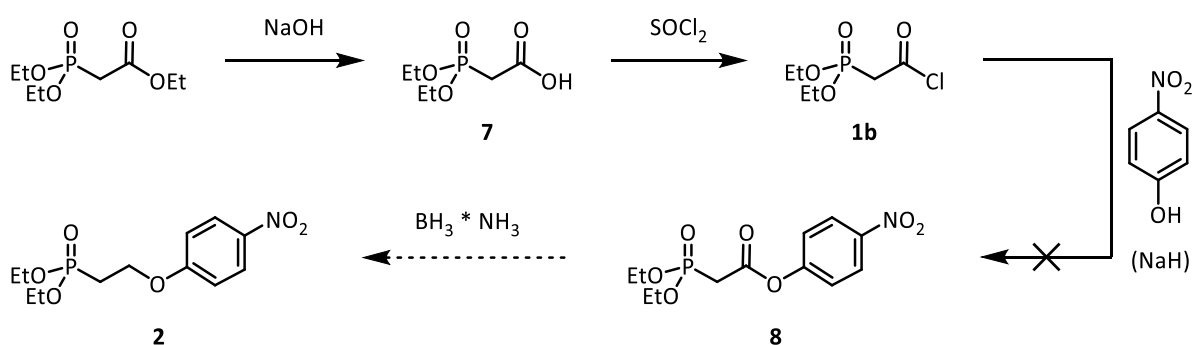
As classical substitutions failed to produce the desired precursor **2**, an altered method using H-phosphonates was studied, inspired by the work of Cohen *et al.*²⁹ They were able to deprotonate dibenzyl phosphite and thus facilitate its reaction with 4-(bromomethyl) toluene. A similar reaction was set up using 1-(2-bromo-ethoxy)-4-nitrobenzene (**5a**), Cs_2CO_3 and TBAI in DMF. The precursor (**5a**) was synthesized according to Ngo *et al* using Cs_2CO_3 as base (Scheme 9).³⁰ Attempts utilizing K_2CO_3 as base failed at producing the desired compound. Despite all efforts, the reaction failed to produce the anticipated phosphonate using different phosphites (diethyl-, diisopropyl-, diphenyl- and dibenzyl phosphite), and bases (triethylamine or Cs_2CO_3) at varied temperatures (0 °C to r.t.). Spectroscopic data of the discussed experiments always indicated the elimination of the bromide and thus formation of alkene **6** [^1H NMR: δ_{H} 6.67 (dd, $J_{\text{HH}} = 6.0, 7.6$ Hz, 1 H, $-\text{O}-\text{CH}=\text{CH}_2$), 4.98 (dd, $J_{\text{HH}} = 2.0, 11.7$ Hz, 1 H, $-\text{CH}=\text{CH}_2$), 4.68 (dd, $J_{\text{HH}} = 2.0, 4.0$ Hz, 1 H, $-\text{CH}=\text{CH}_2$).



Scheme 9 – Attempted synthesis of **NPEP** via the use of an H-phosphonate as starting from nitrophenol.

(2-(4-Nitrophenoxy)ethyl) phosphonic acid – Acyl halide

Most of the above discussed reactions competed with eliminations. Thus, another approach, using diethyl (2-chloro-2-oxoethyl)phosphonate (**1b**) was envisaged, in order to rule out this problem. The acyl halide (**1b**) was synthesized from the corresponding acid **7**, which was obtained by basic hydrolysis of triethyl phosphonoacetate (Scheme 10). This method makes use of the high reactivity of acyl chlorides compared to alkyl halides to form the desired core. In case of success, the resulting ester was planned to be reduced to the corresponding ether using a borane-ammonia complex.³¹

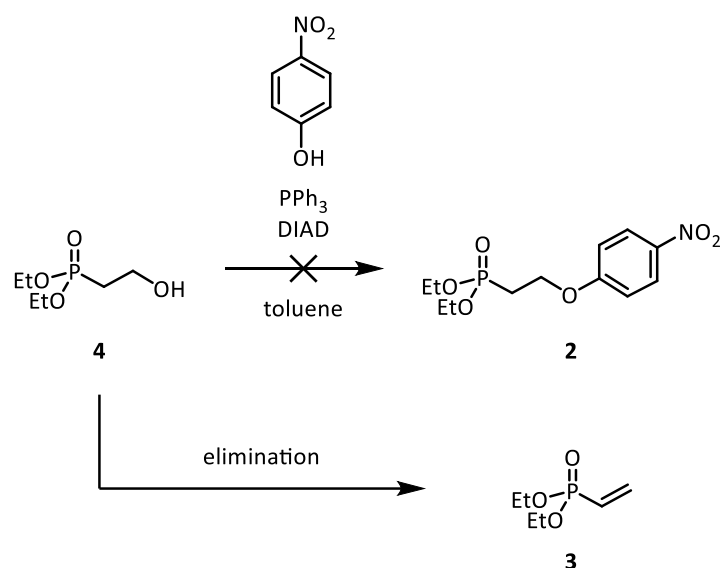


Scheme 10 – Attempted synthesis of ethyl protected **NPEP** (**2**) via an acyl halide.

The nitrophenol-ester formation was tested twice, once without base and once in the presence of NaH. Unfortunately, neither approach furnished ester **8** and the proposed synthetic strategy had thus to be discarded.

(2-(4-Nitrophenoxy)ethyl) phosphonic acid – Mitsunobu

As none of the described direct substitution experiments were successful, a Mitsunobu reaction for the introduction of the *p*-nitrophenolate was attempted using β -hydroxy phosphonate **4** (Scheme 8). The setup was inspired by a publication of Shi *et al.*, who discussed the synthesis of alkyl-aryl esters including phenol derivatives *via* Mitsunobu reactions at elevated temperatures (Scheme 11).³²

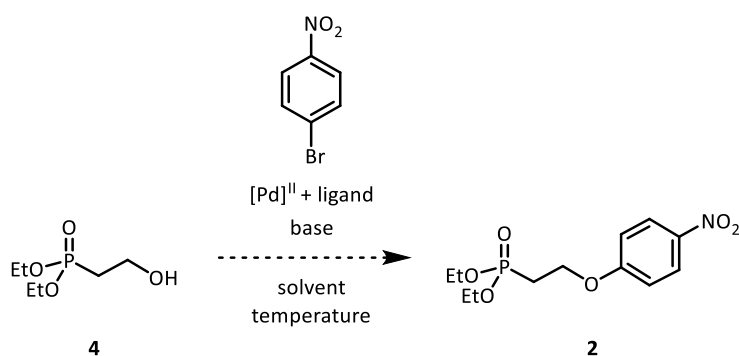


Scheme 11 – Attempted synthesis of **NPEP** via Mitsunobu reaction.

Unfortunately, the reaction again turned out to yield solely elimination product **3**. As the order of addition of reagents plays a crucial role in many cases for Mitsunobu reactions, the order of their addition was altered without noticeable effect. Lowering the reaction temperature to 0 °C in a DCM/toluene mixture (known to counteract the elimination) also led to similar results. Similarly, a base-catalysed Mitsunobu reaction, known to enable the functionalisation of phenols at 0 °C³³ using triethyl amine gave the same results.

(2-(4-Nitrophenoxy)ethyl) phosphonic acid – Pd^{II} catalysis

As elimination was still the major problem, a milder approach, not relying on high temperatures or harsh pH seemed more promising for the synthesis of target compound **2**. Thus, different Pd-catalyzed couplings were tested. These reaction were all carried out in a similar fashion as discussed by Gowrisankar *et al* (Scheme 12).³⁴



Scheme 12 – Attempted synthesis of **NPEP** via palladium catalysis; used ligands and solvents are listed in Table 1.

The reaction of diethyl 2-hydroxyethylphosphonate with 1-bromo-4-nitrobenzene was investigated using palladium (II) acetate in combination with different ligands under basic conditions. Furthermore, varied solvents and temperatures were tested (Table 1).

Table 1 - Reaction conditions of the conducted Pd-catalysed experiments; DavePhos: 2-dicyclohexylphosphino-2'-(*N,N*-dimethylamino)-biphenyl; Tetrakis: tetrakis(triphenylphosphine); DPE: 1,2-Bis(diphenylphosphin)ethane.

Entry	Ligand	Base	Solvent and concentration	Temperature (°C)
1	/	K ₂ CO ₃	Toluene (0.5M)	85
2	DavePhos	CS ₂ CO ₃	Toluene (0.5M)	85
3	/	Ag ₂ CO ₃	THF (0.3M)	85
4	/	CS ₂ CO ₃	THF (0.3M)	r.t.-50
5	Tetrakis	CS ₂ CO ₃	THF (0.3M)	r.t.-50
6	DavePhos	CS ₂ CO ₃	THF (0.3M)	r.t.
7	Tetrakis	CS ₂ CO ₃	THF (0.3M)	r.t.
8	DPE	CS ₂ CO ₃	THF (0.3M)	r.t.

All carried out reactions were primarily analysed *via* NMR spectroscopy. Both reactions performed in toluene (entry 1 and 2) only showed unreacted starting material. However, the proton NMR spectrum of entry 4, carried out in THF, contained a pair of duplicated triplets (δ_{H} 3.58 ppm, J = 18.9 and 6.5 Hz; 3.80 ppm, J = 25.1 and 5.6 Hz; Figure 2). However, the appearance of such a doublet of triplets in the mentioned region hints at the existence of an alkyl phosphonate. Given the difference in intensity, it seemed unlikely that both signals origin from the same molecule. However, the crude mixture was worked up and analysed after

purification attempts by column chromatography. Unfortunately, none of the collected fractions contained the desired product.

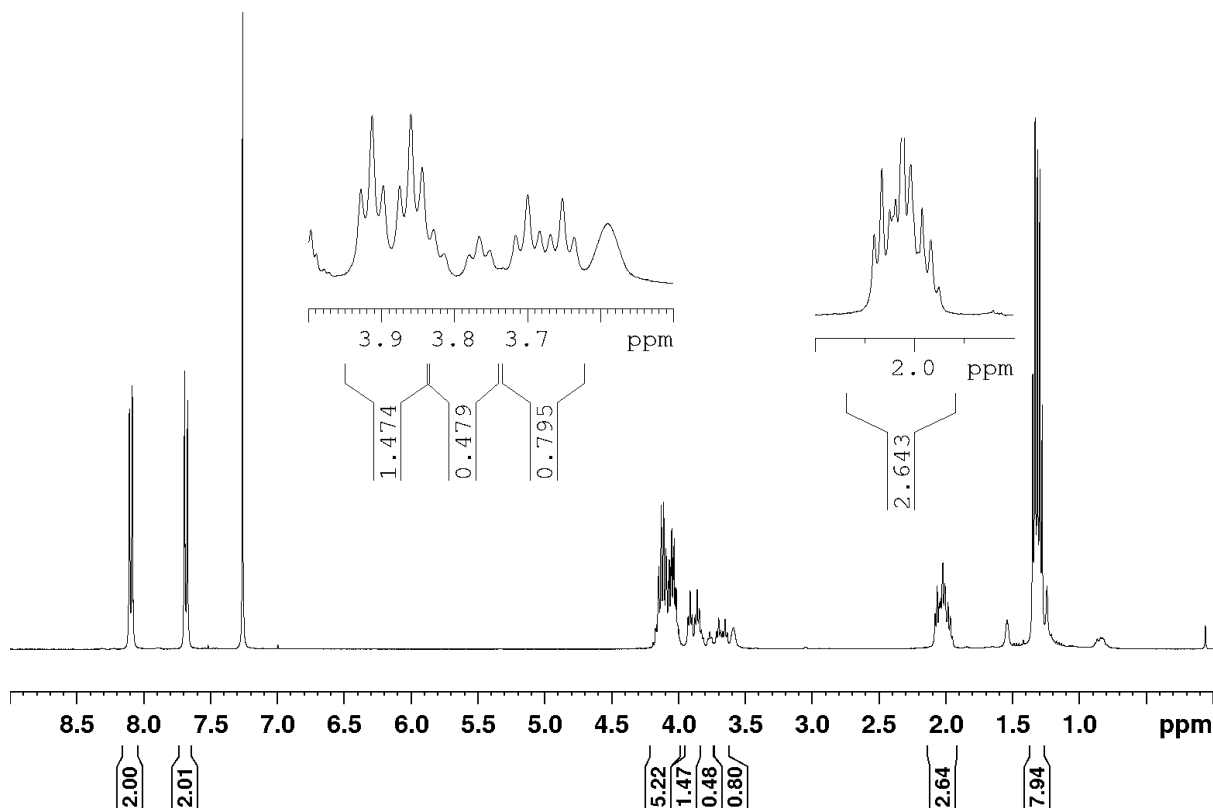


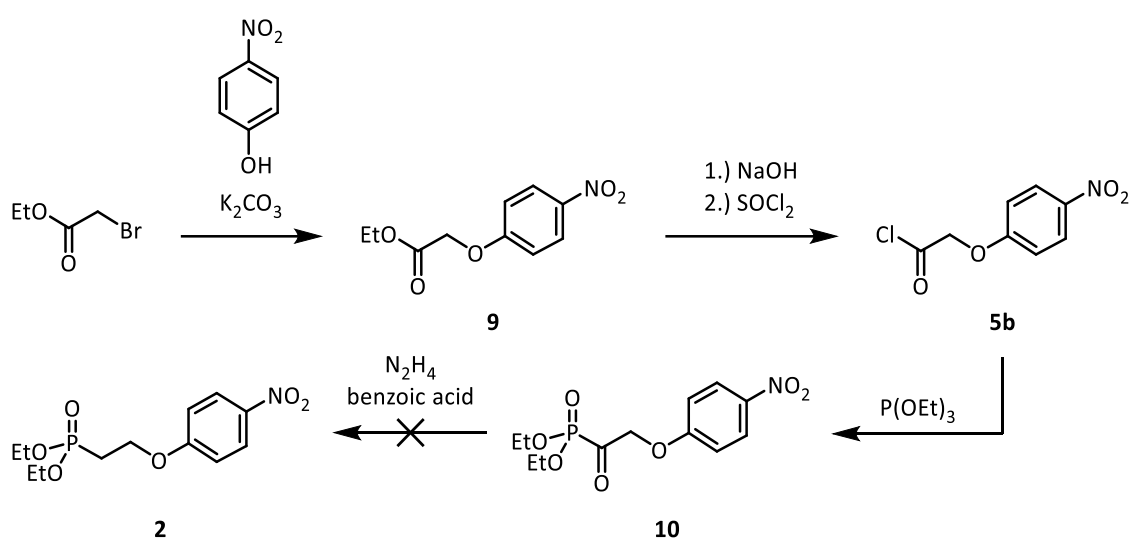
Figure 2 - Crude ^1H NMR spectrum of entry 3 (Table 1).

This intriguing set of dt signals could also be observed in all of the consecutive catalytic experiments (entry 5-8). Failing isolation in addition to mismatching integrals of the two dt led to the conclusion that the observed signals do not origin from the desired compound but rather stem from a catalyst-bound intermediate which decomposes during work-up.

(2-(4-Nitrophenoxy)ethyl)phosphonic acid – Arbuzov reaction

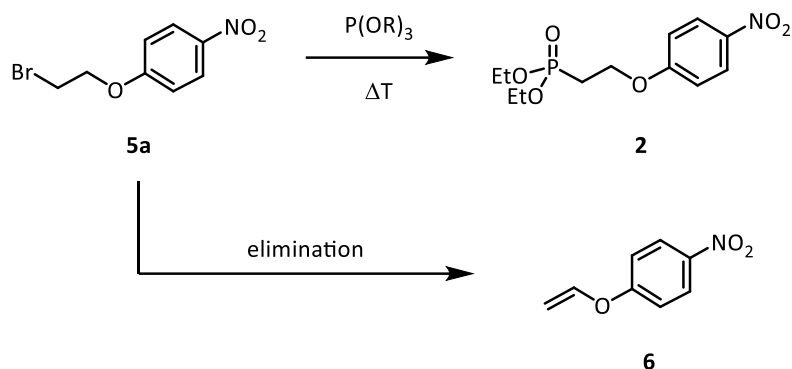
Another very promising approach for the direct synthesis of the desired organophosphonate **2** involves C–P bond formation *via* an Arbuzov reaction between a trialkyl phosphite and either an acyl chloride or an alkyl halide. While the usage of alkyl halides necessitates temperatures north of 140 °C, acyl chlorides are reactive at room temperature but require an additional step for the removal of the α -oxo group next to the phosphorus.³⁵ When using alkyl halides for Arbuzov reactions, both alkyl bromides and chlorides are able to yield the desired phosphonate. Alkyl bromides are nonetheless more reactive and usually lead to higher conversions.^{36,37}

The first approach for the synthesis of **NPEP** via an Arbuzov reaction was performed according to literature using 2-(4-nitrophenoxy)acetyl chloride (**5b**) and triethyl phosphite.³⁵ Acyl chloride **5a** was previously synthesized starting from ethyl 2-bromoacetate and *p*-nitrophenol (Scheme 13)³⁸. The results of the Arbuzov reaction were promising as spectroscopic data implied the presence of the desired compound's keto and enol tautomer in admixture with one major side product (NMR: δ_P –7.12 ppm). Additional 2D NMR experiments revealed this undesired side product to bear a direct bond between the nitrophenol-moiety and a phosphate group. While the formation of the α -oxophosphonate **5a** could be successfully carried out, the hydrazine-mediated reduction was unsuccessful.



Scheme 13- Attempted synthesis of **NPEP** via an acyl chloride-using Arbuzov reaction.

Since the reductive removal of the α -carbonyl from **10** turned out to be troublesome, the Arbuzov reaction between an alkyl bromide and a variety of trialkyl phosphites was attempted next (Scheme 14).



Scheme 14- Attempted synthesis of **NPEP** via an Arbuzov reaction between alkyl bromide **5a** and varied trialkyl phosphites; $R = \text{Me, Et}$.

The reaction using triethyl phosphite and **5a** was tested at 150 and 170 °C, both times resulting in elimination product **6**. Since chlorides are considered to be less prone to elimination, a similar reaction was carried out using the corresponding alkyl chloride **5c**. The reaction was again unsuccessful, leading to elimination as well as decomposition. Thus, the more reactive trimethyl phosphite³⁷ was used instead of triethyl phosphite, again leading to the formation of elimination product **6** as well as widespread decomposition.

In order to prevent eliminations, three different approaches were tested. First, as Arbuzov reactions follow an S_N2 mechanism,³⁷ DMF or *p*-xylene were used as additives to stabilize the intermediate cationic species and prevent elimination. Additionally, lowering the reaction temperature to 130°C or using a high excess of triethyl phosphite (60 eq.) without additional solvent was tested under argon atmosphere. While the reactions in DMF and *p*-xylene did not yield any desired product, high excess of phosphite in combination with a lower reaction temperature showed promising results. ¹H NMR spectroscopy of an experiment using an excess of triethyl phosphite showed a promising set of two dt after 40 minutes reaction time ($\delta_H = 4.26$ ppm, td, $J_{HH} = 7.5$ Hz, $J_{HP} = 18.7$ Hz; 2.27 ppm, dt, $J_{HH} = 7.5$ Hz, $J_{HP} = 11.4$ Hz) (Figure 3).

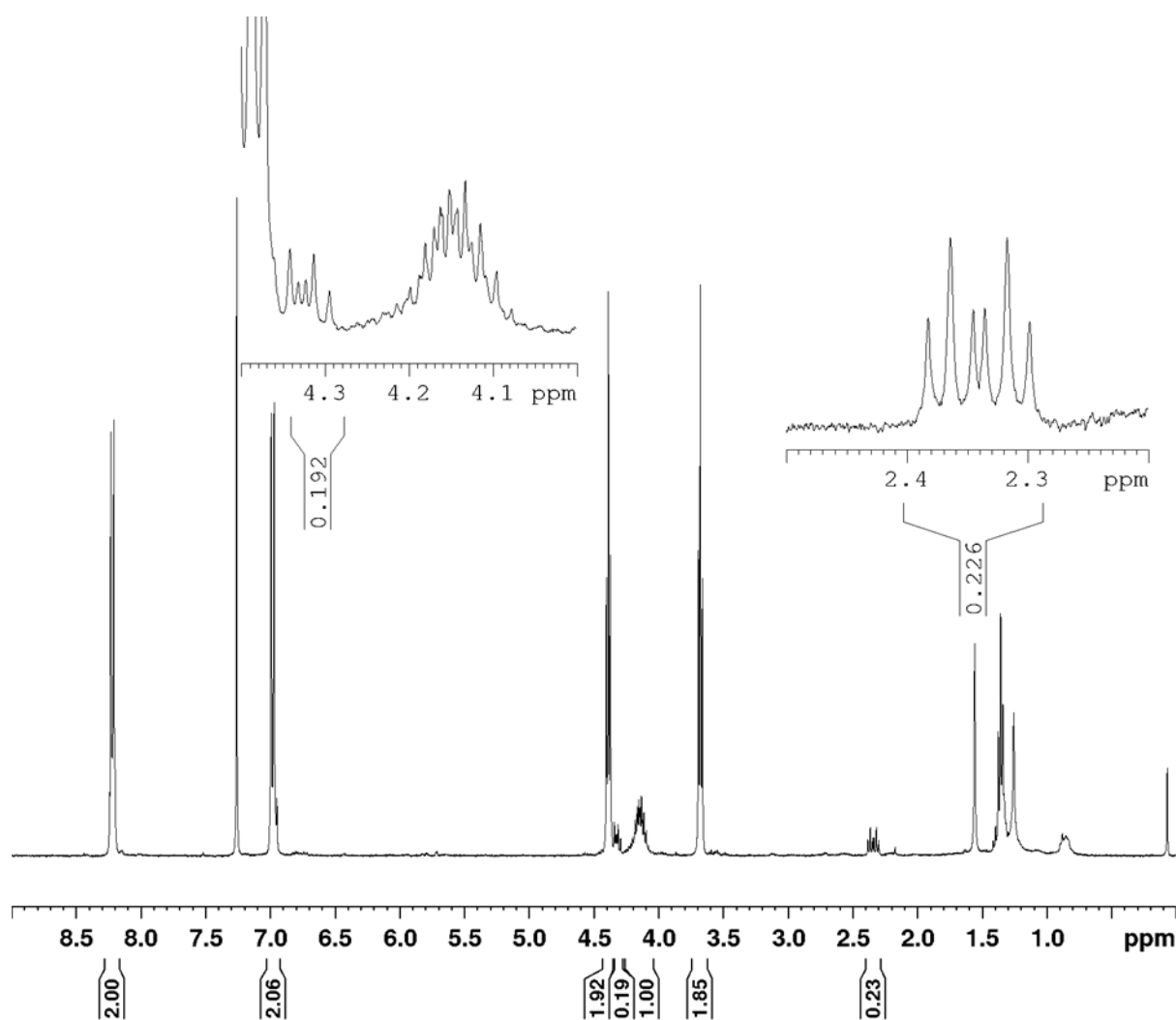


Figure 3 – Crude ^1H NMR spectrum indicating the formation of **2**; close-ups show the relevant signals.

A 50 mg sample of the crude reaction mixture was thus purified using preparative TLC (5% MeOH in DCM, $R_f = 0.71$) and indeed proved the successful synthesis of protected **NPEP (2**, Figure 4).

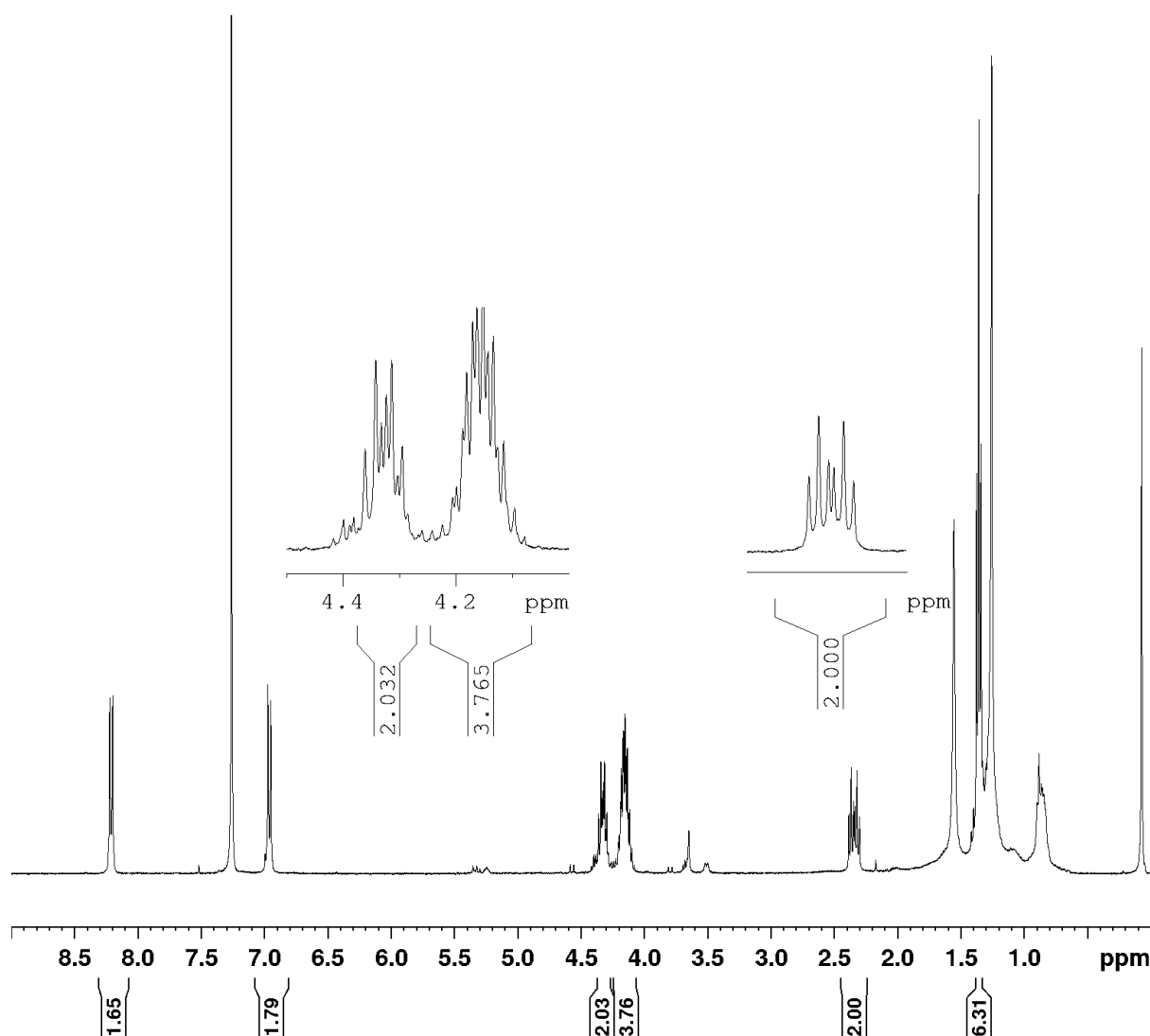


Figure 4 - ^1H NMR spectrum of ethyl protected NPEP (2).

Additional experiments showed inert atmosphere not to be necessary for the reaction. However, after 30-40 minutes the calculated conversion was less than 8 mol% (calculated *via* proton NMR in reference to remaining starting material signals). Thus, longer reaction times were tested to improve conversion rates (Table 2, Figure 5).

Table 2 - Optimization of the Arbuzov reaction for the synthesis of **NPEP** with regard to reaction time; percentages calculated from ^1H NMR.

Entry	time (h)	Starting material (mol%)	Product (mol%)
1	0.5	93	7
2	0.7	81	11
3	1.0	82	17
4	2.0	60	40
5	4.5	35	65

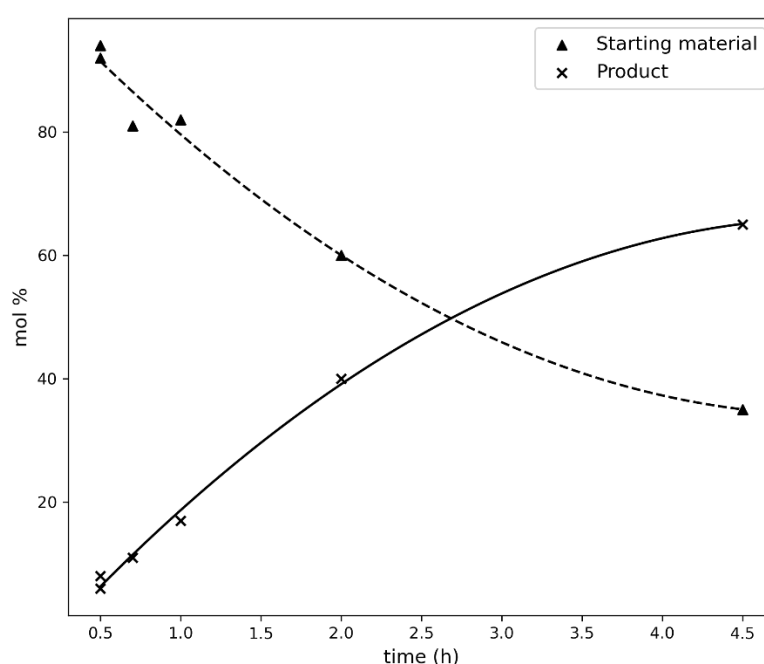


Figure 5 - Scatter plot of the reaction optimization listed in Table 2; $R^2_{(\text{starting material})}$: 0.98; $R^2_{(\text{product})}$: 0.99.

The collected data, as listed in Table 2, suggests that 4.5 h of reaction time yield the highest amounts of product. However, the amount of decomposition products also increases with time. Considering both product formation and accompanying decomposition, it seems likely that the ideal reaction time is between two and three hours. It would be interesting to evaluate the effect of inert atmosphere on the formation of side products, since the ^1H NMR spectrum of the first reaction (Figure 3) shows significantly less decomposition compared to experiments with similar reactions times under normal atmosphere.

As previously stated, purification *via* preparative TLC tremendously increased product purity. Therefore, scalability of purification based on polarity differences was tested by purifying 100

mg of crude reaction mixture by column chromatography. All UV-active fractions contained the desired compound in admixture with excessive amounts of triethyl phosphate (^{31}P NMR: $\delta_{\text{P}} = -0.90$ ppm). The purification was repeated by manual flash column chromatography as the available cartridges for automated chromatography had an insufficient length for this separation. However, using longer chromatography columns (length: 35 cm, $\varnothing$: 8 mm) did not solve the issue.

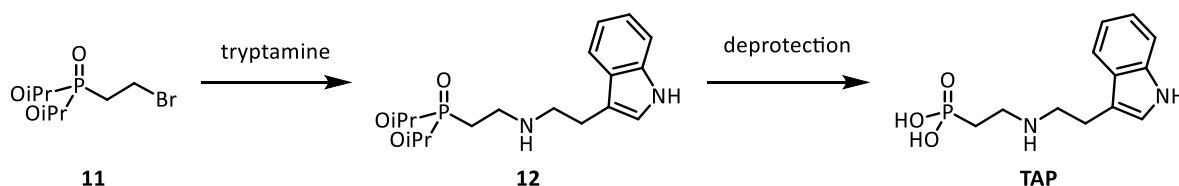
Thus, removal of the phosphate was attempted after final deprotection to give the free phosphonic acid, which is water soluble. This way, all apolar impurities can simply be removed by extraction. Unfortunately, the main impurity, triethyl phosphate gives phosphate under the chosen deprotection conditions and thus remains inseparable from the desired product.

Preliminary experiments were undertaken to investigate the possible purification of the final product through ionic exchange chromatography (IEC). Since this purification technique is based on charge differences rather than on polarity differences, it seems likely that phosphate, with three negative charges, may be separable from **NPEP**.

The final purification of higher substance amounts could not be realized within the scope of this master thesis due to time constraints.

2.2 2-Tryptamine ethyl phosphonic acid

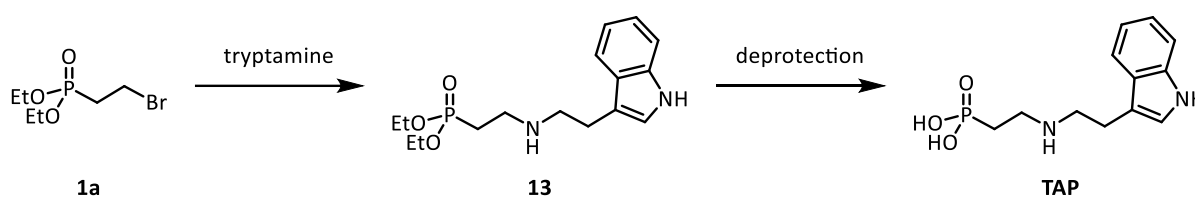
2-Tryptamine ethyl phosphonic acid (**TAP**) was planned to be synthesized according to literature²⁶, starting with an Arbuzov reaction of triisopropyl phosphite and 1,2-dibromoethane to yield phosphonate **11**. Tryptamine was then intended to be introduced into the molecule *via* nucleophilic substitution. Simple deprotection of the phosphonate was then thought to yield the desired phosphonic acid **TAP**.



Scheme 15 - Synthesis of **TAP** starting from isopropyl protected phosphonate **11**.

Synthesis of 2-tryptamine ethyl phosphonic acid

While the synthesis of phosphonate **11** as well as the subsequent functionalization towards compound **12** went smoothly, the deprotection failed as only one isopropyl residue was cleavable at 110 °C over 72 h. A second approach utilizing triethyl phosphate was set up. Both, the addition of tryptamine as well as deprotection of the ethyl groups went smoothly. The final product was purified using a sequence of washing steps with DCM and toluene. As determined by elementary analysis, **TAP** was obtained as a partial hydrobromide adduct with an overall yield of 37%.



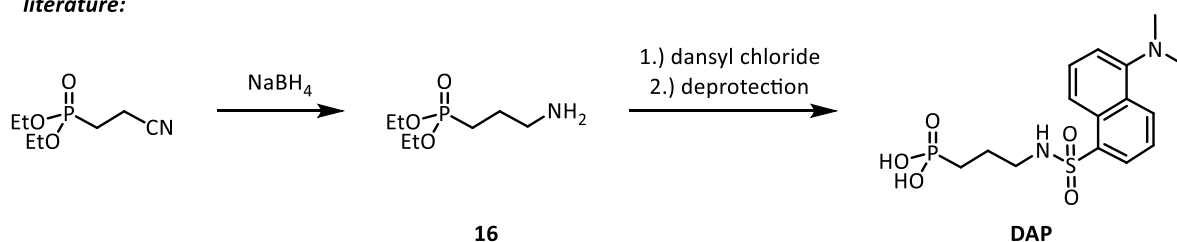
Scheme 16 - Synthesis of **TAP** starting from ethyl protected phosphonate **1a**.

2.3 *N*-Dansyl-3-aminopropyl phosphonic acid

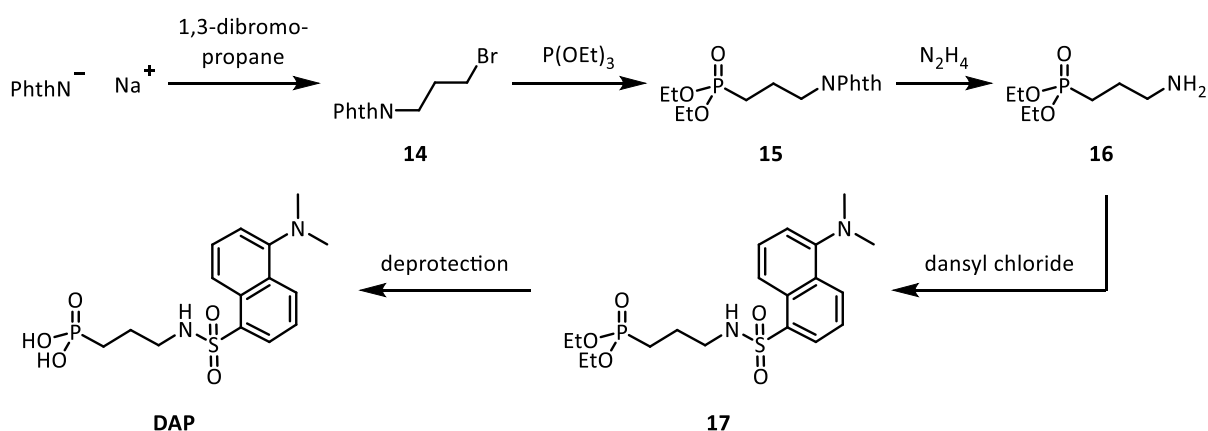
While there is a literature known approach for the synthesis of *N*-dansyl-3-aminopropyl phosphonic acid (**DAP**), a slight deviation from this methodology was planned to be tested as the previously used starting material, diethyl diethyl 2-cyanoethylphosphonate is no longer commercially available. The literature approach starts from diethyl 2-cyanoethylphosphonate which is reduced to the free amine. The resulting aminopropyl phosphonate is subsequently reacted to dansyl chloride and deprotected to yield the desired compound (Scheme 17, top).^{26,27}

The hereby presented approach starts from phthalimide sodium salt and 1,3-dibromopropane, yielding the corresponding protected bromoalkane **14**. A subsequent Arbuzov reaction was planned to be set up to synthesize phthalimide protected aminopropyl phosphonate **15** which could be deprotected using hydrazine. The free amine **16** was planned to be subsequently converted to the desired compound according to the literature procedure (Scheme 17, bottom).

literature:



this work:



Scheme 17 - Intended synthesis of **DAP**; top: literature approach; bottom: this work; Phth = phthalyl-residue.

Synthesis of *N*-dansyl-3-aminopropyl phosphonic acid

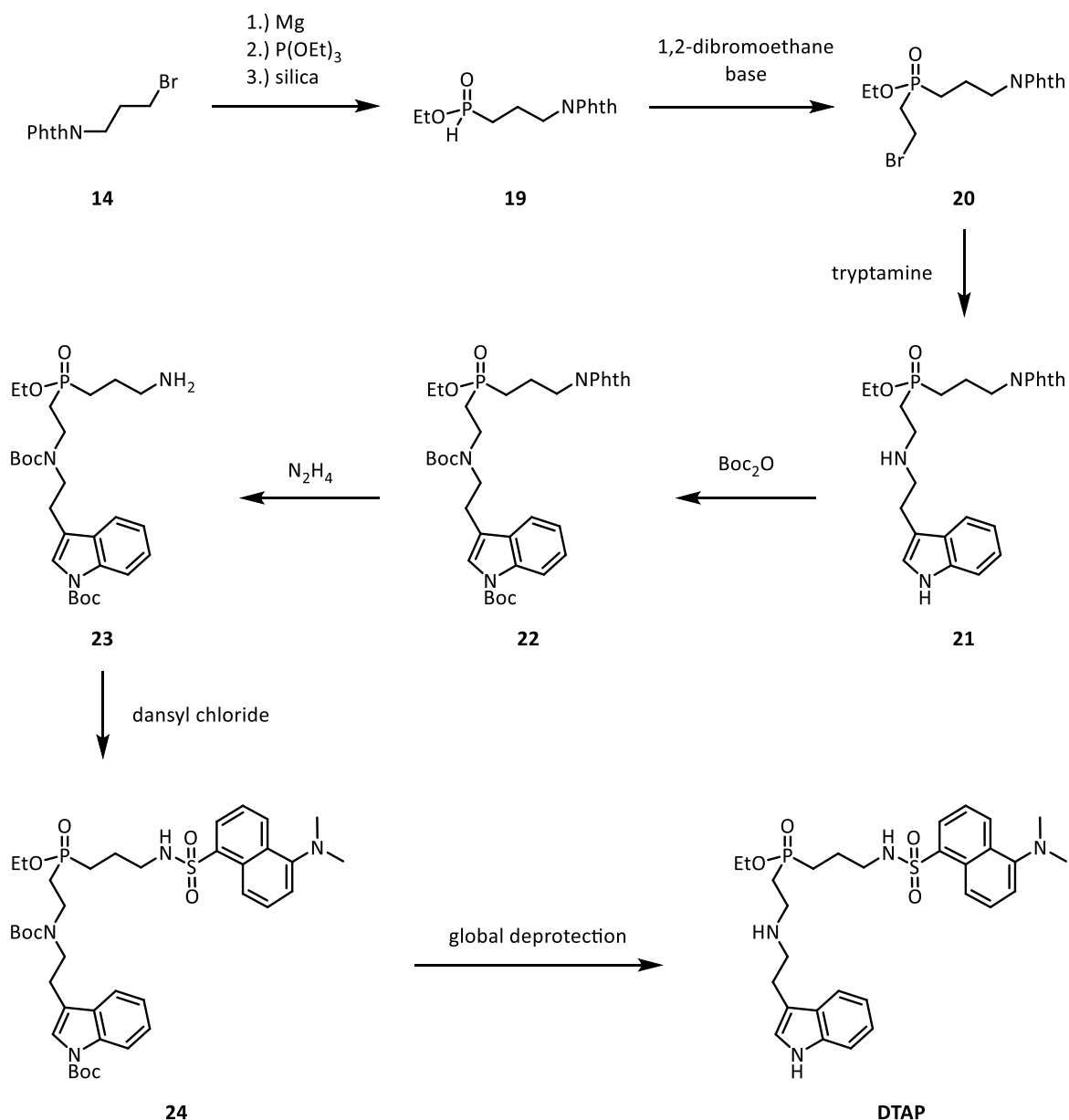
The synthesis of compound **14** as well as the subsequent Arbuzov reaction went smoothly (Scheme 17). Reductive removal of the phthalimide to the free amine **16** unfortunately only yielded 50% of the desired product. Compound **17** was synthesized efficiently through addition of dansyl chloride but purification turned out to be rather difficult. The crude product had to be purified by three successive column chromatographic runs in order to obtain analytically pure **17**. Global deprotection with TMSBr/allylTMS to give the final product **DAP** yielded the desired compound quantitatively. After a few months, most of the substance seemed to have decomposed, suggesting that future batches should be stored as sodium salt in order to increase the life span of this phosphonic acid.

While this novel synthesis requires one additional step, it increases the overall yield of the synthesis of **DAP** to 17% without any optimization. The former approach led to yields of 15%. Diethyl 2-cyanoethylphosphonate is in addition more expensive than the starting materials used in the hereby discussed synthesis route.

2.4 (*N*-Dansyl-3-aminopropyl)2-tryptamine ethyl phosphine

In 2024, Tamara Dinhof, a former group member, described the synthesis of **DTAP** in her doctoral dissertation.²⁶ However the last step as well as the purification of the final compound turned out to be quite tedious and, in the end, not victorious. Furthermore, the synthetic sequence yielded too little product to conduct the appropriate cell studies necessary to determine the scientific value of **DTAP**'s ability to detect C-P lyase activity. Another drawback of the already published methodology is the use of PCl_3 alongside other highly dangerous, halogenated compounds. Additionally, the first steps of this route resulted in rather low yields. Hence, a different approach had to be considered.

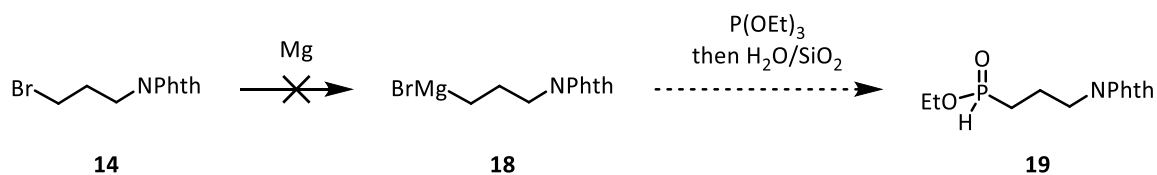
DTAP was projected to be synthesized starting with a Grignard reaction between triethyl phosphite and 3-bromopropane-1-phthalimide to obtain phosphinic acid **19**. The latter was planned to be reacted with 1,2-dibromoethane to produce compound **20**. The obtained key intermediate **20** was then envisaged to react similarly to compound **1a** (see Scheme 15) with tryptamine. The dansyl group was finally planned to be introduced after Boc protection of the amines and reductive cleavage of the phthalimide to the free amine. Global deprotection was then intended to yield the desired compound **DTAP** (Scheme 18).



Scheme 18 - Proposed synthesis of **DTAP**; Phth = Phthalyl, Boc =tert-butyloxycarbonyl.

Synthesis of (N-dansy-3-aminopropyl)2-tryptamine ethyl phosphine

As discussed in chapter 2.4, **DTAP**'s phosphinate moiety center was planned to be synthesized via a Grignard reaction followed by silica mediated reduction (Scheme 19).³⁹

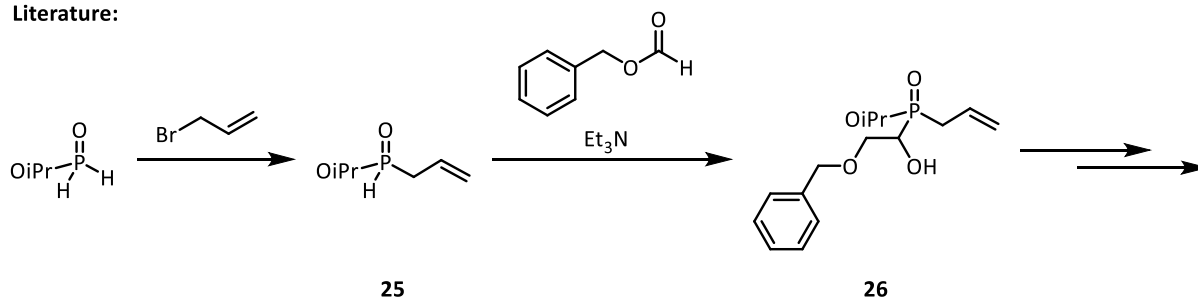


Scheme 19 – Attempted synthesis of the phosphinate core of **DTAP** via a Grignard reaction.

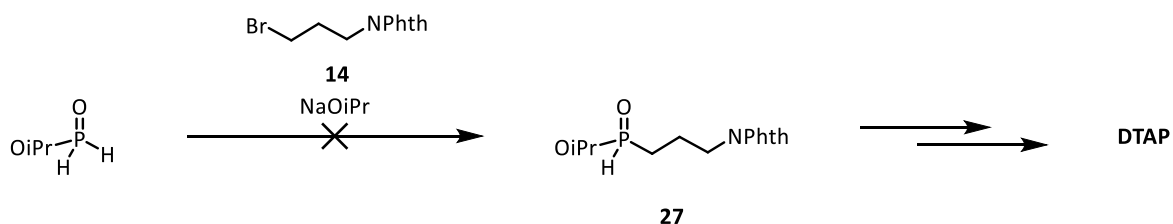
The first attempt was not successful. During the reaction, it seemed probable that the formation of the Grignard reagent was troublesome. The same setup was repeated multiple times, using different activation methods for the magnesium. The malfunction of the formation of the Grignard-reagent indicated that the quality of the used magnesium (0) was not sufficient for the intended purpose. Delivery times for Grignard-grade magnesium are currently above six months; thus, this approach could not be persecuted during the current thesis. Additional attempts are planned for the near future after arrival of the new batch of Mg.

Due to the lack of available magnesium, a different approach inspired by a publication of Dayde *et al.*, starting from phosphinic acid was tested (Scheme 20, top).⁴⁰ Isopropyl protected phosphinic acid was reacted with **14** using NaOiPr as base in analogy to the published method, yet this approach was unsuccessful.

Literature:



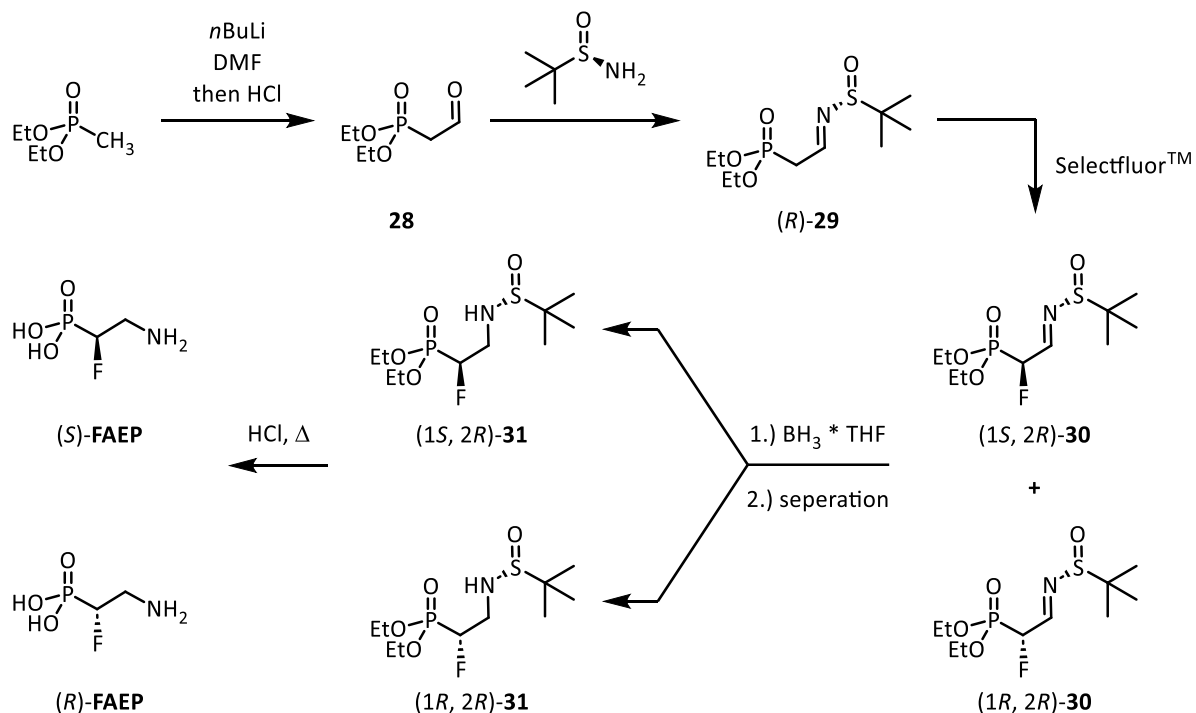
This work:



Scheme 20 – Top: literature known method for the synthesis of a phosphinate starting from phosphinic acid;⁴⁰ bottom: attempted synthesis of **DTAP** starting from phosphinic acid using phtalyl-protected alkylbromide **14**.

2.5 (R)- And (S)-2-amino-1-fluoroethylphosphonate

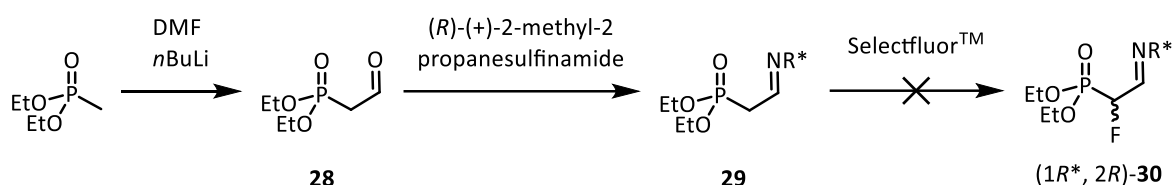
One last goal of the current Master's thesis was the synthesis of the pure enantiomers of **FAEP** for enzyme mechanistic studies. Past experiments with a racemate suggested that only one of the enantiomers might express the desired bioactivity. Thus, pure samples of both enantiomers are necessary to verify this hypothesis. While in theory the two enantiomers might be separable using a chiral stationary phase for column chromatography, it seems more feasibly to obtain both enantiomers by forming diastereomers by introducing a chiral auxiliary on an easily cleavable position. The envisaged approach starts from diethyl methylphosphonate, which can be deprotonated and subsequently reacted with DMF to give aldehyde **28** after acidic work-up. A reductive amination was then planned to be employed in order to introduce (*R*)-(+)-2-methyl-2-propanesulfinamide as chiral auxiliary. Consecutive fluorination *via* SelectfluorTM, an electrophilic fluorination reagent,⁴¹ was planned to yield a diastereomeric pair of (1*R*,2*R*)- and (1*S*,2*R*)-**30**. After reduction of the imine to the amine, the isomers were envisaged to be separated and then deprotected by acidolysis to yield the desired compounds (Scheme 21).



Scheme 21 - Proposed synthesis of (*R*)- and (*S*)-FAEP.

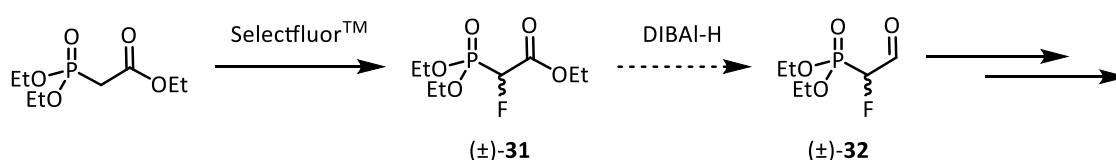
Synthesis of (±)-2-amino-1-fluoroethylphosphonate

Aldehyde **28** was synthesized according to literature.⁴² The reductive amination was inspired by the reaction of Groch *et al.* and proceeded smoothly.⁴³ Compound **29** was then deprotonated using NaH and subsequently reacted with Selectfluor™ to yield the fluorinated diastereomers **30**, however, unsuccessfully (Scheme 22).



Scheme 22 - Attempted synthesis of a diastereomeric pair of **30** as key intermediate for the synthesis of (R)- and (S)-FAEP; NR*: (R)-(+)-2-methyl-2-propanesulfinimine.

Given the failure of the fluorination step, another approach was designed. As the fluorination of triethyl phosphonoacetate has already proven to be successful, fluorinated triethyl phosphonoacetate was planned to be partially reduced using DIBAL-H. The subsequent reductive amination would then again enable the synthesis of the desired pair of diastereomers. However, the fluorination step once again proved to be the bottleneck of this sequence, yielding less than 10% of desired product (Scheme 23).² The partial reduction using DIBAL-H was not attempted as only small amounts of compound (±)-**31** were obtained.

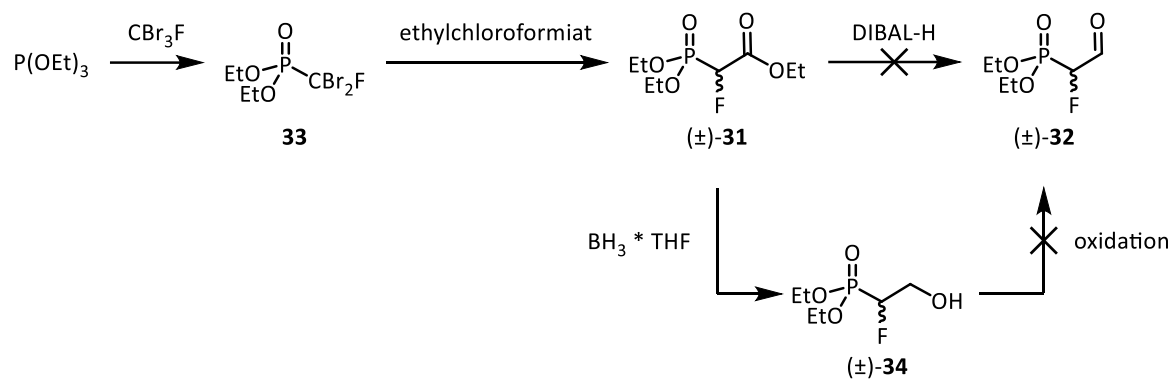


Scheme 23 - Electrophilic fluorination of triethyl phosphonoacetate using Selectfluor™.

The strategy for fluorine incorporation was thus again changed inspired by Patois and Savignac, who started with an Arbuzov reaction between triethyl phosphite and CBr₃F.⁴⁴ The corresponding phosphonate successively attacks ethyl chloroformate to yield (±)-**31**, albeit only in a very low yield of 7% (Scheme 24). The partial reduction of ester (±)-**31** using DIBAL-H did not yield any desired product. As a direct reduction towards the desired aldehyde was not feasible, the phosphonate ester was completely reduced using borane tetrahydrofuran to obtain the corresponding alcohol (±)-**34**. The reaction worked out pleasantly with a yield of

² Isolated yield could not be determined, due to product loss by spilling. ³¹P NMR: δ_P: 10.06 (d, J_{PF} = 72.2 Hz) ppm. ¹⁹F NMR δ_F: -210.44 (d, J_{FP} = 72.2) ppm.

36%, enabling an attempt for the subsequent oxidation to achieve the aldehyde [(±)-**32**]. Unfortunately, neither Dess-Martin-Periodinan-, nor Swern conditions could successfully give (±)-**32** (Scheme 24). Due to time constraints, no further experiments were possible.

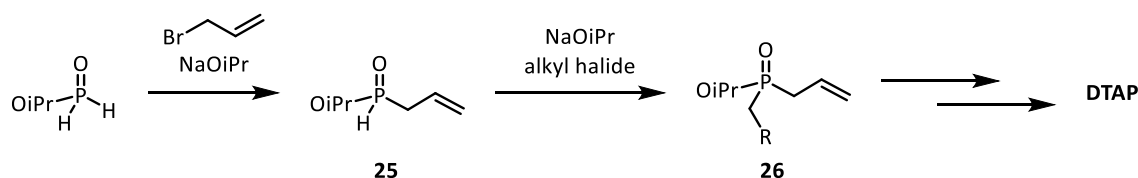


*Scheme 24 - Attempted synthesis of fluorinated aldehyde (±)-**32**; tested oxidation techniques: Dess-Martin-Periodinane and Swern oxidation.*

3 Conclusion and Outlook

Finally, an Arbuzov approach with significantly different conditions to typical standard procedures was able to lead to the desired compound **NPEP**, albeit in admixture with high quantities of triethyl phosphate. The subsequent TMSBr-mediated deprotection also proved to be victorious at yielding **NPEP**. The current methodology struggles however with purification of the final compound as phosphate esters formed during the key step of the synthesis. Future attempts of purification are planned to be executed using an ion exchange resin for chromatographic resolution. Additionally, it seems likely that performing the Arbuzov reaction under inert atmosphere may reduce the formation of impurities. The biological assays planned with this compound, will be performed by the group of Prof. David Zechel (Queen's University, Kingston, Canada) as soon as sufficient amounts of pure **NPEP** are available.

The proposed synthetic strategy towards **DTAP** was not successful, arguably due to the poor condition of the available magnesium. As there are multiple publications discussing Grignard reactions at a phosphorus centre^{39,45,46}, future attempts using a fresh container of Grignard-grade magnesium seem promising. A second approach, starting from phosphinic acid also failed at producing the desired compound. The underlying publication⁴⁰ discusses a procedure starting with the deprotonation of isopropyl-protected phosphinic acid which subsequently attacks allyl bromide. An additional deprotonation facilitates the successive attack of a different alkyl halide (Scheme 25). It is planned to test this methodology again in the near future and afterwards functionalize the two side chains in order to produce the desired compound in sufficient yields for all planned biochemical experiments



*Scheme 25 – Proposed synthesis of **DTAP** starting from phosphinic acid using allyl bromide.*

The two standards, **TAP** and **DAP**, were synthesized successfully. If future attempts for the synthesis of **DTAP** are successful, the corresponding biological studies will be carried out by members of the group of Prof. Ditlev Brodersen (Aarhus University, Denmark).

The synthesis of **FAEP** via the proposed methodology turned out to be troublesome. It seems likely that the fluorination reaction suffers from low yields as a result of steric hindrance as both, the chiral auxiliary as well as SelectfluorTM bear rather large moieties. The tested work-around (Scheme 24) failed at producing the aldehyde necessary for the introduction of the chiral auxiliary. Despite this failure, it seems quite likely that the proposed method might present a promising option for future attempts at synthesizing this substance. Typically reported temperatures for DiBAL-H mediated reductions range from -70 to -80 °C. However, a comparable test-reaction of non-fluorinated triethylphosphonoacetat did only yield the anticipated aldehyde when being warmed to -40 °C. The product, if successfully synthesized in the future, would be biologically evaluated by the group of Prof. Alessio Peracchi (University of Parma, Italy).

4 Experimental

4.1 General statements

All of the used chemicals were obtained from Merck, abcr chemicals, BLDpharm, Tokyo Chemical Industry, Thermo Fisher Scientific and Fluorochem and were used without further purification.

Reactions necessitating elevated temperatures were heated using oil baths. The specified temperatures always refer to the temperature of the oil bath. Dry reagents were obtained by drying the compounds under high vacuum for at least 15 min. Three vacuum/argon purging cycles were performed to create inert conditions (argon atmosphere). All reactions were performed in dry solvents with the exception of water/organic solvent mixtures.

Thin layer chromatography (TLC) was performed using Merck silica gel 60 F₂₅₄ plates (0.25 mm thick coating). Product spots were visualized using UV light ("UV") or a vanillin stain [15 g vanillin in 250 mL EtOH and 2.5 mL H₂SO₄ (conc)]. *R_f* values are listed as follows: *R_f* (mobile phase) = value (visualization technique).

Compound purification by column chromatography was performed using a Biotage Isolera Prime® medium pressure liquid chromatography flash purification system, utilizing either self-filled (silica gel, 240-400 mesh, acquired from Machery-Nagel) or pre-packed Sfär-Duo separation cartridges. The mobile phase composition is typically given by percentage figures. The given value refers to the more polar solvent listed. If not otherwise stated, all fractions of the chromatography were collected and individually checked for their compound content by TLC.

NMR spectra were recorded either on a Bruker AV NEO 400 (¹H: 400.27 MHz, ³¹P: 162.03 MHz) or AV NEO NanoBay 400 (¹H: 400.13 MHz, ³¹P: 161.98 MHz, ¹⁹F: 376.50 MHz) spectrometer and the recorded spectra were referenced as in Table 3 Solvent residual peaks as references for NMR spectra. Coupling constants are given in Hz. Purities of compounds were estimated using ³¹P NMR spectra, or ¹H NMR spectra and were given in mol%; if no percentage is specified, the individual compound has an estimated purity >99 mol%.

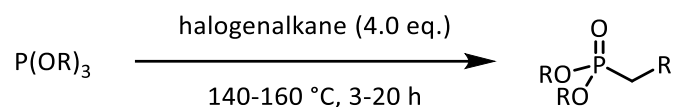
Table 3 Solvent residual peaks as references for NMR spectra.

nucleus	Solvent	Referenced to (residual)	Shift/ppm
^1H	CDCl_3	CHCl_3	7.26
	D_2O	HOD	4.79
	$\text{DMSO-}d_6$	$\text{CD}_3\text{S(O)CD}_2\text{H}$	2.50
^{31}P :	external H_3PO_4	H_3PO_4	0.00
^{19}F :	external CCl_3F	CCl_3F	0.00

Mass spectra were recorded either on an Thermo Scientific UltiMate 3000 RSLCnano system – capillary flow or an Ultimate 3000 HPLC system coupled with Bruker maXis UHR-TOF via electrospray ionization.

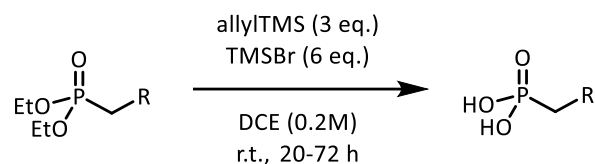
4.2 General Procedures

General procedure A - Arbuzov reactions



One equivalent of the stated trialkyl phosphite was mixed with 4 eq. of the respective haloalkane in a round bottom flask attached to a reflux condenser. The mixture was heated to 140-160 °C for 3 to 20 h as specified for the respective compound. The resulting mixture was placed into a 50-60 °C water bath and was dried in high vacuum (< 1 mbar) for 1 h. The residual crude was further purified as specified for each substance.

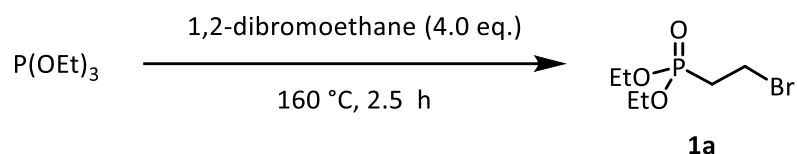
General procedure B - Deprotection of phosphonates



Allyltrimethylsilane (3 eq.) and TMSBr (6 eq.) were added to a stirred solution of phosphonate in 1,2-dichloroethane (DCE, final concentration 0.2M) under argon atmosphere. The mixture was stirred between 20 and 72 h at room temperature (r.t., 18-25 °C) as specified for each compound. The solvent was removed under high vacuum, the crude material was redispersed in dichloromethane (DCM) and subsequently concentrated *in vacuo* again (this step was performed twice). The remaining residue was dissolved in water, filtered through cotton wool, liberated from the solvent and dried by coevaporation with toluene followed by the specified procedure (either high vac or freeze drying). The pure product was obtained after extensive drying.

4.3 2-(4-Nitrophenoxy)ethyl phosphonic acid

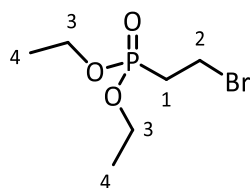
Diethyl 2-bromoethylphosphonate (**1a**)



Compound **1a** was prepared according to general procedure **A** from triethyl phosphite (20 mmol, 3.323 g, 1 eq.) at 160 °C for 2.5 h. The crude mixture was purified via vacuum distillation (b.p. 110 °C / 3 Torr; 160 °C oil bath) to obtain the desired substance (**1a**) as a colorless oil.

Yield: 4.03 g (equal to 16.1 mmol = 81%).

R_f (50% EtOAc in *n*-heptane) = 0.3 (vanillin).

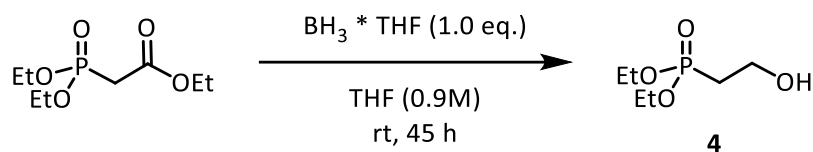


¹H NMR (400.27 MHz, CDCl₃, [42Nov2425/50]): δ_H: 4.11 (m, 4 H, H₃), 3.52 (m, 2 H, H₂), 2.37 (m, 2 H, H₁), 1.32 (m, 6 H, H₄) ppm.

³¹P NMR (162.03 MHz, CDCl₃, [42Nov2425/50]): δ_P 25.56 ppm.

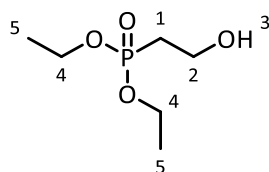
Recorded spectroscopic data are in agreement with those reported in the literature.⁴⁷

Diethyl 2-hydroxyethylphosphonate (**4**)



Borane tetrahydrofuran complex solution (30 mmol, 29.2 g, 33.3 mL, 0.9M in THF, 1 eq.) was added dropwise to triethyl phosphonoacetate (30 mmol, 6.73 g, 5.95 mL, 1 eq.) at room temperature under argon atmosphere. The reaction mixture was stirred for 45 h at room temperature, before the addition of EtOH (until the gas formation stopped) and concentrated after 30 min of stirring. The concentrate was diluted with water, washed twice each with *n*-heptane and diethyl ether. The aqueous phase was concentrated and dried by coevaporation with toluene to give the desired compound (**4**) as a colorless oil.

Yield: 5.993 g (equal to 26.3 mmol = 88%).

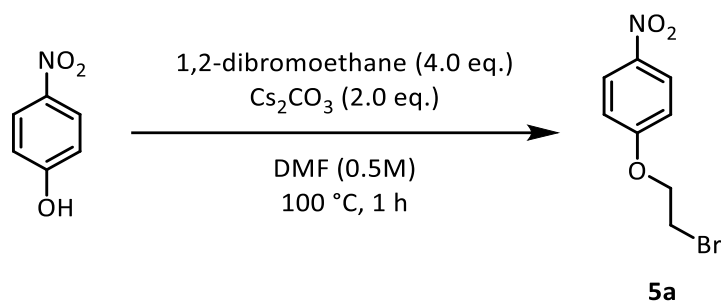


¹H NMR (400.27 MHz, CDCl₃, [42Nov0325/350]): δ_{H} : 4.20-4.04 (m, 4 H, H4), 3.90 (dt, $J_{\text{HP}} = 21.3$, $J_{\text{HH}} = 6.2$, 2 H, H2), 2.72 (broad s, 1 H, H3), 2.05 (dt, $J_{\text{HP}} = 17.3$, $J_{\text{HH}} = 6.2$, 2 H, H1), 1.34 (t, $J_{\text{HH}} = 7.1$, 6 H, H5) ppm.

³¹P NMR (162.03 MHz, CDCl₃, [42Nov0325/351]): δ_{P} 30.35 ppm.

Recorded spectroscopic data are in agreement with those reported in the literature.⁴⁸

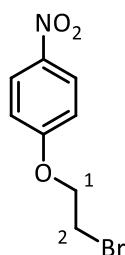
1-(2-Bromoethoxy)-4-nitrobenzene (5a)



1,2-dibromoethane (160 mmol, 30.058 g, 13.8 mL, 4 eq.) was added slowly to a stirred suspension of dry Cs_2CO_3 (80 mmol, 26.086 g, 2 eq.) and *p*-nitrophenol (40 mmol, 5.564 g, 1 eq.) in 80 mL DMF under argon atmosphere. The reaction vessel was heated to 100 °C for 1 h, the mixture was allowed to reach room temperature and was diluted with water. The product was extracted three times using EtOAc and the combined organic phases were washed once with brine, dried with Na_2SO_4 and filtrated. The concentrated product was further purified using column chromatography (50 g silica, 0-7% EtOAc/*n*-heptane in 3.5 CV; 7% for 10 CV; the first UV-active fraction was collected) to yield the desired species (**5a**) in admixture with an unknown impurity (5 mol%) as a slightly yellowish solid.

Yield: 5.91 g (molar purity: 95%, equal to 22.8 mmol = 57%).

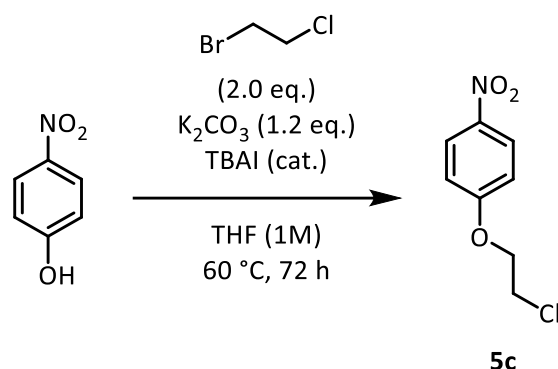
***R*_f** (in 40% EtOAc/ *n*-heptane): 0.58 (UV).



¹H NMR (400.27 MHz, CDCl_3 , [42Nov2525/140]): δ_{H} : 8.22 (m, 2 H, CH arom.), 6.98 (m, 2 H, CH arom.), 4.39 (t, J_{HH} = 6.2, 2 H, H1), 3.67 (t, J_{HH} = 6.2, 2 H, H2), ppm.

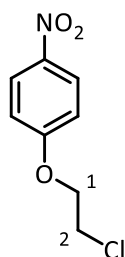
Recorded spectroscopic data are in agreement with those reported in the literature.³⁰

1-(2-Bromoethoxy)-4-nitrobenzene (5c)



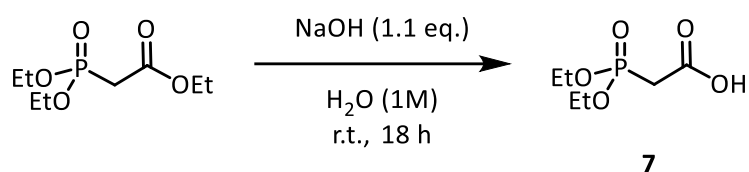
1-Bromo-2-chloroethane (20 mmol, 2.868 g, 1.66 mL, 2 eq.) was added dropwise to dry *p*-nitrophenol (10 mmol, 1.391 g, 0.94 mL, 1 eq.) and potassium carbonate (12 mmol, 1.658 g, 0.68 mL, 1.2 eq.) in 10 mL dry THF under argon atmosphere. After the addition of one spatula tip of TBAI, the mixture was stirred at 60 °C for 72 h. The yellow suspension was diluted with water, the phases separated and the aqueous layer extracted three times with DCM. The combined organic phases were washed once with water, dried using MgSO₄, filtered and concentrated under reduced pressure. The residue was subjected to column chromatography (50 g silica, EtOAc/ *n*-heptane: 1 CV 2%, 3 CV 2→40%, 9 CV 40→60%, fraction size: 18 mL; only UV-active fractions were collected) to afford the product (**5c**) as an off-white solid.

Yield: 707 mg, 3.51 mmol, 35%.



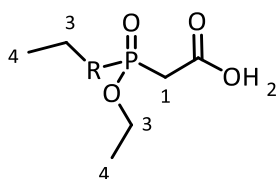
¹H NMR (400.27 MHz, CDCl₃, [42Sep2225/270]): δ_H: 8.22 (m, 2 H, CH arom.), 6.99 (m, 2 H, CH arom.), 4.33 (t, *J*_{HH} = 5.8, 2 H, H1), 3.86 (t, *J*_{HH} = 5.8, 2 H, H2) ppm.

Diethylphosphonoacetic acid (**7**)



NaOH (11 mmol, 440 mg, 1.1 eq.) was added to a solution of triethyl phosphonoacetate (10 mmol, 2.242 g, 1.98 mL, 1 eq.) in 100 mL of a 1:1 water/1,4-dioxane mixture. The mixture was stirred for 18 h before the dropwise addition of an aqueous 2M HCl solution until the suspension reached pH 1. The mixture was extracted three times using EtOAc, washed once with brine and once with water. The combined organic phases were dried using MgSO₄, filtered and concentrated under reduced pressure to afford the product (**7**) as a slightly yellowish oil.

Yield: 945 mg (equal to 4.82 mmol = 48.2%).

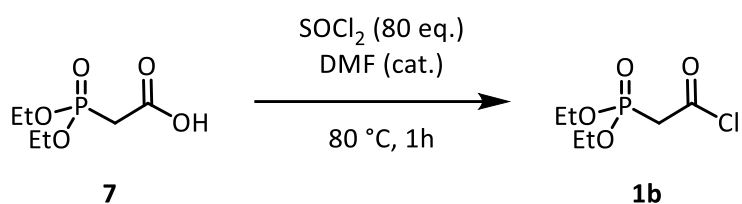


¹H NMR (400.27 MHz, CDCl₃, [42Nov1325/70]): δ_H: 9.73 (broad s, 1 H, H₂), 4.03 (m, 4 H, H₃), 2.99 (d, *J*_{HP} = 21.6, 2H, H₁), 1.35 (t, *J*_{HH} = 7.1, 6 H, H₄) ppm.

³¹P NMR (162.03 MHz, CDCl₃, [42Nov1325/71]): δ_P 21.13 ppm.

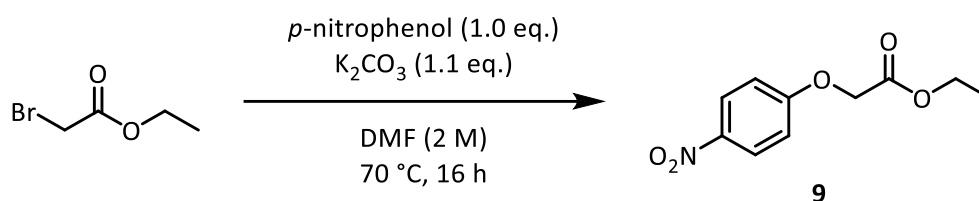
Recorded spectroscopic data are in agreement with those reported in the literature.⁴⁹

Diethyl 2-chloro-2-oxoethylphosphonate (**1b**)



Thionyl chloride (208 mmol, 24.746 g, 15.1 mL, 80 eq.) was added to diethyl phosphonoacetic acid **7** (2.6 mmol, 510 mg, 1 eq.) under argon atmosphere. The reaction mixture was heated to 80 °C for 1 h after the addition of 0.1 mL DMF and concentrated under reduced pressure to give the desired acyl chloride **8** as an orange oil which was directly used for the subsequent step without further purification.

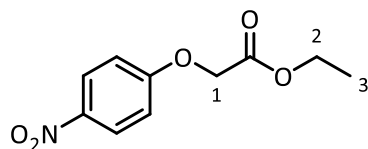
Ethyl 2-(4-nitrophenoxy)acetate (**9**)



Ethyl bromoacetate (20 mmol, 3.340 g, 2.2 mL, 1.0 eq.) was added to a suspension of dry potassium carbonate (22 mmol, 3.41 g, 1.1 eq.) and *p*-nitrophenol (20 mmol, 2.782 g, 1 eq.) in 10 mL dry DMF under argon. The mixture was stirred at 70 °C for 16 h. The yellow suspension was allowed to reach room temperature, diluted with water and the crude was extracted three times using EtOAc. The combined organic phases were washed two times with brine, dried using Na₂SO₄ and concentrated under reduced pressure to give the desired compound (**9**) in admixture with DMF (20 mol%) and starting material (10 mol%) as a colorless oil.

Yield: 4.46 g, (molar purity: 70%, equal to 13.9 mmol = 69%).

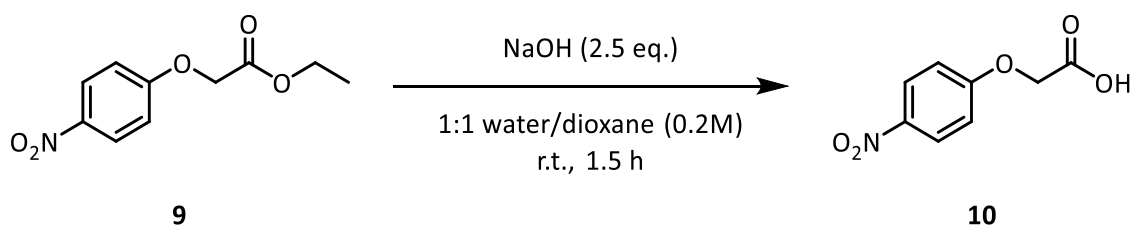
R_f (50% EtOAc in *n*-heptane) = 0.71 (UV).



¹H NMR (400.27 MHz, CDCl₃, [42Sep2525/220]): δ_H: 8.22-6.97 (m, 4 H, CH arom.), 4.72 (s, 2 H, H1), 4.29 (q, *J*_{HH} = 7.1, 2 H, H2), 1.30 (t, *J*_{HH} = 7.1, 3 H, H3) ppm.

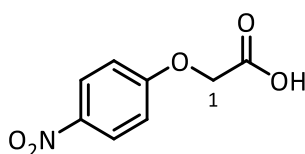
Recorded spectroscopic data are in agreement with those reported in the literature.³⁸

2-(4-Nitrophenoxy)acetic acid (**10**)



NaOH (49.5 mmol, 1.980 g, 2.5 eq.) was added to a solution of **9** (19.8 mmol, 4.459 g, 1.0 eq.) in 100 mL of a 1:1 water/1,4-dioxane mixture and the resulting suspension was stirred for 1.5 h at room temperature. An aqueous 1M HCl solution was added until pH 3 and the aqueous phase was extracted three times using EtOAc. The combined organic phases were washed once with brine and once with H₂O, dried using Na₂SO₄ and concentrated *in vacuo* to afford the product (**10**) as a brown solid.

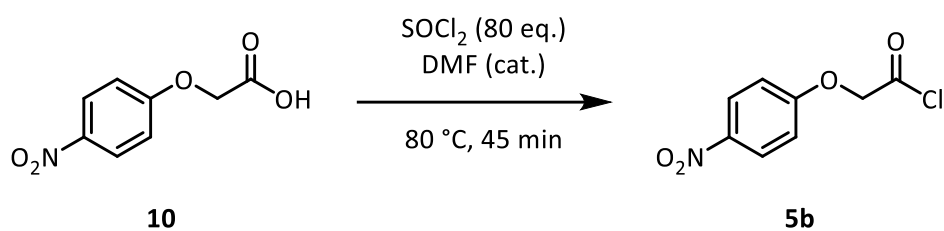
Yield: 3.63 g, 18.4 mmol, 93%.



¹H NMR (400.27 MHz, (CD₃)₂SO, [41Sep2525/360]): δ_H: 8.24-8.17, 7.16-7.10 (symmetrical m, 4 H, CH arom.), 4.88 (s, 2 H, H1).

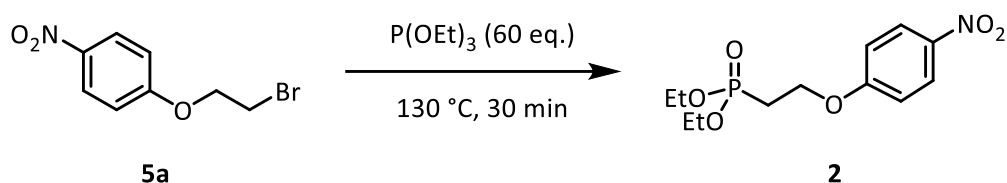
Recorded spectroscopic data are in agreement with those reported in the literature.⁵⁰

2-(4-Nitrophenoxy)acetyl chloride (**5b**)



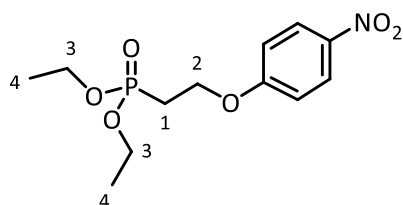
Thionyl chloride (331 mmol, 39.360 g, 24 mL, 80 eq.) as well as 0.1 ml DMF were added to **10** (17 mmol, 3.624 g, 1 eq.) under argon atmosphere and the reaction mixture was heated to 80°C for 1 h. The product **5b** was concentrated *in vacuo* and directly used for the subsequent reaction without purification or characterization.

Diethyl 2-(4-nitrophenoxy)ethylphosphonate (**2**)



5a (1 mmol, 246 mg, 1 eq.) was dissolved in 10.3 mL of triethyl phosphite (60 mmol, 9.970 g, 60 eq.) and the mixture was heated to $130\text{ }^\circ\text{C}$ for 30 min. Remaining triethyl phosphite was removed via distillation (oil bath at $50\text{ }^\circ\text{C}$ at 0.8 mbar) and a 50 mg aliquot of crude product was purified by preparative TLC (5% MeOH in DCM). The purified compound **2** was obtained as a colorless liquid.

Yield (preparative TLC): 2 mg (molar purity: 96%, equal to 0.0063 mmol = 0.63%).

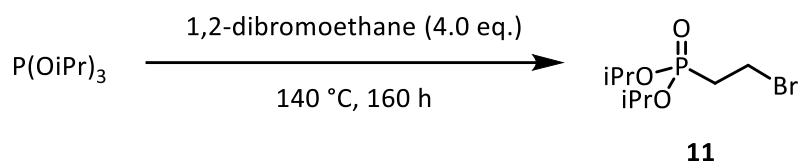


^1H NMR (400.27 MHz, CDCl_3 , [42Dec0225/380]): δ_{H} : 8.25-8.17, 7.01-6.93 (symmetrical m, 4 H, CH arom.), 4.26 (dt, $J_{\text{HP}} = 11.4$, $J_{\text{HH}} = 7.5$, 2 H, H2), 4.09 (m, 4 H, H3), 2.27 (dt, $J_{\text{HP}} = 18.7$, $J_{\text{HH}} = 7.5$, 2 H, H1), 1.29 (t, $J_{\text{HH}} = 7.1$, 6 H, H4) ppm.

^{31}P NMR (162.03 MHz, CDCl_3 , [42Dec0225/381]): δ_{P} 26.49 (product, 96%), -0.98 (Unknown impurity, 4%) ppm.

4.4 2-Tryptamine ethyl phosphonic acid

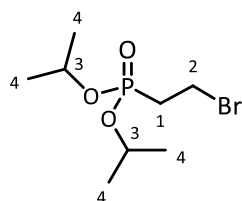
Diisopropyl 2-bromoethylphosphonate (**11**)



Compound **11** was prepared according to general procedure **A** from triisopropyl phosphite [40 mmol, 10 mmol, 2.082 g, 1 equivalent (eq.)] at 140 °C for 16 h. The crude product was purified using column chromatography [50 g silica, EtOAc/*n*-heptane: 1 cartridge volume (CV) 25%, 4 CV 25→100%, fraction size: 18 mL]. The product (**11**) was obtained as a colorless almost watery liquid, in admixture with several unknown impurities (approx. 13 mol%).

Yield: 2.211 g (purity: 87 mol%, equal to 7.04 mmol = 70%).

R_f (100% EtOAc) = 0.48 (vanillin).

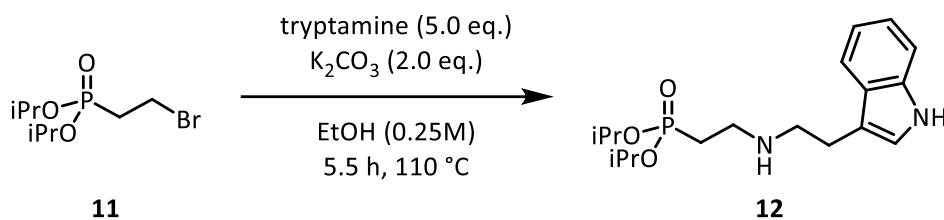


¹H NMR (400.27 MHz, CDCl₃, [42Jun0525/411]): δ_H: 4.70 (m, 2 H, H3), 3.51 (m, 2 H, H2), 2.34 (m, 2 H, H1), 1.32 (d, *J*_{HH} = 6.3, 12 H, H4) ppm.

³¹P NMR (162.03 MHz, CDCl₃, [42Jun0525/410]): δ_P 23.30 (product, 87%), 6.57 (unknown impurity, 3%), 2.32 (unknown impurity, 4%), -3.36 (unknown impurity, 5%) ppm.

Recorded spectroscopic data are in agreement with those reported in the literature.⁵¹

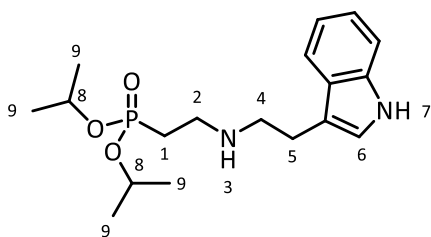
Diisopropyl (2-((2-(1H-indol-3-yl)ethyl)amino)ethyl)phosphonate (**12**)



Potassium carbonate (4 mmol, 553 mg, 2 eq.) was added to a mixture of dry tryptamine (10 mmol, 1.602 g, 5 eq.) and **11** (2 mmol, 546 mg, 1 eq.) in 8 mL EtOH under argon atmosphere. The suspension was refluxed for 5.5 h (110 °C), filtered, and the filter cake was washed twice with EtOH. The combined filtrates were concentrated under reduced pressure, and the resulting oil was diluted with EtOAc and water. The phases were separated and the aqueous phase was extracted three times using EtOAc. The combined organic phases were dried (Na_2SO_4), filtered and the residue was concentrated under reduced pressure. The crude mixture was purified by column chromatography (25 g silica, DCM/MeOH: 1 CV 4%, 10 CV 4→40%; only UV-active fractions were collected) to give the desired product (**12**) as an orange-brown oil in admixture with two unknown impurities (in total 6 mol% impurities).

Yield: 550 mg (purity: 94%, equal to 1.47 mmol = 73%).

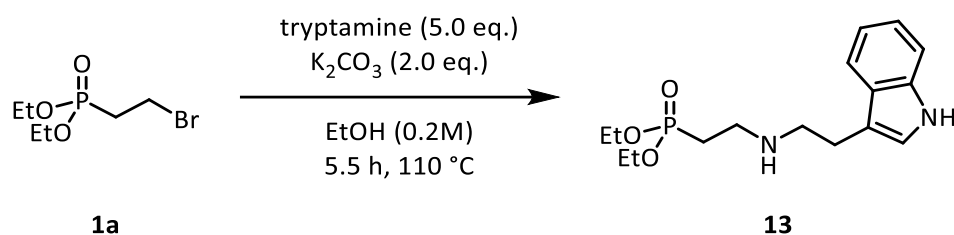
R_f (20% MeOH in DCM)= 0.65 (UV).



¹H NMR (400.27 MHz, CDCl_3 , [41Jun1825/360]): δ_{H} : 8.05 (s, 1 H, H7), 7.61 (m, 1 H, H6), 7.36 (m, 1 H, CH arom.), 7.19 (m, 1 H, CH arom.), 7.10 (m, 1 H, CH arom.), 7.05 (d, 1 H, CH arom.), 4.65 (m, 2 H, H8), 2.96 (m, 4 H, H4 + H5), 2.91 (dt, $J_{\text{HP}} = 13.7$, $J_{\text{HH}} = 6.9$, 2 H, H2), 1.91 (dt, $J_{\text{HP}} = 18.4$, $J_{\text{HH}} = 6.9$, 2 H, H1), 1.27 (m, 12 H, H9) ppm.

³¹P NMR (162.03 MHz, CDCl_3 , [41Jun1825/361]): δ_{P} 28.38 (product, 94%), 26.24 (unknown impurity, 3%), 15.20 (unknown impurity, 0.25%), -3.35 (unknown impurity, 3%) ppm.

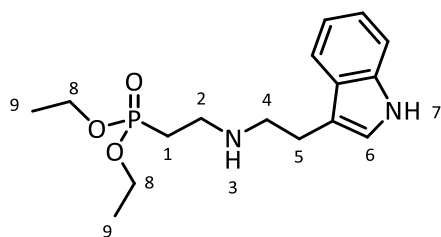
Diethyl (2-((2-(1H-indol-3-yl)ethyl)amino)ethyl)phosphonate (**13**)



Potassium carbonate (21.2 mmol, 2.930 g, 2 eq.) was added to a stirred solution of dry tryptamine (53.4 mmol, 8.554 g, 5 eq.) and **1a** (10.6 mmol, 2.597 g, 1 eq.) under argon atmosphere. The suspension was stirred for 15 h at reflux (110 °C). The cooled mixture was filtrated, and the filter cake was washed with EtOH. The solvent was removed *in vacuo*, and the resulting oil was diluted with EtOAc. The diluted product-containing solution was washed twice with water, and once with brine. The organic phase was dried using Na₂SO₄, filtrated and concentrated under reduced pressure. The crude material was purified using column chromatography (100 g silica, MeOH/DCM: 4 CV 10%, 4.2 CV 10→40%, fraction size: 18 mL; only UV-active fractions were collected) to yield the product (**13**) as a yellow oil as a mixture of rotamers in an estimated ratio of 1: 1.1.

Yield: 2.231 g (equal to 6.88 mmol = 65%).

R_f (4% MeOH in DCM) = 0.69 (UV).

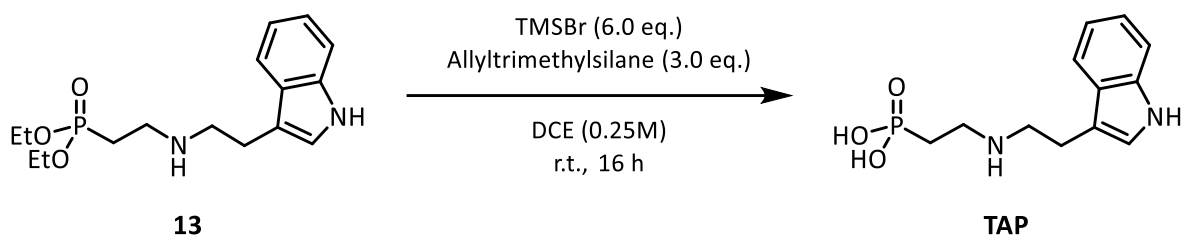


¹H NMR (400.27 MHz, CDCl₃, [42Aug2025/430]): δ_H: 8.09 (broad s, 1 H, H7), 7.62 (m, 1 H, CH arom.), 7.36 (symmetrical m, 1 H, CH arom.), 7.19 (m, 1 H, CH arom.), 7.11 (m, 2 H, CH arom.), 7.05 (m, 1 H, H6), 4.09-3.98 (m, 4 H, H8), 2.97-2.93 (m, 6 H, H2 + H4 + H5), 1.95 (dt, *J*_{HP} = 18.3, *J*_{HH} = 7.2, 2 H, H1), 1.27 (t, *J*_{HH} = 7.2, 6 H, H9) ppm.

³¹P NMR (162.03 MHz, CDCl₃, [42Aug2025/431]): δ_P 30.49 ppm.

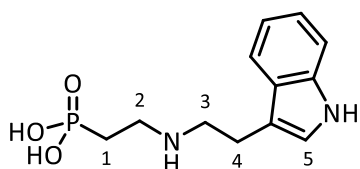
Recorded spectroscopic data are in agreement with those reported in the literature.²⁶

2-Tryptamine ethyl phosphonic acid (TAP)



TAP was prepared according to general procedure **B** from **13** (3.7 mmol, 1.200 g, 1 eq.) for 16 h. The product (**TAP**) was obtained as a mixture of rotamers with an estimated ratio of 1 : 1.1 as a light brown powder after lyophilization from water.

Yield: 836 mg, (equal to 3.12 mmol = 84%).



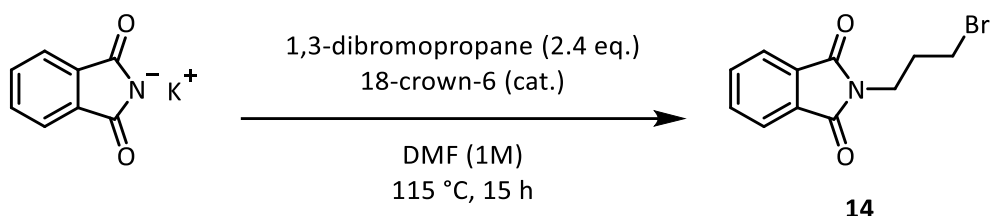
¹H NMR (400.27 MHz, D₂O [42Sep1125/470]): δ_{H} : 7.70 (m, 1 H, CH arom.), 8.12 (m, 1 H, CH arom.), 7.34 (s, 1 H, H5), 7.29 (m, 1 H, CH arom.), 7.21 (m, 1 H, CH arom.), 3.43 (t, $J_{\text{HH}} = 7.1$, 2 H, H4), 3.27 (m, 2 H, H2), 3.22 (m, 2 H, H3), 1.98 (m, 2 H, H1) ppm.

³¹P NMR (162.03 MHz, D₂O, [42Sep1125/471]): δ_{P} 18.74 ppm.

Recorded spectroscopic data are in agreement with those reported in the literature.²⁶

4.5 *N*-Dansyl-3-aminopropyl phosphonic acid

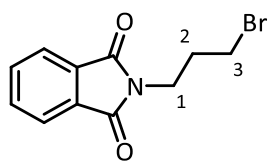
N-(3-Bromopropyl)phthalimide (**14**)



1,2-dibromoethane was added dropwise to a stirred suspension of dry potassium phthalimide (9.85 mmol, 1.824 g, 1 eq.) and one spatula tip of 18-crown-6 (1,4,7,10,13,16-hexaoxacyclooctadecan) in 10 mL dimethyl formamide (DMF) under argon atmosphere. The mixture was stirred for 18 h at 100 °C, filtered through Celite®, diluted with water and extracted three times using DCM. The combined organic phases were washed twice with brine, once with water, dried (Na₂SO₄), filtered and concentrated *in vacuo*. The crude product was purified using column chromatography (100 g silica, EtOAc/*n*-heptane: 5 CV 10→12%, 1 CV 12%, 6 CV 12→30%, 6 CV 30→60%, fraction size: 18 mL) Fractions 2-15 were combined and concentrated under reduced pressure to give the desired product (**14**) as a white fluffy solid.

Yield: 1.86 g, (equal to 6.94 mmol = 71%).

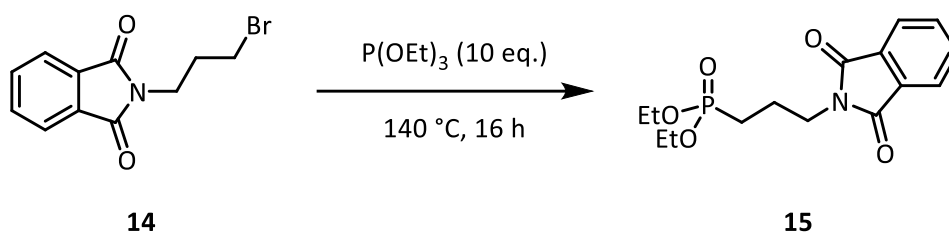
R_f (50% EtOAc in *n*-heptane) = 0.63 (UV).



¹H NMR (400.27 MHz, CDCl₃, [42Aug1425/550]): δ_H: 7.88-7.69 (m, 4 H, CH arom.), 3.84 (t, *J*_{HH} = 6.8, 2 H, H1), 3.42 (t, *J*_{HH} = 6.8, 2 H, H3), 2.26 (q, *J*_{HH} = 6.8, 2 H, H2) ppm.

Recorded spectroscopic data are in agreement with those reported in the literature.⁵²

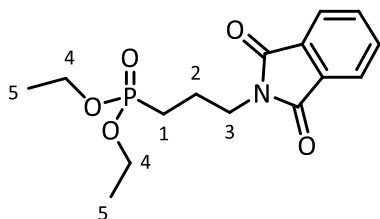
Diethyl (3-(phthalimide)propyl)phosphonate (**15**)



Compound **15** was synthesized according to general procedure A from **14** (5.74 mmol, 1.540 g, 1 eq.) using triethyl phosphite (57.4 mmol, 9.537 g, 9.93 mL, 10 eq.) at 140 °C for 16 h. The crude product was purified *via* column chromatography (100 g silica, EtOAc/*n*-heptane: 5 CV 10→15%, 1 CV 18%, 8 CV 18→70%, 4 CV 70→100%, 3 CV 100%, fraction size: 18 mL; only UV-active fractions were collected) to give the desired product (**15**) alongside an unknown impurity (30 mol%) as an oil.

Yield: 2.070 g (purity: 70 mol%, equal to 4.5 mmol = 78%).

R_f (60% EtOAc in *n*-heptane)= 0.19 (UV).

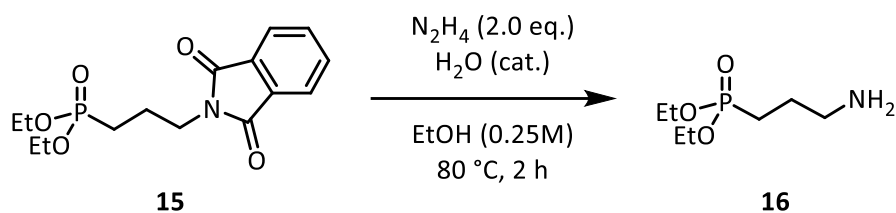


¹H NMR (400.27 MHz, CDCl₃, [41Aug2125/410]): δ_H: 7.87-7.80, 7.75-7.69 (symmetrical m, 4 H, CH arom.), 4.19-4.00 (m, 4 H, H₄), 3.75 (t, *J*_{HH} = 7.1, 2 H, H₃), 2.07-1.93 (m, 2 H, H₁), 1.84-1.67 (m, 2 H, H₂); 1.35-1.28 (m, 6 H, H₅) ppm.

³¹P NMR (162.03 MHz, CDCl₃, [41Aug2125/411]): δ_P 30.72 (product, 70%), 33.63 ppm (unknown impurity, 30%) ppm.

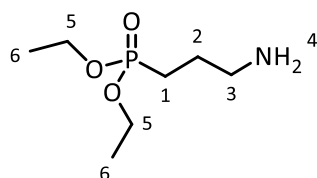
Recorded spectroscopic data are in agreement with those reported in the literature.⁵³

Diethyl 3-aminoethyl phosphonate (**16**)



Hydrazine (10.2 mmol, 511 mg, 1 mL, 50% in water, 2 eq.) was added to **15** (5.1 mmol, 1.659 g, 1 eq.) dissolved in 20 mL EtOH and the mixture was stirred at 80 °C for 2 h. The cooled suspension was filtrated through cotton wool and concentrated under reduced pressure. The remaining liquid was diluted with DCM, filtered and concentrated *in vacuo* to give the desired product (**16**) in admixture with an unknown impurity (12 mol%) as a yellow liquid.

Yield: 549 mg (molar purity: 88%, equal to 2.48 mmol = 49%).

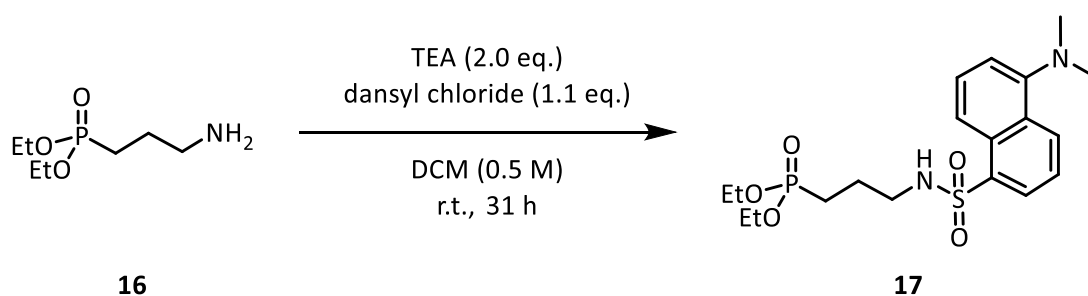


¹H NMR (400.27 MHz, CDCl₃, [42Aug2225/430]): δ_{H} : 4.09 (m, 4H, H5), 2.78 (m, 2 H, H4), 1.85-1.67 (m, 6 H, H1 + H2 + H3), 1.32 (t, $J_{\text{HH}} = 7.1$, 6 H, H6) ppm.

³¹P NMR (162.03 MHz, CDCl₃, [42Aug2225/431]): δ_{P} 33.63 (unknown impurity, 12%) 32.25 (product, 88%) ppm.

Recorded spectroscopic data are in agreement with those reported in the literature.⁵³

Diethyl (N-dansyl)-3-aminoethyl phosphonate (**17**)



Dansyl chloride (2.75 mmol, 742 mg, 1.1 eq.) in 5 mL DCM and triethyl amine (TEA, 5 mmol, 506 mg, 0.07 mL 2 eq.) were added one after the other to dry **16** (2.5 mmol, 488 mg, 1 eq.) in 5 mL DCM under argon atmosphere. After 31 h of stirring at room temperature, water was added, the phases separated and the aqueous phase extracted three times using DCM. The combined organic phases were washed once with brine, dried (Na₂SO₄), filtered and concentrated *in vacuo*. The product was obtained after purification using three consecutive chromatographic separations:

Column 1: 10 g silica, MeOH/DCM: 4 CV 5%, 4 CV 5→10%, 4 CV 10→20%, 4 CV 20→40%; fraction size = 18 mL, only UV-active fractions were collected.

An analytical sample was further purified by two additional chromatographic runs as follows.

Fractions 3-10 contained product $R_f = 0.24$ (100% EtOAc, UV) were concentrated and subjected to a third separation.

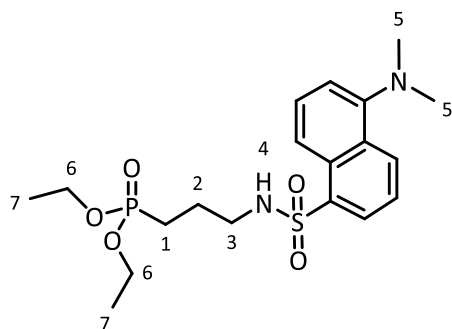
Column 2: 50 g silica, MeOH/DCM: 4 CV 5%, 4 CV 5→10%, 4 CV 10→20%, 4 CV 20→40%; fraction size = 18 mL, only UV-active fractions were collected.

Fractions 6-12 contained product, were concentrated and subjected to a third separation.

Column 3: 100 g silica, EtOAc/*n*-heptane, 20 CV 50-100%; fraction size = 18 mL, only UV-active fractions were collected.

All UV active fractions were collected and concentrated to give the desired product (**17**) as an off-white solid as a mixture of rotamers with an estimated ratio of 1 : 0.8.

Yield: 673 mg (equal to 1.57 mmol = 63%).

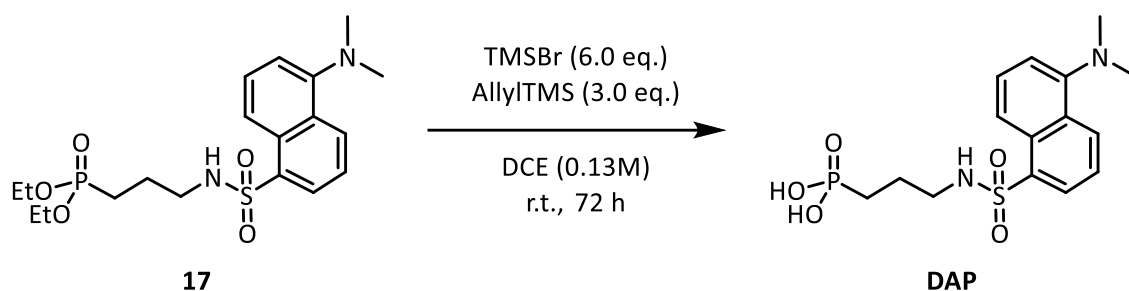


¹H NMR (400.27 MHz, CDCl₃, [42Aug2925/140]): δ_{H} : 8.53 (m, 1 H, CH arom.), 8.28 (m, 1 H, CH arom.), 8.24 (m, 1 H, CH arom.), 7.57 (m, 1 H, CH arom.), 7.51 (m, 1 H, CH arom.), 7.19 (m, 1 H, CH arom.), 5.20 (broad t, $J_{\text{HH}} = 6.2$, 1H, H4), 4.02 (m, 4 H, H6), 2.99 (dt, $J_{\text{HP}} = 6.2$, $J_{\text{HH}} = 6.5$, 2 H, H1), 2.88 (broad s, 6H, H5), 1.80-1.60 (m, 4 H, H3 + H2), 1.27 (t, $J_{\text{HH}} = 7.1$, 6 H, H7) ppm.

³¹P NMR (162.03 MHz, CDCl₃, [42Aug2925/141]): δ_{P} 31.42 ppm.

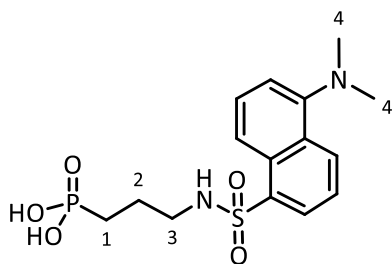
Recorded spectroscopic data are in agreement with those reported in the literature.⁵⁴

N-dansyl-3-aminopropyl phosphonic acid (DAP)



DAP was prepared according to general procedure **B** from **17** (0.83 mmol, 355 mg, 1.0 eq.) for 72 h. The product (**DAP**) was obtained as a salmon-pink, flaky solid as a mixture of rotamers with an estimated ratio of 1 : 1 after lyophilization from water.

Yield: 338 mg (equal to 0.91 mmol, quantitatively).



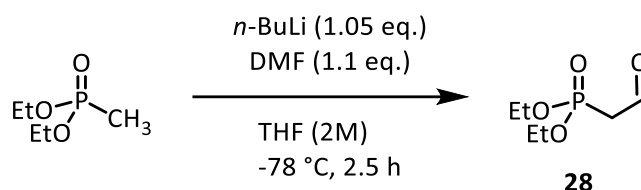
¹H NMR (400.27 MHz, D₂O, [42Sep0125/160]): δ_{H} : 8.82 (m, 1 H, CH arom.), 8.47 (m, 1 H, CH arom.), 8.42 (m, 1 H, CH arom.), 8.11 (m, 1 H, CH arom.), 7.94 (m, 2 H, CH arom.), 3.53 (m, 6 H, H₄), 3.03 (t, J_{HH} = 6.8, 2 H, H₃), 1.57-1.44 (m, 2 H, H₁), 1.38-1.26 (m, 2 H, H₂) ppm.

³¹P NMR (162.03 MHz, D₂O, [42Sep0125/161]): δ_{P} 26.33 ppm.

Recorded spectroscopic data are in agreement with those reported in the literature.²⁷

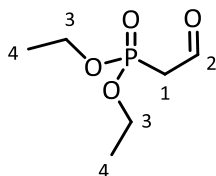
4.6 (*R*)- And (*S*)-2-amino-1-fluoroethylphosphonate

Phosphonoacetaldehyde (**28**)



Diethyl methylphosphonate (30 mmol, 4.56 g, 4.4 mL, 1.0 eq.) was added dropwise to a cooled solution of $n\text{-BuLi}$ (31.5 mmol, 8.73 g, 12.6 mL, 2.5 M, 1.05 eq.) in 13 mL dry THF under argon at $-78\text{ }^{\circ}\text{C}$. After 90 min of constant stirring at $-78\text{ }^{\circ}\text{C}$, DMF (33 mmol, 2.41 g, 1.1 eq.) was added dropwise. The mixture was stirred for additional 30 min (until warmed to $-60\text{ }^{\circ}\text{C}$) before removal of the cooling bath. After reaching room temperature, an aqueous 2 M HCl solution was added until pH 1. The crude was extracted three times using DCM. The combined organic phases were dried over MgSO_4 , filtered and concentrated. Bulb-to-bulb distillation (b.p. $65\text{--}90\text{ }^{\circ}\text{C}$ / 0.37 mbar) afforded the product (**28**) in admixture with starting material (18 mol%) as well as several unknown impurities (in total 20 mol%) as a pinkish oil.

Yield: 4.07 g (molar purity: 77%, equal to 17.4 mmol = 58%).

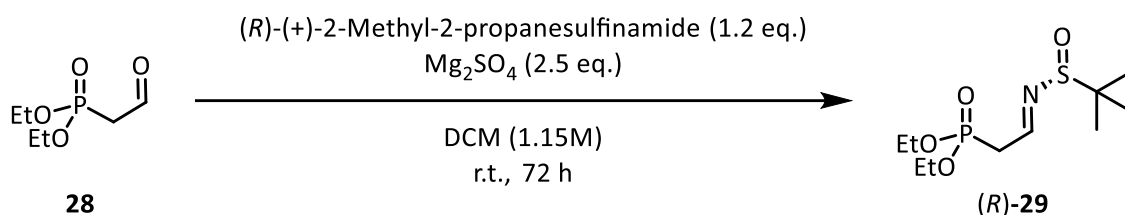


^1H NMR (400.27 MHz, CDCl_3 , [42Oct2425/110]): δ_{H} : 9.67 (m, 1 H, H2), 4.25–4.08 (m, 4 H, H3), 3.10–3.04 (dd, $J_{\text{HP}} = 21.8$, $J_{\text{HH}} = 3.3$, 2 H, H1), 1.35 (m, 6 H, H4) ppm.

^{31}P NMR (162.03 MHz, CDCl_3 , [42Oct2425/111]): δ_{P} : 30.48 (starting material, 18%), 22.18 (unknown impurity, 14%), 18.86 (product, 77%) ppm.

Recorded spectroscopic data are in agreement with those reported in the literature.⁵⁵

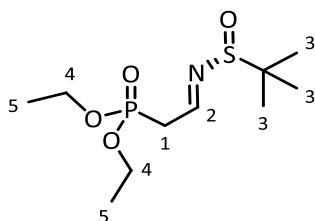
Diethyl (*R*)-(2-((*tert*-butylsulfinyl)imino)ethyl)phosphonate [(*R*)-**29**]



Dry MgSO_4 (27.5 mmol, 3.31 g, 2.5 eq.) as well as **28** (11 mmol, 1.98 g, 1.0 eq.) were dissolved in 9.5 mL DCM under argon before addition to (*R*)-(+)-2-methyl-2-propanesulfinamide (13.2 mmol, 1.63 g, 1.2 eq.). The mixture was stirred for 72 h, filtered over Celite®, concentrated *in vacuo* and purified using column chromatography (25 g silica, 12 CV 100% EtOAc, fraction size: 18 mL). The product (**29**) was obtained as a slightly yellowish oil in admixture with diethyl methyl phosphite (16 mol%) as well as unknown impurities (in total 11 mol%).

Yield: 2.16 g (molar purity: 72 %, equal to 5.48 mmol = 50%).

R_f (100% EtOAc) = 0.21 (UV).

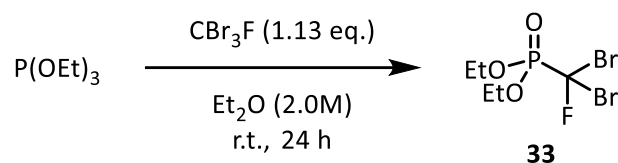


^1H NMR (400.27 MHz, CDCl_3 , [41Oct2725/101]): δ_{H} : 7.99 (m, 1 H, H2), 4.21-4.08 (m, 4 H, H4), 3.22- 3.06 (AB-system; A-part: ddd, $J_{\text{HP}} = 22.5$, $J_{\text{HH}} = 6.0$, $J_{\text{HH}} = 5.5$; B-part: ddd, $J_{\text{HP}} = 22.1$, $J_{\text{HH}} = 6.0$, $J_{\text{HH}} = 6.1$, 2 H, H1), 1.36-1.30 (m, 6 H, H5), 1.32-1.20 (m, 9 H, H3) ppm.

^{31}P NMR (162.03 MHz, CDCl_3 , [41Oct2725/101]): δ_{P} : 30.50 (diethyl methylphosphite, 16%), 22.58 (unknown impurity, 2%), 21.55 (product, 72%), 21.36 (unknown impurity, 2%) ppm.

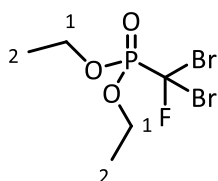
Recorded spectroscopic data are in agreement with those reported in the literature.⁵⁶

Diethyl dibromofluoromethylphosphonate (**33**)



Triethyl phosphite (9.91 mmol, 1.65 g, 1.7 mL, 1.0 eq.) was dissolved in 5 mL Et₂O under argon atmosphere. CBr₃F (11.2 mmol, 3.04 g, 1.1 mL, 1.1 eq.) was added and the mixture was stirred for 24 h at room temperature. The pale-yellow solution was subsequently concentrated under reduced pressure and purified by bulb-to-bulb distillation (b.p. 90-100 °C/3 Torr) to afford the desired product (**33**) in admixture with several unknown impurities (in total 19 mol%) as a yellowish oil.

Yield: 504 mg (molar purity: 82%, equal to 1.26 mmol = 13%).

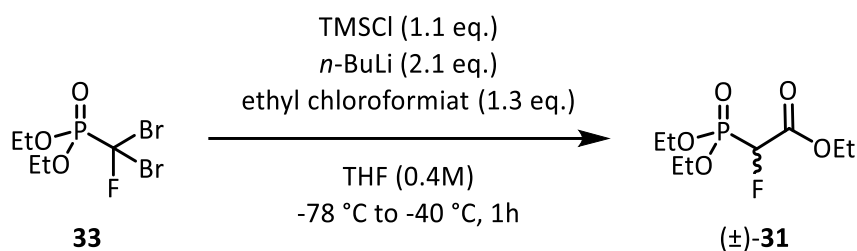


¹H NMR (400.27 MHz, CDCl₃, [42Nov1025/130]): δ_H: 4.49-4.43 (m, 4 H, H1), 1.42 (t, *J*_{HH} = 7.1, 6 H, H2) ppm.

³¹P NMR (162.03 MHz, CDCl₃, [42Nov1025/131]): δ_P: 1.72, 1.25 (product, d, *J*_{PF} = 76.3, 82%), -0.89 ppm (unknown impurity, 13 %), -13.15 (unknown impurity, 6%) ppm.

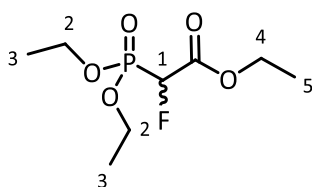
Recorded spectroscopic data are in agreement with those reported in the literature.⁵⁷

(±)-Ethyl 2-(diethoxyphosphoryl)-2-fluoroacetate [(±)-**31**]



n-BuLi (8.4 mmol, 2.328 g, 3.36 mL, 2.5 mol/L in hexane, 2.1 eq.) was added dropwise to a solution of **33** (4 mmol, 1.312 g, 1 eq.) in 10 mL dry THF at -78 °C under argon atmosphere. After 30 min of constant stirring at -78 °C, ethyl chloroformate (5.2 mmol, 1.135 g, 0.495 mL, 1.3 eq.) was added slowly and the mixture was allowed to reach -30 °C in the cooling bath. Then, 1.5 mL EtOH was added, and the suspension was poured on a mixture of ice, 10 mL DCM and 10 mL of an aqueous 2M HCl solution. The phases were separated and the aqueous layer extracted three times using DCM. The aqueous phase was concentrated to half its volume, and the residue was extracted additional three times using DCM. The combined organic phases were dried using MgSO₄, filtered and concentrated under reduced pressure. The crude compound was bulb-to-bulb distilled (b.p. 94-114 °C, 0.61 mbar) to afford the product (±)-**31** as a colorless oil.

Yield: 606 mg (equal to 2.05 mmol = 51%).

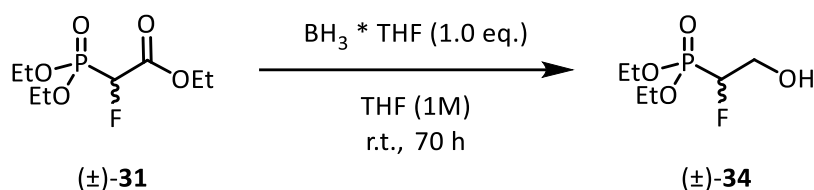


¹H NMR (400.27 MHz, CDCl₃, [42Nov1025/130]): δ_H: 5.20 (dd, *J*_{HF} = 47.01, *J*_{HP} = 12.4, 1 H, H1), 4.34 (q, *J*_{HH} = 7.16, 2 H, H4), 4.25 (m, 4 H, H2), 1.41-1.29 (m, 9 H, H3 + H5) ppm.

³¹P NMR (162.03 MHz, CDCl₃, [42Nov1025/131]): δ_P: 10.28, 9.25 (d, *J*_{PF} = 70.3 Hz) ppm.

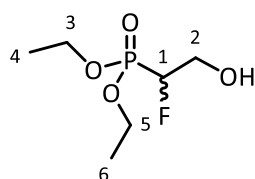
Recorded spectroscopic data are in agreement with those reported in the literature.⁴⁴

(±)-Diethyl (1-fluoro-2-hydroxyethyl)phosphonate [(±)-34]



Borane-THF complex (1.88 mmol, 876 mg, 2.09 mL, 0.9M in THF, 1 eq.) was added dropwise to (±)-**31** (1.88 mmol, 455 mg, 1 eq.) under argon atmosphere. The resulting solution was stirred for 70 h at room temperature. The reaction mixture was concentrated under reduced pressure and diluted with EtOAc and EtOH. After 1 h of stirring, the mixture was again concentrated, diluted with water and washed twice with *n*-heptane as well as diethyl ether before being concentrated. The product (±)-**34** was obtained as a white, almost colorless solid.³

Yield: 150 mg (equal to 0.676 mmol = 36%).



¹H NMR (400.27 MHz, CDCl₃, [41Nov1725/80]): δ_H: 4.89, 4.77 (symmetrical m, 1 H, H1), 4.30-4.17 (m, 4 H, H3 + H5), 1.37 (m, 6 H, H4 + H6) ppm.

³¹P NMR (162.03 MHz, CDCl₃, [41Nov1725/81]): δ_P 15.8 (d, *J* = 72.9) ppm.

³ The product yield as well as spectroscopic data were tentatively assigned as the product contained small amounts of water.

5 References

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