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Stereoselective Synthesis of Pantaphos

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Content

List of Abbreviations

EtOAc – Ethyl acetate

CV - Column volume

TBDMSCl – *tert*-Butyldimethylsilyl chloride

TFA- Trifluoroacetic acid

RT – Room temperature

NBS - *N*-Bromosuccinimide

AIBN - Azobisisobutyronitrile

HBF_4 (48 w/w% in H_2O) - Fluoroboric acid

pTsOH - *Para*-Toluenesulfonic acid

BOMCl - Benzyl chloromethyl ether

LDA - Lithium diisopropylamide

TsCl - 4-Toluenesulfonyl chloride

PG – Protecting group

HWE – Horner-Wadsworth-Emmons

(R,R)-Noyori-type catalyst - RuCl(p-cymene)[(*R,R*)-Ts-DPEN]

ATH - Asymmetric transfer hydrogenation

DMPU - *N,N*'-Dimethylpropylene urea

HMPA - Hexamethylphosphoramide

Abstract

Phosphonates represent a unique class of phosphorus compounds characterised by a direct carbon–phosphorus bond, endowing them with exceptional chemical and enzymatic stability. Among these, pantaphos—a naturally occurring secondary metabolite produced by *Pantoea ananatis*—has gained attention for its potent herbicidal and cytotoxic effects, including selective inhibition of glioma cell proliferation. This master's thesis deals with the stereoselective synthesis of pantaphos with the objective of evaluating the influence of both double bond geometry and the stereochemistry of the α-hydroxyphosphonate moiety on its potential biological activity after successful synthesis.

Thus, a stepwise synthetic route was developed, beginning with the preparation of an aldehyde precursor (**3**) from acetophenone, followed by a sequence of reactions including a Pudovik reaction (to give phosphonate **4**), TBDMS protection (to give TBDMS protected α-hydroxyphosphonate **5**), ozonolysis (to give β-ketophosphonate **6**), Horner–Wadsworth–Emmons olefination (to give diester **7**) to give a racemic α-hydroxyphosphonate (**8**) as key intermediate. Alternative deprotection methods employing TFA, pTsOH, and HBF_4 were explored for removal of the TBDMS ether, albeit without satisfactory outcome. The desired α - hydroxyphosphonate (**8**) was planned to be oxidised (to give α-ketophosphonate **9**), followed by an asymmetric transfer hydrogenation employing a Noyori-type catalyst to afford its optically pure α-hydroxyphosphonate (**8**) equivalent. Despite the successful execution of individual reactions up until the ozonolysis, purification of several intermediates proved challenging due to decomposition on silica during medium-pressure liquid chromatography (MPLC).

While full isolation of enantiomerically pure pantaphos was not achieved, the synthetic work provided valuable insights into the stability, reactivity, and handling of the α - hydroxyphosphonate intermediates presumably needed for its preparation. The study establishes a foundation for future optimization of stereoselective routes toward pantaphos and related bioactive phosphonates. Additionally, an alternative synthetic pathway starting

from maleic acid is proposed to circumvent the instability associated with early introduction of the olefinic moiety.

Deutsche Zusammenfassung

Phosphonate stellen eine einzigartige Klasse von Phosphorverbindungen dar, die durch eine direkte Kohlenstoff-Phosphor-Bindung gekennzeichnet sind, die ihnen eine außergewöhnliche chemische sowie enzymatische Stabilität verleiht. Unter diesen hat Pantaphos – ein natürlich vorkommender Sekundärmetabolit, der von *Pantoea ananatis* produziert wird – aufgrund seiner starken herbiziden und zytotoxischen Wirkung, einschließlich der selektiven Hemmung der Gliomzellproliferation, besondere Aufmerksamkeit erlangt.

Diese Masterarbeit befasst sich mit der stereoselektiven Synthese von Pantaphos mit dem Ziel, den Einfluss sowohl der Doppelbindungsgeometrie als auch der Stereochemie der α - Hydroxyphosphonat-Einheit auf die potenzielle biologische Aktivität nach erfolgreicher Synthese zu untersuchen.

Dazu wurde eine Syntheseroute entwickelt, beginnend mit der Herstellung eines Aldehyd-Vorläufers aus Acetophenon, gefolgt von einer Reaktionssequenz, die nach einer Pudovik-Reaktion (zur Erlangung des Phosphonates **4**) und TBDMS-Schützung des resultierenden racemischen α-Hydroxyphosphonats (**5**) eine wichtige Zwischenstufe lieferte. Diese konnte erfolgreich durch Ozonolyse (zur Erlangung des β-ketophosphonates **6**) sowie nachfolgende Horner-Wadsworth-Emmons-Olefinierung (zur Erlangung des Diesters **7**) zu einem racemischen Schlüsselintermediat umgewandelt werden. An diesem TBDMS-geschützen Diester (**7**) wurden verschiedene alternative Entschützungsmethoden getestet. Unter anderem TFA, pTsOH und HBF_4, jedoch führte keine der getesteten Methoden zu einer zufriedenstellenden Umsetzung und das isolierte Produkt stellte sich als äußerst instabil heraus. Das resultierende Zwischenprodukt, racemisches α-hydroxyphosphonate **8,** sollte anschließend oxidiert und einer asymmetrischen Transferhydrierung mit einem Noyori-artigen Katalysator unterzogen werden, um optisch reine α-Hydroxyphosphonat-

Intermediate zu gewinnen. Trotz des erfolgreichen Verlaufs der Einzelschritte bis zur Ozonolyse erwies sich die Aufreinigung mehrerer Zwischenprodukte aufgrund von Zersetzung auf Kieselgel während der Mitteldruckflüssigkeitschromatographie (MPLC) als schwierig.

Obwohl die Synthese von enantiomerenreinem Pantaphos nicht geglückt ist, lieferte die synthetische Arbeit wertvolle Erkenntnisse über die Stabilität, Reaktivität und Handhabung der α-Hydroxyphosphonat-Zwischenprodukte, die vermutlich für seine Herstellung erforderlich sind. Die Studie legt damit die Grundlage für zukünftige Optimierungen stereoselektiver Syntheserouten für Pantaphos und seiner verwandten Analoga. Letztendlich wird ein alternativer Syntheseweg ausgehend von Maleinsäure vorgeschlagen, um die mit der frühen Einführung der olefinischen Gruppe verbundene Instabilität zu umgehen.

Introduction

Phosphonates

For a long time, research about phosphorus biochemistry was dominated by studies about phosphates, containing four oxygen-phosphorous bonds (fig. 1)[1]. The reason for this bias lies in the importance of phosphates and their esters for higher life-forms, which rely on phosphates as exclusive source for the essential nutrient phosphorus. In contrast, phosphonic acids and their salts, phosphonates[2], were studied less, as their bio(geo)chemical importance was only discovered two decades ago. They are of the same oxidation state as phosphates but are characterised by a stable covalent carbon–phosphorus bond, which also imparts resistance to hydrolysis and phosphatase activity.

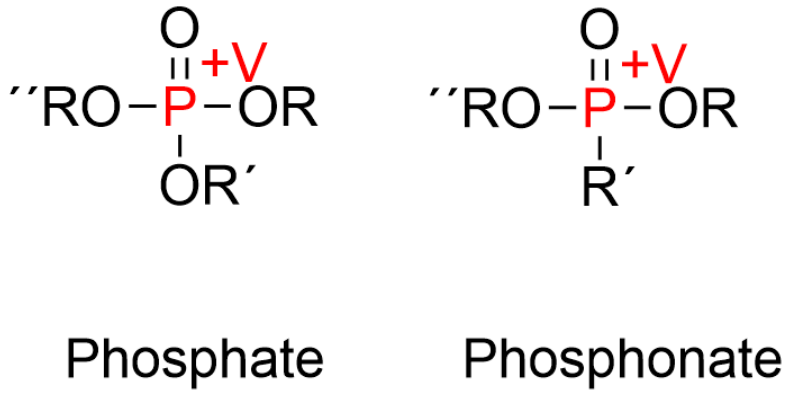


Figure 1 Phosphates (left) represent the fully oxidised phosphorous moiety. In phosphonates (right) one oxygen-phosphorous bond is substituted by a carbon-phosphorous bond.

Apart from their environmental importance as alternative phosphorus source for microbes, phosphonates are also a group of industrially and agriculturally important compounds. They serve as excellent chelating agents which explains their prominent role as industrial complexing agents (16.000 tons in Europe in 1999[3]) or scale inhibitors (12.000-13.000 tons in Europe in 1996[4]). Commonly used phosphonate based complexing agents, such as 1 – hydroxyethane- 1,1-diphosphonic acid (HEDP, fig. 2) are structurally similar to established aminopolycarboxylates like ethylenediaminetetraacetate (EDTA, fig. 2)[2].

In addition to their industrial bulk use relying on their metal ion complexing properties, the stability of phosphonates as well as their similarity to phosphates, which are highly abundant in eukaryotic cells, allows them to interact with numerous biochemical pathways[5]: Thus, phosphonates can also be used as antimetabolites with glyphosate certainly being the most important individual compound on the market (140.000 tons used in Europe in 2017[6])[7]. Glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSP synthase), which is responsible for the synthesis of aromatic amino acids in plants[8].

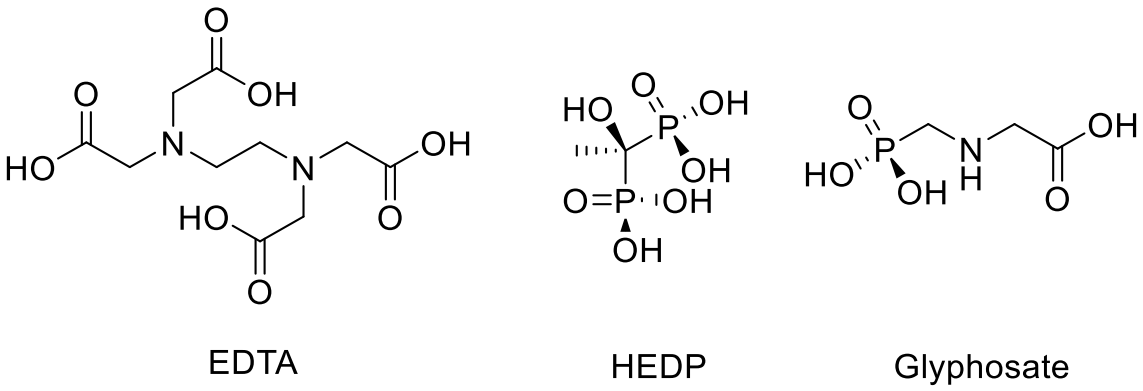


Figure 2 Ethylenediaminetetraacetate (EDTA), 1 – hydroxyethane- 1,1-diphosphonic acid (HEDP) and N-phosphomethylglycine (glyphosate).

Enzyme inhibition by phosphonates is usually specific, in cases where they are acting on enzymes that metabolise homologous phosphate substrates. Their mode of action in enzyme inhibition can however also be based on mimicry of naturally occurring carboxylates and they can serve as analogues of carboxylic acids, amino acids and peptides. Phosphonate moieties also actively imitate the transition state of peptide and ester bond hydrolysis and/or formation[9]. The possibility of potential applications is vast [7] considering the abundance of metabolic pathways involving phosphate esters, carboxylic acids and peptide-bond cleavage or formation in animals and plants.

Pantaphos

Pantaphos (fig. 3) is a microbial secondary metabolite synthesised by the pathogen *Pantoea ananatis* and was identified as the causative agent for tissue necrosis in important crops such as rice, corn, melon, pineapple and onions. In the latter pantaphos was shown to cause a disease known as onion centre rot[7].

An impressive demonstration of both potency and selectivity of pantaphos as an antimetabolite was conducted by *Polidore et al*. in 2021: when tested against 6 cancerous cell lines (human osteosarcoma, ovarian cancer, colon cancer, lung carcinoma, fibroblast cells, glioma cells) all but one (ovarian cancer) showed IC_{50} values between 6.0 µM to 37.0 µM with the glioma cell line being especially sensitive to pantaphos with an IC_{50} of 1.0 µM. Despite pantaphos' potent effect on human cancer cells, it did not influence proliferation of bacteria or fungi in culture media. However, when compared to known herbicides like glyphosate or phosphinothricin, pantaphos achieved similar to superior herbicidal potency[7].

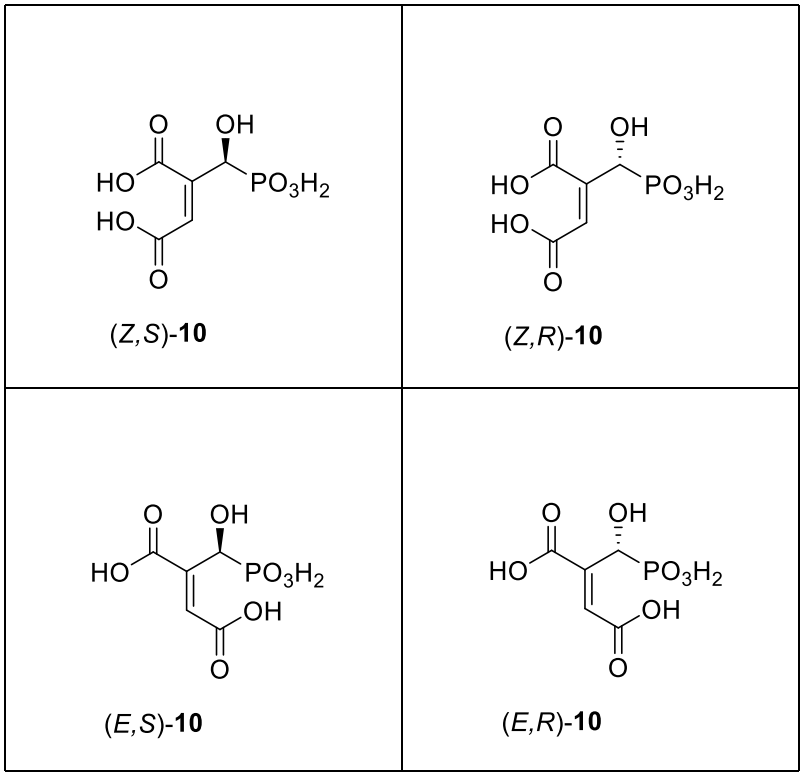


Figure 3 All enantiomeric and geometric permutations of pantaphos: (±)-(E/Z)-Pantaphos

Aims of the thesis

This master's thesis in the field of bioorganic chemistry focuses on the stereoselective synthesis of pantaphos (**10**, fig. 4) to determine the absolute configuration of the natural product and compare the herbicidal potential and pharmacological effects of both geometric isomers in combination with the absolute configuration at the stereocenter next to the phosphorus atom in different biochemical assays.

Results and Discussion

Proposed synthesis

The following chapter describes the envisaged synthetic strategies to yield pantaphos in a stereoselective manner (fig. 4): Pantaphos is an interesting molecule with only five carbon atoms, eight oxygen and one phosphorus atom. It contains a stereocenter and a double bond in conjugation with two carboxylic acids. Prior attempts to synthesise pantaphos showed that the double bond and thus resulting conjugation throughout most of the molecule causes problems. Additionally, the lability of the acid PGs complicated the synthesis by increasing the chance of undesired side product formation. One measure to circumvent the molecules' lability was to replace the ethyl ester groups on the phosphonate with isopropyl esters, which was shown to increase the stability. Additionally, the double bond should be introduced relatively late (**7**) to help in the planned synthesis. The stereocenter poses an additional challenge: when reacting the α -ketophosphonate (**9**) with either a (*R,R*)- or (*S,S*)-Noyori-type catalyst, optically pure alcohols are expected to result. However, to achieve a high ee, the oxidation of the racemic alcohol to the α -ketophosphonate must proceed with high to quantitative yield.

Thus, the planned synthetic pathway includes the following steps: The aldehyde (**3**) will be obtained starting from acetophenone in three steps by published means[10–12]. The aldehyde will then be reacted with phosphite under conventional Pudovik reaction conditions. The obtained phosphonate [(±)-**4**] needs to be protected with TBDMSCl under basic conditions to yield the TBDMS protected ether (**5**) which will further be subjected to ozonolysis to yield the β-ketophosphonate (**6**) and acetophenone as an equimolar side product. During the next step the second carboxylate moiety will be introduced as its corresponding ethyl ester in a HWE reaction to yield the diester (**7**). Deprotection of the TBDMS group is planned to be achieved using BF_3*(OEt_2) to again yield the racemic α -hydroxyphosphonate **8** required for the subsequent oxidation step. After successful oxidation of (±)-**8**, the obtained α - ketophosphonate **9** will be reduced again in a stereoselective way using an (*R,R*)-Noyori- type catalyst to yield the optically pure α-hydroxyphosphonate (**8**). A final global deprotection in 6 M aq. HCl is envisaged to give pantaphos as a single enantiomer (**10**).

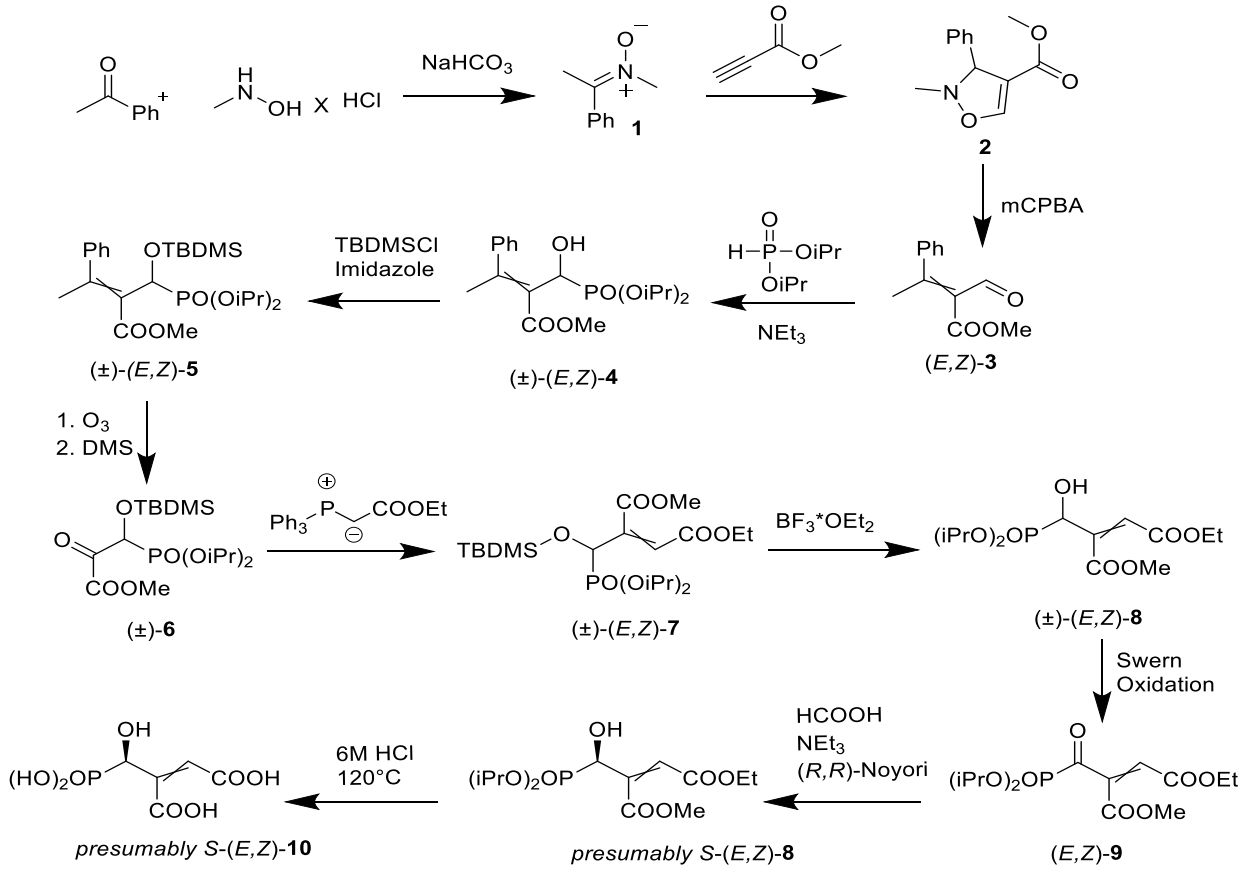


Figure 4 Proposed synthesis to yield (E/Z)-(S)-pantaphos.

Results

The proposed synthesis was successfully executed as outlined above (fig. 4), until the benzyl group was cleaved in the ozonolysis; it was possible to achieve enrichment of geometric isomers after the introduction of the phosphonate group into the precursor (**4**), according to ^{1}H and ^{31}P NMR measurements the ratio of geometric isomers was 61:39 with one isomer presenting as white crystals while the other isomer was a yellow oil even at – 20°C. Due to the lack of vicinal hydrogens at the double bond, no characterisation of E/Z isomers was possible therefore the two geometrical isomers are referred to as isomers A/B. The separation of geometrical isomers proved to be difficult, often requiring more than 20 CV per separation run as well as two consecutive runs with notable loss in yield.

Purification by normal phase MPLC worked as intended until the ozonolysis step giving (±)-**6**, when the crude applied to the MPLC to purify the β-ketophosphonate **6** decomposed on the column bed. The strip of the column bed first exposed to the crude turned dark and would barely migrate through the column, even with large quantities of polar solvent added. An additional finding supported the theory of decomposition, which was the finding of isopropanol throughout most fractions collected, regardless of polarity of the eluent gradient, suggesting that the isopropanol was formed on the column throughout the elution by acid-catalysed cleavage of the phosphonate esters rather than applied to the column as an impurity in the crude. While it is known that acidic silica can degrade phosphonates[13], some findings contradict that the column beds acidity caused the decay of the precursor: in unsuccessful experiments the protected diester **7** was exposed to acids of varying strength and concentration but did not react with any of them but left the PGs (TBDMS-ether and isopropylesters) unchanged.

Interestingly in the first attempt to purify the β-ketophosphonate **6**, the *A*-isomer decomposed nearly completely while the B-isomer stayed mostly intact, but in the next attempt the results were the opposite, contradicting any geometrical effects on the stability of the precursor on a silica bed at this stage. In all attempts to purifying the isomers at this step of the synthesis, some fractions were lavender coloured, the discolouration could not

be traced to an ^{1}H-NMR active impurity and vanished within a day, if left on the bench, however the discolouration persisted when stored at -20°C suggesting an instability of the purple hued impurity to either sunlight or higher temperatures. It is possible that the cleavage of the benzyl group led to a decreased stability of the precursor at this step.

The subsequent HWE reaction proved to be similarly difficult to purify by means of normal phase MPLC, leading to low yields (40%-50%) albeit of good purity (below 5% ^{1}H NMR active impurities).

While the compounds (±)-**6** and (±)-**7** could not be purified by normal phase MPLC on silica, attempts to use neutral bed types (aluminium oxide or C_{18}-modified silica gel) failed too, with the spots either not migrating at all (C_{18}) or the inability to dye the spots as usual (phosphonates are coloured yellow/red when heated in the presence of vanillin on Silica but do not show the same reaction on Al_2O_3) rendering purification attempts difficult due to detection problems.

After olefination, the deprotection of the TBDMS group to give the racemic α- hydroxyphosphonate (±)-**8** proved difficult, with many conventional methods failing: TFA[14] (excess) as well as *p*TsOH[15] (1.1 eq.) showed no conversion at all, while HBF_4[16] as a fluoride donor showed traces of the desired alcohol in the crude NMR spectra of the reaction mixture, which could unfortunately not be isolated. A mixture of NBS/AIBN[17] was tried to deprotect the alcohol too but gave no conversion either. Finally, the TBDMS group was cleaved by BF_3*(OEt_2) and the crude was purified by removal of solvents and evaporation of volatile impurities on the Schlenk line (3 mbar, 60°C oil bath, 15 min), most notably unreacted TBDMSCl or formed TBDMSF leading to low yields (~ 35%) of acceptable purity.

Oxidation attempts of (±)-**8** using either DMP or Cr^{6+} species yielded no **9** as determined by ^{1}H and ^{31}P NMR spectra. Swern oxidation however showed conversion according to crude NMR measurements as the signals formerly attributed to the CH-OH group (at 4.84 and 2.88 ppm, respectively) were missing and the ^{31}P signal shifted from 16.55 ppm on the α - hydroxyphosphonate to 14.65 ppm in the α– ketophosphonate. However, **9** could not be isolated due to aforementioned stability issues on normal phase MPLC. Notably,

α - hydroxyphosphonates usually show higher ^{31}P NMR shifts, ranging from 15.72 - 22.21 ppm[18], however isolated pantaphos was reported to show ^{31}P NMR shifts of around 15 ppm[7].

Due to difficulties in purification of **9**, the crude reaction mixture was used to test the ATH with a Noyori-type catalyst (RuCl(*p*-cymene)[(S,S)-Ts-DPEN]). However, despite a changed crude ^{1}H-NMR spectrum with the signals formerly attributed to the CH-OH group reappearing and the ^{31}P signal shifting to 20.45 ppm after ATH, again no purification was achieved.

Due to all the mentioned problems, another synthetic route was devised to avoid the stability issues presumably caused by the double bond conjugating most of the compound. Instead of introducing the double bond early in the synthesis, it was envisaged to be formed only in the penultimate step of the synthetic sequence.

Starting from malate **11** the hydroxyl-group as well as the CH-acidic proton in α position to the ester were planned to be deprotonated simultaneously by LDA followed by reaction with BOMCl in an Fráter-Seebach-type alkylation. In **12**, the BOM-PG is planned to be substituted by a tosylate to yield **13**. The benzyl group of the remaining BOM-PG would then be cleaved hydrogenolytically and the newly formed alcohol oxidised to the corresponding aldehyde **14**.

After introduction of the phosphorous-carbon bond using dimethyltrimethylsilyl phosphite (**15**) the alcohol would next be oxidised and subsequently selectively reduced by asymmetric transfer hydrogenation to form *R*-alcohol **16**. An elimination of the tosylate would yield **17** and subsequent final global deprotection with half concentrated HCl is envisaged to yield enantiopure pantaphos **18**.

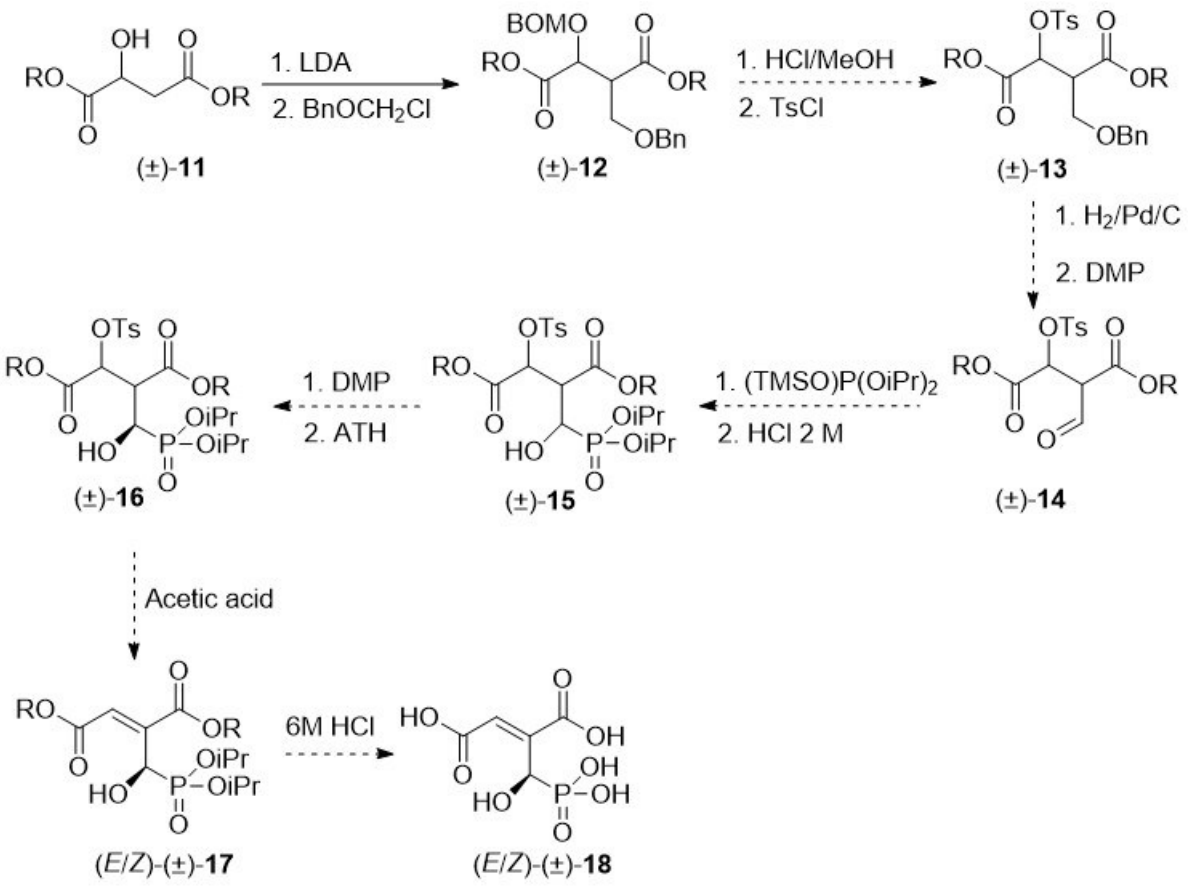


Figure 5 Alternative synthetic route to yield pantaphos starting from (±)-malate. R = isopropyl or ethyl residue.

The second proposed strategy could not be investigated far since the first double deprotonation did not occur as intended: LDA deprotonated the alcohol but failed to deprotonate the CH_2-group-position at the chosen conditions as demonstrated by addition of D_2O to the reaction mixture. Stronger bases (*n*-BuLi) succeeded at deprotonating both positions but ended up deprotonating the BOM PG and ester PGs as well. Weaker bases (NaH) failed to deprotonate the alcohol as well as the α position. Raising the reaction temperature from −78°C to −40°C did not change the reaction outcome. The originally one - pot reaction was split into a two-part reaction to circumvent potential interferences of compounds by first deprotonating and protecting the alcohol and subsequently deprotonating the α position, however this did not help decreases side reactions of deprotected esters or BOM PGs. To stabilize the ester PGs, isopropyl residues were used

instead of ethyl groups, that suffered from the same lability. In the literature HMPA was used as a dipolar aprotic co-solvent that stabilizes cations but not anions[19], thereby enhancing the reactivity of the deprotonated species to yield **12**. Due to HMPAs toxicity, DMPU (7.0 eq.) was used instead but did not yield **12**.

Experimental Section

General experimental

The used chemicals were obtained from abcr chemicals, Merck, TCI, Fluka or Acros. Thin layer chromatography was conducted using Merck silica gel 60 F_{254} glass plates, coated 0.25 mm thick. Visualisation of spots was performed using UV light and/or by dipping the plates in one of the below described stains followed by heating with a heat gun as indicated for the respective compounds.

Table 1 Composition of the used TLC stains.

Vanillin stain	15 g vanillin in 250 mL ethanol and 2.5 mL H_2SO_4 (conc.)
CAM stain	46 g $(NH_4)_6Mo_7O_{24} \times 4\ H_2O$, 2 g $Ce(SO_4)_2 \times 4\ H_2O$ in 1 L 10% H_2SO_4

Compound purification was performed by medium-pressure liquid chromatography (MPLC) using a Biotage Isolera Prime® flash purification system using either pre-packed Sfär®-Duo separation cartridges or self-filled Sfär®-Duo or KP-Sil® cartridges in combination with Machery-Nagel silica gel 60 (230-400 mesh).

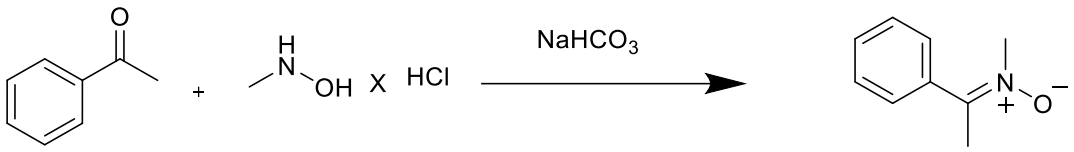
NMR spectra were recorded either on a Bruker BioSpinAV III HD 700 (^{1}H: 700.40 MHz, ^{13}C: 176.12 MHz), AV III 600 (^{1}H: 600.25 MHz, ^{13}C: 150.93 MHz, ^{31}P: 242.99 MHz), AV NEO 400 (^{1}H: 400.27 MHz, ^{31}P: 162.03 MHz) or AV NEO NanoBay 400 (^{1}H: 400.13 MHz, ^{31}P: 161.98 MHz) spectrometer and the recorded spectra were referenced as given in Table 2.

Table 2 Solvent (residual) signals used for calibration of the recorded NMR spectra.

Nucleus	Solvent	referenced to (residual)	Shift/ppm
^{1}H	$CDCl_3$ D_2O	C**H**Cl_3 **H**OD	7.26 4.79
^{13}C	$CDCl_3$	**C**DCl_3	77.16
^{31}P	External H_3PO_4	H_3**P**O_4	0.00

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

Synthesis of Imine **1**



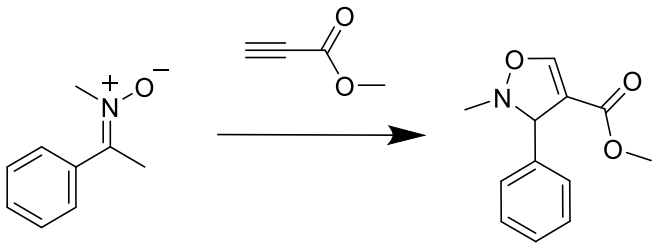
1

10.9 g (1.3 eq., 130 mmol) of methyl-hydroxylamine and 10.9 g (1.3 eq., 130 mmol) of $NaHCO_3$ were dissolved in 60 mL of EtOH under Ar atmosphere. 12.0 g (1 eq., 100 mmol) of acetophenone were added and the mixture stirred at 60°C for 16 h.

The crude was diluted with 100 mL of DCM, washed twice with 25 mL of H_2O, dried with Na_2SO_4 and the solvent removed *in vacuo* to yield 13.8 g (92.5 %) **1** of sufficient purity for the next step.

^{1}H NMR (400.27 MHz, $CDCl_3$, [42Mar2525/290]): δ_H 7.44 – 7.40 (3H, m, arom), 7.27-7.24 (2H, m, arom), 3.65 (3H, q, J = 1.32 Hz, Cq-**CH_3**), 2.44 (3H, q, J = 1.26, N-**CH_3**), 1.80 (1H, bs, **-OH**).

Synthesis of Isoxazoline **2**



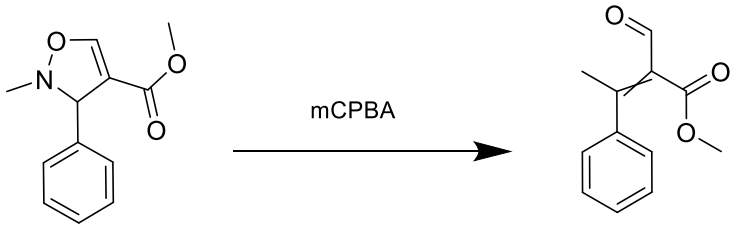
1 **2**

Methylpropiolat (2 eq., 17 g, 200 mmol) was added to a stirred solution of Imine **1** (1 eq., 13.8 g ,100.0 mmol) in dry THF (60 mL) and the resulting mixture was then stirred at 40°C under Ar atmosphere for 3 h. The obtained crude was concentrated *in vacuo* and purified

using MPLC [TLC (EtOAc/n-heptane 1:2), Rf = 0.46 {UV and vanillin active}; gradient: 5 → 100% EtOAc in heptane]

^{1}H NMR (400.27 MHz, $CDCl_3$, [42Mar2825/100]): δ_H 7.51 -7.26 (5 H, m, arom), 3.67 (3H, s, O- **CH_3**), 2.62 (3H, s, N-**CH_3**), 1.85 (3H, s, C-**CH_3**, educt).

Synthesis of Aldehyde (*E/Z*)-**3**

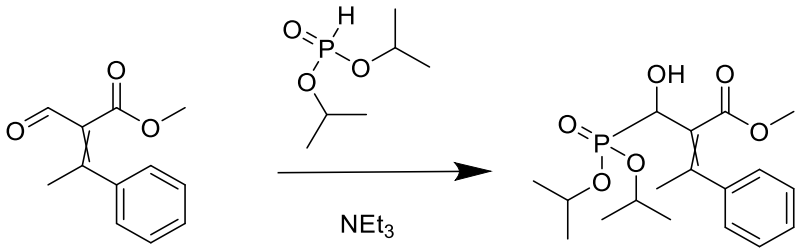


2 (*E,Z*)-**3**

*m*CPBA (1.3 eq., 12.5 g, 72.4 mmol) was added to **2** (1 eq., 13 g, 55.7 mmol) dissolved in DCM under Ar atmosphere at 0°C. After 30 min, a 5 %(w/w) aq. sodium thiosulfate solution was added, and the phases were separated. The aqueous phase was extracted 4 × with 30 mL DCM each. The organic phase was washed neutral with a sat. aq. $NaHCO_3$ solution and subsequently with brine before it was dried with Na_2SO_4. The crude (10.6 g, **3**, A:B= 83:17) was stored at 4°C.

^{1}H NMR (400.27 MHz, $CDCl_3$, [42Mar3125/110]): δ_H 10.09 (1H, s, C**H**O, A), 9.36 (1H, s, C**H**O, B), 7.47-7.38 (10H, m, arom, A/B), 3.89 (3H, s, O-**CH_3**, B), 3.53 (3H, s, O-**CH_3**, A), 2.56 (3H, s, Cq-**CH_3**, A), 2.35 (3H, s, Cq-**CH_3**, B).

Synthesis of phosphonate (±) -(*E/Z*)-**4**



(*E,Z*)-**3** (±)-(*E,Z*)-**4**

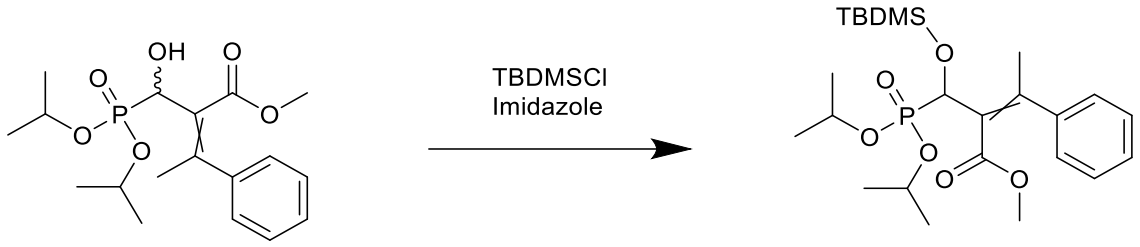
3 (1. eq., 10.6 g, 52.2 mmol), diisopropyl phosphite (1.05 eq., 9.1 g, 54.8 mmol) and NEt_3 (0.5 eq., 2.6 g, 26.1 mmol) were stirred for 1 h at RT under Ar atmosphere, followed by 20 h at 40°C.

The volatiles were removed *in vacuo* at 50°C and the crude was purified via MPLC [TLC (EtOAc/*n*-heptane 1:1), R_f = 0.16 {UV and vanillin active}; gradient: 5 → 100% EtOAc in *n* - heptane] to yield **4** as mixture of geometric isomers A and B (13.5 g, 67.5%, *A:B* = 61:39) as a slightly yellow oil.

^{1}H NMR (400.27 MHz, $CDCl_3$, [42Apr0125/440]): δ_H 7.33 – 7.20 (10H, m, arom, A/B), 4.83 (1H, d, J = 12.39 Hz, **CH**-OH, A), 4.80 (1H, d, J = 12.39 Hz, **CH**-OH, B), 4.73 – 4.58 (4H, m, 4x $(CH_3)_2$-**CH**-O, A/B), 4.51 (2H, dd, J = 9.3 Hz, 1.3 Hz CH-**OH**, A/B), 3.28 (6H, s, COO**CH_3,** A/B), 1.29 (12H, d, **$(CH_3)_2$**-CH-O**,** A), 1.28 (12H, d, **$(CH_3)_2$**-CH-O**,** B)

Decoupled ^{31}P NMR (242.99 MHz, $CDCl_3$), ([42Apr0125_441]): 19.99 (isomer A, 45%), 19.28 (isomer B, 35%), 4.56 (20%, diisopropylphosphit).

Synthesis of TBDMS protected α-hydroxyphosphonate **5**



(±)-(*E,Z*)-**4** (±)-*(E,Z)*-**5**

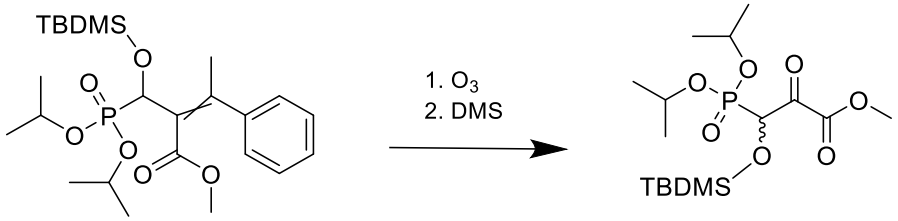
Imidazole (2 eq., 5.0 g, 73.1 mmol) and TBDMSCl (1.2 eq., 6.6 g, 43.9 mmol) were added to *(E/Z)*-**4** (1 eq., 13.5 g, 36.5 mmol) dissolved in dry DCM (50 mL) under Ar atmosphere and the mixture was stirred for 16 h at RT. A sat. aq. NH_4Cl solution was added and the aqueous phase extracted with DCM (3 × 30 mL each). The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure.

Purification of the crude via MPLC [TLC (EtOAc/*n*-heptane 1:1), R_f = 0.38 (**5**), 0.25 (**4**) {both UV and vanillin active}; gradient: 5 →100% EtOAc in n - heptane] yielded the protected product **5** (11.3 g, 23.3 mmol, 64%, A:B = 50:50) as yellow oil and white solid, which could be partly separated.

^{1}H NMR (400.27 MHz, $CDCl_3$, [42Apr0425/80]: δ_H 7.10 – 6.99 (10H, m, arom, A/B), 4.92 (1H, d, J = 18.3 Hz, **CH**-OH, A), 4.92 (1H, d, J = 18.3 Hz, **CH**-OH, B), 4.55 (2H, sep, J = 6.3 Hz, 2x $(CH_3)_2$ -**CH**-O-, A), 4.49 (2H, sep, J = 6.3 Hz, 2x $(CH_3)_2$ -**CH**-O-, B), 3.58 (3H, s, COO**CH$_3$**,B), 3.06 (3H, s, COO**CH$_3$**, A), 1.34 (6H, d, J = 2.61 Hz, **$(CH_3)_2$** -CH-O, A), 1.32 (6H, d, J = 2.61 Hz, **$(CH_3)_2$** -CH-O, A), 1.28 (6H, d, J = 6.18 Hz, **$(CH_3)_2$** -CH-O, B), 1.18 (6H, d, J = 6.18 Hz, **$(CH_3)_2$** -CH-O, B), 0.82 (18H, s, 2x Si-Cq-**$(CH_3)_3$,** A/B), 0.19 (6 H, s, Si(**CH_3**)$_2$, A), - 0.14 (6H, s, Si(**CH_3**), B)

Decoupled ^{31}P NMR (242.99 MHz, $CDCl_3$ [42Apr0425_81]): 18.70 ppm (100%, A/B-**5**).

Synthesis of β-ketophosphonate **6**



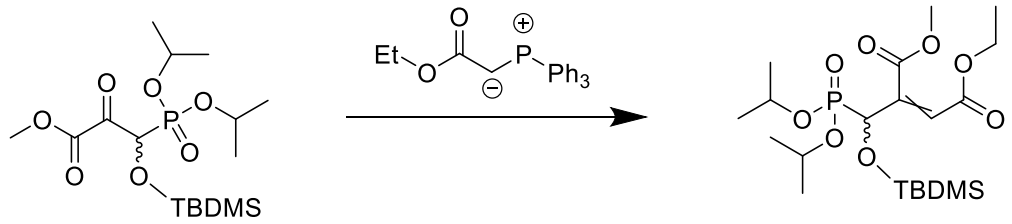
(±)-*(E,Z)*-**5** (±)-**6**

5 (1 eq., 6.0 g, 14.2 mmol) was dissolved in DCM (25 mL), at −5°C and O_3 (flow: 100 N/h, O_3 level: 62%) was bubbled through the reaction mixture for 1 h. Then, O_2 was bubbled through the solution until the blue colour disappeared. Dimethyl sulfide (2 eq., 1.84 mL) was added, the cooling bath was removed and stirring was continued until RT was reached. After removal of the solvent under reduced pressure, the crude was purified via MPLC [TLC (EtOAc/ *n* - heptane 1:1), R_f = 0.44 {UV and vanillin active}; gradient: 10 →100% EtOAc in n - heptane] to yield the ketone **6** (1.80 g, 4.1 mmol, 29 %) as purple oil which could be stored at - 20°C.

^{1}H NMR (400.27 MHz, $CDCl_3$, [42Apr0825_350]: δ_H 5.56 (1H, d, *J* = 22.17 Hz, **CH**-OH), 4.79 (1H, sep, J = 6.2 Hz, $(CH_3)_2$ -**CH**-O-), 4.70 (1H, sep, J = 6.2 Hz, $(CH_3)_2$ -**CH**-O-), 3.87 (3 H, s, CO**CH$_3$**), 1.37-1.27 (12 H, m, 2x **(CH$_3$)$_2$** -CH-), 0.93 (9H, s, Cq(**CH$_3$**)$_3$), 0.11 (6H, s, Si**(CH$_3$)$_2$**).

Decoupled ^{31}P NMR (242.99 MHz, $CDCl_3$ [42Apr0825_351]): 11.23 (100%, **6**).

Synthesis of diester (*E/Z*)-**7**



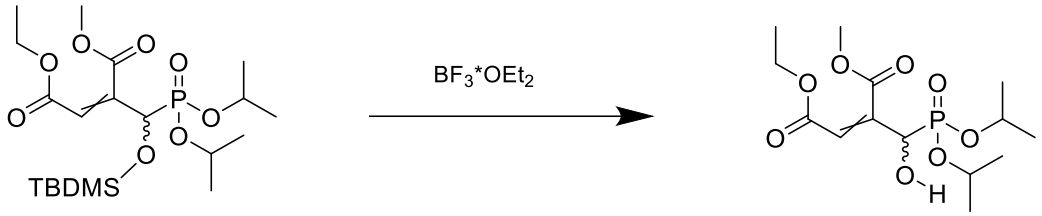
(±)-**6** (±)-(*E,Z*)-**7**

(Carbethoxymethylene)triphenylphosphorane (1.2 eq., 2.11 g, 6.05 mmol) was added to **6** (1 eq., 2 g, 5 mmol), dissolved in THF (17 mL) under Ar atmosphere and the resulting mixture was stirred at 60°C for 16 h, the mixture turned red within minutes. The solvent was removed *in vacuo*, and the crude product was purified by two subsequent MPLC runs [TLC (EtOAc/*n*-heptane 1:1), R_f = 0.57 {UV and vanillin active}; gradient: 10 → 20% EtOAc in n-heptane] to yield **7** (940 mg,2.01 mmol, 44%, A:B = 85:15) as slightly yellow oil.

^{1}H NMR (400.27 MHz, $CDCl_3$, [42Apr1425/130]: δ_H 6.31 (1H, dd, J = 5.7 Hz, 1.75 Hz, C**H**=C_q, A/B), 4.90 (1H, dd, J = 17.9 Hz, 1.75 Hz, **CH**-O-PG, A/B), 4.87 (1H, sep, $(CH_3)_2$ -**CH**, B), 4.79 (1H, sep, $(CH_3)_2$ -**CH**, B), 4.77 (1H, sep, $(CH_3)_2$ -**CH**, A), 4.69 (1H, sep, J = 6.0 Hz, **CH**-O-PG, A), 4.20 (2H, q, J = 6.97 Hz, -**CH_2**CH_3, A/B), 3.78 (3H, s, COO**CH_3** , A/B), 1.38-1.22 (12H, m, 2x **$(CH_3)_2$** -CH, A/B), 1.28 (3 H, t, J = 6.97 Hz, -CH_2**CH_3** , A/B), 0.93 (9H, s, Cq**$(CH_3)_3$**, A/B), 0.19 (3H, s, Si**$(CH_3)_2$** A/B), 0.12 (3H, s, Si**$(CH_3)_2$**, A/B).

Decoupled ^{31}P NMR (242.99 MHz, $CDCl_3$ [42Apr1425/131]): 15.20 ppm (85%, A-**7**), 15.13 ppm (15%, B-**7**).

Synthesis of α-hydroxyphosphonate (E/Z)-(±)-**8**



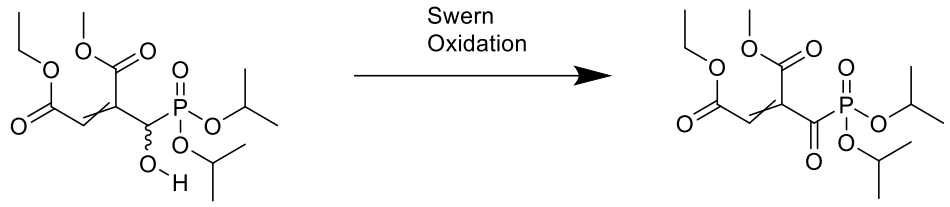
(±)-(*E,Z*)-**7** (±)-(*E,Z*)-**8**

BF_3*OEt_2 (1.9 eq., 54 µL, 0.40 mmol) was added to **7** (1 eq., 96.8 mg, 0.21 mmol) in 1 ml ACN under Ar atmosphere at room temperature and the reaction mixture was stirred for 16 h before addition of a sat. aq. $NaHCO_3$ solution and subsequent extraction of the aqueous phase with DCM. The crude was dried using Na_2SO_4, filtered and concentrated *in vacuo*. The product was dried on the Schlenk line at 10 mbar in a 60°C oil bath to give (E/Z)-(±)- **8** (12.6 mg, 34%, A:B = 86:14) as a colourless oil.

^{1}H NMR (400.27 MHz, $CDCl_3$, [42Jul0725_230]: δ_H 6.38 (1H, dd, J = 5.7 Hz, 1.75 Hz, C**H**=C_q, A), 6.37 (1H, dd, J = 5.7 Hz, 1.75 Hz, C**H**=C_q, B), 5.23 (1H, bs, -O**H,** A), 4.85 (1H, ddd, J = 16.05 Hz, 5.49 Hz, 1.83 Hz, **CH**-OH, A), 4.84 (1H, ddd, J = 16.05 Hz, 5.49 Hz, 1.83 Hz, **CH**-OH, B), 4.76 (2H, sep, J = 6.51 Hz, 2x **CH**-O, A/B), 4.20 (2H, q, J = 6.98 Hz, -**CH_2**CH_3, A/B), 3.81 (3H, s, COO**CH_3**, A/B), 2.88 (1H, bs, -O**H,** B), 1.38-1.22 (12H, m, 2x **$(CH_3)_2$** -CH, A/B), 1.28 (3 H, t, J = 6.98, -CH_2**CH_3**, A/B).

Decoupled ^{31}P NMR (242.99 MHz, $CDCl_3$, [42Jul0725_231): 16.85 ppm (100%, A/B-**8**).

Synthesis of α-ketophosphonate **9**

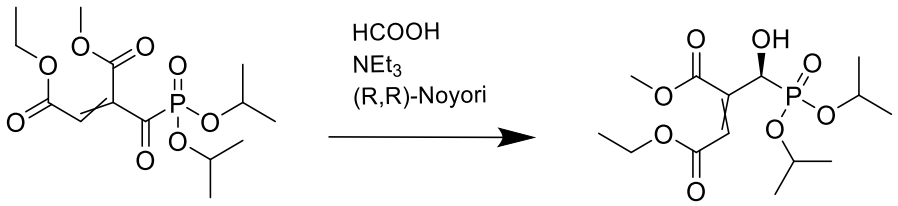


(±)-(*E,Z*)-**8** (*E,Z*)-**9**

Dimethylsulfoxide (2.5 eq., 71µL, 1.0 mmol) in dry DCM (1 mL) was added dropwise to a solution of oxalyl chloride (1.25 eq., 42 µL, 0.5 mmol) in dry DCM (5 mL) at −78 °C. The reaction mixture was stirred for 5 min before a solution of α-hydroxyphosphonate **8** (1 eq., 141 mg, 0.4 mmol) in DCM (1.5 mL) was added followed by triethylamine (5.2 eq., 290 µL, 2.1 mmol) after another 5 min. The reaction mixture was allowed to come to ambient temperature in the ice bath. The reaction mixture was then diluted with diethyl ether and water, and the layers were separated. The organic layer was washed with water and half dilute aq. $NaHCO_3$ (1:1 in H_2O). The combined organic phases were dried with Na_2SO_4; filtered and evaporated *in vacuo*.

Decoupled ^{31}P NMR (242.99 MHz, $CDCl_3$, [42Aug1425/81]: 16.50 ppm (100%, A/B-**9**)

ATH of α-ketophosphonate **8**



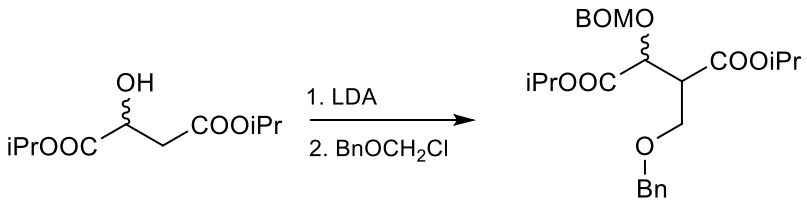
(*E,Z*)-**9** *S*-(*E,Z*)-**8**

Under Argon atmosphere 0.28 mL NEt_3 and 0.12 mL HCOOH were combined in an ice bath.

To a stirred solution of 0.25 mL DCM and dH_2O were added 0.2 mL 0.2 M KOH and 13 mg (0.02 mmol, 0.05 eq.) *R,R*-Noyori catalyst. The resulting purple organic phase was separated from the aqueous phase and dried using CaH_2. The purple organic phase was added under Argon atmosphere to 0.28 mL NEt_3 and 0.12 mL HCOOH and the resulting mixture was cooled on an ice bath. **9** was added and the now orange solution was stirred for 16 h.

Decoupled ^{31}P NMR (242.99 MHz, $CDCl_3$, [42Jul2525/171]: 16.85 ppm (100%, A/B-**8**).

BOMCl functionalization of malate **12**



11 **12**

To a solution of lithium diisopropylamide, prepared from diisopropylamine (2.4 eq., 3.4 mL, 24 mmol) in dry THF (34 mL) and *n*-butyllithium (2.2 eq., 2.5 M in hexane, 8.8 mL , 22 mmol), was added a solution of diisopropyl malate (1 eq., 1.90 g, 10 mmol) in dry THF (3 ml) at −78 °C under nitrogen, and the mixture was stirred for 30 min, and then at −20 °C for 1 h. Then, the solution was cooled again and to the cooled (−78 °C) solution was added benzyl chloromethyl methyl ether (2.2 eq., 4.2 mL, 30 mmol) in 11.5 mL of DMPU over 20 minutes, and the mixture was stirred at −78 °C for 6 h, then at room temperature for 12 h. After 2 h, the reaction was quenched with NH_4Cl (1 mL) then partitioned between EtOAc (200 mL) and saturated NH_4Cl (150 mL). The aqueous layer was extracted with EtOAc (150 mL). The combined organic layer was washed with H_2O and brine, dried over Na_2SO_4, and concentrated. The crude yellow oil was purified by MPLC [TLC (EtOAc/*n*-heptane 1:4), R_f = 0.82 {UV and CAM active, **12**}, 0.79 { CAM active, unknown impurity}, 0.77 { CAM active, unknown impurity }, 0.48 { CAM active, unknown impurity }, 0.35 {UV and CAM active, **11**}, 0.30 {UV and CAM active},; gradient: 10 → 20% EtOAc in n-heptane].

^{1}H NMR (400.27 MHz, $CDCl_3$, [42Jul0725_230]: δ_H 7.24 (17 H, s, unreacted BOMCl CH_2 group), 7.18 (5 H, m, arom), 7.14 (2 H, s, O-CH_2-O), 5.00 (1H, sep, J = 6.24 Hz, $(CH_3)_2$ -C**H**), 4.92 (1H, sep, J = 6.24 Hz, $(CH_3)_2$ -C**H**), 4.58 (16 H, m, 2H from Ph-C**H**$_2$-O, remainder from unreacted BOMCl CH_2), 4.30 (1H, dd, J = 10.94 Hz, 5.54 Hz, C**H**CH_2), 2.68 (1H, dd, J = 16.02 Hz, 4.54 Hz, CHC**H**$_2$ hydrogen 1), 2.62 (1H, dd, J = 16.02 Hz, 5.91 Hz, CHC**H**$_2$ hydrogen 2), 1.14 (12 H, m, (**CH$_3$)$_2$** -CH).

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