

Synthesis and antimycobacterial evaluation of phosphohydroxamic acid double prodrugs

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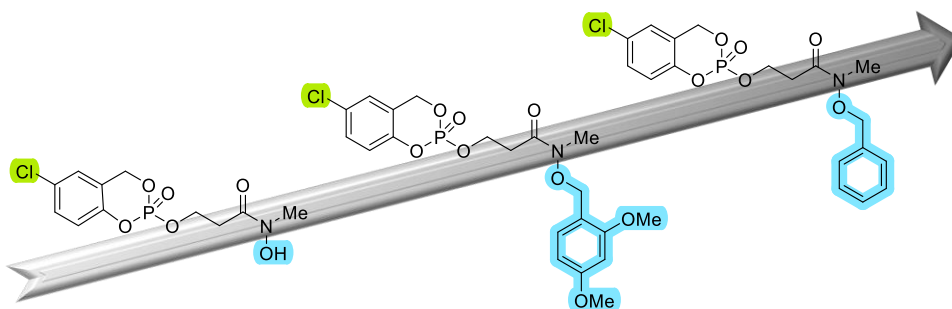
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Abstract

The synthesis and biological evaluation of *cycloSal* double prodrugs designed to enhance intracellular delivery of DXR inhibitors are reported. Two compounds inhibited *Mycobacterium smegmatis* growth outperforming isoniazid in agar diffusion assays. Structure–activity relationship analysis revealed that electron-withdrawing substituents (Cl and CF₃) on the *cycloSal* moiety enhance activity. Overall, hydroxamate protection proved essential, with the *O*-benzyl derivative providing the best balance between membrane permeation and intracellular activation.



Growth inhibition of *Mycobacterium smegmatis*

Keywords: Antimycobacterial, *cycloSal*igenyl prodrugs, isoprenoid biosynthesis, 1-deoxy-D-xylulose 5-phosphate reducto-isomerase

Introduction

Alexander Fleming's discovery of penicillin in 1928 ushered in the era of modern antibiotic therapy, revolutionizing the treatment of bacterial infections. The subsequent golden age of antibiotic discovery (1940–1960) involved extensive exploration of natural products together with the development of synthetic and semi-synthetic compounds. This period led to the identification of major antibiotic classes such as cephalosporins, aminoglycosides, tetracyclines, macrolides, fluoroquinolones, and sulfonamides, many of which remain indispensable in clinical use today. However, the long-term effectiveness of these drugs is increasingly compromised by the remarkable ability of bacteria to adapt and develop resistance. From the 1970s onward, the antibiotic development pipeline slowed dramatically, with only eight new classes of antibiotics approved since that time, while AntiMicrobial Resistance (AMR) continued to rise as a consequence of the widespread misuse and overuse of antibiotics in humans, animals, and agriculture.^{1,2} AMR has consequently emerged as one of the most critical global health challenges of the 21st century. The World Health Organization (WHO) warns that resistance to commonly used antibiotics is now widespread, increasing by over 40% between 2018 and 2023—an average annual rise of 5–15%. Alarming, projections estimate that AMR may account for up to 39 million cumulative deaths by 2050, highlighting its status as an urgent global public health threat.³

In 2024, the World Health Organization (WHO) released an updated list of bacterial priority pathogens, emphasizing the urgent need for new antibacterial agents. *Mycobacterium tuberculosis*, the causative agent of tuberculosis, remains one of the most devastating and concerning pathogens. Despite a modest decline in tuberculosis incidence between 2023 and 2024, the disease caused an estimated 1.23 million deaths and 10.7 million new infections worldwide in 2024. Tuberculosis was thus ranked as the second leading cause of death from infectious disease, surpassed only by COVID-19.⁴

Current tuberculosis therapy relies on first-line antibiotics introduced in the 1960s, such as isoniazid, rifampicin, pyrazinamide, and ethambutol (Figure 1). The therapies require prolonged multidrug regimens that frequently result in poor patient adherence and cumulative toxicities, particularly hepatotoxicity, highlighting the need for shorter, safer treatment options.⁵

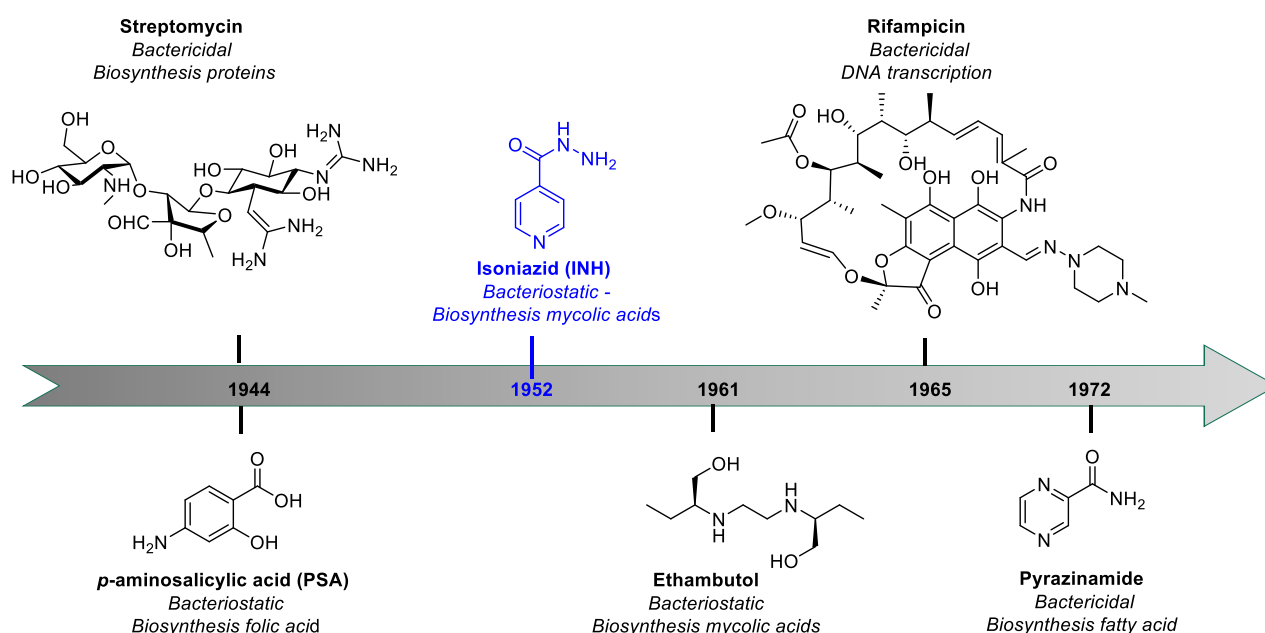
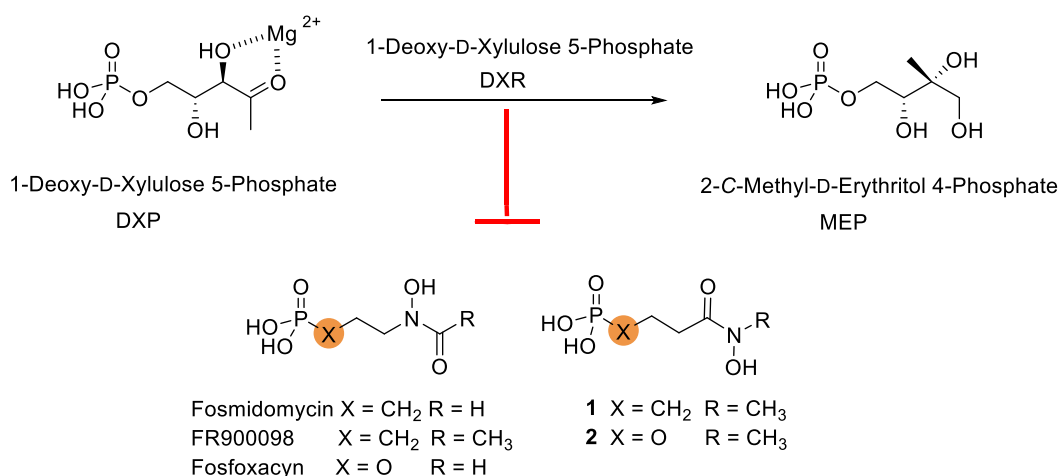


Figure 1. Antitubercular drug timeline.

The discovery of new anti-tuberculosis agents is further complicated by the unique biology of *M. tuberculosis*. Its slow growth rate and its highly impermeable mycolic acid-rich envelope severely limit drug penetration and reduce the effectiveness of many antibacterial compounds. These challenges underscore the urgent need for innovative therapeutic strategies targeting essential bacterial pathways absent in humans. The Methyl Erythritol Phosphate (MEP) pathway, a non-mevalonate route for isoprenoid biosynthesis, represents a particularly attractive target, as it is indispensable for *M. tuberculosis* viability yet completely absent in mammalian cells.^{6,7}

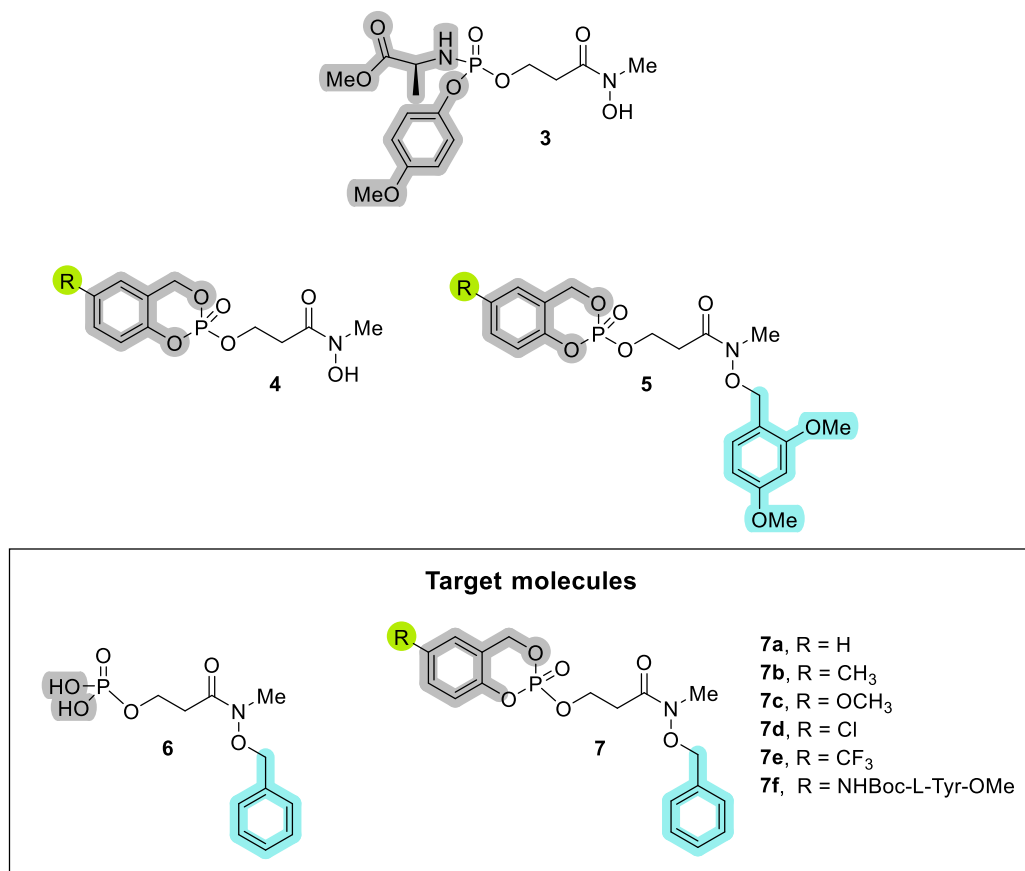
However, the high polarity of known MEP pathway inhibitors often compromises cellular uptake. In this context, prodrug strategies offer a powerful approach to enhance membrane permeability, and improve intracellular drug delivery. Of particular interest is 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR), the second enzyme of the MEP pathway, which is potently inhibited by the natural phosphono-retrohydroxamic acids fosmidomycin and its *N*-methylated analogue FR900098, as well as by the phosphate analogue fosfoxacin and related synthetic phosphono- **1** and phospho-hydroxamic acid derivatives **2** (Scheme 1).⁸



Scheme 1. DXR enzyme and its inhibitors.

Unfortunately, due to a lack of cellular uptake, fosmidomycin and its analogues are inefficient and do not inhibit the growth of mycobacteria. This result is not surprising as mycobacteria, in contrast to many other bacteria, do not possess a transporter for such molecules and are surrounded by a hydrophobic waxy cell wall preventing passive diffusion into the cell.⁹

Phosphorylated compounds are rarely developed as potential antibiotics because of their susceptibility to the hydrolytic degradation of the phosphatases. Therefore, the prodrug approach has been adopted to bypass this lack of uptake and to increase the bioavailability of these hydrophilic compounds by masking the charged phosphate with an aryl phosphoramidate moiety developed by McGuigan *et al.* ("ProTide" approach)¹⁰ and with a *cyclo*Saligenyl group developed by C. Meier *et al.* (Scheme 2).¹¹ We recently reported that the phosphoramidate prodrug **3** may not be suitable for delivering fosfoxacin analogues¹² whereas *cyclo*Saligenyl-based prodrugs **4** and **5** exhibited promising antimycobacterial activity making them interesting candidate compounds.¹³



Scheme 2. Previously reported prodrugs **3-5**, target molecules in current work **6** and **7**.

Additionally, double prodrugs bearing a *p*-DiMethoxyBenzyl (DMB) protecting group on the hydroxamate moiety in **5** were more active than the corresponding unprotected prodrug analogues **4**, likely due to increased lipophilicity and enhanced cellular uptake conferred by the combined DMB and *cycloSal* motifs (Scheme 2). However, it remains unclear whether the DMB group is cleaved to release the active inhibitor and whether *O*-DMB-protected hydroxamates can bind the DXR active site.

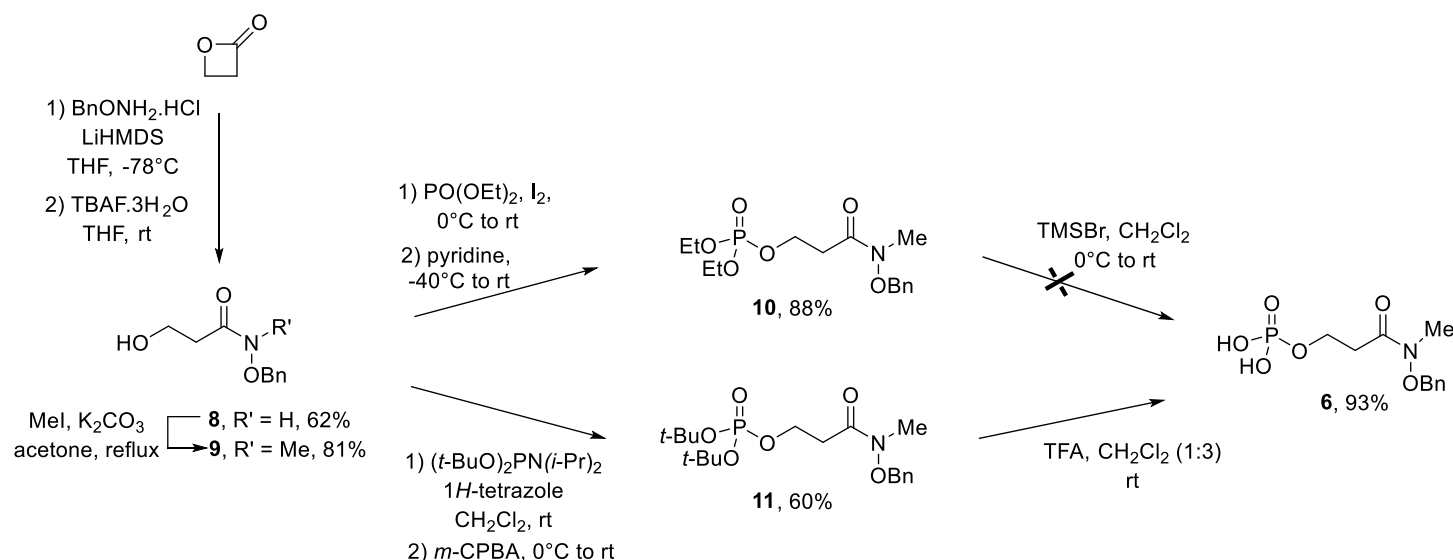
To address these questions, we report herein the synthesis of phosphonohydroxamate analogues bearing a benzyl protecting group on the hydroxamate **6**, together with a series of the corresponding *O*-benzyl protected *cycloSal* double prodrugs **7** (Scheme 2). The inhibitory potency of the parent compounds was evaluated against *M. smegmatis* DXR as well as the antimycobacterial activity of the *cycloSal* prodrugs. The resulting activities were compared with those of the unprotected prodrug and the DMB-protected double prodrug, enabling a systematic assessment of the impact of hydroxamate protection on DXR inhibition and antimycobacterial activity.

Results and Discussion

Synthesis of the *O*-benzylphosphohydroxamate **6**

To synthesize compound **6**, we initially adopted the strategy described by Ghilagaber *et al.*, which consists of synthesizing a diethyl phosphate intermediate followed by deprotection using trimethylsilyl bromide (TMSBr) (Scheme 3).¹⁴ The alcohol **8** was obtained in one step in 62% yield from commercially available β -propiolactone

using commercially *O*-benzyl hydroxylamine hydrochloride in presence of an excess of LiHMDS.¹² Subsequent selective *N*-methylation versus *O*-methylation of **8** was achieved under basic conditions with K₂CO₃ and methyl iodide leading to **9** in 81% yield. In this approach, the phosphorylation was achieved by generating diethylphosphoryl iodide *in situ* from triethyl phosphite and iodine, which subsequently reacted with the primary alcohol **9** in the presence of pyridine to afford the corresponding phosphorylated compounds **10** in 88% yield (Scheme 3). Subsequent cleavage of the ethyl groups using TMSBr in dichloromethane was then attempted. However, instead of yielding the desired phosphate **6**, this reaction led predominantly to products resulting from bromide substitution at the phosphate moiety, thereby preventing access to the target compound. To overcome this limitation, the ethyl protecting groups were replaced by *tert*-butyl groups, which can be removed under acidic conditions using trifluoroacetic acid (TFA).¹⁵ Phosphorylation of alcohol **9** was performed using di-*tert*-butyl *N,N*-diisopropylphosphoramidite and 1*H*-tetrazole, followed by oxidation with purified *m*-CPBA.¹⁶ The di-*tert*-butyl phosphate **11** was obtained in 60% yield. Final acidic deprotection using TFA/CH₂Cl₂ (1:3) afforded the target compounds **6** in 93% yield (Scheme 3).

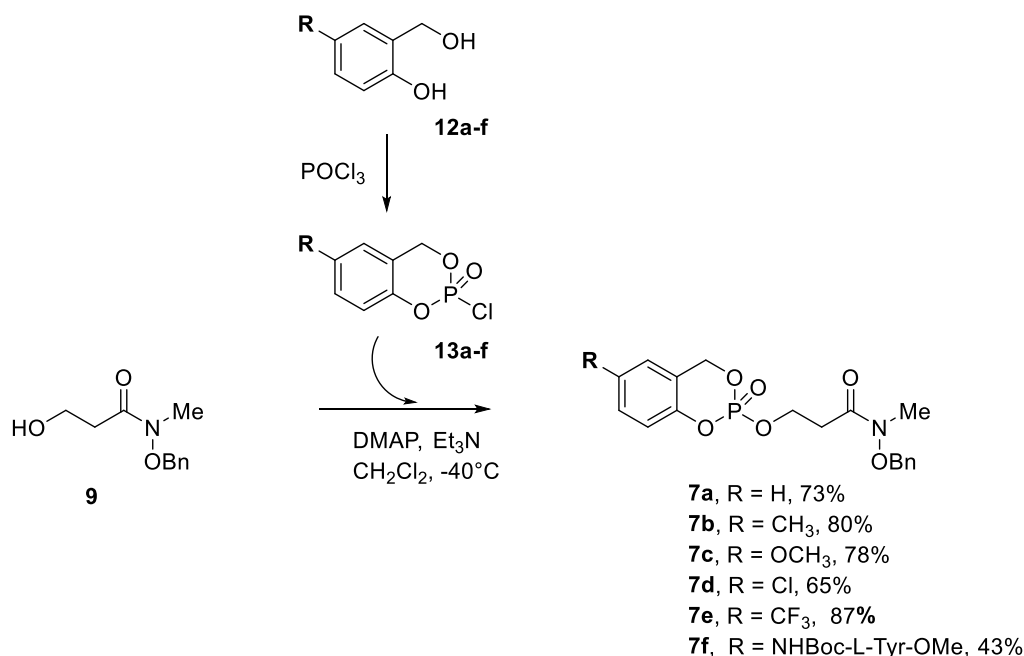


Scheme 3. Synthesis of compound **6**.

Synthesis of the double prodrugs **7a-f**

The sequence to synthesize the *cycloSal* prodrugs **7a-f** is outlined in Scheme 4. The key step is an addition elimination between *O*-protected hydroxamic acids **9** and *cycloSal*phosphochloridate derivatives **13**, obtained by phosphorylation of the differently substituted salicylic alcohols **12** using phosphorus(V) chemistry. As salicylic alcohol **12a** (R = H) is the only commercially available compound, derivative analogues **12b-f** were obtained as previously described.^{13,17}

Coupling of the *O*-protected hydroxamic acid **9** with the *cycloSal*phosphochloridate derivatives **13** was carried out in CH₂Cl₂ at -40 °C in the presence of triethylamine (TEA) and a catalytic amount of *N,N*-dimethylaminopyridine (DMAP). Under these conditions, *cycloSal* prodrugs **7a-f** were obtained in moderate to good yields (Scheme 4).



Scheme 4. Synthesis of double prodrugs **7a-f**.

Biological activity

Mycobacterium smegmatis is a non-pathogenic surrogate for *Mycobacterium tuberculosis*. It shares key physiological and metabolic characteristics with *M. tuberculosis*, including the essential methylerythritol phosphate (MEP) pathway targeted in this study, while offering the advantages of rapid growth and lower biosafety requirements. Therefore, all compounds synthesized in this study were evaluated against *M. smegmatis* as an initial screening model for antimycobacterial activity. Compound **6** was first evaluated *in vitro* against DXR from *M. smegmatis*. No significant inhibition of the enzymatic activity was observed at 100 μ M ($\leq 10\%$ inhibition), indicating that the compound does not efficiently interact with the enzyme under the tested conditions and therefore does not act as a direct DXR inhibitor. This result suggests that intracellular deprotection of the *O*-benzyl group would be necessary—most likely by chemical or enzymatic cleavage—would be required to generate the active inhibitory species. To further investigate its potential biological activity, compound **6** was evaluated against *M. smegmatis* using a standard disk diffusion assay. Briefly, sterile paper disks containing the compound were placed on agar plates previously inoculated with *M. smegmatis*, and antibacterial activity was assessed by the presence of growth inhibition zones around the disks. No growth inhibition was observed for either strain. This lack of activity likely results from the presence of the free, negatively charged phosphate group which severely limits passive diffusion of these molecules across the bacterial membrane.

The growth inhibitory activity of the synthesized double prodrugs **7a-7f** was subsequently evaluated against *M. smegmatis* using the same agar diffusion assay. Cellulose disks (6mm) were loaded with 800 nmoles of each compound. Isoniazid (INH) was used as the reference compound. At 800 nmoles, most prodrugs exhibited inhibition values lower than that of isoniazid (30 nmoles), indicating a lower intrinsic potency relative to the reference drug. However, two derivatives, **7d** and **7e**, displayed superior inhibition values (1.47 and 1.34, respectively), exceeding that of isoniazid under identical experimental conditions. (Figure 2)

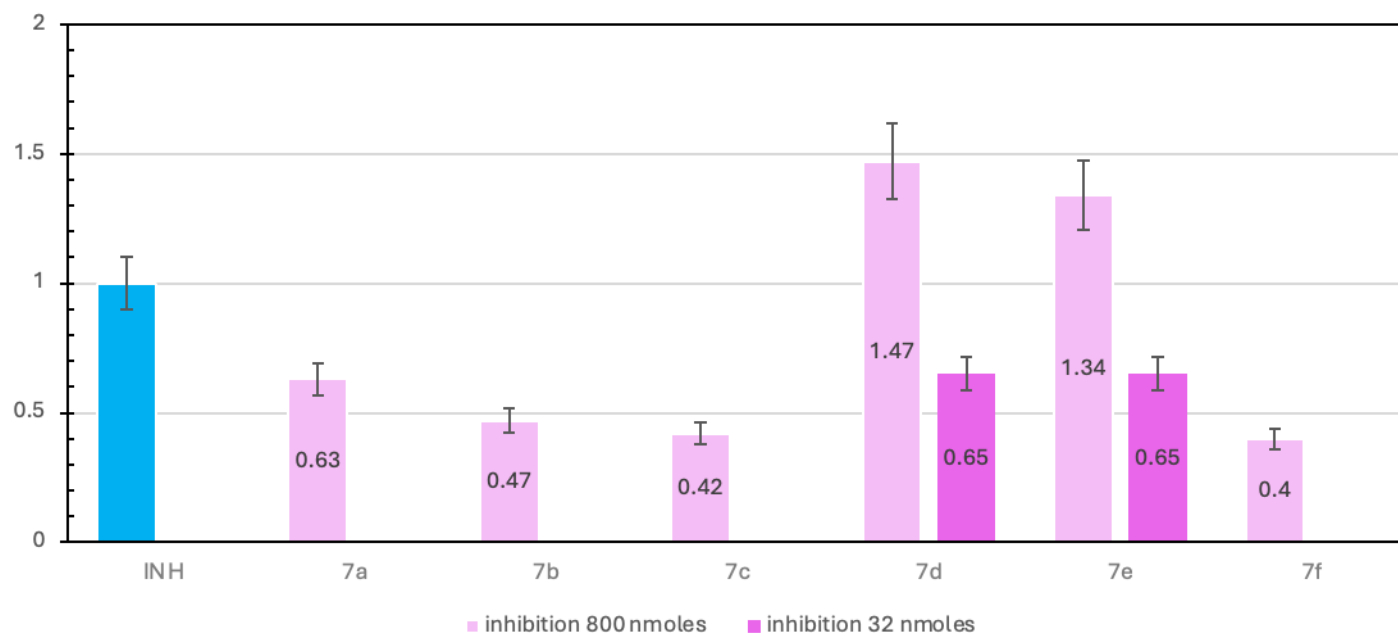


Figure 2. *M. smegmatis* growth inhibition of compounds **7a–7f** compared to reference isoniazid (INH). The inhibitory activity of the compounds was quantified by expressing their inhibition zone diameters as ratios relative to that of isoniazid (30 nmoles), which was set as the reference value of 1 (shown in cyan). In light pink: ratios of compounds at 800 nmoles. In deep pink: ratios of compounds at 32 nmoles. Data are presented as mean $\pm$ 10% standard error (SE).

Based on these promising results, compounds **7d** and **7e** were further evaluated at a reduced dose (32 nmoles), to determine whether their activity could be maintained at a level comparable to isoniazid (30 nmoles). At 32 nmoles, both prodrugs exhibited an inhibition value of 0.65. As expected, the activity decreased at lower concentration. Nevertheless, the persistence of the inhibition activity at lower dose highlights their potential interest. Both compounds exhibited MICs values superior to 2500 μ M.

Structure-activity relationship analysis revealed a clear substituent effect. At 800 nmoles the unsubstituted compound **7a**, and the electron-donating methyl-substituted **7b** and methoxy-substituted **7c** *cycloSal*s derivatives demonstrated a weaker growth inhibition than isoniazid (30 nmoles). Compound **7f**, in which the amine is protected with a Boc group, exhibited approximately half the inhibitory activity of isoniazid against *M. smegmatis*. This lower activity is consistent with the reduced electron-donating character and the increased steric demand of the Boc-protected amine, which are expected to attenuate prodrug hydrolysis. In contrast, compounds **7d** and **7e**, bearing electron-withdrawing substituents (Cl and CF₃ respectively) on the *cycloSal* moieties, exhibited the strongest antibacterial activity. These results suggest that electron-withdrawing groups on the *cycloSal* enhanced growth inhibition of *M. smegmatis*, whereas electron-donating substituents markedly reduce the activity. The presence of electron-withdrawing substituents on the *cycloSal* moiety may facilitate hydrolytic cleavage of **7d** and **7e**, thereby promoting intracellular release of the active phosphate species. In contrast, electron-donating substituents decrease the activation process, resulting in reduced antibacterial activity.¹³ Additionally, the increased lipophilicity conferred by Cl and CF₃ substituents may also contribute to improved membrane permeation and intracellular accumulation.

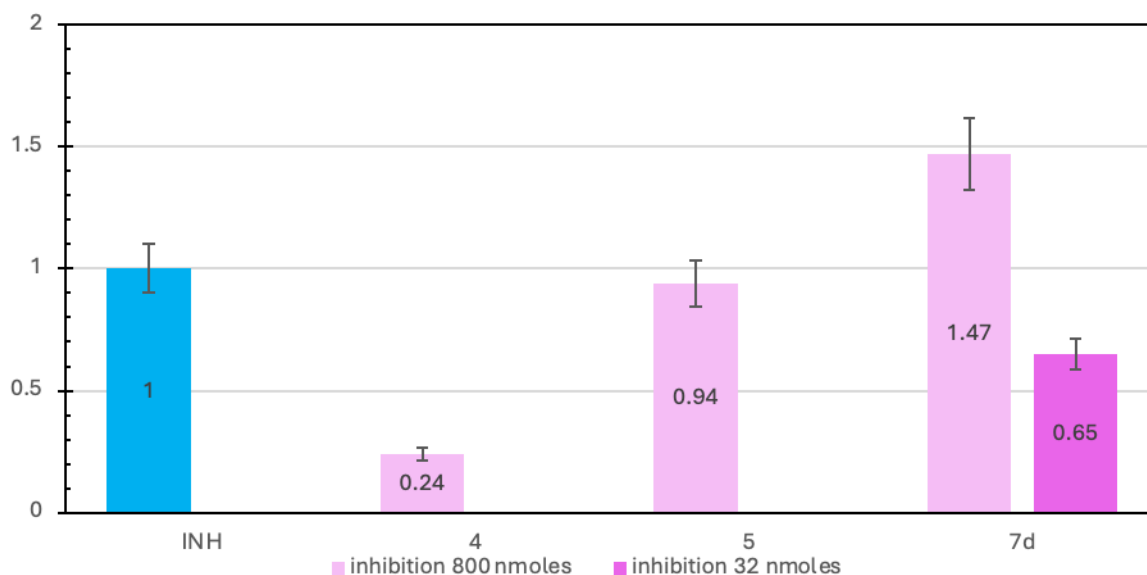


Figure 3. Influence of the hydroxamate protection group on *M. smegmatis* growth inhibition. Compounds **4** (R = Cl, hydroxamate without protecting group), **5** (R = Cl, *O*-2,4-dimethoxybenzyl hydroxamate-protecting group) and **7d** (R = Cl, *O*-benzyl hydroxamate-protecting group) were compared to reference isoniazid (INH). The inhibitory activity of the compounds was quantified by expressing their inhibition zone diameters as ratios relative to that of isoniazid (30 nmoles), which was set as the reference value of 1 (shown in cyan). In light pink: ratios of compounds at 800 nmoles. In deep pink: ratios of compounds at 32 nmoles. Data are presented as mean $\pm$ 10% standard error (SE).

Figure 3 illustrates the influence of the hydroxamate protecting group on the *M. smegmatis* growth inhibition of 5-chloro-*cycloSal* compounds. Interestingly, the *cycloSal* derivative **4** (R = Cl) bearing a free hydroxamate displays significantly lower growth inhibition than the ODMB- and OBn-protected analogues **5** (R = Cl) and **7c** respectively, although the biologically active species on the DXR enzyme is the fully deprotected compound *i.e.* lacking both the *cycloSal* moiety and the hydroxamate protecting group.⁸ This apparent paradox suggests that masking the hydroxamate function plays a crucial role beyond simple release of the active compound. The free hydroxamate is highly polar and potentially partially ionized at physiological pH, which may reduce membrane permeability across the lipid-rich mycobacterial envelope or promote premature interactions and/or efflux before reaching its intracellular target. In contrast, *O*-benzyl protection increases overall lipophilicity and may allow improved cellular penetration and more favorable intracellular distribution prior to enzymatic deprotection. The DMB-protected *cycloSal* **5** (R = Cl) reaches near-reference activity (0.94), and the OBn-protected *cycloSal* **7d** exceeds it (1.47 at 800 nmol). Notably, **7d** still retains significant activity at a lower dose (0.65 at 32 nmol), indicating that the prodrug remains efficient even at reduced concentration.

The superior performance of the OBn-protected *cycloSal* prodrug **7d** compared to the ODMB analogue **5** with R = Cl, likely reflects subtle but critical differences in intracellular activation kinetics rather than variations in lipophilicity as previously mentioned. Although the DMB group is more electron-rich and generally more lipophilic due to its two methoxy substituents, this increased stabilization could render the benzylic position less susceptible to enzymatic oxidation, a plausible first step in intracellular deprotection. In mycobacteria, cleavage of *O*-benzyl protecting groups certainly proceeds through enzyme-mediated oxidative processes. The OBn

group, being less sterically hindered and less electronically stabilized, could undergo this oxidation more readily, leading to a more efficient liberation of the active species. In contrast, the ODMB group may confer excessive stability, slowing the deprotection step and consequently reducing the intracellular concentration of the fully active inhibitor. Additionally, the greater steric bulk of the DMB substituent may affect enzyme recognition or intracellular diffusion. Overall, we suggest that the OBn derivative achieves a better balance between membrane permeation, metabolic stability, and rate of enzymatic activation, resulting in superior antibacterial activity.

Conclusions

A series of *cycloSal* double prodrugs **7** was synthesized to enhance intracellular delivery and enable the controlled release of the active phosphate species. These compounds exhibited greater antibacterial activity than their unprotected prodrug analogues **4**, likely due to the increased lipophilicity and improved cellular uptake conferred by the combined OBn and *cycloSal* motifs. In particular, compounds **7d** and **7e**, bearing electron-withdrawing substituents (Cl and CF₃, respectively) on the *cycloSal* moieties, exhibited the highest antibacterial activity. Comparison of the chloro-*cycloSal* derivatives further revealed that the most active compound was the double prodrug with a *O*-benzyl protected hydroxamate **7d**, which outperformed both the DMB-protected analogue **5** (R = Cl) and the corresponding unprotected derivative **4** (R = Cl). These findings underline the critical influence of the hydroxamate protecting group and highlight the importance of balancing membrane permeability with prodrug activation kinetics in designing DXR-targeted antimycobacterial agents. Unexpectedly, we also demonstrated that the *O*-benzyl phosphohydroxamic acid is neither a substrate nor an inhibitor of DXR. This observation raises important questions regarding the origin of the antibacterial activity. It remains to be established whether intracellular *O*-benzyl hydrolysis regenerates the active phosphohydroxamic acid or whether the compound exerts its antibacterial effect through an alternative mechanism or molecular target. Further studies are needed to determine the underlying mechanism.

Experimental Section

General. All non-aqueous reactions were run in dry solvents under an argon atmosphere. Commercial grade reagents were used without further purification. Petroleum ether 40-60 °C (Carlo-Erba) was used for purification. Flash chromatography was performed on silica gel 60 230–400 mesh with the solvent system as indicated. Automated flash chromatography was performed on a Puriflash® 215 (Interchim). TLC plates were revealed under UV light (254 nm) and/or by spraying with an ethanolic solution of phosphomolybdic acid (20%) or an aqueous solution of potassium permanganate followed by heating. The NMR spectra were recorded on a BRUKER Avance 300 (¹H-NMR: 300 MHz; ¹³C-NMR, 75.5 MHz; ³¹P-NMR 121.5 MHz; ¹⁹F-NMR 282.4 MHz) or a BRUKER Avance 400 (¹H-NMR: 400 MHz; ¹³C-NMR, 100.6 MHz; ³¹P-NMR 162 MHz) or a BRUKER Avance 500 (¹H-NMR: 500 MHz; ¹³C-NMR, 125.8 MHz). ¹H-NMR experiments were performed in CDCl₃, D₂O, CD₃OD in CDCl₃ with CHCl₃ (δ = 7.26 ppm), DHO (δ = 4.79 ppm), CD₂HOD (δ = 3.31 ppm) as internal references. ¹³C-NMR experiments were performed in CDCl₃ with CDCl₃ (δ = 77.23 ppm), CD₃HOD (δ = 49.0 ppm) as internal references. For ³¹P-NMR reference, the spectrometer had an external reference, corresponding to 80% phosphoric acid in D₂O (δ = 0 ppm). The chemical shifts (δ) are expressed in ppm. s, d, t, q, or bs are abbreviations for multiplicity correspond to singlet, doublet, triplet and quadruplet or broad singlet. J-couplings are exposed in Hz. Most of the

hydroxamate are present as two *Z* and *E* conformers in equilibrium. If only one signal is described, it is common to all conformers. The evaluation of the relative amount of the conformers was made by integration of the CH₂CO or CH₂N or OCH₂Bn proton signals. Negative or positive-mode electrospray MS were performed on a Bruker Daltonics microTOF spectrometer (Bruker Daltonik GmbH, Bremen, Germany) equipped with an orthogonal electrospray (ESI) interface. Calibration was performed using a solution of 10 mM sodium formate. Sample solutions were introduced into the spectrometer source with a syringe pump (Harvard type 55 1111: Harvard Apparatus Inc., South Natick, MA, USA) with a flow rate of 5 $\mu\text{L min}^{-1}$.

***N*-(Benzyloxy)-3-hydroxypropanamide (8)**

A stirred suspension of *O*-benzylhydroxylamine hydrochloride (3.83 g, 24 mmol, 1.5 equiv) in dry THF (4.5 mL/mmol) at -78 °C was treated with 1 M solution of LiHMDS (80 mL, 80 mmol, 5 equiv). After 1 h a solution of β -propiolactone (1 mL, 16 mmol, 1 equiv) in a minimum of THF was added. The resulting solution was stirred overnight at rt. The reaction was cooled at -78 °C then was quenched with a saturated aqueous solution of NH₄Cl, warmed to rt, and extracted several times with EtOAc. The collect organic layers were dried over anhydrous Na₂SO₄, filtered, and evaporated to dryness under reduced pressure. The crude product was dissolved in THF and treated with TBAF.(H₂O)₃ (6.3 g, 24 mmol 1.5 equiv). After total consumption of starting material, the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (EtOAc to EtOAc/MeOH, 90:10) to give the product **8** (1.83 g, 62%) as a white solid and a mixture of two conformers *Z* and *E* in a 70:30 ratio respectively.

R_f = 0.19 (EtOAc/petroleum ether, 7:3)

Mp = 83 °C – 84 °C

¹H-NMR (500 MHz, CDCl₃): 2.33 (7/10 of 2H, bs, CH₂CO), 2.50 (3/10 of 2H, bs, CH₂CO), 2.63 (7/10 of 1H, bs, OH), 2.81 (3/10 of 1H, bs, OH), 3.86 (2H, td, ³J = 5.5 Hz, ³J = 5.5 Hz, CH₂OH), 4.83 (3/10 of 2H, bs, OCH₂Ph), 4.94 (7/10 of 2H, bs, OCH₂Ph), 7.39 (5H, s, CH_{Ar}), 7.87 (3/10 of 1H, bs, NH), 8.31 (7/10 of 1H, bs, NH).

¹³C-NMR (125.8 MHz, CDCl₃): 34.0 (CH₂CO), 35.7 (CH₂CO), 58.1 (CH₂OH), 58.7 (CH₂OH), 78.5 (OCH₂Ph), 79.7 (OCH₂Ph), 128.9 (CH_{Ar}), 129.1 (CH_{Ar}), 129.5 (CH_{Ar}), 135.3 (C_{Ar}), 170.2 (CO).

MS (EI)⁺: *m/z* calculated for C₁₀H₁₃NO₃Na [M+Na]⁺ 218.08, found 218.08.

***N*-(Benzyloxy)-3-hydroxy-*N*-methylpropanamide (9)**

To a solution of *N*-H hydroxamic acid (552 mg, 2.83 mmol, 1 equiv), anhydrous K₂CO₃ (782 mg, 5.66 mmol, 2 equiv) in anhydrous acetone (10.5 mL/mmol) was added iodomethane (890 μL , 14.1 mmol, 5 equiv). The resulting mixture was refluxed overnight. The mixture was filtered and acetone was removed under reduced pressure. The resulting oil was dissolved in ether. The organic layer was washed with water and then aqueous layer was saturated with NaCl and extracted with EtOAc. The organic layers were collected, dried over anhydrous Na₂SO₄, filtered and evaporated. The crude product was purified by flash chromatography (EtOAc/petroleum ether, 7:3 to EtOAc) to give **9** as a yellow oil (480 mg, 81%) as a sole *E* conformer

R_f = 0.43 (EtOAc/petroleum ether, 7:3).

¹H-NMR (400 MHz, CDCl₃): 2.62 (2H, t, ³J = 5.2 Hz, CH₂CO), 3.05 (1H, t, ³J = 6.0 Hz, OH), 3.22 (3H, s, NCH₃), 3.82 (2H, td, ³J = 5.6 Hz, ³J = 5.5 Hz CH₂OH), 4.84 (2H, s, OCH₂Ph), 7.38 (5H, m, CH_{Ar}).

¹³C-NMR (125.8 MHz, CDCl₃): 33.4 (NCH₃), 34.4 (CH₂CO), 58.5 (CH₂OH), 76.5 (OCH₂Ph), 128.9 (CH_{Ar}), 129.3 (CH_{Ar}), 129.5 (CH_{Ar}), 134.3 (C_{Ar}), 174.8 (CO).

MS (EI)⁺: *m/z* calculated for C₁₁H₁₅NO₃Na [M+Na]⁺ 232.09, found 232.09.

3-((Benzyloxy)(methyl)amino)-3-oxopropyl diethyl phosphate (10)

Iodine (148 mg, 0.58 mmol, 1.1 equiv) was added to a solution of triethyl phosphite (100 μL , 0.58 mmol, 1.1 equiv) in dry CH₂Cl₂ (2.5 mL/mmol) at 0 °C. The reaction was stirred for 20 min at 0 °C and then for 1 h at rt. This

phosphorylation agent was slowly added to a solution of alcohol **9** (88 mg, 0.53 mmol, 1 equiv) and pyridine (171 μ L, 2.12 mmol, 4 equiv) in dry CH_2Cl_2 (13 mL/mmol) at -40°C . The reaction was allowed to warm up to rt and stirred for 2 h. The mixture was washed with a 3% solution of KHSO_4 , a saturated solution of NaHCO_3 and brine. The organic layer was dried over anhydrous Na_2SO_4 , filtered and evaporated to dryness under reduced pressure. The crude product was purified by automated chromatography (EtOAc/petroleum ether, 80:20 to EtOAc/MeOH, 95:5) to give **10** as a colorless oil (177 mg, 88%) and as the sole *E* conformer.

Rf = 0.20 (EtOAc/petroleum ether, 80:20).

^1H -NMR (300 MHz, CDCl_3): 1.20 (6H, td, $^3J = 7.1$ Hz, $^4J = 0.7$ Hz, $\text{CH}_3\text{CH}_2\text{OP}$), 2.66 (2H, t, $^3J = 6.6$ Hz, CH_2CO), 3.08 (3H, s, NCH₃), 3.93–4.03 (4H, m, $\text{CH}_3\text{CH}_2\text{OP}$), 4.15–4.22 (2H, m, POCH_2), 4.72 (2H, s, OCH_2Ph), 7.26 (5H, s, CH_{Ar}).

^{13}C -NMR (125.8 MHz, CDCl_3): 16.3 (d, $^3J_{\text{C-P}} = 6.7$ Hz, $\text{CH}_3\text{CH}_2\text{OP}$), 33.3 (d, $^3J_{\text{C-P}} = 6.5$ Hz, CH_2CO), 33.6 (NCH₃), 63.2 (d, $^2J_{\text{C-P}} = 5.2$ Hz, POCH_2), 64.0 (d, $^2J_{\text{C-P}} = 5.6$ Hz, $\text{CH}_3\text{CH}_2\text{OP}$), 76.6 (OCH_2Ph), 79.7 (OCH_2Ph), 129.0 (CH_{Ar}), 129.3 (CH_{Ar}), 129.5 (CH_{Ar}), 134.4 (C_{Ar}), 171.7 (CO).

^{31}P -NMR (121.5, CDCl_3): -0.97.

MS (EI)⁺: *m/z* calculated for $\text{C}_{15}\text{H}_{25}\text{NPO}_6$ [$\text{M}+\text{H}$]⁺ 346.14, found 346.15.

3-((Benzyloxy)(methyl)amino)-3-oxopropyl di-*tert*-butyl phosphate (**11**)

To a stirred solution of alcohol **9** (80 mg, 0.38 mmol, 1 equiv) in anhydrous THF (16 mL/mmol) were successively added 0.45M solution of 1*H*-tetrazole in acetonitrile (3.8 mL, 1.71 mmol, 4.5 equiv) and di-*t*-butylisopropylphosphoramidite (265 μ L, 0.84 mmol, 2.2 equiv). The reaction mixture was stirred for 2 h while a white solid was formed. A solution of *m*-chloroperoxybenzoic acid (270 mg, 1.33 mmol, 3.5 equiv) purified according to the method described Armarego¹⁵ was added, the reaction mixture was stirred overnight at rt. CH_2Cl_2 was added and organic layer was washed with a 10% aqueous solution of $\text{Na}_2\text{S}_2\text{O}_5$. The aqueous layer was extracted twice with CH_2Cl_2 and the combined organic layers were washed with a saturated solution of NaHCO_3 and brine. The organic layer was dried over anhydrous Na_2SO_4 , filtered and evaporated to dryness under reduced pressure. The crude product was purified by automated flash chromatography (EtOAc/petroleum ether, 60:40 to EtOAc) to give **11** as a colorless oil (92 mg, 60%) and as the sole conformer *E*. Rf = 0.46 (EtOAc).

^1H -NMR (500 MHz, CDCl_3): 1.47 (18H, s, *t*BuO), 2.79 (2H, t, $^3J = 6.6$ Hz, CH_2CO), 3.20 (3H, s, NCH₃), 4.23 (2H, td, $^3J = 6.9$ Hz, $^3J_{\text{P-H}} = 7.0$ Hz, POCH_2), 4.85 (2H, bs, OCH_2Ph), 7.38 (5H, s, CH_{Ar}).

^{13}C -NMR (125.8 MHz, CDCl_3): 30.4 (d, $^3J_{\text{C-P}} = 4.3$ Hz, *t*BuO), 33.3 (d, $^3J_{\text{C-P}} = 7.3$ Hz, CH_2CO), 33.6 (NCH₃), 62.7 (d, $^2J_{\text{C-P}} = 6.0$ Hz, POCH_2), 76.6 (OCH_2Ph), 82.5 (d, $^2J_{\text{C-P}} = 7.3$ Hz, C_4° of *Ot*Bu), 128.9 (CH_{Ar}), 129.2 (CH_{Ar}), 129.5 (CH_{Ar}), 135.4 (C_{Ar}), 172.1 (CO).

^{31}P -NMR (162.0 MHz, CDCl_3): -10.2.

HRMS (EI)⁺: *m/z* calculated for $\text{C}_{19}\text{H}_{32}\text{NO}_6\text{PNa}$ [$\text{M}+\text{Na}$]⁺ 24.1865, found 24.1816.

3-((Benzyloxy)(methyl)amino)-3-oxopropyl dihydrogen phosphate (**6**)

The protected phosphate **11** (20 mg, 50 μ mol, 1 equiv) was treated with a solution of trifluoroacetic acid (TFA)/ CH_2Cl_2 (1:3 v/v). The reaction mixture was monitoring by TLC. Toluene was added and the mixture was evaporated under reduced pressure to give the desired product. The product **12** was obtained without purification as a colorless oil (12 mg, 93%).

^1H -NMR (300 MHz, CD_3OD): 2.80 (2H, t, $^3J = 6.3$ Hz, CH_2CO), 3.23 (3H, s, NCH₃), 4.19–4.23 (2H, m, POCH_2), 4.98 (OCH_2Ph not visible: within water signal), 7.38–7.46 (5H, s, CH_{Ar}).

^{13}C -NMR (125.8 MHz, CD_3OD): 33.5 (NCH₃), 34.1 (CH_2CO), 63.3 (d, $^2J_{\text{C-P}} = 5.3$ Hz, POCH_2), 77.4 (OCH_2Ph), 129.7 (CH_{Ar}), 130.1 (CH_{Ar}), 130.8 (CH_{Ar}), 135.9 (C_{Ar}), 173.6 (CO).

^{31}P -NMR (121.5 MHz, CD_3OD): 0.1

HRMS (EI)⁺: *m/z* calculated for $\text{C}_{11}\text{H}_{16}\text{NO}_6\text{PNa}$ [$\text{M}+\text{Na}$]⁺ 312.0607, found 312.0590.

General procedure for the synthesis of cycloSal phosphochloridate (13a-f)

A solution of saligenol **12a-f** (1 equiv) and triethylamine (2.5 equiv) in CH₂Cl₂ (C = 0.25 M) was added dropwise to a solution of phosphorous oxichloride (1.1 equiv) in CH₂Cl₂ (C = 0.25 M) at -78°C. The reaction mixture was stirred overnight and allowed to slowly warm to rt. After determination of the proportion of cycloSal phosphochloridate using ³¹P NMR, the crude mixture was directly used in the next step

2-Chloro-4H-benzo[d][1,3,2]dioxaphosphinine 2-oxide (13a)¹³

Using the general procedure, **13a** was synthesized from 2-(hydroxymethyl)phenol (1.024 g, 8.0 mmol, 1 equiv). ³¹P NMR analysis confirmed the formation of **13a** (proportion: 44%).

³¹P-NMR (162 MHz, CDCl₃): δ -6.2.

2-Chloro-6-methyl-4H-benzo[d][1,3,2]dioxaphosphinine 2-oxide (13b)¹³

Using the general procedure, **13b** was synthesized from 2-(hydroxymethyl)-4-methylphenol (500 mg, 3.62 mmol, 1 equiv). ³¹P NMR analysis confirmed the formation of **13b** (proportion: 52%).

³¹P-NMR (162 MHz, CDCl₃): δ -5.9.

2-Chloro-6-methoxy-4H-benzo[d][1,3,2]dioxaphosphinine 2-oxide (13c)¹³

Using the general procedure, **13c** was synthesized from 2-(hydroxymethyl)-4-methoxyphenol (618 mg, 4.01 mmol, 1 equiv). ³¹P NMR analysis confirmed the formation of **13c** (proportion: 57%).

³¹P-NMR (162 MHz, CDCl₃): δ -6.0.

2,6-Dichloro-4H-benzo[d][1,3,2]dioxaphosphinine 2-oxide (13d)¹³

Using the general procedure, **13d** was synthesized from 4-chloro-2-(hydroxymethyl)phenol (570 mg, 3.59 mmol, 1 equiv). ³¹P NMR analysis confirmed the formation of **13d** (proportion: 78%).

³¹P-NMR (162 MHz, CDCl₃): δ -6.7.

2-Chloro-6-(trifluoromethyl)-4H-benzo[d][1,3,2]dioxaphosphinine 2-oxide (13e)¹³

Using the general procedure, **13e** was synthesized from 2-(hydroxymethyl)-4-(trifluoromethyl)phenol (465 mg, 2.42 mmol, 1 equiv). ³¹P NMR analysis confirmed the formation of **13e** (proportion: 70%).

³¹P-NMR (162 MHz, CDCl₃): δ -7.0.

Methyl 2-((tert-butoxycarbonyl)amino)-3-(2-chloro-2-oxido-4H-benzo[d][1,3,2]dioxaphosphinin-6-yl)propanoate (13f)¹⁷

Using the general procedure, **13f** was synthesized from methyl (S)-2-((tert-butoxycarbonyl)amino)-3-(4-hydroxy-3-(hydroxymethyl)phenyl)propanoate (939 mg, 2.89 mmol, 1 equiv). ³¹P NMR analysis confirmed the formation of **13f** (proportion: 40%).

³¹P-NMR (162 MHz, CDCl₃): δ -6.2.

General procedure for the synthesis of cycloSal phosphotriester (7)

A solution of DMAP (0.5 equiv), alcohol **9** (1 equiv) and triethylamine (1.1 equiv) in CH₂Cl₂ (0.3 M) was cooled down to -40°C and a solution of cycloSal phosphochloridate **13** (3 equiv) was added dropwise. The reaction mixture was allowed to warm up to rt and stirred 4h. A saturated solution of NH₄Cl was added and the aqueous layer was extracted three times with CH₂Cl₂. The combined organic layers were dried over Na₂SO₄ and the solvent was evaporated under reduced pressure.

N-(Benzyloxy)-N-methyl-3-((2-oxido-4H-benzo[d][1,3,2]dioxaphosphinin-2-yl)oxy)propenamide (7a)

The general procedure was applied to prepare compound **7a** from **9** (147 mg, 0.70 mmol, 1 equiv) and **13a** (3 equiv). The crude was purified by automated flash chromatography (from EtOAc/petroleum ether, 75:25 to EtOAc/petroleum ether, 80:20). The desired product was afforded as a colorless oil (194 mg, 73%).

R_f = 0.35 (EtOAc/ CH₂Cl₂, 50:50)

¹H-NMR (500 MHz, CDCl₃): δ = 2.71 – 2.87 (m, 2H, CH₂CO), 3.15 (s, 3H, NCH₃), 4.37 – 4.53 (m, 2H, POCH₂), 4.79 (s, 2H, OCH₂Ph), 5.30 (dd, ³J_{H-P} = 17.9, ²J_{H-H} = 14.0 Hz, 1H, 1xArCH₂OP), 5.38 (dd, ²J_{H-H} = 14.0, ³J_{H-P} = 9.1 Hz, 1H,

1xArCH₂OP), 7.02 (dd, *J* = 8.2, 1.1 Hz, 1H, CH_{Ar(cycloSal)}), 7.05 (dd, *J* = 7.7, 1.7 Hz, 1H, CH_{Ar(cycloSal)}), 7.10 (dd, *J* = 7.5, 1.1 Hz, 1H, CH_{Ar(cycloSal)}), 7.28 (dd, *J* = 8.0 Hz, 1H, CH_{Ar(cycloSal)}), 7.31 – 7.41 (m, 5H, CH_{Ar(Bn)}).

¹³C-NMR (125.8 MHz, CDCl₃): δ = 33.1 (d, *J* = 7.0 Hz, CH₂CO), 33.5 (NCH₃), 64.0 (d, *J* = 5.4 Hz, POCH₂), 68.7 (d, *J* = 6.9 Hz, ArCH₂OP), 76.5 (OCH₂Ph), 118.8 (d, *J* = 9.1 Hz, CH_{Ar}C_{Ar}OP), 120.8 (d, *J* = 9.9 Hz, C_{Ar}C_{Ar}OP), 124.3 (CH_{Ar(cycloSal)}), 125.3 (d, *J* = 1.1 Hz, CH_{Ar(cycloSal)}), 128.9 (CH_{Ar(Bn)}), 129.2 (CH_{Ar(Bn)}), 129.4 (CH_{Ar(Bn)}), 129.8 (d, *J* = 1.8 Hz, CH_{Ar(cycloSal)}), 134.2 (C_{Ar(Bn)}), 150.3 (d, *J* = 6.9 Hz, C_{Ar}OP), 171.2 (CO).

³¹P-NMR (202.0 MHz, CDCl₃): -9.5.

HRMS (EI)⁺: *m/z* calculated for C₁₈H₂₁NO₆P [M+H]⁺ 378.1101, found 378.1105.

***N*-(Benzyloxy)-*N*-methyl-3-((6-methyl-2-oxido-4H-benzo[d][1,3,2]dioxaphosphinin-2-yl)oxy)propenamide (7b)**

The general procedure was applied to prepare compound **7b** from **9** (132 mg, 0.63 mmol, 1 equiv) and **13b** (3 equiv). The crude was purified by automated flash chromatography (EtOAc/petroleum ether, 75:25). The desired product was afforded as a colorless oil (196 mg, 80%).

R_f = 0.37 (EtOAc/petroleum ether, 75:25)

¹H-NMR (500 MHz, CDCl₃): δ = 2.29 (s, 3H, CH₃Ar), 2.66 – 2.99 (m, 2H, CH₂CO), 3.16 (s, 3H, NCH₃), 4.36 – 4.53 (m, 2H, POCH₂), 4.80 (s, 2H, OCH₂Ph), 5.21 – 5.38 (m, 2H, ArCH₂OP), 6.84 (s, 1H), 6.91 (d, *J* = 8.3 Hz, 1H, CH_{Ar(cycloSal)}), 7.07 (d, *J* = 8.4 Hz, 1H, CH_{Ar(cycloSal)}), 7.32 – 7.42 (m, 5H, CH_{Ar(Bn)}).

¹³C-NMR (126 MHz, CDCl₃): δ = 20.8 (C_{Ar(cycloSal)}-CH₃), 33.1 (d, *J* = 6.9 Hz, CH₂CO), 33.6 (NCH₃), 64.0 (d, *J* = 5.4 Hz, POCH₂), 68.8 (d, *J* = 6.9 Hz, ArCH₂OP), 76.5 (OCH₂Ph), 118.5 (d, *J* = 9.1 Hz, CH_{Ar}C_{Ar}OP), 120.4 (d, *J* = 9.8 Hz, C_{Ar}CH₂OP), 125.6 (d, *J* = 1.1 Hz, CH_{Ar(cycloSal)}), 128.9 (CH_{Ar(Bn)}), 129.3 (CH_{Ar(Bn)}), 129.5 (CH_{Ar(Bn)}), 130.3 (d, *J* = 1.7 Hz, CH_{Ar(cycloSal)}), 134.0 (C_{Ar(cycloSal)}-CH₃), 134.2 (C_{Ar(Bn)}), 148.2 (d, *J* = 6.9 Hz, C_{Ar(cycloSal)}OP), 171.3 (CO).

³¹P-NMR (202 MHz, CDCl₃): -9.3.

HRMS (EI)⁺: *m/z* calculated for C₁₉H₂₂NO₆PNa [M+Na]⁺ 414.1077, found 414.1079

***N*-(Benzyloxy)-*N*-methyl-3-((6-methoxy-2-oxido-4H-benzo[d][1,3,2]dioxaphosphinin-2-yl)oxy)propenamide (7c)**

The general procedure was applied to prepare compound **7c** from **9** (160 mg, 0.77 mmol, 1 equiv) and **13c** (3 equiv). The crude was purified by automated flash chromatography (from EtOAc/petroleum ether, 60:40 to EtOAc/petroleum ether, 90:10). The desired product was afforded as a colorless oil (247 mg, 78%).

R_f = 0.38 (EtOAc/petroleum ether, 90:10)

¹H-NMR (500 MHz, CDCl₃): δ = 2.72 – 2.88 (m, 2H, CH₂CO), 3.17 (s, 3H, NCH₃), 3.76 (s, 3H, OCH₃), 4.37 – 4.53 (m, 2H, POCH₂), 4.80 (s, 2H, OCH₂Ph), 5.27 (dd, ³J_{H-P} = 17.4, ²J_{H-H} = 14.0 Hz, 1H, 1xArCH₂OP), 5.34 (dd, ²J_{H-H} = 14.1, ³J_{H-P} = 9.3 Hz, 1H, 1xArCH₂OP), 6.55 (d, *J* = 3.0 Hz, 1H, CH_{Ar(cycloSal)}), 6.80 (dd, *J* = 9.0, 3.1, 1H, CH_{Ar(cycloSal)}), 6.95 (d, *J* = 9.0 Hz, 1H, CH_{Ar(cycloSal)}), 7.32 – 7.43 (m, 5H, CH_{Ar(Bn)}).

¹³C-NMR (125.8 MHz, CDCl₃): δ 33.1 (d, *J* = 7.1 Hz, CH₂CO), 33.6 (NCH₃), 55.9 (OCH₃), 64.0 (d, *J* = 5.4 Hz, POCH₂), 68.8 (d, *J* = 6.9 Hz, ArCH₂OP), 76.6 (OCH₂Ph), 110.2 (d, *J* = 1.1 Hz, CH_{Ar(cycloSal)}), 115.1 (d, *J* = 1.6 Hz, CH_{Ar(cycloSal)}), 119.7 (d, *J* = 9.1 Hz, CH_{Ar}C_{Ar}OP), 121.5 (d, *J* = 9.7 Hz, C_{Ar}C_{Ar}OP), 128.9 (CH_{Ar(Bn)}), 129.3 (CH_{Ar(Bn)}), 129.5 (CH_{Ar(Bn)}), 134.2 (C_{Ar(Bn)}), 144.0 (d, *J* = 6.8 Hz, C_{Ar}OP), 156.1 (C_{Ar}OCH₃), 171.4 (CO).

³¹P-NMR (162.0 MHz, CDCl₃): -9.5.

HRMS (EI)⁺: *m/z* calculated for C₁₉H₂₂NO₇PNa [M+Na]⁺ 430.1026, found 430.1017

***N*-(Benzyloxy)-3-((6-chloro-2-oxido-4H-benzo[d][1,3,2]dioxaphosphinin-2-yl)oxy)-*N*-methylpropanamide (7d)**

The general procedure was applied to prepare compound **7d** from **9** (191 mg, 0.93 mmol, 1 equiv) and **13d** (3 equiv). The crude was purified by automated flash chromatography (from EtOAc/CH₂Cl₂, 90:10 to EtOAc/CH₂Cl₂, 50:50). The desired product was afforded as a colorless oil (252 mg, 65%).

R_f = 0.38 (EtOAc/petroleum ether, 90:10)

¹H-NMR (500 MHz, CDCl₃): δ = 2.72 – 2.85 (m, 2H, CH₂CO), 3.17 (s, 3H, NCH₃), 4.39 – 4.53 (m, 2H, POCH₂), 4.80 (s, 2H, OCH₂Ph), 5.25 (dd, ³J_{H-P} = 18.2, ²J_{H-H} = 14.3 Hz, 1H, 1xArCH₂OP), 5.35 (dd, ²J_{H-H} = 14.3, ³J_{H-P} = 8.6 Hz, 1H, 1xArCH₂OP), 6.97 (d, *J* = 8.7 Hz, 1H, CH_{Ar(cycloSal)}), 7.05 (s, 1H, CH_{Ar(cycloSal)}), 7.24 (d, *J* = 8.1 Hz, 1H, CH_{Ar(cycloSal)}), 7.32 – 7.44 (m, 5H, CH_{Ar(Bn)}).

¹³C-NMR (125.8 MHz, CDCl₃): δ = 33.1 (d, *J* = 7.1 Hz, CH₂CO), 33.5 (NCH₃), 64.2 (d, *J* = 5.4 Hz, POCH₂), 68.1 (d, *J* = 7.0 Hz, ArCH₂OP), 76.5 (OCH₂Ph), 120.2 (d, *J* = 9.3 Hz, CH_{Ar}C_{Ar}OP), 122.2 (d, *J* = 9.9 Hz, C_{Ar}CH₂OP), 125.3 (d, *J* = 1.1 Hz, CH_{Ar(cycloSal)}), 128.9 (CH_{Ar(Bn)}), 129.3 (CH_{Ar(Bn)}), 129.5 (CH_{Ar(Bn)}), 129.5 (C_{Ar}Cl), 129.8 (d, *J* = 1.7 Hz, CH_{Ar(cycloSal)}), 134.2 (C_{Ar(Bn)}), 148.8 (d, *J* = 6.8 Hz, C_{Ar(cyclo)}OP), 171.2 (CO).

³¹P-NMR (121.5 MHz, CDCl₃): -10.3

HRMS (EI)⁺: *m/z* calculated for C₁₈H₁₉ClNO₆PNa [M+Na]⁺ 434.0531, found 434.0524

***N*-(Benzyloxy)-*N*-methyl-3-((6-trifluoromethyl-2-oxido-4H-benzo[d][1,3,2]dioxaphosphinin-2-yl)oxy)propenamide (7e)**

The general procedure was applied to prepare compound **7e** from **9** (118 mg, 0.56 mmol, 1 equiv) and **13e** (3 equiv). The crude was purified by automated flash chromatography (from EtOAc/petroleum ether, 50:50 to EtOAc/petroleum ether, 65:35). The desired product was afforded as a colorless oil (219 mg, 87%).

R_f = 0.37 (EtOAc/petroleum ether, 65:35)

¹H-NMR (500 MHz, CDCl₃): δ = 2.72 – 2.88 (m, 2H, CH₂CO), 3.18 (s, 3H, NCH₃), 4.40 – 4.59 (m, 2H, POCH₂), 4.81 (s, 2H, OCH₂Ph), 5.34 (dd, ³J_{H-P} = 18.4, ²J_{H-H} = 14.0 Hz, 1H, 1xArCH₂OP), 5.44 (dd, ²J_{H-H} = 14.2, ³J_{H-P} = 8.7 Hz, 1H, 1xArCH₂OP), 7.14 (d, *J* = 8.6 Hz, 1H, CH_{Ar(cycloSal)}), 7.32 – 7.42 (m, 6H, 5xCH_{Ar(Bn)}+1xCH_{Ar(cycloSal)}), 7.57 (d, *J* = 8.4 Hz, 1H, CH_{Ar(cycloSal)}).

¹³C-NMR (126 MHz, CDCl₃): δ = 33.1 (d, *J* = 6.9 Hz, CH₂CO), 33.6 (NCH₃), 64.4 (d, *J* = 5.3 Hz, POCH₂), 68.3 (d, *J* = 6.9 Hz, ArCH₂OP), 76.5 (OCH₂Ph), 119.6 (d, *J* = 9.4 Hz, CH_{Ar}C_{Ar}OP), 121.4 (d, *J* = 9.9 Hz, C_{Ar}CH₂OP), 123.0 (q, *J* = 3.9 Hz, CH_{Ar(cycloSal)}), 123.6 (q, *J* = 271.9 Hz, C_{Ar}CF₃), 126.9 (q, *J* = 33.5 Hz, C_{Ar}CF₃), 127.1 – 127.2 (m, CH_{Ar(cycloSal)}), 129.0 (CH_{Ar(Bn)}), 129.4 (CH_{Ar(Bn)}), 129.5 (CH_{Ar(Bn)}), 134.1 (C_{Ar(Bn)}), 152.7 (d, *J* = 6.8 Hz, C_{Ar(cycloSal)}OP), 171.2 (CO).

³¹P-NMR (121.5 MHz, CDCl₃): δ -10.6

¹⁹F-NMR (282.4 MHz, CDCl₃): δ -62.2

HRMS (EI)⁺: *m/z* calculated for C₁₉H₁₉F₃NO₆PNa [M+Na]⁺ 468.0794, found 468.0800

***L*-Methyl 3-(2-(3-((benzyloxy)(methyl)amino)-3-oxopropoxy)-2-oxido-4H-benzo[d][1,3,2]dioxaphosphinin-6-yl)-2-((tert-butoxycarbonyl)amino)propanoate (7f)**

The general procedure was applied to prepare compound **7f** from **9** (120 mg, 0.57 mmol, 1 equiv) and **13f** (3 equiv). The crude was purified by automated flash chromatography (from EtOAc/CH₂Cl₂, 90:10 to EtOAc/CH₂Cl₂, 50:50). The desired product was afforded as a white solid (249 mg, 43%). Two diastereoisomers were detected by ¹³C-NMR spectroscopy. Unambiguous assignment of the signals to each individual diastereomer has not been achieved.

R_f = 0.35 (EtOAc/petroleum ether, 50:50)

¹H-NMR (500 MHz, CDCl₃): δ = 1.41 (s, 9H, C(CH₃)₃), 2.73 – 2.89 (m, 2H, CH₂CO), 2.98 (dd, *J* = 13.8, 6.1 Hz, 1H, 1xCHCH₂), 3.08 (dd, *J* = 13.6, 4.9 Hz, 1H, 1xCHCH₂), 3.17 (s, 3H, NCH₃), 3.71 (s, 3H, OCH₃), 4.38 – 4.58 (m, 3H, 2xPOCH₂+CHCH₂), 4.82 (s, 2H, OCH₂Ph), 4.98 (d, *J* = 8.2 Hz, 1H, NH), 5.20 – 5.41 (m, 2H, ArCH₂OP), 6.84 (s, 1H, CH_{Ar(cycloSal)}), 6.95 (d, *J* = 8.4 Hz, 1H, CH_{Ar(cycloSal)}), 7.04 (d, *J* = 8.3 Hz, 1H, CH_{Ar(cycloSal)}), 7.31 – 7.44 (m, 5H, CH_{Ar(Bn)}).

¹³C-NMR (126 MHz, CDCl₃): δ = 28.4 (C(CH₃)₃), 33.2 (d, *J* = 5.9 Hz, CH₂CO), 33.5 (CH₃N, diastereoisomer), 33.6 (CH₃N, diastereoisomer), 37.75 (CH₂CHN, diastereoisomer), 37.77 (CH₂CHN, diastereoisomer), 52.51 (OCH₃, diastereoisomer), 52.52 (CH₃, diastereoisomer), 54.46 (CHN, diastereoisomer), 54.50 (CHN, diastereoisomer), 64.1 (d, *J* = 5.4 Hz, POCH₂), 68.6 (d, *J* = 6.9 Hz, ArCH₂OP), 76.5 (OCH₂Ph), 80.3 (C(CH₃)₃), 118.9 (d, *J* = 9.3 Hz, CH_{Ar}C_{Ar}OP), 120.8 (d, *J* = 9.7 Hz, C_{Ar}CH₂OP), 126.1 (CH_{Ar(cycloSal)}, diastereoisomer), 126.2 (CH_{Ar(cycloSal)},

diastereoisomer), 128.9 ($\text{CH}_{\text{Ar}(\text{Bn})}$), 129.3 ($\text{CH}_{\text{Ar}(\text{Bn})}$), 129.5 ($\text{CH}_{\text{Ar}(\text{Bn})}$), 130.65 (d, $J = 1.7$ Hz, $\text{CH}_{\text{Ar}(\text{cycloSal})}$, diastereoisomer), 130.69 (d, $J = 1.7$ Hz, $\text{CH}_{\text{Ar}(\text{cycloSal})}$, diastereoisomer), 132.5 ($\text{CHCH}_2\text{C}_{\text{Ar}(\text{cycloSal})}$), 134.3 ($\text{C}_{\text{Ar}(\text{Bn})}$), 149.4 (d, $J = 7.1$ Hz, $\text{C}_{\text{Ar}(\text{cycloSal})\text{OP}}$), 155.1 (NCO carbamate), 171.3 (CO), 172.2 (COOCH_3).

^{31}P -NMR (121.5 MHz, CDCl_3): -9.7.

HRMS (EI) $^+$: m/z calculated for $\text{C}_{27}\text{H}_{35}\text{N}_2\text{O}_{10}\text{PNa}$ $[\text{M}+\text{Na}]^+$ 601.1922, found 601.1901

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Supplementary Material

Copies of ^1H , ^{31}P and ^{13}C NMR spectra of for compounds **7a-7f** are available in the supplementary material file associated with this paper.

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