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Article

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KEYWORDS: Phenolic drugs, Propofol, Prodrug strategies, Phosphonamidate Prodrug

ABSTRACT Phosphonamidate **3a** of methoxymethylphosphonic acid (MMPA) with propofol (**1**) and L-alanine ethyl ester was found to be an efficient scaffold for the oral delivery of compound **1**. The synthesis and evaluation of MMPA based phosphonamidates of compound **1**, HSK3486 (**2**) and other phenolic drugs revealed the general application of MMPA as the effective delivery vehicle for phenolic drugs. Based on plasma concentrations of compound **1** and SN38 **(14)**, the oral bioavailability of compound **3a** and **15** in beagle dogs was found to be 97.6% and 34.1% respectively.

INTRODUCTION

We have recently reported that HSK3486[2] (**2**, Figure 1), a close analog of propofol[1] (**1**) now in advanced phase II clinical trial in China, has shown equivalent potency of **1** at 1/5 of the dosage in similar lipid emulsion formulation. Given that compounds **1** and **2** may hold therapeutic potentials in insomnia,[3] migraine,[4] anxiety,[5] and analgesia[6] etc, we have decided to explore the oral delivery of **1** and **2** through prodrug strategy, and evaluate the potential clinical usages of these oral prodrugs. Herein we report the discovery of an efficient oral delivery vehicle for phenolic drugs such as propofol (**1**) and HSK3486 (**2**).

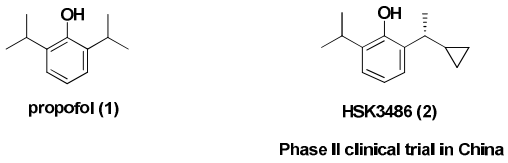


Figure 1. Chemical structures of **1** and **2.**

Previous attempts to make **1** orally bioavailable through formulation and prodrug modifications have met with limited success.[7-11] Our efforts turned to the aryloxyphosphonamidate ProTide technology first reported by the McGuigan group,[12-16] which was used subsequently by Gilead Sciences for the marketed drug tenofovir alafenamide.[17] As illustrated with the prodrug of tenofovir[18] (**A,** Figure 2), oral absorption is facilitated by the neutral phosphonamidate **A** of tenofovir with an amino acid ester and phenol. It was postulated that the release of the phosphonic acid target drug happened first through hydrolysis of the amino acid ester, affording the release of the alcohol fragment **A1**. This was followed by intramolecular lactam formation affording the release of the phenol fragment **A3**. This transient cyclic lactam **A4** was then hydrolyzed through a two step sequence, first the rapid hydrolysis of the P-O bond, followed by a phosphoramidase mediated cleavage[19] of the P-N bond in **A5**, affording the

release of the target drug 9-*R*-(2-phosphonomethoxypropyl)adenine and the amino acid **A6**.

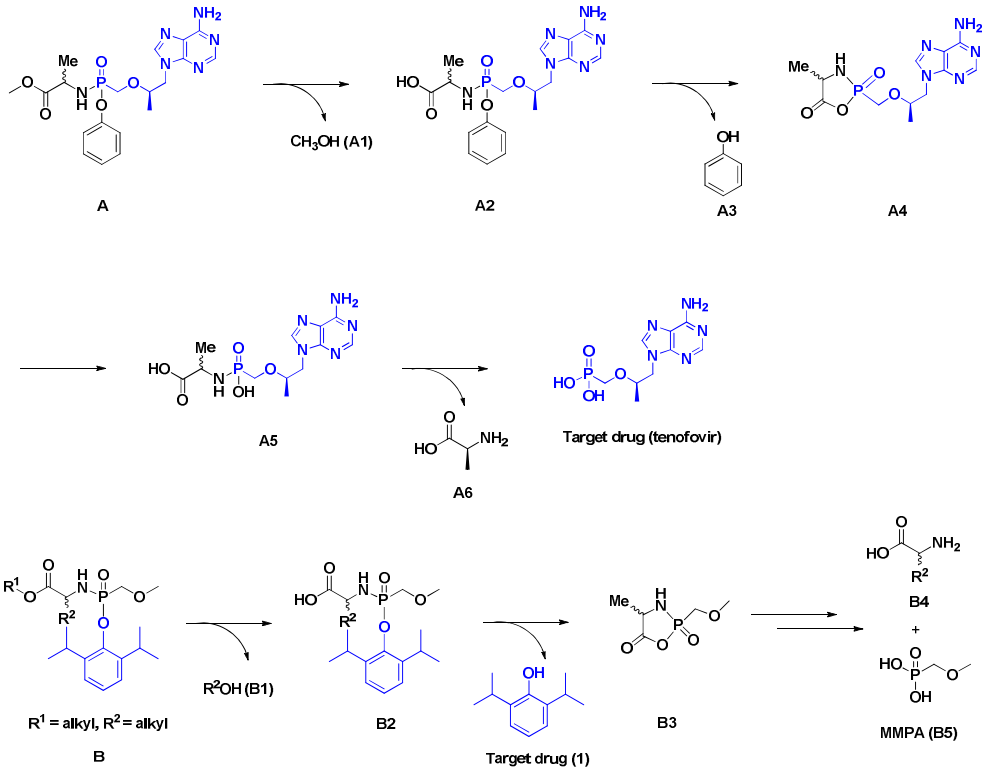


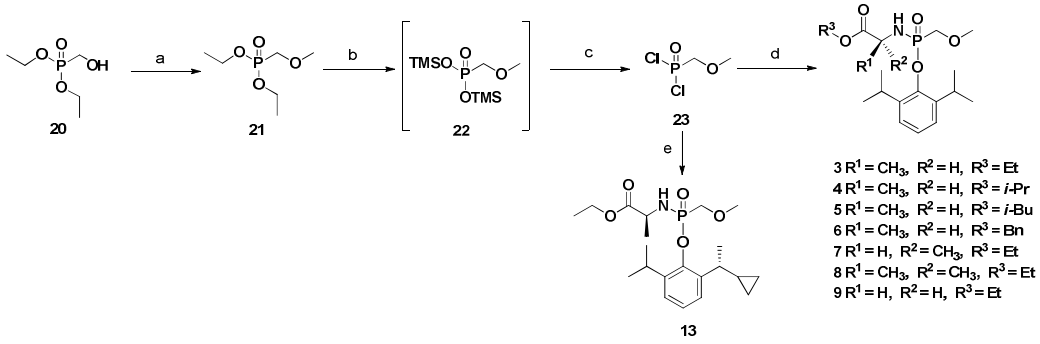
Figure 2. MMPA based oral delivery of **1**.

We reasoned that if this prodrug system was orally bioavailable as a whole, each of its fragments would also be delivered orally just as efficiently, i.e. the alcohol, amino acid and phenol fragments. We now demonstrate for the first time that simple replacement of the phenol (**A3**) with **1** (Figure 2), together with methoxymethylphosphonic acid (**B5**) as the delivery vehicle (Figure 2), afforded the oral delivery of **1** with the release of MMPA (**B5**), alcohol **B1** and amino acid **B4**. Unlike the tenofovir prodrug, oral bioavailability of MMPA prodrug is not dependent upon collapse of transient intermediate **B3** to liberate the target drug. With **1** as the model phenolic drug, we herein report preliminary results of the proof of concept of these MMPA prodrugs with the syntheses and preliminary pharmacological evaluation of a series of phosphonamidate prodrugs.

RESULTS AND DISCUSSION

Chemistry. Given the pivotal role that amino acid ester hydrolysis plays in the MMPA prodrugs as previously discussed, a series of phosphonamidates of MMPA for **1** (compounds **3** to **9**) and **2** (compound **13**) were designed and prepared (Scheme 1). The synthesis started with readily available diethyl (hydroxymethyl)phosphonate **20** that was first methylated using iodomethane and NaH in THF to give diethyl (methoxymethyl)phosphonate **21**. Reaction of diethyl (methoxymethyl)phosphonate **21** with 2 equivalents of bromotrimethylsilane in CH_3CN selectively removed the two ethyl groups to give the crude bistrimethylsilylphosphonate **22** that readily underwent chlorination using oxalyl chloride in CH_2Cl_2 to provide the key intermediate methoxymethylphosphonic acid dichloride[20] **23**, which could be purified by distillation. Reaction of the dichloride with compound **1** and various amino acid esters in Et_3N/CH_2Cl_2 afforded prodrugs **3** to **9** in 30% to 51% yield. The phosphonamidate prodrug **13** of **2** with L-alanine ethyl ester was prepared in a similar manner.

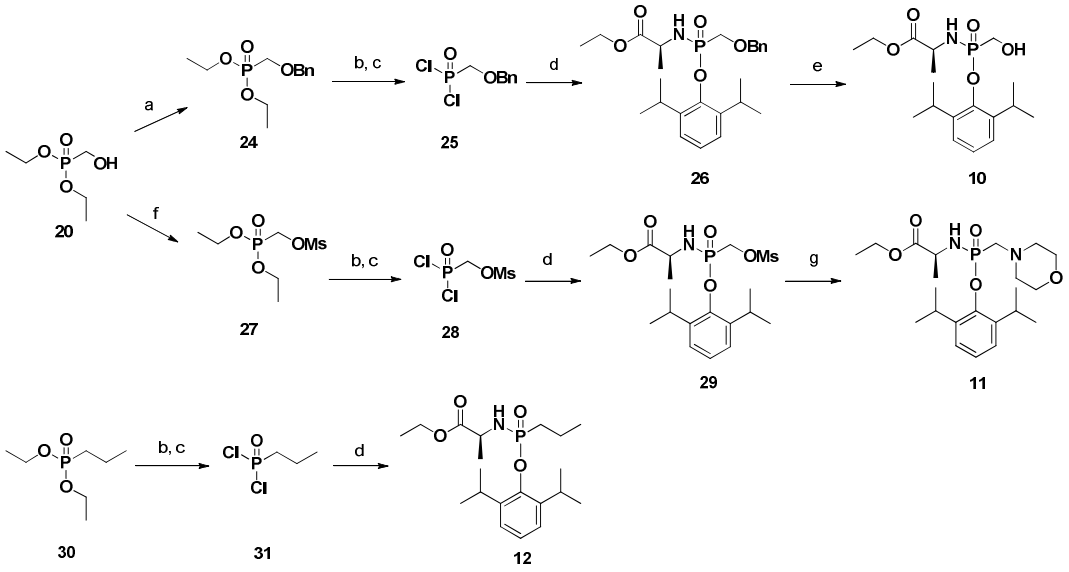
Scheme 1. Synthesis of Phosphonamidates **3**–**9** and **13**



Reagents and conditions: (a) CH_3I, NaH, THF, 0 ℃ to rt, 2 hrs; (b) TMSBr, CH_3CN, 50 ℃, 2 hrs; (c) oxalyl chloride, CH_2Cl_2, 0 ℃ to rt, 2 hrs; (d) **1**, Et_3N, amino acid ester, CH_2Cl_2, -10 ℃ to rt; (e) **2**, Et_3N, L-alanine ethyl ester, CH_2Cl_2, -10 ℃ to rt.

The morpholinomethylphosphonic acid based prodrug of compound **1** containing L-alanine ethyl ester was prepared (Scheme 2) first by converting diethyl hydroxymethylphosphonate **20** into its mesylate **27**. Dichloride **28** was prepared using the previous two step sequence (TMSBr–oxalyl chloride), which was reacted with **1** and L-alanine ethyl ester to give the mesylate intermediate **29**. Displacement of the mesylate **29** with morpholine afforded the morpholinomethylphosphonamidate prodrug **11**. Similarly the benzyloxymethylphosphonamidate intermediate **26** containing **1** and L-alanine ethyl ester was obtained in a similar fashion through the benzyl ether **24** followed by the two step chlorination sequence (TMSBr–oxalyl chloride) and phosphonamidate formation to **26**. Debenzylation of **26** under mild conditions afforded the hydroxymethylphosphonamidate prodrug **10**. Starting with diethyl propylphosphonate **30**, the propylphosphonic acid dichloride **31** was prepared in the same two step sequence (TMSBr–oxalyl chloride) and reacted with **1** and L-alanine ethyl ester to afford the propylphosphonamidate **12**.

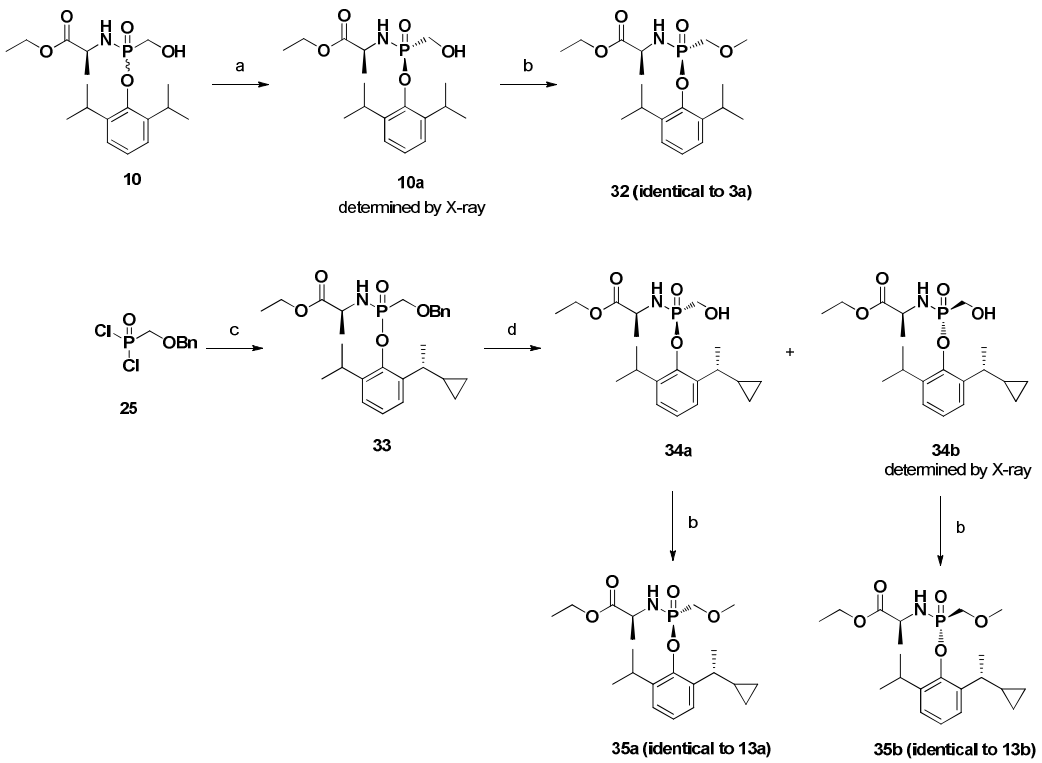
Scheme 2. Synthesis of Phosphonamidates **10**–**12**



Reagents and conditions: (a) BnBr, NaH, THF, 0 ℃ 12 hrs; (b) TMSBr, CH_3CN, 50 ℃, 2–3 hrs; (c) oxalyl chloride, CH_2Cl_2, 0 ℃ to rt, 1–12 hrs; (d) **1**, Et_3N, L-alanine ethyl ester, CH_2Cl_2 -10 ℃ to rt; (e) H_2, 10% Pd/C, CH_2Cl_2, rt, 8 hrs; (f) MsCl, Et_3N, CH_2Cl_2, 0 ℃ to rt, 4 hrs; (g) morpholine, Et_3N, THF, 80 ℃, 8 hrs.

All of the phosphonamidate prodrugs above were isolated as their diastereoisomeric mixtures. Prodrugs **3** and **13** were further purified by SFC (Supercritical Fluid Chromatography) to produce each pair of diastereoisomers **3a**–**3b** and **13a**–**13b** respectively. Since all these compounds were either waxy solids or liquids, the absolute configuration of **3a** was successfully determined through the hydroxymethylphosphonamidate analog **10a** (Scheme 3). Crystallization of the diastereoisomeric mixture of **10** from a mixed solvent (ethyl acetate /n-hexane = 1/10) afforded **10a**, whose absolute stereochemistry was determined by X-ray crystallography to be (*S, S*$_P$). Methylation of compound **10a** under neutral conditions using iodomethane and silver oxide in dichloromethane at room temperature produced compound **32** that has been found to be identical in all aspects to compound **3a**. Similarly, the absolute stereochemistry of compound **13b** was determined through silver oxide mediated methylation of the corresponding hydroxymethylphosphonamidate compound **34b** (*S, S*$_P$) to **35b** (Scheme 3). For a given amino acid ester, the chemical shift of the phosphorus atom of the *S*-stereoisomer has so far been found to be always more upfield than the *R*-stereoisomer in the ^{31}P NMR (see supporting information), which is in accord with the ^{31}P NMR of tenofovir alafenamide.[17]

Scheme 3. Determination of the Absolute Configuration of Compound **3a** and **13b**



Reagents and conditions: (a) recrystallization (ethyl acetate/n-hexane = 1/10); (b) MeI, Ag_2O, CH_2Cl_2, rt, overnight; (c) **2**, Et_3N, L-alanine ethyl ester, CH_2Cl_2, -10 ℃ to rt, 2 hrs; (d) H_2, 10% Pd/C, MeOH, rt, 8 hrs.

Biological Evaluation Initially, the *in vivo* anesthetic effects of phosphonamidate prodrugs were evaluated by loss of righting reflex (LORR) experiments in ICR mice[21] through oral administration. The on-set and duration of anesthesia of phosphonamidate prodrugs allowed preliminary accessment of the impacts of amino acids and esters on the oral delivery of **1** and **2**. Delightfully, all compounds were found to produce moderate to noticeable anesthetic effects, an indication of at least some oral bioavailability for all compounds. Compound **3** produced the most pronounced anesthetic action with an onset of anesthesia (4.50 ± 1.58 min) and duration time (60.31 ± 57.76 min) (Table 1). The bulkier the ester moiety of the prodrug was, the slower was the onset of anesthesia

(compound **3** vs compound **4**, **5** and **6**), which might be the result of the relative rate of hydrolysis of different ester groups (ethyl > isobutyl > benzyl > isopropyl). It is interesting to note that the ester moiety could also affect the duration of anesthetic action, as compound **6** with a benzyl ester exhibited a much longer duration than compound **3**, **4** and **5**.

Furthermore, phosphonamidate prodrugs containing different amino acids were investigated. With the incorporation of an unnatural amino acid, compound **7** showed similar potency when compared with compound **3**. However, it showed a slower onset of anesthetic action than its L-alanine analog. This may be due to the unnatural amino acid used in **7** not being the natural substrate for the esterase responsible in the first step of hydrolysis.

Compound **8** exhibited a 2-fold longer anesthetic duration with a comparable LORR effect to compound **3**. This much longer duration of anesthetic action might be due to steric hindrance of the bulkier dimethyl amino acid in the hydrolysis step. It is somewhat surprising that compound **9** with a less bulky glycine ester produced a significantly slower onset of anesthesia than **3**, which has relatively more steric bulk at the alpha position of the amino acid. This observation is in accord with a report[22] that a substituted amino acid such as alanine is the more preferable substrate than glycine for the esterase responsible in the first step of hydrolysis.

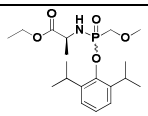
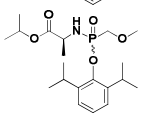
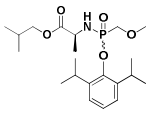
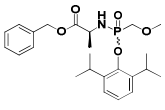
The effect of substituents on the methoxymethylphosphonic acid (MMPA) moiety was also studied. Compounds **10**, **11** and **12** had slower onset of anesthetic action and shorter anesthetic duration times compared with compound **3**. This might indicate that the electronic and relative steric effect of those substituents could also be rate-determining in

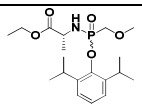
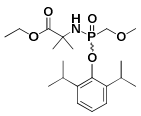
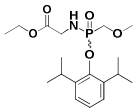
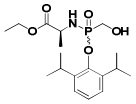
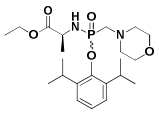
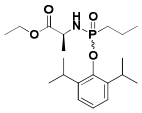
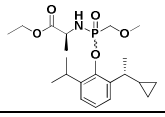
the hydrolysis-cyclization sequence of phosphonamidate prodrugs (Figure 2). More in-depth investigation is required to delineate the scope and utilities for such substitutions.

It is noted that prodrug **13** showed a similar anesthetic action at a lower dose of 100 mg/kg, which could be attributed to the higher potency of **2** compared with compound **1**. Also, the higher ratio of mortality caused by **13** might be due to the lower LD_{50} of **2** compared with **1** as reported in our previous work,[2] and a more detailed dose response study will have to be conducted.

With the results discussed above, we demonstrated that the rate of activation and the duration time of these phosphonamidate prodrugs were tunable through modifications of the ester moiety and the substituents on MMPA. These properties are critical for selecting the right substitutions for the oral delivery of **1** and **2** based on the therapeutic demands. These results have also built the foundation of expanding the scope of this MMPA based prodrug technology onto other phenolic drugs with diverse indications and clinical demands.

Table 1. Anesthetic Effect of Prodrugs in ICR Mice

Compound	Structure	Dose (mg/kg)	Onset (min)	Duration (min)	LORR (%)	mortality (%)
3		200[a]	4.50 ± 1.58	60.31 ± 57.76	100	10
4		200[a]	12.70 ± 4.90	59.38 ± 20.57	40	0
5		200[a]	8.36 ± 3.31	24.47 ± 15.56	100	10
6		200[a]	9.34 ± 5.48	94.61 ± 55.89	50	10

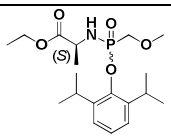
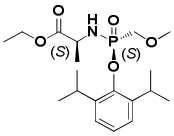
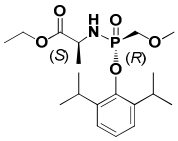
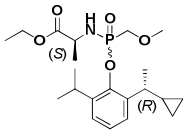
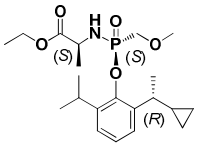
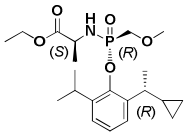
7		200 [a]	9.48 ± 2.46	44.80 ± 35.46	100	10
8		200 [a]	6.74 ± 1.11	115.9 ± 63.56	100	20
9		200 [a]	14.37 ± 7.40	32.48 ± 23.19	70	0
10		200 [a]	6.91 ± 0.42	19.19 ± 3.59	40	10
11		200 [a]	7.65 ± 1.95	16.18 ± 7.49	60	10
12		200[a]	11.21 ± 3.43	36.11 ± 25.10	60	0
13		100 [b]	4.49 ± 2.38	74.44 ± 6.57	100	50

[a]200 mg - eq/kg parent drug,[b]100 mg - eq/kg parent drug

To further investigate the effect of the chirality on the phosphorus, the anesthetic effects of each pair of enantiomeric pure compounds **3a**–**3b, 13a**–**13b** were studied with results shown in Table 2. Compound **3a** (*S, S*$_P$) and **3b** (*S, R*$_P$) produced a similar anesthetic duration time, but a significant difference in the onset of anesthetic action and LORR (**3a**, 4.38 ± 0.55, 100% vs **3b**, 9.68 ± 1.19, 33%). These results indicated that the chirality of the phosphorus had pronounced effect on the pharmacological behavior. Either the esterase mediated hydrolysis or formation of the transient lactam intermediate **B3** or both could have been affected by the chirality on the phosphorus. Meanwhile, compound **13a** (*S, S*$_P$) and **13b** (*S, R*$_P$) showed a slight difference in the onset of anesthetic action and duration. The higher potency of **2** might have masked the difference. The pharmacological difference could be more obvious at lower dose that will be one of

the subjects for further studies.

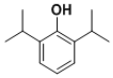
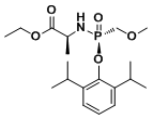
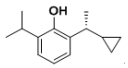
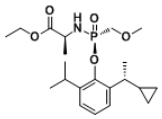
Table 2. Anesthetic Effect of **3a, 3b, 13a** and **13b** in ICR Mice

Compound	Structure	Dose (mg/kg)	Onset (min)	Duration (min)	LORR (%)	mortality (%)
3		200 [a]	4.50 ± 1.58	60.31 ± 57.76	100	10
3a		200[a]	4.38 ± 0.55	40.10 ± 32.51	100	0
3b		200 [a]	9.68 ± 1.19	47.24 ± 19.69	33	0
13		100 [b]	4.49 ± 2.38	74.44 ± 6.57	100	50
13a		100[b]	3.98 ± 0.84	107.68 ± 53.44	100	0
13b		100 [b]	4.35 ± 0.39	85.23 ± 23.70	100	17

[a]200 mg - eq/kg parent drug,[b]100 mg - eq/kg parent drug

The pharmacokinetic profiles of prodrugs **3a** (*S, S*$_P$) and **13a** (*S, S*$_P$) in beagle dogs were investigated (Table 3). Oral bioavailability was calculated as a percentage of the active compound quantitated in plasma after oral dosing of the prodrug versus intravenously administered active compound by measuring plasma concentrations of the active compound. Based on $AUC_{(0\text{-}t)}$, the oral bioavailability (F) of **3a** and **13a** in beagle dog was found to be 97.6 % and 29.5 % respectively.

Table 3. Pharmacokinetic **3a** and **13a** in Beagle Dogs

Compound	Structure	Dose (mg/kg)		$t_{1/2}$ (h)	C_{max} ng/mL	AUC_{0-t} (ng/mL·h)	F (%)
1		IV	3.0	0.20 ± 0.08	2050 ± 815	616 ± 173	/
		PO	6.9	0.64 ± 0.15	97 ± 29	102 ± 34	7.3
3a		PO	25.0	0.71 ± 0.16	1613 ± 889	2316 ± 1422	97.6
2		IV	2.0	0.20 ± 0.05	1611 ± 123	417 ± 27	/
		PO	10.0	0.44 ± 0.21	233 ± 251	162 ± 192	7.7
13a		PO	25.0	1.05 ± 0.18	452 ± 137	764 ± 327	29.5

Based on the pharmacokinetic profile of **3a**, we proposed that compound **3a** might be used for other indications such as insomnia. The hypnotic potency of **3a** was evaluated based on the prolongation of sleep induced by pentobarbital in ICR mice,[23] at the dosages where compound **3a** alone caused no LORR. Compound **3a** produced a dose dependent prolongation of pentobarbital - induced sleep time at dosages of 55, 65 and 75 mg/kg (Figure 3). At dosages of 65.0 and 75.0 mg/kg, compound **3a** remarkably increased sleep duration to 69 ± 20 min and 82 ± 53 min, respectively, compared to the vehicle group of 33 ± 20 min.

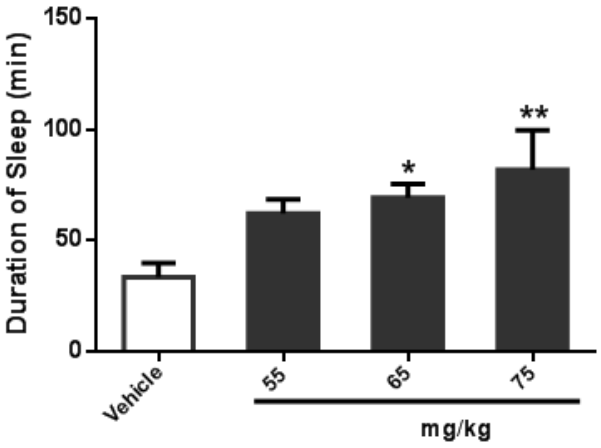


Figure 3. Effects of **3a** on sleep duration in pentobarbital-treated mice (* $p<0.05$, ** $p<0.001$), compared to vehicle group.

The generality of the MMPA platform to other phenolic drugs with more complicated structures was investigated. Phosphonamidate prodrugs of phenolic drugs SN38[24] (**14**), estradiol[25] (**16**) and naloxone[26] (**18**) (Figure 4) were synthesized by using the same method as described for compound **3**–**9** (Scheme 1). The pharmacokinetic profiles of compounds **15**, **17** and **19**, each as a mixture of its diastereoisomeric pair, were evaluated in beagle dogs via oral administration (Table 4). As an active metabolite of irinotecan[27], **14** was not orally active. To our delight, the oral bioavailability of **14** as its MMPA prodrug **15** was found to be 34.1%. Even **16** was taken orally in clinic, its plasma concentration was not measurable in our hands when given orally. However, as its MMPA prodrug **17**, the bioavailability of **16** was found to be 18.6%. The oral bioavailability of **19** as its MMPA prodrug was found to be 15.7%, a moderate increase from 9.6% for compound **18**. These results provide support on the generality of MMPA prodrug platform on phenolic drugs and warrant additional studies for other linking atoms

and methods in this platform.

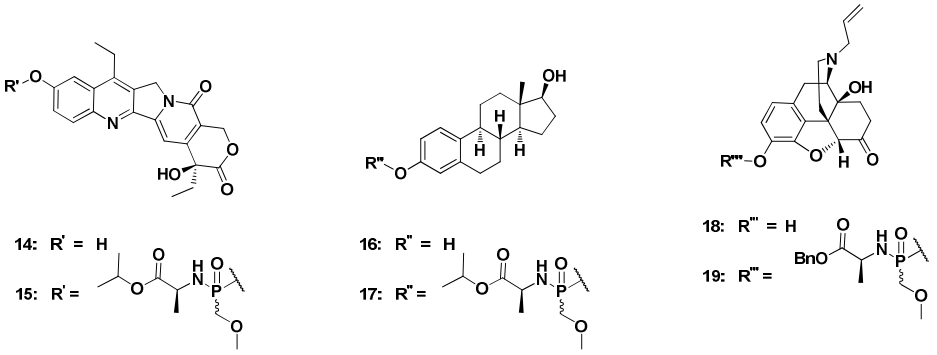


Figure 4. Chemical structures of **14**–**19**.

Table 4. Pharmacokinetic Parameters of Selected Prodrugs (**15**, **17** and **19**) in Beagle Dogs

Compound	Dose (mg/kg)		$t_{1/2}$ (h)	C_{max} ng/mL	AUC_{0-t} (ng/mL·h)	F (%)
14	IV	2.0	2.67 ± 0.22	983 ± 244	410 ± 63	/
	PO	16.0	NA	ND	NA	NA
15	PO	25.0	1.04 ± 0.11	1167 ± 27	1117 ± 182	34.1
16	IV	0.2	0.27 ± 0.08	76 ± 24	28 ± 12	/
	PO	4.4	NA	ND	NA	NA
17	PO	8.0	0.84 ± 0.34	116 ± 17	115 ± 44	18.6
18	IV	1.0	0.38 ± 0.05	196 ± 18	122 ± 16	/
	PO	10.0	8.99 ± 3.43	43 ± 4	117 ± 10	9.6
19	PO	16.4	1.23 ± 0.45	104 ± 26	172 ± 7	15.7

ND: Not Detected; NA: Not Available

CONCLUSION

This work disclosed above has demonstrated how MMPA could be used as delivery vehicle for oral administration of phenolic drugs such as **1** and **2**. The oral bioavailability of **1** will allow further studies for other indications. Prodrugs **3a** and **13a** were chosen for further preclinical development. MMPA platform has shown promising results to other phenolic drugs with more sophisticated structures including **14**, **16** and **18**. MMPA platform may allow oral delivery of drug targets of interest through different linking atoms and linkers. Efforts in exploring different linkers and linkages to different functional groups in the MMPA platform are currently underway as well as the oral delivery of the **B1** alcohol and **B4** amino acid (Figure 2) through this technology. The ongoing mechanistic studies of MMPA platform and all related biological intermediates and metabolites will be reported in due course.

EXPERIMENTAL SECTION

Anesthetic effects Loss of righting reflex (LORR), a validated rodent model of general anesthesia, was applied to evaluate the anesthetic effects of the prodrugs of **1** and **2** in ICR mice. Animals were fasted over night before experiment and administered with prodrugs in 10% of dimethyl sulfoxide, 20% of Solutol HS-15 and 70% of saline by gavage (n = 10/dose group for diastereoisomeric mixtures, n = 6/dose group for enantiopure compounds **3a**, **3b**, **13a** and **13b**). Then the mice were placed on their backs continually until they stopped righting themselves. Anesthetic effects were assessed using onset of LORR and anesthetic duration.

Animals Male and female ICR mice (20 ± 2 g) were purchased from Chengdu Dossy Experimental Animals Co., Ltd. Animal facilities, animal care, and study programs were conducted in conformity with in-house guidelines of Institute of Laboratory Animal Resources, Commission on Life Science, National Research Council (National Academy Press, Washington, DC. 2010). All animals were housed in cages at 21 ± 2 ℃ and provided free access to food and water. Rooms with constant temperature and humidity were in a cycle of 12 hrs of light and 12 hrs of dark.

Pharmacokinetic profiles of prodrugs in beagle dogs were investigated. Parent drugs which were formulated in 5% of dimethyl sulfoxide, 5% of Solutol HS-15 (30%, w/v) and 90% of saline were administered a single i.v. bolus injection to beagle dogs (n=3/group), and blood samples were collected into heparinized tubes at pre–dose and at 2, 5, 10, 20, 30 min, 1, 2, 4, 6, 8, 10, 12, and 24 hrs postdose. After a week washout, the prodrugs which were prepared in 5% of dimethyl sulfoxide, 5% of Solutol HS-15 (30%, w/v) and 90% of saline were administered to the same beagle dogs via i.g. and blood samples were collected into heparinized tubes at pre-dose and at 5, 10, 20, 30 min, 1, 2, 4, 6, 8, 10, 12, and 24 hrs postdose after administration. All blood samples were centrifuged at 5500 rpm for 10 min to obtain plasma at 2–8 ℃. Plasma samples were stored at -80 ℃ until analysis. Parent drugs concentrations in all plasma samples were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Pharmacokinetic parameters were analyzed by the WinNonlin V6.3 software (Pharsight, Mountain View, CA, USA) using non-compartmental analysis.

Pentobarbital–Induced Sleep Hypnotic potency of compound **3a** was evaluated based on the prolongation of sleep induced by pentobarbital in ICR mice. Fasted male and female mice were randomly divided into four groups, including vehicle group and three dose groups (55, 65, 75 mg/kg, n=10/ group). Compound **3a** was formulated in 10% of dimethyl sulfoxide, 20% of Solutol HS-15 (30%, w/v) and 70% of saline, and was administrated orally 5 min before the pentobarbital injection. After the intraperitoneal injection of 40 mg/kg pentobarbital, the mice were observed for measurement of sleep duration. The sleep duration was defined as period from falling into sleep to recovering the righting reflex. Data were expressed as mean ± SEM. Statistical analysis was performed with Sigma Stat software (SPSS Inc., Chicago, USA).

Synthetic Procedures in Chemistry All purchased starting materials were used without further purification. ^{1}H NMR and ^{13}C NMR spectra were acquired at ambient temperature on a Bruker Avance HD 400 spectrometer (400 MHz and 101 MHz) in $CDCl_3$ and CD_3OD with residual solvent signal ($CHCl_3$ and CD_2HOD) as reference (7.26 and 77.16, 3.31 and 49.00 respectively). ^{31}P NMR spectra were recorded at ambient temperature on a Bruker Avance HD 400 spectrometer (162 MHz), with proton decoupling. Chemical shifts were reported relative to external 85 % H_3PO_4. Chemical shifts are expressed in parts per million (ppm, δ units), and coupling constants are reported in hertz (Hz). Hight Resolution Mass Spectra (HRMS) were obtained on LTQ FT Ultra of Thermo Fisher Scientific from the National Center for Organic Mass Spectrometry in Shanghai, Shanghai Institute of Organic Chemistry, Chinese Academic of Science. Mass spectra were obtained on FinniganLCQAd instrument (ESI). Most

masses were reported as those of the protonated parent ions. Preparative column chromatography was performed using 200–300 mesh silica. Purities of title compounds were ≥95 % by HPLC.

Ethyl (2S)-2-[[(2,6-diisopropylphenoxy)-(methoxymethyl)phosphoryl]amino] propanoate (**3**) Under nitrogen atmosphere, to a stirred solution of **23** (4.5 g, 21 mmol) in 50 mL of dichloromethane was added a solution of **1** (3.7 g, 21 mmol) and triethylamine (8.7 g, 86 mmol) in dichloromethane (50 mL) at -10 ℃. The reaction mixture was allowed to warm to room temperature, stirred for 1 hr, and L-alanine ethyl ester (5.0 g, 43 mmol) was then added. After 2 hrs, the reaction mixture was quenched with a saturated solution of sodium dihydrogen phosphate (100 mL), the organic layer was dried with anhydrous sodium sulfate and concentrated *in vacuum*. The residue was purified by flash column chromatography (1:10–1:1 ethyl acetate/hexanes). The title compound was obtained as yellow oil (2.5 g) in 30.9 % yield. R_f = 0.50 (1:2 ethyl acetate/hexanes, TLC), major isomer : minor isomer = 1.18 : 1.00. HRMS (ESI): Calcd for $C_{19}H_{33}NO_5P$ $[M+H]^+$ 386.2091; Found: 386.2090.

The diastereoisomeric mixture was separated by SFC to afford two optical isomers (see supporting information for separation method). Major isomer: chiral HPLC retention time = 18.2 min. ^{1}H NMR (400 MHz, CD_3OD): δ 7.28–6.99 (m, 3H), 4.14–4.02(m, 2H), 3.98 (dq, *J* = 9.2, 7.2 Hz, 1H), 3.94 (dd, *J* = 13.2, 8.8 Hz, 1H), 3.87 (dd, *J* = 13.2, 8.0 Hz, 1H), 3.54 (hept, *J* = 6.8 Hz, 2H), 3.45 (d, *J* = 1.2 Hz, 3H), 1.30 (d, *J* = 7.2 Hz, 3H), 1.22–1.18 (m, 15H). ^{13}C NMR (101 MHz, CD_3OD): δ 174.93 (d, *J* = 5.0 Hz), 146.60 (d, *J* = 11.4 Hz), 141.88 (d, *J* = 3.2 Hz), 126.66 (d, *J* = 1.7 Hz), 125.17 (d, *J* = 1.7 Hz), 69.99 (d, *J* = 157.9 Hz), 62.19, 61.57 (d, *J* = 15.2 Hz), 50.90, 28.21, 24.03, 23.80, 21.28 (d, *J* = 4.4

Hz), 14.46. ^{31}P NMR (162 MHz, CD_3OD): δ 23.69. MS m/z (ESI): 386.2 $[M+H]^+$. Minor isomer: chiral HPLC retention time = 19.1 min. ^{1}H NMR (400 MHz, CD_3OD): δ 7.27–7.03 (m, 3H), 4.20–4.10 (m, 2H), 4.00 (dq, *J* = 7.6, 7.6 Hz, 1H), 4.02–3.90 (m, 2H) , 3.57 (hept, *J* = 6.8 Hz, 2H), 3.48 (d, *J* = 1.2 Hz, 3H), 1.27–1.22 (m, 6H), 1.21 (d, *J* = 6.8 Hz, 12H). ^{13}C NMR (101 MHz, CD_3OD): δ 175.30 (d, *J* = 4.0 Hz), 146.25 (d, *J* = 11.4 Hz), 142.03 (d, *J* = 3.1 Hz), 126.68 (d, *J* = 1.8 Hz), 125.15 (d, *J* = 1.8 Hz), 69.85 (d, *J* = 156.1 Hz), 62.29, 61.66 (d, *J* = 14.1 Hz), 50.91, 28.11, 24.03, 23.95, 20.75 (d, *J* = 5.4 Hz), 14.49. ^{31}P NMR (162 MHz, CD_3OD): δ 24.96. MS m/z (ESI): 386.2 $[M+H]^+$.

Isopropyl (2S)-2[[(2,6-diisopropylphenoxy)(methoxymethyl)phosphoryl]amino] propanoate (**4**) Yellow oil (1.0 g), 40.9 % yield. R_f = 0.60 (1:2 ethyl acetate/hexanes, TLC), major isomer : minor isomer = 1.63 : 1.00. HRMS (ESI): Calcd for $C_{20}H_{35}NO_5P$ $[M+H]^+$ 400.2247. Found: 400.2247. The diastereoisomeric mixture was separated by SFC to afford two optical isomers. Major isomer: ^{1}H NMR (400 MHz, CD_3OD): δ 7.15–7.08 (m, 3H), 4.95 (hept, *J* = 6.4 Hz, 1H), 4.04–3.74 (m, 3H), 3.58 (hept, *J* = 6.8 Hz, 2H), 3.44 (d, *J* = 0.8 Hz, 3H), 1.29 (d, *J* = 7.2 Hz, 3H), 1.21–1.17 (m, 18H). ^{13}C NMR (101 MHz, CD_3OD): δ 174.29 (d, *J* = 5.3 Hz), 146.50 (d, *J* = 11.3 Hz), 141.75 (d, *J* = 3.1 Hz), 126.60 (d, *J* = 1.7 Hz), 125.11 (d, *J* = 1.8 Hz), 69.97 (d, *J* = 157.8 Hz), 69.78, 61.57 (d, *J* = 15.0 Hz), 50.88, 28.10, 24.14, 23.91, 22.04, 21.96, 21.44 (d, *J* = 4.1 Hz). ^{31}P NMR (162 MHz, CD_3OD): δ 23.53. MS m/z (ESI): 400.3 $[M+H]^+$. Minor isomer: ^{1}H NMR (400 MHz, CD_3OD): δ 7.33–6.95 (m, 3H), 5.00 (hept, *J* = 6.4 Hz, 1H), 4.06–3.90 (m, 3H), 3.60 (hept, *J* = 6.8 Hz, 2H), 3.49 (d, *J* = 0.8 Hz, 3H), 1.33–1.12 (m, 21H). ^{13}C NMR (101 MHz, CD_3OD): δ 174.77 (d, *J* = 4.0 Hz), 146.21 (d, *J* = 11.4 Hz), 141.99 (d, *J* = 3.1 Hz), 126.66 (d, *J* = 1.8 Hz), 125.13 (d, *J* = 1.8 Hz), 70.00, 69.84 (d, *J* = 156.1 Hz), 61.66 (d, *J*

= 14.1 Hz), 50.96, 28.07, 24.08, 24.00, 22.01 (d, J = 3.0 Hz), 20.83 (d, J = 5.2 Hz). ^{31}P NMR (162 MHz, CD_3OD): δ 24.89. MS m/z (ESI): 400.3 $[M+H]^+$.

Isobutyl (2S)-2-[[(2,6-diisopropylphenoxy)-(methoxymethyl)phosphoryl]amino] propanoate (**5**). Yellow oil (5.8 g), 37.6 % yield. R_f = 0.70 and 0.6 (1:2 ethyl acetate/hexanes, TLC), major isomer : minor isomer = 1.46 : 1.00. HRMS (ESI): Calcd for $C_{21}H_{37}NO_5P$ $[M+H]^+$ 414.2404. Found: 414.2403. The diastereoisomeric mixture was separated by chiral preparative HPLC to afford two optical isomers. Major isomer: ^{1}H NMR (400 MHz, CD_3OD): δ 7.15–7.09 (m, 3H), 4.01 (dq, J = 9.0, 7.2 Hz,1H), 3.97–3.84 (m, 2H), 3.86 (dd, J = 10.4, 6.8 Hz, 1H), 3.81 (dd, J = 10.4, 6.4 Hz, 1H), 3.56 (hept, J = 6.8 Hz, 2H), 3.44 (d, J = 1.2 Hz, 3H), 1.98–1.75 (m, 1H), 1.31 (d, J = 7.2 Hz, 3H), 1.20 (d, J = 6.8 Hz, 12H), 0.90 (d, J = 6.8 Hz, 6H). ^{13}C NMR (101 MHz, CD_3OD): δ 174.90 (d, J = 5.0 Hz), 146.57 (d, J = 11.3 Hz), 141.82 (d, J = 3.1 Hz), 126.65 (d, J = 1.7 Hz), 125.16 (d, J = 1.7 Hz), 72.16, 69.98 (d, J = 157.8 Hz), 61.59 (d, J = 15.3 Hz), 50.86, 28.85, 28.15, 24.09, 23.86, 21.44 (d, J = 4.4 Hz), 19.38 (d, J = 1.8 Hz). ^{31}P NMR (162 MHz, CD_3OD): δ 23.56. MS m/z (ESI): 414.1 $[M+H]^+$. Minor isomer: ^{1}H NMR (400 MHz, CD_3OD): δ 7.41–6.74 (m, 3H), 4.03 (dq, J = 7.2, 7.2 Hz,1H), 4.01–3.91 (m, 2H), 3.93 (dd, J = 10.6, 6.6 Hz, 1H), 3.87 (dd, J = 10.6, 6.4 Hz, 1H), 3.58 (hept, J = 6.8 Hz, 1H), 3.47 (d, J = 0.8 Hz, 3H), 2.07–1.80 (m, 1H), 1.26 (d, J = 7.2 Hz, 3H), 1.21 (d, J = 6.8 Hz, 12H), 0.93 (d, J = 6.8 Hz, 6H). ^{13}C NMR (101 MHz, CD_3OD): δ 175.29 (d, J = 4.1 Hz), 146.23 (d, J = 11.5 Hz), 142.01 (d, J = 3.1 Hz), 126.67 (d, J = 1.8 Hz), 125.14 (d, J = 1.7 Hz), 72.24, 69.83 (d, J = 156.1 Hz), 61.65 (d, J = 14.3 Hz), 50.89, 28.91, 28.08, 24.05, 23.98, 20.90 (d, J = 5.4 Hz), 19.37 (d, J = 2.4 Hz). ^{31}P NMR (162 MHz, CD_3OD): δ 24.88. MS m/z (ESI): 414.1 $[M+H]^+$.

Benzyl (2S)-2-(((2,6-diisopropylphenoxy)(methoxymethyl)phosphoryl)amino) propanoate (**6**) Yellow oil (5.0 g), 36.4 % yield. R_f = 0.60 and 0.50 (1:2 ethyl acetate/hexanes, TLC), major isomer : minor isomer = 1.40 : 1.00. HRMS (ESI): Calcd for $C_{24}H_{35}NO_5P$ $[M+H]^+$ 448.2247. Found: 448.2247. The diastereoisomeric mixture was separated by chiral preparative HPLC to afford two optical isomers. Major isomer: ^{1}H NMR (400 MHz, CD_3OD): δ 7.45–7.23 (m, 5H), 7.14–7.08 (m, 3H), 5.19–5.08 (m, 2H), 4.07 (dq, *J* = 9.0, 7.2 Hz, 1H), 3.92 (dd, *J* = 13.2, 8.8 Hz, 1H), 3.86 (dd, *J* = 13.2, 7.6 Hz, 1H), 3.57 (hept, *J* = 6.8 Hz, 2H), 3.37 (d, *J* = 1.2 Hz, 3H), 1.32 (d, *J* = 7.2 Hz, 3H), 1.20 (d, *J* = 6.8 Hz, 6H), 1.19 (d, *J* = 6.8 Hz, 6H). ^{13}C NMR (101 MHz, CD_3OD): δ 174.58 (d, *J* = 4.8 Hz), 146.53 (d, *J* = 11.3 Hz), 141.78 (d, *J* = 3.1 Hz), 137.08, 129.49, 129.25, 129.20, 126.63 (d, *J* = 1.7 Hz), 125.14 (d, *J* = 1.7 Hz), 69.94 (d, *J* = 157.6 Hz), 67.73, 61.53 (d, *J* = 15.1 Hz), 50.91, 28.13, 24.07, 23.85, 21.24 (d, *J* = 4.4 Hz). ^{31}P NMR (162 MHz, CD_3OD): δ 23.56. MS m/z (ESI): 448.3 $[M+H]^+$. Minor isomer: ^{1}H NMR (400 MHz, CD_3OD) δ 7.43–7.24 (m, 5H), 7.13–7.11 (m, 3H), 5.19–5.08 (m, 2H), 4.09 (dq, *J* = 7.2, 7.2 Hz, 1H), 3.92 (dd, *J* = 13.2, 8.0 Hz, 1H), 3.88 (dd, *J* = 13.2, 7.6 Hz, 1H), 3.58 (hept, *J* = 6.8 Hz, 2H), 3.40 (d, *J* = 1.0 Hz, 3H), 1.27 (d, *J* = 7.2 Hz, 3H), 1.20 (d, *J* = 6.8 Hz, 12H). ^{13}C NMR (101 MHz, CD_3OD): δ 175.00 (d, *J* = 3.7 Hz), 146.20 (d, *J* = 11.4 Hz), 141.98 (d, *J* = 3.1 Hz), 137.11, 129.53, 129.30, 129.27, 126.66 (d, *J* = 1.8 Hz), 125.13 (d, *J* = 1.8 Hz), 69.79 (d, *J* = 156.0 Hz), 67.93, 61.60 (d, *J* = 14.3 Hz), 50.92, 28.07, 24.03, 23.96, 20.64 (d, *J* = 5.5 Hz). ^{31}P NMR (162 MHz, CD_3OD): δ 24.94. MS m/z (ESI): 448.3 $[M+H]^+$.

Ethyl (2R)-2-[[(2,6-diisopropylphenoxy)-(methoxymethyl)phosphoryl]amino] propanoate (**7**) Yellow oil (6.0 g), 51 % yield. R_f = 0.50 (1:2 ethyl acetate/hexanes, TLC), major isomer : minor isomer = 1.32 : 1.00. HRMS (ESI): Calcd for $C_{19}H_{33}NO_5P$ $[M+H]^+$ 386.2091. Found: 386.2090. The diastereoisomeric mixture was separated by chiral preparative HPLC to afford two optical isomers. Major isomer: ^{1}H NMR (400 MHz, CD_3OD): δ 7.21–7.08 (m, 3H), 4.20–4.10 (m, 2H), 4.00 (dq, *J* = 7.2, 7.2 Hz, 1H), 3.98 (dd, *J* = 13.2, 8.0 Hz, 1H), 3.94 (dd, *J* = 13.2, 8.0 Hz, 1H), 3.57 (hept, *J* = 6.8 Hz, 2H), 3.48 (d, *J* = 1.2 Hz, 3H), 1.27–1.23 (m, 6H), 1.21 (d, *J* = 6.8 Hz, 12H). ^{13}C NMR (101 MHz, CD_3OD): δ 175.31 (d, *J* = 3.8 Hz), 146.25 (d, *J* = 11.4 Hz), 142.03 (d, *J* = 3.2 Hz), 126.69 (d, *J* = 1.8 Hz), 125.16 (d, *J* = 1.8 Hz), 69.85 (d, *J* = 156.1 Hz), 62.29, 61.66 (d, *J* = 14.1 Hz), 50.90, 28.11, 24.03, 23.95, 20.74 (d, *J* = 5.5 Hz), 14.49. ^{31}P NMR (162 MHz, CD_3OD): δ 24.96. MS m/z (ESI): 386.2 $[M+H]^+$. Minor isomer: ^{1}H NMR (400 MHz, CD_3OD): δ 7.15–7.09 (m, 3H), 4.15 – 4.05 (m, 2H), 3.99 (dq, *J* = 9.0, 7.2 Hz, 1H), 3.94 (dd, *J* = 13.2, 8.8 Hz, 1H) , 3.87 (dd, *J* = 13.2, 8.0 Hz, 1H), 3.55 (hept, *J* = 6.8 Hz, 2H), 3.44 (d, *J* = 0.8 Hz, 3H), 1.31 (d, *J* = 7.2 Hz, 2H), 1.21–1.17 (m, 15H). ^{13}C NMR (101 MHz, CD_3OD): δ 174.84 (d, *J* = 5.1 Hz), 146.55 (d, *J* = 11.4 Hz), 141.82 (d, *J* = 3.0 Hz), 126.63 (d, *J* = 1.8 Hz), 125.13 (d, *J* = 1.8 Hz), 69.97 (d, *J* = 158.0 Hz), 62.12, 61.58 (d, *J* = 15.1 Hz), 50.83, 28.15, 24.08, 23.84, 21.32 (d, *J*= 4.3 Hz), 14.49. ^{31}P NMR (162 MHz, CD_3OD): δ 23.62. MS m/z (ESI): 386.2 $[M+H]^+$.

Ethyl 2-[[(2,6-diisopropylphenoxy)-(methoxymethyl)phosphoryl]amino]-2-methyl-propanoate (**8**) Yellow oil (5.0 g), 50.1 % yield. R_f = 0.70 (1:2 ethyl acetate/hexanes, TLC), isomer 1 : isomer 2 = 1.00 : 1.00. HRMS (ESI): Calcd for $C_{20}H_{34}NO_5P$ $[M+H]^+$

400.2247. Found: 400.2246. The diastereoisomeric mixture was separated by chiral preparative HPLC to afford two optical isomers. Isomer 1: ^{1}H NMR (400 MHz, CD_3OD): δ 7.33–6.88 (m, 3H), 4.20–4.01 (m, 2H), 3.94 (dd, *J* = 13.2, 8.0 Hz, 1H), 3.90 (dd, *J* = 13.2, 7.6 Hz, 1H), 3.63 (hept, *J* = 6.8 Hz, 2H), 3.49 (d, *J* = 1.2 Hz, 3H), 1.41(s, 3H), 1.37 (s, 3H), 1.22–1.17 (m, 15H). ^{13}C NMR (101 MHz, CD_3OD): δ 176.84 (d, *J* = 5.4 Hz), 146.17 (d, *J* = 11.6 Hz), 142.14 (d, *J* = 3.1 Hz), 126.63 (d, *J* = 1.8 Hz), 125.08 (d, *J* = 1.8 Hz), 70.27 (d, *J* = 156.9 Hz), 62.58, 61.60 (d, *J* = 14.0 Hz), 58.46, 28.72 (d, *J* = 4.8 Hz), 28.10, 27.90 (d, *J* = 2.2 Hz), 24.06, 23.86, 14.36. ^{31}P NMR (162 MHz, CD_3OD): δ 23.69. MS m/z (ESI): 400.1 $[M+H]^+$. Isomer 2: ^{1}H NMR (400 MHz, CD_3OD): δ 7.18–7.10 (m, 3H), 4.17–4.03 (m, 2H), 3.94 (dd, *J* = 13.2, 8.0 Hz, 1H), 3.90 (dd, *J* = 13.2, 7.6 Hz, 1H), 3.63 (hept, *J* = 6.8 Hz, 2H), 3.49 (d, *J* = 1.2 Hz, 3H), 1.41 (s, 3H), 1.38 (s, 3H), 1.24–1.16 (m, 15H). ^{13}C NMR (101 MHz, CD_3OD): δ 176.81 (d, *J* = 5.5 Hz), 146.15 (d, *J* = 11.7 Hz), 142.11 (d, *J* = 3.1 Hz), 126.62 (d, *J* = 1.8 Hz), 125.07 (d, *J* = 1.8 Hz), 70.26 (d, *J* = 156.9 Hz), 62.56, 61.60 (d, *J* = 14.1 Hz), 58.44, 28.72 (d, *J* = 4.9 Hz), 28.08, 27.90 (d, *J* = 2.0 Hz), 24.07, 23.87, 14.37. ^{31}P NMR (162 MHz, CD_3OD): δ 23.67. MS m/z (ESI): 400.1 $[M+H]^+$

Ethyl 2-[[(2,6-diisopropylphenoxy)-(methoxymethyl)phosphoryl]amino]acetate (9)

Yellow oil (5.0 g), 43.9 % yield. R_f = 0.50 (1:2 ethyl acetate/hexanes, TLC), isomer 1 : isomer 2 = 1.00 : 1.00. HRMS (ESI): Calcd for $C_{18}H_{31}NO_5P$ $[M+H]^+$. 372.1934. Found: 372.1932. The diastereoisomeric mixture was separated by chiral preparative HPLC to afford two optical isomers. Isomer 1: ^{1}H NMR (400 MHz, CD_3OD): δ 7.18–7.10 (m, 3H), 4.16 (q, *J* = 6.8 Hz, 2H), 4.02 (dd, *J* = 13.2, 8.0 Hz, 1H), 3.98 (dd, *J* = 13.2, 8.0 Hz, 1H), 3.81 (dd, *J* = 18.0, 10.0 Hz, 1H), 3.68 (dd, *J* = 18.0, 13.6 Hz, 1H), 3.57 (hept, *J* = 6.8 Hz,

2H), 3.48 (d, *J* = 0.8 Hz, 3H), 1.28–1.18 (m, 15H). ^{13}C NMR (101 MHz, CD_3OD): δ 172.63 (d, *J* = 3.8 Hz), 146.31 (d, *J* = 11.4 Hz), 141.94 (d, *J* = 3.0 Hz), 126.64 (d, *J* = 1.8 Hz), 125.14 (d, *J* = 1.8 Hz), 69.93 (d, *J* = 156.7 Hz), 62.12, 61.65 (d, *J* = 14.3 Hz), 43.44, 28.17, 24.04, 23.98, 14.52. ^{31}P NMR (162 MHz, CD_3OD): δ 25.68. MS m/z (ESI): 372.2 $[M+H]^+$. Isomer 2: ^{1}H NMR (400 MHz, CD_3OD): δ 7.18–7.10 (m, 3H), 4.14 (q, *J* = 7.2 Hz, 2H), 4.02 (dd, *J* = 13.2, 8.0 Hz, 1H), 3.98 (dd, *J* = 13.2, 8.0 Hz, 1H), 3.81 (dd, *J* = 18.0, 10.0 Hz, 1H), 3.70 (dd, *J* = 18.0, 13.6 Hz, 1H), 3.57 (hept, *J* = 6.8 Hz, 2H), 3.48 (d, *J* = 0.8 Hz, 3H), 1.27–1.20 (m, 15H). ^{13}C NMR (101 MHz, CD_3OD): δ 172.62 (d, *J* = 3.9 Hz), 146.30 (d, *J* = 11.4 Hz), 141.92 (d, *J* = 2.9 Hz), 126.64 (d, *J* = 1.8 Hz), 125.14 (d, *J* = 1.8 Hz), 69.92 (d, *J* = 156.8 Hz), 62.12, 61.64 (d, *J* = 14.3 Hz), 43.44, 28.17, 24.04, 23.99, 14.52. ^{31}P NMR (162 MHz, CD_3OD): δ 25.67. MS m/z (ESI): 372.2 $[M+H]^+$.

Ethyl (2S)-2-[[(2,6-diisopropylphenoxy)-(hydroxymethyl)phosphoryl]amino]propanoate (**10**) To a stirred solution of **26** (15.0 g, 32.5 mmol) in 100 mL of dichloromethane was added Pd/C (10.0 g, 10 wt %). The mixture was stirred under hydrogen atmosphere at room temperature for 8 hrs. The resulting solution was filtered. The solvent was removed under reduced pressure. The title compound was obtained as yellow oil (8.0 g) in 66.3 % yield. R_f = 0.40 (1:1 ethyl acetate/hexanes, TLC), major isomer : minor isomer = 1.75 : 1.00. HRMS (ESI): Calcd for $C_{18}H_{30}NO_5P$ $[M+H]^+$ 371.1856. Found: 371.1858. The diastereoisomeric mixture was separated by chiral preparative HPLC to afford two optical isomers. Major isomer: chiral retention time = 7.81 min, ^{1}H NMR (400 MHz, CD_3OD): δ 7.16–7.09 (m, 3H), 4.19–4.11 (m, 2H), 4.10–4.05 (m, 2H), 4.03 (dq, *J* = 7.2, 7.2 Hz, 1H), 3.61 (hept, *J* = 6.8 Hz, 2H), 1.27 (d, *J* = 7.2 Hz, 3H), 1.26 (t, *J* = 7.2 Hz, 3H), 1.23 (d, *J* = 6.8 Hz, 6H), 1.22 (d, *J* =

6.8 Hz, 6H). ^{13}C NMR (101 MHz, CD_3OD): δ 175.85 (d, *J* = 3.9 Hz), 146.17 (d, *J* = 11.7 Hz), 142.04 (d, *J* = 3.0 Hz), 126.58 (d, *J* = 1.8 Hz), 125.11 (d, *J* = 1.8 Hz), 62.43, 59.71 (d, *J* = 152.9 Hz), 50.88, 28.06, 24.07, 23.95, 20.71 (d, *J* = 5.3 Hz), 14.44. ^{31}P NMR (162 MHz, CD_3OD): δ 28.02. MS m/z (ESI): 394.2 $[M+Na]^+$. Minor isomer: chiral HPLC retention time = 12.41 min. This isomer can also be isolated by crystallization method. **10** (3.0 g) was dissolved in 10 mL ethyl acetate/n-hexane (v : v = 1:10) at 70 ℃, cooled down to room temperature and stayed overnight. After filtration, **10a** was obtained as white crystal (0.70 g) in 23.3 % yield. The optical purity of the isolated diastereoisomer was determined by chiral HPLC to be 100 %. ^{1}H NMR (400 MHz, CD_3OD): δ 7.16–7.07 (m, 3H), 4.10–3.98 (m, 5H), 3.57 (hept, *J* = 6.8 Hz, 2H), 1.36 (d, *J* = 6.8 Hz, 3H), 1.21(d, *J* = 6.8 Hz, 12H), 1.19 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD): δ 175.02 (d, *J* = 5.3 Hz), 146.25 (d, *J* = 11.6 Hz), 141.61 (d, *J* = 3.1 Hz), 126.52 (d, *J* = 1.8 Hz), 125.10 (d, *J* = 1.8 Hz), 62.19, 59.74 (d, *J* = 156.2 Hz), 50.82, 28.14, 24.08, 23.82, 21.49 (d, *J* = 4.0 Hz), 14.42. ^{31}P NMR (162 MHz, CD_3OD): δ 27.01. MS m/z (ESI): 394.2 $[M+Na]^+$.

Ethyl (2S)-2-[[(2,6-diisopropylphenoxy)-(morpholinomethyl)phosphoryl]amino] propanoate (**11**) To a stirred solution of **29** (4.94 g, 11.0 mmol) in 20 mL of tetrahydrofuran was added morpholine (2.87 g, 33.0 mmol) and triethylamine (5.67 g, 56.0 mmol). The reaction mixture was heated to 80 ℃ and stirred for 8 hrs. The resulting mixture was concentrated *in vacuum*. The residue was purified by flash column chromatography (1:10–1:1 ethyl acetate/hexanes). The title compound was obtained as yellow oil (4.0 g) in 82.6 % yield. R_f = 0.60 and 0.5 (1:2 ethyl acetate/hexanes, TLC), major isomer : minor isomer = 1.41 : 1.00. HRMS (ESI): Calcd for $C_{22}H_{38}N_2O_5P$ 441.2513 $[M+H]^+$. Found: 441.2509. The diastereoisomeric mixture was separated by

chiral preparative HPLC to afford two optical isomers. Major isomer: ^{1}H NMR (400 MHz, CD_3OD): δ 7.18–7.06 (m, 3H), 4.15–3.95 (m, 2H), 4.07–3.95 (m, 1H), 3.69 (m, 4H), 3.57 (hept, *J* = 6.8 Hz, 2H), 3.04 (dd, *J* = 15.4, 11.2 Hz, 1H), 2.98 (dd, *J* = 15.4, 10.2 Hz, 1H), 2.73 (m, 2H), 2.63 (m 2H), 1.40 (d, *J* = 7.2 Hz, 3H), 1.21 (d, *J* = 6.8 Hz, 12H), 1.19 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD): δ 175.54 (d, *J* = 4.4 Hz), 146.15 (d, *J* = 11.8 Hz), 142.16 (d, *J* = 3.0 Hz), 126.65, 12 5.18, 68.07, 62.28, 57.54 (d, *J* = 155.1 Hz), 56.60 (d, *J* = 10.0 Hz), 51.09, 28.10, 24.12, 23.99, 21.12, 14.52. ^{31}P NMR (162 MHz, CD_3OD): δ 26.58. MS m/z (ESI): 441.3 $[M+H]^+$. Minor isomer: ^{1}H NMR (400 MHz, CD_3OD): δ 7.21–7.08 (m, 3H), 4.27–4.08 (m, 2H), 4.00 (dq, *J* = 7.2, 7.2 Hz, 1H), 3.74–3.66 (m, 4H), 3.55 (hept, *J* = 6.8 Hz, 2H), 3.09 (dd, *J* = 15.4, 10.2 Hz, 1H), 3.01 (dd, *J* = 15.4, 10.8 Hz, 1H), 2.78–2.60 (m, 4H), 1.30–1.17 (m, 18H). ^{13}C NMR (101 MHz, CD_3OD): δ 175.53 (d, *J* = 4.4 Hz), 146.14 (d, *J* = 11.8 Hz), 142.15 (d, *J* = 3.0 Hz), 126.65 (d, *J* = 1.6 Hz), 125.18 (d, *J* = 1.8 Hz), 68.07, 62.28, 57.54 (d, *J* = 155.0 Hz), 56.60 (d, *J* = 9.9 Hz), 51.09, 28.10, 24.13, 24.00, 21.15 (d, *J* = 4.9 Hz), 14.52. ^{31}P NMR (162 MHz, CD_3OD): δ 27.56. MS m/z (ESI): 441.4 $[M+H]^+$.

Isopropyl (2S)-2-[[(2,6-diisopropylphenoxy)-propyl-phosphoryl]amino]propanoate (**12**) Under nitrogen atmosphere, to a stirred solution of **31** (4.00 g，25.0 mmol) in 25 mL of dichloromethane was added a solution of **1** (4.45 g，25.0 mmol) and triethylamine (10.0 g，99.0 mmol) in dichloromethane (20 mL) at -20 ℃, then stirred for 2 hrs. The L-alanine ethyl ester (5.03 g, 43.0 mmol) was then added. The reaction mixture was allowed to warm to room temperature and stirred for 3 hrs, quenched with water (20 mL) and extracted with ethyl acetate (50 mL). The organic layer was washed with sodium dihydrogen phosphate (15 wt %, 20 mL × 3), dried with

anhydrous sodium sulfate and concentrated *in vacuum*. The residue was purified by flash column chromatography (1:9–3:7 ethyl acetate/hexanes). The title compound was obtained as yellow oil (2.0 g) in 20.9 % yield. R_f = 0.60 (1:5 ethyl acetate/hexanes, TLC), major isomer : minor isomer = 1.15 : 1.00. HRMS (ESI): Calcd for $C_{20}H_{35}NO_4P$ $[M+H]^+$ 384.2298. Found: 384.2297. The diastereoisomeric mixture was separated by chiral preparative HPLC to afford two optical isomers. Major isomer: ^{1}H NMR (400 MHz, CD_3OD): δ 7.20–7.07 (m, 3H), 4..22–4.07 (m, 2H), 3.89 (dq, *J* = 7.6, 7.6 Hz, 1H), 3.53 (hept, *J* = 6.8 Hz, 2H), 2.12–1.89 (m, 2H), 1.89–1.66 (m, 2H), 1.30–1.17 (m, 15H), 1.13–1.05 (m, 6H). ^{13}C NMR (101 MHz, CD_3OD): δ 175.63 (d, *J* = 4.1 Hz), 146.76 (d, *J* = 11.5 Hz), 142.10 (d, *J* = 3.1 Hz), 126.45 (d, *J* = 1.9 Hz), 125.06 (d, *J* = 1.8 Hz), 62.16, 50.69, 31.73 (d, *J* = 130.0 Hz), 28.46, 23.96, 23.76, 20.58 (d, *J* = 5.5 Hz), 17.13 (d, *J* = 4.8 Hz), 15.80 (d, *J* = 18.4 Hz), 14.48. ^{31}P NMR (162 MHz, CD_3OD): δ 34.56. MS m/z (ESI): 384.2 $[M+H]^+$. Minor isomer: ^{1}H NMR (400 MHz, CD_3OD): δ 7.17–7.05 (m, 3H), 4.04–3.97 (m, 2H), 3.94 (dq, *J* = 9.6, 7.0 Hz, 1H), 3.49 (hept, *J* = 6.8 Hz, 2H), 2.04–1.87 (m, 2H), 1.76–1.70 (m, 2H), 1.29 (d, *J* = 7.0 Hz, 3H), 1.21 (d, *J* = 6.8 Hz, 6H), 1.20 (d, *J* = 6.8 Hz, 6H), 1.15 (t, *J* = 7.2 Hz, 3H), 1.07 (td, *J* = 7.2, 1.6 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD): δ 174.82 (d, *J* = 4.8 Hz), 147.06 (d, *J* = 11.4 Hz), 141.88 (d, *J* = 3.0 Hz), 126.38 (d, *J* = 1.8 Hz), 125.07 (d, *J* = 1.8 Hz), 62.06, 50.49, 31.45 (d, *J* = 131.3 Hz), 28.50, 23.96, 23.74, 21.53 (d, *J* = 4.6 Hz), 17.25 (d, *J* = 4.5 Hz), 15.71 (d, *J* = 18.2 Hz), 14.42. ^{31}P NMR (162 MHz, CD_3OD): δ 33.57. MS m/z (ESI): 384.2 $[M+H]^+$.

Ethyl (2S)-2-[[[2-[(1R)-1-cyclopropylethyl]-6-isopropyl-phenoxy](methoxymethyl) phosphoryl]amino]propanoate (**13**) Yellow oil (2.5 g), 28.0 % yield. R_f = 0.60 (1:2 ethyl acetate/hexanes, TLC), major isomer : minor isomer = 1.10 : 1.00. HRMS (ESI):

Calcd for $C_{21}H_{35}NO_5P$ $[M+H]^+$ 412.2247. Found: 412.2245. The diastereoisomeric mixture was separated by chiral preparative HPLC to afford two optical isomers. Major isomer: ^{1}H NMR (400 MHz, CD_3OD): δ 7.28 (dd, *J* = 6.2, 3.0 Hz, 1H), 7.20–7.10 (m, 2H), 4.20–4.04 (m, 2H), 3.97 (dq, *J* = 9.0, 7.0 Hz,1H), 3.90 (dd, *J* = 13.4, 8.8 Hz, 1H), 3.84 (dd, *J* = 13.4, 7.8 Hz, 1H), 3.56 (hept, *J* = 6.8 Hz, 1H), 3.44 (d, *J* = 1.0 Hz, 3H), 2.80 (dq, *J* = 9.0, 7.0 Hz, 1H), 1.30 (d, *J* = 7.0 Hz, 3H), 1.28 (d, *J* = 6.8 Hz, 3H), 1.25–1.17 (m, 9H), 1.00–0.88 (m, 1H), 0.58–0.50 (m, 1H), 0.37–0.18 (m, 3H). ^{13}C NMR (101 MHz, CD_3OD): δ 174.81 (d, *J* = 5.2 Hz), 146.76 (d, *J* = 11.4 Hz), 141.69 (d, *J* = 3.0 Hz), 140.77 (d, *J* = 3.2 Hz), 126.54 (d, *J* = 1.7 Hz), 126.17 (d, *J* = 1.7 Hz), 125.15 (d, *J* = 1.7 Hz), 70.03 (d, *J* = 157.3 Hz), 62.16, 61.53 (d, *J* = 15.4 Hz), 50.82, 38.27, 28.15, 24.00, 23.91, 21.47, 21.34 (d, *J* = 4.3 Hz), 19.23, 14.50, 5.42, 4.22. ^{31}P NMR (162 MHz, CD_3OD): δ 23.42. MS m/z (ESI): 412.3 $[M+H]^+$. Minor isomer: ^{1}H NMR (400 MHz, CD_3OD): δ 7.27 (dd, *J* = 6.4, 3.2 Hz, 1H), 7.15–7.11 (m, 2H), 4.22–4.10 (m, 2H), 3.99 (dq, *J* = 7.2, 7.2 Hz, 1H), 3.94 (dd, *J* = 13.2, 8.0 Hz, 1H), 3.90 (dd, *J* = 13.2, 8.0 Hz, 1H), 3.57 (hept, *J* = 6.8 Hz, 1H), 3.47 (d, *J* = 1.0 Hz, 1H), 2.81 (dq, *J* = 9.0, 7.0 Hz, 1H), 1.30 (d, *J* = 7.0 Hz, 3H), 1.27–1.21 (m, 9H), 1.20 (d, *J* = 6.8 Hz, 3H), 0.98–0.88 (m, 1H), 0.59–0.51 (m, 1H), 0.37–0.17 (m, 3H). ^{13}C NMR (101 MHz, CD_3OD): δ 175.24 (d, *J* = 3.8 Hz), 146.39 (d, *J* = 11.4 Hz), 141.81 (d, *J* = 3.1 Hz), 141.08 (d, *J* = 3.1 Hz), 126.57 (d, *J* = 1.8 Hz), 126.17 (d, *J* = 1.7 Hz), 125.09 (d, *J* = 1.8 Hz), 69.81 (d, *J* = 156.0 Hz), 62.27, 61.61 (d, *J* = 14.6 Hz), 50.85, 38.32, 28.12, 24.32, 23.69, 21.53, 20.76 (d, *J* = 5.5 Hz), 19.38, 14.50, 5.46, 4.29. ^{31}P NMR (162 MHz, CD_3OD): δ 24.77. MS m/z (ESI): 434.3 $[M+Na]^+$.

Isopropyl (S)-2-[[[[(S)-4,11-diethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl]oxy](methoxymethyl)phosphoryl]amino]propanoate (**15**) Yellow solid (9.0 g), 95.9 % yield. R_f = 0.60 (10:1 dichloromethane/methanol, TLC), major isomer : minor isomer = 1.40 : 1.00. The diastereoisomeric mixture was separated by SFC to afford two optical isomers. Major isomer: ^{1}H NMR (400 MHz, CD_3OD): δ 8.11 (d, *J* = 9.2 Hz, 1H), 8.09 (t, *J* = 2.0 Hz, 1H), 7.65 (dd, *J* = 9.2, 2.0 Hz, 1H), 7.61 (s, 1H), 5.60 (d, *J* = 16.2 Hz, 1H), 5.39 (d, *J* = 16.2 Hz, 1H), 5.26 (s, 2H), 4.90 (hept, *J* = 6.6 Hz, 1H), 4.05 (dq, *J* = 9.2, 7.0 Hz, 1H), 3.95 (d, *J* = 8.0 Hz, 2H), 3.52 (d, *J* = 1.0 Hz, 3H), 3.25 (dq, *J* = 7.6, 1.8 Hz, 2H), 2.04–1.95 (m, 2H), 1.41 (t, *J* = 7.6 Hz, 3H), 1.34 (d, *J* = 7.0 Hz, 3H), 1.16 (d, *J* = 6.6 Hz, 3H), 1.15 (d, *J* = 6.6 Hz, 3H), 1.01 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD): δ 174.86, 174.70 (d, *J* = 3.6 Hz), 159.25, 152.86, 152.73, 150.62 (d, *J* = 9.6 Hz), 147.72, 147.67, 147.37, 132.63, 129.52, 129.05, 126.28, 120.43, 114.92, 99.59, 74.27, 70.00, 69.44 (d, *J* = 157.2 Hz), 66.77, 61.70 (d, *J* = 14.2 Hz), 51.03, 50.78, 32.16, 23.84, 21.87 (d, *J* = 4.1 Hz), 21.05 (d, *J* = 5.6 Hz), 14.24, 8.15. ^{31}P NMR (162 MHz, CD_3OD): δ 26.15. MS m/z (ESI): 614.2 $[M+H]^+$. Minor isomer: ^{1}H NMR (400 MHz, CD_3OD): δ 8.13 (d, *J* = 9.2 Hz, 1H), 8.03 (t, *J* = 2.0 Hz, 1H), 7.67 (dd, *J* = 9.2, 2.0 Hz, 1H), 7.61 (s, 1H), 5.58 (d, *J* = 16.2 Hz, 1H), 5.38 (d, *J* = 16.2 Hz, 1H), 5.27 (s, 2H), 4.93 (hept, *J* = 6.2 Hz, 1H), 4.02 (dq, *J* = 8.4, 7.2 Hz, 1H), 4.00 (d, *J* = 7.8 Hz, 2H), 3.55 (d, *J* = 1.0 Hz, 3H), 3.22 (q, *J* = 7.6 Hz, 2H), 2.04–1.90 (m, 2H), 1.42 (t, *J* = 7.6 Hz, 3H), 1.28 (d, *J* = 7.2 Hz, 3H), 1.20 (d, *J* = 6.2 Hz, 3H), 1.19 (d, *J* = 6.2 Hz, 3H), 1.01 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD): δ 174.78, 174.69 (d, *J* = 4.0 Hz), 159.13, 152.77, 152.60, 150.45 (d,

J = 9.6 Hz), 147.60, 147.56, 147.15, 132.54, 129.41, 128.91, 126.41, 120.41, 115.00, 99.47, 74.21, 70.15, 69.38 (d, *J* = 155.1 Hz), 66.77, 61.74 (d, *J* = 13.7 Hz), 51.05, 50.70, 32.25, 23.87, 21.90 (d, *J* = 3.5 Hz), 20.68 (d, *J* = 5.6 Hz), 14.19, 8.17. ^{31}P NMR (162 MHz, CD_3OD): δ 27.15. MS m/z (ESI): 614.2 $[M+H]^+$.

Isopropyl (2S)-2-[[[(8R,9S,13S,14S,17S)-17-hydroxyl-13-methyl-6,7,8,9,11,12, 14,15,16,17-decahydrocyclopenta[a]phenanthrene-3-yl]oxy-(methoxymethyl)phosphoryl]amino]propanoate (**17**) Colorless oil (0.50 g), 27.6 % yield. R_f = 0.30 (1:1 ethyl acetate/hexanes, TLC), major isomer : minor isomer = 2.44 : 1.00. The diastereoisomeric mixture was separated by chiral preparative HPLC to afford two optical isomers. Major isomer: ^{1}H NMR (400 MHz, CD_3OD): δ 7.25 (d, *J* = 8.4 Hz, 1H), 6.96 (d, *J* = 8.4 Hz, 1H), 6.92 (s, 1H), 4.93 (hept, *J* = 6.2 Hz, 1H), 3.96 (dq, *J* = 9.2, 7.0 Hz, 1H), 3.85–3.75 (m, 2H), 3.67 (t, *J* = 8.6 Hz, 1H), 3.43 (d, *J* = 1.0 Hz, 3H), 2.91–2.77(m, 2H), 2.40–2.28 (m, 1H), 2.24–2.14 (m, 1H), 2.11–1.93 (m, 2H), 1.93–1.86 (m, 1H), 1.76–1.66 (m, 1H), 1.57–1.50 (m, 1H), 1.50–1.44 (m, 1H),1.44–1.31 (m, 4H), 1.30–1.25 (m, 4H, including 1.29 (d, *J* = 7.0 Hz, 3H)), 1.20 (d, *J* = 6.2 Hz, 3H), 1.19 (d, *J* = 6.2 Hz, 3H), 0.77 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD): δ 174.74 (d, *J* = 3.9 Hz), 149.21 (d, *J* = 9.1 Hz), 139.66, 138.46, 127.58, 121.80 (d, *J* = 4.4 Hz), 118.98 (d, *J* = 4.1 Hz), 82.44, 69.93, 69.20 (d, *J* = 156.6 Hz), 61.55 (d, *J* = 14.2 Hz), 51.33, 51.07, 45.45, 44.34, 40.18, 37.98, 30.73, 30.56, 28.25, 27.46, 24.03, 21.98 (d, *J* = 9.6 Hz), 21.06 (d, *J* = 5.5 Hz), 11.66. ^{31}P NMR (162 MHz, CD_3OD): δ 24.89. MS m/z (ESI): Calcd for $C_{26}H_{41}NO_6P$ $[M + H]^+$ 494.2. Found: 494.2. Minor isomer: ^{1}H NMR (400 MHz, CD_3OD): δ 7.25 (d, *J* = 8.4 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 1H), 6.91 (s, 1H), 4.94 (hept, *J* = 6.2 Hz, 1H), 3.96 (dq, *J* = 8.4, 7.0 Hz, 1H), 3.88–3.78 (m, 2H), 3.66 (t, *J* = 8.6 Hz, 1H), 3.48 (d, *J* = 1.0 Hz, 3H), 2.90–2.77 (m, 2H),

2.39–2.25 (m, 1H), 2.24–2.11 (m, 1H), 2.11–1.93 (m, 2H), 1.94–1.85 (m, 1H), 1.77–1.62 (m, 1H), 1.57–1.49 (m, 1H), 1.49–1.44 (m, 1H), 1.44–1.39 (m, 1H), 1.39–1.30 (m, 2H),1.29–1.24 (m, 4H, including 1.28 (d, J = 7.0 Hz, 3H)), 1.24–1.14 (m, 7H, including 1.21 (d, J = 6.2 Hz, 3H), 1.20 (d, J = 6.2 Hz, 3H)), 0.77 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD): δ 174.83 (d, J = 4.5 Hz), 149.12 (d, J = 9.3 Hz), 139.59, 138.44, 127.55, 121.85 (d, J = 4.3 Hz), 119.05 (d, J = 4.3 Hz), 82.42, 70.01, 69.15 (d, J = 156.8 Hz), 61.57 (d, J = 13.5 Hz), 51.32, 51.00, 45.43, 44.32, 40.15, 37.97, 30.72, 30.57, 28.24, 27.44, 24.03, 21.94 (d, J = 5.9 Hz), 20.73 (d, J = 5.4 Hz), 11.66. ^{31}P NMR (162 MHz, CD_3OD): δ 25.75. MS m/z (ESI): 494.2 $[M+H]^+$.

Benzyl-(2S)-2-[[[[(4R,4aS,7aR,12bS)-3-allyl-4a-hydroxy-7-oxo-2,3,4,4a,5,6,7,7a-octahydro-1H-4,12-methanobenzofuro[3,2-e]isoquinolin-9-yl]oxy](methoxymethyl)-phosphoryl]amino]propanoate (**19**) Yellow oil (4.50 g), 27.6 % yield. R_f = 0.50 (1:5 10:1 dichloromethane/methanol, TLC), major isomer : minor isomer = 1.04 : 1.00. The diastereoisomeric mixture was separated by SFC to afford two optical isomers. Major isomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40–7.24 (m, 5H), 7.07 (dd, J = 8.2, 1.4 Hz, 1H), 6.65 (d, J = 8.2 Hz, 1H), 5.83 (ddt, J = 16.6, 10.2, 6.4 Hz, 1H), 5.27–5.18 (m, 2H), 5.16–5.07 (m, 2H), 4.67 (s, 1H), 4.32–4.21 (m, 1H), 4.00 (dd, J = 13.6, 8.4 Hz, 1H), 3.97 (d, J = 8.2 Hz, 1H), 3.83 (dd, J = 13.6, 7.2 Hz, 1H), 3.48 (d, J = 0.8 Hz, 3H), 3.16 (d, J = 6.4 Hz, 2H), 3.13–2.96 (m, 3H), 2.60 (t, J = 5.4 Hz, 1H), 2.56 (d, J = 4.8 Hz, 1H), 2.38 (td, J = 12.6, 5.0 Hz, 1H), 2.28 (dt, J = 14.2, 3.0 Hz, 1H), 2.08 (td, J = 12.2, 3.6 Hz, 1H), 1.88 (ddd, J = 13.4, 4.8, 3.0 Hz, 1H), 1.59 (td, J = 14.2, 3.6 Hz, 1H), 1.56 (td, J = 10.4, 2.6 Hz, 1H), 1.39 (d, J = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 207.87, 173.62 (d, J = 5.5 Hz), 146.99 (d, J = 4.1 Hz), 135.62, 134.86, 132.11 (d, J = 9.1 Hz), 130.08, 129.41,

128.63, 128.37, 128.16, 123.70 (d, J = 3.3 Hz), 119.91, 118.49, 90.76, 70.22, 68.17 (d, J = 152.7 Hz), 66.95, 62.17, 61.26 (d, J = 13.5 Hz), 57.69, 50.85, 49.47, 43.27, 36.11, 31.34, 30.47, 23.06, 21.58 (d, J = 4.4 Hz). ^{31}P NMR (162 MHz, $CDCl_3$): δ 25.79. MS m/z (ESI): 597.3 $[M+H]^+$. Minor isomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40–7.24 (m, 5H), 7.09 (dd, J = 8.2, 1.6 Hz, 1H), 6.67 (d, J = 8.2 Hz, 1H), 5.83 (ddt, J = 16.6, 10.2, 6.4 Hz, 1H), 5.27–5.17 (m, 2H), 5.27–5.18 (m, 2H), 4.72 (s, 1H), 4.31–4.23 (m, 1H), 4.23–4.13 (m, 1H), 4.03–3.83 (m, 2H), 3.48 (d, J = 1.0 Hz, 3H), 3.22–3.15 (m, 2H), 3.15–3.00 (m, 3H), 2.69–2.51 (m, 2H), 2.42 (td, J = 12.6, 5.2 Hz, 1H), 2.25 (dt, J = 14.0, 3.0 Hz, 1H), 2.11 (td, J = 12.2, 3.8 Hz, 1H), 1.90 (ddd, J = 13.4, 4.6, 3.2 Hz, 1H), 1.62–1.43 (m, 2H), 1.26 (m, 1H), 1.07 (d, J = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 208.77, 173.71 (d, J = 5.6 Hz), 147.40 (d, J = 4.0 Hz), 135.85, 134.82, 132.46 (d, J = 10.6 Hz), 129.90 (d, J = 1.4 Hz), 129.49, 128.59, 128.28, 128.19, 123.53 (d, J = 3.1 Hz), 119.91 (d, J = 1.4 Hz), 118.56, 91.03, 70.25, 68.93 (d, J = 156.6 Hz), 66.92, 62.17, 61.44 (d, J = 12.1 Hz), 57.68, 51.31, 49.62, 43.29, 36.17, 31.74, 30.51, 23.05, 20.39 (d, J = 3.6 Hz). ^{31}P NMR (162 MHz, $CDCl_3$): δ 26.56. MS m/z (ESI): 597.2 $[M+H]^+$.

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ABBREVIATIONS USED

AUC, area under the curve; BnBr, benzyl bromide, CH_3I, iodomethane; DMSO, dimethyl sulfoxide; Et_3N, triethylamine; ESI, electrospray ionization; H_2, hydrogen; HPLC, high-performance liquid chromatography; ICR, Institute of Cancer Research; i.g., intragastric; i.v., intravenous; LD_{50}, median lethal dose; MsCl, methanesulfonyl chloride; NaH, sodium hydride; TLC, thin-layer chromatography; TMSBr, bromotrimethylsilane; THF, tetrahydrofuran.

Supporting Information Available: General chemistry information, absolute configuration determination, ^{31}P NMR chemical shifts for *S*-stereoisomer and *R*-stereoisomer**,** HPLC analytical and preparative separation method and x-ray crystallography data; molecular formula strings. This material is available free of charge via the internet at http://pubs.acs.org.

REFERENCES

(1) Kay, B.; Rolly, G. I.C.I. 35868, A new intravenous induction agent. *Acta Anaesthesiol. Belg.* **1977**, *28*, 303−316.

(2) Qin, L. L.; Ren, L.; Wan, S. L.; Liu, G. L.; Luo, X. F.; Liu, Z. H.; Li, F. Q.; Yu, Y.; Liu, J. Y.; Wei, Y. G. Design, synthesis and evaluation of novel 2, 6-disubstituted phenol derivatives as general anesthetics. *J. Med. Chem.* **2017**, *60*, 3606−3617.

(3) Xu, Z. Q.; Jiang, X. J.; Li, W.; Gao, D.; Li, X. J.; Liu, J. Propofol-induced sleep: efficacy and safety in patients with refractory chronic primary insomnia. *Cell Biochem. Biophys.* **2011**, *60*, 161−166.

(4) (a) Krusz, J. C.; Scott, V.; Belanger, J. Intravenous propofol: unique effectiveness in treating intractable migraine. *Headache* **2000**, *40*, 224−230. (b) Mohseni, M.; Fatehi, F. Propofol alleviates intractable migraine headache: A case report. *Anesthesiol. Pain Med.* **2012**, *2*, 94−96. (c) Dhir, A. Propofol in the treatment of refractory migraine headaches. *Expert Rev. Neurother*. **2016**, *16*, 1007−1011.

(5) Pain, L.; Oberling, P.; Launoy, A.; Di Scala, G. Effect of nonsedative doses of propofol on an innate anxiogenic situation in rats. *Anesthesiology* **1999**, *90*, 191−196.

(6) Zacny, J. P.; Coalson, D. W.; Young, C. J.; Klafta, J. M.; Lichtor, J. L.; Rupani, G. Propofol at conscious sedation doses produces mild analgesia to cold pressor-induced pain in healthy volunteers. *J. Clin. Anesth.* **1996**, *8*, 469−474.

(7) Xu, F.; Gallop, M. A.; Sasikumar, V.; Dilip, U. Serine amino acid derived prodrugs of propofol, compositions, uses and crystalline forms thereof. WO2006071995, 2006.

(8) Gonnissen, Y. R. J. P. Propofol phosphonyl derivatives, synthesis, and use in long acting formulations. WO2010115869, 2010.

(9) Wozniak, K. M.; Vornov, J. J.; Mistry, B. M.; Wu, Y.; Rais, R.; Slusher, B. S. Gastrointestinal delivery of propofol from fospropofol: its bioavailability and activity in rodents and human volunteers. *J. Transl. Med.* **2015***, 13,* 170−183.

(10) Uchegbu, I. F.; Jones, M. C.; Corrente, F.; Godfrey, L.; Laghezza1, D.; Carafa, M.; Holm, P.; Schätzlein, A. G. The oral and intranasal delivery of propofol using chitosan amphiphile nanoparticles. *Pharm. Nanotechnol*. **2014**, *2*, 65−74.

(11) Tsagogiorgas, C.; Theisinger, S.; Holm, P.; Thiel, M.; Quintel, M.; Holm, R. Buccal absorption of propofol when dosed in 1-perfluorobutylpentane to anaesthetised and conscious Wistar rats and Gottingen mini-pigs. *Eur. J. Pharm. Biopharm.* **2013**, *85*, 1310–1316.

(12) McGuigan, C.; Pathirana, R. N.; Mahmood, N.; Devine, K. G.; Hay, A. J. Aryl phosphate derivatives of AZT retain activity against HIV1 in cell lines which are resistant to the action of AZT. *Antiviral Res*. **1992**, *17*, 311−321.

(13) McGuigan, C.; Pathirana, R. N.; Mahmood, N.; Hay, A. J. Aryl phosphate derivatives of AZT inhibit HIV replication in cells where the nucleoside is poorly active. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 701−704.

(14) McGuigan, C.; Pathlrana, R. N.; Choi, S. S. M.; Kinchington, D.; Connor, T. J. Phosphoramidate derivatives of AZT as inhibitors of HIV: studies on the carboxyl terminus. *Antiviral Chem. Chemother*. **1993**, *4*, 97−101.

(15) Cahard, D.; McGuigan, C.; Balzarini, J. Aryloxy phosphoramidate triesters as Pro-Tides. *Mini-Rev. Med. Chem*. **2004**, *4*, 371−381.

(16) Mehellou, Y.; Balzarini, J.; McGuigan, C. Aryloxy phosphoramidate triesters: A technology for delivering monophosphorylated nucleosides and sugars into cells. *ChemMedChem* **2009**, *4*, 1779−1791.

(17) Becker, M. W.; Chapman, H. H.; Cihlar, T.; Eisenberg, E. J.; He, G. X.; Kernan, M. R.; Lee, W. A.; Prisbe, E. J.; Rohloff, J. C.; Sparacino, M. L. Prodrugs of phosphonate

nucleotide analogues and methods for selecting and making same. WO2002008241, 2002.

(18) Ballatore, C.; McGuigan, C.; De Clercq, E.; Balzarini, J. Synthesis and evaluation of novel amidate prodrugs of PMEA and PMPA. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1053−1056.

(19) Brenner, C. Hint, Fhit, and GaIT: function, structure, evolution, and mechanism of three branches of the histidine triad superfamily of nucleotide hydrolases and transferase. *Biochemistry* **2002**, 41, 9003–9014.

(20) Ware, R. W.; Almond, M. R.; Lampert, B. M. Phosphonate ester derivatives and methods of synthesis thereof. WO2012031045, 2012.

(21) Hill-Venning, C.; Peters, J. A.; Callachan, H.; Lambert, J. J.; Gemmell, D. K.; Anderson, A.; Byford, A.; Hamilton, N.; Hill, D. R.; Marshall, R. J.; Campbell, A. C. The anesthetic action and modulation of $GABA_A$ receptor activity by the novel water-soluble aminosteroid Org 20599. *Neuropharmacology* **1996**, *35*, 1209−1222.

(22) Birkus, G.; Wang, R.; Liu, X. H.; Kutty, N.; MacArthur, H.; Cihlar, T.; Gibbs, C.; Swaminathan, S.; Lee, W.; McDermott, M. Cathepsin A is the major hydrolase catalyzing the intracellular hydrolysis of the antiretroviral nucleotide phosphonoamidate prodrugs GS-7340 and GS-9131. *Antimicrob. Agents Chemother*. **2007**, *51*, 543−550.

(23) Forouzanfar, F.; Ghorbani, A.; Hosseini, M.; Rakhshandeh, H. Hydroalcoholic extract of needles of Pinus eldarica enhances pentobarbital-induced sleep: possible involvement of GABAergic system. *Avicenna J. Phytomed.* **2016**, *6*, 449–457.

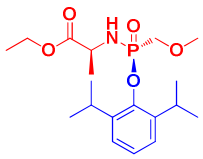
(24) Bala, V.; Rao, S. S.; Boyd, B. J.; Prestidge, C. A. Prodrug and nanomedicine approaches for the delivery of the camptothecin analogue SN38. *J. Controlled Release* **2013**, *172*, 48−61.

(25) Fotherby, k. Bioavailability of orally administered sex steroids used in oral contraception and hormone replacement therapy. *Contraception* **1996**, *54*, 59−69.

(26) Hussain, M. A.; Aungst, B. J.; Kearney, A.; Shefter, E. Buccal and oral bioavailability of naloxone and naltrexone in rats. *Int. J. Pharm*. **1987**, *36*, 127−130.

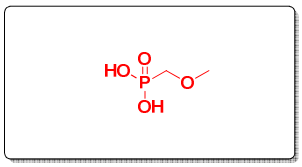
(27) Pommier, Y. Topoisomerase I inhibitors: camptothecins and beyond. *Nat. Rev. Cancer* **2006**, *6*, 789–802.

Table of Contents Graphic.



3a

F in beagle dogs as compound 1: 97.6 %



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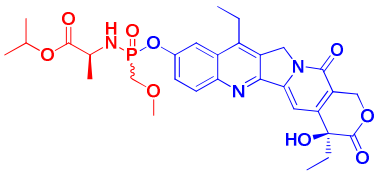

15

F in beagle dogs as compound 14: 34.1 %