Bioorganic & Medicinal Chemistry Letters 19 (2009) 6918–6921

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design, synthesis and evaluation of novel oxazaphosphorine prodrugs of 9-(2-phosphonomethoxyethyl)adenine (PMEA, adefovir) as potent HBV inhibitors

Peng Lu [a], Jiangxia Liu [b], Yuya Wang [a], Xiaoyan Chen [a], Yushe Yang [a,*], Ruyun Ji [a]

[a] State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Shanghai Institute for Biological Sciences, Chinese Academy of Science, Shanghai 201203, China
[b] Key Laboratory of Medical Molecular Viology, Institute of Medical Microbiology, Shanghai Medical College, Fudan University, Shanghai 200032, China

ARTICLE INFO

Article history:
Received 30 August 2009
Revised 14 October 2009
Accepted 16 October 2009
Available online 21 October 2009

Keywords:
Oxazaphosphorine
Prodrug
Hepatitis B virus

ABSTRACT

A series of novel oxazaphosphorine prodrugs of 9-(2-phosphonomethoxyethyl)adenine (PMEA, adefovir) were synthesized and their anti-hepatitis B virus (HBV) activity was evaluated in HepG2 2.2.15 cells, with adefovir dipivoxil as a reference drug. In the cell assays, compounds **7b** and **7d** exhibited anti-HBV activity comparable to that of adefovir dipivoxil, while compound **7c**, with an IC_{50} value of 0.12 μM, was found to be three times more potent than the reference compound. In vitro stability studies showed that (S_P,*S*)-**7c**, the diastereomer of compound **7c**, was stable in human blood plasma but underwent rapid metabolism to release the parent drug PMEA in liver microsomes. The possible metabolic pathway of (S_P,*S*)-**7c** in human liver microsomes was described. These findings suggest that compound (S_P,*S*)-**7c** is a promising anti-HBV drug candidate for further development.

Hepatitis B virus (HBV) infection is a major global health problem. Over 350 million people worldwide are infected chronically with HBV and are therefore at risk of various liver diseases, which results in 0.5–1.2 million deaths each year (the tenth leading cause of death worldwide).[1] Although interferon α and several nucleoside and nucleotide analogues currently are available for the treatment of HBV, they are associated with a low cure rate, viral resistance, cytotoxicity and other limitations.[2] New drugs with better therapeutic profiles are still urgently needed.

Among current anti-HBV agents,[3] nucleotide analog 9-(2-phosphonomethoxyethyl)adenine (PMEA) (**1**, Fig. 1) has shown impressive activity against a broad spectrum of DNA viruses including HBV, and more importantly, it is associated with a low rate of viral resistance. However, the negative charge of the phosphonate moiety significantly impairs its cellular uptake and leads to poor oral bioavailability.[4] One potential strategy for overcoming these limitations is to use prodrugs.[5] One of the best examples is the prodrug adefovir dipivoxil (**2**, Fig. 1), which has been approved by the US FDA for HBV therapy. Adefovir dipivoxil can be converted rapidly into the parent drug PMEA in vivo and suppress replication of HBV that is resistant to other anti-HBV drugs such as lamivudine, emtricitabine and famciclovir.[6] However, the dose-limiting nephrotoxicity of adefovir and its potential to release the carcinogen formaldehyde and toxic pivalic acid have limited its clinical use.[7] To circumvent these therapeutic limitations, several new classes of PMEA prodrugs including acyloxyalkyl esters, *S*-acyl-2-thioethyl (SATE) esters[8] and phosphoramidates[9] have been developed. Although promising, these prodrugs are cleaved by esterases, which results in their rapid breakdown in the blood, and a lack of sustained therapeutic effects. In this Letter, we report our efforts to develop a series of oxazaphosphorine prodrugs of PMEA **7a**–**i** (Fig. 1) with more potent anti-HBV activity, lower cytotoxicity and better plasma stability.

Synthesis of oxazaphosphorine prodrugs of PMEA **7a**–**i** is outlined in Scheme 1. *N*-(3-Hydroxypropyl)-amino acid esters **4a**–**i** were prepared from various commercially available amino acids in 25–34% yields in five steps, according to a previously described method, with minor modifications.[10] N-Protected PMEA dichloride **3** was obtained by treating PMEA with oxalyl chloride in the presence of *N*,*N*-diethylformamide in refluxing CH_2Cl_2, followed by activation with two equivalents of pyridine at 0 °C in CH_2Cl_2.[11] Cyclization of **4a**–**i** with phosphonyl dichlorides **3** at −78 °C, followed by removal of the imine protecting group with acetic acid yielded cyclized oxazaphosphorines **6a**–**i** as oils. Treatment of **6a**–**i** with one equivalent of fumaric acid in refluxing acetonitrile for 2 h provided the final products **7a**–**i** as crystalline salt, with yields of 15–33% for all four steps.[12]

To investigate stereochemistry effects of the phosphine groups on anti-HBV activity, four diastereomerically pure analogs were prepared. As shown in Scheme 2, chromatographic resolution of **5g** afforded diastereomers (S_P,*S*)-**5g** and (R_P,*S*)-**5g**. (S_P,*S*)-**7g** and (R_P,*S*)-**7g** were prepared in two steps from (S_P,*S*)-**5g** and (R_P,*S*)-**5g**, respectively. Using **5i** as the starting material, (R_P,*R*)-**7i** and

* Corresponding author. Tel./fax: +86 21 50806786.
E-mail address: ysyang@mail.shcnc.ac.cn (Y. Yang).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.10.072

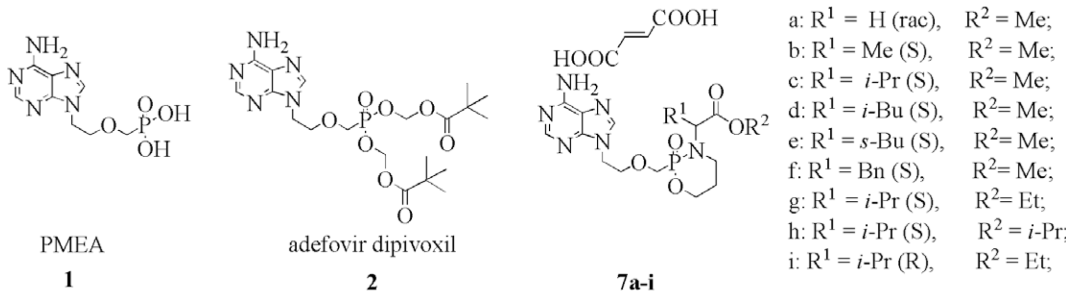
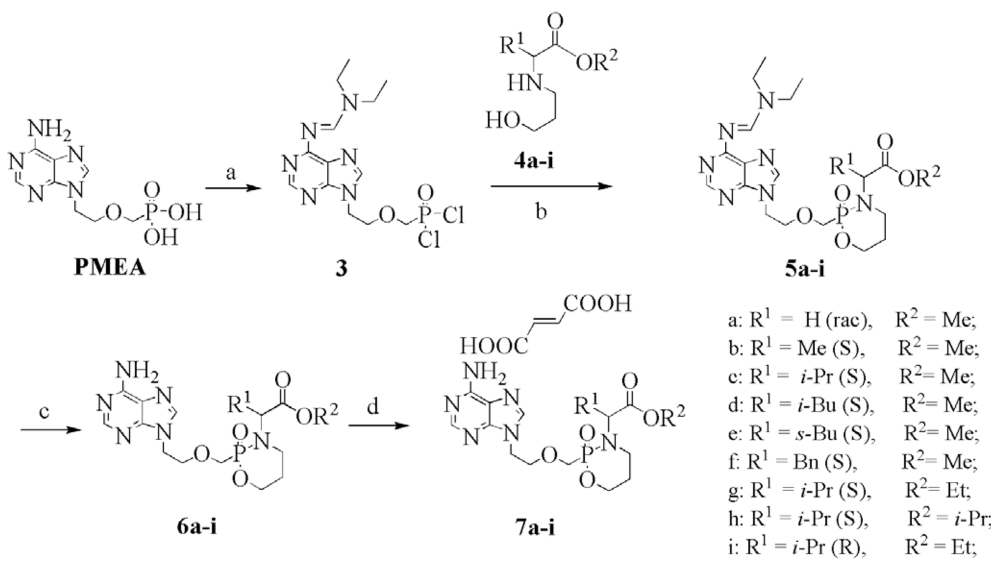
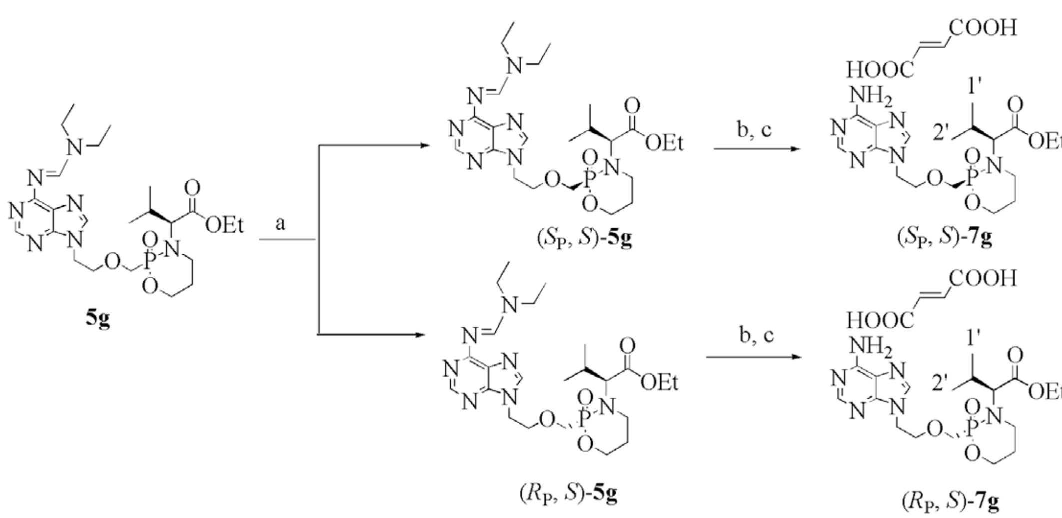


Figure 1. Structures of PMEA (**1**), adefovir dipivoxil (**2**) and compounds **7a**–**i**.



Scheme 1. Reagents and conditions: (a) (i) Et_2NCHO, $(COCl)_2$, CH_2Cl_2, reflux, 3 h, (ii) Py, 0 °C, 20 min; (b) TEA, CH_2Cl_2, −78 °C; (c) AcOH, reflux, 2 h; (d) fumaric acid, MeCN, reflux, 1 h.



Scheme 2. Reagents: (a) chromatographic resolution; (b) AcOH, reflux, 2 h; (c) fumaric acid, MeCN, reflux, 1 h.

(S_P,R)-**7i** were obtained using a similar method to that described for compound **7g**. The spectroscopic data of ^{1}H and ^{31}P NMR were used to assign the configuration of the four diastereomers of **7g** and **7i**.[13] The results are summarized in Table 1.

The target compounds were evaluated for their inhibitory effects on the replication of HBV in HepG2 2.2.15 cells, as described previously.[14] Viral DNA recovered from the secreted particles in culture medium was analyzed by real-time PCR using Icycler (Bio-Rad). Amplification primers were HBVFP: 5′-TGT CCT GGT TAT CGC TGG-3′ and HBVRP: 5′-CAA ACG GGC AAC ATA CCT T-3′. The TaqMan probe was FAM-5′-TGT GTC TGC GGC GTT TTA TCA T-3′-TAMRA. The data was analyzed using Icycler IQ 3.0. IC_{50},

Table 1
Analytical data of diastereomers of compounds **7g** and **7i**

Compound	MS	NMR δ (ppm)		
		^{1}H (C-1′, CH_3)[b]	^{1}H (C-2′, CH_3)[b]	^{31}P[c]
(S_P,S)-**7g**	463.1824[a]	0.94	0.86	21.77
(R_P,S)-**7g**	463.1838[a]	0.82	0.65	22.02
(R_P,R)-**7i**	463.1834[a]	0.94	0.86	21.76
(S_P,R)-**7i**	463.1815[a]	0.82	0.65	21.95

[a] HRMS (FAB), m/z calcd for $C_{18}H_{29}N_6O_5P$ $[M+Na]^+$ 463.1835.
[b] ^{1}H chemical shift was recorded at 300 MHz with a Varian Gemini spectrometer using tetramethylsilane as an internal standard.
[c] ^{31}P chemical shift was recorded at 121 MHz using 85% H_3PO_4 in a coaxial insert as an external standard.

Table 2
Anti-HBV activity of prodrugs **7a–i**

Compound	R[1]	R[2]	IC_{50}[a] (μM)	CC_{50}[b] (μM)	SI[c]
7a	H (rac)	Me	0.88	602	683
(S_P,S)-**7b**	Me	Me	0.51	650	1270
(R_P,S)-**7b**	Me	Me	0.91	590	649
7c	*i*-Pr (*S*)	Me	0.12	577	4800
7d	*i*-Bu (*S*)	Me	0.32	222	694
7e	*s*-Bu (*S*)	Me	2.21	648	293
7f	Bn (*S*)	Me	2.62	726	277
(S_P,S)-**7g**	*i*-Pr (*S*)	Et	3.00	560	187
(R_P,S)-**7g**	*i*-Pr (*S*)	Et	50	468	9
7h	*i*-Pr (*S*)	*i*-Pr	5.76	592	103
(R_P,R)-**7i**	*i*-Pr (*R*)	Et	50	439	9
(S_P,R)-**7i**	*i*-Pr (*R*)	Et	50	400	8
Adefovir dipivoxil			0.33	555	1680

[a] IC_{50} (Concentrations of compounds achieving 50% inhibition of cytoplasmic HBV-DNA synthesis).
[b] CC_{50} (Concentrations of compounds required for 50% extinction of HepG2 2.2.15 cells).
[c] SI (selectivity index) = CC_{50}/IC_{50}.

CC_{50} and SI of these compounds are listed in Table 2. Adefovir dipivoxil was used as a positive control.

In our previous Letter, we reported a series of L-amino acid ester prodrugs of PMEA with enhanced antiviral activity.[15] It is thought that amino acid esters can be delivered efficiently by human peptide transporter 1. To take advantage of the active transporting mechanism of amino acids, various amino acid esters were incorporated into oxazaphosphorine prodrugs of PMEA **7a–i**. In L-amino acid series, all the compounds except **7d** showed less cytotoxicity than adefovir dipivoxil did (Table 2), which suggests that introduction of an L-amino acid ester moiety at the N-3 position reduces cytotoxicity, probably because of production of relatively less toxic metabolites. Incorporation of the unnatural amino acid D-valine led to production of prodrug **7i**, which was more toxic than adefovir dipivoxil. L-valine prodrug **7c** and L-leucine prodrug **7d** exhibited potent anti-HBV activity, with IC_{50} values of 0.12 and 0.32 μM, respectively. The most potent compound **7c** was nearly three times more active than the reference compound adefovir dipivoxil. D-valine prodrug **7i** showed no inhibition of HBV replication. From these results, it appeared that the contribution of amino acid moieties to anti-HBV activity has the following order of magnitude: L-valine > L-leucine > L-alanine > glycine > L-isoleucine > L-phenylalanine > > D-valine. The poor activity of D-valine prodrug **7i** suggests that the configuration of amino acid is an important determinant of anti-HBV activity.

Since the L-valine methyl ester **7c** emerged as the most active compound, the effect of ester groups on anti-HBV activity was examined further. As shown in Table 2, although ethyl ester **7g** and isopropyl ester **7h** showed a similar CC_{50} value to that of the methyl ester prodrug **7c**, their potency as indicated by their IC_{50} values was diminished 25- and 48-fold, respectively. The decreased inhibitory activity was possibly caused by the increased steric hindrance of the ester groups.

To explore the influence of stereochemistry on anti-HBV activity, six diastereomerically pure compounds were obtained by successive applications of column chromatography and crystallization. As shown in Table 2, D-valine (S_P,S)-**7i** and (R_P,S)-**7i** showed no inhibition of HBV replication. For the L-alanine methyl ester analogs, (S_P,S)-**7b** (IC_{50} = 0.51 μM) and (R_P,S)-**7b** (IC_{50} = 0.91 μM), there was a modest preference for the *S*-phosphor diastereomer. The same trend was observed for the L-valine ethyl ester **7g** in that (S_P,S)-**7g** displayed more potent anti-HBV activity than (R_P,S)-**7g**. Based on the data in Table 2, L-valine prodrug **7c** was chosen for further profiling.

It has been reported that adefovir dipivoxil has poor in vitro stability in plasma, which causes rapid decomposition and decreased therapeutic index.[16] Thus, the in vitro stability of **7c** in human blood and liver microsomes was examined further. Both diastereomers of **7c** were stable in plasma ($t_{1/2}$ >6 h), whereas adefovir

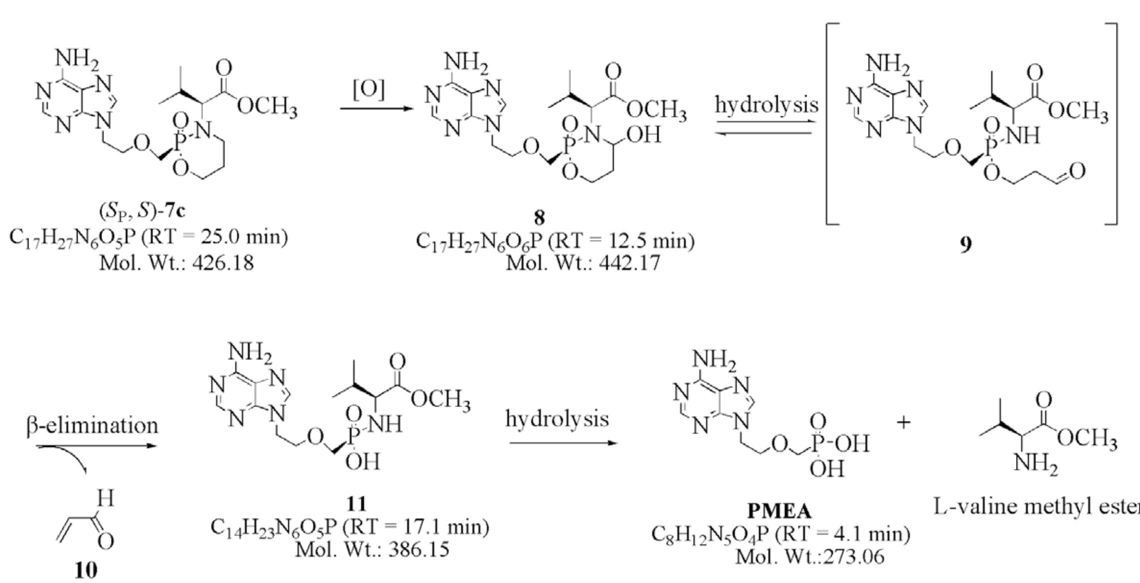


Figure 2. Possible metabolic pathway of (S_P,S)-**7c**.

dipivoxil displayed poor stability with a half-life of 45 min in human plasma and microsomes. Although (R_P,S)-**7c** and (S_P,S)-**7c** have similar stability in plasma, the hydrolysis rate of isomer (S_P,S)-**7c** ($t_{1/2}$ = 3.6 h) in human microsomes was faster than that of (R_P,S)-**7c** ($t_{1/2}$ >6 h). Moreover, diastereomer (S_P,S)-**7c** was more stable than adefovir dipivoxil in human blood and liver microsomes.

The possible metabolic pathway for prodrug (S_P,S)-**7c** was investigated further in human microsomes (Fig. 2). Prodrug (S_P,S)-**7c** was incubated with human liver microsomes for 6 h and the metabolites were identified with LC–MS. Oxidation of the carbon adjacent to the amide led to the intermediate **8** (LC–MS peak at 12.5 min with an observed mass of 443). Subsequent hydrolysis of **8** afforded the second intermediate **9**, which underwent a facile β-elimination to produce the third intermediate **11** (LC–MS peak at 17.1 min with an observed mass of 387) and acrolein **10**, a known glutathione scavenger.[17] Furthermore, **11** was hydrolyzed to the parent compound PMEA, which underwent subsequent phosphorylation to form triphosphate as an HBV polymerase inhibitor.[18] The parent compound PMEA appeared in LC–MS at 4.1 min, which was confirmed by comparison with the authentic sample. Compound **11** was found to be the major metabolite in human microsomes.

In summary, we described the synthesis and structure–activity relationships of a novel class of oxazaphosphorine prodrugs of PMEA, as highly potent anti-HBV agents with excellent stability in human plasma. The possible metabolic pathway in human microsomes for the most potent prodrug **7c** was described. The results suggest that oxazaphosphorine prodrugs of PMEA have significant potential for treatment of HBV infection. A further study of the prodrug (S_P,S)-**7c** is underway in our laboratory and will be reported in due course.

References and notes

1. Malik, A.; Lee, W. *Ann. Intern. Med.* **2000**, *132*, 723.
2. (a) Wong, D. K. H.; Cheung, A. M.; O'Rourke, K.; Naylor, C. D.; Detsky, A. S.; Heathcote, J. *Ann. Intern. Med.* **1993**, *119*, 312; (b) Lok, A. S.; Hussain, M.; Cursano, C.; Margotti, M.; Gramenzi, A.; Grazi, G. L.; Jovine, E.; Benardi, M.; Andreone, P. *Hepatology* **2000**, *32*, 1145; (c) Kim, J. W.; Park, S. H.; Louie, S. G. *Ann. Pharmacother.* **2006**, *40*, 472.
3. (a) De Clercq, E.; Sakuma, T.; Baba, M.; Pauwels, R.; Balzarini, J.; Rosenberg, I.; Holy, A. *Antiviral Res.* **1987**, *8*, 261; (b) Yokota, T.; Mochizuki, S.; Konno, K.; Mori, S.; Shigheta, S.; De Clercq, E. *Antimicrob. Agents Chemother.* **1991**, *35*, 394.
4. Ulrika, E.; John, M. H.; Jae-Seung, K.; Stefanie, M.; Paul, K.; Katherine, Z. B.; Julie, M. B.; John, C. D.; Boris, A. K.; Charles, E. M. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 583.
5. (a) Krise, J. P.; Stella, V. J. *Adv. Drug Delivery Rev.* **1996**, *19*, 287; (b) Schultz, C. *Bioorg. Med. Chem.* **2003**, *11*, 885.
6. (a) Chen, Z.; Zheng, M. *Exp. Opin. Ther. Patents* **2005**, *15*, 1027; (b) Douglas, H. S.; Kevin, T. B.; Sanjay, K. N. *Am. J. Physiol. Renal Physiol.* **2001**, *281*, F197.
7. Speit, G.; Schutz, P.; Merk, O. *Mutagenesis* **2000**, *15*, 85.
8. Lefebvre, I.; Perigaud, C.; Pompon, A.; Aubertin, A. M.; Girarder, J. L.; Kirn, A.; Gosselin, G.; Imbach, J. L. *J. Med. Chem.* **1995**, *38*, 3941.
9. McGuigan, C.; Pathirana, R. N.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1993**, *36*, 1048.
10. Sorensen, M. D.; Blæhr, L. K. A.; Christensen, M. K.; Hoyer, T.; Latini, S.; Hjarnaa, P. V.; Björkling, F. *Bioorg. Med. Chem.* **2003**, *11*, 5461.
11. (a) Erion, M. D.; Reddy, K. R.; Boyer, S. H.; Matelich, M. C.; Gomez-Galeno, J.; Lemus, R. H.; Ugarkar, B. G.; Colby, T. J.; Schanzer, J.; Van Poelje, P. D. *J. Am. Chem. Soc.* **2004**, *126*, 5154; (b) Erion, M. D.; Van Poelje, P. D.; Mackenna, D. A.; Colby, T. J.; Montag, A. C.; Fujitaki, J. M.; Linemeyer, D. L.; Bullough, D. A. *J. Pharmacol. Exp. Ther.* **2005**, *46*, 4321; (c) Hecker, S. J.; Reddy, R.; Van Poelje, P. D.; Sun, Z.; Varkhedkar, W.; Huang, V.; Reddy, M. V.; Fujitaki, J. M.; Olsen, D. B.; Koeplinger, K. A.; Boyer, S. H.; MacCoss, D. L.; Linemeyer, M.; Erion, M. D. *J. Med. Chem.* **2007**, *50*, 3891.
12. *Experimental details and characterization data*. Standard procedure: preparation of oxazaphosphorine prodrugs of PMEA **7a**–**i**: Oxalyl chloride (3.5 equiv, 0.60 mL, 7.0 mmol) was added slowly to a slurry of PMEA (1.0 equiv, 2.0 mmol) and *N*,*N*-diethylformamide (1.1 equiv, 0.24 mL, 2.2 mmol) in CH_2Cl_2 (20 mL), and the resulting mixture was refluxed for 3 h. The reaction was cooled to room temperature and concentrated in vacuo. The resultant yellow foam was dissolved in CH_2Cl_2 (20 mL), cooled to 0 °C, and treated slowly with pyridine (2 equiv, 0.32 mL, 4.0 mmol). The cold solution was then added slowly to a −78 °C solution of *N*-(3-hydroxypropyl)-amino acid esters (**4a**–**i**, 1 equiv, 2 mmol) and triethylamine (6.3 equiv, 1.8 mL, 12.6 mmol) in CH_2Cl_2 (20 mL) at a rate that maintained the internal reaction temperature at −78 °C. The reaction mixture was warmed to 0 °C for 2 h, washed with water (3 × 20 mL), and the organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The resultant residue was purified by chromatography (CH_2Cl_2/MeOH, 20:1) to give N-protected oxazaphosphorine prodrugs of PMEA as white foams.The above prepared foam was dissolved in ethanol (30 mL). Acetic acid (2.2 equiv, 0.25 mL, 4.4 mmol) was added, and the solution was heated at reflux for 2 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The resultant residue was purified by chromatography (CH_2Cl_2/MeOH, 20:1) to give **6a**–**i** as colorless oils.
The oils obtained were redissolved in acetonitrile (10 mL) and treated with appropriate fumaric acid (1 equiv). After being refluxed for 2 h, the solution was allowed to cool to room temperature for 2 h. The solid was filtered, rinsed with ether (5 mL) and dried to give **7a**–**i** fumaric acid salts as crystalline solids.
The first-eluting diastereoisomer (S_P,S)-**7g**: mp 128–129 °C. $[\alpha]_D$ = −3.2° (*c* 0.340, MeOH). ^1H NMR (CD_3OD, 300 MHz) δ 8.21 (s, 1H, 2′-H), 8.16 (s, 1H, 8′-H), 6.75 (s, 2H, CH=CH), 4.46 (m, 2H, NCH_2), 4.23 (m, 2H, OCH_2), 3.84–4.08 (m, 6H, $OCH_2P + OCH_2 + OCH_2CH_3$), 3.53 (t, *J* = 10.2 Hz, 1H, NCHCO), 3.06 (m, 2H, NCH_2), 2.17 (m, 1H, CHH), 1.96 (m, 1H, CHH), 1.67 (m, 1H, $CH(CH_3)_2$), 1.18 (t, *J* = 7.2 Hz, 3H, OCH_2CH_3), 0.94 (d, *J* = 6.6 Hz, 3H, CH_3), 0.86 (d, *J* = 6.6 Hz, 3H, CH_3). ^{13}C NMR (CD_3OD, 75 MHz) δ 172.2 (2C), 168.9, 157.5, 153.8, 151.2, 143.7, 135.8 (2C), 120.4, 72.7, 71.1, 69.5, 68.0, 65.0, 62.4, 45.1, 43.0, 28.0, 27.5, 20.1, 19.7, 15.0. ^{31}P NMR (CD_3OD, 120 MHz) δ 21.77. MS (ESI) *m*/*z* 441.3 $[M+H]^+$, 463.3 $[M+Na]^+$. HRMS calcd for $C_{18}H_{29}N_6O_5P$ $[M+Na]^+$ 463.1824, found 463.1835.
The second-eluting diastereoisomer (R_P,S)-**7g**: mp 231–232 °C. $[\alpha]_D$ = −18.0° (*c* 0.300, MeOH). ^1H NMR (CD_3OD, 300 MHz) δ 8.21 (s, 1H, 2′-H), 8.19 (s, 1H, 8′-H), 6.75 (s, 2H, CH=CH), 4.50 (m, 2H, NCH_2), 4.08–4.29 (m, 4H, $OCH_2 + OCH_2CH_3$), 3.77–3.94 (m, 4H, $OCH_2P + CH_2O$), 3.53 (t, *J* = 9.9 Hz, 1H, NCHCO), 3.40 (m, 2H, NCHH), 3.05 (m, 2H, NCHH), 1.96 (m, 2H, CH_2), 1.62–1.89 (m, 1H, $CH(CH_3)_2$), 1.26 (t, *J* = 7.2 Hz, 3H, OCH_2CH_3), 0.82 (d, *J* = 6.6 Hz, 3H, CH_3), 0.65 (d, *J* = 6.6 Hz, 3H, CH_3). ^{13}C NMR (CD_3OD, 75 MHz) δ 173.8 (2C), 168.8, 157.4, 153.5, 151.3, 143.8, 135.8 (2C), 120.4, 72.7, 71.4, 69.4, 67.8, 65.4, 62.3, 45.2, 42.9, 29.3, 27.9, 20.2, 20.1, 14.9. ^{31}P NMR (CD_3OD, 120 MHz) δ 22.02. MS (ESI) *m*/*z* 441.3 $[M+H]^+$, 463.3 $[M+Na]^+$. HRMS calcd for $C_{18}H_{29}N_6O_5P$ $[M+Na]^+$ 463.1838, found 463.1835.
The first-eluting diastereoisomer (R_P,R)-**7i**: mp 176–177 °C. $[\alpha]_D$ = +2.9° (c 0.340, MeOH). ^1H NMR (CD_3OD, 300 MHz) δ 8.21 (s, 1H, 2′-H), 8.16 (s, 1H, 8′-H), 6.75 (s, 2H, CH=CH), 4.46 (m, 2H, NCH_2), 4.23 (m, 2H, OCH_2), 3.84–4.08 (m, 6H, $OCH_2P + OCH_2 + OCH_2CH_3$), 3.53 (t, *J* = 10.2 Hz, 1H, NCHCO), 3.00–3.13 (m, 2H, NCH_2), 2.17 (m, 1H, CHH), 2.01 (m, 1H, CHH), 1.67 (m, 1H, $CH(CH_3)_2$), 1.18 (t, *J* = 7.2 Hz, 3H, OCH_2CH_3), 0.94 (d, *J* = 6.6 Hz, 3H, CH_3), 0.86 (d, *J* = 6.6 Hz, 3H, CH_3). ^{13}C NMR (CD_3OD, 75 MHz) δ 172.2 (2C), 168.9, 157.5, 153.8, 151.2, 143.7, 135.8 (2C), 120.4, 72.7, 71.1, 69.5, 68.0, 65.0, 62.4, 45.1, 43.0, 28.0, 27.5, 20.1, 19.7, 15.0. ^{31}P NMR (CD_3OD, 120 MHz) δ 21.76. MS (ESI) *m*/*z* 441.3 $[M+H]^+$, 463.3 $[M+Na]^+$. HRMS calcd for $C_{18}H_{29}N_6O_5P$ $[M+Na]^+$ 463.1834, found 463.1835.
The second-eluting diastereoisomer (S_P,R)-**7i**: obtained as a white solid. mp 136–137 °C. $[\alpha]_D$ = +31.1° (*c* 0.325, MeOH). ^1H NMR (CD_3OD, 300 MHz) δ 8.21 (s, 1H, 2′-H), 8.19 (s, 1H, 8′-H), 6.75 (s, 2H, CH=CH), 4.50 (m, 2H, NCH_2), 4.08–4.29 (m, 4H, $OCH_2 + OCH_2CH_3$), 3.77–3.94 (m, 4H, $OCH_2P + CH_2O$), 3.53 (t, *J* = 9.9 Hz, 1H, NCHCO), 3.41 (m, 2H, NCHH), 2.95 (m, 2H, NCHH), 1.96 (m, 2H, CH_2), 1.62–1.89 (m, 1H, $CH(CH_3)_2$), 1.26 (t, *J* = 7.2 Hz, 3H, OCH_2CH_3), 0.82 (d, *J* = 6.6 Hz, 3H, CH_3), 0.65 (d, *J* = 6.6 Hz, 3H, CH_3). ^{13}C NMR (CD_3OD, 75 MHz) δ 173.8 (2C), 168.8, 157.4, 153.5, 151.3, 143.8, 135.8 (2C), 120.4, 72.7, 71.4, 69.4, 67.8, 65.4, 62.3, 45.2, 42.9, 29.3, 27.9, 20.2, 20.1, 14.9. ^{31}P NMR (CD_3OD, 120 MHz) δ 21.99. MS (ESI) *m*/*z* 441.3 $[M+H]^+$, 463.3 $[M+Na]^+$. HRMS calcd for $C_{18}H_{29}N_6O_5P$ $[M+Na]^+$ 463.1815, found 463.1835.
13. (a) Pikul, S.; Dunham, K. L. M.; Almstead, N. G.; De, B.; Natchus, M. G.; Anastasio, M. V.; McPhail, S. J.; Snider, C. E.; Taiwo, Y. O.; Chem, L.; Dunaway, C. M.; Gu, F.; Mieling, G. E. *J. Med. Chem.* **1999**, *42*, 87; (b) Sawa, M.; Kiyoi, T.; Kurokawa, K.; Kumihara, H.; Yamamoto, M.; Miyasaka, T.; Ito, Y.; Hirayama, R.; Inoue, T.; Kirii, Y.; Nishiwaki, E.; Ohmoto, H.; Maeda, Y.; Ishibushi, E.; Inoue, Y.; Yoshino, K.; Kondo, H. *J. Med. Chem.* **2002**, *45*, 919; (c) Mosbo, J.; Verkade, J. G. A. *J. Org. Chem.* **1977**, *42*, 1549; (d) Cooper, D. B.; Harrison, J. M.; Inch, T. D.; Lewis, G. J. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1049.
14. Mary, A. S.; Cheng, M. L.; George, A. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 1005.
15. Fu, X. Z.; Jiang, S. H.; Li, C.; Xin, J.; Yang, Y. S.; Ji, R. Y. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 465.
16. (a) Farquhar, D.; Srivastva, D. N.; Kattesch, N. J.; Saunders, P. P. *J. Pharm. Sci.* **1983**, *72*, 324; (b) Farquhar, D.; Khan, S.; Srivastva, D. N.; Saunder, P. P. *J. Med. Chem.* **1994**, *37*, 3902; (c) Dang, Q.; Brown, B. S.; van Poelje, P. D.; Colby, T. C.; Erion, M. D. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1505.
17. Banijamali, A. R.; Xu, Y.; Dematteo, V.; Strunk, R. J.; Sumner, S. J. *J. Agric. Food. Chem.* **2000**, *48*, 4693.
18. (a) Balzarini, J.; Hao, Z.; Herdewjin, P.; Johns, D.; De Clercq, E. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 1499; (b) Merta, A.; Votruba, I. *J. Biochem. Pharmacol.* **1992**, *44*, 2067; (c) Robbins, B.; Greenhaw, J.; Connelly, M.; Fridland, A. *Antimicrob. Agents Chemother.* **1995**, *39*, 2304.