Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 465–470
Bioorganic & Medicinal Chemistry Letters

Design and synthesis of novel bis(L-amino acid) ester prodrugs of 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA) with improved anti-HBV activity

Xiaozhong Fu,[a] Saihong Jiang,[a] Chuan Li,[a] Jian Xin,[b] Yushe Yang[a,*] and 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]*Shanghai Fudan-Yueda Bio-Tech Co., Ltd, Shanghai 201203, China*

Received 13 July 2006; revised 28 September 2006; accepted 10 October 2006
Available online 12 October 2006

Abstract—A series of novel bis(L-amino acid) ester prodrugs of 9-[2-(phosphonomethoxy)ethyl] adenine (PMEA) was synthesized and their anti-HBV activity was evaluated in HepG 2 2.2.15 cells. Compounds **11**, **12**, **21**, **22**, **26**, and **27** demonstrated more potent anti-HBV activity and higher selective index (SI) than adefovir dipivoxil, which was used as a positive control. Compound **11**, which was found to be the most potent one, was five times more potent than adefovir dipivoxil with EC_{50} value of 0.095 μM and CC_{50} value of 6636 μM. The SI value (>69,000) of compound **11** was 60 times and 24 times higher than those of adefovir dipivoxil and lamivudine, respectively. In vitro stability studies showed that compound **11** was relatively more stable than adefovir dipivoxil with $t_{1/2}$ of 270 min. These findings suggested that compound **11** could be considered as a promising candidate for further in vivo studies.

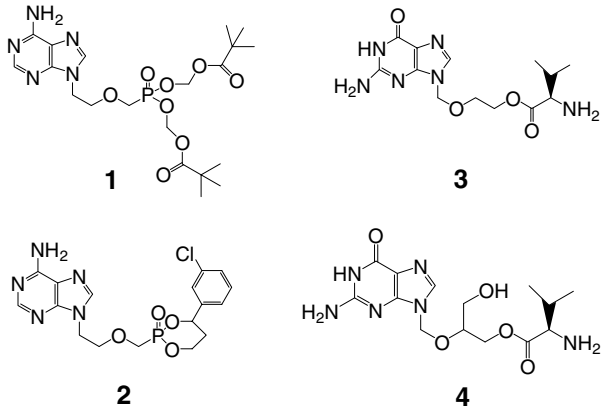
Hepatitis B virus (HBV) can cause both acute and chronic infection. Of the approximately two billion people who have been infected with HBV worldwide, an estimated 350–400 million are chronically infected, with 0.5–1.2 million deaths annually from the resulting cirrhosis, liver failure, and hepatocellular carcinoma.[1] Only a few drugs are currently approved by the FDA for the treatment of chronic HBV infection. They include lamivudine, adefovir dipivoxil, and entecavir. Lamivudine has a low sustained response rate, and drug resistance limits its efficacy.[2] Adefovir dipivoxil (**1**), an ester prodrug of PMEA, has potent in vitro and in vivo activity against HBV. In particular, it has shown impressive ability to suppress replication of HBV resistance to lamivudine, emtricitabine, and famcicloir.[2] However, dose-limiting nephrotoxicity and its potential of releasing toxic formaldehyde and pivalic acid are its primary limitations.[3,4] In order to optimize cellular uptake and antiviral activity, and to reduce cytotoxicity of PMEA, several prodrugs such as bis(SATE)-PMEA,[5] *cyclo* Sal-PMEA,[6] and Hep-direct prodrug remofovir (**2**)[7] have been reported. These new prodrugs have improved the pharmacodynamics and pharmacokinetics of PMEA, but their obvious weakness of instability and cytotoxicity[6,7] makes the development of newer and more effective prodrug strategies imperative.

An active transporting mechanism has been applied in the designing of nucleoside L-amino acid ester prodrugs such as L-valacyclovir (**3**)[8] and L-valganciclovir (**4**).[9] These prodrugs significantly enhance oral bioavailability and anti-viral activity of the parent drugs. This property has been ascribed to their advantages of being able to be efficiently delivered by the human peptide transporter 1(hPepT1).[10,11] Unlike nucleoside agents, acyclic nucleoside phosphonates have the advantage of skipping the requisite first phosphorylation step to reach their active metabolic form. We reasoned that incorporation of appropriate amino acids into the PMEA would produce a new class of prodrugs with improved anti-HBV activity, bioavailability, and reduced cytotoxicity. To our best knowledge, the L-amino acid ester prodrug strategy has not yet been reported in the case of acyclic nucleoside phosphonates. In this article, we report our efforts related to the synthesis of a series of novel bis(L-amino acid) esters

Keywords: Adefovir; Anti-HBV nucleotide; Amino acid; Prodrug.
* Corresponding author. Tel.: +86 21 50806786; e-mail: ysyang@mail.shcnc.ac.cn

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.10.021

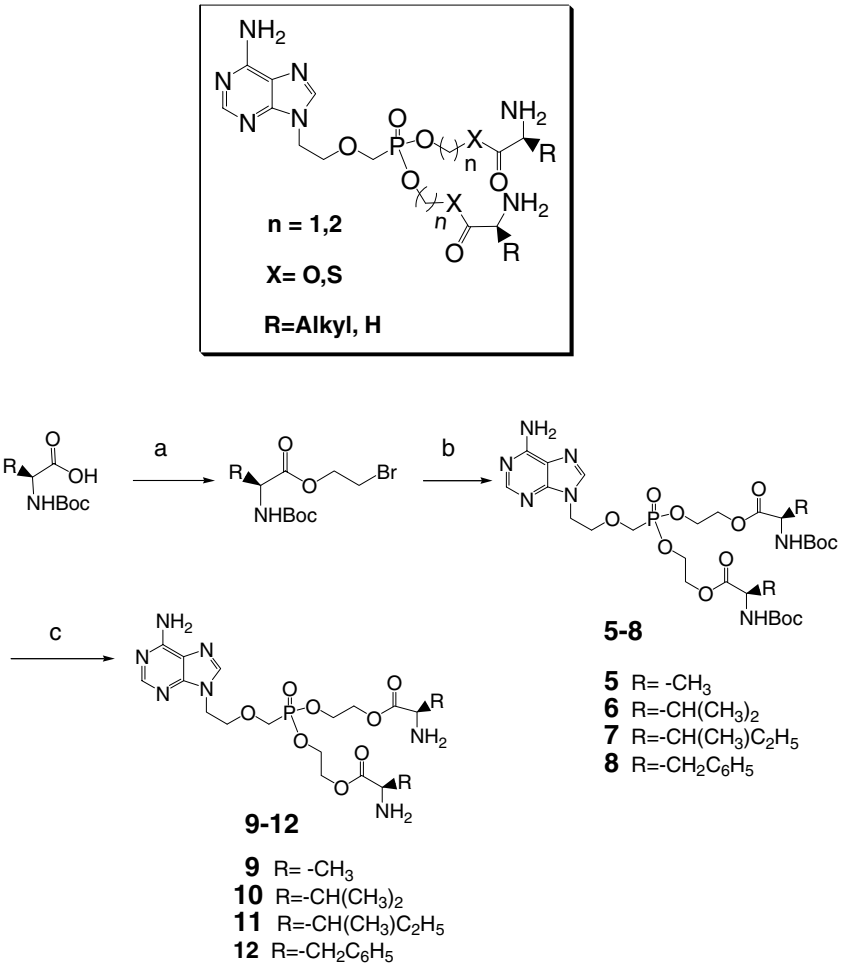
prodrugs of PMEA as anti HBV agents, and evaluations of their anti-HBV activity in HepG2 2.2.15 cells, resulting in the discovery of a promising prodrug **11** with more potent anti-HBV activity, lower cytotoxicity, and higher selective index (SI) than those of adefovir dipivoxil.



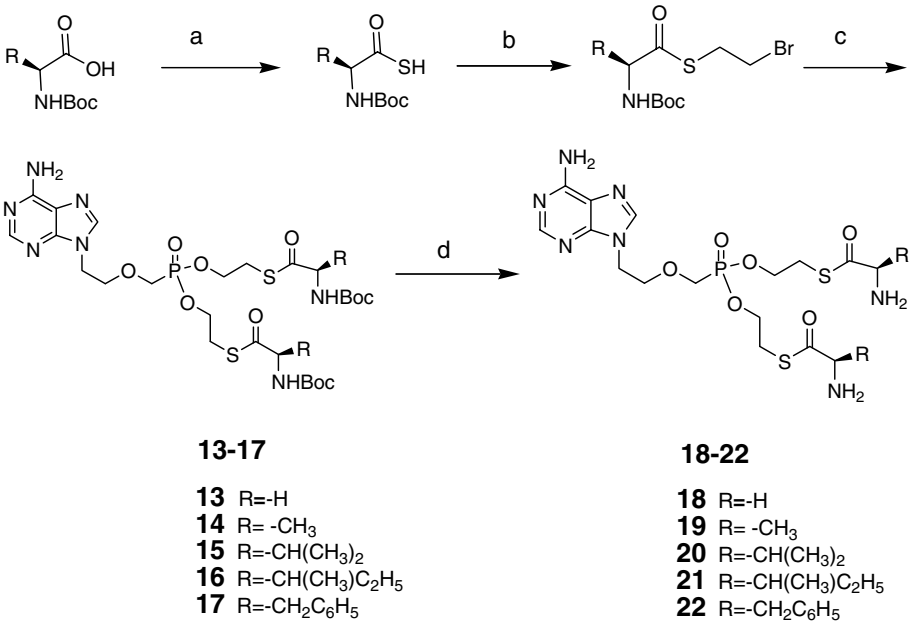
Synthesis of these novel compounds was carried out in a straightforward manner. Thus condensation of *N*-Boc-L-amino acids with 2-bromoethanol in the presence of DCC and DMAP yielded 2-bromoethyl ester of *N*-Boc-L-amino acids,[12] which were coupled with PMEA using *N*,*N*′-dicyclohexyl-4-morpholine carboxamidine (DCMC) as an acid scavenger in anhydrous DMF to yield compounds **5**–**8**. Removal of the *N*-Boc-protecting group in 4*N* hydrochloride-1,4-dioxane solution afforded compounds **9**–**12** as hydrochloride salts (Scheme 1).

The procedure for synthesis of compounds **18**–**22** is outlined in Scheme 2. After activation by isobutyl chloroformate (IBCF), *N*-Boc-L-amino acids reacted with H_2S at −20 to −10 °C to give *N*-Boc-L-amino monothioacids, which then reacted with 1,2-dibromoethane, using NaH as base, to give 2-bromoethyl ester of *N*-Boc-L-amino monothioacids.[13] The ester then reacted with PMEA to afford compounds **13**–**17**. Deprotection of the *N*-protecting group via a procedure similar to that in Scheme 1 generated the desired compounds **18**–**22**.

Condensation of chloromethyl chlorosulfate with *N*-Boc-protected L-amino acids in a dichloromethane–water mixture using *n*-Bu_4NHSO_4 as phase-transfer catalyst[14] smoothly afforded chloromethyl ester of *N*-Boc-L-amino acid. The resulting ester then reacted with PMEA to yield compounds **23**–**25**. Removal of



Scheme 1. Reagents and conditions: (a) 2-bromoethanol, DCC, DMAP, CH_2Cl_2, rt; (b) PMEA, DCMC, DMF, rt–80 °C; (c) 4 N HCl-1,4-dioxane solution, rt.



13-17

13 R=-H
14 R= -CH_3
15 R=-$CH(CH_3)_2$
16 R=-$CH(CH_3)C_2H_5$
17 R=-$CH_2C_6H_5$

18-22

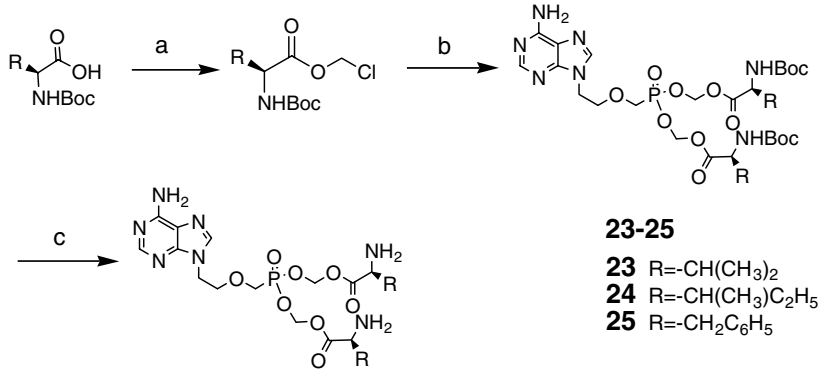
18 R=-H
19 R= -CH_3
20 R=-$CH(CH_3)_2$
21 R=-$CH(CH_3)C_2H_5$
22 R=-$CH_2C_6H_5$

Scheme 2. Reagents and conditions: (a) H_2S, IBCF, NMM, THF, 1 N HCl, −20 to −10 °C; (b) 1,2-dibromoethane, NaH, THF, −20 °C to rt; (c) PMEA, DCMC, DMF, rt to 80 °C; (d) 4N HCl-1,4-dioxane solution, rt.

N-Boc-protecting group as before gave the desired compounds **26**–**28** (Scheme 3).

The structure of the target compounds was confirmed by NMR (^1H, ^{13}C, ^{31}P), HRMS, and elemental analysis.

The synthesized compounds were evaluated for their inhibitory effect on the replication of HBV in HepG2 2.2.15 cell lines according to the reference.[15] Viral DNA recovered from the secreted particles in cultured 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 were analyzed using Icycler IQ 3.0. EC_{50}, CC_{50}, and SI of these compounds are reported in Table 1. Adefovir dipivoxil and lamivudine were used as positive controls. As indicated in Table 1, all synthesized compounds demonstrated potent anti-HBV activity with EC_{50} values of 0.0952–12.5 μM.[16] Compounds **11**, **12**, **21**, **22**, **26**, and **27** exhibited more potent anti-HBV activity than adefovir dipivoxil with EC_{50} values of 0.095, 0.31, 0.21, 0.21, 0.30, and 0.34 μM, respectively. The most active one, compound **11**, was almost as potent as lamivudine and five times more potent than adefovir dipivoxil. The SI value of



23-25

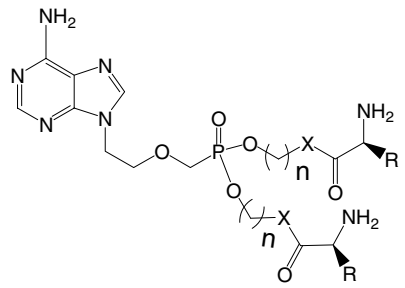
23 R=-$CH(CH_3)_2$
24 R=-$CH(CH_3)C_2H_5$
25 R=-$CH_2C_6H_5$

26-28

26 R=-$CH(CH_3)_2$
27 R=-$CH(CH_3)C_2H_5$
28 R=-$CH_2C_6H_5$

Scheme 3. Reagents and conditions: (a) chloromethyl chlorosulfate, *n*-Bu_4NHSO_4, $NaHCO_3$, CH_2Cl_2–H_2O, rt; (b) PMEA, DCMC, DMF, rt to 80 °C; (c) 4*N* HCl-1,4-dioxane solution, rt.

Table 1. Anti-HBV evaluation of bis(L-amino acid)ester prodrugs of PMEA



Compound[a]	R	X	*n*	EC_{50}[b] (μM)	CC_{50}[c] (μM)	SI[d]	*c*log *P*[e]	$t_{1/2}$[f] (min)
9	Methyl	O	2	12.5	1840	146	−1.75	ND
10	Isopropyl	O	2	1.31	2587	1974	0.100	120
11	2-Methypropyl	O	2	0.0952	6636	69523	1.16	270
12	Benzyl	O	2	0.311	2590	8354	1.08	15
18	H	S	2	10.2	259	25	−2.16	ND
19	Methyl	S	2	1.56	2415	1548	−1.54	ND
20	Isopropyl	S	2	0.752	28712	38167	0.310	ND
21	2-Methylpropyl	S	2	0.211	3409	16129	1.37	ND
22	Benzyl	S	2	0.208	10984	52727	1.29	ND
26	Isopropyl	O	1	0.304	3378	11094	−0.460	ND
27	2-Methylpropyl	O	1	0.341	8560	25111	0.590	ND
28	Benzyl	O	1	0.659	15378	23312	0.510	ND
Adefovir dipivoxil	—	—	—	0.517	540	1044	1.39	45
Lamivudine	—	—	—	0.0807	230	2850	—	—

[a] Obtained as hydrochloride salts.
[b] EC_{50} (Concentrations of compounds achieving 50% inhibition of cytoplasmic HBV-DNA synthesis).
[c] CC_{50} (Concentrations of compounds required for 50% extinction of HepG2 2.2.15 cells).
[d] SI (selective index CC_{50}/EC_{50}).
[e] Calculated using Chemdraw ultra 8.0 program.
[f] In human plasma, and 'ND' indicates not determined.

compound **11** was over 60 times and 24 times higher than those of adefovir dipivoxil and lamivudine, respectively. The SAR research showed that the compounds with the larger bulk of the amino acid alkyl group, **11**, **12**, **21**, and **22**, showed higher anti-HBV activity and SI than that of the corresponding compounds **9**, **10**, **18**, and **19** with the smaller bulk of the amino acid alkyl group. In addition, respective comparisons of **9**, **10** with **19**, **20**, and **11**, **12** with **21**, **22** indicated that substitution of the amino acid esters with their corresponding thioesters greatly improve the anti-HBV activity and SI value of the compounds with a smaller bulk of amino acid alkyl moiety. However, similar trend was not observed for compounds with a larger bulk of amino acid alkyl moiety. Further study of compounds **10**, **11**, **12**, **26**, **27**, and **28** revealed that structure modification of amino acids moiety had less effect on the anti-HBV activity and SI of the bis(L-amino acid) acetal esters of PMEA.

All synthesized compounds, except for compound **18**, had much lower cytotoxicities compared with those of adefovir dipivoxil and lamivudine. Compounds **11**, **20**, **22**, **27**, and **28** exhibited preferable cytotoxicity with CC_{50} values of 6636, 28712, 10984, 8560, and 15378 μM. L-Amino acid ester prodrugs possessed significantly reduced cell toxicity and higher anti-HBV activity compared with those of adefovir dipivoxil, suggesting that replacement of bis (pivaloyloxy methyl) ester with L-amino acid ester is crucial to antiviral activity and cell toxicity. These results suggested that bis(L-amino acid) ester prodrugs of PMEA contrast with many prodrugs of PMEA, which gained antiviral potency and were accompanied by a proportional enhancement of cytotoxicity.

The usefulness of the prodrugs of PMEA should depend not only on the stability of the prodrug for its transport across the cell membrane but also upon its reversion to the parent compound intracellulary, especially in the virally infected cells. The half-life ($t_{1/2}$) of hydrolysis of the esters was therefore determined in human plasma.[17] Data in Table 1 indicated that compounds **10**, **11**, and **12** were susceptible to the action of plasma esterases with $t_{1/2}$ ranging from 15 to 270 min. The results suggested that compound **11** was more stable than adefovir dipivoxil with $t_{1/2}$ of 270 min, a length over six times as long as that of adefovir dipivoxil ($t_{1/2}$ 45 min).

In summary, we described a novel class of L-amino acid ester prodrugs of PMEA as an anti-HBV agent with very potent activity and reduced toxicity. The results suggested that L-amino acid ester strategy has significant potential in the acyclic nucleoside phosphonate prodrug design. Further biological and SAR studies of this new class of anti-HBV agents are ongoing in our laboratories.

References and notes

1. Lavanchy, D. *J. Viral Hepat.* **2004**, *11*, 97.
2. Chen, Z.; Zheng, M. *Expert Opin. Ther. Patents* **2005**, *15*, 1027.
3. Wang, J.-T.; Choi, J.-R. *Drugs Future* **2004**, *29*, 163.
4. Douglsh, H. S.; Kevin, T. B.; Sanjay, K. N. *Am. J. Physiol. Renal. Physiol.* **2001**, *281*, F197.
5. Benzaria, S.; Pelicano, H.; Johnson, R.; Maury, G.; Imbach, J.-L.; Aubertin, A.-M.; Obert, G.; Gosselin, G. *J. Med. Chem.* **1996**, *39*, 4958.
6. Meris, C.; Gorbig, U.; Muller, C.; Balzarini, J. *J. Med. Chem.* **2005**, *48*, 8079.
7. 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.
8. Friedrichsen, G. M.; Chen, W.; Begtrup, M.; Lee, C. P.; Smith, P. L.; Borchardt, R. T. *Eur. J. Pharm. Sci.* **2002**, *16*, 1.
9. Sugawara, M.; Hung, W.; Fei, Y. J.; Leibach, V. G.; Ganapathy, M. E. *J. Pharm. Sci.* **2000**, *89*, 781.
10. Carsten, U. N.; Birger, B.; Flemming, S. J.; Sven, F.; Bente, S. *Expert Opin. Ther. Patents* **2002**, *12*, 1329.
11. Isabel, R. A.; Hannelore, D. *Trends Pharmacol. Sci.* **2003**, *23*, 434.
12. Chaim, G.; Yakir, K. *Tetrahedron Lett.* **1979**, *20*, 3811.
13. Ludwig, B.; Suresh, K. S.; Ghosh, B. K. *J. Org. Chem.* **1965**, *30*, 949.
14. Naoyuki, H.; Mitsuya, H.; Takashi, T.; Takayuki, K.; Tomiki, H.; Kenji, T. *Synth. Commun.* **1994**, *24*, 767.
15. Mary, A. S.; Cheng, M. L.; George, A. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 1005.
16. Experimental details and characterization data.

(a) General. Tetrahydrofuran was dried by distillation from sodium/benzophenone. *N*,*N*-Dimethylformamide was dried by distillation in vacuo from calcium hydride. Other commercially available chemicals and solvents were used without further purification. NMR spectra were recorded on a Varian Mercury-400 MHz spectrometer. Low resolution mass spectra were obtained using a Finnigan MAT 95 mass spectrometer, and high resolution mass spectra were obtained using a Kratos MS80 mass spectrometer. Elemental analysis for carbon, hydrogen, and nitrogen was determined on a Vario EL elemental analyzer. Flash chromatography was carried out on silica gel (200–300 mesh), and chromatographic solvent proportions are expressed on a volume:volume basis.

(b) Detail synthesis and spectroscopic data of compound **11**.

Procedure (1): DCC (2.27 g, 0.011 mol) was added slowly to a solution of *N*-Boc-L-isoleucine (2.31 g, 0.01 mol), 2-bromoethanol (1.24 g, 0.01 mol) and DMAP (1.23 g, 0.01 mol) in CH_2Cl_2 (30 mL) at room temperature. The reaction mixture was stirred at room temperature for 24 h. The *N*,*N*′-dicyclohexyl urea was filtered off, and the filtrate was evaporated to leave a residue which was dissolved in acetic ether, cooled at −10 °C for 6 h, and the resultant solid was filtered off. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography on silica gel with eluent (10:1 petroleum ether/acetic ether) to get (2*S*,3*S*)-2-bromoethyl 3-methyl-2-(pivalamido) pentanoate as a colorless oil (2.48 g, 76.8%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.99 (d, J = 8.15 Hz, 1H, NHCO), 4.48–4.37 (m, 2H, OCH_2CH_2Br), 4.28 (m, 1H, COCH), 3.52 (t, J = 6.07 Hz, 2H, OCH_2CH_2Br), 1.87 (q, J = 4.71 Hz, 1H, C*H*(Me)), 1.42 (s, 9H, $C(Me)_3$), 1.22–1.27 (m, 2H, CH_2CH_3), 0.94–0.87 (m, 6H, 2× CH_3). MS (EI) *m*/*z* (%): 323 (M^+, 15), 57 (100).

Procedure (2): to a 100 mL two-necked flask were added PMEA (0.10 g, 0.36 mmol), *N*,*N*′-dicyclohexyl-4- morpholine carboxamidine (0.21 g, 0.72 mmol), (2S, 3S)-2-bromoethyl 3-methyl-2-(pivalamido) pentanoate (0.58 g, 1.80 mmol), and anhydrous DMF (15 mL). The heterogeneous mixture was stirred at room temperature for 24 h then at 80 °C 5 h. The insolubles were filtered off, and the filtrate was concentrated in vacuo. The residue was partitioned between 1% citric acid aqueous solution (5 mL) and acetic ether (5 mL), the organic layer was separated, and the aqueous layer was extracted with acetic ether (2× 5 mL). The combined organic extracts were washed with water (5 mL) then with brine (5 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel using a mixture of CH_2Cl_2 and MeOH (25:1) to obtain compound **7** (0.052 g, 18.35%) as a white foam solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.32 (s, 1H, 8-H), 7.96 (s, 1H, 2-H), 5.87 (br s, 2H, NH_2), 5.37 (d, J = 7.86 Hz, 1H, NHCO), 5.21 (d, J = 7.86 Hz, 1H, NHCO), 4.39 (2H, t, J = 5.32 Hz, NCH_2), 4.10–4.37 (m, 10H, 2× CH_2CH_2OP, 2× CH_2CH_2OP, 2× COCH), 3.93 (t, J = 5.26 Hz, 2H, OCH_2CH_2N), 3.81 (d, J = 8.41 Hz, 2H, OCH_2P), 1.84 (m, 2H, 2× C*H*(Me)), 1.42 (s, 18H, 2× $C(Me)_3$), 1.05–1.26 (m, 4H, 2× CH_2CH_3), 0.85–0.91 (m, 12H, 4× CH_3). MS (EI) *m*/*z* (%): 787 (M^+, 12), 163 (100). Anal. Calcd for $C_{34}H_{58}N_7O_{12}$: C, 51.84; H, 7.36; N, 12.45. Found: C, 51.79; H, 7.59; N, 12.16.

Procedure (3): to a 10 mL two-necked flask were added compound **7** (0.21 g, 0.27 mmol), 1,4-dioxane (1 mL), and 4 N hydrochloride-1, 4-dioxane solution (1.13 mL, 4.53 mmol). The mixture was stirred at room temperature for 4 h. After solvent was removed under vacuum, the resultant residue was dissolved in ethanol (0.5 mL), and the solution was stirred at 0 °C for 15 min. Ethyl ether (2.0 mL) was added to the solution and further stirred at 0 °C for 30 min. The resultant product was collected by filtration, washed quickly with ethyl ether, and dissolved in water (0.5 mL) dried by lyophilization to get a white foam of the compound **11** as hydrochloride salts (0.15 g, 93.15%). $[\alpha]_D^{20}$ + 14.2 (*c* 0.60, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 8.38 (s, 1H, 8-H), 8.42 (s,1H, 2-H), 4.55 (t, J = 5.08 Hz, 2H, NCH_2), 4.38–4.53 (m, 4H, 2× CH_2CH_2OP), 4.21–4.36 (m, 4H, 2× CH_2CH_2OP), 4.06–4.11 (m, 2H, 2× COCH), 3.98–4.02 (m, 4H, OCH_2P, OCH_2CH_2N), 2.00–2.02 (m, 2H, 2× C*H*(Me)), 1.34 and 1.57 (m, 4H, 2× CH_2CH_3), 0.96–1.06 (m, 12H, 4× CH_3). ^{13}C NMR (100 MHz, CD_3OD): δ 170.3 (2C), 152.2, 151.0, 146.6, 145.8, 120.2, 72.7, 66.6 (2C), 66.1 (2C), 65.1, 58.8 (2C), 45.5, 38.3 (2C), 27.2 (2C), 15.0 (2C), 12.6 (2C). ^{31}P (400 MHz, CD_3OD): δ 24.0. MS (EI) *m*/*z* (%): 587 (M^+, 11), 86 (100). HRMS (EI): Calcd for $C_{24}H_{42}N_7O_8P$: 587.2832. Found: 587.2840 (M^+). Anal. Calcd for $C_{24}H_{42}N_7O_8P.3HCl.2H_2O$: C, 39.31; H, 6.68; N, 13.37. Found: C, 39.17; H, 6.91; N, 13.16.

(c) Spectroscopic data for other newly synthesized compounds.

Compound **9**: $[\alpha]_D^{20}$ + 1.4 (*c* 0.350, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 8.42 (s, 1H, 8-H), 8.38 (s, 1H, 2-H), 4.55 (t, J = 5.08 Hz, 2H, NCH_2), 4.43 (t, J = 4.41 Hz, 4H, 2× CH_2CH_2OP), 4.32 (m, 4H, 2× CH_2CH_2OP), 4.18 (q, J = 7.14 Hz, 2H, 2× COCH), 4.01–4.04 (m, 4H, OCH_2P, OCH_2CH_2N), 1.56 (d, J = 7.28 Hz, 6H, 2× CH_3). MS (EI) *m*/*z* (%): 503 (M^+, 0.5), 143 (100).

Compound **10**: $[\alpha]_D^{20}$ + 11 (*c* 0.286, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 8.41 (s, 1H, 8-H), 8.36 (s, 1H, 2-H), 4.54 (t, J = 4.76 Hz, 2H, NCH_2), 4.40–4.47 (m, 4H, 2× CH_2CH_2OP), 4.31 (m, 4H, 2× CH_2CH_2OP), 4.19 (m, 2H, 2× COCH), 3.98–4.05 (m, 4H, OCH_2P, OCH_2CH_2N),

2.30–2.33 (m, 2H, 2× C*H*(Me)$_2$), 1.13 (d, J = 6.96 Hz, 6H, 2× CH_3), 1.06 (d, J = 7.06 Hz, 6H, 2× CH_3). ^{31}P (400 MHz, CD_3OD): δ 27.1. MS (EI) *m*/*z* (%): 559 (M^+, 0.7), 72 (100). Anal. Calcd for $C_{22}H_{38}N_7O_8P.3HCl.2H_2O$: C, 37.47; H, 6.38; N, 13.91. Found: C, 37.29; H, 6.33; N, 13.79.

Compound **12**: $[\alpha]_D^{20}$ + 7.2 (*c* 0.304, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 8.37 (s, 1H, 8-H), 8.35 (s, 1H, 2-H), 7.25–7.36 (m, 10H, ArH), 4.51 (t, J = 5.09 Hz, 2H, NCH_2), 4.27–4.41 (m, 8H, 2× CH_2CH_2OP, 2× CH_2CH_2OP), 4.22 (m, 2H, 2× COCH), 3.98–4.01 (m, 4H, OCH_2P, OCH_2CH_2N), 3.16–3.28 (m, 4H, 2× CH_2Ph). MS (EI) *m*/*z* (%): 655 (M^+, 1.2),178 (100).

Compound **18**: ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.49 (s, 1H, 8-H), 8.42 (s, 1H, 2-H), 4.54 (t, J = 5.12 Hz, 2H, NCH_2), 4.31 (m, 4H, 2× CH_2CH_2OP), 3.97 (d, J = 7.82 Hz, 2H, OCH_2P), 3.92 (m, 2H, OCH_2CH_2N), 3.85 (s, 4H, 2× $COCH_2$), 3.26 (m, 4H, 2 × SCH_2CH_2). ^{13}C NMR (100 MHz, CD_3OD): δ 169.1 (2C), 152.3, 150.9, 146.6, 146.0, 120.1, 72.7, 66.5 (2C), 66.2, 56.7 (2C), 41.6, 31.3 (2C). MS (ESI) *m*/*z* (%): 508.3 (MH^+, 100). HRMS (ESI): Calcd for $C_{16}H_{27}N_7O_6PS_2$: 508.1124. Found: 508.1139 (MH^+).

Compound **19**: $[\alpha]_D^{20}$ + 2.1 (*c* 0.270, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 8.43 (s, 1H, 8-H), 8.38 (s, 1H, 2-H), 4.55 (t, J = 5.13 Hz, 2H, NCH_2), 4.34 (q, J = 6.97 Hz, 2H, 2× COCH), 4.16–4.20 (m, 4H, 2× CH_2CH_2OP), 4.02–3.98 (m, 4H, OCH_2P, OCH_2CH_2N), 3.29 (m, 4H, 2× SCH_2CH_2), 1.61 (d, J = 6.96 Hz, 6H, 2× CH_3). ^{13}C NMR (100 MHz, CD_3OD): δ 198.4 (2C), 152.7, 151.1, 146.5, 146.3, 120.1, 72.7, 66.5 (2C), 64.9, 56.9 (2C), 45.5, 30.9 (2C), 18.1 (2C). MS (ESI) *m*/*z* (%): 536.1 (MH^+, 100). HRMS (ESI): Calcd for $C_{18}H_{30}N_7O_6PS_2Na$: 558.1334. Found: 558.1359 (MNa^+).

Compound **20**: $[\alpha]_D^{20}$ + 16.1 (*c* 0.267, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 8.43 (s, 1H, 8-H), 8.39 (s, 1H, 2-H), 4.58 (t, J = 4.69 Hz, 2H, NCH_2), 4.14–4.28 (m, 6H, 2× CH_2CH_2OP, 2× COCH), 3.98–4.02 (m, 4H, OCH_2P, OCH_2CH_2N), 3.31 (t, J = 6.26 Hz, 4H, 2× SCH_2CH_2), 2.26–2.41 (m, 2H, 2× C*H*(Me)$_2$), 1.12 (d, J = 6.85 Hz, 6H, 2× CH_3), 1.04 (d, J = 7.04 Hz, 6H, 2× CH_3). MS (ESI) *m*/*z* (%): 592.1 (MH^+, 100).

Compound **21**:$[\alpha]_D^{20}$ + 28 (*c* 0.208, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 8.41 (s, 1H, 8-H), 8.36 (s, 1H, 2-H), 4.56 (t, J = 5.12 Hz, 2H, NCH_2), 4.18–4.25 (m, 6H, 2× CH_2CH_2OP, 2× COCH), 3.91–4.01 (m, 4H, OCH_2P, OCH_2CH_2N), 3.24 (m, 4H, 2× SCH_2CH_2), 2.07 (m, 2H, 2× C*H*(Me)), 1.24 and 1.61 (m, 4H, 2× CH_2CH_3), 0.97–1.08 (m, 12H, 4× CH_3). ^{13}C NMR (100 MHz, CD_3OD): δ 197.2 (2C), 152.2, 151.4, 146.4, 142.4, 119.7, 72.7, 66.6 (2C), 65.4, 57.7 (2C), 45.5, 38.8 (2C), 31.1 (2C), 26.4 (2C), 15.6 (2C), 12.5 (2C). MS (ESI) *m*/*z* (%): 620.2 (MH^+, 100). HRMS (ESI): Calcd for $C_{24}H_{42}N_7O_6PS_2Na$: 642.2273. Found: 642.2319 (MNa^+).

Compound **22**: ^{1}H NMR (400 MHz, CD_3OD): δ 8.38 (s, 1H, 8-H), 8.35 (s, 1H, 2-H), 7.28–7.38 (m, 10H, ArH), 4.56 (m, 2H, NCH_2), 4.05–4.18 (m, 6H, 2× CH_2CH_2OP, 2× COCH), 3.96 (t, J = 4.59 Hz, 2H, OCH_2CH_2N), 3.91 (d, J = 8.25 Hz, 2H, OCH_2P), 3.24 (m, 4H, 2× SCH_2CH_2), 3.13–3.19 (m, 4H, 2× CH_2Ph). ^{13}C NMR (100 MHz, CD_3OD): δ 197.9 (2C), 152.9, 151.6, 146.4, 142.4, 135.6 (2C), 131.4 (4C), 131.0 (4C), 129.8 (2C), 120.2, 72.7, 66.3 (2C), 65.1, 57.2 (2C), 45.4, 39.1 (2C), 31.0 (2C). MS (ESI) *m*/*z* (%): 688.2 (MH^+, 100). HRMS (ESI): Calcd for $C_{30}H_{39}N_7O_6PS_2$: 688.2141. Found: 688.2161 (MH^+).

Compound **26**: $[\alpha]_D^{20}$ + 7.4 (*c* 0.253, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 8.42 (s, 1H, 8-H), 8.37 (s, 1H, 2- H), 5.65–5.91 (m, 4H, 2× OCH_2OP), 4.57 (t, J = 4.89 Hz, 2H, NCH_2), 4.11 (m, 2H, 2× COCH), 4.08 (d, J = 8.22 Hz, 2H, OCH_2P), 4.02 (t, J = 4.89 Hz, 2H, OCH_2CH_2N), 2.14–2.21 (m, 2H, 2× C*H*(Me)$_2$), 1.10 (d, J = 5.48 Hz, 6H, 2× CH_3), 1.07 (d, J = 5.28 Hz, 6H, 2× CH_3). MS (ESI) *m*/*z* (%): 532.1 (MH^+, 100). Anal. Calcd for $C_{20}H_{34}N_7O_8P{\cdot}3HCl{\cdot}1H_2O$: C, 36.45; H, 5.92; N, 14.88. Found: C, 36.17; H, 6.18; N, 15.16.

Compound **27**: ^{1}H NMR (400 MHz, CD_3OD): δ 8.41 (s, 1H, 8-H), 8.36 (s, 1H, 2-H), 5.73–5.89 (m, 4H, 2× OCH_2OP), 4.56 (t, J = 5.13 Hz, 2H, NCH_2), 4.18 (m, 2H, 2× COCH), 4.08 (d, J = 8.07 Hz, 2H, OCH_2P), 4.01 (t, J = 5.14 Hz, 2H, OCH_2CH_2N), 2.05 (m, 2H, 2× C*H*(Me)), 1.32 and 1.61 (m, 4H, 2× CH_2CH_3), 0.97–1.06 (m, 12H, 4× CH_3). ^{31}P (400 MHz, CD_3OD): δ 23.6. ^{13}C NMR (100 MHz, CD_3OD): δ 169.6 (2C), 152.1, 151.0, 146.6, 145.7, 120.0, 84.6 (2C), 72.8, 66.9, 58.7 (2C), 45.4, 38.2 (2C), 27.1 (2C), 15.4 (2C), 12.5 (2C). MS (ESI) *m*/*z* (%): 560.2 (MH^+, 100). HRMS (ESI): Calcd for $C_{22}H_{39}N_7O_8P$: 560.2519. Found: 560.256 (MH^+).

Compound **28**: $[\alpha]_D^{20}$ + 12.5 (*c* 0.277, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 8.35 (s, 1H, 8-H), 8.34 (s, 1H, 2-H), 7.37–7.25 (m, 10H, ArH), 5.85–5.70 (m, 4H, 2× OCH_2OP), 4.53–4.46 (m, 4H, NCH_2, 2× COCH), 4.09 (d, J = 7.70 Hz, 2H, OCH_2P), 4.01 (t, J = 5.13 Hz, 2H, OCH_2CH_2N), 3.21–3.15 (m, 4H, 2× CH_2Ph). ^{13}C NMR (100 MHz, CD_3OD): δ 169.7 (2C), 152.4, 151.3, 146.5, 146.0, 135.8 (2C), 131.1 (4C), 130.8 (4C) 129.5 (2C), 119.6, 84.5 (2C), 72.6, 67.3, 58.2 (2C), 45.5, 37.8 (2C). MS (ESI) *m*/*z* (%): 628.2 (MH^+, 100). HRMS (ESI): Calcd for $C_{28}H_{35}N_7O_8P$: 628.2206. Found: 628.2217 (MH^+).

17. Agarwal, S. K.; Gogu, S. R.; Rangan, S. R. S.; Agrawal, K. C. *J. Med. Chem.* **1990**, *33*, 1505.