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Tetrahedron: *Asymmetry* 22 (2011) 1505–1511

Contents lists available at SciVerse ScienceDirect

Tetrahedron: *Asymmetry*

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

Synthesis of (*R*)- and (*S*)-β-hydroxyphosphonate acyclonucleosides: structural analogues of Adefovir (PMEA)

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ARTICLE INFO

Article history:
Received 20 June 2011
Accepted 11 August 2011
Available online 1 October 2011

ABSTRACT

A synthetic pathway to new acyclonucleoside phosphonates, as analogues of Adefovir, is described. The reduction of an acyclonucleoside β-ketophosphonate, readily available from the nucleobase and benzylacrylate, afforded a mixture of (*R*)- and (*S*)-β-hydroxyphosphonate derivatives which was resolved. The assignment of the absolute configuration was proposed on the basis of NMR studies and was supported by molecular modelling studies.

1. Introduction

Over the past 30 years, antiviral drug discoveries[1,2] have attracted the interest of both organic and medicinal chemists to propose new potential drugs for the treatment of viral infections, including DNA viruses and retroviruses. Among the various classes of antiviral drugs, acyclic nucleoside and nucleotide derivatives[3–5] have received considerable attention due to their ability to inhibit viral DNA polymerases and reverse transcriptases, which play key roles in the viral cycles.

Acyclic nucleoside phosphonates (ANPs) belong to a family of modified nucleotide analogues, in which the sugar moiety has been replaced by a functionalized acyclic chain linking the nucleobase at one end and the phosphonic acid group at the other. Therefore, ANPs are metabolically stable due to the presence of a phosphonate linkage (P–C) instead of a phosphoester (P–O), making these compounds resistant towards phosphatases. Furthermore, they do not require the first intracellular phosphorylation step which is often considered as a limiting-step and essential for the antiviral effect. Indeed, ANPs share their mechanism of action with other nucleoside analogues. Briefly, after two subsequent phosphorylation steps the ANP is converted to its corresponding triphosphate derivative, which can then interfere with nucleic acid biosynthesis as a DNA chain terminator.[5]

Amongst the antiviral agents that exhibited a broad spectrum of activity and bear a phosphonate group, adenine containing ANPs are well represented (Fig. 1). Prodrugs of Adefovir (PMEA, **1**) and Tenofovir (PMPA, **2**) have been approved for the treatment of hepatitis B and HIV infections,[6] respectively. Herein, we aimed to obtain novel ANPs structurally related to Adefovir (PMEA) which contained a carbinol moiety that replaced the oxygen atom of the phosphonomethoxyethyl chain (Fig. 1). The CH(OH) functionality is not an isostere for the C–O–C linkage, however, the resulting β-hydroxyphosphonate derivative may present some structural features (distance between the nucleobase and the phosphonate moiety, flexibility and so on) and the presence of the hydroxyl group may allow the formation of supplementary hydrogen bonds.

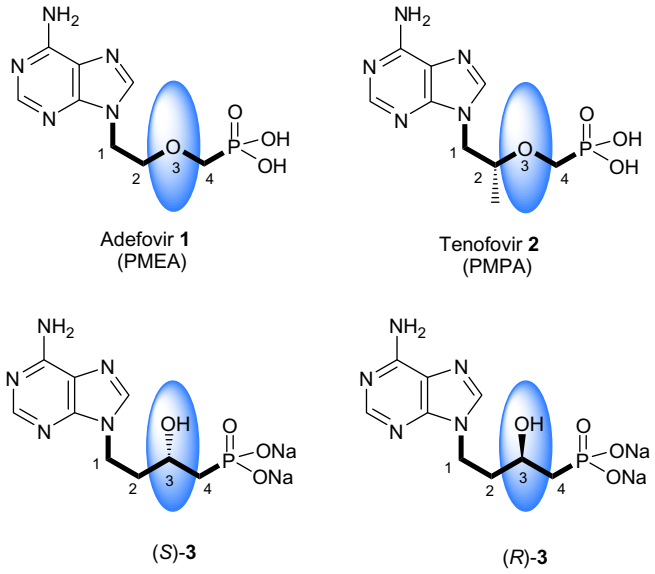

Figure 1. Examples of adenine containing ANPs (PMEA and PMPA) and the structures of targeted analogues.

Enantiomerically pure β-hydroxyphosphonate derivatives have received significant attention due to their potential biological

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doi:10.1016/j.tetasy.2011.08.010

activity.[7] Consequently, several synthetic routes using the corresponding β-ketophosphonates as intermediates have been reported and may involve stereoselective reduction,[8,9] enzymatic reduction,[10] as well as the use of chiral derivatizing agents.[7,11] Herein, we report the synthesis and resolution of enantiomerically pure (*R*)- and (*S*)-β-hydroxyphosphonate bearing adenine as a nucleobase, and the use of a chiral derivatizing agent such as (*S*)-methoxyphenyl acetic acid [(*S*)-MPA].

2. Results and discussion

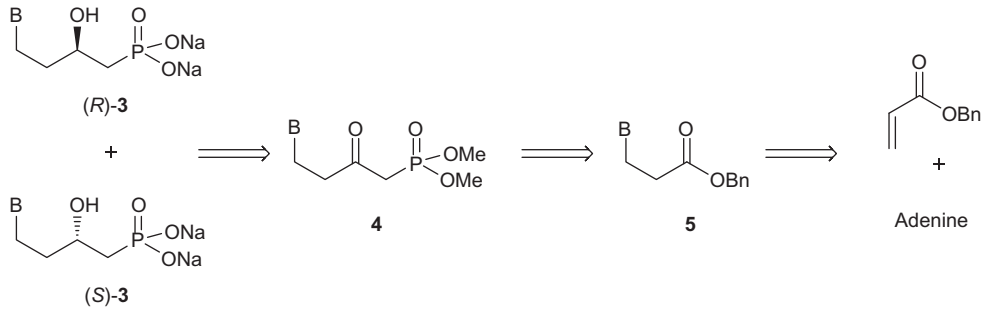
2.1. Chemistry

The retro-synthesis proposed for the preparation of the target β-hydroxyphosphonate derivatives (*R*)-**3** and (*S*)-**3** is shown in Scheme 1. Both isomers may be obtained via reduction of the corresponding β-ketophosphonate intermediate **4** and resolution of the diastereomeric pair with a suitable chiral derivatizing agent. The assignment of the absolute configuration would then be addressed using NMR spectroscopy. The key intermediate **4** could be prepared through treatment of **5** with the lithium salt of dimethylmethylphosphonate. Compound **5** would be available through the Michael addition of adenine to benzyl acrylate as the Michael acceptor.

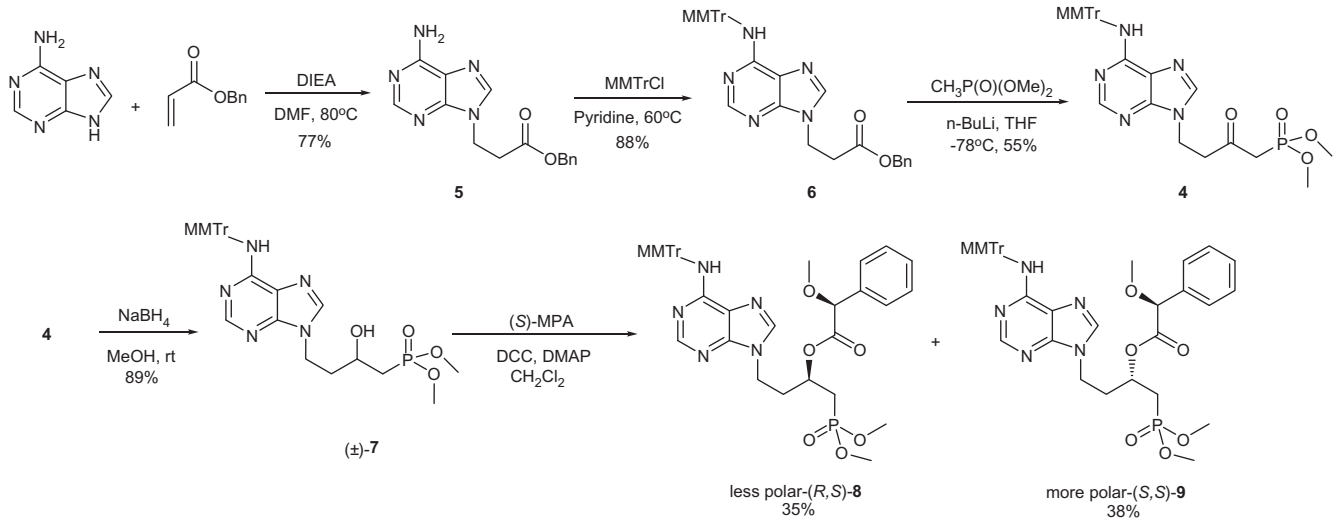
In the literature, such Michael additions suffer from low yields and a lack of regioselectivity, with the formation of both *N*-7 and *N*-9 substituted products. Initially the reaction was conducted in the presence of various bases such as K_2CO_3,[12] Cs_2CO_3, or catalytic Na in methanol.[13] Finally, diisopropylethylamine (DIEA) was selected as a suitable base, allowing the exclusive formation of the *N*-9 alkylated product in good yield (77%). It is noteworthy that this step could be easily scaled up to a 25 g batch. After removal of the solvent, the addition of ethyl acetate to the crude afforded pure **5** as a precipitate without further purification. The exclusive formation of the *N*-9 alkylated product was confirmed by NMR and UV absorption studies.[14]

The exocyclic amino group of **5** was next protected with a monomethoxytrityl (MMTr) group to give **6** in 88% yield (Scheme 2). The MMTr moiety was chosen as the nucleobase protecting group to enhance the solubility of intermediate **6** in the less polar solvents used in the next step of the synthesis (such as THF). Compound **6** was treated with an excess of the lithium salt of dimethylmethylphosphonate at −78 °C in THF and afforded the desired key intermediate β-ketophosphonate **4** in 55% yield. Reduction of β-ketophosphonate **4** was performed with $NaBH_4$ in methanol and led to a racemic mixture of β-hydroxy-phosphonate (±)-**7** in 89% yield.

With the racemic mixture of (±)-**7** in hand, we next resolved the (*R*)- and (*S*)-isomers using (*S*)-methoxyphenylacetic acid [(*S*)-MPA], a well known chiral derivatizing agent. Thus, coupling of (±)-**7** with (*S*)-MPA in the presence of 1,3-dicyclohexylcarbodiimide (DCC) and a catalytic amount of 4-*N*,*N*-(dimethylamino)pyridine (DMAP), in dichloromethane at room temperature, provided an equimolar (a 1:1 ratio was observed in the ^{31}P NMR spectra, Figure 2) mixture of the two mandelates [Scheme 2, (*R*,*S*)-**8** as less polar and (*S*,*S*)-**9** as more polar], which were separated by silica gel



Scheme 1. Proposed retrosynthesis for derivatives (*R*)-**3** and (*S*)-**3**.



Scheme 2. Synthesis of the β-ketophosphonate intermediate **4** and the diastereomeric pair: (*R*,*S*)-**8** and (*S*,*S*)-**9**.

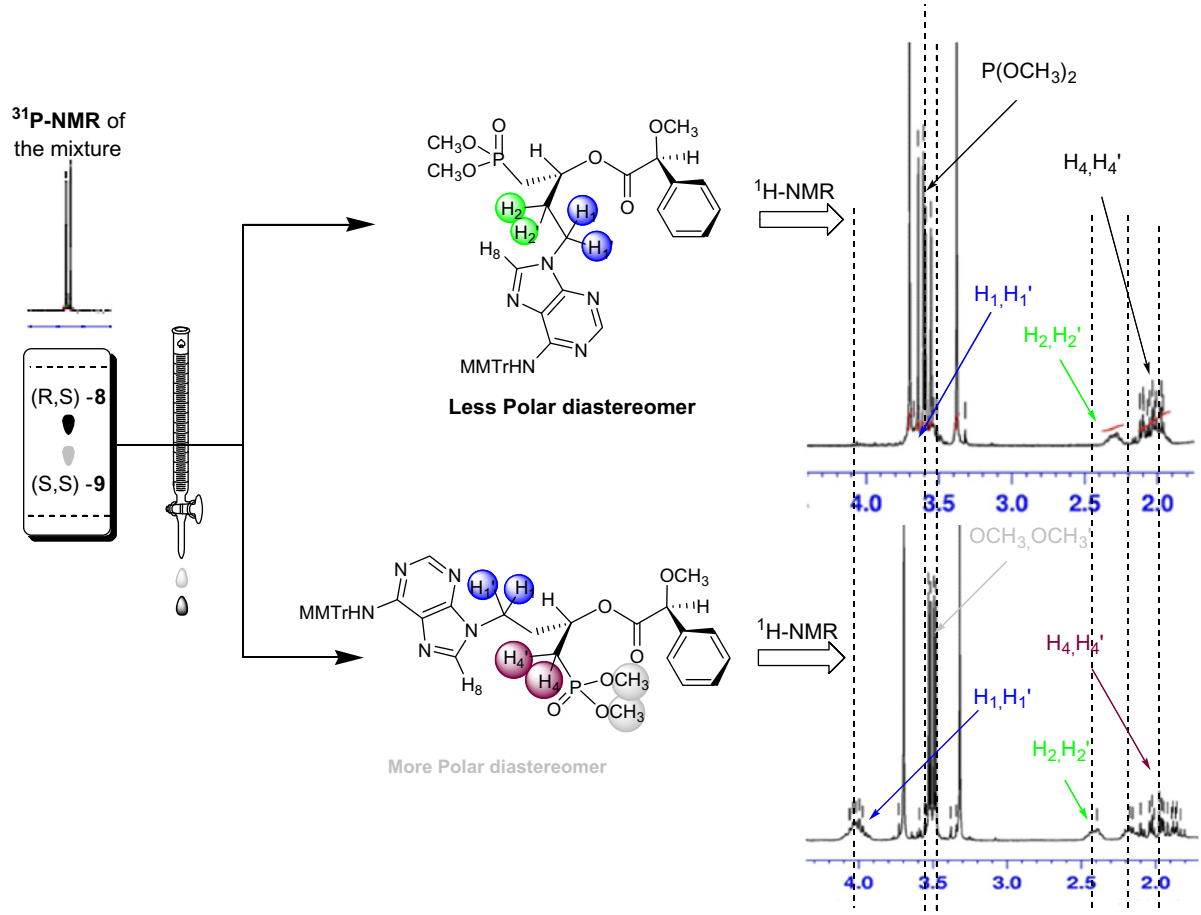
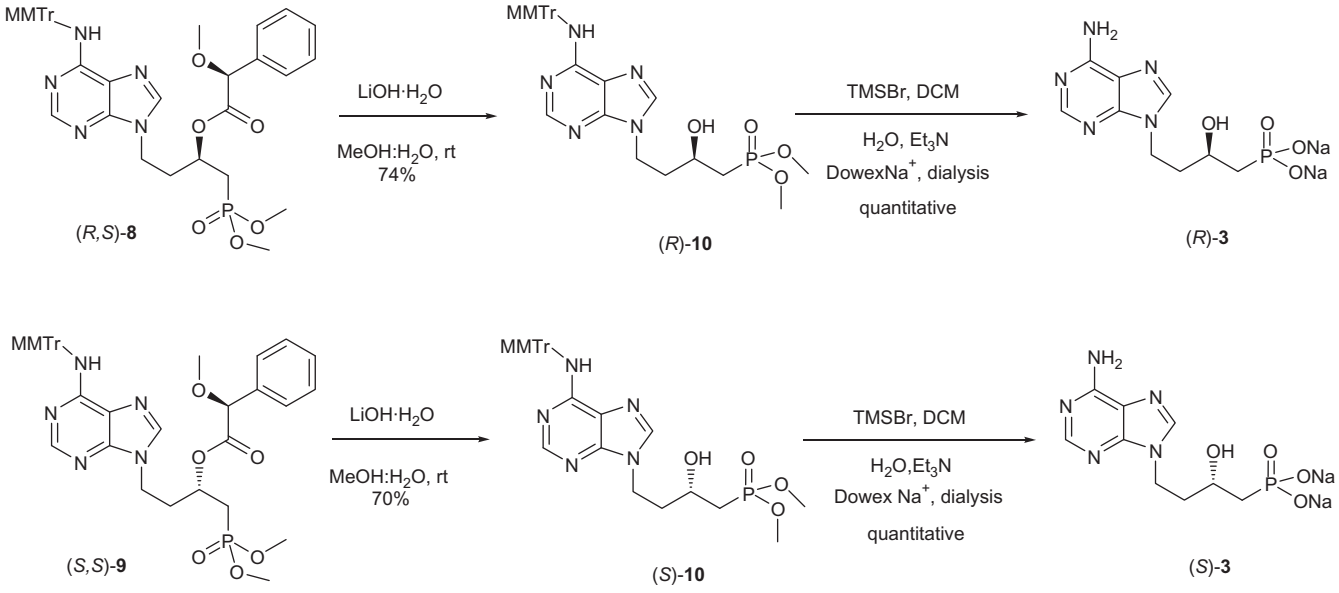


Figure 2. Assignment of the absolute configuration of the diastereomeric pair based on the NMR chemical shift differences due to the shielding effect exerted by the anisotropy cone of the phenyl ring from (*S*)-MPA.

column chromatography. The less polar and more polar diastereomers were obtained in 35% and 38% yields, respectively.

Once the absolute configuration was determined by NMR spectroscopy, the final steps of the synthesis were separately carried out for both derivatives (Scheme 3). Treatment of the mandelates (*R*,*S*)-**8** or (*S*,*S*)-**9** with LiOH·H_2O, in a mixture of MeOH/H_2O (8:2) at room temperature, led to enantiomerically pure (*R*)-**10** or (*S*)-**10** after flash column purification in 74% and 70% yields, respectively. Finally, the (*R*)- and (*S*)-**10** isomers were treated with an excess of TMSBr in dry dichloromethane at room temperature to



Scheme 3. Final steps of the synthesis of acyclic phosphonate analogues (*R*)- and (*S*)-**3**.

Table 1
Chemical shift data of the diastereomeric pair of β-hydroxyphosphonates **8** and **9**

Entry	Group	Less polar (δ_{RS})	More polar (δ_{SS})	$\Delta\delta$
^{1}H NMR (300 MHz, $CDCl_3$)				
1	CH_2P(O)	2.10	1.99	0.11
		2.02	1.87	0.15
2	$(CH_3O)_2$P	3.57	3.49	0.08
		3.61	3.51	0.1
3	Ad–CH_2CH_2	2.16	2.28	0.12
4	Ad–CH_2CH_2	3.59	4.04	0.45
5	H-8 (adenine)	6.94	7.42	0.48
^{13}C NMR (75 MHz, $CDCl_3$)				
7	CH_2P(O)	29.9	29.6	0.3
8	Ad–CH_2CH_2	34.7	34.4	0.3
9	Ad–CH_2CH_2	39.6	40	0.4

remove both the dimethylphosphonate and MMTr protecting groups in a single step, and afford the desired derivatives (*R*)-**3** and (*S*)-**3** in quantitative yields after passing through a Dowex Na^+ ion exchange resin and dialysis.

2.2. Assignment of the absolute configuration by NMR studies

The assignment of the absolute configuration of the diastereomeric pair was achieved by the Trost model,[15] which is based on the chemical shift differences in the NMR spectra between the two diastereomers (Table 1). The origin of the chemical shift difference is attributed to the shielding cone of the phenyl ring from (*S*)-MPA; the signals of the substituents under the shielding cone are moved upfield.[16] When the phenyl group is eclipsed with the adenine–CH_2–CH_2 moiety in the less polar diastereomer (Fig. 2), the

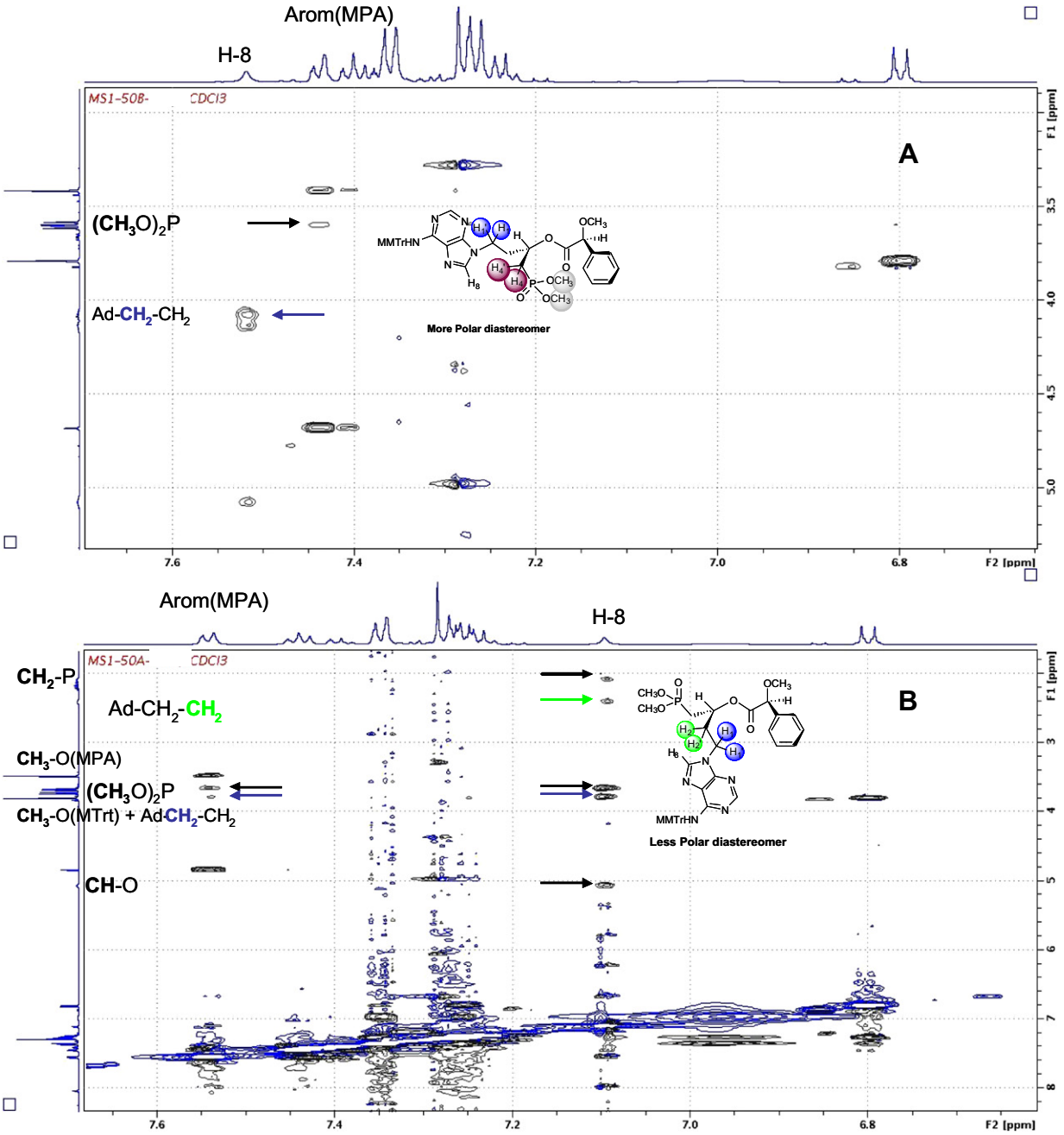


Figure 3. Part of the NOESY spectra of the (A) more polar diastereomer (*S*,*S*)-**9**; (B) less polar diastereomer (*R*,*S*)-**8** in $CDCl_3$.

two methylene groups are shielded and should appear upfield compared to the more polar one. Indeed, we observed that the Ad–CH_2–***CH***$_2$ signal resonates at 2.16 ppm in the less polar diastereomer whereas it resonates at 2.28 ppm in the more polar one ($\Delta\delta$ 0.12 ppm). Similarly the Ad–***CH***$_2$–CH_2 resonates at 3.59 ppm in the less polar diastereomer, whereas it resonates at 4.04 ppm in the more polar one ($\Delta\delta$ 0.45 ppm). On the other hand, when the phenyl group is eclipsed with the $(CH_3O)_2P$ and the $CH_2P(O)$ groups, as in the more polar diastereomer, both the methyl and methylene protons are shielded and should appear upfield with respect to the less polar one. Indeed, the two methyl protons of the $(CH_3O)_2P$ group resonate at 3.51 and 3.49 ppm in the more polar diastereomer (Fig. 2), whereas in the less polar one, they resonate at 3.61 and 3.57 ppm ($\Delta\delta$ 0.1 and $\Delta\delta$ 0.08 ppm, respectively). Similarly, the protons of the $CH_2P(O)$ group resonate at 1.87 and 1.99 ppm in the more polar diastereomer whereas in less polar one, they appeared at 2.02 and 2.1 ppm ($\Delta\delta$ 0.15 and $\Delta\delta$ 0.11 ppm, respectively). In addition, chemical shift differences were also observed in the ^{13}C NMR spectra of the two diastereomers and these are summarized in Table 1.

When the phenyl group was eclipsed with the adenine moiety as in the less polar diastereomer, the H-8 proton of the nucleobase was shielded and resonated at 6.94 ppm whereas in the more polar one, it appeared at 7.42 ppm ($\Delta\delta$ 0.48 ppm). This difference can be attributed to the π-stacking effect between the phenyl ring and the nucleobase. This observation was supported by molecular modelling studies and suggested that the H-8 proton was in close proximity to the phenyl ring. The 2D NMR-NOESY spectra were also obtained and a selected portion is shown in Figure 3. In the case of the less polar diastereomer (*R*,*S*)-**8**, we clearly observed cross peaks between the H-8 proton of the nucleobase and the aromatic protons of MPA with both the adenine–CH_2–CH_2 moiety and the $(CH_3O)_2P$ groups (Fig. 3B). In comparison with an identical part of the 2D NOESY spectrum of the more polar diastereomer, due to the overlapping of the signals of interest, less relevant cross peaks were observed (Fig. 3A). Consequently, the ^1H-, ^{13}C- and ^{31}P-NMR spectra of both isolated diastereoisomers strongly suggested that the absolute configuration of the less polar compound is (*R*,*S*)-**8** while the more polar compound is (*S*,*S*)-**9**.

2.3. Molecular modelling studies

In addition to the NMR experiments, computational calculations were carried out in order to determine the minimum energy conformer for each diastereomeric mandelate. Molecular modelling was achieved using the Biopolymer module implemented in the Insight II suite program (Accelrys). Atomic charges and potentials were assigned using the Gasteiger–Marsili empirical atomic partial charges[17,18] and Charmm22 force field,[19] respectively. Then, four planar angles were selected corresponding to the bonds of the methoxy and carbonyl groups of MPA and the proton at the β-position to the phosphorus atom of each diastereomer, and a systemic conformational search using the AMMP (*Another Molecular Mechanics Program*) program[20] and Charmm22 as Force field was carried out on the diastereomeric pair. Molecular modelling studies supported the NMR chemical shift analysis of each diastereomer due to the geometry predicted by Mosher's approximation[21] in which the methoxy and carbonyl groups of MPA and the proton at the β-position to the phosphorus atom are in almost the same plane for both diastereomers (Fig. 4).

In agreement with the NMR analysis, a π-stacking effect between the adenine moiety and phenyl ring from MPA was also observed in our molecular models. In the case of the lowest energy conformer compound (*R*,*S*)-**8** (Fig. 4A), the H-8 proton was projected to be close to the phenyl ring and resonate at a higher field than for (*S*,*S*)-**9** (Fig. 4B). These theoretical studies supported the experimental results and confirmed our hypothesis with regard to the absolute configurations of both diastereomers.

3. Conclusion

In conclusion, we have synthesized, separated and characterized a new set of enantiomerically pure (*R*)- and (*S*)-β-hydroxyphosphonic acids belonging to the adenine acyclonucleoside phosphonate family. These derivatives were designed as structural analogues of Adefovir (PMEA), a well-known ANP used for the treatment of HBV infections. The newly obtained derivatives were assayed in various cell lines (including HEL, CRFK, Vero, MDCK and HeLa cells) for antiviral activity against a wide variety of DNA and RNA viruses [varicella zoster virus (VZV), cytomegalovirus (CMV), herpes simplex virus type 1 (HSV-1, KOS and TK$^-$ KOS), HSV-2 (G), vaccinia virus and vesicular stomatitis viruses, feline herpes and corona viruses, parainfluenza-3, reovirus-1, Sindbis, Coxsackie B4 and Punta Toro virus, influenza A (H1N1 and H3N2) and influenza B viruses and respiratory syncytial virus]. However, none of them revealed a significant effect up to 100 μM. Cytotoxicity was evaluated on HEL, Vero, HeLa and MDCK cell cultures and the studied compounds were found to be non-toxic (up to 100 μM). Further studies are currently underway to tentatively explain the lack of biological activity.

4. Experimental

Tetrahydrofuran (THF) was distilled from sodium/benzophenone immediately prior to use, DMF and methanol from CaH_2, dichloromethane from P_2O_5, pyridine and Et_3N from KOH. Solids were dried

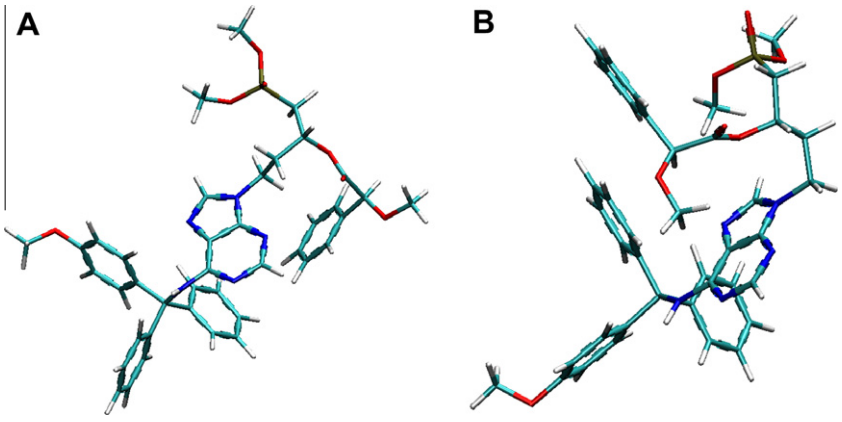


Figure 4. (A) Minimum energy conformer for the less polar diastereomer (*R*,*S*)-**8**. (B) Minimum energy conformer for the more polar diastereomer (*S*,*S*)-**9**.

over P_2O_5 under reduced pressure at rt. Moisture sensitive reactions were performed under argon atmosphere using oven-dried glassware. ^{1}H NMR spectra were recorded at 300 MHz and ^{13}C NMR spectra at 75 MHz with proton decoupling at 25 °C using a Bruker 300 Avance. Chemical shifts are given in δ values referenced to the residual solvent peak ($CHCl_3$ at 7.26 and 77 ppm or DMSO-d_5 at 2.49 and 39.5 ppm) relative to TMS. COSY experiments were performed in order to confirm proton assignments. Coupling constants, *J*, are reported in Hertz (Hz). 2D ^{1}H–^{3}C heteronuclear COSY spectra were recorded for the attribution of ^{13}C signals. ^{31}P NMR spectra were recorded at ambient temperature at 121 MHz with proton decoupling. Chemical shifts are reported relative to external H_3PO_4. The ESI-QTof mass spectra were recorded in the positive-ion or negative-ion modes using a Micromass Q-TOF spectrometer. Specific rotations were measured with a Perkin–Elmer Model 341 spectropolarimeter (path length 1 cm) and are given in units of 10^{-1} deg cm^2 g^{-1}. TLC was performed on pre-coated aluminium sheets of silica gel 60 F_{254} (Merck, Art. 9385), visualization of products being accomplished by UV absorbance followed by charring with 5% ethanolic sulfuric acid and then heating.

4.1. *N*-9-(2-Benzyloxycarbonylethyl)adenine 5

To a suspension of adenine (10.0 g, 74 mmol) in dry DMF (200 mL) diisopropylethylamine (21.48 mL, 148 mmol) was added and the mixture was heated at 80 °C for 1 h under argon. Then, the reaction mixture was cooled to room temperature and benzylacrylate (24.0 g, 148 mmol) was added. The mixture was heated at 80 °C for 3–4 days. The solvent was evaporated and the crude was suspended in ethyl acetate and filtered. The resulting solid was washed with ethyl acetate to give **5** as a fine powder (17 g, 77%). R_f (5% MeOH in DCM) 0.4. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.21 (t, *J* = 6.0 Hz, 2H), 4.59 (t, *J* = 6.0 Hz, 2H), 5.25 (s, 2H), 7.46 (m, 5H, H_{arom}), 8.26 (s, 1H), 8.31 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 33.5 (s, CH_2), 390 (s, CH_2), 65.7 (s, CH_2), 118.7 (s, C5), 127.9, 128.0, 128.3, 135.8, 140.8 (s, C8), 149.4 (s, C2), 152.3 (s, C6), 155.9 (s, C4), 170.5 (C=O). ESI-QTof > 0: *m*/*z* 298.1 $(M+H)^+$, HRMS calcd for $C_{15}H_{15}N_5O_2$: 298.1304, found: 298.1320. UV λ_{max} = 261 nm (ε 21,000), λ_{min} = 230 nm (ε 9000) (EtOH 95).

4.2. [*N*-6-Monomethoxytrityl-*N*-9-(2-benzyloxycarbonyethyl)] adenine 6

To a solution of **5** (12.0 g, 42 mmol) in dry pyridine (240 mL), 4-methoxytrityl chloride (25.94 g, 84 mmol) was added portionwise and the reaction mixture was then heated at 60 °C for 24 h. The solvent was evaporated, and the residue was co-evaporated with a toluene/methanol (1:1) mixture. The crude was dissolved in ethyl acetate and washed with saturated $NaHCO_3$, water, brine and dried over $MgSO_4$, concentrated in vacuo. The crude product was purified by column chromatography (5% acetone in DCM) and the resulting solid was passed through neutral alumina to remove unwanted colouring material to afford **6** (20.4 g, 88%) as a colourless solid. R_f (5% acetone in DCM) 0.37. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.89 (t, *J* = 6.5 Hz, 2H, Ad–CH_2–CH_2), 3.70 (s, 3H, OCH_3), 4.41 (t, *J* = 6.5 Hz, Ad–CH_2–CH_2), 5.03 (s, 2H, CH_2Ph), 6.71 (d, *J* = 15.5 Hz, $2H_{arom}$), 7.12 (br s, 1H, NH), 7.18 (m, $17H_{arom}$), 7.77 (s, H-2), 7.89 (s, H-8). ^{13}C NMR (75 MHz, $CDCl_3$) δ 34.2 (s, Ad–CH_2–CH_2), 39.3 (s, Ad–CH_2–CH_2), 55.2 (s, C_{arom}), 66.9 (s, CH_2–Ph), 70.9 (s, C–Ph_3), 113.1 (s, C_{arom}), 120.9 (s, C-5), 126.9, 127.1, 127.9, 128.4, 128.5, 128.9, 130.2, 135.2, 137.2, 140.5 (s, C_{arom}), 145.2 (s, C-8), 148.7 (s, C-4), 152.2 (s, C-2), 154.1 (s, C-6), 158.30 (s, C_{arom}), 170.8 (s, C=O). ESI-QTof > 0: *m*/*z* 570 $(M+H)^+$, HRMS calcd for $C_{35}H_{31}N_5O_3$: 570.2505, found: 570.2516. UV λ_{max} = 275 nm (ε 18,200), λ_{min} = 246 nm (ε 7000) (EtOH 95).

4.3. [*N*-6-Monomethoxytrityl-*N*-9-(3-oxo-4-dimethylphosphonobutyl)]adenine 4

A solution of dimethylmethylphosphonate (0.473 mL, 4.37 mmol) in dry THF (7.5 mL) was cooled to −78 °C and *n*-BuLi (1.74 mL, 4.37 mmol) was added dropwise. The resulting mixture was stirred at −78 °C for 1 h and a solution of **6** (1.0 g, 1.75 mmol) in THF (5 mL) was added dropwise. The reaction mixture was stirred at −78 °C for 2 h, and then the reaction mixture was added to a cooled saturated NH_4Cl solution. The resulting organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with water, brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography (4% MeOH in DCM) to afford **4** (560 mg, 55%) as a colourless solid. R_f (4% MeOH in DCM) 0.31. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.21 (d, *J* = 23.0 Hz, 2H, CH_2P), 3.4 (t, *J* = 6.0 Hz, 2H, Ad–CH_2–CH_2), 3.77 (d, *J* = 11.0 Hz, 6H, $(CH_3O)_2$P), 3.92 (s, 3H, OCH_3), 4.61 (t, *J* = 6.0 Hz, 2H, Ad–CH_2–CH_2), 6.92 (d, *J* = 8.5 Hz, $2H_{arom}$), 7.38 (m, $12H_{arom}$), 8.02 (s, 1H, H-2), 8.21 (s, 1H, H-8). ^{13}C NMR (75 MHz, $CDCl_3$) δ 39.4 (d, *J* = 199 Hz, CH_2P), 42.7 (d, *J* = 35 Hz, $(CH_3O)_2$P), 70.9 (s, C–Ph_3), 113.1 (s, C_{arom}), 120.7 (s, C-5), 126.8, 127.8, 128.9, 130.2, 137.2, 140.9 (s, C_{arom}) 145.2 (s, C-8), 148.6 (s, C-4), 152.0 (s, C-2), 154.0 (s, C-6), 158.3 (s, C_{arom}), 199.3 (d, *J* = 6.5 Hz, C=O). ^{31}P (121 MHz, $CDCl_3$) δ 21.5. ESI-QTof > 0: *m*/*z* 586 $(M+H)^+$, HRMS calcd for $C_{31}H_{32}N_5O_5P$: 586.2219, found: 586.2204. UV λ_{max} = 275 nm (ε 21,100), λ_{min} = 248 nm (ε 11,700) (EtOH 95).

4.4. (±)-[*N*-6-Monomethoxytrityl-*N*-9-(3-hydroxy-4-dimethyl phosphonobutyl)]adenine 7

To a solution of **4** (1.2 g, 2.04 mmol) in methanol was added sodium borohydride (463 mg, 12.24 mmol) and the resulting mixture was stirred for 1 h. Then, the reaction mixture was cooled to 0 °C and quenched with saturated NH_4Cl. The solvent was removed under reduced pressure, and the crude was dissolved in water and extracted with ethyl acetate. The resulting organic layer was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography (4% MeOH in DCM) to afford (±)-**7** (1.05 g, 87%) as a colourless solid. R_f (4% MeOH in DCM) 0.28. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.86 (d, *J* = 5.5 Hz, 1H, CHP), 1.93 (d, *J* = 8.0 Hz, 1H, CHP), 1.96 (dm, *J* = 41.5 Hz, 2H, Ad–CH_2–CH_2), 3.62 (s, 3H, $(CH_3O)_2$P), 3.66 (s, 3H, $(CH_3O)_2$P), 3.73 (s, 3H, OCH_3), 3.77 (m, 1H, C*H*(OH)), 4.3 (dm, *J* = 38.0 Hz, Ad–CH_2–CH_2), 6.73 (d, *J* = 12.0 Hz, $2H_{arom}$), 6.98 (br s, 1H, NH), 7.21 (m, $12H_{arom}$), 7.76 (s, 1H, H-2), 7.98 (s, 1H, H-8). ^{13}C NMR (75 MHz, $CDCl_3$) δ 32.6 (d, *J* = 138.0 Hz, CH_2P), 38.5 (d, *J* = 15.0 Hz, Ad–CH_2–CH_2), 40.2 (s, Ad–CH_2–CH_2), 52.4 (d, *J* = 6.5 Hz, (CH_3O)P), 52.5 (d, *J* = 6.5 Hz, (CH_3O)P), 52.2 (s, C_{arom}), 62.6 (d, *J* = 4.0 Hz, CH(OH)), 71.0 (s, CPh_3), 113.1 (s, C_{arom}), 120.8 (s, C-5), 126.9, 127.9, 128.9, 130.2, 137.6, (s, C_{arom}), 145.1 (s, C-8), 148.9 (s, C-4), 152.1 (s, C-2), 154.2 (s, C-6), 158.3 (s, C_{arom}). ^{31}P (121 MHz, $CDCl_3$) δ 31.8. ESI-QTof > 0: *m*/*z* 588 $(M+H)^+$, HRMS calcd for $C_{31}H_{34}N_5O_5P$: 588.2376, found: 588.2391. UV λ_{max} = 276 nm (ε 23,900), λ_{min} = 248 nm (ε 11,600) (EtOH 95).

4.5. Derivatization of (±)-7 with (*S*)-MPA

To a solution of (±)-**7** (1 g, 1.7 mmol) and (*S*)-methoxyphenyl acetic acid (0.40 g, 2.38 mmol) in dichloromethane (15 mL) were added 1,3-dicyclohexylcarbodiimide (0.49 g) and a catalytic amount of dimethylaminopyridine at room temperature. The resulting mixture was stirred for 1 h, then filtered and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (5% ethanol in diethyl ether) to afford the less polar compound **8** (430 mg, 35%) and the more polar compound **9** (470 mg, 38%).

4.5.1. Less polar diastereomer (*R*,*S*)-8

R_f (5% ethanol in diethyl ether) 0.2. $[\alpha]_D^{20} = +53.3$ (*c* 0.89, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.1 (dd, *J* = 8.0 Hz, 1H, CHP), 2.02 (dd, *J* = 5.5 Hz, 1H, CHP), 2.16 (dm, *J* = 67.5 Hz, 2H, Ad–CH_2–CH_2), 3.37 (s, 3H, OCH_3), 3.57 (d, *J* = 11.0 Hz, 3H, $(CH_3O)_2P$), 3.59 (m, 2H, Ad–CH_2–CH_2), 3.61 (d, *J* = 11.0 Hz, 3H, $(CH_3O)_2P$), 3.69 (s, 3H, OCH_3), 4.74 (s, 1H, CH), 4.94 (m, 1H, CH), 6.7 (d, *J* = 12.0 Hz, $2H_{arom}$), 6.8 (br s, 1H, NH), 6.94 (s, 1H, H8), 7.26 (m, $12H_{arom}$), 7.98 (s, 1H, H_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 29.9 (d, *J* = 140.0 Hz, CH_2P), 34.7 (d, *J* = 7.5 Hz, Ad–CH_2–CH_2), 39.6 (Ad–CH_2–CH_2), 52.4 (d, *J* = 6.5 Hz, $(CH_3O)_2P$), 52.7 (d, *J* = 6.5 Hz, $(CH_3O)_2P$), 55.2 (s, C_{arom}), 57.4 (OCH_3), 66.9 (d, *J* = 1.5 Hz, C-Ph$_3$), 70.9 (CH(OH)), 82.2 (*C*(OCH_3)), 113.1 (s, C_{arom}), 120.9 (C-5), 126.8, 127.1, 127.3, 127.8, 128.9, 128.9, 129.2, 130.2, 136.5, 139.8 (s, C_{arom}), 145.2 (C-8), 148.7 (C-4), 152.1 (C-2), 154.0 (C-6), 158.3 (s, C_{arom}), 170.1 (C=O). ^{31}P (121 MHz, $CDCl_3$) δ 27.6. ESI-QTof > 0: *m*/*z* 736 $(M+H)^+$, HRMS calcd for $C_{40}H_{42}N_5O_7P$: 736.2900, found: 736.2910. UV λ_{max} = 276 nm (ε 18,700), λ_{min} = 247 nm (ε 7500) (EtOH 95).

4.5.2. More polar diastereomer (*S*,*S*)-9

R_f (5% ethanol in diethyl ether) 0.17. $[\alpha]_D^{20} = +48.6$ (*c* 0.74, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.87 (ddd, *J* = 7.5 Hz, 5.0 Hz, 7.5 Hz, 1H, CHP), 1.99 (ddd, *J* = 5.0 Hz, 10.0 Hz, 5.0 Hz, 1H, CHP), 2.28 (d, *J* = 65.5 Hz, 2H, Ad–CH_2–CH_2), 3.32 (s, 3H, OCH_3), 3.49 (d, *J* = 11.0 Hz, 3H, $(CH_3O)_2P$), 3.51 (d, *J* = 11.0 Hz, 3H, $(CH_3O)_2P$), 3.69 (s, 3H, OCH_3), 4.04 (m, 2H, Ad–CH_2–CH_2), 4.59 (s, 1H, CH), 4.97 (m, 1H, CH), 6.7 (d, *J* = 12.0 Hz, $2H_{arom}$), 6.8 (br s, 1H, NH), 7.24 (m, $12H_{arom}$), 7.42 (s, 1H, H8), 7.98 (s, 1H, H2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 29.6 (d, *J* = 138.5 Hz, CH_2P), 34.4 (d, *J* = 5.5 Hz, Ad–CH_2–CH_2), 40.0 (s, Ad–CH_2–CH_2), 52.4 (d, *J* = 4.0 Hz, $(CH_3O)_2P$), 52.5 (d, *J* = 4.0 Hz, $(CH_3O)_2P$), 55.2 (s, OCH_3), 57.5 (s, OCH_3), 67.9 (s, C–Ph$_3$), 70.9 (CH(OH), 82.4 (*C*(OCH_3)), 113.1 (s, C_{arom}), 120.9 (C-5), 126.8, 127.1, 127.8, 128.8, 129.0, 130.2, 135.2, 137.2, 139.8 (s, C_{arom}), 145.2 (C-8), 148.9 (C-4), 152.2 (C-2), 154.1 (C-6), 158.3 (s, C_{arom}), 170.1 (C=O). ^{31}P (121 MHz, $CDCl_3$), δ 27.5. ESI-QTof > 0: *m*/*z* 736 $(M+H)^+$, HRMS calcd for $C_{40}H_{42}N_5O_7P$: 736.2900, found: 736.2928. UV λ_{max} = 276 nm (ε 21,100), λ_{min} = 247 nm (ε 8200) (EtOH 95).

4.6. (*R*)-[*N*-6-Monomethoxytrityl-*N*-9-(3-hydroxy-4-dimethyl phosphonobutyl)]adenine 10

To a solution of (*R*,*S*)-**8** (280 mg, 0.38 mmol) in methanol/water (8:2, v/v, 10 mL) was added $LiOH{\cdot}H_2O$ (32.47 mg, 0.77 mmol), and the resulting mixture was stirred at room temperature until completion of the reaction was indicated by TLC. Then, the solvents were evaporated under reduced pressure and the crude was dissolved in water and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$ concentrated under reduced pressure. The crude product was purified by flash column chromatography (4% MeOH in dichloromethane) and afforded (*R*)-**10** (165 mg, 74%). $[\alpha]_D^{20} = +28.3$ (*c* 0.46, MeOH). The NMR chemical shifts were identical to (±)-**7**. HRMS calcd for $C_{31}H_{34}N_5O_5P$: 588.2376, found: 588.2380. UV λ_{max} = 276 nm (ε 26,000), λ_{min} = 248 nm (ε 12,800) (EtOH 95).

4.7. (*S*)-[*N*-6-Monomethoxytrityl-*N*-9-(3-hydroxy-4-dimethyl phosphonobutyl)]adenine 10

The above procedure was applied to (*S*,*S*)-**9** to afford (*S*)-**10** (156 mg, 70%). $[\alpha]_D^{20} = -30.4$ (*c* 0.6, MeOH). The NMR chemical shifts were identical to (±)-**7**. HRMS calcd for $C_{31}H_{34}N_5O_5P$: 588.2376, found: 588.2388. UV λ_{max} = 276 nm (ε 17,000), λ_{min} = 246 nm (ε 6500) (EtOH 95).

4.8. (*R*)-9-(3-Hydroxy-4-phosphonobutyl)adenine 3

To a cooled solution of (*R*)-**10** (110 mg, 0.187 mmol) in dry dichloromethane (4 mL) was added trimethylsilylbromide (0.16 mL, 1.22 mmol), and the reaction mixture was stirred at room temperature until the completion of the reaction, as indicated by TLC (isopropanol/water/ammonia 30%, 7:2:1, v/v/v). Next, water was added and the reaction mixture was stirred at room temperature for 1 h. The aqueous layer was separated, washed with diethyl ether and concentrated under high vacuum. After freeze-drying, the crude product was treated with triethylamine until pH 7 and then passed through a Dowex Na^+ ion exchange resin column; the desired fractions were collected and lyophilized to give (*R*)-**3** quantitatively as a hygroscopic salt. R_f (*i*PrOH/H_2O/NH_4OH 30%, 7:2:1, v/v/v) 0.25. $[\alpha]_D^{20} = +10.6$ (*c* 0.47, H_2O). ^{1}H NMR (300 MHz, D_2O) δ 1.54 (m, 2H, CH_2P), 1.9 (dm, *J* = 42.5 Hz, 2H, Ad–CH_2–CH_2), 3.84 (m, 1H, C*H*(OH)), 4.18 (t, *J* = 8.0 Hz, 2H, Ad–CH_2–CH_2), 8.03 (s, 1H, H2), 8.06 (s, 1H, H8). ^{13}C NMR (75 MHz, D_2O) δ 35.2 (s, Ad–CH_2–CH_2), 37.1 (t, *J* = 13.0 Hz, CH_2P), 41.0 (s, Ad–CH_2–CH_2), 66.2 (d, *J* = 3.5 Hz, CH(OH)), 118.4 (s, C-5), 142.6 (s, C-8), 148.7 (s, C-2), 152.1 (s, C-6), 155.3 (s, C-4). ^{31}P (100 MHz, $CDCl_3$) δ 18.5. ESI-QTof > 0: *m*/*z* 332 $(M+H)^+$, HRMS calcd for $C_9H_{12}N_5O_4Na_2P$: 332.0501, found: 332.0490. UV λ_{max} = 261 nm (ε 9000), λ_{min} = 228 nm (ε 1600) (EtOH 95).

4.9. (*S*)-*N*-9-(3-Hydroxy-4-phosphonobutyl)adenine 3

The procedure described above was applied to (*S*)-**10** (120 mg, 0.204 mmol) to afford (*S*)-**3** quantitatively as a hygroscopic salt. The NMR chemical shifts were identical to (*R*)-**3**. $[\alpha]_D^{20} = -10.6$ (*c* 0.47, H_2O). ESI-QTof > 0: *m*/*z* 332 $(M+H)^+$, HRMS calcd for $C_9H_{12}N_5O_4Na_2P$: 332.0501, found: 332.0494. UV λ_{max} = 261 nm (ε 8500), λ_{min} = 228 nm (ε 1400) (EtOH 95).

Acknowledgments

M.K. is grateful to the RTRS-Infectiopôle Sud for a doctoral fellowship. The authors are indebted to the staff of Professor J. Balzarini laboratory for the screening of antiviral activity.

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