Letters in Organic Chemistry, 2018, *15*, 10-14

RESEARCH ARTICLE

Efficient Synthesis and Resolution of Tenofovir Alafenamide

Bin Yang[1], Hongmeng Xie[1], Kerui Ran[1] and Yongjun Gan[2,*]

[1]*Department of Pharmacy, Children's Hospital of Chongqing Medical University, Ministry of Education Key Laboratory of Child Development and Disorders, China International Science and Technology Cooperation Base of Child Development and Critical Disorders, Chongqing Key Laboratory of Pediatrics, 400014 Chongqing, P.R. China;* [2]*Experimental Teaching Center, Chongqing Medical University, 400016 Chongqing, P.R. China*

ARTICLE HISTORY

Received: January 12, 2017
Revised: June 20, 2017
Accepted: August 23, 2017

DOI:
10.2174/1570178614666170907113121

Abstract: ***Background***: Tenofovir alafenamide (TAF) is an oral antiviral prodrug of tenofovir (TFV), we have developed a facial and efficient method for the synthesis and chiral resolution of TAF.

Method: The practical synthetic route of a mixed two diastereomers at phosphorous could start from (R)-9-[2-(Phosphonomethoxy)propyl]adenine (PMPA), the esterification reaction between PMPA and phenol occurred under the catalysis of dicyclohexylcarbodiimide (DCC) in 1-methyl-2-pyrrolidinone (NMP) at the temperature of 100°C to afforded **1**. Phosphonochloridate was synthesized from **1** by chloride acetylation with thionyl chloride, and then react with an excess of L-Alanine isopropyl ester hydrochloride to give the diastereomer mixture of 9-[(R)-2-[[(R,S)-[[(S)-1-(isopropoxycarbonyl)ethyl] amino]-phenoxyphosphinyl]methoxy]propyl]adenine (**2**). The antipodes of **2** were separated in a satisfactory yield and diastereomeric excess (99% de) by resolution *via* formation diastereoisomer salt or inclusion complex to afford the more potent diastereomer (**3**). Tenofovir Alafenamide hemifumarate could be afforded by **3** and fumaric acid in a 1:0.5 ratio.

Results: The diastereomeric excess of **3** could reach to 99% de.

Conclusion: In a word, we have developed an efficient and chromatography-free route for the preparation of TAF. In consideration of the expensive equipment and higher operation cost of SMBC, we chose a traditional resolution route to obtain chiral phosphorus.

Keywords: Chiral phosphorus, inclusion complex, resolution, D-(-)-Tartaric acid, TADDOL, tenofovir alafenamide.

1. INTRODUCTION

Tenofovir alafenamide (TAF) is an oral antiviral prodrug of tenofovir (TFV), which is a HIV-1 nucleotide reverse transcriptase inhibitor. Tenofovir disoproxil fumarate (TDF) is another TFV prodrug developed by Gilead Sciences, was first approved for the prophylaxis or treatment of HIV and HBV in 2001 [1-3]. TAF is mostly converted to TFV after absorbed by human body, compared with the TDF, TAF has a higher tenofovir diphosphate (TFV-DP) levels in peripheral blood mononuclear cells. As a result, TAF has demonstrated potent anti-HIV-1 activity at lower doses than TDF in monotherapy studies [4]. Besides, the high oral bioavailability and favorable tissue-selective distribution of TAF made it a promising candidate for HBV therapy [5].

TAF also known as GS-7340, it has the formula $C_{21}H_{29}N_6O_5P$ (Fig. **1**). The existing synthetic route has non-stereoselective led to the chiral center at phosphorous was racemic [6], so GS-7171 was a 1:1 mixture of two diastereomers be composed with GS-7339 and GS-7340, (Scheme **1**) GS-7340 was about ten-fold more active than GS-7339 *in vitro* anti-HIV-1 activity [7], the process for separation of the diastereomers of GS-7171 involves the use of simulated moving bed chromatography(SMBC) [8], which needs expensive equipment and has a higher operation cost. Thus, a more simple and efficient method is necessary to develop in order to separate and get GS-7340.

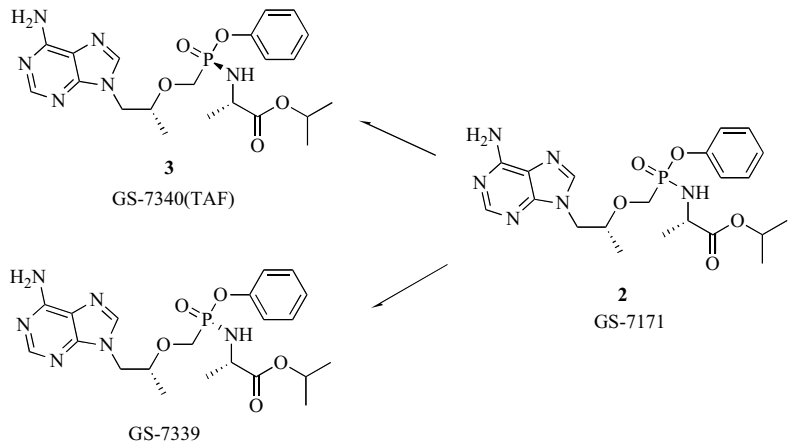
*Address correspondence to this author at the Experimental Teaching Center, Chongqing Medical University, 400016 Chongqing, P.R. China;
Tel: +86 18623627351; E-mail: cqjy0708@126.com

2. EXPERIMENTAL

2.1. General Experimental Procedures

Reactions were monitored by thin layer chromatography with silica gel plates under the UV light. NMR spectra were determined on a Bruck 300 NMR spectrometer with the solvent of D_2O, $CDCl_3$ or DMSO-d6. HPLC purity was determined on Agilent Proshell 120 Bonus-RP column with Agilent 1120. All solvents, reagents, and the starting materials were used as received from commercial vendors.

1875-6255/18 $58.00+.00



Scheme (1). The diastereomers of **2**.

2.1.1. [(R)-2-(Phenylphosphonomethoxy)propyl]adenine(1)

Phenol (131g, 1392 mmol) and triethylamine (115 mL, 831 mmol) were added to a stirred solution of anhydrous PMPA (200 g, 696 mmol) in NMP (520 mL) at room temperature. The mixture was heated to 100°C. A solution of 1, 3-dicyclohexylcarbodiimide (287g, 1.391 mmol) in NMP (20 mL) was then added over 6 hours at 100°C. The mixture was stirred at this temperature for 10 hours more. Water (400 mL) was added after the reaction was cooled to 45°C, then the reaction was cooled to room temperature and stirred for 30 min, the mixture was filtered and solids were rinsed with water (200 mL) to give the filtrate. The filtrate and rinse were combined and evaporated under reduced pressure, after removing the solvent by reduced pressure distillation, the tan slurry obtained finally. Water (300 mL) was added, and adjusted to pH=11 with 25% sodium hydroxide solution. The suspension was removed by filtration through a funnel charged with celite. The filtrate was extracted with ethyl acetate (500 mL*3). Concentrated hydrochloric acid was added into the aqueous solution until the mixture was adjusted to pH=3.1, and then cooled to 0°C and stirred for 2h, the precipitation was isolated by filtration under reduced pressure and washed with methanol (500 mL), and dried to give **1** as a white powder (120 g. 47.6% yield). ^{1}H NMR (300 MHz, D_2O, δ): 1.16 (d, *J* = 3.7 Hz, 3H), 3.54 (m, 2H), 3.71 (dd, *J* = 10.5 Hz, 2H), 4.03 – 3.99 (m, 2H), 4.22-4.10 (m, 2H), 4.45 (dd, J=11.3, 8.3Hz, 2H), 6.59 (d, J =5.2 Hz, 2H), 7.05 (t, J =5.3 Hz, 1H), 7.21 (t, J =5.1 Hz, 2H), 8.05 (s, 1H), 8.16 (s, 1H); ^{13}CNMR (100 MHz, DMSO-d6): δ155.8 (C-6), 152.7(C-2), 151.3(C-19), 149.2(C-4), 140.7(C-8), 130.0 (C-21), 130.0(C-23), 124.9(C-22), 120.6(C-20), 120.6 (C-24), 119.8(C-5), 72.4(C-12), 56.3(C-14), 55.6(C-11), 19.0 (C-25); ^{31}P NMR (72 MHz, D_2O, δ):15.0 (decoupled).

2.1.2. 9-[(R)-2-[[(R,S)-[[(S)-1-(isopropoxycarbonyl)ethyl] amino]-phenoxyphosphinyl]methoxy]propyl]adenine (2)

To a stirred solution of **1** (120g, 331 mmol) in anhydrous acetonitrile (600 mL) thionyl chloride (78.7g, 661 mmol) was added at room temperature in 10 min. The mixture was heated under reflux until solids dissolved, then the solvent was evaporated under reduced pressure to give yellow semi solid. The yellow semi solid was diluted with dichloromethane (400 mL) and the mixture was cooled to −30°C by ice-salt bath. A solution of L-alanine isopropyl ester hydrochloride (83.2g, 497 mmol) in dichloromethane (200 mL) was added dropwise over a period of 1h and control the inside temperature not more than -20°C by ice-salt bath, followed to it was added triethylamine (100.3 g, 993 mmol) over 30 minutes at the same temperature. The reaction liquid was warmed to room temperature after stirred at -20°C for 2h, and then 10% NaH_2PO_4 solution(600 mL) added and separated after the reaction became clarification. The organic solution was dried with Na_2SO_4 after washed with another 600mL 10% NaH_2PO_4 solution, and concentrated to brown oil under reduced pressure. Acetonitrile (600 mL) and fumaric acid (38.4 g, 331 mmol) was added to the reside, the mixture was heated under reflux for an hour, filter while hot and the filtrate cooled to 0°C for 2h. The solid was isolated by filtration, rinsed with 100 mL acetonitrile, and dried to 122 g light brown solid. The solid was dissolved in a mixture of 100 mL ethyl acetate and 100 mL saturated sodium bicarbonate solution, the organic layer was collected and concentrated to pale yellow oil (98 g, 62% yield) under reduced pressure. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.64-1.22 (m, 12H), 3.65 (dd, J=9.8, 8.1Hz, 1H), 3.96-3.84 (m, 5H), 4.2-4.09 (m, 1H), 4.9-4.83 (m, 1H), 6.2 (s, 2H), 7.15-7.07 (m, 5H), 8.0 (s, 1H), 8.27(s, 1H); ^{13}CNMR (100 MHz, DMSO-d6): δ171.9 (C-26), 155.8(C-6), 152.7(C-2), 149.6(C-18), 149.2 (C-4), 140.8(C-8), 129.5(C-20), 129.5(C-22), 125.1(C-21), 121.3(C-19), 121.3(C-23), 119.8(C-5), 72.1(C-12), 68.8 (C-29), 62.0(C-14), 52.9(C-11), 49.5(C-24), 21.7(C-30) 21.7 (C-31), 18.9(C-33), 18.3(C-25); ^{31}P NMR (72 MHz, $CDCl_3$, δ): 21.0, 22.5(decoupled).

2.1.3. 9-[(R)-2-[[(S)-[[(S)-1-(isopropoxycarbonyl)ethyl] amino]-phenoxyphosphinyl]methoxy]propyl]adenine (3)

To a stirred solution of **2** (20 g, 42 mmol) in isopropanol (200 mL) D-(-)-Tartaric acid (3.2 g, 21 mmol) and D-DBTA (7.5 g, 21 mmol) were added at room temperature. The reaction liquid was heated under reflux until solids dissolved, and then cooled to 0°C over a period of 5 h, the mixture was filtered under reduced pressure and the solid rinsed with isopropanol. The solid was dissolved in a mixture of ethyl

acetate (50 mL) and saturated sodium bicarbonate solution (50 mL), the organic layer was separated and concentrated to off-white powder (9.6 g, 48% yield, 99% de).

To a stirred solution of **2** (20 g, 42 mmol) in acetone (100 mL) (R,R)-TADDOL (9.8 g, 84 mmol) was added at room temperature. The mixture was heated under reflux until solids dissolved, and then cooled to 20°C over a period of 2 h, the mixture was filtered under reduced pressure and the solid further purified by one recrystallizations from acetone (50 mL). The dissociation of the complex was using column chromatography (silica gel, 200-300 mesh), the column was eluted with ethyl acetate to get 3 (7.8 g, 39% yield, 99% de, the HPLC column was Agilent Proshell 120 Bonus-RP column 150 mm × 4.6 mm, 2.7 μm, Mobile phase solution A was 0.02% phosphoric acid aqueous, and mobile phase solution B was Methanol, Gradient elution was performed as shown in Table **1** with a run time of 50 min. The flow rate was 0.5 mL/min and the column temperature was 40 °C. The UV detection was performed at 260 nm. The retention time was 25.69 min). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.18 -1.10 (m, 12H), 3.57 (dd, *J*=9.8, 8.0Hz, 1H), 3.86-3.77 (m, 5H), 4.2(dd, J=10.8, 2.3Hz, 1H), 4.7-5.62 (m, 1H), 6.1 (s, 2H), 7.1-7.03 (m, 5H), 8.0 (s, 1H), 8.27 (s, 1H) 13CNMR (100 MHz, DMSO-d6): δ171.9(C-26), 155.8(C-6), 152.7(C-2), 149.6(C-18), 149.2(C-4), 140.8(C-8), 129.5(C-20), 129.5 (C-22), 125.1(C-21), 121.3(C-19), 121.3(C-23), 119.8(C-5), 72.1 (C-12), 68.8(C-29), 62.0(C-14), 52.9(C-11), 49.5 (C-24), 21.7 (C-30)21.7(C-31), 18.9(C-33), 18.3(C-25); 31P NMR (72 MHz, CDCl3, δ): 21.0 (decoupled).

2.1.4. Tenofovir Alafenamide Hemifumarate (4)

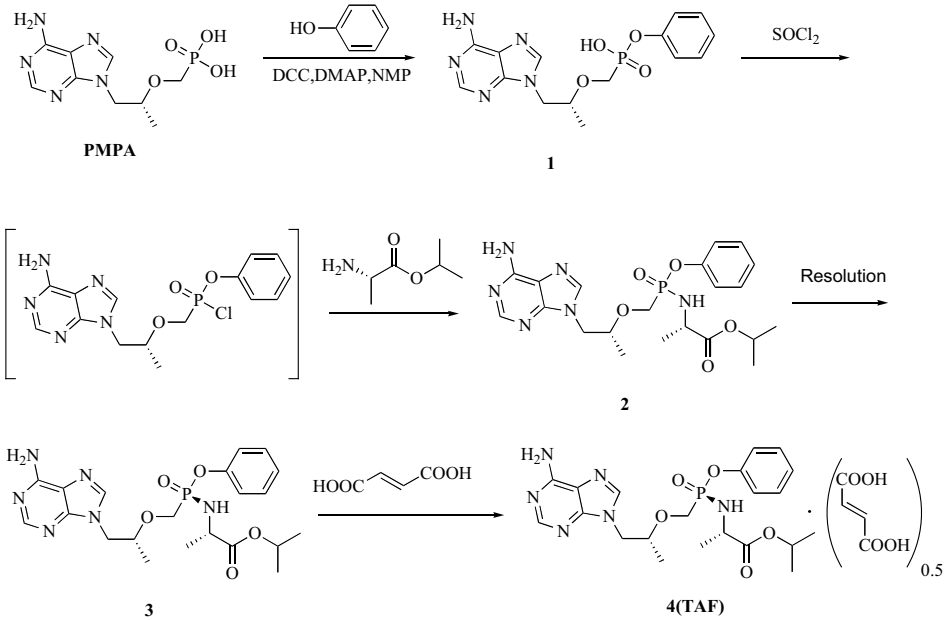
To a stirred solution of **3** (8 g, 16.8 mmol) in acetonitrile fumaric acid (0.97 g, 8.4 mmol) was added. The mixture was heated under reflux until solids were dissolved, filtered while hot to remove any undissolved particulates. The filtered solution was cooled to 55°C and stirred for 2h at this temperature. The mixture was cooled to 0-5°C over 2 hours, filtered under reduced pressure and the solid dried under vacuum at 50°C to provide the TAF (**4**) (8.3 g, 92% yield). δ1.18-1.10 (m, 12H), 3.58 (dd, J =9.8, 8.0Hz, 1H), 3.90-3.84 (m, 2H), 3.94 (m, 1H), 4.14 (dd, J = 5.2, 11.1 Hz, 1H), 4.27(m, 1H), 4.83 (heptet, J= 4.5 Hz, 1H), 5.67 (t, J= 8.4 Hz, 1H), 6.62 (s, 1H), 7.08 (d. J =5.7 Hz, 2H), 7.13 (t, J =5.3 Hz, 1H), 7.22 (s, 2H), 7.28 (t, J = 5.7 Hz, 2H), 8.13 (t, J = 10.4Hz, 2H). ^{13}CNMR (100 MHz, DMSO-d6): δ171.9 (C-26), 167.1(C-36), 155.8(C-6), 152.7(C-2), 149.6(C-18), 149.2(C-4), 140.8 (C-8), 133.8(C-34), 129.5(C-20), 129.5(C-22), 125.1(C-21), 121.3 (C-19), 121.3(C-23), 119.8(C-5), 72.1(C-12), 68.8(C-29), 62.0(C-14), 52.9(C-11), 49.5(C-24), 21.7(C-30) 21.7 (C-31), 18.9(C-33), 18.3(C-25).

3. RESULTS AND DISCUSSION

In our work Scheme (**2**), the commercially available (R)-PMPA side-chain and L-alanine isopropyl ester hydrochloride were homochiral starting materials, the esterification reaction between PMPA and phenol occurred under the catalysis of dicyclohexylcarbodiimide (DCC) in 1-methyl-2-pyrrolidinone (NMP) at the temperature of 100°C to afforded **1**. Phos-

Table 1. The gradient elution of HPLC.

Time (min):	0	25	40	40.1	50
B%:	20	60	60	20	20



Scheme (2). The synthesis of TAF.

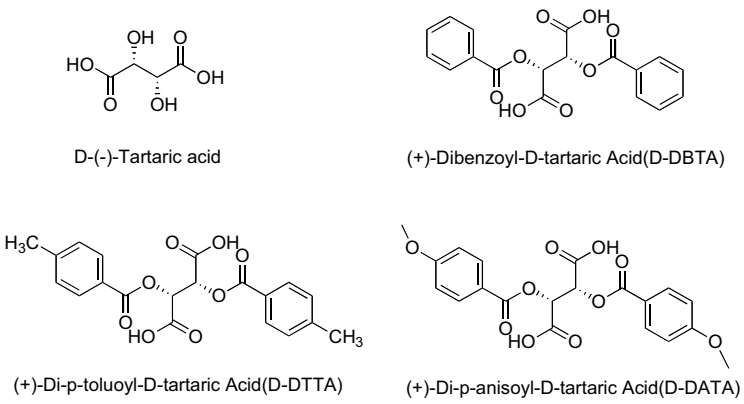


Fig. (1). D-(-)-Tartaric acid and its derivatives.

Table 2. Dutch resolution of 2.

Resolving Agent	Reagent Ratio	Yield%	de%
D-TA/ D -DBTA/**2**	0.5:0.5:1	48	99
D -TA/ D -DTTA/**2**	0.5:0.5:1	44	77
D -TA/ D -DATA/**2**	0.5:0.5:1	33	85
D -TA/ D -DBTA/D -DTTA/**2**	0.5:0.25:0.25:1	41	68
D -TA/ D -DBTA/D -DATA/**2**	0.5:0.25:0.25:1	38	73
D -TA/ D -DTTA/D -DATA/**2**	0.5:0.25:0.25:1	35	87

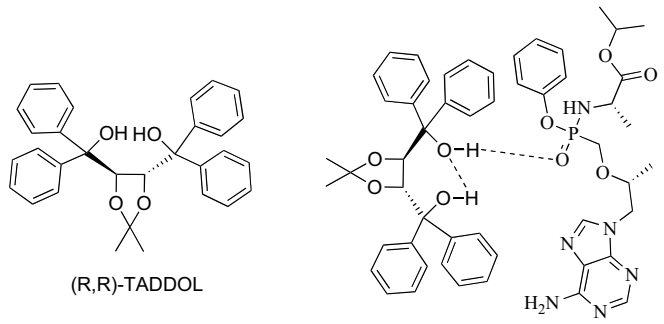


Fig. (2). The formation of the inclusion complex with the basic H-bridge interaction between (R,R)-TADDOL and **2**.

phonochloridate was synthesized from **1** by chloride acetylation with thionyl chloride, and then react with an excess of L-Alanine isopropyl ester hydrochloride to give the diastereomer mixture of 9-[(R)-2-[[(R,S)-[[(S)-1-(isopropoxycarbonyl) ethyl]amino]-phenoxyphosphinyl]methoxy]propyl]adenine (**2**). The antipodes of **2** were separated in a satisfactory yield and diastereomeric excess (99% de) by resolution *via* formation of diastereomeric complexes or inclusion complex, we choose two resolution methods to afford the more potent diastereomer (**3**), the one was the use of mixtures of resolving agents were composed of D-(-)-Tartaric acid and its derivatives, which also known as 'Dutch Resolution' [9], the other resolution agent was (R,R)-TADDOL, an efficient chiral resolving agent of chiral phosphine oxides [10, 11]. Tenofovir Alafenamide hemifumarate could been afforded by **3** and fumaric acid in a 1:0.5 ratio (Fig. **2**).

Our work seems to be deadlocked when we had obtained compounds **2**, because the SMBC method is not what we can afford, so we have to find a more simple and cheaper way to get **3** fortunately, the purine ring of **2** have alkaline amino groups, we found that **2** and fumaric acid can form salt and crystallization from the organic solvent, maybe the tartaric acid could also form a solid and crystallization from organic solvent with **2** because of its structural similarity with fumaric acid, At the same time the difference solubility of the diastereomeric complexes in different solvents maybe significant because the tartaric acid was a chiral compound,

therefore, we have a very good chance to obtain **3** by chiral resolution. Finally, we found that there is an obvious difference between the solubility of D-(-)-Tartaric acid and **2** formation of the diastereomeric salt in isopropanol after a lot of solvent screening experiment, but we can only obtain **3** in a diastereomeric excess of 21% by crystallization one time of the diastereomer mixture of D-(-)-Tartaric acid and **2**,and the diastereomeric excess could reach 99% after three times recrystallization with the yield of 17% (the theoretical yield of chiral resolution was 50%). In order to increase the efficiency of resolution, we tried to use the 'Dutch Resolution' prepare **3**. We studied the effect of resolution between different combinations of D-(-)-Tartaric acid and its derivatives (Fig. **1**), to our great surprise, we can almost complete separation of **3** and GS-7339 when we used to 1:1 proportion of D-(-)-Tartaric acid and D-DBTA as the resolving agent (Table **2**).

At the same time, our another way of separating **3** *via* inclusion complex formation with (R,R)- TADDOL has also made a good progress, the inclusion complex formation may rely on the basic H-bridge interaction between (R,R)-TADDOL and **2** (Fig. **2**), and the (S)-inclusion complex was separated by filtration from acetone, then **3** was isolated from the (S)-inclusion complex with the method of column chromatography(silica gel, ethyl acetate), the diastereomeric excess of **3** could reach 99% after recrystallization with the yield of 39%. In fact, we have developed a resolution procedure that is the first example of TAF *via* inclusion complex formation. A practical method for the resolution of **3** by the separation of the complexes formed by interaction with chiral hosts (R,R)-TADDOL.

CONCLUSION

In a word, we have developed an efficient and chromatography-free route for the preparation of TAF. In consideration of the expensive equipment and higher operation cost of SMBC, we chose a traditional resolution route to obtain chiral phosphorus.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

The authors acknowledge financial support for this work by the Natural Science Foundation of Chongqing (cstc2016shmszx130079).

REFERENCES

[1] Gallant, J.E. *Clin. Infec. Dis*., **2003**, *37*, 944-950.
[2] Jenh, A.M.; Pham, P.A. *Expert Rev. Anti Infe*., **2010**, *8*, 1079-1092.
[3] Duwal, S.; Schutte, C.; Kleist, M.V. *PLoS One*, **2012**, *7*, e40382.
[4] Callebaut, C.; Stepan, G.; Tian, Y.; Miller, M.D. *Antimicrob. Agents Chemother*., **2015**, *59*, 5909-5916.
[5] Ruane, P.J.; DeJesus, E.; Berger, D.; Markowitz, M.; Bredeek, U.F.; Callebaut, C.; Zhong, L.; Ramanathan, S.; Rhee, M.S.; Fordyce, M.W.; Yale, K. *J. Acquir. Immune. Defic. Syndr*., **2013**, *63*, 449-455.
[6] Becker, M.; Chapman, H.; Cihlar, T.; Eisenberg, E.; He, G.X.; Kernan, M.; Lee, W.; Prisbe, E.; Rohloff, J.; Sparacino, M. Produrgs of phosphonate nucleotide analogues and methods for selecting and making same. WO02/08241 A2. 2002.
[7] Lee, W.A.; He, G.X.; Eisenberg, E.; Cihlar, T.; Swaminathan, S.; Mulato, A.; Cundy, K.C. *Antimicrob. Agents Chemother*., **2005**, *49*, 1898-1906.
[8] Chapman, H.; Kernan, M.; Prisbe, E.; Rohloff, J.; Sparacino, M.; Terhorst, T.; Yu, R. *Nucleosides Nucleotides Nucleic Acids,* **2001**, *20*, 621-628.
[9] Kellogg, R.M.; Nieuwenhuijzen, J.W.; Pouwer, K.; Vries, T.R.; Broxterman, Q.B.; Grimbergen, R.F.; Kaptein, B.; La Crois, R.M.; de Wever, E.; Zwaagstra, K.; van der Laan, A.C. *Synthesis*, **2003**, *10*, 1626-1638.
[10] Novák, T.; Schindler, J.; Ujj, V.; Czugler, M.; Fogassy, E.; Keglevich, G. *Tetrahedron: Asymmetry,* **2006**, *17*, 2599-2602.
[11] Novák, T.; Ujj, V.; Schindler, J.; Czugler, M.; Kubinyi, M.; Mayer, Z.A.; Fogassy, E.; Keglevich, G. *Tetrahedron: Asymmetry*, **2007**, *18*, 2965-2972.