

Online Research @ Cardiff

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository: <http://orca.cf.ac.uk/113714/>

This is the author's version of a work that was submitted to / accepted for publication.

Citation for final published version:

Pileggi, Elisa, Serpi, Michaela and Pertusati, Fabrizio 2018. Preparation of pyrimidine alkenyl acyclic nucleoside phosphonoamidates. *Current Protocols in Nucleic Acid Chemistry* 74 (13-14), e56. 10.1002/cpnc.56 file

Publishers page: <https://doi.org/10.1002/cpnc.56> <<https://doi.org/10.1002/cpnc.56>>

Please note:

Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher's version if you wish to cite this paper.

This version is being made available in accordance with publisher policies. See <http://orca.cf.ac.uk/policies.html> for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.



Preparation of pyrimidine alkenyl acyclic nucleoside phosphonoamidates

Elisa Pileggi,¹ Michaela Serpi,¹ Fabrizio Pertusati^{1*}

¹ School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Redwood Building, CF103NB, Cardiff, UK

*Corresponding author

Running title

ProTide of alkenyl ANPs

Significance statement

Acyclic nucleoside phosphonates (ANPs) play a key role in the treatment of a variety of infectious diseases including antiviral, antiparasitic, antimicrobial and antituberculous conditions. Unfortunately, ANPs suffer of poor bioavailability due to the presence of an ionized phosphonic acid group. To circumvent this problem several prodrugs strategies have been evaluated. Among them, the ProTide approach have proved to be one of the most powerful technology with the phosphonoamidate tenofovir alafenamide fumarate (TAF, Vemlidy) approved in 2015 for the treatment of HIV and later in 2016 for HBV infections. Given the tremendous importance of phosphonoamidate prodrugs in the antiviral arena and beyond, the two synthetic methodologies to prepare ProTides of alkenyl ANPs, reported in this unit, are of extremely importance for the drug development of this class of compounds.

Abstract

This synthetic protocol describes two strategies for the preparation of pyrimidine alkenyl acyclic nucleoside phosphonoamidates (ANPs) including linear and trisubstituted alkenyl derivatives. For the first procedure the bis trimethylsilyl ester of the parent alkenyl ANPs is the key intermediate that reacts with the desired amino

acid ester and aryl alcohol. For the second procedure, an allyl phosphonoamidate bearing the ProTide promoieties is the key synthon employed as olefin partner for a cross metathesis reaction with an alkylated nucleobase.

Keywords: ProTide, cross metathesis, acyclic nucleoside phosphonate, allylphosphonoamidate, prodrug, antiviral.

INTRODUCTION

This unit presents two different synthetic strategies for the synthesis of alkenyl acyclic nucleoside phosphonoamidate prodrugs. The first methodology (Basic Protocol 1) consists in the preparation of linear (*E*)-but-2-enyl pyrimidine ProTide via the bis trimethylsilyl ester of the parent alkenyl dimethylphosphonate nucleoside, synthesized following the procedure reported in Basic protocol 1 Unit 14.11 (Bessières et al., 2001). This intermediate, obtained by treatment of the parent nucleoside with an excess of trimethylsilyl bromide (TMSBr) is reacted, without purification, with the desired amino acid ester and an excess of phenol in pyridine in the presence of triethylamine, aldrithiol-2 and triphenylphosphine.

The procedure reported in Basic Protocol 2 involves in the first instance, the preparation of the allylphosphonoamidate intermediate obtained in the same way as in the Basic Protocol 1. This derivative is then reacted with alkylated nucleobase via olefin cross metathesis using second generation Hoveyda-Grubbs catalyst to obtain the branched (*E*)-2-methyl-but-2-enyl pyrimidine ProTide.

NOTE: All glassware should be oven dried, and all reactions should be performed under anhydrous conditions.

CAUTION: All reactions must be run in a suitable fume hood with efficient ventilation. Safety glasses and reagent-impermeable protective gloves should be worn at all time.

Compound characterization. Chemical characterizations data are provided for all compounds. ¹H, ³¹P and ¹³C NMR spectra were recorded in a Bruker Avance 500 spectrometer at 500 MHz, 202 MHz and 125 MHz respectively and auto-calibrated to the deuterated solvent reference peak in case of ¹H and ¹³C NMR and 85% H₃PO₄ for

^{31}P NMR experiments. All ^{31}P and ^{13}C NMR spectra were proton-decoupled. Chemical shifts are given in parts per million (ppm) and coupling constants (J) are measured in Hertz (Hz) and related to multiplicities. Analytical High Performance Liquid Chromatography (HPLC) analysis was performed using Varian Prostar system (LC-Workstation-Varian Prostar 335 LC detector). High resolution mass spectrometry was performed on a Bruker Daltonics MicroTof-LC system (atmospheric pressure ionization, electron spray mass spectroscopy) in positive mode.

BASIC PROTOCOL 1

PREPARATION OF (*E*)-BUT-2-ENYL PHOSPHONOAMIDATE PYRIMIDINE

The synthesis of phosphondiamidate prodrugs of ANPs via bis trimethylsilyl ester has been reported by Holy et al (Jansa et al., 2011) and then successfully adapted by us for the synthesis of adefovir and tenofovir phosphonoamidate prodrugs (Pertusati et al., 2014). In this protocol we are reporting a modification of this methodology (Pertusati et al., 2017) for the synthesis of S_{P} and R_{P} isomers of (*E*)- N^1 -(4'-*O*-phenyl-(neopentyloxy-*L*-alanine)-phosphinyl-but-2-enyl)thymine (**3a** and **3b**). (*E*)- N^1 -(4'-dimethoxyphosphinyl-2'-butenyl) thymine (**1**), prepared according to literature procedures (Topalis et al., 2011), is reacted overnight with TMSBr at room temperature to afford intermediate **2**, which after removal of the volatile is used in the next step without further purification. The mixture of diastereoisomers **3a** and **3b** is obtained by stirring **2** with the desired amino acid ester salt and an excess of phenol in presence of aldrithiol-2 and triphenylphosphine at 50 °C for 16 h. Purification by flash chromatography, followed by preparative HPLC, allows the separation of the two diastereoisomers (**3a** and **3b**).

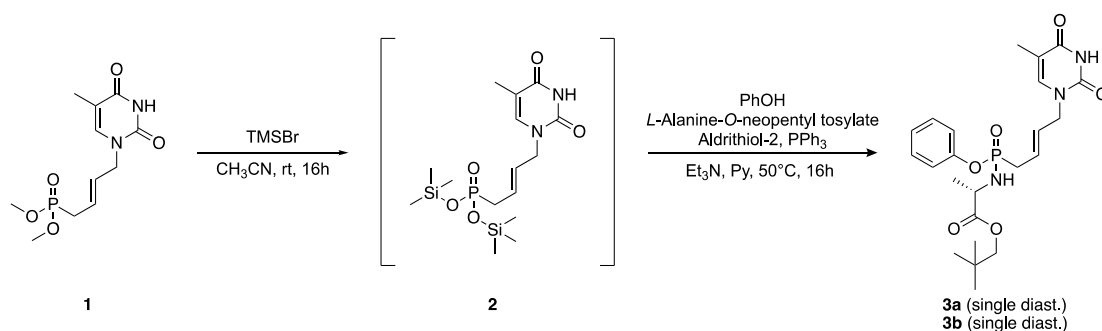


Figure 1.

Synthesis of S_P and R_P isomer of (*E*)- N^1 -(4'-*O*-phenyl-(neopentyloxy-*L*-alanine)-phosphinyl-but-2-enyl)thymine (**3a** and **3b**).

Materials

(*E*)- N^1 -(4'-dimethoxyphosphinyl-2'-butenyl)thymine (see Basic protocol 1 Unit 14.11)

Dry Argon (Ar)

Anhydrous acetonitrile (CH_3CN , Sigma-aldrich)

Bromotrimethylsilane (TMSBr) (Sigma-aldrich)

Anhydrous pyridine (Py, Sigma-aldrich)

L-Alanine neopentyl ester tosylate (see Support protocol unit 15.5.8)

Phenol (PhOH, Sigma-aldrich)

Triethylamine (Et_3N , Sigma-aldrich)

Aldrithiol-2 (Sigma-aldrich)

Triphenylphosphine (PPh_3 , Sigma-aldrich)

Methanol (MeOH, VWR chemicals)

Toluene (VWR chemicals)

Hexane (VWR chemicals)

Dichloromethane (CH_2Cl_2 , VWR chemicals)

Acetonitrile HPLC grade (CH_3CN , VWR chemicals)

Water HPLC grade (VWR chemicals)

Anhydrous MgSO_4 (Sigma-aldrich)

Silica gel (35-70 μ , 60A; Fisher)

Sand (Sigma-Aldrich)

Deuterated methanol (CD_3OD), 99.8% pure (Goss Scientific, CD_3OD is used for NMR characterization).

Deuterated chloroform (CDCl₃), 99.8% pure, (Goss Scientific, CDCl₃ is used for NMR characterization).

Magnetic stirring and heating plate

Oil bath

Condenser/airflux condenser

100-mL round-bottom flask

100 mL separatory funnel

Glass funnel

Analytical TLC plate (aluminum-backed TLC plates, precoated with silica gel 60 F₂₅₄, 0.2 mm; Merck Kieselgel)

Preparative TLC plate (aluminum-backed TLC plates, precoated with silica gel 60 F₂₅₄, 20 x 20, 500-2000 μm; Merck Kieselgel)

Preparative TLC chamber

Rotary evaporator equipped with vacuum pump

Vacuum desiccator

Glass flash chromatography column

Preparative HPLC (Varian Prostar; LC Workstation-Varian Prostar 335 LC detector; Varian Pursuit XRs 5 C18 150 x 21.2 mm reverse phase column)

UV light source

Prepare phosponoamidate **3**

1. Place 0.087 g (0.275 mmol) of (*E*)-*N*¹-(4'-dimethoxyphosphinyl-2'-butenyl)thymine (**1**) in a 100 mL round bottom flask containing a magnetic stir bar and apply an argon atmosphere.
2. Add 10 mL of anhydrous acetonitrile.
3. While stirring, add 0.182 mL (1.38 mmol) of TMSBr at room temperature and continue stirring for 16 h under an argon atmosphere to obtain a brown solution.
4. After this period, evaporate the solvent under reduced pressure on a rotary evaporator without any contact with air to afford **2** as a brown foamy solid crude mixture.
5. Dry the crude residue under vacuum for 1 h (oil pump).
6. Dissolve the solid in 5 mL of anhydrous pyridine under an argon atmosphere.

7. Add 0.091 g (0.275 mmol) of dry *L*-alanine neopentyl ester tosylate, 0.432 g (1.65 mmol) of phenol and 3.4 mL (24.9 mmol) of triethylamine.
8. Place the reaction mixture in an oil bath, heat at 50 °C and stir for 10 min to obtain a yellow solution.
9. In a separate flask, prepare a solution with 0.155 g (1.65 mmol) of aldrithiol-2 and 0.363 g (1.65 mmol) of triphenylphosphine in 5 mL of anhydrous pyridine under an argon atmosphere.
10. Add the aldrithiol/triphenylphosphine solution to the stirring reaction mixture and keep at 50 °C for 4 h.
11. Allow cooling down to room temperature.
12. Evaporate the reaction mixture to dryness using a rotary evaporator under reduced pressure.
13. Add a mixture of methanol, water, toluene and hexane 1:1:1:1 (10/10/10/10 mL) to the residue and transfer the mixture into a 100 mL separatory funnel.
14. Remove the upper layer (hexane/toluene) and wash the lower phase with a mixture of toluene and hexane 1:1 (v/v) three times (3 x 10 mL).
15. Remove the upper layer and extract the lower layer (MeOH/H₂O) three times with CH₂Cl₂ (3 x 20 mL).
16. Combine the CH₂Cl₂ phases, dry over MgSO₄, filter by gravity filtration and then evaporate using a rotary evaporator under reduced pressure.
17. Dissolve the crude product in the minimum amount of CH₂Cl₂ and carefully place the solution on top of a glass flash chromatography column packed with silica gel in CH₂Cl₂. Elute a gradient solution of CH₂Cl₂/MeOH (99:1 to 93:7 v/v).
18. Monitor the fractions by TLC and visualize by UV light, combine the fractions containing the products and evaporate to dryness using a rotary evaporator under reduced pressure.
19. Complete the purification of the products by preparative thin layer chromatography on silica gel.

Dissolve the crude product in the minimum amount of CH₂Cl₂ and apply the sample on a TLC plate about 1.5 cm from the bottom edge and allow the solvent to evaporate. Place the TLC plate in a separation chamber containing 200 mL 95:5 (v/v) CH₂Cl₂/MeOH solution. When the solvent reaches 1.5 cm

from the upper edge remove the TLC plate and allow the solvents to evaporate.

20. Scrape off the backing material of the desired band, visualized using UV light, and extract it with minimal 90:10 (v/v) CH₂Cl₂/MeOH solution. Filter the silica off using a glass filter funnel, wash it using a small amount of 90:10 (v/v) CH₂Cl₂/MeOH solution and concentrate the filtrate using a rotary evaporator under reduced pressure.
21. Dissolve the isomers mixture in MeOH (HPLC grade) and separate them by preparative HPLC (20 ml/min, gradient eluting system CH₃CN/H₂O – from 10/90 to 100/0, 30 min) to afford compounds as foamy solids.
22. Characterise the compound by ³¹P NMR, ¹H NMR, ¹³C NMR and MS.

³¹P NMR spectra documented below were obtained with proton decoupling

(E)-N¹-(4'-O-phenyl-(neopentyloxy-L-alanine)-phosphinyl-but-2-enyl)thymine (3a and 3b).

3a: Yield 0.021 g (16%). *R_f* = 0.32 (CH₂Cl₂/MeOH - 95:5).

³¹P-NMR (202 MHz, CD₃OD) δ_P 29.23.

¹H-NMR (500 MHz, CD₃OD) δ_H 7.41 (1H, d, *J* = 1.1 Hz, H-6), 7.38-7.34 (2H, m, CH-Ph), 7.21-7.18 (3H, m, CH-Ph), 5.83-5.79 (2H, m, NCH₂CH= and =CHCH₂P), 4.36 (2H, t, *J* = 4.8 Hz, CH₂N), 4.05-3.99 (1H, m, CHCH₃), 3.87, 3.77 (2H, AB, *J*_{AB} = 10.5 Hz, CH₂C(CH₃)₃), 2.87 (2H, ddd, *J* = 6.4 and 4.6 Hz, ²*J*_{PH} = 20.5 Hz, CH₂P), 1.87 (3H, d, *J* = 1.2 Hz, CH₃), 1.26 (3H, d, *J* = 7.3 Hz, CHCH₃), 0.96 (9H, s, C(CH₃)₃)

¹³C-NMR (125 MHz, CDCl₃) δ_C 174.05 (d, ³*J*_{PC} = 4.9 Hz, COO), 163.9 (C-4), 150.67 (C-2), 150.38 (d ²*J*_{CP} = 9.1 Hz, C-*ipso* Ph), 139.74 (CH-6), 129.77 (CH-Ph), 129.47 (d, ²*J*_{PC} = 10.7 Hz, =CHCH₂P), 129.21 (d, ³*J*_{PC} = 14.7 Hz, NCH₂CH=), 124.64 (CH-Ph), 120.68 (d, ³*J*_{PC} = 4.5 Hz, CH-Ph), 111.00 (C-5), 74.71 (CH₂C(CH₃)₃), 49.73 (CHCH₃), 49.49 (CH₂N), 32.44 (d, ¹*J*_{PC} = 127.2 Hz, CH₂P), 29.69 (C(CH₃)₃), 26.32 (C(CH₃)₃), 21.53 (d ³*J*_{PC} = 6.3 Hz, CHCH₃), 10.87 (CH₃).

HPLC: Reverse phase HPLC eluting with gradient method CH₃CN/H₂O from 10/90 to 100/0 in 30 min, 1ml/min, λ = 254 nm and 263 nm, showed one peak with *t_R* 16.06 min.

MS(ESI+) *m/z* = 500.2 [*M* + Na⁺] (100%).

3b: Yield 0.013 g (10%). $R_f = 0.29$ ($\text{CH}_2\text{Cl}_2/\text{MeOH} - 95:5$).

^{31}P -NMR (202 MHz, CDCl_3) δ_P 28.51.

^1H -NMR (500 MHz, CD_3OD) δ_H 7.37 (1H, d, $J=1.1$ Hz, H-6), 7.35-7.32 (2H, m, CH-Ph), 7.22-7.17 (3H, m, CH-Ph), 5.79-5.76 (2H, m, $\text{NCH}_2\text{CH=}$ and $=\text{CHCH}_2\text{P}$), 4.35 (2H, m, CH_2N), 3.91, 3.82 (2H, AB, $J_{AB} = 10.5$ Hz, $\text{CH}_2\text{C}(\text{CH}_3)_3$), 3.67-3.60 (1H, m, CHCH_3), 2.86-2.80 (2H, m, CH_2P), 1.87 (3H, s, CH_3), 1.38 (3H, d, $J = 7.2$ Hz, CHCH_3), 0.99 (9H, s, $\text{C}(\text{CH}_3)_3$).

^{13}C -NMR (125 MHz, CDCl_3) δ_C 173.79 (d, $^3J_{PC} = 4.9$ Hz, COO), 164.01 (C-4), 150.72 (C-2), 150.43 (d, $^2J_{CP} = 7.84$ Hz, C-*ipso* Ph), 139.66 (CH-6), 129.77 (CH-Ph), 129.24 (d, $^2J_{PC} = 14.7$ Hz, $=\text{CHCH}_2\text{P}$), 125.16 (d, $^2J_{PC} = 11.0$ Hz, $\text{NCH}_2\text{CH=}$), 124.86 (CH-Ph), 120.47 (d, $^3J_{PC} = 4.9$ Hz, CH-Ph), 111.05 (C-5), 74.78 ($\text{CH}_2\text{C}(\text{CH}_3)_3$), 49.62 (CHCH_3), 49.23 (CH_2N), 32.79 (d, $^1J_{PC} = 130.8$ Hz, CH_2P), 29.70 ($\text{C}(\text{CH}_3)_3$), 29.36 ($\text{C}(\text{CH}_3)_3$), 21.79 (d, $^3J_{PC} = 2.5$ Hz, CHCH_3), 12.30 (CH_3).

HPLC: Reverse phase HPLC eluting with gradient method $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ from 10/90 to 100/0 in 30 min, 1ml/min, $\lambda = 254$ nm and 263 nm, showed one peak with t_R 16.14 min.

MS(ESI+) $m/z = 500.2$ [$M + \text{Na}^+$] (100%).

BASIC PROTOCOL 2

PREPARATION OF (*E*)-2-METHYL-BUT-2-ENYL PHOSPHONOAMIDATE PYRIMIDINE

This protocol describes the preparation of phosphonoamidate prodrugs of trisubstituted alkenyl acyclonucleoside using cross-metathesis reaction. Olefin cross-metathesis methodology has been used for the direct synthesis of a vast array of unsaturated ANPs analogues including bis-POM, bis-POC, and alkoxyesters prodrugs (Hamada et al., 2013; Pradère et al., 2011). Only very recent application of such procedure for the preparation of ProTides has been reported (Bessières et al., 2018). Despite some similarities, the synthetic strategy we are reporting here differs from that published by Agrofoglio *et al.*

This methodology involves first the synthesis of the aryloxy allylphosphonoamidate **6** as the key synthon. Briefly, the commercial dimethyl allylphosphonate **4** is converted into the corresponding silyl ester **5** in presence of an excess of TMSBr and 2,6-lutidine as acid scavenger. After removal of the volatile **5** is used without further

purification and treated with the amino acid ester hydrochloride and an excess of aryl alcohol in presence of aldrithiol-2 and triphenylphosphine at 50°C for 16 h to obtain the desired allylphosphonoamidate **6**.

The second olefin partner for the cross metathesis reaction (**9** and **10**) was synthesized by *N*¹-substitution using 3-bromo-2-methylpropene (Bessieres et al., 2016).

As illustrated in Figure 2, the allylphosphonoamidate intermediate **6** is then sonicated with 2-methylallyl pyrimidines **9** and **10** in presence of Hoveyda-Grubbs second generation catalyst in CH₂Cl₂ at reflux for 24 h, to obtain the final ProTides **11** and **12**.

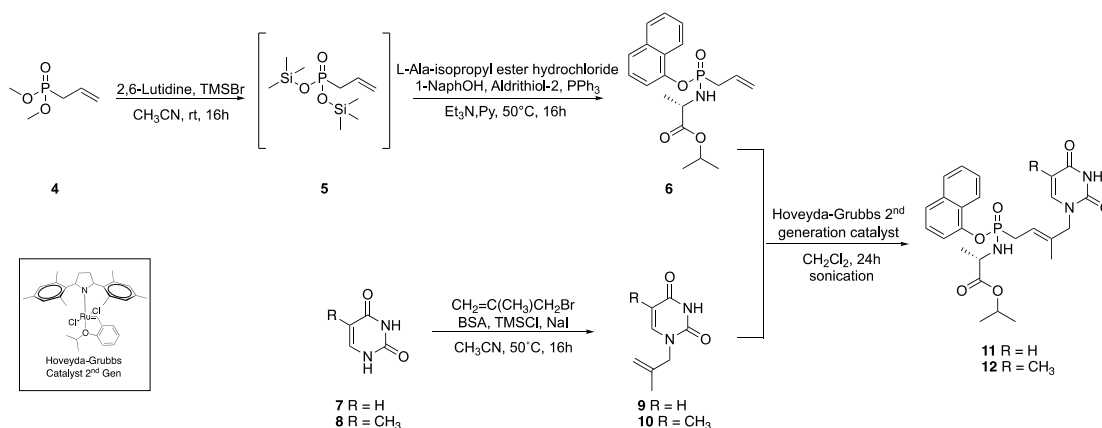


Figure 2. Preparation of (*E*)-*N*¹-(4'-*O*-(1-Naphthyl)-(isopropoxy-*L*-Alanine)-phosphinyl-2'-methyl-but-2'-enyl) pyrimidines (**11** and **12**) via cross metathesis using *O*-(1-naphthyl)-(isopropoxy-*L*-Alanine)-allylphosphonate (**6**) as key synthon.

Materials

Dimethyl allylphosphonate (**4**) (Alfa Aesar)

2,6-Lutidine (Sigma-aldrich)

Dry Argon (Ar)

Anhydrous acetonitrile (CH₃CN, Sigma-aldrich)

Bromotrimethylsilane (TMSBr) (Sigma-aldrich)

Anhydrous pyridine (Py, Sigma-aldrich)

L-Alanine isopropyl ester hydrochloride (Sigma-aldrich)

1-Naphthol (1-NaphOH, Sigma-aldrich)

Triethylamine (Et₃N, Sigma-aldrich)

Aldrithiol-2 (Sigma-aldrich)
Triphenylphosphine (PPh₃, Sigma-aldrich)
Ethyl acetate (EtOAc, VWR chemicals)
Hexane (VWR chemicals)
Methanol (MeOH, VWR chemicals)
Uracil (**7**) (Sigma-aldrich)
Thymine (**8**) (Sigma-aldrich)
N,O-Bis(trimethylsilyl)acetamide (BSA, Sigma-aldrich)
3-Bromo-2-methylpropene (Sigma-aldrich)
Sodium iodide (NaI, Sigma-aldrich)
Chlorotrimethylsilane (TMSCl, Sigma-aldrich)
Anhydrous dichloromethane (CH₂Cl₂, Sigma-aldrich)
Hoveyda-Grubbs Catalys 2nd Generation (Sigma-aldrich)
Dichloromethane (CH₂Cl₂, VWR chemicals)
2-propanol (VWR chemicals)
Acetonitrile HPLC grade (CH₃CN, VWR chemicals)
Water HPLC grade (VWR chemicals)
Anhydrous MgSO₄ (Sigma-aldrich)
Magnetic stirring and heating plate
Oil bath
Condenser/airflux condenser
50-, 100-mL round-bottom flask
Analytical TLC plate (aluminum-backed TLC plates, precoated with silica gel 60 F₂₅₄,
0.2mm; Merck Kieselgel)
250 mL separating funnel
Glass funnel
Filter paper
Rotary evaporator equipped with vacuum pump
Vacuum desiccator
Fisherbrand 11203 Ultrasonic Cleaner
Automatic Flash Chromatography (Biotage Isolera One)
Preparative HPLC (Varian Prostar; LC Workstation-Varian Prostar 335 LC detector;
Varian Pursuit XRs 5 C18 150 x 21.2 mm reverse phase column)
UV light source

Deuterated methanol (CD₃OD), 99.8% pure (Goss Scientific, CD₃OD is used for NMR characterization).

Preparation of allylphosphonoamidate derivative

1. Place 0.500 g (3.3 mmol) of **4** in a 100 mL round bottom flask containing a magnetic stir bar and apply an argon atmosphere.
2. Add 25 mL anhydrous acetonitrile and 1.55 mL (13.3 mmol) of 2,6-lutidine.
3. While stirring add 2.20 mL (16.6 mmol) of TMSBr at room temperature and continue stirring for 16 h under an argon atmosphere to obtain a brown solution.
4. After this period, evaporate the solvent under reduced pressure on a rotary evaporator without any contact with air to afford **5** as a brown foamy solid crude mixture.
5. Dry the crude residue under vacuum for 1 h (oil pump).
6. Dissolve the solid in 10 mL of anhydrous pyridine under an argon atmosphere.
7. Add 0.558 g (3.3 mmol) of dry *L*-alanine isopropyl ester hydrochloride, 2.88 g (19.9 mmol) of dry 1-naphthol and 6.9 mL (49.9 mmol) of triethylamine.
8. Place the reaction mixture in an oil bath, heat at 50 °C and stir for 10 min to obtain a yellow solution.
9. Prepare a solution with 4.40 g (19.9 mmol) of aldrithiol-2 and 5.24 g (19.9 mmol) of triphenylphosphine in 10 mL of anhydrous pyridine under an argon atmosphere.
10. Add the aforementioned solution to the stirring reaction mixture while stirring and keep at 50 °C for 16 h.
11. Allow cooling down to room temperature.
12. Monitor the reaction by TLC using 4:6 (v/v) EtOAc /hexane and visualize by UV light (**6** R_f = 0.58).
13. Evaporate the reaction mixture to dryness using a rotary evaporator under reduced pressure.
14. Purify the residue by Biotage Isolera One
Dissolve the crude product in the minimum amount of CH₂Cl₂/ solution and carefully place into a 100 g SNAP cartridge ULTRA. Purify using 100 ml/min gradient eluent system EtOAc/hexane 10% 1CV, 10-100% 12CV, 100% 2CV.

15. Monitor the fractions by TLC and visualize by UV light, combine the fractions containing the pure product and evaporate to dryness using a rotary evaporator under reduced pressure to afford compound **6** as yellow oil.
16. Characterise the compounds by ^{31}P NMR, ^1H NMR and ^{13}C NMR.

^{31}P NMR spectra documented below were obtained with proton decoupling.

*O-(1-naphthyl)-(isopropoxy-L-Alanine)-allylphosphonate (**6**). Yield 0.940 g (79%).
*R_f: 0.58 (EtOAc/Hexane - 4:6).**

^{31}P NMR (202 MHz, CD_3OD) δ_{P} : 30.01, 29.43.

^1H NMR (500 MHz, CD_3OD) δ_{H} : 8.19 (d, $J = 7.2$ Hz, 1H, ArH), 7.89 (d, $J = 7.9$ Hz, 1H, ArH), 7.71-7.69 (m, 1H, ArH), 7.58-7.40 (m, 4H, ArH), 6.07-5.91 (m, 1H, CH=), 5.38-5.28 (m, 2H, $\text{CH}_2=$), 5.95-4.82 (m, 1H, $\text{CH}(\text{CH}_3)_2$), 3.99-3.97 (m, 1H, CHCH_3 L-Ala), 3.03-2.93 (m, 2H, CH_2P), 1.25 (d, $J = 7.8$ Hz, 1.5H, CHCH_3 L-Ala), 1.21-1.10 (m, 7.5H, CHCH_3 L-Ala, $\text{CH}(\text{CH}_3)_2$).

^{13}C NMR (125 MHz, CD_3OD) δ_{C} : 173.5 (d, $^3J_{\text{C-P}} = 4.2$ Hz, C=O, ester), 173.1 (d, $^3J_{\text{C-P}} = 4.2$ Hz, C=O, ester), 146.4 (d, $^2J_{\text{C-P}} = 8.5$ Hz, C-O, Ph), 146.3 (d, $^2J_{\text{C-P}} = 8.5$ Hz, C-O, Ph), 134.9 (C-Ar), 127.4 ($^2J_{\text{C-P}} = 9.3$ Hz, CH=), 123.3 ($^2J_{\text{C-P}} = 10.9$ Hz, CH=), 126.9 (d, $^3J_{\text{C-P}} = 5.6$ Hz C-Ar), 126.8 (d, $^3J_{\text{C-P}} = 4.9$ Hz C-Ar), 126.3 (CH-Ar), 125.95 (CH-Ar), 125.90 (CH-Ar), 125.1 (CH-Ar), 125.0 (CH-Ar), 124.3 (CH-Ar), 124.2 (CH-Ar), 121.6 (CH-Ar), 121.4 (CH-Ar), 119.7 (d, $^3J_{\text{C-P}} = 14.2$ Hz $\text{CH}_2=$), 119.6 (d, $^3J_{\text{C-P}} = 13.8$ Hz $\text{CH}_2=$), 115.4 (d, $^3J_{\text{C-P}} = 4.1$ Hz CH-Ar), 115.2 (d, $^3J_{\text{C-P}} = 3.4$ Hz CH-Ar), 68.6 ($\text{CH}(\text{CH}_3)_2$), 68.5 ($\text{CH}(\text{CH}_3)_2$), 49.6 (CHCH_3 L-Ala), 49.4 (CHCH_3 L-Ala), 33.7 (d, $^1J_{\text{C-P}} = 129.0$ Hz CH_2P), 33.5 (d, $^1J_{\text{C-P}} = 129.6$ Hz CH_2P), 20.5 ($\text{CH}(\text{CH}_3)_2$), 20.4 ($\text{CH}(\text{CH}_3)_2$), 20.3 ($\text{CH}(\text{CH}_3)_2$), 19.7 (d, $^3J_{\text{C-P}} = 5.4$ Hz, CHCH_3 L-Ala), 19.1 (d, $^3J_{\text{C-P}} = 5.4$ Hz, CHCH_3 L-Ala).

Prepare N^1 -2'-methylallyl-pyrimidines

17. Dissolve 1.5 g of nucleobase (13.3 mmol of **7**, 11.8 mmol of **8**) in 25 mL of anhydrous acetonitrile in a 100 mL round bottom flask containing a magnetic stir bar and apply an argon atmosphere.
18. While stirring add *N,O*-Bis(trimethylsilyl)acetamide (BSA)
 For **9**: 8.18 mL (33.4 mmol) of BSA

- For **10**: 7.20 mL (29.7 mmol) of BSA
- Place the reaction mixture in an oil bath, heat at reflux temperature and stir until a clear solution is observed (usually 10 min).
 - Add under an argon atmosphere 3-bromo-2-methylpropene, NaI and chlorotrimethylsilane
For **9**: 2.40 mL (23.7 mmol) of 3-bromo-2-methylpropene, 1.96 g (13.1 mmol) of NaI, 1.51 mL (11.8 mmol) of chlorotrimethylsilane
For **10**: 2.70 mL (26.7 mmol) of 3-bromo-2-methylpropene, 2.21 g (14.7 mmol) of NaI, 1.70 mL (13.3 mmol) of chlorotrimethylsilane
 - Stir under reflux and under an argon atmosphere for 16 h.
 - Monitor the reaction by TLC and visualize with UV light using 7:3 (v/v) EtOAc/hexane and visualize by UV light (**9** R_f : 0.25; **10** R_f : 0.45).
 - Evaporate the solvent to dryness under reduced pressure on a rotary evaporator.
 - Dissolve the residue in 50 mL of EtOAc and wash the mixture in sequence with 20 mL of NaHCO₃ aqueous saturated solution, 20 mL of Na₂SO₄ aqueous saturated solution and 20 mL of H₂O using a 250 mL separating funnel.
 - Dry the organic phase over anhydrous MgSO₄, filter by gravity filtration and evaporate the solution to dryness using a rotary evaporator under reduced pressure.
 - Purify the residue by Biotage Isolera One
Dissolve the crude product in the minimum amount of CH₂Cl₂ and carefully place into a 50 g SNAP cartridge ULTRA. Purify using a 100 ml/min gradient eluent system EtOAc/hexane 17% 1CV, 17-100% 10CV, 100% 3CV.
 - Monitor the fractions by TLC and visualize by UV light, combine the fractions containing the pure product and evaporate to dryness using a rotary evaporator under reduced pressure to afford compounds **9** and **10** as pale-yellow solids.
 - Characterise the compounds by ¹H NMR.

*N*¹-2'-methylallyl-uracil (**9**). Yield 1.2 g (51%). R_f : 0.25 (EtOAc/Hexane - 7:3).

¹H NMR (500 MHz, CD₃OD) δ_H : 7.50 (d, J = 7.8 Hz, 1H, H-6), 5.71 (d, J = 7.8 Hz, 1H, H-5), 4.98 (s, 1H, CH₂=), 4.81 (s, 1H, CH₂=), 4.33 (s, 2H, CH₂-N), 1.76 (s, 3H, CH₃, alkene).

*N*¹-2'-methylallyl-thymine (**10**). Yield 2.1 g (98%). *R*_f: 0.45 (EtOAc/Hexane - 7:3).
¹H NMR (500 MHz, CD₃OD) δ_H: 7.34 (s, 1H, H-6), 4.98 (s, 1H, CH₂=), 4.80 (s, 1H, CH₂=), 4.30 (s, 2H, CH₂-N), 1.89 (s, 3H, CH₃, base), 1.76 (s, 3H, CH₃, alkene).

Olefin cross metathesis

23. Dissolve 0.150 g of the allylphosphonoamidate **6** (415.0 μmol) in 10 mL of anhydrous dichloromethane in a 50 mL round bottom flask containing a magnetic stir bar and apply an argon atmosphere.
24. Add *N*¹-2'-methylallyl-pyrimidine
For **11**: 0.137 g (830.1 μmol) of **9**.
For **12**: 0.150 g (830.1 μmol) of **10**.
25. Add 0.039 g (62.2 μmol, 15 mol%) of Hoveyda-Grubbs second generation catalyst.
Note: The total amount of second generation Hoveyda-Grubbs catalyst is introduced in three equal portions of 5 mol% at t = 0, 2, 4 h over the course of the reaction.
26. Sonicate the reaction mixture at 37 MHz for 24 h.
27. Monitor the reaction by TLC using 95:5 (v/v) CH₂Cl₂/MeOH and visualize by UV light (**11** *R*_f: 0.22; **12** *R*_f: 0.24).
28. Evaporate the reaction mixture to dryness using a rotary evaporator.
Dissolve the crude product in the minimum amount of 99:1 (v/v) CH₂Cl₂/MeOH solution and carefully place into a 50 g SNAP cartridge ULTRA. Purify using a 100 ml/min gradient eluent system MeOH/CH₂Cl₂ 1% 1CV, 1-10% 12CV, 10% 2CV.
29. Monitor the fractions by TLC and visualize by UV light, combine the fractions containing a mixture of *E* and *Z* isomers of the compound and evaporate to dryness using a rotary evaporator under reduced pressure.
30. Separate the two isomers by reverse phase chromatography.
For **11**: Dissolve the product in MeOH (HPLC gradient) and purify by preparative HPLC (20 ml/min, isocratic eluting system CH₃CN/H₂O - 35/65, 30 min.) to afford the compound as pale yellow foamy solid.
For **12**: Dissolve the product in MeOH and carefully place into a 60 g SNAP cartridge KP-C18-HS, and purify by reverse phase flash chromatography

using 100 ml/min, isocratic eluent system CH₃CN/H₂O 40/60 12CV, to afford the compound as pale yellow foamy solid.

31. Characterise the compounds by ³¹P NMR, ¹H NMR and ¹³C NMR, HRMS and HPLC.

³¹P NMR spectra documented below were obtained with proton decoupling.

(E)-N¹-(4'-O-(1-naphthyl)-(isopropoxy-L-Alanine)-phosphinyl-2'-methyl-but-2'-enyl)uracil (11). Yield 0.028 g (14%). R_f = 0.22 (CH₂Cl₂/MeOH - 95:5).

³¹P NMR (202 MHz, CD₃OD) δ_P: 30.28, 29.49.

¹H NMR (500 MHz, CD₃OD) δ_H: 8.14-8.13 (m, 1H, ArH), 7.88-7.84 (m, 1H, ArH), 7.70-7.67 (m, 1H, ArH), 7.58-7.49 (m, 3H, ArH), 7.44-7.38 (m, 2H, H-6, ArH), 5.61-5.57 (m, 1.5H, CH=, H-5), 5.51-5.47 (m, 0.5H, CH=), 4.93 (sept, J = 6.5 Hz, 0.5H, CH(CH₃)₂), 4.88-4.84 (m, 0.5H, CH(CH₃)₂), 4.33-4.25 (m, 2H, CH₂-N), 4.04-3.97 (m, 1H, CHCH₃ L-Ala), 3.08-2.90 (m, 2H, CH₂P), 1.65 (bs, 3H, CH₃, alkene), 1.27 (d, J = 7.0 Hz, 1.5H, CHCH₃ L-Ala), 1.20 (d, J = 6.2 Hz, 1.5H, CH(CH₃)₂), 1.19 (d, J = 6.2 Hz, 1.5H, CH(CH₃)₂), 1.17 (d, J = 6.9 Hz, 1.5H, CHCH₃ L-Ala), 1.12 (d, J = 6.2 Hz, 1.5H, CH(CH₃)₂), 1.15 (d, J = 6.2 Hz, 1.5H, CH(CH₃)₂).

¹³C NMR (125 MHz, CD₃OD) δ_C: 173.6 (d, ³J_{C-P} = 4.3 Hz, C=O, ester), 173.2 (d, ³J_{C-P} = 4.1 Hz, C=O, ester), 165.17 (C-4), 165.15 (C-4), 151.5 (C-2), 151.4 (C-2), 146.5 (d, ²J_{C-P} = 9.7 Hz, C-O, Ph), 146.3 (d, ²J_{C-P} = 9.7 Hz, C-O, Ph), 145.2 (C-6), 145.1 (C-6), 135.2 (d, ³J_{C-P} = 14.5 Hz, C=), 135.4 (d, ³J_{C-P} = 14.5 Hz, C=), 134.9 (C-Ar), 127.5 (CH-Ar), 127.4 (CH-Ar), 126.8 (d, ³J_{C-P} = 4.9 Hz C-Ar), 126.6 (d, ³J_{C-P} = 5.1 Hz C-Ar), 126.3 (CH-Ar), 126.1 (CH-Ar), 125.2 (CH-Ar), 125.1 (CH-Ar), 124.3 (CH-Ar), 124.2 (CH-Ar), 121.5 (CH-Ar), 121.3 (CH-Ar), 117.4 (²J_{C-P} = 11.0 Hz, CH=), 116.9 (²J_{C-P} = 11.0 Hz, CH=), 115.4 (d, ³J_{C-P} = 3.8 Hz CH-Ar), 115.1 (d, ³J_{C-P} = 3.8 Hz CH-Ar), 101.2 (C-5), 68.69 (CH(CH₃)₂), 68.66 (CH(CH₃)₂), 53.7 (d, ⁴J_{C-P} = 2.3 Hz, CH₂-N), 53.5 (d, ⁴J_{C-P} = 2.3 Hz, CH₂-N), 49.7 (CHCH₃ L-Ala), 49.5 (CHCH₃ L-Ala), 28.3 (d, ¹J_{C-P} = 128.9 Hz CH₂P), 28.1 (d, ¹J_{C-P} = 129.8 Hz CH₂P), 20.6 (CH(CH₃)₂), 20.56 (CH(CH₃)₂), 20.52 (CH(CH₃)₂), 20.4 (CH(CH₃)₂), 19.8 (d, ³J_{C-P} = 5.8 Hz, CHCH₃ L-Ala), 19.1 (d, ³J_{C-P} = 5.5 Hz, CHCH₃ L-Ala), 13.3 (d, ⁴J_{C-P} = 2.4 Hz, CH₃, alkene), 13.2 (d, ⁴J_{C-P} = 2.2 Hz, CH₃, alkene).

HPLC: Reverse phase HPLC eluting with gradient method CH₃CN/H₂O from 10/90 to 100/0 in 30 minutes, 1ml/min, λ = 254 nm and 263 nm, showed one peak with t_R 15.57 min.

HRMS (ESI): m/z [M+Na]⁺ calcd for C₂₅H₃₀N₃O₆P: 522.1770, found: 522.1764.

(E)-N¹-(4'-O-(1-naphthyl)-(isopropoxy-L-Alanine)-phosphinyl-2'-methyl-but-2'-enyl)thymine (**12**). Yield 0.075 g (36%). R_f = 0.24 (CH₂Cl₂/MeOH - 95:5).

³¹P NMR (202 MHz, CD₃OD) δ_P: 30.32, 29.54.

¹H NMR (500 MHz, CD₃OD) δ_H: 8.13-8.12 (m, 1H, ArH), 7.89-7.87 (m, 1H, ArH), 7.71-7.68 (m, 1H, ArH), 7.57-7.48 (m, 3H, ArH), 7.45-7.39 (m, 1H, ArH), 7.27 (s, 0.5H, H-6), 7.26 (s, 0.5H, H-6), 5.61-5.56 (m, 1H, CH=), 4.93-4.84 (m, 1H, CH(CH₃)₂), 4.32-4.26 (m, 2H, CH₂-N), 4.01-3.91 (m, 1H, CHCH₃ L-Ala), 3.08-2.86 (m, 2H, CH₂P), 1.75 (s, 3H, CH₃, base), 1.67 (s, 3H, CH₃, alkene), 1.27 (d, J = 6.9 Hz, 1.5H, CHCH₃ L-Ala), 1.20-1.16 (m, 4.5H, CHCH₃ L-Ala, CH(CH₃)₂), 1.13-1.10 (m, 3H, CH(CH₃)₂).

¹³C NMR (125 MHz, CD₃OD) δ_C: 173.5 (d, ³J_{C-P} = 3.9 Hz, C=O, ester), 173.1 (d, ³J_{C-P} = 3.5 Hz, C=O, ester), 165.34 (C-4), 165.32 (C-4), 151.69 (C-2), 151.61 (C-2), 146.5 (d, ²J_{C-P} = 9.5 Hz, C-O, Ph), 146.3 (d, ²J_{C-P} = 9.5 Hz, C-O, Ph), 140.94 (C-6), 140.92 (C-6), 135.5 (d, ³J_{C-P} = 14.3 Hz, C=), 135.1 (d, ³J_{C-P} = 14.7 Hz, C=), 134.9 (C-Ar), 127.48 (CH-Ar), 127.46 (CH-Ar), 126.7 (d, ³J_{C-P} = 5.1 Hz C-Ar), 126.6 (d, ³J_{C-P} = 5.1 Hz C-Ar), 126.3 (CH-Ar), 126.0 (CH-Ar), 125.16 (CH-Ar), 125.11 (CH-Ar), 124.3 (CH-Ar), 124.2 (CH-Ar), 121.4 (CH-Ar), 121.3 (CH-Ar), 117.1 (²J_{C-P} = 11.1 Hz, CH=), 116.6 (²J_{C-P} = 10.7 Hz, CH=), 115.3 (d, ³J_{C-P} = 3.5 Hz CH-Ar), 115.1 (d, ³J_{C-P} = 3.9 Hz CH-Ar), 110.1 (C-5), 68.69 (CH(CH₃)₂), 68.65 (CH(CH₃)₂), 53.5 (d, ⁴J_{C-P} = 2.7 Hz, CH₂-N), 53.2 (d, ⁴J_{C-P} = 2.3 Hz, CH₂-N), 49.7 (CHCH₃ L-Ala), 49.5 (CHCH₃ L-Ala), 28.3 (d, ¹J_{C-P} = 129.0 Hz CH₂P), 28.1 (d, ¹J_{C-P} = 130.0 Hz CH₂P), 20.55 (CH(CH₃)₂), 20.54 (CH(CH₃)₂), 20.48 (CH(CH₃)₂), 20.40 (CH(CH₃)₂), 19.8 (d, ³J_{C-P} = 5.5 Hz, CHCH₃ L-Ala), 19.1 (d, ³J_{C-P} = 5.9 Hz, CHCH₃ L-Ala), 13.3 (d, ⁴J_{C-P} = 2.3 Hz, CH₃, alkene), 13.2 (d, ⁴J_{C-P} = 2.7 Hz, CH₃, alkene), 10.8 (CH₃, base).

HPLC: Reverse phase HPLC eluting with gradient method CH₃CN/H₂O from 10/90 to 100/0 in 30 minutes, 1ml/min, λ = 254 nm and 263 nm, showed one peak with t_R 16.26 min.

HRMS (ESI): m/z [M+Na]⁺ calcd for C₂₆H₃₂N₃O₆P: 536.1926, found: 536.1921.

COMMENTARY

Background Information

In the last years, the ProTide approach, pioneered by Chris McGuigan's group, has displayed a great deal of success in the development of nucleoside-based antivirals and anticancer drugs. Sofosbuvir (Nakamura et al., 2016) and TAF (Abdul Basit et al., 2017; Ray et al., 2016) on the market for viral infections, and Acelerin (Slusarczyk et al., 2014) and NUC 3373 (McGuigan et al., 2011) in clinical trials (Phase III and Phase I) for patients with advanced solid tumours, are the undeniable proofs of how powerful is this technology.

While there are several efficient procedures to synthesize phosphoroamidate nucleosides, the phosphonoamidate cognate class especially of acyclic nucleoside phosphonates (ANPs) lacks of such plethora of synthetic methodologies (Pradere et al., 2014).

We were able to synthesize prodrugs of adefovir and tenofovir in moderate yield (Pertusati et al., 2014) by adaption of the one-pot procedure for preparing phosphonodiamidate, reported by Jansa et al 2011 (Jansa et al., 2011). Unfortunately, when these conditions were applied on the alkenyl-pyrimidine substrate, only traces of the desired phosphonoamidate product were detected with the phosphonodiamidate being the major product. Increasing the equivalents of the aryl-alcohol (6 equivalents) with respect to the amino acid (1 equivalent) proved necessary to obtain the desired phosphonoamidate in moderate yield as reported in Basic Protocol 1 for the preparation of linear (*E*)-4-phosphonoamidate-but-2'-en-1'-yl pyrimidine (Pertusati et al., 2017). However, we discovered that this methodology suffers from the limitation that only linear olefin must be employed, as with trisubstituted alkenyl derivatives no formation of the desired ProTide was observed in our hand. This finding prompted us to investigate and then develop the methodology reported in Basic Protocol 2 using a cross-metathesis reaction for the direct synthesis of branched unsaturated ANP phosphonoamidates. At the time we started this investigation, no application of such procedure for the synthesis of ProTides was yet reported. However, recently, a paper reporting on the use of the cross metathesis for the synthesis of ProTide derivatives of linear (*E*)-but-2-enyl nucleoside scaffold, was published by Agrofoglio et al. (Bessières et al., 2018)

Both our and Agrofoglio's procedures involves the preparation of the aryl allylphosphonoamidate intermediate to be then reacted with the alkylated nucleobase in the CM reaction. However our synthetic pathway using the one-pot procedure reported by Holi (Jansa et al., 2011) proves to be a shorter and efficient approach for the synthesis of the allylphosphonoamidate synthon. Moreover the cross metathesis conditions appear to be different. Dichloromethane was the solvent of choice in our case with branched alkenyl nucleosides whereas for Agrofoglio linear olefin only water was effective.

Critical Parameters and Troubleshooting

The successful preparation of the silylated intermediates **2** and **5** in both Basic Protocol 1 and 2 is critical for the outcome of the two synthetic procedures. In particular, timing (16 h) is a crucial parameter for this step as in case of too short reaction time only partial dealkylation can be observed. The silyl esters **2** and **5** are air and moisture sensitive compounds and therefore must be kept at all the time under strictly dry atmosphere.

For Basic Protocol 2, the presence of 2,6-lutidine revealed to be essential for the first step as only degradation of the silyl ester intermediate **5** is detected when this acid scavenger is not present.

For the preparation of the aryl phosphonoamidate moiety (ProTide approach) in both Basic Protocols 1 and 2, the aryl-alcohol to amino acid ester ratio needs to be 6 to 1 in favor of the aryloxy reagent to reduce the formation of the bisamidate derivative as byproduct. For the cross metathesis reaction, the sequential catalyst loading is a crucial parameter to afford the desired transalkylidenation product in good yield.

The synthetic procedures described in this unit are intended for use only by persons with prior training in experimental organic chemistry and thus with knowledge of the common chemical laboratory techniques, such as extraction, solvent evaporation, column chromatography, TLC and HPLC. Characterization of the products demands knowledge of monodimensional (^1H , ^{13}C and ^{31}P) and bidimensional (COSY, HSQC, HMBC and NOESY) NMR experiments, as well as of mass spectroscopy. Careful attention to details of basic organic synthesis methodologies is required. General laboratory safety is also of primary concern when hazardous materials are involved. Strict adherence to the reported procedures is therefore highly recommended.

Understanding Result

The approaches applied in both Basic protocol 1 and 2 can be applied to prepare numerous alkenyl acyclic pyrimidine ProTide derivatives with different aryloxy and amino acid ester moieties. Moreover, both the protocols can be adapted to obtain the bis-amidate derivatives when in the first step of the two methodologies only the amino acid ester is employed.

The cross metathesis reaction can be significantly influenced by the length of the acyclic side chain. When the CM procedure reported in Basic Protocol 2 is employed for the preparation of aryl vinylphosphonoamidate derivatives, no formation of the final product is observed.

Time Considerations

According to Basic Protocol 1, two weeks are required for the nucleoside preparation including purification and characterization of the final ProTide. In case of Basic Protocol 2 only one week is needed for the synthesis, characterization of the phosphonoamidate prodrug including the preparation of the two olefins and the cross metathesis reaction.

Reference

- Abdul Basit, S., Dawood, A., Ryan, J., & Gish, R. (2017). Tenofovir alafenamide for the treatment of chronic hepatitis B virus infection. *Expert Rev Clin Pharmacol*, 1-10. doi:10.1080/17512433.2017.1323633
- Bessièrès, M., De Schutter, C., Roy, V., & Agofoglio, L. A. (2001). Olefin Cross-Metathesis for the Synthesis of Alkenyl Acyclonucleoside Phosphonates *Current Protocols in Nucleic Acid Chemistry*: John Wiley & Sons, Inc.
- Bessièrès, M., Hervin, V., Roy, V., Chartier, A., Snoeck, R., Andrei, G., Agrofoglio, L. A. (2018). Highly convergent synthesis and antiviral activity of (E)-but-2-enyl nucleoside phosphonoamidates. *European Journal of Medicinal Chemistry*, 146, 678-686. doi:https://doi.org/10.1016/j.ejmech.2018.01.086
- Bessieres, M., Sari, O., Roy, V., Warszycki, D., Bojarski, A. J., Nolan, S. P., Agrofoglio, L. A. (2016). Sonication-Assisted Synthesis of (E)-2-Methyl-but-2-enyl Nucleoside Phosphonate Prodrugs. *ChemistrySelect*, 1(12), 3108-3113. doi:10.1002/slct.201600879
- Hamada, M., Roy, V., McBrayer, T. R., Whitaker, T., Urbina-Blanco, C., Nolan, S. P., Agrofoglio, L. A. (2013). Synthesis and broad spectrum antiviral evaluation of bis(POM) prodrugs of novel acyclic nucleosides. *European*

- Journal of Medical Chemistry*, 67, 398-408.
doi:10.1016/j.ejmech.2013.06.053
- Jansa, P., Baszczynski, O., Dracinsky, M., Votruba, I., Zidek, Z., Bahador, G., Janeba, Z. (2011). A novel and efficient one-pot synthesis of symmetrical diamide (bis-amidate) prodrugs of acyclic nucleoside phosphonates and evaluation of their biological activities. *European Journal of Medical Chemistry*, 46(9), 3748-3754. doi:10.1016/j.ejmech.2011.05.040
- McGuigan, C., Murziani, P., Slusarczyk, M., Gonczy, B., Vande Voorde, J., Liekens, S., & Balzarini, J. (2011). Phosphoramidate ProTides of the anticancer agent FUDR successfully deliver the preformed bioactive monophosphate in cells and confer advantage over the parent nucleoside. *J Med Chem*, 54(20), 7247-7258. doi:10.1021/jm200815w
- Nakamura, M., Kanda, T., Haga, Y., Sasaki, R., Wu, S., Nakamoto, S., Yokosuka, O. (2016). Sofosbuvir treatment and hepatitis C virus infection. *World J Hepatol*, 8(3), 183-190. doi:10.4254/wjh.v8.i3.183
- Pertusati, F., Hinsinger, K., Flynn, A. S., Powell, N., Tristram, A., Balzarini, J., & McGuigan, C. (2014). PMPA and PMEA prodrugs for the treatment of HIV infections and human papillomavirus (HPV) associated neoplasia and cancer. *European Journal of Medical Chemistry*, 78, 259-268. doi:10.1016/j.ejmech.2014.03.051
- Pertusati, F., Hinsinger, K., Flynn, Á. S., Powell, N., Tristram, A., Balzarini, J., & McGuigan, C. (2014). PMPA and PMEA prodrugs for the treatment of HIV infections and human papillomavirus (HPV) associated neoplasia and cancer. *European Journal of Medicinal Chemistry*, 78, 259-268. doi:http://doi.org/10.1016/j.ejmech.2014.03.051
- Pertusati, F., Serafini, S., Albadry, N., Snoeck, R., & Andrei, G. (2017). Phosphonoamidate prodrugs of C5-substituted pyrimidine acyclic nucleosides for antiviral therapy. *Antiviral Res*, 143, 262-268. doi:http://dx.doi.org/10.1016/j.antiviral.2017.04.013
- Pradère, U., Clavier, H., Roy, V., Nolan, S. P., & Agrofoglio, L. A. (2011). The Shortest Strategy for Generating Phosphonate Prodrugs by Olefin Cross-Metathesis – Application to Acyclonucleoside Phosphonates. *European Journal of Organic Chemistry*, 2011(36), 7324-7330. doi:10.1002/ejoc.201101111
- Pradere, U., Garnier-Amblard, E. C., Coats, S. J., Amblard, F., & Schinazi, R. F. (2014). Synthesis of Nucleoside Phosphate and Phosphonate Prodrugs. *Chemical Reviews*, 114(18), 9154-9218. doi:10.1021/cr5002035
- Ray, A. S., Fordyce, M. W., & Hitchcock, M. J. M. (2016). Tenofovir alafenamide: A novel prodrug of tenofovir for the treatment of Human Immunodeficiency Virus. *Antiviral Res*, 125, 63-70. doi:http://dx.doi.org/10.1016/j.antiviral.2015.11.009
- Slusarczyk, M., Lopez, M. H., Balzarini, J., Mason, M., Jiang, W. G., Blagden, S., McGuigan, C. (2014). Application of ProTide Technology to Gemcitabine: A Successful Approach to Overcome the Key Cancer Resistance Mechanisms Leads to a New Agent (NUC-1031) in Clinical Development. *Journal of Medicinal Chemistry*, 57(4), 1531-1542. doi:10.1021/jm401853a
- Topalis, D., Pradère, U., Roy, V., Caillat, C., Azzouzi, A., Broggi, J., Agrofoglio, L. A. (2011). Novel Antiviral C5-Substituted Pyrimidine Acyclic Nucleoside

Phosphonates Selected as Human Thymidylate Kinase Substrates. *Journal of Medicinal Chemistry*, 54(1), 222-232. doi:10.1021/jm1011462